Synthesis, Biological Evaluation and Molecular Docking Analysis of 2-phenyl-benzofuran-3-carboxamide derivatives as Potential Inhibitors of *Staphylococcus aureus* Sortase A

Wan He, Yong Zhang, Jian Bao, Xinxian Deng, Jennifer Batara, Shawn Casey, Qiuyuan Guo, Faqin Jiang, Lei Fu

PII: S0968-0896(16)31454-7
DOI: http://dx.doi.org/10.1016/j.bmc.2016.12.030
Reference: BMC 13457

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 17 October 2016
Revised Date: 15 December 2016
Accepted Date: 18 December 2016

Please cite this article as: He, W., Zhang, Y., Bao, J., Deng, X., Batara, J., Casey, S., Guo, Q., Jiang, F., Fu, L., Synthesis, Biological Evaluation and Molecular Docking Analysis of 2-phenyl-benzofuran-3-carboxamide derivatives as Potential Inhibitors of *Staphylococcus aureus* Sortase A, *Bioorganic & Medicinal Chemistry* (2016), doi: http://dx.doi.org/10.1016/j.bmc.2016.12.030

Synthesis, Biological Evaluation and Molecular Docking Analysis of 2-phenyl-benzofuran-3-carboxamide derivatives as Potential Inhibitors of *Staphylococcus aureus* Sortase A

Wan He[1,a], Yong Zhang[1,b], Jian Bao[a], Xinxian Deng[a], Jennifer Batara[a], Shawn Casey[a], Qiuyuan Guo[a], Faqin Jiang[*,a], Lei Fu[*,a]

a School of Pharmacy, Shanghai Jiao Tong University, No. 800 Dongchuan Rd., Shanghai, 200240, PR China;

b School of Science and Biotechnology, Shanghai Jiao Tong University, Shanghai, 200240, PR China;

[1] Co-first authors

* Corresponding author. Dr. Faqin Jiang, Tel.:+86 21 34204787, fax:+86 21 34204787, E-mail address :jfq2008@sjtu.edu.cn; Dr. Lei Fu, Tel.:+86 21 34204791, fax:+86 21 34204791, E-mail address: leifu@sjtu.edu.cn.

Abstract:

In Gram-positive bacteria, Sortase A (Srt A) is a critical cysteine transpeptidase that is responsible for recognizing and assembling surface virulence proteins through the recognition of a LPXTG (leucine, proline, X, threonine, and glycine, where X is any amino acid) signal. Mutants lacking genes for Srt A attenuate infections without affecting microbial viability. Here a series of 2-phenyl- benzofuran-3-carboxamide derivatives were synthesized and identified as potent Srt A inhibitors. Activity assays revealed that multiple compounds exhibited excellent inhibitory activity against Srt A compared with known Sortase A inhibitor pHMB (IC_{50}=130 μM). Structural activity relationships (SARs) demonstrated that the amide group at 3-position was essential for inhibitory activity. Replacement of the hydroxyl group at the 2-phenyl position of benzofuran with other substitutions such as a methoxyl, halogen or nitro group reduced the enzyme inhibitory activity in most cases. The compound **Ia-22** was found to be the most potent inhibitor against the enzyme with an IC_{50} value of 30.8 μM. Molecular docking studies showed **Ia-22** shared similar binding pattern with substrate LPXTG in the binding pocket of Srt A (PDB: 2KID) including *i*-butyl stretching,

L-shape pattern kinking, and H-bond interaction with Srt A functional site residues Cys184, Trp194 and Arg197.

Keywords: *Staphylococcus aureus*, Sortase A, Inhibitor, Benzofuran

1. Introduction

Staphylococcus aureus (*S. aureus*) is one of the most common pathogenic bacteria which clinically leads to purulent infections, such as pneumonia, pseudo membranous enteritis, pericarditis, septicemia and sepsis systemic infection.[1,2] With the wide application of antibiotics clinically, *S. aureus* resistant strains appear constantly. An example of a clinically problematic infection is the main pathogenic bacteria of nosocomial infections and multiple drug resistance, *methicillin-resistant S. aureus* (*MRSA*).[3,4] This strain has developed resistance to antibiotics currently in clinical use, including vancomycin, which was once considered the best therapy for *MRSA*. Therefore, in order to cope with the increasingly serious drug resistance problems, it is urgent to develop new antibacterial targets and corresponding inhibitors.

Srt A is a critical cysteine transpeptidase that is responsible for anchoring surface virulence proteins to peptidoglycan through recognizing the LPXTG signal labeled on their C-terminal.[5-7] These surface proteins display a critical role in the infection process, including bacterial adhesion and invasion of host tissues, and evasion of the host immune system. Mutants lacking genes for Srt A display attenuated virulence, but did not affect the growth of the bacteria.[8, 9] Therefore, Srt A has been considered as an ideal target for the design of novel anti-virulence as they may be less likely to induce a selective pressure that leads to drug resistance.

Previously, progress towards this goal has been made and several distinct Srt A inhibitors have been identified, including diarylacrylonitriles,[10,11] aryl(β-amino)ethyl ketones,[12] bis(indole)alkaloids,[13] substrate-modified peptide,[14,15] vinyl sulfones,[16] and other natural

compounds.[17-19] However, these compounds have not been developed into drugs or clinical applications because of their low activity or poor specificity. It is suggested that potent inhibitors may result from utilizing the specific recognition of Srt A to its substrate (LPXTG).[20,21]

Usually, Srt A can specifically recognize a substrate with an L-shape mode, which is afforded by Leucine and Proline side chains. If compounds with an L-shape like the substrate by Leucine/Proline residues can be tolerated by Srt A. Based on the principle, early, we discovered that the benzo[d]oxazole derivatives bearing aryl substituents at the C-2 position and carboxamide at C-7 position exhibited highly inhibitory activity against *S. aureus* Srt A.[22] Based on our published results of benzo[d]oxazole derivatives, *i*-butyl amide group and the benzoxazole core shaped the whole molecule into a L-shape mode to be specific recognition by Srt A. We speculate similar structures should be specific recognized by Srt A and exhibited highly inhibitory activity. Therefore, we designed a series of novel 2-phenyl-benzofuran-3-carboxamide derivatives (**Fig.1**) as potential Srt A inhibitors. The whole molecule is constructed into a kinked pattern like LPATG, with a benzofuran core and a 3-carboxamide mimicking the Proline and Leucine side chains, respectively.

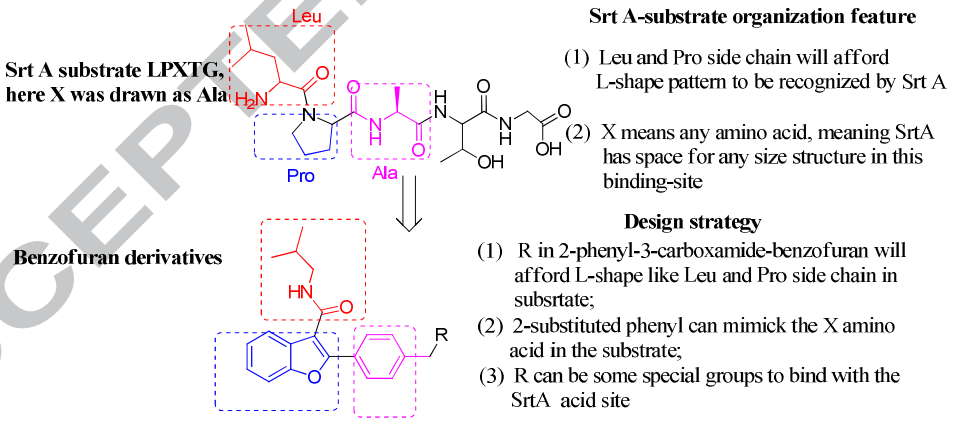


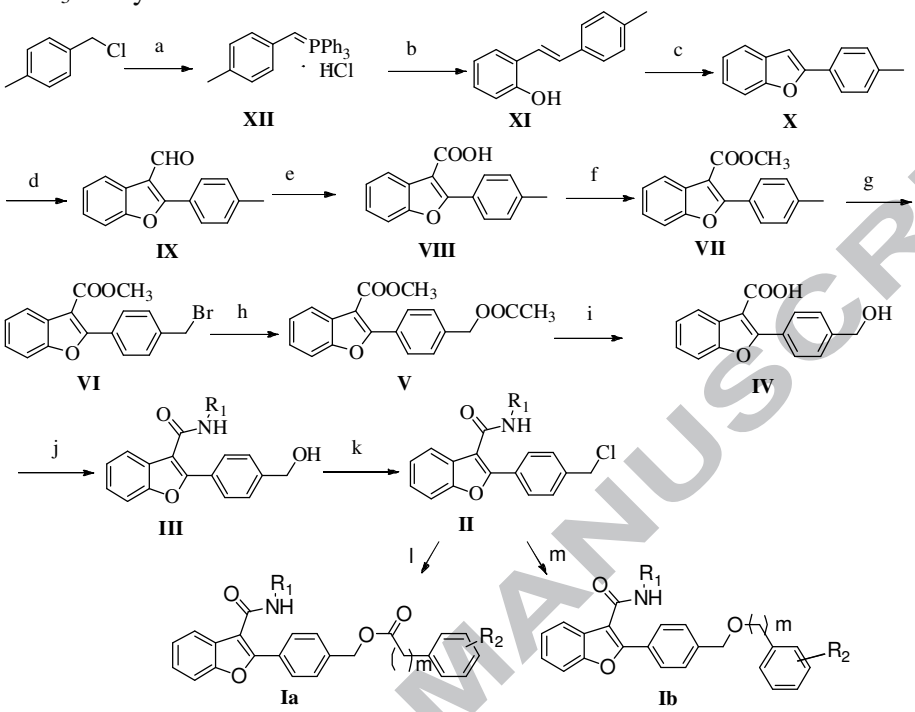
Fig. 1. The design strategy of target compounds.

2. Results and discussion

2.1 Chemistry

The 2,3-substituted-benzofuran derivatives were prepared according to

the synthetic route in **Scheme 1**. Firstly, p-methylbenzyl chloride was used as the starting material, which was then treated with PPh_3 to yield intermediate **XII**. Subsequently, coupling compound **XII** with 2-hydroxybenzaldehyde via a Wittig reaction yielded intermediate **XI**.



Scheme 1 Reagents and conditions: a) PPh_3, CH_3CN, reflux, 70%; b) DBU, CH_3CN, reflux, 61%; c) I_2, K_2CO_3, THF, r.t. 94%; d) DMF, $POCl_3$, 1,2-dichloroethane, reflux, 78.2%; e) NH_2SO_3H, $NaClO_2$, CH_3OH/H_2O, r.t., 86%; f) H_2SO_4, CH_3OH, reflux, 90%; g) NBS, AIBN, CCl_4, reflux, 75%; h) NaOAc, DMF, 90^0C; i) NaOH, CH_3OH/H_2O, reflux, 86%; j) HATU, Et_3N, r.t., 90%; k) $SOCl_2$, CH_2Cl_2, reflux, 78%; l) NaOH, DMF, 45^0C, 30% - 71%; m) K_2CO_3, CH_3CN, reflux, 45% - 90%.

Compound **XI** underwent intra-molecular cyclization in the presence of K_2CO_3 and I_2 to produce benzofuran **X**. Compound **X** was then treated with $POCl_3$ and DMF by a Vilsmeier reaction to produce compound **IX**. Following oxidation, esterification, bromination, hydrolysis, amidation and chloridization reactions, 2,3-substituted intermediate **II** was obtained. Eventually, the target molecules **Ia** and **Ib** were easily synthesized by substitution reaction of an aromatic acid or phenol. The chemical structures were confirmed by spectral data. The ^{1}H NMR, ^{13}C NMR and mass spectra results are consistent with the proposed structures.

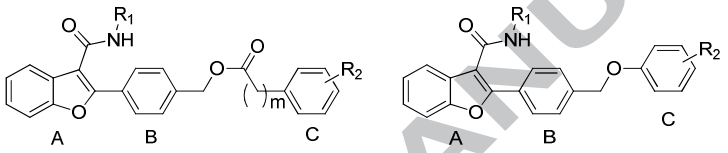
2.2 Inhibition of Sortase A

Thirty-seven new compounds were evaluated for their *in vitro* inhibitory activities against Srt A according to a previously reported HPLC assay procedure with known Sortase A

inhibitor pHMB as positive compound.[23, 24] The inhibitory potency of the tested compounds, presented as IC_{50} values, are shown in the **Table 1** and are compared with that of a known Srt A inhibitor, pHMB.[10] The results demonstrated that most of the compounds exhibited stronger activity than pHMB (130 ± 4.3 μM) against the Srt A of *S. aureus*. Especially, compound **Ia-22** was found to be the most potent inhibitor against the enzyme with an IC_{50} value of 30.8 μM, closely followed by compounds **Ia-4**, **Ia-5** and **Ia-7**.

Table 1. Inhibitory activity of the target compounds against Sortase A

Ia **Ib**



Structure index	R_1	R_2	m[a]	IC_{50} (μM)[b]
pHMB[c]	(positive control)			130.0 ± 4.3
Ia-1	*i*-Bu	H	0	158.4 ± 1.0*
Ia-2	*i*-Bu	2-OH	0	76.4 ± 2.7**
Ia-3	*i*-Bu	3-OH	0	50.6 ± 1.0**
Ia-4	*i*-Bu	4-OH	0	43.0 ± 0.7**
Ia-5	*i*-Bu	3,5-di-OH	0	34.5 ± 2.4**
Ia-6	*i*-Bu	3,5-di-OCH_3	0	62.0 ± 0.8**
Ia-7	*i*-Bu	3,4-di-OH	0	39.6 ± 1.7**
Ia-8	*i*-Bu	3,4,5-tri-OCH_3	0	55.2 ± 4.9**
Ia-9	*i*-Bu	4-$N(CH_3)_2$	0	>200
Ia-10	*i*-Bu	2,4-di-OH	0	57.0 ± 0.7**
Ia-11	*i*-Bu	2-NO_2	0	>200
Ia-12	*i*-Bu	4-F	0	>200
Ia-13	*i*-Bu	4-SH	0	89.1 ± 1.2**
Ia-14	*i*-Bu	3-OH,4-NO_2	0	60.6 ± 0.3**
Ia-15	*i*-Bu	4-NH_2	0	52.3 ± 1.4**
Ia-16	*i*-Bu	2-OH	1	89.0 ± 0.6**
Ia-17	*i*-Bu	3-OH	1	76.3 ± 0.8**
Ia-18	*i*-Bu	4-OH	1	84.0 ± 2.4**
Ia-19	*i*-Bu	4-NO_2	1	>200
Ia-20	*i*-Bu	3-CF_3	1	>200

Ia-21	*i*-Bu	4-CF_3	1	>200
Ia-22	*i*-Bu	3,4-di-OH	1	30.8 ± 2.3**
Ia-23	*i*-Bu	4-OH	(C=C)	158.5 ± 3.8**
Ia-24	*n*-Bu	4-OH	0	186.4 ± 5.8**
Ia-25	*n*-Bu	3,4-di-OH	0	66.0 ± 1.4**
Ib-26	*i*-Bu	3-OH	0	86.4 ± 1.7**
Ib-27	*i*-Bu	4-CH_3	0	>200
Ib-28	*i*-Bu	4-Cl	0	>200
Ib-29	*i*-Bu	4-CH_2OH	0	120.8 ± 2.6*
Ib-30	*i*-Bu	3-CHO	0	89.0 ± 1.3**
Ib-31	*i*-Bu	3-$COCH_3$	0	147.9 ± 0.8*
Ib-32	*i*-Bu	4-CN	0	97.0 ± 0.5**
Ib-33	*i*-Bu	4-SO_3CH_3	0	131.6 ± 1.3
Ib-34	*i*-Bu	3-$NHCOCH_3$	0	148.0 ± 1.8**
Ib-35	*i*-Bu	4-$NHCOCH_3$	0	170.7 ± 4.6*
Ib-36	*i*-Bu	4-$COOCH_3$	0	>200
Ib-37	*n*-Bu	4-SO_3CH_3	0	>200

[a] "m" represents double bond for **Ia-23** and number of carbon for the rest.

[b] IC_{50} values are means ± SD (n = 3). [c] pHMB : p-Hydroxymercuribenzoic acid.

*Significantly different from control, $p < 0.05$; ** Significantly different from control, $p < 0.001$.

Preliminary structural activity relationships were deduced as follows. Firstly, the R_1 substituent at the 3-position of benzofuran has an obvious influence on inhibition. Compounds **Ia-4** (IC_{50}=43.0 μM) and **Ia-7** (IC_{50}=39.6 μM) showed significantly higher inhibitory activity, as compared to those of compounds **Ia-24** (IC_{50} = 186.4 μM) and **Ia-25** (IC_{50} = 60.0 μM). **Ib-33** (IC_{50}=131.6 μM) showed higher activity than **Ib-37**(IC_{50}>200 μM)**.** This indicates that the *i*-butylamide substituent at the 3-position is indispensable for activity which is consistent with our design. Secondly, R_2 substituents at the C ring of benzofuran derivatives have a significant impact on inhibitory activity as well. For example, introduction of a -F, -Cl, -CF_3, -NO_2 or -$COOCH_3$ group instead of a -OH, -SH, -NH_2 or -OCH_3 is significantly detrimental to the inhibitory activity (**Ia-2** vs **Ia-11** and **Ia-12**; **Ia-13** to **Ia-15** vs **Ia-12**; **Ia-16** to **Ia-18** vs **Ia-19** to **Ia-21**; **Ia-7** vs **Ia-14**; **Ib-26** vs **Ib-28** to **Ib-37**). Meanwhile,

we noted that the inhibitory activity is proportional to the number of hydroxyl groups as compounds **Ia-5** and **Ia-22** (IC_{50}=34.5 μM, 30.8 μM), which contain two hydroxyl groups, showed higher activity than compounds **Ia-3** and **Ia-18** (IC_{50}=50.6 μM, 84.0 μM) with a single hydroxyl group. Thirdly, the length of the linker between the B and C rings has little influence on inhibitory activity. The activity slightly decreased with an increase in linker length (**Ia-16** to **Ia-18** vs **Ia-2** to **Ia-4**) except in the case of compound **Ia-22** (IC_{50}=30.8 μM). Finally, introduction of an ester linker between the B and C rings resulted in a boost of inhibitory activity against Srt A compared to an ether linker (**Ia-3** vs **Ib-26**).

2.3 Molecular docking simulation

To further elucidate the possible binding mechanism of our compounds, molecular docking of some of the most potent compounds was done using Gold Suite v5.0.1 (CCDC, Cambridge, U.K., 2010).The initial structure of Srt A was obtained from the 3D-NMR structure of the Srt A - LPAT complex (PDB code: 2KID).[24] The docking conformations of our ligands (**Ia-22** and **Ib-26**) and the overlay of the ligands and LPAT within the binding pocket are shown in Fig. 2.

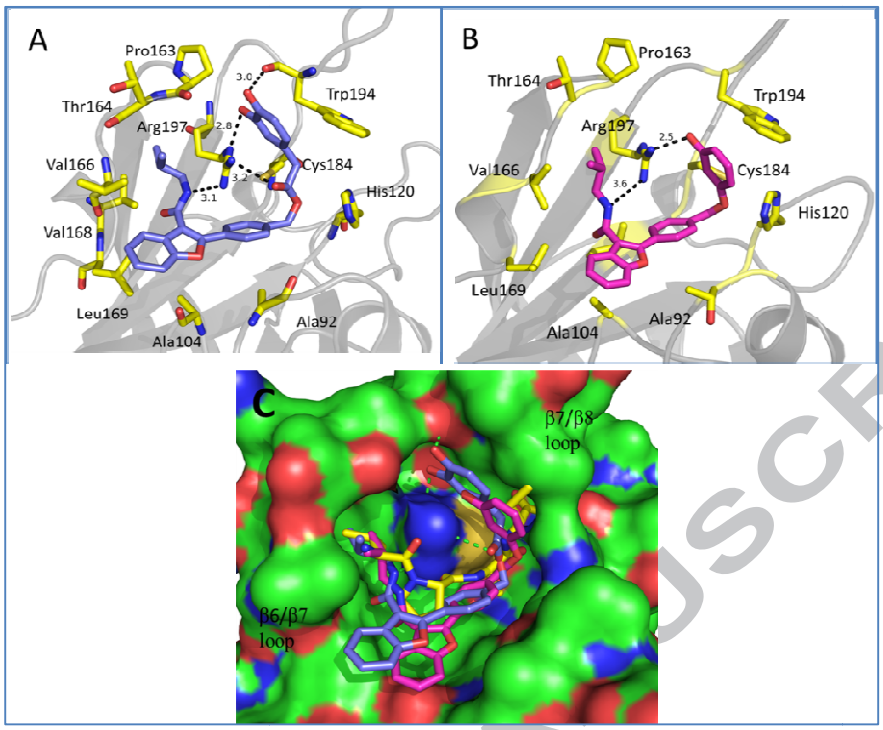


Fig. 2. **A**, **B**: Docking of compounds **Ia-22** and **Ib-26** to Srt A (PBD code: 2KID). Only residues situated less than 4 Å from the inhibitor have been shown and colored yellow; **C**: Overlap of the LPAT and compounds (**Ia-22** and **Ib-26**) in the binding pocket of Srt A. The protein is colorized by electrostatic surface potential. LPAT is colored by yellow, **Ia-22** colored by blue, **Ib-26** colored by magenta. The figures were created using the program PYMOL

The model showed a special L-shape binding mode which was similar to that of the peptide substrate LPAT binding to Srt A.[21] As depicted in Fig. 2C, the two ligands bind to Srt A with a special L-shape pattern similar to that of the LPAT binding. To our knowledge, the Ile of LPAT binding to the β6/β7 loop-β8 channel exerts decisive effect for compound activity. We design our ligands mimicking such component to simulate the similar binding conformation. Indeed, the *i*-butyl amide group stretches to the channel and forms hydrophobic interactions with Thr164, Val166, Val168, Leu169 and Ile182.

The binding arrangement explains the activity change. H-bonds formed between the carbonyl oxygen atom of the ester and the two active site residues, Cys184 and Arg197, may also help to stabilize the key conformation. In addition, two -OH groups substituted at the C ring of compound **Ia-22** formed two additional H-bonds to the residues Trp194 and Arg197. It explains the significant reduction in activity when the

-OH group was replaced with a methyl and halogen. As shown in **Fig. 2B**, the -OH group at the C ring of compound **Ib-26** formed a H-bond to the basic residue Arg197. However, compared to the ester linker of compound **Ia-22**, the ether linker of compound **Ib-26** significantly reduced binding affinity, which led to a slight reduction in activity compared to that of compound **Ia-22**. These results may explain why the ester linker contributes to more positive impacts in activity than that of the ether linker.

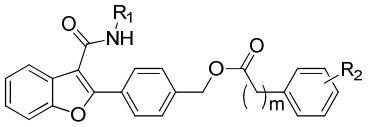
The 2,3-substituted benzofuran plays a key role in shaping the whole molecule in a L-shape conformation which fits the corresponding active site pocket of Srt A. An overlay of compounds **Ia-22**, **Ib-26** and LPAT in the binding pocket (**Fig. 2C**) demonstrates reoccurring binding motifs shared by these ligands and Srt A: *i*-butyl side chain, and the L-shape pattern. Since the disulfide bond between LPAT and Cys184 greatly strengthens its binding, further modification shall focus on the interactions between Cys184 and functional groups of the ligand.

3. Experimental

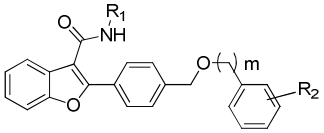
3.1 General methods for synthesis

The moisture sensitive reactions were carried out under a nitrogen atmosphere with the solvents distilled from appropriate drying agents prior to use. Other solvents and commercial chemicals were purchased and used directly without purification. Reactions were monitored by TLC (silica gel aluminum sheets 60 F254) with a UV indicator. The NMR spectra were obtained using an Agilent NMR Systems 400 MHz Spectrometer (^{1}H NMR at 400 MHz; ^{13}C NMR at 100 MHz). Chemical shifts were designated as follows in ppm from the internal standard (chemical shift in δ, ppm). Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; m, multiplet. Coupling constants (J values) were measured in Hertz (Hz). High resolution mass spectra were obtained on an Agilent Technologies Q-TOF 6230. Melting points were determined on a Mel-Temp II melting point apparatus and are uncorrected.

3.1.1 Synthesis procedures and analytical data



Ia-1~Ia-25



Ib-26~Ib-35

Triphenyl-(4-methylbenzyl) phosphonic chloride (**XII**)

To a solution of p-methylbenzyl chloride (50 g, 356 mmol) in CH_3CN (200 mL), triphenylphosphine (93.3 g, 356 mmol) was added and refluxed for 6 h. Then the reaction was cooled to room temperature before filtered. The residue was washed with ether (3×30 mL) and dried *in vacuo* to afford intermediate **XII** as a white solid (102.3 g, 70 % yield) which was used in the next step without further purification.

(*E*)-2-(4-methylstyryl)phenol (**XI**)

To a solution of compound **XII** (100 g, 248.2 mmol) and 2-hydroxybenzaldehyde (30.3 g, 248.2 mmol) in CH_3CN (350 mL), DBU (91.3 mL, 595.7 mmol) was added dropwise slowly and refluxed overnight. The reaction was cooled to room temperature. Subsequently, HCl (1 M) was added to achieve a neutral pH (7) before concentration by pressure reduction. The residue obtained was dissolved in EtOAc (150 mL) and washed with water (2×75 mL), brine (1×75 mL) and dried over anhydrous Na_2SO_4. The drying agent was filtered off and the solvent was removed *in vacuo* to afford the crude product which was purified by column chromatography to give intermediate **XI** as a white solid (31.8 g, 61 % yield); ^{1}H NMR (DMSO-d_6) δ 9.67 (s, 1H), 7.52 (d, *J* = 7.40 Hz, 1H), 7.40 (d, *J* = 7.80 Hz, 2H), 7.32 (d, *J* = 16.82 Hz, 1H), 7.09-7.17 (m, 3H), 7.01 - 7.08 (m, 1H), 6.83 (d, *J* = 7.80 Hz, 1H), 6.77 (t, *J* = 7.40 Hz, 1H), 2.27 (s, 3H).

2-(4-methylphenyl) benzofuran (**X**)

To a solution of compound **XI** (5.4 g, 25.7 mmol) and anhydrous K_2CO_3 (21.2 g, 154.2 mmol) in THF (150 mL), I_2 (39.4 g, 154.2 mmol) was added and stirred at room temperature for 1 h. After the reaction was completed, aqueous $Na_2S_2O_3$ was added to eliminate the remaining I_2. The mixture was concentrated. The residue obtained was dissolved in EtOAc (100 mL) and washed with water (2×50 mL), brine

(1×50 mL) and dried over anhydrous Na_2SO_4. The drying agent was filtered off and the solvent was removed *in vacuo* to afford the crude product which was purified by column chromatography to give intermediate **X** as a white solid (5.1 g, 94 % yield).^{1}H NMR (DMSO-d_6) δ 7.78 (d, *J* = 8.20 Hz, 2H), 7.57 (d, *J* = 8.60 Hz, 1H), 7.60 (d, *J* = 7.40 Hz, 1H), 7.32 (s, 1H), 7.28 (d, *J* = 8.20 Hz, 2H), 7.24 (m, 1H), 7.19 - 7.22 (m, 1H), 2.32 (s, 3H).

2-(4-methylphenyl)benzofuran-3-carbaldehyde (**IX**)

To a solution of DMF (11.8 mL, 153.6 mmol) in 1,2-dichloroethane (50 mL), $POCl_3$ (23.5 g, 153.6 mmol) was added dropwise at 0 ^{0}C. The reactions were allowed to reach room temperature before it was stirred for 1h. Compound **X** (4.0 g, 19.2 mmol) was then added and refluxed overnight. The reaction was cooled to room temperature before poured onto 350 g of crushed ice and stirred for an additional 1 h. The aqueous solution was basified to pH 9 with aqueous NaOH (1M) and extracted with CH_2Cl_2. The combined extracts were dried, concentrated, and purified by column chromatography to give intermediate **IX** as a white solid (3.5 g, 78 % yield). ^{1}H NMR (DMSO-d_6) δ 10.23 (s, 1H), 8.10 - 8.14 (m, 1H), 7.87 (d, *J* = 8.20 Hz, 2H), 7.71 (d, *J* = 7.40 Hz, 1H), 7.36 - 7.46 (m, 4H), 2.39 (s, 3H).

2-(4-methylphenyl)benzofuran-3-carboxylic acid (**VIII**)

To a solution of intermediate **IX** (3.5 g, 14.8 mmol) in acetone (40 mL), $NaClO_2$ (3.3 g, 36.5 mmol) and NH_2SO_3H (2.86 g, 29.5 mmol) were added at 0 ^{0}C and stirred at room temperature for 4 h. Then aqueous $Na_2S_2O_3$ was added to eliminate remaining $NaClO_2$ before being poured onto water (20 mL) which yielded a large amount of solid precipitation. The mixture was filtered to give intermediate **VIII** as yellow solid (3.0 g, 86 % yield) which was used in the next step without further purification.

2-(4-methylphenyl)benzofuran-3-carboxylate (**VII**)

To a solution of intermediate **VIII** (3.0 g, 11.9 mmol) in MeOH (50 mL), concentrated H_2SO_4 (5 mL) was added dropwise at 0 ^{0}C. The reaction was then

refluxed overnight before being cooled to room temperature and concentrated by reduced pressure. Then the residue was dissolved in EtOAc (30 mL) and washed with aqueous $NaHCO_3$, brine and dried over anhydrous Na_2SO_4. The solvent was removed and the residue was concentrated and purified by column chromatography to give intermediate **VII** as a white solid (2.85 g, 90 % yield); ^{1}H NMR (DMSO-d_6) δ 7.94-7.98 (m, 1H), 7.85 (d, *J* = 8.20 Hz, 2H), 7.63 - 7.68 (m, 1H), 7.34 - 7.42 (m, 2H), 7.32 (d, *J* = 7.80 Hz, 2H), 3.83 (s, 3H), 2.36 (s, 3H).

2-(4-bromomethylphenyl)benzofuran-3-carboxylate (**VI**)

To a solution of intermediate **VII** (7.0 g, 26.3 mmol) in CCl_4 (100 mL), NBS (5.14 g, 28.9 mmol) and AIBN (0.2 g, 1.2 mmol) were added and refluxed for 12 h. The reaction was subsequently cooled to room temperature before filtration. The filtrate was extracted with EtOAc and the organic phase was washed with water and brine, dried over anhydrous Na_2SO_4, concentrated and purified by column chromatography to give intermediate **VI** as a white solid (6.8 g, 75 % yield); ^{1}H NMR (DMSO-d_6) δ 8.00 (d, *J* = 5.50 Hz, 1H), 7.95 (d, *J* = 8.60 Hz, 2H), 7.69 (d, *J* = 7.40 Hz, 1H), 7.59 (d, *J* = 8.20 Hz, 2H), 7.36 - 7.44 (m, 2H), 4.77 (s, 2H), 3.85 (s, 3H).

2-(4-(acetoxymethyl)phenyl)benzofuran-3-carboxylate (**V**)

To a solution of intermediate **VI** (1.5 g, 4.3 mmol) in DMF (10 mL), anhydrous NaOAc (1.78 g, 21.5 mmol) was added and heated at 120 ^{0}C for 3 h. After cooling to room temperature the mixture was extracted with EtOAc. The combined extracts were washed with water, brine, dried over anhydrous Na_2SO_4 and concentrated to give intermediate **V** as white solid (1.2 g, 86.3 % yield) which was used in the next step without further purification.

2-(4-(hydroxymethyl)phenyl)benzofuran-3-carboxylic acid (**IV**)

To a solution of intermediate **V** (2.0 g, 6.17 mmol) in THF (10 mL), aqueous NaOH (3M, 10 mL) was added and refluxed for 3 h. The reaction was cooled to room temperature and acidified by HCl (1M) to be pH 4 before being poured onto water (10 ml). The

precipitate was filtered to give intermediate **IV** as yellow solid (1.65 g, 95 % yield) which was used in the next step without further purification.

2-(4-(hydroxymethyl)phenyl)-*N*-isobutyl benzofuran-3-carboxamide (**III**)

To a solution of intermediate **IV** (1.0 g, 3.7 mmol) in CH_2Cl_2 (20 mL), *i*-butylamine (0.54 g, 7.4 mmol), HATU (2.1 g, 5.6 mmol), triethylamine (0.75 g, 14.8 mmol) was added and stirred at room temperature for 2 h. Then HCl (1M) was added to achieve acidic pH 5 and extracted with CH_2Cl_2. The combined extracts were washed with aqueous $NaHCO_3$ and brine, dried, concentrated and purified by column chromatograph to give intermediate **III** as yellow solid (1.2 g, 90 % yield); ^{1}H NMR (DMSO-d_6) δ 7.79 - 7.88 (d, *J* = 8.20 Hz, 2H), 7.64 (d, *J* = 8.20 Hz, 1H), 7.57 (d, *J* = 7.40 Hz, 1H), 7.39 - 7.45 (d, *J* = 8.20 Hz, 2H), 7.27 - 7.39 (m, 2H), 5.30 (br. s., 1H), 4.53 (br. s., 2H), 3.11 (t, *J* = 6.4 Hz, 2H), 1.83 (m, 1H), 0.89 (d, *J* = 6.60 Hz, 6H).

2-(4-(chloromethyl)phenyl)-N-*i*-butylbe nzofuran-3-carboxamide (**IIa**)

To a solution of intermediate **III** (1.0 g, 3.1 mmol) in CH_2Cl_2 (20 mL), $SOCl_2$ (0.78 mL, 9.3 mmol) was added dropwise at 0 ^{0}C and stirred at room temperature for 1 h. Then the mixture was poured onto ice water and extracted with EtOAc. The combined extracts were washed with aqueous Na_2CO_3 and brine, dried, concentrated and purified by column chromatograph to give intermediate **IIa** as yellow solid (525 mg, 40.6 % yield); ^{1}H NMR ($CDCl_3$) δ 8.01 (d, *J* = 8.60 Hz, 2H), 7.78 (d, *J* = 7.40 Hz, 1H), 7.49 - 7.55 (m, 3H), 7.29 - 7.39 (m, 2H), 5.01 (br. s., 1H), 4.27 (s, 2H), 3.31 (t, *J* = 6.4 Hz, 2H), 1.87 (m, 1H), 0.95 (d, *J* = 6.60 Hz, 6H).

2-(4-(chloromethyl)phenyl)-*N*-*n*-butylbe nzofuran-3-carboxamide (**IIb**)

Compound **IIb** was synthesized, following a similar procedure as the one used for preparation of **IIa** as yellow solid (11.3 % totally yield).

4-(3-(isobutylcarbamoyl)benzofuran-2-y l)benzyl benzoate (**Ia-1**)

To a solution of intermediate **IIa** (200 mg, 0.496 mmol) in DMF (10 mL), NaOH (99.2 mg, 2.48 mmol) and

benzoic acid (302.6 mg, 2.48 mmol) was added and stirred at room temperature for 3 h before H_2O (10 mL) was added. The resulting solid was filtered, washed with ethyl acetate and 95 % ethanol, and recrystallized in DMF/H_2O to give compound **Ia-1** as white solid (150 mg, 71.5 % yield); m.p. 168-170 ^{0}C, ^{1}H-NMR ($CDCl_3$) δ 8.07 (m, 2H), 7.94 (d, J=8.4 Hz, 2H), 7.81 (m, 1H), 7.54 (m, 4H), 7.44 (m, 2H), 7.32 (m, 2H), 5.94 (br. s., 1H), 5.41 (s, 2H), 3.28 (t, *J* = 6.4Hz, 2H), 1.82 (m, 1H), 0.91 (d, *J* = 6.60 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 166.3, 164.0, 154.0, 153.7, 137.8, 133.2, 129.8, 129.7, 129.3, 128.4, 128.3, 127.6, 125.4, 123.8, 120.8, 113.3, 111.3, 66.1, 47.1, 28.5, 20.2; HRMS-ESI $C_{27}H_{25}NO_4$ calcd [M+H] 428.1856, found 428.1859.

4-(3-(isobutylcarbamoyl)benzofuran-2-yl)benzyl-2'-hydroxybenzoate (**Ia-2**)

Compound **Ia-2** was synthesized, following a similar procedure as the preparation of **Ia-1** starting from compound **IIa** and 2-hydroxybenzoic acid to obtain the product as yellow solid (55.3 % yield); m.p. 180-182 ^{0}C. ^{1}H NMR ($CDCl_3$) δ 10.70 (s, 1H), 7.97 (d, *J* = 8.20 Hz, 2H), 7.88 (d, *J* = 8.20 Hz, 1H), 7.77 - 7.82 (m, 1H), 7.49 - 7.56 (m, 3H), 7.43 - 7.49 (m, 1H), 7.27 - 7.37 (m, 2H), 6.98 (d, *J* = 8.60 Hz, 1H), 6.88 (t, *J* = 7.60 Hz, 1H), 5.96 (br. s., 1H), 5.41 (s, 2H), 3.28 (t, *J* = 6.46 Hz, 2H), 1.84 (m, 1H), 0.92 (d, *J* = 6.60 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 169.8, 163.9, 161.7, 153.8, 153.7, 136.9, 136.0, 129.9, 129.6, 128.4, 128.4, 127.5, 125.4, 123.8, 120.8, 119.3, 117.6, 113.4, 112.2, 111.3, 66.4, 47.2, 28.5, 20.2; HRMS-ESI $C_{27}H_{25}NO_5$ calcd [M+H] 444.1805, found 444.1801.

4-(3-(isobutylcarbamoyl)benzofuran-2-yl)benzyl-3'-hydroxybenzoate (**Ia-3**)

Compound **Ia-3** was synthesized, following a similar procedure as the preparation of **Ia-1** starting from compound **IIa** and 3-hydroxybenzoic acid to obtain the product as white solid (64 % yield); m.p. 185-187 ^{0}C. ^{1}H NMR (DMSO-d_6) δ 9.81 (s, 1H), 8.60 (t, *J* = 5.60 Hz, 1H), 7.89 (d, *J* = 8.20 Hz, 2H), 7.65 (d, *J* = 7.80 Hz, 1H), 7.54 - 7.59 (m, 3H), 7.24 - 7.45 (m, 5H), 7.01 (d, *J* = 8.60 Hz, 1H), 5.32 (s, 2H), 3.11 (t, *J* = 6.4 Hz, 2H), 1.83 (m, 1H), 0.88 (d, *J* = 6.60 Hz, 6H); ^{13}C NMR (DMSO-d_6) δ 165.9, 163.6, 158.0, 153.3, 152.0, 137.9, 131.1, 130.4, 129.4, 128.8, 128.0, 127.2,

125.9, 124.1, 121.0, 120.9, 120.4, 116.1, 115.0, 111.7, 66.1, 47.1, 28.5, 20.7; HRMS-ESI $C_{27}H_{25}NO_5$ calcd [M+H] 444.1805, found 444.1811.

4-(3-(isobutylcarbamoyl)benzofuran-2-yl)benzyl-4'-hydroxybenzoate (**Ia-4**)

Compound **Ia-4** was synthesized, following a similar procedure as the preparation of **Ia-1** starting from compound **IIa** and 4-hydroxybenzoic acid to obtain the product as white solid (52.6 % yield); m.p. 185-188 ^{0}C. ^{1}H NMR (DMSO-d_6) δ 10.34 (s, 1H), 8.57 (t, *J* = 5.67 Hz, 1H), 7.89 (d, *J* = 8.20 Hz, 2H), 7.84 (d, *J* = 9.00 Hz, 2H), 7.65 (d, *J* = 7.80 Hz, 1H), 7.54 - 7.59 (m, 3H), 7.35 - 7.38 (m, 1H), 7.31 - 7.34 (m, 1H), 6.84 (d, *J* = 8.60 Hz, 2H), 5.32 (s, 2H), 3.11 (t, *J* = 6.4 Hz, 2H), 1.82 (m, 1H), 0.88 (d, *J* = 6.60 Hz, 6H); ^{13}C NMR (DMSO-d_6) δ 163.8, 163.6, 162.6, 153.3, 152.0, 138.2, 132.0, 129.3, 128.6, 128.0, 127.2, 127.1, 126.9, 125.9, 124.1, 120.9, 120.8, 120.5, 115.9, 114.9, 111.7, 65.6, 47.1, 28.5, 20.7; HRMS-ESI $C_{27}H_{25}NO_4$ calcd [M+H] 444.1805, found 444.1807.

4-(3-(isobutylcarbamoyl)benzofuran-2-yl)benzyl-3',5'-dihydroxybenzoate (**Ia-5**)

Compound **Ia-5** was synthesized, following a similar procedure as the preparation of **Ia-1** starting from compound **IIa** and 3,5-dihydroxybenzoic acid to obtain the product as white solid (45.9 % yield); m.p. 184-186 ^{0}C. ^{1}H NMR (DMSO-d_6) δ 9.61 (s, 2H), 8.57 (t, *J* = 5.80 Hz, 1H), 7.90 (d, *J* = 8.20 Hz, 2H), 7.65 (d, *J* = 8.20 Hz, 1H), 7.53 - 7.60 (m, 3H), 7.35 - 7.40 (m, 1H), 7.29 - 7.35 (m, 1H), 6.85 (s, 2H), 6.43 (s, 1H), 5.32 (s, 2H), 3.12 (t, *J* = 6.4 Hz, 2H), 1.83 (m, 1H), 0.89 (d, *J* = 6.65 Hz, 6H); ^{13}C NMR (DMSO-d_6) δ 166.0, 163.6, 159.0, 153.3, 152.0, 137.9, 131.6, 129.4, 128.8, 128.0, 127.3, 125.9, 124.1, 120.9, 115.0, 111.7, 107.8, 107.6, 66.1, 47.1, 28.5, 20.7; HRMS-ESI $C_{27}H_{25}NO_6$ calcd [M+H] 460.1755, found 460.1759.

4-(3-(isobutylcarbamoyl)benzofuran-2-yl)benzyl-3',5'-dimethoxybenzoate (**Ia-6**)

Compound **Ia-6** was synthesized, following a similar procedure as the preparation of **Ia-1** starting from compound **IIa** and 3,5-dimethoxybenzoic acid to obtain the product as white solid (54.5 % yield); m.p. 181-182 ^{0}C. ^{1}H NMR (DMSO-d_6) δ

8.56 (t, *J* = 5.80 Hz, 1H), 7.90 (d, *J* = 8.60 Hz, 2H), 7.65 (d, *J* = 8.20 Hz, 1H), 7.55 - 7.60 (m, 3H), 7.35 - 7.40 (m, 1H), 7.29 - 7.34 (m, 1H), 7.09 (s, 2H), 6.77 (s, 1H), 5.38 (s, 2H), 3.77 (s, 6H), 3.11 (t, *J* = 6.4 Hz, 2H), 1.83 (m, 1H), 0.88 (d, *J* = 6.65 Hz, 6H); ^{13}C NMR (DMSO-d_6) δ 165.7, 163.6, 161.0, 153.3, 152.0, 137.8, 131.9, 129.4, 128.7, 128.0, 127.3, 125.9, 124.0, 120.9, 115.0, 111.7, 107.3, 105.8, 66.3, 56.0, 47.1, 28.4, 20.7; HRMS-ESI $C_{29}H_{29}NO_6$ calcd [M+H] 488.2068, found 488.2060.

4-(3-(isobutylcarbamoyl) benzofuran-2-yl) benzyl-3', 4'-dihydroxybenzoate (**Ia-7**)

Compound **Ia-7** was synthesized, following a similar procedure as the preparation of **Ia-1** starting from compound **IIa** and 3,4-dihydroxybenzoic acid to obtain the product as white solid (34 % yield); m.p. 180-182 ^{0}C. ^{1}H NMR (DMSO-d_6) δ 9.81 (s, 1H), 9.38 (s, 1H), 8.59 (t, *J* = 5.80 Hz, 1H), 7.87 - 7.93 (m, 2H), 7.65 (d, *J* = 7.80 Hz, 1H), 7.52 - 7.60 (m, 3H), 7.29 - 7.40 (m, 4H), 6.80 (d, *J* = 8.20 Hz, 1H), 5.30 (s, 2H), 3.12 (t, *J* = 6.4 Hz, 2H), 1.83 (m, 1H), 0.89 (d, *J* = 6.60 Hz, 6H); ^{13}C NMR (DMSO-d_6) δ 165.9, 163.6, 153.3, 152.0, 151.1, 145.6, 138.3, 129.3, 128.7, 128.0, 127.2, 125.9, 124.1, 122.4, 120.9, 120.7, 116.7, 115.8, 114.9, 111.7, 65.6, 47.1, 28.5, 20.7; HRMS-ESI $C_{27}H_{25}NO_6$ calcd [M+H] 460.1755, found 460.1749.

4-(3-(isobutylcarbamoyl) benzofuran-2-yl) benzyl-3',4',5'-trimethoxybenzoate (**Ia-8**)

Compound **Ia-8** was synthesized, following a similar procedure as the preparation of **Ia-1** starting from compound **IIa** and 3,4,5-trimethoxybenzoic acid to obtain the product as white solid (28.9 % yield); m.p. 190-192 ^{0}C. ^{1}H NMR (DMSO-d_6) δ 8.57 (t, *J* = 5.8 Hz, 1H), 7.90 (d, *J* = 8.20 Hz, 2H), 7.65 (d, *J* = 7.80 Hz, 1H), 7.54 - 7.61 (m, 3H), 7.35 - 7.40 (m, 1H), 7.29 - 7.34 (m, 1H), 7.27 (s, 2H), 5.39 (s, 2H), 3.81 (s, 6H), 3.71 (s, 3H), 3.11 (t, *J* = 6.26 Hz, 2H), 1.82 (m, 1H), 0.88 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR (DMSO-d_6) δ 165.6, 163.6, 153.5, 153.3, 152.0, 142.4, 138.0, 129.3, 128.6, 128.0, 127.3, 125.9, 125.0, 124.1, 120.9, 115.0, 111.7, 107.1, 66.2, 60.6, 56.5, 47.1, 28.4, 20.7;

HRMS-ESI $C_{30}H_{31}NO_7$ calcd [M+H] 518.2173, found 518.2167.

4-(3-(isobutylcarbamoyl) benzofuran-2-yl) benzyl-4'-dimethylaminobenzoate (**Ia-9**)

Compound **Ia-9** was synthesized, following a similar procedure as the one used for preparation of **Ia-1** starting from compound **IIa** and 4-dimethylaminobenzoic acid to obtain the pure product as white solid (65 % yield); m.p. 180-183 ^{0}C. ^{1}H NMR (DMSO-d_6) δ 8.57 (br. s., 1H), 7.89 (d, *J* = 7.80 Hz, 2H), 7.77 - 7.83 (m, 2H), 7.65 (d, *J* = 7.80 Hz, 1H), 7.52 - 7.61 (m, 3H), 7.37 (t, *J* = 7.40 Hz, 1H), 7.32 (t, *J* = 7.20 Hz, 1H), 6.67 - 6.74 (m, 2H), 5.30 (s, 2H), 3.12 (t, *J* = 6.0 Hz, 2H), 2.97 (s, 6H), 1.83 (m, 1H), 0.89 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR (DMSO-d_6) δ 166.1, 163.6, 153.8, 153.3, 152.1, 138.6, 131.4, 129.2, 128.5, 128.1, 127.2, 125.8, 124.0, 120.9, 115.9, 114.9, 111.7, 111.3, 110.0, 65.2, 47.1, 28.5, 20.7; HRMS-ESI $C_{27}H_{25}NO_5$ calcd [M+H] 471.2206, found 471.2249.

4-(3-(isobutylcarbamoyl) benzofuran-2-yl) benzyl-2', 4'-dihydroxybenzoate (**Ia-10**)

Compound **Ia-10** was synthesized, following a similar procedure as the preparation of **Ia-1** starting from compound **IIa** and 2,4-dihydroxylbenzoic acid to obtain the product as white solid (27 % yield); m.p. 173-175 ^{0}C. ^{1}H NMR (DMSO-d_6) δ 10.64 (s, 1H), 10.48 (s, 1H), 8.58 (t, *J* = 5.8 Hz, 1H), 7.90 (d, *J* = 8.20 Hz, 2H), 7.65 (d, *J* = 8.20 Hz, 1H), 7.68 (d, *J* = 9.00 Hz, 1H), 7.54-7.60 (m, 3H), 7.37 (t, *J* = 7.40 Hz, 1H), 7.32 (t, *J* = 7.20 Hz, 1H), 6.36 (d, 8.80 Hz, 1H), 6.29 (s, 1H), 5.37 (s, 2H), 3.11 (t, *J* = 6.4 Hz, 2H), 1.83 (m, 1H), 0.88 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR (DMSO-d_6) δ 169.2, 164.8, 163.6, 163.3, 153.3, 152.0, 137.6, 132.2, 129.4, 128.8, 128.0, 127.3, 125.9, 124.1, 120.9, 115.0, 111.7, 108.9, 104.4, 103.0, 66.0, 47.1, 28.5, 20.7; HRMS-ESI $C_{27}H_{25}NO_6$ calcd [M+H] 460.1755, found 460.1755.

4-(3-(isobutylcarbamoyl) benzofuran-2-yl) benzyl-2'-nitrobenzoate (**Ia-11**)

Compound **Ia-11** was synthesized, following a similar procedure as the preparation of **Ia-1** starting from

compound **IIa** and 2-nitrobenzoic acid to obtain the product as yellow solid (55 % yield); m.p. 173-175 ^{0}C. ^{1}H NMR (DMSO-d_6) δ 8.59 (t, *J* = 5.4 Hz, 1H), 8.06 (d, 8.20 Hz, 2H), 7.90 (d, *J* = 7.80 Hz, 2H), 7.65 (d, *J* = 7.80 Hz, 1H), 7.58 (d, *J* = 8.20 Hz, 3H), 7.29 - 7.41 (m, 4H), 5.38 (s, 2H), 3.11 (t, *J* = 6.4 Hz, 2H), 1.82 (m, 1H), 0.88 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR (DMSO-d_6) δ 166.9, 165.1, 164.4, 163.6, 153.3, 152.0, 137.8, 132.7, 132.6, 129.4, 128.8, 128.0, 127.2, 126.5, 125.9, 124.1, 120.9, 116.5, 116.3, 115.0, 111.7, 66.3, 47.1, 28.5, 20.7; HRMS-ESI $C_{27}H_{24}FNO_4$ calcd [M+H] 473.1634, found 473.1677

4-(3-(isobutylcarbamoyl) benzofuran-2-yl) benzyl-4'-fluorobenzoate (**Ia-12**)

Compound **Ia-12** was synthesized, following a similar procedure as the preparation of **Ia-1** starting from compound **IIa** and 4-fluorobenzoic acid to obtain the product as white solid (55 % yield); m.p. 173-175 ^{0}C. ^{1}H NMR (DMSO-d_6) δ 8.59 (t, *J* = 5.4 Hz, 1H), 8.06 (d, *J* = 8.20 Hz, 2H), 7.90 (d, *J* = 7.80 Hz, 2H), 7.65 (d, *J* = 7.80 Hz, 1H), 7.58 (d, *J* = 8.2 Hz, 3H), 7.29 - 7.41 (m, 4H), 5.38 (s, 2H), 3.11 (t, *J* = 6.4 Hz, 2H), 1.82 (m, 1H), 0.88 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR (DMSO-d_6) δ 166.9, 165.1, 164.4, 163.6, 153.3, 152.0, 137.8, 132.6 (d, J_{C-F} = 9), 129.4, 128.8, 128.0, 127.2, 126.5, 125.9, 124.1, 120.9, 116.5, 116.3, 115.0, 111.7, 66.3, 47.1, 28.5, 20.7; HRMS-ESI $C_{27}H_{24}FNO_4$ calcd [M+H] 446.1762, found 446.1768.

4-(3-(isobutylcarbamoyl) benzofuran-2-yl) benzyl-4'-thiolbenzoate (**Ia-13**)

Compound **Ia-13** was synthesized, following a similar procedure as the preparation of **Ia-1** starting from compound **IIa** and 4-thiolbenzoic acid to obtain the product as white solid (18 % yield); m.p. 179-181 ^{0}C. ^{1}H NMR (DMSO-d_6) δ 8.58 (t, *J* = 5.8 Hz, 1H), 7.71 - 7.86 (m, 4H), 7.62 (d, *J* = 7.8 Hz, 1H), 7.55 (d, *J* = 7.4 Hz, 1H), 7.47 (d, *J* = 8.2 Hz, 2H), 7.27 - 7.38 (m, 2H), 7.23 (d, *J* = 7.8 Hz, 2H), 4.28 (s, 2H), 3.09 (br. s., 2H), 1.81 (m, 1H), 0.87 (d, *J* = 6.26 Hz, 6H); ^{13}C NMR (DMSO-d_6) δ 170.0, 163.7, 153.2, 152.1, 139.6, 137.9, 137.1, 130.1, 129.7, 128.4, 128.1, 127.3, 127.1, 126.5, 125.8, 124.0, 120.8, 114.6, 111.6, 110.0, 66.8, 47.1, 28.5, 20.7;

HRMS-ESI $C_{27}H_{25}NO_4S$ calcd [M+H] 460.1577, found 460.1568.

4-(3-(isobutylcarbamoyl)benzofuran-2-yl)benzyl-3'-hydroxy-4'-nitrobenzoate (**Ia-14**)

Compound **Ia-14** was synthesized, following a similar procedure as the preparation of **Ia-1** starting from compound **IIa** and 3-hydroxy-4-nitrobenzoic acid to obtain the product as white solid (38 % yield); m.p. 199-201 ^{0}C. ^{1}H NMR (DMSO-d_6) δ 8.60 (t, *J* = 5.6 Hz, 1H), 7.87-7.97 (m, 3H), 7.68-7.72 (m, 1H), 7.66 (d, *J* = 8.2 Hz, 1H), 7.55-7.62 (m, 3H), 7.48-7.53 (m, 1H), 7.38 (t, *J* = 7.8 Hz, 1H), 7.32 (t, *J* = 7.2 Hz, 1H), 5.39 (s, 2H), 3.12 (t, *J* = 6.4 Hz, 2H), 1.83 (m, 1H), 0.89 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR (DMSO-d_6) δ 165.5, 163.6, 163.1, 153.3, 152.0, 151.7, 138.3, 138.1, 129.2, 128.6, 128.1, 127.2, 125.8, 124.1, 120.9, 114.9, 113.4, 111.7, 107.6, 65.3, 47.1, 28.5, 20.7; HRMS-ESI $C_{27}H_{24}N_2O_7$ calcd [M+H] 489.1656, found 489.1661.

4-(3-(isobutylcarbamoyl) benzofuran-2-yl) benzyl-4'-aminobenzoate (**Ia-15**)

Compound **Ia-15** was synthesized, following a similar procedure as the preparation of **Ia-1** starting from compound **IIa** and 4-aminobenzoic acid to obtain the product as white solid (45 % yield); m.p. 221-223 ^{0}C. ^{1}H NMR (DMSO-d_6) δ 10.34 (s, 2H), 8.57 (t, *J* = 5.6 Hz, 1H), 7.89 (d, *J* = 8.2 Hz, 2H), 7.84 (d, *J* = 9.00 Hz, 2H), 7.65 (d, *J* = 7.8 Hz, 1H), 7.54-7.59 (m, 3H), 7.35-7.38 (m, 1H), 7.31-7.34 (m, 1H), 6.84 (d, *J* = 8.6 Hz, 2H), 5.32 (s, 2H), 3.11 (t, *J* = 6.4 Hz, 2H), 1.82 (m, 1H), 0.88 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR (DMSO-d_6) δ 165.5, 163.6, 163.1, 153.3, 152.0, 151.7, 138.3, 138.1, 129.2, 128.6, 128.1, 127.2, 125.8, 124.1, 120.9, 114.9, 113.4, 111.7, 107.6, 65.3, 47.1, 28.5, 20.7; HRMS-ESI $C_{27}H_{26}N_2O_4$ calcd [M+H] 443.1965, found 443.1953.

4-(3-(isobutylcarbamoyl)benzofuran-2-yl)benzyl-2'-(2-hydroxyphenyl)acetate (**Ia-16**)

Compound **Ia-16** was synthesized, following a similar procedure as the preparation of **Ia-1** starting from compound **IIa** and 2-(2-hydroxyphenyl)acetic acid to obtain the product as white solid (42.4 % yield);

m.p. 179-181 ^{0}C. ^{1}H NMR ($CDCl_3$) δ 7.87 (d, *J* = 8.2 Hz, 2H), 7.77 (d, *J* = 7.4 Hz, 1H), 7.49 (d, *J* = 7.8 Hz, 1H), 7.40 (d, *J* = 8.2 Hz, 2H), 7.26 - 7.36 (m, 2H), 7.15 (t, *J* = 7.6 Hz, 1H), 7.10 (d, *J* = 7.4 Hz, 1H), 6.81 - 6.94 (m, 2H), 6.01 (br. s., 1H), 5.17 (s, 2H), 3.71 (s, 2H), 3.27 (t, *J* = 6.4 Hz, 2H), 1.84 (m, 1H), 0.92 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 170.7, 161.5, 152.5, 151.5, 151.1, 134.4, 128.5, 126.8, 126.5, 125.8, 125.7, 124.8, 122.8, 121.2, 118.1, 118.0, 114.5, 110.6, 108.8, 64.1, 44.6, 34.9, 25.9, 17.7; HRMS-ESI $C_{28}H_{27}NO_5$ calcd [M+H] 458.1962, found 458.1954.

4-(3-(isobutylcarbamoyl)benzofuran-2-yl)benzyl-2'-(3-hydroxyphenyl)acetate (**Ia-17**)

Compound **Ia-17** was synthesized, following a similar procedure as the preparation of **Ia-1** starting from compound **IIa** and 2-(3-hydroxyphenyl)acetic acid to obtain the product as white solid (39.4 % yield); m.p. 197-199 ^{0}C. ^{1}H NMR (DMSO-d_6) δ 9.40 (br. s., 1H), 8.59 (t, *J* = 5.6 Hz, 1H), 7.80-7.90 (m, 2H), 7.65 (d, *J* = 8.2 Hz, 1H), 7.58 (d, *J* = 7.8 Hz, 1H), 7.41-7.48 (m, 2H), 7.37 (t, *J* = 7.6 Hz, 1H), 7.32 (t, *J* = 7.2 Hz, 1H), 7.08 (t, *J* = 7.8 Hz, 1H), 6.57 - 6.74 (m, 3H), 5.14 (s, 2H), 3.63 (s, 2H), 3.12 (t, *J* = 6.4 Hz, 2H), 1.83 (m, 1H), 0.89 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR (DMSO-d_6) δ 171.4, 163.6, 157.8, 153.3, 151.9, 137.9, 135.8, 129.7, 129.2, 128.5, 128.0, 127.1, 125.9, 124.1, 120.9, 120.3, 116.7, 114.9, 114.3, 111.7, 65.7, 47.1, 40.8, 28.5, 20.7; HRMS-ESI $C_{28}H_{27}NO_5$ calcd [M+H] 458.1962, found 458.1959.

4-(3-(isobutylcarbamoyl)benzofuran-2-yl)benzyl-2'-(4-hydroxyphenyl)acetate (**Ia-18**)

Compound **Ia-18** was synthesized, following a similar procedure as the preparation of **Ia-1** starting from compound **IIa** and 2-(4-hydroxyphenyl)acetic acid to obtain the product as white solid (33.0 % yield); m.p. 212-214 ^{0}C. ^{1}H NMR (DMSO-d_6) δ 9.28 (s, 1H), 8.55 (t, *J* = 5.8 Hz, 1H), 7.81-7.89 (m, 2H), 7.65 (d, *J* = 8.2 Hz, 1H), 7.55-7.61 (m, 1H), 7.40-7.46 (m, 2H), 7.35-7.40 (m, 1H), 7.28-7.34 (m, 1H), 7.02-7.08 (m, 2H), 6.65-6.72 (m, 2H), 5.13 (s, 2H), 3.59 (s, 2H), 3.12 (t, *J* = 6.4 Hz, 2H), 1.83 (m, 1H), 0.90 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR (DMSO-d_6) δ 171.4, 163.6, 157.8, 155.3,

151.9, 137.9, 135.8, 129.7, 129.2, 128.5, 128.0, 127.1, 125.9, 124.1, 120.9, 120.3, 116.7, 112.9, 114.3, 111.7, 65.5, 47.1, 40.8, 28.5, 20.7; HRMS-ESI $C_{28}H_{27}NO_5$ calcd [M+H] 458.1962, found 458.1960.

4-(3-(isobutylcarbamoyl)benzofuran-2-yl)benzyl-2'-(4-nitrophenyl)acetate (**Ia-19**)

Compound **Ia-19** was synthesized, following a similar procedure as the preparation of **Ia-1** starting from compound **IIa** and 2-(4-nitrophenyl)acetic acid to obtain the product as white solid (42.0 % yield); m.p. 233-235 ^{0}C. ^{1}H NMR (DMSO-d_6) δ 8.56 (t, *J* = 5.6 Hz, 1H), 8.13-8.22 (m, 2H), 7.83-7.89 (m, 2H), 7.65 (d, *J* = 8.2 Hz, 1H), 7.57 (d, *J* = 9.00 Hz, 3H), 7.42-7.48 (m, 2H), 7.38 (m, 1H), 7.29-7.35 (m, 1H), 5.17 (s, 2H), 3.97 (s, 2H), 3.11 (t, *J* = 6.4 Hz, 2H), 1.83 (m, 1H), 0.89 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR (DMSO-d_6) δ 170.6, 163.6, 160.6, 153.3, 151.9, 147.0, 142.7, 137.6, 131.4, 129.3, 128.6, 128.0, 127.1, 125.9, 124.1, 123.8, 120.9, 115.0, 111.7, 66.0, 47.1, 40.6, 28.5, 20.7; HRMS-ESI $C_{28}H_{26}N_2O_6$ calcd [M+H] 487.1864, found 487.1862.

4-(3-(isobutylcarbamoyl)benzofuran-2-yl)benzyl-2'-(3-trifloromethyl-phenyl)acetate (**Ia-20**)

Compound **Ia-20** was synthesized, following a similar procedure as the preparation of **Ia-1** starting from compound **IIa** and 2-(3-trifloromethyl-phenyl)acetic acid to obtain the product as white solid (61.5 % yield); m.p. 211-213 ^{0}C. ^{1}H NMR ($CDCl_3$) δ 7.86-7.96 (m, 2H), 7.78 (d, *J* = 7.4 Hz, 1H), 7.43-7.57 (m, 5H), 7.36-7.41 (m, 2H), 7.26-7.36 (m, 2H), 5.99 (br. s., 1H), 5.17 (s, 2H), 3.74 (s, 2H), 3.27 (t, *J* = 6.4 Hz, 2H), 1.83 (m, 1H), 0.92 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 170.5, 164.0, 153.8, 153.7, 137.1, 134.5, 132.8, 129.4, 129.1, 128.3, 128.2, 127.5, 126.2, 126.1, 125.4, 124.1 (q, J_{C-F}=365), 123.8, 120.8, 113.4, 111.5, 111.3, 110.0, 66.3, 47.1, 40.9, 28.5, 20.2; HRMS-ESI $C_{29}H_{26}F_3NO_4$ calcd [M+H] 510.1887, found 510.1876.

4-(3-(isobutylcarbamoyl)benzofuran-2-yl)benzyl-2'-(4-trifloromethyl-phenyl)acetate (**Ia-21**)

Compound **Ia-21** was synthesized, following a similar procedure as the preparation of **Ia-1** starting from

compound **IIa** and 2-(4-trifloromethyl-phenyl)acetic acid to obtain the product as white solid (56.2 % yield); m.p. 213-215 ^{0}C.^{1}H NMR ($CDCl_3$) δ 7.92 (d, *J* = 8.2 Hz, 2H), 7.79 (d, *J* = 7.4 Hz, 1H), 7.58 (d, *J* = 7.8 Hz, 2H), 7.51 (d, *J* = 7.8 Hz, 1H), 7.26 - 7.45 (m, 6H), 5.96 (br. s., 1H), 5.17 (s, 2H), 3.74 (s, 2H), 3.28 (t, *J* = 6.4 Hz, 2H), 1.84 (m, 1H), 0.92 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 170.5, 164.0, 153.8, 153.7, 137.1, 133.6, 129.9, 129.7, 129.5, 128.4, 128.3, 127.5, 125.5(q, J_{C-F}=370), 123.8, 120.7, 113.4, 111.3, 66.3, 47.2, 41.0, 28.5, 20.2; HRMS-ESI $C_{29}H_{26}F_3NO_4$ calcd [M+H] 510.1887, found 510.1877.

4-(3-(isobutylcarbamoyl)benzofuran-2-yl)benzyl-2'-(3,4-dihydroxy-phenyl)acetate (**Ia-22**)

Compound **Ia-22** was synthesized, following a similar procedure as the preparation of **Ia-1** starting from compound **IIa** and 2-(3,4-dihydroxy-phenyl)acetic acid to obtain the product as white solid (23.2 % yield); m.p. 232-234 ^{0}C. ^{1}H NMR (DMSO-d_6) δ 8.87 (s, 1H), 8.78 (s, 1H), 8.60 (t, *J* = 5.6 Hz, 1H), 7.81-7.89 (m, 2H), 7.66 (d, *J* = 8.2 Hz, 1H), 7.58 (d, *J* = 7.4 Hz, 1H), 7.40-7.47 (m, 2H), 7.37 (t, *J* = 7.4 Hz, 1H), 7.32 (t, *J* = 7.4 Hz, 1H), 6.66 (br. s., 1H), 6.64 (d, *J* = 8.2 Hz, 1H), 6.50 (d, *J* = 7.8 Hz, 1H), 5.12 (s, 2H), 3.52 (s, 2H), 3.12 (t, *J* = 6.0 Hz, 2H), 1.83 (m, 1H), 0.89 (d, *J* = 6.6 Hz, 6H);^{13}C NMR (DMSO-d_6) δ 171.8, 163.6, 153.3, 151.9, 145.5, 144.7, 138.0, 129.2, 128.5, 128.1, 127.1, 125.9, 125.2, 124.1, 120.9, 120.5, 117.1, 115.9, 114.9, 111.7, 65.5, 47.1, 40.2, 28.5, 20.7; HRMS-ESI $C_{28}H_{27}NO_6$ calcd [M+H] 474.1911, found 474.1912.

(*E*)-4-(3-(isobutylcarbamoyl)benzofuran-2-yl)benzyl-3'-(4-hydroxyphenyl) acrylate (**Ia-23**)

Compound **Ia-23** was synthesized, following a similar procedure as the preparation of **Ia-1** starting from compound **IIa** and 2-(4-hydroxyphenyl)acetic acid to obtain the product as white solid (37.0 % yield); m. p. 173-175 ^{0}C. ^{1}H NMR (DMSO-d_6) δ 10.02 (s, 1H), 8.60 (t, *J* = 5.8 Hz, 1H), 7.85-7.91 (m, 2H), 7.60-7.69 (m, 2H), 7.57 (d, *J* = 8.6 Hz, 2H), 7.54 (d, *J* = 2.35 Hz, 2H), 7.51 (s, 1H), 7.37 (t, *J* = 7.24 Hz, 1H), 7.32 (t, *J* = 7.4 Hz, 1H),

6.74-6.79 (m, 2H), 6.46 (d, *J* = 15.65 Hz, 1H), 5.23 (s, 2H), 3.11 (t, *J* = 6.4 Hz, 2H), 1.83 (m, 1H), 0.89 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR (DMSO-d_6) δ 166.9, 163.6, 160.4, 153.3, 152.0, 145.7, 138.2, 130.9, 129.2, 128.8, 128.0, 127.2, 125.9, 125.5, 124.1, 120.9, 116.2, 114.9, 114.2, 111.7, 65.3, 47.1, 28.5, 20.7; HRMS-ESI $C_{29}H_{27}NO_5$ calcd [M+H] 470.1962, found 470.1955.

4-(3-(butylcarbamoyl)benzofuran-2-yl)benzyl-4'-hydroxybenzoate (**Ia-24**)

Compound **Ia-24** was synthesized, following a similar procedure as the preparation of **Ia-1** starting from compound **IIb** and 4-hydroxybenzoic acid to obtain the product as white solid (49.6 % yield); m.p. 158-160 ^{0}C. ^{1}H NMR (DMSO-d_6) δ 10.37 (br. s., 1H), 8.55 (t, *J* = 5.6 Hz, 1H), 7.85 (d, *J* = 9.00 Hz, 2H), 7.88 (d, *J* = 8.2 Hz, 2H), 7.65 (d, *J* = 7.8 Hz, 1H), 7.53 - 7.61 (m, 3H), 7.34-7.40 (m, 1H), 7.28-7.34 (m, 1H), 6.84 (d, *J* = 9.00 Hz, 2H), 5.33 (s, 2H), 3.24-3.31 (m, 2H), 1.49 (m, 2H), 1.31 (m, 2H), 0.87 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (DMSO-d_6) δ 165.8, 163.4, 162.9, 153.3, 152.1, 138.2, 132.0, 129.2, 128.9, 128.0, 127.2, 125.9, 124.0, 120.9, 120.5, 116.7, 115.8, 114.8, 111.7, 65.9, 39.1, 31.4, 20.1, 14.1; HRMS-ESI $C_{27}H_{25}NO_5$ calcd [M+H] 444.1805, found 444.1807.

4-(3-(butylcarbamoyl)benzofuran-2-yl)benzyl-3',4'-dihydroxybenzoate (**Ia-25**)

Compound **Ia-25** was synthesized, following a similar procedure as the preparation of **Ia-1** starting from compound **IIb** and 3,4-dihydroxybenzoic acid to obtain the product as white solid (32.0 % yield); m.p. 187-189 ^{0}C. ^{1}H NMR (DMSO-d_6) δ 9.83 (br. s., 1H), 9.39 (br. s., 1H), 8.55 (t, *J* = 5.2 Hz, 1H), 7.89 (d, *J* = 7.8 Hz, 2H), 7.65 (d, *J* = 8.2 Hz, 1H), 7.52-7.61 (m, 3H), 7.28-7.41 (m, 4H), 6.80 (d, *J* = 8.2 Hz, 1H), 5.30 (s, 2H), 3.28 (m, 2H), 1.50 (m, 2H), 1.31 (m, 2H), 0.87 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (DMSO-d_6) δ 165.9, 163.4, 153.3, 152.1, 151.1, 145.6, 138.3, 129.2, 128.6, 128.0, 127.2, 125.9, 124.1, 122.4, 120.9, 120.7, 116.7, 115.8, 114.8, 111.7, 65.6, 39.1, 31.4, 20.1, 14.1; HRMS-ESI $C_{27}H_{25}NO_6$ calcd [M+H] 460.1755, found 460.1754.

2-(4-((3-hydroxyphenoxy)methyl)phenyl)-*N*-isobutylbenzofuran-3-carbox

amide (**Ib-26**)

To a solution of compound **IIa** (250 mg, 0.62 mmol) in CH_3CN (10 mL), 1, 2-dihydroxybenzene (68.2 mg, 0.62 mmol) and anhydrous K_2CO_3 (171.12 mg, 1.24 mmol) were added and refluxed overnight before cooled to room temperature. H_2O (10 mL) was then added. The resulting solid was filtered, washed with ethyl acetate, and recrystallized in MeOH/H_2O to yield compound **Ib-26** as yellow solid (210 mg, 42.5 % yield). m.p. 201-203 ^{0}C. ^{1}H NMR (DMSO-d_6) δ 9.39 (s, 1H), 8.59 (br. s., 1H), 7.89 (d, *J* = 7.8 Hz, 2H), 7.65 (d, *J* = 7.8 Hz, 1H), 7.49 - 7.61 (m, 3H), 7.28 - 7.42 (m, 2H), 7.03 (t, *J* = 7.8 Hz, 1H), 6.30 - 6.48 (m, 3H), 5.08 (br. s., 2H), 3.12 (br. s., 2H), 1.83 (m, 1H), 0.89 (d, *J* = 6.4 Hz, 6H); ^{13}C NMR (DMSO-d_6) δ 170.0, 163.7, 153.2, 152.1, 139.6, 137.9, 137.1, 130.1, 129.7, 128.4, 128.1, 127.3, 127.1, 125.8, 124.0, 120.8, 114.6, 111.6, 47.1, 36.5, 28.5, 20.7; HRMS-ESI $C_{26}H_{25}NO_4$ calcd [M+H] 416.1856, found 416.1832.

2-(4-((4-tolyloxy)methyl)phenyl)-*N*-isob utyl benzofuran-3-carboxamide (**Ib-27**)

Compound **Ib-27** was synthesized, following a similar procedure as the preparation of **Ib-26** starting from compound **IIa** and 4-methylphenol to obtain the product as white solid (82.3 % yield); m.p. 176-179 ^{0}C. ^{1}H NMR (DMSO-d_6) δ 8.60 (t, *J* = 5.8 Hz, 1H), 7.88 (d, *J* = 8.0 Hz, 2H), 7.65 (d, *J* = 8.4 Hz, 1H), 7.50- 7.60 (m, 3H), 7.35-7.40 (m, 1H), 7.29-7.34 (m, 1H), 7.02-7.10 (m, 2H), 6.85-6.92 (m, 2H), 5.10 (s, 2H), 3.12 (t, *J* = 6.4 Hz, 2H), 2.19 (s, 3H), 1.83 (m, 1H), 0.89 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (DMSO-d_6) δ 163.7, 156.5, 153.3, 152.1, 139.1, 130.3, 129.9, 129.0, 128.3, 128.1, 127.1, 125.8, 124.0, 120.9, 115.1, 114.8, 111.7, 69.1, 47.1, 28.5, 20.7, 20.5; HRMS-ESI $C_{27}H_{27}NO_3$ calcd [M+H] 414.2064, found 414.2034.

2-(4-((4-chlorophenoxy)methyl)phenyl)-*N*-isobutylbenzofuran-3-carboxamide(**Ib -28**)

Compound **Ib-28** was synthesized, following a similar procedure as the preparation of **Ib-26** starting from compound **IIa** and 4-chlorophenol to obtain the product as white solid (76.6 % yield); m.p. 183-185 ^{0}C.^{1}H NMR (DMSO-d_6) δ 8.59 (t, *J* = 5.8 Hz, 1H), 7.86-7.92 (m, 2H), 7.65 (d, *J* = 8.4 Hz,

1H), 7.58 (d, *J* = 8.0 Hz, 1H), 7.588-7.533 (d, *J* = 8.2 Hz, 2H), 7.35-7.40 (m, 1H), 7.28-7.35 (m, 3H), 7.00-7.05 (m, 2H), 5.14 (s, 2H), 3.11 (t, *J* = 6.4 Hz, 2H), 1.82 (m, 1H), 0.88 (d, *J* = 6.4 Hz, 6H); ^{13}C NMR (DMSO-d_6) δ 163.6, 157.5, 153.3, 152.0, 138.5, 129.7, 129.6, 129.2, 128.4, 128.1, 127.2, 125.8, 125.0, 124.1, 120.9, 117.3, 117.0, 114.9, 111.7, 110.0, 69.4, 47.1, 28.5, 20.7; HRMS-ESI $C_{26}H_{24}ClNO_3$ calcd [M+H] 434.1517, found 434.1536.

2-(4-((4-(hydroxymethyl)phenoxy) methyl)phenyl)-*N*-isobutylbenzofuran-3-carboxamide (**Ib-29**)

Compound **Ib-29** was synthesized, following a similar procedure as the preparation of **Ib-26** starting from compound **IIa** and 4-hydroxymethylphenol to obtain the product as white solid (33.4 % yield); m.p. 203-205 ^{0}C. ^{1}H NMR (DMSO-d_6) δ 8.60 (t, *J* = 5.8 Hz, 1H), 7.88 (d, *J* = 8.2 Hz, 2H), 7.65 (d, *J* = 7.8 Hz, 1H), 7.52 - 7.60 (m, 3H), 7.37 (m, 1H), 7.32 (m, 1H), 7.18 - 7.23 (m, 2H), 6.93 - 6.97 (m, 2H), 5.13 (s, 2H), 5.03 (t, *J* = 5.6 Hz, 1H), 4.38 (d, *J* = 5.4 Hz, 2H), 3.11 (t, *J* = 6.4 Hz, 2H), 1.83 (m, 1H), 0.89 (d, *J* = 6.4 Hz, 6H); ^{13}C NMR (DMSO-d_6) δ 163.7, 157.5, 153.3, 153.2, 152.1, 139.1, 135.3, 129.0, 128.4, 128.3, 128.2, 128.1, 127.1, 126.9, 125.8, 124.0, 120.9, 114.9, 114.8, 111.7, 69.1, 62.9, 47.1, 28.5, 20.7; HRMS-ESI $C_{27}H_{27}NO_4$ calcd [M+H] 430.2013, found 430.2045.

2-(4-((3-formylphenoxy)methyl) phenyl)-*N*-isobutylbenzofuran-3-carbox amide(**Ib-30**)

Compound **Ib-30** was synthesized, following a similar procedure as the preparation of **Ib-26** starting from compound **IIa** and 3-hydroxybenzaldehyde to obtain the product as white solid (42.1 % yield); m.p. 166-168 ^{0}C, ^{1}H NMR (DMSO-d_6) δ 9.95 (s, 1H), 8.60 (t, *J* = 5.6 Hz, 1H), 7.90 (d, *J* = 8.2 Hz, 2H), 7.65 (d, *J* = 8.2 Hz, 1H), 7.58 (d, *J* = 8.6 Hz, 3H), 7.49 - 7.54 (m, 3H), 7.31 - 7.39 (m, 3H), 5.24 (s, 2H), 3.11 (t, *J* = 6.4 Hz, 2H), 1.82 (m, 1H), 0.88 (d, *J* = 6.4 Hz, 6H); ^{13}C NMR (DMSO-d_6) δ 193.3, 163.6, 159.1, 153.3, 152.0, 138.4, 138.1, 130.9, 129.2, 128.4, 128.1, 127.2, 125.9, 124.1, 123.3, 122.1, 120.9, 114.9, 114.4, 111.7, 69.4, 47.1, 28.5, 20.7; HRMS-ESI $C_{27}H_{25}NO_4$ calcd [M+H] 428.1856, found 428.1831.

2-(4-((3-acetylphenoxy)methyl)phenyl)-*N*-isobutylbenzofuran-3-carboxamide (**Ib-31**)

Compound **Ib-31** was synthesized, following a similar procedure as the preparation of **Ib-26** starting from compound **IIa** and 3-acetylphenol to obtain the product as white solid (47.0 % yield); m.p. 168-170 ^{0}C. ^{1}H NMR (DMSO-d_6) δ 8.60 (t, *J* = 5.8 Hz, 1H), 7.90 (d, *J* = 8.6 Hz, 2H), 7.65 (d, *J* = 8.2 Hz, 1H), 7.58 (d, *J* = 8.6 Hz, 3H), 7.52 - 7.56 (m, 2H), 7.43 (t, *J* = 7.8 Hz, 1H), 7.35 - 7.40 (m, 1H), 7.33 (m, 1H), 7.26 - 7.31 (m, 1H), 5.23 (s, 2H), 3.11 (t, *J* = 6.4 Hz, 2H), 2.55 (s, 3H), 1.82 (m, 1H), 0.88 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (DMSO-d_6) δ 198.1, 163.6, 158.8, 153.3, 152.0, 138.7, 138.6, 130.4, 129.2, 128.4, 128.1, 127.2, 125.9, 124.1, 121.5, 120.9, 120.4, 114.9, 114.2, 111.7, 69.3, 47.1, 28.5, 27.3, 20.7, 47.1, 28.5, 20.7; HRMS-ESI $C_{28}H_{27}NO_4$ calcd [M+H] 442.2013, found 442.2045.

2-(4-((4-cyanophenoxy)methyl)phenyl)-*N*-isobutylbenzofuran-3-carboxamide (**Ib-32**)

Compound **Ib-32** was synthesized, following a similar procedure as the preparation of **Ib-26** starting from compound **IIa** and 4-cyanophenol to obtain the product as white solid (51.0 % yield); m.p. 176-178 ^{0}C. ^{1}H NMR (DMSO-d_6) δ 8.60 (t, *J* = 6.0 Hz, 1H), 7.905 - 7.884 (d, *J* = 8.4 Hz, 2H), 7.774 - 7.752 (d, *J* = 8.8 Hz, 2H), 7.662 - 7.642 (d, *J* = 8.0 Hz, 1H), 7.589 - 7.554 (m, 3H), 7.302 - 7.396 (m, 2H), 7.190 - 7.167 (d, *J* = 9.2 Hz, 1H), 5.251 (s, 2H), 3.11 (t, *J* = 6.4 Hz, 2H), 1.82 (m, 1H), 0.88 (d, *J* = 6.4 Hz, 6H); ^{13}C NMR (DMSO-d_6) δ 163.6, 162.1, 153.3, 152.0, 137.9, 134.7, 129.4, 128.6, 128.0, 127.2, 125.9, 124.1, 120.9, 119.5, 116.4, 115.0, 111.7, 103.6, 69.6, 47.1, 28.5, 20.7; HRMS-ESI $C_{27}H_{24}N_2O_3$ calcd [M+H] 425.1860, found 425.1879.

N-isobutyl-2-(4-((4-(methylsulfonyl) phenoxy)methyl)phenyl)benzofuran-3-carboxamide (**Ib-33**)

Compound **Ib-33** was synthesized, following a similar procedure as the preparation of **Ib-26** starting from compound **IIa** and 4-(methylsulfonyl)phenol to obtain the product as white solid (60.2 % yield); m.p. 203-205 ^{0}C. ^{1}H NMR (DMSO-d_6) δ

8.60 (t, *J* = 5.6 Hz, 1H), 7.911-7.891 (d, *J* = 8.0 Hz, 2H), 7.8430 - 7.8226 (d, *J* = 8.4 Hz 2H), 7.664-7.644 (d, *J* = 8.0 Hz, 1H), 7.588 - 7,569 (d, *J* = 7.6 Hz, 3H), 7.395 - 7.303 (m, 2H), 7.243 - 7.221 (d, *J* = 8.8 Hz, 2H), 5.27 (s, 2H), 3.131 - 3.110 (m, 5H), 1.83 (m, 1H), 0.89 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (DMSO-d_6) δ 163.6, 162.4, 153.3, 152.0, 138.0, 133.3, 129.7, 129.3, 128.5, 128.0, 127.2, 125.9, 124.1, 120.9, 115.8, 114.9, 111.7, 69.6, 47.1, 44.4, 28.5, 20.7; HRMS-ESI $C_{27}H_{27}NO_5S$ calcd [M+H] 478.1683, found 478.1661.

2-(4-((3-acetamidophenoxy)methyl) phenyl)-*N*-isobutylbenzofuran-3-carbox amide(**Ib-34**)

Compound **Ib-34** was synthesized, following a similar procedure as the preparation of **Ib-26** starting from compound **IIa** and 3-acetamidophenol to obtain the product as white solid (45.0 % yield); m.p. 166-168 ^{0}C. ^{1}H NMR (DMSO-d_6) δ 9.91 (br. s., 1H), 8.60 (t, *J* = 5.6 Hz, 1H), 7.89 (d, *J* = 8.6 Hz, 2H), 7.65 (d, *J* = 7.8 Hz, 1H), 7.52 - 7.60 (m, 3H), 7.29 - 7.40 (m, 3H), 7.17 (t, *J* = 8.2 Hz, 1H), 7.08 (d, *J* = 8.2 Hz, 1H), 6.68 (m, 1H), 5.10 (s, 2H), 3.12 (t, *J* = 6.4 Hz, 2H), 2.00 (s, 3H), 1.83 (m, 1H), 0.89 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR (DMSO-d_6) δ 168.8, 163.7, 158.9, 153.3, 152.1, 141.0, 138.9, 129.9, 129.1, 128.3, 128.1, 127.1, 125.8, 124.0, 120.9, 114.8, 112.0, 111.7, 110.0, 109.5, 106.2, 69.0, 47.1, 28.5, 24.5, 20.7; HRMS-ESI $C_{28}H_{28}N_2O_4$ calcd [M+H] 457.2122, found 457.2100.

2-(4-((4-acetamidophenoxy)methyl) phenyl)-*N*-isobutylbenzofuran-3-carbox amide(**Ib-35**)

Compound **Ib-35** was synthesized, following a similar procedure as the preparation of **Ib-26** starting from compound **IIa** and 4-acetamidophenol to obtain the product as white solid (49.0 % yield); m.p. 158-160 ^{0}C. ^{1}H NMR (DMSO-d_6) δ 9.90 (br. s., 1H), 8.60 (br. s., 1H), 7.89 (d, *J* = 8.2 Hz, 2H), 7.65 (d, *J* = 7.8 Hz, 1H), 7.49 - 7.61 (m, 3H), 7.28 - 7.41 (m, 3H), 7.16 (t, *J* = 8.0 Hz, 1H), 7.07 (d, *J* = 7.8 Hz, 1H), 6.68 (d, *J* = 7.4 Hz, 1H), 5.10 (s, 2H), 3.12 (t, *J* = 6.0 Hz, 2H), 2.00 (s, 3H), 1.83 (m, 1H), 0.89 (d, *J* = 6.4 Hz, 6H); ^{13}C NMR (DMSO-d_6) δ 168.8, 163.7, 158.9, 153.3, 152.1, 141.0, 138.9, 129.9, 129.1, 128.3, 128.1, 127.2, 125.8, 124.0, 120.9, 114.8, 112.0, 111.7, 109.5, 106.2, 69.0, 47.1,

28.5, 24.5, 20.7; HRMS-ESI $C_{28}H_{28}N_2O_4$ calcd [M+H] 457.2122, found 457.2178.

N-butyl-2-(4-((4-(methoxycarbonyl) phenoxy)methyl)phenyl)benzofuran-3-carboxamide (**Ib-36**)

Compound **Ib-36** was synthesized, following a similar procedure as the preparation of **Ib-26** starting from compound **IIb** and 4-(methoxycarbonyl)phenol to obtain the product as white solid (37.0 % yield); m.p. 168-170 ^{0}C.^{1}H NMR (DMSO-d_6) δ 8.60 (t, *J* = 5.4 Hz, 1H), 7.90 (d, *J* = 8.2 Hz, 4H), 7.65 (d, *J* = 7.8 Hz, 1H), 7.53 - 7.61 (m, 3H), 7.28 - 7.42 (m, 2H), 7.12 (d, *J* = 8.6 Hz, 2H), 5.23 (s, 2H), 3.78 (s, 3H), 3.12 (t, *J* = 6.4 Hz, 2H), 1.83 (m, 1H), 0.88 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR (DMSO-d_6) δ 166.3, 163.6, 162.5, 153.3, 152.0, 138.2, 131.7, 129.3, 128.5, 128.1, 127.2, 125.9, 124.1, 122.6, 120.9, 115.3, 114.9, 111.7, 110.0, 69.5, 52.3, 47.1, 28.5, 20.7; HRMS-ESI $C_{28}H_{27}NO_5$ calcd [M+H] 458.1962, found 458.1921.

N-butyl-2-(4-((4-(methylsulfonyl)phenoxy)methyl)phenyl)benzofuran-3-carboxamide (**Ib-37**)

Compound **Ib-37** was synthesized, following a similar procedure as the preparation of **Ib-26** starting from compound **IIb** and 4-(methylsulfonyl)phenol to obtain the product as white solid (61.0 % yield); m.p. 234-236 ^{0}C. ^{1}H NMR (DMSO-d_6) δ 8.56 (t, *J* = 5.6 Hz, 1H), 7.90 (d, *J* = 8.2 Hz, 2H), 7.81 - 7.86 (m, 2H), 7.65 (d, *J* = 7.8 Hz, 1H), 7.58 (d, *J* = 8.2 Hz, 3H), 7.34 - 7.40 (m, 1H), 7.29 - 7.34 (m, 1H), 7.20 - 7.26 (m, 2H), 5.28 (s, 2H), 3.28 (m, 2H), 3.13 (s, 3H), 1.50 (m, 2H), 1.26 - 1.38 (m, 2H), 0.88 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (DMSO-d_6) δ 163.4, 162.4, 153.3, 152.0, 138.0, 133.3, 129.7, 129.3, 128.5, 128.0, 127.2, 125.9, 124.1, 120.9, 115.8, 114.9, 111.7, 69.7, 44.4, 39.1, 31.4, 20.1, 14.1; HRMS-ESI $C_{27}H_{27}NO_5S$ calcd [M+H] 478.1683, found 478.1651.

3.2 Biological assays

3.2.1 Purification of Srt A Protein

The Srt A protein was purified according to the known method.[25] The pET28-SrtA△59 was transformed into Escherichia coli strain BL21 (Novagen). The pET28-SrtA59 transformed cells were grown in 1 L of Luria broth media

at 37 °C until the OD600 reached 0.6. The culture was then induced with 1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG; Invitrogen, Carlsbad, CA) and grown for another 8 h at 37 °C. The cells were harvested and incubated on ice for 30 min with lysis buffer (50 mM NaH_2PO_4, 300 mM NaCl, 20 mM imidazole, pH 8.0) containing 1 mg/mL lysozyme. After a brief sonication, the lysate was centrifuged and the supernatant was applied to 0.8 mL of Ni-NTA agarose beads pre-equilibrated with lysis buffer (Qiagen, Madison, WI). After washing off the unbound contaminating proteins, the His6-tagged protein was eluted with elution buffer (50 mM NaH_2PO_4, 300 mM NaCl, 250 mM imidazole, pH 8.0). All samples were analyzed using Coomassie Plus Protein Assay (Pierce Biotechnology, Rockford, IL) measuring the absorbance at wavelength 595 nm to determine the protein concentration.

3.2.2 Inhibition assays

The activities of compounds were tested using previously reported high performance liquid chromatography (HPLC) assay.23, 24 Purified Srt A (5 μM) was breifly pre-incubated with varying concentrations of inhibitor (dissolved in DMSO, final concentration of 12.5~200 μM) in 96-well plates containing assay buffer (150 mM NaCl, 5 mM $CaCl_2$, 50 mM Tris-HCl, 0.05% Tween 20, pH 7.5) at 37 0C for 1 h. Reactions were then initiated by adding substrate Abz-LPATG-Dnp (final concentration 10 μM). Reactions were performed in a total volume of 100 μL with all reagents. 30 min later, the reaction was quenched by adding 50 μL HCl (1 M). 20 μL quenched reaction mixture was then injected onto a reverse phase Agilent XDB-C 18(4.6×150 mm, 3.5 μm) HPLC column. The reaction components were separated using a linear gradient from 20% to 60% acetonitrile/0.1% trifluoroacetic acid applied over a period of 25 mins. For each concentration, the area percentage of G-Dnp compared with those in the absence of inhibitor was calculated at 355 nm. IC_{50} values were calculated as the concentration associated 50% decrease of the area percentage of G-Dnp. Each experiment was repeated three times.

3.3 Docking

Docking of **Ia-22** and **Ib-26** to Sortase A (PDB code: 2KID) were performed using Gold Suite v5.0.1 (CCDC, Cambridge, U.K., 2010) with constrain conditions. 24 Before docking was run, parameters were specially addressed. The binding site was defined to include all residues within a 10.0 Å radius of the Ile182 Cγ carbon atom. Compounds **Ia-22** and **Ib-26** were built and minimized with a MM94 force field. GoldScore was applied as a scoring function and other parameters were set as standard default. 10 conformations were produced after the docking runs were complete. We referred to the 20 LPAT conformations contained in the Srt A crystal and selected the most reasonable docking conformations as our solutions.

4. Conclusions

Thirty-seven novel 2-phenyl-benzofuran-3-carboxamide derivatives were synthesized and evaluated their inhibitory activity against Srt A. Most compounds exhibited remarkable inhibitory activity. The SARs analysis of all tested compounds indicated that substituents on the benzofuran and phenyl rings had significant impacts on the inhibitory activity against Srt A. But, the linker between benzofuran and phenyl rings mildly affected inhibitory activity. Our findings were further confirmed by the molecular docking study of compound **Ia-22** with Srt A (PDB: 2KID). Molecular docking studies showed the active compounds shared similar binding pattern with substrate(LPAT) in the binding pocket of Srt A (PDB: 2KID) including *i*-butyl stretching, L-shape pattern kinking, and H-bond interaction with Srt A functional site residues Cys184, Trp194 and Arg197. The binding model presents a rationale for the activity of these compounds.

5. Acknowledgements

This research was supported by the National Natural Science Foundation, Beijing, China (to Dr. Jiang, No: 81202397), the Shanghai Natural Science Foundation Project, Shanghai, China (to Dr. Jiang, No: 12ZR1415400), and the International S&T Cooperation Program of China (to Dr. Fu, No. 2014DFA41360).

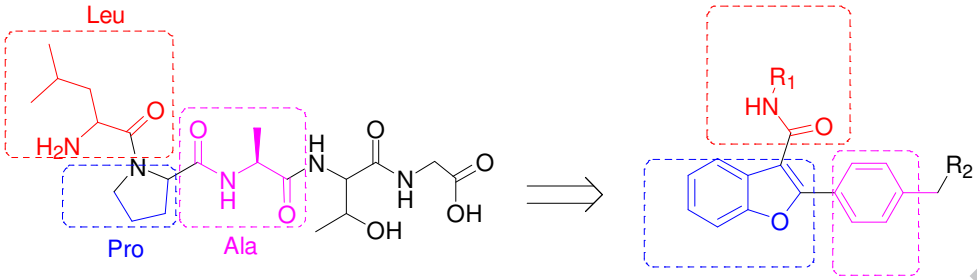
References

1. Bremell, T.; Abdelnour, A.; Tarkowski, A. Infect. Immun. 1992, 60: 2976-2985.
2. Mathew, A.G.; Cissell, R.; Liamthong, S. Foodborne. Pathog. Dis. 2007, 4: 115-133.
3. Date, S.V.; Modrusan, Z.; Lawrence, M.; Morisaki, J.H.; Toy, K.; Shah, I.M.; Kim, J.; Park, S.; Xu, M.; Basuino, L. J. Infect. Dis. 2014, 209: 1542-1550.
4. Ippolito, G.; Leone, S.; Lauria, F.N.; Nicastri, E.; Wenzel, R.P. Int. J. Infect. Dis. 2010, 14: S7-S11.
5. Ton-That, H.; Liu, G.; Mazmanian, S.K.; Faull, K.F.; Schneewind, O. Proc. Natl. Acad. Sci. USA. 1999, 96: 12424-12429.
6. Ilangovan, U.; Ton-That, H.; Iwahara, J.; Schneewind, O.; Clubb, R.T. Proc. Natl. Acad. Sci. USA. 2001, 98: 6056- 6061.
7. Oh, K.B.; Oh, M.N.; Kim, J.G.; Shin, D.S.; Shin, J. Appl. Microbiol. Biotechnol. 2006, 70: 102-106.
8. Weiss, W.J.; Lenoy, E.; Murphy, T.; Tardio, L.; Burgio, P.; Projan, S.J.; Schneewind, O.; Alksne, L. J. Antimicrob. Chemother. 2004, 53: 480-486.
9. Sibbald, M.J.J.B.; Yang, X.M.; Tsompanidou, E.; Qu, D.; Hecker, M.; Becher, D.; Buist, G.; van Dijl, J.M. Proteomics. 2012, 12: 3049-3062.
10. Park, B.S.; Kim, J.G.; Kim, M.R.; Lee, S.E.; Takeoka, G.R.; Oh, K.B.; Kim, J.H. J. Agric. Food Chem. 2005, 53:9005-9009.
11. Maresso, A.W.; Wu, R.; Kern, W.J.; Zhang, R.; Janik, D.; Missiakas, D.M.; Duban, M.-E.; Joachimiak, A.; Schneewind, O. J. Biol. Chem. 2007, 282: 23129-23139.
12. Suree, N.; Yi, S.W.; Thieu, W.; Marohn, M.; Damoiseaux, R.; Chan, A.; Jung, M.E.; Clubb, R.T. Bioorg. Med. Chem. 2009, 17: 7174-7185.
13. Won, T.H.; Jeon, J.; Kim, S.H.; Lee, S.H.; Rho, B.J.; Oh, D.C.; Oh, K.B.; Shin, J. J. Nat. Prod. 2012, 75: 2055-2061.
14. Won, T.H.; Jeon, J.; Lee, S.H.; Rho, B. J.; Oh, K.B.; Shin, J. Bioorg. Med. Chem. 2012, 20: 4082-4087.
15. Frankel, B.A.; Bentley, M.; Kruger,

R.G.; McCafferty, D.G. J. Am. Chem. Soc. 2004, 126: 3404-3405.

16. Hu, P.; Huang, P.; Chen, M.W. Arch. Oral. Biol. 2013, 58: 1343-1348.
17. Oh, I.; Yang, W.Y.; Chung, S.C.; Kim, T.Y.; Oh, K.B.; Shin, J. Arch. Pharm. Res. 2011, 34: 217-222.
18. Oh, K.B.; Mar, W.; Kim, S.; Kim, J.Y.; Oh, M.N.; Kim, J.G.; Shin, D.; Sim, C.J.; Shin, J. Bioorg. Med. Chem. Lett. 2005, 15: 4927-4931.
19. Moritsugu, K.; Terada, T.; Kidera, A. J. Am. Chem. Soc. 2012, 134: 7094-7101.
20. Biswas, T.; Pawale, V.S.; Choudhury, D.; Roy, R.P. Biochem. 2014, 53(15): 2515-2524.
21. Kruger, R.G.; Dostal, P.; McCafferty, D.G. Anal. Biochem. 2004, 326: 42-48.
22. Zhang, Y.; Bao, J.; Deng, X.X.; He,W.; Fan, J.J.; Jiang, F.Q.; Fu, L. Bioorg. Med. Chem. Lett. 2016, 26: 4081-4085.
23. Chan, A.H.; Wereszczynski, J.; Amer, B.R.; Yi, S.W.; Jung, M.E.; McCammon, J.A.; Clubb, R.T. Chem. Biol. Drug. Des. 2013, 82: 418-28.
24. Suree, N.; Liew, C.K.; Villareal, V.A.; Thieu, W.; Fadeev, E.A.; Clemens, J.J.; Jung, M.E.; Clubb, R.T. J. Biol. Chem. 2009, 284: 24465-24477.
25. Lu, C.S.; Zhu, J.; Wang, Y.; Umeda, A.; Cowmeasow, R.B.; Lai, E.; Moreno, G.N.; Person, M.D.; Zhang Z.W. Biochem., 2007, 46, 9346-9354.

Graphical abstract



Srt A substrate LPATG

Potent Sortase A inhibitors