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Discovery of novel quaternary ammonium derivatives of (3*R*)-quinuclidinyl carbamates as potent and long acting muscarinic antagonists

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ABSTRACT

Novel quaternary ammonium derivatives of *N*,*N*-disubstituted (3*R*)-quinuclidinyl carbamates have been identified as potent M_3 muscarinic antagonists with long duration of action in an in vivo model of bronchoconstriction. These compounds have also presented a high level of metabolic transformation (human liver microsomes). The synthesis, structure–activity relationships and biological evaluation of these compounds are reported.

Chronic obstructive pulmonary disease (COPD) is a preventable and treatable lung disease characterised by airflow limitation that is not fully reversible.[1,2] The major reversible component of airway obstruction in COPD is believed to be cholinergic.[3,4] Inhaled anticholinergics, which increase bronchodilation by blocking muscarinic receptors on airway smooth muscle, are established treatment options for COPD.[5] Currently, only two inhaled muscarinic antagonists (Fig. 1) are available for the management of symptomatic COPD patients: ipratropium, a short-acting agent requiring up to four doses per day and tiotropium, a long-acting, once-daily treatment.[5–7]

During the course of the Almirall research program to discover a novel, long-acting muscarinic M_3 antagonist for the inhaled treatment of COPD, with a potentially improved risk-benefit profile compared with currently marketed antimuscarinic agents,[8] new *N*,*N*-disubstituted (3*R*)-quinuclidinyl carbamates and their quaternary ammonium derivatives were identified as potent antimuscarinic compounds.

Several *N*,*N*-disubstituted carbamates of 3-quinuclidinol had been described in the literature as compounds with potent anticholinergic activity.[9] During the 1990's, researchers from Yamanouchi Pharmaceuticals identified different series of quinuclidinyl carbamates, represented by the compounds YM-905 and YM-46303 (Fig. 2), as potent muscarinic antagonists with therapeutic potential for the treatment of smooth muscle contractility and tone, for example in urinary and respiratory disorders.[10–15] These Yamanouchi carbamates were described to have selectivity for the M_3 receptor in front of the M_2 one.[12–14] Compound YM-905, solifenacin, is currently marketed for the treatment of urinary incontinence.[15]

In this publication we will report the synthesis and biological evaluation of a variety of novel *N*,*N*-disubstituted (3*R*)-quinuclidinyl carbamates. These compounds were prepared as tertiary amines and evaluated for their muscarinic binding activities. Quaternary ammonium derivatives of the more interesting examples were prepared to obtain analogues with predicted low oral bioavailability, adequate for inhaled administration.[8,16] Although quaternization limits the absorption across membranes,[8,16] after inhaled administration there could be observed anticholinergic side effects associated with the binding of systemic available

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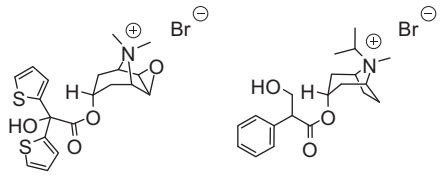


Figure 1. Inhaled muscarinic antagonists available for the treatment of COPD.

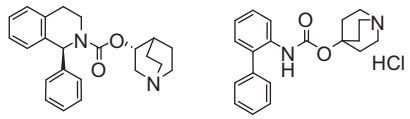
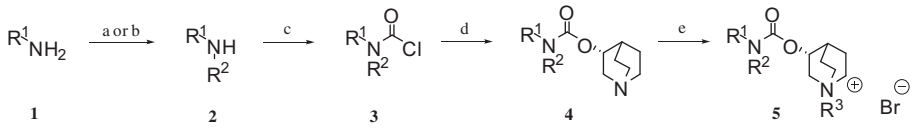


Figure 2. Examples of quinuclidinyl carbamates.

compound to muscarinic receptors outside the respiratory tract. Given the distribution of muscarinic receptors these unwanted physiological effects could be tachycardia, dry mouth, urinary retention and constipation.[17] Whereas dry mouth is the most common one[18,19] as a result of the systemic blockade of M_1 and M_3 receptors,[20] tachycardia which results from blockade of cardiac M_2 receptors[17,21] could be considered the most relevant one. For this reason compounds with selectivity for the receptor M_3 in front of the M_2 one would be safer. The influence of the quaternization in the muscarinic binding activity profile and in the duration of action in an in vivo model of bronchoconstriction will be described. The plasma stability and the metabolism in human liver microsomes of some selected analogues with long duration of action will also be reported.

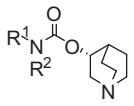
The synthetic route to the quinuclidine carbamate derivatives is outlined in Scheme 1.

Disubstituted amines of general formula **2** that are not commercially available were synthesized from amines of formula **1** according to standard methods, for example by reductive alkylation using the corresponding aldehyde, or by alkylation with the corresponding bromide. Transformation of amines of formula **2** into their carbamoyl chloride derivatives **3**[22] followed by reaction with the sodium salt of (3*R*)-quinuclidinol in refluxing toluene led to carbamates of formula **4.** Quaternary ammonium derivatives of formula **5** were obtained by reaction of selected (3*R*)-quinuclidinyl carbamates with a variety of bromoalkyl or bromoalkylaryl derivatives.



Scheme 1. Reagents and conditions: (a) (i) R^2CHO, EtOH, molecular sieves 0.3 nm, 78 °C, (ii) $NaBH_4$, 65–83%; (b) R^2Br, K_2CO_3, toluene, 110 °C, 18–32%; (c) Triphosgene, CH_2Cl_2, 10 °C to rt, 74–96%; (d) (3*R*)-quinuclidinol, Na, toluene, 110 °C, 18–33%; (e) R^3Br, THF, 66 °C, 48–82%.

Table 1
Effects of R^1 and R^2 substitution on M_3, M_2, M_1 muscarinic receptor binding activities for *N*,*N*-disubstituted-(3*R*)-quinuclidinyl carbamate derivatives



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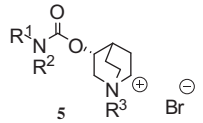
Compd	R^1	R^2	Activity: IC_{50} [a](nM)			Selectivity[b]	
			M_3	M_2	M_1	M_2/M_3	M_1/M_3
4a	Ph–	Ph–	796.8 (43.9)	2779.0[c] (1034.4)	319.4 (6.0)	3.5	0.4
4b	Ph–	Ph-CH_2–	1.2 (0.1)	3.7 (1.2)	0.4 (0.1)	3.1	0.3
4c	Ph–	Ph-$(CH_2)_2$–	3.6 (0.1)	42.1 (3.4)	1 (0.4)	11.7	0.3
4d	Ph–	(2-Th)-CH_2–	0.6 (0.1)	1.8 (0.7)	0.4 (0.3)	3.0	0.7
4e	Ph–	(3-Th)-CH_2–	1.0 (0.3)	4.7 (1.8)	0.4 (0.1)	4.7	0.4
4f	Ph–	*n*-Propyl–	71.1 (5.2)	349.3 (18.8)	26.7 (4.8)	4.9	0.4
4g	Ph–	*n*-Butyl–	4.3 (0.7)	22.9 (10.9)	1.1 (0.1)	5.3	0.2
4h	Ph–	*n*-Pentyl–	1.7 (0.0)	13.6 (5.7)	0.8 (0.4)	8.0	0.5
4i	Ph–	Isopropyl–	659.3 (138.2)	1364.9 (17.2)	428.0 (5.8)	2.1	0.6
4j	(2-Th)-CH_2-	Ph-CH_2–	22.5 (4.0)	88.9 (11.6)	8.8 (0.2)	3.95	0.4
4k	(2-Th)-CH_2–	(2-Th)-CH_2–	5.9 (0.5)	22.7 (3.2)	2.4 (0.4)	3.8	0.4
4l	(2-Th)-CH_2–	*n*-Butyl–	17.7 (1.6)	76.2 (21.9)	0.8 (0.5)	4.3	0.04
4m	(4-Me)-Ph–	Ph-CH_2–	74.7 (32.7)	195.6 (33.2)	39.7 (5.4)	2.6	0.5
4n	(4-F)-Ph–	Ph-CH_2–	15 (2.7)	115.7 (61.0)	8.3 (1.7)	7.7	0.5
4o	(3-F)-Ph–	(3-F)-Ph-CH_2–	2.4 (0.6)	26.8 (15.9)	1.5 (0.3)	11.2	0.6
4p	(3-Me)-Ph–	(2,4,5-tri-F)-Ph-CH_2–	1.4 (0.1)	45.8 (21.0)	1 (0.1)	32.7	0.7
4q	(3-F)-Ph–	(3,4,5-tri-F)-Ph-CH_2–	1.5 (0.1)	97.3 (15.0)	0.9 (0.3)	64.9	0.6
Solifenacin			130.8 (27.1)	573.7 (45.3)	63.5 (8.8)	4.4	0.5
YM-46303			6.0 (1.2)	14.5 (2.6)	3.0 (0.9)	2.4	0.5
Tiotropium bromide			0.35[d] (0.01)	0.21[d](0.04)	0.22[d](0.03)	0.6	0.63
Ipratropium bromide			2.07[d](0.03)	2.24[d](0.16)	2.65[d](0.13)	1.08	1.28

[a] Values shown are the mean, $n = 2$ (SD), standard deviation is given in parentheses.
[b] The selectivity is calculated as the ratio between the IC_{50} values.
[c] The value shown is the mean of $n = 1$ (3510 nM) and $n = 2$ (2047 nM).
[d] Values shown are the mean, $n = 3$ (SD), standard deviation is given in parentheses.

Table 2
Effects of R^3 substitution on the M_3, M_2, M_1 muscarinic receptor binding activities and in vivo duration of action for novel quaternary *N*,*N*-disubstituted-(3*R*)-quinuclidinyl carbamates



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Compd	R^1	R^2	R^3	Activity: IC_{50}[a] (nM)			Selectivity[b]		Duration of action
				M_3	M_2	M_1	M_2/M_3	M_1/M_3	*T* (h)
4b	Ph–	Ph-CH_2–	_	1.2 (0.1)	3.7 (1.2)	0.4 (0.1)	3.1	0.3	1.5[c]
5a	Ph–	Ph-CH_2–	Ph-$(CH_2)_2$–	17.7 (0.8)	50.9 (0.6)	15.1 (3.9)	2.9	0.85	<2[d]
5b	Ph–	Ph-CH_2–	Ph-$(CH_2)_3$–	0.3 (0.0)	4.0 (1.2)	1.5 (0.3)	13.3	5.0	>6[d]
5c	Ph–	Ph-CH_2–	2-Th-$(CH_2)_3$–	0.6 (0.3)	7.6 (0.6)	1.4 (0.8)	12.7	2.3	>6[d]
5d	Ph–	Ph-CH_2–	Ph-O-$(CH_2)_2$–	1.0 (0.0)	3.9 (0.3)	1.7 (0.1)	3.9	1.7	>6[d]
4d	Ph–	(2-Th)-CH_2–	_	0.6 (0.1)	1.8 (0.7)	0.4 (0.3)	3.0	0.7	<4[d]
5e	Ph–	(2-Th)-CH_2–	CH_3–	18.6 (3.9)	33.1 (6.5)	9.2 (2.8)	1.8	0.5	NE[e]
5f	Ph–	(2-Th)-CH_2–	2-Th-$(CH_2)_3$–	0.5 (0.1)	3.2 (0.5)	1.6 (0.7)	6.4	3.2	>6[d]
5g	Ph–	(2-Th)-CH_2–	Ph-O-$(CH_2)_2$–	0.3 (0.0)	1.1 (0.3)	0.4 (0.1)	3.7	1.3	>6[d]
4e	Ph–	(3-Th)-CH_2–	_	1.0 (0.3)	4.7 (1.8)	0.4 (0.1)	4.7	0.4	<4[d]
5h	Ph–	(3-Th)-CH_2–	2-Th-$(CH_2)_3$–	0.6 (0.3)	8.4 (2.6)	2.0 (0.6)	14	3.3	>6[d]
5i	Ph–	(3-Th)-CH_2–	Ph-O-$(CH_2)_2$–	0.2 (0.0)	1.6 (0.2)	0.4 (0.1)	8.0	2	>6[d]
4g	Ph–	*n*-Butyl–	_	4.3 (0.7)	22.9 (10.9)	1.1 (0.1)	5.3	0.2	<1[d]
5j	Ph–	*n*-Butyl–	Ph-$(CH_2)_2$–	19.0 (0.3)	120.8 (28.8)	33.3 (5.2)	6.3	1.7	NE[e]
5k	Ph–	*n*-Butyl–	Ph-$(CH_2)_3$–	0.8 (0.2)	17.7 (3.8)	2.5 (0.9)	22.1	3.1	NE[e]
5l	Ph–	*n*-Butyl–	2-Th-$(CH_2)_3$–	0.6 (0.0)	10.9 (1.8)	2.0 (0.1)	18.2	3.3	>6[d]
5m	Ph–	*n*-Butyl–	Ph-O-$(CH_2)_2$–	0.8 (0.1)	8.9 (0.9)	1.4 (0.0)	11.1	1.7	>6[d]
4p	(3-Me)-Ph–	(2,4,5-tri-F)-Ph-CH_2–	_	1.4 (0.1)	45.8 (21.0)	1 (0.1)	32.7	0.7	NE[e]
5n	(3-Me)-Ph–	(2,4,5-tri-F)-Ph-CH_2–	Ph-$(CH_2)_3$–	0.9 (0.1)	29.8 (5.7)	3.0 (0.3)	33.1	3.3	<6[d]
5o	(3-Me)-Ph–	(2,4,5-tri-F)-Ph-CH_2–	Ph-O-$(CH_2)_2$–	1.2 (0.4)	28.0 (10.1)	2.4 (0.5)	23.3	2.0	<6[d]
4q	(3-F)-Ph–	(3,4,5-tri-F)-Ph-CH_2–	_	1.5 (0.1)	97.3 (25.0)	0.9 (0.3)	64.9	0.6	NE[e]
5p	(3-F)-Ph–	(3,4,5-tri-F)-Ph-CH_2–	Ph-O-$(CH_2)_2$–	0.6 (1.2)	14.4 (2.6)	1.1 (0.9)	24.0	1.8	>6[d]
Tiotropium bromide				0.35[f] (0.01)	0.21[f] (0.04)	0.22[f] (0.03)	0.6	0.63	>6[d]
Ipratropium bromide				2.07[f] (0.03)	2.24[f] (0.16)	2.65[f] (0.13)	1.08	1.28	3.4[g]

[a] Values shown are the mean, *n* = 2 (SD), standard deviation is given in parentheses.
[b] The selectivity is calculated as the ratio between the IC_{50} values.
[c] Administered concentration of 3 mg/mL, *n* = 4–6 animals.
[d] Administered concentration of 1 mg/mL, *n* = 4–6 animals.
[e] NE = not evaluated.
[f] Values shown are the mean, *n* = 3 (SD), standard deviation is given in parentheses.
[g] Administered concentration of 0.3 mg/mL, *n* = 4–6 animals.

The results presented in Table 1 summarize the influence of R^1 and R^2 substituents on the muscarinic binding activities (IC_{50}) for a series of compounds of formula **4**. The IC_{50} values for solifenacin, YM-46303, tiotropium bromide and ipratropium bromide were also determined for reference.[23]

For the group of compounds where R^1 is a phenyl group a significant influence of the R^2 substituent was observed. Compound **4a**, wherein R^2 is a phenyl group, presented low binding potencies at the muscarinic M_3, M_2, M_1 receptors. Replacement of the phenyl substituent in R^2 by a benzyl group (compound **4b**), a 2-thienylmethyl group (compound **4d**) and a 3-thienylmethyl group (compound **4e**) resulted in a great improvement of the activity. In order to understand the behaviour of these compounds, a qualitative 4-point pharmacophore model (two hydrophobic-aromatic rings, one hydrogen bond acceptor and one positive charged, protonated or quaternized, amine) was generated using a training set of reference compounds.[24] Benzyl and thienyl-methyl derivatives presented a good overlap with the pharmacophore. By contrast phenyl derivative lacked one of the pharmacophoric centers. Compound **4d** wherein R^2 is the 2-thienylmethyl substituent showed the highest activity at the M_3 receptor with an IC_{50} M_3 of 0.6 nM, 10-fold lower than YM-46303 and in the range of tiotropium bromide. For the subset of analogues wherein R^2 is an alkyl group, the best values were obtained for compounds **4g** and **4h** (R^2: *n*-butyl and *n*-pentyl, respectively). Both *n*-butyl and *n*-pentyl derivatives presented a good overlap with the pharmacophore model described before, whereas the *n*-propyl (compound **4f**) and more clearly the isopropyl (compound **4i**) derivatives didn't fit with one of the two hydrophobic-aromatic centers. For the group of compounds where R^1 is a 2-thienylmethyl group, compound **4k**, wherein R^2 is also the 2-thienylmethyl substituent, presented the best potency at M_3 receptor (IC_{50} M_3: 5.9 nM). Among the tested substituted analogues of compound **4b,** the results obtained for examples **4m** and **4n** suggested that the *para*-substitution in R^1 was not favourable to activity. Compounds **4o**, **4p**, and **4q** maintained the high M_3 binding activity and their selectivity for the M_3 receptor over the M_2 significantly increased. In general, like the reference carbamates, examples of Table 1 showed less activity at the M_2 receptor in comparison with the activity at the M_3 one[12–14] and the binding potencies for the M_3 and M_1 receptors were in the same range.

To explore the SAR around R^3, we made a selection of the non quaternized derivatives previously described in Table 1. We chose the most active analogues (compounds **4b**, **4d**, and **4e**) and also compound **4g** as a representative example where R^2 is an alkyl group. Muscarinic receptor binding potencies (IC_{50}) for their quaternized derivatives are shown in Table 2.[23]

The evaluated derivatives of compound **4b** and **4g** presented high M_3 binding activities ($IC_{50} \leqslant 1$ nM) when R^3 was a phenylpropyl, 2-thienylpropyl and phenoxyethyl chain. When R^3 was a phenethyl chain a loss of activity was observed suggesting that a distance of three atoms between the nitrogen of the quinucli-

dine and the phenyl or thienyl ring in R^3 could be beneficial to activity. In the case of compound **4d,** its binding activity profile was retained for the quaternized derivatives **5f** and **5g** (R^3: 2-thienylpropyl and phenoxyethyl, respectively). Derivatives of compound **4e** with these two chains also presented high M_3 binding potencies. Among the tested examples, compounds **5b**, **5g**, and **5i** presented IC_{50} M_3 values comparable with that of tiotropium bromide. As in the case of the non quaternized precursors, the quaternary derivatives described in Table 2 showed less activity at the M_2 receptor in comparison with the activity at the M_3 subtype.

The in vivo duration of action was evaluated by measuring the inhibition of bronchospasm induced by acetylcholine in anesthetized guinea pigs at different time points.[25] The results are shown in Table 2 along with data for tiotropium bromide and ipratropium bromide. Duration of action (*T*, h) was defined as the time taken to recover 50% of the maximum inhibitory effect achieved by the test compound.[26]

The non quaternized compounds **4b**, **4d**, **4e**, and **4g** had short duration of action (1.5 h, <4 h, <4 h, <1 h, respectively). Quaternization of compound **4b** to obtain the derivatives **5b, 5c,** and **5d** (R^3: phenylpropyl, 2-thienylpropyl and phenoxyethyl respectively) resulted in a longer duration of action (>6 h) similar to that of tiotropium bromide. Quaternization of compounds **4d, 4e**, and **4g** with the 2-thienylpropyl group, (compounds **5f, 5h**, and **5l**, respectively) and the phenoxyethyl group (compounds **5g, 5i**, and **5m**, respectively) also resulted in a longer duration of action (>6 h). In summary, quaternization with the R^3 chains phenylpropyl, 2-thienylpropyl and phenoxyethyl, appeared to improve the duration of action of these compounds relative to the corresponding unquaternized precursors.

On the basis of the results of M_3 binding potency and duration of action, these preferred R^3 chains were applied to the substituted derivatives of compound **4b** (carbamates **4p** and **4q** described in Table 1). The synthesized quaternary compounds were evaluated for their in vitro and in vivo activities. The results are presented in Table 2. In comparison with their unquaternized precursors, the evaluated quaternized derivatives retained their high activity at the M_3 receptor and their M_2/M_3 binding profile, but only compound **5p** showed a duration of action >6 h.

A selection of compounds of Table 2 with higher M_3 receptor binding potencies and long duration of action were further characterized. To evaluate its potential for low systemic class-related effects in humans, preliminary stability studies in human plasma and metabolism in human liver microsomes were performed. The results are showed in Table 3. Tiotropium bromide and ipratropium bromide were also evaluated for reference. All the quaternized carbamates evaluated showed low level of degradation in human plasma, but the level of transformation in the metabolism assay was higher than for tiotropium bromide and ipratropium bromide in all the examples.

The values of M_3 muscarinic binding potency and duration of action, for highlighted compounds of the Almirall ester series previously reported in the literature[8] and for carbamate derivatives described in Table 3 were comparable.[23,26] A difference was observed in their corresponding M_3, M_2, M_1 binding activity profile. In the case of ester derivatives the IC_{50} values for the three receptors were similar,[8] as in the case of tiotropium and ipratropium, while in the carbamate derivatives certain degree of selectivity for the M_3 receptor in front of the M_2 one was found (a range of M_2/M_3 values from 3.7, compound **5g**, to 18.2, compound **5l**, is shown in Table 3). Regarding to human plasma stability, a wide range of values of percentage of degradation (depending on the structure of the compound) was observed in the ester series[8] while the carbamate derivatives described in Table 3 presented low levels of degradation, however their level of transformation in human liver microsomes was high, from 79% (compound **5h**) to 100% (compound **5b**).

In conclusion, novel quaternary ammonium derivatives of *N*,*N*-disubstituted (3*R*)-quinuclidinyl carbamates have been identified as potent antimuscarinic agents. They show high muscarinic M_3 receptor binding activity, higher M_2/M_3 selectivity ratio than tiotropium and ipratropium, long duration of action (comparable to that of tiotropium bromide in an in vivo model of bronchoconstriction), and higher level of metabolic transformation in human liver microsomes than the reference compounds tiotropium and ipratropium. Their predicted low oral absorption (quaternization of the tertiary amino function minimize absorption across membranes)[8,16] and their observed high level of metabolism would indicate that after inhaled administration the systemic exposition of these compounds could be very limited, and the class-related systemic effects very low.

The potential of this series of compounds for the inhaled treatment of COPD is currently under study and evaluation.

Table 3
In vitro studies of human plasma stability and metabolism in human liver microsomes for selected quaternary carbamates with higher M_3 binding activities and longer in vivo duration of action

Compound	Activity: IC_{50}[a] (nM)			Selectivity[b]		In vivo duration of action	Stability (% degradation 1 h)	Metabolism (% transformation 30 min)
	M_3	M_2	M_1	M_2/M_3	M_1/M_3	*T* (h)	Human plasma[a,c]	Human microsomes[a,d]
5b	0.3 (0.0)	4.0 (1.2)	1.5 (0.3)	13.3	5.0	>6[e]	<5	100 (0.0)
5c	0.6 (0.3)	7.6 (0.6)	1.4 (0.8)	12.7	2.3	>6[e]	<5	93 (0.18)
5d	1.0 (0.0)	3.9 (0.3)	1.7 (0.1)	12.7	2.3	>6[e]	<5	78 (0.41)
5f	0.5 (0.1)	3.2 (0.5)	1.6 (0.7)	6.4	3.2	>6[e]	6.2 (6.0)	97 (1.36)
5g	0.3 (0.0)	1.1 (0.3)	0.4 (0.1)	3.7	1.3	>6[e]	2.7 (4.5)	97 (0.37)
5h	0.6 (0.3)	8.4 (2.6)	2.0 (0.6)	14	3.3	>6[e]	<5	79 (0.07)
5i	0.2 (0.0)	1.6 (0.2)	0.4 (0.1)	8.0	2	>6[e]	<5	92 (0.85)
5l	0.6 (0.0)	10.9 (1.8)	2.0 (0.1)	18.2	3.3	>6[e]	<5	81 (0.06)
Tiotropium bromide	0.35[f] (0.01)	0.21[f] (0.04)	0.22[f] (0.03)	0.6	0.63	>6[e]	11.0 (7.5)	0.55 (0.36)
Ipratropium bromide	2.07[f] (0.03)	2.24[f] (0.16)	2.65[f] (0.13)	1.08	1.28	3.4[g]	<5	2.3 (2.4)

[a] Values shown are the mean, $n = 2$ (SD), standard deviation is given in parentheses.
[b] The selectivity is calculated as the ratio between the IC_{50} values.
[c] Experimental conditions: $T = 37$ °C, compound concentration = 5 μM.
[d] Experimental conditions: $T = 37$ °C, compound concentration = 5 μM, microsomes = 1 mg protein/mL.
[e] Administered concentration of 1 mg/mL, $n = 4$–6 animals.
[f] Values shown are the mean, $n = 3$ (SD), standard deviation is given in parentheses.
[g] Administered concentration of 0.3 mg/mL, $n = 4$–6 animals.

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