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New pyrimido[5,4-*b*]indoles and [1]benzothieno[3,2-*d*]pyrimidines: High affinity ligands for the α_1-adrenoceptor subtypes

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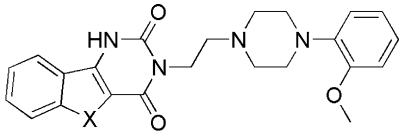
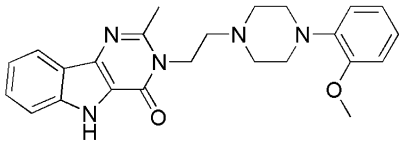
Abstract—A number of new pyrimido[5,4-*b*]indole and [1]benzothieno[3,2-*d*]pyrimidine derivatives were synthesized and evaluated for their binding and functional properties at α_1-adrenergic receptor (α_1-AR) subtypes. They behaved as potent α_1-AR antagonists. In binding experiments, some of them (RC24 and RC23) showed very high affinity for the α_{1D}-AR subtype.

α_1-Adrenergic receptors (α_1-ARs) belong to the seven-transmembrane-domain receptor superfamily and play a primary role in the regulation of several physiological processes, particularly in the cardiovascular system. Up to date, three different α_1-AR subtypes, namely α_{1A}-, α_{1B}-, and α_{1D}-AR, have been cloned and characterized.[1] In the past decades, several non-subtype-selective α_1-AR antagonists, such as prazosin, the prototype of this class of substances, doxazosin and terazosin, have been used as effective antihypertensive drugs and, more recently, in the symptomatic treatment of lower urinary tract symptoms (LUTS) suggestive of benign prostatic hypertrophy (BPH).[2]

Intensive research in academia and pharmaceutical industries has led to the discovery of a number of subtype-selective antagonists, particularly for the α_{1A}-AR which seems the main subtype involved in bladder outlet obstruction in patients with BPH. However, recent evidence has led to a re-evaluation of the role of the α_{1A}-AR subtype in BPH. In clinical trials, highly selective α_{1A}-AR antagonists, although effective in increasing urinary flow rate, failed to provide relief of the subjective irritative and filling symptoms associated with BPH.[3] Thus, there is as yet no clinical validation that such subtype-selective agents will provide any clear therapeutic advantage. On the other hand, increasing evidence has also supported the role of the α_{1D}-AR subtype in mediating some aspects of BPH symptomatology.[4] This has led to the hypothesis that a mixed α_{1A}-/α_{1D}-AR antagonist showing selectivity over α_{1B}-ARs could be a superior therapeutic agent in treating BPH.[5] Moreover, it has been suggested that a selective α_{1D}-AR antagonist could be potentially useful in the treatment of LUTS in women.[4]



1: X = NH (RN5);
2: X = S (RN17)



3 (RA36)

Keywords: α_1-Adrenoceptor; Ligand; Antagonist; Synthesis; α_{1D}-Adrenoceptor subtype.
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In the last decade, we have been involved in the development of new selective α_1-AR ligands characterized by a planar tricyclic system coupled to a pharmacophoric phenylpiperazine (PP) moiety.[6–10] Pyrimido[5,4-*b*]indole-2,4-dione derivative RN5 (**1**) and its [1]benzothieno[3,2-*d*]pyrimido-2,4-dione analogue RN17 (**2**) are two representative examples of such ligands. Although they were not able to discriminate among the α_1-AR subtypes, both showed remarkable affinity with pK_i values in the nanomolar range. Successively, it was observed that the substitution of the carbonyl moiety with a methyl group at the 2-position of the tricyclic system of **1** led to ligands, such as RA36 (**3**), endowed with a certain degree of selectivity for the α_{1D}-AR.[10]

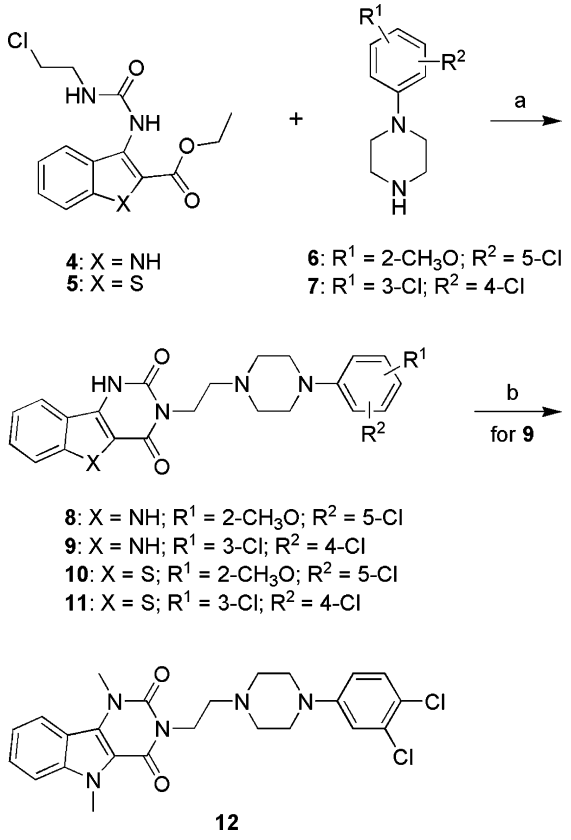
On these premises and with the aim to obtain novel α_1-AR subtype-selective ligands, in particular for the α_{1D}-AR, we synthesized a series of new tricyclic derivatives **8**–**12** and **19**–**22** based on the pyrimido[5,4-*b*]indole and the [1]benzothieno[3,2-*d*]pyrimidine systems.

Like compounds **1**–**3**, new derivatives bear a 2-[4-(substitutedphenyl)piperazin-1-yl]ethyl chain at the 3-position of the tricyclic system. They show a 2-methoxy-5-chloro or a 3,4-dichloro pattern of substitution on the phenyl ring. In α_1-AR ligands belonging to the PP class, nature and position of substituents on the phenyl ring greatly influence their subtype-selectivity. In some cases, it has been observed that the presence of two or more halogen substituents enhances ligand selectivity for α_{1D}-AR over other two α_1-AR subtypes, serotonin, and dopamine receptors.[11,12]

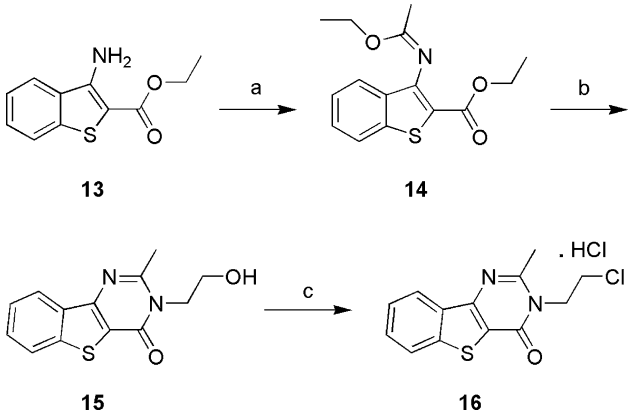
Preparation of tricyclic derivatives **8**–**12** and **19**–**22** was carried out as outlined in Schemes 1–3. Starting *N*-(2-chloroethyl)ureas **4** and **5**,[6,8] obtained by reaction of 2-chloroethyl isocyanate with the suitable indole or benzo[*b*]thiophene amino ester, were caused to react with 1-(5-chloro-2-methoxyphenyl)piperazine[13] (**6**) or 1-(3,4-dichlorophenyl)piperazine (**7**) to give tricyclic compounds **8**–**11** in good yields. Reaction of **9** with iodomethane in dry DMF afforded dimethylated derivative **12** (Scheme 1). In Scheme 2 is outlined the synthetic pathway for the preparation of **16**, a key intermediate for the synthesis of final compounds **21** and **22**. 3-Amino-2-ethoxycarbonylbenzo[*b*]thiophene (**13**) was caused to react with 1,1,1-triethoxyethane to give the iminoether **14**. Then, reaction with 2-ethanolamine transformed **14** to the tricyclic alcohol **15** which, in turn, was converted to the alkyl chloride hydrochloride **16**.

Finally, compounds **19**–**22** were obtained in good yields by heating at 140 °C chlorides **16** and **17** with the suitable piperazines **6**, **7**, and **18** in the absence of solvent (Scheme 3).

Synthesized compounds were characterized by ^{1}H NMR, IR spectra, and elemental analysis; analytical data were consistent with the proposed structures. Detailed synthetic procedures along with analytical data are given for final derivatives **8** and **21**.[14,15]



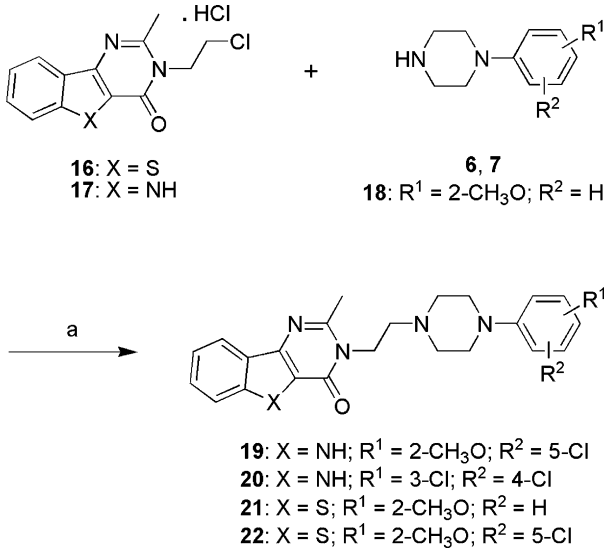
Scheme 1. Reagents and conditions: (a) 140 °C, neat, 1 h; (b) NaH (80% dispersion in mineral oil), iodomethane, dry dimethylformamide, room temperature, 12 h.



Scheme 2. Reagents and conditions: (a) 1,1,1-triethoxy ethane, reflux, 24 h; (b) ethanolamine, reflux, 2 h; (c) thionyl chloride, toluene, reflux, 4 h.

Compounds **8**–**12** and **19**–**22** were evaluated in binding assays on human α_{1A}-AR, α_{1B}-AR, and α_{1D}-AR subtypes stably expressed in HEK293 cells using [^{125}I]BE2254 as radioligand.[10] Their affinity values, expressed as pK_i, are summarized in Table 1. For sake of clarity, affinity values of parent compounds **1**–**3** are also reported.

Derivatives **8**–**12**, having a tricyclic moiety with a 2,4-dione function, showed somewhat different structure–



Scheme 3. Reagents and conditions: (a) 140 °C, neat, 1 h.

Table 1. Binding properties of new tricyclic derivatives

Compound	pK_i[a] (M)		
	α_{1A}-AR	α_{1B}-AR	α_{1D}-AR
8	8.08 ± 0.06	7.97 ± 0.06	8.81 ± 0.09
9[b]	nt	nt	nt
10	7.27 ± 0.06	7.04 ± 0.10	7.87 ± 0.10
11	5.37 ± 0.13	4.72 ± 0.16	6.51 ± 0.13
12	6.74 ± 0.01	6.22 ± 0.08	6.36 ± 0.12
19	8.88 ± 0.04	8.35 ± 0.04	9.78 ± 0.03
20	7.52 ± 0.13	7.08 ± 0.07	8.13 ± 0.05
21	8.83 ± 0.03	7.76 ± 0.03	9.40 ± 0.05
22	8.46 ± 0.05	7.76 ± 0.03	9.08 ± 0.05
1[c]	9.54 ± 0.14	8.74 ± 0.14	9.44 ± 0.11
2	8.48 ± 0.09	8.05 ± 0.08	8.90 ± 0.12
3[c]	8.52 ± 0.03	7.68 ± 0.05	9.39 ± 0.19

[a] Each value is the mean ± SE for data from three different experiments conducted in duplicate.
[b] Not tested (nt) due to its very low solubility.
[c] Binding data from Ref. 10.

affinity relationships with respect to compounds **19**–**22**, bearing a 2-methyl group in place of the carbonyl group.

With reference to the first set of derivatives, insertion of a 5-chloro substituent on the phenyl ring of the PP moiety was detrimental to affinity at all three α_1-AR subtypes. This effect is clearly evidenced by pK_i values of **8** and **10** in comparison with affinities of their parent compounds **1** and **2**. The 3,4-dichloro pattern of substitution on PP moiety also was not well tolerated by receptor binding sites. In fact, derivative **11** showed the worst affinity in the series. Moreover, although it was not possible to test indole analogue **9** due to its very low solubility in the binding medium, the more soluble dimethyl derivative **12** displayed affinity values in the micromolar range only.

Again, among compounds **19**–**22**, the 3,4-dichloro pattern gave a molecule (**20**) which showed to possess modest affinity. On the other hand, 2-methoxy-5-chloro derivative **19** (RC24) emerged as one of the most interesting ligands in the series. In fact, it displayed very high affinity for the α_{1D}-AR subtype ($pK_i = 9.78$). With respect to its de-halogenated parent compound **3**, it presents a higher affinity coupled to a common α_{1D}-AR selectivity. Among tested molecules, 2-methoxy derivative **21** (RC23) is another noteworthy ligand; it can be considered the [1]benzothieno[3,2-*d*]pyrimidine analogue of **3** and shares with the latter a very high affinity and a slight selectivity for the α_{1D}-AR.

Ligands **19**, **21**, and **22**, along with their parent compounds **1**–**3**, were also tested in functional assays for their activity at α_1-AR subtypes in isolated rat prostatic vas deferens (α_{1A}-AR), spleen (α_{1B}-AR), and thoracic aorta (α_{1D}-AR).[16] All tested compounds behaved as antagonists and their pK_b values are summarized in Table 2.

As a general trend, for the α_{1A}- and the α_{1D}-AR subtypes, functional pK_b values were similar or slightly lower (about 10-fold) than the corresponding pK_i values obtained in binding experiments. However, for the α_{1B}-AR subtype, most of pK_b values were higher than the corresponding pK_i. In particular, compound **3** showed an affinity in functional assays ($pK_b = 9.73$) which is two orders of magnitude higher than that obtained in binding assays ($pK_i = 7.68$); similarly, in functional assays compound **22** showed a selectivity for the α_{1B}-AR subtype which was not evident in binding experiments.

Observed discrepancy between pK_b and pK_i values can be ascribed to the obvious different experimental conditions inherent to the two pharmacological assays, particularly in the sources of receptor proteins (human vs rat, cloned vs native). In this context, the recent body of evidence suggesting that α_1-ARs can form homo- and heterodimers is intriguing.[18] Thus, discrepancy in pharmacological data could be also accounted for by a possible different supramolecular organization of homogeneous cloned receptors with respect to the native receptors in tissues.[19] A more detailed pharmacological study on the most interesting molecules is in progress.

In conclusion, a series of new tricyclic pyrimido[5,4-*b*]indole and [1]benzothieno[3,2-*d*]pyrimidine derivatives

Table 2. Antagonist potency, expressed as $pK_b \pm$ SE values, of selected tricyclic derivatives at α_1-AR in isolated rat prostatic vas deferens (α_{1A}-AR), spleen (α_{1B}-AR), and thoracic aorta (α_{1D}-AR)

Compound	pK_b[a]		
	α_{1A}-AR	α_{1B}-AR	α_{1D}-AR
1	8.46 ± 0.10	8.67 ± 0.21	8.71 ± 0.01
2	8.61 ± 0.14	8.44 ± 0.15	8.89 ± 0.04
3	8.53 ± 0.12	9.73 ± 0.10	9.24 ± 0.09
19	7.60 ± 0.19	8.68 ± 0.07	8.39 ± 0.07
21	8.65 ± 0.09	7.63 ± 0.14	8.98 ± 0.11
22	7.52 ± 0.14	8.91 ± 0.09	7.57 ± 0.16

[a] pK_b values were calculated according to van Rossum[17] at a single concentration. Each concentration was investigated at least four times.

were synthesized and tested for their affinity at the three α_1-AR subtypes. In binding assays on human cloned receptors, some new compounds such as **19** (RC24) and **21** (RC23) showed very high affinity and a slight preference for the α_{1D}-subtype. In addition, functional tests in isolated rat tissues evidenced that new compounds act as potent α_1-AR antagonists.

Acknowledgments

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14. Synthesis of 3-[2-[4-(5-chloro-2-methoxyphenyl)piperazin-1-yl]ethyl]-1*H*-pyrimido[5,4-*b*]indole-2,4(3*H*,5*H*)-dione (**8**). A mixture of *N*-(2-chloroethyl)-*N*′-[3-(2-ethoxycarbonyl)indolyl]urea (**4**) (0.30 g, 0.97 mmol) and 1-(5-chloro-2-methoxyphenyl)piperazine (**6**) (1.09 g, 4.84 mmol) in a 10 mL flask was heated in a oil bath at 140 °C for 45 min. After being cooled, the solid mass was suspended in EtOH (10 mL). The solid was filtered off and dried. Then it was recrystallized from dimethylformamide/water to give **8** (0.13 g, 30%) as a pure creamy white powder: mp 301–303 °C; IR (KBr, selected lines) cm^{-1} 3159, 2813, 1709, 1626, 1241, 1018, 788. The ^1H NMR spectrum was recorded at 200 MHz on a Varian Inova Unity 200 spectrometer. ^1H NMR (DMSO-d_6) δ 11.96 (br s, 1H, NH which exchanges with D_2O), 11.76 (br s, 1H, NH which exchanges with D_2O), 8.00–7.90 (m, 1H indole), 7.48–7.31 (m, 2H, indole), 7.18–7.06 (m, 1H, indole), 7.00–6.90 (m, 2H, aromatic), 6.85–6.75 (m, 1H, aromatic), 4.10 (t, J = 6.5 Hz, 2H, $CONCH_2$), 3.77 (s, 3H, CH_3), 3.02–2.88 (m, 4H, piperazine), 2.69–2.55 (m, 4H + 2H, piperazine + $CONCH_2CH_2$).
15. Synthesis of 3-[2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl]-2-methyl[1]benzothieno[3,2-*d*]pyrimidin-4(3*H*)-one (**21**). 3-Amino-1-benzothiophene-2-carboxylic acid ethyl ester (**13**) (1.10 g, 4.98 mmol) was added to 1,1,1-triethoxyethane (4.50 g, 27.7 mmol). The reaction mixture was stirred and refluxed for 24 h and then volatiles were evaporated at reduced pressure. The oily residue (1.40 g) of 3-[(1-ethoxyethylidene)amino][1]benzothiophene-2-carboxylic acid ethyl ester (**14**) was used for the subsequent step without purification. Compound **14** (1.36 g, 4.67 mmol) was dissolved in 2-aminoethanol (19.23 g, 0.315 mol). The reaction mixture was stirred and refluxed for 90 min. After being cooled, water (60 mL) was added to the reaction mixture and a solid slowly precipitated. It was filtered off, washed with water, and dried to give 3-(2-hydroxyethyl)-2-methyl[1]benzothieno[3,2-*d*]pyrimidin-4(3*H*)-one (**15**) (0.60 g, 49%). An analytical sample was recrystallized from ethyl acetate to give pure **15** as a pale yellow powder: mp 168–169 °C; IR (KBr, selected lines) cm^{-1} 3399, 1649, 1550, 1431, 1379, 1059, 756. ^1H NMR (DMSO-d_6) δ 8.29-8.10 (m, 2H, aromatic), 7.71–7.52 (m, 2H, aromatic), 5.05 (broad t, J = 5.8 Hz, 1H, OH which exchanges with D_2O), 4.21 (t, J = 5.4 Hz, 2H, NCH_2), 3.80–3.68 (m, 2H, CH_2OH), 2.77 (s, 3H, CH_3). Alcohol **15** (0.55 g, 2.11 mmol) was suspended in toluene (20 mL) and thionyl chloride (0.50 g, 4.22 mmol) was added. The reaction mixture was stirred and refluxed for 4 h. Volatiles were evaporated under reduced pressure to give a residue (0.59 g, 88%) of crude 3-(2-chloroethyl)-2-methyl[1]benzothieno[3,2-*d*]pyrimidin-4(3*H*)-one hydrochloride (**16**) which was used in the successive step without further purification. A mixture of **16** (0.331 g, 1.05 mmol) and 1-(2-methoxyphenyl)piperazine (**18**) (1.01 g, 5.29 mmol) in a 10 mL flask was heated in a oil bath at 140 °C for 1 h. After being cooled, the solid mass was suspended in EtOH (10 mL). The solid was filtered off and dried. Then it was recrystallized from EtOH to give **21** (0.19 g, 41%) as a pure pale yellow powder: mp 160–161 °C; IR (KBr, selected lines) cm^{-1} 2950, 2827, 1670,1501, 1244, 1148, 750. ^1H NMR (DMSO-d_6) δ 8.27–8.10 (m, 2H, aromatic), 7.71–7.55 (m, 2H, aromatic), 6.95–6.83 (m, 4H, aromatic), 4.28 (t, J = 6.7 Hz, $CONCH_2$), 3.76 (s, 3H, OCH_3), 3.00–2.89 (m, 4H, piperazine), 2.80 (s, 3H, CCH_3), 2.75–2.70 (m, 4H + 2H, piperazine + $CONCH_2CH_2$).
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