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Design, Synthesis, Docking Study and Antiplatelet Evaluation of New Thiosemicarbazide Derivatives,Derived From Captopril

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ABSTRACT

A series of thiosemicarbazide derivatives of captopril, a well-known angiotensin-converting enzyme inhibitor ACEI, have been synthesized by reaction of hydrazide of captopril with different phenylisothiocyanate substituents. The synthesized compounds were characterized using FTIR, ^{1}HNMR and CHNS analysis. The final derivatives were tested for antiplatelet activity using multiplate analyzer and adenosine diphosphate (ADP), arachidonic acid (AA), and collagen, as platelet aggregation inducers. Among tested compounds, derivative 7 and 10 were the most potent inhibitors of platelet aggregation induced by arachidonic acid, with percent inhibition (97.14±0 and 95.71±2.02) and IC_{50} (2.7 and 1.21µg\ml), respectively. Molecular docking study was performed using purino receptor P_2Y_{12}, COX-1, and glycoprotein IIb\IIIa as the target protein, compound 7 has a potential to become as a lead molecule for COX-1 inhibitor with binding energy (-10.67) Kcal/mol. Also, compound 6 was found as the best inhibitor for the glycoprotein IIa/IIIb with percent inhibition (83.9±2.8), and binding energy (-10.05) Kcal/mol.

Keywords: Captopril, ACEI, Antiplatelet, Thiosemicarbazide, COX-1.

INTRODUCTION

Thrombosis is a pathological clot takes a vital role in many diseases as deep venous thrombosis, myocardial infarction, and stroke[1,2]. Haematosis refer to the maintenance of blood fluidity and prevents it lose of after vascular injury[3]. Platelet and coagulation factors have a leading role in haemostatic and thrombotic processes, which are involved in thrombus formation and to avoid haemorrhage through stimulation and stabilization of thrombin[4,5]. One of the rational approaches for prevents and treatment of the cardiovascular diseases associated with thrombosis is using anticoagulant and antiplatelet drugs.

The coagulation system consists of intrinsic and extrinsic pathways the typical final mediated of this system is thrombin, that trigger production of factors (V, VIII, and IX), activation of platelet and cleavage of fibrinogen to fibrin where it remains active after binds to fibrin[6], many approaches for protection or treatment of thromboembolic events depend on inhibition of thrombin formation or block its activity. While the main antiplatelet mechanisms are; first, cycloxygenase-1 (COX-1) inhibitor, likes aspirin, by inhibition of (COX-1), aspirin will reduce the extent of thromboxane A2 formation, and consequently the aggregation of platelets[7], like most NSAIDs, its use is associated with the induce of asthma, gastrointestinal (GIT) disorder, and reduce the number of white cells and platelet[8]. Second, adenosine diphosphate (ADP) antagonist as clopidogrel, which acts by preventing (ADP) stimulates platelet's purinergic receptor P_2Y_{12}, it has more considerable role in treatment of pathological conditions associated with high platelet reactivity, as acute coronary syndrome (ACS), and coronary artery disease (CAD), also its uses associated with GIT adverse effect[9]. Third, platelet membrane glycoprotein IIb/IIIa receptor inhibitor as tirofiban that is used in treating coronary artery bypass grafting (CABG), and atherectomy, bleeding is the significant adverse effect[10].

Thiosemicarbazide and its derivatives have attracted significant attention as versatile reagents in synthetic organic chemistry. It shows a broad spectrum of biological activities, such as antifungal, antibacterial, anticonvulsant[11], antioxidant[12], antitumor[13]. Anti-inflammatory and antithrombotic activity of synthetic compounds contain thiosemicarbazide moiety, make it an attractive unit in developing a new compound for cardiovascular activity[14,15].

The aim of this study, is to synthesize a series of new captopril derivatives, a first oral active angiotensin converting enzyme inhibitor (ACEI) drug, containing thiosemicarbazide moiety, for enhancing the cardiovascular activity of captopril, and avoid the main limitations that associated with common anticoagulant, and antiplatelet drugs.

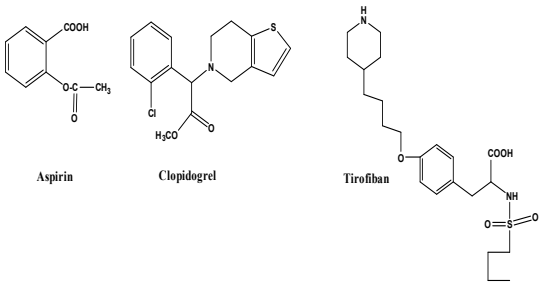


Fig. 1. Chemical structures of some antiplatelet drugs

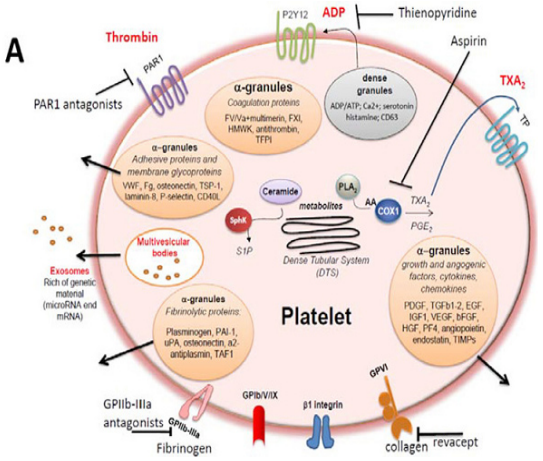


Fig. 2. Platelet receptor-ligand interaction[16]

MATERIALS AND METHODS

All reagents and anhydrous solvents were used as received from the commercial suppliers, (Sigma-Aldrich, Munich, Germany, BDH, Pool Dorset, England and Fluka, Newport News, USA). Captopril as (s,s) isomer was purchased from Sigma-Aldrich (Shanghai, China). Melting points were determined by the capillary method using Electrothermal IA9000, Essex, UK and they are uncorrected. Thin layer chromatography (TLC) was run on silica gel (60) F254 Merck (Germany), exposed to UV254 nm light, and the eluent used is n-hexane: ethyl acetate (1:1) for compounds (1,2) and (5-10), and chloroform: methanol (1:1) for compounds (3) and (4), to check the purity of the compounds, as well as, monitoring the progress of reactions. FT-IR spectra were recorded by using a Shimadzu model (Kyoto, Japan) spectrophotometer on KBr disk, (ν = cm^{-1}).CHNS microanalysis was done by using a Euro EA3000 elemental analyzer (Carlo Erba, Milan, Italy). ^{1}HNMR spectra were recorded on Inova model Ultra shield 500MHz, at (Tehran University, Iran), using tetramethylsilane

(TMS) as an internal standard. The chemical shift was expressed as (δ= ppm), DMSO-d_6 was used as a solvent.

Chemical Synthesis

Chemical synthesis of all new derivatives is depicted in scheme 1.

Synthesis of methyl 1-(3-mercapto-2-methylpropanoyl) pyrrolidine-2-carboxylate (1): [17,18]

To (9.21 mmol, 2.0 g) of cold captopril solution in (20 ml) methanol, (9.21 mmol, 1.8 ml) of thionyl chloride ($SOCl_2$), was added via dropping funnel, over 30 min. The temperature was maintained at 0^{O}C for 1h. The mixture was stirred for 2 h, at r. t and then, 4h at 50-60^{O}C. Excess of solvent and ($SOCl_2$) was removed by rotary evaporator. The product was re-dissolved in (20 ml) ethyl acetate, and extracted with 5% sodium bicarbonate and water (3x20ml), respectively. The organic layer was dried over anhydrous $MgSO_4$, and left the solvent to evaporate.

Colourless oil, yield 50%., B.P 75^{O}C, R_f = 0.5, IR (KBr disc, v= cm^{-1}): 2972.31 and 2875.86 (CH) str of aliph. CH_2 & CH_3; 2549.89 (SH) str of thiol; 1732.08 (C=O) str of ester; 1639.49 (C=O) str of amide of pyrrolidine; 1172.08 asym str (C-O-C); 1043.43 sym (C-O-C) str of OCH_3.

Synthesis of ethyl 1-(3-(ethylthio)-2-methylpropanoyl) pyrrolidine-2-carboxylate (2)[19-21]

To a solution of captopril (9.20 mmol, 2.0g) in (25 ml) of dry acetone, (18.40 mmol, 2.54 g) of anhydrous K_2CO_3 was added. The mixture stirred for 1 h at r.t. Then (18.4 mmol, 5.6 ml,) of ethyl iodide (C_2H_5-I) was added throughout 15 min. The reaction was heated at 50^{O}C for 24 hour. Under reduced pressure; an excess of solvent was removed after filtration, then re-dissolved the product in (20 ml) ethyl acetate, and extracted it with 5%$NaHCO_3$ and water (3x20 ml) respectively, then dried over anhydrous $MgSO_4$, and left it overnight.

Yellow oily, yield 72%, B.P 79^{O}C, R_f = 0.8, IR (KBr disc, v =cm^{-1}): 2974.23, 2931.18 and 2873.94 (C-H) str. of aliph. CH_2 & CH_3; 1728.22 (C=O) str. of ester; 1645.28 (C=O) str of amide pyrrolidine; 1028.06 (C-O-C) str of ester.

General method for the synthesis of 1-(3-mercapto-2-methylpropanoyl)pyrrolidine-2-carbohydrazide (3);and1-(3-(ethylthio)-2-methylpropanoyl) pyrrolidine-2-carbohydrazide (4).[22,23]

To (9.0 mmol), (0.18 g) of compound 1 or (0.20 g) of 2, respectively, in (20 ml) of absolute EtOH, (27 mmol, 0.88 ml) of hydrazine hydrate was added slowly, with stirring for 2 h at r.t then, the mixture was refluxed for 6 h. The solvent was removed under reduced pressure, the white precipitate formed, was recrystallized from methanol: diethyl ether (1:2).

Compound 3

White powder, yield 72%, m.p: 160-162^{O}C, R_f = 0.9. IR (KBr disc, (v=cm^{-1}): 3506.59 and 3460.3 (NH) str of prim. amine; 3332.99 (NH) str of prim. amide; 2970.38, 2947.23 and 2873.94 (C-H) str of aliph. CH_2 & CH_3; 2592.33 str of (SH); 1662 (C=O) str of amide hydrazide; 1635.64 (C=O) str amide of pyrrolidine. Elemental analysis: Calcd. for ($C_9H_{17}N_3O_2S$): C: 46.73; H: 7.41; N: 18.17; S: 13.86. Found: C: 46.20; H: 7.32; N: 17.82; S: 13.11. ^{1}HNMR (500MHz, DMSO-d_6, δ=ppm); 9.22 (1H, s, NH-minor); 8.91 (1H, NH-major); 4.21 (2H, brs, NH_2); 3.55 (2H, m, CH_2-pyrrolidine); 2.45-2.96 (3H, m, CH_2 and CH aliph.); 1.83 (3H, m, CH_2 and CH-pyrrolidine); 1.04 (3H, d, CH_3).

Compound 4

White powder, yield 65%, m. p: 175-177^{O}C, R_f = 0.85, IR (KBr disc, v=cm^{-1}): 3305.99 and 3213.41 (NH) str of prim. amine; 3028.24 (NH) str of prim. amide; 2970.38, 2931.8 and 2873.94 (C-H) str of aliph. CH_2 and CH_3; 1693.3 (C=O) str of amide hydrazide; 1647.21 (C=O) str amide of pryrrolidine. Elemental analysis: calcd. for ($C_{11}H_{21}N_3O_2S$): C: 50.94; H: 8.16; N: 16.20; S: 12.36. Found: C: 50.60; H: 7.91; N: 16.11; S: 11.88. ^{1}HNMR (500MHz, CD_3OD, δ=ppm); 4.38 (1H, dd, CH-pyrrolidine); 3.73 (3H, m, CH_2 and CH-pyrrolidine); 2.61-3.12 (5H, m, 2(CH_2) and CH-aliph.), 1.70-2.25 (3H, m, CH_2 and CH-pyrrolidine); 1.19-1.25 (6H, m, 2CH_3).

General method for the synthesis of non-protected / free (SH)- thiosemicarbazide captopril derivatives (5-8), and synthesis of ethyl protected (SH) thiosemicarbazide captopril derivatives (9-10). [24]

To (1.90 mmol), (0.40 g) of compound 3 or (0.50 g) of 4, in (20 ml) absolute methanol, (1.90 mmol) of phenylisothiocyanate derivatives each was added as dropwise with stirring. The reaction

temperature was maintained at 40°-50°C, and it was monitored by TLC for 4-6 h, using eluent, *n*-hexane: ethyl acetate (1:1). The solid precipitate was filtrated, recrystallized with ethyl acetate, and dried in a vacuum desiccator.

Compound 5: 2-((3-mercapto-2-methylpropanoyl) prolyl)-N-phenylhydrazine-1- carbothioamide

White powder, yield 45%, m.p: 180-185°C, R_f = 0.21, IR (KBr disc, v= cm^{-1}): 3248.13 br. (N-H) str of (1st amide, 2nd amine and NH adjacent to thiocarbonyl); 3055.24 Ar (CH) str; 2974.23, 2951.09 and 2873.94 (C-H) str of aliph. CH_2 & CH_3; 2592.33 str of (SH); 1693.5 (C=O) str of amide hydrazide; 1616.35(C=O) str of amide pyrrolidine; 1546.91, 1500.62 and 1446.61 Ar(C=C) str; 1184.29 str of (C=S). Elemental analysis: Calcd. for ($C_{16}H_{22}N_4O_2S_2$) C: 52.43; H: 6.05; N: 15.29; S: 17.50. Found: C: 52.22; H: 5.77; N: 15.15; S: 17.24. ^{1}HNMR (500MHz, DMSO-$_{d6}$, δ=ppm); 10.38 (1H,s, (O=C)-NH); 9.69 (1H,s, NH-NH-(C=S); 9.18(1H,s, (S=C)-NH-ph); 7.16-7.72 (5H,m, Ar-H); 4.16 (1H,dd, CH-pyrrolidine); 3.59 (2H,m, CH_2-pyrrolidine); 2.78 (3H,m, CH_2 and CH-aliphatic); 1.95 (3H, m, CH_2 and CH-pyrrolidine); 0.99 (3H, d, CH_3).

Compound 6: N-(4-bromophenyl)-2-((3-mercapto-2-methylpropanoyl) prolyl) hydrazine-1-carbothioamide

White powder, yield 69%, m.p: 198-200, R_f = 0.3, IR (KBr disc, v=cm^{-1}): 3232.7 br (NH) str of (1st amide, 2nd amine and NH adjacent to thiocarbonyl); 2974.23, 2931.8 and 2873.94 (C-H) str of aliph.CH_2 & CH_3; 2507.46 cm^{-1} str of(SH); 1685.79 (C=O) str of 1stamide; 1624.06 (C=O) str amide of pyrrolidine; 1180.44 str of (C=S); 1068.56 str of (C-Br). Elemental analysis: Calcd. for ($C_{16}H_{21}BrN_4O_2S_2$) C: 43.15; H: 4.75; N: 12.58; S: 14.40. Found: C: 43.08; H: 4.73; N: 12.52; S: 14.29. ^{1}HNMR (500MHz, DMSO-$_{d6}$, δ=ppm); 10.53 (1H,s, (O=C)-NH); 9.92 (1H, s, NH-NH-(C=S); 9.25 (1H, s, (S=C)-NH-ph); 7.69 (2H, d, 2Ar-H); 7.52 (2H,d, 2Ar-H); 4.17 (1H, dd, CH-pyrrolidine); 3.65 (2H, m, CH_2-pyrrolidine); 2.71-3.03 (3H,m, CH_2 and CH-aliph); 1.03 (3H, d, CH_3).

Compound 7: N-(4-chlorophenyl)-2-((3-mercapto -2-methylpropanoyl)prolyl)hydrazine-1-carbothioamide

Pale white powder, yield 48%, m.p: 190-192C, R_f = 0.21, IR (KBr disk, v =cm^{-1}): 3236.55 br (NH) str of (1st amide, 2nd amine and NH adjacent to thiocarbonyl); 2974.23, 2931.8 and 2873.94 (C-H) str of aliph. CH_2 & CH_3; 2565.33 cm^{-1} str of (SH); 1685.79 (C=O) str of 1st amide; 1620.21(C=O) str of amide pyrrolidine; 1539.2, 1492.9 and 1462.04 str of Ar-(C=C); 1176.58str of (C=S); 1087.85 str of (C-Cl). Elemental analysis: Calcd. for ($C_{16}H_{21}ClN_4O_2S_2$) C: 47.93; H: 5.28; N: 13.97; S: 15.99. Found: C: 47.88; H: 5.24; N: 13.90; S: 15.81. ^{1}HNMR (500MHz, DMSO-$_{d6}$, δ=ppm); 10.52 (1H, s, (C=O)-NH); 9.90 (1H, s, NH-NH-(C=S); 9.25 (1H, s, (C=S)-NH-ph); 7.74 (2H, d, 2Ar-H); 7.39 (2H, d, Ar-H); 4.17(1H,dd, CH-pyrrolidine); 3.65 (2H, m, CH_2-pyrrolidine); 2.71-3.02 (3H, m, CH_2 and CH-aliph.); 1.86-2.15 (4H, m, 2(CH_2)- pyrrolidine; 1.035 (3H, d, CH_3).

Compound 8: 2-((3-mercapto-2-methylpropanoyl)prolyl)-N-(4-methoxyphenyl)hydrazine-1-carbothioamide

Yellow powder, yield 62%, m.p:198-200 °C, R_f = 0.33, IR (KBr disc, v = cm^{-1}): 3224.98 (N-H) str of (1st amide, 2nd amine and NH adjacent to thiocarbonyl); 3124.68 Ar (C-H) str; 2974.23, 2935.66 and 2877.79 (C-H) str of aliph. CH_2 & CH_3; 2557.61 str of(SH); 1681.93 (C=O) str of 1st amide; 1624.06(C=O) str of amide pyrrolidine; 1543.05, 1512.19 and 1462.04 Ar(C=C) str; 1246 asym (C-O) str; 1180.44 str of (C=S); 1029 sym (C-O) str .Elemental analysis: Calcd. for ($C_{17}H_{24}N_4O_3S_2$) C: 51.49; H: 6.10; N: 14.13; S: 16.17. Found: C: 51.38; H: 5.97: N: 14.4; S: 15.97. ^{1}HNMR (500MHz, DMSO-$_{d6}$, δ=ppm); 10.33 (1H, s, (C=O)-NH); 9.57 (1H, s, NH-NH-(C=S); 9.08 (1H, s, (C=S)-NH-ph); 7.44 (2H, d, 2Ar); 6.83 (2H, d, 2Ar-H); 4.15 (1H, dd, CH-pyrrolidine); 3.68 (3H, s, OCH3); 3.59 (2H, m, CH_2 pyrrolidine); 2.42-2.94 (3H, m, CH_2 and CH-aliph.); 1.25-2.25 (3H, m, CH_2 and CH-pyrrolidine); 0.98 (3H, d, CH_3).

Compound 9: 2-((3-(ethylthio)-2-methylpropanoyl) prolyl)-N-phenylhydrazine-1-carbothioamide

White powder, yield 49 %, m.p: 193-196 °C), R_f = 0.47, IR (KBr disc, v = cm^{-1}): 3282.84 overlap (NH) str of (1st amide and 2nd amine); 3244.27 (N-H) str adjacent to thiocarbonyl; 3055.24 Ar(C-H) str; 2974.23, 2931.8 and 2873.94 (C-H) str of aliph. CH_2 & CH_3; 1689.64 (C=O) str of 1st. amide; 1654.92 (C=O) str of amide of pyrrolidine; 1600.92, 1546.91 and 1500.62 Ar (C=C) str; 1184.29 str of (C=S). Elemental analysis: Calcd. for ($C_{18}H_{26}N_4O_2S_2$): C: 54.79; H: 6.64; N: 14.20; S: 16.25. Found: C: 54.70; H: 6.55; N: 13.60; S: 16.05. ^{1}HNMR (500MHz, DMSO-$_{d6}$, δ=ppm); 10.49 (1H, s, (O=C)-NH); 9.79 (1H, s, NH-NH-(C=S); 9.19 (1H, s, (S=C)-NH-ph); 7.12-7.68 (5H, m, 5Ar-H); 4.18

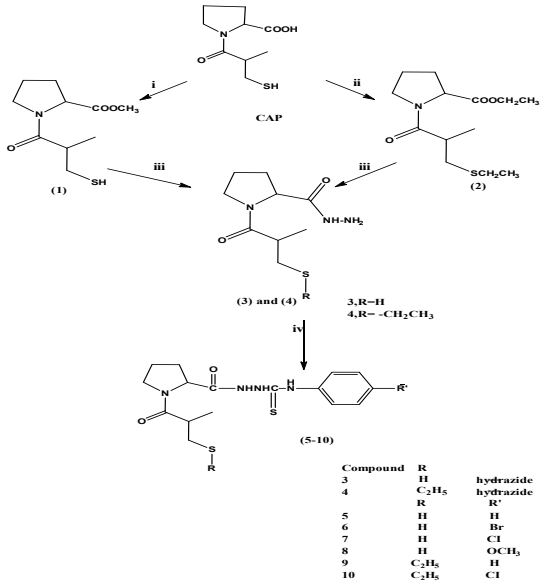
(1H, dd, CH-pyrrolidine); 3.64 (3H, m, CH_2 and CH-pyrrolidine); 2.71-3.02 (3H, m, CH_2 and CH-aliph.); 2.09 (2H, q, CH_2); 1.94(3H,t, CH_3); 1.03 (3H,d, CH_3).

Compound 10: N-(4-chlorophenyl)-2-((3-(ethylthio)-2-methylpropanoyl)prolyl)hydrazine-1-carbothioamide

Pale white powder, yield 52%, m.p: 202-204°C, R_f = 0.4. IR (KBr disc, v = cm^{-1}): 3197.98 overlap str for (NH) of (1st amide and 2nd amine); 3109.25 str for (N-H) adjacent to thiocarbonyl; 3005.1 Ar(C-H) str; 2939.52 (C-H) str of. aliph CH_2 & CH_3; 1678.07 (C=O) str of 1st amide; 1631.78 (C=O) str of amide pyrrolidine; 1534.05, 1508.33 and 1492.90 Ar(C=C) str; 1192.01 str of (C=S); 1087.85 str for (C-Cl). Elemental analysis: Calcd for ($C_{18}H_{25}ClN_4O_2S_2$) C: 50.39; H: 5.87; N: 13.06; S; 14.95; Cl: 8.26. Found: C: 49.49; H: 5.81; N: 13.00; S: 14.65. ^{1}HNMR (500MHz, DMSO-$_{d6}$, δ=ppm); 10.52 (1H, s, (C=O)-NH); 9.91 (1H, s, NH-NH-(C=S); 9.24 (1H,s, (C=S)-NH-ph);7.70 (2H, d, 2Ar-H); 7.51 (2H,d, 2Ar-H); 4.17 (1H,dd, CH-pyrrolidine); 3.75 (4H, m, 2(CH_2) pyrrolidine); 2.71-3.38(3H, m, CH_2 and CH-aliph.); 2.09 (2H,q, CH_2), 1.88 (3H, t, CH_3); 1.03 (3H,d, CH_3).

Antiplatelet Activity

The antiplatelet aggregation activity of the final synthetic CAP derivatives was measured using human plasma. Fresh blood samples were obtained from non-smoker healthy volunteers with a negative history of drug consumption, up to 14 days before the test.



Scheme 1: Synthesis of new CAP derivatives (1-10) (i) $SOCl_2$, CH_3OH (ii) $CHCH_2I$, K_2CO_3, $(CH_3)_2CO$ (iii) NH_2NH_2, CH_3CH_2OH. (iv) R' PhNCS, CH_3OH

The whole blood was collected in sodium citrate (9:1) by volume. Specific weight of each tested compound was dissolved in (DMSO), to prepare different concentrations (25, 50, 100, 150 and 200µg\ml). 500 µl of sample contains 250 µl of normal saline, 125 µl of whole blood, and 125 µl from each concentration of tested compound was incubated for 3 min at 37°C, then 5µl of (ADP) (5 µm), 5µl of arachidonic acid (0.25mM), and 1 µl of collagen (1µg\ml), were added separately, and aggregation was monitored for 6 min, and area under curve (AUC) was recorded. Aspirin was used as a standard drug, and platelet aggregation inhibition percent was calculated, using Equation 1, where IC_{50} was calculated from first order linear equation. Equation 1[25]:

% Inhibition = [1- (D/S)] * 100

Where D: platelet aggregation in the presence of a tested compound, S: platelet aggregation in the presence of a solvent.

Docking Study

Molecular docking is an attractive scaffold of computational modelling, which facilitates the prediction of preferred binding orientation of one molecule to another, as well as, help in the mechanistic study by placing a molecule (ligand) into the preferred binding site of the target specific region of the DNA/protein (receptor), mainly in a non-covalent fashion, to form a stable complex of potential efficacy and more specificity when both interact with each other, in order to form a stable complex.[26,27]

Computer stimulation automated docking study was performed using molecular docking software, Pymol Autodock/vina plugin.The crystallography structure of purino receptor P_2Y_{12}, cyclooxygenase-1 (COX-1), and glycoprotein IIb/IIIa proteins were taken from (PDB) with resolution 1.45, 2.61 and 2.45A° and PDB ID (4XPZ, 1EQG, and 3ZDY), respectively. The target proteins were prepared for docking study using VC5F chimera version 1.11.2 software, where all bonded ligands, water molecules were removed from it.

Gauss view 6.0 software was used for building the 3D structure of starting and designed derivatives and for optimization the energy for flexible

docking. The binding affinity, ordering ligand and inhibition constant (Ki) were performed using pymol Auto dock/ vina plugin, while the type of amino acid in the target protein that involved in the formation of H-bond, and its number, was predicted using Auto dock-tools 1.5.6.

RESULTS AND DISCUSSION

Chemistry

Methyl ester 1 obtained by adding an equal mmole of thionyl chloride to ice solution of captopril in absolute methanol, raise the temperature of the reaction mixture to catalyze the reaction. Compound 1 characterized by IR spectroscopy, due to the absence of broad OH band, and carbonyl band of CAP carboxylic acid, and appearance of a peak of (C=O) ester str, at 1732 cm^{-1}.

Ethyl ester 2 obtained from reaction of two mmoles of ethyl iodide with CAP solution, in dry acetone, for esterification of carboxylic acid group, and ethyl alkylation of the thiol group (SH), in the presence of anhydrous K_2CO_3, as a catalyst. The absence of (SH) peak at 2565 cm^{-1} and appearance of (C=O) ester peak at 1728.22 cm^{-1}, are the main IR characterization.

Compounds 3 and 4 are prepared by refluxing a solution of compounds 1 or 2 in dry EtOH with hydrazine hydrate for 2 h, respectively. The IR spectrum characterized by a band of hydrazide primary amine at 3506, 3460 and 3305, 3213 cm -1 for 3 and 4, respectively, with a distinct peak for (C=O) str. of an amide of hydrazide at 1662 cm^{-1} and 1693.3 cm^{-1}, respectively.

Thiosemicarbazide derivatives of CAP (5-10) were synthesized by stirring a warm solution of compounds 3 or 4 with the corresponding substituted phenyl isothiocyanate derivatives.

IR spectra of (5-10), each displayed distinct broad band at 3248, 3232, 3236, 3224, 3282, and 3197, respectively, indicating overlap of three (NH) bands.

^{1}HNMR spectroscopy displayed characteristic signals for the prepared compounds. Compound 1 showed a distinct singlet peak at δ= 3.55.ppm due to (OCH_3) str, an (indication of methyl ester formation). Hydrazide compound 3, displayed in ^{1}HNMR spectrum a singlet peak, at δ =9.22 and δ=8.91ppm due to major and minor NH group, also another significant peak at δ= 4.21 ppm, as abroad singlet, due to appearance NH_2 of hydrazide.

While compound 4, using CD_3OD as a solvent, recorded a peak as a multiplet at δ=2.55-3.20 ppm integrating for five protons, due to two aliphatic (CH_2) and one (CH) groups, another significant peak at δ=1.19-1.25 ppm, integrating for six protons owing to two aliphatic methyl groups ($2CH_3$).

Compounds (5-8), each displayed three prominent signals, attributed to three NH groups of the thiosemicarbazide derivatives, as follow: (C=O) NH, NHNH-(C=S) and (C=S)-NH-ph: at δ=10.38, 9.69, 9,18 for compound 5; δ=10.53, 9.92, 9.25 for compound 6; δ=10.52, 9.90, 9.25 for compound 7; and δ=10.33 , 9.57, and 9.08 ppm for compound 8, respectively.

Also, compounds (9 &10), each recorded the following signals for the three NH groups at δ= 10.49, 9.79, 9.19 for compound 9 and 10.52, 9.91 and 9.24 ppm for compound 10.

In addition, the S-ethylation group (S-CH_2-CH_3-) of each protected (SH), displayed obviously, for compound 9, (CH_3) recorded as a triplet at δ= 1.94 ppm, and at δ= 2.09 ppm, as a quartet for (CH_2) group, while compound 10 showed a signal at δ= 1.88 ppm, as a triplet, due to (CH_3), and at δ= 2.09 ppm, as a quartet due to (CH_2), respectively.

All the aromatic protons for the compounds (5-10) displayed at their expected aromatic region (see exp. part).

Antiplatelet Activity

The *in- vitro* antiplatelet activity of all final derivatives was assayed on whole human blood using Multiplate® analyzer. Adenosine diphosphate (ADP), arachidonic acid (AA), and collagen were used as inducers for platelet aggregation, where aspirin was used as positive control. The IC_{50} was defined as the concentration of the test compound that inhibits platelet aggregation by 50 percent.

The antiplatelet activity of tested compounds is listed in (Table 1). Data shows that the majority of derivatives inhibit platelet aggregation induced by arachidonic acid and collagen greater than that induced by ADP. Among tested compounds, derivative 7 and 10 were more potent inhibitor of platelet aggregation induced by arachidonic acid with % inhibition (97.14±0) and %(95.71±2.02), and IC_{50} (2.7 and 1.217 µg\ml), respectively, and compound 6 and 10, showed better antiplatelet activity induced by collagen with % inhibition (83.9±2.8 and 78.5±1.44) and IC_{50} (5.27 and 0.817 µg\ml), respectively. Based on these results, it could be suggested that phenyl substitution with electron withdrawal group (EWG) enhances the antiplatelet activity of a synthetic compound in a better manner than unsubstituted, or phenyl substituted with electron donating group (EDG). However, the activity of all synthetic derivatives are still better than that of a parent (captopril), that means, chemical replacement of carboxylic acid of captopril with thiosemicarbazide moiety could enhance antiplatelet activity, while the modification on thiol group, has a little effect.

Table1: Percent inhibition ± SD and IC_{50} of tested compounds (5-10), using ADP, AA, and collagen as inducer agents

Compound	ADP %INH±SD	IC_{50} µg\ml	AA %INH±SD	IC_{50} µg\ml	Collagen %INH±SD	IC_{50} µg\ml
5	42.23±0	259.08	88.5±0	57.98	73.46±0	26.6
6	49.4±0	209.67	91.4±4.04	15.8	83.9±2.8	5.27
7	40.588±0.88	283.15	97.14±0.0	2.7	70.4±1.44	9.6
8	45.88±0	218.17	92.85±2.02	16.01	76.5±1.44	10.6
9	17.64±0	569.1 µg\ml	81.42±2.02	81.89	37.75±1.44	281.19
10	39.41±0.83	257.52 µg\ml	95.71±2.02	1.217	78.5±1.44	0.817
Captopril	41.1±0.0	266.107	75.71±2.02	16.04	50±1.44	199.7
Aspirin	35.88±0.83	326.5	85.71±0	21.73	28.57±0.0	388.23

ADP: Adenosine diphosphate; AA: Arachidonic acid; % INH: Inhibition percent, which calculated as an average of three values of AUC for each concentration; SD: Standard deviation; IC_{50}: The half maximum inhibitory concentration.

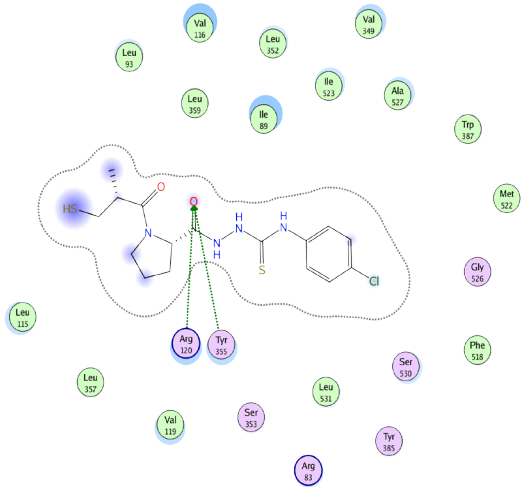
Docking Study

Generally, the docking study was performed to determine the binding score that helps in the prediction of the activity of synthesized derivatives. Several proteins play a role in regulating platelet aggregation, among them purino receptor: P_2Y_{12}, COX-1 and glycoprotein IIb/IIIa that play a vital role, where ADP, arachidonic acid, and collagen act as agonist respectively. The affinity of tested compounds against purinoreceptor P_2Y_{12}:were 8> 6> captopril> 10> 5> 7> 9> aspirin, and the affinity of tested compounds against COX-1 was: 7> 10> 8> 6> 5> 9> captopril> aspirin, while the sequence of compounds against glycoprotein IIb/IIIa was 7> 6> 8> 5> 10> 9> captopril> aspirin according to binding energy values as shown in (Table 2), compound with lower energy value, predicted to has higher activity.

Table 2: Docking study of tested compounds (5-10)

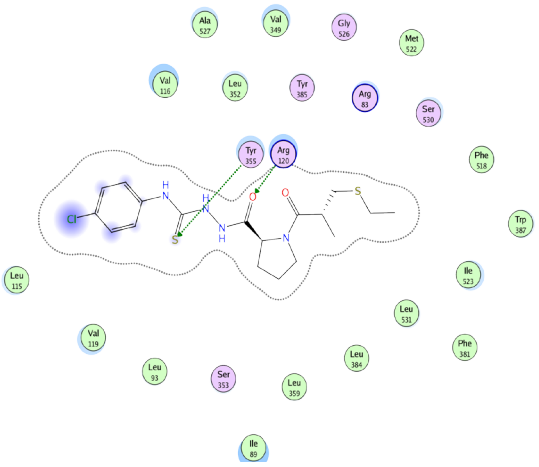
Enzyme		Tested CAP derivatives (5-10)							
		CAP.	Asp.	5	6	7	8	9	10
4XPZ	Binding energy (Kcal)/ mol	-6.57	-0.7	-5.73	-7.94	-5.4	-8.21	-4.03	-6.55
	Ki (µM)	15.25	0.304	63.44	1.54	109.45	0.952	1.02	0.956
	H-bond	2	0	0	0	0	0	0	0
	Amino acid	TYR 209, ASN 235							
1EQG	Binding energy (Kcal)/ mol	-6.84	-6.8	-8	-8.34	-10.67	-10.33	-7.86	-10.66
	Ki (Mm)	9.68	10.314	1.38	0.773	0.0512	0.0266	1.74	0.0207
	H-bond	1	2	0	2	2	1	1	2
	Amino acid	SER530 TRP387	THR206, ASN382		HIS207, TYR 355	ARG 120,	THR206	THR206 TYR355	ARG 120,
3ZDY	Binding energy (Kcal)/ mol	-4.8	0.1	-9.19	-10.05	-10.41	-9.74	-6.64	-8.49
	Ki (µM)	131.944		0.184	0.042	0.023	0.0723	12.94	1.33
	H-bond	1	0	1	2	0	0	0	2
	Amino acid	ALA122		ASP126	ARG B214, ASP A224			ARG B214, ARG B216	

CAP: Captopril; ASP: Aspirin; (4XPZ, 1EQG, and 3ZDY) are PDB ID of purino receptor P_2Y_{12}, cyclooxygenase-1 (COX-1), and glycoprotein IIb/IIIa respectively; Ki: inhibition constant; TYR: Tyrosine; ASN: Asparagine; SER: Serine; THR: Threonine; TRP: Tryptophan; HIS: Histidine; ARG: Arginine; ALA: Alanine; ASP: Aspartic acid.



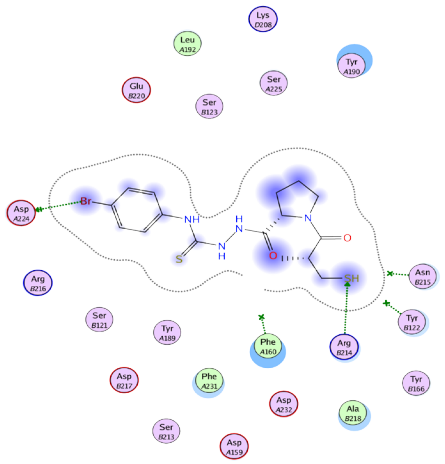
(a)

Fig. 3. Compound 7, binding to 1EQG



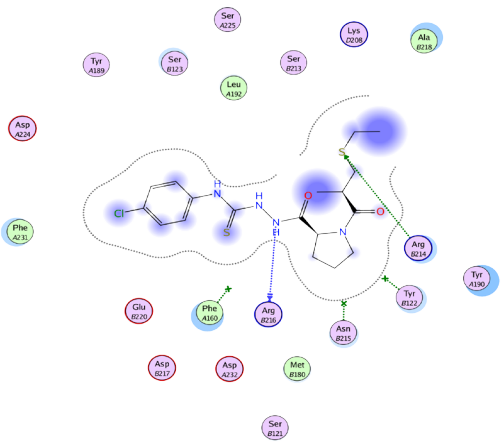
(b)

Fig. 4. Compound 10, binding to 1EQG



(c)

Fig. 5. Compound 6, binding to 3ZDY



(d)

Fig. 6. Compound 10, binding to 3ZDY

CONCLUSION

New thiosemicarbazide derivatives of captopril have been successfully synthesized and characterized. Most CAP thiosemicarbazide derivatives displayed moderate antiplatelet activity through a COX-1 inhibitor pathway, and compound 10 was the best one, with lower IC_{50}. The tested compounds, like 6, also showed antiplatelet activity through glycoprotein IIb/IIIa receptor inhibitor pathway, but lower than that of COX-1, however, the (glycoprotein) pathway, may be considered as a second pathway.

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CONFLICT OF INTEREST

All authors have none to declare

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