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Facile synthesis, molecular docking, and biological screening of 1,3-disubstituted urea derivatives

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ABSTRACT

*A series of 1-phenyl-3-(4-phenylthiazo-2-yl) urea derivatives **3a-f**, **4a-f**, and **5a-f** have been synthesized to meet the structural requirements essential for anti-inflammatory and antimicrobial properties. Target compounds were synthesized according to a new and convenient strategy. The strategy involves the reaction of 2-amino-4-phenylthiazoles **1a-c** with ethyl chloroformate to afford ethyl 4-(substituted)phenylthiazol-2-ylcarbamates **2a-c** followed by reaction with the appropriate amines either in a highly boiling point aprotic solvent or solvent free condition. Most of the target compounds showed potent antibacterial activity that equipotent or higher than ampicillin. Also, they were evaluated for their in vivo anti-inflammatory activities in rats compared to indomethacin. Four compounds **3b, 3e, 4e** and **5e** proved to be the most active anti-inflammatory agents in the present study with superior GI safety profile and good safety margin compared to indomethacin. In abases of molecular modeling; all synthesized 1,3-disubstituted ureas were subjected to docking simulation into active sites of human soluble epoxide hydrolase (sEH).*

Key words: 1,3-disubstituted ureas, green synthesis, antimicrobial activity, anti-inflammatory activity, molecular docking.

INTRODUCTION

Synthesis of heterocyclic compounds as a substituent in 1,3-disubstituted ureas is an attractive field for synthetic chemists due to their diverse range of pharmacological activities. 1,3-Disubstituted ureas possess antimicrobial [1-4], antiviral [5-7], anticancer [8,9], hypoglycemic [10], anti-hyperlipidemic [11], anticonvulsants [12], anti-angiogenics [13], and anti-parkinsonian activity [14] Additionally, several 1,3-disubstituted ureas were reported as much more potent anti-inflammatory. The anti-inflammatory activities of disubstituted ureas were recognized to be due to the inhibition to the soluble epoxide hydrolase enzyme (sEH) [15-17]. The soluble epoxide hydrolase (sEH) enzyme catalyzes the conversion of epoxy-eicosatrienoic acids (EETs) and leukotoxin; the important endogenous signaling lipids, to less active dihydroxy-eicosatrienoic acids (DHETs) [18] and toxic, pro-inflammatory leukotoxin diols [19] respectively. The increase of EETs and other fatty acid epoxides, resulting in anti-inflammatory effect [20]. Further, the importance of 2-aminothiazole and its derivatives is well known. Some antibacterial drugs, e.g., sulphathiazole [21], even a range of cephalosporin [22,23] and monobactam antibiotics [24] contain in its molecule 2-aminothiazole moiety. In addition, 2-aminothiazoles as a unique molecule were reported to have antibacterial, antifungal [25-27] and anti-inflammatory activities [28,29]. According with the aforementioned biological activities of 1,3-disubstituted ureas, and 2-aminothiazoles, the target of the present work was to synthesis various 1,3-disubstituted ureas containing 2-aminothiazole moieties, through a convenient synthetic strategy. Further, studying the molecular docking for these compounds with human sEH, compared with their anti-inflammatory results. Also

to investigate the antimicrobial activities for these compounds, taking in consideration that the compounds treated microbial infections and inflammation is a current trend in treatments.

EXPERIMENTAL SECTION

Chemistry

Melting points were determined on an electrothermal melting point apparatus [Stuart Scientific, model SMP3, Staffordshire, UK] and were uncorrected. Pre-coated silica gel plates (kieselgel 0.25 mm, 60G F254, Merck, Darmstadt, Germany) were used for TLC monitoring of reactions. The developing solvent systems of $CHCl_3/CH_3OH$ (9.5:0.5 and 8:2 v/v) were used and the spots were detected at 254 nm wavelength using ultraviolet lamp (Spectroline, model CM-10, Seattle, USA). IR spectra (KBr discs) were recorded on Thermoscientific, Nicolet-6700 FT-IR spectrometer (Thermo-Fischer Scientific Inc., USA). ^{1}H-NMR Spectra of final compounds **3a-f**, **4a-f**, and **5a-f** were scanned on a JEOL JNM-LA series FT-NMR system (400 MHz, JEOL, Tokyo, Japan) at the Unit of Trace Analyses, Assiut University. Chemical shifts are expressed in δ-value (ppm) relative to TMS as an internal standard, using DMSO-d_6 as a solvent, and deuterium oxide was used for the detection of exchangeable protons. Mass spectra were performed with JEOL-JMS600, at the Unit of Trace Analyses, Assiut University. The microanalyses of final compounds were performed on a Vario elemental analyzer III (Vario, Hanau, Germany) at the unit of Microanalysis, Cairo University. Yields % of final compounds **3a-f**, **4a-f**, and **5a-f** are presented in Table 1. The required chemicals and solvents are of reagents grade. The starting materials 2-amino-4-(substituted)-phenylthiazoles (**1a-c**), and ethyl 4-(substituted)-phenylthiazol-2-ylcarbamates (**2a-c**) were synthesized according to reported procedures [26,30].

General Procedures for Synthesis of Compounds 3a-f, **4a-f**, and **5a-f:**

Method A [31]

A mixture of 2-amino-4-(substituted)-phenylthiazoles (**1a-c**) (0.01 mol), and phenyl- or 4-chlorophenyl isocyanate (0.011 mol) in toluene (30 ml), was heated under reflux for 1 hr. The formed precipitate was filtered while hot, washed three times with hot n-hexane (3 X 20 ml), dried and crystallized from suitable solvent.

Method B

A mixture of ethyl 4-(substituted)-phenylthiazol-2-ylcarbamate (**2a-c**) (0.005 mol), and the appropriate amine (0.015 mol) in *m*-xylene (30 ml), was heated under reflux for 3 hr. The reaction mixture was concentrated near the dryness, cooled, diluted with n-hexane (30 ml), and boiled. The formed precipitate was filtered while hot, washed three times with hot n-hexane (20 ml), dried and crystallized from suitable solvent.

Method C

A mixture of ethyl 4-(substituted) phenylthiazol-2-ylcarbamate (**2a-c**) (0.005 mol), and the appropriate amine (0.015 mol) was heated at 120-130^{o}C for 3 hr. The reaction mixture was cooled, boiled with 5 ml ethanol and poured in dilute hydrochloric acid 50 ml. The formed precipitate was filtered, washed with water, dried and crystallized from suitable solvent.

Characterization for Compounds 3a-f, **4a-f**, and **5a-f:**

1-phenyl-3-(4-phenylthiazol-2-yl)urea (3a) [31] Cryst. solvent, ethanol; m.p. 218-9 ºC (Lit. [31]: 209 ºC); IR (KBr) ύ cm^{-1}: 3388, 3191, 3108, 1714, 1599; ^{1}H-NMR (DMSO-d_6): δ (ppm) 7.02-7.89 (m, 11H, $C_6\underline{H}_5$, $C_6\underline{H}_5$, C5-H thiazole); 8.92 (br. s, 1H, N^{1}H); 10.70 (br. s, 1H, N^{3}H); EI-MS m/z: 295.73 (M$^+$, 0.9%), 202.49 (59.4%), 176.43 (100%).

1-(4-chlorophenyl)-3-(4-phenylthiazol-2-yl)urea (3b). Cryst. solvent, DMF/H_2O; m.p. 246-7 ºC; IR (KBr) ύ cm^{-1}: 3386, 3189, 3106, 1710, 1596; ^{1}H-NMR (DMSO-d_6): δ (ppm) 7.29-7.89 (m, 10H, $C_6\underline{H}_5$, $C_6\underline{H}_4$, C5-H thiazole); 9.05 (br. s, 1H, N^{1}H); 10.78 (br. s, 1H, N^{3}H). EI-MS m/z: 331.81 (M$^+$+2, 0.9%), 329.75 (M$^+$, 2.4%), 183.95 (100%), 175.87 (86.8%); Anal. Calcd. (%) for $C_{16}H_{12}ClN_3OS$: C, 58.27; H, 3.67; N, 12.74. Found: C, 58.63; H, 3.69, N, 12.73.

1-(4-fluorophenyl)-3-(4-phenylthiazol-2-yl)urea (3c). Cryst. solvent, ethanol; m.p. 231-2 ºC; IR (KBr) ύ cm^{-1}: 3286, 3204, 3145, 1692, 1654, 1541; ^{1}H-NMR (DMSO-d_6): δ (ppm) 7.13-7.88 (m, 10H, $C_6\underline{H}_5$, $C_6\underline{H}_4$, C5-H thiazole); 8.93 (br. s, 1H, N^{1}H); 10.70 (br. s, 1H, N^{3}H); EI-MS m/z: 313.57 (M$^+$, 3.2%), 202.47 (28.2%), 176.44 (100%); Anal. Calcd. (%) for $C_{16}H_{12}FN_3OS$: C, 61.33; H, 3.86; N, 13.41. Found: C, 61.58; H, 3.78, N, 13.39.

1-(4-phenylthiazol-2-yl)-3-*p*-tolylurea (3d). Cryst. solvent, ethanol; m.p. 225-6 ºC; IR (KBr) ύ cm^{-1}: 3389, 3189, 1712, 1597; ^{1}H-NMR (DMSO-d_6): δ (ppm) 2.25 (s, 3H, CH_3); 7.04-7.88 (m, 10H, $C_6\underline{H}_5$, $C_6\underline{H}_4$, C5-H thiazole); 8.81 (br. s, 1H, N^{3}H); 10.65 (br. s, 1H, N^{1}H); EI-MS m/z: 309.71 (M$^+$, 0.7%), 202.47 (47.4%), 176.47 (57.3%), 134.30

(69.4%), 133.31(100%); Anal. Calcd. (%) for $C_{17}H_{15}N_3OS$: C, 66.00; H, 4.89; N, 13.58. Found: C, 66.03; H, 4.89, N, 13.28.

1-phenethyl-3-(4-phenylthiazol-2-yl)urea (3e). Cryst. solvent, ethanol/water; m.p. 164-5 ºC; IR (KBr) ύ cm^{-1}: 3379, 3187, 3117, 1666, 1558; ^{1}H-NMR (DMSO-d_6): δ (ppm) 2.77 (t, 2H, C$\underline{H}_2$C$_6$H$_5$, *J*= 7.2); 3.38 (t, 2H, NC$\underline{H}_2$); 6.54 (br. s, 1H, N^{1}H); 7.19-7.84 (m, 10H, C$_6\underline{H}_5$, C$_6\underline{H}_4$, C5-H thiazole); 10.26 (br. s, 1H, N^{3}H); EI-MS m/z: 323.73 (M^+, 0.8%), 202.44 (23.0%), 176.38 (100%); Anal. Calcd. (%) for $C_{18}H_{17}N_3OS$: C, 66.85; H, 5.30; N, 12.99. Found: C, 67.00; H, 5.48, N, 13.05.

1-cyclohexyl-3-(4-phenylthiazol-2-yl)urea (3f). Cryst. solvent, hexane/dioxane; m.p. 152-3 ºC; IR (KBr) ύ cm^{-1}: 3405, 3344, 3169, 1686, 1519; ^{1}H-NMR (DMSO-d_6): δ (ppm) 1.19-1.82 (m, 11H, cyclohexyl-H); 6.48 (d, 1H, N^{1}H, *J*=6.8); 7.29-7.85 (m, 6H, C$_6\underline{H}_5$, C5-H thiazole); 10.33 (br. s, 1H, N^{3}H); EI-MS m/z: 224.47 (M^+-C$_6$H$_5$, 0.8%), 202.55 (13.8%), 176.37 (100%); Anal. Calcd. (%) for $C_{16}H_{19}N_3OS$: C, 63.76; H, 6.35; N, 13.94. Found: C, 63.69; H, 6.48, N, 13.92.

1-[4-(4-chlorophenyl)thiazol-2-yl]-3-phenylurea (4a). Cryst. solvent, ethanol; m.p. 250-2 ºC; IR (KBr) ύ cm^{-1} : 3389, 3184, 3103, 1709, 1598; ^{1}H-NMR (DMSO-d_6): δ (ppm) 7.02-7.91 (m, 10H, C$_6\underline{H}_4$, C$_6\underline{H}_5$, C5-H thiazole); 8.93 (br. s, 1H, N^{3}H); 10.70 (br. s, 1H, N^{1}H); EI-MS m/z: 238.33 (M^++2 - C$_6$H$_5$N, 50.9%), 236.27 (M^+- C$_6$H$_5$N, 51.4%), 212.37 (48.4%), 210.31 (100%); Anal. Calcd. (%) for $C_{16}H_{12}ClN_3OS$: C, 58.27; H, 3.67; N, 12.74. Found: C, 58.42; H, 3.60, N, 12.87.

1-(4-chlorophenyl)-3-[4-(4-chlorophenyl)thiazol-2-yl]urea (4b). Cryst. solvent, DMF/H_2O; m.p. 252-4 ºC; IR (KBr) ύ cm^{-1}: 3388, 3184, 3105, 1709, 1525; ^{1}H-NMR (DMSO-d_6): 7.31-7.91 (m, 9H, C$_6\underline{H}_4$, C$_6\underline{H}_4$, C5-H thiazole); 9.07 (br. s, 1H, N^{1}H); 10.78 (br. s, 1H, N^{3}H); EI-MS m/z: 238.51 (M^++2 - C$_6$H$_6$ClN, 28.0%), 236.52 (M^+-C$_6$H$_6$ClN, 89.7%), 212.41(33.5%), 210.25 (100%); Anal. Calcd. (%) for $C_{16}H_{11}Cl_2N_3OS$: C, 52.76; H, 3.04; N, 11.54. Found: C, 52.61; H, 2.85, N, 11.82.

1-[4-(4-chlorophenyl)thiazol-2-yl]-3-(4-fluoro-phenyl)urea (4c). Cryst. solvent, ethanol; m.p. 266-7 ºC; IR (KBr) ύ cm^{-1}: 3383, 3190, 3105, 1712, 1597; ^{1}H-NMR (DMSO-d_6): δ (ppm) 7.14-7.77 (m, 9H, C$_6\underline{H}_4$, C$_6\underline{H}_4$, C5-H thiazole); 8.95 (br. s, 1H, N^{3}H); 10.70 (br. s, 1H, N^{1}H); Anal. Calcd. (%) for $C_{16}H_{11}ClFN_3OS$: C, 55.25; H, 3.19; N, 12.08. Found: C, 55.51; H, 3.19, N, 12.34.

1-[4-(4-chlorophenyl)thiazol-2-yl]-3-*p*-tolylurea (4d). Cryst. solvent, ethanol; m.p. 271-2 ºC; IR (KBr) ύ cm^{-1}: 3304, 3100, 1640, 1560; ^{1}H-NMR (DMSO-d_6): δ (ppm) 2.25 (s, 3H, CH$_3$); 7.11-7.91 (m, 9H, C$_6\underline{H}_4$, C$_6\underline{H}_4$ and C5-H thiazole); 8.80 (br. s, 1H, N^{3}H); 10.61 (br. s, 1H, N^{1}H); EI-MS m/z: 343.18 (M^+, 1.6%), 237.87 (37.9%), 235.87 (100%); Anal. Calcd. (%) for $C_{17}H_{14}ClN_3OS$: C, 59.39; H, 4.10; N, 12.22. Found: C, 59.44; H, 4.44, N, 12.17.

1-[4-(4-chlorophenyl)thiazol-2-yl]-3-phenethylurea (4e). Cryst. solvent, Ethyl acetate/Hexane; m.p. 175-6 ºC; IR (KBr) (cm^{-1}): 3300, 3260, 3100, 1686, 1558; ^{1}H-NMR (DMSO-d_6): δ (ppm) 2.77 (t, 2H, C$\underline{H}_2$C$_6$H$_5$, *J*= 7.2); 3.36-3.41 (m, 2H, NC$\underline{H}_2$); 6.53 (br. s, 1H, N^{3}H); 7.19-7.88 (m, 10H, C$_6\underline{H}_5$, C$_6\underline{H}_4$, C5-H thiazole); 10.19 (br. s, 1H, N^{1}H); EI-MS m/z: 238.41 (M^++2 - C$_8$H$_{11}$N, 21.1%), 236.42 (M^+ - C$_8$H$_{11}$N, 93.7%), 212.35 (45.7%), 210.26 (100%); Anal. Calcd. (%) for $C_{18}H_{16}ClN_3OS$: C, 60.41; H, 4.51; N, 11.74. Found: C, 60.71; H, 4.82, N, 11.56.

1-[4-(4-chlorophenyl)thiazol-2-yl]-3-cyclohexylurea (4f). Cryst. solvent, Hexane/dioxane; m.p. 165-7 ºC; IR (KBr) ύ cm^{-1}: 3331, 3258, 3104, 1650, 1558; ^{1}H-NMR (DMSO-d_6): δ (ppm) 0.98-1.81 (br. m, 11H, cyclohexyl-H); 6.47 (d, 1H, N^{3}H, *J*= 7.6); 7.35-7.92 (m, 5H, C$_6\underline{H}_4$, C5-H thiazole); 10.36 (br. s, 1H, N^{1}H); Anal. Calcd. (%) for $C_{16}H_{18}ClN_3OS$: C, 57.22; H, 5.40; N, 12.51. Found: C, 57.10; H, 5.50, N, 12.60.

1-phenyl-3-(4-*p*-tolylthiazol-2-yl)urea (5a). Cryst. solvent, DMF/water; m.p. 259-60 ºC; IR (KBr) ύ cm^{-1}: 3390, 3200, 1700, 1589; ^{1}H-NMR (DMSO-d_6): δ (ppm) 2.31 (s, 3H, CH$_3$); 7.02-7.78 (m, 10H, C$_6\underline{H}_4$, C$_6\underline{H}_5$, C5-H thiazole); 8.92 (br. s, 1H, N^{1}H); 10.67 (br. s, 1H, N^{3}H); EI-MS m/z: 309.59 (M^+, 4.2%), 216.43 (81.2%), 190.50 (100%); Anal. Calcd. (%) for $C_{17}H_{15}N_3OS$: C, 66.00; H, 4.89; N, 13.58. Found: C, 65.53; H, 5.79, N, 13.62.

1-(4-chlorophenyl)-3-(4-*p*-tolylthiazol-2-yl)urea (5b). Cryst. solvent, DMF/water; m.p. 276-7 ºC; IR (KBr) ύ cm^{-1}: 3380, 3189, 3104, 1711, 1594; ^{1}H-NMR (DMSO-d_6): δ (ppm) 2.31 (s, 3H, CH$_3$); 7.20-7.77 (m, 9H, C$_6\underline{H}_4$, C$_6\underline{H}_4$, C5-H thiazole); 9.05 (br. s, 1H, N^{1}H); 10.74 (br. s, 1H, N^{3}H); EI-MS m/z: 343.62 (M^+, 3.6%), 216.38 (36.2%), 190.44 (100%); Anal. Calcd. (%) for $C_{17}H_{14}ClN_3OS$: C, 59.39; H, 4.10; N, 12.22. Found: C, 59.36; H, 4.27, N, 12.02.

1-(4-fluorophenyl)-3-(4-*p*-tolylthiazol-2-yl)urea (5c). Cryst. solvent, ethanol; m.p. 273-4 ºC; IR (KBr) ύ cm^{-1}: 3380, 3189, 3104, 1711, 1594; ^{1}H-NMR (DMSO-d_6): δ (ppm) 2.31 (s, 3H, CH_3); 7.14-7.77 (m, 9H, $C_6\underline{H}_4$, $C_6\underline{H}_4$, C5-H thiazole); 8.95 (br. s, 1H, N^1H); 10.71 (br. s, 1H, N^3H). EI-MS m/z: 327.69 (M^+, 7.28%), 216.46 (21.72%), 190.43 (100%); Anal. Calcd. (%) for $C_{17}H_{14}FN_3OS$: C, 62.37; H, 4.31; N, 12.84. Found: C, 62.55; H, 4.60, N, 12.60.

1-*p*-tolyl-3-(4-*p*-tolylthiazol-2-yl)urea (5d). Cryst. solvent, DMF/water; m.p. 244-5 ºC; IR (KBr) ύ cm^{-1}: 3382, 3191, 3104, 1709, 1596; ^{1}H-NMR (DMSO-d_6): δ (ppm) 2.25 (s, 3H, CH_3); 2.31 (s, 3H, CH_3); 7.11-7.77 (m, 9H, $C_6\underline{H}_4$, $C_6\underline{H}_4$, C5-H thiazole); 8.81 (br. s, 1H, N^1H); 10.62 (br. s, 1H, N^3H); EI-MS m/z: 216.38 (M^+ - C_7H_9N, 39.6%), 190.51 (M^+- C_8H_7NO, 100%), 133.27 (40.9%); Anal. Calcd. (%) for $C_{18}H_{17}N_3OS$: C, 66.85; H, 5.30; N, 12.99. Found: C, 66.53; H, 5.26, N, 13.12.

1-phenethyl-3-(4-*p*-tolylthiazol-2-yl)urea (5e). Cryst. solvent, DMF/water; m.p. 173-5 ºC; IR (KBr) ύ cm^{-1}: 3300, 3273, 3123, 1689, 1557; ^{1}H-NMR (DMSO-d_6): δ (ppm) 2.30 (s, 3H, CH_3); 2.76 (t, 2H, $CH_2C_6\underline{H}_5$, *J*= 6.8); 3.36-3.41 (m, 2H, $N\underline{CH}_2$-); 6.52 (br. s, 1H, N^1H); 7.18-7.72 (m, 10H, $C_6\underline{H}_5$, $C_6\underline{H}_4$, C5-H thiazole); 10.46 (br. s, 1H, N^3H). EI-MS m/z: 337.82 (M^+, 1.8%), 215.92 (20.3%), 189.84 (95.7%), 83.97(100%); Anal. Calcd. (%) for $C_{19}H_{19}N_3OS$: C, 67.63; H, 5.68; N, 12.45. Found: C, 67.94; H, 5.39, N, 12.55.

1-cyclohexyl-3-(4-*p*-tolylthiazol-2-yl)urea (5f). Cryst. solvent, hexane/dioxane; m.p. 194-5 ºC; IR (KBr) ύ cm^{-1}: 3407, 3344, 3172, 1689, 1538; ^{1}H-NMR (DMSO-d_6): δ (ppm) 1.17-1.81 (br. m, 11H, cyclohexyl-H); 2.30 (s, 1H, CH_3); 6.49 (d, 1H, N^1H, *J*= 10.8); 7.18-7.75 (m, 5H, $C_6\underline{H}_4$, C5-H thiazole); 10.31 (br. s, 1H, N^3H); EI-MS m/z: 315.60 (M^+, 1.6%), 216.38 (23.1%), 190.52 (100%); Anal. Calcd. (%) for $C_{17}H_{21}N_3OS$: C, 64.73; H, 6.71; N, 13.32. Found: C, 65.02; H, 6.99, N, 13.00.

Biological screening:

Antimicrobial activity:

All final 1,3-disubstituted urea derivatives **3a-f**, **4a-f**, and **5a-f** were tested for their in *vitro* antibacterial and antifungal activities, compared to ampicillin (for antibacterial investigation), and fluconazole (for antifungal investigation) as reference drugs respectively. The investigation was performed, using the standard agar cup diffusion method [32] at a concentration of 100 µmol/ml.

Antibacterial assay. It was done at Microbiology laboratory at Microbiology department, Assiut University. Four pathogenic bacterial species representing both Gram-positive starins *(Staphylococcus aureus, Staph saprophyticus* and *Bacillus species*) and Gram-negative strain (*Escherichia coli)* were selected. All these bacterial isolates were isolated, identified at Microbiology department, Assiut University. For antibacterial investigation; bacterial strains were individually cultured for 48 h in 100 ml conical flasks containing 30 ml nutrient agar medium. Bioassay was done in 10 cm sterile Petri dishes in which 1 ml of bacterial suspension and 15 ml of nutrient agar were poured. Plates were shaken gently to homogenize the inoculate. After solidification of the media, 5 mm cavities were cut in the solidified agar (4 to 6 cavities/plate) using sterile cork borer. The test compounds (**3 a-f**, **4 a-f** and **5 a-f**) and reference drugs were dissolved in DMSO (100 µmol/ml) that served as positive control and were loaded in the cavities. In addition, other cavities were loaded with the solvent DMSO and served as a negative control. The seeded plates were incubated at 28±2 °C for 48 h. The diameters of inhibition zones (in mm) of triplicate sets were measured and the results are cited in **Table 2.**

Antifungal assay. It was done at Mycological Center, Assiut University. For antifungal assay; three human pathogenic fungi species (*Trichophyton rubrum* AUMC 1804, *Candida albicans* AUMC 1299 and *Scopulariopsis brevicaulis* AUMC 361] were used in the present study. For antifungal screening; spore suspension in sterile distilled water was prepared from 7 days old culture of the test fungi growing on Sabouraud's dextrose broth (30 ml) media in 100 ml conical flasks. The final spore concentration was nearly 5×10^4 spores /ml. Bioassay was done in 10 cm sterile Petri dishes in which 1 ml of spore suspension and 15 ml of nutrient agar were poured. Plates were shaken gently to homogenize the inoculate. After solidification of the media, 5 mm cavities were cut in the solidified agar (4 to 6 cavities/plate) using sterile cork borer. The test compounds (**3 a-f**, **4 a-f** and **5 a-f**) and reference drug were dissolved in DMSO (100 µmol/ml). DMSO was served as a negative control. The seeded plates were incubated at 28±2 °C for 7 days.

The minimium inhibitory concentration (MIC_S). Using Agar cup diffusion method, the test compounds giving positive results (with zone of inhibition higher than 17 mm) were diluted with DMSO to prepare a series of descending concentration down to 1.56 µmol /ml. Diluted solutions were added to plates containing different bacterial isolates, then zone of inhibition was measured. Plotting square of diameter (X^2) against Log C, gave a straight line, then by extrapolation of this straight line to cut log C at certain point, this indicates MIC. Results are cited in Table 2.

Anti-inflammatory activity

The anti-inflammatory activity of eighteen final 1,3-disubstituted urea derivatives **3a-f**, **4a-f**, and **5a-f** was evaluated by the carrageenan-induced paw edema method *in vivo* in rats according to a reported method [16], using indomethacin as a reference standard. The thickness of rat paw edema was measured by digital plethysmometer LE7500 (Panlab S.L., Cornella, Barcelona, Spain). Adult albino male rats weighing 150-200 g were divided into twenty groups of 6 animals each. The animals were fed with commercial feed pellets and were given water *ad libitum*. Work was conducted in accordance with the internationally accepted principles for laboratory animals' use and care as found in the European Community Guidelines [33]. Indomethacin and the tested compounds, **3a-f**, **4a-f**, and **5a-f** were suspended in 0.5% sodium carboxymethylcellulose (NaCMC) saline solution and given *orally* by gastric tubes, at a dose level of 28 mmol/kg. Eighteen different groups were treated with a suspension of the tested compounds, and reference group was treated by indomethacin, one hour before induction of inflammation. The control group was given saline solution containing 0.5% NaCMC. After 30 minutes, 0.1 ml of freshly prepared carrageenan solution (1% in normal saline) was injected into the subplantar region of the right hind paw of each rat to induce edema. The thickness of rat paw was measured by digital plethysmometer at different time intervals (three and five hours) after administration of the test samples. The difference between the thicknesses of two paws (right and left) was taken as a measure of edema [16].

The results of the anti-inflammatory activity were expressed as percentage inhibition of edema thickness in treated animals in comparison with the control group according to the following equation:

% Edema inhibition = 100 (1 - Vt /Vc)

Where, Vt = volume of edema in treated group, Vc = volume of the edema in the control group. The anti-inflammatory activities of the tested compounds using carrageenan-induced paw edema in rats, at three and five hours respectively, are shown in Table 3.

Ulcerogenic effect

Scanning electron microscopy of the stomachs of rats was performed on JEOL JSM-5400 LV Scanning microscope (JEOL, Tokyo, Japan) at the unit of Electron Microscope, Assiut University. The ulcerogenic effect was determined for the highly active anti-inflammatory compounds **3b, 3e, 4e,** and **5e**, in albino rats following the previously reported standard method [34]. Adult albino rats weighing 150-200 g were divided into six groups of five animals each. The animals were fasted 24 hours before drug administration. indomethacin (reference standard) and the tested compounds (30 mg/kg) were suspended in saline solution using 0.5% NaCMC and were administered orally for three successive days to fasted rats. The control group animals were given saline solution using 0.5% NaCMC. Six hours following the dose, the animals were sacrifice by cervical dislocation and the stomach was removed, opened along the greater curvature and washed with saline. The Stomachs were examined with a magnifying lens (10x) for the presence of lesions and erosions.

Ulcer was defined as at least one lesion that was 0.5 mm or more in length. All lesions of more than 0.1 mm in length were summed to obtain the ulcer index. Stomachs were kept in 10% w/v formalin solution. After 24 h, the surface of stomachs was examined using scanning electron microscope. The results were listed in Table 4 and some representative photo-micrographs for gastric mucosa of control healthy and treated rats are shown in Figure 1.

Ulcer index

The ulcers were examined on the dissected stomach and the ulcer index was calculated by using the formula, UI = 10/X, Where X= total mucosal area / total ulcerated area.

Acute toxicity

The highly active anti-inflammatory compounds **3b, 3e, 4e,** and **5e** were further investigated for their median lethal dose (LD_{50}) compared to indomethacin (reference standard) in male mice. Twenty five groups of mice each consisting of six animals, were used. The compounds and indomethacin were given orally at doses of 20, 40, 60, 100, and 120 mg/kg. Twenty four hours later, the % mortality in each group and for each compound was recorded. The LD_{50} values were calculated using the method described by Litchfield and Wilcoxon [35].

Molecular Modeling

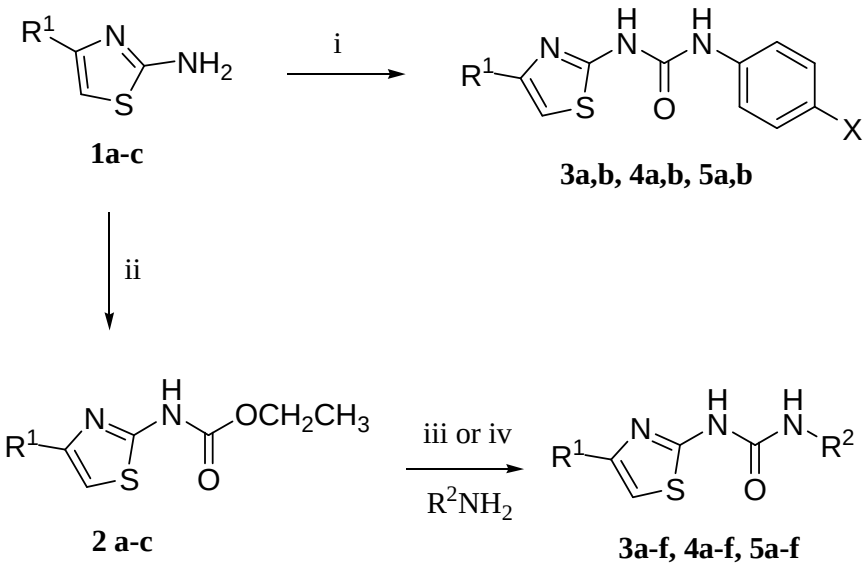
Docking simulation study is performed using Molecular Operating Environment (MOE®) version 10.2010, Chemical Computing Group Inc., Montreal, Canada. The computational software operated under "Windows XP" installed on an Intel Pentium IV PC with a 1.6 GHz processor and 512 MB memory. All the minimizations were performed with MOE until it reached a RMSD gradient of 0.05 Kcal mol^{-1} $A^{\circ-1}$ with MMFF94X force-field. The atomic coordinates of the crystal structures of human sEH complex with CIU (1-cyclohexyl-3-(4-iodophenyl)urea)

was retrieved from Protein Data Bank (entry 1VJ5). All synthesized compounds were docked into the ligand-binding pocket manually by superposition with the parent molecule (CIU) and minimized on MM geometry. The docking results appear in a DBV window (dock.mdb). The (S) field that the docking poses is ranked by the MM/GBVI binding free energy calculation, which is identical to the E_refine score. Also the RMSD field, which is the RMSD of the docking pose compared to the cocrystal ligand position was noted. The Database browser was used to compare the docking poses to the ligand in the co-crystallized structure.

RESULTS AND DISCUSSION

Chemistry

The target compounds 1-aryl (or alkyl)-3-[4-(substituted)phenylthiazo-2-yl] urea **3a-f**, **4a-f**, and **5a-f** were prepared according to Scheme 1.



X = H, Cl; R^1 = Ph, 4-Cl.Ph, 4-CH_3

R^2 = Ph, 4-Cl.Ph, 4-F.Ph, 4-CH_3.Ph, -CH_2CH_2Ph or *cyclo*-C_6H_{11}

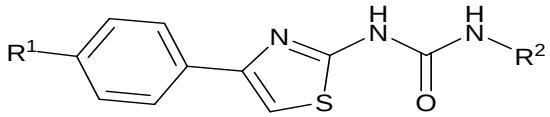
i, phenylisocyanate (or 4-chlorophenylisocyamate), toluene, reflux;
ii, ethyl chloroformate, benzene, triethylamine, reflux; iii, m-xylene, reflux;
iv, heat at 130 °, solvent free

Scheme 1: synthesis of the target compounds

Compounds 2-amino-4-phenylthiazole **1a-c** were prepared according to a reported procedure **[26]** through one pot reaction; by heating a mixture of 4-(substituted)acetophenones, thiourea, and iodine. This was a favourable and simpler method than bromination of acetophenones, followed by reaction with thiourea [30]. The chemical structures of compounds **1a-c** were confirmed by matching their physical and spectral data with the reported ones [25,26,36]. Reaction of 2-amino-4-phenylthiazole **1a-c** with ethyl chloroformate in benzene and in presence of triethylamine afforded ethyl 4-(substituted)phenyl-thiazol-2-ylcarbamates **2a-c**, according to a reported method [**30].** ^{1}H-NMR spectra of compounds **2a-c** revealed the triplet signals of $\underline{CH_3}CH_2$ at δ 0.92-1.03 ppm, and the quartet signal of $CH_3\underline{CH_2}O$ at δ 4.00-4.10 ppm, also the presence of NH broad singlet at δ 10.26-11.01 ppm. IR spectra of compounds **2a-c** showed presence of strong bands at ύ 3167-3154 cm^{-1} and ύ 1720-1717 cm^{-1} which were assigned for the NH and C=O groups, respectively. The preparation of the target 1,3-disubstituted urea was afforded through the reaction of 2-amino-4-phenylthiazole **1a-c** with aryl isocyanate in toluene under reflux (*method A*) **[31].** This reaction is illustrated by reaction of phenyl isocyanate or 4-chlorophenyl isocyanate with 2-amino-4-phenylthiazoles **1a-c** to afford compounds **3a**, **b**; **4a**, **b** and **5a**, **b**. This method was favourable due the simplicity and high yield (90-97%), except it consumed more expensive reagents [2,5]. Another method (*method B*) for preparation of the target compounds, 1,3-disubstituted ureas is the reaction of ethyl 4-(substituted)-phenylthiazol-2-ylcarbamates **2a-c** with the appropriate amines, under reflux in an aprotic, highly boiling point solvent, e.g. toluene or *m*-xylene, to afford the target 1-(substituted)-phenyl-3-[4-(substituted)-phenylthiazol-2-yl] ureas **3a-e**, **4a-e**, and **5a-e** in good yields (65–73%). Alternatively, the reaction of **2a-c** with the appropriate amines at 120-130° under solvent-free condition

was performed for synthesis of the target urea derivatives **3a-f**, **4a-f**, and **5a-f** (*method C*). The key advantages of the later method are that, it doesn't involve any hazardous organic solvent, also high yields (75–90%), simple workup, and easily purification of products. The characterization of compounds **3a-f**, **4a-f**, and **5a-f** is based on their,[1]H-NMR, FT-IR, mass spectroscopy. All elemental analysis of compounds **3b-f**, **4a-f**, and **5a-f** are good agreement with the desired values.

Table 1: Yields % of compounds 3a-f, 4a-f, and 5a-f



No	R^1, R^2	Method, Yield%	No	R^1, R^2	Method, Yield%	No	R^1, R^2	Method, Yield%
3a	H, Ph	**A**, 95 **B**, 70 **C**, 90	**4a**	Cl, Ph	**A**, 96 **B**, 72 **C**, 90	**5a**	CH_3, Ph	**A**, 90 B, 67 C, 86
3b	H, 4-Cl.Ph	**A**, 97 **B**, 73 **C**, 88	**4b**	Cl, 4-Cl.Ph	**A**, 97 **B**, 70 **C**, 88	**5b**	CH_3, 4-Cl.Ph	**A**, 93 **B**, 68 **C**, 88
3c[a]	H, 4-F.Ph	**B**, 70 **C**, 89	**4c**[a]	Cl, 4-F.Ph	**B**, 72 **C**, 88	**5c**[a]	CH_3, 4-F.Ph	**B**, 69 **C**, 85
3d[a]	H, 4-CH_3.Ph	**B**, 68 **C**, 90	**4d**[a]	Cl, 4-CH_3.Ph	**B**, 72 **C**, 87	**5d**[a]	CH_3, 4-CH_3.Ph	**B**, 66 **C**, 84
3e[a]	H, $(CH_2)_2$Ph	**B**, 65 **C**, 84	**4e**[a]	Cl, $(CH_2)_2$Ph	**B**, 70 **C**, 84	**5e**[a]	CH_3, $(CH_2)_2$Ph	**B**, 60 **C**, 80
3f[a, b]	H, *cyclo*-C_6H_{11}	**C**, 80	**4f**[a, b]	Cl, *cyclo*-C_6H_{11}	**C**, 83	**5f**[a, b]	CH_3, *cyclo*-C_6H_{11}	**C**, 75

[a] *Method A was not detected,* [b] *Method B gave tiny yields*

Table 2: *In* vitro antibacterial activity of compounds (3a-f, 4a-f, 5a-f) and ampicillin exhibited by minimum inhibitory concentration (MIC) in µmol/ml[a]

Compound no	(MIC in µmol/ml)			
	Gram-positive			Gram-negative
	S. aureus	***Bacillus spc***	***S. saprophyticus***	***E. coli***
DMSO	-	-	-	
Ampicillin	(3.125)	(6.25)	(6.25)	(12.5)
3a	(37.5)	(25)	(25)	-
4a	-	p.i.[b]	(6.25)	(6.25)
5a	(6.25)	(3.125)	(6.25)	(6.25)
3b	(6.25)	(6.25)	(3.125)	-
4b	(6.25)	(3.125)	(6.25)	p.i.[b]
5b	(18)	(12.5)	(25)	-
3c	(25)	(25)	(25)	(50)
4c	(6.25)	(6.25)	(6.25)	(6.25)
5c	(3.125)	(6.25)	(3.125)	-
3d	(12.5)	(12.5)	(25)	-
4d	-	(50)	(50)	-
5d	-	(25)	(25)	-
3e	-	(50)	-	p.i.[b]
4e	(25)	(12.5)	(12.5)	(3.75)
5e	-	(75)	-	p.i.[b]
3f	(3.125)	(6.25)	(3.125)	(3.125)
4f	(3.125)	(6.25)	(12.5)	(12.5)
5f	(3.125)	(6.25)	12.5	(3.125)

[a] *Antibacterial evaluation was performed at 100 µmol/ml* [b] *(p.i.) partial inhibition; MIC > 100 µmol/ml*

Biological screening

Antimicrobial activity

Investigation was performed, using the standard agar cup diffusion method [**32**] at a concentration of 100 µmol/ml. By comparing the antibacterial activity of the target compounds in Table 2, it was found that, most of the compounds in the three series; **3a-f**, **4a-f**, and **5a-f**, exhibited considerable significant antibacterial activity against tested gram positive strains; *Staph aureus*, *Staph saprophyticus* and *Bacillus species* and negative strain *E coli*. Also, it was obvious that presence of cyclohexyl urea moiety as well as halogens (Cl, F) on phenyl urea moiety enhanced activity markedly against all tested gram positive and gram negative strains, in most cases equal to/ or more potent than reference. Compounds **3f** and **5f** that bearing cyclohexyl urea group and **5c** having fluorophenyl urea moiety exhibited equipotent antibacterial activity with reference against pathogenic *Staph aureus* and *Bacillus species.* In addition to these derivatives, compounds **3b, 4c**, and **5c** having chlorine or fluorine on phenyl urea moiety exhibited

the same potency compared with ampicillin (MIC = 6.25 µmol/ml) against *Bacillus species.* It is noteworthy to mention that compounds **4b** (R^1, R^2 = 4-Cl-Ph) and **5a** (R^1, R^2 = 4-CH_3-Ph, Ph) (MIC = 3.125 µmol/ml) showed two folds higher activity than ampicillin against *Bacillus species.* Regarding correlation of the antimicrobial activity of the 1,3-disubstituted urea derivatives with various substituents, it was found that steric factor plays a significant role; as increasing bulkiness of R^2 by introducing phenethyl- (compounds **3e, 4e** and **5e**) or tolyl groups (compounds **3d, 4d,** and **5d**) decreased or abolished antibacterial activity against pathogenic *Staph aureus* and *Bacillus species* markedly. In addition, inhibitory activity against *Staph. Saprophyticus* strain was significant in case of compounds **3b, 3d** and **5c** (MIC = 3.125 µmol/ml), as they surpassed ampicillin (MIC = 6.25 µmol/ml) by two folds, while, compounds **4a, 4b, 4c**, and **5a** exhibited equal antibacterial activity with reference. Concerning gram negative bacteria, represented by *E coli* strain, it can be inferred that 1,3-disubstituted urea derivatives in our study, showed a promising antibacterial activity that surpassed ampicillin in case of compounds, **3f, 4e**, and **5f** (by 4 folds); **4a, 4d** and **5a** (by 2 folds) while **4f** was equipotent with reference. Remaining test compounds showed partial or no inhibition at a concentration of 100 µmol/ml. On the other hand, none of the tested compounds **3a-f**, **4a-f**, and **5a-f**; had notable fungal growth inhibitory activity against any of the tested pathogenic fungal strains, at a concentration of 100 µmol/ml.

Table 3: *In vivo* Anti-inflammatory activity of compounds (3a-f, 4a-f, 5a-f), and indomethacin and scoring function database of the tested ligands

Compound no	% Inhibition of Edema ± SE[a]		dG (sEH) Kcal/mole (S values)
	3hr	5hr	
Negative Control	0.00	0.00	-
indomethacin	48.88 ± 1.68	76.55 ± 2.19	-
3a	42.07 ± 2.60 [b]	65.26 ± 2.72 [b]	-27.30
4a	40.21 ±2 .02 [b]	60.68 ± 2.28 [b]	-23.76
5a	41.30 ± 1.98 [b]	63.31 ±1.69 [b]	-24.88
3b	43.58 ±1.45 [b]	66.43 ± 2.46 [b]	-28.80
4b	42.67 ± 2.72 [b]	64.61 ± 2.60 [b]	-27.00
5b	43.03 ± 1.88 [b]	65.20 ±2.50 [b]	-26.94
3c	42.21± 2.34 [b]	65.67 ±2.71 [b]	-27.42
4c	40.05 ± 2.19 [b]	64.60 ± 1.93 [b]	-24.95
5c	40.52 ± 2.31 [b]	64.88 ± 2.46 [b]	-25.05
3d	42.36 ± 1.77 [b]	64.68 ± 2.67 [b]	-27.83
4d	41.19 ± 2.55 [b]	62.73 ± 2.51 [b]	-25.97
5d	41.55 ± 3.55 [b]	63.35 ± 2.55 [b]	-25.68
3e	46.24± 1.92 [b]	76.00± 1.73 [b]	-29.68
4e	48.50± 2.84 [b]	78.09 ± 1.27 [b]	-30.94
5e	48.93 ± 1.91 [b]	78.28 ± 2.32 [b]	-30.73
3f	43.20 ± 2.47 [c]	65.90 ± 3.03 [c]	-28.20
4f	41.08 ± 2.27 [b]	61.91 ± 2.03[b]	-25.53
5f	40.84 ± 2.12 [c]	62.46 ± 2.39 [c]	-25.05

[a] All values are expressed as mean ±SE, n =6 animals per each group [b] Significant difference at p <0.01 vs. control (one-way ANOVA)
[c] Significant difference at p < 0.05 vs. control (one-way ANOVA)

Table 4: Ulcerogenic effects of compounds (3b, 3e, 4e, 5e) and indomethacin

Compound no.	Ulcer index (mean ± SE)[a,b]
Control	0.54 ± 0.05
indomethacin	7.2 ± 0.33 [c]
3b	3.1 ± 0.21 [c]
3e	2.16 ± 0.11 [c]
4e	2.5 ± 0.21 [c]
5e	2.56 ± 0.15 [c]

[a] All values are expressed as mean ±SE
[b] n =5 animals per each group
[c] p < 0.001 vs control (one-way ANOVA)

Anti-inflammatory activity

Generally, it has been observed from the obtained results in Table 3, all the tested compounds showed a considerable anti-inflammatory activity, and the activities for these compounds were varied correlated to its substituent of the 1,3-disubstituted ureas. In case of 4-phenylthiazo-2-yl moiety, the anti-inflammatory activity was more or less affected by substitution of phenyl ring, but in general the activities were slightly increased with the order of 4-phenylthiazo-2-yl > 4-methylphenylthiazo-2-yl > 4-chlorophenylthiazo-2-yl for these derivatives. In the case of substituted-phenyl (or alkyl) urea, the activities more pronounced increased in phenethyl moieties than

substituted-phenyl or cyclohexyl moieties, and the activity of cyclohexyl ureas were more or less affected as substituted phenyl. Compounds that have phenethyl moieties **3e, 4e** and **5e** exhibit nearly the same anti-inflammatory properties (46.24 - 48.93% and 76.00 - 78.28% inhibition of edema at three and five hours respectively) as that of the used reference standard indomethacin (48.88 and 76.55 % inhibition of edema at three and five hours respectively).

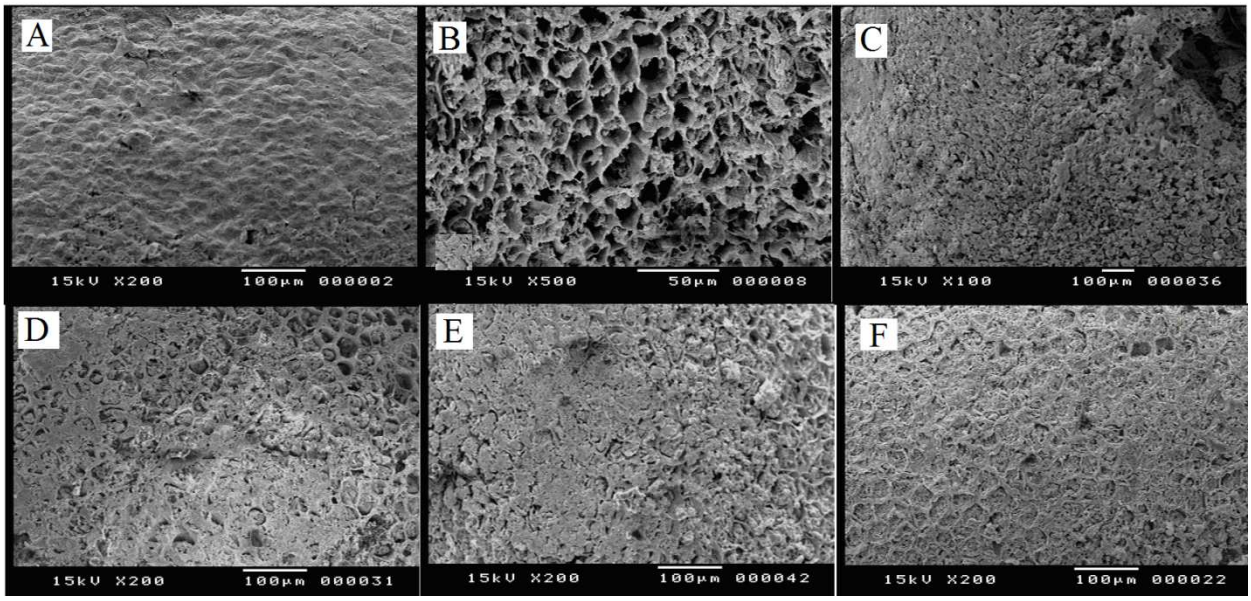


Figure 1: Representative micrographs for gastric mucosa of healthy and treated rats

A: Control Healthy stomach **B: Indomethacin 30 mg/kg** **C: Compound (3b) 30 mg/kg**
D: Compound (3e) 30 mg/kg **E: Compound (4e) 30 mg/kg** **F: Compound (5e) 30 mg/kg**

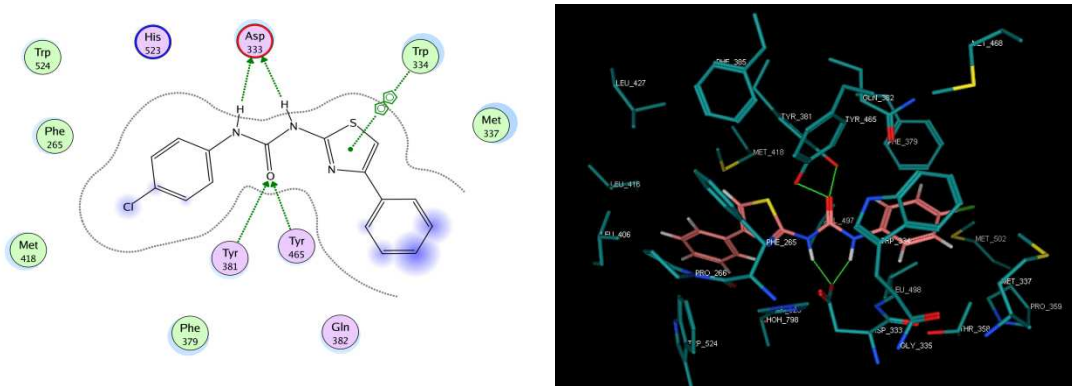


Figure 2: Docked complexes of compound 3b into human sEH

Ulcerogenic effect

Results of ulcerogenic effect revealed that compounds (**3e, 4e** and **5e**) showed superior GI safety profile, since they gave 70, 65, and 64 % protection respectively, compared to indomethacin, in the population of the test animals at oral doses 30 mg/kg. On the other hand, compound **3b** in the same concentration dose gave 57% protection when compared to indomethacin Table 4. Scanning electron microscopy of the control stomach of rat revealed normally, as the stomach lining is protected by a layer of mucus (Figure 1). The stomachs of rats treated with indomethacin showed marked signs of ulceration as abrasion and sloughing of the epithelium (cracked clay appearance) which is obviously reduced in compound **3b**. On the other hand, significant reduction in the gastric mucosal injury and valuable protection of submucosal cells of rats' stomach treated with compounds **3e**, **4e** and **5e** was observed. In this case, the stomach lining covered by a layer of mucus and photomicrographs revealed normal appearance of the rat stomach compared to control group (Figure 1).

Acute toxicity (LD_{50})

LD_{50} investigation results indicated that compounds **3b, 3e, 4e** and **5e** were less toxic and well tolerated by the experimental animals compared to indomethacin (LD_{50} of **3b**, 100 mg/kg; **3e**, **4e**, and **5e**, 120 mg/kg; while LD_{50} of indomethacin, 40 mg/kg).

Molecular Modeling Study

In order to rationalize the obtained anti-inflammatory results and to help in understanding the potential interactions between the ligand and enzyme active site, the synthesized disubstituted ureas have been manually docked into the active site of sEH. According to literature, the primary pharmacophore for sEH inihibitor is the urea group bearing a bulky and/or hydrophobic substituent such as cyclohexyl, alkyl or aryl groups [15,17,37,38]. For this, we used the published X-ray crystal structure of human sEH complexed with a urea-based ligand 1-cyclohexyl-3-(4-iodophenyl)urea, CIU (PDB accession number (1VJ5**)** [39] Molecular docking simulation of the prepared 1,3-disubstituted urea into sEH active site revealed several molecular interactions considered to be responsible for the observed affinity. The interaction between sEH and the prepared 1,3-disubstituted urea, as shown in Figure 2 and Figure 3, was observed in three categories. (i) The urea carbonyl group accepts hydrogen bonds from the phenolic Tyr465 and Tyr381. (ii) Carboxylate Asp333 accepts a hydrogen bond from the urea NH groups. (iii) The disubstituted moieties including 4-phenylthiazo-2-yl; (substituted) phenyl, phenethyl, and cyclohexyl adopt a different conformation with respect to the urea group. This reflects the large size of the hydrophobic pocket that can accommodate large substrate substituents and different conformations of such substituents in this region of the active site. These moieties bind in the hydrophobic channel, making van der Waals' forces with Trp334, Leu498, Met337, Val497, Trp524 and Gln382.

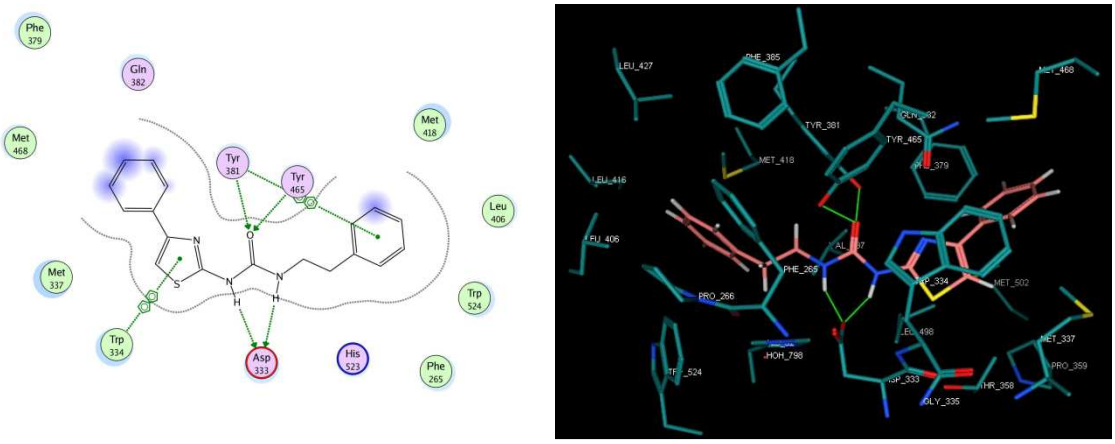


Figure 3: Docked complexes of compound 3e into human sEH

On studying the two plausible binding modes for **3b,** as illustrated in Figure 2, it was found that, urea carbonyl group that binds with phenolic hydrogen from Tyr465 and Tyr381; and carboxylate of Asp333 that binds with urea NH group resulted in steric clashes between the two aryl groups of the inhibitor and the residues of the binding site, such as Trp334, Leu498, Met337, Val497, Trp524 and Gln382. These results might explain the slightly increase in docking with compounds having an ethyl linker such **3e** (Figure 3) suggesting that these compounds, and presumably, other compounds in the same **4e**, and **5e** series could orient themselves to avoid an unfavorable interactions with the residues at the active site.

From the docking data, it was noticed that, the anti-inflammatory activities for all the synthesized 1,3-disubstitruted urea, matched with the docking data, since the S values (Table 3) in case of 3-[4-(substituted)phenylthiazo-2-yl]urea, increased in the order: Ph > 4-CH_3.Ph > 4-Cl Ph. But in the case of 1-substituted phenyl, phenethyl, or cyclohexyl urea, the S values were slightly increased in case of, phenethyl moieties than in substituted phenyl or cyclohexyl moieties.

CONCLUSION

As a part of our ongoing studies in developing new derivatives as dual antimicrobial/anti-inflammatory agents we have described the synthesis 1,3-disubstituted urea derivatives. The reactions of ethyl 4-(substituted)phenylthiazol-2-ylcarbamates with the appropriate amines in an aprotic solvent or a solvent free condition, provides a novel and convenient entry into the synthesis of the target compounds. The advantage of the later environmentally benign procedure is the high yields, short reaction time, and easy workup of the reaction. Compounds **3b**, **3f**, **4a**, **4b**, **4c**, **4e**, **5a**, **5c**, and **5f** exhibited superior antibacterial activity compared to reference, ampicillin. The evaluation of all target

compounds for their *in vivo* anti-inflammatory activities in rats gave significant results comparable to indomethacin. The gastric ulcerogenic effect of most potent anti-inflammatory active compounds (**3b, 3e, 4e,** and **5e**), were examined on gastric mucosa of albino rats and they showed superior GI safety profile compared with indomethacin. LD_{50} of **3b, 3e, 4e** and **5e** were determined in mice; they were less toxic and well tolerated orally up to 100-120 mg/kg. To understand the mode of binding interactions in silico, all target urea derivatives that having anti-inflammatory activity were docked into inhibitor binding cavity of human soluble epoxide hydrolase (sEH) receptor. The results signify that the molecular docking approach is reliable and produces a good correlation between docking score function and anti-inflammatory activity. In conclusion, the synthesized 1,3-disubstituted urea derivatives represent promising dual antibacterial /anti-inflammatory leads.

Acknowledgments
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