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Synthesis and biological evaluation of Esaprazole analogues showing σ_1 binding and neuroprotective properties in vitro

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ABSTRACT

Esaprazole, a molecule previously acknowledged to protect against stomach and intestinal ulcers was surprisingly discovered to have neuroprotective activities and σ_1 binding in vitro. A highly diverse set of Esaprazole analogues **2**–**5** was prepared in order to increase blood–brain barrier penetration. The analogues showed a structure–activity relationship at the σ_1 receptor closely matching already published pharmacophores. Many of the analogues were shown to have neuroprotective properties in two assays using primary cultures of cortical neurons exposed to glutamate and hydrogen peroxide. However, no apparent SAR for these two assays could be developed. Metabolic stability of the analogues were also investigated and the structure of R^1 had a significant bearing on the ADME properties of the compound resulting in two series of compounds. Compounds in which R^1 was a H or acyl group had good metabolic stability in RLM but poor BBB penetration, whereas compounds where R^1 was a cyclo- or bicyclo-alkyl group had poor metabolic stability but good BBB penetration.

1. Introduction

Loss of neurons due to neurodegenerative diseases or brain ischemia is significant causes of death or long-term disability in populations throughout the world. Although significant effort has been made in finding treatments for neurodegenerative diseases and to improve patient prospects after an ischemic attack, the result of most clinical trials has been disappointing. Herein we present in vitro work which potentially could be the first step in finding a compound with neuroprotective effects, high blood–brain barrier (BBB) penetration and metabolic stability.

Esaprazole **1** (Fig. 1), also known as hexaprazole, was developed in the 1980s as a drug for the treatment of gastric and duodenal ulcers.[1] It was shown to have antiulcer, antisecretary and cytoprotective activities.[2] Esaprazole completed phase II clinical trials with only few minor side effects being reported, but was shown to be less effective than Cimetidine and Ranitidine at healing ulcers.[1,3]

The cytoprotective effect of Esaprazole was shown by the inhibition of the necrotizing action of gastric mucosa damaging agents[2] although no specific mechanism of action was disclosed. We speculated whether Esaprazole had more general cytoprotective effects and discovered through testing in receptor binding assays that Esaprazole was a weak σ_1 ligand. High affinity σ_1 binders has previously been shown to have neuroprotective capabilities in animal studies.[4] Further evidence for σ_1 mediated neuroprotective abilities comes from the discovery of mutations in the σ_1 receptor leading to motor neuron degeneration in amyotrophic lateral sclerosis patients.[5] Therefore, we tested whether Esaprazole would show neuroprotective properties in experiments using primary culture of cortical neurons. Compounds with such properties would have a great potential in the treatment of a wide variety of diseases such as cerebral ischemia, multiple sclerosis or Alzheimer's disease. Calculations indicated that Esaprazole is a relatively polar compound ($c\log D$ at pH 7.4 is -1.25[6]) and it was expected that the ability to penetrate the BBB would not be ideal ($\log BB$ -0.03[7]). Thus analogues of Esaprazole were prepared[8] with the intention of creating a SAR around the initial hit and to develop analogues with improved $\log BB$ values.

2. Chemistry

A range of Esaprazole like analogues were prepared based around structure **2** (Table 1) along with the bicyclic, fused and spiro piperazine analogues **3**, **4** and **5**, respectively (Fig. 2). Reaction of acyl halide **6** with an amine (2.1 equiv) at ca. 15 °C in acetonitrile or DCM gave after workup amide **7** in 75–96% yield and generally >95% purity. In cases where the amine was expensive, equal equivalents of amine, acyl halide and Cs_2CO_3 were used in acetonitrile at 5 °C to RT to give amide **7** in 60–99% yield.[9] Amide **7** was then reacted with a variety of amines to give a broad range of Esaprazole analogues **2**–**5**. For example, reaction of amide **7** with *N*-alkyl or acyl (homo) piperazines in ethanol at reflux gave the desired aminoalkamide **2**–**3** ($R^1 \neq H$) in generally >70% yield, although

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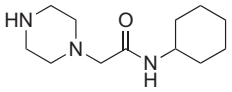
yields were lower in cases of steric hinderance (e.g., $R^3 \neq H$ or $R^4/R^5 \neq H$). If $R^4 = R^5$ = alkyl then the yield was only 14–22%, and modification of the reaction conditions resulted in the preparation of the Favorskii-like rearranged product.[10]

In order to minimise dialkylation of the piperazine moiety, the reaction of amide **7** with piperazine or homopiperazine was carried out in the presence of aqueous HCl[11] to afford aminoalkamide **2** (R^1 = H) or **5** in 38–94% yield. Bicyclic aminoalkamides **4** were formed from the reaction of amide **7** with Boc-(1*S*,4*S*)-(+)-2,5-diazabicyclo[2.2.1]heptane under basic conditions in 30–75% followed by deprotection with HCl to give amine **6**. In both cases the deprotection step proceeded in very poor yield.



1

Figure 1. Structure of Esaprazole **1**.

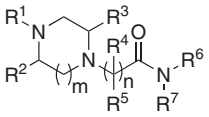
3. Results and discussion

3.1. Binding properties of Esaprazole

Whilst it is known that Esaprazole **1** does not bind to the histamine H_2-receptor,[2] to our knowledge no other receptor binding data has been published and therefore Esaprazole was screened for activity in a wide range of assays, including both receptor-, enzyme- and ion channel assays (a list of assays is given in Supplementary data). Esaprazole was active at only three targets; the σ_1 receptor (IC_{50} 27 μM) and the muscarinic M_3 and M_5 receptors (IC_{50} 87 and 71 μM, respectively) in radioligand binding studies.

A representative selection of nine analogues was tested at the muscarinic M_1, M_2, M_3, M_4, and M_5 and the sigma σ_1 and σ_2 receptors for radioligand binding. Six compounds did not show binding at any muscarinic receptor below 100 μM whilst two others only showed activity at two receptors between 50 and 80 μM (Table 2). In comparison, most of the analogues tested showed binding below 100 μM at the σ_1 receptor and eight of the compounds bound with a 10-fold higher affinity to the σ_1 receptor

Table 1
Esaprazole-like aminoalkamide analogues **2**



2

Compound structure	R^1	R^2	R^3	R^4	R^5	R^6	R^7	*m*	*n*
1	H	H	H	H	H	Cyclohexyl	H	1	1
2a	H	H	H	H	H	*i*-Pr	H	1	1
2b	Me	H	H	H	H	Cyclohexyl	H	2	1
(±)-**2c**	Cyclopentyl	H	H	Et	H	Cyclohexyl	H	1	1
(±)-**2d**	Et	Me	H	H	H	Cyclopentyl	Me	1	1
(±)-**2e**	EtC(O)	H	H	H	H	$-CH_2CH(Me)Et$	H	1	1
2f	H	H	H	H	H	$-CH(Et)_2$	H	2	1
trans-**2g**	H	Me	Me	H	H	*n*-Bu	*n*-Pr	1	1
2h	Cyclopropyl-C(O)	H	H	H	H	Cyclohexyl	H	1	2
2i	H	H	H	H	H	*n*-Bu	*n*-Pr	1	1
(±)-**2j**	H	H	H	*n*-Bu	H	Cyclohexyl	H	1	1
2k	EtC(O)	H	H	H	H	$-CH(Et)_2$	H	1	1
2l	EtC(O)	H	H	H	H	Cyclohexyl	H	1	1
2m	H	H	H	H	H	Cyclopentyl	Me	1	1
2n	Cyclopentyl	H	H	H	H	Cyclohexyl	H	1	1
(±)-**2o**	H	H	H	Me	H	$-(CH_2)_2$-cyclooctane	H	1	1
(±)-**2p**	H	H	H	Me	H	Cyclopentyl	H	2	1
2q	H	H	H	H	H	$-(CH_2)_2$-*t*Bu	H	1	1
2r	H	H	H	H	H	*exo*-Norbornan-2-yl	H	1	1
2s	H	H	H	H	H	(1*S*,2*S*,3*S*,5*R*)-Pinan-3-yl	H	1	1
2t	Cyclohexyl	H	H	Me	Me	*i*-Pr	H	1	1
2u	Cyclohexyl-C(O)	H	H	H	H	CH_2CH_2*t*Bu	H	1	1
2v	Cyclohexyl	H	H	H	H	(1*S*,2*S*,3*S*,5*R*)-Pinan-3-yl	H	1	1
(±)-**2w**	EtC(O)	H	H	Me	H	Cyclopentyl	H	1	1
2x	*i*-Pr	H	H	H	H	(*R*)-3,3-Dimethylbutan-2-yl	H	2	1
2y	Me	H	H	H	H	$-(CH_2)_3$-cyclopentane	H	2	1
2z	H	H	H	H	H	*n*-Pentyl	Me	2	1
2aa	Ac	H	H	H	H	Cycloheptyl	H	2	2
2ab	Cyclopentyl	H	H	Me	Me	Cyclohexyl	H	1	1
(±)-**2ac**	Cyclohexyl-C(O)	H	H	Me	H	$-(CH_2)_2$-cyclooctane	H	1	1

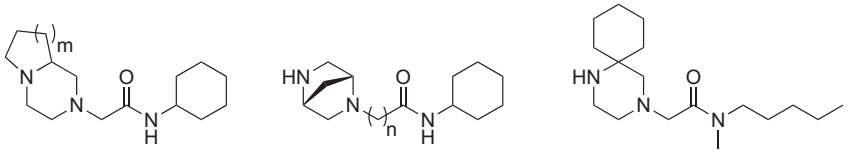


Figure 2. Novel bicyclic analogues **3**, **4** and **5**.

Table 2
Radioligand binding results of muscarinic and sigma receptors for Esaprazole and selected analogues

Compound	IC_{50} (μM)		Ratio	IC_{50} (μM)				
	σ_1	σ_2	σ_1:σ_2	M_1	M_2	M_3	M_4	M_5
Esaprazole **1**	27.2	460	17:1	>100	>100	87	>100	71
2a	>100	>1000	—	>100	>100	83	>100	63
2b	3.25	41	13:1	102	>100	>100	>100	>100
2k	>100	1130	—	>100	>100	>100	>100	>100
2l	>100	>1000	—	>100	>100	>100	>100	>100
2n	0.43	1.45	3.4:1	>100	>100	>100	>100	>100
2t	8.41	104	13:1	>100	>100	>100	>100	>100
2ab	0.50	33	66:1	>100	>100	>100	>100	>100
(*R*)-**3a**	0.39	11	28:1	56	>100	>100	>100	74
(*S*)-**3a**	2.25	51	23:1	50	93	57	73	26

compared to the σ_2 receptor. Due to this and the well documented connection between σ_1 binding and neuroprotective abilities,[12] only the binding to the σ_1 receptor was investigated further.

3.2. Binding to the σ_1 receptor

A great difference in binding affinities to the σ_1 receptor was observed among the analogues and therefore we investigated whether a correlation between binding to the σ_1 receptor and the structure of the Esaprazole analogues existed. About 80 compounds were prepared using the chemistry described in Scheme 1, and a representative sample is presented in Table 3. Examination of the data showed improved binding to the σ_1 receptor when R^1 was an alkyl or cycloalkyl group and that increasing the size and therefore lipophilicity generally increased the binding. For example, increasing the size of R^1 from proton to Me to cyclopentyl increased the binding by 10- and 60-fold, respectively (compare compounds **1**, **2b** and **2n**, respectively). However, compounds of structure **2** in which R^1 was an acyl group, such as acetyl or propionyl (e.g., (±)-**2e**, **2k**, **2l** and (±)-**2w**), generally did not bind to the σ_1 receptor at less than 100 μM. If the length of the linker *n* between the amide and amine group was two though (e.g., compounds **2h** and **2aa**) or the acyl group was large, such as a cyclohexylcarbonyl group (e.g., **2u** and (±)-**2ac**), then activity was restored back to the level of Esaprazole **1**.

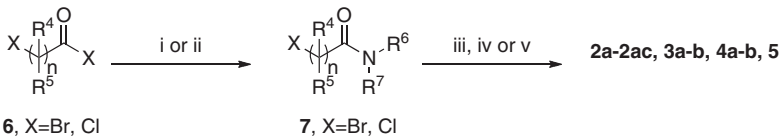
Compounds based around structure **3**, in which the piperazine moeity was replaced by a bicyclic moeity, bound at the σ_1 receptor at around 0.3 μM, ca. 100 times better than Esaprazole **1**. Interestingly, whilst the size of the bicyclic ring in **3** did not significantly alter the binding, the (*R*)-enantiomer of **3a** was about 5-fold more active than the (*S*)-enantiomer. Since the bicyclic analogue was significantly more active, we decided to investigate whether other substituents on the piperazine moeity would also be beneficial. Introducing a small substituent, such as 2-methyl or a *trans*-2,5-dimethyl substitution onto the piperazine ring (compounds (±)-**2d** and *trans*-**2g**, respectively), did not significantly improve binding. However, it was found that spirocycle **5** was one of the most active compounds prepared, binding to the σ_1 receptor at 0.2 μM. Other spirocyclic σ_1 ligands are known but they are structurally dissimilar to the ligands presented here.[13,14] The fact that three structurally different compounds (e.g., **2n**, (*R*)-**3a** and **5**) were all highly active indicated that a large, flexible hydrophobic pocket is available for binding.

Introducing small alkyl groups at R^4 and/or R^5 in structure **2**, such as dimethyl, ethyl or *n*-butyl, did not significantly alter the binding of the analogues regardless of whether R^1 was a proton (such as for or compounds (±)-**2c**, **2n** and **2ab**) or a cyclopentyl group (compare **1** with (±)-**2j**). However, the size of the groups R^6 and R^7 in structure **2** did have a role in how well the analogues bound to the σ_1 receptor. In general, if R^6 was smaller than a cyclohexyl group and R^7 was a also very small (e.g., compounds **2a** or **2m**), then all activity was lost. However, if R^6 was a slightly larger group, such as *n*-pentyl (**2z**) or 3,3-dimethylbutyl (**2q**), then similar activity to the cyclohexyl group was obtained. Bridged groups, such as pinanyl (**2s**) or bicyclo[2.2.1]heptyl (**2r**) either did not give any benefit or made the binding worse, respectively. Larger and/or longer groups, such as cyclooctylethyl or cyclopentylpropyl, did not significantly improve binding (compare **2ac**–**2u** and **2y**–**2b**).

3.3. σ_1 Receptor pharmacophore

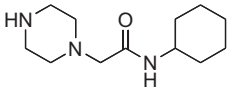
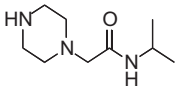
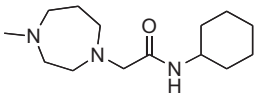
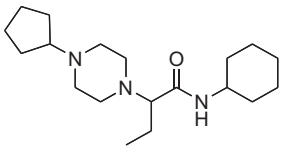
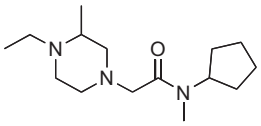
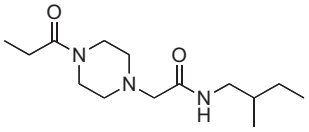
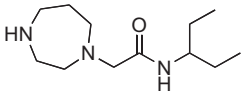
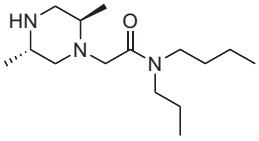
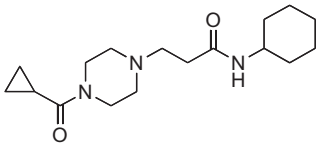
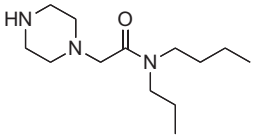
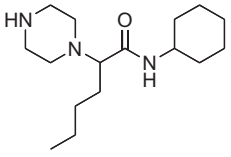
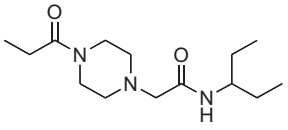
Glennon,[15] Laggner,[16] Pricl[17] and Wünsch[14] have all published pharmacophore models for the σ_1 receptor. Since both the Laggner and Wünsch have been compared to the Glennon model and were found to be similar, we decided to evaluate our results based on the Glennon pharmacophore. This pharmacophore for the σ_1 receptor has an amine binding site flanked by a primary hydrophobic region, 6–10 Å away, and a secondary hydrophobic region 2.5–3.9 Å away (Fig. 3). To obtain affinity in the sub nanomolar range, the optimal distance between the amine binding site and the primary hydrophobic region was about five carbons whereas it was about three carbons to the secondary hydrophobic region. Apart from the chain length having a large effect on compound potencies, Glennon also showed that the σ_1 affinity decreased when the chain linking the hydrophobic region to the amine site was functionalised and not a hydrocarbon linker. Since the compounds presented here have an amide functionality in the linker, this may explain why the majority of compounds shown in Table 3 are significantly less active.

However, even though the activities of our ligands and those presented by Glennon are not of the same order of magnitude, our results still fit the general pharmacophore model proposed by Glennon. In order for our compounds to show good binding, R^6 needs to be a large hydrophobic group so it fits into one hydrophobic region, whilst R^1 needs to be an alkyl or cycloalkyl group to fit into the other hydrophobic region. All of the ligands **1**–**5** prepared contain a piperazine group and provided that R^1 is not an acyl group, either of the two nitrogen atoms N-1 or N-4 could bind to the amine site. This means that binding can theoretically occur in three different binding modes. If the amine binding site binds to N-1, then R^6 will fit into the primary hyrophobic region and R^1 will fit into the secondary hydrophobic region (binding mode 1). Increasing the size of R^1 and/or R^6 will improve the binding in the respective hydrophobic regions and thus will result in more active compounds. Alternatively the amine site can bind to N-4 and either R^6 or R^1 will fit into the primary hyrophobic region (binding



Scheme 1. Reagents and conditions: (i) Amine, MeCN or DCM, 15 °C, 1–2 h; (ii) amine, Cs_2CO_3, MeCN, 5 °C to rt, 1 h; (iii) *N*-alkyl or acyl (homo)piperazine, Na_2CO_3, EtOH, reflux, 2–18 h; (iv) (homo)piperazine, HCl, EtOH, reflux, 3–18 h; (v) Boc-(1*S*,4*S*)-(+)-2,5-diazabicyclo[2.2.1]heptane, Na_2CO_3, EtOH, reflux, 4 h, then HCl, EtOH.

Table 3
Percent reduction in cell death in primary culture of cortical neurons after glutamate or peroxide insult

Compound number	Structure	σ_1 IC_{50} (μM)	Percent reduction in cell death[a]					
			Glutamate intoxication Concentration of test compound			Peroxide intoxication Concentration of test compound		
			1 μM	10 μM	100 μM	1 μM	10 μM	100 μM
1		27.2	6	10*	27***	23**	33***	44***
2a		>100	11*	16**	25***	14***	17***	23***
2b		3.25	8*	12**	19***	3	13*	21***
(±)-**2c**		0.59	28***	39***	39***	3	12*	20***
(±)-**2d**		1.2	9***	14***	[b]	6	19*	[b]
2e		>100	14	9	21**	2	11	28**
2f		15.2	11	15	21**	0	12	33***
trans-**2g**		7.60	11***	15***	21***	8	11	41***
2h		8.83	2	8	21***	4	12*	27***
2i		16.3	−2	3	20***	2	9	25***
(±)-**2j**		11.5	[c]	[c]	[c]	[c]	[c]	[c]
2k		>100	2	7	25***	0	4	17*

(*continued on next page*)

Table 3 (*continued*)

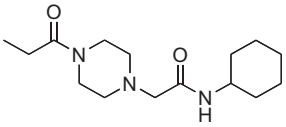
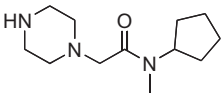
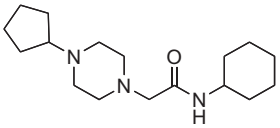
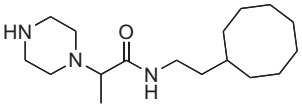
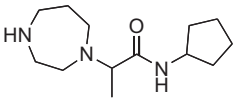
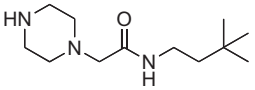
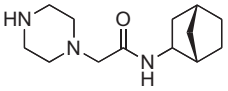
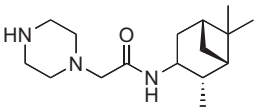
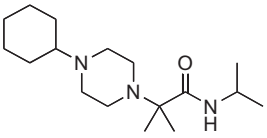
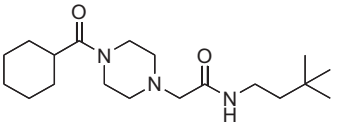
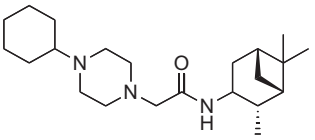
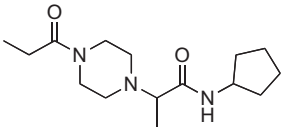
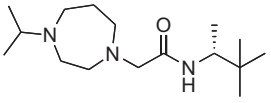
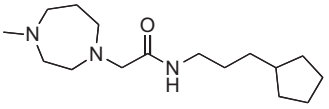
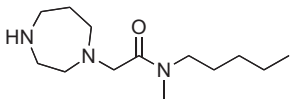
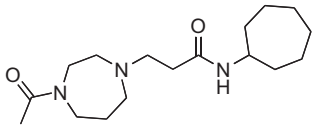
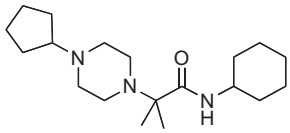
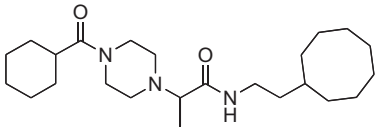
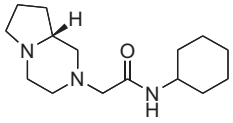
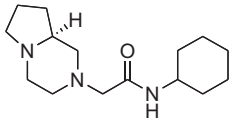
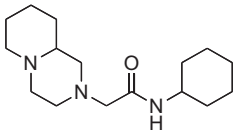
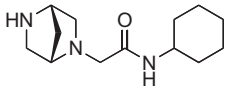
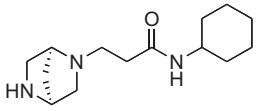
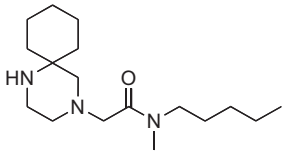
Compound number	Structure	σ_1 IC_{50} (μM)	Percent reduction in cell death[a]					
			Glutamate intoxication Concentration of test compound			Peroxide intoxication Concentration of test compound		
			1 μM	10 μM	100 μM	1 μM	10 μM	100 μM
2l		>100	3	3	14	3	3	24***
2m		>100	−1	7	29***	−3	−1	16*
2n		0.43	3	4	9	9	11	10
(±)-**2o**		7.24	6	4	[b]	9	14**	[b]
(±)-**2p**		NA	−1	1	9**	5	7	12
2q		18.8	3	4	8	[c]	[c]	[c]
2r		86.8	2	2	2	[c]	[c]	[c]
2s		15.8	2	0	−9	[c]	[c]	[c]
2t		8.41	2	7*	9**	6	10	18
2u		21.1	4	5	13**	5	5	2
2v		0.18	8	−3	[b]	[c]	[c]	[c]
(±)-**2w**		>100	3	4	8***	1	3	12
2x		11.8	0	1	5	[c]	[c]	[c]
2y		0.93	2	2	0	[c]	[c]	[c]

Table 3 (*continued*)

Compound number	Structure	σ_1 IC_{50} (μM)	Percent reduction in cell death[a]					
			Glutamate intoxication Concentration of test compound			Peroxide intoxication Concentration of test compound		
			1 μM	10 μM	100 μM	1 μM	10 μM	100 μM
2z		15.2	[c]	[c]	[c]	1	5	16
2aa		18	0	6	12**	3	16**	35***
2ab		0.50	[c]	[c]	[c]	[c]	[c]	[c]
(±)-**2ac**		11.6	[c]	[c]	[c]	[c]	[c]	[c]
(*R*)-**3a**		0.39	[c]	[c]	[c]	[c]	[c]	[c]
(*S*)-**3a**		2.25	[c]	[c]	[c]	[c]	[c]	[c]
(±)-**3b**		0.27	1	3	16***	8	10	27***
4a		NA	2	2	9	7	13*	30***
4b		NA	5	6	10*	6	13	45***
5		0.21	4	6	[b]	3	−1	[b]

[a] Threshold for statistical significance: *P <0.05, **P <0.01, ***P <0.001.
[b] Compound cytotoxic at 100 μM.
[c] nd – Not determined.

modes 2 and 3, respectively). However, for binding modes 2 and 3 this forces either an amine or an amide group into the secondary hydrophobic region which would be electronically disfavoured. It is therefore clear that binding mode 1 best fits the data and this conclusion also fits the data presented by Glennon who showed, by removing either the N-1 or N-4 nitrogen of a piperazine analogue, that it was the N-1 nitrogen which interacted with the amine binding site.

In the cases when R^1 is an acyl group, there is only 1 basic nitrogen, N-4, which is able to interact with the amine binding site. Since the amine group is in the middle of the molecule, there are two possible binding modes such that R^6 can be either located towards the primary hydrophobic region or towards the secondary hydrophobic region. In both cases though, an amide group is forced into the secondary hydrophobic region and R^6 (for binding mode 2) or R^1 (for binding mode 3) has to be quite large to be able to reach the primary hydrophobic region. Since having an amide functionality in a hydrophobic region is unlikely to be favourable, it is therefore not surprising that compounds where R^1 is an acyl group either bind poorly or not at all.

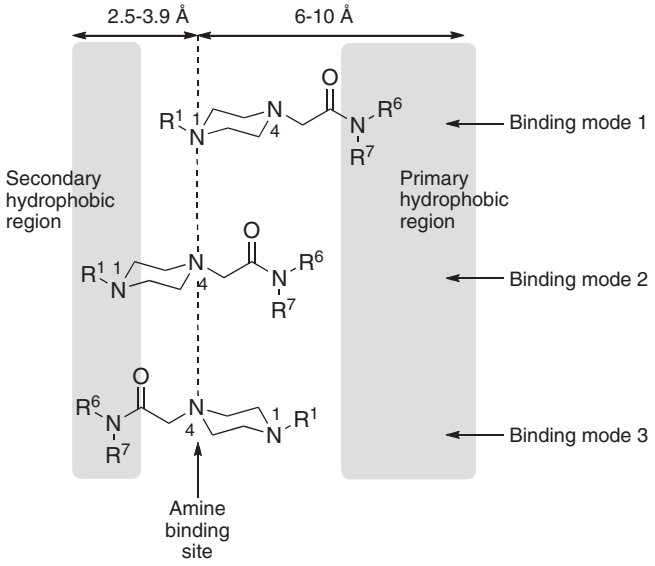


Figure 3. σ_1 Binding model proposed by Glennon[15] when applied to Esaprazole like ligands.

3.4. Activity in primary culture of cortical neurons

Prior to in vitro testing in primary culture of cortical neurons the cytotoxicity of compounds **1**–**5** were evaluated. It was found that none of the compounds showed any cytotoxicity at 10 μM and only 4 out of the 38 compounds tested were cytotoxic even at 100 μM. The compounds were then tested for their ability to prevent cell death in primary cultures of cortical neurons when exposed to glutamate or hydrogen peroxide. In these experiments, primary cultures of cortical neurons consisting of neuronal and glial cells were grown for 10 days. The test compound was added and after 30 min glutamate or peroxide concentrations known to induce cell death in approximately 50% of the cultured cells was added. After the insult, the cells were washed and further incubated with the test compound alone. After 24 h cell death was measured by analyzing the cellular release of LDH.

Esaprazole **1** prevented cell death in a dose dependent manner against both glutamate and peroxide induced cell death, with a 27% and 44% decrease in cell death, respectively (Table 3). These results were confirmed by showing a corresponding increase in viable cell counts against both glutamate and peroxide induced insults (data not shown). Many of the analogues prepared also prevented cell death in the glutamate or peroxide assay. Overall there was no clear correlation between σ_1 binding and prevention of cell death in either the glutamate or the peroxide assay. Whilst the most active analogue in the glutamate assay ((±)-**2c**) was active at the σ_1 receptor at around 1 μM, other analogues with similar activity in the glutamate assay (**2a**, **2k** or **2m**) did not have any significant σ_1 binding. Conversely there were also compounds which did not protect against cell death in the glutamate assay but either bound (**2n**) or did not bind (**2r**) to the σ_1 receptor. For the peroxide assay, weak σ_1 binding analogues (**1**, **2f**, *trans*-**2g** and **2aa**) tended to show the prevention of cell death. However, the weak σ_1 binding analogue **2u** was not neuroprotective and both strong σ_1 binding analogue and non-binding analogues were active ((±)-**2c**, **2a**) and inactive (**2n**, (±)-**2w**).

The results of the peroxide and glutamate assays were also examined to find possible correlations with compound structure (Table 3). The most active compound in the glutamate assay was (±)-**2c** with 39% more neurons surviving at both 10 and 100 μM. The analogue of (±)-**2c** lacking the ethyl group in the linker (**2n**) did not show any significant activity. Investigating this further revealed that most of the other compounds which had a substituent at R^4 and/or R^5 also reduced cell death in a weak, but significant manor ((±)-**2p**, **2t**, (±)-**2w**) at 100 μM in the glutamate assay. In addition, most compounds in which both R^6 and R^7 were an alkyl substituent were also significantly active ((±)-**2d**, *trans*-**2g**, **2i**, **2m**). However, having a substituent at R^4, R^5 and/or R^7 was not a requirement for a compound to have activity since there were compounds lacking both structural features which were also significantly activity (**2b**, **2k**). No single class of substituent at R^1 or R^6 was required for activity. For example, R^1 could be a proton, acyl or alkyl group resulting in both active (**1**, **2b**, **2k**) and inactive compounds (**2q**, **2x**). Likewise R^6 could be a cyclohexyl or an acyclic group again resulting in both active (**1**, **2a**) and inactive analogues (**2x**, **2y**). One structural feature which did not reduce cell death was a bridged ring system at R^6 (**2r**, **2s**, **2v**).

As for the glutamate assay, it was not possible to generate a SAR from the peroxide assay data (Table 3). However, it was possible to find structural features which tended to prevent cell death in the assay. The three most active compounds in the peroxide assay (**1**, **2f**, **4b**) were those compounds where R^6 was cyclohexyl-like and all other R groups were a proton. The chain length, *n*, and ring size, *m*, did not have any effect on the protective effects. Constraining the piperazine ring as a diazabicyclo[2.2.1]heptyl moiety highly significantly reduced cell death, with **4a** and **4b** reducing cell death by 30% and 45% at 100 μM, respectively. Whilst compounds in which R^6 was cyclohexyl and all other R groups were a proton showed significant neuroprotective effects, these structural features were not a prerequisite for significant activity. For example, significant activity could be obtained if R^1 was either an acyl group (**2h**, **2aa**) or some type of alkyl group (**2b**, (±)-**2d**, (±)-**3b**). However, the vast majority of analogues tested in which R^1 was a proton showed some neuroprotective effects in the peroxide assay. Other structural features appear to be less important for imparting activity, and as such there were analogues showing neuroprotective effects when R^6 was a cyclic or non-cyclic alkyl group and/or when R^6 was a proton or an alkyl group (**1**, **2f**, **2i**).

As previously mentioned, defining a SAR for both the glutamate and peroxide assays was not possible. This is probably in part due to assay variability although it may also be that different compounds possess different neuroprotective mechanisms of action. For example, both **4a** and **4b** were significantly active in the peroxide assay and yet showed very little activity in the glutamate assay. It is therefore possible that these two compounds possess abilities directly aimed at the peroxide assay (e.g., antioxidant or scavenging effects) compared to the glutamate assay and that σ_1 binding is not the only important neuroprotective mode of action for this class of compound.

3.5. Metabolic stability and blood–brain barrier penetration

Calculations indicated that Esaprazole is a relatively polar compound and it was expected that the ability to penetrate the blood–brain barrier would not be ideal (Table 4). Therefore the compound library was designed such that the majority of compounds prepared would be more lipophilic (higher *c*log*D*) and would have better blood–brain barrier penetration ability (higher *c*logBB). Two different calculation models were used indicating that the compounds being prepared had a *c*log*D* value of −2.05 to 6.0[6] and *c*logBB value of −0.06 to 1.67.[7]

A number of analogues were also tested in vitro for metabolic stability, blood–brain barrier penetration and whether they were *P*-gp substrates (Table 4). In general the type of substituent present on R^1 dominated the blood–brain barrier potential and efflux ratio, whilst other substitutions had a smaller effect. All of the analogues tested in which R^1 was a proton, regardless of any other structural

Table 4
Blood–brain barrier penetration and metabolic stability in rat liver microsomes

Compound number	$Papp_{A-B}$	$Papp_{B-A}$	Efflux ratio	Half-life in RLM[a] (min)	*c*logBB PA[b]	*c*logBB ACB[c]	*c*log*D* PA[b]	*c*log*D* ACD[c]
1	0.61	5.0	8.2	>60	−0.03	0.29	−1.25	−0.02
2a	0.38	0.45	1.2	>60	−0.06	0.02	−1.89	−1.19
2b	3.1	28	8.8	22	0.25	0.39	−0.56	0.08
(±)-**2c**	5.7	50	8.8	7.0	1	1.14	2.34	3.14
(±)-**2d**	2.6	39	15	21	0.4	0.24	0.53	0.74
2e	[d]	[d]	[d]	[d]	0.1	0.47	0.49	1.81
2f	[d]	[d]	[d]	[d]	0.05	0.36	−2.05	−0.67
trans-**2g**	[d]	[d]	[d]	[d]	0.28	0.83	0.43	1.16
2h	0.69	49	71	>60	0.61	0.16	2.22	1.08
2i	[d]	[d]	[d]	[d]	0.2	0.41	0	0.27
(±)-**2j**	[d]	[d]	[d]	[d]	0.51	1.14	0.85	2.12
2k	2.8	43	16	>60	0.13	0.45	0.71	1.81
2l	6.7	46	6.8	>60	0.23	0.49	1.16	1.93
2m	0.28	3.8	14	>60	−0.03	0.12	−1.89	−0.81
2n	16	44	2.7	9.3	0.8	0.69	1.93	2.12
(±)-**2o**	[d]	[d]	[d]	[d]	0.57	1.45	1.1	2.77
(±)-**2p**	[d]	[d]	[d]	[d]	0.07	0.32	−1.89	−0.79
2q	[d]	[d]	[d]	[d]	0.01	0.36	−0.88	0.22
2r	[d]	[d]	[d]	[d]	−0.02	0.18	−0.75	−0.16
2s	[d]	[d]	[d]	[d]	0.4	0.87	0.6	1.36
2t	8.0	56	7.0	4.9	0.59	0.78	1.44	2.4
2u	12	47	3.9	21	0.74	1.2	2.75	3.69
2v	7.6	54	7.1	3.1	1.34	1.48	3.19	4.07
(±)-**2w**	3.1	50	16	>60	0.13	0.4	0.57	1.71
2x	1.7	41	24	3.2	0.55	0.82	0.02	0.86
2y	5.1	45	8.9	1.8	0.6	0.85	0.75	1.12
2z	0.2	8.1	40	3.4	0.08	0.19	−1.1	−0.73
2aa	0.2	31	137	>60	0.53	0.35	1.97	1.46
2ab	17	44	2.5	1.7	0.95	1.03	2.42	3.01
(±)-**2ac**	[d]	[d]	[d]	[d]	1.38	1.67	4.52	6
(*R*)-**3a**	12	35	3.0	8.2	0.39	0.33	1.06	1.1
(*S*)-**3a**	13	43	3.3	4.2	0.39	0.33	1.06	1.1
(±)-**3b**	14	40	2.9	18	0.56	0.56	1.74	1.69
4a	[d]	[d]	[d]	[d]	0.03	0.18	−1.45	−0.8
4b	0.31	0.41	1.3	45	0.1	−0.02	−0.31	−1.65
5	1.1	57	52	17	0.46	0.68	1.72	0.94

[a] Half life in rat liver microsomes.
[b] Using the algorithm by Pharma Algorithms.
[c] Using the algorithm by Advanced Chemical Development.
[d] nd – Not determined.

feature, had a low brain penetration potential ($Papp_{A-B}$ from 0.2 to 1.1, e.g., **1**, **2z**, **4b**, **5**). In addition, the three analogues containing the longer linker (i.e., $n = 2$, **2h**, **2aa**, **4b**) also had $Papp_{A-B}$ from 0.2 to 0.7. However, for acyl piperazines **2h** and **2aa** this was because they were strong *P*-gp substrates, with efflux ratios of 71 and 137, respectively, rather than poor penetration. It therefore appears that the structural combination of when R^1 is an acyl group and $n = 2$ results in a compound which interacts very strongly with the *P*-gp receptor resulting in the high efflux ratios.

Alkyl or acyl piperazines generally had moderate brain penetration potential ($Papp_{A-B}$ from 2.0 to 12, e.g., **2b**, **2l**, **2u**) and were moderate *P*-gp substrates (efflux ratio 3.9–24). Whilst the substitution of R^1 was the dominating feature, the substituent at R^4 and R^5 also had some effect. If R^5 was a cyclohexyl group it had a higher $Papp_{A-B}$ and lower efflux ratio than if R^5 was a cyclopentyl (compare **2l** vs (±)-**2w**) or acyclic substituent (compare **2k** vs **2l**). However, the compounds with the best brain penetration potential were those where R^1 was a cycloalkyl group (entries **2n**, **2t**) or especially when R^1 cyclised with the piperazine moiety to form a pyrido- or pyrrolo[1,2-*a*]pyrazine. These latter compounds had a $Papp_{A-B}$ around 12 and an efflux of around three (entries (***R***)-**3a**, (***S***)-**3a** and (±)-**3b**). It is interesting to note that whilst there was a difference in the σ_1 binding for the two enantiomers (***R***)-**3a** and (***S***)-**3a**, the $Papp_{A-B}$ and efflux ratio were effectively the same.

We compared the *c*logBB from both prediction algorithms to the measured $Papp_{A-B}$ results (the prediction program does not take into account efflux mechanisms when calculating *c*logBB) and found that there was a rough correlation between the predicted and measured results ($R^2 = 0.42$ for both algorithms). Whilst the program accepts additional training data to improve the prediction, we did not investigate this feature.

The metabolic stability of the analogues was tested using rat liver microsomes. Again, the type of substituent present on R^1 tended to dominate the metabolic stability of the analogue, although not to the same extent as for the blood–brain barrier penetration. Ventura et al.[18,19] have shown in human volunteers that about 85% of Esaprazole is excreted in urine unchanged after 24 h and that the metabolites detected in rat, dog and human are N-formulation, N-actylation and 4-hydroxylation of the cyclohexyl ring. Therefore it could be expected that structurally similar analogues will also have a long half life in rat liver microsomes.

This proved to be the case, with the majority of analogues in which R^1 was either a proton or an acyl group having long half lives ($T_{1/2}$ >60 min) in rat liver microsomes. Interestingly this set of analogues also included a number of structural features which may have been expected to be hydroxylated, such as when R^4 or R^6 was a short alkyl chain (**2k**, (±)-**2w**), or N-demethylated, such as when R^7 was a methyl group (**2m**). However, if R^6 was a longer alkyl chain, then the compound had a short half life (**2z**).

All of the analogues when R^1 was an alkyl, cycloalkyl or bicycloalkyl substituent had poorer half lives than when R^1 was either a proton or an acyl group. If R^1 was a cyclopentyl or cyclohexyl group (**2v**, **2ab** and (±)-**2c**) then the half life was below 10 min. If R^1 contained an alkyl chain and R^6 was a cyclohexyl group then

the half life was around 20 min, but this dropped to only a couple of minutes when R^6 was an alkyl-like chain (compare entries **2b**, **2y**). The bicyclic analogues **3a** and **3b** showed a moderate to poor half life, with the [6.5] ring system (**3b**) being slightly more stable than the [5.5] ring system and the (*R*)-enantiomer of **3a** being slightly more stable than the (*S*)-enantiomer.

We also investigated how much compound penetrated the brain using an in vivo rat model. A single dose of 1600 mg/kg po of **1** or **2l** was given to rats ($n = 2$) and blood samples were taken just prior to dosing, and then after 30, 60 and 120 min at which point the rats were sacrificed and the brains perfused. For **1** the ratio of compound in brain vs. in plasma at 120 min was 0.25 (±0.04) whereas for **2l** the ratio was 0.33 (±0.01). As expected from the in vitro data, **2l** had a higher brain-plasma ratio than **1**, although the difference was not as large as expected. No side effects from this single high dose of **1** or **2l** were observed.

4. Conclusion

We were interested in developing an orally available drug candidate with good blood–brain barrier penetration as a neuroprotective compound for the treatment of neurodegenerative diseases. A structurally broad range of analogues to Esaperazole **1** were prepared and were tested for activity at the σ_1 receptor. A SAR of the analogues was found and which matched previously published σ_1 receptor pharmacophores. However, there did not appear to be any SAR when examining the compounds ability to prevent cell death in a primary culture of cortical neurons against a glutamate or peroxide insult.

Examination of the ADME properties of the analogues showed that there were two series of compounds. One series in which R^1 was H or acyl, resulted in compounds with good metabolic stability ($T_{1/2}$ >60 min) but with poor blood–brain barrier penetration (low $Papp_{A-B}$ and/or moderate to high *P*-gp efflux). The other series was where R^1 was a cyclo- or bicyclo-alkyl group giving compounds with poor metabolic stability ($T_{1/2}$ <20 min) but with good blood–brain barrier penetration (high $Papp_{A-B}$ and low to moderate *P*-gp efflux). In conclusion we have prepared a broad series of Esaprazole analogues with many compounds showing neuroprotective properties in two primary culture of cortical neuron assays. Esaprazole **1** has since been sent for in vivo testing.

5. Experimental section

5.1. Chemistry

5.1.1. General methods

Purity was determined by HPLC and is stated for each example. HPLC was performed on a Waters Alliance e2695 Separations Module with detection using a Waters 2998 photodiode array detector with the wavelength set to 220 nm. The column used was a Waters XBridge C18, 100 × 4.6 mm, 3.5 μm, 135 Å which was kept in a heater unit at 30 °C. Buffer A was 0.10% trifluoroacetic acid in 95% water, 5% acetonitrile. Buffer B was 0.08% trifluoroacetic acid in 10% water, 90% acetonitrile. The flow was 1.0 mL/min. Following an initial hold of 0.5 min at 0% buffer B, the compounds were eluted off using a gradient of 0–40% buffer B over 10.0 min, a gradient of 40–100% buffer B over 2.5 min followed by a hold at 100% buffer B for 2.5 min, and the column was re-equilibrated at 0% buffer B for a total run time of 20 min.

High resolution mass spectra (HRMS) were performed on a Micromass Q-TOF 1.5 mass spectrometer, using polyethylene glycol as an external standard. ^{1}H NMR spectra were run on a Varian Mercury 300 MHz, or a JEOL ECX300 (MHz) or ECX400 (MHz) spectrometer using either $CDCl_3$ or CD_3OD as solvent. The residual solvent signal (δ 7.26 for $CDCl_3$ and δ 3.31 for CD_3OD) was used as reference.

Gas–liquid chromatography (GC) was carried out on an Agilent 6890N instrument with FID detection at 250 °C, H_2 flow 25 ml/min, air flow 450 ml/min. The column used was a Varian VF5 30 m × 0.32 mm × 0.25 μm. The initial temperature was set at 40 °C for 2 min, and the temperature was ramped at 15 °C/min to 250 °C, where it was held for 4 min, for a total run time of 22 min.

Compound purification was carried out using a TeledyneIsco Combiflash Companion. The crude sample was dissolved in DCM, celite 545 (ca. 10 g per gram of crude sample) was added and the solvent removed in vacuo to give a dry powder. The dry powder was loaded into an empty reservoir and gently compacted. The silica column was wetted with A buffer (Et_2O), then the solvent flow was passed through the load and silica column using an increasing ratio of B buffer (0.1 M NH_3 in 1:1 MeOH/Et_2O) to elute off the product.

Esaprazole **1** was purchased from Chess GmbH, Mannheim (cat# 2455) and *N*-isopropyl-1-piperazineacetamide **2a** was purchased from Acros Organics (cat# 205610250).

5.1.2. General procedure 1 (GP1)

The amine (1.0 equiv) in ethanol (1 mL per mmol amine) was added to the alkyl halide (1.1 equiv) and sodium carbonate (3 equiv) in ethanol (2 mL per mmol amine). The reaction was heated to reflux for 2–3 h then water (5 mL per mmol amine) and aqueous sodium carbonate (1 M, 5 mL per mmol amine) were added. The product was extracted with ethyl acetate (3 times, 20 mL per mmol amine) and the organic layers combined.

Dowex® 50WX2 hydrogen form 100–200 mesh (4 g per mmol amine) was washed with methanol (10 mL per gram resin). The reaction mixture was loaded onto the ion exchange resin using gravity filtration and the resin was washed with methanol (10 mL per gram resin). The product was eluted off the resin with ca. 1 M NH_3 in methanol (10 mL per gram resin) and the solution was concentrate to give the product.

5.1.3. General procedure 2 (GP2)

The amine (2.0 equiv) was added to aqueous hydrochloric acid (2.0 equiv, 1.2 M) then the alkyl halide (1.0 equiv) in ethanol (2 mL per mmol amine) was added and the reaction was heated to reflux. The reaction was cooled to room temperature and aqueous sodium carbonate (1 M, 10 mL per mmol alkyl halide) was added. The product was extracted with ethyl acetate (3 times, 20 mL per mmol alkyl halide) and the combined organic layers were dried over $MgSO_4$, filtered and concentrated. The crude material was purified using the Combiflash (A buffer Et_2O, B buffer 0.1 M NH_3 in 1:1 MeOH/Et_2O) and the relevant fractions combined and concentrated to give the product.

5.1.4. Example A.6.1: general procedure 3 (GP3)

The amine (1.0 equiv) in ethanol (1 mL per mmol amine) was added to the alkyl halide (1.1 equiv) and sodium carbonate (3 equiv) in ethanol (2 mL per mmol amine). The reaction was heated to reflux then water (5 mL per mmol amine) and aqueous sodium carbonate (1 M, 5 mL per mmol amine) were added. The product was extracted with ethyl acetate (3 times, 10 mL per mmol amine) and the combined organic layers were dried over $MgSO_4$, filtered and concentrated. The crude material was purified using the Combiflash (A buffer Et_2O, B buffer 0.1 M NH_3 in 1:1 MeOH/Et_2O) and the relevant fractions combined and concentrated to give the product.

5.1.5. General procedure 4 (GP4)

A halide intermediate (1.0 equiv), amine (0.9–10 equiv) and optionally a base (2.7 equiv) and halogenating agent (0.5 equiv) were mixed together in industrial methylated spirits (15 mL per g halide) and heated in a microwave at 150 °C for 1 h, repeating

if neccesary. Upon completion of the reaction, the mixture was passed through a phase seperation cartridge, concentrated and purified.

5.1.6. *N*-Cyclohexyl-2-(4-methyl-1,4-diazepan-1-yl)acetamide (2b)

1-Methyl-1,4-diazepane (30 mmol, 3.43 g), 2-chloro-*N*-cyclohexylacetamide (33 mmol, 5.81 g) and sodium carbonate (90 mmol, 9.60 g) were used and the reaction was heated to 50 °C for 15 h according to GP3. The crude material purified using a 80 g silica column eluting 0% buffer B for 2 min, then 0–100% buffer B over 21 min and 100% buffer B for 4 min to give **2b** (1.59 g, 21%) as a colourless oil; ^{1}H NMR (300 MHz, CD_3OD) δ 3.69 (m, 1H), 3.12 (s, 2H) 2.73 (m, 8H), 2.39 (s, 3H), 1.81 (m, 6H), 1.66 (m, 1H), 1.33 (m, 5H); HPLC R_t 6.68 min (99.8% pure); HRMS (ESI-TOF) calcd for $C_{14}H_{28}N_3O$ (M+H) 254.2232, found 254.2235.

5.1.7. *N*-Cyclohexyl-2-(4-cyclopentylpiperazin-1-yl)butanamide ((±)-2c)

1-Cyclopentylpiperazine (4 mmol, 619 mg), 2-bromo-*N*-cyclohexylbutanamide (4.4 mmol, 1.10 g) and sodium carbonate (12 mmol, 1.33 g) were used and the reaction was heated to reflux for 18 h according to GP3. The crude material purified using a 12 g silica column eluting 0% buffer B for 1 min, then 0–100% buffer B over 11 min and 100% buffer B for 3 min to give (±)-**2c** (953 mg, 74%) as a white powder; ^{1}H NMR (300 MHz, CD_3OD) δ 3.68 (tt, J = 10.5, 3.9 Hz, 1H), 2.76 (dd, J = 8.2, 6.0 Hz, 1H), 2.53 (m, 8H), 2.53 (m, 1H), 1.87 (m, 4H), 1.68 (m, 9H), 1.30 (m, 7H), 0.89 (t, J = 7.4 Hz, 3H); HPLC R_t 9.63 min (94.4% pure); HRMS (ESI-TOF) calcd for $C_{19}H_{35}N_3NaO$ (M+Na) 344.2678, found 344.2686.

5.1.8. *N*-Cyclopentyl-2-(4-ethyl-3-methylpiperazin-1-yl)-*N*-methylacetamide ((±)-2d)

1-Ethyl-2-methylpiperazine (1 mmol, 128 mg), 2-chloro-*N*-cyclopentyl-*N*-methylacetamide (1.1 mmol, 193 mg) and sodium carbonate (3 mmol, 317 mg) were used according to GP1 to give (±)-**2d** as a yellow oil; ^{1}H NMR (300 MHz, CD_3OD, mixture of two rotamers in ca. 2:3 ratio) δ 4.84 (m, 2/5 H), 4.50 (m, 3/5 H), 3.25 (s, 6/5H), 3.19 (s, 4/5H), 2.94 (s, 6/5H), 2.88 (m, 4H), 2.79 (s, 9/5H), 2.53 (m, 1H), 2.38 (m, 3H), 2.03 (m, 1H), 1.88 (m, 1H), 1.75 (m, 3H), 1.63 (m, 4H), 1.07 (t, J = 7.2 Hz, 3H), 1.07 (d, J = 6.3 Hz, 3H); HPLC R_t 6.68 min (98.6% pure); HRMS (ESI-TOF) calcd for $C_{15}H_{29}N_3NaO$ (M+Na) 290.2208, found 290.2205.

5.1.9. *N*-(2-Methylbutyl)-2-(4-propionylpiperazin-1-yl)acetamide ((±)-2e)

1-(Piperazin-1-yl)propan-1-one (1 mmol, 144 mg), 2-chloro-*N*-(2-methylbutyl)acetamide (1.1 mmol, 182 mg) and sodium carbonate (3 mmol, 316 mg) were used according to GP1 to give (±)-**2d** (258 mg, 96%) as a colourless oil; ^{1}H NMR (300 MHz, CD_3OD) δ 3.61 (m, 4H), 3.18 (dd, J = 13.2, 6.2 Hz, 1H), 3.05 (dd, J = 13.1, 7.4 Hz, 1H), 3.05 (s, 2H), 2.52 (m, 4H), 2.41 (q, J = 7.5 Hz, 2H), 1.60 (td, J = 13.4, 6.7 Hz, 1H), 1.42 (m, 1H), 1.20 (m, 1H), 1.11 (dd, J = 8.6, 6.3 Hz, 3H), 0.94 (t, J = 7.3 Hz, 3H), 0.91 (d, J = 6.7 Hz, 3H); HPLC R_t 7.91 min (99.6% pure); HRMS (ESI-TOF) calcd for $C_{14}H_{27}N_3NaO_2$ (M+Na) 292.1995, found 292.1900.

5.1.10. 2-(1,4-Diazepan-1-yl)-*N*-(pentan-3-yl)acetamide (2f)

1,4-Diazepane (2 mmol, 199 mg) was added to aqueous hydrochloric acid (2 mmol, 1.67 mL, 1.2 M) and stirred for ca. 5 min. 2-Chloro-*N*-(pentan-3-yl)acetamide (1.0 mmol, 169 mg) in ethanol (2 mL) was added and the reaction was heated to reflux for 3 h. The reaction was cooled to room temperature and aqueous sodium carbonate (1 M, 10 mL) was added. The product was extracted with ethyl acetate (3 × 20 mL) and the organic layers combined. Dowex® 50WX2 hydrogen form 100–200 mesh (4 g) was washed with methanol (40 mL). The reaction mixture was loaded onto the ion exchange resin using gravity filtration and the resin was washed with methanol (40 mL). The product was eluted off the resin with ca. 1 M NH_3 in methanol (40 mL) and the solution was concentrate to give **2f** (184 mg, 81%) as a colourless oil; ^{1}H NMR (300 MHz, CD_3OD) δ 3.69 (tt, J = 9.0, 4.9 Hz, 1H), 3.19 (s, 2H), 2.92 (m, 4H), 2.77 (m, 4H), 1.81 (m, 2H), 1.58 (m, 2H), 1.44 (m, 2H), 0.91 (t, J = 7.4 Hz, 6H); HPLC R_t 5.67 min (92.8% pure); HRMS (ESI-TOF) calcd for $C_{12}H_{25}N_3NaO$ (M+Na) 250.1895, found 250.1877.

5.1.11. *N*-Butyl-2-(*trans*-2,5-dimethylpiperazin-1-yl)-*N*-propylacetamide (*trans*-2g)

trans-2,5-Dimethylpiperazine (6 mmol, 686 mg), hydrochloric acid (6 mmol, 5 mL) and *N*-butyl-2-chloro-*N*-propylacetamide (2.0 mmol, 385 mg) were used according to GP2. The crude material purified using a 12 g silica column eluting 0% buffer B for 1 min, then 0–100% buffer B over 11 min and 100% buffer B for 3 min to give *trans*-**2g** (135 mg, 50%) as a yellow oil; ^{1}H NMR (300 MHz, CD_3OD) δ 3.74 (dd, J = 14.7, 1.3 Hz, 1H), 3.5–3.2 (m, 4H), 2.99 (m, 3H), 2.86 (dd, J = 11.8, 2.9 Hz, 1H), 2.58 (m, 2H), 2.15 (ddd, J = 11.9, 10.6, 1.4 Hz, 1H), 1.60 (m, 4H), 1.36 (m, 2H), 1.09 (t, J = 6.2 Hz, 6H), 0.95 (m, 6H); HPLC R_t 9.04 min (99.7% pure); HRMS (ESI-TOF) calcd for $C_{15}H_{32}N_3O$ (M+H) 270.2545, found 270.2525.

5.1.12. *N*-Cyclohexyl-3-(4-(cyclopropanecarbonyl)piperazin-1-yl)propanamide (2h)

Cyclopropyl(piperazin-1-yl)methanone hydrochloride (3 mmol, 573 mg), 3-chloro-*N*-cyclohexylpropanamide (3.3 mmol, 638 mg) and sodium carbonate (12 mmol, 1.27 g) were used and the reaction was heated to reflux for 18 h according to GP3. The crude material purified using a 12 g silica column eluting 0% buffer B for 1 min, then 0–50% buffer B over 11 min and 50% buffer B for 10 min to give **2h** (132 mg, 14%) as a white solid; ^{1}H NMR (300 MHz, CD_3OD) δ 3.77 (m, 2H), 3.68 (m, 1H), 3.62 (m, 2H), 2.69 (t, J = 7.1 Hz, 2H), 2.55 (m, 2H), 2.47 (m, 2H), 2.38 (t, J = 7.1 Hz, 2H), 1.96 (m, 1H), 1.85 (m, 2H), 1.76 (m, 2H), 1.64 (m, 1H), 1.30 (m, 5H), 0.85 (m, 4H); HPLC R_t 8.63 min (99.7%); HRMS (ESI-TOF) calcd for $C_{17}H_{30}N_3O_2$ (M+H) 308.2338, found 208.2331.

5.1.13. *N*-Butyl-2-(piperazin-1-yl)-*N*-propylacetamide (2i)

Piperazine (4.5 mmol, 388 mg), hydrochloric acid (4.5 mmol, 3.75 mL) and *N*-butyl-2-chloro-*N*-propylacetamide (2.25 mmol, 431 mg) were used and the reaction was heated to reflux for 5 h according to GP2. The crude material purified using a 12 g silica column eluting 0% buffer B for 1 min, then 0–100% buffer B over 11 min and 100% buffer B for 5 min to give **2i** (480 mg, 88%) as a pale yellow oil; ^{1}H NMR (300 MHz, CD_3OD) δ 3.40–3.20 (m, 6H), 2.97 (m, 4H), 2.58 (m, 4H), 1.59 (m, 4H), 1.35 (m, 2H), 0.99 (t, J = 7.3 Hz, 3H), 0.95 (t, J = 7.2 Hz, 3H); HPLC R_t 8.48 min (98.9% pure); HRMS (ESI-TOF) calcd for $C_{13}H_{28}N_3O$ (M+H) 242.2232, found 242.2239.

5.1.14. *N*-Cyclohexyl-2-(piperazin-1-yl)hexanamide ((±)-2j)

Piperazine (12 mmol, 1.04 g), hydrochloric acid (12 mmol, 10 mL) and 2-bromo-*N*-cyclohexylhexanamide (4 mmol, 1.10 g) were used and the reaction was heated to reflux for 2 days according to GP2. Piperazine (6 mmol, 500 mg) was added and the reaction was heated to reflux for one additional day. The crude material purified using a 12 g silica column eluting 0% buffer B for 1 min, then 0–100% buffer B over 11 min and 100% buffer B for 7 min to give (±)-**2j** (550 mg, 49%) as a white powder; ^{1}H NMR (300 MHz, CD_3OD) δ 3.67 (m, 1H), 2.86 (m, 4H), 2.82 (m, 1H), 2.59 (m, 4H), 1.97–1.50 (m, 7H), 1.50–1.08 (m, 9H), 0.92 (t, J = 7.1 Hz, 3H) HPLC R_t 10.42 min (94.7% pure); HRMS (ESI-TOF) calcd for $C_{16}H_{32}N_3O$ (M+H) 282.2545, found 282.2566.

5.1.15. *N*-(Pentan-3-yl)-2-(4-propionylpiperazin-1-yl)acetamide (2k)

1-(Piperazin-1-yl)propan-1-one (4 mmol, 566 mg), 2-chloro-*N*-(pentan-3-yl)acetamide (4.4 mmol, 726 mg) and sodium carbonate (12 mmol, 1.27 g) were used and the reaction was heated to reflux for 18 h according to GP3. The crude material purified using a 12 g silica column eluting 0% buffer B for 1 min, then 0–100% buffer B over 11 min and 100% buffer B for 3 min to give **2k** (1.04 g, 100%) as a colourless oil; ^{1}H NMR (300 MHz, CD_3OD) δ 3.70 (m, 1H), 3.61 (m, 4H), 3.07 (s, 2H) 2.53 (m, 4H), 2.41 (q, J = 7.5 Hz, 2H), 1.59 (m, 2H), 1.43 (m, 2H), 1.12 (t, J = 7.5 Hz, 3H), 0.91 (t, J = 7.4 Hz, 6H); HPLC R_t 7.49 min (98.8% pure); HRMS (ESI-TOF) calcd for $C_{14}H_{28}N_3O_2$ (M+H) 270.2182, found 270.2198.

5.1.16. *N*-Cyclohexyl-2-(4-propionylpiperazin-1-yl)acetamide (2l)

1-(Piperazin-1-yl)propan-1-one (4 mmol, 569 mg), 2-chloro-*N*-cyclohexylacetamide (4.4 mmol, 777 mg) and sodium carbonate (12 mmol, 1.29 g) were used and the reaction was heated to reflux for 18 h according to GP3. The crude material purified using a 12 g silica column eluting 0% buffer B for 1 min, then 0–100% buffer B over 11 min and 100% buffer B for 3 min to giv **2l** (1.08 g, 96%) as a colourless oil; ^{1}H NMR (300 MHz, CD_3OD) δ 3.70 (m, 1H), 3.61 (m, 4H), 3.03 (s, 2H) 2.51 (m, 4H), 2.41 (q, J = 7.5 Hz, 2H), 1.81 (m, 4H), 1.65 (m, 1H), 1.31 (m, 5H), 1.12 (t, J = 7.5 Hz, 3H), HPLC R_t 8.00 min (99.7%); HRMS (ESI-TOF) calcd for $C_{15}H_{28}N_3O_2$ (M+H) 282.2182, found 282.2218.

5.1.17. *N*-Cyclopentyl-*N*-methyl-2-(piperazin-1-yl)acetamide (2m)

Piperazine (12 mmol, 1.04 g), hydrochloric acid (12 mmol, 10 mL) and 2-chloro-*N*-cyclopentyl-*N*-methylacetamide (4 mmol, 701 mg) were used and the reaction was heated to reflux for 18 h according to GP2. The crude material purified using a 12 g silica column eluting 0% buffer B for 1 min, then 0–100% buffer B over 11 min and 100% buffer B for 7 min to give **2m** (601 mg, 67%) as a pale yellow oil; ^{1}H NMR (300 MHz, CD_3OD, mixture of two rotamers in ca. 1:1 ratio) δ 4.83 (m, 1/2H), 4.50 (m, 1/2H), 3.28 (s, 1H), 3.23 (s, 1H), 2.94 (s, 3/2H), 2.92 (m, 4H), 2.79 (s, 3/2H), 2.55 (m, 4H), 1.91 (m, 1H), 1.77 (m, 3H), 1.63 (m, 4H); HPLC R_t 5.92 min (97.5% pure); HRMS (ESI-TOF) calcd for $C_{12}H_{24}N_3O$ (M+H) 226.1919, found 226.1913.

5.1.18. *N*-Cyclohexyl-2-(4-cyclopentylpiperazin-1-yl)acetamide (2n)

1-Cyclopentylpiperazine (4 mmol, 622 mg), 2-chloro-*N*-cyclohexylacetamide (4.4 mmol, 771 mg) and sodium carbonate (12 mmol, 1.26 g) were used and the reaction was heated to reflux for 4 h according to GP3. The crude material purified using a 12 g silica column eluting 0% buffer B for 1 min, then 0–100% buffer B over 11 min and 100% buffer B for 3 min to give **2n** (1.01 g, 86%) as a white solid; ^{1}H NMR (300 MHz, CD_3OD) δ 3.68 (m, 1H), 2.99 (s, 2H) 2.57 (m, 8H), 2.51 (m, 1H), 1.91 (m, 4H), 1.66 (m, 6H), 1.31 (m, 7H); HPLC R_t 8.84 min (99.1% pure); HRMS (ESI-TOF) calcd for $C_{17}H_{32}N_3O$ (M+H) 294.2545, found 294.2548.

5.1.19. *N*-(2-Cyclooctylethyl)-2-(piperazin-1-yl)propanamide ((±)-2o)

2-Chloro-*N*-(2-cyclooctylethyl)propanamide (4.07 mmol, 1 g), piperazine (40.7 mmol, 3.5 g) and NaI (4.07 mmol, 610 mg) were used according to GP4, purifying by Biotage SP4, to give (±)-**2o** (1.13 g, 94%) as a pale yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.16 (t, J = 6.1 Hz, 1H), 3.24 (dtd, J = 11.0, 7.0, 6.5, 3.8 Hz, 2H), 2.93 (m, 5H), 2.46 (m, 5H), 1.51 (m, 15H), 1.27 (m, 2H), 1.20 (d, J = 7.0 Hz, 3H); HPLC R_t 12.36 min (96.6% pure); GC: R_t 15.95 min; HRMS (ESI-TOF) calcd for $C_{17}H_{34}N_3O$ (M+H) 296.2702, found 296.2726.

5.1.20. *N*-Cyclopentyl-2-(1,4-diazepan-1-yl)propanamide ((±)-2p)

2-Chloro-*N*-cyclopentylpropanamide (5.7 mmol, 1 g) and homopiperazine (57 mmol, 5.7 g) were used according to GP4, purifying by Biotage SP4 (2 cycles) to give (±)-**2p** (668 mg, 49%) as a yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.28 (m, 1H), 4.18 (dp, J = 7.9, 6.7 Hz, 1H), 3.23 (q, J = 6.9 Hz, 1H), 2.90 (m, 4H), 2.64 (m, 4H), 1.94 (m, 2H), 1.67 (m, 4H), 1.36 (m, 2H), 1.20 (d, J = 7.0 Hz, 3H); HPLC R_t 5.75 min (91.7% pure); GC: R_t 12.93 min; HRMS (ESI-TOF) calcd for $C_{13}H_{25}N_3NaO$ (M+Na) 262.1890, found 262.1853.

5.1.21. *N*-(3,3-Dimethylbutyl)-2-(piperazin-1-yl)acetamide (2q)

2-Chloro-*N*-(3,3-dimethylbutyl)acetamide (5.63 mmol, 1 g) and piperazine (56.3 mmol, 4.85 g) were used according to GP4, purifying by Biotage SP4 to give **2q** (1.09 g 94%) as a pale yellow solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.05 (s, 1H), 3.26 (m, 2H), 2.95 (s, 2H), 2.90 (m, 4H), 2.56 (s, 2H), 2.49 (dd, J = 6.2, 3.5 Hz, 4H), 1.39 (m, 2H), 0.91 (s, 9H); HPLC R_t 8.31 min (99.4% pure); GC: R_t 11.69 min; HRMS (ESI-TOF) calcd for $C_{12}H_{26}N_3O$ (M+H) 228.2070, found 228.2045.

5.1.22. *N*-((1*S*,2*S*,4*R*)-rel-Bicyclo[2.2.1]heptan-2-yl)-2-(piperazin-1-yl)acetamide (2r)

N-((1*S*,2*S*,4*R*)-rel-Bicyclo[2.2.1]heptan-2-yl)-2-chloroacetamide (5.35 mmol, 1 g) and piperazine (53.5 mmol, 4.6 g) were used according to GP4, purifying by Biotage SP4 to give **2r** (991 mg, 78%) as a yellow oil which solidified on standing; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.01 (s, 1H), 3.73 (td, J = 8.0, 3.5 Hz, 1H), 2.90 (m, 6H), 2.48 (m, 4H), 2.28 (m, 1H), 2.17 (m, 1H), 1.79 (ddd, J = 13.1, 8.0, 2.2 Hz, 1H), 1.47 (m, 2H), 1.21 (m, 5H); HPLC R_t 7.28 min (97.0% pure); GC: R_t 13.20 min; HRMS (ESI-TOF) calcd for $C_{13}H_{24}N_3O$ (M+H) 238.1919, found 238.1909.

5.1.23. 2-(Piperazin-1-yl)-*N*-((1*S*,2*S*,3*S*,5*R*)-2,6,6-trimethylbicyclo[3.1.1]heptan-3-yl)acetamide (2s)

2-Chloro-*N*-((1*S*,2*S*,3*S*,5*R*)-2,6,6-trimethylbicyclo[3.1.1]heptan-3-yl)acetamide (4.35 mmol, 1 g) and piperazine (43.5 mmol, 3.75 g) were used according to GP4, purifying by Biotage SP4 to give **2s** (1.04 g, 86%) as a viscous yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.01 (s, 1H), 4.25 (tt, J = 9.6, 6.4 Hz, 1H), 2.97 (s, 2H), 2.91 (m, 4H), 2.48 (m, 6H), 2.04 (m, 1H), 1.95 (m, 1H), 1.80 (m, 1H), 1.75 (m, 1H), 1.48 (ddd, J = 13.9, 6.0, 2.4 Hz, 1H), 1.22 (s, 3H), 1.10 (d, J = 7.1 Hz, 3H), 1.04 (s, 3H), 0.86 (d, J = 9.8 Hz, 1H); HPLC R_t 10.95 min (98.0% pure); GC: R_t 14.28 min; HRMS (ESI-TOF) calcd for $C_{16}H_{30}N_3O$ (M+H) 280.2389, found 280.2390.

5.1.24. 2-(4-Cyclohexylpiperazin-1-yl)-*N*-isopropyl-2-methylpropanamide (2t)

Propan-2-amine (210 mmol, 18 ml) was dissolved in DCM (21 mL) and added drop wise to a stirred solution of 2-bromo-2-methylpropanoyl bromide (100 mmol, 23.0 g) in DCM (100 mL) at ca. 10 °C. The reaction was stirred at room temperature for 30 min then concentrated HCl (10 mL) and water (40 mL) were added. The layers were separated and the organic phase was washed with saturated aqueous sodium hydrogen carbonate (30 mL), dried over magnesium sulphate, filtered and concentrated in vacuo to give 2-bromo-*N*-isopropyl-2-methylpropanamide (20.2 g, 97%) as a white solid; ^{1}H NMR (300 MHz, CD_3OD) δ 6.50 (s, 1H), 4.01 (dhept, J 7.8, 6.6 Hz, 1H), 1.95 (s, 6H), 1.20 (d, J = 6.5 Hz, 6H); HPLC R_t 12.06 min.

1-Cyclohexylpiperazine (20 mmol, 3.37 g), 2-bromo-*N*-isopropyl-2-methylpropanamide (48 mmol, 9.92 g) and sodium carbonate (60 mmol, 6.35 g) were used and the reaction was heated to reflux for 16 h according to GP3. The crude material purified using a 80 g silica column eluting 0% buffer B for 2 min, then 0–100% buffer B over 23 min and 100% buffer B for 10 min. The product containing

fractions were repurified using a 80 g silica column eluting 0% buffer B for 2 min, then 0–50% buffer B over 21 min to give **2t** (1.31 g, 22%) as a very pale yellow oil; ^{1}H NMR (300 MHz, CD_3OD) δ 3.94 (hept, J = 6.7 Hz, 1H), 2.66 (s, 4H), 2.55 (m, 4H), 2.25 (m, 1H), 1.97 (m, 2H), 1.83 (m, 2H), 1.66 (m, 1H), 1.26 (m, 5H), 1.17 (s, 6H), 1.15 (d, J = 6.7 Hz, 6H); HPLC R_t 8.53 min (95.8% pure); HRMS (ESI-TOF) calcd for $C_{17}H_{34}N_3O$ (M+H) 296.2702, found 296.2709.

5.1.25. 2-(4-(Cyclohexanecarbonyl)piperazin-1-yl)-*N*-(3,3-dimethylbutyl)acetamide (2u)

2-Chloro-*N*-(3,3-dimethylbutyl)acetamide (5.63 mmol, 1 g), 1-(cyclohexylcarbonyl)-piperazine (5.12 mmol, 1 g) and Na_2CO_3 (15.2 mmol, 1.61 g) were used according to GP4, purifying by Biotage SP4 to give **2u** (1.56 g, 88%) as a pale yellow solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.97 (s, 1H), 3.55 (m, 4H), 3.30 (m, 3H), 3.01 (m, 2H), 2.45 (m, 5H), 1.79 (m, 2H), 1.69 (m, 4H), 1.46 (m, 5H), 1.35–1.25 (m, 3H), 0.94 (s, 9H); HPLC R_t 12.43 min (91.4% pure); GC: R_t 18.14 min; HRMS (ESI-TOF) calcd for $C_{19}H_{35}N_3NaO_2$ (M+Na) 360.2621, found 360.2584.

5.1.26. 2-(4-Cyclohexylpiperazin-1-yl)-*N*-((1*S*,2*S*,3*S*,5*R*)-2,6,6-trimethylbicyclo[3.1.1]heptan-3-yl)acetamide (2v)

2-Chloro-*N*-((1*S*,2*S*,3*S*,5*R*)-2,6,6-trimethylbicyclo[3.1.1]heptan-3-yl)acetamide (4.35 mmol, 1 g), 4-cyclohexylpiperazine (3.96 mmol, 666 mg) and Na_2CO_3 (11.74 mmol, 1.25 g) were used according to GP4, purifying by Biotage SP4 to give **2v** (1.15 g, 87%) as a yellow orange oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.98 (s, 1H), 4.25 (tt, J = 9.6, 6.5 Hz, 1H), 3.48 (s, 1H), 2.98 (s, 2H), 2.59 (m, 8H), 2.42 (dtd, J = 9.7, 6.2, 2.3 Hz, 1H), 2.25 (m, 1H), 1.83 (m, 7H), 1.63 (dt, J = 13.2, 3.0 Hz, 1H), 1.48 (ddd, J = 14.0, 6.0, 2.5 Hz, 1H), 1.21 (m, 7H), 1.10 (d, J = 7.2 Hz, 3H), 1.04 (s, 3H), 0.86 (d, J = 9.7 Hz, 1H); HPLC R_t 13.55 min (97.5% pure); GC: R_t 18.40 min; HRMS (ESI-TOF) calcd for $C_{22}H_{40}N_3O$ (M+H) 362.3171, found 362.3187.

5.1.27. *N*-Cyclopentyl-2-(4-propionylpiperazin-1-yl)propanamide ((±)-2w)

2-Chloro-*N*-cyclopentylpropanamide (5.7 mmol, 1 g), *N*-propionylpiperazine (5.2 mmol, 736 mg) Na_2CO_3 (15 mmol, 1.63 g) and NaI (2.84 mmol, 430 mg) were used according to GP4, purifying by biotage SP4 (2 cycles), to give (±)-**2w** (794 mg, 54%) as a pale yellow solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ6.99 (s, 1H), 4.17 (m, 1H), 3.60 (m, 2H), 3.45 (q, J = 4.8 Hz, 2H), 3.01 (q, J = 7.0 Hz, 1H), 2.47 (m, 4H), 2.31 (q, J = 7.5 Hz, 2H), 1.93 (m, 2H), 1.61 (m, 4H), 1.35 (m, 2H), 1.19 (d, J = 7.0 Hz, 3H), 1.11 (t, J = 7.4 Hz, 3H); HPLC R_t 7.17 min (97.0% pure); GC R_t 15.2 min; HRMS (ESI-TOF) calcd for $C_{17}H_{27}N_3NaO_2$ (M+Na) 304.2001, found 304.1994.

5.1.28. (*R*)-*N*-(3,3-Dimethylbutan-2-yl)-2-(4-isopropyl-1,4-diazepan-1-yl)acetamide (2x)

(*R*)-2-Chloro-*N*-(3,3-dimethylbutan-2-yl)acetamide (5.63 mmol, 1 g), *N*-isopropyl(1,4)-diazapane (5.12 mmol, 0.73 g) and Na_2CO_3 (15.2 mmol, 1.61 g) were used according to GP4, purifying by Biotage SP4 to give **2x** (794 mg, 55%) as a yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32 (s, 1H), 3.83 (m, 1H), 3.10 (s, 2H), 2.89 (m, 1H), 2.69 (m, 8H), 1.77 (m, 2H), 1.04 (d, J = 6.9 Hz, 3H), 0.98 (d, J = 6.9 Hz, 6H), 0.89 (s, 9H); HPLC R_t 7.69 min (95.1% pure); GC: R_t 12.74 min; HRMS (ESI-TOF) calcd for $C_{16}H_{34}N_3O$ (M+H) 284.2702, found 284.2706.

5.1.29. *N*-(3-Cyclopentylpropyl)-2-(4-methyl-1,4-diazepan-1-yl)acetamide (2y)

2-Chloro-*N*-(3-cyclopentylpropyl)acetamide (4.9 mmol, 1 g), 1-methylhomopiperazine (4.47 mmol, 0.56 mL) and Na_2CO_3 (13.23 mmol, 1.4 g) were used according to GP4, purifying by Biotage SP4 to give **2y** (727 mg, 58%) as an orange oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45 (s, 1H), 3.27 (td, J = 7.1, 5.9 Hz, 2H), 3.15 (s, 2H), 2.79 (m, 7H), 2.50 (s, 3H), 1.94 (m, 2H), 1.74 (m, 3H), 1.54 (m, 6H), 1.32 (m, 2H), 1.05 (m, 2H); HPLC R_t 10.33 min (88.4% pure); GC: R_t 14.71 min; HRMS (ESI-TOF) calcd for $C_{16}H_{32}N_3O$ (M+H) 282.2545, found 282.2559.

5.1.30. 2-(1,4-Diazepan-1-yl)-*N*-methyl-*N*-pentylacetamide (2z)

Homopiperazine (12 mmol, 1.19 g), hydrochloric acid (12 mmol, 10 mL) and 2-chloro-*N*-methyl-*N*-pentylacetamide (4 mmol, 709 mg) were used and the reaction was heated to reflux for 3.5 h according to GP2. The crude material purified using a 12 g silica column eluting 0% buffer B for 1 min, then 0-100% buffer B over 11 min and 100% buffer B for 7 min to give **2z** (367 mg, 38%) as a pale yellow oil; ^{1}H NMR (300 MHz, CD_3OD, mixture of 2 rotamers in ca. 1:1 ratio) δ3.41 (s, 1H), 3.39 (s, 1H), 3.36 (m, 2H), 3.08 (s, 3/2H), 2.91 (m, 4H), 2.90 (s, 3/2H), 2.76 (dt, J = 6.8, 2.5 Hz, 4H), 1.82 (m, 2H), 1.60 (m, 2H), 1.34 (m, 4H), 0.95 (t, J = 7.1 Hz, 3/2 H), 0.92 (t, J = 7.1 Hz, 3/2 H); HPLC R_t 7.53 min (99.7% pure); HRMS (ESI-TOF) calcd for $C_{13}H_{28}N_3O$ (M+H) 242.2232, found 242.2218.

5.1.31. 3-(4-Acetyl-1,4-diazepan-1-yl)-*N*-cycloheptylpropanamide (2aa)

1-(1,4-Diazepan-1-yl)ethanone (3 mmol, 426 mg), 3-bromo-*N*-cycloheptylpropanamide (4.5 mmol, 1.12 g), sodium carbonate (6 mmol, 636 mg) and potassium iodide (3.3 mmol, 549 mg) were used and the reaction was heated to reflux for 17 h according to GP3. The crude material purified using a 12 g silica column eluting 0% buffer B for 1 min, then 0–100% buffer B over 11 min and 100% buffer B for 3 min to give **2aa** (661 mg, 71%) as a colourless oil; ^{1}H NMR (300 MHz, CD_3OD) δ3.83 (m, 1H), 3.59 (m, 4H), 2.81 (m, 3H), 2.69 (m, 3H), 2.33 (td, J = 6.9, 1.8 Hz, 2H), 2.11 (s, 3H), 1.86 (m, 4H), 1.56 (m, 10H), HPLC R_t 9.18 min (99.7% pure); HRMS (ESI-TOF) calcd for $C_{17}H_{32}N_3O_2$ (M+H) 310.2495, found 310.2499.

5.1.32. *N*-Cyclohexyl-2-(4-cyclopentylpiperazin-1-yl)-2-methylpropanamide (2ab)

1-Cyclopentylpiperazine (40 mmol, 6.17 g), 2-bromo-*N*-cyclohexyl-2-methylpropanamide (40 mmol, 9.93 g), sodium carbonate (80 mmol, 7.3 g) and potassium iodide (44 mmol, 8.48 g) were used and the reaction was heated to reflux for 2 days according to GP3. The crude material purified using a 80 g silica column eluting 0% buffer B for 2 min, then 0–100% buffer B over 21 min and 100% buffer B for 9 min to give **2ab** (1.78 g, 14%) as a colourless oil; ^{1}H NMR (300 MHz, CD_3OD) δ 3.64 (m, 1H), 2.96–2.17 (m, 9H), 1.91 (m, 2H), 1.68 (m, 9H), 1.34 (m, 7H), 1.18 (s, 6H); HPLC R_t 10.47 min (98.8% pure); HRMS (ESI-TOF) calcd for $C_{19}H_{36}N_3O$ (M+H) 322.2858, found 322.2845.

5.1.33. 2-(4-(Cyclohexanecarbonyl)piperazin-1-yl)-*N*-(2-cyclooctylethyl)propanamide ((±)-2ac)

2-Chloro-*N*-(2-cyclooctylethyl)propanamide (4.07 mmol, 1 g), 1-cylohexylcarbonyl piperazine (3.70 mmol, 727 mg), Na_2CO_3 (10.99 mmol, 1.16 g) and NaI (4.07 mmol, 610 mg) were used according to GP4, purifying by Biotage SP4, to give (±)-**2ac** (1.58 g, 96%) as a yellow oil; ^{1}H NMR 400 MHz ($CDCl_3$) δ 7.03 (s, 1H), 3.62 (m, 2H), 3.49 (m, 2H), 3.26 (tt, J = 7.3, 5.9 Hz, 2H), 3.04 (q, J = 7.0 Hz, 1H), 2.46 (m, 5H), 1.79 (m, 2H), 1.55 (m, 19H), 1.23 (m, 8H); HPLC R_t 14.93 min (94.3% pure); HRMS (ESI-TOF) calcd for $C_{24}H_{44}N_3O_2$ (M+H) 406.3434, found 406.3413.

5.1.34. (*R*)-*N*-Cyclohexyl-2-(hexahydropyrrolo[1,2-*a*]pyrazin-2(1*H*)-yl)acetamide ((*R*)-3a)

(*R*)-Octahydropyrrolo[1,2-*a*]pyrazine (20 mmol, 2.52 g), 2-chloro-*N*-cyclohexylacetamide (22 mmol, 3.86 g) and sodium carbonate (60 mmol, 6.35 g) were used and the reaction was heated to reflux for 16 h according to GP3. The crude material purified using a 80 g silica column eluting 0% buffer B for 2 min, then

0–100% buffer B over 21 min and 100% buffer B for 4 min to give (***R***)-**3a** (3.81 g, 72%) as a white wax; ^{1}H NMR (300 MHz, CD_3OD) δ 3.68 (m, 1H), 3.08 (m, 2H), 3.06 (s, 2H) 2.97 (m, 1H), 2.81 (m, 1H), 2.43 (m, 2H), 2.29 (m, 2H), 2.11 (m, 1H), 1.83 (m, 7H), 1.64 (m, 1H), 1.32 (m, 6H); HPLC R_t 7.82 min (99.6% pure); HRMS (ESI-TOF) calcd for $C_{15}H_{28}N_3O$ (M+H) 266.2232, found 266.2230.

5.1.35. (*S*)-*N*-Cyclohexyl-2-(hexahydropyrrolo[1,2-*a*]pyrazin-2(1*H*)-yl)acetamide ((*S*)-3a)

(*S*)-Octahydropyrrolo[1,2-*a*]pyrazine (20 mmol, 2.53 g), 2-chloro-*N*-cyclohexylacetamide (22 mmol, 3.93 g) and sodium carbonate (60 mmol, 6.41 g) were used and the reaction was heated to reflux for 16 h according to GP3. The crude material purified using a 80 g silica column eluting 0% buffer B for 2 min, then 0–100% buffer B over 21 min and 100% buffer B for 4 min to give (***S***)-**3a** (3.82 g, 72%) as a white wax; ^{1}H NMR (300 MHz, CD_3OD) δ 3.69 (m, 1H), 3.05 (s, 2H) 3.01 (m, 3H), 2.79 (m, 1H), 2.37 (m, 2H), 2.22 (m, 2H), 2.05 (m, 1H), 1.87 (m, 5H), 1.64 (m, 1H), 1.33 (m, 6H); HPLC R_t 7.82 min (99.9% pure); HRMS (ESI-TOF) calcd for $C_{15}H_{28}N_3O$ (M+H) 266.2232, found 266.2241.

5.1.36. *N*-Cyclohexyl-2-(dihydro-1*H*-pyrido[1,2-*a*]pyrazin-2(6*H*,7*H*,8*H*,9*H*,9a*H*)-yl)acetamide (3b)

Octahydro-1*H*-pyrido[1,2-*a*]pyrazine (1 mmol, 141 mg), 2-chloro-*N*-cyclohexylacetamide (1.1 mmol, 194 mg) and sodium carbonate (3 mmol, 321 mg) were used according to GP1 to give **3b** (274 mg, 98%) as a cream solid; ^{1}H NMR (300 MHz, CD_3OD) δ 3.69 (m, 1H), 2.98 (s, 2H), 2.83 (m, 2H), 2.72 (m, 2H), 2.37 (m, 2H), 2.12 (m, 2H), 2.02 (dd, *J* = 19.6, 9.1 Hz, 2H), 1.85–1.7 (m, 5H), 1.65–1.5 (m, 4H), 1.45–1.2 (m, 7H); HPLC R_t 8.16 min (99.5% pure); HRMS (ESI-TOF) calcd for $C_{16}H_{30}N_3O$ (M+H) 280.2389, found 280.2381.

5.1.37. 2-((*1S,4S*)-2,5-Diazabicyclo[2.2.1]heptan-2-yl)-*N*-cyclohexylacetamide (4a)

(*1S,4S*)-*tert*-Butyl 2,5-diazabicyclo[2.2.1]heptane-2-carboxylate (4 mmol, 792 mg), 2-chloro-*N*-cyclohexylacetamide (4.4 mmol, 776 mg) and sodium carbonate (12 mmol, 1.27 g) were used and the reaction was heated to reflux for 4 h according to GP3. The crude material purified using a 12 g silica column eluting 0% buffer B for 1 min, then 0–100% buffer B over 11 min and 100% buffer B for 3 min to give Boc-**4a** (1.04 g, 77%) as a colourless oil; ^{1}H NMR (300 MHz, CD_3OD) δ 4.29 (1H, br s), 3.68 (m, 1H), 3.52 (br s, 1H), 3.45 (m, 1H), 3.26 (d, *J* = 16.0 Hz, 1H), 3.18 (d, *J* = 16.0 Hz, 1H), 2.93 (dd, *J* = 9.6, 2.1 Hz, 1H), 2.63 (m, 1H), 1.90 (m, 2H), 1.78 (m, 4H), 1.65 (m, 1H), 1.47 (s, 9H), 1.32 (m, 5H); HPLC R_t 11.52 min

Boc-**4a** (1.04 g) was dissolved in ethanol (9 mL) then HCl in ether (4.6 mL of 2.0 M solution) was added. The reaction was stirred at room temperature for 8 days then at 60 °C for 18 h. Aqueous Na_2CO_3 (1 M, 20 mL) was added, the product was extracted with EtOAc (3 × 30 mL) and the organic phase dried over $MgSO_4$, filtered and concentrated. The crude material purified using a 4 g silica column eluting 0% buffer B for 1 min, then 0–100% buffer B over 11 min and 100% buffer B for 6 min to give **4a** (17 mg, 2%) as a white precipitate; ^{1}H NMR (300 MHz, CD_3OD) δ 3.69 (m, 1H), 3.62 (br s, 1H), 3.43 (br s, 1H), 3.24 (d, *J* = 16.0 Hz, 1H), 3.17 (d, *J* = 16.0 Hz, 1H), 3.09 (dd, *J* = 10.5, 1.1 Hz, 1H), 2.90 (dd, *J* = 10.0, 2.4 Hz, 1H), 2.82 (dd, *J* = 10.5, 2.4 Hz, 1H), 2.58 (dd, *J* = 10.0, 1.1 Hz, 1H), 1.81 (m, 5H), 1.63 (m, 2H), 1.30 (m, 5H); HPLC R_t 6.62 min (94.7% pure).

5.1.38. 3-((*1S,4S*)-2,5-Diazabicyclo[2.2.1]heptan-2-yl)-*N*-cyclohexylpropanamide (4b)

(*1S,4S*)-*tert*-Butyl 2,5-diazabicyclo[2.2.1]heptane-2-carboxylate (4 mmol, 794 mg), 3-chloro-*N*-cyclohexylpropanamide (4.4 mmol, 837 mg) and sodium carbonate (12 mmol, 1.27 g) were used and the reaction was heated to reflux for 4 h according to GP3. The crude material purified using a 12 g silica column eluting 0% buffer B for 1 min, then 0–100% buffer B over 8 min and 100% buffer B for 3 min to give Boc-**4b** (463 mg, 33%) as a colourless oil; ^{1}H NMR (300 MHz, CD_3OD) δ 4.28 (br s, 1H), 3.64 (tt, *J* = 9.5, 3.2 Hz, 1H), 3.55 (br s, 1H), 3.45 (dd, *J* = 10.4, 4.8 Hz, 1H), 3.18 (ddd, *J* = 10.3, 8.0, 2.2 Hz, 1H), 2.83 (m, 3H), 2.66 (t, *J* = 9.4 Hz, 1H), 2.31 (t, *J* = 6.9 Hz, 2H), 1.84 (m, 3H), 1.73 (m, 3H), 1.64 (m, 1H), 1.46 (s, 9H), 1.28 (m, 5H); HPLC R_t 11.84 min.

Boc-**4b** (463 mg) was dissolved in ethanol (5 mL) then concentrated HCl (5 drops) was added. The reaction was heated to 60 °C for 18 h. Aqueous Na_2CO_3 (1 M, 20 mL) was added, the product was extracted with EtOAc (3 × 30 mL) and the organic phase dried over $MgSO_4$, filtered and concentrated. The crude material purified using a 4 g silica column eluting 0% buffer B for 1 min, then 0–100% buffer B over 11 min and 100% buffer B for 8 min to give **4b** (35 mg, 11%) as a pale yellow oil; ^{1}H NMR (300 MHz, CD_3OD) δ 3.63 (m, 1H), 3.52 (br s, 1H), 3.45 (br s, 1H), 3.09 (dd, *J* = 10.6, 1.3 Hz, 1H), 2.81 (m, 4H), 2.48 (dd, *J* = 10.0, 1.3 z, 1H), 2.31 (t, *J* = 7.1 Hz, 2H), 1.78 (m, 5H), 1.62 (m, 2H), 1.32 (m, 5H); HPLC R_t 7.06 min (99.2% pure).

5.1.39. *N*-Methyl-*N*-pentyl-2-(1,4-diazaspiro[5.5]undecan-4-yl)acetamide (5)

1,4-Diazaspiro[5.5]undecane dihydrochloride (1 mmol, 229 mg) in ethanol (1 mL) was added to 2-chloro-*N*-methyl-*N*-pentylacetamide (1.1 mmol, 197 mg) and sodium carbonate (5 mmol, 530 mg) in ethanol (2 mL) and the reaction was heated to reflux for 3 h. The reaction was cooled to room temperature then water (5 mL) and aqueous Na_2CO_3 (1 M, 5 mL) was added. The product was extracted with EtOAc (3 × 20 mL). Dowex® 50WX2 hydrogen form 100–200 mesh (4 g) was washed with methanol (40 mL). The reaction mixture was filtered then loaded onto the ion exchange resin using gravity filtration. The resin was washed with methanol (40 mL). The product was eluted off the resin with ca. 1 M NH_3 in methanol (20 mL) and the solution was concentrated. The crude material was purified by Combiflash (A buffer Et_2O, B buffer 0.1 M NH_3 in 1:1 MeOH/Et_2O) using a 4 g silica column eluting 0% buffer B for 1 min, then 0–100% buffer B over 11 min and 100% buffer B for 2 min to give **5** (102 mg, 35%) as a yellow oil; ^{1}H NMR (300 MHz,, CD_3OD, mixture of two rotamers in ca. 1:1 ratio) 3.47 (m, 1/2H), 3.36 (m, 1/2H), 3.13 (s, 3H) 3.12 (s, 2/2H), 2.90 (s, 2/2H), 2.87 (m, 2H), 2.37 (m, 4H), 1.66 (m, 3H), 1.56 (m, 5H), 1.35 (m, 11H), 0.95 (t, *J* = 7.1 Hz, 3/2 H), 0.94 (t, *J* = 7.1 Hz, 3/2 H); HPLC R_t 10.26 min (92.5% pure); HRMS (ESI-TOF) calcd for $C_{17}H_{37}N_3O$ (M+H) 296.2702, found 296.2680.

5.2. Biological assays

In vitro ADME properties in terms of blood–brain barrier penetration potential and metabolic stability of the compounds in addition to the sigma and muscarinic bindings were performed as previously described by Elbrønd-Bek et al.[8] Likewise, the methods of the in vitro neuronal damage assays (hydrogen peroxide and glutamate) was outlined by Wellejus et al.[20] All receptor-, enzyme- and ion channel assays were carried out by Ricerca Biosciences, Taipei, Taiwan and the pocedures are available on Ricerca Biosciences homepage.

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penetration and metabolic stability testing; Ricerca Biosciences, Taipei, Taiwan for performing the receptor binding and enzyme inhibition assays and Anette Andersen at the Department of Chemistry, Copenhagen University, Denmark for running the HRMS.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2013.02.058.

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