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Design, synthesis, and biological evaluation of novel 4-alkylamino-1-hydroxymethylimidazo[1,2-*a*]quinoxalines as adenosine A_1 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_1 receptor inhibitory activity in the radioligand binding assays. The compounds were tested for the inhibition percent (IP) and the affinity toward A_1AR (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 $K_i = 7$ nM, 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_1AR.

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.[1,2]

The various types of adenosine A_1 receptor (A_1AR) antagonists have described by other groups[3–7] and were reported to display A_1AR antagonistic structure–activity requirement.[8,9] 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 amino acids, for example, N^1, N^6, N^9, and the three ribose-hydroxyl groups.[10] Therefore we designed 4-alkylamino-1-hydroxymethyl imidazo[1,2-*a*]quinoxalines, these compounds bind to A_1AR 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_1 receptor, and structure–activity relationship (SAR) studies were reported here.

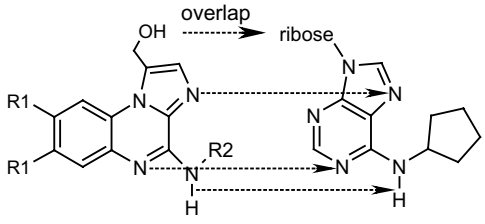

Figure 1. Putative binding mode of 1-hydroxmethyl-4-alkylaminoimidazo[1,2-*a*]quinoxaline: overlap with the agonist N^6-cyclopentyladenosine.

Keywords: Adenosine A_1 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,[11] then treated with $NH_3 \cdot H_2O$ 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 20 h in trichloromethane and Et_3N to transform into intermediates **2a**–**c**.[12,13] **2a**–**c** were oxidated with CrO_3-pyridine or Swern's oxidant to intermediates **3a**–**c**.[14,15] Intermediates **4a**–**c** were prepared from **3a**–**c** in trifluoroacetic acid and trifluoroacetic anhydride.[12] Compounds **5**–**23** were obtained from **4a**–**c** with substituted amine in hexamethyldisilazane.[16]

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.

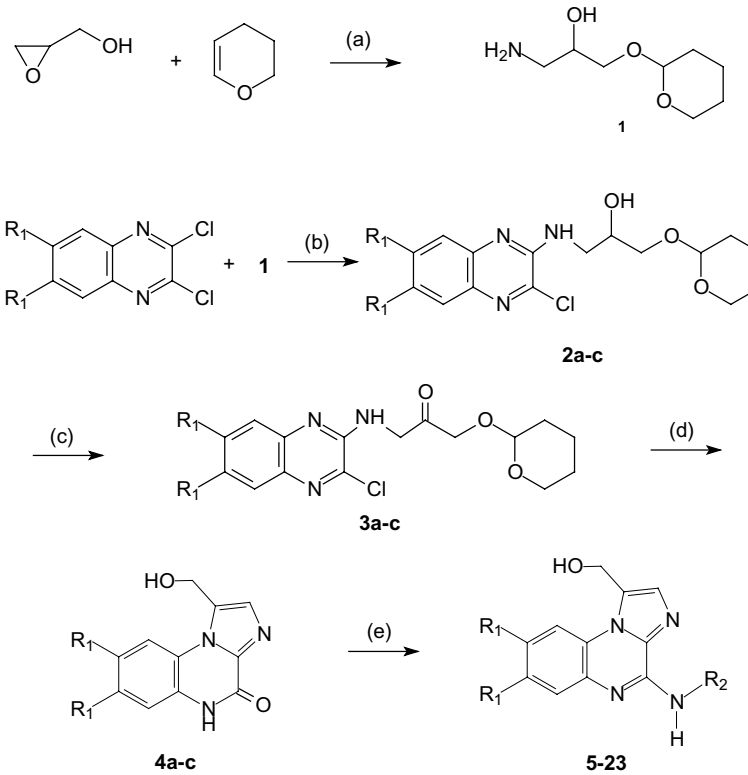
3. Biology

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

4. Results and discussion

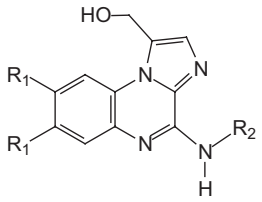
Based on the results of the site-directed mutagenesis studies, the ribose-hydroxy groups of adenosine interacts with Val87, Ser94, Ile274, Leu276, Thr277, and His278 of adenosine A_1 receptor, which were the amino acids shown to influence ligand binding.[10] Therefore, hydroxymethyl group in the compounds was introduced in order to improve the affinities of the compounds for A_1AR. As a result, the affinities of 4-alkylamino-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.[10]

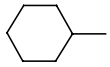
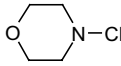
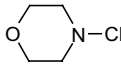
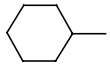
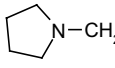
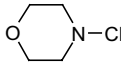
Affinity data reported in Table 1 clearly showed that all the new compounds were characterized by a biological profile. The affinities of compounds **7**, **12**, **17**, **18**, and **19** toward A_1AR were in the nanomolar range. On the basis of both A_1AR affinity data and structural proper-



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.

Table 1. Inhibition of adenosine A_1 receptor, IP ± SEM (%) and K_i (nM)



Compd	R_1	R_2	IP ± SEM (K_i, nM)
IRFI-165[a]			92.0 ± 4.0% (48)
5	H	$(CH_3)_2$–CH–CH_2–	68.5 ± 4.5%
6	H	CH_3–$(CH_2)_2$–CH_2–	75.5 ± 0.5%
7	H		94.0 ± 6.0% (19)
8	H		82.5 ± 3.5%
9	H		21.7 ± 11.3%
10	CH_3–	$(CH_3)_2$–CH–CH_2–	47.0 ± 8.0%
11	CH_3–	CH_3–$(CH_2)_3$–CH_2–	73.5 ± 5.5%
12	CH_3–		92.5 ± 0.5% (13)
13	CH_3–	C_6H_5–CH_2–	44.0 ± 9.0%
14	CH_3–	$(CH_3)_2N$–CH_2–$C(CH_3)_2CH_2$–	32.5 ± 5.5%
15	CH_3–		24.0 ± 11.0%
16	Cl–	CH_3–$(CH_2)_2$–CH_2–	76.5 ± 9.7%
17	Cl–	$(CH_3)_2$–CH–CH_2–	94.5 ± 2.5% (23)
18	Cl–		93.0 ± 7.0% (7)
19	Cl–		93.0 ± 1.0% (87)
20	Cl–	C_6H_5–CH_2–	60.5 ± 3.5%
21	Cl–		77.0 ± 11.4%
22	Cl–	$(CH_3)_2N$–CH_2–$C(CH_3)_2CH_2$–	65.0 ± 20.6%
23	Cl–		72.0 ± 3.0%

[a] 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_1AR. The synthesis was based on intramolecular cyclization of quinoxaline substituted by an aminoketone group. The affinities toward A_1AR 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. ^{1}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 25×75 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% $NH_3 \cdot H_2O$ 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 $CHCl_3$/MeOH/$NH_3 \cdot H_2O$ 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** (16 g, 91.4 mmol), 2,3,6,7-tetrachloroquinxaline (15 g, 66 mmol), and Et_3N (15 mL, 100 mmol) in 120 mL of $CHCl_3$ 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 (9.9 g, 99 mmol) was added to a magnetically stirred solution of pyridine (16 mL, 198 mmol) in 150 mL of dry CH_2Cl_2. The deep burgundy solution was stirred for 15 min at ambient temperature. At the end of this period, a solution of the **2c** (5 g, 12.3 mmol) in 30 mL of CH_2Cl_2 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 $CHCl_3$. 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-(tetrahydropyran-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 (5.4 g, 50 mmol) in dry dichloromethane (20 mL) was cooled to −60 °C, which was added dropwise to a solution of DMSO (7.4 g, 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** (3.7 g, 10 mmol) in dry dichloromethane (10 mL), the mixture was stirred for 1 h, and then triethylamine (10.9 g, 108 mmol) 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** (2.95 g, 7.3 mmol), 0.5 mL of trifluoroacetic acid, and 15 mL of trifluoroacetic anhydride in 25 mL of $CHCl_3$ was refluxed for 40 h. The mixture was evaporated under reduced pressure. The residue was stirred in 30 mL of CH_2Cl_2 and filtered to give 97.0% yield of yellow solid. Mp >300 °C. MS (ESI): 283.9 (M+1). ^{1}H NMR (DMSO-d_6) (δ): 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_2), 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). ^{1}H NMR (DMSO-d_6) (δ): 8.45 (1H, s, ArH), 8.08 (1H, dd, J = 8 Hz, ArH), 7.66 (1H, t, J = 8 Hz, 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_2), 3.66 (2H, t, CH_2), 2.09 (1H, m, CH), 0.93 (6H, d, J = 8 Hz, $2CH_3$). Anal. Calcd for $C_{15}H_{18}N_4O$: 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). ^{1}H NMR (DMSO-d_6) (δ): 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 = 4 Hz, CH_2), 3.60 (2H, m, CH_2), 1.70 (2H, m, CH_2), 1.40 (2H, m, CH_2), 0.95 (3H, t, CH_3). Anal. Calcd for $C_{15}H_{18}N_4O$: 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). ^{1}H NMR (DMSO-d_6) (δ): 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 = 4 Hz, CH_2), 4.58 (1H, m, CH), 1.58–2.03 (8H, m, $4CH_2$). Anal. Calcd for $C_{16}H_{18}N_4O$: 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). ^{1}H NMR (DMSO-d_6) (δ): 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_2), 4.14 (1H, m, CH), 1.15–1.96 (10H, m, $5CH_2$). Anal. Calcd for $C_{17}H_{20}N_4O$: 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*]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). ^{1}H NMR (DMSO-d_6) (δ): 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_2), 3.67 (2H, t, CH_2), 3.58 (4H, t, $2CH_2$), 2.62 (2H, t, CH_2), 2.50 (4H, t, $2CH_2$). Anal. Calcd for $C_{17}H_{21}N_5O_2$: 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*]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). ^{1}H NMR (DMSO-d_6) (δ): 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_2), 3.16 (2H, s, CH_2), 2.25 (3H, s, CH_3), 2.21 (3H, s, CH_3), 2.25–221 (1H, br, OH), 1.98 (1H, m, CH), 0.95 (6H, d, J = 6.8 Hz, $2CH_3$). Anal. Calcd for $C_{17}H_{22}N_4O$: 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). ^{1}H NMR (DMSO-d_6) (δ): 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_2), 3.50 (2H, m, CH_2), 2.34 (3H, s, CH_3), 2.31 (3H, s, CH_3), 1.65 (2H, m, CH_2), 1.34 (4H, m, $2CH_2$) 0.88 (3H, t, CH_3). Anal. Calcd for $C_{17}H_{22}N_4O$: 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 **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). ^{1}H NMR (DMSO-d_6) (δ): 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_2), 4.51 (1H, m, CH), 2.34 (3H, s, CH_3), 2.31 (3H, s, CH_3), 2.09–1.58 (8H, m, $4CH_2$). Anal. Calcd for $C_{18}H_{22}N_4O$: 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 **5**. The product was white solid (Yield: 51.4%). Mp 144–146 °C. MS (ESI): 333.0 (M+1). ^{1}H NMR (DMSO-d_6) (δ): 8.05 (1H, s, ArH), 7.87 (1H, m, NH), 7.20–7.51 (7H, m, 7ArH), 4.97 (2H, s, CH_2), 4.74 (2H, s, CH_2), 2.34 (3H, s, CH_3), 2.29 (3H, s, CH_3). Anal. Calcd for $C_{20}H_{20}N_4O$: 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). ^{1}H NMR (DMSO-d_6) (δ): 8.04 (1H, s, ArH), 7.48 (1H, s, ArH),

7.41 (1H, s, ArH), 4.95 (2H, s, CH_2), 3.46 (2H, s, CH_2), 2.34 (3H, s, CH_3), 2.31 (3H, s, CH_3), 2.29 (6H, s, $2CH_3$), 2.27 (2H, s, CH_2), 0.96 (6H, s, $2CH_3$). 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 **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). ^{1}H NMR (DMSO-d_6) (δ): 8.04 (1H, s, ArH), 7.48 (1H, s, ArH), 7.40 (1H, s, ArH), 4.96 (2H, s, CH_2), 3.63 (2H, t, CH_2), 3.58 (4H, t, $2CH_2$), 2.60 (2H, t, CH_2), 2.50 (4H, t, $2CH_2$), 2.34 (3H, s, CH_3), 2.30 (3H, s, CH_3). Anal. Calcd for $C_{19}H_{25}N_5O_2$: 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 °C. MS (ESI): 339.1 (M+1). ^{1}H NMR (DMSO-d_6) (δ): 8.51 (1H, s, ArH), 7.74 (1H, s, ArH), 7.56 (1H, s, ArH), 4.93 (2H, s, CH_2), 3.54 (2H, t, CH_2), 1.65 (2H, m, CH_2), 1.37 (2H, m, CH_2), 0.93 (3H, t, CH_3). Anal. Calcd for $C_{15}H_{16}Cl_2N_4O$: 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*]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). ^{1}H NMR (DMSO-d_6) (δ): 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_2), 3.38 (2H, m, CH_2), 2.08 (1H, m, CH), 0.92 (6H, m, $2CH_3$). Anal. Calcd for $C_{15}H_{16}Cl_2N_4O$: 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*]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). ^{1}H NMR (DMSO-d_6) (δ): 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_2), 4.56 (1H, m, CH), 2.09–1.57 (8H, m, $4CH_2$). Anal. Calcd for $C_{16}H_{16}Cl_2N_4O$: 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*]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). ^{1}H NMR (DMSO-d_6) (δ): 8.51 (1H, s, ArH), 7.76 (1H, s, ArH), 7.56 (1H, s, ArH), 4.93 (2H, s, CH_2), 4.13 (1H, m, CH), 1.98–1.15 (10H, m, $5CH_2$). 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). ^{1}H NMR (DMSO-d_6) (δ): 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_2), 4.75 (2H, s, CH_2). Anal. Calcd for $C_{18}H_{14}Cl_2N_4O$: 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). ^{1}H NMR (DMSO-d_6) (δ): 8.50 (1H, s, ArH), 7.74 (1H, s, ArH), 7.56 (1H, s, ArH), 4.92 (2H, s, CH_2), 3.65 (2H, t, CH_2), 2.60 (2H, t, CH_2), 1.69 (4H, m, $2CH_2$). Anal. Calcd for $C_{17}H_{19}Cl_2N_5O$: 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 (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). ^{1}H NMR (DMSO-d_6) (δ): 8.50 (1H, s, ArH), 7.75 (1H, s, ArH), 7.57 (1H, s, ArH), 4.92 (2H, s, CH_2), 3.49 (2H, s, CH_2), 2.30 (8H, s, $2CH_3$,CH_2), 0.96 (6H, s, $2CH_3$). Anal. Calcd for $C_{18}H_{23}Cl_2N_5O$: 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 **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). ^{1}H NMR (DMSO-d_6) (δ): 8.52 (1H, s, ArH), 7.76 (1H, s, ArH), 7.58 (1H, s, ArH), 4.93 (2H, s, CH_2), 3.65 (2H, t, CH_2), 3.57 (4H, t, $2CH_2$), 2.60 (2H, t, CH_2), 2.50 (4H, t, $2CH_2$). Anal. Calcd for $C_{17}H_{19}Cl_2N_5O_2$: 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_1AR 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. [^{3}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 [^{3}H]-

DPCPX to synaptosomal membranes from rat brain. Briefly, membrane protein (30 μM, 100 μL) were incubated with test compound solution (10 μM, 100 μL) and [^{3}H]-DPCPX (100 μL) for 30 min at 37 °C. Nonspecific binding was determined in the presence of R-PIA (10 μM, 100 μL). 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_{CPM} is total rate of binding, P_{CMP} is rate of test compounds binding, and N_{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_1AR according to a published procedure[19] and K_i values were obtain from the Cheng–Prusoff equation.[20]

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