

OPEN

Synthesis, Bioevaluation, Structure-Activity Relationship and Docking Studies of Natural Product Inspired (Z)-3-benzylideneisobenzofuran-1(3H)-ones as Highly Potent antioxidants and Antiplatelet agents

Bharti Rajesh Kumar Shyamal¹, Lalit Yadav¹, Mohit K. Tiwari¹, Manas Mathur², Jaroslav I. Prikhodko³, Irina V. Mashevskaya³, Dharmendra K. Yadav^{4*} & Sandeep Chaudhary^{1*}

For the first time, a series of highly potent natural product inspired substituted (Z)-3-benzylideneisobenzofuran-1(3H)-ones 28a-t, embraced with electron-withdrawing groups (EWG) and electron-donating groups (EDG) at site I and site II, were prepared and assessed for their *in vitro* antioxidant activities (DPPH free radical scavenging assay) and arachidonic acid (AA)-induced antiplatelet activities using ascorbic acid (IC₅₀ = 4.57 µg/mL) and aspirin (IC₅₀ = 21.34 µg/mL), as standard references, respectively. In this study, compounds 28f-g, 28k-l and 28q have shown high order of *in vitro* antioxidant activity. Infact, 28f and 28k were found to show **10-folds** and **8-folds** more antioxidant activity than ascorbic acid, respectively and was found to be the most active analogues of the series. Similarly, Compounds 28c-g, 28k-l, 28o and 28q-t were recognized as highly potent antiplatelet agents (upto **6-folds**) than aspirin. Furthermore, *in silico* studies of the most active antioxidants 28f, 28k and 28l and very active antiplatelet molecules 28f, 28k, 28l and 28s were carrying out for the validation of the biological results. This is the first detailed study of the discovery of several (Z)-3-benzylideneisobenzofuran-1(3H)-ones as highly potent antioxidants and antiplatelet agents.

Isobenzofuran-1(3H)-one, commonly known as “phthalide” is found in many naturally-occurring and pharmaceuticals important molecules¹. This benzo-fused heterocyclic class of compounds are blended with several pharmacological properties such as anti-tumor², anti-HIV³, anti-allergic⁴, antifungal⁵, antidiabetic⁶, antispasmodic⁷, anti-inflammatory⁸, pesticidal⁹, COX-2 inhibitor¹⁰, insecticidal¹¹, anti-microbial¹², herbicidal¹³, and anti-cancer¹⁴ etc. Several naturally occurring as well as synthetic molecules having isobenzofuran-1(3H)-ones have also been classified as promising antioxidants 1–7 (Fig. 1)^{15–18}. Similarly, several natural and synthetic molecules 7–15 show promising platelet aggregation inhibitors¹⁵ (Fig. 1). Relevant to the present study, natural product inspired isobenzofuran-1(3H)-ones are known to be potential antioxidant (7) and antiplatelet agents (7, 12–15)^{15,19–21}.

Earlier, Teng and his co-workers carried out extensive platelet aggregation inhibitory studies on butylidene-phthalide 7 triggered by various inducers such as Arachidonic acid (AA), Adenosine diphosphate (ADP), platelet activation factor (PAF), etc. as well as the inhibition of thromboxane B2 (TXB2) formation caused by AA, collagen, ionophore A23187 and thrombin²⁰. In this study, 7 showed significantly higher inhibitory

¹Laboratory of Organic & Medicinal Chemistry, Department of Chemistry, Malaviya National Institute of Technology, Jaipur, 302017, India. ²School of Agriculture, Suresh Gyan Vihar University, Mahal Road, Jagatpura, Jaipur, 302017, India. ³Department of Organic Chemistry, Perm State University, Bukireva Street, Perm, 614990, Russian Federation. ⁴Gachon Institute of Pharmaceutical Sciences and Department of Pharmacy, College of Pharmacy, Gachon University of Medicine and Science, Incheon, 21936, South Korea. *email: dharmendra30oct@gmail.com; schaudhary.chy@mnit.ac.in

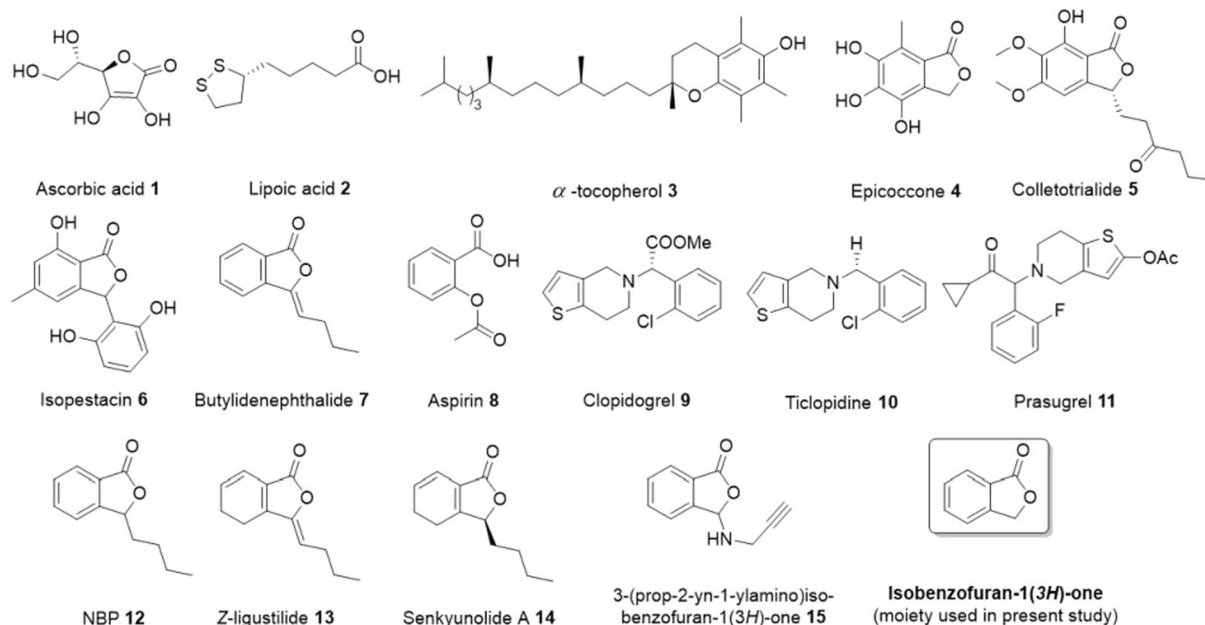


Figure 1. Structure of some naturally occurring/synthetic antioxidants 1–7 and antiplatelet agents 7–15.

efficacy in AA-induced platelet aggregation ($IC_{50} = 70 \mu M$) in comparison to collagen-induced aggregation ($IC_{50} = 120 \mu M$) in washed rabbit platelets. Teng *et al.* also suggested that butylidenephthalide 7 inhibits platelet aggregation mainly by inhibiting cyclooxygenase-1 (COX-1) enzyme leading to the reduction of thromboxane A₂ (TBXA₂) formation. Similar studies further confirms the above facts^{15,21}. Overall, these studies revealed that (*Z*)-3-substituted-isobenzofuran-1(*3H*)-ones class of compounds exhibits AA-induced antiplatelet activities *via* inhibition of COX-1²⁰. Nevertheless, there has been no detailed study on the AA-induced platelet aggregation inhibitory activities of (*Z*)-3-benzylideneisobenzofuran-1(*3H*)-ones. Therefore, there is a scope to develop a more potent isobenzofuran-1(*3H*)-ones having potential antioxidant as well as antiplatelet agents.

Synthetic/naturally isolated isobenzofuran-1(*3H*)-ones and its derivatives have attracted medicinal chemists and pharmacologists due to their pronounced biological activities and their potential applications as antioxidant as well as antiplatelet agents^{15–21}. For example, ascorbic acid 1¹⁵, Pestacin 17¹⁷, Micromeriol 18²², Pulvinate analogue 19²³, 5-(bis(3,4-dimethoxyphenyl)-methylene)furan-2(*5H*)-one 20²⁴, ailanthoidol 21²⁵, etc. were reported as antioxidants. It has been well documented that isobenzofuran-1(*3H*)-ones displayed good AA-induced platelet aggregation inhibitory activity as compared to ADP and collagen-induced factors. However, the AA-induced platelet aggregation inhibiting activities of (*Z*)-3-benzylideneisobenzofuran-1(*3H*)-ones have never been explored. Nevertheless, so far, there is no detailed study done proving its inhibition by cyclooxygenase-1 (COX-1) enzyme.

Therefore, in our endeavor in search for novel bioactive heterocycles and also based on the above facts, we have designed **prototype 16** i.e. C-3 (*Z*)-benzylideneisobenzofuran-1(*3H*)-ones, incorporating similar sub-structural units of 1, 7 and 17–25 (Fig. 2) and assessed their antioxidant and AA-induced platelet aggregation inhibiting activities with the anticipation that the (*Z*)-3-benzylideneisobenzofuran-1(*3H*)-ones would also show promising antioxidant as well as antiplatelet activity. Hence, the designed prototype 16 is derived from sub-structure in which the molecule, as a whole or in part of, is responsible for antioxidant activity. Similarly, butylidenephthalide 7 (dual potential as antioxidant and antiplatelet agent)¹⁵, Justicidin A 22²⁶, prostacyclin (Trade name: Epoprostenol, prostaglandin I₂) 23²⁷, Zontivity (Trade name: Vorapaxar) 24²⁸, 6-bromo-3-butyloisobenzofuran-1(*3H*)-one (bromo derivative of NBP) 25²⁹, etc. were reported to show promising platelet aggregation inhibitory activity (Fig. 2).

In the present study, we report the synthesis a series of functionalized (*Z*)-3-benzylideneisobenzofuran-1(*3H*)-ones 28a–t, their antioxidant and AA-induced antiplatelet activities, and structure-activity relationship (SAR) studies. Although compounds 28a–j, 28m–r and 28t have been prepared earlier by other routes;³⁰ including our route;³¹ however, for the first time, substrate-controlled silver oxide nanoparticle (Ag₂ONPs)-catalyzed synthesis of compound 28a–t have been prepared. Various substituted (*Z*)-3-benzylideneisobenzofuran-1(*3H*)-ones 28a–t have shown high order of antioxidant and AA-induced antiplatelet activities using ascorbic acid and aspirin taken as standard reference, respectively. We also perform the *in silico* studies of most active compounds 28f, 28k, 28l and 28s for the validation of biological results.

Results and Discussion

Recently, an efficient synthesis of (*Z*)-3-benzylideneisobenzofuran-1(*3H*)-ones in a highly regioselective manner in excellent yields has been reported by our group³¹. Ag₂ONPs-mediated reaction of several substituted 2-iodobenzoic acids 26a–f (R¹ = H, Br, CH₃, F, OCH₃; X = Br, I) with various *p*-/*m*-substituted terminal alkynes 27a–f in the presence of pivalic acid as additive in DMF as solvent at 120 °C for 3 h furnished

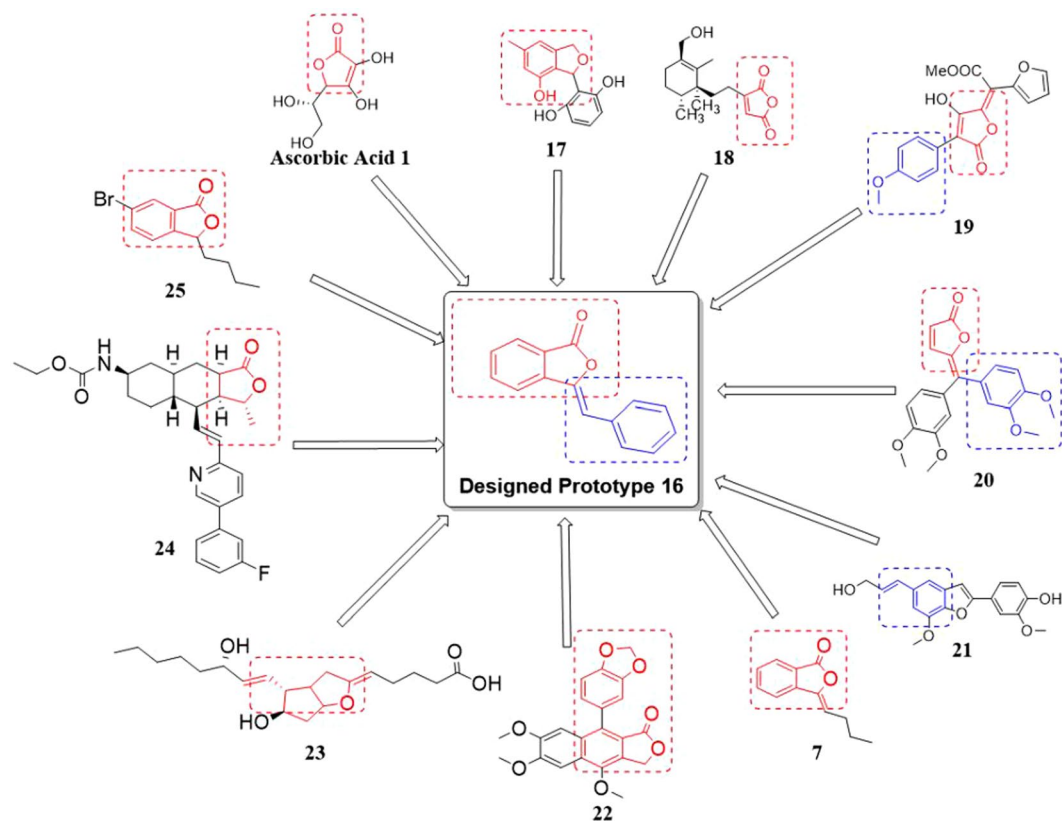


Figure 2. Design strategy for the target compound (*Z*)-3-benzylideneisobenzofuran-1(*3H*)-one **16** as an antioxidant and as an antiplatelet agent.

(*Z*)-3-benzylideneisobenzofuran-1(*3H*)-ones **28a–t** upto 95% yields via 5-*exo-dig* cyclization strategy (Fig. 3). The protocol is operationally simple and show functional group tolerance. The structures of compounds **28a–t** were confirmed by their spectroscopic analytical data (^1H and ^{13}C NMR, FT-IR and HRMS) (Fig. 3).

Then, the functionalized (*Z*)-3-benzylideneisobenzofuran-1(*3H*)-ones **28a–t** were assessed for their *in vitro* antioxidant activity using literature procedure (Fig. 3)^{31,32}. The results are shown in Table 1. Primarily, (*Z*)-3-benzylideneisobenzofuran-1(*3H*)-one **28a**, unsubstituted at site I and II, was assessed for its *in vitro* antioxidant activity. It was observed that **28a** ($\text{IC}_{50} = 14.38 \pm 0.09 \mu\text{g/mL}$), demonstrated reduced potency than the standard reference, ascorbic acid ($\text{IC}_{50} = 4.57 \mu\text{g/mL}$) (Table 1, Entry 1). On the other hand, **28b** ($\text{IC}_{50} = 8.88 \pm 0.12 \mu\text{g/mL}$) and **28c** ($\text{IC}_{50} = 6.33 \pm 0.08 \mu\text{g/mL}$) having *p*-methyl (*p*- CH_3) and *p*-chloro (*p*-Cl) substituents considerably augments the antioxidant activity in comparison to **28a** (Table 1, entry 2–3). Introduction of the substituent having larger size i.e., *p*-Br group in the case of compound **28d** ($\text{IC}_{50} = 52.34 \pm 0.29 \mu\text{g/mL}$), drastically diminishes the antioxidant activity (Table 1, entry 4). Furthermore, incorporation of methoxy (OMe) substituent at site II exhibited incremental effect in the antioxidant activity. Likewise, Compound **28e** ($\text{IC}_{50} = 34.41 \pm 0.94 \mu\text{g/mL}$), having *p*-OMe substitution at site II exhibited good activity as compared to **28d** (Table 1, entry 5); surprisingly, compound **28f** ($\text{IC}_{50} = 0.41 \pm 0.12 \mu\text{g/mL}$), having *m*-methoxy (*m*-OMe) group at site II demonstrated high order of antioxidant activity in comparison with reference standard (Table 1, entry 6). Infact, **28f** displayed 10-folds more activity than ascorbic acid.

Also, parallel trends were observed when **28g–l** having 6-bromo substituent at site I along with H/ CH_3 /Cl/Br/*p*-OMe/*m*-OMe groups at *p*-/*m*-position of site II were analyzed (Table 1, entry 7–12). Sequentially, **28g** ($\text{IC}_{50} = 1.59 \pm 0.55 \mu\text{g/mL}$), 6-Br substitution at site I and unsubstituted at site II, displayed approximately 3-folds greater potency (Table 1, Entry 7). Nonetheless, **28h–j** having bromo substitution at C-6 position of site I and *p*- CH_3 , *p*-Cl and *p*-Br substitution at site II do not have valuable effect on the antioxidant activity (Table 1, entry 8–10). But, compound **28k** ($\text{IC}_{50} = 0.55 \pm 0.15 \mu\text{g/mL}$) and **28l** ($\text{IC}_{50} = 0.732 \pm 0.44 \mu\text{g/mL}$), having bromo substitution at C-6 position of site I and *p*-OMe/*m*-OMe group at site II showed highly potent activity in comparison to ascorbic acid (Table 1, Entry 11–12). Infact, **28k** and **28l**, showed greater than **8-folds** and **7-folds** more activity than the standard reference (Table 1, Entry 11–12).

In parallel, further developments were noticed when compounds **28m–q** having electron-donating group (EDG) i.e., methyl groups at C-6 position of site I along with H/ CH_3 /Cl/OMe groups at *p*-/*m*-position of site II were analyzed (Table 1, entry 13–17). Compound **28m** ($\text{IC}_{50} = 7.23 \pm 0.04 \mu\text{g/mL}$), electron-donating group (CH_3 group) at C-6 position of site I and no substitution at site II, displayed potential activity than ascorbic acid and showed greater activity than that of **28a** (Table 1, Entry13). However, **28n–p** having CH_3 group at C-6 position of site I and *p*- CH_3 , *p*-Cl and *p*-OMe substitutions at site II also showed potency except **28n** (Table 1, entry 14–16). Nonetheless, compound **28q** ($\text{IC}_{50} = 3.83 \pm 0.88 \mu\text{g/mL}$), having CH_3 group at C-6 position of site I and *m*-OMe

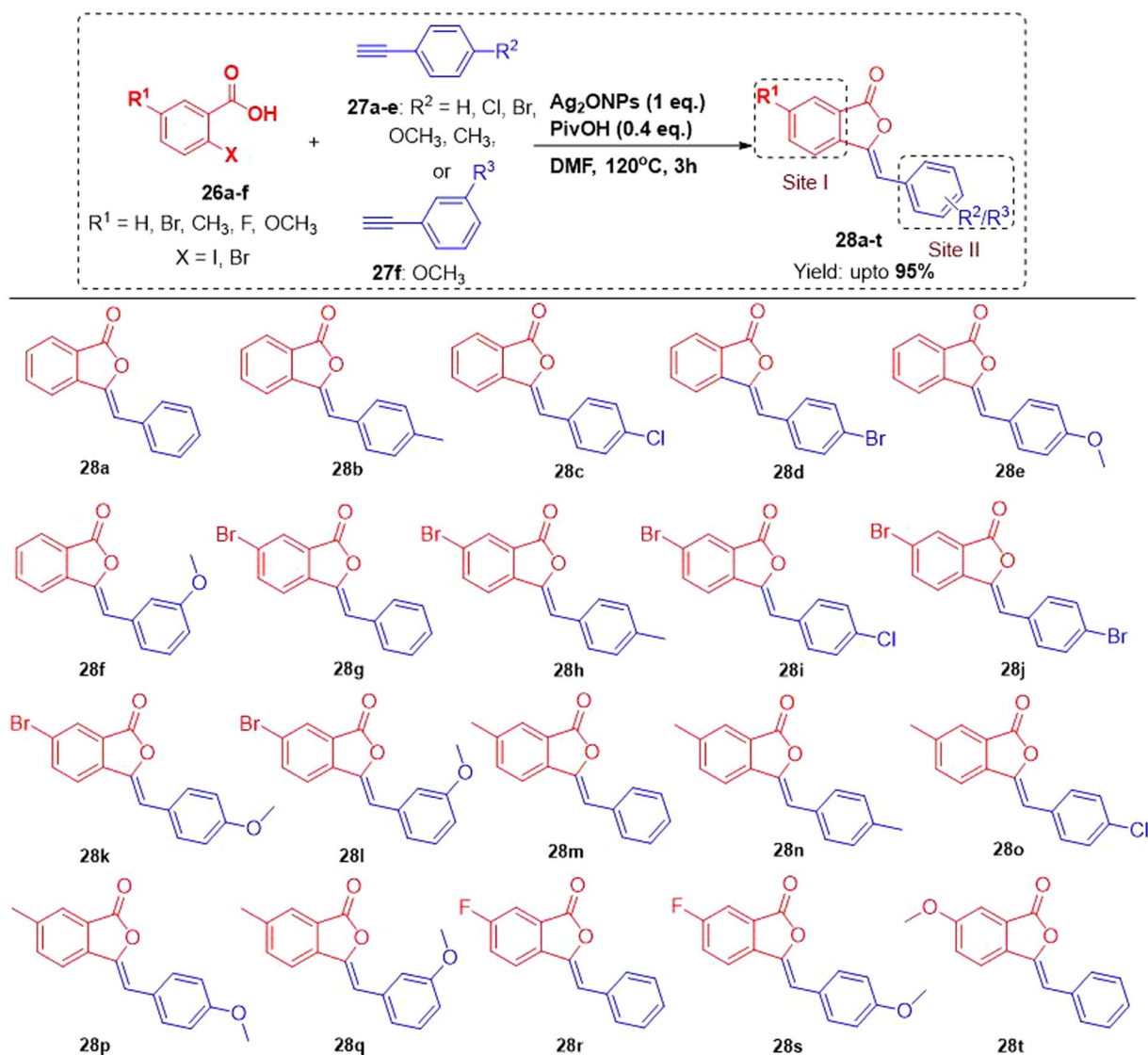


Figure 3. Synthesis of functionalized (Z)-3-benzylideneisobenzofuran-1(3H)-ones **28a-t** using our procedure.

group at site II showed more antioxidant potency than ascorbic acid (Table 1, Entry17). Chronologically, EWG (F) group at C-6 position of site I were also analyzed (**28r-s**) and it was observed that both the compounds exhibited decreased potency than the standard reference, thereby, decreasing the significance of EWG group (entry 18–19, Table 1).

The SAR analysis revealed that the five compounds i.e. **28f-g**, **28k-l** and **28q**, exhibited promising antioxidant activity with the IC_{50} values in the range 0.41–3.83 $\mu\text{g/mL}$. Further, no substitution at C-6 position of site I and *m*-OMe group at site II (i.e. compound **28f**), showed best antioxidant potency than ascorbic acid. However, EDG substitution at site I (Br, CH_3) and *H/p*-OMe/*m*-OMe substitution on site II also accounts for promising activity (i.e. compound **28g**, **28k-l** and **28q**). EWG group (fluoro group) at site I do not show favourable effects on the antioxidant activity [strong EWG-containing phenyl acetylenes (F, NO_2 , CN etc.) do not undergoes reaction under our optimized reaction conditions]. In addition, *m*-OMe group at site II augment antioxidant activity. Therefore, to examine the effect of OMe group at site I, we prepared **28t**. It was found that compound **28t** ($\text{IC}_{50} = 21.21 \pm 0.12 \mu\text{g/mL}$), having OMe group at C-6 position of site I and no substitution at site II showed diminished activity in comparison with the standard reference (Table 1, entry 20). Thus, OMe group showed advantageous effect on site II rather than site I. Finally, the structure-activity relationship studies illustrates that the two compounds, **28f** and **28k**, showed **10-folds** and **8-folds** higher antioxidant potency than commercially used antioxidant referred in the present study.

Since this scaffold have shown promising inhibition of platelet aggregation; compounds **28a-t** were also tested for their AA-induced inhibition of platelet aggregation using aspirin as the standard reference (Table 1)^{33,34}. As it has been observed from Table 1, compounds **28a-f** were prepared having no substitutions at C-6 position of site I and *H/CH₃/Cl/Br/p*-OMe/*m*-OMe substitutions at site II, respectively; **28a** ($\text{IC}_{50} = 64.57 \pm 0.58 \mu\text{g/mL}$) demonstrated significant AA-induced antiplatelet activity in comparison with the standard reference aspirin

Entry. No.	Comp. No.	R ¹	R ²	R ³	Antioxidant Activity ^[a,b] (IC ₅₀ in µg/mL)	AA-induced antiplatelet activity ^[c,d] (IC ₅₀ in µg/mL)
1	28a	H	H	H	14.38 ± 0.09	64.57 ± 0.58
2	28b	H	CH ₃	H	8.88 ± 0.12	44.66 ± 0.41
3	28c	H	Cl	H	6.33 ± 0.08	19.57 ± 0.28
4	28d	H	Br	H	52.34 ± 0.29	12.86 ± 0.11
5	28e	H	OCH ₃	H	34.41 ± 0.94	14.00 ± 0.17
6 ^[e]	28f	H	H	<i>m</i> -OCH ₃	0.41 ± 0.12	4.20 ± 0.28
7	28g	Br	H	H	1.59 ± 0.55	16.28 ± 0.25
8	28h	Br	CH ₃	H	65.91 ± 0.75	29.32 ± 0.38
9	28i	Br	Cl	H	28.61 ± 3.11	31.34 ± 0.36
10	28j	Br	Br	H	20.36 ± 0.27	79.57 ± 0.64
11	28k	Br	OCH ₃	H	0.55 ± 0.15	7.28 ± 0.48
12 ^[e]	28l	Br	H	<i>m</i> -OCH ₃	0.73 ± 0.44	4.20 ± 0.28
13	28m	CH ₃	H	H	7.23 ± 0.04	47.93 ± 0.44
14	28n	CH ₃	CH ₃	H	45.60 ± 0.91	25.60 ± 0.31
15	28o	CH ₃	Cl	H	7.60 ± 0.38	19.64 ± 0.28
16	28p	CH ₃	OCH ₃	H	16.34 ± 0.27	24.64 ± 0.42
17 ^[e]	28q	CH ₃	H	<i>m</i> -OCH ₃	3.83 ± 0.88	9.60 ± 0.62
18	28r	F	H	H	13.20 ± 0.21	11.37 ± 0.67
19	28s	F	OCH ₃	H	25.52 ± 0.30	3.25 ± 0.18
20	28t	OCH ₃	H	H	21.21 ± 0.12	5.16 ± 0.32
21	Ascorbic acid 1	—	—	—	4.57	—
22	Acetyl salicylic acid 8	—	—	—	—	21.34 ± 1.09

Table 1. *In vitro* antioxidant activity (DPPH free radical scavenging assay) and *In vitro* AA-induced antiplatelet activity of functionalized (*Z*)-3-benzylideneisobenzofuran-1(3*H*)-ones **28a-t**. ^[a]The results are articulated as a mean ± standard deviation (n = 3). ^[b]For details, see ref. ³⁷. ^[c]For details, see refs. ^{20,34}. ^[d]A mean of 03 experimental data are determined. ^[e]The OMe group at R³-position of the molecule is present at the *meta*-position; however, all the group at R² are at *para*-position.

(IC₅₀ = 21.34 ± 1.09 µg/mL; Table 1, entry 1). Shifting to CH₃ group at *p*-position of site II (**28b**) improves IC₅₀ value to 44.66 ± 0.41 µg/mL (Table 1, entry 2). However, the antiplatelet activity was improved tremendously when Cl/Br/OMe groups at site II were introduced. Compounds **28c-e** showed IC₅₀ value of 19.57 ± 0.28 µg/mL, 12.86 ± 0.11 µg/mL and 14.00 ± 0.17 µg/mL, respectively which were found to be more active than aspirin (Table 1, entries 3–5). As also been noticed in the case of antioxidant activities of these compounds, **28f** having *m*-OMe group at site II exhibited five-folds more efficacious (IC₅₀ = 4.20 ± 0.28 µg/mL) than the standard reference (Table 1, entry 6). Furthermore, **28g-l** were analyzed having bromo substitution at C-6 position of site I along with H/CH₃/Cl/Br/*p*-OMe/*m*-OMe groups at *p*/*m*-position of site II (Table 1, entry 7–12). It has been interpreted that introduction of bromine group at site I improves antiplatelet activity (IC₅₀ = 16.28 ± 0.25 µg/mL) upto four-folds than **28a** and more active than aspirin (Table 1, entry 7). In contrast, compounds **28h-j** having bromo substitution at C-6 position of site I along with CH₃/Cl/Br groups at *p*-position of site II showed decreased activity i.e., IC₅₀ values of 29.32 ± 0.38 µg/mL, 31.34 ± 0.36 µg/mL and 79.57 ± 0.64 µg/mL, respectively, than the standard reference (Table 1, entry 8–10). The activity has been increased effectively if OMe groups at *p*/*m*-position of site II is introduced. Compounds **28k** (IC₅₀ = 7.28 ± 0.48 µg/mL) and **28l** (IC₅₀ = 4.20 ± 0.28 µg/mL) having bromo substitution at C-6 position of site I along with *p*/*m*-OMe substitutions on site II showed 3–5 folds greater antiplatelet potency than the standard reference, respectively (Table 1, entry 11–12).

Similarly, we analyzed the effect of EDG group at site I and prepared **28m-q** having H/CH₃/Cl/OMe groups at *p*/*m*-position of site II (Table 1, entry 13–17). Introduction of CH₃ group at site I (**28m**) showed more aggregation inhibitory activity (IC₅₀ = 47.93 ± 0.44 µg/mL) than **28a** and less activity than **28g** (Table 1, entry 13). The activity has been found comparable to aspirin if CH₃/Cl substitutions on site II were also introduced (Table 1, entry 14–15). While **28p** (IC₅₀ = 24.64 ± 0.42 µg/mL) with methyl (CH₃) group at C-6 position of site I and *p*-OMe substitutions on site II exhibited comparable activity; Compound **28p** (IC₅₀ = 24.64 ± 0.42 µg/mL) with methyl (CH₃) group at C-6 position of site I and *m*-OMe substitutions on site II showed two-folds more potency than the standard reference (Table 1, entry 16–17). Sequentially, (EWG) group i.e., fluoro groups at C-6 position of site I were also analyzed (**28r-s**) and it was observed that both the compounds, **28r** (IC₅₀ = 11.37 ± 0.67 µg/mL) and **28s** (IC₅₀ = 3.25 ± 0.18 µg/mL), exhibited ~two- to seven-folds more potency than aspirin, respectively (Table 1, entry 18–19). Infact, Compound **28s** showed highest potency among the series. Compound **28t** (IC₅₀ = 5.16 ± 0.32 µg/mL), in contract to the antioxidant activity, showed four-folds more antiplatelet potency than the standard reference (Table 1, entry 20). Overall, it interprets that introduction of EWG or EDG at site I improves antiplatelet activity than aspirin. Similarly, introduction of *p*/*m*-OMe on site II leads to either more activity or comparable to the standard reference.

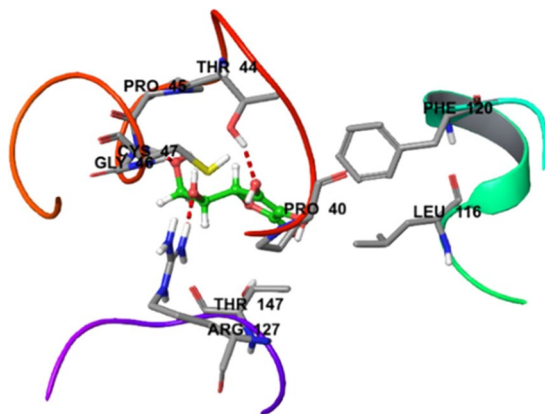


Figure 4. The binding interaction of standard drug **ascorbic acid** (docking score = 3.1764).

Molecular docking studies. All the most active compounds (**28f**, **28k** and **28l**) along with one inactive compound **28d** were further analyzed for their *in silico* studies using the reported protocol where the antioxidant target (PDB ID: 3MNG) were taken to explore the orientations and binding affinities of the target compounds in order to observe the difference in the docking score of active and inactive compounds^{35,36}. Wild type human antioxidant enzyme Peroxiredoxins (Prdxs) was chosen, containing essential cysteine residues as catalyst and thioredoxin as an electron donor, which help in scavenging peroxide and are involved in the metabolic cellular response to reactive oxygen species³⁶. It has been confirmed that the ascorbate-mediated reduction of protein sulfenic acids represents a modification of the peroxiredoxin-thiol-specific antioxidant paradigm, which directly confirms the interlinking of peroxiredoxins with standard drug ascorbic acid (vitamin C)³⁶. Therefore, the interlinking of standard reference ascorbic acid with peroxiredoxins direct us to perform molecular docking studies on this enzyme.

Similarly, to study the binding modes of the five active molecules (**28k**, **28s**, **28f**, **28l** and **28j**) in the cyclooxygenase-1 (COX-1) enzyme against platelet aggregation inhibitory activity, we performed molecular docking study with aspirin (reference compound) on COX-1 domain antiplatelet target (PDB ID: 2OYE) using Surfex-Dock using the reported procedure (see details in supporting information)³⁷.

Antioxidant molecular docking studies. The docking outcomes for the **ascorbic acid** against antioxidant target reflected a high binding affinity (docking score = 3.1764) as shown in Fig. 4. The active compound **28k**, **28f** and **28l** displayed docking results against antioxidant target (PDB ID: 3MNG) exhibited a docking score of 3.9321, 4.6899 and 3.4080, respectively, thereby reflecting their high binding affinity. These values were found to be more than that of ascorbic acid. Therefore, **28k**, **28f** and **28l** showed elevated binding affinity and hydrophobic interaction which are responsible for more stability and activity (Fig. 5A–C). Likewise, the inactive compound **28d** (Fig. 5D) was found to show less binding affinity than the ascorbic acid as indicated by its docking score of 2.9813. This showed low binding affinity and weak hydrophobic interaction which may be responsible for less stability and activity (see details in supporting information).

Antiplatelet molecular docking studies. Likewise, the antiplatelet docking outcomes for aspirin against target (PDB ID: 2OYE) revealed low docking score (total score of 4.4803) thereby reflecting its lower binding affinity (Fig. 6A). The docking outcomes for **28k**, **28s**, **28f**, and **28l** against PDB ID: 2OYE reflected docking score designated by a total score of 5.1953, 5.7131, 5.1010 and 5.1184, respectively thereby indicating a high binding affinities which were found to be more than standard reference aspirin. Therefore, **28k**, **28s**, **28f** and **28l** elevated binding affinity and hydrophobic interaction which are responsible for more stability and activity (Fig. 6B–E). In contrast, the antiplatelet docking outcomes for the inactive compound **28j** against target (PDB ID: 2OYE) showed lower docking score (total score = 4.1714) thereby reflecting lower binding affinity which, in turn, is found to be lesser than the standard reference. Thus, the bound compound **28j** showed weak hydrophobic interaction which leads to low binding affinity thereby responsible for less stability and activity of the molecule (Fig. 6F).

Conclusions

We disclose the first detailed study for the identification of (*Z*)-3-benzylideneisobenzofuran-1(3*H*)-one analogues **28a–t** as highly potent antioxidant and AA-induced antiplatelet agents. Five isobenzofuran-1(3*H*)-one analogues **28f–g**, **28k–l** and **28q** were found to be the active compounds of the series in DPPH assay. Infact, two compounds, **28f** and **28k**, showed **10-folds** and **8-folds** more antioxidant activity than ascorbic acid, respectively. Similarly, twelve isobenzofuran-1(3*H*)-one analogues **28c–g**, **28k–l**, **28o**, and **28q–t** exhibited highly potent (upto **6-folds**) platelet aggregation inhibitors as compared to aspirin in AA-induced antiplatelet biological assay. Furthermore, compounds **28f**, **28k**, **28l** and **28s** were analyzed through docking studies for the justification of the obtained results. These highly active potent molecules were found commendable of additional structural optimization and advancement as potential antioxidant and/or antiplatelet representatives.

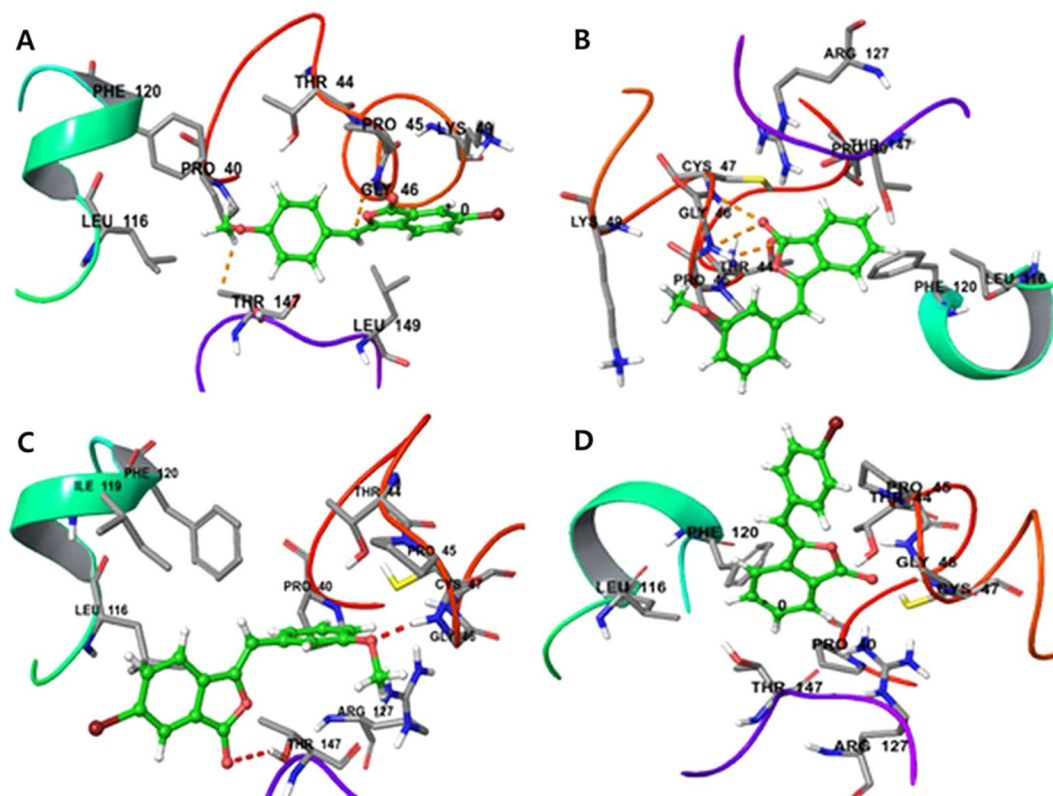


Figure 5. The docking outcomes for compound **28k** (A), **28f** (B) **28l** (C) and **28d** (D) having docking score of 3.9321, 4.6899, 3.4080 and 2.9813 respectively.

Materials and Methods

Chemistry. *General.* All the glass apparatus were oven dried prior to use. Melting points were taken in open capillaries on Sisco melting point apparatus and are presented uncorrected. All the AR grade chemicals were used as supplied from commercial source (Sigma Aldrich, TCI, Alpha Aesar, Spectrochem etc.) and used without further purification. Laboratory grade commercial reagents and solvents were purified by standard procedures prior to use. The silica gel (100–200 Mesh) used for column chromatography were supplied either from Qualigens™ (India) or Rankem (India), unless otherwise noted. UV fluorescence and Iodine vapor served as the visualizing agent for thin layer chromatography (Merck silica gel 60 F₂₅₄ precoated plates (0.25 mm)). ¹H NMR and ¹³C NMR spectral data were recorded on a JEOL ECS-400 (2-channel support with an adaptable broadband RF execution) spectrometer working at 400 MHz for ¹H and 100 MHz for ¹³C) utilizing CDCl₃ as a solvent. The ¹H-NMR (400 MHz) chemical shifts were measured relative to CDCl₃ as the internal reference (CDCl₃: δ = 7.249 ppm). Tetramethylsilane (δ 0.00 ppm) served as an internal standard in ¹H NMR and CDCl₃ (δ 77.0 ppm) in ¹³C NMR. Chemical shifts are reported in parts per million. Splitting patterns are described as singlet (s), doublet (d), double doublet (dd), triplet (t), multiplet (m), and broad (br). Infrared spectra were recorded on a FT-IR Spectrum 2 (Perkin-Elmer) spectrophotometer. Electron Impact Mass Spectroscopy (HR-EIMS) data were obtained from Xevo G2-S-Q-ToF (Waters, USA) compatible with ACQUITY UPLC® and nano ACQUITY UPLC® systems. The BUCHI Rotavapor R-210 was used for drying and concentration of the solvents. All animal experiments were performed in compliance with the relevant laws and guidelines of Suresh Gyan Vihar University, and approved by the institutional animal ethical committee(s).

General procedure (GP) for the synthesis of (Z)-3-benzylideneisobenzofuran-1(3H)-ones 28a-t. Substituted halo-aromatic carboxylic acid **26a-f** (1.21 mmol, 1.0 eq.), substituted terminal alkynes **27a-e/27 f** (1.21 mmol, 1.0 eq.) were dissolved in dry DMF (2 mL) taken in a round-bottom flask; added Ag₂ONPs (1.21 mmol, 1.0 eq.) as well as the PivOH (0.484 mmol, 0.4 eq.) as additive. The reaction mixture was stirred at 120 °C for 3 h. After completion of the reaction, the reaction mixture was cooled to room temperature and dilute it with EtOAc (10 mL) and filtered through a celite bed and then, washed further with EtOAc (15 mL). The combined organic solvents were extracted with EtOAc (3 × 10 mL), washed with water (2 × 20 mL) and then with saturated NaCl solution (20 mL). The organic layer was dried over anhyd. Na₂SO₄ and evaporated under decreased pressure. The crude product was purified by column chromatography over silica gel (100–200 mesh size) using 2% EtOAc: Hexane as an eluant to furnish **28a-t**.

The detailed spectral data of compounds **28a-j**, **28m-r** and **28t** are given in the supporting information.

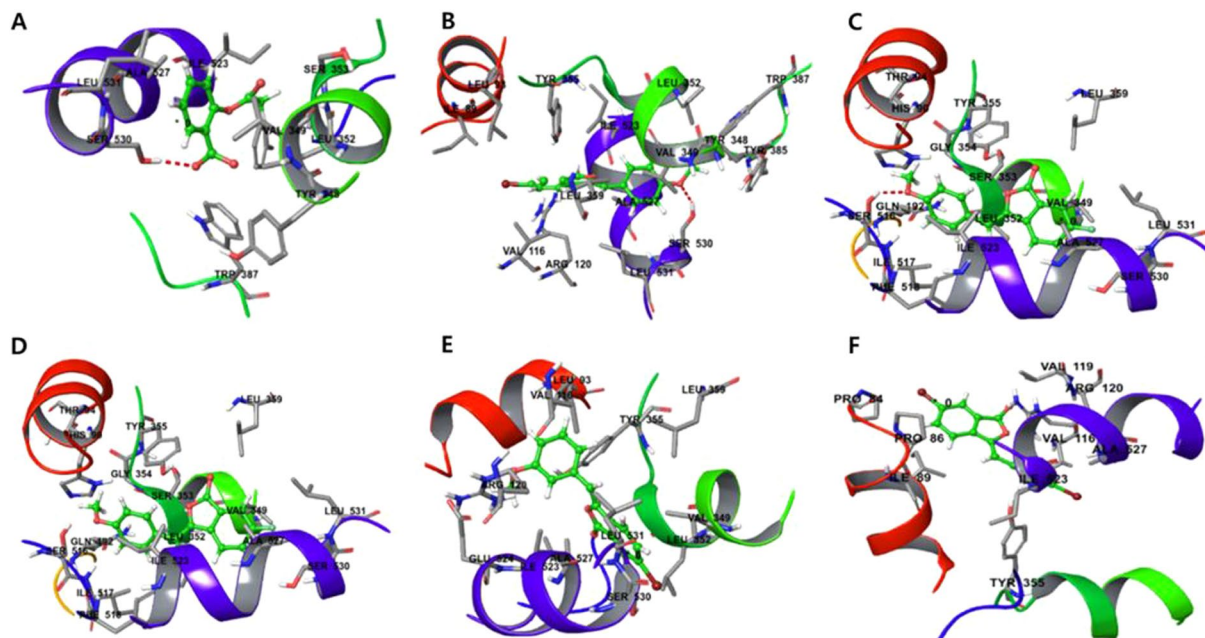


Figure 6. The docking score of **28k** (A), **28s** (B), **28f** (C), **28l** (D), **28j** (E) and **aspirin** (F) showed elevated binding affinity in terms of docking score of 5.1953, 5.7131, 5.1010, 5.1184, 4.1714 and 4.4803 respectively.

(*Z*)-6-bromo-3-(4-methoxybenzylidene) isobenzofuran-1(3*H*)-one (**28k**). Light bluish solid, m.p. 180–184 °C, 61% yield. $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 8.04–8.03 (m, 1 H), 7.80–7.76 (m, 3 H), 7.59 (d, $J = 8.3$ Hz, 1 H), 6.94 (d, $J = 8.8$ Hz, 2 H), 6.37 (s, 1 H), 3.84 (s, 3 H); $^{13}\text{C NMR}$ (100 MHz, CDCl_3) δ 165.81, 160.13, 142.38, 139.50, 137.56, 131.93, 128.52, 125.63, 124.86, 123.15, 121.01, 114.45, 108.00, 55.45; HRMS (ESI) Calculated for $\text{C}_{16}\text{H}_{11}\text{BrO}_3$ $[\text{M} + \text{H}]^+$: 330.9965, found 330.9967; FT-IR (Neat, cm^{-1}): 1752, 1596, 1505, 1450, 1246, 1022, 970, 822, 525.

(*Z*)-6-bromo-3-(3-methoxybenzylidene) isobenzofuran-1(3*H*)-one (**28l**). Light yellow solid, m.p. 158–160 °C, 70% yield. $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 8.05 (s, 1 H), 7.82–7.80 (m, 1 H), 7.63 (d, $J = 8.3$ Hz, 1 H), 7.39–7.38 (m, 2 H), 7.31 (t, $J = 8.1$, 1 H), 6.88 (d, $J = 9.3$ Hz, 1 H), 6.38 (s, 1 H), 3.85 (s, 3 H); $^{13}\text{C NMR}$ (100 MHz, CDCl_3) δ 165.50, 159.90, 144.04, 139.28, 137.71, 134.08, 129.87, 128.62, 125.23, 123.83, 123.01, 121.33, 115.16, 114.90, 107.97, 55.44; HRMS (ESI) Calculated for $\text{C}_{16}\text{H}_{11}\text{BrO}_3$ $[\text{M} + \text{H}]^+$: 330.9965, found 330.9964; FT-IR (Neat, cm^{-1}): 2922, 2852, 1765, 1667, 1584, 1453, 1241, 1171, 1041, 978, 772, 687, 490.

(*Z*)-6-fluoro-3-(4-methoxybenzylidene) isobenzofuran-1(3*H*)-one (**28s**). greenish white solid, m.p. 164–168 °C, 71% yield. $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 7.80–7.78 (d, $J = 12.8$ Hz, 2 H), 7.77–7.71 (m, 1 H), 7.58–7.56 (dd, $J = 7.1$ Hz, 2.1 Hz, 1 H), 7.45–7.40 (m, 1 H), 6.96–6.93 (d, $J = 8.8$ Hz, 2 H), 6.34 (s, 1 H), 3.85 (s, 3 H); $^{13}\text{C NMR}$ (100 MHz, CDCl_3) δ = 166.19, 164.47, ($J_{\text{C-F}} = 250$ Hz), 159.98, 142.38, 136.92, 131.74, 125.72, 123.04 ($J_{\text{C-F}} = 25$ Hz), 121.56, ($J_{\text{C-F}} = 9$ Hz), 114.41, 111.84, ($J_{\text{C-F}} = 24$ Hz), 107.18, 55.44; HRMS (ESI) Calculated for $\text{C}_{15}\text{H}_{10}\text{FO}_3$ $[\text{M} + \text{H}]^+$: 271.0765, found 271.0767; FT-IR (Neat, cm^{-1}): 2921, 2851, 1761, 1602, 1493, 1254, 1164, 982, 807, 538.

Biological methods.

In vitro antioxidant DPPH radical scavenging activity:³⁸ See SI.

Platelet aggregation inhibitory activity evaluation:^{20,34} See SI.

Molecular docking studies:³⁹ see SI.

Data availability

All data generated or analyzed during this study are included in this published article.

Received: 26 September 2019; Accepted: 27 January 2020;

Published online: 11 February 2020

References

- Karmakar, R., Pahari, P. & Mal, D. Phthalides and phthalans: Synthetic methodologies and their applications in the total synthesis. *Chemical Reviews* **114**, 6213–6284, <https://doi.org/10.1021/cr400524q> (2014).
- Zhao, C. *et al.* Arylnaphthalene lactone analogues: Synthesis and development as excellent biological candidates for future drug discovery. *RSC Advances* **8**, 9487–9502, <https://doi.org/10.1039/c7ra13754k> (2018).
- Chang, C.-W. & Chein, R.-J. Absolute configuration of anti-HIV-1 agent (–)-Concentricolide: Total synthesis of (+)-(R)-Concentricolide. *The Journal of Organic Chemistry* **76**, 4154–4157, <https://doi.org/10.1021/jo2004132> (2011).
- Kurume, A. *et al.* Synthesis of 3-substituted isocoumarins and their inhibitory effects on degranulation of RBL-2H3 cells induced by antigen. *Chem. Pharm. Bull* **56**, 1264–1269, <https://doi.org/10.1248/cpb.56.1264> (2008).

5. Cutler, H. G., Cox, R. H., Crumley, F. G. & Cole, P. D. 6-Pentyl- α -pyrone from *Trichoderma harzianum*: Its plant growth inhibitory and antimicrobial properties. *Agricultural and Biological Chemistry* **50**, 2943–2945, <https://doi.org/10.1080/00021369.1986.10867860> (1986).
6. Zhang, H. *et al.* New type of anti-diabetic compounds from the processed leaves of *Hydrangea macrophylla* var. *thunbergii* (*Hydrangeae Dulcis Folium*). *Bioorganic & Medicinal Chemistry Letters* **17**, 4972–4976, <https://doi.org/10.1016/j.bmcl.2007.06.027> (2007).
7. Bellina, F., Ciucci, D., Vergamini, P. & Rossi, R. Regioselective synthesis of natural and unnatural (Z)-3-(1-alkylidene) phthalides and 3-substituted isocoumarins starting from methyl 2-hydroxybenzoates. *Tetrahedron* **56**, 2533–2545, [https://doi.org/10.1016/S0040-4020\(00\)00125-3](https://doi.org/10.1016/S0040-4020(00)00125-3) (2000).
8. Del-Ángel, M., Nieto, A., Ramírez-Apan, T. & Delgado, G. Anti-inflammatory effect of natural and semi-synthetic phthalides. *European Journal of Pharmacology* **752**, 40–48, <https://doi.org/10.1016/j.ejphar.2015.01.026> (2015).
9. Miyazawa, M., Tsukamoto, T., Anzai, J. & Ishikawa, Y. Insecticidal effect of phthalides and furanocoumarins from *angelica acutiloba* against *Drosophila melanogaster*. *Journal of Agricultural and Food Chemistry* **52**, 4401–4405, <https://doi.org/10.1021/jf0497049> (2004).
10. Lv, J.-L., Zhang, L.-B. & Guo, L.-M. Phthalide dimers from *angelica sinensis* and their COX-2 inhibition activity. *Fitoterapia* **129**, 102–107, <https://doi.org/10.1016/j.fitote.2018.06.016> (2018).
11. Farias, E. S., *et al.* Phthalides as promising insecticides against *Tuta absoluta* (Lepidoptera: Gelechiidae). *Journal of Environmental Science and Health, Part B*, 1–8, <https://doi.org/10.1080/03601234.2017.1371556> (2017).
12. Yoshikawa, M. *et al.* B, and F, new antiallergic and antimicrobial principles from *Hydrangeae dulcis Folium*. *Chem. Pharm. Bull.* **40**, 3121–3123, <https://doi.org/10.1248/cpb.40.3121> (1992).
13. Teixeira, M. G., Alvarenga, E. S., Lopes, D. T., & Oliveira, D. F. Herbicidal activity of isobenzofuranones and in silico identification of their enzyme target. *Pest Management Science*, <https://doi.org/10.1002/ps.5456> (2019).
14. Liao, K.-F., *et al.* Anti-cancer effects of radix *angelica sinensis* (Danggui) and n-butylidene-phthalide on gastric cancer: Implications for REDD1 activation and mTOR inhibition. *Cellular Physiology and Biochemistry*, 2231–2246, <https://doi.org/10.1159/000492641> (2018).
15. Lin, G., Chan, S. S.-K., Chung, H.-S., & Li, S.-L. Chemistry and biological activities of naturally occurring phthalides. *Studies in Natural Products Chemistry*, 611–669, [https://doi.org/10.1016/S1572-5995\(05\)80065-1](https://doi.org/10.1016/S1572-5995(05)80065-1) (2005).
16. León, A., Del-Ángel, M., Ávila, J. L., & Delgado, G. Phthalides: Distribution in nature, chemical reactivity, synthesis, and biological activity. *Progress in the Chemistry of Organic Natural Products*, 127–246, https://doi.org/10.1007/978-3-319-45618-8_2 (2017).
17. Harper, J. K. *et al.* Pestacin: a 1,3-dihydro isobenzofuran from *pestalotiopsis microspora* possessing antioxidant and antimycotic activities. *Tetrahedron* **59**, 2471–2476, [https://doi.org/10.1016/S0040-4020\(03\)00255-2](https://doi.org/10.1016/S0040-4020(03)00255-2) (2003).
18. Tianpanich, K. *et al.* Radical scavenging and antioxidant activities of isocoumarins and a phthalide from the endophytic fungus *Colletotrichum* sp. *Journal of Natural Products* **74**, 79–81, <https://doi.org/10.1021/np1003752> (2011).
19. Chen, M. *et al.* Synthesis and biological evaluation of n-butylphthalide derivatives as anti-platelet aggregation agents. *Natural Product Research* **30**, 2716–2719, <https://doi.org/10.1080/14786419.2015.1136907> (2016).
20. Teng, C.-M., Chen, W.-Y., Ko, W.-C. & Ouyang, C. Antiplatelet effect of butylidene-phthalide. *Biochimica et Biophysica Acta (BBA) - General Subjects* **924**, 375–382, [https://doi.org/10.1016/0304-4165\(87\)90151-6](https://doi.org/10.1016/0304-4165(87)90151-6) (1987).
21. Xu, H. L. & Feng, Y. P. Effects of 3-n-butylphthalide on thrombosis formation and platelet function in rats. *Yao Xue Xue Bao.* **36**, 329–33 (2001).
22. Abdelwahab, M. F., Hussein, M. H. & Kadry, H. H. Cytotoxicity and antioxidant activity of new biologically active constituents from *Micromeria nervosa* grown in Egypt. *Bulletin of Faculty of Pharmacy* **53**, 185–194, <https://doi.org/10.1016/j.bfopcu.2015.11.001> (2015).
23. Nadal, B. *et al.* Synthesis and antioxidant properties of pulvinic acids analogues. *Bioorg. Med. Chem.* **18**, 7931–7939, <https://doi.org/10.1016/j.bmc.2010.09.037> (2010).
24. E. Marchal, P. Uriac, Y. Brunel, S. Poigny, EP 2 350 036 B1, Bulletin 2015/16.
25. Rangaswamy, J., Kumar, H. V., Harini, S. T. & Naik, N. Functionalized 3-(benzofuran-2-yl)-5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazole scaffolds: A new class of antimicrobials and antioxidants. *Arabian Journal of Chemistry* **10**, 2685–2696, <https://doi.org/10.1016/j.arabjc.2013.10.012> (2017).
26. Xiong, L., Bi, M.-G., Wu, S. & Tong, Y.-F. Total synthesis of 6'-hydroxyjusticidin A. *Journal of Asian Natural Products Research* **14**, 322–326, <https://doi.org/10.1080/10286020.2011.653561> (2012).
27. Kermodé, J., Butt, W. & Shann, F. Comparison between prostaglandin E1 and epoprostenol (prostacyclin) in infants after heart surgery. *Heart* **66**, 175–178, <https://doi.org/10.1136/hrt.66.2.175> (1991).
28. Barry, K. A. & Somerville, N. J. University of Virginia Health System, Charlottesville, Vorapaxar (Zontivity) for the Prevention of Thrombotic Cardiovascular Events Virginia. *Am Fam Physician.* **92**, 304–305 (2015).
29. Ma, F., Gao, Y., Qiao, H., Hu, X. & Chang, J. Antiplatelet activity of 3-butyl-6-bromo-1(3H)-isobenzofuranone on rat platelet aggregation. *J. Thromb. Thrombolysis.* **33**, 64–73, <https://doi.org/10.1007/s1239-011-0647-9> (2011).
30. Kumar, M. R., Irudayanathan, F. M., Moon, J. H. & Lee, S. Regioselective one-pot synthesis of isocoumarins and phthalides from 2-iodobenzoic acids and alkynes by temperature control. *Advanced Synthesis & Catalysis* **355**, 3221–3230, <https://doi.org/10.1002/adsc.201300561> (2013).
31. Chaudhary, S., Shyamalal, B. R. K., Yadav, L., Tiwari, M. K. & Kumar, K. Ag₂O nanoparticle-catalyzed substrate-controlled regioselectivities: direct access to 3-ylidene-phthalides and isocoumarins. *RSC Advances* **8**, 23152–23162, <https://doi.org/10.1039/c8ra03926g> (2018).
32. Selvaraj, S., Mohan, A., Narayanan, S., Sethuraman, S. & Krishnan, U. M. Dose-dependent interaction of trans-resveratrol with biomembranes: Effects on antioxidant property. *Journal of Medicinal Chemistry* **56**, 970–981, <https://doi.org/10.1021/jm3014579> (2013).
33. Packham, M., Rand, M. & Kinlough-Rathbone, R. Similarities and differences between rabbit and human platelet characteristics and functions. *Comparative Biochemistry and Physiology Part A: Physiology* **103**, 35–54, [https://doi.org/10.1016/0300-9629\(92\)90239-m](https://doi.org/10.1016/0300-9629(92)90239-m) (1992).
34. Chen, K.-S., Ko, F.-N., Teng, C.-M. & Wu, Y.-C. Antiplatelet and vasorelaxing actions of some benzyloquinoline and phenanthrene alkaloids. *Journal of Natural Products* **59**, 531–534, <https://doi.org/10.1021/np960354x> (1996).
35. Copley, S. D., Novak, W. R. P. & Babbitt, P. C. Divergence of function in the thioredoxin fold superfamily: Evidence for evolution of peroxiredoxins from a thioredoxin-like ancestor. *Biochemistry* **43**, 13981–13995, <https://doi.org/10.1021/bi048947r> (2004).
36. Monteiro, G., Horta, B. B., Pimenta, D. C., Augusto, O. & Netto, L. E. S. Reduction of 1-Cys peroxiredoxins by ascorbate changes the thiol-specific antioxidant paradigm, revealing another function of vitamin C. *PNAS* **104**(12), 4886–4891 (2007).
37. Jaiswal, P. K. *et al.* Non-peptide-based new class of platelet aggregation inhibitors: Design, synthesis, biological evaluation, SAR and in silico studies. *Archiv Der Pharmazie* **351**, 1–17, <https://doi.org/10.1002/ardp.201700349> (2018).
38. Hernández-Vázquez, E. *et al.* 1,5-Diarylpyrazole and vanillin hybrids: Synthesis, biological activity and DFT studies. *European Journal of Medicinal Chemistry* **100**, 106–118, <https://doi.org/10.1016/j.ejmech.2015.06.010> (2015).
39. Yadav, D. K., *et al.* Insight into the molecular dynamic simulation studies of reactive oxygen species in native skin membrane. *Frontiers in Pharmacology*, 9, <https://doi.org/10.3389/fphar.2018.00644> (2018).

Acknowledgements

B.R.K.S. acknowledges UGC, Delhi for financial assistance in the form of SRF fellowship. L.Y. and M.K.T. acknowledges DST, New Delhi and CSIR, New Delhi for providing SRF fellowships, respectively. Materials Research Centre (MRC), MNIT, Jaipur is acknowledged for providing analytical facilities. The authors are also thankful to Gachon Institute of Pharmaceutical Science and the Department of Pharmacy, College of Pharmacy, Gachon University of Medicine and Science, Incheon, Korea for providing the computational modeling facility. This study was funded by the Council of Scientific and Industrial Research [CSIR-EMR Grant No. **02 (0189)/14/EMR-II**], New Delhi and the Ministry of Education and Science (Indo-Russian International grant agreement no. **C-26/174.5 dated 31-01-2019**) Perm, Russian Federation. We are also thankful to National Research Foundation of Korea (NRF), the Ministry of Education, Science, and Technology (No: **2017R1C1B2003380**) for providing financial assistance at the Gachon University, Incheon city, Korea.

Author contributions

S.C. and D.K.Y. conceived and designed the study. B.R.K.S., L.Y., M.K.T. and J.I.P. synthesized all the compounds. S.C. and I.V.M. supervised the synthesis of all compounds. MM carried out all the biological testing. S.C., B.R.K.S., L.Y., M.K.T. carried out the S.A.R. analysis. D.K.Y. carried out the *in silico* studies. S.C. and D.F.Y. wrote the manuscript. All authors have read and approved the final version of the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information is available for this paper at <https://doi.org/10.1038/s41598-020-59218-6>.

Correspondence and requests for materials should be addressed to D.K.Y. or S.C.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2020