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Search for the pharmacophore in prazosin for Transport-P

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Abstract—Partial structures of prazosin have been synthesised and tested for inhibition of Transport-P in order to identify the structural features of prazosin, which appear to be involved in binding to the putative transporter. It is shown that the pyrimidinyl 4-amino group is critically important for binding but that the 6,7-dimethoxy and 2-furoyl groups are not essential.

1. Introduction

Transport-P is an antidepressant-sensitive, proton-dependent, V-ATPase-linked uptake process for amines in peptidergic neurones of the hypothalamus. It is unusual in its anatomical location in post-synaptic neurones and in being activated by its ligand prazosin.[1–8] Although prazosin is a potent antagonist at adrenergic α_1 receptors,[9] this uptake effect of prazosin does not appear to involve adrenergic receptors.[7] There are two types of compounds that have so far been identified to act as ligands for Transport-P: Group A compounds, exemplified by prazosin, are accumulated in a cooperative manner and Group B compounds, which include phenylethylamines and some well established antidepressants, are accumulated noncooperatively by the same uptake process. The structural properties of group B have been studied[2] by examining a large series of compounds for their ability to inhibit competitively the uptake of prazosin (1 μM) in immortalised gonadotrophin-releasing hormone neurones (GT1-1 GnRH cells) as previously described in detail.[1]

Those group B compounds, which have the greatest affinity for Transport-P appear to consist of a condensed cyclic structure of 18–20 carbon atoms and a basic amine, which is neither completely neutral nor permanently positively charged. Some examples of the compounds are 4-benzylpiperidine, 4-phenylbutylamine and imipramine, which had IC_{50} values of 4.4 ± 0.5, 2.8 ± 0.6 and 0.23 ± 0.07 μM, respectively[2] (Fig. 1).

Presumably these compounds bind to a putative transporter protein involved in the uptake process. The special interest in characterising this transporter is that it may provide clues for the development of a novel type of antidepressant drug.[10] Since these compounds differ markedly from prazosin in chemical structure it is of interest to examine in detail the extent to which the structure of prazosin is required for the uptake process. Initially, we have concentrated on seeking the structural features, which appear to be involved in binding.

In seeking the pharmacophore for uptake of prazosin (**1**) it is helpful to distinguish four main structural elements of prazosin based on the four different rings that comprise its structure (Fig. 2). These are labelled A: dimethoxy-benzenoid fusion, B: 4-aminopyrimidine, C: piperazine and D: 2-furoyl. Various notional fragmentations

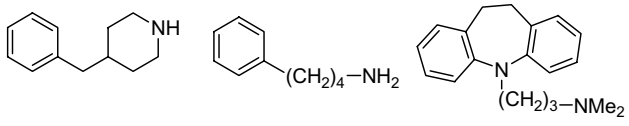

Figure 1. Structures of some inhibitors of Transport-P.

Keywords: Transport-P; Prazosin; Peptidergic neurones; V-ATPase-linked uptake.
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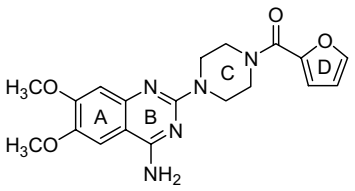

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Figure 2. The four rings, labelled A–D, in prazosin (**1**).

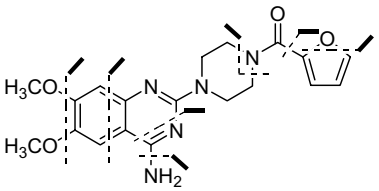


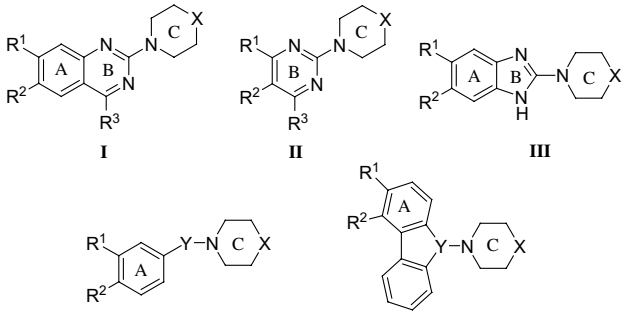
Figure 3. Notional fragmentations of the prazosin structure.

(Fig. 3) have been made to the molecular structure of prazosin to remove functional groups or particular atoms in a stepwise manner. These led to a set of compounds (Table 1), which have been synthesised and tested for their influence on the uptake of prazosin as follows.

The 2-furoyl group (D) has been replaced by benzoyl, acetyl or phenyl to give compounds **2**, **3** and **4**, respectively. In another example the 2-furoyl group (D) has been removed and the amidic N of the piperazine ring has been replaced by CHMe to give the 4-methylpiperidine **5**. The 3-methylpiperidine isomer **6** has also been made. The two methoxy groups on ring A have been removed to afford **7** and then the 2-furoyl group (D) replaced by benzoyl to give **8**; finally the 2-furoyl group (D) has been removed and the amidic N of the piperazine ring C has been replaced by CH_2 to provide a piperidine **9**.

The 4-amino group on ring B has been replaced by a hydrogen atom to give **10**, and also replaced by 4-hydr-

Table 1. Structure and activities of prazosin analogues



Compd	Struct	R^1	R^2	R^3 or Y	X	Inhibition[a] %
1	I	MeO	MeO	NH_2	NCOFur[b]	[c]
2	I	MeO	MeO	NH_2	NCOPh	63 ± 5
3	I	MeO	MeO	NH_2	NCOMe	37 ± 5
4	I	MeO	MeO	NH_2	NPh	93 ± 2[d]
5	I	MeO	MeO	NH_2	CHMe	85 ± 3
6	I	MeO	MeO	NH_2	CHMe[e]	85 ± 3
7	I	H	H	NH_2	NCOFur	64 ± 5
8	I	H	H	NH_2	NCOPh	73 ± 7
9	I	H	H	NH_2	CH_2	83 ± 3
10	I	MeO	MeO	H	NCOFur	5 ± 10
11	I	MeO	MeO	OH	NCOFur	0
12	II	H	H	NH_2	NCOFur	44 ± 4
13	III	H	H		NCOFur	36 ± 6
14	IV	MeO	MeO	CH_2CH_2–	NCOFur	10 ± 13
15	IV	MeO	MeO	CH_2CH_2–	NCOPh	16 ± 7
16	IV	MeO	MeO	CH_2CH_2–	CH_2	91 ± 2[f]
17	IV	MeO	MeO	SO_2	NCOFur	0
18	IV	Cl	Cl	CH_2	NCOFur	47 ± 7
19	V	H	H	CH	NCOFur	5 ± 7

[a] % inhibition of prazosin uptake by 10^{-4} M concentration of the test compound. Values are shown with ±SEM.
[b] Fur =  .
[c] Prazosin.
[d] IC_{50} = 5.2 ± 2 μM.
[e] Isomer of **5** (3-methylpiperidine).
[f] IC_{50} = 23 ± 12 μM.

oxy, which tautomerises to the amide to give **11**. The dimethoxy-benzenoid ring A has been removed to afford the disubstituted pyrimidine **12**. Replacement of dimethoxy-aminoquinazoline (A and B) by benzimidazole gives **13**.

The amidino fragment (N^3=C–NH_2) of the quinazoline in ring B has been removed and the N^1=C replaced by CH_2–CH_2 to provide the arylethylpiperazine **14** which, upon further replacement of furoyl (D) by benzoyl, gives **15**; then replacement of the furoylpiperazine (C and D) by piperidine affords **16**. The linkage has been further shortened using a polar group SO_2 to make a neutral (i.e., nonbasic) molecule **17**. In another approach to reduce the piperazine pK_a but still leave it basic and to explore the chemical space potentially available to contribute hydrophobically to binding, dichlorobenzyl and fluorenyl groups (the latter approximating to a phenyl benzyl group) have been attached directly to the piperazine moiety giving **18** and **19**, respectively.

2. Chemistry

The synthesis of 2-(1-piperazinyl)-4-aminoquinazolines **2**–**9** is summarised in Scheme 1. Treatment of 6,7-dimethoxy-quinazoline-2,4(1*H*,3*H*)-dione (**20a**) with a mixture of $POCl_3$ and PCl_5 afforded the corresponding 2,4-dichloro compound **21a**, which upon reaction with NH_4OH led regioselectively to the 4-amino-2-chloro derivative **22a**. The same sequence starting from quinazoline-2,4(1*H*,3*H*)-dione (**20b**) led to the corresponding quinazoline **22b**. Nucleophilic addition of the appropriate piperazines, piperidines or furfurylamine was effected in refluxing 1-pentanol or isoamyl alcohol to produce the desired final compounds.

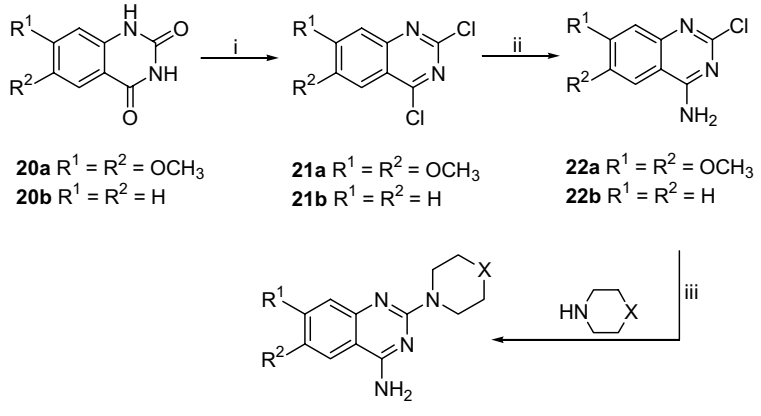
Reduction of 2,4-dichloro-6,7-dimethoxyquinazoline (**21a**) with $NaBH_4$ led to 2-chloro-6,7-dimethoxyquinazoline (**23**), which was then subjected to nucleophilic addition by 1-(2-furoyl)piperazine, obtained by treating piperazine with 2-furoyl chloride, to give **10** (Scheme 2).

Compound **11** was prepared from 2-chloro-4,6,7-trimethoxyquinazoline (**24**) and 1-(2-furoyl)piperazine (Scheme 2). Compound **12** was obtained as shown in Scheme 3 using 4-amino-2-chloropyrimidine as intermediate. Reaction of 2,4-dichloropyrimidine with NH_4OH, followed by chromatographic separation, permitted the isolation of the desired 4-amino-2-chloro isomer, which was subsequently reacted with 1-(2-furoyl) piperazine to give **12**. Benzimidazole **13** was prepared by direct reaction of 2-chloro benzimidazole with 1-(2-furoyl)piperazine (Scheme 3). The 3,4-dimethoxyphenylethyl-piperazines **14**, **15** and 3,4-dimethoxyphenylethyl-piperidine **16** (X = CH_2) were synthesised as depicted in Scheme 4. Bromination of 2-(3,4-dimethoxyphenyl)ethanol (**25**) with *N*-bromosuccinimide in the presence of PPh_3 afforded the corresponding bromo derivative **26**, which was then substituted with the appropriate piperazines or piperidine in the presence of K_2CO_3.

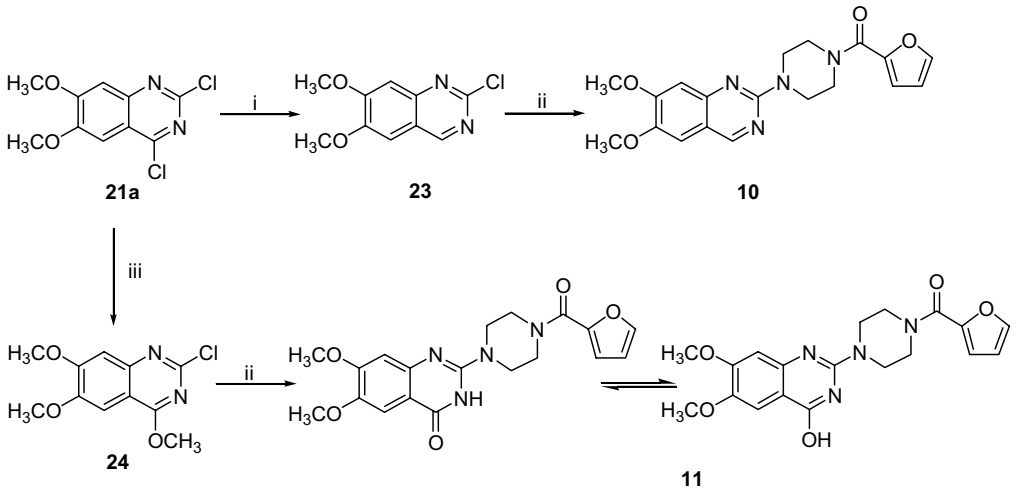
Compounds **17**–**19** were prepared by direct replacement of a chloro or bromo substituent in 3,4-dimethoxybenzene sulfonyl chloride, 3,4-dichlorobenzyl bromide or 9-bromofluorene, respectively, with 1-(2-furoyl)piperazine (Scheme 3).

3. Biological studies

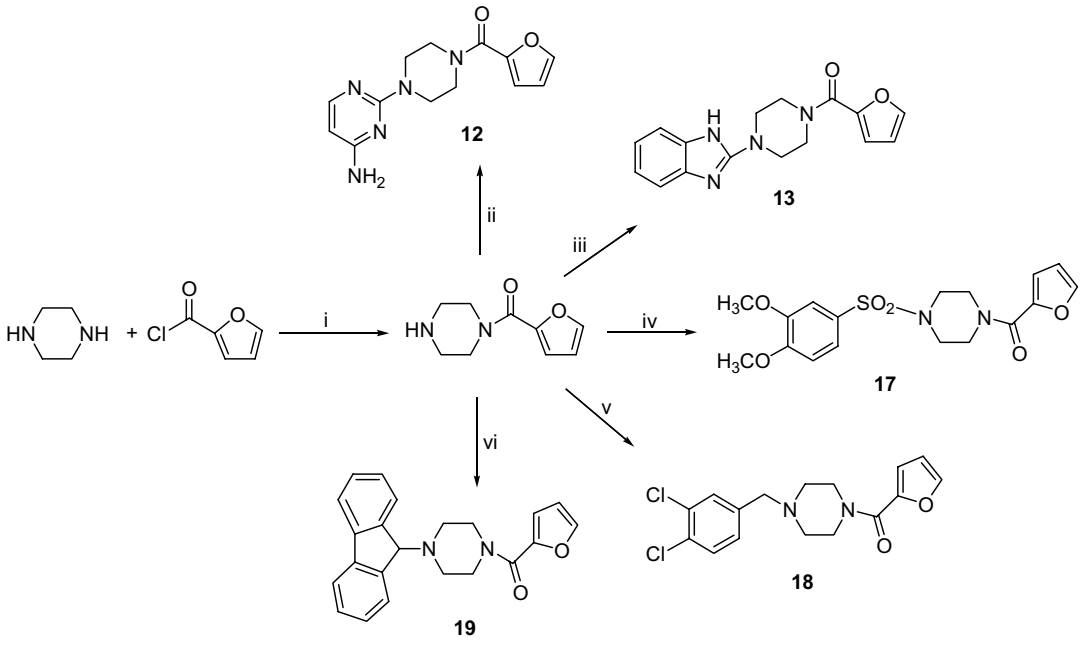
The compounds were examined for their ability to inhibit competitively the uptake of prazosin (1 μM) in immortalised gonadotrophin-releasing hormone neurones (GT1-1 GnRH cells) as previously described in detail.[1] Each compound was tested for its ability to compete with prazosin (at 10^{-6} M; inhibition of Transport-P). Efficacy was defined as % inhibition of the uptake of prazosin (at 10^{-6} M) when the test compound was used in a concentration of 10^{-4} M. Efficacy was expressed as % of the effect of a maximal inhibitory concentration of desipramine (10^{-4} M). IC_{50} values were calculated for compounds, which achieved a maximal inhibitory response, defined as 90% of the inhibitory effect of desipramine 10^{-4} M. Each experimental point



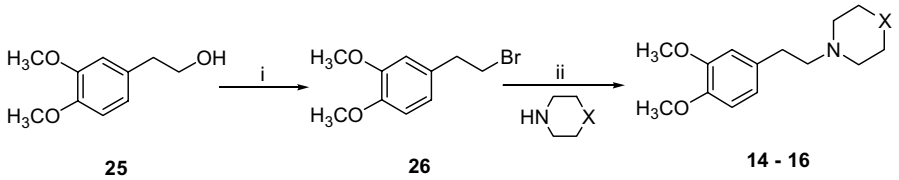
Scheme 1. Experimental conditions: (i) $POCl_3$, PCl_5, $PhN(CH_3)_2$, reflux, 20 h; (ii) 35% NH_4OH, THF, rt, 16 h; (iii) appropriate amine, 1-pentanol or isoamyl alcohol, reflux, 3–20 h.



Scheme 2. Experimental conditions: (i) $NaBH_4$, isopropanol, 70–80 °C, 5 days; (ii) 1-(2-furoyl)piperazine, isoamyl alcohol, 150 °C, 18 h; (iii) $NaOCH_3$, CH_3OH, reflux, 12 h.



Scheme 3. Experimental conditions: (i) 48% HBr, EtOH–H_2O, 80 °C, 1.5 h; (ii) 4-amino-2-chloropyrimidine, 1-pentanol, reflux, 3 h; (iii) 2-chlorobenzimidazole, 1-pentanol, 100 °C, 6 h; (iv) 3,4-dimethoxybenzene sulfonyl chloride, Et_3N, CH_2Cl_2, rt, 12 h; (v) 3,4-dichlorobenzyl bromide, 1-pentanol, reflux, 16 h; (vi) 9-bromofluorene, 1-pentanol, reflux, 12 h.



Scheme 4. Experimental conditions: (i) PPh_3, *N*-bromosuccinimide, toluene, rt; 12 h; (ii) appropriate amine, K_2CO_3, EtOH, reflux; 24 °C.

was carried out in triplicate and each experiment was performed twice; the data are mean ± SEM of six observations for each experimental point. Unlabelled prazosin (at 10^{-6} M) increased the uptake of [^{3}H]prazo-

sin by 126.8 ± 6.3%. Biological data and general structures of title compounds are reported in Table 1.

4. Results and discussion

Previous work has demonstrated that compounds with a basic amino group, which would be protonated (cationic) at the physiological neutral pH of 7.4 are able to inhibit the uptake of prazosin. In the present series, all the compounds except **10**, **11**, **14**, **17** and **19** bind to some extent to the uptake system. Compound **10** (i.e., prazosin minus the quinazoline 4-amino group) was not able to inhibit uptake at 10^{-4} M. Comparison with 2-amino-6,7-dimethoxy-4-methylquinazoline as a model reference structure for the pK_a (reported[11] 5.2) suggests that **10** would probably have <1% of its molecules in the protonated form at pH 7.4. Prazosin, on the other hand (pK_a 6.84)[12] would be approximately 40% cationic (note 2,4-diaminoquinazoline has pK_a 7.96 at 20 °C).[13] Compound **11** would be even less basic than **10**.

Compound **14**, which was not active, and **15**, which gave low inhibition (10–16% at 10^{-4} M), are 1-dimethoxyphenylethyl-4-acyl-piperazine derivatives and the result is surprising in view of the inhibitory activity of the piperidine analogue **16** (91% inhibition at 10^{-4} M). However, this may be accounted for by the differences in pK_a values since acyl piperazines are reported to have substantially lower pK_a's than piperidines (compare 1-methyl-4-benzoyl-piperazine pK_a 6.78 with 1-methylpiperidine pK_a 10.19).[14] Another acylpiperazine that gave low inhibition is the benzimidazole **13** (36% inhibition at 10^{-4} M), which would certainly have a pK_a value below that of 2-aminobenzimidazole (whose pK_a is 7.5 at 20 °C).[15]

For the furoyl analogue, comparing prazosin with **7**, it appears that the dimethoxy groups contribute to affinity. For the benzoylpiperazines, however, (compare **2** and **8**) the dimethoxy groups do not appear to contribute to binding affinity. The benzenoid fusion appears to make a small contribution to affinity when comparing the des-methoxy compounds **7** and **12**. Compound **12**, which, in comparison with prazosin, no longer has the benzenoid fusion or the dimethoxy groups still retains some affinity but the two effects appear to be additive since it has less than 50% affinity. Two compounds (**4** and **16**) were able to achieve a maximal inhibition of prazosin binding and had their IC_{50} values determined as 5.2 ± 2 and 23 ± 12 μM, respectively.

Further studies are in progress to determine the structural features needed for a compound to activate the transport system.

5. Conclusions

1. It is not necessary to retain the dimethoxy groups or even the dimethoxy-benzenoid fusion (A) to achieve 10^{-4} M binding affinity to the putative uptake system.
2. The presence of the 4-amino group in the pyrimidine ring (B) is critically important presumably because it raises the pK_a >7 so that sufficient numbers of molecules are protonated and positively charged at pH 7.4.
3. The piperazine ring (C) can be replaced by piperidine and therefore the 2-furoyl group (D) is not required for binding.
4. If the piperazine ring is retained, then the 2-furoyl group (D) can be replaced by phenyl to achieve μM affinity.

6. Experimental

6.1. Chemistry (general)

Melting points were carried out on an Electrothermal® melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded at 300 or 500 MHz on a Bruker AMX-300 and a Bruker AVANCE-500, respectively. The NMR experiments were carried out in $CDCl_3$, CD_3OD or DMSO-d_6 with tetramethylsilane as an internal reference. Chemical shifts are quoted in parts per million (ppm) and the coupling constants are reported in Hertz (Hz). Analytical HPLC was carried out on a Shimadzu HPLC apparatus with a Kromasil C18 μm reversed column (250 × 4.6 mm) at a flow rate of 1 mL/min and detected at 254 nm. The mobile phases were mixtures of A = methanol + 0.1% trifluoroacetic acid, and B = water + 0.1% trifluoroacetic acid, and are indicated as the ratio A:B. Mass spectra were recorded on a VG 7070H Double Focusing Mass Spectrometer or a VG ZAB-SE Double Focusing Mass Spectrometer, using atmospheric pressure chemical ionisation (APCI), electrospray (ES) or fast atom bombardment (FAB). Elemental analyses were determined on a Perkin Elmer 2400 CHN elemental analyser and were carried out at UCL Departmental Microanalysis Service. Column chromatography was done using Merck silica gel 60 (70–230 mesh). TLC was carried out using Merck Kieselgel 60 F_{254} aluminium sheets, visualised at 254 nm and stained with potassium iodoplatinate or 5% H_2SO_4 in ethanol prior to heating.

6.2. 4-Amino-2-(4-benzoyl-piperazin-1-yl)-6,7-dimethoxyquinazoline hydrochloride hydrate (2)

A mixture of 4-amino-2-chloro-6,7-dimethoxyquinazoline (**22a**) (0.77 g, 3.2 mmol), prepared as previously described[16] and 1-benzoylpiperazine[17] (0.61 g, 3.2 mmol) in 1-pentanol (20 mL) was heated at reflux and stirred for 3 h. A white solid was formed, and after cooling it was filtered and washed with diethyl ether. The solid was recrystallised from methanol to give **2** as white crystals (0.45 g, 32%); mp 288–290 °C; HPLC (A:B = 50:50), R_t 9.33 min (99.7%); ^{1}H NMR (DMSO-d_6, 500 MHz): δ 8.90 (br s, 1H, NH), 8.65 (br s, 1H, NH), 7.74 (s, 1H, quin-8H), 7.53 (s, 1H, quin-5H), 7.45–7.49 (m, 5H, Ph), 3.94–4.01 (br s, 4H, $NCH_2 \times 2$), 3.87 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 3.42–3.76 (br s, 4H, $NCH_2 \times 2$). MS (ESP) *m/z* 394 ($M+1^+$, 100%). Anal. Calcd for $C_{21}H_{23}N_5O_3 \cdot HCl \cdot 0.25H_2O$: C, 58.06; H,

5.68; N, 16.12; Cl, 8.16. Found: C, 58.16; H, 5.43; N, 16.13; Cl, 7.86.

6.3. 2-(4-Acetylpiperazin-1-yl)-4-amino-6,7-dimethoxyquinazoline hydrochloride sesquihydrate (3)

4-Amino-2-chloro-6,7-dimethoxyquinazoline (**22a**) (0.15 g, 0.63 mmol) and 1-acetylpiperazine (0.08 g, 0.63 mmol) were dissolved in isoamyl alcohol (9 mL) and the mixture was stirred overnight at 140 °C under nitrogen. A precipitate was formed and the mixture was cooled and Et_2O was added. The precipitate was filtered off, washed with Et_2O and recrystallised from EtOH/Et_2O to afford **3** as hygroscopic white crystals (0.13 g, 54%); mp 240–244 °C (lit.[18] mp 244–245 °C); HPLC (A:B = 40:60), R_t 9.23 min (100%); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.78 (br s, 2H, NH_2), 7.74 (s, 1H, quin-8H), 7.44 (s, 1H, quin-5H), 3.89 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.80 (br s, 4H, $NCH_2 \times 2$), 3.61 (br s, 4H, $NCH_2 \times 2$), 2.06 (s, 3H, $COCH_3$). MS (FAB+) *m/z* 332 (M^+, 100%). Anal. Calcd. for $C_{16}H_{21}N_5O_3 \cdot HCl \cdot 1.5H_2O$: C, 48.67; H, 6.38; N, 17.74; Cl, 8.98. Found: C, 49.07; H, 6.47; N, 17.38; Cl, 8.58.

6.4. 4-Amino-6,7-dimethoxy-2-(4-phenyl-piperazin-1-yl)quinazoline hydrochloride (4)

A mixture of 4-amino-2-chloro-6,7-dimethoxyquinazoline (**22a**) (0.35 g, 1.46 mmol) and 1-phenylpiperazine (0.24 g, 1.46 mmol) in 1-pentanol (20 mL) was heated under reflux and stirred for 20 h. A solid appeared and after cooling it was filtered and recrystallised from 1-pentanol to give **4** as pale yellow crystals (0.18 g, 30%); mp 283–286 °C; HPLC (A:B = 50:50), R_t = 11.54 min (99.2%); ^{1}H NMR (DMSO-d_6, 500 MHz): δ 8.96 (br s, 1H, NH_2), 8.65 (br s, 1H, NH_2), 7.77 (s, 1H, quin-8H), 7.62 (s, 1H, quin-5H), 7.24 (t, 2H, Ph-2,6H), 7.00 (d, 2H, Ph-3,5H), 6.81 (t, 1H, Ph-4H), 4.01–4.03 (br s, 4H, $NCH_2 \times 2$), 3.87 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.27–3.29 (br s, 4H, $NCH_2 \times 2$). MS (ES) *m/z* 366 $[M+H]^+$. Anal. Calcd for $C_{20}H_{23}N_5O_2 \cdot HCl$: C, 59.77; H, 6.02; N, 17.43; Cl, 8.82. Found: C, 59.55; H, 5.93; N, 17.37; Cl, 8.55.

6.5. 4-Amino-6,7-dimethoxy-2-(4-methylpiperidin-1-yl)quinazoline (5)

A mixture of 4-amino-2-chloro-6,7-dimethoxyquinazoline (**22a**) (0.50 g, 2.09 mmol) and 4-methyl-piperazine (0.41 g, 4.17 mmol) in 1-pentanol (20 mL) was heated at reflux and stirred for 20 h. The solution was evaporated and the amorphous solid was triturated with diethyl ether and filtered. The solid was suspended in water and a saturated solution of $NaHCO_3$ was added until pH 8 with stirring. The resulting solid was filtered, washed with water and recrystallised from EtOH/H_2O to give **5** as pale brown crystals (0.075 g, 12%). The analysis of this material corresponds to the presence of 5% inorganic material; mp 176–178 °C; HPLC (A:B = 70:30), R_t = 47.01 min (98.9%); ^{1}H NMR (DMSO-d_6, 500 MHz): δ 7.43 (s, 1H, quin-8H), 7.21 (br s, 2H, NH_2), 6.78 (s, 1H, quin-5H), 4.69 (d, 2H, NCH_2), 3.83 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 2.78 (t, 2H, NCH_2), 1.59–1.66 (m, 1H), 1.01–1.11 (m, 4H, $NCH_2 \times 2$), 0.92 (d, 3H, CH_3). MS (FAB+), *m/z* 303 $[M+H]^+$. Anal. Calcd for $C_{16}H_{22}N_4O_2$: C, 63.56; H, 7.33; N, 18.53. Calcd for 5% inorganic: C, 60.3; H, 6.96; N, 17.60. Found: C, 60.16; H, 6.95; N, 17.42.

6.6. 4-Amino-6,7-dimethoxy-2-(3-methylpiperidin-1-yl)quinazoline hydrochloride hydrate (6)

A mixture of 4-amino-2-chloro-6,7-dimethoxyquinazoline (**22a**) (0.11 g, 0.45 mmol) and 3-methylpiperidine (0.045 g, 0.45 mmol) in isoamyl alcohol (10 mL) was heated at 140 °C under N_2 and stirred for 12 h. After being cooled, the mixture was diluted with diethyl ether and the resulting white precipitate was collected and washed with diethyl ether before being recrystallised from EtOH/Et_2O to afford a white solid (0.065 g, 42%); mp 246–249 °C; HPLC (A:B = 30:70), R_t = 3.80 min (100%); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.64 (d, 2H, NH_2), 7.72 (s, 1H, quin-8H), 7.54 (s, 1H, quin-5H), 4.51 (d, 2H, NCH_2), 3.87 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 3.06 (t, 2H, NCH_2), 2.78 (m, 1H), 1.42–1.82 (m, 4H), 1.13 (d, 3H). MS (ES) *m/z* 303 $[M+H]^+$. Anal. Calcd for $C_{16}H_{22}N_4O_2 \cdot HCl \cdot 0.25 \cdot H_2O$: C, 55.97; H, 6.90; N, 16.32. Found: C, 55.93; H, 6.89; N, 16.12.

6.7. 4-Amino-2-[4-(2-furoyl)piperazin-1-yl]-quinazoline hydrochloride (7)

Compound **7** was prepared as described for **2**, from 4-amino-2-chloroquinazoline[19] (**22b**) (0.20 g, 1.1 mmol) and 1-(2-furoyl)piperazine (0.22 g, 1.25 mmol), to give **7** (0.25 g, 64%); mp 306–308 °C/decomp.; HPLC (A:B = 50:50), R_t 4.56 min (99.4%); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 9.12 (br s, 1H, NH_2), 8.91 (br s, 1H, NH_2), 8.20 (d, 1H, quin-8H), 7.94 (d, 1H, quin-5H), 7.80 (d, 1H, fur-5H), 7.73 (t, 1H, quin-8H), 7.34 (t, 1H, quin-6H), 7.00 (d, 1H, fur-3H), 6.58 (br s, 1H, fur-4H), 3.96 (br s, 4H, $NCH_2 \times 2$), 3.78 (br s, 4H, $NCH_2 \times 2$). MS (APCI) *m/z* 324.1 (M^+, 100%). Anal. Calcd for $C_{17}H_{17}N_5O_2 \cdot 1.1HCl$: C, 56.18; H, 5.02; N, 19.27; Cl, 10.73. Found: C, 56.00; H, 5.07; N, 19.20; Cl, 10.36.

6.8. 4-Amino-2-(4-benzoylpiperazin-1-yl)-quinazoline hydrochloride (8)

Compound **8** was prepared as described for **2**, from 4-amino-2-chloro-quinazoline (**22b**) (0.24 g, 1.3 mmol) and 1-(2-benzoyl)piperazine (0.25 g, 1.3 mmol), to give **8** (0.31 g, 65%); mp 310–312 °C/decomp.; HPLC (A:B = 50:50), R_t 27.91 min (99.4%); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 9.19 (br s, 1H, NH_2), 9.04 (br s, 1H, NH_2), 8.26 (d, 1H, quin-8H), 7.86 (m, 2H, quin-5,7H), 7.51 (m, 6H, quin-6H + Ph), 4.00 (br s, 4H, $NCH_2 \times 2$), 3.81 (br s, 2H, NCH_2), 3.59 (br s, 2H, NCH_2). MS (APCI) *m/z* 334.2 ($M+1^+$, 100%). Anal. Calcd for $C_{19}H_{19}N_5O \cdot 1.1HCl$: C, 61.10; H, 5.42; N, 18.75; Cl, 10.44. Found: C, 61.13; H, 5.46; N, 18.41; Cl, 10.15.

6.9. 4-Amino-2-(piperidin-1-yl)-quinazoline hydrochloride (9)

Compound **9** was prepared as described for **2**, from 4-amino-2-chloro-quinazoline (**22b**) (0.44 g, 2.44 mmol) and piperidine (0.21 g, 2.44 mmol), to give **9** (0.25 g, 37%); mp 280–282 °C/decomp.; HPLC (A:B = 50:50), R_t 7.17 min (100%); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 9.05 (br s, 1H, NH_2), 8.90 (br s, 1H, NH_2), 8.22 (d, 1H, quin-8H), 7.81 (t, 1H, quin-7H), 7.65 (d, 1H, quin-5H), 7.43 (t, 1H, quin-6H), 3.86 (br s, 4H, $NCH_2 \times 2$), 1.66 (br s, 6H, $CH_2 \times 3$). MS (APCI) *m/z* 229 (M^+). Anal. Calcd for $C_{13}H_{16}N_4 \cdot HCl$: C, 58.98; H, 6.47; N, 21.16; Cl, 13.39. Found: C, 58.82; H, 6.55; N, 20.96; Cl, 13.68.

6.10. 6,7-Dimethoxy-2-[4-(2-furoyl)-piperazin-1-yl]quinazoline hydrogen oxalate (10)

To a solution of 2,4-dichloro-6,7-dimethoxyquinazoline (**21a**) (0.35 g, 1.37 mmol) in isopropanol (15 mL), $NaBH_4$ (0.05 g, 1.37 mmol) was added. The mixture was heated at 70–80 °C for 5 days, until HPLC examination showed the complete transformation of the starting material. The solvent was evaporated and the residue was purified by preparative HPLC using MeOH–H_2O 70:30 as eluant. Evaporation of the appropriate fractions gave an off-yellow residue, which was recrystallised from EtOH to afford 0.043 g (14%) of pure 2-chloro-6,7-dimethoxyquinazoline (**23**); HPLC (A:B = 70:30), R_t 4.13 min; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.02 (s, 1H, quin-4H), 7.25 (s, 1H, quin-8H), 7.09 (s, 1H, quin-5H), 4.02 (s, 3H, OCH_3), 4.00 (s, 3H, OCH_3). MS (FAB+) *m/z* 225 (M^+). To a solution of **23** (0.04 g, 0.19 mmol) in isoamyl alcohol (8 mL), 1-(2-furoyl)piperazine (0.035 g, 0.19 mmol) was added. The mixture was heated at 150 °C for 18 h. The solvent was evaporated under vacuum and the residue was dissolved in saturated $NaHCO_3$ and extracted with CH_2Cl_2. The organic layer was dried (Na_2SO_4) and evaporated to give a residue that was purified by column chromatography using EtOAc–petrol spirit 1:10. The product was then transformed into its oxalate to give **10** as yellow crystals (0.03 g, 34%); mp 204–206 °C; HPLC (A:B = 60:40), R_t 3.02 min (100%); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 9.00 (s, 1H, quin-4H), 7.88 (d, 1H, fur-5H), 7.26 (s, 1H, quin-5H), 7.05 (d, 1H, fur-3H), 6.96 (s, 1H, quin-8H), 6.78 (br s, 1h, fur-3H), 6.66 (dd, 1H, fur-4H), 3.91 (s, 3H, OCH_3), 3.88 (d, 4H), 3.84 (s, 3H, OCH_3). MS (ES+) *m/z* 369.3. Anal. Calcd for $C_{19}H_{20}N_4O_4 \cdot C_2H_2O_4$: C, 55.02; H, 4.84; N, 12.22. Found: C, 54.66; H, 4.79; N, 11.94.

6.11. 6,7-Dimethoxy-2-[4-(2-furoyl)-piperazin-1-yl]quinazoline-4-one hydrate (11)

A mixture of 2-chloro-4,6,7-trimethoxyquinazoline (**24**) (0.069 g, 0.27 mmol) and 1-(2-furoyl)piperazine (0.049 g, 0.27 mmol) in isoamyl alcohol (10 mL) was heated at 150 °C under N_2 and stirred for 12 h. The solution was cooled but no precipitate formed; the solvent was then evaporated to afford an off-white solid that was recrystallised twice from EtOH/Et_2O to give **11** (0.05 g, 54%); mp 266–268 °C; HPLC (A:B = 50:50), R_t = 3.28 min (100%); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.49 (d, 1H, fur-5H), 7.40 (s, 1H, quin-5H), 7.06 (d, 1H, fur-3H), 6.55 (s, 1H, quin-8H), 6.50 (dd, 1H, fur-4H), 3.95 (br s, 7H, OCH_3 + $NCH_2 \times 2$), 3.91 (s, 3H, OCH_3), 3.78 (br s, 4H, $NCH_2 \times 2$). MS (FAB+), *m/z* 385 $[M+H]^+$. Anal. Calcd for $C_{16}H_{22}N_4O_2 \cdot 0.25H_2O$: C, 58.68; H, 5.31; N, 14.41. Found: C, 58.88; H, 5.24; N, 14.41.

6.12. 4-Amino-2-[4-(2-furoyl)piperazin-1-yl]-pyrimidine hydrochloride hydrate (12)

4-Amino-2-chloropyrimidine[20,21] (0.09 g, 0.72 mmol) was heated with 1-(2-furoyl)piperazine (0.13 g, 0.72 mmol) in 1-pentanol (3 mL) under reflux for 3 h. The mixture was then cooled and the filtrate was recrystallised from EtOH–ether, to give **12** (0.14 g, 62%); mp 243–244 °C/decomp.; HPLC (A:B = 30:70), R_t 8.11 min (99.9%); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.89 (d, 1H, pyr-6H), 7.87 (d, 1H, fur-5H), 7.05 (d, 1H, fur-3H), 6.65 (dd, 1H, fur-4H), 6.50 (d, 1H, pyr-5H), 3.81 (br s, 8H, $NCH_2 \times 4$). MS (FAB +) *m/z* 274 ($M+1^+$). Anal. Calcd for $C_{13}H_{15}N_5O_2 \cdot HCl \cdot H_2O$: C, 47.64; H, 5.54; N, 21.37; Cl, 10.82. Found: C, 47.65; H, 5.40; N, 21.27; Cl, 10.49.

6.13. 2-[4-(2-Furoyl)piperazin-1-yl]-benzimidazole hydrochloride hydrate (13)

A suspension of 2-chlorobenzimidazole (0.17 g, 1.15 mmol) and 1-(2-furoyl)piperazine (0.20 g, 1.15 mmol) in 1-pentanol (5 mL) was heated at 100 °C for 6 h. After cooling, the solid was filtered off, washed thoroughly with CH_2Cl_2, then recrystallised twice from EtOH, to give **13** as a white solid (0.22 g, 59%); mp 274–276 °C; HPLC (A:B = 50:50), R_t 4.53 min (99.9%); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.90 (d, 1H, fur-5H), 7.44 (br s, 2H, benzim-6,7H), 7.29 (br s, 2H, benzim-5,6H), 7.12 (d, 1H, fur-3H), 6.68 (dd, 1H, fur-4H), 3.94 (br s, 4H, $NCH_2 \times 2$), 3.80 (m, 4H, $NCH_2 \times 2$). MS (FAB+) *m/z* 297 ($M+1^+$, 100%). Anal. Calcd for $C_{16}H_{15}N_4O_2 \cdot HCl \cdot 0.25H_2O$: C, 57.15; H, 4.95; N, 16.66; Cl, 10.54. Found: C, 57.17; H, 4.81; N, 16.56; Cl, 10.12.

6.14. 1-[2-(3,4-Dimethoxyphenyl)ethyl]-4-(2-furoyl)-piperazine hydrogen oxalate (14)

An externally cooled solution of 2-(3,4-dimethoxyphenyl)ethanol (**25**) (2.80 g, 15.3 mmol) dissolved in toluene (30 mL) was treated with PPh_3 (4.48 g, 17.1 mmol) and *N*-bromosuccinimide (2.85 g, 16 mmol) was added portionwise. After 12 h of stirring at room temperature the mixture was quenched with $Na_2S_2O_3$ (10% w/v, 17 mL). The organic layer was washed twice with NaOH (1 M) and then with water, dried ($MgSO_4$) and evaporated. The residue was treated with diethyl ether, and precipitated Ph_3PO was removed by filtration. Column chromatography purification afforded 4-(2-bromoethyl)-1,2-dimethoxybenzene (**26**) as an oil, which crystallised on standing and was recrystallised from EtOH. A solution of **26** (0.13 g, 0.53 mmol) and 1-(2-furoyl) piperazine (0.11 g, 0.64 mmol) in ethanol (3 mL) was stirred under reflux in the presence of K_2CO_3 (0.04 g, 0.28 mmol)

during 24 h. The reaction mixture was filtered and the filtrate was evaporated in vacuo. The resulting residue was purified by column chromatography using EtOAc–MeOH 50:1 to give an oil, which was converted into the oxalate salt. Recrystallised from EtOH–diethyl ether gave **14** (0.23 g, 99%); mp 135–136 °C; HPLC (A:B = 55:45), R_t 11.2 min; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.87 (br s, 1H, fur-5H), 7.06 (d, 1H, fur-3H), 6.88 (d, 1H, Ph-5H), 6.86 (s, 1H, Ph-2H), 6.75 (dd, 1H, Ph-6H), 6.66 (dd, 1H, fur-4H), 3.81 (br s, 4H, $NCH_2 \times 2$), 3.75 (s, 3H, OCH_3), 3.72 (s, 3H, OCH_3), 2.82 (br s, 6H, $NCH_2 \times 3$), 2.79 (m, 2H, CH_2). MS (APCI) *m/z* 345.2 ($M+1^+$, 100%). Anal. Calcd for $C_{19}H_{24}N_2O_4 \cdot C_2H_2O_4$: C, 58.06; H, 6.03; N, 6.45. Found: C, 57.80; H, 6.00; N, 6.33.

6.15. 1-[2-(3,4-Dimethoxyphenyl)ethyl]-4-benzoyl-piperazine hydrogen oxalate (15)

Compound **15** was prepared as described for **14**, from **26** (0.25 g, 1 mmol) and benzoyl piperazine (0.19 g, 1 mmol). Yield 86%; mp 145–146 °C; HPLC (A:B = 45:55), R_t 13.7 min (100%); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.58 (m, 5H, Ph), 7.00 (d, 1H, Ph-4H), 6.99 (s, 1H, Ph-2H), 6.88 (d, 1H, Ph-5H), 3.87 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.58 (m, 4H, $NCH_2 \times 2$), 3.03 (m, 6H, $NCH_2 \times 3$), 2.82 (m, 2H, CH_2). MS (APCI) *m/z* 355.2 ($M+1^+$, 100%). Anal. Calcd for $C_{21}H_{26}N_2O_3 \cdot C_2H_2O_4$: C, 62.15; H,6.35; N, 6.30. Found: C, 61.73; H, 6.38; N, 6.23.

6.16. 1-[2-(3,4-Dimethoxyphenyl)ethyl]piperidine hydrogen oxalate (16)

Compound **16** was prepared as described for **14** except that no K_2CO_3 was used, reacting **26** (0.25 g, 1 mmol) and piperidine (0.85 g, 10 mmol). Yield 57%; mp 194–196 °C; HPLC (A:B = 65:35), R_t 7.93 min (100%); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 6.90 (d, 1H, Ph-5H), 6.88 (s, 1H, Ph-2H), 6.77 (d, 1H, Ph-4H), 3.72 (s, 3H, OCH_3), 3.59 (s, 3H, OCH_3), 3.18 (m, 6H, $NCH_2 \times 3$), 2.90 (m, 2H, CH_2), 1.74 (m, 4H, $NCH_2 \times 2$), 1.59 (m, 2H, CH_2). MS (ES) *m/z* 250 (M^+, 100%). Anal. Calcd for $C_{15}H_{23}NO_2 \cdot C_2H_2O_4$: C, 60.12; H, 7.42; N, 4.13. Found: C, 59.67; H, 7.49; N, 4.06.

6.17. 1-(3,4-Dimethoxybenzenesulfonyl)-4-(2-furoyl)-piperazine (17)

A mixture of 3,4-dimethoxybenzenesulfonyl chloride (0.37 g, 1.56 mmol), 1-(2-furoyl)piperazine (0.28 g, 1.56 mmol) and triethylamine (2 mL) in anhydrous CH_2Cl_2 (8 mL) was stirred at room temperature for 12 h. After cooling a white precipitate was formed and it was filtered off and the resulting solution evaporated. The oil was crystallised upon addition of EtOH and the precipitate was recrystallised twice to give **17**. Yield 72%; mp 115–116 °C; HPLC (A:B = 50:50), R_t 9.19 min (100%); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.38 (d, 1H, fur-H5), 7.29 (dd, 1H, Ph-2H), 7.12 (d, 1H, fur-3H), 6.93 (d, 1H, Ph-5H), 6.88 (d, 1H, Ph-6H), 6.39 (dd, 1H, fur-H4), 3.87 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.81 (t, 4H, $NCH_2 \times 2$), 3.01 (t, 4H, $NCH_2 \times 2$). MS (APCI) *m/z* 381 ($M+1^+$, 100%). Anal. Calcd for $C_{17}H_{19}N_2O_6S$: C, 53.82; H, 5.55; N, 7.38. Found: C, 53.70; H, 5.55; N, 7.38.

6.18. 1-(3,4-Dichlorobenzyl)-4-(2-furoyl)-piperazine hydrogen oxalate (18)

A solution of 3,4-dichlorobenzyl bromide (0.402 g, 1.67 mmol) and 1-(2-furoyl)piperazine (0.302 g, 1.67 mmol) in 1-pentanol (7 mL) was heated at reflux for 16 h. Then, the solvent was evaporated and the solid dissolved and extracted with NaOH and CH_2Cl_2. The organic phase was dried and evaporated. Oxalic acid was added to an ethanolic solution and the oxalate precipitated upon addition of diethyl ether. The solid was filtered and recrystallised twice from EtOH/diethyl ether to give 18 as white crystals (34%); mp 164–166 °C; HPLC (A:B = 60:40), R_t 4.75 min (100%); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.82 (d, 1H, fur-H5), 7.63 (d, 1H, Ph-2H), 7.61 (s, 1H, Ph-5H), 7.35 (dd, 1H, Ph-6H), 6.98 (d, 1H, fur-H3), 6.62 (dd, 1H, fur-H4), 3.69 (s, 4H, $NCH_2 \times 2$), 2.58 (t, 4H, $NCH_2 \times 2$). MS (FAB+) *m/z* 339 ($M+1^+$, 100%). Anal. Calcd for $C_{16}H_{26}Cl_2N_2O_2 \cdot 1.1C_2H_2O_4$: C, 49.88; H, 4.19; N, 6.39; Cl, 16.18. Found: C, 50.15; H, 4.08; N, 6.41; Cl, 16.0.

6.19. 4-(9-Fluorenyl)-1-(2-furoyl)piperazine (19)

A mixture of 9-bromofluorene (0.32 g, 1.30 mmol) and 1-(2-furoyl)piperazine (0.23 g, 1.30 mmol) in 1-pentanol (5 mL) was heated under reflux and stirred for 12 h. Evaporation of the solvent afforded a yellow sticky oil, which was triturated with petrol spirit (bp 60–80 °C). The resulting precipitate was collected and purified by extraction. It was dissolved in a solution of $NaHCO_3$ and extracted with CH_2Cl_2. Evaporation of the organic solvent gave a white product, which was recrystallised from MeOH to give 0.16 g (13%) of final product; mp 161–163 °C; HPLC (A:B = 70:30), R_t = 4.09 min (100%); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.52–7.62 (d, 4H, Ar-H × 4), 7.20–7.29 (m, 5H, Ar-H × 4 + fur-5H), 6.85 (d, 1H, fur-3H), 6.34 (t, 1H, fur-H4), 4.80 (s, 1H, NCH), 3.68 (br s, 4H, $NCH_2 \times 2$), 2.60 (br s, 4H, $NCH_2 \times 2$). MS (FAB+), *m/z* 385 $[M+H]^+$. Anal. Calcd for $C_{22}H_{20}N_2O_2$: C, 76.41; H, 5.82; N, 8.18. Found: C, 76.72; H, 5.85; N, 8.13.

6.20. Pharmacology

The compounds were tested for their ability to inhibit the uptake of prazosin (at 10^{-6} M) in GT1-1 immortalised gonadotrophin-releasing hormone (GnRH) peptidergic neurones, as has been described in detail.[1] Briefly, the cells were grown at 37 °C in Corning 175 cm^2 flasks in culture medium consisting of Dulbecco's modified Eagle's medium (DMEM) and Ham's F-12 (ratio 1:1) containing 10% foetal bovine serum and sodium bicarbonate 3.7 g/L, in a humidified atmosphere containing 5% CO_2 in air. Culture media were changed at 48-h intervals. After 7 days, the cells were dispersed in the presence of trypsin, deoxyribonuclease I and ethylenediaminetetraacetic acid and incubated in Corning or Nunc 12-well plates ($\approx 2 \times 10^6$ cells/well). The wells had been coated with poly-D-lysine (2.5 μg/cm^2; Sigma P-6407; MW 70,000–

150,000) and laminin (0.25 μg/cm^2; Sigma L-2020). Drugs were dissolved in buffer consisting of DMEM with 25 mM HEPES and 0.5 mM sodium ascorbate, pH 7.4. Uptake studies were performed on the intact cells. After 2–4 days in culture, the cells were washed twice with buffer at 25 °C then incubated at 37 °C for 60 min in the presence of [^{3}H]prazosin 2×10^{-10} M and unlabelled prazosin 10^{-6} M. The test compounds were present in the indicated concentrations. At the end of the incubation period, the culture plates were placed on ice to inhibit the release of amines.[8] After 30 s, the buffer was removed and the cells were washed twice with buffer at 0 °C. The cold buffer was then removed and the cells were solubilised with 2 mL of a warm solution of 0.1% sodium dodecyl sulphate and 0.1 M sodium hydroxide. Fifty microlitres aliquots were removed for protein assay and 10 mL of scintillation liquid was then added to the cell extract, mixed and radioactivity was measured in a scintillation spectrometer with an efficiency of 50%. Protein content was measured by the bicinchoninic acid modification of the biuret reaction using albumin standards (Perbio, Chester, Cheshire, England). Nonspecific uptake was defined as uptake in the presence of the antidepressant desipramine (at 10^{-4} M) and specific (desipramine-sensitive) uptake was obtained by subtracting nonspecific from total uptake. Efficacy was defined as % inhibition of the uptake of prazosin (at 10^{-6} M) when the test compound was used in a concentration of 10^{-4} M. Efficacy was expressed as % of the effect of a maximal inhibitory concentration of desipramine (10^{-4} M). Half-maximal inhibitory concentrations (IC_{50} values) were calculated from the concentration–response curves. IC_{50} values were calculated only for compounds, which achieved a maximal inhibitory response, defined as 90% of the inhibitory effect of desipramine (10^{-4} M). When a compound did not achieve the maximal inhibitory response, IC_{50} values were not calculated and the data were expressed only as efficacy (% inhibition relative to desipramine 10^{-4} M). The experiments were carried out in triplicate and each experiment was performed twice; the data are therefore presented as the mean ± SEM of six observations.

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