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## Design, synthesis, and biological evaluation of novel 4-alkylamino-1-hydroxymethylimidazo[1,2-a]quinoxalines as adenosine A<sub>1</sub> receptor antagonists

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Abstract—A series of 4-alkylamino-1-hydroxymethylimidazo[1,2-*a*]quinoxalines have been synthesized and evaluated for their adenosine A<sub>1</sub> receptor inhibitory activity in the radioligand binding assays. The compounds were tested for the inhibition percent (IP) and the affinity toward A<sub>1</sub>AR ( $K_i$ ) that IP were more than 90% in the nanomolar range. 4-Cyclopentylamino-7,8-dichloro-1-hydroxymethylimidazo[1,2-*a*]quinoxaline **18** is the most potent compound in this series, having  $K_i$ =7nM, which is remarkably higher than that of IRFI-165 ( $K_i$ =48). 1-Hydroxymethyl groups of the tricyclic heteroarmatic compounds displayed the potent affinities toward A<sub>1</sub>AR.

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## 1. Introduction

Adenosine is a neurotransmitter and neuromodulator in the central nervous system. It is now well established that the multiple effects of the nucleoside are mediated by activation of adenosine receptors, which have been classified into four subtypes  $A_1$ ,  $A_{2a}$ ,  $A_{2b}$ , and  $A_3$ , based on biochemical, pharmacological, and molecular cloning properties. The main potential therapeutic indications of adenosine  $A_1$  receptor antagonists are for the treatment of cognitive deficits, renal failure, acute respiratory distress syndrome, cardiac arrhythmias, and asystolic arrest.<sup>1,2</sup>

The various types of adenosine  $A_1$  receptor ( $A_1AR$ ) antagonists have described by other groups<sup>3–7</sup> and were reported to display  $A_1AR$  antagonistic structure–activity requirement.<sup>8,9</sup> Lots of tricyclic heteroaromatic compounds have been synthesized to develop new centrally active  $A_1AR$  antagonists. The six–six–five ring system can overlap with endogenous adenosine, which has several sites that can potentially interact with receptor



Figure 1. Putative binding mode of 1-hydroxmethyl-4-alkylaminoimidazo[1,2-a]quinoxaline: overlap with the agonist  $N^6$ -cyclopentyladenosine.

amino acids, for example,  $N^1$ ,  $N^6$ ,  $N^9$ , and the three ribose-hydroxyl groups.<sup>10</sup> Therefore we designed 4-alkylamino-1-hydroxymethyl imidazo[1,2-*a*]quinoxalines, these compounds bind to A<sub>1</sub>AR by mimicking adenosine derivatives as shown in Figure 1. From this overlap it appears that hydroxymethyl group is overlapped with the ribose-hydroxyl group, which may be an optimal interaction with the binding site of the receptor to increase the affinity. We recently synthesized 19 compounds. All of them were evaluated for IP toward radioligand, and the compounds whose IP were more than 90% were tested for their affinity toward adenosine A<sub>1</sub> receptor, and structure–activity relationship (SAR) studies were reported here.

*Keywords*: Adenosine A<sub>1</sub> receptor; Imidazo[1,2-*a*]quinoxaline; Tricyclic heteroaromatic compounds.

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## 2. Chemistry

Compounds 5–23 were synthesized as outlined in Scheme 1. In detail, the hydroxy group of glycidol was protected with 3,4-2*H*-pyran,<sup>11</sup> then treated with NH<sub>3</sub>·-H<sub>2</sub>O to afford 1-amino-3-(tetrahydropyran-2-yloxy)propan-2-ol 1. Next, a mixture of the intermediate 1 and substituted 2,3-dichloroquinoxalines was refluxed for 20h in trichloromethane and Et<sub>3</sub>N to transform into intermediates 2a–c.<sup>12,13</sup> 2a–c were oxidated with CrO<sub>3</sub>pyridine or Swern's oxidant to intermediates 3a–c.<sup>14,15</sup> Intermediates 4a–c were prepared from 3a–c in trifluoroacetic acid and trifluoroacetic anhydride.<sup>12</sup> Compounds 5–23 were obtained from 4a–c with substituted amine in hexamethyldisilazane.<sup>16</sup>

Compounds 5–23 were purified with up-dry column (UDC). The manipulation of UDC was ruled out. (i) Silica gel (GF254,  $\Phi < 38 \,\mu$ m) was added into a 250×20 mm glass tube under reduced pressure. The sample was added to about 20 mm from the mouth of tube. The tube was sealed with absorbent cotton. (ii) The tube was reversed and immerged into solvent. (iii) After completely evolved, the gel was pushed out, irradiated with ultraviolet lamp, and cut down the region-adsorbed product, which was washed with suitable solvent. The solvent was evaporated under reduced pressure to give pure compounds.

## 3. Biology

The adenosine  $A_1AR$  binding affinities of the 4-alkylamino-1-hydroxymethylimidazo[1,2-a]quinoxalines were determined using standard radioligand binding assay procedures.<sup>2,17–20</sup> Competitive displacement of [<sup>3</sup>H]-1,3-dipropyl-8-cyclopentyxanthine ([<sup>3</sup>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 inhibited percent. The compounds whose IP were more than 90% were tested for their affinity toward A<sub>1</sub>AR, expressed as their  $K_i$  values. The data of IP and  $K_i$  values were listed in Table 1.

### 4. Results and discussion

Based on the results of the site-directed mutagenesis studies, the ribose-hydroxy groups of adenosine interacts with Val87, Ser94, Ile274, Leu276, Thr277, and His278 of adenosine A<sub>1</sub> receptor, which were the amino acids shown to influence ligand binding.<sup>10</sup> Therefore, hydroxymethyl group in the compounds was introduced in order to improve the affinities of the compounds for A<sub>1</sub>AR. As a result, the affinities of 4-al-kylamino-1-hydroxymethyl imidazo[1,2-*a*]quinoxalines (7, **12**, **17**, and **18**) is higher than that of IRFI-165, which was considered the most potent antagonist in the literature.<sup>10</sup>

Affinity data reported in Table 1 clearly showed that all the new compounds were characterized by a biological profile. The affinities of compounds 7, 12, 17, 18, and 19 toward  $A_1AR$  were in the nanomolar range. On the basis of both  $A_1AR$  affinity data and structural proper-



Scheme 1. Reagents and conditions: (a) pyridinium *p*-toluenesulfonate,  $CH_2Cl_2$ ,  $NH_4OH/EtOH$ ; (b)  $Et_3N$ ,  $CH_3CN$ ; (c)  $CrO_3-2Py$  or  $DMSO-(COCl)_2$ ; (d)  $(CF_3CO)_2O-CF_3COOH$ ; (e)  $R_2NH_2$ , hexamethyldisilazane.





Compd	R <sub>1</sub>	R <sub>2</sub>	IP $\pm$ SEM ( $K_i$ , nM)
IRFI-165 <sup>a</sup> 5 6	H H	(CH <sub>3</sub> ) <sub>2</sub> CHCH <sub>2</sub> CH <sub>3</sub> (CH <sub>2</sub> ) <sub>2</sub> CH <sub>2</sub>	92.0 $\pm$ 4.0% (48) 68.5 $\pm$ 4.5% 75.5 $\pm$ 0.5%
7	Н	$\bigcirc -$	94.0±6.0% (19)
8	Н		82.5±3.5%
9	Н	ON-CH <sub>2</sub> CH <sub>2</sub> -	21.7±11.3%
10 11	CH <sub>3</sub> CH <sub>3</sub>	(CH <sub>3</sub> ) <sub>2</sub> -CH-CH <sub>2</sub> - CH <sub>3</sub> -(CH <sub>2</sub> ) <sub>3</sub> -CH <sub>2</sub> -	$47.0 \pm 8.0\%$ $73.5 \pm 5.5\%$
12	CH <sub>3</sub> -	$\sim$	92.5±0.5% (13)
13 14	CH <sub>3</sub> CH <sub>3</sub>	C <sub>6</sub> H <sub>5</sub> -CH <sub>2</sub> - (CH <sub>3</sub> ) <sub>2</sub> N-CH <sub>2</sub> -C(CH <sub>3</sub> ) <sub>2</sub> CH <sub>2</sub> -	$\begin{array}{c} 44.0 \pm 9.0\% \\ 32.5 \pm 5.5\% \end{array}$
15	CH <sub>3</sub> -	O N−CH <sub>2</sub> CH <sub>2</sub> -	24.0±11.0%
16 17	Cl– Cl–	CH <sub>3</sub> -(CH <sub>2</sub> ) <sub>2</sub> -CH <sub>2</sub> (CH <sub>3</sub> ) <sub>2</sub> -CH-CH <sub>2</sub>	76.5±9.7% 94.5±2.5% (23)
18	Cl–	$\frown$	93.0±7.0% (7)
19	Cl-		93.0±1.0% (87)
20	Cl–	C <sub>6</sub> H <sub>5</sub> -CH <sub>2</sub> -	60.5±3.5%
21	Cl–	N-CH <sub>2</sub> CH <sub>2</sub> -	77.0±11.4%
22	Cl–	(CH <sub>3</sub> ) <sub>2</sub> N-CH <sub>2</sub> -C(CH <sub>3</sub> ) <sub>2</sub> CH <sub>2</sub> -	$65.0 \pm 20.6\%$
23	Cl–	ON-CH <sub>2</sub> CH <sub>2</sub> -	72.0±3.0%

<sup>a</sup> IRFI-165: N-Cyclopentyl-1-methylimidazo[1,2-a]quinoxalin-4-amine.

ties of the studied compounds, some suggestions can be ruled out. (i) A common feature of these compounds was the increase in  $A_1$  potency seen with the *N*-cyclopentyl substitution at the exocyclic amine. (ii) Comparison between the dichloride substitution class and the remaining series suggested that the chloride was beneficial to  $A_1AR$  affinities, for example, the affinity of 17 were higher than those of 5 and 10. (iii) The size of substitution at the exocyclic amine was very important for defining toward  $A_1AR$  affinity. The affinities of compounds (9, 15, and 23), which were substituted with 2-(morpholin-4-yl)ethyl group at the exocyclic amine were remarkably lower than those of compounds (6, 11, and 17) substituted with isobutyl group at the exocyclic amine, which suggested the presence of an alkyl binding site with strict steric requirements. A hydrophobic region having a limited degree of lipophilicity accommodating ability has also been suggested.

#### 5. Conclusion

In the present paper, we described the synthesis of 4-alkylamino-1-hydroxymethylimidazo[1,2-a]quinoxalines and their affinity toward A<sub>1</sub>AR. The synthesis was based on intramolecular cyclization of quinoxaline substituted by an aminoketone group. The affinities toward A<sub>1</sub>AR were determined using standard radioligand binding assay procedures. Hydroxymethyl group in position 1 was beneficial to interaction with the binding site of

the receptor. Substitution at the exocyclic amine was previously shown to be essential.

## 6. Experimental

## 6.1. Chemistry

**6.1.1. General procedure.** Melting points were determined using a RY-1 apparatus and are uncorrected. <sup>1</sup>H NMR spectra were recorded on Varian UNITY INOVA 600 MHz and JNM-ECA-400 400 MHz instrument in the solvent indicated below. Chemical shift values are reported in parts per million (ppm) relative to that for tetramethylsilane used as an internal reference standard. Spectral splitting patterns are designated as follows: s, singlet; br, broad; d, doublet; t, triplet; m, multiplet. Mass spectra were obtained from Micromass ZabSpec and API3000 instruments. Elemental analysis was carried at the CarloErba-1106.

All reactions were monitored by TLC on  $25 \times 75$  mm glass sheets precoated with silica gel (GF254) to a thickness of 0.25 mm and viewed at 254 nm UV-light.

**6.1.2. 1-Amino-3-(tetrahydropyran-2-yloxy)propan-2-ol** (1). A mixture of glycidol (29 g, 0.392 mol), 3,4-dihydropyran (61 g, 0.726 mol), and pyridinium *p*-toluenesulfonate (10 g, 0.04 mol) in dry dichloromethane was stirred for 40 h at ambient temperature. Then the solution was diluted with ether and washed once with half saturated brine to remove the catalyst. After evaporation of the solvent, 35 g of the oil was obtained. The oil was added to a solution of 500 mL of 25–28% NH<sub>3</sub>:-H<sub>2</sub>O and 500 mL of ethanol. The solution was stirred for 40 h at 40 °C. The mixture evaporated under reduced pressure and purified by chromatography on silica gel column, eluting with a CHCl<sub>3</sub>/MeOH/NH<sub>3</sub>·H<sub>2</sub>O mixture (3:1:0.01) to give 50.4% yield of colorless oil. MS (ESI): 176.2 (M+1).

**6.1.3. 1-(3,6,7-Trichloroquinoxalin-2-amino)-3-(tetrahydropyran-2-yloxy)propan-2-ol (2c).** A solution of **1** (16g, 91.4 mmol), 2,3,6,7-tetrachloroquinxaline (15g, 66 mmol), and Et<sub>3</sub>N (15 mL, 100 mmol) in 120 mL of CHCl<sub>3</sub> was refluxed under stirring for 30 h. The mixture evaporated under reduced pressure and purified by chromatography on silica gel column, eluting with a petroleum ether/EtOAc mixture (3:1) to give yellow solid (Yield: 74.5%). Mp 114–116 °C. MS (ESI): 406.4 (M+1).

**6.1.4. 1-(3-Chloroquinoxalin-2-amino)-3-(tetrahydropyran-2-yloxy)propan-2-ol (2a). 2a** was prepared from 2,3-dichloroquinxaline following the protocol described for **2c**. The product was yellow oil (Yield: 85.2%). MS (ESI): 338.2 (M+1).

**6.1.5. 1-(6,7-Dimethyl-3-chloroquinoxalin-2-amino)-3-(tetrahydropyran-2-yloxy)propan-2-ol (2b). 2b** was prepared from 2,3-dichloro-6,7-dimethylquinxaline following the protocol described for **2c**. The product was yellow oil (Yield: 68.4%). MS (ESI): 366.1 (M+1). 6.1.6. 1-(3,6,7-Trichloroquinoxalin-2-amino)-3-(tetrahydropyran-2-yloxy)propan-2-one (3c). Chromium trioxide (9.9g, 99mmol) was added to a magnetically stirred solution of pyridine (16mL, 198mmol) in 150mL of dry CH<sub>2</sub>Cl<sub>2</sub>. The deep burgundy solution was stirred for 15min at ambient temperature. At the end of this period, a solution of the 2c (5g, 12.3 mmol) in 30 mL of CH<sub>2</sub>Cl<sub>2</sub> was added in one portion. A tarry, black deposit separated immediately. After stirring an additional 1 h at ambient temperature, the solution was decanted from the residue, which was washed with 200 mL of CHCl<sub>3</sub>. The combined organic solution was washed with three 100 mL portion of 5% aqueous sodium hydroxide and three 100 mL portion of saturated brine, and dried over anhydrous magnesium sulfate. The solution was evaporated under reduced pressure and purified by chromatography on silica gel column, eluting with a petroleum ether/EtOAc mixture (4:1) to give 59.3% yield of white solid. MS (ESI): 404.1 (M+1).

**6.1.7. 1-(3-Chloroquinoxalin-2-amino)-3-(tetrahydropy-ran-2-yloxy)propan-2-one (3a). 3a** was prepared from **2a** following the protocol described for **3c**. The product was yellow oil (Yield: 50.2%). MS (ESI): 336.4 (M+1).

6.1.8. 1-(6,7-Dimethyl-3-chloroquinoxalin-2-amino)-3-(tetrahydropyran-2-yloxy)propan-2-one (3b). A solution of oxalyl chloride (5.4g, 50mmol) in dry dichloromethane (20 mL) was cooled to -60 °C, which was added dropwise to a solution of DMSO (7.4g, 100 mmol) in dry dichloromethane (30 mL) with the temperature maintained below -50 °C. After being stirred at the same temperature for 10 min, to mixture was added dropwise a solution of **2b** (3.7g, 10mmol) in dry dichloromethane (10mL), the mixture was stirred for 1h, and then triethylamine (10.9g, 108mmol) was added dropwise to the mixture with the temperature maintained below -50 °C. After 10 min the reaction mixture was allowed to warm to room temperature and then poured into ice-water (200 mL). The solution was extracted with chloroform, and the organic layers were collected, washed with saturated aqueous NaCl, dried over anhydrous magnesium sulfate. The solution was evaporated under reduced pressure and purified by chromatography on silica gel column, eluting with a petroleum ether/ EtOAc mixture (4:1) to give 40.3% yield of yellow oil. MS (ESI): 364.0 (M+1).

**6.1.9. 7,8-Dichloro-1-hydroxymethylimidazo[1,2-***a***]quinoxalin-4-one (4c). A mixture of 3c (2.95g, 7.3 mmol), 0.5 mL of trifluoroacetic acid, and 15 mL of trifluoroacetic anhydride in 25 mL of CHCl<sub>3</sub> was refluxed for 40 h. The mixture was evaporated under reduced pressure. The residue was stirred in 30 mL of CH<sub>2</sub>Cl<sub>2</sub> and filtered to give 97.0% yield of yellow solid. Mp >300 °C. MS (ESI): 283.9 (M+1). <sup>1</sup>H NMR (DMSO-***d***<sub>6</sub>) (\delta): 12.18 (1H, s, H–N), 8.03 (1H, s, 2-ArH), 7.79 (1H, s, 9-ArH), 7.58 (1H, s, 6-ArH), 6.08 (2H, s, CH<sub>2</sub>), 4.90 (1H, s, –OH).** 

6.1.10. 1-Hydroxymethylimidazo[1,2-*a*]quinoxalin-4-one (4a). 4a was prepared from 3a following the protocol

described for **4c**. The product was yellow solid (Yield: 80.2%). MS (ESI): 216.3 (M+1).

**6.1.11. 7,8-Dimethyl-1-hydroxymethylimidazo**[1,2-*a*]**quinoxalin-4-one (4b). 4b** was prepared from **3b** following the protocol described for **4c**. The product was yellow solid (Yield: 64.6%). MS (FAB): 244.1 (M+1).

**6.1.12.** 1-Hydroxymethyl-4-isobutylaminoimidazo[1,2-*a*]quinoxaline (5). A mixture of 4a (0.5 g, 2.3 mmol), hexamethyldisilazane (5 mL, 21.7 mmol), *p*-toluenesulfonic acid (0.06 g, 032 mmol), and isobutylamine (6 mL, 60.6 mmol) was stirred under stirring for 40 h. The mixture was filtered, and the filtrate was evaporated under reduced pressure and purified by UDC to give 38.2 yield of white solid. Mp 162–164 °C. MS (ESI): 271.4 (M+1). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) ( $\delta$ ): 8.45 (1H, s, ArH), 8.08 (1H, dd, *J*=8Hz, ArH), 7.66 (1H, t, *J*=8Hz, NH),7.56 (1H, m, ArH), 7.37 (1H, m, ArH), 7.26 (1H, m, ArH), 5.34 (1H, br, OH), 4.65 (2H, s, CH<sub>2</sub>), 3.66 (2H, t, CH<sub>2</sub>), 2.09 (1H, m, CH), 0.93 (6H, d, *J*=8Hz, 2CH<sub>3</sub>). Anal. Calcd for C<sub>15</sub>H<sub>18</sub>N<sub>4</sub>O: C, 65.61; H, 6.29; N, 21.86. Found: C, 65.42; H, 6.35; N, 21.97.

**6.1.13. 4-Butylamino-1-hydroxymethylimidazo**[1,2-*a*]**quinoxaline (6). 6** was prepared from **4a** and butylamine following the protocol described for **5**. The product is white solid (Yield: 33.6%). Mp 146–148 °C. MS (ESI): 270.9 (M+1). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) ( $\delta$ ): 8.40 (1H, s, ArH), 8.05 (1H, d, ArH), 7.56 (2H, m, ArH, NH), 7.50 (1H, m, ArH), 7.26 (1H, m, ArH), 5.20 (1H, t, OH), 4.65 (2H, d, *J*=4Hz, CH<sub>2</sub>), 3.60 (2H, m, CH<sub>2</sub>), 1.70 (2H, m, CH<sub>2</sub>), 1.40 (2H, m, CH<sub>2</sub>), 0.95 (3H, t, CH<sub>3</sub>). Anal. Calcd for C<sub>15</sub>H<sub>18</sub>N<sub>4</sub>O: C, 65.61; H, 6.29; N, 21.86. Found: C, 65.73; H, 6.16; N, 21.66.

**6.1.14. 4-Cyclopentylamino-1-hydroxymethylimidazo[1,2***a***]quinoxaline (7).** 7 was prepared from **4a** and cyclopentylamine following the protocol described for **5**. The product was white solid (Yield: 40.5%). Mp 170– 172 °C. MS (ESI): 283.1 (M+1). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) ( $\delta$ ): 8.44 (1H, s, ArH), 8.08 (1H, m, ArH), 7.58 (1H, m, NH), 7.42 (1H, m, ArH), 7.38 (1H, m, ArH), 7.26 (1H, m, ArH), 5.34 (1H, t, OH), 4.65 (2H, d, *J*=4Hz, CH<sub>2</sub>), 4.58 (1H, m, CH), 1.58–2.03 (8H, m, 4CH<sub>2</sub>). Anal. Calcd for C<sub>16</sub>H<sub>18</sub>N<sub>4</sub>O: C, 68.06; H, 6.43; N, 19.84. Found: C, 68.33; H, 6.16; N, 20.06.

**6.1.15. 4-Cyclohexylamino-1-hydroxymethylimidazo[1,2***a***]quinoxaline (8). 8** was prepared from **4a** and cyclohexylamine following the protocol described for **5**. The product was white solid (Yield: 21.5%). Mp 190– 192 °C. MS (ESI): 297.2 (M+1). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) ( $\delta$ ): 8.27 (1H, m, ArH), 7.60 (1H, m, ArH), 7.51 (1H, s, ArH), 7.40 (1H, m, ArH), 7.29 (1H, m, ArH), 4.96 (2H, s, CH<sub>2</sub>), 4.14 (1H, m, CH), 1.15–1.96 (10H, m, 5CH<sub>2</sub>). Anal. Calcd for C<sub>17</sub>H<sub>20</sub>N<sub>4</sub>O: C, 68.90; H, 6.80; N, 18.90. Found: C, 68.78; H, 6.91; N, 18.98.

**6.1.16. 1-Hydroxymethyl-4-[2-(morpholino-4-yl)ethyl]aminoimidazo[1,2-***a***]<b>quinoxaline (9). 9** was prepared from **4a** and 2-(morpholino-4-yl)ethylamine following the protocol described for **5**. The product was white solid (Yield: 60.3%). Mp 146–148 °C. MS (ESI): 328.3 (M+1). <sup>1</sup>H NMR (DMSO- $d_6$ ) ( $\delta$ ): 8.39 (1H, s, ArH), 8.06 (1H, m, ArH), 7.58 (1H, m, ArH), 7.40 (1H, m, ArH), 7.29 (1H, m, ArH), 4.63 (2H, s, CH<sub>2</sub>), 3.67 (2H, t, CH<sub>2</sub>), 3.58 (4H, t, 2CH<sub>2</sub>), 2.62 (2H, t, CH<sub>2</sub>), 2.50 (4H, t, 2CH<sub>2</sub>). Anal. Calcd for C<sub>17</sub>H<sub>21</sub>N<sub>5</sub>O<sub>2</sub>: C, 62.37; H, 6.47; N, 21.39. Found: C, 62.46; H, 6.35; N, 21.45.

**6.1.17. 7,8-Dimethyl-1-hydroxymethyl-4-isobutylaminoimidazo[1,2-***a***]<b>quinoxaline (10). 10** was prepared from **4b** and isobutylamine following the protocol described for **5**. The product was white solid (Yield: 36.2%). Mp 246–248 °C. MS (ESI): 299.1 (M+1). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) ( $\delta$ ): 7.17 (1H, s, ArH), 7.10 (1H, s, NH), 7.07 (1H, s, ArH), 6.90 (1H, s, ArH), 3.24 (2H, m, CH<sub>2</sub>), 3.16 (2H, s, CH<sub>2</sub>), 2.25 (3H, s, CH<sub>3</sub>), 2.21 (3H, s, CH<sub>3</sub>), 2.25–221 (1H, br, OH), 1.98 (1H, m, CH), 0.95 (6H, d, *J*=6.8Hz, 2CH<sub>3</sub>). Anal. Calcd for C<sub>17</sub>H<sub>22</sub>N<sub>4</sub>O: C, 68.43; H, 7.43; N, 18.78. Found: C, 68.55; H, 7.35; N, 18.81.

**6.1.18. 4**-*n*-Amylamino-7,8-dimethyl-1-hydroxymethylimidazo[1,2-*a*]quinoxaline (11). 11 was prepared from 4b and *n*-amylamine following the protocol described for **5**. The product was white solid (Yield: 27.2%). Mp 164–166 °C. MS (ESI): 313.5 (M+1). <sup>1</sup>H NMR (DMSO- $d_6$ ) ( $\delta$ ): 8.04 (1H, s, ArH), 7.46 (1H, s, ArH), 7.41 (1H, s, ArH), 7.16 (1H, d, NH), 4.95 (2H, s, CH<sub>2</sub>), 3.50 (2H, m, CH<sub>2</sub>), 2.34 (3H, s, CH<sub>3</sub>), 2.31 (3H, s, CH<sub>3</sub>), 1.65 (2H, m, CH<sub>2</sub>), 1.34 (4H, m, 2CH<sub>2</sub>) 0.88 (3H, t, CH<sub>3</sub>). Anal. Calcd for C<sub>17</sub>H<sub>22</sub>N<sub>4</sub>O: C, 69.20; H, 7.74; N, 17.93. Found: C, 69.35; H, 7.55; N, 18.19.

**6.1.19. 4-Cyclopentylamino-7,8-dimethyl-1-hydroxymethylimidazo[1,2-***a***]quinoxaline (12). 12 was prepared from <b>4b** and cyclopentylamine following the protocol described for **5**. The product was white solid (Yield: 37.2%). Mp 240–242 °C. MS (ESI): 311.3 (M+1). <sup>1</sup>H NMR (DMSO- $d_6$ ) ( $\delta$ ): 8.04 (1H, s, ArH), 7.46 (1H, s, ArH), 7.41 (1H, s, ArH), 7.16 (1H, m, NH), 5.66 (1H, t, OH), 4.96 (2H, m, CH<sub>2</sub>), 4.51 (1H, m, CH), 2.34 (3H, s, CH<sub>3</sub>), 2.31 (3H, s, CH<sub>3</sub>), 2.09–1.58 (8H, m, 4CH<sub>2</sub>). Anal. Calcd for C<sub>18</sub>H<sub>22</sub>N<sub>4</sub>O: C, 69.65; H, 7.14; N, 18.05. Found: C, 69.30; H, 7.32; N, 18.12.

**6.1.20. 4-Benzylamino-7,8-dimethyl-1-hydroxymethylimidazo[1,2-***a***]quinoxaline (13). 13 was prepared from 4b and benzylamine following the protocol described for <b>5**. The product was white solid (Yield: 51.4%). Mp 144–146 °C. MS (ESI): 333.0 (M+1). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) ( $\delta$ ): 8.05 (1H, s, ArH), 7.87 (1H, m, NH), 7.20–7.51 (7H, m, 7ArH), 4.97 (2H, s, CH<sub>2</sub>), 4.74 (2H, s, CH<sub>2</sub>), 2.34 (3H, s, CH<sub>3</sub>), 2.29 (3H, s, CH<sub>3</sub>). Anal. Calcd for C<sub>20</sub>H<sub>20</sub>N<sub>4</sub>O: C, 72.27; H, 6.06; N, 16.85. Found: C, 72.42; H, 5.98; N, 16.78.

6.1.21. 7,8-Dimethyl-4-(3-dimethylamino-2,2-dimethylpropyl)amino-1-hydroxymethylimidazo[1,2-*a*]quinoxaline (14). 14 was prepared from 4b and *N*,*N*,2,2-tetramethyl-3-aminopropylamine following the protocol described for 5. The product was white solid (Yield: 51.4%). Mp 136–138 °C. MS (ESI): 356.4 (M+1). <sup>1</sup>H NMR (DMSO- $d_6$ ) ( $\delta$ ): 8.04 (1H, s, ArH), 7.48 (1H, s, ArH), 7.41 (1H, s, ArH), 4.95 (2H, s, CH<sub>2</sub>), 3.46 (2H, s, CH<sub>2</sub>), 2.34 (3H, s, CH<sub>3</sub>), 2.31 (3H, s, CH<sub>3</sub>), 2.29 (6H, s, 2CH<sub>3</sub>), 2.27 (2H, s, CH<sub>2</sub>), 0.96 (6H, s, 2CH<sub>3</sub>). Anal. Calcd for  $C_{20}H_{29}N_5O$ : C, 67.58; H, 8.22; N, 19.70. Found: C, 68.66; H, 8.05; N, 19.81.

**6.1.22. 7,8-Dimethyl-1-hydroxymethyl-4-[2-(morpholino-4-yl)ethyl]aminoimidazo[1,2-***a***]quinoxaline (15). 15 was prepared from <b>4b** and 2-(morpholino-4-yl)ethylamine following the protocol described for **5**. The product was white solid (Yield: 61.0%). Mp 128–130 °C. MS (ESI): 356.2 (M+1). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) ( $\delta$ ): 8.04 (1H, s, ArH), 7.48 (1H, s, ArH), 7.40 (1H, s, ArH), 4.96 (2H, s, CH<sub>2</sub>), 3.63 (2H, t, CH<sub>2</sub>), 3.58 (4H, t, 2CH<sub>2</sub>), 2.60 (2H, t, CH<sub>2</sub>), 2.50 (4H, t, 2CH<sub>2</sub>), 2.34 (3H, s, CH<sub>3</sub>), 2.30 (3H, s, CH<sub>3</sub>). Anal. Calcd for C<sub>19</sub>H<sub>25</sub>N<sub>5</sub>O<sub>2</sub>: C, 64.20; H, 7.09; N, 19.70. Found: C, 63.95; H, 7.12; N, 19.81.

**6.1.23. 4-Butylamino-7,8-dichloro-1-hydroxymethylimidazo[1,2-***a***]quinoxaline (16). 16 was prepared from 4c and isobutylamine following the protocol described for 5. The product was white solid (Yield: 32.4%). Mp 210-212 \degree C. MS (ESI): 339.1 (M+1). <sup>1</sup>H NMR (DMSO-***d***<sub>6</sub>) (\delta): 8.51 (1H, s, ArH), 7.74 (1H, s, ArH), 7.56 (1H, s, ArH), 4.93 (2H, s, CH<sub>2</sub>), 3.54 (2H, t, CH<sub>2</sub>), 1.65 (2H, m, CH<sub>2</sub>), 1.37 (2H, m, CH<sub>2</sub>), 0.93 (3H, t, CH<sub>3</sub>). Anal. Calcd for C<sub>15</sub>H<sub>16</sub>Cl<sub>2</sub>N<sub>4</sub>O: C, 53.11; H, 4.75; N, 16.52. Found: C, 53.33; H, 4.65; N, 16.35.** 

**6.1.24. 7,8-Dichloro-1-hydroxymethyl-4-isobutylaminoimidazo[1,2-***a***]<b>quinoxaline (17). 17** was prepared from **4c** and isobutylamine following the protocol described for **5**. The product was white solid (Yield: 32.4%). Mp 210–212 °C. MS (ESI): 339.1 (M+1). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) ( $\delta$ ): 8.52 (1H, s, ArH), 8.03 {1H, t, NH}, 7.75 (1H, s, ArH), 7.56 (1H, s, ArH), 5.84 (1H, t, OH), 4.93 (2H, m, CH<sub>2</sub>), 3.38 (2H, m, CH<sub>2</sub>), 2.08 (1H, m, CH), 0.92 (6H, m, 2CH<sub>3</sub>). Anal. Calcd for C<sub>15</sub>H<sub>16</sub>Cl<sub>2</sub>N<sub>4</sub>O: C, 53.11; H, 4.75; N, 16.52. Found: C, 53.48; H, 4.55; N, 16.55.

**6.1.25. 4-Cyclopentylamino-7,8-dichloro-1-hydroxymethylimidazo[1,2-***a***]<b>quinoxaline (18). 18** was prepared from **4c** and cyclopentylamine following the protocol described for **5**. The product was white solid (Yield: 34.4%). Mp 240–242 °C. MS (ESI): 351.2 (M+1). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) ( $\delta$ ): 8.52 (1H, s, ArH), 7.83 {1H, t, NH}, 7.75 (1H, s, ArH), 7.56 (1H, s, ArH), 5.87 (1H, t, OH), 4.93 (2H, m, CH<sub>2</sub>), 4.56 (1H, m, CH), 2.09– 1.57 (8H, m, 4CH<sub>2</sub>). Anal. Calcd for C<sub>16</sub>H<sub>16</sub>Cl<sub>2</sub>N<sub>4</sub>O: C, 54.71; H, 4.59; N, 15.95. Found: C, 54.48; H, 4.75; N, 15.78.

**6.1.26. 4-Cyclohexylamino-7,8-dichloro-1-hydroxymethylimidazo[1,2-***a***]<b>quinoxaline (19). 19** was prepared from **4c** and cyclohexylamine following the protocol described for **5**. The product was white solid (Yield: 34.4%). Mp 258–260 °C. MS (ESI): 365.3 (M+1). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) ( $\delta$ ): 8.51 (1H, s, ArH), 7.76 (1H, s, ArH), 7.56 (1H, s, ArH), 4.93 (2H, s, CH<sub>2</sub>), 4.13 (1H, m, CH), 1.98–1.15 (10H, m, 5CH<sub>2</sub>). Anal. Calcd for  $C_{17}H_{18}Cl_2N_4O:$  C, 55.90; H, 4.97; N, 15.34. Found: C, 55.67; H, 5.05; N, 15.52.

**6.1.27. 4-Benzylamino-7,8-dichloro-1-hydroxymethylimidazo[1,2-***a***]quinoxaline (20). 20 was prepared from 4c and benzylamine following the protocol described for 5. The product is white solid (Yield: 56.9%). Mp 212– 214 °C. MS (ESI): 373.0 (M+1). <sup>1</sup>H NMR (DMSO-***d***<sub>6</sub>) (\delta): 8.52 (1H, s, ArH), 7.75 (1H, s, ArH), 7.59 (1H, s, ArH), 7.43–7.19 (5H, m, 5ArH), 4.94 (2H, s, CH<sub>2</sub>), 4.75 (2H, s, CH<sub>2</sub>). Anal. Calcd for C<sub>18</sub>H<sub>14</sub>Cl<sub>2</sub>N<sub>4</sub>O: C, 57.92; H, 3.78; N, 15.01. Found: C, 57.73; H, 3.82; N, 15.18.** 

**6.1.28. 7,8-Dichloro-1-hydroxymethyl-4-[2-(pyrrolidin-1-yl)ethyl]aminoimidazo[1,2-***a***]quinoxaline (21). 21 was prepared from 4c and 2-(pyrrolidin-1-yl)ethylamine following the protocol described for 5. The product was white solid (Yield: 52.9\%). Mp 194–196 °C. MS (ESI): 380.2 (M+1). <sup>1</sup>H NMR (DMSO-***d***<sub>6</sub>) (\delta): 8.50 (1H, s, ArH), 7.74 (1H, s, ArH), 7.56 (1H, s, ArH), 4.92 (2H, s, CH<sub>2</sub>), 3.65 (2H, t, CH<sub>2</sub>), 2.60 (2H, t, CH<sub>2</sub>), 1.69 (4H, m, 2CH<sub>2</sub>). Anal. Calcd for C<sub>17</sub>H<sub>19</sub>Cl<sub>2</sub>N<sub>5</sub>O: C, 53.69; H, 5.04; N, 18.42. Found: C, 53.91; H, 4.75; N, 18.55.** 

**6.1.29. 7,8-Dichloro-4-(3-dimethylamino-2,2-dimethylpropyl)amino-1-hydroxymethylimidazo[1,2-***a***]quinoxaline <b>(22). 22** was prepared from **4c** and *N*,*N*,2,2-tetramethyl-3-aminopropylamine following the protocol described for **5**. The product was white solid (Yield: 61.0%). Mp 154–156 °C. MS (ESI): 396.1 (M+1). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) ( $\delta$ ): 8.50 (1H, s, ArH), 7.75 (1H, s, ArH), 7.57 (1H, s, ArH), 4.92 (2H, s, CH<sub>2</sub>), 3.49 (2H, s, CH<sub>2</sub>), 2.30 (8H, s, 2CH<sub>3</sub>,CH<sub>2</sub>), 0.96 (6H, s, 2CH<sub>3</sub>). Anal. Calcd for C<sub>18</sub>H<sub>23</sub>Cl<sub>2</sub>N<sub>5</sub>O: C, 54.55; H, 5.85; N, 17.67. Found: C, 54.66; H, 5.74; N, 17.81.

**6.1.30. 7,8-Dichloro-1-hydroxymethyl-4-[2-(morpholino-4-yl)ethyl]aminoimidazo[1,2-***a***]quinoxaline (23). 23 was prepared from <b>4c** and 2-(morpholino-4-yl)ethylamine following the protocol described for **5**. The product was white solid (Yield: 63.8%). Mp 184–186 °C. MS (ESI): 396.4 (M+1). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) ( $\delta$ ): 8.52 (1H, s, ArH), 7.76 (1H, s, ArH), 7.58 (1H, s, ArH), 4.93 (2H, s, CH<sub>2</sub>), 3.65 (2H, t, CH<sub>2</sub>), 3.57 (4H, t, 2CH<sub>2</sub>), 2.60 (2H, t, CH<sub>2</sub>), 2.50 (4H, t, 2CH<sub>2</sub>). Anal. Calcd for C<sub>17</sub>H<sub>19</sub>Cl<sub>2</sub>N<sub>5</sub>O<sub>2</sub>: C, 51.53; H, 4.83; N, 17.67. Found: C, 51.26; H, 5.01; N, 17.58.

# 6.2. Biochemistry: measurement of A<sub>1</sub>AR binding affinities

**6.2.1. Materials.** Adenosine deaminase from calf intestinal mucosa (ADA) was supplied by Fluka Chemie GmbH.  $N^6$ -(phenylisopropyl)adenosine (R-PIA) was provided by Sigma-Aldrich. [<sup>3</sup>H]-DPCPX (9.25 MBq, 250 µCi) was from Amersham Biosciences.

**6.2.2.** Compounds radioligand binding assay. Compounds were assessed for their ability to inhibit binding of the  $A_1AR$  selective antagonist radioligand [<sup>3</sup>H]-

DPCPX to synaptosomal membranes from rat brain. Briefly, membrane protein  $(30 \,\mu\text{M}, 100 \,\mu\text{L})$  were incubated with test compound solution  $(10 \,\mu\text{M}, 100 \,\mu\text{L})$  and  $[^{3}\text{H}]$ -DPCPX  $(100 \,\mu\text{L})$  for 30 min at 37 °C. Nonspecific binding was determined in the presence of R-PIA  $(10 \,\mu\text{M}, 100 \,\mu\text{L})$ . The incubations were blocked by filtration using a cell harvester and the radioactivity contents were measured by liquid scintillation. All the assays were performed in triplicate or in quadruplicate. The inhibition percent were calculated according to an equation. Here,  $T_{\text{CPM}}$  is total rate of binding,  $P_{\text{CMP}}$  is rate of nonspecific binding.

$$IP = \frac{T_{CPM} - P_{CPM}}{T_{CPM} - N_{CPM}} \times 100\%$$

The compounds whose IP were more than 90% were tested for their affinity toward A<sub>1</sub>AR according to a published procedure<sup>19</sup> and  $K_i$  values were obtain from the Cheng–Prusoff equation.<sup>20</sup>

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