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Original article

High affinity and selectivity of [[(arylpiperazinyl)alkyl]thio]thieno[2,3-d]pyrimidinone derivatives for the 5-HT_{1A} receptor. Synthesis and structure–affinity relationships

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Abstract – In this work we report the affinity of new thienopyrimidinones for 5-HT_{1A}Rs and the selectivity versus α_1ARs. The 3-amino-2-[[3-[4-(2-methoxyphenyl)-1-piperazinyl]propyl]thio]-6-ethyl-thieno[2,3-*d*]pyrimidin-4(3*H*)-one **27** is the most potent and selective (Ki 0.19 nM, selectivity 115). Compound **31** with the N4 piperazine orthonitrophenyl nucleus instead of the orthomethoxyphenyl also shows a good affinity and selectivity (Ki 1.46 nM, selectivity 84). The results of derivatives **28**, **29** and **30** (Ki 3.28, 12.59 and 4.38 nM; selectivity 24, 4 and 5, respectively), which have, respectively, an ethyl, an allyl and an acetylamino group instead of an N3 amino group, indicate the importance of this last group for the interaction with 5-HT_{1A}R. Comparison of the results for the superior homologue **53** (Ki 3.72 nM, selectivity 51) and the inferior homologue **52** (5-HT_{1A} Ki 1 499 nM, α_1A Ki NA) of 2-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-6,7-dimethyl-8*H*-[1,3,4]thiadiazolo[3,2-*a*]thieno[2,3-*d*]pyrimidin-8-one **57** (Ki 23 nM, selectivity 5) shows how important the length of the chain binding the two heterocyclic systems is in the interaction with 5-HT_{1A}Rs and α_1ARs. © 2000 Éditions scientifiques et médicales Elsevier SAS

5-HT_{1A} receptor / ligands / thienopyrimidinones

1. Introduction

In a recent paper [1] we described new arylpiperazinylalkylthiothienopyrimidinone derivatives prepared and tested with the specific aim of developing ligands with higher affinity and selectivity for the 5-HT_{1A} receptors rather than the α_1-adrenoreceptors. Selective ligands for the 5-HT_{1A}R versus the α_1AR might help clarify the structural requirements distinguishing the two receptors, which are representative members of the same G protein superfamily, showing some common features in their binding sites. Moreover antianxiety and antidepressant arylpiperazine compounds such as buspirone or NAN-190 show high affinity for the 5-HT_{1A}R but also act on α_1AR, therefore selective 5-HT_{1A}R ligands might help explain the role of 5-HT_{1A}R in anxiety and depression [1]. Radioligand binding assays showed compound **1** as the derivative with the highest affinity and compound **2** as the one with the best selectivity (Ki 0.16 and 3.72 nM; selectivity 39 and 117, respectively) (*figure 1*). The present paper reports the synthesis and in vitro assays for the 5-HT_{1A}R and the α_1AR of new compounds **3** and **4** (*figure 1*), whose structures and structural variations were referred to compound **1**, chosen as the lead.

The aim is that of obtaining compounds with still high affinity and with an improved selectivity for the 5-HT_{1A}R versus the α_1AR and also of acquiring further structure–affinity relationships.

Our interest is mainly addressed to the non-pharmacophoric part because the interaction of the arylpiperazine pharmacophore with the 5-HT_{1A}R is well known [2]. With reference to the type **3** compounds, modifications performed on lead **1** refer in particular to: i) the variation of substituents 5 and 6 of the thienopyrimidine nucleus; the substitution of the thienopyrimidine

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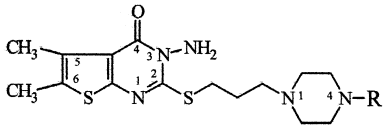
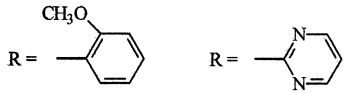
Abbreviations: 5-HT_{1A}Rs: 5-HT_{1A} receptors; α_1ARs: α_1A receptors; SD: standard deviation





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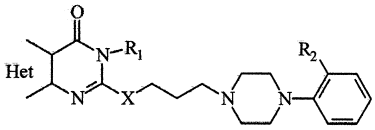

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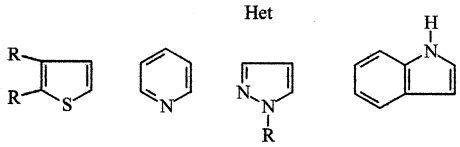
$R_1 = C_2H_5, CH_2CH{=}CH_2, NHCOCH_3, NH_2$;
$R_2 = OCH_3, NO_2$;
X = S, CH_2.



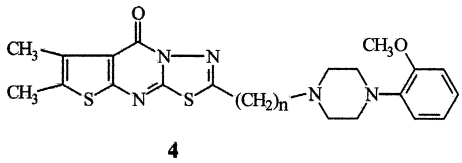


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Figure 1. **1** and **2** are the most interesting compounds, already reported in our previous work [1] and **3** and **4** are the general structures of new compounds (n = 2, is published [1]).

system with other systems such as the pyridopyrimidine, pyrazolopyrimidine and the indolopyrimidine ones; ii) the substitution of the amino group in position 3 of the pyrimidine ring with the ethyl, allyl, and acetylamino groups, to verify and clarify which structural features of the N3 substituents affect the 5-HT_{1A} ligand–receptor interaction and iii) the substitution on the N4 piperazine ring of the orthomethoxyphenyl nucleus with the orthonitrophenyl, bioisoster of the N4 pyrimidine nucleus, present in the most selective compound **2** of the previous work [1]. Moreover, believing that the higher molecule rigidity might privilege structures with higher affinity and selectivity, the influence of the chain length on the thiadiazolothienopyrimidine system (compounds **4**, *figure 1*) was studied.

2. Chemistry

Compounds **6–32** were prepared according to *figure 2*. The potassium salts of the 2-thioxothieno[2,3-*d*]pyrimidine derivatives **13–18** [1, 3, 4] reacted at reflux in ethanol with the appropriate chloroalkyl compounds **23–25** [5] to give the respective 2-(alkylthio)thieno[2,3-

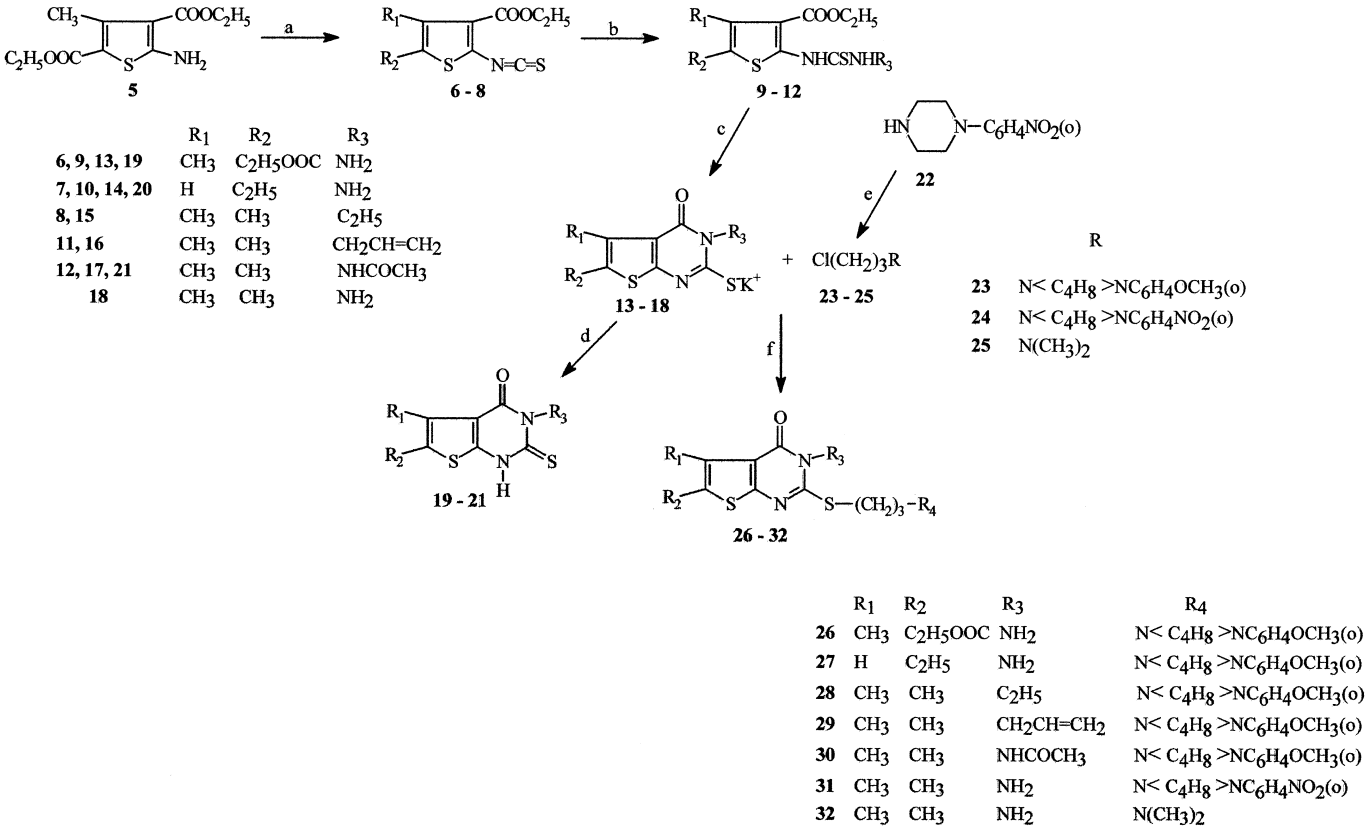


Figure 2. General synthetic procedure for compounds **26–32**. Reagents and conditions: (a) $CSCl_2$, $NaHCO_3$, $CHCl_3/H_2O$; (b) $N_2H_4{\cdot}H_2O$, allylamine or acethydrazine, CH_2Cl_2, r.t.; (c) KOH, EtOH, reflux; (d) HCl, H_2O, r.t.; (e) $Cl(CH_2)_3Br$, K_2CO_3, DMF, r.t.; (f) EtOH, reflux.

d]pyrimidinones **26–32**. The new potassium salts **13**, **14** and **17** were obtained by heating of derivatives **9**, **10** and **12** in an ethanolic potassium hydroxide solution. Compounds **9** and **10** were synthesized from the isothiocyanates **6** and **7** [6] and hydrazine hydrate and compounds **11** and **12** from isothiocyanate **8** and allylamine and acethydrazine, respectively, in dichloromethane at room temperature. Analytical and spectral data of compound **11** were like those of the known compound synthesized with another procedure [3].

Acidification of an aqueous solution of the potassium salts **13**, **14** and **17** gave the thioxo compounds **19–21**, whose structures were substantiated by elemental analyses and IR spectra. Isothiocyanate **6** was obtained by reaction of diethyl ester of 5-amino-3-methyl-2,4-thiophenedicarboxylic acid **5** with thiophosgene in a solution of chloroform/water. The unknown piperazine **24** was obtained by reaction of 1-(2-nitrophenyl)piperazine [7] and 1-bromo-3-chloropropane in dimethylformamide in the presence of potassium carbonate.

The arylpiperazinylalkylthiopyrimidinones **42–45** were prepared by condensation between chloroalkylpiperazine **23** [5] and the monopotassium salts of 3-amino-2-thioxopyrido[2,3-*d*]pyrimidin-4(1*H*)-one **37**, of 5-amino-1,5,6,7-tetrahydro-1-methyl **38** and 5-amino-1,5,6,7-tetrahydro-1-(phenyl-methyl)-6-thioxo-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one **39** [8], and of 3-amino-1,2,3,5-tetrahydro-2-thioxo-4*H*-pyrimido[5,4-*b*]indol-4-one **40** [9] (*figure 3*). The unknown salts **37** and **38** were prepared from isothiocyanates **33** [10] and **35** [11] and hydrazine hydrate in dichloromethane at room temperature, and subsequent treatment with an ethanolic potassium hydroxide solution of the thioxoderivative **34** and of the (hydrazinothioxomethyl)amino derivative **36**. Acidification of an aqueous solution of the salt **38** gave the thioxo

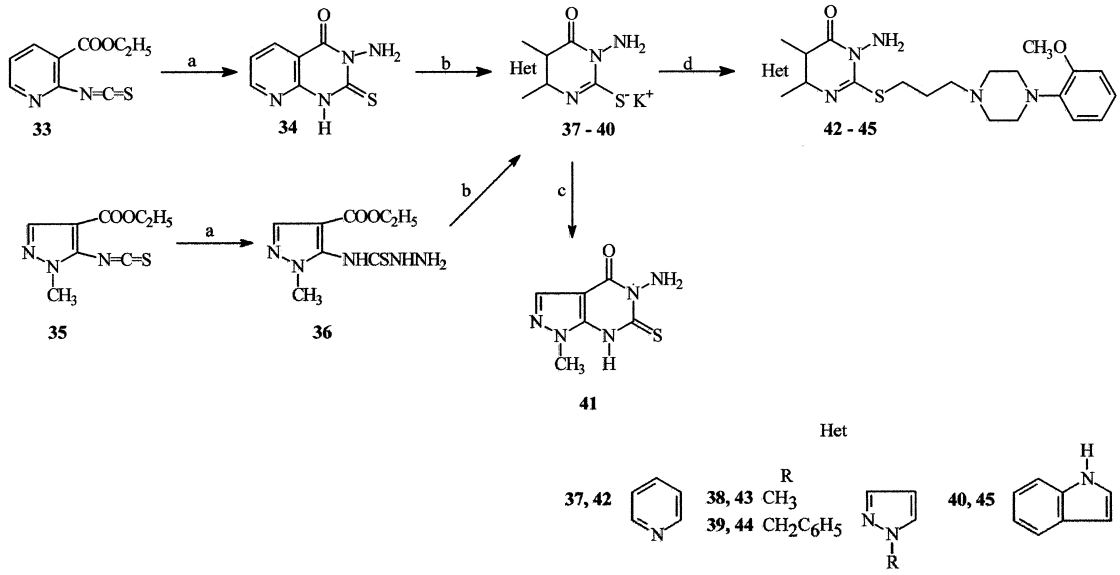


Figure 3. General synthetic procedure for compounds **42**–**45**. Reagents and conditions: (a) $N_2H_4 \cdot H_2O$, CH_2Cl_2, r.t.; (b) KOH, EtOH, reflux; (c) HCl, H_2O, r.t.; (d) 1-(3-chloropropyl)-4-(2-methoxyphenyl)piperazine **23**, EtOH, reflux.

compound **41**, whose structure was substantiated by elemental analysis and IR spectrum (*figure 3*).

Treatment of the amino ester **46** [12] with chlorovaleryl chloride gave the chloroderivative **47**, which reacted with 1-(2-methoxyphenyl)piperazine to give compound **48**; lastly, by reaction between derivative **48** and hydrazine monohydrate at reflux in ethanol was synthesized the alkylpyrimidinone **49** (*figure 4*).

Monopotassium salt **18** with 2-chloroacetylchloride and 4-chlorobutyrylchloride gave the 2-chloromethyl and the 2-chloropropyl-thiadiazolothienopyrimidinones **50** and **51**, respectively, which reacted at 140 °C with 1-(2-methoxyphenyl)piperazine to give compounds **52** and **53** (*figure 5*). The proposed structures of compounds **26**–**32**, **42**–**45**, **49**, **52** and **53** were confirmed by elemental analyses (*tables I* and *II*) and by spectroscopic IR data and ^{1}H-NMR spectra of some representative samples (see the experimental protocols).

3. Pharmacology

The compounds **26–32**, **42–45**, **49**, **52** and **53** were evaluated for in vitro affinity on 5-HT_{1A} and α_1A

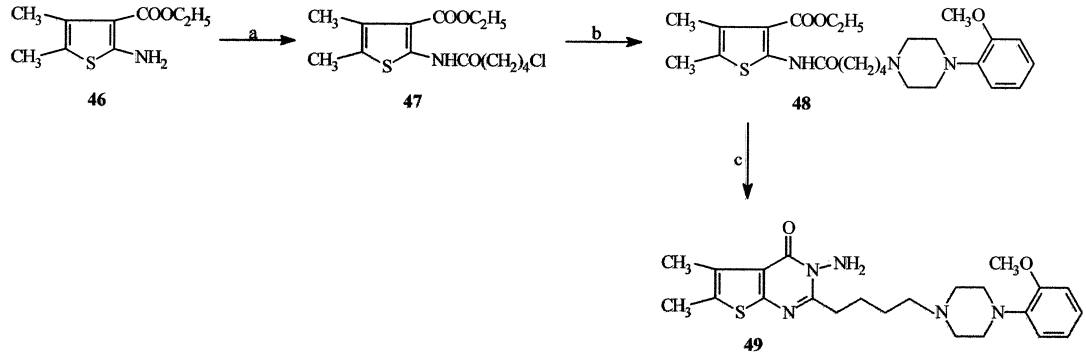


Figure 4. Synthesis of arylpiperazinylalkylthieno[2,3-*d*]pyrimidine **49**. Reagents and conditions: (a) $ClCO(CH_2)_4Cl$, $CHCl_3$, reflux; (b) 1-(2-methoxyphenyl)piperazine, K_2CO_3, DMF, reflux; (c) $N_2H_4 \cdot H_2O$, EtOH, reflux.

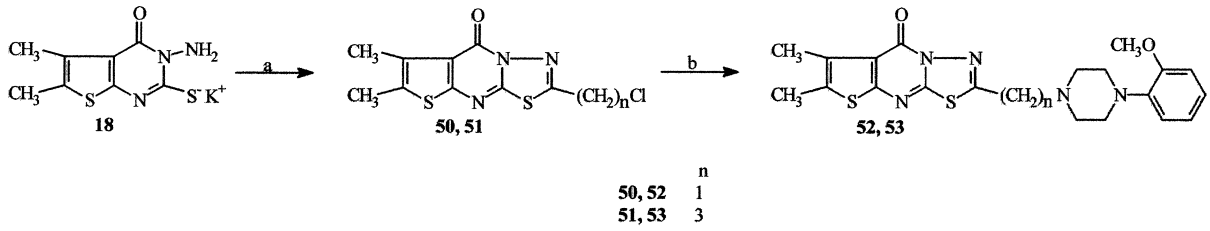


Figure 5. Synthesis of [1,3,4]thiadiazolo[3,2-*a*]thieno[2,3-*d*]pyrimidinones **52** and **53**. Reagents and conditions: (a) $ClCO(CH_2)_nCl$, CH_3SO_3H, P_2O_5; (b) 1-(2-methoxyphenyl)piperazine.

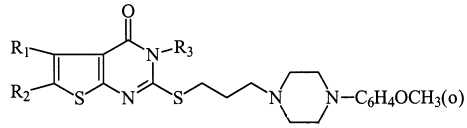
receptors by radioligand binding assays. For comparison data for lead **1** and compounds **2** and **54–58** previously published are included [1]. The results are shown in *tables III* and *IV*.

Table V reports the affinity (Ki) of some structures (**26**, **27**, **31**, **42**, **49** and **53**; representative of those most active on the 5-HT_{1A}R and selective towards the α_1AR, compound **1** was also included for comparison) for other 5-HT receptor subtypes and D_1 and D_2 dopaminergic receptors.

4. Results and discussion

In general, binding assays show that the thienopyrimidine system, with adequate substituents, is the most favourable structure for affinity towards the 5-HT_{1A}Rs, when compared to other systems, except for the pyridopyrimido and the quinazoline [1] moieties. Changes in this system affect affinity and selectivity. In fact, the only compound in the whole **26–32**, **42–45**, **49**, **52** and **53** series with the same affinity of the lead **1** is **27** (Ki 0.19 nM), where a hydrogen and an ethyl replace the two methyls in positions 5 and 6. As for compound **26**, where there is a methyl in position 5 and an ethoxycarbonyl in position 6, the affinity is decreased (Ki 1.36 nM); except for the pyrido derivative **42** (Ki 0.38 nM), the same applies to **43**, **44** and **45**, where the pyrazole and indole heterocycles are used in place of thiophene (Ki 0.82, 2.4 and 3.72 nM). The selectivity of compounds **26**, **42** and especially of compound **27** is better than the lead **1** (selectivity 53, 72, 115 and 39, respectively). These data and that of the quinazoline derivative **58** [1] (Ki 0.27 nM, selectivity 12) show how, in the 5-HT_{1A}R ligand interaction, steric factors play a role on the non-pharmacophoric part condensed with the pyrimidinone moiety rather than

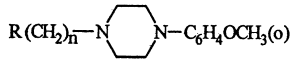
Table I. Physical and chemical properties of compounds **26–32** and **49**.

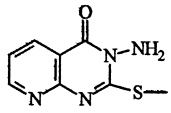
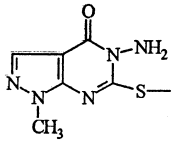
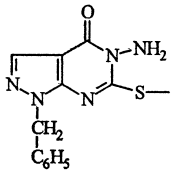
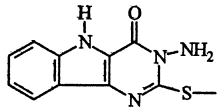
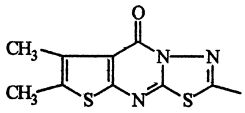
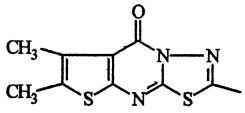


Compound	R_1	R_2	R_3	M.p. °C	Recryst. solv. % yield	Formula
26	Me	$COOC_2H_5$	NH_2	143–144	EtOH 22	$C_{24}H_{31}N_5O_4S_2$
27	H	C_2H_5	NH_2	112–114	EtOH 40	$C_{22}H_{29}N_5O_2S_2$
28	Me	Me	C_2H_5	103–105	cyclohexane 22	$C_{24}H_{32}N_4O_2S_2$
29	Me	Me	$CH_2CH{=}CH_2$	104–105	EtOH 40	$C_{25}H_{32}N_4O_2S_2$
30	Me	Me	$NHCOCH_3$	210–212	EtOH/H_2O 31	$C_{24}H_{31}N_5O_3S_2$
31[a]	Me	Me	NH_2	153–155	EtOH 21	$C_{21}H_{26}N_6O_3S_2$
32[b]	Me	Me	NH_2	108–110	EtOH 24	$C_{13}H_{20}N_4OS_2$
49[c]	Me	Me	NH_2	136–138	EtOH 52	$C_{23}H_{31}N_5O_2S$

[a] The orthomethoxy is replaced by a orthonitro group; [b] The phenylpiperazine system is replaced by an $N(CH_3)_2$ group; [c] The S in the alkyl chain in this case is substituted by a methylene group.

Table II. Physical and chemical properties of compounds **42–45, 52** and **53**.



Compound	R	n	M.p., °C	Recryst. solv. % yield	Formula
42		3	174–176	EtOH 21	$C_{21}H_{26}N_6O_2S$
43		3	155–157	EtOH 39	$C_{20}H_{27}N_7O_2S$
44		3	69–71	EtOH 35	$C_{26}H_{31}N_7O_2S$
45		3	192–193	EtOH/dioxane 40	$C_{24}H_{28}N_6O_2S$
52		1	183–185	EtOH 55	$C_{21}H_{23}N_5O_2S_2$
53		3	144–154	EtOH 59	$C_{23}H_{27}N_5O_2S_2$

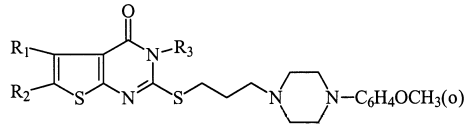
electronic factors, towards which the α_1AR seem to be more sensitive.

The binding tests of compounds **28**–**30**, with the N3 ethyl, allyl and acetylamino groups, indicate the favourable effect of the N3 amino group on the affinity and selectivity towards the 5-HT_{1A}R; these three compounds show less affinity and selectivity (Ki 3.28, 12.59 and 4.38 nM, selectivity 24, 4 and 5, respectively) than the lead **1** and the compound **27**.

Molecular modelling approaches through the utilization of 3-D-QSAR methodologies PARM (Pseudo Atomic Receptor Model), HASL (Hypothetical Active Site Lattice) and CoMFA (Comparative Molecular Field Analysis) upon 23 molecules belonging to the previous work [1], were found to provide predictive 5-HT_{1A} receptor models [13, 14]. The PARM receptor model shows that at the region near the R3 position of the ligands, several negatively charged O and N atoms are present which may act as hydrogen bond acceptors.

A hydrogen bond interaction or even an electrostatic interaction might be supposed, between our N3 substituents and the 5-HT_{1A} receptor site; such an interaction might also be influenced by steric factors (Guccione S. et al., 1999 QSAR, Gordon Research Conference, Tilton, New Hampshire) and is at its maximum level in the amino group, while it decreases in the sequence methyl **55**, ethyl **28**, acetylamino **30**, allyl **29**, hydrogen **54**, and aniline **56** groups (Ki 1.64, 3.28, 4.38, 12.59, 13.14 and 272 nM, selectivity 14, 24, 5, 4, 2.6 and 0.10, respectively). With reference to the α_1AR, the results of these compounds are less explicit; however, an electrostatic interaction at the R3 region seems not as important and

Table III. Affinities of compounds **1**, **2**, **26–32**, **49**, **54–56** and of two reference compounds.



				K (nM)(± SD)[a]		
Compound	R_1	R_2	R_3	5-HT_{1A}[b]	α_1-A[c]	selectivity[d]
1[e]	Me	Me	NH_2	0.16 ± 0.01	6.26 ± 0.85	39
2[e, f]	Me	Me	NH_2	3.72 ± 0.27	434 ± 83	117
26	Me	$COOC_2H_5$	NH_2	1.36 ± 0.2	72.08 ± 21.4	53
27	H	C_2H_5	NH_2	0.19 ± 0.05	21.96 ± 6.16	115
28	Me	Me	C_2H_5	3.28 ± 0.27	79.8 ± 28	24
29	Me	Me	$CH_2CH{=}CH_2$	12.59 ± 7.66	51.48 ± 8.5	4
30	Me	Me	$NHCOCH_3$	4.38 ± 1.09	20.59 ± 5.1	5
31[g]	Me	Me	NH_2	1.46 ± 0.2	123 ± 13	84
32[i]	Me	Me	NH_2	2951 ± 816	NA[h]	/
49[l]	Me	Me	NH_2	0.32 ± 0.02	18.02 ± 4.2	56
54[e]	Me	Me	H	13.14 ± 1.09	34.32 ± 4.29	2.6
55[e]	Me	Me	Me	1.64 ± 0.08	23 ± 4.3	14
56[e]	Me	Me	NHC_6H_5	272 ± 9.3	27 ± 4.3	0.10
Serotonin				1.47 ± 0.1		
Prazosin					3.86 ± 0.68	

[a] Ki values (nM) are followed by SD. [b] Affinity at [^{3}H]-8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT)-labelled 5-HT_{1A} sites. [c] Affinity at [^{3}H]prazosin-labelled α_1-adrenergic sites. [d] Selectivity for 5-HT_{1A} over α_1-adrenergic sites: calculated as Ki (α_1A)/Ki (5-HT_{1A}). [e] Previously published data [1], here reported for comparison as Ki. [f] The orthomethoxyphenyl is replaced by a pyrimidine nucleus. [g] The orthomethoxy is replaced by an orthonitro group. [h] < 50% inhibition at 10^{-5} M. [i] The phenylpiperazine system is replaced by an $N(CH_3)_2$ group. [l] The S in the alkyl chain is substituted by a methylene group.

the steric features of the N3 substituents are less conditioning, as indicated in the α_1AR 3-D-QSAR models [13, 14].

Compound **31**, which has the N4 orthonitrophenyl nucleus in place of the orthomethoxyphenyl, shows an affinity towards the 5-HT_{1A}R lower than that of lead, but a good increase in selectivity (Ki 1.46 nM, selectivity 84). Instead, the few data known in the literature [15, 16] report that the *ortho* substitutions in the N4 phenyl ring by electron withdrawing groups are favourable to both receptors. In our previous study [1], the most selective compound was the N4 pyrimido derivative **2** (Ki 3.72 nM, selectivity 117). These results suggest that, in such structures, the steric and/or electronic properties of the N4 piperazine substituent may affect the interaction of the ligand, especially with α_1AR.

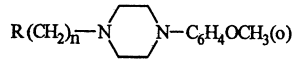
In compound **32**, the substitution of the arylpiperazine nucleus with the dimethylamine group leads to a loss of affinity towards both receptors (5-HT_{1A} Ki 2 951 nM and α_1A Ki NA). This confirms that the arylpiperazine nucleus is the fundamental pharmacophore.

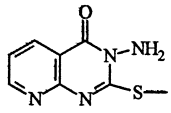
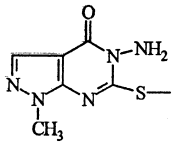
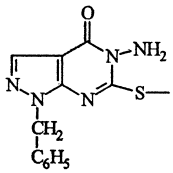
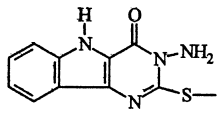
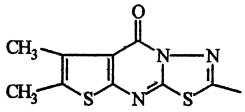
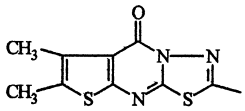
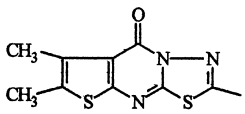
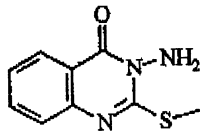
The only substitution in the thioalkyl chain of sulphur with a methylene is not influential; in fact, compound **49** shows a very small decrease in affinity and a slight improvement in selectivity (Ki 0.32 nM; selectivity 56).

In the arylpiperazinylalkylthiadiazolothienopyrimidinone series, the compound **53** shows a greater affinity and selectivity towards the 5-HT_{1A}R (Ki 3.72 nM, selectivity 51) than the inferior homologue **57** (Ki 23 nM; selectivity 5); compound **52**, with only one methylene, has a significant fall in affinity for both the 5-HT_{1A}R (Ki 1 499 nM) and the α_1AR (Ki NA). Consequently, it seems that in these compounds, a chain length of three carbon atoms is optimum for 5-HT_{1A}R affinity and also for selectivity. These three products display not only a lower, but also a less differentiated affinity for α_1AR. In fact, the affinity for the 5-HT_{1A}R as a function of the methylene linker length decreases in the order $3 > 2 >> 1$, while for the α_1AR, the affinity decreases in the order $2 = 3 >> 1$. Moreover these data confirm that the presence of the N3 amino group improves the interaction with the 5-HT_{1A}R binding site.

Compounds **26**, **27**, **31**, **42**, **49** and **53** show good selectivity towards 5-HT_{1B}, 5-HT_{2A}, 5-HT_{2C}, 5-HT_3 and D_1 receptors, with the exception of **53** which has an affinity for the 5-HT_{1B} and the 5-HT_{2A} receptors 24 and

Table IV. Affinities of compounds **42–45**, **52**, **53**, **57**, and **58**.



Compound	R	n	Ki (nM) (±SD)		
			5–HT_{1A}	α_1–A	selectivity
42		3	0.38 ± 0.07	27.28 ± 2.5	72
43		3	0.82 ± 0.07	16.9 ± 4.29	21
44		3	2.4 ± 0.16	11.67 ± 2.83	5
45		3	3.72 ± 0.38	58.95 ± 11.9	16
52		1	1 499 ± 365	NA[a]	/
53		3	3.72 ± 0.2	189 ± 28	51
57[b]		2	23 ± 3.3	107 ± 35	5
58[b]		3	0.27 ± 0.02	3.4 ± 0.7	12

[a] < 50% inhibition at 10^{-5} M. [b] Previously published data [1], here reported for comparison as Ki.

50 times lower, respectively, than for the 5-HT_{1A}R. Compounds **26**, **31**, **49** and **53** show the worst selectivity for the D_2 receptor with an affinity about 8–50 times lower than for 5-HT_{1A}R.

In conclusion, the continuation of our researches on new potent and selective arylpiperazinylalkylthienopirimidinone derivatives for the 5-HT_{1A}R rather than the α_1AR, gives further information about the structure–affinity relationships. The results indicate the importance of the non-pharmacophoric thienopyrimidine portion for affinity and selectivity towards 5-HT_{1A}R. The highest affinity and selectivity is obtained with derivative **27**, structurally similar to the lead **1**, followed by the isosteric pyridopyrimidinone **42**; these compounds also

Table V. Ki (nM) values in binding tests of compounds **1**, **26**, **27**, **31**, **42**, **49** and **53** on 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, 5-HT_{2C} and 5-HT_3 receptors and on D_1 and D_2 receptors.

Compound	5-HT_{1A}	5-HT_{1B}	5-HT_{2A}	5-HT_{2C}	5-HT_3	D_1	D_2
1[a]	0.16	385 (2 406)[b]	170 (1 062)	405 (2 531)	400 (2 500)	35 (219)	27 (169)
26	1.36	316 (232)	299 (220)	1 587 (1 167)	NA[c]	1 397 (1 027)	49 (36)
27	0.19	720 (3 789)	239 (1 258)	1 787 (9 405)	257 (1 353)	296 (1 558)	56 (295)
31	1.46	4 020 (1 472)	219 (80)	831 (304)	2 278 (834)	456 (167)	105 (38)
42	0.38	1 648 (4 337)	460 (1 210)	300 (789)	NA[c]	1 175 (3 092)	115 (303)
49	0.32	949 (2 966)	200 (625)	1 373 (4 291)	NA[c]	978 (3 056)	19 (59)
53	3.72	188[d](24)	382 (50)	5 427[d] (708)	NA[c]	2 591 (338)	63 (8)

[a] Previously published data [1], here reported for comparison as Ki. [b] Values in parentheses are the selectivity for the 5-HT_{1A} receptor vs. the other serotonin and dopamine receptors calculated as Ki (x)/Ki (5-HT_{1A}). [c] < 50% inhibition at 10^{-5} M. For the sake of clarity SD are omitted since they were always less than 10%. [c, d] The dose–response curve was better fitted by a two-component model, with the following results: [c] site I, Ki 49 nM; site II, Ki > 10 000 nM, proportion site II 34%; [d] site I, Ki 628 nM; site II, Ki > 10 000 nM, proportion site II 49%.

show a remarkable selectivity for the 5-HT_{1B}, 5-HT_{2A}, 5-HT_{2C}, 5-HT_3, D_1 and D_2 receptors. The N4 orthonitrophenyl derivative **31** shows a good selectivity for 5-HT_{1A}R over the α_1AR.

The presence in the compounds **28**, **29** and **30** of an ethyl, an allyl and an acetylamino group, instead of the N3 amino group, causes a decrease in both affinity and selectivity, showing the importance of the amino group for the interaction with the 5-HT_{1A} receptor binding site.

The binding data of the thienopyrimidothiadiazole derivatives **52**, **53** and **57** show that the chain length in these structures affects the affinity for both receptors with a diversifying modulation, leading to interesting results.

In order to determine the most critical features to design selective and potent ligands which can improve and provide the 5-HT_{1A} receptor model, non-ambiguous and distinct from the α_1A receptor one, conformational analysis and 3-D-QSAR studies about more significant compounds of this series are in progress.

5. Experimental protocols

5.1. Chemistry

Melting points were determined in open capillary tubes on a Gallenkamp melting point apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 281 spectrometer in KBr (potassium bromide) disks. ^{1}H-NMR spectra were obtained at 200 MHz on a Varian Inova Unity 200 spectrometer in DMSO-d_6 or $CDCl_3$ solution. Chemical shifts (δ) are reported in parts per million relative to tetramethylsilane (TMS) as an internal standard; coupling constants (*J*) are in hertz. Signal multiplicities are presented by s (singlet), d (doublet), t (triplet), q (quartet), br s (broad singlet), and m (multiplet). Elemental analyses for C, H, N, and S were obtained on an EA1108 elemental analyzer Fisons-Carlo Erba instrument and were within ± 0.4% of the theoretical values. The purity of the compounds was checked by thin-layer chromatography (TLC) on Merck silica gel 60 F-254 plates.

5.1.1. 5-Isothiocyanato-3-methyl-2,4-thiophenedicarboxylic acid diethyl ester **6**

A solution of amino ester **5** (12.85 g, 50 mmol) in chloroform (100 mL) was added under stirring to a mixture of thiophosgene (3.8 mL, 50 mmol), sodium hydrogencarbonate (6 g, 71.4 mmol), water (50 mL), and chloroform (200 mL) for 40 min. The mixture was stirred at room temperature for 1.3 h, then the organic layer was separated and washed twice with water. The organic layer was dried over anhydrous sodium sulfate and evaporated under reduced pressure. The solid was purified by column chromatography (silica gel, cyclohexane/ethyl acetate 80:20) to give **6** (6 g, 40%); m.p. 76–78 °C; IR (KBr) 2 120 (N=C=S), 1 730 (C=O) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 1.26–1.39 (m, 6H, CH_2*CH_3*), 2.64 (s, 3H, CH_3), 4.20–4.37 (m, 4H, *CH_2*CH_3). Anal. ($C_{12}H_{13}NO_4S_2$) C, H, N, S.

5.1.2. 5-[(Hydrazinothioxomethyl)amino]-3-methyl-2,4-thiophenedicarboxylic acid diethyl ester **9**

A solution of isothiocyanate **6** (2.8 g, 9.36 mmol) in dichloromethane (30 mL) was added under stirring to a solution of hydrazine monohydrate (0.47 mL, 9.4 mmol) in dichloromethane (20 mL). The suspension obtained was stirred at room temperature for 1 h, and then the solid was collected by filtration and washed with dichloromethane and ethanol. Recrystallization from ethanol/dioxane gave **9** (0.9 g, 29%); m.p. 210–212 °C (dec); IR (KBr) 3 360 and 3 210 (NH), 1 715 and 1 660 (C=O) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ 1.25–1.37 (m, 6H, CH_2*CH_3*), 2.68 (s, 3H, CH_3), 4.20–4.39 (m, 4H, *CH_2*CH_3),

7.83 (br s, 2H, NH), 9.53 (br s, 1H, NH), 10.07 (s, 1H, NH). Anal. ($C_{12}H_{17}N_3O_4S_2$) C, H, N, S.

5.1.3. 2-[(Hydrazinothioxomethyl)amino]-5-ethyl-3-thiophenecarboxylic acid ethyl ester **10**

Compound **10** was prepared in the same manner of **9** from isothiocyanate **7** and hydrazine monohydrate. Recrystallized from ethanol, yield: 1.2 g, 47%; m.p. 198–199 °C; IR (KBr) 3 360, 3 200 and 3 140 (NH), 1 665 (C=O) cm^{-1}. Anal. ($C_{10}H_{15}N_3O_2S_2$) C, H, N, S.

5.1.4. 2-[[(2-Propenylamino)thioxomethyl]amino]-4,5-dimethyl-3-thiophenecarboxylic acid ethyl ester **11**

Compound **11** was prepared in the same manner of **9** from isothiocyanate **8** and allylamine. Recrystallized from ethanol, yield: 0.9 g, 32%; m.p. 132–134 °C; IR (KBr) 3 210 (NH), 1 660 (C=O) cm^{-1}. Anal. ($C_{13}H_{18}N_2O_2S_2$) C, H, N, S.

5.1.5. 2-[[(Acetylamino)thioxomethyl]amino]-4,5-dimethyl-3-thiophenecarboxylic acid ethyl ester **12**

Compound **12** was prepared from isothiocyanate **8** and acethydrazine in the same condition of **9**. The solution was stirred at room temperature for 1 h, then the solvent was removed under reduced pressure, the solid was collected with a small amount of ethanol and dried. Recrystallized from ethanol, yield: 1.21 g, 41%; m.p. 199–201 °C; IR (KBr) 3 340 and 3 160 (NH), 1 660 broad (C=O) cm^{-1}. Anal. ($C_{12}H_{17}N_3O_3S_2$) C, H, N, S.

5.1.6. Monopotassium salt of 3-amino-6-carbethoxy-2,3-dihydro-5-methyl-2-thioxothieno[2,3-d]-pyrimidin-4(1H)-one **13** *and its 2-thioxo derivative* **19**

To a solution of potassium hydroxide (0.76 g, 13.5 mmol) in absolute ethanol (55 mL) compound **9** (4.47 g, 13.5 mmol) was added, and the mixture was refluxed under stirring for 1 h. The suspension was filtered while hot and the solid washed with hot absolute ethanol to give **13** (3.6 g, 82%). A suspension of potassium salt **13** (1.08 g, 3.34 mmol) in water (50 mL) was acidified with concentrated hydrochloric acid and stirred at room temperature for 30 min. The solid was collected by filtration and washed with water. Recrystallization from ethanol gave **19** (0.4 g, 42%); m.p. 234–236 °C (dec); IR (KBr) 3 240 (NH), 1 705 and 1 640 (C=O) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ 1.28 (t, J = 7.2 Hz, 3H, CH_2CH_3), 2.74 (s, 3H, CH_3), 4.27 (q, J = 7.2 Hz, 2H, CH_2CH_3). Anal. ($C_{10}H_{11}N_3O_3S_2$) C, H, N, S.

5.1.7. Monopotassium salt of 3-amino-2,3-dihydro-6-ethyl-2-thioxothieno[2,3-d]-pyrimidin-4(1H)-one **14** *and its 2-thioxo derivative* **20**

These compounds were obtained like compounds **13** and **19**. **14**: yield: 3.1 g, 86%. **20**: recrystallized from ethanol, yield: 0.4 g, 61%; m.p. 231–233 °C (dec); IR (KBr) 3 320 and 3 160 (NH), 1 650 (C=O) cm^{-1}. Anal. ($C_8H_9N_3OS_2$) C, H, N, S.

5.1.8. Monopotassium salt of 3-acetylamino-2,3-dihydro-5,6-dimethyl-2-thioxothieno[2,3-d]-pyrimidin-4(1H)-one **17** *and its 2-thioxo derivative* **21**

These compounds were obtained like compounds **13** and **19**. **17**: yield: 1.44 g, 34%. **21**: recrystallized from ethanol, yield: 0.35 g, 46%; m.p. > 275 °C (dec); IR (KBr) 3 220 and 3 200 (NH), 1 725 and 1 670 (C=O) cm^{-1}. Anal. ($C_{10}H_{11}N_3O_2S_2$) C, H, N, S.

5.1.9. 1-(3-Chloropropyl)-4-(2-nitrophenyl)piperazine **24**

A mixture of 1-(2-nitrophenyl)piperazine **22** (1.54 g, 7.44 mmol), 1-bromo-3-chloropropane (0.9 mL, 9.1 mmol) and of potassium carbonate (1.23 g, 8.9 mmol) was stirred at room temperature for 22 h in dimethylformamide (10 mL). The residue was removed by filtration and the solvent evaporated under reduced pressure to yield **24** (1 g, 47%) as a yellow oil, that was purified by column chromatography (silica gel, ethyl acetate). An analytical sample of the free base was obtained as hydrochloride salt with HCl-saturated methanol, dilution with diethyl ether and recrystallization from absolute ethanol; m.p. 197–199 °C (dec). ^{1}H-NMR ($CDCl_3$) δ 2.50 (m, 2H, $CH_2CH_2CH_2$), 3.05–3.25 (m, 6H, CH_2N and piperazine H), 3.55–3.85 (m, 6H, piperazine H and CH_2Cl), 7.18–7.84 (m, 4H, ArH), 12.97 (br s, 1H, NH^+). Anal. ($C_{13}H_{18}ClN_3O_2 \cdot HCl \cdot 0.5\ H_2O$) C, H, N.

5.1.10. General procedure for compounds **26–32**

A mixture of potassium salts **13–18** (3.6 mmol) and 1-(3-chloropropyl)-4-(2-methoxy or 2-nitrophenyl)-piperazine or 3-dimethylamino-propylchloride hydrochloride **23–25** (4.32 mmol) was stirred at reflux for 6 h in ethanol (15 mL). After the suspension was cooled, the residue was collected, the solvent was removed under reduced pressure, and the solid collected with a small amount of ethanol and recrystallized. For product **28** the solid was collected with diethyl ether, compound **29**, after the suspension was cooled, was obtained as a solid. For compounds **30** and **32**, after cooling, was obtained a solution, that gave the products by dilution with water, for **32** the refluxing time was 2 h (*figure 2* and *table I*). The IR and ^{1}H-NMR spectra of the following compound are reported as a representative sample.

5.1.10.1. 5-Amino-4-oxo-6-[[3-[4-(2-methoxyphenyl)-1-piperazinyl]propyl]thio]-3-methyl-3H-thieno[2,3-d]pyrimidin-2-carboxylic acid ethyl ester **26**

IR (KBr) 3 330 and 3 280 (NH), 1 720 and 1 685 (C=O) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ 1.22 (t, *J* = 7.2 Hz, 3H, CH_2CH_3), 1.78 (m, 2H, $CH_2CH_2CH_2$), 2.38 (t, *J* = 6.6 Hz, 2H, CH_2N), 2.45 (m, 4H, piperazine H), 2.74 (s, 3H, CH_3), 2.92 (m, 4H, piperazine H), 3.01 (t, *J* = 6.6 Hz, 2H, SCH_2), 3.70 (s, 3H, OCH_3), 4.21 (q, *J* = 7.2 Hz, 2H, CH_2CH_3), 5.68 (s, 2H, NH_2, exchanges with D_2O), 6.80–6.86 (m, 4H, ArH).

5.1.11. 3-Amino-2,3-dihydro-2-thioxopyrido[2,3-d]pyrimidin-4(1H)-one **34**

To a solution of hydrazine monohydrate (0.53 mL, 10.6 mmol) in dichloromethane (20 mL) was added a solution of isothiocyanate **33** (2.22 g, 10.67 mmol) in dichloromethane (20 mL). The mixture was stirred at room temperature for 1 h, then the solid was collected and washed with ethanol. Recrystallization from ethanol/dioxane gave **34** (1.2 g, 58%); m.p. > 310 °C; IR (KBr) 3 300 and 3 200 (NH), 1 715 (C=O) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ 6.38 (s, 2H, NH_2), 7.40 (dd, *J* = 4.8, 7.8 Hz, 1H, ArH), 8.37 (d, *J* = 7.8 Hz, 1H, ArH), 8.73 (d, *J* = 4.8 Hz, 1H, ArH), 13.60 (s, 1H, NH). Anal. ($C_7H_6N_4OS$) C, H, N, S.

5.1.12. 5-[(Hydrazinothioxomethyl)amino]-1-methyl-1H-pyrazolo-4-carboxylic acid ethyl ester **36**

A solution of isothiocyanate **35** (1 g, 4.73 mmol) in dichloromethane (20 mL) was added under stirring to a solution of hydrazine monohydrate (0.25 mL, 5 mmol) in dichloromethane (10 mL). The suspension was stirred at room temperature for 30 min, then the solid was collected by filtration, washed with dichloromethane and ethanol. Recrystallization from ethanol gave **36** (0.3 g, 26%); m.p. 191–193 °C (dec); IR (KBr) 3 260 and 3 170 (NH), 1 710 (C=O) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ 1.23 (t, *J* = 7 Hz, 3H, CH_2CH_3), 3.65 (s, 3H, CH_3), 4.14 (q, *J* = 7 Hz, 2H, CH_2CH_3), 6.43 (br s, 3H, NH), 7.77 (s, 1H, pyrazole H), 9.61 (s, 1H, NH). Anal. ($C_8H_{13}N_5O_2S$) C, H, N, S.

5.1.13. Monopotassium salt of 5-amino-1,2,3,5-tetrahydro-1-methyl-6-thioxopyrazolo[3,4-d]-pyrimidin-4(4H)-one **38** *and its 6-thioxo derivative* **41**

Compound **36** (1.65 g, 6.79 mmol) was added to a solution of potassium hydroxide (0.38 g, 6.79 mmol) in absolute ethanol (28 mL)), and the mixture was refluxed under stirring for 1 h. The suspension was filtered while hot and the solid washed with hot absolute ethanol to give **38** (1.4 g, 87%). A suspension of potassium salt **38** (0.5 g, 2.11 mmol) in water (50 mL) was acidified with concentrated hydrochloric acid and stirred at room temperature for 30 min. The solid was collected by filtration and washed with water. Recrystallization from dioxane gave **41** (0.15 g, 36%); m.p. 262–264 °C (dec); IR (KBr) 3 290 and 3 190 (NH), 1 705 (C=O) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ 3.88 (s, 3H, CH_3), 7.99 (s, 1H, pyrazole H). Anal. ($C_6H_7N_5OS$) C, H, N, S.

5.1.14. 3-Amino-2-[[3-[4-(2-methoxyphenyl)-1-piperazinyl]propyl]thio]pyrido[2,3-d]pyrimidin-4(3H)-one **42**

To a solution of potassium hydroxide (0.44 g, 7.9 mmol) in absolute ethanol (32 mL) the 2-thioxoderivative **34** (1.54 g, 7.9 mmol) was added and the mixture was refluxed for 30 min. Then to the hot suspension of potassium salt **37**, 1-(3-chloropropyl)-4-(2-methoxyphenyl)piperazine **23** (2.55 g, 9.5 mmol) was added and the mixture was refluxed for 6 h. The solution was cooled, the solvent was removed under reduced pressure, the solid collected, and washed with a small amount of ethanol (*table II*): IR (KBr) 3 320 and 3 200 (NH), 1 695 (C=O) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ 1.84 (m, 2H, $CH_2CH_2CH_2$), 2.43 (m, 6H, CH_2N and piperazine H), 2.91 (m, 4H, piperazine H), 3.07 (t, *J* = 6.8 Hz, 2H, SCH_2), 3.70 (s, 3H, OCH_3), 5.74 (s, 2H, NH_2), 6.80–6.88 (m, 4H, ArH), 7.38 (dd, *J* = 4.6, 8 Hz, 1H, ArH), 8.40 (d, *J* = 8 Hz, 1H, ArH), 8.81 (d, *J* = 4.6 Hz, 1H, ArH).

5.1.15. General procedure for compounds **43–45**

A mixture of potassium salts **38–40** (3.72 mmol) and 1-(3-chloropropyl)-4-(2-methoxyphenyl)piperazine **23** (3.72 mmol) was stirred at reflux for 6 h in ethanol (15 mL). After the suspension was cooled, the residue was eliminated by filtration, from the solution by dilution with water was obtained the desired product **43**, that was collected by filtration and recrystallized. For **44**, after the suspension was cooled, the residue was removed by filtration and the desired product crystallized from the solution, while **45** was obtained as a solid, from the cooled suspension, collected and recrystallized (*figure 3* and *table II*). The IR and ^{1}H-NMR spectra of the following compound are reported as a representative sample.

5.1.15.1. 5-Amino-6-[[3-[4-(2-methoxyphenyl)-1-piperazinyl]propyl]thio]-1-methyl-pyrazolo[3,4-d]pyrimidin-4(3H)-one **43**

IR (KBr) 3 310 and 3 210 (NH), 1 690 (C=O) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ 1.83 (m, 2H, $CH_2CH_2CH_2$), 2.41 (m, 6H, CH_2N and piperazine H), 2.89 (m, 4H, piperazine H), 3.02 (t, *J* = 6.8 Hz, 2H, SCH_2), 3.69 (s, 3H, OCH_3), 3.80 (s, 3H, NCH_3), 5.58 (s, 2H, NH_2), 6.79–6.88 (m, 4H, ArH), 7.94 (s, 1H, pyrazole H).

5.1.16. 2-[(Chloropentanoyl)amino]-4,5-dimethyl-3-thiophenecarboxylic acid ethyl ester **47**

5-Chlorovalerylchloride (1.3 mL, 10.05 mmol) was added to a solution of amino ester **46** (2 g, 10.05 mmol) in chloroform (20 mL) and the solution was refluxed for 4 h. After cooling, the solution was concentrated under reduced pressure to obtain a dark oil, that, by addition of a small amount of water and ethanol, yielded a solid that was collected and washed with diethyl ether. Recrystallization from ethanol gave **47** (1.1 g, 34%); m.p. 82–84 °C; IR (KBr) 3 250 (NH), 1 660 (C=O) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ 1.25 (t, J = 7.2 Hz, 3H, CH_2CH_3),1.67 (m, 4H, $CH_2CH_2CH_2CH_2$), 2.11 (s, 3H, CH_3), 2.14 (s, 3H, CH_3), 2.47 (t, J = 7 Hz, 2H, $CH_2CH_2CH_2CH_2$), 3.59 (t, J = 6.4 Hz, 2H, CH_2Cl), 4.22 (q, J = 7.2 Hz, 2H, CH_2CH_3), 10.87 (s, 1H, NHCO). Anal. ($C_{14}H_{20}ClNO_3S$) C, H, N, S.

5.1.17. 2-[4-[4-(2-Methoxyphenyl)-1-piperazinyl]pentanoylamino]-4,5-dimethyl-3-thiophenecarboxylic acid ethyl ester **48**

A mixture of derivative **47** (1.24 g, 3.9 mmol), of 1-(2-methoxyphenyl)piperazine (0.75 g, 3.9 mmol), and of potassium carbonate (0.54 g, 3.9 mmol) was refluxed under stirring for 2 h. After cooling, the suspension was filtered and the solution was extracted with chloroform and washed with water. The organic layers were dried over anhydrous sodium sulfate and evaporated under reduced pressure. Recrystallization from ethanol yielded **48** (0.8 g, 43%); m.p. 101–103 °C; IR (KBr) 3 250 (NH), 1 650 and 1 690 (C=O) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ 1.24 (t, J = 7 Hz, 3H, CH_2CH_3), 1.51 (m, 4H, $CH_2CH_2CH_2CH_2$), 2.12 (s, 3H, CH_3), 2.15 (s, 3H, CH_3), 2.26 (t, J = 6.8 Hz, 2H, $COCH_2$), 2.43 (m, 6H, CH_2N and piperazine H), 2.85 (m, 4H, piperazine H), 3.69 (s, 3H, OCH_3), 4.21 (q, J = 7 Hz, 2H, CH_2CH_3), 6.76–6.88 (m, 4H, ArH), 10.90 (s, 1H, NHCO). Anal. ($C_{25}H_{35}N_3O_4S$) C, H, N, S.

5.1.18. 3-Amino-2-[4-[4-(2-methoxyphenyl)-1-piperazinyl]butyl]-5,6-dimethyl-thieno[2,3-d]pyrimidin-4(3H)-one **49**

A mixture of derivative **48** (1 g, 2.1 mmol) and of hydrazine hydrate (4 mL, 80 mmol) was refluxed for 12 h in ethanol (10 mL). After cooling, the product was collected and washed with ethanol (*table II*): IR (KBr) 3 280 and 3 110 (NH), 1 670 (C=O) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ 1.48 (m, 2H, CH_2), 1.68 (m, 2H, CH_2), 2.27 (s, 3H, CH_3), 2.32 (s, 3H, CH_3), 2.43 (m, 6H, CH_2N and piperazine H), 2.85 (m, 6H, CH_2 and piperazine H), 3.69 (s, 3H, OCH_3), 5.66 (s, 2H, NH_2), 6.77–6.85 (m, 4H, ArH).

5.1.19. 2-(1-Chloromethyl)-6,7-dimethyl-8H-[1,3,4]thiadiazolo[3,2-a]thieno[2,3-d]pyrimidin-8-one **50**

To a mixture of potassium salt **18** (1.5 g, 5.66 mmol), P_2O_5 (0.80 g, 5.66 mmol), and CH_3SO_3H (1.83 mL) was added 2-chloroacetylchloride (0.60 mL, 7.43 mmol), and the mixture was stirred for 4 h at 80–90 °C. The cooled reaction mixture was poured into cold water and neutralized with a 10% NaOH solution. The product was filtered and washed with water. Recrystallization from ethanol/dioxane gave **50** (0.5 g, 31%); m.p. 229–231 °C; IR (KBr) 1 690 (C=O) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ 2.36 (s, 3H, CH_3), 2.42 (s, 3H, CH_3), 5.24 (s, 2H, CH_2Cl). Anal. ($C_{10}H_8ClN_3OS_2$) C, H, N, S.

5.1.20. 2-(3-Chloropropyl)-6,7-dimethyl-8H-[1,3,4]thiadiazolo[3,2-a]thieno[2,3-d]pyrimidin-8-one **51**

Compound **51** was prepared in the same condition of **50** from potassium salt **18** and 4-chlorobutyryl chloride. Recrystallized from ethanol, yield: 0.6 g, 34%; m.p. 161–163 °C; IR (KBr) 1 690 (C=O) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ 2.22 (m, 2H, $CH_2CH_2CH_2$), 2.35 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 3.17 (t, J = 7.2 Hz, 2H, CH_2), 3.77 (t, J = 6.4 Hz, 2H, CH_2Cl). Anal. ($C_{12}H_{12}ClN_3OS_2$) C, H, N, S.

5.1.21. 2-[2-[4-(2-Methoxyphenyl)-1-piperazinyl]methyl]-6,7-dimethyl-8H-[1,3,4]-thiadiazolo-[3,2-a]thieno[2,3-d]pyrimidin-8-one **52**

A mixture of the thiadiazolo derivative **50** (1 g, 3.5 mmol) and of 1-(2-methoxyphenyl)piperazine (3.36 g, 17.5 mmol) was heated at 140 °C on an oil bath for 2 h. The melted residue was collected with warm ethanol and recrystallized (*table II*): IR (KBr) 1 685 (C=O) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ 2.36 (s, 3H, CH_3), 2.42 (s, 3H, CH_3), 2.76 (m, 4H, piperazine H), 3.01 (m, 4H, piperazine H), 3.77 (s, 3H, OCH_3), 3.97 (s, 2H, CH_2), 6.87–6.96 (m, 4H, ArH).

5.1.22. 2-[3-[4-(2-Methoxyphenyl)-1-piperazinyl]propyl]-6,7-dimethyl-8H-[1,3,4]-thiadiazolo-[3,2-a]thieno[2,3-d]pyrimidin-8-one **53**

Compound **53** was prepared from thiadiazolo derivative **51** in the same manner as compound **52** (*table II*). IR (KBr) 1 680 (C=O) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ 1.86 (m, 2H, $CH_2CH_2CH_2$), 2.29 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 2.43 (m, 6H, CH_2N and piperazine H), 2.83 (m, 4H, piperazine H), 3.00 (t, J = 7.2 Hz, 2H, $CH_2CH_2CH_2$), 3.68 (s, 3H, OCH_3), 6.72–6.87 (m, 4H, ArH).

5.2. Pharmacology

5.2.1. In vitro experiments

Binding assays were performed on male CRL:CD(SD)BR-COBS rats weighing about 150 g. The animals were killed by decapitation, and their brains were rapidly dissected (hippocampus for 5-HT_{1A}; striatum for 5-HT_{1B}, D_1 and D_2; cortex for 5-HT_{2A}, 5-HT_3 and α_1-adrenergic receptors), frozen, and stored at –80 °C until the day of assay. Pig brains were obtained from a local slaughterhouse, and the cortex (for 5-HT_{2C}) was rapidly removed and stored at –80 °C until assay.

Tissue was homogenized in about 50 volumes of ice-cold 50 mM Tris-HCl buffer (pH 7.4) (or 50 mM Hepes-HCl (pH 7.5) for 5-HT_3 receptors) using an Ultra Turrax TP-1810 (2 × 20 s) and centrifuged at 50 000 *g* for 10 min (Beckman model J-21B refrigerated centrifuge). The pellet was resuspended in the same volume of fresh buffer, incubated at 37 °C for 10 min and centrifuged again at 50 000 *g* for 10 min. The pellet was then washed once by resuspension in fresh buffer and centrifuged as before. The pellet was then resuspended in the appropriate incubation buffer (50 mM Tris-HCl (pH 7.7) for 5-HT_{2A} receptors, with the addition of 10 μM pargyline for the other receptors containing 4 mM $CaCl_2$ for 5-HT_{1A} receptors, 0.1% ascorbic acid for α_1-adrenergic receptors, 4 mM $CaCl_2$ and 0.1% ascorbic acid for 5-HT_{1B} and 5-HT_{2C} receptors; 50 mM Hepes-HCl pH 7.4 containing 10 μM pargyline for 5-HT_3 receptors, 50 mM Tris-HCl pH 7.1 containing 10 μM pargyline, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$ and 0.1% ascorbic acid for D_1 and D_2 receptors) just before the binding assay.

Binding assays were done as previously described [17]. Briefly, the following incubation conditions were used. 5-HT_{1A}: [^{3}H]-8-OH-DPAT (sp act. 157 Ci/mmol, NEN) final concentration 1 nM, 30 min 25 °C (non-specific binding: 5-HT 10 μM). 5-HT_{1B}: [^{3}H]-5-HT (sp act. 14.6 Ci/mmol, NEN) final concentration 2 nM, 30 min 25 °C (non-specific binding: 5-HT 10 μM). 5-HT_{2A}: [^{3}H]ketanserin (sp act. 60.0 Ci/mmol, Amersham) final concentration 0.7 nM, 15 min, 37 °C (non-specific binding: methysergide 1 mM). 5-HT_{2C}: [^{3}H]mesulergine (sp act. 73.0 Ci/mmol, NEN) final concentration 1 nM, 30 min, 37 °C (non-specific binding: mesulergine 10 mM). 5-HT_3: [^{3}H]BRL43694 (sp act. 84.8 Ci/mmol, NEN) final concentration 1 nM, 30 min, 25 °C (non-specific binding: GR38032 10 μM). D_1: [^{3}H]SCH23390 (sp act. 71.1 Ci/mmol, NEN) final concentration 0.4 nM, 15 min, 37 °C (non-specific binding: (–)-*cis*-flupentixol 10 μM). D_2: [^{3}H]spiperone (sp act. 19.0 Ci/mmol, NEN) final concentration 0.2 nM, 15 min, 37 °C (non-specific binding: (–)-sulpiride 100 μM). α_1-Adrenergic: [^{3}H]prazosin (sp act. 71.8 Ci/mmol, NEN) final concentration 0.2 nM, 30 min, 25 °C (non-specific binding: phentolamine 3 μM).

Incubations were stopped by rapid filtration under vacuum through GF/B filters which were then washed with 12 mL (4 × 3 times) of ice-cold 50 mM Tris-HCl (pH 7.4) or 50 mM Hepes-HCl (pH 7.5) using a Brandel M-48R apparatus and counted in 4 mL of Filter Count (Packard) in an LKB 1214 RACKBETA liquid scintillation spectrometer with counting efficiency about 60%. Dose inhibition curves were analysed by the 'Allfit' program to obtain the concentration of unlabelled drugs that inhibited ligand binding by 50%. The Ki values were derived from the IC_{50} values [18].

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