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Scaffold Decoration at Positions 5 and 8 of 1,2,4-Triazolo[1,5-*c*]Pyrimidines to Explore the Antagonist Profiling on Adenosine Receptors: A Preliminary Structure–Activity Relationship Study

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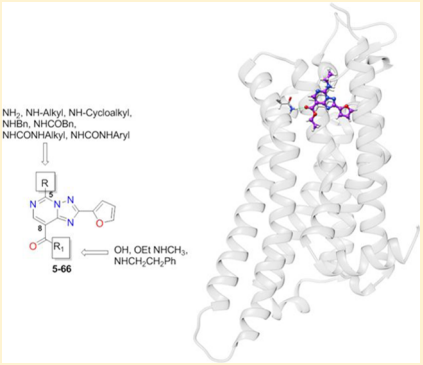
Supporting Information

ABSTRACT: The structure–activity relationship (SAR) of new 5,8-disubstituted-1,2,4-triazolo[1,5-*c*]pyrimidines as adenosine receptors (ARs) antagonists has been explored. All the synthesized compounds show affinity for the hA_{2A} and hA_3 ARs depending on the substitution patterns at the 5 and 8 positions. In particular, a free amino group at the 5 position with an ethoxycarbonyl group at the 8 position leads to potent and quite selective hA_{2A} antagonists (compound **12**: hA_{2A} AR K_i = 3.32 nM; hA_1/hA_{2A} = 55.6; hA_{2A}/hA_3 = 0.01), whereas the introduction of a methylamino function at the 5 position yields a good binding profile at the hA_3 AR (compound **23**: hA_3 AR K_i = 4.14 nM, hA_1/hA_3 = 236; hA_{2A}/hA_3 = 25). Through an in silico receptor-driven approach, we have determined the most favorable orientation of the substitutions at the 5 and 8 positions of the 1,2,4-triazolo[1,5-*c*]pyrimidine (TP) scaffold and, accordingly, we have elucidated the observed SAR.



INTRODUCTION

Activation of adenosine receptors (ARs) is responsible of several effects on different organ systems. On the basis of the widespread, and frequently beneficial, effects attributed to the accumulation of endogenously released adenosine, it is generally accepted that regulation of ARs has great therapeutic potential. In particular, the cardioprotective[1,2] and neuroprotective[3,4] effects associated with AR activation during periods of cardiac and cerebral ischemia, respectively, have been widely investigated. Moreover, it has also been proposed that antagonists for distinct AR subtypes may be used in the treatment of asthma[5,6] or neurological diseases such as Parkinson's disease.[7]

ARs are members of the superfamily of G protein-coupled receptors (GPCRs), with four subtypes currently known, the A_1 AR, A_{2A} AR, A_{2B} AR, and A_3 AR.[8] In the recent decades, numerous medicinal chemistry groups have made intense efforts in developing ideal ligands for these receptor subtypes.[9−16] In particular, the search for selective antagonists held greater appeal than selective agonists, not only for their potential therapeutic applications but also for their preferential use as molecular probes for pharmacological characterization of receptors. Considering all of these aspects, the development of potent and selective ARs antagonists has been one of the most highly investigated areas in medicinal chemistry in recent years.[17]

Our research group and others have extensively investigated the pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine nucleus that, after appropriate modifications and optimization of the N7, N8, and 5 positions, led to very potent and selective human A_{2A} AR antagonists such as compound **1** and preladenant[18−20] or A_3 AR[21−26] antagonists such as compound **2** (Chart 1).

Despite the achieved potency and selectivity, those compounds suffered from poor water solubility and complicated synthetic preparation. Taking into account these drawbacks, in the last years we focused our attention on the synthesis of more simplified heterocyclic derivatives, in particular bicyclic systems such as 1,2,4-triazolo[1,5-*a*]-1,3,5-triazine,[27−29] considering that ZM 241385 has been reported as a potent A_{2A} AR antagonist.[30,31] In addition, this compound binds also with good affinity to the human A_{2B} AR (28 nM), and in its tritiated form, the compound is actually used in radioligand binding studies for this subtype.[32] In particular, at

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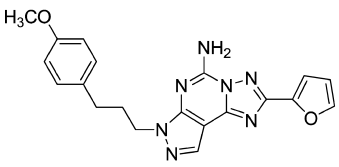
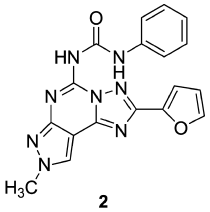
Chart 1. Pyrazolo-triazolo-pyrimidines as Human A_{2A} and A_3 Adenosine Receptor Antagonists



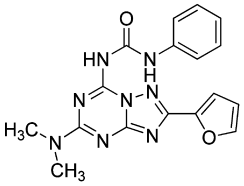
SCH 442416, **1**
hA_1 = 1,111 nM
hA_{2A} = 0.048 nM
hA_{2B} = >10,000
hA_3 = >10,000



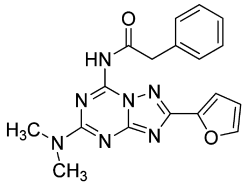
2
hA_1 = 594 nM
hA_{2A} = 381 nM
hA_{2B} = 222 nM
hA_3 = 0.16 nM

the N7 position of the 1,2,4-triazolo-[1,5-*a*]-1,3,5-triazine nucleus, we introduced arylcarbamoyl (**3**) or arylacetyl (**4**) moieties, which gave good results in terms of affinity at the A_3[21−25] and A_{2B}[33] ARs, respectively, when attached to the pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]-pyrimidine nucleus, to obtain corresponding A_{2B} or A_3 AR antagonists with simplified core (Chart 2).[34]

Chart 2. N7 Phenylcarbamoyl and Phenylacetyl Triazolo-triazines



3
rA_1 = 3,150 nM
rA_{2A} = 580 nM
hA_{2B} = > 100,000 nM (functional)
hA_3 = 311 nM



4
rA_1 = 6,080 nM
rA_{2A} = 6,700 nM
hA_{2B} = > 100,000 nM (functional)
hA_3 = 1,050 nM

Unfortunately, both compounds showed poor affinity and selectivity for the hA_3 and hA_{2B} ARs. A possible explanation of this lack of affinity has been given comparing the 1,2,4-triazolo-[1,5-*a*]-1,3,5-triazine derivatives **3**,**4** with the pyrazolo[4,3-*e*]-1,2,4--triazolo[1,5-*c*]-pyrimidine **1**,**2**. We hypothesized that most probably the dimethylamino group in the 1,2,4-triazolo-[1,5-*a*]-1,3,5-triazine nucleus is in a wrong position. In fact, the dimethylamino group lacks correspondence with the N8-methyl group of derivative **2** but is quite similar to its N7 pattern of substitution (e.g., compound **1**), which has been extensively demonstrated to be inactive at the hA_3 ARs.[19,20,33]

With the aim to validate this hypothesis, we decided to translate the substituent to the 4 position of the 1,2,4-triazolo-[1,5-*a*]-1,3,5-triazine nucleus, but the presence of the nitrogen at this position does not allow such a modification (Chart 3).

We therefore designed a new class of compounds on the basis of a 1,2,4-triazolo[1,5-*c*]pyrimidine nucleus which could be considered a deaza analogue of the 1,2,4-triazolo-[1,5-*a*]-1,3,5-triazine core, bearing different substituents at the 5 and 8 positions, and we analyzed their binding profile at the four human ARs.

RESULTS AND DISCUSSION

A. Chemistry. All the designed compounds **5**−**66** have been synthesized as summarized in Schemes 1−4. Condensation between isothiourea **67** and diethyl ethoxymethylene malonate **68** in basic condition led to a pyrimidine sodium salt **69**, which after treatment with $POCl_3$ under reflux afforded chloro derivative **70** that was immediately coupled with 2-furoyl hydrazide in the presence of DBU leading to compound **71** (Scheme 1).[35,36]

By reacting the hydrazide **71** in the presence of P_2O_5/HMDS in dry xylene at reflux, the corresponding triazolo-pyrimidine **5** was obtained. Nucleophilic substitution of methylthio group with veratrylamine at 120 °C in a sealed tube yielded benzylamino derivative **6**, which under basic conditions (LiOH H_2O/EtOH), led to the corresponding carboxylic acid **7**.

By reacting acid derivative **7** with the appropriate amine in the presence of pyridine and $POCl_3$, the corresponding carboxamido derivatives **8**,**9** were obtained, which after amino benzyl deprotection with TFA gave the final compounds **10**,**11** (Scheme 2a). Aminoester derivative **12** could be easily obtained by reacting intermediate **5** with ethanolic ammonia in a sealed tube at 120 °C (Scheme 2b).

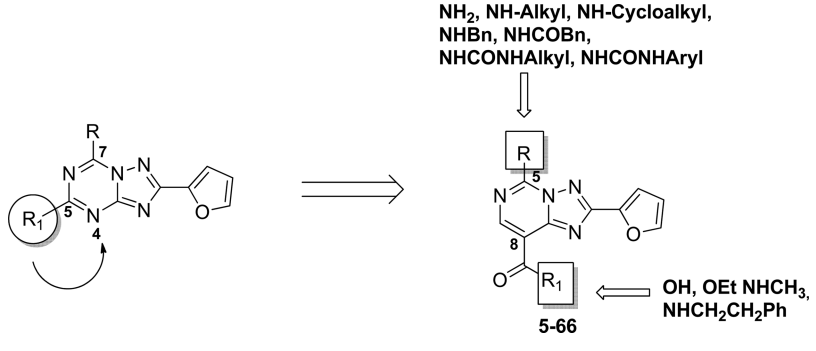
N5-Arylacetyl (**15**, **18**, **21**) or N5 urea (**13**, **14**, **16**, **17**, **19**, **20**) derivatives were obtained by reacting the amino compounds (**10**−**12**) with the appropriate acyl chloride or isocyanate in dry THF at reflux overnight (Scheme 3a). Treatment of compound **20** with LiOH in a mixture EtOH/H_2O led to the corresponding acid derivative **22** (Scheme 3b).

5-Aminoalkyl derivatives (**23**−**50**) were obtained by reacting methylthio intermediate **5** with the appropriate amine in ethanol at 120 °C in a sealed tube (Scheme 4).

Carboxylic acid compounds **51**−**66** were obtained by saponification of the corresponding esters (**23**−**38**) in the presence of LiOH/H_2O/EtOH.

B. Biology. Newly synthesized compounds (**5**−**66**) were tested at the human A_1, A_{2A}, and A_3 receptors expressed in

Chart 3. Rational Design and General Structures of Synthesized Compounds (5−66)



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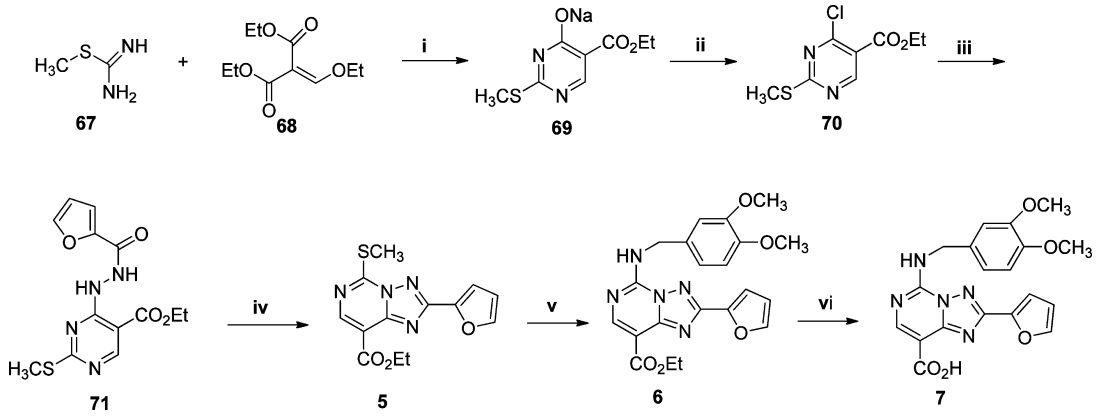
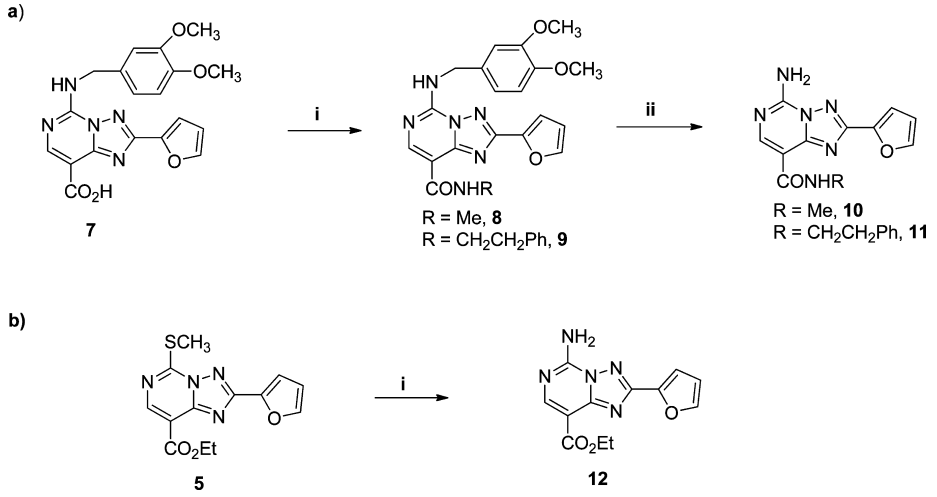
Scheme 1. Synthesis Reagents and Conditions[a]



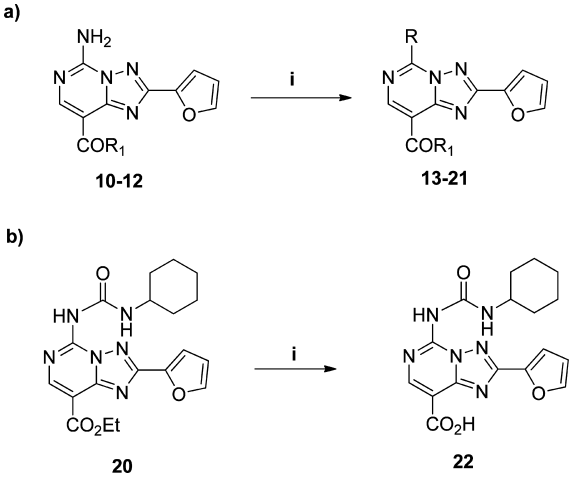
[a](Upper left) i: NaOH, H_2O, EtOH, rt, 24 h; ii: $POCl_3$, reflux, 3 h; iii: 2-furoylhydrazide, DBU, THF, rt, 5 h; v: P_2O_5, HMDS, xylene, 90°C, 2 h, reflux, 48 h; iv: 3,4-dimethoxybenzylamine, EtOH, 120 °C, sealed tube, 2 h; vii: LiOH·H_2O, H_2O/EtOH, 1:40, reflux, 2 h.

Scheme 2. Synthesis Reagents and Conditions[a]



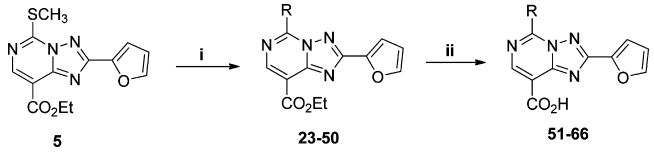
[a](Panel a) i: methylamine or β-phenylethyl amine, dry pyridine, $POCl_3$, rt, 2 h; ii: TFA, trifluoromethanesulfonic acid, anisole, rt, overnight. (Panel b) i: NH_3 7N in MeOH, 120 °C, 2 h.

Scheme 3. Synthesis Reagents and Conditions[a]



[a](Panel a) i: R-NCO, dry THF, reflux, overnight or RCOCl, Et_3N, dry THF reflux, overnight. (Panel b) i: LiOH, H_2O/EtOH 1:40, reflux, 2 h.

Scheme 4. Synthesis Reagents and Conditions[a]



[a](Left) i: R-NH_2 or RR′NH, EtOH, sealed tube, 120 °C, 2 h; ii: LiOH·H_2O, H_2O/EtOH 1:40, reflux, 2 h.

CHO cells: [^{3}H]CCPA (A_1) and [^{3}H]NECA (A_{2A}, A_3) were used as radioligands in binding assays.[37] Inhibition of NECA-stimulated adenylyl cyclase activity was determined as an affinity measurement of the compounds at the A_{2B} AR (Table 1).

Structure–Activity Relationships. As clearly summarized in Table 1, all the synthesized compounds (**5**–**66**) show affinities at the hA_3 and/or hA_{2A} ARs ranging from high nanomolar to nanomolar concentrations, with different degree of selectivity versus the other subtypes. It is quite evident that a nitrogen at the 5 position is fundamental for the affinity, as compound (**5**) bearing a thiomethyl group at the 5 position is inactive at all the

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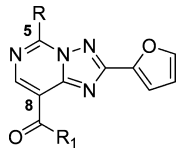
Table 1. Structures and Binding Profile of Synthesized Compounds 5−66



compd	R	R_1	hA_1 (K_i nM)[a]	hA_{2A} (K_i nM)[b]	hA_{2B} (K_inM)[c]	hA_3 (K_i nM)[d]	hA_1/hA_3	hA_{2A}/hA_3	hA_1/hA_{2A}
5	SCH_3	OEt	5720 (3890−8390)	9500 (5360−16 800)	>30 000	2640 (1880−3690)	2.17	3.60	0.60
6	$NHCH_2Ph$-3,4OCH_3	OEt	315 (203−489)	116 (44.3−305)	13 300 (7950−22 200)	38.7 (28.2−53.1)	8.14	3.00	2.71
7	$NHCH_2Ph$-3,4OCH_3	OH	958 (731−1260)	5320 (3680−7700)	>30 000	159 (121−208)	6.02	33.4	5.55
8	$NHCH_2Ph$-3,4OCH_3	$NHCH_3$	428 (271−677)	25.8 (20.2−32.8)	2490 (1730−3580)	282 (182−435)	1.52	0.09	16.6
9	$NHCH_2Ph$-3,4OCH_3	$NHCH_2CH_2Ph$	385 (291−511)	65.2 (43.9−96.9)	>30 000	87.7 (71.7−107)	4.39	0.74	5.90
10	NH_2	$NHCH_3$	135 (104−174)	15.0 (10.6−21.3)	2030 (1470−2796)	609 (459−808)	0.22	0.02	9.00
11	NH_2	$NHCH_2CH_2Ph$	57.1 (38.1−85.5)	11.1 (8.46−14.5)	5320 (4230−6700)	140 (60.9−322)	0.41	0.08	5.14
12	NH_2	OEt	185 (92.5−371)	3.32 (2.03−5.42)	1110 (683−1810)	238 (144−395)	0.78	0.01	55.7
13	NHCONHPh	$NHCH_3$	126 (68−233)	5.76 (3.73−8.91)	1180 (580−2400)	730 (319−1688)	0.17	0.01	21.9
14	$NHCONHcC_6H_{11}$	$NHCH_3$	8990 (4350−17 800)	1520 (618−3750)	>30 000	18 700 (14 200−24 586)	0.48	0.08	5.91
15	$NHCOCH_2Ph$	$NHCH_3$	296 (155−567)	12.4 (10.2−15.0)	4840 (2810−8300)	1190 (796−1769)	0.25	0.01	23.9
16	NHCONHPh	$NHCH_2CH_2Ph$	365 (255−522)	51.4 (22.8−116)	>30 000	1350 (673−2695)	0.27	0.04	7.10
17	$NHCONHcC_6H_{11}$	$NHCH_2CH_2Ph$	>100 000	773 (569−1050)	>30 000	4220 (2107−8468)	>23.7	0.18	>129
18	$NHCOCH_2Ph$	$NHCH_2CH_2Ph$	317 (206−488)	28.1 (20.1−39.3)	>30 000	553 (371−823)	0.57	0.05	11.3
19	NHCONHPh	OEt	322 (133−777)	4.82 (3.21−7.24)	1900 (1240−2930)	357 (257−495)	0.90	0.01	66.8
20	$NHCONHcC_6H_{11}$	OEt	12 700 (2760−58500)	183 (48.4−695)	>30 000	2510 (1300−4865)	5.06	0.07	69.4
21	$NHCOCH_2Ph$	OEt	>100 000	42 700 (33 800−53 900)	>30 000	2590 (1420−4735)	>38.6	16.5	>2.34
22	$NHCONHcC_6H_{11}$	OH	>100 000	24 700 (4480−13 6000)	>30 000	59 400 (25 200−140 113)	>1.68	0.42	>4.05
23	$NHCH_3$	OEt	978 (716−1340)	104 (89.2−122)	2450 (1770−3370)	4.14 (3.53−4.86)	236	25.1	9.40
24	$NHCH_2CH_3$	OEt	162 (157−167)	50.3 (48.8−51.9)	3060 (2230−4200)	3.30 (2.85−3.83)	49.1	15.2	3.22
25	$NHnC_5H_{11}$	OEt	613 (439−856)	50.1 (46.1−54.4)	>10 000	11.3 (9.75−13.1)	54.2	4.43	12.2
26	$NHCH(CH_3)_2$	OEt	40.0 (33.0−48.5)	103 (63.2−167)	~10 000	9.46 (7.70−11.6)	4.23	10.9	0.39
27	$NHCH_2CH(CH_2CH_3)(CH_2)_3CH_3$	OEt	1150 (974−1350)	215 (172−269)	>10 000	39.3 (27.3−56.7)	29.3	5.47	5.35
28	$NHcC_3H_5$	OEt	60.7 (47.7−77.2)	37.4 (31.2−44.8)	2260 (1700−3000)	9.52 (7.24−12.5)	6.38	3.93	1.62
29	$NHcC_5H_9$	OEt	34.4 (33.2−35.7)	43.4 (36.8−51.1)	4620 (3090−6890)	35 (30.2−40.6)	0.98	1.24	0.79
30	$NHCH_2cC_6H_{11}$	OEt	432 (369−507)	121 (75.5−194)	>10 000	41.2 (35−48.4)	10.5	2.94	3.57
31	$NHCH_2Ph$	OEt	940 (643−1380)	32.0 (22.2−46.2)	2510 (1700−3720)	10.0 (8.98−11.2)	94	3.2	29.4
32	$NHCH_2Ph$-4CH_3	OEt	1340 (894−2000)	122 (101−146)	~10 000	56.1 (42.9−73.2)	23.9	2.17	11.0
33	$NHCH_2Ph$-4OCH_3	OEt	1250 (944−1650)	142 (97.8−206)	4810 (2730−8470	57.6 (34.7−95.6)	21.7	2.46	8.80
34	$NHCH_2Ph$-4F	OEt	932 (868−1000)	39.3 (39.2−39.4)	1970 (1270−3060)	46.3 (39.4−54.3)	20.1	0.85	23.7
35	$NHCH_2Ph$-4Cl	OEt	866 (616−1220)	73.2 (51.7−104)	1630 (901−2960)	56.8 (38.8−83.2)	15.2	1.29	11.8
36	$NHCH_2Ph$-4CF_3	OEt	1590 (1510−1670)	209 (159−275)	2040 (1550−2680)	323 (282−370)	4.92	0.65	7.61
37	$NHCH_2Ph$-4-Ph	OEt	890 (701−1130)	187 (150−233)	>30 000	875 (773−990)	1.02	0.21	4.76
38	$NHCHPh_2$	OEt	943 (797−1120)	385 (288−516)	>30 000	336 (289−391)	2.81	1.14	2.45
39	$NHcC_4H_7$	OEt	75.2 (62.5−90.4)	28.9 (13.5−61.6)	>3000	11.7 (9.27−14.8)	6.42	2.47	2.60
40	$NHcC_7H_{13}$	OEt	231 (193−276)	235 (128−433)	>10 000	321 (234−439)	0.72	0.73	0.98
41	$NHcC_8H_{15}$	OEt	176 (130−239)	351 (200−618)	>10 000	517 (360−743)	0.34	0.68	0.50

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Table 1. continued

compd	R	R_1	hA_1 (K_i nM)[a]	hA_{2A} (K_i nM)[b]	hA_{2B} (K_inM)[c]	hA_3 (K_i nM)[d]	hA_1/hA_3	hA_{2A}/hA_3	hA_1/hA_{2A}
42	*N*-pyrrolidinyl	OEt	15 600 (11 500−21 000)	10 200 (9320−11 100)	>10 000	836 (555−1260)	18.7	12.2	1.53
43	*N*-pyperidinyl	OEt	8860 (8780−8930)	8180 (5170−13 000)	>10 000	1770 (1320−2380)	5.00	4.62	1.08
44	$NHCH_2CH_2OCH_3$	OEt	2270 (2000−2590)	292 (161−530)	>10 000	105 (83.8−131)	21.6	2.78	7.77
45	$N(CH_3)_2$	OEt	13 400 (10 100−17 700)	6480 (4030−10 400)	>10 000	275 (214−353)	48.7	23.6	2.07
46	4-morpholinyl	OEt	>100 000	17 600 (16 300−18 800)	>10 000	8700 (6760−11 200)	>11.5	2.02	>5.68
47	$NHcC_6H_{11}$	OEt	141 (126−159)	58.9 (55.0−63.1)	>10 000	131 (115−149)	1.08	0.45	2.39
48	NH-4-morpholinyl	OEt	>100 000	>100 000	>10 000	>30 000			
49	$NHCH_2CH(CH_3)_2$	OEt	45.6 (43.2−48.1)	44.4 (43.0−45.9)	7150 (4670−10 900)	24.4 (18.6−32.1)	1.87	1.82	1.03
50	$NHOCH_3$	OEt	6510 (6330−6680)	210 (152−290)	>10 000	1190 (1000−1420)	5.47	0.18	31.0
51	$NHCH_3$	OH	>100 000	>100 000	>10 000	15 900 (15 400−16 400)	>6.29	>6.29	
52	$NHCH_2CH_3$	OH	11 900 (9460−15 000)	3680 (3000−4520)	>10 000	480 (391−590)	24.8	7.67	3.23
53	$NHnC_5H_{11}$	OH	10 400 (10 100−10 800)	26 900 (23 300−31 100)	>10 000	26 300 (20 700−33 600)	0.39	1.02	0.39
54	$NHCH(CH_3)_2$	OH	4490 (4250−4740)	7660 (5660−10 400)	>10 000	17 500 (12 700−24 200)	0.26	0.44	0.59
55	$NHCH_2CH(CH_2CH_3)(CH_2)_3CH_3$	OH	1780 (1170−2720)	33 800 (27 700−41 100)	>10 000	16 400 (13 400−20 200)	0.11	2.06	0.05
56	$NHcC_3H_5$	OH	1110 (1060−1170)	596 (535−663)	>10 000	165 (136−200)	6.73	3.61	1.86
57	$NHcC_5H_9$	OH	3790 (3440−4170)	37 300 (29 000−47 900)	>10 000	17 700 (15 100−20 700)	0.21	2.11	0.10
58	$NHCH_2cC_6H_{11}$	OH	2740 (2090−3610)	33 900 (32 100−35 700)	>10 000	15 800 (9150−27 300)	0.17	2.14	0.08
59	$NHCH_2Ph$	OH	5090 (4390−5910)	3660 (2430−5510)	>10 000	2230 (1970−2520)	2.28	1.64	1.39
60	$NHCH_2Ph$-4CH_3	OH	2730 (2300−3250)	1190 (850−1660)	>10 000	751 (680−830)	3.63	1.58	2.29
61	$NHCH_2Ph$-4OCH_3	OH	5760 (4090−8100)	12 400 (10 400−14 800)	>30 000	5520 (4120−7390)	1.04	2.25	0.46
62	$NHCH_2Ph$-4F	OH	>100 000	6390 (5150−7930)	>30 000	14 000 (8130−24 200)	>7.14	0.46	>15.6
63	$NHCH_2Ph$-4Cl	OH	>100 000	>100 000	>30 000	11 600 (9040−14 900)	>8.62	>8.62	
64	$NHCH_2Ph$-4CF_3	OH	>100 000	18 000 (16 200−20 000)	>30 000	15 900 (8450−30 000)	>6.29	1.13	>5.55
65	$NHCH_2Ph$-4-Ph	OH	30 100 (19 400−46 800)	>100 000	>30 000	28 300 (25 200−31 700)	1.06	>3.53	<0.30
66	$NHCHPh_2$	OH	2450 (1430−4190)	>100 000	>30 000	2070 (1690−2540)	1.18	>48.0	<0.02

[a]Displacement of specific [^{3}H]-CCPA binding at human A_1 receptors expressed in CHO cells (n = 3−6). [b]Displacement of specific [^{3}H]-NECA binding at human A_{2A} receptors expressed in CHO cells. [c]K_i values of the inhibition of NECA-stimulated adenylyl cyclase activity in CHO cells expressing hA_{2B} receptors. [d]Displacement of specific [^{3}H]-NECA binding at human A_3 receptors expressed in CHO cells. Data are expressed as geometric means, with 95% confidence limits.

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four AR subtypes. Also the presence of a carboxylic function at the 8 position, which improves water solubility, leads to compounds (**7**, **22**, **51**−**66**) exhibiting very poor affinity at all the ARs subtypes. Exceptions are observed for compounds bearing a cyclopropylamino (**56**) or a dimethoxybenzylamino (**7**) groups at the 5 position showing affinity toward the hA_3 AR but poor selectivity versus the other receptor subtypes.

The introduction of a free amino group at the 5 position leads to derivatives (**10**−**12**) with good affinity versus the hA_{2A} AR: In particular, the free amino function at the 5 position combined with a ethoxycarbonyl moiety at the 8 position yields a potent derivative (**12**) selective for the hA_{2A} AR (hA_{2A} AR K_i = 3.32 nM; hA_1/hA_{2A} = 55.7; hA_{2A}/hA_3 = 0.01). The simultaneous introduction at the 8 position of a methyl carboxamido (**10**) or β-phenethylcarboxamido (**11**) groups, instead, produces a reduction of the affinity (3−5-fold) at the hA_{2A} AR with consequent drastic reduction of selectivity (e.g., compound **11**: hA_{2A}AR K_i = 11.1 nM; hA_{2A}/hA_3 = 0.08; hA_1/hA_{2A} = 5.14).

In contrast with our expectations, the introduction at the 5 position of cycloalkylamino/arylureido or arylacetamido moieties does not afford an effect at the hA_3 or hA_{2B} ARs. In fact, the presence of a cyclohexylureido group at the 5 positon (**14**, **17**, **20**) is detrimental for affinity at all the AR subtypes, and only when an ethoxycarbonyl moiety is present at the 8 position is a good affinity for the hA_{2A} AR observed, albeit with poor selectivity versus the other receptor subtypes (compound **20**: hA_{2A} AR K_i = 183 nM; hA_1/hA_{2A} = 69.4; hA_{2A}/hA_3 = 0.07). On the contrary, the introduction of an arylureido moiety at the 5 position (**13**, **16**, **19**) gives different results in terms of affinity and selectivity depending on the substitutions at the 8 position. The simultaneous presence at the 8 position of an ethoxycarbonyl (**19**) or a methyl carboxamido (**13**) function leads to compounds with good affinity and selectivity for the hA_{2A} AR (e.g., compound **19**: hA_{2A} AR K_i = 4.82 nM; hA_{2A}/hA_3 = 0.01; hA_1/hA_{2A} = 66.8), whereas the presence of a β-phenethylcarboxamido group produces a reduction of the affinity (10-fold) at the hA_{2A} AR with a significative loss of selectivity (e.g., compound **16**: hA_{2A} AR K_i = 51.4 nM; hA_{2A}/hA_3 = 0.04; hA_1/hA_{2A} = 7.1).

The introduction of a phenylacetamido group at the 5 position in the presence of a substituted carboxamido group at the 8 position leads to compounds (**15**, **18**) with good hA_{2A} AR affinity and quite good selectivity versus the other AR subtypes (e.g., compound **15**: hA_{2A} AR K_i = 12.4 nM; hA_{2A}/hA_3 = 0.01; hA_1/hA_{2A} = 23.9), although the presence of ethylester at the 8 (**21**) position results in this case detrimental for affinity at all the four AR subtypes (compound **21**: hA_1 AR K_i > 100 000 nM; hA_{2A} AR K_i = 42 700 nM; hA_{2B} AR K_i > 30 000 nM; hA_3 AR K_i = 2590 nM).

A significantly different binding profile is observed when at the 5 position an (ar)alkylamino moiety is introduced in the presence of an ethoxycarbonyl function at the 8 position. In fact, the introduction of a benzylamino group at the 5 position (**31**) leads to an improvement of the affinity at the hA_3 AR, but the selectivity is still low, in particular versus the hA_{2A} AR (hA_3 AR K_i = 10 nM; hA_1/hA_3 = 94; hA_{2A}/hA_3 = 3.2). Substitutions on the para position of phenyl ring (**6**, **32**−**37**) in general reduces the affinity (3−5-fold) at the hA_3 AR with a significant reduction of selectivity (e.g., compound **32**: hA_3 AR K_i = 56.1 nM; hA_1/hA_3 = 23.9; hA_{2A}/hA_3 = 2.17). Substitution of the phenyl ring with a 4-CF_3 (**36**) or 4-Ph (**37**) is detrimental in terms of hA_3 AR affinity and selectivity but shows better affinity for the A_{2A} AR subtype (e.g., compound **37**: hA_3 AR K_i = 875 nM; hA_{2A} AR K_i = 187 nM). An improvement of hA_{2A} AR affinity with respect to hA_3 AR, considering the 5-benzylamino derivatives, is also observed when at the 8 position a substituted carboxamido function (**8**,**9**) is present (e.g., compound **8**: hA_3 AR K_i = 282 nM; hA_{2A} AR K_i = 25.8 nM). Also, introduction of bulkier substituents at the 5 position such as benzhydrylamino group (**38**) leads to a reduction of both affinity and selectivity at hA_{2A} and hA_3 ARs.

Concerning the affinity at the hA_3 AR, promising results are observed when alkylamino substituents are introduced at the 5 position and an ethoxycarbonyl group is present at the 8 position (**23**−**27**, **30**, **49**). Small alkylamino groups such as methylamino (**23**) or ethylamino (**24**) give good affinity and selectivity (e.g., compound **23**: hA_3 AR K_i = 4.14 nM, hA_1/hA_3 = 236; hA_{2A}/hA_3 = 25.1), whereas bulkier and branched chains (**26**, **27**, **49**) lead to a reduction of the affinity with a significant reduction of selectivity versus the other receptor subtypes. This reduction of the affinity is more evident with bulkier substituents such as 2-ethylhexylamino group (compound **27**: hA_3 AR K_i = 39.3 nM; hA_1/hA_3 = 29.3; hA_{2A}/hA_3 = 5.47).

A similar behavior is observed when cycloalkylamino substituents (**28**, **29**, **39**−**41**, **47**) are introduced at the 5 position. Small cycloalkylamino groups, such as cyclopropylamino (**28**) or cyclobutylamino (**39**) show good affinity at the hA_3 AR (e.g., compound **28**: hA_3 AR K_i = 9.52 nM), although bigger cycloalkylamino groups (**29**, **40**, **41**, **47**) are not well-tolerated (e.g., compound **41**: hA_3 AR K_i = 517 nM). Nevertheless, independently from the cycloalkylamino group dimension, the levels of selectivity versus the other receptor subtypes are very low.

Introduction at the 5 position of polar groups such as methoxyamino (**50**) or 2-methoxyethylamino (**44**) results in a drastic reduction of the affinity at the hA_3 AR and low levels of selectivity versus the other receptor subtypes (e.g., compound **44**: hA_3 AR K_i = 105 nM; hA_1/hA_3 = 21.6; hA_{2A}/hA_3 = 2.78). Disubstitution of the nitrogen at the 5 position with methyl groups (**45**) significantly reduces both the affinity and the selectivity at the hA_3 AR (hA_3 AR K_i = 275 nM; hA_1/hA_3 = 48.7; hA_{2A}/hA_3 = 23.6). A more drastic reduction of affinity and selectivity at the hA_3 AR is observed when cyclic amines are introduced at the 5 position: Indeed, pyrrolidine (**42**) or piperidine (**43**) derivatives show affinity for the hA_3 AR in the high nanomolar range (836−1770 nM) and a total absence of selectivity versus other receptor subtypes. More interestingly, the introduction of heterocycles with a heteroatom at the 4 position such as morpholine (**46**) or insertion of other polar functions as in the 4-aminomorpholin derivative (**48**) gives compounds inactivity at all the four ARs, suggesting that polar groups at the 5 position are not well-tolerated.

C. Molecular Modeling. To explain from a molecular point of view the observed binding data, we performed a receptor-based molecular modeling study of the new derivatives by running a previously reported computational protocol.[38] The crystallographic structure of the hA_{2A} AR and hA_3 AR homology model represent the macromolecular starting point of our survey. Selected compounds were docked into the putative TM binding site of the hA_3 AR and the orthosteric pocket of the hA_{2A} AR with the aim to identify their hypothetical binding modes. The outcoming docking poses were selected by taking into account optimal interaction geometries with the residues surrounding the binding site. After the selection of a representative docking pose for each

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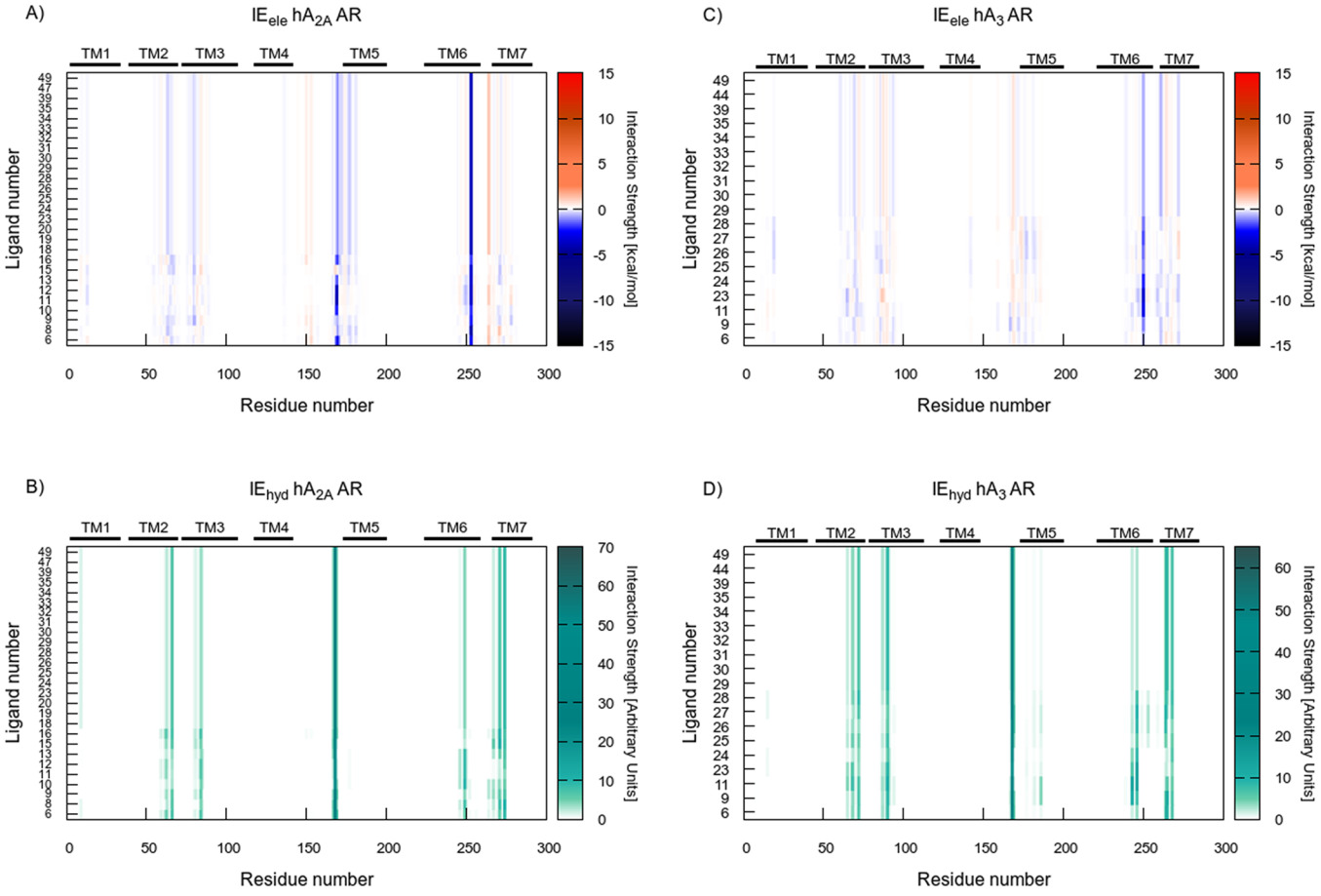

Figure 1. Per residue IEele and IEhyd maps for the most energetically favorable docking poses of compounds with K_i < 100 nM. The maps have been computed for selected poses of the considered compound inside the orthosteric binding site of hA_2 AR (panels A and B) and the putative binding site of hA_{2A} AR (panels C and D). Electrostatic energy contributions are expressed in kcal/mol, whereas hydrophobic contributions are in arbitrary units. Ranges reported in the *x*-axes represent the length of protein sequences, truncated at 300 residues.

compound, we performed a general analysis of the binding modes to identify the residues involved in the binding with the ligands by computing per residue electrostatic and hydrophobic contributions to the interaction energy (IEele and IEhyd, respectively). This semiquantitative analysis was graphically transferred into "Interaction Energy Fingerprints" (IEFs), that is, maps that allow clear recognition of common features and differences among the interaction profiles of the considered compounds. We completed our analysis by the inspection of individual docking poses to explain, from a molecular point of view, the exhibited affinity and selectivity profiles. A detailed description of the methods used is reported in the Experimental Section.

For our molecular modeling investigation we focused on derivatives showing K_i values falling into the low nanomolar range by applying an arbitrary cutoff of 100 nM, whereas a more detailed analysis has been performed for the ligands that display higher activity toward the hA_{2A} (K_i < 15 nM) and hA_3 AR subtypes (K_i < 10 nM).

The IEele map for the hA_{2A} AR subtype (Figure 1A) shows three regions of negative potential energy corresponding to residues mainly located in TM2, TM6, and EL2. Phe168 (EL2) and Asn253 (6.55) take part to the most effective polar interactions, whereas Asp170 (EL2) and to a lesser extent Asn181 (5.42) and the backbones of Ala63 (2.61) and Leu85 (3.33) are engaged in secondary interactions. The IEhyd map (Figure 1B) displays several residues involved in hydrophobic contacts with the ligands, such as Phe168 (EL2) and Leu249 (6.51), for which shared electro-neutral surfaces are broadest. Additionally, Ile66 (2.64), Val84 (3.32), Tyr271 (7.36), and Ile274 (7.39) contribute to the stabilization of ligand−receptor complexes. Similar interaction patterns are also observed for the energetically more favorable poses of the ligands at the hA_3 AR (Figure 1C,D) In particular, the most prominent electrostatic interactions are established with Asn250 (6.55), whereas Gln261 (7.32), Tyr265 (7.36), and His272 (7.43) are engaged in secondary interactions. Hydrophobic contacts include, among others, Phe168 (EL2) and Leu246 (6.51), and to a lesser extent also Val72 (2.64), Leu90 (3.32), Trp243 (6.48), Leu264 (7.35), Tyr265 (7.36), and Ile268 (7.39).

The IEFs analysis (Figure 2A) for the most active compounds at the hA_{2A} AR identifies Asn253 (6.55) and Phe168 (EL2) as the residues mainly contributing to the interaction energy for all the selected derivatives. The interaction with Glu169 (EL2), instead, is a prerogative of compounds bearing a free amino group at the 5 position (**10**, **12**), whereas derivatives bearing arylcarbamoyl (**19**) and phenylacetyl (**15**) groups establish additional hydrophobic contacts with Ile66 (2.64), Val84 (3.32), and Tyr271 (7.36). The corresponding hypothetical binding modes of **12** (Figure 2B) and **19** (Figure 2C) help in explaining these differences: both ligands reside in the upper region of the transmembrane

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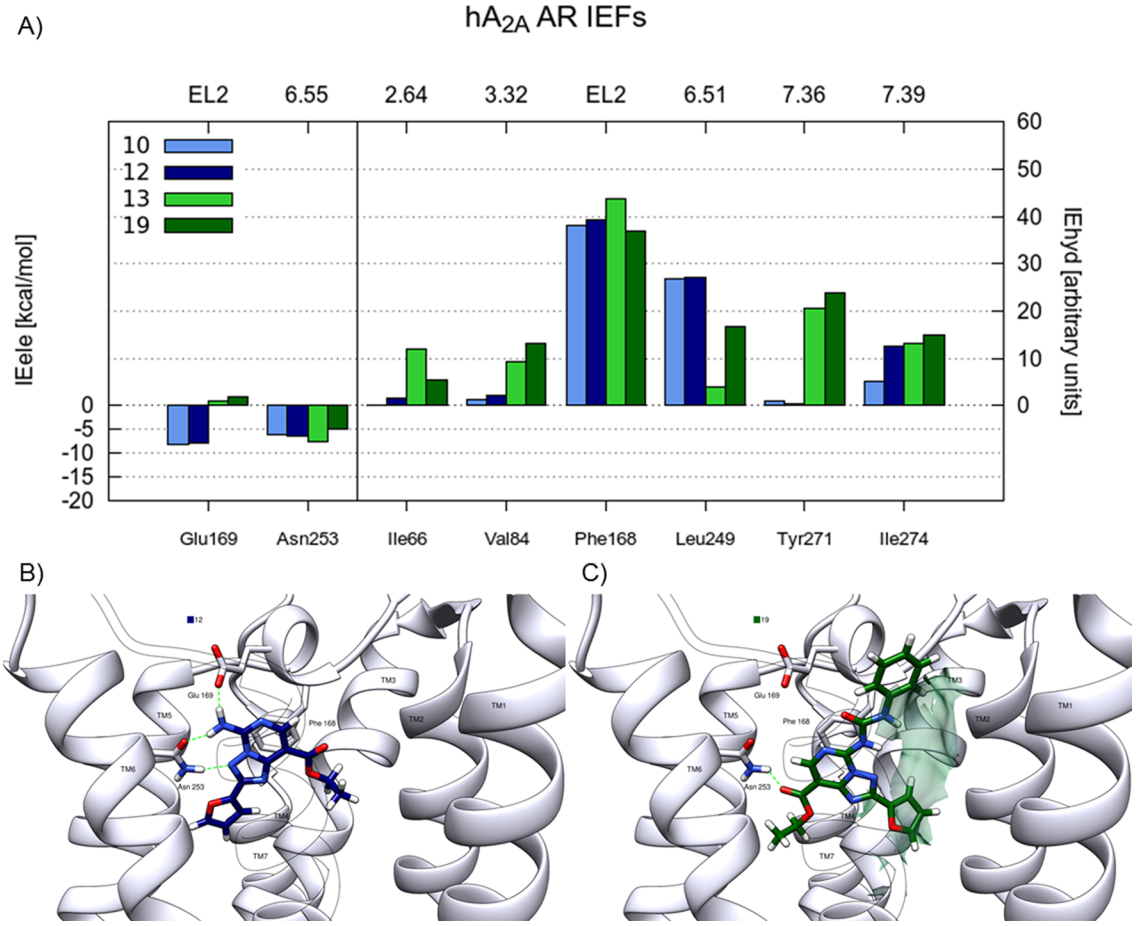

Figure 2. (A) Interaction Energy Fingerprints (IEFs) for the most active compound at the hA_{2A} AR (K_i < 15 nM). Hypothetical binding modes of compounds **12** (B) and **19** (C) inside the orthosteric binding site of hA_{2A} AR. Poses are viewed from the membrane side facing TM6, TM7, and TM1, hydrogen bonds are highlighted as dashed lines, and side chains of Asn253 (6.55), Phe168 (EL2), and Glu169 (EL2) are represented as sticks. In panel C, residues interacting through hydrophobic contacts with compound 19 are displayed as surfaces.

(TM) bundle, with the triazole-pyrimidine (TP) core anchored by an aromatic $\pi-\pi$ stacking interaction with Phe168 (EL2). Compound **12** (hA_{2A} AR K_i = 3.32 nM) is anchored in the cleft by three hydrogen bonds established by the free amino group at the 5 position and the N2 of the triazole ring. The ligand lies in the pocket with the furan ring pointing toward TM6, by adopting a conformation that resembles the crystallographic binding mode of ZM 241385. For compound **19** (hA_{2A} AR K_i = 4.82 nM) the hydrogen bond acceptor role is played by the ethoxycarbonyl group at the 8 position. Therefore, the scaffold is rotated by about 180° with respect to the placement of **12**, and the furan ring is directed toward TM7. In this position the furan ring establishes hydrophobic contacts with Val84 (3.32), whereas the aromatic ring of the arylcarbamoyl moiety interacts with Tyr271 (7.36) and Ile66 (2.64), represented as transparent surfaces in Figure 2C.

The IEFs analysis (Figure 3A) for the most active compounds at the hA_3 AR shows a common interaction pattern for all the considered derivatives, with the main interactions established with Asn250 (6.55) and Phe168 (EL2). A detailed analysis of the individual docking poses, however, reveals that the scaffold is placed at least into two different orientations, as reported in Figure 3B,C, representing the hypothetical binding mode of compounds **24** and **31**, respectively. Derivatives bearing small alkyl and cycloalkyl groups like compound **24** interact with Asn250 (6.55) through the ethoxycarbonyl group at the 8 position, by pointing the furane ring toward TM2 and exposing the alkyl group to the solvent. Conversely, compounds bearing branched chains and benzyl groups like compound **31** interact with Ans250 (6.55) with the N4 of the triazole ring, with the furane ring solvent-exposed and the alkyl/benzyl group projected toward TM2.

A similar trend (e.g., different binding modes depending on the nature and the steric hindrance of the substituents) was also previously observed for 5-alkylaminopyrazolo-[4,3-*e*]1,2,4-triazolo[1,5-*c*]pyrimidines derivatives.[38]

CONCLUSIONS

We have presented a novel series of 5,8-disubstituted-1,2,4-triazolo[1,5-*c*]pyrimidines acting as antagonists of the hA_{2A} and hA_3 ARs. We have analyzed the corresponding SAR and found that the synthesized compounds show affinity for the hA_{2A} and hA_3 ARs depending on the substitution patterns at the 5 and 8 positions. In particular, a free amino group at the 5 position combined with ethoxycarbonyl group at the 8 position affords a potent and quite selective hA_{2A} antagonists (**12**, hA_{2A} AR K_i = 3.32 nM), while the introduction of a methylamino function at the 5 position yields a good binding profile at the hA_3 AR (**23**, hA_3 AR K_i = 4.14 nM).

Using an in silico receptor-driven approach, we have determined the most favorable orientation of the substitutions at the 5 and 8 positions of the TP scaffold at both the

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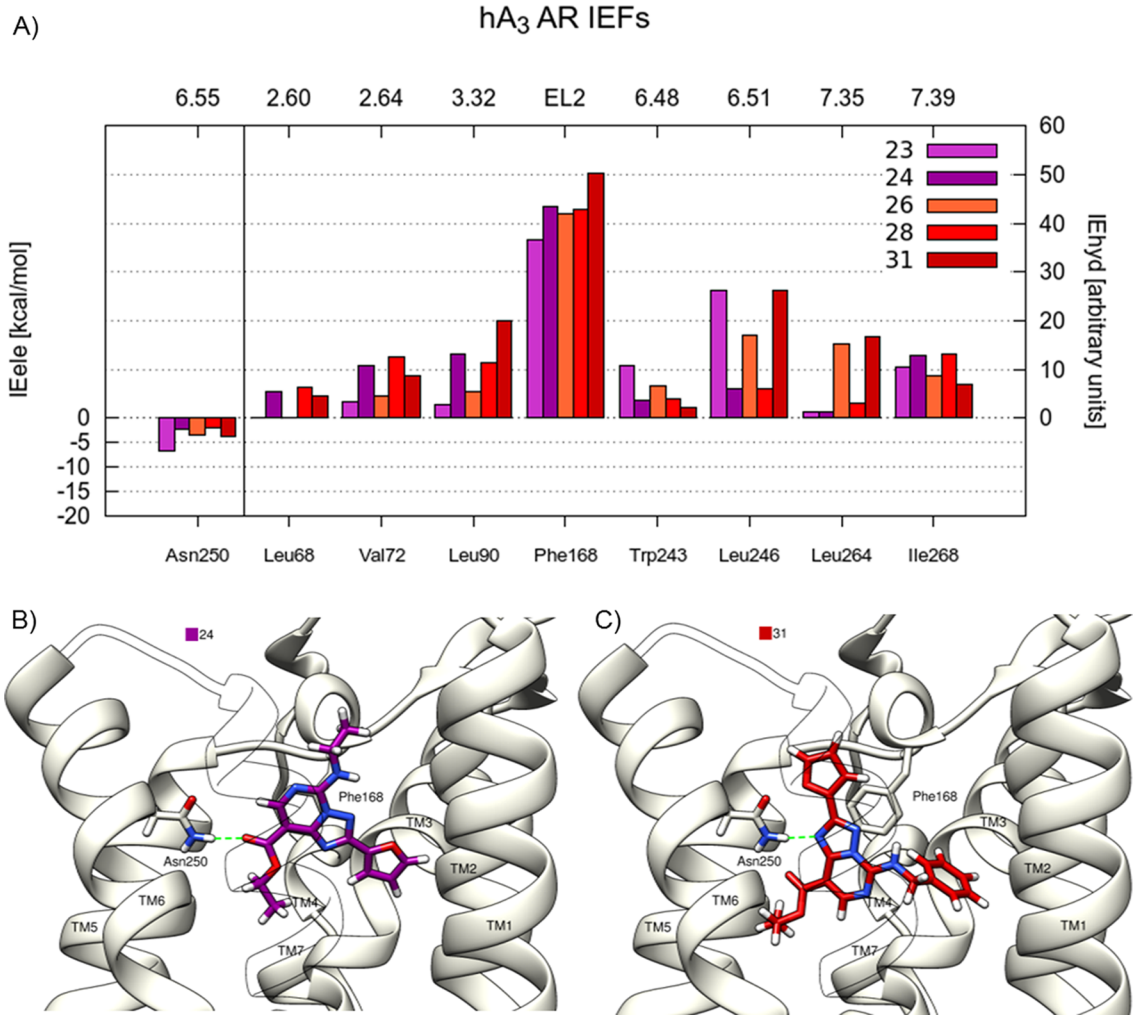

Figure 3. (A) Interaction Energy Fingerprints (IEFs) for the most active compound at the hA_3 AR (K_i < 10 nM). Hypothetical binding modes of compounds **24** (B) and **31** (C) inside the putative binding site of hA_3 AR. Poses are viewed from the membrane side facing TM6, TM7, and TM1, hydrogen bonds are highlighted as dashed lines, and side chains of Asn250 (6.55) and Phe168 (EL2) are represented as sticks.

considered receptor subtypes. At the hA_{2A} AR compounds bearing a free amino group at the 5 position are anchored inside the binding pocket by a tight network of hydrogen bonds, whereas derivatives bearing arylureido and phenylacetamido groups establish several hydrophobic contacts. At the hA_3 AR, instead, although the most active derivatives show a common interaction pattern, the corresponding docking poses suggest at least two different orientations of the TP scaffold into the binding cleft, depending on the steric hindrance of the substituent at the 5 position.

EXPERIMENTAL SECTION

A. Chemistry. Reactions were routinely monitored by thin-layer chromatography (TLC) on silica gel (precoated F_{254} Merck plates). Infrared spectra (IR) were measured on a Jasco FT-IT instrument. ^{1}H NMR were determined in $CDCl_3$ or DMSO-d_6 solutions with a Varian Gemini 200 spectrometer, peaks positions are given in parts per million (δ) downfield relative to the central peak of the solvents, and J values are given in Hz. Electrospray mass spectra were recorded on a PerkinElmer PE SCIEX API 1 spectrometer, and compounds were dissolved in methanol. Light petroleum ether refers to the fractions boiling at 40−60 °C. Melting points were determined on a Buchi−Tottoli instrument and are uncorrected. Flash chromatography was performed using Merck 60−200 mesh silica gel. Elemental analyses were performed by the microanalytical laboratory of Dipartimento di Chimica, University of Trieste, and were within ±0.4% of the theoretical values for C, H, and N. Complete elemental analyses are given in the Supporting Information, and the chemical formula is supplied in the section for each compound.

*4-Hydroxy-2-methylthio-5-carbethoxypyrimidine Sodium Salt (**69**).* To a solution of 34.4 g (0.860 mol) of sodium hydroxide in 215 mL of water at 20 °C were added 59.8 g of 2-methyl-2-thiopseudourea sulfate (**67**, 0.215 mol). The mixture was stirred for 5 min, by which time most of the pseudothiourea had dissolved. Diethyl ethoxymethylenemalonate (**68**, 0.430 mol, 92.9 g), dissolved in 500 mL of ethanol, was added with stirring over a period of 1 h. Stirring was continued for 3 h after the addition was completed. After it was allowed to stand for 24 h, the mixture was filtered to give a pink solid. Yield 60−70%; pale pink solid mp 310 °C. ^{1}H NMR (D_2O) δ: 1.2 (2H, t, J = 7); 2.29 (3H, s); 4.18 (2H, q, J = 7); 8.20 (1H, s); IR (Nujol) cm^{-1}: 3545−3090, 1720, 1610, 1500, 1430. Anal. ($C_8H_9N_2NaOS$) C, H, N.

*4-[N′-(Furan-2-carbonyl)-hydrazino]-2-methylsulfanyl-pyrimidine-5-carboxylic Acid Ethyl Ester (**71**).* To 9 g (38 mmol) of dried **69** was added 27 mL of phosphoryl oxychloride with a rate at which the temperature remained below 50 °C. After the addition was complete, the mixture was refluxed for 3 h. The solvent was then removed under reduced pressure. Ice and diethyl ether were added to the solid residue, and the mixture was shaken until all the solid had dissolved. The organic layer was washed with water, dried over sodium sulfate, and the solvent was removed by evaporation under reduced pressure to give the 4-chloro-2-methylthio-5-carbethoxypyrimidine

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(**70**), which was weighted and readily used in the next step without purification due to stability problems. The 6 g obtained of **70** (25.8 mmol) were dissolved in THF and 5.78 g of 2-furoylhydrazide (45.4 mmol) were added while 6.8 mL of DBU (45.4 mmol) were added dropwise. The mixture was stirred at room temperature for 5 h. The solvent was then removed, the residue was dissolved in dichloromethane, and the resulting solution was washed with water. The organic layer was concentrated, dried and purified by flash chromatography (diethyl ether 6: EtOAc 4). Yield 60−70%; yellow solid (EtOAc-light petroleum) mp 125 °C. ^{1}H NMR ($CDCl_3$) δ: 1.36 (2H, t, J = 7); 2.55 (3H, s); 4.40 (2H, q, J = 7); 6.51 (1H, dd, J = 2, J = 4); 7.20 (1H, d, J = 4); 7.50 (1H, d, J = 2); 8.66 (1H, s); 9.15 (1H, bs), 10.01 (1H, bs); IR (Nujol) cm^{-1}: 3345−3150, 1725, 1685, 1620, 1510, 1410. Anal. ($C_{13}H_{14}N_4O_4S$) C, H, N.

*Ethyl 2-(Furan-2-yl)-5-(methylthio)-[1,2,4]triazolo[1,5-c]-pyrimidine-8-carboxylate (**5**).* To 73 mL of dry xylene were added 2 g of P_2O_5 (14 mmol) and 6.59 mL of hexamethyldisiloxane (30 mmol), and the mixture was heated at 90 °C for 2 h. Three grams of dry compound **71** was then added, and the reaction was refluxed for 2 days. After that, the solvent was removed under reduced pressure, and the residue was dissolved in EtOAc and washed with water. The organic layer was then dried, concentrated and purified by flash chromatography using light petroleum 9: EtOAc 1. Yield 68%; white solid (EtOAc-light petroleum) mp 165 °C. ^{1}H NMR ($CDCl_3$) δ: 1.41 (2H, t, J = 7); 2.82 (3H, s); 4.48 (2H, q, J = 7); 6.61 (1H, dd, J = 2, J = 4); 7.40 (1H, d, J = 4); 7.62 (1H, d, J = 2); 8.89 (1H, s); IR (Nujol) cm^{-1}: 1720, 1620, 1515, 1425; Anal. ($C_{13}H_{12}N_4O_3S$) C, H, N.

*General Procedure for Nucleophilic Substitution with Amines (**6**, **23**−**50**).* To 100 mg (0.328 mmol) of compound **5** dissolved in ethanol, was added the appropriate amine (0.986 mmol). The mixture was heated in a sealed tube at 120 °C for 2 h. The solvent was then removed and the residue was purified by flash chromatography (Light petroleum-EtOAc 8:2 or 7:3).

*5-((3,4-Dimethoxybenzyl)amino)-2-(furan-2-yl)-[1,2,4]triazolo-[1,5-c]pyrimidine-8-carboxylic Acid Ethyl Ester (**6**).* Yield 72%; white solid (EtOAc-light petroleum) mp 140 °C; ^{1}H NMR ($CDCl_3$) δ: 1.34 (2H, t, J = 7); 3.71 (3H, s); 3.72 (3H, s); 4.31 (2H, q, J = 7); 4.67 (2H, s); 6.74 (1H, dd, J = 2, J = 4); 6.82−6.98 (2H, m); 7.07 (1H, s); 7.24 (1H, d, J = 4); 7.98 (1H, d, J = 2); 8.57 (1H, s); 9.54 (1H, bs); IR (Nujol) cm^{-1}: 3345−3170, 1720, 1615, 1520, 1430; Anal. ($C_{21}H_{21}N_5O_5$) C, H, N.

*2-Furan-2-yl-5-methylamino-[1,2,4]triazolo[1,5-c]pyrimidine-8-carboxylic Acid Ethyl Ester (**23**).* Yield 83%; pale yellow solid (EtOAc-light petroleum) mp 220 °C. ^{1}H NMR ($CDCl_3$) δ: 1.45 (3H, t, J = 7); 3.32 (3H, d, J = 4.4); 4.48 (2H, q, J = 7); 6.60 (1H, dd, J = 2, J = 4); 6.70 (1H, bs); 7.41 (1H, d, J = 4); 7.62 (1H, d, J = 2); 8.73 (1H, s); IR (Nujol) cm^{-1}: 3355−31650, 1720, 1615, 1510, 1420. Anal. ($C_{13}H_{13}N_5O_3$) C, H, N.

*5-Ethylamino-2-furan-2-yl-[1,2,4]triazolo[1,5-c]pyrimidine-8-carboxylic Acid Ethyl Ester (**24**).* Yield 78%; pale yellow solid (EtOAc-light petroleum) mp 157 °C. ^{1}H NMR ($CDCl_3$) δ: 1.37−1.48 (6H, m); 3.71−3.86 (2H, m); 4.48 (2H, q, J = 7); 6.60 (1H, dd, J = 2, J = 4); 6.68 (1H, bs); 7.40 (1H, d, J = 4); 7.63 (1H, d, J = 2); 8.71 (1H, s); IR (Nujol) cm^{-1}: 3340−3130, 1720, 1650, 1530, 1410. Anal. ($C_{14}H_{15}N_5O_3$) C, H, N.

*2-Furan-2-yl-5-pentylamino-[1,2,4]triazolo[1,5-c]pyrimidine-8-carboxylic Acid Ethyl Ester (**25**).* Yield 88%; pale yellow solid (EtOAc-light petroleum) mp 100 °C. ^{1}H NMR ($CDCl_3$) δ: 0.93−0.96 (3H, m); 1.41−1.48 (7H, m); 1.72−1.81 (2H, m); 3.67−3.82 (2H, m); 4.47 (2H, q, J = 7); 6.60−6.69 (2H, m); 7.41 (1H, d, J = 4) 7.63 (1H, d, J = 2); 8.71 (1H, s); IR (Nujol) cm^{-1}: 3355−3185, 1725, 1640, 1510, 1435. Anal. ($C_{17}H_{21}N_5O_3$) C, H, N.

*2-Furan-2-yl-5-isopropylamino-[1,2,4]triazolo[1,5-c]pyrimidine-8-carboxylic Acid Ethyl Ester (**26**).* Yield 54%; white solid (EtOAc-light petroleum) mp 125 °C. ^{1}H NMR ($CDCl_3$) δ: 1.41−1.47 (6H, m); 1.67−1.81 (3H, m); 3.65−3.69 (2H, m); 4.62 (2H, q, J = 7); 6.47−6.60 (2H, m); 7.41 (1H, d, J = 4) 7.63 (1H, d, J = 2); 8.70 (1H, s); IR (Nujol) cm^{-1}: 3330−3140, 1725, 1635, 1520, 1415; ES-MS (methanol) m/z: 316.2 (M+1). Anal. ($C_{15}H17N_5O_3$) C, H, N.

*5-(2-Ethyl-hexylamino)-2-furan-2-yl-[1,2,4]triazolo[1,5-c]-pyrimidine-8-carboxylic Acid Ethyl Ester (**27**).* Yield 89%; pale yellow solid (EtOAc-light petroleum) mp 100 °C. ^{1}H NMR ($CDCl_3$) δ: 0.95−0.99 (6H, m); 1.35−1.48 (11H, m); 1.67−1.78 (1H, m); 3.67 (2H, t, J = 6); 4.47 (2H, q, J = 7); 6.56−6.63 (2H, m); 7.38 (1H, d, J = 4) 7.62 (1H, d, J = 2); 8.70 (1H, s); IR (Nujol) cm^{-1}: 3345−3160, 1720, 1650, 1520, 1425; ES-MS (methanol) m/z: 408.2 (M + 23). Anal. ($C_{20}H_{27}N_5O_3$) C, H, N.

*5-Cyclopropylamino-2-furan-2-yl-[1,2,4]triazolo[1,5-c]-pyrimidine-8-carboxylic Acid Ethyl Ester (**28**).* Yield 75%; white solid (EtOAc-light petroleum) mp 137 °C. ^{1}H NMR ($CDCl_3$) δ: 0.83 (2H, bs); 1.05 (2H, d, J = 6); 1.44 (3H, t. J = 7); 3.11 (1H, bs); 4.46 (2H, q, J = 7); 6.59 (1H, dd, J = 2, J = 4); 6.84 (1H, bs); 7.40 (1H, d, J = 4) 7.62 (1H, d, J = 2); 8.77 (1H, s); IR (Nujol) cm^{-1}: 3355−3180, 1715, 1630, 1510, 1410. Anal. ($C_{15}H_{15}N_5O_3$) C, H, N.

*5-Cyclopentylamino-2-furan-2-yl-[1,2,4]triazolo[1,5-c]-pyrimidine-8-carboxylic Acid Ethyl Ester (**29**).* Yield 64%; white solid (EtOAc-light petroleum) mp 127 °C. ^{1}H NMR ($CDCl_3$) δ: 1.44 (3H, t. J = 7); 1.64−1.85 (6H, m); 2.20 (2H, bs); 4.47 (2H, q, J = 7); 4.6 (1H, bs); 6.59 (2H, bs); 7.41 (1H, d, J = 4); 7.63 (1H, d, J = 2); 8.71 (1H, s); IR (Nujol) cm^{-1}: 3340−3165, 1720, 1630, 1515, 1420. Anal. ($C_{17}H_{19}N_5O_3$) C, H, N.

*5-(Cyclohexylmethylamino)-2-furan-2-yl-[1,2,4]triazolo[1,5-c]-pyrimidine-8-carboxylic Acid Ethyl Ester (**30**).* Yield 88%; white solid (EtOAc-light petroleum) mp 127 °C. ^{1}H NMR ($CDCl_3$) δ: 1.02−1.33 (4H,m); 1.44 (3H, t. J = 7); 1.66−1.87 (7H, m); 3.59 (2H, t, J = 6); 4.47 (2H, q, J = 7); 6.60 (1H, dd, J = 2, J = 4); 6.73 (1H, bs); 7.41 (1H, d, J = 4); 7.63 (1H, d, J = 2); 8.70 (1H, s); IR (Nujol) cm^{-1}: 3350−3180, 1715, 1640, 1520, 1430. Anal. ($C_{19}H_{23}N_5O_3$) C, H, N.

*5-Benzylamino-2-furan-2-yl-[1,2,4]triazolo[1,5-c]pyrimidine-8-carboxylic Acid Ethyl Ester (**31**).* Yield 76%; yellow solid (EtOAc-light petroleum) mp 130 °C. ^{1}H NMR ($CDCl_3$) δ: 1.45 (3H, t. J = 7); 4.48 (2H, q, J = 7); 4.92 (2H, d, J = 5); 6.59 (1H, dd, J = 2, J = 4); 6.97 (1H, bs); 7.33−7.49 (6H, m); 7.60 (1H, d, J = 2); 8.74 (1H, s); IR (Nujol) cm^{-1}: 3345−3185, 1725, 1650, 1540, 1430; ES-MS (methanol) m/z: 364.1 (M + 1); 386.1 (M + 23). Anal. ($C_{19}H_{17}N_5O_3$) C, H, N.

*2-Furan-2-yl-5-(4-methyl-benzylamino)-[1,2,4]triazolo[1,5-c]-pyrimidine-8-carboxylic Acid Ethyl Ester (**32**).* Yield 85%; pale yellow solid (EtOAc-light petroleum) mp 104 °C. ^{1}H NMR ($CDCl_3$) δ: 1.45 (3H, t, J = 7); 2.35 (3H, s); 4.48 (2H, q, J = 7); 4.87 (2H, d, J = 5); 6.58 (1H, dd, J = 2, J = 4); 6.93 (1H, bs); 7.24 (4H, dd, J = 8, J = 24); 7.38 (1H, d, J = 4); 7.60 (1H, d, J = 2); 8.74 (1H, s); IR (Nujol) cm^{-1}: 3340−3230, 1725, 1645, 1520, 1415. Anal. ($C_{20}H_{19}N_5O_3$) C, H, N.

*2-Furan-2-yl-5-(4-methoxy-benzylamino)-[1,2,4]triazolo[1,5-c]-pyrimidine-8-carboxylic Acid Ethyl Ester (**33**).* Yield 76%; brown-yellow solid (EtOAc-light petroleum) mp 149 °C. ^{1}H NMR ($CDCl_3$) δ: 1.44 (3H, t. J = 7); 3.81 (3H, s); 4.48 (2H, q, J = 7); 4.84 (2H, d, J = 5); 6.57 (1H, dd, J = 2, J = 4); 6.88−6.92 (3H, m); 7.32−7.36 (3H, m); 7.60 (1H, d, J = 2); 8.74 (1H, s); IR (Nujol) cm^{-1}: 3335−3180, 1715, 1650, 1525, 1420. Anal. ($C_{20}H_{19}N_5O_4$) C, H, N.

*5-(4-Fluoro-benzylamino)-2-furan-2-yl-[1,2,4]triazolo[1,5-c]-pyrimidine-8-carboxylic Acid Ethyl Ester (**34**).* Yield 84%; white solid (EtOAc-light petroleum) mp 150 °C. ^{1}H NMR ($CDCl_3$) δ: 1.45 (3H, t, J = 7); 4.48 (2H, q, J = 7); 4.88 (2H, d, J = 6); 6.59 (1H, dd, J = 2, J = 4); 6.88 (1H, bs); 7.00−7.10 (2H, m); 7.37−7.43 (3H, m); 7.60 (1H, d, J = 2); 8.73 (1H, s); IR (Nujol) cm^{-1}: 3345−3255, 1720, 1645, 1530, 1415; ES-MS (methanol) m/z: 404.1 (M + 23). Anal. ($C_{19}H_{16}FN_5O_3$) C, H, N.

*5-(4-Chloro-benzylamino)-2-furan-2-yl-[1,2,4]triazolo[1,5-c]-pyrimidine-8-carboxylic Acid Ethyl Ester (**35**).* Yield 73%; yellow solid (EtOAc-light petroleum) mp 135 °C. ^{1}H NMR ($CDCl_3$) δ: 1.45 (3H, t, J = 7); 4.49 (2H, q, J = 7); 4.88 (2H, d, J = 6); 6.59 (1H, dd, J = 2, J = 4); 6.96 (1H, bs); 7.30−7.35 (5H, m); 7.37−7.43 (3H, m); 7.60 (1H, d, J = 2); 8.72 (1H, s); IR (Nujol) cm^{-1}: 3350−3195, 1725, 1645, 1540, 1420; ES-MS (methanol) m/z: 398.1 (M + 1); 420.1 (M + 23). Anal. ($C_{19}H_{16}ClN_5O_3$) C, H, N.

*2-Furan-2-yl-5-(4-trifluoromethyl-benzylamino)-[1,2,4]triazolo-[1,5-c]pyrimidine-8-carboxylic Acid Ethyl Ester (**36**).* Yield 85%; white solid (EtOAc-light petroleum) mp 163 °C. ^{1}H NMR ($CDCl_3$) δ: 1.44 (3H, t, J = 7); 4.48 (2H, q, J = 7); 4.94 (2H, d, J = 6); 6.59 (1H, dd, J = 2, J = 4); 7.02 (1H, bs); 7.39−7.66 (6H, m); 8.74 (1H, s); IR

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(Nujol) cm^{-1}: 3325−3150, 1715, 1645, 1530, 1425; ES-MS (methanol) *m/z*: 454.1 (M + 23). Anal. ($C_{20}H_{16}F_3N_5O_3$) C, H, N.

*5-[(Biphenyl-4-ylmethyl)-amino]-2-furan-2-yl-[1,2,4]triazolo[1,5-c]pyrimidine-8-carboxylic Acid Ethyl Ester (**37**).* Yield 87%; orange solid (EtOAc-light petroleum) mp 110 °C. ^1H NMR ($CDCl_3$) δ: 1.45 (3H, t, J = 7); 4.48 (2H, q, J = 7); 4.95 (2H, d, J = 6); 6.59 (1H, dd, J = 2, J = 4); 6.99 (1H, bs); 7.37−7.62 (11H, m); 8.75 (1H, s); IR (Nujol) cm^{-1}: 3330−3190, 1720, 1655, 1550, 1420. Anal. ($C_{25}H_{21}N_5O_3$) C, H, N.

*5-(Benzhydryl-amino)-2-furan-2-yl-[1,2,4]triazolo[1,5-c]-pyrimidine-8-carboxylic Acid Ethyl Ester (**38**).* Yield 71%; pale yellow solid (EtOAc-light petroleum) mp 160 °C. ^1H NMR ($CDCl_3$) δ: 1.42 (3H, t, J = 7); 4.45 (2H, q, J = 7); 6.59 (1H, dd, J = 2, J = 4); 6.71 (1H, d, J = 6); 7.23 (1H, bs); 7.28−7.48 (11H, m); 7.61 (1H, d, J = 2); 8.67 (1H, s); IR (Nujol) cm^{-1}: 3335−3200, 1715, 1650, 1540, 1430. Anal. ($C_{25}H_{21}N_5O_3$) C, H, N.

*5-Cyclobutylamino-2-furan-2-yl-[1,2,4]triazolo[1,5-c]pyrimidine-8-carboxylic Acid Ethyl Ester (**39**).* Yield 76%; yellow solid (EtOAc-light petroleum) mp 116 °C. ^1H NMR ($CDCl_3$) δ: 1.44 (3H, t, J = 7); 1.85−1.88 (2H, m); 2.04−2.28 (2H, m); 2.51 (2H, bs); 4.46 (2H, q, J = 7); 4.72−4.90 (1H, m); 6.60 (1H, dd, J = 2, J = 4); 6.80 (1H, bs); 7.39 (1H, d, J = 4); 7.63 (1H, d, J = 2); 8.68 (1H, s); IR (Nujol) cm^{-1}: 3350−3220, 1725, 1630, 1520, 1430. Anal. ($C_{16}H_{17}N_5O_3$) C, H, N.

*5-Cycloheptylamino-2-furan-2-yl-[1,2,4]triazolo[1,5-c]-pyrimidine-8-carboxylic Acid Ethyl Ester (**40**).* Yield 86%; pale brown solid (EtOAc-light petroleum) mp 123 °C. ^1H NMR ($CDCl_3$) δ: 1.43 (3H, t, J = 7); 1.54−1.80 (10H, m); 2.12 (2H, bs); 4.36−4.59 (3H, m); 6.56−6.66 (2H, m); 7.38 (1H, d, J = 4); 7.62 (1H, d, J = 2); 8.70 (1H, s); IR (Nujol) cm^{-1}: 3335−3180, 1720, 1625, 1515, 1410. Anal. ($C_{19}H_{23}N_5O_3$) C, H, N.

*5-Cyclooctylamino-2-furan-2-yl-[1,2,4]triazolo[1,5-c]pyrimidine-8-carboxylic Acid Ethyl Ester (**41**).* Yield 95%; pale yellow solid (EtOAc-light petroleum) mp 106 °C. ^1H NMR ($CDCl_3$) δ: 1.43 (3H, t, J = 7); 1.63−1.84 (12H, m); 1.98−2.10 (2H, m); 4.41−4.51 (3H, m); 6.56−6.59 (2H, m); 7.37 (1H, d, J = 4); 7.62 (1H, d, J = 2); 8.70 (1H, s); IR (Nujol) cm^{-1}: 3345−3210, 1715, 1625, 1515, 1420. Anal. ($C_{20}H_{25}N_5O_3$) C, H, N.

*2-Furan-2-yl-5-pyrrolidin-1-yl-[1,2,4]triazolo[1,5-c]pyrimidine-8-carboxylic Acid Ethyl Ester (**42**).* Yield 67%; white solid (EtOAc-light petroleum) mp 170 °C. ^1H NMR ($CDCl_3$) δ: 1.43 (3H, t, J = 7); 2.07 (4H, bs); 4.10 (4H, bs); 4.44 (2H, q, J = 7); 6.59 (1H, dd, J = 2, J = 4); 7.29 (1H, d, J = 4); 7.60 (1H, d, J = 2); 8.61 (1H, s); IR (Nujol) cm^{-1}: 1725, 1630, 1520, 1430. Anal. ($C_{16}H_{17}N_5O_3$) C, H, N.

*2-Furan-2-yl-5-piperidin-1-yl-[1,2,4]triazolo[1,5-c]pyrimidine-8-carboxylic Acid Ethyl Ester (**43**).* Yield 77%; pale yellow solid (EtOAc-light petroleum) mp 125 °C. ^1H NMR ($CDCl_3$) δ: 1.43 (3H, t, J = 7); 1.79 (6H, bs); 4.31 (4H, bs); 4.46 (2H, q, J = 7); 6.58 (1H, dd, J = 2, J = 4); 7.32 (1H, d, J = 4); 7.61 (1H, d, J = 2); 8.64 (1H, s); IR (Nujol) cm^{-1}: 1720, 1625, 1515, 1420. Anal. ($C_{17}H_{19}N_5O_3$) C, H, N.

*2-Furan-2-yl-5-(2-methoxy-ethylamino)-[1,2,4]triazolo[1,5-c]-pyrimidine-8-carboxylic Acid Ethyl Ester (**44**).* Yield 92%; white solid (EtOAc-light petroleum) mp 119 °C. ^1H NMR ($CDCl_3$) δ: 1.44 (3H, t, J = 7); 3.42 (3H, s); 3.67 (2H, t, J = 5); 3.93 (2H, q, J = 5); 4.47 (2H, q, J = 7); 6.59 (1H, dd, J = 2, J = 4); 6.95 (1H, bs); 7.37 (1H, d, J = 4); 7.63 (1H, d, J = 2); 8.69 (1H, s); IR (Nujol) cm^{-1}: 3340−3250, 1715, 1640, 1530, 1425. Anal. ($C_{15}H_{17}N_5O_4$) C, H, N.

*5-Dimethylamino-2-furan-2-yl-[1,2,4]triazolo[1,5-c]pyrimidine-8-carboxylic Acid Ethyl Ester (**45**).* Yield 88%; pale yellow solid (EtOAc-light petroleum) mp 139 °C. ^1H NMR ($CDCl_3$) δ: 1.43 (3H, t, J = 7); 3.67 (6H, s); 4.45 (2H, q, J = 7); 6.57 (1H, dd, J = 2, J = 4); 7.31 (1H, d, J = 4); 7.61 (1H, d, J = 2); 8.63 (1H, s); IR (Nujol) cm^{-1}: 1725, 1620, 1530, 1420. Anal. ($C_{14}H_{15}N_5O_3$) C, H, N.

*2-Furan-2-yl-5-morpholin-4-yl-[1,2,4]triazolo[1,5-c]pyrimidine-8-carboxylic Acid Ethyl Ester (**46**).* Yield 82%; white solid (EtOAc-light petroleum) mp 164 °C. ^1H NMR ($CDCl_3$) δ: 1.44 (3H, t, J = 7); 3.94 (4H, bs); 4.38−4.56 (6H, m); 6.59 (1H, dd, J = 2, J = 4); 7.35 (1H, d, J = 4); 7.62 (1H, d, J = 2); 8.65 (1H, s); IR (Nujol) cm^{-1}: 1715, 1630, 1515, 1420. Anal. ($C_{16}H_{17}N_5O_4$) C, H, N.

*5-Cyclohexylamino-2-furan-2-yl-[1,2,4]triazolo[1,5-c]pyrimidine-8-carboxylic Acid Ethyl Ester (**47**).* Yield 86%; pale brown solid (EtOAc-light petroleum) mp 123 °C. ^1H NMR ($CDCl_3$) δ: 1.37−1.51 (7H, m); 1.69−1.88 (4H, m); 2.12−2.17 (2H, m); 4.08−4.18 (1H, m); 4.46 (2H, q, J = 7); 6.52 (1H, d, J = 8); 6.59 (2H, m); 7.38 (1H, d, J = 4); 7.62 (1H, d, J = 2); 8.69 (1H, s); IR (Nujol) cm^{-1}: 3335−3190, 1720, 1625, 1510, 1415. Anal. ($C_{18}H_{21}N_5O_3$) C, H, N.

*2-Furan-2-yl-5-(morpholin-4-ylamino)-[1,2,4]triazolo[1,5-c]-pyrimidine-8-carboxylic Acid Ethyl Ester (**48**).* Yield 75%; white solid (EtOAc-light petroleum) mp 165 °C. ^1H NMR ($CDCl_3$) δ: 1.44 (3H, t, J = 7); 3.91 (4H, t, J = 7); 4.38−4.52 (6H, m); 6.57 (1H, dd, J = 2, J = 4); 7.35 (1H, d, J = 4); 7.61 (1H, d, J = 2); 8.64 (1H, s); IR (Nujol) cm^{-1}: 3355−3230, 1715, 1640, 1520, 1420. Anal. ($C_{16}H_{18}N_6O_4$) C, H, N.

*2-Furan-2-yl-5-isobutylamino-[1,2,4]triazolo[1,5-c]pyrimidine-8-carboxylic Acid Ethyl Ester (**49**).* Yield 56%; sticky foam; ^1H NMR ($CDCl_3$) δ: 0.98 (3H, t, J = 7); 1.32−1.44 (6H, m); 1.70 (2H, m); 4.28−4.45 (3H, m); 6.56 (1H, dd, J = 2, J = 4); 7.35 (1H, d, J = 4); 7.60 (1H, d, J = 2); 8.67 (1H, s); IR (Nujol) cm^{-1}: 3350−3200, 1715, 1620, 1515, 1420. Anal. ($C_{16}H_{19}N_5O_3$) C, H, N.

*2-Furan-2-yl-5-methoxyamino-[1,2,4]triazolo[1,5-c]pyrimidine-8-carboxylic Acid Ethyl Ester (**50**).* Yield 83%; yellow solid (EtOAc-light petroleum) mp 220 °C. ^1H NMR ($CDCl_3$) δ: 1.42 (3H, t, J = 7); 4.02 (3H, s); 4.44 (2H, q, J = 7); 6.55 (1H, dd, J = 2, J = 4); 7.22−7.24 (2H, m); 7.58 (1H, d, J = 2); 8.21 (1H, s); IR (Nujol) cm^{-1}: 3325−3150, 1725, 1620, 1540, 1410; ES-MS (methanol) *m/z*: 326.1 (M + 23). Anal. ($C_{13}H_{13}N_5O_4$) C, H, N.

*General Procedure for the Hydrolysis of Ethyl Ester Compounds (**7**, **22**, **51**−**66**).* To 0.167 mmol of ethyl ester compound (**6**, **20**, **23**−**38**) dissolved in ethanol were added 1.67 mmol of $LiOH·H_2O$ and 1.5 mmol of water. The mixture was refluxed and stirred for 2 h. Then, a small amount of water was added, followed by addition of HCl to make pH 3. The carboxylic acid derivative precipitate and the solid was filtered off.

*5-((3,4-Dimethoxybenzyl)amino)-2-(furan-2-yl)-[1,2,4]triazolo-[1,5-c]pyrimidine-8-carboxylic Acid (**7**).* Yield 78%; white solid (EtOAc-light petroleum) mp 135 °C. ^1H NMR ($CDCl_3$) δ: 3.70 (3H, s); 3.72 (3H, s); 4.68 (2H, d, J = 6); 6.74 (1H, dd, J = 2, J = 4); 6.88−6.98 (2H, m); 7.07 (1H, s); 7.34 (1H, d, J = 4); 7.97 (1H, d, J = 2); 8.49 (1H, s); 9.31 (1H, bs); 13.03 (1H, bs); IR (Nujol) cm^{-1}: 3545−3130, 1700, 1650, 1540, 1420. Anal. ($C_{19}H_{17}N_5O_5$) C, H, N.

*5-(3-Cyclohexylureido)-2-(furan-2-yl)-[1,2,4]triazolo[1,5-c]-pyrimidine-8-carboxylic Acid (**22**).* Yield 91%; pale yellow solid (EtOAc-light petroleum) mp 260 °C. ^1H NMR (d_6-DMSO) δ: 1.13−1.38 (6H, m); 1.52−1.81 (4H, m); 3.73−3.79 (1H, m); 6.64 (1H, dd, J = 2, J = 4); 7.41 (1H, d, J = 4); 7.66 (1H, d, J = 2); 8.50 (1H, bs); 8.78 (1H, s); 8.83 (1H, bs); 13.02 (1H, bs); IR (Nujol) cm^{-1}: 3545−3090, 1700, 1685, 1630, 1540, 1430. Anal. ($C_{17}H_{18}N_6O_4$) C, H, N.

*2-Furan-2-yl-5-methylamino-[1,2,4]triazolo[1,5-c]pyrimidine-8-carboxylic Acid (**51**).* Yield 70%; pale yellow solid (EtOAc-light petroleum) mp 250 °C. ^1H NMR ($CDCl_3$) δ: 3.34 (3H, d, J = 4.8); 6.64−6.51 (2H, m); 7.30 (1H, d, J = 4); 7.66 (1H, d, J = 2); 8.80 (1H, s); IR (Nujol) cm^{-1}: 3555−3100, 1705, 1630, 1515, 1420. Anal. ($C_{11}H_9N_5O_3$) C, H, N.

*5-Ethylamino-2-furan-2-yl-[1,2,4]triazolo[1,5-c]pyrimidine-8-carboxylic Acid (**52**).* Yield 76%; pale yellow solid (EtOAc-light petroleum) mp 240 °C. ^1H NMR ($CDCl_3$) δ: 1.41 (3H, t, J = 7); 3.75−3.88 (2H, m); 6.64 (2H, bs); 7.31 (1H, d, J = 4); 7.66 (1H, d, J = 2); 8.79 (1H, s); IR (Nujol) cm^{-1}: 3560−3120, 1700, 1625, 1515, 1430; ES-MS (methanol) *m/z*: 272.1 (M-1). Anal. ($C_{12}H_{11}N_5O_3$) C, H, N.

*2-Furan-2-yl-5-pentylamino-[1,2,4]triazolo[1,5-c]pyrimidine-8-carboxylic Acid (**53**).* Yield 60%; pale orange solid (EtOAc-light petroleum) mp 140 °C. ^1H NMR ($CDCl_3$) δ: 0.93−0.96 (3H, m); 1.34−1.51 (4H, m); 1.65−1.88 (2H, m); 3.74−3.77 (2H, m); 6.63 (2H, bs); 7.30 (1H, d, J = 4); 7.66 (1H, d, J = 2); 8.78 (1H, s); IR (Nujol) cm^{-1}: 3550−3070, 1695, 1635, 1525, 1400. Anal. ($C_{15}H_{17}N_5O_3$) C, H, N.

*2-Furan-2-yl-5-isopropylamino-[1,2,4]triazolo[1,5-c]pyrimidine-8-carboxylic Acid (**54**).* Yield 60%; pale yellow solid (EtOAc-light petroleum) mp 210 °C. ^1H NMR ($CDCl_3$) δ: 1.42 (3H, d, J = 6.6); 4.49−4.64 (1H, m); 6.48 (1H, d, J = 6.6); 6.64 (1H, dd, J = 2, J = 4); 7.30 (1H, d, J = 4); 7.67 (1H, d, J = 2); 8.78 (1H, s); IR (Nujol) cm^{-1}:

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3545−3050, 1705, 1640, 1515, 1420; ES-MS (methanol) *m/z*: 286.1 (M-1). Anal. ($C_{13}H_{13}N_5O_3$) C, H, N.

*5-(2-Ethyl-hexylamino)-2-furan-2-yl-[1,2,4]triazolo[1,5-c]-pyrimidine-8-carboxylic Acid (**55**).* Yield 76%; pale yellow solid (EtOAc-light petroleum) mp 100 °C. ^{1}H NMR ($CDCl_3$) δ: 0.66−1.11 (6H, m); 1.14−1.57 (8H, m); 1.65−1.82 (1H, m); 3.70 (2H, t, J = 6); 6.60 (2H, m); 7.28 (1H, d, J = 4); 7.65 (1H, d, J = 2); 8.77 (1H, s); IR (Nujol) cm^{-1}: 3530−3030, 1700, 1625, 1530, 1420; ES-MS (methanol) *m/z*: 304.3 (M-23). Anal. ($C_{18}H_{23}N_5O_3$) C, H, N.

*5-Cyclopropylamino-2-furan-2-yl-[1,2,4]triazolo[1,5-c]-pyrimidine-8-carboxylic Acid (**56**).* Yield 81%; white solid (EtOAc-light petroleum) mp 135 °C. ^{1}H NMR ($CDCl_3$) δ: 0.84 (2H, bs); 1.06 (2H, d, J = 6); 3.13 (1H, bs); 6.63 (1H, dd, J = 2, J = 4); 6.78 (1H, bs) 7.27 (1H, d, J = 4); 7.66 (1H, d, J = 2); 8.85 (1H, s); IR (Nujol) cm^{-1}: 3545−3080, 1705, 1620, 1530, 1415. Anal. ($C_{13}H_{11}N_5O_3$) C, H, N.

*5-Cyclopentylamino-2-furan-2-yl-[1,2,4]triazolo[1,5-c]-pyrimidine-8-carboxylic Acid (**57**).* Yield 77%; white solid (EtOAc-light petroleum) mp 250 °C. ^{1}H NMR ($CDCl_3$) δ: 1.60−1.91 (6H, m); 2.21 (2H, bs); 4.65 (1H, bs); 6.50−6.63 (2H, m); 7.31 (1H, d, J = 4); 7.66 (1H, d, J = 2); 8.78 (1H, s); ES-MS (methanol) *m/z*: 312.1 (M-1); IR (Nujol) cm^{-1}: 3560−3090, 1700, 1625, 1530, 1420. Anal. ($C_{15}H_{15}N_5O_3$) C, H, N.

*5-(Cyclohexylmethylamino)-2-furan-2-yl-[1,2,4]triazolo[1,5-c]-pyrimidine-8-carboxylic Acid (**58**).* Yield 70%; white solid (EtOAc-light petroleum) mp 90 °C. ^{1}H NMR ($CDCl_3$) δ: 1.02−1.20 (4H, m); 1.43−1.87 (7H, m); 3.61 (2H, t, J = 6); 6.63 (1H, dd, J = 2, J = 4); 6.70 (1H, bs); 7.30 (1H, d, J = 4); 7.66 (1H, d, J = 2); 8.77 (1H, s); IR (Nujol) cm^{-1}: 3550−3070, 1700, 1640, 1530, 1415. Anal. ($C_{17}H_{19}N_5O_3$) C, H, N.

*5-Benzylamino-2-furan-2-yl-[1,2,4]triazolo[1,5-c]pyrimidine-8-carboxylic Acid (**59**).* Yield 83%; pale yellow solid (EtOAc-light petroleum) mp 205 °C. ^{1}H NMR ($CDCl_3$) δ: 4.94 (2H, d, J = 5); 6.61 (1H, dd, J = 2, J = 4); 6.95 (1H, bs); 7.29−7.40 (6H, m); 7.63 (1H, d, J = 2); 8.82 (1H, s); IR (Nujol) cm^{-1}: 3610−3100, 1705, 1650, 1540, 1420. Anal. ($C_{17}H_{13}N_5O_3$) C, H, N.

*2-Furan-2-yl-5-(4-methyl-benzylamino)-[1,2,4]triazolo[1,5-c]-pyrimidine-8-carboxylic Acid (**60**).* Yield 92%; pale yellow solid (EtOAc-light petroleum) mp 105 °C. ^{1}H NMR ($CDCl_3$) δ: 2.36 (3H, s); 4.88 (2H, bs); 6.61 (1H, dd, J = 2, J = 4); 6.95 (1H, bs); 7.17−7.38 (5H, m); 7.64 (1H, d, J = 2); 8.81 (1H, s); IR (Nujol) cm^{-1}: 3580−3070, 1700, 1655, 1535, 1425; ES-MS (methanol) *m/z*: 350.1 (M+1). Anal. ($C_{18}H_{15}N_5O_3$) C, H, N.

*2-Furan-2-yl-5-(4-methoxy-benzylamino)-[1,2,4]triazolo[1,5-c]-pyrimidine-8-carboxylic Acid (**61**).* Yield 88%; pale brown solid (EtOAc-light petroleum) mp 80 °C. ^{1}H NMR ($CDCl_3$) δ: 3.81 (3H, s); 5.01 (2H, d, J = 5); 6.61 (1H, dd, J = 2, J = 4); 6.89−6.93 (3H, m); 7.27−7.36 (3H, m); 7.62 (1H, d, J = 2); 8.82 (1H, s); IR (Nujol) cm^{-1}: 3575−3060, 1705, 1645, 1545, 1430. Anal. ($C_{18}H_{15}N_5O_4$) C, H, N.

*5-(4-Fluoro-benzylamino)-2-furan-2-yl-[1,2,4]triazolo[1,5-c]-pyrimidine-8-carboxylic Acid (**62**).* Yield 80%; pale yellow solid (EtOAc-light petroleum) mp 120 °C. ^{1}H NMR ($CDCl_3$) δ: 4.91 (2H, d, J = 6); 6.63 (1H, dd, J = 2, J = 4); 6.93 (1H, bs); 7.08 (2H, t, J = 8); 7.29 (1H, d, J = 4); 7.37−7.43 (2H, m); 7.64 (1H, d, J = 2); 8.82 (1H, s); IR (Nujol) cm^{-1}: 3560−3070, 1700, 1640, 1530, 1420; ES-MS (methanol) *m/z*: 352.1 (M-1). Anal. ($C_{17}H_{12}FN_5O_3$) C, H, N.

*5-(4-Chloro-benzylamino)-2-furan-2-yl-[1,2,4]triazolo[1,5-c]-pyrimidine-8-carboxylic Acid (**63**).* Yield 92%; pale yellow solid (EtOAc-light petroleum) mp 280 °C. ^{1}H NMR (d_6-DMSO) δ: 4.74 (2H, d, J = 6); 6.55 (1H, dd, J = 2, J = 4); 7.20 (2H, d, J = 8); 7.35 (2H, d, J = 8); 7.46 (1H, d, J = 4); 7.59 (1H, d, J = 2); 8.61 (1H, s); 8.65 (1H, bs); IR (Nujol) cm^{-1}: 3585−3100, 1700, 1645, 1540, 1435; ES-MS (methanol) *m/z*: 368.0 (M-1). Anal. ($C_{17}H_{12}ClN_5O_3$) C, H, N.

*2-Furan-2-yl-5-(4-trifluoromethyl-benzylamino)-[1,2,4]triazolo-[1,5-c]pyrimidine-8-carboxylic Acid (**64**).* Yield 80%; white solid (EtOAc-light petroleum) mp 250 °C. ^{1}H NMR ($CDCl_3$) δ: 5.02 (2H, d, J = 6); 6.63 (1H, dd, J = 2, J = 4); 7.02 (1H, bs); 7.28 (1H, d, J = 4); 7.52−7.66 (5H, m); 8.80 (1H, s); IR (Nujol) cm^{-1}: 3555−3090, 1695, 1650, 1525, 1430. Anal. ($C_{18}H_{12}F_3N_5O_3$) C, H, N.

*5-[(Biphenyl-4-yl-methyl)-amino]-2-furan-2-yl-[1,2,4]triazolo[1,5-c]pyrimidine-8-carboxylic Acid (**65**).* Yield 94%; pale orange solid (EtOAc-light petroleum) mp 300 °C. ^{1}H NMR ($CDCl_3$) δ: 4.98 (2H, d, J = 6); 6.61 (1H, dd, J = 2, J = 4); 6.97 (1H, bs); 7.29−7.63 (11H, m); 8.84 (1H, s); IR (Nujol) cm^{-1}: 3580−3090, 1700, 1655, 1550, 1420. Anal. ($C_{23}H_{17}N_5O_3$) C, H, N.

*5-(Benzhydryl-amino)-2-furan-2-yl-[1,2,4]triazolo[1,5-c]-pyrimidine-8-carboxylic Acid (**66**).* Yield 88%; pale yellow solid (EtOAc-light petroleum) mp 150 °C. ^{1}H NMR ($CDCl_3$) δ: 6.62 (1H, dd, J = 2, J = 4); 6.72 (1H, d, J = 6); 7.19 (1H, bs); 7.30−7.43 (11H, m); 7.65 (1H, d, J = 2); 8.75 (1H, s); IR (Nujol) cm^{-1}: 3610−3100, 1705, 1650, 1540, 1430. Anal. ($C_{23}H_{17}N_5O_3$) C, H, N.

*General Procedure for the Synthesis of 5-((3,4-Dimethoxybenzyl)amino)-2-(furan-2-yl)-N-substituted-[1,2,4]-triazolo[1,5-c]pyrimidine-8-carboxamides (**8, 9**).* One hundred milligrams of **7** (0.253 mmol) was dissolved in 1 mL of dry pyridine. The mixture was cooled to 0 °C, and 0.1 mL (1.1 mmol) of phosphoryl oxychloride were added dropwise. The reaction was further cooled to 10 °C, and the appropriate amine was added (1.3 mmol of phenethylamine for compound **8**, and a large excess, 4 mmol, of methylamine was used to obtain compound **9**), and then the mixture was left to reach room temperature and stirred for 2 h monitoring by TLC (EtOAc). When the reaction was terminated, ice and water were added to the mixture, and the product was extracted with EtOAc. The organic layer was then washed with a $NaHCO_3$ (aq), dried, and the solvent was removed under reduced pressure. The crude was purified by column chromatography.

*5-((3,4-Dimethoxybenzyl)amino)-2-(furan-2-yl)-N-methyl-[1,2,4]-triazolo[1,5-c]pyrimidine-8-carboxamide (**8**).* Yield 68%; white solid (EtOAc-light petroleum) mp 202 °C. ^{1}H NMR ($CDCl_3$) δ: 3.11 (3H, d, J = 4); 3.66 (3H, s); 3.67 (3H, s); 4.83 (2H, d, J = 6); 6.64 (1H, dd, J = 2, J = 4); 6.67 (1H, bt); 6.74−6.78 (2H, m); 7.01 (1H, s); 7.26 (1H, d, J = 4); 7.63 (1H, d, J = 2); 8.56 (1H, bs); 8.82 (1H, s); IR (Nujol) cm^{-1}: 3350−3130, 1685, 1640, 1525, 1420. Anal. ($C_{20}H_{20}N_6O_4$) C, H, N.

*5-((3,4-Dimethoxybenzyl)amino)-2-(furan-2-yl)-N-β-phenethyl-[1,2,4]triazolo[1,5-c]pyrimidine-8-carboxamide (**9**).* Yield 69%; white solid (EtOAc-light petroleum) mp 130 °C. ^{1}H NMR ($CDCl_3$) δ: 3.02 (2H, t, J = 6); 3.65−3.80 (2H, m); 3.89 (3H, s); 3.90 (3H, s); 4.83 (2H, d, J = 6); 6.63 (1H, dd, J = 2, J = 4); 6.68 (1H, bt); 6.78−6.82 (2H, m); 7.10 (1H, s); 7.21 (1H, d, J = 4); 7.25−7.55 (5H, m); 7.63 (1H, d, J = 2); 8.72 (1H, bs); 8.86 (1H, s); IR (Nujol) cm^{-1}: 3355−3150, 1690, 1655, 1545, 1425. Anal. ($C_{27}H_{26}N_6O_4$) C, H, N.

*General Procedure for the Synthesis of 5-Amino-2-(furan-2-yl)-N-substituted-[1,2,4]triazolo[1,5-c]pyrimidine-8-carboxamides (**10, 11**).* One millimole of 8-carboxamido compound (**8**, **9**) was dissolved in trifluoroacetic acid (10 mL), and 5.5 mmol of trifluoromethanesulfonic acid (0.48 mL) and 3.7 mmol of anisole (0.40 mL) were added. The reaction was stirred at room temperature overnight. Dichloromethane was then added to the mixture, and the mixture was washed three times with water. The organic layer was dried, and the solvent was removed under reduced pressure. The crude was purified by column chromatography (EtOAc-light petroleum 9:1).

*5-Amino-2-(furan-2-yl)-N-methyl-[1,2,4]triazolo[1,5-c]-pyrimidine-8-carboxamide (**10**).* Yield 71%; brown solid (EtOAc-light petroleum) mp 220 °C. ^{1}H NMR ($CDCl_3$) δ: 2.95 (3H, d, J = 4); 6.64 (1H, dd, J = 2, J = 4); 6.77 (1H, bt); 7.40 (1H, d, J = 4); 7.99 (1H, d, J = 2); 8.49 (1H, s); 8.65 (2H, bs); IR (Nujol) cm^{-1}: 3350−3090, 1685, 1635, 1525, 1415. Anal. ($C_{11}H_{10}N_6O_2$) C, H, N.

*5-Amino-2-(furan-2-yl)-N-β-phenethyl-[1,2,4]triazolo[1,5-c]-pyrimidine-8-carboxamide (**11**).* Yield 68%; pale yellow solid (EtOAc-light petroleum) mp 130 °C.^{1}H NMR ($CDCl_3$) δ: 3.05 (2H, t, J = 6); 3.68−3.90 (2H, m); 6.22 (2H, bs); 6.65 (1H, dd, J = 2, J = 4); 7.15 (1H, d, J = 4); 7.22−7.45 (5H, m); 7.66 (1H, d, J = 2); 8.76 (1H, bs); 8.80 (1H, s); IR (Nujol) cm^{-1}: 3360−3140, 1685, 1650, 1540, 1435. Anal. ($C_{18}H_{16}N_6O_2$) C, H, N.

*5-Amino-2-(furan-2-yl)-[1,2,4]triazolo[1,5-c]pyrimidine-8-carboxylic Acid Ethyl Ester (**12**).* One hundred milligrams of **5** (0.33 mmol) was dissolved in ethanol saturated with NH_3 (10 mL), and then the reaction was heated for 2 h. When the compound reacted completely, the solvent was removed under reduced pressure, and the residue was

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recrystallized in EtOAc-light petroleum. Yield 95%; white solid mp 195 °C. ^{1}H NMR ($CDCl_3$) δ: 1.32 (3H, t, J = 7); 4.25 (2H, q, J = 7); 6.48 (2H, bs); 6.63 (1H, dd, J = 2, J = 4); 7.42 (1H, d, J = 4); 7.66 (1H, d, J = 2); 8.69 (1H, s); IR (Nujol) cm^{-1}: 3350−3100, 1720, 1625, 1530, 1415. Anal. ($C_{12}H_{11}N_5O_3$) C, H, N.

*General Procedure for the Synthesis of 5-Ureido Derivatives (**13**, **14**, **16**, **17**, **19**, **20**).* To 0.3 mmol of 5-amino compound (**10**−**12**) dissolved in dry THF was added 3 mmol of the appropriate isocyanate, and the reaction was refluxed overnight. To the mixture was then added water, and it was extracted three times with EtOAc. The organic layers were collected, dried, and the solvent was removed under reduced pressure. The crude was purified by column chromatography.

*2-(Furan-2-yl)-N-methyl-5-(3-phenylureido)-[1,2,4]triazolo[1,5-c]-pyrimidine-8-carboxamide (**13**).* Yield 71%; white solid (EtOAc-light petroleum) mp 150 °C. ^{1}H NMR ($CDCl_3$) δ: 2.95 (3H, d, J = 4); 6.66 (1H, dd, J = 2, J = 4); 7.11−7.52 (6H, bs); 7.87 (1H, d, J = 2); 8.63 (2H, bs); 8.95 (1H, s); 11.08 (1H, bs); IR (Nujol) cm^{-1}: 3350−3180, 1690, 1685, 1645, 1525, 1420. Anal. ($C_{18}H_{15}N_7O_3$) C, H, N.

*5-(3-Cyclohexylureido)-2-(furan-2-yl)-N-methyl-[1,2,4]triazolo-[1,5-c]pyrimidine-8-carboxamide (**14**).* Yield 70%; white solid (EtOAc-light petroleum) mp 199 °C. ^{1}H NMR ($CDCl_3$) δ: 1.03−1.42 (6H, m); 1.60−1.82 (4H, m); 3.02 (3H, d, J = 4); 3.31−3,35 (1H, m); 6.67 (1H, dd, J = 2, J = 4); 7.32 (1H, d, J = 4); 7.71 (1H, d, J = 2); 8.46 (1H, bs); 8.61 (1H, bs); 8.86 (1H, s); 8.92 (1H, bs); IR (Nujol) cm^{-1}: 3350−3170, 1685, 1680, 1625, 1515, 1410. Anal. ($C_{18}H_{21}N_7O_3$) C, H, N.

*2-(Furan-2-yl)-N-phenethyl-5-(3-phenylureido)-[1,2,4]triazolo-[1,5-c]pyrimidine-8-carboxamide (**16**).* Yield 72%; yellow solid (EtOAc-light petroleum) mp 165 °C. ^{1}H NMR ($CDCl_3$) δ: 3.05 (2H, t, J = 6); 3.78−3.92 (2H, m); 6.68 (1H, dd, J = 2, J = 4); 7.08−7.37 (6H, m); 7.63 (1H, d, J = 2); 8.76 (1H, bs); 8.81 (1H, bs); 8.93 (1H, s); 11.08 (1H, bs); IR (Nujol) cm^{-1}: 3345−3130, 1695, 1687, 1650, 1535, 1415. Anal. ($C_{25}H_{21}N_7O_3$) C, H, N.

*5-(3-Cyclohexylureido)-2-(furan-2-yl)-N-β-phenethyl-[1,2,4]-triazolo[1,5-c]pyrimidine-8-carboxamide (**17**).* Yield 69%; white solid (EtOAc-light petroleum) mp 186 °C. ^{1}H NMR ($CDCl_3$) δ: 1.02−1.42 (6H, m); 1.66−1.98 (4H, m); 3.03 (2H, t, J = 6); 3.40−3,53 (1H, m); 3.86−3.91 (2H, m); 6.64 (1H, dd, J = 2, J = 4); 7.16 (1H, d, J = 4); 7,36 (1H, bs); 7.71 (1H, d, J = 2); 8.41 (1H, bs); 8.67 (1H, bs); 8.73 (1H, s); IR (Nujol) cm^{-1}: 3335−3120, 1690, 1685, 1635, 1525, 1410. Anal. ($C_{25}H_{27}N_7O_3$) C, H, N.

*2-(Furan-2-yl)-5-(3-phenylureido)-[1,2,4]triazolo[1,5-c]-pyrimidine-8-carboxylic Acid Ethyl Ester (**19**).* Yield 66%; white solid (EtOAc-light petroleum) mp 170 °C. ^{1}H NMR ($CDCl_3$) δ: 1.47 (3H, t, J = 7); 4.50 (2H, q, J = 7); 6.66 (1H, dd, J = 2, J = 4); 7.15 (1H, d, J = 4); 7.28−7.42 (3H, m); 7.62−7.64 (2H, m); 7.68 (1H, d, J = 2); 8.74 (1H, bs); 8.83 (1H, s); 11.05 (1H, bs); IR (Nujol) cm^{-1}: 3330−3140, 1685, 1680, 1650, 1540, 1415. Anal. ($C_{19}H_{16}N_6O_4$) C, H, N.

*5-(3-Cyclohexylureido)-2-(furan-2-yl)-[1,2,4]triazolo[1,5-c]-pyrimidine-8-carboxylic Acid Ethyl Ester (**20**).* Yield 65%; white solid (EtOAc-light petroleum) mp 172 °C. ^{1}H NMR ($CDCl_3$) δ: 1.10−1.40 (6H, m); 1.47 (3H, t, J = 7); 1.55−1.78 (4H, m); 3.78−3.84 (1H, m); 4.50 (2H, q, J = 7); 6.63 (1H, dd, J = 2, J = 4); 7.42 (1H, d, J = 4); 7.69 (1H, d, J = 2); 8.57 (1H, bs); 8.75 (1H, s); 8.88 (1H, bs); IR (Nujol) cm^{-1}: 3340−3130, 1690, 1685, 1635, 1520, 1415. Anal. ($C_{19}H_{22}N_6O_4$) C, H, N.

*General Procedure for the Synthesis of 5-(2-phenylacetamido) derivatives (**15**, **18**, **21**).* To 0.3 mmol of 5-amino compound (**10**−**12**) dissolved in dry THF (10 mL), 1.8 mmol (0.24 mL) of phenylacetyl chloride and 1.8 mmol (0.25 mL) of Et_3N were added. The reaction was refluxed overnight. The solvent was removed under reduced pressure and the residue was dissolved in EtOAc and washed with water. The organic layer was dried, and the solvent was removed under reduced pressure and the residue was purified by column chromatography (EtOAc-light petroleum).

*2-(Furan-2-yl)-N-methyl-5-(2-phenylacetamido)-[1,2,4]triazolo-[1,5-c]pyrimidine-8-carboxamide (**15**).* Yield 67%; white solid (EtOAc-light petroleum) mp 160 °C. ^{1}H NMR ($CDCl_3$) δ: 3.07 (3H, d, J = 4); 4.37 (2H, s); 6.67 (1H, dd, J = 2, J = 4); 7.22−7.42 (6H, m); 7.71 (1H, d, J = 2); 8.55 (2H, bs); 8.99 (1H, s); 9.11 (1H, bs); IR (Nujol) cm^{-1}: 3335−3120, 1695,1690, 1655, 1530, 1425. Anal. ($C_{19}H_{16}N_6O_3$) C, H, N.

*2-(Furan-2-yl)-N-phenethyl-5-(2-phenylacetamido)-[1,2,4]-triazolo[1,5-c]pyrimidine-8-carboxamide (**18**).* Yield 73%; white solid (EtOAc-light petroleum) mp 83 °C. ^{1}H NMR ($CDCl_3$) δ: 3.01 (2H, t, J = 6); 3.85−3.8 (2H, m); 6.65 (1H, dd, J = 2, J = 4); 7.13 (1H, m, J = 4); 7.28−7.41 (5H, m); 7.70 (1H, d, J = 2); 8.79 (1H, bs); 8.97 (1H, s); 8.12 (1H, s); IR (Nujol) cm^{-1}: 3340−3160, 1690, 1685, 1645, 1540, 1435. Anal. ($C_{26}H_{22}N_6O_3$) C, H, N.

*2-(Furan-2-yl)-5-(2-phenylacetamido)-[1,2,4]triazolo[1,5-c]-pyrimidine-8-carboxylic Acid Ethyl Ester (**21**).* Yield 70%; white solid (EtOAc-light petroleum) mp 243 °C. ^{1}H NMR ($CDCl_3$) δ: 1.32 (3H, t, J = 7); 4.07 (2H, s); 4.54 (2H, q, J = 7); 6.63 (1H, dd, J = 2, J = 4); 7.08−7.44 (6H, m); 7.70 (1H, d, J = 2); 9.49 (1H, s); 9.75 (1H, bs); IR (Nujol) cm^{-1}: 3340−3130, 1720, 1685, 1650, 1530, 1435. Anal. ($C_{20}H_{17}N_5O_4$) C, H, N.

B. Biology. *Binding at Human A_1, A_{2A}, and A_3 ARs.* All pharmacological methods followed the procedures as described earlier.[37] In brief, membranes for radioligand binding were prepared from CHO cells stably transfected with human AR subtypes in a two-step procedure. In a first low-speed step (1000*g*), cell fragments and nuclei were removed. The crude membrane fraction was sedimented from the supernatant at 100 000*g*. The membrane pellet was resuspended in the buffer used for the respective binding experiments, frozen in liquid nitrogen and stored at 80 °C. For the measurement of adenylyl cyclase activity, only one high speed centrifugation of the homogenate was used. The resulting crude membrane pellet was resuspended in 50 mM Tris/HCl, pH 7.4 and immediately used for the cyclase assay.

For radioligand binding at A_1 adenosine receptors 1 nM [^{3}H]CCPA was used, whereas 30 and 10 nM [^{3}H]NECA were used for A_{2A} and A_3 receptors, respectively. Nonspecific binding of [^{3}H]CCPA was determined in the presence of 1 mM theophylline, in the case when [^{3}H]NECA 100 μM R-PIA was used.[39]

Adenylyl Cyclase Activity. The potency of antagonists at the A_{2B} AR was determined in adenylyl cyclase experiments. The procedure was carried out as described previously with minor modifications.[37,40] Membranes were incubated with about 150 000 cpm of [α-^{32}P]ATP for 20 min in the incubation mixture as described without EGTA and NaCl.[37,40] For agonists, the EC_{50} values for the stimulation of adenylyl cyclase were calculated with the Hill equation. Hill coefficients in all experiments were near unity. IC_{50} values for concentration-dependent inhibition of NECA-stimulated adenylyl cyclase caused by antagonists was calculated accordingly. Dissociation constants (K_i) for antagonist were then calculated with the Cheng and Prusoff equation.[41]

C. Molecular Modeling. Energy computation and analyses of docking poses were performed using the MOE suite (Molecular Operating Environment, version 2013.08).[42] The software package MOPAC,[43] implemented in the MOE suite, was utilized for all quantum mechanical calculations. GOLD (Genetic Optimization for Ligand Docking, version 5.1)[44] suite was used to carry out all the docking simulations.

Three-Dimensional Structures of hARs. Among all the currently available crystallographic structures of human A_{2A} AR, we selected for our docking simulations a complex with the selective and high affinity inverse agonist ZM 241385. Among the structures cocrystallized with ZM 241385, we selected the one with the highest resolution and fewest missing atoms (PDB code: 4EIY, 1.80 Å resolution).[45] As, to date, no crystallographic information about the hA_3 AR is available, we used a previously build homology model deposited in our web platform dedicated to ARs, *Adenosiland*.[46] The numbering of the amino acids follows the arbitrary scheme proposed by Ballesteros and Weinstein:[47] each amino acid identifier starts with the helix number (1−7), followed by a dot and the position relative to a reference residue among the most conserved amino acids in that helix, to which the number 50 is arbitrarily assigned.

Molecular Docking. Ligand structures were built using the MOE-builder tool, as part of the MOE suite,[42] and were subjected to a MMFF94x energy minimization until the rms conjugate gradient was <0.05 kcal mol^{-1} Å^{-1}. We used the Protonate 3D methodology, part

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of the MOE suite, for protonation state assignment by selecting a protonation state for each chemical group that minimizes the total free energy of the system (taking titration into account). According to the docking benchmark study recently completed by our research group,[48] we perform the simulations employing the docking tool of GOLD[44] suite as conformational search program and GoldScore as scoring function. Indeed, the latter protocol resulted one of the most successful at reproducing the crystallographic pose of ligand−hA_{2A} AR complexes and the best for the considered crystal structure. For each selected compound, 25 independent docking runs were performed and searching was conducted within a user-specified docking sphere (20 Å radius and centered on the barycenter of the Asn(6.55) residue) with the Genetic Algorithm protocol and the GoldScore scoring function. Prediction of antagonist−receptor complex stability (in terms of corresponding pK_i value) and quantitative analysis for nonbonded intermolecular interactions (H-bonds, transition metal, water bridges, hydrophobic, electrostatic) were calculated and visualized using several tools implemented in the MOE suite.[42] Electrostatic and hydrophobic contributions to the binding energy of individual amino acids have been calculated using the MOE suite. To estimate the electrostatic contributions, atomic partial charges for the ligands were calculated using PM3/ESP methodology. Partial charges for protein amino acids were calculated on the basis of the AMBER99 force field.

Interaction Energy Fingerprints (IEFs). To analyze the ligand−receptor recognition mechanism in a more quantitative manner, we calculated the individual electrostatic and hydrophobic contributions to the interaction energy (hereby denoted as IEele and IEhyd, respectively) of each receptor residue involved in the binding with the ligand. In particular, the IEele was computed on the basis of the nonbonded electrostatic interaction energy term of the force field, whereas the IEhyd contributions was calculated by using the directional hydrophobic interaction term based on contact surfaces as implemented in the MOE scoring function.[42] As a consequence, an energy (expressed in kcal/mol) is associated with the IEele, instead an adimensional score (the higher the better) is related to the IEhyd. The analysis of these contributions have been reported as IEFs, that is, interaction energy patterns (graphically displayed as histograms) reporting the key residues involved in the binding with the considered ligands along with a quantitative estimate of the occurring interactions.

■ ASSOCIATED CONTENT

Supporting Information

Supporting Information contains elemental analyses for the compounds used in this study. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

AR, adenosine receptor; ATP, adenosine triphosphate; CCPA, 2-chloro-N6-cyclopentyladenosine; CHO, chinese hamster ovary; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; DMSO, dimethyl sulfoxide; EGTA, ethylene glycol tetraacetic acid; HMDS, hexamethyldisiloxane; IEele, per residue electrostatic contribution to the interaction energy; IEF, interaction energy fingerprint; IEhyd, per residue hydrophobic contribution to the interaction energy; NECA, 5′-*N*-ethylcarboxamidoadenosine; R-PIA, *R*-(−)-N6-(2-phenylpropyl)adenosine; SAR, structure−activity relationship; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TLC, thin-layer chromatography; TP, 1,2,4-triazolo[1,5-*c*]pyrimidine; ZM 241385, 4-[2-[7-amino-2-(2-furyl)-1,2,4-triazolo[1,5-*a*][1,3,5]triazin-5-yl-amino]ethylphenol; TM, transmembrane; EL2, second extracellular loop

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