

## Supporting Information

### Design, Synthesis, and Structure–Activity Relationships of 1-, 3-, 8-, and 9-Substituted-9-deazaxanthines at the Human A<sub>2B</sub> Adenosine Receptor.

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## Chemistry

General chemistry information was reported in the experimental section.

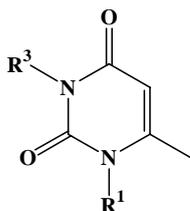
### Synthesis of Uracils.

The syntheses of 1,3-dimethyl-(or -dipropyl)-6-methyl-5-nitrouracil were already described.<sup>1</sup> Nonsymmetrically substituted 1,3-dialkyl-6-methyluracils were prepared by alkylation at position 3 (or 1) of the corresponding 1(3)-alkyl-6-methyluracils<sup>2</sup> or of the commercial 3-isobutyl-6-methyluracil.

### Representative Procedure for the Preparation of 1(3)-Alkyl-3(1),6-dimethyluracils.

NaH (3.6 g, 150 mmol) was added to a solution of the uracil (100 mmol) in anhydrous DMF (300 mL) at 0 °C and the mixture was stirred for 30 min. MeI (9.34 mL, 21.29 g, 150 mmol) was added and the mixture was stirred for additional 2 h at 0 °C. The solvent was evaporated, the residue taken up with water and the solution was extracted with AcOEt. The organic phase was dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated to give a solid compound that was purified by crystallization or flash column chromatography, only in the case of unacceptably low purity.

**Table 10. Experimental Data of New 1,3-Dialkyl-6-methyluracils (198–199).**



Compd	R <sup>1</sup>	R <sup>3</sup>	<sup>1</sup> H-NMR	EI/MS m/e [M] <sup>+</sup>	m.p. (°C) (cryst. solv.)	Yield %
198	CH <sub>3</sub> OCH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub>	CH <sub>3</sub>	CDCl <sub>3</sub> : 5.59 (s, 1H), 3.94 (m, 2H), 3.39 (m, 2H), 3.33 (s, 3H), 3.31 (s, 3H), 2.27 (d, J=1.3 Hz, 3H) 1.93 (m, 2H).	212	56-58 (isopropanol)	69
199	CH <sub>3</sub> OCH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub>	CH <sub>3</sub> CH <sub>2</sub>	CDCl <sub>3</sub> : 5.13 (s, 1H), 3.50 (m, 4H), 3.00 (m, 2H), 2.88 (s, 3H), 1.88 (s, 3H), 1.51 (m, 2H), 0.74 (m, 3H).	226	59-61 (isopropanol)	55

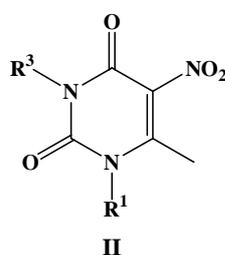
### Synthesis of 3-Isobutyl-1,6-dimethylpyrimidine-2,4(1H,3H) -dione (200).

A 4 N aqueous solution of NaOH (1.18 mL) was added to a mixture of the 3-isobutyl-6-methyluracil (0.82 g, 4.30 mmol) in EtOH (2.6 mL) followed by Me<sub>2</sub>SO<sub>4</sub> (0.69 mL, 7.31 mmol). The mixture was stirred at rt for 48 h. The solvent was evaporated and the solid obtained was suspended in aqueous 10% NaOH and extracted with DCM. The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under vacuum to afford a light brown solid of a sufficient purity for a further synthesis. Yield 81%. <sup>1</sup>H-NMR (δ, ppm, CDCl<sub>3</sub>): 5.80 (s, 1H), 3.90 (m, 2H), 3.15 (s, 3H), 2.15-2.10 (m, 4H), 0.90 (m, 6H).

### Representative Procedure for the Preparation of the Nonsymmetrically Substituted 1,3-Dialkyl-6-methyl-5-nitrouracil derivatives II (see Scheme 1).

A 1:1 mixture of concentrated 98% H<sub>2</sub>SO<sub>4</sub> (20 mL) and fuming HNO<sub>3</sub> (20 mL) was cooled to 0-5 °C, and the appropriate 1,3 dialkyl-6-methyluracil (65 mmol) was gradually added. After standing for 30 min, the mixture was carefully poured on ice and the precipitate was collected by filtration and washed with water to give a yellow solid which presented a sufficient analytical purity to be used as such in the successive syntheses.

Table 11. Experimental data of 1,3-dialkyl-6-methyl-5-nitrouracils II (cps 201–203).



Compd	R <sup>1</sup>	R <sup>3</sup>	<sup>1</sup> HNMR	EI/MS m/e [M] <sup>+</sup>	Yield %
201	CH <sub>3</sub> OCH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub>	CH <sub>3</sub>	CDCl <sub>3</sub> : 4.06 (t, J=7.3 Hz, 2H), 3.44 (t, J=5.5 Hz, 2H), 3.37 (s, 3H), 3.33 (s, 3H), 2.44 (s, 3H), 1.99 (m, 2H).	257	71
202	CH <sub>3</sub> OCH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub>	CH <sub>3</sub> CH <sub>2</sub>	CDCl <sub>3</sub> : 4.01 (m, 4H), 3.48 (m, 2H), 3.38 (s, 3H), 2.45 (s, 3H), 2.03 (m, 2H), 1.29 (m, 3H).	271	88
203	CH <sub>3</sub>	(CH <sub>3</sub> ) <sub>2</sub> CHCH <sub>2</sub>	CDCl <sub>3</sub> : 3.90 (m, 2H), 3.15 (s, 3H), 2.15-2.10 (m, 4H), 0.90 (m, 6H).	242	33

### Synthesis of the Benzaldehydes III (see Scheme 1).

The syntheses of ethyl-(4-formylphenoxy)acetate,<sup>3</sup> 3-formyl-phenoxyacetic acid,<sup>4</sup> ethyl-(4-formyl-3-methoxyphenoxy)acetate<sup>5</sup>, ethyl-(4-formyl-2-methoxyphenoxy)acetate<sup>6</sup> and ethyl-(3-chloro-4-formylphenoxy)acetate<sup>3</sup> were performed according to already published methods, with slight modifications.

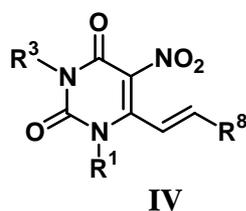
### Synthesis of 3-Chloro-4-formylphenoxyacetic acid 204.

Ethyl (3-chloro-4-formylphenoxy)acetate<sup>3</sup> (2.90 g, 11.95 mmol) was dissolved in a 50% solution of aqueous NaOH 8 N (8 mL) and EtOH (8 mL), followed by stirring at room temperature for 2 h. Water (15 mL) and a 2N aqueous solution of HCl was added to the mixture up to an acidic pH to afford **204** as a yellow crude precipitate that was filtered, dried and used for the next step without further purification (1.97 g, 77% yield) ESI/MS (m/e, [M+H]<sup>+</sup>) 244.

## Synthesis of 4-Formylphenylaminoacetic Acid Ethyl Ester 205.

4-aminobenzaldehyde<sup>7</sup> (0.50 g, 4.12 mmol) was dissolved in DMF (8mL), under an argon atmosphere. NaH (0.20 g, 8.24 mmol) was added at 0 °C followed by stirring at the same temperature for 30 min. Ethyl bromoacetate (1.38 mL, 12.36 mmol) was added and the reaction was heated at 80°C for 16 h. The mixture was cooled, diluted with water and extracted with AcOEt. The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated under vacuum to give a solid residue that was purified by column chromatography on silica gel (AcOEt:hexane 3:7), giving a yellow solid (0.50 g, 58%) that was recrystallized from DCM/hexane m.p.: 90-92 °C. <sup>1</sup>H-NMR (δ, ppm, CDCl<sub>3</sub>): 9.72 (s, 1H), 7.69 (d, J=8.4 Hz, 2H), 6.62 (d, J=8.4, 2H), 5.31 (bs, 1H), 4.24 (q, J=7.1 Hz, 2H), 3.95 (s, 2H), 1.29 (t, J=7.1 Hz, 3H). EI-MS (m/e: [M]<sup>+</sup>) 207.

The representative procedure for the condensation reaction between benzaldehydes **III** and 5-nitro-6-methyl-uracils **II**, leading to compounds of general structure **IV**, has been described in the experimental section of the manuscript.



**Table 12. Experimental Data of 1,3-Dialkyl-6-Styryluracil Derivatives IV (cps 206–216 see Scheme 1).**

Compd	R <sup>1</sup>	R <sup>3</sup>	R <sup>8</sup>	<sup>1</sup> HNMR	EI/MS m/e [M] <sup>+</sup>	Yield %
206	CH <sub>3</sub>	CH <sub>3</sub>		CDCl <sub>3</sub> : 7.43 (d, J=8.8 Hz, 2H), 7.00 (d, J=16.4 Hz, 1H), 6.92 (d, J=8.8 Hz, 2H), 6.52 (d, J=16.4 Hz, 1H), 4.66 (s, 2H), 4.28 (q, J=7.1 Hz, 2H), 3.48 (s, 3H), 3.41 (s, 3H), 1.30 (t, J=7.1 Hz, 3H).	390	50
207	CH <sub>3</sub>	CH <sub>3</sub>		CDCl <sub>3</sub> : 7.33 (d, J=8.5 Hz, 1H), 7.19 (d, J=16.4 Hz, 1H), 6.72 (d, J=16.4 Hz, 1H), 6.54 (d, J=2.2 Hz, 1H), 6.44 (dd, J=8.5, 2.2 Hz, 1H), 4.65 (s, 2H), 4.29 (q, J=7.1 Hz, 2H), 3.86 (s, 3H), 3.49 (s, 3H), 3.41 (s, 3H), 1.31 (t, J=7.1 Hz, 3H).	419	54
208	CH <sub>3</sub>	CH <sub>3</sub>		CDCl <sub>3</sub> : 6.98 (m, 3H), 6.80 (d, J=16.3 Hz, 1H), 6.51 (d, J=16.3 Hz, 1H), 4.73 (s, 2H), 4.27 (m, 2H), 3.93 (s, 3H), 3.50 (s, 3H), 3.43 (s, 3H), 1.30 (t, J=7.1 Hz, 3H).	419	53
209	CH <sub>3</sub>	CH <sub>3</sub>		CDCl <sub>3</sub> : 7.35 (d, J=16.3 Hz, 1H), 7.28 (m, 1H), 7.07 (m, 1H), 7.03 (d, J=16.3 Hz, 1H), 6.85 (m, 1H), 4.66 (s, 2H), 3.94 (m, 4H), 3.86 (s, 3H), 1.70 (m, 4H), 0.96 (t, J=7.4 Hz, 6H).	431	55

*continue*

Compd	R <sup>1</sup>	R <sup>3</sup>	R <sup>8</sup>	<sup>1</sup> HNMR	EI/MS m/e [M] <sup>+</sup>	Yield %
210	CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub>	CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub>		DMSO-d <sub>6</sub> : 10.10 (bs, 1H), 7.61 (d, J=8.6 Hz, 2H), 6.99 (m, 4H), 4.76 (s, 2H), 3.84 (m, 4H), 1.61 (m, 4H), 0.87 (m, 6H). CDCl <sub>3</sub> : 7.30 (m, 1H), 7.15 (d, J=16.4 Hz, 1H), 6.77 (d, J=16.4 Hz, 1H), 6.55 (d, J=2.3 Hz, 1H), 6.45 (m, 1H), 4.66 (s, 2H), 4.28 (q, J=7.1 Hz, 2H), 3.94 (m, 4H), 3.86 (s, 3H), 1.70 (m, 4H), 1.31 (t, J=7.1 Hz, 3H), 0.96 (t, J=7.4 Hz, 6H).	418	72
211	CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub>	CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub>		CDCl <sub>3</sub> : 7.42 (d, J=16.6 Hz, 1H), 7.28 (m, 1H), 7.2 (d, J=16.4 Hz, 1H), 7.07 (m, 1H), 6.85 (m, 1H), 4.66 (s, 2H), 3.94 (m, 4H), 3.86 (s, 3H), 1.70 (m, 4H), 0.96 (t, J=7.4 Hz, 6H). CDCl <sub>3</sub> : 7.31 (d, J=8.6 Hz, 2H), 6.96 (d, J=16.5 Hz, 1H), 6.60 (d, J=8.6 Hz, 2H), 6.37 (d, J=16.5 Hz, 1H), 4.69 (s, 1H), 4.27 (q, J=7.1 Hz, 2H), 3.99-3.83 (m, 6H), 1.78-1.65 (m, 4H), 1.32 (t, J=7.1 Hz, 3H), 0.99-0.94 (m, 6H).	475	95
212	CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub>	CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub>		CDCl <sub>3</sub> : 7.44 (d, J=8.8 Hz, 2H), 7.00 (d, J=16.3 Hz, 1H), 6.92 (d, J=8.8 Hz, 2H), 6.69 (d, J=16.3 Hz, 1H), 4.66 (s, 2H), 4.28 (q, J=7.2 Hz, 2H), 4.04 (t, J=7.2 Hz, 2H), 3.44 (t, J=5.4 Hz, 2H), 3.41 (s, 3H), 3.33 (s, 3H), 2.01 (m, 2H), 1.31 (t, J=7.2 Hz, 3H). CDCl <sub>3</sub> : 7.35 (d, J=8.7 Hz, 2H), 6.90 (d, J=16.3 Hz, 1H), 6.82 (d, J=8.7 Hz, 2H), 6.59 (d, J=16.3 Hz, 1H), 4.57 (s, 2H), 4.17 (q, J=7.1 Hz, 2H), 3.93 (m, 4H), 3.36 (t, J=5.7 Hz, 2H), 3.22 (s, 3H), 1.90 (m, 2H), 1.23-1.13 (m, 3H).	451	32
213	CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub>	CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub>		CDCl <sub>3</sub> : 13.15 (s, 1H), 7.6 (m, 2H), 7.00 (m, 2H), 6.92 (m, 2H), 4.66 (s, 2H), 3.9 (d, 2H), 3.15 (s, 3H), 2.10 (m, 1H), 0.90 (m, 6H).	444	88
214	CH <sub>3</sub>	CH <sub>3</sub> OCH <sub>2</sub> CH <sub>2</sub> H <sub>2</sub>			447	69
215	CH <sub>3</sub> CH <sub>2</sub>	CH <sub>3</sub> OCH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub>			461	25
216	(CH <sub>3</sub> ) <sub>2</sub> CHCH <sub>2</sub>	CH <sub>3</sub>			431	59

The representative procedures for the ring closure of 5-nitro-6-styryluracil **IV** to 9-dAXs **V** (see Scheme 1) were described in the experimental section of the manuscript.

**Representative Procedure for the Hydrolysis of Ethyl esters **V** (Z= Et, see Scheme 1).**

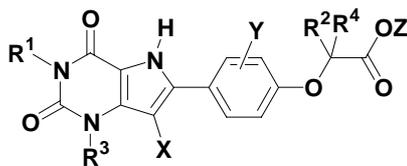
Aqueous NaOH 2N (30 mL) was added to a suspension of the ester **V** (6.00 mmol) in ethanol (30 mL) and the mixture was heated at 50°C for 1 hour. Most of the ethanol was evaporated under vacuum, the remaining aqueous mixture cooled to room temperature and acetic acid was added until an acidic pH was reached. The resulting precipitate was collected by filtration, washed with water and dried to yield the corresponding carboxylic acid **V**, that was purified by column chromatography, or by crystallization (H<sub>2</sub>O /MeOH), only in the case of low purity.

**Representative Procedure for the Synthesis of 9-Halo-9-Deazaxanthines derivatives **VI** (see Scheme 1).**

**9-Chloro Derivatives:** Sulfuryl chloride (10.44 mL, 130.00 mmol) was slowly added to a solution of the appropriate carboxylic acid or ester **V** (15.20 mmol) in glacial acetic acid (30 mL) and the mixture was stirred at room temperature for 4 h. The reaction mixture was carefully poured into ice-brine and extracted with DCM. The organic phase was separated, washed with water, dried over MgSO<sub>4</sub> and evaporated under reduced pressure to yield the expected 9-Cl-1*H*-pyrrolo[3,2-*d*]pyrimidine-2,4(3*H*,5*H*)-dione derivative **VI**, that was eventually purified by column chromatography, or by crystallization (H<sub>2</sub>O /MeOH).

**9-Bromo Derivatives:** Bromine (0.22 mL, 3.63 mmol) diluted in 1mL of acetic acid was slowly added to a solution of the appropriate carboxylic acid or ester **V** (2.6 mmol) in glacial acetic acid (22 mL) and the mixture was stirred at room temperature for 1 h. The reaction mixture was carefully poured into ice-brine and the aqueous suspension was extracted with DCM. The organic phase was separated, washed with water, dried (MgSO<sub>4</sub>) and evaporated under reduced pressure to yield the expected 9-Br-1*H*-pyrrolo[3,2-*d*]pyrimidine-2,4(3*H*,5*H*)-dione derivative **VI**, that was purified by flash column chromatography, or by crystallization (H<sub>2</sub>O /MeOH), when required.

**Table 13. Experimental data of 9-dAXs acid and ester intermediates V and VI (cps 165–196).**



**Structures V and VI (cps 165-196).**

Compd	Yield (%)	Method	m.p. (°C)	Molecular Formula	MS	<sup>1</sup> H-NMR
165	65	B	280-282	C <sub>20</sub> H <sub>23</sub> N <sub>3</sub> O <sub>5</sub>	EI/MS m/e [M] <sup>+</sup> 385	DMSO-d <sub>6</sub> : 12.21 (bs, 1H), 7.85 (d, J=8.8 Hz, 2H), 6.98 (d, J=8.8 Hz, 2H), 6.64 (d, J=2.1 Hz, 1H), 4.74 (s, 2H), 3.87 (m, 4H), 1.62 (m, 4H), 0.90 (m, 6H).
166	48	B	>300	C <sub>18</sub> H <sub>19</sub> N <sub>3</sub> O <sub>5</sub>	ESI/MS m/e [M+H] <sup>+</sup> 358	DMSO-d <sub>6</sub> : 13.01 (bs, 1H), 12.22 (bs, 1H), 7.95 (d, J=8.7 Hz, 2H), 7.03 (d, J=8.8 Hz, 2H), 6.71 (s, 1H), 4.77 (s, 2H), 3.95 (t, J=7.5 Hz, 2H), 3.30 (s, 3H), 1.71 (m, 2H), 0.90 (t, J=7 Hz, 3H).
167	41	B	>300	C <sub>21</sub> H <sub>25</sub> N <sub>3</sub> O <sub>5</sub>	ESI/MS m/e [M+H] <sup>+</sup> 400	DMSO-d <sub>6</sub> : 11.55 (s, 1H), 7.51 (d, J=8.8 Hz, 2H), 7.01 (d, J=8.7 Hz, 2H), 6.15 (s, 1H), 4.08 (t, J=6 Hz, 2H), 3.92 (t, J=6.3 Hz, 2H), 1.85 (s, 3H), 1.70 (m, 4H), 0.91 (m, 6H).
168	60	B	>300	C <sub>18</sub> H <sub>19</sub> N <sub>3</sub> O <sub>5</sub>	ESI/MS m/e [M+H] <sup>+</sup> 358	DMSO-d <sub>6</sub> : 13.52 (bs, 1H), 12.21 (bs, 1H), 7.90 (d, J=8.6 Hz, 2H), 7.12 (d, 2H), 6.82 (s, 1H), 4.82 (s, 2H), 3.95 (t, J=7.1 Hz, 2H), 3.44 (s, 3H), 1.62 (m, 2H), 0.91 (t, J=7 Hz, 3H).
169	30	B	195-197	C <sub>22</sub> H <sub>27</sub> N <sub>3</sub> O <sub>6</sub>	EI/MS m/e [M] <sup>+</sup> 429	CDCl <sub>3</sub> : 7.75 (d, J=8.7 Hz, 2H), 6.98 (d, J=8.7 Hz, 2H), 6.28 (s, 1H), 4.67 (s, 2H), 4.30 (q, J=7.2 Hz, 2H), 4.12 (m, 4H), 3.45 (t, J=5.9 Hz, 2H), 3.35 (s, 3H), 2.06 (m, 2H), 1.35-1.24 (m, 6H).
170	65	B	254-256	C <sub>20</sub> H <sub>23</sub> N <sub>3</sub> O <sub>6</sub>	EI/MS m/e [M] <sup>+</sup> 401	DMSO-d <sub>6</sub> : 7.92 (d, J=8.8 Hz, 2H), 7.06 (d, J=8.8 Hz, 2H), 6.65 (d, J=2.0, 1H), 4.81 (s, 2H), 4.02 (m, 4H), 3.47 (m, 2H), 3.30 (s, 3H), 2.98 (m, 2H), 1.21 (t, J=6.9 Hz, 3H).
171	73	B	>300	C <sub>20</sub> H <sub>22</sub> BrN <sub>3</sub> O <sub>5</sub>	ESI/MS m/e [M+H] <sup>+</sup> 463	DMSO-d <sub>6</sub> : 12.75 (s, 1H), 7.51 (d, 2H), 7.12 (d, J=8.8 Hz, 2H), 6.55 (d, J=2.0, 1H), 4.78 (s, 2H), 4.15 (t, J=7.0 Hz, 2H), 3.82 (t, J=6.5 Hz, 2H), 1.55 (m, 4H), 0.86 (m, 6H).
173	89	B	>300	C <sub>20</sub> H <sub>22</sub> ClN <sub>3</sub> O <sub>5</sub>	ESI/MS m/e [M+H] <sup>+</sup> 421	DMSO-d <sub>6</sub> : 12.75 (s, 1H), 7.61 (d, 2H), 7.04 (d, J=8.9 Hz, 2H), 4.75 (s, 2H), 4.16 (t, J=7 Hz, 2H), 3.92 (t, J=6.8 Hz, 2H), 1.61 (m, 4H), 0.90 (dt, 6H).
174	86	B	>300	C <sub>19</sub> H <sub>21</sub> N <sub>3</sub> O <sub>6</sub>	EI/MS m/e [M] <sup>+</sup> 387	DMSO-d <sub>6</sub> : 12.22 (bs, 1H), 7.94 (d, J=8.8 Hz, 2H), 7.07 (d, J=8.8 Hz, 2H), 6.68 (d, J=2.1, 1H), 4.83 (s, 2H), 4.63 (bs, 1H), 4.05 (t, J=6.8 Hz, 2H), 3.48 (t, J=6.0 Hz, 2H), 3.35 (s, 3H), 3.32 (s, 3H), 1.99 (m, 2H).
175	98	B	>300	C <sub>17</sub> H <sub>17</sub> N <sub>3</sub> O <sub>6</sub>	EI/MS m/e [M] <sup>+</sup> 359	DMSO-d <sub>6</sub> : 12.30 (s, 1H), 7.60 (s, 1H), 7.42 (d, J=8.3 Hz, 1H), 6.91 (d, J=8.3 Hz, 1H), 6.68 (s, 1H), 4.73 (s, 2H), 3.88 (s, 3H), 3.43 (s, 3H), 3.27 (s, 3H).

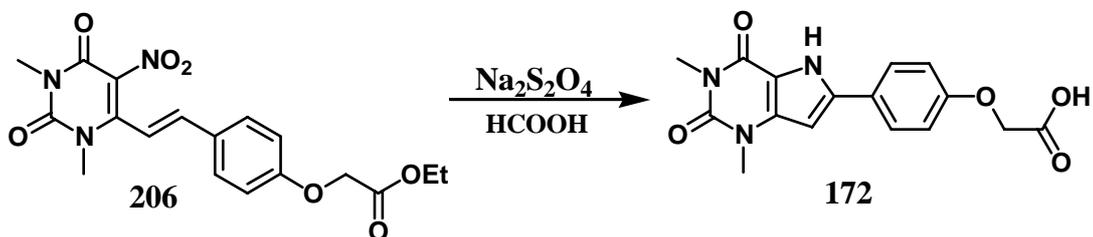
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Compd	Yield (%)	Method	m.p. (°C)	Molecular Formula	MS	<sup>1</sup> H-NMR
176	30	A	243-245	C <sub>18</sub> H <sub>19</sub> N <sub>3</sub> O <sub>5</sub>	ESI/MS m/e [M+H] <sup>+</sup> 357	DMSO-d <sub>6</sub> : 12.45 (bs, 1H), 7.95 (d, J=8.8 Hz, 2H), 7.10 (d, J=8.8 Hz, 2H), 6.72 (s, 1H), 4.94 (s, 2H), 4.28 (q, J=7.1 Hz, 2H), 3.52 (s, 3H), 3.36 (s, 3H), 1.32 (t, J=7.1 Hz, 3H).
177	24	A	177-179	C <sub>21</sub> H <sub>25</sub> N <sub>3</sub> O <sub>6</sub>	EI/MS m/e [M] <sup>+</sup> 415	CDCl <sub>3</sub> : 11.70 (s, 1H), 7.85 (d, J=8.8 Hz, 2H), 6.95 (d, J=8.8 Hz, 2H), 6.46 (d, J=2.1 Hz, 1H), 4.67 (s, 2H), 4.30 (c, J=7.1 Hz, 2H), 4.07 (t, J=6.8 Hz, 2H), 3.48 (s, 3H), 3.43 (m, 2H), 3.34 (s, 3H), 2.05 (m, 2H), 1.32 (t, J=7.1 Hz, 3H).
178	26	A	230-232	C <sub>19</sub> H <sub>21</sub> N <sub>3</sub> O <sub>6</sub>	EI/MS m/e [M] <sup>+</sup> 387	DMSO-d <sub>6</sub> : 12.32 (bs, 1H), 7.60 (s, 1H), 7.42 (d, J=8.3 Hz, 1H), 6.94 (d, J=8.3 Hz, 1H), 6.70 (s, 1H), 4.82 (s, 2H), 4.19 (q, J=7.1 Hz, 2H), 3.88 (s, 3H), 3.43 (s, 3H), 3.27 (s, 3H), 1.23 (t, J=7.1 Hz, 3H).
179	98	A	>300	C <sub>17</sub> H <sub>17</sub> N <sub>3</sub> O <sub>6</sub>	EI/MS m/e [M] <sup>+</sup> 359	DMSO-d <sub>6</sub> : 11.84 (s, 1H), 7.72 (d, J=7.6 Hz, 1H), 6.71 (s, 1H), 6.54 (m, 2H), 4.77 (s, 2H), 3.89 (s, 3H), 3.43 (s, 3H), 3.27 (s, 3H).
180	48	A	234-236	C <sub>19</sub> H <sub>21</sub> N <sub>3</sub> O <sub>6</sub>	EI/MS m/e [M] <sup>+</sup> 387	DMSO-d <sub>6</sub> : 11.75 (s, 1H), 7.66 (d, J=8.6 Hz, 1H), 6.65 (d, J=2.2 Hz, 1H), 6.54 (dd, J=8.6, 2.2 Hz, 1H), 6.48 (s, 1H), 4.81 (s, 2H), 4.14 (q, J=7.1 Hz, 2H), 3.83 (s, 3H), 3.36 (s, 3H), 3.20 (s, 3H), 1.17 (t, J=7.1 Hz, 3H).
181	45	B	>300	C <sub>22</sub> H <sub>23</sub> N <sub>3</sub> O <sub>5</sub>	ESI/MS m/e [M+H] <sup>+</sup> 409	DMSO-d <sub>6</sub> : 13.12 (bs, 1H), 12.35 (bs, 1H), 7.88 (d, J=8.7 Hz, 2H), 7.02 (d, J=8.5 Hz, 2H), 6.72 (s, 1H), 4.76 (s, 2H), 3.81 (m, 4H), 1.25 (m, 2H), 0.38 (m, 8H).
182	65	B	>300	C <sub>22</sub> H <sub>27</sub> N <sub>3</sub> O <sub>5</sub>	ESI/MS m/e [M+H] <sup>+</sup> 413	CDCl <sub>3</sub> : 11.60 (bs, 1H), 7.51 (d, 2H), 7.02 (d, 2H), 4.78 (m, 2H), 4.05 (m, 2H), 3.94 (t, 2H), 2.18 (m, 2H), 1.77 (m, 4H), 1.22 (t, 3H), 0.98 (dt, 6H).
183	62	B	>300	C <sub>20</sub> H <sub>23</sub> N <sub>3</sub> O <sub>5</sub>	ESI/MS m/e [M+H] <sup>+</sup> 385	DMSO-d <sub>6</sub> : 13.12 (bs, 1H), 12.25 (bs, 1H), 7.79 (d, J=8.7 Hz, 2H), 7.82 (d, J=8.5 Hz, 2H), 6.58 (s, 1H), 3.38 (s, 3H), 0.80 (t, J=6 Hz, 2H).
184	90	B	>300	C <sub>26</sub> H <sub>27</sub> N <sub>3</sub> O <sub>5</sub>	ESI/MS m/e [M+H] <sup>+</sup> 462	CDCl <sub>3</sub> : 11.50 (s, 1H), 7.70 (d, 2H), 7.50 (d, 2H), 7.10 (d, 2H), 6.10 (s, 1H), 5.84 (s, 1H), 3.91 (m, 4H), 1.70 (m, 4H), 0.90 (dt, 6H).
186	53	B	>300	C <sub>17</sub> H <sub>17</sub> N <sub>3</sub> O <sub>5</sub>	ESI/MS m/e [M+H] <sup>+</sup> 344	DMSO-d <sub>6</sub> : 12.20 (s, 1H), 7.81 (d, J=8.8 Hz, 2H), 6.90 (d, J=8.6 Hz, 2H), 6.60 (s, 1H), 4.90 (d, J=7.5 Hz, 1H), 3.44 (s, 3H), 3.22 (s, 3H), 1.45 (d, J=7 Hz, 3H).
187	50	B	>300	C <sub>19</sub> H <sub>21</sub> N <sub>3</sub> O <sub>5</sub>	ESI/MS m/e [M+H] <sup>+</sup> 372	DMSO-d <sub>6</sub> : 13.00 (bs, 1H), 12.45 (bs, 1H), 7.95 (m, 2H), 6.90 (m, 2H), 6.72 (s, 1H), 4.74 (s, 2H), 3.72 (d, 2H), 3.26 (s, 3H), 2.10 (m, 1H), 0.90 (d, 6H).
188	25	B	>300	C <sub>22</sub> H <sub>27</sub> N <sub>3</sub> O <sub>5</sub>	ESI/MS m/e [M+H] <sup>+</sup> 414	CDCl <sub>3</sub> : 11.61 (s, 1H), 7.40 (d, 2H), 7.00 (d, 2H), 6.05 (s, 1H), 4.08 (t, 2H), 3.94 (t, 2H), 1.70 (m, 10H), 0.90 (t, 6H).
189	50	B	>300	C <sub>16</sub> H <sub>14</sub> BrN <sub>3</sub> O <sub>5</sub>	ESI/MS m/e [M+H] <sup>+</sup> 407	DMSO-d <sub>6</sub> : 12.80 (s, 1H), 7.62 (d, J=8.7 Hz, 2H), 7.05 (d, J=8.6 Hz, 2H), 4.80 (s, 2H), 3.78 (s, 3H), 3.22 (s, 3H).
190	51	B	>300	C <sub>16</sub> H <sub>14</sub> ClN <sub>3</sub> O <sub>5</sub>	ESI/MS m/e [M+H] <sup>+</sup> 364	DMSO-d <sub>6</sub> : 12.65 (s, 1H), 7.28 (m, 1H), 7.07 (m, 1H), 6.85 (m, 1H), 6.15 (s, 1H), 4.66 (s, 2H), 3.94 (m, 4H), 3.86 (s, 3H), 1.70 (m, 4H), 0.96 (t, J=7.4 Hz, 6H).
191	44	B	>300	C <sub>18</sub> H <sub>14</sub> F <sub>6</sub> N <sub>3</sub> O <sub>5</sub>	ESI/MS m/e [M+H] <sup>+</sup> 466	DMSO-d <sub>6</sub> : 13.10 (s, 1H), 12.60 (s, 1H), 7.81 (d, J=8.8 Hz, 2H), 7.06 (d, J=8.7 Hz, 2H), 6.61 (s, 1H), 4.80 (m, 6H).

*continue*

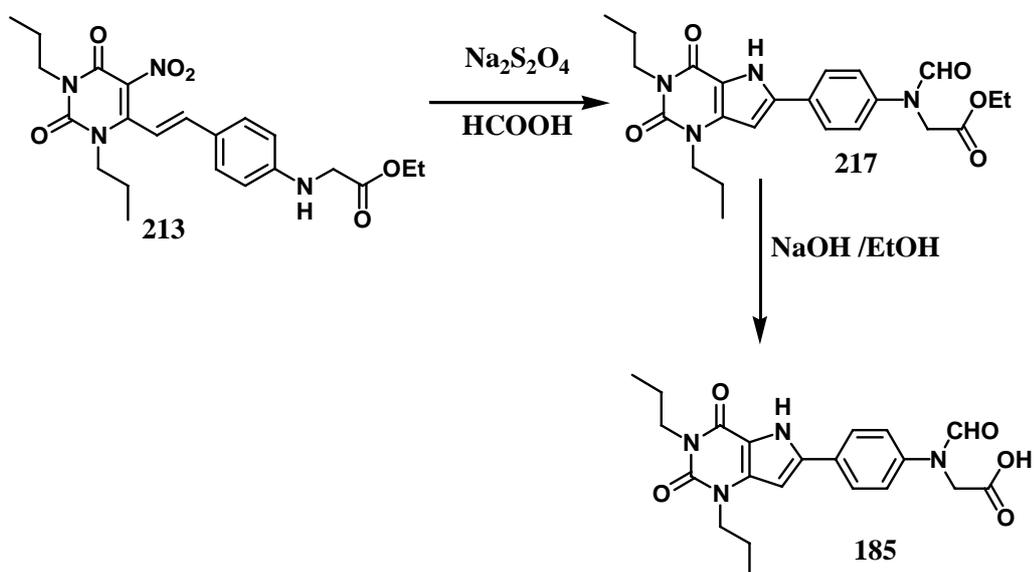
Compd	Yield (%)	Method	m.p. (°C)	Molecular Formula	MS	<sup>1</sup> H-NMR
192	88	B	>300	C <sub>22</sub> H <sub>26</sub> N <sub>4</sub> O <sub>5</sub>	ESI/MS m/e [M+H] <sup>+</sup> 443	CDCl <sub>3</sub> : 9.84 (s, 1H), 7.81 (d, 2H), 6.95 (d, 2H), 6.60 (s, 1H), 4.62 (s, 2H), 3.90 (t, 2H), 3.60 (m, 4H), 3.32 (s, 3H), 2.31 (m, 6H), 1.84 (m, 2H).
193	42	B	>300	C <sub>18</sub> H <sub>19</sub> N <sub>3</sub> O <sub>5</sub>	ESI/MS m/e [M+H] <sup>+</sup> 357	DMSO-d <sub>6</sub> : 13.15 (s, 1H), 12.27 (s, 1H), 7.79 (d, J= 8.8 Hz, 2H), 6.83 (d, J= 8.8 Hz, 2H), 6.60 (s, 1H), 3.39 (s, 3H), 3.23 (s, 3H), 1.52 (s, 6H).
194	30	B	>300	C <sub>22</sub> H <sub>27</sub> N <sub>3</sub> O <sub>7</sub>	ESI/MS m/e [M+H] <sup>+</sup> 446	DMSO-d <sub>6</sub> : 13.10 (bs, 1H), 12.25 (bs, 1H), 7.82 (d, 2H), 7.05 (d, 2H), 6.63 (s, 1H), 4.78 (s, 2H), 4.05 (m, 2H), 3.58 (m, 4H), 3.38 (s, 3H), 3.24 (s, 3H).
195	15	B	209-211	C <sub>20</sub> H <sub>22</sub> ClN <sub>3</sub> O <sub>5</sub>	EI/MS m/e [M] <sup>+</sup> 419	CDCl <sub>3</sub> : 11.61 (s, 1H), 7.48 (d, J=8.5 Hz, 1H), 6.98-6.93 (m, 2H), 6.36 (d, J=2.0 Hz, 1H), 4.82 (s, 2H), 4.07-3.91 (m, 4H), 1.84-1.66 (m, 4H), 1.01-0.88 (m, 6H).
196	45	B	153-155	C <sub>23</sub> H <sub>29</sub> N <sub>3</sub> O <sub>6</sub>	EI/MS m/e [M] <sup>+</sup> 443	DMSO-d <sub>6</sub> : 11.83 (s, 1H), 7.76 (d, J=8.6 Hz, 1H), 6.78 (s, 1H), 6.67 (m, 1H), 6.58 (s, 1H), 4.94 (s, 2H), 4.27 (q, J=7.1 Hz, 2H), 3.94 (m, 7H), 1.71 (m, 4H), 1.31 (t, J=7.1 Hz, 3H), 0.97 (m, 6H).
197	65	B	219-221	C <sub>21</sub> H <sub>25</sub> N <sub>3</sub> O <sub>6</sub>	EI/MS m/e [M] <sup>+</sup> 415	DMSO-d <sub>6</sub> : 11.76 (s, 1H), 7.68 (d, J=8.7 Hz, 1H), 6.69 (d, J=2.2 Hz, 1H), 6.57 (dd, J=8.7, 2.2 Hz, 1H), 6.50 (d, J=2.2 Hz, 1H), 4.75 (s, 2H), 4.59 (s, 1H), 3.87 (m, 7H), 1.63 (m, 4H), 0.89 (m, 6H).

**Synthesis of 4-(1,3-dimethyl-2,4-dioxo-2,3,4,5-tetrahydro-1*H*-pyrrolo[3,2-*d*]pyrimidin-6-yl)phenoxyacetic acid (**172**).**



Ester **206** (0.50 g, 1.28 mmol) was dissolved in formic acid (13 mL),  $\text{Na}_2\text{S}_2\text{O}_4$  (1.11 g, 6.39 mmol) was added and the mixture refluxed for 17 h. Upon cooling to room temperature a precipitate was obtained. It was filtered, washed with water and EtOH, to afford acid derivative **172** (0.25 g, 0.70 mmol). The aqueous layer was evaporated and MeOH was added, to obtain an additional quantity of **172** (0.16 g, 0.45 mmol). Overall yield 90%. m.p. (MeOH/ $\text{H}_2\text{O}$ ): 261-263°C.  $^1\text{H-NMR}$  ( $\delta$ , ppm, 250.13 MHz,  $\text{DMSO-d}_6$ ): 12.89 (bs, 1H), 12.19 (bs, 1H), 7.76 (d,  $J=8.5$  Hz, 2H), 6.89 (d,  $J=8.5$  Hz, 2H), 6.54 (d,  $J=1.6$  Hz, 1H), 4.65 (s, 2H), 3.33 (s, 3H) and 3.17 (s, 3H). Anal. Calcd for  $\text{C}_{16}\text{H}_{15}\text{N}_3\text{O}_5$ : C, 58.36; H, 4.59; N, 12.76. Found: C, 58.45; H, 4.23; N, 12.69. ; EI/MS ( $m/e$ ,  $[\text{M}]^+$ ): 329.

**Synthesis of *N*-[4-(2,4-dioxo-1,3-dipropyl-2,3,4,5-tetrahydro-1*H*-pyrrolo[3,2-*d*]pyrimidin-6-yl)phenyl]-*N*-formylglycine (**185**).**



Glycine ester derivative **213** (0.30 g, 0.67 mmol) was dissolved in formic acid (7 mL),  $\text{Na}_2\text{S}_2\text{O}_4$  (0.58 g, 3.37 mmol) was added and the mixture was refluxed for 24 h. Water was added to the cooled suspension and the precipitate obtained was filtered and washed to afford the 9-dAX derivative **217**. m.p. (MeOH/ $\text{H}_2\text{O}$ ): 267-269°C.  $^1\text{H-NMR}$  ( $\delta$ , ppm,  $\text{CDCl}_3$ ): 11.30 (s, 1H), 8.57 (s, 1H), 7.84 (d,  $J=8.5$  Hz), 7.32 (d,  $J=8.5$  Hz, 2H), 6.28 (s, 1H), 4.53 (s, 2H), 4.23 (q,  $J=7.2$  Hz, 2H), 4.07-3.93 (m, 4H), 1.87-1.66 (m, 4H), 1.30 (t,  $J=7.2$  Hz, 3H) and 1.05-0.92 (m, 6H). EI/MS ( $m/e$ ,  $[\text{M}]^+$ ): 440.

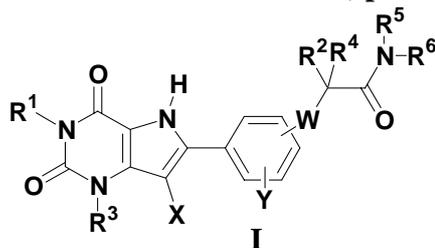
The hydrolysis of **217** to afford *N*-formylglycine derivative **185** was carried out as follows:

The *N*-formylglycine ester derivative **217** (0.20 g, 0.45 mmol) was dissolved in a mixture of EtOH (8 mL) and a 10% aqueous solution of NaOH (8 mL). The mixture was stirred at room temperature for 5 min. Then the solution was acidified with a 10% aqueous solution of HCl and the acidic precipitate **185** was filtered, washed with water and purified by crystallization with MeOH/H<sub>2</sub>O (0.13g, 71% yield) m.p.: 227-229°C. <sup>1</sup>H-NMR (δ, ppm, 250.13 MHz, CDCl<sub>3</sub>): 11.82 (s, 1H), 8.57 (s, 1H), 7.89 (d, J=8.4 Hz, 2H), 7.44 (d, J=8.4 Hz, 2H), 6.27 (d, J=2.2 Hz, 1H), 4.71 (s, 2H), 4.72-3.91 (m, 4H), 1.84-1.64 (m, 4H), 1.03-0.93 (m, 6H). Anal. Calcd. for C<sub>21</sub>H<sub>24</sub>N<sub>4</sub>O<sub>5</sub> : C, 61.15; H, 5.87; N, 13.58. Found: C, 60.88; H, 5.53; N, 13.63 ; EI/MS (m/e, [M]<sup>+</sup>): 412.

The representative procedures for the amidation of oxyacetic acid and oxyacetic ester 9-deazaxanthine derivatives (**V** and **VI**, see Scheme 2) were reported in the Experimental Section of the manuscript.

In **Table 14**, reaction data (yields and methods) were reported, along with some physicochemical, analytical and spectroscopic properties of compounds **7-164**. <sup>1</sup>H-NMR spectra and Elemental Analysis (**Table 15**) were listed only for compounds prepared by traditional solution phase synthesis. For most of the compounds listed in **Table 14**, which were prepared by parallel synthesis, only MW+1 or MW by ESI/MS or EI/MS, respectively, and HPLC retention times were indicated.

**Table 14 Experimental data of 9 dAXs I (cps 7–164 Tables 1–5)**



Compd	Yield (%)	Method	m.p. (°C)	Molecular Formula	MS	R.T. (min)	<sup>1</sup> H-NMR
7	51	B	265-267	C <sub>26</sub> H <sub>27</sub> BrN <sub>4</sub> O <sub>4</sub>	EI/MS m/e [M] <sup>+</sup> 539		CDCl <sub>3</sub> : 8.20 (s, 1H) 7.61 (d, J=8.7 Hz, 2H), 7.47-7.42 (m, 4H), 7.02 (d, J=8.5 Hz, 2H), 6.02 (s, 1H), 4.64 (s, 2H), 3.94-3.82 (m, 4H), 1.74-1.58 (m, 4H), 0.96-0.86 (m, 6H).
8	78	C		C <sub>26</sub> H <sub>27</sub> ClN <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 496	11.0	
9	59	C		C <sub>27</sub> H <sub>29</sub> FN <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 493	11.3	
10	25	B	256-258	C <sub>26</sub> H <sub>27</sub> FN <sub>4</sub> O <sub>4</sub>	EI/MS m/e [M] <sup>+</sup> 478		DMSO-d <sub>6</sub> : 12.21 (s, 1H), 10.18 (s, 1H), 7.89 (m, 2H), 7.63 (m, 2H), 7.12 (m, 4H), 6.67 (s, 1H), 4.77 (s, 2H), 3.84 (m, 4H), 1.61 (m, 4H), 0.91 (m, 6H).
11	77	C		C <sub>28</sub> H <sub>31</sub> FN <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 507	10.9	
12	59	C		C <sub>27</sub> H <sub>30</sub> N <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 475	11.6	
13	52	C		C <sub>28</sub> H <sub>32</sub> N <sub>4</sub> O <sub>5</sub>	ESI/MS m/e [M+H] <sup>+</sup> 505	11.7	
14	65	C		C <sub>28</sub> H <sub>31</sub> BrN <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 569	11.4	
15	35	D		C <sub>27</sub> H <sub>27</sub> F <sub>3</sub> N <sub>4</sub> O <sub>5</sub>	ESI/MS m/e [M+H] <sup>+</sup> 545	11.1	
16	76	C		C <sub>31</sub> H <sub>37</sub> N <sub>5</sub> O <sub>6</sub> S	ESI/MS m/e [M+H] <sup>+</sup> 608	10.1	
17	45	C		C <sub>30</sub> H <sub>36</sub> N <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 517	11.5	
18	47	C		C <sub>28</sub> H <sub>32</sub> N <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 489	10.9	
19	50	C		C <sub>28</sub> H <sub>31</sub> FN <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 507	10.9	
20	41	D		C <sub>33</sub> H <sub>34</sub> N <sub>4</sub> O <sub>5</sub>	ESI/MS m/e [M+H] <sup>+</sup> 567	11.2	
21	48	A	257-259	C <sub>26</sub> H <sub>28</sub> N <sub>4</sub> O <sub>4</sub>	EI/MS m/e [M] <sup>+</sup> 460		DMSO d <sub>6</sub> : 12.20 (s, 1H), 10.10 (s, 1H), 7.87 (d, J=8.6 Hz, 2H), 7.63 (d, J=8.2 Hz, 2H), 7.32 (m, 2H), 7.07 (m, 3H), 6.65 (s, 1H), 4.75 (s, 2H), 3.85 (m, 4H), 1.61 (m, 4H), 0.88 (m, 6H).

*continue*

Compd	Yield (%)	Method	m.p. (°C)	Molecular Formula	MS	R.T. (min)	<sup>1</sup> H-NMR
22	78	C		C <sub>28</sub> H <sub>32</sub> N <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 489	10.8	
23	63	D		C <sub>28</sub> H <sub>29</sub> N <sub>5</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 500	9.9	
24	76	D		C <sub>27</sub> H <sub>30</sub> N <sub>4</sub> O <sub>5</sub>	ESI/MS m/e [M+H] <sup>+</sup> 491	10.3	
25	64	C		C <sub>36</sub> H <sub>39</sub> N <sub>5</sub> O <sub>6</sub>	ESI/MS m/e [M+H] <sup>+</sup> 638	10.5	
26	62	C		C <sub>26</sub> H <sub>26</sub> BrFN <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 558	20.1	
27	60	C		C <sub>28</sub> H <sub>31</sub> N <sub>5</sub> O <sub>5</sub>	ESI/MS m/e [M+H] <sup>+</sup> 518	9.4	
28	38	D		C <sub>27</sub> H <sub>30</sub> N <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 475	10.7	
29	61	D		C <sub>32</sub> H <sub>32</sub> N <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 537	11.4	
30	50	C		C <sub>39</sub> H <sub>32</sub> N <sub>4</sub> O <sub>6</sub> S	ESI/MS m/e [M+H] <sup>+</sup> 537	12.3	
31	60	C		C <sub>27</sub> H <sub>29</sub> ClN <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 510	20.5	
32	44	C		C <sub>26</sub> H <sub>28</sub> N <sub>4</sub> O <sub>5</sub>	ESI/MS m/e [M+H] <sup>+</sup> 477	10.1	
33	44	C		C <sub>26</sub> H <sub>27</sub> IN <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 587	11.2	
34	64	C		C <sub>28</sub> H <sub>30</sub> N <sub>4</sub> O <sub>5</sub>	ESI/MS m/e [M+H] <sup>+</sup> 503	10.1	
35	82	C		C <sub>26</sub> H <sub>26</sub> Cl <sub>2</sub> N <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 530	20.0	
36	35	C		C <sub>26</sub> H <sub>26</sub> BrClN <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 574	21.0	
37	55	C		C <sub>26</sub> H <sub>27</sub> BrN <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 540	20.1	
38	48	C		C <sub>27</sub> H <sub>29</sub> N <sub>5</sub> O <sub>5</sub>	ESI/MS m/e [M+H] <sup>+</sup> 504	9.1	
39	48	C		C <sub>27</sub> H <sub>29</sub> ClN <sub>4</sub> O <sub>5</sub>	ESI/MS m/e [M+H] <sup>+</sup> 526	19.7	
40	65	D		C <sub>26</sub> H <sub>26</sub> ClFN <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 514	20.0	
41	60	D		C <sub>27</sub> H <sub>27</sub> N <sub>5</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 486	10.2	

*continue*

Compd	Yield (%)	Method	m.p. (°C)	Molecular Formula	MS	R.T. (min)	<sup>1</sup> H-NMR
42	85	D		C <sub>28</sub> H <sub>30</sub> N <sub>4</sub> O <sub>6</sub>	ESI/MS m/e [M+H] <sup>+</sup> 519	9.7	
43	28	D		C <sub>26</sub> H <sub>28</sub> N <sub>4</sub> O <sub>5</sub>	ESI/MS m/e [M+H] <sup>+</sup> 477	9.5	
44	65	C		C <sub>31</sub> H <sub>35</sub> N <sub>5</sub> O <sub>5</sub>	ESI/MS m/e [M+H] <sup>+</sup> 558	10.3	
45	72	D		C <sub>26</sub> H <sub>27</sub> ClN <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 495	19.9	
46	52	D		C <sub>28</sub> H <sub>29</sub> ClN <sub>4</sub> O <sub>5</sub>	ESI/MS m/e [M+H] <sup>+</sup> 538	10.8	
47	47	D		C <sub>29</sub> H <sub>32</sub> N <sub>4</sub> O <sub>6</sub>	ESI/MS m/e [M+H] <sup>+</sup> 532	10.6	
48	50	D		C <sub>30</sub> H <sub>34</sub> N <sub>4</sub> O <sub>6</sub>	ESI/MS m/e [M+H] <sup>+</sup> 547	12.1	
49	58	D		C <sub>26</sub> H <sub>26</sub> ClFN <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 513	20.2	
50	47	D		C <sub>27</sub> H <sub>28</sub> N <sub>4</sub> O <sub>5</sub>	ESI/MS m/e [M+H] <sup>+</sup> 489	10.6	
51	80	D		C <sub>33</sub> H <sub>32</sub> N <sub>4</sub> O <sub>5</sub>	ESI/MS m/e [M+H] <sup>+</sup> 565	11.0	
52	74	D		C <sub>26</sub> H <sub>26</sub> Cl <sub>2</sub> N <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 530	21.3	
53	44	D		C <sub>29</sub> H <sub>36</sub> N <sub>5</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 519	7.0	
54	78	D		C <sub>26</sub> H <sub>26</sub> ClFN <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 513	20.2	
55	50	D		C <sub>26</sub> H <sub>29</sub> N <sub>5</sub> O <sub>6</sub> S	ESI/MS m/e [M+H] <sup>+</sup> 540	9.3	
56	93	D		C <sub>32</sub> H <sub>32</sub> N <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 537	11	
57	68	D		C <sub>26</sub> H <sub>27</sub> FN <sub>4</sub> O <sub>5</sub>	ESI/MS m/e [M+H] <sup>+</sup> 495	10.4	
58	79	D		C <sub>26</sub> H <sub>27</sub> FN <sub>4</sub> O <sub>5</sub>	ESI/MS m/e [M+H] <sup>+</sup> 495	10.3	
59	48	D		C <sub>28</sub> H <sub>31</sub> BrN <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 568	11.5	
60	75	D		C <sub>27</sub> H <sub>27</sub> F <sub>3</sub> N <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 529	11.1	

*continue*

Compd	Yield (%)	Method	m.p. (°C)	Molecular Formula	MS	R.T. (min)	<sup>1</sup> H-NMR
61	20	D		C <sub>32</sub> H <sub>31</sub> FN <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 555	11.0	
62	71	B		C <sub>26</sub> H <sub>26</sub> BrClN <sub>4</sub> O <sub>4</sub>	EI/MS m/e [M] <sup>+</sup> 574	9.5	CDCl <sub>3</sub> : 12.22 (s, 1H), 10.22 (s, 1H), 7.57-7.43 (m, 5H), 7.17 (s, 1H), 7.01 (d, J=8.2 Hz, 1H), 6.38 (s, 1H), 4.75 (s, 2H), 3.29 (m, 4H), 1.68-1.48 (m, 4H), 0.82-0.79 (m, 6H).
63	97	B		C <sub>27</sub> H <sub>29</sub> BrN <sub>4</sub> O <sub>5</sub>	EI/MS m/e [M] <sup>+</sup> 569		CDCl <sub>3</sub> : 10.08 (s, 1H), 8.22 (s, 1H), 7.66 (d, J=8.4 Hz), 7.50 (m, 4H), 6.68 (m, 2H), 6.22 (s, 1H), 4.66 (s, 2H), 4.03 (s, 3H), 3.94 (m, 4H), 1.75 (m, 4H), 0.99 (m, 6H).
64	98	B		C <sub>27</sub> H <sub>29</sub> FN <sub>4</sub> O <sub>5</sub>	EI/MS m/e [M] <sup>+</sup> 508		CDCl <sub>3</sub> : 10.07 (s, 1H), 8.21 (s, 1H), 7.67 (d, J=8.4 Hz, 1H), 7.57 (m, 2H), 7.07 (m, 2H), 6.68 (m, 2H), 6.22 (d, J=2.4 Hz, 1H), 4.67 (s, 2H), 4.03 (s, 3H), 3.94 (m, 4H), 1.77 (m, 4H), 0.96 (m, 6H).
65	61	B		C <sub>27</sub> H <sub>30</sub> N <sub>4</sub> O <sub>5</sub>	EI/MS m/e [M] <sup>+</sup> 490		CDCl <sub>3</sub> : 10.07 (s, 1H), 8.21 (s, 1H), 7.63 (m, 3H), 7.38 (m, 2H), 7.18 (m, 1H), 6.67 (m, 2H), 6.21 (d, J=2.1 Hz, 1H), 4.67 (s, 2H), 4.04 (s, 3H), 3.94 (m, 4H), 1.76 (m, 4H), 0.99 (m, 6H).
66	85	A		C <sub>23</sub> H <sub>21</sub> BrN <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 497	8.8	-
67	15	A		C <sub>22</sub> H <sub>19</sub> BrN <sub>4</sub> O <sub>4</sub>	EI/MS m/e [M] <sup>+</sup> 483		CDCl <sub>3</sub> : 7.89 (d, J=8.6 Hz, 2H), 7.19 (d, J=8.6 Hz, 2H), 7.00 (d, J=8.4 Hz, 2H), 6.67 (s, 1H), 6.57 (m, 2H), 4.61 (s, 2H), 3.49 (s, 3H), 3.33 (s, 3H).
68	15	A		C <sub>22</sub> H <sub>19</sub> FN <sub>4</sub> O <sub>4</sub>	EI/MS m/e [M] <sup>+</sup> 423	-	DMSO-d <sub>6</sub> : 12.50 (bs, 1H), 10.36 (bs, 1H), 8.08 (d, J=8.2 Hz, 2H), 7.87 (m, 2H), 7.38 (m, 2H), 7.28 (d, J=8.2 Hz, 2H), 6.83 (s, 1H), 5.00 (s, 2H), 3.53 (s, 3H), 3.46 (s, 3H).
69	70	A		C <sub>23</sub> H <sub>21</sub> BrN <sub>4</sub> O <sub>5</sub>	ESI/MS m/e [M+H] <sup>+</sup> 513	11.0	
70	25	B		C <sub>24</sub> H <sub>22</sub> N <sub>4</sub> O <sub>5</sub>	EI/MS m/e [M] <sup>+</sup> 446		DMSO-d <sub>6</sub> : 8.03 (d, J=8.8 Hz, 2H), 7.93 (d, J=8.8 Hz, 2H), 7.85 (d, J=8.8 Hz, 2H), 7.15 (d, J=8.8 Hz, 2H), 6.68 (s, 1H), 4.89 (s, 2H), 3.49 (s, 3H), 3.33 (s, 3H), 2.60 (s, 3H).
71	15	B		C <sub>23</sub> H <sub>19</sub> N <sub>5</sub> O <sub>4</sub>	EI/MS m/e [M] <sup>+</sup> 429		DMSO-d <sub>6</sub> : 12.18 (bs, 1H), 10.50 (bs, 1H), 7.79 (m, 6H), 7.05 (m, 2H), 6.60 (s, 1H), 4.77 (s, 2H), 3.37 (s, 3H), 3.24 (s, 3H).
72	90	A		C <sub>23</sub> H <sub>21</sub> FN <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 437	9.0	
73	85	A		C <sub>23</sub> H <sub>22</sub> N <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 419	8.8	
74	15	B		C <sub>22</sub> H <sub>19</sub> N <sub>5</sub> O <sub>6</sub>	EI/MS m/e [M] <sup>+</sup> 449		DMSO-d <sub>6</sub> : 12.30 (bs, 1H), 10.80 (bs, 1H), 8.31 (d, J=8.8 Hz, 2H), 7.95 (m, 4H), 7.13 (d, J=8.2 Hz, 2H), 6.69 (s, 1H), 4.91 (s, 2H), 3.51 (s, 3H), 3.31 (s, 3H).
75	75	A		C <sub>22</sub> H <sub>20</sub> N <sub>4</sub> O <sub>4</sub>	EI/MS m/e [M] <sup>+</sup> 404		DMSO-d <sub>6</sub> : 12.30 (bs, 1H), 10.22 (bs, 1H), 7.88 (d, J=8.6 Hz, 2H), 7.66 (d, J=7.8 Hz, 2H), 7.34 (m, 2H), 7.09 (m, 3H), 6.62 (s, 1H), 4.78 (s, 2H), 3.42 (s, 3H), 3.27 (s, 3H).

*continue*

Compd	Yield (%)	Method	m.p. (°C)	Molecular Formula	MS	R.T. (min)	<sup>1</sup> H-NMR
76	37	B		C <sub>25</sub> H <sub>24</sub> N <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 445	16.0	
77	74	A		C <sub>23</sub> H <sub>21</sub> FN <sub>4</sub> O <sub>5</sub>	ESI/MS m/e [M+H] <sup>+</sup> 452	12.0	
78	70	A		C <sub>23</sub> H <sub>21</sub> BrN <sub>4</sub> O <sub>5</sub>	ESI/MS m/e [M+H] <sup>+</sup> 514	10.9	
79	58	A		C <sub>23</sub> H <sub>22</sub> N <sub>4</sub> O <sub>5</sub>	ESI/MS m/e [M+H] <sup>+</sup> 435	10.1	
80	81	B		C <sub>21</sub> H <sub>24</sub> N <sub>4</sub> O <sub>4</sub>	EI/MS m/e [M] <sup>+</sup> 396		DMSO-d <sub>6</sub> : 8.02 (d, J=8.7 Hz, 2H), 7.18 (d, J=8.7 Hz, 2H), 6.69 (s, 1H), 4.68 (s, 2H), 4.27 (m, 1H), 3.60 (s, 3H), 3.45 (s, 3H), 2.03-1.60 (m, 8H).
81	65	D		C <sub>23</sub> H <sub>21</sub> FN <sub>4</sub> O <sub>5</sub>	ESI/MS m/e [M+H] <sup>+</sup> 453	10.1	
82	49	D		C <sub>23</sub> H <sub>22</sub> N <sub>4</sub> O <sub>5</sub>	ESI/MS m/e [M+H] <sup>+</sup> 435	10.0	
83	33	A		C <sub>18</sub> H <sub>21</sub> N <sub>5</sub> O <sub>4</sub>	EI/MS m/e [M] <sup>+</sup> 371		DMSO-d <sub>6</sub> : 7.85 (d, J=8.8 Hz, 2H), 7.01 (d, J=8.8 Hz, 2H), 6.63 (s, 1H), 4.52 (s, 2H), 3.41 (s, 3H), 3.25 (s, 3H), 3.12 (m, 2H), 2.50 (m, 2H).
84	59	D		C <sub>24</sub> H <sub>21</sub> N <sub>5</sub> O <sub>5</sub>	ESI/MS m/e [M+H] <sup>+</sup> 460	9.8	
85	40	D		C <sub>23</sub> H <sub>22</sub> N <sub>4</sub> O <sub>4</sub>	EI/MS m/e [M] <sup>+</sup> 418		CDCl <sub>3</sub> : 10.68 (bs, 1H), 7.64 (d, J=8.7 Hz, 2H), 7.52-7.42 (m, 3H), 7.30-7.28 (m, 2H), 6.83 (d, J=8.7 Hz, 2H), 6.16 (d, J=2.3 Hz, 1H), 4.46 (s, 2H), 3.53 (s, 3H), 3.46 (s, 3H), 3.35 (s, 3H).
86	44	D		C <sub>26</sub> H <sub>26</sub> N <sub>4</sub> O <sub>7</sub>	ESI/MS m/e [M+H] <sup>+</sup> 507	9.6	
87	88	D		C <sub>28</sub> H <sub>28</sub> N <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 485	10.6	
88	86	D		C <sub>30</sub> H <sub>28</sub> N <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 509	10.9	
89	48	D		C <sub>24</sub> H <sub>23</sub> FN <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 451	9.7	
90	31	D		C <sub>24</sub> H <sub>23</sub> IN <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 559	10.2	
91	10	D		C <sub>24</sub> H <sub>24</sub> N <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 433	10.6	
92	78	D		C <sub>24</sub> H <sub>23</sub> FN <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 451	10.8	
93	39	D		C <sub>27</sub> H <sub>28</sub> N <sub>4</sub> O <sub>6</sub>	ESI/MS m/e [M+H] <sup>+</sup> 505	10.2	

*continue*

Compd	Yield (%)	Method	m.p. (°C)	Molecular Formula	MS	R.T. (min)	<sup>1</sup> H-NMR
94	51	D		C <sub>24</sub> H <sub>24</sub> N <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 433	9.6	
95	35	D		C <sub>25</sub> H <sub>23</sub> N <sub>5</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 458	9.4	
96	65	A		C <sub>25</sub> H <sub>26</sub> N <sub>4</sub> O <sub>5</sub>	EI/MS m/e [M] <sup>+</sup> 463		CDCl <sub>3</sub> : 7.71 (d, J=8.5 Hz, 2H), 7.60 (d, J=7.9 Hz, 2H), 7.38 (m, 3H), 7.15 (m, 2H), 6.34 (s, 1H), 4.70 (s, 2H), 4.10 (m, 2H), 3.48 (m, 2H), 3.43 (s, 3H), 3.37 (s, 3H), 2.05 (m, 2H).
97	65	C		C <sub>24</sub> H <sub>24</sub> N <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 433	10.7	
98	49	C		C <sub>28</sub> H <sub>30</sub> N <sub>4</sub> O <sub>6</sub>	ESI/MS m/e [M+H] <sup>+</sup> 519	9.9	
99	54	A		C <sub>24</sub> H <sub>30</sub> N <sub>4</sub> O <sub>5</sub>	EI/MS m/e [M] <sup>+</sup> 454		DMSO-d <sub>6</sub> : 7.83 (d, J=8.8 Hz, 2H), 6.99 (d, J=8.8 Hz, 2H), 6.56 (s, 1H), 4.48 (s, 2H), 4.05 (m, 1H), 3.93 (t, J=6.9 Hz, 2H), 3.55 (m, 2H), 3.39 (s, 3H), 3.37 (s, 3H), 1.92-1.07 (m, 10H).
100	79	C		C <sub>28</sub> H <sub>27</sub> FN <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 503	10.6	
101	68	C		C <sub>28</sub> H <sub>27</sub> BrN <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 564	11.2	
102	84	C		C <sub>26</sub> H <sub>27</sub> BrN <sub>4</sub> O <sub>6</sub>	ESI/MS m/e [M+H] <sup>+</sup> 573	9.9	
103	72	C		C <sub>24</sub> H <sub>23</sub> BrN <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 512	11.6	
104	94	D		C <sub>24</sub> H <sub>17</sub> BrF <sub>6</sub> N <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 620	10.7	
105	85	D		C <sub>26</sub> H <sub>27</sub> FN <sub>4</sub> O <sub>6</sub>	ESI/MS m/e [M+H] <sup>+</sup> 511	9.1	
106	90	D		C <sub>24</sub> H <sub>17</sub> F <sub>7</sub> N <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 559	10.2	
107	90	D		C <sub>26</sub> H <sub>28</sub> N <sub>4</sub> O <sub>6</sub>	ESI/MS m/e [M+H] <sup>+</sup> 493	9.0	
108	88	D		C <sub>24</sub> H <sub>18</sub> FN <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 541	10.1	
109	51	D		C <sub>25</sub> H <sub>26</sub> N <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 447	9.9	
110	13	D		C <sub>27</sub> H <sub>26</sub> N <sub>4</sub> O <sub>5</sub>	ESI/MS m/e [M+H] <sup>+</sup> 487	10.3	
111	80	D		C <sub>32</sub> H <sub>37</sub> FN <sub>6</sub> O <sub>5</sub>	ESI/MS m/e [M+H] <sup>+</sup> 536	9.8	
112	53	D		C <sub>27</sub> H <sub>30</sub> N <sub>4</sub> O <sub>5</sub>	ESI/MS m/e [M+H] <sup>+</sup> 475		DMSO-d <sub>6</sub> : 8.65 (t, J= 6.5, 1H), 7.86 (d, J= 8.8 Hz, 2H), 7.34-7.24 (m, 5H), 7.02 (d, J= 8.8 Hz, 2H), 6.64 (s, 1H), 4.59 (s, 2H), 4.33 (d, J=6.5, 2H), 3.87-3.82 (m, 4H), 1.70-1.50 (m, 4H), 0.92-0.82 (m, 6H).

*continue*

Compd	Yield (%)	Method	m.p. (°C)	Molecular Formula	MS	R.T. (min)	<sup>1</sup> H-NMR
113	47	D		C <sub>28</sub> H <sub>31</sub> ClN <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 524	11.9	
114	55	D		C <sub>23</sub> H <sub>21</sub> ClN <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 453	16.0	
115	30	D		C <sub>23</sub> H <sub>21</sub> FN <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 437	15.0	
116	60	D		C <sub>25</sub> H <sub>25</sub> ClN <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 481	10.0	
117	60	D		C <sub>24</sub> H <sub>24</sub> N <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 433	15.5	
118	36	D		C <sub>25</sub> H <sub>26</sub> N <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 447	16.2	
119	43	D		C <sub>23</sub> H <sub>22</sub> N <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 419	14.7	
120	40	D		C <sub>24</sub> H <sub>24</sub> N <sub>4</sub> O <sub>5</sub>	ESI/MS m/e [M+H] <sup>+</sup> 449	14.8	
121	33	D		C <sub>24</sub> H <sub>24</sub> N <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 433	15.3	
122	70	D		C <sub>27</sub> H <sub>29</sub> ClN <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 510	19.7	
123	61	D		C <sub>24</sub> H <sub>23</sub> ClN <sub>4</sub> O <sub>5</sub>	ESI/MS m/e [M+H] <sup>+</sup> 483	8.4	
124	80	D		C <sub>27</sub> H <sub>28</sub> ClFN <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 528	19.7	
125	58	D		C <sub>24</sub> H <sub>23</sub> ClN <sub>4</sub> O <sub>5</sub>	ESI/MS m/e [M+H] <sup>+</sup> 483	10.3	
126	93	D		C <sub>28</sub> H <sub>32</sub> N <sub>4</sub> O <sub>5</sub>	ESI/MS m/e [M+H] <sup>+</sup> 505	9.7	
127	73	D		C <sub>28</sub> H <sub>31</sub> ClN <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 524	10.6	
128	91	D		C <sub>29</sub> H <sub>32</sub> N <sub>4</sub> O <sub>6</sub>	ESI/MS m/e [M+H] <sup>+</sup> 533	10.3	
129	57	C		C <sub>28</sub> H <sub>30</sub> N <sub>4</sub> O <sub>6</sub>	ESI/MS m/e [M+H] <sup>+</sup> 519	10.2	
130	88	C		C <sub>26</sub> H <sub>28</sub> N <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 461	10.8	
131	47	C		C <sub>25</sub> H <sub>26</sub> N <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 447	10.5	
132	79	C		C <sub>24</sub> H <sub>25</sub> N <sub>5</sub> O <sub>5</sub>	ESI/MS m/e [M+H] <sup>+</sup> 464	8.9	

*continue*

Compd	Yield (%)	Method	m.p. (°C)	Molecular Formula	MS	R.T. (min)	<sup>1</sup> H-NMR
133	40	A		C <sub>27</sub> H <sub>28</sub> BrN <sub>5</sub> O <sub>4</sub>	EI/MS m/e [M] <sup>+</sup> 566		DMSO-d <sub>6</sub> : 8.60 (s, 1H), 7.75 (d, J=8.6 Hz, 2H), 7.50-7.39 (m, 6H), 6.27 (s, 1H), 4.56 (s, 2H), 4.03-3.91 (m, 4H), 1.86-1.65 (m, 4H), 1.05-0.81 (m, 6H).
134	85	B		C <sub>26</sub> H <sub>29</sub> N <sub>5</sub> O <sub>5</sub>	ESI/MS m/e [M+H] <sup>+</sup> 492	10.0	
135	25	B		C <sub>26</sub> H <sub>30</sub> N <sub>6</sub> O <sub>6</sub>	EI/MS m/e [M] <sup>+</sup> 522		DMSO-d <sub>6</sub> : 12.43 (bs, 1H), 11.04 (bs, 1H), 8.06 (d, J=8.5 Hz, 2H), 7.26-7.16 (m, 3H), 6.85 (s, 1H), 5.08 (s, 2H), 4.09-4.07 (m, 10H), 1.95-1.68 (m, 4H), 1.15-1.02 (m, 6H).
136	26	B		C <sub>26</sub> H <sub>29</sub> N <sub>5</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 476	10.5	
137	29	B		C <sub>18</sub> H <sub>16</sub> N <sub>6</sub> O <sub>4</sub> S	EI/MS m/e [M] <sup>+</sup> 412		DMSO-d <sub>6</sub> : 9.23 (s, 1H), 8.64 (s, 1H), 7.93 (d, J=9.0 Hz, 2H), 7.26 (s, 1H), 7.12 (d, J=9.0 Hz, 2H), 6.70 (s, 1H), 5.03 (s, 2H), 3.49 (s, 3H), 3.33 (s, 3H).
138	37	B		C <sub>23</sub> H <sub>26</sub> N <sub>6</sub> O <sub>4</sub>	EI/MS m/e [M] <sup>+</sup> 450		DMSO-d <sub>6</sub> : 12.27 (bs, 1H), 8.11 (d, J=2.2 Hz, 1H), 8.97 (d, J=8.3, 2H), 7.03 (d, J=8.2 Hz, 2H), 6.67 (s, 1H), 6.03 (d, J=1.8 Hz, 1H), 5.84 (s, 2H), 5.44 (s, 2H), 3.92-3.86 (m, 4H), 1.72-1.56 (m, 4H), 0.96-0.87 (m, 6H).
139	84	B		C <sub>23</sub> H <sub>20</sub> N <sub>6</sub> O <sub>4</sub>	EI/MS m/e [M] <sup>+</sup> 444		DMSO-d <sub>6</sub> : 12.12 (bs, 1H), 7.83 (d, J=8.3 Hz, 2H), 7.40 (m, 2H), 7.04 (m, 4H), 6.80 (bs, 1H), 6.58 (s, 1H), 6.04 (bs, 1H), 4.88 (s, 2H), 3.38 (s, 3H), 3.22 (s, 3H).
140	54	B		C <sub>25</sub> H <sub>27</sub> N <sub>5</sub> O <sub>5</sub>	ESI/MS m/e [M+H] <sup>+</sup> 478	10.1	
141	42	B		C <sub>26</sub> H <sub>28</sub> N <sub>6</sub> O <sub>8</sub>	EI/MS m/e [M] <sup>+</sup> 552		DMSO-d <sub>6</sub> : 12.26 (bs, 1H), 11.12 (bs, 1H), 10.94 (bs, 1H), 8.47 (bs, 1H), 7.86 (d, J=8.8 Hz, 2H), 6.92 (d, J=8.7 Hz, 2H), 6.67 (d, J=2.2 Hz, 1H), 5.35 (s, 2H), 3.97-3.87 (m, 4H), 3.33 (s, 3H), 1.81-1.57 (m, 4H), 1.01-0.91 (m, 6H).
142	54	C		C <sub>25</sub> H <sub>27</sub> N <sub>5</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 462	10.1	
143	40	C		C <sub>26</sub> H <sub>29</sub> N <sub>5</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 476	7.6	
144	63	C		C <sub>22</sub> H <sub>19</sub> BrN <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 484	10.9	
145	50	C		C <sub>21</sub> H <sub>20</sub> N <sub>4</sub> O <sub>5</sub>	ESI/MS m/e [M+H] <sup>+</sup> 409	13.6	
146	40	B		C <sub>27</sub> H <sub>30</sub> N <sub>6</sub> O <sub>8</sub>	EI/MS m/e [M] <sup>+</sup> 566		DMSO-d <sub>6</sub> : 12.20 (bs, 1H), 10.84 (bs, 1H), 8.47 (bs, 1H), 7.87 (d, J=8.8 Hz, 2H), 6.93 (d, J=8.7 Hz, 2H), 6.68 (d, J=2.2 Hz, 1H), 5.38 (s, 2H), 3.96-3.90 (m, 4H), 3.70 (s, 3H), 3.32 (s, 3H), 1.80-1.60 (m, 4H), 0.98-0.91 (m, 6H)

*continue*

Compd	Yield (%)	Method	m.p. (°C)	Molecular Formula	MS	R.T. (min)	<sup>1</sup> H-NMR
147	86	C		C <sub>22</sub> H <sub>20</sub> N <sub>4</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 405	10.0	
148	33	A		C <sub>27</sub> H <sub>28</sub> FN <sub>5</sub> O <sub>4</sub>	EI/MS m/e [M] <sup>+</sup> 505		DMSO-d <sub>6</sub> : 8.61 (s, 1H), 7.76 (d, J=8.6 Hz, 2H), 7.67-7.51 (m, 2H), 7.43 (d, J=8.6 Hz, 2H), 7.06-6.99 (m, 2H), 6.28 (s, 1H), 4.58 (s, 2H), 4.03-3.91 (m, 4H), 1.87-1.66 (m, 4H) 1.05-0.96 (m, 6H).
149	70	C		C <sub>25</sub> H <sub>27</sub> N <sub>5</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 462	9.3	
150	26	C		C <sub>24</sub> H <sub>25</sub> N <sub>5</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 448	10.5	
151	36	C		C <sub>25</sub> H <sub>23</sub> N <sub>5</sub> O <sub>5</sub>	ESI/MS m/e [M+H] <sup>+</sup> 450	9.5	
152	88	C		C <sub>29</sub> H <sub>34</sub> N <sub>4</sub> O <sub>5</sub>	ESI/MS m/e [M+H] <sup>+</sup> 519	10.1	
153	80	C		C <sub>26</sub> H <sub>29</sub> N <sub>5</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 476	10.0	
154	51	C		C <sub>26</sub> H <sub>29</sub> N <sub>5</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 476	11.0	
155	20	A		C <sub>26</sub> H <sub>29</sub> N <sub>5</sub> O <sub>4</sub>	EI/MS m/e [M] <sup>+</sup> 476		CDCl <sub>3</sub> : 10.81 (bs, 1H), 8.58-8.56 (m, 2H), 7.76-7.73 (m, 2H), 7.45-7.44 (m, 1H), 7.21-7.20 (m, 2H), 7.02-6.99 (m, 1H), 6.19 (s, 1H), 4.65 (s, 2H), 4.60-4.58 (m, 2H), 4.04-3.94 (m, 4H), 1.77-1.68 (m, 4H), 1.04-0.89 (m, 6H).
156	54	B		C <sub>24</sub> H <sub>26</sub> N <sub>6</sub> O <sub>4</sub>	EI/MS m/e [M] <sup>+</sup> 462		DMSO-d <sub>6</sub> : 12.33 (bs, 1H), 11.05 (bs, 1H), 9.41 (s, 1H), 8.54-8.50 (m, 2H), 7.97 (d, J=8.7 Hz, 2H), 7.14 (d, J=8.8 Hz, 2H), 6.76 (s, 1H), 5.01 (s, 2H), 3.99-3.93 (m, 4H), 1.82-1.61 (m, 4H), 1.04-0.94 (m, 6H).
157	50	C		C <sub>26</sub> H <sub>29</sub> N <sub>5</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 476	10.0	
158	51	C		C <sub>27</sub> H <sub>31</sub> N <sub>5</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 490	11.0	
159	71	A		C <sub>22</sub> H <sub>21</sub> N <sub>5</sub> O <sub>4</sub>	EI/MS m/e [M] <sup>+</sup> 419		DMSO-d <sub>6</sub> : 12.30 (bs, 1H), 8.79 (m, 1H), 8.50 (m, 1H), 7.89 (d, J=8.7 Hz, 2H), 7.25 (d, J=2.9 Hz, 2H), 7.01 (d, J=8.7 Hz, 2H), 6.66 (d, J=2.2 Hz, 1H), 4.68 (s, 2H), 4.38 (d, J=6.0 Hz, 2H), 3.43 (s, 3H), 3.27 (s, 3H).
160	60	B		C <sub>22</sub> H <sub>22</sub> N <sub>6</sub> O <sub>6</sub>	EI/MS m/e [M] <sup>+</sup> 466		DMSO-d <sub>6</sub> : 12.24 (bs, 1H), 10.81 (bs, 1H), 7.81 (d, J=8.8 Hz, 2H), 7.01 (s, 1H), 6.96 (d, J=8.8 Hz, 2H), 6.59 (d, J=2.2 Hz, 1H), 4.84 (s, 2H), 3.86 (s, 3H), 3.84 (s, 3H), 3.37 (s, 3H), 3.22 (s, 3H).
161	35	C		C <sub>24</sub> H <sub>25</sub> N <sub>5</sub> O <sub>4</sub>	ESI/MS m/e [M+H] <sup>+</sup> 448		
162	56	B		C <sub>27</sub> H <sub>29</sub> N <sub>5</sub> O <sub>4</sub>	EI/MS m/e [M] <sup>+</sup> 487		CDCl <sub>3</sub> : 8.61 (s, 1H), 7.75 (d, J=6.6 Hz, 2H), 7.56 (d, 2H), 7.44-7.11 (m, 5H), 6.27 (s, 1H), 4.58 (s, 2H), 4.03-3.80 (m, 4H), 1.83-1.65 (m, 4H), 1.05-0.86 (m, 6H).

*continue*

Compd	Yield (%)	Method	m.p. (°C)	Molecular Formula	MS m/e [M] <sup>+</sup>	R.T. (min)	<sup>1</sup> H-NMR
163	77	B		C <sub>20</sub> H <sub>18</sub> N <sub>6</sub> O <sub>4</sub>	EI/MS 406		DMSO-d <sub>6</sub> : 12.33 (bs, 1H, -NH), 12.07 (bs, 1H, -NH), 9.41 (s, 1H, ArH), 8.55-8.50 (m, 2H, 2xArH), 7.97 (d, J=8.7 Hz, 2H, 2xArH), 7.15 (d, J=8.7 Hz, 2H, 2xArH), 6.73 (s, 1H, -CH=C-), 5.02 (s, 2H, -CH <sub>2</sub> -), 3.52 (s, 3H, -CH <sub>3</sub> ), 3.36 (s, 3H, -CH <sub>3</sub> ).
164	20	B		C <sub>22</sub> H <sub>24</sub> N <sub>4</sub> O <sub>9</sub>	EI/MS 546		DMSO-d <sub>6</sub> : 7.74 (d, J=8.1 Hz, 2H, 2xArH), 7.00 (d, J=8.1 Hz, 2H, 2xArH), 6.50 (s, 1H, -CH=C-), 4.95 (d, J=3.3 Hz, 1H, -CH-), 4.52 (s, 2H, -CH <sub>2</sub> -), 4.46 (s, 1H, -CH-), 3.69-3.36 (m, 5H, 5x-CH-), 3.19 (s, 3H, -CH <sub>3</sub> ), 3.12 (s, 3H, -CH <sub>3</sub> ).

**Table 15. Elemental Analyses.**

Compd	Molecular Formula	C		H		N	
		Theor	Found	Theor	Found	Theor	Found
7	C <sub>26</sub> H <sub>27</sub> BrN <sub>4</sub> O <sub>4</sub>	57.93	57.58	5.05	4.87	10.39	10.56
10	C <sub>26</sub> H <sub>27</sub> FN <sub>4</sub> O <sub>4</sub>	65.32	65.67	5.69	5.48	11.72	11.89
21	C <sub>26</sub> H <sub>28</sub> N <sub>4</sub> O <sub>4</sub>	67.88	68.45	6.13	5.92	12.18	12.38
62	C <sub>26</sub> H <sub>26</sub> BrClN <sub>4</sub> O <sub>4</sub>	54.40	54.13	4.57	4.46	9.76	9.38
63	C <sub>27</sub> H <sub>29</sub> BrN <sub>4</sub> O <sub>5</sub>	57.00	56.89	5.14	4.88	9.85	9.76
64	C <sub>27</sub> H <sub>29</sub> FN <sub>4</sub> O <sub>5</sub>	63.83	63.47	5.75	5.89	11.03	11.35
65	C <sub>27</sub> H <sub>30</sub> N <sub>4</sub> O <sub>5</sub>	66.18	66.50	6.17	6.36	11.43	11.23
67	C <sub>22</sub> H <sub>19</sub> BrN <sub>4</sub> O <sub>4</sub>	54.70	55.05	3.96	4.21	11.60	11.74
68	C <sub>22</sub> H <sub>19</sub> FN <sub>4</sub> O <sub>4</sub>	62.46	62.09	4.53	4.67	13.24	13.58
70	C <sub>24</sub> H <sub>22</sub> N <sub>4</sub> O <sub>5</sub>	64.62	64.25	4.97	4.76	9.42	9.63
71	C <sub>23</sub> H <sub>19</sub> N <sub>5</sub> O <sub>4</sub>	64.39	64.19	4.46	4.50	16.33	16.40
74	C <sub>22</sub> H <sub>19</sub> N <sub>5</sub> O <sub>6</sub>	58.84	58.63	4.26	4.44	15.60	15.51
75	C <sub>22</sub> H <sub>20</sub> N <sub>4</sub> O <sub>4</sub>	65.40	64.17	4.99	5.08	13.87	13.67
80	C <sub>21</sub> H <sub>24</sub> N <sub>4</sub> O <sub>4</sub>	63.69	62.33	6.11	5.98	14.15	13.99
83	C <sub>18</sub> H <sub>21</sub> N <sub>5</sub> O <sub>4</sub>	58.22	58.03	5.66	5.61	18.86	18.64
85	C <sub>23</sub> H <sub>22</sub> N <sub>4</sub> O <sub>4</sub>	66.08	66.35	5.30	5.15	13.40	13.47
96	C <sub>25</sub> H <sub>26</sub> N <sub>4</sub> O <sub>5</sub>	64.25	64.01	5.66	5.60	12.10	12.37
99	C <sub>24</sub> H <sub>30</sub> N <sub>4</sub> O <sub>5</sub>	63.49	63.10	6.66	6.41	12.34	12.15
112	C <sub>27</sub> H <sub>30</sub> N <sub>4</sub> O <sub>5</sub>	68.26	67.89	6.40	6.45	11.79	11.71
133	C <sub>27</sub> H <sub>28</sub> BrN <sub>5</sub> O <sub>4</sub>	57.29	57.03	4.98	4.85	12.37	12.25
135	C <sub>26</sub> H <sub>30</sub> N <sub>6</sub> O <sub>6</sub>	59.82	59.67	5.79	5.70	16.10	16.41
137	C <sub>18</sub> H <sub>16</sub> N <sub>6</sub> O <sub>4</sub> S	52.47	52.21	3.91	3.98	20.40	20.27
138	C <sub>23</sub> H <sub>26</sub> N <sub>6</sub> O <sub>4</sub>	61.38	61.18	5.82	5.89	18.67	18.56
139	C <sub>23</sub> H <sub>20</sub> N <sub>6</sub> O <sub>4</sub>	62.21	61.97	4.54	4.34	18.93	18.76
141	C <sub>26</sub> H <sub>28</sub> N <sub>6</sub> O <sub>8</sub>	56.57	56.34	5.11	5.22	15.23	14.87
146	C <sub>27</sub> H <sub>30</sub> N <sub>6</sub> O <sub>8</sub>	57.29	56.98	5.34	5.38	14.85	14.67
148	C <sub>27</sub> H <sub>28</sub> FN <sub>5</sub> O <sub>4</sub>	64.21	63.98	5.56	5.45	13.86	13.79
155	C <sub>26</sub> H <sub>29</sub> N <sub>5</sub> O <sub>4</sub>	65.60	65.23	6.14	6.27	14.71	14.60
156	C <sub>24</sub> H <sub>26</sub> N <sub>6</sub> O <sub>4</sub>	62.39	62.12	5.67	5.45	18.19	18.25
159	C <sub>22</sub> H <sub>21</sub> N <sub>5</sub> O <sub>4</sub>	63.06	62.74	5.05	5.15	16.72	16.67
160	C <sub>22</sub> H <sub>22</sub> N <sub>6</sub> O <sub>6</sub>	56.70	56.39	4.76	4.91	18.03	17.84
162	C <sub>27</sub> H <sub>29</sub> N <sub>5</sub> O <sub>4</sub>	66.58	66.45	6.00	6.24	14.38	14.15
163	C <sub>20</sub> H <sub>18</sub> N <sub>6</sub> O <sub>4</sub>	59.16	58.89	4.47	4.31	20.70	20.55
164	C <sub>22</sub> H <sub>24</sub> N <sub>4</sub> O <sub>9</sub>	48.40	48.11	4.43	4.52	10.26	10.34
165	C <sub>20</sub> H <sub>23</sub> N <sub>3</sub> O <sub>5</sub>	62.39	62.08	6.02	6.15	10.91	10.72
166	C <sub>18</sub> H <sub>19</sub> N <sub>3</sub> O <sub>5</sub>	60.55	60.17	5.36	5.46	11.77	11.58
167	C <sub>21</sub> H <sub>25</sub> N <sub>3</sub> O <sub>5</sub>	63.21	62.97	6.32	6.51	10.53	10.27
168	C <sub>18</sub> H <sub>19</sub> N <sub>3</sub> O <sub>5</sub>	60.55	60.29	5.36	5.40	11.77	11.64
169	C <sub>22</sub> H <sub>27</sub> N <sub>3</sub> O <sub>6</sub>	61.59	61.24	6.34	6.45	9.79	9.63
170	C <sub>20</sub> H <sub>23</sub> N <sub>3</sub> O <sub>6</sub>	59.90	59.62	5.78	5.83	10.49	10.28
171	C <sub>20</sub> H <sub>22</sub> BrN <sub>3</sub> O <sub>5</sub>	51.99	51.68	5.02	5.10	9.10	8.87
173	C <sub>20</sub> H <sub>22</sub> ClN <sub>3</sub> O <sub>5</sub>	57.19	56.89	5.28	5.33	10.00	9.88
174	C <sub>19</sub> H <sub>21</sub> N <sub>3</sub> O <sub>6</sub>	58.96	58.79	5.47	5.46	10.85	10.76
175	C <sub>17</sub> H <sub>17</sub> N <sub>3</sub> O <sub>6</sub>	56.87	56.49	4.77	4.85	11.70	11.54
176	C <sub>18</sub> H <sub>19</sub> N <sub>3</sub> O <sub>5</sub>	60.72	60.38	5.42	5.53	11.80	11.66
177	C <sub>21</sub> H <sub>25</sub> N <sub>3</sub> O <sub>6</sub>	60.77	60.56	6.07	6.23	10.13	9.96
178	C <sub>19</sub> H <sub>21</sub> N <sub>3</sub> O <sub>6</sub>	58.96	58.63	5.47	5.60	10.85	10.64
179	C <sub>17</sub> H <sub>17</sub> N <sub>3</sub> O <sub>6</sub>	56.87	56.44	4.77	4.68	11.70	11.39
180	C <sub>19</sub> H <sub>21</sub> N <sub>3</sub> O <sub>6</sub>	58.96	58.61	5.47	5.64	10.85	10.75
181	C <sub>22</sub> H <sub>23</sub> N <sub>3</sub> O <sub>5</sub>	64.60	64.29	5.67	5.78	10.27	10.01
182	C <sub>22</sub> H <sub>27</sub> N <sub>3</sub> O <sub>5</sub>	64.13	64.91	6.60	6.69	10.20	10.12
183	C <sub>20</sub> H <sub>23</sub> N <sub>3</sub> O <sub>5</sub>	62.55	62.41	6.04	6.31	10.94	10.78
184	C <sub>26</sub> H <sub>27</sub> N <sub>3</sub> O <sub>5</sub>	67.73	67.38	5.93	6.05	9.11	8.98
186	C <sub>17</sub> H <sub>17</sub> N <sub>3</sub> O <sub>5</sub>	59.52	59.13	4.50	4.67	12.21	12.04
187	C <sub>19</sub> H <sub>21</sub> N <sub>3</sub> O <sub>5</sub>	61.50	61.24	5.71	5.85	11.33	11.17
188	C <sub>22</sub> H <sub>27</sub> N <sub>3</sub> O <sub>5</sub>	63.98	63.88	6.59	6.62	10.17	10.15

*continue*

Compd	Molecular Formula	C		H		N	
		Theor	Found	Theor	Found	Theor	Found
<b>189</b>	C <sub>16</sub> H <sub>14</sub> BrN <sub>3</sub> O <sub>5</sub>	47.21	46.96	3.47	3.24	10.32	10.13
<b>190</b>	C <sub>16</sub> H <sub>14</sub> ClN <sub>3</sub> O <sub>5</sub>	52.93	52.62	3.89	3.68	11.58	11.21
<b>191</b>	C <sub>18</sub> H <sub>14</sub> F <sub>6</sub> N <sub>3</sub> O <sub>5</sub>	46.49	46.21	3.34	3.72	9.04	8.90
<b>192</b>	C <sub>22</sub> H <sub>26</sub> N <sub>4</sub> O <sub>5</sub>	59.78	59.45	5.93	6.01	12.68	12.56
<b>193</b>	C <sub>18</sub> H <sub>19</sub> N <sub>3</sub> O <sub>5</sub>	60.72	60.47	5.38	5.67	11.80	11.72
<b>194</b>	C <sub>22</sub> H <sub>27</sub> N <sub>3</sub> O <sub>7</sub>	59.38	59.05	6.12	6.20	9.44	9.87
<b>195</b>	C <sub>20</sub> H <sub>22</sub> ClN <sub>3</sub> O <sub>5</sub>	57.33	56.98	5.34	5.49	10.03	10.34
<b>196</b>	C <sub>23</sub> H <sub>29</sub> N <sub>3</sub> O <sub>6</sub>	62.35	62.01	6.60	6.77	9.49	9.34
<b>197</b>	C <sub>21</sub> H <sub>25</sub> N <sub>3</sub> O <sub>6</sub>	60.77	60.53	6.72	6.84	10.13	9.97

## Biochemistry and Pharmacology.

### Radioligand binding assays.

Radioligand binding competition assays were performed in vitro using A<sub>1</sub>, A<sub>2A</sub>, A<sub>2B</sub> and A<sub>3</sub> human receptors expressed in transfected CHO (*hA*<sub>1</sub>), HeLa (*hA*<sub>2A</sub> and *hA*<sub>3</sub>) and HEK-293 (*hA*<sub>2B</sub>) cells. The experimental conditions used are summarized in **Table 16**. In each instance aliquots of membranes (15 µg for A<sub>1</sub>, 10 µg for *hA*<sub>2A</sub>, 18 µg for *hA*<sub>2B</sub> and 100 µg for *hA*<sub>3</sub>) in buffer A (see **Table 16**) were incubated for the specified period at 25 °C with the radioligand (2-35 nM) and 6 different concentrations (ranging from 0.1 nM to 1 µM) of the test molecule or standard in a final volume of 200 µl. The binding reaction was stopped by rapid filtration in a multiscreen manifold system (Milipore Iberica, Madrid, Spain). Unbound radioligand was removed by washing 4x with 250 µl ice-cold buffer B for *hA*<sub>1</sub> and *hA*<sub>2A</sub> receptors and 6x 250 µl ice-cold buffer B for *hA*<sub>2B</sub> and *hA*<sub>3</sub> receptors (see table 4). Non specific binding was determined using a 50-1000 µM NECA soln for *hA*<sub>2A</sub> and *hA*<sub>2B</sub> receptors and 10-100 µM R-PIA soln for *hA*<sub>1</sub> and *hA*<sub>3</sub>. Radioactivity retained on filters was determined by liquid scintillation counting using Universol (ICN Biochemicals, Inc.). The binding affinities were determined using [<sup>3</sup>H]-DPCPX as the radioligand for A<sub>1</sub> and A<sub>2B</sub>, [<sup>3</sup>H]-ZM241385 for A<sub>2A</sub> and [<sup>3</sup>H]-NECA for A<sub>3</sub>. The inhibition constant (K<sub>i</sub>) of each compound was calculated by the Cheng-Prusoff expression  $K_i = IC_{50} / (1 + (C/K_D))$ <sup>8</sup>, where IC<sub>50</sub> is the concentration of compound that displaces the binding of radioligand by 50%, C is the free concentration of radioligand and K<sub>D</sub> is the apparent dissociation constant of the radioligand.

### cAMP assays.

These assays were performed on *hA*<sub>2A</sub> and *hA*<sub>2B</sub> receptors transfected in CHO cells by using the method described by Salomon<sup>9</sup>. Briefly, cells were seeded in 12-well culture plates and incubated at 37°C in an atmosphere of 5% CO<sub>2</sub> in Dulbecco's Modified Eagle's Medium Nutrient Mixture F-12 (DMEM F-12), containing 10% Foetal Calf Serum (FCS) and 1% L-Glutamine. This medium was replaced 24 h before the assays by medium containing dialyzed FCS. Prior to the assay, [<sup>3</sup>H]-adenine was added to the medium (3 µCi/mL) and cells were incubated for 2 h in 5% CO<sub>2</sub> atmosphere at 37°C. Cells were washed 3x with 1 mL assay medium (DMEM-F12 and 25 mM HEPES pH=7.4) and pre-incubated with assay medium containing 30 µM rolipram and antagonists at 37°C for 15 min. Agonists were incubated for 15 min at 37°C. Reaction was stopped by adding ice-cold 300 mM perchloric acid containing [<sup>14</sup>C]cAMP (1.1 µCi/mL) and cells were maintained at 4°C for 30 min. The [<sup>3</sup>H]-cAMP elicited in each well was isolated by chromatographic methods and [<sup>14</sup>C]cAMP allowed to calculate the yield of this isolation. The antagonist potency, expressed as the dissociation constant, pK<sub>B</sub>, for one concentration of antagonist, given as pA<sub>2</sub>, was obtained using the following equation:

$$pA_2 = \log ((EC50' / EC50) - 1) / C$$

where EC50' / EC50 is the ratio of concentrations of agonist giving an equal response (50% of the maximal effect) in the presence and in the absence, respectively, of a given concentration of the antagonist (C)<sup>10</sup>.

## Isolated organ assays.

### **A<sub>2A</sub> receptors**

These assays were performed on *hA<sub>2A</sub>* receptors<sup>11</sup> from isolated aorta of 200-250 g male Sprague-Dawley rats. The aorta was rapidly excised and placed in a modified Krebs solution of the following composition (mM): NaCl 118; KCl 4.7; MgSO<sub>4</sub>·7H<sub>2</sub>O 1.2; CaCl<sub>2</sub>·2H<sub>2</sub>O 2.5; KH<sub>2</sub>PO<sub>4</sub> 1.18; NaHCO<sub>3</sub> 25; glucose 11, and the solution was maintained at 37 °C with aeration by carbogen (95% CO<sub>2</sub> + 5% O<sub>2</sub>, pH 7.4±0.1). The vessels were cleaned to remove connective tissues, cut into rings, 4 mm in length, and suspended between stainless steel wires in organ baths containing 20 mL of Krebs solution, under a basal tension of 2 g (kept constant throughout the experiment). The aorta rings were stabilized for 60 min in the modified Krebs solution at 37 ± 0.2°C continuously saturated with carbogen before the start of the assay. During the stabilization time they were washed with new Krebs solution at least three times (15 min each time). All aorta rings were initially exposed to 0.1 μM phenylephrine to elicit a contractile response, and after this the presence of endothelium was confirmed by the addition of acetylcholine (10 μM). Tissues giving less than 25% relaxation of phenylephrine contraction were discarded. After a recovery time of 60 min with successive washes with fresh Krebs solution, a new phenylephrine contraction was elicited and responses to NECA were measured and used to construct cumulative relaxant-response curves. After a new recovery time of 60 min with successive washes with fresh Krebs solution, antagonists were incubated for 30 minutes and then a new phenylephrine contraction was elicited and responses to NECA were measured and used to construct cumulative relaxant-response curves. Potency of antagonists was expressed as pA<sub>2</sub> as for cAMP assays

### **A<sub>2B</sub> receptors**

These assays were performed in A<sub>2B</sub> receptors<sup>12</sup> from isolated aorta of 300-350 g male guinea pigs. The aorta was rapidly excised and placed in modified Krebs solution of the following composition (mM): NaCl 118; KCl 4.7; MgSO<sub>4</sub>·7H<sub>2</sub>O 1.2; CaCl<sub>2</sub>·2H<sub>2</sub>O 2.5; KH<sub>2</sub>PO<sub>4</sub> 1.18; NaHCO<sub>3</sub> 25; glucose 11; indomethacin 0.01, and the solution was maintained at 37 °C under aeration by carbogen. The vessels were cleaned to remove connective tissue and rubbed to remove the endothelium. They were cut into rings, 4 mm in length, that were suspended between stainless steel wires in organ baths containing 20 mL of Krebs solution, under a basal tension of 1 g (maintained constant throughout the experiment). The aorta rings were stabilized for 60 min in the modified Krebs solution at 37 ± 0.2°C continuously saturated with carbogen before the start of the assay, and during the stabilization time they were washed with new Krebs solution at least three times (15 min each time). All aorta rings were initially exposed to 4 μM phenylephrine to elicit a contractile response and after this the absence of endothelium was confirmed by the addition of acetylcholine (10 μM). Tissues giving any relaxation of phenylephrine contraction were discarded. After a recovery time of 60 min with successive washes with fresh Krebs solution, a new phenylephrine contraction was elicited and responses to NECA were measured and used to construct cumulative relaxant-response curves. After a new recovery time of 60 min with successive washes with fresh Krebs solution, antagonists were incubated for 30 minutes and then a new phenylephrine contraction was elicited and responses to NECA were measured and used to construct cumulative relaxant-response curves. Potency of antagonists was expressed as pA<sub>2</sub> as for cAMP assays

**Table 16. Conditions used for radioligand binding assays on A<sub>1</sub>, A<sub>2A</sub>, A<sub>2B</sub>, and A<sub>3</sub> human receptors.**

	A <sub>1</sub>	A <sub>2A</sub>	A <sub>2B</sub>	A <sub>3</sub>
<b>Buffer A</b>	20 mM Hepes, 100 mM NaCl, 10 mM MgCl <sub>2</sub> , 2 units/mL adenosine deaminase (pH=7.4)	50 mM Tris-HCl, 1mM EDTA, 10 mM MgCl <sub>2</sub> , 2 units/mL adenosine deaminase (pH=7.4)	50 mM Tris-HCl, 1mM EDTA, 10 mM MgCl <sub>2</sub> , 0.1mM benzamidine, 2 units/mL adenosine deaminase (pH=6.5)	50 mM Tris-HCl, 1mM EDTA, 5 mM MgCl <sub>2</sub> , 2 units/mL adenosine deaminase (pH=7.4)
<b>Buffer B</b>	20 mM Hepes, 100 mM NaCl, 10 mM MgCl <sub>2</sub> , (pH=7.4)	50 mM Tris-HCl, 1mM EDTA, 10 mM MgCl <sub>2</sub> (pH=7.4)	50 mM Tris-HCl (pH=6.5)	50 mM Tris-HCl (pH=7.4)
<b>Plate</b>	GF/C	GF/C	GF/B	GF/B
<b>Radioligand</b>	[ <sup>3</sup> H]DPCPX 2 nM	[ <sup>3</sup> H]ZM241385 3 nM	[ <sup>3</sup> H]DPCPX 35 nM	[ <sup>3</sup> H]NECA 30 nM
<b>Non-specific binding</b>	10 μM (R)-PIA	50 μM NECA	1000 μM NECA	100 μM (R)-PIA
<b>Incubation</b>	25°C/60 min	25°C/30 min	25°C/30 min	25°C/180 min

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