2018

A reassessment of mycophenolic acid as a lead compound for the development of inhibitors of chikungunya virus replication

Adel Rashad
University of Wollongong, ar917@uowmail.edu.au

Johan Neyts
University of Leuven

Pieter Leyssen
University of Leuven

Paul A. Keller
University of Wollongong, keller@uow.edu.au

Publication Details

Rashad, A. A., Neyts, J., Leyssen, P. & Keller, P. A. (2018). A reassessment of mycophenolic acid as a lead compound for the development of inhibitors of chikungunya virus replication. Tetrahedron, 74 (12), 1294-1306.

A reassessment of mycophenolic acid as a lead compound for the development of inhibitors of chikungunya virus replication

Abstract

Mycophenolic acid (MPA) has been previously reported as an inhibitor of the chikugunya virus (CHIKV) with an EC_{50} value of 0.2 μM. We used MPA as a lead compound designing and synthesizing a series of isatins and benzolactones in a typical medicinal chemistry program. The synthesis and testing of 19 derivatives produced compounds with no desired activity which prompted us to retest the lead compound, MPA. We can reveal that MPA shows no anti-CHIKV activity and therefore needs to be reassessed as a lead compound for this target.

Disciplines

Medicine and Health Sciences | Social and Behavioral Sciences

Publication Details

Rashad, A. A., Neyts, J., Leyssen, P. & Keller, P. A. (2018). A reassessment of mycophenolic acid as a lead compound for the development of inhibitors of chikungunya virus replication. Tetrahedron, 74 (12), 1294-1306.

A Reassessment of Mycophenolic Acid as a Lead Compound For the Development of Inhibitors of Chikungunya Virus Replication

Adel A. Rashad[a], Johan Neyts[b], Pieter Leyssen[b] and Paul A. Keller[a,*]

[a] *School of Chemistry, University of Wollongong, Wollongong 2522, Australia*

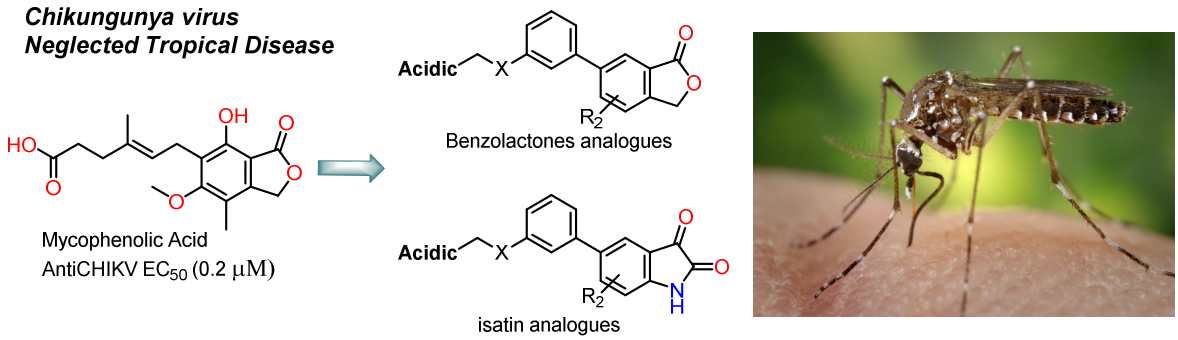
[b] *KU Leuven – University of Leuven, Department of Microbiology and Immunology, Rega Institute for Medical Research, Laboratory of Virology and Chemotherapy, B-3000 Leuven, Belgium*

Keywords

Mycophenolic acid, Chikungunya, Isatin, Benzolactone

Abstract

Mycophenolic acid (MPA) has been previously reported as an inhibitor of the chikugunya virus (CHIKV) with an EC_{50} value of 0.2 µM. We used MPA as a lead compound designing and synthesizing a series of isatins and benzolactones in a typical medicinal chemistry program. The synthesis and testing of 19 derivatives produced compounds with no desired activity which prompted us to retest the lead compound, MPA. We can reveal that MPA shows no anti-CHIKV activity and therefore needs to be reassessed as a lead compound for this target.



Introduction

Chikungunya virus (CHIKV) is a pathogen belonging to the genus Alphavirus, family Togaviridae. It is mainly transmitted through the bite of an infected *Aedes aegypti* or *A. albopictus* mosquito, and the infection is characterized by a typical and devastating arthritis disease. The first report on a patient with chikungunya fever dates from 1952.[1] After five decades of only sporadic cases, a series of large epidemics occurred, starting in la Réunion island (2005-2006) during which almost 1:3 of the population became infected, progressing around the Pacific Ocean through India (1.4 to 6.5 million cases in 2006-2007), in 2009 followed by 3,000 and 42,000 cases in Malaysia and Thailand, respectively.[2,3] In Europe, as well as multiple imported cases, the first patients with indigenous CHIKV transmission were documented in Italy in 2007.[4,5] Presently, CHIKV infections are reported in nearly 40 countries (e.g. Singapore and Italy), in many of which the virus has now become endemic.[6] In 2008, the virus was listed as a category C priority

pathogen by the US National Institute of Allergy and Infectious Diseases (NIAID) due to its high morbidity rate and major health impact.[7,8]

The symptoms of CHIKV infection generally start 4-7 days after the mosquito bite. The course of infection typically has two phases, the first acute phase lasting between 1-10 days, characterized by a painful polyarthralgia, high fever, headache, vomiting, rash, and myalgia. The second is the persistent or chronic phase with disabling polyarthralgia that can last from weeks to years.[9,10] During the recent epidemics, neurological disorders such as encephalitis, peripheral neuropathy and myelopathy have been reported.[11] Cases of multi-organ failure have also been documented[12], and eye infections which may cause neuro retinitis.[13,14] CHIKV-induced mortality is limited (1:1000), with most deaths occurring in neonates, the elderly, and patients with underlying health conditions.

Despite the vast impact of CHIKV infection, a vaccine is still not available. Currently, the treatment options are limited to symptomatic remedies with *e.g.* corticosteroids as no antiviral drug is yet available.[15,16] The development of a chemotherapeutic strategy is considered to be as equally important as the development of a vaccine. In the past years, CHIKV attracted attention from the scientific community following the elucidation of the structure of the viral protease[17] and the viral envelope proteins.[18-20] These reports were followed by *in silico* structure-based drug design efforts to develop small-molecule inhibitors.[21-25] However, only a few lead compounds with acceptable activity have been reported so far.[26] These include mycophenolic acid (MPA **1**),[27] harringtonine **2**[1,28] and 12-*O*-tetradecanoylphorbol 13-acetate (TPA, **3**, Figure 1).[29] TPA **3** has been reported as the most potent compound with an EC_{50} = 0.0029 μM and SI = 1965. However, it is also one of the most potent tumour-promoting agents known to date,[19] and therefore is less likely to be further developed as possible antiviral drug. Harringtonine **2** is a natural product with a complex chemical structure, which complicates further development, however MPA **1**, is a low molecular weight inhibitor and presents as an excellent candidate for further exploration.

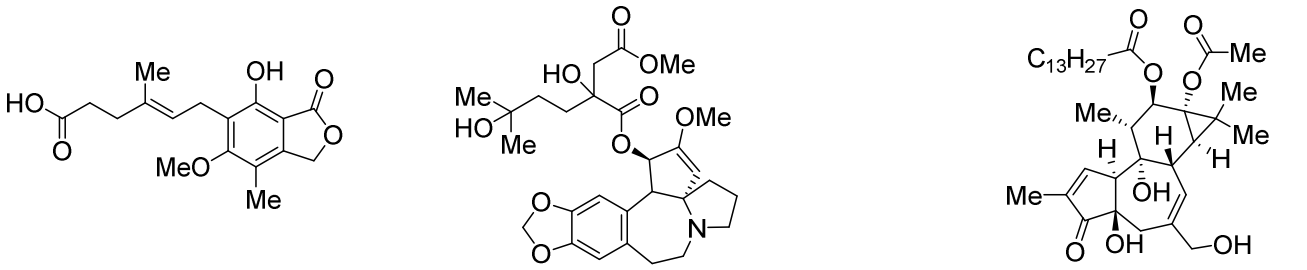


Figure 1 Chemical structure of anti-CHIKV lead compounds with promising antiviral activity.

MPA **1** was reported to inhibit CHIKV replication and virus-induced cell death with an EC_{50} value of 0.2 μM and a selectivity index value of 150,[27] and was found to induce CHIKV apoptosis. It inhibits the cellular inosine monophosphate dehydrogenase (IMPDH), an enzyme that involved in the *de novo*

biosynthesis of guanine nucleotide. It is well known that MPA **1** suffers from a metabolic drawback: rapid conjugation of the C7 phenolic hydroxyl group with glucuronic acid results in more soluble metabolites that are more easily cleared.[20,31-32] Despite this property, MPA **1** is a relatively small molecule with numerous positions available for modification, including the removal or substitution of the phenolic hydroxyl group. This study describes the exploration of MPA **1** as a starting point for the development of compounds with putative anti-CHIKV activity.

Results and Discussions

Design of MPA analogues:

MPA **1** has good drug-like properties with a molecular weight of 320.3, a calculated logP (clogP) value of 2.92, and a calculated logD value of 3.56 (pH = 1-2), 0.67 (pH = 7.4) and 2.52 (pH = 5.5), indicating good blood solubility and oral bioavailability - oral drug absorption occurs in the small intestine (pH ~ 5.5[33,34]). Derivatives of MPA **1**, in particular those for which the metabolic drawbacks are overcome, could possibly lead to an antiviral treatment for CHIKV infection.

MPA **1** can be considered as being composed of three connected moieties (Figure 2): an aromatic head (the 3-oxo-1,3-dihydroisobenzofuran moiety), a rigid linker (the alkene connection) and an acidic tail (the carboxylic acid group). The aromatic head carries two major hydrogen bond acceptors (the carbonyl group and the oxygen atom of the lactone ring). The aromatic benzo moiety carries a hydroxyl group, a methoxy group and a methyl group. In this study, the three components of MPA **1** were investigated as optimizable components, starting from simple core units such as benzoic acids and anilines; Figure 2 shows the possible access to MPA **1** analogues starting from such derivatives. Both the acids and anilines could be converted into the aromatic heads carrying H-bond acceptor features, which could be connected through simple C-C coupling, to the linker and the acidic tail. R groups on the acids and anilines were selected to give similar environment as in **1**.

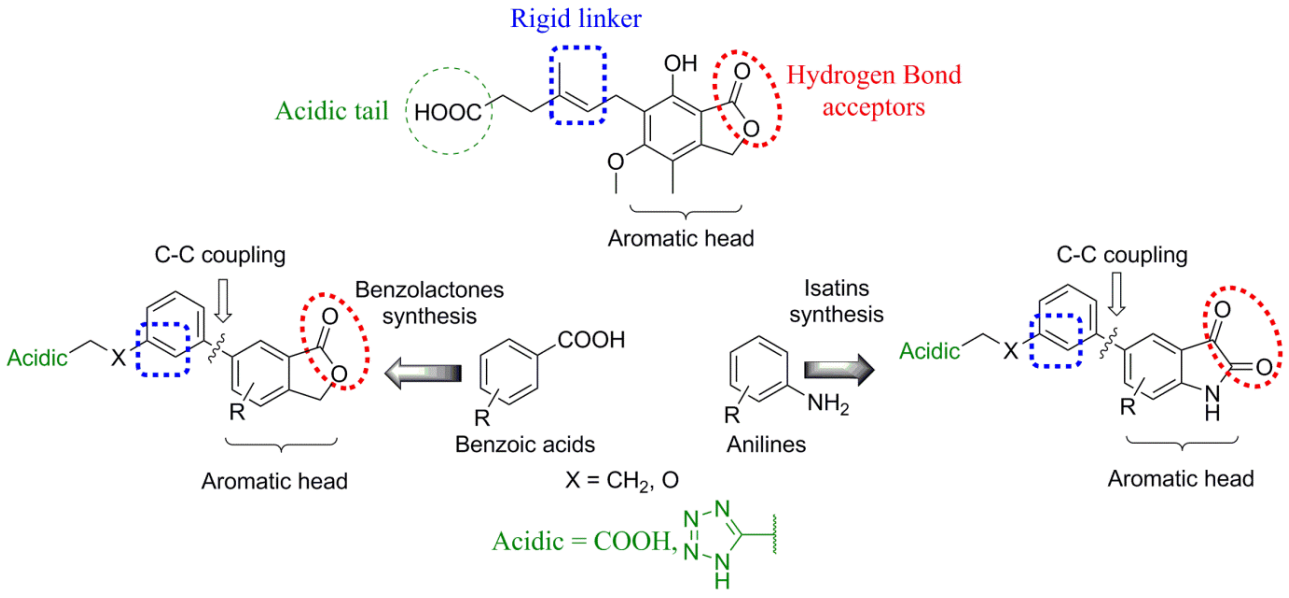


Figure 2. Design of MPA **1** analogues starting from benzoic acids and aniline derivatives. The red circles indicate the HB accepting groups, the blue boxes indicate the rigid linker and the green circle refers to the acidic tail moiety that could be either COOH or the bioisostere tetrazole.

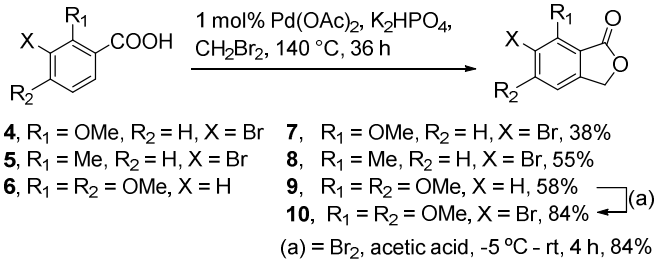
In addition to benzolactones, isatins were also selected as alternative possible replacements for the aromatic head, as they are known to possess various pharmacological properties including anticholinesterase, anticonvulsant, antiinflammatory, antihypertensive, antihypoxic, antimicrobial, and antiviral properties.[35,36] The structure of isatin could participate in the biological activity, with the two carbonyl groups (red circles, Figure 2) accepting H-bonds from the target receptor residues, and the NH donating a H-bond to acceptor residues.[37,38] The aromatic core π-system of isatin was also found to participate through aromatic interactions with some target receptor residues.[39]

Replacing the rigid linker alkene with the phenyl ring (blue boxes, Figure 2) would be an applicable strategy for obtaining an analogous linker between the aromatic head and the acidic tail of MPA analogues. Utilizing cross coupling chemistry[40-42] would facilitate facile access to the bi-aromatic system. This phenyl aromatic linker may also provide a π-electron system for interaction within the yet unknown target site. The acidic tail remained either as a carboxylic group or the bioisostere tetrazole[43] (green, Figure 2). Tetrazole rings have ionisable acidic proton at pH 7.4. They are also planar in structure, and the anion is 10 times[44] more lipophilic than the carboxylate anion and this can enhance drug absorption as a result. Tetrazole rings are also resistant to many of the metabolic reactions that occur to the carboxylic groups.[44,45]

Chemistry:

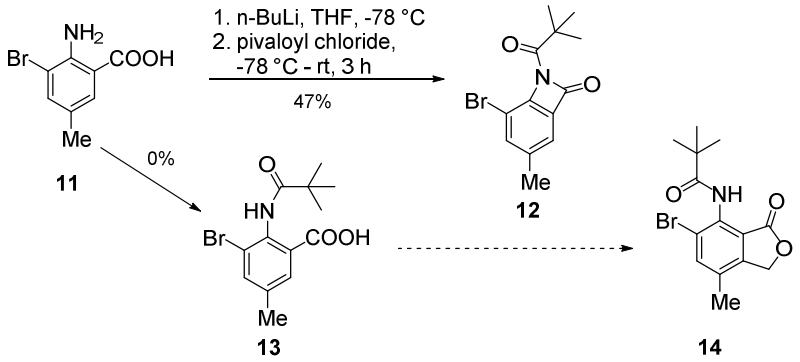
Synthesis of the aromatic heads:

The benzolactone aromatic head synthesis was achieved *via* Pd catalysed chemistry[46] from benzoic acids using dibromomethane as the solvent and carbon donor (Scheme 1). The Br substituent is prepositioned at the benzolactone C6 in preparation for metal catalysed cross coupling.



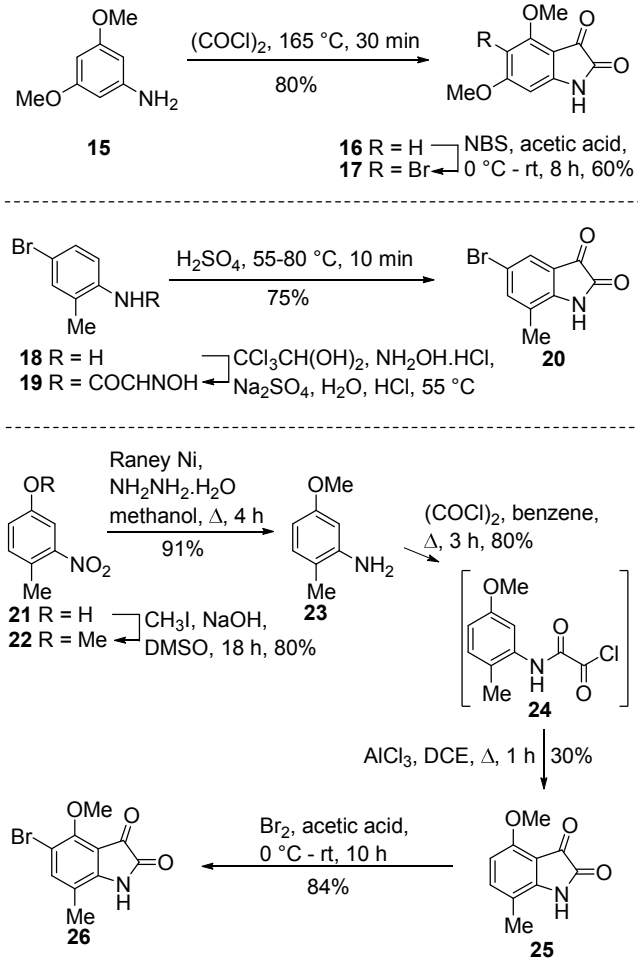
Scheme 1. The synthesis of the benzolactone aromatic heads.

The synthesis of an amino substituted benzolactone was attempted using 2-amino-3-bromo-5-methylbenzoic acid **11**, which then cyclised to afford the aminobenzolactone **14** (Scheme 2). Unsurprisingly, the amino-protected compound **13** was not formed, instead, the β-lactam product **12** was obtained which was then incorporated into the project as an alternative aromatic head.



Scheme 2 The designed synthesis of the aminobenzolactone **14**.

The isatin aromatic heads **16** and **25** were formed by reaction with oxalyl chloride (Scheme 3), followed by the insertion of the C5 bromo substituent using either NBS, or Br_2 in acetic acid producing **17** and **26** respectively. The 7-Me substituted isatin **20** was produced from the aniline **18** with the coupling handle (Br) already in place, using 2,2,2-trichloroethane-1,1-diol and hydroxylamine. These derivatives were selected to demonstrate similarity to the lead MPA **1**, with the OH group masked as methoxy groups to avoid the metabolic liability.



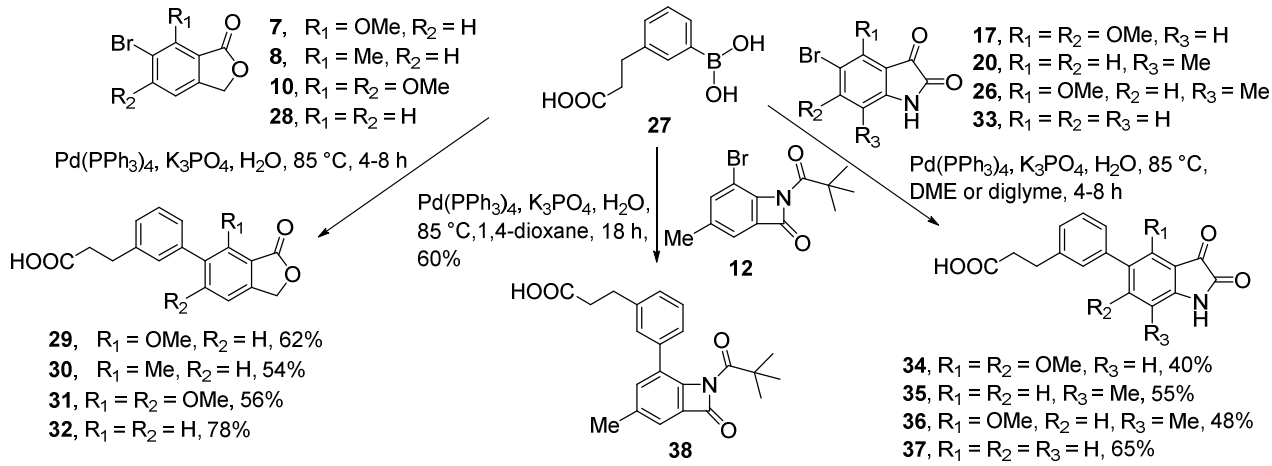
Scheme 3. The synthesis of the isatin aromatic heads.

Synthesis of the acid conjugates:

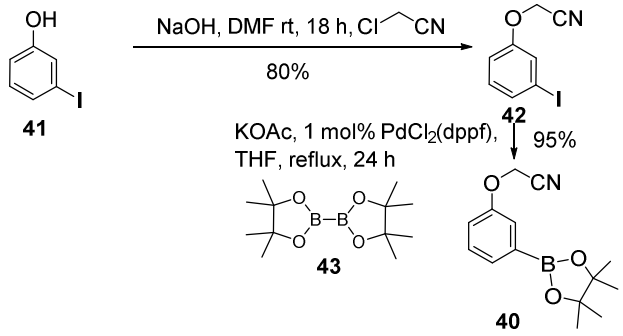
The benzolactone-acid, isatin-acid and β-lactam-acid conjugate syntheses were achieved through the one step C-C cross coupling reaction with the commercially available 3-(3-boronophenyl)propanoic acid **27** (Scheme 4). This facile one step synthesis gave the three main components of the MPA designed analogues, the aromatic head, the rigid linker (phenyl) and the acidic tail (COOH).

Synthesis of the tetrazole conjugates:

Tetrazole conjugates were synthesised from acetonitrile intermediates *via* 3-(3-boronophenyl)propanoic acid **39** in a coupling reaction with the bromobenzolactones. Due to the high cost of **39**, the ester **40** was also synthesized by reacting 3-iodophenol with 2-chloroacetonitrile, followed by a borylation reaction (Scheme 5).



Scheme 4. The synthesis of the acid conjugates.

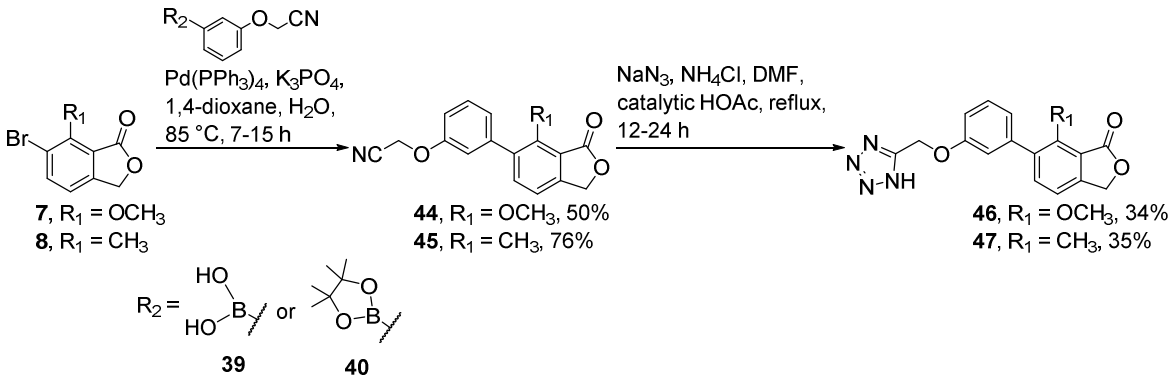


Scheme 5. The synthesis of the boronic ester **40**.

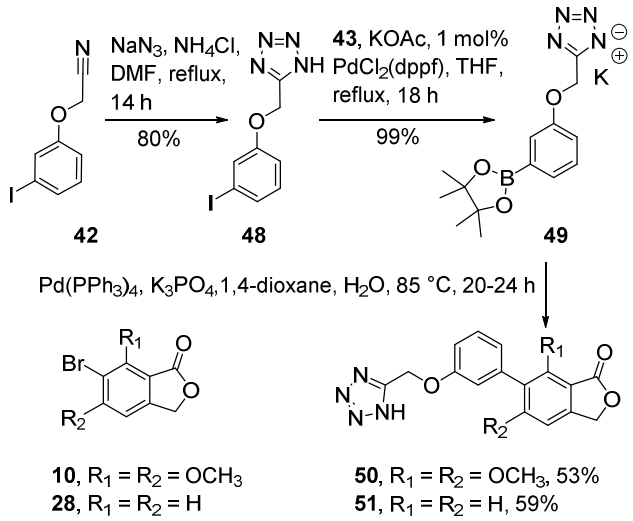
The benzolactone-acetonitrile intermediates **44** and **45** were accessed *via* Suzuki coupling with **39** or **40** (Scheme 6). Subsequent [2 + 3] cycloaddition reaction using NaN_3/NH_4Cl in DMF afforded the tetrazoles **46** and **47** (Scheme 6).

The modest yields (35%) of the tetrazole formation (Scheme 6) were considered insufficient to justify the synthesis of further benzolactone-tetrazole conjugates. An alternative synthetic strategy was devised in

which the tetrazole moiety was attached to the boronic ester, prior to the coupling reaction with the 6-bromobenzolactones. The previously synthesized 2-(3-iodophenoxy)acetonitrile **42** was used to access the intermediate **48** utilizing the azide tetrazole formation *via* mixing with NaN_3 and NH_4Cl in DMF containing few drops of glacial acetic acid (Scheme 7). The tetrazole **48** was then reacted with bis(pinacolato)diboron **43**, potassium acetate and $PdCl_2$(dppf) catalyst in THF (Scheme 7) to afford the tetrazole salt intermediate **49**. The tetrazole conjugates **50** and **51** were synthesized via Suzuki reaction of the tetrazole salt **49** with the remaining 6-bromobenzolactones (Scheme 7).

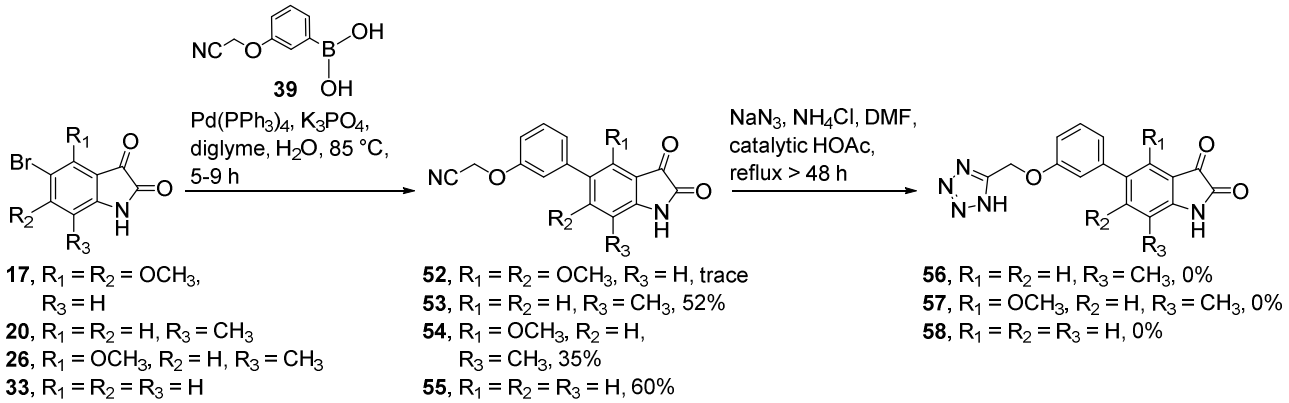


Scheme 6. The synthesis of the tetrazole conjugates **46** and **47**.



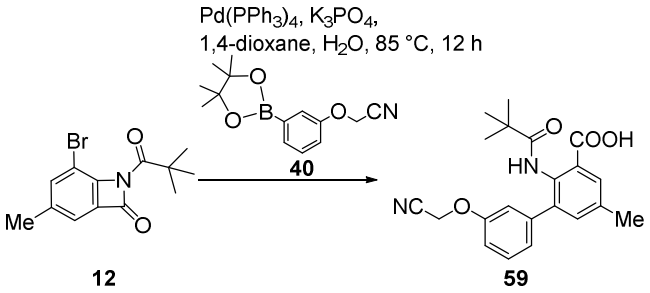
Scheme 7. The synthesis of the tetrazole salt intermediate **49**.

The isatin-acetonitrile intermediates were also accessed through the reaction with **39** (Scheme 8), however, the subsequent [2 + 3] cycloaddition, to form the isatin-tetrazole conjugates, was not successful. The reaction resulted in the disappearance of all starting materials but produced un-identifiable degradation products possibly arising both from the reactivity of isatin core and the interference with the cycloaddition reaction.



Scheme 8. The synthesis of the isatin-acetonitrile intermediates and subsequent attempt to synthesize the isatin-tetrazole conjugates.

Coupling of the β-lactam intermediate **12** with the boronic ester **40** (Scheme 9) formed the biaryl with concurrent β-lactam opening to give the benzoic acid derivative **59**. The difference between the reaction to give **38** (Scheme 4) and that to give **59** (Scheme 9) was the workup, with the former quenched at 0 °C compared to room temperature in the case of **59**. It is believed that during this thermally uncontrolled procedure, the β-lactam ring was hydrolysed to provide the COOH moiety.



Scheme 9. The coupling reaction of the β-lactam **12** with **40**.

Antiviral evaluation

All compounds were evaluated for antiviral activity in a virus-cell-based assay against Chikungunya virus (Indian Ocean strain 899) (Table 1). Compounds for which an EC_{50} value could be calculated from the antiviral dose-response curve, were subjected to a similar experiment on treated, uninfected cells to assess the effect of the compound on host cell metabolism and to determine its selectivity.

Overall, few compounds elicited an antiviral effect in this assay, and the compounds that did, only showed a limited selectivity (EC_{50}/CC_{50}). Furthermore, microscopic inspection of the assay conditions at which some inhibition of virus-induced cell death was detected by the MTS/PMS readout method revealed that none of the compounds matched the hit selection criteria (100% inhibition of virus-induced cell death without any apparent change in host cell or monolayer morphology). Therefore, none of these compounds could be considered to be selective inhibitors of CHIKV replication.

This surprising lack of activity for all our synthesized analogues turned our attention back to the original lead compound MPA **1**, questioning the reported activity against CHIKV. Therefore, in order to test the accuracy of the original finding, we purchased and retested MPA **1**, and subjected it to the same anti-CHIKV assay. To our dismay, it also came up as being not active (Table 1).

In order to reason these outcomes, we examined the assay conditions described in the original manuscript that defined MPA as an anti-CHIKV agent. The reported incubation time of the assays was restricted to 48

Table 1. Antiviral activity of compounds in a virus-cell-based assay against Chikungunya virus (strain 899).

Compound	EC_{50} (μM)	CC_{50} (μM)
1	11 ± 1	28 ± 10
29	NA	ND
30	NA	ND
31	NA	ND
32	NA	ND
34	NA	ND
35	NA	ND
36	NA	ND
37	NA	ND
38	NA	ND
44	NA	ND
45	NA	ND
46	NA	ND
47	NA	ND
50	NA	ND
51	NA	ND
53	84 ± 14	88 ± 18
54	NA	ND
55	NA	ND
59	NA	ND

NA = not active; ND = not determined; EC50 = concentration of compound that inhibits virus-induced cell death with 50%; CC_{50} = concentration of compound that reduces the metabolic activity of treated, uninfected cells with 50%; results are reported as median and standard deviation from a series of at least 2 independent experiments.

hours – in this scenario, MPA reduces the GTP concentration, and as CHIKV replicates much slower, it has not had the opportunity to kill the cells at this 48 hours post-infection timepoint. If a compound is a true antiviral, then it should show antiviral activity even five days post-infection. Therefore, in Chikungunya antiviral testing, it is imperative to incubate the assay for five days to allow sufficient time to show a time-dependency of the antiviral effect. In our five-day anti-CHIKV assays, we have shown that there is no lasting antiviral effect of MPA against CHIKV. Therefore, MPA is likely a 'braking' compound, applying the ‘brakes’ on the replication of CHIKV, but not completely stopping it from multiplying. Therefore, MPA **1** cannot be considered a lead compound targeting the CHIKV and must be reassessed as a lead compound.

Conclusions

To summarize, using MPA as a lead compound for the design and synthesis of potential compounds targeting the CHIKV, we synthesized a series of isatin and bezolactone derivatives. Subsequent testing revealed no activity which led to retesting of the lead MPA **1**. We found that it did not possess any anti-CHIKV activity and therefore we conclude that it cannot be considered as an inhibitor of CHIKV.

Experimental section

Chemical synthesis

Unless otherwise stated, chemicals were purchased from Sigma Aldrich (Australia). (3-(Cyanomethoxy)phenyl)boronic acid was purchased from Combi-Blocks, Inc., USA. All ^{1}H, ^{13}C and spectra were recorded at 500 and 125 MHz, respectively on a Varian Inova 500 MHz spectrometer. Chemical shifts (δ) are reported in parts per million relative to TMS using $CDCl_3$ as a solvent unless otherwise noted. The solvents used for ^{13}C NMR were the same used for the ^{1}H NMR unless otherwise noted. Coupling constants (*J*) are reported in Hertz (Hz). Multiplicities are reported as singlet (s), broad (br), doublet (d), doublet of doublets (dd), doublet of doublets of doublets (ddd), doublet of triplet (dt), multiplet (m), pentet (p), sextet (sex) or septet (sp). Electron impact (EI) and electrospray (ES) mass spectra (MS) were recorded on a Shimadzu QP-5000 spectrometer and high resolution (HR) on a VG AutoSpec spectrometer. Electrospray (ESI) mass spectra were recorded on a Micromass Platform LCZ spectrometer and high resolution on a Micromass QTOF2 spectrometer. Ion mass to charge (*m/z*) values are stated with their relative abundances as a percentage in parentheses. Peaks assigned to the molecular ion are denoted by M^+ or M^- (when using the ESI^-). Thin Layer Chromatography (TLC) was performed using Merck Silica Gel F_{254} aluminium sheets. Column chromatography purifications were performed using Flash Silica Gel. Infrared (IR) spectra were recorded on a (Shimadzu FT-IR spectrometer) fitted with a Smart Omni-Sampler germanium crystal accessory. All IR spectra were recorded on neat samples with strong, medium and weak peaks are assigned as s, m and w, respectively. Melting points were determined using a Gallenkamp (Griffin) melting point apparatus. Temperatures are expressed in degrees Celsius (ºC) and are uncorrected. HPLC purity check was performed using Waters 1525 HPLC pump, WatersTm 486 absorbance detector (using the wavelength, 254 nm) and Nova-Pak® Silica 3.9 x 150 mm C18 column. HPLC solvents used:

solvent A hexane, solvent B isopropanol containing 0.1% TFA and solvent C isopropanol. Retention times in minutes for the HPLC results are expressed as R_t.

*General procedure 1: synthesis of the isobenzofuran-1(3H)-ones (**7**-**10**)*

Under a N_2 atmosphere, a sealed tube was charged with the appropriate benzoic acid (1 mmol), palladium acetate (22.4 mg, 0.1 mmol), dipotassium phosphate (K_2HPO_4, 522.5 mg, 3 mmol) and dibromomethane (4 mL). The contents of the tube were degassed by flushing N_2 through a needle for 10 min. The tube was then sealed, and heated in an oil bath at 140 ºC for 36 h. The tube was then allowed to cool to room temperature, the content was diluted with CH_2Cl_2 (20 mL), and filtered through celite. The filtrate was washed with 1 M HCl (20 mL) and brine (2 x 15 mL), and was dried ($MgSO_4$). The solvent was evaporated under reduced pressure and the residue was either subjected to flash column chromatography (10% methanol in chloroform) or recrystallised from chloroform/hexane mixture (1:3).

*6-Bromo-7-methoxyisobenzofuran-1(3H)-one (**7**)*

Following the general procedure 1 using 3-bromo-2-methoxybenzoic acid **4** (231 mg), the benzofuran **7** was isolated after column chromatography, as a white solid (92 mg, 38%), mp: 102-103 ºC; IR (cm^{-1}): υ 1752 (s, C=O), 1288 (m, C-O), 1064 (w, C-O), 1111 (w, C-Br). ^{1}H NMR ($CDCl_3$), δ: 7.84 (d, *J* = 7.9 Hz, 1H, H5), 7.06 (d, *J* = 7.9 Hz, 1H, H4), 5.23 (s, 2H, CH_2), 4.16 (s, 3H, OCH_3). ^{13}C NMR, δ: 167.5 (C=O), 156.3 (C7), 148.3 ($C3_a$), 139.3 (C5), 118.6 (C4), 117.9 ($C7_a$), 117.2 (C6), 68.7 (CH_2), 62.9 (CH_3) ppm. EI-MS *m/z* 244 (M^+ ^{81}Br, 28), 242 (M^+ ^{79}Br, 30), 215 (31), 213 (33), 198 (100%), 196 (98), 185 (17), 183 (15). HRMS (ESI) calcd for $C_9H_8{}^{79}BrO_3$ (MH^+), 242.9649; found, 242.9657.

*6-Bromo-7-methylisobenzofuran-1(3H)-one (**8**)*

Following the general procedure 1 using 3-bromo-2-methylbenzoic acid **5** (215 mg), the benzofuran **8** was isolated after recrystallisation, as a pale yellow solid (124 mg, 55%), mp: 91-92 ºC; IR (cm^{-1}): υ 1739 (s, C=O), 1358 (w, C-O), 1069 (w, C-Br). ^{1}H NMR ($CDCl_3$), δ: 7.79 (d, *J* = 8.0 Hz, 1H, H5), 7.18 (d, *J* = 8.0 Hz, 1H, H4), 5.20 (s, 2H, CH_2), 2.74 (s, 3H, CH_3). ^{13}C NMR, δ: 170.1 (C=O), 146.1 ($C3_a$), 139.8 (C5), 137.7 (C4), 126.3 (C7), 124.7 (C7a), 120.6 (C6), 68.2 (CH_2), 16.6 (CH_3) ppm. EI-MS *m/z* 228 (M^+ ^{81}Br, 54), 226 (M^+ ^{79}Br, 55), 199 (100%), 197 (99), 171 (32), 169 (30). HRMS (ESI) calcd for $C_9H_8{}^{79}BrO_2$ (MH^+), 226.9708; found, 226.9705.

5,7-Dimethoxyisobenzofuran-1(3H)-one[41] *(**9**)*

Following the general procedure 1 using 2,4-dimethoxybenzoic acid **6** (182 mg). The benzofuran **9** was isolated after column chromatography as a white solid (112.5 mg, 58%). Spectral data of the isolated benzofuran **9** are in agreement with that reported.[41]

*6-Bromo-5,7-dimethoxyisobenzofuran-1(3H)-one (**10**)*

In a round bottom flask (50 mL), 5,7-dimethoxyisobenzofuran-1(3*H*)-one **9** (150 mg, 0.773 mmol) was dissolved in glacial acetic acid (20 mL) with vigorous stirring and the flask was cooled to -5 - 0 ºC using an

ice bath. A solution of bromine (136 mg, 0.851 mmol) in glacial acetic acid (10 mL) was added dropwise over 20 min. The reaction was allowed to warm up slowly to room temperature and was then allowed to stir for 4 h. The formed solid was filtered and washed with dilute acetic acid, and was then subjected to column chromatography (60% CH_2Cl_2 in hexane) to afford the benzofuran **10** (176.7 mg, 84%) as a white solid. It could also be recrystallised from 1,4-dioxane, mp: 189-190 ºC; IR (cm^{-1}): υ 1700 (s, C=O), 1316 (w, C-O), 1071 (w, C-O), 1036 (w, C-Br). ^{1}H NMR ($CDCl_3$), δ: 6.46 (s, 1H, H4), 5.07 (s, 2H, CH_2), 4.02 (s, 6H, OCH_3). ^{13}C NMR, δ: 168.4 (C=O), 162.2 (C7), 159.5 (C5), 150.5 ($C3_a$), 107.8 ($C7_a$), 96.2 (C6), 95.1 (C4), 69.3 (CH_2), 57.1 (C7-OCH_3), 56.6 (C5-OCH_3) ppm. EI-MS *m/z* 274 (M^+ ^{81}Br, 38), 272 (M^+ ^{79}Br, 40), 256 (18), 254 (20), 245 (32), 243 (30), 228 (100%), 226 (100), 215 (20), 213 (21). HRMS (ESI) calcd for $C_{10}H_{10}{}^{79}BrO_4$ (MH^+), 272.9762; found, 272.9754.

*6-Bromoisobenzofuran-1(3H)-one (**28**)*

This compound was prepared according to reported procedures.[42]

*5-Bromo-3-methyl-7-pivaloyl-7-azabicyclo[4.2.0]octa-1,3,5-trien-8-one (**12**)*

In an oven dried 50 mL flask and under a N_2 atmosphere, 2-amino-3-bromo-5-methylbenzoic acid **11** (750 mg, 3.26 mmol) was dissolved in dry THF (20 mL), and the flask was then sealed with a rubber cap with fitted with a N_2 balloon, and cooled to -78 ºC. *n*-BuLi (4.08 mL of 2 M solution, 8.15 mmol) was added dropwise through a needle and the mixture was stirred for 20 min at -78 ºC before adding pivaloyl chloride (0.48 mL, 3.91 mmol) dropwise. The reaction was then allowed to warm to room temperature and stirred for 3 h. The reaction mixture was then diluted with ethyl acetate (10 mL) and water (10 mL), the organic layer was separated and washed with water (2 x 20 mL), brine (2 x 10 mL) and dried ($MgSO_4$). The combined organic layers were concentrated under reduced pressure to yield the β-lactam derivative **12** as a colourless oil (452 mg, 47%), IR (cm^{-1}): υ 1745 (s, C=O), 1634 (s, C=O), 1465 (w, C-N), 1059 (w, C-Br). ^{1}H NMR ($CDCl_3$), δ: 7.94 (s, 1H, H4), 7.86 (s, 1H, H2), 2.45 (s, 3H, CH_3), 1.42 (s, 9H, pivaloyl 3xCH_3). ^{13}C NMR, δ: 168.1 (pivaloyl C=O), 159.7 (C6), 142.3 (C=O), 140.9 (C4), 139.3 (C1), 127.4 (C2), 121.8 (C3), 117.8 (C5), 38.3 ($C(CH_3)_3$), 27.6 (pivaloyl 3xCH_3), 20.9 (CH_3) ppm. EI-MS *m/z* 297 (M^+, ^{81}Br, 33), 295 (M^+, ^{79}Br, 35), 282 (98), 280 (100%), 255 (18), 253 (20), 240 (35), 238 (37). HRMS (ESI) calcd for $C_{13}H_{15}BrNO_2$ (MH^+), 296.0286; found, 296.0294.

*4,6-Dimethoxyindoline-2,3-dione[43] (**16**)*

This compound was prepared following the reported procedure.[43] The 4,6-dimethoxyindole-2,3-dione **16** was obtained as a yellow solid (5.2 g, 80%).

*5-Bromo-4,6-dimethoxyindoline-2,3-dione (**17**)*

In a 100 mL round bottom flask, a solution of 4,6-dimethoxyindole-2,3-dione **16** (143 mg, 0.5 mmol) in acetic acid (10 mL) was stirred at room temperature until all the isatin was completely dissolved before being cooled to 0 ºC using an ice bath. *N*-Bromosuccinimide (97.9 mg, 0.55 mmol) was added portion wise

over 15 min. After addition of the NBS, the reaction mixture started to solidify, and the flask was removed from the ice bath and was allowed to warm to room temperature and then stirred for 8 h. The yellow solid was filtered from the mother liquor to which cold water (10 mL) was added to precipitate the remaining soluble product. The combined solids were washed with saturated $NaHCO_3$ solution (2 x 15 mL) and with 1:1 water ethanol mixture (25 mL) and then vacuum dried. Recrystallisation from 1,4-dioxane yielded the 5-bromo-4,6-dimethoxyindoline-2,3-dione **17** as a yellow solid (118 mg, 60%), mp: >250 ºC; IR (cm^{-1}): υ 3195 (m, NH), 1718 (s, C=O), 1264 (m, C-O). ^{1}H NMR (DMSO-d_6), δ: 11.14 (s, NH), 6.36 (s, H7), 3.99 (s, 3H, C6-OCH_3), 3.94 (s, 3H, C4-OCH_3). ^{13}C NMR, δ: 178.4 (C3), 165.5 (C2), 161.7 (C4), 160.4 (C6), 151.4 ($C7_a$), 102.7 ($C3_a$), 91.8 (C7), 86.2 (C5), 58.1 (C4-OCH_3), 57.2 (C6-OCH_3) ppm. EI-MS *m/z* 287 (M^+ ^{81}Br, 40), 285 (M^+ ^{79}Br, 42), 259 (98), 257 (100%), 215 (6), 213 (7). HRMS (ESI) calcd for $C_{10}H_9N^{79}BrNO_4$ (MH+), 285.9715; found, 285.9719.

5-Bromo-7-methylindoline-2,3-dione[44] ***(20)***

4-Bromo-2-methylaniline **18** (6.88 g, 37 mmol) was added to a 500 mL flask and was reacted with chloral hydrate (7.35 g, 44.4 mmol), hydroxylamine hydrochloride (9.25 g, 133 mmol), sodium sulfate (42 g, 295 mmol) in water (250 mL) and 2 M HCl (12.5 mL), by heating the mixture at 55 ºC with vigorous stirring for 14 h. After cooling to room temperature, the aqueous layer was decanted from the formed gummy residue which was further washed with cold water and dried under vacuum. The flask was then cooled to -5 - 0 ºC using ice/salt bath before adding cold sulfuric acid (22.5 mL). The flask was allowed to warm to room temperature before heating the reaction mixture gradually to 70 ºC where the mixture started to get darker in colour. When the colour change became stable after almost 30 min, the reaction was heated to 80 ºC for 10 min. After cooling to room temperature, the mixture was poured onto crushed ice (150 mL) and stirred for 1 h. The separated solid was then filtered and washed with water (3 x 100 mL) to give a red solid of 5-bromo-7-methylindoline-2,3-dione **20** (6.65 g, 75%) and was sufficiently pure for the next step. The spectral data were consistent with the reported literature.[44]

4-Methoxy-1-methyl-2-nitrobenzene[45] ***(22)***

This compound was synthesized following the reported procedure from 4-methyl-3-nitrophenol **21**.[45] The 4-methoxy-1-methyl-2-nitrobenzene **22** was isolated as a white crystalline solid (5.34 g, 80%).

5-Methoxy-2-methylaniline[46] ***(23)***

To a solution of 4-methoxy-1-methyl-2-nitrobenzene **22** (5.1 g, 30.5 mmol) in methanol (35 mL) under a N_2 atmosphere was added Raney Nickel (0.1 mol %) and the mixture was vigorously stirred at room temperature until the H_2 ceased to evolve. Hydrazine monohydrate (2.0 g, 41 mmol) in methanol (10 mL) was added through a syringe slowly over 10 min. The mixture was then heated at reflux for 4 h. The reaction mixture was filtered hot through celite, and the filtrate was concentrated under reduced pressure. The obtained residue was recrystallised from petroleum spirit to yield 5-methoxy-2-methylaniline **23** as

white solid (3.8 g, 91%). Spectral data for this aniline derivative **23** were in agreement with the reported literature.[46]

*4-Methoxy-7-methylindoline-2,3-dione (**25**)*

In a 250 mL round bottom flask, 5-methoxy-2-methylaniline **23** (2.0 g, 15 mmol) was dissolved in anhydrous benzene (20 mL), and oxalyl chloride (4.12 g, 2.8 mL, 32.4 mmol) was added dropwise. When the HCl ceased to evolve, the reaction was heated at reflux for 3 h. The benzene and oxalyl chloride were then removed by distillation, and the residue was quickly suspended in dichloroethane (20 mL) and then cooled to 0 ºC in an ice bath. Aluminium chloride (2.1 g, 15.5 mmol) as a suspension in dichloroethane (10 mL) was added portion wise with vigorous stirring and the reaction mixture was allowed to warm to room temperature over 3 h. The reaction mixture was then heated at reflux for 40 min. After cooling to room temperature, the mixture was poured onto crushed ice, portioned between 0.1 M HCl and ethyl actetate. The aqueous layer was further extracted with ethyl acetate (2 x 100 mL). The combined organic layers were washed with brine (100 mL), dried ($MgSO_4$) and evaporated under reduced pressure. The resulting orange solid was subjected to a flash column chromatography and elution with 30% ethyl acetate in petroleum spirit yielded 4-methoxy-7-methylindoline-2,3-dione **25** as an orange solid (0.84 g, 30%), mp: 235-236 ºC; IR (cm^{-1}): υ 3195 (m, NH), 1635 (s, C=O), 1250 (m, C-O). ^{1}H NMR (DMSO-d_6), δ: 10.99 (s, NH), 7.36 (d, J = 8.7, 1H, H6), 6.61 (d, J = 8.7, 1H, H5), 3.83 (s, 3H, OCH_3), 2.09 (s, 3H, CH_3). ^{13}C NMR, δ: 180.9 (C2), 160.1 (C3), 156.4 (C4), 149.1 ($C7_a$), 141.8 (C6), 112.9 (C7), 106.5 (C5), 105.8 ($C3_a$), 55.8 (OCH_3), 14.6 (CH_3) ppm. EI-MS *m/z* 191 (M^+, 100%), 163 (50), 149 (10), 134 (7), 121 (7), 106 (93). HRMS (ESI) calcd for $C_{10}H_{12}NO_3$ (MH^+), 192.0661; found, 192.0653.

*5-Bromo-4-methoxy-7-methylindoline-2,3-dione (**26**)*

In a 100 mL round bottom flask, 4-methoxy-7-methylindoline-2,3-dione **25** (440 mg, 2.3 mmol) was dissolved in glacial acetic acid (15 mL) and the mixture was stirred until the solid dissolved completely. The flask was then cooled to 0 ºC using an ice bath. A solution of bromine (405 mg, 2.5 mmol) in glacial acetic acid (5 mL) was then added dropwise over 10 min. The flask was allowed to warm to room temperature and was stirred for 2 h. The resulting solid was filtered and cold water (15 mL) added to the filtrate and the resulting solid was filtered. The combined solids were washed with saturated $NaHCO_3$ (2 x 25 mL) and water (2 x 25 mL), and recrystallised from acetic acid to yield 5-bromo-4-methoxy-7-methylindoline-2,3-dione **26** as an orange solid (520.5 mg, 84%), mp: >250 ºC; IR (cm^{-1}): υ 3162 (m, NH), 1696 (s, C=O), 1248 (w, C-O). ^{1}H NMR (DMSO-d_6), δ: 11.20 (s, NH), 7.67 (s, H6), 3.97 (s, 3H, OCH_3), 2.12 (s, 3H, CH_3). ^{13}C NMR, δ: 180.7 (C2), 159.2 (C3), 152.6 (C4), 148.9 ($C7_a$), 142.6 (C6), 117.7 (C7), 110.1 (C3a), 108.1 (C5), 61.8 (OCH_3), 14.6 (CH_3) ppm. EI-MS *m/z* 271 ($M^{+\ 81}$Br, 90), 269 ($M^{+\ 79}$Br, 92), 243 (98), 241 (100%), 228 (20), 226 (21), 185 (80). HRMS (ESI) calcd for $C_{10}H_9{}^{79}BrNO_3$ (MH^+), 269.9766; found, 269.9773.

*General procedure 2: Suzuki coupling of 6-bromobenzolactone derivatives **7**, **8**, **10** and **28** with 3-(3-boronophenyl)propanoic acid **27***

Under a N_2 atmosphere, a 25 mL flask was charged with the bromoisobenzofuran-1(3*H*)-one (1 equiv), 3-(3-boronophenyl)propanoic acid **27** (1.2 equiv), tetrakis(triphenylphosphine)palladium(0) (5 mol%) and 1,4-dioxane (5 mL). The flask was sealed with a rubber cap. The reaction mixture was degassed by flushing N_2 through a needle for 10 min. The flask was heated in an oil bath at 85 ºC. When the solid dissolved, a N_2 degassed solution of potassium phosphate (3 equiv) in water (3 mL) was added to the reaction mixture and heating at 85 ºC continued for 4-6 h. After cooling to room temperature, the solvent was concentrated under reduced pressure, and 2 M HCl (15 mL) was added to the residue. The suspended solid was either collected by filtration or extracted with ethyl acetate (2 x 25 mL). The combined ethyl acetate extracts were dried ($MgSO_4$) and evaporated under reduced pressure. The obtained residues were dissolved in ethanol and were subjected to PLC (10% methanol in CH_2Cl_2, 0.5% acetic acid).

*3-(3-(4-Methoxy-3-oxo-1,3-dihydroisobenzofuran-5-yl)phenyl)propanoic acid (**29**)*

Following the general procedure 2 using 6-bromo-7-methoxyisobenzofuran-1(3*H*)-one **7** (110 mg, 0.453 mmol), 3-(3-boronophenyl)propanoic acid **27** (105.5 mg, 0.544 mmol), tetrakis(triphenylphosphine)palladium(0) (26.2 mg, 0.023 mmol) and potassium phosphate (288.5 mg, 1.359 mmol) and heating for 5 h, the acid **29** was isolated as a white solid (87.5 mg, 62%), mp: 116-117 ºC; IR (cm^{-1}): υ 2989 (m, OH), 1755 (s, C=O), 1718 (s, C=O), 1216 (m, C-O). ^{1}H NMR (acetone-d_6), δ: 10.54 (bs, 1H, COOH), 7.73 (d, 1H, *J* = 7.5 Hz, isobenzofuran_6H), 7.45 (s, 1H, phenyl_2H), 7.41 (d, 1H, *J* = 7.7 Hz, phenyl_4H), 7.40-7.37 (m, 2H, phenyl_5,6 H), 7.30 (d, 1H, *J* = 7.5 Hz, isobenzofuran_7 H), 5.37 (s, 2H, isobenzofuran CH_2), 3.88 (s, 3H, OCH_3), 2.99 (t, 2H, *J* = 6.7 Hz, COOH-CH_2-CH_2), 2.68 (t, 2H, *J* = 6.7 Hz, COOH-CH_2-CH_2). ^{13}C NMR ($CDCl_3$), δ: resonance attributed to COOH not observed, 168.9 (C=O), 156.9 (isobenzofuran C4), 148.4 (phenyl C1), 140.5 (isobenzofuran $C7_a$), 137.8 (phenyl C5), 137.1 (isobenzofuran C6), 135.7 (phenyl C6), 129.5 (phenyl C3), 128.7 (phenyl C2), 127.9 (isobenzofuran C5), 127.5 (phenyl C4), 118.0 (isobenzofuran $C3_a$), 117.1 (isobenzofuran C7), 68.9 (isobenzofuran C1), 62.7 (OCH_3), 47.4 (COOH-CH_2-CH_2), 30.8 (COOH-CH_2-CH_2) ppm. EI-MS *m/z* 312 (M^+, 75), 295 (20), 266 (17), 239 (100%), 223 (50), 208 (15), 195 (51). HRMS (ESI) calcd for $C_{18}H_{17}O_5$ (MH^+), 313.1076; found, 313.1078. HPLC purity: 97.4%, R_t=8.245 (1% solvent B in solvent A).

*3-(3-(4-Methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)phenyl)propanoic acid (**30**)*

Following the general procedure 2 using 6-bromo-7-methylisobenzofuran-1(3*H*)-one **8** (95 mg, 0.418 mmol), 3-(3-boronophenyl)propanoic acid **27** (97.3 mg, 0.502 mmol), tetrakis(triphenylphosphine)palladium(0) (24.2 mg, 0.021 mmol) and potassium phosphate (266.2 mg, 1.254 mmol) and heating for 4 h, the acid **30** was isolated as a white solid (66.8 mg, 54%), mp: 110-111 ºC; IR (cm^{-1}): υ 2989 (m, OH), 1731 (s, C=O), 1696 (s, C=O). ^{1}H NMR ($CDCl_3$), δ: 7.55 (d, 1H, *J* = 7.8 Hz, isobenzofuran_6 H), 7.50 (d, 1H, *J* = 7.3 Hz, phenyl_4 H), 7.37 (t, 1H, *J* = 7.3 Hz, phenyl_5 H), 7.26 (d,

1H, *J* = 7.3 Hz, phenyl_6 H), 7.20 (s, 1H, phenyl_2 H), 7.16 (d, 1H, *J* = 7.3 Hz, isobenzofuran_7 H), 5.28 (s, 2H, isobenzofuran CH_2), 3.02 (t, 2H, *J* = 7.3 Hz, COOH-CH_2-CH_2), 2.74 (t, 2H, *J* = 7.3 Hz, COOH-CH_2-CH_2), 2.59 (s, 3H, CH_3). ^{13}C NMR, δ: resonance attributed to COOH not observed, 171.6 (C=O), 146.3 (phenyl C1), 143.5 (isobenzofuran C7a), 140.5 (phenyl C3), 140.3 (isobenzofuran C4), 137.6 (phenyl C2), 135.7 (isobenzofuran C5), 132.3 (phenyl C5), 129.5 (phenyl C6), 128.7 (isobenzofuran C6), 127.6 (phenyl C4), 123.8 (isobenzofuran C3a), 119.1 (isobenzofuran C7), 68.5 (isobenzofuran C1), 35.7 (COOH-CH_2-CH_2), 30.7 (COOH-CH_2-CH_2), 14.9 (CH_3) ppm. EI-MS *m/z* 296 (M^+, 100%), 278 (54), 260 (46), 250 (80), 136 (60), 223 (40). HRMS (ESI) calcd for $C_{18}H_{17}O_4$ (MH^+), 297.1127; found, 297.1129. HPLC purity: 97.9%, R_t=5.571 (1% solvent B in solvent A).

*3-(3-(4,6-Dimethoxy-3-oxo-1,3-dihydroisobenzofuran-5-yl)phenyl)propanoic acid (**31**)*

Following the general procedure 2 using 6-bromo-5,7-dimethoxyisobenzofuran-1(3*H*)-one **10** (80 mg, 0.293 mmol), 3-(3-boronophenyl)propanoic acid **27** (68.2 mg, 0.352 mmol), tetrakis(triphenylphosphine)palladium(0) (16.9 mg, 0.015 mmol) and potassium phosphate (186.6 mg, 0.879 mmol) and heating for 6 h, the acid **31** was isolated as a pale yellow oil (56 mg, 56%), IR (cm^{-1}): υ 2989 (m, OH), 1750 (s, C=O), 1739 (s, C=O), 1208 (w, C-O). ^{1}H NMR ($CDCl_3$), δ: 7.33 (t, 1H, *J* = 7.9 Hz, phenyl_5 H), 7.18 (d, 1H, *J* = 7.9 Hz, phenyl_4 H), 7.12-7.10 (m, 2H, phenyl_2,6 H), 6.50 (s, 1H, isobenzofuran_7 H), 4.98 (s, 2H, isobenzofuran CH_2), 4.03 (s, 3H, isobenzofuran_4 OCH_3), 3.85 (s, 3H, isobenzofuran_6 OCH_3), 2.97 (t, 3H, *J* = 7.6 Hz, COOH-CH_2-CH_2), 2.66 (t, 3H, *J* = 7.6 Hz, COOH-CH_2-CH_2). ^{13}C NMR, δ: 177.2 (COOH), 169.0 (C=O), 162.8 (isobenzofuran C6), 159.4 (isobenzofuran C4), 149.3 (phenyl C1), 140.7 (isobenzofuran C7a), 133.8 (phenyl C3), 129.3 (phenyl C2), 128.7 (phenyl C5), 127.7 (phenyl C4), 127.4 (phenyl C6), 117.5 (isobenzofuran C3a), 106.1 (isobenzofuran C5), 95.5 (isobenzofuran C7), 68.3 (isobenzofuran C1), 56.3 (isobenzofuran_6 OCH_3), 56.2 (isobenzofuran_4 OCH_3), 35.6 (COOH-CH_2-CH_2), 30.7 (COOH-CH_2-CH_2) ppm. EI-MS *m/z* 342 (M^+, 100), 326 (25), 296 (26). HRMS (ESI) calcd for $C_{19}H_{18}O_6$ (MH^+), 343.1182; found, 343.1190. HPLC purity: 98.7%, R_t=13.234 (5% solvent B in solvent A).

*3-(3-(3-Oxo-1,3-dihydroisobenzofuran-5-yl)phenyl)propanoic acid (**32**)*

Following the general procedure 2 using 6-bromoisobenzofuran-1(3*H*)-one **28** (90 mg, 0.422 mmol), 3-(3-boronophenyl)propanoic acid **27** (98.2 mg, 0.506 mmol), tetrakis(triphenylphosphine)palladium(0) (24.4 mg, 0.021 mmol) and potassium phosphate (268.7 mg, 1.266 mmol) and heating for 4 h, the acid **28** was isolated as a white solid (92.8 mg, 78%), mp: 118-119 ºC; IR (cm^{-1}): υ 2972 (m, OH), 1743 (s, C=O), 1699 (s, C=O). ^{1}H NMR ($CDCl_3$), δ: 8.11 (s, 1H, isobenzofuran_4 H), 7.90 (d, 1H, *J* = 8.0 Hz, phenyl_4 H), 7.55 (d, 1H, *J* = 7.9 Hz, phenyl_6 H), 7.47 (s, 1H, phenyl_2 H), 7.46 (d, 1H, *J* = 6.2 Hz, isobenzofuran_6 H), 7.41 (t, 1H, *J* = 8.0 Hz, phenyl_5 H), 7.27 (d, 1H, *J* = 6.7 Hz, isobenzofuran_7 H), 5.37 (s, 2H, isobenzofuran CH_2), 3.05 (t, 2H, *J* = 7.6 Hz, COOH-CH_2-CH_2), 2.75 (t, 2H, *J* = 7.6 Hz, COOH-CH_2-CH_2). ^{13}C NMR, δ: 178.3 (COOH), 171.4 (C=O), 145.5 (phenyl C1), 142.8 (isobenzofuran $C7_a$), 141.4 (phenyl

C2), 139.9 (isobenzofuran C5), 133.4 (phenyl C3), 129.6 (phenyl C5), 128.3 (phenyl C6), 127.6 (isobenzofuran C6), 126.7 (isobenzofuran $C3_a$), 125.6 (phenyl C4), 124.3 (isobenzofuran C4), 122.7 (isobenzofuran C7), 69.9 (isobenzofuran C1), 35.6 (COOH-CH_2-CH_2), 30.8 (COOH-CH_2-CH_2) ppm. EI-MS *m/z* 282 (M^+, 70), 253 (20), 237 (100%), 223 (16). HRMS (ESI) calcd for $C_{17}H_{15}O_4$ (MH^+), 283.0970; found, 283.0974. HPLC purity: 99.6%, R_t=6.285 (2% solvent B in solvent A).

*General procedure 3: Suzuki coupling of 5-bromoisatin derivatives **17**, **20**, **26** and **33** with 3-(3-boronophenyl)propanoic acid **27***

Under a N_2 atmosphere, a 25 mL flask was charged with the 5-bromoisatin derivative (1 equiv), 3-(3-boronophenyl)propanoic acid **27** (1.2 equiv), tetrakis(triphenylphosphine)palladium(0) (5 mol%) and DME (3 mL) or diglyme (3 mL), was sealed with a rubber cap. The reaction mixture was degassed by flushing N_2 through a needle for 10 min, and was then heated in an oil bath at 85 ºC. When the solid dissolved, a N_2 degassed solution of potassium phosphate (3 equiv) in water (3 mL) was added to the reaction mixture and heating continued at 85 ºC for 4-6 h. After cooling to room temperature, the solvent was concentrated under reduced pressure. A solution of HCl 2 M (15 mL) was added, and the resulting suspended solid was either collected by filtration or extracted with ethyl acetate (2 x 25 mL). The combined organic layers were dried ($MgSO_4$) and evaporated under reduced pressure. The combined residues were then dissolved in ethanol and were subjected to PLC (10% methanol in CH_2Cl_2, 0.5% acetic acid) to yield the isatin-acid conjugates **34**-**37**.

*3-(3-(4,6-Dimethoxy-2,3-dioxoindolin-5-yl)phenyl)propanoic acid (**34**)*

Following the general procedure 3 using 5-bromo-4,6-dimethoxyindoline-2,3-dione **17** (100 mg, 0.349 mmol), 3-(3-boronophenyl)propanoic acid **27** (81.2 mg, 0.419 mmol), tetrakis(triphenylphosphine)palladium(0) (20.2 mg, 0.017 mmol) and potassium phosphate (222.2 mg, 1.047 mmol) and heating for 6 h, the acid **34** was isolated as a yellow solid (49.5 mg, 40%), mp: 175-176 ºC; IR (cm^{-1}): υ 3185 (m, NH), 2978 (m, OH), 1718 (s, C=O), 1696 (s, C=O), 1290 (w, C-O). ^{1}H NMR (DMSO-d_6), δ: 12.09 (s, 1H, COOH), 10.36 (s, NH), 7.31 (t, *J* = 7.5 Hz, 1H, phenyl_5 H), 7.20 (d, *J* = 7.5 Hz, 1H, phenyl_4 H), 7.08-7.06 (m, 2H, phenyl_2,6 H), 6.35 (s, 1H, isatin_7 H), 3.97 (s, 3H, isatin_4 OCH_3), 3.84 (s, 3H, isatin_C6 OCH_3), 2.85 (t, *J* = 7.5 Hz, 2H, COOH-CH_2-CH_2), 2.58 (t, *J* = 7.5 Hz, 2H, COOH-CH_2-CH_2). ^{13}C NMR, δ: 178.1 (COOH), 174.0 (isatin C2), 166.6 (isatin C3), 161.5 (isatin C6), 160.1 (isatin C4), 149.2 (phenyl C1), 141.0 (isatin $C7_a$), 131.2 (phenyl C3), 130.3 (phenyl C5), 128.3 (double height, phenyl C4 and C6), 127.4 (phenyl C2), 108.2 (isatin C7), 100.6 (isatin C5), 90.1 (isatin $C3_a$), 56.7 (isatin_4 OCH_3), 56.3 (isatin_6 OCH_3), 35.0 (COOH-CH_2-CH_2), 30.3 (COOH-CH_2-CH_2) ppm. EI-MS *m/z* 355 (M^+, 40), 299 (4), 254 (100%), 191 (45). HRMS (ESI) calcd for $C_{19}H_{18}NO_6$ (MH^+), 356.1134; found, 356.1116. HPLC purity: 99.3%, R_t=35.814 (3% solvent B in solvent A).

*3-(3-(7-Methyl-2,3-dioxoindolin-5-yl)phenyl)propanoic acid (**35**)*

Following the general procedure 3 using 5-bromo-7-methylindoline-2,3-dione **20** (100 mg, 0.416 mmol), 3-(3-boronophenyl)propanoic acid **27** (96.8 mg, 0.499 mmol), tetrakis(triphenylphosphine)palladium(0) (24 mg, 0.021 mmol) and potassium phosphate (264.9 mg, 1.248 mmol) and heating for 4 h, the acid **35** was isolated as a red solid (70 mg, 55%), mp: 238-239 ºC; IR (cm^{-1}): υ 3172 (m, NH), 2989 (m, OH), 1731 (s, C=O), 1696 (s, C=O). ^{1}H NMR (acetone-d_6), δ: 9.94 (s, NH), 7.81 (s, 1H, isatin_4 H), 7.66 (s, 1H, phenyl_2 H), 7.60 (s, 1H, isatin_6 H), 7.50 (d, *J* = 7.6 Hz, 1H, pheny_6 H), 7.40 (t, *J* = 7.6 Hz, 1H, phenyl_5 H), 7.29 (d, *J* = 7.6 Hz, 1H, phenyl_4 H), 3.03 (t, *J* = 7.3 Hz, 2H, COOH-CH_2-CH_2), 2.71 (t, *J* = 7.3 Hz, 2H, COOH-CH_2-CH_2), 2.41 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6), δ: 184.8 (COOH), 173.9 (isatin C3), 160.1 (isatin C2), 148.5 (isatin $C7_a$), 141.7 (phenyl C3), 138.7 (phenyl C1), 137.6 (isatin C6), 134.8 (isatin C5), 128.9 (phenyl C5), 127.4 (phenyl C6), 126.5 (phenyl C2), 123.8 (phenyl C4), 122.1 (isatin C4), 119.8 (isatin $C3_a$), 118.1 (isatin C7), 35.3 (COOH-CH_2-CH_2), 30.4 (COOH-CH_2-CH_2), 15.5 (CH_3) ppm. EI-MS *m/z* 309 (M^+, 100%), 277 (25), 253 (80), 207 (79), 193 (30). HRMS (ESI) calcd for $C_{18}H_{16}NO_4$ (MH^+), 310.1079; found, 310.1071. HPLC purity: 98.1%, R_t=5.339 (1% solvent B in solvent A).

*3-(3-(4-Methoxy-7-methyl-2,3-dioxoindolin-5-yl)phenyl) propanoic acid (**36**)*

Following the general procedure 3 using 5-bromo-4-methoxy-7-methylindoline-2,3-dione **26** (100 mg, 0.370 mmol), 3-(3-boronophenyl)propanoic acid **27** (86.1 mg, 0.444 mmol), tetrakis(triphenylphosphine)palladium(0) (21.4 mg, 0.019 mmol) and potassium phosphate (235.6 mg, 1.11 mmol) and heating for 5 h, the acid **36** was isolated as a red solid (60 mg, 48%), mp: 219-220 ºC; IR (cm^{-1}): υ 3182 (m, NH), 2982 (m, OH), 1749 (s, C=O), 1692 (s, C=O), 1287 (w, C-O). ^{1}H NMR (DMSO-d_6), δ: 11.16 (s, NH), 7.40 (s, 1H, isatin_6 H), 7.13 (t, *J* = 7.4 Hz, 1H, phenyl_5 H), 7.26 (s, 1H, phenyl_2 H), 7.22 (d, *J* = 7.4 Hz, 1H, pheny_6 H), 7.19 (d, *J* = 7.4 Hz, 1H, phenyl_4 H), 3.81 (s, 3H, OCH_3), 2.86 (t, *J* = 7.4 Hz, 2H, COOH-CH_2-CH_2), 2.56 (t, *J* = 7.4 Hz, 2H, COOH-CH_2-CH_2), 2.41 (s, 3H, CH_3). ^{13}C NMR, δ: 182.8 (COOH), 174.5 (isatin C2), 160.5 (isatin C3), 154.6 (isatin C4), 149.3 (isatin $C7_a$), 142.6 (phenyl C1), 141.5 (isatin C6), 137.4 (phenyl C3), 129.5 (phenyl C2), 129.1 (phenyl C5), 128.8 (isatin C7), 127.7 (phenyl C6), 127.3 (phenyl C4), 116.5 (isatin C5), 110.3 (isatin $C3_a$), 62.3 (OCH_3), 35.9 (COOH-CH_2-CH_2), 31.0 (COOH-CH_2-CH_2), 15.6 (CH_3) ppm. EI-MS *m/z* 339 (M^+, 100%), 311 (75), 293 (10), 277 (77). HRMS (ESI) calcd for $C_{19}H_{18}NO_5$ (MH^+), 340.1185; found, 340.1187. HPLC purity: 99.4%, R_t=5.579 (1% solvent B in solvent A).

*3-(3-(2,3-Dioxoindolin-5-yl)phenyl)propanoic acid (**37**)*

Following the general procedure 3 using 5-bromoindoline-2,3-dione **33** (100 mg, 0.442 mmol), 3-(3-boronophenyl)propanoic acid **27** (102.9 mg, 0.530 mmol), tetrakis(triphenylphosphine)palladium(0) (25.5 mg, 0.022 mmol) and potassium phosphate (281.5 mg, 1.326 mmol) and heating for 4 h, the acid **37** was isolated as an orange solid (84.8 mg, 65%), mp: 182-183 ºC; IR (cm^{-1}): υ 3185 (m, NH), 2989 (m, OH), 1714 (s, C=O), 1684 (s, C=O). ^{1}H NMR (DMSO-d_6), δ: 11.14 (s, NH), 7.89 (dd, *J* = 8.2, 1.8 Hz, 1H, isatin_6 H), 7.77 (d, *J* = 1.4 Hz, 1H, isatin_4 H), 7.53 (s, 1H, phenyl_2 H), 7.46 (d, *J* = 7.6 Hz, 1H,

phenyl_4 H), 7.35 (t, *J* = 7.5 Hz, 1H, phenyl_5 H), 7.21 (d, *J* = 7.5 Hz, 1H, phenyl_6 H), 6.99 (d, *J* = 7.9 Hz, 1H, isatin_7 H), 2.88 (t, *J* = 7.6 Hz, 2H, COOH-CH_2-CH_2), 2.59 (t, *J* = 7.5 Hz, 2H, COOH-CH_2-CH_2). ^{13}C NMR, δ: 184.9 (COOH), 174.4 (isatin C3), 160.0 (isatin C2), 150.4 (isatin $C7_a$), 142.2 (phenyl C3), 139.1 (phenyl C1), 136.9 (isatin C6), 135.4 (isatin C5), 129.4 (phenyl C5), 127.9 (phenyl C6), 126.7 (phenyl C2), 124.4 (phenyl C4), 122.9 (isatin C4), 118.9 (isatin $C3_a$), 113.1 (isatin C7), 35.7 (COOH-CH_2-CH_2), 30.9 (COOH-CH_2-CH_2) ppm. EI-MS *m/z* 295 (M^+, 40), 267 (100%), 250 (95), 207 (20), 193 (25). HRMS (ESI) calcd for $C_{17}H_{14}NO_4$ (MH^+), 296.0914; found, 296.0912. HPLC purity: 98.2%, R_t=7.237 (1% solvent B in solvent A).

*3-(3-(3-Methyl-8-oxo-7-pivaloyl-7-azabicyclo[4.2.0]octa-1,3,5-trien-5-yl)phenyl)propanoic acid (**38**)*

Under a N_2 atmosphere, a 25 mL round bottom flask was charged with the β-lactam derivative **12** (100 mg, 0.339 mmol), 3-(3-boronophenyl)propanoic acid **27** (72.3 mg, 0.372 mmol), tetrakis(triphenylphosphine)palladium(0) (19.6 mg, 0.016 mmol) and potassium phosphate (215.9 mg, 1.02 mmol). A mixture of 1,4-dioxane (10 mL) and water (3 mL) was added and the reaction mixture was degassed by flushing N_2 through a needle for 10 min. The flask was then sealed with a rubber cap, heated at 85 ºC for 18 h. The solvent was then concentrated under reduced pressure and the flask was cooled to 0 ºC. Chilled 1 N HCl (10 mL) was added dropwise with stirring, and the resulting solid was extracted with ethyl acetate (2 x 15 mL). The combined organic layers were washed with water (15 mL), brine (15 mL) and dried ($MgSO_4$) and concentrated under reduced pressure. The resulting residue was subjected to a flash column chromatography (50% CH_2Cl_2, 50% ethyl acetate and 1% acetic acid) to afford **38** as a pale yellow oil (74.2 mg, 60%), IR (cm^{-1}): υ 2959 (m, OH), 1696 (s, C=O), 1662 (s, C=O), 1481 (m, C-N). ^{1}H NMR ($CDCl_3$), δ: 8.56 (s, 1H, COOH), 7.77 (s, 1H, H4), 7.32-7.29 (m, 2H, phenyl_5 H, H2), 7.20 (d, 1H, *J* = 7.5 Hz, phenyl_6 H), 7.19 (s, 1H, phenyl_2 H), 7.15 (d, 1H, *J* = 7.7 Hz, phenyl_4 H), 2.97 (t, 2H, *J* = 7.5 Hz, COOH-CH_2-CH_2), 2.68 (t, 2H, *J* = 7.6 Hz, COOH-CH_2-CH_2), 2.37 (s, 3H, CH_3), 1.03 (s, 9H, pivaloyl 3xCH_3). ^{13}C NMR, δ: 178.4 (pivaloyl C=O), 176.9 (COOH), 171.9 (azabicyclo C=O), 140.4 (phenyl C1), 140.1 (azabicyclo C6), 139.8 (phenyl C3), 136.4 (phenyl C5), 135.8 (azabicyclo C4), 133.1 (azabicyclo C2), 130.9 (azabicyclo C3), 128.8 (azabicyclo C1), 128.7 (phenyl C2), 127.3 (phenyl C6), 126.8 (azabicyclo C5), 124.8 (phenyl C4), 39.3 ($C(CH_3)_3$), 35.8 (COOH-CH_2-CH_2), 30.9 (COOH-CH_2-CH_2), 27.2 (pivaloyl 3xCH_3), 21.0 (CH_3) ppm. EI-MS *m/z* 365 (M^+, 60), 350 (70), 320 (50), 299 (25), 277 (100%), 235 (26), 220 (23), 193 (52). HRMS (ESI) calcd for $C_{22}H_{24}NO_4$ (MH^+), 366.1705; found, 366.1717. HPLC purity: 98.9%, R_t=27.924 (3% solvent B in solvent A).

*2-(3-Iodophenoxy)acetonitrile (**42**)*

A solution of 3-iodophenol **41** (2.0 g, 9.09 mmol) and NaOH (0.91 g, 22.73 mmol) in dry DMF (25 mL) was stirred vigorously for 30 min until the solution became pale brown. 2-Chloroacetonitrile (1.03 g, 0.86 mL, 13.64 mmol) was then added dropwise through a syringe and the mixture was further stirred for 18 h at

room temperature. The resulting brown solution was then poured onto crushed ice (100 mL), stirred for 30 min, and the resulting oil was extracted with ethyl acetate (2 x 25 mL). The combined organic layers were washed with water (2 x 25 mL), brine (2 x 25 mL) and dried ($MgSO_4$). The organic fraction was then triturated with charcoal (0.5 g) and filtered through celite, and evaporated under reduced pressure to afford 2-(3-iodophenoxy)acetonitrile **42** as a yellow oil (1.88 g, 80%), IR (cm^{-1}): υ 2942 (m, C-H aliphatic), 2246 (s, CN), 1203 (w, C-O). ^{1}H NMR (CDCl3), δ: 7.37 (d, 1H, *J* = 7.7 Hz, phenoxy_6 H), 7.29 (s, 1H, phenoxy_2 H), 7.02 (t, 1H, *J* = 7.9 Hz, phenoxy_5 H), 6.90 (d, 1H, *J* = 8.0 Hz, phenoxy_4 H), 4.68 (s, 2H, CH_2). ^{13}C NMR, δ: 157.1 (phenoxy C1), 132.5 (phenoxy C5), 131.6 (phenoxy C4), 124.7 (phenoxy C2), 115.3 (CN), 114.6 (phenoxy C6), 94.9 (phenoxy C3), 54.0 (CH2) ppm. EI-MS m/z 259 (M+, 100%), 219 (27), 203 (5). HRMS (ESI) calcd for C_8H_7INO (MH^+), 259.9572; found, 259.9565.

*2-(3-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)phenoxy)acetonitrile (**40**)*

Under a N_2 atmosphere, an oven dried 100 mL flask was charged with 2-(3-iodophenoxy)acetonitrile **42** (1.16 g, 4.48 mmol), bis(pinacolato)diboron **43** (4.55 g, 17.92 mmol), potassium acetate (1.76 g, 17.92 mmol) and [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II) ($PdCl_2$(dppf)) (32.8 mg, 1 mol%). Dry THF (50 mL) was added to the mixture, and the flask was heated at reflux for 18 h under N_2 atmosphere. The solvent was then evaporated under reduced pressure, ethyl acetate (30 mL) was added and the mixture was sonicated for 10 min and filtered. The filtrate was triturated with $MgSO_4$ and charcoal (0.5 g), and filtered through a short pad of celite. The filtrate was then concentrated under reduced pressure and the resulting residue was recrystallised from a mixture of ethyl acetate/hexane (3:2) to give the boronic ester **40** as a pink solid (1.1 g, 95%), mp: 40-41 ºC; IR (cm^{-1}): υ 2980 (m, C-H aliphatic), 2152 (m, CN), 1128 (w, C-O). ^{1}H NMR ($CDCl_3$), δ: 7.43 (d, 1H, *J* = 7.6 Hz, phenoxy_6 H), 7.39 (s, 1H, phenoxy_2 H), 7.08 (t, 1H, *J* = 7.9 Hz, phenoxy_5 H), 6.96 (d, 1H, *J* = 8.0 Hz, phenoxy_4 H), 4.76 (s, 2H, CH_2), 1.26 (s, 12H, 4xCH_3). ^{13}C NMR, δ: 157.1 (phenoxy C1), 132.6 (phenoxy C3), 131.4 (phenoxy C5), 124.7 (phenoxy C4), 114.8 (CN), 114.6 (phenoxy C2), 94.6 (phenoxy C6), 83.7 (dioxaborolan C4, C5), 53.9 (CH_2), 25.2 (4xCH_3) ppm. ESI-MS *m/z* 259 (M^+). HRMS (ESI) calcd for $C_{14}H_{19}BNO_3$ (MH^+), 260.1458; found, 260.1462.

*General procedure 4: Suzuki coupling of 6-bromobenzolactone derivatives **7** and **8** with (3-(cyanomethoxy)phenyl)boronic acid **39** or ester **40***

A round bottom 25 mL flask was charged with the appropriate dihydroisobenzofuran derivative (1 equiv), (3-(cyanomethoxy)phenyl)boronic acid **39** (1 equiv) or 2-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenoxy)acetonitrile **40** (1.2 equiv), tetrakis(triphenylphosphine)palladium(0) (5 mol%), 1,4-dioxane (10 mL) was added and the flask was sealed with a rubber cap. The reaction mixture was degassed by flushing N_2 through a needle for 10 min. The flask was heated in an oil bath at 85 ºC. When all the solid was dissolved, potassium phosphate (3 mmol, 636.8 mg) dissolved in water (5 mL) was added to the reaction *via* a needle. The flask was then heated at 85 ºC for 7-15 h. The solvent was concentrated under reduced

pressure, and was added 1 M HCl (10 mL), the reaction mixture turned to a white turbid solution which was then extracted with ethyl acetate (2 x 30 mL), washed with brine (2 x 10 mL) and dried ($MgSO_4$). The solvent was evaporated and the residue was subjected to flash column chromatography (40% ethyl acetate in petroleum spirit).

*2-(3-(4-Methoxy-3-oxo-1,3-dihydroisobenzofuran-5-yl)phenoxy)acetonitrile (**44**)*

Following the general procedure 4 using 6-bromo-7-methoxyisobenzofuran-1(3*H*)-one **7** (100 mg, 0.411 mmol), (3-(cyanomethoxy)phenyl)boronic acid **39** (72 mg, 0.411 mmol), tetrakis(triphenylphosphine)palladium(0) (23.7 mg, 0.021 mmol) and heating for 15 h, the acetonitrile intermediate **44** was isolated as a white solid (60.7 mg, 50%), mp: 103-104 ºC; IR (cm^{-1}): υ 2920 (w, C-H aliphatic), 2165 (m, CN), 1759 (s, C=O), 1212 (w, C-O). ^{1}H NMR ($CDCl_3$), δ: 7.74 (d, 1H, *J* = 7.7 Hz, isobenzofuran_6 H), 7.43 (t, 1H, *J* = 8.0 Hz, phenyl_5 H), 7.25-7.23 (m, 2H, phenyl_4 H, isobenzofuran_7 H), 7.19 (s, 1H, phenyl_2 H), 7.02 (d, 1H, *J* = 8.2 Hz, phenyl_6 H), 5.30 (s, 2H, isobenzofuran CH_2), 4.83 (s, 2H, CN-CH_2), 3.90 (s, 3H, OCH_3). ^{13}C NMR, δ: 168.5 (C=O), 156.7 (phenyl C1), 156.4 (isobenzofuran C4), 148.7 (isobenzofuran $C7_a$), 138.7 (isobenzofuran C6), 137.4 (phenyl C5), 134.7 (isobenzofuran C5), 129.8 (phenyl C4), 124.1 (phenyl C3), 117.9 (phenyl C6), 117.1 (phenyl C2), 116.1 (CN), 115.1 (isobenzofuran $C3_a$), 114.5 (isobenzofuran C7), 68.7 (isobenzofuran C1), 62.7 (CN-CH_2), 53.7 (OCH_3) ppm. EI-MS *m/z* 295 (M^+, 15), 255 (100%), 237 (13), 208 (10), 180 (16). HRMS (ESI) calcd for $C_{17}H_{14}NO_4$ (MH^+), 296.0914; found, 296.0913. HPLC purity: 98.3%, R_t=16.676 (1% solvent B in solvent A).

*2-(3-(4-Methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)phenoxy)acetonitrile (**45**)*

Following the general procedure 4 using 6-bromo-7-methoxyisobenzofuran-1(3*H*)-one **8** (100 mg, 0.358 mmol), 2-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenoxy) acetonitrile **40** (111.4 mg, 0.430 mmol), tetrakis(triphenylphosphine)palladium(0) (20.7 mg, 0.018 mmol) and heating for 7 h, the acetonitrile intermediate **45** was isolated as white solid (93.4 mg, 76%), mp: 99-100 ºC; IR (cm^{-1}): υ 2972 (w, C-H aliphatic), 2169 (m, CN), 1743 (s, C=O). ^{1}H NMR ($CDCl_3$), δ: 7.51 (d, 1H, *J* = 7.7 Hz, isobenzofuran_6 H), 7.43 (t, 1H, *J* = 7.9 Hz, phenyl_5 H), 7.33 (d, 1H, *J* = 7.7 Hz, isobenzofuran_7 H), 7.02 (d, 2H, *J* = 8.0 Hz, phenyl_4,6 H), 6.93 (s, 1H, phenyl_2 H), 5.28 (s, 2H, isobenzofuran CH_2), 4.83 (s, 2H, CN-CH_2), 2.60 (s, 3H, CH_3). ^{13}C NMR, δ: 171.4 (C=O), 156.7 (phenyl C1), 146.7 (isobenzofuran $C7_a$), 142.8 (isobenzofuran C4), 142.1 (isobenzofuran C5), 137.6 (phenyl C3), 135.6 (phenyl C5), 130.1 (isobenzofuran $C3_a$), 124.4 (phenyl C4), 124.1 (isobenzofuran C6), 119.4 (isobenzofuran C7), 116.4 (phenyl C2), 115.3 (CN), 114.1 (phenyl C6), 68.6 (isobenzofuran C1), 53.9 (CN-CH_2), 15.0 (CH_3) ppm. EI-MS *m/z* 279 (M^+, 90), 239 (100%), 223 (15), 195 (12), 165 (24). HRMS (ESI) calcd for $C_{17}H_{14}NO_3$ (MH^+), 280.0974; found, 280.0966. HPLC purity: 98.1%, R_t=11.021 (1% solvent B in solvent A).

*General procedure 5: synthesis of the benzolactone-tetrazole conjugates **46** and **47** using sodium azide method*

Under a N_2 atmosphere, a round bottom flask (25 mL) was charged with the appropriate acetonitrile derivative **44-45** (0.186 mmol), sodium azide (48.4 mg, 0.744 mmol), amonium chloride (39.4 mg, 0.744 mmol), DMF (10 mL) and a catalytic amount of glacial acetic acid (5-10 drops), and the reaction mixture was then heated at reflux for 24 h. DMF was concentrated under reduced pressure and the residue was dried by vacuum. Ethanol was added to the resulting dry residue and the mixture was sonicated for 10 min, filtered and the ethanol solution was concentrated and subjected to PLC (90% CH_2Cl_2, 10% ethanol and 0.1% TFA) to yield the tetrazole conjugate.

*6-(3-((1H-Tetrazol-5-yl)methoxy)phenyl)-7-methoxyisobenzofuran-1(3H)-one (**46**)*

Following the general procedure 5 using 2-(3-(4-methoxy-3-oxo-1,3-dihydroisobenzofuran-5-yl)phenoxy)acetonitrile **44** (55 mg), the tetrazole conjugate **46** was isolated as a pale yellow solid (21.4 mg, 34%), mp: 130-131 ºC; IR (cm^{-1}): υ 3404 (w, NH), 2907 (w, C-H aliphatic), 1751 (s, C=O), 1660 (m, C=N), 1134 (w, C-O). ^{1}H NMR (CD_3OD), δ: 7.72 (d, 1H, *J* = 7.7 Hz, isobenzofuran_5 H), 7.42 (t, 1H, *J* = 7.9 Hz, phenyl_5 H), 7.37 (d, 1H, *J* = 7.7 Hz, isobenzofuran_4 H), 7.22-7.17 (m, 2H, phenyl_2,4 H), 7.10 (d, 1H, *J* = 8.2 Hz, phenyl_6 H), 5.50 (s, 2H, isobenzofuran CH_2), 5.34 (s, 2H, OCH_2), 3.78 (s, 3H, OCH_3). ^{13}C NMR, δ: 169.7 (C=O), 157.9 (phenyl C3), 156.5 (isobenzofuran C7), 149.6 (tetrazole C), 138.7 (isobenzofuran C3a), 137.7 (isobenzofuran C5), 135.1 (phenyl C5), 129.5 (isobenzofuran C6), 129.4 (phenyl C6), 123.0 (phenyl C1), 117.6 (phenyl C4), 117.5 (phenyl C2), 115.9 (isobenzofuran $C7_a$), 113.9 (isobenzofuran C4), 69.3 (isobenzofuran C3), 61.7 (tetrazole-CH_2-O), 59.9 (OCH_3) ppm. EI-MS *m/z* 338 (M^+, 14), 255 (100%), 239 (10), 211 (12), 195 (11). HRMS (ESI) calcd for $C_{17}H_{15}N_4O_4$ (MH^+), 339.1093; found, 339.1107. HPLC purity: 96.3%, R_t=16.339 (5% solvent B in solvent A).

*6-(3-((1H-Tetrazol-5-yl)methoxy)phenyl)-7-methylisobenzofuran-1(3H)-one (**47**)*

Following the general procedure 5 using 2-(3-(4-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)phenoxy)acetonitrile **45** (51.9 mg), the final tetrazole conjugate **47** was isolated as a pale yellow gummy solid (21 mg, 35%), IR (cm^{-1}): υ 3392 (m, NH), 2988 (w, C-H aliphatic), 1741 (s, C=O), 1668 (m, C=N), 1212 (w, C-O). ^{1}H NMR (CD_3OD), δ: 7.58 (d, 1H, *J* = 7.7 Hz, isobenzofuran_5 H), 7.48 (d, 1H, *J* = 7.7 Hz, isobenzofuran_4 H), 7.44 (t, 1H, *J* = 7.9 Hz, phenyl_5 H), 7.12 (d, 1H, *J* = 7.9 Hz, phenyl_6 H), 7.03 (s, 1H, phenyl_2 H), 6.99 (d, 1H, *J* = 7.5 Hz, phenyl_4 H), 5.51 (s, 2H, isobenzofuran CH_2), 5.36 (s, 2H, O-CH_2), 2.55 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6), δ: 170.9 (C=O), 162.4 (phenyl C3), 157.3 (tetrazole C), 147.2 (isobenzofuran $C3_a$), 142.1 (isobenzofuran C7), 141.1 (isobenzofuran C6), 135.7 (phenyl C5), 135.4 (isobenzofuran C5), 129.7 (phenyl C1), 122.9 (isobenzofuran $C7_a$), 122.7 (phenyl C6), 120.8 (isobenzofuran C4), 115.8 (phenyl C2), 114.1 (phenyl C4), 68.5 (isobenzofuran C3), 59.3 (tetrazole-CH_2-O), 14.4 (CH_3) ppm. EI-MS *m/z* 322 (M^+, 50), 239 (75), 223 (26), 195 (60), 165 (100%). HRMS (ESI)

calcd for $C_{17}H_{15}N_4O_3$ (MH^+), 323.1144; found, 323.1138. HPLC purity: 98.7%, R_t=10.695 (5% solvent B in solvent A).

*5-((3-Iodophenoxy)methyl)-1H-tetrazole (**48**)*

In a round bottom 50 mL flask, a solution of 2-(3-iodophenoxy)acetonitrile **42** (1.6 g, 6.18 mmol), sodium azide (1.6 g, 24.7 mmol) and amonium chloride (1.3 g, 24.7 mmol) in dry DMF (25 mL), containing few drops of glacial acetic acid, was heated at reflux for 14 h under a N_2 atmosphere. The solvent was then concentrated under reduced pressure, and the resulting residue was dried under vacuum. Ethanol (25 mL) was added to the residue and the mixture was sonicated for 10 min, filtered and ethanol was evaporated under reduced pressure resulting in a white fluffy solid of the tetrazole intermediate **48** (1.5 g, 80%), mp: 99-100 ºC; IR (cm^{-1}): υ 2995 (w, NH), 1589 (m, C=N), 1240 (w, C-O). ^{1}H NMR (CD_3OD), δ: 7.46 (s, 1H, phenoxy_2 H), 7.39 (t, 1H, *J* = 7.0 Hz, phenoxy_5 H), 7.09-7.08 (m, 2H, phenoxy_4,6 H), 5.47 (s, 2H, CH_2). ^{13}C NMR, δ: 159.7 (phenoxy C1), 155.3 (tetrazole C), 132.4 (phenoxy C4), 132.2 (phenoxy C5), 125.4 (phenoxy C2), 115.3 (phenoxy C6), 94.9 (phenoxy C3), 61.1 (CH_2) ppm. EI-MS *m/z* 302 (M^+, 100%), 220 (80), 203 (10). HRMS (ESI) calcd for $C_8H_8IN_4O$ (MH^+), 302.9743; found, 302.9734.

*Potassium 5-((3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenoxy)methyl)tetrazol-1-ide (**49**)*

Under a N_2 atmosphere, an oven dried 100 mL flask was charged with 5-((3-iodophenoxy)methyl)-1*H*-tetrazole **48** (960 mg, 3.179 mmol), bis(pinacolato)diboron **43** (3.2 g, 12.72 mmol), potassium acetate (1.25 g, 12.72 mmol) and $PdCl_2$(dppf) (23.3 mg, 1 mol%). Dry THF (30 mL) was added to the mixture, and the flask was heated at reflux for 18 h under a N_2 atmosphere. The solvent was then concentrated under reduced pressure. Ethanol (30 mL) was added to the resulting residue and the mixture was further heated at reflux for 10 min, filtered hot and concentrated under reduced pressure to afford a white solid of the boronic ester potassium salt **49** (1.1 g, 99%), mp: >250 ºC ; IR (cm^{-1}): υ 2983 (s, C-H aliphatic), 1680 (m, C=N), 1233 (w, C-O). ^{1}H NMR (CD_3OD), δ: 7.38 (s, 1H, phenoxy_2 H), 7.28 (d, 1H, *J* = 7.1 Hz, phenoxy_6 H), 7.04-6.99 (m, 2H, phenoxy_4,5 H), 5.25 (s, 2H, CH_2), 1.90 (s, 12H, 4xCH_3). ^{13}C NMR, δ: 178.5 (phenoxy C1), 159.5 (tetrazole C), 158.3 (phenoxy C3), 130.8 (phenoxy C5), 130.2 (phenoxy C4), 124.2 (phenoxy C6), 114.2 (phenoxy C2), 93.6 (dioxaborolan C4, C5), 61.2 (CH_2), 22.6 (4xCH_3) ppm. ESI-MS *m/z* 301.1 ($M+K^+$).

*General procedure 6: synthesis of the benzolactone-tetrazole conjugates **50** and **51** using the coupling method:*

Under a N_2 atmosphere, a mixture of the appropriate bromoisobenzofuran-1(3*H*)-one derivative (1 mmol) and potassium 5-((3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenoxy)methyl)tetrazol-1-ide **49** (374 mg, 1.1 mmol), tetrakis(triphenylphosphine)palladium(0) (80.9 mg, 7 mol%) and potassium phosphate (636.8 mg, 3 mmol) was added to a solvent mixture of 1,4-dioxane (25 mL) and water (10 mL) in a 50 mL

round bottom flask. The reaction mixture was degassed by flushing N_2 through a needle for 10 min, the flask was then sealed with a rubber cap, heated at 85 ºC for 20-24 h. The solvent was then concentrated under reduced pressure. Saturated $NaHCO_3$ (25 mL) was added to the resulting residues, and the mixture sonicated for 15 min, before the turbid solution was washed with CH_2Cl_2 (25 mL). The aqueous layer was filtered, cooled to 0 ºC using an ice bath, and the filtrate was then neutralized with 2 M HCl. The resulting white solid was collected by vacuum filtration, washed with chilled methanol and dried under vacuum to afford the tetrazoles.

*6-(3-((1H-Tetrazol-5-yl)methoxy)phenyl)-5,7-dimethoxyisobenzofuran-1(3H)-one (**50**)*

Following the general procedure 6 using 6-bromo-5,7-dimethoxyisobenzofuran-1(3*H*)-one **10** (1 mmol, 273 mg) and heating for 24 h, the final tetrazole conjugate **50** was isolated as a white solid (195 mg, 53%), mp: charring; IR (cm^{-1}): υ 3204 (w, NH), 1740 (s, C=O), 1614 (m, C=N), 1218 (w, C-O). ^{1}H NMR (DMSO-d_6), δ: 7.36 (t, 1H, *J* = 7.9 Hz, phenyl_5 H), 7.05 (d, 1H, *J* = 8.0 Hz, phenyl_6 H), 7.00 (s, 1H, phenyl_2 H), 6.93 (d, 1H, *J* = 7.7 Hz, phenyl_4 H), 6.82 (s, 1H, isobenzofuran_4 H), 5.49 (s, 2H, isobenzofuran CH_2), 5.12 (s, 2H, OCH_2), 3.90 (s, 3H, isobenzofuran_7 OCH_3), 3.89 (s, 3H, isobenzofuran_5 OCH_3). ^{13}C NMR, δ: 167.9 (C=O), 162.6 (isobenzofuran C5), 158.9 (phenyl C3), 157.4 (isobenzofuran C7), 153.6 (tetrazole C), 149.2 (isobenzofuran $C3_a$), 134.9 (phenyl C5), 129.5 (phenyl C6), 122.8 (phenyl C4), 115.9 (phenyl C10), 115.7 (phenyl C11), 114.1 (isobenzofuran $C7_a$), 104.7 (isobenzofuran C4), 96.2 (isobenzofuran C6), 67.8 (isobenzofuran C3), 59.2 (tetrazole-CH_2-O), 56.6 (isobenzofuran_7 OCH_3), 56.1 (isobenzofuran_5 OCH_3) ppm. ESI-MS *m/z* 367.1 ($M-H^+$). HRMS (ESI) calcd for $C_{18}H_{15}N_4O_5$ ($M-H^+$), 367.1042; found, 367.1038. HPLC purity: 95.2%, R_t=18.896 (10% solvent B in solvent A).

*6-(3-((1H-Tetrazol-5-yl)methoxy)phenyl)isobenzofuran-1(3H)-one (**51**)*

Following the general procedure 6 using 6-bromoisobenzofuran-1(3*H*)-one **28** (213 mg, 1 mmol) and heating for 20 h, the final tetrazole conjugate **51** was isolated as a white solid (181 mg, 59%), mp: charring; IR (cm^{-1}): υ 3195 (w, NH), 1751 (s, C=O), 1653 (m, C=N). ^{1}H NMR (CD_3OD), δ: 8.07 (s, 1H, isobenzofuran_7 H), 7.99 (d, 1H, *J* = 8.0 Hz, isobenzofuran_5 H), 7.67 (d, 1H, *J* = 8.0 Hz, isobenzofuran_4 H), 7.38 (t, 1H, *J* = 7.9 Hz, phenyl_5 H), 7.33 (s, 1H, phenyl_2 H), 7.24 (d, 1H, *J* = 7.5 Hz, phenyl_6 H), 7.09 (d, 1H, *J* = 8.0 Hz, phenyl_4 H), 5.42 (s, 2H, isobenzofuran CH_2), 5.37 (s, 2H, O-CH2). ^{13}C NMR, δ: 171.9 (C=O), 159.3 (phenyl C3), 146.3 (tetrazole C), 142.4 (isobenzofuran $C3_a$), 140.9 (isobenzofuran C6), 133.2 (phenyl C1), 129.7 isobenzofuran C5), 125.9 (phenyl C5), 122.9 (phenyl C6), 122.7 (isobenzofuran C7), 122.6 (isobenzofuran $C7_a$), 119.7 (phenyl C2), 114.4 (isobenzofuran C4), 113.7 (phenyl C4), 69.9 (isobenzofuran C3), 61.1 (tetrazole-CH_2-O) ppm. ESI-MS *m/z* 307.1 ($M-H^+$). HRMS (ESI) calcd for $C_{16}H_{11}N_4O_3$ ($M-H^+$), 307.0831; found, 307.0826. HPLC purity: 98.8%, R_t=13.175 (5% solvent B in solvent A).

*General procedure 7: Suzuki coupling of the 5-bromoisatin derivatives **17**, **20**, **26** and **33** with (3-(cyanomethoxy)phenyl)boronic acid **39**:*

Under a N_2 atmosphere, a 25 mL flask was charged with the 5-bromoisatin derivative (1 equiv), (3-(cyanomethoxy)phenyl)boronic acid **39** (1 equiv), tetrakis(triphenylphosphine)palladium(0) (5 mol%), potassium phosphate (3 equiv) and a mixture of diglyme (3 mL) and water (3 mL), and the reaction mixture was degassed by flushing N_2 through a needle for 10 min. The flask was then sealed and heated at 85 ºC for 5-9 h. After cooling to room temperature, the solvent was concentrated under reduced pressure, and 1 N HCl (15 mL) was added. The suspended solid was either collected by filtration or extracted with ethyl acetate (2 x 25 mL). The combined organic layers were dried ($MgSO_4$) and evaporated under reduced pressure. The combined residues were adsorbed onto silica and subjected to flash column chromatography (40% ethyl acetate in petroleum spirit) to yield the acetonitrile derivatives.

*2-(3-(4,6-Dimethoxy-2,3-dioxoindolin-5-yl)phenoxy)acetonitrile (**52**)*

Following the general procedure 7 using 5-bromo-4,6-dimethoxyindoline-2,3-dione **17** (40 mg, 0.139 mmol), (3-(cyanomethoxy)phenyl)boronic acid **39** (24.6 mg, 0.139 mmol), tetrakis(triphenylphosphine)palladium(0) (8 mg, 0.007 mmol), potassium phosphate (88.5 mg, 0.417 mmol) and heating for 9 h, the acetonitrile intermediate **52** could not be isolated. Analysis of the crude mixture: EI-MS *m/z* 338 (M^+, 17), 309 (7), 382 (66), 257 (100%), 225 (25). HRMS (ESI) calcd for $C_{18}H_{15}N_2O_5$ (MH^+), 339.0981; found, 339.0980. This sample was collected from the reaction mixture before purification, but could not be further purified and was not sufficiently stable to perform NMR experiments.

*2-(3-(7-Methyl-2,3-dioxoindolin-5-yl)phenoxy)acetonitrile (**53**)*

Following the general procedure 7 using 5-bromo-7-methylindoline-2,3-dione **20** (100 mg, 0.416 mmol), (3-(cyanomethoxy)phenyl)boronic acid **39** (73.6 mg, 0.416 mmol), tetrakis(triphenylphosphine)palladium(0) (24 mg, 0.021 mmol), potassium phosphate (264.9 mg, 1.248 mmol) and heating for 5 h, the acetonitrile intermediate **53** was isolated as a red solid (63.2 mg, 52%), mp: 208-209 ºC; IR (cm^{-1}): υ 3185 (w, NH), 2365 (m, CN), 1750 (s, C=O), 1587 (s, C=O). ^{1}H NMR (acetone-d_6), δ: 10.13 (s, NH), 7.83 (s, 1H, isatin_4 H), 7.68 (s, 1H, isatin_C6), 7.47 (t, *J* = 7.8 Hz, 1H, phenoxy_5 H), 7.40-7.38 (m, 2H, phenoxy_2,4 H), 7.09 (d, *J* = 8.0 Hz, 1H, phenoxy_6 H), 5.21 (s, 2H, CH_2), 2.39 (s, 3H, CH_3). ^{13}C NMR, δ: 185.1 (isatin C3), 160.5 (phenoxy C1), 158.6 (isatin C2), 149.8 (isatin $C7_a$), 142.4 (phenoxy C3), 138.9 (isatin C7), 136.4 (isatin C6), 131.3 (isatin C5), 123.3 (phenoxy C5), 121.8 (isatin C4), 121.2 (phenoxy C4), 119.5 (phenoxy C2), 116.7 (phenoxy C6), 115.3 (CN), 114.1 (isatin $C3_a$), 54.7 (CH2), 15.8 (CH_3) ppm. EI-MS *m/z* 292 (M^+, 93), 264 (80), 236 (100%), 196 (17), 180 (11). HRMS (ESI) calcd for $C_{17}H_{13}N_2O_3$ (MH^+), 293.0926; found, 293.0931. HPLC purity: 97.9%, R_t=13.070 (2.5% solvent C in solvent A).

*2-(3-(4-Methoxy-7-methyl-2,3-dioxoindolin-5-yl)phenoxy)acetonitrile (**54**)*

Following the general procedure 7 using 5-bromo-4-methoxy-7-methylindoline-2,3-dione **26** (100 mg, 0.370 mmol), (3-(cyanomethoxy)phenyl)boronic acid **39** (65.5 mg, 0.370 mmol), tetrakis(triphenylphosphine)palladium(0) (21.4 mg, 0.019 mmol), potassium phosphate (235.6 mg, 1.11 mmol) and heating for 8 h, the acetonitrile intermediate **54** was isolated as a red solid (41.7 mg, 35%), mp: 179-180 ºC; IR (cm^{-1}): υ 3190 (m, NH), 2166 (w, CN), 1706 (s, C=O), 1611 (s, C=O), 1294 (w, C-O). ^1H NMR (CD_3OD), δ: 7.40 (s, 1H, isatin_6 H), 7.38 (t, *J* = 8.2 Hz, 1H, phenoxy_5 H), 7.12 (d, *J* = 8.2 Hz, 1H, phenoxy_4 H), 7.11 (s, 1H, phenoxy_2 H), 7.01 (d, *J* = 8.3 Hz, 1H, phenoxy_6 H), 5.00 (s, 2H, CH_2), 3.91 (s, 3H, OCH_3), 2.22 (s, 3H, CH_3). ^{13}C NMR, δ: 160.2 (isatin C3), 156.7 (phenoxy C1), 154.9 (isatin C2), 148.6 (isatin C4), 142.1 (isatin $C7_a$), 138.9 (isatin C6), 137.8 (phenoxy C3), 129.2 (phenoxy C5), 123.4 (isatin C5), 120.5 (isatin C7), 115.9 (phenoxy C4), 115.7 (phenoxy C6), 115.5 (phenoxy C2), 113.7 (CN), 109.5 (isatin $C3_a$), 61.1 (CH_2), 53.3 (OCH_3), 13.7 (CH_3) ppm. EI-MS *m/z* 322 (M^+, 74), 294 (68), 280 (4), 254 (7), 226 (100%), 211 (11). HRMS (ESI) calcd for $C_{18}H_{15}N_2O_4$ (MH^+), 323.1032; found, 323.1018. HPLC purity: 99.5%, R_t=11.766 (2.5% isopropanol in solvent A).

*2-(3-(2,3-Dioxoindolin-5-yl)phenoxy)acetonitrile (**55**)*

Following the general procedure 7 using 5-bromoindoline-2,3-dione **33** (100 mg, 0.442 mmol), (3-(cyanomethoxy)phenyl)boronic acid **39** (78.2 mg, 0.442 mmol), tetrakis(triphenylphosphine)palladium(0) (25.5 mg, 0.022 mmol), potassium phosphate (281.5 mg, 1.326 mmol) and heating for 5 h, the acetonitrile intermediate **55** was isolated as a red solid (73.7 mg, 60%), mp: 205-206 ºC; IR (cm^{-1}): υ 3168 (w, NH), 2166 (m, CN), 1732 (s, C=O), 1621 (s, C=O). ^1H NMR (acetone-d_6), δ: 10.04 (s, NH), 7.95 (d, *J* = 8.2, 1H, phenoxy_4 H), 7.83 (s, 1H, phenoxy_2 H), 7.47 (t, *J* = 8.2 Hz, 1H, phenoxy_5 H), 7.39-7.38 (m, 2H, isatin_4,7H), 7.14 (d, *J* = 8.2 Hz, phenoxy_6H), 7.09 (d, *J* = 8.6 Hz, 1H, isatin_6H), 5.21 (s, 2H, CH_2). ^{13}C NMR, δ: 184.1 (isatin C3), 159.6 (phenoxy C1), 157.8 (isatin C2), 150.4 (isatin $C7_a$), 141.5 (phenoxy C3), 137.0 (phenoxy C4), 135.7 (phenoxy C2), 130.6 (phenoxy C5), 123.1 (isatin C5), 121.0 (isatin C7), 118.9 (phenoxy C6), 116.0 (CN), 114.6 (isatin $C3_a$), 113.1 (isatin C4), 112.9 (isatin C6), 53.8 (CH_2) ppm. EI-MS *m/z* 278 (M^+, 46), 250 (100%), 207 (5), 182 (49). HRMS (ESI) calcd for $C_{16}H_{11}N_2O_3$ (MH^+), 279.0770; found, 279.0759. HPLC purity: 99.7%, R_t=20.046 (2.5% solvent C in solvent A).

*3'-(Cyanomethoxy)-5-methyl-2-pivalamido-[1,1'-biphenyl]-3-carboxylic acid (**59**)*

Under a N_2 atmosphere, a 25 mL round bottom flask was charged with the β-lactam derivative **12** (100 mg, 0.339 mmol), the boronic ester **40** (96.6 mg, 0.373 mmol), tetrakis(triphenylphosphine)palladium(0) (19.6 mg, 0.016 mmol) and potassium phosphate (215.9 mg, 1.02 mmol). A mixture of 1,4-dioxane (10 mL) and water (3 mL) was added and the reaction mixture was degassed by flushing N_2 through a needle for 10 min. The flask was then sealed with a rubber cap, heated at 85 ºC for 12 h. The solvent was then concentrated under reduced pressure and 1 N HCl (10 mL) was added dropwise to the resulting residue which was extracted with ethyl acetate (2 x 15 mL). The combined organic layers were washed with water (15 mL),

brine (15 mL) and dried ($MgSO_4$). The mixture was then concentrated under reduced pressure, and the resulting residue was subjected to a flash column chromatography and elution with 90% CH_2Cl_2, 10% ethanol and 0.1% TFA yielded **59** as a pale yellow oil (59.4 mg, 48%), IR (cm^{-1}): υ 3328 (m, NH), 2967 (m, OH), 2200 (m, CN), 1646 (w, NH), 1411 (w, C-N). ^{1}H NMR ($CDCl_3$), δ: 8.61 (s, 1H, COOH), 7.53 (s, 1H, phenyl_6 H), 7.32 (t, 1H, *J* = 7.7 Hz, phenyl_5' H), 7.15 (s, 1H, phenyl_4 H), 7.01 (d, 1H, *J* = 7.5 Hz, phenyl_6'H), 6.92-6.90 (m, 2H, phenyl_2', 4'), 6.47 (s, 1H, NH), 4.73 (s, 2H, CH_2), 2.28 (s, 3H, CH_3), 0.97 (s, 9H, pivaloyl 3xCH_3). ^{13}C NMR, δ: 177.7 (pivaloyl C=O), 176.7 (COOH), 173.8 (C3'), 156.4 (C2), 141.7 (C5), 137.8 (C1), 135.7 (C6'), 133.2 (C4), 130.5 (C6), 129.7 (C1'), 129.6 (C6'), 123.4 (C4'), 115.6 (CN), 115.1 (C3), 113.3 (C2'), 53.5 (CH_2), 38.9 ($C(CH_3)_3$), 26.9 (pivalamido 3xCH_3), 20.8 (CH_3) ppm. ESI-MS *m/z* 365.0 (M-H^+). HRMS (ESI) calcd for $C_{21}H_{23}N_2O_4$ (MH^+), 367.1658; found, 367.1656. HPLC purity: 99.4%, R_t=17.599 (2% solvent B in solvent A).

Antiviral assay

CHIKV Indian Ocean strain 899 (Genbank FJ959103.1) was generously provided by Prof. S. Günther (Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany) (Panning M et al, Emerging Infectious Diseases 2008). BGM cells were maintained in cell growth medium composed of minimum essential medium (MEM Rega-3, Gibco, Belgium) supplemented with 10% Foetal Bovine Serum (FBS, Integro, The Netherlands), 1% L-glutamine (Gibco), and 1% sodium bicarbonate (Gibco). The antiviral assays were performed in virus growth medium which is the respective cell growth medium supplemented with 2% (instead of 10%) FBS. Cell cultures were maintained at 37 °C in an atmosphere of 5% CO2 and 95-99% humidity.

BGM cells were seeded in 96-well tissue culture plates (Becton Dickinson, Aalst, Belgium) at a density of 2.5 × 104 cells/well in 100 µl assay medium and were allowed to adhere overnight. Next, a compound dilution series was prepared in the medium on top of the cells after which the cultures were infected with 0.001 MOI of CHIKV 899 inoculum in 100 µl assay medium. On day 5 post-infection (p.i.), the plates were processed using the MTS/PMS method as described by the manufacturer (Promega, The Netherlands). The 50% effective concentration (EC_{50}), which is defined as the compound concentration that is required to inhibit virus-induced cell death by 50%, was determined using logarithmic interpolation. Potential cytotoxic/cytostatic effects of selected compounds were also evaluated in uninfected cells by means of the MTS/PMS method. The 50% cytotoxic concentration (CC_{50}; i.e., the concentration that reduces the overall metabolic activity of the cells by 50%) was calculated using logarithmic interpolation. All assay wells were checked microscopically for minor signs of virus-induced CPE or possible alterations to the cell or monolayer morphology caused by the compound.

Acknowledgements

AAR thanks the University of Wollongong for a scholarship.

Supplementary material

The ^{1}H and ^{13}C NMR spectra. This material is available free of charge *via* the Internet at http://pubs.acs.org.

REFERENCES

1. Robinson, M. C. *Trans. R. Soc. Trop. Med. Hyg.* **1955**, *49*, 28-32.
2. Outbreak news. Chikungunya, India. *Wkly. Epidemiol. Rec.* **2006**, *81*, 409-410.
3. Saxena, S. K.; Singh, M.; Mishra, N.; Lakshmi, V. *Euro Surveill.* **2006**, *11*, E060810 060812.
4. Sambri, V.; Cavrini, F.; Rossini, G.; Pierro, A.; Landini, M. P. *New Microbiol.* **2008**, *31*, 303-304.
5. Enserink, M. *Science* **2007**, *318*, 1860-1861.
6. Her, Z.; Kam, Y. W.; Lin, R. T.; Ng, L. F. *Microb. Infect.* **2009**, *11*, 1165-1176.
7. Powers, A. M.; Logue, C. H. *J. Gen. Virol.* **2007**, *88*, 2363-2377.
8. Schwartz, O.; Albert, M. L. *Nat. Rev. Microbiol.* **2010**, *8*, 491-500.
9. Ziegler, S. A.; Lu, L.; da Rosa, A. P.; Xiao, S. Y.; Tesh, R. B. *Am. J. Trop. Med. Hyg.* **2008**, *79*, 133-139.
10. Santhosh, S. R.; Dash, P. K.; Parida, M. M.; Khan, M.; Ti-wari, M.; Lakshmana Rao, P. V. *Virus Res.* **2008**, *135*, 36-41.
11. Chandak, N. H.; Kashyap, R. S.; Kabra, D.; Karandikar, P.; Saha, S. S.; Morey, S. H.; Purohit, H. J.; Taori, G. M.; Daginawala, H. F. *Neurol. India* **2009**, *57*, 177-180.
12. Pialoux, G.; Gauzere, B. A.; Jaureguiberry, S.; Strobel, M. *Lancet Infect. Dis.* **2007**, *7*, 319-327.
13. Mahesh, G.; Giridhar, A.; Shedbele, A.; Kumar, R.; Saikumar, S. J. *Indian J. Ophthalmol.* **2009**, *57*, 148-150.
14. Nair, A. G.; Biswas, J.; Bhende, M. P. *J. Ophthalmic. Inflamm. Infect.* **2012**, *2*, 39-40.
15. Briolant, S.; Garin, D.; Scaramozzino, N.; Jouan, A.; Crance, J. M. *Antiviral Res.* **2004**, *61*, 111-117.
16. de Lamballerie, X.; Ninove, L.; Charrel, R. N. *Infect. Disord. Drug Targets* **2009**, *9*, 101-104.
17. Bassetto, M.; De Burghgraeve, T.; Delang, L.; Massarotti, A.; Coluccia, A.; Zonta, N.; Gatti, V.; Colombano, G.; Sorba, G.; Silvestri, R.; Tron, G. C.; Neyts, J.; Leyssen, P.; Brancale, A. *Antiviral Res.* **2013**, *98*, 12-18.
18. Rashad, A. A.; Keller, P. A. *J. Mol. Graphics Model.* **2013**, *44*, 241-252.
19. Voet, D. V., J. G. In Biochimie; de Boeck, Ed., Université: Bruxelles: 2005; p 712.
20. Ando, K.; Suzuki, S.; Arita, M. *J. Antibiot.* **1970**, *23*, 408-413.
21. Nguyen, P. T. V.; Yu, H.; Keller, P. A. *J. Mol. Model.* **2014**, *20*, 2216.
22. Nguyen, P. T. V.; Yu, H.; Keller, P. A. *J. Mol. Graph. Model.* **2015**, *57*, 1-8.
23. Singh, K. H. Dhanachandra; Kirubakaran, P.; Nagarajan, S.; Sakkiah, S.; Muthusamy, K.; Velmurgan, D.; Jeyakanthan, J. *J. Mol. Model.* **2012**, *18*, 39-51.
24. Satheesh, G.; Prabhu, N. P.; Venkataramana, M. *Curr. Comput. Aided Drug Des.* **2014**, *10*, 361-373.
25. Oliferenko, P. V.; Oliferenko, A. A.; Poda, G. I.; Osolod-kin, Dmitry I.; Pillai, G. G.; Bernier, U. R.; Tsikolia, M.; Agramonte, N. M.; Clark, G. G.; Linthicum, K. J.; Katritzky, A. R. *PLoS One* **2013**, *8*, e64547
26. Rashad, A. A.; Mahalingam, S.; Keller, P. A. *J. Med. Chem.* **2014**, *57*, 1147-1166.
27. Khan, M.; Dhanwani, R.; Patro, I. K.; Rao, P. V. L.; Parida, M. M. *Antiviral Res.* **2011**, *89*, 1-8.
28. Kaur, P.; Thiruchelvan, M.; Lee, R. C.; Chen, H.; Chen, K. C.; Ng, M. L.; Chu, J. J. *Antimicrob. Agents Chemother.* **2013**, *57*, 155-167.
29. Bourjot, M.; Delang, L.; Nguyen, V. H.; Neyts, J.; Gueritte, F.; Leyssen, P.; Litaudon, M. *J. Nat. Prod.* **2012**, *75*, 2183-2187.
30. Nery, R.; Nice, E. *J. Pharm. Pharmacol.* **1971**, *23*, 842-847.
31. Sweeney, M. J.; Hoffman, D. H.; Esterman, M. A. *Cancer Res.* **1972**, *32*, 1803-1809.
32. Bopp, R. J.; Schirmer, R. E.; Meyers, D. B. *J. Pharm. Sci.* **1972**, *61*, 1750-1753.
33. Bhal, S. K.; Kassam, K.; Peirson, I. G.; Pearl, G. M. *Mol. Pharm.* **2007**, *4*, 556-560.
34. Tetko, I. V.; Poda, G. I. *J. Med. Chem.* **2004**, *47*, 5601-5604.

35. da Silva, J. F. M.; Garden, S. J.; Pinto, A. C. *J. Brazil. Chem. Soc.* **2001**, *12*, 273-U286.
36. Candido-Bacani Pde, M.; Mori, M. P.; Calvo, T. R.; Vile-gas, W.; Varanda, E. A.; de Syllos Colus, I. M. *J. Toxicol. Environ. Health A* **2013**, *76*, 354-362.
37. Binda, C.; Li, M.; Hubalek, F.; Restelli, N.; Edmondson, D. E.; Mattevi, A. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 9750-9755.
38. Cao, J. R.; Gao, H.; Bemis, G.; Salituro, F.; Ledeboer, M.; Harrington, E.; Wilke, S.; Taslimi, P.; Pazhanisamy, S.; Xie, X. L.; Jacobs, M.; Green, J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2891-2895.
39. Hubalek, F.; Binda, C.; Khalil, A.; Li, M.; Mattevi, A.; Castagnoli, N.; Edmondson, D. E. *J. Biol. Chem.* **2005**, *280*, 15761-15766.
40. Miyaura, N. *Adv. Synth. Catal.* **2004**, *346*, 1522-1523.
41. Yin, L. X.; Liebscher, J. *Chem. Rev.* **2007**, *107*, 133-173.
42. Alonso, F.; Beletskaya, I. P.; Yus, M. *Tetrahedron* **2008**, *64*, 3047-3101.
43. Biot, C.; Bauer, H.; Schirmer, R. H.; Davioud-Charvet, E. *J. Med. Chem.* **2004**, *47*, 5972-5983.
44. Patrick, G. L. An Introduction to Medicinal Chemistry 4th ed.; Oxford University Press: 2009.
45. Liljebris, C.; Larsen, S. D.; Ogg, D.; Palazuk, B. J.; Bleasdale, J. E. *J. Med. Chem.* **2002**, *45*, 1785-1798.
46. Zhang, Y. H.; Shi, B. F.; Yu, J. Q. *Angew. Chem. Int. Ed. Eng.* **2009**, *48*, 6097-6100.