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Novel 2- and 4- Substituted 1*H*-Imidazo[4,5-*c*]quinolin-4-amine Derivatives as Allosteric Modulators of the A_3 Adenosine Receptor

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Abstract

4-Arylamino and 2- cycloalkyl (including amino substitution) modifications were made in a series of 1*H*-imidazo-[4,5-*c*]quinolin-4-amine derivatives as allosteric modulators of the human A_3 adenosine receptor (AR). In addition to allosteric modulation of the maximum functional efficacy (in [^{35}S]GTPγS G protein binding assay) of the A_3AR agonist Cl-IB-MECA (**15**), some analogues also weakly inhibited equilibrium radioligand binding at ARs. 4-(3,5-Dichlorophenylamino) (**6**) or 2-(1-adamantyl) (**20**) substitution produced allosteric enhancement (twice the maximal agonist efficacy), with minimal inhibition of orthosteric AR binding. 2-(4-Tetrahydropyranyl) substitution abolished allosteric enhancement but preserved inhibition of orthosteric binding. Introduction of nitrogen in the six-membered ring at 2 position, to improve aqueous solubility and provide a derivatization site, greatly reduced the allosteric enhancement. 2-(4-(Benzoylamino)cyclohexyl) analogues **23** and **24** were weak negative A_3AR modulators. Thus, consistent with previous findings, the allosteric and orthosteric inhibitory A_3AR effects in imidazoquinolines are structurally separable, suggesting the possible design of additional derivatives with enhanced positive or negative allosteric A_3AR activity and improved selectivity in comparison to inhibition of orthosteric binding.

Keywords

nucleoside; G protein-coupled receptor; allosterism; adenosine receptor; radioligand binding; imidazoquinolines

Introduction

The adenosine receptors (ARs), of which A_1, A_{2A}, A_{2B}, and A_3 subtypes have been defined, represent a physiologically important family of G protein-coupled receptors (GPCRs).[1,2] ARs are important pharmacological targets in the treatment of a variety of diseases because of their key roles in controlling numerous cellular processes. For example, A_3AR agonists are of

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Supporting Information **Available:** Selected ^{1}H and 2D COSY and NOESY spectra with peak assignments. This material is available free of charge via the Internet at http://pubs.acs.org/jmc.

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interest for the treatment of cardiac ischemia, bowel inflammation, protection of skeletal muscle, cancer, and rheumatoid arthritis.[3-7] However, therapeutic intervention using a selective AR agonist is subject to side effects related, in part, to the widespread occurrence of the corresponding receptor throughout the body.

Native agonists of a given GPCR bind at a principal (orthosteric) site on the receptor protein to effect its activation. However, an allosteric modulator would bind to a distinct site on the receptor to either enhance (positive modulator) or impede (negative modulator) the action of a native agonist.[8-11] The therapeutic application of allosteric modulation has advantages over directly-acting orthosteric GPCR agonists. In a particular disease state, the effect of an endogenous agonist, which may be insufficient to fully compensate for an imbalance, may be magnified in a temporally and/or spatially specific manner by a positive allosteric modulator for therapeutic benefit.[12] The allosteric modulator theoretically would have no effect of its own on the unoccupied receptor. An additional advantage of allosteric modulators is that they typically are found to display higher subtype-selectivity than orthosteric ligands of the same receptor. Thus, allosteric action that is dependent on the simultaneous presence of an endogenous ligand ideally can produce a more selective drug action and prevent side effects and possible over-dosage associated with the administration of a conventional orthosteric agonist.

Allosteric modulators of several subtypes of ARs have been reported and their structure-activity relationships (SARs) explored.[8,13,14] *N*-(3,4-Dichlorophenyl)-2-cyclohexyl-1*H*-imidazo[4,5-*c*]quinolin-4-amine (LUF6000, **3**, Chart 1)[15-17] is an allosteric modulator of the human A_3AR that increases the maximum efficacy of the agonist 2-chloro-N^6-(3-iodobenzyl) adenosine-5′-*N*-methylcarboxamide (Cl-IB-MECA, **15**). A six-membered ring provided the optimal A_3AR enhancement in the homologous series of 2-cycloalkyl derivatives **1–4**. Compound **3** enhanced the A_3AR agonist efficacy in a functional assay and decreased the agonist dissociation rate without influencing agonist potency, because of decreased interaction with the orthosteric binding site on the A_3AR. Since the structural requirements for allosteric enhancement at the A_3AR are distinct from the requirements to inhibit equilibrium binding, structural manipulation of this family of imidazoquinolines might achieve even greater selectivity.

In the present study we have extended our search for highly effective allosteric enhancer ligands for the A_3AR, by modifying the substitutions around the 4-arylamino and 2-cycloalkyl moieties of the imidazoquinoline scaffold. We have also identified weak negative allosteric modulators in the same structural series.

Results and Discussion

The novel 4-substituted imidazoquinoline derivatives **5–14** (Scheme 1) were prepared from a key 4-chloro-2-cyclohexyl intermediate **29**. Oxidation of **27**[15] with 3-chloroperoxybenzoic acid (*m*-CPBA) afforded the 5-oxide derivative **28**, which was subsequently converted with phosphorus oxychloride into a 4-chloro species **29**.[15,18] Reaction of **29** with the appropriately substituted aniline provided the desired 4-amino derivatives **5–14** in varying yields (4–100%) where the 3-aminopyridyl derivative **14** being the lowest. Attempts to prepare an *ortho*-pyridyl derivative (i.e., using 2-aminopyridine) under the same reaction condition with **29** failed.

The novel 2-substituted imidazoquinoline derivatives **16–26** (Scheme 2) were prepared similarly. However, here different 2-cycloalkyl groups were appended, each as its carboxylic acid form, to a common intermediate **30** in an earlier synthetic step to give the corresponding imidazoquinoline derivatives **31a-f**. In fact, compound **30** also served as a precursor for **27**.[15] Next, compounds **31a-f** were treated with *m*-CPBA to afford 5-oxide derivatives **32a-f**, followed by phosphorus oxychloride to give the respective 4-chloro compounds **33a-f**.[15,18]

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Reaction of the 4-chloro-2-cycloalkyl derivatives **33a−f** with 3,4-dichloroaniline afforded the desired compounds (**16−21, 23**). Aniline was used instead in the synthesis of compound **24**.

Several alkylamino derivatives, **22** and **26**, were included. In the case of a 2-(4-*cis*-aminocyclohexyl) analogue **22**, it was necessary to remove the amine-protecting group of **21** in order to obtain the final compound. The deprotection was accomplished using methylamine in ethanol. In the case of the piperidine derivative **26**, the reduction of the carbonyl group of **23** with lithium aluminum hydride afforded the *N*-benzyl derivative **25**, which was subsequently converted into the desired compound **26** by hydrogenation.

To obtain a general procedure for the aniline substitution reaction, we first attempted the synthesis following the method recently described by Göblyös et al.,[15] using a microwave reactor (in ethanol under nitrogen, pre-stirring 60 sec, at 120 °C for 40 min, normal sample absorption, fixed hold time). Although the reaction was performed on a very small scale, we were able to determine preferred conditions to be *N,N*-dimethylformamide (DMF) as a solvent and using 2−3 equivalents of corresponding aniline with heating at 140 °C overnight under a N_2 atmosphere in a tightly sealed Biotage reaction vial. In an earlier attempted reaction at 105 °C under the same conditions, no desired product was obtained.[18]

The structures of the imidazoquinoline derivatives were analyzed by NMR in dimethyl sulfoxide (DMSO)-d_6. In all final compounds except the 2-*exo*-norbornyl-4-(3,4-dichlorophenyl)amino analogue **18** that was attached in an axial position, the central imidazoquinoline system was clearly attached to the six-membered ring in an equatorial position. This was demonstrated based on the *J* coupling of the proton at the junction (J_{ax-ax} = ca. 12−11 and J_{ax-eq} = ca. 5−3). We also noted the fact that potentially there are two possible annular tautomers (1*H* or 3*H* at the imidazole ring), which may affect the GPCR binding equilibrium depending on the stability of each form in the aqueous media. 2D NOESY experiments in DMSO-d_6 (see Supporting Information) suggested the 3*H*-tautomer as an exclusive form for compounds **10** and **11**. Interestingly, for compound **14**, two tautomers were observed as correlated by NOE cross-peaks (1*H*-/3*H*- ≈ 88:12 by NMR integration in DMSO-d_6). Unlike other compounds, detection of two competing tautomers on the NMR time-scale could be due to the formation of a hydrogen bond between the pyridyl nitrogen (acceptor) and the imidazole NH (donor) as a less preferred 3*H*-tautomer.

All of the imidazoquinoline derivatives were evaluated for interaction with the human A_3 receptors and two other ARs as listed in Tables 1 and 2. We first tested the effect of these compounds on the equilibrium binding at A_1, A_{2A}, and A_3ARs using standard agonist radioligands[19-21] [^{3}H]2-chloro-N^6-cyclopentyladenosine ([^{3}H]CCPA, **34**), [^{3}H]2-[4-(2-carboxylethyl)phenylethylamino]-5′-*N*-ethylcarboxamidoadenosine ([^{3}H]CGS21680, **35**), and [^{125}I]N^6-(4-amino-3-iodobenzyl)adenosine-5′-*N*-methylcarboxamide ([^{125}I]I-AB-MECA, **36**), respectively. For compounds **5−14** and **16−26**, only the percent inhibition of orthosteric radioligand binding was reported rather than K_i values, because the affinity at all three subtypes is weak and close to the solubility limits of the compounds. It is unknown whether the observed inhibition is of an allosteric or non-allosteric character.

Ability to allosterically modulate the A_3AR was determined using two methods: effects of the imidazoquinoline on the dissociation rate of **36** ([^{125}I]I-AB-MECA) and on the binding to the G protein of the stable GTP analogue [^{35}S]guanosine-5′-(γ-thiotriphosphate) ([^{35}S]GTPγS).[22] Depending on the functional assay used, **15** (Cl-IB-MECA) may appear to be either a full[15] or partial agonist[17,23-25] at the A_3AR. The earlier series of imidazoquinoline derivatives displayed dual and apparently opposite actions as positive allosteric modulators of agonist action and as inhibitors of radioligand binding.[15] In the previous study, compound **3** induced a substantial functional enhancement of the effects of the A_3AR agonist **15** as determined using an agonist radioligand dissociation kinetic assay and a cyclic AMP assay,[15,26] while there was only a weak inhibition of equilibrium radioligand binding at ARs at the concentration used.

The potentiation of the maximum efficacy of the agonist **15** by arylhalo derivatives **5−9** (Table 1) was high and similar to that observed with the lead compound **3**. The percent of maximal functional effect ranged from 171% for **9** to 209% for **6**. Among these halo analogues, **6** had the least inhibition of orthosteric binding to ARs. The degree of enhancement of [^{35}S]GTPγS binding by **6** over a range of concentrations of **15** up to 10 μM was indistinguishable from the effect of the lead compound **3** (Figure 1A). Similarly, the marked decrease in dissociation rate of the A_3AR agonist radioligand [^{125}I]**36** produced by **6** was indistinguishable from the effect of compound **3** (Figure 1B). A 3,5-di-(trifluoromethyl) analogue **10** and another aniline derivative **11** that was disubstituted with electron withdrawing groups displayed an intermediate degree of allosteric enhancement. Other 3,4-disubstituted anilines **12** and **13** that contained electron-donating groups displayed a high degree of allosteric enhancement, but also substantial inhibition of orthosteric binding to the A_3AR and other ARs. The 3-pyridinylamine **14** had a low degree of allosteric enhancement.

Highly variable biological activities were observed for the 2 position derivatives **16−26** (Table 2). Replacement of the distal methylene group of the 2-cyclohexyl ring of **3** with an ether oxygen in the tetrahydropyranyl derivative **16** abolished allosteric modulation of the A_3AR, but retained a similar degree of orthosteric inhibition. Inclusion of a methylene bridge across the 2-cyclohexyl ring of **3** in **17** and **18** resulted in considerable allosteric potentiation. Multiply bridged cycloalkyl substitution in the 2-(1-adamantyl)-4-(3,4-dichlorophenyl)amino analogue **20** resulted in high allosteric potentiation with minimal effects on the binding of orthosteric ligands at A_1, A_{2A}, and A_3ARs. The 2-*exo*-noradamantyl-4-(3,4-dichlorophenyl)amino analogue **19** displayed only moderate allosteric potentiation. The (decreasing) order of allosteric enhancement by these bridged cycloalkyl derivatives was: **20, 17** > **18** > **19**. The adamantyl derivative **20** increased [^{35}S]GTPγS binding over a range of concentrations of **15** up to 10 μM in a manner indistinguishable from the effect of **3** (Figure 2A).

Introduction of a nitrogen atom in (or as substituted at) the six-membered ring in **21−26** in an attempt to improve aqueous solubility and to provide a site for further derivatization greatly reduced the allosteric enhancement of this series. Variable degrees of inhibition of orthosteric binding at the AR were observed in this group with the most pronounced inhibition found for **21** and **24** at the A_{2A}AR and for **25** and **26** at the A_3AR. The slightly decreased agonist efficacy (91−93% of control in both functional assays, Table 2) of 2-(*N*-benzoyl-4-piperidinyl) analogues **23** and **24** was suggestive of possible negative allosteric modulation of the A^3AR. The effect of compound **24** on the dissociation kinetics of the agonist radioligand was only slightly different from control (Figure 2B).

Conclusions

In this study, structural modification of the 1*H*-imidazo-[4,5-*c*]quinolin-4-amine **3** has demonstrated that a limited set of substituents at the 2 and 4 positions are tolerated to preserve the allosteric enhancement of agonist action at the A_3AR. Notably, the haloanilino derivatives **5, 6**, and **8** and the bridged 2-cycloalkyl analogues **17** and **20** approximately doubled the maximum efficacy of the agonist **15** in the [^{35}S]GTPγS assay. The highest potentiation of the maximum efficacy of the agonist **15**, without increased inhibition of orthosteric binding, was observed for the 2-(1-adamantyl)-4-(3,4-dichlorophenyl)amino analogue **20**, with 210% activity of control in the [^{35}S]GTPγS binding assay. Compounds **6** and **20** were preferred as selective allosteric enhancers of the A_3AR due to the minimal effect on binding at the orthosteric sites of the three ARs examined. Substitution of a 4-tetrahydropyran moiety at the 2 position completely abolished allosteric enhancement but preserved inhibition of othosteric binding. Thus, as extension of previous findings, the allosteric and orthosteric inhibitory effects at the A_3AR in this series of imidazoquinolines are structurally separable. These biological results suggest that it will be possible to design additional derivatives that would display enhanced positive or negative allosteric activity at this receptor and improved selectivity in comparison to inhibition of orthosteric binding.

Experimental Procedures

General

Glassware was oven-dried and cooled in a desiccator before use. All reactions were carried out under a dry nitrogen atmosphere. Solvents were purchased as anhydrous grade and used without further purification. Suppliers of some commercial compounds are listed as follows: 3,4-diaminoquinoline (**30**) was purchased from Tyger Scientific, Inc.; 3,5-bis(trifluoromethyl) aniline and 4-aminophthalonitrile were purchased from Acros Organics; polyphosphoric acid, *m*-CPBA, phosphorus oxychloride ($POCl_3$), chloroform ($CHCl_3$), methylene chloride (CH_2Cl_2), DMF, toluene, diisopropyl ether, methanol (MeOH), tetrahydrofuran (THF), and most of other reagents and solvents were purchased from Sigma-Aldrich; dimethyl sulfoxide (DMSO)-d_6, chloroform-*d* ($CDCl_3$), and CD_3OD were purchased from Cambridge Isotope Laboratories. All reagents were of commercial grade and were used without further purification unless otherwise noted.

NMR spectra were recorded on either a Varian Inova/Gemini 300 or a Bruker DRX-600 spectrometer at 25.0 °C under an optimized parameter setting for each sample, unless otherwise mentioned. For compounds **5−14, 28**, and **29**, ^1H NMR chemical shifts were measured relative to the residual solvent peak at 2.50 ppm in DMSO-d_6 and at 3.31 ppm in CD_3OD or in a mixture of $CD_3OD/CDCl_3$. For compounds **16−26, 31a−f, 32a−f**, and **33a−f**, ^1H NMR chemical shifts were measured relative to tetramethylsilane at 0.00 ppm in $CDCl_3$ and the residual water peak at 3.30 ppm in CD_3OD. ^{13}C NMR chemical shifts were measured relative to the residual solvent peak at 49.15 ppm in CD_3OD or in a mixture of $CD_3OD/CDCl_3$. Suggested NMR peak assignments of some target compounds are shown in Supporting Information based on 2D COSY and NOESY experiments.

Analytical or preparative thin layer chromatography (TLC) was performed on either 0.2 mm silica coated sheets with F_{254} indicator (Sigma-Aldrich) or 0.2 mm reversed-phase C18 silica coated sheets (pore size: 60 Å, Whatman Inc.). Visualization of the products on the TLC plate was aided by the use of UV light, ninhydrin, or potassium permanganate. Column chromatography was performed on 230−400 mesh silica gel (pore size: 60 Å, Sigma-Aldrich). The tested imidazoquinoline derivatives were confirmed by HPLC to possess a ≥ 96% purity.

The electrospray ionization (ESI) MS experiments were performed on a Micromass/Waters LCT Premier Electrospray time-of-flight (TOF) mass spectrometer coupled with a Waters HPLC system at the Mass Spectrometry Facility, NIDDK, NIH.

General procedure for 2-substituted 1*H*-imidazo[4,5-*c*]quinoline (31a−f)

Polyphosphoric acid (1.3 mL/mmol) was added to 3,4-diaminoquinoline (**30**) (100 mg, 0.63 mmol) and the appropriate carboxylic acid (1.2 equiv). The mixture was stirred at 100 °C for 5 h. Then the reaction was cooled to 0 °C and to it was slowly added NH_4OH till pH = 8−9. The mixture was extracted with ethyl acetate (3 × 15 mL), the combined organic extracts were washed with water, brine, and again with water, and then dried over $MgSO_4$. The solution was filtered, the solvent was evaporated, and the residue was dried *in vacuo*. The residue obtained was subjected to preparative silica gel column chromatography (100:1 to 8:1 $CHCl_3$/MeOH).

2-(Tetrahydro-2H-pyran-4-yl)-1*H*-imidazo[4,5-*c*]quinoline (31a)

Yield: 107.6 mg (67%). ^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$) δ 13.40 (s, 1H), 9.13 (s, 1H), 8.35 (s, 1H), 8.08 (m, 1H), 7.65 (m, 2H), 3.98 (m, 2H), 3.35 (dd, 2H, J = 11.4, 10.2 Hz), 3.28 (t, 1H, J = 11.3 Hz), 2.05 (m, 2H), 1.95 (dd, 1H, J = 11.6, 4.2 Hz), 1.93 (dd, 1H, J = 11.6, 4.2 Hz); ^{13}C NMR (75 MHz, $CD_3OD/CDCl_3$) δ 143.4, 129.5, 126.3, 121.6, 66.7, 31.07; HRMS (ESI) calcd for $C_{15}H_{16}N_3O^+$ (M + H$^+$): 254.1288, found: 254.1293.

2-(2-Norbornanyl)-1*H*-imidazo[4,5-*c*]quinoline (31b)

Yield: 86.43 mg (52%). ^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$) δ 13.33 (bs, 1H), 9.11 (s, 1H), 8.32 (s, 1H), 8.08 (m, 1H), 7.64 (m, 2H), 3.45 (m, 1H), 3.36 (s, 2H), 3.17 (s, 2H), 3.06 (s, 1H), 2.72 (m, 1H), 2.58 (m, 1H), 2.50 (s, 1H), 2.38 (m, 1H), 1.44 (m, 7H); ^{13}C NMR (75 MHz, $CD_3OD/CDCl_3$) δ 143.85, 129.57, 125.98, 121.43, 48.59, 42.59, 36.61, 35.78, 29.10, 28.59, 23.60; HRMS (ESI) calcd for $C_{17}H_{18}N_3^+$ (M + H$^+$): 264.1495, found: 264.0561.

2-(4-(1,3-Dioxoisoindolin-2-yl)cyclohexyl)-1*H*-imidazo[4,5-*c*]quinoline (31c)

Yield: 88.0 mg (35%). ^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$) δ 13.35 (bs, 1H), 9.13 (s, 1H), 8.35 (s, 1H), 8.07 (m, 1H), 7.69 (m, 6H), 4.16 (m, 1H), 3.06 (t, 1H, J = 12.2 Hz), 2.36 (m, 4H), 1.88 (m, 4H); ^{13}C NMR (75 MHz, $CD_3OD/CDCl_3$) δ 167.9, 167.7, 143.4, 134.3, 129.5, 126.1, 122.9, 121.7, 49.5, 36.9, 30.7, 28.8; HRMS (ESI) calcd for $C_{24}H_{21}N_4O_2^+$ (M + H$^+$): 397.1659, found: 397.1234.

2-(1-Benzoylpiperidin-4-yl)-1*H*-imidazo[4,5-*c*]quinoline (31d)

Yield: 142 mg (67%). ^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$) δ 13.38 (s, 1H), 9.13 (s, 1H), 8.35 (s, 1H), 8.09 (s, 1H), 7.66 (s, 1H), 7.46 (s, 1H), 4.56 (s, 1H), 3.71 (s, 1H), 3.13 (s, 1H), 2.17 (s, 1H), 1.91 (s, 1H); ^{13}C NMR (75 MHz, $CD_3OD/CDCl_3$) δ 169.1, 143.4, 136.3, 129.4, 128.5, 126.6, 121.4, 59.8, 46.8, 41.2, 35.8, 30.4; HRMS (ESI) calcd for $C_{22}H_{21}N_4O^+$ (M + H$^+$): 357.1710, found: 357.1732.

2-(3-Noradamantanyl)-1*H*-imidazo[4,5-*c*]quinoline (31e)

Yield: 118.7 mg (68%). ^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$) δ 8.98 (s, 1H), 8.36 (s, 1H), 8.03 (d, 1H, J = 7.8 Hz), 7.52 (m, 2H), 2.77 (t, 1H, J = 6.9 Hz), 2.33 (s, 2H), 2.20 (m, 2H), 2.06 (m, 3H), 1.99 (m, 2H), 1.68 (m, 4H); ^{13}C NMR (75 MHz, $CD_3OD/CDCl_3$) δ 162.7, 143.2, 128.3, 121.5, 45.6, 43.6, 38.1, 37.8, 37.5, 37.3, 34.3, 30.4; HRMS (ESI) calcd for $C_{19}H_{20}N_3^+$ (M + H$^+$): 290.1652, found: 290.1670.

2-(1-Adamantanyl)-1*H*-imidazo[4,5-*c*]quinoline (31f)

Yield: 75.8 mg (39%). ^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$) δ 9.11 (s, 1H), 8.30 (s, 1H), 8.15 (d, 1H, J = 8.4 Hz), 7.53 (t, 1H, J = 7.2 Hz), 7.43 (d, 1H, J = 7.2 Hz), 2.21 (s, 6H), 2.05 (s, 4H), 1.72 (m, 6H); ^{13}C NMR (75 MHz, $CD_3OD/CDCl_3$) δ 143.6, 129.1, 127.4, 126.6, 121.9, 41.6, 36.5, 36.0, 228.3, 1.2; HRMS (ESI) calcd for $C_{20}H_{22}N_3^+$ (M + H$^+$): 304.1808, found: 304.1823.

General procedure for 2-substituted 1*H*-imidazo[4,5-*c*]quinoline-5-oxide (28, 32a−f)

The appropriate 2-substituted 1*H*-imidazo[4,5-*c*]quinoline derivatives (**27, 31a−f**) in a mixture of $CHCl_3$ (2.5 mL/mmol), CH_2Cl_2 (2.5 mL/mmol) and MeOH (0.25 mL/mmol) was heated for dissolution. To this mixture was added *m*-CPBA (2.5 equiv), which was then refluxed for 30 min. The reaction was cooled to rt, Na_2CO_3 (0.04 g/mmol) was added in one portion as a solid, and then the mixture was refluxed for 1 h. The solvent was removed *in vacuo*, and the crude product was chromatographed on silica gel (20:1 to 7:1 CH_2Cl_2/MeOH for **28**; 100:1 to 8:1 $CHCl_3$/MeOH for **32a−f**) to give the desired compound.

2-Cyclohexyl-1*H*-imidazo[4,5-*c*]quinoline 5-oxide (28).[15]

Scale: 0.612 mmol. Yield: 150 mg (91%). R_f: 0.43 [silica gel, 10:1 CH_2Cl_2/MeOH]; ^{1}H NMR (300 MHz, CD_3OD) δ 9.03 (s, 1H), 8.78−8.72 (m, 1H), 8.47 (m, 1H), 7.89−7.83 (m, 2H), 3.06 (tt, 1H, J = 11.8, 3.5 Hz), 2.20−2.14 (m, 2H), 1.98−1.91 (m, 2H), 1.86−1.69 (m, 3H), 1.60−1.32 (m, 3H); ^{13}C NMR (75 MHz, CD_3OD) δ 130.9, 130.6, 130.4, 129.0, 123.5 121.2, 40.2, 32.9, 27.2, 27.0; HRMS (ESI) calcd for $C_{16}H_{18}N_3O$ (M + H^+): 268.1450, found: 268.1451.

2-(Tetrahydro-2*H*-pyran-4-yl)-1*H*-imidazo[4,5-*c*]quinoline-5-oxide (32a)

Scale: 0.42 mmol. Yield: 65 mg (57%). ^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$) δ 9.18 (s, 1H), 8.80 (m, 1H), 8.53 (m, 1H), 7.85 (m, 2H), 4.17 (m, 2H), 3.68 (m, 2H), 3.36 (m, 1H), 2.15 (m, 4H); HRMS (ESI) calcd for $C_{15}H_{16}N_3O_2^+$ (M + H^+): 270.1237, found: 270.1032.

2-(2-Norbornanyl)-1*H*-imidazo[4,5-*c*]quinoline-5-oxide (32b)

Scale: 0.25 mmol. Yield: 44.4 mg (60%). ^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$) δ 8.77 (s, 1H), 8.05 (s, 1H), 7.95 (s, 1H), 7.81 (m, 2H), 3.13 (mm, 1H), 2.81 (m, 1H), 2.68 (s, 1H), 2.52 (s, 1H), 2.14 (m, 1H), 1.93 (m, 1H), 1.58 (m, 7H); HRMS (ESI) calcd for $C_{17}H_{18}N_3O^+$ (M + H^+): 280.1444, found: 280.1443.

2-(4-(1,3-Dioxoisoindolin-2-yl)cyclohexyl)-1*H*-imidazo[4,5-*c*]quinoline-5-oxide (32c)

Scale: 0.40 mmol. Yield: 65 mg (44%). ^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$) δ 8.96 (s, 1H), 8.63 (m, 1H), 8.32 (m, 1H), 7.75 (m, 6H), 4.18 (m, 1H), 3.04 (t, 1H, J = 11.6 Hz), 2.24 (m, 4H), 1.84 (m, 4H); HRMS (ESI) calculated for $C_{24}H_{21}N_4O_2^+$ (M + H^+): 413.1608, found: 413.1463.

2-(1-Benzoylpiperidin-4-yl)-1*H*-imidazo[4,5-*c*]quinoline-5-oxide (32d)

Scale: 0.30 mmol. Yield: 74 mg (67%). ^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$) δ 8.79 (s, 1H), 8.57 (m, 1H), 8.18 (bs, 1H), 7.76 (s, 1H), 7.68 (m, 1H), 7.54 (m, 5H), 4.59 (m, 1H), 3.76 (m, 1H), 2.93 (m, 2H), 2.02 (m, 2H), 1.85 (m, 4H); HRMS (ESI) calcd for $C_{22}H_{20}N_4O_2^+$ (M + H^+): 372.1586, found: 373.1665.

2-(3-Noradamantanyl)-1*H*-imidazo[4,5-*c*]quinoline-5-oxide (32e)

Scale: 0.41 mmol. Yield: 89 mg (71%). ^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$) δ 8.77 (s, 1H), 8.52 (m, 1H), 8.29 (s, 1H), 7.71 (m, 1H), 7.53 (m, 2H), 2.67 (t, 1H, J = 6.7 Hz), 2.27 (s, 2H), 2.09 (d, 2H, J = 11.4 Hz), 1.95 (d, 1H, J = 10.8 Hz), 1.87 (m, 3H), 1.58 (m, 4H); HRMS (ESI) calcd for $C_{19}H_{20}N_3O^+$ (M + H^+): 306.1601, found: 306.1606.

2-(1-Adamantanyl)-1*H*-imidazo[4,5-*c*]quinoline-5-oxide (32f)

Scale: 0.25 mmol. Yield: 63 mg (78%). ^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$) δ 8.91 (m, 1H), 8.60 (m, 1H), 8.31 (m, 1H), 7.90 (s, 1H), 7.80 (d, 1H, J = 7.5 Hz), 7.54 (m, 1H), 2.08 (s, 10H), 1.76 (s, 6H); HRMS (ESI) calcd for $C_{20}H_{22}N_3O^+$ (M + H^+): 320.1757, found: 320.1753.

4-Chloro-2-cyclohexyl-1*H*-imidazo[4,5-*c*]quinoline (29).[15]

Compound **28** (150 mg, 560 μmol) was suspended in a mixture of toluene (0.30 mL) and DMF (0.60 mL), and then was treated with 140 μL (1.50 mmol) of $POCl_3$ at 0 °C with stirring. The ice bath was removed, and the reaction was heated at 100 °C for 1.5 h. The mixture was cooled to rt, and a few small pieces of ice were added with stirring. Subsequently, the pH was adjusted to 6−7 with solid $NaHCO_3$. The mixture was sonicated and filtered, rinsing with water and diisopropyl ether, and the collected solid was dried *in vacuo* to give 97.1 mg (340 μmol, 61%) of **29** as a beige solid. R_f: 0.56 [silica gel, 10:1 CH_2Cl_2/MeOH]; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.38−8.35 (m, 1H), 8.02−7.99 (m, 1H), 7.71−7.62 (m, 2H), 3.07 (tt, 1H, J = 12.0, 3.5

Hz), 2.16−2.10 (m, 2H), 1.97−1.91 (m, 2H), 1.88−1.75 (m, 3H), 1.59−1.35 (m, 3H); ^{13}C NMR (75 MHz, CD_3OD) δ 129.5, 129.3, 128.2, 122.8, 40.4, 33.0, 27.4, 27.0; HRMS (ESI) calcd for $C_{16}H_{17}ClN_3$ (M + H^+): 286.1111, found: 286.1106.

General procedure for 2-substituted 4-chloro-1*H*-imidazo[4,5-*c*]quinolines (33a−f)

A mixture of toluene (0.45 mL/mmol) and DMF (0.90 mL/mmol) was cooled in an ice bath, and phosphorus oxychloride (2.6 eq.) was added. After 10 min, the appropriate 1*H*-imidazo [4,5-*c*]quinolin-5-oxide was added, and the solution was stirred at rt for 10 min. Subsequently, the solution was heated to 100 °C for 30 min. Upon cooling, the solvent was evaporated, and the resulting syrup was poured on chipped ice while stirring. The mixture was then warmed to rt and carefully adjusted to pH 6−7 with solid $NaHCO_3$. After 2 h, the formed solid was filtered off, washed with water and diisopropyl ether and subsequently dried. The residue obtained was subjected to preparative silica gel column chromatography (100:1 to 8:1 $CHCl_3$/MeOH).

4-Chloro-2-(tetrahydro-2H-pyran-4-yl)-1*H*-imidazo[4,5-*c*]quinoline (33a)

Scale: 0.27 mmol. Yield: 41 mg (53%). ^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$) δ 13.66 (br s, 1H), 8.35 (m, 1H), 8.02 (m, 1H), 7.70 (m, 2H), 4.04 (m, 2H), 3.52 (dd, 2H, J = 11.1, 10.8 Hz), 3.25 (m, 1H), 1.95 (m, 4H); ^{13}C NMR (75 MHz, $CD_3OD/CDCl_3$) δ 143.2, 128.1, 126.8, 121.2, 67.6, 36.1, 31.1; HRMS (ESI) calcd for $C_{15}H_{15}ClN_3O^+$ (M + H^+): 288.0898, found: 288.0713.

4-Chloro-2-(2-norbornanyl)-1*H*-imidazo[4,5-*c*]quinoline (33b)

Scale: 0.25 mmol. Yield: 44.4 mg (60%). ^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$) δ 8.29 (m, 1H), 7.96 (m, 2H), 7.56 (m, 2H), 3.31 (m, 1H), 3.05 (s, 2H), 2.91 (s, 2H), 2.83 (m, 2H), 2.41 (m, 1H), 2.06 (m, 1H), 1.62 (m, 7H); ^{13}C NMR (75 MHz, $CD_3OD/CDCl_3$) δ 143.0, 127.8, 126.5, 121.4, 42.8, 42.0, 36.3, 36.1, 29.6, 28.6; HRMS (ESI) calcd for $C_{17}H_{17}ClN_3^+$ (M + H^+): 298.1106, found: 298.1104.

4-Chloro-2-(4-(1,3-dioxoisoindolin-2-yl)-1*H*-imidazo[4,5-*c*]quinoline (33c)

Scale: 0.27 mmol. Yield: 87 mg (74%). ^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$) δ 8.56 (m, 1H), 8.22 (d, 1H, J = 7.9 Hz), 8.09 (d, 1H, J = 8.5 Hz), 7.70 (m, 6H), 4.25 (m, 1H), 3.35 (m, 1H), 2.42 (m, 4H), 1.85 (m, 41H); ^{13}C NMR (75 MHz, $CD_3OD/CDCl_3$) δ 168.5, 158.7, 144.2, 143.6, 134.1, 132.5, 132.3, 132.2, 129.0, 128.8, 123.3, 50.0, 37.8, 31.3, 29.3; HRMS (ESI) calcd for $C_{24}H_{20}ClN_4O_2^+$ (M + H^+): 431.1269, found: 431.1260.

4-Chloro-2-(1-benzoylpiperidin-4-yl)-1*H*-imidazo[4,5-*c*]quinoline (33d)

Scale: 0.20 mmol. Yield: 42.4 mg (54%). ^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$) δ 7.99 (s, 1H), 7.86 (d, 1H, J = 7.5 Hz), 7.48 (m, 3H), 7.26 (m, 5H), 4.63 (m, 1H), 3.76 (m, 1H), 3.18 (m, 1H), 2.87 (m, 1H), 2.05 (m, 2H), 1.92 (m, 4H); ^{13}C NMR (75 MHz, $CD_3OD/CDCl_3$) δ 135.9, 129.9, 128.6, 128.3, 128.0, 127.0, 126.7, 121.4, 53.6, 36.8, 30.6; HRMS (ESI) calcd for $C_{22}H_{20}ClN_4O^+$ (M + H^+): 391.1320, found: 391.1317.

4-Chloro-2-(3-noradamantanyl)-1*H*-imidazo[4,5-*c*]quinoline (33e)

Scale: 0.23 mmol. Yield: 60 mg (79%). ^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$) δ 8.37 (s, 1H), 8.10 (d, 1H, J = 7.8 Hz), 7.78 (m, 1H), 7.38 (m, 2H), 2.67 (m, 1H), 2.21 (s, 2H), 2.08 (m, 2H), 1.90 (m, 5H), 1.54 (s, 4H); ^{13}C NMR (75 MHz, $CD_3OD/CDCl_3$) δ 162.9, 142.9, 128.2, 127.7, 121.4, 45.5, 43.5, 38.1, 37.7, 37.4, 37.2, 36.4, 34.3, 31.2; HRMS (ESI) calcd for $C_{19}H_{19}ClN_3^+$ (M + H^+): 324.1262, found: 324.1268.

4-Chloro-2-(1-adamantanyl)-1*H*-imidazo[4,5-*c*]quinoline (33f)

Scale: 0.20 mmol. Yield: 39 mg (60%). ^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$) δ 8.34 (s, 1H), 7.95 (m, 2H), 7.45 (m, 2H), 2.15 (m, 10H), 1.78 (m, 6H); ^{13}C NMR (75 MHz, $CD_3OD/CDCl_3$) δ 163.1, 143.0, 132.6, 127.8, 126.5, 121.5, 40.8, 36.4, 36.2, 35.8, 32.2, 28.1; HRMS (ESI) calcd for $C_{20}H_{21}ClN_3^+$ (M + H$^+$): 338.1419, found: 338.1414.

General procedure for *N*-substituted 2-cyclohexyl-1*H*-imidazo[4,5-*c*]quinolin-4-amine (5−14)

A solution of compound **29** and an appropriate aniline in DMF was heated at 140 °C overnight under a dry nitrogen atmosphere in a tightly sealed Biotage microwave vial (size: 0.2−0.5 mL or 0.5−2.0 mL) equipped with a magnetic stir bar. The reaction mixture was cooled, the solvent was removed *in vacuo*, and the crude product was purified by a preparative TLC.

N-(3-Chlorophenyl)-2-cyclohexyl-1*H*-imidazo[4,5-*c*]quinolin-4-amine (5)

Compound **29** (6.7 mg, 23 μmol) was reacted with 3-chloroaniline (10 μL, 94 μmol) in DMF (50 μL). The crude product was chromatographed on silica gel (3:1 petroleum ether/EtOAc) to give 5.5 mg (15 μmol, 62%) of **5**. R_f: 0.43 [silica gel, 2:1 petroleum ether/EtOAc]; ^{1}H NMR (600 MHz, 4:1 $CDCl_3/CD_3OD$) δ 8.30 (s, 1H), 7.94 (d, 1H, J = 7.4 Hz), 7.89 (d, 1H, J = 8.5 Hz), 7.75 (d, 1H, J = 8.0 Hz), 7.48 (t, 1H, J = 7.5 Hz), 7.38 (t, 1H, J = 7.3 Hz), 7.24 (t, 1H, J = 7.9 Hz), 6.94 (d, 1H, J = 8.3 Hz), 2.92 (t, 1H, J = 11.9 Hz), 2.13 (d, 2H, J = 12.6 Hz), 1.90 (d, 2H, J = 13.3 Hz), 1.78 (d, 1H, J = 12.6 Hz), 1.67 (q, 2H, J = 11.7 Hz), 1.44 (q, 2H, J = 12.7 Hz), 1.32 (q, 1H, J = 12.9 Hz); ^{13}C NMR (150 MHz, 4:1 $CDCl_3/CD_3OD$) δ 147.1, 143.9, 134.7, 130.1, 127.7, 127.6, 123.3, 121.8, 120.7, 118.8, 117.0, 115.7, 38.8, 32.2, 26.4, 26.1; HRMS (ESI) calcd for $C_{22}H_{22}ClN_4$ (M + H$^+$): 377.1533, found: 377.1528.

2-Cyclohexyl-*N*-(3,5-dichlorophenyl)-1*H*-imidazo[4,5-*c*]quinolin-4-amine (6)

Compound **29** (7.4 mg, 26 μmol) was reacted with 3,5-dichloroaniline (13 mg, 80 μmol) in DMF (50 μL). The crude product was chromatographed on silica gel (3:1 petroleum ether/EtOAc) to give 6.0 mg (15 μmol, 56%) of **6**. R_f: 0.50 [silica gel, 2:1 petroleum ether/EtOAc]; ^{1}H NMR (600 MHz, 4:1 $CDCl_3/CD_3OD$) δ 8.05 (s, 2H), 7.95 (d, 1H, J = 7.6 Hz), 7.91 (d, 1H, J = 8.3 Hz), 7.49 (t, 1H, J = 7.6 Hz), 7.32 (t, 1H, J = 7.4 Hz), 6.94 (s, 1H), 2.91 (t, 1H, J = 12.0 Hz), 2.13 (d, 2H, J = 11.9 Hz), 1.89 (d, 2H, J = 13.9 Hz), 1.78 (d, 1H, J = 12.2 Hz), 1.66 (q, 2H, J = 12.5 Hz), 1.44 (q, 2H, J = 13.0 Hz), 1.32 (q, 1H, J = 13.1 Hz); ^{13}C NMR (150 MHz, 4:1 $CDCl_3/CD_3OD$) δ 158.1, 146.7, 143.7, 143.2, 135.1, 134.8, 127.8, 127.7, 126.7, 123.6, 121.4, 120.8, 116.9, 115.8, 38.8, 32.2, 26.4, 26.1; HRMS (ESI) calcd for $C_{22}H_{21}Cl_2N_4$ (M + H$^+$): 411.1143, found: 411.1140.

2-Cyclohexyl-*N*-(2,4-dichlorophenyl)-1*H*-imidazo[4,5-*c*]quinolin-4-amine (7)

Compound **29** (8.2 mg, 29 μmol) was reacted with 2,4-dichloroaniline (14 mg, 87 μmol) in DMF (50 μL). The crude product was chromatographed on silica gel (3:1 hexane/EtOAc) to give 5.0 mg (12 μmol, 42%) of **7**. R_f: 0.63 [silica gel, 2:1 petroleum ether/EtOAc]; ^{1}H NMR (600 MHz, 4:1 $CDCl_3/CD_3OD$) δ 8.89 (d, 1H, J = 9.4 Hz), 7.98 (d, 1H, J = 7.7 Hz), 7.84 (d, 1H, J = 8.1 Hz), 7.47 (t, 1H, J = 7.6 Hz), 7.39 (d, 1H, J = 2.2 Hz), 7.33 (t, 1H, J = 7.4 Hz), 7.27 (dd, 1H, J = 8.6, 2.3 Hz), 2.96 (tt, 1H, J = 11.9, 3.5 Hz), 2.11 (d, 2H, J = 12.1 Hz), 1.89 (d, 2H, J = 13.7 Hz), 1.77 (d, 1H, J = 12.3 Hz), 1.70 (q, 2H, J = 12.3 Hz), 1.44 (q, 2H, J = 12.8 Hz), 1.32 (q, 1H, J = 12.7 Hz); ^{13}C NMR (150 MHz, 4:1 $CDCl_3/CD_3OD$) δ 146.7, 136.1, 135.0, 129.0, 127.7, 127.6 (127.615), 127.6 (127.570), 123.6, 122.1, 120.9, 116.1, 39.1, 32.2, 26.4, 26.1; HRMS (ESI) calcd for $C_{22}H_{21}Cl_2N_4$ (M + H$^+$): 411.1143, found: 411.1132.

2-Cyclohexyl-*N*-(3,4-difluorophenyl)-1*H*-imidazo[4,5-*c*]quinolin-4-amine (8)

Compound **29** (7.0 mg, 24 μmol) was reacted with 3,4-difluoroaniline (20 μL, 200 μmol) in DMF (30 μL). The crude product was chromatographed on silica gel (7:4 petroleum ether/EtOAc) to give 7.1 mg (19 μmol, 77%) of **8**. R_f: 0.36 [silica gel, 2:1 petroleum ether/EtOAc]; ^{1}H NMR (600 MHz, 4:1 $CDCl_3/CD_3OD$) δ 8.31 (m, 1H), 7.93 (d, 1H, J = 7.9 Hz), 7.87 (d, 1H, J = 8.4 Hz), 7.47 (t, 1H, J = 7.7 Hz), 7.44 (d, 1H, J = 8.8 Hz), 7.30 (t, 1H, J = 7.4 Hz), 7.09 (q, 1H, J = 9.3 Hz), 2.91 (t, 1H, J = 11.8 Hz), 2.13 (d, 2H, J = 11.9 Hz), 1.89 (d, 2H, J = 13.2 Hz), 1.78 (d, 1H, J = 12.7 Hz), 1.66 (q, 2H, J = 11.4 Hz), 1.44 (q, 2H, J = 12.8 Hz), 1.32 (q, 1H, J = 13.0 Hz); ^{13}C NMR (150 MHz, 4:1 $CDCl_3/CD_3OD$) δ 175.5, 163.8, 158.0, 147.1, 143.9, 134.7, 127.6, 123.2, 120.8, 117.2, 117.1, 115.7, 114.4, 108.3, 108.1, 38.8, 32.2, 26.4, 26.1; HRMS (ESI) calcd for $C_{22}H_{21}F_2N_4$ (M + H^+): 379.1734, found: 379.1739.

2-Cyclohexyl-*N*-(3,5-difluorophenyl)-1*H*-imidazo[4,5-*c*]quinolin-4-amine (9)

Compound **29** (5.1 mg, 18 μmol) was reacted with 3,5-difluoroaniline (9.5 mg, 72 μmol) in DMF (50 μL). The crude product was chromatographed on silica gel (20:1 CH_2Cl_2/MeOH, 20:1 $CHCl_3$/MeOH, and then 15:1 CH_2Cl_2/MeOH) to give 7.4 mg (20 μmol, 100%) of **9**. R_f: 0.54 [silica gel, 20:1 CH_2Cl_2/MeOH]; ^{1}H NMR (600 MHz, DMSO-d_6) δ 13.19 (s, 1H), 9.58 (s, 1H), 8.16 (m, 3H), 7.83 (d, 1H, J = 8.2 Hz), 7.53 (t, 1H, J = 7.7 Hz), 7.41 (t, 1H, J = 8.0 Hz), 6.73 (t, 1H, J = 8.8 Hz), 3.00 (tt, 1H, J = 11.7, 3.3 Hz), 2.08 (d, 2H, J = 12.2 Hz), 1.87 (d, 2H, J = 13.1 Hz), 1.77−1.71 (m, 3H), 1.44 (qt, 2H, J = 12.8, 3.0 Hz), 1.32 (qt, 1H, J = 12.5, 3.3 Hz); ^{13}C NMR (150 MHz, 4:1 $CDCl_3/CD_3OD$) δ 164.6, 164.4, 162.9, 162.8, 146.8, 143.7, 143.6, 134.8, 127.8, 127.7, 123.5, 120.8, 115.8, 101.6, 101.4, 96.8, 96.6, 96.5, 38.8, 32.2, 26.4, 26.1; HRMS (ESI) calcd for $C_{22}H_{21}F_2N_4$ (M + H^+): 379.1734, found: 379.1740.

N-(3,5-Bis(trifluoromethyl)phenyl)-2-cyclohexyl-1*H*-imidazo[4,5-*c*]quinolin-4-amine (10)

Compound **29** (4.35 mg, 15.2 μmol) was reacted with 3,5-bis(trifluoromethyl)aniline (10.0 μL, 62.7 μmol) in DMF (50 μL). The crude product was chromatographed on silica gel (2:1 hexane/EtOAc, 30:1 $CHCl_3$/MeOH, 80:20:1 hexane/EtOAc/triethylamine, 5:2 hexane/EtOAc, and 35:1 $CHCl_3$/MeOH with a trace amount of citric acid) to give 1.40 mg (2.93 μmol, 19%) of **10**. R_f: 0.43 [silica gel, 2:1 hexane/EtOAc]; ^{1}H NMR (600 MHz, DMSO-d_6) δ 13.25 (s, 1H), 10.00 (s, 1H), 9.17 (s, 2H), 8.19 (d, 1H, J = 7.7 Hz), 7.76 (d, 1H, J = 8.2 Hz), 7.58 (s, 1H), 7.56 (t, 1H, J = 7.9 Hz), 7.44 (t, 1H, J = 7.6 Hz), 3.02 (tt, 1H, J = 11.8, 3.4 Hz), 2.11 (d, 2H, J = 11.9 Hz), 1.88 (d, 2H, J = 13.5 Hz), 1.78−1.72 (m, 3H), 1.45 (qt, 2H, J = 12.8, 3.3 Hz), 1.32 (qt, 1H, J = 12.6, 3.3 Hz); ^{13}C NMR (150 MHz, 4:1 $CDCl_3/CD_3OD$) δ 143.6, 142.9, 135.0, 128.0, 127.8, 123.8, 120.8, 118.2, 115.9, 114.3, 38.8, 32.2, 26.4, 26.1; HRMS (ESI) calcd for $C_{24}H_{21}F_6N_4$ (M + H^+): 479.1670, found: 479.1680.

4-(2-Cyclohexyl-1*H*-imidazo[4,5-*c*]quinolin-4-ylamino)phthalonitrile (11)

Compound **29** (4.50 mg, 15.7 μmol) was reacted with 4-aminophthalonitrile (11.6 mg, 78.8 μmol) in DMF (50 μL). The crude product was chromatographed on reversed-phase C18 silica gel (9:1 MeOH/H_2O) and normal-phase silica gel (15:1 $CHCl_3$/MeOH) to give 0.45 mg (1.1 μmol, 7.3%) of **11**. R_f: 0.80 [silica gel, 10:1 $CHCl_3$/MeOH]; ^{1}H NMR (600 MHz, DMSO-d_6) δ 13.30 (s, 1H), 10.19 (s, 1H), 9.07 (s, 1H), 8.78 (d, 1H, J = 7.6 Hz), 8.21 (d, 1H, J = 7.2 Hz), 8.04 (d, 1H, J = 8.9 Hz), 7.89 (d, 1H, J = 8.2 Hz), 7.58 (t, 1H, J = 7.6 Hz), 7.48 (t, 1H, J = 7.1 Hz), 3.02 (tt, 1H, J = 12.1, 3.4 Hz), 2.09 (d, 2H, J = 10.7 Hz), 1.87 (d, 2H, J = 13.2 Hz), 1.78−1.72 (m, 3H), 1.45 (qt, 2H, J = 12.8, 3.3 Hz), 1.32 (qt, 1H, J = 12.9, 3.5 Hz); ^{13}C NMR (150 MHz, 4:1 $CDCl_3/CD_3OD$) δ 158.5, 146.2, 145.7, 143.3, 134.6, 130.0, 128.2, 128.0, 124.4, 122.6, 121.8, 120.9, 116.9, 116.7, 116.5, 116.1, 38.8, 32.2, 26.4, 26.1; HRMS (ESI) calcd for $C_{24}H_{21}N_6$ (M + H^+): 393.1828, found: 393.1835.

2-Cyclohexyl-*N*-(3,4-dimethoxyphenyl)-1*H*-imidazo[4,5-*c*]quinolin-4-amine (12)

Compound **29** (7.8 mg, 27 μmol) was reacted with 3,4-dimethoxyaniline (13 mg, 83 μmol) in DMF (50 μL). The crude product was chromatographed on silica gel (2:3 hexane/EtOAc) to give 2.5 mg (6.2 μmol, 23%) of **12**. R_f: 0.33 [silica gel, 1:1 petroleum ether/EtOAc]; ^{1}H NMR (600 MHz, 4:1 $CDCl_3/CD_3OD$) δ 7.98 (s, 1H), 7.92 (d, 1H, J = 6.6 Hz), 7.80 (d, 1H, J = 8.6 Hz), 7.45 (t, 1H, J = 7.6 Hz), 7.33 (d, 1H, J = 9.7 Hz), 7.27 (t, 1H, J = 7.1 Hz), 6.87 (d, 1H, J = 8.1 Hz), 3.95 (s, 3H), 3.84 (s, 3H), 2.92 (t, 1H, J = 11.9 Hz), 2.13 (d, 2H, J = 11.0 Hz), 1.89 (d, 2H, J = 13.2 Hz), 1.78 (d, 1H, J = 11.6 Hz), 1.67 (q, 2H, J = 12.1 Hz), 1.44 (q, 2H, J = 13.0 Hz), 1.32 (q, 1H, J = 13.0 Hz); ^{13}C NMR (150 MHz, 4:1 $CDCl_3/CD_3OD$) δ 149.3, 144.3, 144.1, 135.1, 127.5, 127.3, 122.7, 120.7, 112.4, 111.0, 104.5, 56.6, 56.0, 38.9, 32.2, 26.4, 26.1; HRMS (ESI) calcd for $C_{24}H_{27}N_4O_2$ (M + H^+): 403.2134, found: 403.2100.

N-(Benzo[*d*][1,3]dioxol-5-yl)-2-cyclohexyl-1*H*-imidazo[4,5-*c*]quinolin-4-amine (13)

Compound **29** (13.7 mg, 47.9 μmol) was reacted with 3,4-(methylenedioxy)aniline (16.3 mg, 115 μmol) in DMF (100 μL). The crude product was chromatographed on silica gel (2:3 and 1:1 hexane/EtOAc) to give 5.4 mg (14 μmol, 29%) of **13**. R_f: 0.59 [silica gel, 1:1 hexane/EtOAc]; ^{1}H NMR (600 MHz, 4:1 $CDCl_3/CD_3OD$) δ 7.91 (br s, 1H), 7.82 (d, 1H, J = 8.6 Hz), 7.80 (s, 1H), 7.44 (t, 1H, J = 7.7 Hz), 7.26 (t, 1H, J = 6.8 Hz), 7.21 (d, 1H, J = 7.3 Hz), 6.78 (d, 1H, J = 8.5 Hz), 5.91 (s, 2H), 2.91 (t, 1H, J = 11.6 Hz), 2.12 (d, 2H, J = 12.0 Hz), 1.89 (d, 2H, J = 13.5 Hz), 1.77 (d, 1H, J = 13.4 Hz), 1.66 (q, 2H, J = 11.9 Hz), 1.43 (q, 2H, J = 12.7 Hz), 1.31 (q, 1H, J = 12.8 Hz); ^{13}C NMR (150 MHz, 4:1 $CDCl_3/CD_3OD$) δ 147.9, 144.0, 135.6, 127.5, 127.2, 122.8, 120.7, 115.6, 112.1, 108.5, 102.2, 101.2, 38.9, 32.2, 26.4, 26.1; HRMS (ESI) calcd for $C_{23}H_{23}N_4O_2$ (M + H^+): 387.1821, found: 387.1814.

2-Cyclohexyl-*N*-(pyridin-3-yl)-1*H*-imidazo[4,5-c]quinolin-4-amine (14)

Compound **29** (6.67 mg, 23.3 μmol) was reacted with 3-aminopyridine (11.0 mg, 116 μmol) in DMF (50 μL). The crude product was chromatographed on normal-phase silica gel (10:1 and 3:1 $CHCl_3$/MeOH, and 90:3:1 $CHCl_3$/MeOH/triethylamine), on reversed-phase C18 silica gel (4:1 MeOH/H_2O), and again on normal-phase silica gel (15:1 $CHCl_3$/MeOH) to give 0.35 mg (0.99 μmol, 4.2%) of **14**. R_f: 0.57 [silica gel, 200:10:1 $CHCl_3$/MeOH/triethylamine]; ^{1}H NMR (600 MHz, DMSO-d_6) [major tautomer] δ 13.16 (s, 1H), 9.36 (d, 1H, J = 2.6 Hz), 9.31 (s, 1H), 8.73 (ddd, 1H, J = 8.2, 2.5, 1.3 Hz), 8.17 (dd, 1H, J = 4.7, 1.5 Hz), 8.14 (dd, 1H, J = 8.2, 1.0 Hz), 7.78 (d, 1H, J = 7.7 Hz), 7.50 (td, 1H, J = 7.7, 1.7 Hz), 7.38 (td, 1H, J = 7.4, 1.1 Hz), 7.35 (dd, 1H, J = 8.3, 4.7 Hz), 3.00 (tt, 1H, J = 12.1, 3.5 Hz), 2.09 (d, 2H, J = 12.7 Hz), 1.87 (dt, 2H, J = 13.2, 3.4 Hz), 1.78−1.72 (m, 3H), 1.45 (qt, 2H, J = 13.0, 3.2 Hz), 1.32 (qt, 1H, J = 12.8, 3.3 Hz); ^{13}C NMR (150 MHz, 2:1 $CDCl_3/CD_3OD$) δ 158.4, 142.0, 140.3, 139.0, 137.2, 131.5, 130.4, 130.2, 127.9, 127.8, 126.7, 124.5, 123.7, 121.1, 116.1, 39.1, 32.4, 26.6, 26.3; HRMS (ESI) calcd for $C_{21}H_{22}N_5$ (M + H^+): 344.1875, found: 344.1873.

General procedure for *N*-substituted 1*H*-imidazo[4,5-*c*]quinolin-4-amine (16−21, 23)

A solution of the appropriate 4-chloro-1*H*-imidazo[4,5-*c*]quinoline and 3,4-dichloroaniline in DMF was heated at 140 °C overnight under a dry nitrogen atmosphere. The reaction mixture was cooled, and the solvent was evaporated. The residue obtained was subjected to preparative silica gel column chromatography (100:1 to 8:1 $CHCl_3$/MeOH).

N-(3,4-Dichlorophenyl)-2-(tetrahydro-2*H*-pyran-4-yl)-1*H*-imidazo[4,5-*c*]quinolin-4-amine (16)

Scale: 0.14 mmol. Yield: 20 mg (35%). ^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$) δ 8.44 (s, 1H), 7.89 (d, 2H, J = 9.0 Hz), 7.74 (dd, 1H, J = 9.0, 2.5 Hz), 7.50 (m, 1H), 7.37 (m, 2H), 4.10 (tdt, 2H, J = 11.4, 3.3 Hz), 3.59 (m, 2H), 3.21 (m, 1H), 2.04 (m, 4H); ^{13}C NMR (75 MHz, $CD_3OD/CDCl_3$) δ 143.4, 140.4, 132.2, 127.4, 127.2, 124.0, 123.2, 120.4, 119.9, 117.9, 115.3, 67.5, 35.5, 31.1; HRMS (ESI) calcd for $C_{21}H_{19}Cl_2N_3O^+$ (M + H^+): 413.0930, found: 413.0952.

N-(3,4-Dichlorophenyl)-2-(exo-norbornanyl)-1*H*-imidazo[4,5-*c*]quinolin-4-amine (17)

Scale: 0.139 mmol. Yield: 6.4 mg (11%). ^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$) δ 9.54 (s, 1H), 8.49 (s, 1H), 8.00 (d, 1H, J = 8.1 Hz), 7.83 (t, 1H, J = 9.6 Hz), 7.57 (t, 2H, J = 8.4 Hz), 7.31 (m, 1H), 7.27 (m, 12H), 3.99 (m, 1H), 3.59 (s, 1H), 3.42 (dd, 1H, J = 11.4, 6.0 Hz), 2.71 (s, 1H), 2.47 (s, 1H), 2.09 (m, 2H), 1.47 (m, 5H); ^{13}C NMR (75 MHz, $CD_3OD/CDCl_3$) δ 130.4, 127.8, 123.4, 120.6, 120.0, 118.4, 42.3, 41.2, 40.5, 37.3, 33.5, 29.9, 24.2; HRMS (ESI) calcd for $C_{23}H_{21}Cl_2N_4^+$ (M + H$^+$): 423.1138, found: 423.1140.

N-(3,4-Dichlorophenyl)-2-(endo-norbornanyl)-1*H*-imidazo[4,5-*c*]quinolin-4-amine (18)

Scale: 0.139 mmol. Yield: 12.6 mg (21%). ^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$) δ 9.81 (s, 1H), 8.44 (s, 1H), 7.98 (d, 1H, J = 8.4 Hz), 7.78 (m, 2H), 7.55 (t, 1H, J = 8.5 Hz), 7.36 (s, 1H), 2.99 (dd, 1H, J = 8.7, 4.9 Hz), 2.60 (s, 1H), 2.48 (s, 1H), 2.23 (m, 1H), 1.82 (m, 1H), 1.66 (m, 2H), 1.30 (m, 5H); ^{13}C NMR (75 MHz, $CD_3OD/CDCl_3$) δ 130.2, 127.4, 123.1, 120.1, 118.1, 42.7, 41.9, 36.4, 36.3, 36.1, 29.7, 28.9; HRMS (ESI) calcd for $C_{23}H_{21}Cl_2N_4^+$ (M + H$^+$): 423.1138, found: 423.1151.

N-(3,4-Dichlorophenyl)-2-(3-noradamantanyl)-1*H*-imidazo[4,5-*c*]quinolin-4-amine (19)

Scale: 0.186 mmol. Yield: 58.2 mg (70%). ^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$) δ 8.23 (s, 1H), 8.06 (d, 1H, J = 8.4 Hz), 7.80 (d, 1H, J = 8.4 Hz), 7.63 (dd, 1H, J = 8.7, 2.4 Hz), 7.43 (m, 1H), 7.31 (m, 2H), 2.76 (t, 1H, J = 6.3 Hz), 2.38 (s, 2H), 2.20 (d, 2H, J = 10.5 Hz), 2.08 (m, 2H), 2.02 (m, 4H), 1.70 (m, 4H); ^{13}C NMR (75 MHz, $CD_3OD/CDCl_3$) δ 160.5, 145.9, 142.0, 139.9, 139.8, 132.4, 132.4, 130.3, 127.6, 125.9, 124.9, 123.5, 120.9, 118.8, 115.5, 45.8, 43.8, 37.6, 34.5; HRMS (ESI) calcd for $C_{25}H_{23}Cl_2N_4^+$ (M + H$^+$): 449.1294, found: 449.1291.

N-(3,4-Dichlorophenyl)-2-(1-adamantanyl)-1*H*-imidazo[4,5-*c*]quinolin-4-amine (20)

Scale: 0.115 mmol. Yield: 38 mg (71%). ^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$) δ 8.33 (s, 1H), 8.04 (d, 1H, J = 7.5 Hz), 7.81 (d, 1H, J = 8.4 Hz), 7.66 (m, 1H), 7.42 (m, 1H), 7.31 (m, 3H), 2.09 (s, 6H), 1.78 (s, 4H); ^{13}C NMR (75 MHz, $CD_3OD/CDCl_3$) δ 161.3, 146.1, 142.4, 140.1, 132.3, 124.5, 120.6, 41.0, 36.3, 35.5, 28.6, 27.6; HRMS (ESI) calcd for $C_{26}H_{25}Cl_2N_4^+$ (M + H$^+$): 463.1451, found: 463.1456.

N-(3,4-Dichlorophenyl)-2-((1s,4s)-4-(1,3-dioxoisoindolin-2-yl)cyclohexyl)-1*H*-imidazo[4,5-*c*]quinolin-4-amine (21)

Scale: 0.044 mmol. Yield: 40 mg (78%). ^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$) δ 8.47 (m, 1H), 8.23 (s, 1H), 7.78 (m, 7H), 7.39 (m, 14H), 4.28 (m, 1H), 3.09 (m, 1H), 2.52 (m, 4H), 1.91 (m, 4H); ^{13}C NMR (75 MHz, $CD_3OD/CDCl_3$) δ 168.5, 160.1, 143.3, 140.4, 134.0, 132.0, 131.6, 130.2, 129.9, 127.2, 122.9, 121.3, 120.3, 119.8, 118.9, 117.8, 100.2, 37.0, 30.9, 28.9; HRMS (ESI) calcd for $C_{30}H_{24}Cl_2N_5O_2^+$ (M + H$^+$): 556.1302, found: 556.1290.

N-(3,4-Dichlorophenyl)-2-(1-benzoylpiperidin-4-yl)-1*H*-imidazo[4,5-*c*]quinolin-4-amine (23)

Scale: 0.108 mmol. Yield: 25 mg (44%). ^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$) δ 8.37 (s, 1H), 7.85 (d. 1H, J = 7.3 Hz), 7.82 (d, 1H, J = 8.4 Hz), 7.66 (dd, 1H, J = 8.7, 2.4 Hz), 7.43 (m, 1H), 7.31 (m, 7H), 4.69 (m, 12H), 3.82 (m, 2H), 3.17 (m, 1H), 3.00 (s.1H), 1.92 (m, 4H); ^{13}C NMR (75 MHz, $CD_3OD/CDCl_3$) δ 171.2, 143.5, 140.4, 135.3, 132.2, 130.1, 128.6, 127.5, 127.3, 126.5, 124.0, 123.2, 120.4, 119.9, 117.9, 42.1, 36.4, 31.0, 30.5; HRMS (ESI) calcd for $C_{28}H_{24}Cl_2N_5O^+$ (M + H$^+$): 516.1352, found: 516.1352.

N-Phenyl-2-(1-benzoylpiperidin-4-yl)-1*H*-imidazo[4,5-*c*]quinolin-4-amine (24)

A solution of **33d** (61.4 mg, 0.156 mmol) and aniline (43 μL, 0.47 mmol) in DMF (0.8 mL) was heating at 140 °C overnight under N_2 atmosphere. The reaction mixture was cooled, and the solvent was evaporated. The residue obtained was subjected to preparative silica gel column chromatography (100:1 to 8:1 $CHCl_3$/MeOH). Yield: 40 mg (57%). ^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$) δ 7.92 (d, 1H, J = 7.5 Hz), 7.81 (s, 2H), 7.75 (d. 1H, J = 8.1 Hz), 7.33 (dd, 1H, J = 7.2, 1.5 Hz), 7.21 (t, 1H, J = 8.0 Hz), 7.12 (d, 1H, J = 7.3 Hz), 6.86 (t, 1H, J = 7.5 Hz), 4.62 (m, 12H), 3.60 (m, 2H), 3.07 (d, 1H, J= 11.4 Hz), 1.92 (m, 4H); ^{13}C NMR (75 MHz, $CD_3OD/CDCl_3$) δ 170.7, 154.8, 143.6, 140.6, 135.6, 129.8, 128.7, 128.5, 127.2, 127.1, 126.6, 122.5, 121.6, 120.5, 118.7, 53.4, 20.8, 13.9; HRMS (ESI) calcd for $C_{28}H_{26}N_5O^+$ (M + H$^+$): 448.2132, found: 448.2137.

2-((1s,4s)-4-Aminocyclohexyl)-*N*-(3,4-dichlorophenyl)-1*H*-imidazo[4,5-*c*]quinolin-4-amine (22)

A 33% solution of methylamine in absolute ethanol (0.5 mL) is added to a stirred solution of **21** (10 mg, 0.017 mmol) in ethanol (0.25 mL). The solution was refluxed for 2 h. The mixture was then cooled to rt and the solvent was evaporated under reduced pressure. The residue was purified by flash column chromatography using a mixture of 10:1 EtOAc/MeOH, by volume. Yield: 5 mg (70%). ^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$) δ 8.40 (s, 1H), 8.08 (d, 1H, J = 8.1 Hz), 7.87 (d, 1H, 8.7 Hz), 7.75 (dd, 1H, J = 8.7, 2.5 Hz), 7.47 (t, 2H, J = 6.9 Hz), 7.29 (m, 4H), 3.40 (m, 1H), 3.12 (m, 1H), 2.27 (m, 2H), 1.95 (m, 2H), 1.89 (m, 4H); ^{13}C NMR (75 MHz, $CD_3OD/CDCl_3$) δ 143.4, 140.4, 132.1, 130.0, 127.4, 124.0, 123.2, 120.8, 119.9, 117.9, 110.7, 105.7, 31.6, 27.4, 25.6; HRMS (ESI) calcd for $C_{22}H_{22}Cl_2N_5^+$ (M + H$^+$): 426.1247, found: 426.4203.

2-(1-Benzylpiperidin-4-yl)-*N*-(3,4-dichlorophenyl)-1*H*-imidazo[4,5-*c*]quinolin-4-amine (25)

A solution of compound **23** (18 mg, 0.035 mmol) in dry THF (0.18 mL) was added dropwise to a stirred slurry of lithium aluminum hydride (0.89 mL of 0.2 M in THF, 0.175 mmol). After heating under reflux overnight, decomposition of excess hydride was effected by cautious addition of water. The inorganic solids were removed by filtration, the organic layer dried over anhydrous sodium sulfate and filtered, and the solvent was removed under reduced pressure. The final residue was purified by flash column chromatography using a mixture of 10:1 EtOAc/ MeOH, by volume. ^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$) δ 11.79 (s, 1H), 8.50 (s, 1H), 8.01 (m, 2H), 7.87 (dd, 1H, J = 9.0, 2.6 Hz), 7.78 (m, 1H), 7.53 (m, 7H), 7.37 (s, 2H), 3.59 (m, 2H), 3.09 (m, 2H), 2.06 (m, 2H), 1.76 (s, 4H); ^{13}C NMR (75 MHz, $CD_3OD/CDCl_3$) δ 140.4, 136.4, 129.4, 128.1, 127.8, 123.0, 120.3, 119.8, 117.8, 100.2, 61.6, 52.9, 30.1, 28.8; HRMS (ESI) calcd for $C_{28}H_{26}Cl_2N_5^+$ (M + H$^+$): 502.1560, found: 502.1541.

N-(3,4-Dichlorophenyl)-2-(piperidin-4-yl)-1*H*-imidazo[4,5-*c*]quinolin-4-amine (26)

A solution of **25** (6 mg, 0.011 mmol) in dry MeOH (0.5 mL) was hydrogenated at 1 atm in the presence of palladium black (0.011 mg). After 2 h, the catalyst was removed by filtration, and the solvent was evaporated under reduced pressure. The final residue was purified by flash column chromatography using a mixture of 10:1:0.1 EtOAc/MeOH/NH_4OH, by volume. ^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$) δ 8.51 (m, 1H), 8.03 (m, 2H), 7.72 (m, 1H), 7.60 (m, 1H), 7.43 (m, 2H), 3.38 (m, 2H), 2.35 (m, 2H), 1.19 (m, 12H), 0.90 (m, 12H); HRMS (ESI) calcd for $C_{21}H_{20}Cl_2N_5^+$ (M + H$^+$): 412.1090, found: 412.1086.

HPLC analysis of compounds 5−14 and 16−26

Purity of compounds was checked using a Hewlett-Packard 1100 HPLC equipped with a Zorbax SB-Aq 5 μ analytical column (50 × 4.6 mm; Agilent Technologies, Palo Alto, CA). System A: linear gradient solvent system; 5 mM TBAP (tetrabutylammonium dihydrogenphosphate) - CH_3CN from 50:50 to 0:100 in 13 min; the flow rate was 0.5 mL/min. System B: linear gradient solvent system; 10 mM TEAA (triethylammonium acetate)-CH_3CN from 65:35 to 0:100 in 13 min; the flow rate was 0.5 mL/min. Peaks were detected by UV absorption with a diode array detector. The compounds eluted at the following retention times: **5**, 7.0 min (system A), 9.4 min (system B); **6**, 8.9 min (system A), 10.44 min (system B); **7**, 9.2 min (system A), 10.9 min (system B); **8**, 7.5 min (system A), 9.3 min (system B); **9**, 7.3 min (system A), 10.1 min (system B); **10**, 9.4 min (system A), 11.7 min (system B); **11**, 7.1 min (system A), 9.8 min (system B); **12**, 3.3 min (system A), 8.0 min (system B); **13**, 4.7 min (system A), 8.6 min (system B); **14**, 7.2 min (system A), 4.7 min (system B); **16**, 5.7 min (system A), 8.6 min (system B); **17**, 8.4 min (system A), 10.9 min (system B); **18**, 8.7 min (system A), 11.2 min (system B); **19**, 9.3 min (system A), 11.3 min (system B); **20**, 9.7 min (system A), 12.0 min (system B); **21**, 9.2 min (system A), 11.5 min (system B); **22**, 2.4 min (system A), 10.3 min (system B); **23**, 6.5 min (system A), 9.6 min (system B); **24**, 2.9 min (system A), 6.9 min (system B). **25**, 7.06 min (system A), 10.8 min (system B); **26**, 1.93 min (system A), 10.6 min (system B).

Pharmacological Methods

[^{3}H]**35** (47 Ci/mmol) was from GE Healthcare Bio-Sciences Corp. (Piscataway, NJ). [^{3}H]**34** (CCPA, 42.6 Ci/mmol), [^{125}I]**36** (I-AB-MECA, 2000 Ci/mmol), and [^{35}S]GTPγS (1068 Ci/mmol) were from Perkin Elmer Life Sciences (Waltham, MA).

Cell culture and membrane preparation

CHO (Chinese hamster ovary) cells expressing the recombinant human ARs (HEK-293 cells were used for the human A_{2A}AR) were cultured in DMEM and F12 (1:1) supplemented with 10% fetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin and 2 μ mol/ml glutamine. Cells were harvested by trypsinization. After homogenization and suspension, cells were centrifuged at 1000 × *g* for 10 min, and the pellet was re-suspended in 50 mM Tris·HCl buffer (pH 7.4) containing 10 mM $MgCl_2$. The suspension was homogenized with an electric homogenizer for 10 sec, and was then re-centrifuged at 20,000 × *g* for 20 min at 4°C. The resultant pellets were resuspended in buffer in the presence of 3 Units/mL adenosine deaminase, and the suspension was stored at −80°C until the binding experiments. The protein concentration was measured using the Bradford assay.[27]

Binding to the Human A_1AR and the A_{2A}AR

For binding to the human A_1AR, [^{3}H]**34** (2 nM) was incubated with membranes (40 μg/tube) from CHO cells stably expressing the human A_1AR at 25 °C for 60 min in 50 mM Tris·HCl buffer (pH 7.4; $MgCl_2$, 10 mM) in a total assay volume of 200 μL. [19] Nonspecific binding was determined using 10 μ M of N^6-cyclopentyladenosine. For human A_{2A}AR binding, membranes (20 μg/tube) from HEK-293 cells stably expressing the human A_{2A}AR were incubated with 15 nM [^{3}H]**35** at 25 °C for 60 min in 200 μL of 50 mM Tris·HCl, pH 7.4, containing 10 mM $MgCl_2$.[20] 5′-*N*-Ethylcarboxamidoadenosine (NECA, **37**, 10 μM) was used to define nonspecific binding. Reaction was terminated by filtration with GF/B filters.

Binding to the Human A_3AR

Each tube in the competitive binding assay contained 100 μL membrane suspension (20 μg protein), 50 μL [^{125}I]**36** (0.5 nM),[21] and 50 μL of increasing concentrations of the test ligands in Tris·HCl buffer (50 mM, pH 7.4) containing 10 mM $MgCl_2$, 1 mM EDTA. Nonspecific binding was determined using 10 μM of **37** (NECA) in the buffer. The mixtures were incubated at 25°C for 60 min. Dissociation was started by the addition of 10 μM **15** in the absence or presence of 10 μ M of each allosteric modulator. Binding reactions were terminated by filtration through Whatman GF/B filters under reduced pressure using a MT-24 cell harvester (Brandell, Gaithersburgh, MD, USA). Filters were washed three times with 9 mL ice-cold buffer. Radioactivity was determined in a Beckman 5500B γ-counter.

[^{35}S]GTPγS binding assay

The preparation of membranes from CHO cells stably expressing human A_3AR was as previously described.[22] [^{35}S]GTPγS binding was measured in 200 μL buffer containing 50 mM Tris·HCl (pH 7.4), 1 mM EDTA, 1 mM $MgCl_2$, 1 μM GDP, 1 mM dithiothreitol, 100 mM NaCl, 3 Units/mL adenosine deaminase, 0.2 nM [^{35}S]GTPγS, 0.004% 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate (CHAPS), and 0.5% bovine serum albumin. Incubations were started by addition of the membrane suspension (10 μg protein/tube) to the test tubes, and carried out in duplicate for 30 min at 25°C. The reaction was stopped by rapid filtration through Whatman GF/B filters, pre-soaked in 50 mM Tris·HCl, 5 mM $MgCl_2$ (pH 7.4) containing 0.02% CHAPS. The filters were washed twice with 3 mL of the buffer mentioned before, and retained radioactivity was measured using liquid scintillation counting. Non-specific binding of [^{35}S]GTPγS was measured in the presence of 10 μM unlabelled GTPγS.

Statistical analysis

Binding and functional parameters were calculated using Prism 5.0 software (GraphPAD, San Diego, CA, USA). IC_{50} values obtained from competition curves were converted to K_i values using the Cheng-Prusoff equation.[28] Data were expressed as mean ± standard error.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

AR, adenosine receptor
Cl-IB-MECA, 2-chloro-*N*-^{6}-(3-iodobenzyl)adenosine-5′-*N*-methylcarboxamide
CCPA, 2-chloro-N^6-cyclopentyladenosine
CHAPS, 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate
DMEM, Dulbecco's modified Eagle's medium
DMF, *N*,*N*-dimethylformamide
DMSO, dimethyl sulfoxide
ESI, electrospray ionization
GPCR, G protein-coupled receptor
GTPγS, guanosine-5′-(γ-thiotriphosphate)
I-AB-MECA, N^6-(4-amino-3-iodobenzyl)adenosine-5′-*N*-methylcarboxamide
m-CPBA, 3-chloroperoxybenzoic acid
NECA, 5′-*N*-ethylcarboxamidoadenosine
SAR, structure-activity relationship
TLC, thin layer chromatography

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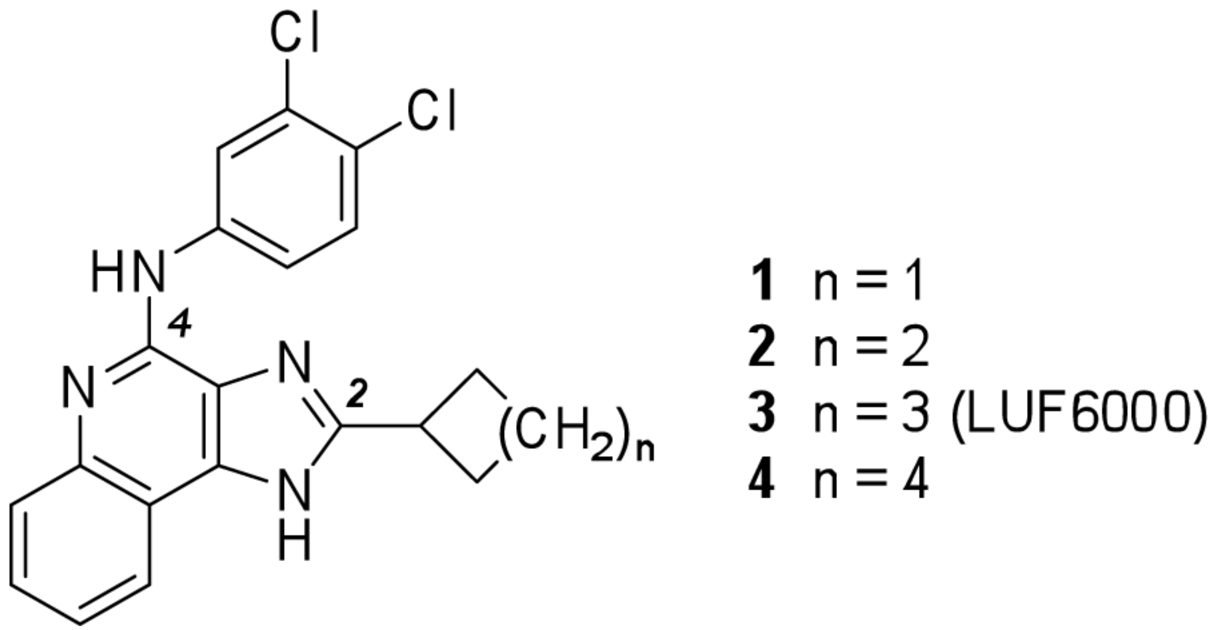
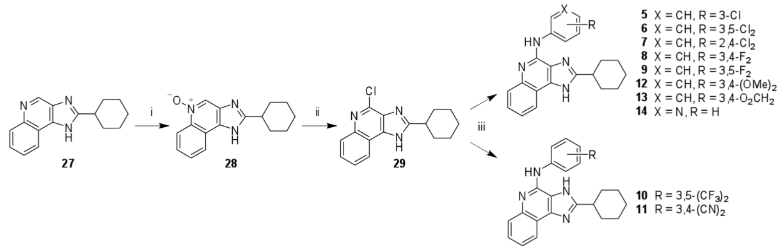


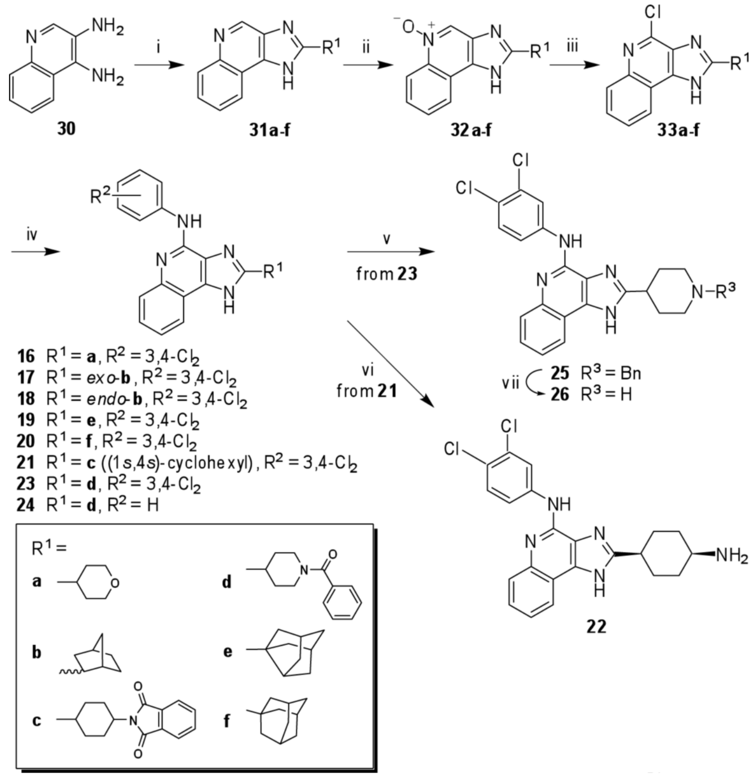
Chart 1.
Structures of a series of 2-cycloalkyl imidazoquinoline derivatives previously found to be positive allosteric modulators of the human A_3AR.



Scheme 1.

Synthesis of novel 1*H*-imidazo-[4,5-*c*]quinolin-4-amine derivatives with structural variation at the 4 position.[a]

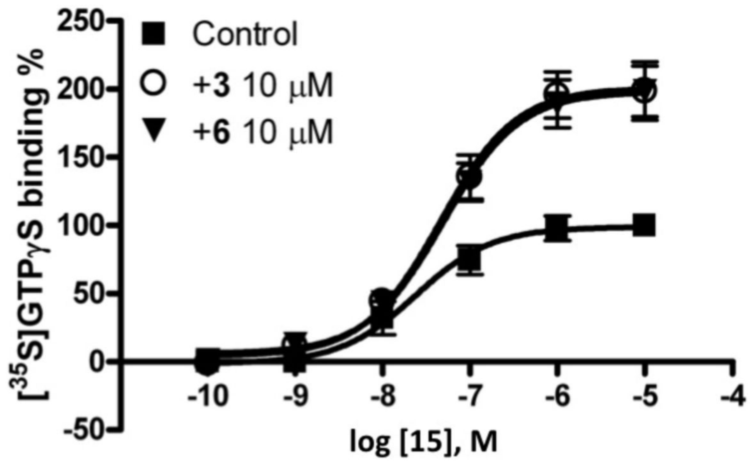
[a] Reagents: (i) *m*-CPBA, $CHCl_3/CH_2Cl_2$/MeOH; (ii) $POCl_3$, toluene/DMF; (iii) R-$PhNH_2$, DMF.



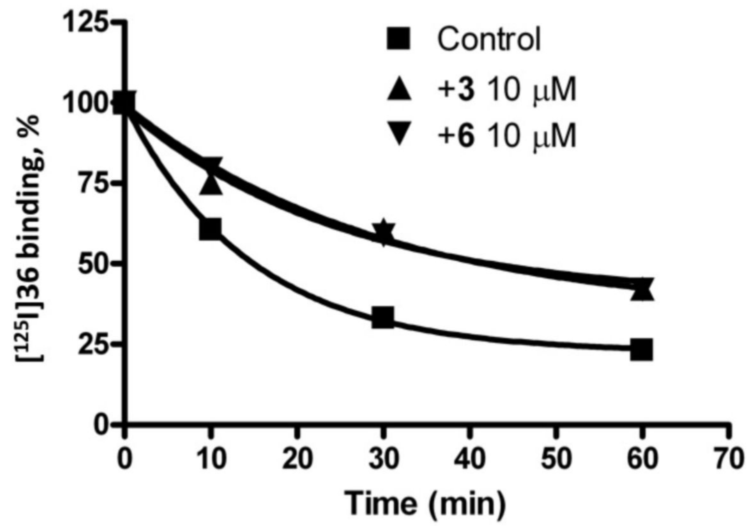
Scheme 2.

Synthesis of novel 1*H*-imidazo-[4,5-*c*]quinolin-4-amine derivatives with structural variation at the 2 position.[a]

[a] Reagents: (i) polyphosphoric acid, R^1CO_2H; (ii) *m*-CPBA, $CHCl_3/CH_2Cl_2$/MeOH; (iii) $POCl_3$, toluene/DMF; (iv) R^2-$PhNH_2$, DMF; (v) $LiAlH_4$, THF; vi) $MeNH_2$, EtOH; (vii) H_2/Pd, MeOH.



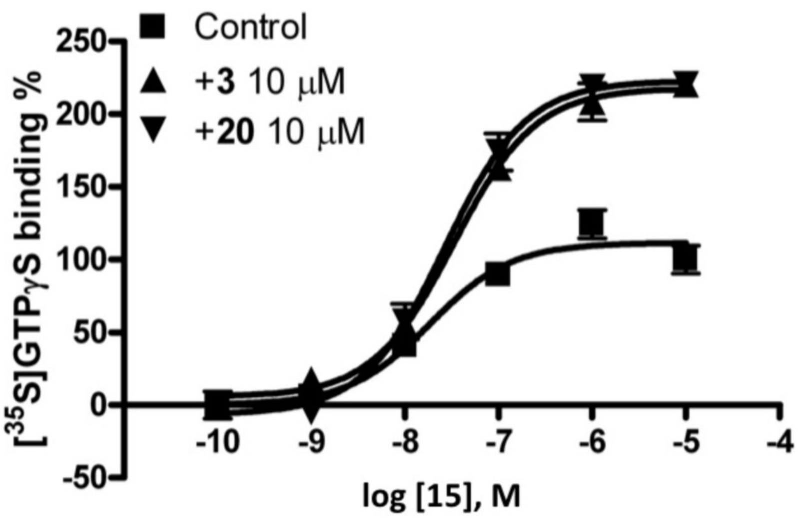
A.



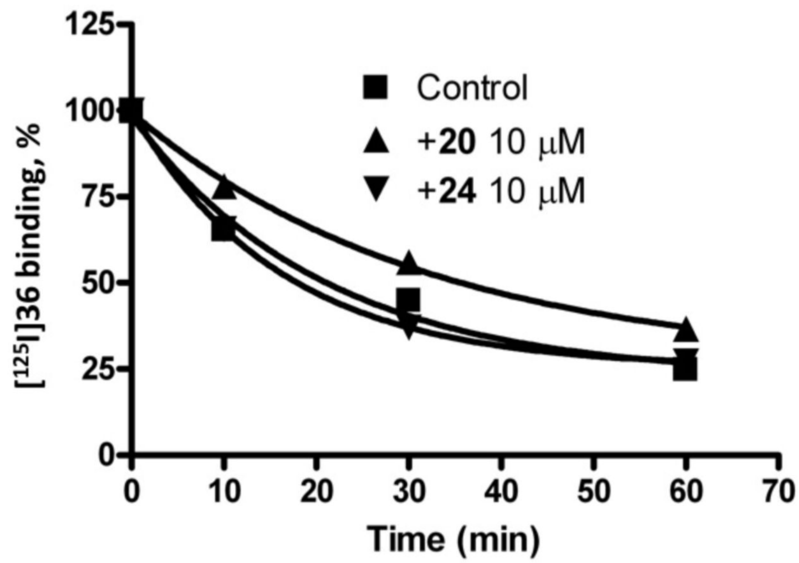
B.

Figure 1.

Allosteric modulation of the human A_3AR by compound **6**. A) Functional assay of the human A_3AR. The % stimulation of binding of $[^{35}S]$GTPγS by increasing concentrations of **15** under control conditions or in the presence of 10 μM of compound **3** or **6**. B) Radioligand binding studies on the human A_3AR. Study of the dissociation kinetics of the agonist radioligand $[^{125}I]$**36** under control conditions and in the presence of 10 μM of compound **3** or **6**.



A.



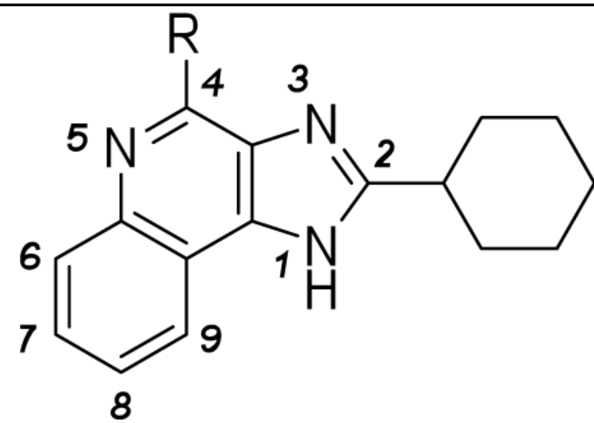
B.

Figure 2.

Allosteric modulation of the human A_3AR by compound **20** or **24**. A) Functional assay of the human A_3AR. The % stimulation of binding of [^{35}S]GTPγS by increasing concentrations of **15** under control conditions or in the presence of 10 μM of compound **3** or **20**. B) Radioligand binding studies on the human A_3AR. Study of the dissociation kinetics of the agonist radioligand [^{125}I]**36** under control conditions and in the presence of 10 μM of compound **20** or **24**.

Table 1

Potency of 4-anilino substituted 1*H*-imidazo-[4,5-*c*]quinolin-4-amine derivatives in binding assays at the human A_1 and A_3ARs expressed in CHO cells and at the human A_{2A} in HEK-293 cells and the allosteric effects at the human A_3AR.[a]



No.	R =	A_1AR, %displ. at 10 μM	A_{2A}AR, %displ. at 10 μM	A_3AR, %displ. at 10 μM	A_3AR ag. dissociation,[b] % at 10 μM	[^{35}S]GTPγS binding in A_3AR cells,[c] % at 10 μM
3	3,4-Cl_2-PhNH	37±9	−11.2±2.8	40.5±13.4	192±7	208±14
5	3-Cl-PhNH	53.3±3.8	42.8±3.7	79.2±1.3	194±3	200±13
6	3,5-Cl_2-PhNH	16.7±2.9	−11.4±10.9	32.7±4.8	184±5	209±22
7	2,4-Cl_2-PhNH	48.0±1.1	67.2±2.4	43.9±0.1	174±5	187±20
8	3,4-F_2-PhNH	14.6±4.8	26.5±7.3	68.8±1.2	187±1	197±17
9	3,5-F_2-PhNH	36.6±5.3	17.2±2.1	70.1±7.9	188±3	171±7
10	3,5-$(CF_3)_2$-PhNH	7.2±2.3	15.6±0.3	21.8±1.4	137±5	143±3
11	3,4-$(CN)_2$-PhNH	33.1±4.1	37.2±1.5	42.2±2.9	129±7	138±7
12	3,4-$(OMe)_2$-PhNH	75.6±2.1	93.8±1.6	98.4±0.1	173±1	182±14
13	3,4-O_2CH_2-PhNH	65.4±3.3	49.0±0.7	83.9±1.1	185±6	166±11
14	3-pyridyl-NH	73.1±2.5	78.1±1.8	64.8±6.9	114±5	119±1

[a] All experiments were performed using adherent mammalian cells stably transfected with cDNA encoding the human ARs. Binding at human A_1, A_{2A}, and A_3ARs in this study was carried out as described in the Experimental Procedures using [^{3}H]**34,** [^{3}H]**35**, or [^{125}I]**36** as a radioligand.[19-21] Values from the present study are expressed as mean ± s.e.m., n = 3−5. Percentage inhibition at A_1, A_{2A}, or A_3 receptors is expressed as the mean value from 2−4 separate experiments with similar results performed in duplicate.

[b] Dissociation: % decrease of [^{125}I]**36** dissociation at 60 min (control = 100%).

[c] Increase of efficacy in the stimulation of the binding of [^{35}S]GTPγS: compared to maximal effect induced by 10 μM **15** alone (set to 100%). It should be noted that the E_{max} of **15** in this functional assay was recently demonstrated to be about 50% of that of **37**.[17] In the adenylate cyclase assay, **15** and **37** were both full agonists.[15]

Table 2

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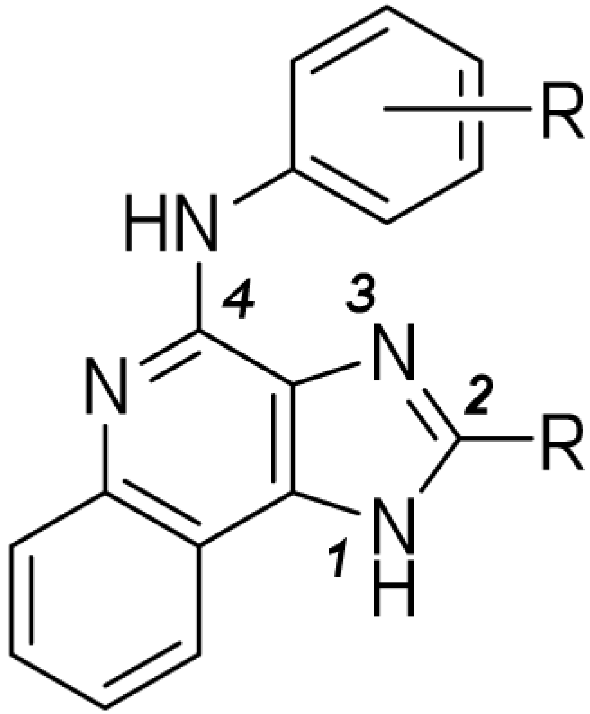
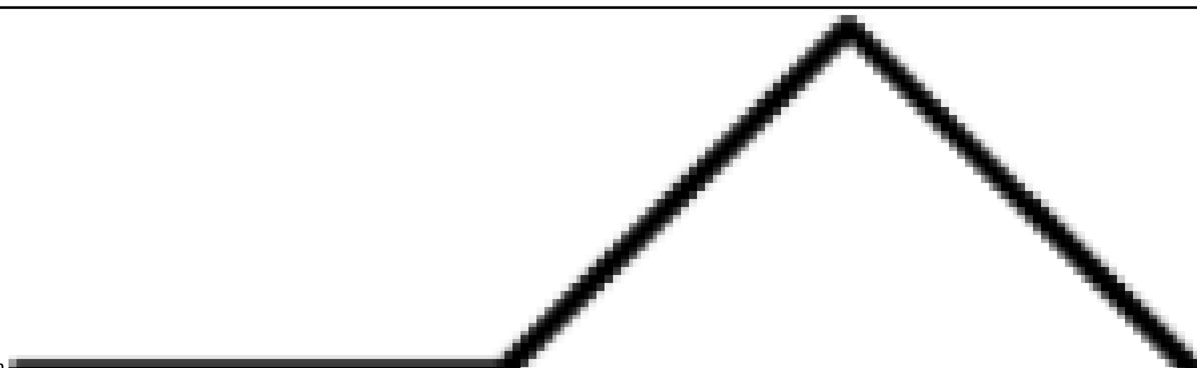
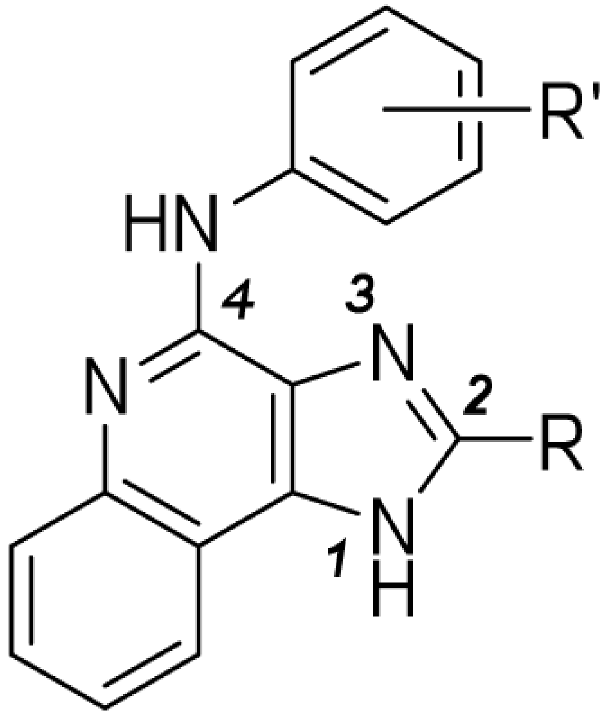
Potency of 2-cycloalkyl substituted 1*H*-imidazo-[4,5-*c*]quinolin-4-amine derivatives in binding assays at the human A_1 and A_3ARs expressed in CHO cells and at the human A_{2A} in HEK-293 cells and the allosteric effects at the human A_3AR.[a]

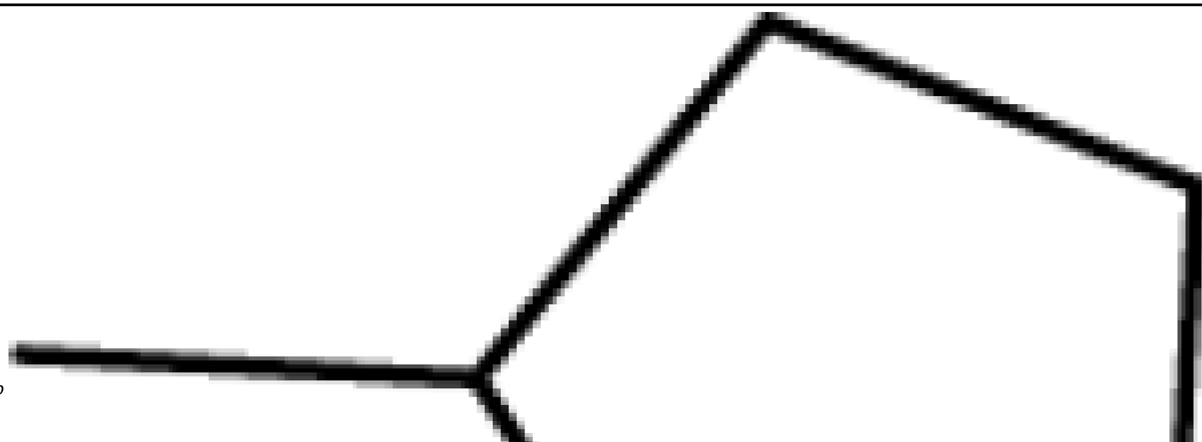


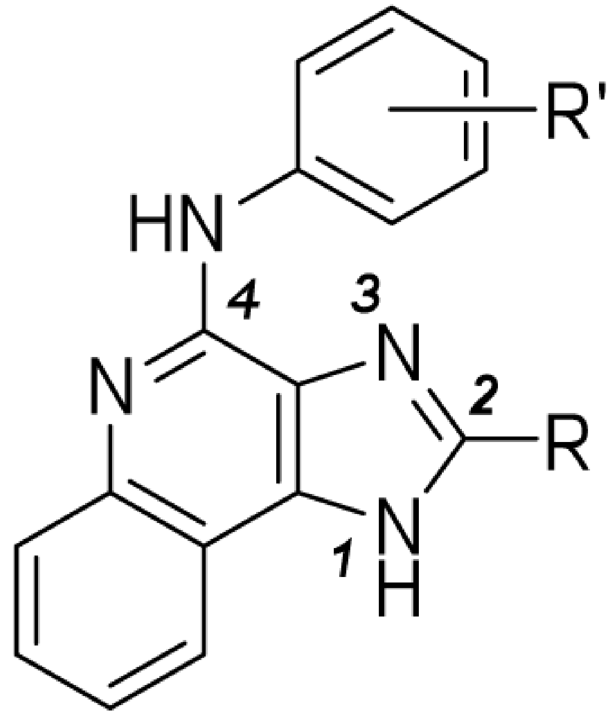
R' = 3,4-dichloro, unless otherwise noted

No.	R =	A_1AR, % displ. at 10 μM	A_{2A}AR, %displ. at 10 μM	A_3AR, % displ. at 10 μM	A_3AR ag. dissociation,[c] % at 10 μM	[^{35}S] GTPγS binding in A_3AR cells,[d] % at 10 μM
1[b]		−5.2	0.4	52	116±3	126±3[b]

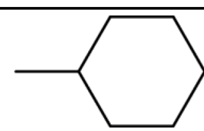


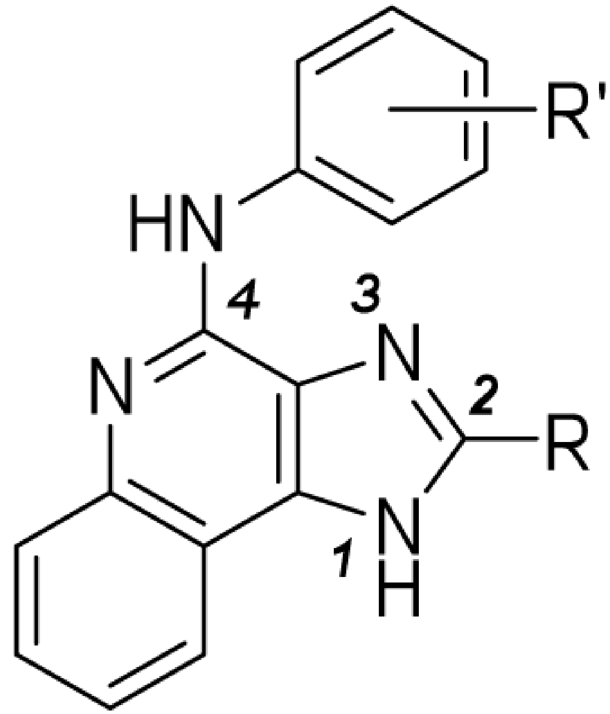
R' = 3,4-dichloro, unless otherwise noted

No.	R =	A_1AR, % displ. at 10 μM	A_{2A}AR, %displ. at 10 μM	A_3AR, % displ. at 10 μM	A_3AR ag. dissociation,[c] % at 10 μM	[^{35}S] GTPγS binding in A_3AR cells,[d] % at 10 μM
2[b]		15	0	67[e]	144±9	141±5[b]

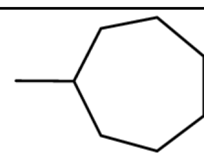


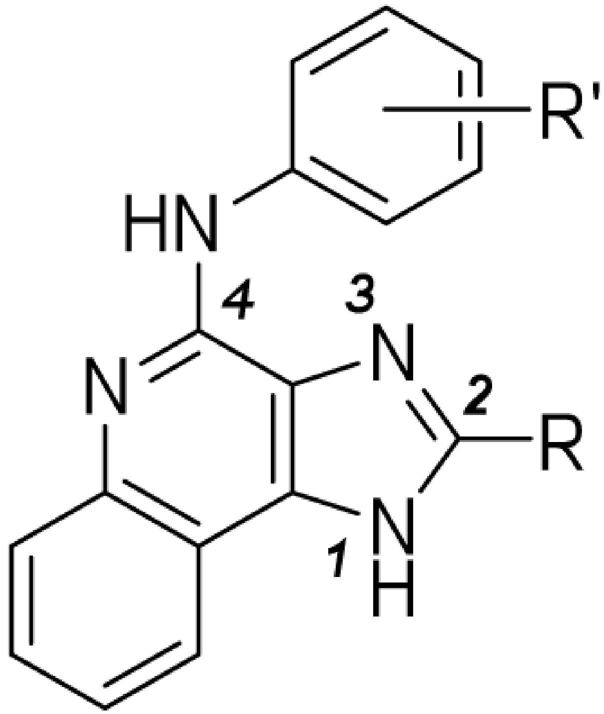
R' = 3,4-dichloro, unless otherwise noted

No.	**R =**	**A_1AR, % displ. at 10 μM**	**A_{2A}AR, %displ. at 10 μM**	**A_3AR, % displ. at 10 μM**	**A_3AR ag. dissociation,[c] % at 10 μM**	**[^{35}S] GTPγS binding in A_3AR cells,[d] % at 10 μM**
3		37±9	−11.2±2.8	40.5±13.4	192±7	208±14

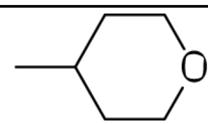


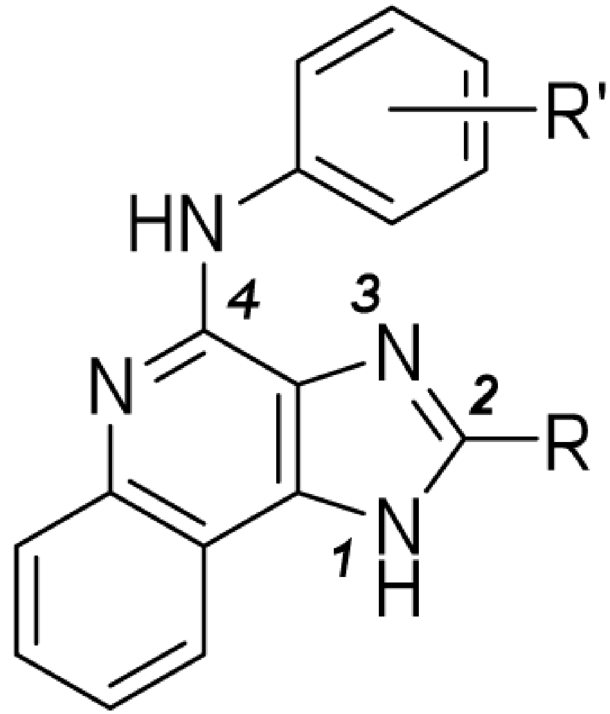
R' = 3,4-dichloro, unless otherwise noted

No.	R =	A_1AR, % displ. at 10 μM	A_{2A}AR, %displ. at 10 μM	A_3AR, % displ. at 10 μM	A_3AR ag. dissociation,[c] % at 10 μM	[^{35}S] GTPγS binding in A_3AR cells,[d] % at 10 μM
4[b]		−4.2	−2.2	68	130±2	115±7[b]

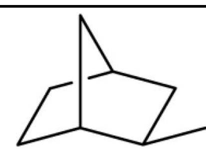


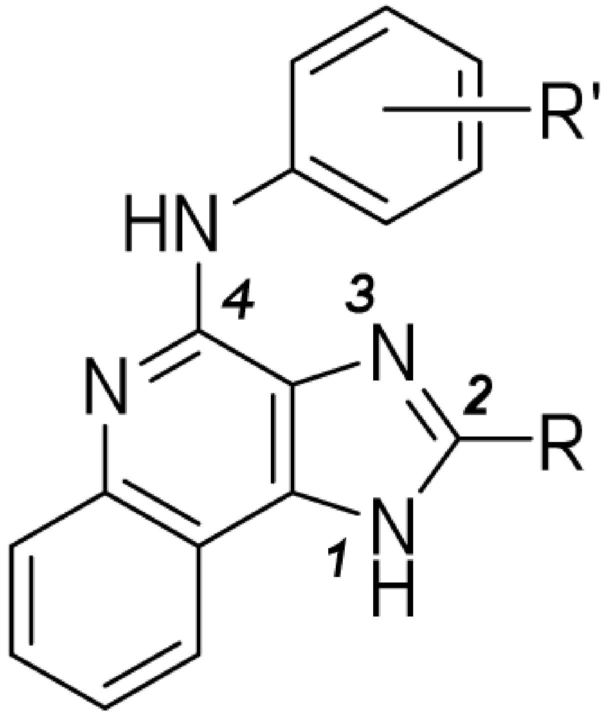
R' = 3,4-dichloro, unless otherwise noted

No.	R =	A_1AR, % displ. at 10 µM	$A_{2A}AR$, %displ. at 10 µM	A_3AR, % displ. at 10 µM	A_3AR ag. dissociation,[c] % at 10 µM	[^{35}S] GTPγS binding in A_3AR cells,[d] % at 10 µM
16		37.2±8.6	−11.9±2.8	69.6±1.4	115±12	114±7

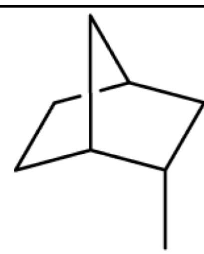


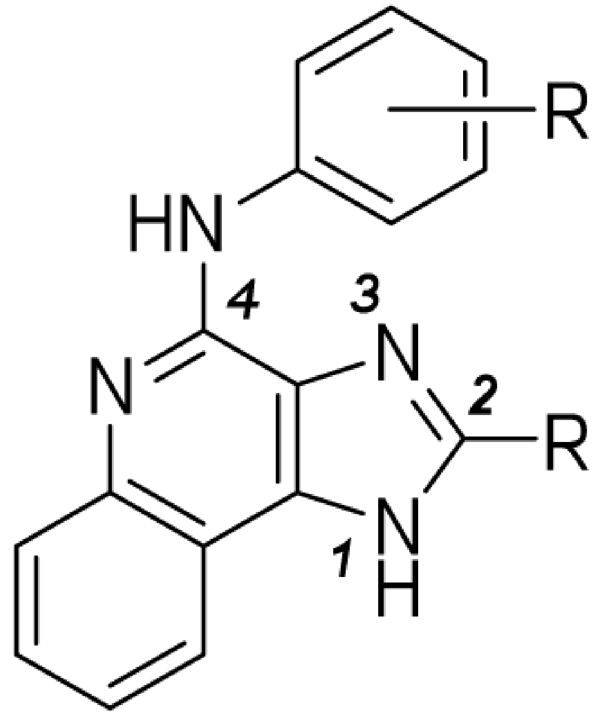
R' = 3,4-dichloro , unless otherwise noted

No.	R =	A_1AR, % displ. at 10 μM	$A_{2A}AR$, %displ. at 10 μM	A_3AR, % displ. at 10 μM	A_3AR ag. dissociation,[c] % at 10 μM	[^{35}S] GTPγS binding in A_3AR cells,[d] % at 10 μM
17		77.9±12.2	5.8±1.0	61.2±1.4	187±16	201±26

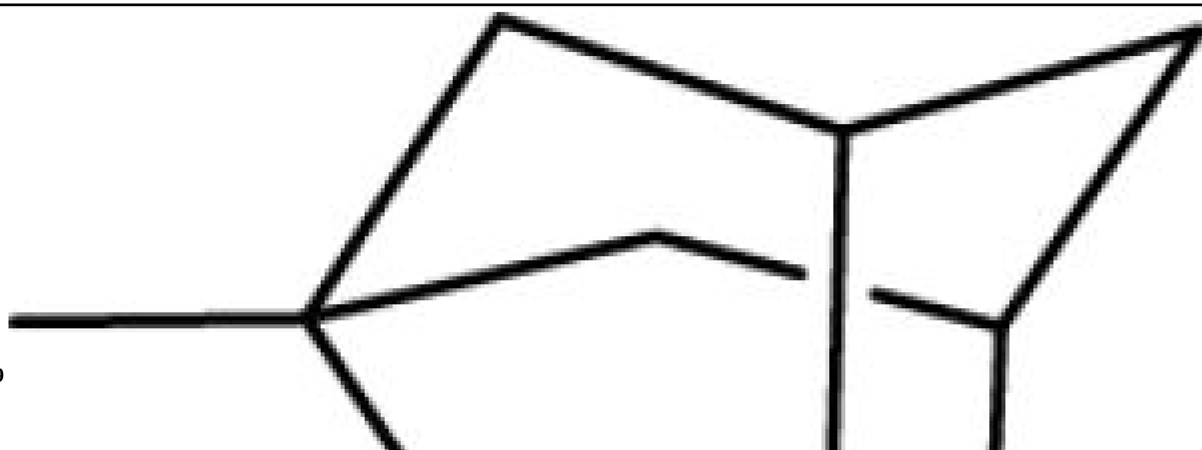


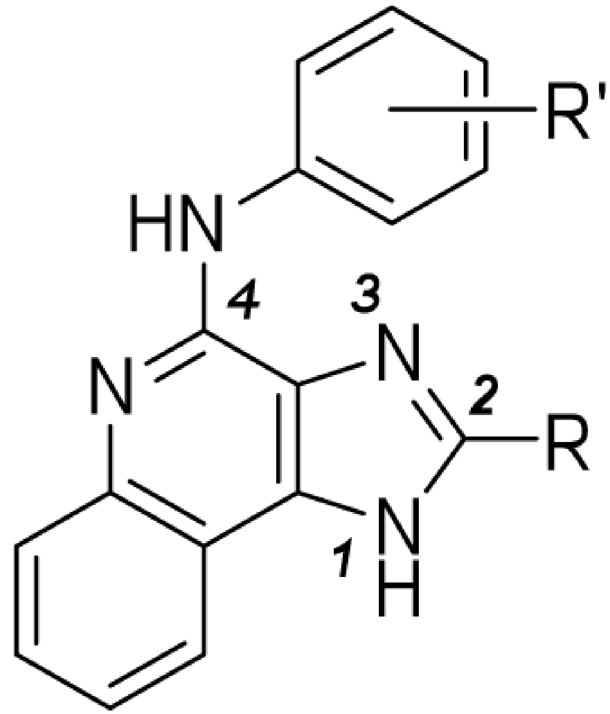
R' = 3,4-dichloro, unless otherwise noted

No.	R =	A_1AR, % displ. at 10 μM	$A_{2A}AR$, %displ. at 10 μM	A_3AR, % displ. at 10 μM	A_3AR ag. dissociation,[c] % at 10 μM	[^{35}S] GTPγS binding in A_3AR cells,[d] % at 10 μM
18		44.8±10.5	39.4±1.9	50.5±5.5	168±8	168±13



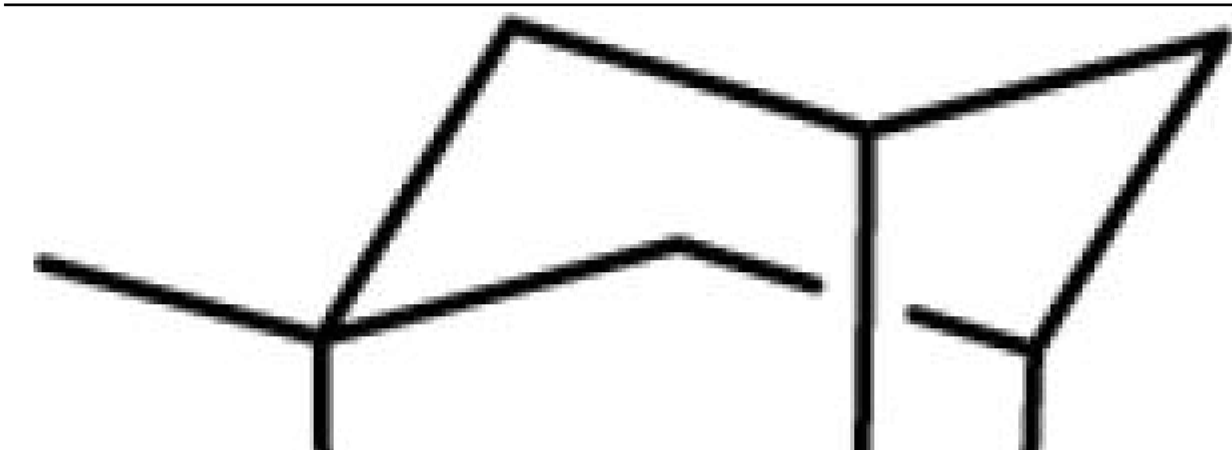
R' = 3,4-dichloro, unless otherwise noted

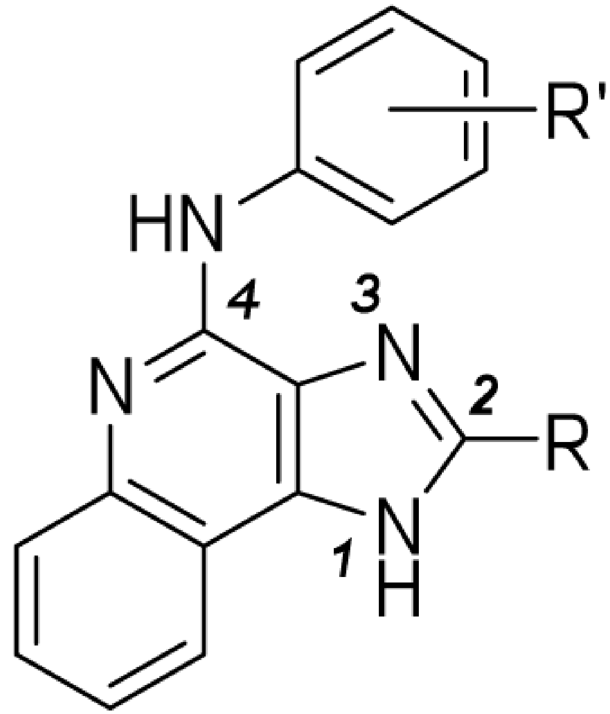
No.	R =	A_1AR, % displ. at 10 µM	$A_{2A}AR$, %displ. at 10 µM	A_3AR, % displ. at 10 µM	A_3AR ag. dissociation,[c] % at 10 µM	[^{35}S] GTPγS binding in A_3AR cells,[d] % at 10 µM
19		14.5±2.7	−17.3±6.9	3.3±4.1	139±10	156±2



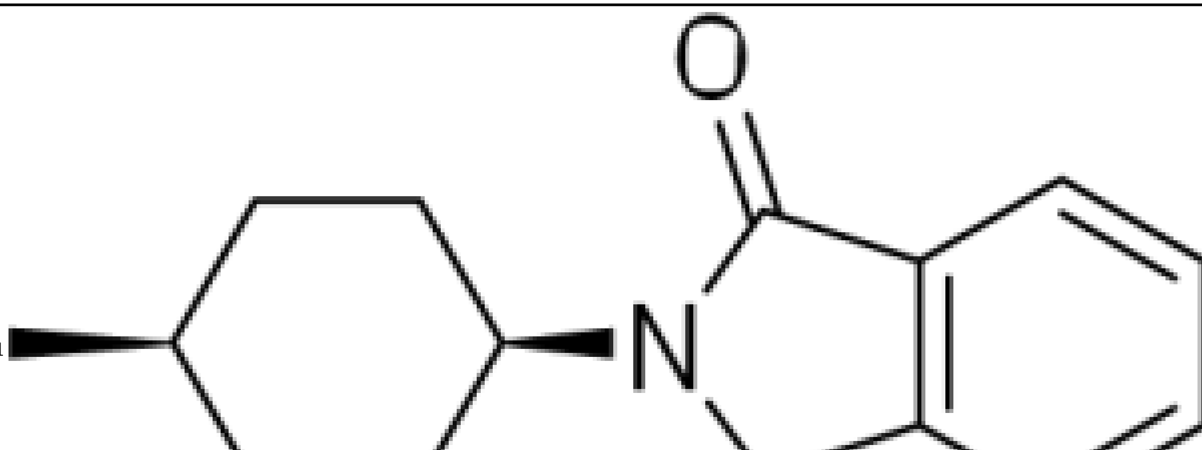
R' = 3,4-dichloro , unless otherwise noted

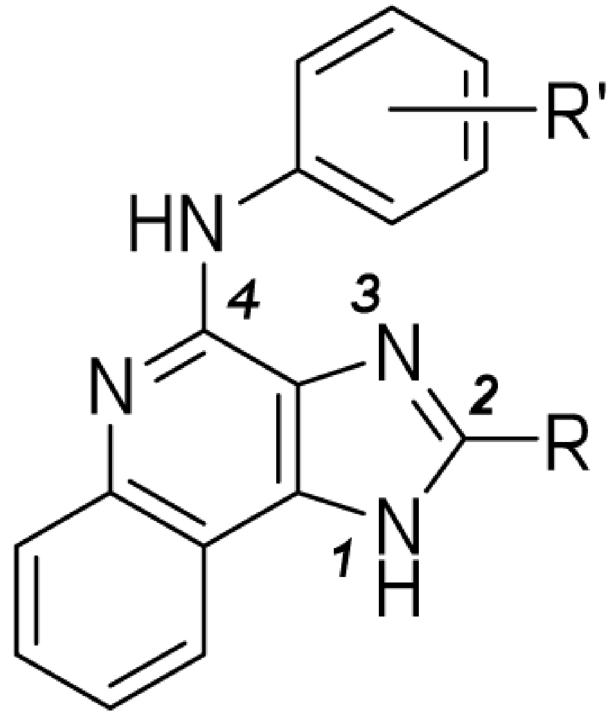
No.	R =	A_1AR, % displ. at 10 μM	$A_{2A}AR$, %displ. at 10 μM	A_3AR, % displ. at 10 μM	A_3AR ag. dissociation,[c] % at 10 μM	[^{35}S] GTPγS binding in A_3AR cells,[d] % at 10 μM



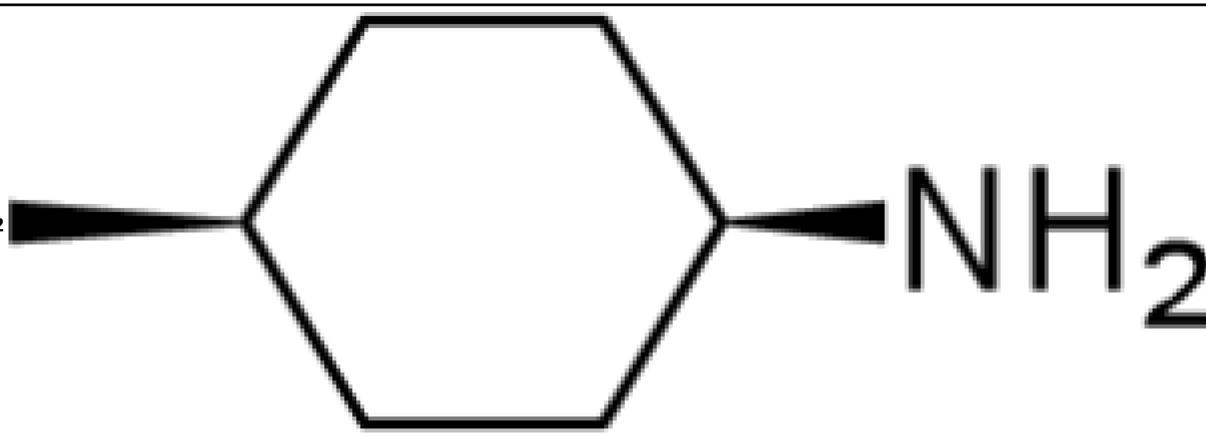


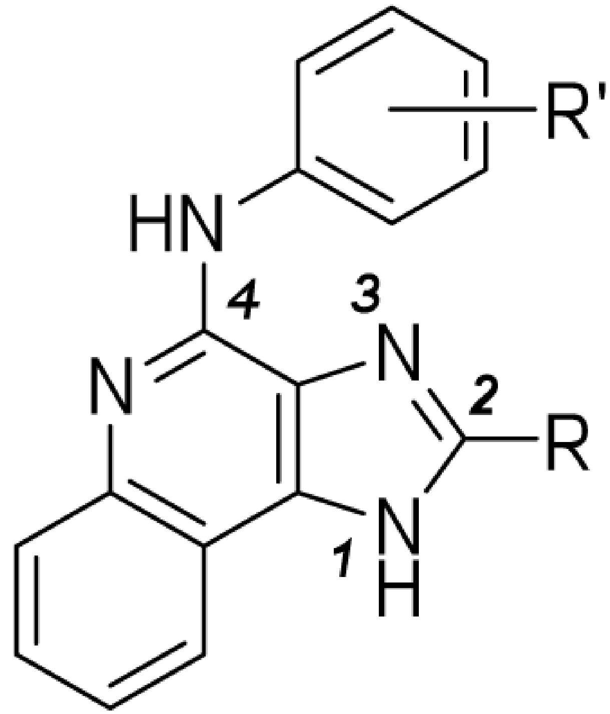
R' = 3,4-dichloro, unless otherwise noted

No.	R =	A_1AR, % displ. at 10 μM	$A_{2A}AR$, %displ. at 10 μM	A_3AR, % displ. at 10 μM	A_3AR ag. dissociation,[c] % at 10 μM	[^{35}S] GTPγS binding in A_3AR cells,[d] % at 10 μM
21		21.4±4.3	56.8±1.7	5.6±4.9	102±5	96±5

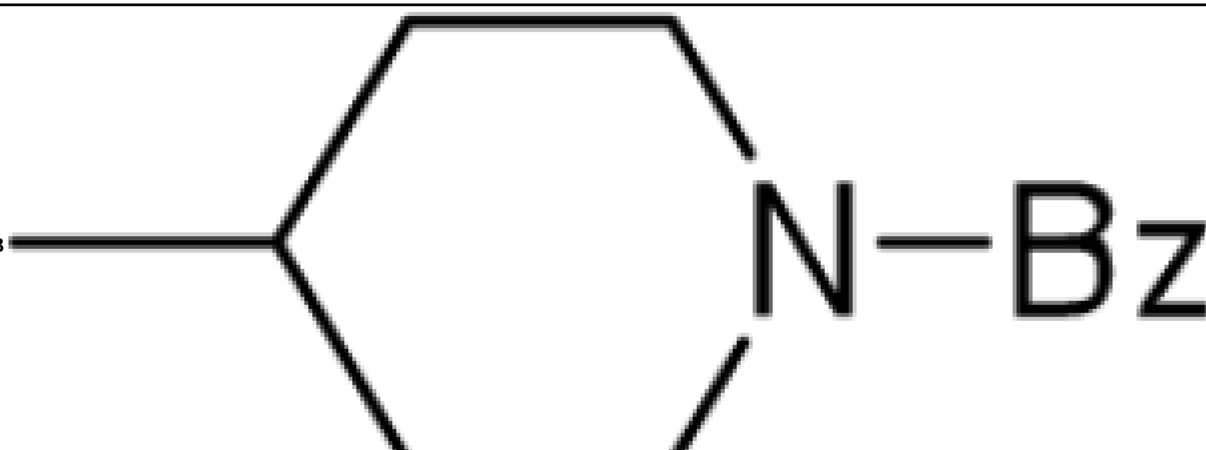


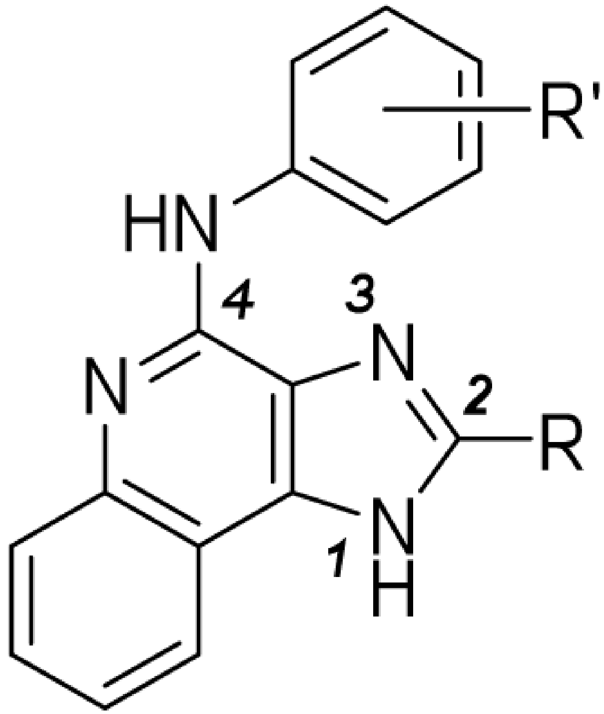
R' = 3,4-dichloro, unless otherwise noted

No.	R =	A_1AR, % displ. at 10 µM	$A_{2A}AR$, %displ. at 10 µM	A_3AR, % displ. at 10 µM	A_3AR ag. dissociation,[c] % at 10 µM	[^{35}S] GTPγS binding in A_3AR cells,[d] % at 10 µM
22		31.7±16.2	15.8±1.2	4.2±6.1	107±11	111±6

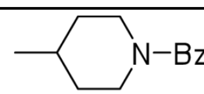


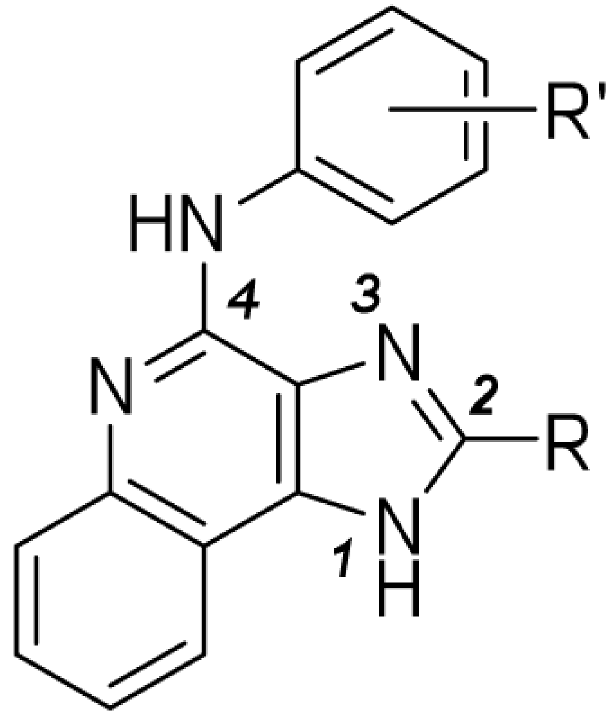
R' = 3,4-dichloro, unless otherwise noted

No.	R =	A_1AR, % displ. at 10 μM	A_{2A}AR, %displ. at 10 μM	A_3AR, % displ. at 10 μM	A_3AR ag. dissociation,[c] % at 10 μM	[^{35}S] GTPγS binding in A_3AR cells,[d] % at 10 μM
23		20.2±10.1	8.8±1.9	29.8±0.6	91±9	93±7

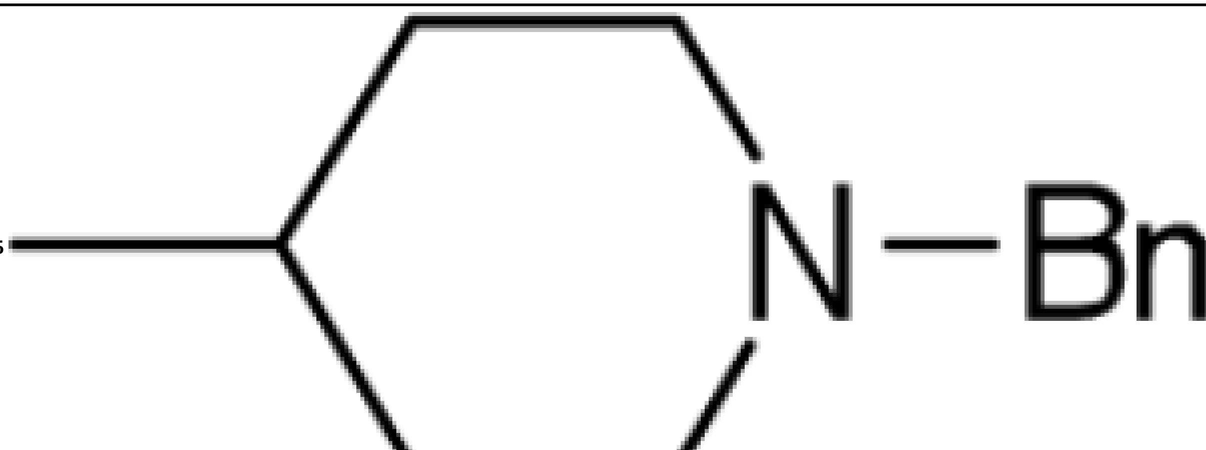


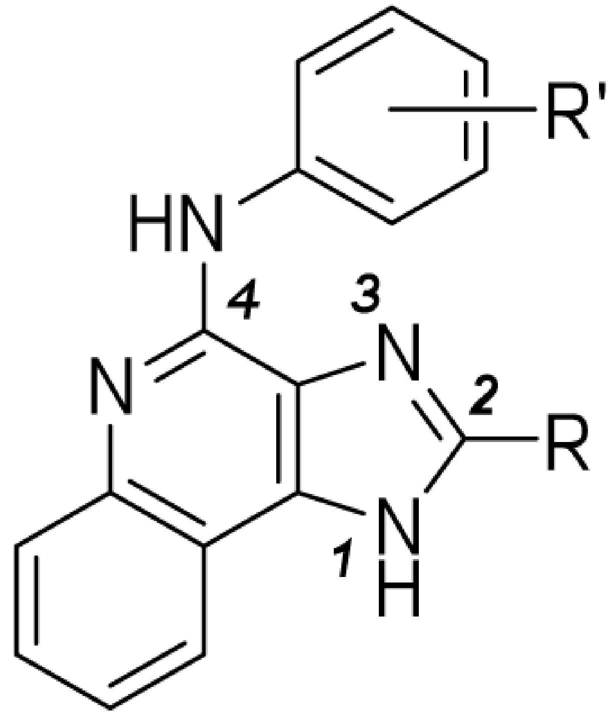
R' = 3,4-dichloro, unless otherwise noted

No.	R =	A_1AR, % displ. at 10 μM	$A_{2A}AR$, %displ. at 10 μM	A_3AR, % displ. at 10 μM	A_3AR ag. dissociation,[c] % at 10 μM	[^{35}S] GTPγS binding in A_3AR cells,[d] % at 10 μM
24	 R' = H	12.4±1.1	43.4±5.5	35.3±1.5	92±5	93±1

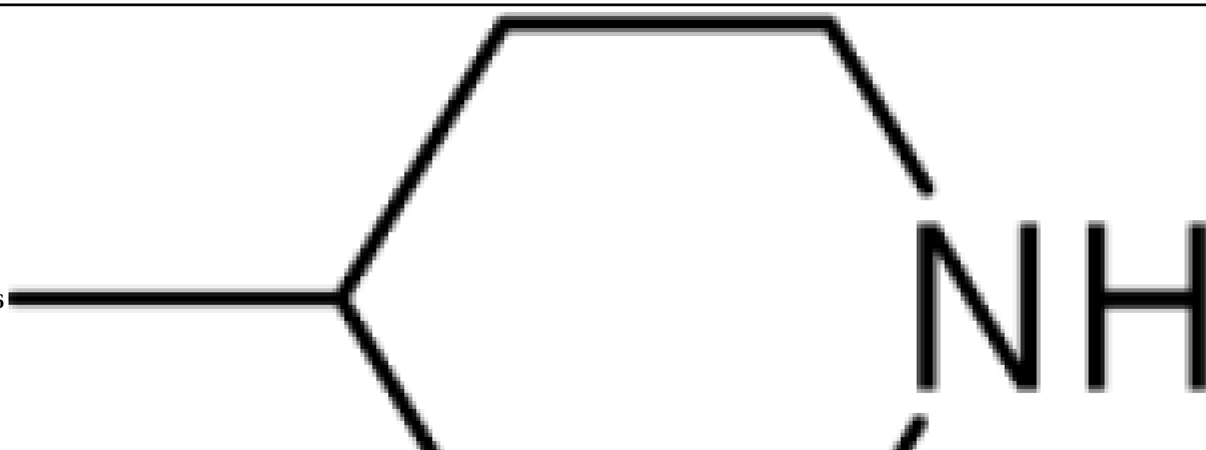


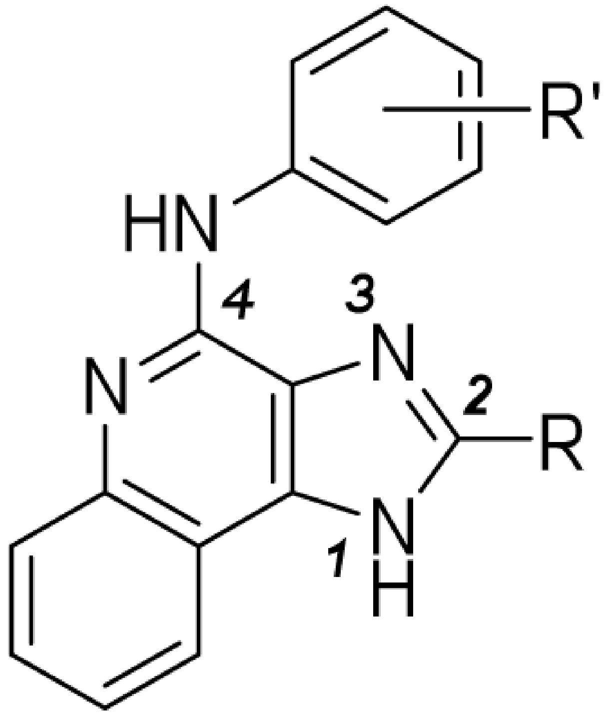
R' = 3,4-dichloro, unless otherwise noted

No.	R =	A_1AR, % displ. at 10 μM	A_{2A}AR, %displ. at 10 μM	A_3AR, % displ. at 10 μM	A_3AR ag. dissociation,[c] % at 10 μM	[^{35}S] GTPγS binding in A_3AR cells,[d] % at 10 μM
25		17.8±3.6	−3.1±1.6	46.3±0.8	93±6	97±5



R' = 3,4-dichloro, unless otherwise noted

No.	R =	A_1AR, % displ. at 10 μM	$A_{2A}AR$, %displ. at 10 μM	A_3AR, % displ. at 10 μM	A_3AR ag. dissociation,[c] % at 10 μM	[^{35}S] GTPγS binding in A_3AR cells,[d] % at 10 μM
26		10.9±8.6	7.0±0.7	52.8±1.1	101±7	99±3



R' = 3,4-dichloro, unless otherwise noted

No.	R =	A_1AR, % displ. at 10 μM	$A_{2A}AR$, %displ. at 10 μM	A_3AR, % displ. at 10 μM	A_3AR ag. dissociation,[c] % at 10 μM	[^{35}S] GTPγS binding in A_3AR cells,[d] % at 10 μM

[a]All experiments were performed using adherent mammalian cells stably transfected with cDNA encoding the human ARs. Binding at human A_1, A_{2A} and A_3ARs in this study was carried out as described in the Experimental Procedures using [^{3}H]**34,** [^{3}H]**35,** or [^{125}I]**36** as a radioligand.[19-21] Values from the present study are expressed as mean ± s.e.m., n = 3−5. Percentage inhibition at A_1, A_{2A}, or A_3 receptors is expressed as the mean value from 2−4 separate experiments with similar results performed in duplicate.

[b]Values from Göblyös et al,[15] the functional enhancement of which was measured with the cyclic AMP assay.[26]

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[c]Dissociation: % decrease of [^{125}I]**36** dissociation at 60 min (control = 100%).

[d]Increase of efficacy in the stimulation of the binding of [^{35}S]GTPγS: compared to maximal effect induced by 10 μM **15** alone (set to 100%). It should be noted that the E_{max} of **15** in this functional assay was recently demonstrated to be about 50% of that of **37**.[17] In the adenylate cyclase assay, **15** and **37** were both full agonists.[15]

[e]K_i value in a binding assay[15] = 4690±970 nM.