European Journal of Medicinal Chemistry 130 (2017) 195–208

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

Discovery of benzofuran-3(2*H*)-one derivatives as novel DRAK2 inhibitors that protect islet β-cells from apoptosis

Sheng Wang [a, 1], Lei Xu [b, 1], Yu-Ting Lu [b], Yu-Fei Liu [a], Bing Han [d], Ting Liu [a], Jie Tang [a, c], Jia Li [b], Jiangping Wu [d, ****], Jing-Ya Li [b, ***], Li-Fang Yu [a, **], Fan Yang [a, *]

[a] Shanghai Engineering Research Center of Molecular Therapeutics and New Drug Development, School of Chemistry and Molecular Engineering, East China Normal University, 3663 North Zhongshan Road, Shanghai 200062, China
[b] Chinese National Center for Drug Screening, State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 189 Guoshoujing Road, Zhangjiang Hi-Tech Park, Shanghai 201203, China
[c] Shanghai Key Laboratory of Green Chemistry and Chemical Process, School of Chemistry and Molecular Engineering, East China Normal University, 3663 North Zhongshan Road, Shanghai 200062, China
[d] Laboratory of Immunology and Cardiovascular Research, Centre Hospitalier de l'Université de Montréal, 900 rue St-Denis, Montréal, Québec, Canada

ARTICLE INFO

Article history:
Received 11 January 2017
Received in revised form
17 February 2017
Accepted 18 February 2017
Available online 21 February 2017

Keywords:
DRAK2 inhibitor
Diabetes
Islet β-cell protection
Apoptosis

ABSTRACT

Death-associated protein kinase-related apoptosis-inducing kinase-2 (DRAK2) is a serine/threonine kinase that plays a key role in a wide variety of cell death signaling pathways. Inhibition of DRAK2 was found to efficiently protect islet β-cells from apoptosis and hence DRAK2 inhibitors represent a promising therapeutic strategy for the treatment of diabetes. Only very few chemical entities targeting DRAK2 are currently known. We carried out a high throughput screening and identified compound **4** as a moderate DRAK2 inhibitor with an IC_{50} value of 3.15 μM. Subsequent SAR studies of hit compound **4** led to the development of novel benzofuran-3(2*H*)-one series of DRAK2 inhibitors with improved potency and favorable selectivity profiles against 26 selected kinases. Importantly, most potent compounds **40** (IC_{50} = 0.33 μM) and **41** (IC_{50} = 0.25 μM) were found to protect islet β-cells from apoptosis in dose-dependent manners. These data support the notion that small molecule inhibitors of DRAK2 represents a promising strategy for the treatment of diabetes.

1. Introduction

Death-associated protein kinase-related apoptosis-inducing kinase-2 (DRAK2, also known as STK17B) is a serine/threonine kinase, belonging to the family of death-associated protein kinase (DAPK). DAPK plays a key role in a wide variety of cell death signaling pathways. In addition to DRAK2, there are four other DAP kinase family members identified to date, including DAPK1, DAPK2, DAPK3, and DRAK1 [1]. The sequence homology of the DAPK family members is restricted to the N-terminal kinase domain, while the C-terminal kinase domains are diverse and relate to their different regulation of signal transduction pathways [2].

DRAK2 is constitutively expressed in multiple tissues and organs, such as the thymus, suprachiasmatic nuclei, pituitary, olfactory lobes, adrenal gland medulla, stomach, skin and testes [3]. It is also rapidly induced in islet β-cells by inflammatory cytokines [4] and free fatty acids [5]. It is conceivable that such induced expression could occur in other types of cells or tissues. Therefore, the initial notion that DRAK2 has T cell-specific expression is incorrect. Inflammatory cytokines are pathogenic in type I diabetes. We have demonstrated that transgenic DRAK2 overexpression along with the cytokine assaults leads to aggravated apoptosis of β-cells [4]. Free fatty acids (FFA) cause insulin resistance and islet β-cell apoptosis in type II diabetes. DRAK2 overexpression increases β-cell apoptosis after FFA stimulation [5]. Conversely, the cytokine and FFA-induced β-cell apoptosis can be inhibited by DRAK2 small interfering RNA (siRNA) [4,5]. Our *in vivo* studies confirm that the DRAK2 transgenic mice are prone to low-dose streptozotosin-induced diabetes (a type I diabetes model) [4] and high-fat diet-induced glucose intolerance (a type II diabetes model) [5]. In

* Corresponding author.
** Corresponding author.
*** Corresponding author.
**** Corresponding author.
E-mail addresses: jianping.wu@umontreal.ca (J. Wu), jyli@simm.ac.cn (J.-Y. Li), lfyu@sat.ecnu.edu.cn (L.-F. Yu), fyang@chem.ecnu.edu.cn (F. Yang).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.ejmech.2017.02.048
0223-5234/

consistent with our findings in transgenic mice, DRAK2 deficient mice are less prone to type I diabetes [3]. Collectively, these results indicate that DRAK2 inhibition can improve islet survival and DRAK2 is a therapeutic target for early stage type I and type II diabetes.

Only very few inhibitors targeting DRAK2 have been reported to date. SC82510 was reported to be a DRAK2 inhibitor that induced axon branching of adult sensory neurons at a low concentration of 1 nM [6]. The thieno [2,3-b]pyridine (**1**) were identified as dual DRAK1 and DRAK2 inhibitors with IC_{50} values of 2.25 and 0.82 μM respectively (Fig. 1) [7]. 5-Arylthieno [2,3-b]pyridine (**2**) was reported to be a more potent inhibitor of DRAK2 with an IC_{50} value of 29 nM. Meanwhile, compound **2** was also a strong binder of DRAK1 (K_d = 99 nM) and DAPK1 (K_d = 54 nM) [8]. The indirubin **3** was recently reported as a high-efficient DRAK2 inhibitor with an IC_{50} value of 3 nM, while showing a high selectivity over the other 4 kinase members in DAPK family and selected protein kinases, such as, tyrosine-protein kinase Met (c-MET), anaplastic lymphoma kinase (ALK), activated CDC42 kinase 1 (Ack1), maternal embryonic leucine zipper kinase (MELK), inwardly rectifying potassium channels IRK, and epidermal growth factor receptor (EGFR) [9].

In our efforts to discover novel DRAK2 inhibitors, we identified 2-(3,4-dihydroxybenzylidenebenzofuran-3(2*H*)-one (**4**) which displays a moderate DRAK2 inhibitory activity with an IC_{50} value of 3.15 μM. Compound **4** belongs to the family of aurones which have been shown to have a wide range of biological and pharmacological activities. With the aim to develop potent and selective novel DRAK2 inhibitors, we carried out structure-activity relationship (SAR) studies. Selected compounds were further assessed for their selectivity against 26 kinases and effects on the glucose-stimulated insulin secretion of primary islets.

1.1. Chemistry

The synthetic routes for compounds **4**, **7**–**20**, **23**, **26**, and **29** were described in Scheme 1. Benzofuran-3(2*H*)-one (**6**) was synthesized from commercially available hydroxyacetophenone (**5**) using a reported procedure [10]. Aldol condensation reaction of **6** with appropriate substituted benzaldehyde in the presence of aluminum oxide [11] or sodium hydroxide [12] afforded compounds **7**–**20**. Indan-1-one **23** was synthesized in two steps according to the reported procedure using 3-phenylpropanoic acid [13,14]. 1-(3,4-Dihydroxyphenyl)ethan-1-one (**24**) was brominated to afford compound **25** [15] which underwent cyclization reaction with 3,4-dihydroxybenzaldehyde in the presence of potassium carbonate to afford compound **26**. Condensation of the benzyl protected 3,4-dihydroxybenzaldehyde (**28**) [16] with compound **6** and subsequent hydrogenation reaction afforded compound **29**.

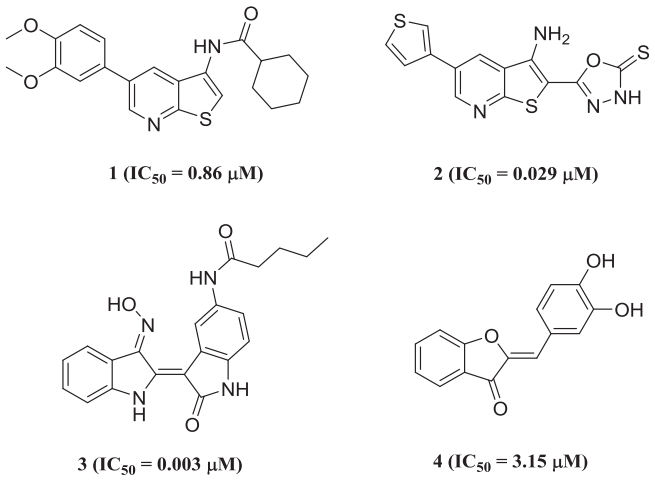


Fig. 1. Structures of selected DRAK2 inhibitors.

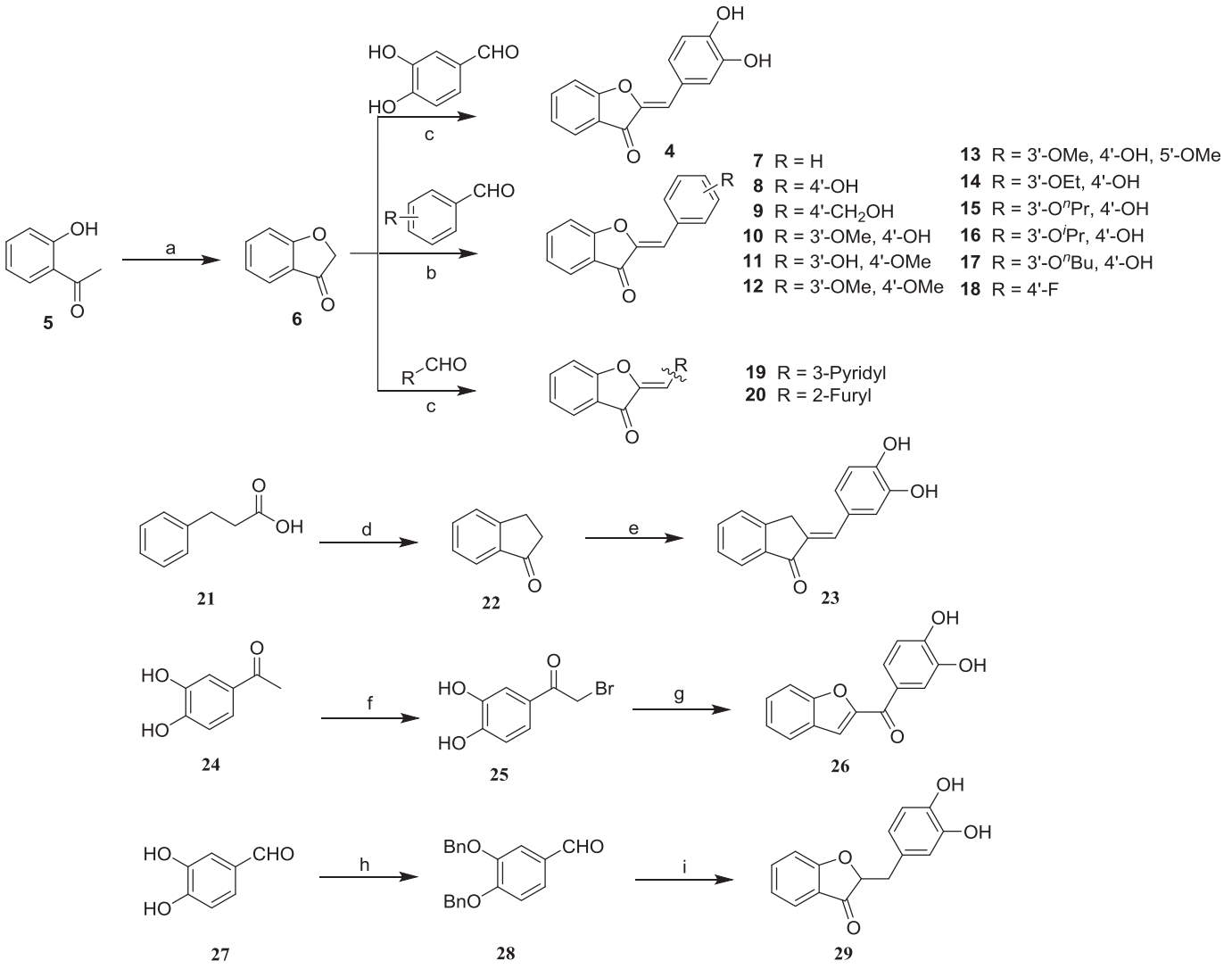
The synthetic routes for 4- or 5-substituted 2-benzylidenebenzofuran-3(2*H*)-one derivatives were listed in Scheme 2. 4-Methoxybenzofuran-3(2*H*)-one (**32**) was obtained from commercially available 1-(2,6-dihydroxyphenyl) ethan-1-one (**30**) via reported cyclization and methylation reactions [17,18]. Compound **33** was obtained via condensation reaction of **32** with 3-ethoxy-4-hydroxybenzaldehyde in the presence of aluminum oxide in dichloromethane at room temperature. Similarly, 5-methoxybenzofuran-3(2*H*)-ones (**40**–**43**) were obtained from 5-methoxy, 5-hydroxy, or 5-OAc substituted benzofuran-3(2*H*)-ones (**37**–**39**) in the presence of aluminum oxide in dichloromethane or potassium hydroxide in methanol and water under reflux condition. Compound **39** was obtained from **38** under a standard acylation conditions. Compounds **37** and **38** were synthesized from commercially available 4-methoxyphenol **34** in 2–3 steps utilizing reported procedures. Likewise, compounds **45**–**47** were synthesized from the corresponding 4-ethoxyphenol, 4-butoxyphenol, and 4-methyphenol. 4-Substituted anisole (**48**–**52**) underwent a Friedel-Crafts acylation with chloroacetyl chloride in the presence of aluminum chloride followed by cyclization with alkali to give 5-substituted benzofuran-3(2*H*)-ones (**53**–**57**) [19]. Nitro compound **57** was reduced with iron powder in glacial acetic acid to afford aniline **58** [10]. Condensation reaction of compounds **53**–**56**, **58** with 3-ethoxy-4-hydroxybenzaldehyde afforded final compounds **59**–**63** respectively.

The procedures for the synthesis of 6- and 7-substituted 2-benzylidenebenzofuran-3(2*H*)-ones were listed in Scheme 3. 6-Hydroxybenzofuran-3(2*H*)-one (**65**) was synthesized from commercially available resorcinol (**64**) using a reported procedure [20]. Subsequent methylation and condensation reaction with 3-ethoxy-4-hydroxybenzaldehyde or 3,4-dihydroxybenzaldehyde in the presence of aluminum oxide in dichloromethane or potassium hydroxide in methanol and water under reflux condition afforded compounds **67** and **68**. Compounds **73** and **74** were synthesized from commercially available 1-(4-fluoro-2-hydroxyphenyl)ethan-1-one (**69**) and 1-(4-chloro-2-hydroxyphenyl)ethan-1-one (**70**) utilizing similar procedures as those for compound **7**. Friedel-Crafts acylation of carboxylic acid **76** [21], followed by a condensation reaction with 3-ethoxy-4-hydroxybenzaldehyde afforded (*Z*)-2-(3-ethoxy-4-hydroxybenzylidene)-7-methoxybenzofuran-3(2*H*)-one (**78**).

All final 2-benzylidenebenzofuran-3(2*H*)-one compounds were prepared via condensation reactions of benzofuran-3(2*H*)-ones with benzaldehydes in the presence of aluminum oxide in dichloromethane, or potassium hydroxide in methanol and water. These conditions are prone to yield mainly the *Z* isomers according to the literature [22–27]. The δ values of olefinic carbon for *Z* configurations determined by ^{13}C NMR spectroscopy were reported around 111 ppm, while the olefinic carbon for *E* configurations were generally between 120 and 130 ppm. In the case of 2-benzylidenebenzofuran-3(2*H*)-ones presented in this work, δ values for olefinic carbons were 101–114 ppm, suggesting *Z* geometries.

2. Results and discussion

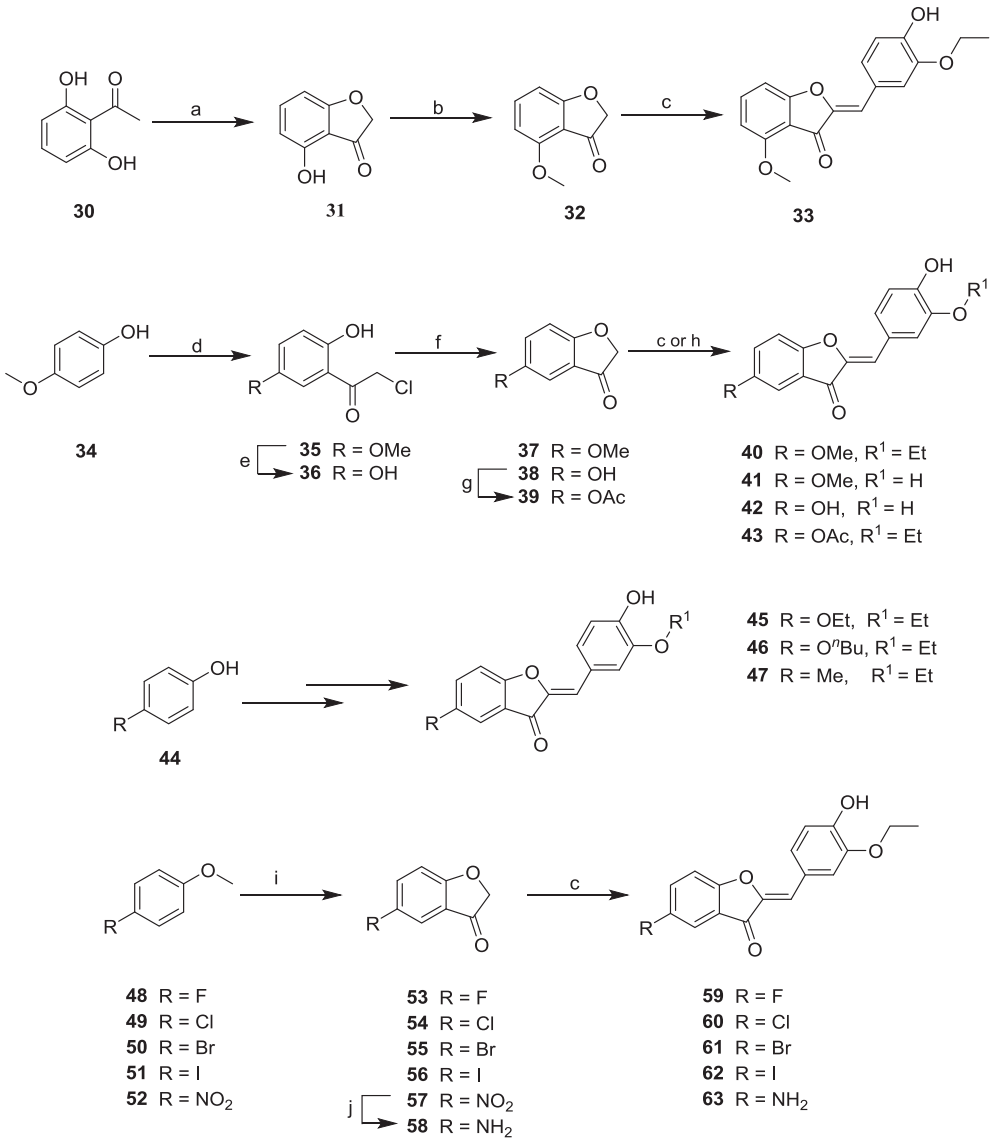
All final compounds were initially screened using a DRAK2-GST autophosphorylation assays for their DRAK2 inhibitory activities and their IC_{50} values were listed in Tables 1 and 2. Keeping the 2-benzylidenebenzofuran-3(2*H*)-one skeleton intact, we first



Scheme 1. Reagents and conditions: (a) 1) $CuBr_2$, $CHCl_3$/EtOAc (1:1), reflux, 10 h; 2) Et_3N, CH_3CN, rt, 5 h; (b) Al_2O_3, CH_2Cl_2, rt, 5 h; (c) KOH, MeOH, H_2O, reflux, 3 h; (d) TfOH, CH_2Cl_2, 0 °C to rt, 6 h; (e) 3,4-dihydroxybenzaldehyde, NaOH, MeOH, rt, 5 h (f) $CuBr_2$, EtOAc, reflux, 5 h; (g) 2-hydroxybenzaldehyde, K_2CO_3, acetone, reflux, 20 h; (h) BnBr, K_2CO_3, DMF, 70 °C, 6 h; (i) 1) benzofuran-3(2*H*)-one, Al_2O_3, CH_2Cl_2, rt, 5 h; 2) cat. Pd/C, H_2, MeOH, rt, 5 h.

focused on assessing the effects of substitutions on the benzene ring. Deletion of 3′-hydroxyl (**8**) or both 3′- and 4′-hydroxyl groups (**7**) resulted in an 18- or 8-fold decreased in inhibitory activities compared to hit compound **4**. Methylation of 4′-hydroxyl and 3′,4′-dihydroxyl groups resulted in a 4–7-fold decrease in activity, indicating the importance of the hydrogen bond donor at position 4′. Alkylation of the 3′-hydroxyl group with methyl (**10**) or ethyl (**14**) maintained the activity, while bulkier groups such as n-propyl, i-propyl, *n*-butyl, as seen in compounds **15**–**17**, were found to be detrimental to the activity compared to smaller groups. Replacement of 4′-hydroxyl with fluoro or hydroxymethyl decreased the inhibitory activity against DRAK2. A decrease of activity was also found when the 3,4-dihydroxybenzyl moiety was replaced by other heterocycles such as 3′-pyridyl (**19**) and 2′-furyl (**20**).

We next looked at the effects of linker part on the inhibitory activity. Replacing the oxygen of benzofuran-3(2*H*)-one compound **4** with CH_2 afforded a weaker inhibitor **23** with a 4-fold drop in IC_{50} value. Benzofuran-2-methanone (**26**) and 2-(3,4-dihydroxybenzyl) benzofuran-3(2*H*)-one (**29**) showed weak inhibitory activity with IC_{50} values over 20 μM. We then examined the effects of electron withdrawing group (EWG) and electron donating group (EDG) substituents at the benzofuran-3(2*H*)-one ring on DRAK2 inhibition (Table 2). A 4-methoxy substitution yielded a 2-fold reduction of activity compared to the unsubstituted compound **14**, while introduction of a methoxy group at 5-, 6-, and 7-position (**40**, **41**, **68**, and **78**) of benzofuran-3(2*H*)-one resulted in an increase in activities (IC_{50} = 0.25–2.84 μM) with compounds **40** and **41** displaying the best activities with IC_{50} values of 0.33 and 0.25 μM. More EDGs and EWGs at 5-position were next accessed including OH (**42**), $OCOCH_3$ (**43**), OCH_2CH_3 (**45**), O^nBu (**46**), CH_3 (**47**), F (**59**), Cl (**60**), Br (**61**), and I (**62**). EDGs were generally found to be beneficial to the activities with the exception of amino compound **63** which showed a 2-fold decrease in activity compared to the corresponding unsubstituted compound **14,** indicating the lack of importance of hydrogen bond donors at this position. Increasing steric bulk from methoxy to ethoxy and acetoxy resulted in submicromolar inhibitors (**41, 43, 45**) but the *n*-butoxy analog was found to be 10-fold less potent, with an IC_{50} value of 3.66 μM. The same trend was observed in the 5-halogen substituted analogs **59**–**62**, where the iodo-substituted compound **62** was the least potent among them and the 5-fluoro substituted compound **59** showing a 3-fold increase in activity (IC_{50} = 1.05 μM). It was found that as the



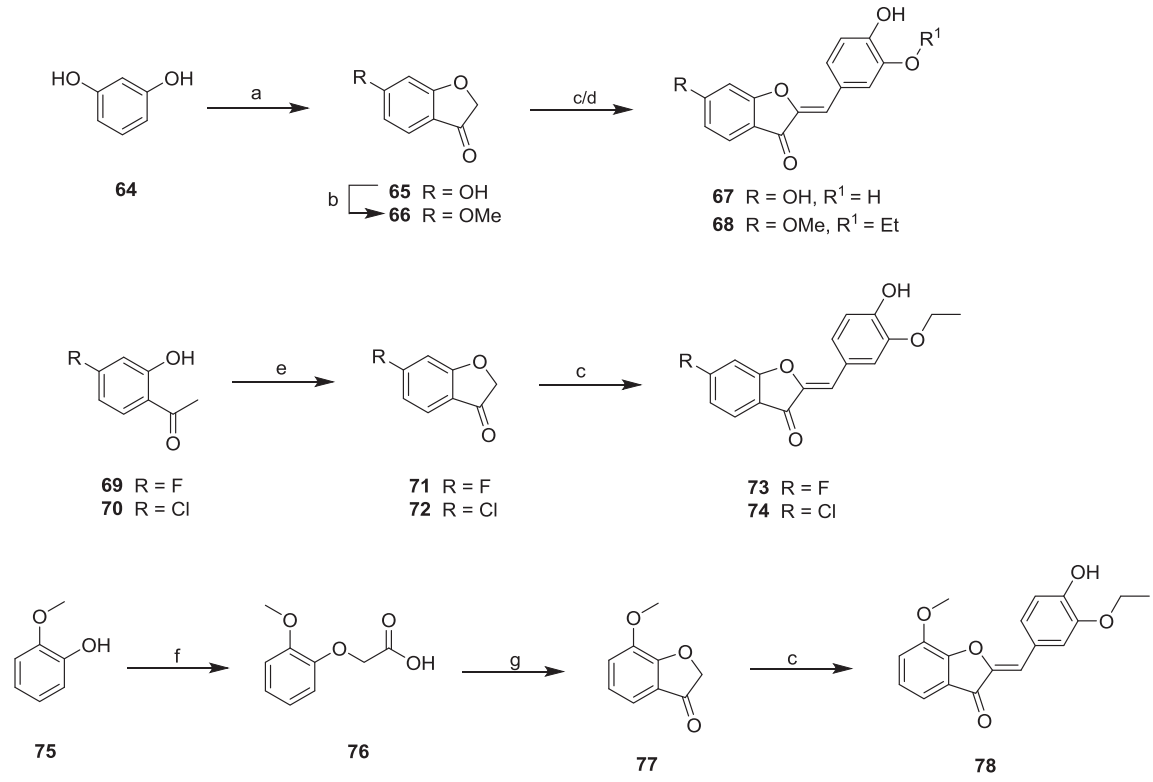
Scheme 2. Reagents and conditions: (a) 1) $(CH_3)_3SiCl$, Lithium diisopropylamide (LDA), THF, −78 °C, 4 h; 2) NBS, −78 °C to rt, 1.5 h; 3) NaOH, H_2O, rt, overnight; (b) CH_3I, K_2CO_3, DMF, 80 °C, 3 h; (c) 3-ethoxy-4-hydroxybenzaldehyde, Al_2O_3, CH_2Cl_2, rt, 5 h; (d) BCl_3/CH_2Cl_2, $ClCH_2CN$, $AlCl_3$, 0 °C–25 °C, 6 h; (e) BBr_3/CH_2Cl_2, CH_2Cl_2, −78 °C–0 °C, 2 h; (f) AcONa, EtOH, reflux, 2 h; (g) Ac_2O, Et_3N, CH_2Cl_2, rt, 1 h; (h) 3,4-dihydroxybenzaldehyde, KOH, MeOH, H_2O, reflux, 3 h; (i) 1) $ClCH_2COCl$, $AlCl_3$, CH_2Cl_2, 25 °C–40 °C to 25 °C, 72 h; 2) AcONa, EtOH, reflux, 2 h; (j) Fe, AcOH, 70 °C, 3 h.

electron-donating ability goes up (OCH_3 > $OCOCH_3$ > CH_3), the inhibitory activities against DRAK2 also increased in sequence (**40** > **43** > **47**). At 6-position, the EWGs F and were found to be detrimental to the activities with IC_{50} values of 60 and 40 μM. Respectively, while the 6-methoxy compounds 67 and 68 maintained activities (IC_{50} values of 1.02 and 2.44 μM).

2.1. Selectivity studies at 26 kinases

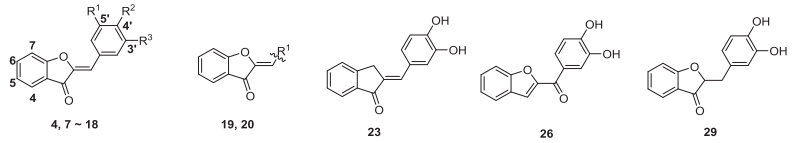
DRAK2 belongs to DAPK family, which consists of DAPK1 DAPK2, DAPK3, DRAK1 and DRAK2. Most potent compounds **40**, **41**, and a natural product sulphuretin (**67**), were selected to evaluate their ability to inhibit DRAK1, DAPK1, DAPK2, and DAPK3 in enzyme assays. As shown in Table 3, compound **1** showed equal potency for both DRAK1 (IC_{50} = 1.72 μM) and DRAK2 (IC_{50} = 1.31 μM) with very weak inhibitory activities if any, for DAPK1, DAPK2, and DAPK3, which were consistent to the reported data [7]. Compounds **40** and **41** showed similar selectivity profiles with moderate to good selectivity for DRAK2 over the other four kinases (>4 fold for compound **40**; >23 fold for compound **41**), while compound **67** showed extensive inhibitory activities against the other kinases of DAPK family.

We further investigated the selectivity of compounds **40**, **41**, and **67** against protein kinases available in house (Table 3, including B lymphoid tyrosine kinase (BLK), IL2-inducible T-cell kinase (ITK), tunica interna endothelial cell kinase (TEK), Janus kinase (JAK), spleen tyrosine kinase (SYK), checkpoint kinases 1 (CHK1), v-akt murine thymoma viral oncogene homolog 1 (AKT1), Aurora kinase A (Aurora-A), EGFR, Bruton agammaglobulinemia tyrosine kinase (BTK), cyclin-dependent kinase (CDK), maternal embryonic leucine zipper kinase (MELK), inhibitor of nuclear factor kappa-B kinase subunit beta (IKKβ), glycogen synthase kinase 3 beta (GSK3β), mitogen-activated protein kinase kinase 1 (MEK1), mitogen-activated protein kinase 3 (ERK1). Sulphuretin **67** was found to be a highly promiscuous compound with extensive activities against these protein kinases. Compound **41** showed moderate inhibitory



Scheme 3. Reagents and conditions: (a) 1) $ClCH_2CN$, $ZnCl_2$, HCl (g), Et_2O, 4 h; 2) H_2O, reflux, 5 h; 3) AcONa, EtOH, reflux, 2 h; (b) CH_3I, K_2CO_3, DMF, 80 °C, 3 h; (c) 3-ethoxy-4-hydroxybenzaldehyde, Al_2O_3, CH_2Cl_2, rt, 5 h; (d) 3,4-dihydroxybenzaldehyde, KOH, MeOH, H_2O, reflux, 3 h; (e) 1) $CuBr_2$, $CHCl_3$/EtOAc (1:1), reflux, 10 h; 2) Et_3N, CH_3CN, rt, 5 h; (f) $ClCH_2COOH$, NaH, DMF, 0 °C to rt, 12 h; (g) 1) $SOCl_2$, reflux; 2) $AlCl_3$, CH_2Cl_2, 0 °C to rt, 1.5 h.

Table 1
The inhibitory activities of compounds **1**, **4**, **7**–**20**, **23**, **26**, and **29** against DRAK2.[a]

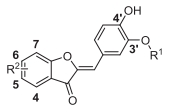


Compound	R^1	R^2	R^3	DRAK2 IC_{50}, μM
1	–	–	–	1.31 ± 0.08
4	OH	OH	H	3.08 ± 0.40
7	H	H	H	24.64 ± 8.65
8	H	OH	H	53.03 ± 10.46
9	H	CH_2OH	H	11.09 ± 1.45
10	OMe	OH	H	3.11 ± 1.90
11	OH	OMe	H	12.10 ± 2.19
12	OMe	OMe	H	21.43 ± 0.28
13	OMe	OH	OMe	6.65 ± 1.77
14	OEt	OH	H	3.24 ± 0.05
15	O^{n}Pr	OH	H	5.37 ± 0.17
16	O^{i}Pr	OH	H	17.39 ± 1.95
17	O^{n}Bu	OH	H	29.10 ± 9.02
18	H	F	H	40.55 ± 14.37
19	3-pyridyl	–	–	39.39 ± 8.85
20	2-furyl	–	–	63.96 ± 14.21
23	–	–	–	13.29 ± 1.59
26	–	–	–	22.91 ± 1.05
29	–	–	–	27.81 ± 1.99

[a] See Experimental Section.

activities against BLK, ITK, TEK, and Aurora-A, with IC_{50} values of 0.5–5 μM. Compound **40** showed greatest selectivity among three tested compounds with relatively weak inhibitory activities against BLK, Aurora-A, BTK, IKKβ, and GSK3β, with IC_{50} values no less than 5 μM.

Table 2
The inhibitory activities of compounds **33**, **40**–**43**, **45**–**47**, **59**–**63**, **67**, **68**, **73**, **74**, and **78** against DRAK2 kinase.[a]



Compound	R^1	R^2	DRAK2 IC_{50}, μM
1	–	–	1.31 ± 0.80
33	Et	4-OMe	7.26 ± 3.93
40	Et	5-OMe	0.33 ± 0.10
41	H	5-OMe	0.25 ± 0.01
42	H	5-OH	0.99 ± 0.07
43	Et	5-OAc	0.69 ± 0.23
45	Et	5-OEt	0.41 ± 0.02
46	Et	5-O^{n}Bu	3.66 ± 0.20
47	Et	5-Me	0.96 ± 0.12
59	Et	5-F	1.05 ± 0.06
60	Et	5-Cl	13.71 ± 0.92
61	Et	5-Br	28.15 ± 4.61
62	Et	5-I	42.52 ± 4.09
63	Et	5-NH_2	7.16 ± 2.23
67	H	6-OH	1.02 ± 0.06
68	Et	6-OMe	2.44 ± 0.56
73	Et	6-F	60.40 ± 13.23
74	Et	6-Cl	40.54 ± 10.69
78	Et	7-OMe	1.29 ± 0.10

[a] See Experimental Section.

2.2. Effect of DRAK2 inhibitors on the glucose-stimulated insulin secretion of primary islets and apoptosis protection of INS-1 cell

Type I and type II diabetes share a common pathological feature of pancreatic β cell apoptosis and hyperglycemia-induced β cell apoptosis has been implicated mainly in type II diabetes. Palmitate, as a kind of free fatty acid, caused mitochondria functional impairment then induced β-cell dysfunction and apoptosis via ER stress [28,29]. And previously studies shown that palmitate-induced β-cell death was caused by Ca^{2+} influx; the Ca^{2+} would trigger apoptotic signals subsequently [30]. Palmitate lipotoxicity mediated β cell death involving caspase activation and subsequentially β cell failure [31]. When human islets were cultured with palmitate together, the intracellular insulin content would decreased as the extension of time and cleaved-caspase 3 increased in the process [32]. Thus palmitate would induce β cell apoptosis and damaged insulin secretion capacity of β cell. When treated islets with FFA, the mRNA level and protein level of DRAK2 significantly increased and promotes the apoptosis of β cell; further more β cell apoptosis were aggravated in DRAK2 transgenic mice islets. If knockdown DRAK2 in NIT-1 cell, it can effectively reduce the FFA induced apoptosis [5].

In our study, two most potent compounds **40** and **41** were selected to assess their effect on the glucose-stimulated insulin secretion of primary islets. Isolated islets (10 islets/well) were cultured in RPMI 1640 with 10% FBS, palmitate (PA, 0.4 mM) was added to the wells at the beginning of the culture. The glucose-stimulated insulin secretion (GSIS) ability of islets were significantly impaired by palmitate incubation as shown in the reduction of 16.7 mM glucose stimulated insulin secretion compared to the dramastic response after 16.7 mM glucose stimulated in control group (Fig. 2A); while adding compound **40** or **41** to the palmitate co-culture islets, the reduced GSIS were partially recovered in a dose-dependent manner compared to the DMSO-treated control, suggesting that compounds **40** and **41** may protect islets function from palmitate-impairment. The result suggests that the palmitate-induced GSIS capacity of primary islets could be reversed by the DRAK2 inhibitors.

To explore the possible mechanism of protection from palmitate impairment, INS-1 cells were applied to co-culture with palmitate

Table 3
The inhibitory activities of 4 selected compounds against the in house protein kinases.[a]

Compound		1	40	41	67
IC_{50}, μM	DRAK2	1.31 ± 0.08	0.33 ± 0.10	0.25 ± 0.01	1.02 ± 0.06
	DRAK1	1.72 ± 0.03	1.41 ± 0.16	5.81 ± 0.77	5.85 ± 1.37
	DAPK1	NA[b]	NA	NA	13.15 ± 0.71
	DAPK2	55.00 ± 8.54	22.72 ± 2.40	6.34 ± 1.62	3.07 ± 0.30
	DAPK3	39.82 ± 0.96	18.88 ± 2.85	12.15 ± 1.94	2.33 ± 0.15
	BLK	NT[c]	5.26 ± 0.32	3.05 ± 0.78	2.88 ± 0.92
	ITK	NT	NA	2.19 ± 0.15	2.02 ± 0.16
	TEK	NT	NA	0.51 ± 0.01	0.95 ± 0.17
	JAK1	NT	NA	NA	NA
	JAK2	NT	NA	NA	9.32 ± 2.87
	JAK3	NT	NA	NA	4.26 ± 0.32
	SYK	NT	NA	NA	4.63 ± 1.38
	CHK1	NT	NA	NA	NA
	AKT1	NT	NA	NA	NA
	Aurora-A	NT	9.92 ± 1.75	1.05 ± 0.20	0.85 ± 0.02
	EGFR	NT	NA	NA	NA
	BTK	NT	5.00 ± 0.23	5.03 ± 0.43	6.02 ± 0.02
	CDK2	NT	NA	NA	NA
	CDK4	NT	NA	NA	2.82 ± 0.27
	CDK5	NT	NA	8.84 ± 0.52	1.50 ± 0.10
	CDK6	NT	NA	NA	2.44 ± 0.03
	CDK9	NT	NA	NA	2.16 ± 0.16
	MELK	NT	NA	NA	NA
	IKKβ	NT	6.23 ± 1.00	NA	NA
	GSK3β	NT	5.01 ± 0.01	5.30 ± 0.98	1.95 ± 0.21
	MEK1	NT	NA	NA	NA
	ERK1	NT	NA	NA	NA

[a] See Experimental Section.
[b] NA: not active, defined as < 50% inhibitory activity at 10 μM in the primary assay.
[c] NT: not tested.

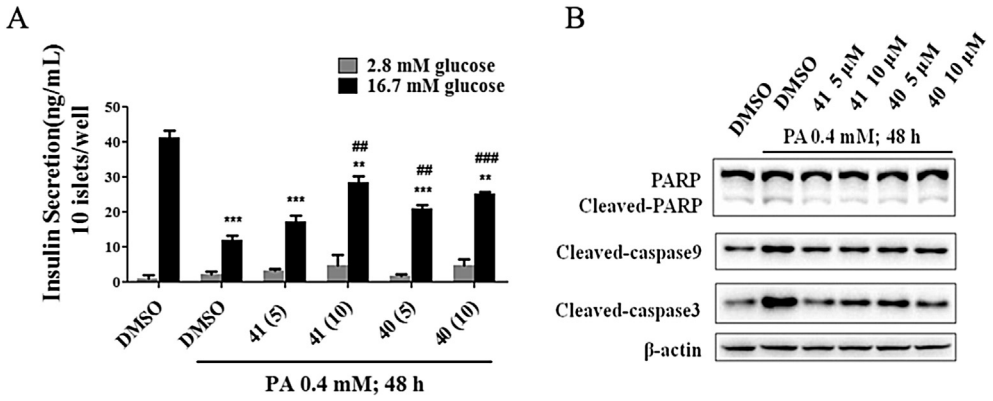


Fig. 2. Effect of DRAK2 inhibitors on the glucose-stimulated insulin secretion of primary islets and apoptosis protection of INS-1 cell. Asterisks indicate p values (*P < 0.05, **P < 0.01, ***P < 0.001) of control versus treated groups, Student's *t*-test. Croisillons indicate p-values ($^{\#}$P < 0.05, $^{\#\#}$P < 0.01, $^{\#\#\#}$P < 0.001) of PA and compounds co-treated groups versus PA treated DMSO group, Student's *t*-test. All datas were shown as means ± SD of three replications.

for 48 h to trigger the INS-1 apoptosis. As shown in Fig. 2B, the cleaved poly (ADP-ribose) polymerase (PARP), cleaved-caspase 9 and cleaved-caspase 3 were increased with palmitate co-culture compared to the control group without palmitate incubation. Adding the tested compound **40** or **41** together with palmitate, the above cleaved-PARP, -caspase 3, and -caspase 9 content were decreased compared to the palmitate co-cultured control. These data suggest that compounds **40** and **41** may protect INS-1 cells from palmitate-induced impairment by reducing cell apoptosis. The partially function recovery of primary islets may be rendered from the inhibition of DRAK2.

3. Conclusions

A total of 36 compounds were synthesized and their inhibitory activities against DRAK2 were evaluated. Key SAR findings included that 1) 3′,4′-dihydroxyl phenyl was crucial for the DRAK2 inhibitory activity with 3′-methoxy or 3′-ethoxy analog tolerated; 2) introduction of a methoxy group at 5-, 6-, and 7-position of benzofuran-3(2*H*)-one resulted in a significant increase in activities. The most potent compounds **40** and **41** exhibited favorable selectivity profiles against the other protein kinases of DAPK family as well as 22 other in house protein kinases. In a GSIS assay, compounds **40** and **41** were found to protect islet β-cell from palmitate impairment in a dose-dependent manner. Western blotting studies in INS-1 cells showed elevated cleavage of PARP, caspase 9 and 3, which can be inhibited by these two novel DRAK2 inhibitors dose-dependently. Overall, this study supports the notion that DRAK2 inhibitors could be a promising strategy for the treatment of diabetes. Continued medicinal chemistry efforts should be made to obtain potent and selective drug-like DRAK2 inhibitors for potential use in clinic.

4. Experimental

4.1. General methods

All reagents and solvents (analytical grade) were purchased from commercial suppliers and were used directly without further purification in addition to the special instructions. All reactions were carried out under nitrogen atmosphere in addition to the special instructions. The progress of reactions was monitored by silica gel thin layer chromatography (TLC), visualized under ZF-20D black box ultraviolet analyzer. Flash column chromatography was performed using Yantai Kangbinuo silica gel (200–300). IR spectra were recorded on a Nicolet Nexus 670 Fourier transform infrared spectrometer using KBr discs. ^{1}H and ^{13}C NMR spectra were tested with a Bruker Avance 400 spectrometer with tetramethylsilane (TMS) as an internal standard (400 MHz for ^{1}H, 100 MHz for ^{13}C). ^{1}H and ^{13}C chemical shifts were reported in parts per million (ppm, δ). The high-resolution mass spectra were recorded on a Bruker ESI-TOF high-resolution mass spectrometer. Melting points of the products were recorded on a WRR-Y drug melting point measurement apparatus and were uncorrected. The purities of the final compounds were recorded with Waters e2695 HPLC system with a ZORBAX SB-C18 column, with detection at 260 nm and 360 nm on a variable wavelength detector 2998 PDA; Gradient I of 50–95% acetonitrile in water in 21min, flow rate = 1.2 mL/min. Gradient II of 10–90% acetonitrile in water (5% formic acid in water) in 23min, flow rate = 1.0 mL/min.

4.1.1. General procedure for the synthesis of 2-benzylidenebenzofuran-3(2H)-one (method A)

To a solution of benzofuran-3(2*H*)-one (1.0 mmol) and benzaldehyde (1.0 mmol) in dichloromethane (6 mL) was added aluminum oxide (30.0 mmol) at room temperature. After stirring for 6 h, the reaction mixture was filtered off. The filtrate was concentrated under vacuum and the residue was purified by flash chromatography on silica gel to give the desired compound.

4.1.2. General procedure for synthesis of 2-benzylidenebenzofuran-3(2H)-one (method B)

To a solution of 3,4-dihydroxybenzaldehyde (1.0 mmol) and the benzofuran-3(2*H*)-one derivatives (1.0 mmol) in methanol (20 mL) was added an aqueous solution of 50% potassium hydroxide (15.0 mmol). After refluxing for 3 h, the reaction mixture was cooled to rt and concentrated under vacuum. The residue was diluted with water (40 mL) and extracted with ethyl acetate (3 × 50 mL). The combined organic phases were washed with brine, dried over anhydrous Na_2SO_4 and concentrated under vacuum. The residue was purified by flash chromatography on silica gel to provide the desired compound.

4.1.3. (Z)-2-(3,4-Dihydroxybenzylidene)benzofuran-3(2H)-one (**4**)

This compound was obtained from **6** [10] and 3,4-dihydroxybenzaldehyde employing method B. Yellow solid, yield 45%; purity (gradient II) 99.1%; m.p. 223–225 °C; IR (KBr) ν_{max} 3440, 3276, 3038, 1697, 1648, 1584, 1529, 1449, 1298, 1259, 1188, 1162, 1134, 1101, 886, 809, 744 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.79 (s, 1H), 9.32 (s, 1H), 7.83–7.75 (m, 2H), 7.54–7.51 (m, 2H),

7.37–7.27 (m, 2H), 6.87 (d, J = 8.2 Hz, 1H), 6.82 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.0, 164.9, 148.6, 145.6, 144.6, 137.1, 125.1, 124.1, 123.7, 123.2, 121.3, 118.2, 116.0, 113.9, 113.0; HRMS (ESI): calcd for $C_{15}H_{10}NaO_4$ $[M+Na]^+$, 277.0477; found, 277.0463.

4.1.4. (Z)-2-Benzylidenebenzofuran-3(2H)-one (**7**)

This compound was obtained from **6** and benzaldehyde employing method A. Yellow solid, yield 88%; purity (gradient I) 95.1%; m.p. 106–107 °C; IR (KBr) ν_{max} 3057, 3022, 1709, 1658, 1600, 1478, 1458, 1301, 1191, 1127, 1098, 883, 744, 686, 558 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (s, 1H), 7.92 (s, 1H), 7.81 (d, J = 7.6 Hz, 1H), 7.70–7.61 (m, 1H), 7.52–7.44 (m, 2H), 7.43–7.37 (m, 1H), 7.33 (d, J = 8.3 Hz, 1H), 7.25–7.18 (m, 1H), 6.90 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 184.8, 166.2, 146.9, 136.9, 132.3, 131.6 (2C), 129.9, 128.9 (2C), 124.7, 123.5, 121.6, 113.1, 113.0; HRMS (ESI): calcd for $C_{15}H_{10}NaO_2$ $[M+Na]^+$, 245.0578; found, 245.0582.

4.1.5. (Z)-2-(4-Hydroxybenzylidene)benzofuran-3(2H)-one (**8**)

This compound was obtained from **6** and 4-hydroxybenzaldehyde employing method A. Yellow solid, yield 85%; purity (gradient II) 98.7%; m.p. 252–254 °C; IR (KBr) ν_{max} 3135, 3019, 2954, 1687, 1639, 1613, 1571, 1510, 1449, 1288, 1172, 1130, 1101, 886, 828, 754 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.25 (s, 1H), 7.89 (d, J = 8.4 Hz, 2H), 7.79 (d, J = 7.1 Hz, 2H), 7.56 (d, J = 8.4 Hz, 1H), 7.38–7.26 (m, 1H), 7.01–6.80 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.1, 165.0, 159.7, 144.7, 137.1, 133.7 (2C), 124.1, 123.7, 122.8, 121.2, 116.1 (2C), 113.4, 113.1; HRMS (ESI): calcd for $C_{15}H_{10}NaO_3$ $[M+Na]^+$, 261.0528; found, 261.0526.

4.1.6. (Z)-2-(4-(Hydroxymethyl)benzylidene)benzofuran-3(2H)-one (**9**)

This compound was obtained from **6** and 4-(hydroxymethyl) benzaldehyde employing method A. Yellow solid, yield 70%; purity (gradient II) 98.8%; m.p. 118–121 °C; IR (KBr) ν_{max} 3392, 3321, 2929, 2868, 1703, 1651, 1597, 1478, 1458, 1301, 1188, 1130, 1040, 886, 818, 757 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 7.92 (d, J = 7.8 Hz, 2H), 7.84–7.66 (m, 2H), 7.58–7.36 (m, 3H), 7.35–7.20 (m, 1H), 6.87 (s, 1H), 4.65 (s, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 186.2, 167.5, 148.1, 145.4, 138.7, 132.8 (2C), 132.4, 128.3 (2C), 125.4, 124.9, 122.6, 114.2, 114.1, 64.8; HRMS (ESI): calcd for $C_{16}H_{12}NaO_3$ $[M+Na]^+$, 275.0684; found, 275.0693.

4.1.7. (Z)-2-(4-Hydroxy-3-methoxybenzylidene)benzofuran-3(2H)-one (**10**)

This compound was obtained from **6** and 4-hydroxy-3-methoxybenzaldehyde employing method A. Yellow solid, yield 73%; purity (gradient I) 98.9%; m.p. 200–201 °C; IR (KBr) ν_{max} 3312, 3061, 2935, 1690, 1639, 1581, 1523, 1365, 1301, 1204, 1117, 1034, 886, 809, 754, 574 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.91 (s, 1H), 7.88–7.75 (m, 2H), 7.63 (s, 1H), 7.58 (d, J = 8.5 Hz, 2H), 7.39–7.29 (m, 1H), 7.10–6.84 (m, 2H), 3.90 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.0, 164.9, 149.4, 147.8, 144.8, 137.0, 126.0, 124.0, 123.7, 123.2, 121.2, 116.1, 115.3, 113.7, 113.2, 55.6; HRMS (ESI): calcd for $C_{16}H_{12}NaO_4$ $[M+Na]^+$, 291.0633; found, 291.0633.

4.1.8. (Z)-2-(3-Hydroxy-4-methoxybenzylidene)benzofuran-3(2H)-one (**11**)

This compound was obtained from **6** and 3-hydroxy-4-methoxybenzaldehyde employing method A. Yellow solid, yield 72%; purity (gradient II) 96.1%; m.p. 185–187 °C; IR (KBr) ν_{max} 3244, 3019, 2958, 2839, 1693, 1639, 1590, 1503, 1455, 1278, 1246, 1188, 1127, 1098, 1024, 879, 802, 754 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.35 (s, 1H), 7.89–7.74 (m, 2H), 7.60–7.51 (m, 2H), 7.44 (d, J = 8.4 Hz, 1H), 7.36–7.29 (m, 1H), 7.07 (d, J = 8.4 Hz, 1H), 6.86 (s, 1H), 3.86 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.2, 165.0, 150.0, 146.6, 145.0, 137.2, 124.8, 124.6, 124.1, 123.7, 121.2, 117.6, 113.3, 113.0, 112.1, 55.6; HRMS (ESI): calcd for $C_{16}H_{12}NaO_4$ $[M+Na]^+$, 291.0633; found, 291.0628.

4.1.9. (Z)-2-(3,4-Dimethoxybenzylidene)benzofuran-3(2H)-one (**12**)

This compound was obtained from **6** and 3,4-dimethoxybenzaldehyde employing method A. Yellow solid, yield 77%; purity (gradient I) 95.1%; m.p. 147–149 °C; IR (KBr) ν_{max} 3061, 2999, 2938, 2835, 1703, 1645, 1594, 1523, 1462, 1327, 1298, 1272, 1204, 1153, 1124, 1014, 883, 805, 757, 558 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.85–7.76 (m, 2H), 7.69–7.61 (m, 2H), 7.58 (d, J = 8.4 Hz, 1H), 7.36–7.29 (m, 1H), 7.12 (d, J = 8.4 Hz, 1H), 6.95 (s, 1H), 3.86 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.2, 165.1, 150.8, 148.8, 145.2, 137.2, 125.7, 124.5, 124.1, 123.8, 121.1, 114.4, 113.2, 113.1, 112.00, 55.6, 55.5; HRMS (ESI): calcd for $C_{17}H_{14}NaO_4$ $[M+Na]^+$, 305.0790; found, 305.0794.

4.1.10. (Z)-2-(4-Hydroxy-3,5-dimethoxybenzylidene)benzofuran-3(2H)-one (**13**)

This compound was obtained from **6** and 4-hydroxy-3,5-dimethoxybenzaldehyde employing method A. Yellow solid, yield 69%; purity (gradient II) 98.6%; m.p. 151–153 °C; IR (KBr) ν_{max} 3511, 3070, 3009, 2938, 2842, 1687, 1642, 1606, 1513, 1455, 1294, 1220, 1134, 1111, 879, 838, 747, 564 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.29 (s, 1H), 7.85–7.75 (m, 2H), 7.59 (d, J = 8.4 Hz, 1H), 7.38 (s, 2H), 7.35–7.28 (m, 1H), 6.93 (s, 1H), 3.86 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.00, 164.9, 148.00 (2C), 144.9, 138.7, 137.1, 124.0, 123.7, 122.0, 121.2, 114.0, 113.3, 109.6 (2C), 56.0 (2C); HRMS (ESI): calcd for $C_{17}H_{14}NaO_4$ $[M+Na]^+$, 321.0739; found, 321.0733.

4.1.11. (Z)-2-(3-Ethoxy-4-hydroxybenzylidene)benzofuran-3(2H)-one (**14**)

This compound was obtained from **6** and 3-ethoxy-4-hydroxybenzaldehyde employing method A. Yellow solid, yield 77%; purity (gradient I) 99.9%; m.p. 121–122 °C; IR (KBr) ν_{max} 3305, 3061, 2980, 2868, 1690, 1642, 1584, 1513, 1478, 1442, 1285, 1253, 1220, 1182, 1130, 1101, 876, 805, 754, 574 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (dd, J = 7.6, 1.4 Hz, 1H), 7.68–7.61 (m, 1H), 7.52–7.44 (m, 2H), 7.35–7.28 (m, 1H), 7.24–7.18 (m, 1H), 7.03–6.98 (m, 1H), 6.86 (s, 1H), 6.08 (s, 1H), 4.22 (q, J = 7.0 Hz, 2H), 1.51 (t, J = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 184.5, 165.7, 147.9, 146.0, 145.7, 136.5, 126.5, 124.8, 124.6, 123.3, 121.9, 114.9, 114.2, 114.0, 112.8, 64.6, 14.8; HRMS (ESI): calcd for $C_{17}H_{14}NaO_4$ $[M+Na]^+$, 305.0790; found, 305.0792.

4.1.12. (Z)-2-(4-Hydroxy-3-propoxybenzylidene)benzofuran-3(2H)-one (**15**)

This compound was obtained from **6** and 4-hydroxy-3-propoxybenzaldehyde employing method A. Yellow solid, yield 74%; purity (gradient I) 96.9%; m.p. 105–107 °C; IR (KBr) ν_{max} 3389, 3064, 2961, 2922, 2851, 1703, 1642, 1584, 1513, 1462, 1282, 1191, 1124, 883, 805, 747 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84–7.78 (m, 1H), 7.67–7.60 (m, 1H), 7.52–7.45 (m, 2H), 7.34–7.29 (m, 1H), 7.25–7.18 (m, 1H), 7.04–6.98 (m, 1H), 6.86 (s, 1H), 6.06 (s, 1H), 4.11 (t, J = 6.6 Hz, 2H), 1.96–1.85 (m, 2H), 1.09 (t, J = 7.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 183.4, 164.7, 147.0, 145.1, 144.7, 135.5, 125.4, 123.8, 123.6, 122.3, 120.9, 113.9, 113.3, 113.0, 111.8, 69.5, 21.5, 9.5; HRMS (ESI): calcd for $C_{18}H_{16}NaO_4$ $[M+Na]^+$, 319.0946; found, 319.0934.

4.1.13. (Z)-2-(4-Hydroxy-3-isopropoxybenzylidene)benzofuran-3(2H)-one (**16**)

This compound was obtained from **6** and 4-hydroxy-3-isopropoxybenzaldehyde employing method A. Yellow solid, yield

70%; purity (gradient I) 97.4%; m.p. 110–111 °C; IR (KBr) ν_{max} 3386, 3067, 2964, 2913, 2871, 1703, 1651, 1603, 1581, 1513, 1462, 1298, 1275, 1191, 1124, 883, 805, 744 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, J = 7.4 Hz, 1H), 7.69 (d, J = 2.1 Hz, 1H), 7.67–7.62 (m, 1H), 7.38–7.31 (m, 2H), 7.24–7.18 (m, 1H), 6.91 (d, J = 8.4 Hz, 1H), 6.84 (s, 1H), 5.78 (s, 1H), 4.75–4.64 (m, 1H), 1.42 (d, J = 6.0 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 184.6, 165.9, 146.5, 146.4, 146.1, 136.6, 125.6, 125.3, 124.6, 123.3, 121.9, 117.0, 113.6, 113.0, 112.6, 71.7, 22.1 (2C); HRMS (ESI): calcd for $C_{18}H_{16}NaO_4$ $[M+Na]^+$, 319.0946; found, 319.0940.

4.1.14. (Z)-2-(3-Butoxy-4-hydroxybenzylidene)benzofuran-3(2H)-one (**17**)

This compound was obtained from **6** and 3-butoxy-4-hydroxybenzaldehyde employing method A. Yellow solid, yield 65%; purity (gradient I) 98.9%; m.p. 126–128 °C; IR (KBr) ν_{max} 3212, 2954, 2922, 2864, 1693, 1642, 1577, 1510, 1436, 1288, 1179, 1130, 883, 815, 760 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 9.76 (s, 1H), 7.84–7.74 (m, 2H), 7.59 (d, J = 2.0 Hz, 1H), 7.58–7.50 (m, 2H), 7.34–7.29 (m, 1H), 6.99–6.88 (m, 2H), 4.06 (t, J = 6.5 Hz, 2H), 1.83–1.70 (m, 2H), 1.56–1.43 (m, 2H), 0.97 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 183.4, 164.7, 146.9, 145.1, 144.7, 135.5, 125.4, 123.8, 123.6, 122.3, 120.9, 113.9, 113.2, 113.0, 111.8, 67.8, 30.1, 18.2, 12.8; HRMS (ESI): calcd for $C_{19}H_{18}NaO_4$ $[M+Na]^+$, 333.1103; found, 333.1081.

4.1.15. (Z)-2-(4-Fluorobenzylidene)benzofuran-3(2H)-one (**18**)

This compound was obtained from **6** and 4-fluorobenzaldehyde employing method A. Yellow solid, yield 76%; purity (gradient I) 97.3%; m.p. 156–157 °C; IR (KBr) ν_{max} 3064, 1713, 1658, 1597, 1510, 1458, 1301, 1236, 1188, 1124, 1098, 879, 831, 751, 696, 571, 503 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 8.13–8.02 (m, 2H), 7.88–7.76 (m, 2H), 7.57 (d, J = 8.3 Hz, 1H), 7.44–7.29 (m, 3H), 6.99 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.5, 165.4, 162.7 ($J_{C\text{-}F}$ = 250.8 Hz, 1C), 146.0 ($J_{C\text{-}F}$ = 2.6 Hz, 1C), 137.7, 133.7 ($J_{C\text{-}F}$ = 8.7 Hz, 2C), 128.6 ($J_{C\text{-}F}$ = 3.2 Hz, 1C), 124.3, 124.0, 120.8, 116.1 ($J_{C\text{-}F}$ = 11.8 Hz, 2C), 113.2, 111.1; HRMS (ESI): calcd for $C_{15}H_9FNaO_2$ $[M+Na]^+$, 263.0484; found, 263.0480.

4.1.16. (Z)-2-(Pyridin-3-ylmethylene)benzofuran-3(2H)-one (**19**)

This compound was obtained from **6** and nicotinaldehyde employing method B. Yellow solid, yield 83%; purity (gradient I) 99.1%; m.p. 123–124 °C; IR (KBr) ν_{max} 3041, 1713, 1664, 1600, 1478, 1455, 1423, 1298, 1179, 1117, 1024, 883, 760, 696, 561 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 9.11 (s, 1H), 8.62 (d, J = 4.7 Hz, 1H), 8.40 (d, J = 8.0 Hz, 1H), 7.98–7.72 (m, 2H), 7.69–7.48 (m, 2H), 7.43–7.29 (m, 1H), 7.01 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.4, 165.5, 151.9, 150.1, 147.4, 137.9, 137.6, 128.2, 124.4, 124.1, 124.0, 120.6, 113.3, 108.6; HRMS (ESI): calcd for $C_{14}H_{10}NO_2$ $[M+H]^+$, 224.0712; found, 224.0700.

4.1.17. (Z)-2-(Furan-2-ylmethylene)benzofuran-3(2H)-one (**20**)

This compound was obtained from **6** and furan-2-carbaldehyde employing method B. Yellow solid, yield 80%; purity (gradient I) 98.6%; m.p. 118–119 °C; IR (KBr) ν_{max} 3131, 3106, 3057, 1706, 1651, 1600, 1468, 1298, 1201, 1114, 1101, 1018, 963, 879, 747, 696 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 8.03 (s, 1H), 7.88–7.75 (m, 2H), 7.56 (d, J = 8.3 Hz, 1H), 7.38–7.29 (m, 1H), 7.25 (d, J = 3.4 Hz, 1H), 6.92 (s, 1H), 6.79 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.9, 165.1, 148.0, 146.8, 144.1, 137.4, 124.1, 123.9, 121.2, 118.2, 113.5, 113.1, 100.8; HRMS (ESI): calcd for $C_{13}H_8NaO_3$ $[M+Na]^+$, 235.0371; found, 235.0376.

4.1.18. 2-(3,4-Dihydroxybenzylidene)-2,3-dihydro-1H-inden-1-one (**23**)

To a solution of 3,4-dihydroxybenzaldehyde (2.0 mmol) and 1-indanone [13] (**22**, 2.0 mmol) in ethanol (30 mL) was added 5% aqueous solution of sodium hydroxide (10.0 mmol) dropwise at room temperature. After stirring for 5 h, the reaction mixture was diluted with water (40 mL) and extracted with ethyl acetate (3 × 30 mL). The combined organic phases were washed with brine, dried over anhydrous Na_2SO_4 and evaporated under vacuum. The residue was purified by flash chromatography on silica gel (petroleum ether/ethyl acetate = 1/1) to provide the title compound. Yellow solid, yield 42%; purity (gradient II) 95.2%; m.p. > 250 °C; IR (KBr) ν_{max} 3463, 3215, 3048, 1677, 1594, 1571, 1532, 1446, 1333, 1291, 1265, 1179, 1124, 1108, 986, 928, 786, 735, 677, 564 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 9.64 (s, 1H), 9.35 (s, 1H), 7.80 (d, J = 7.5 Hz, 1H), 7.71 (s, 2H), 7.57–7.47 (m, 1H), 7.43 (s, 1H), 7.27 (s, 1H), 7.16 (d, J = 8.6 Hz, 1H), 6.91 (d, J = 8.4 Hz, 1H), 4.06 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 193.1, 149.7, 148.0, 145.6, 137.6, 134.4, 133.8, 131.3, 127.5, 126.6, 126.4, 124.3, 123.3, 117.5, 116.0, 32.0; HRMS (ESI): calcd for $C_{16}H_{11}O_3$ $[M-H]^-$, 251.0708; found, 251.0711.

4.1.19. Benzofuran-2-yl(3,4-dihydroxyphenyl)methanone (**26**)

To a solution of 2-bromo-1-(3,4-dihydroxyphenyl) ethan-1-one (**25**, 1.0 mmol) and 2-hydroxybenzaldehyde (1.0 mmol) in acetone (40 mL) was added anhydrous potassium carbonate (3.0 mmol). After refluxing for 20 h the reaction mixture was cooled to rt concentrated under vacuum. The residue was added water (30 mL) and extracted with ethyl acetate (3 × 20 mL). The combined organic phases were washed with brine, dried over anhydrous Na_2SO_4 and evaporated under vacuum. The residue was purified by flash chromatography on silica gel to provide the title compound (dichloromethane/methanol = 80/1). Brown solid, yield 35%; purity (gradient II) 98.3%; m.p. 165–168 °C; IR (KBr) ν_{max} 3463, 3305, 3119, 1594, 1545, 1446, 1294, 1191, 937, 847, 773, 738 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 10.05 (s, 1H), 9.54 (s, 1H), 7.86 (d, J = 7.8 Hz, 1H), 7.76 (d, J = 8.5 Hz, 1H), 7.72 (s, 1H), 7.63–7.46 (m, 3H), 7.44–7.33 (m, 1H), 6.94 (d, J = 8.2 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 181.6, 154.9, 152.0, 151.1, 145.4, 128.0, 127.9, 126.8, 123.9, 123.5, 122.8, 116.3, 115.3, 115.2, 112.1; HRMS (ESI): calcd for $C_{15}H_{10}NaO_4$ $[M+Na]^+$, 277.0477; found, 277.0464.

4.1.20. 2-(3,4-Dihydroxybenzyl)benzofuran-3(2H)-one (**29**)

To a solution of **6** (1.0 mmol) and 3,4-bis(benzyloxy)benzaldehyde (**28**, 1.0 mmol) in dichloromethane (6 mL) was added aluminum oxide (30.0 mmol) at room temperature. After stirring for 5 h, the reaction mixture was filtered off. The filtrate was concentrated under vacuum to give crude product. In a round-bottom flash, the residue was dissolved in methanol (20 mL), and 10% Pd/C (0.1 mmol) was added. A H_2 balloon was connected after the atmosphere was exchanged three times with nitrogen. After stirring for 5 h, the catalyst was filtered off. The filtrate was concentrated in vacuo and the residue was purified by flash chromatography on silica gel (petroleum ether/ethyl acetate = 3/1) to afford the title compound. Light yellow solid, yield 72%; purity (gradient II) 96.4%; m.p. 130–132 °C; IR (KBr) ν_{max} 3299, 2919, 1693, 1610, 1516, 1481, 1462, 1330, 1288, 1195, 1114, 764 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 8.74 (s, 1H), 8.68 (s, 1H), 7.72–7.64 (m, 1H), 7.62–7.53 (m, 1H), 7.25–7.18 (m, 1H), 7.13–7.04 (m, 1H), 6.64 (d, J = 2.1 Hz, 1H), 6.60–6.55 (m, 1H), 6.52–6.47 (m, 1H), 4.96 (dd, J = 7.8, 4.0 Hz, 1H), 3.08 (dd, J = 14.7, 4.0 Hz, 1H), 2.80 (dd, J = 14.7, 7.8 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 203.6, 174.4, 146.0, 145.2, 139.5, 128.4, 124.8, 122.9, 122.3, 122.0, 117.8, 116.1, 114.5, 87.4, 37.6; HRMS (ESI): calcd for $C_{15}H_{12}NaO_4$ $[M+Na]^+$, 279.0633; found, 279.0627.

4.1.21. 4-Methoxybenzofuran-3(2H)-one (**32**)

To a solution of 4-hydroxybenzofuran-3(2*H*)-one (**31**, 2.0 mmol) in anhydrous DMF (10 mL) was added successively anhydrous

potassium carbonate (3.0 mmol) and iodomethane (2.2 mmol). After stirring for 3 h at 80 °C, the reaction mixture was cooled to rt, quenched with water (30 mL), and extracted with dichloromethane (3 × 20 mL). The combined organic phases were washed with brine, dried over anhydrous Na_2SO_4 and evaporated under vacuum. The residue was purified by flash chromatography on silica gel (petroleum ether/ethyl acetate = 30/1) to afford the title compound. Bright yellow solid, yield 93%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49–7.40 (m, 1H), 6.61 (d, J = 8.4 Hz, 1H), 6.40 (d, J = 8.4 Hz, 1H), 4.52 (s, 2H), 3.89 (s, 3H).

4.1.22. (Z)-2-(3-Ethoxy-4-hydroxybenzylidene)-4-methoxybenzofuran-3(2H)-one (**33**)

This compound was obtained from **32** and 3-ethoxy-4-hydroxybenzaldehyde employing method A. Yellow solid, yield 73%; purity (gradient I) 97.3%; m.p. 177–178 °C; IR (KBr) ν_{max} 3302, 3009, 2977, 2925, 1693, 1642, 1581, 1510, 1491, 1433, 1294, 1256, 1169, 1079, 886, 796, 760 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.69 (s, 1H), 7.77–7.65 (m, 1H), 7.54 (s, 1H), 7.48 (d, J = 8.2 Hz, 1H), 7.04 (d, J = 8.2 Hz, 1H), 6.92 (d, J = 8.2 Hz, 1H), 6.82 (d, J = 8.2 Hz, 1H), 6.76 (s, 1H), 4.11 (q, J = 6.9 Hz, 2H), 3.93 (s, 3H), 1.39 (t, J = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 180.5, 165.8, 157.8, 149.2, 146.8, 144.7, 138.6, 125.5, 123.3, 116.4, 116.1, 112.1, 110.1, 105.8, 104.6, 63.9, 56.0, 14.7; HRMS (ESI): calcd for $C_{18}H_{16}NaO_5$ [M+Na]$^+$, 335.0895; found, 335.0890.

4.1.23. 2-Chloro-1-(2-hydroxy-5-methoxyphenyl)ethan-1-one (**35**)

To a dichloromethane solution of boron trichloride (12.0 mmol) was added dropwise a solution of 4-methoxyphenol (**34**, 10.0 mmol) in dichloromethane (20 mL) at 0 °C under nitrogen. And then, chloroacetonitrile (12.0 mmol) was added dropwise, followed by aluminum chloride (5.0 mmol) in one portion. The reaction mixture was allowed to warm to room temperature and was stirred for 6 h until the starting material disappeared completely. The reaction mixture was quenched with 1 N hydrochloric acid at 0 °C. After stirring for 10 min, the aqueous layer was extracted with dichloromethane (3 × 30 mL). The combined organic layers were washed with brine, dried with anhydrous Na_2SO_4 and evaporated under reduced pressure. The crude mixture was purified by silica gel column chromatography (petroleum ether/ethyl acetate = 30/1) to afford the desired compound **35**. Yellow solid, yield 66%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.32 (s, 1H), 7.18 (dd, J = 9.1, 2.8 Hz, 1H), 7.11 (d, J = 2.4 Hz, 1H), 6.98 (dd, J = 9.1, 1.9 Hz, 1H), 4.70 (s, 2H), 3.81 (s, 3H).

4.1.24. 3-Oxo-2,3-dihydrobenzofuran-5-yl acetate (**39**)

To a solution of 5-hydroxybenzofuran- 3(2*H*)-one (**38**, 1.0 mmol) in dichloromethane (20 mL) was added acetic anhydride (1.5 mmol) and triethylamine (2.0 mmol) at the rt. After stirring for 1 h, the reaction mixture was poured into water (30 mL) and extracted with ethyl acetate (3 × 15 mL). The combined organic phases were washed with saturated sodium bicarbonate solution, brine, dried over anhydrous Na_2SO_4, and evaporated under vacuum. The residue was purified by flash chromatography on silica gel (petroleum ether/ethyl acetate = 20/1) to afford the title compound. Yellow solid, yield 88%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 (d, J = 2.5 Hz, 1H), 7.33 (dd, J = 8.9, 2.6 Hz, 1H), 7.14 (d, J = 8.9 Hz, 1H), 4.67 (s, 2H), 2.31 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.1, 171.4, 169.5, 145.2, 131.7, 121.5, 116.3, 114.3, 75.6, 21.0.

4.1.25. (Z)-2-(3-Ethoxy-4-hydroxybenzylidene)-5-methoxybenzofuran-3(2H)-one (**40**)

This compound was obtained from **37** and 3-ethoxy-4-hydroxybenzaldehyde employing method A. Yellow solid, yield 78%; purity (gradient I) 98.0%; m.p. 149–151 °C; IR (KBr) ν_{max} 3527, 3064, 2980, 2935, 1690, 1642, 1581, 1516, 1484, 1310, 1275, 1195, 1117, 905, 809, 735 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.77 (s, 1H), 7.57 (s, 1H), 7.54–7.45 (m, 2H), 7.40–7.33 (m, 1H), 7.26–7.20 (m, 1H), 6.92 (dd, J = 8.3, 2.6 Hz, 1H), 6.88 (d, J = 2.6 Hz, 1H), 4.11 (q, J = 6.7 Hz, 2H), 3.82 (s, 3H), 1.38 (t, J = 6.7 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.0, 159.8, 155.7, 150.4, 147.0, 145.4, 126.2, 125.4, 122.8, 121.5, 116.6, 116.3, 114.1, 114.0, 105.2, 63.9, 55.8, 14.7; HRMS (ESI): calcd for $C_{18}H_{16}NaO_5$ [M+Na]$^+$, 335.0895; found, 335.0881.

4.1.26. (Z)-2-(3,4-Dihydroxybenzylidene)-5-methoxybenzofuran-3(2H)-one (**41**)

This compound was obtained from **37** and 3,4-dihydroxybenzaldehyde employing method B. Yellow solid, yield 33%; purity (gradient II) 99.8%; m.p. 234–236 °C; IR (KBr) ν_{max} 3466, 3312, 2977, 2938, 2845, 1684, 1639, 1584, 1526, 1487, 1429, 1320, 1278, 1201, 1166, 1127, 1021, 899, 809, 741 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.76 (s, 1H), 9.25 (s, 1H), 7.68 (d, J = 8.5 Hz, 1H), 7.47 (s, 1H), 7.31 (d, J = 8.3 Hz, 1H), 7.08 (s, 1H), 6.95–6.78 (m, 2H), 6.71 (s, 1H), 3.93 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.1, 159.9, 155.7, 148.5, 145.6, 145.4, 125.5, 125.1, 123.3, 121.5, 118.2, 116.0, 113.9 (2C), 105.3, 55.8; HRMS (ESI): calcd for $C_{16}H_{12}NaO_5$ [M+Na]$^+$, 307.0582; found, 307.0582.

4.1.27. (Z)-2-(3,4-Dihydroxybenzylidene)-5-hydroxybenzofuran-3(2H)-one (**42**)

This compound was obtained from **38** and 3,4-dihydroxybenzaldehyde employing method B. Yellow solid, yield 28%; purity (gradient II) 96.2%; m.p. >270 °C; IR (KBr) ν_{max} 3447, 3250, 1690, 1639, 1590, 1513, 1494, 1391, 1291, 1230, 1191, 1159, 1121, 895, 818 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.76 (s, 2H), 9.31 (s, 1H), 7.48 (d, J = 2.1 Hz, 1H), 7.35 (d, J = 8.8 Hz, 1H), 7.29 (dd, J = 8.3, 2.1 Hz, 1H), 7.21 (dd, J = 8.8, 2.7 Hz, 1H), 7.01 (d, J = 2.7 Hz, 1H), 6.85 (d, J = 8.3 Hz, 1H), 6.74 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.3, 158.8, 153.6, 148.4, 145.5, 145.4, 125.2, 125.0, 123.3, 121.6, 118.1, 116.0, 113.6, 113.4, 107.5; HRMS (ESI): calcd for $C_{15}H_9O_5$ [M−H]$^-$, 269.0450; found, 269.0453.

4.1.28. (Z)-2-(3-Ethoxy-4-hydroxybenzylidene)-3-oxo-2,3-dihydrobenzofuran-5-yl acetate (**43**)

This compound was obtained from **39** and 3-ethoxy-4-hydroxybenzaldehyde employing method A. Yellow solid, yield 63%; purity (gradient I) 99.1%; m.p. 185–187 °C; IR (KBr) ν_{max} 3466, 3070, 2974, 2922, 2877, 1751, 1697, 1642, 1581, 1516, 1478, 1368, 1301, 1265, 1227, 1175, 1111, 1040, 908, 828 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.83 (s, 1H), 7.85–7.35 (m, 5H), 7.07–6.80 (m, 2H), 4.10 (q, J = 7.0 Hz, 2H), 2.28 (s, 3H), 1.37 (t, J = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.5, 169.3, 162.2, 149.8, 146.9, 146.2, 145.3, 130.8, 126.2, 123.1, 121.7, 116.7 (2C), 116.2, 114.4, 114.0, 63.9, 20.8, 14.7; HRMS (ESI): calcd for $C_{19}H_{16}NaO_6$ [M+Na]$^+$, 363.0845; found, 363.0841.

4.1.29. (Z)-5-Ethoxy-2-(3-ethoxy-4-hydroxybenzylidene) benzofuran-3(2H)-one (**45**)

This compound was synthesized from 4-ethoxyphenol and 3-ethoxy-4-hydroxybenzaldehyde according to the methodology described for **40**. Yellow solid, yield 27%; purity (gradient I) 99.0%; m.p. 164–166 °C; IR (KBr) ν_{max} 3537, 3070, 2980, 2929, 2874, 1684, 1639, 1577, 1516, 1491, 1468, 1310, 1278, 1208, 1182, 1114, 1047, 908, 818, 738, 503 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.79 (s, 1H), 7.56 (d, J = 2.0 Hz, 1H), 7.52 (dd, J = 8.2, 2.0 Hz, 1H), 7.49–7.42 (m, 1H), 7.37–7.29 (m, 1H), 7.19 (d, J = 2.8 Hz, 1H), 6.94 (d, J = 8.3 Hz, 1H), 6.87 (s, 1H), 4.17–4.00 (m, 4H), 1.46–1.29 (m, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.1, 159.8, 154.9, 149.6, 146.9, 145.6, 126.0, 125.9, 123.3, 121.5, 116.6, 116.1, 114.1, 113.7, 105.9, 63.9, 63.9, 14.7, 14.5; HRMS (ESI): calcd for $C_{19}H_{18}NaO_5$ [M+Na]$^+$, 349.1052; found,

349.1036.

*4.1.30. (Z)-5-Butoxy-2-(3-ethoxy-4-hydroxybenzylidene) benzofuran-3(2H)-one (**46**)*

This compound was obtained from 4-butoxyphenol and 3-ethoxy-4-hydroxybenzaldehyde according to the methodology described for **40**. Yellow solid, yield 25%; purity (gradient I) 98.8%; m.p. 182–185 °C; IR (KBr) ν_{max} 3389, 3340, 3057, 2961, 2929, 2871, 1703, 1645, 1584, 1520, 1487, 1426, 1394, 1282, 1182, 1108, 1043, 912, 828 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 9.73 (s, 1H), 7.69–7.41 (m, 3H), 7.38–7.30 (m, 1H), 7.19 (d, J = 2.7 Hz, 1H), 6.94 (d, J = 8.4 Hz, 1H), 6.86 (s, 1H), 4.10 (q, J = 7.2 Hz, 2H), 4.00 (t, J = 6.5 Hz, 2H), 1.79–1.61 (m, 2H), 1.51–1.31 (m, 5H), 0.93 (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.1, 159.8, 155.1, 149.9, 146.9, 145.5, 126.0, 125.9, 123.1, 121.5, 116.6, 116.2, 114.1, 113.7, 105.8, 68.0, 63.9, 30.6, 18.7, 14.7, 13.6; HRMS (ESI): calcd for $C_{21}H_{22}NaO_5$ $[M+Na]^+$, 377.1365; found, 377.1362.

*4.1.31. (Z)-2-(3-Ethoxy-4-hydroxybenzylidene)-5-methylbenzofuran-3(2H)-one (**47**)*

This compound was obtained from p-cresol and 3-ethoxy-4-hydroxybenzaldehyde according to the methodology described for **40**. Yellow solid, yield 28%; purity (gradient I) 99.1%; m.p. 172–174 °C; IR (KBr) ν_{max} 3530, 3073, 2977, 2938, 1700, 1639, 1613, 1577, 1513, 1487, 1471, 1301, 1278, 1191, 1153, 1124, 1043, 908, 802, 735, 500 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 9.78 (s, 1H), 7.65–7.48 (m, 4H), 7.47–7.38 (m, 1H), 6.94 (d, J = 8.2 Hz, 1H), 6.86 (s, 1H), 4.12 (q, J = 6.9 Hz, 2H), 2.37 (s, 3H), 1.40 (t, J = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.0, 163.3, 149.9, 146.9, 145.1, 137.9, 132.9, 126.0, 123.4, 123.1, 121.2, 116.5, 116.2, 113.5, 112.7, 63.9, 20.2, 14.7; HRMS (ESI): calcd for $C_{18}H_{16}NaO_4$ $[M+Na]^+$, 319.0946; found, 319.0951.

*4.1.32. 5-Fluorobenzofuran-3(2H)-one (**53**)*

To a solution of 1-fluoro-4-methoxybenzene (**48**, 10.0 mmol) in dichloromethane (30 mL) was added chloroacetyl chloride (11.0 mmol) at 0 °C. After stirring for 15 min, the reaction mixture was added anhydrous aluminium chloride (11.0 mmol) over 30 min. After stirring for 6 h at 40 °C, the reaction mixture was cool to rt. After stirring for 72 h at rt, the reaction mixture was poured into ice water (50 mL) and extracted with dichloromethane (3 × 30 mL). The combined organic phases were washed with brine, dried over anhydrous Na_2SO_4, and evaporated under vacuum without further purification. To a solution of the residue in ethanol (30 mL) was added anhydrous sodium acetate (20.0 mmol). After refluxing for 2 h, the reaction mixture was cooled to rt and concentrated under vacuum. The residue was dissolved in water (50 mL) and extracted with dichloromethane (3 × 40 mL). The combined organic phases were washed with brine, dried over anhydrous Na_2SO_4, and evaporated under vacuum. The crude mixture was purified by flash chromatography on silica gel (petroleum ether/ethyl acetate = 40/1) to afford the title compound. Bright yellow solid, yield 60%; ^1H NMR (400 MHz, $CDCl_3$) δ 7.36–7.16 (m, 2H), 7.04 (d, J = 8.8 Hz, 1H), 4.61 (s, 2H).

*4.1.33. 5-Chlorobenzofuran-3(2H)-one (**54**)*

This compound was obtained from **49** according to the methodology described for **53**. Bright yellow solid, yield 55%; ^1H NMR (400 MHz, $CDCl_3$) δ 7.64 (d, J = 2.4 Hz, 1H), 7.56 (dd, J = 8.8, 2.3 Hz, 1H), 7.11 (d, J = 8.8 Hz, 1H), 4.67 (s, 2H).

*4.1.34. 5-Bromobenzofuran-3(2H)-one (**55**)*

This compound was obtained from **50** according to the methodology described for **53**. Bright yellow solid, yield 53%; ^1H NMR (400 MHz, $CDCl_3$) δ 7.79 (d, J = 2.2 Hz, 1H), 7.69 (dd, J = 8.8, 2.2 Hz, 1H), 7.06 (d, J = 8.8 Hz, 1H), 4.67 (s, 2H).

*4.1.35. 5-Iodobenzofuran-3(2H)-one (**56**)*

This compound was obtained from **51** according to the methodology described for **53**. Bright yellow solid, yield 62%; ^1H NMR (400 MHz, $CDCl_3$) δ 7.98 (d, J = 2.0 Hz, 1H), 7.85 (dd, J = 8.7, 2.0 Hz, 1H), 6.96 (d, J = 8.7 Hz, 1H), 4.65 (s, 2H).

*4.1.36. 5-Nitrobenzofuran-3(2H)-one (**57**)*

This compound was obtained from **52** according to the methodology described for **53**. Bright yellow solid, yield 22%; ^1H NMR (400 MHz, DMSO-d_6) δ 8.56 (dd, J = 9.2, 2.5 Hz, 1H), 8.40 (d, J = 2.5 Hz, 1H), 7.52 (d, J = 9.1 Hz, 1H), 5.04 (s, 2H).

*4.1.37. 5-Aminobenzofuran-3(2H)-one (**58**)*

To a solution of 5-nitrobenzofuran-3(2*H*)-one (**57**, 2.0 mmol) in acetic acid (20 mL) was added iron (10.0 mmol). After stirring for 3 h at 70 °C, the reaction mixture was cooled to rt and filtered off. The filtrate was neutralized by adding the saturated sodium bicarbonate solution until pH = 10 and extracted with ethyl acetate (3 × 40 mL). The combined organic phases were washed with brine, dried over anhydrous Na_2SO_4, and evaporated under vacuum. The crude mixture was purified by flash chromatography on silica gel (petroleum ether/ethyl acetate = 4/1) to afford the title compound. Yellow solid, yield 77%; ^1H NMR (400 MHz, DMSO-d_6) δ 7.08–6.94 (m, 2H), 6.69 (d, J = 2.3 Hz, 1H), 5.06 (s, 2H), 4.66 (s, 2H).

*4.1.38. (Z)-2-(3-Ethoxy-4-hydroxybenzylidene)-5-fluorobenzofuran-3(2H)-one (**59**)*

This compound was obtained from **53** and 3-ethoxy-4-hydroxybenzaldehyde employing method A. Yellow solid, yield 78%; purity (gradient I) 99.3%; m.p. 157–158 °C; IR (KBr) ν_{max} 3517, 2987, 2942, 2893, 1693, 1645, 1577, 1516, 1481, 1436, 1307, 1285, 1259, 1175, 1105, 1037, 902, 812, 783, 738, 503 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 9.87 (s, 1H), 7.74–7.48 (m, 5H), 7.01–6.87 (m, 2H), 4.12 (q, J = 6.9 Hz, 2H), 1.39 (t, J = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.5 ($J_{C\text{-}F}$ = 3.2 Hz, 1C), 161.1, 158.2 ($J_{C\text{-}F}$ = 242.1 Hz, 1C), 149.8, 146.9, 145.4, 126.2, 124.3 ($J_{C\text{-}F}$ = 26.1 Hz, 1C), 123.1, 122.0 ($J_{C\text{-}F}$ = 8.3 Hz, 1C), 116.6, 116.1, 114.9, 114.8 ($J_{C\text{-}F}$ = 8.3 Hz, 1C), 109.6 ($J_{C\text{-}F}$ = 24.4 Hz, 1C), 63.9, 14.7; HRMS (ESI): calcd for $C_{17}H_{13}FNaO_4$ $[M+Na]^+$, 323.0696; found, 323.0702.

*4.1.39. (Z)-5-Chloro-2-(3-ethoxy-4-hydroxybenzylidene) benzofuran-3(2H)-one (**60**)*

This compound was obtained from **54** and 3-ethoxy-4-hydroxybenzaldehyde employing method A. Yellow solid, yield 24%; purity (gradient I) 99.9%; m.p. 202–203 °C; IR (KBr) ν_{max} 3389, 3070, 2971, 2925, 2880, 1706, 1648, 1577, 1513, 1458, 1317, 1262, 1175, 1127, 1047, 918, 805, 709, 625, 502 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 9.86 (s, 1H), 7.78 (s, 2H), 7.69–7.42 (m, 3H), 6.92 (s, 2H), 4.10 (q, J = 7.4 Hz, 2H), 1.37 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 181.8, 163.3, 149.9, 146.9, 145.0, 136.4, 127.9, 126.3, 123.3, 123.0, 122.7, 116.7, 116.2, 115.1, 114.9, 63.9, 14.7; HRMS (ESI): calcd for $C_{17}H_{13}ClNaO_4$ $[M+Na]^+$, 339.0400; found, 339.0407.

*4.1.40. (Z)-5-Bromo-2-(3-ethoxy-4-hydroxybenzylidene) benzofuran-3(2H)-one (**61**)*

This compound was obtained from **55** and 3-ethoxy-4-hydroxybenzaldehyde employing method A. Yellow solid, yield 30%; purity (gradient I) 99.0%; m.p. 207–209 °C; IR (KBr) ν_{max} 3376, 3067, 2971, 2925, 2877, 1709, 1648, 1600, 1581, 1513, 1462, 1282, 1201, 1182, 1130, 1047, 921, 805, 696, 622 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.92 (d, J = 2.2 Hz, 1H), 7.72 (dd, J = 8.7, 2.2 Hz, 1H), 7.51–7.39 (m, 2H), 7.23 (d, J = 8.7 Hz, 1H), 7.00 (d, J = 8.3 Hz, 1H), 6.87 (s, 1H), 6.06 (s, 1H), 4.21 (q, J = 7.0 Hz, 2H), 1.51 (t, J = 7.0 Hz,

3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 181.6, 163.6, 149.9, 146.9, 144.8, 139.1, 126.3 (2C), 123.2, 123.0, 116.6, 116.1, 115.6, 115.5, 114.9, 63.8, 14.7; HRMS (ESI): calcd for $C_{17}H_{13}BrNaO_4$ $[M+Na]^+$, 382.9895; found, 382.9897.

4.1.41. (Z)-2-(3-Ethoxy-4-hydroxybenzylidene)-5-iodobenzofuran-3(2H)-one (**62**)

This compound was obtained from **56** and 3-ethoxy-4-hydroxybenzaldehyde employing method A. Yellow solid, yield 24%; purity (gradient I) 95.0%; m.p. 215–216 °C; IR (KBr) ν_{max} 3534, 3070, 2974, 2929, 1700, 1642, 1597, 1581, 1516, 1458, 1265, 1195, 1175, 1127, 1040, 918, 812, 693, 587, 500 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.88 (s, 1H), 8.14–7.98 (m, 2H), 7.58 (s, 1H), 7.52 (d, J = 8.3 Hz, 1H), 7.43 (d, J = 8.5 Hz, 1H), 6.93 (s, 2H), 4.11 (q, J = 7.0 Hz, 2H), 1.39 (t, J = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 181.4, 164.2, 149.9, 146.9, 144.7, 144.4, 132.1, 126.3, 123.7, 123.0, 116.5, 116.1, 115.8, 114.6, 87.3, 63.9, 14.7; HRMS (ESI): calcd for $C_{17}H_{13}INaO_4$ $[M+Na]^+$, 430.9756; found, 430.9734.

4.1.42. (Z)-5-Amino-2-(3-ethoxy-4-hydroxybenzylidene) benzofuran-3(2H)-one (**63**)

This compound was obtained from **58** and 3-ethoxy-4-hydroxybenzaldehyde employing method A. Yellow solid, yield 60%; purity (gradient II) 97.4%; m.p. 213–216 °C; IR (KBr) ν_{max} 3386, 3308, 2980, 2909, 2874, 1693, 1642, 1584, 1513, 1494, 1436, 1391, 1288, 1259, 1198, 1153, 1105, 1047, 895, 860, 812, 786, 738 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.70 (s, 1H), 7.55 (s, 1H), 7.52–7.45 (m, 1H), 7.25 (d, J = 8.7 Hz, 1H), 7.08–7.00 (m, 1H), 6.92 (d, J = 8.2 Hz, 1H), 6.83 (d, J = 2.3 Hz, 1H), 6.77 (s, 1H), 5.23 (s, 2H), 4.10 (q, J = 7.0 Hz, 2H), 1.38 (t, J = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.7, 157.7, 149.2, 146.8, 145.6, 145.3, 125.6, 124.2, 123.6, 121.4, 116.4, 116.1, 113.1, 112.4, 105.3, 63.9, 14.7; HRMS (ESI): calcd for $C_{17}H_{15}NNaO_4$ $[M+Na]^+$, 320.0899; found, 320.0895.

4.1.43. (Z)-2-(3,4-Dihydroxybenzylidene)-6-hydroxybenzofuran-3(2H)-one (**67**)

This compound was obtained from **65** and 3,4-dihydroxybenzaldehyde employing method B. Yellow solid, yield 44%; purity (gradient II) 98.0%; m.p. > 270 °C; IR (KBr) ν_{max} 3305, 3154, 1680, 1597, 1584, 1510, 1307, 1282, 1191, 1130, 1108, 989, 892, 831, 767, 661, 513 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.11 (s, 1H), 9.68 (s, 1H), 9.28 (s, 1H), 7.61 (d, J = 8.4 Hz, 1H), 7.46 (s, 1H), 7.25 (d, J = 8.2 Hz, 1H), 6.85 (d, J = 8.2 Hz, 1H), 6.80–6.67 (m, 2H), 6.64 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 181.1, 167.4, 166.0, 147.9, 145.6, 145.5, 125.7, 124.5, 123.4, 117.9, 116.0, 113.2, 112.8, 111.9, 98.3; HRMS (ESI): calcd for $C_{15}H_9O_5$ $[M-H]^-$, 269.0450; found, 269.0454.

4.1.44. (Z)-2-(3-Ethoxy-4-hydroxybenzylidene)-6-methoxybenzofuran-3(2H)-one (**68**)

This compound was obtained from **66** and 3-ethoxy-4-hydroxybenzaldehyde employing method A. Yellow solid, yield 72%; purity (gradient I) 99.7%; m.p. 182–183 °C; IR (KBr) ν_{max} 3215, 2971, 2922, 1687, 1642, 1594, 1510, 1429, 1301, 1272, 1191, 1137, 1098, 1011, 912, 821 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75–7.67 (m, 1H), 7.47 (dd, J = 8.3, 1.9 Hz, 1H), 7.44–7.39 (m, 1H), 6.99 (d, J = 8.3 Hz, 1H), 6.83–6.70 (m, 3H), 6.01 (s, 1H), 4.22 (q, J = 7.0 Hz, 2H), 3.93 (s, 3H), 1.51 (t, J = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.8, 167.1, 166.1, 146.7, 145.6, 144.9, 125.1, 124.7, 123.8, 114.1, 113.9, 113.2, 111.7, 111.0, 95.6, 63.6, 55.0, 13.8; HRMS (ESI): calcd for $C_{18}H_{16}NaO_5$ $[M+Na]^+$, 335.0895; found, 335.0877.

4.1.45. 6-Fluorobenzofuran-3(2H)-one (**71**)

This compound was obtained from **69** according to the methodology described for the synthesis of **6**. Yellow solid, yield 42%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.77–7.66 (m, 1H), 7.28–7.19 (m, 1H), 7.10–6.91 (m, 1H), 4.86 (s, 2H).

4.1.46. 6-Chlorobenzofuran-3(2H)-one (**72**)

This compound was obtained from **70** according to the methodology described for the synthesis of **6**. Yellow solid, yield 45%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (d, J = 8.3 Hz, 1H), 7.17 (d, J = 1.6 Hz, 1H), 7.08 (dd, J = 8.3, 1.6 Hz, 1H), 4.66 (s, 2H).

4.1.47. (Z)-2-(3-Ethoxy-4-hydroxybenzylidene)-6-fluorobenzofuran-3(2H)-one (**73**)

This compound was obtained from **71** and 3-ethoxy-4-hydroxybenzaldehyde employing method A. Yellow solid, yield 80%; purity (gradient I) 96.6%; m.p. 141–142 °C; IR (KBr) ν_{max} 3553, 3488, 3090, 2977, 2797, 1693, 1645, 1600, 1590, 1516, 1436, 1410, 1288, 1265, 1172, 1124, 1095, 1043, 963, 895, 809, 760, 657, 509 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88–7.71 (m, 1H), 7.54–7.36 (m, 2H), 7.10–6.88 (m, 3H), 6.85 (s, 1H), 6.06 (s, 1H), 4.22 (q, J = 7.0 Hz, 2H), 1.52 (t, J = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.7, 167.1 ($J_{C\text{-}F}$ = 243.5 Hz, 1C), 165.8, 147.1, 145.1, 145.0, 125.6, 125.4 ($J_{C\text{-}F}$ = 11.8 Hz, 1C), 123.4, 117.6, 114.0, 113.3, 113.2, 110.8 ($J_{C\text{-}F}$ = 24.0 Hz, 1C), 99.7 ($J_{C\text{-}F}$ = 26.7 Hz, 1C), 63.7, 13.8; HRMS (ESI): calcd for $C_{17}H_{13}FNaO_4$ $[M+Na]^+$, 323.0696; found, 323.0676.

4.1.48. (Z)-6-Chloro-2-(3-ethoxy-4-hydroxybenzylidene) benzofuran-3(2H)-one (**74**)

This compound was obtained from **72** and 3-ethoxy-4-hydroxybenzaldehyde employing method A. Yellow solid, yield 80%; purity (gradient I) 99.1%; m.p. 175–176 °C; IR (KBr) ν_{max} 3534, 3067, 2977, 2929, 1690, 1645, 1603, 1581, 1513, 1423, 1304, 1269, 1191, 1137, 1121, 1063, 921, 857, 805, 770, 622, 500 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (d, J = 8.1 Hz, 1H), 7.53–7.39 (m, 2H), 7.34 (s, 1H), 7.20 (d, J = 8.2 Hz, 1H), 7.00 (d, J = 8.1 Hz, 1H), 6.86 (s, 1H), 6.08 (s, 1H), 4.21 (q, J = 6.9 Hz, 2H), 1.51 (t, J = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.9, 165.8, 148.2, 146.0, 145.8, 142.4, 126.7, 125.3, 124.4, 124.2, 120.6, 115.0, 114.8, 114.2, 113.5, 64.7, 14.8; HRMS (ESI): calcd for $C_{17}H_{13}ClNaO_4$ $[M+Na]^+$, 339.0400; found, 339.0401.

4.1.49. 7-Methoxybenzofuran-3(2H)-one (**77**)

2-(2-Methoxyphenoxy) acetic acid (**76**, 5.0 mmol) was dissolved in thionyl chloride (15 mL) at rt. After refluxing for 4 h, the reaction mixture was cooled to rt and concentrated under vacuum. The residue was dissolved in dichloromethane (50 mL) and aluminum chloride (10.0 mmol) was added slowly at 0 °C. After stirring for 0.5 h at 0 °C and 1 h at rt, the reaction mixture was then poured into ice water (60 mL) and extracted with ethyl acetate (3 × 30 mL). The combined organic phases were washed with brine, dried over anhydrous Na_2SO_4, and evaporated under vacuum. The residue was purified by flash chromatography on silica gel (petroleum ether/ethyl acetate = 30/1) to afford the title compound. Bright yellow solid, yield 27%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.22–7.17 (m, 1H), 7.05 (d, J = 7.9 Hz, 1H), 7.00–6.93 (m, 1H), 4.61 (s, 2H), 3.89 (s, 3H).

4.1.50. (Z)-2-(3-Ethoxy-4-hydroxybenzylidene)-7-methoxybenzofuran-3(2H)-one (**78**)

This compound was obtained from **77** and 3-ethoxy-4-hydroxybenzaldehyde employing method A. Yellow solid, yield 69%; purity (gradient I) 99.8%; m.p. 155–157 °C; IR (KBr) ν_{max} 3427, 3073, 2971, 2925, 2832, 1703, 1651, 1603, 1581, 1516, 1310, 1272, 1208, 1124, 1069, 912, 879, 805, 744 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 (d, J = 1.9 Hz, 1H), 7.47 (dd, J = 8.3, 1.9 Hz, 1H), 7.39 (dd, J = 6.8, 2.0 Hz, 1H), 7.18–7.09 (m, 2H), 7.00 (d, J = 8.3 Hz, 1H), 6.88 (s, 1H), 6.04 (s, 1H), 4.24 (q, J = 7.0 Hz, 2H), 4.02 (s, 3H), 1.51 (t, J = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.1, 154.4, 149.7, 146.9, 145.5, 144.8, 126.0, 124.2, 123.2, 122.6, 119.1, 116.6, 116.2, 114.9, 114.2, 63.9, 56.4, 14.7; HRMS (ESI): calcd for $C_{18}H_{16}NaO_5$

$[M+Na]^+$, 335.0895; found, 335.0906.

4.1.51. DRAK2 in vitro kinase assay

Recombinant GST fused DRAK2 protein were prepared as previous reported [6].DRAK2 kinase assays was carried out based on ADP produced during the DRAK2 autophosphorylation process, with Promega ADP-Glo kits (Promega, Madison, WI). The kinase reaction were conducted in a volume of 5 μL 1X Buffer (HEPES 50 mM (pH7.0), NaN_3 0.02%, Orthovanadate 0.1 mM, 5 mM $MgCl_2$, 0.01% (w/v) bovine serum albumin), contained 0.5 μg DRAK2-GST, 20 μM ATP, and was reacted for 2 h at room temperature. The florescence signal was read on an EnVision® Multilabel Reader to calculate the DRAK2 kinase activity. Inhibition data was shown as means ± SD of three replicates.

4.1.52. Protein kinase profiling

Protein kinase profiling of compounds **1**, **40, 41,** and **67** was performed within a panel of 27 human protein kinases. Percentage of kinase activities were determined for compounds **1**, **40, 41** and **67** at 10 μM. The percentage inhibition was calculated relative to an enzyme control without inhibitor. IC_{50} values were calculated via four-parameter nonlinear regression using Prism 6 software. Profiling data was shown as means ± SD of three replicates.

4.1.53. Primary pancreatic islet isolation

Mouse pancreatic islets were isolated using the Liberase digestion method [33]. Euthanize C57BL/6J mice (20–24 g) by cervical dislocation, saturate the mouse torso with 70% EtOH, open abdominal cavity completely. Find the hepatic artery, portal vein and bile duct bundle, tie tight close to the liver as close as possible, insert the cannula into the bile duct up to half the length of the bile duct, and then gently pull the suture tight around the cannula. Digestion solution (2 mL HBSS containing 20 mM HEPES and 1 mg/mL collagenase P (Roche) enzyme solution) was injected into the common bile duct. After inflation of the pancreas is completed, remove the pancreas from spleen. Harvest the pancreas and transfer it to a 15 mL tube containing 2 mL cold collagenase P enzyme solution. Incubate 10 min in a 37 °C water bath. After incubation, shake the tube by hand in 37 °C water bath for about 8 min. Wash the conical tube with 5 mL Quenching buffer (HBSS containing 10% FBS), spin at RT 300 g for 3 min. Take out supernatant and wash pellet with 10 mL cold Quenching buffer transfer the mixture into a 15 mL plastic tube. Spin at RT 300 g for 3 min. Add 2 mL of 25% Ficoll solution to the pellet and mix by pipette up and down. Slowly add 1 mL each of the next three layers one by one: 23%, 21%, 13% to form the gradient. Put tube on ice until all of the tubes are ready to spin. Spin at 2500 g for 20 min at 4 °C. The islet layer should be visible between the 13% and 21% gradient which near the 4.5 mL mark on the tube. Collect islets and place it into a 5–10 cm cell culture dish with 3–5 mL regular cell culture media. Wash the islet layer in 5 mL HBSS buffer, 1000 g 5 min. Resuspended the islets in 1 mL RPMI 1640 complete medium, and then used for experiment.

4.1.54. Cell culture

The rat insulinoma INS-1 cell line was kindly provided by Dr. Liu Yong (Institute for Nutritional Sciences, Chinese Academy of Sciences) and maintained in RPMI 1640 as described [34]. INS-1 cells between passages 20–30 were used.

4.1.55. Treatment of islets and INS-1 with palmitate

Isolated islets were cultured in 48-well plates for about 10 islets/well in RPMI 1640 with 10% FBS. INS-1 cells were cultured in 48-well plates in RPMI 1640 with 10% FBS. Palmitate (0.4 mM; Sigma-Aldrich) was added to the wells to induce the islets function impairment as indicated in each experiment.

4.1.56. Insulin secretion/content measurement

Glucose-stimulated insulin secretion was determined as previously described in detail [35]. Islets were cultured for 48 h in complete RPMI 1640 medium with 10% FBS in the absence or presence of various stimulants; then pick out 10 islets/well transferred to 48-well plates. The islets were incubated in Kreb's buffer (135 mM NaCl, 3.6 mM KCl, 5 mM NaH_2PO_4, 0.5 mM $MgCl_2$, 1.5 mM $CaCl_2$, 2 mM $NaHCO_3$, 10 mM HEPES (pH 7.4), and 0.1% BSA) for 1 h, and then incubated in Kreb's buffer containing 2.8 mM or 16.7 mM glucose for another hour at 37 °C. One hundred microliters of supernatant was removed for determination of insulin levels. Insulin was determined by HTRF kit (Cisbio).

4.1.57. Antibodies and immunoblotting

Cleaved PARP, cleaved-caspase3 and cleaved-caspase9 antibodies were purchased from Cell Signaling Technologies. β-actin antibody was purchased from ABGENT (San Diego, CA, USA). Western immunoblotting was performed as described previously [36].

Acknowledgments

This work was supported by grants from the National Key Technology R&D Program (2015BAK45B00), Science and Technology Commission of Shanghai Municipality (16430711700 and 16JC1405000), and Chinese Academy of Sciences (CASIMM0120162021), and Shanghai Science and Technology Council (No 16DZ2280100 and 15DZ2291600). This work was also supported by Juvenile Diabetes Foundation (17-2013-440) and J.-Louis Levesque Foundation to J.W. We also thank the Laboratory of Organic Functional Molecules, Sino-French Institute of ECNU for support.

Abbreviations used

c-MET	tyrosine-protein kinase Met
ALK	anaplastic lymphoma kinase
Ack1	activated CDC42 kinase 1
MELK	maternal embryonic leucine zipper kinase
IRK	inwardly rectifying potassium channels
mTORC1	mammalian target of rapamycin complex 1
Bcl	B-cell lymphoma
Bcl-xl	B-cell lymphoma-extra large
Flip	FLICE inhibitory protein
LDA	Lithium diisopropylamide
DMF	*N*,*N*-dimethylformamide
NBS	*N*-bromobutanimide
THF	tetrahydrofuran
rt	room temperature
DRAK1	DAP kinase-related apoptosis-inducing protein kinase
DAPK1	death-associated protein kinase 1
DAPK2	death-associated protein kinase 2
DAPK3	death-associated protein kinase 3
BLK	B lymphoid tyrosine kinase
ITK	IL2-inducible T-cell kinase
TEK	tunica interna endothelial cell kinase
JAK	Janus kinase
SYK	spleen tyrosine kinase
CHK1	checkpoint kinases 1
AKT1	v-akt murine thymoma viral oncogene homolog 1
Aurora-A	aurora kinase A
EGFR	epidermal growth factor receptor
BTK	bruton agammaglobulinemia tyrosine kinase

CDK	cyclin-dependent kinase
MELK	maternal embryonic leucine zipper kinase
IKKβ	inhibitor of nuclear factor kappa-B kinase subunit beta
GSK3β	glycogen synthase kinase 3 beta
MEK1	mitogen-activated protein kinase kinase 1
ERK1	mitogen-activated protein kinase 3
HBSS	Hank's balanced salt solution
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
FBS	fetal bovine serum
RPMI	roswell park memorial institute
INS-1	insulin-1
BSA	bovine serum albumin
HTRF	homogeneous time resolved fluorescence
PARP	poly-ADP-ribose polymerase
GSIS	glucose stimulated insulin secretion

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2017.02.048.

References

[1] R. Shiloh, S. Bialik, A. Kimchi, The DAPK family: a structure-function analysis, Apoptosis 19 (2014) 286–297.
[2] D. Kogel, J.H.M. Prehn, K.H. Scheidtmann, The DAP kinase family of pro-apoptotic proteins: novel players in the apoptotic game, BioEssays 23 (2001) 352–358.
[3] J. Mao, X. Qiao, H. Luo, J. Wu, Transgenic drak2 overexpression in mice leads to increased T cell apoptosis and compromised memory T cell development, J. Bio. Chem. 281 (2006) 12587–12595.
[4] J. Mao, H. Luo, B. Han, R. Bertrand, J. Wu, Drak2 is upstream of p70S6 kinase: its implication in cytokine-induced islet apoptosis, diabetes, and islet transplantation, J. Immunol. 182 (2009) 4762–4770.
[5] J. Mao, H. Luo, J. Wu, Drak2 overexpression results in increased beta-cell apoptosis after free fatty acid stimulation, J. Cell. Biochem. 105 (2008) 1073–1080.
[6] L. Marvaldi, B. Hausott, M. Auer, J. Leban, L. Klimaschewski, A novel DRAK inhibitor, SC82510, promotes axon branching of adult sensory neurons in vitro, Neurochem. Res. 39 (2014) 403–407.
[7] L.J. Gao, S. Kovackova, M. Sala, A.T. Ramadori, S. De Jonghe, P. Herdewijn, Discovery of dual death-associated protein related apoptosis inducing protein kinase 1 and 2 inhibitors by a scaffold hopping approach, J. Med. Chem. 57 (2014) 7624–7643.
[8] P. Leonczak, L.J. Gao, A.T. Ramadori, E. Lescrinier, J. Rozenski, S. De Jonghe, P. Herdewijn, Synthesis and structure-activity relationship studies of 2-(1,3,4-oxadiazole-2(3H)-thione)-3-amino-5-arylthieno[2,3-b]pyridines as inhibitors of DRAK2, ChemMedChem 9 (2014) 2587–2601.
[9] M.E. Jung, B.J. Byun, H.M. Kim, J.Y. Lee, J.H. Park, N. Lee, Y.H. Son, S.U. Choi, K.M. Yang, S.J. Kim, K. Lee, Y.C. Kim, G. Choi, Discovery of indirubin derivatives as new class of DRAK2 inhibitors from high throughput screening, Bioorg. Med. Chem. Lett. 26 (2016) 2719–2723.
[10] N. Zhang, S. Ayral-Kaloustian, J.T. Anderson, T. Nguyen, S. Das, A.M. Venkatesan, N. Brooijmans, J. Lucas, K. Yu, I. Hollander, R. Mallon, 5-ureidobenzofuranone indoles as potent and efficacious inhibitors of PI3 kinase-alpha and mTOR for the treatment of breast cancer, Bioorg. Med. Chem. Lett. 20 (2010) 3526–3529.
[11] V. Wallez, S. Durieux-Poissonnier, P. Chavatte, J.A. Boutin, V. Audinot, J.P. Nicolas, C. Bennejean, P. Delagrange, P. Renard, D. Lesieur, Synthesis and structure-affinity-activity relationships of novel benzofuran derivatives as MT2 melatonin receptor selective ligand, J. Med. Chem. 45 (2002) 2788–2800.
[12] R. Haudecoeur, A. Ahmed-Belkacem, W. Yi, A. Fortune, R. Brillet, C. Belle, E. Nicolle, C. Pallier, J.M. Pawlotsky, A. Boumendjel, Discovery of naturally occurring aurones that are potent allosteric inhibitors of hepatitis C virus RNA-dependent RNA polymerase, J. Med. Chem. 54 (2011) 5395–5402.
[13] M. Oliverio, M. Nardi, P. Costanzo, L. Cariati, G. Cravotto, S.V. Giofre, A. Procopio, Non-conventional methodologies in the synthesis of 1-indanones, Molecules 19 (2014) 5599–5610.
[14] V.N. Korotchenko, M. Saydmohammed, L.L. Vollmer, A. Bakan, K. Sheetz, K.T. Debiec, K.A. Greene, C.S. Agliori, I. Bahar, B.W. Day, A. Vogt, M. Tsang, In vivo structure-activity relationship studies support allosteric targeting of a dual specificity phosphatase, Chembiochem a Euro. J. Chem. Bio. 15 (2014) 1436–1445.
[15] G. Amari, M. Riccaboni, S. Bossolo, Preparation of Alkaloid Aminoester Derivatives as Muscarinic Receptor Antagonists, US20110311458 A1, Dec. 22, 2011.
[16] B. Dario, B. Luisella, E. Antonella, G. Markus, M. Maria, M. Jurgen, V. Ermes, Preparation of Alkylidenebenzofuranones as Telomerase Inhibitors, WO2002083123 A1, Oct. 24, 2002.
[17] C.M. Merie, F.A.F. Josef, G.R. James, O.C. John, Therapeutic Diphenyl Ether Ligands and Their Preparation, Pharmaceutical Compositions, and Use as Serotonin (5HT) Receptor Ligands for Treatment of Nervous System Disorders, WO2006027684 A1, Mar. 16, 2006.
[18] B. Chantal, M. Anne-Marie, B. Ahcene, An efficient synthesis of 4,6 dimethoxyaurones, Heterocycles 55 (2011) 967–972.
[19] T. Peter, R. Jozsef, B. Gabor, S. Gyula, New Process for the Preparation of Racemic ([2s[2r*[r[r*]]]] and ([2r[2s*[s[s*]]]]-(±)- α,α′ -[imino-bis(methylene)] bis[6-fluoro-chroman-2-methanol] and its Pure [2s[2r*[r[r*]&, WO2004041805 A1, May. 21, 2004.
[20] W. Luo, T. Wang, C. Hong, Y.C. Yang, Y. Chen, J. Cen, S.Q. Xie, C.J. Wang, Design, synthesis and evaluation of 4-dimethylamine flavonoid derivatives as potential multifunctional anti-Alzheimer agents, Euro. J. Med. Chem. 122 (2016) 17–26.
[21] K. Manjulatha, S. Srinivas, N. Mulakayala, D. Rambabu, M. Prabhakar, K.M. Arunasree, M. Alvala, M.V. Basaveswara Rao, M. Pal, Ethylenediamine diacetate (EDDA) mediated synthesis of aurones under ultrasound: their evaluation as inhibitors of SIRT1, Bioorg. Med. Chem. Lett. 22 (2012) 6160–6165.
[22] M. Hoffmann, S. Miaskiewicz, J.-M. Weibel, P. Pale, A. Blanc, Assigning regioisomeric or diastereoisomeric relations of problematic trisubstituted double-bonds through heteronuclear 2D selective J-resolved NMR spectroscopy, RSC Adv. 5 (2015) 37138–37148.
[23] J.S. Hastings, H.G. Heller, The stereochemistry of aurones [2-(substituted benzylidene)-3(2H)-benzofuranones], J. Chem. Soc. Perkin Trans. 1 17 (1972) 2128–2132.
[24] B.A. Brady, J.L. Kennedy, W.I. O'Sullivan, The configuration of aurones, Tetrahedron 29 (1973) 359–362.
[25] F. Belluti, A. Rampa, L. Piazzi, A. Bisi, S. Gobbi, M. Bartolini, V. Andrisano, A. Cavalli, M. Recanatini, P. Valenti, Cholinesterase inhibitors: xanthostigmine derivatives blocking the acetylcholinesterase-induced β-amyloid aggregation, J. Med. Chem. 48 (2005) 4444–4456.
[26] H.M. Sim, C.Y. Lee, P.L. Ee, M.L. Go, Dimethoxyaurones: potent inhibitors of ABCG2 (breast cancer resistance protein), Euro. J. Pharm. Sci. 35 (2008) 293–306.
[27] C.Y. Lee, E.H. Chew, M.L. Go, Functionalized aurones as inducers of NAD(P)H: quinone oxidoreductase 1 that activate AhR/XRE and Nrf2/ARE signaling pathways: synthesis, evaluation and SAR, Euro.J. Med. Chem. 45 (2010) 2957–2971.
[28] L.D. Ly, S. Xu, S.K. Choi, C.M. Ha, T. Thoudam, S.K. Cha, A. Wiederkehr, C.B. Wollheim, I.K. Lee, K.S. Park, Oxidative stress and calcium dysregulation by palmitate in type 2 diabetes, Exp. Mol. Med. 49 (2017) e291.
[29] M. Maris, S. Robert, E. Waelkens, R. Derua, M.H. Hernangomez, W. D'Hertog, M. Cnop, C. Mathieu, L. Overbergh, Role of the saturated nonesterified fatty acid palmitate in beta cell dysfunction, J. Proteome Res. 12 (2013) 347–362.
[30] S.E. Choi, H.E. Kim, H.C. Shin, H.J. Jang, K.W. Lee, Y. Kim, S.S. Kang, J. Chun, Y. Kang, Involvement of Ca2+-mediated apoptotic signals in palmitate-induced MIN6N8a beta cell death, Mol. Cell. Endocrinol. 272 (2007) 50–62.
[31] N. Hirota, S. Otabe, H. Nakayama, X. Yuan, K. Yamada, Sequential activation of caspases and synergistic beta-cell cytotoxicity by palmitate and anti-Fas antibodies, Life Sci. 79 (2006) 1312–1316.
[32] J. Staaf, S.J. Ubhayasekera, E. Sargsyan, A. Chowdhury, H. Kristinsson, H. Manell, J. Bergquist, A. Forslund, P. Bergsten, Initial hyperinsulinemia and subsequent beta-cell dysfunction is associated with elevated palmitate levels, Pediatr. Res. 80 (2016) 267–274.
[33] Y. Cui, L. Huang, F. Elefteriou, G. Yang, J.M. Shelton, J.E. Giles, O.K. Oz, T. Pourbahrami, C.Y. Lu, J.A. Richardson, G. Karsenty, C. Li, Essential role of STAT3 in body weight and glucose homeostasis, Mol. Cell. Biol. 24 (2004) 258–269.
[34] L. Yang, P. Huang, F. Li, L. Zhao, Y. Zhang, S. Li, Z. Gan, A. Lin, W. Li, Y. Liu, c-Jun amino-terminal kinase-1 mediates glucose-responsive upregulation of the RNA editing enzyme ADAR2 in pancreatic beta-cells, PloS One 7 (2012) e48611.
[35] L. Yang, L. Zhao, Z. Gan, Z. He, J. Xu, X. Gao, X. Wang, W. Han, L. Chen, T. Xu, W. Li, Y. Liu, Deficiency in RNA editing enzyme ADAR2 impairs regulated exocytosis, FASEB J. 24 (2010) 3720–3732.
[36] Y. Qiu, T. Mao, Y. Zhang, M. Shao, J. You, Q. Ding, Y. Chen, D. Wu, D. Xie, X. Lin, X. Gao, R.J. Kaufman, W. Li, Y. Liu, A crucial role for RACK1 in the regulation of glucose-stimulated IRE1alpha activation in pancreatic beta cells, Sci. Signal. 3 (2010) ra7.