8-Substituted 1,3-dimethyltetrahydropyrazino[2,1-*f*]purinediones: Water-soluble adenosine receptor antagonists and monoamine oxidase B inhibitors

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1. Introduction

Multiple target-directed approaches have recently gained considerable attention, in particular for the treatment of brain disorders.[1–7] Complex neurodegenerative diseases, e.g., Parkinson's (PD)[¶] and Alzheimer's disease (AD), may profit from drugs that exhibit both, symptomatic and neuroprotective effects by interacting with more than one target structure.[8–11]

Monoamine oxidase B (MAO-B) inhibitors are current standard therapeutics for PD. They are often combined with levodopa and increase dopamine levels in the brain by inhibiting the oxidative metabolism of dopamine; at the same time they reduce the production of hydrogen peroxide and may therefore display neuroprotective properties. Selectivity versus the other MAO subtype, MAO-A, is required since simultaneous inhibition of MAO-A and ingestion of biogenic amines, e.g. tyramine has been reported to potentially result in a hypertensive crisis, the so-called 'cheese effect'.[12]

Recently, several A_{2A} adenosine receptor (AR) antagonists have been clinically evaluated as novel therapeutics for PD, and istradefylline (Nouriast®) has been the first A_{2A} antagonist to be approved as a drug in Japan.[13] A_{2A} AR antagonists positively modulate dopamine D_2 receptor function.[14–16] In addition they have shown neuroprotective properties in animal studies.[17–20] Both, MAO-B

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‡ Deceased on July 7, 2011.
¶ *Abbreviations:* AD, Alzheimer's disease; AR, adenosine receptor; MAO, monoamino oxidase; PD, Parkinson's disease.

inhibitors as well as A_{2A} AR antagonists may exhibit disease-modifying properties in PD as well as in AD and perhaps other neurodegenerative diseases.[21] Four AR subtypes exist, A_1, A_{2A}, A_{2B}, and A_3, of which only A_1 and A_{2A} are highly expressed in the brain.[22] While the A_{2A} AR is found predominantly in the caudate-putamen, the A_1 AR shows high expression levels in many brain regions including cortex. Antagonists selective for the A_1 AR subtype have been reported to show cognitive-enhancing effects.[23]

Caffeine (**1**, Table 1), a nonselective AR antagonists, is a potent cognitive enhancer and, as shown in epidemiological studies, provides some protection from AD and PD.[24] Therefore, dual-target drugs, A_1/A_{2A} AR antagonists on the one hand, and A_{2A} AR antagonists/MAO-B inhibitors on the other hand have been developed. Very recently, the first compounds blocking all three targets of interest, A_1 and A_{2A} ARs as well as MAO-B, have been developed: 8-benzyl-1,3-dimethyl-6,7,8,9-tetrahydro**pyrazino**[2,1-*f*]purine-2,4(1*H*,3*H*)-diones of the general structure **2** (Fig. 1) that are annelated tricyclic xanthine derivatives.[25] The **pyrazino**[2,1-*f*]purine scaffold is related to the isomeric **pyrimido**[2,1-*f*]purinedione scaffold **3** (Fig. 1) that had previously been exploited to design adenosine receptor antagonists.[26–32] The shift of the nitrogen atom from position 9 (**3**, Fig. 1) to position 8 (**2**, Fig. 1) was intended to improve the compounds' water-solubility by increasing the basicity of the nitrogen atom in the saturated, annelated ring. The parent compound of this new series, 8-benzyl-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (**2a**, Tables 1 and 3), showed submicromolar affinity for the human A_1 AR and micromolar affinity for the human A_{2A} AR, although no inhibition of MAO-B was observed. Extensive exploration of the 8-benzyl-substitution pattern then led to the identification of a moderately potent triple-acting A_1 and A_{2A} antagonist with MAO-B inhibitory potency (**2b**, Tables 1 and 3) that displayed promising pharmacokinetic properties.[25]

In the present study we investigated the structure–activity relationships (SARs) of a new series of compounds derived from structure **2** at the four human ARs and at human MAO-A and MAO-B. Moreover, we studied potential species differences by determining affinities for the rat A_1 and A_{2A} ARs and determined aqueous solubility. In this new series we replaced the (substituted) benzyl residue of **2** by a broad range of (substituted) aromatic residues that were either attached directly or via different linker moieties to the *N*8 of the pyrazinopurine scaffold.

2. Results and discussion

2.1. Chemistry

The synthetic routes towards *N*8-substituted 1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purinediones **6**–**68** are depicted in Schemes 1–3. Compounds **6**–**63** were obtained from 7-(2-bromoethyl)-8-(hydroxymethyl)-1,3-dimethylpurine-2,4-dione (**4**) according to a recently described procedure (Scheme 1):[25] Briefly, the 8-hydroxymethyl group of compound **4**[25] was converted to the corresponding bromide with phosphorus tribromide and the obtained 7-(2-bromoethyl)-8-(bromomethyl)-1,3-dimethylpurine-2,4-dione (**5**) was subsequently reacted—without prior purification—with the appropriate amines under basic conditions affording the desired *N*8-substituted tetrahydropyrazino[2,1-*f*]purinediones **6**–**63**.

The *N*8-propargyl substituted compound **63** was subsequently used for further derivatization. It served to synthesize the 1,2,3-triazole derivative **65** via a Cu(I)-catalyzed 1,3-dipolar cycloaddition[33,34] with 4-chlorophenylazide (**64**) which had been freshly prepared from 4-chloroaniline in analogy to a protocol described by Hu et al. (Scheme 2).[35]

Furthermore, the terminal alkyne function of compound **63** was reacted through a Pd-catalyzed Sonogashira reaction with aryl iodides to yield the 8-(3-arylprop-2-ynyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purinediones **66**–**68** (Scheme 3).[36]

The structures of all final products were confirmed by proton and carbon NMR spectra. Melting points were determined for all novel compounds. The purity of the tested compounds was confirmed by high-performance liquid chromatography (HPLC) coupled to electrospray ionization mass spectrometry (ESI-MS) using two different methods (for details, see Section 5) and it was generally found that these compounds displayed a purity of greater than 95%, except for compounds **10**, **13**, and **14** whose purity was greater than 92%.

2.2. Biological evaluation

The adenosine receptor binding affinities of compounds **6**–**63** and **65**–**68** were determined in radioligand binding assays (Tables 1 and 2, S1 and S2). All compounds were initially tested at the A_1 AR of rat brain cortical membrane and at the A_{2A} AR of rat brain striatal membrane preparations, because proof-of-principle studies of the newly proposed dual- or triple-target concept will initially be performed in rodents, and substantial species differences had previously been reported for AR antagonists.[37] Selected compounds were further tested for their affinity to human A_1 and A_{2A} ARs recombinantly expressed in Chinese hamster ovary (CHO) cells. They were additionally investigated for their affinity to human A_{2B} and A_3 ARs expressed in CHO cells in order to determine their AR subtype selectivity. Data from standard adenosine receptor antagonists are included for comparison (Tables 1 and S1). [^{3}H]2-Chloro-N^6-cyclopentyladenosine ([^{3}H]CCPA),[38] [^{3}H]1-propargyl-3-(3-hydroxypropyl)-7-methyl-8-(3-methoxystyryl)xanthine ([^{3}H]MSX-2),[39] [^{3}H]8-(4-(4-(4-chlorophenyl)piperazine-1-sulfonyl)phenyl)-1-propylxanthine ([^{3}H]PSB-603),[40] and [^{3}H]2-phenyl-8-ethyl-4-methyl-(8*R*)-4,5,7,8-tetrahydro-1*H*-imidazo[2,1-*i*]purine-5-one ([^{3}H]PSB-11)[41] were used as radioligands in the A_1, A_{2A}, A_{2B}, and A_3 adenosine receptor binding studies, respectively. Selected compounds were additionally investigated in functional (cAMP accumulation) studies at human A_1 and A_{2A} ARs. All new compounds were tested for inhibitory potency at human MAO-B (Table 3). Potent MAO-B inhibitors were additionally tested for selectivity versus human MAO-A (Table 3). Data of standard ligands are included for comparison.

2.3. Structure–activity relationships at adenosine receptors

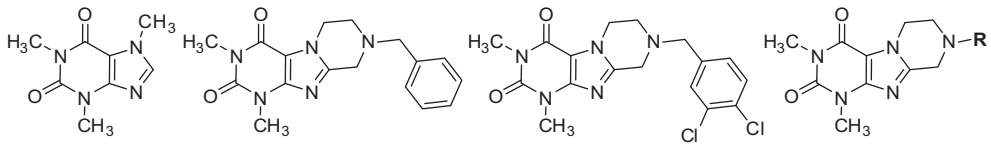
2.3.1. *N*8-Aryl-substituted 1,3-dimethyltetrahydropyrazino[2,1-*f*]purinediones

Within the series of the *N*8-phenyl-substituted 1,3-dimethyltetrahydropyrazino[2,1-*f*]purinediones (**6**–**13**, Table 1) the *N*8-*meta*-chlorophenyl-substituted compound **9** displayed K_i values in the nanomolar range at both rat A_1 and A_{2A} ARs, but dramatically reduced affinity at the respective human receptors. Most compounds within this series showed affinity between 1 and 10 μM for the rat A_{2A} receptor and preference versus the rat A_1 receptor (**6**–**8**, **11**–**13**). However, none of them bound to the human A_{2B} and A_3 ARs at the highest tested concentration (see Table S1 for human A_{2B} and A_3 binding data).

2.3.2. *N*8-2-Phenylethyl-substituted 1,3-dimethyltetrahydropyrazino[2,1-*f*]purinediones

In a second series of compounds we explored the elongation of the C1 linker in the parent structure **2** (Fig. 1) to an ethylene linker between *N*8 of the pyrazino[2,1-*f*]purine scaffold and the (hetero) aromatic substituent (**14**–**39**, Tables 1 and S1). The *N*8-phenethyl-substituted compound **14** can be viewed as the parent

Table 1
A_1 and A_{2A} adenosine receptor affinities of standard inhibitors **1**, **2a**, **2b**, and 8-phenethyl-substituted 1,3-dimethyltetrahydro-pyrazino[2,1-*f*]purinediones **6**–**48**



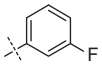
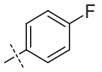
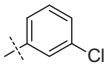
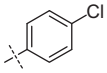
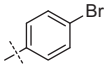
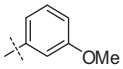
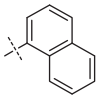
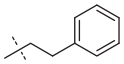
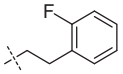
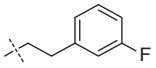
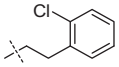
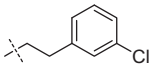
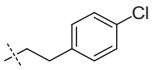
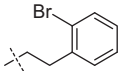
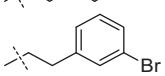
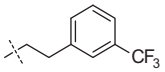
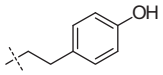
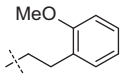
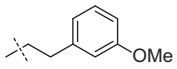
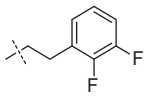
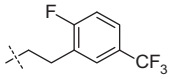
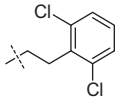
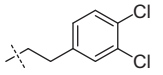
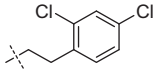
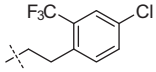
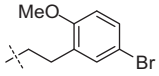
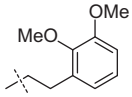
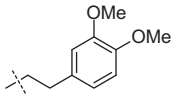
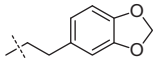
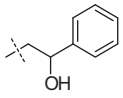
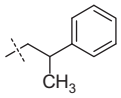
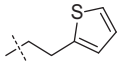
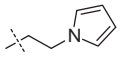
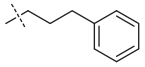
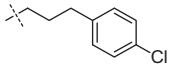
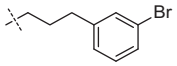
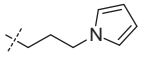
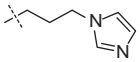
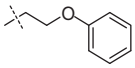
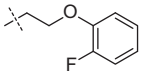
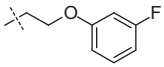
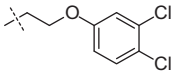
Compd	R	K_i ± SEM (μM); *human*; rat	
		A_1 versus [^{3}H]CCPA[a]	A_{2A} versus [^{3}H]MSX-2[a]
1		*44.9*[b]	*23.4*[b]
		41.0[b]	32.5[b]
2a		*0.265 ± 0.068*[c]	*1.06 ± 0.30*[c]
		0.0793 ± 0.0120[c]	0.598 ± 0.102[c]
2b		*0.791 ± 0.110*[c]	*1.51 ± 0.310*[c]
		0.351 ± 0.057[c]	0.322 ± 0.129[c]
N8-Aryl-substituted 1,3-dimethyltetrahydropyrazino[2,1-f]-purinediones			
6		>1.50 (12%)[d]	3.83 ± 0.790
7		>1.50 (20%)[d]	2.92 ± 0.390
8		>1.50 (4%)[d]	2.13 ± 0.340
9		*⩾10.0 (37%)*[d] 0.159 ± 0.033	*8.86 ± 1.98* 0.557 ± 0.460
10		>1.50 (1%)[d]	>10.0 (34%)[d]
11		>1.50 (15%)[d]	3.90 ± 0.890
12		>1.50 (16%)[d]	1.01 ± 0.230
13		>1.50 (17%)[d]	2.94 ± 1.09
N8-2-Phenylethyl-substituted 1,3-dimethyltetrahydropyrazino[2,1-f]-purinediones			
14		>1.50 (9%)[d]	9.61 ± 1.63
15		>1.50 (24%)[d]	5.63 ± 1.08
16		>1.50 (6%)[d]	1.50 ± 0.050
17		>1.50 (32%)[d]	2.73 ± 0.410
18		>1.50 (14%)[d]	1.31 ± 0.35
19		>1.50 (22%)[d]	4.96 ± 0.64
20		>1.50 (10%)[d]	>1.00 (27%)[d]
21		>1.50 (26%)[d]	2.12 ± 0.26
22		>1.50 (6%)[d]	2.58 ± 0.47

Table 1 (*continued*)

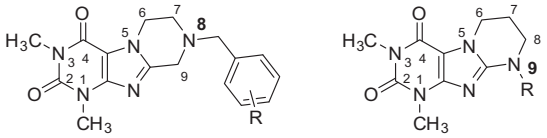
Compd	R	K_i ± SEM (μM); *human*; rat	
		A_1 versus [^{3}H]CCPA[a]	A_{2A} versus [^{3}H]MSX-2[a]
23		>1.50 (11%)[d]	6.04 ± 1.01
24		>1.50 (9%)[d]	>1.00 (15%)[d]
25		>1.50 (17%)[d]	>1.00 (31%)[d]
26		>1.50 (10%)[d]	>1.00 (19%)[d]
27		>1.50 (4%)[d]	>1.00 (25%)[d]
28		>1.50 (34%)[d]	3.05 ± 0.67
29		>1.50 (35%)[d]	2.85 ± 0.14
30		*3.52 ± 0.84* 0.416 ± 0.114	2.25 ± 0.82
31		*0.553 ± 0.180* 0.138 ± 0.020	2.91± 0.71
32		>1.50 (3%)[d]	>1.00 (27%)[d]
33		>1.50 (5%)[d]	>1.00 (30%)[d]
34		>1.50 (−2%)[d]	>1.00 (16%)[d]
35		>1.50 (5%)[d]	6.17 ± 0.60
36		>1.50 (7%)[d]	7.11 ± 2.88
37		>1.50 (27%)[d]	4.52 ± 0.63
38		>1.50 (15%)[d]	1.59 ± 0.60
39		>1.50 (7%)[d]	>1.00 (8%)[d]
N8-3-Propylaryl- and N8-3-ethoxyphenyl-substituted 1,3-dimethyltetrahydropyrazino[2,1-f]purinediones			
40		>1.50 (12%)[d]	1.84 ± 0.300
41		*0.0655 ± 0.0080* 0.352 ± 0.060	*0.230 ± 0.051* 0.316 ± 0.034
42		*3.88 ± 0.58* >1.50 (17%)[d]	*0.512 ± 0.026* 0.450 ± 0.060
43		>1.50 (−4%)[d]	>1.00 (10%)[d]

(*continued on next page*)

Table 1 (*continued*)

Compd	R	K_i ± SEM (μM); *human*; rat	
		A_1 versus [^{3}H]CCPA[a]	A_{2A} versus [^{3}H]MSX-2[a]
44		>1.50 (13%)[d]	>1.00 (13%)[d]
45		>1.50 (−1%)[d]	>1.00 (24%)[d]
46		>1.50 (5%)[d]	>1.00 (23%)[d]
47		>1.50 (8%)[d]	>1.00 (31%)[d]
48		1.25 ± 0.240	2.42 ± 0.940

[a] $n = 3$.
[b] Literature data taken from Ref. 41.
[c] Literature data taken from Ref. 25.
[d] Inhibition of radioligand binding at indicated concentration.



2 **3**

Figure 1. Structures of tricyclic xanthine derivatives.

compound of this series. It showed affinity for the rat A_{2A} receptor in the range of 10 μM. Introduction of a small halide substituent (F, Cl) in the *ortho*-position of the aromatic ring of the phenethyl residue (**15**, **17**) increased the rat A_{2A} AR affinity by 2-3fold whereas the bulky bromo-substituent was not tolerated in that position (**20**). The *meta*-position showed a better tolerance for substitution, and halides (F, Cl, Br) as well as a trifluoromethyl moiety (**16**, **18**, **21**, **22**) increased rat A_{2A} AR affinity yielding AR antagonists with K_i values in the range of 1–10 μM. Single methoxy substituents (**24**, **25**) were not tolerated, whereas a *para*-hydroxy group yielded a rat A_{2A} receptor ligand with micromolar affinity (**23**). None of the compounds showed affinity for rat A_1 or human A_{2B} or A_3 receptors. Introduction of two substituents into the *N*8-phenethyl residue yielded compounds with nanomolar affinity (2,4-dichloro, **30**; 2-trifluoromethyl-4-chloro, **31**) determined at the rat A_1 AR, but displayed significantly decreased affinity at the humanA_1 AR. Generally, introduction of a second substituent did not result in an increase in affinity for the rat A_{2A} AR as compared to the mono-substituted phenethyl series. Also, none of the compounds showed potent affinity at the human A_{2B} or A_3 ARs. The introduction of a methyl substituent in the 2-position of the ethylene linker slightly increased affinity for the rat A_{2A} AR (**14** vs **37**). We had observed a similar effect of a branched substituent also in the recently published *N*8-benzyl substituted series.[25] Bioisosteric replacement of the phenethyl ring by a thiophenylethyl ring resulted in a 6-fold increase in A_{2A} affinity at the rat receptor (**14** vs **38**).

2.3.3. *N*8-3-Propylaryl- and *N*8-3-ethoxyphenyl-substituted 1,3-dimethyltetrahydropyrazino[2,1-*f*]purine-diones

Elongation of the linker to propylene (**40**–**44,** Tables 1 and S1) turned out to be a promising strategy to improve affinity for A_1 and A_{2A} ARs and selectivity versus human A_{2B} or A_3 receptors. Whereas the unsubstituted compound **40** was a micromolar antagonist at the rat A_{2A} AR, substitution of the phenyl ring with *para*-chloro yielded the dual A_1/A_{2A} receptor antagonist **41** with nanomolar affinity (K_i (hA_1) 65 nM, (hA_{2A}) 230 nM). A *meta*-bromo substituent in the phenyl ring as in **42** decreased affinity for the hA_1 AR dramatically but for the hA_{2A} AR only a slight decrease was observed (K_i (hA_1) 3,880 nM, (hA_{2A}) 512 nM). Replacement of the phenyl ring by a pyrrol-1-yl or imidazol-1-yl residue (**40** vs **43**, **44**) or changing the linker from propylene to oxyethylene (**40** vs **45**) resulted in a massive loss of affinity at all AR subtypes.

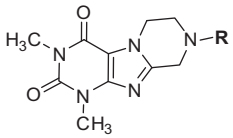
2.3.4. *N*8-Bicyclo-substituted 1,3-dimethyltetrahydro-pyrazino [2,1-*f*]purinediones

A further strategy was to attach bicyclic substituents to the *N*8 of the pyrazino[2,1-*f*]purinedione scaffold (**49**–**54**, Tables 2 and S2). Within this series compound **53**, bearing a 1-tetrahydronaphthyl moiety at position 8, represented a dual hA_1/hA_{2A} AR antagonist (K_i (hA_1) 393 nM, (hA_{2A}) 595 nM) with selectivity versus human A_{2B} and A_3 ARs. Replacing the 1-tetrahydronaphthyl substituent by slightly smaller dihydroinden-1- or -2-yl derivatives (**51**–**54**), reduced the affinity to rat A_1 and A_{2A} ARs by at least 7- to 10-fold.

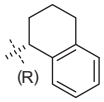
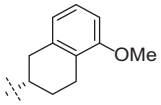
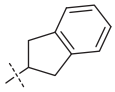
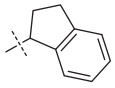
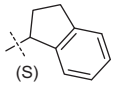
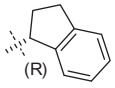
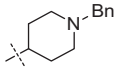
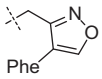
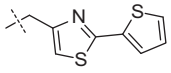
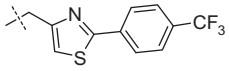
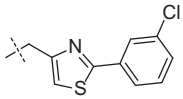
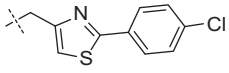
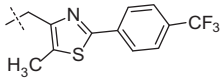
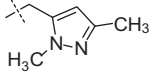
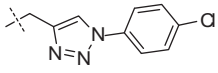
2.3.5. 1,3-Dimethyltetrahydro-pyrazino[2,1-*f*]purinediones with *N*8-heterocyclic linkers

Introduction of more bulky heterocycles as linkers between the *N*8-position of the pyrazino[2,1-*f*]purinediones and different aryl moieties yielded dual A_1/A_{2A} AR antagonists with nanomolar affinity (Tables 2 and S2). Within this series of compounds, 2-arylthiazol-4-ylmethyl-substituted compounds **57**–**60** represented potent dual A_1/A_{2A}ARs antagonists with K_i values in the higher nanomolar range (**58**, K_i (hA_1) 236 nM, (hA_{2A}) 217 nM, **59**; *meta*-chloro, K_i (hA_1) 73 nM, (hA_{2A}) 363 nM) and selectivity versus the human A_{2B} and A_3 AR subtypes. The dramatic effect of minor changes in the substitution pattern is illustrated by compound **61** which differs from the nanomolar A_1/A_{2A} antagonist **58** by an additional methyl group in position 5 of the thiazole moiety. That substitution resulted in a complete loss of A_1 and A_{2A} AR affinity indicating steep SARs for this class of compounds. Replacement of the thiazole by a triazole ring resulted in a large decrease in A_1 and A_{2A} affinity (**65** vs **60**). Also an *N*-substituted 4-piperidinyl linker was not tolerated by the ARs (**55**).

Table 2
A_1 and A_{2A} adenosine receptor affinities of compounds **49**–**63** and **65**–**68**

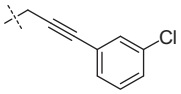
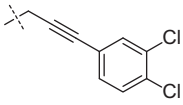
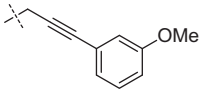


49-68

Compd	R	K_i ± SEM (μM); *human*; rat	
		A_1 versus [^{3}H]CCPA[a]	[^{3}H]MSX-2[a] A_{2A} versus [^{3}H]MSX-2[a]
N8-Bicyclo-substituted 1,3-dimethyltetrahydro-pyrazino[2,1-f]purinediones			
49		*0.393 ± 0.101* 0.100 ± 0.010	*0.595 ± 0.051* 0.510 ± 0.190
50		>1.50 (11%)[d]	>1.00 (11%)[d]
51		>1.50 (12%)[d]	>10.0 (26%)[d]
52		>1.50 (32%)[d]	4.21 ± 0.480
53		>1.50 (29%)[d]	2.48 ± 0.900
54		>1.50 (28%)[d]	5.08 ± 0.150
1,3-Dimethyltetrahydro-pyrazino[2,1-f]purinediones with N8-heterocyclic linkers			
55		>1.50 (7%)[d]	>1.00 (5%)[d]
56		>1.50 (21%)[d]	>1.00 (5%)[d]
57		*0.642 ± 0.049* 0.166 ± 0.037	*0.203 ± 0.022* 0.121 ± 0.022
58		*0.236 ± 0.051* 0.157 ± 0.133	*0.217 ± 0.051* 0.355 ± 0.023
59		*0.073 ± 0.016* 0.437 ± 0.027	*0.363 ± 0.098* 0.160 ± 0.020
60		*0.492 ± 0.017* 0.076 ± 0.002	*0.346 ± 0.060* 0.062 ± 0.012
61		>1.50 (25%)[d]	>1.00 (19%)[d]
62		>1.50 (15%)[d]	>1.00 (14%)[d]
65		>1.50 (36%)[d]	>1.00 (26%)[d]
N8-Propynyl-substituted 1,3-dimethyltetrahydro-pyrazino[2,1-f]purinediones			
63		>1.50 (13%)[d]	>1.00 (28%)[d]

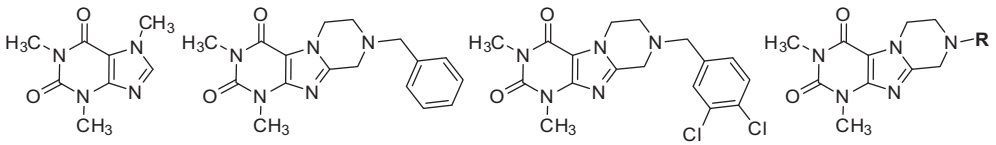
(*continued on next page*)

Table 2 (*continued*)

Compd	R	K_i ± SEM (μM); *human*; rat	
		A_1 versus [^{3}H]CCPA[a] [^{3}H]MSX-2[a]	A_{2A} versus [^{3}H]MSX-2[a]
66		>1.50 (17%)[d]	>1.00 (42%)[d]
67		>1.50 (29%)[d]	>1.00 (31%)[d]
68		>1.50 (17%)[d]	>1.00 (32%)[d]

[a] $n = 3$.
[b] Literature data taken from Ref. 41.
[c] Literature data taken from Ref. 25.
[d] Inhibition of radioligand binding at indicated concentration.

Table 3
MAO-A and MAO-B inhibitory potencies of standard compounds and 1,3-dimethyltetrahydropyrazino[2.1-*f*]purinediones **6**–**68**

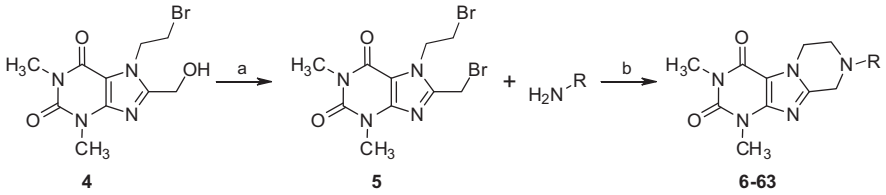


Compd	IC_{50} ± SEM[a] (μM)	
	Human MAO-A	Human MAO-B
1	>50.0 (33%)[b]	>50.0 (16%)[b]
2a	nd[c]	>10.0 (36%)[b]
2b	>10.0 (5%)[b]	0.197 ± 0.025[b]
N8-Aryl-substituted 1,3-dimethyltetrahydro-pyrazino[2,1-f]purinediones		
6	>10.0 (−1%)[b]	ca. 10.0 (61%)[d]
7	>10.0 (8%)[b]	1.50 ± 0.05
8	nd	2.23 ± 0.43
9	>10.0 (4%)[b]	0.385 ± 0.090[c]
10	nd	>10.0 (15%)[d]
11	nd	0.132 ± 0.023
12	>10.0 (3%)[d]	1.16 ± 0.28
13	>10.0 (16%)[d]	0.828 ± 0.106
N8-2-Phenylethyl-substituted 1,3-dimethyltetrahydro-pyrazino[2,1-f]purinediones		
14	>10.0 (−12%)[d]	>10.0 (39%)[d]
15	nd	ca. 10.0 (65%)[d]
16	>10.0 (12%)[d]	>10.0 (35%)[d]
17	>10.0 (11%)[d]	0.723 ± 0.118
18	>10.0 (13%)[d]	ca. 10.0 (61%)[d]
19	nd	>10.0 (36%)[d]
20	nd	0.970 ± 0.059
21	nd	3.65 ± 1.02
22	nd	ca. 10.0 (63%)[d]
23	nd	>10.0 (15%)[d]
24	nd	>10.0 (4%)[d]
25	nd	>10.0 (10%)[d]
26	nd	ca. 10.0 (60%)[d]
27	nd	ca. 10.0 (58%)[d]
28	nd	0.932 ± 0.184
29	>10.0 (14%)[d]	0.802 ± 0.024
30	>10.0 (15%)[d]	2.45 ± 0.10
31	nd	0.425 ± 0.045
32	>10.0 (34%)[d]	>10.0 (3%)[d]
33	nd	>10.0 (1%)[d]
34	nd	>10.0 (13%)[d]
35	nd	>10.0 (24%)[d]
36	nd	>10.0 (3%)[d]
37	>10.0 (23%)	>10.0 (11%)[d]
38	>10.0 (5%)[b]	>10.0 (21%)[d]
39	>10.0 (14%)[b]	>10.0 (19%)[d]

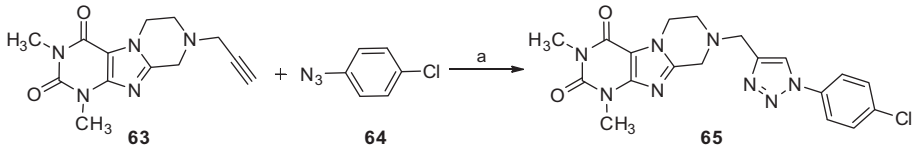
Table 3 (*continued*)

Compd	IC_{50} ± SEM[a] (μM)	
	Human MAO-A	Human MAO-B
N8-3-Propylaryl- and N8-3-ethoxyphenyl-substituted 1,3-dimethyltetrahydropyrazino[2,1-f]purinediones		
40	>10.0 (9%)[b]	>10.0 (14%)[d]
41	>10.0 (12%)[b]	>10.0 (44%)[d]
42	>10.0 (16%)[b]	>10.0 (26%)[d]
43	>10.0 (7%)[b]	>10.0 (27%)[d]
44	nd	>10.0 (2%)[d]
45	>10.0 (11%)[b]	>10.0 (16%)[d]
46	nd	>10.0 (24%)[d]
47	nd	>10.0 (22%)[d]
48	>10.0 (14%)[b]	>10.0 (27%)[d]
N8-Bicyclo-substituted 1,3-dimethyltetrahydro-pyrazino[2,1-f]purinediones		
49	>10.0 (14%)[d]	0.210 ± 0.041[f]
50	>10.0 (2%)[d]	>10.0 (7%)[d]
51	nd	7.59 ± 0.05
52	nd	2.13 ± 0.53
53	nd	>10.0 (22%)[d]
54	nd	2.15 ± 0.38
N8-Hetero(bi-)aryl-substituted 1,3-dimethyltetrahydro-pyrazino[2,1-f]purinediones		
55	nd	>10.0 (2%)[d]
56	nd	>10.0 (39%)[d]
57	nd	>10.0 (33%)[d]
58	nd	ca. 10.0 (54%)[d]
59	>10.0 (−13%)[d]	>10.0 (−2%)[d]
60	>10.0 (3%)[d]	ca. 10.0 (47%)[d]
61	nd	ca. 10.0 (67%)[d]
62	nd	>10.0 (−17%)[d]
65	nd	>10.0 (12%)[d]
N8-Propynyl-substituted 1,3-dimethyltetrahydro-pyrazino[2,1-f]purinediones		
63	nd	>10.0 (27%)[d]
66	>10.0 (19%)[d]	>10.0 (5%)[d]
67	nd	>10.0 (31%)[d]
68	nd	>10.0 (13%)[d]

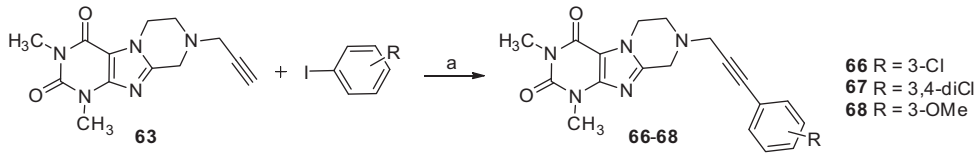
[a] $n = 3$.
[b] Data taken from Ref. 25.
[c] Not determined.
[d] %Inhibition at the indicated concentration.
[e] Rat MAO-B 251 nM.
[f] Rat MAO-B 100 nM.



Scheme 1. Synthesis of *N*8-substituted 1,3-dimethyltetrahydropyrazino[2,1-*f*]purinediones **10**–**67**. Reagents and conditions: (a) PBr_3, CH_2Cl_2, 0 °C to rt, 1 h; (b) dimethoxyethane, *N*,*N*-diisopropylethylamine, rt, 16 h. For R, see Tables 1 and 2.



Scheme 2. Synthesis of 8-(1-((4-chlorophenyl)-1*H*-1,2,3-triazol-4-yl)methyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (**65**) by Huisgen reaction. Reagents and conditions: (a) CuI, sodium ascorbate, *N*,*N*′-dimethylethylenediamine, *tert*-butanol/H_2O (5 mL, 4:1, V:V), 65 °C, 3 h. Compound **64** was prepared in situ from *para*-chloroaniline by reaction with $NaNO_2$, 5 N aq HCl solution, 0 °C, 5 min, followed by NaN_3, 5 min 0 °C, and 1 h, rt.



Scheme 3. Synthesis of 8-(3-arylprop-2-ynyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-diones **66**–**68** by Sonogashira reaction. Reagents and conditions: (a) $Pd(Ph_3P)_4$, CuI, *N*,*N*-diisopropylethylamine, dry DMF, 80 °C, 16 h.

2.3.6. *N*8-Propynyl-substituted 1,3-dimethyltetrahydro-pyrazino[2,1-*f*]purinediones

The final strategy was to substitute the flexible alkyl linkers by a more rigid alkynyl linker as represented by compounds **63** and **66**–**68**. This modification did not yield compounds with notable affinity for any of the AR subtypes (Tables 2 and S2).

2.4. Functional assays

Selected potent compounds (**41**, **49**, **57**, **58**) were investigated in cAMP accumulation assays at the human G_i-coupled A_1 and the G_s-coupled A_{2A} ARs. As expected based on the compounds' structures and the known SAR of AR agonists, most of which are adenosine derivatives, none of the investigated compounds was able to activate human A_1 or A_{2A} ARs (see Figs. 2 and 3). This clearly indicates that the compounds, which show high affinity for the receptors, act as AR antagonists.

2.5. Structure–activity relationships of pyrazinopurinediones as MAO-B inhibitors

All of the final compounds (**6**–**63**, **65**–**68**) were tested for their ability to inhibit human MAO-B, and selected compounds were additionally tested for selectivity versus MAO-A (Table 3). A number of inhibitors of MAO-B with IC_{50}-values in the nanomolar range could be identified, whereas none of the selected compounds tested for MAO-A inhibition did exert inhibition at the maximal test concentration of 10 μM. In the series of 8-phenyl-substituted pyrazino[2,1-*f*]purinediones (**6**–**13**), we identified two nanomolar inhibitors of human MAO-B, the *meta*-chloro-substituted compound **9** (IC_{50} human MAO-B 385 nM, rat MAO-B 251 nM) and the *para*-bromo-substituted compound **11** (IC_{50}human MAO-B 132 nM, rat MAO-B 100 nM). Introduction of an ethylene linker between the heterocyclic scaffold and the aryl moiety (**14**–**39**) turned out to be largely detrimental to MAO-B inhibitory potency as the whole series of investigated 8-phenethylpyrazino[2,1-*f*]purinediones yielded only very few MAO-B inhibitors, all of them with potency in the high nanomolar to low micromolar range. The majority of the active compounds in this series displayed an *ortho*-halide substituent (**17**, **20**, **28**, **31**), the most potent MAO-B inhibitor being the 4-chloro-2-trifluoromethylphenethyl-substituted compound **31** (IC_{50} 425 nM). Further elongation of the linker to propylene confirmed the observation that linker elongation is detrimental to MAO-B inhibitory potency: the series of 8-phenylpropyl-substituted derivatives did not yield a single active MAO-B inhibitor (**40**–**48**). Also, neither the bulky biarylmethyl substituents placed in the 8-position of the heterocyclic scaffold (in **55**–**62** and **65**) nor the rigid propynyl linker in compounds **63** and **66**–**68** was tolerated by the enzyme. On the other hand, introduction of bicyclic substituents in the 8-position (**49**–**54**) yielded a number of potent MAO-B inhibitors. The most potent of these was the tetrahydronaphth-1-yl-substituted compound **49** that inhibited MAO-B with a submicromolar IC_{50} value (IC_{50} 210 nM) showing high selectivity versus MAO-A.

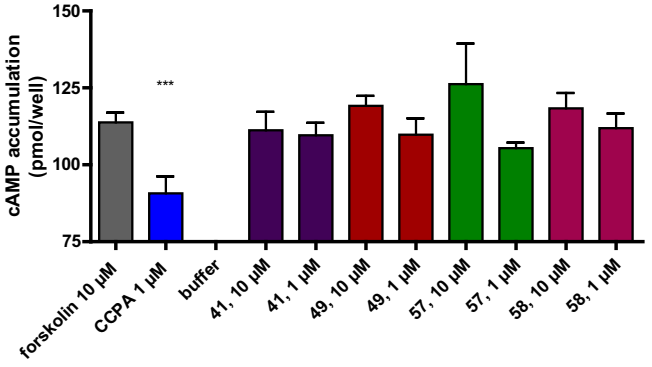


Figure 2. cAMP accumulation assays at CHO cells recombinantly expressing the human A_1 AR. Forskolin (10 μM) induced an increase in the intracellular cAMP concentration by direct activation of adenylate cyclase, which was inhibited by the A_1 AR agonist 2-chloro-N^6-cyclopentyladenosine (CCPA). Non of the selected compounds (tested at 1 and 10 μM concentration) led to a significant change in forskolin-induced cAMP accumulation indicating that they were not acting as A_1 AR agonists.

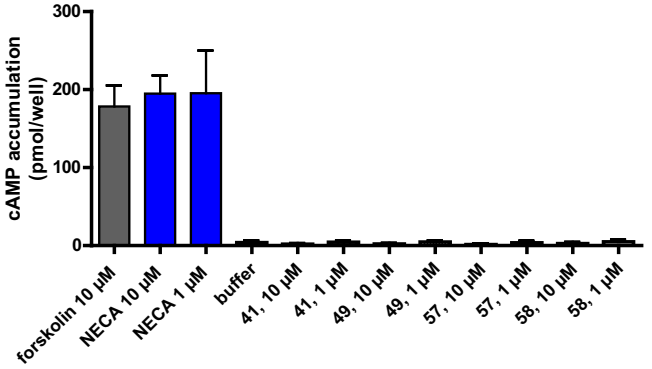


Figure 3. cAMP accumulation assays at CHO cells recombinantly expressing the human A_{2A} AR. Forskolin (10 μM) and the AR agonist NECA (1 and 10 μM) led to an increase in intracellular cAMP concentration. None of the selected compounds (tested at 1 and 10 μM) increased cAMP concentrations indicating that they did not activate the A_{2A}R.

2.6. Water-solubility of selected compounds

The water solubility of selected compounds was tested by thermodynamic solubility measurements at different pH values (Table S3, see Supporting information).[25] Most of these compounds such as the dual hA_1/A_{2A}-antagonist **58** showed good solubility at pH 1 likely due to protonation of *N*8. Compounds **36**, **39**, **55**, **57**, **62** and **63** revealed good solubility also at pH 7.4. In case of **36** the improved solubility at pH 7.4 is probably due to the additional hydroxyl function in position 2 of the ethylene linker. Compounds **39** and **62** display small heterocyclic substituents at the *N*8-position. Compound **55** has a basic piperidinyl moiety as a linker structure. Unlike all other pyrazino[2,1-*f*]purinediones, compound **63** has no aromatic but a simple *N*8-propargyl substituent. The relatively high solubility of compound **57** displaying a biaryl-like thienothiazolyl residue came as a surprise but shows that increased water-solubility is achievable within this series even with compounds having large aromatic residues.

3. Analysis of structure–activity relationships at the different targets

We exploited a recently published synthetic route[25] to generate a large series of *N*8-substituted pyrazino[2,1-*f*]purinediones. The new compounds were designed to explore the effect of different linkers connecting aryl moieties to the *N*8-position of the pyrazino[2,1-*f*]purinedione core on A_1/A_{2A} AR affinities and MAO-B inhibitory potency. Our aim was to identify compounds that hit a combination or even all three targets to enable a polypharmacological approach for the treatment of PD and potentially other neurodegenerative diseases.

A global analysis of the structure–activity relationships (SARs) (Fig. 4) indicates divergent SARs of MAO-B inhibition and A_1/A_{2A} antagonism in this series of *N*8-substituted pyrazino[2,1-*f*]purine-

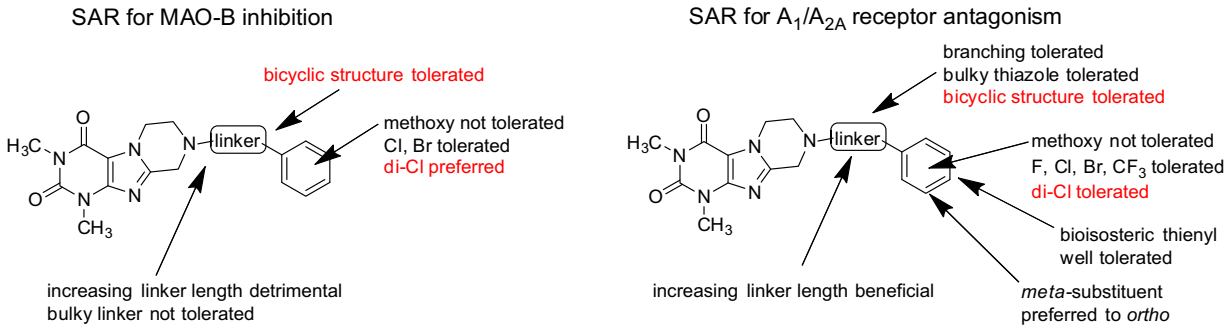


Figure 4. Structure–activity relationships of the *N*8-substituted pyrazino[2,1-*f*]purinediones.

diones. Within the compounds with an ethylene linker we identified mostly micromolar A_1/A_{2A} receptor antagonists and MAO-B inhibitors. The fraction of the more potent, i.e. submicromolar A_1 and A_{2A} AR antagonists is highest among the compounds that display long, flexible propyl (2 out of 5 compounds tested (2/5)) or bulky heteroaromatic linkers (4/7) between the tricyclic core structure and the aromatic residue whereas potent MAO B inhibitors are rather found among the *N*8-aryl-substituted pyrazino[2,1-*f*] purinediones (3/8). Not a single compound of the whole library bound potently to the human A_{2B} or A_3 ARs or inhibited MAO A.

With the linkerless *para*-bromophenyl-substituted compound **11** we obtained a novel potent and selective MAO-B inhibitor (IC_{50} (hMAO-B) 132 nM) that is a weakly active A_{2A} antagonist (K_i (rA_{2A}) 3,900 nM). In the series of C2-linker-substituted compounds we identified the 4-chloro-2-trifluoromethyl substituted compound **31** as a dual hA_1-antagonist/MAO-B inhibitor (K_i (hA_1) 553 nM, IC_{50} (hMAO-B) 425 nM). Further elongation of the linker to three C-atoms appears to be a promising avenue towards dual nanomolar hA_1/A_{2A}-antagonists whose target-binding profile can be tuned through substitution of the aryl moiety: the *para*-chloro substituted compound **41** bound to both A_1 and A_{2A} ARs with nanomolar affinity and a slight, 4-fold preference for the A_1 receptor (K_i (hA_1) 65 nM, (hA_{2A}) 230 nM). The *meta*-bromo substituent on the phenyl ring of **42** decreased affinity for the hA_1 receptor dramatically but only slightly, by two-fold for the hA_{2A} receptor (K_i (hA_1) 3880 nM, (hA_{2A}) 512 nM). The introduction of a 2-aryl-substituted thiazol-4-ylmethyl moiety at the 8-position of the pyrazino[2,1-*f*]purinedione scaffold represents a further access to dual hA_1/A_{2A}-antagonists as demonstrated by compound **58** (K_i (hA_1) 236 nM, (hA_{2A}) 217 nM). Finally, we identified one compound with balanced potency at all three targets, the 1-tetrahydronaphthyl-substituted compound **49** (K_i (hA_1) 393 nM, (hA_{2A}) 595 nM, IC_{50} (MAO-B) 210 nM). Compound **49** shows the same MAO-B inhibitory potency as compound **2b** from the previous series of *N*8-benzyl-substituted pyrazino[2,1-*f*]purinediones but represents a more potent antagonist at the rat A_1 AR and human A_1/A_{2A} ARs.

4. Conclusions

A large series of 8-substituted pyrazino[2,1-*f*]purinediones has been synthesized. Biological evaluation provided valuable insights into the SARs of this so far poorly studied scaffold with respect to AR-antagonistic and MAO-inhibitory potencies as a basis for the further development of this class of compounds. As compared to the well-studied pyrimido[2,1-*f*]purinediones the isomeric pyrazino[2,1-*f*]purinediones offer increased water-solubility which is beneficial for in vitro and especially in vivo studies. Structural modifications have led to promising ligands targeting simultaneously several targets relevant for the therapy of PD and other neurodegenerative diseases.

5. Experimental section

5.1. Chemistry

5.1.1. Material and methods

All commercially available reagents and solvents were used without further purification. The reactions were monitored by thin layer chromatography (TLC) using aluminum sheets coated with silica gel 60 F_{254} (Merck). Melting points were determined on a Büchi 530 melting point apparatus and are uncorrected. Preparative HPLC was performed on a C18 column (250 × 20 mm, particle size 10 μm, Eurospher 100) using a mixture of MeOH and H_2O as eluent at a flow rate of 20 mL/min. NMR data were recorded on a 500 MHz spectrometer (Bruker Advance) at ambient temperature. Shifts are given in ppm relative to the remaining protons of the deuterated solvents. Mass spectra were recorded on an API 2000 mass spectrometer (electron spray ion source, Applied Biosystems, Darmstadt, Germany) coupled with an Agilent 1100 HPLC system using a Phenomenex Luna HPLC C18 column (50 × 2.00 mm, particle size 3 μm). The purity of the tested compounds was determined by HPLC–UV obtained on an LC–MS instrument (Applied Biosystems API 2000 LC-MS/MS, HPLC Agilent 1100) using the procedure as follows: dissolving of the compounds at a concentration of 1.0 mg/mL in methanol and if necessary sonication to complete dissolution. Then, 10 μL of the substance solution was injected into a Phenomenex Luna C18 HPLC column (50 × 2.00 mm, particle size 3 μm) and elution was performed for 30 min at a flow rate of 250 μL/min with a gradient of water: methanol either containing 2 mM ammonium acetate from 90:10 up to 0:100, starting the gradient after 10 min (system A) or containing 2 mM ammonium acetate and 0.1% formic acid from 90:10 up to 0:100, starting the gradient after 10 min (system B) or containing 2 mM ammonium acetate from 60:40 up to 0:100 for 30 min, starting the gradient after 0 min and ending after 20 min (system C). UV absorption was detected from 220 to 400 nm using a diode array detector.

5.1.2. General procedure of the synthesis of tetrahydropyrazino [2,1-*f*]purinediones 6–63[25]

7-(2-Bromoethyl)-8-(hydroxymethyl)-1,3-dimethyl-1*H*-purine-2,6(3*H*,7*H*)-dione (**4**) (100 mg, 0.32 mmol) was dissolved in dry CH_2Cl_2 (30 mL) and cooled to 0 °C. A solution of PBr_3 (90 μL, 0.94 mmol) in dry CH_2Cl_2 (20 mL) was added dropwise. The solution was allowed to warm to rt and stirred for 1 h. Then it was cooled to 0 °C again and the excess of PBr_3 was carefully hydrolyzed by slow addition of saturated aq $NaHCO_3$ solution (5 mL). The pH was set at 8 by addition of more saturated aq $NaHCO_3$ solution. Then, the organic layer was separated and the aqueous layer extracted with CH_2Cl_2 (2 × 50 mL). The combined organic extracts were dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude 7-(2-bromoethyl)-8-bromo-1,3-dimethylpurine-2,4-dione

(**5**) was dissolved in a mixture of dimethoxyethane (10 mL) and diisopropylethylamine (DIPEA) (0.5 mL). Then, the appropriate amine (0.64 mmol) was added and the solution was stirred overnight at rt. The volatiles were removed under reduced pressure and tetrahydropyrazino[2,1-*f*]purinediones **6**–**63** precipitated upon addition of H_2O (20 mL). For purification, the compounds were either filtered off and washed with H_2O (3 × 5 mL) and subsequently with diethylether (3 × 10 mL), or subjected to column chromatography (silica gel, gradient of CH_2Cl_2/MeOH 1:0 to 40:1).

5.1.2.1. 1,3-Dimethyl-8-phenyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (6). Yield: 52%; mp: 208 °C; ^{1}H NMR ($CDCl_3$) δ 7.33–7.30 (m, 2H, C3-/C5-H, phe), 6.98–6.94 (m, 3H, C2-/C4-/C6-H, phe), 4.50 (s, 2H, C9-H_2), 4.45 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 3.72 (t, ^3J = 5.4 Hz, 2H, C7-H_2), 3.56 (s, 3H, N1-CH_3), 3.38 (s, 3H, N3-CH_3). ^{13}C NMR ($CDCl_3$) δ 159.0 (C9a), 157.1 (C4), 151.7 (C2), 148.4 (C10a), 143.4 (C1, phe), 126.2 (C3/C5, phe), 122.8 (C4, phe), 117.3 (C2/C6, phe), 105.9 (C4a), 48.9 (C7), 47.6 (C9), 44.0 (C6), 29.9 (N1-CH_3), 27.9 (N3-CH_3). ESI-MS: positive mode 312.1 $[M+H]^+$. HPLC: 99.9% (A) and 99.9% (B)

5.1.2.2. 8-(3-Fluorophenyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4-(1*H*,3*H*)-dione (7). Yield: 48%; mp: 284 °C; ^{1}H NMR ($CDCl_3$) δ 7.29–7.25 (m, 1H, C5-H, phe), 6.75–6.73 (m, 1H, C4-H, phe), 6.69–6.63 (m, 2H, C2-/C6-H, phe), 4.53 (s, 2H, C9-H_2), 4.48 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 3.76 (t, ^3J = 5.4 Hz, 2H, C7-H_2), 3.58 (s, 3H, N1-CH_3), 3.40 (s, 3H, N3-CH_3). ^{13}C NMR ($CDCl_3$) δ 163.9 (d, J = 245.3 Hz, C3, phe), 155.0 (C9a), 151.7 (C4), 150.5 (d, J = 9.7 Hz, C4, phe), 148.6 (C2), 146.8 (C10a), 130.8 (d, J = 9.9 Hz, C5, phe), 111.4 (d, J = 2.3 Hz, C6, phe), 107.6 (d, J = 21.4 Hz, C4, phe), 106.2 (C4a), 103.4 (d, J = 25.4 Hz, C2, phe), 47.6 (C7), 46.1 (C9), 43.8 (C6), 29.8 (N1-CH_3), 27.9 (N3-CH_3). ESI-MS: negative mode 328.0 $[M-H]^-$, positive mode 330.5 $[M+H]^+$. HPLC: 99.2% (A) and 99.4% (B).

5.1.2.3. 8-(4-Fluorophenyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4-(1*H*,3*H*)-dione (8). Yield: 55%; mp: 229 °C; ^{1}H NMR ($CDCl_3$) δ 7.02 (dd, ^3J = 8.2 Hz, ^4J = 2.2 Hz, 2H, C3-/C5-H, phe), 6.94 (dd, ^3J = 6.9 Hz, ^4J = 2.5 Hz, 2H, C2-/C6-H, phe), 4.45 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 4.42 (s, 2H, C9-H_2), 3.64 (t, ^3J = 5.4 Hz, 2H, C7-H_2), 3.57 (s, 3H, N1-CH_3), 3.38 (s, 3H, N3-CH_3). ^{13}C NMR ($CDCl_3$) δ 158.1 (d, J = 241.4 Hz, C4, phe), 155.0 (C9a), 151.7 (C4), 148.4 (C2), 147.1 (C10a), 145.4 (d, J = 2.1 Hz, C1, phe), 118.7 (d, J = 7.8 Hz, C^2/C6, phe), 116.2 (d, J = 22.5 Hz, C^3/C5, phe), 106.7 (C4a), 47.6 (C7), 46.0 (C9), 44.0 (C6), 29.9 (N1-CH_3), 27.9 (N3-CH_3). ESI-MS: negative mode 328.0 $[M-H]^-$, positive mode 330.5 $[M+H]^+$. HPLC: 99.2% (A) and 99.3% (B).

5.1.2.4. 8-(3-Chlorophenyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4-(1*H*,3*H*)-dione (9). Yield: 59%; mp: 299 °C; ^{1}H NMR ($CDCl_3$) δ 7.24–7.22 (m, 1H, C5-H, phe), 6.91–6.82 (m, 3H, C^4-/C5-/C6-H, phe), 4.50–4.46 (m, 4H, C6-H_2, C9-H_2), 3.72 (t, ^3J = 5.4 Hz, 2H, C7-H_2), 3.56 (s, 3H, N1-CH_3), 3.38 (s, 3H, N3-CH_3). ^{13}C NMR ($CDCl_3$) δ 159.0 (C9a), 155.0 (C4), 149.9 (C2), 148.4 (C10a), 146.8 (C1, phe), 135.4 (C3, phe), 130.6 (C5, phe), 121.0 (C6, phe), 116.3 (C2, phe), 114.2 (C4, phe), 105.9 (C4a), 47.6 (C7), 46.1 (C9), 43.8 (C6), 29.8 (N1-CH_3), 27.9 (N3-CH_3). ESI-MS: negative mode 344.0 $[M-H]^-$, positive mode 346.5 $[M+H]^+$. HPLC: 99.9% (A) and 99.9% (B).

5.1.2.5. 8-(4-Chlorophenyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4-(1*H*,3*H*)-dione (10). Yield: 42%; mp: 297 °C; ^{1}H NMR ($CDCl_3$) δ 7.27 (d, ^3J = 8.2 Hz, 2H, C3-/C5-H, phe), 6.89 (d, ^3J = 8.2 Hz, 2H, C2-/C6-H, phe), 4.45 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 4.42 (s, 2H, C9-H_2), 3.64 (t, ^3J = 5.4 Hz, 2H, C7-H_2), 3.57 (s, 3H, N1-CH_3), 3.38 (s, 3H, N3-CH_3). ^{13}C NMR ($CDCl_3$) δ 156.7 (C9a), 155.0 (C4), 151.7 (C2), 147.5 (C10a), 146.9 (C1, phe), 129.5 (C3/C5, phe), 126.4 (C4, phe), 117.7 (C2/C6, phe), 105.9 (C4a), 48.0 (C7), 46.6 (C9), 43.9 (C6), 29.8 (N1-CH_3), 27.9 (N3-CH_3). ESI-MS: negative mode 344.3 $[M-H]^-$, positive mode 346.3 $[M+H]^+$. HPLC: 93.2% (A) and 95.1% (B).

5.1.2.6. 8-(4-Bromophenyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4-(1*H*,3*H*)-dione (11). Yield: 61%; mp: 287 °C; ^{1}H NMR ($CDCl_3$) δ 7.41 (d, ^3J = 9.2 Hz, 2H, C3-/C5-H, phe), 6.84 (d, ^3J = 9.2 Hz, 2H, C2-/C6-H, phe), 4.47 (s, 2H, C9-H_2), 4.45 (t, ^3J = 5.7 Hz, 2H, C6-H_2), 3.69 (t, ^3J = 6.0 Hz, 2H, C7-H_2), 3.56 (s, 3H, N1-CH_3), 3.39 (s, 3H, N3-CH_3). ^{13}C NMR ($CDCl_3$) δ 155.0 (C9a), 151.7 (C4), 148.5 (C2), 147.9 (C10a), 146.9 (C1, phe), 132.4 (C3/C5, phe), 118.0 (C2/C6, phe), 113.7 (C4, phe), 106.7 (C4a), 47.9 (C7), 46.5 (C9), 43.9 (C6), 29.8 (N1-CH_3), 27.9 (N3-CH_3). ESI-MS: negative mode 390.0 and 392.0 $[M-H]^-$, positive mode 392.0 and 394.0 $[M+H]^+$. HPLC: 98.3% (A) and 98.5% (B).

5.1.2.7. 8-(3-Methoxyphenyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4-(1*H*,3*H*)-dione (12). Yield: 41%; mp: 216 °C; ^{1}H NMR ($CDCl_3$) δ 7.23–7.21 (m, ^3J = 8.9 Hz, 1H, C5-H, phe), 6.59–6.58 (m, 1H, C6-H, phe), 6.53–6.51 (m, 2H, C^2-/C4-H, phe), 4.51 (s, 2H, C9-H_2), 4.46 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 3.79 (s, 3H, OCH_3), 3.64 (t, ^3J = 5.4 Hz, 2H, C7-H_2), 3.57 (s, 3H, N1-CH_3), 3.38 (s, 3H, N3-CH_3). ^{13}C NMR ($CDCl_3$) δ 160.8 (C3, phe), 159.0 (C9a), 157.1 (C4), 151.7 (C2), 148.4 (C10a), 147.9 (C1, phe), 130.4 (C5, phe), 109.1 (C4, phe), 106.2 (C6, phe), 106.2 (C4a), 103.2 (C2, phe), 55.3 (OCH_3), 48.0 (C7), 46.6 (C9), 43.9 (C6), 29.9 (N1-CH_3), 27.9 (N3-CH_3).ESI-MS: positive mode 342.4 $[M+H]^+$. HPLC: 97.4% (A) and 97.6% (B).

5.1.2.8. 1,3-Dimethyl-8-(naphtha-1-yl)-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4-(1*H*,3*H*)-dione (13). Purification by column chromatography. Yield: 37%; ^{1}H NMR ($CDCl_3$) δ 8.16–8.14 (m, 1H), 7.87–7.85 (m, 1H), 7.51–7.49 (d, ^3J = 8.2 Hz, 1H), 7.51–7.49 (m, 2H), 7.41 (dd, ^3J = 8.1 Hz, ^3J = 7.5 Hz, 1H), 7.11 (dd, ^3J = 7.4 Hz, ^4J = 0.8 Hz, 1H), 4.54 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 4.46 (br s, 2H, C9-H_2), 3.64 (t, ^3J = 5.4 Hz, 2H, C7-H_2), 3.58 (s, 3H, N1-CH_3), 3.41 (s, 3H, N3-CH_3). ^{13}C NMR ($CDCl_3$) δ 155.1, 151.8, 148.5, 148.1, 146.7, 134.8, 128.6, 126.4, 126.2, 125.6, 125.2, 122.8, 106.9 (C4a), 51.1 (C7), 49.8 (C9), 44.5 (C6), 29.9 (N1-CH_3), 27.9 (N3-CH_3). ESI-MS: negative mode 360.0 $[M-H]^-$, positive mode 362.0 $[M+H]^+$. HPLC: 92.4% (A) and 92.6% (B).

5.1.2.9. 1,3-Dimethyl-8-phenethyl-6,7,8,9-tetrahydropyrazino [2,1-*f*]purine-2,4(1*H*,3*H*)-dione (14). Yield: 65%; mp: 214 °C; ^{1}H NMR ($CDCl_3$) δ 7.30–7.19 (m, 5H, phe), 4.37 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 3.87 (s, 2H, C9-H_2), 3.64 (t, ^3J = 5.4 Hz, 2H, C7-H_2), 3.53 (s, 3H, N1-CH_3), 3.37 (s, 3H, N3-CH_3) 3.00 (t, ^3J = 6.9 Hz, 2H, N8-CH_2), 2.89 (t, ^3J = 7.3 Hz, 2H, N8-CH_2-CH_2). ^{13}C NMR ($CDCl_3$) δ 159.0 (C9a), 157.1 (C4), 151.7 (C2), 148.4 (C10a), 140.8 (C1, phe), 128.6 (C3/C5, phe), 128.5 (C2/C6, phe), 126.6 (C4, phe), 106.6 (C4a), 59.0 (N8-CH_2), 49.0 (C7), 47.6 (C9), 44.0 (C6), 33.4 (N8-CH_2-CH_2), 29.9 (N1-CH_3), 27.9 (N3-CH_3). ESI-MS: positive mode: 340.5 $[M+H]^+$. HPLC: 92.4% (A) and 92.6% (B).

5.1.2.10. 8-(2-Fluorophenethyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4-(1*H*,3*H*)-dione (15). Purification by column chromatography. Yield: 39%; mp: 201 °C; ^{1}H NMR ($CDCl_3$) δ 7.26–7.20 (m, 2H, C^3-/C4-H, phe), 7.07 (dd, ^3J = 7.6 Hz, ^4J = 1.0 Hz, 1H, C6-H, phe), 7.02 (ddd, ^3J = 8.2 Hz, ^3J = 8.6 Hz, ^4J = 1.6 Hz, 1H, C5-H, phe), 4.49 (t, ^3J = 6.4 Hz, 2H, C6-H_2), 3.99 (s, 2H, C9-H_2), 3.53 (s, 3H, N1-CH_3), 3.37 (s, 3H, N3-CH_3), 3.15 (t, ^3J = 6.4 Hz, 2H, C7-H_2), 3.06–2.99 (m, 4H, N8-CH_2-

CH_2). ^{13}C NMR ($CDCl_3$) δ 161.1 (d, J = 245.3 Hz, C2, phe), 155.0 (C9a), 151.7 (C4), 148.5 (C2), 147.6 (C10a), 131.0 (d, J = 3.9 Hz, C6, phe), 128.7 (d, J = 7.7 Hz, C1, phe), 124.4 (d, J = 3.2 Hz, C5, phe), 123.0 (d, J = 16.7 Hz, C4, phe), 115.5 (d, J = 21.8 Hz, C3, phe), 106.5 (C4a), 57.2 (N8-CH_2), 51.1 (C7), 49.0 (C9), 43.1 (C6), 31.9 (N8-CH_2-CH_2), 29.7 (N1-CH_3), 27.8 (N3-CH_3). ESI-MS: positive mode 358.3 $[M+H]^+$. HPLC: 98.6% (A) and 98.9% (B).

5.1.2.11. 8-(3-Fluorophenethyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4-(1*H*,3*H*)-dione (16). Yield: 42%; mp: 173 °C; ^1H NMR ($CDCl_3$) δ 7.24–7.20 (m, 1H, C5-H, phe), 6.97–6.95 (m, 1H, C2-H, phe), 6.90–6.86 (m, 2H, C4-/C6-H, phe), 4.33 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 3.82 (s, 2H, C9-H_2), 3.52 (s, 3H, N1-CH_3), 3.35 (s, 3H, N3-CH_3), 2.97 (t, ^3J = 5.4 Hz, 2H, C7-H_2), 2.90–2.84 (m, 4H, N8-CH_2-CH_2). ^{13}C NMR ($CDCl_3$) δ 162.9 (d, J = 245.9 Hz, C3, phe), 154.9 (C9a), 151.7 (C4), 148.4 (C2), 147.6 (C10a), 141.7 (d, J = 5.9 Hz, C1, phe), 130.0 (d, J = 8.3 Hz, C5, phe), 124.9 (d, J = 2.6 Hz, C6, phe), 115.5 (d, J = 21.1 Hz, C2, phe), 113.2 (d, J = 21.1 Hz, C4, phe), 106.5 (C4a), 58.6 (N8-CH_2), 51.3 (C7), 49.1 (C9), 44.0 (C6), 33.2 (N8-CH_2-CH_2), 29.7 (N1-CH_3), 27.8 (N3-CH_3). ESI-MS: positive mode 358.3 $[M+H]^+$. HPLC: 97.7% (A) and 97.6% (B).

5.1.2.12. 8-(2-Chlorophenethyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4-(1*H*,3*H*)-dione (17). Yield: 65%; mp: 265 °C; ^1H NMR ($CDCl_3$) δ 7.45 (dd, ^3J = 7.3 Hz, ^4J = 2.3 Hz, 1H, C3-H, phe), 7.24–7.21 (m, 3H, C4-/C5-/C6-H, phe), 4.33 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 3.83 (s, 2H, C9-H_2), 3.53 (s, 3H, N1-CH_3), 3.37 (s, 3H, N3-CH_3), 2.98 (t, ^3J = 5.4 Hz, 2H, C7-H_2), 2.92–2.84 (m, 4H, N8-CH_2-CH_2). ^{13}C NMR (125 MHz, $CDCl_3$) δ 154.9 (C-9a), 151.7 (C-4), 148.4 (C-2), 147.6 (C-10a), 147.5 (C-1, phenyl), 134.4 (C-2, phenyl), 129.4 (C-3, phenyl), 128.4 (C4, C-5 and C-6, phenyl), 106.5 (C-4a), 58.6 (N8-CH_2), 51.3 (C-7), 49.1 (C-9), 44.0 (C-6), 33.2 (N8-CH_2-CH_2), 29.7 (N1-CH_3), 27.8 (N3-CH_3). ESI-MS: positive mode 374.3 $[M+H]^+$. HPLC: 99.3% (A) and 99.4% (B).

5.1.2.13. 8-(3-Chlorophenethyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4-(1*H*,3*H*)-dione (18). Purification by column chromatography. Yield: 41%; mp: 193 °C; ^1H NMR ($CDCl_3$) δ 7.22–7.17 (m, 3H, C2-/C4-/C5-H, phe), 7.08–7.07 (m, 1H, C6-H, phe), 4.34 (t, ^3J = 5.1 Hz, 2H, C6-H_2), 3.82 (s, 2H, C9-H_2), 3.53 (s, 3H, N1-CH_3), 3.37 (s, 3H, N3-CH_3), 2.97 (t, ^3J = 5.4 Hz, 2H, C7-H_2), 2.83 (br s, 4H, N8-CH_2-CH_2). ^{13}C NMR ($CDCl_3$) δ 155.0 (C9a), 151.7 (C4), 148.4 (C2), 147.7 (C10a), 141.2 (C1, phe), 134.3 (C3, phe), 129.8 (C5, phe), 128.7 (C2, phe), 126.8 (C4, phe), 126.6 (C6, phe), 106.6 (C4a), 58.6 (N8-CH_2), 51.3 (C7), 49.1 (C9), 44.1 (C6), 33.2 (N8-CH_2-CH_2), 29.7 (N1-CH_3), 27.7 (N3-CH_3). ESI-MS: positive mode: 374.3 $[M+H]^+$. HPLC: 99.3% (C).

5.1.2.14. 8-(4-Chlorophenethyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4-(1*H*,3*H*)-dione (19). Purification by column chromatography. Yield: 68%; mp: 193 °C; ^1H NMR ($CDCl_3$) δ 7.28 (d, ^3J = 8.5 Hz, 2H, C3-/C5-H, phe), 6.75 (d, ^3J = 8.6 Hz, 2H, C2-/C6-H, phe), 4.56 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 4.01 (s, 2H, C9-H_2), 3.53 (s, 3H, N1-CH_3), 3.37 (s, 3H, N3-CH_3), 3.05 (t, ^3J = 5.4 Hz, 2H, C7-H_2), 2.95 (br s, 4H, N8-CH_2-CH_2). ^{13}C NMR ($CDCl_3$) δ 159.0 (C9a), 154.9 (C4), 151.6 (C2), 148.5 (C10a), 141.1 (C1, phe), 132.2 (C4, phe), 130.0 (C2/C6, phe), 129.0 (C3/C5, phe), 105.9 (C4a), 58.4 (N8-CH_2), 51.0 (C7), 48.8 (C9), 42.6 (C6), 34.8 (N8-CH_2-CH_2), 29.8 (N1-CH_3), 27.9 (N3-CH_3). ESI-MS: positive mode: 374.3 $[M+H]^+$. HPLC: 95.4% (A) and 95.6% (B).

5.1.2.15. 8-(2-Bromophenethyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4-(1*H*,3*H*)-dione (20). Purification by column chromatography. Yield: 74%; mp: 160 °C; ^1H NMR ($CDCl_3$) δ 7.53 (d, ^3J = 7.6 Hz, 1H, C3-H, phe), 7.25–7.21 (m, 2H, C5-/C6-H, phe), 7.08 (dd, ^3J = 8.2 Hz, ^3J = 6.0 Hz, 1H, C4-H, phe), 4.35 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 3.88 (s, 2H, C9-H_2), 3.54 (s, 3H, N1-CH_3), 3.37 (s, 3H, N3-CH_3), 3.03–2.99 (m, 4H, C7-H_2, N8-CH_2), 2.84 (t, ^3J = 7.3 Hz, 2H, N8-CH_2-CH_2). ^{13}C NMR ($CDCl_3$) δ 154.9 (C9a), 151.7 (C4), 148.4 (C2), 147.6 (C10a), 138.4 (C1, phe), 132.9 (C3, phe), 130.7 (C6, phe), 128.2 (C4, phe), 127.6 (C5, phe), 122.4 (C2, phe), 106.5 (C4a), 57.3 (N8-CH_2), 51.3 (C7), 49.0 (C9), 44.2 (C6), 33.7 (N8-CH_2-CH_2), 29.7 (N1-CH_3), 27.8 (N3-CH_3). ESI-MS: positive mode 418.3 and 420.3 $[M+H]^+$. HPLC: 98.6% (A) and 98.3% (B).

5.1.2.16. 8-(3-Bromophenethyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4-(1*H*,3*H*)-dione (21). Yield: 71%; mp: 191 °C; ^1H NMR ($CDCl_3$) δ 7.34–7.33 (m, 2H, C^2-/C^5-H, phe), 7.16–7–11 (m, 2H, C4-/C6-H, phe), 4.34 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 3.83 (s, 2H, C9-H_2), 3.53 (s, 3H, N1-CH_3), 3.37 (s, 3H, N3-CH_3), 2.97 (br s, 2H, C7-H_2), 2.84 (br s, 4H, N8-CH_2-CH_2). ^{13}C NMR ($CDCl_3$) δ 155.0 (C9a), 151.7 (C4), 148.5 (C2), 147.6 (C10a), 140.1 (C1, phe), 132.1 (C6, phe), 131.0 (C3, phe), 128.9 (C5, phe), 125.2 (C2, phe), 123.0 (C4, phe), 106.5 (C4a), 58.7 (N8-CH_2), 51.3 (C7), 49.1 (C9), 44.1 (C6), 33.2 (N8-CH_2-CH_2), 29.7 (N1-CH_3), 27.8 (N3-CH_3). ESI-MS: positive mode 419.3 and 421.1 $[M+H]^+$. HPLC: 97.5% (A) and 97.5% (B).

5.1.2.17. 1,3-Dimethyl-8-(3-(trifluoromethyl)phenethyl)-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (22). Yield: 55%; mp: 154 °C; ^1H NMR ($CDCl_3$) δ 7.47–7.45 (m, 1H, C5-H, phe), 7.40–7.38 (m, 2H, C4-/C6-H, phe), 4.33 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 3.83 (s, 2H, C9-H_2), 3.53 (s, 3H, N1-CH_3), 3.37 (s, 3H, N3-CH_3), 2.98 (t, ^3J = 5.4 Hz, 2H, C7-H_2), 2.92–2.84 (m, 4H, N8-CH_2-CH_2). ^{13}C NMR ($CDCl_3$) δ 155.0 (C9a), 151.7 (C4), 148.5 (C2), 147.6 (C10a), 140.1 (C1, phe), 132.1 (C6, phe), 130.9 (q, $^2J_{C,F}$ = 32.1 Hz, C3, phe), 128.9 (C5, phe), 125.3 (q, $^3J_{C,F}$ = 3.7 Hz, C2, phe), 124.1 (q, $^1J_{C,F}$ = 272.3 Hz, CF_3), 123.0 (q, $^3J_{C,F}$ = 3.2 Hz, C4, phe), 106.5 (C4a), 58.6 (N8-CH_2), 51.3 (C7), 49.1 (C9), 44.1 (C6), 33.7 (N8-CH_2-CH_2), 29.7 (N1-CH_3), 27.8 (N3-CH_3). ESI-MS: negative mode 406.1 $[M-H]^-$, positive mode 408.3 $[M+H]^+$. HPLC: 97.7% (A) and 97.6% (B).

5.1.2.18. 8-(4-Hydroxyphenethyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4-(1*H*,3*H*)-dione (23). Purification by column chromatography. Yield: 35%; mp: 270 °C; ^1H NMR ($CDCl_3$) δ 7.07 (d, ^3J = 8.5 Hz, 2H, C2-/C6-H, phe), 6.75 (d, ^3J = 8.5 Hz, 2H, C3-/C5-H, phe), 4.73 (s, 1H, OH), 4.33 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 3.81 (s, 2H, C9-H_2), 3.54 (s, 3H, N1-CH_3), 3.31 (s, 3H, N3-CH_3), 2.96 (t, ^3J = 5.4 Hz, 2H, C7-H_2), 2.79 (br s, 4H, N8-CH_2-CH_2). ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.0 (C-9a), 157.1 (C-4), 156.0 (C-4, phenyl), 151.7 (C-2), 148.4 (C-10a), 138.8 (C-1, phenyl), 129.8 and 131.6 (C-2 and C-6, phenyl), 115.4 and 113.2 (C-3 and C-5, phenyl), 105.9 (C-4a), 59.3 (N8-CH_2), 55.9 (C-7), 51.4 (C-9), 49.1 (C-6), 32.8 (N8-CH_2-CH_2), 29.9 (N1-CH_3), 27.9 (N3-CH_3). ESI-MS: negative mode 354.1 $[M-H]^-$, positive mode 356.3 $[M+H]^+$. HPLC: 98.1% (A) and 98.3% (B).

5.1.2.19. 8-(2-Methoxyphenethyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (24). Yield: 60%; mp: 228 °C; ^1H NMR ($CDCl_3$) δ 7.20–7.17 (m, 1H, C4-H, phe), 7.13 (d, ^3J = 7.3 Hz, 1H, C^1-H, phe), 6.86 (dd, ^3J = 7.4 Hz, ^3J = 7.4 Hz, 1H, C5-H, phe), 6.83 (d, ^3J = 8.1 Hz, 1H, C3-H, phe), 4.33 (br s, 2H, C6-H_2), 3.84 (s, 2H, C9-H_2), 3.82 (s, 3H, OCH_3), 3.53 (s, 3H, N1-CH_3), 3.37 (s, 3H, N3-CH_3), 2.97 (br s, 2H, C7-H_2), 2.87–2.85 (br s, 2H, N8-CH_2), 2.81–2.79 (br s, 2H, N8-CH_2-CH_2). ^{13}C NMR ($CDCl_3$) δ 157.4 (C2, phe), 155.0 (C9a), 151.7 (C4), 148.5 (C2), 147.6 (C10a), 130.2 (C4, phe), 127.5 (C1, phe), 120.6 (C5, phe), 110.3 (C3, phe), 106.5 (C4a), 55.5 (N8-CH_2), 55.3 (OCH_3), 51.4 (C7), 49.0 (C9), 44.2 (C6), 29.7 (N1-CH_3), 28.0 (N8-CH_2-CH_2), 27.8 (N3-CH_3). ESI-MS: positive mode 370.1 $[M+H]^+$. HPLC: 99.7% (A) and 99.3% (B).

5.1.2.20. 8-(3-Methoxyphenethyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4-(1*H*,3*H*)-dione (25). Yield: 41%; mp: 212 °C; ^{1}H NMR ($CDCl_3$) δ 7.25–7.23 (m, 1H, C5-H, phe), 6.84–6.81 (m, 3H, C2-/C4-/C6-H, phe), 4.46 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 3.79 (s, 3H, OCH_3), 3.51 (s, 3H, N1-CH_3), 3.42 (s, 2H, C9-H_2), 3.37 (s, 3H, N3-CH_3), 3.08 (br s, 6H, C7-H_2, N8-CH_2, N8-CH_2-CH_2). ^{13}C NMR ($CDCl_3$) δ 160.8 (C3, phe), 159.0 (C9a), 157.1 (C4), 151.7 (C2), 148.4 (C10a), 139.9 (C1, phe), 130.4 (C5, phe), 120.8 (C6, phe), 114.2 (C4, phe), 113.2 (C2, phe), 106.2 (C4a), 58.6 (N8-CH_2), 55.4 (OCH_3), 48.0 (C7), 45.9 (C9), 43.9 (C6), 33.2 (N8-CH_2-CH_2), 29.9 (N1-CH_3), 27.9 (N3-CH_3). ESI-MS: positive mode 370.4 $[M+H]^+$. HPLC: 96.6% (A) and 96.8% (B).

5.1.2.21. 8-(3,4-Difluorophenyl)ethyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4-(1*H*,3*H*)-dione (26). Yield: 75%; mp: 178 °C; ^{1}H NMR ($CDCl_3$) δ 7.02–6.94 (m, 3H, C^2-/C5-/C6-H, phe), 4.32 (t, ^3J = 5.1 Hz, 2H, C6-H_2), 3.82 (s, 2H, C9-H_2), 3.54 (s, 3H, N1-CH_3), 3.37 (s, 3H, N3-CH_3), 2.97 (t, ^3J = 5.4 Hz, 2H, C7-H_2), 2.91 (t, ^3J = 7.9 Hz, 2H, N8-CH_2), 2.83 (t, ^3J = 7.6 Hz, 2H, N8-CH_2-CH_2). ^{13}C NMR ($CDCl_3$) δ 155.0 (C9a), 151.7 (C4), 150.6 (dd, $^1J_{C,F}$ = 248.1 Hz, $^2J_{C,F}$ = 13.0 Hz, C3, phe), 148.9 (dd, $^1J_{C,F}$ = 245.8 Hz, $^2J_{C,F}$ = 12.0 Hz, C4, phe), 148.5 (C2), 147.8 (C10a), 128.7 (d, $J_{C,F}$ = 12.7 Hz, C1, phe), 125.4 (dd, $J_{C,F}$ = 6.4 Hz, $J_{C,F}$ = 3.2 Hz, C2, phe), 124.0 (dd, $J_{C,F}$ = 6.7 Hz, $J_{C,F}$ = 4.7 Hz, C3, phe), 115.5 (d, $J_{C,F}$ = 17.2 Hz, C6, phe), 106.6 (C4a), 57.3 (N8-CH_2), 50.9 (C7), 48.6 (C9), 44.2 (C6), 29.7 (N1-CH_3), 27.8 (N3-CH_3), 26.8 (N8-CH_2-CH_2). ESI-MS: positive mode 376.0 $[M+H]^+$. HPLC: 97.8% (A) and 96.2% (B).

5.1.2.22. 8-(2-Fluoro-5-(trifluoromethyl)phenethyl)-1,3-dimethyl-6,7,8,9-tetrahydropyra-zino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (27). Yield: 61%; mp: 226 °C; ^{1}H NMR ($CDCl_3$) δ 7.72–7.70 (m, 1H, C6-H, phe), 7.59–7.56 (m, 1H, C4-H, phe), 7.19–7.17 (m, 1H, C5-H, phe), 4.38 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 3.81 (s, 2H, C9-H_2), 3.52 (s, 3H, N1-CH_3), 3.37 (s, 3H, N3-CH_3), 3.02 (t, ^3J = 5.4 Hz, 2H, C7-H_2), 2.83 (br s, 4H, N8-CH_2-CH_2). ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.0 (d, $^1J_{C,F}$ = 251.3 Hz, C-6, phenyl), 155.0 (C-9a), 151.7 (C-4), 148.5 (C-2), 147.6 (C-10a), 128.7 (C-6, phenyl), 127.3 (C-5, phenyl), 126.8 (C-1, phenyl), 126.2 (q, $^1J_{C,F}$ = 270.4 Hz, CF_3), 124.4 (C-1, phenyl), 116.4 (C-3, phenyl), 106.5 (C-4a), 53.9 (N8-CH_2), 50.9 (C-7), 48.6 (C-9), 44.2 (C-6), 29.7 (N1-CH_3), 27.8 (N3-CH_3). ESI-MS: positive mode 426.0 $[M+H]^+$. HPLC: 99.6% (A) and 98.3% (B).

5.1.2.23. 8-(2,6-Dichlorophenethyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4-(1*H*,3*H*)-dione (28). Yield: 74%; mp: 221 °C; ^{1}H NMR ($CDCl_3$) δ 7.33 (d, ^3J = 7.9 Hz, 2H, C3-/C5-H, phe), 7.09–7.08 (m, 1H, C4-H, phe), 4.40 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 3.95 (s, 2H, C9-H_2), 3.53 (s, 3H, N1-CH_3), 3.37 (s, 3H, N3-CH_3), 3.24 (t, ^3J = 7.3 Hz, 2H, N8-CH_2), 3.09 (t, ^3J = 5.4 Hz, 2H, C7-H_2), 2.84 (t, ^3J = 7.3 Hz, 2H, N8-CH_2-CH_2). ^{13}C NMR ($CDCl_3$) δ 155.0 (C9a), 151.7 (C4), 148.5 (C2), 147.6 (C10a), 143.1 (C1, phe), 135.5 (C2/C6, phe), 128.3 (C^3/C^4/C5, phe), 106.5 (C4a), 55.0 (N8-CH_2), 51.1 (C7), 49.0 (C9), 44.1 (C6), 29.7 (N1-CH_3), 28.6 (N8-CH_2-CH_2), 27.8 (N3-CH_3). ESI-MS: positive mode 408.3 $[M+H]^+$. HPLC: 99.2% (A) and 99.1% (B).

5.1.2.24. 8-(3,4-Dichlorophenethyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4-(1*H*,3*H*)-dione (29). Yield: 81%; mp: 177 °C; ^{1}H NMR ($CDCl_3$) δ 7.33 (d, ^3J = 8.2 Hz, 1H, C5-H, phe), 7.29 (d, ^4J = 2.0 Hz, 1H, C2-H, phe), 7.02 (dd, ^3J = 8.2 Hz, ^4J = 2.0 Hz, 1H, C6-H, phe), 4.34 (t, ^3J = 5.1 Hz, 2H, C6-H_2), 3.81 (s, 2H, C9-H_2), 3.54 (s, 3H, N1-CH_3), 3.38 (s, 3H, N3-CH_3), 2.97 (t, ^3J = 5.1 Hz, 2H, C7-H_2), 2.82 (br s, 4H, N8-CH_2-CH_2). ^{13}C NMR ($CDCl_3$) δ 155.0 (C9a), 151.7 (C4), 148.4 (C2), 147.6 (C10a), 139.4 (C1, phe), 132.5 (C3, phe), 130.6 (C5, phe), 130.5 (C4, phe), 130.5 (C2, phe), 128.1 (C6, phe), 106.6 (C4a), 58.4 (N8-CH_2), 51.2 (C7), 49.2 (C9), 44.1 (C6), 32.6 (N8-CH_2-CH_2), 29.8 (N1-CH_3), 27.9 (N3-CH_3). ESI-MS: positive mode 408.3 $[M+H]^+$. HPLC: 99.2% (A) and 99.1% (B).

5.1.2.25. 8-(2,4-Dichlorophenethyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4-(1*H*,3*H*)-dione (30). Yield: 70%; mp: 185 °C; ^{1}H NMR ($CDCl_3$) δ 7.36 (d, ^4J = 1.1 Hz, ^4J = 1.1 Hz, 1H, C3-H, phe), 7.17–7.16 (m, 2H, C5-/C6-H, phe), 4.34 (br s, 2H, C6-H_2), 3.87 (s, 2H, C9-H_2), 3.53 (s, 3H, N1-CH_3), 3.37 (s, 3H, N3-CH_3), 3.02 (br s, 2H, C7-H_2), 2.97 (t, ^3J = 6.9 Hz, 2H, N8-CH_2), 2.84 (t, ^3J = 7.3 Hz, 2H, N8-CH_2-CH_2). ^{13}C NMR ($CDCl_3$) δ 155.0 (C9a), 151.7 (C4), 148.5 (C2), 147.6 (C10a), 135.3 (C1, phe), 134.6 (C2, phe), 133.0 (C4, phe), 131.5 (C6, phe), 129.4 (C3, phe), 127.3 (C5, phe), 106.5 (C4a), 56.9 (N8-CH_2), 51.1 (C7), 49.0 (C9), 44.1 (C6), 30.6 (N8-CH_2-CH_2), 29.7 (N1-CH_3), 27.8 (N3-CH_3). ESI-MS: positive mode 408.3 $[M+H]^+$. HPLC: 98.9% (A) and 99.0% (B).

5.1.2.26. 8-(4-Chloro-2-(trifluoromethyl)phenethyl)-1,3-dimethyl-6,7,8,9-tetrahydropyr-azino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (31). Purification by column chromatography. Yield: 60%; mp: 195 °C; ^{1}H NMR ($CDCl_3$) δ 7.61 (d, ^4J = 2.2 Hz, 1H, C3-H, phe), 7.44 (dd, ^3J = 8.2 Hz, ^4J = 1.9 Hz, 1H, C5-H, phe), 7.29 (d, ^3J = 8.2 Hz, 1H, C6-H, phe), 4.34 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 3.84 (s, 2H, C9-H_2), 3.54 (s, 3H, N1-CH_3), 3.38 (s, 3H, N3-CH_3), 3.02–2.97 (m, 4H, C7-H_2, N8-CH_2), 2.83–2.80 (m, 2H, N8-CH_2-CH_2). ^{13}C NMR (125 MHz, $CDCl_3$) δ 155.0 (C-9a), 151.7 (C-4), 148.5 (C-2), 147.6 (C-10a), 134.4 (C-4, phenyl), 133.7 (C-5, phenyl), 130.7 (C-6, phenyl), 130.5 (C-1, phenyl), 130.2 (C-2, phenyl), 126.4 (C-3, phenyl), 126.3 (q, $^1J_{C,F}$ = 272.6 Hz, CF_3), 106.5 (C-4a), 56.9 (N8-CH_2), 51.4 (C-7), 49.0 (C-9), 44.4 (C-6), 29.8 (N1-CH_3), 27.9 (N3-CH_3). ESI-MS: positive mode 442.0 $[M+H]^+$. HPLC: 99.9% (A) and 99.1% (B).

5.1.2.27. 8-(2-(5-Bromo-2-methoxyphenyl)ethyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazi-no[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (32). Purification by column chromatography. Yield: 36%; mp: 198 °C; ^{1}H NMR ($CDCl_3$) δ 7.29 (d, ^3J = 8.9 Hz, 1H, C4-H, phe), 7.26 (s, 1H, C6-H, phe), 6.72 (d, ^3J = 8.5 Hz, 1H, C3-H, phe), 4.36 (br s, 2H, C6-H_2), 3.86 (s, 2H, C9-H_2), 3.82 (s, 3H, OCH_3), 3.53 (s, 3H, N1-CH_3), 3.37 (s, 3H, N3-CH_3), 3.00 (br s, 2H, C7-H_2), 2.84–2.81 (m, 4H, N8-CH_2-CH_2). ^{13}C NMR ($CDCl_3$) δ 156.5 (C2, phe), 155.0 (C9a), 151.7 (C4), 148.5 (C2), 147.6 (C10a), 132.8 (C6, phe), 130.2 (C5, phe), 129.9 (C4, phe), 112.6 (C1, phe), 112.0 (C3, phe), 106.5 (C4a), 58.7 (N8-CH_2), 55.5 (OCH_3), 51.3 (C7), 48.9 (C9), 44.2 (C6), 29.7 (N1-CH_3), 27.8 (N3-CH_3), 27.7 (N8-CH_2-CH_2). ESI-MS: positive mode 450.1 and 452.1 $[M+H]^+$. HPLC: 99.2% (A) and 99.3% (B).

5.1.2.28. 8-(2-(2,3-Dimethoxyphenyl)ethyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (33). Purification by column chromatography. Yield: 39%; mp: 180 °C; ^{1}H NMR ($CDCl_3$) δ 7.00–6.97 (m, 1H, C5-H, phe), 6.81–6.78 (m, 2H, C4-/C6-H, phe), 4.40 (br s, 2H, C6-H_2), 3.85–3.84 (s, 8H, C^9-H_2, 2 × OCH_3), 3.53 (s, 3H, N1-CH_3), 3.37 (s, 3H, N3-CH_3), 3.06–2.87 (m, 6H, C7-H_2, N8-CH_2-CH_2). ^{13}C NMR ($CDCl_3$) δ 155.0 (C9a), 152.8 (C3, phe), 151.7 (C4), 150.0 (C, phe), 148.5 (C2), 147.6 (C10a), 147.1 (C2, phe), 124.2 (C1, phe), 124.1 (C6, phe), 123.6 (C5, phe), 111.8 (C4, phe), 106.5 (C4a), 60.7 (N8-CH_2), 55.7 (2 × OCH_3), 51.4 (C7), 49.0 (C9), 44.2 (C6), 29.7 (N1-CH_3), 28.0 (N8-CH_2-CH_2), 27.9 (N3-CH_3). ESI-MS: positive mode 400.5 $[M+H]^+$. HPLC: 97.5% (A) and 99.6% (B).

5.1.2.29. 8-(2-(3,4-Dimethoxyphenyl)ethyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4-(1*H*,3*H*)-dione (34). Yield: 48%; mp: 219 °C; ^{1}H NMR ($CDCl_3$) δ 6.89–6.83 (m, 3H, C^2-/C5-/C6-H, phe), 4.46 (br s, 2H, C6-H_2), 3.95 (s, 2H, C9-H_2), 3.86 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.53 (s, 3H, N1-CH_3), 3.37 (s, 3H, N3-CH_3),

3.10–2.89 (br s, 6H, C^3-H_2, N8-CH_2-CH_2). ^{13}C NMR ($CDCl_3$) δ 155.0 (C9a), 151.7 (C4), 149.1 (C3, phe), 148.5 (C2), 147.9 (C4, phe), 147.8 (C10a), 129.2 (C1, phe), 120.5 (C6, phe), 111.9 (C5, phe), 111.4 (C2, phe), 106.5 (C4a), 60.7 (N8-CH_2), 55.9 (OCH_3), 55.9 (OCH_3), 51.4 (C7), 49.0 (C9), 44.2 (C6), 29.7 (N1-CH_3), 28.0 (N8-CH_2-*CH_2*), 27.9 (N3-CH_3). ESI-MS: positive mode 400.5 $[M+H]^+$. HPLC: 96.7% (A) and 96.9% (B).

5.1.2.30. 8-(2-(Benzo[*d*][1,3]dioxol-5-yl)ethyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino-[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (35). Yield: 45%; mp: 286 °C; ^{1}H NMR ($CDCl_3$) δ 6.72 (d, ^3J = 7.9 Hz, 1H, C5-H, phe), 6.68 (d, ^4J = 1.3 Hz, 1H, C2-H, phe), 6.63 (dd, ^3J = 8.2 Hz, ^4J = 1.6 Hz, 1H, C6-H, phe), 5.91 (s, 2H, O-CH_2-O), 4.33 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 3.81 (s, 2H, C9-H_2), 3.54 (s, 3H, N1-CH_3), 3.37 (s, 3H, N3-CH_3), 2.95 (t, ^3J = 5.4 Hz, 2H, C7-H_2), 2.78 (br s, 4H, N8-CH_2-CH_2). ^{13}C NMR ($CDCl_3$) δ 155.0 (C9a), 151.7 (C4), 148.5 (C2), 147.9 (C10a), 147.7 (C3, phe), 146.1 (C4, phe), 133.0 (C1, phe), 121.5 (C6, phe), 108.9 (C5, phe), 108.3 (C2, phe), 106.6 (C4a), 100.9 (O-CH_2-O), 58.6 (N8-CH_2), 51.3 (C7), 49.1 (C9), 44.0 (C6), 33.2 (N8-CH_2-*CH_2*), 29.7 (N1-CH_3), 27.8 (N3-CH_3). ESI-MS: positive mode 384.3 $[M+H]^+$. HPLC: 96.5% (A) and 96.7% (B).

5.1.2.31. 8-(2-Hydroxy-2-phenylethyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4-(1*H*,3*H*)-dione (36). Purification by column chromatography. Yield: 45%; mp: 194 °C; ^{1}H NMR ($CDCl_3$) δ 7.38–7.33 (m, 4H, phe), 7.31–7.28 (m, 1H, phe), 4.89 (d, ^3J = 3.8 Hz, 1H, N8-CH_2-*CH*), 4.41 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 4.06 (d, ^3J = 16.4 Hz, 1H, C^9-H), 3.89 (d, ^3J = 16.4 Hz, 1H, C^9-H), 3.53 (s, 3H, N1-CH_3), 3.37 (s, 3H, N3-CH_3), 3.22 (t, ^3J = 6.0 Hz, 2H, C7-H_2), 2.80 (t, ^3J = 3.5 Hz, 2H, N8-CH_2), OH not observed. ^{13}C NMR ($CDCl_3$) δ 155.0 (C9a), 151.7 (C4), 148.5 (C2), 147.6 (C10a), 141.0 (C1, phe), 128.6 (C3/C5, phe), 127.9 (C4, phe), 125.8 (C2/C6, phe), 106.5 (C4a), 69.8 (N8-CH_2-*CH*), 65.1 (N8-CH_2), 51.1 (C7), 49.0 (C9), 43.9 (C6), 29.8 (N1-CH_3), 27.9 (N3-CH_3). ESI-MS: negative mode 354.3 $[M-H]^-$, positive mode 356.3 $[M+H]^+$. HPLC: 99.8% (A) and 99.7% (B).

5.1.2.32. 1,3-Dimethyl-8-(2-methyl-2-phenylethyl)-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4-(1*H*,3*H*)-dione (37). Yield: 50%; mp: 175 °C; ^{1}H NMR ($CDCl_3$) δ 7.36–7.33 (m, 2H, phe), 7.28–7.26 (m, 3H, phe), 4.49 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 3.99 (s, 2H, C9-H_2), 3.53 (s, 3H, N1-CH_3), 3.37 (s, 3H, N3-CH_3), 3.13–3.07 (m, 5H, C7-H_2, N8-CH_2, N8-CH_2-*CH*), 1.37 (t, ^3J = 7.0 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ 155.0 (C9a), 151.7 (C4), 148.5 (C2), 147.6 (C10a), 143.5 (C1, phe), 129.2 (C3/C5, phe), 127.0 (C4, phe), 126.9 (C2/C6, phe), 106.8 (C4a), 63.9 (N8-CH_2), 51.1 (C7), 49.0 (C9), 43.9 (C6), 37.0 (N8-CH_2-*CH*), 29.8 (N1-CH_3), 27.9 (N3-CH_3), 18.8 (β-CH_3). ESI-MS: positive mode 354.4 $[M+H]^+$. HPLC: 99.9% (A) and 99.8% (B).

5.1.2.33. 1,3-Dimethyl-8-(2-(thiophen-2-yl)ethyl)-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine2,4-(1*H*,3*H*)-dione (38). Purification by column chromatography. Yield: 28%; mp: 150 °C; ^{1}H NMR ($CDCl_3$) δ 7.13 (dd, ^3J = 5.0 Hz, ^4J = 0.9 Hz, 1H, C^3-H, thiophe), 6.91 (dd, ^3J = 5.1 Hz, ^3J = 3.5 Hz, 1H, C^4-H, thiophe), 6.63 (dd, ^3J = 3.5 Hz, ^4J = 0.9 Hz, 1H, C5-H, thiophe), 4.38 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 3.87 (s, 2H, C9-H_2), 3.54 (s, 3H, N1-CH_3), 3.38 (s, 3H, N3-CH_3), 3.11 (t, ^3J = 5.4 Hz, 2H, C7-H_2), 3.00 (br s, 2H, N8-CH_2), 2.91 (t, ^3J = 7.0 Hz, 2H, N8-CH_2-*CH_2*). ^{13}C NMR ($CDCl_3$) δ 155.0 (C9a), 151.7 (C4), 148.5 (C2), 148.5 (C10a), 136.0 (C1, thiophe), 126.8 (C5, thiophe), 125.1 (C4, thiophe), 123.9 (C3, thiophe), 106.6 (C4a), 58.6 (N8-CH_2), 51.2 (C7), 49.0 (C9), 44.0 (C6), 33.2 (N8-CH_2-*CH_2*), 29.7 (N1-CH_3), 27.8 (N3-CH_3). ESI-MS: positive mode 364.4 $[M+H]^+$. HPLC: 97.7% (A) and 97.9% (B).

5.1.2.34. 8-(2-(1*H*-Pyrrol-1-yl)ethyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (39). Yield: 48%; mp: 171 °C; ^{1}H NMR ($CDCl_3$) δ 6.67–6.66 (m, 2H, C^2-/C^5-H, pyrrol), 6.14–6.13 (m, 2H, C^3-/C^4-H, pyrrol), 4.47 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 4.06 (t, ^3J = 6.3 Hz, 2H, N8-CH_2-*CH_2*), 3.79 (s, 2H, C9-H_2), 3.52 (s, 3H, N1-CH_3), 3.37 (s, 3H, N3-CH_3), 2.93 (t, ^3J = 6.3 Hz, 2H, N8-CH_2), 2.78 (t, ^3J = 5.4 Hz, 2H, C7-H_2). ^{13}C NMR ($CDCl_3$) δ 155.0 (C9a), 151.7 (C4), 148.5 (C2), 122.2 (C^2/C^5, pyrrol), 109.1 (C^3/C^4, pyrrol), 106.5 (C4a), 58.7 (N8-CH_2), 51.4 (C7), 49.0 (C9), 46.4 (N8-CH_2-*CH_2*), 44.2 (C6), 29.7 (N1-CH_3), 27.9 (N3-CH_3). ESI-MS: positive mode 329.3 $[M+H]^+$. HPLC: 98.5% (A) and 99.1% (B).

5.1.2.35. 1,3-Dimethyl-8-(3-phenylpropyl)-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (40). Yield: 81%; mp: 148 °C; ^{1}H NMR ($CDCl_3$) δ 7.28–7.16 (m, 5H, phe), 4.32 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 3.74 (s, 2H, C9-H_2), 3.53 (s, 3H, N1-CH_3), 3.37 (s, 3H, N3-CH_3), 2.89 (t, ^3J = 5.4 Hz, 2H, C7-H_2), 2.67 (t, ^3J = 7.3 Hz, 2H, N8-CH_2) 2.57 (t, ^3J = 7.3 Hz, 2H, N8-CH_2-CH_2-*CH_2*), 1.87 (tt, ^3J = 7.3 Hz, ^3J = 7.3 Hz, 2H, N8-CH_2-*CH_2*). ^{13}C NMR ($CDCl_3$) δ 159.0 (C9a), 154.9 (C4), 151.6 (C2), 148.5 (C10a), 141.5 (C1, phe), 128.5 (C3/C5, phe), 128.4 (C2/C6, phe), 126.0 (C4, phe), 106.5 (C4a), 56.4 (C7), 51.9 (C9), 48.8 (N8-CH_2), 47.5 (C6), 31.3 (N8-CH_2-CH_2-*CH_2*), 29.8 (N1-CH_3), 28.3 (N8-CH_2-*CH_2*), 27.8 (N3-CH_3). ESI-MS: positive mode: 354.1 $[M+H]^+$. HPLC: 99.9% (A) and 99.8% (B).

5.1.2.36. 8-(3-(4-Chlorophenyl)propyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (41). Purification by column chromatography. Yield: 81%; mp: 124 °C; ^{1}H NMR ($CDCl_3$) δ 7.24–7.20 (m, 2H, C3-/C5-H, phe), 7.09–7.06 (m, 2H, C2-/C6-H, phe), 4.32 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 3.72 (s, 2H, C9-H_2), 3.53 (s, 3H, N1-CH_3), 3.37 (s, 3H, N3-CH_3), 2.88 (t, ^3J = 5.4 Hz, 2H, C7-H_2), 2.68–2.66–2.63 (m, 2H, N8-CH_2-CH_2-*CH_2*), 2.55 (tt, ^3J = 7.2 Hz, 2H, N8-CH_2), 1.87–1.82 (m, 2H, N8-CH_2-*CH_2*). ^{13}C NMR ($CDCl_3$) δ 159.0 (C9a), 154.9 (C4), 151.6 (C2), 148.5 (C10a), 139.9 (C1, phe), 131.7 (C4, phe), 129.7 (C2/C6, phe), 128.4 (C3/C5, phe), 106.5 (C4a), 56.4 (C7), 51.9 (C9), 48.8 (N8-CH_2), 47.5 (C6), 31.3 (N8-CH_2-CH_2-*CH_2*), 29.8 (N1-CH_3), 28.3 (N8-CH_2-*CH_2*), 27.8 (N3-CH_3). ESI-MS: positive mode: 388.3 $[M+H]^+$. HPLC: 98.6% (A) and 99.2% (B).

5.1.2.37. 8-(3-(3-Bromophenyl)propyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4-(1*H*,3*H*)-dione (42). Yield: 71%; mp: 164 °C; ^{1}H NMR ($CDCl_3$) δ 7.31–7.30 (m, 2H, C^2-/C^5-H, phe), 7.15–7.11 (m, 1H, C4-H, phe), 7.09–7.07 (m, 1H, C6-H, phe), 4.34 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 3.73 (s, 2H, C9-H_2), 3.53 (s, 3H, N1-CH_3), 3.37 (s, 3H, N3-CH_3), 2.73 (t, ^3J = 5.4 Hz, 2H, C7-H_2), 2.64 (t, ^3J = 8.3 Hz, 2H, N8-CH_2), 2.55 (t, ^3J = 7.0 Hz, 2H, N8-CH_2-CH_2-*CH_2*), 1.88–1.82 (m, 2H, N8-CH_2-*CH_2*). ^{13}C NMR ($CDCl_3$) δ 155.0 (C9a), 151.7 (C4), 148.5 (C2), 147.6 (C10a), 143.9 (C1, phe), 131.5 (C6, phe), 130.0 (C3, phe), 129.1 (C5, phe), 127.1 (C2, phe), 122.5 (C4, phe), 106.6 (C4a), 56.4 (N8-CH_2), 51.3 (C7), 49.1 (C9), 44.1 (C6), 32.7 (N8-CH_2-CH_2-*CH_2*), 29.8 (N1-CH_3), 28.2 (N8-CH_2-*CH_2*), 27.8 (N3-CH_3). ESI-MS: positive mode 433.0 and 435.1 $[M+H]^+$. HPLC: 99.7% (A) and 98.1% (B).

5.1.2.38. 8-(3-(1*H*-Pyrrol-1-yl)propyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4-(1*H*,3*H*)-dione (43). Yield: 69%; mp: 171 °C; ^{1}H NMR ($CDCl_3$) δ 6.62–6.61 (m, 2H, C^2-/C^5-H, pyrrol), 6.13–6.12 (m, 2H, C^3-/C^4-H, pyrrol), 4.32 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 4.06 (t, ^3J = 6.7 Hz, 2H, N8-CH_2-CH_2-*CH_2*), 3.72 (s, 2H, C9-H_2), 3.53 (s, 3H, N1-CH_3), 3.37 (s, 3H, N3-CH_3), 2.87 (t, ^3J = 5.4 Hz, 2H, C7-H_2), 2.48 (t, ^3J = 6.7 Hz, 2H, N8-CH_2), 1.97 (tt, ^3J = 6.6 Hz, ^3J = 6.7 Hz, 2H, N8-CH_2-*CH_2*). ^{13}C NMR ($CDCl_3$) δ 155.0 (C9a), 151.7 (C4), 148.5 (C2), 120.6 (C^2/C^5, pyrrol), 108.2 (C^3/C^4, pyrrol), 106.5 (C4a), 53.9 (N8-CH_2), 51.3 (C7), 49.2 (C9), 46.6

(N8-CH_2-CH_2-CH_2), 44.2 (C6), 28.7 (N8-CH_2-CH_2), 29.8 (N1-CH_3), 27.8 (N3-CH_3). ESI-MS: positive mode 343.1 $[M+H]^+$. HPLC: 99.7% (A) and 99.4% (B).

5.1.2.39. 8-(3-(1*H*-Imidazol-1-yl)propyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (44). Purification by column chromatography. Yield: 55%; mp: 170 °C; ^{1}H NMR ($CDCl_3$) δ 7.46 (s, 1H, C2-H, imidazolyl), 7.07–7.06 (m, 1H, C4-H, imidazolyl), 6.90–6.89 (m, 1H, C5-H, imidazolyl), 4.34 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 4.05 (t, ^3J = 7.3 Hz, 2H, N8-CH_2-CH_2-CH_2), 3.74 (s, 2H, C9-H_2), 3.53 (s, 3H, N1-CH_3), 3.37 (s, 3H, N3-CH_3), 2.98 (t, ^3J = 5.4 Hz, 2H, C7-H_2), 2.50 (t, ^3J = 7.3 Hz, 2H, N8-CH_2), 2.03–1.98 (m, 2H, N8-CH_2-CH_2). ^{13}C NMR ($CDCl_3$) δ 159.0 (C9a), 154.9 (C4), 151.6 (C2), 148.5 (C10a), 137.3 (C2, imidazolyl), 129.7 (C4, imidazolyl), 128.8 (C5, imidazolyl), 106.5 (C4a), 53.5 (C7), 51.2 (C9), 49.3 (N8-CH_2), 44.2 (C6), 34.6 (N8-CH_2-CH_2-CH_2), 29.8 (N1-CH_3), 28.0 (N8-CH_2-CH_2), 27.8 (N3-CH_3). ESI-MS: positive mode: 344.3 $[M+H]^+$. HPLC: 99.9% (A) and 98.8% (B).

5.1.2.40. 1,3-Dimethyl-8-(2-phenoxyethyl)-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (45). Purification by column chromatography. Yield: 49%; mp: 168 °C; ^{1}H NMR ($CDCl_3$) δ 7.27–7.23 (m, 2H, C3-/C5-H, phe), 6.95–6.92 (dd, ^3J = 7.3 Hz, ^3J = 7.3 Hz, 1H, C4-H, phe), 6.84 (d, ^3J = 7.8 Hz, 2H, C2-/C6-H, phe), 4.47 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 4.22 (t, ^3J = 5.4 Hz, 2H, N8-CH_2-CH_2), 3.84 (s, 2H, C9-H_2), 3.51 (s, 3H, N1-CH_3), 3.37 (s, 3H, N3-CH_3), 3.12 (t, ^3J = 5.4 Hz, 2H, N8-CH_2), 3.08 (t, ^3J = 5.4 Hz, 2H, C7-H_2). ^{13}C NMR ($CDCl_3$) δ 158.3 (C1, phe), 155.0 (C9a), 151.7 (C4), 148.5 (C2), 147.6 (C10a), 129.5 (C3/C5, phe), 121.2 (C4, phe), 114.5 (C2/C6, phe), 106.5 (C4a), 66.0 (N8-CH_2–CH_2), 57.4 (N8-CH_2), 51.7 (C7), 49.5 (C9), 44.2 (C6), 29.7 (N1-CH_3), 27.8 (N3-CH_3). ESI-MS: positive mode 356.4 $[M+H]^+$. HPLC: 97.5% (A) and 96.9% (B).

5.1.2.41. 8-(2-(2-Fluorophenoxy)ethyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (46). Yield: 50%; mp: 160 °C; ^{1}H NMR ($CDCl_3$) δ 7.07–7.01 (m, 2H, C3-/C5-H, phe), 6.97–6.96 (m, 1H, C4-H, phe), 6.92–6.87 (m, 1H, C6-H, phe), 4.47 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 4.22 (t, ^3J = 5.4 Hz, 2H, N8-CH_2-CH_2), 3.95 (s, 2H, C9-H_2), 3.51 (s, 3H, N1-CH_3), 3.37 (s, 3H, N3-CH_3), 3.12 (t, ^3J = 5.4 Hz, 2H, N8-CH_2), 3.07 (t, ^3J = 5.4 Hz, 2H, C7-H_2). ^{13}C NMR ($CDCl_3$) δ 155.0 (C9a), 152.9 (d, $^1J_{C,F}$ = 246.0 Hz, C2, phe), 151.7 (C4), 148.5 (C2), 147.6 (C10a), 146.4 (d, $^2J_{C,F}$ = 10.7 Hz, C1, phe), 124.3 (d, $^4J_{C,F}$ = 3.8 Hz, C5, phe), 121.9 (d, $^3J_{C,F}$ = 6.9 Hz, C4, phe), 116.4 (d, $^2J_{C,F}$ = 18.2 Hz, C3, phe), 115.5 (d, $^3J_{C,F}$ = 0.8 Hz, C6, phe), 106.5 (C4a), 67.8 (N8-CH_2–CH_2), 56.0 (N8-CH_2), 51.7 (C7), 49.5 (C9), 44.2 (C6), 29.7 (N1-CH_3), 27.8 (N3-CH_3). ESI-MS: positive mode 374.4 $[M+H]^+$. HPLC: 96.3% (A) and 97.8% (B).

5.1.2.42. 8-(2-(3-Fluorophenoxy)ethyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (47). Yield: 63%; mp: 204 °C; ^{1}H NMR ($CDCl_3$) δ 7.22–7.19 (m, 1H, C4-H, phe), 6.69–6.66 (m, 2H, C^2-/C^5-H, phe), 6.62–6.59 (m, 1H, C6-H, phe), 4.47 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 4.22 (t, ^3J = 5.1 Hz, 2H, N8-CH_2-CH_2), 3.95 (s, 2H, C9-H_2), 3.53 (s, 3H, N1-CH_3), 3.37 (s, 3H, N3-CH_3), 3.09 (t, ^3J = 5.4 Hz, 2H, C7-H_2), 3.06 (t, ^3J = 5.1 Hz, 2H, N8-CH_2). ^{13}C NMR ($CDCl_3$) δ 163.6 (d, $^1J_{C,F}$ = 245.7 Hz, C3, phe), 159.7 (d, $^3J_{C,F}$ = 10.8 Hz, C1, phe), 155.0 (C9a), 151.7 (C4), 148.5 (C2), 147.6 (C10a), 130.4 (d, $^3J_{C,F}$ = 10.0 Hz, C5, phe), 110.3 (d, $^4J_{C,F}$ = 2.7 Hz, C6, phe), 108.1 (d, $^2J_{C,F}$ = 21.3 Hz, C4, phe), 106.5 (C4a), 102.2 (d, $^2J_{C,F}$ = 25.9 Hz, C2, phe), 66.3 (N8-CH_2–CH_2), 56.1 (N8-CH_2), 51.7 (C7), 49.6 (C9), 44.1 (C6), 29.8 (N1-CH_3), 27.9 (N3-CH_3). ESI-MS: positive mode 374.4 $[M+H]^+$. HPLC: 96.3% (A) and 97.8% (B).

5.1.2.43. 8-(2-(3-Fluorophenoxy)ethyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (48). Purification by column chromatography. Yield: 10%; mp: 194 °C; ^{1}H NMR ($CDCl_3$) δ 7.31 (d, ^3J = 8.9 Hz, 1H, C5-H, phe), 6.99 (d, ^4J = 2.9 Hz, 1H, C2-H, phe), 6.75 (dd, ^3J = 8.6 Hz, ^4J = 2.2 Hz, 1H, C6-H, phe), 4.36 (t, ^3J = 5.5 Hz, 2H, C6-H_2), 4.12 (t, ^3J = 5.2 Hz, 2H, N8-CH_2-CH_2), 3.92 (s, 2H, C9-H_2), 3.53 (s, 3H, N1-CH_3), 3.37 (s, 3H, N3-CH_3), 3.08–3.06 (m, 2H, C7-H_2), 3.03 (t, ^3J = 5.2 Hz, 2H, N8-CH_2). ^{13}C NMR ($CDCl_3$) δ 157.4 (C1, phe), 155.0 (C9a), 151.7 (C4), 148.5 (C2), 147.6 (C10a), 133.0 (C3, phe), 130.8 (C5, phe), 124.6 (C4, phe), 116.4 (C2, phe), 114.5 (C6, phe), 106.6 (C4a), 66.8 (N8-CH_2–CH_2), 56.0 (N8-CH_2), 51.7 (C7), 49.6 (C9), 44.2 (C6), 29.8 (N1-CH_3), 27.9 (N3-CH_3). ESI-MS: positive mode 424.3 $[M+H]^+$. HPLC: 100% (C).

5.1.2.44. (*R*)-1,3-Dimethyl-8-(2,1,3,4-tetrahydronaphthalen-1-yl)-6,7,8,9-tetrahydropyra-zino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (49). Purification by column chromatography. Yield: 24%; mp: 202 °C; ^{1}H NMR ($CDCl_3$) δ 7.75 (br s, 1H, tetrahydronaphthyl), 7.21–7.19 (m, 2H, tetrahydronaphthyl), 7.14–7.11 (m, 1H, tetrahydronaphthyl), 4.58–4.57 (m, 1H, C^1-H, tetrahydronaphthyl), 4.47 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 4.08 (s, 2H, C9-H_2), 3.52 (s, 3H, N1-CH_3), 3.37 (s, 3H, N3-CH_3), 3.18 (br s, 2H, C7-H_2), 2.88–2.76 (m, 2H, C^4-H_2, tetrahydronaphthyl), 2.13–1.75 (m, 4H, C^2-/C^3-H_2, tetrahydronaphthyl). ^{13}C NMR ($CDCl_3$) δ 159.0 (C9a), 155.0 (C4), 151.7 (C2), 148.5 (C10a), 138.9.1 (C^{4a}/C^{8a}, tetrahydronaphthyl), 129.6 (C^6/C^7, tetrahydronaphthyl), 126.7 (C^5/C^8, tetrahydronaphthyl), 106.8 (C4a), 63.0 (C1, tetrahydronaphthyl), 49.6 (C7), 47.3 (C9), 44.2 (C6), 29.9 (N1-CH_3), 29.3 (C4, tetrahydronaphthyl), 27.9 (N3-CH_3), 22.5 (C2, tetrahydronaphthyl), 21.0 (C3, tetrahydronaphthyl). ESI-MS: positive mode 366.0 $[M+H]^+$. HPLC: 98.0% (A) and 97.8% (B).

5.1.2.45. (S)-8-(5-Methoxy-2,1,3,4-tetrahydronaphth-2-yl)-1,3-dimethyl-6,7,8,9-tetra-hydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (50). Purification by column chromatography. Yield: 31%; mp: 280 °C; ^{1}H NMR ($CDCl_3$) δ 7.09 (dd, ^3J = 7.9 Hz, ^3J = 7.9 Hz, 1H, C7-H, tetrahydronaphthyl), 6.69 (d, ^3J = 7.8 Hz, 1H, C8-H, tetrahydronaphthyl), 6.66 (d, ^3J = 7.9 Hz, 1H, C6-H, tetrahydronaphthyl), 3.99 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 4.42 (s, 2H, C9-H_2), 3.80 (3H, s, OCH_3), 3.53 (s, 3H, N1-CH_3), 3.37 (s, 3H, N3-CH_3) 3.14–2.87 (m, 6H, C1-/C4-H_2, tetrahydronaphthyl, C7-H_2), 2.61–2.54 (m, 1H, C2-H, tetrahydronaphthyl), 1.72–1.63 (m, 2H, C3-H2, tetrahydronaphthyl). ^{13}C NMR ($CDCl_3$) δ 159.0 (C9a), 157.1 (C4), 154.9 (C6, tetrahydronaphthyl), 151.7 (C2), 148.5 (C10a), 135.8 (C8a, tetrahydronaphthyl), 126.5 (C7, tetrahydronaphthyl), 124.6 (C4a, tetrahydronaphthyl), 121.5 (C8, tetrahydronaphthyl), 107.3 (C6, tetrahydronaphthyl), 106.5 (C4a), 59.3 (C2, tetrahydronaphthyl), 55.2 (OCH_3), 47.7 (C7), 45.7 (C9), 44.7 (C6), 31.9 (C1, tetrahydronaphthyl), 29.7 (N1-CH_3), 27.9 (N3-CH_3), 25.6 (C3, tetrahydronaphthyl), 22.9 (C4, tetrahydronaphthyl). ESI-MS: positive mode 396.1 $[M+H]^+$. HPLC: 97.6% (A) and 97.4% (B).

5.1.2.46. 8-(2,3-Dihydro-1*H*-inden-2-yl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4-(1*H*,3*H*)-dione (51). Purification by column chromatography. Yield: 32%; mp: 255 °C; ^{1}H NMR ($CDCl_3$) δ 7.21–7.15 (m, 4H, C4-/C5-/C6-/C7-H, indanyl), 4.40 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 3.88 (s, 2H, C9-H_2), 3.57 (s, 3H, N1-CH_3), 3.37 (s, 3H, N3-CH_3), 3.19 (t, ^3J = 5.4 Hz, 2H, C7-H_2), 3.04 (br s, 5H, C^1-/C^3-H_2, C2-H, indanyl). ^{13}C NMR ($CDCl_3$) δ 159.0 (C9a), 154.9 (C4), 151.7 (C2), 148.4 (C10a), 140.5 (C3a/C7a, indanyl), 126.9 (C^4/C^7, indanyl), 124.5 (C^5/C^6, indanyl), 106.5 (C4a), 65.9 (N8-CH), 49.6 (C7), 47.3 (C9), 44.2 (C6), 36.9 (C^1/C^3,

indanyl), 29.9 (N1-CH_3), 27.9 (N3-CH_3). ESI-MS: positive mode 352.3 $[M+H]^+$. HPLC: 97.1% (A) and 97.0% (B).

5.1.2.47. (R,S)-8-(2,3-Dihydro-1H-inden-1-yl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino [2,1-*f*]purine-2,4(1*H*,3*H*)-dione (52). Purification by column chromatography. Yield: 40%; mp: 174 °C; ^{1}H NMR ($CDCl_3$) δ 7.26 (br s, 4H, C4-/C5-/C6-/C7-H, indanyl), 4.61 (br s, 1H, C^1-H, indanyl), 4.40 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 3.80 (s, 2H, C9-H_2), 3.51 (s, 3H, N1-CH_3), 3.37 (s, 3H, N3-CH_3), 2.97 (br s, 4H, C^3-H_2, indanyl, C7-H_2), 2.25 (br s, 2H, C^2-H_2, indanyl). ^{13}C NMR ($CDCl_3$) δ 159.0 (C9a), 155.0 (C4), 151.7 (C2), 148.5 (C10a), 144.1 (C^{3a}, indanyl), 140.9 (C^{7a}, indanyl), 126.9 (C^5/C^6, indanyl), 124.5 (C^4/C^7, indanyl), 106.5 (C4a), 69.6 (C1, indanyl), 49.6 (C7), 47.3 (C9), 44.2 (C6), 30.8 (C3, indanyl), 29.9 (N1-CH_3), 27.9 (N3-CH_3), 24.9 (C2, indanyl). ESI-MS: positive mode 352.0 $[M+H]^+$. HPLC: 99.5% (A) and 99.4% (B).

5.1.2.48. (*S*)-8-(2,3-Dihydro-1*H*-inden-1-yl)-1,3-dimethyl-6,7, 8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (53). Purification by column chromatography. Yield: 36%; mp: 186 °C; ^{1}H NMR ($CDCl_3$) δ 7.28–7.24 (m, 4H, C4-/C5-/C6-/C7-H, indanyl), 4.61 (br s, 1H, C^1-H, indanyl), 4.40 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 3.80 (s, 2H, C9-H_2), 3.51 (s, 3H, N1-CH_3), 3.37 (s, 3H, N3-CH_3), 2.97 (br s, 4H, C^3-H_2, indanyl, C7-H_2), 2.25 (br s, 2H, C^2-H_2, indanyl). ^{13}C NMR ($CDCl_3$) δ 159.0 (C9a), 155.0 (C4), 151.7 (C2), 148.5 (C10a), 144.1 (C^{3a}, indanyl), 140.9 (C^{7a}, indanyl), 126.9 (C^5/C^6, indanyl), 124.5 (C^4/C^7, indanyl), 106.5 (C4a), 69.6 (C1, indanyl), 49.6 (C7), 47.3 (C9), 44.2 (C6), 30.8 (C3, indanyl), 29.9 (N1-CH_3), 27.9 (N3-CH_3), 24.9 (C2, indanyl). ESI-MS: positive mode 352.0 $[M+H]^+$. HPLC: 96.8% (A) and 97.0% (B).

5.1.2.49. (*R*)-8-(2,3-Dihydro-1H-inden-1-yl)-1,3-dimethyl-6,7, 8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (54). Yield: 52%; mp: 183 °C; ^{1}H NMR ($CDCl_3$) δ 7.28–7.24 (m, 4H, C4-/C5-/C6-/C7-H, indanyl), 4.61 (br s, 1H, C^1-H, indanyl), 4.40 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 3.80 (s, 2H, C9-H_2), 3.51 (s, 3H, N1-CH_3), 3.37 (s, 3H, N3-CH_3), 2.97 (br s, 4H, C^3-H_2, indanyl C7-H_2), 2.25 (br s, 2H, C^2-H_2, indanyl). ^{13}C NMR ($CDCl_3$) δ 159.0 (C9a), 155.0 (C4), 151.7 (C2), 148.5 (C10a), 144.1 (C^{3a}, indanyl), 140.9 (C^{7a}, indanyl), 126.9 (C^5/C^6, indanyl), 124.5 (C^4/C^7, indanyl), 106.5 (C4a), 69.6 (C1, indanyl), 49.6 (C7), 47.3 (C9), 44.2 (C6), 30.8 (C3, indanyl), 29.9 (N1-CH_3), 27.9 (N3-CH_3), 24.9 (C2, indanyl). ESI-MS: positive mode 352.0 $[M+H]^+$. HPLC: 99.3% (A) and 99.3% (B).

5.1.2.50. 8-(1-Benzylpiperidin-4-yl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4-(1*H*,3*H*)-dione (55). Yield: 58%; mp: 168 °C; ^{1}H NMR ($CDCl_3$) δ 7.25–7.19 (m, 5H, phe), 4.24 (t, ^3J = 5.1 Hz, 2H, C6-H_2), 3.82 (s, 2H, C9-H_2), 3.48 (s, 3H, N1-CH_3), 3.44 (s, 2H, phenyl-CH_2), 3.32 (s, 3H, N3-CH_3), 2.95–2.88 (m, 4H, C2-/C6-H, piperidine, C7-H_2), 2.45–2.41 (m, 1H, C^4-H, piperidine), 1.97–1.93 (m, 2H, C2-/C6-H, piperidine), 1.76–1.74 (m, 2H, C3-/C5-H, piperidine), 1.61–1.54 (m, 2H, C3-/C5-H, piperidine). ^{13}C NMR ($CDCl_3$) δ 155.0 (C9a), 151.7 (C4), 148.5 (C2), 147.9 (C10a), 138.3 (C1, phe), 129.0 (C2/C6, phe), 128.2 (C3/C5, phe), 127.0 (C4, phe), 106.4 (C4a), 62.9 (C4, piperidine), 61.2 (phenyl-CH_2), 52.7 (C7), 47.8 (C9), 45.5 (C2/C6, piperidine), 44.3 (C6), 29.7 (N1-CH_3), 28.2 (C3/C5, piperidine), 27.9 (N3-CH_3). ESI-MS: positive mode 409.0 $[M+H]^+$. HPLC: 99.3% (A) and 99.9% (B).

5.1.2.51. 1,3-Dimethyl-8-((4-phenylisoxazol-3-yl)methyl)-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (56). Yield: 41%; mp: 200 °C; ^{1}H NMR ($CDCl_3$) δ 7.76–7.74 (m, 2H, C2-/C6-H, phe), 7.45–7.42 (m, 3H, C^3-/C^4-/C^5-H, phe), 6.54 (s, 1H, C5-H, isoxazolyl), 4.35 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 3.77 (s, 2H, N8-CH_2), 3.76 (s, 2H, C9-H_2), 3.52 (s, 3H, N1-CH_3), 3.37 (s, 3H, N3-CH_3), 2.97 (t, ^3J = 5.1 Hz, 2H, C7-H_2). ^{13}C NMR ($CDCl_3$) δ 155.0 (C9a), 152.1 (C5, isoxazolyl), 151.8 (C4), 149.3 (C3, isoxazolyl), 148.5 (C2), 148.0 (C10a), 130.4 (C1, phe), 129.1 (C3/C5, phe), 129.0 (C4, phe), 125.8 (C2/C6, phe), 106.8 (C4a), 99.2 (C4, isoxazolyl), 52.5 (N8-CH_2), 51.3 (C7), 48.8 (C9), 44.3 (C6), 29.8 (N1-CH_3), 27.9 (N3-CH_3). ESI-MS: positive mode 393.4 $[M+H]^+$. HPLC: 96.4% (A) and 95.9% (B).

5.1.2.52. 1,3-Dimethyl-8-((2-(thiophen-2-yl)thiazol-4-yl) methyl)-6,7,8,9-tetrahydropyra-zino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (57). Yield: 82%; mp: 182 °C; ^{1}H NMR ($CDCl_3$) δ 7.54 (dd, ^3J = 3.5 Hz, ^4J = 1.0 Hz, 1H, C^3-H, thienyl), 7.30 (dd, ^3J = 5.1 Hz, ^4J = 1.0 Hz, 1H, C5-H, thienyl), 7.10 (s, 1H, C5-H, thiazolyl), 7.06 (dd, ^3J = 5.1 Hz, ^3J = 3.8 Hz, 1H, C^4-H, thienyl), 4.35 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 3.92 (s, 2H, N8-CH_2), 3.78 (s, 2H, C9-H_2), 3.52 (s, 3H, N1-CH_3), 3.37 (s, 3H, N3-CH_3), 3.06 (t, ^3J = 5.4 Hz, 2H, C7-H_2). ^{13}C NMR ($CDCl_3$) δ 162.2 (C2, thiazolyl), 155.0 (C9a), 152.8 (C4, thiazolyl), 151.7 (C4), 148.4 (C2), 148.0 (C10a), 127.8 (C^2/C^5, thienyl), 126.8 (C^3/C^4, thienyl), 106.5 (C4a), 56.6 (N8-CH_2), 50.1 (C7), 48.5 (C9), 42.9 (C6), 29.8 (N1-CH_3), 27.9 (N3-CH_3). ESI-MS: positive mode 415.0 $[M+H]^+$. HPLC: 99.9% (A) and 99.9% (B).

5.1.2.53. 1,3-Dimethyl-8-((2-(4-(trifluoromethyl)phenyl)thiazol-4-yl)methyl)-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4 (1*H*,3*H*)-dione (58). Yield: 82%; mp: 178 °C; ^{1}H NMR ($CDCl_3$) δ 8.00 (d, ^3J = 8.5 Hz, 2H, C2-/C6-H, phe), 7.64 (d, ^3J = 8.5 Hz, 2H, C3-/C5-H, phe), 7.24 (s, 1H, C5-H, thiazolyl), 4.35 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 3.96 (s, 2H, N8-CH_2), 3.87 (s, 2H, C9-H_2), 3.48 (s, 3H, N1-CH_3), 3.33 (s, 3H, N3-CH_3), 3.05 (t, ^3J = 5.4 Hz, 2H, C7-H_2). ^{13}C NMR ($CDCl_3$) δ 166.8 (C2, thiazolyl), 155.0 (C9a), 153.7 (C4, thiazolyl), 151.7 (C4), 148.4 (C2), 148.0 (C10a), 147.8 (C1, phe), 131.7 (q, ^2J = 32.7 Hz, C4, phe), 126.8 (C2/C6, phe), 125.9 (q, ^2J = 3.7 Hz, C^3/C5, phe), 123.8 (q, $^1J_{C,F}$ = 272.3 Hz, CF_3), 118.0 (C5, thiazolyl), 106.5 (C4a), 57.0 (N8-CH_2), 50.1 (C7), 48.5 (C9), 42.9 (C6), 29.8 (N1-CH_3), 27.9 (N3-CH_3). ESI-MS: positive mode 477.3 $[M+H]^+$. HPLC: 99.9% (A) and 99.9% (B).

5.1.2.54. 8-((2-(3-Chlorophenyl)thiazol-4-yl)methyl)-1,3-dimethyl-6,7,8,9-tetrahydro-pyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (59). Yield: 80%; mp: 183 °C; ^{1}H NMR ($CDCl_3$) δ 7.95–7.94 (m, 1H, C2-H, phe), 7.79–7.77 (m, 1H, C5-H, phe), 7.37–7.33 (m, 2H, C4-/C6-H, phe), 7.22 (s, 1H, C5-H, thiazolyl), 4.38 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 3.97 (s, 2H, N8-CH_2), 3.89 (s, 2H, C9-H_2), 3.52 (s, 3H, N1-CH_3), 3.37 (s, 3H, N3-CH_3), 3.08 (t, ^3J = 5.4 Hz, 2H, C7-H_2). ^{13}C NMR ($CDCl_3$) δ 167.0 (C2, thiazolyl), 155.0 (C9a), 153.7 (C4, thiazolyl), 151.8 (C4), 148.5 (C2), 148.0 (C10a), 135.1 (C3, phe), 135.0 (C1, phe), 130.2 (C5, phe), 130.1 (C6, phe), 126.5 (C4, phe), 124.8 (C2, phe), 117.5 (C5, thiazolyl), 106.5 (C4a), 57.0 (N8-CH_2), 51.2 (C7), 48.9 (C9), 44.3 (C6), 29.8 (N1-CH_3), 27.9 (N3-CH_3). ESI-MS: positive mode 443.3 $[M+H]^+$. HPLC: 98.0% (A) and 96.9% (B).

5.1.2.55. 8-((2-(4-Chlorophenyl)thiazol-4-yl)methyl)-1,3-dimethyl-6,7,8,9-tetrahydro-pyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (60). Yield: 75%; mp: 154 °C; ^{1}H NMR ($CDCl_3$) δ 7.86 (d, ^3J = 8.5 Hz, 2H, C3-/C5-H, phe), 7.38 (d, ^3J = 8.6 Hz, 2H C2-/C6-H, phe), 7.19 (s, 1H, C5-H, thiazolyl), 4.39 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 3.97 (s, 2H, N8-CH_2), 3.90 (s, 2H, C9-H_2), 3.52 (s, 3H, N1-CH_3), 3.33 (s, 3H, N3-CH_3), 3.07 (t, ^3J = 5.4 Hz, 2H, C7-H_2). ^{13}C NMR ($CDCl_3$) δ 167.4 (C2, thiazolyl), 155.0 (C9a), 153.3 (C4, thia-

zolyl), 151.8 (C4), 148.5 (C2), 147.9 (C10a), 136.2 (C1, phe), 131.8 (C4, phe), 129.2 (C2/C6, phe), 127.8 (C3/C5, phe), 117.2 (C5, thiazolyl), 106.5 (C4a), 57.0 (N8-CH_2), 51.1 (C7), 48.9 (C9), 44.2 (C6), 29.7 (N1-CH_3), 27.8 (N3-CH_3). ESI-MS: positive mode 443.3 $[M+H]^+$. HPLC: 99.9% (A) and 99.8% (B).

5.1.2.56. 1,3-Dimethyl-8-((2-(4-(trifluoromethyl)phenyl)-5-methylthiazol-4-yl)methyl)-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (61). Yield: 80%; mp: 236 °C; ^{1}H NMR ($CDCl_3$) δ 7.96 (d, ^3J = 8.2 Hz, 2H, C2-/C6-H, phe), 7.64 (d, ^3J = 8.2 Hz, 2H, C3-/C5-H, phe), 7.24 (s, 1H, C5-H, thiazolyl), 4.35 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 3.91 (s, 2H, N8-CH_2), 3.83 (s, 2H, C9-H_2), 3.51 (s, 3H, N1-CH_3), 3.37 (s, 3H, N3-CH_3), 3.01 (t, ^3J = 5.4 Hz, 2H, C7-H_2), 2.45 (s, 3H, C5-CH_3, thiazolyl). ^{13}C NMR ($CDCl_3$) δ 164.6 (C2, thiazolyl), 155.0 (C9a), 151.8 (C4, thiazolyl), 151.7 (C4), 148.4 (C2), 148.0 (C10a), 147.6 (C1, phe), 131.5 (q, ^2J = 32.6 Hz, C4, phe), 126.4 (C2/C6, phe), 126.0 (q, ^2J = 3.7 Hz, C^3/C5, phe), 123.7 (q, $^1J_{C,F}$ = 272.3 Hz, CF_3), 121.4 (C5, thiazolyl), 106.6 (C4a), 52.9 (N8-CH_2), 51.1 (C7), 48.6 (C9), 44.3 (C6), 29.7 (N1-CH_3), 27.9 (N3-CH_3), 15.5 (C^5-CH_3, thiazolyl). ESI-MS: positive mode 491.4 $[M+H]^+$. HPLC: 97.7% (A) and 99.2% (B).

5.1.2.57. 8-((1,3-Dimethyl-1H-pyrazol-5-yl)methyl)-1,3-dimethyl-6,7,8,9-tetrahydro-pyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (62). Yield: 80%; mp: 159 °C; ^{1}H NMR ($CDCl_3$) δ 5.94 (s, 1H, C^4-H, pyrazolyl), 4.35 (br s, 2H, C6-H_2), 3.77 (s, 3H, N1-CH_3, pyrazolyl), 3.73 (s, 2H, N8-CH_2), 3.68 (s, 2H, C9-H_2), 3.51 (s, 3H, N1-CH_3), 3.36 (s, 3H, N3-CH_3), 2.91 (t, ^3J = 5.4 Hz, 2H, C7-H_2), 2.21 (s, 3H, C^3-CH_3, pyrazolyl). ^{13}C NMR ($CDCl_3$) δ 159.9 (C3, pyrazolyl), 155.0 (C9a), 151.7 (C4), 148.4 (C2), 147.4 (C10a), 147.2 (C3, pyrazolyl), 137.4 (C5, pyrazolyl), 107.1 (C4, pyrazolyl), 106.6 (C4a), 51.9 (N8-CH_2), 51.1 (C7), 48.7 (C9), 44.0 (C6), 36.3 (N1-CH_3, pyrazolyl), 29.7 (N1-CH_3), 27.8 (N3-CH_3), 13.3 (C3-CH_3, pyrazolyl). ESI-MS: positive mode 344.1 $[M+H]^+$. HPLC: 99.0% (A) and 99.0% (B).

5.1.2.58. 1,3-Dimethyl-8-(propyn-2-yl)-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (63). Yield: 81%; mp: 201 °C; ^{1}H NMR ($CDCl_3$) δ 4.35 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 3.88 (s, 2H, N8-CH_2), 3.55 (s, 2H, C9-H_2), 3.54 (s, 3H, N1-CH_3), 3.36 (s, 3H, N3-CH_3), 3.00 (t, ^3J = 5.4 Hz, 2H, C7-H_2), 2.33 (s, 1H, N8-CH_2-C≡*CH*). ^{13}C NMR ($CDCl_3$) δ 155.0 (C9a), 151.7 (C4), 148.7 (C2), 147.5 (C10a), 106.5 (C4a), 78.1 (N8-CH_2-C≡CH), 74.8 (N8-CH_2-C≡*CH*), 49.8 (C7), 47.8 (C9), 46.2 (N8-CH_2), 44.2 (C6), 29.8 (N1-CH_3), 27.8 (N3-CH_3). ESI-MS: positive mode: 274.1 $[M+H]^+$. HPLC: 95.1% (A) and 95.2% (B).

5.1.2.59. 8-(1-((4-Chlorophenyl)-1*H*-2,1,3-triazol-4-yl)methyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (65)[33–35]. At 0 °C, 4-chloroaniline (1 mmol, 127 mg) was dissolved in 5 N-aq HCl (5 mL). To this solution was added $NaNO_2$ (1 mmol, 69 mg) dissolved in H_2O (1 mL) and the reaction mixture was stirred for 5 min. Then, NaN_3 (1.2 mmol, 78 mg), dissolved in H_2O (1 mL) was added. The reaction was stirred for 5 min at 0 °C and 1 h at rt. The solution was extracted with diethylether (3 × 20 mL). The organic layers were combined, dried over Na_2SO_4 and the solvent was removed by rotary evaporation. The product, 4-chlorophenylazide (**64**), was isolated (yield: 115 mg, 75%) as a deep red oil and used directly in the next step without further purification.

In a vessel, 1,3-dimethyl-8-(propyn-2-yl)-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (**63**) (82 mg, 0.30 mmol), CuI (12 mg, 0.06 mmol), sodium ascorbate (12 mg, 0.06 mmol) were dissolved in mixture of *tert*-butanol and H_2O (5 mL, 4:1). *N*,*N*′-Dimethylethylenediamine (8 mg, 0.09 mmol) and 4-chlorophenylazide (**64**) (115 mg, 0.75 mmol) dissolved in 1 mL of *tert*-butanol were added and the solution was stirred at 65 °C for 3 h under argon atmosphere. The volatiles were removed and the product was purified by flash-chromatography (silica gel, CH_2Cl_2/MeOH 1:0 to 40:1).

Yield: 91%; mp: 244 °C; ^{1}H NMR ($CDCl_3$) δ 7.94 (s, 1H, C5-H, triazolyl), 7.23 (d, ^3J = 8.8 Hz, 2H, C3-/C5-H, phe), 7.08 (d, ^3J = 8.5 Hz, 2H, C2-/C6-H, phe), 4.37 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 3.93 (s, 2H, N8-CH_2), 3.74 (s, 2H, C9-H_2), 3.51 (s, 3H, N1-CH_3), 3.35 (s, 3H, N3-CH_3), 3.04 (t, ^3J = 5.4 Hz, 2H, C3-H2). ^{13}C NMR ($CDCl_3$) δ 155.0 (C9a), 151.7 (C4), 148.4 (C2), 147.5 (C10a), 144.3 (C1, phe), 135.3 (C4, triazolyl), 134.7 (C4, phe), 130.0 (C3/C5, phe), 121.6 (C2/C6, phe), 120.9 (C5, triazolyl), 106.5 (C4a), 51.1 (N8-CH_2), 48.7 (C7), 46.1 (C9), 44.2 (C6), 29.7 (N1-CH_3), 27.8 (N3-CH_3). ESI-MS: positive mode 427.4 $[M+H]^+$. HPLC: 99.9% (A) and 99.8% (B).

5.1.3. Synthesis of 8-(3-arylprop-2-ynyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-diones 66–68 by Sonogashira cross-coupling reaction[36]

In a dry vessel, 1,3-dimethyl-8-(prop-2-ynyl)-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (**63**) (82 mg, 0.3 mmol), $Pd(Ph_3P)_4$ (34 mg, 0.03 mmol) and CuI (6 mg, 0.03 mol) were combined and dry DMF (2 mL), DIPEA (130 mg) and a corresponding iodoarene (0.4 mmol) was added under argon atmosphere. The reaction mixture was stirred for 16 h at 80 °C under argon atmosphere. The volatiles were removed in vacuo and the product was purified by flash-chromatography (silica gel, CH_2Cl_2/MeOH 1:0 to 40:1).

5.1.3.1. 8-(3-(3-Chlorophenyl)prop-2-ynyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (66). Yield: 52%; mp: 221 °C; ^{1}H NMR ($CDCl_3$) δ 7.37 (dd, ^4J = 1.9 Hz, ^4J = 1.9 Hz, 1H, C2-H, phe), 7.28–7.26 (m, 2H, C4-/C6-H, phe), 7.20 (dd, ^3J = 7.9 Hz, ^3J = 8.5 Hz, 1H, C5-H, phe), 4.38 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 3.95 (s, 2H, N8-CH_2), 3.76 (s, 2H, C9-H_2), 3.53 (s, 3H, N1-CH_3), 3.36 (s, 3H, N3-CH_3), 3.06 (t, ^3J = 5.4 Hz, 2H, C7-H_2). ^{13}C NMR ($CDCl_3$) δ 155.0 (C9a), 151.7 (C4), 148.5 (C2), 147.8 (C10a), 132.5 (C3, phe), 131.6 (C2, phe), 129.8 (C5, phe), 129.6 (C6, phe), 128.4 (C4, phe), 124.0 (C1, phe), 106.6 (C4a), 85.4 (N8-CH_2-C≡*C*), 83.6 (N8-CH_2-*C*≡C), 50.0 (N8-CH_2), 48.1 (C7), 46.9 (C9), 44.2 (C6), 29.8 (N1-CH_3), 27.8 (N3-CH_3). ESI-MS: positive mode 384.1 $[M+H]^+$. HPLC: 99.2% (A) and 99.3% (B).

5.1.3.2. 8-(3-(3,4-Dichlorophenyl)prop-2-ynyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino-[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (67). Yield: 86%; mp: 212 °C; ^{1}H NMR ($CDCl_3$) δ 7.46 (d, ^4J = 1.9 Hz, 1H, C2-H, phe), 7.34 (d, ^3J = 8.2 Hz, 1H, C5-H, phe), 7.20 (dd, ^3J = 8.2 Hz, ^4J = 2.4 Hz, 1H, C6-H, phe), 4.37 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 3.93 (s, 2H, N8-CH_2), 3.74 (s, 2H, C9-H_2), 3.51 (s, 3H, N1-CH_3), 3.35 (s, 3H, N3-CH_3), 3.04 (t, ^3J = 5.4 Hz, 2H, C7-H_2). ^{13}C NMR ($CDCl_3$) δ 155.0 (C9a), 151.7 (C4), 149.5 (C2), 147.6 (C10a), 133.3 (C4, phe), 132.5 (C3, phe), 132.0 (C2, phe), 130.4 (C6, phe), 128.4 (C5, phe), 122.2 (C1, phe), 106.5 (C4a), 84.5 (2 × C, N8-CH_2-*C*≡*C*), 49.9 (N8-CH_2), 48.1 (C7), 46.9 (C9), 44.1 (C6), 29.7 (N1-CH_3), 27.9 (N3-CH_3). ESI-MS: positive mode 418.3 $[M+H]^+$. HPLC: 99.6% (A) and 99.5% (B).

5.1.3.3. 8-(3-(3-Methoxyphenyl)prop-2-ynyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino-[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (68). Yield: 33%; mp: 210 °C; ^{1}H NMR ($CDCl_3$) δ 7.21–7.18 (m, 1H, C5-H, phe), 7.01–6.99 (m, 1H, C6-H, phe), 6.93–6.92 (m, 1H, C2-H, phe), 6.88–6.86 (m, 1H, C4-H, phe), 4.40 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 3.97 (s, 2H, N8-CH_2), 3.78 (s, 2H, C9-H_2), 3.77 (s, 3H, OCH_3), 3.54 (s, 3H, N1-CH_3), 3.38 (s, 3H, N3-CH_3), 3.08 (t, ^3J = 5.4 Hz, 2H, C7-H_2). ^{13}C NMR ($CDCl_3$) δ 166.1 (C3, phe), 155.0 (C9a), 151.7 (C4), 149.5 (C2), 147.6 (C10a),

129.5 (C5, phe), 124.3 (C1, phe), 116.7 (C2, phe), 115.0 (C^4/C6, phe), 106.5 (C4a), 86.8 (N8-CH_2-C≡C), 82.0 (N8-CH_2-C≡C), 55.3 (OCH_3), 50.0 (N8-CH_2), 48.1 (C7), 47.0 (C9), 44.1 (C6), 29.7 (N1-CH_3), 27.9 (N3-CH_3). ESI-MS: positive mode 380.1 $[M+H]^+$. HPLC: 96.0% (A) and 95.1% (B).

5.2. Biological testing

5.2.1. Radioligand binding assays at adenosine receptors

The radioligands were obtained from the following sources: [^{3}H]CCPA from Amersham Biosciences (58 Ci/mmol), [^{3}H]MSX-2 from Amersham Biosciences (84 Ci/mmol), [^{3}H]PSB-603 from Amersham Biosciences (73 Ci/mmol) and [^{3}H]PSB-11 (53 Ci/mmol) from Quotient Bioresearch. The non-radioactive precursors of [^{3}H]MSX-2,[38] [^{3}H]PSB-603[39] and [^{3}H]PSB-11[40] were synthesized in our laboratory. Membrane preparations and radioligand binding assays at rat A_1 (rat brain cortex) and rat A_{2A} (rat brain striatum) were performed as previously described.[43,44] For assays at human A_1, A_{2A}, A_{2B} and A_3ARs, CHO cell membranes expressing one of the human ARs were used as previously reported.[42]

5.2.2. cAMP accumulation assays

Effects of test compounds on forskolin-induced cAMP accumulation at CHO cells recombinantly expressing the human A_1 AR, and on cAMP accumulation in CHO cells recombinantly expressing the human A_{2A} AR were performed as previously described.[37,45]

5.2.3. Monoamine oxidase assays

The determination of MAO-A and MAO-B inhibition was performed using commercially available recombinant human MAO-A and MAO-B enzymes expressed in baculovirus-infected insects cells (Sigma–Aldrich, M7316 and M7441) applying the commercially available Amplex® Red monoamine oxidase assay kit (Invitrogen A12214). The assays were performed as previously described.[11]

6. Water solubility[25]

Water solubility (in mg/mL) was determined using the following procedure: at rt, 500 µl of buffer was added to precisely weighed 1 mg of compound and the mixture was sonicated for 30 s. The pH-value was checked and adjusted if deviating by more than 0.2 pH units from the target value. The solution was left for 1 h at rt and then filtered through a 0.45 µm filter. The reference solution of precisely weighed 1 mg of the same compound dissolved in methanol was prepared. Both solutions were injected into a Chromatographic Acquity UPLC System (from Waters) equipped with a BEH RP18 column (2.1 × 50 mm 1.7 µM) and coupled to a PDA detector (200–400 nm). The elution was performed for 3.33 min at a flow rate of 750 µL/min at a column temperature of 65 °C with a gradient of water/acetonitrile/trifluoroacetic acid (95/5/0.05): acetonitrile from 90:10 to 10:90, starting the gradient after 0.33 min and ending after 2.00 min.

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2016.09.003.

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