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Synthesis, bioactivity and Docking Study of Some New Indole-hydrazone Derivatives

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ABSTRACT

In the present study, a series of new indole-hydrazone derivatives were synthesized. The structures of the synthesized compounds were established on the basis of elemental and spectral (IR and ^{1}H-NMR) studies. All synthesized compounds were screened for their anti-inflammatory against carrageenan induced oedema in albino rats at a dose of 0.2 mmol/kg using indomethacin as a standard drug. The reduction in oedema formation was ranged between 72.3 – 89.3 % for all the synthesized compounds compared to indomethacin (46 % inhibition). Moreover, their anticancer activity against breast cancer cell line (MCF-7) at a dose of 100µg/ml was evaluated. The compounds **2f** and **2j** showed mild anticancer activity (61% and 68% inhibition, respectively) compared to doxorubicin. Furthermore, all molecules were docked to the active site of both cox-1 and cox-2 using the docking program Molego virtual docker. All prepared compounds showed high docking score against both cox-1 and cox-2. The dock scores and binding energy were found to be in good agreement with the pharmacological results. Finally, the drug likeness and bioactivity were predicted using Molinspiration software. The results revealed that all new compounds show good drug likeness score and bioactivity score.

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are a diverse class of drugs commonly used for the treatment of inflammatory conditions, analgesia and fever with little side effects to gastrointestinal tract as well as relieving the pains of everyday life. Therefore, there is an increasing interest in the research and development of selective COX- 2 inhibitors (Dannhandt and Kiefer, 2001; Pratico and Digne, 2005). Notably, epidemiological and clinical studies suggest that non-steroidal anti-inflammatory drugs (NSAIDs), including, cyclooxygenase (COX)-2 selective inhibitors, reduce the risk of developing cancer (Chan, 2002; Thun *et al*, 2002). Moreover, selective COX-2 inhibitors may demonstrate new important therapeutic benefits as anticancer agents, as well as preventing premature labor and perhaps even retarding the progression of Alzheimer's disease (Aisen, 2002). Literature survey reveals that hydrazones and their derivatives are providing valuable therapeutic class in organic chemistry. These compounds have interesting biological properties, such as anti-inflammatory, analgesic, anticonvulsant, anti-tuberculous, antitumor, anti-HIV and antimicrobial activities (Rollas and Kucuk Guzel, 2007; Babahan *et al*., 2013). Further, It is evident that the C=N linkage in azomethine derivatives is essential structure requirement for biological activity (Vinusha, 2015) such as, antimicrobial (da Silva *et al*., 2011), antibacterial (Vashi and Naik, 2004), anticancer (Kuz'min *et al*., 2005). It has been reported that indole and its derivatives are an important class of bioactive molecules in the field of drugs and pharmaceuticals areas including, anti-inflammatory (Sondhi *et al*., 2006, Bhati and Kumar, 2008; Hemalatha *et al*., 2013), antimalarial (Santos, 2015), antimicrobial (Karekal *et al*., 2013; Bhaskar *et al*., 2007), antiviral (Tichý *et al*., 2012), antibacterial (Tiwari *et al*., 2006), antifungal (Guiqing *et al*., 2016), anticancer (Kumar and Kumer, 2013; Macdonough *et al*., 2013) and antioxidant activities (Estevão *et al*., 2010).

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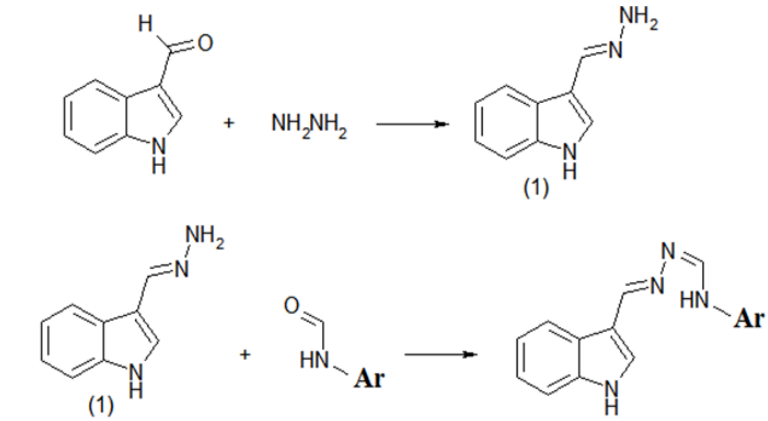

Ar =	2a	2b	2c	2d	2e	2f
Ar =	2g	2h	2i	2j	2k	2l

Scheme 1: The synthetic route for preparation of a series of new indole-hydrazone derivatives (2a-2l)

Keeping in view of biological importance of the three molecular moieties viz., indole, hydrazone and azomethine group, it has been felt worthwhile to synthesize as depicted in **Scheme I**. We wish in the present work to generate new leads by synthesize indole-hydrazone derivatives in the hope of obtaining better anti-inflammatory and anticancer agents. Further, the molecular docking were studied to help in understanding the various interactions between our derivatives and enzyme active sites (cox-1 and cox-2) as well as the correlation between docking studies and the in vivo/*in vitro* studies. In addition, the drug likeness and bioactivity were evaluated for all prepared compounds.

MATERIAL AND METHODS

Chemistry

All melting points were determined with a *Kofler* Block apparatus. The progress of the reactions was monitored by thin layer chromatography (TLC) using aluminum silica gel plates 60 F 245 and further purification was performed using column chromatography (silica gel, 70–230 mesh). IR spectra were recorded on a Perking Elmer 1430 ratio recording infrared spectrophotometer with CDS data station using KBr Wafer technique at the Central Laboratory, Tanta University, and The ^{1}H-NMR spectra were recorded on a Varian Gemini-300 BB Spectrometer at 300 MHz with TMS as a standard, and Elemental Analysis at the Micro Analytical Center, Cairo University, Cairo, Egypt. Chemicals and solutions were purchased from Merck Co. and they were in reactive grade.

1H -indole-3-carbaldehyde hydrazone (1)

Equimolar quantities of hydrazine hydrate (1 ml, 0.02mol) and indole-3-carbaldehyde (2.9 g, 0.02mol) were dissolved in 50 ml of absolute ethanol. The mixture was refluxed for 9 hr.

After the completion of the reaction (as monitored by TLC), the excess of solvent was removed under reduced pressure. The obtained product was recrystallized with ethanol (Lohitha, *et al*., 2011).

General procedure

Suspend 1H-indole-3-carboxaldhyde hydrazone (**1)** (0.01 mol) in absolute ethanol (20 - 40 ml). To the previous mixture N-substituted formamide (0.01mol) was added gradually. The mixture was refluxed with stirring for 16 - 30 hr. After TLC monitoring using chloroform : ethyl acetate (9:1) or n-hexane : ethyl acetate 8:2 (v/v) as eluent, the solution was cooled to 5 ˚C in ice bath.

The product formed was filtered, washed with cold water, purified by silica gel column chromatography using chloroform as eluent, then the solvent was evaporated and the product was recrystallized from ethanol to yield the corresponding compounds.

N'-((1H-indol-3-yl)methylene)-N-phenylformohydrazon-amide (2a)

The compound **2a** was obtained as yellow powder, yield of 78%; M.p.> 300 ˚C; IR (KBr, ν_{max} (cm^{-1})): 1577 (C=C), 1620 (C=N), 3214 (NH); ^{1}H-NMR (300 MHz, *DMSO*-d_6, δ (ppm)): 7.151-7.486 (m, 9H, Ar-H), 8.335 (d, 2H, , *J*= 7.5Hz, Ar-NH, N-CH=N), 8.904 (s, 1H,indole H-2), 11.652 (s, 2H, indole NH, CH=N); Elemental analysis: Calc. for $C_{16}H_{14}N_4$: C, 73.26; H, 5.38; N, 21.36; found: C, 73.38; H, 5.10; N, 19.82%.

N'-((1H-indol-3-yl) methylene)-N-chlorophenyl)formo-hydrazonamide (2b)

The compound **2b** was obtained in the yield of 85%; M.p. 300 °C; IR (KBr, ν_{max} (cm^{-1})): 1577 (C=C), 1620 (C=N), 3215 (NH); ^{1}H-NMR (300 MHz, *DMSO*-d_6, δ (ppm)): 7.174-7.911 (m, 8H, Ar-H), 8.339 (d, 2H, , *J*= 7.5Hz, Ar-NH, N-CH=N), 8.907 (s, 1H,indole H-2), 11.657 (s, 2H, indole NH, CH=N) ; Elemental analysis: Calc. for $C_{16}H_{13}ClN_4$: C, 64.76; H, 4.42; N, 18.88; found: C, 65.02; H, 4.63; N, 19.10%.

N'-((1H-indol-3-yl) methylene)-N-(4-chlorophenyl)-formohydrazonamide (2c)

The compound **2c** was obtained in the yield of 80%; M.p. 298 °C; IR (KBr, ν_{max} (cm^{-1})): 1577 (C=C), 1619 (C=N), 3213 (NH); ^{1}H-NMR (300 MHz, *DMSO*-d_6, δ (ppm)): 7.172-7.910 (m, 9H, Ar-H), 8.331 (d, 2H, , *J*= 7.5Hz, Ar-NH, N-CH=N), 8.899 (s, 1H,indole H-2), 11.649 (s, 2H, indole NH, CH=N) ;Elemental analysis: Calc. for $C_{16}H_{13}ClN_4$: C, 64.76; H, 4.42; N, 18.88; found: C, 64.94; H, 4.43; N, 18.97%.

N'-((1H-indol-3-yl) methylene)-N-(3-nitrophenyl)formo-hydrazonamide (2d)

Compound **2d** was obtained in the yield of 85% as yellow crystalline product; M.p. 300 °C; IR (KBr, ν_{max} (cm^{-1})): 1576 (C=C), 1620 (C=N), 3191 (NH); ^{1}H-NMR (300 MHz, *DMSO*-d_6, δ (ppm)): 7.149-7.910 (m, 9H, Ar-H), 8.333 (d, 2H, , *J*= 7.5Hz, Ar-NH, N-CH=N), 8.904 (s, 1H,indole H-2), 11.651 (s, 2H, indole NH, CH=N) ;Elemental analysis: Calc. for $C_{16}H_{13}N_5O_2$: C, 62.53; H, 4.38; N, 22.79; found: C, 62.81; H, 4.38; N, 22.98%.

N'-((1H-indol-3-yl) methylene)-N-(4-nitrophenyl)formo-hydrazonamide (2e)

Compound **2e** was obtained in the yield of 89% as yellow crystalline product; M.p. 295 °C; IR (KBr, ν_{max} (cm^{-1})): 1577 (C=C), 1619 (C=N), 3215 (NH); ^{1}H-NMR (300 MHz, *DMSO*-d_6, δ (ppm)): 7.177-7.911 (m, 9H, Ar-H), 8.339 (d, 2H, , *J*= 7.5Hz, Ar-NH, N-CH=N), 8.907 (s, 1H,indole H-2), 11.653 (s, 2H, indole NH, CH=N) ;Elemental analysis: Calc. for $C_{16}H_{13}N_5O_2$: C, 62.53; H, 4.38; N, 22.79; found: C, 62.63; H, 4.27; N, 22.82%.

N'-((1H-indol-3-yl) methylene)-N-(3-methoxyphenyl)-formohydrazonamide (2f)

Compound **2f** was obtained in the yield of 75% as yellow color product; M.p. > 300 °C; IR (KBr, ν_{max} (cm^{-1})): 1576 (C=C), 1619 (C=N), 3213 (NH); ^{1}H-NMR (300 MHz, *DMSO*-d_6, δ (ppm)): 4.087 (s,3H,OCH3), 7.197-8.020 (m, 9H, Ar-H), 8.189(d, 2H, , *J*= 7.5Hz, Ar-NH, N-CH=N), 8.048 (s, 1H,indole H-2), 11.952 (s, 2H, indole NH, CH=N) ;Elemental analysis: Calc. for $C_{17}H_{16}N_4O$: C, 69.85; H, 5.52; N, 19.17; found: C, 70.05; H, 5.64; N, 19.31%.

N'-((1H-indol-3-yl) methylene)-N-(4-methoxyphenyl)-formohydrazonamide (2g)

Compound **2g** was obtained in the yield of 70% as yellow crystals; M.p. 290 °C; IR (KBr, ν_{max} (cm^{-1})): 1576 (C=C), 1619 (C=N), 3191 (NH); ^{1}H-NMR (300 MHz, *DMSO*-d_6, δ (ppm)): 3.805 (s,3H,OCH3), 7.175-7.709 (m, 9H, Ar-H), 8.361 (d, 2H, , *J*= 7.5Hz, Ar-NH, N-CH=N), 8.908 (s, 1H,indole H-2), 11.653 (s, 2H, indole NH, CH=N) ;Elemental analysis: Calc. for $C_{17}H_{16}N_4O$: C, 69.85; H, 5.52; N, 19.17; found: C, 70.11; H, 5.63; N, 19.35%.

N'-((1H-indol-3-yl) methylene)-N-(3-methylphenyl-) formohydrazonamide (2h)

Compound **2h** was obtained in the yield of 67% as yellow crystalline product; M.p. 300 °C; IR (KBr, ν_{max} (cm^{-1})): 1577 (C=C), 1620 (C=N), 3214 (NH); ^{1}H-NMR (300 MHz, *DMSO*-d_6, δ (ppm)): 3.312 (s,3H, CH3),7.148-7.812 (m, 8H, Ar-H), 8.189 (d, 2H, , *J*= 7.5Hz, Ar-NH, N-CH=N), 8.900 (s, 1H,indole H-2), 11.652 (s, 2H, indole NH, CH=N) ;Elemental analysis: Calc. for $C_{17}H_{16}N_4$: C, 73.89; H, 5.84; N, 20.27; found: C, 74.13; H, 6.05; N, 20.41%.

N'-((1H-indol-3-yl) methylene)-N-(4-methylphenyl)-formohydrazonamide (2i)

The Compound **2i** was obtained in the yield of 71% as yellow crystalline product; M.p. 312 °C; IR (KBr, ν_{max} (cm^{-1})): 1576 (C=C), 1620 (C=N), 3212 (NH); ^{1}H-NMR (300 MHz, *DMSO*-d_6, δ (ppm)): 3.312 (s,3H, CH3),7.177-7.709 (m, 8H, Ar-H), 8.361 (d, 2H, , *J*= 7.5Hz, Ar-NH, N-CH=N), 8.905 (s, 1H,indole H-2), 11.652 (s, 2H, indole NH, CH=N) ;Elemental analysis: Calc. for $C_{17}H_{16}N_4$: C, 73.89; H, 5.84; N, 20.27; found: C, 74.11; H, 6.20; N, 20.43%.

N'-((1H-indol-3-yl) methylene)-N-benzylformohydrazon-amide (2j)

The Compound **2j** was obtained in the yield of 63% as yellow crystalline product; M.p. 310 °C; IR (KBr, ν_{max} (cm^{-1})): 1578 (C=C), 1620 (C=N), 3217 (NH); ^{1}H-NMR (300 MHz, *DMSO*-d_6, δ (ppm)): 3.312 (s,2H, CH2),7.177-7.709 (m, 9H, Ar-H), 8.361 (d, 2H, , *J*= 7.5Hz, Ar-NH, N-CH=N), 8.905 (s, 1H,indole H-2), 11.652 (s, 2H, indole NH, CH=N) ;Elemental analysis: Calc. for $C_{17}H_{16}N_4$: C, 73.89; H, 5.84; N, 20.27; found: C, 74.01; H, 5.99; N, 20.42%.

N'-((1H-indol-3-yl) methylene)-N-(naphthalen-1-yl)formohydrazonamide (2k)

Compound **2k** was obtained in the yield of 70 % as yellow crystalline product; M.p. 315 °C; IR (KBr, ν_{max} (cm^{-1})): 1577 (C=C), 1620 (C=N), 3216 (NH); ^{1}H-NMR (300 MHz, *DMSO*-d_6, δ (ppm)): 7.148-7.488 (m, 9H, Ar-H), 8.361 (d, 2H, , *J*= 7.5Hz, Ar-NH, N-CH=N), 8.905 (s, 1H,indole H-2), 11.651 (s, 2H, indole NH, CH=N) ;Elemental analysis: Calc. for $C_{20}H_{16}N_4$: C, 76.90; H, 5.16; N, 17.94; found: C, 77.14; H, 5.27; N, 17.89%.

N'-((1H-indol-3-yl) methylene)-N-(quinolin-8-yl) formo-hydrazonamide (2l)

The Compound **2l** was obtained in the yield of 72 % as yellow crystalline product; M.p. 311 °C; IR (KBr, ν_{max} (cm^{-1})): 1577 (C=C), 1620 (C=N), 3216 (NH); ^{1}H-NMR (300 MHz, *DMSO*-d_6, δ (ppm)): 7.150-7.487 (m, 9H, Ar-H), 8.334 (d, 2H, , *J*= 7.5Hz, Ar-NH, N-CH=N), 8.902 (s, 1H,indole H-2), 11.654 (s, 2H, indole NH, CH=N) ;Elemental analysis: Calc. for $C_{19}H_{15}N_5$: C, 72.83; H, 4.82; N, 22.35; found: C, 73.92; H, 5.61; N, 23.03%.

Anti-inflammatory Activity

Animals

Animals were obtained from the animal house of the Faculty of Pharmacy, Tanta University, Tanta Egypt; all animals were allowed free access to water and were kept on a constant standard diet. All procedures involving animals were carried out in accordance with the guidelines for the care and use of laboratory animals and were approved by the Ethics Committee of the Faculty of Pharmacy, Tanta University, Tanta, Egypt. Adult Wistar strain rats of male sex, weighing 100–140 g, were used for anti-inflammatory activity. The animals were allowed food and water ad libitum except during the experiments. They were housed in wire- mesh cages at 25 ± 2°C, with 50 ± 5% relative humidity and 12 hr light/dark cycle. The animals were randomly allocated into groups and fasted for 12–24 hours before the experimental study and used for determining the anti-inflammatory activity. All test compounds and the reference drug were administered I.P, suspended in dimethylsulfoxide (DMSO) solution. This study was done by following the procedure of Winter *et al.* (Winter, *et al*., 1962). The rats were divided into three groups (control, tested compounds and standard drug) of six animals each. A freshly prepared suspension of carrageenan (1.5% in 0.9% saline), 0.1 mL was injected S.C. into the subplantar region of the right hind paw of each rat. The test compounds and standard drug were administered I.P at the dose of 0.2 mmole/Kg to the animals of tested derivatives groups and the standard drug group, respectively, 2 hr before the carrageenan injection. The paw weight of each rat was measured after 4hr of carrageenan treatment with the help of a Plethysmometer. The percent anti-inflammatory activity was calculated according to the formula given below.

$$\% \text{ Inhibition} = \frac{\text{Mean edema of control group} - \text{Mean edema of test}}{\text{Mean edema of control group}} \text{x}100$$

Evaluation of cytotoxic activity of target compound

Anticancer activity evaluation using the Human breast carcinoma cell line (MCF7) was performed in the National Cancer Institute, Cairo University, Cairo, Egypt, using the method of (Skehan, *et al*., 1990).

Cells were plated in 96-mutiwell plate (10 cells/well) for 24 hr before treatment with the compounds to allow attachment of cell to the wall of the plate. The 100 µg/ml of each compound under test was added to the cell monolayer triplicate wells. Monolayer cells were incubated with each compound for 48 hr at 37ºC and in atmosphere of 5% CO_2. After 48 hr, cells were fixed, washed and stained with Sulfo-Rhodamine-B stain. Excess stain was washed with acetic acid and attached stain was recovered with Tris EDTA buffer. Color intensity was measured in an ELISA reader.

Evaluation of drug likeness

The drug likeliness was evaluated using the Lipinski rule of 5 via Lipinski drug filter protocol (Lipinski, 2004) using Molinspiration software (Molinspiration, 2012). Our synthesized target compounds were passed the Lipinski rule of 5 and have properties that would make it a likely orally active drug in humans. The predicted drug likeness score of new compounds were compared with standard drugs indomethacin and doxorubicin as shown in **Table 3**.

Bioactivity score

Bioactivity of prepared compounds was checked by calculating the activity score toward G protein coupled receptors (GPCRs) ligand, ion channel modulator, nuclear receptor legend, kinase inhibitor, protease inhibitor and enzyme inhibitor. All the parameters were checked with the help of software Molinspiration drug-likeness score online (Verma, 2012). The predicted bioactivity score of synthesized compounds and indomethacin, doxorubicin as reference drugs were illustrated in **Table 4**.

Molecular docking studies

Protein Preparation

The three-dimensional crystal structure of enzymes taken from Protein Data Bank (PDB) (http://www.rcsb.org/) is as follows: enzymes COX-1 (PDBID: 3N8Y) (Sidhu, *et al*., 2010) and COX-2 (PDB ID: 3LN1) (Wang, *et al*., 2010). All the PDB's were loaded in the Molegro virtual docker (MVD) (Thomsen, *et al*., 2009) with the removal of all water molecules and cofactors. The standard Molegro algorithm was utilized for rendering the missing charges, protonation states, and assigning of polar hydrogen to the receptor.

Ligands preparation

The Structures of ligands were drawn using marvin sketch and energy minimization was done. Energy minimization was done to help the docking program, Molegro Virtual Docker (MVD), (Thomsen, *et al*., 2009) to identify the bioactive conformer from the local minima. One major advantage of Molegro Virtual Docker (MVD) is that it helps in assigning the missing bond orders, charges, bonds, and hybridization states of the imported ligands.

Molecular docking

Flexible ligand models were used for docking and post-docking geometry optimizations. The post-docking geometry

optimizations were carried out using Molegro Virtual Docker (MVD), Simulations the ligand was binding with site of enzymes COX-1 (PDBID: 3N8Y) and COX-2 (PDB ID: 3LN1). A docking sphere (15 Å radius) was placed on the binding sites of each protein structure in order to allow different orientations of each ligand to be searched in the binding cavities and for multiple protein-ligand poses to be returned. The Root Mean Square Deviation (RMSD) threshold for multiple cluster poses was set at <1.00 Å.

The docking algorithm was set at maximum restoration, of 1500 with a simple evolution population size of 50 and a minimum of 30 runs for each ligand. Each binding site of oligomeric structures was searched, and the lowest energy pose (based on the mol Dock Score and re-rank scores) for each ligand across enzymes COX-1 (PDBID: 3N8Y) and COX-2 (PDB ID: 3LN1) structures. The predicted mol Dock Score and re-rank scores for all prepared compounds as lowest energy pose are presented in **Table 5**.

RESULTS AND DISCUSSION

The synthetic route used to synthesize title compounds was outlined in **Scheme 1**. Hyrazone (1), the starting material was prepared according to the method reported in literature (Lohitha, *et al*., 2011). Hydrazone was condensed with different substituted formamides in ethanol to give the corresponding compounds in good yield.

Reaction of hydrazone with electron withdrawing group at para- position of aromatic formamide was faster and gave better yield than the corresponding meta- substituted ones. Accordingly. the derivatives with electron withdrawing group at para- position for example; p-nitro **(2e)** had better yield and short refluxing time than p-methoxy and p-chloro (**2g** and **2c**).

Synthesized compounds were purified by column chromatography on silica gel. The IR spectra of synthesized compounds exhibited very similar features as the expected bands for the characteristic groups such as NH, C=N, and disappearance of NH_2 stretching vibrations. Presence of peak in the region 3191-3217 cm^{-1}indicates the existence of NH moiety and that of peak in the region 1619–1620 cm^{-1} indicates C=N, in all the compounds.

In the ^{1}H-NMR spectral data, all protons were seen according to the expected chemical shift and integral values, the aromatic protons appeared as a multiplet peaks within the range 6.8-7.8 δ ppm, doublet signals for Ar-NH and N-CH=N appeared at 8.361 δ ppm. Signals belonging to $–NHNH_2$ of hydrazone are disappeared indicating that functionalization of hydrazone with N substituted formamide. Elemental analysis data were also in line with theoretical values.

Anti-inflammatory activity

All the newly synthesized compounds **2a-2l** were evaluated for their anti-inflammatory activity against carrageenin-induced paw edema method in rats, using indomethacin as reference drug at a dose of 0.2 mmol/kg I.P. Percent of the edema inhibition was calculated after 4 hr of carrageenin treatment. The tested compounds showed good anti-inflammatory activity ranging between 72.3 – 89.3%. The results were tabulated in **Table1.**

Table1: Anti-inflammatory activity of the tested compounds.

Comp. No.	Dose (mmol/Kg)	Anti-inflammatory activity(% inhibition ±SEM)
2a	0.2	72.30 ± 1.51
2b	0.2	76.00 ± 1.82
2c	0.2	76.50 ± 1.78
2d	0.2	87.70 ± 1.52
2e	0.2	89.30 ± 1.53
2f	0.2	79.50 ± 1.65
2g	0.2	80.70 ± 1.75
2h	0.2	74.90 ± 1.69
2i	0.2	75.20 ± 1.56
2j	0.2	73.50 ± 1.95
2k	0.2	72.65 ± 1.56
2l	0.2	72.50 ± 1.52
Indomethacin	0.2	46.00 ± 1.92
Control	0.1 ml DMS	---

On comparison, standard drug indomethacin showed 46% inhibition after 4 hr of treatment. Among the tested compounds, **2d** (m-NO_2), **2e** (p-NO_2) and **2g** (p-OCH_3) may be considered as potent anti-inflammatory agents (edema reduction percent: 89.3%, 87.7% and 80.7% respectively), while indomethacin was 46%. On the other hand, the derivative with electron withdrawing group at para-position such as **2e (p-NO_2)** showed somewhat high anti-inflammatory activity (89.3%) compared with the other position such as meta- positions (87.7%) e.g. **2d (m-NO_2)**. These gave an indication that, the nature and the position of substituent effect on the activities of the prepared compounds.

Evaluation of cytotoxic activity of target compounds

The synthesized target compounds were evaluated for their cytotoxic activity *in vitro* against Human breast carcinoma cell line (MCF7) using doxorubicin as a reference compound. The results of single dose experiment (100µg/ml) of synthesized compounds performed on Human breast carcinoma cell line (MCF7) were represented in **Table 2.**

From the results found that the compounds **2f** and **2j** exhibited the percent of inhibition 61.5 and 68 % respectively at the dose 100 µg/ml. While, the rest of compounds showed less activity (the percent of inhibition were ranged between 0 - 20.5 %). Also, the derivatives at meta- position had higher activity than derivatives at para-position. For example, the m-methoxy derivative (**2f**) has inhibition percent at 100 µg/ml equal 61.5 %; while the p-methoxy derivative **(2g)** has inhibition percent = 0 % at the same dose.

Table 2: Cytotoxic activity screening on MCF-7 cell line (100µg/ml).

Sample No.	2a	2b	2c	2d	2e	2f	2g	2h	2i	2j	2k	2l
Inhibition%	20.5	15	5	20	17	61.5	0	5	15	68	15	13

Table 3: Drug likeness score for the tested compounds compared with indomethacin and doxorubicin.

Compd.No.	TPSA (A^2)	n.rotb	NA	miLogP	n ON	n OHNH	NV	MW
Rule	< 160	$< 10^1$	$20 – 70^2$	≤ 5	< 10	< 5	≤ 1	< 500
2a	52.55	4	20	3.23	4	2	0	262.32
2b	52.55	4	21	3.89	4	2	0	296.76
2c	52.55	4	21	3.91	4	2	0	296.76
2d	98.37	5	23	3.17	7	2	0	**307.31**
2e	98.37	5	23	3.19	7	2	0	**307.31**
2f	61.78	5	22	3.27	5	2	0	**292.34**
2g	61.78	5	22	3.29	**5**	2	0	**292.34**
2h	52.55	4	21	3.66	4	2	0	**276.34**
2i	52.55	4	21	3.68	4	2	0	**276.34**
2j	52.55	5	21	2.94	4	2	0	**276.34**
2k	52.55	4	24	4.39	4	2	0	**312.38**
2l	65.44	4	24	3.19	**5**	2	0	**313.36**
Indomethacin	59.30	3	23	3.95	4	1	0	327.77
Doxorubicin	206.08	5	39	0.57	12	7	3	543.52

1 = (Veber, et al., 2002), 2 = (dProperties user`s manual, 2011)

Notes: TPSA: Total molecular polar surface area; n.rotb:number of rotatable bonds; N A:number of atoms; Milog P: partition coefficient; n ON: number of hydrogen acceptor; nONH: number of hydrogen donor, NV: number of violation of five Lipinsky rules; MW: molecular weight and DLMS: Drug likness model score.

Table 4: Bioactivity score of the tested compounds compared with indomethacin and doxorubicin.

Compound	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
2a	-0.1955	-0.4883	-0.2808	-0.4442	-0.4834	-0.0614
2b	-0.1671	-0.4775	-0.2481	-0.4203	-0.5089	-0.1214
2c	-0.1553	-0.4724	- 0.2578	-0.4132	-0.4709	-0.098
2d	-0.2783	-0.5087	-0.3325	-0.462	-0.4873	-0.1772
2e	-0.2617	-0.4796	-0.3451	-0.4074	-0.4711	-0.1631
2f	-0.1919	-0.549	-0.237	-0.3866	-0.455	-0.1309
2g	-0.1761	-0.5382	-0.2477	-0.3575	-0.4337	-0.1143
2h	-0.2169	-0.5696	-0.2871	-0.4252	-0.4974	-0.1485
2i	-0.2036	-0.5556	-0.2906	-0.4222	-0.4881	-0.129
2j	-0.0445	-0.3596	-0.2094	-0.3164	-0.3376	-0.0658
2k	-0.0427	-0.3124	-0.1174	-0.2695	-0.2607	-0.0127
2l	-0.0119	-0.3371	-0.1352	-0.3534	-0.2913	0.024
Indomethacin	0.30	-0.25	-0.11	-0.44	-0.07	0.36
Doxorubicin	0.20	-0.20	-0.07	0.32	0.67	0.66

>0 - active, -5.0 - 0.0 - moderately active, < -5.0 – inactive.

Evaluation of drug likeliness

Lipinski's rule of five or simply the Rule of five (RO5) is a rule of survey and to evaluate drug likeness (physical-chemical properties) or determine if a chemical compound will be orally bioavailable (Lipinski, 2004). The drug likeness was calculated and discussed on the basis of Lipinski's rule and its component for all prepared compounds using Molinspiration software "on-line test". The results were summarized in **Table 3**. The physical-chemical properties including: an octanol-water partition coefficient **(Milog P)** < 5 that means these shows good permeability across cell membrane, polar surface area **(TPSA)** < 160 Å2 which shown to be a very good descriptor characterizing drug absorption, number of violation **(*n* violations)** =1 or < 0 it means compound easily bind to receptor, molecular weight **(MW)** < 500, number of rotatable bonds ***(n* rotb)** < 10 this measures molecular flexibility (Veber, *et al*., 2002), number hydrogen bond donors **(n OHNH)** ≤ 5 (The sum of OHs and NHs), number (MW) > 500, total molecular polar surface area (TPSA) > $160A^{o2}$ and hydrogen bond acceptors **(nON)** > 7, so doxorubicin has n violations **(NV) = 3,** as shown in **Table 3**. From the results reveal that these compounds are orally bioactive because they possess groups which act as substrate for transporter.

Bioactivity score

Similarly, all compounds were taken for calculation of bioactivity score towards G protein–coupled receptors (GPCR) ligands, ion channel modulator, kinase inhibitors, nuclear receptor inhibitors and other enzyme targets based on Molinspiration software "on-line test". These bioactivity results were summarized in **Table 4.** The scores allowed adequate identification of active, moderately active or inactive molecules. If the bioactivity scores is (≥ 0.00) may refer to considerable biological activities, if the bioactivity scores (-5.0 to 0.0) it is moderately active and finally if the bioactivity scores (< -5.0) it is inactive. Consequently, the

results showed the following observations: a) GPCR ligand: all our compounds were found to be moderately bioactive, the bioactivity scores (-0.0119 to -0.2783) comparing with indomethacin and doxorubicin (0.30 and 0.20) respectively. b) ion channel modulator: all our compounds were found to be better bioactive, the bioactivity scores (-0.3124 to -0.5696) comparing with indomethacin and doxorubicin (-0.25 and -0.20) respectively. c) kinase inhibitors: all our compounds were found to be moderately bioactive the bioactivity scores (-0.1174 to -0.3451) comparing with indomethacin and doxorubicin (-0.11 and -0.07) respectively. d) nuclear receptor inhibitors: in comparison with indomethacin and doxorubicin the bioactivity scores were -0.44 and 0.32 respectively whereas our compounds were found to be moderately bioactive with the bioactivity scores (-0.2695 to -0.4252). e) Protease inhibitor: all our compounds were found to be moderately bioactive, the bioactivity scores (-0.2607 to -0.5089), while indomethacin and doxorubicin were -0.07 and 0.67 respectively. f) Enzyme inhibitor: all our compounds were found to be better bioactive; the bioactivity scores were ranged between 0.024 to -0.1772, whereas indomethacin and doxorubicin were 0.36 and 0.66 respectively.

The results herein are well below the limits of bioactivity score (-0.5089 to 0.024) **(Table 4)**. In addition, the designed molecules obeyed the Lipinski rule of five. So the designed molecules may be useful as a lead compound for various diseases like depression, anti inflammatory, cancer and others diseases.

Molecular docking studies

Molecular docking study was performed to investigate the binding affinities and interaction modes between our compounds and the target enzymes using the Molegro Virtual Docker (MVD). All prepared compounds were incorporated in the active site of the enzymes isoform COX-1 (PDBID: 3N8Y) and isoform COX-2 (PDB ID: 3LN1). The docking scores were expressed in negative energy terms; the lower binding free energy is the better binding affinity. In addition, the docking scoring function of Mol Dock is an extension of the piecewise linear potential (PLP) including new hydrogen bonding and electrostatic terms. To further improve docking accuracy, a re-ranking scoring function is introduced. Subsequently, Mol Dock score and re-rank score were indicated in **Table 5**. The docking study displayed that most of the prepared compounds showed promising affinity to inhibit both cox-1 and cox-2. Thus, the synthesized compounds showed good docking scores ranged from -129.601 to -148.276 with cox-1 and from -92.797 to -108.757 with cox-2. From **Table 5** appears that the compounds **2d** and **2e** are considered as good anti-inflammatory agents because they have good mol dock scores of -146.71 and -148.276 with the active site of cox-1 and -104.223, and -108.757 with cox-2 enzymes respectively. While indomethacin showed dock score of -131.578 and 92.5925 with cox-1 and cox-2 respectively. According to docking analysis the most active ligand showed good binding interactions in terms of hydrogen bond and hydrophobic interactions with the residues of proteins amino acids. From the results, compound **2d** indicated hydrogen bond interaction with Arg-374, Arg-376, Asp-375 and Phe-142 and compound **2e** displayed hydrogen bond interaction with Arg-374, Arg-376, Arg-375 and Gly-225 with cox-1, whereas, the reference compounds (indomethacin) showed hydrogen bond with Arg-374, Arg-376 and electrostatic with Arg-374, Arg-376 as represented in **Fig.1 (a)**. While with cox-2, compound **2d** showed hydrogen bond interaction with Lys-239, Ser-549, and His-228 and Lys-346, while compound **2e** showed hydrogen bond interaction with Ser-549 and His-228 and Lys-239, whereas, the reference compounds (indomethacin) showed hydrogen bond with Lys-346, Lys-328, Phe-347 and Asn-546 as indicated in **Fig.1 (b)**. These results are further supported to the anti-inflammatory activity in vivo as illustrated in **Table1** which revealed that the most active compounds (2d and 2e) showed edema reduction percent of 89.3%, 87.7%. So, all the compounds exhibited a good docking score which were agreed and supported to the in- vivo anti inflammatory activity of these compounds.

Table 5: Docking parameters in active site of the enzymes COX-1 and COX-2.

Comp. No.	COX1 (PDBID:3N8Y)			COX2 (PDBID: 3LN1)		
-------	MolDock Score	Rerank Score	HBond	MolDock Score	Rerank Score	HBond
2a	-135.364	-101.479	-3.49179	-95.6503	-77.5339	-3.58658
2b	-135.473	-107.778	-6.58101	-98.1905	-81.0321	-1.24095
2c	-145.331	-109.715	-12.6375	-100.149	-79.33533	-2.38313
2d	-146.71	-117.051	-1.97263	-104.223	-83.358	-2.24342
2e	-148.276	-120.943	-4.33694	-108.757	-86.7309	-4.61197
2f	-147.244	-120.904	-2.89968	-101.766	-79.7038	-2.44933
2g	-146.609	-115.266	-1.37099	-103.341	-85.4336	-4.9669
2h	-132.632	-98.1926	-1.66236	-101.556	-83.7274	-2.5
2i	-133.938	-105.889	-5.76098	-99.8144	-76.3131	-1.89478
2j	-129.601	-104.951	-1.18736	-92.7975	-77.0203	-2.29506
2k	-134.909	-108.209	-1.24076	-108.672	-91.1389	-2.79118
2l	-137.025	-110	-0.926841	-129.807	-111.192	-3.13262
Indomethacin	-131.578	-89.1398	-2.54039	-92.5925	-75.9437	-1.44338

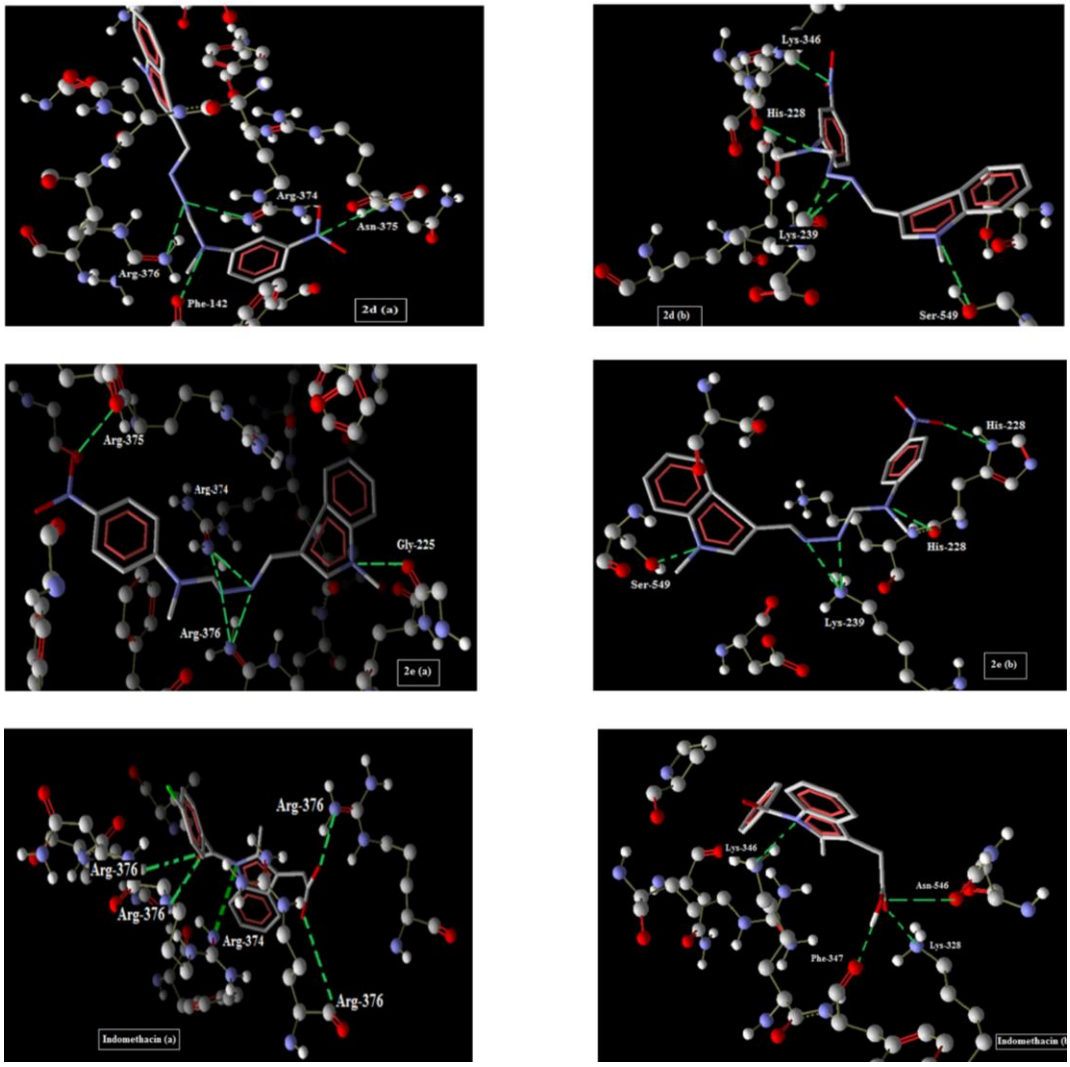

Fig. 1: The Interaction between the compounds 2d, 2e and infomethacin with the active site of cox-1 (a) and cox-2 (b).

CONCLUSIONS

We described herein the synthesis and characterization of indole-hydrazone derivatives. The structures of the new compounds were confirmed by means of different spectroscopic methods and by elemental analyses. On the basis of preliminary screening data for these new compounds, *in vivo* anti inflammatory and cytotoxic activity, were evaluated. Results revealed that the most compounds have remarkable anti inflammatory activity, edema reduction percent ranged between 72.3 – 89.3%, while compounds **2f** and **2j** showed mild cytotoxic activity. Moreover, to help understand the interactions between the ligands and enzyme active sites, the molecular docking studies were carried out for all the synthesized compounds toward active site of cox-1 and cox-2 enzymes and compared the docking score with references drug (indomethcin). The results appeared that, all the compounds exhibited good docking score which are agreed and supported to the in vivo anti inflammatory activity of these compounds. In addition, to recognize the relationship between the physicochemical properties and bioactivity observed for these compounds the drug likeness and bioactivity are calculated using Molinspiration software. All synthesized compounds showed a good drug likeness and bioactivity score comparing with indomethacin and doxorubicin.

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