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PAPER

Synthesis of novel chromene scaffolds for adenosine receptors†

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A one-pot procedure was developed for the synthesis of novel 3-[amino(methoxy)methylene]-2-oxo-3,4-dihydro-2*H*-chromen-4-yl)-3-cyanoacetamides and chromeno[3,4-*c*]pyridine-1-carbonitriles from the reaction of 2-oxo-2*H*-chromene-3-carbonitriles and cyanoacetamides. These chromene derivatives were identified as new scaffolds for adenosine receptors and the hits **3a**, **3c**, **5a**, and **5b** were found.

Introduction

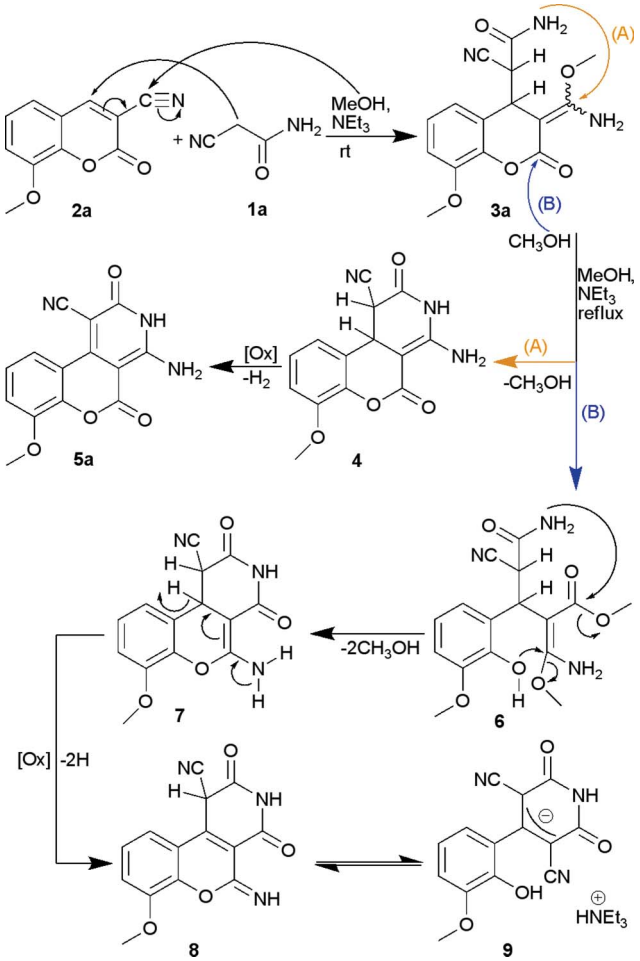
Adenosine receptors are distributed throughout the body, regulating different cellular functions and can be considered attractive targets for therapeutic agents.[1,2] Different compounds proved to be active on these receptors, displaying pharmacological activity namely for the treatment of cardiovascular, inflammatory or neurodegenerative diseases and cancer.[1,2] The active molecules usually belong to the purine family, but compounds with the pyrazolo-triazolo-pyrimidine, dihydropyridine and quinazoline-urea core unit were also identified as active.[1] To our knowledge, the interaction of chromene derivatives with adenosine receptors was never reported.

The chromene scaffold is present in a variety of biologically active compounds[3] and their synthesis has been widely explored in the literature.[4] The 2-oxo-2*H*-chromene-3-carbonitriles, in particular, are usually prepared from the Knoevenagel condensation of salicylaldehydes with malononitrile, a reaction that can be performed in aqueous base followed by acidic treatment.[5] Only a few studies have been reported on the reactivity of these compounds with nucleophiles[5a] and the reaction with cyanoacetamides was never explored.

Results and discussion

Chemical synthesis

Preliminary studies on the reaction of 2-oxochromene **2a**, prepared according to a previously reported procedure[5c] with the commercially available 2-cyanoacetamide **1a**, were performed in methanol and triethylamine (Scheme 1). After 2 days at room temperature, the white solid that precipitated from solution was collected and identified by ^{1}H NMR as a 1 : 1 : 1 mixture of compounds **3a**, **5a**, and **9**. The pure compound **3a** was isolated from the mother liquor in 22% yield. The solid mixture containing **3a**, **5a** and **9** evolved to a 1 : 1 mixture of **5a** and **9** after reflux in methanol and triethylamine



Scheme 1 Proposed mechanism for the formation of compounds **3a**, **5a** and **9** in the reaction of chromene **2a** with 2-cyanoacetamide **1a**.

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† Electronic supplementary information (ESI) available: copies of ^{1}H and ^{13}C NMR spectra of all new compounds. See DOI: 10.1039/c1ob05305a

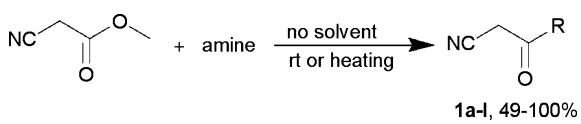
for 2.5 h. Compound **5a** (8.5%) was separated from this mixture upon stirring at room temperature in a combination of ethanol, water and acetic acid. Compound **9** (5.4%) was also isolated from the mother liquor after partial evaporation of the solvent.

Previous work on the condensation of a 3-cyanochromene with ethylcyanoacetate in aqueous ammonia reported the synthesis of a molecule analogous to **5a**.[6] The product, isolated in 25% yield, was characterized by elemental analysis, mass spectrometry and IR spectroscopy.

The results of our preliminary study suggest that compound **3a** is the first product generated in solution and may evolve to **5a** by intramolecular cyclization between the amide nitrogen and the substituent on C-3 (Pathway A). Alternatively, product **9** can be generated after reflux in methanol and triethylamine, probably arising from ring-opening of the chromene moiety, induced by the nucleophilic solvent, to generate intermediate **6**. Two sets of intramolecular cyclizations lead to compound **7** which oxidizes to **8**. Depending on the substituents in the ring, 2-iminochromenes can equilibrate with the phenolic nitrile by ring opening of the pyrane unit, leading to the formation of **9**.

Compounds **3a** and **5a**, isolated in modest yield in this synthetic study, proved to be active on adenosine receptors, encouraging the search for a selective experimental procedure to generate these scaffolds.

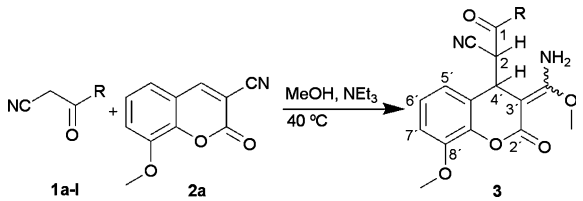
To prepare the target compounds **3a–l**, several amides **1b–l** were synthesized by the direct combination of methyl-2-cyanoacetate and primary or secondary amines, using a previously described procedure (Scheme 2).[4k]

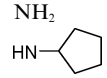
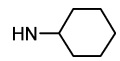
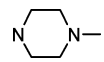
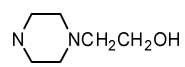
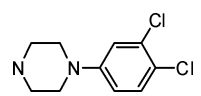
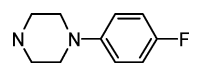
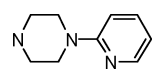
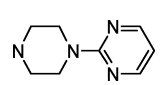


Scheme 2 Reaction of methyl cyanoacetate with primary or secondary amines.

Amides **1a–l** were isolated in 49–100% yield and reacted with 8-methoxy-2-oxo-2*H*-chromene-3-carbonitriles **2a**, under the reaction conditions identified in Table 1. Compounds **3a–l** were isolated in 22–80% yield. The use of ethanol as solvent failed to lead to the analogous structures **3a–l** when the reaction was performed in the presence of triethylamine at 40 °C. The NMR spectra for compound **3a** showed two sets of bands assigned to two diastereomers mainly on the basis of the vicinal coupling constants between the protons on the side chain methyne group (CH–CN) and the proton on the ring carbon C-4′. The values for J = 4.1 Hz and J = 6.3 Hz reveal a different stereochemical disposition of these substituents with dihedral angles around 45°/135° and 30°/145° respectively, according to the Karplus equation.[7] The H–C correlation spectra (HMBC and HMQC) allowed the complete identification of the proton and carbon signals for each diastereomer. Compounds **3b–l**, incorporating a substituted amide, led to a single species in the NMR spectra. In this case, it is possible that the volume of the amide substituent hampers the formation of the most hindered diastereomer. For compounds **3b** and **3c**, where a primary amide is present, the value of the coupling constant between the methylene C–H and the C_4–H (J = 7.2 and 7.6 Hz) indicated a dihedral angle around 20°/155°. The bulkier secondary amine in compounds **3d–l** resulted in coupling constants of around 4 Hz and dihedral angles close to 45°/130°.

Table 1 Synthesis of chromene derivatives **3**

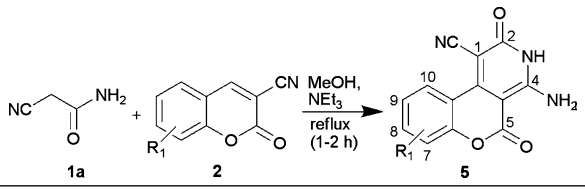


Entry	Compound **1**	*R*	Product **3**, yield
1	**1a**	NH_2	**3a**, 27%
2	**1b**		**3b**, 27%
3	**1c**		**3c**, 33%
4	**1d**		**3d**, 30%
5	**1e**		**3e**, 63%
6	**1f**		**3f**, 34%
7	**1g**		**3g**, 80%
8	**1h**		**3h**, 42%
9	**1i**		**3i**, 60%
10	**1j**		**3j**, 31%
11	**1k**		**3k**, 37%
12	**1l**		**3l**, 80%

The stereochemistry of the amino(methoxy)methylene side chain was not assigned with certainty. The push-pull effect, associated with the presence of two electron-donating substituents conjugated with the carbonyl group, was considered responsible for a low rotational barrier of the exocyclic double bond.

Compounds **5a–d** were isolated in 26–36% yield when the commercially available 2-cyanoacetamide **1a** was combined with 2-oxo-2*H*-chromenes **2** in methanol and the mixture was refluxed for 1–2 h (Table 2). The uncertainty in the relative position of the amino and methoxy substituents favoured the intramolecular cyclization of intermediate **3** under these mild temperature conditions. All compounds were fully characterized by ^{1}H NMR, ^{13}C NMR, correlation techniques (HMBC and HMQC), infrared spectroscopy and elemental analysis (or high-resolution mass spectra).

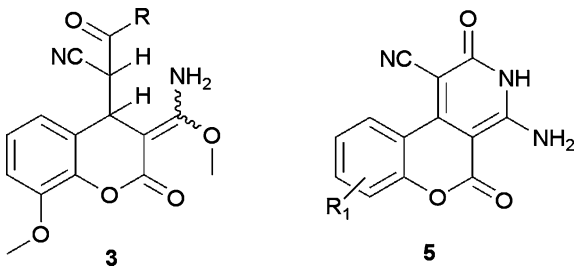
Table 2 Synthesis of chromene derivatives **5**



1a 2 5

Entry	R_1	Product **5**, yield
1	7-OCH_3	**5a**, 26%
2	7-OH	**5b**, 36%
3	9-Cl	**5c**, 35%
4	9-Br	**5d**, 32%

Table 3 Binding affinities of the new compounds at human A_1, A_{2A}, A_{2B} and A_3 receptors ($\mathbf{p}K_i$ or inhibition percentage at 10 μM)



3 5

		Adenosine receptors			
Entry	Compd	A_1	A_{2A}	A_{2B}	A_3
1	**3a**	**6.8 ± 0.1**	**6.6 ± 0.1**	**6.6 ± 0.3**	29 ± 4%
2	**3b**	35 ± 1%	46 ± 5%	39 ± 2%	23 ± 4%
3	**3c**	**6.1 ± 0.2**	49 ± 2%	**5.8 ± 0.2**	41 ± 1%
4	**3d**	61 ± 1%	49 ± 2%	57 ± 5%	26 ± 4%
5	**3e**	14 ± 1%	24 ± 5%	25 ± 5%	14 ± 1%
6	**3f**	38 ± 2%	65 ± 3%	1 ± 5%	12 ± 4%
7	**3g**	29 ± 2%	37 ± 5%	24 ± 5%	17 ± 5%
8	**3h**	20 ± 1%	35 ± 1%	47 ± 5%	36 ± 1%
9	**3i**	31 ± 3%	41 ± 4%	48 ± 4%	21 ± 1%
10	**3j**	18 ± 3%	26 ± 2%	40 ± 2%	26 ± 5%
11	**3k**	20 ± 1%	30 ± 4%	39 ± 4%	27 ± 5%
12	**3l**	58 ± 2%	35 ± 3%	18 ± 5%	13 ± 5%
13	**5a**	**6.9 ± 0.2**	**6.9 ± 0.3**	**5.9 ± 0.4**	13 ± 3%
14	**5b**	**6.1 ± 0.3**	**5.5 ± 0.2**	**6.0 ± 0.2**	**6.3 ± 0.3**
15	**5c**	51 ± 1%	22 ± 1%	29 ± 1%	18 ± 1%
16	**5d**	n.d.[a]	n.d.[a]	n.d.[a]	n.d.[a]

[a] n.d. – not determined

Pharmacological evaluation

A complete *in vitro* evaluation of the affinity of the novel compounds at human adenosine A_1, A_{2A}, A_{2B} and A_3 was carried out by radioligand binding assays at receptors heterologously expressed in mammalian cells lines. All the 15 chromenes tested displayed affinity for adenosine receptors at the concentration of 10 μM, displacing the radioligand binding to a different extent as shown in Table 3.

The pK_i values were calculated for 4 hits identified as compounds showing an inhibition percentage of the radioligand binding ($\%_{inhib.}$) above 70% at the concentration of 10 μM (Table 3). The data revealed 3-[amino(methoxy)methylene]-2-oxo-3,4-dihydro-2*H*-chromen-4-yl)-3-cyanoacetamides **3a** and **3c** and chromeno[3,4-*c*]pyridine-1-carbonitriles **5a** and **5b** as the most active compounds, showing affinities in the submicromolar range at adenosine receptors.

Chromene **3a** presented pK_i values between 6.6 and 6.8 for adenosine A_1, A_{2A} and A_{2B} receptors. Chromene **3c** showed pK_i values of 6.1 and 5.8 at A_1 and A_{2B} receptors, respectively. Chromene **5a** presented a pK_i value of 6.9 for both A_1 and A_{2A} receptors and lower affinity (5.9) for A_{2B} receptor. Chromene **5b** presented pK_i values in the range of 6.0–6.3 for A_1, A_{2B} and A_3 receptors and a lower affinity (5.5) for A_{2A}.

When we compare the affinities of the chromenes **3a** and **3c** for A_1, A_{2A} and A_{2B} receptors, we observe higher affinities for compound **3a**, which leads us to suggest that the existence of a primary amino group in the 3-[amino(methoxy)methylene] group of these chromenes is important for their affinity for these receptor subtypes.

In chromenes **5**, the replacement of the 7-OCH_3 group in chromene **5a** by a 7-OH group in chromene **5b** led to a decrease in affinity for A_1 and A_{2A} receptors and an increase for the A_3 receptor.

Among these 4 hits, chromenes **3a**, **5a** and **5b** show affinities at submicromolar concentrations for both A_1 and A_{2A} receptors. This pharmacological profile can be of therapeutic interest in the light of the efficacy shown by compounds with dual affinity at A_1 and A_{2A} receptors in animal models of Parkinson's disease.[8]

Conclusions

In summary, we developed a one-pot procedure for the synthesis of novel 3-[amino(methoxy)methylene]-2-oxo-3,4-dihydro-2*H*-chromen-4-yl)-3-cyanoacetamides **3** and chromeno[3,4-*c*]pyridine-1-carbonitriles **5** from readily available cyanoacetamides and 2-oxo-2*H*-chromene-3-carbonitriles in methanol and triethylamine. The substituted 2-oxochromene **3** was isolated when the reaction was performed at 40 °C and reflux conditions resulted in the formation of the tricyclic product **5**. These new chromene scaffolds proved to be active at adenosine receptors and 4 hits were identified in this study with affinities in the submicromolar range.

Experimental

General

Chemicals and organic solvents were purchased from commercial sources and used without further purification. All new compounds were fully characterized by elemental analysis and spectroscopic data. The NMR spectra were recorded at room temperature on a Varian Unity Plus (^{1}H: 300 MHz, ^{13}C: 75 MHz) and a Bruker Avance 3400 (^{1}H: 400 MHz, ^{13}C: 100 MHz), including the ^{1}H–^{13}C correlation spectra (HMQC and HMBC) and deuterated DMSO was used as solvent. Chemical shifts (δ) were reported in parts per million (ppm) and the coupling constants, J, are reported in hertz (Hz). The melting points were determined on a Stuart SMP3 melting point apparatus and are uncorrected. The purities of all tested compounds are higher than 95% by elemental analysis, which were performed on a LECO CHNS-932 instrument and were reported to be within 0.4% of calculated values. High resolution mass spectra (HRMS) were obtained from the C.A.C.T.I. – Universidade de Vigo.

Compound **1a** is commercially available. The synthesis of compounds **1b–e** are reported in reference 4k and compounds **1f–l** were synthesized using the method described in this reference. Compounds **2** were prepared according to previously reported procedures[5c] and the synthesis of compound **2** with R_1 = 9-Cl was previously reported.[5b]

3-Oxo-3-(pyrrolidin-1-yl)propanenitrile 1f. White solid, 88% yield. Mp 76–78 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.76 (q, J = 7.2 Hz, 2H, CH_2), 1.85 (q, J = 6.3 Hz, 2H, CH_2), 3.89 (s, 2H, CN-CH_2), 3.28 (t, J = 6.9 Hz, 2H, CH_2), 4.35 (q, J = 7.2 Hz, 2H, CH_2). ^{13}C NMR (75 MHz, DMSO-d_6) δ 24.0 (CH), 25.4 (CN-*C*H), 25.5 (CH), 45.8 (CH), 46.1 (CH), 116.0 (CN), 160.8 (C═O). IR (Nujol mull) ν 3084, 2256, 1651, 1597, 1556, 1521 cm^{-1}. Anal. Calcd for $C_7H_{10}N_2O{\cdot}0.05H_2O$: C, 60.48; H, 7.27; N, 20.16. Found: C, 60.38; H, 7.24; N, 20.17.

3-(Morpholin-4-yl)-3-oxopropanenitrile 1g. Orange solid, 93% yield. Mp 82–84 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.32 (t, J = 4.8 Hz, 2H, CH_2), 3.51–3.59 (m, 4H, 2 CH_2), 3.42 (t, J = 4.5 Hz, 2H, CH_2), 4.02 (s, 2H, CN-CH_2). ^{13}C NMR (75 MHz, DMSO-d_6) δ 24.64 (CN-*C*H), 42.03 (CH), 45.76 (CH), 65.76 (CH), 65.83 (CH), 116.13 (CN), 161.71 (C═O). IR (Nujol mull) ν 2264, 1652, 1618, 1532 cm^{-1}. Anal. Calcd for $C_7H_{10}N_2O_2$: C, 54.54; H, 6.49; N, 18.18. Found: C, 54.53; H, 6.40; N, 18.16.

3-[4-(2-Hydroxyethyl)piperazin-1-yl]-3-oxopropanenitrile 1h. Orange solid, 100% yield. Mp 78–80 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.31–2.42 (m, 6H, 3 CH_2), 3.30 (t, J = 4.8 Hz, 2H, CH_2), 3.42 (t, J = 5.1 Hz, 2H, CH_2), 3.48 (t, J = 6.6 Hz, 2H, NCH_2CH$_2$), 4.01 (s, 2H, CN-CH_2), 4.20–4.60 (brs, 1H, OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ 24.71 (CN-*C*H), 41.70 (CH), 45.37 (CH), 52.57 (CH), 52.95 (CH), 58.47 (*N*-*C*H$_2$-CH$_2$), 59.96 (*C*H$_2$-OH), 116.22 (CN), 161.36 (C═O). IR (Nujol mull) ν 3500–3050 (br), 2258, 1652, 1612 cm^{-1}. Anal. Calcd for $C_9H_{15}N_3O_2{\cdot}0.2H_2O$: C, 53.84; H, 7.68; N, 20.94. Found: C, 53.92; H, 7.59; N, 20.81.

3-[4-(3,4-Dichlorophenyl)piperazin-1-yl]-3-oxopropane-nitrile 1i. White solid, 49% yield. Mp 131–133 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.57 (t, J = 3.9 Hz, 2H, CH_2), 3.19 (t, J = 4.2 Hz, 2H, CH_2), 3.24 (t, J = 4.5 Hz, 2H, CH_2), 3.46 (t, J = 3.3 Hz, 2H, CH_2), 4.08 (s, 2H, CN-CH_2), 6.94 (dd, J = 6.8 Hz, 2.1 Hz, 1H, Ar–H), 7.15 (d, J = 2.1 Hz, 1H, Ar–H), 7.40 (d, J = 6.9 Hz, 1H, Ar–H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 24.75 (CN-*C*H), 41.13 (CH), 44.76 (CH), 47.10 (CH), 47.40 (CH), 115.66 (Ar-*C*H), 116.55 (CN), 116.74 (Ar-*C*H), 119.97 (Ar-*C*-Cl), 130.50 (Ar-*C*H), 131.53 (Ar-*C*-Cl), 150.23 (Ar-*C*), 161.61 (C═O). IR (Nujol mull) ν 3100, 2258, 1653, 1586, 1546 cm^{-1}. Anal. Calcd for $C_{13}H_{13}N_3OCl_2{\cdot}0.1H_2O$: C, 51.97; H, 4.41; N, 13.99. Found: C, 51.97; H, 4.51; N, 13.72.

3-[4-(4-Fluorophenyl)piperazin-1-yl]-3-oxopropanenitrile 1j. Orange solid, 78% yield. Mp 130–132 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.58 (t, J = 4.8 Hz, 2H, CH_2), 3.01–3.12 (m, 4H, 2 CH_2), 3.48 (t, J = 4.8 Hz, 2H, CH_2), 4.09 (s, 2H, CNCH_2), 6.97 (d, J = 9.3 Hz, 2H, 2 Ar–H), 7.04 (d, J = 8.7 Hz, 2H, 2 Ar–H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 24.80 (CN-*C*H), 41.51 (CH), 45.19 (CH), 48.82 (CH), 49.16 (CH), 115.39 (2 Ar-*C*H), 116.19 (CN), 117.77 (2 Ar-*C*H), 147.54 (Ar-*C*), 156.33 (Ar-*C*-F), 161.55 (C═O). IR (Nujol mull) ν 3080, 2259, 1650, 1594, 1511 cm^{-1}. Anal. Calcd for $C_{13}H_{14}N_3OF$: C, 63.16; H, 5.67; N, 17.00. Found: C, 63.21; H, 5.62; N, 17.04.

3-Oxo-3-[4-(pyridin-2-yl)piperazin-1-yl]propanenitrile 1k. Off-white solid, 90% yield. Mp 101–103 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.40–3.60 (m, 8H, 4 CH_2), 4.09 (s, 2H, CN-CH_2), 6.84 (d, J = 8.4 Hz, 1H, Ar–H), 6.65 (t, J = 5.7 Hz, 1H, Ar–H), 7.54 (td, J = 7.8 Hz, 1.8 Hz, 1H, Ar–H), 8.11 (dd, J = 4.8 Hz, 1.8 Hz, 1H, Ar–H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 24.85 (CN-*C*H), 41.29 (CH), 44.15 (CH), 44.33 (CH), 44.89 (CH), 107.32 (Ar-*C*H), 113.37 (Ar-*C*H), 137.66 (Ar-*C*H), 147.56 (Ar-*C*H), 158.55 (Ar-*C*), 161.68 (C═O). IR (Nujol mull) ν 3050, 2256, 1660, 1592, 1567 cm^{-1}. Anal. Calcd for $C_{12}H_{14}N_4O{\cdot}0.2H_2O$: C, 61.64; H, 6.16; N, 23.97. Found: C, 61.80; H, 6.09; N, 23.94.

3-Oxo-3-[4-(pyrimidin-2-yl)piperazin-1-yl]propanenitrile 1l. Yellow solid, 88% yield. Mp 143–145 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.50–3.60 (m, 2H, CH_2), 3.68–3.80 (m, 4H, 2 CH_2), 3.40–3.46 (m, 2H, CH_2), 4.08 (s, 2H, CN-CH_2), 6.66 (t, J = 4.8 Hz, 1H, Ar–H), 8.38 (d, J = 4.8 Hz, 2H, 2 Ar–H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 24.86 (CN-*C*H), 41.40 (CH), 42.82 (CH), 43.02 (CH), 44.98 (CH), 110.52 (Ar-*C*H), 157.99 (2 Ar-*C*H), 161.02 (Ar-*C*), 161.73 (C═O). IR (Nujol mull) ν 2254, 1655, 1583, 1548 cm^{-1}. Anal. Calcd for $C_{11}H_{13}N_5O$: C, 57.14; H, 5.62; N, 30.30. Found: C, 57.00; H, 5.88; N, 30.06.

General procedure for the synthesis of compounds 3a–l

Compound **1** (0.48 mmol) was added to a suspension of 8-methoxy-2-oxo-2*H*-chromene-3-carbonitrile **2a** (0.09 g, 0.45 mmol) in methanol (3.5 mL) and triethylamine (0.7 mL) and the reaction mixture was heated at 40 °C, in a water bath, for 30 min to 1 h and 45 min. For **3a–i** and **3l**, the reaction mixture was concentrated in the rotary evaporator and cooled in an ice bath for a few minutes. Diethyl ether (1–2 mL) was added and the solid was filtered and washed with diethyl ether. For **3j** and **3k** the solvents were removed in the rotary evaporator and the residue purified by flash chromatography using dichloromethane as eluent. The solvent was removed in the rotary evaporator and a few drops of methanol and diethyl ether (3 mL) were added to the oil to precipitate the product. The solid was filtered and washed with diethyl ether.

2-(3-[Amino(methoxy)methylene]-8-methoxy-2-oxo-3,4-dihydro-2*H*-chromen-4-yl)-2-cyanoacetamide 3a. Off-white solid, 22% yield. Mp 181–183 °C. Two sets of bands were present in the NMR spectra and were assigned to diastereomers **A** and **B** in a 1 : 1 ratio. ^{1}H NMR, **3a(A)** (300 MHz, DMSO-d_6) δ 3.64 (s, 3H, OCH_3), 3.82 (s, 6H, Ar-OCH_3),* 3.92 (d, J = 4.1 Hz, 1H, C_2–H), 4.50 (d, J = 4.1 Hz, 1H, $C_{4'}$–H), 6.82 (dd, J = 7.1 Hz, 2.1 Hz, 1H, $C_{5'}$–H), 7.00–7.05 (m, 2H, $C_{7'}$–H),* 7.06 (t, J = 8.1 Hz, 1H, $C_{6'}$–H), 7.40 (s, 1H, C_1-NH_2), 7.52 (s, 1H, C_1-NH_2), 7.82 (s, 2H, $C_{3'}$-NH_2). ^{13}C NMR, **3a(A)** (75 MHz, DMSO-d_6) δ 35.47 ($C_{4'}$), 47.49 (C_2), 50.72 (OCH_3), 55.66 (2 Ar–OCH_3),* 71.81 ($C_{3'}$), 111.68 ($C_{7'}$), 117.45 (CN), 120.00 ($C_{5'}$), 124.00 ($C_{4a'}$), 124.34 ($C_{6'}$), 139.42 ($C_{8a'}$), 146.98 (2 $C_{8'}$),* 162.60 ($C_{2'}$), 165.39 (C_1), 167.96 (*C*-$NH_2(OCH_3)$). ^{1}H NMR, **3a(B)** (300 MHz, DMSO-d_6) δ 3.57 (s, 3H, OCH_3), 3.61 (d, J = 6.3 Hz, 3H, C_2–H), 3.82 (s, 6H, Ar-OCH_3),* 4.44 (d, J = 6.3 Hz, 1H, $C_{4'}$–H), 6.91 (dd, J = 7.2 Hz, 1.2 Hz, 1H, $C_{5'}$–H), 7.00–7.05 (m, 2H, $C_{7'}$–H),* 7.09 (t, J = 7.5 Hz, 1H), 7.29 (s, 1H, $C_{6'}$–H, C_1-NH_2), 7.54 (s, 1H, C_1-NH_2), 7.81 (s, 2H, $C_{3'}$-NH_2).

^{13}C NMR, **3a(B)** (75 MHz, DMSO-d_6) δ 35.66 ($C_{4'}$), 46.94 (C_2), 50.15 (O*C*H$_3$), 55.66 (2 Ar–O*C*H$_3$),* 71.29 ($C_{3'}$), 111.46 ($C_{7'}$), 117.30 (CN), 119.74 ($C_{5'}$), 121.99 ($C_{4a'}$), 124.07 ($C_{6'}$), 139.36 ($C_{8a'}$), 146.98 (2 $C_{8'}$),* 162.73 ($C_{2'}$), 165.34 (C_1), 168.73 (*C*-NH$_2$(OCH$_3$)). *The chemical shift for each isomer overlaps in a single signal. IR (Nujol mull) ν 3402, 3299, 2258, 1693, 1667, 1632, 1591, 1523 cm^{-1}. HRMS (FAB) *m*/*z*: Calc. for $C_{15}H_{15}N_3O_5$ 318.10910 [M+H]$^+$, found: 318.10845 [M+H]$^+$.

2-(3-[Amino(methoxy)methylene]-8-methoxy-2-oxo-3,4-dihydro-2*H*-chromen-4-yl)-2-cyano-*N*-cyclopentylacetamide 3b. White solid, 27% yield. Mp 173–175 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.10 (m, 2H, CH_2), 1.40–1.50 (m, 4H, 2 CH_2), 1.62 (m, 2H, CH_2), 3.51 (d, *J* = 7.2 Hz, 1H, C_2–H), 3.60 (s, 3H, OCH$_3$), 3.82 (s, 3H, Ar-OCH$_3$), 3.83–3.90 (m, 1H, NH-*CH*-C_4H_8), 4.38 (d, *J* = 7.2 Hz, 1H, $C_{4'}$–H), 6.80 (dd, *J* = 7.4 Hz, 1.6 Hz, 1H, $C_{5'}$–H), 7.01 (dd, *J* = 7.6 Hz, 1.6 Hz, 1H, $C_{7'}$–H), 7.06 (t, *J* = 8.4 Hz, 1H, $C_{6'}$–H), 7.80 (s, 2H, NH_2), 7.93 (d, *J* = 7.2 Hz, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 23.20 (CH), 23.24 (CH), 31.87 (CH), 31.90 (CH), 35.75 ($C_{4'}$), 46.06 (C_2), 50.36 (O*C*H$_3$), 50.67 (NH-*C*H), 55.64 (Ar–O*C*H$_3$), 71.78 ($C_{3'}$), 111.45 ($C_{7'}$), 117.34 (CN), 119.71 ($C_{5'}$), 123.76 ($C_{4a'}$), 124.14 ($C_{6'}$), 139.33 ($C_{8a'}$), 146.95 ($C_{8'}$), 162.57 ($C_{2'}$), 162.77 (C_1), 168.06 (*C*-NH$_2$(OCH$_3$)). IR (Nujol mull) ν 3470, 3303, 2240, 1692, 1650, 1607, 1588, 1548, 1513 cm^{-1}. Anal. Calcd for $C_{20}H_{23}N_3O_5$: C, 62.34; H, 5.97; N, 10.91. Found: C, 62.34; H, 5.67; N, 10.87.

2-(3-[Amino(methoxy)methylene]-8-methoxy-2-oxo-3,4-dihydro-2*H*-chromen-4-yl)-2-cyano-*N*-cyclohexylacetamide 3c. Yellow solid, 33% yield. Mp 169–171 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.90–1.24 (m, 5H, 2 CH_2 + CH), 1.55–1.65 (m, 5H, 2 CH_2 + CH), 3.37–3.42 (m, 1H, NH-*CH*-C_5H_{10}), 3.54 (d, *J* = 7.6 Hz, 1H, C_2–H), 3.60 (s, 3H, OCH$_3$), 3.81 (s, 3H, Ar-OCH$_3$), 4.38 (d, *J* = 7.2 Hz, 1H, $C_{4'}$–H), 6.80 (dd, *J* = 7.4 Hz, 1.2 Hz, 1H, $C_{5'}$–H), 7.01–7.06 (m, 2H, $C_{6'}$–H + $C_{7'}$–H), 7.80 (s, 2H, NH_2), 7.86 (d, *J* = 8.0 Hz, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 24.17 (CH), 25.03 (CH), 31.74 (CH), 31.96 (CH), 35.72 ($C_{4'}$), 45.93 (C_2), 50.34 (O*C*H$_3$), 47.87 (NH-*C*H), 55.63 (Ar–O*C*H$_3$), 71.84 ($C_{3'}$), 111.44 ($C_{7'}$), 117.39 (CN), 119.78 ($C_{5'}$), 123.76 ($C_{4a'}$), 124.12 ($C_{6'}$), 139.35 ($C_{8a'}$), 146.94 ($C_{8'}$), 162.24 (C_1), 162.81 ($C_{2'}$), 168.07 (*C*-NH$_2$(OCH$_3$)). IR (Nujol mull) ν 3469, 3305, 2242, 1692, 1651, 1622, 1602, 1587, 1546, 1510 cm^{-1}. Anal. Calcd for $C_{21}H_{25}N_3O_5$: C, 63.16; H, 6.27; N, 10.53. Found: C, 63.18; H, 6.35; N, 10.54.

2-(3-[Amino(methoxy)methylene]-8-methoxy-2-oxo-3,4-dihydro-2*H*-chromen-4-yl)-3-oxo-3-piperidin-1-ylpropane-nitrile 3d. Yellow solid, 30% yield. Mp 161–163 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.40–1.70 (m, 6H, 3 CH_2), 3.29–3.40 (m, 1H, CH), 3.45–3.60 (m, 2H, CH_2), 3.65 (s, 3H, OCH$_3$), 3.66–3.76 (m, 1H, CH), 3.83 (s, 3H, Ar-OCH$_3$), 4.22 (d, *J* = 3.9 Hz, 1H, $C_{4'}$–H), 4.57 (d, *J* = 3.6 Hz, 1H, C_2–H), 6.50–6.69 (m, 1H, $C_{5'}$–H), 7.00–7.05 (m, 2H, $C_{6'}$–H + $C_{7'}$–H), 7.83 (s, 2H, NH_2). ^{13}C NMR (75 MHz, DMSO-d_6) δ 23.79 (CH), 25.21 (CH), 26.06 (CH), 36.06 ($C_{4'}$), 42.68 (CH), 46.23 (C_2 + CH), 50.62 (O*C*H$_3$), 55.64 (Ar–O*C*H$_3$), 71.58 ($C_{3'}$), 111.69 ($C_{7'}$), 117.56 (CN), 120.40 ($C_{5'}$), 121.10 ($C_{4a'}$), 123.84 ($C_{6'}$), 139.53 ($C_{8a'}$), 146.88 ($C_{8'}$), 162.29 (C_1), 162.75 ($C_{2'}$), 167.77 (*C*-NH$_2$(OCH$_3$)). IR (Nujol mull) ν 3372, 3261, 2246, 1684, 1638, 1610, 1590, 1548, 1523 cm^{-1}. Anal. Calcd for $C_{20}H_{23}N_3O_5$: C, 62.34; H, 5.97; N, 10.91. Found: C, 62.11; H, 5.88; N, 11.18.

2-(3-[Amino(methoxy)methylene]-8-methoxy-2-oxo-3,4-dihydro-2*H*-chromen-4-yl)-3-(4-methylpiperazin-1-yl)-3-oxopropanenitrile 3e. White solid, 63% yield. Mp 170–172 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.21 (s, 3H, CH_3), 2.28–2.40 (m, 2H, CH_2), 2.41–2.46 (m, 2H, CH_2), 3.28 (m, 1H, CH), 3.48 (m, 2H, CH_2), 3.65 (s, 3H, OCH$_3$), 3.66 (m, 1H, CH), 3.83 (s, 3H, Ar-OCH$_3$), 4.23 (d, *J* = 3.9 Hz, 1H, $C_{4'}$–H), 4.59 (d, *J* = 3.9 Hz, 1H, C_2–H), 6.55–6.59 (m, 1H, $C_{5'}$–H), 7.00–7.05 (m, 2H, $C_{6'}$–H + $C_{7'}$–H), 7.84 (s, 2H, NH_2). ^{13}C NMR (75 MHz, DMSO-d_6) δ 36.17 ($C_{4'}$), 41.76 (2 CH), 45.27 (2 CH), 45.53 (*C*H$_3$), 46.02 (C_2), 50.66 (O*C*H$_3$), 55.65 (Ar–O*C*H$_3$), 71.52 ($C_{3'}$), 111.73($C_{7'}$), 117.45 (CN), 120.41 ($C_{5'}$), 121.08 ($C_{4a'}$), 123.88 ($C_{6'}$), 139.54($C_{8a'}$), 146.89 ($C_{8'}$), 162.60 ($C_{2'}$),* 162.77 (C_1),* 167.75 (*C*-NH$_2$(OCH$_3$)). *Assignments interchangeable. IR (Nujol mull) ν 3349, 3271, 2247, 1685, 1650, 1638, 1619, 1595 cm^{-1}. Anal. Calcd for $C_{20}H_{24}N_4O_5$: C, 60.00; H, 6.00; N, 14.00. Found: C, 60.00; H, 5.94; N, 14.07.

2-(3-[Amino(methoxy)methylene]-8-methoxy-2-oxo-3,4-dihydro-2*H*-chromen-4-yl)-3-oxo-3-pyrrolidin-1-ylpropane-nitrile 3f. Green solid, 35% yield. Mp 159–162 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.70–2.00 (m, 4H, 2 CH_2), 3.25–3.40 (m, 2H, CH_2), 3.55–3.60 (m, 2H, CH_2), 3.64 (s, 3H, OCH$_3$), 3.83 (s, 3H, Ar-OCH$_3$), 4.34 (d, *J* = 3.9 Hz, 1H, C_2–H),* 4.36 (d, *J* = 3.9 Hz, 1H, $C_{4'}$–H),* 6.56–6.65 (m, 1H, $C_{5'}$–H), 6.90–7.10 (m, 2H, $C_{6'}$–H + $C_{7'}$–H), 7.84 (s, 2H, NH_2). *Assignments interchangeable. ^{13}C NMR (75 MHz, DMSO-d_6) δ 23.73 (CH), 25.86 (CH), 35.34 ($C_{4'}$), 46.27 (2 CH), 47.06 (C_2), 50.58 (O*C*H$_3$), 55.65 (Ar–O*C*H$_3$), 71.68 ($C_{3'}$), 111.62 ($C_{7'}$), 117.38 (CN), 120.92 ($C_{5'}$), 121.41 ($C_{4a'}$), 123.76 ($C_{6'}$), 139.57 ($C_{8a'}$), 146.80 ($C_{8'}$), 162.15 (C_1), 162.86 ($C_{2'}$), 167.80 (*C*-NH$_2$(OCH$_3$)). IR (Nujol mull) ν 3413, 3315, 2255, 1677, 1640, 1609, 1592, 1521 cm^{-1}. Anal. Calcd for $C_{19}H_{21}N_3O_5$: C, 61.46; H, 5.66; N, 11.32. Found: C, 61.39; H, 5.61; N, 11.29.

2-(3-[Amino(methoxy)methylene]-8-methoxy-2-oxo-3,4-dihydro-2*H*-chromen-4-yl)-3-morpholin-4-yl-3-oxopropane-nitrile 3g. Yellow solid, 80% yield. Mp 186–188 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.46–3.49 (m, 2H, CH_2), 3.60–3.70 (m, 9H, 3 CH_2 + OCH$_3$), 3.83 (s, 3H, Ar-OCH$_3$), 4.24 (d, *J* = 3.9 Hz, 1H, $C_{4'}$–H), 4.59 (d, *J* = 3.9 Hz, 1H, C_2–H), 6.57–6.62 (m, 1H, $C_{5'}$–H), 7.02–7.05 (m, 2H, $C_{6'}$–H + $C_{7'}$–H), 7.84 (s, 2H, NH_2). ^{13}C NMR (75 MHz, DMSO-d_6) δ 36.11 ($C_{4'}$), 42.24 (CH), 45.83 (C_2), 45.98 (CH), 50.67 (O*C*H$_3$), 55.66 (Ar–O*C*H$_3$), 65.98 (2 CH), 71.49 ($C_{3'}$), 111.73 ($C_{7'}$), 117.73 (CN), 120.61 ($C_{5'}$), 121.04 ($C_{4a'}$), 123.88 ($C_{6'}$), 139.54 ($C_{8a'}$), 146.87 ($C_{8'}$), 162.77 ($C_{2'}$),* 162.85 (C_1),* 167.76 (*C*-NH$_2$(OCH$_3$)). *Assignments interchangeable. IR (Nujol mull) ν 3382, 3273, 2248, 1678, 1641, 1614, 1593, 1537 cm^{-1}. Anal. Calcd for $C_{19}H_{21}N_3O_6$: C, 58.92; H, 5.43; N, 10.85. Found: C, 58.89; H, 5.23; N, 10.99.

2-(3-[Amino(methoxy)methylene]-8-methoxy-2-oxo-3,4-dihydro-2*H*-chromen-4-yl)-3-[4-(2-hydroxyethyl)piperazin-1-yl]-3-oxopropanenitrile 3h. White solid, 42% yield. Mp 129–131 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.36–2.62 (m, 6H, 3 CH_2), 3.28–3.44 (m, 2H, CH_2-*CH*$_2$-OH + *CH*$_2$-CH_2OH), 3.46–3.60 (m, 3H, CH_2 + *CH*$_2$-CH_2OH), 3.66–3.78 (m, 1H, CH_2-*CH*$_2$-OH), 3.65 (s, 3H, OCH$_3$), 3.83 (s, 3H, Ar-OCH$_3$), 4.23 (d, *J* = 3.9 Hz, 1H, $C_{4'}$–H), 4.45 (t, *J* = 5.1 Hz, 1H, OH), 4.58 (d, *J* = 3.9 Hz, 1H, C_2–H), 6.58 (dd, *J* = 5.7 Hz, 3.3 Hz, 1H, $C_{5'}$–H), 7.02–7.04 (m, 2H, $C_{6'}$–H + $C_{7'}$–H), 7.83 (s, 2H, NH_2). ^{13}C NMR (75 MHz, DMSO-d_6) δ 36.17 ($C_{4'}$), 41.90 (*C*H$_2$-CH_2OH), 45.43

(CH_2-*C*H_2-OH), 45.98 (C_2), 50.67 (O*C*H_3), 52.72 (CH), 53.26 (CH), 55.66 (Ar–O*C*H_3), 58.50 (CH), 59.95 (CH), 71.53 ($C_{3'}$), 111.73 ($C_{7'}$), 117.45 (CN), 120.42 ($C_{5'}$), 121.09 ($C_{4a'}$), 123.88 ($C_{6'}$), 139.54 ($C_{8a'}$), 146.89 ($C_{8'}$), 162.54 ($C_{2'}$),* 162.76 (C_1),* 167.75 (*C*-NH_2(OCH_3)). *Assignments interchangeable. IR (Nujol mull) ν 3500–3200, 2255, 1681, 1664, 1614, 1592, 1534 cm^{-1}. Anal. Calcd for $C_{21}H_{26}N_4O_6 \cdot 0.6H_2O$: C, 57.17; H, 6.17; N, 12.70. Found: C, 57.02; H, 5.97; N, 12.87.

2-(3-[Amino(methoxy)methylene]-8-methoxy-2-oxo-3,4-dihydro-2*H*-chromen-4-yl)-3-[4-(3,4-dichlorophenyl)-piperazin-1-yl]-3-oxo-propanenitrile 3i. Yellow solid, 60% yield. Mp 164–166 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.25 (t, J = 5.2 Hz, 2H, CH_2), 3.30–3.40 (m, 2H, CH_2), 3.52–3.61 (m, 1H, CH_2), 3.62–3.65 (m, 1H, CH_2), 3.72–3.78 (m, 1H, CH_2), 3.83–3.91 (m, 1H, CH_2), 3.71 (s, 3H, OCH_3), 3.82 (s, 3H, Ar-OCH_3), 4.28 (d, J = 4.0 Hz, 1H, $C_{4'}$–H), 4.65 (d, J = 4.0 Hz, 1H, C_2–H), 6.58–6.62 (m, 1H, $C_{5'}$–H), 6.98–7.05 (m, 3H, $C_{6'}$–H + $C_{7'}$–H + Ar–H), 7.21 (d, J = 2.8 Hz, 1H, Ar–H), 7.41 (d, J = 8.8 Hz, 1H, Ar–H), 7.85 (s, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 36.18 ($C_{4'}$), 41.13 (CH), 44.83 (CH), 46.14 (C_2), 47.40 (CH), 47.78 (CH), 50.74 (O*C*H_3), 55.64 (Ar–O*C*H_3), 71.50 ($C_{3'}$), 111.74 ($C_{7'}$), 115.64 (Ar-CH), 116.69 (Ar-CH), 117.37 (CN), 120.04 (Ar-*C*-Cl), 120.61 ($C_{5'}$), 120.93 ($C_{4a'}$), 124.29 ($C_{6'}$), 130.57 (Ar-CH), 131.52 (Ar-*C*-Cl), 139.53 ($C_{8a'}$), 146.84 ($C_{8'}$), 150.32 (Ar-*C*), 162.74 ($C_{2'}$ + C_1), 167.77 (*C*-NH_2(OCH_3)). IR (Nujol mull) ν 3468, 3307, 2246, 1686, 1643, 1611, 1591, 1558, 1516 cm^{-1}. Anal. Calcd for $C_{25}H_{24}N_4O_5Cl_2$: C, 56.50; H, 4.52; N, 10.55. Found: C, 56.49; H, 4.51; N, 10.69.

2-(3-[Amino(methoxy)methylene]-8-methoxy-2-oxo-3,4-dihydro-2*H*-chromen-4-yl)-3-[4-(4-fluorophenyl)piperazin-1-yl]-3-oxo-propanenitrile 3j. White solid, 31% yield. Mp 161–163 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.08–3.20 (m, 4H, 2 CH_2), 3.54–3.61 (m, 1H, CH_2), 3.60–3.70 (m, 2H, CH_2), 3.85–3.92 (m, 1H, CH_2), 3.70 (s, 3H, OCH_3), 3.83 (s, 3H, Ar-OCH_3), 4.29 (d, J = 3.6 Hz, 1H, $C_{4'}$–H), 4.66 (d, J = 3.9 Hz, 1H, C_2–H), 6.58–6.63 (m, 1H, $C_{5'}$–H), 6.90–7.15 (m, 6H, $C_{6'}$–H + $C_{7'}$–H + 4 Ar–H), 7.85 (s, 2H, NH_2). ^{13}C NMR (75 MHz, DMSO-d_6) δ 36.21 ($C_{4'}$), 41.72 (CH), 45.23 (CH), 46.08 (C_2), 48.92 (CH), 49.41 (CH), 50.72 (O*C*H_3), 55.64 (Ar–O*C*H_3), 71.52 ($C_{3'}$), 111.75 ($C_{7'}$), 115.33 (2 Ar-CH), 117.42 (CN), 117.82 (2 Ar-CH), 120.55 ($C_{5'}$), 121.02 ($C_{4a'}$), 123.86 ($C_{6'}$), 139.54 ($C_{8a'}$), 146.87 ($C_{8'}$), 147.55 (Ar-*C*), 156.34 (Ar-*C*-F), 162.68 ($C_{2'}$),* 162.77 (C_1),* 167.48 (*C*-NH_2(OCH_3)). *Assignments interchangeable. IR (Nujol mull) ν 3475, 3318, 2249, 1684, 1650, 1614, 1591, 1510 cm^{-1}. Anal. Calcd for $C_{25}H_{25}N_4O_5F$: C, 62.50; H, 5.21; N, 11.67. Found: C, 62.46; H, 5.16; N, 11.65.

2-(3-[Amino(methoxy)methylene]-8-methoxy-2-oxo-3,4-dihydro-2*H*-chromen-4-yl)-3-oxo-3-(4-pyridin-2-ylpiperazin-1-yl)propanenitrile 3k. White solid, 37% yield. Mp 146–148 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.57–3.61 (m, 4H, 2 CH_2), 3.63–3.65 (m, 4H, 2 CH_2), 3.70 (s, 3H, OCH_3), 3.83 (s, 3H, Ar-OCH_3), 4.29 (d, J = 3.6 Hz, 1H, $C_{4'}$–H), 4.68 (d, J = 3.9 Hz, 1H, C_2–H), 6.58–6.63 (m, 1H, $C_{5'}$–H), 6.68 (t, J = 6.6 Hz, 1H, Ar–H), 6.91 (d, J = 8.4 Hz, 1H, Ar–H), 6.98–7.20 (m, 2H, $C_{6'}$–H + $C_{7'}$–H), 7.57 (t, J = 7.2 Hz, 1H, Ar–H), 7.86 (s, 2H, NH_2), 8.14 (d, J = 3.9 Hz, 1H, Ar–H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 36.17 ($C_{4'}$), 41.61 (CH), 44.21 (CH), 44.85 (CH), 44.94 (CH), 46.19 (C_2), 50.75 (O*C*H_3), 55.65 (Ar–O*C*H_3), 71.52 ($C_{3'}$), 107.48 (Ar-CH), 111.73 ($C_{7'}$), 113.46 (Ar-CH), 117.48 (CN), 120.64 ($C_{5'}$), 121.02 ($C_{4a'}$), 123.87 ($C_{6'}$), 137.67 (Ar-CH), 139.54 ($C_{8a'}$), 146.86 ($C_{8'}$), 147.57 (Ar-CH), 158.69 (Ar-*C*), 162.82 ($C_{2'}$ + C_1), 167.80 (*C*-NH_2(OCH_3)). IR (Nujol mull) ν 3402, 3292, 2248, 1681, 1645, 1614, 1594, 1567, 1527 cm^{-1}. Anal. Calcd for $C_{24}H_{25}N_5O_5 \cdot 0.2H_2O$: C, 61.72; H, 5.44; N, 15.00. Found: C, 61.72; H, 5.43; N, 15.05.

2-(3-[Amino(methoxy)methylene]-8-methoxy-2-oxo-3,4-dihydro-2*H*-chromen-4-yl)-3-oxo-3-(4-pyrimidin-2-ylpiperazin-1-yl) propanenitrile 3l. Yellow solid, 80% yield. Mp 180–182 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.57 (t, J = 5.2 Hz, 2H, CH_2), 3.70–3.81 (m, 4H, 2 CH_2), 3.84–3.88 (m, 2H, CH_2), 3.69 (s, 3H, OCH_3), 3.83 (s, 3H, Ar-OCH_3), 4.29 (d, J = 4.0 Hz, 1H, $C_{4'}$–H), 4.67 (d, J = 4.0 Hz, 1H, C_2–H), 6.60–6.63 (m, 1H, $C_{5'}$–H), 6.68 (t, J = 4.8 Hz, 1H, Ar–H), 7.02–7.04 (m, 2H, $C_{6'}$–H + $C_{7'}$–H), 7.86 (s, 2H, NH_2), 8.40 (d, J = 5.2 Hz, 2H, 2 Ar–H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 36.14 ($C_{4'}$), 41.67 (CH), 42.87 (CH), 43.43 (CH), 44.97 (CH), 46.11 (C_2), 50.67 (O*C*H_3), 55.65 (Ar–O*C*H_3), 71.52 ($C_{3'}$), 110.60 (Ar-CH), 111.75 ($C_{7'}$), 117.41 (CN), 120.66 ($C_{5'}$), 121.05 ($C_{4a'}$), 123.84 ($C_{6'}$), 139.54 ($C_{8a'}$), 146.84 ($C_{8'}$), 158.00 (2 Ar-CH), 161.08 (Ar-*C*), 162.81 (C_1), 162.91 ($C_{2'}$), 167.75 (*C*-NH_2(OCH_3)). IR (Nujol mull) ν 3357, 3271, 2247, 1689, 1673, 1633, 1612, 1589, 1552, 1538, 1505 cm^{-1}. Anal. Calcd for $C_{23}H_{24}N_6O_5$: C, 59.48; H, 5.17; N, 18.10. Found: C, 59.43; H, 5.07; N, 18.27.

General Procedure for the synthesis of compounds 5a–d

2-Cyanoacetamide **1a** (0.10 g, 1.19 mmol) was added to a suspension of the appropriate 2-oxo-2*H*-chromene-3-carbonitrile **2** (1.09 mmol) in methanol (4 mL) and triethylamine (0.7 mL) and the reaction mixture was refluxed for 1–2 h. For **5c** and **5d**, the suspension was cooled to room temperature and kept in an ice bath for a few minutes. The solid was filtered and washed with water. For **5a** and **5b**, the solvent was removed in the rotary evaporator and a mixture of ethanol (2 mL), water (4 mL) and acetic acid (1 mL) was added to the oil. The reaction mixture was stirred at room temperature for 3–5 h, when the solid was filtered and washed with water.

4-Amino-7-methoxy-2,5-dioxo-3,5-dihydro-2*H*-chromeno-[3,4-*c*]pyridine-1-carbonitrile 5a. Green solid, 26% yield. Mp higher than 300 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.94 (s, 3H, OCH_3), 7.42–7.50 (m, 2H, C_8–H + C_9–H), 8.73 (dd, J = 7.6 Hz, 2.0 Hz, 1H, C_{10}–H), 10.26 (s, 1H, NH_2), 10.37 (s, 1H, NH_2), 11.61 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 56.31 (O*C*H_3), 80.85 (C_1),[a] 87.44 (C_{4a}),[a] 116.19 (C_8), 116.44 (C_{10}), 116.66 (C_{10a}), 118.66 (CN), 125.45 (C_9), 139.30 (C_{6a}), 146.10 (C_{10b}), 147.57 (C_7), 163.24 (C_2),[b] 163.85 (C_5).[b a,b]Assignments interchangeable. IR (Nujol mull) ν 3565, 3396, 3274, 2214, 1687, 1642, 1536, 1504 cm^{-1}. Anal. Calcd for $C_{14}H_9N_3O_4 \cdot 0.8H_2O$: C, 56.49; H, 3.56; N, 14.12. Found: C, 56.21; H, 3.66; N, 13.91.

4-Amino-7-hydroxy-2,5-dioxo-3,5-dihydro-2*H*-chromeno-[3,4-*c*]pyridine-1-carbonitrile 5b. Green solid, 36% yield. Mp higher than 300 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.26 (dd, J = 8.0 Hz, 1.6 Hz, 1H, C_8–H), 7.31 (t, J = 8.0 Hz, 1H, C_9–H), 8.46 (dd, J = 8.2 Hz, 1.2 Hz, 1H, C_{10}–H), 10.36 (brs, 3H, OH + NH_2), 11.58 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 80.67 (C_1),[a] 87.42 (C_{4a}),[a] 115.27 (C_{10}), 116.98 (C_{10a}), 118.76 (C_8), 119.83 (CN), 125.39 (C_8), 138.67 (C_{6a}), 145.96 (C_7), 146.43 (C_{10b}), 163.31 (C_2),[b] 163.92 (C_5).[b a,b]Assignments interchangeable. IR (Nujol mull) ν

3345, 3260, 3144, 2217, 1681, 1600, 1522, 1509 cm^{-1}. Anal. Calcd for $C_{13}H_7N_3O_4 \cdot 0.4H_2O$: C, 56.48; H, 2.82; N, 15.21. Found: C, 56.55; H, 2.92; N, 14.98.

4-Amino-9-chloro-2,5-dioxo-3,5-dihydro-2*H*-chromeno[3,4-*c*]pyridine-1-carbonitrile 5c. Yellow solid, 32% yield. Mp higher than 300 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.50 (d, J = 9.0 Hz, 1H, C_7–H), 7.81 (dd, J = 8.8 Hz, 2.4 Hz, 1H, C_8–H), 8.98 (d, J = 2.4 Hz, 1H, C_{10}–H), 10.25 (s, 1H, NH_2), 10.37 (s, 1H, NH_2), 11.64 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 80.84 (C_1),[a] 87.46 (C_{4a}),[a] 117.40 (C_{10a}), 118.42 (CN), 119.61 (C_7), 124.92 (C_{10}), 129.58 (C_9), 133.81 (C_8), 144.56 (C_{10b}), 148.18 (C_{6a}), 162.95 (C_2),[b] 163.58 (C_5).[b a,b]Assignments interchangeable. IR (Nujol mull) ν 3353, 3160, 2204, 1683, 1615, 1598, 1520 cm^{-1}. Anal. Calcd for $C_{13}H_6N_3O_3Cl$: C, 54.26; H, 2.09; N, 14.61. Found: C, 54.52; H, 2.33; N, 14.40.

4-Amino-9-bromo-2,5-dioxo-3,5-dihydro-2*H*-chromeno-[3,4-*c*]pyridine-1-carbonitrile 5d. Yellow solid, 35% yield. Mp higher than 300 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.45 (d, J = 8.8 Hz, 1H, C_7–H), 7.94 (dd, J = 8.8 Hz, 2.0 Hz, 1H, C_8–H), 9.16 (d, J = 2.0 Hz, 1H, C_{10}–H), 10.24 (s, 1H, NH_2), 10.38 (s, 1H, NH_2), 11.66 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 80.83 (C_1),[a] 87.52 (C_{4a}),[a] 117.45 (C_{10a}), 117.88 (C_9), 118.46 (CN), 119.85 (C_7), 127.94 (C_{10}), 136.63 (C_8), 144.55 (C_{10b}), 148.63 (C_{6a}), 163.00 (C_2),[b] 163.60 (C_5).[b a,b]Assignments interchangeable. IR (Nujol mull) ν 3323, 3145, 2204, 1693, 1611, 1599, 1579, 1504 cm^{-1}. Anal. Calcd for $C_{13}H_6N_3O_3Br \cdot 0.2H_2O$: C, 46.48; H, 1.91; N, 12.52. Found: C, 46.55; H, 1.97; N, 12.77.

Synthesis of the ammonium salt of 4-(2-hydroxy-3-methoxy-phenyl)-2,6-dioxo-1,2,3,6-tetrahydropyridine-3,5-dicarbonitrile 9

2-Cyanoacetamide **1a** (0.06 g, 0.68 mmol) was added to a suspension of 2-oxo-8-methoxy-2*H*-chromene-3-carbonitrile **2a** (0.13 g, 0.67 mmol) in methanol (3 mL) and triethylamine (0.7 mL) and the reaction mixture was stirred at room temperature. After 5 h a white solid started to precipitate from solution and after 2 days the solid was filtered and washed with methanol and identified by ^{1}H NMR as a 1 : 1 : 1 mixture of compounds **3a**, **5a**, and **9**. The mother liquor was kept at 0° C for 4 days and a white solid precipitated and was filtered and washed with methanol. The solid was identified by ^{1}H NMR as the pure compound **3a** (22% yield). The solid mixture containing **3a**, **5a** and **9** (in a 1 : 1 : 1 ratio) was refluxed in methanol (3 mL) and triethylamine (0.4 mL) for 2.5 h. The isolated solid was identified as a 1 : 1 mixture of **5a** and **9**. This mixture was stirred at room temperature for 35 min in a combination of ethanol (2 mL), water (4 mL) and acetic acid (1 mL). Compound **5a** precipitated from this reaction mixture and was isolated in 8.5% yield. Compound **9** (5.4%) was also isolated as a white solid from the mother liquor after partial evaporation of the solvent. Mp higher than 300 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.05–1.20 (m, 9H, 3 CH_3), 3.00–3.20 (m, 6H, 3 CH_2), 3.82 (s, 3H, OCH_3), 6.63 (d, J = 7.2 Hz, 1H, $C_{6'}$–H), 6.79 (t, J = 8.1 Hz, 1H, $C_{5'}$–H), 6.95 (d, J = 7.5 Hz, 1H, $C_{4'}$–H), 8.40–9.20 (brs, 1H, NH^+-(CH_2CH_3)$_3$), 8.79 (s, 1H, OH), 10.42 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ 8.65 (3 CH_3), 45.76 (3 CH_2), 55.64 (OCH_3), 82.58 (C_3 + C_5), 111.78 ($C_{4'}$), 118.64 ($C_{5'}$), 1118.75 (2 CN), 120.51 ($C_{6'}$), 124.24 ($C_{1'}$), 142.82 ($C_{2'}$), 147.45 ($C_{3'}$), 160.05 (C_4), 163.50 (C_2 + C_6). IR (Nujol mull) ν 3427, 3113, 2208, 1625, 1516 cm^{-1}. Anal. Calcd for $C_{20}H_{24}N_4O_4 \cdot 0.3H_2O$: C, 61.63; H, 6.32; N, 14.38. Found: C, 61.55; H, 6.12; N, 14.33.

Radioligand binding assays

The inhibition percentage of the compounds was assayed at the concentration of 10 μM at all adenosine receptors following the conditions stated above. Competition binding curves at all receptors were carried out by assaying 6 different concentrations (range from 10 nM to 100 μM) for all the compounds showing an inhibition percentage above 70%. The –log of the inhibition constant (pK_i) of each compound was calculated by the Cheng–Prusoff equation, $K_i = IC_{50}/(1 + [L]/K_D)$, where IC_{50} is the concentration of compound that displaces the binding of the radioligand by 50%, $[L]$ is the free radioligand concentration and K_D is the dissociation constant of each radioligand. IC_{50} values were obtained by non-linear regression fitting the data, using Prism 2.1 software (GraphPad, San Diego, CA).

Human A_1 receptors

Adenosine A_1 receptor competition binding experiments were carried out in membranes from CHO-A_1 cells (Euroscreen, Gosselies, Belgium). On the day of the assay, membranes were defrosted and re-suspended in incubation buffer 20 mM Hepes, 100 mM NaCl, 10 mM $MgCl_2$, 2 UI ml^{-1} adenosine deaminase (pH = 7.4). Each reaction well of a GF/C multiscreen plate (Millipore, Madrid, Spain), prepared in duplicate, contained 15 μg of protein, 2 nM [^{3}H]DPCPX and the test compound. Non-specific binding was determined in the presence of 10 μM (*R*)-PIA. The reaction mixture was incubated at 25 °C for 60 min, after which the samples were filtered and measured in a microplate beta scintillation counter (Microbeta Trilux, Perkin Elmer, Madrid, Spain).

Human A_{2A} receptors

Adenosine A_{2A} receptor competition binding experiments were carried out in membranes from HeLa-A_{2A} cells. On the day of the assay, membranes were defrosted and re-suspended in incubation buffer 50 mM Tris-HCl, 1 mM EDTA, 10 mM $MgCl_2$ and 2 UI mL^{-1} adenosine deaminase (pH = 7.4). Each reaction well of a GF/C multiscreen plate (Millipore, Madrid, Spain), prepared in duplicate, contained 10 μg of protein, 3 nM [^{3}H]ZM241385 and the test compound. Non-specific binding was determined in the presence of 50 μM NECA. The reaction mixture was incubated at 25 °C for 30 min, after which samples were filtered and measured in a microplate beta scintillation counter (Microbeta Trilux, Perkin Elmer, Madrid, Spain).

Human A_{2B} receptors

Adenosine A_{2B} receptor competition binding experiments were carried out in membranes from HEK-293-A_{2B} cells (Euroscreen, Gosselies, Belgium) prepared following the provider's protocol. On the day of the assay, membranes were defrosted and re-suspended in incubation buffer 50 mM Tris-HCl, 1 mM EDTA, 10 mM $MgCl_2$, 0.1 mM benzamidine, 10 μg mL^{-1} bacitracine and 2 UI mL^{-1} adenosine deaminase (pH = 6.5). Each reaction well prepared in duplicate, contained 18 μg of protein, 35 nM

[^{3}H]DPCPX and the test compound. Non-specific binding was determined in the presence of 400 μM NECA. The reaction mixture was incubated at 25 °C for 30 min, after which samples were filtered through a multiscreen GF/C microplate and measured in a microplate beta scintillation counter (Microbeta Trilux, Perkin Elmer, Madrid, Spain).

Human A_3 receptors

Adenosine A_3 receptor competition binding experiments were carried out in membranes from HeLa-A_3 cells. On the day of the assay, membranes were defrosted and re-suspended in incubation buffer 50 mM Tris-HCl, 1 mM EDTA, 5 mM $MgCl_2$ and 2 UI mL^{-1} adenosine deaminase (pH = 7.4). Each reaction well of a GF/B multiscreen plate (Millipore, Madrid, Spain), prepared in triplicate, contained 90 μg of protein, 30 nM [^{3}H]NECA and the test compound. Non-specific binding was determined in the presence of 100 μM (*R*)-PIA. The reaction mixture was incubated at 25 °C for 180 min, after which samples were filtered and measured in a microplate beta scintillation counter (Microbeta Trilux, Perkin Elmer, Madrid, Spain).

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