



Novel N-substituted indole hydrazones as potential antiplatelet agents: synthesis, biological evaluations, and molecular docking studies

Navid Tavili¹, Shaya Mokhtari^{2,3}, Hafezeh Salehabadi¹, Marjan Esfahanizadeh⁴, and Shohreh Mohebbi^{1,*}

¹Department of Medicinal Chemistry, School of Pharmacy, Zanjan University of Medical Sciences, Zanjan, I.R. Iran.

²Central Research Laboratories, Shahid Beheshti University of Medical Sciences, Tehran, I.R. Iran.

³Phytochemistry Research Center, Shahid Beheshti University of Medical Sciences, Tehran, I.R. Iran.

⁴Department of Medicinal Chemistry, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, I.R. Iran.

Abstract

Background and purpose: Antiplatelet agents can diminish the chance of coronary heart diseases due to the prevention of unusual clotting in the arteries by inhibiting platelet aggregation and avoiding the formation of a blood clot. This mechanism can help to prevent ischemic stroke likewise. To improve the activity of these drugs and reduce their side effects, further studies are required.

Experimental approach: Based on the previous studies representing the promising antiplatelet activity of indole hydrazones, a series of their homologs containing twenty-one compounds were prepared in two steps. First, alkylation reaction on the nitrogen of the indole ring, and second, chif base formation by condensation of a primary amine and N-substituted indole-3 carbaldehyde. Consequently, their platelet anti-aggregation activity was evaluated based on the Born turbidimetric method.

Findings/Results: Most of the compounds exhibited noticeable activity against platelet aggregation induced by arachidonic acid. Amongst them, two compounds 2e and 2f showed higher activity with IC₅₀ values that made comparable to indomethacin and acetylsalicylic acid as standard drugs and had no toxicity on platelets.

Conclusion and implications: The synthesized compounds exhibited promising activity against arachidonic acid-induced aggregation; however, none of them showed noticeable antiplatelet activity induced by adenosine di-phosphate. Chemical structure comparison of the prepared derivatives indicated the existence of a lipophilic medium-sized group on the phenyl ring increased their activity. In addition, the docking studies confirmed this hydrophobic interaction in the lipophilic pocket of cyclooxygenase-1 enzyme suggesting that hydrophobicity of this region plays a pivotal role in the anti-platelet activity of these compounds. To prove this finding, the enzymatic evaluation with the target enzyme is required.

Keywords: Antiplatelet aggregation; Indole; Synthesis; Turbidimetric assay.

INTRODUCTION

Thromboembolic disorders including cardiovascular and cerebrovascular events are one of the main causes of mortality worldwide (1,2). It has been revealed that increased self-affinity and aggregation of platelets play an important role in the pathogenesis of atherothrombosis. Antiplatelet agents are therefore considered a significant tool in the treatment or prevention of cardiovascular thrombotic disease. Notwithstanding the

benefits of antiplatelet drugs, there are some limitations to using them such as bleeding, gastric ulcers, drug interactions, and resistance (3,4). Therefore, access to new antiplatelet drugs with improved efficacy and fewer side effects is essential.

Access this article online



Website: <http://rps.mui.ac.ir>

DOI: 10.4103/1735-5362.329926

*Corresponding author: Sh. Mohebbi
Tel: +98-98-2433473635, Fax: +98-2433473639
Email: shmohebbi@zums.ac.ir

A review of compounds with antiplatelet aggregation activity discloses that the presence of the hydrazone moiety is an important factor for being active which the most active antiplatelet compounds reported in previous studies were depicted in Fig. 1. Chelucci *et al.* reported five nonsteroidal anti-inflammatory drugs (NSAID) derivatives with an N-acyl hydrazone subunit that revealed a promising antiplatelet activity (5). A series of 1,2,4 triazole hydrazone derivatives were prepared by Khalid *et al.* that inhibited the platelets aggregation which was induced by arachidonic acid (AA), adenosine diphosphate (ADP), and collagen (6). Ramzan *et al.* reported 1,3,4-Oxadiazoles derivatives that exhibited platelet aggregation inhibition against both AA and collagen (7). Silva *et al.* synthesized and evaluated some phenothiazine bound to acyl hydrazone derivatives for antiplatelet activity. In this study, a novel potent compound was reported for inhibition of cyclooxygenase-1 (COX-1) enzyme (8). Mashayekhi *et al.* investigated the synthesis and anti-platelet aggregation activity of several indole hydrazones and reported one potent compound which well inhibited the platelet aggregation (9). In another study, Lima *et al.* reported the preparation and antiplatelet activity of some

arylsulfonate-acylhydrazone derivatives that two of them showed promising antiplatelet activity (10).

Over the past years, our research team started several studies with emphasis on the chemical structures bearing hydrazone moiety as potential antiplatelet agents. Intriguingly, we discovered that hydrazones of substituted phenyl reveal a notable inhibition of AA-induced platelet aggregation with IC_{50} values comparable to that of indomethacin as a standard inhibitor (11-13).

Considering these experiences, in the present study, we synthesized a new series of N-substituted indole hydrazone derivatives. They were evaluated for their inhibitory activities against AA and ADP-induced platelet aggregation, platelet itself toxicity (platelet membrane leakage assay), and normal cell line toxicity. In addition, a docking study was performed on the COX-1 enzyme as a target for these compounds. As shown in Fig. 1, the synthesized derivatives possess hybridized structures of indole ring and hydrazone moiety as a homologs series (2a-u) to provide the assessment of the relationship between different physicochemical parameters and the experimental bioactivities.

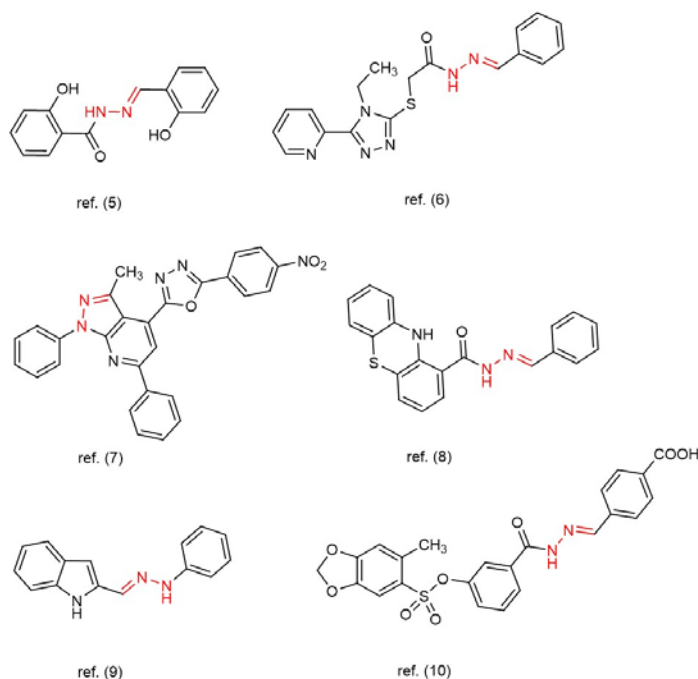


Fig. 1. Chemical structures of the hydrazone derivatives with remarkable antiplatelet activity in the published articles.

MATERIALS AND METHODS

Evaporations of the reaction mixtures were performed with a rotary evaporator. Melting points were determined on an electrothermal method by capillary method. Infrared spectra were recorded by Bruker Tensor 27 (Germany) Fourier-transform infrared (FT-IR) as skinny films of KBr plates with Umax in inverse centimeters. Proton nuclear magnetic resonance spectra (^1H NMR) were measured on a Bruker DRX-Avance (400 MHz) spectrometer (Germany). Chemical shifts values are reported in ppm (parts per million) relative to tetramethylsilane (TMS) as internal standard; s: singlet, d: doublet, dd: double doublet, t: triplet, q: quartet, m: multiplet, br s: broad singlet. Thin-layer chromatography (TLC) was performed on Whatman Sil F/UV 254 silica gel plates with fluorescent indicator and the spots were visualized under 254 and 366 nm illumination. Mass detection of compounds was performed with an Agilent 6400 series mass spectrometer equipped with an electrospray ionization source (USA). Our chemical and reagents were obtained from Merck. The global physicochemical parameters were calculated with ChemDraw Ultra 8.0.

General procedure for the synthesis of N-substituted indole-3-carboxaldehydes (1a-c)

In a 50-mL round bottom flask, indol-3-carboxaldehyde (2.0 mmol), relevant alkyl halides (3.0 mmol), anhydrous K_2CO_3 (2.4 mmol), and KI (0.4 mmol) were dissolved in acetonitrile (12 mL). The mixture was stirred forcefully and heated under reflux, and the reaction progress was controlled by TLC. When the reaction was completed, the inorganic salts (K_2CO_3 and KI) were removed by filtration. Afterward, the solvent was evaporated under reduced pressure and the residue was washed by hexane and recrystallized from n-hexane/ethyl acetate with a 10:1 ratio (14-16).

General procedures for the synthesis of phenylhydrazone derivatives (2a-u)

Indole-3-carboxaldehyde derivatives (1a-c, 1.0 mmol) and suitable phenylhydrazines (1.3 mmol) were added to a mixture of absolute

ethanol (10 mL) and glacial acetic acid (0.28 mL) (17,18). The ratio of ethanol to acetic acid was almost 33:1. The resultant mixture was heated under reflux and the reaction improvement was controlled by TLC. A solid crude was provided by cooling down the mixture and sometimes the evaporation of the solvent worked. The solid was washed with n-hexane and recrystallized from n-hexane/ethyl acetate. The structure of the synthesized compounds was confirmed by different spectral methods such as mass, ^1H NMR and IR spectroscopy.

Evaluation of antiplatelet aggregation activity

The prepared compounds of this study were assessed for the inhibitory activity against AA and ADP platelet aggregation. The inhibitory activity of the final compounds was evaluated by human platelet-rich plasma (PRP) on an APACT 4004 aggregometer through Born's reported turbidimetric method (19). ADP (5 μM), and AA (1.25 mg/mL) were used as inducers of platelet aggregation. IC_{50} was calculated as the concentration of the test compound that inhibits the platelet aggregation by 50%. The detailed procedure was described in our previous works (20).

Cytotoxicity assay

The synthesized compounds were assessed for the probable toxicity on fibroblast L929 cell line by 3-(4,5-dimethylthiazol-2-yl)-2,5-tetrazolium bromide) method (MTT). At the beginning, the compounds were tested at 100 μM and the IC_{50} , calculated for those, showed more than 50% toxicity on the normal cell line. The detailed procedure was also explained in our previous work (11).

Platelet lactate dehydrogenase assay

To evaluate lactate dehydrogenase (LDH) assay, samples of platelet-rich plasma (200 μL) were kept in an incubator with test solutions (1 μL , final concentration of 100 μM) for 30 min. The samples were then, centrifuged at 11000 rpm for 5 min and the supernatant was separated. In order to evaluate the activity of lactate dehydrogenase, an LDH assay kit (Pishtazteb, Iran) was used following the

instructions provided by the manufacturer. DMSO and 1% Triton™ X-100 were used as the vehicle and positive control, respectively. The data was reported as the proportion of released LDH following exposure to the most potent derivatives (2e and 2f) to the total LDH content measured by the addition of 1% Triton™ X-100.

Docking simulation strategy

AutoDock 4.2 was used for the docking study to clarify the binding modes of the selected compounds as COX-1 inhibitors (21). All input files were prepared using AutoDockTools (ADT; version 1.5.6). The optimized structure of the ligands and protein were used as the input for the consequent docking studies. The crystal structure of COX-1 (PDB code: 3N8Y) was achieved from the Protein Data Bank (www.rcsb.org). All water molecules were removed from the protein pdb file, as well as all other heteroatoms including ligands and ions. After adding polar hydrogens, the Kollman-united charges method was assigned for all atoms of the enzyme to calculate the partial atomic charges, and grid maps were generated by AutoGrid 4.2. Three-dimensional structures of ligands were generated by MarvinSketch software version 5.7.1, (ChemAxon Ltd. www.chemaxon.com), and the atomic charge was determined according to Gasteiger-Marsili charge. Each docking job was carried out by 100 runs. All other parameters and the docking validation were performed using previously published methods (22).

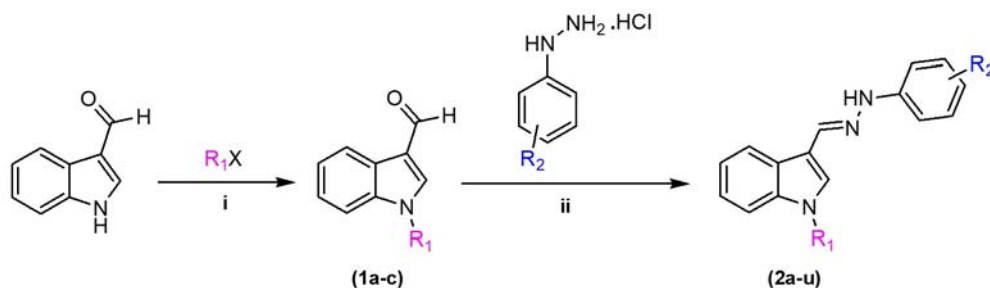
RESULTS

Chemistry

N1-substituted indole intermediates (1a-c) were prepared by the reaction of indole 3 carboxaldehyde with methyl iodide, ethyl iodide, and n-propyl chloride, respectively. The final hydrazones were then synthesized by the reaction of the intermediates with different substituted-phenylhydrazines as presented in Scheme 1.

The final designed compounds were synthesized in good yields with excellent analytical purity as monitored by TLC and CHN elemental analysis. The structures of the compounds were all confirmed by different spectroscopy methods such as IR, ¹HNMR, and mass spectrometry. The spectral data were in line with the structure of the derivatives completely. In ¹HNMR spectra, the hydrogen of the hydrazone moiety resonated by a distinctive pattern as exchangeable hydrogen at 9-10 ppm. In all compounds, indole C2-H appeared as a singlet in 7.51-7.76 ppm. In propylated-indole derivatives, the aliphatic protons of the n-propyl unit appeared at 0.86, 1.81, and 4.16 ppm. Two sets of doublets assigned to the hydrogens of C4 and C7 of the indole ring appeared at 7.46 ppm and 8.30 ppm, respectively.

The analysis of the molecular mass of the compounds performed by ESI-MS and the molecular ions observed as M⁺H. Likewise, the IR spectra were used for the identification of N-H and C=N bonds in all structures which were appeared at 3344 and 1597 cm⁻¹.



Scheme 1. Reagents and conditions: (i) R₁: alkyl homologs, X: (Cl and I), K₂CO₃, KI, acetonitrile, reflux; (ii) substituted-phenylhydrazines, ethanol, and glacial acetic acid.

2-(2-chlorophenyl)-1-((1-methyl-1H-indole-3-yl)methylene)hydrazine (2a)

Yield 89%, m.p. 218-221 °C. ¹HNMR (400 MHz, DMSO-d₆) δ: 3.84 (s, 3H, CH₃), 6.75 (dt, *J* = 8.0 Hz, *J* = 1.6 Hz, 1H, Ar H), 7.14-7.21 (m, 2H, indole C5, 6-H), 7.29 (m, 2H, Ar H), 7.42 (dd, *J* = 6.8 Hz, *J* = 2.0 Hz, 1H, indole C7-H), 7.54 (d, *J* = 7.6 Hz, 1H, Ar H), 7.65 (s, 1H, indole C2-H), 8.26 (d, *J* = 7.6 Hz, 1H, indole C4-H), 8.49 (s, 1H, imine H), 9.44 (s, 1H, NH); IR (cm⁻¹): 3344, 1597, 1438, 1240, 736. ESI-MS *m/z*: 284 (M⁺ H⁺). Anal. Calcd for C₁₆H₁₄ClN₃: C, 67.72; H, 4.97; N, 14.81. Found: C, 67.68; H, 4.93; N, 14.77.

2-(4-chlorophenyl)-1-((1-methyl-1H-indole-3-yl)methylene)hydrazine (2b)

Yield 77%, m.p. 203-205 °C. ¹HNMR (400 MHz, DMSO-d₆) δ: 3.82 (s, 3H, CH₃), 7.02-7.10 (m, 2H, Ar-H), 7.19-7.29 (m, 4H, Ar-H), 7.49 (d, 1H, *J* = 8 Hz, Ar-H), 7.66 (s, 1H, indole C2-H), 8.10 (s, 1H, imine-H), 8.23 (d, 1H, *J* = 8 Hz, Ar-H), 9.83 (s, 1H, NH); IR (cm⁻¹): 3285, 1610, 1511, 1441, 1240, 822. ESI-MS *m/z*: 284 (M⁺ H⁺). Anal. Calcd for C₁₆H₁₄ClN₃: C, 67.72; H, 4.97; N, 14.81. Found: C, 67.65; H, 4.90; N, 14.82.

2-(4-bromophenyl)-1-((1-methyl-1H-indole-3-yl)methylene)hydrazine (2c)

Yield 91%, m.p. 196-198 °C. ¹HNMR (400 MHz, DMSO-d₆) δ: 3.83 (s, 3H, CH₃), 7.02-7.10 (m, 2H, Ar-H), 7.19-7.29 (m, 2H, Ar-H), 7.37 (d, 2H, *J* = 8 Hz, Ar-H), 7.49 (d, 1H, *J* = 8 Hz, Ar-H), 7.66 (s, 1H, indole C2-H), 8.10 (s, 1H, imine-H), 8.23 (d, 1H, *J* = 8 Hz, Ar-H), 10.02 (s, 1H, NH); IR (cm⁻¹): 3266, 1615, 1540, 1436, 1220, 824. ESI-MS *m/z*: 328 (M⁺ H⁺). Anal. Calcd for C₁₆H₁₄BrN₃: C, 58.55; H, 4.30; N, 12.80. Found: C, 55.62; H, 4.26; N, 12.83.

2-(4-fluorophenyl)-1-((1-methyl-1H-indole-3-yl)methylene)hydrazine (2d)

Yield 63%, m.p. 162-164 °C. ¹HNMR (400 MHz, DMSO-d₆) δ: 3.82 (s, 3H, CH₃), 7.02-7.10 (m, 4H, Ar-H), 7.18-7.28 (m, 2H, Ar-H), 7.48 (d, *J* = 8 Hz, 1H, Ar H), 7.63 (s, 1H, indole C2-H), 8.08 (s, 1H, imine H), 8.24 (d, *J* = 7.6 Hz, 1H, indole C4-H), 9.83

(s, 1H, NH); IR (cm⁻¹): 3344, 1597, 1438, 1240, 736. ESI-MS *m/z*: 268 (M⁺ H⁺). Anal. Calcd for C₁₆H₁₄FN₃: C, 71.89; H, 5.28; N, 15.72. Found: C, 71.81; H, 5.12; N, 15.68.

*1-((1-methyl-1H-indole-3-yl)methylene)-2-*o*-tolylhydrazine (2e)*

Yield 54%, m.p. 125-127 °C ¹HNMR (400 MHz, DMSO-d₆) δ: 2.22 (s, 3H, CH₃), 3.83 (s, 3H, CH₃), 6.78 (d, *J* = 8.4 Hz, 2H, Ar C3,5-H), 7.04 (d, *J* = 8.4 Hz, 2H, Ar C2,6-H), 7.13-7.20 (m, 2H, indole C5,6-H), 7.42 (d, *J* = 6.8 Hz, 1H, indole C7-H), 7.68 (s, 1H, indole C2-H), 8.13 (d, *J* = 7.2 Hz, 1H, indole C4-H), 8.29 (s, 1H, imine H), 9.09 (s, 1H, NH); IR (cm⁻¹): 3278, 1616, 1602, 1463, 1243, 812.264 ESI-MS *m/z*: 328 (M⁺ H⁺). Anal. Calcd for C₁₇H₁₇N₃: C, 77.54; H, 6.51; N, 15.96. Found: C, 77.68; H, 6.47; N, 15.80.

*1-((1-methyl-1H-indole-3-yl)methylene)-2-*p*-tolylhydrazine (2f)*

Yield 43%, m.p. 180-182 °C ¹HNMR (400 MHz, DMSO-d₆) δ: 2.24 (s, 3H, CH₃), 3.93 (s, 3H, CH₃), 6.69 (d, *J* = 8.4 Hz, 2H, Ar C3,5-H), 7.07 (d, *J* = 8.4 Hz, 2H, Ar C2,6-H), 7.11-7.19 (m, 2H, indole C5,6-H), 7.47 (d, *J* = 6.8 Hz, 1H, indole C7-H), 7.71 (s, 1H, indole C2-H), 8.18 (d, *J* = 7.2 Hz, 1H, indole C4-H), 8.25 (s, 1H, imine H), 9.36 (s, 1H, NH); IR (cm⁻¹): 3287, 1645, 1609, 1498, 1323, 822.264 ESI-MS *m/z*: 328 (M⁺ H⁺). Anal. Calcd for C₁₇H₁₇N₃: C, 77.54; H, 6.51; N, 15.96. Found: C, 77.55; H, 6.45; N, 15.91.

2-(4-methoxyphenyl)-1-((1-methyl-1H-indole-3-yl)methylene)hydrazine (2g)

Yield 78%, m.p. 203-205 °C. ¹HNMR (400 MHz, DMSO-d₆) δ: 3.68 (s, 3H, OCH₃), 3.84 (s, 3H, CH₃), 6.86 (d, *J* = 8.4 Hz, 2H, Ar C3,5-H), 6.96 (d, *J* = 8.4 Hz, 2H, Ar C2,6-H), 7.10-7.18 (m, 2H, indole C5,6-H), 7.39 (d, *J* = 6.8 Hz, 1H, indole C7-H), 7.57 (s, 1H, indole C2-H), 8.06 (s, 1H, imine H), 8.23 (d, *J* = 7.2 Hz, 1H, indole C4-H), 9.53 (s, 1H, NH); IR (cm⁻¹): 3285, 1610, 1511, 1441, 1240, 822. ESI-MS *m/z*: 280 (M⁺ H⁺). Anal. Calcd for C₁₇H₁₇N₃O: C, 73.10; H, 6.13; N, 15.04. Found: C, 72.98; H, 6.22; N, 15.08.

2-(2-chlorophenyl)-1-((1-ethyl-1H-indole-3-yl)methylene)hydrazine (2h)

Yield 71%, m.p. 121-123 °C. ¹HNMR (400 MHz, DMSO-d₆) δ: 1.40 (t, *J* = 7.2 Hz, 3H, CH₃), 4.25 (q, 2H, *J* = 7.2 Hz, CH₂), 6.75 (dt, *J* = 8.0 Hz, *J* = 1.6 Hz, 1H, Ar H), 7.20-7.33 (m, 4H, Ar-H), 7.55 (t, *J* = 7.2 Hz, 2H, indole C7-H), 7.75 (s, 1H, indole C2-H), 8.26 (d, *J* = 7.6 Hz, 1H, indole C4-H), 8.49 (s, 1H, imine H), 9.44 (s, 1H, NH); IR (cm⁻¹): 3054, 1682, 1470, 1455, 1233, 843. ESI-MS m/z: 298 (M⁺ H⁺). Anal. Calcd for C₁₇H₁₆ClN₃: C, 68.57; H, 5.42; N, 14.11. Found: C, 68.60; H, 5.44; N, 14.08.

2-(4-chlorophenyl)-1-((1-ethyl-1H-indol-3-yl)methylene)hydrazine (2i)

Yield 79%, m.p. 209-212 °C. ¹HNMR (400 MHz, DMSO-d₆) δ: 1.40 (t, *J* = 7.2 Hz, 3H, CH₃), 4.23 (q, *J* = 7.2 Hz, 2H, CH₂), 7.03-7.06 (m, 2H, Ar-H), 7.18-7.28 (m, 4H, Ar-H), 7.53 (d, 1H, *J* = 8 Hz, Ar-H), 7.73 (s, 1H, indole C2-H), 8.12 (s, 1H, imine-H), 8.24 (d, 1H, *J* = 8 Hz, Ar-H), 9.91 (s, 1H, NH); IR (cm⁻¹): 3175, 1681, 1472, 1495, 1197, 903. ESI-MS m/z: 298 (M⁺ H⁺). Anal. Calcd for C₁₇H₁₆ClN₃: C, 68.57; H, 5.42; N, 14.11. Found: C, 68.53; H, 5.47; N, 14.16.

2-(4-bromophenyl)-1-((1-ethyl-1H-indole-3-yl)methylene)hydrazine (2j)

Yield 92%, m.p. 183-185 °C. ¹HNMR (400 MHz, DMSO-d₆) δ: 1.38 (t, *J* = 7.2 Hz, 3H, CH₃), 4.23 (q, 2H, *J* = 7.2 Hz, CH₂), 6.98-7.01 (m, 2H, Ar-H), 7.18-7.27 (m, 2H, Ar-H), 7.35-7.39 (m, 2H, Ar-H), 7.53 (d, 1H, *J* = 8 Hz, Ar-H), 7.73 (s, 1H, indole C2-H), 8.12 (s, 1H, imine-H), 8.23 (d, 1H, *J* = 8 Hz, Ar-H), 9.89 (s, 1H, NH); IR (cm⁻¹): 3154, 1671, 1490, 1267, 899. ESI-MS m/z: 342 (M⁺ H⁺). Anal. Calcd for C₁₇H₁₆BrN₃: C, 59.66; H, 4.71; N, 12.28. Found: C, 59.69; H, 4.65; N, 12.36.

2-(4-fluorophenyl)-1-((1-ethyl-1H-indole-3-yl)methylene)hydrazine (2k)

Yield 71%, m.p. 163-165 °C. ¹HNMR (400 MHz, DMSO-d₆) δ: 1.41 (t, *J* = 7.2 Hz, 3H, CH₃), 4.23 (q, 2H, *J* = 7.2 Hz, CH₂), 7.02-7.10 (m, 4H, Ar-H), 7.19-7.25 (m, 2H, Ar-H), 7.53 (d, *J* = 8 Hz, 1H, ArH), 7.71 (s, 1H, indole C2-

H), 8.09 (s, 1H, imine H), 8.24 (d, *J* = 7.6 Hz, 1H, indole C4-H), 10.01 (s, 1H, NH); IR (cm⁻¹): 3243, 1808, 1493, 1301, 907. ESI-MS m/z: 282 (M⁺ H⁺). Anal. Calcd for C₁₇H₁₆FN₃: C, 72.58; H, 5.73; N, 14.94. Found: C, 72.62; H, 5.76; N, 14.89.

*1-((1-ethyl-1H-indole-3-yl)methylene)-2-*o*-tolylhydrazine (2l)*

Yield 41%, m.p. 119-121 °C. ¹HNMR (400 MHz, DMSO-d₆) δ: 1.41 (t, *J* = 7.2 Hz, 3H, CH₃), 2.26 (s, 3H, CH₃), 4.27 (q, 2H, *J* = 7.2 Hz, CH₂), 6.81 (d, *J* = 8.4 Hz, 2H, Ar C3,5-H), 7.02 (d, *J* = 8.4 Hz, 2H, Ar C2,6-H), 7.11-7.16 (m, 2H, indole C5,6-H), 7.54 (d, *J* = 6.8 Hz, 1H, indole C7-H), 7.62 (s, 1H, indole C2-H), 8.11 (d, *J* = 7.2 Hz, 1H, indole C4-H), 8.32 (s, 1H, imine H), 9.43 (s, 1H, NH); IR (cm⁻¹): 3189, 1711, 1490, 1298, 905. ESI-MS m/z: 278 (M⁺ H⁺). Anal. Calcd for C₁₈H₁₉N₃: C, 77.95; H, 6.90; N, 15.15. Found: C, 77.92; H, 6.91; N, 15.21.

*1-((1-ethyl-1H-indole-3-yl)methylene)-2-*p*-tolylhydrazine (2m)*

Yield 53%, m.p. 144-146 °C. ¹HNMR (400 MHz, DMSO-d₆) δ: 1.44 (t, *J* = 7.2 Hz, 3H, CH₃), 4.33 (q, 2H, *J* = 7.2 Hz, CH₂), 7.27-7.29 (m, 2H, Ar-H), 7.34-7.35 (m, 2H, Ar-H), 7.65 (d, 1H, *J* = 8 Hz, Ar-H), 7.73 (s, 1H, indole C2-H), 8.23 (d, *J* = 7.2 Hz, 1H, indole C4-H), 8.29 (s, 1H, imine H), 9.87 (s, 1H, NH); IR (cm⁻¹): 3201, 1754, 1496, 1245, 879. ESI-MS m/z: 278 (M⁺ H⁺). Anal. Calcd for C₁₈H₁₉N₃: C, 77.95; H, 6.90; N, 15.15. Found: C, 77.89; H, 6.87; N, 15.16.

2-(4-methoxyphenyl)-1-((1-ethyl-1H-indole-3-yl)methylene)hydrazine (2n)

Yield 45%, m.p. 133-135 °C. ¹HNMR (400 MHz, DMSO-d₆) δ: 1.41 (t, *J* = 7.2 Hz, 3H, CH₃), 3.70 (s, 3H, OCH₃), 4.25 (q, 2H, *J* = 7.2 Hz, CH₂), 6.87 (d, *J* = 8.4 Hz, 2H, Ar C3,5-H), 6.99 (d, *J* = 8.4 Hz, 2H, Ar C2,6-H), 7.11-7.19 (m, 2H, indole C5,6-H), 7.42 (d, *J* = 6.8 Hz, 1H, indole C7-H), 7.51 (s, 1H, indole C2-H), 8.11 (s, 1H, imine H), 8.26 (d, *J* = 7.2 Hz, 1H, indole C4-H), 9.72 (s, 1H, NH); IR (cm⁻¹): 3221, 1804, 1475, 1235, 887. ESI-MS m/z: 294 (M⁺ H⁺). Anal. Calcd for C₁₈H₁₉N₃O: C, 73.69; H, 6.53; N, 14.32. Found: C, 73.68; H, 6.49; N, 14.36.

2-(2-chlorophenyl)-1-((1-propyl-1H-indole-3-yl)methylene)hydrazine (2o)

Yield 82%, m.p. 99-101 °C. ¹HNMR (400 MHz, DMSO-d₆) δ: 0.86 (t, *J* = 7.2 Hz, 3H, CH₃), 1.81 (q, 2H, *J* = 7.2 Hz, CH₂), 4.16 (t, *J* = 7.2 Hz, 2H, CH₂), 6.77 (dt, *J* = 8.0 Hz, *J* = 1.6 Hz, 1H, Ar H), 7.20-7.26 (m, 4H, Ar-H), 7.55 (t, *J* = 7.2 Hz, 2H, indole C7-H), 7.73 (s, 1H, indole C2-H), 8.27 (d, *J* = 7.6 Hz, 1H, indole C4-H), 8.49 (s, 1H, imine H), 9.44 (s, 1H, NH); IR (cm⁻¹): 3078, 1672, 1480, 1410, 1250, 795. ESI-MS *m/z*: 312 (M⁺ H⁺). Anal. Calcd for C₁₈H₁₈ClN₃: C, 69.34; H, 5.82; N, 13.48. Found: C, 69.37; H, 5.78; N, 13.53.

2-(4-chlorophenyl)-1-((1-propyl-1H-indole-3-yl)methylene)hydrazine (2p)

Yield 67%, m.p. 179-181 °C. ¹HNMR (400 MHz, DMSO-d₆) δ: 0.86 (t, *J* = 7.2 Hz, 3H, CH₃), 1.82 (q, 2H, *J* = 7.2 Hz, CH₂), 4.24 (t, *J* = 7.2 Hz, 2H, CH₂), 7.03-7.05 (m, 2H, Ar-H), 7.21-7.24 (m, 4H, Ar-H), 7.54 (d, 1H, *J* = 8 Hz, Ar-H), 7.72 (s, 1H, indole C2-H), 8.12 (s, 1H, imine-H), 8.23 (d, 1H, *J* = 8 Hz, Ar-H), 9.92 (s, 1H, NH); IR (cm⁻¹): 3205, 1692, 1488, 1432, 1171, 893. ESI-MS *m/z*: 312 (M⁺ H⁺). Anal. Calcd for C₁₈H₁₈ClN₃: C, 69.34; H, 5.82; N, 13.48. Found: C, 69.40; H, 5.82; N, 13.44.

2-(4-bromophenyl)-1-((1-propyl-1H-indole-3-yl)methylene)hydrazine (2q)

Yield 43%, m.p. 182-184 °C. ¹HNMR (400 MHz, DMSO-d₆) δ: 0.87 (t, *J* = 7.2 Hz, 3H, CH₃), 1.81 (q, 2H, *J* = 7.2 Hz, CH₂), 4.18 (t, *J* = 7.2 Hz, 2H, CH₂), 6.98 (dt, *J* = 8.0 Hz, *J* = 1.6 Hz, 1H, Ar H), 7.36-7.38 (m, 4H, Ar-H), 7.54 (t, *J* = 7.2 Hz, 2H, indole C7-H), 7.72 (s, 1H, indole C2-H), 8.12 (s, *J* = 7.6 Hz, 1H, indole C4-H), 8.23 (d, 1H, imine H), 9.92 (s, 1H, NH); IR (cm⁻¹): 3165, 1677, 1485, 1255, 905. ESI-MS *m/z*: 356 (M⁺ H⁺). Anal. Calcd for C₁₈H₁₈BrN₃: C, 60.68; H, 5.09; N, 11.79. Found: C, 60.61; H, 5.15; N, 11.83.

2-(4-fluorophenyl)-1-((1-propyl-1H-indole-3-yl)methylene)hydrazine (2r)

Yield 51%, m.p. 211-213 °C. ¹HNMR (400 MHz, DMSO-d₆) δ: 0.89 (t, *J* = 7.2 Hz, 3H, CH₃), 1.83 (q, 2H, *J* = 7.2 Hz, CH₂), 4.14 (t, *J* = 7.2 Hz, 2H, CH₂), 7.03-7.10 (m, 4H, Ar-H), 7.17-7.23 (m, 2H, Ar-H), 7.59 (d, *J* = 8 Hz, 1H, Ar H), 7.75 (s, 1H, indole C2-H), 8.13 (s, 1H,

imine H), 8.32 (d, *J* = 7.6 Hz, 1H, indole C4-H), 9.96 (s, 1H, NH); IR (cm⁻¹): 3220, 1791, 1450, 1298, 893. ESI-MS *m/z*: 296 (M⁺ H⁺). Anal. Calcd for C₁₈H₁₈FN₃: C, 73.20; H, 6.14; N, 14.23. Found: C, 73.23; H, 6.19; N, 14.20.

*1-((1-propyl-1H-indole-3-yl)methylene)-2-*o*-tolylhydrazine (2s)*

Yield 63%, m.p. 135-137 °C. ¹HNMR (400 MHz, DMSO-d₆) δ: 0.84 (t, *J* = 7.2 Hz, 3H, CH₃), 1.83 (q, 2H, *J* = 7.2 Hz, CH₂), 4.21 (t, *J* = 7.2 Hz, 2H, CH₂), 6.84 (d, *J* = 8.4 Hz, 2H, Ar C3,5-H), 7.07 (d, *J* = 8.4 Hz, 2H, Ar C2,6-H), 7.13-7.17 (m, 2H, indole C5,6-H), 7.62 (d, *J* = 6.8 Hz, 1H, indole C7-H), 7.66 (s, 1H, indole C2-H), 8.21 (d, *J* = 7.2 Hz, 1H, indole C4-H), 8.31 (s, 1H, imine H), 9.89 (s, 1H, NH); IR (cm⁻¹): 3206, 1746, 1498, 1277, 903. ESI-MS *m/z*: 292 (M⁺ H⁺). Anal. Calcd for C₁₉H₂₁N₃: C, 78.32; H, 7.26; N, 14.42. Found: C, 78.35; H, 7.31; N, 14.39.

*1-((1-propyl-1H-indole-3-yl)methylene)-2-*p*-tolylhydrazine (2t)*

Yield 58%, m.p. 192-194 °C. ¹HNMR (400 MHz, DMSO-d₆) δ: 0.87 (t, *J* = 7.2 Hz, 3H, CH₃), 1.81 (q, 2H, *J* = 7.2 Hz, CH₂), 2.24 (s, 3H, CH₃), 4.22 (t, *J* = 7.2 Hz, 2H, CH₂), 6.86 (d, *J* = 8.4 Hz, 2H, Ar C3,5-H), 7.17 (d, *J* = 8.4 Hz, 2H, Ar C2,6-H), 7.11-7.19 (m, 2H, indole C5,6-H), 7.69 (d, *J* = 6.8 Hz, 1H, indole C7-H), 7.76 (s, 1H, indole C2-H), 8.33 (d, *J* = 7.2 Hz, 1H, indole C4-H), 8.45 (s, 1H, imine H), 9.91 (s, 1H, NH); IR (cm⁻¹): 3158, 1750, 1505, 1276, 905. ESI-MS *m/z*: 292 (M⁺ H⁺). Anal. Calcd for C₁₉H₂₁N₃: C, 78.32; H, 7.26; N, 14.42. Found: C, 78.38; H, 7.36; N, 14.47.

2-(4-methoxyphenyl)-1-((1-propyl-1H-indole-3-yl)methylene)hydrazine (2u)

Yield 82%, m.p. 166-168 °C. ¹HNMR (400 MHz, DMSO-d₆) δ: 0.86 (t, *J* = 7.2 Hz, 3H, CH₃), 1.85 (q, 2H, *J* = 7.2 Hz, CH₂), 3.72 (s, 3H, OCH₃), 4.23 (t, *J* = 7.2 Hz, 2H, CH₂), 6.93 (d, *J* = 8.4 Hz, 2H, Ar C3,5-H), 6.99 (d, *J* = 8.4 Hz, 2H, Ar C2,6-H), 7.12-7.19 (m, 2H, indole C5,6-H), 7.43 (d, *J* = 6.8 Hz, 1H, indole C7-H), 7.57 (s, 1H, indole C2-H), 8.13 (s, 1H, imine H), 8.29 (d, *J* = 7.2 Hz, 1H, indole C4-H), 9.67 (s, 1H, NH); IR (cm⁻¹): 3220, 1798, 1460, 1251, 893. ESI-MS *m/z*: 308 (M⁺ H⁺). Anal. Calcd for C₁₉H₂₁N₃O: C, 74.24; H, 6.89; N, 13.67. Found: C, 74.20; H, 6.93; N, 13.62.

Biological activity

The antiplatelet activity of the prepared derivatives was evaluated conforming to Born's turbidometric method (23) using AA and ADP as inducers of the platelet aggregation. The IC₅₀ values are given in Table 1 as the mean ± SEM of three independent experiments.

Besides this experiment, the safety of the two most active compounds (2e and 2f) was evaluated on platelet cells by the analysis of LDH leakage. The Effect of different concentrations of these compounds on the release of LDH from the platelets is shown in Fig. 2. Furthermore, the cytotoxicity of the two mentioned compounds was tested on L929

normal cells to evaluate their safety on the cell lines which is a pivotal step for the lead compounds to pass during the drug discovery process. Hopefully, despite their notable inhibition activities against platelet aggregation (17.7 and 17.8 μg/mL), the IC₅₀ values for cytotoxicity assay were calculated > 100 μg/mL which showed low toxicity to fibroblast cells.

For these two derivatives (2e and 2f), analysis of Lipinski's rule of five was performed using OSIRIS DataWarrior (version 4.2.2) to indicate their ability for oral administration and their drug-likeness (24). The result is illustrated in Table 2.

Table 1. Antiplatelet activity of the derivatives in presence of arachidonic acid and adenosine diphosphate as inducers of platelet aggregation.

Compounds	R1	R2	Arachidonic acid		Adenosine diphosphate	
			Inhibition (%) at 1 mM	IC ₅₀ (μM)	Inhibition (%) at 5 μM	IC ₅₀ (μM)
2a	CH3	2-Cl	100	85.1 ± 7.3	40.4 ± 3.8	> 500
2b	CH3	4-Cl	100	38.3 ± 2.8	44.4 ± 3.6	> 500
2c	CH3	4-Br	100	38.4 ± 2.4	56.2 ± 4.3	> 500
2d	CH3	4-F	100	38.8 ± 3.1	72.8 ± 7	> 500
2e	CH3	2-CH3	100	17.7 ± 1.2	100	421 ± 9.2
2f	CH3	4-CH3	100	17.8 ± 1.5	78.3 ± 5.5	> 500
2g	CH3	4-OCH3	100	38.1 ± 2.5	62.7 ± 5.3	> 500
2h	C2H5	2-Cl	100	84.5 ± 6.8	64.2 ± 5.8	> 500
2i	C2H5	4-Cl	100	38.4 ± 2.5	68 ± 6.1	> 500
2j	C2H5	4-Br	100	> 100	40.8 ± 3.8	> 500
2k	C2H5	4-F	100	44.3 ± 2.9	92.1 ± 8.3	> 500
2l	C2H5	2-CH3	100	38.2 ± 2.7	67.5 ± 6.2	> 500
2m	C2H5	4-CH3	100	> 100	80 ± 7.1	> 500
2n	C2H5	4-OCH3	93 ± 8.4	40.6 ± 2.9	50.5 ± 4.3	> 500
2o	n-C3H7	2-Cl	100	> 100	68.1 ± 5.4	> 500
2p	n-C3H7	4-Cl	100	80.7 ± 6.5	71.2 ± 6	> 500
2q	n-C3H7	4-Br	100	> 100	80 ± 6.9	> 500
2r	n-C3H7	4-F	100	39.2 ± 3.1	68.9 ± 5.9	> 500
2s	n-C3H7	2-CH3	100	38.2 ± 2.9	67.5 ± 5.5	> 500
2t	n-C3H7	4-CH3	100	> 100	80 ± 6.4	> 500
2u	n-C3H7	4-OCH3	100	> 100	50.5 ± 4	> 500
Indomethacin	-	-	-	1.6 ± 0.47	42 ± 1.2	> 500
Acetylsalicylic acid	-	-	-	0.3 ± 0.02	22.1 ± 0.8	> 500

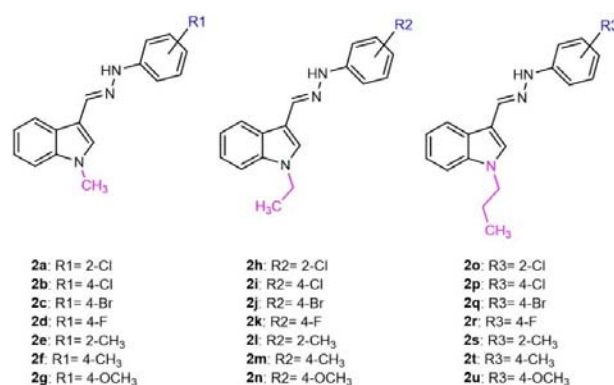


Fig. 2. General structure of the synthesized derivatives.

Table 2. Calculated parameters of Lipinski's rule of five for the compounds 2e and 2f.

Compounds	R1	R2	MW (Da)	cLogP	HBA	HBD
2a	CH3	2-Cl	283	3.75	3	1
2b	CH3	4-Cl	283	3.75	3	1
2c	CH3	4-Br	328	4.02	3	1
2d	CH3	4-F	267	3.35	3	1
2e	CH3	2-CH3	263	3.68	3	1
2f	CH3	4-CH3	263	3.68	3	1
2g	CH3	4-OCH3	279	3.07	4	1
2h	C2H5	2-Cl	297	4.09	3	1
2i	C2H5	4-Cl	297	4.09	3	1
2j	C2H5	4-Br	342	4.36	3	1
2k	C2H5	4-F	281	3.69	3	1
2l	C2H5	2-CH3	277	4.02	3	1
2m	C2H5	4-CH3	277	4.02	3	1
2n	C2H5	4-OCH3	293	3.41	4	1
2o	n-C3H7	2-Cl	311	4.58	3	1
2p	n-C3H7	4-Cl	311	4.58	3	1
2q	n-C3H7	4-Br	356	4.85	3	1
2r	n-C3H7	4-F	295	4.18	3	1
2s	n-C3H7	2-CH3	291	4.51	3	1
2t	n-C3H7	4-CH3	291	4.51	3	1
2u	n-C3H7	4-OCH3	307	3.89	4	1

MW, Molecular weight; cLogP, partition coefficient; HBA, number of hydrogen bond acceptors; HBD, number hydrogen bond donors.

DISCUSSION

COX-1 is one of the major enzymatic pathways in AA metabolism. Aspirin and indomethacin which were used as positive controls in this study inhibit the COX pathway and consequently diverted AA metabolites to the Lipoxygenase pathway. Since a majority of synthesized compounds exhibited remarkable inhibitory activity against AA pathway, we decided to choose COX-1 as a target enzyme in the docking study.

Herein, potent compounds (2e and 2f) in the antiplatelet aggregation test were followed by molecular docking studies to predict their binding mode in the COX-1 active site as a possible target. The docking strategy was initially validated *via* the docking of diclofenac over the COX-1 enzyme, and its modes were compared with the crystallographic structure and interactions in the PDB: 3N8Y. As shown in Fig. 3, diclofenac strongly interacts with COX-1 by forming hydrophobic bonds with Val349, Tyr385, Trp387, Ile523, Gly526, Ala527, and hydrogen bonds with Tyr385 and Ser530. Docking results were completely consistent with the crystallographic structure in terms of the ligand orientation, position, and interactions with the key residues in the COX-1 binding pocket. The docking protocol was then similarly used for compounds 2e and 2f.

The docking results showed that the aromatic rings moiety of compounds 2e and 2f penetrate deep into a hydrophobic pocket of COX-1 and provide appropriate interactions with hydrophobic residues such as Val349, Leu352, Ile 523, Gly526, and Ala527. Furthermore, compound 2 was stabilized by forming a hydrogen bond with Tyr385 (Fig. 4). The hydrophobic binding of mentioned amino acids with different COX-1 inhibitors has been previously reported (25,26). Specific hydrophobic interactions of these compounds with the COX-1 active site confirmed the inhibitory activity of both of them (Fig. 5). However, they need to be confirmed by enzymatic and *in vitro* assays.

At first glance, it is worth mentioning that the synthesized compounds entirely showed considerable antiplatelet activities while AA was the inducer of platelet aggregation. Despite the high inhibition percentage of some derivatives against ADP-induced platelet aggregation, their IC₅₀ values were unremarkable (> 500 μM). Comparative analysis of activity data revealed that the antiplatelet effects were related to the size of alkyls substituted on the nitrogen of the indole ring. Thereby, propyl substitution caused weaker activity which could be considered due to the lack of enough space around indole nitrogen.

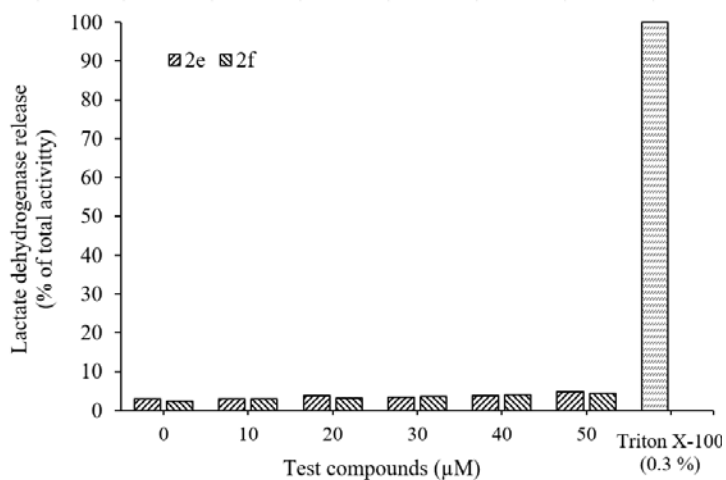


Fig. 3. Effects of different concentrations of compounds 2e and 2f on the release of lactate dehydrogenase from the platelets.

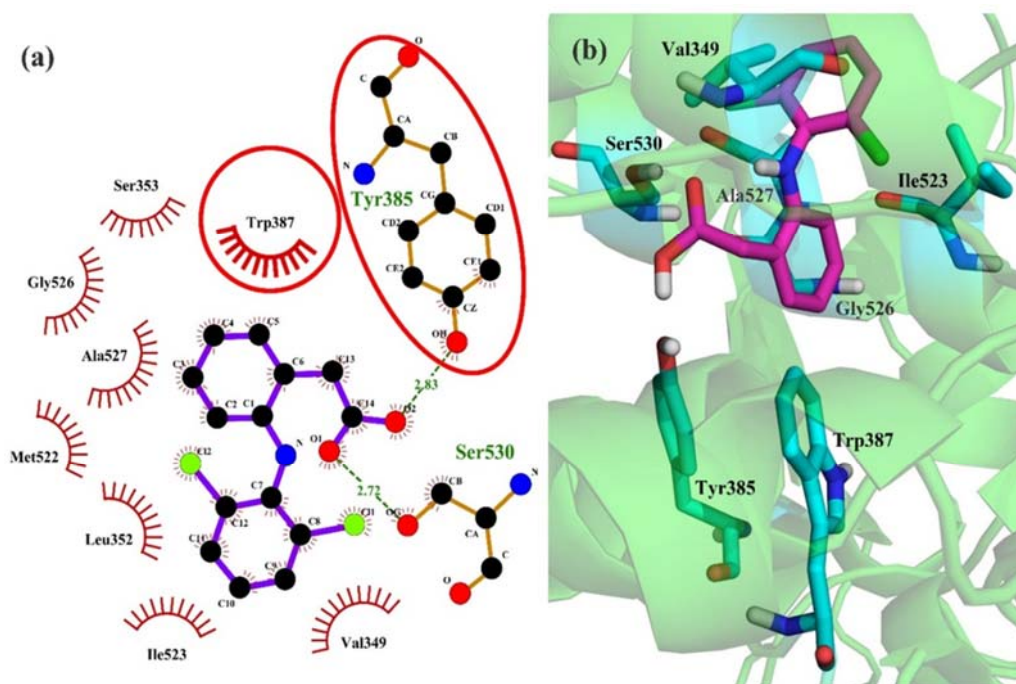


Fig. 4. Interaction of the diclofenac and cyclooxygenase-1. (a) Two-dimensional representation; olive green arrow: H bond interaction, brick red representation: hydrophobic site. Nitrogen is blue, oxygen is red, carbon is black, and chlorine is green. (b) Three-dimensional representation; the ligand is presented in magenta, the residues in blue.

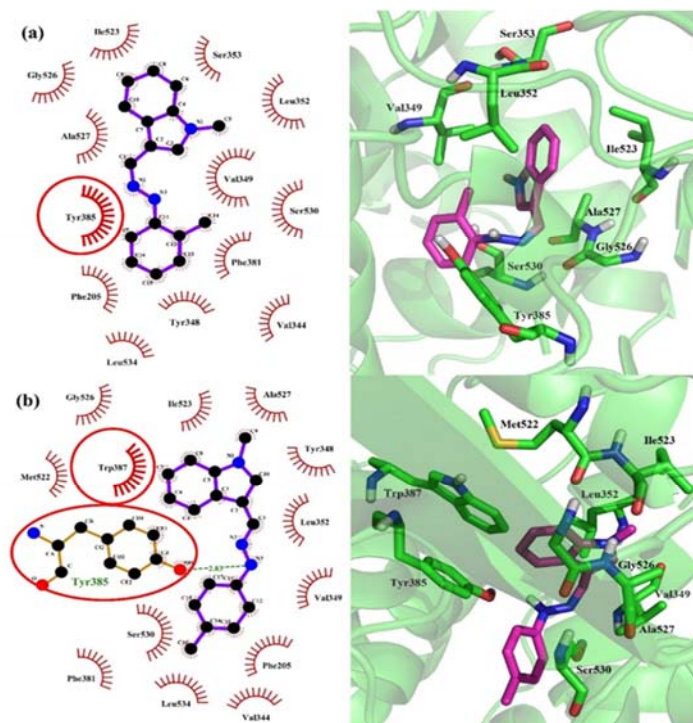


Fig. 5. 2D and 3D plots of the interaction between ligands and cyclooxygenase-1. (a) Compound 2e and (b) compound 2f.

Besides, the substitutions larger than methyl was not tolerated at the ortho position of the phenyl ring to the side chain. The proof of this claim is the difference between the activity data of two chlorine-substituted hydrazines at the positions of ortho and para of the phenyl ring (2a and 2b). As a consequence, it is either changing the angle of the phenyl ring with the side chain or space constraints around that region. The presence of lipophilic substitutions at the para position of the phenyl ring increased the antiplatelet activity and the activity did not change with different halogen sizes from fluorine to bromine. Furthermore, electronic effects of halogens were not responsible for the activity of compounds 2b, 2c, and 2d due to the similar effect that has been shown by methoxy substitution (2g).

Another interesting point was reducing the inhibition activity of compounds bearing methoxy at the para position of their hydrazone moiety (2g, 2n, 2u, respectively) against AA-induced platelet aggregation that was based on the size of the substitution replaced on the nitrogen of indole ring. This decreasing trend of the activity with para methoxy substitution that was confirmed by our previous study,

elucidates the possible existence of the different types of interaction at the para region of the phenyl ring (11). It may happen that getting larger of N1-substitution withdraws a hydrogen binding interaction through methoxy group due to the change of phenyl position in the active site.

It could be considered in Table 1, the most potent derivatives were 2e and 2f bearing two methyl substitutions, one on the phenyl ring (ortho or para) and the other on the nitrogen of the indole ring, compared with the related compounds bearing methyl phenyl on the hydrazone moiety (1g and 1h) which had been synthesized in our previous study (11), methylation of nitrogen in the indole ring (2e and 2f) in this study increased the antiplatelet activity significantly. This finding indicated the probable presence of a small lipophilic pocket around the nitrogen of indole at the receptor. In opposition, this trend was not observed in halogenated phenyl hydrazones (Cl or Br) which came to mind the steric hindrance of halogens has changed the conformation of the compounds and spatial constraints around nitrogen ring should be considered in this case.

Likewise, the molecular study indicated the presence of the specific hydrophobic interactions of the compounds 2e and 2f in the COX-1 active site that encourages the enzymatic evaluation of them in our future studies.

In accordance with their cLogP value shown in Table 2, the chemical structures of compounds 2e and 2f are able to pass through the biological membranes and likewise, the other parameters follow the rule of five. Hence, they could be potentially considered as suitable drug candidates. Hopefully, the safety assay of the two most active compounds on the platelets revealed exposing the test compounds to the platelets did not lead to a remarkable LDH release as compared to the vehicle.

CONCLUSION

To sum up, a series of N1-substituted indole hydrazones were prepared and evaluated for their antiplatelet activity. Most derivatives showed remarkable activity against AA-induced aggregation; conversely, none of them exhibited promising antiplatelet activity while ADP was the inducer. Structure-activity relationship study of the synthesized compounds revealed that lipophilicity of the para-substituted phenyl ring played a major role to improve the antiplatelet activity. The docking studies of the two most active compounds confirmed this conclusion by considering their interaction in the hydrophobic pocket of COX-1 as a target enzyme. Even though, this correlation between docking study and *in vitro* assay needs the enzymatic evaluation. To this end, this finding is a valuable clue for directing lead optimization studies to find out new antiplatelet derivatives with enhanced activity.

Acknowledgements

This study was financially supported by the Research Deputy of Zanjan University of Medical Sciences, Zanjan, I.R. Iran, through Grant No. A-12-613-10.

Conflicts of interest statement

The authors declared no conflict of interest in this study.

Authors' contribution

Sh. Mohebbi contributed to the design and synthesis of the compounds, N. Tavili contributed to synthesis and evaluation of biological activities of the compounds, Sh. Mokhtari synthesized the compounds and carried out the cell culture experiments, H. Salehabadi did the docking study, M. Esfahanizadeh contributed to the evaluation of the antiplatelet activity of the synthesized compounds. The final version of the manuscript was approved by all authors.

REFERENCES

- Mendis S, Puska P, Norrving B. Global Atlas on Cardiovascular Disease Prevention and Control. Geneva: World Health Organization; 2011. pp: 3-12.
- Stegner D, Klaus V, Nieswandt B. Platelets as modulators of cerebral ischemia/reperfusion injury. *Front Immunol.* 2019;10:2505,1-10. DOI: 10.3389/fimmu.2019.02505.
- Hall R, Mazer CD. Antiplatelet drugs: a review of their pharmacology and management in the perioperative period. *Anesth Analg.* 2011;112(2):292-318. DOI: 10.1213/ane.0b013e318203f38d.
- Guthrie R. Review and management of side effects associated with antiplatelet therapy for prevention of recurrent cerebrovascular events. *Adv Ther.* 2011;28:473-482. DOI: 10.1007/s12325-011-0026-0.
- Chelucci RC, Dutra LA, Lopes Pires ME, De Melo TRF, Bosquesi PL, Chung MC, *et al.* Antiplatelet and antithrombotic activities of non-steroidal anti-inflammatory drugs containing an *N*-acyl hydrazone subunit. *Molecules.* 2014;19(2):2089-2099. DOI: 10.3390/molecules19022089.
- Khalid W, Badshah A, Khan AU, Nadeem H, Ahmed S. Synthesis, characterization, molecular docking evaluation, antiplatelet and anticoagulant actions of 1,2,4 triazole hydrazone and sulphonamide novel derivatives. *Chem Cent J.* 2018;12(1):11-26. DOI: 10.1186/s13065-018-0378-5.
- Ramzan A, Nazeer A, Irfan A, Al-Sehemi A, Verpoort F, Khatak Z, *et al.* Synthesis and antiplatelet potential evaluation of 1,3,4-oxadiazoles derivatives. *Z Phys Chem.* 2019;233(12):1741-1759. DOI: 10.1515/zpch-2018-1316.
- Silva GA, Costa LMM, Brito FCF, Miranda ALP, Barreiro EJ, Fraga CA. New class of potent antinociceptive and antiplatelet 10*H*-phenothiazine-1-acylhydrazone derivatives. *Bioorg Med Chem.* 2004;12(12):3149-3158. DOI: 10.1016/j.bmc.2004.04.009.
- Mashayekhi V, Tehrani KHME, Amidi S, Kobarfard F. Synthesis of novel indole hydrazine derivatives and evaluation of their antiplatelet aggregation

- activity. *Chem Pharm Bull (Tokyo)*. 2013;61(2):144-150.
DOI: 10.1248/cpb.c12-00597.
10. Lima LA, Frattani FS, Dos Santos JL, Castro HC, Fraga CA, Zingali RB, *et al.* Synthesis and antiplatelet activity of novel arylsulfonate-acylhydrazone derivatives, designed as antithrombotic candidates. *Eur J Med Chem*. 2008;43(2):348-356.
DOI: 10.1016/j.ejmech.2007.03.032.
 11. Tehrani KHME, Esfahanizadeh M, Mashayekhi V, Hashemi M, Kobarfard F, Gharebaghi F, *et al.* Synthesis, antiplatelet activity and cytotoxicity assessment of indole-based hydrazine derivatives. *Iran J Pharm Res*. 2015;14(4):1077-1086.
PMID: 26664374.
 12. Kalhor N, Mardani M, Abdollahzadeh S, Vakof M, Esfahanizadeh M, Tehrani KHME, *et al.* Novel *N*-substituted ((1*H*-indol-3-yl)methylene) benzohydrazides and ((1*H*-indol-3-yl)methylene)-2-phenylhydrazines: synthesis and antiplatelet aggregation activity. *Bull Korean Chem Soc*. 2015;36(11):2632-2639.
DOI: 10.1002/bkcs.10531.
 13. Esfahanizadeh M, Mohebbi S, Bozorg BD, Amidi S, Gudarzi A, Ayatollahi SA, *et al.* Synthesis and antiplatelet aggregation activity evaluation of some 2-aminopyrimidine and 2-substituted-4,6-diaminopyrimidine derivatives. *Iran J Pharm Res*. 2015;14(2):417-427.
PMID: 25901148.
 14. Sum TJ, Sum TH, Galloway WRJD, Twigg DG, Ciardiello JJ, Spring DR. Synthesis of structurally diverse biflavonoids. *Tetrahedron*. 2018;74(38):5089-5101.
DOI: 10.1016/j.tet.2018.05.003.
 15. Chemate S, Sekar N. Indole-based NLOphoric donor- π -acceptor styryl dyes: synthesis, spectral properties and computational studies. *J Fluoresc*. 2016;26(6):2063-2077.
DOI: 10.1007/s10895-016-1901-5.
 16. Li W, Qi YY, Wang YY, Gan YY, Shao LH, Zhang LQ, *et al.* Design, synthesis, and biological evaluation of sorafenib derivatives containing indole (ketone) semicarbazide analogs as antitumor agents. *J Heterocyclic Chem*. 2020;57(6):2548-2560.
DOI: 10.1002/jhet.3972.
 17. Hassan M, Ghaffari R, Sardari S, Farahani YF, Mohebbi S. Discovery of novel isatin-based thiosemicarbazones: synthesis, antibacterial, antifungal, and antimycobacterial screening. *Res Pharm Sci*. 2020;15(3):281-290.
DOI: 10.4103/1735-5362.288435.
 18. Rezaee Nasab R, Mansourian M, Hassanzadeh F. Synthesis, antimicrobial evaluation and docking studies of some novel quinazolinone Schiff base derivatives. *Res Pharm Sci*. 2018;13(3):213-221.
DOI: 10.4103/1735-5362.228942.
 19. Born GV. Aggregation of blood platelets by adenosine diphosphate and its reversal. *Nature*. 1962;194:927-929.
DOI: 10.1038/194927b0.
 20. Eskandariyan Z, Esfahanizadeh M, Tehrani KHME, Mashayekhi V, Kobarfard F. Synthesis of thioether derivatives of quinazoline-4-one-2-thione and evaluation of their antiplatelet aggregation activity. *Arch Pharm Res*. 2014;37(3):332-339.
DOI: 10.1007/s12272-013-0192-5.
 21. Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, *et al.* AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility. *J Comput Chem*. 2009;30(16):2785-2791.
DOI: 10.1002/jcc.21256.
 22. Abbasi M, Sadeghi-Aliabadi H, Hassanzadeh F, Amanlou M. Prediction of dual agents as an activator of mutant p53 and inhibitor of Hsp90 by docking, molecular dynamic simulation and virtual screening. *J Mol Graph Model*. 2015;61:186-195.
DOI: 10.1016/j.jmgm.2015.08.001.
 23. Sander T, Freyss J, von Korff M, Rufener C. DataWarrior: an open-source program for chemistry aware data visualization and analysis. *J Chem Inf Model*. 2015;55(2):460-473.
DOI: 10.1021/ci500588j.
 24. Moraes AdTO, Miranda MDS, Jacob ÍTT, Amorim CAD, Moura ROD, Silva SASd, *et al.* Synthesis, *in vitro* and *in vivo* biological evaluation, COX-1/2 inhibition and molecular docking study of indole-*N*-acylhydrazone derivatives. *Bioorg Med Chem*. 2018;26(20):5388-5396.
DOI: 10.1016/j.bmc.2018.07.024.
 25. Borges A, Casoti R, Silva MLA, Cunha NL, Rocha Pissurno AP, Kawano DF, *et al.* COX inhibition profiles and molecular docking studies of the lignan hinokinin and some synthetic derivatives. *Mol Inform*. 2018;37(12):e1800037.
DOI: 10.1002/minf.201800037.
 26. Abdel-Aziz AAM, Abou-Zeid LA, ElTahir KEH, Mohamed MA, Abu El-Enin MA, El-Azab AS. Design, synthesis of 2,3-disubstitued 4(3*H*)-quinazolinone derivatives as anti-inflammatory and analgesic agents: COX-1/2 inhibitory activities and molecular docking studies. *Bioorg Med Chem*. 2016;24(16):3818-3828.
DOI: 10.1016/j.bmc.2016.06.026.