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Enhanced bioavailability of a new class of dopamine D-1 antagonists following oral administration of their carbamic acid ester prodrugs to dogs

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Summary

Using specific high-pressure liquid chromatography (HPLC) methods, the pharmacokinetics of NNC 0112 and NNC 0756 were studied in mongrel dogs. Both compounds have a low oral bioavailability of 5.4 ± 3.6% (mean ± SD, $n = 4$) and 6.0 ± 0.5% ($n = 4$), respectively, due to a large first-pass metabolism. Both compounds were rapidly cleared from the body with a total body clearance of 26.0–26.5 ml/min per kg, and terminal elimination half-lives of approx. 2 h. The high first-pass metabolism could be reduced using various mono- and disubstituted carbamate ester prodrugs previously characterized in vitro. The isopropyl monosubstituted carbamate ester increased the oral bioavailability of NNC 0112 3-fold, whereas the *N,N*-dimethyl-substituted carbamate ester improved the bioavailability to approx. 20%. The dimethyl- and diethyl-substituted carbamate esters of NNC 0756 increased the bioavailability to 30 and 14%, respectively, and a novel prodrug form based on the methyl ester of *N*-methylalanine improved the oral bioavailability of NNC 0756 approx. 3-fold.

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Abbreviations: SCH 23390, (+)-*R*-8-chloro-7-hydroxy-5-phenyl-3-methyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine; NNC 0112, (+)-8-chloro-7-hydroxy-5-(benzofuran-7-yl)-3-methyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine; NNC 0756, (+)-8-chloro-7-hydroxy-5-(2,3-dihydrobenzofuran-7-yl)-3-methyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine.

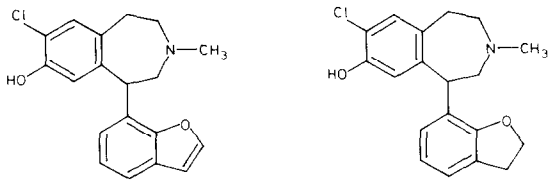
Symbols defined as follows: AUC_{0-t}, concentration of drug in plasma integrated over time (from zero to time t) after a single dose; $AUC_{t-\infty}$, concentration of drug in plasma integrated over time (from time t to infinity) after a single dose; Cl, ratio of the overall elimination rate of a drug to its concentration in plasma; F, relative amount of drug which enters the systemic circulation following oral administration; λ_1, rate constant (first order) for distribution of a drug in the body after administration; λ_2, rate constant (first order) for the elimination processes of the drug following distribution in the body; V_c, hypothetical volume of body fluid that would be required to dissolve the total amount of drug at the same concentration as that found in plasma.

Introduction

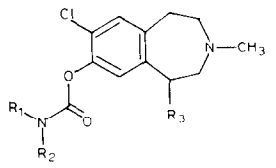
The new class of 5-substituted 8-chloro-7-hydroxy-3-methyl-2,3,4,5-tetrahydro-1*H*-3-benzazepines like SCH 23990, NNC 0112 and NNC 0756 are believed to have potential antipsychotic effect without the hormonal and neuronal side-effects known from classical neuroleptics like haloperidol (Waddington, 1988; Coffin et al., 1989; Waddington and O'Boyle, 1989; Christensen, 1990). Despite the high potency of these drugs in vitro the oral activity is low (Barnett, 1986), indicating a low bioavailability after oral administration. Studies in rats with NNC 0112 have shown that first-pass metabolism in the gut and/or liver is responsible for its low oral activity. The major metabolic inactivation was shown to be conjugation of the phenolic group at the 7-position with β-D-glucuronic acid to yield a pharmacologically inactive β-glucuronide which is excreted in the bile and urine.

It is well-known that extensive first-pass metabolism occurs to a large number of phenolic drugs or endogenous substances like morphinans, steroids and salicylates, the major metabolic pathways being glucuronidation or sulphation (George, 1981; Pond and Tozer, 1984). In a number of cases, bioreversible derivatization of the metabolically labile phenol group to produce prodrugs forms has proved to be a useful approach to reduce the extent of first-pass metabolism, and examples like the salicylate and anthranilate esters of nalbuphine (Aungst et al., 1987), naltrexone (Hussain et al., 1987; Hussain and Shefter, 1988) and β-estradiol (Hussain et al., 1988), various carbamate esters of the dopamine agonists (−)-3-PPP (Thorsberg et al., 1987) and fenoldopam (Brooks et al., 1990), and the bis(*N*,*N*-dimethyl)carbamate ester of terbutaline (Bambuterol) (Olsson and Svensson, 1984; Svensson, 1987; Svensson and Tunek, 1988; Tunek et al., 1988; Lindberg et al., 1989) have been described in the literature.

Especially interesting are disubstituted carbamate esters, since they exhibit potent inhibition of esterases. As a consequence, the labile phenol group is protected from glucuronosyl or sulphate transferase conjugation reactions during the passage of the intestinal epithelial cells which normally possess both hydrolytic activity and high metabolic capacity for conjugative reactions towards phenols (Svensson, 1987). Disubstituted carbamate esters can be bioactivated in the liver by an initial hydroxylation catalyzed by microsomal enzymes to form a chemically unstable *N*-α-hydroxyalkyl derivative which in the case of *N*,*N*-dimethylsubstituted carbamate esters spontaneously releases formaldehyde to form a monosubstituted carbamate ester. This ester is then readily hydrolyzed by plasma esterases to the parent phenol compounds (Svensson and Tunek, 1988). This is also the case for compounds **2–5** (Fig. 1) which are very potent inhibitors of plasma butyrylcholinesterase in vitro and are bioconverted exclusively to NNC 0112 or NNC 0756 by an NADPH-dependent reaction in liver microsomes from mice and rats (Hansen et al., 1991). In mice, the bioconversion could be increased by



NNC 0112 NNC 0756



	R_1	R_2	R_3
1	H	$CH(CH_3)_2$	benzofuran-7-yl
2	CH_3	CH_3	do.
3	CH_3	CH_3	2,3-dihydrobenzofuran-7-yl
4	C_2H_5	C_2H_5	do.
5	CH_3	$^{a}CH(CH_3)COOCH_3$	do.

[a] S-configuration

Fig. 1. Structural formulas of NNC 0112, NNC 0756 and the carbamate esters **1–5**.

pretreatment of the animals with phenobarbital, indicating that microsomal cytochrome P-450 enzymes from the P-450 IIB sub-family are involved in the activation (Hansen et al., 1991).

The potential of the carbamate ester prodrugs **1–5** (Fig. 1) to increase the low bioavailability of NNC 0112 and NNC 0756 in vivo was therefore investigated. In this paper, we describe the pharmacokinetics and oral bioavailability of the two 7-hydroxy-3-benzazepines following oral administration of their carbamate ester prodrugs to mongrel dogs.

Materials and Methods

Chemicals

All chemicals and solvents used were of analytical or HPLC grade. NNC 0112 and NNC 0756 were synthesized in our laboratories by Dr L.B. Hansen. The carbamate ester prodrugs **1–5** were prepared as previously described (Hansen et al., 1991).

Animal experiments

Each dog (weight 22.5–25.5 kg) was fasted overnight with free access to drinking water before dosing. Using a butterfly cannula the dogs were cannulated with a Venflon^{R} polyethylene catheter in the hindleg saphena vein for i.v. dosing and foreleg radialis vein for blood sampling. Solutions for i.v. dosing of NNC 0112 and NNC 0756 were made in 0.05 M tartaric acid containing 5% v/v propylene glycol in a total volume of 20 ml which was dosed through the i.v. catheter over a period of 2 min. Normal doses for i.v. experiments were 0.5–1.0 mg/kg. Oral dosing solutions were made in 50 ml of 0.001 N HCl with 5% of ethanol and Cremophor EL (BASF, Germany), respectively. Each dog received 2.0–4.0 mg/kg NNC 0112 or NNC 0756 and 2.0–4.0 mg/kg of compounds **1–5** (molar equivalent dose of NNC 0112 and NNC 0756, respectively) by oral gavage using a rubber stomach tube. Blood samples (10 ml) were collected into polyethylene Minisorb vials (Nunc, Denmark) from the femoral foreleg vein via the Venflon^{R} catheter. In the case of blood samples from dogs dosed with compounds **1–5**, each vial contained 1 mg of sodium fluoride to inhibit plasma esterases. The vials were placed on ice for a few minutes whereafter plasma was separated by centrifugation for 5 min at $3000 \times g$ at 4 °C. Plasma was stored frozen at −20 °C until HPLC analysis. To correct for the formation of parent compound during sample preparation and analysis a blank blood sample was spiked with the actual prodrug and then processed as described below.

Extraction of dog plasma

Method A NNC 0112 was extracted from 1.0 ml of plasma by mixing with 0.5 ml 0.5 M borate buffer pH 9.0, 0.5 ml of 80% w/v ammonium sulphate, 0.1 ml internal standard (400 ng/ml Nomifensine in 0.01 N HCl) and 4.5 ml hexane-*n*-butanol (9 : 1 v/v) in a 10 ml Minisorb vial end over end for 10 min at 30 rpm. The two phases were then separated by centrifugation for 10 min at $2000 \times g$. An aliquot (4.0 ml) of the organic phase was transferred to a 5 ml Minisorb vial and evaporated to dryness in a Hetovac vacuum concentrator (Technunc, Denmark). The residue was dissolved in 75 μl of acetonitrile-water (60 : 40 v/v) and 50 μl of the solution injected into HPLC system 1 described below.

Method B 1.0 ml plasma and 1.0 ml Krebs-Henseleit buffer pH 7.4 were mixed and applied to a Bond Elut CH (200 mg, Analytichem International) cartridge previously activated with, successively, 2.5 ml methanol, 2.5 ml ice-cold water and 2.5 ml ice-cold Krebs-Henseleit buffer. The cartridge was washed with 2.5 ml ice-cold buffer, water and 50% methanol (total volume 7.5 ml). The prodrug and parent compound were eluted with 2.0 ml acidified (acetic acid) methanol into a 5 ml Minisorb vial. Each vial was then evaporated to dryness in a Hetovac vacuum concentrator (Technunc, Denmark). The residue was dissolved in 75 μl of HPLC eluent (see description in HPLC method 2 below) and 50 μl was injected into the HPLC system. Plasma from dogs dosed orally with prodrugs **1** and **2** was extracted by this method.

Method C The procedure was the same as described for method B except that the elution from the Bond Elut CH cartridge was performed

with 2.0 ml methanol without acetic acid. Plasma from dogs dosed with NNC 0756 and compounds **3–5** was extracted by this procedure.

HPLC methods

The chromatographic system consisted of two high-pressure solvent delivery pumps (model 6000A, Waters Instruments), a UV detector (model 481, Waters Instruments) operated at 283 nm, an M730 integrator and a 720 system controller (both Waters Instruments).

HPLC method 1 NNC 0112 was chromatographed on a Spherisorb Phenyl (5 μm, 4.6 $\times$ 200 mm, Phase Separations, U.K.) analytical column using a Supelcoguard LC8-DB (5 μm, 4.6 $\times$ 10 mm, Supelco Inc., U.S.A.) as a guard column. Prior to use, the analytical column was deactivated by running an eluent containing 0.2% decylamine through the column for several hours (1 ml/min). Elution was achieved using a linear gradient of perchloric acid from 1.8 to 4.2 mM over 7 min in acetonitrile-water (80:20 v/v) at a flow rate of 1.0 ml/min. Under these conditions, NNC 0112 and Nomifensine (I.S.) eluted at 8.7 and 9.7 min, respectively. The concentration of NNC 0112 was calculated using a standard curve of the ratio between the peak height of NNC 0112 and internal standard (I.S.) using spiked plasma standards, giving a day-to-day accuracy and precision of 102.8 and 6.9% (CV) at 50 ng/ml ($n = 9$) and 98.2 and 6.8% (CV) at 250 ng/ml ($n = 4$), respectively. The detection limit was 2 ng/ml.

HPLC method 2 NNC 0756 and the carbamate esters **1–6** were chromatographed on a 4.6 $\times$ 100 mm Knauer stainless-steel cartridge packed with Spherisorb silica (3 μm, Mikrolab, Denmark) using an eluent comprising 3.9% v/v methanol, 28.8% v/v acetonitrile, 28.8% v/v tetrahydrofuran and 38.5% v/v of 5 mM sodium phosphate buffer pH 6.5. The flow rate was 1 ml/min. The use of similar mixtures of aqueous buffer and organic solvents on silica solid-phase supports has recently been described for the separation of other lipophilic basic amines (e.g. Bidlingmeyer et al., 1982; Law, 1987; Shi et al., 1987; Smith et al., 1987; Webb and Eldon, 1987). Plasma concentrations of NNC 0112, NNC 0756 and prodrug were calculated using an external standard curve of either compound in plasma.

Data analysis

A two-compartment open model with constant rate input (2 min) using an iterative non-linear regression procedure was fitted to the data following i.v. administration as previously described (Jansen et al., 1984). The absolute oral bioavailability (F) of NNC 0112 or NNC 0756 after administration of the compounds per se or after oral administration of their prodrugs was calculated using Eqn 1:

$$F = \frac{AUC_{p.o.}/D_{p.o.}}{AUC_{i.v.}/D_{i.v.}} \quad (1)$$

where $AUC_{p.o.}$ is the area under the plasma concentration-time curve of the parent compound following oral administration, $AUC_{i.v.}$ the area under the plasma concentration-time curve after i.v. dosing, and $D_{p.o.}$ and $D_{i.v.}$ the doses given orally and intravenously, respectively. $AUC_{p.o.}$ was calculated according to the trapezoidal rule, and the residual $AUC_{t\to\infty}$ was estimated using the concentration in the last plasma sample and the terminal elimination rate constant λ_2. $AUC_{i.v.}$ was obtained from the fitted curve by non-linear regression and the clearance was then calculated using the relation between dose (D) and AUC ($AUC_{i.v.} = D/Cl$).

Results

In a cross-over study, four dogs received either NNC 0112 or NNC 0756 i.v. and p.o., separated by a wash-out period of 1 week. Following i.v. administration the plasma concentration kinetics of both compounds were biphasic as shown in Fig. 2. Both compounds had a terminal half-life of approx. 2 h. The total body clearance of NNC 0112 and NNC 0756 was calculated to be 26.5 and 26.0 ml/min per kg, respectively (Table 1). Following oral administration, only low plasma concentrations of both compounds were detected (Fig. 2). NNC 0112 was found to have a bioavail-

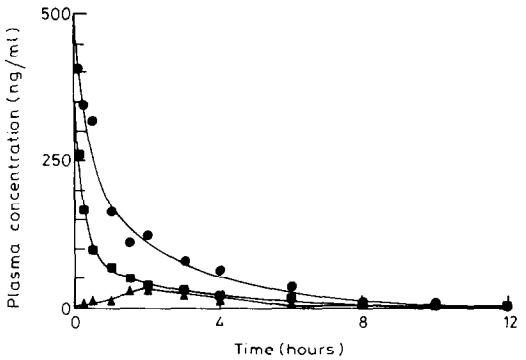


Fig. 2. Plasma concentrations of NNC 0112 from four dogs after i.v. [0.5 mg/kg (■), 1.0 mg/kg (●)] and oral administration [4 mg/kg (▲)]. The i.v. data represent the mean of two dogs at each dose level.

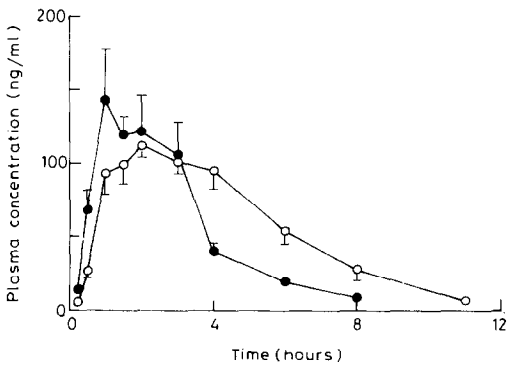


Fig. 3. Plasma concentrations of NNC 0756 (○) and 3 (●) after oral administration of **3** (equivalent to 4 mg/kg NNC 0756). Data are means (± SE) of four dogs.

ability of 5.4 ± 3.6% (mean ± SD), whereas that of NNC 0756 was equally low but with less variability, 6.0 ± 0.5%. The same dogs as used to estimate the bioavailability of these compounds were employed in the determination of the intact fraction absorbed following oral administration of their carbamate ester prodrugs **1–5** (Fig. 3). The absolute bioavailabilities determined for NNC 0112 or NNC 0756 following the administration of their prodrugs are listed in Table 2. $AUC_{i.v.}$ values for each individual from the initial pharmacokinetic study were also used to estimate the bioavailability of the carbamate ester prodrugs. This procedure was justified by comparing the area under the plasma concentration-time curve from two separate i.v. experiments that showed a negligible intra-individual variation in the pharmacokinetics of the parent compounds in all four dogs.

The monosubstituted carbamate ester **1** increased the bioavailability of NNC 0112 from 5 to 15% (Table 2). The prodrug could be detected in plasma only up to 2 h after administration and the concentrations were very low (less than 20 ng/ml plasma). In contrast, the disubstituted carbamate esters **2–5** were eliminated more slowly and could be detected in plasma up to 12 h after oral administration (Fig. 3). Following oral administration of **3–5** there was a significant increase in the plasma concentrations of NNC 0112 or NNC 0756 as compared to those after adminis-

TABLE 1

Mean (± SD) pharmacokinetic parameters for NNC 0112 and NNC 0756 after administration of a single 100 mg oral or a 12.5 or 25 mg intravenous dose to four dogs

Parameter [a]	NNC 0112	NNC 0756
V_c (l/kg) [b]	1.79 (0.54)	2.5
λ_1 (h^{-1}) [b]	3.72 (1.88)	n.d.
λ_2 (h^{-1}) [b]	0.36 (0.05)	0.43 (0.12)
$T_{1/2}$ (h) [b]	1.96 (0.29)	1.6 (0.5)
CL (ml/min per kg) [b]	26.5 (4.43)	26.0 (7.8)
F [c]	0.054 (0.036)	0.06 (0.005)

[a] Symbols: definitions as given in the final footnote on the title page.
[b] i.v. administration.
[c] Oral administration.
n.d. not determined.

TABLE 2

Absolute bioavailability (F) of NNC 0122 and NNC 0756 after oral administration of the parent compounds of their carbamate ester prodrugs 1–5 to dogs [mean (± SD)]

Compound	F (%) [a]
NNC 0112	5.4 (3.6)
NNC 0756	6.0 (0.5)
1	15 [b]
2	20 [b]
3	33.0 (5.0)
4	14 [b]
5	18 [b]

[a] $n = 4$.
[b] $n = 2$.

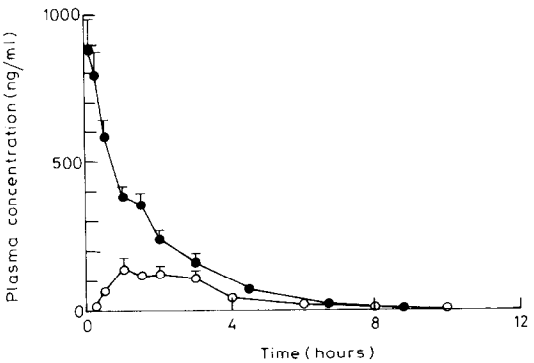


Fig. 4. Plasma concentrations of **3** following i.v. [1.0 mg/kg (●)] and oral [4.0 mg/kg (○)] administration. Data are means (±SE) of four dogs.

tration of the parent compounds (Table 2). The highest bioavailability was achieved with the *N*,*N*-dimethyl-substituted carbamate esters **2** and **3**, a prodrug form previously shown to increase the bioavailability of the drug terbutaline in dogs and humans (Olsson and Svensson, 1984; Svensson and Tunek, 1988). Furthermore, in the case of **3**, the intra-subject variation in the bioavailability of NNC 0756 was less than 15% (CV, $n = 4$) (Table 2). The first-pass metabolism of the prodrug **3** itself was examined by dosing dogs both i.v. and p.o. (1.0 mg/kg body weight). The prodrug was extensively metabolized during absorption and the bioavailability was determined to 10% after oral administration. The half-life could be calculated to be 1.4 h using the data from i.v. administration (Fig. 4).

Discussion

Both mono- and disubstituted carbamate esters were able to reduce the first-pass metabolism of the dopaminergic drugs NNC 0112 and NNC 0756 in dogs. In the case of **1** this was somewhat surprising, since the compound is chemically unstable in aqueous buffer solutions at pH 7.4 and 37 °C, the half-life being 28 min (Hansen et al., 1991). Furthermore, this degradation was shown to be catalyzed by plasma enzymes, the half-life of **1** in dog plasma being only 12.4 min (Hansen et al., 1991). However, presystemic chemical hydrolysis in the intestine could have been decreased by administration of the drugs in aqueous solutions of low pH (0.001 N HCl, pH 3.0), and a substantial fraction of the dose absorbed intact before the pH was raised by pancreatic juices. In fact, similar monosubstituted carbamate esters of the experimental drug (−)-3-PPP have been reported to increase the plasma levels of the parent phenol after oral administration to rats (Thorsberg et al., 1987). In addition, the low plasma levels of **1** observed in dogs agreed with its rapid hydrolysis in plasma. However, the results indicate that a fraction of the administered prodrug leaves the liver intact, i.e. not as glucuronidated parent compound, and then cleaves off NNC 0112 following distribution in the body.

In contrast to the monosubstituted ester **1**, the disubstituted carbamate ester prodrugs **2**–**5** could be detected in plasma for a longer period of time (Figs 3 and 4). The reason for this is most probably the high resistance of these esters to undergoing enzymatic hydrolysis combined with the fact that these compounds are extremely potent inhibitors of the major esterase in plasma, butyrylcholinesterase, with K_i values in the low nanomolar range (Hansen et al., 1991). Besides the prodrugs, prolonged plasma profiles of the parent compounds NNC 0112 and NNC 0756 were observed after oral administration of **2**–**5** (Fig. 3).

The postulated bioactivation of disubstituted carbamate esters in vivo involves an initial hydroxylation at a carbon atom in the α-position to the nitrogen atom in the carbamic acid moiety. This bioactivation mechanism has been demonstrated for the *N*,*N*-dimethyl-substituted carbamate ester of terbutaline (bambuterol) and for **3** in mouse and rat liver microsomes (Svensson and Tunek, 1988; Lindberg et al., 1989; Hansen et al., 1991). The *N*-hydroxymethyl-*N*-methyl carbamate formed then decomposes spontaneously with the release of formaldehyde. The resulting monosubstituted carbamate ester is subsequently hydrolyzed in plasma by esterases to form the parent compound (Hansen et al., 1991). First-pass metabolism of **3** itself is therefore of some importance if the metabolism does not involve the

favorable hydroxylation as the first step in bioactivation, i.e. the prodrug is metabolized at a different position in the molecule resulting in the formation of an inactive metabolite of the parent compound following hydrolysis of the prodrug. However, the substantial first-pass effect of the prodrug did not influence the bioavailability of NNC 0756, since the variation was less than 15% (CV) in the four dogs examined (Table 2). Furthermore, this first-pass effect of the prodrug **3** was shown to undergo only small variations in time when comparing bioavailability results from two independent dosage sessions using the same dogs.

Compound **5** is a novel prodrug form of phenols using *N*-methyl derivatives of naturally occurring amino acids. Several monosubstituted carbamate ester prodrugs formed with amino acid esters were shown to be highly unstable in aqueous buffer and plasma with half-lives less than 11 min. However, substitution of the N-H hydrogen atom with a methyl group as in **5** resulted in chemically stable compounds (Hansen et al., 1991). This approach is new and could open a new avenue of development for prodrug forms of phenolic compounds based on *N*-methyl L-amino acid esters. Furthermore, if the bioactivation is similar to the mechanism described for *N,N*-dimethyl-substituted carbamate esters with release of the *N*-methyl group as formaldehyde and subsequent hydrolysis to from an L-amino acid ester, it could lead to the formation of non-toxic promoieties in vivo. Actually, the resulting amino acid ester could be excreted as such or further metabolized to the natural L-amino acid. The possibility also exists that the methyl ester group in **5** is initially hydrolyzed, yielding a carbamate ester of *N*-methylalanine.

These data indicate that *N,N*-dimethyl-substituted carbamate esters can act as a prodrug form of phenols independently of the chemical structure of the parent compound. Interestingly, the closely related *N,N*-diethyl-substituted analogue **4** only increased the bioavailability of NNC 0756 approx. 3-fold, as compared to the 6-fold increase after oral administration of **3**. The reason for this difference is unknown, but it could be speculated that at least one methyl group should be present in the carbamic acid moiety for optimal bioactivation.

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