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# Imidazo[1,2-*a*]quinoxalin-4-amines: A novel class of nonxanthine $A_1$-adenosine receptor antagonists[1]

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**Abstract** – The syntheses and $A_1$ adenosine receptor affinities of a number of imidazo[1,2-*a*]quinoxalin-4-amines are reported. Structure–activity relationships within the series and in comparison with other similar tricyclic nonxanthine adenosine antagonists are discussed, leading to a putative common binding mode of these nitrogen-containing heterocycles to $A_1$ adenosine receptors. Secondary amino compounds displayed the best affinities toward $A_1$ receptors, while the tertiary amines were almost devoid of activity, thus suggesting a crucial role for the hydrogen bond-forming 4-NH group. Remarkably higher potencies for 1-methyl and *N*-cyclopentyl derivatives were also found. 4-Cyclopentylamino-1-methylimidazo[1,2-*a*]quinoxaline (IRFI 165) is the most potent compound in this series, having $K_i(A_1)$ = 7.9 nM. It is also provided with a good $A_1$ selectivity both versus $A_{2a}$ and $A_3$ subtypes and was selected for further pharmacological studies. © Elsevier, Paris

**adenosine receptors / imidazo[1,2-*a*]quinoxaline / tricyclic heteroaromatic systems / nonxanthine $A_1$-adenosine antagonists / IRFI 165**

## 1. Introduction

The nucleoside adenosine is involved in the regulation of several physiological functions which are mediated by the interaction with four distinct adenosine receptor (AR) subtypes, designated $A_1$, $A_{2a}$, $A_{2b}$, and $A_3$ [1, 2, 3]. The search for subtype- and/or tissue-selective ligands of AR as novel pharmacological tools has been an active area of investigation, which has resulted in the synthesis and evaluation of thousands of new chemical entities as agonists, partial agonists, and antagonists [4-6]. The main potential therapeutic indications of $A_1$AR antagonists are for the treatment of cognitive deficits [7], renal failure [8], acute respiratory distress syndrome [9], cardiac arrhythmias [10] and asystolic cardiac arrest [11]. The naturally occurring xanthines, caffeine and theophylline, were the prototypic AR antagonists. The many attempts to improve their potency and selectivity have resulted in the preparation of a large number of xanthine derivatives, and much is known now in terms of their structure–activity relationships (SAR) as well as of their pharmacological activity [12, 13]. In contrast, the structural requirements for receptor binding among the numerous different classes of nonxanthine AR antagonists are still not well defined [14].

In the course of a research program aimed to develop new centrally active $A_1$AR antagonists [15], our efforts were directed toward the synthesis of tricyclic heteroaromatic compounds containing an exocyclic amine on a six–six–five ring system. Some of these structures *(figure 1)* have been described by other groups [16, 17, 18] and were reported to display AR antagonistic properties, with $A_1$-selectivity being generally observed for *N*-cycloalkylamino derivatives. Among them, we focused our attention on the [1,2,4]triazolo[4,3-*a*]quinoxalin-4-amines described by Sarges et al. [19], who suggested that these compounds bind to AR by mimicking adenosine derivatives as shown in *figure 2*. From this overlap it appears that the 2-nitrogen atom may be unnecessary in order to achieve a good fitting and thus an optimal

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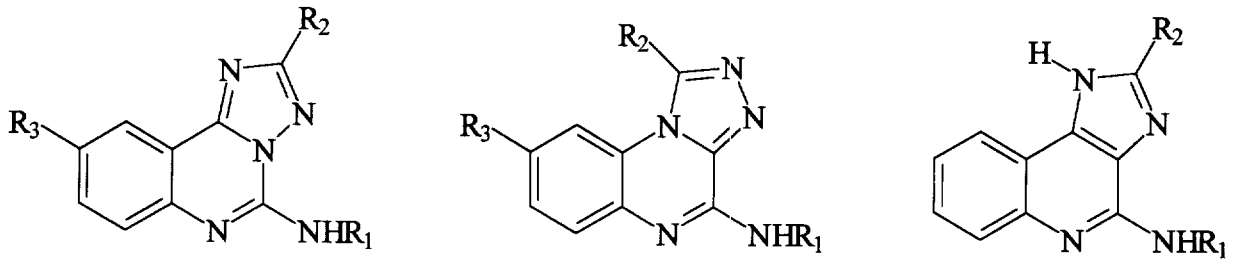



**Figure 1.** Basic structures of representative tricyclic nonxanthine adenosine antagonists.

interaction with the binding site of the receptor. Therefore, we synthesized a number of imidazo- and 1-methylimidazo[1,2-*a*]quinoxalin-4-amines (*figure 3*, compounds **1–26**) and assayed them for $A_1$AR affinity.

## 2. Chemistry

Monosubstitution of 2,3-dichloroquinoxaline (**31a**) with aminoacetaldehyde dimethylacetal or propargylamine gave the respective 2-(substituted) amino-3-chloroquinoxaline **27a** and **28a**, which, after treatment with strong mineral acid, underwent cyclization to imidazo- (**29a**) and 1-methylimidazo[1,2-*a*]quinoxalin-4(5*H*)-one (**30a**) in moderate to good yields (*figure 4*).

The above oxo heterocycles were converted to the final 4-amino derivatives via chlorination with $POCl_3$ or alternatively by an already described single-step silylation–amination procedure [20] using hexamethyldisilazane and the appropriate amine (*figure 5*).

Compounds **9–16** and **24–26** having chloro or fluoro substituents on the carbocyclic ring were prepared as described above from the corresponding 6-halo- and 6,7-dihalo-2,3-dichloroquinoxalines **31b–e**, which were synthesized by a known route [21] from the appropriate *o*-phenylenediamine as illustrated in *figure 6*. The location of the halogen atom in the monochloro and monofluoro derivatives was unequivocally ascertained by 2-D NOE-ROESY experiments carried out on compounds **9**

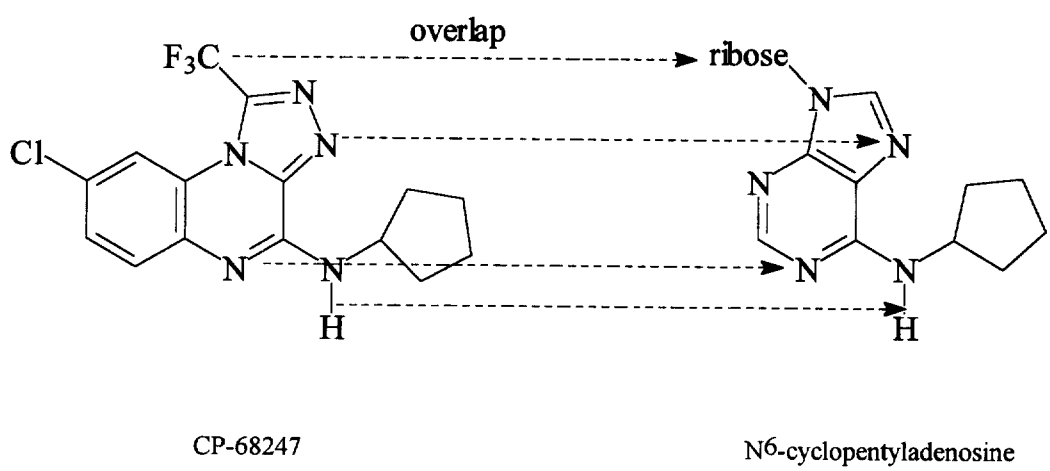
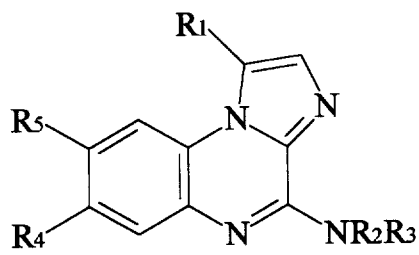



**Figure 2.** Putative binding mode of [1,2,4]triazolo[4,3-*a*]quinoxalin-4-amines: overlap with the agonist $N^6$-cyclopentyladenosine (drawn from [19]).



$R_1$ = H, $CH_3$;
$NR_2R_3$ = $NH_2$, alkylamino, $NH(CH_2)_2OH$, cycloalkylamino, piperidino, morpholino, 4-methylpiperazino;
$R_4$, $R_5$ = H, Cl, F.

**Figure 3.** Imidazo[1,2-*a*]quinoxalin-4-amines assayed for $A_1$AR receptor affinity.

and **12** (as well as on the corresponding dihalogen derivatives **11** and **14** for comparison), which evidenced sharp negative crosspeaks due to a dipolar contact between the 1-H and the *meta*-coupled proton (9-H) adjacent to the halogen substituent.

## 3. Pharmacology

The adenosine $A_1$AR binding affinities of the imidazo[1,2-*a*]quinoxalin-4-amines were determined using standard radioligand binding assay procedures [22]. Competitive displacement of [$^3$H]-1,3-dipropyl-8-cyclopentylxanthine ([$^3$H]DPCPX) in rat brain membranes was used to determine a full concentration–inhibition curve for each compound. The binding potencies of the tested compounds, expressed as their $K_i$ values, are listed in *tables I* and *II*. 4-Cyclopentylamino-1-methylimidazo[1,2-*a*]quinoxaline (**20**, IRFI 165) was also assayed for its $A_{2a}$AR and $A_3$AR binding affinities against [$^3$H]-2-[[4-(2-carboxyethyl)phenethyl]amino]-5'-(*N*-ethylcarboxamido)adenosine ([$^3$H]CGS21680) using rat striatal membranes [23] and against [$^{125}$I]-$N^6$-(4-amino-3-iodobenzyl)adenosine-5'-*N*-methyluronamide ([$^{125}$I]AB-MECA) [24] using human recombinant (HEK-293) cells [25], respectively.

## 4. Results

*Table I* shows that the 1-unsubstituted imidazo[1,2-*a*]quinoxalin-4-amines displayed $K_i(A_1)$ values ranging from 23 nM to 5.6 μM, with the exception of the tertiary amines (including *N*-piperidinyl, *N*-morpholinyl and *N'*-methyl-*N*-piperazinyl derivatives) **1–4** which were almost

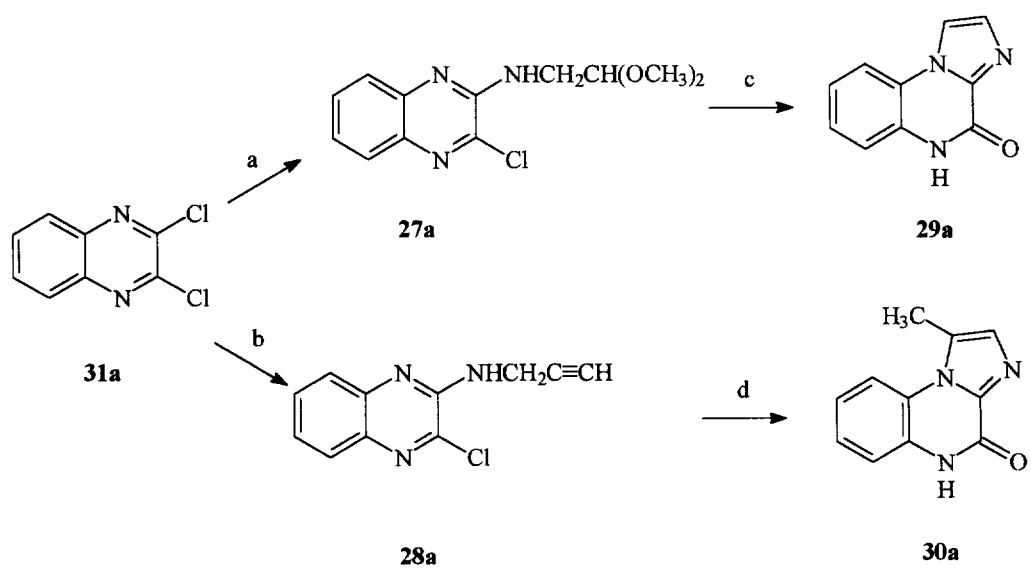



**Figure 4.** (a) $(H_3CO)_2CHCH_2NH_2$, EtOH, reflux; (b) propargylamine, $Et_3N$, EtOH, reflux; (c) 48% HBr (aq.), reflux; (d) conc. $H_2SO_4$, 90 °C.

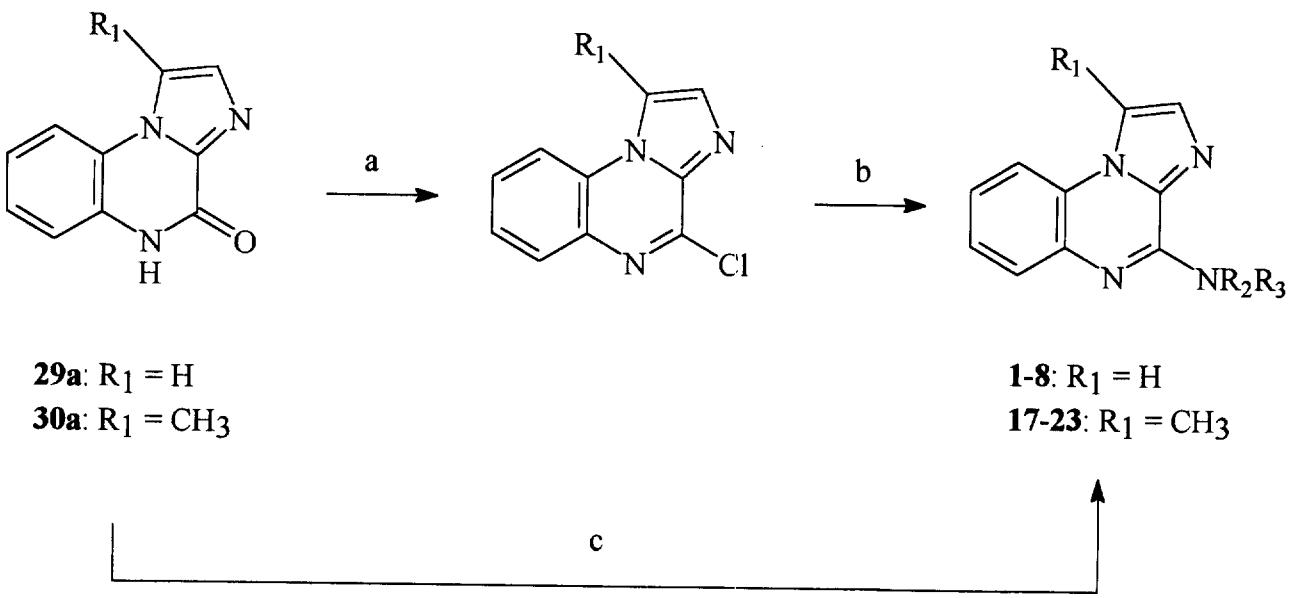
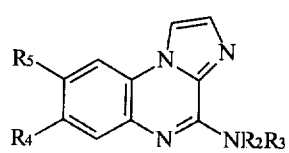



**Figure 5**. (a) $POCl_3$, $C_6H_5N(CH_3)_2$, reflux; (b) $NHR_2R_3$, EtOH, reflux; (c) HMDS, $(NH_4)_2SO_4$, $NHR_2R_3$, reflux.

**Table I.** $A_1AR$ receptor affinities of the reported 1-(unsubstituted)-imidazo[1,2-*a*]quinoxalin-4-amines.




| Compound | $R_2$ | $R_3$ | $R_4$ | $R_5$ | $K_i(A_1)$, nM |
|---|---|---|---|---|---|
| **1** | $CH_2CH_3$ | $CH_2CH_3$ | H | H | ≈ 25,000 |
| **2** | $-(CH_2)_5-$ | | H | H | ≈ 64,000 |
| **3** | $-CH_2CH_2OCH_2CH_2-$ | | H | H | ≈ 18,000 |
| **4** | $-CH_2CH_2N(CH_3)CH_2CH_2-$ | | H | H | > 50,000 |
| **5** | H | $CH(CH_3)_2$ | H | H | 1,430 |
| **6** | H | c-$C_5H_9$ | H | H | 175 |
| **7** | H | c-$C_6H_{11}$ | H | H | 1,015 |
| **8** | H | $CH_2CH_2OH$ | H | H | 1,139 |
| **9** | H | c-$C_5H_9$ | H | Cl | 23.5 |
| **10** | H | c-$C_6H_{11}$ | H | Cl | 394 |
| **11** | H | c-$C_5H_9$ | Cl | Cl | 26.5 |
| **12** | H | c-$C_5H_9$ | H | F | 84.5 |
| **13** | H | c-$C_6H_{11}$ | H | F | 427 |
| **14** | H | c-$C_5H_9$ | F | F | 298 |
| **15** | H | c-$C_6H_{11}$ | Cl | Cl | 5,601 |
| **16** | H | $CH(CH_2CH_3)_2$ | H | Cl | 301.5 |

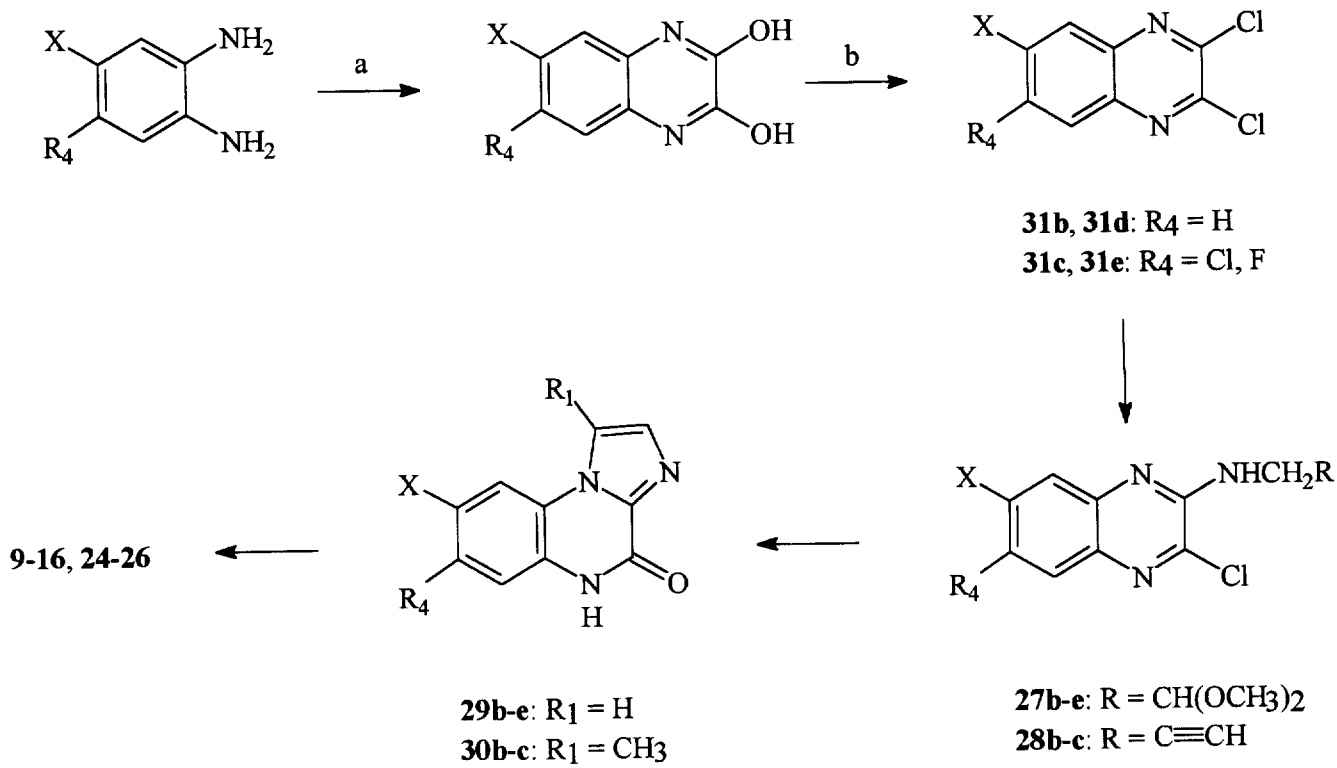
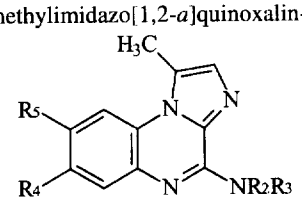



**Figure 6.** (a) Diethyl oxalate, reflux; (b) $POCl_3$, reflux.

**Table II.** $A_1AR$ receptor affinities of the reported 1-methylimidazo[1,2-*a*]quinoxalin-4-amines.



| Compound | $R_2$ | $R_3$ | $R_4$ | $R_5$ | $K_i(A_1)$, nM |
|---|---|---|---|---|---|
| **17** | $-(CH_2)_5-$ | | H | H | > 30,000 |
| **18** | $-CH_2CH_2N(CH_3)CH_2CH_2-$ | | H | H | > 30,000 |
| **19** | H | H | H | H | 160.5 |
| **20** (IRFI 165) | H | c-$C_5H_9$ | H | H | 7.9 |
| **21** | H | c-$C_6H_{11}$ | H | H | 114 |
| **22** | H | $CH_2CH_2OH$ | H | H | 193.5 |
| **23** | H | $CH(CH_2CH_3)_2$ | H | H | 54 |
| **24** | H | c-$C_5H_9$ | H | Cl | 48 |
| **25** | H | c-$C_6H_{11}$ | H | Cl | 55.5 |
| **26** | H | c-$C_5H_9$ | Cl | Cl | 77 |

devoid of affinity. It is noteworthy that the most potent compounds within this subgroup are the 4-cyclopentylamino derivatives, with compound **9** having the lowest $K_i$ value. A significantly decreased affinity with respect to their lower homologues may be noticed for the cyclohexylamino derivatives (about 10-fold), while the open-chain analogue of **9** (**16**) also showed a reduced potency.

The 1-methylimidazo[1,2-*a*]quinoxalin-4-amines proved to be more potent than the corresponding 1-unsubstituted derivatives *(table II)*, with the exception of compounds **24** and **26**, both bearing a chloro substituent in the 8-position. 4-*N*-Piperidinyl and 4-*N'*-methyl-*N*-piperazinyl derivatives (**17** and **18**) showed again no activity at all, whereas the primary amine **19** exhibited a moderate affinity at $A_1$AR. The most potent compound among the entire series is **20** (IRFI 165) which combined 4-cyclopentylamino and 1-methyl substitutions; it has a $K_i(A_1)$ of 7.9 nM. This compound was also shown to be about 300-fold $A_1$ selective versus $A_{2a}$AR, its $K_i(A_{2a})$ being 2.5 μM. In addition, a primary screen on human recombinant $A_3$AR evidenced for IRFI 165 a 69% inhibition of the radioligand binding at 10 μM, thus suggesting a similarly reduced affinity also for this receptor subtype.

## 5. Discussion

The structure–activity analysis of the tested compounds is consistent with the findings emerging from the study of similar tricyclic heteroaromatic systems such as those depicted in *figure 1*. In particular, the loss of potency for all the tertiary amines **1–4**, **17** and **18** clearly suggests the occurrence of a strong interaction between the 4-$NHR_3$ substituent and a hydrogen-bond acceptor in the active binding site of the $A_1$AR. Another common feature in the SAR patterns of these tricyclic systems is the increase in $A_1$ potency and selectivity seen with the *N*-cyclopentyl substitution at the exocyclic amine, and this finding is again confirmed by our results. Furthermore, the increase in $A_1$ affinity observed for 1-methyl derivatives in the present series parallels similar enhancements found with the introduction of lipophilic substituents at the C-1 position of [1,2,4]triazolo [4,3-*a*]quinoxalin-4-amines [17] and at the C-2 position of 1*H*-imidazo[4,5-*c*]quinolin-4-amines [18]. In our series, however, a lack of additivity of the effects of 1-methyl and 8-chloro substituents may be noticed, suggesting that these nearby groups may occupy, at least in part, the same subregion of the active binding site. Alternatively, it may be argued that a limited overall lipophilicity in the substituents at C-1, C-8 and, possibly, C-7 is required for an optimal interaction with the receptor.

The comparable $A_1$ binding potencies between the [1,2,4]triazolo[4,3-*a*]quinoxalin-4-amines described by Sarges et al. [19] and their 2-deaza analogues of the present study, as well as the similarities in the SAR of both series, show that the binding mode depicted in *figure 2* may readily be shared by these tricyclic systems, thus confirming that the 2-nitrogen is unlikely to be crucially involved in the interaction with the receptor. It is noteworthy that the fitting shown in *figure 2* has recently been supported by a theoretical SAR study indicating that this receptor-bound orientation give both electrostatic potential and shape similarities to a number of different classes of adenosine receptor ligands [26].

Thus, on the basis of the binding data and in close analogy with the tentative map of the binding site for the $A_1$ receptor proposed in the above-mentioned study, we were able to draw a putative pharmacophore accommodating 4-(cyclopentylamino)imidazo[1,2-*a*]quinoxalines *(figure 7)*. Besides the H-bond acceptor and the H-bond donor respectively interacting with the 4-NHR and the N-3, which are likely to be essential for the binding, the presence of a cycloalkyl binding site with apparently strict steric requirements and an hydrophobic region having a limited degree of lipophilicity accommodating ability has also been suggested. In agreement with the previously reported models [26, 27], we tentatively propose for IRFI 165 the superimposition with the reference xanthine antagonist DPCPX illustrated in *figure 8*. In this fit the 4-NH of IRFI 165 overlaps with the N7-H of xanthines (which was shown also by our group to be equally essential for binding [28]), carbonyl oxygen in 6-position of xanthine occupies the same position as the N-3, the bridging nitrogen of the imidazoquinoxaline corresponds to the N-1 of DPCPX and, interestingly, the 1-methyl substituent is quite well superimposed with the N1-propyl of the xanthine.

The findings of the present study differ substantially from those of Colotta et al. [29], who assayed a limited number of imidazo[1,2-*a*]quinoxalines having 2-aryl substituents and a $NH_2$ group at position 4 and found a reduced $A_1$AR affinity. However, we believe that the reason for this discrepancy has to be ascribed to the different nature of the substituents, particularly at the exocyclic amine.

The antagonistic character of the imidazo[1,2-*a*]quinoxalin-4-amines described in the present study may be easily understood from the close structural relationships with the known antagonists depicted in *figure 1*. Moreover, IRFI 165, as well as several of its analogues, showed a potent anti-immobility activity on

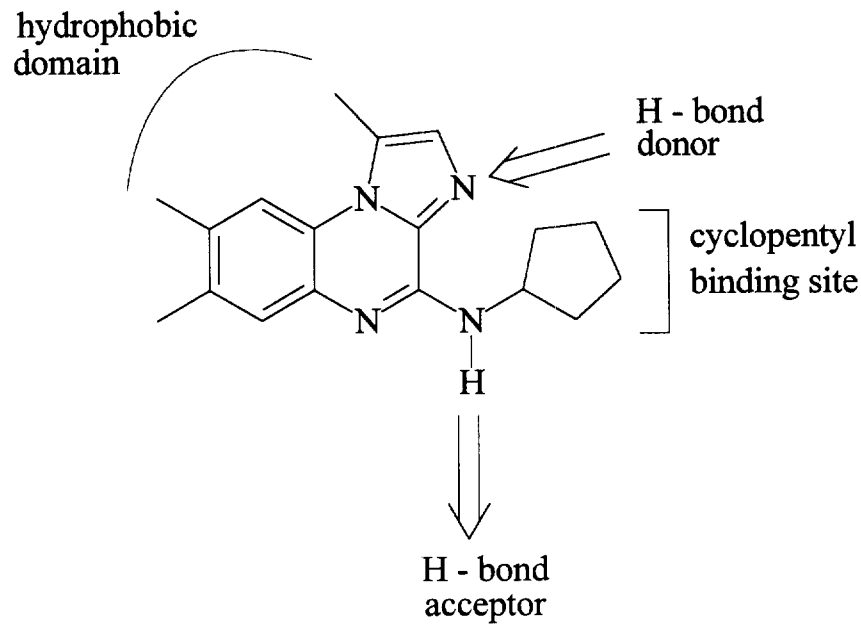



**Figure 7**. Suggested pharmacophore model accommodating 4-(cyclopentylamino)imidazo[1,2-*a*]quinoxalines.

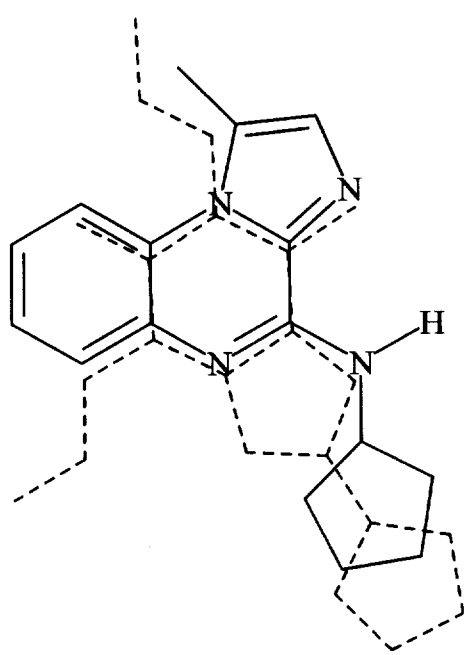


**Figure 8**. Proposed receptor-bound orientation of compound **20**, fitted against 1,3-dipropyl-8-cyclopentylxanthine.

the Porsolt's forced swimming test in rodents [30], an effect which is likely to be mediated by an $A_1$-antagonist induced enhancement of the release of various central neurotransmitters.

In conclusion, this study has provided further insights in the definition of the SAR of nonxanthine heteroaromatic $A_1$AR antagonists, allowing a greater understanding of the structural requisites for maximizing $A_1$ affinity. In addition, a new potent and selective $A_1$AR ligand (i.e. compound **20**, IRFI 165) has been identified and selected for further evaluation. The in vivo pharmacological characterization of IRFI 165 will be fully described elsewhere.

## 6. Experimental protocols

### *6.1. Chemical syntheses*

Melting points were determined by differential scanning calorimetry (DSC) on a Perkin Elmer DSC 7 instrument. Microanalytical data were taken on a Carlo Erba 1106 analyzer or were obtained from Redox snc, Cologno M., Italy. Analyses indicated by the symbols of the elements were within ±0.4% of theoretical values. Infrared (IR) spectra were recorded on a Perkin Elmer 881 spectrophotometer as KBr pellets. $^1$H-NMR spectra were obtained on a Varian EM 360L instrument or on a Bruker AM400 spectrometer using tetramethylsilane

(TMS, δ 0.00) or $CHCl_3$ contained in the deuterated solvent (δ 7.24) as the internal standard. Silica-gel plates (Merck $F_{254}$) were used for analytical TLC. Chromatographic separations were performed on silica gel 60 (Merck, 230–400 mesh) in a preparative medium pressure liquid chromatographic apparatus (Gilson 305) equipped with UV (254 nm) detector (Gilson 112).

2,3,6-Trichloroquinoxaline (**31b**) was obtained from Acros Organics; all the other fine chemicals were from Aldrich.

*6.1.1. Imidazo[1,2-a]quinoxalin-4(5H)-ones (**29a–e**)*

A mixture of 5.0 g (25 mmol) of 2,3-dichloroquinoxaline (**31a**) and 5.5 mL (50 mmol) of aminoacetaldehyde dimethyl acetal in 75 mL of absolute ethanol was refluxed for 4 h. Evaporation of the organic solvent under reduced pressure gave a residue which was dissolved in ethyl acetate. The organic solution was washed with water and brine, dried (anhydrous $Na_2SO_4$) and evaporated at reduced pressure to afford a crude product which was purified by column chromatography (eluant: $CH_2Cl_2$), obtaining 5.0 g of 2-chloro-3-(2,2-dimethoxyethylamino)quinoxaline **27a** (IR: 3347, 2936, 1580, 1523, 1129 $cm^{-1}$).

Compound **27a** (4.5 g, 16.8 mmol) was treated with 20 mL of 48% aqueous HBr and the mixture was refluxed for 4 h. Upon cooling the mixture was neutralized by aqueous NaOH and the resulting precipitate was filtered under suction and dried to obtain 3.2 g of imidazo[1,2-*a*]quinoxalin-4(5*H*)-one (**29a**) [31] which was recrystallized from water. M.p. 329.1 °C (DSC onset); IR (KBr): 3105 (NH), 1685 $cm^{-1}$ (C=O); $^1$H-NMR (DMSO-$d_6$): δ 12.2 (1H, sb), 8.5 (1H, d, H-1, $J$ = 2 Hz) 8.2–7.85 (1H, m), 7.5 (1H, d, H-2, $J$ = 2 Hz), 7.4–7.05 (3H, m); UV (DMSO): $\lambda_{max}$ = 284, 307, 320 nm. Anal. $C_{10}H_7N_3O$ (C, H, N): calc. C 64.86, H 3.81, N 22.69%; found C 64.64, H 3.79, N 22.65%.

The following compounds were prepared in an analogous manner:

*8-Chloroimidazo[1,2-a]quinoxalin-4(5H)-one (**29b**)* (prepared from 2,3,6-trichloroquinoxaline (**31b**); recrystallized from DMF). M.p. > 350 °C (DSC); IR (KBr): 3144 (NH), 1724, 1684 $cm^{-1}$ (C=O); UV (DMSO): $\lambda_{max}$ = 315, 327 nm. Anal. $C_{10}H_6N_3ClO$ (C, H, N): calc. C 54.69, H 2.75, N 19.13%; found C 54.37, H 2.75, N 19.11%.

*7,8-Dichloroimidazo[1,2-a]quinoxalin-4(5H)-one (**29c**)* [32] (prepared from 2,3,6,7-tetrachloroquinoxaline (**31c**); recrystallized from DMF). M.p. > 350 °C (DSC); IR (KBr): 3069 (NH), 3027 (CH), 1711 $cm^{-1}$ (C=O); $^1$H-NMR (400 MHz, $CDCl_3$/DMSO-$d_6$): δ 12.25 (1H, sb), 8.70 (1H, d, H-1, $J$ = 2 Hz), 8.63 (1H, s), 7.74 (1H, d, H-1, $J$ = 2 Hz), 7.66 (1H, s); UV (DMSO): $\lambda_{max}$ = 289, 318, 331 nm. Anal. $C_{10}H_5N_3Cl_2O$ (C, H, N): calc. C 47.27, H 1.98, N 16.54%; found C 47.37, H 2.02, N 16.45%.

*8-Fluoroimidazo[1,2-a]quinoxalin-4(5H)-one (**29d**)* (prepared from 2,3-dichloro-6-fluoroquinoxaline **31d**; recrystallized from absolute ethanol). IR (KBr): 3131 (NH), 1698 $cm^{-1}$ (C=O); $^1$H-NMR (DMSO-$d_6$): δ 12.2 (1H, sb), 8.7 (1H, d, H-1, $J$ = 2 Hz), 8.3 (1H, dd, H-9, $J_{o\text{-HF}}$ = 10 Hz), 7.8 (1H, d, H-1, $J$ = 2 Hz), 7.6–7.2 (2H, m).

*7,8-Difluoroimidazo[1,2-a]quinoxalin-4(5H)-one (**29e**)* (prepared from 2,3-dichloro-6,7-difluoroquinoxaline (**31e**)). IR (KBr): 3127 (NH), 1683 $cm^{-1}$ (C=O); $^1$H-NMR (DMSO-$d_6$): δ 12.6 (1H, sb), 8.6 (1H, d, H-1, $J$ = 2 Hz), 8.35–7.9 (2H, m), 7.7 (1H, dd, H-6, $J_{o\text{-HF}}$ = 10 Hz, $J_{m\text{-HF}}$ = 7 Hz).

*6.1.2. 1-Methylimidazo[1,2-a]quinoxalin-4(5H)-ones (**30a–c**)*

A mixture of 2,3-dichloroquinoxaline (10.0 g, 50 mmol), propargylamine (4.5 mL, 65 mmol), triethylamine (10.5 mL, 75 mmol) in 50 mL of absolute ethanol was heated at reflux for 4 h. Evaporation of the organic solvent under reduced pressure gave a residue which was purified by column chromatography (eluant: $CH_2Cl_2$), obtaining 7.0 g of 2-chloro-3-(propargylamino) quinoxaline **28a** (IR: 3441, 3282, 1518 $cm^{-1}$).

This compound (7.0 g, 32 mmol) was treated with 10 mL of concentrated $H_2SO_4$ and the mixture was stirred at 90 °C for 1 h. Upon cooling the mixture was cautiously neutralized by aqueous NaOH and the resulting precipitate was filtered under suction, dried, decolorized and recrystallized from DMF to obtain 2.4 g of 1-methylimidazo[1,2-*a*]quinoxalin-4(5*H*)-one (**30a**). M.p. > 320 °C (DSC); IR (KBr): 3132 (NH), 2977 (CH), 1691 $cm^{-1}$ (C=O); $^1$H-NMR (DMSO-$d_6$): δ 11.8 (1H, sb), 8.1–7.95 (1H, m), 7.4–7.1 (4H, m), 2.8 (3H, s); UV (DMSO): $\lambda_{max}$ = 263, 292, 304 nm. Anal. $C_{11}H_9N_3O$ (C, H, N): calc. C 66.32, H 4.55, N 21.09%; found C 66.22, H 4.62, N 20.89%.

The following compounds were prepared in an analogous manner:

*8-Chloro-1-methylimidazo[1,2-a]quinoxalin-4(5H)-one (**30b**)* (prepared from 2,3,6-trichloroquinoxaline **31b**; recrystallized from DMF). IR (KBr): 3044 (CH), 1689 $cm^{-1}$ (C=O); $^1$H-NMR (DMSO-$d_6$): δ 11.8 (1H, sb), 8.0 (1H, d, H-9, $J$ = 2 Hz), 7.4–7.2 (3H, m), 2.85 (3H, s).

*7,8-Dichloro-1-methylimidazo[1,2-a]quinoxalin-4(5H)-one (**30c**)* (prepared from 2,3,6,7-tetrachloroquinoxaline **31c**; recrystallized from *N,N*-dimethylacetamide). IR (KBr): 1684 $cm^{-1}$ (C=O); $^1$H-NMR (DMSO-$d_6$): δ 12.3 (1H, sb), 8.1 (1H, s), 7.6 (1H, s), 7.25 (1H, s), 2.8 (3H, s).

*6.1.3. 4-Diethylaminoimidazo[1,2-a]quinoxaline (1)*

Compound **29a** (0.47 g, 2.5 mmol) was treated with $POCl_3$ (5.6 mL) and *N,N*-dimethylaniline (0.42 mL) and the mixture was refluxed for 2 h. Excess reagents were removed under vacuum and the residue was taken up in $CHCl_3$ and washed with water until neutral reaction, dried, evaporated and recrystallized from *n*-hexane/$CHCl_3$, affording 0.29 g of 4-chloroimidazo[1,2-*a*]quinoxaline. M.p. (DSC onset) 192.3 °C; IR (KBr): 3064, 1503 $cm^{-1}$; $^1$H-NMR ($CDCl_3$): δ 8.2 (1H, d, H-1, *J* = 2 Hz), 8.1–7.85 (1H, m), 7.8 (1H, d, H-2, *J* = 2 Hz), 7.75–7.4 (3H, m); UV (EtOH): $\lambda_{max}$ = 247, 315, 330 nm. Anal. $C_{10}H_6N_3Cl$ (C, H, N): calc. C 58.98, H 2.97, N 20.64%; found C 58.75, H 2.98, N 20.57%.

A mixture of the above compound (2.8 g, 13.7 mmol) and diethylamine (9.8 mL, 94.7 mmol) in 40 mL of absolute ethanol was refluxed for 4 h. Evaporation of the organics under reduced pressure gave a residue which was taken up in $CHCl_3$ and washed with water and brine, dried, evaporated, purified by column chromatography (eluant: $CH_2Cl_2$) and recrystallized from *n*-hexane to obtain 1.3 g of the title compound. M.p. (DSC onset) 91.7 °C; IR (KBr): 2976, 1518, 1425 $cm^{-1}$; $^1$H-NMR ($CDCl_3$): δ 7.9 (1H, s), 7.7–7.4 (3H, m), 7.35–7.05 (2H, m), 4.15 (4H, q), 1.3 (6H, t); UV (EtOH): $\lambda_{max}$ = 231, 250, 293, 305, 332, 348 nm. Anal. $C_{14}H_{16}N_4$ (C, H, N): calc. C 69.97, H 6.71, N 23.31%; found C 69.99, H 6.80, N 23.13%.

The following compounds were similarly prepared from the appropriate imidazo- (**29a–e**) or 1-methylimidazo[1,2-*a*]quinoxalin-4(5*H*)-one (**30a–c**).

*6.1.4. 4-(N-piperidinyl)imidazo[1,2-a]quinoxaline (2)*

Recryst. solvent: ethanol/water. M.p. (DSC onset) 108.2 °C; IR (KBr): 3107, 2935, 1517 $cm^{-1}$; $^1$H-NMR ($CDCl_3$): δ 7.9 (1H, d, H-1, *J* = 2 Hz), 7.75–7.4 (3H, m), 7.4–7.1 (2H, m), 4.3 (4H, t), 1.9–1.6 (6H, m); UV (EtOH): $\lambda_{max}$ = 231, 249, 293, 305, 333 nm. Anal. $C_{15}H_{16}N_4$ (C, H, N): calc. C 71.40, H 6.39, N 22.20%; found C 71.38, H 6.63, N 22.61%.

*6.1.5. 4-(N-morpholinyl)imidazo[1,2-a]quinoxaline (3)*

Recryst. solvent: absolute ethanol. M.p. (DSC onset) 142.8 °C; IR (KBr): 3016, 2962, 1517 $cm^{-1}$; $^1$H-NMR ($CDCl_3$): δ 8.0 (1H, s), 7.8–7.5 (3H, m), 7.5–7.2 (2H, m), 4.4 (4H, t), 3.9 (4H, t); UV (EtOH): $\lambda_{max}$ = 230, 248, 292, 304, 330 nm. Anal. $C_{14}H_{14}N_4O$ (C, H, N): calc. C 66.13, H 5.55, N 22.03%; found C 65.43, H 5.47, N 22.25%.

*6.1.6. 4-(N'-Methyl-N-piperazinyl)imidazo[1,2-a]quinoxaline (4)*

This compound was obtained as the dihydrochloride by treatment of the free base with ethanolic HCl. M.p. (DSC onset) 305.6 °C; IR (KBr): 2697, 1560, 1508 $cm^{-1}$; $^1$H-NMR (DMSO-$d_6$/$CD_3OD$): δ 8.7 (1H, sb), 8.3–8.0 (1H, m), 7.8–7.35 (4H, m), 5.5 (4H, t), 3.7–3.3 (4H, m), 2.8 (3H, s); UV (EtOH): $\lambda_{max}$ = 230, 291, 304, 320 nm. Anal. $C_{15}H_{17}N_5 \cdot 2HCl$ (C, H, N): calc. C 52.95, H 5.63, N 20.58%; found C 52.81, H 5.78, N 20.13%.

*6.1.7. 4-Isopropylaminoimidazo[1,2-a]quinoxaline (5)*

Recryst. solvent: *n*-hexane. M.p. (DSC onset) 102.7 °C; IR (KBr): 3230, 2966, 1559 $cm^{-1}$; $^1$H-NMR (DMSO-$d_6$): δ 8.55 (1H, s), 8.2–7.95 (1H, m), 7.6–7.4 (2H, m), 7.4–7.2 (3H, m), 4.5 (1H, m), 1.25 (6H, d); UV (EtOH): $\lambda_{max}$ = 227, 244, 285, 297, 318, 332 nm. Anal. $C_{13}H_{14}N_4$ (C, H, N): calc. C 69.00, H 6.23, N 24.76%; found C 69.17, H 6.70, N 25.05%.

*6.1.8. 4-Cyclopentylaminoimidazo[1,2-a]quinoxaline (6)*

Recryst. solvent: DMF/water. M.p. (DSC onset) 114.3 °C; IR (KBr): 3419, 2962, 1543 $cm^{-1}$; $^1$H-NMR ($CDCl_3$): δ 7.9 (1H, s), 7.8–7.0 (5H, m), 6.2 (1H, d), 4.7 (1H, m), 2.3–2.05 (2H, m), 2.0–1.3 (6H, m); UV (EtOH): $\lambda_{max}$ = 228, 244, 285, 297, 319, 333 nm. Anal. $C_{15}H_{16}N_4$ (C, H, N): calc. C 71.40, H 6.39, N 22.20%; found C 71.13, H 7.30, N 22.29%.

*6.1.9. 4-Cyclohexylaminoimidazo[1,2-a]quinoxaline (7)*

Recryst. solvent: *n*-hexane/ethyl acetate. M.p. (DSC onset) 94.0 °C; IR (KBr): 3227, 2924, 1560 $cm^{-1}$; $^1$H-NMR ($CDCl_3$): δ 7.85 (1H, d, H-1, *J* = 2 Hz), 7.8–7.4 (3H, m), 7.4–7.1 (2H, m), 6.0 (1H, d), 4.2 (1H, m), 2.3–1.95 (2H, m), 1.95–0.9 (8H, m); UV (EtOH): $\lambda_{max}$ = 225, 242, 283, 294, 316, 330 nm. Anal. $C_{16}H_{18}N_4$ (C, H, N): calc. C 72.15, H 6.81, N 21.03%; found C 72.25, H 7.04, N 21.19%.

*6.1.10. 4-(2-Hydroxyethylamino)imidazo[1,2-a]quinoxaline (8)*

Recryst. solvent: isopropyl acetate. M.p. (DSC onset) 150.8 °C; IR (KBr): 3312, 1598, 1564 $cm^{-1}$; $^1$H-NMR ($CDCl_3$/$CD_3OD$): δ 8.2 (1H, s), 8.1–7.55 (2H, m), 7.55 (1H, s), 7.4–7.2 (2H, m), 3.8 (4H, s); UV (EtOH): $\lambda_{max}$ = 227, 285, 297, 317, 330 nm. Anal. $C_{12}H_{12}N_4O$ (C, H, N): calc. C 63.14, H 5.30, N 24.55%; found C 63.16, H 5.40, N 24.99%.

*6.1.11. 8-Chloro-4-cyclopentylaminoimidazo[1,2-a]quinoxaline (9)*

Recryst. solvent: *n*-hexane/ethyl acetate. M.p. (DSC onset) 140.1 °C; IR (KBr): 3401, 2955, 1554 $cm^{-1}$; $^1$H-NMR (400 MHz, $CDCl_3$): δ 7.87 (1H, d, H-1, *J* = 2 Hz), 7.68–7.62 (2H, m), 7.53 (1H, d, H-2, *J* = 2 Hz), 7.35 (1H, dd, H-7), 6.10 (1H, s, N*H*), 4.68–4.58 (1H, m), 2.25–2.13 (2H, m), 1.85–1.55 (6H, m); UV (EtOH): $\lambda_{max}$ = 229,

245, 289, 301, 326, 340 nm. Anal. $C_{15}H_{15}ClN_4$ (C, H, N): calc. C 62.83, H 5.27, N 19.54%; found C 63.04, H 5.36, N 19.64%.

*6.1.12. 8-Chloro-4-cyclohexylaminoimidazo[1,2-a] quinoxaline (10)*

Recryst. solvent: ethanol/water. M.p. (DSC onset) 126.7 °C; IR (KBr): 3413, 2926, 1555 $cm^{-1}$; $^1$H-NMR ($CDCl_3$): δ 7.85 (1H, s), 7.7–7.2 (4H, m), 6.1 (1H, d), 4.2 (1H, m), 2.3–2.0 (2H, m), 2.0–1.2 (8H, m); UV (EtOH): $\lambda_{max}$ = 229, 245, 289, 301, 326, 340 nm. Anal. $C_{16}H_{17}ClN_4$ (C, H, N): calc. C 63.89, H 5.70, N 18.63%; found C 64.07, H 5.79, N 18.80%.

*6.1.13. 4-Cyclopentylamino-7,8-dichloroimidazo[1,2-a]quinoxaline (11)*

Recryst. solvent: ethanol/water. M.p. (DSC onset) 139.4 °C; IR (KBr): 3247, 2961, 1589, 1556 $cm^{-1}$; $^1$H-NMR (400 MHz, $CDCl_3$): δ 7.85 (1H, d, H-1, $J$ = 2 Hz), 7.82 (1H, s, H-9), 7.73 (1H, s, H-6), 7.55 (1H, d, H-2, $J$ = 2 Hz), 6.22 (1H, d, N*H*), 4.65–4.55 (1H, m), 2.22–2.12 (2H, m), 1.85–1.55 (6H, m); UV (EtOH): $\lambda_{max}$ = 235, 274, 292, 304, 330, 345 nm. Anal. $C_{15}H_{14}Cl_2N_4$ (C, H, N): calc. C 56.09, H 4.39, N 17.44%; found C 56.13, H 4.41, N 17.52%.

*6.1.14. 4-Cyclopentylamino-8-fluoroimidazo[1,2-a] quinoxaline (12)*

Recryst. solvent: ethanol/water. M.p. (DSC onset) 85.7 °C; IR (KBr): 3255, 2964, 1551 $cm^{-1}$; $^1$H-NMR (400 MHz, $CDCl_3$): δ 7.83 (1H, d, H-1, $J$ = 2 Hz), 7.68 (1H, dd, H-6, $J_{m\text{-HF}}$ = 6 Hz), 7.54 (1H, d, H-2, $J$ = 2 Hz), 7.34 (1H, dd, H-9, $J_{o\text{-HF}}$ = 9 Hz), 7.17–7.11 (1H, m, H-7), 6.02 (1H, d, N*H*), 4.67–4.58 (1H, m), 2.23–2.12 (2H, m), 1.86–1.56 (6H, m); UV (EtOH): $\lambda_{max}$ = 226, 268, 285, 296, 323, 336 nm. Anal. $C_{15}H_{15}FN_4$ (C, H, N): calc. C 66.65, H 5.59, N 20.73%; found C 66.36, H 5.66, N 20.86%.

*6.1.15. 4-Cyclohexylamino-8-fluoroimidazo[1,2-a]quinoxaline (13)*

Recryst. solvent: ethanol. M.p. (DSC onset) 157.6 °C; IR (KBr): 3415, 2927, 1556 $cm^{-1}$; $^1$H-NMR ($CDCl_3$): δ 7.8 (1H, dd, $J$ = 2 Hz), 7.7–7.35 (1H, dd, $J_{HF}$ = 16 Hz), 7.55 (1H, d, $J$ = 2 Hz), 7.55–7.25 (1H, dd, $J_{HF}$ = 16 Hz), 7.05 (1H, dd), 6.0 (1H, d), 4.25 (1H, m), 2.35–1.2 (10H, m); UV (EtOH): $\lambda_{max}$ = 226, 239, 285, 297, 323, 337 nm. Anal. $C_{16}H_{17}FN_4$ (C, H, N): calc. C 67.59, H 6.03, N 19.71%; found C 67.31, H 6.04, N 19.70%.

*6.1.16. 4-Cyclopentylamino-7,8-difluoroimidazo[1,2-a]quinoxaline (14)*

Recryst. solvent: ethanol/water. M.p. (DSC onset) 146.0 °C; IR (KBr): 3263, 2955, 1554 $cm^{-1}$; $^1$H-NMR (400 MHz, $CDCl_3$): δ 7.78 (1H, d, H-1, $J$ = 2 Hz), 7.54 (1H, d, H-2, $J$ = 2 Hz), 7.51 (1H, dd, H-9, $J_{o\text{-HF}}$ = 10 Hz, $J_{m\text{-HF}}$ = 7 Hz), 7.44 (1H, dd, H-6, $J_{o\text{-HF}}$ = 10 Hz, $J_{m\text{-HF}}$ = 7 Hz), 6.12 (1H, d, N*H*), 4.65–4.55 (1H, m), 2.22–2.12 (2H, m), 1.85–1.05 (6H, m); UV (EtOH): $\lambda_{max}$ = 225, 241, 270, 295, 323, 337 nm. Anal. $C_{15}H_{14}F_2N_4$ (C, H, N): calc. C 62.49, H 4.89, N 19.43%; found C 62.46, H 5.03, N 19.65%.

*6.1.17. 4-Cyclohexylamino-7,8-dichloroimidazo[1,2-a]quinoxaline (15)*

Recryst. solvent: ethanol/water. M.p. (DSC onset) 162.3 °C; IR (KBr): 3332, 2929, 1587, 1550 $cm^{-1}$; $^1$H-NMR ($CDCl_3$): δ 7.8 (2H, m), 7.7 (1H, s), 7.55 (1H, d, $J$ = 2 Hz), 6.2 (1H, d), 4.4 (1H, m), 2.3–1.2 (10H, m); UV (EtOH): $\lambda_{max}$ = 234, 274, 292, 304, 330, 345 nm. Anal. $C_{16}H_{16}Cl_2N_4 \cdot 1/2H_2O$ (C, H, N): calc. C 55.82, H 4.98, N 16.28%; found C 55.83, H 5.00, N 16.31%.

*6.1.18. 1-Methyl-4-(N-piperidinyl)imidazo[1,2-a]quinoxaline (17)*

Recryst. solvent: ethanol/water. M.p. (DSC onset) 78.9 °C; IR (KBr): 3018, 2927, 1502 $cm^{-1}$; $^1$H-NMR ($CDCl_3$): δ 8.05 (1H, dd), 7.65 (1H, dd), 7.5–7.0 (3H, m), 4.25 (4H, t), 2.85 (3H, s), 1.9–1.6 (6H, m); UV (EtOH): $\lambda_{max}$ = 249, 281, 297, 310, 329 nm. Anal. $C_{16}H_{18}N_4$ (C, H, N): calc. C 72.15, H 6.81, N 21.03%; found C 71.84, H 7.09, N 20.70%.

*6.1.19. 1-Methyl-4-(N'-methyl-N-piperazinyl)imidazo [1,2-a]quinoxaline (18)*

Recryst. solvent: *n*-hexane/isopropyl acetate. M.p. (DSC onset) 108.0 °C; IR (KBr): 2928, 1535, 1510 $cm^{-1}$; $^1$H-NMR ($CDCl_3$): δ 8.1 (1H, dd), 7.7 (1H, dd), 7.4–7.0 (3H, m), 4.35 (4H, t), 2.85 (3H, s), 2.6 (4H, t), 2.3 (3H, s); UV (EtOH): $\lambda_{max}$ = 228, 248, 279, 309, 325 nm. Anal. $C_{16}H_{19}N_5$ (C, H, N): calc. C 68.30, H 6.81, N 24.89%; found C 68.37, H 7.15, N 25.05%.

*6.1.20. 1-Methylimidazo[1,2-a]quinoxalin-4-amine (19)*

Recryst. solvent: *n*-hexane/ethyl acetate. M.p. (DSC onset) 245.0 °C; IR (KBr): 3391, 1641, 1517 $cm^{-1}$; $^1$H-NMR (400 MHz, $CDCl_3$/DMSO-$d_6$): δ 8.35 (1H, dd), 7.87 (1H, dd), 7.66 (1H, s), 7.65–7.56 (2H, m), 3.03 (3H, s); UV (EtOH): $\lambda_{max}$ = 258, 268, 301, 313 nm. Anal. $C_{11}H_{10}N_4$ (C, H, N): calc. C 66.65, H 5.08, N 28.26%; found C 66.56, H 5.10, N 28.21%.

*6.1.21. 4-(2-Hydroxyethylamino)-1-methylimidazo [1,2-a]quinoxaline (22)*

Recryst. solvent: ethanol/water. M.p. (DSC onset) 173.4 °C; IR (KBr): 3415, 3217, 1558 $cm^{-1}$; $^1$H-NMR ($CDCl_3$): δ 8.0 (1H, dd), 7.7–7.2 (3H, m), 7.2 (1H, s), 6.6 (1H, m), 5.3 (1H, sb), 3.85 (4H, m), 2.8 (3H, s); UV (EtOH): $\lambda_{max}$ = 225, 242, 271, 301, 314, 327 nm. Anal. $C_{13}H_{14}N_4O$ (C, H, N): calc. C 64.45, H 5.82, N 23.12%; found C 64.29, H 5.91, N 23.13%.

*6.1.22. 4-(1-Ethylpropylamino)-1-methylimidazo[1,2-a]quinoxaline (23)*

Recryst. solvent: *n*-hexane. M.p. (DSC onset) 75.9 °C; IR (KBr): 3231, 2965, 1546 $cm^{-1}$; $^1$H-NMR ($CDCl_3$): δ 8.0 (1H, dd), 7.7 (1H, dd), 7.4–7.1 (3H, m), 5.9 (1H, d), 4.2 (1H, m), 2.85 (3H, s), 1.65 (4H, m), 0.95 (6H, t); UV (EtOH): $\lambda_{max}$ = 221, 241, 268, 298, 313 nm. Anal. $C_{16}H_{20}N_4$ (C, H, N): calc. C 71.61, H 7.51, N 20.83%; found C 71.12, H 7.53, N 20.79%.

*6.1.23. 8-Chloro-4-cyclopentylamino-1-methylimidazo [1,2-a]quinoxaline (24)*

Recryst. solvent: petroleum ether/ethyl acetate. M.p. (DSC onset) 130.5 °C; IR (KBr): 3421, 2954, 1555 $cm^{-1}$; $^1$H-NMR ($CDCl_3$): δ 7.95 (1H, d, $J$ = 2 Hz), 7.6 (1H, d, $J$ = 9 Hz), 7.35–7.15 (2H, m), 6.1 (1H, d), 4.5 (1H, m), 2.8 (3H, s), 2.4–2.05 (2H, m), 2.0–1.3 (6H, m); UV (EtOH): $\lambda_{max}$ = 227, 251, 276, 305, 323, 336 nm. Anal. $C_{16}H_{17}ClN_4$ (C, H, N): calc. C 63.89, H 5.70, N 18.63%; found C 63.79, H 5.79, N 18.58%.

*6.1.24. 8-Chloro-4-cyclohexylamino-1-methylimidazo [1,2-a]quinoxaline (25)*

Recryst. solvent: *n*-hexane. M.p. (DSC onset) 130.4 °C; IR (KBr): 3410, 2927, 1551 $cm^{-1}$; $^1$H-NMR ($CDCl_3$): δ 7.95 (1H, d, $J$ = 2 Hz), 7.6 (1H, d, $J$ = 9 Hz), 7.4–7.2 (2H, m), 6.1 (1H, d), 4.2 (1H, m), 2.8 (3H, s), 2.3–1.1 (10H, m); UV (EtOH): $\lambda_{max}$ = 227, 250, 276, 305, 323, 336 nm. Anal. $C_{17}H_{19}ClN_4$ (C, H, N): calc. C 64.86, H 6.08, N 17.80%; found C 64.86, H 6.21, N 17.91%.

*6.1.25. 4-Cyclopentylamino-7,8-dichloro-1-methylimidazo[1,2-a]quinoxaline (26)*

Recryst. solvent: *n*-hexane/ethyl acetate. M.p. (DSC onset) 213.5 °C; IR (KBr): 3411, 2960, 1548 $cm^{-1}$; $^1$H-NMR ($CDCl_3$): δ 8.0 (1H, s), 7.75 (1H, s), 7.25 (1H, s), 6.2 (1H, d), 4.6 (1H, m), 2.8 (3H, s), 2.3–1.3 (8H, m); UV (EtOH): $\lambda_{max}$ = 233, 279, 308, 327, 341 nm. Anal. $C_{16}H_{16}Cl_2N_4$ (C, H, N): calc. C 57.33, H 4.81, N 16.71%; found C 57.41, H 4.82, N 16.68%.

*6.1.26. 8-Chloro-4-(1-ethylpropylamino)imidazo[1,2-a]quinoxaline (16)*

A mixture of compound **29b** (2.66 g, 12 mmol), hexamethyldisilazane (8.9 mL, 42 mmol), ammonium sulfate (0.32 g, 2.4 mmol) and 1-ethylpropylamine (7.0 mL, 60 mmol) was stirred at 120 °C in a Dean–Stark apparatus for 18 h. Upon cooling, the mixture was concentrated in vacuo to give a residue which was taken up in ethyl acetate plus water. The biphasic mixture contained a solid (consisting mainly of unreacted imidazoquinoxalinone) which was filtered away; the filtrate was collected and the organic layer was washed with brine, dried and evaporated, thus obtaining the crude title compound (0.97 g). Column chromatography (eluant: $CH_2Cl_2$) and recrystallization from *n*-hexane afforded pure **16**. M.p. (DSC onset) 125.1 °C; IR (KBr): 3406, 3105, 2964, 1532 $cm^{-1}$; $^1$H-NMR ($CDCl_3$/$CD_3OD$): δ 8.4 (1H, s), 8.05 (1H, d, $J$ = 2 Hz), 7.8–7.4 (3H, m), 3.85 (1H, m), 1.65 (4H, m), 0.95 (6H, t); UV (EtOH): $\lambda_{max}$ = 230, 245, 289, 301, 326 nm. Anal. $C_{15}H_{17}ClN_4$ (C, H, N): calc. C 62.39, H 5.93, N 19.40%; found C 62.17, H 6.02, N 19.46%.

The following compounds were similarly prepared from 1-methylimidazo[1,2-*a*]quinoxalin-4(5*H*)-one (**30a**) and the appropriate amine.

*6.1.27. 4-Cyclopentylamino-1-methylimidazo[1,2-a] quinoxaline (20)*

Recryst. solvent: ethyl acetate. M.p. (DSC onset) 167.3 °C; IR (KBr): 3294, 2948, 1544 $cm^{-1}$; $^1$H-NMR ($CDCl_3$): δ 8.0 (1H, dd), 7.7 (1H, dd), 7.4–7.05 (3H, m), 6.0 (1H, d), 4.6 (1H, m), 2.8 (3H, s), 2.3–2.05 (2H, m), 2.0–1.4 (6H, m); UV (EtOH): $\lambda_{max}$ = 225, 243, 272, 301, 316, 329 nm. Anal. $C_{16}H_{18}N_4$ (C, H, N): calc. C 72.15, H 6.81, N 21.03%; found C 71.86, H 6.81, N 20.79%.

*6.1.28. 4-Cyclohexylamino-1-methylimidazo[1,2-a] quinoxaline (21)*

Recryst. solvent: *n*-hexane/ethyl acetate. M.p. (DSC onset) 126.5 °C; IR (KBr): 3345, 2938, 1546 $cm^{-1}$; $^1$H-NMR ($CDCl_3$): δ 8.0 (1H, dd), 7.7 (1H, dd), 7.5–7.1 (3H, m), 6.0 (1H, d), 4.2 (1H, m), 2.85 (3H, s), 2.3–1.95 (2H, m), 1.95–0.9 (8H, m); UV (EtOH): $\lambda_{max}$ = 225, 243, 272, 301, 316, 329 nm. Anal. $C_{17}H_{20}N_4$ (C, H, N): calc. C 72.83, H 7.19, N 19.98%; found C 72.21, H 7.54, N 20.14%.

*6.1.29. General procedure for the synthesis of 2,3,6,7-tetrachloro- (31c), 2,3-dichloro-6-fluoro- (31d) and 2,3-dichloro-6,7-difluoroquinoxaline (31e)*

The title compounds were prepared according to literature methods [21] from the appropriate 1,2-phenylenediamine, which in turn was commercially available (4,5-dichloro-1,2-phenylenediamine) or was

obtained by classical $SnCl_2$/HCl reduction of the corresponding 2-nitroaniline. Briefly, a mixture of the phenylenediamine (10 mmol) and diethyl oxalate (60 mmol) was refluxed for 1 day. Upon cooling a solid precipitated; this was collected, washed with cold ethanol and dried to obtain the (substituted)-2,3-dihydroxyquinoxaline (yields 85–95%) which was subsequently treated with 5-fold molar excess of $POCl_3$. The mixture was refluxed for 16 h; evaporation of the excess $POCl_3$ at reduced pressure afforded a residue which was taken up with $CHCl_3$. The resulting suspension was filtered and the filtrate was washed with water and brine, then dried and evaporated to dryness to obtain the title compounds **31c–e** (yields 75–90%).

### *6.2. Biochemistry*

Compounds were assessed for their ability to inhibit binding of the $A_1$AR selective antagonist radioligand [$^3$H]DPCPX to synaptosomal membranes from rat brain according to a published procedure [22]. Briefly, 200 μg of membrane proteins were incubated with test compound solution (at least ten different concentrations) and 0.3 nM [$^3$H]DPCPX in 400 μL of 50 mM Tris.HCl, pH 7.4 for 1 h at 25 °C. Nonspecific binding was determined in the presence of 10 μM (R)-$N^6$-phenylisopropyladenosine (R-PIA). The incubations were blocked by filtration using a cell harvester and, upon separation of the bound from the free, the radioactivity contents were measured by liquid scintillation. All the assays were performed in triplicate or in quadruplicate. Data were fitted by nonlinear regression analysis (Allfit) and the $K_i$ values were calculated according to the Cheng–Prusoff equation [33].

Compound **20** was assessed for its ability to inhibit binding of the $A_{2a}$AR selective agonist radioligand [$^3$H]CGS21680 to rat striatal membranes according to a published procedure [23]. Briefly, 200 μg of membrane proteins were incubated with the test compound solution (twelve different concentrations) and 5 nM [$^3$H]CGS21680 in the incubation buffer (50 mM Tris.HCl pH 7.4) for 1 h at 25 °C. Nonspecific binding was determined in the presence of 25 μM $N^6$-cyclopentyladenosine (CPA). Subsequent harvesting of the assay and analysis of the data were identical with the $A_1$ assay.

The primary screen for the evaluation of $A_3$AR binding affinity of compound **20** was performed by MDS Panlabs, Taipei, ROC, according to standardized procedures, using human recombinant HEK-293 cells [25] as the source, 0.4 nM [$^{125}$I]AB-MECA [24] as radioligand, and 10 μM 5'-(*N*-ethylcarbamoyl)adenosine (NECA) for nonspecific binding.

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