Chroman-4-one derivatives targeting pteridine reductase 1 and showing anti-parasitic activity

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Characterization of compounds 1-3

6-hydroxy-2-(3-hydroxyphenyl)chroman-4-one (1) was isolated as a yellow solid in a 26% yield. Mp 240 °C. ^{1}H NMR (DMSO, 400 MHz) δ ppm: 9.51 (br s, 1H, 3′-OH), 9.42 (br s, 1H, 6-OH), 7.20 (dd, 1H, $J_{5',4'}$ = 8.2 Hz, $J_{5',6'}$ = 7.4 Hz, H-5′), 7.12 (d, 1H, $J_{5,7}$ = 2.9 Hz, H-5), 7.04 (dd, 1H, $J_{7,8}$ = 8.8 Hz, $J_{7,5}$ = 2.9 Hz, H-7), 6.95 (d, 1H, $J_{8,7}$ = 8.8 Hz, H-8), 6.91 (m, 2H, H-6′ + H-2′), 6.76 (d, 1H, $J_{4',5'}$ = 8.2 Hz, H-4′), 5.47 (dd, 1H, $J_{2,3b}$ = 12.7 Hz, $J_{2,3a}$ = 2.6 Hz, H-2), 3.10 (dd, 1H, $J_{3b,3a}$ = 16.8 Hz, $J_{3b,2}$ = 12.7 Hz, Hb-3), 2.76 (dd, 1H, $J_{3a,3b}$ = 16.8 Hz, $J_{3a,2}$ = 2.6 Hz, Ha-3). ^{13}C NMR (DMSO, 100 MHz) δ ppm: 192.17, 157.90, 154.81, 152.03, 141.11, 129.99, 124.98, 121.33, 119.46, 117.43, 115.69, 113.79, 110.39, 79.08, 44.22. ESI-HRMS calcd for $C_{15}H_{13}O_4$ $[M+H]^+$257.0808, found 257.0808.

6-hydroxy-2-(4-hydroxyphenyl)chroman-4-one (2) was isolated as a yellow solid in a 31% yield. Mp 230 °C. ^{1}H NMR (CD_3OD, 400 MHz) δ ppm: 7.33 (d, 2H, $J_{2',3'/5',6'}$ = 8.6 Hz, H-2′ + H-6′), 7.22 (d, 1H, $J_{5,7}$ = 3.0 Hz, H-5), 7.04 (dd, 1H, $J_{7,8}$ = 8.9 Hz, $J_{7,5}$ = 3.0 Hz, H-7), 6.90 (d, 1H, $J_{8,7}$ = 8.9 Hz, H-8), 6.84 (d, 2H, $J_{3',2'/5',6'}$ = 8.6 Hz, H-3′ + H-5′), 5.34 (dd, 1H, $J_{2,3b}$ = 13.3 Hz, $J_{2,3a}$ = 2.8 Hz, H-2), 3.08 (dd, 1H, $J_{3b,3a}$ = 17.0 Hz, $J_{3b,2}$ = 13.3 Hz, Hb-3), 2.74 (dd, 1H, $J_{3a,3b}$ = 17.0 Hz, $J_{3a,2}$ = 2.8 Hz, Ha-3). ^{13}C NMR (CD_3OD, 100 MHz) δ ppm: 193.46, 157.50, 155.63, 151.51, 130.09, 127.59 (2C), 124.55, 120.77, 118.76, 114.90 (2C), 109.95, 79.45, 44.00. ESI-HRMS calcd for $C_{15}H_{13}O_4$ $[M+H]^+$257.0808, found 257.0805.

2-(3,4-dihydroxyphenyl)-6-hydroxychroman-4-one (3) was isolated as an orange solid in a 26% yield. Mp 220 °C. ^{1}H NMR (DMSO, 400 MHz) δ ppm: 9.35 (br s, 1H, OH), 8.90 (br s, 2H, OH), 7.11 (d, 1H, $J_{5,7}$ = 3.0 Hz, H-5), 7.02 (dd, 1H, $J_{7,8}$ = 8.8 Hz, $J_{7,5}$ = 3.0 Hz, H-7), 6.91 (d, 1H, $J_{8,7}$ = 8.8 Hz, H-8), 6.90 (m, 1H, H-2′), 6.75 (m, 2H, H-5′ + H-6′), 5.35 (dd, 1H, $J_{2,3b}$ = 12.8 Hz, $J_{2,3a}$ = 2.6 Hz, H-2), 3.10 (dd, 1H, $J_{3b,3a}$ = 16.8 Hz, $J_{3b,2}$ = 12.8 Hz, Hb-3), 2.68 (dd, 1H, $J_{3a,3b}$ = 16.8 Hz, $J_{3a,2}$ = 2.6 Hz, Ha-3). ^{13}C NMR (DMSO, 100 MHz) δ ppm: 192.52, 154.99, 151.90, 146.01, 145.62, 130.49, 124.93, 121.26, 119.42, 118.27, 115.77, 114.74, 110.36, 79.22, 44.13. ESI-HRMS calcd for $C_{15}H_{13}O_5$ $[M+H]^+$273.0757, found 273.0759.

Table S1. Inhibitory activity of compounds against*Tb*PTR1 and *Lm*PTR1.

The control compound was pyrimetamine (100% of inhibition at 50 μMagainst both enzymes).

The SD values of IC_{50}s agreed to ± 10%.

Comp.	% Inhibition *Lm*PTR1 (50 μM)	SD % Inhibition *Lm*PTR1	IC_{50} *Lm*PTR1 (μM)	% Inhibition *Tb*PTR1 (50 μM)	STD % Inhibition *Tb*PTR1	IC_{50} *Tb*PTR1 (μM)
1	50.4	1.6	57.0	81.3	0.6	31.0
2	56.0	3.4	35.0	17.7	1.6	133.0
3	69.0	0.3	36.0	34.6	1.9	82.0
1A	86.2	0.4	12.5	96.1	0.1	4.3
2A	10.8	1.3	-	2.6	1.5	-
3A	75.4	0.6	35.0	53.1	1.0	38.0

Table S2. Data collection and processing statistics. Values for the outer shell are given in parentheses.

	***Lm*PTR1+NADP$^+$**		***Tb*PTR1+NADP$^+$**
	Compound 1	**Compound 3**	**Compound 1**
PDB code	**5L4N**	**5L42**	**5K6A**
Diffraction source	DLS-I04	XRD-1	XRD-1
Wavelength (Å)	0.9795	1.0000	1.0000
Temperature (K)	100	100	100
Detector	Pilatus 6M-F	Pilatus 2M	Pilatus 2M
Rotation range per image (°)	0.2	1	1
Exposure time per image (s)	0.2	35	15
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_1$
No. of subunit in ASU	4	4	4
a, *b*, *c* (Å) β (°), for monoclinic cell	94.70, 104.25, 137.04	94.70, 104.19, 137.04	74.72, 89.89, 82.68 115.75
Resolution range (Å)	94.70-2.35 (2.48-2.35)	47.35-2.10 (2.21-2.10)	41.74-1.70 (1.79-1.70)
Total No. of reflections	186708 (26441)	328980 (47347)	225595 (31568)
No. of unique reflections	54151 (7950)	79156 (11386)	100585 (14665)
Completeness (%)	95.3 (97.1)	99.5 (99.5)	93.0 (93.3)
Redundancy	3.4 (3.3)	4.2(4.2)	2.2 (2.2)
$\langle I/\sigma(I)\rangle$	7.5 (2.7)	5.6 (2.9)	6.8 (1.9)
R_{rmeas}‡	15.3 (51.6)	15.3 (33.1)	11.2 (42.4)
Overall *B* factor from Wilson plot (Å^2)	17.8	13.6	7.1

Table S3. Structure solution and refinement. Values for the outer shell are given in parentheses.

	LmPTR1+NADP$^+$		**TbPTR1+NADP$^+$**
	Compound 1	**Compound 3**	**Compound 1**
PDB code	**5L4N**	**5L42**	**5K6A**
Resolution range (Å)	82.97-2.35 (2.41-2.35)	47.35-2.10 (2.16-2.10)	41.74-1.70 (1.74-1.70)
Completeness (%)	94.61 (96.86)	99.22 (99.36)	92.66 (91.37)
No. of reflections, working set	51393 (3869)	75089 (5450)	95660 (6902)
No. of reflections, test set	2714 (168)	3968 (289)	4859 (365)
Final R_{cryst}	16.6 (22.4)	16.7 (18.3)	18.5 (30.9)
Final R_{free}	21.8 (26.9)	21.4 (21.7)	22.7 (32.5)
Cruickshank DPI	0.28	0.17	0.12
No. of non-H atoms			
Protein	7739	7828	7336
Ligand	266	354	276
Water	563	843	775
Total	8568	9025	8387
R.m.s. deviations			
Bonds (Å)	0.017	0.019	0.017
Angles (°)	1.878	1.955	1.897
Average *B* factors (Å^2)	24.15	22.84	19.86
Estimate error on coordinates based on R value (Å)	0.28	0.17	0.12
Ramachandran plot			
Most favored (%)	95.0	95.0	97.0
Allowed (%)	5.0	5.0	3.0

Table S4. ADME-Tox data.

* A549 and W1-38 cell growth: 100% = not cytotoxic, 0% = cytostatic. ** Mitochondrial toxicity:100% = mitotoxic, 0% = not mitotoxic.

NI = no inhibition

- Not measured

The SD values of IC_{50}s agreed to ± 10%.

Comp.	% Cell growth A549* (10 μM)	SD % Cell growth A549	GIC_{50} A549 (μM)	% Cell growth W1-38* (10 μM)	SD % Cell growth W1-38	GIC_{50} W1-38 (μM)
1	137	7	> 100	108	29	84
2	105	9	> 100	91	11	> 100
3	124	4	93	86	6	41

Comp.	% Mitochondrial toxicity **	SD % Mitochondrial toxicity (10 μM)	IC_{50} Mitochondria (μM)	% Inhibition Aurora B kinase (10 μM)	SD % Inhibition Aurora B kinase
1	6	4	> 100	NI	-
2	NI	-	> 100	NI	-
3	NI	-	> 100	NI	-

Comp.	% Inhibition hERG (10 μM)	SD % Inhibition hERG	IC_{50} hERG (μM)	% Inhibition CYP1A2 (10 μM)	SD % Inhibition CYP1A2	IC_{50} CYP1A2 (μM)
1	7	2	> 100	3	6	29
2	14	8	> 100	NI	-	59
3	20	8	64	NI	-	62

Comp.	% Inhibition CYP2C9 (10 μM)	SD % Inhibition CYP2C9	IC_{50} CYP2C9 (μM)	% Inhibition CYP2C19 (10 μM)	SD % Inhibition CYP2C19	IC_{50} CYP2C19 (μM)
1	NI	-	72	77.15	3.06	1
2	NI	-	> 100	44.54	5.51	4
3	NI	-	4	2.68	11.85	5

Comp.	% Inhibition CYP2D6 (10 μM)	SD % Inhibition CYP2D6	IC_{50} CYP2D6 (μM)	% Inhibition CYP3A4 (10 μM)	SD % Inhibition CYP3A4	IC_{50} CYP3A4 (μM)
1	NI	-	85	NI	-	80
2	NI	-	94	NI	-	> 100
3	NI	-	> 100	NI	-	65

Table S5. Anti-parasitic activity of compounds **1-3** against *T. brucei* at 10 μM and *L. infantum* at 50 μM, EC_{50} towards *T. brucei* and NOAEL. The reference compounds were pentamidine (IC_{50} = 1.55 ± 0.24 nM) for *T. brucei* and miltefosine (IC_{50} = 2.65 ± 0.40 μM) for *L. infantum*. The SD values of IC_{50}s agreed to ± 10%. ND: Not Determined

Comp.	% inh. *L. infantum* (50 μM)	% inh. *T. brucei* (10 μM)	EC_{50} *T. brucei*	CC_{50} ± SD or NOAEL
1	31	44	12.58 ± 1.69	>100
2	29	49	13.02 ± 1.82	>100
3	3	14	34.82 ± 1.10	>100