Med Chem Res (2014) 23:2676–2689
DOI 10.1007/s00044-013-0848-1

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

# Synthesis, characterization, and computational studies on phthalic anhydride-based benzylidene-hydrazide derivatives as novel, potential anti-inflammatory agents

**Anu Kajal · Suman Bala · Sunil Kamboj · Vipin Saini**

Received: 19 July 2013 / Accepted: 14 October 2013 / Published online: 31 October 2013


**Abstract** A series of phthalic anhydride-based substituted benzylidene-hydrazide derivatives (**3a–i**) was synthesized. The synthesized derivatives were authenticated by TLC, UV–visible, FTIR, NMR, and mass spectroscopic techniques and further screened for in vivo anti-inflammatory and analgesic activities by carrageenan-induced rat paw oedema and tail immersion methods, respectively, using diclofenac sodium as standard drug. The derivatives **3d**, **3e**, and **3h** were found to be most active anti-inflammatory and analgesic agents among all the synthesized derivatives. The physico-chemical similarity of the derivatives with standard drugs was assessed by calculating various physicochemical properties using software programs. The percent similarity of synthesized derivatives was found to be good except **3i**. The derivatives were subjected to QSAR by multilinear regression using Analyze it version 3.0 software and two statistically sound models were developed with $R^2$ (0.933–0.960), $R^2_{adj}$ (0.595–0.762) and $Q^2$ (0.999) with good $F$ (2.76–4.84) values. Molecular docking studies were performed by MVD software (version 2012.5.0.0). The derivative **3h** has emerged out as most potent anti-inflammatory agent with highest dock score, i.e., **−93.64**.

**Keywords** Phthalic anhydride · Benzylidene-hydrazide · Analgesic activity · Anti-inflammatory activity · QSAR · Molecular docking

A. Kajal · S. Bala (✉) · S. Kamboj · V. Saini
M. M. College of Pharmacy, Maharishi Markandeshwar University, Mullana, Ambala, Haryana 133207, India
e-mail: sumankmj7@gmail.com

## Introduction

Cyclooxygenase (COX) is the key enzyme in the synthesis of main mediators (prostaglandins) of inflammation and algesia (Botting, 2006). Inflammation is considered as a protective response of our body to prevent infections and by combating the foreign substances by releasing mediators (Borne, 2002). COX enzyme has two isoforms: COX-1 and COX-2. COX-2 is an inducible enzyme and most of the classical NSAIDs actually inhibit COX-1 instead of COX-2 which results in gastrointestinal injury by gastric ulceration, suppression of $TXA_2$ formation and platelet aggregation (Husain *et al.*, 2005). The gastric ulceration is due to free carboxylic group in parent drugs (Akhter *et al.*, 2010). Studies suggest that the derivatization of carboxylic functionality with hydrazone moiety in compounds have such a pharmacophoric effect which leads to inhibition of COX with minimal side effects like ulcerogenicity (Hunashal, 2011; Mehtap *et al.*, 2009).

Within the scope of rational drug design, computational methods have gain more and more importance to design work flows that are faster, more efficient, and cheaper. Computational studies have been developed as important contributors in drug designing. The term quantitative structure–activity relationship (QSAR) implies the empirical relationships that use molecular parameters to quantify a pharmacological or chemical property for a set of molecules. These studies permit complex biological systems to be modeled successfully using simple structural parameters and to predict substituent effects for a series of biologically active compounds (Wang *et al.*, 2006).

Docking is defined as a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex (Kitchen *et al.*, 2004). A main principle in drug discovery and

development is the interaction between receptors and enzymes with their ligands (Nunez *et al.*, 2012). Docking of a ligand is typically achieved by generating a number of orientations (or poses) of a ligand within the active site and scoring of poses, to identify one or more that closely approximate the bioactive conformation determined by X-ray crystallography (Regina *et al.*, 2008). This is a computational method to determine possible binding modes of a ligand to the active site of a receptor. It makes an image of the active site with interaction points known as grid. Then it fits the ligand in the binding site either by grid search or energy search.

The present work describes the synthesis of benzylidene-hydrazide derivatives and focused on the investigation of anti-inflammatory and analgesic activities of synthesized derivatives. Additional efforts have been made for computational studies which include QSAR studies and in silico docking studies to the target COX-2 enzyme.

## Materials and methods

### Experimental

Melting points of newly synthesized benzylidene-hydrazide derivatives were determined on digital melting point apparatus (Flora; Perfit India) and were found uncorrected. Silica gel G plates of 3 × 15 cm were used for TLC and spots were located by iodine chamber (Table 1). The structures of the synthesized derivatives were confirmed by spectral data. The $\lambda_{max}$ was calculated by using double beam UV–visible 1800 Shimadzu spectrophotometer. The IR spectra were recorded on FTIR-Shimadzu spectrometer using Nujol method. $^{1}$H NMR and $^{13}$C NMR spectra were recorded on BRUKER AVANCE II 400 NMR spectrometer using DMSO as solvent and TMS as internal standard, values were expressed in δ ppm. Mass spectra were obtained with Vg-11-250 J70S spectrophotometer at 70 eV using electron ionization (EI source). For mass spectra, solutions were made in HPLC grade methanol. Structural similarity studies were performed by using Chem 3D Ultra (version 10) (Nikolova and Jaworska, 2003). QSAR studies were performed by multilinear regression using Analyze it version 3.0 software. Molecular docking studies were performed by Molegro Virtual Docker software (version 2012.5.0.0).

### General methods

A series of phthalic anhydride-based substituted benzylidene-hydrazide derivatives were synthesized as outlined in Fig. 1. Phthalic anhydride and glycine were used as starting materials. First of all, acid was synthesized by fusion of both the starting materials. The acid was then subjected to chlorination by using thionyl chloride. After chlorination, ester (**1**) was synthesized by reacting with ethanol. The ester so formed was reacted with hydrazine hydrate in presence of ethanol to get corresponding hydrazide (**2**). The hydrazide derivative was then reacted with different substituted aromatic aldehydes in presence of methanol and glacial acetic acid to yield the 2-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-*N*'-(substituted benzylidene)acetohydrazide derivatives (**3a–i**). The synthesized benzylidene-hydrazide derivatives were characterized on the basis of the spectral and analytical studies (Salimon *et al.*, 2010; Mehtap *et al.*, 2009; Bhandari *et al.*, 2008; Ahluwalia and Aggarwal, 2000).

#### *(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)acetic acid*

A mixture of phthalic anhydride (0.06 M) and glycine (0.06 M) was placed in a 100-ml round bottom flask along with calcium chloride drying tube. The reaction mixture was fused for 20–30 min at 160–190 °C. The product

**Table 1** Physical data of synthesized compounds

| Compounds | Molecular formula | %Age yield | Molecular weight | Solubility | $\lambda_{max}$ | $R_f$ value |
|---|---|---|---|---|---|---|
| **1** | $C_{12}H_{11}NO_4$ | 91.8 | 233.22 | DMSO, EtOH, $CHCl_3$ | 299 | 0.78 |
| **2** | $C_{10}H_9N_3O_3$ | 65.7 | 219.2 | DMSO, EtOH, $CHCl_3$ | 261 | 0.31 |
| **3a** | $C_{17}H_{13}N_3O_3$ | 90.2 | 307.31 | DMSO, EtOH, $CHCl_3$ | 232 | 0.41 |
| **3b** | $C_{17}H_{12}N_4O_5$ | 88.4 | 352.308 | DMSO, EtOH, $CHCl_3$ | 271 | 0.39 |
| **3c** | $C_{17}H_{12}N_4O_5$ | 80.3 | 352.308 | DMSO, EtOH, $CHCl_3$ | 260 | 0.40 |
| **3d** | $C_{17}H_{12}ClN_3O_3$ | 90.0 | 341.755 | DMSO, EtOH, $CHCl_3$ | 259 | 0.38 |
| **3e** | $C_{18}H_{15}N_3O_4$ | 89.9 | 337.337 | DMSO, EtOH, $CHCl_3$ | 254 | 0.39 |
| **3f** | $C_{17}H_{13}N_3O_4$ | 78.8 | 323.31 | DMSO, EtOH, $CHCl_3$ | 328 | 0.50 |
| **3g** | $C_{18}H_{15}N_3O_3$ | 75.9 | 321.337 | DMSO, EtOH, $CHCl_3$ | 261 | 0.41 |
| **3h** | $C_{18}H_{15}N_3O_5$ | 83.6 | 353.336 | DMSO, EtOH, $CHCl_3$ | 248 | 0.40 |
| **3i** | $C_{20}H_{19}N_3O_6$ | 79.4 | 397.389 | DMSO, EtOH, $CHCl_3$ | 292 | 0.36 |

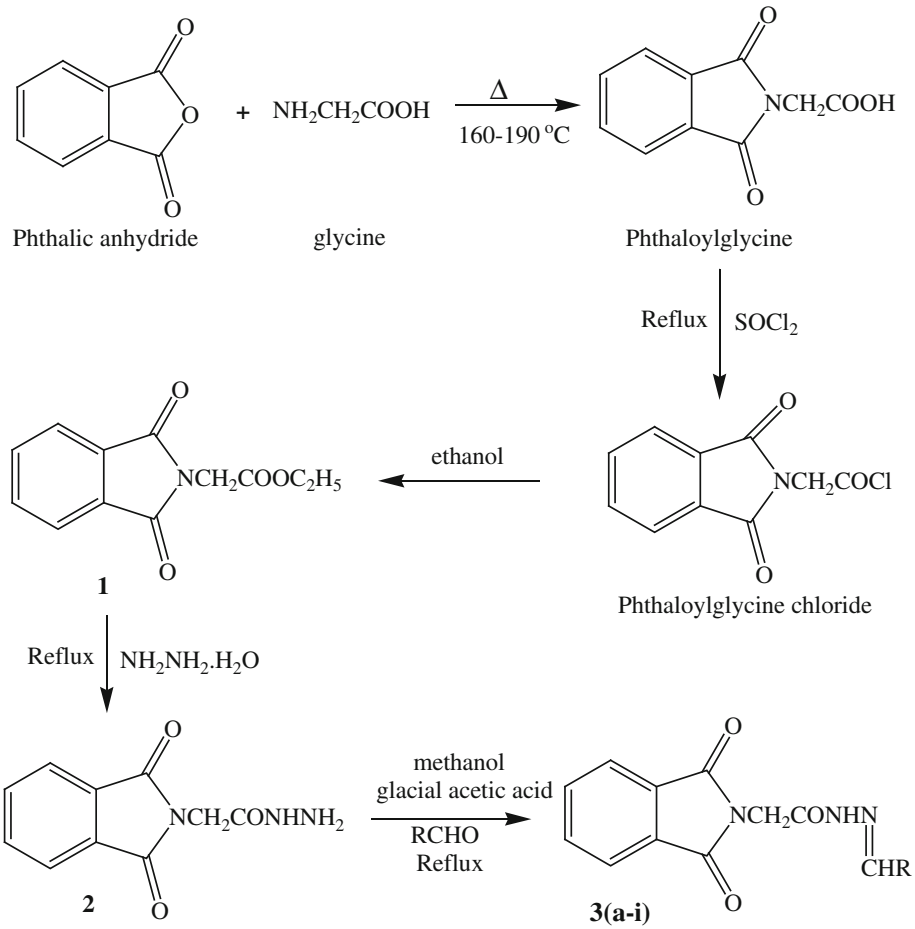



**Fig. 1** Synthesis of (1,3-dioxo-1,3-dihydro-isoindol-2-yl)-acetic acid (substituted benzylidene)-hydrazide derivatives (**3a**–**i**)

obtained was cooled and crystallized from water. $^{1}$H NMR (DMSO-$d_6$ 400 MHz): 11.0 (s, 1H, OH), 7.99 (m, 2H, Ar–H), 7.55 (m, 2H, Ar–H), 4.52 (s, 2H, –$CH_2$); $^{13}$C NMR (DMSO-$d_6$); 167.0 (C-2, 5), 131.9 (C-3,4), 127.8 (C-6,9), 132.0 (C-7,8), 47.2 (C-10), 176.0 (C-11).

*(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)acetyl chloride*

(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)acetic acid (0.039 M) was placed in 250-ml round bottom flask then thionyl chloride (16 ml) was added carefully in the flask and reflux gently for 30–40 min having a calcium chloride drying tube on the top. Contents of the flask were shaken from time-to-time to ensure thorough mixing. Excess thionyl chloride was removed by distillation under reduced pressure. The residual phthaloylglycine chloride was crystallized from petroleum ether. $^{1}$H NMR (DMSO-$d_6$ 400 MHz): 7.92 (m, 2H, Ar–H), 7.45 (m, 2H, Ar–H), 5.18 (s, 2H, –$CH_2$); $^{13}$C NMR (DMSO-$d_6$ 100 MHz); 166.7 (C-2, 5), 131.3 (C-3,4), 127.0 (C-6,9), 132.1 (C-7,8), 58.4 (C-10), 172.5 (C-11).

*Ethyl(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)acetate (**1**)*

Phthaloylglycine chloride obtained was dissolved in ethanol. The mixture was slightly warmed and filtered immediately. Filterate was kept in undisturbed condition for the formation of (**1**). Mp 78–80 °C. IR (Nujol): 3011.93, 2832.59, 1737.94, 1480.43, 1405.20, 1329.20, 1216.17, 705.01 cm$^{-1}$; $^{1}$H NMR (DMSO-$d_6$ 400 MHz): 7.91 (m, 2H, Ar–H), 7.38 (m, 2H, Ar–H), 4.48 (s, 2H, –$CH_2$), 4.12 (s, 2H, –$CH_2$), 1.30 (t, 3H, –$CH_3$); $^{13}$C NMR (DMSO-$d_6$); 166.9 (C-2, 5), 132.3 (C-3,4), 127.4 (C-6,9), 132.9 (C-7,8), 44.9 (C-10), 171.0 (C-11), 59.2 (C-12), 13.6 (C-13).

*2-(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)acetohydrazide (**2**)*

This compound was prepared by refluxing hydrazine hydrate (0.05 M) with (**1**) (0.05 M) in ethanol (15 ml) at 75–80 °C for 2–3 h. The reaction was monitored by TLC. The white colored crystals were separated out by filtration on cooling and recrystallized from ethanol. Mp 240–245 °C. IR (Nujol): 3252.12, 3052.48, 2871.12,

1727.33, 1540.23, 1500.68, 1305.86, 854.50 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$ 400 MHz): 8.21 (br, s, 1H, –NH), 7.82 (m, 2H, Ar–H), 7.76 (m, 2H, Ar–H), 4.45 (s, 2H, $–CH_2$), 3.8 (br, s, 2H, $–NH_2$); $^{13}C$ NMR (DMSO-$d_6$); 166.4 (C-2, 5), 132.0 (C-3,4), 127.1 (C-6,9), 132.0 (C-7,8), 47.3 (C-10), 170.3 (C-11).

*N'-Benzylidene-2-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)acetohydrazide (**3a**)*

Hydrazide (**2**) (0.002 M) was dissolved in methanol (15 ml). To this benzaldehyde (0.002 M) and a few drops of glacial acetic acid were added and mixture was refluxed for 2 h. The reaction was monitored by TLC. The white-colored crude product was filtered out and recrystallized by ethanol. Mp 243–246 °C. IR(Nujol): 3256.63, 3056.34, 2838.12, 1758.19, 1658.54, 1544.08, 1502.61, 1023.28, 860.29 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$ 400 MHz): 9.62 (s, 1H, –NH–N=), 8.21 (s, 1H, –N=CH-), 7.84 (m, 2H, Ar–H), 7.78 (m, 2H, Ar–H), 7.59 (m, 2H, Ar–H), 7.46 (m, 3H, Ar–H), 4.56 (s, 2H, $–CH_2$); $^{13}C$ NMR (DMSO-$d_6$); 167.9 (C-2, 5), 132.3 (C-3,4), 127.4 (C-6,9), 132.9 (C-7,8), 47.4 (C-10), 173.0 (C-11), 154.7 (C-17), 131.2 (C-18), 129.0 (C-19,23), 128.6 (C-20,22), 130.8 (C-23); MS: *m/z* 307.10, 308.10 (M+1), 309.10 (M+2).

*2-(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)-N'-(4-nitrobenzylidene)acetohydrazide (**3b**)*

Hydrazide (**2**) (0.002 M) was dissolved in methanol (15 ml). To this 4-nitrobenzaldehyde (0.002 M) and a few drops of glacial acetic acid was added and mixture was refluxed for 3 h. The reaction was monitored by TLC. The pale yellow-colored crude product was filtered out and recrystallized by ethanol. Mp 245–247 °C. IR(Nujol): 3302.06, 3056.34, 2830.16, 1755.30, 1662.30, 1656.92, 1585.55, 1544.08, 1322.26, 1302.01, 822.68 $cm^{-1}$; $^1H$ NMR(DMSO-$d_6$ 400 MHz): 9.68 (s, 1H, –NH–N=), 8.39 (d, 2H, Ar–H, $J = 8.3$ Hz), 7.98 (s, 1H, –N=CH–), 7.9 (m, 2H, Ar–H), 7.7 (m, 2H, Ar–H), 7.32 (d, 2H, Ar–H, $J = 8.3$ Hz), 4.75 (s, 2H, $–CH_2$); $^{13}C$ NMR (DMSO-$d_6$); 166.4 (C-2,5), 132.5 (C-3,4), 127.4 (C-6,9), 132.0 (C-7,8), 47.6 (C-10), 173.0 (C-11), 154.3 (C-17), 137.3 (C-18), 129.9 (C-19,23), 123.7 (C-20,22), 150.7 (C-21); MS: *m/z* 352.08, 353.08 (M+1), 354.09 (M+2).

*2-(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)-N'-(2-nitrobenzylidene)acetohydrazide (**3c**)*

Hydrazide (**2**) (0.002 M) was dissolved in methanol (15 ml). To this 2-nitrobenzaldehyde (0.002 M) and a few drops of glacial acetic acid was added and mixture was refluxed for 1.5 h. The reaction was monitored by TLC. The orange-colored crude product was filtered out and recrystallized by ethanol. Mp 246–248 °C. IR(Nujol): 3270.21, 3055.38, 2872.61, 1776.08, 1642.46, 1638.09, 1537.33, 1530.58, 1503.54, 1305.86, 1303.94, 859.32 $cm^{-1}$; $^1H$ NMR: 9.59 (s, 1H, –NH–N=), 8.2 (m, 1H, Ar–H), 8.01 (s, 1H, –N=CH–), 7.89 (m, 2H, Ar–H), 7.78 (m, 2H, Ar–H), 7.75 (m, 1H, Ar–H), 7.64 (m, 1H, Ar–H), 7.53 (m, 1H, Ar–H), 4.68 (s, 2H,-$CH_2$); $^{13}C$ NMR (DMSO-$d_6$); 166.2 (C-2, 5), 132.5 (C-3,4), 127.1 (C-6,9), 132.9 (C-7,8), 47.4 (C-10), 173.1 (C-11), 154.7 (C-17), 126.3 (C-18), 129.9 (C-19), 134.7 (C-20), 131.7 (C-21), 123.7 (C-22), 148.9 (C-23); MS: m/z 352.08, 353.08 (M+1), 354.09 (M+2).

*2-(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)-N'-(4-chlorobenzylidene)acetohydrazide (**3d**)*

Hydrazide (**2**) (0.002 M) was dissolved in methanol (15 ml). To this 4-chlorobenzaldehyde (0.002 M) and a few drops of glacial acetic acid was added and mixture was refluxed for 3–4 h. The reaction was monitored by TLC. The white-colored crude product was filtered out and recrystallized by ethanol. Mp 237–239 °C. IR(Nujol); 3320.61, 3053.45, 2871.15, 1776.52, 1647.58, 1630.61, 1538.30, 1530.58, 1306.83, 862.22, 667.44 $cm^{-1}$; $^1H$ NMR: 9.72 (s, 1H, –NH–N=), 7.98 (s, 1H, –N=CH–), 7.92 (m, 2H, Ar–H), 7.79 (m, 2H, Ar–H), 7.5 (d, 2H, Ar–H, $J = 8.2$ Hz), 7.41 (d, 2H, Ar–H, $J = 8.2$ Hz), 4.48 (s, 2H, $–CH_2$); $^{13}C$ NMR (DMSO-$d_6$); 166.2 (C-2, 5), 132.5 (C-3,4), 127.1 (C-6,9), 132.9 (C-7,8), 47.4 (C-10), 173.1 (C-11), 154.7 (C-17), 129.3 (C-18), 130.4 (C-19,23), 129.0 (C-20,22), 136.1 (C-21); MS: *m/z* 341.06, 343.05 (M+1), 342.06 (M+2).

*2-(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)-N'-(3-methoxybenzylidene)acetohydrazide (**3e**)*

Hydrazide (**2**) (0.002 M) was dissolved in methanol (15 ml). To this 3-methoxybenzaldehyde (0.002 M) and a few drops of glacial acetic acid was added and mixture was refluxed for 4 h. The reaction was monitored by TLC. The white-colored crude product was filtered out and recrystallized by ethanol. Mp 240–242 °C. IR(Nujol); 3310.16, 3054.41, 2854.69, 1763.61, 1662.52, 1636.52, 1541.19, 1235.76, 1045.19, 863.18 $cm^{-1}$; $^1H$ NMR: 9.76 (s, 1H, –NH–N=), 8.23 (s, 1H, –N=CH–), 7.9 (m, 2H, Ar–H), 7.74 (m, 2H, Ar–H), 7.21 (m, 1H, Ar–H), 7.11 (m, 1H, Ar–H), 6.99 (m, 1H, Ar–H), 6.79 (m, 1H, Ar–H), 4.56 (s, 2H, $–CH_2$), 3.82 (s, 3H, $–OCH_3$); $^{13}C$ NMR (DMSO-$d_6$); 166.2 (C-2, 5), 132.5 (C-3,4), 127.1 (C-6,9), 132.9 (C-7,8), 47.4 (C-10), 173.1 (C-11), 154.7 (C-17), 132.2 (C-18), 114.6 (C-19), 162.1 (C-20), 116.4 (C-21), 129.6 (C-22), 121.3 (C-23), 56.0 (C-24); MS: *m/z* 337.11 338.11 (M+1), 339.11 (M+2).

*2-(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)-N'-(4-hydroxybenzylidene)acetohydrazide (**3f**)*

Hydrazide (**2**) (0.002 M) was dissolved in methanol (15 ml). To this 4-hydroxybenzaldehyde (0.002 M) and a few drops of glacial acetic acid was added and mixture was refluxed for 4.5 h. The reaction was monitored by TLC. The off white-colored crude product was filtered out and recrystallized by ethanol. Mp 241–244 °C. IR(Nujol): 3627.29, 3261.77, 3131.57, 2844.49, 1770.64, 1645.35, 1630.61, 1533.63, 1350.27, 860.29 $cm^{-1}$; $^{1}H$ NMR: 9.71 (s, 1H, –NH–N=), 8.25 (s, 1H, –N=CH–), 7.98 (m, 2H, Ar–H), 7.81 (m, 2H, Ar–H), 7.31 (d, 2H, Ar–H, $J = 8.1$ Hz), 6.64 (d, 2H, Ar–H, $J = 8.1$ Hz), 5.2 (s, 1H, –OH), 4.52 (s, 2H, $–CH_2$); $^{13}C$ NMR (DMSO-$d_6$); 166.2 (C-2, 5), 132.5 (C-3, 4), 127.1 (C-6, 9), 132.9 (C-7,8), 47.4 (C-10), 173.1 (C-11), 154.7 (C-17), 123.8 (C-18), 130.4 (C-19,23), 115.8 (C-20,22), 159.6 (C-21); MS: *m/z* 323.09, 324.09 (M+1), 325.10 (M+2).

*2-(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)-N'-(4-methylbenzylidene)acetohydrazide (**3g**)*

Hydrazide (**2**) (0.002 M) was dissolved in methanol (15 ml). To this 4-methylbenzaldehyde (0.002 M) and a few drops of glacial acetic acid was added and mixture was refluxed for 3 h. The reaction was monitored by TLC. The pale yellow-colored crude product was filtered out and recrystallized by ethanol. Mp 242–243 °C. IR(Nujol): 3255.02, 3056.34, 2853.64, 1783.27, 1646.32, 1632.35, 1544.08, 1462.15, 1322.26, 1302.33, 862.68 $cm^{-1}$; $^{1}H$ NMR: 9.62 (s, 1H, –NH–N=), 8.14 (s, 1H, –N=CH–), 7.72 (m, 2H, Ar–H), 7.64 (m, 2H, Ar–H), 7.5 (d, 2H, Ar–H, $J = 7.8$ Hz), 7.24 (d, 2H, Ar–H, $J = 7.8$ Hz), 5.2 (s, 2H, $–CH_2$), 2.7(s, 3H, $-CH_3$); $^{13}C$ NMR (DMSO-$d_6$); 166.2 (C-2, 5), 132.5 (C-3, 4), 127.1 (C-6, 9), 132.9 (C-7,8), 47.4 (C-10), 173.1 (C-11), 154.7 (C-17), 128.2 (C-18), 128.9 (C-19,23), 129.3 (C-20,22), 140 (C-21), 20.9 (C-24); MS: *m/z* 321.11, 322.11 (M+1), 323.12 (M+2).

*2-(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)-N'-(4-hydroxy-3-methoxybenzylidene)acetohydrazide (**3h**)*

Hydrazide (**2**) (0.002 M) was dissolved in methanol (15 ml). To this 4-hydroxy-3-methoxy benzaldehyde (0.002 M) and a few drops of glacial acetic acid was added and mixture was refluxed for 6 h. The reaction was monitored by TLC. The white-colored crude product was filtered out and recrystallized by ethanol. Mp 248–249 °C. IR(Nujol): 3726.63, 3282.36, 3055.38, 2849.62, 1748.12, 1629.44, 1644.39, 1542.15, 1502.61,1321.30, 1238.21, 1218.21, 1042.51, 860.29 $cm^{-1}$; $^{1}H$ NMR: 9.74 (s, 1H, –NH–N=), 8.19 (s, 1H, –N=CH–), 7.96 (m, 2H, Ar–H), 7.79 (m, 2H, Ar–H), 7.10 (m, 1H, Ar–H), 6.98 (m, 1H, Ar–H), 6.72 (m, 1H, Ar–H), 5.3 (s, 1H, –OH), 4.62 (s, 2H, $–CH_2$), 3.81 (s, 3H, $–OCH_3$); $^{13}C$ NMR (DMSO-$d_6$); 165.2 (C-2,5), 132.8 (C-3,4), 129.1 (C-6,9), 132.5 (C-7,8), 45.1 (C-10), 175 (C-11), 154.9 (C-17), 124.8 (C-18), 116.0 (C-19), 149.3 (C-20), 145.2 (C-21), 116.8 (C-22), 112.7 (C-23), 56.3 (C-26); MS: *m/z* 353.10, 354.10 (M+1), 355.11 (M+2).

*2-(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl)-N'-(3,4,5-trimethoxybenzylidene)acetohydrazide (**3i**)*

Hydrazide (**2**) (0.002 M) was dissolved in methanol (15 ml). To this 3,4,5-trimethoxybenzaldehyde (0.002 M) and a few drops of glacial acetic acid was added and mixture was refluxed for 2 h. The reaction was monitored by TLC. The white-colored crude product was filtered out and recrystallized by ethanol. Mp 246–247 °C. IR(Nujol): 3330.12, 3053.45, 2843.63, 1764.22, 1647.28, 1640.81, 1538.30, 1528.65, 1248.11, 1040.32, 822.68 $cm^{-1}$; $^{1}H$ NMR: 9.68 (s, 1H, –NH–N=), 8.14 (s, 1H, –N=CH–), 7.8 (m, 2H, Ar–H), 7.73 (m, 2H, Ar–H), 6.62 (m, 2H, Ar–H), 4.43 (s, 2H, $–CH_2$), 3.8 (s, 9H, $–OCH_3$); $^{13}C$ NMR (DMSO-$d_6$); 166.2 (C-2,5), 132.3 (C-3,4), 127.4 (C-6,9), 132.0 (C-7,8), 47.6 (C-10), 173 (C-11), 154.7 (C-17), 125.7 (C-18), 107.9 (C-19,23), 148.7 (C-20,22), 135.5 (C-21), 56.3 (C-26,28), 56.6 (C-29); MS: *m/z* 397.13, 398.13 (M+1), 399.13 (M+2).

## Pharmacological evaluation

### *Anti-inflammatory activity*

The synthesized benzylidene-hydrazide derivatives (**3a–i**) were screened for anti-inflammatory activity by carrageenan-induced rat paw oedema method. In carrageenan model, rat paw edema was induced by injection of 0.1 ml of freshly prepared carrageenan (1 % w/v) in subplantar region of the left hind paw of rats and paw was marked at the level of lateral malleolus (Winter *et al.,* 1962). The different groups of rats were pre-treated with their respective doses. After 1 h, the rats were subjected to injection of carrageenan and paw volume was measured by plethysmometer (model pth-7070, sr.no.pt 070509, Medicad system) after 1, 2, and 3 h. Mean ± SEM for treated and control animal groups was calculated and compared for each time interval and statistically analyzed. Percent inhibition of inflammation after test/standard was calculated using the formula,

$$\% \text{ inhibition } = V_c - V_t/V_c \times 100, \quad (1)$$

where $V_t$ is the of paw oedema volume (ml) in test/standard compound at corresponding time, and $V_c$ is paw oedema

**Table 2** Anti-inflammatory activity of synthesized benzylidene-hydrazide derivatives

| Groups | Change in paw oedema volume (ml) | | | | | |
|---|---|---|---|---|---|---|
| | 1 h | Percent inhibition | 2 h | Percent inhibition | 3 h | Percent inhibition |
| Control | 0.56 ± 0.03 | – | 0.69 ± 0.04 | – | 0.75 ± 0.04 | – |
| Diclofenac sodium (10 mg/kg) | 0.34 ± 0.01 | 39.8 | 0.27 ± 0.02 | 60.8 | 0.24 ± 0.01 | 68.0 |
| **3a** | 0.48 ± 0.01 | 14.2 | 0.58 ± 0.05 | 15.9 | 0.62 ± 0.05 | 17.3 |
| **3b** | 0.45 ± 0.02 | 19.6 | 0.39 ± 0.03* | 43.4 | 0.34 ± 0.02* | 54.6 |
| **3c** | 0.39 ± 0.01* | 30.3 | 0.35 ± 0.01* | 49.2 | 0.32 ± 0.01* | 57.3 |
| **3d** | 0.41 ± 0.01 | 26.7 | 0.36 ± 0.01* | 47.8 | 0.31 ± 0.01* | 58.6 |
| **3e** | 0.40 ± 0.02 | 28.5 | 0.35 ± 0.01* | 49.2 | 0.29 ± 0.02* | 61.4 |
| **3f** | 0.43 ± 0.01 | 23.2 | 0.39 ± 0.02* | 43.4 | 0.37 ± 0.02* | 50.6 |
| **3g** | 0.46 ± 0.02 | 17.8 | 0.52 ± 0.03 | 24.6 | 0.56 ± 0.04 | 25.3 |
| **3h** | 0.38 ± 0.02* | 32.1 | 0.32 ± 0.01* | 53.6 | 0.27 ± 0.02* | 64.0 |
| **3i** | 0.41 ± 0.01 | 26.7 | 0.41 ± 0.02* | 40.5 | 0.38 ± 0.01* | 49.4 |

All values represent mean ± SEM ($n = 6$)

* $P < 0.05$ when experimental groups were compared with control

volume (ml) in control. The paw volume and percentage inhibition data for synthesized benzylidene-hydrazide derivatives at 1, 2, and 3 h are summarized in Table 2 and Fig. 2.

*Analgesic activity*

Analgesic activity of synthesized benzylidene-hydrazide derivatives (**3a–i**) was carried out by using tail immersion method. Swiss albino mice were divided into 11 groups each containing six animals. The lower 5-cm portion of the tail was marked. This part of the tail was immersed in a cup of freshly filled water of exactly 55 °C. Within a few seconds, the mice reacted by withdrawing the tail. Tail withdrawal from hot water was taken as end point. The dependent variable in this test is the time taken by animal to flick its tail. Normally, a mice withdraws its tail within 3 to 5 s and withdrawal time more than 5 s is considered as positive response. A cut of period of 10–12 s was observed to prevent damage to the tail. Then the animals were treated with different doses. The animals were submitted to the same procedure and reaction time was noted before and after 30, 60, and 90 min. The average values of reaction time after each time interval was calculated and compared statistically between treated groups and control group (Vogel, 2002). The relative latency of tail flick response for synthesized benzylidene-hydrazide derivatives at 30, 60, and 90 min are summarized in Table 3 and Fig. 3.

The experimental protocol was approved by Institutional Animals Ethical Committee (IAEC) and animal care was done as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Govt. of India (Protocol no. MMCP/IAEC/12/09).

*Similarity studies*

Assessments of structural similarity of benzylidene-hydrazide derivatives **3a–i** was compared to that of standard compounds. Assessment of structural similarity studies was performed by means of physicochemical similarity between the standard drugs and new analogs designed. The information was used for prediction of biological activity of important target compounds for novel drug discovery. Therefore, we calculated a number of parameters for the synthesized derivatives using Chem 3D Ultra (version 10) and compared them to the values obtained for standard compounds.

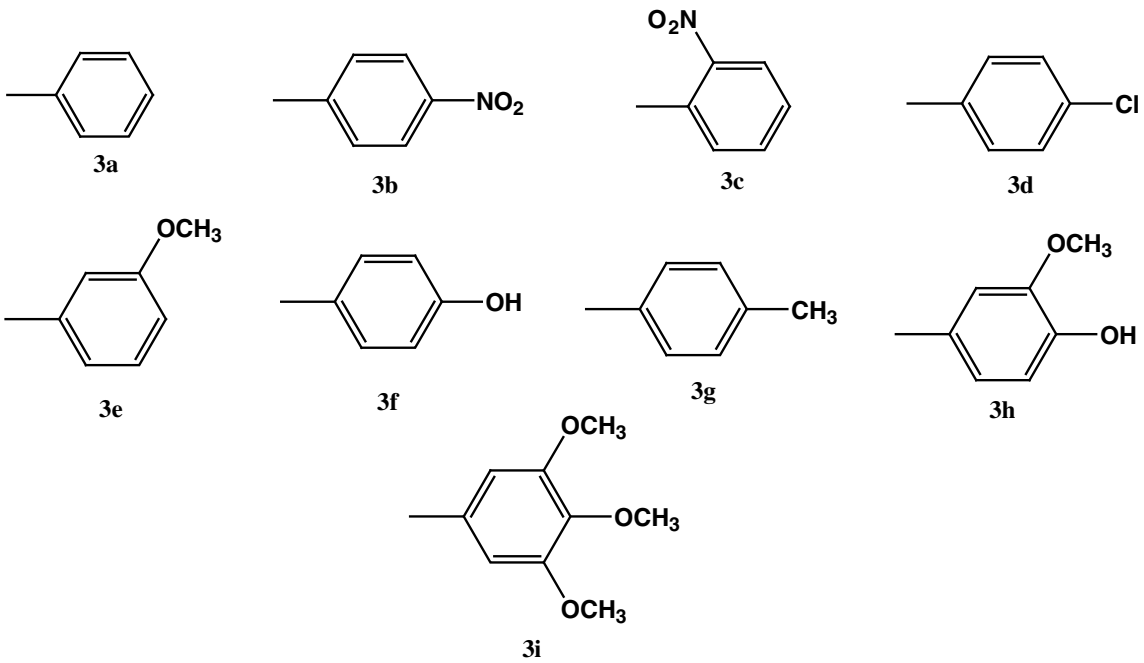
The standard drugs used for assessment of similarity with synthesized derivatives were taken from literature as: sulindac, indomethacin, and aceclofenac. Various set of parameters were used for calculations as given in Table 4.

The distance $d_i$ of a particular target compound $i$ can presented as:

$$d_i^2 = \sum_{j=1}^{n} \frac{\left(1 - X_{i,j}/X_{i,\text{standard}}\right)^2}{n}, \quad (2)$$

where $X_{i,j}$ is value of molecular parameters $i$ for compound $j$, $X_{i,\text{standard}}$ is the value of the same molecular parameter $i$ for standard drug, and $n$ is the total number of the considered molecular parameters.

The similarity of the compounds can be calculated as:



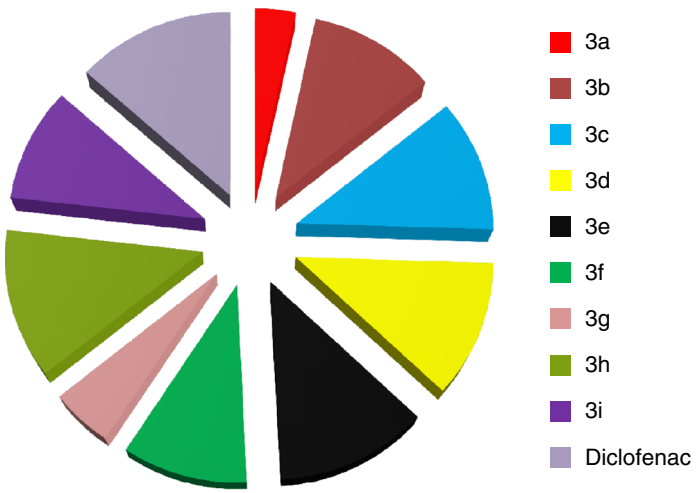




**Fig. 2** Graphical representation of anti-inflammatory activity of synthesized benzylidene-hydrazide derivatives

$$\%\text{Age similarity} = (1 - R) \times 100, \quad (3)$$

where $R$ is quadratic mean also known as the root mean square (RMS), and $R$ can be calculated as:

$$R = \sqrt{d_i^2}. \quad (4)$$

Assessment of structural similarities of synthesized derivatives with standard drugs showed that all the derivatives have good % age similarity except **3i** and **3d** was found to have excellent percentage of similarity (>90 %) with all the standard drugs (Table 5).

*Quantitative structure–activity relationship (QSAR)*

The QSAR analysis was carried out on a series of nine substituted benzylidene-hydrazide derivatives using multi-linear regression. For this purpose, various physicochemical parameters were calculated and correlated with biological activities, i.e., anti-inflammatory (Bhatia *et al.*, 2010) and analgesic activities to obtain QSAR models. The physicochemical parameters were computed using Chem 3D Ultra after energy minimization to minimum RMS gradient of 0.100 kcal/mole Å by MOPAC software

**Table 3** Analgesic activity of synthesized benzylidene-hydrazide derivatives

| Groups | Reaction time (s) | | |
|---|---|---|---|
| | (After 30 min) | (After 60 min) | (After 90 min) |
| Control | 2.13 ± 0.03 | 3.01 ± 0.04 | 3.87 ± 0.10 |
| Diclofenac sodium (10 mg/kg) | 3.06 ± 0.05 | 5.06 ± 0.08 | 10.93 ± 0.01 |
| **3a** | 2.83 ± 0.02 | 3.22 ± 0.04 | 3.89 ± 0.02 |
| **3b** | 5.39 ± 0.03 | 6.45 ± 0.05* | 6.83 ± 0.01* |
| **3c** | 5.35 ± 0.04 | 6.29 ± 0.10* | 6.88 ± 0.06* |
| **3d** | 5.61 ± 0.04 | 7.13 ± 0.19* | 8.91 ± 0.21* |
| **3e** | 3.21 ± 0.06 | 3.94 ± 0.04 | 4.92 ± 0.03 |
| **3f** | 3.42 ± 0.08 | 3.83 ± 0.03 | 4.12 ± 0.03 |
| **3g** | 3.32 ± 0.07 | 3.86 ± 0.02 | 4.50 ± 0.18 |
| **3h** | 4.53 ± 0.10 | 6.22 ± 0.07* | 9.09 ± 0.03* |
| **3i** | 3.83 ± 0.07 | 4.04 ± 0.05 | 4.21 ± 0.05 |

All values represent mean ± SEM ($n = 6$)

* $P < 0.05$ when experimental groups were compared with control

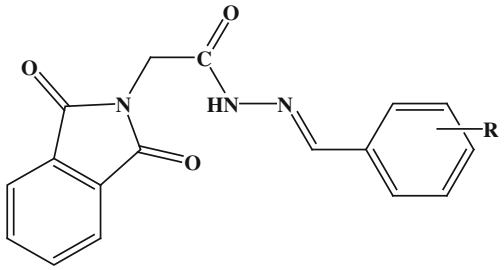
package (Han *et al.*, 2012). Out of all the physicochemical parameters (Table 4), following five parameters were selected for QSAR studies (Log $P$, connolly solvent accessible surface area (SAS), molar refractivity (MR), ovality and molecular weight (MW) which have highly significant effect on biological activity.

Biological activity data was converted to the logarithmic values. For anti-inflammatory activity, percent inhibition ($P$) was converted to $\log(P/100 - P)$ (Bhatia *et al.*, 2010) and for analgesic activity relative latency (%) for 90 min was expressed as [(value of the drug − value of the control)/value of the control] × 100 was taken as biological activity for QSAR model development.

## Statistical analysis

The statistical significance of the models was assessed on the basis of various parameters such as $R^2$ (coefficient of correlation), $R^2_{adj}$ (coefficient of determination), $Q^2$ (cross validates $R^2$) and $F$ (Fischer statistics), considering all the parameters in the model significant only at 95 % confidence level ($p < 0.05$).

Basic structure of synthesized substituted benzylidene-hydrazide derivative




*QSAR models for anti-inflammatory activity*

$$\begin{aligned}\mathrm{Log}(P/100-P) &= -0.04069(\log P) + 0.02529(\mathrm{MW}) \\ &\quad - 0.03073(\mathrm{SAS}) + 0.002801(\mathrm{MR}) \\ &\quad + 23.04(\mathrm{Ovality}) - 27.41\end{aligned}$$

$$N = 9,\ R^2 = 0.642,\ R^2_{adj} = 0.046,\ \mathrm{Press} = 0.002,\ Q^2 = 0.996,\ F = 1.08,\ S = 0.311 \quad (5)$$

Here and thereafter,

$R^2$: coefficient of correlation, $R^2_{adj}$: coefficient of determination, $F$: Fischer statistics, $N$: number of test compounds, Press: predictive error sum of squares, $Q^2$: cross validated $R^2$, BA: Biological activity, $S$: Standard error of estimation.

On exclusion of **3b** and **3c**, Eq. (4) was obtained with better statistical validation:

$$\begin{aligned}\mathrm{Log}(P/100-P) &= 0.6998(\log P) + 0.06842(\mathrm{MW}) \\ &\quad - 0.02632(\mathrm{SAS}) - 0.7417(\mathrm{MR}) \\ &\quad + 133(\mathrm{Ovality}) - 153.6\end{aligned}$$

$$N = 7,\ R^2 = 0.960,\ R^2_{adj} = 0.762,\ \mathrm{Press} = 0.0002,\ Q^2 = 0.999,\ F = 4.844,\ S = 0.173 \quad (6)$$

The observed and predicted anti-inflammatory activity of synthesized benzylidene-hydrazide derivatives is summarized in Table 6, and plot of calculated and

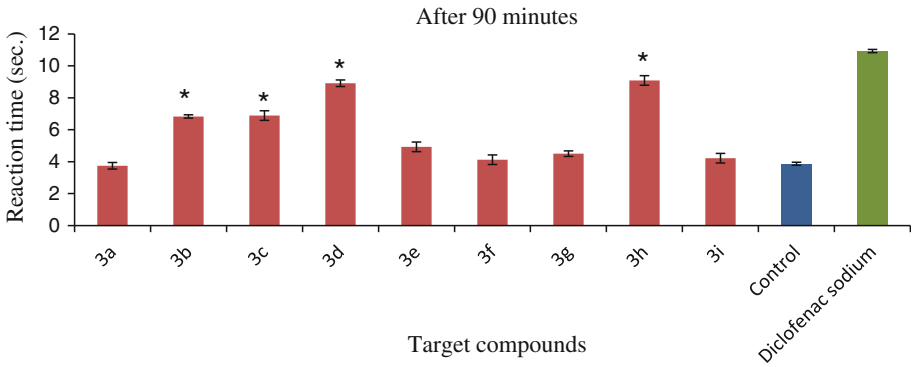



**Fig. 3** Graphical representation of analgesic activity of synthesized benzylidene-hydrazide derivatives. * $p < 0.05$ when experimental groups were compared with control

**Table 4** Calculation of various physicochemical properties of synthesized benzylidene-hydrazide derivatives

| Compounds | SAS[a] (Å$^2$) | MSA[b] (Å$^2$) | SEV[c] (Å$^3$) | Ovality | MR[d] | MTI[e] | WI[f] | BI[g] | MW[h] | Log *P* |
|---|---|---|---|---|---|---|---|---|---|---|
| **3a** | 548.748 | 285.398 | 232.695 | 1.5599 | 84.22 | 10,029 | 1,351 | 384,784 | 307.31 | 2.0271 |
| **3b** | 595.471 | 315.85 | 264.148 | 1.5865 | – | 14,118 | 1,980 | 714,244 | 352.308 | 1.165 |
| **3c** | 584.965 | 308.19 | 254.099 | 1.5885 | – | 13,458 | 1,878 | 678,468 | 352.308 | 1.165 |
| **3d** | 572.5 | 300.2 | 247.109 | 1.5764 | 89.03 | 10,910 | 1,544 | 477,303 | 341.755 | 2.585 |
| **3e** | 593.296 | 312.125 | 257.202 | 1.5958 | 90.69 | 12,526 | 1,727 | 577,890 | 337.337 | 1.9007 |
| **3f** | 556.94 | 290.474 | 236.958 | 1.5685 | 85.92 | 11,104 | 1,544 | 477,303 | 323.31 | 1.6376 |
| **3g** | 579.038 | 304.328 | 250.143 | 1.5851 | 89.27 | 11,492 | 1,544 | 477,303 | 321.337 | 2.5142 |
| **3h** | 599.231 | 316.339 | 261.027 | 1.6015 | 92.38 | 13,637 | 1,927 | 695,793 | 353.336 | 1.5112 |
| **3i** | 677.692 | 365.062 | 308.473 | 1.6535 | 103.61 | 18,064 | 2,565 | 1,144,506 | 397.389 | 1.6479 |
| Std.1 | 580.517 | 317.84 | 282.584 | 1.52626 | 99.250 | 10,917 | 1,517 | 506,021 | 356.420 | 2.2525 |
| Std.2 | 582.618 | 318.50 | 284.198 | 1.52364 | 96.211 | 10,017 | 1,424 | 474,843 | 356.807 | 3.2216 |
| Std.3 | 522.949 | 289.25 | 264.743 | 1.45068 | 86.31 | 8,482 | 1,278 | 465,387 | 354.191 | 3.6459 |

[a] Connolly solvent accessible surface area

[b] Connolly molecular surface area

[c] Connolly solvent excluded volume

[d] Molar refractivity

[e] Molecular topological index

[f] Wiener index

[g] Balaben index

[h] Molecular weight, Std.1—sulindac, Std.2—indomethacin, Std.3—aceclofenac

**Table 5** Assessment of structural similarity of synthesized benzylidene-hydrazide derivatives with standard drugs

| Compounds | Sulindac $(1 - R) * 100$ | Indomethacin $(1 - R) * 100$ | Aceclofenac $(1 - R) * 100$ |
|---|---|---|---|
| **3a** | 64.4 | 45.7 | 82.6 |
| **3b** | 85 | 78.8 | 54.07 |
| **3c** | 92.3 | 88.8 | 64.7 |
| **3d** | 94.3 | 90.3 | 91.7 |
| **3e** | 96.4 | 96.6 | 72.5 |
| **3f** | 75.3 | 75.8 | 98.1 |
| **3g** | 93.9 | 90.8 | 86.9 |
| **3h** | 84.6 | 81.3 | 55 |
| **3i** | 8.02 | 39.9 | 31.9 |

observed anti-inflammatory activity is given in Fig. 4 for Eq. (6).

*QSAR models for analgesic activity*

$$\begin{aligned}\text{Log BA} &= 1.905(\log P) + 0.04127(\text{MW}) - 0.0564(\text{SAS}) \\ &\quad - 0.3592(\text{MR}) + 127.8((\text{Ovality}) - 154.2 \\ N = 9,\ R^2 &= 0.702,\ R^2_{adj} = 0.205,\ \text{Press} = 0.0026, \\ Q^2 &= 0.999,\ F = 1.41,\ S = 0.679\end{aligned} \tag{7}$$

On exclusion of **3b** and **3c**, Eq. (7) was obtained with better statistical validation:

**Table 6** Observed and predicted anti-inflammatory activity of synthesized benzylidene-hydrazide derivatives

| Compounds | Anti-inflammatory activity | | | |
|---|---|---|---|---|
| | Activity % inhibition (*P*) | Observed activity log(*P*/100 − *P*) | Calculated activity | Residuals |
| **3a** | 17.4 | −0.67643 | −0.59761 | −0.07882 |
| **3b** | 54.6 | – | – | – |
| **3c** | 57.3 | – | – | – |
| **3d** | 58.6 | 0.150897 | 0.151309 | −0.00041 |
| **3e** | 61.4 | 0.201581 | 0.171784 | 0.029797 |
| **3f** | 50.6 | 0.010424 | −0.10816 | 0.118586 |
| **3g** | 25.3 | −0.4702 | −0.48822 | 0.018024 |
| **3h** | 64.0 | 0.249877 | 0.342281 | −0.0924 |
| **3i** | 49.4 | −0.01042 | −0.02633 | 0.015911 |

Compounds are not included in QSAR model development

$$\begin{aligned}\text{Log BA} &= 2.215(\log \text{P}) + 0.05131(\text{MW}) - 0.2212(\text{SAS}) \\ &\quad - 0.1456(\text{MR}) + 304.6(\text{Ovality}) - 361.8 \\ N = 7,\ R^2 &= 0.933, R^2_{adj} = 0.595, Press = 0.002, \\ Q^2 &= 0.999, F = 2.76, S = 0.209\end{aligned} \tag{8}$$

The observed and predicted analgesic activity of synthesized benzylidene-hydrazide derivatives is summarized in Table 7 and plot of calculated and observed biological activity is given in Fig. 4 for Eq. (8).

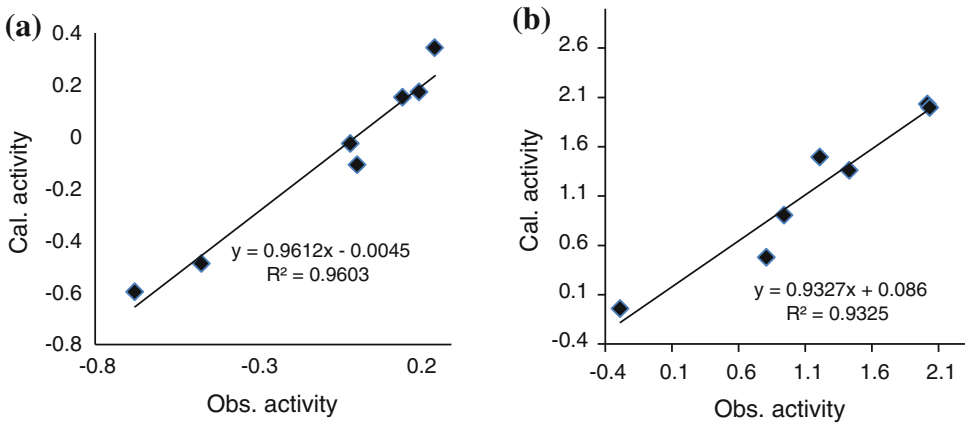



**Fig. 4** Plot between the calculated and observed activities: **a** anti-inflammatory activity and **b** analgesic activity of synthesized benzylidene-hydrazide derivatives

**Table 7** Observed and predicted analgesic activity of synthesized benzylidene-hydrazide derivatives

| Compounds | Analgesic activity | | |
|---|---|---|---|
| | Observed | Calculated | Residual |
| **3a** | −0.28668 | −0.04185 | −0.24483 |
| **3b** | – | – | – |
| **3c** | – | – | – |
| **3d** | 2.01867 | 2.032896 | −0.01423 |
| **3e** | 1.433478 | 1.357953 | 0.075526 |
| **3f** | 0.810229 | 0.47634 | 0.333889 |
| **3g** | 1.21163 | 1.493453 | −0.28182 |
| **3h** | 2.037601 | 1.997297 | 0.040305 |
| **3i** | 0.943768 | 0.905142 | 0.038626 |

Compounds are not included in QSAR model development

## Molecular docking studies

### *Docking studies of synthesized benzylidene-hydrazide derivatives with (COX-2) enzyme*

The synthesized benzylidene-hydrazide derivatives (**3a–i**) were computationally designed and optimized for their interaction with enzyme COX-2 (Pdb-1cx2). The result of their interaction were compared with binding energies of standard drugs (diclofenac sodium, sulindac and SC-S58, where SC-S58 is a selective COX-2 inhibitor, co-crystallized ligand belonging to the vicinal diaryl heterocyclic class and is also used as reference ligand) and summarized in terms of dock score in Table 8 by using following methodology:

- Ligand preparation
- Protein preparation and detecting cavities of protein molecules
- Executing a docking set up through docking wizard panel
- Poses of protein–ligand complex

The binding modes of active derivatives and standard drugs with the active sites of enzyme are shown in Figs. 5 and 6, respectively.

## Result and discussion

### Chemistry

The structures of synthesized derivatives were supported by means of chromatographic and spectroscopic methods. Both analytical and spectral data (IR and $^{1}$H NMR) of all the synthesized derivatives were in full agreement with the proposed structures. The structures of the synthesized derivatives were proven by the spectroscopic method. The structures assigned to (**3a–i**) were supported by IR spectra showing absorption bands at 3,330–3,252 $cm^{-1}$ due to N–H stretching. Stretching vibrations due to phthalyl C=O were observed at 1,783–1,748 $cm^{-1}$. Carbonyl stretch was observed at 1,658–1,629 $cm^{-1}$. Stretching vibrations due to C=N and C=C (aromatic) were observed at 1,668–1,642 and 1,544–1,502 $cm^{-1}$, respectively. Band at 1,503–1,502 $cm^{-1}$ was appeared due to asymmetric N=O stretch. Bands at 1,238–1,235 $cm^{-1}$ indicated C–O–C stretch. Bands at 863–822 $cm^{-1}$ indicated out of plane aromatic stretch. The proton NMR of these derivatives revealed the presence of singlet 9.59–9.76 ppm for –NH–N=. The –N=CH– proton was observed as broad singlet 7.98–8.25 ppm. A 2 protons singlet at 4.42–4.68 ppm appeared to methylene group. All the other aliphatic and aromatic protons were observed within the expected regions. This part concluded the synthesis of benzylidene-hydrazide derivatives.

### Anti-inflammatory activity

The main side effect associated with commonly available NSAIDs is gastric ulceration, which is due to free carboxylic group in the parent drug. In the present study,

**Table 8** Ligand–receptor interaction of synthesized benzylidene-hydrazide derivatives compounds to target COX-2 enzyme

| Ligand | Docking score (binding energy) | Distance (Å) | Amino acid | Group involved in interaction with receptor |
|---|---|---|---|---|
| **3d** | −82.49 | 2.69 | Lys 485 | Carbonyl oxygen of five membered ring of phthalic anhydride |
| | | 2.90 | Glu 479 | Nitrogen of –CONH group |
| **3e** | −91.87 | 3.11 | Lys 485 | Carbonyl oxygen of five membered ring of phthalic anhydride |
| | | 3.34 | Ser 477 | Nitrogen of five membered ring of phthalic anhydride |
| | | 2.80 | Thr 476 | Carbonyl oxygen of –CONH group |
| | | 3.07 | Thr 476 | Carbonyl oxygen of five membered ring of phthalic anhydride |
| | | 3.53 | Ile 498 | Oxygen of methoxy group |
| **3h** | −93.64 | 2.77 | Lys 485 | Carbonyl oxygen of five membered ring of phthalic anhydride |
| | | 2.84 | Thr 476 | Carbonyl oxygen of five membered ring of phthalic anhydride |
| | | 3.27 | Thr 476 | Carbonyl oxygen of –CONH group |
| | | 3.15 | Ser 477 | Nitrogen of five membered ring of phthalic anhydride |
| | | 3.28 | Glu 479 | Nitrogen of –CONH group |
| | | 2.84 | Ser 496 | Oxygen of hydroxyl group |
| Sulindac | −77.424 | 3.06 | Lys 492 | Oxygen of hydroxyl group |
| | | 2.57 | Ser 477 | Oxygen of $-SOCH_3$ group |
| | | 3.15 | Thr 476 | Oxygen of $-SOCH_3$ group |
| Diclofenac | −58.259 | 2.69 | Lys 492 | Carbonyl oxygen of −COOH group |
| SC-S58 | −91.779 | 3.02 | Ile 112 | Nitrogen of $-SO_2NH_2$ |
| | | 2.71 | Arg 120 | Nitrogen of diazole ring |
| | | 3.28 | Ser 119 | Nitrogen of diazole ring |

derivatization of the carboxylate functionality by hydrazone moiety was carried out to get better anti-inflammatory agents. The anti-inflammatory activity data in terms of percent inhibition of benzylidene-hydrazide derivatives (**3a-i**) is given in Table 2. The results obtained (Fig. 2) indicated the effect of various substituents on the anti-inflammatory activity of synthesized derivatives in given order as **3h** (*p*-hydroxy-*m*-methoxy phenyl) > **3e** (*m*-methoxy phenyl) > **3d** (*p*-chloro phenyl) > **3c** (*o*-nitro phenyl) > **3b** (*p*-nitro phenyl) > **3f** (*p*-hydroxy phenyl) > **3i** (3,4,5-trimethoxy phenyl) > **3g** (tolyl) > **3a** (phenyl).

The results suggested that the derivatives **3d**, **3e**, and **3h** have shown highest activity. The derivatives **3b**, **3c**, **3f**, and **3i** possess good anti-inflammatory activity, while **3a** and **3g** have not shown significant anti-inflammatory activity.

The detailed concern of activity and structure of the synthesized derivatives resolved that:

- Bulkier groups in test derivatives have directed to increased lipophilicity which in turn enhanced the permeability across the biological membrane to produce the desired action.
- The electron withdrawing groups in test derivatives have improved the biological activity.
- The hydroxyl (–OH) and methoxy ($-OCH_3$) groups were found to be involved in terms of virtuous hydrogen bond interaction with receptor.

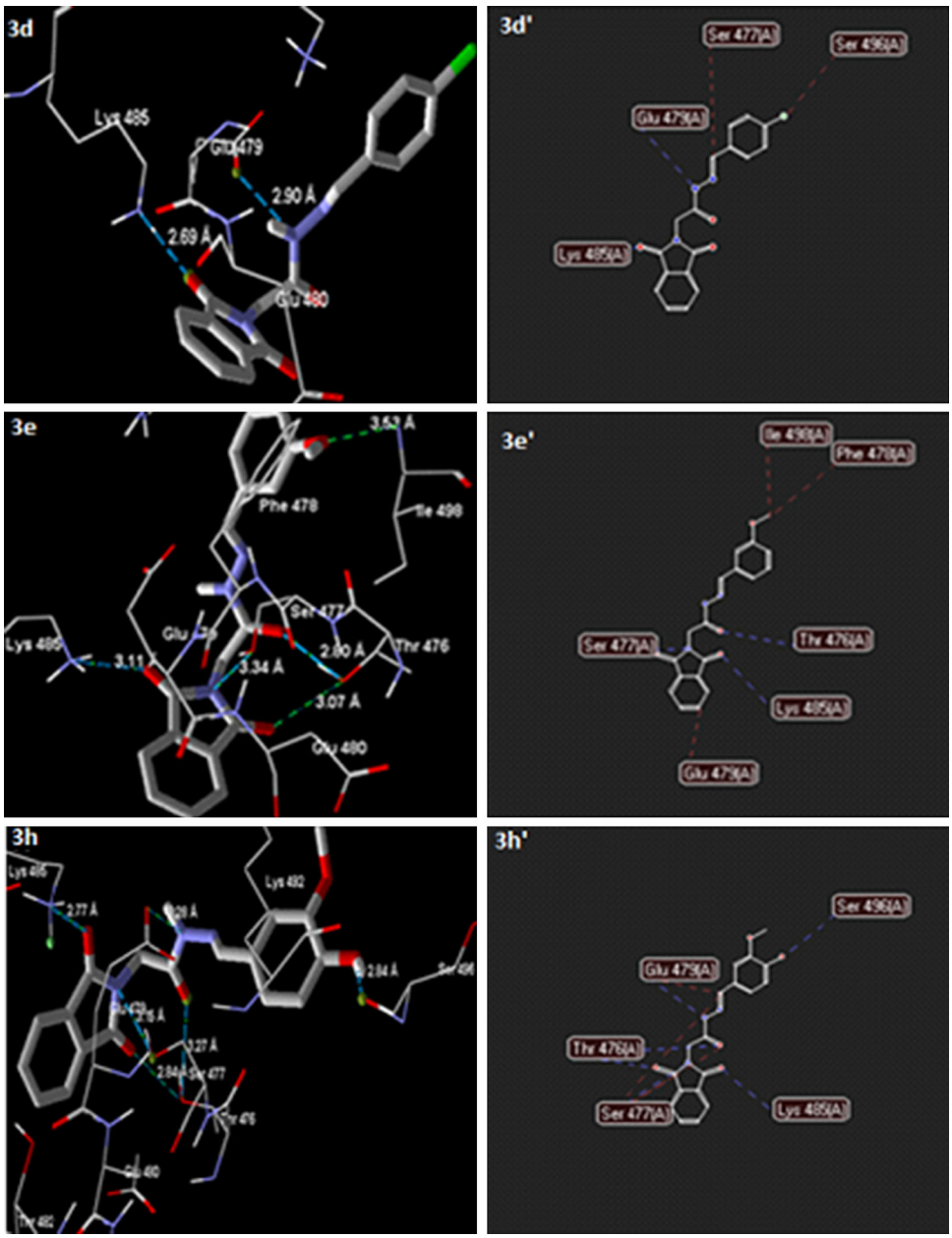



**Fig. 5** Binding modes of **3d**, **3e**, and **3h** (**3d**, **3e**, and **3h** as docking view and **3d′**, **3e′**, and **3h′** as interaction view) with COX-2, where *blue* and *red lines* represent hydrogen bonding and favorable steric interaction, respectively

## Analgesic activity

The analgesic activity data in terms of relative latency of benzylidene-hydrazide derivatives (**3a-i**) is given in Table 3. The reaction time (in s) for the derivatives **3d** (substituted with *p*-chloro phenyl) and **3h** (substituted with *p*-hydroxyl along with *m*-methoxy group) was found to be **8.91 ± 0.21** and **9.09 ± 0.03,** respectively, after 90 min which is comparable with reaction time of diclofenac sodium (**10.93 ± 0.01**) after 90 min. It indicated that these derivatives possess potent analgesic activity. The derivatives **3a**, **3e**, **3f**, **3g**, **3i** were not found to be significantly active.

## Similarity studies

Assessment of structural similarities of synthesized benzylidene-hydrazide derivatives with anti-inflammatory and analgesic drugs (sulindac, indomethacin, and aceclofenac) indicated that all the derivatives have shown good percentage of similarity ranging from **54** to **98.1 %** except **3i**, which has shown less percentage of similarity with all three standard drugs ranging from (**8.02** to **39.9 %**) in Table 5. The derivative **3d** was found to have excellent percentage of similarity (**>90 %**) with all standard drugs. The structural similarity of all the derivatives with standard drugs varies from one to another may be due to the more

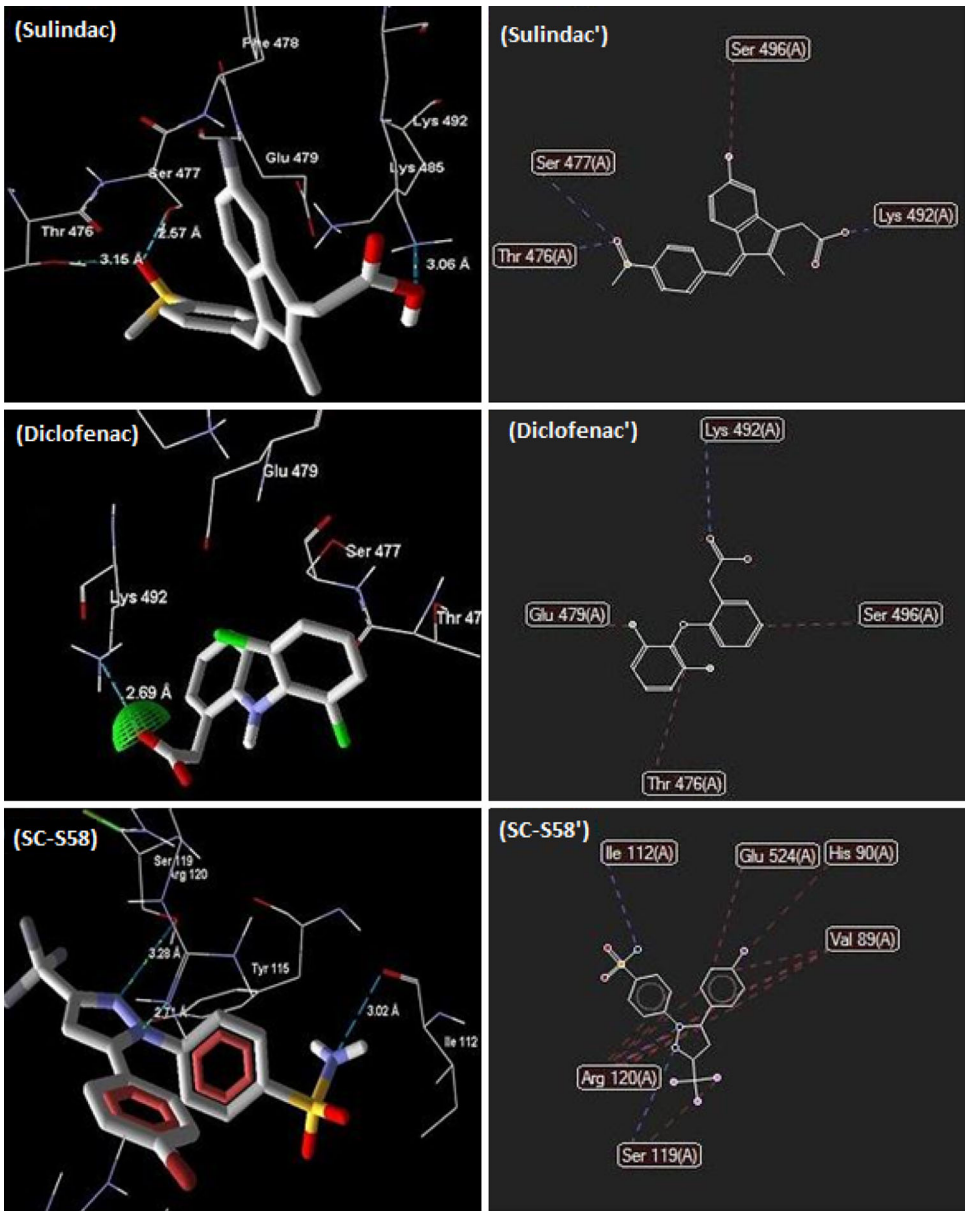



**Fig. 6** Binding modes of sulindac, diclofenac, and SC-S58 (sulindac, diclofenac, and SC-S58 as docking view and sulindac′, diclofenac′, and SC-S58′ as interaction view) with COX-2, where *blue* and *red lines* represent hydrogen bonding and favorable steric interaction, respectively

difference between the values of physicochemical parameters calculated for the synthesized derivatives as well as standard drugs. However, it is not always necessary that good structural similarity of a compound lead to (Kubinyi, 1998) good therapeutic activity as derivative **3f** has shown an excellent percentage of similarity (**75–98 %**) but it was found to be less active as analgesic agent.

## Quantitative structure activity relationship (QSAR) analysis

The results suggested that percent inhibition (anti-inflammatory activity) and relative latency (analgesic activity) were highly dependent on Log *P*, SAS, MR, ovality, and MW. In order to quantify the contribution of these structural features to the benzylidene-hydrazide derivatives used in the study, various physicochemical properties were calculated with the help of various chemoinformatic softwares as listed in the experimental section. Log *P* is a measure of lipophilicity, which is important for penetration, distribution as well as the interaction of drug with receptor. Therefore, it is suggested that lipophillic property has to be investigated for designing of potent anti-inflammatory and analgesic agents. Ovality is another physicochemical parameter which involves a combined effect of size as well as surface area of the substituents present in structure. It characterizes deformation of molecular electron distribution. Connolly solvent accessible surface area (SAS) is indicative of surface properties, molar refractivity (MR) is a measure of the volume occupied by an atom or

group and molecular weight (MW) parameter is an indicative of the size of the molecule. All the developed models have good coefficient of correlation (0.933–0.960), coefficient of determination (0.595–0.762) and cross validated $R^2$ (0.999) with good Fischer statistics (2.76–4.84).

### Molecular docking studies

In the present study, molecular docking studies were performed using MVD software (Version 2012.5.0.0). The synthesized derivatives were subjected to molecular docking studies and possible interactions of target derivative with cyclooxygenase-2 (COX-2) were studied. The derivative **3h** was found to be most potent anti-inflammatory compound having high docking score (binding energy), i.e., −93.64 (high as compared to all standard drugs) with hydrogen bond distance 2.77–3.28 Å (Table 8). The results revealed that hydrogen bond distance is important in docking studies. The distance more than 3.2 Å indicates weak hydrogen bonding between ligand and receptor, 2.6–3.2 Å represent good hydrogen bonding and less than 2.5 Å indicates strong hydrogen bonding (Jeffrey 1997). Almost all the active derivatives have shown good hydrogen bonding with enzymes. The hydroxyl and methoxy group in **3h** act as donors played a very important role for the formation of six hydrogen bonds with COX-2 enzyme and emerged out as potential COX-2 inhibitor. These results could be used for the development of novel, potent, and effective COX-2 inhibitors.

## Conclusion

From the results of anti-inflammatory activity, it was concluded that the derivatives **3d**, **3e,** and **3h** have potent anti-inflammatory activity. In analgesic activity, the derivatives **3d** and **3h** were found to be potent analgesic agents. The derivatives **3b** and **3c** also have shown good analgesic activity. The derivative **3d** was found to have excellent percentage of similarity (>90 %) with all standard drugs. The results of QSAR studies suggested that anti-inflammatory, analgesic activities are highly dependent on physicochemical parameters such as Log *P*, connolly solvent accessible surface area (SAS), molar refractivity (MR), ovality, and molecular weight (MW). The five parameters correlation equations for anti-inflammatory and analgesic activities could be used for the prediction of biological activities of unknown and unavailable compounds of this class. The derived model could be used in future for designing of more potent and specific COX-2 inhibitors against inflammation and pain.

**Acknowledgments** Authors are thankful to M. M. College of Pharmacy, M. M. University, Mullana, Ambala for providing support and facility to carry out research.

## References

Ahluwalia VK, Aggarwal R (2000) Comprehensive practical organic chemistry; preparation and qualitative analysis. Universities Press (India), Hydrebad, pp 229–230

Akhter M, Husain A, Azad B, Ajmal M (2010) Aroylpropionic acid based 2,5-disubstituted-1,3,4-oxadiazoles: synthesis and their anti-inflammatory and analgesic activities. Eur J Med Chem 44:2372–2378

Bhandari SV, Bothara KG, Raut MK, Patil AJ, Sarkate AP, Mokale VJ (2008) Design, synthesis and evaluation of anti-inflammatory, analgesic and ulcerogenicity studies of novel s-substituted phenacyl-1,3,4-oxadiazole-2-thiol and schiff bases of diclofenac acid as nonulcerogenic derivatives. Bioorg Med Chem 16:1822–1831

Bhatia NM, Mahadik KR, Bhatia MS (2010) Exploring the influence of steric, electronic and lipophillic descriptors of 1,3-diarylpropenones on their anti-inflammatory activity. DARU 18:230–236

Borne RF (2002) Non-steroidal anti-inflammatory drugs. In: Williams DA, Lemke TL (eds) Foye's principles of medicinal chemistry, 5th edn. Lippincott Williams & Wilkins, New York, pp 751–793

Botting RM (2006) Cyclooxygenase: past, present and future. J Therm Biol 31:208–219

Han WS, Lee JK, Lee JS, Hahn HG, Yoon CN (2012) Study of thiazoline derivatives for the design of optimal fungicidal compounds using multiple linear regressions (MLR). Bull Korean Chem Soc 33:1703–1706

Hunashal RD (2011) Synthesis, anti-inflammatory and analgesic activity of 2-[4-(substituted benzylideneamino)-5-(substituted phenoxymethyl)-4H-1,2,4-triazol-3-yl thio] acetic acid derivatives. Arab J Chem. doi:10.1016/j.arabjc.2011.01.003

Husain MSYK, Hasan SM, Alam MM (2005) 2-Arylidene-4-(4-phenoxy-phenyl)but-3-en-4-olides: synthesis, reactions and biological activity. Eur J Med Chem 40:1394–1404

Jeffrey GA (1997) An introduction to hydrogen bonding. Oxford University Press, New York

Kitchen DB, Decornez H, Furr JR, Bajorath J (2004) Docking and scoring in virtual screening for drug discovery: methods and applications. Nat Rev Drug Discov 3(11):935–949

Kubinyi H (1998) Similarity and dissimilarity: a medicinal chemist's view, in 3D QSAR in drug design. Kluwer, Dordrecht

Mehtap G, Semra U, Esra K (2009) Synthesis and analgesic and anti-inflammatory activities-6-substituted-3(2H)-pyridazinone-2-acetyl-2-(*p*-substituted/nonsubstituted benzal) hydrazone derivatives. Eur J Med Chem 44:3760–3764

Nikolova N, Jaworska J (2003) Approaches to measure chemical similarity-a review. QSAR Comb Sci 22:1006–1026

Nunez S, Venhorst J, Kruse CG (2012) Target–drug interactions: first principles and their application to drug discovery. Drug Discov Today 17:10–22

Regina GL, Romano S, Valerio G, Antonio L, Ettore N, Olivia B, Paola T, Enzo A (2008) Synthesis, structure–activity relationship and molecular modeling studies of new indole inhibitors of monoamine oxidase A and B. Bioorg Med Chem 22:9729–9740

Salimon J, Salih N, Yousif E, Hameed A, Kreem A (2010) Synthesis and pharmacological evaluation of 9(10H)-acridone bearing 1,3,4-oxadiazole derivatives as antimicrobial agents. Arab J Chem 3:205–210

Vogel HG (2002) Drug discovery and evaluation: pharmacological assays. Springer, New York

Wang X, Tang Y, Xie Q, Qiu Z (2006) QSAR study of 4-phenylpiperidine derivatives as mu opioid agonists by neural network method. Eur J Med Chem 41:226–232

Winter A, Risley EA, Nuss GW (1962) Carrageenan-induced oedema in hind paws of the rat as an assay for anti-inflammatory drugs. Proc Soc Exp Biol 111:544–547