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

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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 (IC\(_{50}\) = 4.57 µg/mL) and aspirin (IC\(_{50}\) = 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. In fact, 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(3\(\text{H}\))-one, commonly known as “phthalide” is found in many naturally-occurring and pharmaceuticals important molecules. This benzo-fused heterocyclic class of compounds are blended with several pharmacological properties such as anti-tumor, anti-HIV, anti-allergic, antifungal, antidiabetic, antispasmodic, anti-inflammatory, pesticidal, COX-2 inhibitor, insecticidal, anti-microbial, herbicidal, and anti-cancer 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(3\(\text{H}\))-ones are known to be potential antioxidant (7) and antiplatelet agents (7, 12–15)15,18–21.

Earlier, Teng and his co-workers carried out extensive platelet aggregation inhibitory studies on butylideneephthalide 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 thrombin. In this study, 7 showed significantly higher inhibitory
efficacy in AA-induced platelet aggregation (IC$_{50}$ = 70 µM) in comparison to collagen-induced aggregation (IC$_{50}$ = 120 µM) in washed rabbit platelets. Teng et al. also suggested that butylidenephthalide inhibits platelet aggregation mainly by inhibiting cyclooxygenase-1 (COX-1) enzyme leading to the reduction of thromboxane A2 (TXA2) formation. Similar studies further confirmed the above facts. Overall, there has been no detailed study on the AA-induced platelet aggregation inhibitory activities of (Z)-3-benzylidinesobenzofuran-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 agent. For example, ascorbic acid, Pestacin, Micromeriol, Pulvinate analogue, 5-(bis(3,4-dimethoxyphenyl)-methylene)fur-2(5H)-one, ailanthoidol, 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-benzylidinesobenzofuran-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)-benzylidene-isobenzofuran-1(3H)-ones, incorporating similar sub-structural units of 1, 7 and 17–25 (Fig. 2) and assessed their antioxidant and AA-induced antiplatelet activities with the anticipation that the (Z)-3-benylidene-isobenzofuran-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 (dual potential as antioxidant and antiplatelet agent), Justicidin A, prostacyclin (Trade name: Epoprostenol, prostaglandin I$_2$), Zontivity (Trade name: Vorapaxar), 6-bromo-3-butylisobenzofuran-1(3H)-one (bromo derivative of NBP), 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-benylidinesobenzofuran-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, however, for the first time, substrate-controlled silver oxide nanoparticle (Ag$_2$ONPs)-catalyzed synthesis of compound 28a-t have been prepared. Various substituted (Z)-3-benzylidinesobenzofuran-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-benylidinesobenzofuran-1(3H)-ones in a highly regioselective manner in excellent yields has been reported by our group. Ag$_2$ONPs-mediated reaction of several substituted 2-iodobenzoic acids $^{26a-f}$ (R$_1$ = H, Br, CH$_3$, F, OCH$_3$; 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...
(Z)-3-benzylidenesobenzofuran-1-(3H)-ones 28a-t were assessed for their in vitro antioxidant activity using literature procedure (Fig. 3)\textsuperscript{31,32}. The results are shown in Table 1. Primarily, (Z)-3-benzylidenesobenzofuran-1-(3H)-one 28a, unsubstituted at site I and II, was assessed for its in vitro antioxidant activity. It was observed that 28a (IC\textsubscript{50} = 14.38 ± 0.09 \mu g/mL), demonstrated reduced potency than the standard reference, ascorbic acid (IC\textsubscript{50} = 4.57 \mu g/mL) (Table 1, Entry 1). On the other hand, 28b (IC\textsubscript{50} = 8.88 ± 0.12 \mu g/mL) and 28c (IC\textsubscript{50} = 6.33 ± 0.08 \mu g/mL) having p-methyl (p-CH\textsubscript{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 (IC\textsubscript{50} = 52.34 ± 0.29 \mu 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 (IC\textsubscript{50} = 34.41 ± 0.94 \mu g/mL), having p-OMe substitution at site II exhibited good activity as compared to 28d (Table 1, entry 5); surprisingly, compound 28f (IC\textsubscript{50} = 0.41 ± 0.12 \mu 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\textsubscript{3}/Cl/Br/p-OMe/m-OMe groups at p-/m-position of site II were analyzed (Table 1, entry 7–12). Sequentially, 28g (IC\textsubscript{50} = 1.59 ± 0.55 \mu 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\textsubscript{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 (IC\textsubscript{50} = 0.55 ± 0.15 \mu g/mL) and 28l (IC\textsubscript{50} = 0.732 ± 0.44 \mu 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\textsubscript{3}/Cl/OMe groups at p-/m-position of site II were analyzed (Table 1, entry 13–17). Compound 28m (IC\textsubscript{50} = 7.23 ± 0.04 \mu g/mL), electron-donating group (CH\textsubscript{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\textsubscript{3} group at C-6 position of site I and p-CH\textsubscript{3}, p-Cl and p-OMe substitutions at site II also showed potency except 28n (Table 1, entry 14–16). Nonetheless, compound 28q (IC\textsubscript{50} = 3.83 ± 0.88 \mu g/mL), having CH\textsubscript{3} group at C-6 position of site I and m-OMe...
group at site II showed more antioxidant potency than ascorbic acid (Table 1, Entry 17). 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-I and 28q, exhibited promising antioxidant activity with the IC_{50} values in the range 0.41–3.83 µg/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, CH₃) and H/p-OMe/m-OMe substitution on site II also accounts for promising activity (i.e. compound 28g, 28k-I and 28q). EWG group (fluoro group) at site I do not show favourable effects on the antioxidant activity [strong EWG-containing phenyl acetylenes (F, NO₂, 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 (IC_{50} = 21.21 ± 0.12 µg/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 refered 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 (IC_{50} = 64.57 ± 0.58 µg/mL) demonstrated significant AA-induced antiplatelet activity in comparison with the standard reference aspirin.
Substitutions on site II exhibited comparable activity; Compound entry 14–15). 

Table 1. In vitro antioxidant activity (DPPH free radical scavenging assay) and In vitro AA-induced antiplatelet activity of functionalized (Z)-3-benzyldeneisobenzofuran-1(3H)-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.

(IA\textsubscript{50} = 21.34 ± 1.09 µg/mL; Table 1, entry 1). Shifting to CH\textsubscript{3} group at p-position of site II (28b) improves IA\textsubscript{50} 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 IA\textsubscript{50} 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 (IA\textsubscript{50} = 4.20 ± 0.28 µg/mL) than the standard reference (Table 1, entry 6). Furthermore, 28g-h were analyzed having bromo substitution at C-6 position of site I along with H/CH\textsubscript{3}/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 (IA\textsubscript{50} = 16.28 ± 0.25 µg/mL) up to 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 CH\textsubscript{3}/Cl/Br groups at p-position of site II showed decreased activity i.e., IA\textsubscript{50} 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 (IA\textsubscript{50} = 7.28 ± 0.48 µg/mL) and 28l (IA\textsubscript{50} = 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/CH\textsubscript{3}/Cl/Ome groups at p-/m-position of site II (Table 1, entry 13–17). Introduction of CH\textsubscript{3} group at site I (28m) showed more aggregation inhibitory activity (IA\textsubscript{50} = 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 CH\textsubscript{3}/Cl substitutions on site II were also introduced (Table 1, entry 14–15). While 28p (IA\textsubscript{50} = 24.64 ± 0.42 µg/mL) with methyl (CH\textsubscript{3}) group at C-6 position of site I and p-Ome substitutions on site II exhibited comparable activity; Compound 28p (IA\textsubscript{50} = 24.64 ± 0.42 µg/mL) with methyl (CH\textsubscript{3}) 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 (IA\textsubscript{50} = 11.37 ± 0.67 µg/mL) and 28s (IA\textsubscript{50} = 3.25 ± 0.18 µg/mL), exhibited -two- to seven-folds more potency than aspirin, respectively (Table 1, entry 18–19). 

Table 1. In vitro antioxidant activity (DPPH free radical scavenging assay) and In vitro AA-induced antiplatelet activity of functionalized (Z)-3-benzyldeneisobenzofuran-1(3H)-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.
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. 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 species. 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). 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 Surflex-Dock using the reported procedure (see details in supporting information).

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 lower 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(3H)-one analogues 28a-t as highly potent antioxidant and AA-induced antiplatelet agents. Five isobenzofuran-1(3H)-one analogues 28f-g, 28k-l and 28q were found to be the active compounds of the series in DPPH assay. In fact, two compounds, 28f and 28k, showed 10-folds and 8-folds more antioxidant activity than ascorbic acid, respectively. Similarly, twelve isobenzofuran-1(3H)-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.
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 QualigensTM (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 × 20 mL), washed with water (2 × 20 mL) and then 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 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.
(Z)-6-bromo-3-(4-methoxybenzylidene) isobenzofuran-1(3H)-one (28k). Light bluish solid, m.p. 180–184 °C, 61% yield. 1H NMR (400 MHz, CDCl3) δ 8.04–8.03 (m, 1 H), 7.80–7.76 (m, 3 H), 7.59 (d, J = 8.8 Hz, 1 H), 6.94 (d, J = 8.8 Hz, 2 H), 6.37 (s, 1 H), 3.84 (s, 3 H); 13C NMR (100 MHz, CDCl3) δ 165.81, 160.13, 142.38, 139.50, 137.56, 131.93, 128.52, 125.63, 123.15, 121.01, 114.45, 108.00, 55.45; HRMS (ESI) Calculated for C16H11BrO3 [M + 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(3H)-one (28l). Light yellow solid, m.p. 158–160 °C, 70% yield. 1H NMR (400 MHz, CDCl3) 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); 13C NMR (100 MHz, CDCl3) 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 C16H11BrO3 [M + 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(3H)-one (28s). Greenish white solid, m.p. 164–168 °C, 71% yield. 1H NMR (400 MHz, CDCl3) 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); 13C NMR (100 MHz, CDCl3) δ 166.19, 164.47, (JCF = 250 Hz), 159.98, 142.38, 136.92, 131.74, 123.03, 121.56, (JCF = 9 Hz), 114.41, 111.84, (JCF = 24 Hz), 107.18, 55.44; HRMS (ESI) Calculated for C15H10FO3 [M + 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: See SI.
Platelet aggregation inhibitory activity evaluation: See SI.
Molecular docking studies: See SI.

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

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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
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