



Pergamon

Quinazolines as Adenosine Receptor Antagonists: SAR and Selectivity for A_{2B} Receptors

Thomas R. Webb,^{a,*} Dmitriy Lvovskiy,^a Soon-Ai Kim,^b Xiao-duo Ji,^b
Neli Melman,^b Joel Linden^c and Kenneth A. Jacobson^{a,b,*}

^aChemBridge Corporation, 16981 Via Tazon, Suite G, San Diego, CA 92127, USA

^bMolecular Recognition Section, Laboratory of Bioorganic Chemistry, NIDDK, NIH, Bldg. 8A, Rm. B1A 17,
Bethesda, MD 20892-0810, USA

^cUniversity of Virginia, Charlottesville, VA, USA

Received 14 February 2002; accepted 11 July 2002

Abstract—We have recently reported the discovery of numerous new compounds that are selective inhibitors of all of the subtypes of the adenosine receptor family via a pharmacophore database searching and screening strategy. During the course of this work we made the unexpected discovery of a potent A_{2B} receptor antagonist, 4-methyl-7-methoxyquinazoly-2-(2'-amino-4'-imidazolinone) (**38**, CMB 6446), which showed selectivity for this receptor and functioned as an antagonist, with a binding K_i value of 112 nM. We explored the effects of both substituent- and ring-structural variations on the receptor affinity in this series of derivatives, which were found to be mostly non-selective adenosine receptor ligands with K_i values in the micromolar range. Since no enhancement of A_{2B} receptor affinity of **38** was achieved, the previously reported pharmacophore-based searching strategy yielded the most potent and selective structurally-related hit in the database originally searched.

© 2002 Elsevier Science Ltd. All rights reserved.

Introduction

The adenosine receptors (ARs) are G protein-coupled receptors consisting of four subtypes, A₁, A_{2A}, A_{2B}, and A₃.¹ Until recently,² no selective antagonists of the A_{2B} subtype were known, among either xanthines or non-xanthines. It has been difficult to characterize the pharmacological actions of this AR subtype using only non-selective agonists and antagonists.

Our approach to the identification of novel AR antagonists has been unified pharmacophore-based screening.³ During this study we discovered one compound that showed significant binding to all AR subtypes at 10 micromolar concentration (**38**, CMB 6446), but also showed some selectivity for the A_{2B}AR. In order to determine whether this compound that bound to the A_{2B}AR was already optimized for potency among the members of this chemical series present in the database

originally searched, we conducted substructure-based searching and AR assays on other substructure 'hits'. These other 'hits' provided a wide range of substituents on the quinazoline ring. These experiments would give us information regarding the overall quality of the pharmacophore-based queries and structure–activity relationship data. We synthesized five additional compounds for the purpose of testing the importance on one of the ring nitrogens for adenosine receptor recognition.

Few A_{2B}AR subtype-selective antagonists are known, but the biological interest in this subtype is growing. The alkylxanthine theophylline is a weak, non-selective AR antagonist, used therapeutically for the treatment of asthma. The use of theophylline has been associated with unpleasant side effects, such as insomnia and diuresis. The development of a theophylline-like drug with reduced side effects is desirable. The mechanism of action of theophylline in asthma has been controversial for decades,⁴ and only recently did antagonism of the A_{2B}AR subtype become a likely mechanism.^{5,6} The A_{2B}AR is expressed in some mast cells, such as canine mastocytoma cells and human HMC-1 cells, in which it is responsible for triggering acute Ca²⁺ mobilization.

*Corresponding authors. Tel.: +1-301-496-9024; fax: +1-301-480-8422; e-mail: twebb@chembridge.com (T. R. Webb); tel.: +1-858-451-7400; fax: +1-858-451-4701; e-mail: kajacobs@helix.nih.gov (K. A. Jacobson).

Thus, a novel nonpurine A_{2B}AR antagonist, to complement the recently reported A_{2B}AR selective xanthines,² would be useful as a probe of the relationship of this receptor subtype to disease states.

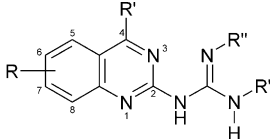
Results

To further understand the structure–activity relationships of the most potent A_{2B} antagonist discovered (**38**, CMB 6446) in our previously reported pharmacophore searching efforts,³ and to explore structural modifications that might lead to enhanced selectivity we set about the iterative collection, synthesis and assay of structurally related compounds. In order to facilitate this process we used 2-guanidinyquinazoline substructure searches in ISISBase to search a large available database of compounds (~100,000 from Express-Pick™, available from ChemBridge corporation) the same database that was originally used for the pharmacophore based queries (CNS-Set™). Compounds that matched the 2-guanidinyquinazoline-substructure query from either database were selected for assay.

In addition to **38**, the additional quinazoline derivatives, **3–53**, were tested for affinity in radioligand binding assays at ARs as shown in Tables 1–5. Compounds **3–48** were obtained from the database, and the synthetic procedures for compounds not previously reported are provided. Scheme 1 shows an overview of the chemistry that was used in the synthesis of these compounds. Substituted anilines of general structure I were subjected to acetone in the presence of iodine to give substituted 1,2-dihydro-2,2,4-trimethylquinoline derivatives of general structure II.⁹ Derivatives of this type were treated with cyanoguanidine to give substituted 4-methylquinazoly-2-guanidines (III),¹⁰ and then further derivatized to give compounds of the general structure IV.¹¹ Alternately compounds of general structure III could be selectively mono- or di-alkylated or acylated to give derivatives of the general structure V.¹²

We also prepared a series of new substituted quinoline derivatives (see Scheme 2). Compounds **49–53** were prepared in order to test the requirement for the N3 nitrogen in AR binding. These substituted 4-methylquinoline derivatives were prepared from 2-amino-4-

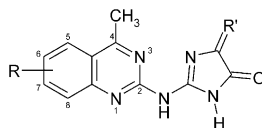
Table 1. Affinities of quinazoline derivatives in radioligand binding assays at A₁, A_{2A}, and A_{2B} adenosine receptors^{a–c}

							
Compd	R	R'	R''	rA ₁ ^a	rA _{2A} ^b	hA _{2B} ^c	rA ₁ /hA _{2B}
3	—	CH ₃	H, H	3.77±0.84	5.05±0.62	36±7% (10 ⁻⁵)	<1
4	—	CH ₃	CH ₃ CH ₂ CO, H	2.13±0.31	4.28±1.15	39±0% (10 ⁻⁵)	<1
5	—	CH ₃	C ₆ H ₅ CO, H	0.988±0.171	0.296±0.063	35±2% (10 ⁻⁵)	<1
6	—	CH ₃	CH ₃ CO, CH ₃ CO	57% (10 ⁻⁴)		<10% (10 ⁻⁵)	
7	—	OH	H, H	36±4% (10 ⁻⁴)	46.0±13.0	<10% (10 ⁻⁵)	
8	5-OCH ₃	CH ₃	H, H	5.16±1.32	8.41±2.66	10±4% (10 ⁻⁵)	<1
9	5-OCH ₃	CH ₃	CH ₃ CO, CH ₃ CO	23.2±8.7	<10% (10 ⁻⁴)		
10	6-CH ₃	CH ₃	H, H	1.22±0.14	2.94±1.22	39±5% (10 ⁻⁵)	<1
11	6-CH ₃	CH ₃	CH ₃ CH ₂ CO, H	7.33±2.20	9.22±1.59	27±9% (10 ⁻⁵)	
12	6-CH ₃	OCH ₃	H, H	43	8.41±2.72	50±1% (10 ⁻⁵)	—
13	6-OCH ₃	CH ₃	CH ₃ , H	12.2±2.9	1.58±0.30	53±2% (10 ⁻⁵)	
14	6-OCH ₃	CH ₃	CH ₃ CO, H	<10% (10 ⁻⁵)		50±1% (10 ⁻⁵)	>1
15	6-OCH ₃	CH ₃	2:CH ₃ CH ₂ CO	11.7±3.1	34±2% (10 ⁻⁵)	1.97±0.09	5.9
16	6-OCH ₃	CH ₃	C ₆ H ₅ CO, H	<10% (10 ⁻⁵)		34±4% (10 ⁻⁵)	>1
17	6-OCH ₂ CH ₃	CH ₃	CH ₃ CO, CH ₃ CO	9.54±3.41	17.2±8.5	<10% (10 ⁻⁵)	
18	6-CH ₃ , 7-CH ₃	CH ₃	H, H	2.30±0.37	6.8±1.8	3.44±0.18	0.67
19	6-CH ₃ , 7-CH ₃	CH ₃	CH ₃ CO, H	2.49±0.43	3.90±1.76	41±2% (10 ⁻⁵)	<1
20	6-CH ₃ , 7-CH ₃	CH ₃	2: CH ₃ CH ₂ CO,	38±3% (10 ⁻⁴)		<10% (10 ⁻⁵)	
21	6-CH ₃ , 8-CH ₃	CH ₃	H, H	2.01±0.03	2.2±0.5	45±6% (10 ⁻⁵)	<1
22	7-CH ₃	CH ₃	CH ₃ CH ₂ CO, H	10.1±5.2	22.5±8.1	32±7% (10 ⁻⁵)	
23	7-CH ₃	CH ₃	CH ₃ CO, CH ₃ CO	9.22±2.77	21.4±5.7	<10% (10 ⁻⁵)	
24	7-CH ₃	CH ₂ CH ₃	H, H	0.359±0.095	0.551±0.102	1.36±0.31	0.26
25	7-OCH ₃	CH ₃	H, H	1.62±0.13	2.14±0.56	0.215±1.040	7.5
26	7-OCH ₃	CH ₃	C ₆ H ₅ CO, H	14.2±3.3	12.4±7.0	<10% (10 ⁻⁵)	
27	7-OCH ₃	CH ₃	CH ₃ CO, CH ₃ CO	47±5% (10 ⁻⁴)		<10% (10 ⁻⁵)	
28	7-OCH ₂ CH ₃	CH ₃	CH ₃ CO, H	21.5±10.2	32	<10% (10 ⁻⁵)	
29	8-CH ₃	CH ₃	H, H	3.96±1.22	1.87±0.96	47±1% (10 ⁻⁵)	<1
30	8-OCH ₃	CH ₃	H, H	0.413±0.087	0.258±0.069	0.592±0.120	0.70
31	8-OCH ₂ CH ₃	CH ₃	H, H	1.33±0.61	0.624±0.250	1.78±0.03	0.75

^aDisplacement of specific [³H]R-PIA binding in rat brain membranes, expressed as K_i±SEM in μM (n=3–5), or as a percentage of specific binding displaced at the indicated concentration (M).

^bDisplacement of specific [³H]CGS 21680 binding in rat striatal membranes. Expressed a K_i±SEM in μM (n=3–6), or as a percentage of specific binding displaced at the indicated concentration (M).

^cDisplacement of specific [³H]ZM241,385 binding at human A_{2B} receptors expressed in HEK cells, in membranes, expressed as K_i±SEM in μM (n=3–4), or as a percentage of specific binding displaced at the indicated concentration (M).

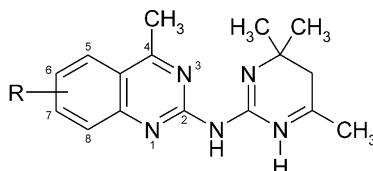
Table 2. Affinities of 4-methylquinazoline imidazolinone derivatives in radioligand binding assays A₁, A_{2A}, and A_{2B} receptors^{a–c}

Compd	R	R'	rA ₁ ^a	rA _{2A} ^b	hA _{2B} ^c	rA ₁ /hA _{2B}
32	—	H, H	10.2±2.3	23.8±4.0	45% (10 ^{−5})	—
33	6-CH ₃	H, H	1.44±0.31	5.74±1.05	0.446±0.096	3.2
34	6-CH ₃	=CHCOOCH ₃	2.91±0.38	1.49±0.40	38±8% (10 ^{−5})	<1
35	6-OCH ₃	H, H	12.7±1.7	17.7±11.9	9.44±0.11	1.3
36	6-OCH ₂ CH ₃	H, H	5.58±1.95	24±2% (10 ^{−4})	14±3% (10 ^{−5})	<1
37	7-CH ₃	H, H	1.35±0.26	12.9±4.1	0.292±0.070	4.6
38	7-OCH ₃	H, H	1.29±0.43	2.4±0.3	0.112±0.015	12

^aDisplacement of specific [³H]R-PIA binding in rat brain membranes, expressed as $K_i \pm \text{SEM}$ in μM ($n=3-5$).

^bDisplacement of specific [³H]CGS 21680 binding in rat striatal membranes. Expressed as $K_i \pm \text{SEM}$ in μM ($n=3-6$), or as a percentage of specific binding displaced at the indicated concentration (M).

^cDisplacement of specific [³H]ZM241,385 binding at human A_{2B} receptors expressed in HEK cells, in membranes, expressed as $K_i \pm \text{SEM}$ in μM ($n=3-4$), or as a percentage of specific binding displaced at the indicated concentration (M).

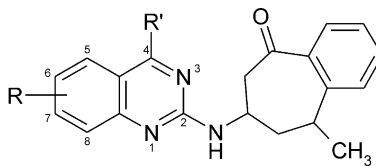
Table 3. Affinities of 4-methylquinazoline dihydropyrimidine derivatives in radioligand binding assays A₁, A_{2A}, and A_{2B} receptors^{a–c}

Compd	R	rA ₁ ^a	rA _{2A} ^b	hA _{2B} ^c	rA ₁ /hA _{2B}
39	—	1.89±0.12	5.17±1.89	38±3% (10 ^{−5})	<1
40	6-CH ₃ , 7-CH ₃	3.89±1.15	8.4±0.4	31±1% (10 ^{−5})	<1
41	7-OCH ₃	0.648±0.050	19.3±8.3	4.04±0.05	0.16

^aDisplacement of specific [³H]R-PIA binding in rat brain membranes, expressed as $K_i \pm \text{SEM}$ in μM ($n=3-5$).

^bDisplacement of specific [³H]CGS 21680 binding in rat striatal membranes. Expressed as $K_i \pm \text{SEM}$ in μM ($n=3-6$).

^cDisplacement of specific [³H]ZM241,385 binding at human A_{2B} receptors expressed in HEK cells, in membranes, expressed as $K_i \pm \text{SEM}$ in μM ($n=3-4$), or as a percentage of specific binding displaced at the indicated concentration (M).

Table 4. Affinities of 4-methylquinazoline benzoheptanone derivatives (R' = methyl) in radioligand binding assays A₁, A_{2A}, and A_{2B} receptors^{a–c}

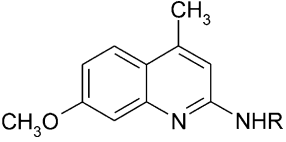
Compd	R	rA ₁ ^a	rA _{2A} ^b	hA _{2B} ^c	rA ₁ /hA _{2B}
42	—	2.68±0.13	3.1±1.0	44±2% (10 ^{−5})	<1
43	6-CH ₃	1.65±0.24	1.50±0.79	46±4% (10 ^{−5})	<1
44	6-OCH ₃	3.41±0.82	8.4±2.9	25±4% (10 ^{−5})	<1
45	6-OCH ₂ CH ₃	2.88±0.06	5.71±2.35	<10% (10 ^{−5})	<1
46	6-CH ₃ , 7-CH ₃	1.95±0.19	2.08±0.27	42±8% (10 ^{−5})	<1
47	7-CH ₃	0.334±0.038	1.44±0.07	4.3±2.4	0.23
48	7-OCH ₃	1.04±0.04	1.57±0.35	1.66±0.18	0.63

^aDisplacement of specific [³H]R-PIA binding in rat brain membranes, expressed as $K_i \pm \text{SEM}$ in μM ($n=3-5$).

^bDisplacement of specific [³H]CGS 21680 binding in rat striatal membranes. Expressed as $K_i \pm \text{SEM}$ in μM ($n=3-6$).

^cDisplacement of specific [³H]ZM241,385 binding at human A_{2B} receptors expressed in HEK cells, in membranes, expressed as $K_i \pm \text{SEM}$ in μM ($n=3-4$), or as a percentage of specific binding displaced at the indicated concentration (M).

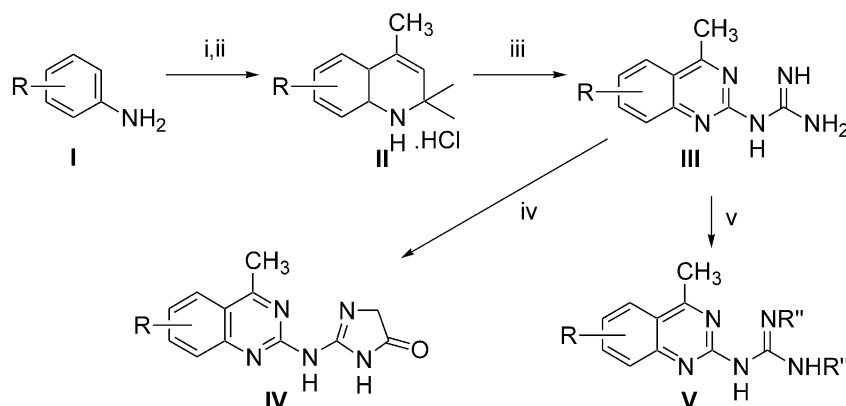
Table 5. Affinities of 4-methylquinoline derivatives in radioligand binding assays A₁, A_{2A}, and A_{2B} receptors^{a,b,c}

				
Compd	R	rA ₁ ^a	rA _{2A} ^b	hA _{2B} ^c
49	COCH ₃	6.30 ± 1.13	45.7 ± 4.6	< 10% (10 ⁻⁵)
50	COOCH ₃	21.1 ± 7.3	< 10% (10 ⁻⁴)	< 10% (10 ⁻⁵)
52	C(NH)NH ₂	19.8 ± 2.6	17.2 ± 1.9	< 10% (10 ⁻⁵)
53	C(NH)NH-CO ₂ C(CH ₃) ₃	8.75 ± 2.77	5.06 ± 1.69	< 10% (10 ⁻⁵)
51	C(NH)N (CO ₂ C(CH ₃) ₃) ₂	< 10% (10 ⁻⁵)	40.7 ± 10.1	< 10% (10 ⁻⁵)

^aDisplacement of specific [³H]R-PIA binding in rat brain membranes, expressed as $K_i \pm \text{SEM}$ in μM ($n = 3-5$), or as a percentage of specific binding displaced at the indicated concentration (M).

^bDisplacement of specific [³H]CGS 21680 binding in rat striatal membranes. Expressed as $K_i \pm \text{SEM}$ in μM ($n = 3-6$), or as a percentage of specific binding displaced at the indicated concentration (M).

^cDisplacement of specific [³H]ZM241,385 binding at human A_{2B} receptors expressed in HEK cells, in membranes, expressed as $K_i \pm \text{SEM}$ in μM ($n = 3-4$), or as a percentage of specific binding displaced at the indicated concentration (M).



Scheme 1. General synthetic method for analogues in the quinazoline series. R = alkyl, alkoxy. Reagents: (i) iodine/acetone, reflux; (ii) HCl gas; (iii) cyanoguanidine; (iv) ethyl bromoacetate/DMF or glycine/water reflux; (v) acyl anhydride/DMSO.

methyl-7-methoxyquinoline (Scheme 2). Compounds **49** and **50** were synthesized with typical acylation methods. Compound **51** was prepared using di-Boc-protected triethylguanidine,²⁰ and the deprotection of one or both of the Boc groups gave a mixture of compounds **52** (30%) and **53** (57%).

The potency of test compounds at the human A_{2B}AR was evaluated in binding assays and in a functional assay. As an initial indication of selectivity, the binding was compared at rat A₁ and A_{2A}ARs. There appeared to be a great freedom of substitution of both the quinazoline ring system and the pendant guanidino group, as judged from retention of binding at these three ARs.

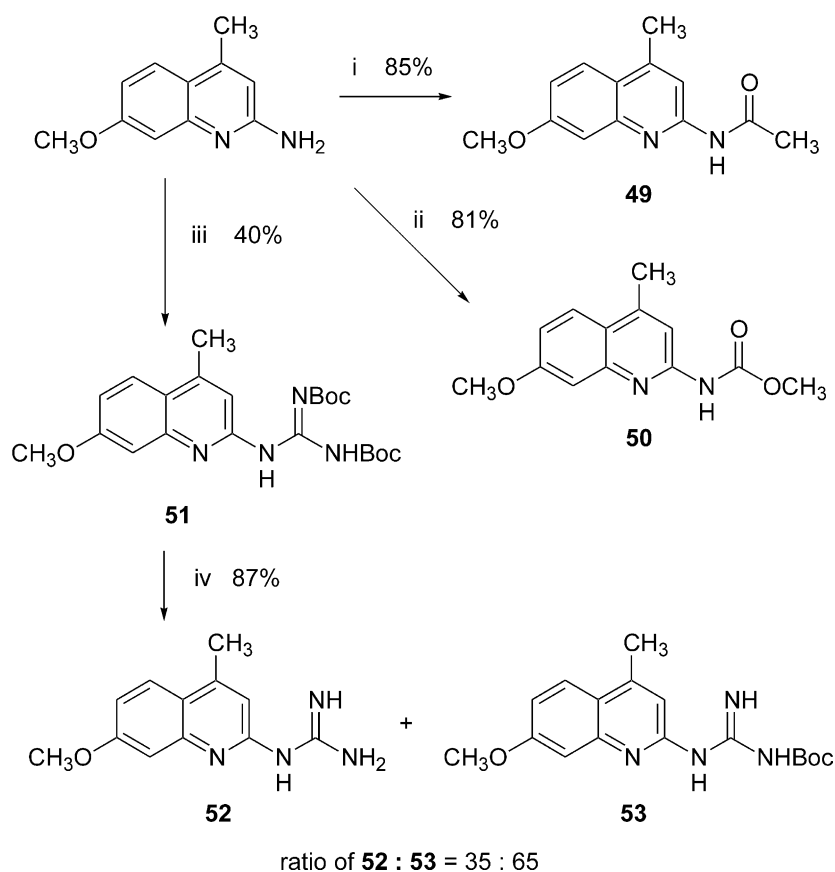
Compound **38**, the previously-identified lead, was 10.6- and 21.4-fold selective for human A_{2B} versus rat A₁ and A_{2A}ARs, with a K_i value of 112 nM. At the human A₃AR a K_i value of $1.85 \pm 0.62 \mu\text{M}$ was determined,⁸ thus selectivity with respect to this subtype was 16.5-fold. A related derivative, **37**, was slightly less selective for the A_{2A}AR. The N3 was found to be necessary for recognition by the A_{2B}AR, since 3-deaza analogues did not bind at that subtype (cf., **25** and its deaza analogue **51**). Nevertheless, analogues lacking the N3 retained affinity for A₁ and A_{2A}ARs.

In addition to binding effects, the functional effect of **38** in inhibiting the A_{2B}AR-mediated rise in intracellular calcium in HEK-A_{2B} cells was examined. At 100 nM, **38** inhibited calcium mobilization stimulated by NECA (100 nM) by roughly 70%.

Discussion

There is evidence that both the A_{2B}AR and A₃AR may play a role in asthma.^{5,13-15} The A₃AR mediates the degranulation of rat RBL mast-like cells and is present in high density in human blood eosinophils. The availability of antagonists selective for the A_{2B}AR shall provide an opportunity to explore the importance of these two AR subtypes in asthma and other inflammatory diseases.

Xanthine derivatives displaying selectivity for the A_{2B} subtype in binding assays were recently reported.^{2,6} Several such xanthines were found to have antagonistic effects in functional assays.^{2,16} In addition to xanthine antagonists of ARs, there is need to identify non-xanthine structures. We initially set out to use information derived from known A₃AR antagonists both to identify



Scheme 2. The synthesis of the quinoline derivatives. Reagents: (i) acetic anhydride, TEA, cat. DMAP in CH_2Cl_2 ; (ii) methyl chloroformate, TEA, CH_2Cl_2 ; (iii) di-Boc-*N*-triflylguanidine, TEA, CH_2Cl_2 ; (iv) 5% TFA/ CH_2Cl_2 .

new leads for this receptor family and to explore the pharmacophore relationships within the receptor family. Further, we also wished to explore the utility of pharmacophore database queries for the discovery of new leads.³ An unexpected added benefit of our approach was the discovery of new selective leads for related AR subtypes, A_1 , A_{2A} , and A_{2B} . This ability to find new structural series from pharmacophore information for an existing bioactive structural series is advantageous in the development of new drug leads.

Pharmacophore-based screening of a diverse chemical library has identified 4-methylquinazoline derivatives as AR antagonists. Analogues of 4-substituted quinazolines have been selected via 2D substructure queries or synthesized, then tested in receptor binding, and found to be generally non-selective. These derivatives distantly resemble isoquinolines reported as A_3 AR antagonists.¹⁹ Most of the structural variation occurred at the 2-position, with guanidino and substituted guanidino groups. Simple functional group substitution of the phenyl ring was included. Nearly all of the compounds bound to A_1 , A_{2A} , and A_{2B} ARs in the micromolar range. Dramatic flexibility of substitution of the 2-guanidino group was possible with retention of affinity. Bulky substituents (such as dihydropyrimidines **39–41** and benzocycloheptanones **42–48**) did not prevent the binding to ARs. However the di-Boc derivative **51** did not bind appreciably at any of the ARs examined.

One imidazolinone derivative, **38**, was roughly an order of magnitude selective for human A_{2B} versus rat A_1 and A_{2A} ARs. Further binding and functional studies within the same species will be required to rigorously establish the true selectivity of this compound. Interestingly this compound was selected in the initial pharmacophore-based searching.³ The full characterization of this compound will require comparative binding studies within the same species. This derivative functionally antagonized the Ca^{2+} response mediated by the recombinant human A_{2B} AR expressed in HEK293 cells.

In addition to **38**, potent binding to the human A_{2B} AR ($K_i < 1 \mu\text{M}$) was noted for the unsubstituted guanidine derivatives **25** and **30** and for the other unsubstituted imidazolinone derivatives **33** and **37**. Compound **25**, which displayed a low degree of selectivity for the A_{2B} AR, was similar to **38** in the position of substitution of the quinazoline, that is, a 7-methyl group replaced the 7-methoxy group. Other analogues substituted at the 7-position were not A_{2B} -selective. The *N*-benzoyl derivative **5** and the unsubstituted guanidine **24** were potent at A_1 and A_{2A} ARs and less potent at the A_{2B} AR. The *N*-propionyl derivative **15** was more potent at the human A_{2B} AR than at rat A_1 and A_{2A} ARs. The quinazoline N3 was found to be necessary for recognition by the A_{2B} AR but not by A_1 and A_{2A} ARs.

The lack of high affinity binding to ARs or distinct SAR patterns within the series suggests that there may be

novel mode of binding. Further experiments will be needed to define the competitive nature of the antagonism by these quinazoline derivatives.

Thus, at least in this case, the pharmacophore-based query selected the most potent and selective compound in this structural series present in the entire database (of 9600 compounds) that was originally searched. The search of a larger database (~100,000 compounds) using 2D sub-structure queries based on **38** did not identify more potent or selective compounds within this particular series. Further experiments will need to be performed to determine if the results obtained from these types of queries against other targets may also yield analogous results. Refinement of searching methods will be of increasing value to researchers in medicinal chemistry with the recent advent of substructure- and similarity-based compound acquisition via a web browser interface and the availability of larger numbers of diverse compounds from combinatorial and parallel synthesis. Further experiments of this type with purine receptors, as well as application of the search strategies that we have been developing, will be the topic of future publications.

Experimental

Chemistry

All proton NMR spectra were acquired on a Bruker AC-300 spectrometer using DMSO- d_6 as the solvent, unless otherwise indicated, and reported as δ from TMS as the internal standard. Other than compounds **49–53** (see detailed experimental and procedures below) the compounds used in this study were obtained from the ChemBridge Corporation collection, described at <http://www.hit2lead.com>. General procedures and experimental details are provided below. Following the procedures described and substituting the corresponding substituted anilines (Formula I, Scheme 1) for *p*-methoxyaniline, substituted quinolines (Formula II) were obtained.⁸ Compounds **3**,¹⁷ (**10**, **13**, **14**, **16**, **17**),¹² (**18**, **25**),¹⁸ (**32**, **33**, **35**, **36**, **37**, **38**)¹¹ may be prepared according to the literature procedures. The combustion analytical data were obtained on new compounds from NuMega Resonance Labs (San Diego, CA, USA).

Preparation of substituted quinazolines

4-Methyl-6-methoxyquinazolyl-2-guanidine. A solution was prepared of 0.1 mol 1,2-dihydro-2,2,4-trimethyl-6-methoxyquinoline hydrochloride in 100 mL of water, and was treated with 0.1 mol of dicyandiamide. The resulting mixture was refluxed for approximately four hours until the evolution of gas ceased. The hot reaction mixture was decanted from oils, cooled and treated with concentrated KOH with constant stirring until a pH of between 10 and 11 was obtained. The precipitate was isolated by filtration, washed several times with isopropanol, recrystallized from DMSO, then washed with isopropanol and dried under vacuum. The desired product of 4-methyl-6-methoxy quinazolyl-2-guanidine was obtained at a yield of 84%, a melting point of 298–

300 °C, Anal. (calcd/found): 57.14/57.12, H: 57.14/57.12, and N: 30.30/30.32.

General procedure for the synthesis of substituted 4-methylquinazolyl-2-guanidines

Following the procedure described above for 4-methyl-6-methoxy quinazolyl-2-guanidine and substituting the following substituted quinoline hydrochlorides (Formula II, Scheme 1) for 1,2-dihydro-2,2,4-trimethyl-6-methoxyquinoline hydrochloride, the corresponding substituted quinazolyl-2-guanidine compounds (Formula III) were obtained.

4-Hydroxyquinazolyl-2-guanidine (7). ¹H NMR: δ 7.15 (dd, 1H, J =8.0, 8.0 Hz), 7.35 (d, 1H, J =8.0 Hz), 7.5 (brs, 4H), 7.55 (dd, 1H, J =8.0, 8.0 Hz), 7.9 (d, 1H, J =8.0 Hz), 10.95 (brs, 1H). Mp=286–287 °C. Anal. calcd for C₉H₉N₅O·H₂O: C, 53.20; H, 4.46; N, 34.46. Found: C, 52.76; H, 4.42; N, 33.40.

4,6,8-Trimethylquinazolyl-2-guanidine (21). ¹H NMR: δ 1.08 (t, 6H, J =7.2 Hz), 2.43 (s, 3H), 2.45 (s, 3H), 2.59 (br, 4H), 2.82 (s, 3H), 7.57 (s, 1H), 7.95 (s, 1H), 11.6–12.0 (br, 2H). Mp=228–230 °C.

4-Ethyl-7-methylquinazolyl-2-guanidine (24). ¹H NMR: δ 1.35 (t, 3H, J =7.2 Hz), 2.55 (s, 3H), 3.29 (q, 2H, J =7.2 Hz), 7.46 (dd, 1H, J =8.4, 2.0 Hz), 7.74 (brd, 1H, J =2.0 Hz), 8.16 (d, 1H, J =8.4 Hz), 8.5 (br, 4H). Mp=274–276 °C.

4-Methyl-8-methylquinazolyl-2-guanidine (29). ¹H NMR: δ 2.48 (s, 3H), 2.71 (s, 3H), 7.15 (br, ~4H), 7.17 (dd, 1H, J =8.1, 6.9 Hz), 7.56 (d, 1H, J =6.9 Hz), 7.81 (d, 1H, J =8.1 Hz). Mp=252–253 °C.

4-Methyl-8-methoxyquinazolyl-2-guanidine (30). ¹H NMR: δ 2.69 (s, 3H), 3.91 (s, 3H), 7.17 (dd, 1H, J =7.5, 2.5 Hz), 7.19 (dd, 1H, J =7.5, 6.8 Hz), 7.21 (br, 4H), 7.50 (dd, 1H, J =6.8, 2.5 Hz). Mp=179–181 °C.

4-Methyl-8-ethoxyquinazolyl-2-guanidine (31). ¹H NMR: δ 1.42 (t, 3H, J =7.0 Hz), 2.71 (s, 3H), 4.16 (q, 2H, J =7.0 Hz), 7.17 (dd, 1H, J =7.5, 2.7 Hz), 7.20 (dd, 1H, J =7.5, 6.4 Hz), 7.33 (br, ~4H), 7.52 (dd, 1H, J =6.4, 2.7 Hz). Mp=218–219 °C.

General procedure for the synthesis of 4-methyl-6-methoxyquinazolyl-2-*N*-acylguanidines

A suspension of 4-methyl-6-methoxyquinazolyl-2-guanidine (0.01 mol) was prepared in 30 mL DMSO at ambient temperature. The suspension was treated with the corresponding anhydride (0.005 mol) and allowed to stir for 2 h. The reaction mixture was filtered and the residue recrystallized from dioxane yielding the desired 4-methyl-6-methoxyquinazolyl-2-*N*-acylguanidine.

General procedure for the preparation of substituted 4-methylquinazolyl-2-*N*-acylguanidines

Following the procedures described for 4-methyl-6-methoxyquinazolyl-2-*N*-acylguanidine above and substituting

the following substituted quinazolyl-2-guanidines (Formula III, Scheme 1) for 4-methyl-6-methoxyquinazolyl-2-guanidine, the corresponding substituted 4-methylquinazolyl-2-*N*-acylguanidines (Formula V) were obtained with the listed physical and spectral properties.

4-Methylquinazolyl-2-*N*-propionylguanidine (4). ¹H NMR: two forms: δ 1.07 (t, 3H, $J=7.5$ Hz), 2.56 (br, 2H), 2.87 and 2.92 (s, 3H), 7.58 and 7.64 (dd, 1H, $J=8.2, 7.5$ Hz), 7.75–8.0 (m, 2H), 8.20 and 8.25 (d, 1H, $J=8.2$ Hz), 8.5 (br, ~ 2 H), 11.1–11.6 (br, ~ 1 H). Mp = 153–155 °C.

4-Methylquinazolyl-2-*N*-benzoylguanidine (5). ¹H NMR: δ 2.92 (s, 3H), 7.43–7.46 (m, 2H), 7.51 (dd, 1H, $J=8.5, 7.5$ Hz), 7.60 (dd, 1H, $J=8.0, 7.5$ Hz), 7.91–7.97 (m, 2H), 8.18–8.24 (m, 3H), 9.3 (bs, 1H), 10.0 (bs, 1H), 12.1 (bs, 1H). Mp = 170–172 °C. Anal. calcd for C₁₇H₁₅N₅O: C, 66.87; H, 4.95; N, 22.94. Found: C, 66.37; H, 5.09; N, 22.60.

4,6-Dimethylquinazolyl-2-*N*-propionylguanidine (11). ¹H NMR: δ 1.06 (t, 3H, $J=7.5$ Hz), 2.35 (q, 2H, $J=7.5$ Hz), 2.49 (s, 3H), 2.81 (s, 3H), 7.64–7.69 (m, 2H), 7.86 (s, 1H), 9.9–11.5 (br, ~ 3 H). Mp = 179–180 °C. Anal. calcd for C₁₄H₁₇N₅O: C, 61.98; H, 6.32; N, 25.81. Found: C, 61.86; H, 6.01; N, 25.56.

4,6,7-Trimethylquinazolyl-2-*N*-acetylguanidine (19). ¹H NMR, two forms: δ 2.28 (bs, 3H), 2.44 (s, 3H), 2.46 (s, 3H), 2.83 and 2.87 (s, 3H), 7.59 and 7.71 (s, 1H), 7.94 and 7.99 (s, 1H), 8.4 (bs, ~ 2 H). MS (EI) m/z 271 [M]⁺. Mp = 248–249 °C.

4,7-Dimethylquinazolyl-2-*N*-propionylguanidine (22). ¹H NMR: δ 1.06 (t, 3H, $J=7.2$ Hz), 2.35 (q, 2H, $J=7.2$ Hz), 2.49 (s, 3H), 2.79 (s, 3H), 7.33 (d, 1H, $J=8.4$ Hz), 7.58 (s, 1H), 8.00 (d, 1H, $J=8.4$ Hz), 9.9–11.1 (br, ~ 3 H). MS (EI) m/z 271 [M]⁺. Mp = 159–161 °C.

4-Methyl-7-methoxyquinazolyl-2-*N*-benzoylguanidine (26). ¹H NMR: δ 2.91 (s, 3H), 3.95 (s, 3H), 7.51 (d, 1H, $J=2.5$ Hz), 7.64 (dd, 1H, $J=9.0, 2.5$ Hz), 7.7 (bs, 1H), 7.89 (d, 1H, $J=9.0$ Hz), 8.1–8.35 (br, 4H), 10.9–13.4 (br, ~ 2 H). MS (EI) m/z 335 [M]⁺. Mp = 236–237 °C.

4-Methyl-7-ethoxyquinazolyl-2-*N*-acetylguanidine (28). ¹H NMR: δ 1.40 (t, 3H, $J=7.2$ Hz), 2.05 (s, 3H), 2.80 (s, 3H), 4.19 (q, 2H, $J=7.2$ Hz), 7.39 (d, 1H, $J=2.5$ Hz), 7.50 (dd, 1H, $J=9.0, 2.5$ Hz), 7.73 (d, 1H, $J=9.0$ Hz), 9.8 (br, ~ 3 H). Mp = 154–155 °C. Anal. calcd for C₁₄H₁₇N₅O₂·1/3H₂O: C, 57.33; H, 6.07; N, 23.88. Found: C, 57.17; H, 5.60; N, 23.51.

General procedure for the preparation of substituted 4-methylquinazolyl-2-*N,N*-diacylguanidines.

Following the procedures described for 4-methylquinazolyl-2-*N*-acylguanidines except 0.002 mol of the anhydride was used, and substituting the following substituted quinazolyl-2-guanidines (Formula III, Scheme 1) for 4-methyl-6-methoxyquinazolyl-2-guanidine, the corresponding substituted 4-methylquinazolyl-2-*N,N*-diacylguanidines (Formula V) were obtained with the listed spectral and physical properties.

4-Methylquinazolyl-2-*N,N*-diacetylguanidine (6). ¹H NMR: δ 2.26 (bs, 6H), 2.88 (s, 3H), 7.60 (dd, 1H, $J=8.0, 7.5$ Hz), 7.81 (d, 1H, $J=8.6$ Hz), 7.92 (dd, 1H, $J=8.6, 7.5$ Hz), 8.21 (d, 1H, $J=8.0$ Hz), 11.4–11.9 (br, ~ 2 H). Mp = 196–197 °C. Anal. calcd for C₁₄H₁₅N₅O₂: C, 58.94; H, 5.30; N, 24.55. Found: C, 58.59; H, 5.23; N, 24.43.

6-Methoxy-4-methylquinazolyl-2-*N,N*-dipropionylguanidine (15). ¹H NMR: δ 1.10 (t, 6H, $J=7.2$ Hz), 2.62 (bs, 4H), 2.70 (s, 3H), 3.97 (s, 3H), 7.12–8.15 (m, 3H), 11.6–12.0 (br, 2H). Mp = 156–158 °C.

4,6,7-Trimethylquinazolyl-2-*N,N*-dipropionylguanidine (20). ¹H NMR: δ 1.08 (t, 6H, $J=7.2$ Hz), 2.43 (s, 3H), 2.45 (s, 3H), 2.59 (br, 4H), 2.82 (s, 3H), 7.57 (s, 1H), 7.95 (s, 1H), 11.6–12.0 (br, 2H). ¹H NMR (DMSO-*d*₆): 2.25 (bs, 6H), 2.49 (s, 3H), 2.84 (s, 3H), 7.43 (d, 1H, $J=8.4$ Hz), 7.60 (s, 1H), 8.09 (d, 1H, $J=8.4$ Hz), 11.6 (br, ~ 2 H). MS (EI) m/z 299 [M]⁺. Mp = 169–171 °C. Anal. calcd for C₁₈H₂₃N₅O₂: C, 63.32; H, 6.79; N, 20.51. Found: C, 63.20; H, 6.59; N, 20.46.

4,7-Dimethylquinazolyl-2-*N,N*-diacetylguanidine (23). ¹H NMR: δ 2.25 (bs, 6H), 2.49 (s, 3H), 2.84 (s, 3H), 7.43 (d, 1H, $J=8.4$ Hz), 7.60 (s, 1H), 8.09 (d, 1H, $J=8.4$ Hz), 11.6 (br, ~ 2 H). MS (EI) m/z 299 [M]⁺. Mp = 180–182 °C. Anal. calcd for C₁₅H₁₇N₅O₂: C, 60.19; H, 5.72; N, 23.40. Found: C, 59.80; H, 5.61; N, 23.13.

4-Methyl-7-methoxyquinazolyl-2-*N,N*-diacetylguanidine (27). ¹H NMR: δ 2.28 (bs, 6H), 2.92 (s, 3H), 3.96 (s, 3H), 7.13 (s, 1H), 7.17 (d, 1H, $J=8.4$ Hz), 8.09 (d, 1H, $J=8.4$ Hz), 11.5–12.1 (br, ~ 2 H). Mp = 176–178 °C.

Preparation of 4-methyl-6-methoxyquinazolyl-2-(2'-amino-4'-imidazolinone) (35).

A mixture of 4-methyl-6-methoxyquinazolyl-2-guanidine (0.01 mole) and ethyl 2-bromoacetate (0.006 mole) in 50 mL of dimethylformamide was refluxed for 4 to 6 h, allowed to cool and poured into water. The resulting precipitate was filtered and the residue recrystallized from dioxane yielding the desired 4-methyl-6-methoxyquinazolyl-2-(2'-amino-4'-imidazolinone) with the reported physical and spectroscopic properties.¹⁷

General procedure for the preparation of substituted 4-methylquinazolyl-2-(2'-amino-4'-imidazolinones).

Following the procedures described above for **35** and substituting the following substituted quinazolyl-2-guanidines (Formula III, Scheme 1) for 4-methyl-6-methoxyquinazolyl-2-guanidine, the corresponding substituted 4-methylquinazolyl-2-(2'-amino-4'-imidazolinone) (**32**, **33**, **36**, **37**, and **38**) were obtained with the reported spectral and physical properties.¹⁰

4-Methyl-6-methoxyquinazolyl-2-*N*-methylguanidine (13). A mixture of 4-methyl-6-methoxyquinazolyl-2-guanidine (0.01 mol) and iodomethane (0.015 mol) in 30 mL of dry dimethylformamide was heated under reflux for 10 h. The mixture was allowed to cool, the precipitate

was isolated by filtration and washed with acetone yielding the desired product.¹¹

6-Methyl-4-methoxyquinazolyl-2-guanidine. A suspension of 4-hydroxy-6-methylquinazolyl-2-guanidine (5 g) in 100 mL of methanol was treated with 100 mg of *p*-toluenesulfonic acid and refluxed for 15 h. The precipitate was isolated by filtration, washed with water, dried and recrystallized from dimethylformamide yielding 4 g (80% yield) of the desired 6-methyl-4-methoxyquinolyl-2-guanidine. Mp 265–266 °C.

Preparation of substituted quinolines

***N*-(7-Methoxy-4-methyl-quinolin-2-yl)-acetamide (49).** To a solution of 2-amino-4-methyl-7-methoxyquinoline (9.4 mg, 0.04 mmol) in CH₂Cl₂ (1 mL) was sequentially added triethylamine (14 μL, 0.10 mmol) and acetic anhydride (6.6 μL, 0.07 mmol) and DMAP (1 mg, 0.008 mmol). The reaction mixture was stirred at room temperature for 1 h, and the solvent was removed under a stream of nitrogen. The residue was purified by preparative thin layer chromatography (ethyl acetate/hexanes/methanol=10/10/1) to give 49 (9.8 mg, 84%) as a white solid. ¹H NMR (CDCl₃) δ 2.25 (s, 3H), 2.68 (s, 3H), 3.94 (s, 3H), 7.13 (s, 1H), 7.82 (dd, 1H, *J*=1.1, 8.4 Hz), 8.17 (s, 1H), 9.16 (bs, 1H). MS (CI/NH₃) *m/z* 231 (M+H⁺)⁺. FAB+ exact mass: calcd for C₁₃H₁₅N₂O₂ 231.1134. Found: 231.1138.

***N*-(7-Methoxy-4-methyl-quinolin-2-yl)-carbamic acid methyl ester (50).** To a solution of 2-amino-4-methyl-7-methoxyquinoline (10 mg, 0.053 mmol) in CH₂Cl₂ (1 mL) was added triethylamine (14 μL, 0.1 mmol) and methyl chloroformate (6 μL, 0.08 mmol). The reaction mixture was stirred at room temperature for 1 h, and the solvent was removed by a nitrogen stream. The residue was purified by preparative thin layer chromatography (ethyl acetate:hexanes:methanol=10:15:1) to give 50 (10.5 mg, 81%) as a white solid. ¹H NMR (CDCl₃) δ 2.67 (d, 3H, *J*=0.8 Hz), 3.83 (s, 3H), 3.93 (s, 3H), 7.09 (dd, 1H, *J*=2.6, 9.1 Hz), 7.14 (d, 1H, *J*=2.5 Hz), 7.59 (bs, 1H), 7.81 (d, 1H, *J*=9.1 Hz), 7.94 (s, 1H). MS (CI/NH₃) *m/z* 247 (M+H⁺)⁺. FAB+ exact mass: calcd for C₁₃H₁₅N₂O₃ 247.1083. Found: 247.1072.

***N,N'*-Di-*tert*-butyloxycarbonyl-*N'*-(7-Methoxy-4-methyl-quinolin-2-yl)-guanidine (51).** 2-Amino-4-methyl-7-methoxyquinoline (19 mg, 0.1 mmol) was added to a solution of di-BOC-protected-triflylguanidine (36 mg, 0.1 mmol) and triethylamine (16 μL, 0.12 mmol) in CH₂Cl₂ (1 mL), and the mixture was stirred at refluxing temperature until all triflylguanidine was consumed (the reaction was monitored by TLC). After completion of the reaction, the mixture was diluted with CH₂Cl₂ (2 mL) and the organic layer was washed with 2 M sodium bisulfate, saturated sodium bicarbonate, brine, and dried over sodium sulfate, filtered, concentrated in vacuo. The residue was purified by preparative thin layer chromatography (ethyl acetate/hexanes/methanol=10:30:1) to give 51 (16 mg, 40%) as a white solid. ¹H NMR (DMSO-*d*₆) δ 1.52 (s, 9H), 2.63 (s, 3H), 3.89 (s, 3H), 7.15–7.19 (m, 1H), 7.19 (s, 1H), 7.93 (s, 1H), 7.94 (d, 1H,

J=9.6 Hz), 10.81 (s, 1H), 11.95 (s, 1H). MS (CI/NH₃) *m/z* 431 (M+H⁺)⁺.

***N*-(7-Methoxy-4-methyl-quinolin-2-yl)-guanidine (52) and *N-tert*-butyloxycarbonyl-*N'*-(7-Methoxy-4-methyl-quinolin-2-yl)-guanidine (53).** A solution of 51 (8 mg, 0.019 mmol) in 5% trifluoroacetic acid in CH₂Cl₂ (1 mL) was stirred for 2 h at room temperature. The solvent was evaporated under reduced pressure and the residue was purified by preparative thin-layer chromatography (ethyl acetate/hexanes/methanol=10:10:1) to give 52 (0.8 mg, 30%) and 53 (1.6 mg, 57%) as a white solid.

52. ¹H NMR (CD₃OD) δ 2.68 (s, 3H), 3.95 (s, 3H), 6.82 (d, 1H, *J*=0.8 Hz), 7.19 (dd, 1H, *J*=2.6, 9.2 Hz), 7.38 (d, 1H, *J*=2.6 Hz), 7.95 (d, 1H, *J*=9.2 Hz). MS (CI/NH₃) *m/z* 231 (M+H⁺)⁺. FAB+ exact mass: calcd for C₁₂H₁₅N₄O 231.1246. Found: 231.1238.

53. ¹H NMR (CD₃OD) δ 1.55 (s, 9H), 2.66 (s, 3H), 3.97 (s, 3H), 6.85 (s, 1H), 7.15 (dd, 1H, *J*=2.6, 9.2 Hz), 7.32 (d, 1H, *J*=2.6 Hz), 7.91 (d, 1H, *J*=9.2 Hz). MS (CI/NH₃) *m/z* 331 (M+H⁺)⁺. FAB+ exact mass: calcd for C₁₇H₂₃N₄O₃ 331.1770. Found: 331.1761.

Pharmacology

R-PIA, and 2-chloroadenosine were purchased from Sigma-RBI (St. Louis, MO, USA). Other synthetic reagents were purchased from Aldrich (Milwaukee, WI, USA).

K_i values of quinazoline derivatives were determined in displacement of binding of the non-selective radioligand [³H]ZM241385 (4 - (2 - [7 - amino - 2 - {furyl}{1,2,4}-triazolo{2,3 - *a*}{1,3,5}triazin - 5 - ylaminioethyl) - phenol (Tocris, Ballwin, MO, USA) at the human A_{2B}AR stably expressed in HEK-293 cell membranes.⁷ In order to determine selectivity, the compounds were evaluated using standard binding assays at A₁, A_{2A}, and A₃ARs. The screening² utilized rat brain A₁/A_{2A}ARs (with radioligands [³H]*R*-PIA, Amersham, Arlington Heights, IL, USA and [³H]CGS21680, Perkin-Elmer, Boston, MA, USA⁸

The functional effects in inhibiting the rise in intracellular calcium elicited by the non-selective agonist NECA in HEK cells expressing the human AAR were examined, by methods described.⁶ The test compound was incubated with cells at 37° for 2 min. Then the cells (1 million in 2 mL) were transferred to a stirred cuvette maintained at 37 °C within an Aminco SLM 8000 spectrofluorometer (SML instruments, Urbana, IL, USA). The ratios of indo-1 fluorescence obtained at 400 and 485 nm (excitation, 332 nm) was recorded using a slit width of 4 nm. NECA was added after a 100 s equilibration period.

Acknowledgements

We are grateful for the help of Sergey Shorshnev of ChemBridge Moscow for his help with NMR spectral interpretation.

References and Notes

1. Jacobson, K. A.; Knutsen, L. J. S. In *P1 and P2 Purine and Pyrimidine Receptors*; Abbracchio, M. P., Williams, M., Eds.; *Handbook of Experimental Pharmacology, Volume 151/I: Purinergic and Pyrimidinergic Signaling I*; Springer: Berlin, Germany, 2001; p 129.
2. Kim, Y.-C.; Ji, X.-D.; Melman, N.; Linden, J.; Jacobson, K. A. *J. Med. Chem.* **2000**, *43*, 1165.
3. Webb, T. R.; Melman, N.; Lvovskiy, D.; Ji, X.-D.; Jacobson, K. A. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 31.
4. Feoktistov, I.; Polosa, R.; Holgate, S. T.; Biaggioni, I. *Trends Pharmacol. Sci.* **1998**, *19*, 148.
5. Kirshenbaum, A. S.; Hettinger, B.; Day, Y.-J.; Gilfillan, A. M.; Metcalfe, D. D.; Kim, Y.-C.; Linden, J.; Jacobson, K. A. 2001, *Amer. Acad. Allergy, Asthma and Immunol.*, 57th Annual Meeting, 2001 (abstr 2000803).
6. Auchampach, J. A.; Jin, J.; Wan, T. C.; Caughey, G. H.; Linden, J. *Mol. Pharmacol.* **1998**, *52*, 846.
7. Ji, X.-D.; Jacobson, K. A. *Drug Des. Discov.* **1999**, *16*, 217.
8. Olah, M. E.; Gallo-Rodriguez, C.; Jacobson, K. A.; Stiles, G. L. *Mol. Pharmacol.* **1994**, *45*, 978.
9. Cliffe, W. H. *J. Chem. Soc.* **1933**, *35*, 1327.
10. Knövenagel, E. *Ber.* **1921**, *54B*, 1722.
11. Shikhaliev, Kh. S.; Falaleyev, A. V.; Ermolova, G. I.; Solov'ev, A. S. *Chem. Heterocycl. Comp.* **1999**, *35*, 818.
12. Shikhaliev, Kh. S.; Falaleyev, A. V.; Ermolova, G. I.; Solov'ev, A. S. *Khim. Khim. Tekhnol.* **1998**, *41*, 116.
13. Fozard, J. R.; Hannon, J. P. *Pulm. Pharmacol. Ther.* **1999**, *12*, 111.
14. Salvatore, C. A.; Tilley, S. L.; Latour, A. M.; Fletcher, D. S.; Koller, B. H.; Jacobson, M. A. *J. Biol. Chem.* **2000**, *275*, 4429.
15. Feoktistov, I.; Polosa, R.; Holgate, S. T.; Biaggioni, I. *Trends Pharmacol. Sci.* **1998**, *19*, 148.
16. Jacobson, K. A.; IJzerman, A. P.; Linden, J. *Drug Devel. Res.* **1999**, *47*, 45.
17. Arya, V. P.; David, J.; Grewal, R. S.; Marathe, S. B.; Patil, S. D. *Indian J. Chem.* **1977**, *15B*, 1129.
18. Rosowsky, A.; Nadel, M. E.; Modest, E. J. *J. Heterocycl. Chem.* **1972**, *9*, 625.
19. van Muijlwijk-Koezen, J. E.; Timmerman, H.; Link, R.; van der Goot, H.; Ijerman, A. P. *J. Med. Chem.* **1998**, *41*, 3994.
20. Feichtinger, K.; Zapf, C.; Sings, H. L.; Goodman, M. *J. Org. Chem.* **1998**, *63*, 3804.