

Supporting Information

Multi-Objective Evolutionary Design of Selective Adenosine Receptor Ligands

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Binding assays Adenosine A₁/A_{2A}/A_{2B}/A₃ receptors.

References

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Computational

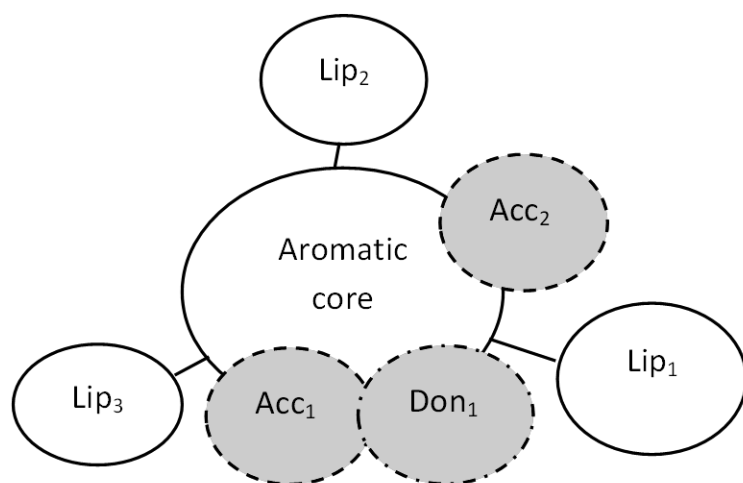
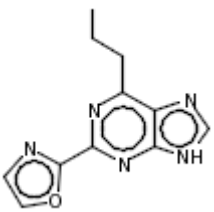
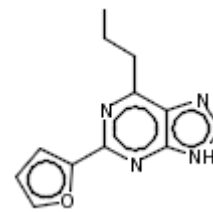
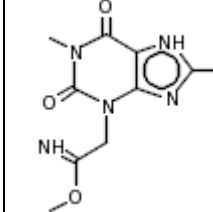
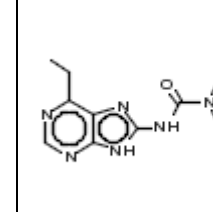
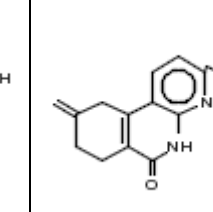
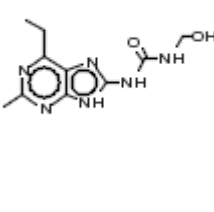
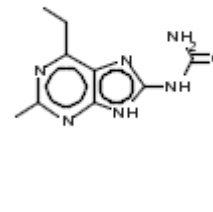
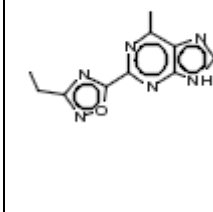
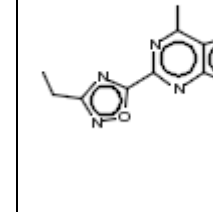
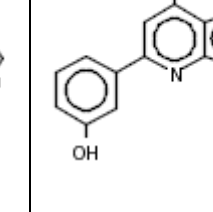
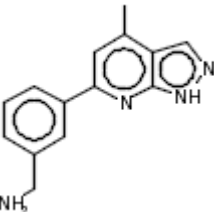
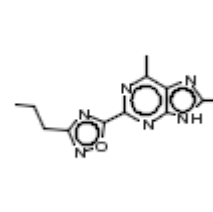
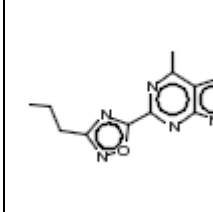
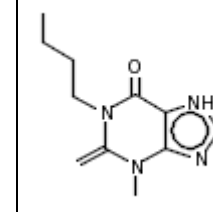
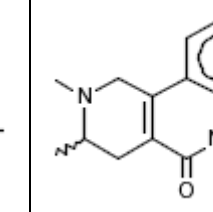
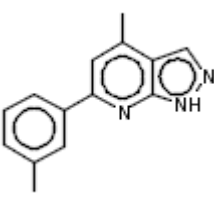
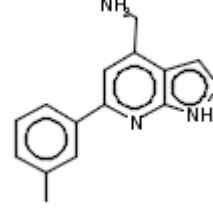
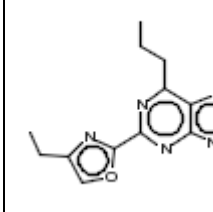
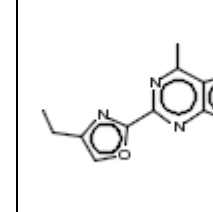
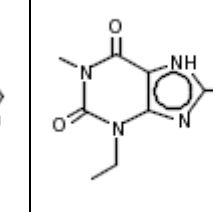


Figure S1. Schematic diagram of pharmacophore proposed by Chang et al (J Med Chem 47 (2004) 6529). At the center is the aromatic core which is surrounded by three lipophilic regions, Lip₁, Lip₂, and Lip₃. Below the aromatic core are the hydrogen bond acceptor and donor region, Acc₁ and Don₁, respectively. On the right of the aromatic core, a second hydrogen bond acceptor, Acc₂, is located.

Table S1. Generated compounds that exhibit adenosine scaffolds or ring systems. The pharmacophore score is provided under each compound.

				
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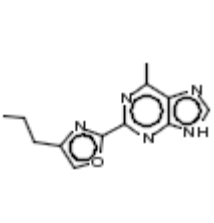
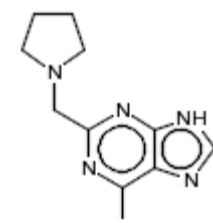
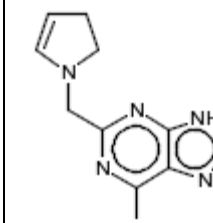
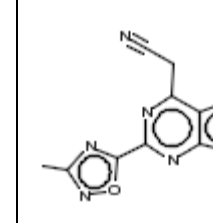
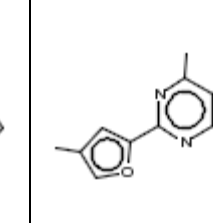
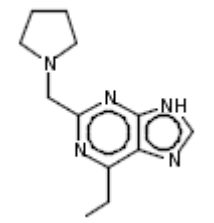
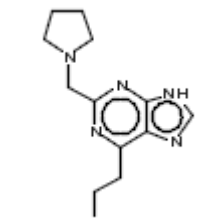
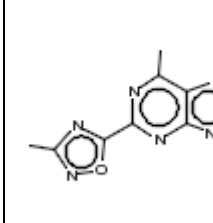
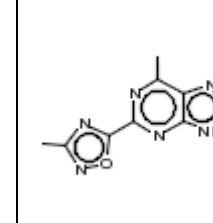
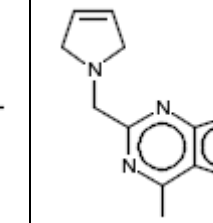
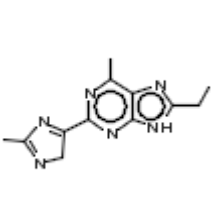
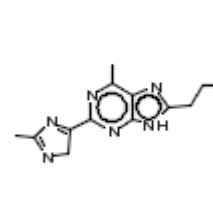
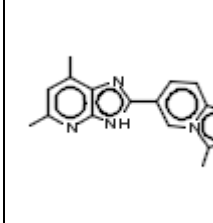
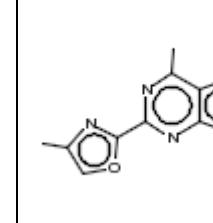
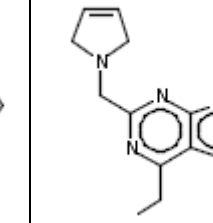
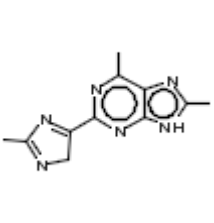
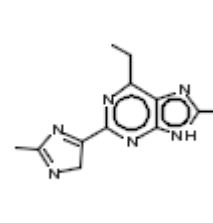
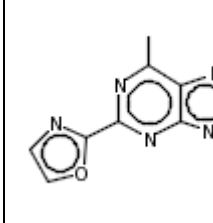
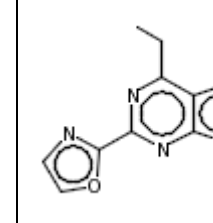
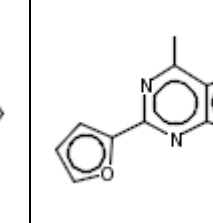
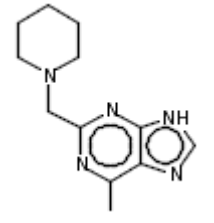
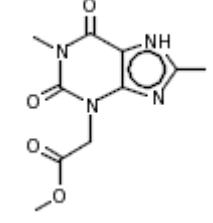
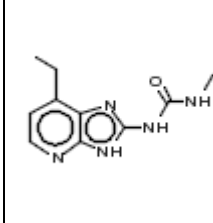
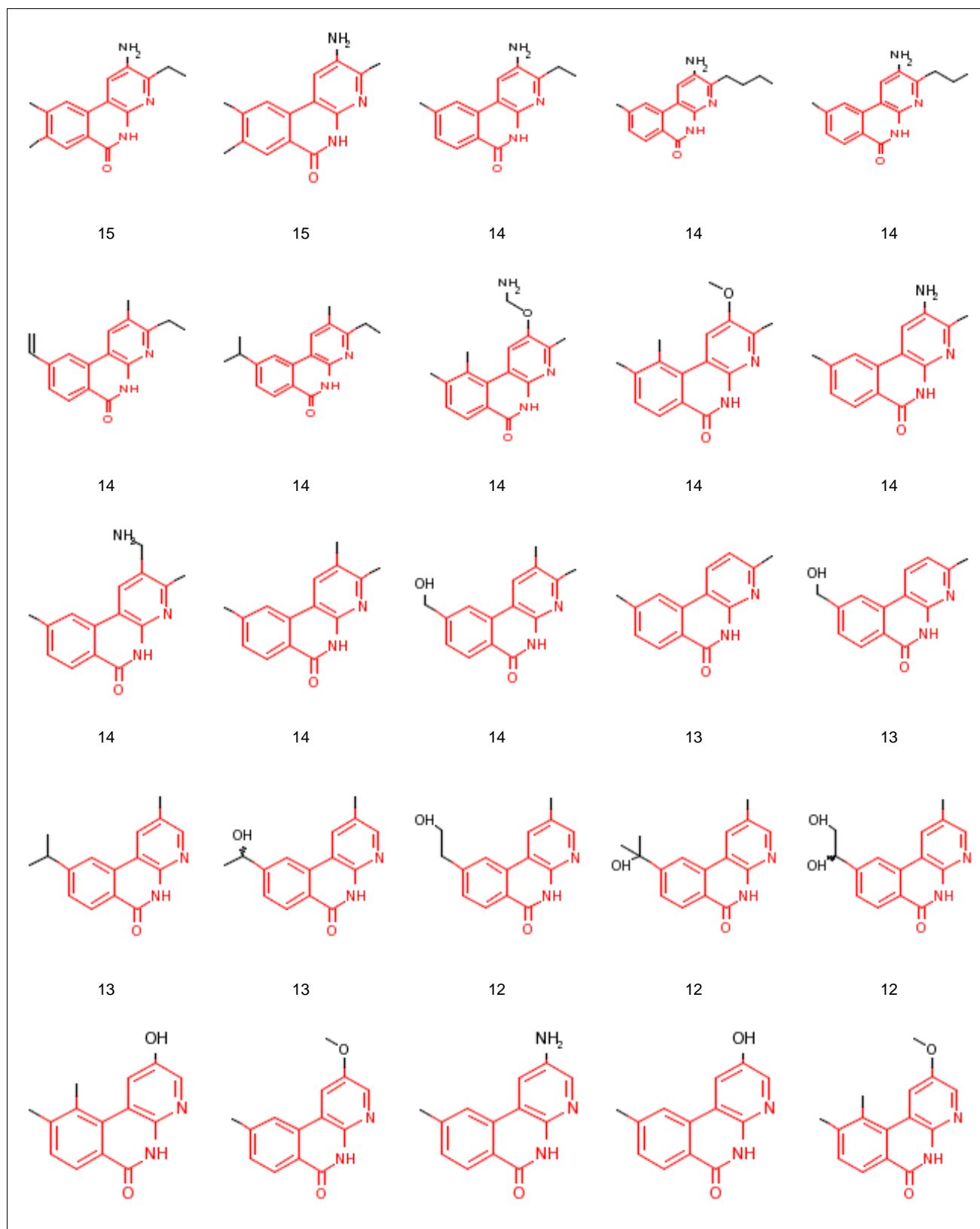
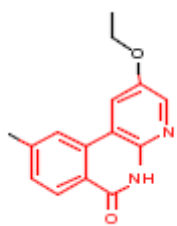
				
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Table S2. Generated compounds that exhibit adenosine scaffolds or ring systems. The pharmacophore score is provided under each compound. The common scaffold of a group of compounds is indicated in red. Compounds are ranked left to right, top to bottom; ‘cont.’ indicates that the group continues from the previous row.

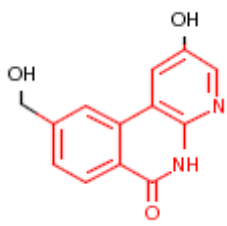


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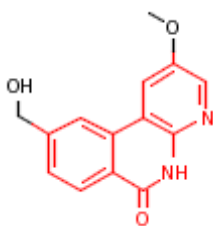
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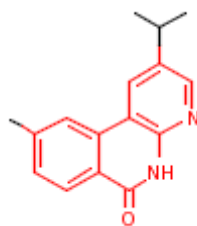
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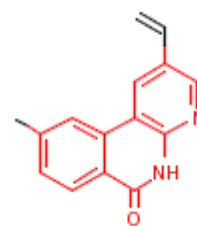
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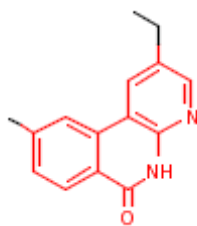
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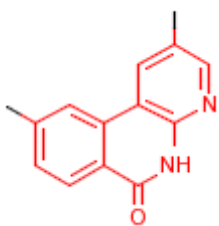
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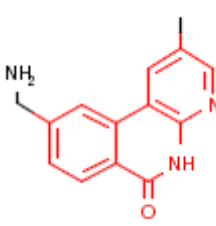
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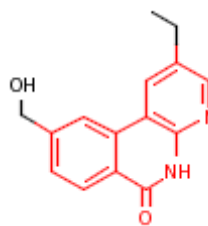
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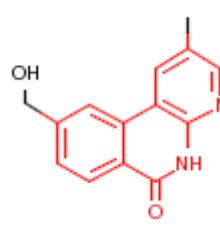
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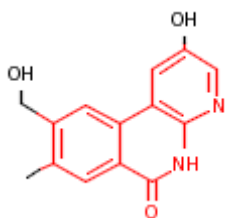
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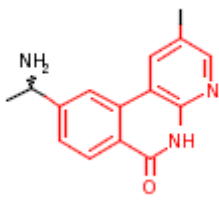
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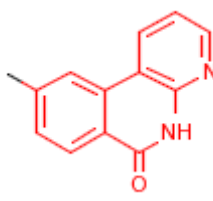
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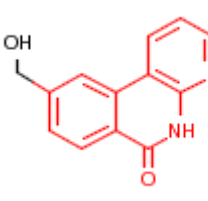
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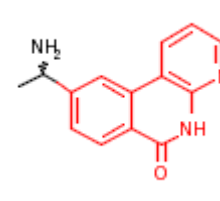
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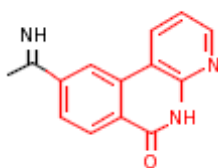
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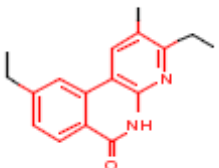
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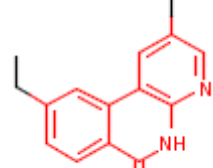
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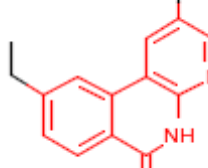
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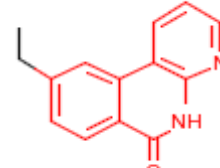
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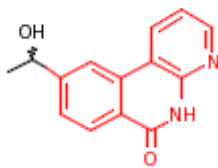
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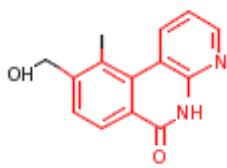
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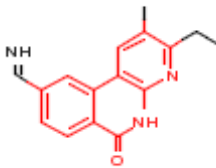
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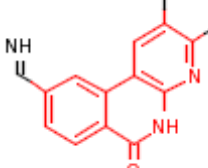
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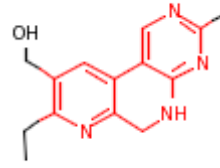
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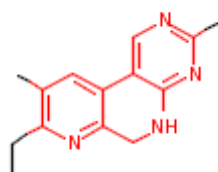


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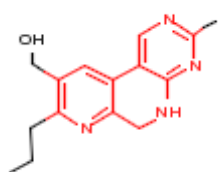
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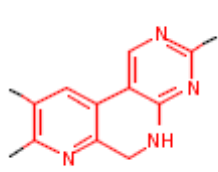
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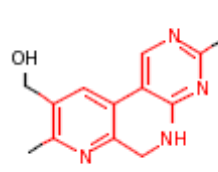
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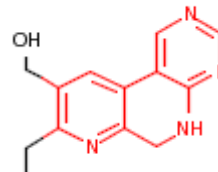
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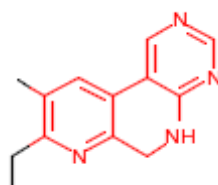
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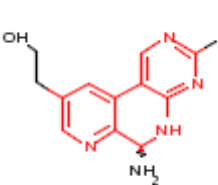
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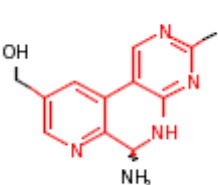
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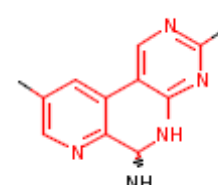
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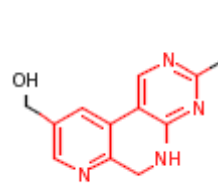
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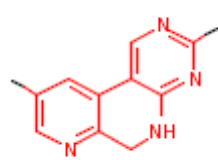
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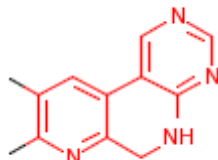
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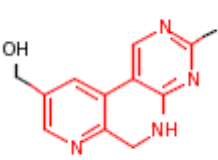
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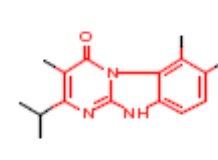
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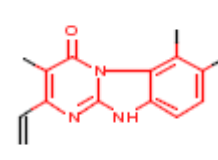
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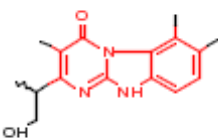
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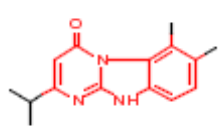
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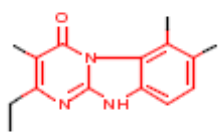
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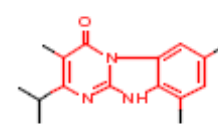
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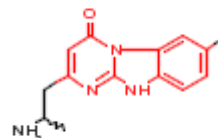
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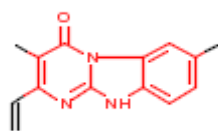
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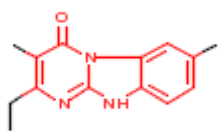
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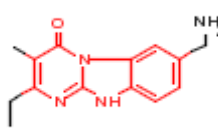
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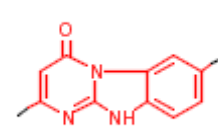
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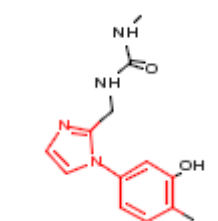
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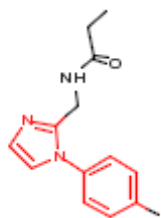
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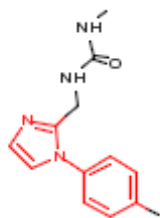
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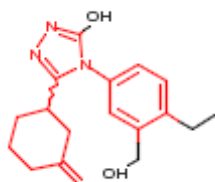
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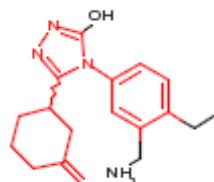
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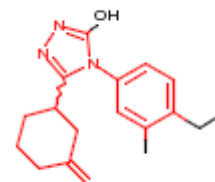
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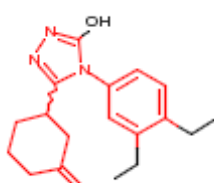
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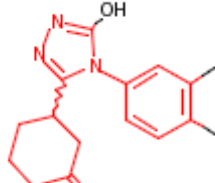
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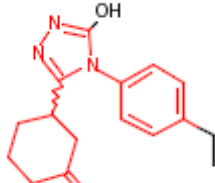
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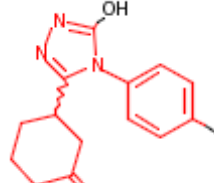
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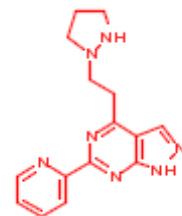
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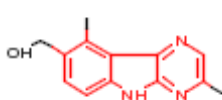
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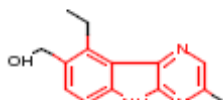
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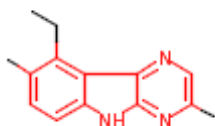
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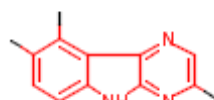
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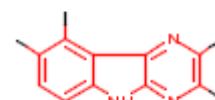
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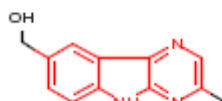
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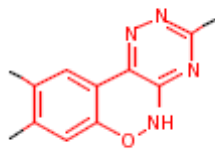
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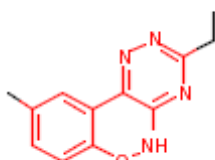
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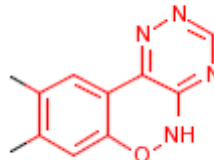
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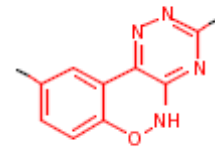
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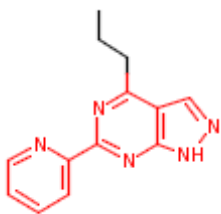
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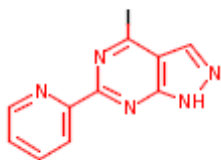
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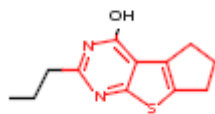
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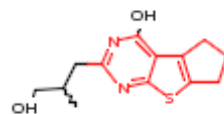
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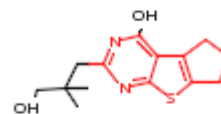
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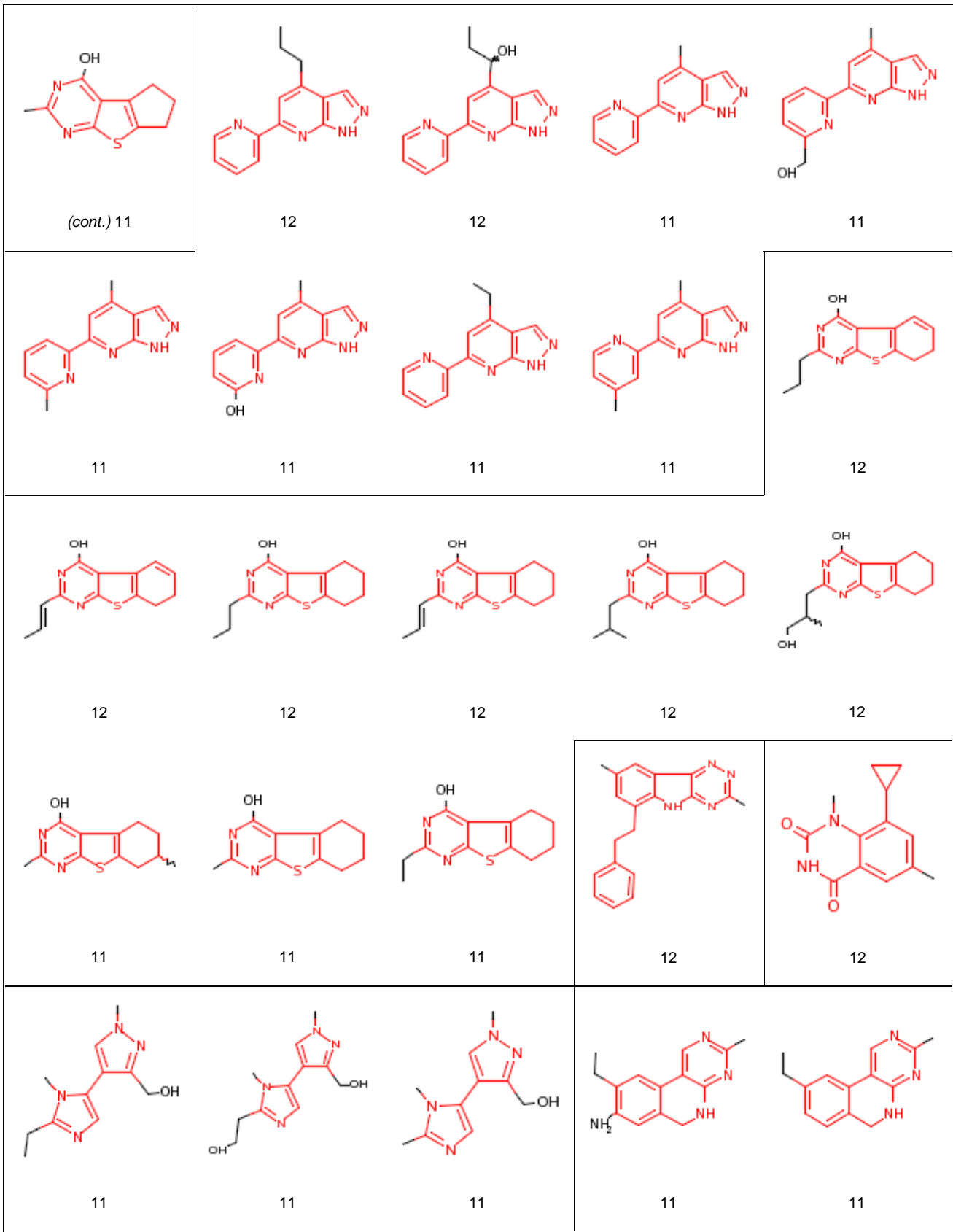
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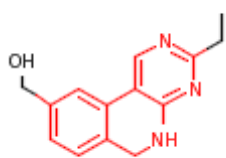


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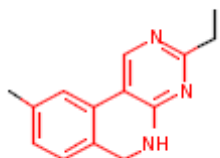


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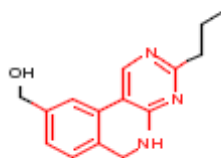




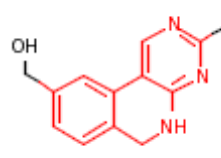
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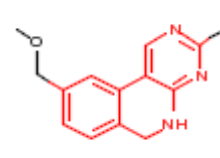
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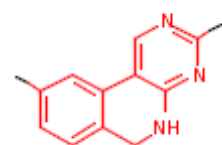
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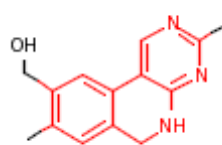
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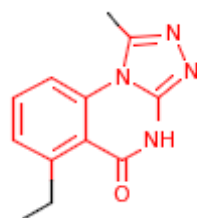
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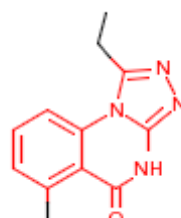
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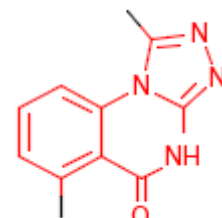
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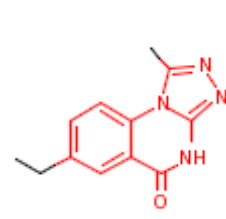
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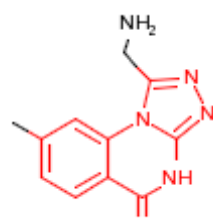
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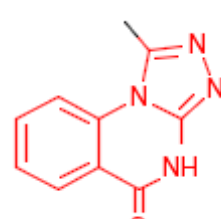
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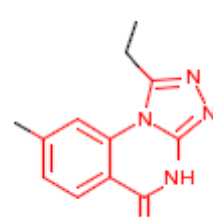
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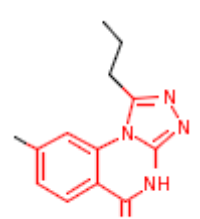
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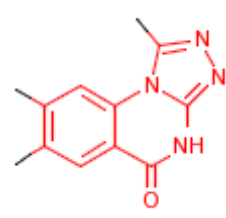
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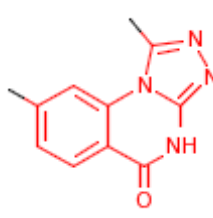
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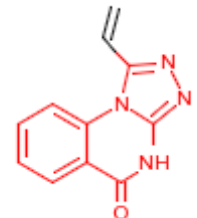
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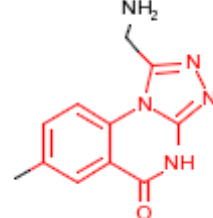
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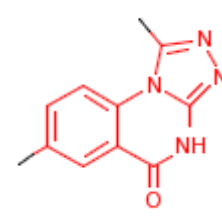
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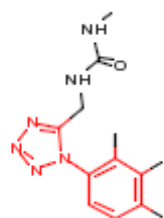
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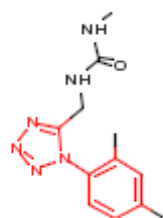
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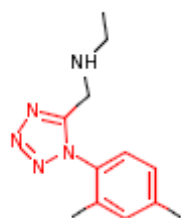
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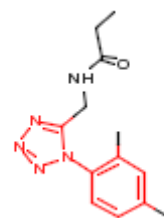
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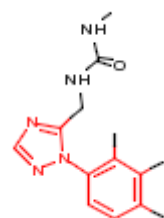
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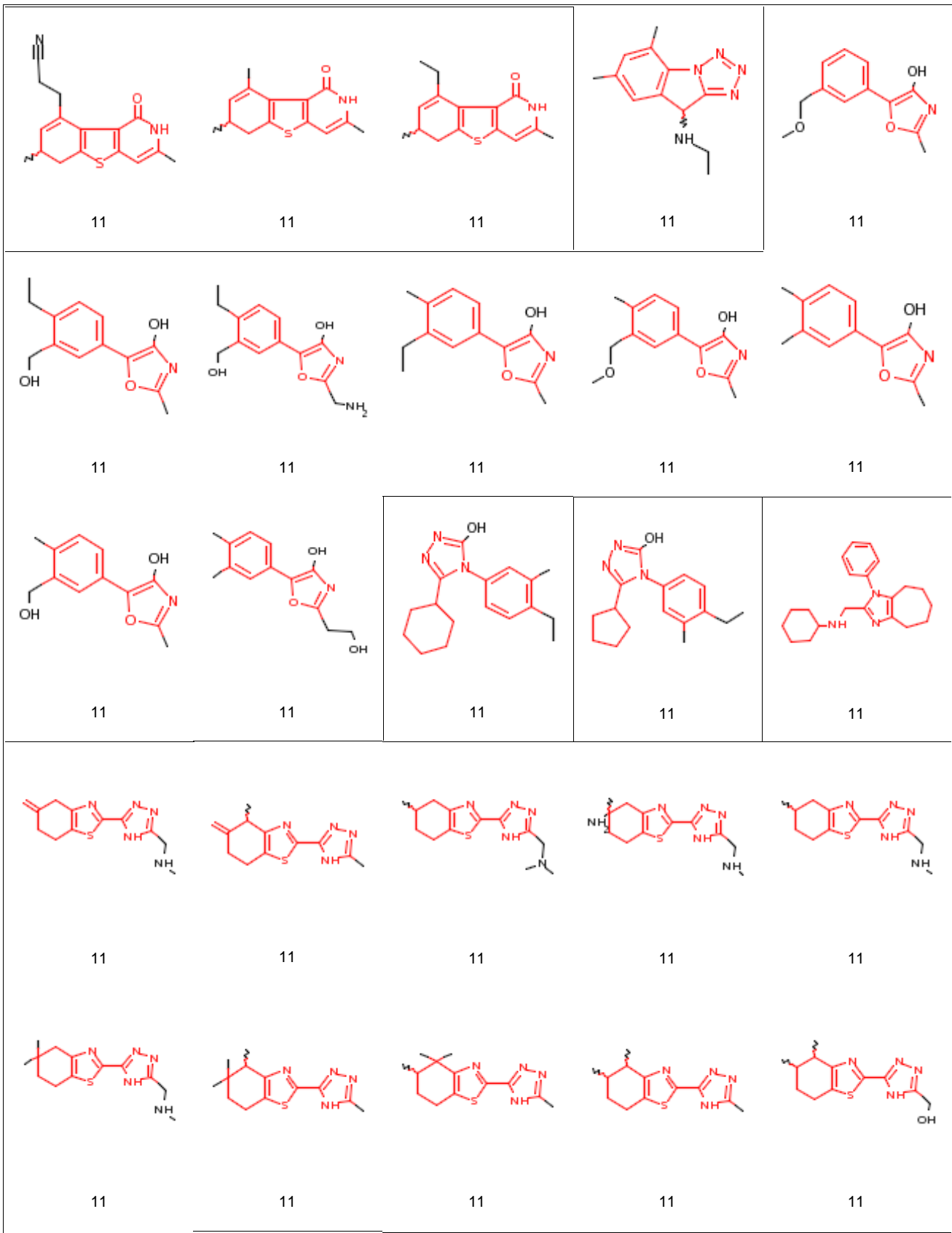
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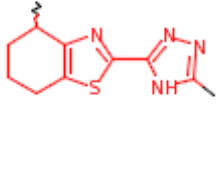
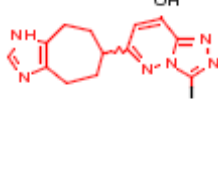
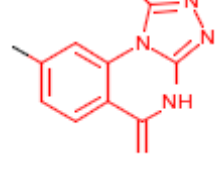
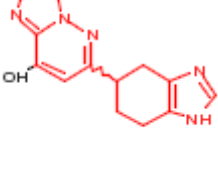
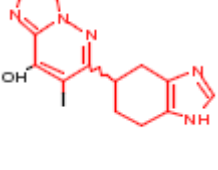
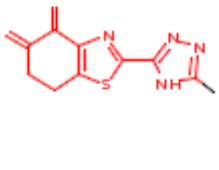
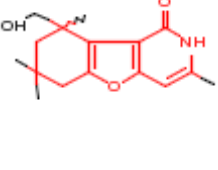
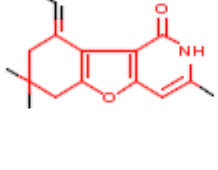
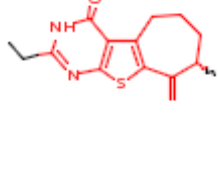
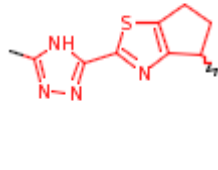
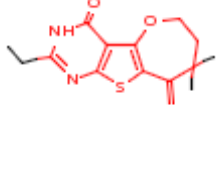
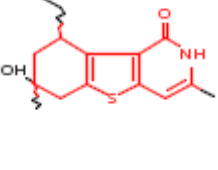
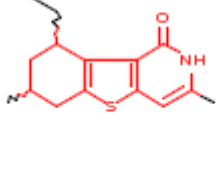
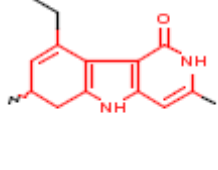
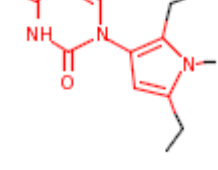
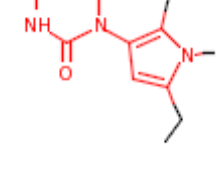
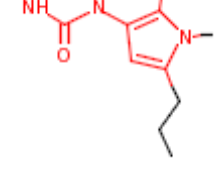
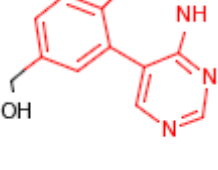
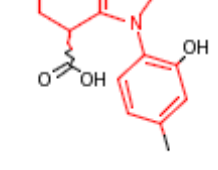
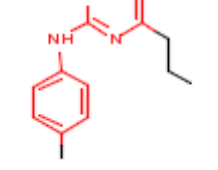
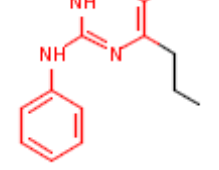
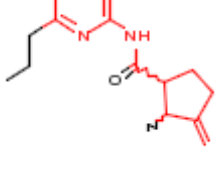
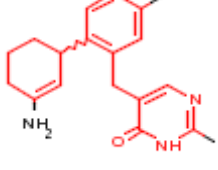
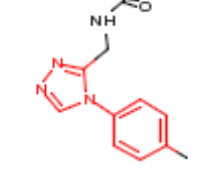


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11



 <p>(cont.) 11</p>	 <p>11</p>	 <p>11</p>	 <p>11</p>	 <p>11</p>
 <p>11</p>	 <p>11</p>	 <p>11</p>	 <p>11</p>	 <p>11</p>
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 <p>(cont.) 11</p>	 <p>11</p>	 <p>11</p>	 <p>11</p>	 <p>11</p>
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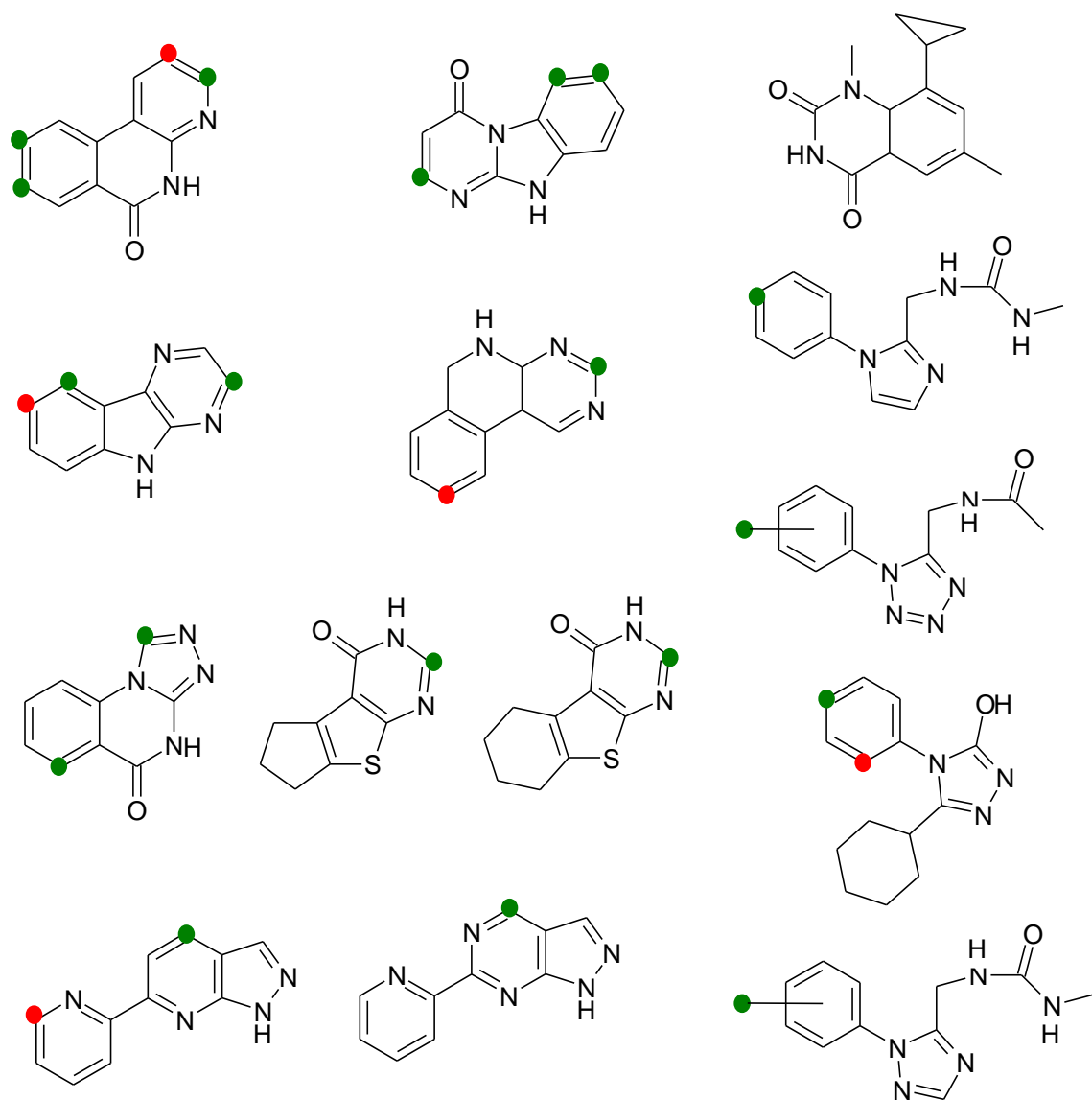
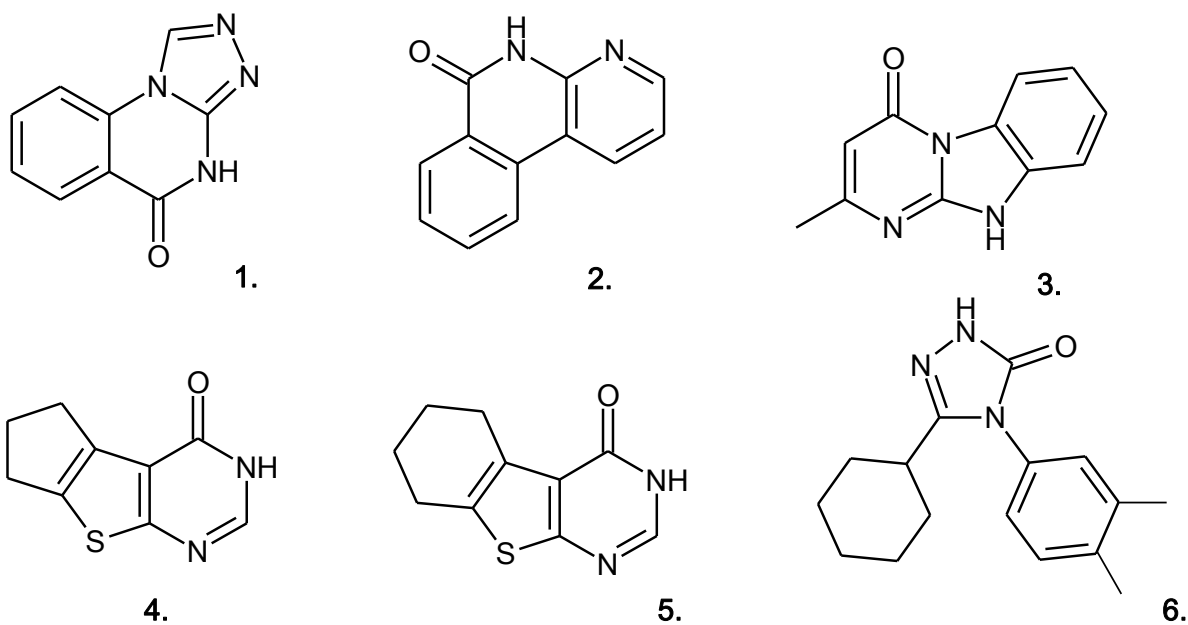


Figure S2. Chemical structures of scaffolds generated by multi-objective evolutionary design. Green spheres denote hydrophobic substituents (mostly alkyl chains) and red spheres denote hydrogen donor groups (mostly amino and hydroxyl groups).

Experimental Section

Abbreviations:

CPA, N⁶-cyclopentyladenosine; R-PIA, (*R*)-N⁶-phenylisopropyladenosine; NECA, 5'-(N-ethylcarboxamido)adenosine; CGS21680, 2-*p*-(2-carboxyethyl)phenethylamino-5'-N-ethylcarboxamidoadenosine; [¹²⁵I]-AB-MECA, N⁶-(4-amino-3-iodobenzyl)-5'-N-methylcarbamoyladenosine; [³H]ZM241385, 4-(2-[7-amino-2-{2-furyl}{1,2,4}triazolo{2,3-*a*}{1,3,5}-triazin-5-yl-amino]ethyl)phenol; [³H]DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; [³H]PSB-11, 2-phenyl-8-ethyl-4-methyl-(8*R*)-4,5,7,8-tetrahydro-1*H*-imidazo[2,1-*i*]purin-5-one; [³H]PSB-603, 8-(4-(4-(4-chlorophenyl)piperazine-1-sulfonyl)phenyl)-1-propylxanthine; GPCR, G protein-coupled receptor; PSA, polar surface area; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; ADA, adenosine deaminase.

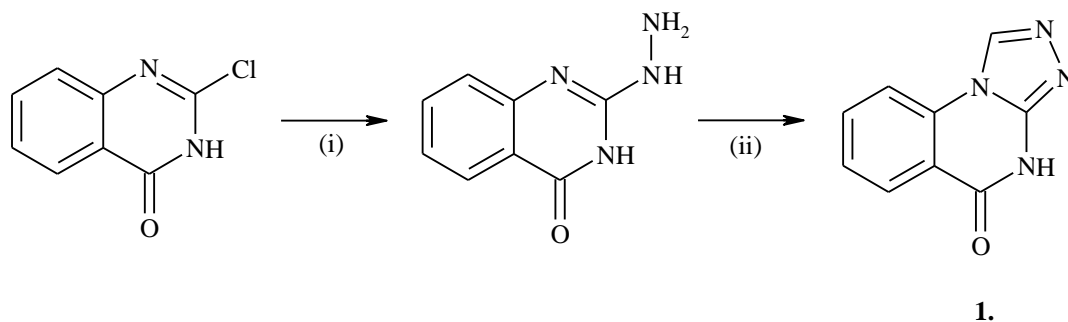


Duplicated from Figure 3 (main text). Chemical structures of selected scaffolds generated using the multi-objective evolutionary design method: [1,2,4]Triazolo[4,3-*a*]quinazolin-5-one (1), 4-Aza-5[*H*]-phenanthridin-6-one (2), 2-Methylpyrimido[1,2-*a*]benzimidazol-4(10*H*)-one (3), 3,5,6,7-Tetrahydro-4*H*-cyclopenta[4,5]thieno[2,3-*d*]pyrimidin-4-one (4), 5,6,7,8-Tetrahydro[1]benzothieno[2,3-*d*]pyrimidin-4(3*H*)-one (5), 5-Cyclohexyl-4-(3,4-dimethylphenyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one (6).

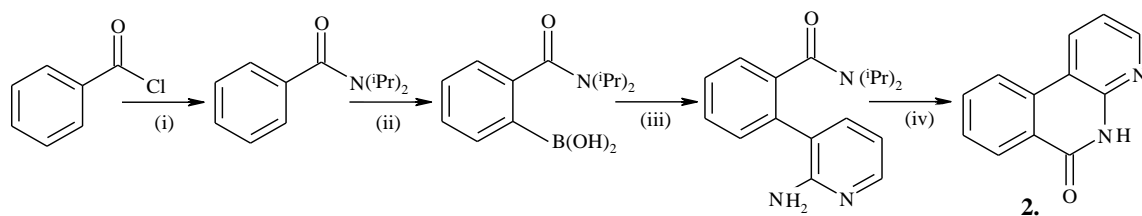
Chemistry – Synthesis Results

The 6:6:5 fused heteroaromatic system [1,2,4]triazolo[4,3-*a*]quinazolin-5-one (**1**) was synthesized as previously reported,¹ by the cyclization of 2-hydrazinoquinazolin-4-one² in formic acid. Compound **2** was synthesized using a slightly modified reported method starting from the synthesis of the 2-(*N,N*-diisopropylcarboxamido)phenylboronic acid,³ which was then used in a Suzuki-Miyaura cross-coupling reaction with 2-amino-3-bromopyridine under microwave conditions. Subsequently, cyclization of 2-(2-aminopyridin-3-yl)-*N,N*-diisopropylbenzamide using lithium diisopropylamide yielded the desired compound **2**. The 6:5:6 fused heteroaromatic system 2-methylpyrimido[1,2-*a*]benzimidazol-4(10*H*)-one (**3**) was obtained by heating a mixture of 2-aminobenzimidazole and ethyl acetoacetate in the presence of phosphoryl chloride and phosphoric acid. The free base (**3**) was liberated by the addition of NaHCO₃. Both substituted 2-aminothiophenes (2-amino-5,6-dihydro-4*H*-cyclopenta[*b*]thiophene-3-carbonitrile and 2-amino-4,5,6,7-tetrahydro-1-benzothiophene-3-carbonitrile) were synthesized using Gewald's method, which involves condensation of the corresponding ketone (cyclopentanone or cyclohexanone) with an activated nitrile and elemental sulfur in the presence of KF/alumina as catalyst.⁴ Cyclization of 2-amino-5,6-dihydro-4*H*-cyclopenta[*b*]thiophene-3-carbonitrile and 2-amino-4,5,6,7-tetrahydro-1-benzothiophene-3-carbonitrile in formic acid at 110 °C yielded compounds **4** and **5**, respectively. Compound **6** was synthesized starting from the commercially available 3,4-dimethylphenylisocyanate, which was reacted with hydrazine hydrate to obtain the corresponding semicarbazide, and was subsequently condensed with cyclohexanecarboxaldehyde to afford the corresponding semicarbazone. Finally, alkaline potassium ferricyanide oxidation of this semicarbazone yielded the desired compound **6**. Schemes showing the synthesis path and reaction conditions are in Schemes S1-S5.

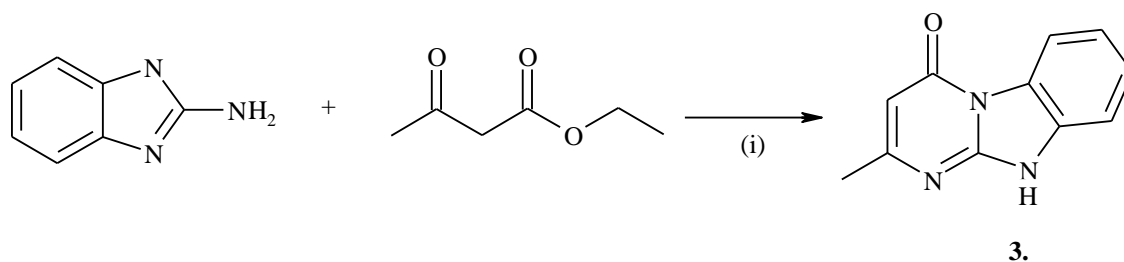
Scheme S1. Synthetic route to [1,2,4]Triazolo[4,3-*a*]quinazolin-5-one (**1**) (i) Hydrazine hydrate, K₂CO₃, 2h reflux, pH 7 with AcOH (ii) Formic acid, 95 °C, 4 h.



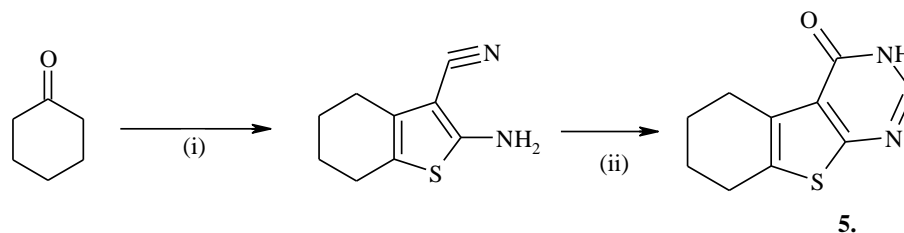
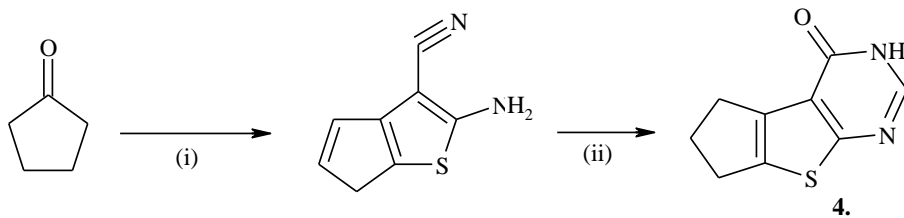
Scheme 2. Synthetic route to 4-Aza-5[H]-phenanthridin-6-one (**2**); (i) $\text{NH}(\text{iPr})_2$, Et_3N , Et_2O , $0\text{ }^\circ\text{C}$; (ii) $s\text{-BuLi}$, THF, $\text{B}(\text{OMe})_3$, $-78\text{ }^\circ\text{C}$; (iii) $\text{Pd}(\text{OAc})_2$, PPh_3 , K_2CO_3 , 2-amino-3-bromopyridine, toluene/EtOH MW $160\text{ }^\circ\text{C}$, 60 min; (iv) LDA, THF, $-78\text{ }^\circ\text{C}$.



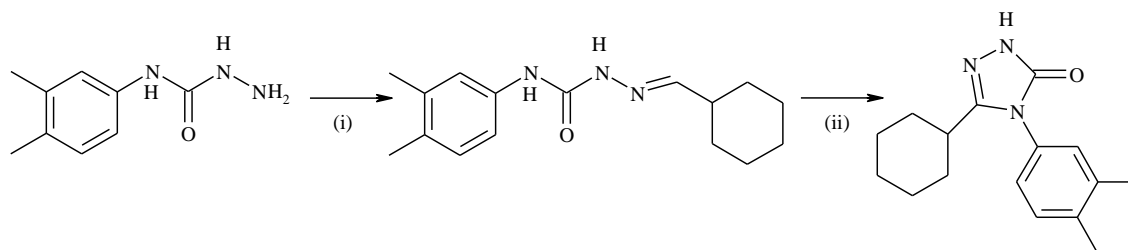
Scheme S3. Synthetic route to 2-methylpyrimido[1,2-*a*]benzimidazol-4(10*H*)-one (**3**) (i) POCl_3 / Phosphoric acid (PPA), $130\text{ }^\circ\text{C}$.



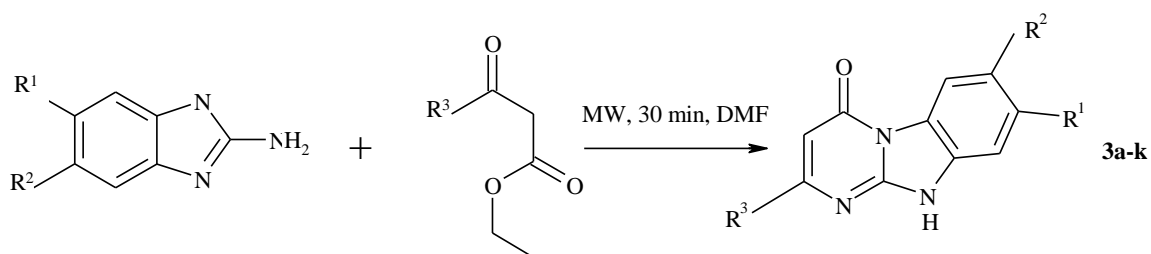
Scheme S4. Synthetic route to 3,5,6,7-tetrahydro-4*H*-cyclopenta[4,5]thieno[2,3-*d*]pyrimidin-4-one (**4**) and 5,6,7,8-tetrahydro[1]benzothieno[2,3-*d*]pyrimidin-4(3*H*)-one (**5**) (i) KF /alumina, elemental S, malonitrile, EtOH, $100\text{ }^\circ\text{C}$, 5 min; (ii) formic acid $110\text{ }^\circ\text{C}$ MW 5 min.



Scheme S5. Synthetic route to 5-cyclohexyl-4-(3,4-dimethylphenyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one (**6**); (i) cyclohexanecarbaldehyde, EtOH reflux; (ii) 100 °C 50% ethanolic NaOH solution, $K_3[Fe(CN)_6]$ 90 min.



S3. General procedure for the preparation of compounds **3a-3k** under microwave conditions.



To a solution of 2-aminobenzimidazole or 2-amino-5,6-dimethylbenzimidazole in DMF (3 mL), the corresponding β -keto-ester was added in equimolar amounts. The reaction mixture was stirred and heated at 165 °C under microwave conditions for 30 min. A solid was formed upon cooling, which was filtered and washed with cold H_2O , MeOH and Et_2O . The filtrate was concentrated under reduced pressure and extracted with EtOAc. Subsequently, the organic layers were combined, dried over $MgSO_4$, concentrated and purified using chromatography on SiO_2 .

2-Isopropylpyrimido[1,2-*a*]benzimidazol-4(10*H*)-one (3a) Yield: 40% (127 mg) 1H NMR (DMSO- d_6) δ = 12.85 (s br, 1H), 8.39 (d, J = 8.0 Hz, 1H), 7.49-7.41 (m, 2H), 7.29 (d, J = 7.2 Hz, 1H), 2.83 (m, 1H), 1.23 (d, J = 6.8 Hz, 6H) ppm. ^{13}C -NMR (DMSO- d_6) δ = 159.7, 148.9, 126.2, 121.5, 115.3, 112.5, 96.4, 34.4, 21.4 ppm. Anal. Calcd for $[C_{13}H_{13}N_3O]$: C, 68.7; H, 5.8; N, 18.5. Found C, 68.6; H, 5.9; N, 18.6. HRMS (m/z) = 228.11309 calculated for $[M+H]^+$ = 228.11314.

2-Propylpyrimido[1,2-*a*]benzimidazol-4(10*H*)-one (3b) Yield: 43% (137 mg) 1H NMR (DMSO- d_6) δ = 12.73 (s br, 1H), 8.37 (d, J = 8.0 Hz, 1H), 7.50 (d, J = 8.0 Hz, 1H), 7.43 (t, J = 7.2 Hz, 1H), 7.28 (t, J = 8.0 Hz, 1H), 5.84 (s, 1H), 2.53 (t, J = 7.2 Hz, 2H), 1.69 (m, 2H), 0.93 (t, J = 7.6 Hz, 3H) ppm. ^{13}C -NMR (DMSO- d_6) δ = 159.4, 148.6, 125.6, 121.4, 115.2, 113.2, 98.2, 37.3, 21.1, 13.5 ppm. HRMS (m/z) = 228.11310 calculated for $[M+H]^+$ = 228.11314. Purity (HPLC) 99%

2-Cyclohexylpyrimido[1,2-*a*]benzimidazol-4(10*H*)-one (3c) Yield: 57% (147 mg) 1H NMR (DMSO- d_6) δ = 12.81 (s br, 1H), 8.38 (d, J = 8.0 Hz, 1H), 7.48 (d, J = 7.6 Hz, 1H), 7.43 (t, J = 7.2 Hz, 1H), 7.28 (t, J = 7.2 Hz, 1H), 5.83 (s, 1H), 1.82 (dd, J = 12.0, 4H), 1.68 (d, J = 11.2 Hz, 1H), 1.45 (q, 2H), 1.30 (q,

2H), 1.22 (m, 2H)ppm. ^{13}C -NMR (DMSO- d_6) δ = 159.9, 148.9, 125.9, 121.6, 115.4, 112.7, 96.8, 65.0, 49.8, 46.8, 44.4, 31.4, 27.8, 25.9, 25.6, 25.1 ppm. HRMS (m/z) = 268.14448 calculated for $[\text{M}+\text{H}]^+$ = 268.14444. Purity (HPLC) 99%

2-Phenylpyrimido[1,2-*a*]benzimidazol-4(10*H*)-one (3d) Yield: 31% (302 mg) ^1H NMR (DMSO- d_6) δ = 13.1 (s br, 1H), 8.47 (d, J = 8.0 Hz, 1H), 8.11 (m, 2H), 7.49 (m, 5H), 7.35 (m, 1H), 6.62 (s, 1H) ppm. ^{13}C -NMR (DMSO- d_6) δ = 159.8, 149.6, 136.9, 130.3, 128.7, 126.7, 126.2, 121, 8, 115.7, 111.02, 97.1 ppm. HRMS (m/z) = 262.09752 calculated for $[\text{M}+\text{H}]^+$ = 262.09749. Purity (HPLC) 99%

2-(4-pyridyl)pyrimido[1,2-*a*]benzimidazol-4(10*H*)-one (3e) Yield: 59% (350 mg) ^1H NMR (DMSO- d_6) δ = 13.2 (s br, 1H), 8.72 (d, J = 6.4 Hz, 2H), 8.48 (d, J = 10.4 Hz, 1H), 8.06 (d, J = 6.4 Hz, 2H), 7.51 (d, J = 5.2 Hz, 2H), 7.36 (m, 1H), 6.83 (s, 1H) ppm. ^{13}C -NMR (DMSO- d_6) δ = 159.8, 158.1, 150.4, 149.8, 144.4, 130.7, 126.5, 125.8, 122.1, 121.1, 115.8, 111.2, 98.5 ppm. HRMS (m/z) = 263.09263 calculated for $[\text{M}+\text{H}]^+$ = 263.09274. Purity (HPLC) 99%

2-Methyl-7,8-dimethylpyrimido[1,2-*a*]benzimidazol-4(10*H*)-one (3f) Yield: 26% (71 mg) ^1H NMR (DMSO- d_6) δ = 12.6 (s br, 1H), 8.16 (s, 1H), 7.29 (s, 1H), 5.81 (s, 1H), 2.33 (s, 6H), 2.27 (s, 3H) ppm. ^{13}C -NMR (DMSO- d_6) δ = 159.2, 148.3, 134.3, 129.8, 124.8, 115.6, 113.3, 98.6, 22.3, 20.0, 19.8 ppm. Anal. Calcd for $[\text{C}_{13}\text{H}_{13}\text{N}_3\text{O}]$: C, 68.7; H, 5.8, N, 18.5. Found C, 68.2; H, 5.6, N, 18.4. HRMS (m/z) = 228.11307 calculated for $[\text{M}+\text{H}]^+$ = 228.11307.

2-Isopropyl-7,8-dimethylpyrimido[1,2-*a*]benzimidazol-4(10*H*)-one (3g) Yield: 30% (91 mg) ^1H NMR (DMSO- d_6) δ = 12.72 (s br, 1H), 8.18 (s, 1H), 7.25 (s, 1H), 5.85 (s, 1H), 2.80 (m, 1H), 2.34 (s, 6H), 1.22 (d, J = 6.8Hz, 6H) ppm. ^{13}C -NMR (DMSO- d_6) δ = 159.6, 134.5, 129.9, 115.7, 96.5, 34.7, 21.5, 19.9, 19.8 ppm. HRMS (m/z) = 256.14446 calculated for $[\text{M}+\text{H}]^+$ = 256.14444. Purity (HPLC) 99%

2-Propyl-7,8-dimethylpyrimido[1,2-*a*]benzimidazol-4(10*H*)-one (3h) Yield: 41% (124 mg) ^1H NMR (DMSO- d_6) δ = 12.63 (s br, 1H), 8.16 (s, 1H), 7.26 (s, 1H), 5.81 (s, 1H), 2.33 (s, 6H), 1.67 (m, 2H), 0.92 (t, J = 7.6 Hz, 3H) (peak below dmsso corresponding to CH_2) ppm. ^{13}C -NMR (DMSO- d_6) δ = 159.3, 134.3, 129.8, 115.6, 98.4, 37.9, 21.2, 19.9, 19.8, 13.6 ppm. HRMS (m/z) = 256.14445 calculated for $[\text{M}+\text{H}]^+$ = 256.14444. Purity (HPLC) 98%

2-Cyclohexyl-7,8-dimethylpyrimido[1,2-*a*]benzimidazol-4(10*H*)-one (3i) Yield: 37% (132 mg) ^1H NMR (CDCl_3) δ = 8.39 (s, 1H), 7.29 (s, 1H), 6.01 (s, 1H), 2.64 (t, J = 10.8 Hz, 1H), 2.08 (d, J = 13.2 Hz, 2H), 1.90 (d, J = 12.4 Hz, 2H), 1.79 (d, J = 12.8 Hz, 1H), 1.48 (m, 4H), 1.31 (t, J = 11.6 Hz, 1H) ppm. ^{13}C -NMR (CDCl_3) δ = 159.9, 148.4, 133.8, 129.4, 123.9, 115.6, 111.4, 96.6, 44.6, 31.1, 25.4, 25.2, 19.6, 19.2 ppm. HRMS (m/z) = 296.17586 calculated for $[\text{M}+\text{H}]^+$ = 296.17574. Purity (HPLC) 96%

2-Phenyl-7,8-dimethylpyrimido[1,2-*a*]benzimidazol-4(10*H*)-one (3j) Yield: 37% (130 mg) ^1H NMR (DMSO- d_6) δ = 12.9 (s br, 1H), 8.26 (s, 1H), 8.10 (d, J = 6.0 Hz, 2H), 7.50 (d, J = 6.8 Hz, 3H), 7.29 (s, 1H), 6.60 (s, 1H), 2.37 (s, 6H) ppm. ^{13}C -NMR (DMSO- d_6) δ = 159.7, 135.0, 130.3, 130.2, 128.7, 126.9, 116.0, 111.4, 96.8, 19.9, 19.8 ppm. HRMS (m/z) = 290.12883 calculated for $[\text{M}+\text{H}]^+$ = 290.12879. Purity (HPLC) 98%

2-(4-pyridyl)-7,8-dimethylpyrimido[1,2-*a*]benzimidazol-4(10*H*)-one (3k) Yield: 68% (365 mg) ^1H NMR (DMSO- d_6) δ = 13.1 (s br, 1H), 8.72 (d, J = 5.1 Hz, 2H), 8.30 (s, 1H), 8.05 (d, J = 4.8 Hz, 2H), 7.31 (s, 1H), 6.79 (s, 1H), 8.27 (s, 6H) ppm. ^{13}C -NMR (DMSO- d_6) δ = 149.3, 134.1, 129.4, 120.0, 115.1, 110.5, 97.2, 19.0, 18.8 ppm. HRMS (m/z) = 291.12394 calculated for $[\text{M}+\text{H}]^+$ = 291.12404. Purity (HPLC) 96%.

Chemistry - Materials and Methods

All reagents were obtained from commercial sources and all solvents were of analytical grade. ^1H and ^{13}C NMR spectra were recorded on a Bruker AC 400 spectrometer or on a 300 MHz Bruker DPX300 spectrometer. Chemical shifts are reported in δ (ppm) and the following abbreviations are used: s = singlet, d = doublet, dd = double doublet, dt = double triplet, t = triplet, td = triple doublet, m = multiplet, br = broad. C, H and N analyses were performed with a Perkin-Elmer 2400 series II analyzer and are within 0.5% of the theoretical values unless otherwise stated. Reactions were routinely monitored by TLC using Merck silica gel F254 plates. Microwave reactions were performed on an Emrys Optimizer (Biotage AB). Wattage was automatically adjusted to maintain the desired temperature. HPLC purity of compounds was measured with a reverse-phase column (RP18, 125 x 4 mm, 5 μm , 254 nm and diode array, 210-360 nm) with two diverse solvent systems; A: Water, 10% acetonitrile, 10 mM HOAc, 5 mM SDS. B: Water, 90% acetonitrile, 10 mM HOAc, 5 mM SDS. Samples were eluted by a gradient between 100% A and 100% B at a flow rate of 0.6 mL/min. Reverse-phase HPLC revealed that all samples submitted for assay accounted for > 95% of the UV absorbing material.

[1,2,4]Triazolo[4,3-a]quinazolin-5-one (1).¹

2-Hydrazinoquinazolin-4-one (1 g, 5.7 mmol), synthesized as previously reported,² was heated in formic acid (6 mL) for 4 h on a steam bath. Subsequently, the reaction mixture was cooled and poured into water to precipitate the desired compound. Yield 39% (413 mg). ^1H NMR (DMSO- d_6) δ = 12.82 (s, br, 1H, NH), 9.41 (s, 1H), 8.16 (m, 2H), 7.92 (t, J = 7.6 Hz, 1H), 7.57 (t, J = 7.6 Hz, 1H) ppm. ^{13}C -NMR δ (DMSO- d_6) δ = 159.7, 146.7, 134.9, 133.2, 128.6, 126.5, 117.2, 115.9 ppm. Anal. for $[\text{C}_9\text{H}_6\text{N}_4\text{O}]$ found (calculated) %: C 57.8 (58.1), H 3.4 (3.3), N 30.0 (30.1). HRMS (m/z) = 187.06129 calculated for $[\text{M}+\text{H}]^+ = 187.06144$.

4-Aza-5[H]-phenanthridin-6-one (2).⁵

Suzuki-Miyaura cross-coupling reaction between 2-(*N,N*-diisopropylcarboxamido)phenylboronic acid and 2-amino-3-bromopyridine under microwave conditions was performed to obtain 2-(2-aminopyridin-3-yl)-*N,N*-diisopropylbenzamide, with subsequent cyclization affords the entitle compound 2. Briefly, 2-(*N,N*-diisopropylcarboxamido)phenylboronic acid (600 mg, 2.4 mmol), prepared according to the literature,³ was added to a solution of 2-amino-3-bromopyridine (380 mg, 2.2 mmol), potassium carbonate (400 mg), palladium(II) acetate (98 mg, 0.44 mmol) and triphenylphosphine (231 mg, 0.88 mmol) in toluene/ethanol/water (2:1:1) mixture. The reaction vial was then sealed and heated at 160 $^\circ\text{C}$ for 30 min. Upon completion the reaction mixture was poured into water and the product was extracted with ethyl acetate. The organic phases were collected and dried with MgSO_4 and concentrated to yield 2-(2-aminopyridin-3-yl)-*N,N*-diisopropylbenzamide as a yellow solid that was triturated with diethyl ether. The cyclization of 2-(2-aminopyridin-3-yl)-*N,N*-diisopropylbenzamide (195 mg, 0.7 mmol) was performed as previously reported,⁵ with lithium diisopropylamide (1.6 M, 1.1 mL) dissolved in 9 mL of dry THF at -78 $^\circ\text{C}$. The reaction mixture was warmed to room temperature and stirred overnight. The reaction mixture was concentrated in vacuo and suspended in water (20 mL). The solid was filtered off and triturated with ethyl acetate. The resulting solid was dried yielding the desired compound. Yield

60% (82 mg). ^1H NMR (DMSO- d_6) δ = 12.05 (s br, 1H, NH), 8.84 (dd, J = 6.4 Hz, J = 1.6 Hz, 1H), 8.55 (d, J = 8.4 Hz, 1H), 8.50 (dd, J = 3.2 Hz, J = 1.6 Hz, 1H), 8.33 (dd, J = 6.8 Hz, J = 0.8 Hz, 1H), 7.89 (td, J = 8.2 Hz, J = 1.2 Hz, 1H), 7.69 (td, J = 7.2 Hz, 1H), 7.33 (m, 1H) ppm. ^{13}C NMR (DMSO- d_6) δ = 165.7, 163.8, 141.2, 134.9, 132.7, 128.5, 127.9, 125.9, 72.9, 22.1 ppm. Anal. for $[\text{C}_{12}\text{H}_8\text{N}_2\text{O}]\cdot\text{H}_2\text{O}$ found (calculated) %: C 67.6 (67.3), H 4.2 (4.7), N 12.9 (13.1). HRMS (m/z) = 197.07084 calculated for $[\text{M}+\text{H}]^+$ = 197.07094.

2-Methylpyrimido[1,2-*a*]benzimidazol-4(10*H*)-one (3).⁶

A mixture of 2-aminobenzimidazole (1.33 g, 0.01 mol) and ethyl acetoacetate (1.27 mL, 0.01 mol) was heated at 130 °C in phosphoryl chloride (6.13 g, 0.04 mol) and phosphoric acid (700 mg) until evolution of HCl(g) ceased. The reaction was cooled to 80 °C and subsequently ethanol was added. The reaction mixture was cooled to 0 °C to obtain a precipitate. The solid was filtered off and the free base was liberated by the addition of NaHCO_3 solution. The resulting solid was recrystallized from ethanol yielding the desired compound. Yield: 34% (670 mg) ^1H NMR (DMSO- d_6) δ = 8.35 (d, J = 7.6 Hz, 1H), 7.50 (d, J = 8.0 Hz, 1H), 7.39 (td, J = 8.4 Hz, J = 1.2 Hz, 1H), 7.24 (td, J = 8.0 Hz, J = 1.2 Hz, 1H), 5.78 (s, 1H), 2.31 (s, 3H) ppm. ^{13}C -NMR (DMSO- d_6) δ = 165.7, 163.8, 141.2, 134.9, 132.7, 128.5, 127.9, 125.9, 72.9, 22.1 ppm. HRMS (m/z) = 200.08173 calculated $[\text{M}+\text{H}]^+$ = 200.08184. Purity (HPLC) 98%.

Compounds **3a-3k** were synthesized under microwave conditions, which is further detailed in the Supporting Information.

3,5,6,7-Tetrahydro-4*H*-cyclopenta[4,5]thieno[2,3-*d*]pyrimidin-4-one (4).

2-Amino-5,6-dihydro-4*H*-cyclopenta[*b*]thiophene-3-carbonitrile was prepared following the typical Gewald reaction between cyclopentanone (10 mmol), malononitrile (10 mmol), sulfur (10 mmol) and KF/alumina as catalyst in EtOH under microwave conditions.⁴ 2-Amino-5,6-dihydro-4*H*-cyclopenta[*b*]thiophene-3-carbonitrile (129 mg, 0.8 mmol) and formic acid (5 mL) were heated under microwave condition for 3 min at 110 °C. The reaction mixture was cooled, poured into water, filtered and dried. Yield 41% (63 mg). ^1H NMR (DMSO- d_6) δ = 11.77 (s, 1H, NH), 8.44 (s, 1H), 2.90 (t, J = 7.2 Hz, 2H), 2.79 (t, J = 7.2 Hz, 2H), 2.35 (m, 2H) ppm. ^{13}C -NMR (DMSO- d_6) δ = 157.6, 30.0, 28.7, 28.3 ppm. Anal. for $[\text{C}_9\text{H}_8\text{N}_2\text{SO}]\cdot\text{H}_2\text{O}$ found (calculated) %: C 50.8 (51.4), H 4.9 (4.8), N 13.4 (13.3), S 15.3 (15.3). HRMS (m/z) = 211.05365 calculated for $[\text{M}+\text{H}_2\text{O}+\text{H}]^+$ = 211.05357.

5,6,7,8-Tetrahydro[1]benzothieno[2,3-*d*]pyrimidin-4(3*H*)-one (5).

2-Amino-4,5,6,7-tetrahydro-1-benzothiophene-3-carbonitrile was prepared following the typical Gewald reaction between cyclohexanone (0.01 mol), malononitrile (0.01 mol), sulfur (0.01 mol) and KF/alumina as catalyst in EtOH under microwave conditions.⁴ 2-Amino-4,5,6,7-tetrahydro-1-benzothiophene-3-carbonitrile (200 mg, 1.1 mmol) and formic acid (5 mL) were heated under microwave condition for 3 min at 110 °C. The reaction mixture was cooled, poured into water, filtered and dried. Yield 45% (103 mg). ^1H NMR (DMSO- d_6) δ = 11.34 (s, 1H, NH), 8.37 (s, 1H), 2.63 (m, 4H), 1.72 (m, 4H) ppm. ^{13}C -NMR (DMSO- d_6) δ = 157.6, 26.6, 24.7, 22.7 ppm. Anal. for $[\text{C}_{10}\text{H}_{10}\text{N}_2\text{SO}]\cdot\text{H}_2\text{O}$ found (calculated) %: C, 53.4 (53.6), H 5.3 (5.4), N 12.6 (12.5), S 13.8 (14.3). HRMS (m/z) = 225.06935 calculated for $[\text{M}+\text{H}_2\text{O}+\text{H}]^+$ = 225.06922.

5-Cyclohexyl-4-(3,4-dimethylphenyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one (6) .

Commercially available 3,4-dimethylphenylisocyanate (1 g, 6.8 mmol) was added to a solution of hydrazine hydrate (10 mmol) in 25 mL of dichloromethane at -60 °C. The reaction mixture was allowed to reach room temperature and stirred for additional 4 h. The precipitate was filtered and washed with cold dichloromethane yielding the corresponding semicarbazide. This semicarbazide (500 mg, 2.8 mmol) was then mixed with cyclohexanecarboxaldehyde (2.5 mmol, 194 μL) in ethanol (15 mL) and

heated under reflux for 2 h. Subsequently, the reaction mixture was filtered and the ethanol was removed under vacuo yielding the corresponding cyclohexanecarboxaldehyde *N*-phenylsemicarbazone. Finally, the latter (153 mg, 0.6 mmol) was heated at 100 °C in 50% ethanolic NaOH solution (10 M, 10%), followed by the addition of K₃Fe(CN)₆ in water (10 M, 10%). The reaction mixture was heated at 100 °C for 90 min. Subsequently, the reaction mixture was cooled in an ice bath and the solution was acidified with HCl (3 M) to afford the entitled compound **6**. Yield 45% (72 mg) ¹H NMR (DMSO-*d*₆) δ = 11.54 (s, 1H), 7.27 (d, *J* = 7.6 Hz, 1H), 7.12 (d, 1H), 7.05 (dd, *J* = 6.0 Hz, *J* = 2.0 Hz, 1H), 2.38 (tt, 1H), 2.27 (d, *J* = 4 Hz, 6H), 1.66 (m, 4H), 1.33, (m, 2H), 1.09 (m, 3H). ¹³C-NMR (Acetone-*d*₆) δ = 138.7, 138.1, 132.3, 131.2, 129.5, 125.9, 35.7, 30.9, 26.5, 26.3, 19.7, 19.5. HRMS (*m/z*) 272.17576 calculated for [M+H]⁺ = 272.17574. Purity (HPLC) 96%.

Biology - Binding Studies.

[³H]DPCPX (8-cyclopentyl-1,3-dipropylxanthine) and [³H]ZM241385 (4-(2-[7-amino-2-{2-furyl}{1,2,4}triazolo{2,3-*a*}{1,3,5}-triazin-5-yl-amino]ethyl)phenol) were purchased from ARC Inc. St Louis, USA. [¹²⁵I]-AB-MECA (N⁶-(4-amino-3-iodobenzyl)-5'-N-methylcarbamoyladenine) was obtained from PerkinElmer Singapore PTE, Ltd. [³H]PSB603 and [³H]PSB11 were custom made for one of us (C.E.M.).

CHO cells expressing the human adenosine A₁ receptor were provided by Dr. Andrea Townsend-Nicholson, University College of London, UK. HEK 293 cells stably expressing the human adenosine A_{2A} receptor were a gift from Dr. Wang (Biogen). CHO cells expressing the human A_{2B} receptor were provided by Dr. Steve Rees (GlaxoSmithKline, UK), those expressing the human A₃ receptor by Dr. K.-N. Klotz (University of Würzburg, Germany).

All compounds made were tested in radioligand binding assays to determine their affinities at the human adenosine A₁, A_{2A}, A_{2B}, and the A₃ receptors.

Adenosine A₁ Receptor. Affinity at the A₁ receptor was determined on membranes from CHO cells expressing the human receptors, using [³H]DPCPX as the radioligand. Membranes containing 5 μg of protein were incubated in a total volume of 100 μL of 50 mM Tris-HCl (pH 7.4) and [³H]DPCPX (final concentration 1.6 nM) for 1 h at 25 °C in a shaking water bath. Nonspecific binding was determined in the presence of 100 μM CPA. The incubation was terminated by filtration over pre-wetted Whatman GF/B filters under reduced pressure with a Brandel harvester. Filters were washed three times with ice-cold buffer and placed in scintillation vials. Emulsifier Safe (3.5 mL) was added, and after 2 h radioactivity was counted in a TriCarb 2900TR liquid scintillation counter.

Adenosine A_{2A} Receptor. At the A_{2A} receptor, affinity was determined on membranes from HEK 293 cells stably expressing this human receptor, using [³H]ZM241385 as the radioligand. Membranes containing 40 μg of protein were incubated in a total volume of 100 μL of 50 mM Tris-HCl (pH 7.4) and [³H]ZM241385 (final concentration 2.0 nM) for 2 h at 25 °C in a shaking water bath. Nonspecific binding was determined in the presence of 100 μM CGS. The incubation was terminated by filtration over pre-wetted Whatman GF/B filters under reduced pressure with a Brandel harvester. Filters were washed three times with ice-cold buffer and placed in scintillation vials. Emulsifier Safe (3.5 mL) was added, and after 2 h radioactivity was counted in a TriCarb 2900TR liquid scintillation counter.

Adenosine A_{2B} Receptor. At the A_{2B} receptor, radioligand displacement was determined on membranes from CHO cells stably transfected with human A_{2B} receptor, using [³H]PBS603 as the radioligand. Membranes containing 15 µg of protein were incubated in a total volume of 100 µL of 50 mM Tris·HCl (pH 7.4), 1U/mL ADA, 0.1 w/v % CHAPS (pH 8.2 at 5 °C), and [³H]PBS603 (final concentration 1.0 nM) for 2 h at 25 °C in a shaking water bath. Nonspecific binding was determined in the presence of 100 µM NECA. The incubation was terminated by filtration over pre-wetted Whatman GF/C filters under reduced pressure with a Brandel harvester. Filters were washed five times with ice-cold 50 mM Tris·HCl, pH7.4 + 0.1% BSA buffer and placed in scintillation vials. Emulsifier Safe (3.5 mL) was added, and after 5 h radioactivity was counted in a TriCarb 2900TR liquid scintillation counter.

Adenosine A₃ Receptor. The affinity at the A₃ receptor was measured on membranes from CHO cells stably expressing the human A₃ receptor, using [³H]PSB11 or [¹²⁵I]AB-MECA as the radioligand.

In the binding assays using [³H]PSB11 as radioligand (final concentration 4 nM), membranes containing 50 µg of protein were incubated in a total volume of 100 µL of 50 mM Tris-HCl with 1 mM EDTA (pH 8 at 25°C) for 2 h at 25 °C in a shaking water bath. Nonspecific binding was determined in the presence of 100 µM NECA.

In the binding assays using [¹²⁵I]AB-MECA as radioligand (final concentration 0.10 nM), membranes containing 25 µg of protein were incubated in a total volume of 100 µL of 50 mM Tris·HCl, 10 mM MgCl₂, 1 mM EDTA, 0.01% CHAPS (pH 7.4) for 1 h at 37 °C in a shaking water bath. Nonspecific binding was determined in the presence of 100 µM R-PIA. The incubation was terminated by filtration over pre-wetted Whatman GF/B filters under reduced pressure with a Brandel harvester. Filters were washed three times with ice-cold buffer and radioactivity was counted in a TriCarb 2900 TR liquid scintillation counter or in a Wallac 1470 Wizard gamma counter for [³H]PSB11 or [¹²⁵I]AB-MECA, respectively.

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