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Synthesis, Molecular Modeling and Biological Assessment of Aryloxymethyl 1,3,4-Thiadiazole and 1,2,4-Triazole-5-Thione Derivatives as Potential COX Inhibitors

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Abstract

Objectives: Thiadiazole and triazole-5-thione derivatives are five membered heterocyclic compounds showing cyclooxygenase inhibition activities. Based on this observation a series of novel aryloxymethyl 1,3,4-thiadiazole and 1,2,4-triazole-5-thione derivatives were synthesized and evaluated their biological activities.

Material and Methods: The suggested chemical structure of synthesized compounds were confirmed using FT-IR, Mass and ^{1}H-NMR and ^{13}C-NMR spectrometric methods and elemental analysis. The biological activity or potency of synthesized compounds were evaluated using COX inhibitor screening assay kit (Cayman Chemical Company), Indomethacin, and NS398 as standard compounds.

Results: Compounds 2b and 3b showed good inhibition activity against COX-2 and COX-1 enzymes respectively.

Conclusion: The obtained data indicate that compound 2b is more selective to COX-2 and compound 3b is more selective to COX-1 compared with other synthesized compounds. These twe compounds show promising selectivity and could be a starting point for future research in this area.

Keywords: Thiadiazole, Triazole; Cyclooxygenase; Inflammation; Docking

Introduction

Inflammation is a localized bodily condition that occurs ensuing a scratch, cut, burn, or other types of tissue injuries. It causes swelling, redness, warmth, and itching of the affected area. The cyclooxygenase 1 (COX-1) and cyclooxygenase 2 (COX-2) isozymes are the chief bio-ingredients liable for inflammation. Thus, non-steroidal anti-inflammatory drugs (NSAIDs) have a

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fundamental role in treating inflammatory diseases; particularly function via blocking up of the Coxs active sites. NSAIDs illustrate different potencies against COX-1 and COX-2 depending on their chemical structures. This ultimately leads to diversifying their efficiencies and side effects. NSAIDs with low potency against COX-1, lower COX-2/COX-1 activity ratio, and higher potency against COX-2 potentially have a better anti-inflammatory activity along with fewer adverse effects on the human body [1]. Nowadays, the selectivity of NSAIDs against Coxs enzymes has become more attractive due to their inclining anti-inflammatory usages and introducing new NSAIDs with low adverse effects [1]. Thus, conventional NSAIDs are obsoleting, while selective COX-2 inhibitors with appealing drug profiles and diminished adverse effects are of interest. Subsequent to higher interest in selective inhibitors, researchers have recently developed a new generation of compounds with high selectivity for COX-2 along with higher anti-inflammatory and lower ulcerogenic effects. Substituted thiosemicarbazides (known as thiourea derivatives) are invaluable ingredients for the synthesis of several five-membered heterocycles components such as 1,2,4-triazole-3-thiones, 1,3,4-thiadiazoles, and 1,3,4-oxadiazoles. Besides, evidence also proved the anti-microbial efficiencies of these compounds. In addition to anti-inflammatory impact the analgesic, antibacterial, antifungal, anti-tuberculosis, antiviral, anticonvulsant, and anticancer activities of 2-substituted amino-1,3,4-thiadiazole and 4-substituted-1,2,4-triazole-5-thiones compounds are well-versed. In the last decade, most studies focus on enhancing the performance and safety profile of new NSAIDs. Structure-activity relationship (SAR) studies have been performed on a wide range of COX inhibitory compounds. Compounds carrying 2-substituted amino-5-aryl-1,3,4-thiadiazole and 3-aryl-4-substituted-1,2,4-triazole-5-thione rings address higher-level analgesic and anti-inflammatory activities. The current study aimed to design and synthesis a series of novel 1,3,4-thiadiazole and 1,2,4-triazole-5-thione derivatives to procure new selective COX-2 inhibitors. The chemical structure characterization, as well as the inhibitory impact of the compounds on COX-1 and COX-2, also considered. Besides, molecular docking performed to assess the interaction of each molecule with the active site of COX-2

Materials and Methods

All applied chemicals reagents and solvents were provided from Aldrich, Merck and Fluka companies. Melting points (MP) were determined in a (Thomas Hoover) apparatus. UV and IR were done in a Shimadzu UV-160A UV–Visible spectrophotometer and Perkin–Elmer FT-IR 1720x spectrophotometer, respectively. 1H-NMR spectra obtained using a Varian Mercury 400, 400 MHz. High Performance Digital NMR spectrometer and all chemical shifts were reported as (ppm) values [2]. The Mass spectra was studied in Micromass ZQ LC-MS Spectrometer with ESI+ method. Elemental analysis was studied at the Central Laboratory of Faculty of Pharmacy, Ankara University with LECO CHN 932. The accuracy was 0.4%.

Synthesis of 2-(7-methoxy-2-naphthyloxy) acetyl hydrazide (Compound c)

7-Methoxy-2-naphthol 1.44 g (10 mmol), K2CO3 and ethyl bromoacetate 1.67g (10 mmol) in 50 ml acetone were refluxed in oil bath for 6 h. Then the mixture was filtered and the extra solvent was eliminated by distillation. The residue and 1.00 g (20 mmol) hydrazine monohydrate were dissolved in ethanol (50 ml) and refluxed for 1 h. The precipitated solid was filtered, dried and crystallized from ethanol.

Synthesis of thiosemicarbazide derivatives (Compound 1a-d)

1-(2-naphthyloxyacetyl)hydrazine (10 mmol) and suitable substituted isothiocyanate derivatives (10 mmol) was dissolved in EtOH (30 ml) and refluxed 4 h in water bath. The precipitated crude product (on cooling), was filtered, washed with diethyl ether, dried, and crystallized from dioxane–water.

1-(2-(7-Methoxy-2-naphthyloxy)acetyl)-4-methyl-3-thiosemicarbazide (Compound 1a)

Yield 84%, mp.193-5 0C. FT-IR (cm-1); 3338, 3223 (N-H stretching); 3062, 3006 (C-H stretching, aromatic); 2965, 2935 (C-H stretching, aliphatic); 1699 (C=O stretching, amide); 1572 (C=C stretching, aromatic); 1474, 1384 (C-H bending, CH3, CH2);

1212 (C=S stretching); 1178, 1162 (C-O stretching, Ar-C-O) and 836, 812 (C-H bending, 1,2,4-trisubstituted benzene). 1H-NMR (DMSO-d6, ppm); δ 2.80 (3H, d, -NHCH3); 3.85 (3H, s, -OCH3); 4.66 (2H, s, -CH2); 7.00-7.80 (6H, m, aromatic protons); 8.00 (1H, d, -NHCH3); 9.32 (1H, s, NHNHCSNH) and 10.10 (1H, s, -NHNHCSNH). MS (m/z); 320 [M+H]+; 342 [M+Na]+, 102 (100%), 175, 118, 87, 74, 57. Elemental analysis; for C15H17N3O3S, (MW: 319.1 g/mol); C, 56.41; H, 5.37; N, 13.16; S, 10.04. Found: C, 56.01, H, 5.36; N, 13.24; S, 9.97.

1-(2-(7-Methoxy-2-naphthyloxy)acetyl)-4-ethyl-3-thiosemicarbazide (Compound 1b)

Yield 72%, mp. 185-7 0C. FT-IR (cm-1); 3263, 3200 (N-H stretching), 3066, 3026 (C-H stretching, aromatic); 2968 (C-H stretching, aliphatic); 1694 (C=O stretching, amide); 1546 (C=C stretching, aromatic); 1464, 1387 (C-H bending, CH3, CH2); 1209 (C=S stretching); 1177,1026 (C-O stretching, Ar-C-O); 859, 832 (C-H bending, 1,2,4-trisubstituted benzene). 1H-NMR (DMSO-d6, ppm); 1.07 (3H, t,-NHCH2CH3); 3.60 (2H, m, -NHCH2CH3); 3.85 (3H, s, -OCH3); 4.67 (2H, s, -CH2); 7.00-7.80 (6H, m, aromatic protons); 8.02 (1H, t, -NHCH2CH3); 9.25 (1H, s,-NHNHCSNH) and 10.10 (1H, s, -NHNHCSNH). MS (m/z); 334 [M+H]+; 355 [M+Na]+, 269, 175, 118, 87 and 57 (100%). Elemental analysis; for C16H19N3O3S, (MW: 333.41 g/mol); C, 57.64; H, 5.74; N, 12.60; S, 9.62. Found: C, 57.65; H, 5.54; N, 11.70; S, 8.83.

1-(2-(7-Methoxy-2-naphthyloxy)acetyl)-4-allyl-3-thiosemicarbazide (Compound 1c)

Yield 81%, mp. 188-190 0C. FT-IR (cm-1); 3305, 3148 (N-H stretching); 3002 (C-H stretching, aromatic); 2965, 2939 (C-H stretching, aliphatic); 1695 (C=O stretching, amide); 1549, 1513 (C=C stretching, allyl and aromatic); 1480, 1383 (C-H bending, CH3, CH2); 1208 (C=S stretching); 1182, 1166 (C-O stretching, Ar-C-O); 860, 831 (C-H bending, 1,2,4-trisubstituted benzene). 1H-NMR (DMSO-d6, ppm); 3.85 (3H, s, -OCH3); 4.10 (2H, t,-NHCH2CH=CH2); 4.67 (2H, s, -CH2); 5.05 (1H, dd, -NHCH2CH=CH2; HA; JAB: 1.6 Hz, JAX: 10 Hz); 5.15 (1H, dd, -NHCH2CH=CH2; Hb; JAB: 1.6 Hz, JBX: 17.2 Hz); 5.80 (1H, m, -NHCH2CH=CH2); 7.00-7.80 (6H, m, aromatic protons); 8.20 (1H, bs,-NHCH2); 9.37 (1H, s, -NHNHCSNH) and 10.16 (1H, bs, -NHNHCSNH). MS (m/z); 346 [M+H]+; 368 [M+Na]+, 311, 175, 118, 87 and 57 (100%). Elemental analysis; for C17H19N3O3S, (MW: 345.42 g/mol); C, 59.11; H, 5.54; N, 12.17; S, 9.28. Found: C, 59.42; H, 5.31; N, 11.78; S, 8.94.

1-(2-(7-Methoxy-2-naphthyloxy)acetyl)-4-phenyl-3-thiosemicarbazide (Compound 1d)

Yield 81%, mp. 170-2 0C. FT-IR (cm-1); 3560, 3440, 3184 (N-H stretching); 3041 (C-H stretching, aromatic); 3041 (C-H stretching, aliphatic); 1681 (C=O stretching, amide); 1633 (N-H bending); 1547 (C=C stretching, aromatic); 1469, 1395 (C-H bending, CH3, CH2), 1216 (C=S stretching); 1178, 1156 (C-O stretching, Ar-C-O); 867, 820, 746, 698 (C-H bending, 1,2,4-trisubstituted and monosubstituted benzene). 1H-NMR (DMSO-d6, ppm); 3.83 (3H, s, -OCH3); 4.73 (2H, s, -CH2); 7.00-7.80 (11H, m, aromatic protons); 9.71 (2H, s, -NHNHCSNH) and 10.36 (1H, s, -NHNHCSNH). MS (m/z); 382 [M+H]+; 404 [M+Na]+, 311, 269, 229, 175, 105, 73 and 57 (100%). Elemental analysis; for C20H19N3O3S, (MW: 381.45 g/mol); C, 62.97; H, 5.02; N, 11.02; S, 8.41. Found: C, 62.69; H, 5.08; N, 11.14; S, 8.39.

Synthesis of 1,3,4-thiadiazole derivatives (Compound 2a-d)

POCl3 5ml was added on the suitable thiosemicarbazide derivatives (1 mmol) and refluxed in a oil bath (3 h). After pouring into ice-water of the resulting solution a precipitated product obtained, filtered and crystallized from suitable solvents (8).

5-((7-Methoxy-2-naphthyloxy)methyl)-2-methylamino-1,3,4-thiadiazole (Compound 2a)

Yield 68%, mp. 147-9 0C. FT-IR (cm-1); 3202 (N-H stretching); 3040 (C-H stretching aromatic); 2938 (C-H stretching aliphatic); 1634 (C=N stretching, thiadiazole); 1585 (C=C stretching, aromatic); 1454, 1409, 1347 (C-H bending, CH3, CH2); 1263, 1158 (C-O stretching, Ar-O-C); 871, 825 (C-H bending, 1,2,4-trisubstituted benzene). 1H-NMR (DMSO-d6, ppm); 2.90 (3H, d, -CH3); 3.90 (3H, s, -OCH3); 5.40 (2H, s, -CH2); 7.00-7.80 (6H, m, aromatic protons) and 7.90 (1H, q, NH). MS (m/z);

302 [M+H]+(100%); 324 [M+Na]+, 175, 128, 101, 87 and 60. Elemental analysis; for C15H15N3O2S, (MW: 301.36 g/mol); C, 59.78; H, 5.02; N, 13.94; S, 10.64. Found: C, 60.02; H, 5.31; N, 13.84; S,10.36.

5-((7-Methoxy-2-naphthyloxy)methyl)-2-ethylamino-1,3,4-thiadiazole (Compound 2b)

Yield 55%, mp. 160-2 0C. FT-IR (cm-1); 3178 (N-H stretching); 3050 (C-H stretching, aromatic); 2967 (C-H stretching, aliphatic); 1633 (C=N stretching, thiadiazole); 1538 (C=C stretching, aromatic); 1469, 1453, 1344 (C-H bending, CH3, CH2); 1261, 1026 (C-O stretching, Ar-O-C); 871, 825 (C-H bending, 1,2,4-trisubstituted benzene). 1H-NMR (DMSO-d6, ppm); 1.20 (3H, t, -CH2CH3); 3.20 (2H, m, -CH2CH3); 3.80 (3H, s, - OCH3); 5.40 (2H, s, -CH2); 7.00-7.80 (6H, m, aromatic protons) and 7.9 (1H, t, NH). MS (m/z); 316 [M+H]+ (100%); 338 [M+Na]+, 174, 142, 119, 87 and 60. Elemental analysis; for C16H17N3O2S, (MW: 315.39 g/mol); C, 60.93; H, 5.43; N,13.32; S,10.17. Found: C, 60.59; H, 5.40; N, 13.01; S, 9.87.

5-((7-Methoxy-2-naphthyloxy)methyl)-2-allylamino-1,3,4-thiadiazole (Compound 2c)

Yield 70%, mp.158-160 0C. FT-IR (cm-1); 3306 (N-H stretching); 3150, 3006 (C-H stretching, aromatic); 2960, 2940 (C-H stretching, aliphatic); 1696 (C=N stretching, thiadiazole); 1551, 1514 (C=C stretching, allyl, aromatic); 1384, 1300 (C-H bending, CH3, CH2, CH); 1260, 1056 (C-O stretching, Ar-O-C); 860, 832, 724 (C-H bending, 1,2,4-trisubstituted benzene). 1H-NMR (DMSO-d6, ppm); 3.70 (3H, s, -OCH3); 5.40 (2H, s, -CH2); 5.05 (1H, dd, -NHCH2CH=CH2; HA; JAB:1.6 Hz, JAX: 9.0 Hz); 5.15 (1H, dd, -NHCH2CH=CH2; HB; JAB:1.6 Hz, JBX:31.0 Hz); 5.90 (1H, m, -CH2CH=CH2); 3.80 (2H,t, -CH2-CH=CH2); 7.0-7.8 (6H, m, aromatic protons) and 8.0 (1H, t, NH). 13C-NMR (DMSO-d6, ppm); 47.55 (C21), 55.79 (C19), 64.93 (C6), 106.07 (C15), 107.96 (C17), 116.42 (C23), 116.87 (C9), 116.98 (C13), 124.82 (C11), 129.75 (C10), 129.95 (C12), 135.02 (C22), 136.18 (C16), 154.47 (C14), 156.57 (C8), 158.55 (C5, thiadiazole), 170.38 (C2, thiadiazole). MS (m/z); 328 [M+H]+ (100%); 350 [M+Na]+, 174, 154, 102, 88 and 60. Elemental analysis; for C17H17N3O2S, (MW: 327.31 g/mol); C, 62.36; H, 5.23; N, 12.83; S, 9.79. Found: C, 62.36; H, 5.23; N, 12.83; S, 9.79.

5-((7-Methoxy-2-naphthyloxy)methyl)-2-phenylamino-1,3,4-thiadiazole (Compound 2d)

Yield 68%, mp. 210-212 0C. FT-IR (cm-1); 3258 (N-H stretching); 3194, 3135 (C-H stretching, aromatic); 3053 (C-H stretching, aliphatic); 1633 (C=N stretching, thiadiazole); 1556 (C=C stretching, aromatic); 1495, 1446 (C-H bending, CH3, CH2); 1216, 1027 (C-O stretching, Ar-O-C); 874, 830, 751 (C-H bending, 1,2,4-trisubstituted benzene, monosubstituted benzene). 1H-NMR (DMSO-d6, ppm); 3.90 (3H, s, -OCH3); 5.40 (2H, s, -CH2); 7.00-7.90 (11H, m, aromatic protons) and 10.40 (1H, s, NH). MS (m/z); 364 [M+H]+; 386 [M+Na]+ (100%), 218, 190, 136, 73 and 56. Elemental analysis; for C20H17N3O2S, (MW: 363.43 g/mol); C, 66.10; H, 4.71; N, 11.56; S, 8.82. Found. C, 66.15; H, 4.60; N, 11.61; S, 8.71.

Synthesis of 1,2,4-triazole-3–thione derivatives (Compound 3a-d)

Thiosemicarbazide derivatives (4 mmol) in 40 ml, 1N aqueous NaOH were refluxed for 8 h. The acidification of resulting mixture to pH 2 was done with 1N HCl. The acidic solution was extracted with 25ml ethyl acetate 3 times, then the organic phase dehydrated with anhydride sodium sulfate, filtered and distilling off the organic phase and the residue recrystallized from suitable solvents (8).

3-((7-Methoxy-2-naphthyloxy)methyl)-4-methyl-1,2,4-triazole-5-thione (Compound 3a)

Yield 55%, mp. 221-3 0C. FT-IR (cm-1); 3153 (N-H stretching, triazole); 3052 (C-H stretching, aromatic); 2921, 2765 (C-H stretching, aliphatic); 1633 (C=N stretching, triazole); 1585 (C=C stretching); 1395 (C-N stretching, triazole); 1396, 1341 (C-H bending, CH3, CH2); 1258 (C=S stretching); 1215, 1158 (C-O stretching, Ar-O-C); 825, 792 (C-H bending, 1,2,4-trisubstituted benzene). 1H-NMR (DMSO-d6, ppm); 3.50 (3H, s, -CH3), 3.90 (3H, s, -OCH3), 5.30 (2H, s, -CH2), 7.00-7.80 (6H, m, aromatic) and 13.85 (1H, s, NH, triazole). MS (m/z); 302 [M+H]+; 324 [M+Na]+ (100%), 302, 175, 102, 87, 71 and 57. Elemental

analysis; for C15H15N3O2S, (MW: 301.36 g/mol); C, 59.78;H, 5.02; N, 13.94; S, 10.64. Found: C, 59.67; H, 5.22; N, 13.85; S, 10.47.

3-((7-Methoxy-2-naphthyloxy)methyl)-4-ethyl-1,2,4-triazole-5-thione (Compound 3b)

Yield 59.3%, mp. 204-6 0C. FT-IR (cm-1); 3091 (N-H stretching, triazole); 3050 (C-H stretching, aromatic); 2911, 2762 (C-H stretching, aliphatic); 1633 (C=N stretching, triazole); 1606, 1582 (N-H bending, C=C stretching, aromatic); 1497, 1462, 1350 (C-H bending, CH3, CH2); 1367 (C-N stretching, triazole); 1257 (C=S stretching); 1213, 1029 (C-O stretching, Ar-O-C); 826, 787 (C-H bending, 1,2,4-trisubstituted benzene).1H-NMR (DMSO-d6, ppm); 1.30 (3H, t, -CH2CH3); 3.90 (3H, s, -OCH3); 4.00 (2H, q, -CH2CH3); 5.40 (2H, s, -CH2); 7.00-7.80 (6H, m, aromatic protons) and 13.90 (1H, s, NH, triazole). 13C-NMR (DMSO-d6, ppm); 14.09 (C21); 41.00 (C20); 55.82 (C19); 60.66 (C6); 106.08 (C15); 107.95 (C17); 116.15 (C9); 117.14 (C13); 124.93 (C11); 129.82, 130.06 (C10, C12); 136.15 (C16); 148.59 (C3 triazole); 156.41 (C14); 158.61 (C8); 167.87 (C5 triazole). MS (m/z); 316 [M+H]+; 338 [M+Na]+, 301, 175, 142, 102, 87, 74 and 56 (100%).Elemental analysis; for C16H17N3O2S, (MW: 315.39 g/mol); C, 60.93; H, 5.43; N, 13.32, S, 10.17. Found: C, 60.83; H, 5.38; N, 13.09; S, 9.90.

3-((7-Methoxy-2-naphthyloxy)methyl)-4-allyl-1,2,4-triazole-5-thione (Compound 3c)

Yield 47.5%, mp. 148-150 0C. FT-IR (cm-1); 3092 (N-H stretching, triazole); 3049 (C-H stretching, aromatic); 2924, 2763 (C-H stretching, aliphatic); 1633 (C=N stretching, triazole); 1583 (C=C stretching); 1394 (C-H bending); 1349 (C-N stretching, triazole); 1229 (C=S stretching); 1212, 1158 (C-O stretching, Ar-O-C); 827, 760 (C-H bending, 1,2,4-trisubstituted benzene). 1H-NMR (DMSO-d6, ppm); 3.85 (3H, s, -OCH3); 4.70 (2H, d, -CH2CH=CH2); 5.10 (1H, dd, -NHCH2CH=CH2; HA; JAB: 1.6 Hz, JAX: 17 Hz); 5.20 (1H, dd, -NHCH2CH=CH2; HB; JAB: 1.2 Hz, JBX: 10.2 Hz); 5.30 (2H, s, -CH2); 5.90 (1H, m, -CH2CH=CH2); 7.00-7.80 (6H, m, aromatic protons) and 13.95 (1H, s,NH triazole). MS (m/z); 328 [M+H]+; 349 [M+Na]+, 301, , 175, 154, 102 (100%), 87, 74 and 58. Elemental analysis; for C17H17N3O2S, (MW: 327.4 g/mol). C, 62.36; H, 5.23; N, 12.83; S, 9.79. Found: C, 62.08; H, 5.25; N, 12.72; S, 9.63.

3-((7-Methoxy-2-naphthyloxy)methyl)-4-phenyl-1,2,4-triazole-5-thione (Compound 3d)

Yield 55%, mp. 191-3 0C. FT-IR (cm-1); 3080 (N-H stretching, triazole); 3034 (C-H stretching, aromatic); 2909, 2757 (C-H stretching, aliphatic); 1627 (C=N stretching, triazole); 1494 (C=C stretching, aromatic); 1386 (C-N stretching, triazole); 1330 (C-H bending); 1254 (C=S stretching); 1210, 1026 (C-O stretching, Ar-O-C); 840, 760 (C-H bending, 1,2,4-trisubstituted benzene, monosubstituted benzene). 1H-NMR (DMSO-d6, ppm); 3.85 (3H, s, -OCH3); 5.05 (2H, s, -CH2); 6.80-7.70 (11H, m, aromatic protons) and 14.10 (1H, s, NH, triazole). MS (m/z); 363 [M+H]+ (100%); 386 [M+Na]+, 335, 301, 190 , 175, 104, 87 and 73. Elemental analysis; for C20H17N3O2S, (MW: 363.43 g/mol); C, 66.10; H, 4.71; N, 11.56; S, 8.82. Found: C, 65.73; H, 5.22; N, 11.64; S, 8.83.

Biological Activity

The biological assessment of compounds (2a-d and 3a-d) against COX-1 and COX-2 was assessed using the COX (ovine) Assay kit (Inhibition assay kit of Cayman). The procedure implied based on company prescription. This screening assay straight measures the PGF2α concentration. PGF2α obtained by reduction of PGH2 in presence of SnCl2 via COX enzymatic activity in each reaction vessel. Ensuing the preparation of PGF2α synthesizing reaction, compounds (2a-d and 3a-d) separately were added to the reaction vessels. Indomethacin and NS-398 were used as the standard inhibitor for COX-1 and COX-2 respectively. The IC50 values (potency) of synthesized products were determined and compared with the references [3].

Docking studies

The inhibitory effect of synthesized compounds on COX-2 was also tested via molecular docking study using Molecular

Operating Environment (MOE) to determining the interaction stance of compounds with the active site of the enzyme (Fig. 1). Ligands with different configurations were prepared using the MOE builder tool. Subsequently, the molecules which required the lowest energy for stability were selected for docking (MMFF94x, gradient: 0.05). The x-ray crystallographic structure of Cox-2 enzyme in combination with phenylsulfonamide-3-trifluoromethyl-5-(4-bromophenyl) pyrazole SC-558 molecule was obtained from the protein data bank (PDB: 1CX2) [4]. The molecule replaced with target ligands in the MOE environment. Required modification and error correction was done subsequently. Afterward, the addition of hydrogens was done and the partial charges were calculated (Gasteiger methodology). Finally, docking was applied using the triangle matcher placement method. The GBVI/WSA dG scoring function was used to calculate the ligand-enzyme complex S scores. The complexes with the lowest S score were selected ultimately.

Result and Discussion

The methods that were used to synthesize the aryloxymethyl 1,3,4-thiadiazole and 1,2,4-triazole-5-thione derivatives are outlined in Scheme 1 (Reagents and conditions: (i) K2CO3, EtOCOCH2Br, acetone, refluxed 6 h; (ii) NH2-NH2.H2O, Ethanol, refluxed 1 h; (iii) RNCS, Ethanol, reflux 4 h; (iv) POCl3, reflux 3 h; (v) 1N NaOH (aq), reflux 8 h.). The chemical structures of the compounds 2a-d and 3a-d were determined by using spectral methods (IR, 1H,13C NMR, ESI-MS) and elemental analysis. In the IR spectra of 2a-d and 3a-d, afforded N-H stretching (3300-3200 cm-1), C-H stretching (3100-3000 cm-1), aliphatic C-H stretching (2950-2900 cm-1), thiadiazole and triazole C=N stretching, (1700-1630 cm-1), aromatic ring C=C stretching (1580-1500 cm-1). In the 1H NMR spectra, the NH protons of secondary amine as singlet peaks at 7.9-8.0ppm. The methylene protons as singlet at 5.3-5.4 ppm and the NH protons of traizole-thione as singlet at 13.85-14.10 ppm according to the solvent used [5].

The formation of the thiadiazole ring was determined by the observation of the peaks 158 and 170 (C2 and C5 respectively) ppm and for the triazole ring at 156 and 167 (C3 and C5 respectively) ppm in the 13C NMR. In the ESI Mass spectra of compound 2a-d and compound 3a-d, as well as [M+H] and [M+Na], fragments formed by the cleavage of synthesized products were observed. The elemental analysis of compounds were in an acceptable with the proposed structure of the compounds.

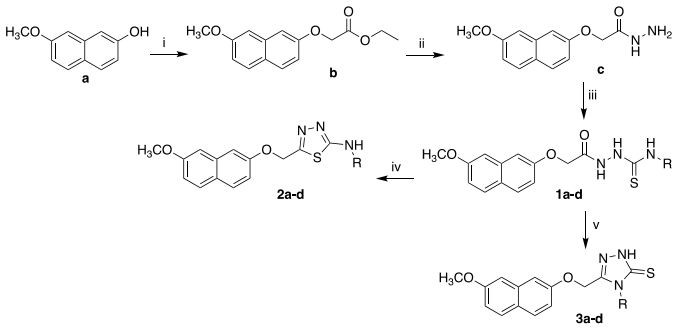

FIG 1. **Synthesis of target compounds 2a-d and 3a-d. Reagents and conditions: (i) K2CO3, EtOCOCH2Br, acetone, reflux 6 h; (ii) NH2NH2.H2O, EtOH, reflux 1 h; (iii) RNCS, EtOH, reflux 4 h; (iv) POCl3, reflux 3 h; (v) 1N NaOH (aq), reflux 8h**.

Biological Activity

1,3,4-thiadiazole and 1,2,4-triazole-5-thione derivatives (Compounds 2a-d and 3a-d) were screened for their ability to inhibit COX-2 and COX-1 enzymatic activity using a COX inhibitor screening assay kit. The potency (IC50 values) of test compounds was determined and compared to that of the reference molecules NS-398 (selective COX-2 inhibitor) and indomethacin

(selective COX-1 inhibitor). Additionally, to understand the the interaction of Compound 2b, which showed more selectivity on COX-2, with COX-2 enzyme a docking study was carried out. The inhibitory effects of compounds on COX-1 and COX-2 enzymes were lower than standards (NS-398 and indomethacin). However, among the compounds screened for COX-1 and COX-2 inhibition activity, Compound 2b was shown a selective inhibitory effect on COX-2 enzyme than the rest of compounds. Also, Compounds 3b and 3d were shown selective inhibitory effect on COX-1 enzyme then the other compounds.

TABLE 1. **The information in column four above, shows the in-vitro test compound concentration required to produce 50% inhibition of enzymatic activity**

Compound	**R**	**COX-1 IC_{50}(μM)[a]**	**COX-2 IC_{50}(μM)[a]**	**Selectivity Index (SI[b])**
2a	CH_3	>250	>250	n.d.[c]
2b	C_2H_5	>250	**150.2**	>1.6
2c	C_3H_5	>250	230.2	>1.1
2d	C_6H_5	136.3	>250	<0.5
3a	CH_3	154.5	>250	<0.6
3b	C_2H_5	**45.6**	176.5	0.3
3c	C_3H_5	**31.8**	>250	<0.1
3d	C_6H_5	166.4	>250	<0.7
NS-398		213.2	2.1	101.5
Indomethacin		0.67	18.5	0.036

Molecular docking studies demonstrated that the naphthyl ring that exists in the synthesized compounds was fitted into the hydrophobic cavity in the active site of Cox-2 enzyme formed via Val349, Tyr355, Leu359, and Leu531 amino acid residues. A hydrogen bond formed between the carbonyl group of Leu 352 residues and the methylene group of compound 2b. The orientation of compound 2b in the active site of COX-2 illustrated in figure 1. (compound 2b is displayed in yellow, residues are shown in white). Although thiadiazole moiety did not fill the adjunct pocket, thiadiazole ring formed an arene-cation interaction with His90. The selectivity of this compound may be due to the presence of this interaction. Based on Docking studies, in the 1,3,4-thiadiazole derivatives, ethyl substitution to 2-amino group increase the inhibitory activity and COX-2 selectivity.

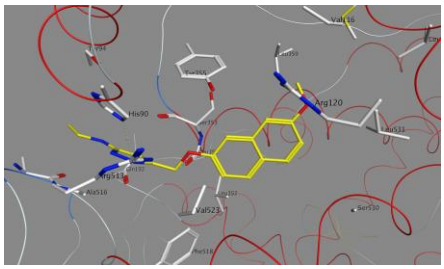

FIG 2. **The orientation of compound 2b in the active site of COX-2 (compound 2b is displayed as yellow, residues are**

shown as white)

Optimizing all the parameters of synthetic methods required to achieve a good yield of compounds by using the classic method is time-consuming and costly (8). As such, the Palaska design and employed the methods for synthesis of 1,3,4-thiadiazole and 1,2,4-triazole-5-thione derivatives with good yield, in this study the yield was also in good and acceptable limitations. All chemical structures of compounds were characterized by using IR, 1H-NMR and 13C-NMR and Mass spectroscopic methods as well as elemental analysis. The obtained data from the efficacy of compounds on both COX enzyme were lower than standards (NS-398 and indomethacin). However, among the compounds screened for their inhibition activity, compound 2b demonstrated a stronger selective inhibitory effect on COX-2 than the rest of the compounds. In addition, compounds 3b and 3d showed a stronger selectivity on COX-1 then the other compounds. Based on Docking studies, in the 1,3,4-thiadiazole derivatives, ethyl substitution to 2-amino group increase the inhibitory activity and COX-2 selectivity. With the aim of getting insights into the structural basis for its activity, Compound 2a was docked into the active site of COX-2 enzyme by using MOE software program. Docking studies showed that the naphthyl ring of the compounds fitted into the hydrophobic cavity formed Val349, Tyr355, Leu359 and Leu531. A hydrogen bond occurred between C=O moiety of Leu352 and methylene group of Compound 2b. Although thiadiazole moiety did not fill the adjunct pocket, but thiadiazole ring formed an arene-cation interaction with His90. The selectivity of this compound may be due to the presence of this interaction

Conclusion

In this study, a series of 1,3,4-thiadiazole and 1,2,4-triazole-5-thione derivatives were synthesized and evaluated for their inhibition activities against COX-2 and COX-1 enzymes. The obtained data shows that compound 2b compared with others has selective inhibition activity on COX-2. The inhibition activity on COX-2 and COX-1 of all compounds showed less than the standard controls (Indomethacin and NS-938). Thus, we can conclude that these thiadiazole derivatives with ethyl functional group could be an excellent starting point to design new selective COX-2 inhibitor agents. This could be a promising lead compound for further studies.

Acknowledgment

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References

1. Ricciotti E, FitzGerald A. Prostaglandins and Inflammation. Arterioscler Thromb Vasc Biol. 2011; 31:986-1000.
2. DEWitt DL. COX-2-Selective Inhibitors: The New Super Aspirins. Mol Pharmacol. 1999; 55: 625-631.
3. Botting RM. Inhibitors of Cyclooxygenases: Mechanism, Selectivity and Uses. J Physiol Pharmacol. 2006; 57: 113-124.
4. Dannhardt G, Kiefer W. Cyclooxygenase Inhibitors–Current Status and Future Prospects. Eur J Med Chem. 2001; 36: 109-126.
5. Blobaum AL, Marnett LJ. Structural and Functioanl Basis of Cyclooxygenase Inhibition. J Med Chem. 2007; 50: 1425-1441.