レミマゾラムベシル酸塩
アネレム静注用 50mg

第 2 部 CTD の概要
2.6 非臨床試験の概要文及び概要表
2.6.1 緒言

ムンディファーマ株式会社

略号一覧表

略語・用語	内容
$GABA_A$受容体	A 型 γ-アミノ酪酸受容体

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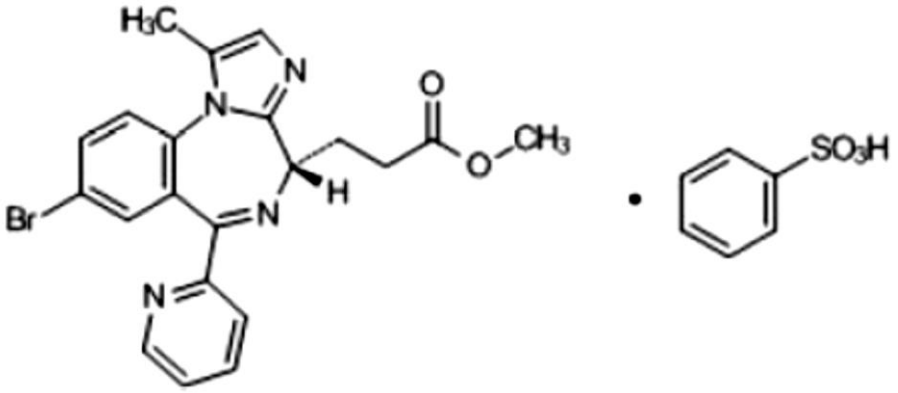
2.6 非臨床試験の概要文及び概要表

2.6.1 緒言

(1) 医薬品に関する情報

レミマゾラムベシル酸塩は全身麻酔の導入及び維持に用いる短時間作用型の新規ベンゾジアゼピン系薬剤であり、静脈内投与剤として開発されている。本薬の化学名は、Methyl 3-{(4*S*)-8-bromo-1-methyl-6-pyridin-2-yl-4*H*-imidazo[1,2-*a*][1,4]benzodiazepin-4-yl} propanoate monobenzensulfonate である。化学構造式を以下に示す（図 2.6.1-1）



レミマゾラム　　ベシル酸

図 2.6.1-1 レミマゾラムベシル酸塩（CNS 7056B/ ONO2745BS）の化学構造式

(2) 薬力学的特性

レミマゾラムは、$GABA_A$ 受容体のベンゾジアゼピン結合部位に作用することで麻酔・鎮静作用を発現する。エステル構造を有し、主に肝臓のカルボキシエステラーゼにより速やかに薬理活性のない CNS7054 に代謝される。また、レミマゾラムの受容体結合能及び特異性を *in vitro* で評価した結果、$GABA_A$ 受容体を除き、各種受容体、酵素及びイオンチャンネルに対する親和性は認められなかった。

なお、本剤の効能・効果、用法・用量は添付文書（案）（CTD 1.8）に記載した。

レミマゾラムベシル酸塩
アネレム静注用 50mg

第 2 部 CTD の概要
2.6 非臨床試験の概要文及び概要表
2.6.2 薬理試験の概要文

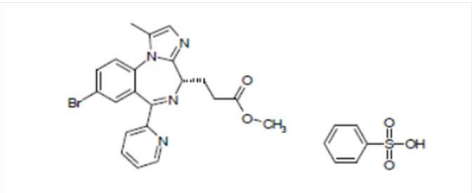
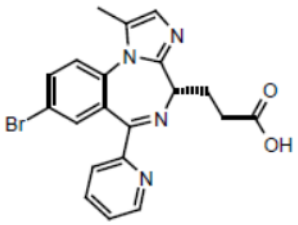
ムンディファーマ株式会社

略号一覧表

略語・用語	内容
ADP	アデノシン 5'-二リン酸ナトリウム塩（adenosine 5'-diphosphate sodium salt）
APD_{30}	30%再分極時の活動電位持続時間（30% action potential duration）
APD_{50}	50%再分極時の活動電位持続時間（50% action potential duration）
APD_{90}	90%再分極時の活動電位持続時間（90% action potential duration）
ECG	心電図（electrocardiogram）
EC_{50}	50%有効濃度（50% effective concentration）
$ED_{20(BP)}$	平均血圧を 20%低下させる投与量（dose at which 20% drop in mean blood pressure was observed）
ED_{50}	50%有効用量（50% effective dose）
$ED_{50(LRR)}$	50%の動物に LRR を発現する投与量（dose at which 50% of animals had LRR）
E_{max}	最大効力（maximum effect）
GABA	γ-アミノ酪酸 (gamma-aminobutyric acid)
HEK293	ヒト胎児腎細胞 293（human embryonic kidney cells 293）
hERG	ヒト ether-a-go-go 関連遺伝子（human ether-a-go-go-related gene）
IC_{25}	25%阻害濃度（25% inhibitory concentration）
IC_{50}	50%阻害濃度（50% inhibitory concentration）
Ki	阻害定数（inhibition constant）
LD_{50}	50%致死量（50% lethal dose）
LRR	正向反射消失（loss of righting reflex）
NOAEL	無毒性量（no observed adverse effect level）
NOEL	無影響量（no observed effect level）
pKi	阻害定数 Ki の値の常用対数の絶対値（-log 10 of inhibition constant Ki）
pCO_2	二酸化炭素分圧（carbon dioxide partial pressure）
pO_2	酸素分圧（oxygen partial pressure）
QTc	補正 QT（QT interval corrected）
QTcB	心拍数補正 QT（QT interval corrected for heart rate）
RMP	静止膜電位（resting membrane potential）
SD	Sprague Dawley
SNpr	黒質網様部（substantia nigra pars reticulata）
SO_2	酸素飽和度（oxygen saturation）

レミマゾラム及び類縁物質一覧表

表 レミマゾラム及び類縁物質の科学名と化学構造式

略称	化学名	化学構造式
レミマゾラムベシル酸塩 CNS 7056 besylate ONO-2745BS ONO-IN-251BS	Methyl 3-[(4*S*)-8-bromo-1-methyl-6-pyridin-2-yl-4*H*-imidazo[1,2-*a*][1,4]benzodiazepin-4-yl]propanoate monobenzenesulfonate	
[redacted]	[redacted]	[redacted]
レミマゾラムの主代謝物 CNS 7054 ONO-IN-252	3-[(4S)-8-bromo-1-methyl-6-pyridin-2-yl-4H-imidazo[1,2-a][1,4]benzodiazepin-4-yl]propanoic acid	

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2.6.2 薬理試験の概要文

2.6.2.1 まとめ

レミマゾラムベシル酸塩（以下、レミマゾラム）はベンゾジアゼピン系薬剤であるが、この薬物クラスの他の薬剤とは代謝様式及び代謝速度が異なっている。レミマゾラムは脳内のベンゾジアゼピン結合部位［γ-アミノ酪酸（GABA）$_A$受容体］へ高親和性に結合するアゴニストとして働き、off-target 活性は認められていない。レミマゾラムの鎮静作用評価、呼吸系及び心血管系に対する潜在的作用の検討及び臨床でレミマゾラムと併用が想定される薬剤との薬力学的薬物相互作用の同定のため、げっ歯類、ミニブタ、ヒツジ及びサルを用いた多数の試験を実施した。

以下、本項の各試験における本剤の投与量は、特に記載のない限りレミマゾラム遊離塩基量として記載した。

レミマゾラムは試験で使用した全動物種において、発現が速やかで持続が短い鎮静作用を示した。レミマゾラムによる鎮静作用はフルマゼニルで回復した。[redacted] 又はカルボン酸代謝物である CNS 7054 は試験で用いた用量において鎮静作用を示さなかった。

検討した濃度範囲で、レミマゾラムは $GABA_A$ 受容体以外の各種受容体、トランスポーター及びイオンチャネルに対して明らかな作用を示さなかった。主代謝物である CNS 7054 は、検討した濃度範囲で各種受容体、トランスポーター及びイオンチャネルに対して明らかな作用を示さなかった。レミマゾラムはミダゾラムと同様、ラット受動回避反応試験において健忘作用を示した。

レミマゾラムは *in vitro* で $GABA_A$ 受容体を活性化するのに必要な濃度及び毒性試験又は臨床試験で達成し得る血漿中濃度よりもはるかに高濃度において、ヒト ether-a-go-go 関連遺伝子（hERG）電流に対する弱い阻害作用を示した。

レミマゾラムは、ミニブタ、ヒツジ及びサルにおいて、呼吸系又は心血管系への過度な抑制を示すことなく鎮静作用を示した。レミマゾラムはラットの血圧を低下させた。

臨床で併用が想定される抗不安薬、抗コリン薬、オピオイド系鎮痛薬、その他の鎮静薬及び麻酔薬がレミマゾラムの鎮静作用に及ぼす作用について評価した。その結果、抗コリン薬のアトロピン及びベンゾジアゼピン系薬剤のミダゾラムを除き、レミマゾラムはほとんどの薬剤との併用で相乗的な鎮静作用を示した。レミマゾラムにより生じる血圧低下は、ドパミン及びフェニレフリンとの併用で減弱し、ランジオロール、ミルリノン、ニカルジピン、ニトログリセリン、アルプロスタジル及びセボフルランとの併用で増強し、リドカイン及びジゴキシンとの併用では影響を受けなかった。しかしながら、セボフルランとの併用において鎮静作用を発現する用量と血圧低下が増強される用量には十分な乖離が認められた。レミマゾラムはラットの心拍数を増加させた。この心拍数増加はドパミン、フェニレフリン、リドカイン、ランジオロール、ジゴキシン及びニカルジピンとの併用で減弱し、ミルリノン、ニトログリセリン及びアルプロスタジルとの併用では影響を受けなかった。

2.6.2.2 効力を裏付ける試験

2.6.2.2.1 *In vitro* 試験

レミマゾラムは体内で急速に分解されるように設計されたベンゾジアゼピンエステル誘導体である。レミマゾラム及び主代謝物 CNS 7054 の活性を確認するため、*in vitro* 受容体結合試験を実施した。

2.6.2.2.1.1 $GABA_A$ 受容体ベンゾジアゼピン結合部位への結合

試験番号：[redacted]（参考資料）、記載箇所：4.2.1.1.1
試験番号：[redacted]（参考資料）、記載箇所：4.2.1.1.2
試験番号：[redacted]（評価資料）、記載箇所：4.2.1.1.3
試験番号：[redacted]（評価資料）、記載箇所：4.2.1.1.4
試験番号：[redacted]（参考資料）、記載箇所：4.2.1.1.5

$GABA_A$ 受容体ベンゾジアゼピン結合部位に対するレミマゾラムの結合親和性を評価した結果、10 nmol/L～10 µmol/L の濃度での評価で、50%阻害濃度（IC_{50}）= 10.5 nmol/L を示した（[redacted]）。レミマゾラムの主代謝物である CNS 7054（10 µmol/L）は、$GABA_A$ 受容体ベンゾジアゼピン結合部位に対して明らかな作用を示さなかった（[redacted]）。$GABA_A$ 受容体ベンゾジアゼピン結合部位に対するレミマゾラム、CNS 7054 及びミダゾラムの IC_{50} 値はそれぞれ 30.5 nmol/L、5130 nmol/L 及び 6.58 nmol/L であり、阻害定数（Ki）はそれぞれ 26.3 nmol/L、4420 nmol/L 及び 5.66 nmol/L であった（[redacted]）。以上の結果から、レミマゾラムは $GABA_A$ 受容体ベンゾジアゼピン結合部位に高い結合親和性を示し、主代謝物である CNS 7054 は約 170 倍低い結合親和性を示すと結論された（表 2.6.2-1）。追加試験（[redacted]）でも、レミマゾラムは放射能標識ムシモールの $GABA_{A1}$ への結合を 62.1%抑制したが、CNS 7054 は意味のある作用（Control-specific binding が 50%を超える阻害又は亢進）を示さず、同様の結果を得た。

表 2.6.2-1 ラット脳 $GABA_A$ 受容体のベンゾジアゼピン結合部位に対する結合親和性

Remimazolam Ki (nmol/L)	CNS 7054 Ki (nmol/L)	Separation ratio
26.3	4420	~170-fold

n = 2
Separation ratio was calculated from Ki of CNS 7054/ Ki of remimazolam.
Data from[redacted]

別試験（[redacted]）でもレミマゾラムは $GABA_A$ 受容体ベンゾジアゼピン結合部位に高い結合親和性を示した。ヒト、ミニブタ及びラットの脳組織を用いた放射性リガンド［^3H］フルニトラゼパムとの競合的結合阻害試験でのレミマゾラムの Ki 値は約 30 nmol/L［Ki の常用対数の絶対値（pKi）：約 7.5］であった（表 2.6.2-2）。レミマゾラムの主代謝物である CNS 7054 は顕著な低親和性を示し、Ki 値は約 10000 nmol/L（pKi 値：約 5.0）であった。レミマゾラム及び主代謝物である CNS 7054 の親和性の乖離はヒトで 410 倍、ラット及びブタで 320 倍程度であった。

表 2.6.2-2 ［^{3}H］フルニトラゼパム結合に対する競合的結合阻害

Species	Remimazolam pKi	CNS 7054 pKi	Separation ratio
Human	7.53 ± 0.10	4.91 ± 0.22	410-fold
Rat	7.50 ± 0.09	4.99 ± 0.06	320-fold
Yucatan Micropig	7.56 ± 0.10	5.05 ± 0.04	326-fold

Results are expressed as mean ± standard error of the mean of 4-8 separate experiments.
Separation was calculated from Ki of CNS 7054/ Ki of remimazolam.
Data from [redacted]

2.6.2.2.1.2 GABA$_A$ 受容体サブタイプ遺伝子導入細胞の GABA 電流に対する調節作用

試験番号：[redacted]（参考資料）、記載箇所：4.2.1.1.6

種々のラット GABA$_A$ 受容体サブタイプ（α1β2γ2、α2β2γ2、α3β2γ2、α5β2γ2）を遺伝子導入した Ltk 細胞（マウス線維芽細胞）の GABA 電流に対するレミマゾラムの調節作用を、ホールセルパッチクランプ法を用いて評価した（[redacted]）。ミダゾラム（0.03～3 μmol/L）と同様に、レミマゾラム（0.1～10 μmol/L）は評価した全ての GABA$_A$ 受容体サブタイプ遺伝子導入細胞の GABA 電流を濃度依存的に増強した。両薬剤の 50%有効濃度（EC_{50}）及び最大効力（E_{max}）を表 2.6.2-3 に示す。ミダゾラムと同様に、レミマゾラムは GABA$_A$ 受容体サブタイプ間で明確な選択性を示さなかった。

表 2.6.2-3 GABA$_A$ 受容体サブタイプ遺伝子導入 Ltk 細胞の GABA 電流（2 μmol/L GABA）に対するレミマゾラム及びミダゾラムの促進作用

	Remimazolam		Midazolam	
Subtype	EC_{50} (nmol/L)	E_{max}	EC_{50} (nmol/L)	E_{max}
α1β2γ2	327	371%	138	295%
α2β2γ2	565	184%	254	192%
α3β2γ2	899	199%	264	290%
α5β2γ2	1426	277%	124	110%

n = 3~7 individual cells
Data from [redacted]

2.6.2.2.2 *In vivo* 試験

2.6.2.2.2.1 マウスでの鎮静作用

試験番号：[redacted]（評価資料）、記載箇所：4.2.1.1.7
試験番号：[redacted]（評価資料）、記載箇所：4.2.1.1.8

正向反射消失（LRR）を鎮静作用の指標として、レミマゾラム（15～30 mg/kg）を雄 Rj: NMRI マウスに急速静注した場合の作用を評価した（[redacted] および [redacted]）。投与量の増加と共に LRR 発現数は増加し、LRR 発現までの時間は短縮した。LRR 持続時間は最高用量でも短かった（< 10 分間）。レミマゾラム（30 mg/kg、急速静注）投与の 15 分前にベンゾジアゼピン拮抗薬であるフルマゼニル（20 mg/kg）を腹腔内投与しておくと、LRR 持続時間が短縮し、LRR 発現数が減少した。[redacted] 又は CNS 7054 は検討した投与量（30～100 mg/kg、急速静注）では LRR を発現しなかった（表 2.6.2-4）。フルマゼニル前投与の有無に関わらず、レミマゾラム処置による死亡は認められなかった。

比較に用いたミダゾラム（15～50 mg/kg、急速静注）は 40 及び 50 mg/kg の投与量で持続時間の長い LRR（32～65 分間）を発現した。フルマゼニルはミダゾラムによる鎮静作用を減弱させる

傾向を示したが、統計学的有意差は認められなかった。ミダゾラムは 50 mg/kg を単剤で投与した場合に 16 匹中 1 匹、フルマゼニルとの併用で 16 匹中 4 匹の死亡が認められた。

プロポフォール（10～40 mg/kg、急速静注）は 20～40 mg/kg で速やかに用量依存的な鎮静作用を発現し、LRR 持続時間は 10～22 分間であった。

要約すると、レミマゾラムはマウスにおいて LRR の発現が速やかで、持続時間が短い鎮静作用を示した。フルマゼニルはレミマゾラムによる鎮静作用に対して有意な拮抗作用を示すが、ミダゾラムによる鎮静作用に対しては有意な拮抗作用を示さなかった。また、ミダゾラムは単独投与又はフルマゼニルとの併用投与で計 5 匹が死亡したのに対し、レミマゾラム投与による死亡は認められなかったことから、少なくともマウスにおいて、レミマゾラムの安全上の優位性が示唆されたと考えられる。

表 2.6.2-4　マウスでのレミマゾラム及び比較対照薬の鎮静作用

Treatment	Dose (mg/kg)	No. of animals with LRR / Total animals	Latency to LRR (min, mean ± SEM)	Duration of LRR (min, mean ± SEM)	Study No.
Remimazolam	30	6 / 8	0.4 ± 0.1	8.4 ± 2.6	a
	30	6 / 8	1.2 ± 0.2	3.6 ± 1.8	b
[redacted]	30	0 / 8	-	0.0 ± 0.0	b
CNS 7054	100	0 / 8	-	0.0 ± 0.0	b
Midazolam	50	7 / 8	1.2 ± 0.4	37.4 ± 3.5	a
	50	6 / 8	1.2 ± 0.3	65.1 ± 14.3	b
Propofol	20	8 / 8	0.0 ± 0.0	10.0 ± 0.6	b

a: [redacted] (results of Experiment 3)
b: [redacted]
LRR: loss of righting reflex
SEM: standard error of the mean

2.6.2.2.2.2　ラットでの鎮静作用

試験番号：[redacted]（評価資料）、記載箇所：4.2.1.1.9
試験番号：[redacted]（参考資料）、記載箇所：4.2.1.1.10
試験番号：[redacted]（参考資料）、記載箇所：4.2.1.1.11
試験番号：[redacted]（参考資料）、記載箇所：4.2.1.1.12
試験番号：[redacted]（参考資料）、記載箇所：4.2.1.1.13
試験番号：[redacted]（参考資料）、記載箇所：4.2.1.1.14
試験番号：[redacted]（参考資料）、記載箇所：4.2.1.1.15
試験番号：[redacted]（参考資料）、記載箇所：4.2.1.1.16
試験番号：[redacted]（参考資料）、記載箇所：4.2.3.1.2
試験番号：[redacted]（評価資料）、記載箇所：4.2.1.1.17
試験番号：[redacted]（参考資料）、記載箇所：4.2.1.1.18
試験番号：[redacted]（評価資料）、記載箇所：4.2.1.1.19
試験番号：[redacted]（評価資料）、記載箇所：4.2.1.1.20
試験番号：[redacted]（参考資料）、記載箇所：4.2.1.4.2
試験番号：[redacted]（参考資料）、記載箇所：4.2.1.4.4
試験番号：[redacted]（参考資料）、記載箇所：4.2.1.4.7
試験番号：[redacted]（参考資料）、記載箇所：4.2.1.4.8

試験番号：[redacted]（参考資料）、記載箇所：4.2.1.4.10
試験番号：[redacted]（参考資料）、記載箇所：4.2.1.4.12

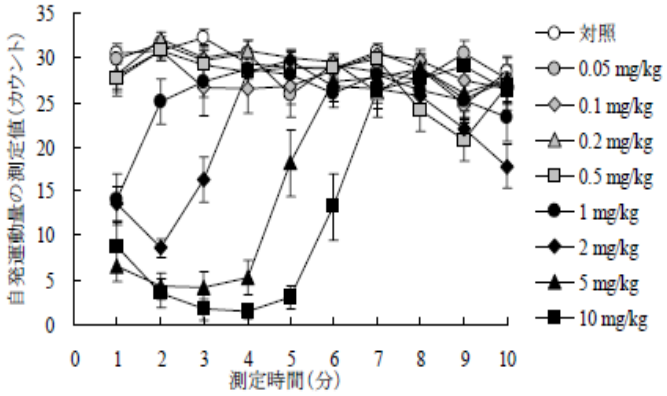
LRR、運動失調及び自発運動量をパラメータとしてラットでのレミマゾラムの鎮静作用を評価した（[redacted]、[redacted]）。対照薬として、プロポフォール、ミダゾラム及びデクスメデトミジンを用いた。

レミマゾラム（0.05～10 mg/kg、急速静注）では用量依存的な自発運動量の低下が認められた（図 2.6.2-1）。本試験（[redacted]）及びその予備検討試験（[redacted]）において、自発運動量の有意な低下は≥1 mg/kg で認められ、低下作用の持続時間は短かった（最高用量で最大 7 分間）。プロポフォール（0.2～10 mg/kg、急速静注）は 5 mg/kg 以上で有意な自発運動量の低下が短時間認められた。ミダゾラム（≥0.05 mg/kg）及びデクスメデトミジン（≥0.2 μg/kg）は有意な自発運動量の低下が長時間認められた。

ベンゾジアゼピン系薬剤は $GABA_A$ 受容体で神経伝達物質の GABA の働きを増強することにより中枢神経系に作用する。GABA はラット脳の黒質網様部（SNpr）の神経活動を迅速に阻害し、ベンゾジアゼピン系薬剤はこの阻害作用を増強する。雄 Sprague Dawley（SD）ラット（n = 7～8／群）において、レミマゾラム（0.05～8 mg/kg、急速静注）は SNpr 細胞発火をベースライン発火頻度の 20%まで阻害し、50%有効用量（ED_{50}）は 0.83 mg/kg であった。レミマゾラムの最大阻害作用は従来のベンゾジアゼピン系薬剤の作用（最大阻害はベースラインから 40%～50%）を上回った。レミマゾラム（8 mg/kg、急速静注）により阻害された SNpr 細胞発火は、投与 7 分後に完全に回復した（ベースライン発火頻度の 98%）（[redacted]）。

なお、ラットを用いた鎮静作用の評価に先立ち、試験条件設定のため以下に示す予備試験を実施した：E07CS027 試験（ベンゾジアゼピン拮抗薬により阻害されるレミマゾラムの鎮静作用、レミマゾラムでは認められないミダゾラムによる再鎮静作用）、[redacted] 試験（同用量のミダゾラムと比較してより短いレミマゾラムの鎮静作用の持続及び回復時間）、及び[redacted] 試験［鎮静作用評価に最適な溶媒及びラット系統の検討（pH 3.3 に調整した生理食塩液及び SD ラットを選定）、レミマゾラムの致死量の検討（100 mg/kg と判断）］。

図 2.6.2-1　ラット自発運動量に対するレミマゾラムの効果



Mean values ± standard error of the mean (SEM) of 10 rats per group are shown. Data from [redacted]

[redacted] 試験では、レミマゾラム、ミダゾラム、プロポフォール及びデクスメデトミジン投与後、用量依存的に LRR を発現する動物数が増加し、溶媒対照群と比べて、それぞれ≥5 mg/kg、

≥20 mg/kg、≥5 mg/kg 及び≥10 μg/kg で有意な作用が認められた。レミマゾラム（20 mg/kg）、ミダゾラム（50 mg/kg）、プロポフォール（10 mg/kg）及びデクスメデトミジン（20 μg/kg）が全ての動物に LRR を発現する最小用量において、LRR を発現するまでの時間はそれぞれ 0.8、0.6、0.3 及び 2.3 分間で、LRR の持続時間はそれぞれ 6.8、49.2、10.7 及び 61.9 分間であった。さらに、いずれの薬剤も用量依存的に運動失調を発現する動物数が増加し、溶媒対照群と比べて、それぞれ≥0.5 mg/kg、≥0.2 mg/kg、≥2 mg/kg 及び≥5 μg/kg で有意な作用が認められた。同様の結果は予備試験（[redacted]）でも認められた。致死性については 2.6.6.2.1 項に詳細を記載した。

これらの化合物の ED_{50} 値及び 50%致死量（LD_{50}）をそれぞれ LRR 発現匹数及び死亡匹数から算出し、有効用量と致死量間の安全域（LD_{50}/ED_{50}）を算出した（表 2.6.2-5）。レミマゾラムはプロポフォール及びミダゾラムより優れた安全域を示した。

表 2.6.2-5　ラットでのレミマゾラム及び比較対照薬（急速静注）の ED_{50}、LD_{50} 及び安全域

	ED_{50} (mg/kg)	LD_{50} (mg/kg)	Safety margin (LD_{50} / ED_{50})	Study No.
Remimazolam	3.9 4.7 4.3	90 > 100*	23-fold ≥ 23-fold	[redacted]
Midazolam	14 15	≥ 100	≥ 7.1-fold	[redacted]
Propofol	4.4 4.7	22	5-fold	[redacted]
Dexmedetomidine	0.0086	≥ 1	≥ 120-fold	[redacted]

*: dose with 4/12 lethal incidences (LD_{50} not calculated, highest dose tested)

レミマゾラム（0.5～100 mg/kg、11 用量）とミダゾラム（10 mg/kg）の鎮静作用を比較した試験（13-0291PD）では、雌雄 SD ラット（12 匹／群）にレミマゾラム、ミダゾラム又は生理食塩液を尾静脈内投与した。LRR 発現数から算出した ED_{50} 値はレミマゾラムで 4.32 mg/kg であった。LRR の発現が認められた最小用量は 2 mg/kg であり（12 匹中 2 匹）、統計学的有意差は 5 mg/kg から認められた（12 匹中 7 匹）。運動失調は評価しなかった。同用量（10 mg/kg）において、レミマゾラムの LRR 発現作用（12 匹中 11 匹）はミダゾラム（12 匹中 3 匹）よりも顕著であった。最大非致死量は 80 mg/kg であった（詳細は 2.6.6.2.1 項）。

4 種類の鎮静剤を比較して異なる特性が明確になった（[redacted]、[redacted]）。レミマゾラム及びプロポフォールの LRR 発現時間及び持続時間は短く、デクスメデトミジンの LRR 発現時間は最も長かった。またミダゾラム及びデクスメデトミジンはレミマゾラム及びプロポフォールと比べて明らかに長い LRR 持続時間を示した。LRR からの回復に続く運動失調からの回復を含めた LRR からの完全回復時間は、レミマゾラムが最も短く、次にプロポフォールが続いた。ミダゾラムの LRR からの完全回復時間は非常に長かった。各薬剤において全動物で LRR の発現が認められた最小用量での LRR 発現時間及び持続時間並びに LRR からの回復時間を示す（表 2.6.2-6）。

表 2.6.2-6　全動物で LRR 発現が認められた最小用量でのレミマゾラム及び比較対照薬の LRR 発現及び持続時間並びに LRR からの回復時間

Treatment	Dose (mg/kg, i.v.)	Onset of LRR (min, mean ± SEM)	Duration of LRR (min, mean ± SEM)	Recovery from ataxia after LRR (min, mean ± SEM)	Study No.
Remimazolam	20	0.8 ± 0.34	6.8± 0.46	9.6 ± 0.69	a
		0.4 ± 0.12	4.5± 0.90	9.9 ± 0.38	b
Midazolam	50	0.6 ± 0.07	49.2± 9.69#	120.0 ± 0.00	a
		0.3 ± 0.08	37.4± 4.24	221.7 ± 6.96	b
Propofol	10	0.3 ± 0.01	10.7 ± 0.72	14.0 ± 0.68	a
		0.1 ± 0.03	7.9 ± 0.86	12.1 ± 0.58	b
Dexmedetomidine hydrochloride	0.02	2.3 ± 0.52	61.9 ± 5.73	74.6 ± 8.91	a
		4.2 ± 0.88	42.7 ± 4.86	67.6 ± 6.94	b

LRR: loss of righting reflex, i.v.: intravenous
#: animals with no confirmed recovery from LRR or ataxia within 120 min after onset of LRR were included. The data of these animals was defined as 120 min.
SEM: standard error of the mean, n=10
a: [redacted]
b: [redacted]

レミマゾラム（25 mg/kg、急速静注）により LRR を発現した 6 匹中 6 匹では Haffner 法による疼痛反射、角膜反射及び耳介反射がいずれも認められなかったが、ミダゾラム（25 mg/kg、急速静注）により LRR を発現した 6 匹中 2 匹では Haffner 法による疼痛反射が認められた（[redacted]）。ミダゾラムと比較してレミマゾラムの LRR からの回復時間（それぞれ 25 及び 10 分間）及び運動失調からの回復時間（それぞれ 132 及び 12 分間）は明らかに短かった。

溶媒検討の結果、25 mmol/L クエン酸緩衝液 pH 3.8 に 25.86% w/v β-シクロデキストリン（Captisol®）を添加してもレミマゾラムの鎮静作用に影響は認められなかった（[redacted]）。Lot no. [redacted] のレミマゾラム原薬で検出された新たな不純物はレミマゾラムの鎮静作用に影響しなかった。不純物の一つである [redacted] の鎮静作用強度はレミマゾラムの 25%程度と推定された（[redacted]）。原薬における含量は 0.10%以下に管理されていることから、臨床における鎮静作用への影響はないと判断した。

マウスの試験結果と同様に、ベンゾジアゼピン拮抗薬であるフルマゼニルはラットでのレミマゾラムの鎮静作用を回復させた（[redacted]）。

レミマゾラム（30 mg/kg）を急速静注して LRR の発現を確認した後、レミマゾラム投与 2 分後にフルマゼニル（0.05～1 mg/kg）を急速静注すると、フルマゼニル投与 1 分後に回復する動物数は用量依存的に増加し、0.2 mg/kg 以上の群で溶媒群と比較して有意な増加が認められた。またレミマゾラム投与群において、フルマゼニル投与 20 分後までに LRR の再発現は認められなかった。非ベンゾジアゼピン系薬剤プロポフォール（10 mg/kg）の鎮静作用はフルマゼニル（10 mg/kg、急速静注）で回復しなかった。ミダゾラム（50 mg/kg、急速静注）により発現した LRR は 5 及び 10 mg/kg のフルマゼニルで有意に回復したが、全てのフルマゼニル投与群（2、5 及び 10 mg/kg、急速静注）で LRR の再発現が認められた。

以上のことから、レミマゾラムの鎮静作用はフルマゼニルにより用量依存的に回復すること、またミダゾラムと比べて低用量のフルマゼニルで回復することが明らかになった。また、フルマゼニルに対する反応性の違いから、レミマゾラムはミダゾラム及びプロポフォールと比較して、鎮静作用を急遽中断する必要性が生じた場合の対応処置において、より安全性の高い薬剤になる可能性が考えられた。

レミマゾラムの作用が最も低用量から認められる運動失調（よろめき歩行又は腹臥位の状態）を指標として、レミマゾラム及び[redacted]並びに主代謝物である CNS 7054 の鎮静作用を低用量で比較した（[redacted]）。投与後 10 分間以内の運動失調発現匹数に統計学的有意差が認められる最小用量を算出し、各薬物の鎮静作用を比較した。レミマゾラム、[redacted] 及び CNS 7054 は用量依存的に運動失調を発現し、それぞれ 0.5、20 及び 100 mg/kg で有意な作用を示した。[redacted]は 100 mg/kg で運動失調を発現しなかった。したがって、CNS 7054 及び[redacted] の鎮静作用強度は、レミマゾラムと比較するとそれぞれ 1/200 及び 1/40 であると考えられた[redacted] は投与可能な最大用量である 100 mg/kg でも鎮静作用を示さなかった。

ラットを用いた種々の試験間での鎮静作用を比較するため、各薬力学試験及び薬物相互作用試験においてレミマゾラムが 50%の動物に LRR を発現する投与量［$ED_{50(LRR)}$］を表 2.6.2-7 にまとめた。全試験でのレミマゾラムの $ED_{50(LRR)}$値の平均値 ± 標準偏差は 3.71 ± 0.95 mg/kg であった。

表 2.6.2-7　ラットでのレミマゾラム及び比較対照薬（急速静注）の $ED_{50(LRR)}$値

Treatment	$ED_{50(LRR)}$ (mg/kg)	Study No.
Remimazolam	3.9	[redacted]
	4.7	[redacted]
	4.1	[redacted]
	2.1 and 2.2*	[redacted]
	4.3	[redacted]
	3.7	[redacted]
	4.3	[redacted]
	4.7	[redacted]
	3.1	[redacted]
Midazolam	14	[redacted]
	15	[redacted]
	15	[redacted]
Propofol	4.4	[redacted]
	4.7	[redacted]
	3.6	[redacted]

*: 2 different lots

2.6.2.2.2.3　ミニブタでの鎮静作用

試験番号：[redacted]（評価資料）、記載箇所：4.2.1.1.21
試験番号：[redacted]（参考資料）、記載箇所：4.2.1.4.20
試験番号：[redacted]（参考資料）、記載箇所：4.2.1.1.22
試験番号：[redacted]（参考資料）、記載箇所：4.2.1.3.13
試験番号：[redacted]（評価資料）、記載箇所：4.2.1.1.23
試験番号：[redacted]（参考資料）、記載箇所：4.2.1.1.24
試験番号：[redacted]（評価資料）、記載箇所：4.2.1.1.25

ミニブタを用いた試験では動物数が少なく ED_{50} 値が算出できないため、試験間及び系統間でのレミマゾラムの鎮静作用の評価は完全な鎮静作用を発現するのに必要な最小用量（鎮静用量）を基準とした。一般鎮静症状スコアにおける症状 4 の「背部又は腹臥位」（スコア 4）を完全な鎮静作用発現と評価した。レミマゾラムの鎮静用量は 1～3 mg/kg の間で変動し、系統間（ユカタン系及び NIBS 系）で明らかな違いは認められなかった。ミダゾラムの鎮静用量はユカタン系と比べて NIBS 系で低かった。プロポフォールは NIBS 系を用いた 1 試験のみで評価され、その鎮静用

量はレミマゾラム及びミダゾラムと比べて、高かった（表 2.6.2-8）。

表 2.6.2-8　NIBS 及びユカタン系ミニブタでのレミマゾラム及び比較対照薬の鎮静作用

Study No. (Strain); method of administration	Remimazolam	Midazolam	Propofol
	Sedative dose (mg/kg or mg/kg/h)		
[redacted] (NIBS); i.v. bolus	1*	-	-
[redacted] (NIBS): i.v. infusion	3.2(n = 2)*, 6.4(n = 1)*	0.1*	6.4*
[redacted] (Yucatan)[a]; i.v. bolus	1 (score 4-5[b]) 3 (score 5[b])	3 (score 4[b])	- -

*: the lowest dose needed to reach a general sedation score of 4, n=3
a: estimated values because report only presents graphic results of a composite score (general sedation, ptosis measure and pinna reflex)
b: maximum, 5.3
-: not tested

ユカタン系ミニブタ（n = 3／群）にレミマゾラム（0.05～3 mg/kg）を急速静注後、検討した全ての用量で速やかに鎮静作用を発現した（[redacted]）。

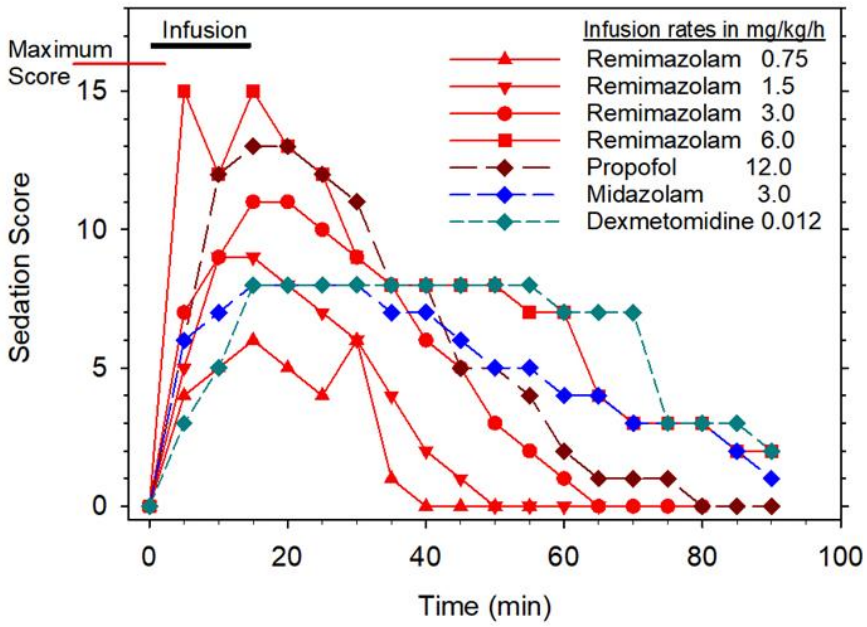
鎮静スコアリングシステム（最大スコア：5.3）において、レミマゾラムは深鎮静を発現し、3 mg/kg の鎮静スコアは 5 に達した。回復まで最大 60 分間かかった最高用量（3 mg/kg）を除き、レミマゾラムによる鎮静作用は速やかに（15～30 分）回復した。ミダゾラム（0.05～3 mg/kg、急速静注）はレミマゾラムと同様に速やかな鎮静作用を発現し、3 mg/kg の鎮静スコアは 4 に達したが、回復までにかかる時間はレミマゾラムより長かった（投与量に依存して 45～90 分）。

NIBS 系ミニブタで同様の鎮静スコアリングシステム（最大スコア：5.3）を用いた試験でも同様の結果が認められ、レミマゾラム（0.1、0.3 及び 1.0 mg/kg、急速静注）による用量依存的な鎮静作用（投与 2 分後の平均鎮静スコアはそれぞれ 1.4、1.9 及び 2.7）及び回復時間（それぞれ 20、45 及び 60 分）が認められた（[redacted]）。

ユカタン系ミニブタにおいて、レミマゾラム 26.4 μg/kg/min（14 分間）及びミダゾラム 45 μg/kg/min（19 分間）の静脈内持続投与は、同程度の深い鎮静作用（鎮静スコア：5.3）を発現した（[redacted]）。レミマゾラムは 30 分、ミダゾラムは 90 分以内に鎮静からの回復が認められ､急速静注の場合と同様にレミマゾラムはミダゾラムと比較して鎮静からの速い回復を示した。

各用量のレミマゾラム（最高 100 μg/kg/min、15 分間）を NIBS 系ミニブタ（n = 3／群）に静脈内持続投与し、鎮静作用を評価した（[redacted]）。比較対照薬としてプロポフォール、ミダゾラム及びデクスメデトミジンを用いた。鎮静スコアリングシステム（最大スコア：16）を用いた評価系において、レミマゾラムは用量依存性の鎮静作用を発現した。レミマゾラムの鎮静作用は発現及び消失がいずれも速やかであり、プロポフォールの鎮静作用プロファイルと類似していた。ミダゾラム及びデクスメデトミジンでは、鎮静からの回復により長い時間を要した。過用量 100 μg/kg/min のレミマゾラムは投与開始 5 分後から投与終了（投与開始 15 分後）まで平均スコア 15 を示し、投与終了 75 分後でも鎮静スコアの平均値は 2 を示した（図 2.6.2-2）。

図 2.6.2-2　NIBS 系ミニブタでのレミマゾラム及び比較対照薬（静脈内持続投与）の鎮静作用



Mean values of 3 male micropigs are shown in each group, comparing sedation scores of all tested remimazolam groups with those of the high dose groups of propofol, midazolam, and dexmedetomidine. A composite score was used combining assessment of sedative state, eyelid closure, and pinna reflex (maximum score = 16). Scores in the control group were zero in each instance.　Data from [redacted]

NIBS 系ミニブタを用いた試験で、レミマゾラムの静脈内持続投与により投与量に応じた一定の鎮静深度を維持できることが確認された（[redacted]、[redacted]、[redacted]、[redacted]）（表 2.6.2-9）。特に長期試験において一定の鎮静深度を維持するため投与量の調整が必要であった（[redacted]、[redacted]）。鎮静深度は、筋緊張、探索行動、背景刺激に対する反応及び歩行／姿勢を評価した一般鎮静症状スコアを測定し、各項目を 0 から 4 でスコア化した。

レミマゾラムの 28 日間静脈内持続投与は、ミニブタで安定した鎮静作用を 28 日間維持した（[redacted]）。長期投与期間中、鎮静維持投与量は徐々に増え、投与 28 日目でレミマゾラム及びミダゾラムの増加の程度は平均 2.0 及び 3.1 倍となった。鎮静作用の蓄積はなく、持続投与終了後に鎮静作用の延長は認められなかった。

レミマゾラムの鎮静導入血漿中濃度（導入投与終了時点）及び鎮静維持血漿中濃度（維持投与終了時点）の平均はそれぞれ 1670 及び 542 ng/mL であり、鎮静回復血漿中濃度（維持投与終了後、一般鎮静症状スコアが 3 を示した時点）は 318 ng/mL であった（[redacted]）。28 日間の静脈内持続投与期間中、動脈血ガス分圧［酸素分圧（pO_2）及び二酸化炭素分圧（pCO_2）］はミニブタの正常範囲を維持し、レミマゾラム及びミダゾラムとも異常は認められなかった（[redacted]）。

急速静注、短期又は長期静脈内持続投与のいずれの投与方法でも、レミマゾラムの鎮静作用は速やかな作用発現、投与中の安定した作用発現及び投与量に依存した速やかな回復を示した。

表 2.6.2-9　NIBS 系ミニブタでのレミマゾラム及びミダゾラム（静脈内持続投与）の鎮静作用（平均値）

Treatment	Sedation level [general sedation score]	Length of infusion	Dose needed for induction [mg/kg]	Maintenance dose [mg/kg/h]	Time until recovery (1 score below maintenance level / full recovery) [min]	No. of animals	Study No.
Remimazolam	4	70-148 min	1.1[a]	1.4[b]	16 / ND	6	[redacted]
	≥ 2	240 min	-	0.6 [c]	8 / 20	3	[redacted]
	≥ 3	240 min	-	1.8 [c]	15 / 50	3	
	4	240 min	-	6.0 [c]	35 / 90	3	
	2	7 days	0.3[d]	0.6 (day 1) to 1.3 (day 7)	ND	2	[redacted]
	2	28 days	0.3[d]	0.56 (day 1) to 1.16 (day 28)	ND / 67	6	[redacted]
Midazolam	2	7 days	0.03[e]	0.12 (day 1) to 0.15 (day 7)	ND	2	[redacted]
	2	28 days	0.03[e]	0.13 (day 1) to 0.39 (day 28)	ND / 110	6	[redacted]

a: Induction dosing was carried out at 0.6 mg/kg/min until general sedation score reached 4.
b: Maintenance dosing was started at 4.0 mg/kg/h and decreased at 30 min intervals to find the lowest dose required to maintain a general sedation score of 4.
c: At a given dose, a score of 4 had to be maintained for 30 min to qualify that dose as the maintenance dose. This does not exclude temporary attainment of score 4 at lower doses.
d: Induction dosing was carried out at 0.3 mg/kg/min until general sedation score reached 3.
e: Induction dosing was repeated at 0.03 mg/kg over 1 min every 5 min until general sedation score reached 3.
ND: Not determined
-: Not tested

2.6.2.2.2.4　サルでの鎮静作用

試験番号：[redacted]（評価資料）、記載箇所：4.2.1.3.14
試験番号：[redacted]（評価資料）、記載箇所：4.2.1.3.15
試験番号：[redacted]（評価資料）、記載箇所：4.2.3.2.7
試験番号：[redacted]（参考資料）、記載箇所：4.2.3.2.6
試験番号：[redacted]（評価資料）、記載箇所：4.2.3.1.5
試験番号：[redacted]（評価資料）、記載箇所：4.2.3.2.4
試験番号：[redacted]（参考資料）、記載箇所：4.2.3.2.8
試験番号：[redacted]（評価資料）、記載箇所：4.2.3.6.10
試験番号：[redacted]（評価資料）、記載箇所：4.2.3.1.6

カニクイザルにおけるレミマゾラムの鎮静作用に関しては、独立した各種薬力学的試験ではなく、安全性薬理試験及び毒性試験結果を用いて評価した。

投与した群内の全ての動物が、鎮静状態（横臥位、腹臥位、座位、起立不能、部分的又は完全な閉眼、刺激に対する反応性消失及び傾眠など）を示す最小用量を鎮静用量と定義した。レミマゾラムを急速静注した 3 試験での鎮静用量はいずれも 2 mg/kg 以上であった（表 2.6.2-10）。

表 2.6.2-10　カニクイザルでのレミマゾラム（急速静注）の鎮静作用

Mode of administration	No. and sex per group	Main sedation characteristics during or immediately after dosing	Sedative dose	Study No.
Single bolus	5 M	Ananastasia, lateral or prone position	2 mg/kg[a]	[redacted]
Single bolus	4 M	Eyelid closure, disappearance of touch response, somnolence	5 mg/kg[a]	[redacted]
4 week-RDT, 1 bolus/day	3 M/3 F or 5 M/5 F	lateral or prone position, unresponsiveness, ataxia	5 mg/kg[b]	[redacted]
4 week-RDT, 1 bolus/week	4 M/4 F	Eyelid closure, muscle relaxation	12.5 mg/kg[b]	[redacted]

F: female, M: male, RDT: repeated dose toxicity study
a: highest dose tested
b: lowest dose tested

レミマゾラムによる鎮静作用発現からの回復時間を 2 試験で検討した。鎮静症状は複数の時点で観察した。[redacted] 試験では、レミマゾラム 5 mg/kg 急速静注後、ビデオ観察による鎮静作用が 2～92 分間確認された。閉眼及び筋弛緩を鎮静作用の指標として用いた[redacted] 試験では、レミマゾラム 12.5 mg/kg 急速静注の 2～24 分後に鎮静作用が認められた。その後の静脈内持続投与（12.5 mg/kg/2h）終了後、完全な回復は 8～89 分後に認められた。

サルの静脈内持続投与試験では、投与時間、投与量範囲及び投与間隔が試験間で大きく異なるため、鎮静作用発現の投与速度及び投与量はミニブタの場合と同様に、投与中に観察される症状を指標に判断した（表 2.6.2-11）。下記に示した 8 試験では、鎮静症状を発現するレミマゾラムの持続投与速度は 0.75～3 mg/kg/h、総投与量は 6～30 mg/kg とばらつきが認められた。しかし、鎮静用量は主に体位及び歩行に対する作用として定義しており、鎮静作用を示す最小用量を検討する評価項目としては深い鎮静レベルを設定した可能性を考慮する必要がある。

表 2.6.2-11　カニクイザルでのレミマゾラム（静脈内持続投与）の鎮静作用

Mode of administration	No. and sex per group	Main Sedation characteristics during or immediately after dosing	Sedative infusion rate (dose)	Study No.
6 h-infusion	2 M	Slight sedation, sitting position, ataxic gait, incomplete eyelid opening, decrease in spontaneous activity	1 mg/kg/h (6 mg/kg)	[redacted]
2 week-RDT, 9 h/day	3 M/3 F or 5 M/5 F	Sitting position, incomplete eyelid opening or eyelid closure, decrease in spontaneous activity, sedation, ataxic gait	0.75 mg/kg/h[b] (6.75 mg/kg)	[redacted]
6 h-infusion	5 M	Crouching, lateral or prone position	3 mg/kg/h (18 mg/kg)	[redacted]
4-day RDT, 4 h/day	3 M	Sedation, ataxic gait, incomplete eyelid opening or eyelid closure, somnolence	1.6 mg/kg/h[a] (6.4 mg/kg)	[redacted]
6 h-infusion	4 M	Sitting or lateral position, eyelid closure, disappearance of touch response	3 mg/kg/h (18 mg/kg)	[redacted]
4 h-infusion	5 M	Sedation, incomplete eyelid opening or eyelid closure, ataxic gait	1.6 mg/kg/h[a] (6.4 mg/kg)	[redacted]
24 h-infusion	3 M/3 F	Sedation, sitting position, partial or eyelid closure	1.25 mg/kg/h[b] (30 mg/kg)	[redacted]
4 week-RDT, 2 h/day once a week	4 M/4 F	Eyelid closure, muscle relaxation (only signs monitored)	6.25 mg/kg/h[b] (12.5 mg/kg)	[redacted]

F: female, M: male, RDT: repeated dose toxicity study
a: highest dose tested
b: lowest dose tested

2.6.2.3 副次的薬理試験

2.6.2.3.1 選択性プロファイル

試験番号：[redacted]（参考資料）、記載箇所：4.2.1.1.1
試験番号：[redacted]（参考資料）、記載箇所：4.2.1.1.2
試験番号：[redacted]（評価資料）、記載箇所：4.2.1.1.3
試験番号：[redacted]（評価資料）、記載箇所：4.2.1.1.4

各種受容体、トランスポーター及びイオンチャンネルに対するレミマゾラム（10 μmol/L）の親和性を評価した。$GABA_A$ 受容体ベンゾジアゼピン結合部位以外に明らかな結合親和性は認められなかった（[redacted]）。同様の試験をレミマゾラムの主代謝物である CNS 7054（10 μmol/L）に対しても実施し、検討したいずれの標的に対しても明らかな結合親和性は認められなかった（[redacted]）。

$GABA_A$ 受容体ベンゾジアゼピン結合部位を含めた 40 種類の受容体、トランスポーター及びイオンチャネルに対して実施した別試験（[redacted]）でも、レミマゾラム（$GABA_A$ 受容体ベンゾジアゼピン結合部位に対して 0.3 nmol/L～3 μmol/L、その他は 10 μmol/L）及び CNS 7054（$GABA_A$ 受容体ベンゾジアゼピン結合部位に対して 0.03～300 μmol/L、その他は 10 μmol/L）は同様の結果を示した。レミマゾラムは $GABA_A$ 受容体ベンゾジアゼピン結合部位を除き、特異的な結合を示さなかった。別の試験（[redacted]）では、$GABA_{A1}$ 受容体を含む 29 種類の関連受容体、イオンチャネル及びトランスポーターに対するレミマゾラム又は CNS 7054 の結合親和性を評価した。レミマゾラムが放射能標識ムシモールの $GABA_{A1}$ への結合を 62.1%抑制した以外、レミマゾラム及び CNS 7054 はいずれの標的に対しても意味のある作用（control-specific binding が 50%を超える阻害又は亢進）を示さなかった。各評価結果を表 2.6.2-12 に示す。

表 2.6.2-12　各種受容体、トランスポーター及びイオンチャネルに対するレミマゾラム及び CNS 7054 の結合親和性

Target	Source	Radioligand	%inhibition		Study No.
			Remimazolam 10 μmol/L	CNS 7054 10 μmol/L	
Adenosine A_1	Rat whole brain	[^{3}H]DPCPX	10.6 6	0.2 9	[redacted]
Adenosine A_{2A}	Human recombinant	[^{3}H]CGS21680	3.8 20	3.2 -4	[redacted]
Adenosine A_3	Human recombinant	[^{125}I]AB-MECA	0.5 0	1.8 -5	[redacted]
α_1-Adrenergic, Non-selective	Rat whole brain	[^{3}H]Prazosin	6.8 20	1.2 6	[redacted]
α_2-Adrenergic, Non-selective	Rat cerebral cortex	[^{3}H]Rauwolscine	2.4 9	0.0 13	[redacted]
β_1-Adrenergic	Human recombinant	[^{3}H]CGP-12177 [^{125}I]Cyanopindolol	6.5 -4	1.4 -12	[redacted]
β_2-Adrenergic	Human recombinant	[^{3}H]CGP-12177	0.5 -6	4.3 -9	[redacted]
Adrenergic NE Transporter	Human recombinant	[^{125}I]RTI-55	-9	 -13	[redacted]
Calcium Channel, Type L, Dihydropyridine	Rat cerebral cortex	[^{3}H]PN200-110 [^{3}H]Nitrendipine	0.0 -5	0.0 2	[redacted]
CB_1	Human recombinant (CHO cells)	[^{3}H]CP55940	-33.0	-11.2	[redacted]
CB_2	Human recombinant (CHO cells)	[^{3}H] WIN 55212-2	1.2	3.0	[redacted]
Cholecystokinin CCK_A	Human recombinant	[^{125}I]Cholecystokinin Octapeptide [^{3}H]Me-N-(±)L364,718	2.7 -9	0.7 6	[redacted]
Cholecystokinin CCK_B	Human recombinant	[^{125}I]Cholecystokinin Octapeptide [^{3}H]CCK-8	0.1 11	2.8 10	[redacted]
Dopamine D_1	Human recombinant	[^{3}H]SCH23390	5.9 16	11.2 -10	[redacted]
Dopamine D_{2L}	Human recombinant	[^{3}H]Spiperone	10.1 -1	1.3 -16	[redacted]
Dopamine D_{2S}	Human recombinant (HEK-293 cells)	[^{3}H]7-OH-DPAT	-21.9	-4.7	[redacted]
Dopamine D_3	Human recombinant (CHO cells)	[^{3}H]methyl-spiperone	1.2	-0.5	[redacted]
Dopamine $D_{4.2}$	Human recombinant (CHO-K1 cells)	[^{3}H]Spiperone	-7	-2	[redacted]
Dopamine D_5	Human recombinant (GH4 cells)	[^{3}H]SCH 23390	8.6	-11.8	[redacted]

表 2.6.2-12　各種受容体、トランスポーター及びイオンチャネルに対するレミマゾラム及び CNS 7054 の結合親和性（続き）

Target	Source	Radioligand	%inhibition		Study No.
			Remimazolam 10 μmol/L	CNS 7054 10 μmol/L	
Dopamine Transporter	Human recombinant	[^{3}H]WIN35428 [^{125}I]RTI-55	1.2 -7	0.6 -10	[redacted]
Endothelin ET_A	Human recombinant Rat A10 cells	[^{125}I]Endothelin-1	0.5 4	3.7 -1	[redacted]
Endothelin ET_B	Human recombinant	[^{125}I]Endothelin-1	0.6 9	0.0 2	[redacted]
Estrogen	Rat uterus Calf uterus	[^{3}H]Estradiol	2.3 1	2.1 1	[redacted]
$GABA_A$, Agonist Site	Rat cerebellum Rat whole brain	[^{3}H]Muscimol	0.0 -10	0.0 -3	[redacted]
$GABA_{A1}$ (α1, β2, γ2)	Human recombinant (CHO cells)	[^{3}H]Muscimol	-62.1	-5.1	[redacted]
$GABA_A$, Benzodiazepine, Central	Rat whole brain	[^{3}H]Flunitrazepam	101	 34	[redacted]
$GABA_A$, Chloride Channel	Rat cerebral cortex	[^{3}H]EBOB [^{3}H]TBOB	0.0 19	0.0 -7	[redacted]
$GABA_B$	Rat cerebellum	[^{3}H]Aminobutyric Acid [^{3}H]GABA	4.7 15	5.2 13	[redacted]
GABA transporter	Rat cerebral cortex	[^{3}H]GABA (+10 μmol/L isoguvacine) (+10 μmol/L baclofen)	-9.4	-7.1	[redacted]
Glutamate, AMPA	Rat cerebral cortex	[^{3}H]AMPA	-4.6	-8.8	[redacted]
Glutamate, kainate	Rat cerebral cortex	[^{3}H]kainic acid	-4.6	-3.4	[redacted]
Glutamate, NMDA, Glycine	Rat cerebral cortex	[^{3}H]MDL 105,519	6	-7	[redacted]
Glutamate, NMDA, Phencyclidine	Rat cerebral cortex	[^{3}H]TCP	-8	-9	[redacted]
Glutamate, Non-selective	Rat cerebral cortex/Rat whole brain	[^{3}H]L-Glutamic acid	8.4 -11	0.1 -16	[redacted]
Histamine H_1, Peripheral	Guinea pig lung	[^{3}H]Pyrilamine	5.8 7	22.1 12	[redacted]
Imidazoline I_2, Central	Rat cerebral cortex	[^{3}H]RX781094 [^{3}H]Idazoxan	0.4 -10	3.0 4	[redacted]
Insulin	Rat liver	[^{125}I]Insulin (Porcine)	2.3 -8	9.5 5	[redacted]
Potassium Channel [K_{ATP}]	Rat whole brain Syrian hamster pancreatic beta cells	[^{3}H]Glibenclamide [^{3}H]Glyburide	0.0 6	0.2 17	[redacted]

表 2.6.2-12　各種受容体、トランスポーター及びイオンチャネルに対するレミマゾラム及び CNS 7054 の結合親和性（続き）

Target	Source	Radioligand	%inhibition		Study No.
			Remimazolam 10 μmol/L	CNS 7054 10 μmol/L	
Leukotriene D_4	Guinea pig lung	[^{3}H]Leukotriene D_4	0.0 13	0.0 2	[redacted]
Muscarinic M_2	Human recombinant	[^{3}H]Scopolamine Methyl Chloride [^{3}H]NMS	0.0 23	0.9 13	[redacted]
Muscarinic M_3	Human recombinant	[^{3}H]Scopolamine Methyl Chloride [^{3}H]NMS	7.3 24	0.3 -5	[redacted]
Muscarinic, Non-selective	Rat cerebral cortex	[^{3}H]Quinuclidinyl Benzilate	0.0 6	0.8 0	[redacted]
Norepinephrine Transporter	Human recombinant	[^{3}H]Nisoxetine Hydrochloride	1.9	0.0	[redacted]
Nicotinic, Acetylcholine Central	Rat cerebral cortex	[^{3}H]Nicotine [^{3}H]Cytisine	19.3 9	3.4 14	[redacted]
Nicotinic Acetylcholine α7, Bungarotoxin	Human recombinant (SH-SY5Y cells)	[^{125}I]α-Bungarotoxin	10	8	[redacted]
Nicotinic neuronal α4β2	Human recombinant (SH-SY5Y cells)	[^{3}H]cytisine	6.7	9.1	[redacted]
Opiate, δ (DOP)	Human recombinant	[^{3}H]DADLE	19.8	-0.1	[redacted]
Opiate κ (OP2, KOP)	Human recombinant (HEK-293 cells)	[^{3}H]U-69593	3	3	[redacted]
Opiate, μ (MOP)	Human recombinant (HEK-293 cells)	[^{3}H]DAMGO	-4.5	1.6	[redacted]
Opiate, Non-selective	Rat cerebral cortex Rat whole brain	[^{3}H]Naloxone	2.4 4	4.4 20	[redacted]
Orexin, OX_1	Human recombinant (CHO cells)	[^{125}I]orexin-A	14.9	10.0	[redacted]
Orexin, OX_2	Human recombinant (HEK-293 cells)	[^{125}I]orexin-A	-0.7	-8.2	[redacted]
Platelet Activating Factor (PAF)	Rabbit platelets	[^{3}H]PAF	0.0 17	0.0 20	[redacted]
Progesterone	Rabbit uterus Calf uterus	[^{3}H]Progesterone [^{3}H]R-5020	0.0 9	2.4 10	[redacted]
Serotonin 5-HT_1	Rat striatum Rat cerebral cortex	[^{3}H]Hydroxytryptamine Creatinine Sulfate [^{3}H]5-HT	14.5 23	2.0 3	[redacted]
Serotonin, 5-HT_{1A}	Human recombinant (HEK-293 cells)	[^{3}H]8-OH-DPAT	13.0	-4.5	[redacted]

表 2.6.2-12　各種受容体、トランスポーター及びイオンチャネルに対するレミマゾラム及び CNS 7054 の結合親和性（続き）

Target	Source	Radioligand	%inhibition		Study No.
			Remimazolam 10 µmol/L	CNS 7054 10 µmol/L	
Serotonin, 5-HT_2	Rat whole brain	[^{3}H]Ketanserin	-7	-9	[redacted]
Serotonin, 5-HT_{2A}	Rat cerebral cortex Human recombinant (HEK-293 cells)	[^{3}H]Ketanserin Hydrochloride [^{125}I](±)DOI	0.0 -3.5	0.0 -8.2	[redacted]
Serotonin, 5-HT_{2B}	Human recombinant (CHO cells)	[^{125}I](±)DOI	-11.9	-11.5	[redacted]
Serotonin, 5-HT_{2C}	Human recombinant (HEK-293 cells)	[^{125}I](±)DOI	-1.0	0.4	[redacted]
Serotonin, 5-HT_3	Human recombinant (CHO cells)	[^{3}H]BRL 43694	5.2	-1.5	[redacted]
Serotonin, 5-HT_{5a}	Human recombinant (HEK-293 cells)	[^{3}H]LSD	-7.8	-3.3	[redacted]
Serotonin, 5-HT_6	Human recombinant (CHO cells)	[^{3}H]LSD	-4.5	-14.1	[redacted]
Serotonin, 5-HT_7	Human recombinant (CHO cells)	[^{3}H]LSD	2.3	-6.6	[redacted]
Serotonin Transporter	Human recombinant	[^{3}H]Imipramine Hydrochloride [^{125}I]RTI-55	2.8 -7	2.3 2	[redacted]
Sigma 1	Jurkat cells (endogenous)	[^{3}H](+)pentazocine	-3.5	1.0	[redacted]
Sigma 2	Jurkat cells (endogenous)	[^{3}H]DTG (+1 µmol/L (+) Pentazocine)	-15.2	-11.7	[redacted]
Sigma, Non-selective	Guinea pig whole brain	[^{3}H]DTG	0.6 4	0.4 4	[redacted]
Sodium Channel, Site 2	Rat whole brain	[^{3}H]Batrachotoxin A 20-alpha-Benzoate [^{3}H]Batrachotoxin	0.8 17	0.0 7	[redacted]
Testosterone	Human receptor Rat ventral prostate	[^{3}H]Methyltrienolone [^{3}H]Mibolerone	0.0 13	0.5 8	[redacted]

CHO: Chinese hamster ovary, HEK: human embryonic kidney
n = 2 experiments

2.6.2.3.2　健忘作用

試験番号：[redacted]（参考資料）、記載箇所：4.2.1.2.1

レミマゾラムの健忘作用をプロポフォールと比較するために、ラット受動回避反応試験で明室滞在時間を評価指標として評価した（n = 10／群）。レミマゾラム（0.2、0.5 及び 1 mg/kg、急速静注）及びプロポフォール（1、2 及び 5 mg/kg、急速静注）は用量依存的に明室滞在時間を短縮した。レミマゾラムは 0.5 mg/kg 以上、プロポフォールは 2 mg/kg 以上で対照群と比較して有意差が認められた。試験系の妥当性を確認するために陽性対照薬であるミダゾラム（0.02、0.2 及び 2 mg/kg、急速静注）の作用を検討した結果、用量依存的に明室滞在時間が短縮し、0.2 mg/kg 以

上で対照群と比較して有意差が認められた（n = 5／群）。

レミマゾラム及びプロポフォールの健忘作用の ED_{50} 値は、それぞれ 0.68 及び 2.5 mg/kg であった（表 2.6.2-13）。鎮静作用の ED_{50} 値は同一試験施設において評価した LRR に対する ED_{50} 値を用いた。レミマゾラムにおいて、鎮静作用はプロポフォールと同程度の用量から認められるが、健忘作用はプロポフォールより低用量から認められた。

表 2.6.2-13　レミマゾラム及びプロポフォールの健忘作用及び鎮静作用の ED_{50} 値、用量比

Treatment	ED_{50} value of amnestic effect (95% confidential interval)	ED_{50} value of sedative effect (95% confidential interval)	Dose ratio
Remimazolam	0.68 mg/kg (0.53-0.87)	3.9 mg/kg (2.6-5.8)	5.7 times
Propofol	2.5 mg/kg (1.90-3.20)	4.4 mg/kg *	1.8 times

*: 95% confidential interval could not be calculated.
Data from [redacted]

2.6.2.4 安全性薬理試験

2.6.2.4.1 中枢神経系に及ぼす影響

中枢神経系への影響について、ラットを用いた Irwin 試験（2.6.2.4.1.1 項）並びに単回及び反復投与毒性試験結果の評価を実施した（2.6.2.4.1.2 項）。なお、身体依存性及び乱用傾向に対する影響については、2.6.6.8.3 項に記載した。また、本剤の主作用である鎮静作用については、2.6.2.2.2 項に記載した。

2.6.2.4.1.1 ラットでの中枢神経系に及ぼす影響（Irwin 試験）

試験番号：[redacted]（評価資料）、記載箇所：4.2.1.3.1

ラットの行動及び生理学的状態に対する作用を検出できる Irwin 試験を用いて、レミマゾラムの中枢神経系に対する作用を評価した（表 2.6.2-14）。雄 SD ラット（n = 6／群）にレミマゾラム（10、20 及び 30 mg/kg）を静脈内投与 5 分後、群居性消失、自発運動量及び驚愕反応の減少並びに握力及び体緊張の低下として検出される鎮静作用が誘導された。これらの症状は速やかに減弱し、投与 45 分後までには正常に回復していた。10 及び 30 mg/kg では投与 45 分後に体温低下が認められた。

全ての症状の変化は、中枢神経系抑制薬、特にベンゾジアゼピン系薬剤で予期されるものであった。本剤の特徴である非常に速い鎮静作用からの回復と一致して、全ての影響は一時的で、発現後速やかに消失した。予期されるリバウンド効果としての興奮や活動量の増加は認められなかった。投与 10 及び 20 分後に接触応答の増加を示す動物もいたが、同様の影響が溶媒投与群にも認められたことから、薬剤ではなく、試験条件に起因するものと考えられた。

表 2.6.2-14　Irwin 試験において観察された行動のまとめ

Treatment	Observation time points			
	5 min	10 min	20 min	45 min
Vehicle	↑LA, ↑TR-5, PA-5	↑LA, ↑TR-3, PA-5	PA-5	PA-5
Remimazolam 10 mg/kg, i.v.	DC, ↓LA, ↓AL-6, ↓SR, ↓TR-3, ↓F-2, C-2, PA-6, ↓BT-5, ↓GS-6, PY-5 $	↑TR-3, PA-6, ↓BT-2, V-3	↑TR-2, PA-6, V-3	PA-6
Remimazolam 20 mg/kg, i.v.	DC, ↓LA, ↓AL-2, ↓SR, ↓TR-2, PA-5, ↓BT-3, ↓GS-4, L-3 (5) * #	↑TR-2, PA-5 (5) *	PA-4, L-3 (5) *	PA-2, NAD-3 (5) *
Remimazolam 30 mg/kg, i.v.	DC, ↓LA, ↓AL-6, ↓SR, LRR-2, ↓TR-5, ↓F-4, C-4, PA-6, ↓BT-6, ↓GS-6, PY-6, L-2 $	AG(T)-2, ↑TR-2, PA-6	↑TR-3, PA-6, V-4	PA-6, D-3
Reference chlorpromazine 3 mg/kg, i.v.	DC, ↓LA, A-3, ↓AL-3, ↓SR, AC(F)-3, AG(Sp)-3, ↓TR-4, ↓F-4, ↓PR-3, ↓CR-3, C-3, PA-6, ↓BT-4, PY-3, L-2, PT-4, ↓G-4	DC, ↓LA, A-3, ↓AL-3, ↓SR, AC(F)-3, AG(Sp)-3, ↓TR-5, ↓F-4, ↓PR-3, ↓CR-3, C-3, PA-6, ↓BT-3, ↓GS-2, PY-3, L-5, PT-4, ↓G-3	DC, ↓LA, A-3, ↓AL-6, ↓SR, AC(F)-3, AG(Sp)-3, ↓TR-6, ↓F-5, ↓PR-3, ↓CR-3, C-3, PA-6, ↓BT-3, ↓GS-4, PY-2, L-3, PT-4, ↓G-4	DC, ↓LA, A-2, T-2, ↓AL-5, ↓SR, AC(F)-4, AG(Sp)-3, ↓TR-5, ↓F-4, ↓PR-3, ↓CR-3, C-3, PA-6, ↓BT-4, ↓GS-4, PY-3, L-6, PT-4, ↑U-2, ↓G-4

A: Apathy, AC(F): Abnormal body carriage (flat), AG(Sp): Abnormal gait (spread), AG(T): Abnormal gait (walking on toes), ↓AL: decreased alertness, ↓BT: decreased body tone, C: catalepsy, ↓CR: Decreased corneal reflex, D: diarrhea, DC: dispersion in cage, ↓F: decreased fearfulness, ↓G: Decreased grooming, ↓GS: decreased grip strength, L: lacrimation, ↑LA: increased locomotor activity, ↓LA: decreased locomotor activity, LRR: loss of righting reflex, NAD: no abnormalities detected, PA: passivity, PY: paralysis, ↓PR: Decreased pinna reflex, PT: Ptosis, ↓SR: decreased startle response, T: Tremor, ↑TR: increased touch response, ↓TR: decreased touch response, ↑U: Increased urination, V: vocalization. All values represent the number of rats showing the observation at a respective time point where the observation occurred in >1 rat. n = 6 animals per group unless indicated in parenthesis.
*: Animal 2 died during dosing due to suffocation in the restraint tube.
#: Animal 5 was asleep during observations.
$: At the time of group observation, all rats were sleeping. By the time of the last individual observation, all rats were awake and mobile.
Data from [redacted]

2.6.2.4.1.2　毒性試験における中枢神経系に関連した全身状態の変化及び病理組織所見

試験番号：[redacted]（評価資料）、記載箇所：4.2.3.1.1
試験番号：[redacted]（評価資料）、記載箇所：4.2.3.1.5
試験番号：[redacted]（評価資料）、記載箇所：4.2.3.1.6
試験番号：[redacted]（参考資料）、記載箇所：4.2.3.2.1
試験番号：[redacted]（評価資料）、記載箇所：4.2.3.2.2
試験番号：[redacted]（参考資料）、記載箇所：4.2.3.2.3
試験番号：[redacted]（評価資料）、記載箇所：4.2.3.2.4
試験番号：[redacted]（評価資料）、記載箇所：4.2.3.2.5
試験番号：[redacted]（参考資料）、記載箇所：4.2.3.2.6
試験番号：[redacted]（評価資料）、記載箇所：4.2.3.2.7
試験番号：[redacted]（参考資料）、記載箇所：4.2.3.2.8

中枢神経系への影響について、単回及び反復投与毒性試験（主要な試験及び補足的な試験）の報告書について、下記の項目に関して再評価を行った。

a) 中枢神経系抑制薬の予想される影響に関連する全身状態の変化
b) 中枢神経系抑制薬の予想される影響に関連しない中枢神経系への影響（逆説的影響）
c) 中枢神経系での病理組織的な影響

とりわけ、逆説的な影響の可能性、及び／又は、薬剤投与中止後に起きる影響（リバウンド又は再鎮静）について重点的に評価した。これらの再評価結果を表 2.6.2-15 に示す。

毒性試験の再評価結果から、予想されたとおり、過度の中枢神経系抑制作用が用量制限事項として確認された。高用量での長期投与後においても、明確なリバウンド作用は認められなかった。他のベンゾジアゼピン系薬剤で知られている嘔吐や興奮のような有害作用は、ほとんどの試験において認められなかった。また、依存性試験（2.6.6.8.3 項）において、ベンゾジアゼピン系薬剤の薬理作用として知られている薬物耐性や薬物依存性が認められた。

表 2.6.2-15　毒性試験における中枢神経系に関連した全身状態の変化及び病理組織所見

Species	Doses (mg/kg)	Method of administration	Related changes to the expected clinical signs	Unrelated changes to the expected clinical signs	Pathological changes in central nervous system	Study No.
Acute toxicity studies						
Mouse	40, 50, 65, 100, 125	single bolus i.v. administration	subdued behavior, piloerection, abnormal breathing, abnormal gait, prostration and lip licking	None observed	Not done	[redacted]
Monkey	6, 18, 60, 150	6 h i.v. infusion	ataxic gait, incomplete eyelid opening, eyelid closure, decrease in spontaneous activity, sedation, somnolence, coma, sitting position, and lateral position. There were more pronounced in the higher dose groups. Clinical signs associated with the desired effects were maintained after cessation of dosing in higher doses. No rebound effects were observed such as hyperactivity or agitation.	Vomiting was observed in 1 animal in each of the 6, 60, and 150 mg/kg groups during the dosing or on Day 1 after dosing.	Not done	[redacted]
Monkey	30, 60, 120	24 h i.v. infusion	ataxic gait, a decrease in spontaneous activity, somnolence, sitting position, eyelid closure, sedation, lateral position and coma	None observed	None observed	[redacted]

表 2.6.2-15　毒性試験における中枢神経系に関連した全身状態の変化及び病理組織所見（続き）

Species	Doses (mg/kg)	Method of administration	Related changes to the expected clinical signs	Unrelated changes to the expected clinical signs	Pathological changes in central nervous system	Study No.
Repeated-dose toxicity studies						
Rat	15, 30, 50	bolus i.v. administration 7 days	death, prostration, lip licking, abnormal gait, subdued behavior, abnormal breathing, hunched posture, eyelid closure and dilated pupils	sporadic finding of agitation	None observed	[redacted]
Rat	10, 20, 30	bolus i.v. administration 28 days	prostration, abnormal gait, subdued behavior and abnormal breathing	None observed	None observed	[redacted]
Monkey	20	bolus i.v. administration 14 days	sedation, recumbency, incoordination, unsteady gait, tremors and hunched posture	None observed	Not done	[redacted]
Monkey	6.75, 9.0, 11.25, 22.5	9 h i.v. infusion, 14 days	eyelid closure, decreased spontaneous activity, sedation, somnolence, ataxic gait, sitting and/or lateral position	sporadic findings of vomiting	None observed	[redacted]
Monkey	12, 30, 60	12 h i.v. infusion, 28 days	ataxic gait, decreased spontaneous activity, imcomplete eyelid opening, sedation, sitting position, somnolence, and coma	None observed	None observed	[redacted]
Monkey	12.5, 25, 50	2.08 h i.v. infusion, 1/week, 5 times	eyelid closure and muscle relaxation	None observed	None observed	[redacted]

表 2.6.2-15　毒性試験における中枢神経系に関連した全身状態の変化及び病理組織所見（続き）

Species	Doses (mg/kg)	Method of administration	Related changes to the expected clinical signs	Unrelated changes to the expected clinical signs	Pathological changes in central nervous system	Study No.
Repeated-dose toxicity studies (cont.)						
Monkey	5, 10, 20	bolus i.v. administration 28 days	Recumbency, unresponsiveness, drowsiness and abnormal gait	None observed	None observed	[redacted]
Monkey	1.6, 3.2, 6.4	4 h i.v. infusion, 5 days	eyelid closure, sedation, somnolence and ataxic gait	None observed	Not done	[redacted]

i.v.: intravenous

2.6.2.4.2 心血管系及び呼吸系に及ぼす影響

2.6.2.4.2.1 hERG チャネル発現 HEK293 細胞に対する作用（*in vitro* 試験）

試験番号：[redacted]（参考資料）、記載箇所：4.2.1.3.2
試験番号：[redacted]（参考資料）、記載箇所：4.2.1.3.3
試験番号：[redacted]（参考資料）、記載箇所：4.2.1.3.4
試験番号：[redacted]（評価資料）、記載箇所：4.2.1.3.5

薬剤誘導性 QT 間隔延長は心室不整脈及び突然死をもたらす可能性がある。典型的な QT 間隔延長は、速やかな再分極を引き起こす遅延整流性カリウム電流 I_{kr} が通過する hERG カリウムチャネルの間接的な阻害に起因する。レミマゾラムと CNS 7054 の hERG 電流に対する作用は、hERG を安定的に発現させたヒト胎児腎細胞 293（HEK293）（n = 3～4／濃度）を用いたホールセルパッチクランプ法により測定した（[redacted]、[redacted]、[redacted] 及び[redacted]）。

レミマゾラム、その主代謝物である CNS 7054、ミダゾラム及び K^+チャネルブロッカーである E-4031（逆頻度依存性の I_{kr} チャネル阻害剤）について hERG 電流に対する作用を評価し、以下の結果を得た。レミマゾラムは 3 μmol/L まで hERG 電流に対する阻害作用を示さず、CNS 7054 は 0.3 μmol/L まで 50%を超える阻害作用を示さなかったが、ミダゾラム及び E-4031 は濃度依存性に阻害作用を示し、IC_{50} 値はそれぞれ 7.6 μmol/L 及び 16 nmol/L であった（[redacted]）。

CNS 7054 は 3 μmol/L まで hERG 電流に対する阻害作用を示さなかった（溶媒対照群に対する%の平均 ± 標準誤差は、0.03、0.3 及び 3 μmol/L でそれぞれ 87.7 ± 5.4%、87.5 ± 5.1%及び 83.4 ± 5.3%）（[redacted]）。

3 μmol/L の濃度で E-4031 及び消化管運動機能改善薬であるシサプリドは hERG 電流に対してほぼ 100%の阻害作用（それぞれ 99.7 ± 0.2%及び 98.8 ± 0.8%）を示したのに対し、レミマゾラム及び CNS 7054 はそれぞれ 24.3 ± 4.9%及び 18.8 ± 10.4%の阻害作用を示した（[redacted]）。

CNS 7054 は 100 μmol/L まで hERG 電流に対する阻害作用を示さなかった（[redacted]）。レミマゾラムは濃度依存性に hERG 電流に対する阻害作用を示し、25%阻害濃度（IC_{25}）及び IC_{50} 値はそれぞれ 62 及び 207 μmol/L であった（[redacted]）。これらの濃度は *in vitro* での $GABA_A$ 受容体活性化に必要な濃度（2.6.2.2.1.1 項）をはるかに超えた濃度であり、非臨床薬理及び毒性試験又は臨床試験で到達した血漿中遊離型薬物濃度よりもかなり高濃度である。

2.6.2.4.2.2 モルモット摘出乳頭筋の活動電位に対する作用（*in vitro* 試験）

試験番号：[redacted]（評価資料）、記載箇所：4.2.1.3.6
試験番号：[redacted]（評価資料）、記載箇所：4.2.1.3.7

レミマゾラム（[redacted]）及び主代謝物である CNS 7054（[redacted]）の心筋活動電位に対する作用をモルモット乳頭筋で評価した（n = 5／群）。

レミマゾラムは 10 及び 30 μmol/L で各パラメータに対して溶媒対照群と比較して有意な変化は認められなかった（表 2.6.2-16）。100 及び 300 μmol/L では、カルシウムチャネルの抑制に起因する 30%及び 50%再分極時の活動電位持続時間（APD_{30} 及び APD_{50}）の短縮が認められた。

さらに、最高濃度の 300 μmol/L では静止膜電位（RMP）の上昇及び 90%再分極時の活動電位持続時間（APD_{90}）の延長も認められが、作用は限定的であった（レミマゾラム：ΔAPD_{90} = 5.4 ms、対照群：ΔAPD_{90} = -1.8 ms）。これに対し、陽性対照群であるソタロール（30 μmol/L）では 37 ms まで延長が認められた。以上の結果から、レミマゾラムは 100 μmol/L 以上の濃度でカルシウムチャネルを阻害し、さらに 300 μmol/L で内向き整流性及び遅延整流性カリウムチャネルを阻害する

と考えられる。

CNS 7054 は 10、30 及び 100 μmol/L で心筋活動電位に作用しなかった。300 μmol/L で APD_{30} 及び APD_{50} の短縮が認められたが、APD_{30}（96.2%）は対照群と比較して有意な差ではなかった。

表 2.6.2-16　モルモット乳頭筋の心筋活動電位に対するレミマゾラム、CNS 7054 及びソタロールの作用

Test article	Dose (μmol/L)	RMP (%)	APA (%)	Vmax (%)	APD_{30} (%)	APD_{50} (%)	APD_{90} (%)
Vehicle control 0.1% DMSO	-	100.7 ± 0.3	100.5 ± 0.2	102.2 ± 1.3	99.0 ± 1.4	98.9 ± 0.9	99.0 ± 0.7
Remimazolam	10	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	30	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	100	n.s.	n.s.	n.s.	***88.2 ± 1.3	***90.9 ± 0.9	n.s.
	300	***94.9 ± 1.8	n.s.	n.s.	***81.1 ± 2.8	***88.1 ± 1.5	*103.5 ± 2.2
Sotalol	30	#99.6 ± 0.2	n.s.	n.s.	###111.3 ± 2.0	###119.2 ± 2.3	###121.9 ± 2.5
Vehicle control	-	100.6 ± 0.3	100.0 ± 0.2	102.4 ± 0.8	100.0 ± 1.4	99.9 ± 0.9	99.6 ± 0.5
CNS 7054	10	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	30	*99.6 ± 0.2	n.s.	n.s.	n.s.	n.s.	n.s.
	100	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	300	**99.2 ± 0.4	n.s.	n.s.	n.s.	*96.1 ± 0.5	n.s.
Sotalol	30	#99.8 ± 0.2	n.s.	n.s.	##111.2 ± 1.9	###118.9 ± 2.2	###121.0 ± 2.4

RMP: resting membrane potential, APA: action potential amplitude, Vmax : maximal upstroke velocity, APD_{30}, APD_{50} and APD_{90} : action potential duration at 30%, 50% and 90% repolarization, respectively.
Each value represents percentage change of post value (post value/pre value x 100); the mean ± standard error (S.E.) of 5 preparations.
n.s.: not significantly different from the vehicle control values, numerical values are not mentioned in this case
*: p<0.05, **: p<0.01, ***: p<0.001; significantly different from the vehicle control values (one-way ANOVA / Dunnett's test).
#: p<0.05, ##: p<0.01, ###: p<0.001; significantly different from the vehicle control values (F-test / Student t-test or Welch test).
Data from [redacted] and [redacted]

以上より、内向き整流性及び遅延整流性カリウムチャネル阻害に対する無影響量（NOEL）は、レミマゾラムは 100 μmol/L、CNS 7054 は 300 μmol/L であった。カルシウムチャネルに対する NOEL は、それぞれ 30 及び 100 μmol/L であった。

2.6.2.4.2.3　血液学的機能に及ぼす影響（*in vitro* 試験）

試験番号：[redacted]（参考資料）、記載箇所：4.2.1.3.8
試験番号：[redacted]（評価資料）、記載箇所：4.2.1.3.9
試験番号：[redacted]（評価資料）、記載箇所：4.2.1.3.10

ラット血漿を用いた試験で、レミマゾラムの血小板凝集（単独での凝集誘発作用、アデノシン 5'-二リン酸ナトリウム塩［ADP］及びコラーゲン誘発凝集に対する作用）及び血液凝固系（プロトロンビン時間及び活性化部分トロンボプラスチン時間）に対する作用は、それぞれ、最終濃度 333 μg/mL 及び 1000 μg/mL まで認められなかった（[redacted]）。

ヒト血漿（n = 5／濃度）を用いて、レミマゾラム（[redacted]）及び CNS 7054（[redacted]）（いずれも 3、10、30、100 及び 300 μmol/L）の血小板凝集、凝固系及び線溶系に及ぼす影響を、溶媒対照群並びに各試験の陽性対照薬であるアルプロスタジル（500 nmol/L）、ヘパリン（0.3、5 又は 7 U/mL）及びガベキサート（100 μmol/L）と比較して評価した。なお、血小板凝集に対する影響は各化合物単独での凝集及び ADP 又はコラーゲン誘発凝集、凝固系に対する影響はプロトロンビン時間及び活性化部分トロンボプラスチン時間、線溶系に対する影響はプラスミン活性を指標に検討した。

各試験での陽性対照薬の作用は確認されたが、レミマゾラム及び CNS 7054 は検討した最高濃度 300 μmol/L まで血小板凝集、凝固系及び線溶系に影響しなかった。

2.6.2.4.2.4 ラットでの心血管系に及ぼす影響

試験番号：[redacted]（参考資料）、記載箇所：4.2.1.3.11

レミマゾラムの血行力学的作用の可能性について、覚醒下のテレメトリー装着雄 SD ラット（n = 5／群）を用いて単回急速静注で評価した（[redacted]）。クロスオーバー法を用いて 5 匹のラットに 0、20 及び 30 mg/kg を投与した。投与間のウォッシュアウト期間は 48 時間以上とした。1 匹はカテーテル装着の失敗により除外した。投与前 2 時間以上及び投与後 4 時間まで継続的に収縮期、拡張期及び平均の動脈圧並びに心拍数をモニターし、データを収集した。鎮静状態は回復が認められるまで継続的に観察した。

レミマゾラム投与直後から全ての動物で活動性低下が認められ、各動物は投与後約 5～10 分間鎮静状態が継続した。レミマゾラム投与群では、各時点の溶媒対照群と比較して持続した血圧上昇がみられ、平均動脈圧の最大変動は投与約 15 分後に認められた。20 及び 30 mg/kg での平均動脈圧変化はそれぞれ約 15%及び 22%であった。血圧は投与後 1.25 時間以内に各時点の溶媒対照群と同程度まで回復した。拘束及び投与操作の影響で、溶媒投与群を含む全群で投与直前から血圧上昇及び心拍数増加がみられた。

レミマゾラム投与群では、各時点の溶媒対照群と比べて持続した心拍数増加を示した。20 及び 30 mg/kg では、投与後約 15 分で最大変化が認められ、それぞれ約 18%及び 34%増加した。心拍数は投与後 1.25 時間以内に各時点の溶媒対照群と同程度まで回復した。レミマゾラムの静脈内投与時の無毒性量（NOAEL）は 30 mg/kg 以上であることが確認された。

2.6.2.4.2.5 ウサギでの心血管系に及ぼす影響

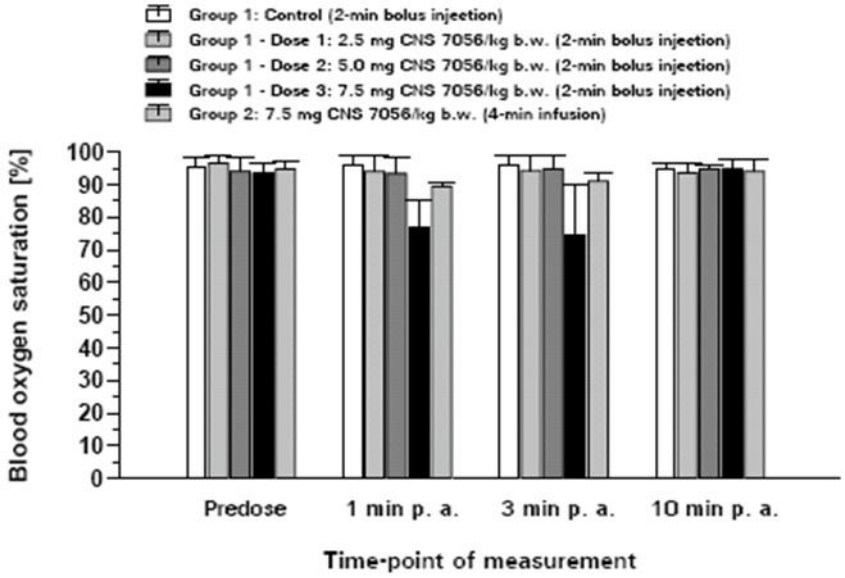
試験番号：[redacted]（参考資料）、記載箇所：4.2.1.3.12

パルスオキシメトリーで非侵襲的に測定した酸素飽和度（SO_2）に対するレミマゾラムの作用を全 8 匹の雌ウサギで評価した（[redacted]）。また、心拍数を記録し、鎮静状態に特に留意して一般状態を観察した。クロスオーバー法で 4 匹のウサギに 0、2.5、5 及び 7.5 mg/kg を 2 分間かけて急速静注した。投与間のウォッシュアウト期間は 2 日間以上とした。他の 4 匹のウサギには 7.5 mg/kg を 4 分間かけて静脈内持続投与した。

レミマゾラムは、局所刺激性及び心拍数の有意な変動を示さなかった。レミマゾラム 7.5 mg/kg 急速静注時の SO_2 は投与前値と比較して投与 1 分後で 18%まで、投与 3 分後で 20%まで低下したが、統計学的に有意ではなかった（図 2.6.2-3）。7.5 mg/kg 静脈内持続投与では、統計学的有意差が認められた投与 3 分後（投与前値と比較して 4%低下）を除き、SO_2 の有意な変動は認められなかった。

レミマゾラム 2.5～7.5 mg/kg の 2 分間急速静注及び 7.5 mg/kg の 4 分間静脈内持続投与で、投与 30 分後に鎮静及び腹臥位として確認される全身性所見が認められた。また、5 及び 7.5 mg/kg 投与後に呼吸数の低下が確認された。全ての一般症状は遅くとも投与 4 時間後までに回復した。

図 2.6.2-3　酸素飽和度に対するレミマゾラムの作用



CNS 7056: Remimazolam. Results are expressed as mean+standard deviation.
Data from [redacted]

2.6.2.4.2.6　ミニブタでの心血管系及び呼吸系に及ぼす影響

試験番号：[redacted]（参考資料）、記載箇所：4.2.1.3.13

レミマゾラムの心血管系及び呼吸系に及ぼす影響を評価するために、雄ミニブタ（n＝3／群）にレミマゾラムを静脈内投与して血圧、心拍数、心電図（ECG）パラメータ（PR、QRS、QT 及び QTc 間隔）及び動脈血液ガス（pH、pO_2、pCO_2 及び SO_2）を測定した。レミマゾラムの作用は全身性麻酔薬であるプロポフォール、ミダゾラム及びデクスメデトミジンと比較した（[redacted]）。

最高用量において、プロポフォール（0.2 mg/kg/min）は最も深い鎮静作用を示し、以降鎮静レベルはレミマゾラム（50 μg/kg/min）、デクスメデトミジン（0.2 μg/kg/min）の順であった。ミダゾラムの鎮静作用は同投与量（50 μg/kg/min）のレミマゾラムの約 3/4 程度であった。レミマゾラムは検討した薬剤の中で最も速く鎮静作用を発現した。

レミマゾラムは 50 μg/kg/min の投与期間中に平均血圧の増加（10%～12%）を示し、12.5～50 μg/kg/min の投与期間中及び投与後に心拍数増加（20%～51%）及び PR 間隔の短縮傾向を示した。これらの変化は麻酔導入時及び回復時に認められた。

レミマゾラム（12.5～100 μg/kg/min、15 分間）の静脈内持続投与期間中及び投与後 75 分間に ECG パラメータの異常な変化は観察されなかった。また、レミマゾラム投与に起因する動脈血液ガスの変化及び呼吸系に対する有害作用も認められなかった。過剰量での作用評価を目的とした高用量（100 μg/kg/min）では、レミマゾラムは血液ガスの変動は誘導しなかったが、麻酔導入時に血圧（10%～14%）及び心拍数（22%～27%）を低下させた。

プロポフォール、ミダゾラム及びデクスメデトミジンは動脈血液ガスに影響しなかったが、麻酔誘導時及び／又は麻酔からの回復時の興奮に伴って、血圧、心拍数及び ECG パラメータを変動させた。プロポフォールは比較的弱い鎮静作用を示す 0.05 mg/kg/min で血圧低下作用を示したことは重要な知見である。また、デクスメデトミジンは 0.2 μg/kg/min で投与期間中及び投与後の血圧及び心拍数を増加させ、投与後の QT 及び QTc 間隔を著明に延長させた。

以上より、ミニブタにおいてレミマゾラムは呼吸系又は心血管系に重篤な作用を及ぼすことな

く、他の麻酔薬 3 剤と比較してより速やかな鎮静作用発現を示すことが確認された。

2.6.2.4.2.7 ヒツジでの心血管系及び呼吸系に及ぼす影響

試験番号：[redacted]（参考資料）、記載箇所：4.2.2.3.2

雌ヒツジ 3 匹にレミマゾラム（最高用量 12 mg/kg、2 分間）を急速静注すると、用量依存的な一過性の平均動脈圧の低下が認められた（最大低下 12%）。全投与量で中心静脈圧が低下（最大低下 34%）したことから、静脈貯留が示唆された。また、心拍出量が変化することなく、代償性に心拍数が増加した（最大増加 25%）。雌ヒツジ 6 匹にレミマゾラム（0.5、1 及び 2 mg/kg）を急速静注すると、鎮静深度に比例して用量依存的な心血管系の抑制作用が認められた（[redacted]）。

雌ヒツジ 3 匹にレミマゾラム（0.05～12 mg/kg）を急速静注すると、呼吸数の減少（最大減少 33%）及び pCO_2 の上昇（最大上昇 12%）を伴う用量依存的な呼吸抑制作用が認められた。また、一過性だが、有意で用量依存的な pO_2 の低下（最大低下 34%）及び SO_2 の低下（最大低下 4%）が認められた（SAIVS 07-002）。

ヒツジを用いた別試験 [1)]で、レミマゾラムの作用をミダゾラム及びプロポフォールの作用と比較した。3 つの薬剤はいずれも用量依存的な呼吸抑制作用を示し、中程度（ミダゾラム及びプロポフォール）から強度（レミマゾラム）の鎮静作用が認められる投与量で呼吸抑制が認められた。

2.6.2.4.2.8 サルでの心血管系、呼吸系及び一般症状に及ぼす影響

試験番号：[redacted]（評価資料）、記載箇所：4.2.1.3.14
試験番号：[redacted]（評価資料）、記載箇所：4.2.3.2.4
試験番号：[redacted]（参考資料）、記載箇所：4.2.3.2.6
試験番号：[redacted]（評価資料）、記載箇所：4.2.3.2.7
試験番号：[redacted]（評価資料）、記載箇所：4.2.1.3.15

安全性薬理試験において、レミマゾラムを覚醒下のテレメトリー装着雄カニクイザル（n＝5）に急速静注（0.5、1、2 及び 5 mg/kg）又は静脈内持続投与（6、18、30 及び 60 mg/kg、6 時間）を 7 日間間隔で計 5 回行った（[redacted]）。テレメトリーシステムを用いて ECG パラメータに対する作用をモニターした。

急速静注では、2 及び 5 mg/kg で一過性の軽度な血圧の低下が投与直後に観察され、投与 1 時間後にそれぞれ最大 0.58°C 及び 1.28°C の腹腔内体温の低下が確認された。検討した投与量において、心拍数、QT 及び補正 QT（QTc）間隔を含む ECG パラメータ並びに呼吸数に対する影響は認められなかった。対照薬である抗ヒスタミン剤アステミゾール（10 mg/kg、経口投与）は心拍数に影響しなかったが、QT 及び QTc 間隔の延長が認められ、投与 2 時間後に最大の延長を示した（投与前と比較して 122%）。

6 時間の静脈内持続投与では、検討した投与量で血圧、心拍数及び呼吸数に影響を及ぼさなかった。18 mg/kg 以上で腹腔内体温の低下（18、30 及び 60 mg/kg でそれぞれ 1.76、2.02 及び 2.50°C）が観察された。18 mg/kg 以上で認められた QTc 間隔のわずかな延長（8%～10%）は、体温低下の結果であると考えられる（2.6.6.9 項）。

反復急速静注後の一般症状は投与した全動物で認められ、0.5 mg/kg で座位、起立困難、自発運動量減少及び失調歩行、2 及び 5 mg/kg でうずくまり、横臥位、腹臥位、接触に対する反応性消失及び閉眼が観察され、投与量に依存した症状の発現が認められた。

2 週間の反復投与毒性試験（[redacted]）では、カニクイザルに 1 日 1 回 9 時間 6.75、9.0、11.25、及び 22.5 mg/kg のレミマゾラムを静脈内持続投与した（2 低用量群では各群雌雄 3 匹、対照群及び 2 高用量群では各群雌雄 5 匹）。血圧、ECG パラメータ及び体温を投与 12 日目及び回復期 12 日目に測定し、いずれの群にも異常は認められなかった。

4 週間の反復投与毒性試験（[redacted]）では、レミマゾラムを 12.5、25 及び 50 mg/kg の投与量でカニクイザル（各群雌雄 4 匹）に週 1 回、4 週間（合計 5 回）静脈内投与した。各薬剤は投与速度 48 mL/kg/h で 5 分間投与後、8 mL/kg/h で 2 時間持続投与した。

Day 1（初回投与日）、15 及び 29 に体温及び心拍数の低下並びに QT 間隔の延長が投与開始 1 時間後に認められた。Day 1 及び 29 では QTc 間隔の延長がみられ、陰性対照群と比較して統計学的有意差が認められた［Day 1 : 全レミマゾラム投与群で有意差を認めたが、用量依存性はなかった、Day 29 : 低用量のレミマゾラム投与群で有意差を認め、用量依存性は認めなかった］。しかし、Day 15 では有意な延長は認められなかった。また投与終了 2～4 時間後では、有意な延長は認められなかった。薬物投与日に陰性対照群の動物に異常は認められなかった。毒性に関する詳細な知見は 2.6.6.3.6 項に記載した。

別の反復投与毒性試験（[redacted]）では、レミマゾラムを 5、10 及び 20 mg/kg/day で 1 日 1 回 28 日間カニクイザル（対照群及び高用量群では各群雌雄 5 匹、低及び中用量群では各群雌雄 3 匹）に急速静注した。溶媒（25.86% w/v β-シクロデキストリン含有 25 mmol/L クエン酸緩衝液、pH 3.8）投与群を陰性対照群とした。ECG 測定は投与開始前及び 4 週目の最終投与前日の投与前及び投与 15 分後に実施した。

投与開始前値と比較して、4 週目の投与前及び投与後の非補正 QT 間隔は雄雌 5、10 及び 20 mg/kg/day 群で延長が認められたが、心拍数補正 QT（QTcB）を用いると、同期間で対照群でも同程度の変化が認められることから、投与開始前値からの 4 週目投与前値の変化に投薬の関連性はないと考えられた。したがって、4 週目投与前の QTcB 値は、4 週目投与後の値の比較のためのベースラインとして適切であると判断した。この比較において、5 及び 10 mg/kg/day 群での雌雄における平均 QTcB 値の延長（約+10 ms 以下）は、重要な変化ではないと考えられた。20 mg/kg/day 群での平均 QTcB 値の延長は雄でのみ顕著（約+25 ms）であったが、これは雄 5 匹中 1 匹で QTcB 値が約 90 ms 延長したことが主に影響していた。この外れ値を除くと、平均値は約+10 ms 未満の増加であった。一貫した用量相関性が認められないことから、これらの変化は心拍数及び体温の変化による二次的なものと考えられる。

呼吸系の影響を検討した安全性薬理試験（[redacted]）で、覚醒下の雄カニクイザル（n = 8）にレミマゾラムを単回急速静注又は静脈内持続投与した。血液分析装置を用いて動脈血の pH、pO_2、pCO_2 及び SO_2 を測定した。レミマゾラムの単回急速静注時の投与量は 0.5、1、2 及び 5 mg/kg で、6 時間の単回静脈内持続投与は 6、18、30 及び 60 mg/kg（1、3、5 及び 10 mg/kg/h）であった。

単回急速静注において、レミマゾラムは検討した投与量で動脈血 pH、pO_2 又は SO_2 に対して影響しなかったが、5 mg/kg では投与 20 分後に pCO_2 が約 5 mmHg 増加した。別試験（[redacted]）において、同用量のレミマゾラムは呼吸数に影響しなかった。

静脈内持続投与において、3 mg/kg/h 以上で投与開始 1 時間後から pCO_2 が増加し、投与後 6 時間以内に pCO_2 は元のレベルまで回復した。最大変化は投与開始 1～3 時間後に観察され、ベースラインからの増加量は 3 及び 5 mg/kg/h で約 6 mmHg、10 mg/kg/h で約 10 mmHg であった。

2.6.2.5 薬力学的薬物相互作用

2.6.2.5.1 鎮静作用

試験番号：[redacted]（参考資料）、記載箇所：4.2.1.4.1
試験番号：[redacted]（参考資料）、記載箇所：4.2.1.4.2
試験番号：[redacted]（参考資料）、記載箇所：4.2.1.4.3
試験番号：[redacted]（参考資料）、記載箇所：4.2.1.4.4
試験番号：[redacted]（参考資料）、記載箇所：4.2.1.4.5
試験番号：[redacted]（参考資料）、記載箇所：4.2.1.4.6
試験番号：[redacted]（参考資料）、記載箇所：4.2.1.4.7
試験番号：[redacted]（参考資料）、記載箇所：4.2.1.4.8
試験番号：[redacted]（参考資料）、記載箇所：4.2.1.4.9
試験番号：[redacted]（参考資料）、記載箇所：4.2.1.4.10
試験番号：[redacted]（参考資料）、記載箇所：4.2.1.4.11
試験番号：[redacted]（参考資料）、記載箇所：4.2.1.4.12
試験番号：[redacted]（参考資料）、記載箇所：4.2.1.4.20
試験番号：[redacted]（評価資料）、記載箇所：4.2.1.4.21

雄 SD 又は Wistar ラットを用いて、抗不安薬、抗コリン薬、オピオイド系鎮痛薬、その他の鎮静薬及び麻酔薬とレミマゾラムとの薬力学的薬物相互作用を評価した。吸入麻酔薬のセボフルラン及びヒドロキシジン（皮下投与）を除き、薬剤は尾静脈より静脈内投与した。評価系の妥当性を確認するため、レミマゾラム単剤、対照化合物及び併用投与する薬剤単剤での鎮静作用を評価した。鎮静作用は LRR を示した動物数により評価した。ほとんどの試験では 50%の動物に LRR を発現する投与量［$ED_{50\ (LRR)}$］を算出して比較した。探索試験において、各薬剤の併用試験のため、最適な試験条件及びパラメータを検討した（[redacted]）。

全ての試験において、溶媒投与で LRR は発現しなかったが、レミマゾラムは用量依存的な鎮静作用を示した。単剤投与の場合は、2～30 mg/kg のレミマゾラムを投与した。併用投与には、より低用量で効果を示さない 0.1～2 mg/kg を主に用いた。特に言及しない場合は、併用する薬剤は効果を示さない投与量（最大無作用量又は最小有効用量の半分量）を探索試験の評価用量として用いた（表 2.6.2-17）。

NIBS 系ミニブタを用いてオピオイド系鎮痛薬との相互作用を評価した（[redacted]）。なお、本試験でのレミマゾラム単剤による鎮静作用は 2.6.2.2.2.3 項に記載した。フェンタニル又はレミフェンタニルの単剤投与時に、特異的な行動変化が観察された。自発運動量及び匂いかぎ行動の増加などの興奮性症状は、溶媒、レミマゾラム、プロポフォール又はミダゾラムとの併用に関係なく両オピオイド系鎮痛薬投与後に認められたことから、ミニブタを用いた評価系はオピオイド系鎮痛薬との併用作用を評価するには不適当であると考えられた。

フェンタニル及びレミフェンタニルの鎮痛作用に対するレミマゾラムの影響は、熱回避反応潜時を指標としたテイルフリック 3 試験により評価した（[redacted]、[redacted]、[redacted]）。

表 2.6.2-17　薬力学的薬物相互作用（鎮静作用及び鎮痛作用）

Co-administration with	Effects on	Species	Method of admin.	Doses Test Article	Gender and No. per Group	Noteworthy Findings	Study No.
Fentanyl (7, 10 µg/kg, i.v.)	Sedation [(loss of lighting reflex (LRR)]	Rat	intravenous (i.v.)	0, 2, 5, 10, 20, 30 mg/kg (depending on experiment)	male (M) n = 8	Remimazolam (≥20 mg) alone dose-dependently induced LRR. Fentanyl significantly enhanced this effect. A similar enhancing effect was observed with midazolam (10, 30 mg/kg) and co-administered fentanyl.	[redacted]
Fentanyl (5 µg/kg, i.v.)	Sedation (LRR)	Rat	i.v.	0, 2, 5, 10 mg/kg (single); 0, 0.1, 0.2, 0.5, 1 mg/kg (co-administration)	M n = 10	The sedative effect of remimazolam was synergistically enhanced by co-administration of fentanyl, the 50% effective dose (ED_{50}) was reduced by 91%. The ED_{50} of midazolam and propofol dropped by 95 and 60%, respectively.	[redacted]
Remifentanil (5 µg/kg, i.v.)	Sedation (LRR)	Rat	i.v.	0, 2, 5, 10 mg/kg (single); 0, 0.2, 0.5, 1, 2 mg/kg (co-administration)	M n = 10	The sedative effect of remimazolam was synergistically enhanced by co-administration of remifentanil, the ED_{50} value was reduced by 89%. The ED_{50} of midazolam and propofol dropped by 93 and 58%, respectively.	[redacted]
Propofol (2 mg/kg, i.v.) Dexmedetomidine (5 µg/kg, i.v.) Thiamylal (5 mg/kg, i.v.) Midazolam (10 mg/kg, i.v.)	Sedation (LRR)	Rat	i.v.	0, 2, 5, 10 mg/kg (single); 0, 0.1, 0.2, 0.5, 1, 2 mg/kg (co-administration)	M n = 10	The sedative effect of remimazolam was synergistically enhanced by co-administration of propofol, dexmedetomidine and thiamylal, the ED_{50} values decreased by 86%, 95%, and 86%, respectively. No synergistic potentiation of the sedative effect was observed with midazolam, presumably due to the same mechanism of action.	[redacted]
Hydroxyzine [70 mg/kg, subcutaneous (s.c.)]	Sedation (LRR)	Rat	i.v.	0, 2, 5, 10 mg/kg (single); 0, 0.2, 0.5, 1, 2 mg/kg (co-administration)	M n = 10	The sedative effect of remimazolam was synergistically enhanced by co-administration of hydroxyzine, the ED_{50} value decreased by 77%.	[redacted]
Atropine (1 mg/kg, i.v.)	Sedation (LRR)	Rat	i.v.	0, 2, 5, 10 mg/kg (single); 0, 1, 2, 5, 10 mg/kg (co-administration)	M n = 10	Co-administration of atropine did not alter the sedative dose of remimazolam indicating lacking interaction.	[redacted]

表 2.6.2-17　薬力学的薬物相互作用（鎮静作用及び鎮痛作用）（続き）

Co-administration with	Effects on	Species	Method of admin.	Doses Test Article	Gender and No. per Group	Noteworthy Findings	Study No.
Ketamine (2 mg/kg, i.v.)	Sedation (LRR)	Rat	i.v.	0, 2, 5, 10 mg/kg (single); 0, 0.1, 0.2, 0.5, 1 mg/kg (co-administration)	M n = 10	The sedative effect of remimazolam was synergistically enhanced by co-administration of ketamine, the ED_{50} value decreased by 93%.	[redacted]
Sevoflurane [0.4% (MAC); 0.4% (LRR) via inhalation]	Anesthesia (MAC); Sedation (LRR)	Rat	i.v.	0, 10, 20 mg/kg (single); 0, 1, 2, 5 mg/kg (MAC) (co-administration) 0, 2, 5, 10 mg/kg (single); 0, 0.1, 0.2, 0.5 mg/kg (LRR) (co-administration)	M n = 5-6	Preliminary study to optimize conditions and parameters for anesthetic and sedative effects of remimazolam in combination with sevoflurane using minimum alveolar concentration (MAC) and LRR evaluation.	[redacted]
Sevoflurane (0.4% via inhalation)	Sedation (LRR)	Rat	i.v.	0, 2, 5, 10 mg/kg (single); 0, 0.1, 0.2, 0.5 mg/kg (co-administration)	M n = 10	The sedative effect of remimazolam was synergistically enhanced by co-administration of sevoflurane. The ED_{50} of remimazolam decreased by 94% from 3.1 (alone) to 0.19 mg/kg upon sevoflurane co-administration.	[redacted]
Fentanyl (1, 2, 5 μg/kg, i.v.)	Analgesia (heat avoidance latency)	Rat	i.v.	0, 5 mg/kg	M n = 8	Remimazolam did not influence the analgesic effect of fentanyl.	[redacted]
Remifentanil (1, 2, 5 μg/kg, i.v.)	Analgesia (heat avoidance latency)	Rat	i.v.	0, 5 mg/kg	M n = 8	Remimazolam did not influence the analgesic effect of remifentanil	[redacted]
Remifentanil (1, 2, 5, 10 μg/kg, i.v.)	Analgesia (heat avoidance latency)	Rat	i.v.	0, 5 mg/kg	M n = 8	Remimazolam did not influence the analgesic effect of remifentanil.	[redacted]

表 2.6.2-17　薬力学的薬物相互作用（鎮静作用及び鎮痛作用）（続き）

Co-administration with	Effects on	Species	Method of admin.	Doses Test Article	Gender and No. per Group	Noteworthy Findings	Study No.
Fentanyl (7.5, 12, 25 μg/kg/h, i.v.) Remifentanil (7.5, 15 μg/kg/h, i.v.)	Sedation (general sedation and ptosis score, pinna reflex)	Micropig	i.v.	0, 0.4, 0.8, 1.6, 3.2, 6.4 mg/kg/h dose escalation every 30 min	M n = 2-3	Administration of fentanyl or remifentanil led to changes in excitation-like symptoms (increased motor activity and sniffing behavior), irrespective of co-administration of remimazolam, propofol, or midazolam. Therefore, the test system was deemed inappropriate to investigate interactions with opioid analgesics.	[redacted]
Remifentanil (35 μg/kg/h, i.v.)	Sedation (sedation and ptosis score)	Cynomol-gus monkey	i.v.	5, 10, 15, 20, 35, 50 mg/kg/h (single); 0, 0.5, 1, 2, 5, 10 mg/kg/h (co-administration); dose escalation every 20 min	M n = 6	The sedative effect of remimazolam was synergistically enhanced by co-administration of remifentanil, the sedative dose was reduced by 92%. Sedative doses of midazolam and propofol decreased by 98 and 58%, respectively.	[redacted]

以上より、ラットを用いた薬力学的薬物相互作用試験で、オピオイド系鎮痛薬、ベンゾジアゼピン結合部位（$GABA_A$受容体）以外に活性部位を有する鎮静剤又は麻酔剤との併用投与で、レミマゾラムの鎮静作用に相乗効果が認められた。フェンタニル、レミフェンタニル、プロポフォール、デクスメデトミジン、チアミラール、ヒドロキシジン、ケタミン又はセボフルランとの併用により、レミマゾラムの鎮静作用に対するED_{50}値は77%～95%減少した。ベンゾジアゼピン系薬剤ミダゾラム又は抗コリン薬アトロピンとの併用では、レミマゾラムの鎮静作用に相乗効果は認められなかった。カニクイザルでは、レミフェンタニルの併用によりレミマゾラムが鎮静作用を誘発する投与量は92%まで減少し、相乗効果が認められた。結論として、オピオイド系鎮痛薬又は非ベンゾジアゼピン系薬剤との併用によりレミマゾラムの鎮静作用が増強される可能性が示された。また、フェンタニル及びレミフェンタニルの鎮痛作用は、レミマゾラムの併用投与により影響しないことが示された。

2.6.2.5.2 心血管系に対する作用

試験番号：[redacted]（参考資料）、記載箇所：4.2.1.4.13
試験番号：[redacted]（参考資料）、記載箇所：4.2.1.4.14
試験番号：[redacted]（参考資料）、記載箇所：4.2.1.4.15
試験番号：[redacted]（参考資料）、記載箇所：4.2.1.4.16
試験番号：[redacted]（参考資料）、記載箇所：4.2.1.4.17
試験番号：[redacted]（参考資料）、記載箇所：4.2.1.4.18
試験番号：[redacted]（参考資料）、記載箇所：4.2.1.4.19

臨床で併用投与が想定される薬剤とレミマゾラムとの薬物相互作用について、血圧及び心拍数（[redacted]）並びに筋収縮（[redacted]）に対する作用を指標に評価した。各試験において、薬剤は覚醒下（[redacted]及び[redacted]は麻酔下）の雄SDラットに尾静脈より静脈内投与（セボフルランの場合は吸入投与）した。実験条件及び結果を表 2.6.2-18 にまとめた。

表 2.6.2-18　薬力学的薬物相互作用（心血管系に及ぼす影響）

Co-administration with	Effects on	Species	Method of admin.	Doses Test Article	Gender and No. per Group	Noteworthy Findings	Study No.
Sevoflurane (1.2, 2.4% via inhalation)	Blood pressure, heart rate	Rat	intravenous (i.v.)	0, 5, 10 mg/kg	male (M) n = 7	Remimazolam (5 and 10 mg/kg) alone caused a mild decrease in blood pressure (< 10%) and increased heart rate. Sevoflurane 1.2% had no effect whereas 2.4% enhanced the remimazolam induced decrease of blood pressure. The increase of heart rate was significantly attenuated by both sevoflurane concentrations.	[redacted]
				0, 10, 20 mg/kg midazolam	n = 4	Midazolam (10 and 20 mg/kg) alone decreased blood pressure (> 10%) and increased heart rate. Sevoflurane did not modify the decrease on blood pressure, but attenuated the increase of heart rate.	
Fentanyl (5 µg/kg, i.v.) Remifentanil (5 µg/kg, i.v.)	Blood pressure, heart rate	Rat	i.v.	0, 1, 2, 5, 10, 20, 30 mg/kg (depending on co-administration)	M n = 8	When remimazolam or propofol was administered alone, the doses which elicited a 20% drop in mean blood pressure ($ED_{20(BP)}$) were ≥ 30 and 9.4 mg/kg, respectively. When remimazolam and propofol were co-administered with fentanyl, the $ED_{20(BP)}$ values were 8.2 and 3.3 mg/kg, respectively, or when co-administered with remifentanil, ≥ 20 and 6.8 mg/kg, respectively. The separation ratios between the effective dose for a sedative effect ($ED_{50(LRR)}$), calculated from ED_{50} values for loss of righting reflex in an earlier study and the dose which elicited a decrease in blood pressure ($ED_{20(BP)}$) was ≥ 7.7- and 2.1-fold (remimazolam or propofol alone), 19- and 1.7-fold (co-administration with fentanyl), or ≥ 43- and 4.5-fold (co-administration with remifentanil), respectively. It was concluded, that the separation ratio for remimazolam was larger than that for propofol, and similar for that following co-administration with an analgesic (fentanyl and remifentanil).	[redacted]

表 2.6.2-18　薬力学的薬物相互作用（心血管系に及ぼす影響）（続き）

Co-administration with	Effects on	Species	Method of admin.	Doses Test Article	Gender and No. per Group	Noteworthy Findings	Study No.
Dopamine (30 µg/kg, i.v.) Phenylephrine (3 µg/kg, i.v.)	Blood pressure, heart rate	Rat	i.v.	0, 10 mg/kg	M n = 8	Remimazolam alone decreased slightly the blood pressure by 1.7 and 0.5%, whereas after combination with dopamine or phenylephrine the blood pressure was significantly increased by 13.2 and 14.1%, respectively. Remimazolam alone produced an increased heart rate (23.6 or 15.0%), whereas the change (%) in heart rate after the combination with dopamine (5.3%) or with phenylephrine (-3.0%) was significantly lower. It was concluded, that the decreased blood pressure and increased heart rate induced by remimazolam were attenuated by combination with dopamine or phenylephrine.	[redacted]
Landiolol (30 mg/kg, i.v.) Lidocaine (5 mg/kg, i.v.)	Blood pressure, heart rate	Rat	i.v.	0, 10 mg/kg	M n = 8	The decreased blood pressure induced by remimazolam (-4.3%) was synergistically enhanced by the co-administration with landiolol (-10.4%). There were no effects of the combined administration with lidocaine (-9.4%) compared to remimazolam alone (-10.4%). The increased heart rate after remimazolam alone (15.0 or 13.4%) was attenuated by the combined administration with landiolol (-1.3%) and lidocaine (2.9%), respectively.	[redacted]

表 2.6.2-18　薬力学的薬物相互作用（心血管系に及ぼす影響）（続き）

Co-administration with	Effects on	Species	Method of admin.	Doses Test Article	Gender and No. per Group	Noteworthy Findings	Study No.
Milrinone (100 µg/kg, i.v.) Digoxin (1 mg/kg, i.v.)	Blood pressure, heart rate	Rat	i.v.	0, 10 mg/kg	M n = 8	The decrease of blood pressure (-10.2%) produced by co-administration of remimazolam with milrinone was suggested as being synergistic because neither the administration of remimazolam (1.0%) nor of milrinone (-2.6%) caused a significant effect. There were no significant effects of the combined administration with digoxin (-8.2%) compared to remimazolam alone (-0.4%). The increased heart rate after remimazolam alone (25.4%) was attenuated by digoxin (7.5%), whereas milrinone (20.3% vs. remimazolam alone 19.8%) was ineffective.	[redacted]
Nicardipine (100 µg/kg, i.v.) Nitroglycerin (300 µg/kg, i.v.) Alprostadil (30 µg/kg, i.v.)	Blood pressure, heart rate	Rat	i.v.	0, 10 mg/kg	M n = 8	The decreased blood pressure induced by remimazolam (-2.4, -2.1 or -1.4%) was synergistically enhanced by the co-administration with nicardipine (-24.9%), nitroglycerin (-23.1%) and alprostadil (-27.0%), respectively. The increased heart rate after remimazolam alone (13.4%) was attenuated by nicardipine (8.0%), whereas nitroglycerin (15.5% vs. remimazolam alone 12.1%) or alprostadil (31.3% vs. remimazolam alone 26.5%) were ineffective.	[redacted]

表 2.6.2-18　薬力学的薬物相互作用（心血管系に及ぼす影響）（続き）

Co-administration with	Effects on	Species	Method of admin.	Doses Test Article	Gender and No. per Group	Noteworthy Findings	Study No.
Rocuronium (0.5, 1, 2 mg/kg, i.v.) Suxamethonium (0.1, 0.2, 0.5 mg/kg, i.v.) Rocuronium (4 mg/kg, i.v.) + neostigmine (0.1 mg/kg, i.v.) Rocuronium (4 mg/kg, i.v.) + sugammadex (10 mg/kg, i.v.)	Muscle contraction (electrical stimulation of sciatic nerve)	Rat	i.v.	0, 5, 10, 20 mg/kg	M n = 2-6	Preliminary study to set conditions for a test for evaluating muscle relaxant action. Remimazolam (5-20 mg/kg) alone did not affect the muscle contraction and showed no muscle relaxant action. Rocuronium (0.5-2 mg/kg) and also suxamethonium (0.1-0.5 mg/kg) induced dose dependent muscle relaxant action which were reversed by neostigmine and sugammadex, respectively. Remimazolam (10 mg/kg) does not influence the muscle relaxant action of rocuronium or suxamethonium and the muscle relaxant reversal action of neostigmine or sugammadex.	[redacted]

レミマゾラム単剤投与によりラットで血圧低下が認められた。この血圧低下作用はドパミン及びフェニレフリンとの併用で減弱し、ランジオロール、ミルリノン、ニカルジピン、ニトログリセリン、アルプロスタジル及びセボフルランとの併用で増強し、リドカイン及びジゴキシンとの併用では影響しなかった。しかし、セボフルランとの併用下において、レミマゾラムの鎮静作用が増強される投与量と血圧低下が増強される投与量との間には十分な乖離があると考えられた（[redacted]、[redacted]、[redacted]、[redacted]、[redacted]）。同様に、オピオイド系鎮痛薬フェンタニル及びレミフェンタニルはレミマゾラムの血圧低下作用を増強した（[redacted]）。

本試験における平均血圧を 20%低下させる投与量［$ED_{20(BP)}$］及び別試験における 50%の動物に LRR を誘導する投与量［$ED_{50(LRR)}$］との用量比を算出し、表 2.6.2-19 に示した。プロポフォールは比較対照薬として用いた。用量比［$ED_{20(BP)}$／$ED_{50(LRR)}$］の結果から、レミマゾラムはプロポフォールと比較して血圧低下作用及び鎮静作用の用量がより乖離していることが示された。フェンタニル又はレミフェンタニルと併用した場合でも、同様にレミマゾラムはより高い用量比を示した。レミマゾラムの $ED_{20(BP)}$値はフェンタニル又はレミフェンタニルを併用した場合に明らかに低下した。しかしながら、レミマゾラム単剤投与試験（30 mg/kg）及びレミフェンタニルとの併用試験（20 mg/kg）は最高用量のレミマゾラムでも $ED_{20(BP)}$値に達していないため、正確な用量比を確定することはできなかった。

表 2.6.2-19　レミマゾラム及びプロポフォールの単剤投与又はオピオイド系鎮痛薬との併用投与後の $ED_{20(BP)}$及び $ED_{50(LRR)}$値

Experimental Group		$ED_{50(LRR)}$ mg/kg	$ED_{20(BP)}$ mg/kg	Separation Ratio
Single-drug administration	Remimazolam (10, 20, 30 mg/kg)	3.9	≥ 30	≥ 7.7-fold
	Propofol (5, 10, 20 mg/kg)	4.4	9.4	2.1-fold
Co-administration with 5 μg/kg fentanyl	Remimazolam (1, 2, 5, 10 mg/kg)	0.44	8.2	19-fold
	Propofol (1, 2, 5, 10 mg/kg)	1.9	3.3	1.7-fold
Co-administration with 5 μg/kg remifentanil	Remimazolam (2, 5, 10, 20 mg/kg)	0.46	≥ 20	≥ 43-fold
	Propofol (1, 2, 5, 10 mg/kg)	1.5	6.8	4.5-fold

$ED_{50(LRR)}$: Dose at which 50% of animals had loss of righting reflex (LRR) ([redacted])
$ED_{20(BP)}$: Dose at which 20% drop in mean blood pressure (BP) was observed ([redacted])
Separation ratio was calculated from $ED_{20(BP)}$/ $ED_{50(LRR)}$

レミマゾラムの単剤投与により心拍数増加が認められた。この心拍数増加作用は、ドパミン、フェニレフリン、リドカイン、ランジオロール、ジゴキシン及びニカルジピンとの併用で減弱し、ミルリノン、ニトログリセリン及びアルプロスタジルとの併用で場合は影響を受けなかった（[redacted]、[redacted]、[redacted]、[redacted]、[redacted]）。

レミマゾラムの単剤投与は筋収縮に影響しなかった。レミマゾラムを併用投与しても、ロクロニウム又はスキサメトニウムの筋弛緩作用に影響せず、ロクロニウム及びネオスチグミンの両剤併用投与、又はロクロニウム及びスガマデクスの両剤併用投与による作用に影響しなかった（[redacted]）。

2.6.2.6 考察及び結論

レミマゾラムは脳内のベンゾジアゼピン結合部位（$GABA_A$ 受容体）へ高親和性に結合し、off-target に対する非特異的な活性はレミマゾラム及びその主代謝物である CNS 7054 で認められていない。レミマゾラムは試験で使用した全動物種において、作用発現が速く、早期に回復する鎮静作用を示した。ミニブタ、ヒツジ及びサルにおいて、レミマゾラムは、呼吸系又は心血管系を過度に抑制せずに深鎮静を誘導した。レミマゾラムは、急速静注後に短時間の鎮静作用を示し、長期静脈内持続投与により持続的な鎮静作用を示した。静脈内持続投与では一定の鎮静深度を維持できた。レミマゾラムの鎮静作用はフルマゼニルによって速やかに回復したことから、鎮静作用を急遽中断する必要性があった場合に対応しやすいと考えられた。

[redacted]又は主代謝物である CNS 7054 は、マウスにおいて LRR を指標とした鎮静作用を示さなかった。運動失調を指標としたより低用量からのラットを用いた鎮静作用評価でも、[redacted]及び CNS 7054 の鎮静作用強度は、レミマゾラムと比較してそれぞれ 1/40 及び 1/200 であった。以上より、血漿中濃度を考慮しても[redacted]及び CNS 7054 による鎮静作用は無視できる程度であると考えられる。

鎮静作用の速やかな発現及び回復に加えて、有効用量と致死量間の安全域（LD_{50}/ED_{50}）はミダゾラム（≥ 7.1 倍）及びプロポフォール（5 倍）と比べてレミマゾラム（23 倍）は極めて大きく、良好な安全性プロファイルを有することが示された。デクスメデトミジンはより広い安全域（≥ 120 倍）を有するが、鎮静作用発現後の回復時間が極めて長いことの懸念が上回る。

ラット受動回避反応試験では、レミマゾラムはミダゾラムと同等の用量で健忘作用を発現したことから、臨床で問題になっている術中覚醒による外傷後ストレス障害の予防に有用である可能性が示唆された。

レミマゾラムは、*in vitro* で $GABA_A$ 受容体を活性化させるのに必要な濃度及び毒性試験や臨床試験で到達しうる血漿中遊離型薬物濃度をはるかに上回る高濃度において、hERG 電流に対する弱い阻害作用を示した。

他のベンゾジアピン系薬剤と同様に [2)]、レミマゾラム単剤投与で血圧低下及び心拍数増加が認められた。ラットでのレミマゾラムによる血圧低下作用は、ドパミン及びフェニレフリンとの併用で減弱し、ランジオロール、ミルリノン、ニカルジピン、ニトログリセリン、アルプロスタジル及びセボフルランとの併用で増強し、リドカイン及びジゴキシンとの併用では影響されなかった。しかし、セボフルランとの併用下において、レミマゾラムの鎮静作用が増強される投与量と、血圧低下が増強される投与量との間には十分な乖離があると考えられた。レミマゾラムによる心拍数増加作用は、ドパミン、フェニレフリン、リドカイン、ランジオロール、ジゴキシン及びニカルジピンと併用投与した場合に減弱し、ミルリノン、ニトログリセリン及びアルプロスタジルと併用投与した場合は影響を受けなかった。

ラットにおいて、ベンゾジアゼピン結合部位（$GABA_A$ 受容体）以外に活性部位を有する鎮静剤（プロポフォール、デクスメデトミジン、チアミラール及びヒドロキシジン）と同様に、オピオイド系鎮痛薬フェンタニル及びレミフェンタニルとの併用投与でもレミマゾラムの鎮静作用に対する相乗効果が認められた。ベンゾジアゼピン系薬剤ミダゾラム又は抗コリン薬アトロピンとの併用投与では、レミマゾラムの鎮静作用に対する相乗効果は認められなかった。ケタミンと併用すると、レミマゾラムの鎮静作用に相乗効果が認められた。同様な結果はセボフルランとの併用時に得られた。同様に、サルにおいてレミフェンタニルとの併用投与でレミマゾラムの鎮静作用

に相乗効果が認められた。

以上、レミマゾラムは、速やかな作用発現、短い持続時間及び速やかな回復を特徴とする鎮静作用プロファイルを有するとともに、鎮静作用量において過度の呼吸抑制又は心血管系の抑制を誘導しなかった。このことから、レミマゾラムは呼吸機能や循環動態が不安定になりやすい患者も含め、広く有用な鎮静剤となる可能性が示唆された。

2.6.2.7 図表

図表は本文中に記載した。

2.6.2.8 参考文献

1) Upton RN, Martinez AM, Grant C. Comparison of the sedative properties of CNS 7056, midazolam, and propofol in sheep. Br J Anaesth. 2009; 103(6): 848-57.
2) Olkkola KT, Ahonen J. Midazolam and other benzodiazepines. Handb Exp Pharmacol. 2008; 182: 335-60.

レミマゾラムベシル酸塩
アネレム静注用 50mg

第 2 部 CTD の概要
2.6 非臨床試験の概要文及び概要表
2.6.3 薬理試験概要表

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2.6.3 薬理試験概要表

2.6.3.1 薬理試験：一覧表

Test Article: Remimazolam

Type of Study	Test System	Meth. of Admin	Testing Facility	Study Number	Location in CTD
Primary Pharmacodynamics					
Selectivity profile	Various receotors, transporters and ion channels	*in vitro*	[redacted]	[redacted]	4.2.1.1.1
Selectivity profile	Various receotors, transporters and ion channels	*in vitro*	[redacted]	[redacted]	4.2.1.1.2
Gamma-aminobutyric acid (GABA) receptor binding	Various receotors, transporters and ion channels	*in vitro*	[redacted]	[redacted]	4.2.1.1.3
Selectivity profile	Various receotors and ion channels	*in vitro*	[redacted]	[redacted]	4.2.1.1.4

2.6.3.1 薬理試験：一覧表（続き）

Test Article: Remimazolam

Type of Study	Test System	Meth. of Admin	Testing Facility	Study Number	Location in CTD
Primary Pharmacodynamics (cont.)					
Selectivity profile	Various receotors	*in vitro*	[redacted]	[redacted]	4.2.1.1.4
GABA receptor binding	Various receotors	*in vitro*	[redacted]	[redacted]	4.2.1.1.5
$GABA_A$ receptor sub-type modulation	Ltk cells (mouse fibroblast)	*in vitro*	[redacted]	[redacted]	4.2.1.1.6
Induction of sedation, reversal by flumazenil	Mouse	i.v.	[redacted]	[redacted]	4.2.1.1.7
Effects of [redacted] and CNS 7054 on loss of righting reflex (LRR)	Mouse	i.v.	[redacted]	[redacted]	4.2.1.1.8
Sedative effect, comparison with existing drugs, dose response relationship	Rat	i.v.	[redacted]	[redacted]	4.2.1.1.9
Optimal test conditions to evaluate the sedative profiles	Rat	i.v.	[redacted]	[redacted]	4.2.1.1.10
Induction of sedation, midazolam and propofol as comparators	Rat	i.v.	[redacted]	[redacted]	4.2.1.1.11

2.6.3.1 薬理試験：一覧表（続き）

Test Article: Remimazolam

Type of Study	Test System	Meth. of Admin	Testing Facility	Study Number	Location in CTD
Primary Pharmacodynamics (cont.)					
In vivo electrophysiology	Rat	i.v.	[redacted]	[redacted]	4.2.1.1.12
Optimal test conditions to evaluate the influence of flumazenil	Rat	i.v.	[redacted]	[redacted]	4.2.1.1.13
Sedative profile compared to midazolam	Rat	i.v.	[redacted]	[redacted]	4.2.1.1.14
Assessment of vehicles and animal strains, comparison with control substances	Rat	i.v.	[redacted]	[redacted]	4.2.1.1.15
Additional analysis of 50% effective dose (ED_{50}) values and 50% lethal dose (LD_{50}) values	Rat	i.v.	[redacted]	[redacted]	4.2.1.1.16
Potential effect of vehicle on LRR induction	Rat	i.v.	[redacted]	[redacted]	4.2.1.1.17
Sedative effect of lot no. YMK110831 with new impurities (ONO-G0000-854-01)	Rat	i.v.	[redacted]	[redacted]	4.2.1.1.18
Influence of flumazenil, comparison with existing drugs	Rat	i.v.	[redacted]	[redacted]	4.2.1.1.19
Sedative effect of metabolite [redacted]	Rat	i.v.	[redacted]	[redacted]	4.2.1.1.20

2.6.3.1 薬理試験：一覧表（続き）

Test Article: Remimazolam

Type of Study	Test System	Meth. of Admin	Testing Facility	Study Number	Location in CTD
Primary Pharmacodynamics (cont.)					
Sedative effect in micropigs (bolus/infusion)	Micropig	i.v.	[redacted]	[redacted]	4.2.1.1.21
Sedative effect in micropigs	Micropig	i.v.	[redacted]	[redacted]	4.2.1.1.22
Sedative effects in micropigs (induction and maintenance)	Micropig	i.v.	[redacted]	[redacted]	4.2.1.1.23
Test conditions to maintain mild sedation by long-term infusion	Micropig	i.v.	[redacted]	[redacted]	4.2.1.1.24
Sedative effects by long-term infusion	Micropig	i.v.	[redacted]	[redacted]	4.2.1.1.25
Secondary Pharmacodynamics					
Amnestic effect	Rat	i.v.	[redacted]	[redacted]	4.2.1.2.1

2.6.3.1 薬理試験：一覧表（続き）

Test Article: Remimazolam

Type of Study	Test System	Meth. of Admin	Testing Facility	Study Number	Location in CTD
Safety Pharmacology					
Irwin screen test	Rat	i.v.	[redacted]	[redacted]	4.2.1.3.1
Human ether-a-go-go-related gene (hERG) study	Human	*in vitro*	[redacted]	[redacted]	4.2.1.3.2
hERG study, rapid screening technology	Human	*in vitro*	[redacted]	[redacted]	4.2.1.3.3
hERG study	Human	*in vitro*	[redacted]	[redacted]	4.2.1.3.4
hERG study	Human	*in vitro*	[redacted]	[redacted]	4.2.1.3.5
CNS papillary muscle	Guinea pig	*in vitro*	[redacted]	[redacted]	4.2.1.3.6
CNS papillary muscle	Guinea pig	*in vitro*	[redacted]	[redacted]	4.2.1.3.7
Effects on hematic functions	Rat	*in vitro*	[redacted]	[redacted]	4.2.1.3.8

2.6.3.1 薬理試験：一覧表（続き）

Type of Study	Test System	Meth. of Admin	Testing Facility	Study Number	Location in CTD
Safety Pharmacology (cont.)					
Effects on hematic functions	Human	*in vitro*	[redacted]	[redacted]	4.2.1.3.9
Effects on hematic functions	Human	*in vitro*	[redacted]	[redacted]	4.2.1.3.10
Cardiovascular effects	Rat	i.v.	[redacted]	[redacted]	4.2.1.3.11
Blood oxygen saturation	Rabbit	i.v.	[redacted]	[redacted]	4.2.1.3.12
Cardiovascular & respiratory effects	Micropig	i.v.	[redacted]	[redacted]	4.2.1.3.13
Cardiovascular & respiratory effects	Cynomolgus monkey	i.v.	[redacted]	[redacted]	4.2.1.3.14
Respiratory effects	Cynomolgus monkey	i.v.	[redacted]	[redacted]	4.2.1.3.15

2.6.3.1 薬理試験：一覧表（続き）

Test Article: Remimazolam

Type of Study	Test System	Meth. of Admin	Testing Facility	Study Number	Location in CTD
Pharmacodynamic Drug Interactions					
Fentanyl, effects on sedation (LRR)	Rat	i.v.	[redacted]	[redacted]	4.2.1.4.1
Fentanyl, effects on sedation (LRR)	Rat	i.v.	[redacted]	[redacted]	4.2.1.4.2
Fentanyl, effects on analgesia (heat avoidance latency)	Rat	i.v.	[redacted]	[redacted]	4.2.1.4.3
Remifentanil, effects on sedation (LRR)	Rat	i.v.	[redacted]	[redacted]	4.2.1.4.4
Remifentanil, effects on analgesia (heat avoidance latency)	Rat	i.v.	[redacted]	[redacted]	4.2.1.4.5
Remifentanil, effects on analgesia (heat avoidance latency)	Rat	i.v.	[redacted]	[redacted]	4.2.1.4.6
Propofol, midazolam, dexmedetomodine, and thiamylal, effects on sedation (LRR)	Rat	i.v.	[redacted]	[redacted]	4.2.1.4.7
Hydroxyzine, effects on sedation (LRR)	Rat	i.v.	[redacted]	[redacted]	4.2.1.4.8

2.6.3.1 薬理試験：一覧表（続き）

Test Article: Remimazolam

Type of Study	Test System	Meth. of Admin	Testing Facility	Study Number	Location in CTD
Pharmacodynamic Drug Interactions (cont.)					
Atropine, effects on sedation (LRR)	Rat	i.v.	[redacted]	[redacted]	4.2.1.4.9
Ketamine, effects on sedation (LRR)	Rat	i.v.	[redacted]	[redacted]	4.2.1.4.10
Sevoflurane, effects on anesthesia (minimal alveolar concentration [MAC]) and sedation (LRR)	Rat	i.v.	[redacted]	[redacted]	4.2.1.4.11
Sevoflurane, effects on sedation (LRR)	Rat	i.v.	[redacted]	[redacted]	4.2.1.4.12
Sevoflurane effects on blood pressure (BP) and heart rate (HR)	Rat	i.v.	[redacted]	[redacted]	4.2.1.4.13
Fentanyl or remifentanil effects on BP and HR	Rat	i.v.	[redacted]	[redacted]	4.2.1.4.14
Dopamine or phenylephrine effects on BP and HR	Rat	i.v.	[redacted]	[redacted]	4.2.1.4.15

2.6.3.1 薬理試験：一覧表（続き）

Test Article: Remimazolam

Type of Study	Test System	Meth. of Admin	Testing Facility	Study Number	Location in CTD
Pharmacodynamic Drug Interactions (cont.)					
Landiolol or lidocaine effects on BP and HR	Rat	i.v.	[redacted]	[redacted]	4.2.1.4.16
Milrinone or digoxin effects on BP and HR	Rat	i.v.	[redacted]	[redacted]	4.2.1.4.17
Hypotensive agents effects on BP and HR	Rat	i.v.	[redacted]	[redacted]	4.2.1.4.18
Muscle relaxants effects on BP and HR	Rat	i.v.	[redacted]	[redacted]	4.2.1.4.19
Fentanyl, effects on sedation (sedation and ptosis scores, pinna reflex)	Micropig	i.v.	[redacted]	[redacted]	4.2.1.4.20
Remifentanil, effects on sedation (sedation and ptosis scores)	Monkey	i.v.	[redacted]	[redacted]	4.2.1.4.21

2.6.3.2 効力を裏付ける試験

Table 2.6.3.2-01	Primary Pharmacodynamics	Test Article: Remimazolam
Report Title: [redacted] Custom Screen Report		
Route of Administration: *in vitro*	Location in CTD: 4.2.1.1.1	Study Number: [redacted]
GLP Compliance: non-GLP		
Aim of Study: To evaluate the activity of remimazolam in radioligand binding assays. Study Design: Radioligand binding assays using a wide array of receptors or membrane preparations from several species. Reference compounds were tested as positive control as an integral part of each assay.		
Species (Gender, Strain, Supplier, n/Group): Insect, rat, syrian hamster, guinea pig, rabbit, calf, human, n = 2 experiments Age/Body Weight: no data Conditioning and Surgical Pre-treatment: Preparation of membranes for binding studies		
Test Compounds (Batch No) and Test Conditions (Formulation, Doses, Route of Administrations):		
Remimazolam	Batch No: no data, dissolved in 0.5% DMSO, concentration 10 μmol/L against various types of receptors. 0.01, 0.1, 1 and 10 μmol/L against gamma-aminobutyric acid $(GABA)_A$ benzodiazepine, Cen. receptor.	
Additional compounds	Specific antagonists at the respective receptor according to the [redacted] reference compound data list.	
Method of Evaluation: The amount of specific binding was determined by subtracting the amount of nonspecific binding from the amount of total binding. In the case of $GABA_A$, benzodiazepine, Cen. receptor the reference was [^{3}H] Flunitrazepam (dissociation constant: Kd = 4.4 nmol/L). Statistical Analysis: No statistical analysis was performed.		
Results: No detectable affinity was seen at any site other than the $GABA_A$ receptor (50% inhibitory concentration: IC_{50} of remimazolam = 10.5 nmol/L).		

Table Summary of binding affinity of remimazolam to various receptors and others

Target	Source	Radioligand	% inhibition (Remimazolam 10 μmol/L)
Adenosine A_1	Rat brain	[^{3}H]DPCPX	6
Adenosine A_{2A}	Human recombinant, mammalian	[^{3}H]CGS21680	20
Adenosine A_3	Human recombinant, mammalian	[^{125}I]AB-MECA	0
Adrenergic α_1, Non-selective	Rat brain	[^{3}H]Prazosin	20
Adrenergic α_2, Non-selective	Rat cortex	[^{3}H]Rauwolscine	9
Adrenergic β_1	Human recombinant, mammalian	[^{125}I]Cyanopindolol	-4
Adrenergic β_2	Human recombinant, mammalian	[^{3}H]CGP-12177	-6
Adrenergic NE Transporter	Human recombinant, mammalian	[^{125}I]RTI-55	-9
Calcium Channel, Type L, Dihydropyridine	Rat cerebral cortex	[^{3}H]Nitrendipine	-5
Cholecystokinin CCK_A	Human recombinant	[^{3}H]Me-N-(±)L364,718	-9
Cholecystokinin CCK_B	Human recombinant	[^{3}H]CCK-8	11
Dopamine D_1	Human recombinant, mammalian	[^{3}H]SCH23390	16
Dopamine D_{2L}	Human recombinant, mammalian	[^{3}H]Spiperone	-1
Dopamine Transporter	Human recombinant, mammalian	[^{125}I]RTI-55	-7

2.6.3.2 効力を裏付ける試験（続き）

Table 2.6.3.2-01 (cont.)	Primary Pharmacodynamics	Test Article: Remimazolam
Report Title: [redacted] Custom Screen Report		
Route of Administration: *in vitro*	Location in CTD: 4.2.1.1.1	Study Number: [redacted]
GLP Compliance: non-GLP		

Results:

Table Summary of binding affinity of remimazolam to various receptors and others (cont.)

Target	Source	Radioligand	% inhibition (Remimazolam 10 µmol/L)
Endothelin ET_A	Rat A10 cells	[^{125}I]Endothelin-1	4
Endothelin ET_B	Human recombinant, mammalian	[^{125}I]Endothelin-1	9
Estrogen	Calf uterus	[^{3}H]Estradiol	1
$GABA_A$, Agonist Site	Rat brain	[^{3}H]Muscimol	-10
$GABA_A$, Bezodiazepine, Central	Rat brain	[^{3}H]Flunitrazepam	101
$GABA_A$, Chloride Channel	Rat cerebral cortex	[^{3}H]TBOB	19
$GABA_B$	Rat cerebellum	[^{3}H]GABA	15
Glutamate, Non-selective	Rat brain	[^{3}H]L-Glutamate	-11
Histamine H_1, Peripheral	Guinea pig lung	[^{3}H]Pyrilamine	7
Imidazoline I_2, Central	Rat brain cortex	[^{3}H]Idazoxan	-10
Insulin	Rat liver	[^{125}I]Insulin	-8
Leukotriene D_4	Guinea pig lung	[^{3}H]Leukotriene D_4	13
Muscarinic M_2	Human recombinant, insect	[^{3}H]NMS	23
Muscarinic M_3	Human recombinant, insect	[^{3}H]NMS	24
Muscarinic, Non-selective	Rat cortex	[^{3}H]QNB	6
Nicotinic Acetylcholine, Central	Rat cortex	[^{3}H]Cytisine	9
Opiate, Non-selective	Rat brain	[^{3}H]Naloxone	4
Platelet Activating Factor	Rabbit platelets	[^{3}H]PAF	17
Potassium Channel [K_{ATP}]	Syrian hamster pancreatic beta cells	[^{3}H]Glyburide	6
Progesterone	Calf uterus	[^{3}H]R-5020	9
Serotonin 5 HT_1	Rat cerebral cortex	[^{3}H]5-HT	23
Serotonin 5 HT_2	Rat brain	[^{3}H]Ketanserin	-7
Serotonin Transporter	Human recombinant, mammalian	[^{125}I]RTI-55	-7
Sigma, Non-selective	Guinea pig brain	[^{3}H]DTG	4
Sodium Channel, Site 2	Rat brain	[^{3}H]Batrachotoxin	17
Testosterone	Rat ventral prostate	[^{3}H]Mibolerone	13

Conclusion: Remimazolam has high binding affinity for the benzodiazepine site of the $GABA_A$ receptor.

2.6.3.2 効力を裏付ける試験（続き）

Table 2.6.3.2-02	Primary Pharmacodynamics	Test Article: CNS 7054
Report Title: [redacted] CustomScreen Report		
Route of Administration: *in vitro*	Location in CTD: 4.2.1.1.2	Study Number: [redacted]
GLP Compliance: non-GLP		
Aim of Study: To evaluate the activity of test compound CNS 7054 in radioligand binding assays. Study Design: Radioligand binding assays using a wide array of receptors or membrane preparations from several species. Reference compounds were tested as positive control as an integral part of each assay.		
Species (Gender, Strain, Supplier, n/Group): Insect, rat, syrian hamster, guinea pig, rabbit, calf, human, n = 2 experiments Age/Body Weight: no data Conditioning and Surgical Pre-treatment: Preparation of membranes for binding studies		
Test Compounds (Batch No) and Test Conditions (Formulation, Doses, Route of Administrations):		
CNS 7054	Batch No: no data, dissolved in 0.5% DMSO, concentration 10 µmol/L against various types of receptors.	
Additional compounds	Specific antagonists at the respective receptor according to the [redacted] reference compound data list.	
Method of Evaluation: The amount of specific binding was determined by subtracting the amount of nonspecific binding from the amount of total binding. Statistical Analysis: No statistical analysis was performed.		
Results: No significant responses were observed in any primary assay.		

Table Summary of binding affinity of CNS 7054 to various receptors and others

Target	Source	Radioligand	% inhibition (CNS 7054 10 µmol/L)
Adenosine A_1	Rat brain	[^{3}H]DPCPX	9
Adenosine A_{2A}	Human recombinant, mammalian	[^{3}H]CGS21680	-4
Adenosine A_3	Human recombinant, mammalian	[^{125}I]AB-MECA	-5
Adrenergic α_1, Non-selective	Rat brain	[^{3}H]Prazosin	6
Adrenergic α_2, Non-selective	Rat cortex	[^{3}H]Rauwolscine	13
Adrenergic β_1	Human recombinant, mammalian	[^{125}I]Cyanopindolol	-12
Adrenergic β_2	Human recombinant, mammalian	[^{3}H]CGP-12177	-9
Adrenergic NE Transporter	Human recombinant, mammalian	[^{125}I]RTI-55	-13
Calcium Channel, Type L, Dihydropyridine	Rat cerebral cortex	[^{3}H]Nitrendipine	2
Cholecystokinin CCK_A	Human recombinant	[^{3}H]-Me-N-(±)L364,718	6
Cholecystokinin CCK_B	Human recombinant	[^{3}H]-CCK-8	10
Dopamine D_1	Human recombinant, mammalian	[^{3}H]SCH23390	-10
Dopamine D_{2L}	Human recombinant, mammalian	[^{3}H]Spiperone	-16
Dopamine Transporter	Human recombinant, mammalian	[^{125}I]RTI-55	-10
Endothelin ET_A	Rat A10 cells	[^{125}I]Endothelin-1	-1
Endothelin ET_B	Human recombinant, mammalian	[^{125}I]Endothelin-1	2

2.6.3.2 効力を裏付ける試験（続き）

Table 2.6.3.2-02 (cont.)	Primary Pharmacodynamics	Test Article: CNS 7054
Report Title: [redacted] CustomScreen Report		
Route of Administration: *in vitro*	Location in CTD: 4.2.1.1.2	Study Number: [redacted]
GLP Compliance: non-GLP		

Results:

Table Summary of binding affinity of CNS 7054 to various receptors and others (cont.)

Target	Source	Radioligand	% inhibition (CNS 7054 10 µmol/L)
Estrogen	Calf uterus	[^{3}H]Estradiol	1
$GABA_A$, Agonist Site	Rat brain	[^{3}H]Muscimol	-3
$GABA_A$, Bezodiazepine, Central	Rat brain	[^{3}H]Flunitrazepam	34
$GABA_A$, Chloride Channel	Rat cerebral cortex	[^{3}H]TBOB	-7
$GABA_B$	Rat cerebellum	[^{3}H] GABA	13
Glutamate, Non-selective	Rat brain	[^{3}H]L-Glutamate	-16
Histamine H_1, Peripheral	Guinea pig lung	[^{3}H]Pyrilamine	12
Imidazoline I_2, Central	Rat brain cortex	[^{3}H]Idazoxan	4
Insulin	Rat liver	[^{125}I]Insulin	5
Leukotriene D_4	Guinea pig lung	[^{3}H]Leukotriene D_4	2
Muscarinic M_2	Human recombinant, insect	[^{3}H]NMS	13
Muscarinic M_3	Human recombinant, insect	[^{3}H]NMS	-5
Muscarinic, Non-selective	Rat cortex	[^{3}H]QNB	0
Nicotinic Acetylcholine, Central	Rat cortex	[^{3}H]Cytisine	14
Opiate, Non-selective	Rat brain	[^{3}H]Naloxone	20
Platelet Activating Factor	Rabbit platelets	[^{3}H]PAF	20
Potassium Channel [K_{ATP}]	Syrian hamster pancreatic beta cells	[^{3}H]Glyburide	17
Progesterone	Calf uterus	[^{3}H]R-5020	10
Serotonin 5 HT_1,	Rat cerebral cortex	[^{3}H]5-HT	3
Serotonin 5 HT_2	Rat brain	[^{3}H]Ketanserin	-9
Serotonin Transporter	Human recombinant, mammalian	[^{125}I]RTI-55	2
Sigma, Non-selective	Guinea pig brain	[^{3}H]DTG	4
Sodium Channel, Site 2	Rat brain	[^{3}H]Batrachotoxin	7
Testosterone	Rat ventral prostate	[^{3}H] Mibolerone	8

Conclusion: CNS 7054, the acid metabolite of Remimazolam, showed no detectable affinity at any site examined.

2.6.3.2 効力を裏付ける試験（続き）

Table 2.6.3.2-03	Primary Pharmacodynamics	Test Article: Remimazolam, CNS 7054
試験表題：ONO-2745BS 及び代謝物 ONO-IN-252 の各種受容体等に対する結合親和性の検討		
Route of Administration: *in vitro*	Location in CTD: 4.2.1.1.3	Study Number: [redacted]
GLP Compliance: non-GLP		
Aim of Study: To evaluate the binding affinity of remimazolam and the metabolite CNS 7054 to 40 various receptors, transporters and ion channels including to the benzodiazepine binding site of the $GABA_A$ receptor (hereafter $GABA_A$, BZ Central receptor). Study Design: Radioligand binding assays using a wide range of receptors, transporters and ion channels using standard selective radioligands for each binding site. Standard reference compounds for each binding site were used as positive controls as an integral part of each assay.		
Species (Gender, Strain, Supplier, n/Group): Rat, guinea pig, rabbit, human, n = 2 experiments Age/Body Weight: not applicable Conditioning and Surgical Pre-treatment: Using commercially available preparations and preparation of membranes from living tissues of various species		
Test Compounds (Batch No) and Test Conditions (Formulation, Doses, Route of Administrations):		
Remimazolam	Lot No: SOL12621/6, dissolved in DMSO, serially diluted with DMSO and further diluted with Millli-Q water to get the final concentrations: 0.3, 1, 3, 10, 30, 100, 300 nmol/L, 1 and 3 µmol/L against $GABA_A$, BZ Central receptor and 10 µmol/L against other receptors, respectively.	
CNS 7054	Lot No: YSD070912-1, dissolved in DMSO, serially diluted with DMSO and further diluted with Millli-Q water to get the final concentrations. 0.03, 0.1, 0.3, 1, 3, 10, 30, 100, and 300 µmol/L against $GABA_A$, BZ Central receptor and 10 µmol/L against other receptors, respectively.	
Midazolam	Positive compound for $GABA_A$, BZ Central receptor, lot No. STJ5796, final concentrations: 0.1, 0.3, 1, 3, 10, 30, 100, 300 nmol/L and 1 µmol/L.	
Additional compounds	Positive compounds of each binding site according to the list given in study conditions (see report chapter 6); the final concentrations: 1 µmol/L (for Endothelin ET_A and ET_B, Insulin and Leukotriene D_4 receptors) or 10 µmol/L (for other receptors).	
Method of Evaluation: The amount of specific binding was determined by subtracting the amount of nonspecific binding from the amount of total binding. Inhibition constant (Ki) values, IC_{50} values and Kd values were calculated (Scatchard analysis) only in the case of $GABA_A$, BZ Central receptors Statistical Analysis: No statistical analysis was performed.		
Results: Except for the $GABA_A$, BZ Central receptor, no specific binding was found. The 50% inhibitory concentration (IC_{50}) values of remimazolam and the metabolite CNS 7054 against the $GABA_A$, BZ Central receptor were 30.5 and 5130 nmol/L, respectively. The IC_{50} value of midazolam was 6.58 nmol/L. Inhibition constant (Ki) values of remimazolam and metabolite CNS 7054 against the $GABA_A$, BZ Central receptor were 26.3 and 4420 nmol/L, respectively. The Ki value of midazolam was 5.66 nmol/L.		

Table Summary of binding affinity of remimazolam and CNS 7054 to various receptors and others

Target	Source	Radioligand	% inhibition (Remimazolam 10 µmol/L)	% inhibition (CNS 7054 10 µmol/L)
Adenosine A_1	Rat whole brain	[^{3}H(N)]DPCPX	10.6	0.2
Adenosine A_{2A}	Human recombinant	[^{3}H(N)]CGS21680	3.8	3.2
Adenosine A_3	Human recombinant	[^{125}I]AB-MECA	0.5	1.8

2.6.3.2 効力を裏付ける試験（続き）

Table 2.6.3.2-03 (cont.)	Primary Pharmacodynamics	Test Article: Remimazolam, CNS 7054
試験表題：ONO-2745BS 及び代謝物 ONO-IN-252 の各種受容体等に対する結合親和性の検討		
Route of Administration: *in vitro*	Location in CTD: 4.2.1.1.3	Study Number: [redacted]
GLP Compliance: non-GLP		

Results:

Table Summary of binding affinity of remimazolam and CNS 7054 to various receptors and others (cont.)

Target	Source	Radioligand	% inhibition (Remimazolam 10 μmol/L)	% inhibition (CNS 7054 10 μmol/L)
α_1-Adrenergic, Non-selective	Rat whole brain	[^{3}H]Prazosin	6.8	1.2
α_2-Adrenergic, Non-selective	Rat cerebral cortex	[^{3}H]Rauwolscine	2.4	0.0
β_1-Adrenergic	Human recombinant	[^{3}H]CGP-12177	6.5	1.4
β_2-Adrenergic	Human recombinant	[^{3}H]CGP-12177	0.5	4.3
Calcium Channel, Type L, Dihydropyridine	Rat cerebral cortex	[^{3}H]PN200-110	0.0	0.0
CCK_A	Human recombinant	[^{125}I]Cholecystokinin Octapeptide	2.7	0.7
CCK_B	Human recombinant	[^{125}I]Cholecystokinin Octapeptide	0.1	2.8
Dopamine D_1	Human recombinant	[^{3}H]SCH23390	5.9	11.2
Dopamine D_2 Long	Human recombinant	[^{3}H]Spiperone	10.1	1.3
Dopamine Transporter	Human recombinant	[^{3}H]WIN35,428	1.2	0.6
Estrogen	Rat uterus	[^{3}H(N)]Estradiol	2.3	2.1
Endothelin ET_A	Human recombinant	[^{125}I]Endothelin-1	0.5	3.7
Endothelin ET_B	Human recombinant	[^{125}I]Endothelin-1	0.6	0.0
$GABA_A$, Agonist Site	Rat cerebellum	[^{3}H(N)]Muscimol	0.0	0.0
$GABA_A$, Chloride Channel	Rat cerebral cortex	[^{3}H]EBOB	0.0	0.0
$GABA_B$	Rat cerebellum	[^{3}H(N)]Aminobutyric Acid	4.7	5.2
Glutamate, Non-selective	Rat cerebral cortex	[^{3}H]L-Glutamic acid	8.4	0.1
Histamine H_1, Peripheral	Guinea pig lung	[^{3}H]Pyrilamine	5.8	22.1
Imidazoline, Central	Rat cerebral cortex	[^{3}H]RX781094	0.4	3.0
Insulin	Rat liver	[^{125}I]Insulin (Porcine)	2.3	9.5
Potassium Channel [K_{ATP}]	Rat whole brain	[^{3}H(N)]Glibenclamide	0.0	0.2
Leukotriene D_4	Guinea pig lung	[^{3}H]Leukotriene D_4	0.0	0.0
Muscarinic, Non-selective	Rat cerebral cortex/ Rat brain	[^{3}H(N)]Quinuclidinyl Benzilate	0.0	0.8
Muscarinic M_2	Human recombinant	[^{3}H]Scopolamine Methyl Chloride	0.0	0.9

2.6.3.2 効力を裏付ける試験（続き）

Table 2.6.3.2-03 (cont.)	Primary Pharmacodynamics	Test Article: Remimazolam, CNS 7054
試験表題：ONO-2745BS 及び代謝物 ONO-IN-252 の各種受容体等に対する結合親和性の検討		
Route of Administration: *in vitro*	Location in CTD: 4.2.1.1.3	Study Number: [redacted]
GLP Compliance: non-GLP		

Results:

Table Summary of binding affinity of remimazolam and CNS 7054 to various receptors and others (cont.)

Target	Source	Radioligand	% inhibition (Remimazolam 10 μmol/L)	% inhibition (CNS 7054 10 μmol/L)
Muscarinic M_3	Human recombinant	[^{3}H]Scopolamine Methyl Chloride	7.3	0.3
Sodium Channel, Site 2	Rat whole brain	[^{3}H]Batrachotoxinin A 20-alpha-Benzoate	0.8	0.0
Norepinephrine Transporter	Human recombinant	[^{3}H]Nisoxetine Hydrochloride	1.9	0.0
Nicotinic, Neuronal	Rat cerebral cortex	[^{3}H]Nicotine	19.3	3.4
Opiate, Non-selective	Rat cerebral cortex	[^{3}H]Naloxone	2.4	4.4
Platelet Activating Factor (PAF)	Rabbit platelets	[^{3}H(N)]PAF	0.0	0.0
Progesterone	Rabbit uterus	[^{3}H(N)]Progesterone	0.0	2.4
Serotonin 5 HT_1, Non-selective	Rat striatum	[^{3}H(N)]Hydroxytryptamine Creatinine Sulfate	14.5	2.0
Serotonin 5 HT_{2A}	Rat cerebral cortex	[^{3}H]Ketanserin Hydrochloride	0.0	0.0
Serotonin Transporter	Human recombinant	[^{3}H(N)]Imipramine Hydrochloride	2.8	2.3
Sigma, Non-selective	Guinea pig whole brain	[^{3}H]DTG	0.6	0.4
Testosterone	Human receptor	[^{3}H]Methyltrienolone	0.0	0.5

Conclusion: Remimazolam demonstrated a high binding affinity to the $GABA_A$, BZ Central receptor, whereas the binding affinity of its metabolite CNS 7054 was approximately 170 times less. Except for the $GABA_A$, BZ Central receptor, no further specific binding was found.

2.6.3.2 効力を裏付ける試験（続き）

Table 2.6.3.2-04	Primary Pharmacodynamics	Test Article: Remimazolam, CNS 7054
Report Title: *In Vitro* Pharmacology – Study of CNS7054 and Remimazolam		
Route of Administration: *in vitro*	Location in CTD: 4.2.1.1.4	Study Number: [redacted]
GLP Compliance: non-GLP		
Aim of Study: To evaluate the activity of test compounds remimazolam and CNS 7054 in radioligand binding assays. Study Design: Radioligand binding assays using a wide array of receptors or membrane preparations. Reference compounds were tested as positive control as an integral part of each assay.		
Species (Gender, Strain, Supplier, n/Group): Human recombinant or endogenous receptors expressed in human cells and rat cerebral cortex preparations, n = 2 experiments, Age/Body Weight: not applicable Conditioning and Surgical Pre-treatment: Preparation of membranes for binding studies		
Test Compounds (Batch No) and Test Conditions (Formulation, Doses, Route of Administrations):		
CNS 7054	Batch No: 80337_03-02, dissolved in DMSO, concentration 10 µmol/L against various types of receptors.	
Remimazolam	Batch No: 80431_08-01, dissolved in DMSO, concentration 10 µmol/L against various types of receptors.	
Additional compounds	In each experiment and if applicable, the respective reference compound was tested concurrently with CNS 7054 and Remimazolam.	
Method of Evaluation: The amount of specific binding was determined by subtracting the amount of nonspecific binding from the amount of total binding. Statistical Analysis: No statistical analysis was performed.		
Results: No significant responses (>50% inhibition at 10 µmol/L) were observed in any primary assay except for the $GABA_{A1}$ (α1, β2, γ2) receptor (62.1% inhibition at 10 µmol/L remimazolam)		

Table Summary of binding affinity of remimazolam and CNS 7054 to various receptors and others

Target	Source	Radioligand	% inhibition (Remimazolam 10 µmol/L)	% inhibition (CNS 7054 10 µmol/L)
CB_1	Human recombinant (CHO cells)	[^{3}H]CP 55940	-33.0	-11.2
CB_2	Human recombinant (CHO cells)	[^{3}H]WIN 55212-2	1.2	3.0
D_{2S}	Human recombinant (HEK-293 cells)	[^{3}H]7-OH-DPAT	-21.9	-4.7
D_3	Human recombinant (CHO cells)	[^{3}H]methyl-spiperone	1.2	-0.5
D_5	Human recombinant (GH4 cells)	[^{3}H]SCH 23390	8.6	-11.8
$GABA_{A1}$ (α1, β2, γ2)	Human recombinant (CHO cells)	[^{3}H]muscimol	-62.1	-5.1
GABA transporter	Rat cerebral cortex	[^{3}H] GABA (+10 µmol/L isoguvacine) (+10 µmol/L baclofen)	-9.4	-7.1
Glutamate, AMPA	Rat cerebral cortex	[^{3}H]AMPA	-4.6	-8.8
Glutamate, kainate	Rat cerebral cortex	[^{3}H]kainic acid	-4.6	-3.4
N neuronal α4β2	Human recombinant (SH-SY5Y cells)	[^{3}H]cytisine	6.7	9.1
δ (DOP)	Human recombinant (Chem-1)	[^{3}H]DADLE	19.8	-0.1

2.6.3.2 効力を裏付ける試験（続き）

Table 2.6.3.2-04 (cont.)	Primary Pharmacodynamics	Test Article: Remimazolam, CNS 7054
Report Title: *In Vitro* Pharmacology – Study of CNS7054 and Remimazolam		
Route of Administration: *in vitro*	Location in CTD: 4.2.1.1.4	Study Number: [redacted]
GLP Compliance: non-GLP		

Results:

Table Summary of binding affinity of remimazolam and CNS 7054 to various receptors and others (cont.)

Target	Source	Radioligand	% inhibition (Remimazolam 10 μmol/L)	% inhibition (CNS 7054 10 μmol/L)
μ (MOP)	Human recombinant (HEK-293 cells)	[^{3}H]DAMGO	-4.5	1.6
OX_1	Human recombinant (CHO cells)	[^{125}I]orexin-A	14.9	10.0
OX_2	Human recombinant (HEK-293 cells)	[^{125}I]orexin-A	-0.7	-8.2
5-HT_{1A}	Human recombinant (HEK-293 cells)	[^{3}H]8-OH-DPAT	13.0	-4.5
5-HT_{2A}	Human recombinant (HEK-293 cells)	[^{125}I](±)DOI	-3.5	-8.2
5-HT_{2B}	Human recombinant (CHO cells)	[^{125}I](±)DOI	-11.9	-11.5
5-HT_{2C}	Human recombinant (HEK-293 cells)	[^{125}I](±)DOI	-1.0	0.4
5-HT_3	Human recombinant (CHO cells)	[^{3}H]BRL 43694	5.2	-1.5
5-HT_{5a}	Human recombinant (HEK-293 cells)	[^{3}H]LSD	-7.8	-3.3
5-HT_6	Human recombinant (CHO cells)	[^{3}H] LSD	-4.5	-14.1
5-HT_7	Human recombinant (CHO cells)	[^{3}H] LSD	2.3	-6.6
sigma 1	Jurkat cells (endogenous)	[^{3}H](+)pentazocine	-3.5	1.0
sigma 2	Jurkat cells (endogenous)	[^{3}H] DTG (+1 μmol/L (+) Pentazocine)	-15.2	-11.7

Conclusion: Remimazolam and CNS 7054 showed no detectable affinity at any site examined except for remimazolams binding to its primary target the $GABA_{A1}$ (α1, β2, γ2) receptor.

2.6.3.2 効力を裏付ける試験（続き）

Table 2.6.3.2-05	Primary Pharmacodynamics	Test Article: Remimazolam, CNS 7054
Report Title: *In Vitro* Pharmacology – Study of CNS7054 and Remimazolam		
Route of Administration: *in vitro*	Location in CTD: 4.2.1.1.4	Study Number: [redacted]
GLP Compliance: non-GLP		
Aim of Study: To evaluate the activity of test compounds remimazolam and CNS 7054 in radioligand binding assays. Study Design: Radioligand binding assays using a wide array of receptors or membrane preparations. Reference compounds were tested as positive control as an integral part of each assay.		
Species (Gender, Strain, Supplier, n/Group): Human recombinant or endogenous receptors expressed in rat cerebral cortex preparations, n = 2 experiments, Age/Body Weight: not applicable Conditioning and Surgical Pre-treatment: Preparation of membranes for binding studies		
Test Compounds (Batch No) and Test Conditions (Formulation, Doses, Route of Administrations):		
CNS 7054	Batch No: 80337_03-02, dissolved in DMSO, concentration 10 µmol/L against various types of receptors.	
Remimazolam	Batch No: 80431_08-01, dissolved in DMSO, concentration 10 µmol/L against various types of receptors.	
Additional compounds	In each experiment and if applicable, the respective reference compound was tested concurrently with CNS7054 and Remimazolam.	
Method of Evaluation: The amount of specific binding was determined by subtracting the amount of nonspecific binding from the amount of total binding. Statistical Analysis: No statistical analysis was performed.		
Results: No significant responses (>50% inhibition at 10 µmol/L) were observed in any primary assay.		

Table Summary of binding affinity of remimazolam and CNS 7054 to various receptors

Target	Source	Radioligand	% inhibition (Remimazolam 10 µmol/L)	% inhibition (CNS 7054 10 µmol/L)
Dopamine $D_{4.2}$	Human recombinant CHO-K1 cells	[^{3}H]Spiperone	-7	-2
Glutamate, NMDA, Glycine	Wistar Rat cerebral cortex	[^{3}H]MDL 105,519	6	-7
Glutamate, NMDA, Phencyclidine	Wistar Rat cerebral cortex	[3H]TCP	-8	-9
Nicotinic Acetylcholine α7, Bungarotoxin	Human recombinant SH-SY5Y cells	[^{125}I] α-Bungarotoxin	10	8
Opiate κ (OP2, KOP)	Human recombinant HEK-293 cells	[^{3}H]U-69593	3	3

Conclusion: Remimazolam and CNS 7054 showed no detectable affinity at any site examined.

2.6.3.2 効力を裏付ける試験（続き）

Table 2.6.3.2-06	Primary Pharmacodynamics	Test Article: Remimazolam, CNS 7054
Report Title: Competition for [^{3}H]flunitrazepam binding to homogenates of rat, pig and human brain tissue by CNS 7056X, CNS 7054X and midazolam.		
Route of Administration: *in vitro*	Location in CTD:4.2.1.1.5	Study Number: [redacted]
GLP Compliance: non-GLP		
Aim of Study: To evaluate the affinity of remimazolam and its metabolite, CNS 7054 to bind to the benzodiazepine site of the $GABA_A$ receptor in tissue derived from the brains of rats, pigs and man. Study Design: Binding study in isolated cerebral cortex membrane preparation of rat, pig and human brain. Midazolam was employed as a standard reference compound and [^{3}H]-Flunitrazepam as the radioligand.		
Species (Gender, Strain, Supplier, n/Group): rat (Sprague-Dawley), pig (Yucatan micropig) and human brain membranes obtained from Analytical Biological Services Inc., USA, 3 values/point in the curve Age/Body Weight: no data Conditioning and Surgical Pre-treatment: Preparation of membranes for binding studies		
Test Compounds (Batch No) and Test Conditions (Formulation, Doses, Route of Administrations):		
Remimazolam	Batch No: no data, dissolved in DMSO at a concentration of 50 mmol/L, 12 concentrations (1×10^{-4} – 5.6×10^{-10} mol/L) diluting in assay buffer	
CNS 7054	Batch No: no data, dissolved in DMSO at a concentration of 50 mmol/L, 12 concentrations (1×10^{-4} – 5.6×10^{-10} mol/L) diluting in assay buffer	
Midazolam	Batch No: no data, dissolved in DMSO at a concentration of 50 mmol/L, 12 concentrations (1×10^{-5} – 5.6×10^{-11} mol/L) diluting in assay buffer	
[^{3}H]-Flunitrazepam	Batch No: no data, diluted with assay buffer to give the desired concentration of ^{3}H ligand (~30,000 cpm, 2.51 nmol/L). 50 µl of the mixture was added to each well of the plates containing test compounds.	
Control(s)	No data	
Method of Evaluation: The amount of specific binding was determined by subtracting the amount of nonspecific binding from the amount of total binding. Data was plotted as % displacement of [^{3}H]-flunitrazepam vs compound concentration. RoboSage Microsoft Excel add-in (Glaxo Wellcome) for calculation of IC_{50} values, method of Cheng & Prusoff (1973) for determination of pKi values. Statistical Analysis: No statistical analysis was performed.		
Results: The inhibition constant (Ki) for competition with the radioligand, [^{3}H]-flunitrazepam, binding to tissue homogenates from human, rat and pig brain was approximately 30 nmol/L (pKi ~7.5). The acid metabolite of remimazolam, CNS 7054, showed a markedly lower affinity, with Ki values of approximately 10,000 nmol/L (pKi ~5.0). Midazolam revealed a pKi of ~8.5. The separation between the affinity of remimazolam and its metabolite CNS 7054 ranged from 410-fold in human brain tissue to 320-fold in rat brain tissue.		
Conclusion: Remimazolam shows a high binding affinity for the benzodiazepine site of the $GABA_A$ receptor in tissue derived from the brains of rats, pigs and human.		

2.6.3.2 効力を裏付ける試験（続き）

Table 2.6.3.2-07	Primary Pharmacodynamics	Test Article: Remimazolam
Report Title: CNS 7056X-besylate: Modulatory Effects on Recombinant GABA Currents recorded from Stably Transfected Ltk Cells		
Route of Administration: *in vitro*	Location in CTD: 4.2.1.1.6	Study Number: [redacted]
GLP Compliance: non-GLP		
Aim of Study: To evaluate the modulatory effects of remimazolam on the rat $GABA_A$ receptor subtypes α1β2γ2, α2β2γ2, α3β2γ2, α5β2γ2. Study Design: Using the whole-cell patch-clamp technique submaximal GABA currents were recorded from stably transfected Ltk (mouse fibroblast) cells expressing various rat $GABA_A$ receptor subtypes. The test system was validated using the reference compound midazolam.		
Species (Gender, Strain, Supplier, n/Group): Ltk cells stably expressing rat $GABA_A$ receptor subtypes; supplied by bSys GmbH, Switzerland; n = 3 Age/Body Weight: not applicable Conditioning and Surgical Pre-treatment: Ltk cells were stably transfected (calciumphosphate precipitation) with recombinant $GABA_A$ receptor cDNAs (rat).		
Test Compounds (Batch No) and Test Conditions (Formulation, Doses, Route of Administrations):		
Remimazolam	Batch No: LJC-105-004-01, stock solution: 10 mmol/L dissolved in DMSO, final concentrations 0.1, 0.3, 1, 3 and 10 µmol/L, diluted from the stock formulation using submaximal GABA solution (2 µmol/L corresponding to most EC_{50}) , patch-clamp technique	
Midazolam	Dormicum, Batch No: B1310, stock solution: 50 mg/ml dissolved in DMSO, final concentrations 0.03, 0.1, 0.3, 1 and 3 µmol/L, diluted from the stock solution using submaximal GABA solution, patch-clamp technique	
Method of Evaluation: Complete cumulative dose response analysis per cell (5 test item or reference concentrations). Dose-response curves were fitted from data of at least 3 individual cells. EC_{50} values were calculated using a sigmoidal equation. Statistical Analysis: No statistical analysis was performed.		
Results: Remimazolam dose dependently stimulated submaximal GABA currents recorded in Ltk cells stably transfected with rat cDNA encoding the α1β2γ2, α2β2γ2, α3β2γ2, α5β2γ2 $GABA_A$ receptor subtypes, the corresponding 50% effective concentration (EC_{50}) values were 327, 565, 899 nmol/L and 1.426 µmol/L, respectively. The EC_{50} values of midazolam were 138, 254, 264 and 124 nmol/L. Maximum effect (E_{max}) values were 371%, 184%, 199% and 277% for remimazolam, respectively and 295%, 192%, 290% and 110% for midazolam, respectively.		
Conclusion: Remimazolam enhanced GABA currents in cells transfected with all tested subtypes of the $GABA_A$ receptor. Remimazolam, like midazolam, did not show clear selectivity between the subtypes of the $GABA_A$ receptor.		

2.6.3.2 効力を裏付ける試験（続き）

Table 2.6.3.2-08	Primary Pharmacodynamics	Test Article: Remimazolam
Report Title: Evaluation of CNS7056X besylate salt for sleep induction (loss of righting reflex) and reversal by flumazenil		
Route of Administration: i.v.	Location in CTD: 4.2.1.1.7	Study Number: [redacted]
GLP Compliance: non GLP		
Aim of Study: To evaluate the potential to induce loss of righting reflex (LRR) of remimazolam besylate salt after i.v. administration and its reversal by flumazenil after i.p. administration in the mouse Study Design: Induction of LRR by remimazolam administered as an i.v. bolus was measured via latency and duration of LRR and the effect of flumazenil pre-treatment 30 or 15 min prior to remimazolam administration was determined. In the original report, the doses of remimazolam are expressed in mg/kg of salt but in this document all doses are expressed in mg/kg of base.		
Species (Gender, Strain, Supplier, n/Group): Mouse (male, Rj:NMRI, Elevage Janvier, France, n = 8) Age/Body Weight: 21-26 g Conditioning and Surgical Pre-treatment: Stabilized for at least 5 days on wood litter with free access to food and water		
Test Compounds (Batch No) and Test Conditions (Formulation, Doses, Route of Administrations):		
Remimazolam	([redacted], batch no. AE-123-01), white powder, dissolved in physiological saline; 15, 20, 25 and 30 mg/kg (Exp. 1), 25 mg/kg (Exp. 2), 30 mg/kg (Exp. 3), administered i.v. immediately before the test	
Flumazenil	([redacted], batch no. 102K0972) white powder, dispersed in 0.2% hydroxypropylmethylcellulose in physiological saline; 20 mg/kg administered i.p. 30 min (Exp. 2) or 15 min (Exp. 3) before the test	
Midazolam	([redacted], batch no. 4532027B), colorless liquid, diluted with physiological saline; 15, 20, and 30 mg/kg (Exp. 1) and 50 mg/kg (Exp. 2 and 3), administered i.v. immediately before the test	
Control(s)	Vehicle (physiological saline)	
Method of Evaluation: Determination of latency and duration of LRR induced by remimazolam alone or after pre-treatment with flumazenil in comparison to vehicle control and midazolam. Statistical Analysis: Mann-Whitney U test, Fisher's Exact test and Chi-Square test		

2.6.3.2 効力を裏付ける試験（続き）

Table 2.6.3.2-08 (cont.)	Primary Pharmacodynamics	Test Article: Remimazolam
Report Title: Evaluation of CNS7056X besylate salt for sleep induction (loss of righting reflex) and reversal by flumazenil		
Route of Administration: i.v.	Location in CTD: 4.2.1.1.7	Study Number: [redacted]
GLP Compliance: non GLP		
Results: Experiment 1 (Sleep induction) Remimazolam dose-dependently and significantly induced LRR as compared with vehicle control. 2/8, 5/8, 6/8, and 6/8 animals experienced LRR at 15, 20, 25, and 30 mg/kg, respectively. The onset of LRR was rapid (3.0, 1.8, and 1.6 min at 20, 25, and 30 mg/kg, respectively) and the duration of LRR was short (1.7, 4.9, 6.0, and 7.3 min at 15, 20, 25, and 30 mg/kg, respectively). Midazolam (15; 20 and 30 mg/kg) did not clearly induce LRR (1/8 at highest dose). Experiment 2 (Reversal by flumazenil administered 30 min before the test) Remimazolam (25 mg/kg) induced LRR in 2 of 8 mice. Flumazenil (20 mg/kg), administered i.p. 30 min before the test, completely abolished remimazolam-induced LRR. Midazolam (50 mg/kg), induced LRR in 8 of 8 mice. Flumazenil (20 mg/kg), administered i.p. 30 min before the test, tended to decrease midazolam-induced LRR, although the effects were not statistically significant. Experiment 3 (Reversal by flumazenil administered 15 min before the test) Remimazolam (30 mg/kg), induced LRR in 6 of 8 mice. Flumazenil (20 mg/kg), administered i.p 15 min before the test, nearly abolished remimazolam-induced LRR (1/8 mice, p = 0.0128). Midazolam (50 mg/kg) induced LRR in 7 of 8 mice. Flumazenil (20 mg/kg), administered i.p 15 min before the test, tended to decrease midazolam-induced LRR, although the effects were not statistically significant. Mortality No deaths occurred with remimazolam treatments. Midazolam alone resulted in 1/8 deaths (Exp 3) and in combination with flumazenil, 2/8 deaths occurred in each Exp 2 and 3.		
Conclusion: Remimazolam induced LRR with a rapid onset and short duration over a dose range of 20 to 30 mg/kg. In the same test, the comparator substance midazolam had similar effects at 50 mg/kg but not at lower doses. Flumazenil (20 mg/kg i.p.) clearly antagonized remimazolam-induced LRR in experiment 3. It also tended to decrease midazolam-induced LRR, although the effects did not reach statistical significance.		

2.6.3.2 効力を裏付ける試験（続き）

Table 2.6.3.2-09	Primary Pharmacodynamics	Test Article: (S)-Remimazolam, (R)-Remimazolam, CNS 7054
Report Title: Evaluation of (S)-CNS 7056X besylate, (R)-CNS 7056X besylate and CNS 7054X for sleep induction (loss of righting reflex) in the mouse		
Route of Administration: i.v.	Location in CTD: 4.2.1.1.8	Study Number: [redacted]
GLP Compliance: non GLP		
Aim of Study: To evaluate the potential to induce loss of righting reflex (LRR) of [redacted] remimazolam and the main degradation product CNS 7054 after i.v. administration in the mouse Study Design: Latency and duration of sleep (LRR) after i.v. administration of [redacted]-remimazolam, or CNS 7054 were compared to vehicle control, midazolam and propofol. Each drug was administered immediately before the test at 4 different doses to 8 mice per group. In the original report, the doses of remimazolam are expressed in mg/kg of salt but in this document all doses are expressed in mg/kg of base.		
Species (Gender, Strain, Supplier, n/Group): Mouse (male, Rj:NMRI, Elevage Janvier, France, n = 8) Age/Body Weight: 22-27 g Conditioning and Surgical Pre-treatment: Stabilized for at least 5 days on wood litter with free access to food and water		
Test Compounds (Batch No) and Test Conditions (Formulation, Doses, Route of Administrations):		
[redacted]	([redacted], batch no. AE-123-01), white powder, dissolved in physiological saline; 15, 20, 25 and 30 mg/kg, i.v.	
[redacted]	([redacted], batch no. AW/245/90/40), yellow powder, dissolved in physiological saline; 15, 20, 25 and 30 mg/kg, i.v.	
CNS 7054	([redacted], batch no. SC0964/14 1), white powder, dissolved in physiological saline, adjusted to pH 9-10; 30, 50, 70, 100 mg/kg, i.v.	
Midazolam	([redacted], batch no. 4200474), colorless liquid, diluted with physiological saline; 20, 30, 40, 50 mg/kg, i.v.	
Propofol (Rapinovet®)	([redacted], batch no. 027091), white liquid, diluted with 10% intralipid; 10, 20, 30, 40 mg/kg, i.v. and compared to propofol vehicle (10% intralipid)	
Control(s)	Vehicle (physiological saline) and 10% intralipid, i.v.	
Method of Evaluation: Latency and duration of sleep (LRR) were recorded for all treated animals and compared to the respective vehicle controls and subsequently the comparators midazolam and propofol. Statistical Analysis: Analysis of variance (ANOVA) followed by Mann Whitney U tests for the comparison of treated groups to vehicle controls regarding latency and duration of LRR; regarding the number of mice losing righting reflex in comparison to vehicle controls, Fisher's Exact Probability tests or Chi-Square tests. Significance was evaluated using Dunnett's t test.		

2.6.3.2 効力を裏付ける試験（続き）

Table 2.6.3.2-09 (cont.)	Primary Pharmacodynamics	Test Article: (S)-Remimazolam, (R)-Remimazolam, CNS 7054
Report Title: Evaluation of (S)-CNS 7056X besylate, (R)-CNS 7056X besylate and CNS 7054X for sleep induction (loss of righting reflex) in the mouse		
Route of Administration: i.v.	Location in CTD: 4.2.1.1.8	Study Number: [redacted]
GLP Compliance: non GLP		
Results: (S)-remimazolam (20, 25 and 30 mg/kg) dose-dependently induced a short lasting loss of righting reflex in mice (2, 4 and 6 showing LRR out of 8 tested at 20, 25 and 30 mg/kg, respectively, compared to 0 in vehicle controls), significantly so at 30 mg/kg ($p<0.01$). The same tendency was also observed - although not clearly dose-dependently - on the duration of LRR (1.6, 1.1, and 3.6 min, respectively). It had no effects at 15 mg/kg. [redacted] (15, 20, 25 and 30 mg/kg) did not induce LRR (1/32 mice). CNS 7054 (30, 50, 70 and 100 mg/kg) did not induce LRR (0/32). Midazolam at 20 and 30 mg/kg did not induce LRR. At 40 and 50 mg/kg, LRR was induced in 7 and 6 of 8 mice, respectively, ($p<0.01$) and lasted 49.3 and 65.1 min, respectively ($p<0.01$). Propofol (10, 20, 30 and 40 mg/kg) dose-dependently induced LRR, as measured by the number of mice with LRR (1, 8, 8 and 8 out of 8 tested at 10, 20, 30 and 40 mg/kg, respectively), significantly so at 20, 30 and 40 mg/kg ($p<0.001$). It had a similar effect on the duration of LRR (0.2, 10.0, 17.7, and 22.3 min, respectively), significantly so at 20, 30, and 40 mg/kg ($p<0.01$).		
Conclusion: The results demonstrate that (S)-remimazolam induces LRR over the dose-range of 20-30 mg/kg, while the [redacted] and the metabolite CNS 7054 were devoid of effects over the dose ranges of 15-30 mg/kg and 30-100 mg/kg, respectively. The comparison substances midazolam and propofol induced LRR over the dose-ranges of 40-50 mg/kg and 20-40 mg/kg, respectively. Duration of LRR at each highest dose was shortest with (S)-remimazolam (3.6±1.8 min), longer with propofol (22.3±1.0 min) and even longer with midazolam (65.1±14.3 min).		

2.6.3.2 効力を裏付ける試験（続き）

Table 2.6.3.2-10	Primary Pharmacodynamics	Test Article: Remimazolam
試験表題：ONO-2745BS のラット鎮静作用の検討（既存薬物との比較および用量反応性）		
Route of Administration: i.v.	Location in CTD: 4.2.1.1.9	Study Number: [redacted]
GLP Compliance: non GLP		
Aim of Study: To compare the sedative effects of remimazolam with existing drugs (midazolam, propofol, dexmedetomidine hydrochloride) as measured by loss of righting reflex (LRR), ataxia and decrease in spontaneous locomotor activity (SLA). Further to characterize and compare sedative effects in terms oflatency and duration of LRR and ataxia and minimum effective dose (MED) and lowest lethal dose (LD_{LO}) regarding each parameter. Study Design: After i.v. administration into the tail vein, sedative effects were assessed in a blinded manner in two different dose range settings. In experiment 1, LRR (number of animals produce LRR, latency and duration of LRR) and LD_{LO} that is the lowest dose level of the test compound that produces death were evaluated for each test compound. In experiment 2, ataxia (number of animals produce ataxia and incidence) and decrease in SLA (% of the maximum reduction) were determined.		
Species (Gender, Strain, Supplier, n/Group): Rats (male, Crl:CD(SD), Charles River Laboratories Japan, n=10) Age/Body Weight: 6 weeks Conditioning and Surgical Pre-treatment: Acclimation period of 6–10 days on wood litter with free access to food and water		
Test Compounds (Batch No) and Test Conditions (Formulation, Doses, Route of Administrations):		
Remimazolam	(ONO-2745BS, [redacted], lot no.SOL12621/6, purity 95% or more), dissolved in physiological saline adjusted to pH 3.3. 1, 2, 5, 10, 20, 50 and 100 mg/kg (Exp1) and 0.05, 0.1, 0.2, 0.5, 1, 2, 5, 10 mg/kg (Exp2) i.v. into the tail vein	
Midazolam	([redacted], Lot no. LTE0854), dissolved in physiological saline adjusted to pH 3.3. 5, 10, 20, 50 and 100 mg/kg (Exp1) and 0.02, 0.05, 0.1, 0.2, 0.5, 1, 2, 5 mg/kg (Exp2) i.v. into the tail vein	
Propofol	(1%Diprivan® Injection, [redacted], serial no. 11370), diluted with physiological saline. 1, 2, 5, 10, 20, 50 mg/kg (Exp1) and 0.2, 0.5, 1, 2, 5, 10 mg/kg (Exp2) i.v. into the tail vein	
Dexmedetomidine hydrochloride	(Precedex® injection 200 µg “Maruishi”, [redacted], serial no. 7814), diluted with physiological saline. 2, 5, 10, 20, 50, 100, 200, 500 and 1000 µg/kg (Exp1) and 0.2, 0.5, 1, 2, 5 µg/kg (Exp2) i.v. into the tail vein	
Control(s)	Physiological saline (pH 3.3)	
Method of Evaluation: An animal was considered to have LRR when it failed to return to a prone position within 15 sec after being placed in a supine position. Recovery from LRR was reached when the animal returned to a prone position three consecutive times. In experiment 1 with higher dose range, LRR (the number of animals with LRR, incidence, latency and duration) was evaluated and MED that is the lowest dose level of the test compound that produces statistically significant response was also determined. Furthermore, duration of ataxia following LRR and LD_{LO} were evaluated for each test compound. Recovery from ataxia was reached when the animal showed no staggering gait or prone position and successfully climbed a metal mesh three consecutive times. Parameters of mortality were number of dead animals and LD_{LO}. In experiment 2 using a lower dose range, ataxia (number of animals with ataxia, incidence and MED) was evaluated. SLA was measured every minute for 10 min after administration using an infrared sensor system (MDC system, BrainScience idea Co, Ltd., Japan). Measured values, maximum decrease (%) and MED were determined. Statistical Analysis: One-tailed Fisher’s exact test with closed testing procedure for comparison between the test compound and control, one-tailed Cochran-Armitage trend test (significance level of 5%) for incidence of LRR and ataxia or two-tailed Jonckheere-Terpstrsa test for decrease of SLA (significance level of 5%) to confirm dose-relationships.		

2.6.3.2 効力を裏付ける試験（続き）

Table 2.6.3.2-10 (cont.)	Primary Pharmacodynamics	Test Article: Remimazolam
試験表題：ONO-2745BS のラット鎮静作用の検討（既存薬物との比較および用量反応性）		
Route of Administration: i.v.	Location in CTD: 4.2.1.1.9	Study Number: [redacted]
GLP Compliance: non GLP		

Results: In experiment 1, the number of animals experiencing LRR increased dose-dependently with remimazolam, midazolam, propofol and dexmedetomidine hydrochloride and MEDs were 5, 20, 5 mg/kg and 10 μg/kg, respectively. Maximum effective dose (ED_{max}) that the lowest dose levels of remimazolam, midazolam, propofol and dexmedetomidine hydrochloride that produces LRR in all animals were 20, 50, 10 mg/kg and 20 μg/kg, respectively. In experiment 2, remimazolam, midazolam, propofol and dexmedetomidine hydrochloride produced ataxia dose-dependently and MEDs were 0.5, 0.2, 2 mg/kg, and 5 μg/kg, respectively. Remimazolam, midazolam, propofol and dexmedetomidine hydrochloride dose-dependently decreased in SLA and MEDs were 1, 0.05, 5 mg/kg and <0.2 μg/kg, respectively. Endpoints evaluated in the study are summarized in the table below.

Test compound	Letency of LRR at ED_{max}	Duration of LRR at ED_{max}	Duration of ataxia after LRR at ED_{max}	MED			LD_{LO}	Safety margin (LD_{LO}/MED for incidence of LRR)
				Incidence of LRR	Incidence of Ataxia	Decrase of SLA		
Remimazolam	0.8 min	6.8 min	9.6 min	5 mg/kg	0.5 mg/kg	1 mg/kg	100 mg/kg	20-fold
Midazolam	0.6 min	49.2 min	120.0 min	20 mg/kg	0.2 mg/kg	0.05 mg/kg	100 mg/kg	5-fold
Propofol	0.3 min	10.7 min	14.0 min	5 mg/kg	2 mg/kg	5 mg/kg	10 mg/kg	2-fold
Dexmedetomidine hydrochloride	2.3 min	61.9 min	74.6 min	10 μg/kg	5 μg/kg	<0.2 μg/kg	500 μg/kg	50-fold

Conclusion: This study proved the sedative effect of remimazolam via three different parameters (LRR, ataxia and decrease in SLA). Remimazolam's rapid onset of sedation was comparable to that of midazolam and propofol, while duration of sedation was significantly shorter than that of midazolam and dexmedetomidine hydrochloride but similar to propofol. Both remimazolam and midazolam induced ataxia and decrease in SLA at lower doses than LRR possibly caused by their common mechanism of action as benzodiazepines. The safety margin (LD_{LO} /MED for LRR) was significantly larger for remimazolam than for midazolam and propofol suggesting a good dosing safety in addition to a rapid on- and offset of sedation.

2.6.3.2 効力を裏付ける試験（続き）

Table 2.6.3.2-11	Primary Pharmacodynamics	Test Article: Remimazolam
試験表題：ONO-2745BS のラット鎮静作用を評価するための試験条件設定		
Route of Administration: i.v.	Location in CTD: 4.2.1.1.10	Study Number: [redacted]
GLP Compliance: non GLP		
Aim of Study: To determine the optimal test conditions such as doses, parameters and methods to evaluate the sedative profile of remimazolam for the main study by comparing with those of midazolam, propofol, and dexmedetomidine hydrochloride. Study Design: Immediately after receiving the test compound i.v. into the tail vein, sedative effects were evaluated via recording latency and duration of loss of righting reflex (LRR) and ataxia, the latter both with high and low dose ranges. Ataxia was defined by staggering gait or prone position. Recovery from LRR was reached when the animal was able to right itself three consecutive times after losing its righting reflex. Full recovery was achieved when the animal walked without ataxia and was able to pull itself up three consecutive times when suspended from a horizontal wire. Spontaneous locomotor activity (SLA) every minute was measured for 30 min using an infrared sensor system (MDC system, BrainScience idea Co, Ltd., Japan) .		
Species (Gender, Strain, Supplier, n/Group): Rats (male, Crl:CD(SD), Charles River Japan, Inc., n = 8-10) Age/Body Weight: 6-7 weeks Conditioning and Surgical Pre-treatment: Stabilized for at least 5 days on wood litter with free access to food and water		
Test Compounds (Batch No) and Test Conditions (Formulation, Doses, Route of Administrations):		
Remimazolam	(ONO-2745BS, Lot SOL12621/5, [redacted]), diluted with saline (pH 3.3) LRR and ataxia: 1, 2, 5, 10, 20, 50 and 100 mg/kg, i.v. into the tail vein locomotor activity/ataxia: 0.1, 0.2, 0.5, 1, 2, 5 and 10 mg/kg, i.v. into the tail vein	
Propofol	(1%Diprivan® injection, serial no.45360, [redacted]), diluted with saline LRR and ataxia: 1, 2, 5, 10, 20, and 50 mg/kg, i.v. into the tail vein locomotor activity/ataxia: 0.5, 1, 2, 5 and 10 mg/kg, i.v. into the tail vein	
Midazolam	(Lot LTE0854, [redacted]), dissolved in 1 mol/L HCl and diluted with saline (pH 3.3) LRR and ataxia: 2, 5, 10, 20, 50 and 100 mg/kg, i.v. into the tail vein locomotor activity/ataxia: 0.05, 0.1, 0.2, 0.5, 1, 2 and 5 mg/kg, i.v. into the tail vein	
Dexmedetomidine hydrochloride	(Precedex injection® 200 μg “Maruishi”, serial no.7613, [redacted], 100 μg/mL), diluted with saline LRR and ataxia: 2, 5, 10, 20, 50, 100, 200, 500, 1000 and 2000 μg/kg, i.v. into the tail vein locomotor activity/ataxia: 0.1, 0.2, 0.5, 1, 2 and 5 μg/kg, i.v. into the tail vein	
Control 1	Saline adjusted to pH 3.3, i.v. into the tail vein	
Control 2	Physiological saline (for dexmedetomidine hydrochloride experiment only)	
Method of Evaluation: LRR was evaluated by determining the number of rats with LRR and the corresponding incident rate (%), latency to LRR and duration of LRR. Ataxia was evaluated by determining the number of rats with ataxia and the corresponding incident rate (%), latency to ataxia and duration of ataxia. SLA per minute was measured with sensor counts for 30 min and the maximum reduction (% of control) was calculated. Number of deaths and latency to death was recorded. Statistical Analysis: one-tailed Fisher’s exact test for the number of animals with LRR or ataxia, two-tailed t-test for the maximum reduction of SLA		

2.6.3.2 効力を裏付ける試験（続き）

Table 2.6.3.2-11 (cont.)	Primary Pharmacodynamics	Test Article: Remimazolam
試験表題：ONO-2745BS のラット鎮静作用を評価するための試験条件設定		
Route of Administration: i.v.	Location in CTD: 4.2.1.1.10	Study Number: [redacted]
GLP Compliance: non GLP		

Results:

Remimazolam (5-100 mg/kg) induced LRR dose-dependently. The maximal number of sedated animals was reached at 20 mg/kg. At that dose, duration of LRR was 4.5±0.90 min and ataxia lasted 9.9±0.38 min. All animals administered 100 mg/kg died. At lower doses (0.1-10 mg/kg), dose-dependent onset of ataxia became apparent with the maximal number of ataxic animals at 1 mg/kg. At that dose, ataxia lasted 4.2±0.75 min. Reduction of SLA was induced dose-dependently (0.2-10 mg/kg).

Propofol (5-50 mg/kg) induced LRR dose-dependently. The maximal number of sedated animals was reached at 10 mg/kg. At that dose, duration of LRR was 7.9±0.86 min and ataxia lasted 12.1±0.58 min. Deaths occurred in 4/10 and 9/9 animals treated with 20 and 50 mg/kg, respectively. At 1-10 mg/kg, ataxia occurred dose-dependently with the maximal number of ataxic animals reached at 2 mg/kg. At that dose, ataxia lasted 3.6±0.44 min. Reduction of SLA was induced nearly dose-dependently (1-10 mg/kg).

Midazolam (20-100 mg/kg) induced LRR dose-dependently. The maximal number of sedated animals was reached at 50 mg/kg. At that dose, duration of LRR was 37.4±4.24 min and ataxia lasted 221.7±6.96 min (5/10 animals still ataxic at the end of observation at 4 hours). Deaths occurred in 9/10 animals at 100 mg/kg. At 0.1-5 mg/kg, ataxia occurred dose-dependently with the maximal number of ataxic animals reached at 0.5 mg/kg. At that dose, ataxia lasted 23.4±2.60 min. Reduction of SLA was induced dose-dependently (0.1-5 mg/kg).

Dexmedetomidine hydrochloride (10-1000 μg/kg) induced LRR dose-dependently. The maximal number of sedated animals was reached at 20 μg/kg. At that dose, duration of LRR was 42.7±4.86 min and ataxia lasted 67.6±6.94 min. Deaths occurred in 2/10, 2/10 and 6/10 animals administered 200, 500 and 1000 μg/kg, respectively. At 1-5 μg/kg, ataxia occurred dose-dependently with the maximal number of ataxic animals reached at 5 μg/kg. At that dose, ataxia lasted 28.4±1.45 min. Reduction of SLA was induced dose-dependently (1-5 μg/kg).

For all four test compounds, no observed effect level (NOEL) that is the highest dose level of the test compound that produces no LRR and ataxia or no significant reduction of SLA, maximum effective dose (ED_{max}) that is the lowest dose level of the test compound that produces LRR and ataxia in all animals or shows the maximum reduction of SLA, as well as lowest lethal dose (LD_{LO}) that is the lowest dose level of the test compound that produces death, and lowest lethal dose causes >50% death for mortality ($LD_{>50\%}$) are determined and summarized in the table below:

Endpoint	NOEL/ED_{max}/LD_{LO}/$LD_{>50\%}$	Remimazolam	Propofol	Midazolam	Dexmedetomidine hydrochloride
LRR	NOEL	2 mg/kg	2 mg/kg	10 mg/kg	5 μg/kg
	ED_{max}	20 mg/kg	10 mg/kg	50 mg/kg	20 μg/kg
Ataxia	NOEL	<0.1 mg/kg	<0.5 mg/kg	0.05 mg/kg	0.5 μg/kg
	ED_{max}	1 mg/kg	2 mg/kg	0.5 mg/kg	5 μg/kg
Reduction of SLA	NOEL	0.1 mg/kg	2 mg/kg	0.05 mg/kg	0.5 μg/kg
	ED_{max}	10 mg/kg	10 mg/kg	5 mg/kg	5 μg/kg
Mortality	LD_{LO}	100 mg/kg	20 mg/kg	100 mg/kg	200 μg/kg
	$LD_{>50\%}$	100 mg/kg	50 mg/kg	100 mg/kg	1000 μg/kg

2.6.3.2 効力を裏付ける試験（続き）

Table 2.6.3.2-11 (cont.)	Primary Pharmacodynamics	Test Article: Remimazolam
試験表題：ONO-2745BS のラット鎮静作用を評価するための試験条件設定		
Route of Administration: i.v.	Location in CTD: 4.2.1.1.10	Study Number: [redacted]
GLP Compliance: non GLP		

Conclusion:

The sedative effect of remimazolam in rats can be evaluated in comparison to other sedatives via LRR (latency and duration), ataxia (latency and duration) and reduction in SLA as parameters each with 10 animals per group. The optimal observation period of SLA was the first 10 min after drug administration because control groups showed reduced to no activity at later time points (14-30 min after administration). The optimal dose ranges for evaluation of LRR and lethal dose were 1-100 mg/kg for remimazolam, 5-100 mg/kg for midazolam, 1-50 mg/kg for propofol, and 2-1000 μg/kg for dexmedetomodine hydrochloride. The optimal dose ranges for assessment of ataxia and reduction in SLA were considered 0.05-10 mg/kg for remimazolam, 0.02-5 mg for midazolam, 0.2-10 mg/kg for propofol, and 0.2-5 μg/kg for dexmedetomidine hydrochloride.

2.6.3.2 効力を裏付ける試験（続き）

Table 2.6.3.2-12	Primary Pharmacodynamics	Test Article: Remimazolam
Report Title: The sedative effect of acute intravenous administration of CNS 7056X and midazolam in the rat		
Route of Administration: i.v.	Location in CTD: 4.2.1.1.11	Study Number: [redacted]
GLP Compliance: non GLP		
Aim of Study: To evaluate the sedative profile of a high dose of remimazolam in the rat and to compare with the same dose of midazolam Study Design: After i.v. drug administration into the tail vein, time to onset of loss of righting reflex (LRR), to recovery and full recovery from LRR were recorded and compared to vehicle controls and midazolam. Recovery from LRR was reached when the animal was able to right itself three consecutive times after losing its righting reflex. Full recovery was achieved when the animal walked without ataxia and was able to pull itself up three consecutive times when suspended from a horizontal wire. For animals demonstrating LRR, reflex responses to corneal touch, ear touch (Pinna reflex) and pinching a rear hind foot (Haffner reflex) were measured.		
Species (Gender, Strain, Supplier, n/Group): Rat (male, Wistar, n = 6) Age/Body Weight: 300 – 450 g Conditioning and Surgical Pre-treatment: not described		
Test Compounds (Batch No) and Test Conditions (Formulation, Doses, Route of Administrations):		
Remimazolam	(batch no. U12438/54/1) dissolved in physiological saline and diluted to 10 mg/mL, 25 mg/kg, i.v. into the tail vein	
Midazolam	(Versed®, 5 mg/mL) 25 mg/kg, i.v. into the tail vein	
Method of Evaluation: Latency and duration of LRR were recorded for all treated animals and compared to vehicle control and midazolam. Mean time±SEM to onset of LRR, to recovery from LRR and to total recovery were determined (n = 6). Statistical Analysis: No statistical analysis was performed.		
Results: Intravenous administration of both midazolam (25 mg/kg) and remimazolam (25 mg/kg) led to an immediate LRR in rats. Recovery from the LRR was observed after 9.61±0.53 and 24.62±2.46 (mean±standar error of the mean) min for rats treated with remimazolam and midazolam, respectively. Full recovery was observed after 11.60±0.48 and 132.34±6.25 min for rats treated with remimazolam and midazolam, respectively. When tested during LRR, none of the rats receiving remimazolam exhibited a Haffner, corneal or Pinna reflex. In contrast, 2 of 6 rats treated with midazolam exhibited the Haffner reflex and none exhibited the corneal or Pinna reflex.		
Conclusion: After intravenous injection at equal doses of 25 mg/kg, both remimazolam and midazolam caused a rapid LRR in rats. A rapid recovery from the sedative effects was observed with remimazolam while recovery from the sedative effects of midazolam took substantially longer. It is concluded that remimazolam has a significantly shorter duration of sedative effect than midazolam in the rat.		

2.6.3.2 効力を裏付ける試験（続き）

Table 2.6.3.2-13	Primary Pharmacodynamics	Test Article: Remimazolam
Report Title: *In vivo* Electrophysiological Evaluation of Putative Benzodiazepine Agonists		
Route of Administration: i.v.	Location in CTD: 4.2.1.1.12	Study Number: [redacted]
GLP Compliance: non-GLP		
Aim of Study: Extracellular single cell recording studies to evaluate the ability of 4 novel benzodiazepines to inhibit substantia nigra pars reticulata (SNpr) neurons. Study Design: Using an *in vivo* electrophysiological assay where the drugs inhibit the activity of SNpr neurons by potentiating their responses to a tonic, endogenous GABAergic input.		
Species (Gender, Strain, Supplier, n/Group): Rats (male, Sprague Dawley, Taconic, U.S.A, n=7-8), 6-10 dose-response curves for each drug Age/Body Weight: 225-350 g Conditioning and Surgical Pre-treatment: Anaesthetised (chloral hydrate, 400 mg/kg, i.p.) rats were placed in a stereotaxic instrument and the skull was surgically exposed. An electrode was lowered through a small burr hole to the level of the substantia nigra for recording the neuronal activity.		
Test Compounds (Batch No) and Test Conditions (Formulation, Doses, Route of Administrations):		
Remimazolam	GW-502056, Batch No: no data, formulation: no data, increasing doses of 0.05 – 8 mg/kg, i.e. each dose doubled the previously administered cumulative dose, i.v. via lateral tail vein	
GW-439178	Batch No: no data, formulation: no data, increasing doses of 0.05-8 mg/kg, i.v. via lateral tail vein	
GW-480473	Batch No: no data, formulation: no data, increasing doses of 0.05-8 mg/kg, i.v. via lateral tail vein	
GW-481757	Batch No: no data, formulation: no data, increasing doses of 0.05-8 mg/kg, i.v. via lateral tail vein	
Method of Evaluation: For each SNpr neuron, the firing rate after each dose was averaged and compared to the mean baseline rate before drug administration to generate a percentage change in firing at each dose. EC_{50} values ± standard error of the mean (SEM) (using GraphPad Pism), the maximum inhibitory effect, and the time for recovery form inhibition to baseline firing rates. Statistical Analysis: No statistic analysis was performed.		
Results: After i.v. administration remimazolam (named GW-502056 in this study) was found to inhibit neuronal firing of electrophysiologically well characterized neurons of the SNpr with an 50% effective dose (ED_{50}) of 0.83±0.01 mg/kg (n = 7) and inhibited firing to 20% of baseline firing rates. The other test compounds inhibited the firing of the neurons with the following ED_{50} values: 1.32±0.01 mg/kg (GW-439178, n = 8), 2.12±0.01 mg/kg (GW-480473, n = 8) and 4.87±0.01 mg/kg (GW-481757, n = 7). SNpr cell firing recovered to 98% of baseline in 7 min for remimazolam, to >100% of baseline in 13 min for GW-439178, to 92 of baseline in 16 min for GW-480473, and to 96% of baseline in 22 min for GW-481757.		
Conclusion: Remimazolam was the most potent agonist of the series regarding inhibition of firing rates. Its inhibition produced by 8 mg/kg remimazolam within 7 min was the fastest recovery to baseline rates of the series and much faster than that of diazepam and flurazepam (30-50 min), seen in earlier studies.		

2.6.3.2 効力を裏付ける試験（続き）

Table 2.6.3.2-14	Primary Pharmacodynamics	Test Article: Remimazolam
試験表題：ベンゾジアゼピン拮抗薬投与による ONO-2745BS の鎮静作用を評価するための試験条件設定		
Route of Administration: i.v.	Location in CTD: 4.2.1.1.13	Study Number: [redacted]

GLP Compliance: non GLP

Aim of Study: Preliminary study to set endpoints, methods and conditions for comparison of remimazolam sedative action with existing drugs and to evaluate the effect of flumazenil (benzodiazepine antagonist) against the sedative actions.

Study Design: Rats received remimazolam, midazolam, propofol, or dexmedetomidine hydrochloride intravenously into the tail vein and occurrence of loss of righting reflex (LRR) was monitored. Effects of pre- or post administration of flumazenil were evaluated via LRR.

Experiment 1: Flumazenil (1, 2, 5, 10 mg/kg) was administered 2.5 min before midazolam (50 mg/kg, n = 10 per group); flumazenil (10 mg/kg) was administered 2.5 min before propofol (10 mg/kg, n = 6 per group) and dexmedetomidine hydrochloride (20 μg/kg, n = 6 per group)

Experiment 2: Flumazenil (1, 2, 5, 10 mg/kg) was administered 2 min after midazolam (50 mg/kg, n = 5-8 per group). Changing the time point of post treatment, flumazenil (0.1, 0.2, 0.5, 1, 2, 5, 10 mg/kg) was administered 5 min after midazolam (50 mg/kg, n = 9-10 per group). Flumazenil (10 mg/kg) was administered 2 min after propofol (10 mg/kg, n = 6 per group) and 10 min after dexmedetomidine hydrochloride (20 μg/kg, n = 3 per group)

Experiment 3: Flumazenil (1, 2, 5, 10 mg/kg) was administered 2 min after midazolam (50 mg/kg, n = 10 per group).

Experiment 4: 0.01 mg/kg flumazenil was administered 2 min before 20 mg/kg remimazolam (n = 7 per group), all following flumazenil doses (0.02, 0.05, 0.1, 0.2, 0.5, 1 mg/kg) were administered 2 min after 20 mg/kg remimazolam (n = 4-8 per group). To test higher doses of remimazolam, the following pairings were performed.

30 mg/kg remimazolam, 2 min later flumazenil (0.05, 0.1, 0.2, 0.5 mg/kg) (n = 5 per group), 40 mg/kg remimazolam, 2 min later flumazenil (0.1, 0.2, 0.5 mg/kg) (n = 6 per group), 50 mg/kg remimazolam, 2 min later flumazenil (0.5, 1, 2, 5, 10 mg/kg) (n = 5-6 per group).

Vehicle served as negative control in each single experiment.

Species (Gender, Strain, Supplier, n/Group): Rats [ale, Crl:CD (SD), Charles River Laboratories Japan, Inc., n = 3 to 10 animals per group depending on experiment and group]; Age/Body Weight: 6 or 7 weeks old at use; Conditioning and Surgical Pre-treatment: at least 5 days of adaptation on wood litter with food and water access at libitum, 6 animals per cage (size: W345 × L403 × H177 mm)

Test Compounds (Batch No) and Test Conditions (Formulation, Doses, Route of Administrations):

Remimazolam	(ONO-2745BS, Lots SOL12621/5 and SOL12621/6), dissolved in saline (pH 3.3) to 10 mg/mL, diluted to 2, 3, 4, 5 mg/mL with saline (pH 3.3)
Midazolam	(Dormicum® Injection, serial no. T010Y04, 5 mg/mL or midazolam, Lot LTE0854), undiluted use or diluted to 5 mg/mL with saline (pH 3.3)
Propofol	(1%Diprivan® Injection, serial no. 45360, 10 mg/mL), diluted with saline to 1 mg/mL
Dexmedetomidine hydrochloride	(Precedex® Injection 200 μg 'Maruishi', serial no. 7613, 100 μg/mL), diluted with saline to 2 μg/mL
Flumazenil	(Lot 027K1401), dissolved in polyethylene glycol / propylene glycol (1:1 v/v) to 6 mg/mL, further diluted with saline to 1 mg/mL

Method of Evaluation: Endpoint of evaluation were incidence, onset and duration LRR. Onset of LRR was defined by the animal being unable to return from supine to prone position after ≥15 seconds. After a first spontaneous return to prone position after LRR, reversal was achieved when the animal was able to return from evaluator-imposed supine position to prone position on 3 consecutive occasions. Microsoft Excel 2002 SP-2 (Microsoft Corp.) was used to calculate means and standard deviations and to create tables and graphs.

Statistical Analysis: Significance tests were performed using SAS 9.1.3 Service Pack 4 (SAS Institute Japan) and the linked system EXSAS Ver.7.1.0 (Arm-Systech). Fisher's exact test with closed testing procedure for comparison between the test compound and control.

2.6.3.2 効力を裏付ける試験（続き）

Table 2.6.3.2-14 (cont.)	Primary Pharmacodynamics	Test Article: Remimazolam
試験表題：ベンゾジアゼピン拮抗薬投与による ONO-2745BS の鎮静作用を評価するための試験条件設定		
Route of Administration: i.v.	Location in CTD: 4.2.1.1.13	Study Number: [redacted]
GLP Compliance: non GLP		

Results: Exp. 1: Midazolam at 50 mg/kg induced LRR in all animals (10/10) and duration of LRR was 28.5±3.01 min. Pre-treatment with flumazenil (≥5 mg/kg) significantly antagonized the incidence of LRR (5 and 10 mg/kg flumazenil prevented LRR in 6/10 and 8/10 animals, respectively). Propofol and dexmedetomidine hydrochloride induced LRR in all animals irrespective of a pre-treatment with solvent or flumazenil.

Exp. 2 and 3: Whether flumazenil was administered 2 min or 5 min after midazolam, dose-dependent reversal of LRR was seen from 1 min after flumazenil administration. From 3 up to 20 min after flumazenil administration, some animals showed repeat LRR after initial reversal of LRR. Therefore, reversal of LRR was measured at 1 min after addition of flumazenil and repeat LRR thereafter until 20 min. The number of animals with midazolam-induced LRR that showed reversal of LRR after flumazenil post-treatment increased in a dose dependent manner, with significant differences seen at doses of ≥5 mg/kg in the flumazenil 2 min post-treatment evaluation system, and at doses of ≥0.5 mg/kg in the flumazenil 5 min post-treatment evaluation system. Flumazenil had no effect on propofol-induced LRR. With dexmedetomidine hydrochloride, all animals (3/3) showed reversal of LRR with solvent administration, reflecting a known reversal upon stimulation, in this case probably by solvent administration.

Exp. 4: Remimazolam induced LRR in all animals at 20 mg/kg but for only 4.5±0.90 min which was considered to be too short to evaluate antagonistic effects of flumazenil. At 30, 40, and 50 mg/kg, all animals showed LRR when flumazenil was administered 2 min after remimazolam administration. Flumazenil induced reversal of LRR at all remimazolam dose levels, and the lowest dose of flumazenil inducing reversal of LRR in ≥50% of animals was 0.02, 0.2, 0.5 and 2 mg/kg, respectively, in remimazolam 20, 30, 40 and 50 mg/kg treatment groups. 3 of 38 animals died in the remimazolam 50 mg/kg group. Remimazolam dose levels of 30 and 40 mg/kg were therefore considered to be optimal, because the duration of LRR was comparatively long (9.0 min, 11.0 min, respectively) and no deaths occurred at these doses.

The no observed effect level of flumazenil against midazolam-induced LRR was 1 mg/kg, and the effective dose exhibiting significant antagonistic action was 5 mg/kg. The no observed effect level of flumazenil against LRR induced by remimazolam 30 mg/kg was 0.05 mg/kg, and dose level producing reversal in ≥50% animals was 0.2 mg/kg.

Conclusion: In pivotal studies, the sedative action of remimazolam will be investigated based on the endpoint of reversal of LRR, using a flumazenil post-treatment evaluation system including evaluation of repeat LRR. The dose levels of remimazolam, midazolam and propofol will be set at 30, 50 and 10 mg/kg, respectively. The dose levels of flumazenil will be set at 0.05, 0.1, 0.2, 0.5 and 1 mg/kg in the remimazolam treatment group, 1, 2, 5 and 10 mg/kg in the midazolam treatment group and 10 mg/kg in the propofol treatment group. In pivotal studies, 10 animals per group will be used.

2.6.3.2 効力を裏付ける試験（続き）

Table 2.6.3.2-15	Primary Pharmacodynamics	Test Article: Remimazolam
試験表題：化合物 S の鎮静作用の検討		
Route of Administration: i.v.	Location in CTD: 4.2.1.1.14	Study Number: [redacted]
GLP Compliance: non GLP		
Aim of Study: To evaluate the sedative profile of remimazolam and compare it with the same dose of midazolam Study Design: After i.v. drug administration of remimazolam into the tail vein, time to onset of loss of righting reflex (LRR), to recovery and full recovery from LRR was recorded and compared to vehicle controls and midazolam. Recovery from LRR was reached when the animal was able to right itself three consecutive times after losing its righting reflex. Full recovery was achieved when the animal walked without ataxia and was able to pull itself up three consecutive times when suspended from a horizontal wire.		
Species (Gender, Strain, Supplier, n/Group): Rats (male, Crlj:Wistar, Charles River Japan, Inc., n = 10) Age/Body Weight: 8 weeks / 286.8 – 334.8 g Conditioning and Surgical Pre-treatment: Stabilized for at least 5 days on wood litter with free access to food and water		
Test Compounds (Batch No) and Test Conditions (Formulation, Doses, Route of Administrations):		
Remimazolam	besylate salt (Lot LJC-039-081-1), dissolved in a citric acid monohydrate and tri-sodium dehydrate buffer (0.15 M, pH 3.5), 25 mg/kg i.v.	
Midazolam	(Dormicum® Injection, 5 mg/mL, [redacted]), 25 mg/kg i.v.	
Control(s)	Vehicle	
Method of Evaluation: Latency and duration of LRR as well as time to recovery and full recovery from LRR were recorded compared to vehicle control and midazolam. Mean time±standard error of the mean (SEM) to onset of LRR, to recovery from LRR and to total recovery were determined (n = 10). Statistical Analysis: No statistical analysis was performed.		
Results: Remimazolam induced LRR in all 10 animals immediately after administration, while midazolam immediately induced ataxia and LRR in 9/10 rats with latency of 2.05±0.34 min. One rat treated with midazolam did not show any sedation and was disregarded for evaluation. Recovery from LRR occurred after 8.36±0.42 min for remimazolam (n = 10) treated rats and 28.13±5.83 min for midazolam treated animals (n = 9), full recovery was observed after 12.98±0.52 min and 135.56±11.03 min, respectively.		
Conclusion: Remimazolam and midazolam both induced LRR, the latter with ataxia before onset of LRR. Recovery from sedative effects was rapid with remimazolam and took markedly longer with midazolam.		

2.6.3.2 効力を裏付ける試験（続き）

Table 2.6.3.2-16	Primary Pharmacodynamics	Test Article: Remimazolam
試験表題：ONO-2745 および対照薬の鎮静作用における溶媒および動物の系統の比較検討		
Route of Administration: i.v.	Location in CTD: 4.2.1.1.15	Report Number: [redacted]
GLP Compliance: non GLP		
Aim of Study: To choose the optimum solvent for remimazolam and the optimal rat strain for evaluating sedative action. Study Design: Solubility of remimazolam was assessed at room temperature by adding the respective solvent to remimazolam until dissolution was confirmed visually. To assess tolerability of solvents, SD rats (n = 2-3) were injected one solvent i.v. into the tail vein and behavior of the animals was monitored during administration and 1 h later. Normal saline, saline (pH 2.5–4.0) and lactose solvent (pH 3.1) were administered at 10 mL/kg, and citrate buffer (pH 3.8) and citrate buffer containing Sulfobutylether β-Cyclodextrin (Captisol®; SEB-β-CD) (pH 3.8) were administered at 10, 20 and 30 mL/kg. To rule out an effect of pH, motor activity was compared between SD rats injected either with control substances normal saline or saline at pH 3.3 (n = 4). For comparison of the sedative action of remimazolam in different solvents and different rat strains, SD and Wistar rats intravenously received either 10 or 30 mg/kg remimazolam solved in saline, citrate buffer containing SEB-β-CD, or lactose solvent (n = 4). Onset and recovery of ataxia and loss of righting reflex (LRR) were recorded. Additionally, 1 animal was administered 80 mg/kg and 3 animals received 100 mg/kg remimazolam. LRR and deaths were noted. For comparison of sedative action of propofol and midazolam in different rat strains ,SD and Wistar rats intravenously received 5 and 10 mg/kg propofol (n = 4), or 25 and 50 mg/kg midazolam (n = 5). In addition, 100 mg/kg midazolam were administered to both strains (n = 2). LRR, ataxia, and lethality were determined.		
Species (Gender, Strain, Supplier, n/Group): Rats (male, Crl:CD (SD) and Crlj:WI (Wistar), Charles River Laboratories Japan, Inc., n=4) Age/Body Weight: 6-7 weeks old at use Conditioning and Surgical Pre-treatment: 3–6 animals per cage were acclimatized for at least 5 days with food and water ad libitum.		
Test Compounds (Batch No) and Test Conditions (Formulation, Doses, Route of Administrations):		
Remimazolam Test Solvents	(Lot No: SOL12621/5, [redacted].), dissolved to 10 mg/mL and further diluted to 1 and 3 mg/mL with each of the test solvents below; (1) normal saline (2) saline (pH 2.5–4.0) (3) citrate buffer (pH 3.8) (4) citrate buffer containing SEB-β-CD (pH 3.8) (5) lactose solvent (pH 3.1).	
Midazolam	(Lot No: T010Y04, [redacted]), dissolved in 1 mol/L hydrochloric acid, diluted to 10 mg/mL and further diluted to 2.5, 5, and 10 mg/mL with normal saline and adjusted to pH 3.3.	
Propofol	(1%Diprivan® Injection, Lot No: 45360, [redacted]), diluted with normal saline to 1 and 5 mg/mL	

2.6.3.2 効力を裏付ける試験（続き）

Table 2.6.3.2-16 (cont.)	Primary Pharmacodynamics	Test Article: Remimazolam
試験表題：ONO-2745 および対照薬の鎮静作用における溶媒および動物の系統の比較検討		
Route of Administration: i.v.	Location in CTD: 4.2.1.1.15	Report Number: [redacted]
GLP Compliance: non GLP		

Method of Evaluation: Solubility of remimazolam was assessed at room temperature by adding the respective solvent to remimazolam until dissolution was confirmed visually. Tolerability of solvents was assessed by visual monitoring of behavior during injection and 1 hour later. To assess motor activity, each animal was placed into a cage equipped with an infrared sensor and cumulative motor activity per min was measured for 30 min (n = 4/group).
Ataxia was defined as the state in which the animal was unable to clamber normally when suspended 3 consecutive times on the wire mesh. LRR was defined as the state in which the animal was unable to return its head to normal position 3 consecutive times while in supine position. The variables evaluated were number of animals experiencing LRR, onset of LRR, duration of ataxia and LRR, and number of dead animals.
Statistical Analysis: No statistical analysis was performed.

Results: Solubility of remimazolam at room temperature was 14, 5, 20, and 16 mg/mL in normal saline (pH ≈ 3), citrate buffer (pH ≈ 4), citrate buffer containing SEB-β-CD (pH ≈ 4), and lactose solvent (pH ≈ 3), respectively. Saline at pH 3.1–4.0 had no effect on behavior, though with saline at pH 2.5 squealing and struggling was observed during injection. Since solubility of citrate buffers was low, 20 and 30 mL/kg in addition to 10 mg/kg were administered. In citrate buffer, no effects were observed at 10 mL/kg, 2/2 animals showed transient lower limb paralysis at 20 mL/kg, 1/2 animal died at 30 mL/kg. In citrate buffer containing SEB-β-CD, 2/2 animals showed slight struggling at 10 mL/kg, 2/2 animals showed transient lower limb paralysis at 20 mL/kg and 2/2 animals died at 30 mL/kg. Neither normal saline nor pH 3.3 saline administered into the tail vein induced ataxia or changed motor activity.
None of the solvents [normal saline, citrate buffer containing SEB-β-CD (pH 3.8), lactose solvent (pH 3.1)] differed in terms of sedative action of remimazolam (number of animals with LRR, onset and duration of LRR, duration of ataxia). The same is true for the two rat strains. 1/1 animal treated with 80 mg/kg remimazolam experienced LRR for 10.3 min and survived while 3/3 animals treated with 100 mg/kg developed dyspnea immediately after administration and died.
No difference between rat strains regarding sedation and mortality. Therefore, the table below includes data of SD rats only and also integrates sedation data of remimazolam.

2.6.3.2 効力を裏付ける試験（続き）

Table 2.6.3.2-16 (cont.)	Primary Pharmacodynamics	Test Article: Remimazolam
試験表題：ONO-2745 および対照薬の鎮静作用における溶媒および動物の系統の比較検討		
Route of Administration: i.v.	Location in CTD: 4.2.1.1.15	Report Number: [redacted]
GLP Compliance: non GLP		

Conclusion: On the basis of the above results, it was decided to use normal saline as the remimazolam solvent and SD as the rat strain in the next efficacy pharmacology studies. It was decided to dissolve remimazolam in normal saline to a concentration of 10 mg/mL and then to dilute with saline (pH 3.3).

Table Sedative action of remimazolam, midazolam, and propofol in SD rats (mean±SEM)

Test Article	Dose (mg/kg, i.v.)	No. of Deaths	Incidence of LRR	Onset of LRR (min)	Duration of LRR (min)	Duration of Ataxia (min)
Remimazolam (solvent: normal saline)	10	0/4	4/4	1.3±0.1	2.7±0.6	6.3±0.3
	30	0/4	4/4	0.3±0.1	8.7±0.6	10.6±0.4
	100	3/3	3/3	-	-	-
Midazolam	25	0/5	4/5	10.1±4.0	25.2±12.8	85.6±12.4
	50	0/5	5/5	1.4±1.1	43.7±9.1	131.3±16.6
	100	1/2	2/2	0	127.4	214.3
Propofol	5	0/4	2/4	0.3	0.6	4.2±0.6
	10	0/4	4/4	0	7.7±1.2	10.3±0.9

2.6.3.2 効力を裏付ける試験（続き）

Table 2.6.3.2-17	Primary Pharmacodynamics	Test Article: Remimazolam
試験表題：ONO-2745BS のラット鎮静作用の検討（既存薬物との比較および用量反応性）－ED_{50} 値と LD_{50} 値に関する追加解析－		
Route of Administration: i.v.	Location in CTD: 4.2.1.1.16	Study Number: [redacted]
GLP Compliance: non GLP		
Aim of Study: To calculate and compare 50% effective dose (ED_{50}) for loss of righting reflex (LRR) and 50% lethal dose (LD_{50}) values for remimazolam, midazolam, propofol and dexmedetomidine hydrochloride using data from study [redacted] (Table 2.6.3.2-16) Study Design: Based on the data of number of animals occured LRR and number of deaths in study E08QA005, the ED_{50} and the LD_{50} were calculated and the margins between effective and lethal doses were determined as the ratio of LD_{50}/ED_{50}.		
Species (Gender, Strain, Supplier, n/Group): Refere to Study [redacted] Age/Body Weight: Refere to Study [redacted] Conditioning and Surgical Pre-treatment: Refere to Study [redacted]		
Test Compounds (Batch No) and Test Conditions (Formulation, Doses, Route of Administrations):		
Remimazolam	Refere to Study [redacted]	
Midazolam	Refere to Study [redacted]	
Propofol	Refere to Study [redacted]	
Dexmedetomidine hydrochloride	Refere to Study [redacted]	
Control(s)	Refere to Study [redacted]	
Method of Evaluation: Based on the results of study [redacted], the ED_{50} and the LD_{50} were calculated and the margins between effective and lethal doses (the ratio of LD_{50}/ED_{50}) were determined. ED_{50} values and LD_{50} values were calculated by the probit method and 95% confidence intervals were determined where possible. Statistical Analysis: No statistical analysis was performed.		
Results: For remimazolam, midazolam, propofol and dexmedetomidine hydrochloride, the ED_{50} were 3.9, 14, 4.4 mg/kg and 8.6 μg/kg, respectively. The LD_{50} were 90, ≥100, 22 mg/kg and ≥1000 μg/kg, respectively. Accordingly, the ratios of LD_{50}/ED_{50} were 23, ≥7.1, 5.0 and ≥120-fold, respectively. The 95% confidence intervals for the the LD_{50} of remimazolam and the ED_{50} of propofol could not be determined. The LD_{50} of midazolam and dexmedetomidine hydrochloride could not be determined because number of deaths were less than 5 of 10 animals even at the highest dose.		
Conclusion: The study showed that remimazolam exhibited larger margin between effective and lethal doses than propofol.		

2.6.3.2 効力を裏付ける試験（続き）

Table 2.6.3.2-18	Primary Pharmacodynamics	Test Article: Remimazolam
Report Title: Evaluation of CNS7056X.besylate in two vehicles for sleep induction (Loss of Righting Reflex) in the rat.		
Route of Administration: i.v.	Location in CTD: 4.2.1.1.17	Study Number: [redacted]
GLP Compliance: non GLP		
Aim of Study: To evaluate the potential to induce loss of righting reflex (LRR) of remimazolam after i.v. administration in two different vehicles in the rat. Study Design: Rats (n = 8) received remimazolam i.v. at 3 different doses in 2 different vehicles. Duration of LRR and number of animals showing LRR were recorded and compared between the two vehicles.		
Species (Gender, Strain, Supplier, n/Group): Rat (male, Rj: Sprague-Dawley, Elevage Janvier, France, n = 8) Age/Body Weight: 200-246 g Conditioning and Surgical Pre-treatment: Stabilized for at least 5 days on wood litter with free access to food and water		
Test Compounds (Batch No) and Test Conditions (Formulation, Doses, Route of Administrations):		
Remimazolam	CNS7056X.besylate (CNS 7056B), [redacted], batch no. [redacted] white powder, dispersed in vehicle 1 or dissolved in vehicle 2 10, 20 and 30 mg/kg of base, i.v.	
Vehicle 1	25.86% w/v β-cyclodextrin (Captisol®) in 25 mmol/L citrate buffer solution (pH = 3.8), i.v.	
Vehicle 2	25 mmol/L citrate buffer solution (pH = 3.8), i.v.	
Method of Evaluation: The latency and duration of LRR (maximum: 90 minutes after administration) were recorded in rats received either vehicle or test substance by the intravenous route. The righting reflex test was performed as soon as the animals appear sedated, approximately every 20-30 seconds. Once the righting reflex was absent, duration of LRR was measured by testing for the return of the righting reflex approximately every 20-30 seconds thereafter. Statistical Analysis: Fisher's Exact test for quantal data (number of rats showing LRR) and analysis of variance (ANOVA) followed by Dunnett's t test for quantitative data (latency and duration of LRR)		
Results: Remimazolam prepared in vehicle 1 significantly and dose-dependently induced short lasting LRR. Out of 8 rats, 5 ($p<0.05$), 7 ($p<0.01$) and 8 ($p<0.001$) rats showed LRR upon administration of 10, 20 and 30 mg/kg, respectively, and duration of LRR increased correspondingly [2.3 min ($p<0.05$), 4.9 min ($p<0.01$) and 8.9 min ($p<0.01$)]. Remimazolam prepared in vehicle 2 significantly and dose-dependently induced a short lasting LRR in 6 rats ($p<0.01$), 8 rats ($p<0.001$) and 8 rats, ($p<0.001$) showed LLR out of 8 rats after administration of 10, 20 and 30 mg/kg, respectively. Duration of LRR increased correspondingly [1.9 min (not significant), 6.0 min ($p<0.01$), 8.1 min ($p<0.01$)].		
Conclusion: Remimazolam had similar effects when prepared in vehicle 1 or vehicle 2, indicating that the effects of remimazolam were not affected by the presence of β-cyclodextrin (Captisol®).		

2.6.3.2 効力を裏付ける試験（続き）

Table 2.6.3.2-19	Primary Pharmacodynamics	Test Article: Remimazolam (lot no. [redacted]), Remimazolam (lot no. [redacted]), [redacted]
試験表題：ONO-2745BS（Lot 番号 YMK110831）のラット鎮静作用		
Route of Administration: i.v.	Location in CTD: 4.2.1.1.18	Study Number: [redacted]
GLP Compliance: non GLP		
Aim of Study: To compared the sedative efficacy of remimazolam (lot no. SOL12621/6) used in preclinical studies with that of remimazolam (lot no. [redacted]) containing new impurities in order to confirm if one of new impurities [redacted] had no impact on the sedative effect of remimazolam. Study Design: After i.v. administration of the test substance into the tail vein, animals were each placed into a plastic container and checked for loss of righting reflex (LRR) in a blinded manner.		
Species (Gender, Strain, Supplier, n/Group): Rats [male, Crl:CD(SD), Charles River Laboratories Japan, n = 10 for remimazolam (lot no. [redacted] and [redacted]) and n = 6 for [redacted]] Age/Body Weight: 6 weeks Conditioning and Surgical Pre-treatment: Acclimation period of 6-8 days on wood litter with free access to food and water		
Test Compounds (Batch No) and Test Conditions (Formulation, Doses, Route of Administrations):		
Remimazolam (lot no. [redacted])	(ONO-2745BS, [redacted] lot no. [redacted], purity 94.6%), dissolved in physiological saline, 1, 2, 5, and 10 mg/kg, i.v. into the tail vein	
Remimazolam (lot no. [redacted])	(ONO-2745BS, [redacted]., lot no. [redacted], purity no less than 95%), dissolved in physiological saline, 1, 2, 5, and 10 mg/kg, i.v. into the tail vein	
[redacted]	([redacted], lot no. [redacted], purity no less than 95%), dissolved in physiological saline, (Exp 1) 10, 20, and 50 mg/kg, i.v. into the tail vein (Exp 2) 0.2, 0.5, 1, 2, 5, 10, and 20 mg/kg, i.v. into the tail vein	
Control(s)	Physiological saline	
Method of Evaluation: The primary endpoints for sedative effect were the number of animals exhibiting LRR (defined as the failure of an animal return to the prone position within 15 sec after being placed in a supine position), the latency and duration of LRR. An animal was considered to have recovered from LRR if it successfully returned to a prone position three consecutive times. As a secondary endpoint the ED_{50} for number of animals showing LRR was calculated. Further endpoints for experiments with [redacted] were ataxia (staggering gait or prone position) and mortality. Statistical Analysis: Tests of significant differences were conducted with SAS 9.2 TS2M3 (SAS Institute Japan Ltd.) and its cooperative system EXSUS Ver. 7.7.1 (CAC Corp.). One-tailed Fisher's exact test with testing closed procedure (significance level of 5%) for comparison between the control and remimazolam groups (lot no. [redacted] or [redacted]). One-tailed Cochran-Armitage trend test (significance level of 5%) to confirm dose-relationships in remimazolam groups.		

2.6.3.2 効力を裏付ける試験（続き）

Table 2.6.3.2-19 (cont.)	Primary Pharmacodynamics	Test Article: Remimazolam (lot no. [redacted]), Remimazolam (lot no. [redacted]), [redacted]
試験表題：ONO-2745BS（Lot 番号 YMK110831）のラット鎮静作用		
Route of Administration: i.v.	Location in CTD: 4.2.1.1.18	Study Number: [redacted]
GLP Compliance: non GLP		

Results: All parameters investigated (incidence of LRR, latency and duration of LRR) were similar between remimazolam (lot no. [redacted], with new impurities) and remimazolam (lot no. [redacted], used for preclinical purposes). Both lots induced dose-dependent LRR and significant effects were observed from a dose of 5 mg/kg. The ED_{50} values of lot no. [redacted] and [redacted] were 2.1 and 2.2 mg/kg, respectively.
In experiment 1, high doses of [redacted] (10, 20, and 50 mg/kg) induced LRR at doses of 20 (4/6 animals) and 50 mg/kg (6/6 animals) as well as ataxia at all doses tested (3/6 animals at 10 mg/kg, 6/6 animals at 20 and 50 mg/kg). Duration of LRR was 1.2±0.38 min in the 20 mg/kg group and 6.9±1.73 min in the 50 mg/kg group. 1, 2 and 3 out of 6 animals died at 10, 20 and 50 mg/kg, respectively. In the cased of 50 mg/kg group, animals showed LRR and decrease of respiratory rate immediately after administration and died 3 or 9 min after administration due to respiratory arrest. In the case of 20 mg/kg group, 1 out of 2 animals was once recovered from LRR but found dead in a housing cage 15 min after administration. Other animals (one in each 10 and 20 mg/kg group) was found dead in housing cages next morning although the existence was confirmed until 1 hour after administration.
In experiment 2, low doses of [redacted] (0.2 to 20 mg/kg) resulted in LRR at the highest dose of 20 mg/kg (5/6 animals) with latency of 1.1±0.27 min, as well as ataxia at doses of 5 mg/kg (1/6 animals), 10 mg/kg (4/6 animals) and 20 mg/kg (6/6 animals). 3 out of 6 animals in the 20 mg/kg group died. 2 out of 3 deaths were confirmed at 1 or 5 min after administration, and 1 out of 3 deaths was found the next morning of the study in an individual cage . At necropsy of the rat died the next morning of the study, dark red color in the lungs and dark red pleural fluid were observed. It was suggested the cause of deaths was the effects on the cardiovascular and cardiopulmonary systems. No deaths were observed at doses of 10 mg/kg or lower. Necropsy was performed in all survival animals the next day of the study (about 24 hours after administration) and no abnormalities were not observed.

Conclusion: Comparison of the two lots of remimazolam (lot no. [redacted] and [redacted]) showed that the new impurities contained in lot no. [redacted] had no impact on the sedative effect of remimazolam.
One of the new impurities [redacted] induced LRR in more than half of animals received the doses of 20 mg/kg or more. As remimazolam induced LRR in more than half of animals received the doses of 5 mg/kg or more, the sedative potency of [redacted] was estimated to be one forth of that of remimazolam. Because one death was found the next morning of the study despite individual housing in experiment 2, it was considered that two deaths found the next morning in experiment 1 were due to the direct effect of [redacted]. The minimum lethal dose of [redacted] by bolus injection was 10 mg/kg. Further investigation was considered necessary in regards of the safety of [redacted].

2.6.3.2 効力を裏付ける試験（続き）

Table 2.6.3.2-20	Primary Pharmacodynamics	Test Article: Remimazolam
試験表題：ベンゾジアゼピン受容体拮抗剤投与による ONO-2745BS および既存薬物の鎮静作用		
Route of Administration: i.v.	Location in CTD: 4.2.1.1.19	Study Number: [redacted]
GLP Compliance: non GLP		
Aim of Study: To examine whether the sedative effect of remimazolam was antagonized by the benzodiazepine receptor antagonist flumazenil Study Design: In a blinded manner, remimazolam or control drugs midazolam and propofol were administered to rats and the induction of loss of righting reflex (LRR) was confirmed. Flumazenil was administered 2 min after administration of the sedative drugs. The recovery from LRR was assessed at 1 min after administration of flumazenil. For animals recovered from LRR, no re-appearance of LRR was confirmed up to 20 min after administration of flumazenil.		
Species (Gender, Strain, Supplier, n/Group): Rats (male, Crl:CD(SD), Charles River Laboratories Japan, n = 10) Age/Body Weight: 6 weeks Conditioning and Surgical Pre-treatment: Acclimation period of 8-9 days on wood litter with free access to food and water		
Test Compounds (Batch No) and Test Conditions (Formulation, Doses, Route of Administrations):		
Remimazolam	(ONO-2745BS, [redacted], lot no. [redacted], purity 95% or more), dissolved in physiological saline adjusted to pH 3.3 30 mg/kg, i.v. into the tail vein	
Midazolam	([redacted]., Dormicum® Injection, serial no. A001A01), undiluted use, 50 mg/kg, i.v. into the tail vein	
Propofol	(1%Diprivan® Injection, [redacted], serial no. 11570), diluted with physiological saline, 10 mg/kg, i.v. into the tail vein	
Flumazenil	([redacted]., Lot No. 027K1401, purity 95% or more), first dissolved in a mixture of polyethylene glycol and propylene glycol (1:1 vol) to 6 mg/mL, further diluted with physiological saline; 0.05, 0.1, 0.2, 0.5, 1 mg/kg in combination with remimazolam, 1, 2, 5, 10 mg/kg in combination with midazolam, 10 mg/kg in combination with propofol, i.v. into the tail vein	
Method of Evaluation: Primary endpoint was the number of animals recovered from LRR at 1 min after administration of flumazenil. Secondary endpoint was the number of animals showing re-appearance of LRR after the recovery from LRR. Statistical Analysis: Statistical analysis was conducted with SAS 9.1.3 Service Pack 4 (SAS Institute Japan Ltd.) and its cooperative system EXSAS Ver. 7.5.2 (Arm Systex Co, Ltd.). One-tailed Fisher's exact test (significance level of 5%) with closed testing procedure was performed for comparison between the test compounds and flumazenil or vehicle. No statistical analysis was performed for propofol because of the same results between flumazenil and vehicle groups. One-tailed Cochran-Armitage trend test (significance level of 5%) was performed to confirm dose-relationship of flumazenil in theremimazolam and midazolam groups.		

2.6.3.2 効力を裏付ける試験（続き）

Table 2.6.3.2-20 (cont.)	Primary Pharmacodynamics	Test Article: Remimazolam
試験表題：ベンゾジアゼピン受容体拮抗剤投与による ONO-2745BS および既存薬物の鎮静作用		
Route of Administration: i.v.	Location in CTD: 4.2.1.1.19	Study Number: [redacted]
GLP Compliance: non GLP		
Results: In remimazolam groups, flumazenil increased the number of animals recovering from LRR dose-dependently with significant increases at doses of 0.2 mg/kg or more, while vehicle did not produce recovery in any of the animals. The number of animals with recovery from LRR/total number of animals was 0/10, 2/10, 5/10, 10/10, 10/10 at flumazenil doses of 0.05, 0.1, 0.2, 0.5, and 1 mg/kg, respectively. No re-appearance of LRR occurred in remimazolam groups. In midazolam groups, flumazenil increased the number of animals with recovery from LRR dose-dependently, with significant increases at doses of 5 mg/kg or more, while vehicle did not produce recovery in any of the animals. The number of animals with recovery from LRR/total number of animals was 0/10, 2/10, 7/10, and 9/10 at flumazenil doses of 1, 2, 5, and 10 mg/kg, respectively. Re-appearance of LRR was observed in 2/2, 5/7, and 2/9 animals that had recovered from sedation at 2, 5, and 10 mg/kg of flumazenil, respectively. In propofol groups, no recovery from LRR was observed in any of the animals after administration of vehicle or flumazenil at a dose of 10 mg/kg. Six animals were excluded due to technical problems such as loss of catheter or incomplete administration needle misplacement.		
Conclusion: The sedative effect of remimazolam was dose-dependently reversed by flumazenil without any case of re-appearance of LRR and the doses needed for this effect were lower than those necessary for reversal of midazolam-induced sedation. Based on the differences in responsiveness to flumazenil, remimazolam was considered to have different characteristics from midazolam or propofol, suggesting that remimazolam might have a higher safety profile than these existing drugs.		

2.6.3.2 効力を裏付ける試験（続き）

Table 2.6.3.2-21	Primary Pharmacodynamics	Test Article: Remimazolam, R-Remimazolam, CNS 7054, R-CNS 7054
試験表題：ONO-2745BS の代謝物および[redacted]のラット鎮静作用		
Route of Administration: i.v.	Location in CTD: 4.2.1.1.20	Study Number: [redacted]
GLP Compliance: non GLP		
Aim of Study: To compare the sedative effects of the metabolite CNS 7054, the [redacted] of CNS 7054 ([redacted]) and the [redacted] of remimazolam ([redacted]) with that of remimazolam by means of the evaluation of ataxia. Study Design: After i.v. administration of the test compounds to rats via the tail vein, the occurrence of ataxia was investigated for 10 min in a blinded manner. Due to a low solubility of the metabolites, two experiments were performed using either solubilizier (experiment 1) or saline adjusted to pH 3.3 (experiment 2) as vehicle.		
Species (Gender, Strain, Supplier, n/Group): Rats [male, Crl:CD(SD), Charles River Laboratories Japan, n = 10] Age/Body Weight: 6 weeks Conditioning and Surgical Pre-treatment: Acclimatisation period of 8-9 days in econ cages on bedding with free access to food and water in groups of 5 animals		
Test Compounds (Batch No) and Test Conditions (Formulation, Doses, Route of Administrations):		
CNS 7054	(ONO-IN-252, Lot No. YSD070912-1, purity 95% or more; [redacted]) dissolved in 4 vol% WellSolve® (Celeste Corporation, Japan) in phosphate buffered salts (PBS), 20, 50, and 100 mg/kg, i.v. into tail vein	
[redacted]	([redacted], Lot No. YN080118-1, purity 95% or more; [redacted]) dissolved in 4 vol% WellSolve® (Celeste Corporation, Japan) in PBS, 100 mg/kg, i.v. into the tail vein	
Remimazolam	(ONO-2745BS, Lot No. SOL12621/6, purity 95% or more; [redacted]) dissolved in 4 vol% WellSolve® (Celeste Corporation, Japan) in PBS for experiment 1 or saline adjusted to pH 3.3 for experiment 2, 0.2, 0.5, and 1 mg/kg, i.v. into the tail vein	
[redacted]	([redacted], Lot No. YN080110-1, purity 95% or more; [redacted]) dissolved in saline adjusted to pH 3.3, 5, 10 and 20 mg/kg, i.v. into the tail vein	
Control 1	4 vol%WellSolve® ([redacted]) in PBS, i.v. into the tail vein	
Control 2	saline adjusted to pH 3.3, i.v. into the tail vein	
Method of Evaluation: Minimum effective dose (MED) that is the lowest dose level of the test compound that produces statistically significant number of animals showing ataxia within 10 min after dosing was determined as the primary endpoint. Statistical Analysis: Statistical analysis was performed using SAS 9.1.3 Service Pack 4 (SAS Institute Japan Ltd.) and its cooperative system EXSAS Version 7.5.2 (Arm Systex Co., Ltd.). One-tailed Fisher's exact probability tests with closed testing procedure for comparison between control 1 and either CNS 7054 or remimazolam as well as control 2 and either [redacted] or remimazolam. One-tailed Cochran-Armitage trend tests to confirm dose-relationships of CNS 7054, [redacted] or remimazolam. No statistical tests were performed for [redacted] because the result of [redacted] was the same as that of control 1. Results were considered significant when $p<0.05$.		

2.6.3.2 効力を裏付ける試験（続き）

Table 2.6.3.2-21 (cont.)	Primary Pharmacodynamics	Test Article: Remimazolam, R-Remimazolam, CNS 7054, R-CNS 7054
試験表題：ONO-2745BS の代謝物および[redacted]のラット鎮静作用		
Route of Administration: i.v.	Location in CTD: 4.2.1.1.20	Study Number: [redacted]
GLP Compliance: non GLP		
Results: Experiment 1 compared ataxia induction by [redacted] the metabolite, control 1 and remimazolam: In the CNS 7054 groups, a dose-dependent increase of animals with ataxia was observed, being 0/10, 3/10, and 7/10 in the 20, 50, and 100 mg/kg groups, respectively. The increase in the 100 mg/kg group was significant ($p<0.01$) compared to the control, where no ataxia occurred. With the [redacted] of the metabolite ([redacted]), no ataxia was induced at 100 mg/kg. With remimazolam, ataxia occurred dose-dependently in 1/10, 7/10, and 10/10 animals that had received 0.2, 0.5, and 1 mg/kg, respectively. The increase was significant with 0.5 mg/kg ($p<0.01$) or 1 mg/kg ($p<0.001$). Experiment 2 compared ataxia induction by control 2, [redacted] and remimazolam. No ataxia occurred in the control group. In the [redacted] groups, a dose-dependent increase of animals with ataxia was observed, being 0/10, 3/10, and 9/10 animals in the 5, 10, and 20 mg/kg groups, respectively. The increase in the 20 mg/kg group was significant ($p<0.001$). In the remimazolam groups, a dose-dependent increase in ataxia was observed, with the number of animals with ataxia in the 0.2, 0.5, and 1 mg/kg groups being 0/10, 6/10, and 10/10 animals, respectively. Increases were significant at doses of 0.5 mg/kg ($p<0.01$) or 1 mg/kg ($p<0.001$). MEDs of CNS 7054, [redacted], and remimazolam were 100, 20, and 0.5 mg/kg, respectively. MEDs of remimazolam for ataxia in both experiments were 0.5 mg/kg regardless of using different vehicles.		
Conclusion: The sedative effect of CNS 7054, a major metabolite of remimazolam, was investigated. It induced ataxia dose-dependently but its sedative effect was significantly weaker and reached only 1/200 of the effect achieved with remimazolam based on MEDs. Therefore, rapid metabolisation of remimazolam to CNS 7054 results in its characteristic rapid offset of the sedative effect. Investigation of [redacted] remimazolam ([redacted]) and its metabolite CNS 7054 ([redacted]) revealed no sedative activity at all for the latter ([redacted]) and a weak sedative effect of [redacted] reaching 1/40 of the effect of remimazolam. Therefore, it was concluded that [redacted] are unlikely to have an effect on the sedative action of remimazolam.		

2.6.3.2 効力を裏付ける試験（続き）

Table 2.6.3.2-22	Primary Pharmacodynamics	Test Article: Remimazolam
試験表題：ONO-IN-251 のミニブタ鎮静作用及び血中動態の検討（急速単回投与、持続投与）		
Route of Administration: i.v.	Location in CTD: 4.2.1.1.21	Study Number: [redacted]
GLP Compliance: non GLP		
Aim of Study: To assess the sedative effect of remimazolam in micropigs by i.v. bolus or infusion and collect plasma samples for plasma pharmacokinetics of remimazolam. Study Design: Micropigs received bolus administrations (100, 300, 1000 μg/kg) or infusion (0, 30, 100 μg/kg/min) of remimazolam and the sedative effects were evaluated before and up to 240 min after administration using sedation scoring systems. During the observation period, arterial and venous blood samples were collected at 13 and 11 times, respectively. Animals were defined as recovered as soon as a score of 0 was reached. Continuous infusions were given for 240 min with concurrent and subsequent assessment of sedation for further 240 min. During the entire observation period of 480 min, arterial and venous blood was collected 18 and 16 times, respectively.		
Species (Gender, Strain, Supplier, n/Group): Micropig (male, NIBS, obtained from Nisseiken Co., n = 4) Age/Body Weight: 5 months/ 14.3-15.7 kg on the day of receipt. Conditioning and Surgical Pre-treatment: Quarantine for 7 days was applied followed by acclimation for 11 days. The animals were housed individually in cages with free access to water and fed 400 g of solid diet per day. Catheters were implanted into the superior vena cava for administration and the femoral vein or artery for blood collection.		
Test Compounds (Batch No) and Test Conditions (Formulation, Doses, Route of Administrations):		
Remimazolam	(ONO-IN-251, lot no. SOL12621/6, [redacted]), diluted with saline Bolus administration: 100, 300, 1000 μg/kg, i.v. via catheter Infusion: 10, 30, 100 μg/kg/min, i.v. via catheter	
Control	Vehicle (saline)	
Method of Evaluation: Sedation levels were evaluated by sedation scoring system before and at various time points up to 240 min after administration. The mean effect score (sedation score) was calculated as the sum of the effect score for general sedation (muscle tone, explorative behavior, responsiveness to background stimuli and gait/posture, score: 0-4 for each symptom), ptosis (score: 0-4) and pinna reflex (score: 0-8), devided by three. Arterial and venous blood samples were collected before and at various time points up to 240 min after i.v. bolus or infusion. Statistical Analysis: No statistical analysis was performed.		
Results: Remimazolam at 100, 300, and 1000 μg/kg via bolus administration caused dose-dependent sedation, which was assessed using the scoring system mentioned above. Marked sedative effects indicated as 3 or higher scores for general sedation and ptosis were noted at 1000 μg/kg. Sedation lasted for 15 min after administration, and the scores returned to normal by 60 min after administration. Pinna reflex did not disappear even at 1000 μg/kg. Remimazolam at 10, 30, and 100 μg/kg/min by continuous administration caused dose-dependent sedation assessed by the same scoring system as above. Marked and stable sedative effects indicated as 3 or higher scores for general sedation and ptosis were noted at 100 μg/kg/min from 5 min after the start of infusion to the end of infusion. Sedation lasted 25 min after the end of infusion, and the scores returned to normal by 105 min after the end of infusion. Pinna reflex was not affected even at 100 μg/kg/min.		
Conclusion: Remimazolam exhibits dose-dependent sedative effects when administered either by bolus or by 240-minute continuous administration in micropigs. Furthermore, the sedative effects of remimazolam do not accumulate, and recovery from sedation by remimazolam is not delayed even after 240-minute continuous administration.		

2.6.3.2 効力を裏付ける試験（続き）

Table 2.6.3.2-23	Primary Pharmacodynamics	Test Article: Remimazolam
Report Title: The sedative effect of intravenous administration of CNS 7056X and midazolam in the Yucatan micropig		
Route of Administration: i.v.	Location in CTD: 4.2.1.1.22	Study Number: [redacted]
GLP Compliance: non GLP		
Aim of Study: To evaluate the sedative effects of remimazolam and midazolam in the Yucatan micropig Study Design: In the first set of experiments, micropigs received i.v. bolus injections of either remimazolam or midazolam (0.05-3 mg/kg) into the femoral vein and were observed for behavioral changes. In a second set of experiments, continuous infusions were conducted with remimazolam (26.4 µg/kg/min) and midazolam (30 or 45 µg/kg/min) until a constant and equivalent deep level of sedation was observed. Infusion was discontinued as soon as the animal reached maximal sedation and time to full recovery was determined. The behavioral changes were assessed during the experiments using a scoring system (see below). Heart rate and blood pressure were monitored in one experiment.		
Species (Gender, Strain, Supplier, n/Group): Micropig (female, Yucatan, n = 3) Age/Body Weight: 31-51 kg Conditioning and Surgical Pre-treatment: The right femoral artery and vein were cannulated. The access ports were exposed on the back of the pigs. The animals were trained for a sling and for handling.		
Test Compounds (Batch No) and Test Conditions (Formulation, Doses, Route of Administrations):		
Remimazolam	CNS 7056X (GW502056) plus NaCl (sufficient to provide a 0.9% solution) were dissolved in 0.1 mol/L HCl and 0.1 mol/L NaOH (2.03:1, volume:volume) to provide a solution of 10 mg/mL Bolus administration: 0.05-3 mg/kg, i.v. via femoral catheter Infusion: 26.4 µg/kg/min, i.v. via femoral catheter	
Midazolam	(Versed®, 5 mg/mL) Bolus administration: 0.05-3 mg/kg, i.v. via femoral catheter Infusion: 30 or 45 µg/kg/min, i.v. via femoral catheter	
Method of Evaluation: Sedation levels were evaluated using sedation scoring system at various time points. The mean effect score was calculated as the sum of the effect score for general sedation (gait/posture, muscle tone, responsiveness to background stimuli and food, explorative behavior, score: 0-4 for each symptom), ptosis (score: 0-4) and responsiveness to ear blast (score: 0-8), divided by three. The maximum possible score was 5.3. Statistical Analysis: No statistical analysis was performed.		
Results: Intravenous injection of both midazolam and remimazolam had sedative effects in the Yucatan micropig after bolus dosing. Onset of sedation was fast for both drugs. The depth of sedation increased dose-dependently for both compounds. With continuous infusion, a constant and equivalent deep level of sedation (score 5.3) was observed by infusion of remimazolam (26.4 µg/kg/min for 14 min) or midazolam (45 µg/kg/min for 19 min). Recovery from sedation induced by remimazolam was seen within 30 min. Recovery from sedation by midazolam was seen after approximately 90 min. The effects of remimazolam (26.4 µg/kg/min) and midazolam (30 µg/kg/min) on heart rate and blood pressure were also monitored. Remimazolam caused a similar reduction in heart rate and blood pressure to that seen with midazolam. Both drugs showed a similar recovery from reduced blood pressure while recovery from reduced heart rate appeared more rapid for remimazolam.		
Conclusion: Remimazolam more potently and rapidly induced sedation in micropigs than midazolam and exhibited no major effect on heart rate and blood pressure. Recovery from sedation induced by remimazolam was markedly shorter than seen after intravenous administration of midazolam.		

2.6.3.2 効力を裏付ける試験（続き）

Table 2.6.3.2-24	Primary Pharmacodynamics	Test Article: Remimazolam
試験表題：ミニブタにおける ONO-IN-251 の導入及び維持投与での鎮静作用		
Route of Administration: i.v.	Location in CTD: 4.2.1.1.23	Study Number: [redacted]
GLP Compliance: non GLP		
Aim of Study: To investigate the dose levels of remimazolam to induce and maintain sedation (general sedation score of 4) and collect blood samples to determine the effective plasma concentration. Study Design: Remimazolam (bolus, 0.6 mg/kg/min) was administered intravenously for induction of sedation until general sedation score of 4 was achieved. The bolus injection was then stopped and infusion of remidazolam was started at the highest dose for 30 min (4.0 mg/kg/h) for maintenance of sedation. Subsequently, the doses of infusion (2.5, 1.6, 1.0, 0.6, 0.4 mg/kg/h) were reduced in stages every 30 min until the general sedation score was reached 3. The maintenance dose to maintain general sedation score of 4 for 30 min was determined.		
Species (Gender, Strain, Supplier, n/Group): Micropig (male, NIBS, Nisseiken Co. Ltd., n = 6 per group) Age/Body Weight: 5-6 months, 17.0-19.6 kg Conditioning and Surgical Pre-treatment: Quarantine for 7 days were applied followed by acclimation for 4 days. The animals were housed individually in cages with free access to water and fed 400 g of solid diet per day. Catheters were implanted into the superior vena cava for administration and the right femoral artery for blood collection.		
Test Compounds (Batch No) and Test Conditions (Formulation, Doses, Route of Administrations):		
Remimazolam	ONO-IN-251BS (lot no. SOL12621/6), dissolved in physiological saline. Doses were expressed as the free base. induction dose: 0.6 mg/kg/min, until general sedation score 4 was reached, i.v. bolus via catheter maintenance dose: 4.0, 2.5, 1.6, 1.0, 0.6, 0.4 mg/kg/h (decreasing at 30 min intervals), i.v. infusion via catheter	
Control(s)	Vehicle (saline solution, lot no. 9K81N, Otsuka Pharmaceutical Co., Ltd.)	
Method of Evaluation: Sedation levels were evaluated using sedation scoring system at before/after induction of sedation and every 15 min during infusion for maintenance. Scores for general sedation (muscle tone, explorative behavior, responsiveness to background stimuli and gait/posturee: 0-4 for each symptom) and ptosis (score: 0-4) were recorded. General sedation score was defined as the highest score in each of symptoms. The sedation induction dose was calculated from the duration of initial dosing (0.6 mg/kg/min) until general sedation score 4 was reached. The maintenance dose was defined as the final dose of infusion to maintain general sedation score of 4 for 30 min. Recovery from sedation was defined as the decrease of thegeneral sedation score from 4 to 3 after the end of infusion. Arterial blood samples were collected at various time points after induction of sedation until animals were recovered from sedation. Data were presented as mean values with standard deviation Statistical Analysis: No statistical analysis was performed.		
Results: The mean doses of remimazolam required for induction and for maintenance of sedation at general sedation score 4 were 1.1±0.14 mg/kg and 1.4±0.38 mg/kg/h, respectively. The mean recovery time lasted 15.7±6.98 min. Mean plasma concentrations for induction and maintenance were 1670±328 ng/mL and 542±243 ng/mL, respectively. For recovery, plasma concentrations decreased to a mean 318±183 ng/mL.		
Conclusion: Constant and deep sedation of micropigs at a general sedation level of 4 can be achieved with an initial and subsequent continuous infusion of remimazolam. The mean doses (minimum dose-maximum dose) required to induce and maintain sedation were 1.1 (0.6-1.4) mg/kg and 1.4 (0.6-2.5) mg/kg/h, respectively.		

2.6.3.2 効力を裏付ける試験（続き）

Table 2.6.3.2-25	Primary Pharmacodynamics	Test Article: Remimazolam
試験表題：ミニブタにおける ONO-IN-251BS の長期持続投与での鎮静作用を評価するための条件設定		
Route of Administration: i.v.	Location in CTD: 4.2.1.1.24	Study Number: [redacted]
GLP Compliance: non GLP		
Aim of Study: Preliminary study to optimize study condition utilizing midazolam in order to evaluate sedative effects of long-term sedation by remimazolam. Study Design: The sedation maintenance dose leading to a mild sedation (general sedation score 2 of possible 0 to 4) by midazolam and remimazolam was to be determined. General sedation and respiratory functions (pO_2 and pCO_2) were observed during the 28-day sedation. During sedation, animals were fed via high-calorie infusion into the double lumen catheter or by enteral nutrition via a gastric tube.		
Species (Gender, Strain, Supplier, n/Group): Micropig (male, NIBS, Nisseiken Co. Ltd., n = 2-3) Age/Body Weight: 6 to 15 months old, 21.3-22.5 kg at time of delivery Conditioning and Surgical Pre-treatment: Animals were acclimated and housed thereafter individually in cages (630 × 1130 × 710 mm) with free access to water and 400 g solid feed in the morning. For infusions, a double lumen catheter was inserted in thesuperior vena cava. For blood collections, a catheter was inserted into a branch of the femoral artery and located at the abdominal artery. For enteral nutrition, a gastric tube was inserted from the greater curvature of the stomach.		
Test Compounds (Batch No) and Test Conditions (Formulation, Doses, Route of Administrations):		
Remimazolam	(ONO-IN-251BS, lot no. SOL12621/6, [redacted]), diluted with physiological saline induction dose: 0.3 mg/kg/min, until general sedation score 3 was reached i.v. bolus via catheter maintenance dose: 0.6, 0.75, 0.9, 1.15, 1.40 mg/kg/h (varying to maintain general sedation score at 2), i.v. infusion via catheter	
Midazolam	(Dormicum® Injection 10 mg, lot no. C004A04, 1A600, C015A01, [redacted].), diluted with physiological saline induction dose: 0.03 mg/kg repeatedly over 1 min every 5 min until general sedation score 3 was reached, i.v. bolus via catheter maintenance doses: 0.10, 0.12, 0.15 mg/kg/h (varying to maintain general sedation score at 2), i.v. infusion via catheter	
Vehicle control	Physiological saline (Lot no. 1B79N, [redacted].), 0.25 mL/kg/h, i.v. via catheter	
Method of Evaluation: General sedation was assessed using general sedation score from 0 to 4 that accounted for muscle tone, explorative behavior, response to background stimulus and gait/body position. Assessment took place at short intervals (30 to 60 min) on day1 after start of infusion and, thereafter, 4 times daily. Blood collection was performed for arterial blood gas analysis before infusion, 8 hours after onset of infusion on day1 and, thereafter, once every 7 days. Respiratory functions (pO_2 and pCO_2) were evaluated using a blood analysis system (Roche OPTI CCA; Roche). Statistical Analysis: No statistical analysis was performed.		
Results: Sedation maintenance dose for midazolam was 0.12 mg/kg/h at day 1, raised to 0.24-0.36 mg/kg/h on day 28. The way of nourishment had to be changed from high-calorie infusion to enteral nutrition because both control and midazolam groups showed abnormalities in arterial partial pressure of gases and some animals exhibited hematochezia with high-calorie parental nutrition With enteral nutrition, no abnormalities or sedative effects were observed during 28 days in the control group. Subsequently, maintenance doses were determined for midazolam and remimazolam and tested during a 7-day infusion in 2 animals. Both animals receiving midazolam showed general sedation score 2 with maintenance doses of 0.12 mg/kg/h at day 1 to 0.15 mg/kg/h at day 7. With remimazolam, sedation was kept steady at score 2 with 0.60 mg/kg/h at day 1 up to 1.40 and 1.15 mg/kg/h at day 7. In order to get a clear start of sedation, induction doses were set at 0.3 mg/kg/min and 0.03 mg/kg for remimazolam and midazolam, respectively.		
Conclusion: Based on the preliminary studies, in the main study ([redacted]), mild sedation will be obtained using 0.3 mg/kg/min remimazolam for induction and 0.60 mg/kg/h for maintenance during 28 days with enternal nutrition. Midazolam will be used at 0.03 mg/kg for induction and at 0.10 mg/kg/h for sedation maintenance. The number of animals will be raised to 6.		

2.6.3.2 効力を裏付ける試験（続き）

Table 2.6.3.2-26	Primary Pharmacodynamics	Test Article: Remimazolam
試験表題：ミニブタにおける ONO-IN-251BS の長期持続投与での鎮静作用		
Route of Administration: i.v.	Location in CTD: 4.2.1.1.25	Study Number: [redacted]
GLP Compliance: non GLP		
Aim of Study: Sedative effect of remimazolam in long-term continuous administration to evaluate and compare with midazolam to evaluate the daily dose needed to maintain the mild level of sedation. Study Design: Sedation by remimazolam and midazolam was induced with a dose leading to a general sedation score of 3 of possible 0 to 4 and subsequently maintained on a mild sedation level of 2 during 28 days. General sedation and respiratory functions (pO_2 and pCO_2) were observed during the 28-day sedation. During the experiment, animals were fed by enteral nutrition via a gastric tube.		
Species (Gender, Strain, Supplier, n/Group): Micropigs (male, NIBS, Nisseiken Co., Ltd. n = 6 per group) Age/Body Weight: 5-6 months / 14.1 to 16.1 kg at the time of receipt Conditioning and Surgical Pre-treatment: Seven days of quarantine were followed by 11 days of acclimation. The animals were housed individually in cages (630 × 1130 × 710 mm) with free access to water and 400 g solid feed per day. For drug application a catheter was inserted into the superior vena cava and sutured to the neck. A catheter for blood collection was inserted into a branch of the right femoral artery until the tip reached the abdominal artery. A gastric tube was inserted from the greater curvature of the stomach. Purse-string suture was performed to fix the stomach and the catheter; then, the catheter was exposed from the subcutaneous layer of the stomach, and the skin was sutured.		
Test Compounds (Batch No) and Test Conditions (Formulation, Doses, Route of Administrations):		
Remimazolam	(ONO-IN-251BS, lot no. 27000984, [redacted]), diluted with physiological saline induction dose: 0.3 mg/kg/min, until general sedation score 3 was reached, i.v. bolus via catheter maintenance dose: 0.6 mg/kg/h (varying to maintain general sedation score of 2 in the range between 0.36 and 1.80 mg/kg/h), i.v. infusion via catheter	
Midazolam	(Dormicum® Injection 10 mg, lot no. E007A01, E008A04, E010A01, [redacted].), diluted with physiological saline induction dose: 0.03 mg/kg repeatedly over 1 min until general sedation score 3 was reached, i.v. bolus via catheter maintenance doses: 0.12 mg/kg/h (varying to maintain general sedation score at 2 in the range between 0.12 and 0.45 mg/kg/h), i.v. infusion via catheter	
Vehicle control	Physiological saline (Lot no. 0K79N, [redacted].), i.v. via catheter	
Method of Evaluation: General sedation was assessed using a general sedation score from 0 to 4 that accounted for muscle tone, explorative behavior, response to background stimulus and gait/body position. Assessment took place at short intervals (30 to 60 min) until sedation level 2 was reached on day1 and, thereafter, 4 times daily on day 2-28. Weight was measured on the day before infusion and once daily during infusion. The time until recovery from sedation (score 0) was measured after the completion of the 28-day infusion. Blood collection for arterial blood gas analysis was performed before infusion and on days 7, 14, 21 and 28. Respiratory functions (pO_2 and pCO_2) were evaluated using a blood analysis system (Roche OPTI CCA; Roche). Plasma concentration of remimazolam was determined on days 1, 7, 14, 21, and 28. Statistical Analysis: No statistical analysis was performed		

2.6.3.2 効力を裏付ける試験（続き）

Table 2.6.3.2-26 (cont.)	Primary Pharmacodynamics	Test Article: Remimazolam
試験表題：ミニブタにおける ONO-IN-251BS の長期持続投与での鎮静作用		
Route of Administration: i.v.	Location in CTD: 4.2.1.1.25	Study Number: [redacted]
GLP Compliance: non GLP		

Results:

The doses of remimazolam and of midazolam had to be increased from day 1 to day 28 to maintain a sedation level with general sedation score of 2 as shown in the table below.

Days	Remimazolam Dose: mg/kg/h (mean±SEM)	Remimazolam Plasma concentration (ng-mL±SD)	Midazolam Dose: mg/kg/h (mean±SEM)
Day 1	0.56±0.04	142±17	0.13±0.00
Day 7	0.68±0.06	129 ±70	0.16±0.01
Day 14	0.83±0.12	198±100	0.25±0.02
Day 21	0.96±0.17	-	0.31±0.02
Day 28	1.16±0.24	205±150	0.39±0.03

SE: standard error of the mean, SD: standard deviation

Sedation recovery time for remimazolam, shown as mean±SEM (min-max), was 66.7±5.58 (50-90) min, which was shorter than that of midazolam, at 110.0±8.16 (90-140) min.

Conclusion:

Remimazolam was administered to micropigs for 28 days as continuous intravenous administration to evaluate the daily dose needed to maintain a mild level of sedation equivalent to that obtained during artificial ventilation in intensive treatment. As a result, this maintenance dose of remimazolam increased with long-term administration, as seen for midazolam. However, the degree of increase by remimazolam was equivalent or less than that by midazolam.

2.6.3.3 副次的薬理試験

Table 2.6.3.3-01	Secondary Pharmacodynamics	Test Article: Remimazolam
試験表題：ラットにおける ONO-2745BS の健忘作用の検討		
Route of Administration: i.v.	Location in CTD: 4.2.1.2.1	Study Number: [redacted]
GLP Compliance: non GLP		
Aim of Study: The amnestic effect was investigated using light latency in the passive avoidance study as an index. Study Design: The amnestic effect was evaluaed usingstep-through passive avoidance task. The chamber was devided into a light compartment and a dark compartment, with a gate between the two. Rats were placed in the light compartment and electric shocks were given through the grid of the floor when the rats entered the dark compartment immediately after drug administration (acquisition trial). In retention trial performed 24 hours later, rats were again placed in the light chamber after drug treatment and the light latency, the time until rats entered the dark compartment, was measured again.		
Species (Gender, Strain, Supplier, n/Group): Rats (male, Crl:CD (SD), Charles River Laboratories Japan, Inc), 10/group (remimazolam, propofol) and 5/group (midazolam) Age/Body Weight: 6-8 weeks Conditioning and Surgical Pre-treatment: Animals were acclimated in econ cages (4 to 5 animals/cage) with bedding (Paper Clean; Japan SLC, Inc.) for 7 or more days. CRF-1 pelletized diet and tap water were supplied ad libitum.		
Test Compounds (Batch No) and Test Conditions (Formulation, Doses, Route of Administrations):		
Remimazolam	ONO-2745BS, Lot No:SOL12621/6, dissolved in physiological saline, 0.2, 0.5 and 1.0 mg/kg i.v. in a dose volume of 10 mL/kg immediately before acquisition trial.	
Midazolam	Dormicum® Injection 10 mg, [redacted], Batch No: no data, to confirm the validity of the system, diluted with physiological saline to the concentration corresponding to the dose, 0.02, 0.2 and 2 mg/kg i.v. in a dose volume of 10 mL/kg immediately before acquisition trial.	
Propofol	1% Diprivan® Injection, [redacted], Batch No: no data, given as reference drug, diluted with physiological saline to the concentration corresponding to the dose, 1, 2 and 5 mg/kg i.v. in a dose volume of 10 mL/kg immediately before acquisition trial.	
Control(s)	Physiological saline solution, given in a dose volume of 10 mL/kg.	
Method of Evaluation: The light latency was measured as an index of amnestic effect of drugs. f the memory of electric shocks was lost due to the amnestic effect of the drug, the light latency become shorter, compared with the control group. In addition, the dose at which light latency is shortened by 50% (ED_{50} value), and dose ratio (times) of ED_{50} values of sedative effect and amnestic effect were calculated. ED_{50} value of sedative effect was quoted from the in-house document. Data were presented as means and standard errors (Microsoft Office Excel 2007 SP1, Microsoft Corporation). Statistical Analysis: Significance test and linear regression analysis (SAS 9.2 TS2M3, SAS Institute Japan Inc. and its cooperative system, EXSUS Ver.7.7.1, CAC EXICARE Corporation), comparison between the control group and the test substance group (two-tailed Steel's multiple comparison test, with significance level of 5%), ED_{50} values of amnestic effect (linear regression analysis using the percentage of shortening at each dose)		
Results: In the remimazolam group, the light latency was shortened in a dose-dependent manner; significant differences were observed at doses of 0.5 and 1.0 mg/kg compared with the control group. In the propofol group, the light latency was shortened in a dose-dependent manner; significant differences were observed at doses of 2 and 5 mg/kg compared with the control group. ED_{50} values of amnestic effect of remimazolam and propofol were 0.68 mg/kg and 2.5 mg/kg, respectively. The ratio of ED_{50} of the sedative effect and ED_{50} of the amnestic effect for remimazolam and propofol were 5.7- and 1.8-fold, respectively based on in-house date of the sedative effect (ED_{50}: 3.9 mg/kg for remimazolam and 4.4 mg/kg for propofol).		
Conclusion: For remimazolam, while sedative effect is observed with a similar dose level as propofol, according to the previous investigations, an amnestic effect was observed with a lower dose than propofol.		

2.6.3.4 安全性薬理試験

2.6.3.4.1 安全性薬理試験一覧表

Organ Systems Evaluated	Species/ Strain	Method of Admin.	Doses	Gender and No. per Group	Noteworthy Findings	GLP Compliance	Study Number	Location in CTD
CNS	Rat	intravenous (i.v.)	0, 5, 10,20, 30 mg/kg	6 males (M)	Remimazolam: sedative effects (≥10 mg/kg) as dispersion in cage, decreased locomotor activity, alertness, startle response, grip strength and body tone from 5 min post-dose. were rapidly diminished within 45 min post-dose. Decrease in body temperature (10 and 30 mg/kg) at 45 min post-dose.	Yes	[redacted]	4.2.1.3.1
Heart (hERG encoded channel from stably transfected HEK293 cells)	Human	*in vitro*	0.03, 0.3, 3 µmol/L	3	Remimazolam: no inhibition of human ether-a-go-go-related gene (hERG) tail current up to 3 µmol/L. CNS 7054: insufficient inhibition at concentrations up to 0.3 µmol/L.	No.	[redacted]	4.2.1.3.2
Heart (hERG encoded channel from stably transfected HEK293 cells)	Human	*in vitro*	0.03, 0.3, 3 µmol/L	3	CNS 7054: no inhibition of hERG tail current up to 3 µmol/L.	No.	[redacted]	4.2.1.3.3
Heart (hERG encoded channel from stably transfected HEK293 cells)	Human	*in vitro*	3 µmol/L	3	Remimazolam and CNS 7054 blocked hERG tail current with 24.3% and 18.8%, respectively.	No.	[redacted]	4.2.1.3.4
Heart (hERG encoded channel from stably transfected HEK293 cells)	Human	*in vitro*	0, 10, 30, 100, 300 µmol/L 10, 30, 100 µmol/L in case of CNS 7054	4	Remimazolam inhibited hERG tail current concentration-dependently [estimated 25% inhibitory concentration (IC_{25}) and 50% inhibitory concentration (IC_{50}) values: 62 and 207 µmol/L; concentrations are much higher than plasma levels reached in any pharmacodynamic or toxicological study]. CNS 7054: no inhibition up to 100 µmol/L.	Yes	[redacted]	4.2.1.3.5

2.6.3.4.1 安全性薬理試験一覧表（続き）

Organ Systems Evaluated	Species/ Strain	Method of Admin.	Doses	Gender and No. per Group	Noteworthy Findings	GLP Compliance	Study Number	Location in CTD
Heart (papillary muscle)	Guinea pig	*in vitro*	0, 10, 30, 100, 300 µmol/L	5	At 100 and 300 µmol/L, the 30% and 50% action potential duration (APD_{30} and APD_{50}) decreased and at 300 µmol/L the resting membrane potential (RMP) increased and the 90% action potential duration (APD_{90}) was prolonged. Thus, the no observed effect level (NOEL) for inhibition of the inwardly and the delayed rectifying potassium channels was 100 µmol/L and the NOEL for inhibition of calcium channels was 30 µmol/L.	Yes	[redacted]	4.2.1.3.6
Heart (papillary muscle)	Guinea pig	*in vitro*	0, 10, 30, 100, 300 µmol/L	5	CNS 7054: no effect on myocardial action potential (≤100 µmol/L). Rise in RMP and decrease of APD_{30} and APD_{50} at 300 µmol/L. Thus, the NOELs for inhibition of the inwardly and the delayed rectifying potassium channels was 300 µmol/L and the NOEL for inhibition of calcium channels was 100 µmol/L.	Yes	[redacted]	4.2.1.3.7
Blood (hematic functions)	Rat	*in vitro*	0, 33, 100, 333 µg/mL(platelet aggregation) 0, 100, 300, 1000 µg/mL (coagulation)	3	No effect on platelet aggregation (maximum aggregation rate, adenosine 5'-diphosphate sodium salt (ADP) or collagen-induced platelet aggregation), coagulation [prothrombin time (PT) or activated partial thromboplastin time (APTT)]	No	[redacted]	4.2.1.3.8
Blood (hematic functions)	Human	*in vitro*	0, 3, 10, 30, 100, 300 µmol/L	5	No effect on platelet aggregation (maximum aggregation rate, ADP- or collagen-induced platelet aggregation), coagulation (PT or APTT) or fibrinolysis (≥3 µmol/L).	Yes	[redacted]	4.2.1.3.9

2.6.3.4.1 安全性薬理試験一覧表（続き）

Organ Systems Evaluated	Species/ Strain	Method of Admin.	Doses	Gender and No. per Group	Noteworthy Findings	GLP Compliance	Study Number	Location in CTD
Blood (hematic functions)	Human	*in vitro*	0, 3, 10, 30, 100, 300 µmol/L	5	CNS 7054: no effect on platelet aggregation (maximum aggregation rate, ADP- or collagen-induced platelet aggregation), coagulation (PT or APTT) or fibrinolysis (≥3 µmol/L).	Yes	[redacted]	4.2.1.3.10
Cardiovascular system	Rat	i.v.	0, 20, 30 mg/kg	5 M	Sedation, lasting for ≤10 min post-dose. Slight transient increase of blood pressure and heart rate, which were estimated as being not relevant with respect to the basic hemodynamic endpoints. Thus, the NOEL was at least 30 mg/kg.	No	[redacted]	4.2.1.3.11
Blood	Rabbit	i.v. injection i.v. infusion	0, 2.5, 5, 7.5 mg/kg 0, 7.5 mg/kg	4 females (F) 4 F	No signs of local intolerance, no variations of heart rate and no changes of oxygen saturation (except for 4% reduction of oxygen saturation 3 min post infusion). Systemic intolerance signs as sedation and abdominal position in all animals after injection (≥2.5 mg/kg) and after infusion (7.5 mg/kg). A reduced respiratory rate after 5.0 and 7.5 mg/kg in all animals. All clinical symptoms, also the nystagmus and mydriasis after i.v. infusion had subsided ≤4 h post-dose.	No	[redacted]	4.2.1.3.12

2.6.3.4.1 安全性薬理試験一覧表（続き）

Organ Systems Evaluated	Species/ Strain	Method of Admin.	Doses	Gender and No. per Group	Noteworthy Findings	GLP Compliance	Study Number	Location in CTD
Cardiovascular & respiratory	Micropig	i.v. infusion	12.5, 25.0, 50.0, 100.0 μg/kg/min	4 M	Sedation (≥12.5 μg/kg/min) and increase of mean blood pressure (50 μg/kg/min). Increase of heart rate and tended to shorten PR interval during and after administration (12.5-50 μg/kg/min). No abnormal changes in the electrocardiogram (ECG), also, neither changes in arterial blood gas nor any adverse effects on the respiratory system (12.5-100 μg/kg/min). Decreased blood pressure and heart rate (100 mg/kg/min).	No	[redacted]	4.2.1.3.13
Cardiovascular & respiratory	Cynomolgus monkey	i.v. injection i.v. infusion	0, 0.5, 1, 2, 5 mg/kg 0, 6, 18, 30, 60 mg/kg	5 M 5 M	After injection: transient and slight decrease in blood pressure and decrease in intra-abdominal body temperature (≥2 mg/kg); no effects on heart rate, any ECG parameter including QT interval and QTc or respiratory rate (≥0.5 mg/kg). After infusion: a decrease in intra-abdominal body temperature and prolongation of QTc based on it (≥18 mg/kg). No effects on blood pressure, heart rate, any ECG parameter or respiratory rate (≥6 mg/kg).	Yes	[redacted]	4.2.1.3.14
Respiratory	Cynomolgus monkey	i.v. injection i.v. infusion	0, 0.5, 1, 2, 5 mg/kg 0, 6, 18, 30, 60 mg/kg/6h	4 M 4 M	After injection: slight increase in arterial carbon dioxide tension (5 mg/kg). No effect on arterial blood pH, arterial oxygen tension, or hemoglobin oxide saturation (≥0.5 mg/kg). After infusion: slight increase in arterial carbon dioxide tension (≥18 mg/kg/6h). No effect on arterial blood pH, arterial oxygen tension, or hemoglobin oxygen saturation (≥6 mg/kg/6h).	Yes	[redacted]	4.2.1.3.15

2.6.3.4.2 安全性薬理試験

Table 2.6.3.4-01	Safety Pharmacology	Test Article: Remimazolam
Report Title: Effects of CNS7056X.besylate salt (CNS 7056B) in the Irwin Test in Rats		
Route of Administration: i.v.	Location in CTD: 4.2.1.3.1	Study Number: [redacted]
GLP Compliance: GLP complaint		
Aim of Study: To assess the effects of remimazolam in a 'Primary Observation Test' designed to detect effects on the gross behavioural and physiological state of rats. Study Design: In a blinded study the parameters outlined in the Irwin Test were systematically evaluated for each rat 5, 10, 20 and 45 min post-dose. Following the final observation, the core temperature of each rat was measured from the rectum by means of a thermocouple connected to a digital thermometer. On conclusion of the testing period all of the animals were terminated by cervical dislocation.		
Species (Gender, Strain, Supplier, n/Group): Rats (male, Sprague-Dawley, Harlan UK Ltd.), 6/group, 5 treatment groups Age/Body Weight: 9-10 weeks/ 220-252 g Conditioning and Surgical Pre-treatment: Animals were acclimatized in solid-bottom cages with bedding (sawdust) for 9 days until use. An expanded rodent diet and mains tap water were supplied ad libitum. Animals were weighed prior to testing, and body weights recorded, on the same day as the administration of substances.		
Test Compounds (Batch No) and Test Conditions (Formulation, Doses, Route of Administrations):		
Remimazolam	Batch No:SOL12621/5, 10, 20 and 30 mg/kg (free-base), given i.v. via tail vein in a dose volume of 3 mL/kg 5 min before the first observation.	
Chlorpromazin HCL	Batch No: 016K1127, used as reference substance in a dose of 3 mg/kg, given i.v. in a dose volume of 3 mL/kg 5 min before the first observation.	
Control	Vehicle: 25.86% w/v Captisol® (Batch No: NC-04A-05009) in pH 3.8, 25 mM citrate buffer solution, 3 mL/kg; i.v.	
Method of Evaluation: The observation parameters were scored on an arbitrary scale from 0 to 8 (factors already present in the animal were scored as '4', with potentiation or depression being scored as higher or lower, respectively. Exception are lethality ('0' – alive or '1' – dead). Only changes from normal were noted; all other cells in the table were left blank. If there were no changes from normal, the NAD (No Abnormalities Detected) box was checked. Statistical Analysis: The choice of parametric or non-parametric test was based on whether the groups to be compared satisfied the homogeneity of variance criterion (evaluated by the Levene Mean test or F-test). The test substance body temperature data satisfied the criterion and were therefore compared to vehicle group data using analysis of variance (ANOVA) and Dunnett's test. Reference substance body temperature data failed to satisfy the homogeneity of variance criteria and were compared to vehicle group data using Mann-Whitney U-test. Summarised data are presented as mean±s.e. mean for the treatment groups. Statistical significance was assumed when $P<0.05$. As the data generated during the Irwin test were only partly quantitative, no statistical analysis was performed.		

2.6.3.4.2 安全性薬理試験（続き）

Table 2.6.3.4.2-01 (cont.)	Safety Pharmacology	Test Article: Remimazolam
Report Title: Effects of CNS7056X.besylate salt (CNS 7056B) in the Irwin Test in Rats		
Route of Administration: i.v.	Location in CTD: 4.2.1.3.1	Study Number: [redacted]
GLP Compliance: GLP complaint		
Results: Remimazolam caused rats to exhibit the following changes from normal after 10 mg/kg: dispersion in cage, catalepsy, passivity, paralysis, decrease of locomotor activity, alertness, startle response, touch response, fearfulness, grip strength and body tone at 5 min post-dose mostly in at least 2/6 animals. An increase of touch response was seen in 3/6 animals at 10 min post-dose and in 2/6 at 20 min post-dose. This dose caused a significant decrease in the mean body temperature of rats at 45 min post-dose when compared to vehicle group data. 20 mg/kg produced the following effects: dispersion in cage, passivity, lacrimation, decrease of locomotor activity, alertness, startle response, touch response, grip strength and body tone at 5 min post-dose mostly in at least 2/5 animals. A decrease of touch response was seen in 2/5 at 5 min post-dose and an increase of touch response in 2/5 animals at 10 min post-dose. In addition, animal 2 died during dose administration due to suffocation in the restraint tube; this is considered to be unrelated to the administration of test substance. The mean body temperature was not significantly affected. 30 mg/kg produced the following effects: dispersion in cage, catalepsy, loss of righting reflex, passivity, paralysis, lacrimation, decrease of locomotor activity, alertness, startle response, fearfulness, body tone and grip strength at 5 min post-dose mostly in at least 2/6 animals. A decrease of touch response was seen in 5/6 at 5 min post-dose, an increase of touch response in 2/6 animals at 10 min post-dose and in 3/6 at 20 min post-dose. This dose caused a significant decrease in the mean body temperature of rats at 45 min post-dose when compared to vehicle group data. Any other observations were limited to 1 out of 6 rats and were not considered to be test substance related. Chlorpromazin, 3 mg/kg i.v. produced observations and changes in body temperature that were consistent with chlorpromazine’s known pharmacological actions as a CNS depressant. The vehicle caused rats to exhibit the following changes from normal: increased locomotor activity (5 and 10 min post-dose), increased touch response (5/6 at 5 min post-dose and 3/6 at 10 min post-dose) and passivity (5/6 at all time points post-dose). Any other observations were limited to 1 out of 6 rats and were not considered to be vehicle related.		
Conclusion: Intravenous administration of remimazolam (10, 20 and 30 mg/kg) caused sedative effects 5 minutes post-dose, which rapidly diminished, so that most animals were back to normal by 45 minutes post-dose. At doses of 10 and 30 mg/kg, a decrease in body temperature was noted 45 minutes post-dose.		

2.6.3.4.2 安全性薬理試験（続き）

Table 2.6.3.4.2-02	Safety Pharmacology	Test Article: Remimazolam, CNS 7054
Report Title: Effect of CeNeS Limited Test Substances on HERG Tail Current Recorded from Stably Transfected HEK293 Cells		
Route of Administration: *in vitro*	Location in CTD: 4.2.1.3.2	Study Number: [redacted]
GLP Compliance: non GLP		
Aim of Study: To assess the effects of remimazolam and CNS 7054 on human ether-a-go-go-related gene (hERG)-encoded channel tail current recorded from stably transfected HEK293 cells. Study Design: Ion channel evaluated: hERG potassium channel current. Cell line used: HEK293 stable cell line. Voltage profile: Holding -80 mV, step -50 mV (200 msec), step +20 mV (4.8 sec), step -50 mV (5 sec), return to holding potential. Stimulation frequency: every 15 sec (0.067 Hz). Exposure time: 5 min/concentration. The following steps were carried out: whole cell – wash – vehicle – treatment 1 – treatment 2 – treatment 3 – wash. Vehicle group and positive control not required (see Appendix 1, p 11/48).		
Species (Gender, Strain, Supplier, n/Group): hERG transfected HEK293 cell line, n=3 cells/concentration Age/Body Weight: not applicable Conditioning and Surgical Pre-treatment: no data		
Test Compounds (Batch No) and Test Conditions (Formulation, Doses, Route of Administrations):		
Remimazolam	Compound A, Batch No: LJC-039-083-1, off-white solid (powder), formulated in water (pH 7.4) to produce stock concentrations of 0.1, 1 and 10 mmol/L (see Appendix 1, p 11/48), respectively. Stock solutions were diluted to achieve test concentrations of 0.03, 0.3 and 3 µmol/L (see report p. 2/48), respectively.	
CNS 7054	Compound B, Batch No: 1639-00090 (received 13.5.2005), yellow powder, formulated in water (pH 7.4) to produce stock concentrations of 0.1, 1 and 10 mmol/L (see Appendix 1, p 11/48), respectively. Stock solutions were diluted to achieve test concentrations of 0.003, 0.03 and 0.3 µmol/L (see report p. 2/48), respectively.	
E-4031	Compound C, Batch No: no data, formulated in water (pH 7.4) to produce stock concentrations of 0.001, 0.01 and 0.1 mmol/L (see Appendix 1, p 11/48), respectively. Stock solutions were diluted to achieve test concentrations of 0.003, 0.03 and 0.3 µmol/L.	
Midazolam	Hypnovel (liquid 10 mg/2 mL), Batch No: B1111, formulated in water (pH 7.4) to produce stock concentrations of 0.1, 1 and 10 mmol/L (see Appendix 1, p 11/48). Stock solutions were diluted to achieve test concentrations of 0.1, 1 and 10 µmol/L (see report p 2/48), respectively.	
Control	Vehicle: water (pH 7.4).	
Method of Evaluation: no data. Statistical Analysis: IC_{50} values were estimated.		
Results: Remimazolam: no inhibition of hERG tail current was observed. In the CNS 7054 treated cells, insufficient inhibition of hERG tail current observed to estimate IC_{50}. E-4031 inhibited hERG tail current with an IC_{50} of 16 nmol/L. Midazolam inhibited hERG tail current with an IC_{50} of 7.6 µmol/L.		
Conclusion: Remimazolam did not inhibit hERG tail current in concentrations up to 3 µmol/L. CNS 7054 produced insufficient inhibition of hERG tail current at concentrations up to 0.3 µmol/L.		

2.6.3.4.2 安全性薬理試験（続き）

Table 2.6.3.4.2-03	Safety Pharmacology	Test Article: CNS 7054
Report Title: Effect of CNS7054X on HERG tail current recorded from stably transfected HEK293 cells		
Route of Administration: *in vitro*	Location in CTD: 4.2.1.3.3	Study Number: [redacted]
GLP Compliance: non GLP		
Aim of Study: Due to ambiguous results obtained during an earlier examination of CNS 7054, this compound was re-tested in this supplementary experiment to assess the effects of CNS 7054 on human ether-a-go-go-related gene (hERG)-encoded channel tail current recorded from stably transfected HEK293 cells. Study Design: Ion channel evaluated: hERG potassium channel current. Cell line used: HEK293 stable cell line.		
Species (Gender, Strain, Supplier, n/Group): hERG transfected HEK293 cell line, n=3 cells/concentration Age/Body Weight: not applicable Conditioning and Surgical Pre-treatment: no data		
Test Compounds (Batch No) and Test Conditions (Formulation, Doses, Route of Administrations):		
CNS 7054	Batch No: 1639-00090, formulated in dimethyl sulfoxide (DMSO) to obtain stock solutions of 0.03, 0.3 and 3 mmol/L. These stock solutions were then diluted in bath solution to provide final perfusion concentrations of 0.03, 0.3 and 3 µmol/L CNS 7054 with a corresponding vehicle concentration of 0.1% DMSO.	
Control	Vehicle: DMSO, 0.1%	
Method of Evaluation: no data Statistical Analysis: not applicable.		
Results: After CNS 7054 no inhibition of hERG tail current was observed.		
Conclusion: CNS 7054 did not inhibit hERG tail current in concentrations up to 3 µmol/L.		

2.6.3.4.2 安全性薬理試験（続き）

Table 2.6.3.4.2-04	Safety Pharmacology	Test Article: Remimazolam, CNS 7054
Report Title: A study of the effects of CENES compounds A-D on hERG currents		
Route of Administration: *in vitro*	Location in CTD: 4.2.1.3.4	Study Number: [redacted]
GLP Compliance: non GLP		
Aim of Study: To assess the effects of 4 compounds on hERG currents recorded from stably expressed in HEK293 cells. Study Design: hERG currents were recorded using the whole-cell voltage-clamp technique using a Multiclamp 700B amplifier (Axon Instruments). Pulse protocol was continually repeated every 15 sec during the entire experiment. hERG currents were recorded in control conditions (black traces) and during the application of test compound.		
Species (Gender, Strain, Supplier, n/Group): hERG transfected HEK293 cell line, n=3 cells/concentration Age/Body Weight: not applicable Conditioning and Surgical Pre-treatment: Cells were maintained in cell media that contained 90% Iscove's Modified Dulbecco's Medium, 10% fetal bovine serum, 2 mmol/L L-Glutamine, 1 mmol/L Na-Pyruvate, penicillin G sodium 100 units/mL, streptomycin sulfate 100 µg/mL and geneticin 500 µg/mL. The flasks were incubated with VERSENE (EDTA) at 1:5000 for 5 min at 37°C to detach the cells from the flasks. Cells used were plated on glass coverslips 8-12 h prior to use. Borosilicate glass patch pipettes were filled with (mmol/L): KCl 140, $CaCl_2$ 1, EGTA 5, $MgCl_2$ 2, and HEPES 10 (pH 7.2) and were pulled to a tip resistance of 3 to 4 MΩ. External bath solution consisted of (mmol/L) NaCl 140, $CaCl_2$ 2, KCl 2.5, $MgCl_2$ 2, glucose 10, sucrose 23.5 and HEPES 10 (pH 7.4). Currents were stable for up to 30 min at room temperature.		
Test Compounds (Batch No) and Test Conditions (Formulation, Doses, Route of Administrations): All compounds were prepared as a 3 mmol/L stock solusion in DMSO and stored at -20°C. On the day of experiments, the stock solution was diluted 1:1000 in assay buffer to give a final concentration of 3 µmol/L.		
Remimazolam	compound D, Batch No: no data, test conditions: see above	
CNS 7054	compound C, Batch No: no data, test conditions: see above	
Cisapride	compound B, Batch No: no data, test conditions: see above	
E-4031	compound A, Batch No: no data, test conditions: see above	
Control	Vehicle: Dimethyl sulfoxide (DMSO); vehicle group not used.	
Method of Evaluation: Voltage clamp protocols were controlled using pClamp 8.2 (Axon Instruments) acquisition and analysis software. For block of hERG current mean values and SEM were calculated. Statistical Analysis: no statistical analysis was performed.		
Results: hERG tail currents were almost completely blocked by E-4031 and cisapride (99.7% and 98.8% respectively) and were blocked by approximately 25% by CNS 7054 and remimazolam (18.8% and 24.3% respectively), all at a concentration of 3 µmol/L.		
Conclusion: At a concentration of 3 µmol/L remimazolam blocked 24.3% and CNS 7054 blocked 18.8% of HERG tail current, whereas E-4031 and cisapride blocked almost 100% of hERG currents.		

2.6.3.4.2 安全性薬理試験（続き）

Table 2.6.3.4.2-05	Safety Pharmacology	Test Article: Remimazolam
Report Title: Effect of CNS 7056X.besylate salt and CNS 7054X on hERG Tail Current Recorded from Stably Transfected HEK293 Cells		
Route of Administration: *in vitro*	Location in CTD: 4.2.1.3.5	Study Number: [redacted]
GLP Compliance: GLP complaint		
Aim of Study: To assess the effects of remimazolam and CNS 7054 on human ether-a-go-go-related gene (hERG)-encoded channel tail current recorded from HEK293 cells stably transfected with hERG cDNA. Study Design: HEK293 cells stably transfected with hERG cDNA were used as test system. After the whole-cell recording configuration had been established, the voltage protocol was run a minimum of 10 times at 15 sec intervals to allow a stable recording to be achieved. The test substance was allowed to equilibrate for approximately 10 min or until steady-state tail current had been achieved. During the equilibration phase the voltage protocol was run and the resultant current recorded.		
Species (Gender, Strain, Supplier, n/Group): hERG.T.HEKB (HEK293 cells stably transfected with hERG cDNA), obtained from the University of Wisconsin, n = 4 cells/concentration of remimazolam, CNS 7054, n = 4 cells for DMSO, n = 4 cells for E-4031 (2 vehicle treated cells and 2 treated cells with 100 µmol/L CNS 7054). Age/Body Weight: not applicable Conditioning and Surgical Pre-treatment: Cells were continuously maintained in and passaged (n passage = 34 to 37) using minimum essential medium supplemented with 10% foetal bovine serum, 1% non-essential amino acids, 1% sodium pyruvate and 0.4 mg/mL geneticin. For electrophysiology studies, the cells were seeded onto sterile glass coverslips in 35 mm^2 dishes (containing 3 mL medium, without geneticin) at a density of 0.5×10^5 cells per dish and stored in a humidified, gassed (5% CO_2) incubator at 37°C.		
Test Compounds (Batch No) and Test Conditions (Formulation, Doses, Route of Administrations):		
Remimazolam	Batch No: SOL12621/5, formulated in DMSO at a concentration of 300 mmol/L and serially diluted in DMSO to produce further stock solutions of 100, 30 and 10 mmol/L. Aliquots of the stock solutions were diluted in bath solutions to achieve final perfusion solutions of 10, 30, 100 and 300 µmol/L, respectively maintaining a corresponding vehicle concentration of 0.1% DMSO.	
CNS 7054	Batch No: SOL13231/1, 10, 30 and 100 µmol/L, formulated in DMSO at a concentration of 100 mmol/L and serially diluted in DMSO to produce further stock solutions of 30 and 10 mmol/L. Aliquots of the stock solutions were diluted in bath solutions to achieve final perfusion solutions of 10, 30 and 100 µmol/L, respectively maintaining a corresponding vehicle concentration of 0.1% DMSO.	
E-4031	Batch No: WKN7006, used as hERG current blocking reference substance which was prepared as a 100 µmol/L stock solution in reverse osmosis water. Aliquots were added to bath solution to achieve final perfusion solutions of 100 nmol/L.	
Control	Vehicle: Dimethyl sulfoxide (DMSO); Batch No: 056K0172, diluting aliquots of DMSO in bath solution to achieve final perfusion solutions of 0.1%.	

2.6.3.4.2 安全性薬理試験（続き）

Table 2.6.3.4.2-05 (cont.)	Safety Pharmacology	Test Article: Remimazolam
Report Title: Effect of CNS 7056X.besylate salt and CNS 7054X on hERG Tail Current Recorded from Stably Transfected HEK293 Cells		
Route of Administration: *in vitro*	Location in CTD: 4.2.1.3.5	Study Number: [redacted]
GLP Compliance: GLP complaint		
Method of Evaluation: For each cell the magnitude of the tail current after approximately 10 min exposure to test substance or vehicle was compared to the tail current obtained under control (pretreatment) conditions and the residual current (% of control) was calculated. The change in the magnitude of the tail current in the vehicle treated cells was used to give an indication of any current rundown that was observed. The data capture and primary analysis were accomplished using Axon Instruments pCLAMP (ver. 8.1) software. Compounds that inhibit hERG current have been shown to prolong the cardiac action potential and hence QT interval in man. Statistical Analysis: Statistical analysis and graphical representation were performed using GraphPad Prism (ver. 2.01). The test substance groups were compared to the vehicle group using one-way analysis of variance (ANOVA), followed by Dunnett's t-test. Statistical significance was assumed when $P<0.05$. IC_{25} and IC_{50} values were estimated from the sigmoidal function fit to the data.		
Results: In the vehicle treated cells, approximately 10 min exposure to 0.1% DMSO produced a residual tail current of 89.9±3.9% when compared to control values. This equates to a decrease in tail current of 10.1%. Remimazolam inhibited hERG tail current in a concentration-dependent manner, with a statistically significant inhibition of tail current observed at concentrations of 30 µmol/L and above ($P<0.05$, compared to vehicle). The estimated IC_{25} andIC_{50} values for remimazolam inhibition of hERG tail current were 62 and 207 µmol/L, respectively. In the CNS 7054 treated cells, approximately 10 min exposure to 10, 30 and 100 µmol/L CNS 7054 produced residual tail currents of 80.1±1.5%, 90.0±0.9% and 88.7±2.9%, respectively, when compared to control values. This equates to decreases in tail current of 19.9%, 10.0% and 11.3%. When compared to the vehicle treated cells, a statistically significant reduction in hERG tail current ($P<0.05$) was observed upon exposure to 10 µmol/L CNS 7054. However, this is attributed to current rundown rather than to any inhibitory effect since no statistically significant difference between the vehicle and CNS 7054 treated groups was observed at the higher concentrations of 30 and 100 µmol/L ($P>0.05$). Effect of E-4031: When administered to 2 vehicle treated cells and 2 cells treated with 100 µmol/L CNS 7054, 100 nmol/L E-4031 (approximately 10 min exposure) inhibited hERG tail current in all cells tested. Combining the data from all 4 cells, 100 nmol/L E-4031 inhibited hERG tail current by 91.2%.		
Conclusion: CNS 7054 produced no inhibition of hERG tail current at concentrations up to 100 µmol/L. Remimazolam inhibited hERG tail current in a concentration-dependent manner with estimated IC_{25} and IC_{50} values of 62 and 207 µmol/L, respectively.		

2.6.3.4.2 安全性薬理試験（続き）

Table 2.6.3.4.2-06	Safety Pharmacology	Test Article: Remimazolam
試験表題：ONO-2745BS のモルモット摘出乳頭筋の活動電位に対する作用		
Route of Administration: *in vitro*	Location in CTD: 4.2.1.3.6	Study Number: [redacted]
GLP Compliance: GLP complaint		
Aim of Study: To assess the effect of remimazolam on action potential of isolated guinea pig papillary muscles. Study Design: The study was performed by perfusing papillary muscle specimens in an organ bath with perfusions solution (Tyrode's solution). The perfusion rate was 5.0 or 5.1 mL/min. Action potential waveforms were analyzed in real-time, using the action potential analysis module (APA II module) of the HEM system to measure the resting membrane potential (RMP), action potential amplitude (APA), maximum velocity of the increase in amplitude (Vmax), and 30%, 50%, and 90% action potential duration (APD_{30}, APD_{50}, and APD_{90}, respectively). The temperature of the perfusion solution in the organ bath has to be maintained at 36.8 to 37.2°C during the acquisition of pre-values and post-values, with the difference in temperature between these acquisition periods being not more than 0.2°C. Each specimen was only treated with 1 dose.		
Species (Gender, Strain, Supplier, n/Group): Guinea pig (male, Slc:Hartley, Japan SLC, Inc, 5/group). Age/Body Weight: 4-6 weeks / 291.8-426.5 g Conditioning and Surgical Pre-treatment: Fresh prepared isolated papillary muscle specimens were stimulated with rectangular waves for at least 30 min until the response became stable. Action potentials were monitored using an oscilloscope via the microelectrode amplifier and a multifunction filter and input into a high-end data acquisition real-time analysis system (HEM ver. 3.5).		
Test Compounds (Batch No) and Test Conditions (Formulation, Doses, Route of Administrations):		
Remimazolam	Batch No: SOL12621/6, white crystalline powder, dissolved in dimethyl sulfoxide (DMSO) to prepare a 300 mmol/L test solution. This was serially diluted with DMSO to prepare 100, 30, and 10 mmol/L test solutions. For measurement of action potential, 250 µL of test article solution were added to 250 mL (dilution 1/1000) of the Tyrode's solution to obtain final concentrations of 10, 30, 100 and 300 µmol/L in the organ bath.	
(±)-Sotalol hydrochloride	Batch No: 087K4065, 250 µL of a 30 mmol/L solution in DMSO was added to 250 mL (1/1000) of the Tyrode's solution to obtain a final concentration of 30 µmol/L.	
Control	Vehicle: DMSO, batch No: CDQ4850 and PEH6238, 0.1 vol%.	
Method of Evaluation: Continuous stable waveforms obtained over a 10 sec period were used to calculate the pretreatment value (pre-value) for the vehicle control, test article, or positive control article. Continuous stable waveforms obtained over a 10 sec period at 30 to 35 min after the start of perfusion with the perfusion solution for application were used to calculate the posttreatment value (post-value). The mean ± standard error of the % pre-value of each parameter was calculated for each group. Statistical Analysis: One-way analysis of variance was performed to compare the % pre-values among the ONO-2745BS and vehicle control groups. Each ONO-2745BS group was compared with the vehicle control group using Dunnett's test for RMP, APD_{30}, APD_{50}, and APD_{90} that showed significant differences. The F-test was performed for the positive control group and the vehicle control group. RMP, APA, Vmax, APD_{30} and APD_{50} that showed homogeneity of variance were assessed using Student t-test, and APD_{90}, whose variance was not homogenous, was assessed using Welch test. In all cases, the level of significance was set at $p<0.05$ (2-tailed).		

2.6.3.4.2 安全性薬理試験（続き）

Table 2.6.3.4.2-06 (cont.)	Safety Pharmacology	Test Article: Remimazolam
試験表題：ONO-2745BS のモルモット摘出乳頭筋の活動電位に対する作用		
Route of Administration: *in vitro*	Location in CTD: 4.2.1.3.6	Study Number: [redacted]
GLP Compliance: GLP complaint		
Results: The RMP, APA, Vmax, APD_{30}, APD_{50}, and APD_{90} in the vehicle control group were 100.7, 100.5, 102.2, 99.0, 98.9, and 99.0%, respectively. Remimazolam did not show any significant changes in any parameter at 10 and 30 μmol/L in comparison with the vehicle control group. However, APD_{30} and APD_{50} were shortened at 100 μmol/L (88.2% and 90.9%, respectively). Furthermore, a rise in RMP and prolongation of APD_{90} (94.9% and 103.5%, respectively) were observed in addition to the shortening in APD_{30} and APD_{50} (81.1 and 88.1, respectively) at the highest concentration of 300 μmol/L. In contrast, the RMP, APD_{30}, APD_{50} and APD_{90} were 99.6%, 111.3%, 119.2%, and 121.9%, respectively, in the positive control group (sotalol hydrochloride: 30 μmol/L), thereby showing significant changes in comparison with the vehicle control group.		
Conclusion: For remimazolam, at 10 and 30 μmol/L, there were no significant changes in the various study parameters compared with changes in the vehicle control group. At concentrations of 100 and 300 μmol/L, the 30% and 50% action potential duration (APD_{30} and APD_{50}) decreased. In addition, an increase in the resting membrane potential and a prolongation of the 90% action potential duration (APD_{90}) were observed at the highest concentration of 300 μmol/L. This prolongation was still of limited magnitude (remimazolam, ΔAPD_{90} = 5.4 msec; controls, ΔAPD_{90} = -1.8 msec). By contrast, sotalol (30 μmol/L), used as a positive control, produced an increase by 37 msec. Based on these results, it was considered that remimazolam inhibited calcium channels at 100 μmol/L or higher and, in addition, inhibited the inwardly and the delayed rectifying potassium channels at 300 μmol/L. Thus, the NOEL for inhibition of the inwardly and the delayed rectifying potassium channels was 100 μmol/L and the NOEL for inhibition of calcium channels was 30 μmol/L.		

2.6.3.4.2 安全性薬理試験（続き）

Table 2.6.3.4.2-07	Safety Pharmacology	Test Article: CNS 7054
試験表題：ONO-IN-252（ONO-2745BS 代謝物）のモルモット摘出乳頭筋の活動電位に対する作用		
Route of Administration: *in vitro*	Location in CTD: 4.2.1.3.7	Study Number: [redacted]
GLP Compliance: GLP complaint		
Aim of Study: To assess the effect of CNS 7054 (remimazolam principal metabolite) on action potential of isolated guinea pig papillary muscles. Study Design: The study was performed by perfusing papillary muscle specimens in an organ bath with perfusions solution (Tyrode's solution). The perfusion rate was 5.0 or 5.1 mL/min. Action potential waveforms were analyzed in real-time, using the action potential analysis module (APA II module) of the HEM system to measure the resting membrane potential (RMP), action potential amplitude (APA), maximum velocity of the increase in amplitude (Vmax), and 30%, 50%, and 90% action potential duration (APD_{30}, APD_{50}, and APD_{90}, respectively). The temperature of the perfusion solution in the organ bath has to be maintained at 36.9 to 37.2°C during the acquisition of pre-values and post-values, with the difference in temperature between these acquisition periods being not more than 0.1°C. Each specimen was only treated with 1 dose.		
Species (Gender, Strain, Supplier, n/Group): Guinea pig (male, Slc:Hartley, Japan SLC, Inc, 5/group). Age/Body Weight: 4-6 weeks / 278.1-397.5 g Conditioning and Surgical Pre-treatment: Fresh prepared isolated papillary muscle specimens were stimulated with rectangular waves for at least 30 min until the response became stable. Action potentials were monitored using an oscilloscope via the microelectrode amplifier and a multifunction filter and input into a high-end data acquisition real-time analysis system (HEM ver. 3.5).		
Test Compounds (Batch No) and Test Conditions (Formulation, Doses, Route of Administrations):		
CNS 7054	Batch No: MMK090616, white powder, dissolved in dimethyl sulfoxide (DMSO) to prepare a 300 mmol/L test solution. This was serially diluted with DMSO to prepare 100, 30, and 10 mmol/L test solutions. For measurement of action potential, 250 µL of test article solution were added to 250 mL (dilution 1/1000) of the Tyrode's solution to obtain final concentrations of 10, 30, 100 and 300 µmol/L.	
(±)-Sotalol hydrochloride	Batch No: 039K4094, 250 µL of a 30 mmol/L solution in DMSO was added to 250 mL (1/1000) of the Tyrode's solution to obtain a final concentration of 30 µmol/L.	
Control	Vehicle: DMSO, batch No: PEH6238 and CDL2209, 0.1 vol%.	
Method of Evaluation: Continuous stable waveforms obtained over a 10 s period were used to calculate the pretreatment value (pre-value) for the vehicle control, test article, or positive control article. Continuous stable waveforms obtained over a 10 s period at 30 to 35 min after the start of perfusion with the perfusion solution for application were used to calculate the posttreatment value (post-value). The mean ± standard error of the % pre-value of each parameter was calculated for each group. Statistical Analysis: One-way analysis of variance was performed to compare the % pre-values among the CNS 7054 and vehicle control groups. Each CNS 7054 group was compared with the vehicle control group using Dunnett's test for RMP, APD_{50}, and APD_{90} that showed significant differences. The F-test was performed for the positive control group and the vehicle control group. RMP, APA, Vmax, APD_{30} and APD_{50} that showed homogeneity of variance were assessed using Student t-test, and APD_{90}, whose variance was not homogenous, was assessed using Welch test. In all cases, the level of significance was set at $p<0.05$ (2-tailed).		

2.6.3.4.2 安全性薬理試験（続き）

Table 2.6.3.4.2-07 (cont.)	Safety Pharmacology	Test Article: CNS 7054
試験表題：ONO-IN-252（ONO-2745BS 代謝物）のモルモット摘出乳頭筋の活動電位に対する作用		
Route of Administration: *in vitro*	Location in CTD: 4.2.1.3.7	Study Number: [redacted]
GLP Compliance: GLP complaint		
Results: The RMP, APA, Vmax, APD_{30}, APD_{50}, and APD_{90} in the vehicle control group were 100.6%, 100.0%, 102.4%, 100.0%, 99.9%, and 99.6%, respectively. CNS 7054 did not show any significant changes at 10 and 100 µmol/L in comparison with the vehicle control group. However, a significant rise in RMP and shortening in APD_{50} (99.2% and 96.1%, respectively) compared with the vehicle control group were observed at the highest concentration of 300 µmol/L. Even though the difference was not significant, APD_{30} also was shortened (96.2%) at the highest concentration of 300 µmol/L. A significant rise in RMP was observed at 30 µmol/L as compared to the vehicle control group. However, no rise was observed at 100 µmol/L, thereby indicating no dose relationship. Therefore, this change was considered to be accidental. In contrast, the RMP, APD_{30}, APD_{50}, and APD_{90} were 99.8%, 111.2%, 118.9%, and 121.0%, respectively, in the positive control group (sotalol hydrochloride: 30 µmol/L), thereby indicating significant changes in comparison with the vehicle control group.		
Conclusion: For CNS 7054, at concentrations of 10, 30, and 100 µmol/L, the principal metabolite of remimazolam did not affect the myocardial action potential. There were decreases of the APD_{30} and APD_{50} at 300 µmol/L, which were attributed to inhibition of calcium channels. Thus, the NOELs for inhibition of the inwardly and the delayed rectifying potassium channels was 300 µmol/L for CNS 7054. NOEL for inhibition of calcium channels was 100 µmol/L.		

2.6.3.4.2 安全性薬理試験（続き）

Table 2.6.3.4.2-08	Safety Pharmacology	Test Article: Remimazolam
試験表題：ONO-2745BS のラットにおける血液機能に対する作用検討（*in vitro*）		
Route of Administration: *in vitro*	Location in CTD: 4.2.1.3.8	Study Number: [redacted]
GLP Compliance: non GLP		
Aim of Study: To assess the influence of remimazolam on hematic functions (platelet aggregation and coagulation system) using rat plasma. Study Design: The effects of remimazolam on hematic functions were examined in comparison with that of the reference drug.		
Species (Gender, Strain, Supplier, n/Group): Rats [male, Crl:CD(SD), Charles River Laboratories Japan, n = 9] Age/Body Weight: no data Conditioning and Surgical Pre-treatment: Platelet-rich plasma (PRP) and platelet-poor plasma (PPP) prepared from blood of 3 rats for 3 sets.		
Test Compounds (Batch No) and Test Conditions (Formulation, Doses, Route of Administrations):		
Remimazolam	Batch No: SOL12621/6, dissolved and diluted with physiological saline	
Midazolam	Dormicum Injection 5 mg/mL, [redacted]., Batch No: no data	
Control	physiological saline	
Method of Evaluation: The mean and standard error of each of the parameters of hematic functions were calculated for each group and evaluated compared with vehicle control group [induction of platelet aggregation, inhibitory effect on 5'-diphosphate sodium salt (ADP)- or collagen-induced platelet aggregation, and influence on coagulation, indicated as prothrombin time (PT) and activated partial thromboplastin time (APTT)]. Statistical Analysis: One-way analysis of variance was performed to compare the vehicle control group with the remimazolam groups. If a significant difference was observed, each remimazolam group was compared with the vehicle control group by using Dunnett's test. The F-test was performed between the positive control group and the vehicle control group. If the data showed homogeneity of variance, t-test was performed, while Welch's test was used when the variance was not homogenous. In all cases, the level of significance was set at $p<0.05$ (two-tailed).		
Results: No effect of remimazolam nor midazolam on platelet aggregation (maximum aggregation rate, ADP- or collagen-induced platelet aggregation) and coagulation (PT or APTT) were observed.		
Conclusion: Remimazolamhad no effect on the platelet aggregation and the blood coagulation system up to the each highest concentration of 333 and 1000 μg/mL, respectively.		

2.6.3.4.2 安全性薬理試験（続き）

Table 2.6.3.4.2-09	Safety Pharmacology	Test Article: Remimazolam
試験表題：ONO-2745BS のヒトにおける血液機能に対する作用（*in vitro*）		
Route of Administration: *in vitro*	Location in CTD: 4.2.1.3.9	Study Number: [redacted]
GLP Compliance: GLP complaint		
Aim of Study: To assess the influence of remimazolam on hematic functions (platelet aggregation, the coagulation system, and the fibrinolysis system) in human plasma. This study was performed as a supplementary safety pharmacology study. Study Design: The effect of remimazolam on hematic functions, its influence on platelet aggregation [platelet aggregation induced by remimazolam alone and modulation of aggregation induced by adenosine 5'-diphosphate sodium salt (ADP) or collagen], the coagulation system [prothrombin time (PT) and activated partial thromboplastin time (APTT)], and the fibrinolysis system (plasmin activity) was investigated by using human plasmin comparison with that of the vehicle control and test method specific reference drugs.		
Species (Gender, Strain, Supplier, n/Group): Human [Platelet-rich plasma (PRP) and platelet-poor plasma (PPP) prepared from human blood, donated from fasted non-smoking healthy volunteers, 5/concentration group] Age/Body Weight: not applicable Conditioning and Surgical Pre-treatment: The blood, collected from the median cubital vein, was mixed with the anticoagulant sodium citrate at a ratio of 1:9 and then centrifuged for 10 min at about 250 g (for obtaining the supernatant, used as PRP) or 1600 g (for obtaining the supernatant, used as PPP)		
Test Compounds (Batch No) and Test Conditions (Formulation, Doses, Route of Administrations):		
Remimazolam	Batch No: SOL12621/6, white crystalline powder, diluted with DMSO to get final concentrations of 3, 10, 30, 100, and 300 µmol/L, *in vitro*, added to plasma with a microsyringe).	
Alprostadil alfadex	Prostandin 500 (subsequently abbreviated as PGE_1), positive control for ADP- or collagen-induced platelet aggregation, Batch No: 743FA, dissolved in a sufficient volume of physiological saline to get final concentration of 500 nmol/L, *in vitro*.	
Heparin sodium	Heparin Sodium Injection "Ajinomoto"; positive control for coagulation parameters PT and APTT, respectively, Batch No: 70261, diluted with a sufficient volume of physiological saline to get final concentration of 7 U/mL and 0.3 U/mL, respectively, *in vitro*.	
Gabexate mesilate	Brand name: FOY500, positive control for fibrinolysis, Batch No: 749FA, dissolved in a sufficient volume of physiological saline to get a final concentration of 100 µmol/L, *in vitro*.	
Control	Dimethyl sulfoxide (subsequently abbreviated as DMSO), vehicle control for remimazolam, Batch No: CDQ4850, final concentration of 0.5 vol%.	

2.6.3.4.2 安全性薬理試験（続き）

Table 2.6.3.4.2-09 (cont.)	Safety Pharmacology	Test Article: Remimazolam
試験表題：ONO-2745BS のヒトにおける血液機能に対する作用（*in vitro*）		
Route of Administration: *in vitro*	Location in CTD: 4.2.1.3.9	Study Number: [redacted]
GLP Compliance: GLP complaint		
Method of Evaluation: The mean and standard error of each of the parameters of hematic functions were calculated for each group and evaluated compared with vehicle control group (induction of platelet aggregation, inhibitory effect on adenosine 5'-diphosphate sodium salt (ADP)- or collagen-induced platelet aggregation, influence on coagulation, indicated as prothrombin time (PT) and activated partial thromboplastin time (APTT), and on fibrinolysis). Statistical Analysis: One-way analysis of variance was performed to compare the vehicle control group with the remimazolam groups. If a significant difference was observed, each remimazolam group was compared with the vehicle control group by using Dunnett's test. The F-test was performed between the positive control group and the vehicle control group. If the data showed homogeneity of variance, t-test was performed, while Welch's test was used when the variance was not homogenous. In all cases, the level of significance was set at $p<0.05$ (two-tailed).		
Results: Platelet aggregation: Maximum aggregation rate: In the vehicle control group, the maximum aggregation rate was 1.2%. In the 3, 10, 30, 100, and 300 μmol/L remimazolam groups, the maximum aggregation rate was 1.2%, 1.2%, 0.8%, 1.2%, and 1.6%, respectively; i.e. that remimazolam did not induce aggregation when added up to a concentration of 300 μmol/L. ADP-induced platelet aggregation: There were no significant differences between the vehicle control group (maximum aggregation rate 72.2%) and any of the 5 remimazolam groups with maximum aggregation rates of 70.6%, 71.2%, 70.6%, 70.0%, and 70.6%, respectively. In the positive control group (PGE_1 at a concentration of 500 nmol/L), the maximum aggregation rate was 7.0%, and there was significant inhibition of platelet aggregation compared with the vehicle control group. Collagen-induced platelet aggregation: There were no significant differences between the vehicle control group (maximum aggregation rate 74.0%) and any of the remimazolam groups with maximum aggregation rates of 73.4%, 74.4%, 73.6%, 74.0%, and 72.4%, respectively. PGE_1 inhibited significantly the maximum aggregation rate, which was 11.6%. Coagulation: PT: There were no significant differences between the vehicle control group (PT 12.9 sec) and any of the 5 remimazolam groups with PT values of 13.1, 13.1, 13.1, 13.2, and 13.4 sec, respectively. In the positive control group (heparin at a concentration of 7 U/mL), the PT (38.7 sec) was no significant different from the vehicle control group, although the PT was three times as long as that of control group. APTT: There were no significant differences between the vehicle control group (APTT 34.6 sec) and any of the 5 remimazolam groups with APTT values of 36.1, 35.0, 35.7, 35.6, and 35.5 sec, respectively. Heparin at a concentration of 0.3 U/mL induced a significant prolongation of APTT (APTT = 95.1 sec). Fibrinolysis: There were no significant differences between the vehicle control group (plasmin activity 82.2%) and any of the 5 remimazolam groups with plasmin activities of 84.4%, 83.8%, 83.2%, 84.8%, and 87.0%, respectively. In the positive control group (gabexate mesilate at a concentration of 100 μmol/L), the plasmin activity (24.8%) was decreased significantly.		
Conclusion: Remimazolam, added to human plasma, had no effect up to the highest tested concentration of 300 μmol/L on platelet aggregation, coagulation or fibrinolysis.		

2.6.3.4.2 安全性薬理試験（続き）

Table 2.6.3.4.2-10	Safety Pharmacology	Test Article: CNS 7054
試験表題：ONO-IN-252（ONO-2745BS 代謝物）のヒトにおける血液機能に対する作用（*in vitro*）		
Route of Administration: *in vitro*	Location in CTD: 4.2.1.3.10	Study Number: [redacted]
GLP Compliance: GLP complaint		
Aim of Study: To assess the influence CNS 7054 on hematic functions (platelet aggregation, the coagulation system, and the fibrinolysis system) in human plasma. This study was performed as a supplementary safety pharmacology study. Study Design: The effect of CNS 7054 on hematic functions, its influence on platelet aggregation [platelet aggregation induced by CNS 7054 alone and modulation of aggregation induced by adenosine 5'-diphosphate sodium salt (ADP) or collagen], the coagulation system [prothrombin time (PT) and activated partial thromboplastin time (APTT)], and the fibrinolysis system (plasmin activity) was investigated by using human plasma in comparison with that of the vehicle control and test method specific reference drugs.		
Species (Gender, Strain, Supplier, n/Group): Platelet-rich plasma (PRP) and platelet-poor plasma (PPP) prepared from human blood, donated from fasted nonsmoking healthy volunteers, 5/concentration group Age/Body Weight: not applicable Conditioning and Surgical Pre-treatment: The blood, collected from the median cubital vein, was mixed with the anticoagulant sodium citrate at a ratio of 1:9 and then centrifuged for 10 min at about 250 g (for obtaining the supernatant, used as PRP) or 1600 g (for obtaining the supernatant, used as PPP)		
Test Compounds (Batch No) and Test Conditions (Formulation, Doses, Route of Administrations):		
CNS 7054	Batch No: MMK090616, white powder, diluted with DMSO to get final concentrations of 3, 10, 30, 100, and 300 µmol/L, *in vitro*, added to plasma with a microsyringe.	
Alprostadil alfadex	Prostandin 500 (subsequently abbreviated as PGE_1), positive control for ADP- or collagen-induced platelet aggregation, Batch No: 849FA, dissolved in a sufficient volume of physiological saline to get final concentration of 500 nmol/L, *in vitro*.	
Heparin sodium	Heparin Sodium Injection "Ajinomoto", positive control for coagulation parameters PT and APTT, respectively, Batch No: 70261, diluted with a sufficient volume of physiological saline to get final concentration of 5 U/mL and 0.3 U/mL, respectively, *in vitro*.	
Gabexate mesilate	Brand name: FOY500, positive control for fibrinolysis, Batch No: 749FA, dissolved in a sufficient volume of physiological saline to get a final concentration of 100 µmol/L, *in vitro*.	
Control	Dimethyl sulfoxide (subsequently abbreviated as DMSO), vehicle control for CNS 7054, Batch No: CDL2209, final concentration of 0.5 vol%.	

2.6.3.4.2 安全性薬理試験（続き）

Table 2.6.3.4.2-10 (cont.)	Safety Pharmacology	Test Article: CNS 7054
試験表題：ONO-IN-252（ONO-2745BS 代謝物）のヒトにおける血液機能に対する作用（*in vitro*）		
Route of Administration: *in vitro*	Location in CTD: 4.2.1.3.10	Study Number: [redacted]
GLP Compliance: GLP complaint		
Method of Evaluation: The mean and standard error of each of the parameters of hematic functions were calculated for each group and evaluated compared with vehicle control group (induction of platelet aggregation, inhibitory effect on ADP- or collagen-induced platelet aggregation, influence on coagulation, indicated as PT and APTT, and on fibrinolysis). Statistical Analysis: One-way analysis of variance was performed to compare the vehicle control group with the CNS 7054 groups. If a significant difference was observed, each CNS 7054 group was compared with the vehicle control group by using Dunnett's test. The F-test was performed between the positive control group and the vehicle control group. If the data showed homogeneity of variance, t-test was performed, while Welch's test was used when the variance was not homogenous. In all cases, the level of significance was set at $p<0.05$ (two-tailed).		
Results: Platelet aggregation: Maximum aggregation rate: In the vehicle control group, the maximum aggregation rate was 2.2%. In the 3, 10, 30, 100, and 300 μmol/L CNS 7054 groups, the maximum aggregation rate was 1.6%, 1.8%, 1.4%, 1.2%, and 1.6%, respectively; i.e. that CNS 7054 did not induce aggregation when added up to a concentration of 300 μmol/L. ADP-induced platelet aggregation: There were no significant differences between the vehicle control group (maximum aggregation rate 73.2%) and any of the 5 CNS 7054 groups with maximum aggregation rates of 76.4%, 75.2%, 74.0%, 77.6%, and 76.6%, respectively. In the positive control group (PGE_1 at a concentration of 500 nmol/L), the maximum aggregation rate was 7.4%, and there was significant inhibition of platelet aggregation compared with the vehicle control group. Collagen-induced platelet aggregation: There were no significant differences between the vehicle control group (maximum aggregation rate 78.8%) and any of the CNS 7054 groups with maximum aggregation rates of 79.0%, 80.2%, 77.8%, 79.6%, and 78.0%, respectively. PGE_1 inhibited significantly the maximum aggregation rate, which was 5.6%. Coagulation: PT: There were no significant differences between the vehicle control group (PT 12.5 sec) and any of the 5 CNS 7054 groups with PT values of 12.7, 12.7, 12.7, 12.7 and 12.8 sec, respectively. In the positive control group (heparin at a concentration of 5 U/mL), the PT (19.4 sec) was prolongated significantly compared with the vehicle control group. APTT: There were no significant differences between the vehicle control group (APTT 35.0 sec) and any of the 5 CNS 7054 groups with APTT values of 35.5, 35.4, 34.9, 35.2, and 35.3 sec, respectively. Heparin at a concentration of 0.3 U/mL induced a significant prolongation of APTT (APTT = 76.9 sec). Fibrinolysis: There were no significant differences between the vehicle control group (plasmin activity 116.6%) and any of the 5 CNS 7054 groups with plasmin activities of 117.4%, 111.6%, 109.0%, 112.2%, and 111.2%, respectively. In the positive control group (gabexate mesilate at a concentration of 100 μmol/L), the plasmin activity (27.4%) was decreased significantly.		
Conclusion: CNS 7054, added to human plasma, had no effect up to the highest tested concentration of 300 μmol/L on platelet aggregation, coagulation or fibrinolysis.		

2.6.3.4.2 安全性薬理試験（続き）

Table 2.6.3.4.2-11	Safety Pharmacology	Test Article: Remimazolam
Report Title: Blood pressure and heart rate hemodynamic evaluation of CNS7056 in conscious telemetrized male Sprague-Dawley rats following a single intravenous bolus administration		
Route of Administration: i.v.	Location in CTD: 4.2.1.3.11	Study Number: [redacted]
GLP Compliance: non GLP		
Aim of Study: To assess the potential hemodynamic effects of remimazolam in conscious, telemetrized, male Sprague-Dawley rats following a single i.v. bolus administration. Study Design: In a cross-over design, five rats were treated with doses of 0, 20 and 30 mg/kg with a wash out between the doses of at least 48 h. One rat (number 104) was disregarded due to a failure of the blood pressure catheter. Systolic, diastolic, and mean arterial blood pressures and heart rate were monitored in conscious, freely-moving rats and collected continuously for at least 2 h prior to dosing and for at least 4 h post-dose. The state of sedation was observed continuously until recovery was evident. The animals were not handling during this time.		
Species (Gender, Strain, Supplier, n/Group): Rats (male, Sprague-Dawley), 5/group, 3 treatment groups Age/Body Weight: 409-465 g Conditioning and Surgical Pre-treatment: Prior to study, the animals were surgically instrumented with radio-telemetry transmitters for measurement of blood pressure and heart rate. Rats were unrestrained and monitored for selected hemodynamic parameters. For experimentation, the transmitter was activated, the receiver was placed beneath the animal's cage, and data acquisition was initiated.		
Test Compounds (Batch No) and Test Conditions (Formulation, Doses, Route of Administrations):		
Remimazolam	Batch No: no data, 20 and 30 mg/kg, i.v. via tail vein over approximately 2 min in a dose volume of 6 mL/kg after the monitoring phase prior to dosing.	
Control	Vehicle: 0.9% sodium chloride (NaCl) for injection, Batch No: no data, 6 mL/kg, i.v.	
Method of Evaluation: Observations for mortality, morbidity, injury, and the availability of food and water were conducted twice daily for all animals. A detailed clinical examination of each animal was performed prior to dosing, at 30 min post-dose, and following completion of the telemetry monitoring session (at approximately 4 h post-dose). The observations conducted during this time focused on the principal pharmacologic effect of the test article (i.e. sedation), which was expected to occur immediately following the injection, and with animals recovering within 30 min. The state of sedation was observed continuously after the injection until recovery was evident, and the onset and disappearance of these findings were documented appropriately. Any abnormalities noted during this period were documented as unscheduled clinical observations. Hemodynamic responses to 20 and 30 mg/kg of i.v. administered remimazolam were compared to the responses of the vehicle control. Statistical Analysis: no statistical analysis was performed.		

2.6.3.4.2 安全性薬理試験（続き）

Table 2.6.3.4.2-11 (cont.)	Safety Pharmacology	Test Article: Remimazolam
Report Title: Blood pressure and heart rate hemodynamic evaluation of CNS7056 in conscious telemetrized male Sprague-Dawley rats following a single intravenous bolus administration		
Route of Administration: i.v.	Location in CTD: 4.2.1.3.11	Study Number: [redacted]
GLP Compliance: non GLP		
Results: Clinical findings: Decreased activity was noted in all animals immediately following remimazolam administration, with each animal remaining in a sedative state for approximately 5 to 10 min post-dose. Cardiovascular effects: Immediately prior to dose administration the blood pressure began to increase in all groups, including the vehicle group, as a result of the restraint and test article administration procedures. Blood pressure values following the vehicle control dose normalized within an expected time frame and were maintained within a normal range for rats throughout the monitoring period. In remimazolam-dosed animals, blood pressure remained elevated compared to the time-matched vehicle controls, with maximal changes in mean arterial pressure observed at approximately 15 min post-dose. For the two dose levels of 20 and 30 mg/kg, the mean arterial pressure changes amounted to approximately 15% and 22%, respectively. Blood pressure returned to values comparable to those of time-matched vehicle controls within 1.25 h post-dose. Heart rate: Immediately prior to dose administration, heart rates began to increase in all groups, including the vehicle control group, as a result of the restraint and dose administration procedures. With remimazolam, the rates remained elevated compared to the time-matched vehicle control values. Maximal changes were observed at approximately 15 min post-dose, with increases of approximately 18% and 34% (20 and 30 mg/kg, respectively). Within 1.25 h post-dose, values were comparable to those observed in the time-matched vehicle controls.		
Conclusion: With respect to the basic hemodynamic endpoints evaluated in this study, a single i.v. administration of remimazolam produced no adverse effects in rats at 20 or 30 mg/kg. A no observed adverse effect level (NOAEL) of at least 30 mg/kg has been established for i.v. administrated remimazolam.		

2.6.3.4.2 安全性薬理試験（続き）

Table 2.6.3.4.2-12	Safety Pharmacology	Test Article: Remimazolam
Report Title: Study of blood oxygen saturation in rabbits with CNS 7056 by intravenous administration (non-GLP)		
Route of Administration: i.v.	Location in CTD: 4.2.1.3.12	Study Number: [redacted]
GLP Compliance: non GLP		
Aim of Study: To assess the influence on blood oxygen levels of remimazolam administered intravenously (bolus injection and infusion) to adult female rabbits. Study Design: Oxygen saturation of the blood was monitored by non-invasive pulse oximetry using an artery as point of access. In addition, the heart rate was recorded. The clinical status was observed, with particular attention to sedation.		
Species (Gender, Strain, Supplier, n/Group): Rabbits (female, Himalayan, LPT, Löhndorf branch Breeding station), a total of 8 rabbits: group 1 (n = 4) and group 2 (n = 4) Age/Body Weight: 5-6 months / 2.15-2.58 kg (group 1) and 2.49-2.78 kg (group 2) Conditioning and Surgical Pre-treatment: Prior to study, the animals were adapted for 20 days in cages with wire floors with free access to food and water.		
Test Compounds (Batch No) and Test Conditions (Formulation, Doses, Route of Administrations):		
Remimazolam	Batch No: P02308, white to off-white, lyophilised lactose formulation, provided in sterile vials. Group 1: 0, 2.5, 5.0 and 7.5 mg/kg cross over with a wash out period between the doses of 2 or 3 days; i.v. 2 min-bolus injection into a medial ear vein. Group 2: 7.5 mg/kg, 4-min i.v. infusion into a medial ear vein, each animal in each group in a dose volume of 2 mL/kg.	
Control	Vehicle: 0.9% NaCl solution for injection (Batch No: 28706701), 2 mL/kg; i.v.	
Method of Evaluation: A detailed clinical examination of each animal was performed prior to dosing, approximately 30 min post-dose, and following completion of the monitoring period (at approximately 4 h post-dose). The observations conducted during this time focused on the principal pharmacologic effect of the test article (i.e. sedation), which was expected to occur almost immediately after injection, with a recovery not later than 30 min post injection. The state of consciousness was monitored continuously after injection until recovery was evident, and the onset and disappearance of sedation, along with other effects as tremors, convulsions, reactivity to handling and bizarre behaviour was documented appropriately. Potential respiratory and circulatory effects were documented if noted during this time. Statistical Analysis: For each dosage group the values obtained were compared to the start value by means of Student's paired t-test ($p \leq 0.01$ corresponding to $t = 5.8409$ for 3 degrees of freedom (df = total number of animals per group – number of groups compared)		

2.6.3.4.2 安全性薬理試験（続き）

Table 2.6.3.4.2-12 (cont.)	Safety Pharmacology	Test Article: Remimazolam
Report Title: Study of blood oxygen saturation in rabbits with CNS 7056 by intravenous administration (non-GLP)		
Route of Administration: i.v.	Location in CTD: 4.2.1.3.12	Study Number: [redacted]
GLP Compliance: non GLP		
Results: Clinical findings: Local tolerance: After 2-min bolus injection no signs were noted after 2.5, 5.0 and 7.5 mg remimazolam/kg. After i.v. infusion no signs were noted. Systemic tolerance: After 2-min bolus injection of remimazolam all animals treated with 2.5, 5.0 and 7.5 mg/kg revealed sedation and abdominal position at 30 min post-dose. The respiratory rate after 2.5 mg/kg was reduced in 3 animals and increased in 1 animal, whereas after 5.0 and 7.5 mg/kg the respiratory rate was reduced in all animals within 5 min post-dose and had subsided at 4 h post-dose at the latest. Tremor was noted 30 min post-dose for 1 animal after 7.5 mg/kg. No signs of systemic intolerance reactions were noted for the control. After i.v. infusion of 7.5 mg remimazolam/kg all animals revealed sedation and abdominal position at 30 min post-dose. In addition a reduced respiratory rate, nystagmus, and mydriasis were noted. The symptoms had started within 5 min post-dose. For animal no. 5, abdominal position was noted again starting 1 to 2 h post-dose. All clinical symptoms observed had subsided at 4 hours post-dose at the latest. Blood oxygen saturation: After 2-min bolus injection of 2.5 or 5.0 mg remimazolam/kg or with the control no influence was noted at any time point of measurement. 7.5 mg/kg caused a reduction of the mean values by 18% 1 min post-dose and by 20% 3 min post-dose compared to the pre-dose values. The changes were not statistically significant. No difference to the pre-dose values was noted at 10 min post-dose. After i.v. infusion a reduction by 6% 1 min post-dose and a significant reduction by 4% 3 min post-dose compared to the pre-dose values was noted, lasted no longer than 10 min. Heart rate: After 2-min bolus injection no test item related influence was noted at any time point. After i.v. infusion reductions by 16% 1 min and 3 min post-dose and by 26% 10 min post-dose were noted, which were not significant compared to the pre-dose values.		
Conclusion: Remimazolam produced neither signs of local intolerance, significant variations of heart rate nor significant changes of oxygen saturation (except for 4% reduction of oxygen saturation 3 min after i.v. infusion, which was statistically significant). Remimazolam induced 30 min post-dose systemic intolerance signs as sedation and abdominal position in all animals after 2-min bolus injection of 2.5, 5.0 and 7.5 mg/kg and after i.v. infusion of 7.5 mg/kg, and a reduced respiratory rate in all animals after 5.0 and 7.5 mg/kg. All clinical symptoms, also the nystagmus and mydriasis after i.v. infusion of 7.5 mg/kg had subsided at 4 h post-dose at the latest.		

2.6.3.4.2 安全性薬理試験（続き）

Table 2.6.3.4.2-13	Safety Pharmacology	Test Article: Remimazolam
Report Title: Effects of ONO-IN-251 on the Respiratory and Cardiovascular Systems in Micropigs		
Route of Administration: i.v.	Location in CTD: 4.2.1.3.13	Study Number: [redacted]
GLP Compliance: non GLP		
Aim of Study: To assess the effects of remimazolam on the respiratory and cardiovascular systems administered once intravenously to micropigs. Study Design: The effect of remimazolam on blood pressure, heart rate, electrocardiogram parameters (ECG) and arterial blood gas was compared with those of 3 other systemic anesthetics. The micropigs were also observed for symptoms, and relationships between the effects of these test articles on the respiratory and cardiovascular systems and their sedative effects (effective doses, sedative activity, and/or sedative duration) were examined.		
Species (Gender, Strain, Supplier, n/Group): Micropigs (male, NIBS, supplier: Nisseiken Co., Ltd.), a total of 4 micropigs: 4 used for the preliminary study, 3 used for the sighting study treated at different dose levels. Age/Body Weight: 5 months / 13.3-14.8 kg Conditioning and Surgical Pre-treatment: The animals were housed individually in stainless steel cages with free access to tap water, solid feed were given. Prior to preparation of animals for measurement of test parameters, surgical operation for implanting catheters were performed to secure the vein to be used for postoperative management or for administration. The surgical operation (with antibiotic treatment for 3 days) took place for preparing animals to be used for measurement (12 days after surgical operation or thereafter) of blood pressure, ECG (PR interval, QRS duration, QT interval and QTc interval) and arterial blood gas.		
Test Compounds (Batch No) and Test Conditions (Formulation, Doses, Route of Administrations):		
Remimazolam	Batch No: AE-123-01, dosing: 12.5, 25.0, 50.0 and 100.0 μg/kg/min i.v. infusion in a volume of 1 mL/kg/15 minover a period of 15 min.	
Propofol	1% Diprivan® injection, Positive control, Batch No: 36950, dosing: 0.05, 0.1 and 0.2 mg/kg/min i.v. infusion in a volume of 1 mL/kg/15 min over a period of 15 min.	
Midazolam	Dormicum®, Positive control, Batch No: T009Y02 and T011Y03, dosing: 12.5, 25.0 and 50.0 μg/kg/min i.v. infusion in a volume of 1 mL/kg/15 min over a period of 15 min.	
Dexmedetomidine	Precedex® Positive control, Batch No: 19340, dosing: 0.05, 0.1 and 0.2 μg/kg/min i.v. infusion in a volume of 1 mL/kg/15 min over a period of 15 min.	
Control 1 Control 2	Vehicle for remimazolam: citric acid buffer Vehicle for propofol, midazolam, and dexmedetomidine infusion dosing: 0.9% NaCl solution	

2.6.3.4.2 安全性薬理試験（続き）

Table 2.6.3.4.2-13 (cont.)	Safety Pharmacology	Test Article: Remimazolam
Report Title: Effects of ONO-IN-251 on the Respiratory and Cardiovascular Systems in Micropigs		
Route of Administration: i.v.	Location in CTD: 4.2.1.3.13	Study Number: [redacted]
GLP Compliance: non GLP		
Method of Evaluation: Depending on the test parameters measurements were performed at different times before, during or after administration. Individual scores of symptoms at each time of observation were totaled, and group mean values and standard errors were calculated. Individual values obtained in the measurements were totaled at each time of measurement, and group mean values and standard errors were calculated. Statistical Analysis: No statistical analysis was performed		
Results: At the highest dose levels, sedation by propofol (0.2 mg/kg/min) was the deepest followed by remimazolam (50 μg/kg/min) and dexmedetomidine (0.2 μg/kg/min). The depth of sedation caused by midazolam was about three quarters of that by remimazolam at the same dose level (50 μg/kg/min). Remimazolam sedated the animals more quickly than any of the other drugs. Remimazolam at 50 μg/kg/min increased mean blood pressure (10%-12%) during administration, and at 12.5-50 μg/kg/min it increased heart rate (20%-51%) and tended to shorten PR interval during and after administration. These changes were noted at induction of and recovery from anesthesia. No abnormal changes were observed in the ECG during or for 75 min after infusion of remimazolam (12.5-100 μg/kg/min; 15 min). Also, neither changes in arterial blood gas attributable to the drugs, nor any adverse effects on the respiratory system were noted. At a higher dose (100 μg/kg/min), tested to evaluate the effects of an overdose, remimazolam caused no changes in arterial blood gas but decreased blood pressure (10%-14%) and heart rate (22%-27%) at the onset of anesthesia. Propofol, midazolam, and dexmedetomidine had no effects on arterial blood gas, but caused some changes in blood pressure, heart rate, and electrocardiographic parameters accompanying anesthesia induction and/or excitation during recovery from anesthesia. Of note, propofol decreased blood pressure at 0.05 mg/kg/min, at which its sedative effects were relatively weak, and dexmedetomidine at 0.2 μg/kg/min increased blood pressure and heart rate during and after administration and markedly prolonged QT interval and QTc interval after administration.		
Conclusion: In conclusion, remimazolam, at an effective dose, sedated the animals more quickly than the three comparator anesthetics, without major effect on the respiratory or cardiovascular systems.		

2.6.3.4.2 安全性薬理試験（続き）

Table 2.6.3.4.2-14	Safety Pharmacology	Test Article: Remimazolam
試験表題：ONO-2745BS の覚醒下カニクイザルにおける心血管及び呼吸系に対する安全性薬理試験		
Route of Administration: i.v.	Location in CTD: 4.2.1.3.14	Study Number: [redacted]
GLP Compliance: GLP complaint		
Aim of Study: To assess the effects of remimazolam on the cardiovascular and respiratory systems (only respiratory rate) administered intravenously to conscious cynomolgus monkeys using different dosing techniques. Study Design: The effect of remimazolam on blood pressure, heart rate, electrocardiogram (ECG), respiratory rate and intra-abdominal body temperature, monitored via a telemetry system, was compared to vehicle control and to the positive control astemizole. In addition, clinical signs were observed as cage-side and as video observation. Remimazolam was administered at 4 different doses in the bolus and infusion dosing group. The study was conducted using a Latin square design in both groups.		
Species (Gender, Strain, Supplier, n/Group): Cynomolgus monkeys (male, purpose-bred, suppliers: China National Scientific Instruments & Materials Import /Export Corporation, Guangzhou Kesen Imports & Exports CO., LTD, Guangxi Grandforest Scientific Primate Company, Ltd., Guangdong Scientific Instruments & Materials Import / Export Corporation), a total of 10 monkeys: group 1 (n = 5) and group 2 (n = 5) Age/Body Weight: 3-5 years / 3.39-4.37 kg Conditioning and Surgical Pre-treatment: Prior to study, implantation takes place (of a telemetry transmitter, a catheter for the measurement of blood pressure and lead wires for the recording of the ECG) and an antibiotic and buprenorphine hydrochloride were administered IM for 3 d, including the day of surgery, to prevent infection and to palliate pain, respectively. During the recovery period [from the surgery to the day before the first dosing day (Day 1)] of 21 to 23 days, the animals were observed for clinical signs once daily, and intra-abdominal body temperature, blood pressure, and ECG waveforms were visually confirmed and briefly (at least 10 min) recorded once using the telemetry system		
Test Compounds (Batch No) and Test Conditions (Formulation, Doses, Route of Administrations):		
Remimazolam	Batch No: SOL12621/6, white crystalline powder. Doses group 1 (i.v. bolus injection 3 mL/min): 0, 0.5, 1.0, 2.0 and 5.0 mg/kg in a volume of 2.5 mL/kg; Group 2 (infusion 6 mL/kg/6h): 0, 6, 18, 30 and 60 mg/kg. Dosing was performed with a wash out period between the doses of 7 days (day 1, 8, 15, 22 and 29).	
Astemizole	Positive control, Batch No: 113K0704, diluted in 0.5w/v% methylcellulose aqueous solution. 10 mg/kg, 5 mL/kg administered orally (in the bolus dosing group on day 36)	
Control 1 Control 2	Vehicle for bolus dosing: 25.86 w/v% sulfobutyl ether ß-cyclodextrin solution, i.v., 2.5 mL/kg, speed: 3 mL/min Vehicle for infusion dosing: 0.9% NaCl solution (Batch No: 7K99N and 8A94N), i.v., 6 mL/kg/6h	

2.6.3.4.2 安全性薬理試験（続き）

Table 2.6.3.4.2-14 (cont.)	Safety Pharmacology	Test Article: Remimazolam
試験表題：ONO-2745BS の覚醒下カニクイザルにおける心血管及び呼吸系に対する安全性薬理試験		
Route of Administration: i.v.	Location in CTD: 4.2.1.3.14	Study Number: [redacted]
GLP Compliance: GLP complaint		
Method of Evaluation: Blood pressure, heart rate, ECG, respiratory rate, and intra-abdominal body temperature were analysed, using a telemetry system, 2 and 1 h before dosing and in the bolus group immediately, 5 and 20 min and 1, 2, 6, and 12 h after dosing and in the infusing group 0.5, 1, 3 and 6 h after the start and 0.5, 1, 2 and 6 h after the end of dosing. The actual values and the % change from the pre-dosing values (or differences from the pre-dosing values of intra-abdominal body temperature) were expressed as mean value ± standard error (S.E.) at each time point. Statistical Analysis: ANOVA was performed using DOSE as a factor, between control and test article at each time point of the actual values. If significant differences were observed in DOSE, Dunnett's test was performed between the control and test article (at a 2-sided significance level of 5%). A paired t-test was performed between control 1 and the positive control to compare the actual values at each time point (2-sided 5% level) for the bolus dosing group. Statistical analysis was not performed on clinical signs.		
Results: Bolus dosing: Remimazolam had no effect on heart rate, any ECG parameter (PR interval, QRS duration, RR interval, QT interval, and QTc), or respiratory rate at any dose level. At 2 and 5 mg/kg, there were transient and slight but statistically significant decreases in systolic, diastolic (only 5 mg/kg) and mean blood pressure immediately after dosing when compared with control article-1. In addition, there were statistically significant decreases in intra-abdominal body temperature 20 min and 1 h after dosing at these dose levels when compared with control article-1 with maximum changes of 0.58°C at 2 mg/kg and 1.28°C at 5 mg/kg at 1 h post-dose. In clinical signs, difficulty in standing up, ataxic gait, decrease in spontaneous activity (slight) were observed at 0.5 mg/kg and higher. Incomplete eyelid opening or retchingwere observed at 1 mg/kg and higher. Crouching, lateral position, prone position, disappearance of touch response, or eyelid closure were observed at 2 and 5 mg/kg. At 10 mg astemizole/kg, prolongations of QT interval and QTc were observed and QTc showed the maximum prolongation at 2 h after dosing (122.0% of pre-dosing value). Infusion dosing: Remimazolam had no effects on blood pressure, heart rate, or respiratory rate at any dose level. There were statistically significant decreases in intra-abdominal body temperature between 1 h after the start of dosing and 0.5 h or 1 h after the end of dosing (6.5 or 7 h after the start of dosing) at 18 mg/kg and higher when compared with control-2. Decreases in intra-abdominal body temperature showed the maximum changes at 3 h after the start of dosing at these dose levels, and there with the were decreases of 1.76°C at 18 mg/kg, 2.02°C at 30 mg/kg, and 2.50°C at 60 mg/kg when compared pre-dosing values. At 18 mg/kg and higher, prolongation of QTc based on decrease in intra-abdominal body temperature was observed; however, remimazolam had no direct effects on any ECG parameter. In clinical signs, crouching, difficulty in standing up, ataxic gait, decrease in spontaneous activity (slight), or vomiting was observed at 6 mg/kg and higher. Sitting, lateral and prone positions, or suppressed response to stimulation was observed at 1 mg/kg and higher. Supine position, disappearance of touch response, somnolence, or retching was observed at 30 and 60 mg/kg.		
Conclusion: After rapid administration (bolus group) remimazolam produced a transient and slight decrease in blood pressure and decrease in intra-abdominal body temperature at 2 mg/kg and higher. There were no effects on heart rate, any ECG parameter including QT interval and QTc or respiratory rate at any dose level. Likewise, i.v. infusion (6, 18, 30 and 60 mg/kg over 6 h) was not associated with effects on blood pressure, heart rate, any ECG parameter or respiratory rate at any dose level. A decrease in intra-abdominal body temperature and prolongation of QTc based on it were observed at 18 mg/kg and higher.		

2.6.3.4.2 安全性薬理試験（続き）

Table 2.6.3.4.2-15	Safety Pharmacology	Test Article: Remimazolam
試験表題：ON0-2745BS の覚醒下カニクイザルにおける呼吸系に対する安全性薬理試験		
Route of Administration: i.v.	Location in CTD: 4.2.1.3.15	Study Number: [redacted]
GLP Compliance: GLP complaint		
Aim of Study: To assess the effects of remimazolam on the respiratory systems (blood gas parameters) administered intravenously to conscious cynomolgus monkeys using different dosing techniques. Study Design: The effect of remimazolam on the blood gas parameters (arterial blood pH, arterial oxygen, carbon dioxide tensions, and hemoglobin oxygen saturation) measured by using a clinical analyzer was compared to vehicle control. In addition, clinical signs were observed as cage-side observation. Remimazolam was administered in 4 different doses in the bolus and infusion dosing group, using a crossover design in both groups.		
Species (Gender, Strain, Supplier, n/Group): Cynomolgus monkeys (male, purpose-bred, supplier: Guangxi Grandforest Scientific Primate Company, Ltd, a total of 8 monkeys: group 1 (n = 4) and group 2 (n = 4)) Age/Body Weight: 4-5 years / 4.05-4.76 kg Conditioning and Surgical Pre-treatment: Prior to study, a catheter was implanted and an antibiotic and buprenorphine hydrochloride were administered IM for 3 d, including the day of surgery, to prevent infection and to palliate pain, respectively. During the recovery period (from the surgery day to the day before the first dosing day) of 9 to 10 days the animals were observed for clinical signs (including at the surgery sites) once daily.		
Test Compounds (Batch No) and Test Conditions (Formulation, Doses, Route of Administrations):		
Remimazolam	Batch No: SOL12621/6, white crystalline powder. Doses group 1 (i.v. bolus injection 3 mL/min): 0, 0.5, 1.0, 2.0 and 5.0 mg/kg and a volume of 2.5 mL/kg; Group 2 (infusion 6 mL/kg/6h): 0, 6, 18, 30 and 60 mg/kg. Dosing was performed with a wash out period between the doses of 7 days.	
Control 1 Control 2	Vehicle for bolus dosing: 25.86 w/v% sulfobutyl ether ß-cyclodextrin solution, i.v., 2.5 mL/kg, speed: 3 mL/min Vehicle for infusion dosing: 0.9% NaCl solution (Batch No: 9C88N), i.v., 6 mL/kg/6h	
Method of Evaluation: Blood gas parameters were analysed immediately after sampling, once before dosing and in the bolus group immediately, 5 and 20 min and 1 and 6 h after dosing and in the infusing group 1, 3, 6, 6.5 and 12 h after the start of dosing. The values for blood gas parameters are expressed as mean value ± standard error (S.E.) for each time point. Statistical Analysis: ANOVA was performed using DOSE as a factor, to make a comparison between control article and test article at each time point. When a significant difference was observed with DOSE, Dunnett's test was performed to compare control article and test article. These tests were conducted at a 2-sided significance level of 5%. Statistical analysis was not performed on clinical signs.		

2.6.3.4.2 安全性薬理試験（続き）

Table 2.6.3.4.2-15 (cont.)	Safety Pharmacology	Test Article: Remimazolam
試験表題：ON0-2745BS の覚醒下カニクイザルにおける呼吸系に対する安全性薬理試験		
Route of Administration: i.v.	Location in CTD: 4.2.1.3.15	Study Number: [redacted]
GLP Compliance: GLP complaint		
Results: Bolus dosing: Remimazolam had no effect on arterial blood pH, arterial oxygen tension, or hemoglobin oxide saturation at any dose level. However, at 5 mg/kg, carbon dioxide gas partial pressure increased by approximately 5 mmHg ($p<0.01$) at 20 min post-dosing. The slight increase of approximately 3 mmHg ($p<0.05$) at 0.5 mg/kg was considered to be not test article related since the values were within the range of variation in arterial carbon dioxide tension at dosing of the control article in all animals. In clinical signs observations, incomplete eyelid opening, eyelid closure, suppressed response to stimulation, disappearance of touch response, and/or somnolence were observed at 0.5 mg/kg and greater. Ataxic gait was observed at 2 mg/kg and greater, sitting position and difficulty in standing up were observed at 5 mg/kg. Infusion dosing: Remimazolam had no effects on arterial blood pH, arterial oxygen tension, or hemoglobin oxygen saturation at any dose level. Arterial carbon dioxide tension increased slightly at 18 mg/kg/6h and greater. In clinical signs observations, ataxic gait was observed at 6 mg/kg/6h and greater, sitting position, lateral position, difficulty in standing up, eyelid closure, suppressed response to stimulation, disappearance of touch response, and/or somnolence were observed at 18 mg/kg/6h and greater.		
Conclusion: After rapid and continuous administration remimazolam produced a slight increase in arterial carbon dioxide tension at 5 mg/kg and 18 mg/kg/6h and greater, respectively. However, it was considered that the effect on the respiratory system was weak since remimazolam had no effect on any other respiratory parameter.		

2.6.3.5 薬力学的薬物相互作用試験

Co-dministration with	Effects on	Species	Method of Admin.	Doses test article	Gender and No. per Group	Noteworthy Findings	Study Number	Location in CTD
Fentanyl (7, 10 µg/kg, i.v.)	Sedation (LRR)	Rat	i.v.	0, 2, 5, 10, 20 mg/kg, or 2, 10, 30 mg/kg	M n = 8	Remimazolam (≥20 mg) alone dose-dependently induced LRR. Fentanyl significantly enhanced this effect. A similar enhancing effect was observed with midazolam (10, 30 mg/kg) and co-administered fentanyl.	[redacted]	4.2.1.4.1
Fentanyl (5 µg/kg, i.v.)	Sedation (LRR)	Rat	i.v.	0, 0.1, 0.2, 0.5, 1 mg/kg	M n = 10	The sedative effect of remimazolam was synergistically enhanced by co-administration of fentanyl, the ED_{50} was reduced by 91%. The ED_{50} of midazolam and propofol dropped by 95 and 60%, respectively.	[redacted]	4.2.1.4.2
Fentanyl (1, 2, 5 µg/kg, i.v.)	Analgesia (heat avoidance latency)	Rat	i.v.	0, 5 mg/kg	M n = 8	Remimazolam did not influence the analgesic effect of fentanyl.	[redacted]	4.2.1.4.3
Remifentanil (5 µg/kg, i.v.)	Sedation (LRR)	Rat	i.v.	0, 0.2, 0.5, 1, 2 mg/kg	M n = 10	The sedative effect of remimazolam was synergistically enhanced by co-administration of remifentanil, the ED_{50} was reduced by 89%. The ED_{50} of midazolam and propofol dropped by 93 and 58%, respectively.	[redacted]	4.2.1.4.4
Remifentanil (1, 2, 5 µg/kg, i.v.)	Analgesia (heat avoidance latency)	Rat	i.v.	0, 5 mg/kg	M n = 8	Remimazolam did not influence the analgesic effect of remifentanil.	[redacted]	4.2.1.4.5

2.6.3.5 薬力学的薬物相互作用試験（続き）

Co-dministration with	Effects on	Species	Method of Admin.	Doses test article	Gender and No. per Group	Noteworthy Findings	Study Number	Location in CTD
Remifentanil (1, 2, 5, 10 μg/kg, i.v.)	Analgesia (heat avoidance latency)	Rat	i.v.	0, 5 mg/kg	M n = 8	Remimazolam did not influence the analgesic effect of remifentanil.	[redacted]	4.2.1.4.6
Propofol (2 mg/kg, i.v.), Dexmedetomidine (5 μg/kg, i.v.) Thiamylal (5 mg/kg, i.v.) Midazolam (10 mg/kg, i.v.)	Sedation (LRR)	Rat	i.v.	0, 0.1, 0.2, 0.5, 1, 2 mg/kg	M n = 10	The sedative effect of remimazolam was synergistically enhanced by co-administration of propofol, dexmedetomidine and thiamylal, the ED_{50} values decreased by 86%, 95%, and 86%, respectively. No synergistic potentiation of the sedative effect was observed with midazolam, probably due to the same mechanism of action.	[redacted]	4.2.1.4.7
Hydroxyzine (70 mg/kg, s.c.)	Sedation (LRR)	Rat	i.v.	0, 0.1, 0.2, 0.5, 1, 2 mg/kg	M n = 10	The sedative effect of remimazolam was synergistically enhanced by co-administration of hydroxyzine, the ED_{50} value decreased by 77%.	[redacted]	4.2.1.4.8
Atropine (1 mg/kg, i.v.)	Sedation (LRR)	Rat	i.v.	0, 1, 2, 5, 10 mg/kg	M n = 10	Co-administration of atropine did not alter the sedative dose of remimazolam indicating lacking interaction.	[redacted]	4.2.1.4.9
Ketamine (2 mg/kg, i.v.)	Sedation (LRR)	Rat	i.v.	0, 0.1, 0.2, 0.5, 1 mg/kg	M n = 10	The sedative effect of remimazolam was synergistically enhanced by co-administration of ketamine, the ED_{50} value decreased by 93%.	[redacted]	4.2.1.4.10
Sevoflurane [0.4% (MAC); 0.4% (LRR) via inhalation]	Anesthesia (MAC); Sedation	Rat	i.v.	0, 1, 2, 5 mg/kg (MAC); 0, 0.1, 0.2, 0.5 mg/kg (LRR)	M n = 5-6	Preliminary study to optimize conditions and parameters for anesthetic and sedative effects using using MAC and LRR for evaluation, and propofol and midazolam as control substances.	[redacted]	4.2.1.4.11

2.6.3.5 薬力学的薬物相互作用試験（続き）

Co-dministration with	Effects on	Species	Method of Admin.	Doses test article	Gender and No. per Group	Noteworthy Findings	Study Number	Location in CTD
Sevoflurane 0.4% via inhalation)	Sedation (LRR)	Rat	i.v.	0, 0.1, 0.2, 0.5 mg/kg	M n = 10	The sedative effect of remimazolam was synergistically enhanced by co-administration of sevoflurane. The $ED_{50(LRR)}$ of remimazolam decreased by 94% from 3.1 (alone) to 0.19 upon sevoflurane co-administration.	[redacted]	4.2.1.4.12
Sevoflurane (1.2% or 2.4% via inhalation)	Blood pressure, heart rate	Rat	i.v.	0, 5, 10 mg/kg	M n = 7	Remimazolam (5 and 10 mg/kg) alone decreased blood pressure and increased heart rate. Sevoflurane 1.2% had no effect whereas 2.4% enhanced the remimazolam induced decrease of blood pressure. The increase of heart rate was significantly attenuated by both sevoflurane concentrations.	[redacted]	4.2.1.4.13
				0, 10, 20 mg/kg midazolam	n = 4	Midazolam (10 and 20 mg/kg) alone decreased blood pressure and increased heart rate. Sevoflurane did not modify the decrease on blood pressure, but attenuated the increase of heart rate.		

2.6.3.5 薬力学的薬物相互作用試験（続き）

Co-dministration with	Effects on	Species	Method of Admin.	Doses test article	Gender and No. per Group	Noteworthy Findings	Study Number	Location in CTD
Fentanyl (5 µg/kg, i.v.) Remifentanil (5 µg/kg, i.v.)	Blood pressure, heart rate	Rat	i.v.	0, 1, 2, 5, 10, 20, 30 mg/kg (depending on co-admin-istration)	M n = 8	When remimazolam or propofol was administered alone, the doses which elicited the $ED_{20(BP)}$ were ≥30 and 9.4 mg/kg, respectively. When remimazolam and propofol were co-administered with fentanyl, the $ED_{20(BP)}$ values were 8.2 and 3.3 mg/kg, respectively, or when co-administered with remifentanil, ≥20 and 6.8 mg/kg, respectively. The separation ratios between the $ED_{50(LRR)}$ and the $ED_{20(BP)}$ were ≥7.7- and 2.1-fold (remimazolam or propofol alone), 19- and 1.7-fold (co-administration with fentanyl), or ≥43- and 4.5-fold (co-administration with remifentanil), respectively. It was concluded, that the separation ratio for remimazolam was larger than that for propofol, and similar for that following co-administration with an analgesic (fentanyl and remifentanil).	[redacted]	4.2.1.4.14

2.6.3.5 薬力学的薬物相互作用試験（続き）

Co-dministration with	Effects on	Species	Method of Admin.	Doses test article	Gender and No. per Group	Noteworthy Findings	Study Number	Location in CTD
Dopamine (30 µg/kg, i.v.) Phenylephrine (3 µg/kg, i.v.)	Blood pressure, heart rate	Rat	i.v.	0, 10 mg/kg	M n = 8	Remimazolam alone decreased slightly the blood pressure by 1.7% and 0.5%, whereas after combination with dopamine or phenylephrine the blood pressure was significantly increased by 13.2% and 14.1%, respectively. Remimazolam alone produced an increased heart rate (23.6% or 15.0%), whereas the change (%) in heart rate after the combination with dopamine (5.3%) or with phenylephrine (-3.0%) was significantly lower. It was concluded, that the decreased blood pressure and increased heart rate induced by remimazolam were attenuated by combination with dopamine or phenylephrine.	[redacted]	4.2.1.4.15
Landiolol (30 mg/kg, i.v.) Lidocaine (5 mg/kg, i.v.)	Blood pressure, heart rate	Rat	i.v.	0, 10 mg/kg	M n = 8	The decreased blood pressure induced by remimazolam (-4.3%) was synergistically enhanced by the co-administration with landiolol (-10.4%). There were no effects of the combined administration with lidocaine (-9.4%) compared to remimazolam alone (-10.4%). The increased heart rate after remimazolam alone (15.0% or 13.4%) was attenuated by the combined administration with landiolol (-1.3%) and lidocaine (2.9%), respectively. The increased heart rate after remimazolam alone (15.0% or 13.4%) was attenuated by the combined administration with landiolol (-1.3%) and lidocaine (2.9%), respectively.	[redacted]	4.2.1.4.16

2.6.3.5 薬力学的薬物相互作用試験（続き）

Co-dministration with	Effects on	Species	Method of Admin.	Doses test article	Gender and No. per Group	Noteworthy Findings	Study Number	Location in CTD
Milrinone (100 µg/kg, i.v.) Digoxin (1 mg/kg, i.v.)	Blood pressure, heart rate	Rat	i.v.	0, 10 mg/kg remimazolam	M n = 8	The decrease of blood pressure (-10.2%) produced by co-administration of remimazolam with milrinone was suggested as being synergistic because neither the administration of remimazolam (1.0%) nor of milrinone (-2.6%) caused a significant effect. There were no significant effects of the combined administration with digoxin (-8.2%) compared to remimazolam alone (-0.4%). The increased heart rate after remimazolam alone (25.4%) was attenuated by digoxin (7.5%), whereas milrinone (20.3% vs. remimazolam alone 19.8%) was ineffective.	[redacted]	4.2.1.4.17
Nicardipine (100 µg/kg, i.v.) Nitroglycerin (300 µg/kg, i.v.) Alprostadil (30 µg/kg, i.v.)	Blood pressure, heart rate	Rat	i.v.	0, 10 mg/kg remimazolam	M n = 8	The decreased blood pressure induced by remimazolam (-2.4%, -2.1% or -1.4%) was synergistically enhanced by the co-administration with nicardipine (-24.9%), nitroglycerin (-23.1%) and alprostadil (-27.0%), respectively. The increased heart rate after remimazolam alone (13.4%) was attenuated by nicardipine (8.0%), whereas nitroglycerin (15.5% vs. remimazolam alone 12.1%) or alprostadil (31.3% vs. remimazolam alone 26.5%) were ineffective.	[redacted]	4.2.1.4.18

2.6.3.5 薬力学的薬物相互作用試験（続き）

Co-dministration with	Effects on	Species	Method of Admin.	Doses test article	Gender and No. per Group	Noteworthy Findings	Study Number	Location in CTD
Rocuronium (0.5, 1, 2 mg/kg, i.v.) Suxamethonium (0.1, 0.2, 0.5 mg/kg, i.v.) Suxamethonium (0.1, 0.2, 0.5 mg/kg, i.v.) Rocuronium (4 mg/kg, i.v.)+ neostigmine (0.1 mg/kg, i.v.) Rocuronium (4 mg/kg, i.v.)+ sugammadex (10 mg/kg, i.v.)	Muscle contraction (electrical stimulation of sciatic nerve)	Rat	i.v.	0, 5, 10, 20 mg/kg	M n = 2-6	Preliminary study to set conditions for a test for evaluating muscle relaxant action. Remimazolam (5-20 mg/kg) alone did not affect the muscle contraction and showed no muscle relaxant action. Rocuronium (0.5-2 mg/kg) and also suxamethonium (0.1-0.5 mg/kg) induced dose dependent muscle relaxant action which were reversed by neostigmine and sugammadex, respectively. Remimazolam (10 mg/kg) does not influence the muscle relaxant action of rocuronium or suxamethonium and the muscle relaxant reversal action of neostigmine or sugammadex.	[redacted]	4.2.1.4.19
Fentanyl (7.5, 12, 25 μg/kg/h, i.v.) Remifentanil (7.5, 15 μg/kg/h, i.v.)	Sedation (general sedation and ptosis score, pinna reflex)	Micropig	i.v.	0, 0.4, 0.8, 1.6, 3.2, 6.4 μg/kg/h dose escalation every 30 min	M n = 2	Administration of fentanyl or remifentanil led to changes in excitation-like symptoms (increased motor activity and sniffing behavior), irrespective of co-administration of remimazolam, propofol, or midazolam. Therefore, the test system was deemed inappropriate to investigate interactions with opioid analgesics.	[redacted]	4.2.1.4.20
Remifentanil (35 μg/kg/h, i.v.)	Sedation (sedation and ptosis score)	Cynomolgus monkey	i.v.	0, 0.5, 1, 2, 5, 10 mg/kg/h dose escalation every 20 min	M n = 6	The sedative effect of remimazolam was synergistically enhanced by co-administration of remifentanil, the sedative dose was reduced by 92%. Sedative doses of midazolam and propofol decreased by 98% and 58%, respectively.	[redacted]	4.2.1.4.21

*: GLP not required

$ED_{20\ (BP)}$: dose at which 20% drop in mean blood pressure was observed, $ED_{50(LRR)}$: dose at which 50% of animals had LRR, i.v.: intravenous, LRR: loss of righting reflex, M: male, MAC: minimal alveolar concentration, s.c.: subcutaneous.