

Supporting Information

Design and Synthesis of Novel Tricyclic Benzoxazines as Potent 5-HT_{1A/B/D} Receptor Antagonists Leading to the Discovery of 6-{2-[4-(2-methyl-5-quinolinyl)-1-piperazinyl]ethyl}-4*H*-imidazo[5,1-*c*][1,4]benzoxazine-3-carboxamide (GSK588045)

Steven M. Bromidge,^{a,} Roberto Arban,^b Barbara Bertani,^b Silvia Bison,^b Manuela Borriello,^b Paolo Cavanni,^b Giovanna Dal Forno,^b Romano Di-Fabio,^b Daniele Donati,^{b,§} Stefano Fontana,^b Massimo Gianotti,^b Laurie J. Gordon,^a Enrica Granci,^b Colin P. Leslie,^b Luca Moccia,^b Alessandra Pasquarello,^b Ilaria Sartori,^b Anna Sava,^b Jeannette M. Watson,^a Angela Worby,^a Laura Zonzini,^b Valeria Zucchelli^b*

Neurosciences CEDD, GlaxoSmithKline, ^aNew Frontiers Science Park, Third Avenue, Harlow, Essex CM19 5AW, UK ^bMedicines Research Centre, Via A. Fleming 4, Verona 37135, Italy

AUTHOR EMAIL: stevebromidge@gmail.com

*To whom correspondence should be addressed. Phone +44 (0)1279 627031. Fax +44 (0)1279 622371.

E-mail: stevebromidge@gmail.com.

§Present address: Nerviano Medical Sciences Srl, Via Pasteur 10, 20014 Nerviano, Milan, Italy.

Experimental and Analytical Data for Compounds 7-9, 15-18 and 21-29.

General Methods.

Proton Magnetic Resonance (NMR) spectra were recorded either on Varian instruments at 300, 400 or 500 MHz, or on a Bruker instrument at 300 MHz. Chemical shifts are reported in ppm (δ) using the residual solvent line as internal standard. Splitting patterns are designed as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; b, broad. The NMR spectra were recorded at a temperature ranging from 25 to 90°C. When more than one conformer was detected the chemical shifts for the most abundant one is reported. Mass spectra (MS) are typically taken on a 4 II triple quadrupole Mass Spectrometer (Micromass UK) or on a Agilent MSD 1100 Mass Spectrometer, operating in ES (+) and ES (-) ionization mode or on an Agilent LC/MSD 1100 Mass Spectrometer, operating in ES (+) and ES (-) ionization mode coupled with HPLC instrument Agilent 1100 Series [LC/MS - ES (+):analysis performed on a Supelcosil ABZ +Plus (33x4.6 mm, 3 μ m) (mobile phase: 100% [water +0.1% HCO₂H] for 1 min, then from 100% [water +0.1% HCO₂H] to 5% [water +0.1% HCO₂H] and 95% [CH₃CN] in 5 min, finally under these conditions for 2 min; T=40°C; flux= 1 mL/min; LC/MS - ES (-):analysis performed on a Supelcosil ABZ +Plus (33x4.6 mm, 3 μ m) (mobile phase: 100% [water +0.05% NH₃] for 1 min, then from 100% [water +0.05% NH₃] to 5% [water +0.05% NH₃] and 95% [CH₃CN] in 5 min, finally under these conditions for 2 min; T=40°C; flux= 1 mL/min] ; in the mass spectra only one peak in the molecular ion cluster is reported.

DAD chromatographic traces, mass chromatograms and mass spectrums were taken on a UPLC/MS Acquity™ system coupled with a Micromass ZQ™ mass spectrometer operating in ESI positive or negative. The phases used are: A) H₂O/ACN 95/5 + 0.1% TFA; B) H₂O/ACN 5/95 + 0.1% TFA. The gradient was: t=0min) 95%A 5%B, t=0.25) 95%A 5%B, t=3.30) 100%B, t=4.0) 100%B, followed by 1 min of reconditioning. Flash silica gel chromatography was carried out on silica gel 230-400 mesh (supplied by Merck AG Darmstadt, Germany) or over Varian Mega Be-Si pre-packed cartridges or over pre-packed Biotage silica cartridges. SPE-SCX cartridges are ion exchange solid phase extraction columns supplied by Varian. The eluent used with SPE-SCX cartridges is methanol followed by 2N

ammonia solution in methanol. In a number of preparations, purification was performed using either Biotage manual flash chromatography (Flash+) or automatic flash chromatography (Horizon, SP1) systems. All these instruments work with Biotage Silica cartridges. SPE-Si cartridges are silica solid phase extraction columns supplied by Varian. Microwave reactions were carried out in a Personal Chemistry EmrysTM Optimiser (300W). All reactions were carried out under anhydrous nitrogen or argon atmosphere using standard Schlenk techniques. Most chemicals and solvents were analytical grade and used without further purification.

The purity of the compounds reported in the manuscript was established through HPLC methodology. All the compounds reported in the manuscript have a purity >95%.

Preparation of Compound 7.

4-(2-Oxopropyl)-8-(2-propen-1-yl)-2H-1,4-benzoxazin-3(4H)-one (36). NaH (159 mg of a 60% w/w suspension in mineral oil, 3.98 mmol) was added in portions to a solution of intermediate **30** (500 mg, 2.65 mmol) and 1-chloro-2-propanone (253 μ L, 3.17 mmol) in dry DMF (6 mL) at 0°C. The reaction mixture was allowed to warm to room temperature, stirred for 7 h and then quenched with water (10 mL) and extracted with AcOEt (3 x 30 mL). The combined organic layers were dried over Na₂SO₄ and concentrated in vacuo to give the crude product which was purified by flash chromatography on silica gel, eluting with 5% acetone in CH₂Cl₂ to afford the title compound (314 mg, 49%) as a white solid. ¹H-NMR (300 MHz, CDCl₃) δ : 6.95-6.85 (m, 2H), 6.49 (m, 1H), 5.95 (m, 1H), 5.08-5.01 (m, 2H), 4.67 (s, 4H), 3.39 (m, 2H), 2.54 (s, 3H). MS *m/z* 246.20 [M+H]⁺.

2-Methyl-6-(2-propen-1-yl)-4H-imidazo[2,1-*c*][1,4]benzoxazine (37). NH₄OAc (396 mg, 5.14 mmol) was added to a solution of intermediate **36** (63 mg, 0.257 mmol) in glacial AcOH (1 mL). The mixture was irradiated in a microwave reactor (300W, 150°C, 10 min), then diluted with AcOEt (10 mL), poured into ice-water (10 mL) and basified with aqueous 1N NH₄OH solution (3 mL). The mixture was extracted with AcOEt (3 x 20 mL), the combined organics were dried over Na₂SO₄ and the solvent

was removed under reduced pressure. The crude material was purified by flash chromatography on silica gel, eluting with 5% acetone in CH₂Cl₂ to afford the title compound (47 mg, 81%). ¹H-NMR (300 MHz, CDCl₃) δ: 7.09-6.94 (m, 4H), 5.95 (m, 1H), 5.19 (s, 2H), 5.06-5.00 (m, 2H), 3.39 (m, 2H), 2.26 (s, 3H). MS *m/z* 227.10 [M+H]⁺.

(2-Methyl-4*H*-imidazo[2,1-*c*][1,4]benzoxazin-6-yl)acetaldehyde (38). Osmium tetroxide (0.405 mL of a 4% by wt. solution in water, 0.125 eq) was added to a stirred solution of intermediate **37** (115 mg, 0.509 mmol) in THF/water (2:1; 12 mL). After 10 min. sodium periodate (272 mg, 1.27 mmol) was added and the reaction mixture was stirred for 2 h. After evaporation of THF the residue was partitioned between water (10 mL) and AcOEt (3 x 30 mL). The organic layers were combined, dried over Na₂SO₄ and concentrated in vacuo. The title compound (42 mg, 36% yield) was isolated by flash chromatography on silica gel using AcOEt/cyclohexane (1/1) as eluent. ¹H-NMR (300 MHz, CDCl₃) δ: 9.77 (s, 1H), 7.22-6.91 (m, 4H), 5.24 (s, 2H), 3.77 (s, 2H), 2.31 (s, 3H). MS *m/z* 229.20 [M+H]⁺.

2-Methyl-6-{2-[4-(2-methyl-5-quinolinyl)-1-piperazinyl]ethyl}-4*H*-imidazo[2,1-*c*][1,4]benzoxazine dihydrochloride (7). A mixture of intermediate **38** (42 mg, 0.18 mmol) and 2-methyl-5-piperazin-1-yl-quinoline (63 mg, 0.28 mmol, 1.5 eq) in dry 1,2-dichloroethane (4 mL) was stirred at room temperature for 30 min. before sodium triacetoxyborohydride (58 mg, 0.28 mmol, 1.5 eq) was added. The resulting mixture was stirred for 3 h, quenched with saturated aqueous NaHCO₃ (10 mL) and extracted with CH₂Cl₂ (3 x 10 mL). The combined organic layers were dried over Na₂SO₄ and concentrated in vacuo. The crude product was purified by SPE-SI cartridge eluting with a gradient of 1-3% MeOH in CH₂Cl₂ to afford the corresponding free base of the title compound (21 mg, 26%) as a white solid. To a solution of the free base (21 mg, 0.05 mmol) in 3:1 MeOH/CH₂Cl₂ (4 mL), HCl (84 μL of a 1.25 M solution in MeOH, 0.105 mmol, 2.2eq) was slowly added at 0°C. After 2 h stirring at room temperature evaporation of the volatiles gave the title compound (23 mg) as a yellow solid. ¹H-NMR (500 MHz, DMSO-*d*₆) δ: 10.47 (bs, 1H), 8.55 (bs, 1H), 7.75 (bs, 2H), 7.68 (s, 1H), 7.60 (m, 2H), 7.29 (bs, 1H), 7.2 (m, 2H), 5.36 (s, 2H), 3.75-3.00 (m, 12H), 2.74 (s, 3H), 2.21 (s, 3H). MS *m/z* 440.30

[M+H]⁺.

Preparation of Compound 8.

4-(1-Methyl-2-oxopropyl)-8-(2-propen-1-yl)-2H-1,4-benzoxazin-3(4H)-one (39). A mixture of intermediate **30** (500 mg, 2.65 mmol), 3-chloro-2-butanone (294 μ l, 2.91 mmol, 1.1eq) and K₂CO₃ (402 mg, 2.91 mmol, 1.1eq) in dry acetone (15 mL) was heated at reflux for 4 h. Further 3-chloro-2-butanone (294 μ l, 2.91 mmol, 1.1eq) and K₂CO₃ (402 mg, 2.91 mmol, 1.1 eq) were added and the reaction mixture was heated at reflux for 18 h. The mixture was cooled to room temperature, solids were filtered-off and washed with acetone (50 mL) and the combined filtrates concentrated in vacuo. The crude material was purified by flash chromatography on silica gel, eluting with 5% acetone in CH₂Cl₂ to afford the title compound (626 mg, 91% yield) as a pale yellow viscous oil. ¹H-NMR (300 MHz, CDCl₃) δ : 6.96-6.85 (m, 2H), 6.66-6.58 (m, 1H), 5.93 (m, 1H), 5.16-5.00 (m, 3H), 4.70-4.52 (m, 2H), 3.38 (m, 2H), 2.12 (s, 3H), 1.51 (m, 3H). MS *m/z* 260.2 [M+H]⁺.

[4-(1-Methyl-2-oxopropyl)-3-oxo-3,4-dihydro-2H-1,4-benzoxazin-8-yl]acetaldehyde (40). The title compound was prepared in 64% yield according to the procedure of intermediate **38** (D28) starting from intermediate **39** (195 mg, 0.753 mmol). The product was purified by flash chromatography on silica gel using AcOEt/cyclohexane (1/1) as eluent. ¹H-NMR (300 MHz, CDCl₃) δ : 9.72 (s, 1H), 6.93 (m, 1H), 6.85 (m, 1H), 6.68 (m, 1H), 5.12 (m, 1H), 4.59 (m, 2H), 3.71 (s, 2H), 2.11 (s, 3H), 1.50 (m, 3H).

4-(1-Methyl-2-oxopropyl)-8-{2-[4-(2-methyl-5-quinolinyl)-1-piperazinyl]-ethyl}-2H-1,4-benzoxazin-3(4H)-one (41). The title compound was isolated as a white solid in 92% yield according to the general procedure described for **6** (**E8**) starting from intermediate **40** (127 mg, 0.487 mmol). The product was purified by flash chromatography on silica gel eluting with a gradient of 1-2% MeOH in CH₂Cl₂. ¹H-NMR (500 MHz, DMSO-d₆) δ : 8.36 (d, 1H), 7.60 (m, 2H), 7.40 (d, 1H), 7.12 (dd, 1H), 7.04 (m, 3H), 4.95 (q, 1H), 4.7 (dd, 2H), 3.05 (bs, 4H), 2.85 (t, 2H), 2.75 (bs, 4H), 2.65 (m, 5H), 2.08 (s,

3H), 1.41 (d, 3H). MS m/z 473.40 [M+H]⁺.

1,2-Dimethyl-6-{2-[4-(2-methyl-5-quinolinyl)-1-piperazinyl]ethyl}-4H-imidazo[2,1-c][1,4]-benzoxazine dihydrochloride (8). NH₄OAc (163 mg, 2.12 mmol, 20 eq) was added to a solution of intermediate **41** (50 mg, 0.106 mmol) in glacial AcOH (1 mL). The mixture was irradiated in a microwave reactor (300W, 150°C, 10 min), then diluted with AcOEt (10 mL), poured into ice-water (10 mL) and basified with an aqueous solution of 1N NH₄OH (3 mL). The mixture was extracted with AcOEt (3 x 30 mL). The combined organics were dried over Na₂SO₄ and the solvent was removed under reduced pressure. The crude material was purified by flash chromatography on silica gel eluting with a gradient of 1 to 3% MeOH in CH₂Cl₂ to afford the free base of the title compound (44 mg, 92%) which was dissolved in 3:1 MeOH/CH₂Cl₂ (4 mL) and treated with HCl (2.2 eq. of 1.25 M solution in MeOH) at 0°C. The resulting mixture was stirred at room temperature for 2 h before evaporation of the volatiles to give the title compound (44 mg) as a yellow solid. ¹H-NMR (500 MHz, DMSO-d₆) δ: 10.65 (bs, 1H), 8.64 (bs, 1H), 7.79 (bs, 2H), 7.68 (d, 1H), 7.61 (bs, 1H), 7.32 (bs, 1H), 7.27 (d, 1H), 7.20 (t, 1H), 5.27 (s, 2H), 3.75 (d, 2H), 3.70-3.00 (m, 10H), 2.77 (s, 3H), 2.48 (s, 3H), 2.18 (s, 3H). MS m/z 454.40 [M+H]⁺.

Preparation of Compound 9.

Methyl [3-oxo-8-(2-propen-1-yl)-2,3-dihydro-4H-1,4-benzoxazin-4-yl]acetate (42). The title compound was prepared in 95% yield according to the procedure described for intermediate **36**. Thus, intermediate **30** (1.0 g, 5.29 mmol) in DMF (15 mL) was treated with methyl bromoacetate (651 μL, 6.88 mmol, 1.3 eq) and NaH (318 mg of 60% w/w suspension in mineral oil, 7.94 mmol, 1.5 eq). The mixture was stirred for 6 h and the crude product was purified by flash chromatography on silica gel using AcOEt/cyclohexane (1/4) as eluent. ¹H-NMR (300 MHz, CDCl₃) δ: 6.96-6.90 (m, 2H), 6.65-6.60 (m, 1H), 5.95 (m, 1H), 5.10-5.01 (m, 2H), 4.67 (s, 2H), 4.66 (s, 2H), 3.78 (s, 3H), 3.39 (m, 2H). MS m/z 262.2 [M+H]⁺.

Methyl [3-oxo-8-(2-oxoethyl)-2,3-dihydro-4H-1,4-benzoxazin-4-yl]acetate (43). The title

compound was prepared in 66% yield according to the general procedure described for **38** starting from intermediate **42** (1.0 g, 3.83 mmol). The product was purified by flash chromatography on silica gel using AcOEt/cyclohexane (3/7) as eluent. ¹H-NMR (300 MHz, CDCl₃) δ: 9.74 (m, 1H), 6.98 (m, 1H), 6.88 (m, 1H), 6.71 (m, 1H), 4.68 (s, 2H), 4.67 (s, 2H), 3.79 (s, 3H), 3.74 (m, 2H).

Methyl (8-{2-[4-(2-methyl-5-quinolinyl)-1-piperazinyl]ethyl}-3-oxo-2,3-dihydro-4H-1,4-benzoxazin-4-yl)acetate (44). The title compound was prepared in 90% yield according to the procedure of **6** starting from intermediate **43** (300 mg, 1.14 mmol). The product was purified by flash chromatography on silica gel eluting with a gradient of 1-2% MeOH in CH₂Cl₂. ¹H-NMR (300 MHz, CDCl₃) δ: 8.37 (m, 1H), 7.71 (m, 1H), 7.58 (m, 1H), 7.24 (m, 1H), 7.07 (m, 1H), 6.95 (m, 2H), 6.64 (m, 1H), 4.70 (s, 2H), 4.66 (s, 2H), 3.79 (s, 3H), 3.16 (bs, 4H), 2.98-2.74 (m, 8H), 2.73 (s, 3H). MS *m/z* 475.20 [M+H]⁺.

(8-{2-[4-(2-Methyl-5-quinolinyl)-1-piperazinyl]ethyl}-3-oxo-2,3-dihydro-4H-1,4-benzoxazin-4-yl)acetic acid (45). To a suspension of intermediate **44** (300 mg, 0.633 mmol) in methanol (5 mL) was added 2N NaOH solution in MeOH (5 mL). The mixture was allowed to stir at room temperature for 4 h before addition of water (10 mL). The MeOH was evaporated under reduced pressure and the residue was acidified with 6N aqueous HCl (5 mL). This mixture was applied to a SPE-SCX cartridge (eluting with 1:1 water/MeOH then 2N ammonia solution in MeOH) to afford the title compound (290 mg, 99%) as a white solid. ¹H-NMR (300 MHz, DMSO-d₆) δ: 8.30 (m, 1H), 7.58-7.52 (m, 2H), 7.34 (m, 1H), 7.07 (m, 1H), 6.90-6.80 (m, 2H), 6.75 (m, 1H), 4.60 (s, 2H), 4.21 (s, 2H), 3.01 (bs, 4H), 2.84-2.53 (m, 11H). MS *m/z* 461.2 [M+H]⁺.

8-{2-[4-(2-Methyl-5-quinolinyl)-1-piperazinyl]ethyl}-4-(3,3,3-trifluoro-2-oxopropyl)-2H-1,4-benzoxazin-3(4H)-one (46). To a suspension of intermediate **45** (D35) (115 mg, 0.25 mmol) in toluene (10 mL) at room temperature was added oxalyl chloride (64 μL, 0.75 mmol, 3 eq) and a drop of DMF. The suspension was heated to 60°C for 3 h. Removal of the solvent under reduced pressure gave the crude acid chloride which was used directly in the subsequent reaction. To a suspension of this crude

acid chloride (120 mg, 0.25 mmol) in CH₂Cl₂ (10 mL) at room temperature were added trifluoroacetic anhydride (315 μ l, 1.5 mmol, 6 eq) and pyridine (162 μ l, 2 mmol, 8 eq). After stirring for 2 h, water (15 mL) was added before the reaction mixture was basified by the addition of saturated aqueous NaHCO₃ (10 mL). The organic phase was separated and the remaining aqueous phase was extracted with CH₂Cl₂ (3 x 50 mL). The combined organic phases were dried over MgSO₄ and evaporated under reduced pressure to give a crude product which was subjected to flash column chromatography on silica gel eluting with 3% MeOH in CH₂Cl₂ to afford the title compound (79 mg, 60%) as a yellow solid (hydrate of the ketone). ¹H-NMR (300 MHz, DMSO-d₆) δ : 8.37 (m, 1H), 7.70 (m, 1H), 7.58 (m, 1H), 7.23 (m, 1H), 7.08-6.81 (m, 4H), 5.15-4.20 (m, 4H), 3.14 (bs, 4H), 2.95-2.60 (m, 11H). MS *m/z* 531.1 [M+H]⁺.

6-{2-[4-(2-Methyl-5-quinolinyl)-1-piperazinyl]ethyl}-2-(trifluoromethyl)-4H-imidazo[2,1-c][1,4]benzoxazine dihydrochloride (9). Intermediate **46** (79 mg, 0.149 mmol) was reacted with NH₄OAc (230 mg, 2.98 mmol, 20 eq.) in AcOH (2 mL) in a similar way to the preparation of **8**. The crude product was purified by flash chromatography on silica gel eluting with a gradient of 1 to 2% MeOH in CH₂Cl₂ to afford the title compound (42 mg, 58%) as the free base. This was treated with HCl (2.2 eq. of 1.25N solution in MeOH) to afford the title compound as a yellow solid. ¹H-NMR (500 MHz, DMSO-d₆) δ : 10.52 (bs, 1H), 8.74 (s, 1H), 8.63 (bs, 1H), 7.79 (m, 3H), 7.61 (bs, 1H), 7.30 (m, 2H), 7.22 (t, 1H), 5.47 (s, 2H), 3.75 (d, 2H), 3.70-3.10 (m, 10H), 2.77 (s, 3H). MS *m/z* 494.30 [M+H]⁺.

Preparation of Compounds 17 and 24.

***N,N*-Dimethyl-6-(2-propen-1-yl)-4H-imidazo[5,1-c][1,4]benzoxazine-3-carboxamide (52a).** 1-Hydroxybenzotriazole (80 mg, 0.59 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (113 mg, 0.59 mmol) were added to a solution of intermediate **48** (150 mg, 0.59 mmol) and dimethylamine (0.35 mL of a 2M sol. in THF, 0.7 mmol) in a 2:1 solution of DMF/ CH₂Cl₂ (6 mL). The reaction mixture was stirred at room temperature for 4 h, DMF was removed by SPE-SCX cartridge (eluting with MeOH followed by 2N ammonia solution in MeOH). The crude product was then purified by SPE-SI cartridge eluting with 5% MeOH in CH₂Cl₂ to afford the title compound (68 mg, 41%) as a

white solid. ¹H-NMR (300 MHz, CDCl₃) δ: 7.85 (s, 1H), 7.35 (d, 1H), 7.05 (d, 1H), 6.95 (t, 1H), 5.95 (m, 1H), 5.5 (s, 2H), 5.05 (m, 2H), 3.6 (s, 3H), 3.45 (d, 2H), 3.1 (s, 3H). MS *m/z* 284.2 [M+H]⁺.

***N*-Cyclopentyl-6-(2-propen-1-yl)-4*H*-imidazo[5,1-*c*][1,4]benzoxazine-3-carboxamide (52b).** The title compound was prepared in 21% yield according to the general procedure described above for **52a** from intermediate **48** (150 mg, 0.59 mmol) and cyclopentylamine (70 μL, 0.07 mmol). ¹H-NMR (300 MHz, CDCl₃) δ: 7.85 (s, 1H), 7.3 (d, 1H), 6.9-7.1 (m, 3H), 5.95 (m, 1H), 5.55 (s, 2H), 5.05 (m, 2H), 4.35 (q, 1H), 3.45 (d, 2H), 2-2.1 (m, 2H), 1.45-1.8 (m, 6H). MS *m/z* 324 [M+H]⁺.

***N,N*-Dimethyl-6-(2-oxoethyl)-4*H*-imidazo[5,1-*c*][1,4]benzoxazine-3-carboxamide (53a).** 4-Methylmorpholine *N*-oxide (50 mg, 0.42 mmol) and osmium tetroxide (50 μl of a 4% by wt. solution in water, 0.035 eq) were added to a solution of intermediate **52a** (D8) (60 mg, 0.21 mmol) in a 8:1 mixture of acetone/water (2.25 mL). The reaction mixture was stirred for 4 h and then quenched with a saturated aqueous solution of Na₂SO₃ (10 mL). After 30 min. stirring the mixture was extracted with AcOEt (3 x 10 mL). The combined organic layers were dried over Na₂SO₄ and concentrated in vacuo to give the intermediate 6-(2,3-dihydroxypropyl)-*N,N*-dimethyl-4*H*-imidazo[5,1-*c*][1,4]benzoxazine-3-carboxamide (40 mg, 0.14mmol, 60%) which was taken-up in a 1:1 mixture of THF/water (2 mL) without further purification. Sodium periodate (43 mg, 0.21 mmol) was added and the mixture stirred for 1 h. After evaporation of THF, the residue was partitioned between water (10 mL) and CH₂Cl₂ (3 x 10 mL). The combined organic layers were dried over Na₂SO₄ and concentrated in vacuo. The crude material was purified by chromatography eluting with 2% MeOH in CH₂Cl₂ to give the corresponding 6-[2,2-bis(methyloxy)ethyl]-*N,N*-dimethyl-4*H*-imidazo[5,1-*c*][1,4]benzoxazine-3-carboxamide (25 mg). Hydrolysis of the acetal with HCl (0.15 mL, 1 M solution in Et₂O) and further evaporation of the volatiles gave the title compound (20 mg, 33%) as a white solid. ¹H-NMR (300 MHz, CDCl₃) δ: 9.75 (s, 1H), 7.8 (s, 1H), 7.45 (t, 1H), 7.05 (d, 1H), 6.75 (d, 1H), 5.5 (s, 2H), 3.55 (s, 3H), 3.8 (s, 2H), 3.1 (s, 3H).

***N*-Cyclopentyl-6-(2-oxoethyl)-4*H*-imidazo[5,1-*c*][1,4]benzoxazine-3-carboxamide (53b).**

Intermediate **52b** (D10) (37 mg, 0.12 mmol) was treated with 4-methylmorpholine *N*-oxide (28 mg, 0.24 mmol) and osmium tetroxide (29 μ l of a 4% by wt. solution in water, 0.035 eq) according to the method described for **53a** to afford the title compound as a white solid (25 mg, 67%) which was used without further purification; $^1\text{H-NMR}$ (300 MHz, CDCl_3) δ : 9.8 (s, 1H), 7.8 (s, 1H), 7.45 (t, 1H), 6.7-7.1 (m, 3H), 5.5 (s, 2H), 4.4 (q, 1H), 3.8 (s, 2H), 1.4-1.9 (m, 8H).

***N,N*-Dimethyl-6-{2-[4-(2-methyl-5-quinolinyl)-1-piperazinyl]ethyl}-4*H*-imidazo[5,1-*c*][1,4]benzoxazine-3-carboxamide dihydrochloride (17)**. The title compound was prepared in 60% yield following the general procedure described for **6** starting from intermediate **53a** (20 mg, 0.07 mmol) and 2-methyl-5-piperazin-1-yl-quinoline (19 mg, 0.084 mmol). $^1\text{H-NMR}$ (500 MHz, DMSO-d^6) δ : 11 (bs, 1H), 8.77 (bs, 1H), 8.57 (s, 1H), 7.9 (bs+d, 3H), 7.69 (bs, 1H), 7.36 (bs, 1H), 7.25 (d, 1H), 7.15 (t, 1H), 5.54 (s, 2H), 3.7-3.2 (m, 12H), 2.96-2.82 (2s, 6H), 2.5 (m, 3H). MS m/z 497.3 $[\text{M}+\text{H}]^+$.

***N*-Cyclopentyl-6-{2-[4-(2-methyl-5-quinolinyl)-1-piperazinyl]ethyl}-4*H*-imidazo[5,1-*c*][1,4]benzoxazine-3-carboxamide dihydrochloride (24)**. The title compound was prepared in 40% yield following the general procedure described for **6** starting from intermediate **53b** (25 mg, 0.08 mmol) and 2-methyl-5-piperazin-1-yl-quinoline (20 mg, 0.09 mmol). $^1\text{H-NMR}$ (500 MHz, DMSO-d^6) δ : 10.79 (bs, 1H), 8.67 (bs, 1H), 8.58 (s, 1H), 7.9 (d, 1H), 7.8 (d+bs, 3H), 7.6 (bs, 1H), 7.32 (bs, 1H), 7.24 (d, 1H), 7.15 (t, 1H), 5.59 (s, 2H), 4.18 (m, 1H), 3.7-3 (m, 12H), 2.78 (bs, 3H), 1.83 (m, 2H), 1.67 (m, 2H), 1.52 (m, 4H). MS m/z 537.3 $[\text{M}+\text{H}]^+$.

Preparation of Compound 18.

6-{2-[4-(2-Methyl-5-quinolinyl)-1-piperazinyl]ethyl}-4*H*-imidazo[5,1-*c*][1,4]benzoxazine-3-carbonitrile dihydrochloride (18). To an ice cooled solution of the free base of **20** (23 mg, 0.049 mmol) in THF (0.5 mL) and pyridine (0.008 mL, 0.098 mmol), trifluoroacetic anhydride (0.008 mL, 0.054 mmol) was added and the resulting solution was stirred at 0°C for 1 h. The crude reaction mixture was applied to a SPE-SCX cartridge (eluting with MeOH followed by 2*N* ammonia solution in MeOH). After evaporation of solvent from the ammonia fractions, the resulting mixture was further purified by

SPE cartridge (silica gel, 2g) eluting with 2% MeOH in CH₂Cl₂ to afford the free base of the title compound (7 mg, 32%) as an off-white solid. The free base was treated with HCl (2.1 eq. of 1N solution in Et₂O) in dry MeOH at 0°C. Evaporation of solvent and trituration with Et₂O gave the title compound as a yellow solid. ¹H-NMR (500 MHz, DMSO-d⁶) δ: 10.93 (bs, 1H), 8.92 (bs, 1H), 8.79 (s, 1H), 7.95 (bs, 2H), 7.91 (d, 1H) 7.83 (bs, 1H), 7.46 (bs, 1H), 7.34 (d, 1H), 7.24 (t, 1H), 5.56 (s, 2 H), 4-3 (m, 12H), 2.91(bs, 3H). MS *m/z* 451.3 [M+H]⁺.

Compounds 21-23 and 25-29: General procedure for amide formation

To a stirred solution of intermediate **65** (1 eq) and DIPEA (1.1 eq) in DMF was added TBTU (1.1 eq) and the resulting solution was stirred for 1 h at room temperature. The desired amine (1.1 eq) was added and the solution stirred for 1 h. The crude solution was applied to a SPE-SCX cartridge (eluting with MeOH followed by 2N ammonia solution in MeOH). After evaporation of solvent from the ammonia fractions and trituration with Et₂O, the free base of the desired compound was isolated in pure form. The free base was dissolved in dry MeOH and treated with HCl (2.1 eq. of 1N solution in Et₂O) at 0°C. Evaporation of solvent and trituration with Et₂O gave the final compounds in pure form.

***N*-(1-Methylethyl)-6-{2-[4-(2-methyl-5-quinolinyl)-1-piperazinyl]ethyl}-4*H*-imidazo[5,1-*c*][1,4]benzoxazine-3-carboxamide dihydrochloride (**21**)**. The title compound was prepared in 83% yield from intermediate **65** following the general procedure for amide formation using isopropylamine. ¹H-NMR (300 MHz, DMSO-d⁶) δ: 11.41 (bs, 1H), 9.07 (d, 1H), 8.61 (s, 1H), 8.09 (d, 1H), 8.02 (t, 1H), 7.92 (d, 1H), 7.87 (s, 1H), 7.85 (dd, 1H), 7.51 (d, 1H), 7.25 (d, 1H), 7.14 (t, 1H), 5.59 (s, 2H), 4.11-4.01 (m, 1H), 3.75-3.71 (bd, 2H), 3.53-3.49 (bd, 4H), 3.43-3.39 (bd, 4H), 3.22-3.15 (m, 2H), 2.98 (s, 3H), 1.15 (d, 6H). MS *m/z* 511.4 [M+H]⁺.

***N*-Cyclopropyl-6-{2-[4-(2-methyl-5-quinolinyl)-1-piperazinyl]ethyl}-4*H*-imidazo[5,1-*c*][1,4]benzoxazine-3-carboxamide dihydrochloride (**22**)**. The title compound was prepared in 60% yield from intermediate **65** following the general procedure for amide formation using cyclopropylamine. ¹H-NMR (300 MHz, DMSO-d⁶) δ: 11.24 (bs, 1H), 9.06 (d, 1H), 8.58 (s, 1H), 8.18

(d, 1H), 8.05(s, 1H), 8.01 (t, 1H), 7.91 (d, 1H), 7.85 (dd, 1H), 7.50 (d, 1H), 7.25 (d, 1H), 7.14 (t, 1H), 5.59 (s, 2H), 4.11-3.71 (m, 2H), 3.50-3.45 (bd, 4H), 3.42-3.38 (bd, 4H), 3.22-3.15 (m, 2H), 2.98 (s, 3H), 2.82 (m, 1H), 0.65-0.62 (m, 6H). MS m/z 509.4 [M+H]⁺.

***N*-Cyclobutyl-6-{2-[4-(2-methyl-5-quinolinyl)-1-piperazinyl]ethyl}-4*H*-imidazo[5,1-*c*][1,4]benzoxazine-3-carboxamide dihydrochloride (23).** The title compound was prepared in 83% yield from intermediate **65** following the general procedure for amide formation using cyclobutylamine. ¹H-NMR (300 MHz, DMSO-*d*⁶) δ : 11.39 (bs, 1H), 9.07 (d, 1H), 8.62 (s, 1H), 8.37 (d, 1H), 8.11 (d, 1H), 8.01 (t, 1H), 7.91 (d, 1H), 7.85 (dd, 1H), 7.50 (d, 1H), 7.25 (d, 1H), 7.14 (t, 1H), 5.59 (s, 2H), 4.46-4.35 (m, 1H), 3.78-3.71 (bm, 2H), 3.56-3.49 (bd, 4H), 3.43-3.39 (bd, 4H), 3.22-3.15 (m, 2H), 2.98 (s, 3H), 2.17-2.10 (m, 4H), 1.65-1.58 (m, 2H). MS m/z 523.4 [M+H]⁺.

(Cyclopropylmethyl)-6-{2-[4-(2-methyl-5-quinolinyl)-1-piperazinyl]ethyl}-4*H*-imidazo[5,1-*c*][1,4]benzoxazine-3-carboxamide dihydrochloride (25). The title compound was prepared in 60% yield from intermediate **65** following the general procedure for amide formation using cyclopropanemethylamine. ¹H-NMR (300 MHz, DMSO-*d*⁶) δ : 11.50 (bs, 1H), 9.05 (d, 1H), 8.60 (s, 1H), 8.21 (bs, 1H), 8.01 (t, 1H), 7.91 (d, 1H), 7.85 (dd, 1H), 7.50 (d, 1H), 7.25 (d, 1H), 7.14 (t, 1H), 5.59 (s, 2H), 3.74 (bd, 2H), 3.53-3.49 (bd, 4H), 3.42-3.38 (bd, 4H), 3.22-3.15 (m, 2H), 3.10 (t, 2H), 2.97 (s, 3H), 1.04-1.00 (m, 1H), 0.40 (d, 2H), 0.22 (d, 2H). MS m/z 523.4 [M+H]⁺.

3-(1-Azetidinylcarbonyl)-6-{2-[4-(2-methyl-5-quinolinyl)-1-piperazinyl]ethyl}-4*H*-imidazo[5,1-*c*][1,4]benzoxazine dihydrochloride (26). The title compound was prepared in 48% yield from intermediate **65** following the general procedure for amide formation using cyclobutylamine. ¹H-NMR (300 MHz, DMSO-*d*⁶) δ : 10.98 (bs, 1H), 8.98 (bd, 1H), 8.57 (s, 1H), 7.98-7.96 (bd, 2H), 7.87-7.81 (m, 2H), 7.47 (bt, 1H), 7.24 (dd, 1H), 7.15 (t, 1H), 5.59 (s, 2H), 4.54 (t, 2H), 4.00 (t, 2H), 3.75-3.34 (m, 10H), 3.20-3.15 (m, 2H), 2.92 (s, 3H), 1.96 (m, 2H). MS m/z 509.4 [M+H]⁺.

6-{2-[4-(2-Methyl-5-quinolinyl)-1-piperazinyl]ethyl}-3-(1-pyrrolidinyl-carbonyl)-4*H*-imidazo[5,1-*c*][1,4]benzoxazine dihydrochloride (27). The title compound was prepared in 81% yield

from intermediate **65** following the general procedure for amide formation using pyrrolidine. ¹H-NMR (300 MHz, DMSO-d⁶) δ: 11.56 (bs, 1H), 9.06 (d, 1H), 8.57 (s, 1H), 8.10 (d, 1H), 8.02 (t, 1H), 7.91 (d, 1H), 7.82 (dd, 1H), 7.51 (d, 1H), 7.24 (dd, 1H), 7.15 (t, 1H), 5.59 (s, 2H), 4.01 (t, 2H), 3.72 (bd, 2H), 3.53-3.35 (m, 10H), 3.21-3.17 (m, 2H), 2.98 (s, 3H), 1.94-1.75 (m, 4H). MS *m/z* 523.4 [M+H]⁺.

6-{2-[4-(2-Methyl-5-quinolinyl)-1-piperazinyl]ethyl}-3-(1-piperidinyl-carbonyl)-4*H*-imidazo[5,1-*c*][1,4]benzoxazine dihydrochloride (28). The title compound was prepared in 94% yield from intermediate **65** following the general procedure for amide formation using piperidine. ¹H-NMR (300 MHz, DMSO-d⁶) δ: 11.55 (bs, 1H), 9.06 (d, 1H), 8.57 (s, 1H), 8.10 (d, 1H), 8.02 (t, 1H), 7.91 (d, 1H), 7.82 (dd, 1H), 7.51 (d, 1H), 7.24 (dd, 1H), 7.15 (t, 1H), 5.53 (s, 2H), 4.46-4.05 (m, 2H), 3.72 (bd, 2H), 3.53-3.49 (bd, 4H), 3.44-3.35 (bd, 6H), 3.21-3.17 (bm, 2H), 2.98 (s, 3H), 1.63-1.62 (bd, 2H), 1.54-1.53 (bd, 4H). MS *m/z* 537.4 [M+H]⁺.

6-{2-[4-(2-Methyl-5-quinolinyl)-1-piperazinyl]ethyl}-3-(4-morpholinyl carbonyl)-4*H*-imidazo[5,1-*c*][1,4]benzoxazine dihydrochloride (29). The title compound was prepared in 33% yield from intermediate **65** following the general procedure for amide formation using morpholine. ¹H-NMR (300 MHz, DMSO-d⁶) δ: 11.46 (bs, 1H), 9.04 (d, 1H), 8.59 (s, 1H), 8.06 (d, 1H), 8.00 (t, 1H), 7.90-7.83 (m, 2H), 7.48 (d, 1H), 7.26 (d, 1H), 7.15 (t, 1H), 5.56 (s, 2H), 4.31 (m, 4H), 3.93-3.56 (m, 6H), 3.53-3.49 (bd, 4H), 3.42-3.38 (bd, 4H), 3.22-3.17 (m, 2H), 2.95 (s, 3H). MS *m/z* 537.4 [M+H]⁺.

Preparation of Compounds 15 and 16.

***N*-Methyl-*N*-(methoxy)-6-{2-[4-(2-methyl-5-quinolinyl)-1-piperazinyl]ethyl}-4*H*-imidazo[5,1-*c*][1,4]benzoxazine-3-carboxamide (66).** To a stirred solution of intermediate **65** (54 mg, 0.115 mmol) and DIPEA (0.24 mL, 0.138 mmol) in DMF (1 mL) at room temperature was added TBTU (41 mg, 0.12 mmol). After stirring for 1 h *N,O*-dimethylhydroxylamine hydrochloride amine (13.5 mg, 0.138 mmol) was added and the reaction mixture was stirred for a further 1 h. The reaction mixture was applied to a SPE-SCX cartridge (eluting with MeOH followed by 2N ammonia solution in MeOH) to afford, after triturating with Et₂O, the title compound (40 mg, 68%). ¹H-NMR (300 MHz, DMSO-d⁶) δ: 8.38 (d, 1H),

7.93 (s, 1H), 7.72 (d, 1H), 7.57 (t, 1H), 7.32 (d, 1H), 7.26 (bd, 1H), 7.15 (d, 1H), 7.09-6.99 (m, 2H), 5.53 (s, 2 H), 3.84 (bs, 3H), 3.55 (bs, 3H), 3.15 (bs, 4H), 2.99-2.94 (m, 2H), 2.85 (m, 4H), 2.75-2.72 (m, 5H). MS m/z 513.4 $[M+H]^+$.

1-(6-{2-[4-(2-Methyl-5-quinolinyl)-1-piperazinyl]ethyl}-4H-imidazo[5,1-c][1,4]benzoxazin-3-yl)ethanone hydrochloride (15). To an ice cooled stirred solution of intermediate **66** (40 mg, 0.078 mmol) in THF (1 mL), methyl magnesium bromide (0.03 mL of a 3M solution in Et₂O, 0.09 mmol) was added and the resulting solution was stirred for 1 h at 0°C. The reaction mixture was poured into cold aqueous HCl (2 mL of 2.5N solution), then treated with NaHCO₃ (15 mL) and extracted with CH₂Cl₂ (3 x 15 mL). The combined organic layers were dried over Na₂SO₄ and evaporated in vacuo to give a brown oil that was purified by SPE cartridge (silica gel, 2 g) eluting with 2% MeOH in CH₂Cl₂ to afford the free base of the title compound (22 mg, 60%) as a white solid. The free base was treated with HCl (2.1 eq. of 1N solution in Et₂O) in dry MeOH at 0°C. Evaporation of solvent and trituration with Et₂O gave the title compound as a yellow solid. ¹H-NMR (500 MHz, DMSO-d₆) δ: 11.41 (bs, 1H), 9.08 (d, 1H), 8.66 (d, 1H), 8.2-7.08 (m, 4H) 7.52 (dd, 1H), 7.3 (dd, 1H), 7.19 (t, 1H), 5.62 (s, 2 H), 4.2-3.2 (m, 12H), 2.99 (s, 3H), 2.5 (s, 3H). MS m/z 468.4 $[M+H]^+$.

***E/Z*-1-(6-{2-[4-(2-Methyl-5-quinolinyl)-1-piperazinyl]ethyl}-4H-imidazo[5,1-c][1,4]benzoxazin-3-yl)ethanone O-methyloxime dihydrochloride (16).** A solution of the free base of compound **15** (75 mg, 0.160 mmol), pyridine (4 mL) and methoxylamine hydrochloride (27 mg, 0.32 mmol) in 95% EtOH (4 mL) was stirred at reflux for 2 h. The solvent was evaporated *in vacuo* and the residue dissolved in water and extracted with CH₂Cl₂ (3 x 15 mL). The combined organic layers were dried over Na₂SO₄ and evaporated in vacuo and the resulting brown oil was purified by SPE-SiI cartridge (2 g) eluting with 4% MeOH in CH₂Cl₂ to afford the free base of the title compound (80 mg, 100%) as a white solid. The free base was treated with HCl (2.1 eq. of 1N solution in Et₂O) in dry MeOH at 0°C. Evaporation of solvent and trituration with Et₂O gave the title compound as a yellow solid (mixture of *E/Z* isomers with 85/15 ratio). ¹H-NMR (500 MHz, DMSO-d₆) δ: 11.29 (bs, 1H), 8.95 (bd, 1H), 8.58 (s, 1H), 7.99 (m, 2H), 7.84

(m, 2H), 7.46 (bd, 1H), 7.3-7.1 (m, 2H), 5.48 (s, 2 H), 3.89 (s, 3H), 3.8-3.1 (m, 12H), 2.92 (s, 3H), 2.2 (s, 3H). MS m/z 497.4 [M+H]⁺.