Bioorganic & Medicinal Chemistry 16 (2008) 7254–7263

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Molecular features of the prazosin molecule required for activation of Transport-P

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ARTICLE INFO

Article history:
Received 21 February 2008
Revised 19 June 2008
Accepted 19 June 2008
Available online 25 June 2008

Keywords:
Transport-P
Prazosin
Peptidergic neurones
Quinazoline
Furan
Antidepressant
Amine uptake

ABSTRACT

Closely related structural analogues of prazosin have been synthesised and tested for inhibition and activation of Transport-P in order to identify the structural features of the prazosin molecule that appear to be necessary for activation of Transport-P. So far, all the compounds tested are less active than prazosin. It is shown that the structure of prazosin appears to be very specific for the activation. Only quinazolines have been found to activate, and the presence of the 6,7-dimethoxy and 4-amino groups appears to be critically important.

1. Introduction

Transport-P is an uptake process for amines that is located in post-synaptic peptidergic neurones.[1] It accumulates amines in acidified vesicles in the peptidergic neurones.[2,3] Transport-P has functional properties which have not been described for any other membrane transport system. In particular, the α_1 adrenergic ligand prazosin, which is a substrate for this uptake process, accumulates in a non-linear manner in the peptidergic neurones that possess Transport-P. In the concentration range 10^{-10} to 10^{-7} M, accumulation of prazosin via Transport-P is linear, but at concentrations of prazosin greater than 10^{-7} M uptake becomes exponential. This is seen as a paradoxical increase[1,4] in the accumulation of [^{3}H]prazosin at concentrations of unlabelled prazosin greater than 10^{-7} M. The prazosin paradox is attributable to activation of a cooperative uptake process, which accumulates prazosin in a sigmoidal manner, that cannot be described by the Michaelis–Menten model. The paradoxical increase in accumulation of [^{3}H]prazosin and the exponential increase in prazosin uptake are abolished by antidepressants such as desipramine.[5] This antidepressant-sensitive increase in accumulation of the radioligand is not seen in presynaptic neurones or in non-neuronal cells, such as kidneys cells or muscle cells.[2,4] This indicates that Transport-P is a specialised function of peptidergic neurones.

Although prazosin is a potent adrenergic α_1 receptor antagonist,[6] this uptake effect of prazosin does not appear to involve adrenergic receptors.[4] Two types of compounds 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 antidepressant drugs, are accumulated non-cooperatively by the same uptake process. The structural properties of Group B have been investigated[7] by testing a 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.[7] These compounds presumably bind to a putative transporter protein involved in the uptake process. The special interest in characterising this transporter is that it may provide an approach for the development of a novel type of antidepressant drug.[9]

Previously, we reported[10] our study of partial structures of prazosin as part of a search for the pharmacophore of prazosin for Transport-P. We now describe our studies to determine the structural features of the prazosin molecule **1** (Fig. 1) that appear to be required for activation of Transport-P. Four major features, A–D (Fig. 2) of the prazosin structure have been investigated, namely: A, the furoyl system (compounds **2–8**), B, the 6,7-dimethoxy groups

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✠ Deceased. This paper is a tribute to him and his pioneering discovery of Transport-P.

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doi:10.1016/j.bmc.2008.06.037

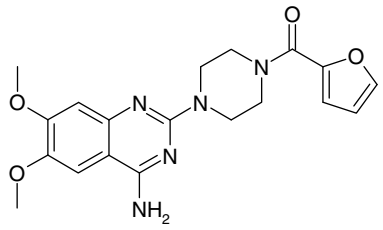

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Figure 1. The structure of prazosin (**1**).

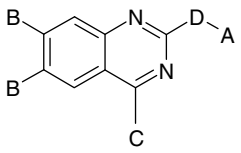


Figure 2. Structural features (A–D) studied for the prazosin molecule; where (A) represents the 2-furoyl moiety, (B) represents 6,7-dialkoxy, (C) represents 4-amino and (D) represents the 2-piperazino group.

(compounds **9**–**11**), C, the 4-amino group (compounds **12**–**19**), and D, the 2-(*N*-piperazino-linking moiety (compounds **20** and **21**–**23**; in these latter structures the furoyl group has also been removed). This approach has given rise to the synthesis of a set of compounds (Table 1) which have been tested for their influence on the uptake of prazosin.

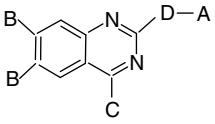
The 2-furoyl group A has been replaced by 2-thienoyl, 2-tetrahydrofuranoyl, benzoyl, acetyl and trifluoroacetyl to give compounds **2**, **3**, **4**, **5** and **6**, respectively. In two other examples, it has been replaced by benzyloxycarbonyl or phenyl giving **7** and **8**. In the last example, therefore, the carbonyl group is no longer present.

In some other compounds, the 6,7-dimethoxy groups B of prazosin have been fused into a dioxalane ring giving **9** or replaced by hydrogen atoms to give the desmethoxy analogue **10** of prazosin or the desmethoxy analogue **11** of the benzoyl compound **4**.

The ring 4-amino group C has been acetylated, methylated, benzylated or phenylated giving compounds **12**, **13**, **14** and **15**, respectively. Both amino-hydrogen atoms of the 4-amino group have also been replaced by ethyl (**16**) or by incorporating the amino group into a morpholine ring (**17**). The amino group has also been removed (**18**), that is, replaced by H at the ring 2-position, or replaced by hydroxyl to give **19**, which tautomerises to the ring amide.

Finally, the six-membered 2-(*N*-piperazino)ring D has been enlarged to a seven-membered 1,4-diazepane in compound **20**.

Table 1
Structures and synthesis schemes of prazosin analogues



Compound	A	B	B	C	D	Scheme
1[a]	2-Furanyl	MeO	MeO	NH_2	*N*-Piperazino-CO[b]	
2	2-Thienyl	MeO	MeO	NH_2	*N*-Piperazino-CO[b]	1
3[c]	2-Tetrahydro- furanyl	MeO	MeO	NH_2	*N*-Piperazino-CO[b]	1
4	Ph	MeO	MeO	NH_2	*N*-Piperazino-CO[b]	[d]
5	CH_3	MeO	MeO	NH_2	*N*-Piperazino-CO[b]	[d]
6	CF_3	MeO	MeO	NH_2	*N*-Piperazino-CO[b]	1
7	—OCH_2Ph	MeO	MeO	NH_2	*N*-Piperazino-CO[b]	1
8	Ph	MeO	MeO	NH_2	—N N—	[d]
9	2-Furanyl	OCH_2——CH_2O		NH_2	*N*-Piperazino-CO[b]	1
10	2-Furanyl	H	H	NH_2	*N*-Piperazino-CO[b]	[d]
11	Ph	H	H	NH_2	*N*-Piperazino-CO[b]	[d]
12	2-Furanyl	MeO	MeO	$NHCOCH_3$	*N*-Piperazino-CO[b]	2
13	2-Furanyl	MeO	MeO	NHMe	*N*-Piperazino-CO[b]	3
14	2-Furanyl	MeO	MeO	$NHCH_2Ph$	*N*-Piperazino-CO[b]	3
15	2-Furanyl	MeO	MeO	NHPh	*N*-Piperazino-CO[b]	3
16	2-Furanyl	MeO	MeO	NEt_2	*N*-Piperazino-CO[b]	3
17	2-Furanyl	MeO	MeO	—N O	*N*-Piperazino-CO[b]	3
18	2-Furanyl	MeO	MeO	H	*N*-Piperazino-CO[b]	[d]
19	2-Furanyl	MeO	MeO	OH	*N*-Piperazino-CO[b]	[d]
20	2-Furanyl	MeO	MeO	NH_2	—N N—C(=O)	4
21	2-Furanyl	MeO	MeO	NH_2	—$NHCH_2$—	4
22	*N*-Piperidinyl	MeO	MeO	NH_2	—$NHCH_2CH_2$—	4
23		MeO	MeO	NH_2	—N ~CH_3	[d]

[a] Prazosin.
[b] *N*-Piperazino-CO:— N N—C(=O).
[c] Terazosin[12].
[d] Zunszain et al.[10]

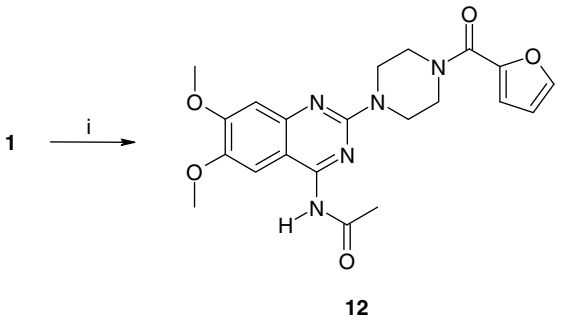
Otherwise, it has been replaced by 2-furylmethylamino (in **21**), 2-(*N*-piperidinoethyl)amino (in **22**) and 4-methyl-*N*-piperidino (in **23**).

2. Chemistry

The syntheses of 2-(*N*-piperazinyl)-4-aminoquinazolines **2**, **3**, **6**, **7**, **9** are summarised in Scheme 1. Treatment of 6,7-ethylenedioxy-quinazoline-2,4(1*H*,3*H*)-dione (**24a**) or 6,7-dimethoxy-quinazoline-2,4(1*H*,3*H*)-dione (**24b**) with $POCl_3$ in the presence of *N*,*N*-dimethylaniline afforded the corresponding 2,4-dichloro compounds (**25a** and **25b**) which, upon reaction with NH_4OH, led regioselectively to the 4-amino-2-chloro derivatives **26a** and **26b**. Nucleophilic substitution by the appropriate N-substituted piperazine was effected in boiling 1-pentanol under reflux to yield the desired final products.

Compound **12** was obtained from prazosin by base catalysed condensation with methyl acetate (Scheme 2). The synthesis of compounds **13**–**17** is outlined in Scheme 3. Treatment of 2,4-dichloro-6,7-dimethoxyquinazoline (**25b**) with the appropriate amines gave the corresponding 2-chloro-4-substituted-amino-6,7-dimethoxyquinazolines (**27**). These were then subjected to nucleophilic substitution of the 2-chloro group with *N*-(2-furoyl-piperazine) in boiling 1-pentanol under reflux to furnish the corresponding required products.

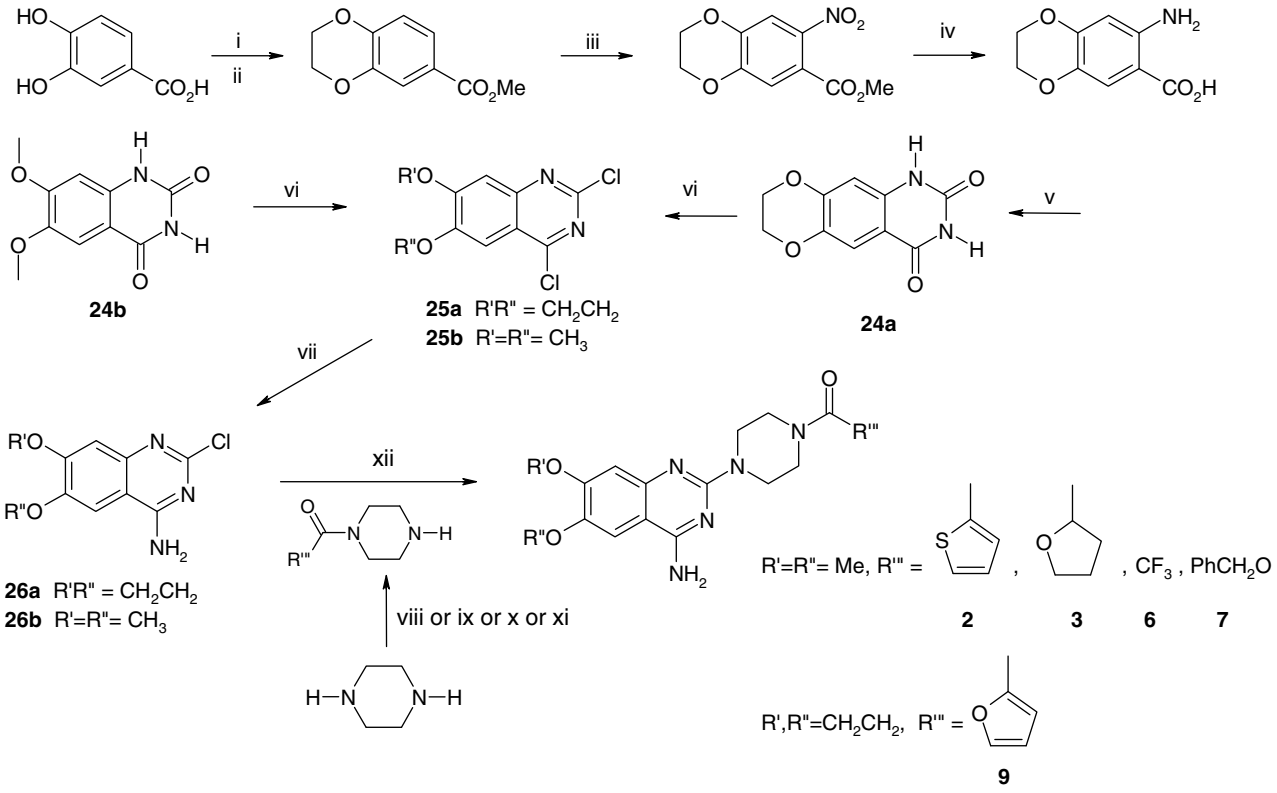
1,4-Diazepane was condensed with ethyl 2-furoate (Scheme 4) to yield the corresponding 2-furoic acid amide which was then treated with 2-chloro-4-amino-6,7-dimethoxyquinazoline (**26b**) to furnish compound **20**. Compounds **21** and **22** were similarly obtained from **26b** and 2-furfurylamine or *N*-(2-aminoethyl)piperidine, respectively (Scheme 4).



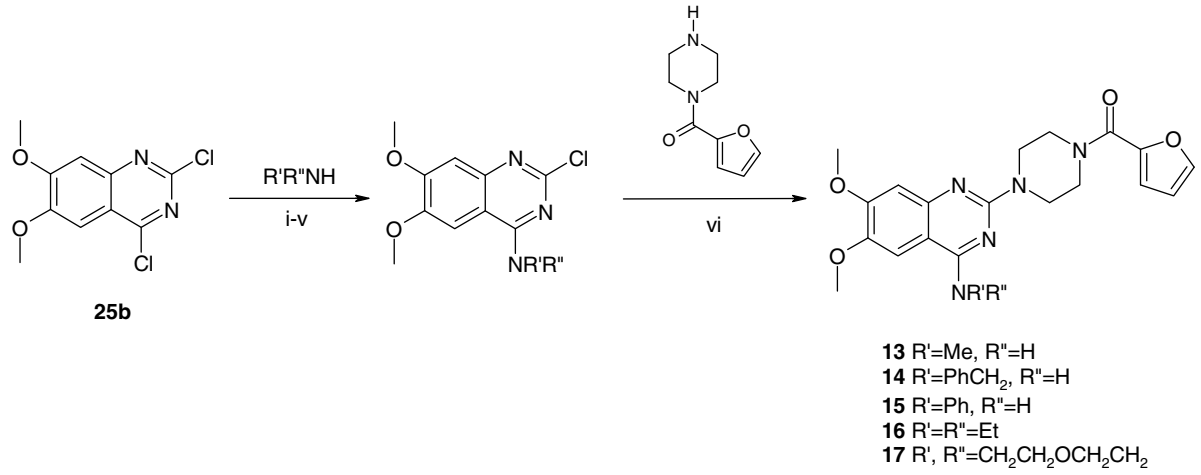
Scheme 2. Reagent and condition: (i) Ac_2O, reflux, 1,5 h.

3. Biological studies

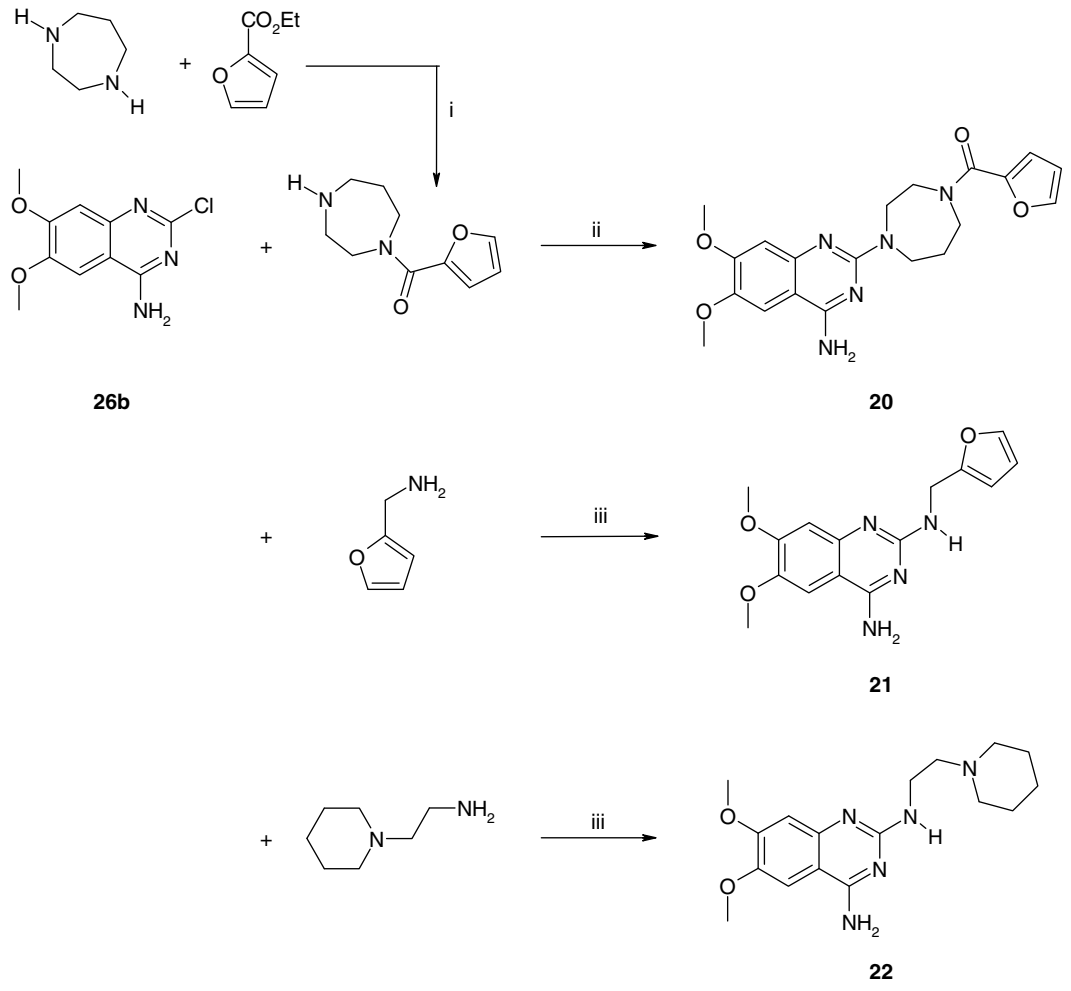
The compounds were examined for their ability to inhibit competitively the uptake of prazosin (1 μM) in immortalised gonadotrophin-releasing hormone neurones (GT1-1 GnRH cells), as previously described in detail.[7] Each compound was tested for its ability to compete with prazosin (at 10^{-6} M; inhibition of Transport-P). Efficacy was defined from the inhibition of the uptake of prazosin (at 10^{-6} M) when the test compound was used in a concentration of 10^{-4} M, and was expressed as percentage of the effect of a maximal inhibitory concentration of desipramine (10^{-4} M). IC_{50} values were calculated for the new compounds which were clearly not activators. Each experimental point was carried out in triplicate, and each experiment was performed twice; the data are mean ± SEM of six observations for each experimental point.



Scheme 1. Reagents and conditions: (i), $SOCl_2$, MeOH; (ii) $BrCH_2CH_2Br$, MeOH, KOH; (iii) HNO_3; (iv) $N_2H_4{\cdot}H_2O$, Pd/C, MeOH; (v) KNCO, AcOH, then NaOH; (vi) $PhNMe_2$, $POCl_3$, reflux; (vii) 35% NH_4OH, THF; (viii) 2-thienyl chloride,110 °C, 16 h then 150 °C, 4 h; (ix) methyl 2-tetrahydrofuroate,110 °C, 5 h; (x) ethyl trifluoroacetate, THF; (xi) CbzCl at pH 4.5 in MeOH/toluene/H_2O; (xii) 1-pentanol, reflux.



Scheme 3. Reagents and conditions: (i) $MeNH_2$, aq EtOH; (ii) $PhCH_2NH_2$, MeOH, rt; (iii) $PhNH_2$, KF, 18-C-6, MeCN; (iv) Et_3N, EtOH, reflux; (v) morpholine, MeOH, rt; (vi) 1-pentanol, reflux.



Scheme 4. Reagents and conditions: (i) 115 °C, 5 h; (ii) 3-methyl-1-butanol, reflux, 115 °C, 5 h; (iii) 3-methyl-1-butanol, reflux under N_2.

Each compound was also tested for its ability to activate Transport-P (increasing the uptake of a tracer dose of 2×10^{-10} M [^{3}H]prazosin). Unlabelled prazosin at 10^{-6} M increased the uptake of [^{3}H]prazosin by 126.8 ± 6.3% (derived from 70 independent experiments). Compounds were tested at three different concentrations, that is, at 10^{-6}, 10^{-5} and 10^{-4} M. Compound structures are in Table 1; biological results are given in Table 2.

Table 2
Activities of prazosin analogues on Transport-P

Compound[a]	Inhibition[b] (%)	Activation[c]		
		10^{-6} M	10^{-5} M	10^{-4} M
2	Insol.	57 ± 20	161 ± 16	Insol.
3	41 ± 3	0	0	38 ± 6
4	63 ± 5[d]	0	40 ± 8	47 ± 7
5	37 ± 5[d]	0	0	61 ± 12
6	31 ± 6	0	0	26 ± 8
7	75 ± 2	0	6 ± 4	0
8	93 ± 2[d,e]	0	13 ± 5	0
9	42 ± 9	0	74 ± 14	163 ± 33
10	64 ± 5[d]	0	0	22 ± 14
11	73 ± 7[d]	0	0	0
12	0	0	35 ± 8	206 ± 22
13	0	0	55 ± 16	229 ± 43
14	69 ± 3[f]	0	0	0
15	80 ± 8[g]	0	0	0
16	55 ± 5[h]	0	0	0
17	61 ± 3[i]	0	0	0
18	5 ± 10[d]	0	0	0
19	0[d]	0	0	0
20	82 ± 3	0	31 ± 5	0
21	71 ± 3	26 ± 3	43 ± 7	0
22	52 ± 3	13 ± 8	29 ± 10	37 ± 10
23	85 ± 3[d]	0	13 ± 5	0

[a] Compound structures are in Table 1.
[b] Inhibition of prazosin uptake by 10^{-4} M concentration of the test compound expressed as percentage of the effect of a maximal inhibitory concentration (10^{-4} M) of desipramine. Values are shown with ±SEM of three replicates.
[c] % activation of [^{3}H]prazosin uptake (at 2×10^{-10} M) at the indicated concentrations of compound.
[d] Zunszain et al.[10]
[e] IC_{50} = 5.2 ± 2.0 μM[d].
[f] IC_{50} = 5.4 ± 0.8 μM.
[g] IC_{50} = 4.5 ± 2.0 μM.
[h] IC_{50} = 80 μM.
[i] IC_{50} = 81 ± 7 μM.

4. Results and discussion

Our studies have previously shown that compounds that possess a basic amino group, which would be protonated at the physiological neutral pH of 7.4, are able to inhibit the uptake of prazosin. Most of the compounds in the current series bind to the uptake system to some extent. The thienoyl analogue **2** was too insoluble to be tested at 10^{-4} M. Two compounds lacking a 4-amino group **18** and **19** did not bind, as has been previously reported; it is believed that this is a consequence of their having low basicities, as has been previously argued. Two other compounds, in the range of concentrations used, also did not appear to bind; these are the *N*-acetyl derivative **12** of prazosin, and *N*-methylprazosin (**13**). This is presumably because they activate uptake rather than inhibiting it.

Other N-monosubstituted compounds such as *N*-benzyl (**14**) and *N*-phenyl (**15**) prazosin bind well, having IC_{50}s of 5.4 ± 0.8 and 4.5 ± 2.0 μM, respectively. The tertiary amines diethylamino (**16**) and morpholino (**17**) bind less well than the latter two, having IC_{50}s of 80 and 81 μM, respectively. Some of the other close structural analogues showed less than 50% binding at 10^{-4} M. These are compounds **3**, **5** and **6**, where the furoyl moiety in prazosin is replaced by tetrahydrofuranoyl, acetyl or trifluoroacetyl, respectively, and **9** where the 6,7-dimethoxy group is replaced by ethylenedioxy.

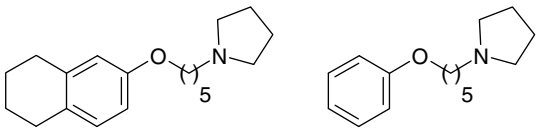


Figure 3. Most active compounds in Ref. 8, inhibiting the uptake of prazosin.

These results stand in contrast with our previous finding[10] that it is not necessary to have a structure resembling prazosin in order to achieve good inhibition of prazosin binding. Simple aromatic alkylamines whether primary, secondary or tertiary were shown to have IC_{50}s in the low micromolar range of concentrations. The most potent of this series were *N*-[5-(1,2,3,4-tetrahydronaphthalen-6-yloxy)pentyl]pyrrolidine (IC_{50} = 0.19 μM) and *N*-(5-phenoxypropyl)pyrrolidine (IC_{50} = 0.46 μM) (Fig. 3).

More important for our current purpose is to uncover the structural features that are required for activating uptake. Here, we appear to require structures close to that of prazosin. Compound **2**, the thienoyl analogue was active at 10^{-6} M and showed a dose-related increase in activation at 10^{-5} M; it is not as potent as prazosin itself, which shows 132% activation at 10^{-6} M, but was the most potent of the compounds tested.

Other acyl groups for A (Table 1) such as tetrahydrofuranyl (**3**), acetyl (**5**) and trifluoroacetyl (**6**) were some 100-fold less effective than **2**, and benzoate (**7**) was not active. The benzoyl analogue **4** appeared to be 10 times more potent than these (activation at 10^{-5} M) but had a rather shallow dose–response relationship. Removing the carbonyl group from the piperazine ring (**8**) also removed the activating activity relative to benzoyl (**4**).

Removing the 6,7-dimethoxy groups from prazosin (**1**) or from the benzoyl analogue (**4**), in **10** and **11**, respectively, removed the ability of the compounds to activate uptake. On the other hand, fusing the two methoxy groups into a dioxalane ring (**9**) gave a compound that was able to activate uptake, but was more than 10 times less potent than prazosin (10^{-4} to 10^{-5} M).

As mentioned above, the N-substituted prazosin derivatives with *N*-acetyl (**12**) or *N*-methyl (**13**) were able to activate prazosin uptake at 10^{-4} to 10^{-5} M. Although weaker than prazosin in activating Transport-P, these compounds appear to retain the full maximum ability to activate the uptake process. For example, at 10^{-4} M, **13** increased the uptake of [^{3}H]prazosin by 229%, which is similar to the maximal activating effect of prazosin (which is exerted at 3×10^{-6} M). In contrast, N-substituted derivatives having a larger *N*-substituent such as benzyl (**14**) or phenyl (**15**) were not active. Similarly, the tertiary amines diethylamino (**16**) and morpholino (**17**) were not active.

Enlarging the piperazine ring D (Table 1) of prazosin to 1,4-diazepane (**20**) also has a profound effect on ability to activate Transport-P, and **20** has only weak activity. Surprisingly, replacing the carbonyl piperazine by methylamino (**21**), or replacing furoylpiperazine by 2-(*N*-piperidino)ethylamino (**22**), provides compounds that retain some activating effect. Methylpiperidine on its own (**23**), that is without a group A, showed very weak activity (at 10^{-5} M).

Compound **21** appeared to have a bell-shaped concentration–response relationship for activation of Transport-P, since it was apparently inactive at 10^{-4} M yet showed concentration-dependent activation at 10^{-6} to 10^{-5} M. The precise reason for this is not known, but it is probably related to the inhibitory effect which was evident at 10^{-4} M.

Three other compounds (**8**, **20**, and **23**), which were able to activate Transport-P at 10^{-5} M but unable to activate at 10^{-4} M also demonstrated inhibition at 10^{-4} M. The situation is complex, since some compounds (**3**–**6**, **9**, **10 and 22**) are able to both activate and inhibit at 10^{-4} M; **22** has a surprisingly shallow concentration–response relationship. On the other hand, compounds **12** and **13,** which demonstrated a high level of activation at 10^{-4} M, did not inhibit Transport-P.

5. Conclusions

1. The structure of prazosin appears to be very specific for the activation of uptake-P and to date no compound has been found to be more potent than prazosin. The most active compound so far

is the thienoyl analogue. Compounds with other acyl groups for A were much less effective, but it is of interest to note that a benzoyl group afforded a better analogue than did tetrahydrofuranoyl, acetyl or trifluoroacetyl.
2. The presence of the 6,7-dimethoxy groups B appears to be very necessary for activation of uptake-P and it will be of importance to explore alternatives.
3. The 4-amino group C can accept a small substituent such as acetyl or methyl but with a substantial loss in activity. Larger groups such as diethyl, benzyl or phenyl cannot be accommodated.
4. The piperazine ring D seems to have a critical geometry in so far as enlarging it to a 1,4-diazepane greatly reduces activating activity. Surprisingly, however, the furoylpiperazine can be replaced by furfurylamine or 2-(*N*-piperidinyl)ethylamine to give compounds which retain some ability to activate Transport-P at 10^{-5} to 10^{-6} M.

6. Experimental

6.1. Chemistry (general)

Melting points were determined on an Electrothermal® melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded at 300 and 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. For HPLC I 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; for HPLC II the mobile phases were mixtures of A = acetonitrile + 0.1% trifluoroacetic acid, and B = water + 0.1% trifluoroacetic acid; eluent gradient: 10:90 to 100:0 in 13 min at 1.5 mL/min. 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 ionization (APCI), electrospray (ES) or fast atom bombardment (FAB). Elemental analyses were determined on a Perkin-Elmer 2400 CHN elemental analyzer 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} aluminum sheets, visualized at 254 nm and stained with potassium iodoplatinate (KIP) or 5% H_2SO_4 in ethanol prior to heating.

6.1.1. 4-Amino-6,7-dimethoxy-2-(4-(2-thiophene-carbonyl)-piperazin-1-yl)quinazoline hydrochloride hydrate (2)

Under a nitrogen atmosphere, ethyl thiophene-2-carboxylate (1.34 mL, 9.96 mmol) and piperazine (1.72 g, 20 mmol) were stirred at 110 °C for 25 h. The temperature was then raised to 150 °C for a further 5 h, and the mixture then left to cool to room temperature. The residue was partitioned between CH_2Cl_2 (20 mL) and 0.5 N HCl (20 mL), and the aqueous layer was adjusted to pH 10 with saturated aqueous K_2CO_3 and the free base extracted with CH_2Cl_2 (20 mL). The organic layer was then washed with water (20 mL), dried over Na_2SO_4 and concentrated in vacuo to yield a brown coloured oil (0.274 g, 14% yield) of *N*-(thiophene-2-carbonyl)piperazine. R_f = 0.05 (EtOAc, KIP); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.84 (t, J = 5.1 Hz, 4H, pip), 3.65 (t, J = 5.1 Hz, 4H, pip), 6.97 (t, J = 4.3 Hz, 1H, thi-4H), 7.21 (dd, J = 2.3, 1.38 Hz, 1H, thi-3H), 7.37 (dd, J = 1.1, 3.9 Hz, 1H, thi-5H); MS (FAB) *m/z* 197 (M^+ 35%) 111 ($M^+-C_4H_9N_2$ 100%).

Under a nitrogen atmosphere a mixture of 4-amino-2-chloro-6,7-dimethoxy-quinazoline (**26b**), (0.27 g, 1.11 mmol) prepared as previously described,[11] *N*-(thiophene-2-carbonyl)piperazine (0.22 g, 1.11 mmol) and 3-methyl-1-butanol (5 mL) was stirred and boiled under reflux (~170 °C) for 5 h. The mixture was cooled to 0–5 °C and left to rest for approximately 30 min. The cream precipitate was then collected by filtration, washed with diethyl ether (2× 10 mL), recrystallised twice from methanol by precipitation with diethyl ether to yield an off white crystalline product **2** (0.21 g, 48%); mp = 265–267 °C; R_f = 0.68 (EtOAc); HPLC I (MeOH/H_2O 50:50) t_R 6.87 min (100%); ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.84 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), behind methoxy 3.84 (br s, 4H, pip-3,3′ + 5,5′H), 3.97 (m, 4H, pip-2,2′ + 6,6′H), 7.17 (t, J = 4.4 Hz, 1H, thi-4H), 7.52 (d, J = 3.2 Hz, 1H, thi-3H), 7.55 (s, 1H, Ar-5H), 7.77 (s, 1H, Ar-8H), 7.81 (d, J = 4.7 Hz, 1H, thi-5H); MS (FAB) *m/z* 400 (M^+ 100%). Anal. Calcd for $C_{19}H_{21}N_5O_3S{\cdot}HCl{\cdot}H_2O$: C, 50.27; H, 5.33; N, 15.93; Cl, 7.81. Found: C, 50.14; H, 4.94; N, 15.52; Cl, 8.17.

6.1.2. *rac*-4-Amino-6,7-dimethoxy-4-(2-tetrahydrofuranoyl-piperazin-1-yl)quinazoline hydrochloride monohydrate (3)

To a mixture of tetrahydro-2-furoic acid (5 mL, 0.05 mol) and methanol (10 mL, 0.21 mol), sulfuric acid (1 mL) was added slowly down the walls of the flask and the contents swirled gently. The mixture was heated under reflux for 40 min and left to cool. The mixture was quenched with ice (22 g) and stirred until all the ice has melted. The organic product was extracted with ether (35 mL) and washed successively with water (25 mL), saturated sodium carbonate solution (25 mL) and brine (25 mL), dried over $MgSO_4$ and concentrated in vacuo, to give methyl tetrahydrofuran-2-carboxylate as a sweet smelling transparent oil (1.52 g, 23% yield).

Under a nitrogen atmosphere, methyl tetrahydrofuran-2-carboxylate (1.03 g, 7.90 mmol) and piperazine (0.82 g, 9.48 mmol) were heated at 110 °C for 5 h. The mixture was cooled to room temperature and partitioned between chloroform (20 mL) and saturated aqueous sodium bicarbonate (20 mL). The organic layer was washed with water (2× 10 mL) and dried over Na_2SO_4. The extract was concentrated and chromatographed on a silica gel column (EtOAc/MeOH, 4:1). When the first set of spots emerged the column was flushed with 100% MeOH to give *N*-(tetrahydrofuran-2-carbonyl)piperazine (0.24 g, 16%).

Under a nitrogen atmosphere, 4-amino-2-chloro-6,7-dimethoxy-quinazoline (0.14 g, 0.59 mmol) and *N*-(tetrahydrofuran-2-carbonyl)piperazine (0.11 g, 0.59 mmol) in 3-methyl-1-butanol (5 mL) were heated under reflux for $4\frac{1}{2}$ h with stirring. The mixture was cooled to 0–5 °C and left to stand for approximately 30 min. The white precipitate was collected by filtration and washed with acetone (2× 10 mL), before being recrystallised twice from methanol and precipitated using diethyl ether, to yield the product **3** as a white crystalline solid (0.17 g, 72%); mp = 276–280 °C; lit.[12] mp 278–279 °C, for the hydrochloride dihydrate. R_f = 0.095 (EtOAc) ; HPLC I (MeOH/H_2O 50 :50), t_R 5.47 min (100%); ^{1}H NMR (500 MHz, CD_3OD): δ 1.98 (m, 2H, thf-4,4′H), 2.08 (m, 1H, thf-3H), 2.25 (m, 1H, thf-3H), 3.66–4.02 (m, 10H, thf-5,5′H and pip-H), 3.92 (s, 3H, OCH_3), 3.98 (s, 3H, OCH_3), 4.80 (m, 1H, thf-2H), 7.16 (s, 1H, Ar-5H), 7.55 (s, 1H, Ar-8H); MS (FAB) *m/z* 388 (M^+ 100%). Anal. Calcd for $C_{19}H_{25}N_5O_4{\cdot}0.9HCl{\cdot}0.8H_2O$: C, 52.5; H, 6.38; N, 16.11; Cl, 7.34. Found: C, 52.14; H, 6.21; N, 15.94; Cl, 7.60.

6.1.3. 4-Amino-6,7-dimethoxy-2-(4-trifluoroacetylpiperazin-1-yl)quinazoline hydrochloride (6)

A mixture of 4-amino-2-chloro-6,7-dimethoxyquinazoline[11] (0.45 g, 1.9 mmol) and 1-trifluoroacetylpiperazine (prepared according to the method of Xu et al.[13]) (0.35 g, 1.9 mmol) in 1-

pentanol (10 mL) was heated under reflux for 1 h. After cooling, the solid formed was filtered and recrystallised twice from methanol, yielding 4-amino-6,7-dimethoxy-2-(4-trifluoroacetylpiperazin-1-yl)quinazoline hydrochloride (**6**) as a white solid (0.52 g, 66%); mp = 287–288 °C; HPLC II *tR* = 6.52 min (98.4%); ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.62 (br s, 4H, pip-H), 3.75 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 4.08 (br s, 4H, pip-H), 7.54 (s, 1H, Ar-H_5), 7.66 (s, 1H, Ar-H_8), 8.70 (br s, 1H, NH), 8.95 (br s, 1H, NH); MS (CI^+, CH_4): *m/z* 386 (M+1, 100%); Anal. Calcd for $C_{16}H_{18}N_5O_3F_3{\cdot}HCl{\cdot}1.3H_2O$: C, 43.15; H, 4.85; N, 15.73; Cl, 7.98. Found: C, 43.01; H, 4.63; N, 15.54; Cl, 8.01.

6.1.4. 4-(4-Amino-6,7-dimethoxyquinazolin-2-yl)-piperazine-1-carboxylic acid benzyl ester hydrochloride (7)

A mixture of 4-amino-2-chloro-6,7-dimethoxyquinazoline (1 g. 4.2 mmol) and benzyl 1-piperazinecarboxylate[14] (0.92 g, 4.2 mmol) in 10 mL of 1-pentanol was heated under reflux for 3 h. After cooling, the solid formed was filtered, washed with Et_2O and recrystallised from MeOH, yielding 4-(4-amino-6,7-dimethoxyquinazolin-2-yl)-piperazine-1-carboxylic acid benzyl ester hydrochloride (**7**) as a white solid (1.57 g, 86%); mp = 249–251 °C; HPLC II *tR* = 7.88 min (98%); MS (FAB^+) m/z 424 (M+1, 82%), base peak: *m/z* 154; ^{1}H NMR (300 MHz, DMSO-d_6 + 1 drop D_2O): δ 3.48 (br s, 4H, pip-H), 3.81 (br s + s, 7H, pip-H + OCH_3), 3.86 (s, 3H, OCH_3), 5.10 (s, 2H, CH_2Ph), 7.20 (s, 1H, Ar-H_5), 7.33 (s, 5H, C_6H_5), 7.63 (s, 1H, Ar-H_8); Anal. Calcd for $C_{22}H_{25}N_5O_4{\cdot}1.25HCl$: C, 56.33; H, 5.60; N 14.93; Cl 9.47. Found: C 56.31; H 5.59; N 14.70; Cl 9.64.

6.1.5. [4-(4-Amino-7,8-dihydro-[1,4]dioxino[2,3g]quinazolin-2-yl)piperazin-1-yl]-furan-2-yl methanone hydrochloride (9)

A mixture of 7-amino-1,4-benzodioxan-6-carboxylic acid[15,16] (0.9 g, 4.6 mmol) and potassium cyanate (1.5 g, 18.5 mmol) in 30 mL of water containing 1.5 mL of glacial acetic acid was stirred for 4 h. Then, NaOH (11.2 g, 280 mmol) was added and the reaction was heated under reflux for 2 h. After cooling, the reaction medium was acidified with concd HCl and the solid formed was filtered and recrystallised from MeOH, yielding 7,8-dihydro-1H-[1,4]dioxino[2,3g]quinazoline-2,4-dione **(24a)** as a white solid (1.0 g, 99%), mp = 364–365 °C (lit.[15] 364–366 °C), ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.23 (br s, OCH_2) + 4.31 (br s, OCH_2) (4H), 6.58 (s, 1H), 7.23 (s, 1H).

A mixture of **24a** (0.65 g, 2.95 mmol), 5 mL of $POCl_3$ and 0.4 mL of freshly distilled *N,N*-dimethylaniline was heated under reflux for 4 h. After cooling, it was poured into ice, stirred vigorously and the solid formed was filtered and recrystallised twice from Et_2O/MeOH, yielding 2,4-dichloro-7,8-dihydro-1H-[1,4]dioxino[2,3g]quinazoline (**25a**) (0.4 g, 53%), mp = 215–218 °C (lit.[15] 221–223 °C).

A mixture of 2,4-dichloro-7,8-dihydro-1H-[1,4]dioxino[2,3g]quinazoline (0.26 g, 1.01 mmol), 10 mL of THF and 5 mL of aq NH_4OH 35% was stirred at room temperature for 16 h. The solvent was then removed under reduced pressure and water was added to the solid obtained, which was filtered and recrystallised from MeOH to yield 4-amino-2-chloro-7,8-dihydro-1H-[1,4]dioxino[2,3g]quinazoline (**26a**) as a white solid (0.19 g, 79%), mp = 279–280 °C (d); ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.33 (m, 4H), 6.99 (s, 1H), 7.71 (s, 1H), 7.99 (br s, 2H).

A mixture of 4-amino-2-chloro-7,8-dihydro-1H-[1,4]dioxino[2,3g]quinazoline (**26a**) (0.05 g, 0.22 mmol) and 1-(2-furoyl)piperazine[11] (0.05 g, 0.28 mmol) in 5 mL of 1-pentanol was heated under reflux for 1 h. After cooling, the solid formed was filtered and recrystallised from MeOH, yielding [4-(4-amino-7,8-dihydro-[1,4]dioxino[2,3g]quinazolin-2-yl)piperazin-1-yl]-furan-2-yl methanone hydrochloride (**9**) as a white solid (0.08 g, 89%), mp = 198–200 °C, HPLC II *tR* = 6.12 min (100%); ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.85 (br s, 4H, pip-H), 3.94 (br s, 4H, pip-H), 4.32 (d, 2H, OCH_2), 4.40 (d, 2H, OCH_2), 6.65 (dd, 1H, fur-H_4), 7.08 (d, 1H, fur-H_3), 7.36 (s, 1H, Ar-H_5), 7.80 (s, 1H, Ar-H_8); 7.87 (d, 1H, fur-H_5), 8.76 (br s, 1H, NH), 8.80 (br s, 1H, NH); MS (FAB^+): 382 (M+1, <5%), base peak: 154; Anal. Calcd for $C_{19}H_{19}N_5O_4{\cdot}HCl{\cdot}1.5H_2O$: C, 51.25; H, 5.18; N, 15.73; Cl, 7.98. Found: C, 50.96; H, 4.79; N, 15.98; Cl, 8.05.

6.1.6. 4-Acetamido-6,7-dimethoxy-2-[4-(2-furoyl)piperazin-1-yl]quinazoline (12)

To a warm suspension of 4-amino-6,7-dimethoxy-(2-(2-furoyl)piperazin-1-yl) quinazoline hydrochloride hydrate (prazosin)[17] (0.1 g, 0.26 mmol) in propan-2-ol was added 0.019 g, 0.36 mmol of CH_3ONa. The solution was cooled, dried (Na_2SO_4) and evaporated, and the resulting free base was dried in vacuo. Acetic anhydride (2 mL) was added and the mixture was heated under reflux under nitrogen. After 1.5 h, the mixture was treated with 10 mL of water and basified with 8 mL of 12 N NaOH, which was added dropwise in 5 min. After cooling the mixture to room temperature the compound was extracted into $CHCl_3$ (3× 20 mL). The organic layers were combined, washed with water and dried (Na_2SO_4). Evaporation of the solvent under reduced pressure afforded yellow crystals of product **12** that was then recrystallised from MeOH; mp 245–247 °C; HPLC I (MeOH/H_2O 50:50) t_R 5.54 min (100%); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.61 (s, 3H, CH_3), 3.91 (br m, 8H, pip-H), 3.93 (s, 3H, OCH_3), 3.97 (s, 3H, OCH_3), 6.49 (dd, *J* = 1.8, 3.4 Hz, 1H, fur-H^4), 6.89 (s, 1H, Ar-H), 6.90 (s, 1H, Ar-H), 7.04 (d, *J* = 3.35 Hz, 1H, fur-H^3), 7.50 (d, *J* = 1.8 Hz, 1H, fur-H^5), 8.07 (br s, 1H, N-H); MS (FAB^+) m/z 426. Anal. Calcd for $C_{21}H_{23}N_5O_5$: C, 59.29; H, 5.45; N, 16.46. Found: C, 58.80; H, 5.49; N, 16.42.

6.1.7. 6,7-Dimethoxy-4-methylamino-2-(4-(2-furoyl)piperazin-1-yl)quinazoline hydrochloride (13)

A mixture of 2,4-dichloro-6,7-dimethoxyquinazoline[18] (**25b**, 0.3 g, 1.16 mmol) and 1 mL of aq methylamine (40% wt) (11.6 mmol) in 20 mL of ethanol was heated under reflux for 1 h. After cooling, the solvent was removed under reduced pressure and water was added to the remaining oil, yielding a solid that was filtered, recrystallised from water and air dried, furnishing 2-chloro-6,7-dimethoxy-4-methylaminoquinazoline as a white solid (0.2 g, 69%). Mp = 219–220 °C. ^{1}H NMR (DMSO-d_6): δ 2.96 (d, 3H), 3.80 (s, 3H), 3.84 (s, 3H), 7.03 (s, 1H), 7.42 (s, 1H), 8.36 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 27.7, 55.7, 55.9, 102.0, 106.4, 106.9, 146.7, 148.3, 154.2, 155.2, 160.4.

A mixture of 2-chloro-6,7-dimethoxy-4-methylaminoquinazoline (0.15 g, 0.52 mmol), 1-(2-furoyl)piperazine (0.1 g, 0.55 mmol) and 10 mL of 1-pentanol was heated under reflux for 1.5 h. After cooling, the solid formed was filtered and recrystallised from methanol, yielding 6,7-dimethoxy-4-methylamino-2-(4-(2-furoyl)piperazin-1-yl)quinazoline hydrochloride (**13**) as a white solid (0.18 g, 75%), mp = 198–200 °C; HPLC II *tR* = 6.99 min (99.9%); ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.03 (s, 3H, NCH_3), 3.83 (br s, 4H, pip-H) + 3.85 (2 s, 6H, OCH_3), 3.99 (br s, 4H, pip-H), 6.66 (d, 1H, fur-H_4), 7.08 (d, 1H, fur-H_3), 7.54 (s, 1H, Ar-H_5), 7.89 (d, 1H, fur-H_5), 8.02 (s, 1H, Ar-H_8), 9.53 (br s, 1H, NH); MS (CI^+, CH_4): 398 (M+1, 5%), Base peak: 181; Anal. Calcd for $C_{20}H_{23}N_5O_4{\cdot}1.1HCl{\cdot}1.8H_2O$: C, 51.11; H, 5.90; N, 14.91; Cl, 8.32. Found: C, 50.85; H, 5.36; N, 14.81; Cl, 8.36.

6.1.8. 4-Benzylamino-6,7-dimethoxy-2-(4-(2-furoyl)piperazin-1-yl)quinazoline hydrochloride (14)

A mixture of 2,4-dichloro-6,7-dimethoxyquinazoline[15] (**25b**, 0.26 g, 1 mmol) and benzylamine (0.5 mL, 4.6 mmol) in 5 mL of methanol was stirred at room temperature for 1 h. After cooling, diethyl ether (5 mL) was added and the reaction medium was kept

in the fridge, leading to the formation of a white solid that was filtered and recrystallised from petroleum ether and ethyl acetate, yielding 4-benzylamino-2-chloro-6,7-dimethoxyquinazoline as a white solid (0.26 g, 79%). Mp = 229–231 °C. ^{1}H NMR (DMSO-d_6): δ 3.86 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 4.73 (d, 2H, $NHCH_2Ph$), 7.08 (s, 1H, Ar-H_5), 7.31 (m, 5H, $NHCH_2C_6H_5$), 7.70 (s, 1H, Ar-H_8), 8.92 (t, 1H, N*H*CH_2Ph).

A mixture of 4-benzylamino-2-chloro-6,7-dimethoxyquinazoline (0.161 g, 0.52 mmol), 1-(2-furoyl)piperazine (0.094 g, 0.52 mmol) and 10 mL of 1-pentanol was heated under reflux for 3 h. After cooling, the solid formed was filtered and recrystallised from methanol, yielding 4-benzylamino-6,7-dimethoxy-2-(4-(2-furoyl)piperazin-1-yl)quinazoline hydrochloride (**14**) as a white solid (0.08 g, 32%), mp = 267–268 °C; HPLC II *tR* = 8.03 min (100%); ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.54 (br s, 4H, pip-H), 3.78 (s, 6H, OCH_3), 3.89 (br s, 4H, pip-H), 4.61 (d, 2H, NCH_2Ph), 6.32 (dd, 1H, fur-H_4), 6.84 (d, 1H, fur-H_3), 7.10 (m, 5H, C_6H_5), 7.33 (d, 1H, fur-H_5), 7.79 (s, 1H, Ar-H_5), 8.03 (s, 1H, Ar-H_8), 9.49 (t, 1H, NH); MS (FAB$^+$): 474 (M+1, 40%), base peak: 154; Anal. Calcd for $C_{26}H_{27}N_5O_4$·1.18HCl: C, 60.46; H, 5.46; N, 13.56; Cl, 8.12. Found: C, 60.69; H, 5.57; N, 13.60; Cl, 8.32.

6.1.9. 6,7-Dimethoxy-4-phenylamino-2-(4-(2-furoyl)piperazin-1-yl)quinazoline hydrochloride (15)

A mixture of 2,4-dichloro-6,7-dimethoxyquinazoline (25 b, 0.26 g, 1 mmol), anhydrous KF (0.25 g. 4.3 mmol) and 18-crown-6 (1.13 g, 4.3 mmol) in 5 mL of anhydrous acetonitrile was stirred at room temperature and N_2 atmosphere for 10 min. Aniline (0.2 mL, 2.15 mmol) was then added, and the reaction was stirred for 24 h. The solvent was then removed under reduced pressure, and the resulting solid was dissolved with ethyl acetate and water (10 mL of each). The layers were separated and the organic layer was dried over anhydrous sodium sulphate, filtered and the solvent was removed under reduced pressure. The solid obtained was recrystallised from AcOEt/MeOH (1:1), yielding 2-chloro-6,7-dimethoxy-4-phenylamino-quinazoline (0.139 g, 0.44 mmol) as a white solid, mp = 218–220 °C; ^{1}H NMR (300 MHz, $(CD_3)_2CO$): δ 3.83 (s, 3H, O*C*H_3), 3.93 (s, 3 H, O*C*H_3), 7.1–7.2 (m, 2H, NHPh-H_4 + Ar-H_5), 7.39 (t, 2H, NHPh-H_3), 7.73 (s, 1H, NHPh-H_2), 7.81 (d, 2H, Ar-H_8).

A mixture of 2-chloro-6,7-dimethoxy-4-phenylamino-quinazoline (0.09 g, 0.26 mmol) and 1-(2-furoyl)piperazine (0.05 g, 0.28 mmol) in 5 mL of 1-pentanol was heated under reflux for 1 h. After cooling, the solid formed was filtered and recrystallised twice from methanol, yielding 6,7-dimethoxy-4-phenylamino-2-(4-(2-furoyl)piperazin-1-yl)quinazoline hydrochloride (**15**, 0.071 g, 55%) as a white solid, mp = 209–210 °C; HPLC II *tR* = 9.09 min (99.1%); ^{1}H NMR (300 MHz, $CDCl_3$ + drops DMSO-d_6): δ 3.68 (br s, 4H, pip-H), 3.76 + 3.79 (2s, 6H, OCH_3), 3.85 (br s, 4H, pip-H), 6.28 (dd, 1H, fur-H_4), 6.80 (d, 1H, fur-H_3), 7.17 (t, 1H, NPh-H_4), 7.20 (t, 2H, NPh-H_3), 7.26 (s, 1H, Ar-H_5), 7.31 (d, 1H, fur-H_5), 7.36 (d, 2H, NPh-H_2), 7.68 (s, 1H, Ar-H_8), 7.95 (br s, 1H, N**H** Ph); MS (FAB$^+$): 460 (M+1, 100%); Anal. Calcd for $C_{25}H_{25}N_5O_4$·1.1HCl·1.7H_2O (MW = 529.75): C, 56.63; H, 5.57; N, 13.21; Cl, 7.37. Found: C, 56.62; H, 5.34; N, 13.14; Cl, 7.32 .

6.1.10. 4-Diethylamino-6,7-dimethoxy-2-(4-(2-furoyl)piperazin-1-yl)quinazoline hydrochloride (16)

A mixture of 2,4-dichloro-6,7-dimethoxyquinazoline (**25b**, 0.26 g, 1 mmol) and diethylamine (1 mL, 9 mmol) in 10 mL of ethanol was heated under reflux for 1 h. After cooling, the solvent was removed under reduced pressure and water was added to the remaining oil, yielding a solid that was filtered, recrystallised from water and air dried, yielding 2-chloro-4-diethylamino-6,7-dimethoxyquinazoline as a white solid (0.25 g, 84%), mp = 147–148 °C (lit.[19] 148–149 °C). ^{1}H NMR (DMSO-d_6): δ 1.22 (t, 6H, NCH_2CH_3), 3.65 (q, 4H, NCH_2CH_3), 3.87 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 7.10 (s, 1H, Ar-H_5), 7.18 (s, 1H, Ar-H_8).

A mixture of 2-chloro-4-diethylamino-6,7-dimethoxyquinazoline (0.2 g, 0.7 mmol) and 1-(2-furoyl)piperazine[11] (0.13 g, 0.7 mmol) in 10 mL of 1-pentanol was heated under reflux for 4 h. After cooling, the solid formed was filtered and recrystallised from methanol, yielding 4-diethylamino-6,7-dimethoxy-2-(4-(2-furoyl)piperazin-1-yl)quinazoline hydrochloride (**16**) as a white solid (0.15 g, 45%), mp = 223–224 °C; HPLC II *tR* = 7.69 min (100%); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.44 (t, 6H, NCH_2CH_3); 3.76 (q, 2H, NCH_2CH_3), 3.81 (s, 3H, OCH_3), 3.96 (br s, 4H, pip-H), 4.04 (s, 3H, OCH_3), 4.20 (br s, 4H, pip-H), 6.48 (dd, 1H, fur-H_4), 7.05 (d, 1H, fur-H_3), 7.09 (s, 1H, Ar-H_5), 7.50 (d, 1H, fur-H_5), 8.42 (s, 1H, Ar-H_8); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.9, 45.8, 56.1, 57.1, 101.5, 102.4, 105.3, 111.4, 117,4, 144.3, 146.4; MS (CI$^+$, CH_4): 440 (M+1, 100%); Anal. Calcd for $C_{23}H_{29}N_5O_4$·1.1HCl·0.7H_2O: C, 56.12; H, 6.40; N, 14.23; Cl, 7.94. Found: C, 56.03; H, 6.44; N, 14.25; Cl, 8.11.

6.1.11. 6,7-Dimethoxy-4-(*N*-morpholino)-2-(4-(2-furoyl)piperazin-1-yl)quinazoline hydrochloride (17)

A mixture of 2,4-dichloro-6,7-dimethoxyquinazoline (**25b**, 0.26 g, 1 mmol) and morpholine (1 mL, 11.5 mmol) in 10 mL of methanol was stirred at room temperature for 1 h. After cooling, diethyl ether (5 mL) was added and the reaction medium was kept in the fridge, leading to the formation of a white solid that was filtered and recrystallised from petroleum ether and ethyl acetate, yielding 2-chloro-6,7-dimethoxy-4-(*N*-morpholino)quinazoline (0.3 g, 97%) as a white solid, mp = 221–222 °C. ^{1}H NMR (DMSO-d_6): δ 3.71–3.75 (m, 8H), 4.00 (d, 6H), 7.1–7.2 (2 s, 2H); Anal. Calcd for $C_{14}H_{16}N_3O_3Cl$·0.1HCl (MW = 313.15): C, 53.66; H, 5.46; N, 13.41; Cl, 12.47. Found: C, 53.87; H, 5.25; N, 13.39; Cl, 12.61.

A mixture of 2-chloro-6,7-dimethoxy-4-(*N*-morpholino)quinazoline (0.161 g, 0.52 mmol) and 1-(2-furoyl)piperazine[11] (0.094 g, 0.52 mmol) in 10 mL of 1-pentanol was heated under reflux for 3 h. After cooling, the solid formed was filtered and recrystallised from methanol, yielding the product **17** (0.08 g, 32%) as a white solid, mp = 235–235.5 °C; HPLC II = 99.7%; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.73 (br s, 4H, NCH_2CH_2O), 3.88 (2s, 6H, OCH_3) + 3.99 (br s, 4H, NCH_2CH_2O), 4.01 (br s, 8H, pip-H), 6.65 (dd, 1H, fur-H_4), 7.01 (d, 1H, fur-H_3), 7.28 (d, 1H, fur-H_5), 7.53 (s, 1H, Ar-H_5), 7.88 (s, 1H, Ar-H_8); MS (CI$^+$, CH_4): 454 (M+1, 100%); Anal. Calcd for $C_{23}H_{27}N_5O_5$·HCl·0.8H_2O (MW = 503.9): C, 54.77; H, 5.87; N, 14.89; Cl, 7.04. Found C, 54.77; H, 5.68; N, 13.84; Cl, 7.10.

6.1.12. 4-Amino-6,7-dimethoxy-2-[2-furoyl-4-(1,4-diazepan-1-yl)]quinazoline (20)

Under a nitrogen atmosphere, a mixture of ethyl 2-furoate (3.309 g, 0.0236 mmol) and 1,4-diazepane (7.096 g, 0.0708 mmol) was heated at 115 °C for 5 h, to give a golden brown oil. The product was extracted with CH_2Cl_2 (20 mL) and 2 M HCl (20 mL, pH 2). The aqueous layer was then basified to pH 10 with saturated K_2CO_3, and the product extracted with CH_2Cl_2 (2× 20 mL) and dried over Na_2SO_4. The organic product was filtered and concentrated in vacuo to give a brown coloured oil, which was purified by column chromatography (CH_2Cl_2/MeOH 60:1) to yield *N*-(furan-2-carbonyl)1,4-diazepane as a golden coloured oil (1.471 g, 32%); R_f = 0.61 (MeOH, KIP); ^{1}H NMR (300 MHz $CDCl_3$-D_2O shake): δ 2.09 (m, 2H, diazep), 3.10 (t, *J* = 5.79 Hz, 2H, diazep), 3.22 (br s, 2H, diazep), 3.95 (br s, 2H, diazep), 4.03 (br s, 2H, diazep), 6.68 (m, 1H, fur-4H), 7.23 (d, *J* = 3.3 Hz, 1H, fur-3H), 7.68 (br s, 1H, fur-5H); MS (FAB) 195 (M$^+$ 100%) 95 (M$^+$−$C_5H_{11}N_2$ 95%).

Under a nitrogen atmosphere, a mixture of 2-chloro-4-amino-6,7-dimethoxy-quinazoline (**26b**) (0.411 g, 1.72 mmol) and *N*-(furan-2-carbonyl)-1,4-diazepane (**28**) (0.333 g, 1.72 mmol) in 3-methyl-1-butanol (5 mL) was heated under reflux for $5\frac{1}{2}$ h. The mixture was then left to cool between 0 and 5 °C for approximately 30 min. The white solid precipitate was collected by filtration, washed with diethyl ether and recrystallised twice from methanol (precipitated with diethyl ether) to yield a white solid (0.416 g, 61%); mp = 270–271 °C; R_f 0.72 (EtOAc); HPLC I (MeOH/H_2O 1:1) t_R 6.05 min (100%); ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.98 (m, 2H, diazep), 3.73 (br t, J = 5.43 Hz, 2H, diazep), 3.86 (s 3H, OCH_3), 3.89 (s 3H, OCH_3) 3.93 (br t, J = 5.43 Hz, 2H, diazep), 3.97 (br t, J = 5.93 Hz, 2H, diazep), 4.09 (br t, J = 5.58 Hz, 2H, diazep) 6.56 (dd, J = 1.72 and 1.68 Hz, 1H, fur-4H), 6.92 (d, J = 3.34 Hz, 1H, fur-3H), 7.73 (s and d, 2H, Ar-5H and fur-5H overlap), 7.75 (s, 1H, Ar-8H). Anal. Calcd for $C_{20}H_{23}N_5O_4 \cdot 0.95HCl \cdot 0.5H_2O$: C, 54.46; H, 5.7; N, 15.88; Cl, 7.69. Found: C, 54.54; H, 5.64; N, 15.94; Cl, 7.46.

6.1.13. 4-Amino-6,7-dimethoxy-2-(furfurylamino)quinazoline hydrochloride (21)

A mixture of 2-chloro-4-amino-6,7-dimethoxy-quinazoline (0.211 g, 0.88 mmol), furfuryl amine (0.08 mL, 0.88 mmol) and 3-methyl-1-butanol (5 mL) was heated at reflux (~180 °C) for 5 h. After cooling to 0–5 °C, the mixture was left to rest for approximately 30 min, and the precipitate was collected by filtration and washed with acetone. The hydrochloride salt was then recrystallised from methanol twice, and on the second occasion it was precipitated with ether, to yield white crystals (0.151 g, 57%); mp 249–251 °C; R_f = 0.09 (EtOAc); HPLC I (MeOH/H_2O 1:1) t_R 9.09 min (100%); ^{1}H NMR (300 MHz, CD_3OD): δ 4.07 (s, 3H, OCH_3), 4.11 (s, 3H, OCH_3), 4.84 (s, 2H, CH_2), 6.52 (m, 2H, furan-3H, 4H), 7.10 (s, 1H, Ar-5H), 7.60 (t, J = 1.26 Hz, 1H, furan, 5H), 7.69 (s, 1H, Ar-8H); MS (FAB) *m/z* 301 (M^+, 100%). Anal. Calcd for $C_{15}H_{16}N_4O_3 \cdot HCl \cdot 0.1H_2O$: C, 53.21; H, 5.06; N, 16.55; Cl, 10.47. Found: C, 53.15; H, 5.14; N, 16.71; Cl, 10.04.

6.1.14. 4-Amino-6,7-dimethoxy-2-[*N*-(2-aminoethyl)piperidinyl]quinazoline dihydrochloride (22)

Under a nitrogen atmosphere, a mixture of 2-chloro-4-amino-6,7-dimethoxy-quinazoline (0.14 g, 0.56 mmol), *N*-(2-aminoethyl)piperidine (0.08 mL, 0.56 mmol) and 3-methyl-1-butanol (5 mL) was heated under reflux (~180 °C) for 5 h. The mixture was then cooled to 0–5 °C and left to rest for approximately 30 min. The white precipitate was collected by filtration and washed with 3-methyl-1-butanol (5 mL) and acetone (5 mL). The hydrochloride salt was then recrystallised twice from methanol (precipitated with diethyl ether) to yield small white (powdery) crystals (0.04 g, 20% yield); mp = 266–269 °C; R_f = 0.36 (MeOH/EtOAc 1:1); HPLC I (MeOH/H_2O 1:1) t_R 8.34 min (100%); ^{1}H NMR (300 MHz, CD_3OD): δ 1.69 (br s, 2H, pip), 1.91 (t, J = 5.65 Hz, 4H, pip), 3.35 (o, 4H, pip), 3.38 (t, J = 5.99 Hz, 2H, CH_2), 3.92 (s, 3H, OCH_3), 3.93 (o, 2H, CH_2), 3.97 (s, 3H, OCH_3), 6.97 (s, 1H, Ar-5H), 7.56 (s, 1H, Ar-8H); MS (FAB) *m/z* 332 (M^+, 100%). Anal. Calcd for $C_{17}H_{25}N_5O_2 \cdot 2HCl \cdot 2H_2O$: C, 46.18; H, 7.11; N, 15.84. Found: C, 45.81; H, 7.12; N, 15.67.

6.2. Pharmacology: inhibition of uptake

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.[7] 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% fetal 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 seven days, the cells were dispersed in the presence of trypsin, deoxyribonuclease I and ethylenediamine- tetraacetic acid and incubated in Corning or Nunc 12-well plates (approximately 2×10^6 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.[20] 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 microlitre 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). Non-specific 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 non-specific from total uptake. Efficacy was defined from the inhibition of the uptake of prazosin (at 10^{-6} M) when the test compound was used in a concentration of 10^{-4} M and expressed as percentage 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. Where IC_{50} values were not calculated, the data were expressed only as efficacy (percentage 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.

6.3. Pharmacology: activation of uptake

The compounds were tested for their ability to increase the uptake of a tracer dose of [^{3}H]prazosin in the peptidergic neurones. The cells were incubated in the presence of [^{3}H]prazosin 2×10^{-10} M and concentrations of the test compounds, ranging from 10^{-9} M to 10^{-4} M. Each experiment included a positive control (unlabelled prazosin 10^{-6} M). Basal uptake was defined as the amount of [^{3}H]prazosin accumulated by the cells in the presence of [^{3}H]prazosin 2×10^{-10} M. For the purpose of these experiments, activation of Transport-P was defined as the increase of uptake of [^{3}H]prazosin which is caused by unlabelled prazosin 10^{-6} M; the value for activation of Transport-P was therefore obtained by subtracting basal uptake from uptake in the presence of unlabelled prazosin 10^{-6} M. The increase in uptake of [^{3}H]prazosin was expressed as percentage of the basal value. Each experimental point was carried out in triplicate and each experiment was carried out twice.

The compounds were dissolved in water, uptake/binding buffer (vide supra) or DMSO. When DMSO was used as a solvent, it was present in all solutions to 1:1000. In six consecutive experiments, DMSO had no effect on the accumulation of [^{3}H]prazosin when the cells were exposed to this radioligand at a concentration of 2×10^{-10} M (DMSO: 26.9 ± 1.8 fmol/mg protein; water: 26.5 ± 1.9 fmol/mg protein). However DMSO diminished the activation of Transport-P, which was caused by unlabelled prazosin 10^{-6} M. In

six consecutive experiments, the increase in uptake in the presence of DMSO was 19.8 ± 2.7 fmol/mg protein, representing 76 ± 12% increase above basal; in the absence of DMSO, the increase in uptake was 34.3 ± 4.0 fmol/mg protein, representing 132 ± 16% increase above basal. Despite this effect of DMSO, there were no qualitative differences in the experiments and the conclusions were the same, regardless of whether DMSO was included or not.

Acknowledgments

We warmly thank Cesare Federico for the synthesis of compound **12** and CAPES (Brazil) for financial assistance to J.F.M.S.

References

1. Al-Damluji, S.; Krsmanovic, L. Z.; Catt, K. J. *Br. J. Pharmacol.* **1993**, *109*, 299.
2. Al-Damluji, S.; Kopin, I. J. *Br. J. Pharmacol.* **1996**, *117*, 111.
3. Al-Damluji, S.; Porter, D.; Krsmanovic, L. Z.; Knutson, J. R.; Kopin, I. J. *Br. J. Pharmacol.* **1997**, *120*, 876.
4. Al-Damluji, S.; Shen, W. B.; White, S.; Barnard, E. A. *Br. J. Pharmacol.* **2001**, *132*, 336.
5. Al-Damluji, S.; Kopin, I. J. *Br. J. Pharmacol.* **1996**, *117*, 811.
6. Campbell, S. F.; Davey, M. J.; Hardstone, J. D.; Lewis, B. N.; Palmer, M. J. *J. Med. Chem.* **1987**, *30*, 49.
7. Al-Damluji, S.; Kopin, I. J. *Br. J. Pharmacol.* **1998**, *124*, 693.
8. Al-Damluji, S.; Ganellin, C. R. Abstracts of Papers, British Pharmacological Society Meeting, April 2002, Hatfield, UK *Br. J. Pharmacol.*, **2002**, *136*, 20 p.
9. Al-Damluji, S. *Curr. Drug Targets CNS Neurol. Disord.* **2004**, *3*, 201.
10. Zunszain, P. A.; Federico, C.; Sechi, M.; Al-Damluji, S.; Ganellin, C. R. *Bioorg. Med. Chem.* **2005**, *13*, 3681.
11. Althuis, T. H.; Hess, H.-J. *J. Med. Chem.* **1977**, *20*, 146.
12. Titmarsh, S.; Monk, J. P. *Drugs* **1987**, *33*, 461; Roteman, R. U.S.Patent, 4,251,532, 1981 to Abbott Laboratories
13. Xu, D.; Prasad, K.; Repic, O.; Blacklock, T. J. *Tetrahedron Lett.* **1995**, *36*, 7357.
14. Goldman, L.; Williams, J. H. *J. Org. Chem.* **1953**, *18*, 815.
15. Hess, H.-J.; Cronin, T. H.; Scriabine, A. *J. Med. Chem.* **1968**, *11*, 130.
16. Cava, M. P.; Dalton, D. R. *J. Org. Chem.* **1966**, *31*, 1281.
17. Hess, H. J. U.S. Patent, 3,511,836 (1970), 3, 935,213 (1973).; Honkanen, E.; Pippuri, A.; Kairisalo, P.; Thaler, H.; Koivisto, M.; Tuomi, S. *J. Heterocycl. Chem.* **1980**, *17*, 797.
18. Curd, F. H. S.; Landquist, J. K.; Rose, F. L. *J. Chem. Soc.* **1948**, 1759.
19. Millen, J.; Riley, T. N.; Waters, I. W.; Hamrick, M. E. *J. Med. Chem.* **1985**, *28*, 12.
20. Al-Damluji, S.; Shen, W. B. *Br. J. Pharmacol.* **2001**, *132*, 851.