

Structural reassignment of a dibenz[*b,f*][1,4]oxazepin-11(10*H*)-one with potent anti*giardial* activity

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ABSTRACT

A screen for compounds with anti*giardial* activity in the Compounds Australia Scaffolds library identified SN00797640 (supplied structure being 8-acylamino-dibenzoxazepinone **1**) as a hit compound with potent anti-parasitic activity (concentration for 50% growth inhibition of *Giardia duodenalis*, IC₅₀ 0.18 µM). To further explore the structure–activity relationships in this series, compound **1** and analogues, including its 7-acylamino-dibenzoxazepinone regioisomer (**2**), were synthesized and assessed for anti-*Giardia* activity. While regioisomer **2** demonstrated anti*giardial* activity, resynthesized **1** and other 8-acylamino-dibenzoxazepinone analogues were inactive. Comparison of spectroscopic and physical properties demonstrated the correct structure of SN00797640 to be 7-acylamino regioisomer **2**. These results highlight the importance of independent synthesis in verifying the structure and activity of screening hits.

Keywords: anti*giardial*, dibenzoxazepinones, *Giardia*, giardiasis, medicinal chemistry, organic synthesis, parasite, screening, structural reassignment.

Introduction

A range of analytical, synthetic and medicinal chemistry techniques are required in the triage of drug screening hits, particularly in deciding which hit compounds should progress into a medicinal chemistry hit-to-lead program.^[1] Many issues can be identified on analysis of an initial set of hits, including that library compounds plated out for screening have degraded over time or have been misidentified, false positives can originate from unknown contaminants or assay interference, and certain types of compounds are promiscuous, so-called frequent hitters.^[2] The purification or resynthesis of hits enables their activity to be confirmed, leading to prioritization of the hit or, conversely, can reveal issues with quality, identity or inconsistent activity between batches that can lead to de-prioritization of the hit or at least demand further enquiry prior to progression.^[3] In previous work on fragment-based screening against HIV integrase, we noted the isomerization of a hit compound occurring during testing that, once clarified, enabled development of more strongly binding compounds.^[4] We now describe how the resynthesis of an assay hit from a phenotypic screen of a library of commercial compounds for anti*giardial* activity enabled reassignment of the supplied structure of the active compound.

In the search for new compounds with activity against the important intestinal parasite *Giardia duodenalis*,^[5] a series of acylamino-dibenz[*b,f*][1,4]oxazepin-11(10*H*)-ones (Compounds Australia Scaffold Series CL9406) were identified as potent hits in a growth inhibition assay (Hart CJS, Riches AG, Tiash S, Clapper E, Ramu S, Zuegg J, Ryan JH, Skinner-Adams TS. unpubl. data). The commercially sourced compound SN00797640, with assigned structure **1** (Fig. 1), was the most potent hit (*G. duodenalis* mean + s.d. 50% growth inhibition (inhibitory concentration, IC₅₀) = 0.18 ± 0.05 µM) in this series. To access additional material for further evaluation and to investigate structure–activity relationships (SARs) within the Scaffold Series CL9406, we resynthesized compound **1**. However, the newly synthesized material as well as several analogues of **1** were found to be

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inactive. Herein, we describe how analytical and synthetic chemistry were used to reassign the structure of the active compound as the regioisomeric 7-acylamino **2**.

Results and discussion

Synthesis

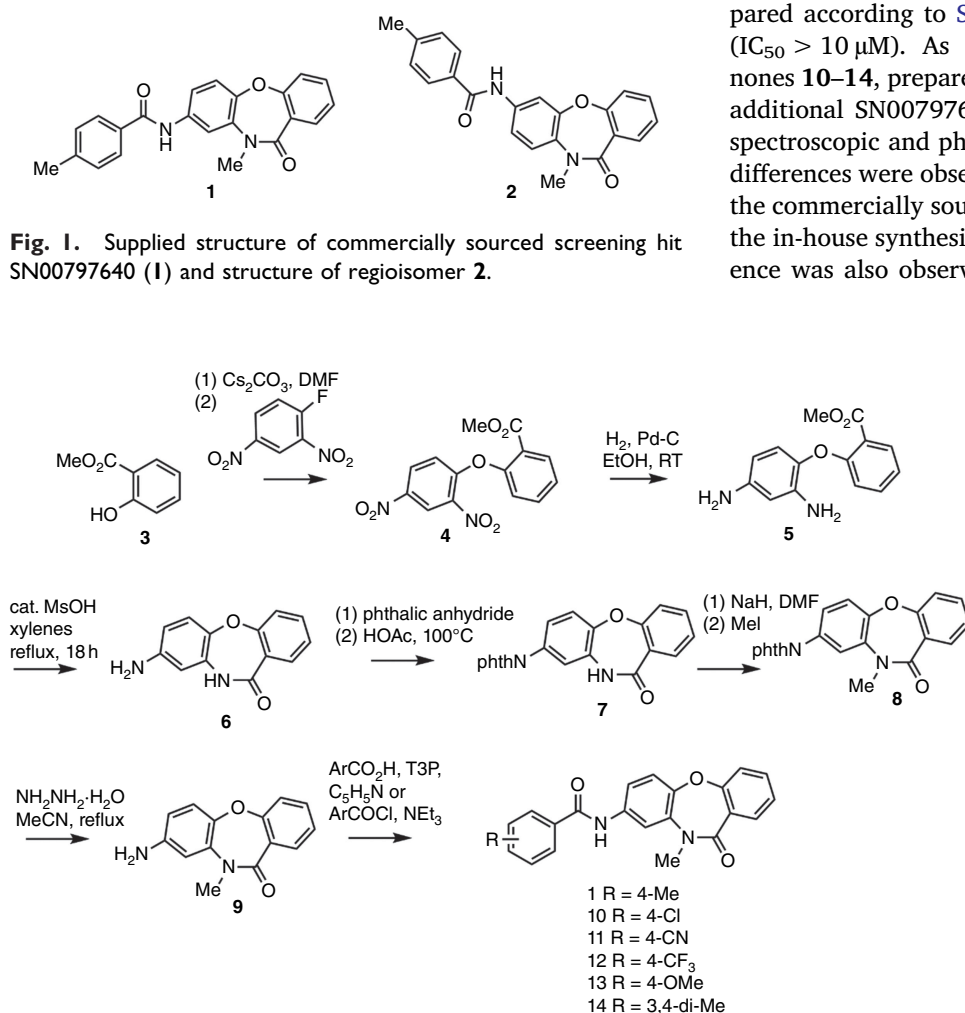
Despite compound **1** being commercially available, a literature search found no reported methods for its preparation. We therefore developed our own synthesis of **1** (Scheme 1). Reaction of 2,4-dinitrofluorobenzene with the anion of methyl salicylate **3** gave biaryl ether **4**, previously obtained from reaction of **3** with 2,4-dinitrochlorobenzene.^[6,7] Catalytic hydrogenation/hydrogenolysis reduced both nitro groups of **4** to give diamine **5**, which appears in a 2018 patent without any characterization data.^[8] Acid-catalyzed lactamization of **5** then gave dibenzoxazepinone **6**, which was reported in a 1974 paper as the product of thermally mediated cyclization of the ethyl ester analogue of **5**, also without accompanying physical or spectroscopic data.^[9] The

amino group of **6** was protected as the novel phthalimide **7**, which enabled selective *N*-methylation of the lactam, affording the previously unreported **8**. Subsequent hydrazinolysis of the phthalimide **8** gave aniline **9**. A literature search found no previous reports of compound **9**. The coupling of **9** with *p*-toluic acid gave 8-acylamino dibenzoxazepinone **1**. Additional analogues **10–14** were prepared by coupling amine **9** with other carboxylic acids or acid chlorides, and are characterized here for the first time.

The synthesis of the regioisomeric 7-acylamino dibenzoxazepinone **2** (Scheme 2) employed a tandem S_NAr -Smiles rearrangement sequence to give tricyclic ring system **16** in one operation from **15** and 3,4-difluoronitrobenzene.^[10] Hydrogenation of **16** then gave the 7-aminodibenzoxazepinone **17**.^[11] Amide coupling of **17** with *p*-toluic acid gave the 7-acylamino dibenzoxazepinone **2**. A literature search found no reports describing either the preparation or physical or spectroscopic characterization of compound **2**.

Biological activity and structural characterization

In contrast to the original commercially sourced compound, SN00797640 ($IC_{50} = 0.18 \pm 0.05 \mu M$), compound **1** prepared according to Scheme 1 was inactive against *Giardia* ($IC_{50} > 10 \mu M$). As additional 8-acylamino dibenzoxazepinones **10–14**, prepared to examine SARs, were also inactive, additional SN00797640 was purchased from ChemDiv for spectroscopic and physical property assessment. Significant differences were observed between the 1H NMR spectrum of the commercially sourced sample (SN00797640) and that of the in-house synthesized sample of **1** (Fig. 2). A large difference was also observed between the melting points of the



Scheme 1. Synthesis of 8-acylamino dibenz[b,f][1,4]oxazepin-11(10H)-ones. T3P, 2,4,6-triisopropyl-1,3,5,2,4,6-trioxatriphosphorinane-2,4,6-trioxide.

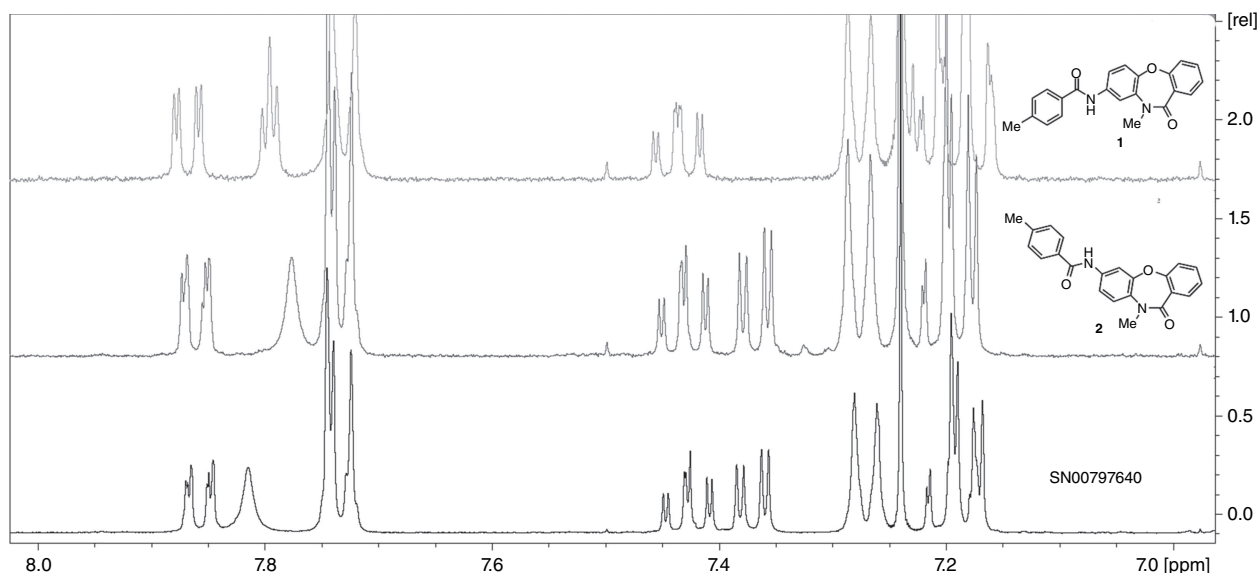
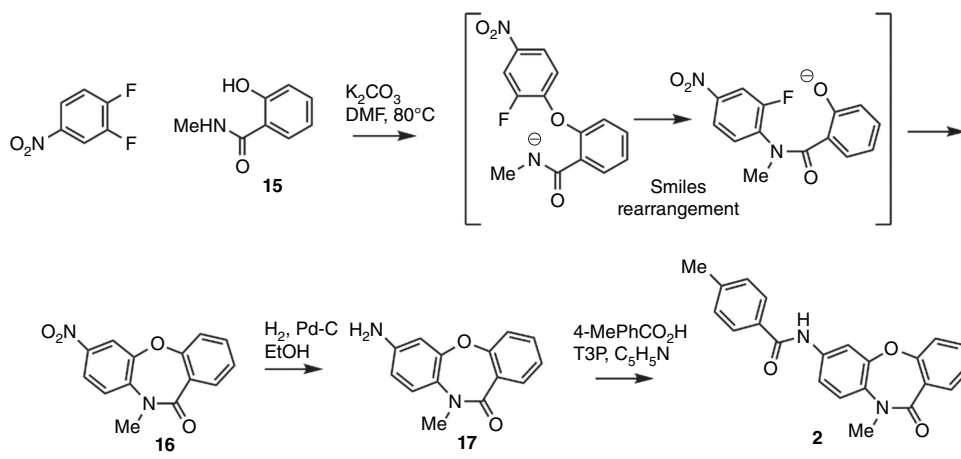


Fig. 2. Aromatic region of ^1H NMR spectra (400 MHz, CDCl_3) of **1**, **2** and SN00797640.

commercially sourced sample of SN00797640 (mp 211–212°C) and in-house synthesized sample of **1** (mp 255–257°C). Conversely, compound **2** exhibited a ^1H NMR spectrum and melting point (mp 211–213°C) in accord with SN00797640 (Fig. 2). Compound **2** also demonstrated potent *in vitro* activity against *Giardia* parasites ($\text{IC}_{50} = 0.28 \pm 0.04 \mu\text{M}$) similar to that observed for SN00797640. When notified of this discrepancy, ChemDiv did not have any SN00797640 (ChemDiv code: MO13-0086) in stock with which they could independently verify its regiosomeric identity. However, they provided confirmation (by 2D NOESY spectroscopy) that other available members of the CL9406 series were 7-acylamino-dibenzoxazepinones, rather than the corresponding 8-acylamino isomers, as originally specified in ChemDiv documentation (pers. comm.). The initial misassignment of the structure of SN00797640 serves as a reminder of the importance of using independent synthesis

to verify the structure and activity of screening hits prior to undertaking more extensive SAR studies of a hit series.^[1,2]

Conclusion

Synthesis of compounds **1** and **2** enabled reassignment of the structure of the anti-giardial screening hit SN00797640 from the commercially supplied structure **1** to structure **2**. In contrast to the 7-benzamido regioisomer **2**, the 8-benzamido regioisomer **1** was inactive against *Giardia duodenalis* *in vitro*. The revision of this structure led to review of the CL9406 series by ChemDiv and confirmation that this series comprise all 7-acylamino isomers. With the structure of the hit compound confirmed, analogues of **2** can be synthesized and evaluated for anti-giardial activity, enabling development of robust SARs.

Experimental

General chemistry experimental

Melting points were determined on a Büchi B-545 digital melting point apparatus and are uncorrected. NMR spectra were recorded on Bruker Avance 400 and 500 MHz NMR spectrometers at 25°C. ^1H spectra in CDCl_3 and d_6 -DMSO are referenced to residual solvent (δ_{H} 7.24 and 2.50 ppm respectively). ^{13}C NMR spectra in CDCl_3 and d_6 -DMSO are referenced to the central resonance of the CDCl_3 'triplet' (δ_{C} 77.23 ppm) and the d_6 -DMSO septet (δ_{C} 39.51 ppm) respectively. Thin layer chromatography (TLC) was performed on Merck pre-coated 0.25 mm silica F₂₅₄ aluminium-backed plates (no. 5554). Column chromatography was performed using Merck (no. 9385, 230–400 mesh) silica gel 60. Analytical LCMS was performed on a Waters Acquity I Class with an Acquity UPLC BEH C₁₈ 1.7 mm 2.1 × 50 mm column with Photodiode Array (PDA) (254 nm) and Quadrupole Dalton (QDa) detection. Mobile phase: H_2O (0.1% formic acid)/ACN (0.1% formic acid) 95:5 → 100:0 over 7 min and a flow rate of 0.4 mL/min with an injection volume of 1.00 μL . Mass spectrometric analyses were performed on a Thermo Scientific Q Exactive mass spectrometer fitted with an Atmospheric-Pressure Chemical Ionization (APCI) ion source or an Atmospheric Solids Analysis Source (ASAP) ion source. Positive and/or negative ions were recorded in an appropriate mass range at 140 000 mass resolution. The APCI probe was used without flow of solvent. The nitrogen nebulizing/desolvation gas used for vaporization was heated to 450°C in these experiments. The sheath gas flow rate was set to 25 and the auxiliary gas flow rate to 2 (all arbitrary units). The discharge current was 4 mA and the capillary temperature was 320°C. All anhydrous reactions were performed under a dry nitrogen atmosphere. All inorganic solutions are aqueous unless otherwise specified.

Synthesis

Methyl 2-(2,4-dinitrophenoxy)benzoate 4: A stirred mixture of 2,4-dinitrofluorobenzene (4.08 g, 21.9 mmol), methyl salicylate **3** (3.33 g, 21.9 mmol), caesium carbonate (7.14 g, 21.9 mmol) and DMF (15 mL) was heated to 60°C until TLC analysis (EtOAc/heptane, 20:80) showed complete reaction (30 min). The mixture was filtered, evaporated under reduced pressure and the residue partitioned between 1:1 heptane/EtOAc and 2 M Na_2CO_3 . The organic phase was washed with water (×2), then brine, dried (Na_2SO_4) and evaporated under reduced pressure. The crude product was recrystallized from EtOAc/heptane to give **4** (6.11 g, 88%) as an off-white solid. Mp 90–91°C (lit.^[6] 88°C, lit.^[7] 85°C). NMR data have not been previously reported: ^1H NMR (400 MHz, CDCl_3) δ 8.86 (d, J = 2.8 Hz, 1H), 8.25 (dd, J = 9.3, 2.8 Hz, 1H), 8.09 (dd, J = 7.8, 1.7 Hz, 1H), 7.70–7.65 (m, 1H), 7.44 (apparent dt, J = 7.7, 1.1 Hz, 1H), 7.25 (dd, J = 8.1, 1.1 Hz, 1H), 6.79, d, J = 9.3 Hz, 1H), 3.73

(s, 3H). ^{13}C NMR (125 MHz, CDCl_3) δ 164.4, 157.0, 152.5, 141.3, 139.0, 135.0, 133.2, 128.9, 127.4, 123.8, 123.7, 122.3, 117.3, 52.7.

Methyl 2-(2,4-diaminophenoxy)benzoate 5: Methyl 2-(2,4-dinitrophenoxy)benzoate **4** (3.25 g, 10.2 mmol) was hydrogenated (1 atm H_2 , balloon) over 10% Pd/C (50% H_2O , 1.09 g, 0.511 mmol) in ethanol (100 mL) and the mixture stirred vigorously at room temperature for 3 h (reaction complete by TLC analysis), filtered through a pad of Celite and evaporated under reduced pressure to give a brown solid. The crude product was dissolved in EtOAc and filtered through a short plug of silica to remove baseline impurities to give diamine **5** (2.53 g, 96%) as a tan solid. Mp 128–131°C. HRMS (high-resolution mass spectrometry, APCI, +ve ion) m/z calcd for $\text{C}_{14}\text{H}_{15}\text{O}_3\text{N}_2$ [$\text{M} + \text{H}^+$] 259.1077, found 259.1077. ^1H NMR (400 MHz, CDCl_3) δ 7.77 (dd, J = 7.7, 1.8 Hz, 1H), 7.36–7.30 (m, 1H), 7.03–6.98 (m, 1H), 6.87 (dd, J = 8.4, 0.8 Hz, 1H), 6.76 (d, J = 8.4 Hz, 1H), 6.14 (d, J = 2.6 Hz, 1H), 6.06 (dd, J = 8.4, 2.6 Hz, 1H), 3.92 (broad s, 2H), 3.89 (s, 3H), 3.49 (br. s, 2H). ^{13}C NMR (125 MHz, CDCl_3) δ 167.2, 158.2, 144.6, 140.2, 135.0, 133.5, 131.5, 122.9, 121.8, 120.9, 116.1, 105.4, 103.3, 52.4.

8-Aminodibenzo[b,f][1,4]oxazepin-11(10H)-one 6: A mixture of methyl 2-(2,4-diaminophenoxy)benzoate **5** (2.39 g, 9.25 mmol), methanesulfonic acid (50 μL) and xylenes (50 mL) was refluxed for 20 h. After cooling, the solvent was evaporated under reduced pressure and the residue was triturated with EtOAc to give the 8-aminodibenzo[b,f][1,4]oxazepinone **6** (1.78 g, 85%) as a yellow powder. Mp 236–240°C (dec.). HRMS (APCI, +ve ion) m/z calcd for $\text{C}_{13}\text{H}_{11}\text{N}_2\text{O}_2$ [$\text{M} + \text{H}^+$] 227.0814, found 227.0815. ^1H NMR (400 MHz, d_6 -DMSO) δ 10.34 (broad s, 1H), 7.73 (dd, J = 7.7, 1.7 Hz, 1H), 7.59–7.54 (m, 1H), 7.30–7.23 (m, 2H), 7.00 (d, J = 8.5 Hz, 1H), 6.41 (d, J = 2.6 Hz, 1H), 6.36 (dd, J = 8.5, 2.6 Hz, 1H) (NH_2 signal not observed). ^{13}C NMR (125 MHz, d_6 -DMSO) δ 166.2, 159.6, 146.6, 141.2, 134.0, 131.3, 131.1, 126.1, 124.8, 121.2, 120.3, 110.2, 105.8.

2-(11-Oxo-10,11-dihydrodibenzo[b,f][1,4]oxazepin-8-yl)-isoindoline-1,3-dione 7: The 8-aminodibenzo[b,f][1,4]oxazepinone **6** (1.45 g, 6.41 mmol) was dissolved in dry DMF (20 mL) and phthalic anhydride (949 mg, 6.41 mmol) was added. After stirring at room temperature for 2 h, no starting amine was detected by TLC. The solvent was evaporated under reduced pressure, acetic acid (30 mL) was added, and the mixture was heated at reflux for 3 h, then evaporated. Trituration of the residue with EtOAc gave **7** (1.84 g, 81%) as a colourless solid. Mp 298–301°C. HRMS (APCI, +ve ion) m/z calcd for $\text{C}_{21}\text{H}_{13}\text{N}_2\text{O}_4$ [$\text{M} + \text{H}^+$] 357.0870, found 357.0871. ^1H NMR (400 MHz, d_6 -DMSO) δ 10.71 (broad s, 1H), 7.98–7.87 (m, 4H), 7.79 (dd, J = 7.8, 1.7 Hz, 1H), 7.67–7.62 (m, 1H), 7.49 (d, J = 8.4 Hz, 1H), 7.41–7.32 (m, 2H), 7.26–7.21 (m, 2H). ^{13}C NMR (125 MHz, d_6 -DMSO) δ 166.8, 165.7, 158.6, 149.8, 134.7, 134.6, 131.6, 131.5, 131.5, 129.3, 125.7, 125.6, 124.3, 123.5, 121.7, 120.6, 120.5.

2-(10-Methyl-11-oxo-10,11-dihydrodibenzo[b,f][1,4]oxazepin-8-yl)isoindoline-1,3-dione 8: Sodium hydride (60% in oil, 104 mg, 2.60 mmol) was added to a stirred suspension of the lactam **7** (841 mg, 2.36 mmol) in DMF (10 mL). After 15 min, the bubbling had subsided to give a clear solution, and methyl iodide (1.67 g, 11.8 mmol) was added and the mixture was stirred at room temperature for 2 h to give a thick slurry. The DMF was evaporated under reduced pressure and the residue was mixed with 1 M HCl and the solid collected by filtration, dried at the pump, and washed with methyl *tert*-butyl ether to give the *title compound 8* (760 mg, 87%) as a tan powder. Mp 241–242°C. HRMS (APCI, +ve ion) *m/z* calcd for $C_{22}H_{15}N_2O_4^+$ [$M + H^+$] 371.1026, found 371.1024. 1H NMR (400 MHz, d_6 -DMSO) δ 8.01–7.88 (m, 4H), 7.78 (dd, $J = 7.8, 1.7$ Hz, 1H), 7.65–7.60 (m, 2H), 7.55 (d, $J = 8.5$ Hz, 1H), 7.41 (broad d, $J = 8.0$ Hz, 1H), 7.37–7.30 (m, 2H), 3.48 (s, 3H). ^{13}C NMR (125 MHz, d_6 -DMSO) δ 166.8, 165.1, 159.7, 152.3, 135.7, 134.8, 134.2, 131.9, 131.5, 129.6, 125.7, 125.7, 125.6, 123.5, 122.5, 121.5, 120.0, 36.3.

10-Methyl-8-aminodibenz[b,f][1,4]oxazepin-11(10H)-one 9: Hydrazine hydrate (1.03 g, 20.5 mmol) was added to a suspension of phthalimide **8** (759 mg, 2.05 mmol) in acetonitrile. The mixture was refluxed for 1 h, during which time it became homogeneous, before a voluminous precipitate appeared. After cooling to room temperature, the solid was removed by filtration and the filtrate was evaporated to give a tan solid. Column chromatography (EtOAc/CHCl₃, 0:100 → 10:90) gave the amine **9** (445 mg, 90%) as a colourless oil that solidified on standing. Mp 230–238°C (dec.). HRMS (ESI, +ve ion) *m/z* calcd for $C_{14}H_{13}O_2N_2^+$ [$M + H^+$] 241.0972, found 241.0970. 1H NMR (400 MHz, CDCl₃) δ 7.84 (dd, $J = 7.8, 1.7$ Hz, 1H), 7.43–7.38 (m, 1H), 7.19–7.11 (m, 2H), 7.00 (d, $J = 8.6$ Hz, 1H), 6.49 (d, $J = 2.7$ Hz, 1H), 6.40 (dd, $J = 8.6, 2.7$ Hz, 1H), 3.62 (broad s, 2H), 3.52 (s, 3H). ^{13}C NMR (125 MHz, CDCl₃) δ 166.7, 161.0, 146.1, 144.2, 136.3, 133.4, 132.2, 126.4, 124.9, 121.8, 119.6, 112.4, 108.5, 36.5.

4-Methyl-N-(10-methyl-11-oxo-10,11-dihydrodibenzo[b,f][1,4]oxazepin-8-yl)benzamide 1: A vial was charged with amine **9** (30 mg, 0.125 mmol), 4-methylbenzoic acid (19 mg, 0.137 mmol) and pyridine (200 μ L). After 10 min, 2,4,6-tripropyl-1,3,5,2,4,6-trioxatriphosphorinane-2,4,6-trioxide (T3P) (50% solution in EtOAc, 112 μ L, 0.188 mmol) was added dropwise and the mixture was stirred at room temperature for 3 h. The mixture was partitioned between 1 M HCl and EtOAc. The organic phase was washed successively with sat. aq. NaHCO₃ and brine, dried (MgSO₄) and evaporated. Column chromatography (EtOAc/CHCl₃, 10:90 → 30:70) gave amide **1** (20 mg, 45%) as a colourless oil, which crystallized on standing. Mp 255–257°C. LCMS: t_R 2.99 min. (98.3% purity). HRMS (ESI, +ve ion) *m/z* calcd for $C_{22}H_{19}N_2O_3^+$ [$M + H^+$] 359.1390, found 359.1391. 1H NMR (500 MHz, CDCl₃) δ 7.87 (dd, $J = 7.8, 1.7$ Hz, 1H), 7.81–7.78 (m, 1H), 7.75–7.71 (m, 2H), 7.74 (broad s, 1H),

7.46–7.41 (m, 1H), 7.30–7.25 (m, 2H), 7.24–7.15 (m, 4H), 3.60 (s, 3H), 2.41 (s, 3H). ^{13}C NMR (125 MHz, CDCl₃) δ 166.4, 165.6, 160.5, 150.0, 142.8, 136.4, 135.7, 133.5, 132.4, 131.5, 129.5, 127.0, 126.2, 125.2, 121.6, 119.7, 117.6, 114.7, 36.8, 21.5.

4-Chloro-N-(10-methyl-11-oxo-10,11-dihydrodibenzo[b,f][1,4]oxazepin-8-yl)benzamide 10: Prepared as for **1** from aryl amine **9** (29 mg, 0.120 mmol) and 4-chlorobenzoic acid (21 mg, 0.132 mmol) using T3P. The crude product was purified by column chromatography (CHCl₃) to give product **10** (32 mg, 96%) as a colourless solid. Mp 256–257°C. LCMS: t_R 3.08 min. (99.9% purity). HRMS (ESI, +ve ion) *m/z* calcd for $C_{21}H_{16}O_3N_2^{35}Cl^+$ [$M + H^+$] 379.0844, found 379.0844. 1H NMR (400 MHz, CDCl₃) δ 7.85 (dd, $J = 7.7, 1.6$ Hz, 1H), 7.83 (broad s, 1H), 7.80–7.75 (m, 2H), 7.75 (brd, $J = 1.9$ Hz, 1H), 7.46–7.41 (m, 3H), 7.22–7.15 (m, 4H), 3.61 (s, 3H). ^{13}C NMR (125 MHz, d_6 -DMSO) δ 165.3, 164.4, 159.9, 148.8, 136.8, 136.6, 135.2, 134.0, 133.2, 131.9, 129.6, 128.5, 125.8, 125.5, 121.1, 119.9, 118.2, 114.9, 36.2.

4-Cyano-N-(10-methyl-11-oxo-10,11-dihydrodibenzo[b,f][1,4]oxazepin-8-yl)benzamide 11: Aryl amine **9** (50 mg, 0.208 mmol) was dissolved in pyridine (0.5 mL) and 4-cyanobenzoyl chloride (38 mg, 0.229 mmol) was added and the mixture stirred at room temperature for 18 h. The resulting suspension was diluted with methyl *tert*-butyl ether (MTBE) and the solid was collected by filtration to give amide **11** (42 mg, 55%) as a pale grey solid. Mp 267–269°C. LCMS: t_R 2.73 min. (96.1% purity). HRMS (ESI, +ve ion) *m/z* calcd for $C_{22}H_{16}O_3N_3^+$ [$M + H^+$] 370.1186, found 370.1187. 1H NMR (400 MHz, d_6 -DMSO) δ 10.60 (broad s, 1H), 8.11–8.07 (m, 2H), 8.05–8.02 (m, 2H), 7.86 (d, $J = 2.4$ Hz, 1H), 7.76 (dd, $J = 7.7, 1.7$ Hz, 1H), 7.62–7.57 (m, 2H), 7.40–7.28 (m, 3H), 3.50 (s, 3H). ^{13}C NMR (125 MHz, d_6 -DMSO) δ 165.3, 164.2, 159.9, 149.0, 138.5, 136.6, 135.3, 134.0, 132.5, 131.9, 128.5, 125.8, 125.5, 121.2, 119.9, 118.3, 118.2, 115.0, 114.0, 36.2.

N-(10-Methyl-11-oxo-10,11-dihydrodibenzo[b,f][1,4]oxazepin-8-yl)-4-(trifluoromethyl)benzamide 12: Prepared as for **11** from aryl amine **9** (50 mg, 0.208 mmol) and 4-trifluoromethylbenzoyl chloride (52 mg, 0.249 mmol). The mixture was poured into 1 M HCl and extracted with EtOAc ($\times 3$). The combined extracts were washed with H₂O then brine, dried (MgSO₄) and evaporated. The crude product was purified by column chromatography (EtOAc/CHCl₃, 10:90 → 20:80) to give amide **12** (75 mg, 87%) as an off-white solid. Mp 224–231°C. LCMS: t_R 3.23 min. (98.5% purity). HRMS (ESI, +ve ion) *m/z* calcd for $C_{22}H_{16}O_3N_2F_3^+$ [$M + H^+$] 413.1108, found 413.1109. 1H NMR (500 MHz, d_6 -DMSO) δ 10.59 (broad s, 1H), 8.16–8.12 (m, 2H), 7.94–7.90 (m, 2H), 7.87 (d, $J = 2.4$ Hz, 1H), 7.77 (dd, $J = 7.8, 1.7$ Hz, 1H), 7.63–7.57 (m, 2H), 7.38 (d, $J = 8.8$ Hz, 1H), 7.35 (dd, $J = 8.1, 0.9$ Hz, 1H), 7.33–7.29 (m, 1H), 3.51 (s, 3H). ^{13}C NMR (125 MHz, d_6 -DMSO) δ 165.8, 164.9, 160.4, 149.4, 138.8, 137.1,

135.8, 134.5, 132.4, 132.0 (q, $^2J_{CF}$ = 32 Hz), 129.0, 126.3, 126.0–125.9 (m), 124.4 (q, $^1J_{CF}$ = 272 Hz), 121.7, 120.3, 118.7, 115.4, 36.7.

4-Methoxy-N-(10-methyl-11-oxo-10,11-dihydrodibenzo[b,f][1,4]oxazepin-8-yl)benzamide 13. Prepared as for **1** from aryl amine **9** (28 mg, 0.117 mmol) and *p*-anisic acid (20 mg, 0.128 mmol) using T3P. The crude product was purified by column chromatography (CHCl₃) to give product **13** (38 mg, 87%) as a colourless solid. Mp 253–255°C. LCMS: t_R 2.79 min. (96.9% purity). HRMS (ESI, +ve ion) m/z calcd for C₂₂H₁₉O₄N₂⁺ [M + H]⁺ 375.1339, found 375.1340. ¹H NMR (400 MHz, CDCl₃) δ 7.88–7.78 (m, 5H), 7.45–7.40 (m, 1H), 7.22–7.15 (m, 4H), 6.98–6.92 (m, 2H), 3.85 (s, 3H), 3.59 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 166.7, 165.5, 162.9, 160.7, 150.1, 136.5, 136.0, 133.8, 132.6, 129.1, 126.7, 126.4, 125.5, 121.8, 119.9, 117.9, 115.0, 114.3, 55.7, 37.0.

3,4-Dimethyl-N-(10-methyl-11-oxo-10,11-dihydrodibenzo[b,f][1,4]oxazepin-8-yl)benzamide 14. Prepared as for **1** from aryl amine **9** (50 mg, 0.208 mmol) and 3,4-dimethylbenzoic acid (34 mg, 0.229 mmol) using T3P. The crude product was purified by column chromatography (MeOH/CHCl₃, 0:100 → 2:98) to give amide **14** (47 mg, 61%) as an off-white solid. Mp 242–244°C. LCMS: t_R 3.15 min (96.2% purity). HRMS (ESI, +ve ion) m/z calcd for C₂₃H₂₁O₃N₂⁺ [M + H]⁺ 373.1547, found 373.1542. ¹H NMR (400 MHz, *d*₆-DMSO) δ 10.24 (broad s, 1H), 7.87 (d, J = 2.4 Hz, 1H), 7.76 (dd, J = 7.7, 1.7 Hz, 1H), 7.75–7.72 (m, 1H), 7.68 (dd, J = 7.8, 1.7 Hz, 1H), 7.64–7.56 (m, 2H), 7.37–7.27 (m, 4H), 3.50 (s, 3H), 2.30 (s, 3H), 2.29 (s, 3H). ¹³C NMR (*d*₆-DMSO, 125 MHz) δ 165.5, 165.3, 160.0, 148.6, 140.6, 137.2, 136.4, 135.2, 134.0, 132.0, 131.9, 129.4, 128.6, 125.9, 125.4, 125.1, 121.0, 119.8, 118.0, 114.7, 36.2, 19.4.

10-Methyl-7-nitrodibenz[b,f][1,4]oxazepin-11(10H)-one 16: This method was adapted from the protocol previously described by Liu *et al.*^[10] 2-Hydroxy-*N*-methylbenzamide **15** (240 mg, 1.59 mmol) was dissolved in DMF (10 mL), then 3,4-difluoronitrobenzene (176 μ L, 1.59 mmol) and K₂CO₃ (549 mg, 3.98 mmol) were added. The heterogeneous mixture was stirred vigorously for 1 h at 80°C. The yellow mixture was cooled and evaporated under reduced pressure and the residue was partitioned between EtOAc and water. The organic phase was washed with brine, dried (MgSO₄), then concentrated to afford a light-yellow oil. Purification by flash column chromatography (CH₂Cl₂/heptane, 2:1) yielded **16** (313 mg, 73%) as a white solid. Mp 156–158°C (lit.^[10] 158–163°C). ¹H NMR (400 MHz, CDCl₃) δ 8.12 (d, J = 2.6 Hz, 1H), 8.08 (dd, J = 8.9, 2.6 Hz, 1H), 7.90–7.86 (m, 1H), 7.53–7.49 (m, 1H), 7.34 (d, J = 8.9 Hz, 1H), 7.28–7.24 (m, 2H), 3.60 (s, 3H).

7-Amino-10-methyldibenzo[b,f][1,4]oxazepin-11(10H)-one 17: The nitro compound **16** (313 mg, 1.16 mmol) was hydrogenated (balloon) over 10% Pd/C (50% H₂O) (63 mg, 0.030 mmol) in a mixture of ethanol (100 mL) and EtOAc (5 mL) at room temperature for 2 h. The mixture was filtered through Celite and evaporated under reduced pressure.

The residue was dissolved in EtOAc and filtered through a plug of silica gel to give **17** (249 mg, 1.04 mmol, 90%) as a pale-yellow solid. Mp 188–192°C (lit.^[11] 194–196°C). ¹H NMR (400 MHz, CDCl₃) δ 7.84 (dd, J = 7.8, 1.7 Hz, 1H), 7.43–7.37 (m, 1H), 7.21–7.15 (m, 1H), 7.12 (dd, J = 8.1, 1.0 Hz, 1H), 6.98 (d, J = 8.6 Hz, 1H), 6.55 (d, J = 2.6 Hz, 1H), 6.47 (dd, J = 8.6, 2.6 Hz, 1H), 3.67 (broad s, 2H), 3.51 (s, 3H). ¹³C NMR (125 MHz, CDCl₃) δ 166.4, 160.4, 154.7, 145.1, 133.1, 132.1, 126.8, 126.6, 125.1, 123.4, 119.8, 112.1, 107.3, 36.7.

4-Methyl-N-(10-methyl-11-oxo-10,11-dihydrodibenzo[b,f][1,4]oxazepin-7-yl)benzamide 2: Aryl amine **17** (50 mg, 0.208 mmol) and *p*-toluic acid (31.2 mg, 0.229 mmol) were dissolved in pyridine (1 mL). After 10 min, T3P (50% w/w in EtOAc, 0.186 mL, 0.312 mmol) was added dropwise. The mixture was stirred at room temperature for 3 h, then poured into 1 M HCl (15 mL) and extracted with EtOAc (3 × 15 mL). The combined extracts were washed with brine, dried (MgSO₄) and evaporated under reduced pressure. Silica gel chromatography (EtOAc/CHCl₃, 10:90) afforded **2** (55 mg, 74%) as a colourless solid. Mp 211–213°C. LCMS: t_R 2.96 min (99.6% purity). m/z 359.31 [M + H]⁺. HRMS (APCI, +ve ion) m/z calcd for C₂₂H₁₉N₂O₃⁺ [M + H]⁺ 359.1390, found 359.1388. ¹H NMR (400 MHz, CDCl₃) δ 7.88–7.84 (m, 1H); 7.77 (broad s, 1H), 7.76–7.71 (m, 3H), 7.46–7.40 (m, 1H), 7.37 (dd, J = 8.8, 2.5 Hz, 1H), 7.30–7.26 (m, 2H), 7.22–7.17 (m, 3H), 3.56 (s, 3H), 2.41 (s, 3H). ¹³C NMR (125 MHz, CDCl₃) δ 166.4, 165.6, 160.4, 153.9, 142.8, 136.2, 133.5, 132.2, 132.1, 131.6, 129.5, 127.0, 126.2, 125.3, 122.7, 120.0, 117.2, 113.2, 36.8, 21.5. A purchased (ChemDiv) sample of SN00797640 gave mp 211–212°C and spectral data in agreement with compound **2**.

Cell lines and cultivation

Metronidazole-sensitive *Giardia duodenalis* assemblage B (BRIS/91/HEPU/1279)^[12] parasites were maintained essentially as previously described in 8 mL borosilicate culture tubes, using modified Keister's TYI-S-33 medium supplemented with 10% heat-inactivated fetal bovine serum, 100 units/mL penicillin, and 100 μ g/mL streptomycin.^[13]

Giardia dose-response assays

The activity of compounds was assessed by dose response to determine IC₅₀ values as previously described.^[14] In brief, compounds were serially diluted in triplicate, in DMSO-controlled media, and all test wells were inoculated with parasites (1.5 × 10⁴ parasites/well; 100 μ L/test). Albendazole (positive control) plates were prepared identically and run alongside each experiment. All plates were sealed in culture chambers, gassed with 3% O₂, 5% CO₂ in N₂ and incubated at 37°C, with parasites imaged and enumerated at 48 h.^[14] Growth inhibition was calculated as a percentage relative to vehicle controls minus any

background, and IC₅₀ values were determined using log-linear interpolation as previously described.^[15] All assays were performed at least twice.

Supplementary material

Copies of ¹H NMR, ¹³C NMR spectra and LCMS traces are available [online](#). Supplementary material is available [online](#).

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