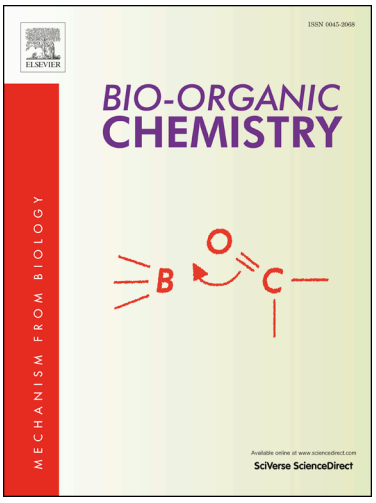

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Madlen B. Labib, Souty M.Z. Sharkawi, Mahmoud El-Daly

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Design, synthesis of novel isoindoline hybrids as COX-2 inhibitors: anti-inflammatory, analgesic activities and docking study

Madlen B. Labib[a,*], Souty M.Z. Sharkawi[b] and Mahmoud El-Daly[c]

[a] *Department of Pharmaceutical Organic Chemistry, Faculty of pharmacy, Beni-Suef University, Beni-Suef 62514, Egypt, E-mail: madlenwannas@gmail.com*

[b]*Department of pharmacology & Toxicolgy, Faculty of Pharmacy, Beni-Suef University, Beni-Suef 62514, Egypt, E-mail: drsoutyph@yahoo.com*

[c]*Department of Pharmacology & Toxicology, Faculty of Pharmacy, Minia University, El-Minia Egypt, E-mail address: eldaly_m@mu.edu.eg*

**To whom correspondence should be addressed.*

Madlen B. Labib, Ph.D.

Address: Department of Pharmaceutical Organic Chemistry, Faculty of Pharmacy, Beni-Suef University, Beni-Suef 62514, Egypt.

E-mail address: madlenwannas@gmail.com

Official E-mail address: madlen.wanas@pharm.bsu.edu.eg

Tel.: (002)-01224299119

Abstract

A group of novel isoindoline hybrids incorporating oxime, hydrazone, pyrazole, chalcone or aminosulfonyl pharmacophores (**9-14**) was designed and characterized by spectral data and elemental analyses results. All newly synthesized compounds were evaluated as COX-2 inhibitors, anti-inflammatory and analgesic agents. Six hybrid derivatives (**10b, 10c**, **11a, 11d**, **13, 14**) were moderate COX-2 inhibitors (IC_{50} = 0.11-0.18 µM) close to standard celecoxib (IC_{50} = 0.09 µM). The most active compounds showed outstanding *in vivo* anti-inflammatory activity (% edema inhibition = 41.7-50, 1h; 40.7-67.4, 3h; 20-46.7, 6h) better than reference drug diclofenac (% edema inhibition = 29.2, 1h; 22.2, 3h; 20, 6h). Most compounds showed significant peripheral and/or central analgesic activity. The moderate selective COX-2 inhibitor; dimethoxychalcone **11d** (SI = 103) displayed excellent anti-inflammatory activity (% edema inhibition = 45.8-59.3) and increased thermal pain threshold (50-92.85%) comparable to piroxicam (75%). Molecular docking studies have been established.

Keywords*:* Isoindoline; Hybrids; COX-2; Analgesic; Anti-Inflammatory.

1- Introduction

Non steroidal anti-inflammatory drugs (NSAIDs) are the most prescribed drugs for treatment of inflammation and pain associated with various pathological disorders. The mechanism of action of NSAIDs is attributed to inhibition of cyclooxygenase (COX) enzymes which catalyze prostaglandins (PGs) biosynthesis from arachidonic acid [1-3]. There are two known forms of COX enzymes. COX-1 is a constitutive enzyme responsible for production of cytoprotective PG in gastric and bowel mucosa, normal renal functions and haemostasis while COX-2 is an inducible enzyme induced for inflammatory response and other pathological conditions [4,5].

Traditional NSAIDs are non selective inhibitors of both the "housekeeping" COX-1 and the inflammatory response of COX-2 enzymes leading to various side effects such as gastric ulceration, bleeding and renal dysfunction [6]. Finding clinically useful NSAIDs *via* selective inhibition of COX-2 enzyme is a goal for medicinal chemists to alleviate inflammation without interrupting normal body functions [7]. Highly selective COX-2 inhibitors such as valdecoxib (Bextra)^{TM} proved to cause cardiac toxicity and were withdrawn from market [8] while moderate selective COX-2 inhibitors as celecoxib (Celebrex)^{TM} are considered as safe anti-inflammatory drugs [9].

Structure activity relationship studies identified the diverse chemical structures of reported COX-2 inhibitors. Generally, they possess two aryl ring substitution on a central scaffold. The central system is either carbo/heterocyclic ring system, or acyclic core system with 2 or 3 membered chain structure as iminic olefinic, azo, acetylenic or α,β-unsaturated ketone structures [10-12].

Isoindoline-1,3-dione derivatives (phthalimides) are nitrogen containing heterocycls that have been utilized extensively as building blocks in organic synthesis

owing to their varied biological activities [13-18]. Also, *N*-functionalized isoindolines have received great attention due to their COXs inhibitory activity, anti-inflammatory and analgesic properties [19-22]. This diversity of pharmacological activities may be due to the lipophilic nature of isoindoline moiety as it possesses a hydrophobic structural feature [O=C-N(R)-C=O] that facilitates crossing various biological membranes *in vivo* [23]. Further, a number of research articles reported the synthesis of compounds (**1-5**) endowed with the pharmacologically-interesting pharmacophores like phthalimide moiety [24], oxime group [25], hydrazono-bridge [26,27], chalcone moiety [28], pyrazole ring [26] and aminosulfonyl moiety [26,27] as selective COX-2 inhibitors with anti-inflammatory potential comparable with common drugs (Figure 1). Also, it is worthy to note that the bulky bi-cyclic isoindoline ring system may enhance COX-2 selectivity as it maximizes the hydrophobic interactions within COX-2 active site [29].

[Please insert Figure 1 about here]

Appreciation of these findings and in continuation with our previous work [26, 30,31] to develop active anti-inflammatory agents, we report herein the synthesis of some novel *N*-functionalized isoindoline-1,3-diones using the concept of pharmacophore hybridization [32] to obtain multiple-ligands compounds combining two functionalities; isoindoline-1,3-dione with either oxime (**9**), hydrazono (**10a-d**), chalcone (**11a-d**), pyrazole (**12-14**) or aminosulfonyl (**10c** and **14**) moieties (Figure 2A) in one compound in order to investigate the COX-1/COX-2 enzyme inhibition, *in vivo* anti-inflammatory and analgesic activities. Also, designed compounds (Figure 2B) possessed either acyclic iminic (**10a-d**), acyclic α,β-unsaturated ketone (**11a-d**) or heterocyclic pyrazole ring (**12-14**) as central core structures substituted with two aryl rings in order to fulfill the common structural features of selective COX-2 inhibitors

[10]. Finally, molecular docking study was applied to examine the probable binding modes of designed compounds inside COX-2 enzyme active site.

[Please insert Figure 2A about here]

[Please insert Figure 2B about here]

2- Results and Discussion

2.1. Chemistry

The synthetic steps adopted for the target compounds (**9, 10a-d, 11a-d, 12, 13, 14**) were outlined in (Schemes 1 and 2). 2-(3-Acetylphenyl)isoindoline-1,3-dione (**8**) was prepared according to previously reported method describing the reaction of phthalic anhydride with different amines [33]. Condensation of **8** with hydroxylamine hydrochloride gave oxime **9** in good yield (1.76 g, 63%). The structure of oxime **9** was confirmed by spectroscopic and elemental analyses. IR spectrum of compound **9** displayed OH stretching vibration at 3243 cm^{-1} while the ^{1}H NMR spectrum showed a new D_2O exchangeable singlet signal at δ 11.34 ppm due to hydroxyl proton which confirmed the structure. Also, disappearance of ($\underline{CO}CH_3$) peak in ^{13}C NMR spectrum of oxime **9** proved formation of the compound.

Different substituted phenyl hydrazine hydrochlorides namely: phenylhydrazine hydrochloride, 4-hydrazinylbenzoic acid hydrochloride, 4-hydrazinylbenzenesulfonamide hydrochloride and 4-methanesulfonylphenylhydrazine hydrochloride were subjected to react with **8** to afford the corresponding hydrazones **10a-d**. Spectroscopic data (IR, ^{1}H NMR, ^{13}C NMR and MS) and elemental analysis of compounds **10a**–**d** confirmed their structures. IR spectrum of **10a-d** showed an absorption band at the range 3346-3449 cm^{-1} indicating NH group. Benzoic acid derivative **10b** displayed carboxylic OH group at 3422 cm^{-1} while aminosulfonyl hydrazone **10c** showed additional two characteristic sharp peaks at 1328, 1148 cm^{-1}

indicating SO_2 group. ^{1}H NMR of **10a-d** showed the appearance of the signal at δ 9.36-9.98 ppm corresponding to NH proton while ^{13}C NMR spectrum revealed the disappearance of (<u>*CO*</u>CH_3) peak of the starting ketone and displayed C=N peak at δ 146.11-156.02 ppm.

Also, ^{1}H NMR spectrum of benzoic acid derivative **10b** exhibited the carboxylic proton at δ 12.28 ppm. ^{13}C NMR confirmed the presence of benzoic acid moiety by the presence of C=O peak at δ 172.02 ppm. The ^{1}H NMR spectrum of benzene sulfonamide derivative **10c** displayed a singlet D_2O exchangeable peak of two protons intensity at δ 7.08 ppm due to NH_2 protons. Additionally, NMR spectra of **10d** proved the structure *via* presence of a singlet signal of three protons integration at δ 3.11 ppm in ^{1}H NMR and a peak at δ 44.71 ppm in ^{13}C NMR due to methyl group of methanesulfonyl moiety. Also, the mass spectrum of **10a** showed the molecular ion peak [M^+] at (*m*/*z* 355) corresponding to the formula $C_{22}H_{17}N_3O_2$ (Scheme 1).

Claisen-Schmidt condensation between ketone **8** and different aromatic aldehydes in 5% methanolic potassium hydroxide solution afforded chalcones **11a-d** (Scheme 1). ^{1}H NMR spectrum of **11a-d** revealed the disappearance of the signal corresponding to CH_3 protons of starting ketone **8** and appearance of two doublets with a high coupling constant (J = 15.6 Hz) corresponding to olefinic protons of the formed α,ß-unsaturated ketones; (COC<u>*H*</u>=CH) and (COCH=C<u>*H*</u>) at δ 7.54-7.89 and 7.74-8.14 ppm, sequentially. Also, ^{13}C NMR spectra of chalcones **11a-d** showed the presence of three distinctive peaks at δ 124.00-124.49, 130.35-145.20 and 167.87-172.22 ppm corresponding to (CO<u>*CH*</u>=CH), (COCH=<u>*CH*</u>) and (<u>*CO*</u>CH=CH) respectively which confirmed the predicted structure.

[Please insert Scheme 1 about here]

Treatment of 4-nitrophenyl chalcone **11a** with excess hydrazine hydrate 99% either in absolute ethanol or glacial acetic acid at reflux temperature provided 2-{3-[5-(4-nitrophenyl)-4,5-dihydro-1*H*-pyrazol-3-yl]phenyl}isoindoline-1,3-dione (**12,** 44%) and *N*-acetyl derivative; 2-{3-[1-acetyl-5-(4-nitrophenyl)-4,5-dihydro-1*H*-pyrazol-3-yl]phenyl}isoindoline-1,3-dione (**13**, 46%) respectively (Scheme 2). IR spectrum of dihydropyrazole **12** revealed the presence of NH stretching band at ν 3432 cm^{-1} while the ^{1}H NMR spectrum showed a D_2O exchangeable signal at δ 6.90 ppm due to the NH proton. The structure of *N*-acetyl pyrazole **13** was confirmed through elemental and spectral analyses. ^{1}H NMR displayed a singlet signal at δ 2.33 ppm due to acetyl protons (CO$\underline{CH_3}$) while ^{13}C NMR showed two peaks at δ 22.04 and δ 169.22 ppm corresponding to (CO$\underline{C}H_3$) and ($\underline{CO}CH_3$) consequentially. Also, the mass spectrum of **13** exhibited the molecular ion peak at *m/z* 454 confirming its molecular formula $C_{25}H_{18}N_4O_5$.

Furthermore, reaction of chalcone **11a** with 4-hydrazinylbenzenesulfonamide hydrochloride under reflux in absolute ethanol afforded the aminosulfonyl derivative **14** . IR spectrum of compound **14** showed absorption bands at 3452, 3426 cm^{-1} and 1340, 1158 cm^{-1} corresponding to NH_2 and SO_2 groups in sequent. All collected data for compounds **12-14** were in accord with the assumed structure. ^{1}H NMR of target compounds; 3,5-diarylpyrazolines **12**, **13** and 1,3,5-triarylpyrazoline **14** revealed the presence of three characteristic doublet of doublet signals (dd), each of one proton intensity due to three pyrazoline protons which confirmed formation of pyrazoline ring. The two methylene protons at C-4 displayed two signals; one at δ 2.77-3.15 and the second at δ 3.47-3.92 with two distinctive *J* values. The higher *J* values indicate coupling of the two protons at C-4 with each other while the lower *J* value is due to coupling of C-4 protons with methine proton at C-5. Also, methine proton at C-5

resonates at δ 4.95-5.69 with two J values due to coupling with two methylene protons at C-4. Moreover, ^{13}C NMR spectra of compounds **12- 14** showed three characteristic peaks at δ 38.49-42.31, 59.51-62.18 and 151.68-157.80 ppm corresponding to dihydropyrazoline C-4, C-5, C-3 respectively which confirmed the structure (Scheme 2).

[Please insert Scheme 2 about here]

2.2. Biological Activity

2.2.1. *In vitro* cyclooxygenases (COX-1, COX-2) inhibitory activity:

The ability of synthesized compounds to inhibit ovine COX-1 and COX-2 enzymes was determined *via* measuring their peroxidase activity using colorimetric enzyme immune assay (EIA) kit. The inhibitory activities of tested compounds, celecoxib, diclofenac and indomethacin were expressed as IC_{50} values (concentration causing 50% enzyme inhibition). Also, COX-2 selectivity index (SI) values were calculated as [IC_{50} (COX-1) / IC_{50} (COX-2)] and tabulated (Table 1).

Data obtained from colorimetric assays demonstrated the weak COX-1 inhibitory activity (IC_{50} = 6.98 — 11.33 μM) of prepared compounds relative to the selective COX-1 inhibitor (indomethacin, IC_{50} = 0.04 μM) and the non selective COX inhibitor (diclofenac, IC_{50} = 5.1 μM). Consequently, the prepared isoindoline derivatives can be considered as safe anti-inflammatory agents. Furthermore, all synthesized compounds showed moderate COX-2 inhibitory activity (IC_{50} = 0.11 — 0.38 μM) compared to the selective COX-2 inhibitor (celecoxib, IC_{50} = 0.09 μM) and diclofenac (IC_{50} = 0.84 μM). Six compounds (**10b**, **10c**, **11a**, **11d**, **13** and **14**) exhibited potent COX-2 inhibition (IC_{50} = 0.11 — 0.18 μM) close to celecoxib while compounds (**9**, **10a**, **11b**, **11c**, **11d** and **12**) inhibited COX-2 with less potency possessing IC_{50} values in range of 0.24 to 0.38 μM.

Regarding COX-2 selectivity index, dimethoxy chalcone **11d** and *N*-acetyl pyrazole **13** showed the best values (SI = 103, 101.9 respectively) while benzoic acid hydrazone **10b**, nitrophenyl chalcone **11a** and compounds possessing aminosulfonyl pharmacophore (**10c**, **14**) showed moderate COX-2 selectivity indices (SI = 86.83 — 51.27). On the other hand, oxime **9**, phenylhydrazone **10a,** methoxy chalcones (**11b**, **11c**) and pyrazole **12** possessed lower SI values in range of (37.37 to 22.5). The least SI value was gained by the hydrazone **10d** (SI = 18.36). Collectively, all prepared isoindolines showed weak COX-1 inhibition and moderate COX-2 inhibition resulting in reasonable COX-2 selectivity index values.

[Please insert Table 1 about here]

2.2.2. *In vivo* anti-inflammatory activity

In vivo anti-inflammatory activity of all target compounds was evaluated adopting formalin-induced rat paw edema assay using diclofenac as a reference drug. Comparing paw-volume change (% edema inhibition) produced by tested compounds and diclofenac (10 mg/kg) after 1, 3 and 6 h from induction of inflammation *via* subcutaneous formalin injection (Table 2), showed a wide range of anti-inflammatory activity (8.3-50%; 1 h), (14.8-67.4%; 3 h) and (6.7-46.7%; 6 h) relative to the reference drug diclofenac (29.2%; 1 h, 22.2%; 3 h, 20%; 6 h).

After 1 h, six compounds showed superior anti-inflammatory activities and were more potent than diclofenac where both aminosulfonylphenyl derivatives **10c** and **14** possessed potent activity (50%). Chalcone **11d** and *N*-acetyl pyrazole **13** displayed % edema inhibition of (45.8%) while hydrazone **10b** and chalcone **11a** displayed lower potency (41.7%). Oxime **9**, hydrazone **10a**, chalcone **11c** and pyrazole **12** displayed moderate activity (25-33.3%) and were more active than compounds **10a** and **11b** (8.3 and 12.5%). After 3 h, compounds (**10b**, **10c**, **11a, 11d**,

13, 14) displayed potent anti-inflammatory activity (% edema inhibition = 40.7 - 67.4%) in order of (**10c** > **11d** =**13** > **11a** > **10b** > **14**) while compounds (**9, 10a, 10d, 11b, 11c, 12)** showed moderate activity ranged between (14.8 and 37%). After 6 h, chalcones **11a**, **11d** were the most potent compounds with equal percentage activities (46.7%). Hydrazone **10c** and pyrazole **13** showed (43.3% and 40%) edema inhibition in sequent. Four compounds (**11b**, **11c**, **12** and **14**) were of equal potency (30%) higher than diclofenac (20%).

Collectively, six compounds (**10b**, **10c, 11a, 11d, 13** and **14**) exhibited promising anti-inflammatory activity (43.3-67.4%) at the three time intervals (1, 3 and 6 h) and were more potent than diclofenac (20-29.2%). In conclusion, the results showed that molecular hybridization of isoindoline moiety with hydrazone, chalcone and/or pyrazole pharmacophores produced active compounds as anti-inflammatory agents.

[Please insert Table 2 about here]

2.2.3. Analgesic activity

Analgesic activities of all prepared compounds were evaluated using two different models; acetic acid induced writhing test and the hotplate latency test.

1- Acetic acid-induced writhing test: All test compounds produced peripheral analgesic effect against acetic acid induced writhing behavior relative to vehicle-treated mice except compound **14**. A significant reduction in the writhing response was observed in the hydrazono derivative **10c** (42.5%), nitrophenyl chalcone **11a** (48.9), dimethoxy analogue **11d** (53.6%) and the *N*-acetyl pyrazole **13** (44.6%). Furthermore, the most active compounds (**10c**, **11a, 11d** and **13**) showed better analgesic activities than control drug piroxicam (61.7%) as illustrated in Figure 3.

[Please insert Figure 3 about here]

2- Hot plate latency test: Oral administration of all test compounds increased the latency time in comparison with basal values. The results showed that compounds (**10b**, **10c**, **11a**, **11d**, **13** and **14**) produced (41-92%) increase in pain threshold after 1h and (35-50%) after 2h. In addition, the chalcone **11d** exhibited superior central analgesic activity (92.85%) at 1 h more than the control drug, piroxicam (75%) while the *N*-acetyl pyrazole **13** was equipotent to piroxicam (Table 3). The obtained results were in accordance with the *in vitro* COXs data as compounds **11d** and **13** showed potent COX-2 inhibitory activity (IC_{50} = 0.11 μM). These results showed a parallel correlation between the analgesic and anti-inflammatory activities of tested compounds.

[Please insert Table 3 about here]

2.3. Docking study

To investigate the possible binding interactions of synthesized compounds inside COX-2 enzyme active site and to predict their mechanism of action as anti-inflammatory agents, molecular docking study was performed. MOE 2008.10 program (Molecular Operating Environment, Chemical Computing Group, Canada) was used as modeling software. X-ray crystal structure of enzyme COX-2 active site in complex with the selective COX-2 inhibitor, SC-558 was downloaded from the protein data bank (PDB code: 1CX2) [38]. The ligand, SC-558 was found to form 2 hydrogen bonding interactions with His-90 and Arg-513 amino acid residues inside COX-2 active site with distance of (2.35 and 2.47 A^o) and binding energy (E-score = -15.6068 Kcal/mol). Docked compounds formed both hydrogen bonding and arene-cation interactions with affinity (E-score = -11.2962 – -17.4061 Kcal/mol). Binding interactions, docking scores, amino acid residues and hydrogen bond lengths are summarized in Table 4.

[Please insert Table 4 about here]

All designed compounds made 1 to 3 hydrogen bonding interactions in addition to arene-cation interactions with His-90, Arg-513, Arg-120 and Tyr-355 amino acids. In particular, the most active derivatives (**10b**, **10c**, **11a**, **11d**, **13** and **14**) showed the best affinity range from -15.7074 to -17.4061 Kcal/mol comparable to SC-558 and formed 1 to 3 hydrogen bonds with His-90, Arg-513, Arg-120 and Tyr-355 amino acid residues inside COX-2 active site with a distance range of (2.44 – 2.95 A^o). Compounds (**9, 10a, 10d, 11b, 11c, 12**) displayed lower affinity than ligand (E-score = -11.2962 - -14.9380 Kcal/mol). 2D and 3D interactions of the proposed binding mode for ligand SC-558 and chalcone **11d** that showed the highest COX-2 SI value (SI = 103) with amino acid residues forming H-bonds inside the enzyme active site are displayed in Figures 4 and 5.

[Please insert Figure 4 about here]

[Please insert Figure 5 about here]

3- Conclusion

This study describes the synthesis of new isoindoline-1,3-dione hybrids endowed with oxime group (**9**), hydrazono-bridge (**10a-d**), chalcone moiety (**11a-d**), pyrazole ring (**12-14**) or aminosulfonylphenyl (**10c** and **14**) pharmacophores as COX-2 inhibitors with dual anti-inflammatory and analgesic activities. Target compounds were screened for their COX-1/COX-2 inhibition and *in vivo* anti-inflammatory activity applying formalin-induced rat paw edema assay. Analgesic activities were evaluated adopting two screening models; acetic acid induced writhing test and the hotplate latency test.

All target compounds were more selective to COX-2 than COX-1 with a wide selectivity index (SI) range of (18.36-103). The most potent COX-2 enzyme inhibitors

(IC_{50} = 0.11 — 0.18 μM) were: hydrazones (**10b**, **10c**), chalcones (**11a**, **11d**) and pyrazoles (**13, 14**) comparable to celecoxib (IC_{50} = 0.09 μM). The most active compounds possessed superior central analgesic activity (35-92.85%) relative to reference drug piroxicam (75%) and displayed percentage edema inhibition (43.3-67.4%) at three time intervals (1h, 3h, 6h) relative to reference drug diclofenac (20-29.2%) indicating promising anti-inflammatory activities. All synthesized compounds were subjected to molecular docking study and showed perfect docking scores and effective binding interaction with amino acid residues inside COX-2 active site.

A common structural feature in the active compounds (**10b**, **10c**, **11a**, **11d**, **13** and **14**) is the presence of either acyclic central systems such as iminic structure (**10b**, **10c**), α,β-unsaturated ketone (**11a**, **11d**) or central cyclic five membered pyrazole core ring (**13**, **14**). In addition, presence of aminosulfonyl group at the para position of phenyl ring contributes for the activity in compounds (**10c**, **14**) while the presence of a hydrogen acceptor group such as methoxy substituent in **11d** and acetyl group in **13** that mimic aminosulfonyl pharmacophore improved their COX-2 selectivity (SI = 103, 101.9 in sequent) and potency of enzyme inhibition (IC_{50} = 0.11 μM).

In conclusion, molecular hybridization of isoindoline, hydrazone, chalcone and/or pyrazole pharmacophores constitutes a useful implement to produce effective hybrid scaffolds as COX-2 inhibitors with improved anti-inflammatory activity and analgesic potential.

4- Experimental

4.1. Chemistry

General: Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. Infrared (IR) spectra were recorded as films on KBr plates using a Nicolet 550 Series II Magna FT-IR spectrometer (Middleton, WI). ^{1}H NMR and ^{13}C

NMR spectra were measured on a Bruker Avance III 400MHz (BrukerBioSpin AG, Fa¨llanden, Switzerland) for ^{1}H and 100MHz for ^{13}C with BBFO Smart Probe and Bruker 400 AEON Nitrogen-Free Magnet, Faculty of Pharmacy, Beni-Suef University, Egypt, in DMSO-d_6 with TMS as the internal standard, where *J* (coupling constant) values are estimated in Hertz (Hz) and chemical shifts were recorded in ppm on δ scale. Mass spectra (MS) were recorded on a Hewlett Packard 5988 spectrometer (Palo Alto, CA). Microanalyses for C, H and N were carried out on Perkin-Elmer 2400 analyzer (Perkin-Elmer, Norwalk, CT) at the Micro analytical unit of Cairo University, Egypt, and all compounds were within ± 0.4% of the theoretical values. All other reagents, purchased from the Aldrich Chemical Company (Milwaukee, WI), were used without further purification.

Procedure for synthesis of *2-(3-acetylphenyl)isoindoline-1,3-dione (**8**)*:

A mixture of phthalic anhydride (**6**) (1.48 g, 0.01 mol) and 3-aminoacetophenone (**7**, 1.35 g, 0.01 mol) in glacial acetic acid (20 mL) was heated under reflux for 12 h. The separated solid on hot was filtered, dried and crystallized from aqueous ethanol as white crystals; 82% (2.17 g) yield; mp 247-249 ^{o}C; IR (KBr, υ cm^{-1}): 3063 (C-H aromatic), 2923 (C-H aliphatic), 1770, 1724, 1669 (3C=O); ^{1}H NMR (DMSO-d_6, δ ppm): 2.50 (s, 3H, CH_3), 7.70-7.75 (m, 2H, phenyl H-4, H-5), 7.91-7.93 (m, 2H, isoindoline H-5, H-6), 7.98-8.00 (m, 2H, isoindoline H-4, H-7), 8.03-8.05 (m, 2H, phenyl H-2, H-6); ^{13}C NMR (DMSO-d_6, δ ppm): 27.19 (CH_3), 124.00 (CH, phenyl C-2), 127.17 (CH, phenyl C-6), 128.49 (CH, phenyl C-4), 129.97 (CH, isoindoline C-4, C-7), 131.82 (C, phenyl C-3), 132.44 (CH, phenyl C-5), 132.69 (C, isoindoline C-3a, C-7a), 135.35 (CH, isoindoline C-5, C-6), 137.90 (C, phenyl C-1), 167.49 (C, isoindoline 2C=O), 198.21 (C, *CO*CH_3). Anal. Calcd. for $C_{16}H_{11}NO_3$: C, 72.45; H, 4.18; N, 5.28. Found: C, 72.53; H, 4.23; N, 5.18

Procedure for synthesis of *(ZE)-2-[3-(1-hydroxyiminoethyl)phenyl]isoindoline-1,3-dione (**9**):*

A mixture of compound **8** (2.65 g, 0.01 mol) and hydroxylamine hydrochloride (0.07 g, 0.01 mol) in ethanol (15 mL) was heated under reflux for 2 h. After cooling to room temperature, the resultant solid product was collected by filtration, dried and crystallized from ethanol to give compound **9** as white solid; 63% (1.76 g) yield; mp 280-282 ^{o}C; IR (KBr, υ cm^{-1}): 3243 (OH), 3052 (C-H aromatic), 2920 (C-H aliphatic), 1767, 1712 (2C=O), 1600 (C=N); ^{1}H NMR (DMSO-d_6, δ ppm): 2.18 (s, 3H, CH_3), 7.46 (d, J = 8 Hz, 1H, phenyl H-4), 7.55 (t, J =8 Hz, 1H, phenyl H-5), 7.72 (d, J = 8 Hz, 1H, phenyl H-6), 7.75 (s, 1H, phenyl H-2), 7.90-7.94 (m, 2H, isoindoline H-5, H-6), 7.96-8.00 (m, 2H, isoindoline H-4, H-7), 11.34 (s, 1H, OH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, δ ppm): 12.01 (CH_3), 123.90 (CH, phenyl C-2), 125.01 (CH, phenyl C-6), 125.66 (CH, phenyl C-4), 128.40 (CH, isoindoline C-4, C-7), 129.37 (CH, phenyl C-5), 132.04 (C, phenyl C-3), 132.53 (C, isoindoline C-3a, C-7a), 135.18 (CH, isoindoline C-5, C-6), 138.22 (C, phenyl C-1), 152.76 (C, C=N), 167.48 (C, isoindoline 2C=O); MS (m/z, %): 280 ($M^{+\cdot}$, 70.36); Anal. Calcd. for $C_{16}H_{12}N_2O_3$: C, 68.56; H, 4.32; N, 9.99. Found: C, 68.45; H, 4.45; N, 10.08

General procedure for synthesis of compounds **10a-d**

A mixture of 2-(3-acetylphenyl)isoindoline-1,3-dione (**8**, 2.65 g, 0.01 mol) and the appropriate phenyl hydrazine hydrochloride (0.01 mol) in ethanol was heated under reflux for 14 h. After cooling, the reaction was poured onto crushed ice; the resulting precipitate was filtered, crystallized from ethanol to give hydrazones **10a-d** in good yield (70-80%).

*2-{3-[1-(Phenylhydrazono)ethyl]phenyl}isoindoline-1,3-dione (**10a**):*Yellow solid; 78% (2.76 g) yield; mp 182-184 ^{o}C; IR (KBr, υ cm^{-1}): 3346 (NH), 3058 (C-H

aromatic), 2922 (C-H aliphatic), 1770, 1710 (2C=O), 1593 (C=N); ^{1}H NMR (DMSO-d_6, δ ppm): 2.29 (s, 3H, CH_3), 6.76 (t, J = 7.2 Hz, 1H, aminophenyl H-4), 7.19-7.27 (m, 4H, aminophenyl H-2, H-3, H-5, H-6), 7.37 (d, J = 8Hz, 1H, phenyl H-4), 7.54 (t, J = 8 Hz, 1H, phenyl H-5), 7.83 (d, J = 8Hz, 1H, phenyl H-6), 7.91-7.93 (m, 3H, isoindoline H-5, H-6 and phenyl H-2), 7.97-7.99 (m, 2H, isoindoline H-4, H-7), 9.36 (s, 1H, NH, D_2O exchangeable) ; ^{13}C NMR (DMSO-d_6, δ ppm): 13.12 (CH_3), 113.27 (CH, aminophenyl C-2, C-6), 119.76 (CH, aminophenyl C-4), 123.93 (CH, phenyl C-2), 124.45 (CH, phenyl C-6), 125.45 (CH, phenyl C-4), 129.02 (CH, isoindoline C-4, C-7), 129.46 (CH, phenyl C-5), 131.67 (C, phenyl C-3), 132.30 (C, isoindoline C-3a, C-7a), 132.58 (CH, aminophenyl C-3, C-5), 135.38 (CH, isoindoline C-5, C-6), 137.85 (C, phenyl C-1), 140.54 (C, aminophenyl C-1), 146.11 (C, C=N), 167.79 (C, isoindoline 2C=O); MS (m/z, %): 355 ($M^{+\cdot}$, 100); Anal. Calcd. for $C_{22}H_{17}N_3O_2$: C, 74.35; H, 4.82; N, 11.82. Found: C, 74.42; H, 4.85; N, 12.03

*4-(N'-{1-[3-(1,3-Dioxo-1,3-dihydroisoindol-2-yl)phenyl]ethylidene}hydrazino)benzoic acid (**10b**):* Yellow solid; 71% (2.83 g) yield; mp 270-272 ^{o}C; IR (KBr, υ cm^{-1}): 3422 (OH), 3360 (NH), 3074 (C-H aromatic), 2921 (C-H aliphatic), 1771, 1718, 1665 (3C=O), 1602 (C=N); ^{1}H NMR (DMSO-d_6, δ ppm): 2.32 (s, 3H, CH_3), 7.30 (d, J = 8 Hz, 2H, benzoic acid, H-3, H-5), 7.41 (d, J = 8 Hz, 1H, phenyl H-4), 7.56 (t, J = 8 Hz, 1H, phenyl H-5), 7.81 (d, J = 8 Hz, 2H, benzoic acid H-2, H-6), 7.87 (d, J = 8 Hz, 1H, phenyl H-6), 7.93-7.95 (m, 3H, isoindoline H-5, H-6, phenyl H-2), 7.98-8.01 (m, 2H, isoindoline H-4, H-7), 9.82 (s, 1H, NH, D_2O exchangeable), 12.28 (s, 1H, CO*OH*, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, δ ppm): 13.64 (CH_3), 115.07 (CH, benzoic acid C-2, C-6), 123.92 (CH, phenyl C-2), 125.07 (CH, phenyl C-6), 125.69 (CH, phenyl C-4), 127.53 (CH, isoindoline C-4, C-7), 129.37 (CH, phenyl C-5), 132.11 (C, phenyl C-3), 132.60 (C,

isoindoline C-3a, C-7a), 135.18 (CH, benzoic acid C-2, C-6), 135.58 (CH, isoindoline C-5, C-6), 138.15 (C, phenyl C-1), 140.83 (C, benzoic acid C-4), 147.32 (C, benzoic acid C-1), 156.02 (C, C=N), 167.62 (C, isoindoline 2C=O), 172.02 (C, *CO*OH); MS (m/z, %): 399 ($M^{+\cdot}$, 86.22); Anal. Calcd. for $C_{23}H_{17}N_3O_4$: C, 69.17; H, 4.29; N, 10.52. Found: C, 68.96; H, 4.46; N, 10.34

*4-(N'-{1-[3-(1,3-Dioxo-1,3-dihydroisoindol-2-yl)phenyl]ethylidene}hydrazino) benzenesulfonamide (**10c**):* Off white solid; 70% (3.03 g) yield; mp 258-260 ^{o}C; IR (KBr, υ cm^{-1}): 3449 (NH), 3325, 3239 (NH_2), 3077 (C-H aromatic), 2922 (C-H aliphatic), 1774, 1703 (2C=O), 1594 (C=N), 1328, 1148 (SO_2); ^{1}H NMR (DMSO-d_6, δ ppm): 2.32 (s, 3H, CH_3), 7.08 (s, 2H, NH_2, D_2O exchangeable), 7.34 (d, J = 8.8 Hz, 2H, aminosulfonylphenyl H-3, H-5), 7.41 (d, J = 8.4 Hz, 1H, phenyl H-4), 7.56 (t, J = 8 Hz, 1H, phenyl H-5), 7.66 (d, J = 8.8 Hz, 2H, aminosulfonylphenyl H-2, H-6), 7.86 (d, J = 8 Hz, 1H, phenyl H-6), 7.92-7.95 (m, 3H, isoindoline H-5, H-6 and phenyl H-2), 7.98-8.01 (m, 2H, isoindoline H-4, H-7), 9.81 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, δ ppm): 13.69 (CH_3), 112.56 (CH, aminosulfonylphenyl C-3, C-5), 123.92 (CH, phenyl C-2), 125.11 (CH, phenyl C-6), 125.68 (CH, phenyl C-4), 127.53 (CH, isoindoline C-4, C-7), 127.68 (CH, aminosulfonylphenyl C-2, C-6), 129.33 (CH, phenyl C-5), 132.14 (C, aminosulfonylphenyl C-1), 132.62 (C, phenyl C-3), 134.28 (C, isoindoline C-3a, C-7a), 135.17 (CH, isoindoline C-5, C-6), 140.15 (C, phenyl C-1), 142.73 (C, aminosulfonylphenyl C-4), 148.85 (C, C=N), 167.59 (C, isoindoline 2C=O); MS (m/z, %): 434 ($M^{+\cdot}$, 10.83); Anal. Calcd. for $C_{22}H_{18}N_4O_4S$: C, 60.82; H, 4.18; N, 12.90. Found: C, 61.06; H, 4.16; N, 12.74

*2-(3-{1-[(4-Methanesulfonylphenyl)hydrazono]ethyl}phenyl)isoindolin-1,3-dione (**10d**):* Off white solid; 65% (2.81 g) yield; mp 303-305 ^{o}C; IR (KBr, υ cm^{-1}): 3438

(NH), 3070 (C-H aromatic), 2925 (C-H aliphatic), 1776, 1706 (2C=O), 1593 (C=N), 1386, 1140 (SO_2); ^{1}H NMR (DMSO-d_6, δ ppm): 2.34 (s, 3H, CH_3), 3.11 (s, 3H, $SO_2C\underline{H_3}$), 7.40-7.44 (m, 3H, methyllsulfonylphenyl H-2, H-6 and phenyl H-4) 7.57 (t, J = 8 Hz, 1H, phenyl H-5), 7.73 (d, J = 8.4 Hz, 2H, methylsulfonylphenyl H-3, H-5), 7.89 (d, J = 8 Hz, 1H, phenyl H-6), 7.92-7.94 (m, 2H, isoindoline H-5, H-6), 7.99-8.06 (m, 3H, isoindoline H-4, H-7 and phenyl H-2), 9.98 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, δ ppm): 13.80 (CH_3), 44.71 ($SO_2\underline{CH_3}$), 112.90 (CH, methanesulfonylphenyl C-3, C-5), 123.93 (CH, phenyl C-2), 125.20 (CH, phenyl C-6), 125.78 (CH, phenyl C-4), 127.70 (CH, isoindoline C-4, C-7), 129.12 (CH, methanesulfonylphenyl C-2, C-6), 129.38 (CH, phenyl C-5), 130.29 (C, methanesulfonylphenyl C-1), 132.12 (C, phenyl C-3), 132.64 (C, isoindoline C-3a, C-7a), 135.19 (CH, isoindoline C-5, C-6), 140.03 (C, phenyl C-1), 142.70 (C, methanesulfonylphenyl C-4), 150.23 (C, C=N), 167.58 (C, isoindoline 2C=O); Anal. Calcd. for $C_{23}H_{19}N_3O_4S$: C, 63.73; H, 4.42; N, 9.69. Found: C, 63.58; H, 4.53; N, 9.74

Procedure for synthesis of chalcones **11a-d**

A mixture of 2-(3-acetylphenyl)isoindoline-1,3-dione **(8,** 2.65 g 0.01 mol) and the appropriate aromatic aldehyde (0.01 mol) were dissolved in 5% methanolic potassium hydroxide solution (15 mL). The reaction mixture was kept on stirring for 24 h at room temperature. The obtained solid was filtered, washed with water and crystallized from methanol to give chalcones **11a-d**.

(E)-2-{3-[3-(4-Nitrophenyl)acryloyl]phenyl}isoindoline-1,3-dione ***(11a):*** Yellow solid; 64% (2.54 g) yield; mp 318-320 ^{o}C; IR (KBr, υ cm^{-1}): 3073 (C-H aromatic), 1740, 1663, 1642 (3C=O); ^{1}H NMR (DMSO-d_6, δ ppm): 7.76-7.82 (m, 3H, phenyl H-4, H-5, H-6), 7.89 (d, J = 15.6 Hz, 1H, COC$\underline{H}$=CH), 7.94-7.97 (m, 2H, isoindoline H-5, H-6), 8.00- 8.03 (m, 2H, isoindoline H-4, H-7), 8.14 (d, J = 15.6 Hz, 1H,

COCH=C*H*), 8.19 (d, *J* = 8.4 Hz, 2H, nitrophenyl H-2, H-6), 8.29-8.31 (m, 3H, nitrophenyl H-3, H-5 and phenyl H-2); ^{13}C NMR (DMSO-d_6, δ ppm): 119.36 (CH, phenyl C-2), 124.08 (CH, phenyl C-6), 124.43 (CO*C*H=CH), 124.89 (CH, nitrophenyl C-3, C-5), 126.75 (CH, phenyl C-4), 129.70 (CH, isoindoline C-4, C-7), 129.90 (CH, phenyl C-5), 130.31 (CH, nitrophenyl C-2, C-6), 130.35 (COCH=*C*H), 130.58 (CH, isoindoline C-5, C-6), 132.47 (C, phenyl C-3), 138.21 (C, isoindoline C-3a, C-7a), 141.20 (C, phenyl C-1), 141.61 (C, nitrophenyl C-4), 148.56 (C, nitrophenyl C-1), 166.90 (C, isoindoline 2C=O), 172.22 (C, *CO*CH=CH); MS (m/z, %): 398 ($M^{+\cdot}$, 15.55); Anal. Calcd. for $C_{23}H_{14}N_2O_5$: C, 69.34; H, 3.54; N, 7.03. Found: C, 69.58; H, 3.65; N, 7.24

(E)-2-{3-[3-(4-Methoxyphenyl)acryloyl]phenyl}isoindoline-1,3-dione ***(11b)****:* Yellow solid; 68% (2.60 g) yield; mp 184-186 ^{o}C; IR (KBr, υ cm^{-1}): 3071 (C-H aromatic), 2925 (C-H aliphatic), 1710, 1674, 1648 (3C=O); ^{1}H NMR (DMSO-d_6, δ ppm): 3.83 (s, 3H, OCH_3), 7.03 (d, *J* = 8.4 Hz, 2H, methoxyphenyl H-3, H-5), 7.54 (t, *J* = 8 Hz, 1H, phenyl H-5), 7.76-7.82 (d, *J* = 7.2 Hz, 1H, phenyl H-4), 7.63 (d, *J* = 15.6 Hz, 1H, COC*H*=CH), 7.66-7.68 (m, 2H, isoindoline H-5, H-6), 7.74 (d, *J* = 15.6 Hz, 1H, COCH=C*H*), 7.82 (d, *J* = 8.4 Hz, 2H, methoxyphenyl H-2, H-6), 7.86-7.91 (m, 2H, isoindoline H-4, H-7), 7.98 (d, *J* = 7.6 Hz, 1H, phenyl H-6), 8.35 (s, 1H, phenyl H-2); ^{13}C NMR (DMSO-d_6, δ ppm): 55.89 (OCH_3), 115.00 (CH, methoxyphenyl C-3, C-5), 120.26 (CH, phenyl C-2), 124.00 (CO*C*H=CH), 124.34 (CH, phenyl C-4), 127.74 (C, methoxyphenyl C-1), 128.23 (CH, phenyl C-6), 129.56 (CH, isoindoline C-4, C-7), 129.98 (CH, phenyl C-5), 130.50 (C, isoindoline C-3a, C-7a), 131.11 (CH, methoxyphenyl C-2, C-6), 132.17 (CH, isoindoline C-5, C-6), 138.91 (C, phenyl C-3), 140.41 (C, phenyl C-1), 144.57 (COCH=*C*H), 161.95 (C, methoxyphenyl C-4),

167.86 (C, isoindoline 2C=O), 168.14 (C, <u>CO</u>CH=CH); Anal. Calcd. for $C_{24}H_{17}NO_4$: C, 75.19; H, 4.47; N, 3.65. Found: C, 75.08; H, 4.60; N, 3.64.

(E)-2-{3-[3-(2,3-Dimethoxyphenyl)acryloyl]phenyl}isoindoline-1,3-dione (***11c***)*:* Off white solid; 68% (2.80 g) yield; mp 250-252 ^{o}C; IR (KBr, υ cm^{-1}): 3069 (C-H aromatic), 2938 (C-H aliphatic), 1716, 1662, 1640 (3C=O); ^{1}H NMR (DMSO-d_6, δ ppm): 3.82 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 7.16-7.19 (m, 2H, dimethoxyphenyl, H-4, H-5), 7.24 (d, *J* = 7.6 Hz, 1H, dimethoxyphenyl H-6), 7.53-7.55 (m, 2H, isoindoline H-5, H-6), 7.56-7.58 (m, 2H, isoindoline H-4, H-7), 7.61 (d, *J* = 6.8 Hz, 1H, phenyl H-6), 7.67 (d, *J* = 7.6 Hz, 1H, phenyl H-4), 7.81 (d, *J* = 15.6 Hz, 1H, COC<u>*H*</u>=CH), 7.89 (t, *J* = 6.4 Hz, 1H, phenyl H-5), 7.98 (d, *J* = 15.6 Hz, 1H, COCH=C<u>*H*</u>), 8.39 (s, 1H, phenyl H-2); ^{13}C NMR (DMSO-d_6, δ ppm): 56.32 (OCH_3), 61.45 (OCH_3), 115.62 (CH, dimethoxyphenyl C-4), 119.70 (CH, dimethoxyphenyl C-6), 123.52 (CH, phenyl C-2), 124.13 (CH, dimethoxyphenyl C-5), 124.49 (CO<u>*C*</u>H=CH), 124.92 (CH, phenyl C-4), 128.24 (CH, phenyl C-6), 128.23 (C, dimethoxyphenyl C-1), 129.74 (CH, isoindoline C-4, C-7), 130.06 (CH, phenyl C-5), 130.56 (C, phenyl C-3), 132.31 (CH, isoindoline C-5, C-6), 134.15 (C, isoindoline C-3a, C-7a), 138.97 (COCH=<u>*C*</u>H), 139.08 (C, phenyl C-1), 140.19 (C, dimethoxyphenyl C-2), 153.72 (C, dimethoxyphenyl C-3), 161.24 (C, isoindoline 2C=O), 167.87 (C, <u>CO</u>CH=CH); Anal. Calcd. for $C_{25}H_{19}NO_5$: C, 72.63; H, 4.63; N, 3.39. Found: C, 72.69; H, 4.59; N, 3.35.

(E)-2-{3-[3-(3,4-Dimethoxyphenyl)acryloyl]phenyl}isoindoline-1,3-dione (***11d***)*:* Yellow solid; 68% (2.80 g) yield; mp 162-164 ^{o}C; IR (KBr, υ cm^{-1}): 3068 (C-H aromatic), 2936 (C-H aliphatic), 1717, 1655, 1642 (3C=O); ^{1}H NMR (DMSO-d_6, δ ppm): 3.83 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 7.04 (d, *J* = 8.4 Hz, 1H, dimethoxyphenyl H-5), 7.15-7.18 (m, 2H, isoindoline H-5, H-6), 7.23-7.27 (m, 2H,

isoindoline H-4, H-7), 7.40 (d, *J* = 8.4 Hz, 1H, dimethoxyphenyl H-6), 7.54 (d, *J* = 15.6 Hz, 1H, COC*H*=CH), 7.59 (d, *J* = 6.4 Hz, 1H, phenyl H-4), 7.68 (t, *J* = 8.4 Hz, 1H, phenyl H-5), 7.74 (s, 1H, dimethoxyphenyl H-2), 7.93 (d, *J* = 15.6 Hz, 1H, COCH=C*H*), 7.99 (d, *J* = 7.6 Hz, 1H, phenyl H-6), 8.37 (s, 1H, phenyl H-2); ^{13}C NMR (DMSO-d_6, δ ppm): 55.92 (OCH_3), 56.07 (OCH_3), 111.26 (CH, dimethoxyphenyl C-2), 112.07 (CH, dimethoxyphenyl C-5), 119.50 (CH, dimethoxyphenyl C-6), 120.20 (CH, phenyl C-2), 124.28 (CO*C*H=CH), 125.78 (CH, phenyl C-4), 127.88 (C, dimethoxyphenyl C-1), 128.23 (CH, phenyl C-6), 129.37 (CH, isoindoline C-4, C-7), 129.58 (CH, phenyl C-5), 130.05 (C, isoindoline C-3a, C-7a), 132.29 (CH, isoindoline C-5, C-6), 138.88 (C, phenyl C-3), 140.38 (C, phenyl C-1), 145.20 (COCH=*C*H), 149.48 (C, dimethoxyphenyl C-4), 151.80 (C, dimethoxyphenyl C-3), 167.91 (C, isoindoline 2C=O), 168.21 (C, *CO*CH=CH); Anal. Calcd. for $C_{25}H_{19}NO_5$: C, 72.63; H, 4.63; N, 3.39. Found: C, 72.71; H, 4.68; N, 3.42.

Procedure for synthesis of *2-{3-[5-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-3-yl]phenyl}isoindoline-1,3-dione (**12**):*

A mixture of (*E*)-2-{3-[3-(4-nitrophenyl)acryloyl]phenyl}isoindoline-1,3-dione (**11a**, 3.98 g, 0.01 mol) and hydrazine hydrate (1.28 g, 0.04 mol) in absolute ethanol (25 mL) was heated under reflux for 8 h. The separated solid on hot was collected, dried and crystallized from benzene to obtain compound **12** as dark yellow solid; 44% (1.81 g) yield; mp 198-200 ^{o}C; IR (KBr, υ cm^{-1}): 3432 (NH), 3060 (C-H aromatic), 2925 (C-H aliphatic), 1723, 1670 (2C=O), 1601 (C=N); ^{1}H NMR (DMSO-d_6, δ ppm): 2.77 (dd, *J* = 16.4, 10.4 Hz, 1H, pyrazole H-4), 3.47 (dd, *J* = 16.8, 12.4 Hz, 1H, pyrazole H-4), 4.95 (dd, *J* = 12.4, 10.4 Hz, 1H, pyrazole H-5), 6.54 (d, *J* = 7.6 Hz, 1H, phenyl H-4), 6.77 (d, *J* = 7.6 Hz, 1H, phenyl H-6), 6.90 (s, 1H, NH, D_2O exchangeable), 7.02 (t, *J* = 7.6 Hz, 1H, phenyl H-5), 7.54-7.63 (m, 2H,

isoindoline H-5, H-6), 7.66 (d, *J* = 8.8 Hz, 2H, nitrophenyl H-2, H-6), 7.69-7.74 (m, 3H, isoindoline H-4, H-7 and phenyl H-2), 8.23 (d, *J* = 8.8 Hz, 2H, nitrophenyl H-3, H-5); ^{13}C NMR (DMSO-d_6, δ ppm): 41.32 (CH_2, pyrazole C-4), 62.18 (CH, pyrazole C-5), 111.20 (CH, phenyl C-2), 114.19 (CH, phenyl C-6), 114.81 (CH, nitrophenyl C-3, C-5), 124.09 (CH, phenyl C-4), 124.37 (CH, isoindoline C-4, C-7), 128.40 (CH, phenyl C-5), 128.99 (CH, nitrophenyl C-2, C-6), 129.42 (CH, isoindoline C-5, C-6), 133.81 (C, phenyl C-3), 140.76 (C, isoindoline C-3a, C-7a), 147.05 (C, phenyl C-1), 149.06 (C, nitrophenyl C-4), 149.98 (C, nitrophenyl C-1), 151.68 (C, pyrazole C-3), 167.52 (C, isoindoline 2C=O); MS (m/z, %): 412 ($M^{+\cdot}$, 10.44); Anal. Calcd. for $C_{23}H_{16}N_4O_4$: C, 66.99; H, 3.91; N, 13.59. Found: C, 66.87; H, 3.78; N, 13.65

Procedure for synthesis of *2-{3-[1-acetyl-5-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-3-yl]phenyl}isoindoline-1,3-dione (**13**)*:

A mixture of chalcone **11a** (3.98 g, 0.01 mol) and hydrazine hydrate (1.28 g, 0.04 mol) in glacial acetic acid (25 mL) was heated under reflux for 8 h. The solid separated on hot was collected, dried and crystallized from benzene to obtain compound **13** as white solid; 46% (2.08 g) yield; mp 149-151 ^{o}C; IR (KBr, υ cm^{-1}): 3025 (C-H aromatic), 2926 (C-H aliphatic), 1784, 1690, 1644 (3C=O), 1580 (C=N); ^{1}H NMR (DMSO-d_6, δ ppm): 2.33 (s, 3H, CO$\underline{CH_3}$), 3.15 (dd, *J* = 10.0, 5.2 Hz, 1H, pyrazole H-4), 3.92 (dd, *J* = 18.0, 12.0 Hz, 1H, pyrazole H-4), 5.69 (dd, *J* = 12.0, 5.2 Hz, 1H, pyrazole H-5), 7.37-7.39 (m, 3H, phenyl H-4, H-5, H-6), 7.49 (d, *J* = 8.4 Hz, 2H, nitrophenyl H-2, H-6), 7.69-7.72 (m, 2H, isoindoline H-5, H-6), 7.89-7.94 (m, 2H, isoindoline H-4, H-7), 8.07 (s, 1H, phenyl H-2), 8.20 (d, *J* = 8.4 Hz, 2H, nitrophenyl H-3, H-5); ^{13}C NMR (DMSO-d_6, δ ppm): 22.04 (CO$\underline{CH_3}$), 42.31 (CH_2, pyrazole C-4), 59.51 (CH, pyrazole C-5), 117.09 (CH, phenyl C-2), 121.57 (CH, phenyl C-6), 122.29 (CH, nitrophenyl C-3, C-5), 123.20 (CH, phenyl C-4), 124.43

(CH, isoindoline C-4, C-7), 137.42 (CH, phenyl C-5), 129.74 (CH, nitrophenyl C-2, C-6), 131.67 (C, phenyl C-3), 132.09 (C, isoindoline C-3a, C-7a), 134.81 (CH, isoindoline C-5, C-6), 140.02 (C, phenyl C-1), 147.13 (C, nitrophenyl C-4), 150.07 (C, nitrophenyl C-1), 154.69 (C, pyrazole C-3), 168.29 (C, isoindoline 2C=O), 169.22 (C, <u>*CO*</u>CH_3); MS (m/z, %): 454 ($M^{+\cdot}$, 10.39); Anal. Calcd. for $C_{25}H_{18}N_4O_5$: C, 66.08; H, 3.99; N, 12.33. Found: C, 65.94; H, 4.09; N, 12.09

Procedure for synthesis of *4-[3-[3-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)phenyl]-5-(4-nitrophenyl)-4,5-dihydropyrazol-1-yl]benzenesulfonamide (**14**):*

A mixture of the chalcone **11a**, (3.98 g, 0.01 mol) and 4-hydrazinylbenzenesulfonamide hydrochloride (2.23 g, 0.01 mol) in ethanol (25 mL) was heated under reflux for 12 h. The reaction mixture was poured onto crushed ice. The resulting precipitate was filtered off; the crude product obtained was crystallized from benzene to afford compound **14** as orange solid; 51% (2.89 g) yield; mp 240-242 ^{o}C; IR (KBr, υ cm^{-1}): 3452, 3426 (NH_2), 3070 (C-H aromatic), 2965 (C-H aliphatic), 1703, 1640 (2C=O), 1603 (C=N), 1340, 1158 (SO_2); ^{1}H NMR (DMSO-d_6, δ ppm): 2.78 (dd, *J* = 16.4, 10.4 Hz, 1H, pyrazole H-4), 3.48 (dd, *J* = 16.4, 10.8 Hz, 1H, pyrazole H-4), 4.96 (dd, *J* = 10.8, 10.4 Hz, 1H, pyrazole H-5), 7.36 (d, *J* = 8.8 Hz, 2H, aminosulfonylphenyl H-3, H-5), 7.47 (s, 2H, NH_2, D_2O exchangeable), 7.59-7.69 (m, 2H, phenyl H-4, H-5), 7.70-7.75 (m, 3H, aminosulfonylphenyl H-2, H-6 and phenyl H-6), 7.83 (d, *J* = 8.4 Hz, 2H, nitrophenyl H-2, H-6), 7.92-7.95 (m, 2H, isoindoline H-5, H-6), 7.97-8.00 (m, 3H, isoindoline H-4, H-7 and phenyl H-2), 8.04 (d, *J* = 8.8 Hz, 2H, nitrophenyl H-3, H-5); ^{13}C NMR (DMSO-d_6, δ ppm): 38.49 (CH_2, pyrazole C-4), 61.66 (CH, pyrazole C-5), 123.99 (CH, aminosulfonylphenyl C-3, C-5), 125.92 (CH, phenyl C-2), 127.25 (CH, phenyl C-6), 128.05 (CH, nitrophenyl C-3, C-5), 128.58 (C, phenyl C-4), 129.54 (C, aminosulfonylphenyl C-2, C-6), 129.91

(CH, isoindoline C-4, C-7), 131.46 (CH, phenyl C-5), 131.69 (C, aminosulfonylphenyl C-1), 131.91 (C, phenyl C-3), 132.45 (CH, nitrophenyl C-2, C-6), 132.77 (C, isoindoline C-3a, C-7a), 135.32 (CH, isoindoline H-5, H-6), 137.92 (C, phenyl C-1), 143.67 (C, nitrophenyl C-4), 144.48 (C, aminosulfonylphenyl C-4), 148.82 (C, nitrophenyl C-1), 157.80 (C, pyrazole C-3), 167.44 (C, isoindoline 2C=O); MS (m/z, %): 567 ($M^{+\cdot}$, 20.06); Anal. Calcd. for $C_{29}H_{21}N_5O_6S$: C, 61.37; H, 3.73; N, 12.34. Found: C, 61.62; H, 3.58; N, 12.48.

4.2. Biological evaluation

***Animals*:** Adult male Swiss albino mice (20–25 g) and Wistar rats (150–175 g) were used throughout the study and were housed at controlled conditions at a temperature of 24±1 °C and relative humidity of 40–80% with free access to standard pellet diet and tap water. The animals were allowed to adapt to the experimental environment for 7 days before experimentation. All experimental procedures and animal handling were performed according to guidelines of the Research Ethical Committee of the Faculty of Pharmacy, Beni-Suef University, Egypt.

***Drugs and Chemicals*:** Piroxicam was obtained from Sigma-Aldrich (St. Louis, MO, USA). Diclofenac was purchased from Merck (Rahway, NJ, Germany). All chemicals and solvents used in the current study were obtained from authorized suppliers and were of analytical grade.

4.2.1. *In vitro* cyclooxygenases (COX-1, COX-2) inhibitory activity

The ability of prepared compounds to inhibit COX-1 and COX-2 enzymes (IC_{50} value) was determined using the colorimetric enzyme immune assay (EIA) kit, catalog no. 560131, Cayman Chemical, Ann Arbor, MI, USA [34].

4.2.2. *In vivo* Anti-inflammatory activity

Anti-inflammatory potential of test compounds was evaluated using the *in vivo* formalin-induced rat foot paw edema model [35]. Test compounds (10 mg/kg), vehicle or the reference drug diclofenac (10 mg/kg) were administered *via* oral route just prior to induction of inflammation, which was performed using 6% formalin solution as a subcutaneous injection on the plantar surface of the left hind-paw. The anti-inflammatory activity was then calculated based on paw-volume changes at 1, 3 and 6 h after formalin injection using plethysmometer. The right hind-paw served as a reference for comparison with the opposite limb. Results (% edema inhibition) were expressed as percentage paw-volume change (mL).

4.2.3. Analgesic activity

Acetic acid induced writhing test

The acetic acid-induced writhing induction tests were carried according to the previously described method [36]. Briefly, vehicle, piroxicam (reference standard, 10 mg/kg) or test compounds (10 mg/kg) were administered orally 30 min before intra-peritoneal injection of 0.7% acetic acid solution (10 mL/kg). The mice were then kept individually in glass cages for observation, and the number of writhing movements (abdominal constriction followed by dorsiflexion and extension) was counted for the next 30 min beginning 5 min after acetic acid injection. The results are expressed as the number of writhes per 30 min period.

Hot plate latency test

The hot plate latency test was performed as described earlier [37]. Hot plate latency (seconds) was evaluated in animals receiving normal saline or test agents (10 mg/kg) at 0, 1 and 2 h after administration. Piroxicam (10 mg/kg) was used as a reference standard. Test compounds were administered using an oral gavage 1 h before starting the experimental protocol. Response latency was determined as the

difference in time between placement of the mouse on the hot plate and occurrence of the licking of hind-paws at 50 ^{o}C. A cut-off latency of 30 seconds was used to prevent heat-induced tissue damage.

Statistical analysis

Statistical comparisons between different groups were analyzed for statistical significance using one-way ANOVA test followed by Dunnett's post-hoc test, with a value of $p < 0.05$ considered significant. Data were expressed as the mean value ± standard deviation (SD).

4.3. Molecular docking study

Docking study was performed for all target compounds inside COX-2 enzyme active site using Molecular Operating Environment (MOE version 2008.10; Chemical Computing Group, Canada) to operate docking calculations. Enzyme COX-2, in complex with SC-558 (PDB code ICX2) crystal structure was downloaded from the protein data bank [38]. Docking of the ligand inside enzyme active site was accomplished to determine the binding energy score, root mean standard deviation (RMSD = 1.0042 A^{o}) and amino acids interactions. London-DG force was used to carry out docking and refinement of the results was performed using force field energy. 3D structures of the synthesized compounds were used to fulfill docking study and docking protocol was then applied. Binding energy scores, amino acids residues forming hydrogen bonding interactions and their lengths were measured and tabulated (Table 3).

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Declaration of interest

The authors have declared no conflict of interest.

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Figures, schemes and table captions

Figure 1. Chemical structures of reported anti-inflammatory compounds as selective COX-2 inhibitors: valdecoxib, celecoxib, isoindoline (**1**), oxime (**2**), hydrazono-pyrazole (**3**), hydrazone (**4**) and chalcone (**5**) derivatives

Figure 2A. Design of the new compounds (**9, 10a-d, 11a-d** and **12—14**)

Figure 2B: Structural similarity between celecoxib and designed compounds

Figure 3. Analgesic effect of test compounds and piroxicam on acetic acid induced writhing response in mice. Data represent the mean value ± SD of four mice per group. Statistical comparisons were analyzed using one-way ANOVA followed by Dunnett's test and denoted by $p < 0.05$

Figure 4. a) 2D interaction and **b)** 3D interaction of ligand SC-558 inside COX-2 enzyme active site

Figure 5. a) 2D interaction of the proposed binding mode and **b)** 3D interaction of compound **11d** (shown in green) facing the ligand (shown in red) inside COX-2 enzyme active site

Scheme 1. Synthesis of compounds (**8, 9, 10a-d** and **11a-d**)

Scheme 2: Synthesis of compounds **(12, 13** and **14**)

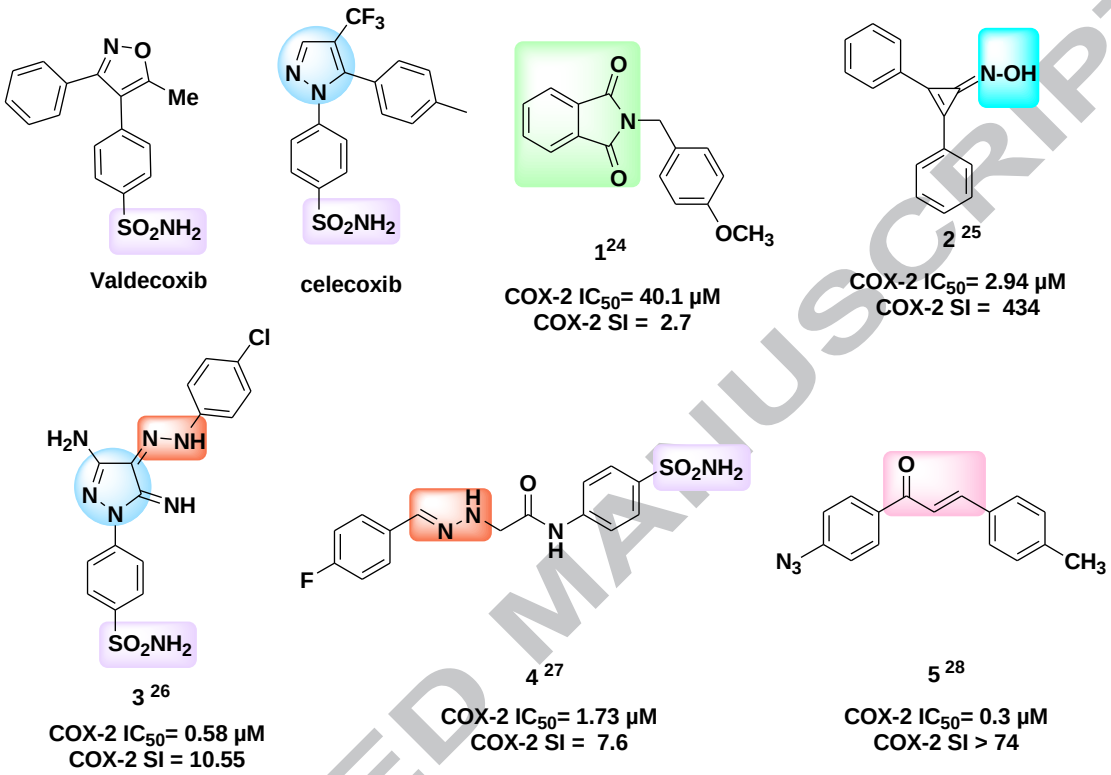
Table 1. *In vitro* COX-1 and COX-2 enzyme inhibition of compounds (**9-14**), celecoxib, diclofenac and indomethacin

Table 2. Anti-inflammatory activity of test compounds (**9- 14**) and reference drug diclofenac

Table 3: Analgesic effect of compounds (**9-14**) compared to reference drug piroxicam on thermal pain induced by hot plate in mice

Table 4. Docking study data for SC-558 and newly synthesized compounds (**9-14**) into COX-2 active site

Figure 1. Chemical structures of reported anti-inflammatory compounds as selective COX-2 inhibitors: valdecoxib, celecoxib, isoindoline (**1**), oxime (**2**), hydrazono-pyrazole (**3**), hydrazone (**4**) and chalcone (**5**) derivatives



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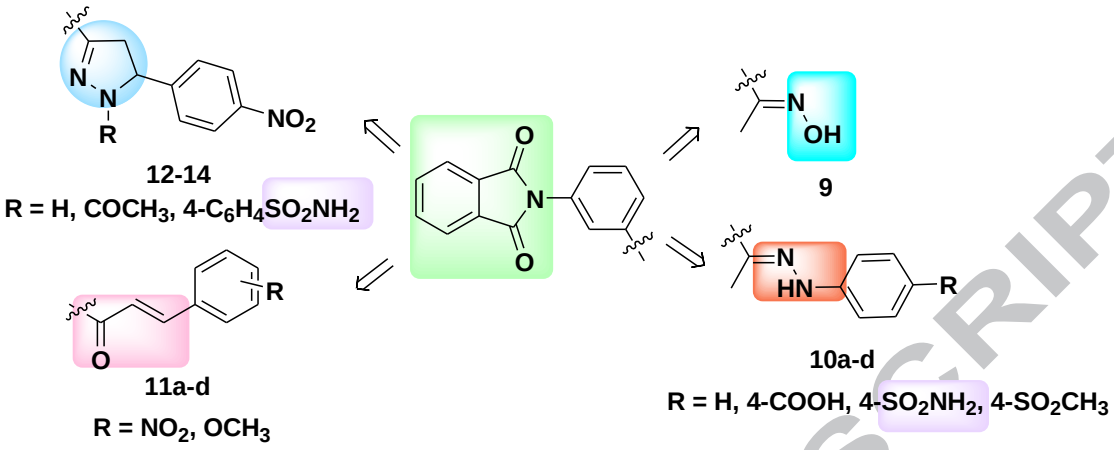
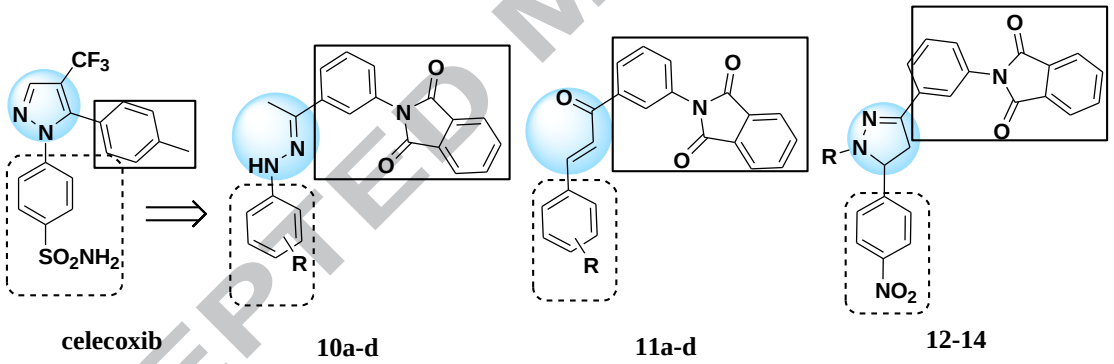


Figure 2B. Structural similarity between celecoxib and designed compounds .



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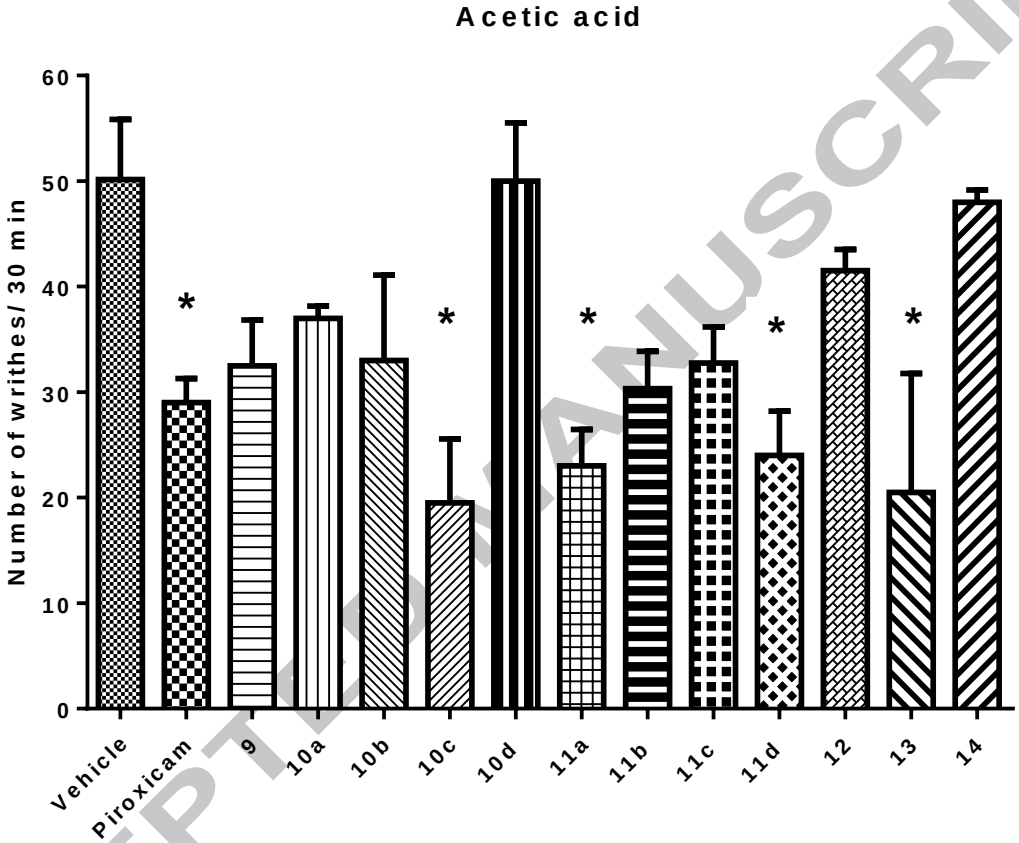


***** Significantly different from normal control group at $p < 0.05$.

Figure 4. **a)** 2D interaction and **b)** 3D interaction of ligand SC-558 inside COX-2 enzyme active site

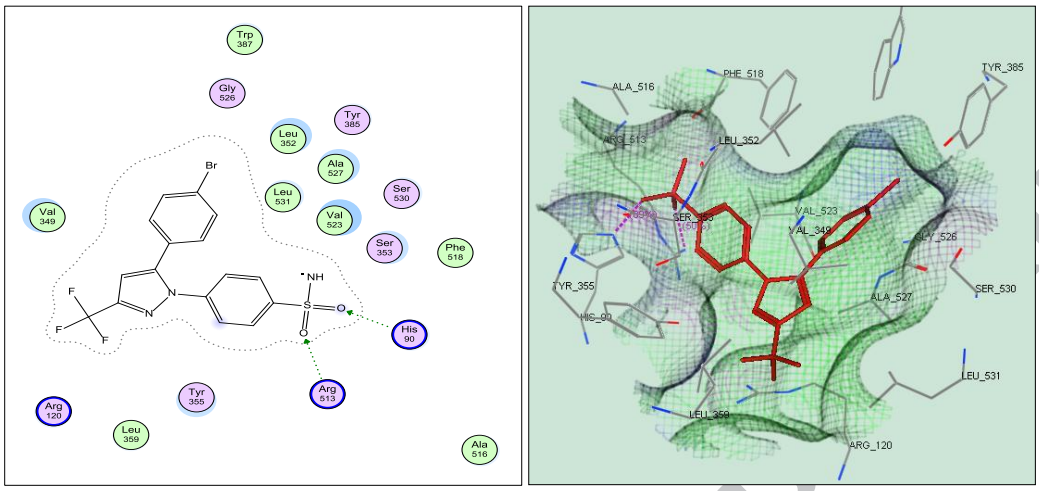
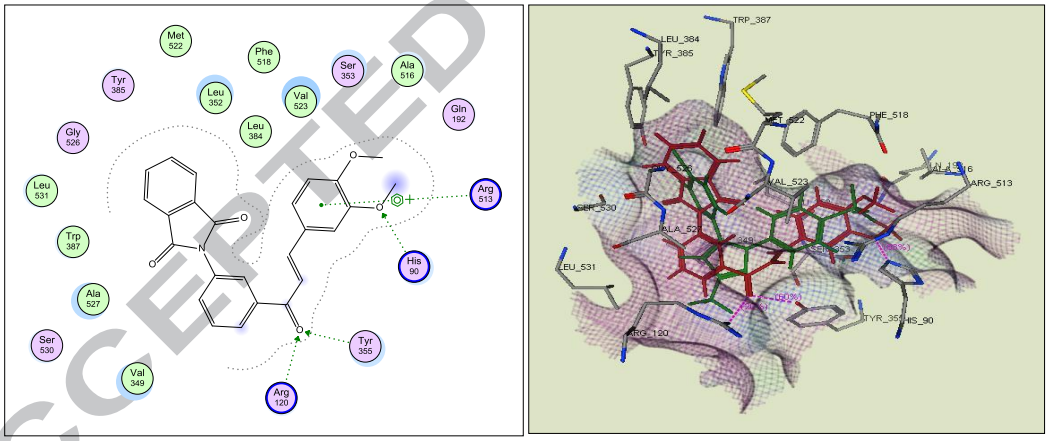
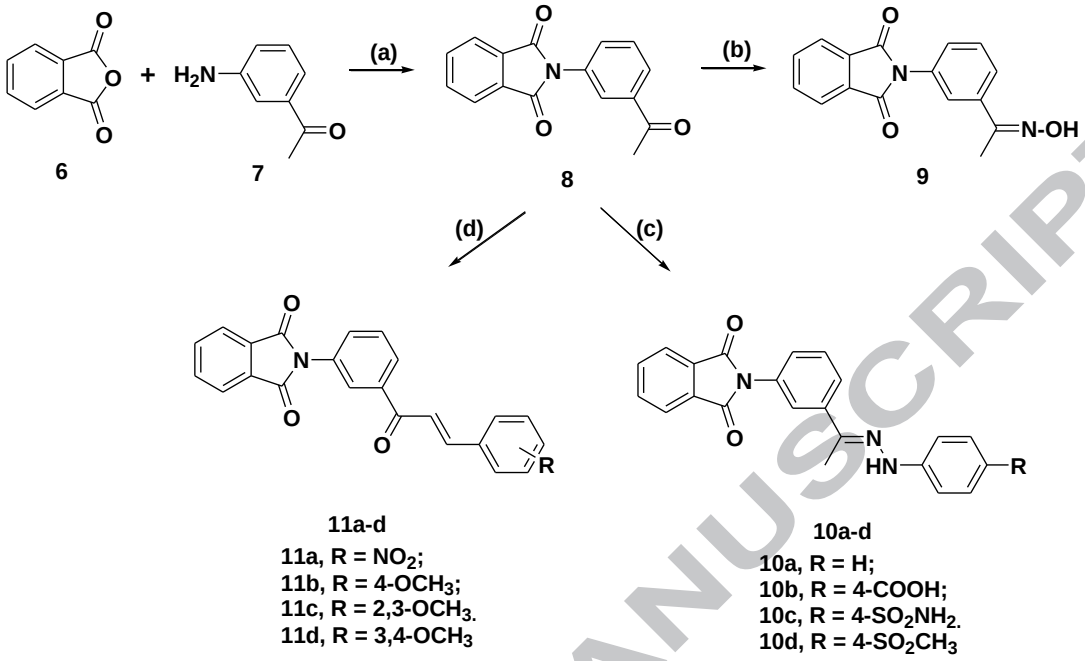


Figure 5. **a)** 2D interaction of the proposed binding mode and **b)** 3D interaction of compound **11d** (shown in green) facing the ligand (shown in red) inside COX-2 enzyme active site

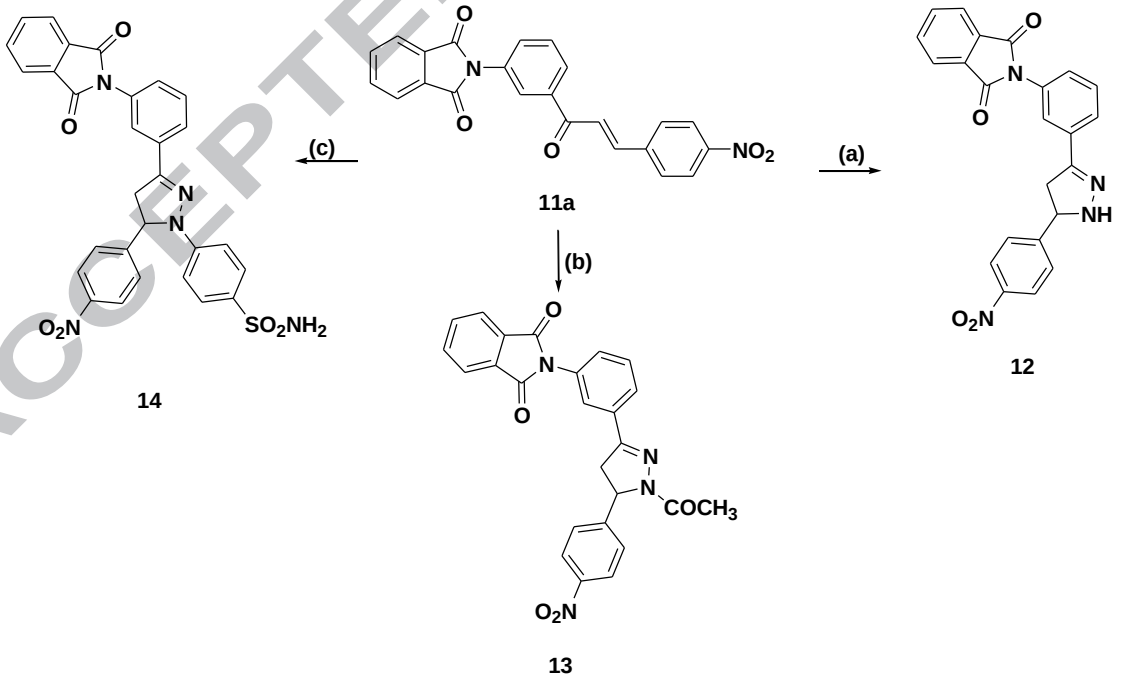


Scheme 1. Synthesis of compounds (**8, 9, 10a-d** and **11a-d**)



Scheme 1. *Reagents and conditions:* (a) glacial acetic acid, reflux, 12 h; (b) EtOH, $NH_2OH.HCl$, reflux, 2 h; (c) EtOH, phenylhydrazine hydrochloride derivatives, reflux, 14 h; (d) MeOH, KOH, ArCHO, r.t., 24 h.

Scheme 2: Synthesis of compounds **(12, 13** and **14**)



Scheme 2. *Reagents and conditions:* (a) EtOH, N_2H_4, reflux, 8 h; (b) glacial acetic acid, N_2H_4, reflux, 8 h; (c) EtOH, 4-hydrazinylbenzenesulfonamide hydrochloride , reflux, 12 h.

Table 1. *In vitro* COX-1 and COX-2 enzyme inhibition of compounds (**9-14**), celecoxib, diclofenac and indomethacin

Compound no	IC_{50} (μM)[a]		COX-2 SI[b]
	COX-1	COX-2	
Celecoxib	15.1	0.09	167.78
Diclofenac sodium	5.1	0.84	6.07
Indomethacin	0.04	0.51	0.08
9	7.42	0.26	28.53
10a	8.97	0.24	37.37
10b	11.33	0.17	66.6
10c	10.23	0.13	78.69
10d	6.98	0.38	18.36
11a	10.42	0.12	86.83
11b	9.34	0.29	32.2
11c	7.65	0.34	22.5
11d	11.33	0.11	103
12	9.52	0.36	26.4
13	11.21	0.11	101.9
14	9.23	0.18	51.27

[a] *In vitro* test compound concentration that produce 50% inhibition of COX-1and COX-2 enzymes, the result (IC_{50}, μM) is the mean of two values obtained using an ovine COX-1/COX-2 assay Kits (Cayman Chemicals Inc., Ann Arbor, MI, USA). the deviation from the mean is <10% of the mean value.

[b] The *in vitro* COX-2 selectivity index (COX-1 IC_{50}/COX-2 IC_{50}).

Table 2. Anti-inflammatory activity of test compounds (**9- 14**) and reference drug diclofenac

	Paw volume change (mL)			% Edema inhibition		
Treatment	**1 h**	**3 h**	**6 h**	**1 h**	**3 h**	**6 h**
9	1.8 ± 0.30	2.3 ± 0.20	2.8 ± 0.43	25.00	14.80	06.70
10a	1.6 ± 0.02*	2.0 ± 0.35	2.8 ± 0.28	33.30	25.90	06.70
10b	1.4 ± 0.10*	1.5 ± 0.16*	2.4 ± 0.16	41.70	44.40	20.00
10c	1.2 ± 0.08*	0.9 ± 0.13*	1.7 ± 0.20*	50.00	67.40	43.30
10d	2.2 ± 0.22	2.1 ± 0.27	2.5 ± 0.18	8.30	22.20	16.70
11a	1.4 ± 0.10 *	1.4 ± 0.06*	1.6 ± 0.36*	41.70	48.10	46.70
11b	2.1 ± 0.15	1.8 ± 0.13	2.1 ± 0.18*	12.50	33.30	30.00
11c	1.6 ± 0.11*	1.7 ± 0.16*	2.1 ± 0.17	33.30	37.00	30.00
11d	1.3 ± 0.09*	1.1 ± 0.13*	1.6 ± 0.36*	45.80	59.30	46.70
12	1.8 ± 0.06	1.9 ± 0.34	2.1 ± 0.26*	25.00	29.60	30.00
13	1.3 ± 0.04*	1.1 ± 0.13*	1.8 ± 0.2*	45.80	59.30	40.00
14	1.2 ± 0.20*	1.6 ± 0.27*	2.1 ± 0.05*	50.00	40.70	30.00
Vehicle	2.4 ± 0.30	2.7 ± 0.09	3.0 ± 0.37	0	0	0
Diclofenac	1.7 ± 0.20*	2.1 ± 0.34	2.4 ± 0.46	29.20	22.20	20.00

Values are means ± SEM (n = 3–4).

Table 3: Analgesic effect of compounds (**9-14**) compared to reference drug piroxicam on thermal pain induced by hot plate in mice

		1 h		2 h	
Treatments	**Basal**	**Latency time (s)***	**% latency change**	**Latency time (s)***	**% latency change**
Saline	19 ± 5.1	18 ± 5.8	0	16 ± 1.2	0
Piroxicam	16 ± 5.1	28 ± 2.4*	75.00	27 ± 2.6*	68.75
9	19 ± 7.1	23 ± 3.3	21.05	23 ± 5.4	21.05
10a	21 ± 3.6	29 ± 4.7*	38.10	28 ± 5.2*	33.33
10b	17 ± 4.2	24 ± 4.2	41.18	24 ± 7.3	41.18
10c	20 ± 4.0	29 ± 2.5*	45.00	27 ± 3.9*	35.00
10d	15 ± 4.8	18 ± 1.0	20.00	17 ± 1.0	13.33
11a	20 ± 7.0	29 ± 1.5*	45.00	27 ± 3.2*	35.00
11b	16 ± 4.6	18 ± 2.4	25.00	14 ± 1.7	25.00
11c	14 ± 3.9	20 ± 2.4*	42.85	17 ± 2.4*	21.42
11d	14 ± 3.6	27 ± 1.0*	92.85	21 ± 2.2*	50.00
12	23 ± 7.l	29 ± 1.0*	26.10	27 ± 3.9*	17.39
13	16 ± 4.0	28 ± 1.8*	75.00	24 ± 6.5*	50.00
14	20 ± 2.3	29 ± 1.0*	45.00	29 ± 1.0*	45.00

*Significantly different from normal control group at $p < 0.05$.

Values are means ± SD of four mice per group. Statistical comparisons between basal and post-drug values were analyzed for statistical significance using one-way ANOVA test followed by Dunnett's test and denoted by $p < 0.05$.

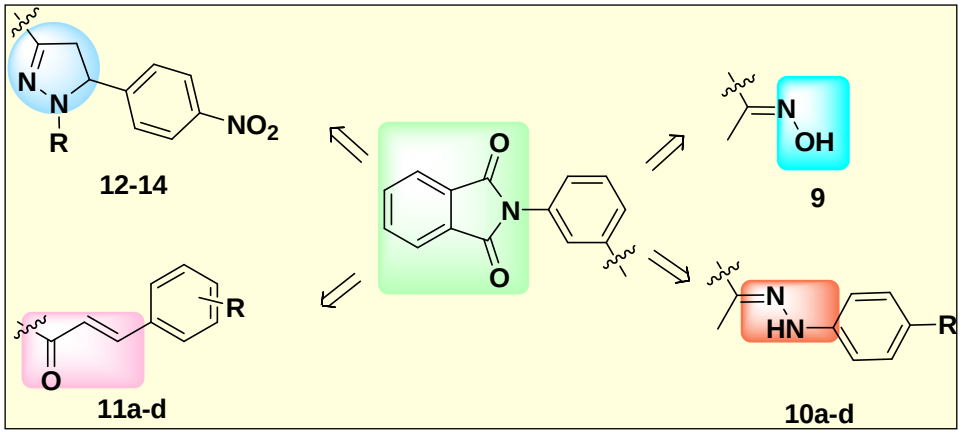
Table 4. Docking study data for SC-558 and newly synthesized compounds (**9-14**) into COX-2 active site

Compound no.	E- Score (Kcal/mol)	H-bonds residue	Distance (A^{o})	Arene-cation interaction
9	-11.2962	Arg-513	2.68	--
10a	-12.3229	Arg-513	2.29	--
10b	-16.4148	His-90	2.44	--
10c	-15.7132	His-90	2.92	--
10d	-13.6194	His-90	2.53	--
11a	-15.7074	Tyr-355 Arg-513	2.95 2.84	--
11b	-13.9021	Arg-513	2.13	His-90, Isoindole ring
11c	-14.9380	Tyr-355 His-90	2.57 2.78	Arg-513, dimethoxyphenyl ring
11d	-17.4061	Arg-120 Tyr-355 His-90	2.54 2.39 2.32	Arg-513, dimethoxyphenyl ring
12	-14.7760	Tyr-355	2.75	His-90, nitrophenyl ring
13	-15.9909	Arg-513	2.47	His-90, Isoindole ring
14	-16.8758	Tyr-355	2.63	Arg-513, nitrophenyl ring
SC-558	-15.6068	His-90 Arg-513	2.35 2.47	--

GRAPHICAL ABSTRACT

Design, synthesis of novel isoindoline hybrids as COX-2 inhibitors: anti-inflammatory, analgesic activities and docking study

Madlen B. Labib[1,*], Souty M.Z. Sharkawi[2] and Mahmoud El-Daly[3]



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Highlights

- Novel isoindoline-1,3-dione hybrids were designed and synthesized
- Designed compounds were screened for COXs inhibition and were more selective towards COX-2
- Six hybrid derivatives showed high potency as anti-inflammatory agents over diclofenac
- Most compounds displayed promising peripheral and/or central analgesic activities