Development of benzo[1,4]oxazines as potent biofilm inhibitors and dispersal agents against *Vibrio cholerae*.

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**Contents**

General remarks S2  
Complete list of compounds tested S3 - S5  
BIC$_{50}$ curves for active compounds S6 – S7  
EC$_{50}$ curves of the antibiotics used in the co-dosing experiments S8 - S10  
Flow cell experiments of oxazine 25 S11  
BioMAP antibacterial profiling of oxazine 25 S12  
CFU analysis of oxazine 25 S13  
HeLa cell line toxicity study of oxazine 25 S14  
Stability study of oxazine 25 in culture media S15  
Experimental procedures S16 - S32  
$^1$H and $^{13}$C NMR spectra S33 – S73  
References S74
**General remarks**

All reactions were performed in an open flask using acetone washed, oven dried glassware with magnetic stirring and if required heated through the use of Dry Syn™ blocks. All reagents used were acquired from chemical supply companies or, as indicated in the individual experimental details, prepared within the laboratory. Reactions that were performed at 0 °C were done so using water/ice baths. All solvents used in the course of the project were obtained from the departmental Grubbs solvent system.

Analytical thin layer chromatography (TLC) was carried out utilizing aluminium backed Merck TLC plates (silica gel 60 F254) and visualized with UV light (254 nm) or basic KMnO₄ solution. Flash column chromatography was performed using Alfa Aesar, silica gel 60, 0.032 – 0.063 mm (230 – 450 mesh) as the stationary phase. Columns were typically packed as a slurry, with the eluent used for a particular purification noted within the individual experimental details for each reaction.

All "H and "C NMR spectra were obtained on either a Varian Unity 500+ or a Varian Inova 600 MHz spectrometer equipped with a 5 mm HCN triple resonance cryoprobe. Chemical shifts are expressed in parts per million (ppm) downfield from tetramethylsilane (TMS). All coupling constants given are in Hz. High resolution mass spectrometry was performed on an Agilent 6230 electrospray ionization (ESI) accurate-mass time-of-flight (TOF) liquid chromatograph/ mass spectrometer.

All procedures for determination of the biofilm inhibitory concentration (BIC₅₀) occurred as previously described.¹ See individual experimental details for antibiotic and co-dosing procedure. All BIC₅₀ and BDC₅₀ reported are the result of three biological replicates each consisting of two technical replicates.
**Table 1.** A complete list of the compounds screened as biofilm inhibitors against *Vibrio cholerae*

| Compound | Structure | BIC<sub>50</sub> / µM | BDC<sub>50</sub> / µM | Compound | Structure | BIC<sub>50</sub> / µM | BDC<sub>50</sub> / µM |
|----------|-----------|------------------------|------------------------|----------|-----------|------------------------|------------------------|
| 1        | ![Structure 1](image1) | 63                     | -                      | 15       | ![Structure 15](image15) | 122                    | -                      |
| 8        | ![Structure 8](image8) | >500                   | -                      | 16<sup>c</sup> | ![Structure 16c](image16c) | 200                    | -                      |
| 9        | ![Structure 9](image9) | >500                   | -                      | 17<sup>c</sup> | ![Structure 17c](image17c) | 195                    | -                      |
| 10       | ![Structure 10](image10) | >500                   | -                      | 18       | ![Structure 18](image18) | >500                   | -                      |
| 11       | ![Structure 11](image11) | >500                   | -                      | 19       | ![Structure 19](image19) | >500                   | -                      |
| 12       | ![Structure 12](image12) | >500                   | -                      | 20       | ![Structure 20](image20) | >500                   | -                      |
| 13<sup>b</sup> | ![Structure 13b](image13b) | 175                    | -                      | 21       | ![Structure 21](image21) | 45                     | -                      |
| 14<sup>c</sup> | ![Structure 14c](image14c) | 95                     | -                      | 22       | ![Structure 22](image22) | 13                     | -                      |
Table 1 continued. A complete list of the compounds screened as biofilm inhibitors against *Vibrio cholerae*

| Compound | Structure | BIC<sub>50</sub> / µM | BDC<sub>50</sub> / µM | Compound | Structure | BIC<sub>50</sub> / µM | BDC<sub>50</sub> / µM |
|----------|----------|------------------------|-----------------------|----------|----------|------------------------|-----------------------|
| 24       | ![Structure](image1) | >500 -                |                      | 30       | ![Structure](image2) | >500 -                |                      |
| 23       | ![Structure](image3) | >500 -                |                      | 31       | ![Structure](image4) | >500 -                |                      |
| 25       | ![Structure](image5) | 6 13                  |                      | 32       | ![Structure](image6) | >500 -                |                      |
| 26       | ![Structure](image7) | >500 -                |                      | 33       | ![Structure](image8) | >500 -                |                      |
| 27       | ![Structure](image9) | >500 -                |                      | 34       | ![Structure](image10) | >500 -                |                      |
| 28       | ![Structure](image11) | >500 -                |                      | 35       | ![Structure](image12) | >500 -                |                      |
| 29       | ![Structure](image13) | >500 -                |                      | 36       | ![Structure](image14) | >500 -                |                      |
Table 1 continued. A complete list of the compounds screened as biofilm inhibitors against *Vibrio cholerae*

| compound | structure | BIC_{50} / \mu M | BDC_{50} / \mu M | compound | structure | BIC_{50} / \mu M | BDC_{50} / \mu M |
|----------|-----------|----------------|----------------|----------|-----------|----------------|----------------|
| 37       | ![Structure 37](image1) | >500            | -              | 3        | ![Structure 3](image2) | >500            | -              |
| 38       | ![Structure 38](image3) | >500            | -              | 4        | ![Structure 4](image4) | >500            | -              |
| 39       | ![Structure 39](image5) | >500            | -              | 5        | ![Structure 5](image6) | >500            | -              |
| 40       | ![Structure 40](image7) | >500            | -              | 6        | ![Structure 6](image8) | >500            | -              |
| 41       | ![Structure 41](image9) | >500            | -              | 7        | ![Structure 7](image10) | >500            | -              |

[a] BIC_{50} and BDC_{50} determined with 3 biological replicates each consisting of two technical replicates. For the biofilm dispersal assay, the appropriate compound, antibiotic or DMSO control was pinned into the well following two hours of incubation and then subsequently incubated for a further 4 hours.

[b] Major isomer shown. Stereochemistry of the major isomer determined by long range nOe interaction as shown in subsequent section of SI.

[c] Major isomer assumed based upon nOe interaction observed in oxazine 13.
Supporting Information

BIC$_{50}$ and BDC$_{50}$ curves for active biofilm inhibitors and dispersal agents
Supporting Information

EC₅₀ curves of the antibiotics used in the co-dosing experiments

Preformed Biofilm Screening Overview

Experimental procedure

The preformed biofilm screen followed the general experimental procedure developed in P. aeruginosa.¹ For the V. cholerae biofilm dispersal assay (BDC₅₀), compound, antibiotic or DMSO control were pinned into the screening plate following two hours of incubation and incubated for a further 4 hours at 32°C. An identical washing and analytical procedure to that reported in the literature was performed.¹ For the antibiotic co-dosing experiments, both oxazine 25 and the relevant antibiotic were added after 2 hours of incubation and OD₆₀₀ readings immediately taken to determine initial OD₆₀₀ values. After incubation, OD₆₀₀ readings were acquired, and the change in OD₆₀₀ values used as a measure of cell growth for each well. Immediately following OD₆₀₀ readings, the plates were washed, PBS buffer added, and each well imaged using our standard protocol to determine biofilm coverage.

Data interpretation

In both the biofilm inhibition and dispersal assays, four outcomes are possible for any assay well. In the case of strong antibiotic activity, both planktonic and attached cells are eliminated, and the resulting screening images are blank, with low OD₆₀₀ readings (Image A). For compounds capable of eradicating only the planktonic cells without impacting attached cells a lower OD₆₀₀ reading would be expected, but with retention of large biofilm colonies in the image (Image B). If the compound has no effect on planktonic or biofilm-associated cells, then both the OD₆₀₀ and biofilm coverage are high (Image C). Finally if the compound is capable of only inducing detachment of the bacteria with no bactericidal effects then an OD₆₀₀ reading of close to 1.0 would be expected and cellular imaging would show only planktonic cells, without the presence of large mature biofilm colonies (Image D).
Supporting Information

Addition of antibiotic at $t = 0$

[Images and graphs showing the effect of various antibiotics on cell growth]
Addition of antibiotic at $t = 2$
Flow cell experiments of oxazine 25

Overnight culture of rugose wild type *V. cholerae* (A1552 harboring a Tn7GFP insertion) was diluted 200-fold into 2% LB medium containing either the indicated concentration of test compound or an equal volume of DMSO as a vehicle control and inoculated into an Ibidi µ 0.46 6-well flow cell. After 1 hour of static incubation at room temperature, flow of 2% LB containing test compound or DMSO was initiated at 7.5 mL/minute at room temperature for 6 hours. Flow cells were imaged on a Zeiss LSM5 confocal microscope. Z-projections of Z-stacks were created with the FIJI build of Image J. Quantitative analysis of images was performed with COMSTAT.²

| Compound 25 / µM | 0   | 63  | 100 | 125 | 200 | 250 |
|------------------|-----|-----|-----|-----|-----|-----|
| Mean Biomass (µm²/µm²) | 2.939 | 1.866 | 1.411 | 1.480 | 0.463 | 0.880 |
| Std. Deviation   | 0.294 | 0.206 | 0.234 | 0.208 | 0.281 | 0.173 |
| Fold reduction   | 1.58 | 2.08 | 2.09 | 6.35 | 3.34 |
BioMAP antibacterial profiling of oxazine

The antibacterial profiling of oxazine followed that previously reported in the literature. In brief, the screening panel consisted of six Gram-positive strains (BSL1: *Bacillus subtilis* 168, *Staphylococcus epidermidis* [ATCC 14990], *Enterococcus faecium* [ATCC 6569], *Listeria ivanovii* [BAA-139]; BSL2: *S. aureus* [ATCC 29213], methicillin-resistant *S. aureus* (MRSA) [BAA-44] and nine Gram-negative strains (BSL1: *Escherichia coli* K12 [BW 25113], *Acinetobacter baumanii* [NCIMB 12457], *Enterobacter aerogenes* [ATCC 35029], *Ochrobactrum anthropi* [ATCC 49687], *Providencia alcalifaciens* [ATCC 9886]; BSL2: *Yersinia pseudotuberculosis* [IP2666 pIBI], *Pseudomonas aeruginosa* [ATCC 27835], *Salmonella typhimurium* LT2, *Vibrio cholerae* O1 [biotype El Tor A1552, smooth variant (Fy_Vc_1)].

All staphylococcal strains, *L. ivanovii* and *E. faecium* cultures were grown in 10 mL of tryptic soy broth (17 g tryptone, 3 g soytone, 2.5 g dextrose, 5 g NaCl and 2.5 g dipotassium phosphate in 1 L distilled water; pH 7.5). *P. alcalifaciens*, *O. anthropi*, *E. aerogenes* and *A. baumanii* were grown in nutrient broth (Difco, USA) while *B. subtilis*, *E. coli*, *V. cholerae*, *S. typhimurium*, *P. aeruginosa* and *Y. pseudotuberculosis* cultures were grown in Luria Broth (10 g tryptone, 5 g yeast extract and 10 g NaCl in 1 L distilled water; pH 7.5). All three media were autoclaved at 121°C for 30 min. Inoculated cultures were grown overnight in a shaker (200 rpm; 30°C).

Overnight saturated cell cultures of pathogenic strains were diluted 1:1000 with fresh media and 30 µL of culture was dispensed into each well of sterile clear 384-well plates. 200 nL of DMSO prefraction stock solutions were pinned into screening plates using a Perkin Elmer Janus MDT robot. After inoculation, screening plates were stacked in a plate reader/shaker (Perkin Elmer EnVision) and OD\textsubscript{600} readings taken once per hour for 24 h. Computer generated growth curves for serially diluted pure compounds were used to determine MIC values by correlating the OD\textsubscript{600} reading at the pre-exponential phase of the bacteria to the concentrations in individual wells.

| Pathogen                        | MIC of oxazine 25 / µM<sup>a</sup> |
|---------------------------------|-------------------------------------|
| *Bacillus subtilis*             | >200                                |
| *Staphylococcus epidermidis*    | >200                                |
| *Enterococcus faecium*          | >200                                |
| *Listeria ivanovii*             | >200                                |
| *S. aureus*                     | >200                                |
| MRSA                            | >200                                |
| *Escherichia coli*              | >200                                |
| *Acinetobacter baumanii*        | >200                                |
| *Enterobacter aerogenes*        | >200                                |
| *Ochrobactrum anthropi*         | >200                                |
| *Providencia alcalifaciens*     | >200                                |
| *Yersinia pseudotuberculosis*   | >200                                |
| *Pseudomonas aeruginosa*        | >200                                |
| *Salmonella typhimurium*        | >200                                |
| *Vibrio cholerae*               | >200                                |
CFU analysis of oxazine 25

Overnight grown cultures of *V. cholerae* O1, El Tor A1552, rugose variant (Fy_Vc_2) were diluted 1:1000 in the presence of 200 µM, 50 µM and 6 µM of oxazine 25 in LB medium. Cultures were incubated at 30 °C with shaking at 200 rpm. Samples were harvested at specific time points and plated to enumerate CFU/ml. A negative control of doxycycline at 10 µM was also utilized. In all instances growth of *V. cholerae* in the presence of the oxazine 25 was comparable to that of the DMSO control vehicle. It should be noted that at the 24 hour time point a depreciation in CFU is observed. This is typical for such experiments.
HeLa cell line toxicity study of oxazine 25

Cytological profiling was performed as previously described. Plates were imaged using an ImageXpress Micro epifluorescent microscope (Molecular Devices, LLC) with a 10× Nikon objective lens. Images were analysed using MetaXpress (Molecular Devices, LLC). In all instances up to 200 μM, oxazine 25 exerted no toxicity toward HeLa cells, with comparable cellular counts compared to the DMSO control vehicle (see below). White bar indicates a distance of 100 μm.
Stability study of oxazine 25 in culture media

Oxazine 25 (5 mg, 0.02 mmol) was dissolved in DMSO (100 µl) and added in a single portion to the appropriate culture media and, if appropriate, heated to 37 °C. In all instances agitation of the mixture was obtained by mechanical stirring. Following overnight incubation, the solution was diluted with methanol (5 mL) and subjected to reverse-phase HPLC using a Phenomenex Synergy-A 10µ fusion C18 column. An isocratic gradient of 6:4 Methanol/H2O (acidified with 0.02% of formic acid) was used as the solvent system. A wavelength of $\lambda = 254$ nm was used in all instances. The oxazine 25 was identified to have a retention time of 7.0 minutes.
Experimental Details

Methyl-3,5-dimethoxy-2-nitrobenzoate 3

![Methyl-3,5-dimethoxy-2-nitrobenzoate 3](image)

Methyl-3, 5-dimethoxybenzoate 2 (3.0 g, 12.4 mmol) was dissolved in acetic anhydride (20 cm³) and the resulting solution cooled to 0 °C. 70% Nitric acid (1.2 cm³, 18.8 mmol) was introduced dropwise and the subsequent mixture warmed to room temperature and stirred for 15 minutes. The precipitate was filtered, washed with water (3 × 10 cm³) and dried overnight. Recrystallization of the crude material from methanol afforded the title compound as a pale yellow crystalline solid (2.8 g, 94%). δ_H (500 MHz, CDCl₃) 3.80 (3H, s, OC₃H₃), 3.87 (3H, s, OC₃H₃), 3.90 (3H, s, OC₃H₃), 6.96 (1H, d, J 5.0, ArCH), 7.06 (1H, d, J 5.0, ArCH). All data is in accordance with that of the literature.

Methyl-3-hydroxy-5-methoxy-2-nitrobenzoate 4

![Methyl-3-hydroxy-5-methoxy-2-nitrobenzoate 4](image)

Aluminium chloride (2.2 g, 16.8 mmol) was added portionwise to a solution of the ester 2 (1.0 g, 4.2 mmol) in DCM (20 cm³) at 0 °C over a period of 90 minutes. The resulting blood red solution warmed to room temperature and stirred for a further 60 minutes. The reaction mixture was poured into a slurry of 1N HCl (50 cm³) and ice (100 g) and the aqueous phase extracted with ethyl acetate (3 × 50 cm³). The organic layers were combined, washed with brine (50 cm³) and dried over magnesium sulfate. Removal of the solvent in vacuo afforded the title compound as a pale yellow solid (860 mg, 89%). δ_H (500 MHz, CDCl₃) 3.86 (3H, s, OC₃H₃), 3.89 (3H, s, OC₃H₃), 6.52 (1H, s, ArCH), 6.55 (1H, s, ArCH), 10.89 (1H, s, O_H); δ_C (125 MHz, CDCl₃) 53.6, 56.6, 102.9, 110.2, 125.4, 133.5, 158.2, 165.9, 166.9; m/z (ESI-TOF) 228.0603 (100%, MH⁺, C₉H₁₀NO₆ requires 228.0508).

Methyl 3-(benzyloxy)-5-methoxy-2-nitrobenzoate 5

![Methyl 3-(benzyloxy)-5-methoxy-2-nitrobenzoate 5](image)

Nitrophenol 4 (500 mg, 2.2 mmol) was added to a suspension of potassium carbonate (1.2 g, 8.8 mmol) and benzyl bromide (1.0 cm³, 8.8 mmol) in a 1:1 mixture of methanol (8 cm³) and dichloromethane (8 cm³). The mixture was heated at reflux for 3 hours before being cooled to room temperature and poured into a 1N HCl solution (10 cm³). The aqueous phase was extracted with ethyl acetate (3 × 10 cm³) and the organic layers combined, washed with brine (10 cm³) and dried over magnesium sulfate. Removal of the solvent in vacuo yielded a dark orange oil. The oil was triturated with hexane (10 cm³)
and the resultant solid filtered, washed with hexane (10 cm³) and dried to afford the title compound as a pale yellow solid (616 mg, 97%). \( \delta_H \) (500 MHz, CDCl\(_3\)) 3.81 (3H, s, OCH\(_3\)), 3.86 (3H, s, OCH\(_3\)), 5.14 (2H, s, CH\(_2\)), 6.70 (1H, d, J 2.3, ArCH), 6.98 (1H, d, J 2.3, ArCH), 7.28 – 7.36 (5H, m, ArCH); \( \delta_C \) (125 MHz, CDCl\(_3\)) 53.4, 56.2, 71.6, 105.3, 106.3, 125.9, 127.3, 128.1, 128.7 (2 × ArCH), 129.0 (2 × ArCH), 135.3, 151.7, 161.1, 164.1; m/z (ESI-TOF) 318.0979 (100%, MH\(^+\), C\(_{16}\)H\(_{16}\)NO\(_6\) requires 318.0978).

Methyl-2-amino-3-(benzylloxy)-5-methoxybenzoate 6

The benzyl protected nitro aromatic (500 mg, 1.6 mmol) \( 5 \) was added to a suspension of SnCl\(_2\).2H\(_2\)O (1.4 g, 6.4 mmol) in a 3: 1 mixture of ethanol (12 cm³) and 6N HCl (4 cm³) and the mixture heated at reflux for 4 hours. Upon cooling to room temperature, the solid hydrochloride salt was filtered, re-dissolved in ethyl acetate and washed with a saturated aqueous solution of Na\(_2\)CO\(_3\) (15 cm³). The organic layer was separated and the aqueous phase extracted with extracted with ethyl acetate (3 × 15 cm³). The organic layers were combined, washed with brine (10 cm³) and dried over magnesium sulfate. Removal of the solvent in vacuo afforded the title compound as a brown solid that required no further purification (360 mg, 81%). \( \delta_H \) (500 MHz, CDCl\(_3\)) 3.74 (3H, s, OCH\(_3\)), 3.87 (3H, s, OCH\(_3\)), 5.05 (2H, s, CH\(_2\)), 5.73 (2H, s, NH\(_2\)), 6.63 (1H, d, J 2.7, ArCH), 6.94 (1H, d, J 2.7, ArCH), 7.32 – 7.44 (5H, m, ArCH); \( \delta_C \) (125 MHz, CDCl\(_3\)) 51.8, 56.0, 71.0, 103.3, 106.1, 110.0, 127.9 (2 × ArCH), 128.5, 128.9 (2 × ArCH), 136.6, 137.3, 147.5, 149.8, 168.7; m/z (ESI-TOF) 288.1235 (100%, MH\(^+\), C\(_{16}\)H\(_{18}\)NO\(_4\) requires 288.1236).

Methyl 3-(benzylloxy)-5-methoxy-2-(2-oxopropanamido)benzoate 7

Aniline 6 (500 mg, 1.6 mmol) was dissolved in dichloromethane (5 cm³) and added dropwise to a solution of pyruvoyl chloride\(^6\), pyridine (0.4 cm³, 5 mmol) and dichloromethane (10 cm³) at 0 °C. The mixture was warmed to room temperature and stirred for 15 minutes before being poured into an aqueous solution of 0.1M HCl solution (15 cm³). The organic layer was separated, washed with brine (10 cm³) and dried over magnesium sulfate. Removal of the solvent in vacuo afforded a crude orange oil. The oil was dissolved in ethanol (5 cm³) and stored at -20 °C for 30 minutes. The resultant solid was filtered, washed with cold ethanol (5 cm³) and air dried to afford the title compound as a pale yellow solid (234 mg, 65%). \( \delta_H \) (500 MHz, CDCl\(_3\)) 2.53 (3H, s, CH\(_3\)), 3.83 (3H, s, OCH\(_3\)), 3.90 (3H, s, OCH\(_3\)), 5.14 (2H, s, CH\(_2\)), 6.75 (1H, d, J 2.4, ArCH), 7.01 (1H, d, J 2.4, ArCH), 7.32 – 7.45 (5H, m, ArCH), 9.27 (1H, s, NH); \( \delta_C \) (125 MHz, CDCl\(_3\)) 24.7, 52.8, 56.0,
Supporting Information

71.4, 105.0, 106.0, 118.5, 127.2, 127.5 (2 × ArCH), 128.4, 128.9 (2 × ArCH), 136.3, 153.7, 158.2, 158.4, 167.1, 196.8; m/z (ESI-TOF) 358.1295 (100%, MH+, C₁₉H₂₀NO₆ requires 358.1291).

Methyl-2-hydroxy-7-methoxy-2-methyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazine-5-carboxylate 8

1,4-Cyclohexadiene (400 mg, 0.5 cm³, 5 mmol) was added to a suspension of the α-ketoamide 7 (357 mg, 1 mmol) and Pearlman’s catalyst (7 mg, 2 mol% wt) in ethanol (5 cm³). The resulting mixture was heated at 50 ºC for 5 minutes and then cooled to room temperature. Filtration of the suspension through a cotton wool plug afforded the hemi-acetal 8 as a white solid that required no further purification (220 mg, 81%). δH (500 MHz, CDCl₃) 1.80 (3H, s, CH₃), 3.55 (1H, s, OH), 3.83 (3H, s, OCH₃), 3.96 (3H, s, OCH₃), 6.87 (1H, d, J 2.8, ArCH), 7.24 (1H, d, J ArCH), 10.24 (1H, s, NH); δC (125 MHz, CDCl₃) 23.5, 52.8, 56.1, 96.0, 108.9, 110.2, 114.6, 123.2, 143.1, 155.3, 163.7, 167.2; m/z (ESI-TOF) 268.0825 (100%, MH+, C₁₂H₁₄NO₆ requires 268.0821).

Methyl-7-methoxy-2-methylene-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazine-5-carboxylate 1⁷

Methanesulfonyl chloride (171 mg, 0.12 cm³, 1.5 mmol) was added dropwise to a solution of hemi-acetal 8 (265 mg, 1 mmol) and N,N'-di-iso-propylethylamine (258 mg, 0.35 cm³, 2 mmol) in dichloromethane (5 cm³) at 0 ºC. The solution was stirred for 90 minutes before being warmed to room temperature and poured into water (5 cm³). The aqueous phase was extracted with ethyl acetate (3 × 5 cm³) and the organic layers combined, washed with brine (10 cm³) and dried over magnesium sulfate. Removal of the solvent in vacuo yielded an off white solid that was determined to be >95% pure by LC-MS analysis. To obtain a sample for analytical purposes, the oxazine 1 was purified by flash column chromatography on silica gel using an eluent of 20% ethyl acetate: petroleum ether 40 – 60 ºC (240 mg, 82%). δH (500 MHz, CDCl₃) 3.78 (3H, s, OCH₃), 3.92 (3H, s, OCH₃), 5.07 (1H, dd, J 1.5 1.0, 1 × C=CH₂), 5.62 (1H, d, J 1.5, 1 × C=CH₂), 6.77 (1H, dd, J 2.2 1.0, ArCH), 7.17 (1H, d, J 2.2, ArCH), 10.4 (1H, s, NH). All data is in accordance with that of the literature.
Supporting Information

Methyl-5-methoxy-3-(2-methoxy-2-oxoethoxy)-2-nitrobenzoate 36

![Chemical Structure](image)

Methyl-2-bromoacetate (0.15 cm³, 0.5 mmol) was added to a suspension of nitrophenol 5 (110 mg, 0.5 mmol) and potassium carbonate (220 mg, 1.5 mmol) in acetone (10 cm³). The mixture was heated at reflux for 3 hours before being cooled to room temperature and filtered. Removal of the solvent \textit{in vacuo} yielded the title compound as an off white solid that required no further purification (120 mg, 76%). $\delta_{\text{H}}$ (500 MHz, CDCl$_3$) 3.81 (3H, s, OC$_3$H$_3$), 3.87 (3H, s, OC$_3$H$_3$), 3.90 (3H, s, OC$_3$H$_3$), 4.72 (2H, s, CH$_2$), 6.61 (1H, d, $J$ 2.6, ArCH), 7.06 (1H, d, $J$ 2.6, ArCH); $\delta_{\text{C}}$(125 MHz, CDCl$_3$) 52.9, 53.5, 56.4, 66.7, 105.2, 107.3, 126.4, 151.2, 161.2, 162.7, 164.1, 168.3; $m/z$ (ESI-TOF) 300.0723 (100%, MH$^+$, C$_{12}$H$_{14}$NO$_8$ requires 300.0719).

Methyl-5-methoxy-3-(1-ethoxy-1-oxopropan-2-yloxy)-2-nitrobenzoate 35

![Chemical Structure](image)

Ethyl-2-bromopropanoate (0.06 cm³, 0.5 mmol) was added to a suspension of nitrophenol 5 (110 mg, 0.5 mmol) and potassium carbonate (220 mg, 1.5 mmol) in acetone (10 cm³). The reaction mixture was heated at reflux for 3 hours before being cooled to room temperature and filtered. Removal of the solvent \textit{in vacuo} yielded the title compound as an off white solid that required no further purification (135 mg, 92%). $\delta_{\text{H}}$ (500 MHz, CDCl$_3$) 1.25 (3H, t, $J$ 6.8, OCH$_2$CH$_3$), 1.62 (3H, d, $J$ 6.8, CHCH$_3$), 3.85 (3H, s, OCH$_3$), 3.89 (3H, s, OCH$_3$), 4.22 (2H, q, $J$ 7.1, OCH$_2$CH$_3$), 4.76 (1H, q, $J$ 6.8, CHCH$_3$), 6.60 (1H, d, $J$ 2.3, ArCH), 7.04 (1H, d, $J$ 2.3, ArCH); $\delta_{\text{C}}$(125 MHz, CDCl$_3$) 14.3, 18.4, 53.4, 56.3, 62.0, 75.0, 105.7, 107.2, 126.1, 136.0, 151.1, 161.0, 164.0, 170.7; $m/z$ (ESI-TOF) 314.0874 (100%, MH$^+$, C$_{13}$H$_{16}$NO$_8$ requires 314.0876).

\textbf{General procedure A for the formation of the oxazine substrates 9 and 10 via a platinum(IV) oxide catalysed hydrogenation.}

The nitrophenol 5 (0.2 mmol) was added in a single portion to a suspension of platinum(IV) oxide (10% wt) in ethanol (5 cm³). The system was evacuated and backfilled with hydrogen gas five times. Following completion of the final cycle, the mixture was stirred for 4 hours. The suspension was filtered and the solvent removed \textit{in vacuo} to afford the title compound.
Methyl-7-methoxy-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazine-5-carboxylate 9

Prepared in accordance to general procedure A using nitro-ester 36 (60 mg, 0.2 mmol) and platinum(IV) oxide (6 mg). Removal of the solvent in vacuo afforded the title compound as an off white solid (29 mg, 50%). $\delta_H$ (500 MHz, CDCl$_3$) 3.82 (3H, s, OCH$_3$), 3.96 (3H, s, OCH$_3$), 4.64 (2H, s, CH$_2$), 6.80 (1H, d, J 2.8, ArCH$_3$), 7.19 (1H, d, J 2.8, ArCH$_3$), 10.26 (1H, s, NH); $\delta_C$ (125 MHz, CDCl$_3$) 52.8, 56.0, 67.3, 108.3, 109.3, 114.6, 123.5, 145.0, 155.1, 164.4, 167.2; m/z (ESI-TOF) 238.0717 (100%, MH$^+$, C$_{11}$H$_{12}$NO$_5$ requires 238.0715).

Methyl-7-methoxy-2-methyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazine-5-carboxylate 10

Prepared in accordance to general procedure A using nitro-ester 35 (50 mg, 0.2 mmol) and platinum(IV) oxide (5 mg). Removal of the solvent in vacuo afforded the title compound as an off white solid (35 mg, 75%). $\delta_H$ (500 MHz, CDCl$_3$) 1.60 (3H, d, J 6.9, CH$_3$), 3.82 (3H, s, OCH$_3$), 3.96 (3H, s, OCH$_3$), 4.67 (1H, q, J 6.8, CH$_3$), 6.81 (1H, d, J 2.8, ArCH$_3$), 7.18 (1H, d, J 2.8, ArCH$_3$), 10.13 (1H, s, NH); $\delta_C$ (125 MHz, CDCl$_3$) 16.7, 52.8, 56.0, 73.6, 104.8, 108.2, 109.5, 114.3, 123.9, 144.7, 155.0, 166.9; m/z (ESI-TOF) 252.0875 (100%, MH$^+$, C$_{12}$H$_{14}$NO$_5$ requires 252.0872).

**General Procedure B for the sulphuric acid catalysed addition of an alcohol to the oxazine 1**

The alcohol (1 mmol) was added to a solution of oxazine 1 (62 mg, 0.3 mmol) and sulphuric acid (3 drops) in THF (1 cm$^3$) at 0 ºC. The resulting solution was stirred for 12 hours and the volatiles removed in vacuo to afford the crude product. Purification of the crude material occurred as described in the individual experimental details.

Methyl-2,7-dimethoxy-2-methyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazine-5-carboxylate 11

According to general procedure B using methanol (0.1 cm$^3$, 3 mmol). Removal of the solvent in vacuo afforded the title compound as a white solid that required no further purification (20 mg, 90%). $\delta_H$ (500 MHz, CDCl$_3$) 1.76 (3H, s, CH$_3$), 3.34 (3H, s, OCH$_3$), 3.81 (3H, s, OCH$_3$), 3.93 (3H, s, OCH$_3$), 6.85 (1H, d, J 2.9, ArCH$_3$), 7.21 (1H, d, J 2.9, ArCH$_3$), 10.22 (1H, s, NH); $\delta_C$ (125 MHz, CDCl$_3$) 18.9, 50.2, 52.8, 56.0, 99.3, 108.5, 110.0, 114.3, 123.9, 143.0, 155.0, 162.9, 167.2; m/z (ESI-TOF) 281.0901 (100%, MH$^+$, C$_{13}$H$_{15}$NO$_6$ requires 281.0899).
Supporting Information

Methyl-7-methoxy-2-methyl-2-(octyloxy)-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazine-5-carboxylate 31

 Prepared in accordance with general procedure B using 1-octanol (0.2 cm³, 1 mmol). Removal of the solvent in vacuo yielded a pale yellow oil. Purification of the crude material by flash column chromatography on silica gel using an eluent of 10% ethyl acetate: n-hexanes afforded the title compound as a white solid (32 mg, 35%). δH (500 MHz, CDCl₃) 0.85 (3H, t, J 7.1, CH₂CH₃), 1.06 – 1.18 (8H, m, 4 × CH₂), 1.20 – 1.27 (2H, m, CH₂), 1.37 – 1.44 (2H, m, CH₂), 1.76 (3H, s, CH₃), 3.46 – 3.55 (2H, m, OCH₂), 3.73 (3H, s, OCH₃), 3.86 (3H, s, OCH₃), 6.75 (1H, dd, J 2.8 0.6, ArCH), 7.12 (d, J 2.8, ArCH), 10.17 (1H, s, NH); δC (125 MHz, CDCl₃) 14.3, 19.4, 22.9, 26.2, 29.4, 29.7, 32.0, 52.7, 56.0, 62.8, 99.1, 105.4, 108.2, 110.1, 114.1, 124.1, 143.2, 154.9, 163.2, 167.3; m/z (ESI-TOF) 380.2076 (100%, MH⁺, C₂₀H₂₉NO₆ requires 380.2073).

Methyl-2-(but-3-yn-1-yloxy)-7-methoxy-2-methyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazine-5-carboxylate 30

 Prepared in accordance with general procedure B using but-3-yn-1-ol (0.1 cm³, 1 mmol). Removal of the solvent in vacuo afforded an orange oil. Purification of the crude material by flash column chromatography on silica gel afforded the title compound as a white solid (16 mg, 63%). δH (500 MHz, CDCl₃) 1.78 (3H, s, CH₃), 1.84 (1H, s, C≡CH), 2.16 – 2.43 (2H, m, CH₂), 3.70 – 3.76 (2H, m, OCH₂), 3.81 (3H, s, OCH₃), 3.94 (3H, s OCH₃), 6.84 (1H, d, J 2.8, ArCH), 7.20 (1H, d, J 2.8, ArCH), 10.21 (1H, s, NH); δC (125 MHz, CDCl₃) 19.5, 20.0, 52.8, 56.0, 61.0, 69.8, 80.5, 99.1, 108.5, 110.1, 114.3, 123.9, 142.9, 155.0, 162.7, 167.2; m/z (ESI-TOF) 320.1137 (100%, MH⁺, C₁₆H₁₈NO₆ requires 320.1134).

Methyl-2-(hex-3-yn-1-yloxy)-7-methoxy-2-methyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazine-5-carboxylate 29

 Prepared in accordance with general procedure B using hex-3-yn-1-ol (1 cm³, 1 mmol). Removal of the solvent in vacuo yielded a crude yellow oil. Purification of the crude material by flash column chromatography on silica gel using an eluent of 20% ethyl acetate: n-hexanes afforded the title compound as a white solid (18 mg, 63%). δH (500 MHz, CDCl₃) 1.04 (3H, t, J 7.5, CH₂CH₃), 1.78 (3H, s, CH₃), 2.03 – 2.08 (2H, m, CH₂), 2.21 – 2.35 (2H, m, CH₂), 3.63 – 3.69 (2H, m, OCH₂), 3.81 (3H, s, OCH₃), 3.93 (3H, s, OCH₃), 6.84 (1H, d, J 2.8, ArCH), 7.19 (1H, d, J 2.8, ArCH), 10.20 (1H, s, NH); δC (125
Methyl-2-(but-3-en-1-yloxy)-7-methoxy-2-methyl-3-oxo-3, 4-dihydro-2H-benzo[b][1,4]oxazine-5 carboxylate 34

Prepared in accordance to general procedure B using 6-hexene-1-ol (0.1 cm³, 1 mmol). Removal of the solvent in vacuo yielded a crude yellow oil. Purification of the crude material by flash column chromatography on silica gel using an eluent of 10% ethyl acetate: petroleum ether 40 – 60 ºC afforded the title compound as a white solid (22 mg, 88%). δH (500 MHz, CDCl₃) 1.76 (7H, s, 2 × CH₂ and CH₃), 2.15 – 2.24 (2H, m, CH₂CH=CH₂), 3.59 – 3.69 (2H, m, OC₃H₂), 3.81 (3H, s, OC₃H₃), 3.93 (3H, s, OCH₃), 4.88 – 4.96 (2H, m, CH=CH₂), 5.49 – 5.62 (1H, m, CH=CH₂), 6.81 (1H, d, J 2.6, ArCH), 7.19 (1H, d, J 2.6, ArCH), 10.19 (1H, s, NH); δC (125 MHz, CDCl₃) 12.5, 14.3, 19.4, 20.5, 52.7, 56.0, 61.7, 75.3, 83.4, 99.0, 108.3, 110.0, 114.2, 123.9, 142.9, 154.9, 162.8, 167.2; m/z (ESI-TOF) 348.1448 (100%, MH⁺, C₁₈H₂₂NO₆ requires 348.1147).

General procedure C for the formation of α-ketoamides 37 - 41 from the aniline 6

The α-ketoacid chloride (2 mmol) was added in a single portion to a solution of aniline (1 mmol) and pyridine (237 mg, 0.3 cm³, 3 mmol) in DCM (10 cm³) at 0 ºC. The resulting mixture was stirred for 1 hour before being quenched through addition of a saturated aqueous solution of NaHCO₃ (10 cm³). The organic layer was separated and the aqueous phase extracted with dichloromethane (3 × 10 cm³). The organic layers were combined, washed with brine (10 cm³) and dried over magnesium sulfate. Removal of the solvent in vacuo afforded the crude α-ketoamide. Purification of the crude material occurred as described in the individual experimental details.

Methyl-3-(benzyloxy)-5-methoxy-2-(2-oxobutanamido)benzoate 39

Prepared in accordance with general procedure C using 2-oxobutanoyl chloride (238 mg, 2 mmol). Removal of the solvent in vacuo yielded a brown oil. Purification of the crude material by recrystallization from ethanol at -20 ºC afforded the title compound as an off white solid (267 mg, 72%). δH (500 MHz, CDCl₃) 1.6 (3H, t, J 7.2, CH₂CH₃), 2.97 (2H, q, J 7.2, CH₂CH₃), 3.80 (3H, s, OCH₃), 3.88 (3H, s, OCH₃), 6.72 (1H, d, J 2.2, ArCH), 6.99 (1H, d, J 2.2, ArCH), 7.32 – 7.42 (5H, m, ArCH), 9.27 (1H, s, NH); δC (125 MHz, CDCl₃) 7.4, 30.6, 52.8, 56.0, 71.4, 105.0, 105.9, 118.6, 127.5, 128.4, 128.9, 153.7, 158.2, 158.3, 167.1, 199.4; m/z (ESI-TOF) 372.1449 (100%, MH⁺, C₂₀H₂₂NO₆ requires 372.1447).
Methyl 3-(benzyloxy)-5-methoxy-2-(2-oxopropanamido)benzoate 40

Prepared in accordance with general procedure C using 3-methyl-2-oxobutanoic chloride\(^8\) (268 mg, 2 mmol). Removal of the solvent \textit{in vacuo} yielded an orange oil. Purification of the crude material by recrystallization from ethanol at -20 °C afforded the title compound as an off white solid (250 mg, 65%). \(\delta_{\text{H}}(500 \text{ MHz, CDCl}_3)\) 1.62 (5.4H, d, \(J\ 6.9, 2 \times \text{CH}_2\) major rotamer), 1.66 (0.6H, d, \(J\ 6.9, 2 \times \text{CH}_2\) minor rotamer), 3.63 [0.9H, septet, \(J\ 6.9, \text{CH}_3\) major rotamer], 3.84 (2.7H, s, \(\text{OCH}_3\) major rotamer), 3.88 (0.3H, s, \(\text{OCH}_3\) minor rotamer), 5.14 (1.8H, s, \(\text{CH}_2\) major rotamer), 5.16 (0.2H, s, \(\text{CH}_2\) minor rotamer), 6.74 (0.1H, d, \(J\ 2.7, \text{ArCH}\) minor rotamer), 6.77 (0.9H, s, d, \(J\ 2.7, \text{ArCH}\) major rotamer), 6.99 (0.1H, d, \(J\ 2.7, \text{ArCH}\) minor rotamer), 7.02 (0.9H, d, \(J\ 2.7, \text{ArCH}\) major rotamer), 7.33 – 7.45 (5H, m, \(\text{ArCH}\) major and minor rotamer); \(\delta_{\text{C}}\) (125 Hz, CDCl\(_3\)) 17.9, 34.5, 52.8, 56.0, 71.4, 104.9, 105.9, 118.6, 127.2, 127.6 (2 \(\times\) ArCH), 128.4, 128.9 (2 \(\times\) ArCH), 136.2, 153.7, 158.0, 158.3, 167.1, 201.9; \(m/z\) (ESI-TOF) 384.1605 (100%, MH\(^+\), \(C_{21}H_{24}NO_6\) requires 384.1604). Only major rotamer \(^{13}\text{C}\) values are reported.

Methyl 3-(benzyloxy)-5-methoxy-2-(4-methyl-2-oxopentanamido)benzoate 37

Prepared in accordance with general procedure C using 4-methyl-2-oxopentanoic chloride\(^8\) (296 mg, 2 mmol). Removal of the solvent \textit{in vacuo} yielded brown solid. Purification of the crude material by recrystallization from ethanol at -20 °C afforded the title compound as a pale yellow solid (252 mg, 60%). \(\delta_{\text{H}}(500 \text{ MHz, CDCl}_3)\) 0.93 [6H, d, \(J\ 5.8, 2 \times \text{CH}(\text{CH}_3)_2\)], 2.15 [1H, net, \(J\ 5.8, \text{CH}(\text{CH}_3)_2\)], 2.79 [2H, d, \(J\ 5.8, \text{CH}_2\text{CH}(\text{CH}_3)_2\)], 3.79 (3H, s, \(\text{OCH}_3\)), 3.86 (3H, s, \(\text{OCH}_3\)), 5.09 (2H, s, \(\text{CH}_2\)), 6.71 (1H, d, \(J\ 2.2, \text{ArCH}\)), 6.97 (1H, d, \(J\ 2.2, \text{ArCH}\)), 7.28 – 7.40 (5H, m, ArCH), 9.23 (1H, s, NH); \(\delta_{\text{C}}\) (125 Hz, CDCl\(_3\)) 22.5, 24.5, 45.2, 52.5, 55.7, 71.0, 104.7, 105.6, 118.3, 127.0, 127.2 (2 \(\times\) ArCH), 128.1, 128.6 (2 \(\times\) ArCH), 136.0, 153.4, 158.1, 166.8, 198.4; \(m/z\) (ESI-TOF) 400.1764 (100%, MH\(^+\), \(C_{22}H_{26}NO_6\) requires 400.1760).
Methyl 3-(benzyloxy)-5-methoxy-2-(2-oxo-2-phenylacetamido)benzoate 38

Prepared in accordance with general procedure C using 2-oxo-2-phenylacetamide\(^8\) (336 mg, 2 mmol). Removal of the solvent \textit{in vacuo} yielded a brown oil. Purification of the crude material by recrystallization from ethanol at -20 °C afforded the title compound as a yellow solid (274 mg, 60%). \(\delta\)\(^H\) (500 MHz, CDCl\(_3\)) 3.83 (3H, s, OCH\(_3\)), 3.93 (3H, s, OCH\(_3\)), 5.15 (2H, s, ArCH), 6.79 (1H, d, J 2.4, ArCH), 7.05 (1H, d, J 2.4, ArCH), 7.33 – 7.43 (5H, m, ArCH), 7.47 – 7.48 (2H, m, ArCH), 7.59 – 7.62 (1H, m, ArCH), 8.23 – 8.24 (2H, m, ArCH), 9.49 (1H, s, NH); \(\delta\)\(^C\) (125 MHz, CDCl\(_3\)) 52.9, 56.0, 71.4, 105.1, 106.0, 119.0, 126.9, 127.8 (2 × ArCH), 128.5, 128.7 (2 × ArCH), 128.9 (2 × ArCH), 131.5 (2 × ArCH), 133.5, 134.5, 136.3, 153.8, 158.4, 160.0, 167.2, 188.0; \(m/z\) (ESI-TOF) 420.1449 (100%, MH\(^+\), C\(_{24}\)H\(_{22}\)NO\(_6\) requires 420.1447).

Methyl-3-(benzyloxy)-5-methoxy-2-(2-oxo-2-(p-tolyl)acetamido)benzoate 41

Prepared in accordance with general procedure C using 2-oxo-2-(p-tolyl)acetamide\(^8\) (364 mg, 2 mmol). Removal of the solvent \textit{in vacuo} yielded a brown oil. Purification of the crude material by recrystallization from ethanol at -20 °C afforded the title compound as a yellow solid (210 mg, 73%). \(\delta\)\(^H\) (500 MHz, CDCl\(_3\)) 2.42 (3H, s, ArC\(_{6}\)H\(_3\)), 3.83 (3H, s, OCH\(_3\)), 3.92 (3H, s, OCH\(_3\)), 5.15 (2H, s, CH\(_2\)), 6.78 (1H, d, J 2.7, ArCH), 7.04 (1H, d, J 2.7, ArCH), 7.23 (2H, d, J 8.0, ArCH), 7.33 – 7.39 (3H, m, ArCH), 7.45 – 7.47 (2H, m, ArCH), 8.18 (2H, d, J 8.0, ArCH), 9.47 (1H, s, NH); \(\delta\)\(^C\) (125 MHz, CDCl\(_3\)) 22.1, 52.9, 56.0, 71.4, 105.1, 106.0, 119.0, 126.9, 127.8 (2 × ArCH), 128.4, 128.9 (2 × ArCH), 129.5 (2 × ArCH), 131.0, 131.6 (2 × ArCH), 136.3, 145.7, 153.8, 158.3, 160.3, 167.2, 187.4; \(m/z\) (ESI-TOF) 434.1605 (100%, MH\(^+\), C\(_{25}\)H\(_{24}\)NO\(_6\) requires 434.1604).

General procedure D for the formation of the oxazines 13 - 15 from the \(\alpha\)-keto amides 37, 39 and 40

The \(\alpha\)-keto amide 38, 40 or 41 (1 mmol) was added to a suspension of 20% Pd(OH)\(_2\)/C (2 mol %) and 1,4-cyclohexadiene (400 mg, 0.5 cm\(^3\), 5 mmol) in ethanol (5 cm\(^3\)). The reaction mixture was heated at reflux for 5 minutes before being cooled to room temperature. The suspension was filtered through a cotton wool plug and the solvent removed \textit{in vacuo} to afford the hemi-acetal intermediate. The hemi-acetal intermediate was re-dissolved in THF (5 cm\(^3\)) and added to a suspension of \(p\)-toluenesulfonic acid (2 equivalents) in THF (5 cm\(^3\)). The mixture was heated at reflux for 90 minutes before being cooled...
to room temperature. Removal of the volatiles \textit{in vacuo} yielded the crude oxazine. Purification of the crude material occurred as described in the individual experimental details.

Methyl-(Z)-2-ethyldene-7-methoxy-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazine-5-carboxylate 14

Prepared in accordance with general procedure D using α-keto amide 39 (371 mg, 1 mmol), 20% Pd(OH)$_2$/C (7 mg, 2 mol %) and \( p \)-toluenesulfonic acid (260 mg, 1.2 mmol). Removal of the solvent \textit{in vacuo} yielded a yellow oil as a 9:1 mixture of isomers. Purification and isolation of the major isomer by flash column chromatography on silica gel using an eluent of 5% ethyl acetate: \( n \)-hexanes afforded the title compound as a white solid (120 mg, 45%). \( \delta_H \) (600 MHz, CDCl$_3$) 1.87 (3H, \textit{d}, \( J \) 7.3, \( CH_3 \)), 3.84 (3H, s, OCH$_3$), 3.96 (3H, s, OCH$_3$), 6.11 (1H, \textit{q}, \( J \) 7.3, \( C=CH \)), 6.85 (1H, \textit{d}, \( J \) 2.8, ArCH), 7.18 (1H, \textit{d}, \( J \) 2.8, ArCH), 10.28 (1H, s, \( NH \)); \( \delta_C \) (150 MHz, \textit{d}_6-Acetone) 9.2, 52.1, 55.3, 107.2, 108.3, 111.0, 113.7, 121.9, 142.2, 142.8, 154.7, 155.2, 167.0; m/z (ESI-TOF) 264.0875 (100%, MH$^+$, C$_{13}$H$_{14}$NO$_5$ requires 264.0872).

Methyl-7-methoxy-3-oxo-2-(propan-2-ylidene)-3,4-dihydro-2H-benzo[b][1,4]oxazine-5-carboxylate 15

Prepared in accordance with general procedure D using α-keto amide 40 (385 mg, 1 mmol), 20% Pd(OH)$_2$/C (8 mg, 2 mol %) and \( p \)-toluenesulfonic acid (190 mg, 1 mmol). Removal of the solvent \textit{in vacuo} yielded a brown oil. Purification of the crude material by flash column chromatography on silica gel using an eluent of 10% ethyl acetate: \( n \)-hexanes afforded the title compound as a white solid (83 mg, 30%). \( \delta_H \) (600 MHz, \textit{d}_6-Acetone) 1.94 (3H, s, \( CH_3 \)), 2.23 (3H, s, \( CH_3 \)), 3.81 (3H, s, OCH$_3$), 3.93 (3H, s, OCH$_3$), 6.93 (1H, \textit{d}, \( J \) 2.8, ArCH), 7.12 (1H, \textit{d}, \( J \) 2.8, ArCH), 9.87 (1H, s, \( NH \)); \( \delta_C \) (150 MHz, \textit{d}_6-Acetone) 18.5, 19.1, 52.0, 55.3, 107.2, 107.7, 113.2, 123.0, 128.5, 135.7, 144.1, 154.6, 157.3, 166.9; m/z (ESI-TOF) 278.1030 (100%, MH$^+$, C$_{14}$H$_{16}$NO$_5$ requires 278.1028).

Methyl-(Z)-7-methoxy-2-(2-methylpropylidene)-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazine-5-carboxylate 13

Prepared in accordance with general procedure D using α-keto amide 37 (399 mg, 1 mmol), 20% Pd(OH)$_2$/C (8 mg, 2 mol %) and \( p \)-toluenesulfonic acid (258 mg, 1.6 mmol). Removal of the solvent \textit{in vacuo} yielded a crude orange oil. Purification of the crude material by flash column chromatography on silica gel using an eluent of 10% ethyl acetate: \( n \)-hexanes
afforded the title compound as a white solid (99 mg, 34%). \( \delta_H \) (600 MHz, \( d_6\)-DMSO) 1.07 [6H, d, J 7.3, CH(CH₃)$_2$], 2.92 – 2.99 [1H, m, CH(CH₃)$_2$], 3.77 (3H, s, OCH$_3$), 3.89 (3H, s, OCH$_3$), 5.83 [1H, d, J 9.6, C=CHCH(CH$_3$)$_2$], 7.05 (1H, d, J 2.8, ArCH), 7.09 (1H, d, J 2.8, ArCH), 10.09 (1H, s, NH); \( \delta_C \) (150 MHz, \( d_6\)-Acetone) 21.5, 24.2, 52.4, 55.3, 107.2, 108.4, 113.7, 121.9, 122.8, 140.0, 142.8, 154.7, 155.4, 167.0; \( m/z \) (ESI-TOF) 292.1188 (100%, MH$^+$, C$_{13}$H$_{18}$NO$_3$ requires 292.1185).

Methyl-2-hydroxy-7-methoxy-3-oxo-2-phenyl-3,4-dihydro-2H-benzo[b][1,4]oxazine-5-carboxylate 28 and methyl-7-methoxy-3-oxo-2-phenyl-3,4-dihydro-2H-benzo[b][1,4]oxazine-5-carboxylate 12

1,4-Cyclohexadiene (400 mg, 0.5 cm$^3$, 5 mmol) was added to a suspension of the \( \alpha \)-ketoamide 38 (419 mg, 1 mmol) and 20% Pd(OH)$_2$/C (8 mg, 2 mol %) and the resulting mixture heated at 50°C for 2 hours. Upon cooling to room temperature, TLC analysis revealed the presence of two compounds, the hemiacetal 26 (R$_f$ 0.15, 20% EtOAc: n-hexanes) and the fully hydrogenated phenyl oxazine 12 (R$_f$ 0.3, 20% EtOAc: n-hexanes). Removal of the solvent in vacuo afforded a 1:1 mixture of the two compounds as determined by analysis of the crude $^1$H NMR spectroscopy data. Purification and isolation of both compounds by flash column chromatography on silica gel using an eluent of 20% ethyl acetate: n-hexanes afforded the hemiacetal 26 as an orange solid (118 mg, 36%) and the phenyl oxazine 12 (98 mg, 31%) as a yellow solid. Hemi-acetal 26: \( \delta_H \) (500 MHz, CDCl$_3$) 3.70 (3H, s, OCH$_3$), 3.83 (3H, s, OCH$_3$), 5.25 (1H, s, OH), 6.69 (1H, d, J 3.0, ArCH), 7.06 (1H, d, J 3.0, ArCH), 7.25 – 7.33 (3H, m, ArCH), 7.43 – 7.45 (2H, m, ArCH), 11.66 (1H, s, NH); \( \delta_C \) (125 MHz, CDCl$_3$) 53.0, 55.9, 75.3, 109.4, 110.7, 120.8, 121.3, 129.1, 129.3, 138.9, 151.5, 158.0, 168.4, 172.5; \( m/z \) (ESI-TOF) 330.0980 (100%, MH$^+$, C$_{13}$H$_{18}$NO$_3$ requires 330.0978). Phenyl Oxazine 12: \( \delta_H \) (500 MHz, CDCl$_3$) 3.95 (1.5H, s, OCH$_3$, rotamer A), 3.98 (1.5H, s, OCH$_3$, rotamer B), 4.05 (1.5H, s, OCH$_3$, rotamer A), 4.08 (1.5H, s, OCH$_3$, rotamer B), 6.94 (0.5H, d, J 2.8, CH rotamer A), 7.32 (0.5H, d, J 2.8, CH, rotamer B), 7.39 (0.5H, d, J 2.5, ArCH, rotamer A), 7.49 – 7.54 (1.5H, m, ArCH, rotamer A and B), 7.58 – 7.61 (1H, m, ArCH, rotamer A and B), 7.69 – 7.72 (0.5H, m, ArCH, rotamer A), 7.78 (0.5H, d, J 2.3, ArCH, rotamer B), 8.29 – 8.41 (1H, m, rotamer A and B), 8.65 – 8.67 (1H, m, rotamer A and B); \( \delta_C \) (125 MHz, CDCl$_3$) 52.9 (rotamer A), 53.1 (rotamer B), 56.5 (rotamer A), 56.6 (rotamer B), 100.2 (rotamer A), 103.2 (rotamer B), 114.3 (rotamer A), 117.5 (rotamer B), 124.4 (rotamer A), 124.7 (rotamer B), 128.6 (2 × rotamer A), 128.9 (2 × rotamer B), 129.7 (2 × rotamer A), 131.4 (2 × rotamer B), 131.5 (rotamer A), 132.5 (rotamer B), 133.8 (rotamer A), 134.4 (rotamer B), 134.6 (rotamer A), 135.2 (rotamer B) 147.6 (rotamer A), 147.6 (rotamer B), 148.5 (rotamer B), 151.8 (rotamer A), 152.4 (rotamer B), 160.2 (rotamer A), 161.4 (rotamer B), 165.1 (rotamer A), 166.4 (rotamer B), 180.0 (rotamer A), 180.1 (rotamer B); \( m/z \) (ESI-TOF) 314.1032 (100%, MH$^+$, C$_{13}$H$_{18}$NO$_3$ requires 314.1028).
General procedure E for the palladium catalysed Heck reaction between the oxazine 1 and an aryl bromide

N, N-diisopropylethylamine (5 µl, 3 mg, 30 µmol) was added to a suspension of Palladium acetate (1 mg, 4 µmol), triphenylphosphine (2 mg, 8 µmol), oxazine 1 (5 mg, 20 µmol) and the aryl bromide (20 µmol) in toluene (3 cm³). The mixture was heated at reflux for 2 hours and then cooled to room temperature. The residue was purified as described in the individual experimental details.

Methyl 2-benzylidene-7-methoxy-3-oxo-4-dihydro-2H-benzo[b][1,4]oxazine-5-carboxylate 16

Prepared in accordance with general procedure E using bromobenzene (2 µl, 3 mg, 20 µmol). The residue was purified by flash column chromatography on silica gel using an eluent of 20% ethyl acetate: n-hexane to afford the title compound as a pale yellow solid (4 mg, 62%). δ<sup>H</sup> (500 MHz, d<sub>6</sub>-DMSO) 3.84 (3H, s, OC<sub>3</sub>H<sub>3</sub>), 3.93 (3H, s, OCH<sub>3</sub>), 6.83 – 6.85 (1H, m, ArCH), 7.16 (1H, d, J 2.8, ArCH), 7.35 (1H, d, J 2.8, ArCH), 7.37 – 7.40 (1H, m, ArCH), 7.47 (2H, t, J 7.5, ArCH), 7.93 – 7.94 (1H, m, ArCH); δ<sup>C</sup> (125 MHz, d<sub>6</sub>-DMSO) 53.6, 56.7, 108.2, 109.8, 112.5, 114.8, 121.6, 129.3, 129.5 (2 × ArCH), 130.8 (2 × ArCH), 133.5, 141.4, 142.6, 155.1, 156.0, 167.1; m/z (ESI-TOF) 326.1029 (100%, MH<sup>+</sup>, C<sub>18</sub>H<sub>16</sub>NO<sub>5</sub> requires 326.1028).

Methyl-(Z)-7-methoxy-2-(4-nitrobenzylidene)-3-oxo-4-dihydro-2H-benzo[b][1,4]oxazine-5-carboxylate 17

Prepared in accordance with general procedure E using 4-nitrobromobenzene (3 mg, 20 µmol). The residue was purified by flash column chromatography on silica gel using an eluent of 20% ethyl acetate: n-hexane to afford the title compound as a bright yellow solid (4 mg, 62%). δ<sup>H</sup> (500 MHz, CDCl<sub>3</sub>) 3.83 (3H, s, OCH<sub>3</sub>), 3.94 (3H, s, OCH<sub>3</sub>), 6.94 (1H, s, C=CH), 7.20 (1H, d, J 2.3, ArCH), 7.31 – 7.34 (1H, m, ArCH), 7.39 – 7.42 (2H, m, ArCH), 7.80 – 7.82 (2H, m, ArCH), 10.41 (1H, s, NH); δ<sup>C</sup> (125 MHz, CDCl<sub>3</sub>) 52.6, 55.9, 107.8, 108.9, 113.2, 113.8, 121.9, 128.5 (2 × ArCH), 128.6, 130.1 (2 × ArCH), 133.2, 140.6, 142.4, 154.7, 156.4, 166.8; m/z (ESI-TOF) 371.0881 (100%, MH<sup>+</sup>, C<sub>15</sub>H<sub>18</sub>N<sub>2</sub>O<sub>7</sub> requires 371.0879).
Methyl-7-methoxy-4-methyl-2-methylene-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazine-5-carboxylate 18

Iodomethane (6 µl, 0.1 mmol) was added in a single portion to a suspension of oxazine 1 (15 mg, 0.06 mmol) and potassium carbonate (14 mg, 0.1 mmol) in DMF (1 cm³). The mixture was stirred vigourously overnight before being poured into a solution of 1N HCl (10 cm³) and ethyl acetate (10 cm³). The organic layer was extracted, washed with brine (5 × 10 cm³) and dried over magnesium sulfate. Removal of the solvent yielded a yellow oil. Purification of the crude material by flash column chromatography on silica gel using an eluent of 10% ethyl acetate: n-hexane afforded the title compound as a colourless oil (10 mg, 66%). δH (500 MHz, CDCl₃) 3.28 (3H, s, NCH₃), 3.83 (3H, s, OC₃H₃), 3.95 (3H, s, OC₃H₃), 5.14 (1H, d, J 1.8, 1 × C=C₆H₂), 5.64 (1H, d, J 1.8, 1 × C=C₆H₂), 6.75 (1H, d, J 2.9, ArCH), 6.84 (1H, d, J 2.9, ArCH); δC (125 MHz, CDCl₃) 34.7, 53.1, 56.1, 100.7, 105.2, 109.6, 122.2, 145.5, 155.8, 158.6, 168.0, 191.1; m/z (ESI-TOF) 264.0875 (100%, MH⁺, C₁₃H₁₄NO₅ requires 264.0872).

7-methoxy-2-methylene-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazine-5-carboxylic acid 19

Lithium hydroxide monohydrate (43 mg, 1 mmol) was added in a single portion to a solution of oxazine 1 (63 mg, 0.5 mmol) in THF (2 cm³), MeOH (2 cm³) and water (2 cm³). The mixture was stirred for 90 minutes before being acidified to pH 1 through addition of an aqueous solution of 1N hydrochloric acid (3 cm³). The precipitated solid was filtered, washed with cold methanol and air dried to afford the title compound as a white solid (23 mg, 36%). δH (500 MHz, d₆-DMSO) 3.77 (3H, s, OC₃H₃), 5.14 (1H, d, J 2.4, 1 × C=C₆H₂), 5.48 (1H, d, J 2.4, 1 × C=C₆H₂), 7.01 (1H, d, J 2.4, ArCH), 7.14 (1H, d, J 2.4, ArCH), 10.55 (1H, s, CO₂H); δC (125 MHz, d₆-DMSO) 56.5, 98.3, 99.3, 107.6, 109.6, 115.6, 121.8, 142.7, 155.0, 155.1, 169.0; m/z (ESI-TOF) 235.0562 (100%, MH⁺, C₁₁H₁₀NO₅ requires 235.0559).

General Procedure F for the condensation of either an alcohol or an amine with the carboxylic acid 19

Oxalyl chloride (8 µl, 0.1 mmol) was added to a suspension of the acid 19 (10 mg, 0.04 mmol) and DMF (1 drop) in DCM (5 cm³). The mixture was heated at reflux for 4 hours before being cooled to room temperature. Removal of the volatiles in vacuo afforded the acid chloride as a yellow solid. The solid was dissolved in DCM (2 cm³) and added in a single portion to a solution of the nucleophile (0.1 mmol) and pyridine (16 mg, 20 µl, 0.2 mmol) in DCM (5 cm³) at 0 °C. The mixture was warmed to room temperature and stirred for 90 minutes before being poured into water (10 cm³). The aqueous phase was extracted with ethyl acetate (3 × 15 cm³) and the organic layers combined, washed with brine (10 cm³) and dried over magnesium sulphate. Removal of the solvent in vacuo afforded the crude ester or amide. Purification of the crude material occurred as described in the individual experimental procedure.
Phenyl-7-methoxy-2-methylene-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazine-5-carboxylate 21

Prepared in accordance with general procedure F using phenol (9 mg, 0.1 mmol). Removal of the solvent in vacuo yielded a crude yellow oil. Purification of the crude material by flash column chromatography on silica gel using an eluent of 15% ethyl acetate: n-hexanes afforded the title compound as a white solid (9 mg, 75%). $\delta^H$ (600 MHz, $d_6$-Acetone) 3.88 (3H, s, OCH$_3$), 5.09 (1H, d, $J$ 2.1, 1 $\times$ C=C-H$_2$), 5.52 (1H, d, $J$ 2.1, 1 $\times$ C=C-H$_2$), 7.01 (1H, d, $J$ 2.7, ArCH), 7.32 – 7.36 (2H, m, ArCH), 7.47 – 7.52 (4H, m, ArCH), 10.13 (1H, s, NH); $\delta^C$ (150 MHz, $d_6$-Acetone) 55.5, 98.2, 107.9, 109.1, 113.4, 121.8 (2 $\times$ ArCH), 122.4, 126.3, 129.5 (2 $\times$ ArCH), 142.6, 148.0, 150.5, 154.6, 165.2; m/z (ESI-TOF) 312.0874 (100%, MH$^+$, C$_{17}$H$_{14}$NO$_5$ requires 312.0872).

2,4,5-Trichlorophenyl-7-methoxy-2-methylene-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazine-5-carboxylate 22

Prepared in accordance with general procedure F using 2, 4, 5-trichlorophenol (20 mg, 0.1 mmol). Removal of the solvent in vacuo yielded a crude yellow oil. Purification of the crude material by flash column chromatography on silica gel using an eluent of 10% ethyl acetate: n-hexanes afforded the title compound as a white solid (8 mg, 50%). $\delta^H$ (600 MHz, $d_6$-Acetone) 3.83 (3H, s, OCH$_3$), 5.11 (1H, d, $J$ 1.7, 1 $\times$ C=C-H$_2$), 5.53 (1H, d, $J$ 1.7, 1 $\times$ C=C-H$_2$), 7.06 (1H, d, $J$ 2.7, ArCH), 7.48 (1H, d, $J$ 2.7, ArCH), 7.85 (1H, s, ArCH), 7.92 (1H, s, ArCH), 9.89 (1H, s, NH); $\delta^C$ (150 MHz, $d_6$-Acetone) 55.6, 98.4, 108.5, 109.2, 112.1, 122.7, 125.2, 125.9, 126.3, 130.6, 131.2, 142.7, 145.7, 147.8, 154.6, 155.0, 163.8; m/z (ESI-TOF) 413.9705 (100%, MH$^+$, C$_{17}$H$_{14}$Cl$_3$NO$_5$ requires 413.9703).

4-Methoxyphenyl-7-methoxy-2-methylene-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazine-5-carboxylate 25

Prepared in accordance with general procedure F using 4-methoxyphenol (12 mg, 0.1 mmol). Removal of the solvent in vacuo yielded a yellow oil. Purification of the crude material by flash column chromatography on silica gel using an eluent
of 20% ethyl acetate: n-hexanes afforded the title compound as a white solid (6 mg, 52%). \( \delta_H \) (600 MHz, \( d_6 \)-Acetone) 3.82 (3H, s, OCH\(_3\)), 3.88 (3H s, OCH\(_2\)H), 5.10 (1H, d, J 2.1, 1 × C=CH\(_2\)), 5.53 (1H, d, J 2.1, 1 × C=CH\(_2\)), 7.01 – 7.04 (3H, m, ArCH), 7.23 – 7.26 [2H, (AX)\(_2\), ArCH], 7.45 (1H, d, J 2.8, ArCH), 10.15 (1H, s, NH); \( \delta_C \) (150 MHz, CDCl\(_3\)) 55.6, 55.9, 99.6, 108.3, 108.8, 114.6 (2 × ArCH), 114.8, 116.0, 122.3 (2 × ArCH), 142.7, 143.4, 147.5, 149.4, 154.8, 157.7, 165.6; m/z (ESI-TOF) 342.0980 (100%, MH\(^+\), C\(_{18}\)H\(_{16}\)NO\(_6\) requires 342.0978).

7-Methoxy-2-methylene-3-oxo-3,4-dihydro-2\( H \)-benzo[b]1,4]oxazine-5-carboxamide 20

![Structure of 7-Methoxy-2-methylene-3-oxo-3,4-dihydro-2\( H \)-benzo[b]1,4]oxazine-5-carboxamide 20](image)

Prepared in accordance with general procedure F using ammonium hydroxide (4 µl, 0.1 mmol). Removal of the solvent in vacuo afforded an orange solid. The solid was triturated with cold acetone to afford the title compound as an off white solid (2 mg, 25%). \( \delta_H \) (600 MHz, \( d_6 \)-DMSO) 3.84 (3H, s, OCH\(_3\)), 5.18 (1H, d, J 2.2, 1 × C=CH\(_2\)), 5.50 (1H, d, J 2.2, 1 × C=CH\(_2\)), 6.99 (1H, d, J 2.2, ArCH), 7.26 (1H, d, J 2.3, ArCH), 7.87 (1H, s, 1 × NH\(_2\)), 8.37 (1H, s, 1 × NH\(_2\)), 11.49 (1H, s, NH); \( \delta_C \) (150 MHz, \( d_6 \)-DMSO) 56.6, 98.8, 105.6, 108.2, 115.3, 121.0, 127.9, 142.7, 148.5, 154.9, 170.0; m/z (ESI-TOF) 235.0720 (100%, MH\(^+\), C\(_{11}\)H\(_{11}\)N\(_2\)O\(_4\) requires 235.0719).

7-Methoxy-N-(4-methoxyphenyl)-2-methylene-3-oxo-3,4-dihydro-2\( H \)-benzo[b][1,4]oxazine-5-carboxamide 24

![Structure of 7-Methoxy-N-(4-methoxyphenyl)-2-methylene-3-oxo-3,4-dihydro-2\( H \)-benzo[b][1,4]oxazine-5-carboxamide 24](image)

Prepared in accordance with general procedure F using 4-methoxyaniline (12 mg, 0.1 mmol). Removal of the solvent in vacuo yielded a crude orange oil. Purification of the crude material by flash column chromatography on silica gel using an eluent of 30% ethyl acetate: n-hexanes afforded the title compound as an off white solid (8 mg, 63%). \( \delta_H \) (500 MHz, CDCl\(_3\)) 3.85 (3H, s, OCH\(_3\)), 3.86 (3H, s, OCH\(_3\)), 5.10 (1H, d, J 2.1, 1 × C=CH\(_2\)), 5.65 (1H, d, J 2.1, 1 × C=CH\(_2\)), 6.78 (1H, d, J 2.6, ArCH), 6.82 (1H, d, J 2.6, ArCH), 6.93 – 6.97 [2H, (AX)\(_2\), ArCH], 7.47 – 7.52 [2H, (AX)\(_2\), ArCH], 7.77 (1H, s, NH), 10.51 (1H, s, NH); \( \delta_C \) (150 MHz, \( d_6 \)-DMSO) 56.0, 56.6, 95.0, 101.9, 114.9 (2 × ArCH), 117.5, 120.0, 129.6 (2 × ArCH), 133.6, 141.4, 148.1, 155.5, 156.3, 159.1, 160.0, 163.3; m/z (ESI-TOF) 341.1140 (100%, MH\(^+\), C\(_{19}\)H\(_{17}\)N\(_2\)O\(_5\) requires 341.1137).
7-Methoxy-N-phenyl-2-methylene-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazine-5-carboxamide 23

Prepared in accordance with general procedure F using aniline (10 µl, 0.1 mmol). Removal of the solvent in vacuo yielded a crude orange oil. Purification of the crude material by flash column chromatography on silica gel using an eluent of 30% ethyl acetate: n-hexanes afforded the title compound as a white solid (7 mg, 58%). δH (500 MHz, CDCl3) 3.76 (3H, s, OCH3), 5.01 (1H, d, J = 2.3, 1 × C=CH2), 5.56 (1H, d, J = 2.1, 1 × C=CH2), 6.70 (1H, d, J = 2.1, 1 × C=CH2), 7.13 (1H, app. t, J = 7.3, ArC_H), 7.33 (2H, app. t, J = 7.3, ArC_H), 7.50 (2H, d, J = 7.3, ArC_H), 7.78 (1H, s, N_H), 10.36 (1H, s, N_H); δC (125 MHz, CDCl3) 56.3, 99.5, 104.9, 105.2, 106.5, 121.0, 125.7, 129.5, 137.1, 143.6, 148.1, 149.5, 155.3, 155.6, 165.3; m/z (ESI-TOF) 311.1036 (100%, MH+), C17H15N2O4 requires 311.1032).

N-Heptyl-7-methoxy-2-methylene-3-oxo-3, 4-dihydro-2H-benzo[b][1,4]oxazine-5-carboxamide 27

Prepared in accordance with general procedure F using 1-aminoheptane (8 mg, 100 µl, 0.8 mmol). Removal of the solvent in vacuo yielded a yellow oil. Purification of the crude material by flash column chromatography on silica gel using an eluent of 15% ethyl acetate: n-hexanes afforded the title compound as a white solid (8 mg, 75%). δH (500 MHz, CDCl3) 0.89 (3H, t, J = 6.8, CH3), 1.26 – 1.36 (8H, m, 4 × CH2), 1.58 – 1.63 (2H, m, CH2), 3.41 (2H, q, J = 7.1, CH2N), 3.80 (3H, s, OCH3), 5.05 (1H, d, J = 1.6, 1 × C=CH2), 5.61 (1H, d, J = 1.6, 1 × C=CH2), 6.20 (1H, s, NH), 6.64 (1H, d, J = 2.6, ArCH), 6.70 (1H, d, J = 2.6, ArCH), 10.65 (1H, s, NH); δC (500 MHz, CDCl3) 14.3, 22.8, 27.2, 29.2, 29.7, 32.0, 40.3, 56.2, 95.0, 99.2, 104.6, 106.4, 118.7, 120.4, 143.4, 148.2, 155.2, 167.0; m/z (ESI-TOF) 291.1348 (100%, MH+), C15H19N2O4 requires 291.1345).

Methyl-2-acetoxy-7-methoxy-2-methyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazine-5-carboxylate 26

Triethylamine (12 µl, 80 µmol) was added dropwise to a solution of hemi-acetal 8 (10 mg, 400 µmol), acetyl chloride (6 µl, 800 µmol) and DMAP (1 mg, 8 µmol) in DCM (2 cm³) at -10 ºC. The reaction mixture was stirred for 1 hour before the volatiles were removed in vacuo. The crude residue was purified by flash column chromatography on silica gel using...
an eluent of 15% ethyl acetate: n-hexanes to afford the title compound as a white solid (7 mg, 50%). δ_H (500 MHz, CDCl₃) 1.88 (3H, s, CH₃), 2.08 (3H, s, CH₃), 3.79 (3H, s, OCH₃), 3.93 (3H, s, OCH₃), 6.73 (1H, d, J 3.0, ArCH), 7.18 (1H, d, J 3.0, ArCH), 10.39 (1H, s, NH); δ_C (500 MHz, CDCl₃) 21.2, 24.2, 52.8, 56.0, 98.0, 108.4, 108.5, 114.1, 122.5, 143.0, 155.0, 162.2, 167.3, 169.5; m/z (ESI-TOF) 310.0930 (100%, MH⁺, C₁₄H₁₆NO₇ requires 310.0927).

Methyl 4-benzoyl-2-(benzoyloxy)-7-methoxy-2-methyl-3-oxo-3,4-dihydro-2H-benzo[bf]1,4]oxazine-5-carboxylate 32

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\text{Methyl 4-benzoyl-2-(benzoyloxy)-7-methoxy-2-methyl-3-oxo-3,4-dihydro-2H-benzo[bf]1,4]oxazine-5-carboxylate 32}
\]

Benzoyl chloride (20 µl, 0.16 mmol) was added to a solution of oxazine 1 (10 mg, 0.08 mmol), DMAP (0.1 mg, 0.1 µmol) and triethylamine (0.01 cm³, 10 mg, 0.1 mmol) in DCM (2 cm³) at 0 ºC. The reaction mixture was stirred for 2 hours before being poured into an aqueous 1N HCl solution (10 cm³). The organic phase was extracted with EtOAc (3 × 5 cm³) and the organic layers combined, washed with brine (10 cm³) and dried over magnesium sulfate. Removal of the solvent in vacuo yielded a yellow oil. Purification of the crude material by flash column chromatography on silica gel using an eluent of 15% ethyl acetate: n-hexanes afforded the title compound as a colourless oil (11 mg, 25%). δ_H (500 MHz, CDCl₃) 2.10 (3H, s, CH₃), 3.40 (3H, s, OCH₃), 3.88 (3H, s, OCH₃), 6.97 (1H, d, J 3.0, ArCH), 7.24 (1H, d, J 3.0, ArCH), 7.40 – 7.42 (2H, m, ArCH), 7.41 – 7.65 (4H, m, ArCH), 7.85 (2H, dd, J 8.3 1.3, ArCH), 8.00 (2H, dd, J 8.4 1.1, ArCH); δ_C (125 MHz, CDCl₃) 20.7, 52.3, 56.2, 100.0, 108.1, 111.9, 119.8, 124.2, 125.9, 128.5 (2 × ArCH), 128.8 (2 × ArCH), 130.0 (2 × ArCH), 130.2 (2 × ArCH), 133.6, 134.1, 135.0, 145.5, 157.5, 161.9, 164.3, 165.7, 173.6; m/z (ESI-TOF) 476.1345 (100%, MH⁺, C₂₆H₂₂NO₈ requires 476.1345).

Methyl 2-(2-chloroacetoxy)-7-methoxy-2-methyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazine-5-carboxylate 33

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\text{Methyl 2-(2-chloroacetoxy)-7-methoxy-2-methyl-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazine-5-carboxylate 33}
\]

Triethylamine (12 µl, 0.08 mmol) was added dropwise to a solution of hemi-acetal 8 (10 mg, 0.04 mmol), chloroacetyl chloride (63 µl, 0.08 mmol) and DMAP (1 mg, 8 µmol) in DCM (5 cm³) at -10 ºC. The mixture was stirred for 1 hour before the volatiles were removed in vacuo to yield a yellow oil. Purification of the crude material by flash column chromatography on silica gel using an eluent of 10% ethyl acetate: n-hexanes afforded the title compound as a white solid (3 mg, 25%). δ_H (600 MHz, CDCl₃) 1.95 (3H, s CH₃), 3.80 (3H, s OCH₃), 3.95 (3H, s OCH₃), 4.08 (2H, s, CH₂Cl), 6.75 (1H, d, J 2.8, ArCH), 7.21 (1H, d, J 2.8, ArCH), 10.48 (1H, s, NH); δ_C (150 MHz, CDCl₃) 23.6, 40.6, 62.6, 65.8, 99.1, 108.3, 114.0, 121.9, 142.2, 154.9, 160.7, 165.5, 166.9, 172.7; m/z (ESI-TOF) 344.0538 (100%, MH⁺, C₁₄H₁₅ClNO₇ requires 344.0537).
nOe spectra of oxazine 13

Key nOe interactions of irradiated proton ($\delta_H = 2.91$, highlighted in blue) with protons highlighted in red.
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