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Letter

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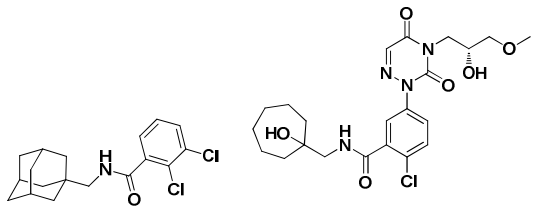
ABSTRACT: We describe the synthesis and SAR of 1,2,3-triazolopiperidines as a novel series of potent, brain penetrant P2X7 antagonists. Initial efforts yielded a series of potent human P2X7R antagonists with moderate to weak rodent potency, some CYP inhibition, poor metabolic stability and low solubility. Further work in this series, which focused on the SAR of the *N*-linked heterocycle, not only increased the potency at the human P2X7R but also provided compounds with good potency at the rat P2X7R. These efforts eventually delivered a potent rat and human P2X7R antagonist with good physicochemical properties, an excellent pharmacokinetic profile, good partitioning into the CNS and demonstrated *in vivo* target engagement after oral dosing.

Introduction. Over the past few years, numerous reports have appeared in the literature that implicate cytokines in depression and in particular that cytokines play a role in treatment resistant depression.[1, 2] As such, several laboratories have reported that reduction of IL-1β levels in mice correlates to symptom improvement in a random stress model of depression.[3] Since the activation of the P2X7 receptor (P2X7R) results in the production of IL-1β,[4] antagonists of P2X7R, which are known to block IL-1β release, are hypothesized to be useful drugs for the treatment of depression.[5]

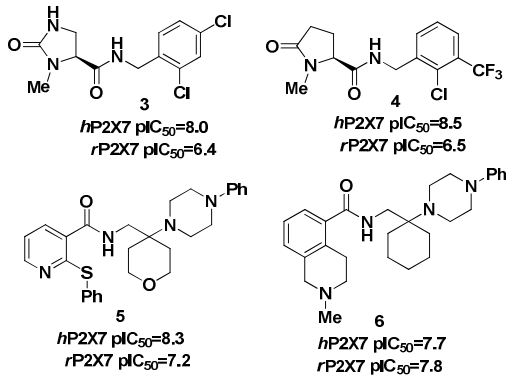
There have been numerous reports of P2X7R antagonists in the literature,[6-10] most notably the benzamide class of compounds from Astra-Zeneca (**1**)[11, 12] and a structurally distinct benzamide from Pfizer, CE-224,535 (**2**).[13] The Pfizer benzamide was tested in human clinical trials where it was shown to lower peripheral levels of IL-1β in a rheumatoid arthritis trial.[14] However, since CE-224,535 is not known to penetrate into the CNS, the effect on reduced CNS levels of IL-1β are not currently unknown. Herein, we describe a series of potent, brain penetrant P2X7R antagonists that show robust P2X7R target engagement in rodents.

Figure 1: Previously Reported P2X7 Antagonists



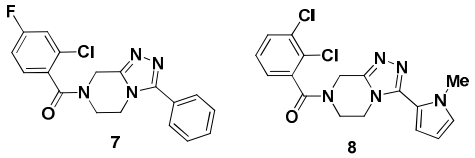
Results and Discussion. Glaxo SmithKline recently disclosed a series of brain penetrant P2X7R antagonists (**3, 4**)[6, 7] that had good affinity for the human P2X7R but only modest affinity for the rat P2X7R. We recently disclosed two selective brain penetrant P2X7R antagonists (**5, 6**) with appreciable affinity for the rat P2X7R and subsequently demonstrated robust P2X7R target engagement in the CNS of the rat as measured by ex-vivo autoradiography.[15, 16]

Figure 2: Brain Penetrant P2X7R Antagonists



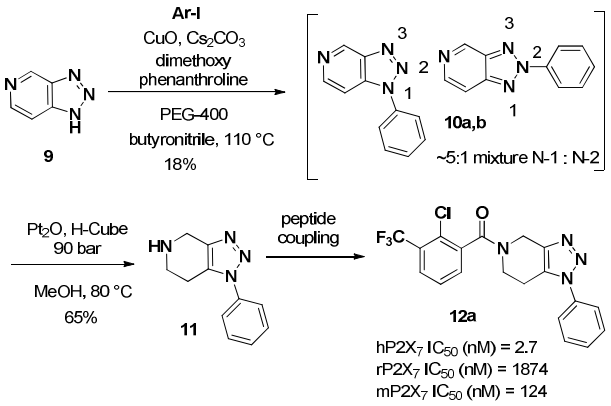
In addition to the various P2X7R chemotypes in Figure 2, we were aware of the 1,2,4-triazolopiperazines disclosed in 2010 (Figure 3).[17] In a quest to explore other heterocyclic cores that could serve as competent P2X7R antagonists we decided to embark on a campaign to discover novel heterocycles which were potent P2X7 antagonists and this work eventually led us to the 1,2,3-triazolopiperidines series disclosed in this report.

Figure 3: 1,2,4-Triazolopiperazine P2X7R Antagonists



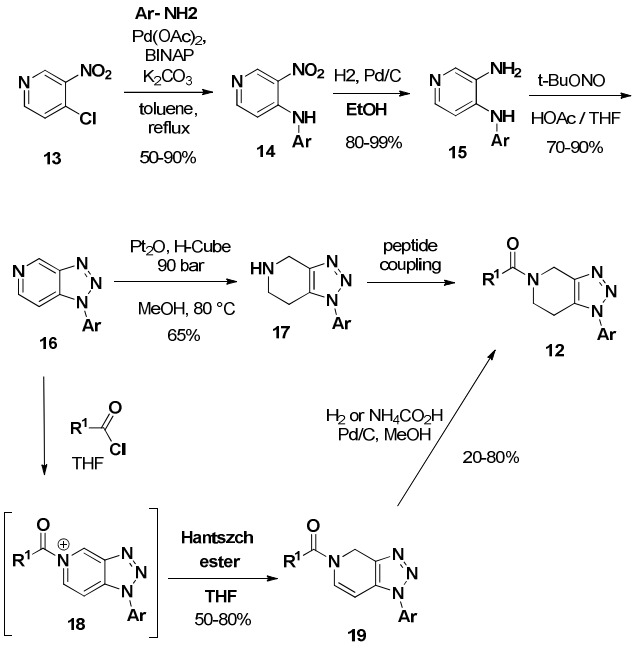
Chemistry. Our initial synthesis began with commercially available 1*H*-[1,2,3]triazolo[4,5-c]pyridine (**9**) (Scheme 1). Although arylation of the 1*H*-[1,2,3]-triazolo-[4,5-c]-pyridine had the potential to give several regioisomers, we anticipated that the electronic effect of the pyridyl nitrogen would favor arylation to the 1-position. In practice, the use of Buchwald's copper mediated arylation[18, 19] furnished the desired arylated products in a 5:1 ratio favoring the *N-1* aryl regioisomer albeit in low yield and as an inseparable mixture.[20] Subsequent hydrogenation of the *N*-1, *N*-2 mixture (H-cube, Pt_2O, 90 bar, MeOH) did furnish a small amount the 1,2,3-triazolopiperidine however this method suffered from incomplete / irreproducible conversion, even after conducting the reaction in continuous flow mode. Regardless, the regioisomers were separated at the 1,2,3-triazolopiperidine stage and then coupled with the corresponding benzoic acids to furnish the desired product(s) (Scheme 1). As the first analog made (**12a**) exhibited an hP2X7R IC_{50} = 2.7 nM, our interest in this series grew.

Scheme 1: First Generation Synthesis of 1,2,3-Triazolopiperidines: Triazole Arylation Route



The low yield and irreproducibility of the hydrogenation reaction coupled with difficulties encountered with the purification of the regioisomers prompted us to look at alternative methods to more efficiently prepare these compounds. The regiochemical issue was addressed by using 4-chloro-3-nitropyridine as the starting material as shown in Scheme 2. An amino heterocycle displacement of chloro-nitro-pyridine (**13**), was followed by reduction which furnished the diamino pyridine (**15**), which was subsequently converted to the 1,2,3-triazolopyridine core (**16**) after treatment with t-butyl nitrite. Reduction as in Scheme 1 provided **17**, which was coupled to the desired carboxylic acid or acid chloride to provide compounds **12**.

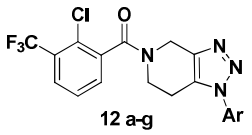
Scheme 2: Synthetic Route to 1,2,3-Triazolopiperidines



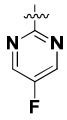
Alternatively, we were aware that an acyl pyridinium[21, 22] species (**18**) could enable the synthesis of the final products directly. As such, the acyl pyridinium species (**18**) was formed by the addition of an acid chloride to the triazolopyridine **16** in THF, however, this species (**18**) was unstable and readily decomposed in the presence of protic solvents such as methanol. Treatment of the intermediate acyl pyridinium (**18**) with sodium or lithium borohydride did not result in reduction to the desired product. However, use of the Hantzsch ester[23] cleanly provided the partially reduced derivatives (**19**); which were subsequently reduced with hydrogen over Pd/C in ethanol to provide final products (**12**). In some cases the pendant heterocycles were cleaved and a transfer hydrogenation with ammonium formate and Pd/C was more productive.

The first entry into this series of antagonists was compound **12a** (Table 1), which had a potent hP2X7 FLIPR IC_{50} of 2.7 nM, but was significantly less potent at the rat P2X7R with a IC_{50} = 1900 nM. In addition, compound **12a** was rapidly metabolized in-vitro and was a CYP 2C19 inhibitor with an IC_{50} of 0.1 μM. Compound **12a** was not cross reactive in a commercial panel of 50 receptor, ion channel and transporter assays (Eurofins-CEREP, http://www.eurofins.com/) at a screening concentration of 1.0 μM. Since compound **12** had a clogP of ~4.0, we reasoned that reducing lipophilicity (lower cLogP) could address both the metabolism issues and the CYP profile.

Table 1: SAR for *N1*-Aryl 1,2,3-Triazolopiperidines



12 a-g

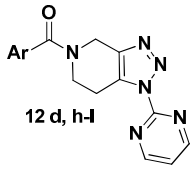
#	Ar	*hP2X7* IC_{50} *(nM)*[a]	*rP2X7* IC_{50} *(nM)*[b]	*HLM/ RLM ER*[c]	*CYP 2C19* *(IC50) μM*[d]	*cLogP*
12a		**2.7**	**1900**	**0.94 / 0.92**	**0.10**	**4.0**
12b		**3.8**	**1400**	**0.90 / >0.92**	**0.30**	**3.2**
12c		**2.3**	**1900**	**0.80 / 0.86**	**3.2**	**2.3**
12d		**4.2**	**6.8**	**0.61 / 0.82**	**8.5**	**2.3**
12e		**2.1**	**30**	**0.85 / 0.89**	**1.3**	**3.3**
12f		**24**	**3000**	**0.54 / 0.65**	**>10**	**2.5**
12g		**39**	**209**	**0.70 / 0.82**	**>10**	**2.9**

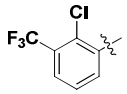
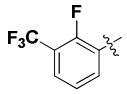
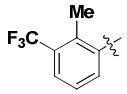
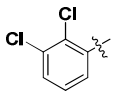
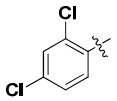
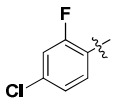
[a]human FLIPR pIC_{50} measured in a Ca^{2+} flux assay, [b]rat FLIPR pIC_{50} measured in a Ca^{2+} flux assay, all data are the result of at least three assays run in triplicate with the mean value reported; The standard deviation is all cases was less than two fold. [c]HLM and RLM refer to human and rat liver microsomal preparations with data reported as the extraction ratio (ER), [d] CYP2C19 data was obtained from human liver microsomes. Details of all assay conditions are provided in the supplemental information.

Indeed, alternative heterocycles proved to be a fruitful expedition (Table 1). Changing from a phenyl (**12a**) to a pyridyl (**12b**) maintained affinity at the human receptor (IC_{50} = 3.8 nM) and provided a slight improvement in the CYP 2C19 inhibition (IC_{50} = 0.3 μM) though the rat affinity and metabolic stability were not improved. The addition of a second nitrogen, as in the pyrimidine (**12c**), also maintained affinity for the human receptor (IC_{50} = 2.3 nM), showed a further improvement in the CYP 2C19 inhibition (IC_{50} = 3.2 μM), and a modest improvement in the human and rat microsomal stability. The pyrimidine compound (**12d**) was especially gratifying as it was potent at both the human (IC_{50} = 4.2 nM) and rat (IC_{50} = 6.8 nM) P2X7R in addition to further improving the CYP inhibition (IC_{50} = 8.5 μM) and the microsomal stability. This was one of the first compounds in this series that showed substantial affinity for the rat P2X7R. The 4-fluoropyridine (**12e**) also had significantly improved potency at the rat receptor (IC_{50} = 30 nM), relative to the pyridyl parent (**12b**, IC_{50} = 1400 nM), and reduced CYP inhibition (IC_{50} = 1.3 μM), but was still rapidly metabolized. The 5-fluoro pyrimidine (**12f**) had good human potency (IC_{50} = 24 nM), improved CYP inhibition (IC_{50} = >10 μM), and microsomal stability when compared to the parent pyrimidine (**12d**) though the rat affinity (IC_{50} = 3000 nM) was reduced. Finally, the pyrazole (**12g**) had good human affinity (IC_{50} = 39 nM), reduced rat affinity (IC_{50} = 209 nM), moderate microsomal stability and no CYP inhibition (IC_{50} = >10 μM).

After optimizing the *N-1*-aryl portion of the molecule, we prepared a limited number of benzamides while keeping the aryl group as a pyrimidine (Table 2). The 2-chloro-3-trifluoromethyl (**12d**) had an IC_{50} = 4.2 nM. Replacing the chloro (**12c**) with a fluoro (**12h**) provided a compound with reduced potency at the human (IC_{50} = 22 nM) and rat (IC_{50} = 258 nM) receptor, though human microsomal stability and CYP 2C19 liability were slightly improved. The 2-methyl (**12h**) analog was slightly more potent at the human receptor (IC_{50} = 2.2 nM), though significantly less potent at the rat receptor (349 nM). Human and rat microsomal stability and CYP 2C19 inhibition were also improved. The 2,3-dichloro derivative (**12j)** was potent at the human receptor (IC_{50} = 2.0 nM), less potent at rat receptor (IC_{50} = 53 nM), had improved microsomal stability, but slightly worse in the CYP 2C19 assay. Finally, both of the 2,3-bis halo derivatives (**12k, l**) were less active.

Table 2: SAR for Benzamide of 1,2,3-Triazolopiperidines

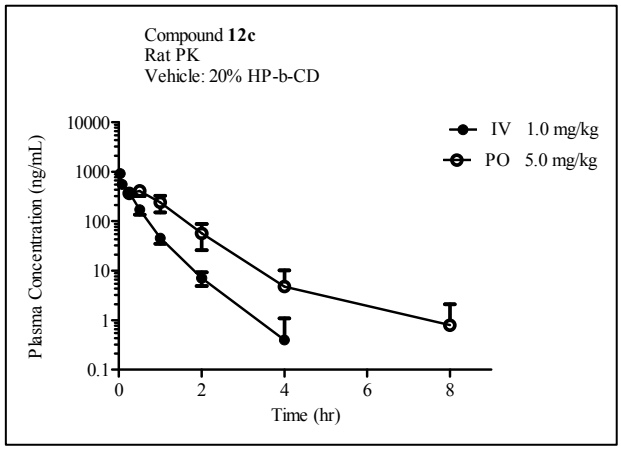


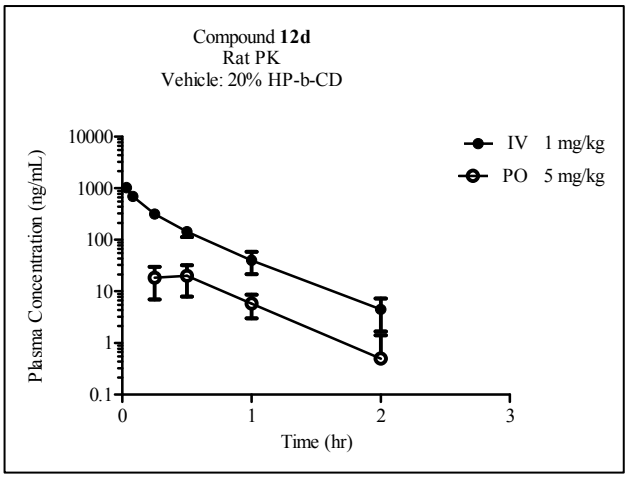
#	Ar	*hP2X7* IC_{50} *(nM)*[a]	*rP2X7* IC_{50} *(nM)*[b]	*HLM/ RLM ER*[c]	*CYP 2C19* $(IC_{50})_d$ *μM*	*cLogP*
12d		**4.2**	**6.8**	**0.61 / 0.82**	**8.5**	**2.3**
12h		**22**	**258**	**0.50 / 0.85**	**>10**	**1.9**
12i		**2.2**	**349**	**0.47 / 0.72**	**>10**	**2.3**
12j		**2.0**	**53**	**0.49 / 0.82**	**6.3**	**2.2**
12k		**387**	**4300**	**N/D**	**N/D**	**2.3**
12l		**255**	**1300**	**N/D**	**N/D**	**1.7**

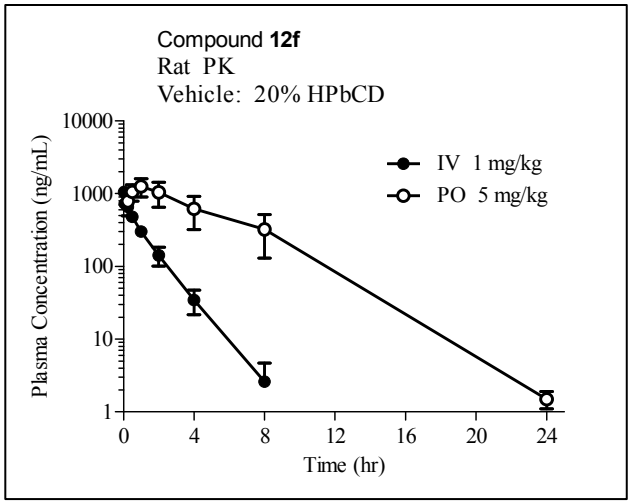
[a-d] See Table 1

Since we had now generated potent P2X7 compounds in this series, we were interested to see how these compounds behaved in-vivo, and in particular if the compounds would distribute efficiently into the CNS. Although we were especially interested in compound (**12d**), as it was active at both the human and the rat P2X7R, we obtained additional in-vitro ADME characterization of three of the more promising compounds (**12c, 12d,** and **12f**). The profiles of those three compounds are shown in Table 3 and 4. In general all three compounds have reasonable physical properties, including solubility, permeability and protein binding to warrant in-vivo testing.

Figure 4: Rat PK Data for compound **12c, 12d,** and **12f**.



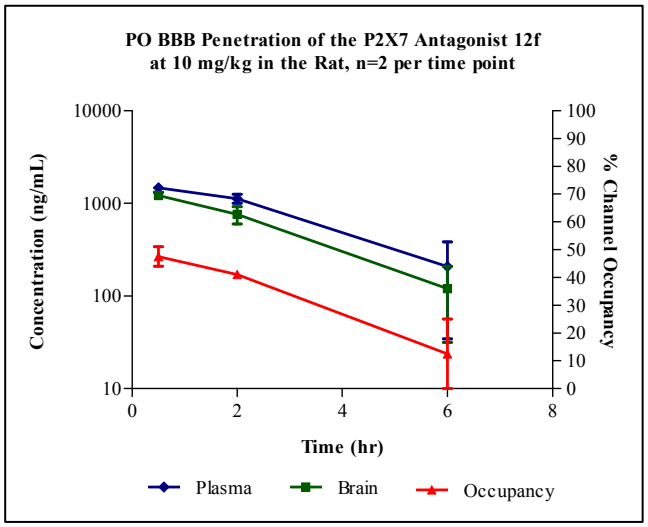




The rat pharmacokinetic data for all three compounds is shown in Figure 4. The pyrazine (**12c**) had bioavailability of 33% and reached a Cmax of 1.0 μM. The half-life of 0.7 h is consistent with the relatively high microsomal extraction ratio (0.82) as clearance of **12c** was near the rat hepatic blood flow. Disappointingly, compound **12d**

which had the best rat P2X7R affinity (4.2 nM) had the lowest oral exposure of all three compounds with a bioavailability of 1%. Compound **12f** proved to have the best PK with measured bioavailability greater than 100%, and a half-life of 2.3 hrs, which is slightly longer than predicted by the rat extraction ratio (0.65). Although we do not know the reason for the low bioavailability of compound **12d**, it is interesting to note that **12d** has the lowest solubility of the three compounds.

Figure 5: Rat P2X7 Receptor Occupancy Data for **12f**.



Compound **12f** was also tested in a rat ex-vivo autoradiography (ARG) experiment to assess the brain P2X7 receptor occupancy (Figure 5**)**. When **12f** was dosed at 10 mg/Kg PO in the rat it achieved a maximum occupancy of 45% at 30 minutes. The occupancy decreased over time in conjunction with decreasing concentrations of **12f** in the brain and plasma, which were measured in the same experiment. Therefore, compound **12f** demonstrated proof of concept that the 1,2,3-triazolopiperidines are a new class of brain penetrant P2X7R antagonists that are capable of occupying the receptor when dosed orally.

Conclusions.

We describe the synthesis and SAR of 1,2,3-triazolopiperidines as a new series of potent and brain penetrant P2X7 antagonists. The prototype *N-1*-phenyl triazole (**12a**) was potent at the human P2X7R, but had poor affinity for the rat P2X7R, poor microsomal stability, was a strong inhibitor of CYP 2C19, and had a high cLogP. By replacing the phenyl group with various heterocycles we discovered compound **12f**, a potent human and rat P2X7R ligand with sufficiently good physicochemical properties to enable oral dosing in rats and provide high levels of the compound in the plasma and the brain, and demonstrated target engagement as measured by the rat ARG. Additional studies of this novel class of P2X7R antagonists will be the subject of future publications.

Acknowledgements.

The authors would like to thank Professor David MacMillan for useful discussions toward optimizing the synthetic strategy, and especially the use of the Hantzsch ester reduction of the acyl pyridinium species.

Supporting Information Available: Supporting information for "Synthesis, SAR and Pharmacological Characterization of Brain Penetrant P2X7 Receptor Antagonists" including synthesis, characterization and assay conditions is available free of charge via the Internet at http://pubs.acs.org.

Table 3: In Vitro Pharmacology for Compounds **12c, 12d,** and **12f**.

	Human FLIPR IC_{50}[a]	rat FLIPR IC_{50}[b]	mouse FLIPR IC_{50}[c]	human K_i	rat K_i	hERG[d] IC_{50} (μM)	human WB IC_{50}[e]
12c	2.3 nM	1.9 μM	15 nM	23	5.0	>10	316 nM
12d	4.2 nM	6.8 nM	4.0 nM	16	2.8	>10	79 nM
12f	24 nM	3.0 μM	588 nM	32	20	>10	251 nM

[a]human FLIPR pIC_{50} measured in a Ca^{2+} flux assay, [b]rat FLIPR pIC_{50} measured in a Ca^{2+} flux assay, [c]mouse FLIPR pIC_{50} measured in a Ca^{2+} flux assay, [d]hERH IC_{50} determined in a tritiated dofetilide binding assay, [e]Human blood was primed with lipopolysaccharide (LPS; 30 ng/ml) followed by addition of the test antagonist or vehicle with the final P2X7 stimulus of Bz-ATP. Test compounds were added and incubated for an additional 30 minutes. The P2X7 agonist, Bz-ATP (1 mM) was finally added and incubated for 1.5 hours at 37°C. Post incubation, the plates were centrifuged (low-speed spin) and the supernatant collected for IL-1□ ELISA analysis as per manufacturer's protocol (human IL-1□: Thermo Scientific; catalogue # EH2IL1B5;). All data are the result of at least three assays run in triplicate with the mean value reported.

Table 4: Physical Properties and In Vitro DMPK Parameters for Compounds **12c, 12d,** and **12f**.

	MW	clog P	human ER[a]	rat ER[a]	mouse ER[a]	human/rat ppb[b]	Caco-2 A to B/B to A[c]	brain pb[d]	Solubility pH2/pH7[e]
12c	408.7	2.3	0.80	0.86	0.84	89.6 / 91.6	81.2 / 38.4	94.0	304 / 235
12d	408.8	2.3	0.61	0.82	0.89	84.3 / 88.6	71.4 / 45.4	90.9	48 / 50
12f	426.8	2.5	0.54	0.65	0.87	83.4 / 83.9	75.5 / 55.4	92.5	49 / 202

[a]human, rat or mouse extraction ratio as measured in a microsomal preparation, [b]human or rat protein binding reported as % bound, [c]P_{app} reported in units of cm/sec x 10^{-6}, [d]rat brain protein binding reported as % bound, [e]Reported in μM.

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