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Synthesis, Biological Evaluation and Molecular Modeling Studies of Some 5-Methylisoxazole Derivatives as Anti-Inflammatory Agents

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ABSTRACT

Some novel 4-(5-methylisoxazol-3-ylamino) thiazole derivatives incorporated with different heterocyclic moieties were synthesized and screened for their anti-inflammatory activity. Compounds (1, 2, 10, 11, 12, 13) screened for their anti-inflammatory activity. Among them, compound 13 showed the greatest anti-inflammatory potency. Furthermore, compound 2 showed the least anti-inflammatory potency. Molecular modeling simulation was done to explore the binding mode of these compounds within active sites of trypsin and bovine serum albumin.

Keywords: 5-methylisoxazol; Anti-inflammatory activity; Molecular modeling simulation

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INTRODUCTION

The importance of heterocycles in pharmacology and nature product chemistry drive the search for new methods for the construction of heterocyclic unit such as isoxazoles. Isoxazole derivatives reported as an important class of bioactive molecules, which exhibit remarkable_activities such as antifungal [1], Aβ precursor protein [2], protein tyrosine phosphatase 1B inhibitors [3], antiviral [4], anti-inflammatory [5], anticonvulsant [6, 7], antitubercular [8] and immunomodulatory [9]. Trypsin, is a class of serine protease, has a molecular mass of 23,300 Da and consists of 223 amino residues [10]. Due to the great use of trypsin, many studies have been made [11]. Serum albumin is the principal extracellular protein of the circulatory system, and also accounts for about 60% of the total plasma proteins [12]. Great studies about the interaction between serum albumin, commercial drugs and natural or synthetic compounds showing biological activity have been made [13]. Inflammation is a bodily reaction to injury, infection or destruction featuring resulting in heat, redness, swelling and disordered physiological functions. When the human body injured, a set of chemical changes occurs in the injured area. Anti-inflammatory means the property of a substance to reduce inflammation or swelling. So, in order to treat inflammatory diseases, anti-inflammatory and analgesic drugs are required. NSAIDs are usually used drugs for inflammation such as aspirin, diclofenac, indomethacin, and also ibuprofen [14]. There is excessive activation of phagocytes [mast cells, neutrophils, mononuclear cells] in inflammatory disorders. Neutrophils are known to be a rich source of proteinase that holds in their lysosomal granules many serine proteinases. It was reported that leukocytes proteinase play a significant role in the development of tissue damage during inflammatory reactions and a great level of protection was provided by proteinase inhibitors [15]. Based on the above literature's importance we here report some new isoxazole derivatives.

MATERIALS AND METHODS

Chemistry

NMR spectra were recorded on a General Electric QE 300 instrument and chemical shifts were given with respect to TMS. IR spectra were recorded on a Perkin-Elmer 1420 spectrometer and a Broad FTS7 (KBr). Mass spectra were

obtained on a Jeol JMS D-300 spectrometer operating at 70 eV. Microanalysis was conducted using an Elemental analyzer 1106. Melting points were determined on a Reichet Hot Stage and uncorrected.

7-Chloro-*N*-(5-methylisoxazol-3-yl)-2*H*-benzo[*b*][1,4]oxazin-3-amine (3):
(0.01 mol) of **2** [16] was mixed with (0.01 mol) of 2-amino-4-chlorophenol, followed by (2 mL) of triethylamine in (5 mL) of absolute ethanol. The reaction mixture was refluxed for 1 h. The formed precipitate was filtered, washed with water, air dried, and crystallized from ethanol.
Yield: 75%, m.p.≈ 185-187°C. IR (KBr) ν, cm^{-1}: 3225 (NH), 1629 (C=N), 1605 (C=C). ^{1}H NMR spectrum (d_6-DMSO, δ ppm): 2.36 (s, 3H, CH_3 of isoxazole), 3.88 (s, 2H, CH_2 of oxazine), 6.61 (s, 1H, CH of isoxazole), 7.01-7.45 (m, 3H, Ar), 9.74 (s, 1H, NH, D_2O exchangeable). MS m/z: M^+.263 (1.68%), M^{+2}.265 (4%). Anal. Calcd. for $C_{12}H_{10}ClN_3O_2$ (263.68): C, 54.66; H, 3.82; N, 15.94%; found: C, 54.50; H, 3.41; N, 15.51%.

2-Hydrazinyl-*N*-(5-methylisoxazol-3-yl)acetamide (4):
A mixture of compound **2** [16] (0.01 mol) and hydrazine hydrate 99 % (0.1 mol) in absolute ethanol (10 mL) refluxed for 14 h. The reaction mixture was cooled, and the precipitated solid was collected by filtration, washed with water, air dried, and crystallized from DMF.
Yield: 60%, m.p.≈ 202-204°C. IR (KBr) ν, cm^{-1}: 3421, 3379, 3242, 3187 (NH_2, 2NH), 1683 (C=O). ^{1}H NMR spectrum (d_6-DMSO, δ ppm): 2.38 (s, 3H, CH_3 of isoxazole), 3.55 (s, 2H, CH_2), 6.54 (s, 1H, CH of isoxazole), 8.91, 9.01, 9.52 (s, 4H, NH_2, 2NH, D_2O exchangeable). MS m/z: M^+.170 (3.90%). Anal. Calcd. for $C_6H_{10}N_4O_2$ (170.17): C, 42.35; H, 5.92; N, 32.92%; found: C, 42.01; H, 5.78; N, 32.85%.

***N*-(2*H*-benzo[*b*][1,4]thiazin-3-yl)-5-methylisoxazol-3-amine (5):**
(0.01 mol) of **2** [16] was mixed with (0.01 mol) of 2-aminothiophenol, followed by (2 mL) of triethylamine in (5 mL) of absolute ethanol. The reaction mixture was refluxed for 1 h. The formed precipitate was filtered, washed with water, air dried, and crystallized from benzene.
Yield: 70%, m.p.>300 °C. IR (KBr) ν, cm^{-1}: 3295 (NH), 1630 (C=N), 1595 (C=C). ^{1}H NMR spectrum (d_6-DMSO, δ ppm): 2.40 (s, 3H, CH_3 of isoxazole), 4.01 (s, 2H, CH_2 of thiazine), 6.42 (s, 1H, CH of isoxazole), 7.10-7.75 (m, 4H, Ar), 8.31 (s, 1H, NH, D_2O exchangeable). MS m/z: M^+.245 (6%). Anal. Calcd. for $C_{12}H_{11}N_3OS$ (245.30): C, 58.76; H, 4.52; N, 17.13%; found: C, 58.61; H, 4.45; N, 16.95%.

***N*-hexyl-5-methylisoxazol-3-amine (6):**
A mixture of compound **1** (0.005 mol) and 1-iodohexane (0.005 mol) in (10 mL) absolute ethanol containing KOH (0.005 mol) was refluxed for 6 h. The reaction mixture was concentrated and acidified with diluted HCl. The separated solid was filtered off and crystallized from ethanol.
Yield: 60%, m.p.> 300 °C. IR (KBr) ν, cm^{-1}: 3245 (NH), 1621 (C=N), 1601 (C=C). ^{1}H NMR spectrum (d_6-DMSO, δ ppm): 1.09 (t, 3H, CH_3 of hexyl), 1.28 (p, 2H, CH_2 of hexyl), 1.30 (p, 2H, CH_2 of hexyl), 1.39 (p, 2H, CH_2 of hexyl), 1.62 (m, 2H, CH_2 of hexyl), 2.35 (s, 3H, CH_3 of isoxazole), 3.22 (t, 2H, CH_2-N), 6.35 (s, 1H, CH of isoxazole), 9.11 (s, 1H, NH, D_2O exchangeable). MS m/z: M^+.182 (47.54%). Anal. Calcd. for $C_{10}H_{18}N_2O$ (182.26): C, 65.90; H, 9.95; N, 15.37%; found: C, 65.85; H, 9.81; N, 15.21%.

2-(5-Amino-4-cyano-1*H*-pyrazol-1-yl)-*N*-(5-methylisoxazol-3-yl)acetamide (7):
A mixture of compound **4** (0.001 mol), ethoxymethylenemalononitrile (0.001 mol) and anhydrous potassium carbonate (0.0015 mol) in absolute ethanol (15 mL) was heated under reflux for 8 h. The solvent was evaporated under reduced pressure and the remaining solid was crystallized from DMF.
Yield: 80%, m.p.> 300 °C. IR (KBr) ν, cm^{-1}: 3427, 3370, 3187 (NH_2, NH), 2204 (C≡N), 1665 (C=O). ^{1}H NMR spectrum (d_6-DMSO, δ ppm): 2.35 (s, 3H, CH_3 of isoxazole), 4.41 (s, 2H, CH_2), 6.51 (s, 1H, CH of isoxazole), 5.50 (s, 2H, NH_2, D_2O exchangeable), 7.51 (s, 1H, CH of pyrazole), 9.45 (s, 1H, NH, D_2O exchangeable). MS m/z: M^+.246 (10%). Anal. Calcd. for $C_{10}H_{10}N_6O_2$ (246.23): C, 48.78; H, 4.09; N, 34.13%; found: C, 48.71; H, 3.92; N, 33.91%.

Ethyl-5-hydroxy-1-(2-((5-methylisoxazol-3-yl)amino)-2-oxoethyl)-1*H*-pyrazole-4-carboxylate (8):
A mixture of compound **4** (0.001 mol), diethyl ethoxymethylenemalonate (0.001 mol) and anhydrous potassium carbonate (0.0015 mol) in acetonitrile (15 mL) was heated under reflux for 12 h. After cooling, the solution was acidified with dilute hydrochloric acid and the precipitate was collected by filtration, air dried and crystallized from ethanol.
Yield: 75%, m.p.≈ 255-257°C. IR (KBr) ν, cm^{-1}: 3423, 3271 (OH, NH), 1685, 1664 (2C=O). ^{1}H NMR spectrum (d_6-DMSO, δ ppm): 1.20 (t. 3H, CH_3 of ethyl), 2.37 (s, 3H, CH_3 of isoxazole), 4.25 (q, 2H, CH_2 of ethyl), 4.71 (s, 2H,

CH_2), 6.30 (s, 1H, CH of isoxazole), 6.56 (s, 1H, CH of pyrazole), 9.33 (s, 1H, NH, D_2O exchangeable), 10.50 (s, 1H, OH, D_2O exchangeable). MS m/z: M^+.294 (12%). Anal. Calcd. for $C_{12}H_{14}N_4O_5$ (294.26): C, 48.98; H, 4.80; N, 19.04%; found: C, 48.72; H, 4.72; N, 18.85%.

2-(3,5-Dimethyl-1*H*-pyrazol-1-yl)-*N*-(5-methylisoxazol-3-yl)acetamide (9):
A mixture of compound **4** (0.001 mol) and acetylacetone (0.001 mol) in glacial acetic acid (15 mL) was heated under reflux for 10 h. The reaction mixture was cooled and the precipitate was collected by filtration, washed with water, air dried and crystallized from isopropanol.
Yield: 75%, m.p.> 300 °C. IR (KBr) ν, cm^{-1}: 3435 (NH), 1706 (C=O). ^{1}H NMR spectrum (d_6-DMSO, δ ppm): 2.21 (s, 3H, CH_3 of pyrazole), 2.35 (s, 3H, CH_3 of isoxazole), 2.41 (s, 3H, CH_3 of pyrazole), 4.82 (s, 2H, CH_2), 6.21 (s, 1H, CH of pyrazole), 6.36 (s, 1H, CH of isoxazole), 9.32 (s, 1H, NH, D_2O exchangeable). MS m/z: M^+.234 (4.03%). Anal. Calcd. for $C_{11}H_{14}N_4O_2$ (234.25): C, 56.40; H, 6.02; N, 23.92%; found: C, 56.31; H, 5.92; N, 23.89%.

2-(3-Methyl-5-oxo-4,5-dihydro-1*H*-pyrazol-1-yl)-*N*-(5-methylisoxazol-3-yl)acetamide (10):
A mixture of compound **4** (0.001 mol), ethyl acetoacetate (0.001 mol) and anhydrous potassium carbonate (0.0015 mol) in absolute ethanol (15 mL) was heated under reflux for 10 h. The reaction mixture was cooled and the precipitate was collected by filtration, washed with water, air dried and crystallized from ethanol.
Yield: 85%, m.p.≈ 236-238°C. IR (KBr) ν, cm^{-1}: 3230 (NH), 1697, 1672 (2C=O). ^{1}H NMR spectrum (d_6-DMSO, δ ppm): 1.84 (s, 3H, CH_3 of pyrazole), 2.20 (s, 2H, CH_2 of pyrazole), 2.37 (s, 3H, CH_3 of isoxazole), 4.95 (s, 2H, CH_2), 6.41 (s, 1H, CH of isoxazole), 9.15 (s, 1H, NH, D_2O exchangeable). MS m/z: M^+.236 (7%). Anal. Calcd. for $C_{10}H_{12}N_4O_3$ (236.23): C, 50.84; H, 5.12; N, 23.72%; found: C, 50.74; H, 5.03; N, 23.66%.

2-((1,3-Dioxoisoindolin-2-yl)amino)-*N*-(5-methylisoxazol-3-yl)acetamide (11):
A solution of phthalic anhydride (0.01 mol) in acetic acid (15 mL) was added to **4** (0.005 mol). The reaction mixture was stirred and refluxed for 4-8 h, poured onto ice cold water and the resulting precipitate filtered off by suction and crystallized from DMF.
Yield: 69%, m.p.> 300 °C. IR (KBr) ν, cm^{-1}: 3300, 3150 (2NH), 1705, 1689, 1674 (3C=O). ^{1}H NMR spectrum (d_6-DMSO, δ ppm): 2.37 (s, 3H, CH_3 of isoxazole), 5.01 (s, 2H, CH_2), 6.35 (s, 1H, CH of isoxazole), 7.01-7.81 (m, 4H, Ar), 9.15, 9.35 (s, 2H, 2NH, D_2O exchangeable). MS m/z: M^+.300 (1.56%). Anal. Calcd. for $C_{14}H_{12}N_4O_4$ (300.27): C, 56.00; H, 4.03; N, 18.66%; found: C, 55.85; H, 3.93; N, 18.70%.

Potassium 2-(2-((5-methylisoxazol-3-yl)amino)-2-oxoethyl)hydrazinecarbodithioate (12):
A solution of potassium hydroxide (0.015 mol) in water (10 mL) was added to a mixture of compound **4** (0.01 mol) and carbon disulfide (5 mL) in absolute ethanol (20 mL). The mixture was stirred at room temperature for 8 h. The resulting precipitate filtered off by suction and crystallized from ethanol.
Yield: 70%, m.p.≈ 210-212°C. IR (KBr) ν, cm^{-1}: 3374, 3210, 3100 (3NH), 1682 (C=O). ^{1}H NMR spectrum (d_6-DMSO, δ ppm): 2.34 (s, 3H, CH_3 of isoxazole), 4.93 (s, 2H, CH_2), 6.41 (s, 1H, CH of isoxazole), 9.11, 9.36, 9.78 (s, 3H, 3NH, D_2O exchangeable). MS m/z: M^+.284 (3.23%). Anal. Calcd. for $C_7H_9KN_4O_2S_2$ (284.40): C, 29.56; H, 3.19; N, 19.70%; found: C, 29.50; H, 3.01; N, 19.62%.

***N*-(5-methylisoxazol-3-yl)-2-(2-(phenylcarbamothioyl)hydrazinyl)acetamide (13):**
A mixture of compound **4** (0.001 mol) and phenyl isothiocyanate (0.001 mol) in absolute ethanol (15 mL) was heated under reflux for 10 h. The precipitated solid was collected by filtration, washed with water, air dried and crystallized from ethanol.
Yield: 80%, m.p.≈ 226-228°C. IR (KBr) ν, cm^{-1}: 3380, 3230, 3185, 3010 (4NH), 1691 (C=O). ^{1}H NMR spectrum (d_6-DMSO, δ ppm): 2.36 (s, 3H, CH_3 of isoxazole), 4.92 (s, 2H, CH_2), 6.32 (s, 1H, CH of isoxazole), 7.21-7.82 (m, 5H, Ar), 9.12, 9.41, 9.70, 10.21 (s, 4H, 4NH, D_2O exchangeable). MS m/z: M^+.305 (18.31%). Anal. Calcd. for $C_{13}H_{15}N_5O_2S$ (305.36): C, 51.13; H, 4.95; N, 22.94%; found: C, 51.02; H, 4.90; N, 22.90%.

Chemicals:
3-Amino-5-methyl isoxazole **(1)** from (**New Lab Company**), 15th of May City, Cairo, Egypt

Molecular docking study
All docking studies were performed using "Internal Coordinate Mechanics" (Molsoft ICM 3.5-0a).

Preparation of small molecule

Compounds (**1, 2, 10, 11, 12, 13)** were built in Chem Draw Ultra version 11.0 and their energy minimized through Chem3D Ultra version 11.0/MM2, Jop Type: minimum RMS Gradient of 0.100 and saved as MDL Mol File (*.Mol).

Generation of Ligand and Enzyme Structures

The crystal structures of trypsin (PDB code: 2ZQ1) and Bovine Serum Albumin (BSA) (PDB code: 4F5S) [17] complex were retrieved from the RCSB Protein Data Bank (http://www.rcsb.org/pdb/ home/home.do). In our investigation, the 3D-coordinates in X-ray crystal structure of trypsin and BSA in complex with their legend were used as the receptor model in trypsin and BSA docking simulation (Figure 1, 4). All bound waters ligands and cofactors were removed from the protein.

Docking using Molsoft ICM 3.5-0a program

The conversion of our PDB file into an ICM object involves the addition of hydrogen bonds, assignment of atom types and charges from the residue templates, then perform ICM small molecule docking through setup the receptor, review and adjust binding site makes receptor maps, then start docking simulation, followed by displaying the results. ICM stochastic global optimization algorithm attempts to find the global minimum of the energy function that includes five grid potentials describing the interaction of the flexible ligand with the receptor and internal conformational energy of the ligand, during this process a stack of alternative low energy conformations is saved. All inhibitors were compared according to the best binding free energy (minimum) obtained among all the run.

Pharmacology

Proteinase inhibitory activity:

The test was performed according to Sakat [18] with minor modifications. The reaction mixture (2 ml) was containing 0.06 mg trypsin, 20 mM Tris HCl buffer (pH 7.4) and 1 ml test sample (250 µ gm/ml for each compound). The mixture was incubated for 5 min and then 1 ml of 0.8% (w/v) casein was added. The mixture was incubated for an additional 20 min. 1 ml of 10% Perchloric acid was added to arrest the reaction. Cloudy suspension was centrifuged, and the absorbance of the supernatant was read at 280 nm. The experiment was performed in triplicates. Percentage inhibition of protein denaturation was calculated by using the following formula: Percent inhibition = [(A control − A test)/A control] × 100. Where A control is the absorbance of the control reaction and A test is the absorbance of the control reaction with extract.

Inhibition of albumin denaturation:

The anti-inflammatory activity of different compounds was studied by inhibition of albumin denaturation technique according to the method of Mizushima et al., [19] with minor modifications.

- Control solution (5 ml): 0.2 ml from 1% aqueous solution bovine serum albumin, 2.8 ml of phosphate buffer (pH 6.4), and 2 ml DMSO
- Standard solution (5 ml): 0.2 ml from 1% aqueous solution bovine serum albumin, 2.8 ml of phosphate buffer, and 2 ml from standard drug [250 µg aspirin /ml DMSO].
- Test solution (5 ml): 0.2 ml from 1% aqueous solution bovine serum albumin, 2.8 ml of phosphate buffer, and 2 ml of 250 µg/ml of test sample dissolved in DMSO. All of the above solutions were adjusted to pH 6.4 using a small amount of 1 N HCl. The samples were incubated at 37°C for 15 minutes and heated at 70°C for 5 minutes. After cooling, the absorbance of the above solutions was measured using ultraviolet-visible spectrophotometer at 660 nm.

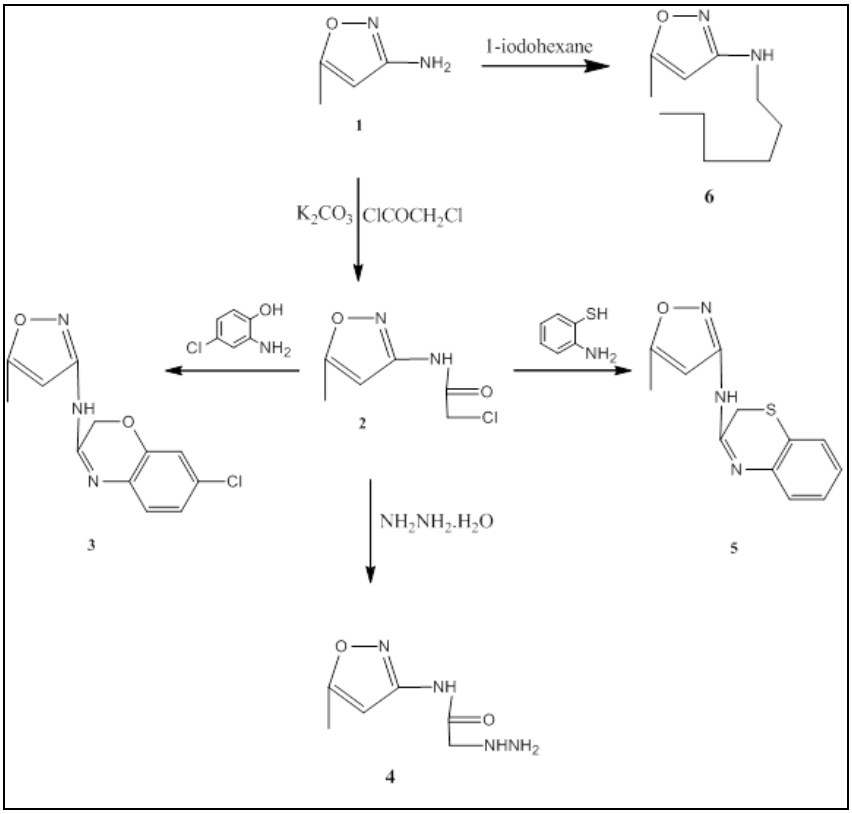
The percentage inhibition of protein denaturation was calculated using the following formula. Percentage inhibition = (Abs control − Abs sample) × 100/Abs control.

RESULTS AND DISCUSSION

Chemistry

Compound 3 was prepared by cyclization of 2 with 2-amino-4-chlorophenol (Scheme 1). ^{1}H NMR (DMSO-d_6) spectra of 3 revealed signals at 2.36 (s, 3H, CH_3 of isoxazole), 3.88 (s, 2H, CH_2 of oxazine), 6.61 (s, 1H, CH of isoxazole), 7.01-7.45 (m, 3H, Ar), 9.74 (s, 1H, NH, D_2O exchangeable). Compound 4 was prepared by the reaction of compound 2 with hydrazine hydrate (Scheme 1). IR spectra of compound 4 exhibited bands at 3421, 3379, 3242, 3187 (NH_2, 2NH), 1683 (C=O). Compound 5 prepared by cyclization of 2 with 2-aminothiophenol (Scheme 1). ^{1}H NMR (DMSO-d_6) spectra of 5 revealed signals at 2.40 (s, 3H, CH_3 of isoxazole), 4.01 (s, 2H, CH_2 of thiazine), 6.42 (s, 1H, CH of isoxazole), 7.10-7.75 (m, 4H, Ar), 8.31 (s, 1H, NH, D_2O exchangeable). Compound 1 reacted with 1-

iodohexane to give 6 (Scheme1). ^{1}H NMR (DMSO -d_6) spectra of 6 revealed signals at 1.09 (t, 3H, CH_3 of hexyl), 1.28 (p, 2H, CH_2 of hexyl), 1.30 (p, 2H, CH_2 of hexyl), 1.39 (p, 2H, CH_2 of hexyl), 1.62 (m, 2H, CH_2 of hexyl), 2.35 (s, 3H, CH_3 of isoxazole), 3.22 (t, 2H, CH_2-N), 6.35 (s, 1H, CH of isoxazole), 9.11 (s, 1H, NH, D_2O exchangeable). Compounds 7 and 8 were prepared by the reaction of compound 4 with ethoxymethylenemalononitrile and diethyl ethoxymethylenemalonate, respectively (Scheme 2). IR spectra of 7 exhibited bands at 3427, 3370, 3187 (NH_2, NH), 2204 (C≡N), 1665 (C=O).^{1}H NMR (DMSO-d_6) spectra of 8 revealed signals at 1.20 (t. 3H, CH_3 of ethyl), 2.37 (s, 3H, CH_3 of isoxazole), 4.25 (q, 2H, CH_2 of ethyl), 4.71 (s, 2H, CH_2), 6.30 (s, 1H, CH of isoxazole), 6.56 (s, 1H, CH of pyrazole), 9.33 (s, 1H, NH, D_2O exchangeable), 10.50 (s, 1H, OH, D_2O exchangeable). Compounds 9 and 10 were prepared by reaction of 4 with acetylacetone and ethyl acetoacetate, respectively (Scheme 2).

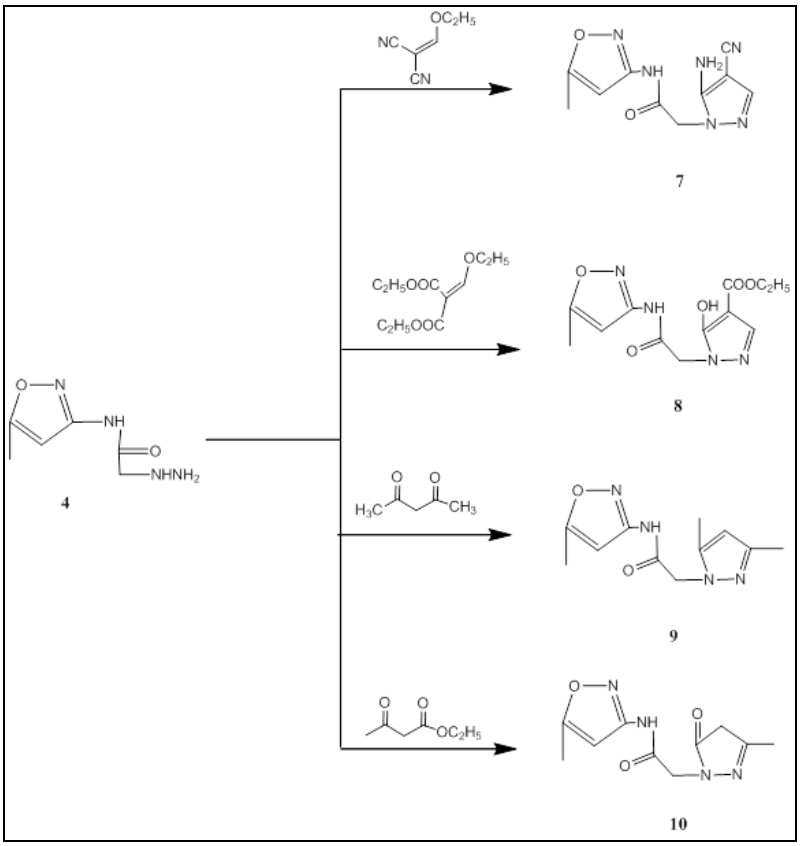


Scheme 1: Synthesis of 5-methyllisoxazol derivatives (1-6)

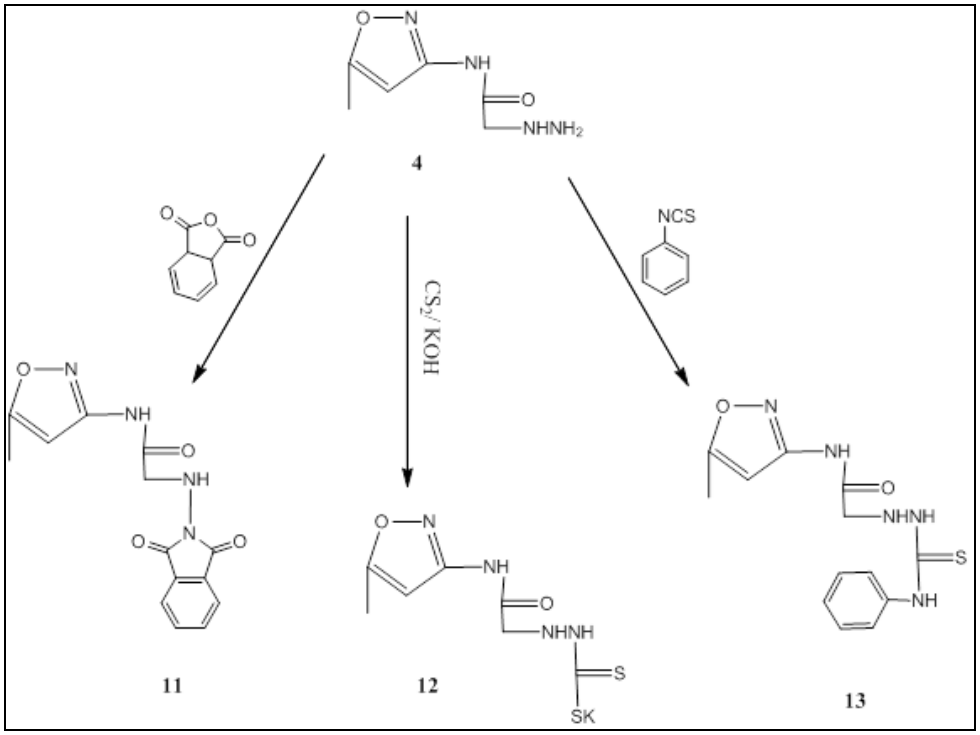
^{1}H NMR (DMSO-d_6) spectra of 9 revealed signals at 2.21 (s, 3H, CH_3 of pyrazole), 2.35 (s, 3H, CH_3 of isoxazole), 2.41 (s, 3H, CH_3 of pyrazole), 4.82 (s, 2H, CH_2), 6.21 (s, 1H, CH of pyrazole), 6.36 (s, 1H, CH of isoxazole), 9.32 (s, 1H, NH, D_2O exchangeable). IR spectra of 10 exhibited bands at 3230 (NH), 1697, 1672 (2C=O). 11, 12 and 13 can be formed via the reaction of 4 with phthalic anhydride, carbon disulfide and phenyl isothiocyanate, respectively (Scheme 3). IR spectra of 11 exhibited bands at 3300, 3150 (2NH), 1705, 1689, 1674 (3C=O). ^{1}H NMR (DMSO-d_6) spectra of 12 revealed signals at 2.34 (s, 3H, CH_3 of isoxazole), 4.93 (s, 2H, CH_2), 6.41 (s, 1H, CH of isoxazole), 9.11, 9.36, 9.78 (s, 3H, 3NH, D_2O exchangeable). ^{1}H NMR (DMSO-d_6) spectra of compound 13 revealed signals at 2.36 (s, 3H, CH_3 of isoxazole), 4.92 (s, 2H, CH_2), 6.32 (s, 1H, CH of isoxazole), 7.21-7.82 (m, 5H, Ar), 9.12, 9.41, 9.70, 10.21 (s, 4H, 4NH, D_2O exchangeable).

Docking analysis

Compounds (1, 2, 10, 11, 12, 13) were used for docking study. All calculations were performed using "Internal Coordinate Mechanics" (Molsoft ICM 3.5-0a). Molecular modeling docking studies were performed and ICM score values [20-22] combined with hydrogen bonds formed with the surrounding amino acid residues helped us to predict the correct binding geometry for each binder at the active site.



Scheme 2: Synthesis of 5-methyllisoxazol derivatives (7-10)



Scheme 2: Synthesis of 5-methyllisoxazol derivatives (11-13)

As shown in (Table 1) (S)-N-(4-carbamimidoylbenzyl)-1-(2-(cyclo hexylamino)ethanoyl)pyrrolidine-2-carboxamide (ligand of trypsin) reveals ICM score of -117.49 and forms 6 H bonds with Q193, D189, S214, W215 and G219 (Figure 1), the target compounds elicited binding affinities (ICM scores range from -70.34 to -38.36). Compound 13 showed high activity probably due to their high ICM scores (-70.34) (Figure 2), however, compounds (12, 10, 11, 1) are biologically moderate as they have moderate ICM scores of ranges from (-60.17 to -39.66). Compound 2 showed less activity due to its lowest ICM scores (- 38.36) (Figure 3).

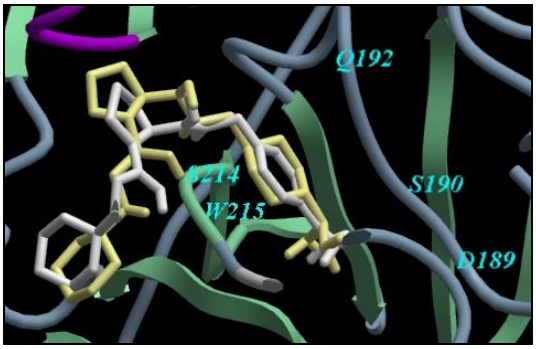


Figure 1: Binding model of the ligand of trypsin into the active pocket of trypsin receptor

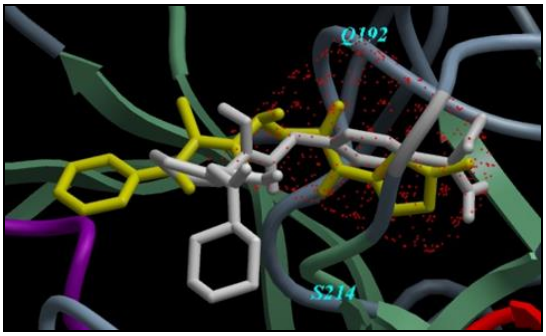


Figure 2: Binding mode of compound 13 (the most active compound) with trypsin. For clarity, only interacting residues are displayed. Ligand of trypsin is represented as white balls and sticks models, compound 13 represented as yellow balls and sticks models and the red dots show the binding sites of trypsin

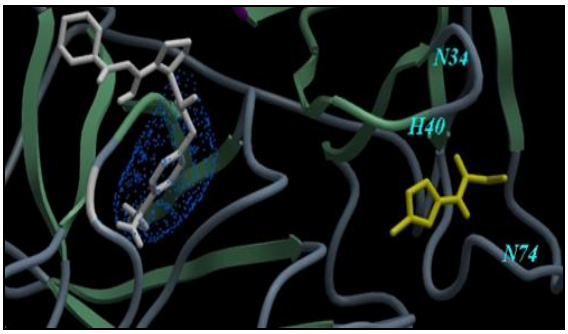


Figure 3: Binding mode of compound 2 (the less active compound) with trypsin. For clarity, only interacting residues are displayed. Ligand of trypsin is represented as white balls and sticks models, compound 2 represented as yellow balls and sticks models and the blue dots show the binding sites of trypsin.

Also, as shown in (Table 2) triethyleneglycol (ligand of BSA) reveals ICM score of - 49.68 and forms 9 H bonds with Y147, F148, S192, R458 and D108 (Figure 4), the target compounds elicited binding affinities (ICM scores range from -71.36 to - 47.25). Compounds (13, 12, 1, 10, 11) showed activity probably due to their high ICM scores which ranged from (-71.36 to -55.24). Compound 13 showed high activity probably due to their high ICM scores (-71.36) (Figure 5), however, compound 2 is biologically moderate as they have moderate ICM scores (- 47.25) (Figure 6).

Table 1: Docking of compounds (1, 2, 10, 11, 12, 13) on trypsin

High Activity
Low Activity

Cpd No	ICM score (ΔG)	No. of H-bonds	Atom of ligand involved	Amino acid residues forming the hydrogen bonds	Length of H-bond Å
The ligand of trypsin	-117.49	6	m of Mo2	Q192	2.11
			m of Mh39	D189	2.25
			m of Mh37	D189	2.69
			m of Mh26	S214	2.27
			m of Mh36	W215	2.21
			m of Mh38	G219	2.19
1	-39.66	2	m of Mh6	D189	2.35
			m of Mh5	G219	2.2
2	-38.36	5	m of Mo1	N34	2.7
			m of Mo1	N34	1.9
			m of Mo2	H40	2.1
			m of Mn1	H40	2.09
			m of Mh5	N74	2.02
10	-53.14	3	m of Mo2	S202	2.54
			m of Mo3	S202	2.15
			m of Mo2	G203	2.1
11	-45.72	9	m of Mo2	K230	1.74
			m of Mn1	K230	2.21
			m of Mo2	K230	1.69
			m of Mo4	K230	2.55
			m of Mo1	K230	2.77
			m of Mn1	K230	1.85
			m of Mo2	K230	2.5
			m of Mh5	D165	2.54
			m of Mh5	D165	1.14
12	-60.17	6	m of Mo2	N34	2.23
			m of Mn1	N34	2.35
			m of Mn1	H40	2.75
			m of Mo1	H40	1.68
			m of Mn3	R66	2.21
			m of Mo2	R66	2.45
13	-70.34	2	m of Mn4	Q192	2.35
			m of Mh5	S214	2.52

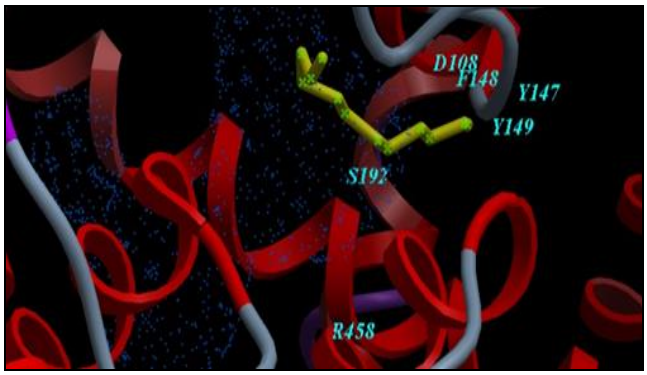


Figure 4: Binding model of the ligand of BSA into the active pocket of BSA receptor

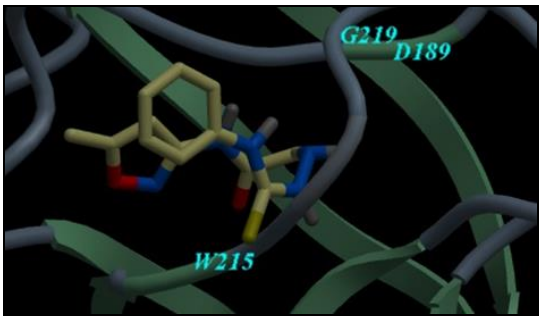


Figure 5: Binding mode of compound 13 (the most active compound) with BSA. For clarity, only interacting residues are displayed. Compound 13 represented as balls and sticks models

Table 2: Docking of compounds (1, 2, 10, 11, 12, 13) on BSA

High Activity
Low Activity

Cpd No	ICM score (ΔG)	No. of H-bonds	Atom of ligand involved	Amino acid residues forming the hydrogen bonds	Length of H-bond Å
			m of Mo3	Y147	1.95
			m of Mo3	F148	2.32
			m of Mo4	S192	2.25
			m of Mo1	R458	2.15
			m of Mo1	R458	1.4
			m of Mo2	R458	2.78
			m of Mo2	R458	2.56
			m of Mh3	D108	1.72
			m of Mh10	Y147	1.44
1	-60.21	2	m of Mh6	D189	2.35
			m of Mh5	G219	2.22
2	-47.25	1	m of Mh5	G219	2.18
10	-59.37	4	m of Mo2	H40	1.45
			m of Mo1	R66	2.28
			m of Mo3	N74	1.46
			m of Mh5	N74	2.17
11	-55.24	8	m of Mo1	Y20	1.94
			m of Mn1	Y20	2.71
			m of Mo1	K159	2.72
			m of Mn1	K159	1.75
			m of Mo2	K159	2.33
			m of Mo2	K159	2.67
			m of Mo2	K159	1.4
			m of Mo4	S202	1.84
12	-69.07	3	m of Mn1	Q192	2.22
			m of Mh8	C191	2.18
			m of Mh5	S214	1.64
13	-71.36	3	m of Mh14	D189	2.31
			m of Mh15	W215	2.19
			m of Mh8	G219	2.24

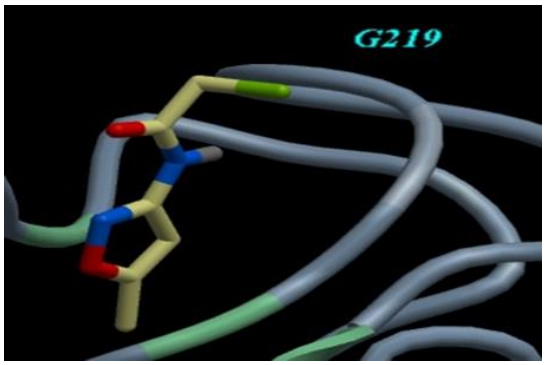


Figure 6: Binding mode of compound 2 (the less active compound) with BSA. For clarity, only interacting residues are displayed. Compound 2 represented as balls and sticks models

Pharmacology

Some synthesized compounds (1, 2, 10, 11, 12, 13) were evaluated for their anti-inflammatory activity. The inhibitory effect of compounds (1, 2, 10, 11, 12, 13) on albumin denaturation and proteinase inhibitory action was shown in (Table 3). From (Table 3) it was noticed that compound 13 showed the greatest anti-inflammatory potency, while compound 2 showed the least anti-inflammatory potency.

Table 3: Anti-inflammatory activity of compounds (1, 2, 10, 11, 12, 13)

High Activity
Low Activity

Samples	Albumin denaturation inhibition	Proteinase inhibition
1	93	60.6
2	71.87	59.6
10	90.1	63.1
11	84.1	60.3
12	93	67.3
13	97.7	95.8

Structure-activity relationship (SAR):

The results of the anti-inflammatory screening promoted some specific and remarkable data about the structure-activity relationship (SAR) of the synthesized molecules. By investigating the variation in the selectivity of the tested compounds it was revealed that:

i) Compound 13 which has CSNHPh group was found to be more active in the biological activities discussed in this paper than the other compounds.

ii) As shown for anti-inflammatory screening, chlorine reduces the anti-inflammatory activity of compound **2** as it has a higher electronegativity that attracts the electrons towards itself. Furthermore, the electronic effects promoted by the chlorine insertion in molecule modify its electronic distribution, which directly influences the binding of the compound to biological targets. This fact was verified by the decrease in the anti-inflammatory effect [23].

CONCLUSION

A novel series of some new 5-methylisoxazol-3-yl derivatives was synthesized and evaluated as anti-inflammatory agents. Anti-inflammatory activity results exhibited that, compound 13 showed the greatest anti-inflammatory potency (Table 3). On the other hand, compound 2 showed the least potency (Table 3). The docking study was achieved to predict the binding modes, affinities and orientations of compounds (1, 2, 10, 11, 12, 13) at the active sites of the used enzymes.

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