Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Structure-Based Drug Design of Chromone Antagonists of the Adenosine A_{2A} Receptor

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SUPPORTING INFORMATION

Contents

1. Compound Quality Control & Biological Testing

The following screening compounds were commercially available: compound **1**, from Chembridge; compounds **3**, **6**, **10**, **11**, **12**, **13** and **15** from InterBioScreen; compound **14** from Otava and compound **16** from ChemDiv.

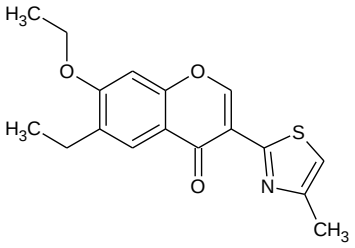
Compounds **2**, **4**, **5**, **7**, **8** and **9** were synthesised as detailed in section 2.

LCMS analysis of all test compounds **1**-**16** was carried out at Heptares under the following conditions: Instruments: Waters Alliance 2795, Waters 2996 PDA detector, Micromass ZQ. Column: Waters X-Bridge C-18, 2.5micron, 2.1 x 20mm or Phenomenex Gemini-NX C-18, 3 micron, 2.0 x 30mm. Gradient [time (min)/solvent D in C (%)]: 0.00/2, 0.10/2, 8.40/95, 9.40/95, 9.50/2, 10.00/2 (solvent C = 1.58g ammonium formate in 2.5L water + 2.7mL ammonia solution; solvent D = 2.5L Acetonitrile + 132mL (5%) solvent C + 2.7mL ammonia solution). Injection volume 5 uL; UV detection 230 to 400nM; column temperature 45^{o}C; 1.5 mL/min. LCMS results:

Compound Ref	MW	Retention Time	ion observed (ES+ mode)
1	331.34	1.42	332.16
2	344.43	4.07	345.24
3	359.4	4.43	360.17
4	327.4	5.17	328.17
5	339.41	5.13	340.22
6	315.34	4.41	316.26
7	273.31	1.33	274.21
8	259.28	1.03	260.16
9	287.33	1.74	288.27
10	301.36	1.99	302.26
11	329.41	2.55	330.18
12	273.31	1.08	274.21
13	343.4	4.72	344.17
14	343.4	4.82	344.17
15	329.37	4.40	330.18
16	315.34	4.00	316.18

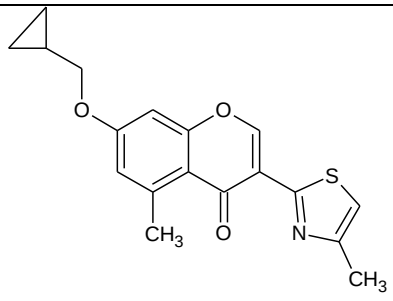
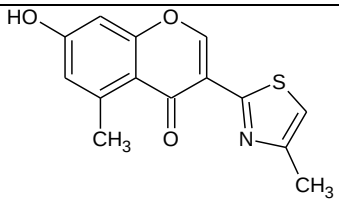
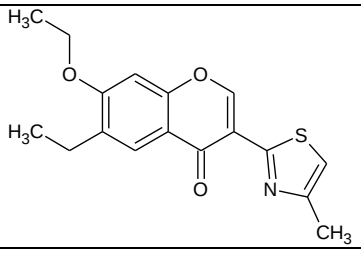
Radioligand binding assays with A_{2A} receptor and A_1 receptor were performed at Heptares, as described in: M. Congreve, S. P. Andrews, A. S. Dore, K. Hollenstein, E. Hurrell, C. J. Langmead, J. S. Mason, I. W. Ng, B. Tehan, A. Zhukov, M. Weir, and F. H. Marshall, *J. Med. Chem.*, 2012**, 55**, 1898.

Radioligand binding assays with A_{2B} and A_3 receptors were performed at MDS Pharma Services, under the following conditions: A_{2B}: HEK293 recombinant cells were incubated for 90 minutes at 25 ^{o}C, pH 6.5 with 1.6 nM [^{3}H]-MRS1754, 50 mM Tris-HCl, 5 mM $MgCl_2$, 1 mM EDTA, 0.01% Bacitracin and varying concentrations of test compound. A_3: CHO-K1 recombinant cells were incubated for 60 minutes at 25 ^{o}C, pH 7.4 with 0.5 nM [^{125}I]-AB-MECA, 25 mM HEPES, 5 mM $MgCl_2$, 1 mM $CaCl_2$, 0.1% BSA and varying concentrations of test compound.



Receptor	**Affinity**
A_{2A}	pK_i 7.4
A_1	pK_i 5.3
A_{2B}	pIC_{50} <5
A_3	pIC_{50}<5

Compound stability in rat liver microsomes was measured at Cyprotex and the results are reported as half-lives ($T_{1/2}$) in minutes. Pooled microsomes from male Sprague Dawley rats (final protein concentration 0.5 mg/mL), 0.1M phosphate buffer solution and test compound (final concentration 3 µM; final DMSO concentration 0.25%) were pre-incubated at 37 ^{o}C prior to the addition of NADPH (final concentration 1 mM) to initiate the reaction. Experiments were run over 0, 5, 15, 30 and 45 minute time courses and were terminated by the addition of methanol containing an internal standard. Quantitative analysis was then performed by LCMS.

Compound	**Rat liver microsome $T_{1/2}$ (mins)**
 **(4)**	18
 **(7)**	14
	16

2. Experimental Procedures for Chemical Synthesis

2.1 General Experimental Conditions

All non-commercial compounds were prepared in India by Piramal (formerly Oxygen Healthcare). Commercial reagents were utilized without further purification. Room temperature refers to 25-27 ^{o}C.

HPLC purities were measured under the following conditions:

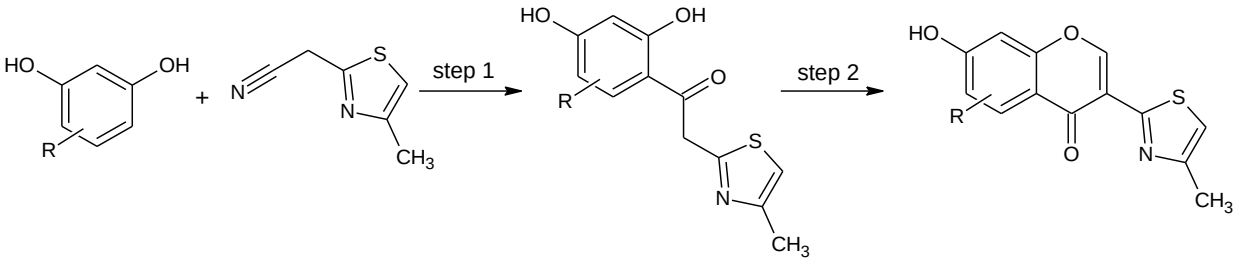
Instrument: Waters Alliance 2695. Column: Sunfire C-18, 250 x 4.6 mm, 5 µm, or equivalent. Gradient [time (min)/% solvent B in A]: 0.00/10, 9.00/90, 11.00/100, 20.00/100, 20.01/10, 25.00/10 (solvent A = 0.1% formic acid in water; solvent B = 0.1% formic acid in acetonitrile). 1 mL/min; detection wavelength specified for each compound in the detailed experimental section.

Mass spectroscopy was carried out on a Shimadzu LCMS-2010 EV, using electrospray conditions as specified for each compound in the detailed experimental section.

^{1}H-NMR spectra were recorded at 400 MHz on a Bruker instrument. Chemical shift values are expressed in parts per million, i.e. (δ)-values. The following abbreviations are used for the multiplicity for the NMR signals: s=singlet, b=broad, d=doublet, t=triplet, dd=doublet of doublets, m=multiplet. Chromatography refers to column chromatography performed using 60-120 mesh silica gel and executed under nitrogen pressure (flash chromatography) conditions.

2.2 The Preparation of 7-Hydroxychromone Derivatives

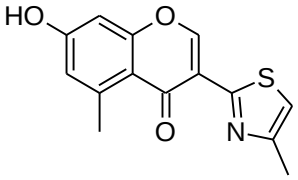
Typical Procedure



Step 1: A resorcinol derivative (39.4 mmol) and (4-methyl-1,3-thiazol-2-yl)acetonitrile (5.5 g, 39.4 mmol) were added to borontrifluoride diethyletharate (70 mL, 45% in ether) and the resulting mixture was stirred for 10 minutes at room temperature. Dry HCl gas was then bubbled through the mixture for 6 h at 30-40 ºC and the resulting mixture was stirred for 24 hrs at room temperature, diluted with water (300 mL), and then refluxed for 1 hour. The pH of the mixture was then adjusted to pH 3 with ammonia solution and the resulting precipitate was filtered and washed with water (56 mL). The crude compound, Intermediate A, was used in the next step without any further purification.

Step 2: A solution of Intermediate A (32.3 mmol) in pyridine (34 mL) was treated with triethylorthoformate (19.1 g, 129 mmol) and piperidine (1.0 mL) and heated for 4 hrs at 70-80 ºC. Upon completion, the mixture was concentrated *in vacuo* and purified by gradient flash chromatography, affording the 7-hydroxychromone derivative.

Preparation of 7-Hydroxy-5-methyl-3-(4-methyl-1,3-thiazol-2-yl)-4H-chromen-4-one (7)



Step 1: 1-(2,4-Dihydroxy-6-methylphenyl)-2-(4-methyl-1,3-thiazol-2-yl)-ethanone (8.50 g, 80%) was prepared from 5-methylresorcinol monohydrate (5.6 g, 39.4 mmol) and 2-(4-methyl-1,3-thiazol-2-yl)acetonitrile (5.5 g, 39.4 mmol) according to the typical procedure.

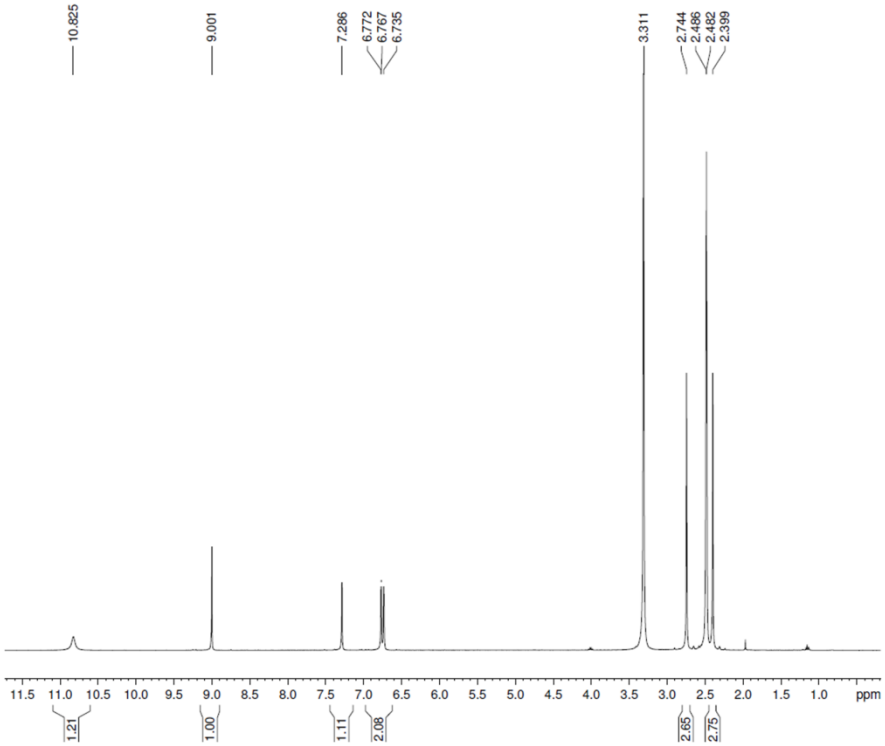
Mass spectroscopy: (ESI +ve) 263.9 $[M+H]^+$.

Step 2: 7-Hydroxy-5-methyl-3-(4-methyl-1,3-thiazol-2-yl)-4*H*-chromen-4-one (1.45 g, 14%) was prepared from 1-(2,4-dihydroxy-6-methyl-phenyl)-2-(4-methyl-1,3-thiazol-2-yl)-ethanone (8.50 g, 32.3 mmol) and triethylorthoformate (19.1 g, 129 mmol) according to the typical procedure.

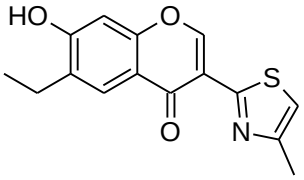
HPLC: 99.2% (284 nm)

Mass spectroscopy: (ESI +ve) 273.9 $[M+H]^+$.

^{1}H NMR: (400 MHz, d_6-DMSO) δ: 2.39 (s, 3H), 2.74 (s, 3H), 6.74 (m, 1H), 6.77 (m, 1H), 7.29 (s, 1H), 9.00 (s, 1H), 10.83 (bs, 1H).



Preparation of 6-Ethyl-7-hydroxy-3-(4-methyl-1,3-thiazol-2-yl)-4*H*-chromen-4-one (9)



Step-1: 1-(5-Ethyl-2,4-dihydroxyphenyl)-2-(4-methyl-1,3-thiazol-2-yl)-ethanone (1.65 g, 80%) was prepared from 4-ethylresorcinol (1.0 g, 7.23 mmol) and 2-(4-methyl-1,3-thiazol-2-yl)acetonitrile (1.0 g, 7.23 mmol) according to the typical procedure.

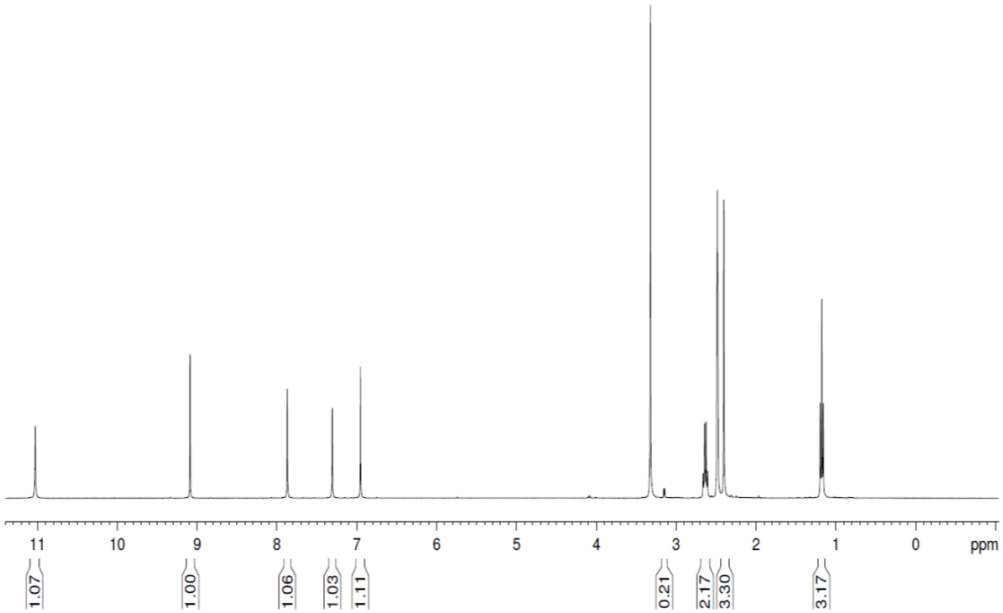
Mass spectroscopy: (ESI +ve) 277.9 $[M+H]^+$.

Step-2: 6-Ethyl-7-hydroxy-3-(4-methyl-1,3-thiazol-2-yl)-4*H*-chromen-4-one (0.65 g, 40%) was prepared from 1-(5-ethyl-2,4-dihydroxyphenyl)-2-(4-methyl-1,3-thiazol-2-yl)-ethanone (1.65 g, 5.95 mmol) and triethyl orthoformate (3.53 g, 23.8 mmol) according to the typical procedure.

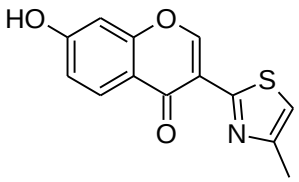
HPLC: 96.6% (289 nm)

Mass spectroscopy: (ESI +ve) 287.9 $[M+H]^+$.

^{1}H NMR: (400 MHz, d_6-DMSO) δ: 1.11 (t, *J* 7.6, 3H), 2.48 (s, 3H), 2.55 (q, *J* 7.6, 2H), 6.90 (s, 1H), 7.36 (s, 1H), 7.80 (s, 1H), 9.10 (s, 1H), 11.04 (s, 1H).



Preparation of 7-hydroxy-3-(4-methyl-1,3-thiazol-2-yl)-4H-chromen-4-one (8)



Step-1: 1-(2,4-dihydroxyphenyl)-2-(4-methyl-1,3-thiazol-2-yl)ethanone (1.65 g, 73%) was prepared from resorcinol (1.00 g, 9.09 mmol) and 2-(4-methyl-3-thiazol-2yl) acetonitrile (1.25 g, 9.09 mmol) according to the typical procedure.

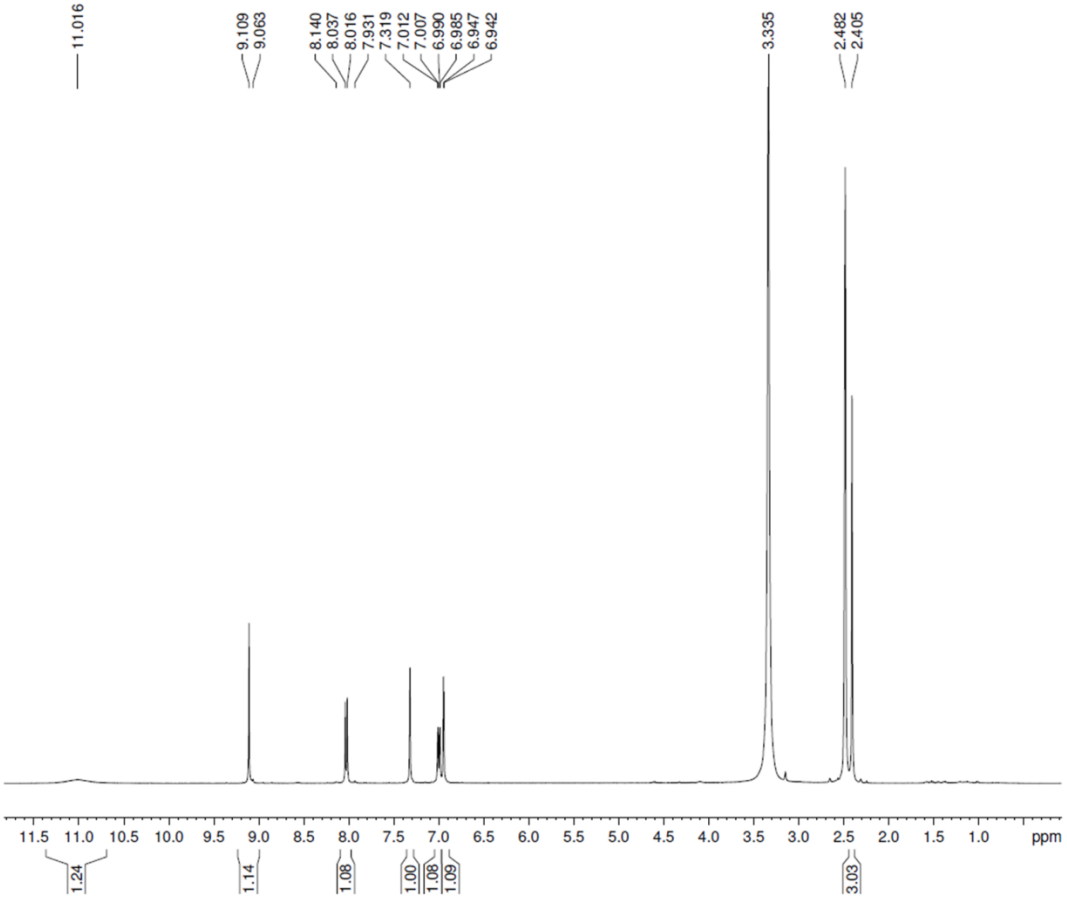
Mass spectroscopy: (ESI +ve) 249.9 $[M+H]^+$.

Step-2: 7-hydroxy-3-(4-methyl-1,3-thiazol-2-yl)-4H-chromen-4-one (0.25 g, 14%) was prepared from 1-(2,4-dihydroxyphenyl)-2-(4-methyl-1,3-thiazol-2-yl)ethanone (1.65 g, 6.61 mmol) and triethyl orthoformate (3.90 g, 26.5 mmol) according to the typical procedure.

HPLC: 99.5% (286 nm)

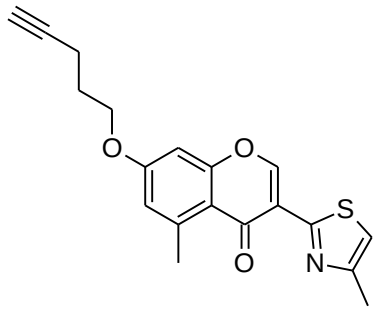
Mass spectroscopy: (ESI +ve) 259.9 $[M+H]^+$.

^{1}H NMR: (400 MHz, d_6-DMSO) δ: 2.41 (s, 3H), 6.94 (m, 1H), 7.00 (m, 1H), 7.32 (s, 1H), 8.03 (m, 1H), 9.10 (s, 1H), 11.02 (bs, 1H).



2.3 *O*-Alkylations of 7-hydroxy-5-methyl-3-(4-methyl-1,3-thiazol-2-yl)-4*H*-chromen-4-one

Preparation of 5-methyl-3-(4-methyl-1,3-thiazol-2-yl)-7-(pent-4-yn-1-yloxy)-4*H*-chromen-4-one (5)

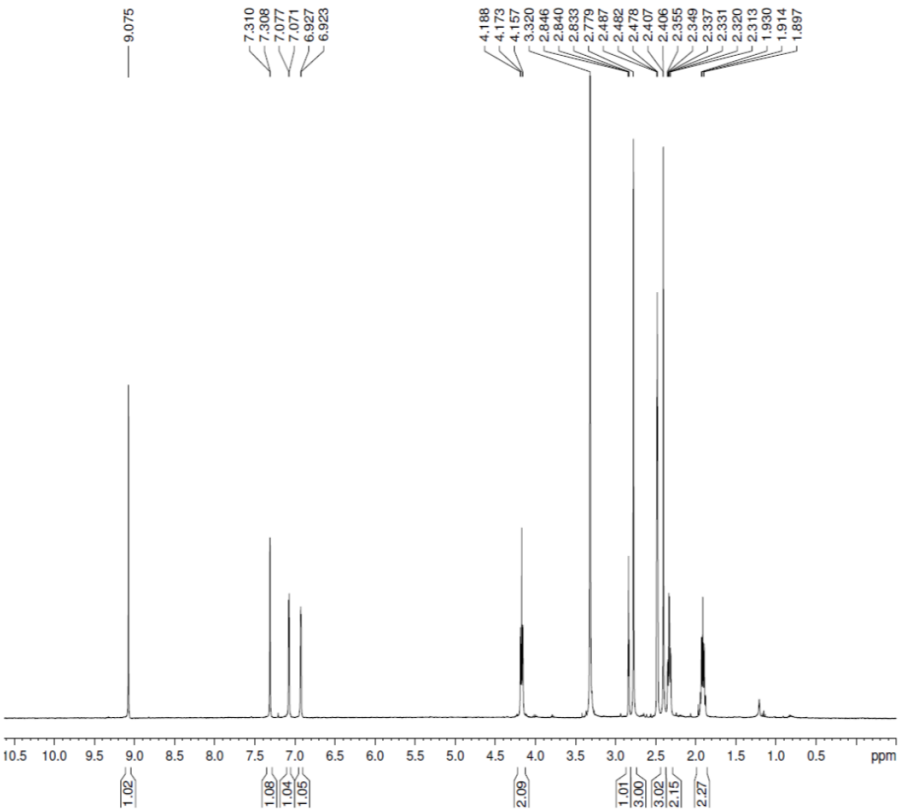


7-hydroxy-5-methyl-3-(4-methyl-1,3-thiazol-2-yl)-4*H*-chromen-4-one (0.250 g, 0.91 mmol), 5-chloro-1-pentyne (0.103 g, 1.37 mmol), potassium iodide (5 mg) and K_2CO_3 (0.190 g, 1.37 mmol) were refluxed in acetone (20mL) for 6 hours. After completion of the reaction, the solvent was removed *in vacuo* and the crude residue was partitioned between water (30 mL) and ethylacetate (30 mL). The separated aqueous phase was back-extracted with ethylacetate (2 × 20 mL) and all organic extracts were then combined, dried over Na_2SO_4 and concentrated *in vacuo*. The crude compound was purified by gradient flash chromatography with ethyl acetate/isohexane mixtures, affording 5-methyl-3-(4-methyl-1,3-thiazol-2-yl)-7-(pent-4-yn-1-yloxy)-4*H*-chromen-4-one (0.130 g, 42 %).

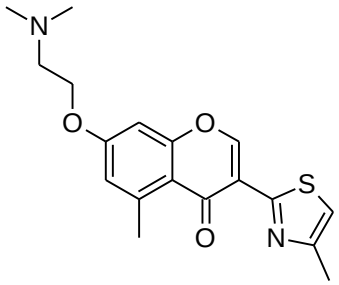
HPLC: 94.4% (283 nm)

Mass spectroscopy: (ESI +ve) 399.9 $[M+H]^+$.

^{1}H NMR: (400 MHz, d_6-DMSO) δ: 1.91 (m, 2H), 2.33 (m, 2H), 2.40 (m, 3H), 2.78 (s, 3H), 2.84 (m, 1H), 4.17 (m, 2H), 6.92 (m, 1H), 7.07 (m, 1H), 7.31 (m, 1H), 9.07 (s, 1H).



Preparation of 7-[2-(dimethylamino)ethoxy]-5-methyl-3-(4-methyl-1,3-thiazol-2-yl)-4*H*-chromen-4-one (2)

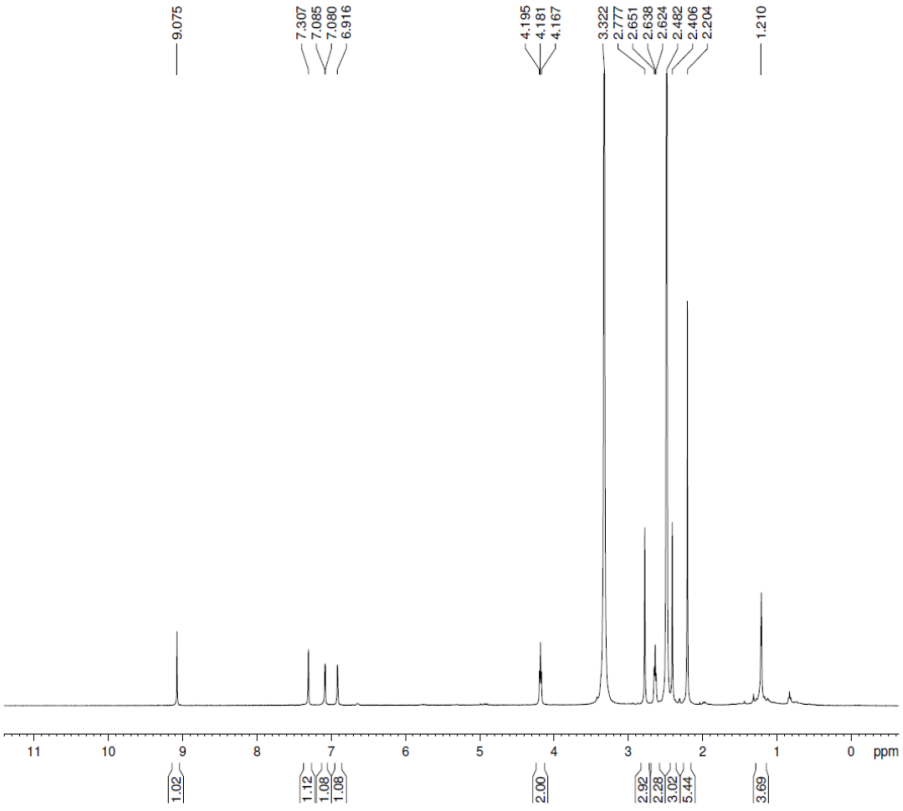


7-[2-(dimethylamino)ethoxy]-5-methyl-3-(4-methyl-1,3-thiazol-2-yl)-4*H*-chromen-4-one (18 mg, 15 %) was prepared from 7-hydroxy-5-methyl-3-(4-methyl-1,3-thiazol-2-yl)-4*H*-chromen-4-one (0.10 g, 0.36 mmol), 2-chloro-*N*,*N*-dimethylethanamine (0.16 g, 1.10 mmol), potassium iodide (5 mg) and potassium carbonate (0.152 g 1.10 mmol), according to the procedure used for the preparation of 5-methyl-3-(4-methyl-1,3-thiazol-2-yl)-7-(pent-4-yn-1-yloxy)-4*H*-chromen-4-one.

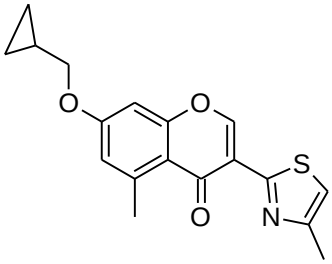
HPLC: 98.9 % (283 nm)

Mass spectroscopy: (ESI +ve) 345.0 $[M+H]^+$.

H NMR: (400 MHz, d_6-DMSO) δ: 2.20 (s, 6H), 2.41 (s, 3H), 2.64 (t, *J* 5.4, 2H), 2.77 (s, 3H), 4.18 (t, *J* 5.4, 2H), 6.91 (s, 1H), 7.08 (m, 1H), 7.30 (s, 1H), 9.07 (s, 1H).



Preparation of 7-(Cyclopropylmethoxy)-5-methyl-3-(4-methyl-1,3-thiazol-2-yl)-4*H*-chromen-4-one (4)

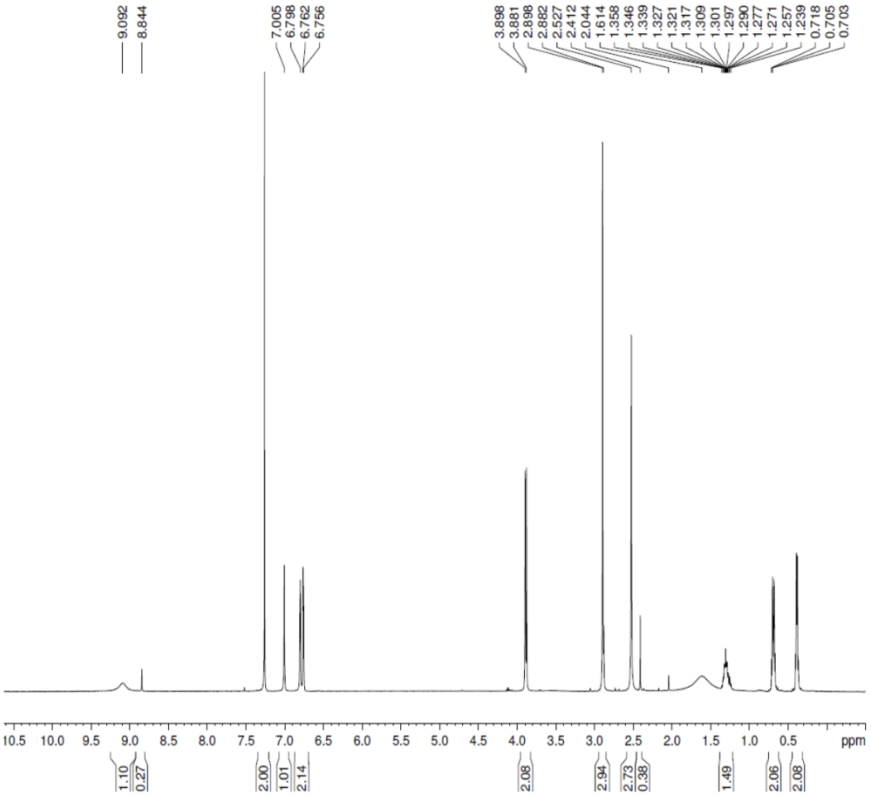


A solution of 7-hydroxy-5-methyl-3-(4-methyl-1,3-thiazol-2-yl)-4*H*-chromen-4-one (0.20 g, 0.73 mmol) in *N,N*-dimethylacetamide (1.5 mL) was treated with (bromomethyl)-cyclopropane (0.11 g, 0.82 mmol) and K_2CO_3 (0.11 g, 0.80 mmol), then stirred for 90 minutes at 90 ºC. After completion of the reaction, the mixture was poured into water (30 mL) and extracted with ethylacetate (3 × 20 mL); the organic extracts were combined, dried over Na_2SO_4 and concentrated *in vacuo*. The crude compound was purified by gradient flash chromatography with ethyl acetate/isohexane mixtures, affording 7-Cyclopropylmethoxy-5-methyl-3-(4-methyl-1,3-thiazol-2-yl)-4*H*-chromen-4-one (0.10 g, 42%).

HPLC: 99.9% (283 nm)

Mass spectroscopy: (ESI +ve) 327.9 $[M+H]^+$.

H NMR: (400 MHz, $CDCl_3$) δ: 0.40 (m, 2H), 0.71 (m, 2H), 1.30 (m, 1H), 2.52 (s, 3H), 2.89 (d, *J* 2.4, 3H), 3.88 (m, 2H), 6.76 (d, *J* 2.4, 1H), 6.80 (s, 1H), 7.00 (s, 1H), 9.09 (bs, 1H).



3. Computational Methods

WaterMap

WaterMap is computational software from Schrodinger LLC (www.schrodinger.com) that exploits an all atom explicit solvent molecular dynamics simulation followed by a statistical thermodynamic analysis of water clusters. The Grand Canonical method computes the free energy of water binding to proteins by equilibrating concentrations between a reference state and a simulation cell that includes waters bound to a protein active site. The GCMC method locates ensembles of water positions consistent with a selected free-energy level. We tested the suitability of using these methods as an implicit solvent alternative, in linear combination with a molecular mechanic force field (WNP-MMSA), for the quantitative prediction of ligand free energy of binding. See references and discussion in A. Bortolato, B. G. Tehan , M. S. Bodnarchuk, J. W. Essex and J. S. Mason, *J. Chem. Inf. Model*., 2013, **53**, 1700–1713.

WaterFLAP

WaterFLAP is computational software from Molecular Discovery Ltd. (www.moldiscovery.com) that is an extension under development of the new FLAP software that uses GRID in an iterative manner using hotspots from the water probe to build a complete network of waters in a binding site, with or without a ligand present. See: P. J. Goodford, *J.Med.Chem.*, 1985**, 28**, 849.

The starting water network at 10 Å from the ligand has been created using WaterFLAP using a GRID step of 0.5 Å and a maximum water probe GRID energy of -2 kcal/mol. After 200 steps steepest descend minimization the resulting water network has been equilibrated in the presence of the protein-ligand complex using GROMACS molecular dynamics for 20 ps. After a final minimization the waters have been scored using the OH2 and CRY GRID probes. The sorting takes into account the presence of other water molecules as well as the ligand and protein.

Adenosine A_1 Receptor Homology Modelling

Homology models were constructed from two adenosine A_{2A} receptor structure templates, a high-resolution 1.8 Å structure binding the high-affinity ligand ZM241385 (PDB:4EIY) and a 3.6 Å structure binding the low affinity ligand caffeine (PDB:3RFM). Maestro software (Schrodinger LLC (www.schrodinger.com), PRIME package) was used for the homology modelling, preserving side-chain rotations for all conserved residues.