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Synthesis, Molecular Docking and Biological Evaluation of Napthyl *N*-Acyl Hydrazone Derivatives

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A series of 2-(benzamido)-*N*′-((naphthalen-1-yl)methylene)-3-(substituted phenyl)acrylo hydrazides **(4a-m**, **5a-b)** were synthesized by condensation of 2-(benzamido)-3-(substituted phenyl)acrylohydrazides with 1-naphthaldehyde in presence of few drops of acetic acid in ethanol. Structural elucidation of final compounds was confirmed by spectral data. Title compounds were evaluated for antioxidant and antibacterial activities and subjected to *in silico* studies and molecular docking studies with cyclooxygenase-II (COX-II, PDB I'd: 3LN1) and active compounds from docking studies were selected for *in vivo* evaluation of their antiinflammatory activity. Among the series, compound **4i** showed good antioxidant activity; **5b** showed good antibacterial activity. All the six derivatives with good docking scores were selected for *in vivo* evaluation of their antiinflammatory activity. Among the selected active compounds from docking studies, compound **4m** showed good antiinflammatory activity which is comparable with that of standard drug diclofenac. *In silico* studies indicated that all the compounds followed Lipinski's rule and exhibited good oral absorption and bioavailability.

Keywords: *N*-acyl hydrazones, *in silico* studies, Biological activity, ADME properties.

INTRODUCTION

Hydrazone and *N*-acyl hydrazone nucleus possess very pharmacophoric cores for new drug development [1]. The hydrazone contains two nucleophilic nitrogen atoms and one carbon atom with both electrophillic and nucleophilic character [2]. *N*-Acyl hydrazone moiety reported to possess diverse pharmacological activities such as antiinflammatory, analgesic, antioxidant, antiplatelet [3], vasoactive, cardiac stimulant [4], antiparkinsonism [5], antitumor [6], antibacterial [7] and antinociceptive activities [8]. Various approved drugs contain *N*-acyl hydrazine moiety such as nitrofurazone, nifuroxazide, carbazochrome, nitrofurantoin, azumolene and dantrolene [9]. Drugs like iproniazide, isoniazid and isocarboxazide containing *N*-acyl hydrazone moiety were used to treat tuberculosis and also showed antidepressant activity. Nifuroxazide used as an intestinal antiseptic [1,10], whereas 2-chloroquinolinyl hydrazone derivatives are anticonvulsants and ribavirin hydrazone derivatives are anticancer agents [11].

The hydrazone derivative flosulide from natural safrole exhibits antiinflammatory activity. Furacilin, ftivazide and furazolidone bearing hydrazone pharmacophore group are well-known antimicrobial agents [12]. Derivatives of pyrazine *N*-acyl hydrazone exhibited good anti-inflammatory activity [13]. Bacterial resistance to antibiotics and side effects of existing NSAID's is a growing concern for development of newer therapeutic agents. Hence, present study aimed towards synthesis of novel *N*-acyl hydrazone derivatives containing naphthyl moiety and all synthesized derivatives were subjected to molecular docking studies, physico-chemical parameters, toxicity risks, bioactivity score, absorption, distribution, metabolism and excretion prediction using online tools. Further, title compounds were evaluated for antioxidant, antibacterial and antiinflammatory activities.

EXPERIMENTAL

Melting points of the derivatives were determined using Tempo melting point apparatus in an open capillary tube. Purity of the compounds was determined by using precoated TLC plates (E. Merck Silica Gel 60 F_{254}). The FT-IR spectra were recorded on Bruker Alpha-T FT-IR spectrophotometer using KBr Pellet tchnique. ^{1}H NMR spectra (chemical shift in δ ppm) were recorded at 400 MHz on Bruker NMR spectrometer using DMSO as solvent. LC-MS, Agilent 1200 infinity series was used for recording mass spectra.

Synthesis of 4-benzylidene-2-(phenyloxazol)-5(4*H*)-one (2a-m): 4-Benzylidene-2-(phenyloxazol)-5(4H)-ones were synthesized according to the procedure reported earlier [14].

Synthesis of 2-(benzamido)-3phenylacrylohydrazide (3a-m): 2-(Benzamido)-3-phenylacrylohydrazides **(3a-m)** were synthesized according to reported method [14] and used for subsequent reaction after drying.

Synthesis of 2-(benzamido)-*N*′-((naphthalen-1-yl)-methylene)-3-phenylacrylohydrazide (4a-m): Different substituted 2-(benzamido)-*N*′-((naphthalen-1-yl)methylene)-3-phenyl acrylohydrazides were synthesized by refluxing equimolar amounts of 2-(benzamido)-3-phenylacrylohydrazide (0.003 mol) and 1-naphthaldehyde (0.003 mol) at 60 ºC with few drops of acetic acid in ethanol for 1 h (**Scheme-I**). Then, the reaction mixture was cooled to room temperature and solid obtained was filtered and recrystallized from methanol [14].

Synthesis of 2-(benzamido)-*N*′((substituted naphthalen-1-yl)methylene)-3-phenylacrylohydrazide (5a-b): Equimolar amounts of 2-(benzamido)-3-phenylacrylohydrazide (0.003 mol) and substituted naphthaldehydes (0.003 mol) were refluxed at 60 ºC with few drops of acetic acid in ethanol for 1 h (**Scheme-I**). Then the reaction mixture was cooled to room temperature and solid obtained was filtered and recrystallized from methanol [14].

2-(Benzamido)-*N*′-((naphthalen-1-yl)methylene)-3-phenylacrylohydrazide (4a): m.f.: $C_{27}H_{21}N_3O_2$; yield: 80%; m.p.: 106-108 ºC; IR (KBr, ν_{max}, cm^{-1}): 3413 (NH), 2984 (Ar-H), 2874 (C-H), 1598 (C=O), 1584 (C=N), 1468(C=C). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 7.24 (s, 1H, C=CH), 7.33-8.85 (m, 16H, Ar-H), 8.87 (s, 1H, N=CH), 10.21(s, 1H, NHCO), 11.87 (s, 1H, CONHN=).

2-(Benzamido)-*N*′-((naphthalen-1-yl)methylene)-3-*p*-tolylacrylohydrazide (4b): m.f.: $C_{28}H_{23}N_3O_2$; yield: 65%; m.p.: 126-128 ºC; IR (KBr, ν_{max}, cm^{-1}): 3224 (NH), 2960 (Ar-H), 2836 (C-H), 1645 (C=O), 1603 (C=N), 1472(C=C). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.42 (s, 3H, CH_3), 7.21 (s, 1H, C=CH) 7.52-8.31 (m, 16H, Ar-H), 8.61 (s, 1H, N=CH), 9.47 (s, 1H, NHCO), 10.37 (s, 1H, CONHN=). Mass (*m/z*): 432 $(M-H)^-$.

2-(Benzamido)-3-(4-hydroxyphenyl)-*N*′-((naphthalen-1-yl)methylene)acrylohydrazide (4c): m.f.: $C_{27}H_{21}N_3O_3$; yield: 56%; m.p.: 107-108 ºC; IR (KBr, ν_{max}, cm^{-1}): 3213 (NH), 3045 (Ar-H), 2920 (C-H), 1644 (C=O), 1551 (C=N), 1475 (C=C). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 5.02 (s, 1H, OH), 7.19 (s, 1H, C=CH) 7.48-8.35 (m, 16H, Ar-H), 8.75 (s, 1H, N=CH), 9.65 (s, 1H, NHCO), 10.57 (s, 1H, CONHN).

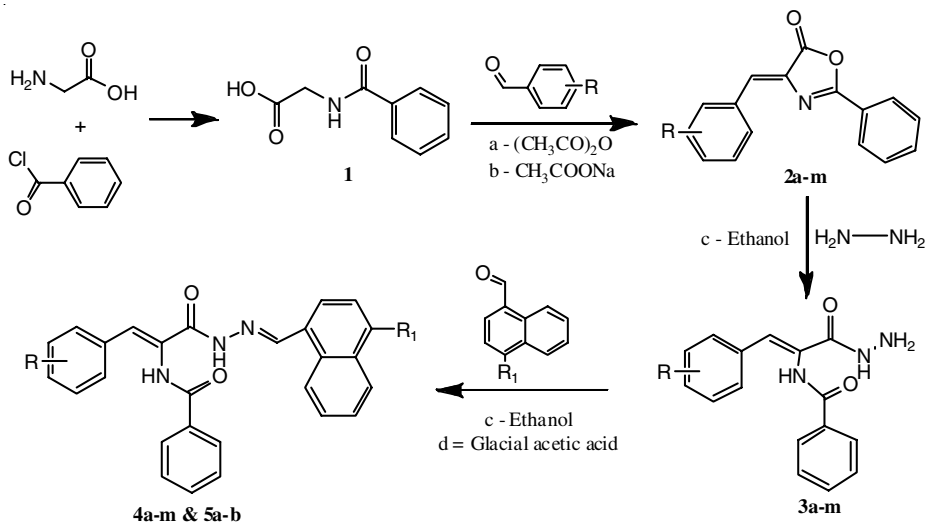
2-(Benzamido)-3-(4-(dimethylamino)phenyl)-*N*′-((naphthalen-1-yl)methylene)acrylohydrazide (4d): m.f.: $C_{29}H_{26}N_4O_2$; yield: 72%; m.p.: 198-199 ºC; IR (KBr, ν_{max}, cm^{-1}): 3215 (NH), 3049 (Ar-H), 2899 (C-H), 1642 (C=O), 1528 (C=N), 1480 (C=C). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.90 (s, 6H, $(CH_3)_2N$), 7.01 (s, 1H, C=CH), 7.20-8.18 (m, 16H, Ar-H), 8.98 (s, 1H, N=CH), 10.11 (s, 1H, NHCO), 11.33 (s, 1H, CONHN=).

2-(Benzamido)-3-(4-methoxyphenyl)-*N*′-((naphthalen-1-yl)methylene)acrylohydrazide (4e): m.f.: $C_{28}H_{23}N_3O_3$; yield: 71%; m.p.: 118-120 ºC ; IR (KBr, ν_{max}, cm^{-1}): 3228 (NH), 2936 (Ar-H), 2835 (C-H), 1655 (C=O), 1583 (C=N), 1479 (C=C). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 3.78 (s, 3H, OCH_3), 6.98 (s, 1H, C=CH), 7.00-8.84 (m, 16H, Ar-H), 9.86 (s, 1H, N=CH), 10.11(s, 1H, NHCO), 11.71 (s 1H, CONHN=).

2-(Benzamido)-3-(3,4-dimethoxyphenyl)-*N*′-((naphthalen-1-yl)methylene)acrylohydrazide (4f): m.f.: $C_{29}H_{25}N_3O_4$; yield: 58%; m.p.: 202-203 ºC; IR (KBr, ν_{max}, cm^{-1}): 3219 (NH), 2959 (Ar-H), 2835 (C-H), 1643 (C=O), 1511 (C=N), 1472 (C=C). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 3.58-3.89 (s, 6H, $(OCH_3)_2$), 6.99 (s, 1H, C=CH), 7.26-8.12 (m, 15H, Ar-H), 8.57 (s, 1H, N=CH), 9.26 (s, 1H, NHCO), 10. 41 (s 1H, CONHN=). Mass (*m/z*): 478 $(M-H)^-$.

2-(Benzamido)-3-(3,4,5-trimethoxyphenyl)-*N*′-((naphthalen-1-yl)methylene)acrylohydrazide (4g): m.f.: $C_{30}H_{27}N_3O_5$; yield: 56%; m.p.: 140-142 ºC; IR (KBr, ν_{max}, cm^{-1}): 3219 (NH), 2941 (Ar-H), 2835 (C-H), 1639 (C=O), 1578 (C=N), 1472 (C=C). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 3.28-3.95 (s, 9H, $(OCH_3)_3$), 6.84 (s, 1H, C=CH), 7.16-8.34 (m, 14H, Ar-H), 8.98 (s, 1H, N=CH), 9.87 (s, 1H, NHCO), 10.91 (s, 1H, CONHN=).

2-(Benzamido)-3-(4-hydroxy-3-methoxyphenyl)-*N*′-((naphthalen-1yl)methylene)acrylohydrazide (4h): m.f.: $C_{28}H_{23}N_3O_4$; yield: 67%; m.p.: 150-151 ºC ; IR (KBr, ν_{max}, cm^{-1}):



Comp. code	R	R_1
4a	H	H
4b	4-CH_3	H
4c	4-OH	H
4d	4-$N(CH_3)_2$	H
4e	4-OCH_3	H
4f	3,4-$(OCH_3)_2$	H
4g	3,4,5-$(OCH_3)_3$	H
4h	4-OH, 3-OCH_3	H
4i	4-OH, 3,5$(OCH_3)_2$	H
4j	4-NO_2	H
4k	4-F	H
4l	4-$NHCOCH_3$	H
4m	4-$CH_3(CH_3)_2$	H
5a	H	4-CH_3
5b	H	4-OH

Scheme-I: Synthesis of napthyl *N*-acyl hydrazone derivatives

3213 (NH), 3052 (Ar-H), 2874 (C-H), 1643 (C=O), 1510 (C=N), 1474 (C=C). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 3.93 (s, 3H, OCH_3), 5.02 (s, 1H, OH), 6.89 (s, 1H, C=CH), 6.98-8.12 (m, 15H, Ar-H), 8.87 (s, 1H, N=CH), 9.66 (s, 1H, NHCO), 10.89 (s, 1H, CONHN=).

2-(Benzamido)-3-(4-hydroxy-3,5-dimethoxyphenyl)-*N′*-((naphthalen-1-yl)methylene)acrylohydrazide (4i): m.f.: $C_{29}H_{25}N_3O_5$; yield: 68%; m.p.: 108-109 ºC; IR (KBr, ν_{max}, cm^{-1}): 3235 (NH), 2941 (Ar-H), 2839 (C-H), 1651 (C=O), 1511 (C=N), 1474 (C=C). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 3.85 (s, 6H $(OCH_3)_2$), 5.23 (s, 1H, OH), 6.99 (s, 1H, C=CH), 7.28-8.62 (m, 14H, Ar-H), 8.98 (s, 1H, N=CH), 10.22 (s, 1H, NHCO), 11.19 (s, 1H, CONHN=).

2-(Benzamido)-*N′*-((naphthalen-1-yl)methylene)-3-(4-nitrophenyl)acrylohydrazide (4j): m.f.: $C_{27}H_{20}N_4O_4$; yield: 68%; m.p.: 182-183 ºC; IR (KBr, ν_{max}, cm^{-1}): 3316 (NH), 3060 (Ar-H), 2844 (C-H), 1655 (C=O), 1589 (C=N), 1479 (C=C). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 6.78 (s, 1H, C=CH), 7.02-8.25 (m, 16H, Ar-H), 9.01 (s, 1H, N=CH), 10.37 (s, 1H, NHCO), 11.12 (s, 1H, CONHN=).

2-(Benzamido)-3-(4-fluorophenyl)-*N′*-((naphthalen-1-yl)methylene)acrylohydrazide (4k): m.f.: $C_{27}H_{20}N_3O_2F$; yield: 85%; m.p.: 124-125 ºC; IR (KBr, ν_{max}, cm^{-1}): 3219 (NH), 3051 (Ar-H), 2855 (C-H), 1642 (C=O), 1509 (C=N), 1473(C=C). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 6.87 (s, 1H, C=CH), 7.19-8.45 (m, 16H, Ar-H), 9.31 (s, 1H, N=CH), 10.28 (s, 1H, -NHCO), 11.34 (s 1H, CONHN=).

3-(4-Acetamidophenyl)-2-(benzamido)-*N′*-((naphthalen-1-yl)methylene)acrylohydrazide (4l): m.f.: $C_{29}H_{24}N_4O_3$; yield: 55%; m.p.: 138-139 ºC; IR (KBr, ν_{max}, cm^{-1}): 3244 (NH), 3050 (Ar-H), 2874 (C-H), 1647 (C=O), 1513 (C=N), 1405 (C=C). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.06 (s, 3H, CH_3), 7.22 (s, 1H, C=CH), 7.55-8.85 (m, 16H, Ar-H), 8.87 (s, 1H, N=CH), 9.11 (s, 1H, $NHCOCH_3$), 10.15 (s, 1H, NHCO), 11.76 (s, 1H, CONHN=).

2-(Benzamido)-3-(4-isopropylphenyl)-*N′*-((naphthalen-1-yl)methylene)acrylohydrazide (4m): m.f.: $C_{30}H_{27}N_3O_2$; yield: 85%; m.p.: 120-121 ºC; IR (KBr, ν_{max}, cm^{-1}): 3230 (NH), 2960 (Ar-H), 2871 (C-H), 1639 (C=O), 1575 (C=N), 1475 (C=C). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 1.14-1.16 (s, 6H, $(CH_3)_2$), 2.86 (s, 3H, CH_3), 7.18 (s, 1H, C=CH), 7.23-8.04 (m, 16H, Ar-H), 8.81 (s, 1H, N=CH), 9.04 (s, 1H, NHCO), 10.14 (s, 1H, CONHN=).

2-(Benzamido)-*N′*-((1-methylnaphthalen-4-yl)methylene)-3-phenyl acrylohydrazide (5a): m.f.: $C_{28}H_{23}N_3O_2$; yield: 79%, m.p.: 209-210 ºC; IR (KBr, ν_{max}, cm^{-1}): 3252 (NH), 2982 (Ar-H), 2875 (C-H), 1643 (C=O), 1573 (C=N), 1493 (C=C). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 1.24 (s, 3H, CH_3), 6.90 (s, 1H, C=H), 7.03-8.29 (m, 16H, Ar-H), 8.31 (s, 1H, N=CH), 9.31 (s, 1H, NHCO), 10.19 (s, 1H, CONHN=).

2-(Benzamido)-*N′*-((1-hydroxynaphthalen-4-yl)-methylene)-3-phenylacrylohydrazide (5b): m.f.: $C_{27}H_{21}N_3O_3$; yield: 78%, m.p.: 108-109 ºC; IR (KBr, ν_{max}, cm^{-1}): 3413 (NH), 2982 (Ar-H), 2873 (C-H), 1584 (C=O), 1550 (C=N), 1469 (C=C). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 5.54 (s, 1H, OH), 7.01 (s, 1H, C=CH), 7.45-8.39 (m, 16H, Ar-H), 8.91 (s, 1H, N=CH), 10.33 (s, 1H, NHCO), 11.37 (s, 1H, CONHN=).

Interaction with stable DPPH•: The DPPH radical scavenging activity of the synthesized compounds was performed according to reported method [17]. Final compounds or standard (2 mL of 100 μM) were added to 2 mL of DPPH (100 μM) ethanolic solutions. Ethanol (2 mL) was added to 2 mL of 100 μM DPPH ethanolic solution, treated as negative control. The tubes were covered with aluminum foil to protect from light and kept at an ambient temperature for 30 min. Then, the absorbance was read at 517 nm using ethanol as blank. Ascorbic acid was used as standard.

Assay of nitric oxide (NO) scavenging activity: Sodium nitroprusside (10 μM) in phosphate buffer pH 7.4 and 100 μM concentrations of the synthesized compounds/standard dissolved in methanol were incubated for 120 min at 25 ºC. Control experiment also carried out in the same manner (without test compound). After incubation, 2 mL of solution was taken out and diluted using Griess reagent (2 mL). The absorbance of pink colour chromophore, which is formed by diazotization and coupling reaction involved in this assay was measured at 546 nm [16].

Antibacterial activity: Synthesized compounds (**4a-m** & **5a-b**) were evaluated for their antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus* (Gram-positive) and *Escherichia coli*, *Pseudomonas aeruginosa* (Gram-negative) by cup plate method at a concentration of 100 μg/mL. Amoxicillin and streptomycin were used as standard drugs. Nutrient agar was used as culture medium for bacterial strains. DMSO was used as solvent control. Bacterial innoculum prepared by transferring the stock culture into the nutrient broth in a conical flask and incubated at 35-37 ºC for 24 h before analysis. All the glassware, inoculums, media and reagents were placed in sterilized laminar airflow cabinet following all the aseptic conditions. Sterile hot agar medium (25 mL) was poured in each plate and allowed to harden. The agar plates were inoculated with test innoculums using sterile cotton swab by even streaking of the swab in three directions over total surface of the plate. Cups of 6 mm diameter were made using a sterile cork borer in the agar plate after the inoculums had dried. All the synthesized were added to these cups with micropipette and incubated at 37 ºC for 24 h and zone of inhibition was measured [17].

Molecular docking studies: Molecular docking studies were performed using online software SwissDock. The crystal structure of target protein COX-II (PDB ID: 3LN1) was retrieved from Swiss target database and protein preparation including elimination of water molecules, native ligand and other hetero atoms was done using Chimera before starting molecular docking process in order to avoid hinderence in simulation. The structures of ligands were drawn in chemdraw ultra and energy minimization was done using Chemdraw 3D and saved in mol2 format. Docking was done by using swissdock server (http://www.swissdock.ch/docking) by uploading the structures of protein and ligands. Visualization of docking results was done in the UCSF Chimera molecular viewer.

Antiinflammatory activity: The *in vivo* antiinflammatory activity of selected active compounds from docking studies was tested by following method of Winter *et al.* [18]. Selected test

compounds (100 mg/kg body wt.) were given orally to groups of male wistar albino rats (150-180 g) 1 h prior to injection of 0.05 mL 1% carrageenan suspension into the subplantar region of rat hind paw. Other groups were similarly treated with standard drug 100 mg/kg diclofenac (positive controls) or 0.5% sodium carboxy methylcellulose (vehicle controls). Injected paw volume was measured immediately after injecting carrageenan suspension and again after 3 h by water displacement using plethysmograph. Average edema volumes for test compounds and positive controls were statistically compared with that of the vehicle controls and expressed as % edema inhibition which was calculated using the following formula:

$$\text{Edema inhibition (\%)} = 100\left(1 - \frac{V_t}{V_c}\right)$$

where, V_c = edema volume in the control group; V_t = edema volume in the test compounds treated group.

***In silico* studies:** Different substituted hydrazone derivatives were designed and screened for their physico-chemical parameters and prediction of toxicity risks using online OSIRIS property explorer, bioactivity score by Molinspiration tool and ADME properties were predicted using preADMET. Osiris online software tool was used to predict the important physicochemical properties including lipophilicity (cLog P), solubility, molecular weight (MW), topological molecular polar surface area (TPSA), number of hydrogen acceptors (nALH), number of hydrogen-donors (nDLH), drug-likeness and drug score of the compounds to determine oral bioavailability. According to Lipinski's rule of five orally bioavailable drugs must fulfil the following criteria: cLog p ≤ 5, MW ≤ 500, nALH ≤ 10, and nDLH ≤ 5 [19,20]. The % of absorption was determined using the formula:

$$\text{ABS (\%)} = 109 - (0.345 \times \text{TPSA})$$

Toxicity like mutagenic, tumorigenic, irritant and reproductive risks were also predicted using OSIRIS property explorer through comparison of chemical structure of new derivatives with molecular fragments whose toxicities are defined in a database [21]. Bioactivity scores of the compounds were predicted using Molinspiration 2011.06 [22]. Pharmacokinetic properties like ADME profiling of final compounds were determined using preADMET online server [23].

RESULTS AND DISCUSSION

A series of fifteen 2-(benzamido)-*N*′-((naphthalen-1-yl)-methylene)-3-(substituted phenyl)acrylohydrazides (**4a-m** & **5a-b**) were synthesized by refluxing equimolar amounts of 2-(benzamido)-3-(substituted phenyl)acrylohydrazides (**3a-m**) with 1-naphthaldehyde in presence of glacial acetic acid (**Scheme-I**). Structures of the synthesized compounds were confirmed by FT-IR, ^{1}H NMR and mass spectra. Completion of reactions were monitored by TLC. The FT-IR spectra of final compounds showed infrared absorption at 1493-1405 cm^{-1} indicating presence of C=C functional group, while the bands observed at 1603-1509 cm^{-1} due to C=N, 1655-1598 cm^{-1} corresponded with C=O linkage and 3413-3213 cm^{-1} observed due to -NH group.^{1}H NMR spectra were taken for title compounds, which also supported the structures assigned. The compounds showed singlet at δ 6.78-7.24 ppm due to styryl protons, singlet at δ 8.31-9.86 ppm due to N=CH and singlets at δ 10.14-11.87 ppm due to -CONH and all aromatic protons were found as singlet, doublet & triplets between δ 6.98-8.85 ppm. Structures of the synthesized compounds were further confirmed by mass spectral data.

DPPH (2,2-diphenyl-1-picrylhydrazyl) assay: All the title compounds (**4a-m** & **5a-b**) were evaluated for antioxidant studies by DPPH assay at 100 µM concentration (Table-1). It is interesting to note that among the series, compound **4i** showed the equipotent activity to that of standard compound ascorbic acid (91%). Compounds **4h** and **5a** elicited good antioxidant activity (89% and 85%, respectively). This result highlight that methoxy substitution at 3rd and 5th position in the scaffold plays important role and it is analogous to the previous reports on the antioxidant properties of hydrazones [24]. Compounds **4l** and **4c** derivatives showed moderate activity (49% and 40%) when compared with standard.

TABLE-1
ANTIOXIDANT ACTIVITY OF 2-(BENZAMIDO)-*N*′-((NAPHTHALEN-1-YL)METHYLENE)-3-PHENYLACRYLOHYDRAZIDES

Compd. code	Inhibition (%)	
	DPPH radical scavenging activity	Nitric oxide scavenging activity
4a	10	34
4b	15	42
4c	40	17
4d	–	35
4e	10	44
4f	13	31
4g	10	36
4h	89	35
4i	91	54
4j	18	34
4k	16	39
4l	49	32
4m	12	22
5a	85	53
5b	16	52
Ascorbic acid	91	–
Tocoferol	–	55

Reduction of DPPH free radial by test compounds at 100 µM was estimated in alcoholic solution and the absorbance was measured at 517 nm.

Assay of nitric oxide (NO) scavenging activity: All the synthesized compounds were evaluated for their nitric oxide scavenging activity at 100 µM concentration and results are shown in Table-1. It is interesting to note that all the compounds were found to be active comparable to the standard compound. Among the series, compounds **4i, 5a** and **5b** derivatives exhibited good antioxidant activity (54%, 53% and 52%, respectively) comparable to that of standard tocoferol (55%). The good activity of compound **4i** might be due to methoxy and hydroxy groups [25]. Compounds **4e**, **4b** and **4k** showed moderate activity (44%, 42% and 39%).

Antibacterial activity: All the compounds (**4a-m** & **5a-b**) were evaluated for *in vitro* antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus* (Gram-positive) *Escherichia coli* and *Pseudomonas aeruginosa* (Gram-negative) bacterial strains at concentration of 100 µg by using cup-plate method. After 24 h, zone of inhibitions of test compounds was measured and compared with that of standard antibacterial agents such as streptomycin for Gram-negative and amoxicillin for Gram-positive strains (Table-2). Among the series, compounds **5b** and **4k** elicited good antibacterial activity (29 mm and 28 mm) against the Gram-positive bacterial strain *B. subtilis.* All title compounds displayed moderate antibacterial activity against Gram-positive bacterial strain *P. aeruginosa*. Compounds **5b**, **4l** and **4f** exhibited good inhibitory activity (22 mm, 21 mm and 17 mm) against Gram-negative bacterial strain *E. coli*.

TABLE-2
ANTIBACTERIAL ACTIVITY OF 2-(BENZAMIDO)-*N*'-((NAPHTHALEN-1-YL) METHYLENE)-3-PHENYLACRYLOHYDRAZIDES

Compd. code	Zone of inhibition (mm)			
	Gram-positive		Gram-negative	
	B. subtilis	*S. aureus*	*E. coli*	*P. aeruginosa*
4a	19	15	17	13
4b	24	16	20	15
4c	17	15	15	14
4d	21	17	13	12
4e	20	19	18	11
4f	20	18	21	15
4g	20	15	17	13
4h	16	15	14	12
4i	24	17	18	14
4j	21	18	16	15
4k	28	15	17	14
4l	23	18	21	17
4m	25	17	17	16
5a	21	16	16	16
5b	29	16	22	17
Amoxicillin	33	31	–	–
Streptomycin	–	–	32	34

Molecular docking studies: Molecular docking study was performed to investigate the protein-ligand interactions of the title compounds with COX-II (PDB ID: 3LN1) and results are given in Table-3. All the derivatives in this series exhibited good interactions with COX-2 and docking scores of these compounds are in the range of -9.09 to -7.55 Kcal/mol. Among the series, trimethoxy (**4g**), fluoro (**4k**) and isopropyl (**4m**) derivatives showed high binding affinity towards COX-2 (Fig. 1). Compounds which exhibited good binding affinity towards COX-2 in molecular docking studies were selected for *in vivo* antiinflammatory evaluation.

***In vivo* antiinflammatory activity:** Selected active compounds from docking studies were evaluated for antiinflammatory activity by carrageenan-induced rat paw-edema model. Results indicated that antiinflammatory activity of isopropyl derivative **4m** (69%) is so close to that of standard diclofenac (70%). Compounds **4h**, **4k**, **4e** and **4g** showed good activity (54-62%) (Table-4). Statistical analysis was performed by one-way ANOVA.

TABLE-3
MOLECULAR DOCKING SCORES OF TITLE COMPOUNDS WITH COX-II (PDB id -3LN1)

Compd. code	Docking scores (Kcal/mol)		Interacting amino acids	Bond distance (Å)
	Energy (ΔG)	Full fitness		
4a	-8.32	-2179.86	HEME TYR	2.643 2.010
4b	-7.92	-2179.76	GLN ILE	2.696 2.799
4c	-7.99	-2192.17	LYS ILE	2.698 3.050
4d	-7.22	-2184.12	TYR ALA	2.673 2.970
4e	-8.47	-2180.95	PRO TYR	2.573 2.865
4f	-7.81	-2163.07	HSE TYR	2.547 2.690
4g	-9.09	-2155.94	ILE GLN	3.514 2.100
4h	-8.35	-2182.80	VAL GLN	3.018 2.385
4i	-7.55	-2164.58	HSE ILE	2.136 2.689
4j	-7.57	-2167.10	HEME ALA	2.556 2.947
4k	-8.85	-2181.96	LYS ILE	2.621 2.963
4l	-8.05	-2192.71	GLN GLN	2.659 2.550
4m	-8.57	-2181.47	LYS TYR	2.926 2.550
5a	-7.69	-2174.72	LEU LYS	7.729 2.545
5b	-7.67	-2181.46	LYS ILE	2.598 3.150
Diclofenac	-5.4	-2238.8	PHE	2.1

TYR = Tyrosine; GLN = Glutamine; VAL = Valine; ILE = Isoleucine; LYS = Lysine; ALA = Alanine; PRO = Proline; HSE = homoserine; LEU = Leucine.

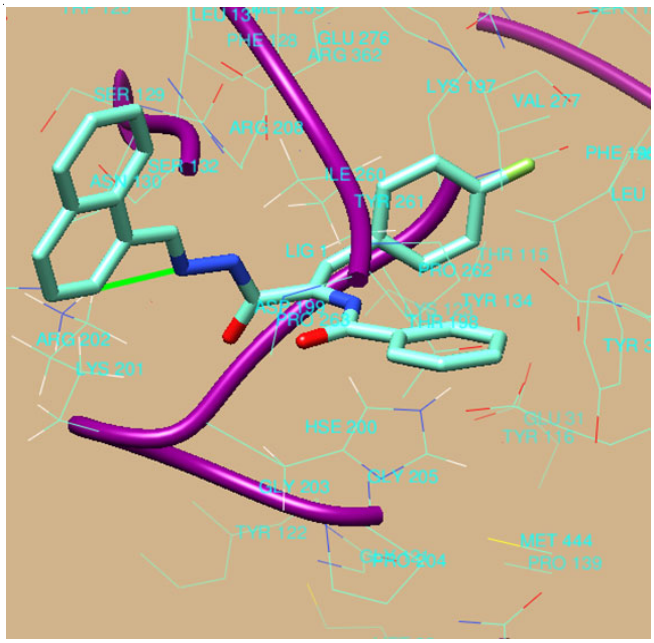


Fig. 1. Molecular docking interactions of 2-(benzamido)-3-(4-fluorophenyl) *N*'-((naphthalen-1-yl)methylene)acrylo hydrazide (**4k**) with COX-2

TABLE-4
ANTIINFLAMMATORY ACTIVITY OF SELECTED 2-(BENZAMIDO)-*N*′-((NAPHTHALEN-1-YL)METHYLENE)-3-PHENYLACRYLOHYDRAZIDES (**4a, 4e, 4g, 4h, 4k** AND **4m**)

Compd. code	Edema volume (Mean ± SD)	Inhibition (%)
Control	0.78 ± 0.05	0
Diclofenac	0.23 ± 0.04	70
4a	0.40 ± 0.03	49
4e	0.36 ± 0.05	54
4g	0.36 ± 0.01	54
4h	0.30 ± 0.04	62
4k	0.32 ± 0.04	59
4m	0.24 ± 0.03	69

Values were expressed as Mean ± SD (n = 5); Analyzed by one-way ANOVA followed by post hoc Dunnetts test.

Molecular descriptors: All the molecular properties were calculated by using Osiris property explorer (Table-5). All the synthesized compounds followed Lipinski's rule except compound **4g** (*m.w.* 509.5) and showed good % of oral absorption (71.45-84.66%). Among the derivatives, compound **5a** showed the highest oral absorption and good bioavailability followed by **4a**, **4b**, **4c** and **4d**. All the compounds showed log S values -6.77 to -7.41, indicating the good oral bioavailability. All the derivatives exhibited favourable drug-likeness (5.05-7.00).

Toxicity prediction: Toxicity like mutagenic, tumorigenic, irritant and reproductive risks of all the title compounds were predicted using OSIRIS Property Explorer (Table-6). Results indicated that most of the derivatives are free from the risks of mutagenic, reproductive and irritant except compounds **4g**, **4h**, **4l** having mutagenic toxicity, while compound **4e** shows reproductive risk.

Prediction of bioactivity score: Molinspiration 2011.06 was used to predict bioactivity scores of the synthesized compounds and results indicated that all compounds in the series are moderately active as protease inhibitors, kinase inhibitors, ion channel modulators, nuclear receptor ligands and inactive as GPCR ligands and enzyme inhibitors (Table-7).

TABLE-6
TOXICITY PREDICTION OF 2-(BENZAMIDO)-*N*′-((NAPHTHALEN-1-YL)METHY LENE)-3-PHENYLACRYLOHYDRAZIDES

Compd. code	Mutagenic	Tumorigenic	Reproductive	Irritant
4a	None	High	None	None
4b	None	High	None	None
4c	None	High	None	None
4d	None	High	None	None
4e	None	High	High	None
4f	None	High	None	None
4g	High	High	None	None
4h	High	High	None	None
4i	None	High	None	None
4j	None	High	None	None
4k	None	High	None	None
4l	High	High	None	None
4m	None	High	None	None
5a	Low	High	None	None
5b	None	High	None	None

ADME prediction: Pharmacokinetic properties of the synthesized compounds were evaluated using PreADMET. All the derivatives showed % HIA ranging from 94.86% to 96.42%, and moderate Caco2 cell permeability. All the compounds exhibited high % plasma protein binding (90.04-99.83%) except compounds **4g** and **4i** (88.91% and 87.90%). Compounds **4b, 4c, 4k, 4m, 5a** and **5b** showed optimum penetration into CNS *via* the blood brain barrier (Table-8).

Conclusion

2-(Benzamido)-*N*′-((naphthalen-1-yl)methylene)-3-(substituted phenyl)acrylohydrazides **(4a-m** and **5a-b)** were synthesized and screened for antioxidant, antibacterial and anti-inflammatory activities. Most of the synthesized compounds exhibited significant antioxidant and antibacterial activity. Among the series, compound **4i** showed good antioxidant activity in both *in vitro* methods, compound **5b** exhibited good antibacterial activity against Gram-positive and Gram-negative

TABLE-5
MOLECULAR PROPERTIES OF 2-(BENZAMIDO)-*N*′-((NAPHTHALEN-1-YL) METHYLENE)-3-PHENYLACRYLOHYDRAZIDES

Compd. code	MW	cLog P	cLog S	nAH	nDH	TPSA	DL	%Abs
4a	419.4	5.09	-6.77	5	2	70.56	6.39	84.65
4b	433.5	5.49	-6.79	5	2	70.56	5.05	84.65
4c	435.4	5.52	-6.79	5	2	70.56	6.33	84.65
4d	462.5	5.59	-6.81	5	2	70.56	6.09	84.65
4e	449.5	5.59	-7.07	6	3	90.79	6.37	77.67
4f	479.5	5.66	-7.08	6	2	79.79	6.35	81.47
4g	509.5	5.71	-7.09	6	2	73.8	7.02	83.53
4h	465.5	5.80	-7.10	6	2	73.8	7.78	83.53
4i	495.5	5.90	-7.12	7	3	100.0	6.34	74.5
4j	464.4	6.01	-7.41	7	2	89.02	6.35	78.28
4k	437.4	6.11	-7.41	7	3	99.66	6.75	74.61
4l	476.5	6.35	-7.53	8	3	109.2	6.34	71.32
4m	461.5	6.72	-7.70	8	2	98.25	6.35	75.10
5a	433.5	6.35	-7.41	5	2	70.56	6.33	84.66
5b	435.4	5.66	-6.77	6	3	90.79	6.37	77.67

%ABS, percentage of absorption; MW, molecular weight; nDH, number of H-bond donors; nAH, number of H-bond acceptors; TPSA, topological polar surface area; cLog P, Partition coefficient; cLog S, Solubility; DL, drug-likeness.

TABLE-7
BIOACTIVITY SCORES OF 2-(BENZAMIDO)-*N*′-((NAPHTHALEN-1-YL)METHYLENE)-3-PHENYL ACRYLOHYDRAZIDES

Compd. code	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
4a	-0.31	-0.66	-0.48	-0.68	-0.37	-0.30
4b	-0.34	-0.69	-0.50	-0.69	-0.41	-0.34
4c	-0.27	-0.60	-0.43	-0.56	-0.35	-0.26
4d	-0.29	-0.63	-0.42	-0.62	-0.37	-0.30
4e	-0.33	-0.68	-0.48	-0.66	-0.40	-0.33
4f	-0.32	-0.68	-0.46	-0.64	-0.40	-0.31
4g	-0. 31	-0.73	-0.44	-0.66	-0.40	-0.31
4h	-0.30	-0.65	-0.44	-0.60	-0.41	-0.28
4i	-0.30	-0.67	-0.41	-0.59	-0.38	-0.25
4j	-0.40	-0.65	-0.54	-0.70	-0.46	-0.36
4k	-0.30	-0.65	-0.44	-0.65	-0.38	-0.31
4l	-0.33	-0.68	-0.43	-0.70	-0.39	-0.33
4m	-0.29	-0.62	-0.47	-0.59	-0.36	-0.28
5a	-0.34	-0.71	-0.49	-0.68	-0.42	-0.34
5b	-0.28	-0.61	-0.42	-0.55	-0.36	-0.24

TABLE-8
ADME PROPERTIES OF 2-(BENZAMIDO)-*N*′-((NAPHTHALEN-1-YL) METHYLENE)-3-PHENYLACRYLOHYDRAZIDES

Compd. code	HIA (%)	*In vitro* $Caco_2$	MDCK	PPB	BBB
4a	96.13	21.86	8.39	94.09	0.87
4b	96.19	22.06	0.83	92.50	1.60
4c	94.86	21.26	0.06	97.02	1.07
4d	96.40	24.84	0.05	93.25	0.73
4e	96.36	24.18	0.10	92.74	0.32
4f	96.42	26.60	0.08	90.04	0.14
4g	96.35	29.45	0.06	88.91	0.07
4h	95.02	21.78	0.06	91.07	0.63
4i	95.00	22.50	0.05	87.90	0.35
4j	95.47	20.36	0.04	99.83	0.04
4k	96.13	24.66	0.07	97.22	1.12
4l	95.32	21.06	0.04	93.96	0.23
4m	96.30	23.02	0.16	94.81	3.15
5a	96.19	22.24	9.31	92.46	1.54
5b	94.86	20.22	0.12	96.11	1.08

HIA (%): Percentage human intestinal absorption; PCaco2 (nm/s): Caco2 cell permeability in nm/s; MDCK (nm/sec): Madin-Darby canine kidney cell permeabity in nm/sec; PPB(%): *in vitro* plasma protein Binding.

stains comparable to that of standard drugs amoxycillin and streptomycin. However, isopropyl derivative **4m** exhibited strong antiinflammatory activity which is comparable to that of standard diclofenac. Docking studies also revealed that compound **4m** exhibited good binding interactions with COX-2. *In silico* studies indicated that all compounds in this series followed Lipinski's rule and exhibited good oral absorption and good bioavailability.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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