HETEROCYCLES, Vol. 102, No. 8, 2021, pp. 1536 - 1551. © 2021 The Japan Institute of Heterocyclic Chemistry
Received, 22nd April, 2021, Accepted, 31st May, 2021, Published online, 8th June, 2021
DOI: 10.3987/COM-21-14481

SYNTHESIS, CHARACTERIZATION, AND DRAK2 INHIBITORY ACTIVITIES OF HYDROXYAURONE DERIVATIVES

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Abstract – We reported the synthesis of 25 derivatives of hydroxyaurone, which were characterized by ^{1}H NMR, ^{13}C NMR, high resolution mass spectrum, and single crystal X-ray diffraction analysis. Their activities on the death-associated protein kinase-related apoptosis-inducing kinase-2 (DRAK2) were evaluated by kinase detection kit at a dosage of 5 μM. Most of the synthetic hydroxyaurones exhibited moderate to good inhibitory activities. The IC_{50} value ranged from 0.81 to 2.42 μM when the structure of aurone's B ring was kept unchanged and its A ring was substituted by hydroxyl groups. On the contrary, modification of the aurone's B-ring with hydroxyl groups lead to the IC_{50} value ranging from 1.15 to 17.5 μM. This indicates presence of hydroxyl group of the B ring is crucial for aurone's DRAK2 kinase inhibitors. Therefore, hydroxyaurone may serve as a new possible lead compound for the discovery of DRAK2. Further pharmacological investigations are underway and will be reported in due course.

INTRODUCTION

The aurone (2-benzylidenebenzofuran-3(2*H*)-ones) is a simple flavonoid, isolated from *Coreopsis tinctoria* Nutt., *Smilax riparia* and other plants,[1,2] occurring in *Z* configuration. Aurones possess a wide

range of biological activities, including as inhibitors of cyclin-dependent kinases, inhibitors of chorismate synthase, oxidant resistance, inhibitors of tyrosinase, and as drug candidates to treat cancer, inflammation, diabetes, and Alzheimer's disease.[3-16] Aurone has A, B, and C tricyclic rings as shown in Figure **1**. Previously, most of the research was focused on the substituent modification of the tricyclic of aurone.[17,18] However, the hydroxylation on both A and B rings was rarely reported. The death-associated protein kinase-related apoptosis-inducing kinase-2 (DRAK2), also known as serine/threonine kinase (STK 17B) is related to various diseases such as diabetic, leukemia, calcium neuromodulation, autoimmune diseases, and tumor.[5,19-25,26] A number of inhibitors of DRAK2 were discovered including compounds **I**, **II**, **III**, **IV** (alstonlarsine A) and **V** (Figure **2**).[5,27-31] Among them compound **III** is an aurone derivative. Recently, our group confirmed that the natural product **6i**, which was isolated from *Coreopsis tinctoria* Nutt., was also an aurone compound. Compounds **6i** and **III** are both hydroxyaurones. The results of the activity showed that it was a moderate DRAK2 inhibitor with IC_{50} value of 2.44 ± 0.04 μM.[3]

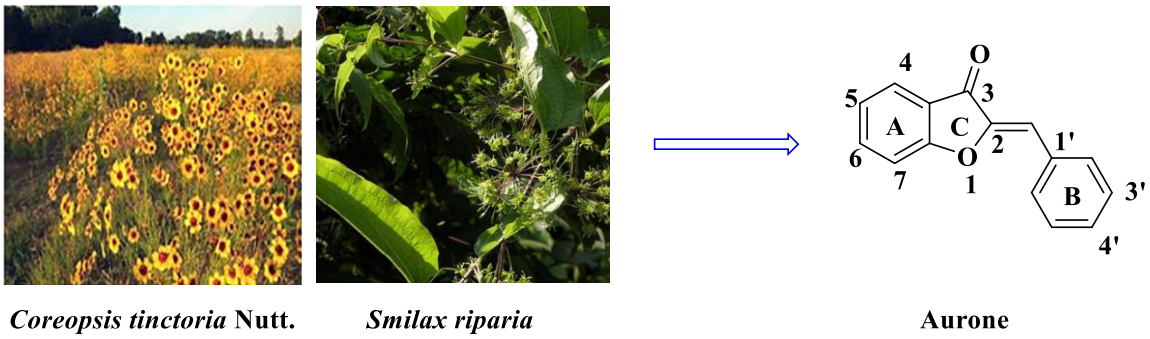


Figure 1. Aurone from *Coreopsis tinctoria* Nutt. & *Smilax riparia*

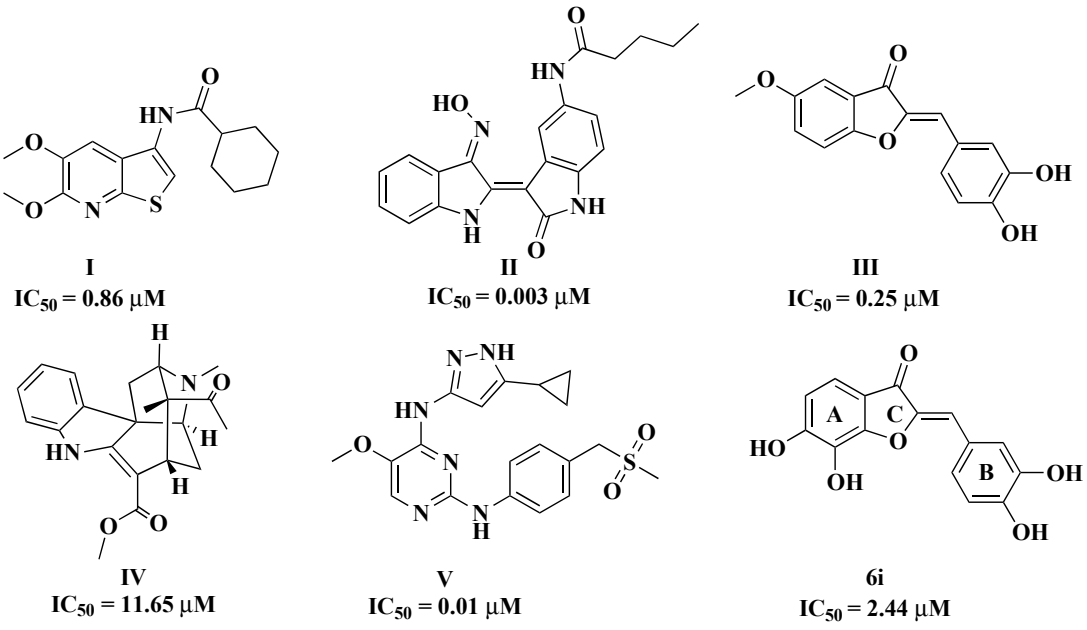


Figure 2. Structure and IC_{50} value of DRAK2 inhibitors

Molecular docking of compound **6i** with 6ZJF by means of discovery studio 2016 software was investigated and the result was shown as Figure **3**. The binding affinity of compound **6i** is mainly attributed to several strong hydrogen bonds with Ala113, Glu111, and Lys62. These results encourage more structural modification of **6i** in order to obtain compounds with better activity.

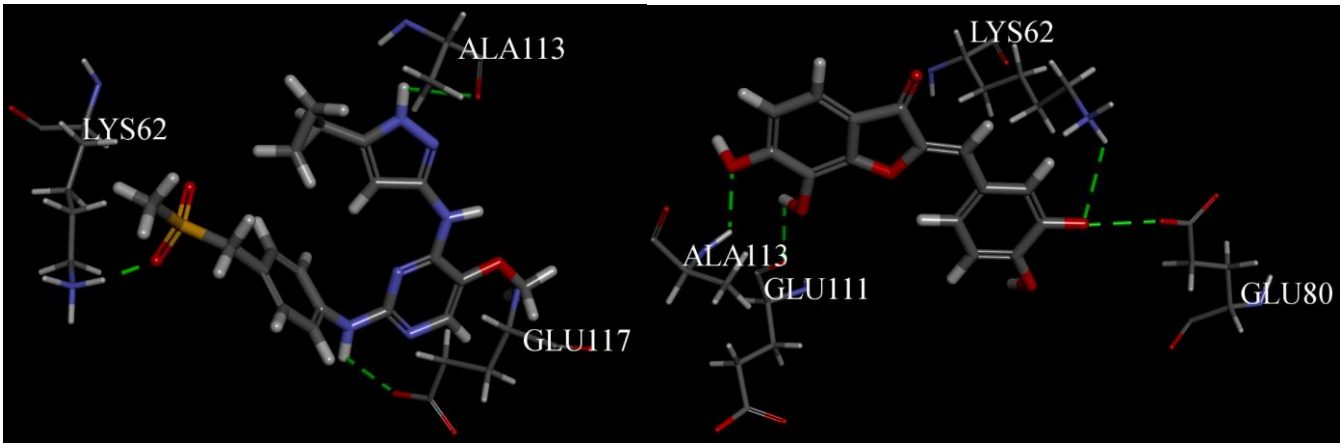


Figure 3. Hydrogen bonding interactions are depicted in green dots.
Proposed binding mode of **5** and **6i** in the DRAK2

RESULTS AND DISCUSSION

Synthesis. The synthetic routes for compounds **6a~6t** are described in Scheme **1**. Chalcone derivatives **3a-3t** were synthesized from commercially available methoxyacetophenone (**1a-1h**) and appropriately substituted benzaldehydes (**2a-2n**) using the reported aldol condensation method.[4,8,32] In the presence of anhydrous $AlCl_3$, the *ortho*-methyl group of the carbonyl group was removed to obtain the key intermediate hydroxychalcone derivatives **4a-4t**.[15,33] Subsequently, compounds **4a-4t** underwent ring-closure reaction in the presence of mercury acetate at 110 ^{o}C to obtain methoxy aurone compounds **5a-5t**.[15] Finally, upon the treatment of BBr_3, all the hydroxyl protecting groups of the compounds **5a-5t** were removed to obtain the target compounds **6a-6t**.[4,34,35] The final compounds were characterized by ^{1}H NMR, ^{13}C NMR, and HRMS analysis. The double bond configuration of **6b** (CCDC Deposition Number 2075041) was further determined by single crystal X-ray diffraction analysis (Figure **4**).

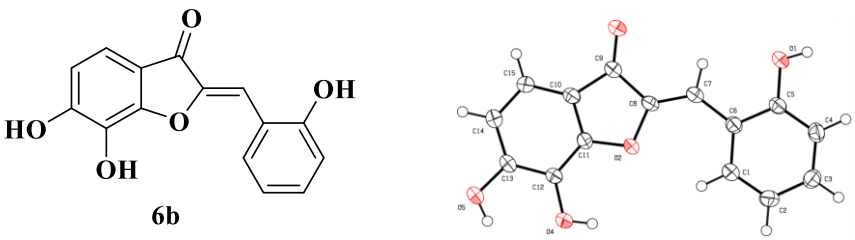
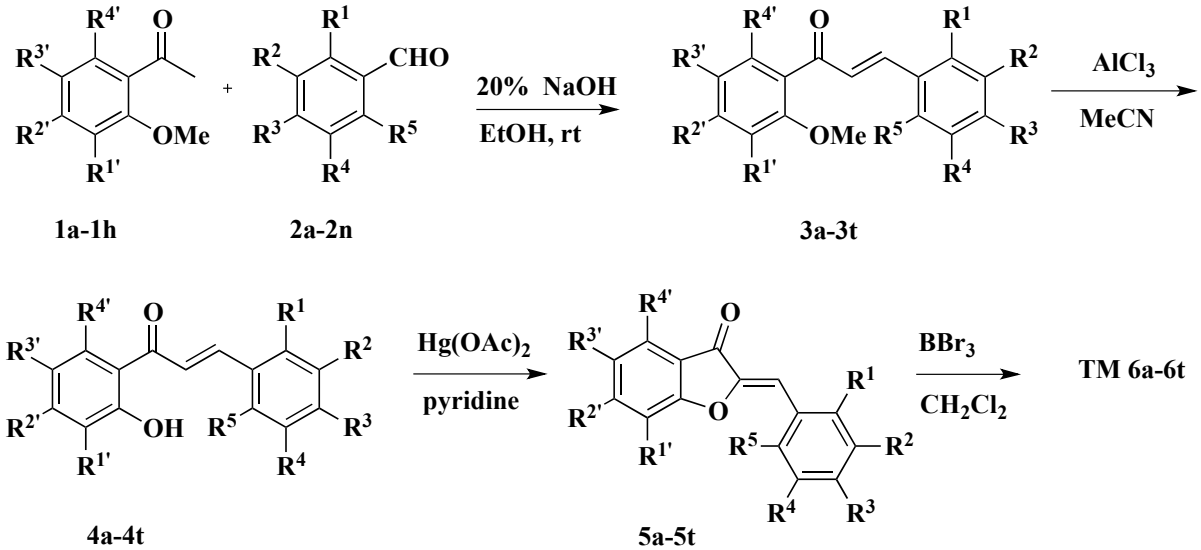


Figure 4. Single crystal structure of **6b** (CCDC Deposition Number 2075041)



1a-1h **2a-2n** **3a-3t**

4a-4t **5a-5t**

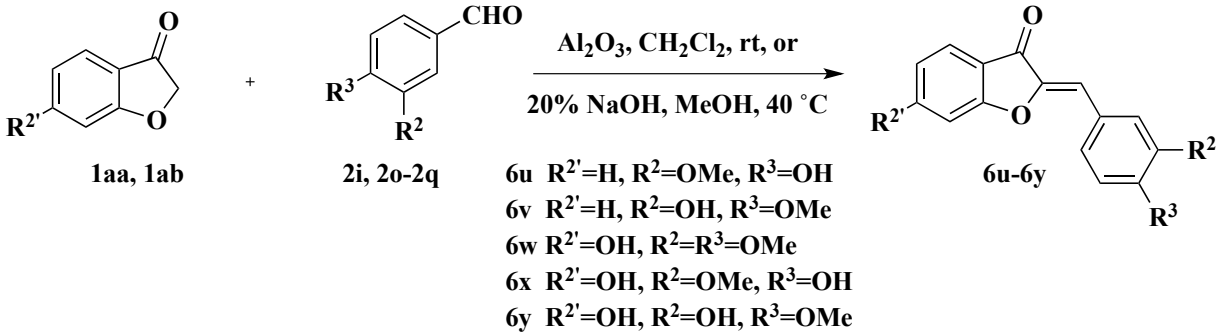
3a~5n	$R^{1'}=R^{2'}$=OMe, $R^{3'}=R^{4'}$=H	**6a~6n**	$R^{1'}=R^{2'}$=OH, $R^{3'}=R^{4'}$=H
3a, 4a, 5a	$R^1=R^2=R^3=R^4=R^5$=H	**6a**	$R^1= R^2=R^3=R^4=R^5$=H
3b, 4b, 5b	$R^2=R^3=R^4=R^5$=H, R^1=OMe	**6b**	$R^2=R^3=R^4=R^5$=H, R^1=OH
3c, 4c, 5c	$R^1=R^3=R^4=R^5$=H, R^2=OMe	**6c**	$R^1=R^3=R^4=R^5$=H, R^2=OH
3d, 4d, 5d	$R^1=R^2=R^4=R^5$=H, R^3=OMe	**6d**	$R^1= R^2=R^4=R^5$=H, R^3=OH
3e, 4e, 5e	$R^3=R^4=R^5$=H, $R^1= R^2$=OMe	**6e**	$R^3=R^4=R^5$=H, $R^1= R^2$=OH
3f, 4f, 5f	$R^2=R^4=R^5$=H, $R^1=R^3$=OMe	**6f**	$R^2=R^4=R^5$=H, $R^1=R^3$=OH
3g, 4g, 5g	$R^2=R^3=R^5$=H, $R^1=R^4$=OMe	**6g**	$R^2=R^3=R^5$=H, $R^1=R^4$=OH
3h, 4h, 5h	$R^2=R^3=R^4$=H,$R^1=R^5$=OMe	**6h**	$R^2=R^3=R^4$=H, $R^1=R^5$=OH
3i, 4i, 5i	$R^1=R^4=R^5$=H, $R^2=R^3$=OMe	**6i**	$R^1=R^4=R^5$=H, $R^2=R^3$=OH
3j, 4j, 5j	$R^1=R^3=R^5$=H, $R^2=R^4$=OMe	**6j**	$R^1=R^3=R^5$=H, $R^2=R^4$=OH
3k, 4k, 5k	$R^4=R^5$=H, $R^1= R^2=R^3$=OMe	**6k**	$R^4=R^5$=H, $R^1= R^2=R^3$=OH
3l, 4l, 5l	$R^2=R^5$=H, $R^1=R^3=R^4$=OMe	**6l**	$R^2=R^5$=H, $R^1=R^3=R^4$=OH
3m, 4m, 5m	$R^2=R^4$=H, $R^1=R^3=R^5$=OMe	**6m**	$R^2=R^4$=H, $R^1=R^3=R^5$=OH
3n, 4n, 5n	$R^1=R^5$=H, $R^2=R^3=R^4$=OMe	**6n**	$R^1=R^5$=H, $R^2=R^3=R^4$=OH
3o~5t	$R^1=R^4=R^5$=H, $R^2=R^3$=OMe	**6o~6t**	$R^1=R^4=R^5$=H, $R^2=R^3$=OH
3o, 4o, 5o	$R^{1'}=R^{2'}=R^{3'}=R^{4'}$=H	**6o**	$R^{1'}=R^{2'}=R^{3'}=R^{4'}$=H
3p, 4p, 5p	$R^{1'}=R^{3'}=R^{4'}$=H,$R^{2'}$=OMe	**6p**	$R^{1'}=R^{3'}=R^{4'}$=H,$R^{2'}$=OMe
3q, 4q, 5q	$R^{1'}=R^{2'}=R^{4'}$=H, $R^{3'}$=OMe	**6q**	$R^{1'}=R^{2'}=R^{4'}$=H, $R^{3'}$=OH
3r, 4r, 5r	$R^{1'}=R^{2'}=R^{3'}$=H, $R^{4'}$=OMe	**6r**	$R^{1'}=R^{2'}=R^{3'}$=H, $R^{4'}$=OH
3s, 4s, 5s	$R^{1'}=R^{4'}$=H, $R^{2'}=R^{3'}$=OMe	**6s**	$R^{1'}=R^{4'}$=H, $R^{2'}=R^{3'}$=OH
3t, 4t, 5t	$R^{1'}=R^{3'}$=H, $R^{2'}=R^{4'}$=OMe	**6t**	$R^{1'}=R^{3'}$=H, $R^{2'}=R^{4'}$=OH

Scheme 1. Syntheses of hydroxyaurones

The selective removal of the hydroxyl protecting group adjacent to the carbonyl group was achieved under the condition of $AlCl_3$. Except **5p**, all protection groups of the phenolic hydroxyl in the compounds **5a~5t** can be removed under the condition of BBr_3. This may be due to the lack of electrons in the A ring, which makes it difficult for oxygen atoms to interact with boron atoms. It is noted that when the deprotection reaction was quenched by methanol, the product would diminish or even totally disappear during the work-up process. However, if the reaction was quenched with ice-cold water, the target

compounds were obtained in good yield.

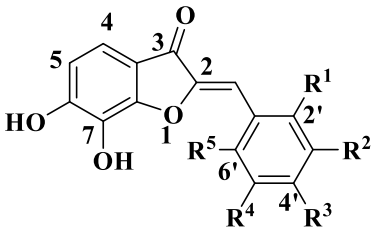
The synthetic routes for aurone derivatives **6u-6y** are listed in Scheme **2**. (*Z*)-2-(4-Hydroxy-3-methoxybenzylidene)benzofuran-3(2*H*)-one derivatives (**6u-6y**) were obtained from commercially available 3-coumaranone (**1aa**) and appropriately substituted benzaldehydes via reported aldol condensation reactions.[11,14]



Scheme 2. Syntheses of compounds **6u-6y**

DRAK2 Inhibitory activities. Their death-associated protein kinase-related apoptosis-inducing kinase-2 (DRAK2) activities were evaluated in Envision (PerkinElmer, USA) by kinase detection kit applying the previously reported procedure.[36] The results are listed in Table **1~3**.

Table 1. The inhibitory activities of compounds **6a-6n** against DRAK2 kinase

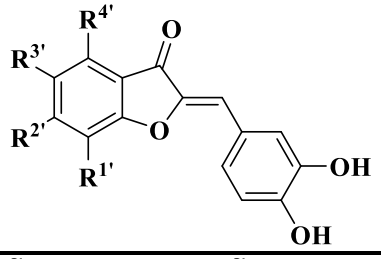


Compound No.	R^1	R^2	R^3	R^4	R^5	DRAK2 IC_{50} (μM)
Natural 6i	H	OH	OH	H	H	2.44 ± 0.04
6a	H	H	H	H	H	1.63 ± 2.16
6b	OH	H	H	H	H	1.89 ± 0.10
6c	H	OH	H	H	H	1.38 ± 0.06
6d	H	H	OH	H	H	1.15 ± 0.05
6e	OH	OH	H	H	H	3.07 ± 0.19
6f	OH	H	OH	H	H	1.75 ± 0.04
6g	OH	H	H	OH	H	7.36 ± 0.27
6h	OH	H	H	H	OH	14.31 ± 1.61
6i	H	OH	OH	H	H	2.44 ± 0.11
6j	H	OH	H	OH	H	1.61 ± 0.10

6k	OH	OH	OH	H	H	3.27 ± 0.24
6l	OH	H	OH	OH	H	4.49 ± 0.25
6m	OH	H	OH	H	OH	17.5 ± 0.77
6n	H	OH	OH	OH	H	3.17 ± 0.43

See Experimental Section.

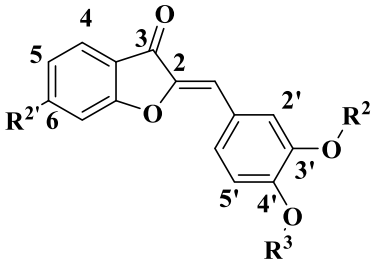
Table 2. The inhibitory activities of compounds **6o-6t** against DRAK2 kinase



Compound No.	**$R^{1'}$**	**$R^{2'}$**	**$R^{3'}$**	**$R^{4'}$**	**DRAK2 IC_{50} (μM)**
Natural 6i	OH	OH	H	H	2.44 ± 0.04
6o	H	H	H	H	1.02 ± 0.12
6p	H	OMe	H	H	1.35 ± 0.94
6q	H	H	OH	H	0.81 ± 0.08
6r	H	H	H	OH	2.42 ± 1.58
6s	H	OH	OH	H	1.31 ± 0.69
6t	H	OH	H	OH	1.47 ± 0.87

See Experimental Section.

Table 3. The inhibitory activities of compounds **6u-6y** against DRAK2 kinase



Compound No.	**$R^{2'}$**	**R^{2}**	**R^{3}**	**DRAK2 IC_{50} (μM)**
Natural 6i	-	-	-	2.44 ± 0.04
6u	H	Me	H	9.5 ± 0.27
6v	H	H	Me	NR
6w	OH	Me	Me	NR
6x	OH	Me	H	9.66 ± 7.10
6y	OH	H	Me	NR

See Experimental Section.

As shown in Table **1**, all the compounds with B ring modified showed inhibiting moderate to good activity on DRAK2. Among them, compounds **6b** (1.89 ± 0.10 μM), **6c** (1.38 ± 0.06 μM), **6d** (1.15 ± 0.05

μM), **6f** (1.75 ± 0.04 μM), and **6j** (1.61 ± 0.10 μM) were more potent than natural product **6i** (2.44 ± 0.04 μM). Although compound **6a** without hydroxyl substitution in B ring has activity, its error is large and it can not be proved to be better than the positive control. Therefore, hydroxyl substitution in B ring is very important for DRAK2 inhibition. However, when more hydroxyl groups were introduced to B ring, the inhibitory activity does not improve. It was clear that the presence of hydroxyl groups on 2′ and 6′-positions of B ring remarkably decreased the interaction with the DRAK2 (**6h** and **6m**). It may be that since the planarity of ring-A, B, and C through the overlapping of pi-orbital might be lost by 2′, 6′-disubstituted phenyl ring-B. This indicated that the position of hydroxyl groups was more favorable than the number of hydroxyl groups.

To investigate the effect of hydroxyl group on A ring, aurone derivatives **6o-6t** were prepared. As shown in Table **2**, the inhibitory activity was not enhanced significantly (IC_{50} was 0.81 to 2.42 μM) when the pattern of the hydroxyl group on A ring changed, which implied that the hydroxyl of A ring had little influence on inhibitory effect. Compound **6q** has no hydroxyl group at 6,7, but its activity is best, which may be due to the fact that the 5 position is more important than the 6,7 position.

In addition, methoxy groups were introduced to aurone to obtain compounds **6u-6y**. As shown in Table **3**, only compounds **6u** and **6x** would block the action of the kinase, with worse potency than the positive control. Thus, it was suspected that methylation of the aurone's hydroxyl probably cause a decline in the activity.

CONCLUSIONS

A total of 25 compounds were synthesized and their inhibitory activities against DRAK2 were evaluated. Key SAR findings included that 1) presence of hydroxyl group of the B ring is significant for aurone's DRAK2 inhibitory activities, which 4′-hydroxylated phenyl was crucial; 2) introduction of dihydroxyl group at 2′- and 6′-position of aurone resulted in a significant lose in activities. The most potent compound **6q** exhibited favorable potence. Overall, this study supports the notion that hydroxyaurone could be a promising strategy for DRAK2 inhibitors. Continued medicinal chemistry efforts should be made to obtain better potent compounds.

EXPERIMENTAL

General methods. All reagents and solvents (analytical grade) were purchased from commercial suppliers (Tansoole.com) and were used directly without further purification unless otherwise mentioned. Except for the first step, all reactions are carried out under argon atmosphere. 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

(100-200). ^{1}H and ^{13}C NMR spectra were achieved with a VARIAN MR 400 and BRUKER AVANCE NEO 600 spectrometer with tetramethylsilane (TMS) as an internal standard (600 and 400 MHz for ^{1}H, 150 and 100 MHz for ^{13}C). ^{1}H and ^{13}C chemical shifts were reported in parts per million (ppm, d). The high-resolution mass spectra were recorded on Bruker ESI-TOF high-resolution mass spectrometer. Melting points of the products was recorded on a WRR-Y drug melting point measurement apparatus and were uncorrected.

General procedure for the synthesis of compounds 6a-6t (method A). General procedure for the synthesis of compounds 3a-3t. 20% NaOH (10 mL) was added dropwise to a pre-cooled mixture of 10 mmol of selected acetophenone and 15 mmol of selected benzaldehyde in EtOH (50 mL) under stirring. The mixture was stirred at room temperature for 3-12 h. After completion of the reaction as indicated by TLC, the mixture was poured into water. The product was extracted with ethyl acetate (EA) (3×30 mL) then dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel to give the desired compound.

General procedure for the synthesis of compounds 4a-4t. To a solution of substrate (**3a-3t,** 5.0 mmol) in MeCN (50 mL) was added $AlCl_3$ (850 mg, 7.5 mmol) at room temperature. After being stirred, it was refluxed at 80 °C for 4 h. The reaction mixture was then poured into cold water and extracted with EA (3×30 mL). The combined organic layer was dried with Na_2SO_4. The solvent was evaporated under vacuum and the crude product was purified by column chromatography on silica gel eluting with the mixture of petroleum ether and EA.

General procedure for the synthesis of compounds 5a-5t. A mixture of an appropriate compound (**4a-4t,** 4.5 mmol) was added to the solution of mercuric acetate (5.4 mmol) in 30 mL of pyridine and stirred for 3-6 h at 110 °C. Upon completion, as determined by TLC, the mixture was poured into ice cold water and extracted with dichloromethane (DCM) (3×50 mL). The combined organic portion was washed with saturated aqueous $CuSO_4$ solution until no pyridine left and dried with anhydrous Na_2SO_4. The solvent was evaporated under vacuum and the crude product was purified by column chromatography on silica gel eluting with a mixture of DCM and EA.

General procedure for the synthesis of compounds 6a-6t. To a DCM solution of BBr_3 (4-10 mmol) was added dropwise a solution of compound (**5a-5t**, 1 mmol) in DCM (5 mL) at 0 ^{o}C under nitrogen. The reaction mixture was heated to room temperature and was stirred for 24 h until the starting material disappeared completely. The reaction mixture was treated by activated carbon for 0.5 h, and concentrated under vacuum. The residue was purified by flash chromatography on C-(18) reversed-phase silica gel to give the desired compounds **6a-6t**.

General procedure for the synthesis of compounds 6u-6y (method B or method C). Compounds **6u-6y** were synthesized by the reported method, while the aldol reagent for compounds **6u** and **6v** was

alumina oxide (method B),[4] and the aldol reagent for compounds **6w-6y** was 20% NaOH (method C).[14]

(*Z*)-2-Benzylidene-6,7-dihydroxybenzofuran-3(2*H*)-one (6a): This compound was obtained from (*Z*)-2-benzylidene-6,7-dimethoxybenzofuran-3(2*H*)-one (**5a**) employing method A. Yellow solid, yield 74.2%; mp 219.4-220.3 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.05 (d, *J* = 7.2 Hz, 2H), 7.50 (t, *J* = 7.4 Hz, 2H), 7.43 (t, *J* = 7.3 Hz, 1H), 7.16 (d, *J* = 8.3 Hz, 1H), 6.78 (s, 1H), 6.75 (d, *J* = 8.3 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.36, 155.34, 154.94, 147.66, 132.26, 131.32, 130.16, 129.67, 128.99, 115.69, 114.10, 112.93, 110.29. HRMS)ESI): calcd for $C_{15}H_{10}O_4$ [M-H]$^-$ 253.0506, found 253.0503.

(*Z*)-6,7-Dihydroxy-2-(2-hydroxybenzylidene)benzofuran-3(2*H*)-one (6b): This compound was obtained from (*Z*)-6,7-dimethoxy-2-(2-methoxybenzylidene)benzofuran-3(2*H*)-one (**5b**) employing method A. Yellow solid, yield 85.9%; mp 237.9-238.9 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.27 (dd, *J* = 8.0, 1.3 Hz, 1H), 7.29 - 7.20 (m, 1H), 7.12 (d, *J* = 8.3 Hz, 1H), 7.09 (s, 1H), 6.94 (dt, *J* = 7.1, 3.0 Hz, 2H), 6.71 (d, *J* = 8.3 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.16, 157.14, 155.74, 155.01, 147.36, 131.44, 131.26, 130.42, 119.68, 119.19, 115.70, 115.52, 113.85, 113.03, 104.23. HRMS)ESI): calcd for $C_{15}H_{10}O_5$ [M-H]$^-$ 269.0455, found 269.0452.

(*Z*)-6,7-Dihydroxy-2-(3-hydroxybenzylidene)benzofuran-3(2*H*)-one (6c): This compound was obtained from (*Z*)-6,7-dimethoxy-2-(3-methoxybenzylidene)benzofuran-3(2*H*)-one (**5c**) employing method A. Yellow solid, yield 33.7%; mp 271.8-272.1 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.80 (s, 1H), 9.67 (s, 2H), 7.47 (d, *J* = 7.8 Hz, 1H), 7.42 – 7.38 (m, 1H), 7.29 (t, *J* = 7.9 Hz, 1H), 7.15 (d, *J* = 8.3 Hz, 1H), 6.85 (dd, *J* = 7.8, 2.0 Hz, 1H), 6.75 (d, *J* = 8.3 Hz, 1H), 6.67 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.40, 157.63, 155.41, 154.90, 147.53, 133.32, 130.24, 129.91, 122.49, 117.68, 117.06, 115.65, 114.18, 112.83, 110.61. HRMS)ESI): calcd for $C_{15}H_{10}O_5$ [M-H]$^-$ 269.0455, found 269.0452.

(*Z*)-6,7-Dihydroxy-2-(4-hydroxybenzylidene)benzofuran-3(2*H*)-one (6d): This compound was obtained from (*Z*)-6,7-dimethoxy-2-(4-methoxybenzylidene)benzofuran-3(2*H*)-one (**5d**) employing method A. Yellow solid, yield 43.5%; mp 281.7-282.4 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.90 (d, *J* = 8.7 Hz, 2H), 7.11 (d, *J* = 8.3 Hz, 1H), 6.89 (d, *J* = 8.7 Hz, 2H), 6.72 (d, *J* = 9.5 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.07, 159.27, 154.95, 154.35, 145.97, 133.51, 130.10, 123.22, 116.03, 115.30, 114.51, 112.71, 111.31. HRMS)ESI): calcd for $C_{15}H_{10}O_5$ [M-H]$^-$ 269.0455, found 269.0458.

(*Z*)-2-(2,3-Dihydroxybenzylidene)-6,7-dihydroxybenzofuran-3(2*H*)-one (6e): This compound was obtained from (*Z*)-2-(2,3-dimethoxybenzylidene)-6,7-dimethoxybenzofuran-3(2*H*)-one (**5e**) employing method A. Yellow solid, yield 63.8%; mp 292.0-293.0 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.89 (dd, *J* = 7.8, 1.5 Hz, 1H), 7.38 (s, 1H), 7.20 (d, *J* = 8.4 Hz, 1H), 6.84 (dd, *J* = 7.8, 1.6 Hz, 1H), 6.79 (t, *J* = 7.9 Hz, 1H), 6.72 (d, *J* = 8.3 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 185.53, 157.08, 156.30, 149.10, 147.55, 146.36, 131.42, 123.90, 121.03, 120.66, 117.71, 117.10, 116.21, 113.69, 108.21. HEMS)ESI): calcd for $C_{15}H_{10}O_6$ [M-H]$^-$ 285.0405, found 285.0400.

(*Z*)-2-(2,4-Dihydroxybenzylidene)-6,7-dihydroxybenzofuran-3(2*H*)-one (6f): This compound was obtained from (*Z*)-2-(2,4-dimethoxybenzylidene)-6,7-dimethoxybenzofuran-3(2*H*)-one (**5f**) employing method A. Yellow solid, yield 54.2%; mp 227.8-228.8 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 8.28 (d, *J* = 8.7 Hz, 1H), 7.36 (s, 1H), 7.18 (d, *J* = 8.4 Hz, 1H), 6.71 (d, *J* = 8.3 Hz, 1H), 6.45 (dd, *J* = 8.7, 2.3 Hz, 1H), 6.35 (d, *J* = 2.3 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 185.30, 162.66, 160.97, 156.62, 155.65, 147.48, 134.96, 131.31, 116.80, 116.65, 113.55, 112.96, 109.64, 109.39, 103.01. HRMS)ESI): calcd for $C_{15}H_{10}O_6$ $[M-H]^-$ 285.0405, found 285.0400.

(*Z*)-2-(2,5-Dihydroxybenzylidene)-6,7-dihydroxybenzofuran-3(2*H*)-one (6g): This compound was obtained from (*Z*)-2-(2,5-dimethoxybenzylidene)-6,7-dimethoxybenzofuran-3(2*H*)-one (**5g**) employing method A. Yellow solid, yield 42.4%; mp 270.4-271.3 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.62 (d, *J* = 2.7 Hz, 1H), 7.13 (d, *J* = 8.3 Hz, 1H), 7.04 (s, 1H), 6.78 – 6.69 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.30, 155.23, 154.75, 150.33, 149.99, 146.96, 130.34, 119.30, 119.09, 116.60, 116.27, 115.46, 114.38, 112.68, 105.00. HRMS)ESI): calcd for $C_{15}H_{10}O_6$ [$M-H]^-$ 285.0405, found 285.0400.

(*Z*)-2-(2,6-Dihydroxybenzylidene)-6,7-dihydroxybenzofuran-3(2*H*)-one (6h): This compound was obtained from (*Z*)-2-(2,6-dimethoxybenzylidene)-6,7-dimethoxybenzofuran-3(2*H*)-one (**5h**) employing method A. Yellow solid, yield 22.1%; mp 268.3-269.3 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.19 (d, *J* = 8.4 Hz, 1H), 7.12 – 7.05 (m, 2H), 6.68 (d, *J* = 8.3 Hz, 1H), 6.44 (d, *J* = 8.2 Hz, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 183.10, 156.77, 154.83, 147.56, 130.97, 115.74, 112.60, 107.88, 107.05, 105.30. HRMS)ESI): calcd for $C_{15}H_{10}O_6$ $[M- H]^-$ 285.0405, found 285.0397.

(*Z*)-2-(3,4-Dihydroxybenzylidene)-6,7-dihydroxybenzofuran-3(2*H*)-one (6i): This compound was obtained from (*Z*)-2-(3,4-dimethoxybenzylidene)-6,7-dimethoxybenzofuran-3(2*H*)-one (**5i**) employing method A. Orange yellow solid, yield 63.7%; mp 295.3-296.2 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.44 (d, *J* = 2.0 Hz, 1H), 7.38 (dd, *J* = 8.3, 2.0 Hz, 1H), 7.12 (d, *J* = 8.3 Hz, 1H), 6.85 (d, *J* = 8.2 Hz, 1H), 6.73 (d, *J* = 8.3 Hz, 1H), 6.62 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.08, 155.06, 154.32, 148.01, 145.94, 145.54, 130.23, 124.59, 123.60, 118.43, 116.02, 115.27, 114.63, 112.62, 111.82. HRMS)ESI): calcd for $C_{15}H_{10}O_6$ $[M-H]^-$ 285.0405, found 285.0401.

(*Z*)-2-(3,5-Dihydroxybenzylidene)-6,7-dihydroxybenzofuran-3(2*H*)-one (6j): This compound was obtained from (*Z*)-2-(3,5-dimethoxybenzylidene)-6,7-dimethoxybenzofuran-3(2*H*)-one (**5j**) employing method A. Yellow solid, yield 20.2%; mp 325.6-326.6 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.84 (s, 1H), 9.49 (s, 3H), 7.14 (d, *J* = 8.3 Hz, 1H), 6.86 (d, *J* = 1.9 Hz, 2H), 6.75 (d, *J* = 8.3 Hz, 1H), 6.53 (s, 1H), 6.33 (t, *J* = 2.0 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.43, 158.56, 155.46, 154.84, 147.38, 133.51, 130.30, 115.59, 114.24, 112.72, 111.04, 109.46, 104.58. HRMS)ESI): calcd for $C_{15}H_{10}O_6$ $[M-H]^-$ 285.0405, found 285.0400.

(*Z*)-6,7-Dihydroxy-2-(2,3,4-trihydroxybenzylidene)benzofuran-3(2*H*)-one (6k): This compound was obtained from (*Z*)-6,7-dimethoxy-2-(2,3,4-trimethoxybenzylidene)benzofuran-3(2*H*)-one (**5k**) employing method A. Orange solid, yield 18.8%; mp 210.3 ºC carbonization; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.71 (d, *J* = 8.7 Hz, 1H), 7.11 – 7.08 (m, 2H), 6.73 (d, *J* = 8.3 Hz, 1H), 6.50 (d, *J* = 8.7 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 181.97, 154.83, 154.01, 148.87, 145.74, 132.89, 130.16, 122.66, 115.14, 114.85, 112.64, 111.89, 108.24, 106.49. HRMS)ESI): calcd for $C_{15}H_{10}O_7$ [M-H]$^-$ 301.0354, found 301.0346.

(*Z*)-6,7-Dihydroxy-2-(2,4,5-trihydroxybenzylidene)benzofuran-3(2*H*)-one (6l): This compound was obtained from (*Z*)-6,7-dimethoxy-2-(2,4,5-trimethoxybenzylidene)benzofuran-3(2*H*)-one (**5l**) employing method A. Yellow solid, yield 46.8%; mp 236.4 ºC carbonization; ^{1}H NMR (400 MHz, CD_3OD) δ 7.84 (s, 1H), 7.36 (s, 1H), 7.18 (d, *J* = 8.3 Hz, 1H), 6.71 (d, *J* = 8.4 Hz, 1H), 6.39 (s, 1H).^{13}C NMR (100 MHz, CD_3OD) δ 185.32, 156.71, 155.55, 154.61, 151.49, 147.31, 140.07, 131.57, 118.55, 116.88, 116.82, 113.49, 112.16, 110.20, 103.75. HRMS (ESI) *m/z*: calcd for $C_{15}H_{10}O_7$ [M-H]$^-$ 301.0354, found 301.0346.

(*Z*)-6,7-Dihydroxy-2-(2,4,6-trihydroxybenzylidene)benzofuran-3(2*H*)-one (6m): This compound was obtained from (*Z*)-6,7-dimethoxy-2-(2,4,6-trimethoxybenzylidene)benzofuran-3(2*H*)-one (**5m**) employing method A. Yellow solid, yield 20.9%; mp 219.4 ºC carbonization; ^{1}H NMR (400 MHz, CD_3OD) δ 7.24 (s, 1H), 7.19 (d, *J* = 8.4 Hz, 1H), 6.71 (d, *J* = 8.4 Hz, 1H), 5.97 (s, 1H). ^{13}C NMR (100 MHz, CD_3OD) δ 182.86, 161.69, 158.38, 154.37, 154.15, 144.88, 129.83, 115.37, 115.20, 112.26, 106.88, 100.28, 95.05. HRMS (ESI) *m/z*: calcd for $C_{15}H_{10}O_7$ [M-H]$^-$ 301.0354, found 301.0348.

(*Z*)-6,7-Dihydroxy-2-(3,4,5-trihydroxybenzylidene)benzofuran-3(2*H*)-one (6n): This compound was obtained from (*Z*)-6,7-dimethoxy-2-(3,4,5-trimethoxybenzylidene)benzofuran-3(2*H*)-one (**5n**) employing method A. Yellow solid, yield 31.7%; mp 290 ºC carbonization; ^{1}H NMR (400 MHz, CD_3OD) δ 7.18 (d, *J* = 8.3 Hz, 1H), 7.07 (s, 2H), 6.72 (d, *J* = 8.3 Hz, 1H), 6.63 (s, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 185.33, 156.98, 155.92, 148.08, 147.05, 137.72, 131.53, 124.51, 116.91, 116.38, 115.17, 113.50, 112.47. HRMS)ESI): calcd for $C_{15}H_{10}O_7$ [M-H]$^-$ 301.0354, found 301.0348.

(*Z*)-2-(3,4-Dihydroxybenzylidene)benzofuran-3(2*H*)-one (6o): This compound was obtained from (*Z*)-2-(3,4-dimethoxybenzylidene)benzofuran-3(2*H*)-one (**5o**) employing method A. Orange yellow solid, yield 93.7%; mp 225.4-226.0 ºC; ^{1}H NMR (400 MHz, CD_3OD) δ 7.80 – 7.71 (m, 2H), 7.57 (d, *J* = 2.0 Hz, 1H), 7.42 (d, *J* = 8.3 Hz, 1H), 7.32 – 7.25 (m, 2H), 6.87 (d, *J* = 8.3 Hz, 1H), 6.83 (s, 1H). ^{13}C NMR (100 MHz, CD_3OD) δ 182.86, 161.69, 158.38, 154.37, 154.15, 144.88, 129.83, 115.37, 115.20, 112.26, 106.88, 100.28, 95.05. HRMS (ESI) *m/z*: calcd for $C_{15}H_{10}O_4$ [M-H]$^-$ 253.0506, found 253.0505.

(*Z*)-2-(3,4-Dihydroxybenzylidene)-6-methoxybenzofuran-3(2*H*)-one (6p): This compound was obtained from (*Z*)-2-(3,4-dimethoxybenzylidene)-6-methoxybenzofuran-3(2*H*)-one(**5p**) employing method A. yellow solid, yield 52.3%; mp 221.7-222.4 ºC; ^{1}H NMR (400 MHz, CD_3OD) δ 7.64 (d, *J* = 8.6 Hz, 1H), 7.49 (d, *J* = 2.0 Hz, 1H), 7.25 (dd, *J* = 8.3, 2.0 Hz, 1H), 6.90 (d, *J* = 2.1 Hz, 1H), 6.83 (d, *J* = 8.3

Hz, 1H), 6.80 (dd, *J* = 8.6, 2.1 Hz, 1H), 6.69 (s, 1H), 3.93 (s, 3H).^{13}C NMR (100 MHz, CD_3OD) δ 184.53, 169.74, 169.42, 149.57, 147.62, 146.80, 126.55, 126.41, 125.47, 119.14, 116.71, 115.88, 115.18, 113.68, 97.58, 56.79. HRMS (ESI) *m/z*: calcd for $C_{16}H_{12}O_5$ $[M-H]^-$ 283.0612, found 283.0612.

(*Z*)-2-(3,4-Dihydroxybenzylidene)-5-hydroxybenzofuran-3(2*H*)-one (6q): This compound was obtained from (*Z*)-2-(3,4-dimethoxybenzylidene)-5-methoxybenzofuran-3(2*H*)-one (**5q**) employing method A. Orange solid, yield 55.7%; mp 308.7-309.4 ºC; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.74 (s, 2H), 9.29 (s, 1H), 7.48 (d, *J* = 2.0 Hz, 1H), 7.34 (d, *J* = 8.8 Hz, 1H), 7.29 (dd, *J* = 8.3, 2.0 Hz, 1H), 7.20 (dd, *J* = 8.8, 2.7 Hz, 1H), 7.01 (d, *J* = 2.7 Hz, 1H), 6.85 (d, *J* = 8.2 Hz, 1H), 6.74 (s, 1H).^{13}C NMR (150 MHz, DMSO-d_6) δ 183.45, 158.89, 153.75, 148.47, 145.65, 145.56, 125.34, 125.06, 123.47, 121.75, 118.23, 116.12, 113.71, 113.50, 107.64. HRMS (ESI) *m/z*: calcd for $C_{15}H_{10}O_5$ $[M-H]^-$ 269.0455, found 269.0455.

(*Z*)-2-(3,4-Dihydroxybenzylidene)-4-hydroxybenzofuran-3(2*H*)-one (6r): This compound was obtained from (*Z*)-2-(3,4-dimethoxybenzylidene)-4-methoxybenzofuran-3(2*H*)-one (**5r**) employing method A. Orange solid, yield 45.6%; mp 253.3 ºC carbonization; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.02 (s, 1H), 9.65 (s, 1H), 9.26 (s, 1H), 7.51 (t, *J* = 8.1 Hz, 1H), 7.44 (d, *J* = 1.8 Hz, 1H), 7.24 (dd, *J* = 8.3, 1.8 Hz, 1H), 6.84 (d, *J* = 8.2 Hz, 1H), 6.81 (d, *J* = 8.0 Hz, 1H), 6.62 (d, *J* = 8.9 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 181.11, 165.75, 157.00, 148.03, 145.59, 144.87, 138.20, 124.53, 123.54, 117.95, 116.08, 111.56, 110.37, 109.50, 102.35. HRMS (ESI) *m/z*: calcd for $C_{15}H_{10}O_5$ $[M-H]^-$ 269.0455, found 269.0456.

(*Z*)-2-(3,4-Dihydroxybenzylidene)-5,6-dihydroxybenzofuran-3(2*H*)-one (6s): This compound was obtained from (*Z*)-2-(3,4-dimethoxybenzylidene)-5,6-dimethoxybenzofuran-3(2*H*)-one (**5s**) employing method A. Orange solid, yield 30.4%; mp 229.0 ºC carbonization; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.72 (s, 4H), 7.42 (d, *J* = 1.9 Hz, 1H), 7.21 (dd, *J* = 8.3, 1.9 Hz, 1H), 6.95 (s, 1H), 6.82 (d, *J* = 8.2 Hz, 1H), 6.75 (s, 1H), 6.55 (s, 1H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 182.00, 161.91, 156.46, 148.26, 146.43, 145.96, 143.68, 124.83, 124.05, 118.29, 116.45, 112.18, 111.67, 107.51, 98.94. HRMS (ESI) *m/z*: calcd for $C_{15}H_{10}O_6$ $[M-H]^-$ 285.0405, found 285.0405.

(*Z*)-2-(3,4-Dihydroxybenzylidene)-4,6-dihydroxybenzofuran-3(2*H*)-one (6t): This compound was obtained from (*Z*)-2-(3,4-dimethoxybenzylidene)-4,6-dimethoxybenzofuran-3(2*H*)-one (**5t**) employing method A. Orange solid, yield 67.4%; mp 230 ºC carbonization; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.82 (s, 2H), 9.37 (d, *J* = 208.7 Hz, 2H), 7.38 (d, *J* = 2.0 Hz, 1H), 7.17 (dd, *J* = 8.3, 2.0 Hz, 1H), 6.81 (d, *J* = 8.2 Hz, 1H), 6.44 (s, 1H), 6.17 (d, *J* = 1.7 Hz, 1H), 6.06 (d, *J* = 1.7 Hz, 1H), ^{13}CNMR (150 MHz, DMSO-d_6) δ 179.06, 167.56, 167.01, 158.21, 147.49, 145.94, 145.49, 123.97, 123.71, 117.62, 116.00, 109.62, 102.90, 97.66, 90.34. HRMS (ESI) *m/z*: calcd for $C_{15}H_{10}O_6$ $[M-H]^-$ 285.0405, found 285.0404.

(*Z*)-2-(4-Hydroxy-3-methoxybenzylidene)benzofuran-3(2*H*)-one (6u): This compound was obtained from benzofuran-3(2*H*)-one and 4-hydroxy-3-methoxybenzaldehyde (**5u**) employing method B. Yellow

solid; yield 63.0%; mp 196.6-197.2 ºC; ^{1}H NMR (400 MHz,$CDCl_3$) δ 7.81 (d, *J* = 7.6 Hz, 1H), 7.67 – 7.62 (m, 1H), 7.49 (dd, *J* = 6.3, 1.9 Hz, 2H), 7.31 (d, *J* = 8.3 Hz, 1H), 7.22 (t, *J* = 7.5 Hz, 1H), 7.00 (d, *J* = 8.8 Hz, 1H), 6.87 (s, 1H), 6.02 (s, 1H), 3.99 (s, 3H).^{13}C NMR (100 MHz, $CDCl_3$) δ 184.63, 165.87, 147.95, 146.82, 145.90, 136.67, 126.75, 124.98, 124.73, 123.48, 122.05, 115.12, 114.06, 113.46, 112.98, 56.12. HRMS (ESI) *m/z*: calcd for $C_{16}H_{12}O_4$ [M-H]$^-$ 267.0663, found 267.0663.

(*Z*)-2-(3-Hydroxy-4-methoxybenzylidene)benzofuran-3(2*H*)-one (6v): This compound was obtained from benzofuran-3(2*H*)-one and 3-hydroxy-4-methoxybenzaldehyde employing method B. Yellow solid; yield 63.2%; mp 187.6-188.5 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.36 (s, 1H), 7.79 (d, *J* = 7.5 Hz, 2H), 7.56 – 7.51 (m, 2H), 7.42 (dd, *J* = 8.5, 2.0 Hz, 1H), 7.31 (t, *J* = 7.4 Hz, 1H), 7.06 (d, *J* = 8.5 Hz, 1H), 6.85 (s, 1H), 3.85 (s, 3H).^{13}C NMR (100 MHz, DMSO-d_6) δ 183.26, 165.13, 150.06 , 146.73, 145.13, 137.34, 124.86, 124.66, 124.23, 123.83, 121.26, 117.70, 113.42, 113.13, 112.24, 55.69. HRMS (ESI) *m/z*: calcd for $C_{16}H_{12}O_4$ [M-H]$^-$ 267.0663, found 267.0664.

(*Z*)-2-(3,4-Dimethoxybenzylidene)-6-hydroxybenzofuran-3(2*H*)-one (6w): This compound was obtained from 6-hydroxybenzofuran-3(2*H*)-one and 3,4-dimethoxybenzaldehyde employing method C. Yellow solid 93.5%; mp 218.9-219.6 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.14 (s, 1H), 7.61 (d, *J* = 8.4 Hz, 1H), 7.56 (d, *J* = 7.5 Hz, 2H), 7.07 (d, *J* = 8.6 Hz, 1H), 6.80 (d, *J* = 1.8 Hz, 1H), 6.76 (s, 1H), 6.71 (dd, *J* = 8.5, 1.9 Hz, 1H), 3.83 (s, 3H), 3.82 (s, 3H).^{13}C NMR (100 MHz, DMSO-d_6) δ 181.30, 167.66, 166.32, 150.44, 148.80, 146.30, 125.84, 125.15, 124.82, 114.27, 113.12, 112.99, 112.01, 111.24, 98.70, 55.64, 55.59. HRMS (ESI) *m/z*: calcd for $C_{17}H_{14}O_5$ [M-H]$^-$ 297.0768, found 297.0768.

(*Z*)-6-Hydroxy-2-(4-hydroxy-3-methoxybenzylidene)benzofuran-3(2*H*)-one (6x): This compound was obtained from 6-hydroxybenzofuran-3(2*H*)-one and 4-hydroxy-3-methoxybenzaldehyde employing method C. Yellow solid; yield 25.7%; mp 261.2-262.1 ºC; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.09 (s, 1H), 9.74 (s, 1H), 7.60 (d, *J* = 8.4 Hz, 1H), 7.53 (d, *J* = 2.0 Hz, 1H), 7.49 – 7.47 (m, 1H), 6.90 (d, *J* = 8.2 Hz, 1H), 6.80 (d, *J* = 1.9 Hz, 1H), 6.74 (s, 1H), 6.71 (dd, *J* = 8.4, 2.0 Hz, 1H), 3.84 (s, 3H).^{13}C NMR (150 MHz, DMSO-d_6) δ 181.23, 167.53, 166.18, 148.92, 147.80, 145.85, 125.76, 125.48, 123.49, 116.11, 115.19, 113.22, 112.91, 111.78, 98.65, 55.72. HRMS (ESI) *m/z*: calcd for $C_{16}H_{12}O_5$ [M-H]$^-$ 283.0612, found 283.0611.

(*Z*)-6-Hydroxy-2-(3-hydroxy-4-methoxybenzylidene)benzofuran-3(2*H*)-one (6y): This compound was obtained from 6-hydroxybenzofuran-3(2*H*)-one and 3-hydroxy-4-methoxybenzaldehyde employing method C. Yellow solid; yield 86.2%; mp 254.9-255.5 ºC; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.14 (s, 1H), 9.28 (s, 1H), 7.61 (d, *J* = 8.4 Hz, 1H), 7.49 (d, *J* = 2.1 Hz, 1H), 7.35 (dd, *J* = 8.4, 2.0 Hz, 1H), 7.03 (d, *J* = 8.4 Hz, 1H), 6.76 (d, *J* = 1.9 Hz, 1H), 6.71 (dd, *J* = 8.4, 1.9 Hz, 1H), 6.67 (s, 1H), 3.83 (s, 3H).^{13}C NMR (150 MHz, DMSO-d_6) δ 181.29, 167.61, 166.29, 149.61, 146.65, 146.13, 125.89, 124.83, 124.27,

117.42, 113.15, 112.97, 112.22, 111.40, 98.46, 55.66. HRMS (ESI) *m/z*: calcd for $C_{16}H_{12}O_5$ $[M-H]^-$ 283.0612, found 283.0610.

DRAK2 Inhibitor Assays. To identify small‑molecule DRAK2 inhibitors, we used the ADP-Glo™ Kinase Assay kit (Promega) to identify compounds inhibiting DRAK2 in vitro. DRAK2 activity was measured and calculated from with the mount of ADP generated from the enzyme reaction. Compounds were dissolved in DMSO. DRAK2 protein and substrate ATP was diluted in 1X Buffer (HEPES 50 mM pH 7.0, NaN_3 0.02%, Orthovanadate 0.1 mM, $MgCl_2$ 5 mM, Bovine serum albumin 0.01% (w/v). The kinase reaction containing 1 μL of compound solution and 2 μL of DRAK2 (400 nM) were inniated by adding 2 μL of substrate solution (50 μM ATP) and incubated for 2.0 h at room temperature. Then, 2.5 μL of ADP-Glo™ Reagent was added and incubated for 2.0 h to deplete the remaining ATP. Finally, 5 μL of Kinase Detection Reagent was added to convert ADP to ATP with luciferase reaction, which be detected using an EnVision multilabel plate reader. The inhibitor compound **6i** was used as a positive control in the assay.

ACKNOWLEDGEMENTS

This work was supported by the National Key R&D Program of China (No. 2020YFE0205600), the Central Asian Center of Drug Research and Development, Chinese Academy of Sciences (No. CAM201904&CAM202001), and One Belt One Road Youth Exchange Program of Shanghai Municipal Committee of Science and Technology (19430742000).

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