Toxoflavins and deazaflavins as the first reported selective small molecule inhibitors of tyrosyl-DNA phosphodiesterase II

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Supporting Information

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LC–MS methods and solvent gradients

LC–MS analyses were performed on a Waters Acquity UPLC system fitted with BEH C18 1.7 uM columns (2.1 × 50 mm) and with a UV detector. Positive and negative mass ion detection was performed using a Waters SQD detector. Analyses were performed with either buffered acidic or basic solvents and gradients as detailed below:

Low pH:

Solvent A – Water + 10mM ammonium formate + 0.1% formic acid

Solvent B – Acetonitrile + 5% water + 0.1% formic acid

High pH:

Solvent A – Water + 10mM ammonium hydrogen carbonate + 0.1% ammonia solution

Solvent B – Acetonitrile + 0.1% ammonia solution

Gradient:

Time	Flow rate ($mL\ min^{-1}$)	% Solvent A	% Solvent B
0	0.6	95	5
1.2	0.6	5	95
1.7	0.6	5	95
1.8	0.6	95	5

Preparative HPLC instrument and solvent gradients

Several compounds were purified by preparative HPLC on a Waters FractionLynx MS autopurification system, with a Waters XBridge 5 DM C18, 100 mm × 19 mm i.d. column, running at a flow rate of 20 mL min^{-1} with UV diode array detection (210–400nm) and mass-directed collection using both positive and negative mass ion detection. Purifications were performed using acidic or basic solvent systems as appropriate. Compound retention times on the system were routinely assessed using a 30–50 uL test injection and a standard gradient, and then purified using an appropriately selected focussed gradient as detailed below, based upon observed retention time.

Low pH:

Solvent A – Water + 10mM ammonium formate + 0.1% formic acid

Solvent B – Acetonitrile + 5% water +0.1% formic acid

High pH:

Solvent A – Water + 10mM ammonium formate + 0.1% ammonia solution

Solvent B – Acetonitrile + 5% water + 0.1% ammonia solution

Standard Gradient:

Time	Flow rate (mL min^{-1})	% Solvent A	% Solvent B
0	20	90	10
0.3	20	90	10
8.5	20	2	98
12	20	2	98
12.5	0	2	98

Focussed Gradients:

		% Solvent B				
		Retention time on standard gradient (min.)				
Time	Flow rate (mL min^{-1})	0–5.2	4.9–6.6	6.3–7.5	7.3–9.5	9.3–12
0	20	10	10	10	10	10
0.25	20	10	10	10	10	10
0.35	20	10	20	35	45	60
10	20	45	55	65	75	98
12	20	98	98	98	98	98
12.5	0	98	98	98	98	98

Preparative methods and spectroscopic data for intermediates 2–24, 34–36, 45–49, 72, 75, 76, 79, 84, 85, 89, 90–92, 107–127

6-[[(E)-(4-Chlorophenyl)methyleneamino]-methyl-amino]-3-methyl-1H-pyrimidine-2,4-dione 2. Adapting the procedure from the literature,[Error! Bookmark not defined.] a suspension of 6-chloro-3-methyluracil **1** (308 mg, 1.92 mmol) in EtOH (5 mL) was treated with methylhydrazine (0.22 mL, 4.22 mmol) and heated to 100 °C for 10 min under microwave irradiation. The clear solution was treated whilst still warm with 4-chlorobenzaldehyde (404 mg, 2.87 mmol). This was heated for a further 10 min at 50 °C under microwave irradiation. The resultant thick yellow precipitate was diluted with EtOH (2 mL) and filtered. The solid was washed with EtOH (2 × 5 ml) and dried to afford **2** (white solid, 480 mg, 85%).

3-Methyl-1H-pyrimidine-2,4-diones 3–23 were prepared in a manner analogous to that of **2**. Table 1 details a list of yields and LC–MS data (high pH).

6-[Ethyl-[(E)-(4-ethylphenyl)methyleneamino]amino]-3-methyl-1H-pyrimidine-2,4-dione 24. A mixture of 6-chloro-3-methyluracil (2.0 g, 12.5 mmol), ethylhydrazine ethanedioate (1:1) (2.8 g, 18.7 mmol) and N,N-diisopropylethylamine (4.8 g, 37.4 mmol) in EtOH (20 mL) was stirred and heated at reflux for 16 h. The reaction mixture was allowed to cool to ambient temperature, concentrated and triturated with EtOH to give a white solid (1.8 g, 78%). A portion of this (300 mg, 1.63 mmol) and *p*-ethylbenzaldehyde (328 mg, 2.44 mmol) in EtOH (5 mL) was heated at 100 °C under microwave irradiation for 10 min. The reaction mixture was concentrated to afford a white paste. The crude material was purified by trituration (EtOH) and filtered to yield **24** (white solid, 270 mg, 55%); LC–MS *m/z* 301.5 $[M + H]^+$, 74% purity.

6-[Cyclopropylmethyl-[(E)-(4-ethylphenyl)methyleneamino]amino]-3-methyl-1H-pyrimidine-2,4-dione 35. A solution of cyclopropylmethylhydrazine dihydrochloride (1.5 g,

9.34 mmol), Et_3N (3.9 mL, 28.0 mmol) and 6-chloro-3-methyluracil **1** (1.5 g, 9.34 mmol) in EtOH (30 mL) was agitated at reflux for 16 h. *p*-Ethylbenzaldehyde (1.3 mL, 9.34 mmol) was added sub-surface to the mixture at reflux, and the resulting mixture was stirred at reflux for a further 16 h, after which a precipitate had formed. The mixture was cooled to ambient temperature and filtered, and the filter cake was washed with EtOH (2 × 20 mL) and dried to afford **35** (pale yellow solid, 1.2 g, 39%). LC–MS *m/z* 327.6 $[M + H]^+$, 100% purity.

6-[[(E)-(4-Ethylphenyl)methyleneamino]-propyl-amino]-3-methyl-1H-pyrimidine-2,4-dione 34. The title compound was prepared in a manner analogous to that of **35**, using propylhydrazine oxalate; (pale yellow solid, 750 mg, 27%). LC–MS 1.10 min, *m/z* 315.6 $[M + H]^+$, 100% purity.

6-(N-[(E)-(4-Ethylphenyl)methyleneamino]anilino)-3-methyl-1H-pyrimidine-2,4-dione 36. The title compound was prepared in a manner analogous to that of **35** (orange oil, 504 mg, 22%). The product was unstable and therefore used in the subsequent step without further purification; LC–MS *m/z* 347.4 $[M - H]^+$, <20% purity.

3-Benzyl-6-chloro-1H-pyrimidine-2,4-dione 46 and **6-chloro-3-phenyl-1H-pyrimidine-2,4-dione 45** were prepared following literature conditions.[Error! Bookmark not defined.]

3-Benzyl-6-[[(E)-(4-ethylphenyl)methyleneamino]-methyl-amino]-1H-pyrimidine-2,4-dione 49. Adapting the procedure from the literature,[Error! Bookmark not defined.] a mixture of **46** (150 mg, 0.63 mmol) and methylhydrazine (64 mg, 1.39 mmol) in EtOH (3 mL) was heated under microwave irradiation at 100 °C for 10 min. 4-Ethylbenzaldehyde (136 mg, 1.01 mmol) was added to the clear solution, and the resulting mixture was heated under microwave irradiation at 50 °C for a further 10 min. The resulting precipitate was collected by filtration, washed with EtOH (2 × 5 mL) and dried to afford **49** (off-white solid, 180 mg, 78%); LC–MS *m/z* 363.6 $[M + H]^+$, 100% purity.

6-[[(E)-(4-Ethylphenyl)methyleneamino]-methyl-amino]-3-phenyl-1H-pyrimidine-2,4-dione 48. The title compound was prepared in a manner analogous to that of **49**; (white solid, 185 mg, 69%); LC–MS *m/z* 349.6 $[M + H]^+$, 100% purity.

6-[[(E)-(4-Ethylphenyl)methyleneamino]-methyl-amino]-1H-pyrimidine-2,4-dione 47. The title compound was prepared in a manner analogous to that of **49**; (white solid, 240 mg, 86%); LC–MS *m/z* 273.5 $[M + H]^+$, 100% purity.

6-amino-5-[(E)-(4-ethylphenyl)methyleneamino]-1,3-dimethyl-pyrimidine-2,4-dione 72. Compound was prepared according to the literature,[3] (yellow solid, 1.1 g, 63% yield); LC–MS *m/z* 285.6 $[M + H]^+$, 98% purity.

2-Amino-6-[amino(methyl)amino]-1H-pyrimidin-4-one 75.[4] A suspension of 2-amino-6-chloro-1H-pyrimidin-4-one **74** (6.0 g, 41.2 mmol) in EtOH (120 mL) was treated with methylhydrazine (5.8 mL, 110 mmol), and the mixture was heated to reflux for 4 h and then cooled to ambient temperature (stood overnight). The solid was collected by filtration, washed with EtOH (2 × 30 mL) and dried to afford **75** (off-white solid, 5.1g, 80%); ^{1}H NMR (DMSO-d_6): δ 9.71 (br. s, 1H), 6.17 (br. s, 2H), 4.99 (s, 1H), 4.45 (br. s, 2H), 3.11 (s, 3H).

2-Amino-6-[methyl-[(E)-(4-methyl-2-thienyl)methyleneamino]amino]pyrimidin-4-ol 76. A suspension of **75** (300 mg, 1.93 mmol) in EtOH (5 mL) was treated with 4-methylthiophene-2-carbaldehyde (0.24 mL, 1.93 mmol) and heated to 50 °C for 10 min under microwave irradiation. After cooling to ambient temperature, the solid was collected by filtration, washed with EtOH (2 × 5 mL) and dried to afford **76 (**yellow solid, 384 mg, 75%); LC–MS *m/z* 264.5 $[M + H]^+$, 100% purity.

5-Benzyloxy-1-methyl-6-oxo-pyrimidine-4-carboxylic acid 79. The title compound was prepared according to the patent procedure.[5] ^{1}H NMR (DMSO-d_6): δ 13.6 (br. s, 1H), 8.32 (s,

1H), 7.30–7.47 (m, 5H), 5.13 (s, 2H), 3.47 (s, 3H); LC–MS no mass ion observed, 94% purity.

1-[4-(3,4-dimethoxyphenyl)thiophene-2-carbonyl]piperidine-3-carbonyl chloride 85. Lithium hydroxide (39.2 mg, 1.64 mmol) was added to a solution of ethyl 1-[4-(3,4-dimethoxyphenyl)thiophene-2-carbonyl]piperidine-3-carboxylate **84** (300 mg, 0.74 mmol) in THF (5 mL) and water (5 mL), and the resultant solution was allowed to stir at ambient temperature for 2 h. The reaction mixture was concentrated under reduced pressure to remove the THF before diluting with water (10 mL) and washing with EtOAc (5 mL). The aqueous layer was acidified with 1N HCl (aq) (5 mL) and extracted in EtOAc (10 mL), dried and concentrated to afford the intermediate carboxylic acid ((yellow oil, 278 mg, 100% yield); (LC–MS *m/z* 376.6 $[M + H]^+$, 89% purity)), which was treated with oxalyl chloride (0.14 mL, 1.64 mmol) in DCM (5 mL) under an atmosphere of nitrogen followed by a drop of DMF, and the resultant solution was allowed to stir at ambient temperature for 16 h. This mixture was concentrated to furnish product **85** (cream oil, 443 mg, 96% yield), which was used in the subsequent step without further purification; LC–MS inconclusive.

Ethyl 1-[4-(3,4-dimethoxyphenyl)thiophene-2-carbonyl]piperidine-3-carboxylate 84. 1,1'-Carbonyldiimidazole (279.2 mg, 1.72 mmol) was added to a solution of the commercially available 4-(3,4-dimethoxyphenyl)thiophene-2-carboxylic acid **83** (350 mg, 1.32 mmol) in DMF (10 mL) under an atmosphere of nitrogen, and the reaction mixture was allowed to stir at ambient temperature for 2 h. Ethyl piperidine-3-carboxylate (270.6 mg, 1.72 mmol) was added, and the mixture was stirred for 16 h before heating at 100 °C for 16 h. The product mixture was cooled to ambient temperature, diluted with EtOAc (20 mL), washed with water (3 × 10 mL) followed by brine (10 mL). The organic layer was dried and concentrated to afford product **84** (red oil, 532 mg, 100% yield); LC-MS *m/z* 404.5 $[M + H]^+$, 87% purity. The product was used in the subsequent step without further purification.

3-[4-(3,4-dimethoxyphenyl)-2-thienyl]benzoic acid 91. Lithium hydroxide (23.5 mg, 0.98 mmol) was added to a suspension of methyl 3-[4-(3,4-dimethoxyphenyl)-2-thienyl]benzoate (290 mg, 0.82 mmol) in methanol (4 mL) and water (4 mL). The reaction was heated to reflux and stirred for 5 h before cooling to ambient temperature, then was diluted with EtOAc (5 mL) and extracted into saturated sodium carbonate (10 mL). The aqueous layer was acidified to pH 1 with 2N HCl (aq) (10 mL) and extracted into EtOAc (3 × 10 mL). The combined organic layers were washed with brine (10 mL), dried and concentrated to furnish **91** (white solid, 203.1 mg, 69%). The product was used in the subsequent step without further purification; LC–MS *m/z* 341.5 $[M + H]^+$, 100% purity.

Methyl 3-[4-(3,4-dimethoxyphenyl)-2-thienyl]benzoate 89. Prepared according to the literature[6] (yellow solid, 340 mg, 22% yield); LC–MS *m/z* 355.5 $[M + H]^+$, 96% purity.

4-[4-(3,4-dimethoxyphenyl)-2-thienyl]benzoic acid 92. The title compound was prepared in a manner analogous to that of **91** (white solid, 93.2 mg, 77%). The product was used in the subsequent step without further purification; LC–MS *m/z* 341.5 $[M + H]^+$, 100% purity.

Methyl 4-[4-(3,4-dimethoxyphenyl)-2-thienyl]benzoate 90. Prepared according to the literature[6] (white solid, 90 mg, 19% yield); LC–MS *m/z* 355.5 $[M + H]^+$, 97% purity.

Intermediates **72**, **84**, **85** and **91**: Table 2 details a list of yields and LC–MS data (high pH).

6-Chlorouracil 47.[7] 2,4,6-Trichloropyrimidine **105** (15.7 mL, 136 mmol) was added to a solution of sodium hydroxide (27.3 g, 682 mmol) and water (20 mL). The reaction mixture was heated at reflux for 2 h. The solution was cooled, and the pH was adjusted to approximately pH 2 using concentrated HCl (50 mL) while cooling in an ice bath. The resulting precipitate was isolated by filtration, washed with hot water and dried to give the product **47** as a white powder (19.5 g, 133 mmol, 98%). LC–MS *m/z* 147.5 $[M + H]^+$, 100% purity.

6-(Cyclopropylamino)-1H-pyrimidine-2,4-dione 109. The procedure was adapted from the literature.[7] A suspension of 6-chlorouracil **1** (500 mg, 3.41 mmol) and cyclopropylamine (0.28 mL, 4.09 mmol) in 1-propanol (5 mL) was heated by microwave irradiation at 150 °C for 30 min. *iso*-Hexane (5 mL) was added to the reaction mixture, and the resulting precipitate was obtained by filtration and dried under vacuum to yield the product **109** as a beige solid (258.4 mg, 45%). The product was used in the next step without further purification.

6-(Amino)-1H-pyrimidine-2,4-diones 107-127 were prepared in a manner analogous to that of **109**. Table 3 details a list of yields and LC–MS data (high pH).

Summary of purity data for intermediates 2–23, **72**, **84**, **85**, **91** and **107–127**

Table 1. Yields and LC–MS data (high pH) for **3-methyl-1H-pyrimidine-2,4-diones 2**–**23**.

Number	Name	Yield (%)	Purity (%)	Mass ion	Adduct	Retention time (min)
2[Error! Bookmark not defined.]	**6-[[(E)-(4-Chlorophenyl)methyleneamino]-methyl-amino]-3-methyl-1H-pyrimidine-2,4-dione**	85	100	293.4	$[M + H]^+$	0.89
3[8]	**6-[[(E)-Benzylideneamino]-methyl-amino]-3-methyl-1H-pyrimidine-2,4-dione**	84	100	259.5	$[M + H]^+$	0.78
4	**6-[[(E)-Butylideneamino]-methyl-amino]-3-methyl-1H-pyrimidine-2,4-dione**	92	100	225.5	$[M + H]^+$	0.73
5	**6-[[(E)-(4-Ethylphenyl)methyleneamino]-methyl-amino]-3-methyl-1H-pyrimidine-2,4-dione**	81	100	287.5	$[M + H]^+$	0.98
6[Error! Bookmark not defined.]	**3-Methyl-6-[methyl-[(E)-p-tolylmethyleneamino]amino]-1H-pyrimidine-2,4-dione**	100	100	273.5	$[M + H]^+$	0.91
7	**3-Methyl-6-[methyl-[(E)-[4-(trifluoromethoxy)phenyl]methyleneamino]amino]-1H-pyrimidine-2,4-dione**	75	100	343.5	$[M + H]^+$	0.98
8	**3-Methyl-6-[methyl-[(E)-[4-(trifluoromethyl)phenyl]methyleneamino]amino]-1H-pyrimidine-2,4-dione**	79	100	327.5	$[M + H]^+$	0.89
9[9]	**6-[[(E)-(3,4-Dimethoxyphenyl)methyleneamino]-methyl-amino]-3-methyl-1H-pyrimidine-2,4-dione**	62	100	319.5	$[M + H]^+$	0.71

Number	Name	Yield (%)	Purity (%)	Mass ion	Adduct	Retention time (min)
10	**3-Methyl-6-[methyl-[(E)-o-tolylmethyleneamino]amino]-1H-pyrimidine-2,4-dione**	78	100	273.5	$[M + H]^+$	0.83
11	**3-Methyl-6-[methyl-[(E)-m-tolylmethyleneamino]amino]-1H-pyrimidine-2,4-dione**	77	100	273.5	$[M + H]^+$	0.86
12[4]	**3-Methyl-6-[methyl-[(E)-phenethylideneamino]amino]-1H-pyrimidine-2,4-dione**	50	98	273.5	$[M + H]^+$	0.83
13 Error! Bookmark not defined.	**6-[[(E)-Cyclohexylmethyleneamino]-methyl-amino]-3-methyl-1H-pyrimidine-2,4-dione**	46	97	265.6	$[M + H]^+$	0.97
14 Error! Bookmark not defined.	**3-Methyl-6-[methyl-[(E)-3-phenylpropylideneamino]amino]-1H-pyrimidine-2,4-dione**	65	100	287.5	$[M + H]^+$	0.92
15	**3-Methyl-6-[methyl-[(E)-thiazol-4-ylmethyleneamino]amino]-1H-pyrimidine-2,4-dione**	81	94	266.4	$[M + H]^+$	0.56
16	**6-[[(E)-(4-Bromo-2-thienyl)methyleneamino]-methyl-amino]-3-methyl-1H-pyrimidine-2,4-dione**	89	93	343.4, 345.4	$[M + H]^+$	0.83
17	**3-Methyl-6-[methyl-[(E)-2-naphthylmethyleneamino]amino]-1H-pyrimidine-2,4-dione**	92	100	309.6	$[M + H]^+$	0.94
18	**3-Methyl-6-[methyl-[(E)-(4-methyl-2-thienyl)methyleneamino]amino]-1H-pyrimidine-2,4-dione**	83	100	279.5	$[M + H]^+$	0.80
19	**3-Methyl-6-[methyl-[(E)-(4-phenyl-2-thienyl)methyleneamino]amino]-1H-pyrimidine-2,4-dione**	60	100	341.6	$[M + H]^+$	0.96

Number	Name	Yield (%)	Purity (%)	Mass ion	Adduct	Retention time (min)
20	**3-Methyl-6-[methyl-[(E)-tetrahydropyran-4-ylmethyleneamino]amino]-1H-pyrimidine-2,4-dione**	61	100	267.6	$[M + H]^+$	0.57
21	**6-[[(E)-Cyclopentylmethyleneamino]-methyl-amino]-3-methyl-1H-pyrimidine-2,4-dione**	43	64	249.6	$[M - H]^-$	0.92
22 Error! Bookmark not defined.	Not isolated, taken through to next stage	-	93	221.5	$[M - H]^-$	0.65
23	**6-[[(E)-[4-(3,4-Dimethoxyphenyl)-2-thienyl]methyleneamino]-methyl-amino]-3-methyl-1H-pyrimidine-2,4-dione**	32	100	401.5	$[M + H]^+$	0.88

Table 2. Yields and LC–MS data (high pH) for intermediates **72**, **84**, **85** and **91**.

Number	Name	Yield (%)	Purity (%)	Mass ion	Adduct	Retention time (min)
72	**6-amino-5-[(E)-(4-ethylphenyl)methyleneamino]-1,3-dimethyl-pyrimidine-2,4-dione**	63	98	285.6	$[M + H]^+$	1.11
84	**Ethyl 1-[4-(3,4-dimethoxyphenyl)thiophene-2-carbonyl]piperidine-3-carboxylate**	100	87	404.5	$[M + H]^+$	1.13
85	**1-[4-(3,4-dimethoxyphenyl)thiophene-2-carbonyl]piperidine-3-carbonyl chloride**	96	Inconclusive, used in subsequent step			
91	**3-[4-(3,4-dimethoxyphenyl)-2-thienyl]benzoic acid**	69	100	341.5	$[M + H]^+$	0.81

Table 3. Yields and LC–MS data (high pH) for **6-(Amino)-1H-pyrimidine-2,4-diones 107–127**.

Number	Name	Yield (%)	Purity (%)	Mass ion	Adduct	Retention time (min)
107[7]	**6-Anilino-1H-pyrimidine-2,4-dione**	90	93	202.5	$[M - H]^-$	0.48
108[10]	**6-(Methylamino)-1H-pyrimidine-2,4-dione**	96	100	142.5	$[M + H]^+$	0.27
109	**6-(Cyclopropylamino)-1H-pyrimidine-2,4-dione**	45	100	168.5	$[M + H]^+$	0.26
110[11]	**6-(Cyclohexylamino)-1H-pyrimidine-2,4-dione**	quant.	100	208.5	$[M - H]^-$	0.64
111	***t*-Butyl 4-[(2,4-dioxo-1H-pyrimidin-6-yl)amino]piperidine-1-carboxylate**	quant.	96	309.5	$[M - H]^-$	0.64
112[12]	**6-(4-Hydroxyanilino)-1H-pyrimidine-2,4-dione**	quant.	100	220.5	$[M + H]^+$	0.23
113[13]	**6-(4-Methoxyanilino)-1H-pyrimidine-2,4-dione**	71	100	234.5	$[M + H]^+$	0.24
114 Error! Bookmark not defined.	**6-(3-Hydroxyanilino)-1H-pyrimidine-2,4-dione**	25	100	220.5	$[M + H]^+$	0.25
115[14,15]	**6-(2-Hydroxyanilino)-1H-pyrimidine-2,4-dione**	88	100	220.5	$[M + H]^+$	0.24

Number	Name	Yield (%)	Purity (%)	Mass ion	Adduct	Retention time (min)
116[12,16]	**6-[3-(Hydroxymethyl)anilino]-1H-pyrimidine-2,4-dione**	78	Compound was not analyzed and was taken directly into the next step			
117	**6-(3-Methoxyanilino)-1H-pyrimidine-2,4-dione**	81	Compound was not analyzed and was taken directly into the next step			
118	**6-(3-Hydroxy-4-methoxy-anilino)-1H-pyrimidine-2,4-dione**	86	Compound was not analyzed and was taken directly into the next step			
119[7]	**6-(3-Fluoroanilino)-1H-pyrimidine-2,4-dione**	83	100	222.5	$[M + H]^+$	0.23
120[7]	**6-(4-Bromoanilino)-1H-pyrimidine-2,4-dione**	65	95	282.4, 284.5	$[M + H]^+$	0.55
121	***t*-Butyl N-[3-[(2,4-dioxo-1H-pyrimidin-6-yl)amino]phenyl]carbamate**	64	90	263.4	$[M - t\text{-Bu}]^+$	0.64
122	**N-[3-[(2,4-Dioxo-1H-pyrimidin-6-yl)amino]phenyl]acetamide**	83	93	261.5	$[M + H]^+$	0.41
123	**N-[3-[(2,4-Dioxo-1H-pyrimidin-6-yl)amino]phenyl]methanesulfonamide**	68	95	297.5	$[M + H]^+$	0.29
124	**3-[(2,4-Dioxo-1H-pyrimidin-6-yl)amino]benzenesulfonamide**	76	89	283.5	$[M + H]^+$	0.21
125	**6-(1H-Indazol-6-ylamino)-1H-pyrimidine-2,4-dione**	91	85	244.6	$[M + H]^+$	0.31
126	**6-(1H-Indazol-4-ylamino)-1H-pyrimidine-2,4-dione**	63	83	244.7	$[M + H]^+$	0.30

Number	Name	Yield (%)	Purity (%)	Mass ion	Adduct	Retention time (min)
127	**6-[3-(1H-Tetrazol-5-yl)anilino]-1H-pyrimidine-2,4-dione**	75	84	272.5	$[M + H]^+$	0.49

Summary of purity data for final compounds 24–31, 54–70, 73, 86, 93, 94, 97–104, 130, and 134–163.

Table 4. LC–MS data (high pH) for final compounds.

Number	Name	Purity (%)	Mass ion	Adduct	Retention time (min)
24	**3-(4-Chlorophenyl)-1,6-dimethyl-pyrimido[5,4-e][1,2,4]triazine-5,7-dione**	100	304.5	[M+H]+	0.97
25	**1,6-Dimethyl-3-phenyl-pyrimido[5,4-e][1,2,4]triazine-5,7-dione**	>95	270.5	[M+H]+	0.87
26	**3-(4-Ethylphenyl)-1,6-dimethyl-pyrimido[5,4-e][1,2,4]triazine-5,7-dione**	100	298.5	[M+H]+	1.04
27	**1,6-Dimethyl-3-[4-(trifluoromethyl)phenyl]pyrimido[5,4-e][1,2,4]triazine-5,7-dione**	100	338.5	[M+H]+	1.03
28	**1,6-Dimethyl-3-[4-(trifluoromethoxy)phenyl]pyrimido[5,4-e][1,2,4]triazine-5,7-dione**	100	354.5	[M+H]+	1.05
29	**1,6-Dimethyl-3-(p-tolyl)pyrimido[5,4-e][1,2,4]triazine-5,7-dione**	100	284.5	[M+H]+	0.96

Number	Name	Purity (%)	Mass ion	Adduct	Retention time (min)
30	**1,6-Dimethyl-3-(m-tolyl)pyrimido[5,4-e][1,2,4]triazine-5,7-dione**	100	284.5	[M+H]+	0.94
31	**1,6-Dimethyl-3-(o-tolyl)pyrimido[5,4-e][1,2,4]triazine-5,7-dione**	100	284.5	[M+H]+	0.91
54	**1,6-Dimethyl-3-propyl-pyrimido[5,4-e][1,2,4]triazine-5,7-dione**	>95	236.5	[M+H]+	0.74
55	**3-Cyclopropyl-1,6-dimethyl-pyrimido[5,4-e][1,2,4]triazine-5,7-dione**	>95	234.5	[M+H]+	0.64
56	**3-Cyclopentyl-1,6-dimethyl-pyrimido[5,4-e][1,2,4]triazine-5,7-dione**	>95	262.2	[M+H]+	0.85
57	**3-Cyclohexyl-1,6-dimethyl-pyrimido[5,4-e][1,2,4]triazine-5,7-dione**	>95	276.5	[M+H]+	0.96
58	**1,6-Dimethyl-3-tetrahydropyran-4-yl-pyrimido[5,4-e][1,2,4]triazine-5,7-dione**	>95	278.5	[M+H]+	0.59
59	**3-Benzyl-1,6-dimethyl-pyrimido[5,4-e][1,2,4]triazine-5,7-dione**	>95	284.5	[M+H]+	0.87
60	**1,6-Dimethyl-3-phenethyl-pyrimido[5,4-e][1,2,4]triazine-5,7-dione**	>95	298.5	[M+H]+	0.92
61	**1,6-Dimethyl-3-(2-naphthyl)pyrimido[5,4-e][1,2,4]triazine-5,7-dione**	>95	320.6	[M+H]+	1.02
62	**3-(3,4-Dimethoxyphenyl)-1,6-dimethyl-pyrimido[5,4-e][1,2,4]triazine-5,7-dione**	90–95	330.5	[M+H]+	0.79

Number	Name	Purity (%)	Mass ion	Adduct	Retention time (min)
63	**1,6-Dimethyl-3-thiazol-4-yl-pyrimido[5,4-e][1,2,4]triazine-5,7-dione**	>95	277.5	[M+H]+	0.58
64	**1,6-Dimethyl-3-(4-methyl-2-thienyl)pyrimido[5,4-e][1,2,4]triazine-5,7-dione**	>95	290.5	[M+H]+	0.89
65	**3-(4-Bromo-2-thienyl)-1,6-dimethyl-pyrimido[5,4-e][1,2,4]triazine-5,7-dione**	>95	356.4	[M+H]+	0.94
66	**1,6-Dimethyl-3-(4-phenyl-2-thienyl)pyrimido[5,4-e][1,2,4]triazine-5,7-dione**	>95	352.5	[M+H]+	1.07
67	**3-[4-(3,4-Dimethoxyphenyl)-2-thienyl]-1,6-dimethyl-pyrimido[5,4-e][1,2,4]triazine-5,7-dione**	85–90	412.5	[M+H]+	0.97
70	**1-Ethyl-3-(4-ethylphenyl)-6-methyl-pyrimido[4,5-c]pyridazine-5,7-dione**	100	312.5	$[M + H]^+$	1.14–1.16
73	**6-(4-Ethylphenyl)-3,8-dimethyl-pteridine-2,4-dione**	100	297.6	$[M + H]^+$	1.17
86	**[4-(3,4-Dimethoxyphenyl)-2-thienyl]-(3-dimethoxyphosphorylcarbonyl-1-piperidyl)methanone**	97	468.5	$[M + H]^+$	0.70–0.72
93	**3-[4-(3,4-Dimethoxyphenyl)-2-thienyl]-N-methylsulfonyl-benzamide**	100	418.5	$[M + H]^+$	0.85
94	**4-[4-(3,4-dimethoxyphenyl)-2-thienyl]-N-methylsulfonyl-benzamide**	94	418.5	$[M + H]^+$	0.84
96	**N-[4-(3,4-dimethoxyphenyl)-2-thienyl]-3,5-dimethyl-isoxazole-4-carboxamide**	100	359.5	$[M + H]^+$	1.02

Number	Name	Purity (%)	Mass ion	Adduct	Retention time (min)
97	**N-[4-(3,4-dimethoxyphenyl)-2-thienyl]benzamide**	100	340.4	$[M + H]^+$	1.11
98	**N-[4-(3,4-dimethoxyphenyl)-2-thienyl]-1H-imidazole-2-carboxamide**	100	330.6	$[M + H]^+$	0.91
99	**N-[4-(3,4-dimethoxyphenyl)-2-thienyl]-1-methyl-imidazole-2-carboxamide**	100	344.6	$[M + H]^+$	1.02
100	**N-[4-(3,4-dimethoxyphenyl)-2-thienyl]-3-methyl-isoxazole-5-carboxamide**	92	345.5	$[M + H]^+$	0.97
101	**N-[4-(3,4-dimethoxyphenyl)-2-thienyl]-1-methyl-pyrazole-3-carboxamide**	100	344.5	$[M + H]^+$	0.94
102	**N-[4-(3,4-dimethoxyphenyl)-2-thienyl]-4-methyl-thiadiazole-5-carboxamide**	100	362.6	$[M + H]^+$	0.94
103	**N-[4-(3,4-dimethoxyphenyl)-2-thienyl]-2,4-dimethyl-6-oxo-pyran-3-carboxamide**	98	386.5	$[M + H]^+$	0.78
104	**1-[4-(3,4-dimethoxyphenyl)-2-thienyl]-3-phenyl-urea**	100	355.5	$[M + H]^+$	1.14
130	**10-Methylpyrimido[4,5-b]quinoline-2,4-dione**	>95	228.5	$[M + H]^+$	0.61
134	**9-Chloro-10-phenyl-pyrimido[4,5-b]quinoline-2,4-dione**	>95	324.5, 326.5	$[M + H]^+$	0.88
135	**7-Chloro-10-phenyl-pyrimido[4,5-b]quinoline-2,4-dione**	>95	324.5, 326.5	$[M + H]^+$	0.93

Number	Name	Purity (%)	Mass ion	Adduct	Retention time (min)
136	**6-Chloro-10-phenyl-pyrimido[4,5-b]quinoline-2,4-dione**	85–90	324.5, 326.5	$[M + H]^+$	0.87
137	**8-Chloro-10-methyl-pyrimido[4,5-b]quinoline-2,4-dione**	>95	262.5, 264.5	$[M + H]^+$	0.72
138	**8-Chloro-10-cyclopropyl-pyrimido[4,5-b]quinoline-2,4-dione**	>95	288.5, 290.5	$[M + H]^+$	0.77
139	**8-Chloro-10-cyclohexyl-pyrimido[4,5-b]quinoline-2,4-dione**	>95	248.4, 250.5	$[M - CyHx]^+$	1.02
140	**8-Chloro-10-(4-piperidyl)pyrimido[4,5-b]quinoline-2,4-dione**	90–95	331.5, 333.5	$[M + H]^+$	1.02
141	**8-Chloro-10-(4-hydroxyphenyl)pyrimido[4,5-b]quinoline-2,4-dione**	>95	340.2, 342.5	$[M + H]^+$	0.77
142	**8-Chloro-10-(4-methoxyphenyl)pyrimido[4,5-b]quinoline-2,4-dione**	>95	354.5, 356.5	$[M + H]^+$	0.94
143	**8-Chloro-10-(3-hydroxyphenyl)pyrimido[4,5-b]quinoline-2,4-dione**	>95	340.5, 342.4	$[M + H]^+$	0.78
144	**8-Chloro-10-(2-hydroxyphenyl)pyrimido[4,5-b]quinoline-2,4-dione**	90–95	340.5, 342.5	$[M + H]^+$	0.73
145	**10-Phenyl-8-(trifluoromethyl)pyrimido[4,5-b]quinoline-2,4-dione**	>95	356.5	$[M - H]^-$	1.00
146	**2,4-Dioxo-10-phenyl-pyrimido[4,5-b]quinoline-8-carbonitrile**	>95	315.5	$[M + H]^+$	0.77

Number	Name	Purity (%)	Mass ion	Adduct	Retention time (min)
147	**10-(3-Hydroxyphenyl)-2,4-dioxo-pyrimido[4,5-b]quinoline-8-carbonitrile**	>95	331.5	$[M + H]^+$	0.66
148	**10-(4-Hydroxyphenyl)-2,4-dioxo-pyrimido[4,5-b]quinoline-8-carbonitrile**	>95	331.5	$[M + H]^+$	0.65
149	**10-[3-(Hydroxymethyl)phenyl]-2,4-dioxo-pyrimido[4,5-b]quinoline-8-carbonitrile**	100	343.5	$[M - H]^-$	0.69
150	**10-(3-Methoxyphenyl)-2,4-dioxo-pyrimido[4,5-b]quinoline-8-carbonitrile**	90–95	343.5	$[M - H]^-$	0.85
151	**10-(4-Methoxyphenyl)-2,4-dioxo-pyrimido[4,5-b]quinoline-8-carbonitrile**	>95	345.5	$[M + H]^+$	0.81
152	**10-(3-Hydroxy-4-methoxy-phenyl)-2,4-dioxo-pyrimido[4,5-b]quinoline-8-carbonitrile**	>95	361.5	$[M + H]^+$	0.66
153	**10-(3-Fluorophenyl)-2,4-dioxo-pyrimido[4,5-b]quinoline-8-carbonitrile**	100	333.4	$[M + H]^+$	0.8
154	**10-(4-Bromophenyl)-2,4-dioxo-pyrimido[4,5-b]quinoline-8-carbonitrile**	90–95	393.4	$[M + H]^+$	0.87
155	**10-(3-Aminophenyl)-2,4-dioxo-pyrimido[4,5-b]quinoline-8-carbonitrile**	>95	330.5	$[M + H]^+$	0.69
156	**N-[3-(8-Cyano-2,4-dioxo-pyrimido[4,5-b]quinolin-10-yl)phenyl]acetamide**	100	370.5	$[M - H]^-$	0.68
157	**N-[3-(8-Cyano-2,4-dioxo-pyrimido[4,5-b]quinolin-10-yl)phenyl]methanesulfonamide**	100	408.5	$[M + H]^+$	0.55

Number	Name	Purity (%)	Mass ion	Adduct	Retention time (min)
158	**N-[3-(2,4-Dioxopyrimido[4,5-b]quinolin-10-yl)phenyl]methanesulfonamide**	90–95	383.5	$[M + H]^+$	0.66
159	**N-[3-(7-Chloro-2,4-dioxo-pyrimido[4,5-b]quinolin-10-yl)phenyl]methanesulfonamide**	90–95	417.5	$[M + H]^+$	0.77
160	**3-(8-Cyano-2,4-dioxo-pyrimido[4,5-b]quinolin-10-yl)benzenesulfonamide**	100	394.5	$[M + H]^+$	0.62
161	**10-(1H-Indazol-6-yl)-2,4-dioxo-pyrimido[4,5-b]quinoline-8-carbonitrile**	100	355.5	$[M + H]^+$	0.68
162	**10-(1H-Indazol-4-yl)-2,4-dioxo-pyrimido[4,5-b]quinoline-8-carbonitrile**	100	355.5	$[M + H]^+$	0.70
163	**2,4-Dioxo-10-[3-(1H-tetrazol-5-yl)phenyl]pyrimido[4,5-b]quinoline-8-carbonitrile**	90–95	381.5	$[M - H]^-$	0.58

Table 5. LC–MS data (low pH) for final compounds.

Number	Name	Purity (%)	Mass ion	Adduct	Retention time (min)
24	**3-(4-Chlorophenyl)-1,6-dimethyl-pyrimido[5,4-e][1,2,4]triazine-5,7-dione**	>95	304.5	[M+H]+	0.97
25	**1,6-Dimethyl-3-phenyl-pyrimido[5,4-e][1,2,4]triazine-5,7-dione**	>95	270.5	[M+H]+	0.91

Number	Name	Purity (%)	Mass ion	Adduct	Retention time (min)
26	**3-(4-Ethylphenyl)-1,6-dimethyl-pyrimido[5,4-e][1,2,4]triazine-5,7-dione**	100	298.5	[M+H]+	1.06
27	**1,6-Dimethyl-3-[4-(trifluoromethyl)phenyl]pyrimido[5,4-e][1,2,4]triazine-5,7-dione**	100	338.4	[M+H]+	1.07
28	**1,6-Dimethyl-3-[4-(trifluoromethoxy)phenyl]pyrimido[5,4-e][1,2,4]triazine-5,7-dione**	100	354.5	[M+H]+	1.06
29	**1,6-Dimethyl-3-(p-tolyl)pyrimido[5,4-e][1,2,4]triazine-5,7-dione**	100	284.4	[M+H]+	0.96
30	**1,6-Dimethyl-3-(m-tolyl)pyrimido[5,4-e][1,2,4]triazine-5,7-dione**	100	284.5	[M+H]+	0.98
31	**1,6-Dimethyl-3-(o-tolyl)pyrimido[5,4-e][1,2,4]triazine-5,7-dione**	>95	284.5	[M+H]+	0.95
54	**1,6-Dimethyl-3-propyl-pyrimido[5,4-e][1,2,4]triazine-5,7-dione**	>95	236.5	[M+H]+	0.71
55	**3-Cyclopropyl-1,6-dimethyl-pyrimido[5,4-e][1,2,4]triazine-5,7-dione**	90–95	234.5	[M+H]+	0.65
56	**3-Cyclopentyl-1,6-dimethyl-pyrimido[5,4-e][1,2,4]triazine-5,7-dione**	>95	262.2	[M+H]+	0.86
57	**3-Cyclohexyl-1,6-dimethyl-pyrimido[5,4-e][1,2,4]triazine-5,7-dione**	>95	276.5	[M+H]+	1.00
58	**1,6-Dimethyl-3-tetrahydropyran-4-yl-pyrimido[5,4-e][1,2,4]triazine-5,7-dione**	>95	278.5	[M+H]+	0.59

Number	Name	Purity (%)	Mass ion	Adduct	Retention time (min)
59	**3-Benzyl-1,6-dimethyl-pyrimido[5,4-e][1,2,4]triazine-5,7-dione**	>95	284.5	[M+H]+	0.88
60	**1,6-Dimethyl-3-phenethyl-pyrimido[5,4-e][1,2,4]triazine-5,7-dione**	>95	298.5	[M+H]+	0.94
61	**1,6-Dimethyl-3-(2-naphthyl)pyrimido[5,4-e][1,2,4]triazine-5,7-dione**	>95	320.6	[M+H]+	1.04
62	**3-(3,4-Dimethoxyphenyl)-1,6-dimethyl-pyrimido[5,4-e][1,2,4]triazine-5,7-dione**	90–95	330.5	[M+H]+	0.82
63	**1,6-Dimethyl-3-thiazol-4-yl-pyrimido[5,4-e][1,2,4]triazine-5,7-dione**	>95	277.5	[M+H]+	0.57
64	**1,6-Dimethyl-3-(4-methyl-2-thienyl)pyrimido[5,4-e][1,2,4]triazine-5,7-dione**	>95	290.5	[M+H]+	0.9
65	**3-(4-Bromo-2-thienyl)-1,6-dimethyl-pyrimido[5,4-e][1,2,4]triazine-5,7-dione**	>95	356.4	[M+H]+	0.96
66	**1,6-Dimethyl-3-(4-phenyl-2-thienyl)pyrimido[5,4-e][1,2,4]triazine-5,7-dione**	>95	352.5	[M+H]+	1.10
67	**3-[4-(3,4-Dimethoxyphenyl)-2-thienyl]-1,6-dimethyl-pyrimido[5,4-e][1,2,4]triazine-5,7-dione**	95	412.5	[M+H]+	0.99
70	**1-Ethyl-3-(4-ethylphenyl)-6-methyl-pyrimido[4,5-c]pyridazine-5,7-dione**	100	312.5	$[M + H]^+$	1.16, 1.21
73	**6-(4-Ethylphenyl)-3,8-dimethyl-pteridine-2,4-dione**	100	297.5	$[M + H]^+$	1.21

Number	Name	Purity (%)	Mass ion	Adduct	Retention time (min)
86	**[4-(3,4-Dimethoxyphenyl)-2-thienyl]-(3-dimethoxyphosphorylcarbonyl-1-piperidyl)methanone**	94	468.6	$[M + H]^+$	0.92-0.98
93	**3-[4-(3,4-Dimethoxyphenyl)-2-thienyl]-N-methylsulfonyl-benzamide**	100	418.5	$[M + H]^+$	1.15
94	**4-[4-(3,4-dimethoxyphenyl)-2-thienyl]-N-methylsulfonyl-benzamide**	91	418.5	$[M + H]^+$	1.14
96	**N-[4-(3,4-dimethoxyphenyl)-2-thienyl]-3,5-dimethyl-isoxazole-4-carboxamide**	100	359.9	$[M + H]^+$	1.11
97	**N-[4-(3,4-dimethoxyphenyl)-2-thienyl]benzamide**	100	340.5	$[M + H]^+$	1.19
98	**N-[4-(3,4-dimethoxyphenyl)-2-thienyl]-1H-imidazole-2-carboxamide**	100	330.6	$[M + H]^+$	0.99
99	**N-[4-(3,4-dimethoxyphenyl)-2-thienyl]-1-methyl-imidazole-2-carboxamide**	100	344.5	$[M + H]^+$	1.10
100	**N-[4-(3,4-dimethoxyphenyl)-2-thienyl]-3-methyl-isoxazole-5-carboxamide**	92	345.6	$[M + H]^+$	1.10
101	**N-[4-(3,4-dimethoxyphenyl)-2-thienyl]-1-methyl-pyrazole-3-carboxamide**	100	344.6	$[M + H]^+$	1.05
102	**N-[4-(3,4-dimethoxyphenyl)-2-thienyl]-4-methyl-thiadiazole-5-carboxamide**	100	360.4	$[M - H]^-$	1.14
103	**N-[4-(3,4-dimethoxyphenyl)-2-thienyl]-2,4-dimethyl-6-oxo-pyran-3-carboxamide**	100	386.5	$[M + H]^+$	1.14

Number	Name	Purity (%)	Mass ion	Adduct	Retention time (min)
104	**1-[4-(3,4-dimethoxyphenyl)-2-thienyl]-3-phenyl-urea**	100	353.5	$[M - H]^-$	1.16
130	**10-Methylpyrimido[4,5-b]quinoline-2,4-dione**	>95	228.5	$[M + H]^+$	0.62
134	**9-Chloro-10-phenyl-pyrimido[4,5-b]quinoline-2,4-dione**	>95	324.5, 326.5	$[M + H]^+$	0.93
135	**7-Chloro-10-phenyl-pyrimido[4,5-b]quinoline-2,4-dione**	>95	324.5, 326.5	$[M + H]^+$	0.95
136	**6-Chloro-10-phenyl-pyrimido[4,5-b]quinoline-2,4-dione**	>95	324.5, 326.5	$[M + H]^+$	0.92
137	**8-Chloro-10-methyl-pyrimido[4,5-b]quinoline-2,4-dione**	85–90	262.5, 264.5	$[M + H]^+$	0.74
138	**8-Chloro-10-cyclopropyl-pyrimido[4,5-b]quinoline-2,4-dione**	>95	288.5, 290.5	$[M + H]^+$	0.81
139	**8-Chloro-10-cyclohexyl-pyrimido[4,5-b]quinoline-2,4-dione**	>95	248.4, 250.4	$[M - CyHx]^+$	1.08
140	**8-Chloro-10-(4-piperidyl)pyrimido[4,5-b]quinoline-2,4-dione**	90–95	331.5, 333.5	$[M + H]^+$	0.62
141	**8-Chloro-10-(4-hydroxyphenyl)pyrimido[4,5-b]quinoline-2,4-dione**	>95	340.5, 342.5	$[M + H]^+$	0.84
142	**8-Chloro-10-(4-methoxyphenyl)pyrimido[4,5-b]quinoline-2,4-dione**	>95	354.5, 356.5	$[M + H]^+$	1.00

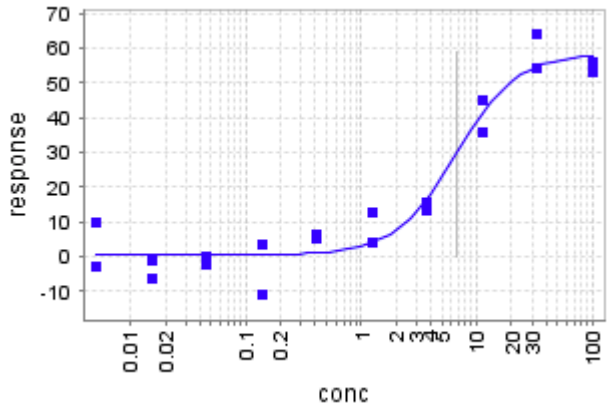
Number	Name	Purity (%)	Mass ion	Adduct	Retention time (min)
143	**8-Chloro-10-(3-hydroxyphenyl)pyrimido[4,5-b]quinoline-2,4-dione**	>95	340.5, 342.5	$[M + H]^+$	0.86
144	**8-Chloro-10-(2-hydroxyphenyl)pyrimido[4,5-b]quinoline-2,4-dione**	90–95	340.5, 342.5	$[M + H]^+$	0.88
145	**10-Phenyl-8-(trifluoromethyl)pyrimido[4,5-b]quinoline-2,4-dione**	>95	358.5	$[M + H]^+$	1.02
146	**2,4-Dioxo-10-phenyl-pyrimido[4,5-b]quinoline-8-carbonitrile**	>95	315.5	$[M + H]^+$	0.81
147	**10-(3-Hydroxyphenyl)-2,4-dioxo-pyrimido[4,5-b]quinoline-8-carbonitrile**	>95	331.5	$[M + H]^+$	0.74
148	**10-(4-Hydroxyphenyl)-2,4-dioxo-pyrimido[4,5-b]quinoline-8-carbonitrile**	>95	331.5	$[M + H]^+$	0.73
149	**10-[3-(Hydroxymethyl)phenyl]-2,4-dioxo-pyrimido[4,5-b]quinoline-8-carbonitrile**	100	343.5	$[M - H]^-$	0.68
150	**10-(3-Methoxyphenyl)-2,4-dioxo-pyrimido[4,5-b]quinoline-8-carbonitrile**	90–95	343.5	$[M - H]^-$	0.84
151	**10-(4-Methoxyphenyl)-2,4-dioxo-pyrimido[4,5-b]quinoline-8-carbonitrile**	90–95	345.5	$[M + H]^+$	0.85
152	**10-(3-Hydroxy-4-methoxy-phenyl)-2,4-dioxo-pyrimido[4,5-b]quinoline-8-carbonitrile**	>95	361.5	$[M + H]^+$	0.75
153	**10-(3-Fluorophenyl)-2,4-dioxo-pyrimido[4,5-b]quinoline-8-carbonitrile**	100	333.4	$[M + H]^+$	0.84

Number	Name	Purity (%)	Mass ion	Adduct	Retention time (min)
154	**10-(4-Bromophenyl)-2,4-dioxo-pyrimido[4,5-b]quinoline-8-carbonitrile**	>95	393.4, 395.4	$[M + H]^+$	0.94
155	**10-(3-Aminophenyl)-2,4-dioxo-pyrimido[4,5-b]quinoline-8-carbonitrile**	>95	330.5	$[M + H]^+$	0.73
156	**N-[3-(8-Cyano-2,4-dioxo-pyrimido[4,5-b]quinolin-10-yl)phenyl]acetamide**	100	370.5	$[M - H]^-$	0.71
157	**N-[3-(8-Cyano-2,4-dioxo-pyrimido[4,5-b]quinolin-10-yl)phenyl]methanesulfonamide**	>95	408.2	$[M + H]^+$	0.76
158	**N-[3-(2,4-Dioxopyrimido[4,5-b]quinolin-10-yl)phenyl]methanesulfonamide**	>95	383.5	$[M + H]^+$	0.74
159	**N-[3-(7-Chloro-2,4-dioxo-pyrimido[4,5-b]quinolin-10-yl)phenyl]methanesulfonamide**	>95	417.5	$[M + H]^+$	0.84
160	**3-(8-Cyano-2,4-dioxo-pyrimido[4,5-b]quinolin-10-yl)benzenesulfonamide**	>95	394.5	$[M + H]^+$	0.70
161	**10-(1H-Indazol-6-yl)-2,4-dioxo-pyrimido[4,5-b]quinoline-8-carbonitrile**	100	355.5	$[M + H]^+$	0.75
162	**10-(1H-Indazol-4-yl)-2,4-dioxo-pyrimido[4,5-b]quinoline-8-carbonitrile**	100	355.5	$[M + H]^+$	0.71
163	**2,4-Dioxo-10-[3-(1H-tetrazol-5-yl)phenyl]pyrimido[4,5-b]quinoline-8-carbonitrile**	>95	381.5	$[M - H]^-$	0.67

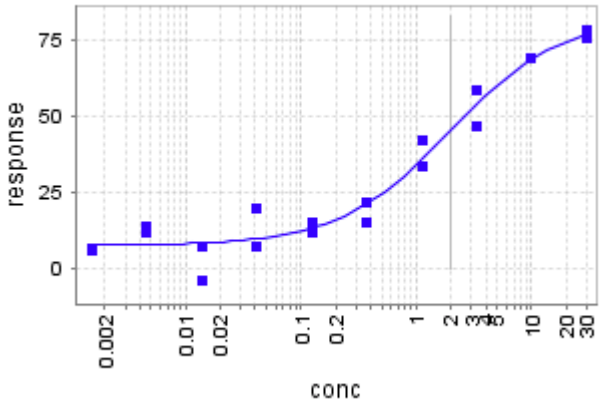
Dose response curves for active deazaflavins and select toxoflavins in the TDP2 chromogenic assay

Figure 1. Deazaflavins

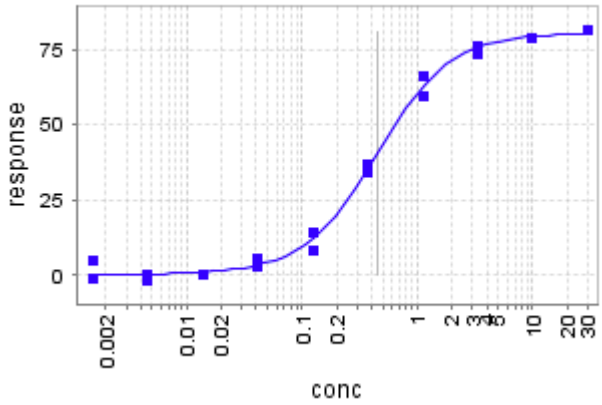
Compound **128** (EC_{50} 7.36 µM)



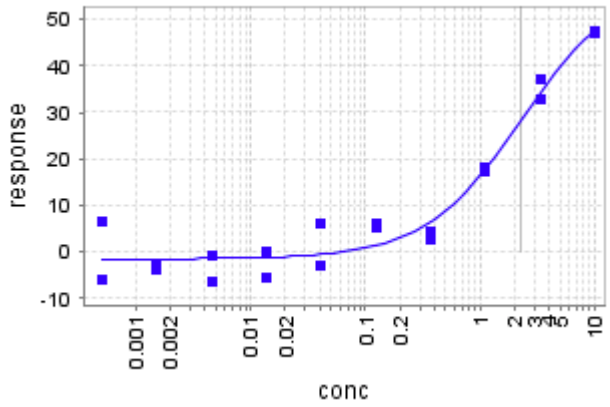
Compound **141** (EC_{50} 1.66 µM)



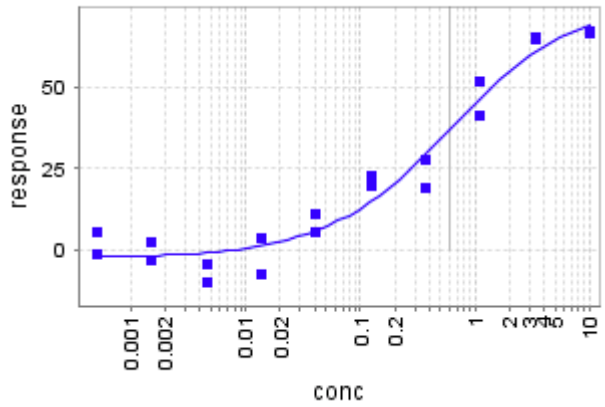
Compound **143** (EC_{50} 0.48 µM)



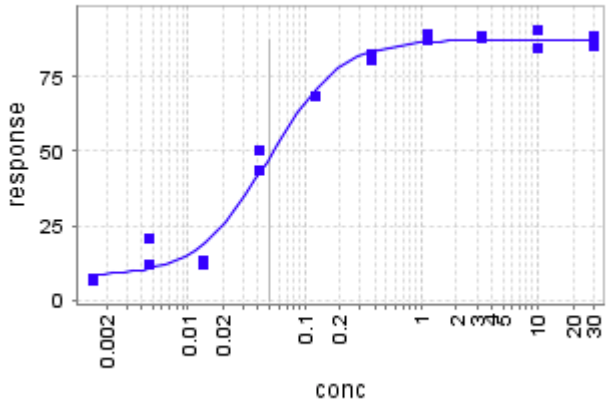
Compound **145** (EC_{50} 2.87 µM)



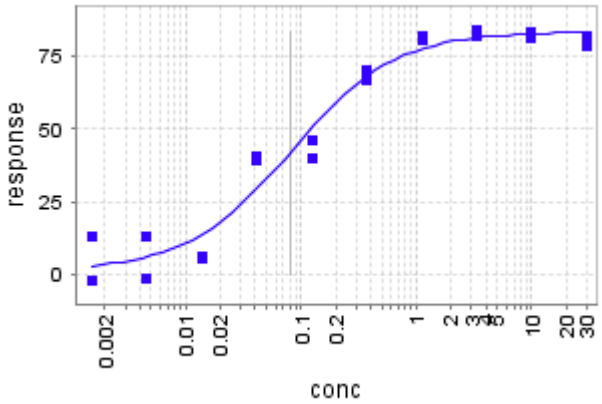
Compound **146** (EC_{50} 0.50 µM)



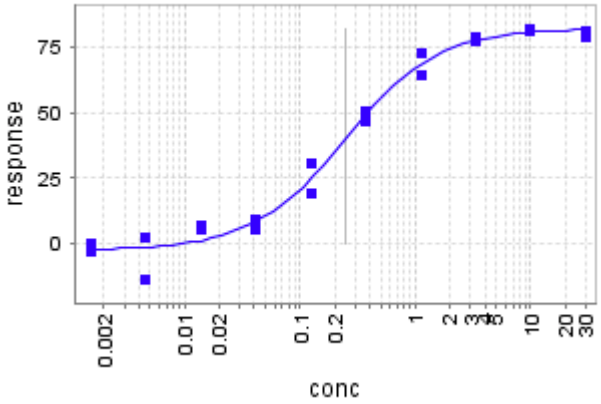
Compound **147** (EC_{50} 0.05 µM)



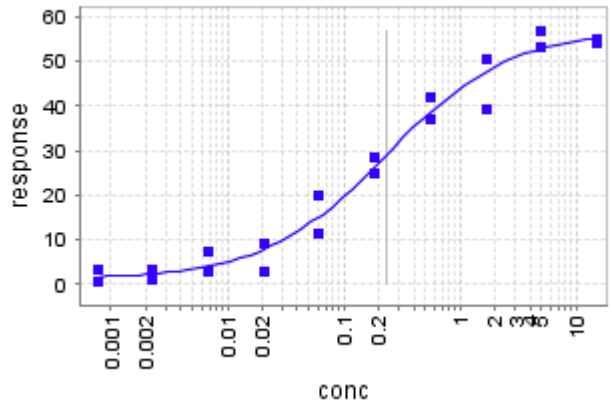
Compound **148** (EC_{50} 0.09 µM)



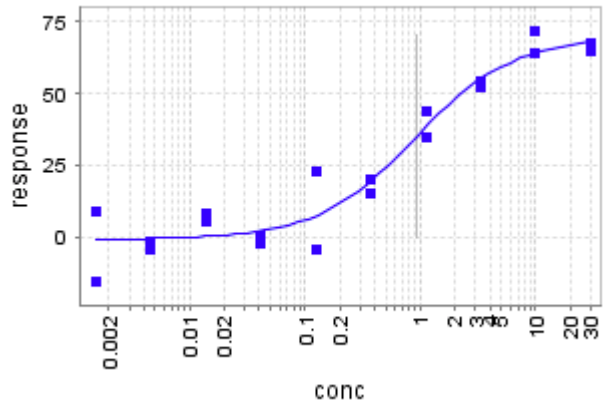
Compound **149** (EC_{50} 0.25 µM)



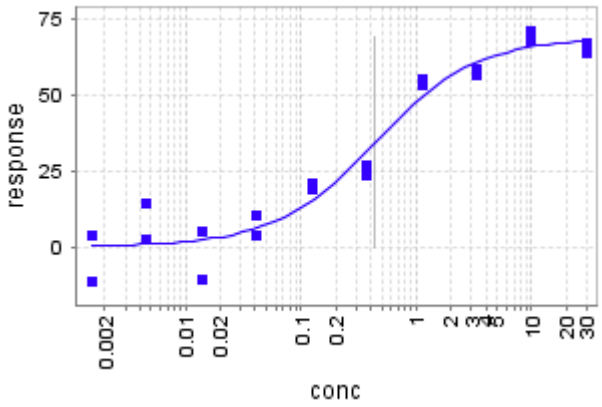
Compound **150** (EC_{50} 0.47 µM)



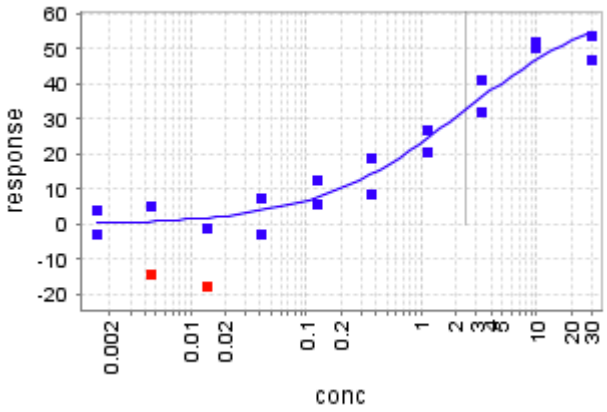
Compound **151** (EC_{50} 1.04 µM)



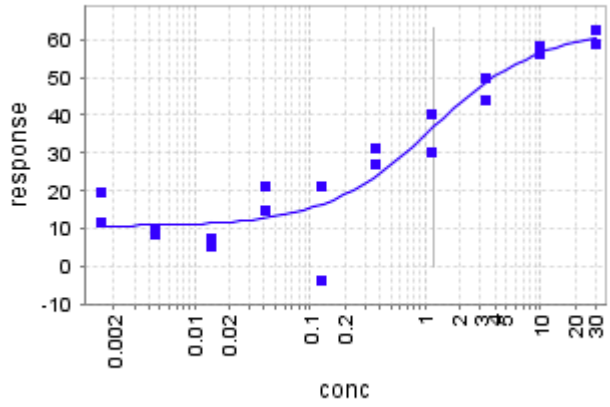
Compound **152** (EC_{50} 0.32 µM)



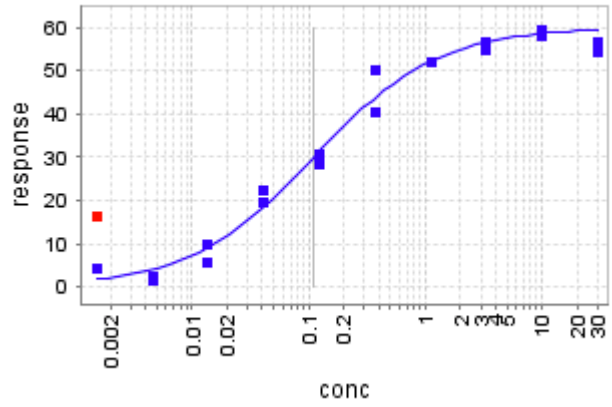
Compound **153** (EC_{50} 3.39 µM)



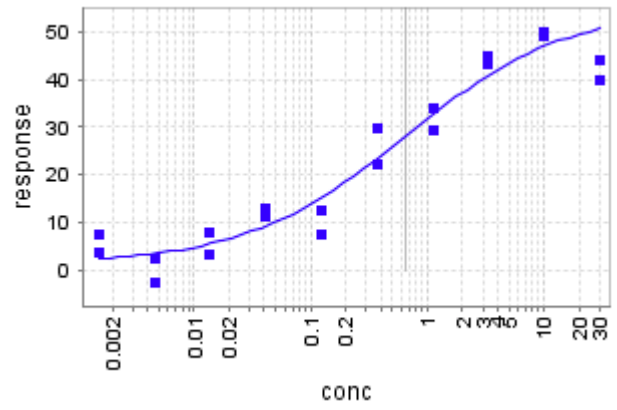
Compound **154** (EC_{50} 2.74 µM)



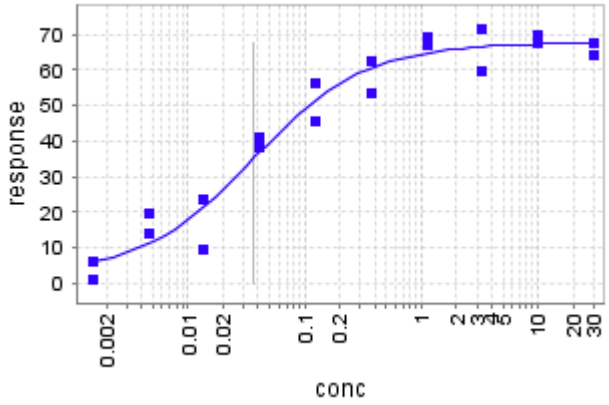
Compound **155** (EC_{50} 0.09 µM)



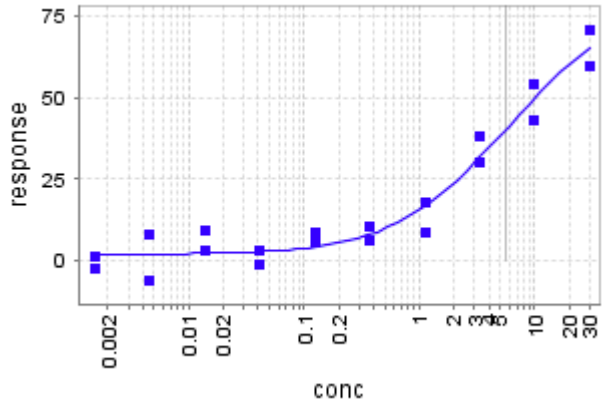
Compound **156** (EC_{50} 0.72 µM)



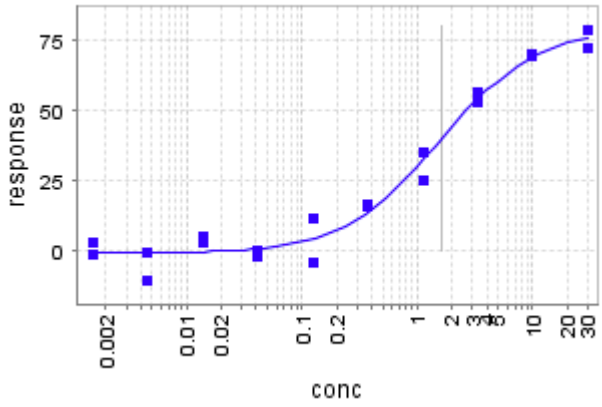
Compound **157** (EC_{50} 0.03 µM)



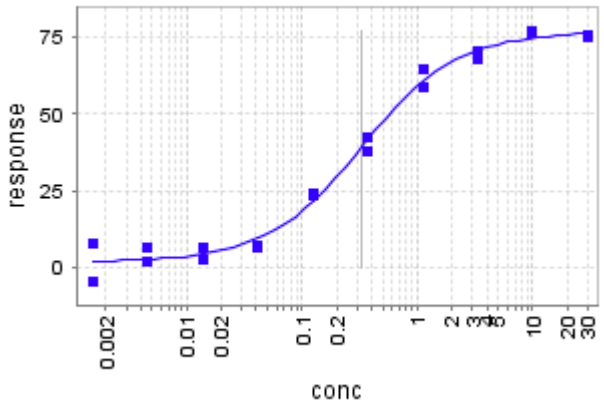
Compound **158** (EC_{50} 4.66 µM)



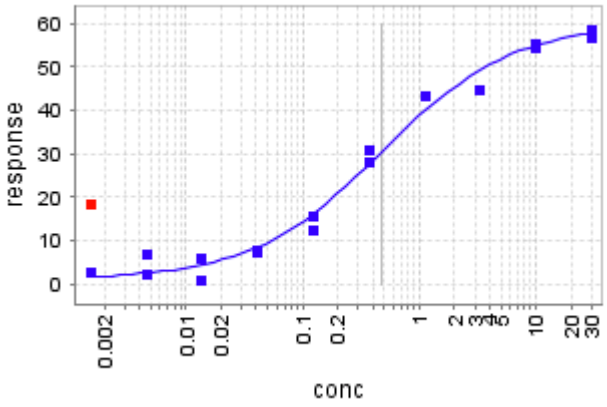
Compound **159** (EC_{50} 1.37 µM)



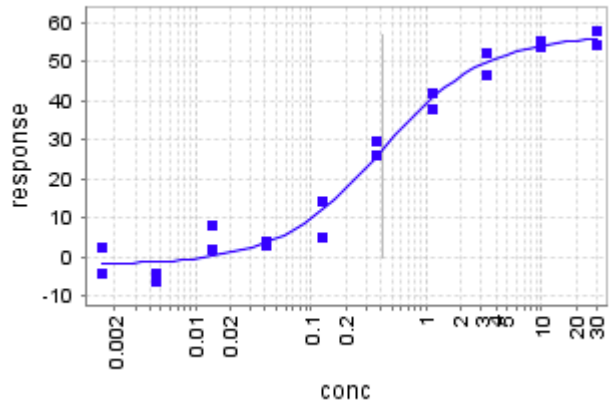
Compound **160** (EC_{50} 0.29 µM)



Compound **161** (EC_{50} 0.64 µM)



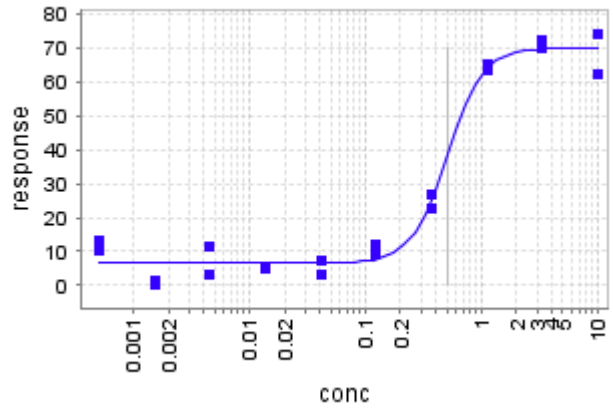
Compound **162** (EC_{50} 0.37 µM)



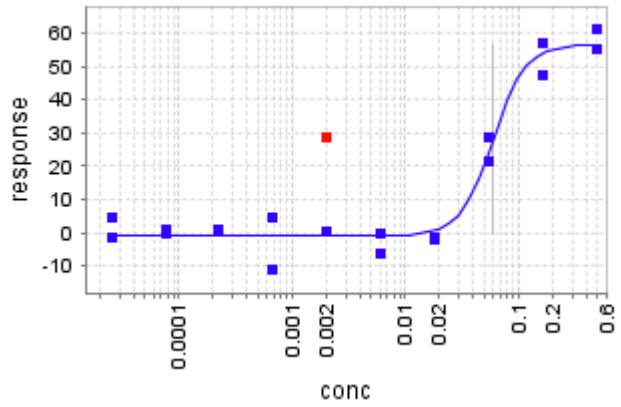
The y-axis response is percent inhibition and the x-axis conc is concentration in micromolar.

Figure 2. Toxoflavins

Compound **26** (EC_{50} 0.28 µM)



Compound **67** (EC_{50} 0.05 µM)



The y-axis response is percent inhibition and the x-axis conc is concentration in micromolar.

Computational docking

TDP2 protein structure (PDB 4GZ1, chains A, C and D) was prepared using the Protein Preparation Wizard within Maestro version 9.3.5 (Schrödinger Suite 2012). Following deletion of the DNA chains and solvent, a grid box was generated using Glide (version 5.8) centred on the magnesium ion, extending the inner grid box length to the maximum value of 14 Å. Deazaflavin ligands were prepared for docking using LigPrep, generating ionization states at pH 7 (Epik) and including the *Add metal binding states* option to generate both neutral and anionic (deprotonated) species. Ligand docking was performed using Glide SP (standard precision), XP (extra precision) and Induced Fit Docking protocols, using default docking parameters. Selected docked poses were refined using molecular mechanics and dynamics protocols within MacroModel in order to evaluate the robustness of the binding modes while allowing localised relaxation of the protein structure.

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