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# Structure–activity relationship studies of CNS agents. Part 29. *N*-Methylpiperazino-substituted derivatives of quinazoline, phthalazine and quinoline as novel $\alpha_1$, 5-$HT_{1A}$ and 5-$HT_{2A}$ receptor ligands

JL Mokrosz[1], B Duszyńska[1], S Charakchieva-Minol[1], AJ Bojarski[1], MJ Mokrosz[1], RL Wydra[2], L Janda[2], L Strekowski[2]

[1]*Department of Medicinal Chemistry, Institute of Pharmacology, Polish Academy of Sciences, 12, Smętna St, 31-343 Kraków, Poland;*
[2]*Department of Chemistry, Georgia State University, Atlanta, GA 30303, USA*

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**Summary** — New *N*-methylpiperazino-substituted quinazolines **8** and **9**, phthalazine **13**, and quinoline **19** have been synthesized. The receptor binding profiles ($\alpha_1$, 5-$HT_{1A}$, 5-$HT_{2A}$) of these compounds and their analogs (**7–22**) have been determined. It has been demonstrated that orientation of a local dipole moment of the heteroaromatic ring system affects both the $\alpha_1$ and 5-$HT_{2A}$ affinity of the investigated class of ligands. Distortion of the coplanar unfused heteroaromatic ring system results in a decreased 5-$HT_{2A}$ affinity. 4-(4-Methylpiperazino)-2-(2-thienyl)quinoline **18** is the most active and selective $\alpha_1$ ligand ($K_i$ = 4.9 nM) with a much lower affinity for 5-$HT_{1A}$ ($K_i$ = 3420 nM) and 5-$HT_{2A}$ ($K_i$ = 211 nM) receptors.

***N*-methylpiperazine / prazosin analog / $\alpha_1$ receptor ligands / 5-$HT_{1A}$ receptor ligands / 5-$HT_{2A}$ receptor ligands / structure–affinity relationships**

## Introduction

Prazosin **1** and doxazosin **4** are regarded as parent compounds of a vast number of different $\alpha_1$-adrenergic receptor ligands [1]. Compounds **1** and **4** are highly potent and selective $\alpha_1$ ligands ($K_i$ = 0.19 and 1.1 nM, respectively) [2, 3] and are classified as $\alpha_1$-adrenoreceptor antagonists [1–3]. The basic structure of prazosin (2-4-diamino-6,7-dimethoxyquinazoline) has served as the core of a large number of derivatives (eg, **2–6**, fig 1). Chern et al [4] showed that the 6,7-dimethoxy substituents of the quinazoline nucleus are not necessary for the formation of a complex of derivatives **2** and **3** with $\alpha_1$ receptors. They also found that various structural modifications of **2** and **3** affected the $\alpha_1$ affinity of the particular derivatives. The highest $\alpha_1$ affinity ($K_i$ ~0.07 nM) was observed for derivatives of **2** with $R^1 = CH_3$ and $R^2 = 2\text{-}OCH_3$ and an arylpiperazine fragment attached to the heterocyclic ring system in position 4, and **3** with $X = OCH_3$ and $R = 2\text{-}OCH_3$. The lowest affinity ($K_i$ > 10 nM) was found for **2** with $R^1 = CH_3$ and $R^2 = H$, and the arylpiperazine fragment at position 5, and for **3** with $X = SCH_3$ and $R = H$. Campbell et al [3] reported on the structure–affinity relationships of doxazosin modified in the benzodioxane and piperazine portions (**4** and **5**, fig 1). The majority of derivatives of **5** showed very high affinity ($0.7 \le K_i \le 7.8$ nm) for $\alpha_1$ receptors, except for a single derivative ($R^1$ = 6,7-di-Cl, $R^2$ = H, X = Y = $CH_2$, $K_i$ = 13.3 nM). Other structural modifications (X, Y, $R^1$) had a small effect on affinity [3]. Alabaster et al [2] analyzed the $\alpha_1$-receptor affinity of a series of 1-amino-6,7-dimethoxyquinazolines substituted at position 2 with a complex piperidine fragment (**6**). All derivatives containing $XCONR^1R^2$ substituents at position 4 of the piperidine ring showed a high $\alpha_1$ affinity ($0.1 \le K_i \le 1.73$ nM). Modifications of the $XCONR^1R^2$ substituent at position 3 of the piperidine ring resulted in a dramatic loss of affinity ($K_i$ = 117 nM for 3-$CON(C_2H_5)_2$) [2].

Many complex derivatives of prazosin show a high affinity for $\alpha_1$ receptors ($K_i$ < 10 nM). The applied modifications of the structure include, in general, substituents at the 2-amino function of the 2,4-diamino-6,7-dimethoxyquinazoline skeleton. A 4-amino-2-(*N*-piperazino)-6,7-dimethoxyquinazoline core of the class

*Abbreviations:* 2-PP: 2-(*N*-piperazino)pyrimidine; 4-Me-2-PP: 2-(4-methylpiperazino)pyrimidine; NOE: nuclear Overhauser effect; 8-OH-DPAT: 8-hydroxy-2-(di-*n*-propylamino)tetralin.

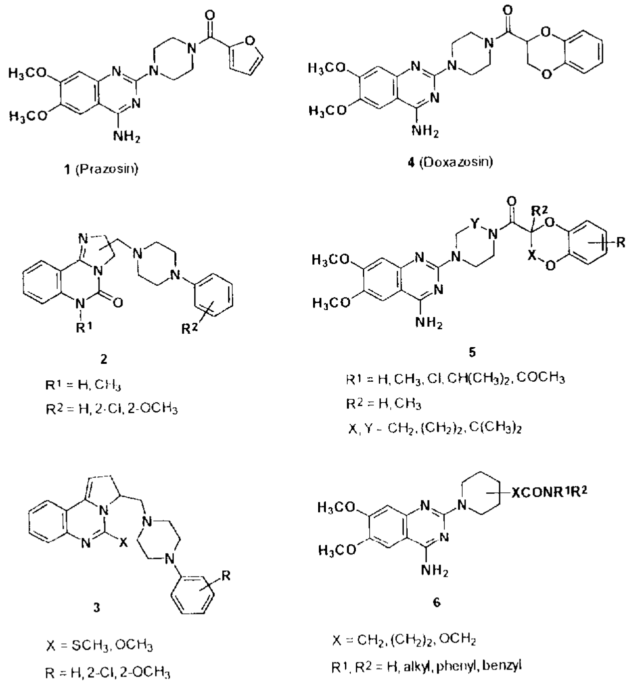



**Fig 1.** Structures of prazosin (**1**) and doxazosin (**4**), and their derivatives and analogs (**2**–**6**).

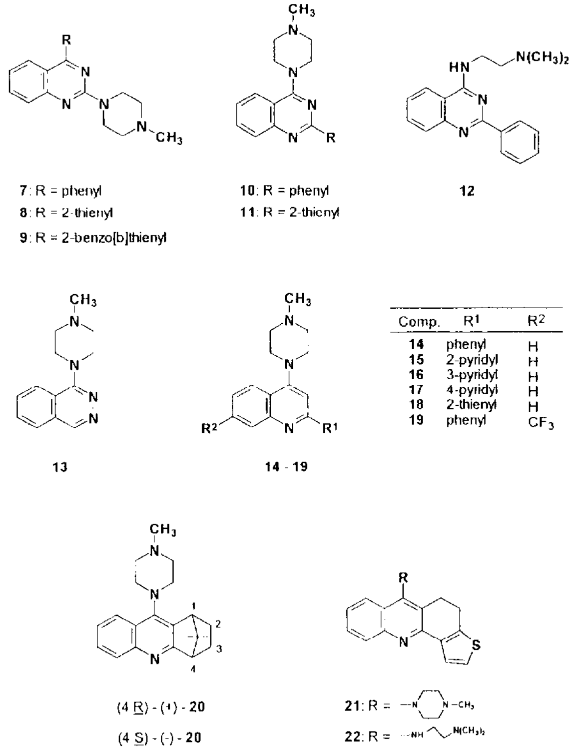


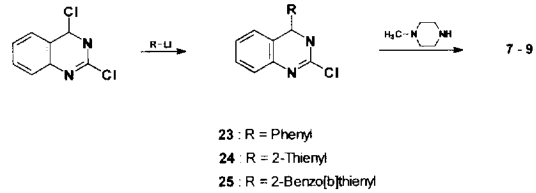

**Fig 2.** Structures of compounds **7**–**22**.

of $\alpha_1$ ligands under discussion may also be regarded as an analog of 2-(*N*-piperazino)pyrimidine (2-PP). Also, it is well documented that a number of typical 5-$HT_{1A}$ ligands of the 1-arylpiperazine class show a significant or even high $\alpha_1$ receptor affinity [5–10]. Therefore, in the present paper we discuss the fundamental structural requirements responsible for the receptor binding profile ($\alpha_1$, 5-$HT_{1A}$ and 5-$HT_{2A}$) of simple, model analogs of 2-(4-methylpiperazino)pyrimidine (4-Me-2-PP) which contain the quinazoline, phthalazine or quinoline ring system instead of a 2-pyrimidinyl moiety (fig 2).

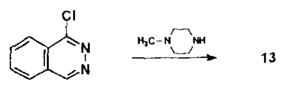
## Chemistry

The structures of compounds **7**–**22** used in this work are given in figure 2. We have shown previously that the reaction of aryllithium and heteroaryllithium reagents with 2,4-dichloroquinazoline is regioselective, resulting in the predominant substitution of the chlorine at position 4 [11]. This reaction is illustrated in scheme 1 by the synthesis of known quinazoline derivatives **23** and **24**, and a new compound **25**. Treatment of **23**–**25** with *N*-methylpiperazine furnished the corresponding 2-(*N*-methylpiperazino)quinazolines **7**–**9**.

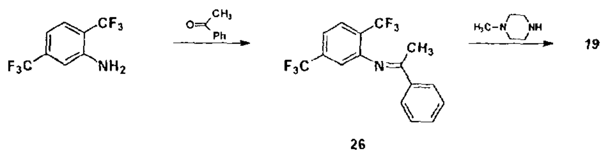
A phthalazine **13** was obtained by a similar nucleophilic displacement of a chlorine atom in 1-chlorophthalazine (scheme 2). Synthesis of quinazolines **10**–**12** [12], quinolines **14**–**18** [13] and fused quinolines **20** [14], **21** [15], and **22** [16] has been reported by us previously. A new quinoline derivative **19** was prepared in a similar fashion (scheme 3). Thus, condensation of 2,5-bis(trifluoromethyl)aniline with acetophenone was followed by lithium *N*-methylpiperazide-mediated cyclization of the resultant Schiff base **26** to give the desired compound **19**. The ketimine **26** and similar ketimines derived from aniline and aryl methyl ketones are thermodynamic mixtures of a major *E* dia-




**Scheme 1.**



Scheme 2.



Scheme 3.

stereomer, as shown for **26** in scheme 3, and a minor *Z* diastereomer [14, 17]. Analytically pure (*E*)-**26** was obtained by chromatography, and the suggested stereochemistry was fully consistent with the results of the proton NOE experiment. As expected, irradiation of the methyl singlet at δ 2.24 resulted in a strong singlet at δ 7.05 for H6 of the aniline proton and a two-proton doublet at δ 7.97 for H2 and H6 of the phenyl group.

## Pharmacology

All compounds **7–22** as well as 2-PP (**27**) and 4-Me-2-PP (**28**) were evaluated for their receptor binding profile ($\alpha_1$, 5-$HT_{1A}$, 5-$HT_{2A}$). The receptor affinities of the investigated compounds were determined in the competition experiments using the following radioligands and the rat brain membranes: [$^3$H]prazosin (cortex), [$^3$H]-8-OH-DPAT (hippocampus), and [$^3$H]ketanserin (cortex) for $\alpha_1$, 5-$HT_{1A}$ and 5-$HT_{2A}$ receptors, respectively. The affinities of prazosin for $\alpha_1$ ($K_i$ = 0.23 ± 0.03 nM), 8-OH-DPAT for 5-$HT_{1A}$ ($K_i$ = 1.43 ± 0.21 nM), and ritanserin for 5-$HT_{2A}$ ($K_i$ = 1.14 ± 0.13 nM) receptors were also determined and they serve as a standard in the conducted biding studies. The results are shown in table I.

## Results and discussion

All investigated compounds show diverse $\alpha_1$, 5-$HT_{1A}$ and 5-$HT_{2A}$ receptor affinities which are within a range of $10^{-9}$ to $10^{-5}$ M, $10^{-8}$ to $10^{-5}$ M and $10^{-7}$ to $10^{-5}$ M, respectively (table I). Derivative **7** exhibits a significantly higher affinity than its parent compounds 2-PP (**27**) and 4-Me-2-PP (**28**) for $\alpha_1$, 5-$HT_{1A}$ and 5-$HT_{2A}$ receptors. 4-Phenyl- and 4-(2-thienyl)-2-(4-methylpiperazino)quinazolines **7** and **8** show the same $\alpha_1$ affinity within experimental error (see table I), whereas extension of the 4-substituent results in the completely inactive 4-(2-benzo[*b*]thienyl) derivative **9**. Permutation of substituents between positions 2 and 4 (cf, **10** vs **7**) significantly enhances the $\alpha_1$ affinity of **10** in relation to **7**. Furthermore, replacement of the *N*-methylpiperazine fragment in compound **10** with a flexible *N,N*-dimethylethylenediamine chain (**12**) does not affect the $\alpha_1$ affinity. The phthalazine derivative **13** has a low, micromolar $\alpha_1$ affinity (table I). Thus, a comparison of the $\alpha_1$-binding data for **7–10**, **12** and **13** may suggest that the orientation of a local dipole moment of the unfused heteroaromatic systems plays some role in stabilization of the ligand-$\alpha_1$ receptor complex.

In order to verify the above hypothesis we have analyzed the $\alpha_1$ affinities of a series of 4-(4-methylpiperazino)quinolines **14–21**. The results obtained are meaningful. Replacement of the N3 atom of the quinazoline system in **10** ($K_i$ = 290 nM) by the C-$sp^2$ aro-

**Table I.** $\alpha_1$, 5-$HT_{1A}$ and 5-$HT_{2A}$ receptor affinities of compounds **7–22**, **27** and **28**.

| *Compound* | $K_i$ ± *SEM(nM)*[a] | | |
|---|---|---|---|
| | $\alpha_1$ | 5-$HT_{1A}$ | 5-$HT_{2A}$ |
| **7** | 1070 ± 160 | 545 ± 22 | 1190 ± 11 |
| **8** | 1340 ± 220 | 290 ± 13 | 468 ± 5 |
| **9** | >50 000 | 43 ± 4 | 256 ± 3 |
| **10** | 290 ± 26 | 924 ± 45 | 612 ± 29 |
| **11** | ND | 409 ± 6 | 476 ± 7 |
| **12** | 335 ± 33 | 4870 ± 70 | 2480 ± 40 |
| **13** | 9700 ± 900 | 1545 ± 80 | 4470 ± 185 |
| **14** | 17 ± 2 | 3710 ± 130 | 276 ± 37 |
| **15** | 292 ± 22 | 3800 ± 210 | 1425 ± 85 |
| **16** | 1280 ± 100 | 6140 ± 280 | 10 300 ± 600 |
| **17** | 161 ± 14 | 3380 ± 210 | 280 ± 9 |
| **18** | 4.9 ± 1.4 | 3420 ± 90 | 211 ± 11 |
| **19** | 264 ± 15 | 9500 ± 1000 | 222 ± 7 |
| (4*R*)-(+)-**20** | 30 000 ± 3300 | 43 400 ± 2600 | 45 000 ± 6000 |
| (4*S*)-(–)-**20** | 6400 ± 350 | >50 000 | 30 800 ± 1700 |
| **21** | 2140 ± 430 | 3650 ± 280 | 8090 ± 300 |
| **22** | 446 ± 32 | ND | 2370 ± 140 |
| **27**[b] | 5970 ± 620 | 1430[c] | 29 500[c] |
| **28**[b] | 6100 ± 1150 | 2180[c] | 19 700[c] |

[a]Mean values from at least three independent experiments; [b]**27**: 2-PP, **28**: 4-Me-2-PP; [c]data taken from reference [18]; ND: not determined.

matic atom strongly increases the $\alpha_1$ affinity of the resultant quinoline **14** ($K_i$ = 17.5 nM). Further modifications of **14** culminated in a 4-(4-methylpiperazino)-2-(2-thienyl)quinoline (**18**), which is the most active $\alpha_1$ ligand ($K_i$ = 4.9 nM) of all compounds under investigation (table I). Moreover, derivative **18** is the most selective $\alpha_1$ ligand as its 5-$HT_{1A}$ and 5-$HT_{2A}$ affinities are 700- and 43-fold lower, respectively. The determined $\alpha_1$ affinity of **15–17** is at least tenfold lower than that of **14**. On the other hand, the 2-pyridyl derivative **15** shows the same $\alpha_1$ affinity as quinazoline **10**; the affinity of the 3-pyridyl isomer **16** is lower, whereas the $K_i$ values of the 4-pyridyl derivative **17** increase slightly in relation to **15** and **10** (table I). Again, it can be suggested that the orientation of a local dipole moment of the unfused heteroaromatic systems controls the $\alpha_1$ affinity of the investigated compounds. A $CF_3$ group in position 7 of the quinoline skeleton significantly decreases the $\alpha_1$ affinity of **19** as compared to the parent compound **14**. Annelation of the quinoline skeleton with bicyclo[2.2.1]-heptane yields enantiomers of **20**, which bind to $\alpha_1$ receptors in the micromolar range ($10^{-6}$ to $10^{-5}$ M) only. Weak enantioselectivity, however, is observed, as the (4*S*)-(–)-**20** enantiomer shows an $\alpha_1$ affinity at least fourfold higher than its (4*R*)-(+)-**20** counterpart.

Our earlier conformational analysis of unfused heteroaromatic ring systems clearly indicated that 4-(2-thienyl)- and 4-(2-furyl)pyrimidine exist predominantly in the opposite coplanar conformations, *s-cis* and *s-trans*, respectively [18–23]. In this work, we carried out a conformational analysis of several representatives of **7–21**, using the recommended and previously applied semi-empirical PM3 method [22, 24, 25]. The results of the PM3 calculations for 2-phenyl-, 2-(2-pyridyl)-, 2-(3-pyridyl)-, and 2-(2-thienyl)-4-(4-methylpiperazino)quinolines **14–16** and **18**, respectively, are shown in figure 3. It was found that the PM3 method prefers coplanar conformations of the heteroaromatic fragments of **14**, **16** and **18**. The calculated differences between *s-cis* and *s-trans* conformers ($\tau_1$ = 0° and 180°, respectively) for **16** and **18** are small and do not exceed 0.14 kcal/mol. Furthermore, the conformers *s-cis* and *s-trans* of **14** are equipopulated as their heats of formation are the same. As expected, two low-energy conformations of a 2-(4-pyridyl)quinoline **17** are only slightly deviated from coplanarity (data not shown). In marked contrast, 2-(2-pyridyl) derivative **15** exists predominantly in *s-trans* conformations, and the rotation barrier ($\Delta E$ = 1.88 kcal/mol) is significantly higher than that calculated for other quinoline derivatives ($\Delta E$ = 0.63, 0.92 and 0.87 kcal/mol for **14**, **16** and **18**, respectively). The *s-cis* conformations of **15** ($-30° < \tau_1 < 30°$) are apparently destabilized by an unfavorable orientation of dipole moment of the quinoline and pyridine subunits. On the other hand, our earlier studies have shown that the attractive S···N interactions additionally stabilize the *s-cis* conformation of the 4-(2-thienyl)pyrimidine fragment [19]. It appears that the same effect is responsible for stabilization of the calculated *s-cis* conformation of the unfused 2-(2-thienyl)quinoline ring system in **18**.

In order to verify our hypothesis that the orientation of the unfused heteroaromatic fragment controls the $\alpha_1$-receptor affinity of **14–18**, we analyzed two additional derivatives, **21** and **22**. These compounds contain a 2-(3-thienyl)quinoline fragment in the fixed *s-trans* conformation due to a rigid ethylene bridge in their structure, and they differ in the conformational freedom of the amino fragment. The PM3-optimized geometry of **21** is shown in figure 4. The $\alpha_1$ affinity data (table I) clearly indicate that the *s-trans* conformation of **21** is unfavorable for interaction with the receptor. Furthermore, the flexible *N,N*-dimethylethylenediamine chain permits a slightly different orientation of **22** at the receptor, though its observed $\alpha_1$ affinity ($K_i$ = 446 nM) is considerably lower than that of **14** and **18**, and is not more than two to three times different from the affinities of **15–17**.

The 5-$HT_{1A}$ affinity of derivatives **7** and **8** (table I) is of the same order as that reported by Glennon et al for 2-(*N*-piperazino)naphthalene ($K_i$ = 265 nM) and 2-(*N*-piperazino)quinoline ($K_i$ = 230 nM) [26]. Surprisingly, derivative **9** is the most active 5-$HT_{1A}$ ligand ($K_i$ = 43 nM) of all investigated compounds. A comparison of the reported 5-$HT_{1A}$ affinity of 1-(*N*-piperazino)naphthalene ($K_i$ = 5 nM) [26] with that of its phthalazine analog **13** ($K_i$ = 9700 nM) indicates that the presence of two adjacent nitrogen atoms in the ring structure is a highly undesirable feature. Derivatives **14–21** show a low or very low 5-$HT_{1A}$ affinity (table I). These findings also agree with the results reported by Glennon, who suggested a region of limited bulk tolerance at the 5-$HT_{1A}$ receptor [27, 28]. Indeed, relatively small $R^1$-substituents of the investigated quinolines may reach that region at 5-$HT_{1A}$ receptors, but these receptors do not tolerate large substituents.

The investigated compounds show a moderate ($211 \leq K_i \leq 280$ nM for **9**, **14** and **17–19**), low ($468 \leq K_i \leq 1425$ nM for **7**, **8**, **10**, **11** and **15**) or even very low ($K_i >$ 2000 nM for **12**, **13**, **16** and **20–22**) affinity for 5-$HT_{2A}$ receptors (table I). In previous studies we have proposed a pharmacophore which is responsible for the formation of a complex between 4,6-di(heteroaryl)-2-(4-methylpiperazino)pyrimidines and 5-$HT_{2A}$ receptors [18, 22]. We have also defined three crucial distances, $d_1$, $d_2$ and $d_3$ (for their definition see table II), and their optimal ranges necessary for high affinity ($K_i$ = $10^{-9}$ to $10^{-8}$ M) of this class of 5-$HT_{2A}$ ligands (table II). The $d_1$, $d_2$ and $d_3$ parameters of the analyzed *N*-methylpiperazines **7–11** and **13–21** are within typical ranges, except for the $d_2$ = 9.53 Å value for derivative

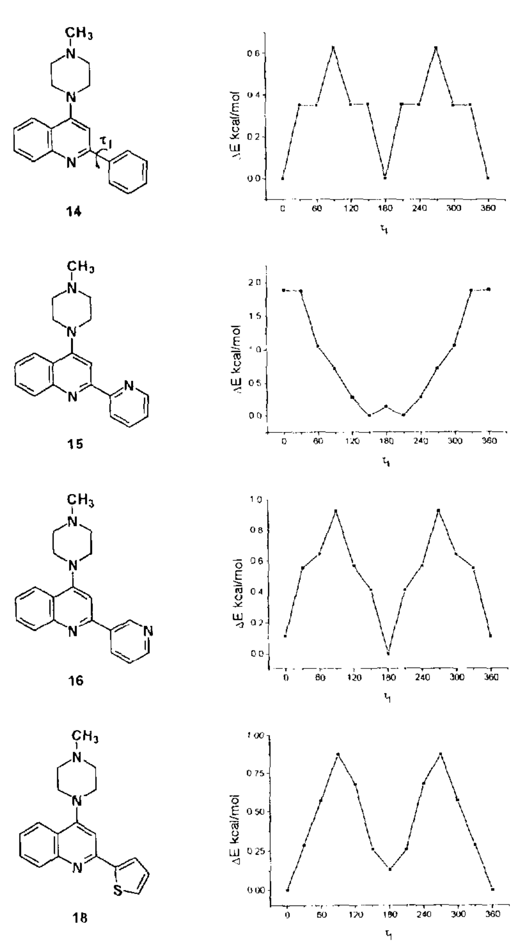



Fig 3. Conformation energy profiles upon rotation ($\tau_1$) of the inter-ring C2-C1'(2') bond calculated by the PM3 method.

9, which reaches the critical, upper limit of this distance (fig 5, table II) [22]. Compound **13** has a very low 5-$HT_{2A}$ affinity, since its structure meets only in part the pharmacophore requirements (table II).

Our previous studies have clearly indicated that the most active 5-$HT_{2A}$ ligands, such as 4,6-di(2-thienyl)-2-(4-methylpiperazino)pyrimidine and its 4,6-di(heteroaryl) analogs, adopt favorable coplanar conformations of both the piperazinopyrimidine subunit and the unfused, tricyclic heteroaromatic system [18, 22]. We have also demonstrated that distortion of the coplanar heteroaromatic ring system significantly decreases the observed 5-$HT_{2A}$ affinity [22]. The same effect is observed for derivatives **7–9**. Their unfused, heteroaromatic ring system exists predominantly in the twisted conformations shown in figure 6. Further-

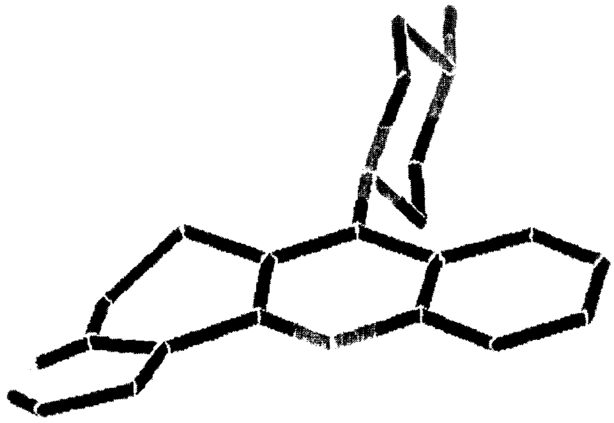


**Fig 4.** The lowest-energy conformer of **21** calculated by the PM3 method.

more, an excellent qualitative relationship between the population of the twisted conformations and the 5-$HT_{2A}$ affinity of **7–9** was observed: the lower the $\Delta E_{180°-60°}$, the higher the 5-$HT_{2A}$ affinity (fig 6, table I).

The PM3 calculations show that arylpiperazine fragments of **7–11** and **13–21** adopt different conformations. While coplanar conformations are favored

**Table II.** The distances $d_1$, $d_2$ and $d_3$ defining the 5-$HT_{2A}$ receptor pharmacophore of the investigated compound.

| *Compound* | $d_1$ (Å)[a] | $d_2$ (Å)[b] | $d_3$ (Å)[c] |
|---|---|---|---|
| Optimal values[d] | 5.2–8.4 | 5.7–8.5 | 4.6–7.3 |
| **7** | 7.83 | 8.47 | 5.03 |
| **8** | 7.83 | 8.37 | 4.91 |
| **9** | 7.83 | 9.53 | 5.87 |
| **11**[e] | 5.98–6.61 | 8.00–8.66 | 6.29 |
| **13**[e] | 5.98–6.48 | – | – |
| **14**[e] | 5.97–6.58 | 8.21–8.94 | 6.44 |
| **15**[e] | 5.97–6.58 | 8.19–8.93 | 6.42 |
| **18**[e] | 5.84–6.62 | 8.07–8.91 | 6.29 |
| **21**[e] | 5.95–6.58 | 8.11–8.78 | 6.25 |

[a]Distance between N4-piperazine atom and a center of the fused benzene ring; [b]distance between N4-piperazine atom and a center of the aromatic R or $R^1$ substituent; [c]distance between centers of the fused benzene ring and the aromatic R or $R^1$ substituent; [d]optimal values of $d_1$, $d_2$ and $d_3$ parameters taken for comparison from references [18] and [22]; [e]ranges of $d_1$ and $d_2$ parameters measured for the opposite conformations of the *N*-methylpiperazine fragment as shown in figure 5.

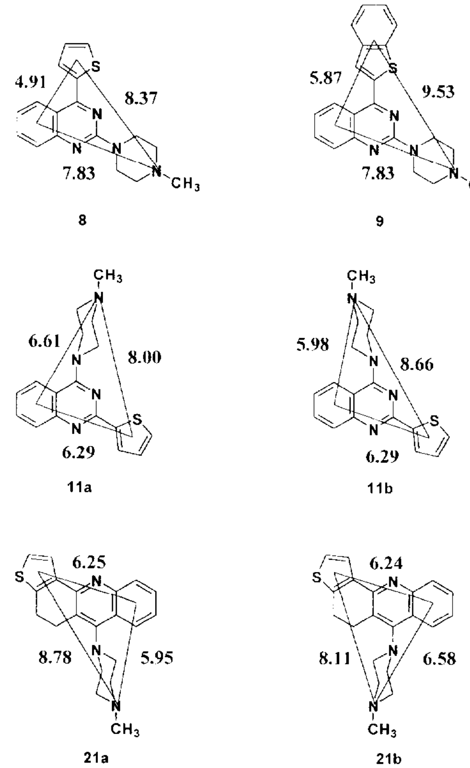



**Fig 5.** Distances defining the 5-$HT_{2A}$ receptor pharmacophore for **7** and **8** in the coplanar conformation of the arylpiperazine ring system, and for **11** and **21** (where **a** and **b** refer to either of the two energy minima of the arylpiperazine fragment in twisted conformations).

for **7–9**, twisted or even orthogonal conformations are definitely more populated in the case of **10**, **11** and **13–21** (fig 7). It should be stressed that our results for the conformational analysis of **7–9** are fully consistent with those reported by others for 2-(4-methylpiperazino)pyrimidine [18, 22, 25]. It may therefore be anticipated that the conformation of the arylpiperazine fragment is not critical for the ability to form a complex, as some derivatives with different conformations of this fragment have similar 5-$HT_{2A}$ affinities (eg, **8** and **11**). On the other hand, all analyzed 4-(4-methylpiperazino)quinolines **14–19** have basically the same conformation energy profiles as that shown for **14** in figure 7b, whereas their 5-$HT_{2A}$ affinities are within a wide $K_i$ range for different $R^1$ substituents (table I).

It can be concluded that orientation of the local dipole moment and planarity of the unfused heteroaromatic ring system are the most important structural features of the investigated class of ligands, which are responsible for the observed 5-$HT_{2A}$ affinity changes. Furthermore, it should be stressed again that the conclusions derived from the 5-$HT_{2A}$ affinity studies are fully consistent with our previous findings and serve as an additional verification of our topographic model of 5-$HT_{2A}$ sites [18, 22].

## Experimental protocols

### *Chemistry*

2,4-Dichloroquinoline [29] and 1-chlorophthalazine [30] were prepared as described. Melting points (mp, Pyrex capillary) are not corrected. $^1$H-NMR spectra were obtained on a Varian-400 (400 MHz) instrument at 23 °C with tetramethylsilane as an internal standard. Crude reaction mixtures were analyzed, and mass spectra of pure components were obtained on a Hewlett-Packard GC-MS instrument equipped with an on-column injector, a poly(dimethylsiloxane)-coated capillary, and a mass selective detector operating at 70 eV. Hydrobromide salts were obtained by using a general procedure [13] and the salts were crystallized from 95% ethanol. Elemental analyses indicated by the symbols of the elements were within ±0.3% of the theoretical values.

*4-(2-Benzo[b]thienyl)-2-chloroquinazoline* ***25***
This compound was obtained in the reaction of 2,4-dichloroquinazoline with 2-benzo[*b*]thienyllithium [31] by using a general procedure reported previously for the preparation of **23** and **24** [11]. After crystallization from dichloromethane/hexane

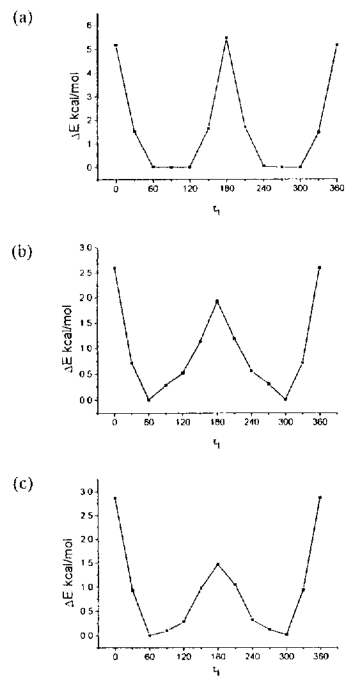



**Fig 6.** Conformation energy profiles upon rotation ($\tau_1$) of the inter-ring C2-C1'(2') bond for **7** (a), **8** (b) and **9** (c) calculated by the PM3 method.

(1:9) the yield was 56%; mp 149–150 °C. $^1$H-NMR ($CDCl_3$) δ: 7.46 (t, $J$ = 8 Hz, 1H); 7.49 (t, $J$ = 8 Hz, 1H); 7.74 (t, $J$ = 8 Hz, 1H); 7.96 (m, 3H); 8.06 (d, $J$ = 8 Hz, 1H); 8.15 (s, 1H); 8.61 (d, $J$ = 8 Hz, 1H). MS *(m/z)*: 296 (100, $M^+$), 298 (35, $M^+$). Anal $C_{16}H_9ClN_2S$ (C, H, N).

*A general method for the preparation of **7–9** and **13***
A solution of **23–25** (1 mmol) or 1-chlorophthalazine (164 mg, 1 mmol) in *N*-methylpiperazine (2 mL) was heated under reflux for 2 h. After cooling the mixture was treated with water (3 mL) and extracted with ether (3 × 25 mL). The extract was dried with $Na_2SO_4$, concentrated on a rotary evaporator, and the residue was subjected to chromatography on silica gel with hexane/triethylamine/ethanol (7:2:1) as an eluent.

*2-(4-Methylpiperazino)-4-phenylquinazoline **7**.* After crystallization from ethanol/hexane (1:9) this compound was obtained in a 90% yield; mp 98–100 °C, reported mp 97–98 °C [32].

*2-(4-Methylpiperazino)-4-(2-thienyl)quinazoline **8**.* This compound was obtained in an 85% yield; an oil. $^1$H-NMR ($CDCl_3$) δ: 2.37 (s, 3H); 2.54 (t, $J$ = 5 Hz, 4H); 4.03 (t, $J$ = 5 Hz, 4H); 7.23 (m, 2H); 7.58 (d, $J$ = 5 Hz, 1H); 7.65 (m, 2H); 7.79 (d, $J$ = 4 Hz, 1H); 8.23 (d, $J$ = 8 Hz, 1H). MS *(m/z)*: 240 (100), 310 (20, $M^+$). **8**•2HBr: mp >310 °C. Anal $C_7H_{18}N_4S$•2HBr (C, H, N).

*4-(2-Benzo[b]thienyl)-2-(4-methylpiperazino)quinazoline **9**.* This compound was obtained in a 58% yield; an oil. **9**•2HBr•$H_2O$: mp >310 °C. $^1$H-NMR (DMSO-$d_6$) δ: 2.87 (s, 3H); 3.17 (m, 2H); 3.44 (m, 2H); 3.60 (m, 2H); 4.92 (m, 2H); 5.60 (br, exchangeable with $D_2O$); 7.51 (m, 3H); 7.71 (d, $J$ = 8 Hz, 1H); 7.88 (d, $J$ = 8 Hz, 1H); 8.09 (m, 2H); 8.50 (s, 1H); 8.54 (d, $J$ = 8 Hz, 1H). Anal $C_{21}H_{20}N_4S$•2HBr• $H_2O$ (C, H, N).

*1-(4-Methylpiperazino)phthalazine **13**.* This compound was obtained in a 73% yield; an oil. $^1$H-NMR ($CDCl_3$) : 2.42 (s, 3H); 2.72 (t, $J$ = 5 Hz, 4H); 3.60 (t, $J$ = 5 Hz, 4H); 7.82 (m, 2H); 7.88 (m, 1H); 8.05 (m, 1H); 9.17 (d, $J$ = 1 Hz, 1H). MS *(m/z)*: 158 (100), 228 (3, $M^+$). **13**•2HBr: mp 273–275 °C. Anal $C_{13}H_{16}N_4$•2HBr (C, H, N).

*(E)-N-(1-Phenylethylidene)-2,5-bis(trifluoromethyl)aniline **26***
Condensation of 2,5-bis(trifluoromethyl)aniline with acetophenone was conducted by using a general procedure [13, 14]. Compound **26** was obtained in an 85% yield after distillation (128–130 °C/3.6 mmHg) on a Kugelrohr. $^1$H-NMR ($CDCl_3$) : 2.24 (s, 3H); 7.05 (s, 1H); 7.42 (d, $J$ = 8 Hz, 1H); 7.48 (m, 3H); 7.80 (d, $J$ = 8 Hz, 1H); 7.97 (d, $J$ = 8 Hz, 2H). MS *(m/z)*: 316 (100), 331 (30, $M^+$). Anal $C_{16}H_{11}F_6N$ (C, H, N).

*4-(4-Methylpiperazino)-2-phenyl-7-(trifluoromethyl)quinoline **19***
Heterocyclization of ketimine **26** with lithium *N*-methylpiperazide was conducted by using a general procedure [13, 14]. Chromatography on silica gel with hexane/triethylamine/ethanol (7:2:1) as an eluent was followed by crystallization of **19** from hexane. Yield 67%; mp 141–142 °C. $^1$H-NMR ($CDCl_3$) δ: 2.47 (s, 3H); 2.78 (m, 4H); 3.36 (m, 4H); 7.39 (s, 1H); 7.52 (m, 3H); 7.63 (d, $J$ = 8 Hz, 1H); 8.11 (m, 3H); 8.44 (s, 1H). MS *(m/z)*: 70 (100), 371 (40, $M^+$). Anal $C_{21}H_{20}F_3N_3$ (C, H, N). **19**•2HBr•1.5$H_2O$: mp 268–270 °C. Anal $C_{21}H_{20}F_3N_3$•2HBr• 1.5$H_2O$ (C, H, N).

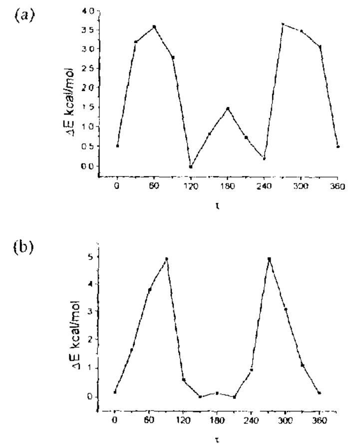



**Fig 7.** Conformation energy profiles upon rotation (τ = lone pair-N1-C1′ -N(or C)2′) of the *N*-methylpiperazine fragment versus heteroaromatic moiety for **10** (a) and **14** (b).

*Pharmacology*

Radioligand binding experiments were conducted for 5-$HT_{1A}$ receptors in the hippocampus of the rat brain, and in the cortex for 5-$HT_{2A}$ receptors, according to the published procedure [33]. [$^3$H]-8-OH-DPAT (190 Ci/mmol, Amersham) and [$^3$H]-ketanserin (60 Ci/mmol, NEN Chemicals) were used for labelling 5-$HT_{1A}$ and 5-$HT_{2A}$ receptors, respectively. The $K_i$ values were determined on the basis of at least three competition binding experiments in which 10–14 drug concentrations ($10^{-10}$ to $10^{-3}$ M), run in triplicate, were used.

*$\alpha_1$-Receptor binding experiments*
[$^3$H]Prazosin (26 Ci/mmol, NEN Chemicals) was used for labelling $\alpha_1$ receptors. The membrane preparation and assay procedure were carried out according to the published procedures [34, 35] with slight modifications. The cortex tissue of the rat brain was homogenized in 20 vol (w/v) of ice-cold Tris-HCl buffer (50 mM, pH = 7.4) with an Ultra Turrax homogenizer. The homogenate was centrifuged at 25 000 × *g* for 10 min, and the resulting pellet was suspended in the same volume of Tris-HCl buffer, and was recentrifuged. The final pellet was resuspended in 170 vol (w/v) of Tris-HCl buffer (50 nM, pH = 7.4). [$^3$H]Prazosin in a volume of 100 µl was added to aliquots (1.7 mL) of the membrane suspension, and the samples were incubated at 25 °C for 30 min. The total incubation volume of 2 mL was filtered through Whatman GF/B glass filters, and was then washed with a cold buffer (3 × 5 mL) using a Brandel cell harvester. Non-specific binding of [$^3$H]prazosin was obtained in the presence of phentolamine (200 µL, final concentration $10^{-6}$ M). The final [$^3$H]prazosin concentration was $3 \times 10^{-10}$ M and the concentration of the analyzed compounds ranged from $10^{-10}$ to $10^{-3}$ M. $K_i$ values were determined from at least three independent experiments, run in triplicate.

*Molecular modeling*

All the molecular modeling experiments were conducted using a Sybyl 6.03 package (Tripos Associates, Inc), installed on an ESV 10/33 workstation. PM3 calculations were conducted using a Mopac 5.0 (QCPE) program implanted into the Sybyl

6.03. Full geometry optimization and gradient norm <0.1 kcal/mol/Å were setup during calculations of low-energy conformations. To investigate the rotational energy barriers, 12 conformations were generated by a step-wise rotation of 30° around the inter-ring bonds. Next, each of these conformations was optimized using a PM3 method over all internal coordinates except for those that define the relative orientation of the respective substituent (gradient norm <0.1 kcal/mol/Å).

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## References

1 Timmermans PBMWM (1989) In: *Receptor Pharmacology and Function* (Williams M, Glennon RA, Timmermans PBMWM, eds) Marcel Dekker, New York, 173–205
2 Alabaster VA, Campbell SF, Danilewicz JC, Greengrass CW, Plews RM (1987) *J Med Chem* 30, 999–1003
3 Campbell SF, Davey MJ, Hardson JD, Lewis BN, Palmer MJ ( 1987) *J Med Chem* 30, 49–57
4 Chern JW, Tao PL, Yen MH (1993) *J Med Chem* 36, 2196–2207
5 Raghupathi RK, Rydelek-Fitzgerald L, Teitler M, Glennon RA (1991) *J Med Chem* 34, 2633–2638
6 El-Bermawy M, Raghupathi R, Ingher SP et al (1992) *Med Chem Res* 2, 88–95
7 Melchiorre C, Minarini A, Tumiatti V et al (1993) *Med Chem Res* 4, 140–145
8 Anzini M, Corelli F, Cagnotto A, Skorupska M (1993) *Med Chem Res* 3, 249–256
9 Paluchowska MH, Dereń-Wesołek A, Charakchieva-Minol S, Chojnacka-Wójcik E, Mokrosz JL (1995) *Med Chem Res* 5, 479–486
10 Mokrosz JL, Paluchowska MH, Kłodzińska A, Charakchieva-Minol S, Chojnacka-Wójcik E (1995) *Arch Pharm (Weinheim)* 328, 770–774
11 Harden DB, Mokrosz MJ, Strekowski L (1988) *J Org Chem* 53, 4137–4140
12 Patterson SE, Janda L, Strekowski L ( 1992) *J Heterocycl Chem* 29, 703–706
13 Strekowski L, Mokrosz JL, Honkan VA et al (1991) *J Med Chem* 34, 1739–1746
14 Strekowski L, Patterson SE, Janda L et al (1992) *J Org Chem* 57, 196–201
15 Strekowski L, Wydra RL, Cegla MT et al (1990) *J Org Chem* 55, 4777–4779
16 Wilson WD, Zhao M, Patterson SE et al (1992) *Med Chem Res* 2, 102–110
17 Strekowski L, Cegla MT, Harden DB, Kong SB (1989) *J Org Chem* 54, 2464–2466
18 Mokrosz JL, Strekowski L, Duszyńska B et al (1994) *Pharmazie* 49, 801–806
19 Strekowski L, Wilson WD, Mokrosz JL et al (1988) *Anticancer Drug Des* 2, 387–398
20 Strekowski L, Mokrosz JL, Tanious FA et al (1988) *J Med Chem* 31, 1231–1239
21 Wilson WD, Tanious FA, Watson RA et al (1989) *Biochemistry* 28, 1984–1992
22 Mokrosz MJ, Strekowski L, Kozak WX et al (1995) *Arch Pharm (Weinheim)* 328, 659–666
23 Mokrosz JL, Bojarski AJ, Harden DB, Strekowski L (1996) *J Heterocycl Chem*, in press
24 Stewart JJP (1989) *J Comp Chem* 10, 221–264
25 Dijkstra GDH (1993) *Rec Trav Chim Pays-Bas* 112, 151–160
26 Glennon RA, Slusher RM, Lyon RA, Titeler M, McKenney JD (1986) *J Med Chem* 29, 2375–2380
27 Glennon RA, Chaurasia C, Titeler M (1990) *J Med Chem* 33, 2777–2784
28 Glennon RA, Westkaemper RB, Bartyzel P (1991) In: *Serotonin Receptor Subtypes: Basic and Clinical Aspects* (Peroutka SJ, ed) Wiley-Liss, New York, 19–64
29 Butler K, Patridge MW (1959) *J Chem Soc*, 1512–1515
30 Atkinson CMA, Brown CW, Simpson JCE (1956) *J Chem Soc* 1081–1086
31 Strekowski L, Wydra RL, Cegla MT, Czarny A, Patterson SE ( 1989) *J Org Chem* 54, 6120–6123
32 Parra VG, Madronero R, Vega S (1977) *Synthesis* 345–346
33 Bojarski AJ, Cega MT, Charakchieva-Minol S et al (1993) *Pharmazie* 48, 289–294
34 Maj J, Klimek V, Nowak G (1985) *Eur J Pharmacol* 119, 113–116
35 Mogilnicka E, Nielsen M (1986) *Eur J Pharmacol* 122, 369–372