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Synthesis, Molecular Modeling Study and Anti-inflammatory Activity of Novel Benzimidazole Derivatives with Promising Cyclooxygenase Inhibitory Properties

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ABSTRACT

*A simple and efficient synthesis of a series of acetohydrazones (**5a-l, 6a-l, 7a-l**), 1,3-dioxoisoindolines (**8a-c**) derivatives and 1,2,4-triazole (**9**) have been attained starting from the key precursors acetohydrazides (**4a-c**). In addition, 1,2,4-triazole (**9**) have been employed for additional cyclization to afford [1,2,4]triazolo[3,4-b][1,3,4]thiadiazine derivatives (**10a-c**) as well as in synthesis of hydrazones (**11a-f**). Structures of the newly synthesized compounds have been confirmed by physical and spectral data (IR, ^{1}H-NMR, M.S and ^{13}C-NMR (APT)) in addition to elemental analysis. All the newly synthesized compounds were investigated for their in vivo anti-inflammatory activity using the carrageenan-induced rat paw edema model. Most of the newly synthesized compounds demonstrated exceptionally high in vivo anti-inflammatory activity as compared to INM. The in vitro inhibitory activity of the most active compounds towards ovine cyclooxygenase COX-1 and human recombinant COX-2 was also assessed. All the bioactive compounds showed high affinity and selectivity towards COX-2 isozyme compared to the reference drug diclofenac sodium with IC_{50} values ranging from 0.28-0.81 µmole versus 0.80 µmole respectively. Molecular docking study was done and revealed a relationship between docking affinity and biological results.*

Key words: benzimidazole, acetohydrazides, anti-inflammatory, COX enzyme, molecular docking.

INTRODUCTION

In the recent years, pain and inflammation are known as a great burden to the healthcare status of our population and the underlying basis of a significant number of diseases [1]. One of the therapeutic targets for the anti-inflammatory activity is lowering prostaglandin (PG) production through the inhibition of cyclooxygenase (COX) enzyme; a membrane-bound hemeprotein which exists in two distinct isoforms, a constitutive COX-1 and inducible COX-2 which are regulated differently. COXs catalyze the biological oxidation of arachidonic acid to prostaglandin and thromboxane. COX-1 is responsible for the cytoprotective prostaglandins production in the gastrointestinal tract and pro-aggregator thromboxane in blood platelets, whereas COX-2 is expressed in response to the pro-inflammatory mediators [2-4]. Therefore, the drugs that inhibit COX-2 enzyme selectively are better anti-inflammatory agents in terms of GI tolerability. Benzimidazoles have been investigated as modulators of pain and inflammation showing excellent therapeutic potency as anti-inflammatory agents [5]. Further, such nucleus was reported as a key pharmacophore for binding to and inhibiting human COX-1 as well as COX-2 [6]. One of the keys for developing COX-2 selective drugs is the larger active site of COX-2 which makes it possible for too large molecules to fit into COX-2 active site [7]. Additionally, there are moieties revealed enhanced anti-inflammatory activity relative to that of the parent compounds e.g., acetohydrazones [8, 9], heterocyclic rings such as 1,2,4-triazoles [10-15], 1,3-dioxoisoindolines [16-18], and 1,3,4-thiadiazines [19, 20].

Enlightened by the abovementioned studies, the present work aims for the synthesis of new 2-alkyl/ aralkyl substituted 2-mercapto-benzimidazoles that incorporate acetohydrazones; as compounds (**5a-l, 6a-l, 7a-l**) or heterocyclic moieties; as compounds (**8a-c**), (**9**), (**10a-c**) and (**11a-f**) , in order to evaluate their plausible pharmacophoric contribution of such moieties in the *in vivo* anti-inflammatory activity. Also, it was of interest to examine the inhibitory effects of the synthesized compounds on COX-1 and COX-2 enzymes and measure their selectivity towards COX-2 enzyme over COX-1, if any. Molecular docking study is performed to interpret and explain the possible mode of binding of the active compounds.

2.1. Chemistry

Melting points were determined on an electrothermal melting point apparatus [Stuart Scientific, model SMP3, UK], and were uncorrected. Pre-coated silica gel plates (kieselgel 0.25 mm, 60G F254, Merck, Germany) were used for TLC monitoring of the chemical reactions using chloroform:methanol (9.5:0.5 v/v) as mobile phase and spots were detected by using ultraviolet lamp at 254 nm wavelength (Spectroline, model CM-10, USA). IR spectra (KBr discs) were recorded on thermo scientific nicolet 6700 FT IR spectrometer (thermo Fischer scientific, USA) for compounds (**5a-j**, **6c-d**, **6f**, **6i**, **6k**, **7a-j**) except for compounds (**5k-l**, **6a-b**, **6e**, **6g-h**, **6j**, **6l**, **7k-l**, **8a-c**, **9**, **10a-c**, **11a-f**) that was done on thermo scientific nicolet IS10 FT IR spectrometer (thermo Fischer scientific, USA). Data acquisition was performed on Omnic software. ^{1}H-NMR Spectra were scanned on a Varian EM-360 L NMR spectrometer (60 MHz, Varian, CA, USA). Coupling constants (*J*) are in Hertz (Hz). Deuterium oxide was used for the detection of exchangeable protons. Chemical shifts are expressed in δ-values (ppm) relative to TMS as an internal standard, using the appropriate solvent as specified. Deuterium oxide was used for the detection of exchangeable protons. Attached proton test (APT) spectrum of compounds (**9**, **10c**) was performed on Brucker Avance III 400MHz (^{1}H-NMR 400 MHz, ^{13}C-NMR 100MHz) Switzerland. Mass spectra were recorded with a (Shimadzu, Kyoto, Japan) for compounds (**5f**, **8a, 9**) and on Direct Probe Controller Inlet Part TO Single Quadropole mass analyzer in Thermo Scientific GCMS model ISQ LT using Thermo X-Calibur software for compounds (**6b**, **7b, 10c, 11a, 11e**). Elemental microanalyses were performed on a Vario elemental analyzer III Vario, Germany) for compounds (**5a-e, 5g-l**, **6b, 6d-e, 6h-l**, **7a-l**, **8b**) except for compounds (**5f, 6a, 6c, 6f-g**, **8a**, **8c**, **9**, **10a-c**, **11a-f**) whose elemental microanalyses were performed on elemental analyzer model flash 2000 thermo fisher. Compounds 1H-benzo[d]imidazole-2(3H)-thione [21] (**1**), 2-(substituted thio)-1H-benzo[d]imidazole [22, 23, 24] (**2a-c**), methyl 2-(2-(substituted thio)-1H-benzo[d]imidazol-1-yl)acetate [25] (**3a-c**) and 2-(2-(methylthio)-1H-benzo[d]imidazol-1-yl)acetohydrazide [25] (**4a-c**) were prepared according to reported methods [21-25]; **Scheme 1**. The required phenacyl bromide derivatives were prepared according to reported procedure [26, 27].

2.1.1. N'-(4-(un)substituted (arylmethylidene or 1-arylethylidene)-2-(2-(substituted thio)-1H-benzo[d]imidazol-1-yl))acetohydrazide, **5a-l**, **6a-l**, **7a-l**

To a suspension of 2-(2-(substituted thio)-1H-benzo[d]imidazole-1-yl)acetohydrazide, compounds **(4a-c)** (0.005 mole) in absolute ethanol (15 ml) and the appropriate aryl aldehydes or acetophenone derivatives (0.005 mole), 2 drops of glacial acetic acid were added. The reaction mixture was refluxed for 4 h then left to cool. The precipitated product was filtered, dried and recrystallized from ethanol.

2.1.1.1. *N'-benzylidene-2-(2-(methylthio)-1H-benzo[d]imidazol-1-yl)acetohydrazide* ***5a***

White crystals, m.p. 214-6ºC, yield 70.5%, IR (KBr) ύ (cm^{-1}) 3189 (N-H); 1686 (C=O); 1614, 1478, 1450 (C=N & C=C); ^{1}H-NMR (60 MHz, $CDCl_3$): 2.83 (3H; s; $SC\underline{H}_3$), 5.40 (2H; s; $NC\underline{H}_2$), 7.10 - 8.10 (10H; m; $\underline{H}C$=N, $C_6\underline{H}_4$ & $C_6\underline{H}_5$), 10.83 (1H; br s; N$\underline{H}$). Anal. Calc. (%) for $C_{17}H_{16}N_4OS$: C, 62.94; H, 4.97; N, 17.27; Found: C, 63.20; H, 5.09; N, 16.99.

2.1.1.2. *N'-(4-chlorobenzylidene)-2-(2-(methylthio)-1H-benzo[d]imidazol-1-yl)acetohydrazide* ***5b***

White crystals, m.p. 248-50ºC, yield 86.5%, IR (KBr) ύ (cm^{-1}) 3181 (N-H); 1676 (C=O); 1615, 1595, 1488, 1478, 1454 (C=N & C=C); ^{1}H-NMR (60 MHz, $CDCl_3$/DMSO-d_6 3:1): 2.85 (3H; s; $SC\underline{H}_3$), 5.45 (2H; s; $NC\underline{H}_2$), 7.10 - 8.43 (9H; m; $\underline{H}C$=N, $C_6\underline{H}_4$ & $C_6\underline{H}_4$), 12.08 (1H; br s; N$\underline{H}$). Anal. Calc. (%) for $C_{17}H_{15}ClN_4OS$: C, 56.90; H, 4.21; N, 15.61; Found: C, 56.80; H, 4.23; N, 15.60.

2.1.1.3. *N'-(4-methylbenzylidene)-2-(2-(methylthio)-1H-benzo[d]imidazol-1-yl)acetohydrazide* ***5c***

White crystals, m.p. 235-237ºC, yield 77.1%, IR (KBr) ύ (cm^{-1}) 3186 (N-H); 1678 (C=O); 1616, 1454 (C=N & C=C); ^{1}H-NMR (60 MHz, $CDCl_3$/DMSO-d_6 3:1): 2.45 (3H; s; $C\underline{H}_3C_6H_4$), 2.81 (3H; s; $SC\underline{H}_3$), 5.41 (2H; s; $NC\underline{H}_2$), 7.06 - 8.36 (9H; m; $\underline{H}C$=N, $C_6\underline{H}_4$ & $C_6\underline{H}_4$), 11.86 (1H; br s; N$\underline{H}$). Anal. Calc. (%) for $C_{18}H_{18}N_4OS$: C, 63.88; H, 5.36; N, 16.56; Found: C, 64.00; H, 5.17; N, 16.34.

2.1.1.4. *N'-(4-methoxybenzylidene)-2-(2-(methylthio)-1H-benzo[d]imidazol-1-yl)acetohydrazide* ***5d***

White crystals, m.p. 215-216ºC, yield 67.0%, IR (KBr) ύ (cm^{-1}) 3191 (N-H); 1678 (C=O); 1603, 1574, 1518, 1478, 1449 (C=N & C=C); 1251, 1027 (C-O); ^{1}H-NMR (60 MHz, $CDCl_3$/DMSO-d_6 3:1): 2.86 (3H; s; $SC\underline{H}_3$), 3.93 (3H; s;

OC$\underline{H}_3$), 5.38 (2H; s; NC$\underline{H}_2$), 6.76 - 8.30 (9H; m; $\underline{H}$C=N, C$_6\underline{H}_4$ & C$_6\underline{H}_4$), 11.71 (1H; br s; N$\underline{H}$). Anal. Calc. (%) for $C_{18}H_{18}N_4O_2S$: C, 61.00; H, 5.12; N, 15.81; Found: C, 61.04; H, 5.35; N, 16.06.

2.1.1.5. *N'-(4-methoxybenzylidene)-2-(2-(methylthio)-1H-benzo[d]imidazol-1-yl)acetohydrazide* ***5e***
Yellow crystals, m.p. 236-238ºC, yield 41.1%, IR (KBr) ύ (cm^{-1}) 3180 (N-H); 1681 (C=O); 1615, 1587, 1522, 1478, 1451 (C=N & C=C); 1552, 1341 (NO_2); ^{1}H-NMR (60 MHz, $CDCl_3$/DMSO-d_6 3:1): 2.81 (3H; s; SC$\underline{H}_3$), 5.38 (2H; s; NC$\underline{H}_2$), 7.00 - 8.46 (9H; m; $\underline{H}$C=N, C$_6\underline{H}_4$ & C$_6\underline{H}_4$), 12.21 (1H; br s; N$\underline{H}$). Anal. Calc. (%) for $C_{18}H_{18}N_4O_2S$: C, 55.27; H, 4.09; N, 18.96; Found: C, 55.40; H, 4.07; N, 19.19.

2.1.1.6. *N'-(4-hydroxybenzylidene)-2-(2-(methylthio)-1H-benzo[d]imidazol-1-yl)acetohydrazide* ***5f***
White crystals, m.p. 238-240ºC, yield 61.0%, IR (KBr) ύ (cm^{-1}) 3445 (OH); 3180 (N-H); 1682 (C=O); 1605, 1583, 1466, 1440 (C=N & C=C); 1241 (C-O); ^{1}H-NMR (60 MHz, $CDCl_3$/DMSO-d_6 3:2): 2.76 (3H; s; SC$\underline{H}_3$), 5.33 (2H; s; NC$\underline{H}_2$), 6.66 - 8.23 (9H; m; $\underline{H}$C=N, C$_6\underline{H}_4$ & C$_6\underline{H}_4$), 9.61 (1H; br s; O$\underline{H}$), 11.36 (1H; br s; N$\underline{H}$). EI-MS: *m/z*: 342.00 ($M^{\cdot+}$+2, 8.84), 341.00 ($M^{\cdot+}$+1, 9.56), 340.00 ($M^{\cdot+}$, 21.79), 77.00 (100.00). Anal. Calc. (%) for $C_{17}H_{16}N_4O_2S$: C, 59.98; H, 4.74; N, 16.46; Found: C, 60.14; H, 4.74; N, 16.29.

2.1.1.7. *2-(2-(methylthio)-1H-benzo[d]imidazol-1-yl)-N'-(1-phenylethylidene)acetohydrazide* ***5g***
White crystals, m.p. 198-199 ºC, yield 60.2%, IR (KBr) ύ (cm^{-1}) 3185 (N-H); 1683 (C=O); 1557, 1505, 1446 (C=N & C=C); ^{1}H-NMR (60 MHz, $CDCl_3$): 2.05 (3H; s; N=CC$\underline{H}_3$), 2.85 (3H; s; SC$\underline{H}_3$), 5.40 (2H; s; NC$\underline{H}_2$), 7.20 - 8.06 (9H; m; C$_6\underline{H}_4$ & C$_6\underline{H}_5$), 10.15 (1H; br s; N$\underline{H}$). Anal. Calc. (%) for $C_{18}H_{18}N_4OS$: C, 63.88; H, 5.36; N, 16.56; Found: C, 63.86; H, 5.36; N, 16.60.

2.1.1.8. *N'-(1-(4-chlorophenyl)ethylidene)-2-(2-(methylthio)-1H-benzo[d]imidazol-1-yl) aceto-hydrazide* ***5h***
White crystals, m.p. 233-235 ºC, yield 82.0%, IR (KBr) ύ (cm^{-1}) 3185 (N-H); 1682 (C=O); 1613, 1446 (C=N & C=C); ^{1}H-NMR (60 MHz, $CDCl_3$): 1.98 (3H; s; N=CC$\underline{H}_3$), 2.83 (3H; s; SC$\underline{H}_3$), 5.38 (2H; s; NC$\underline{H}_2$), 7.03 - 8.00 (8H; m; C$_6\underline{H}_4$ & C$_6\underline{H}_4$), 10.16 (1H; br s; N$\underline{H}$). Anal. Calc. (%) for $C_{18}H_{17}ClN_4OS$: C, 57.98; H, 4.60; N, 15.03; Found: C, 58.01; H, 4.50; N, 14.85.

2.1.1.9. *2-(2-(methylthio)-1H-benzo[d]imidazol-1-yl)-N'-(1-p-tolylethylidene)acetohydrazide* ***5i***
White crystals, m.p. 216-218 ºC, yield 40.1%, IR (KBr) ύ (cm^{-1}) 3184 (N-H); 1683 (C=O); 1616, 1477, 1446 (C=N & C=C); ^{1}H-NMR (60 MHz, $CDCl_3$): 2.00 (3H; s; N=CC$\underline{H}_3$), 2.43 (3H; s; C$\underline{H}_3$C$_6$H$_4$), 2.83 (3H; s; SC$\underline{H}_3$), 5.38 (2H; s; NC$\underline{H}_2$), 7.00 - 8.00 (8H; m; C$_6\underline{H}_4$ & C$_6\underline{H}_4$), 10.16 (1H; br s; N$\underline{H}$). Anal. Calc. (%) for $C_{19}H_{20}N_4OS$: C, 64.75; H, 5.72; N, 15.90; Found: C, 64.58; H, 5.74; N, 15.82.

2.1.1.10. *N'-(1-(4-methoxyphenyl)ethylidene)-2-(2-(methylthio)-1H-benzo[d]imidazol-1-yl) aceto-hydrazide* ***5j***
White crystals, m.p. 200-202 ºC, yield 83.5%, IR (KBr) ύ (cm^{-1}) 3188 (N-H); 1682 (C=O); 1605, 1572, 1511, 1477, 1448 (C=N & C=C); 1249, 1230 (C-O); ^{1}H-NMR (60 MHz, $CDCl_3$/DMSO-d_6 3:1): 2.35 (3H; s; N=CC$\underline{H}_3$), 2.86 (3H; s; SC$\underline{H}_3$), 3.91 (3H; s; OC$\underline{H}_3$), 5.43 (2H; s; NC$\underline{H}_2$), 6.76 - 8.13 (8H; m; C$_6\underline{H}_4$ & C$_6\underline{H}_4$), 10.88 (1H; br s; N$\underline{H}$). Anal. Calc. (%) for $C_{19}H_{20}N_4O_2S$: C, 61.94; H, 5.47; N, 15.21; Found: C, 61.64; H, 5.83; N, 15.33.

2.1.1.11. *2-(2-(methylthio)-1H-benzo[d]imidazol-1-yl)-N'-(1-(4-nitrophenyl)ethylidene)aceto-hydrazide* ***5k***
Yellow crystals, m.p. 239-241 ºC, yield 77.5%, IR (KBr) ύ (cm^{-1}) 3210 (N-H); 1684 (C=O); 1612, 1596, 1580, 1516, 1478, 1444 (C=N & C=C); 1516, 1344 (NO_2); ^{1}H-NMR (60 MHz, $CDCl_3$/DMSO-d_6 4:1): 2.40 (3H; s; N=CC$\underline{H}_3$), 2.81 (3H; s; SC$\underline{H}_3$), 5.41 (2H; s; NC$\underline{H}_2$), 7.05 - 8.46 (8H; m; C$_6\underline{H}_4$ & C$_6\underline{H}_4$), 11.03 (1H; br s; N$\underline{H}$). Anal. Calc. (%) for $C_{18}H_{17}N_5O_3S$: C, 56.38; H, 4.47; N, 18.27; Found: C, 56.63; H, 4.37; N, 18.36.

2.1.1.12. *N'-(1-(4-hydroxyphenyl)ethylidene)-2-(2-(methylthio)-1H-benzo[d]imidazol-1-yl)aceto-hydrazide* ***5l***
White crystals, m.p. 238-239 ºC, yield 54.0%, IR (KBr) ύ (cm^{-1}) 3445 (OH); 3206 (N-H); 1681 (C=O); 1607, 1580, 1515, 1445 (C=N & C=C); 1242 (C-O); ^{1}H-NMR (60 MHz, $CDCl_3$/DMSO-d_6 4:1): 2.26 (3H; s; N=CC$\underline{H}_3$), 2.76 (3H; s; SC$\underline{H}_3$), 5.33 (2H; s; NC$\underline{H}_2$), 6.63 - 8.06 (8H; m; C$_6\underline{H}_4$ & C$_6\underline{H}_4$), 9.53 (1H; br s; O$\underline{H}$), 10.80 (1H; br s; N$\underline{H}$). Anal. Calc. (%) for $C_{18}H_{18}N_4O_2S$: C, 61.00; H, 5.12; N, 15.81; Found: C, 60.97; H, 5.11; N, 15.87.

2.1.1.13. *N'-benzylidene-2-(2-(isopropylthio)-1H-benzo[d]imidazol-1-yl)acetohydrazide* ***6a***
White crystals, m.p. 111-113 ºC, yield 60.0%, IR (KBr) ύ (cm^{-1}) 3204 (N-H); 1689 (C=O); 1611, 1573, 1480, 1462, 1446 (C=N & C=C); ^{1}H-NMR (60 MHz, $CDCl_3$/DMSO-d_6 4:1): 1.43 (6H; d; J = 7.2 Hz, CH(C$\underline{H}_3$)$_2$), 3.76 - 4.25 (1H; m; C$\underline{H}$(CH$_3$)$_2$), 5.33 (2H; s; NC$\underline{H}_2$), 6.90 - 8.26 (9H; m; C$_6\underline{H}_4$ & C$_6\underline{H}_5$) 8.00 (1H; s; $\underline{H}$C=N) and 11.85 (1H; br s; N$\underline{H}$). Anal. Calc. (%) for $C_{19}H_{20}N_4OS$: C, 64.75; H, 5.72; N, 15.90; Found: C, 64.87; H, 5.72; N, 15.70.

2.1.1.14. *N'-(4-chlorobenzylidene)-2-(2-(isopropylthio)-1H-benzo[d]imidazol-1-yl)acetohydrazide* ***6b***
White crystals, m.p. 229-231 ºC, yield 89.2%, IR (KBr) ύ (cm^{-1}) 3196 (N-H); 1696 (C=O); 1611, 1596, 1480, 1461 (C=N & C=C); ^{1}H-NMR (60 MHz, $CDCl_3$): 1.39 (6H; d; *J* = 7.8 Hz, CH(C$\underline{H}_3$)$_2$), 3.81 - 4.33 (1H; m; C$\underline{H}$(CH$_3$)$_2$), 5.35 (2H; s; NC$\underline{H}_2$), 7.13 - 7.96 (9H; m; C$_6\underline{H}_4$, C$_6\underline{H}_4$ & $\underline{H}$C=N) and 10.90 (1H; br s; N$\underline{H}$). EI-MS: *m/z*: 388.16 (M^++2, 9.75), 387.17 (M^++1, 9.19), 386.14 (M^+, 23.54), 163.08 (100.00). Anal. Calc. (%) for $C_{19}H_{19}ClN_4OS$: C, 58.98; H, 4.95; N, 14.48; Found: C, 58.71; H, 4.74; N, 14.81.

2.1.1.15. *2-(2-(isopropylthio)-1H-benzo[d]imidazol-1-yl)-N'-(4-methylbenzylidene)acetohydrazide* ***6c***
White crystals, m.p. 155-157 ºC, yield 89.5%, IR (KBr) ύ (cm^{-1}) 3185 (N-H); 1682 (C=O); 1603, 1470, 1463, 1447 (C=N & C=C); ^{1}H-NMR (60 MHz, $CDCl_3$): 1.40 (6H; d; *J* = 6.6 Hz, CH(C$\underline{H}_3$)$_2$), 2.36 (3H; s; *p*-C$\underline{H}_3$C$_6$H$_4$), 3.78 - 4.30 (1H; m; C$\underline{H}$(CH$_3$)$_2$), 5.33 (2H; s; NC$\underline{H}_2$), 6.96 - 8.00 (9H; m; C$_6\underline{H}_4$, C$_6\underline{H}_4$ & $\underline{H}$C=N) and 10.81 (1H; br s; N$\underline{H}$). Anal. Calc. (%) for $C_{20}H_{22}N_4OS$: C, 65.55; H, 6.05; N, 15.29; Found: C, 65.79; H, 6.12; N, 15.43.

2.1.1.16. *2-(2-(isopropylthio)-1H-benzo[d]imidazol-1-yl)-N'-(4-methoxybenzylidene)acetohydrazide* ***6d***
White crystals, m.p. 196-197 ºC, yield 88.5%, IR (KBr) ύ (cm^{-1}) 3190 (N-H); 1681 (C=O); 1600, 1570, 1510, 1485, 1460, 1446 (C=N & C=C); 1249, 1030 (C-O) ^{1}H-NMR (60 MHz, $CDCl_3$): 1.46 (6H; d; *J* = 7.8 Hz, CH(C$\underline{H}_3$)$_2$), 3.90 (3H; s; OC$\underline{H}_3$), 3.76 - 4.40 (1H; m; C$\underline{H}$(CH$_3$)$_2$), 5.40 (2H; s; NC$\underline{H}_2$), 6.80 - 8.03 (9H; m; C$_6\underline{H}_4$, C$_6\underline{H}_4$ & $\underline{H}$C=N) and 10.98 (1H; br s; N$\underline{H}$). Anal. Calc. (%) for $C_{20}H_{22}N_4O_2S$: C, 62.80; H, 5.80; N, 14.65; Found: C, 62.90; H, 5.60; N, 14.80.

2.1.1.17. *2-(2-(isopropylthio)-1H-benzo[d]imidazol-1-yl)-N'-(4-nitrobenzylidene)acetohydrazide* ***6e***
Yellow crystals, m.p. 254-255 ºC, yield 94.5%, IR (KBr) ύ (cm^{-1}) 3196 (N-H); 1694 (C=O); 1586, 1480, 1450 (C=N & C=C); 1515, 1336 (NO_2); ^{1}H-NMR (60 MHz, $CDCl_3$/DMSO-d_6 1:1): 1.46 (6H; d; *J* = 7.8 Hz, CH(C$\underline{H}_3$)$_2$), 3.80 - 4.31 (1H; m; C$\underline{H}$(CH$_3$)$_2$), 5.41 (2H; s; NC$\underline{H}_2$), 7.01 - 8.56 (9H; m; $\underline{H}$C=N, C$_6\underline{H}_4$ & C$_6\underline{H}_4$), 12.21 (1H; br s; N$\underline{H}$). Anal. Calc. (%) for $C_{19}H_{19}N_5O_3S$: C, 57.42; H, 4.82; N, 17.62; Found: C, 57.09; H, 5.11; N, 17.72.

2.1.1.18. *N'-(4-hydroxybenzylidene)-2-(2-(isopropylthio)-1H-benzo[d]imidazol-1-yl)acetohydrazide* ***6f***
White crystals, m.p. 207-208 ºC, yield 79.1%, IR (KBr) ύ (cm^{-1}) 3411 (OH); 3209 (N-H); 1674 (C=O); 1580, 1515, 1464, 1433 (C=N & C=C); 1241 (C-O); ^{1}H-NMR (60 MHz, $CDCl_3$/DMSO-d_6 3:1): 1.41 (6H; d; *J* = 6.0 Hz, CH(C$\underline{H}_3$)$_2$), 3.63 - 4.30 (1H; m; C$\underline{H}$(CH$_3$)$_2$), 5.35 (2H; s; NC$\underline{H}_2$), 6.66 - 8.30 (10H; m; C$_6\underline{H}_4$, C$_6\underline{H}_5$ & $\underline{H}$C=N), 9.23 - 9.83 (1H; br s; OH), 11.40 (1H; br s; N$\underline{H}$). Anal. Calc. (%) for $C_{19}H_{20}N_4O_2S$: C, 61.94; H, 5.47; N, 15.21; Found: C, 62.18; H, 5.55; N, 15.09.

2.1.1.19. *2-(2-(isopropylthio)-1H-benzo[d]imidazol-1-yl)-N'-(1-phenylethylidene)acetohydrazide* ***6g***
White crystals, m.p. 166-168 ºC, yield 65.0%, IR (KBr) ύ (cm^{-1}) 3192 (N-H); 1686 (C=O); 1479, 1463, 1434 (C=N & C=C); ^{1}H-NMR (60 MHz, $CDCl_3$): 1.43 (6H; d; *J* = 7.2 Hz, CH(C$\underline{H}_3$)$_2$), 1.96 (3H; s; N=CC$\underline{H}_3$), 3.86 - 4.26 (1H; m; C$\underline{H}$(CH$_3$)$_2$), 5.30 (2H; s; NC$\underline{H}_2$), 7.06 - 7.80 (9H; m; C$_6\underline{H}_4$ & C$_6\underline{H}_5$) and 10.08 (1H; br s; N$\underline{H}$). Anal. Calc. (%) for $C_{20}H_{22}N_4OS$: C, 65.55; H, 6.05; N, 15.29; Found: C, 65.81; H, 6.25; N, 14.99.

2.1.1.20. *N'-(1-(4-chlorophenyl)ethylidene)-2-(2-(isopropylthio)-1H-benzo[d]imidazol-1-yl)aceto-hydrazide* ***6h***
White crystals, m.p. 223-225 ºC, yield 79.3%, IR (KBr) ύ (cm^{-1}) 3191 (N-H); 1685 (C=O); 1611, 1462, 1445 (C=N & C=C); ^{1}H-NMR (60 MHz, $CDCl_3$): 1.39 (6H; d; *J* = 7.2 Hz, CH(C$\underline{H}_3$)$_2$), 1.86 (3H; s; N=CC$\underline{H}_3$), 3.66 - 4.33 (1H; m; C$\underline{H}$(CH$_3$)$_2$), 5.35 (2H; s; NC$\underline{H}_2$), 7.06 - 7.96 (8H; m; C$_6\underline{H}_4$ & C$_6\underline{H}_4$) and 10.53 (1H; br s; N$\underline{H}$). Anal. Calc. (%) for $C_{20}H_{21}ClN_4OS$: C, 59.91; H, 5.28; N, 13.97; Found: C, 59.61; H, 5.40; N, 13.95.

2.1.1.21. *2-(2-(isopropylthio)-1H-benzo[d]imidazol-1-yl)-N'-(1-p-tolylethylidene)acetohydrazide* ***6i***
White crystals, m.p. 218-220 ºC, yield 80.1%, IR (KBr) ύ (cm^{-1}) 3184 (N-H); 1682 (C=O); 1615, 1514, 1461, 1446 (C=N & C=C); ^{1}H-NMR (60 MHz, $CDCl_3$): 1.45 (6H; d; *J* = 6.0 Hz, CH(C$\underline{H}_3$)$_2$), 1.96 (3H; s; N=CC$\underline{H}_3$), 2.40 (3H, s, C$\underline{H}_3$C$_6$H$_4$), 3.83 - 4.36 (1H; m; C$\underline{H}$(CH$_3$)$_2$), 5.40 (2H; s; NC$\underline{H}_2$), 6.90 - 7.96 (8H; m; C$_6\underline{H}_4$ & C$_6\underline{H}_4$) and 10.23 (1H; br s; N$\underline{H}$). Anal. Calc. (%) for $C_{21}H_{24}N_4OS$: C, 66.29; H, 6.36; N, 14.72; Found: C, 66.10; H, 6.43; N, 14.72.

2.1.1.22. *2-(2-(isopropylthio)-1H-benzo[d]imidazol-1-yl)-N'-(1-(4-methoxyphenyl)ethylidene)aceto-hydrazide* ***6j***
White crystals, m.p. 204-206 ºC, yield 78.5%, IR (KBr) ύ (cm^{-1}) 3185 (N-H); 1682 (C=O); 1609, 1514, 1447 (C=N & C=C); 1252, 1031 (C-O); ^{1}H-NMR (60 MHz, $CDCl_3$): 1.46 (6H; d; *J* = 7.8 Hz, CH(C$\underline{H}_3$)$_2$), 1.93 (3H; s; N=CC$\underline{H}_3$), 3.66 - 4.36 (1H; m; C$\underline{H}$(CH$_3$)$_2$), 3.83 (3H; s; OC$\underline{H}_3$), 5.40 (2H; s; NC$\underline{H}_2$), 6.71 - 7.93 (8H; m; C$_6\underline{H}_4$ & C$_6\underline{H}_4$) and 10.23 (1H; br s; N$\underline{H}$). Anal. Calc. (%) for $C_{21}H_{24}N_4O_2S$: C, 63.61; H, 6.10; N, 14.13; Found: C, 63.52; H, 6.17; N, 14.13.

2.1.1.23. *2-(2-(isopropylthio)-1H-benzo[d]imidazol-1-yl)-N'-(1-(4-nitrophenyl)ethylidene)aceto-hydrazide* ***6k***
Yellow crystals, m.p. 216-218 ºC, yield 83.5%, IR (KBr) ύ (cm^{-1}) 3193 (N-H); 1690 (C=O); 1597, 1586, 1479, 1438 (C=N & C=C); 1518, 1342 (NO_2); ^{1}H-NMR (60 MHz, $CDCl_3$): 1.45 (6H; d; *J* = 6.6 Hz, CH($C\underline{H}_3$)$_2$), 2.00 (3H; s; N=CC$\underline{H}_3$), 3.76 - 4.38 (1H; m; C$\underline{H}$(CH_3)$_2$), 5.43 (2H; s; NC$\underline{H}_2$), 7.05 - 8.45 (8H; m; $C_6\underline{H}_4$ & $C_6\underline{H}_4$) and 10.66 (1H; br s; N$\underline{H}$). Anal. Calc. (%) for $C_{20}H_{21}N_5O_3S$: C, 58.38; H, 5.14; N, 17.02; Found: C, 58.11; H, 5.36; N, 17.13.

2.1.1.24. *N'-(1-(4-hydroxyphenyl)ethylidene)-2-(2-(isopropylthio)-1H-benzo[d]imidazol-1-yl)aceto-hydrazide* ***6l***
White crystals, m.p. 226-228 ºC, yield 72.0%, IR (KBr) ύ (cm^{-1}) 3424 (OH); 3204 (N-H); 1683 (C=O); 1606, 1516, 1435 (C=N & C=C); 1240 (C-O); ^{1}H-NMR (60 MHz, $CDCl_3$/DMSO-d_6 4:1): 1.49 (6H; d; *J* = 7.8 Hz, C($C\underline{H}_3$)$_2$), 2.30 (3H; s; N=CC$\underline{H}_3$), 3.73 - 4.40 (1H; m; C$\underline{H}$(CH_3)$_2$), 5.43 (2H; s; NC$\underline{H}_2$), 6.73 - 7.93 (8H; m; $C_6\underline{H}_4$ & $C_6\underline{H}_4$), 8.60 - 9.86 (1H, br s; O$\underline{H}$), 10.70 (1H; br s; N$\underline{H}$). Anal. Calc. (%) for $C_{20}H_{22}N_4O_2S$: C, 62.80; H, 5.80; N, 14.65; Found: C, 62.97; H, 5.92; N, 14.68.

2.1.1.25. *N'-benzylidene-2-(2-(benzylthio)-1H-benzo[d]imidazol-1-yl)acetohydrazide* ***7a***
White crystals, m.p. 221-223ºC, yield 66.1%, IR (KBr) ύ (cm^{-1}) 3206 (N-H); 1697 (C=O); 1608, 1495, 1478, 1463 (C=N & C=C); ^{1}H-NMR (60 MHz, $CDCl_3$/DMSO-d_6 3:1): 4.61 (2H; s; C$\underline{H}_2C_6H_5$), 5.28 (2H; s; NC$\underline{H}_2$), 6.85 – 7.88 (14H; m; $C_6\underline{H}_4$, $C_6\underline{H}_5$ & $C_6\underline{H}_5$), 8.05 (1H; s; $\underline{H}$C=N), 11.48 (1H; br s; N$\underline{H}$). Anal. Calc. (%) for $C_{23}H_{20}N_4OS$: C, 68.98; H, 5.03; N, 13.99; Found: C, 69.26; H, 5.24; N, 14.14.

2.1.1.26. *2-(2-(benzylthio)-1H-benzo[d]imidazol-1-yl)-N'-(4-chlorobenzylidene)acetohydrazide* ***7b***
White crystals, m.p. 237-239ºC, yield 54.1%, IR (KBr) ύ (cm^{-1}) 3190 (N-H); 1697 (C=O); 1602, 1488, 1465, 1439 (C=N & C=C); ^{1}H-NMR (60 MHz, $CDCl_3$/DMSO-d_6 3:1): 4.61 (2H; s; C$\underline{H}_2C_6H_5$), 5.31 (2H; s; NC$\underline{H}_2$), 6.96 - 8.43 (13H; m; $C_6\underline{H}_5$, $C_6\underline{H}_4$ & $C_6\underline{H}_4$), 8.10 (1H; s; $\underline{H}$C=N), 12.06 (1H; br s; N$\underline{H}$). EI-MS: *m/z*: 434.23 ($M^{\cdot+}$, 0.98), 91.11 (100.00). Anal. Calc. (%) for $C_{23}H_{19}ClN_4OS$: C, 63.51; H, 4.40; N, 12.88; Found: C, 63.84; H, 4.33; N, 12.56.

2.1.1.27. *2-(2-(benzylthio)-1H-benzo[d]imidazol-1-yl)-N'-(4-methylbenzylidene)acetohydrazide* ***7c***
White crystals, m.p. 217-219ºC, yield 80.0%, IR (KBr) ύ (cm^{-1}) 3203 (N-H); 1697 (C=O); 1610, 1495, 1479, 1464, 1441 (C=N & C=C); ^{1}H-NMR (60 MHz, $CDCl_3$/DMSO-d_6 3:1): 2.53 (3H; s; *p*-C$\underline{H}_3C_6H_4$), 4.68 (2H; s; C$\underline{H}_2C_6H_5$), 5.35 (2H; s; NC$\underline{H}_2$), 7.00 - 8.33 (13H; m; $C_6\underline{H}_5$, $C_6\underline{H}_4$ & $C_6\underline{H}_4$), 8.06 (1H; s; $\underline{H}$C=N), 11.86 (1H; br s; N$\underline{H}$). Anal. Calc. (%) for $C_{24}H_{22}N_4OS$: C, 69.54; H, 5.35; N, 13.52; Found: C, 69.19; H, 4.98; N, 13.67.

2.1.1.28. *2-(2-(benzylthio)-1H-benzo[d]imidazol-1-yl)-N'-(4-methoxybenzylidene)acetohydrazide* ***7d***
White crystals, m.p. 214-215 ºC, yield 85.5%, IR (KBr) ύ (cm^{-1}) 3190 (N-H); 1694 (C=O); 1518, 1504, 1481, 1466, 1454 (C=N & C=C); 1253, 1031 (C-O); ^{1}H-NMR (60 MHz, $CDCl_3$/DMSO-d_6 1:1): 3.88 (3H; s; OCH_3), 4.61 (2H; s; C$\underline{H}_2C_6H_5$), 5.26 (2H; s; NC$\underline{H}_2$), 6.80 - 8.10 (13H; m; $C_6\underline{H}_5$, $C_6\underline{H}_4$ & $C_6\underline{H}_4$), 7.98 (1H; s; $\underline{H}$C=N), 11.60 (1H; br s; N$\underline{H}$). Anal. Calc. (%) for $C_{24}H_{22}N_4O_2S$: C, 66.96; H, 5.15; N, 13.01; Found: C, 67.08; H, 5.05; N, 13.22.

2.1.1.29. *2-(2-(benzylthio)-1H-benzo[d]imidazol-1-yl)-N'-(4-nitrobenzylidene)acetohydrazide* ***7e***
Yellow crystals, m.p. 234-236 ºC, yield 91.3%, IR (KBr) ύ (cm^{-1}) 3216 (N-H); 1698 (C=O); 1612, 1585, 1495, 1478, 1463, 1441 (C=N & C=C); 1517, 1342 (NO_2); ^{1}H-NMR (60 MHz, $CDCl_3$/DMSO-d_6 1:1): 4.58 (2H; s; C$\underline{H}_2C_6H_5$), 5.35 (2H; s; NC$\underline{H}_2$), 6.96 - 8.63 (14H; m; $\underline{H}$C=N, $C_6\underline{H}_5$, $C_6\underline{H}_4$ & $C_6\underline{H}_4$), 12.10 (1H; br s; N$\underline{H}$). Anal. Calc. (%) for $C_{23}H_{19}N_5O_3S$: C, 62.01; H, 4.30; N, 15.72; Found: C, 62.08; H, 4.22; N, 15.98.

2.1.1.30. *2-(2-(benzylthio)-1H-benzo[d]imidazol-1-yl)-N'-(4-hydroxybenzylidene)acetohydrazide* ***7f***
White crystals, m.p. 144-146 ºC, yield 70.2%, IR (KBr) ύ (cm^{-1}) 3486 (OH); 3209 (N-H); 1675 (C=O); 1604, 1513, 1494, 1480, 1463, 1441 (C=N & C=C); 1241 (C-O); ^{1}H-NMR (60 MHz, $CDCl_3$/DMSO-d_6 4:1): 2.93 - 4.03 (1H; br s; OH), 4.61 (2H; s; C$\underline{H}_2C_6H_5$), 5.28 (2H; s; NC$\underline{H}_2$), 6.66 - 8.25 (14H; m; $\underline{H}$C=N, $C_6\underline{H}_4$, $C_6\underline{H}_4$ & $C_6\underline{H}_5$), 11.46 (1H; br s; N$\underline{H}$). Anal. Calc. (%) for $C_{23}H_{20}N_4O_2S$: C, 66.33; H, 4.84; N, 13.45; Found: C, 66.04; H, 5.10; N, 13.22.

2.1.1.31. *2-(2-(benzylthio)-1H-benzo[d]imidazol-1-yl)-N'-(1-phenylethylidene)acetohydrazide* ***7g***
White crystals, m.p. 189-191 ºC, yield 86.4%, IR (KBr) ύ (cm^{-1}) 3182 (N-H); 1682 (C=O); 1614, 1597, 1461, 1439 (C=N & C=C); ^{1}H-NMR (60 MHz, $CDCl_3$): 1.96 (3H; s; N=CC$\underline{H}_3$), 4.55 (2H; s; C$\underline{H}_2C_6H_5$), 5.25 (2H; s; NC$\underline{H}_2$), 7.00 - 8.16 (14H; m; $C_6\underline{H}_4$, $C_6\underline{H}_5$ & $C_6\underline{H}_5$), 9.95 (1H; br s; N$\underline{H}$). Anal. Calc. (%) for $C_{24}H_{22}N_4OS$: C, 69.54; H, 5.35; N, 13.52; Found: C, 69.70; H, 5.22; N, 13.48.

2.1.1.32. *2-(2-(benzylthio)-1H-benzo[d]imidazol-1-yl)-N'-(1-(4-chlorophenyl)ethylidene)aceto-hydrazide* ***7h***
White crystals, m.p. 159-161 ºC, yield 83.0%, IR (KBr) ύ (cm^{-1}) 3184 (N-H); 1687 (C=O); 1606, 1557, 1539, 1493, 1462 (C=N & C=C); ^{1}H-NMR (60 MHz, $CDCl_3$): 1.91(3H; s; N=CC$\underline{H}_3$), 4.56 (2H; s; C$\underline{H}_2C_6H_5$), 5.21(2H; s; NC$\underline{H}_2$), 7.05 - 8.13 (13H; m; $C_6\underline{H}_5$, $C_6\underline{H}_4$ & $C_6\underline{H}_4$), 10.11 (1H; br s; N$\underline{H}$). Anal. Calc. (%) for $C_{24}H_{21}ClN_4OS$: C, 64.20; H, 4.71; N, 12.48; Found: C, 64.00; H, 4.49; N, 12.57.

2.1.1.33. *2-(2-(benzylthio)-1H-benzo[d]imidazol-1-yl)-N'-(1-p-tolylethylidene)acetohydrazide* ***7i***
White crystals, m.p. 181-183 ºC, yield 83.1%, IR (KBr) ύ (cm^{-1}) 3186 (N-H); 1682 (C=O); 1614, 1513, 1494, 1478, 1462, 1442 (C=N & C=C); ^{1}H-NMR (60 MHz, $CDCl_3$): 2.05 (3H; s; N=CC$\underline{H}_3$), 2.46 (3H; s; C$\underline{H}_3C_6H_4$), 4.63 (2H; s; C$\underline{H}_2C_6H_5$), 5.31(2H; s; NC$\underline{H}_2$), 6.88 - 8.00 (13H; m; $C_6\underline{H}_5$, $C_6\underline{H}_4$ & $C_6\underline{H}_4$), 10.20 (1H; br s; N$\underline{H}$). Anal. Calc. (%) for $C_{25}H_{24}N_4OS$: C, 70.07; H, 5.64; N, 13.07; Found: C, 70.12; H, 5.61; N, 12.82.

2.1.1.34. *2-(2-(benzylthio)-1H-benzo[d]imidazol-1-yl)-N'-(1-(4-methoxyphenyl)ethylidene)aceto-hydrazide* ***7j***
White crystals, m.p. 161-163 ºC, yield 40.2%, IR (KBr) ύ (cm^{-1}) 3193 (N-H); 1679 (C=O); 1573, 1514, 1495, 1439 (C=N & C=C); 1247, 1033 (C-O); ^{1}H-NMR (60 MHz, $CDCl_3$): 1.90 (3H; s; N=CC$\underline{H}_3$), 3.81 (3H; s; OC$\underline{H}_3$), 4.55 (2H; s; C$\underline{H}_2C_6H_5$), 5.23 (2H; s; NC$\underline{H}_2$), 6.73 - 7.90 (13H; m; $C_6\underline{H}_5$, $C_6\underline{H}_4$ & $C_6\underline{H}_4$), 9.93 (1H; br s; N$\underline{H}$).Anal. Calc. (%) for $C_{25}H_{24}N_4O_2S$: C, 67.54; H, 5.44; N, 12.60; Found: C, 67.41; H, 5.31; N, 12.80.

2.1.1.35. *2-(2-(benzylthio)-1H-benzo[d]imidazol-1-yl)-N'-(1-(4-nitrophenyl)ethylidene)aceto-hydrazide* ***7k***
Yellow crystals, m.p. 190-192 ºC, yield 85.1%, IR (KBr) ύ (cm^{-1}) 3200 (N-H); 1693 (C=O); 1597, 1582, 1494, 1479, 1461 (C=N & C=C); 1516, 1343 (NO_2); ^{1}H-NMR (60 MHz, $CDCl_3$): 2.43 (3H; s; N=CC$\underline{H}_3$), 4.65 (2H; s; C$\underline{H}_2C_6H_5$), 5.35 (2H; s; NC$\underline{H}_2$), 7.00 - 8.48 (13H; m; $C_6\underline{H}_5$, $C_6\underline{H}_4$ & $C_6\underline{H}_4$), 11.06 (1H; br s; N$\underline{H}$). Anal. Calc. (%) for $C_{24}H_{21}N_5O_3S$: C, 62.73; H, 4.61; N, 15.24; Found: C, 62.94; H, 4.49; N, 15.01.

2.1.1.36. *2-(2-(benzylthio)-1H-benzo[d]imidazol-1-yl)-N'-(1-(4-hydroxyphenyl)ethylidene)aceto-hydrazide* ***7l***
White crystals, m.p. 203-205 ºC, yield 75.2%, IR (KBr) ύ (cm^{-1}) 3486 (OH); 3181 (N-H); 1675 (C=O); 1607, 1580, 1518, 1494, 1463 (C=N & C=C); 1243 (C-O); ^{1}H-NMR (60 MHz, $CDCl_3$/DMSO-d_6 4:1): 2.23 (3H; s; N=CC$\underline{H}_3$), 4.60 (2H; s; C$\underline{H}_2C_6H_5$), 5.30 (2H; s; NC$\underline{H}_2$), 6.70 - 7.86 (13H; m; $C_6\underline{H}_4$, $C_6\underline{H}_4$ & $C_6\underline{H}_5$), 9.20 - 9.60 (1H; br s; OH), 10.50 (1H; br s; N$\underline{H}$). Anal. Calc. (%) for $C_{24}H_{22}N_4O_2S$: C, 66.96; H, 5.15; N, 13.01; Found: C, 66.84; H, 5.83; N, 12.86.

2.1.2. *2-(2-(substituted thio)-1H-benzo[d]imidazol-1-yl)-N-(1,3-dioxoisoindolin-2-yl)acetamide,* ***(8a-c)***
To a solution of 2-(2-(substituted thio)-1H-benzo[d]imidazole-1-yl)acetohydrazide, compounds **(4a-c)** (0.005 mole) in glacial acetic acid (20 mL), phthalic anhydride (0.005 mole) was added. The reaction mixture was refluxed for 5 h, left to cool and the precipitated product was filtered, dried and recrystallized from ethanol.

2.1.2.1. *N-(1,3-dioxoisoindolin-2-yl)-2-(2-(methylthio)-1H-benzo[d]imidazol-1-yl)acetamide* ***8a***
White crystals, m.p. 278-280 ºC, yield 50.1%, IR (KBr) ύ (cm^{-1}) 3100 (N-H); 1791, 1734, 1698 (C=O); 1611, 1513, 1477, 1463, 1448 (C=N & C=C); ^{1}H-NMR (60 MHz, $CDCl_3$/DMSO-d_6 3:1): 2.76 (s; 3H; C$\underline{H}_3$), 5.08 (s; 2H; N-C$\underline{H}_2$), 7.00 - 8.00 (m; 8H; 2$C_6\underline{H}_4$), 11.30 (br s, 1H, N$\underline{H}$). EI-MS: *m/z*: 367.95 ($M^{\cdot+}$+2, 5.13), 366.90 ($M^{\cdot+}$+1, 15.44), 365.95 ($M^{\cdot+}$,63.13), 204.95 (100.00). Anal. Calc. (%) for $C_{18}H_{14}N_4O_3S$: C, 59.01; H, 3.85; N, 15.29; Found: C, 59.24; H, 3.83; N, 15.28.

2.1.2.2. *N-(1,3-dioxoisoindolin-2-yl)-2-(2-(isopropylthio)-1H-benzo[d]imidazol-1-yl)acetamide* ***8b***
White crystals, m.p. 244-246 ºC, yield 40.3%, IR (KBr) ύ (cm^{-1}) 3122 (N-H); 1796, 1742, 1701 (C=O); 1613, 1537, 1481, 1462 (C=N & C=C); ^{1}H-NMR (60 MHz, $CDCl_3$/DMSO-d_6 3:1): 1.49 (d; J = 7.8 Hz; 6H; 2C$\underline{H}_3$), 4.30 - 3.56 (m; 1H; C$\underline{H}$), 5.15 (s; 2H; N-C$\underline{H}_2$), 7.03 - 8.10 (m; 8H; 2$C_6\underline{H}_4$), 11.36 (br s, 1H, N$\underline{H}$). Anal. Calc. (%) for $C_{20}H_{18}N_4O_3S$: C, 60.90; H, 4.60; N, 14.20; Found: C, 61.18; H, 4.69; N, 13.90.

2.1.2.3. *2-(2-(benzylthio)-1H-benzo[d]imidazol-1-yl)-N-(1,3-dioxoisoindolin-2-yl)acetamide* ***8c***
White crystals, m.p. 238-240 ºC, yield 70.0%, IR (KBr) ύ (cm^{-1}) 3147 (N-H); 1795, 1739 (C=O); 1611, 1557, 1495, 1479, 1466 (C=N & C=C); ^{1}H-NMR (60 MHz, $CDCl_3$/DMSO-d_6 3:2): 4.56 (s; 2H; S-C$\underline{H}_2$), 5.00 (s; 2H; N-C$\underline{H}_2$), 6.93 - 8.00 (m; 13H; 2$C_6\underline{H}_4$ & $C_6\underline{H}_5$), 11.00 - 11.40 (br s, 1H, N$\underline{H}$). Anal. Calc. (%) for $C_{24}H_{18}N_4O_3S$: C, 65.14; H, 4.10; N, 12.66; Found: C, 64.95; H, 3.85; N, 12.40.

2.1.3. *4-amino-3-((2-(methylthio)-1H-benzo[d]imidazol-1-yl)methyl)-1H-1,2,4-triazole-5(4H)-thione* ***9***
To a stirred ice-cooled solution of 2-(2-(methylthio)-1H-benzo[d]imidazole-1-yl)acetohydrazide **(4a)** (0.005 mole, 1.2g) and potassium hydroxide (1.68 g, 0.03 mole) in absolute ethanol (50 mL), carbon disulfide (0.03 mole, 1.8 mL) was added. The reaction mixture was stirred at room temperature for 16 h. Dry ether (30 mL) was added; the precipitated solid was filtered, washed with dry ether, and dried. A suspension of the produced potassium dithiocarbazate salt (0.01 mole) (without any further purification) in hydrazine hydrate 99% (0.02 mole, 1 mL), and water (2 mL) was heated under reflux with stirring for 2 h. The reaction mixture was cooled, diluted with cold water, and acidified with conc. hydrochloric acid till pH 7-8. The obtained precipitate was filtered, washed with cold water, dried, and recrystallized from ethanol. Greenish white crystals, m.p. 175-177 ºC, yield 48.6%, IR (KBr) ύ (cm^{-1}) 3435, 3319 (NH & NH_2); 1497, 1479, 1463, 1444 (C=N, C=C); ^{1}H-NMR (60 MHz, $CDCl_3$/DMSO-d_6 4:1): 2.70 (s;

3H; C$\underline{H}_3$), 5.06 (br s, 2H, N$\underline{H}_2$, exchangeable with D_2O), 5.36 (s; 2H; C$\underline{H}_2$), 7.03-7.78 (m; 4H; $C_6\underline{H}_4$), 13.43-14.06 (br s, 1H, N$\underline{H}$). EI-MS: *m/z*: 294.05 (M^++2, 10.21%), 293.05 (M^++1, 13.27%), 292.05 (M^+,83.88%), 64.00 (100.00%). Anal. Calc. (%) for $C_{11}H_{12}N_6S_2$: C, 45.19; H, 4.14; N, 28.74; Found: C, 45.42; H, 4.14; N, 28.97. ^{13}C-NMR (APT) (100 MHz, δ ppm DMSO-d_6): 15.12 (S$\underline{C}H_3$), 38.65 (N$\underline{C}H_2$), 110.25 – 122.22 (CH of benzimidazole), 136.87 & 143.35 (C of benzene ring of benzimidazole), 148.04 ($\underline{C}$-S of imidazole), 153.35 & 167.35 (C of triazole).

2.1.4. *3-((2-(methylthio)-1H-benzo[d]imidazol-1-yl)methyl)-6-(4-(un)substituted phenyl)-7H-[1,2,4]triazolo [3,4-b][1,3,4]thiadiazine* ***10a-c***

A mixture of 4-amino-3-((2-(methylthio)-1H-benzo[d]imidazol-1-yl)methyl)-1H-1,2,4-triazole-5(4H)-thione**,** compound **(9)**, (0.001mole, 2.92g), appropriate phenacyl bromides (0.001mole) and sodium acetate (0.006 mole, 0.5g) in absolute ethanol (25 ml) was heated under reflux with stirring for 10-12 h. The mixture then left to cool, concentrated, under vacuum then filtered, dried and recrystallized from ethanol.

2.1.4.1. *3-((2-(methylthio)-1H-benzo[d]imidazol-1-yl)methyl)-6-phenyl-7H-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazine* ***10a***

White crystals, m.p. 242-244 ºC, yield 75.2%, IR (KBr) ύ (cm^{-1}) 1610, 1520, 1459, 1446 (C=N & C=C); ^{1}H-NMR (60 MHz, $CDCl_3$/DMSO-d_6 4:1): 2.76 (s; 3H; SC$\underline{H}_3$), 4.10 (s; 2H; SC$\underline{H}_2$), 5.66 (s; 2H; NC$\underline{H}_2$), 7.06 - 8.10 (m; 9H; $C_6\underline{H}_4$ & $C_6\underline{H}_5$). Anal. Calc. (%) for $C_{19}H_{16}N_6S_2$: C, 58.14; H, 4.11; N, 21.41; Found: C, 58.38; H, 4.19; N, 21.60.

2.1.4.2. *6-(4-chlorophenyl)-3-((2-(methylthio)-1H-benzo[d]imidazol-1-yl)methyl)-7H-[1,2,4]-triazolo[3,4-b][1,3,4]thiadiazine* ***10b***

White crystals, m.p. 212-214 ºC, yield 40.7%, IR (KBr) ύ (cm^{-1}) 1587, 1556, 1493, 1458, 1447 (C=N & C=C); ^{1}H-NMR (60 MHz, $CDCl_3$/DMSO-d_6 4:1): 2.76 (s; 3H; SC$\underline{H}_3$), 4.06 (s; 2H; SC$\underline{H}_2$), 5.61 (s; 2H; NC$\underline{H}_2$), 7.03 - 8.00 (m; 8H; 2$C_6\underline{H}_4$). Anal. Calc. (%) for $C_{19}H_{15}ClN_6S_2$: C, 53.45; H, 3.54; N, 19.68; Found: C, 53.60; H, 3.50; N, 19.94.

2.1.4.3. *3-((2-(methylthio)-1H-benzo[d]imidazol-1-yl)methyl)-6-p-tolyl-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine* ***10c***

White crystals, m.p. 151-153 ºC, yield 62.0%, IR (KBr) ύ (cm^{-1}) 1606, 1589, 1479, 1463, 1444 (C=N & C=C); ^{1}H-NMR (60 MHz, $CDCl_3$): 2.41 (s; 3H; *p*-$C_6H_4C\underline{H}_3$), 2.76 (s; 3H; SC$\underline{H}_3$), 3.83 (s; 2H; SC$\underline{H}_2$), 5.60 (s; 2H; NC$\underline{H}_2$), 7.00 - 7.86 (m; 8H; 2$C_6\underline{H}_4$). EI-MS: *m/z*: 408.10 (M^++2, 1.17%), 407.24 (M^++1, 1.61%), 406.22 (M^+,6.85%), 91.10 (100.00%) Anal. Calc. (%) for $C_{20}H_{18}N_6S_2$: C, 59.09; H, 4.46; N, 20.67; Found: C, 59.35; H, 4.51; N, 20.88. **^{13}C-NMR (APT)** (100 MHz, δ ppm $CDCl_3$): 15.28 (S-CH_3), 21.58 (*p*-CH_3), 23.34 (S-CH_2), 38.53 (N-CH_2), 109.87 – 129.72 (aromatic CH), 130.11 – 153.96 (aromatic quaternary C).

2.1.5. *4-(4-(un)substituted benzylideneamino)-3-((2-(methylthio)-1H-benzo[d]imidazol-1-yl)methyl)-1H-1,2,4-triazole-5(4H)-thione* ***11a-f***

To a solution of 4-amino-3-((2-(methylthio)-1H-benzo[d]imidazol-1-yl)methyl)-1H-1,2,4-triazole-5(4H)-thione, compound **9** (0.005 mole, 1.46 g) in glacial acetic acid (20 ml), the appropriate aryl aldehydes (0.005 mole) were added. The reaction mixture was heated under reflux with good stirring for 10 h, then left to cool and poured onto (40 ml) cold water. The precipitated product was filtered, dried and recrystallized from ethanol.

2.1.5.1. *4-(benzylideneamino)-5-((2-(methylthio)-1H-benzo[d]imidazol-1-yl)methyl)-4H-1,2,4-triazole-3-thiol* ***11a***

White crystals, m.p. 219-221 ºC, yield 73.0%, IR (KBr) ύ (cm^{-1}) 3434 (NH); 1610, 1571, 1499, 1480, 1463, 1444 (C=N & C=C); ^{1}H-NMR (60 MHz, $CDCl_3$/DMSO-d_6 1:1): 2.70 (s; 3H; SC$\underline{H}_3$), 3.26 - 5.10 (br s, 1H, N$\underline{H}$), 5.46 (s; 2H; NC$\underline{H}_2$), 6.90 - 8.00 (m; 9H; $C_6\underline{H}_4$ & $C_6\underline{H}_5$) 10.25 (s; 1H; C$\underline{H}$=N). EI-MS: *m/z* (%): 380.02 (M^+,0.96%), 57.11 (100.00%). Anal. Calc. (%) for $C_{18}H_{16}N_6S_2$: C, 56.82; H, 4.24; N, 22.09; Found: C, 56.59; H, 4.38; N, 22.34.

2.1.5.2. *4-(4-chlorobenzylideneamino)-5-((2-(methylthio)-1H-benzo[d]imidazol-1-yl)methyl)-4H-1,2,4-triazole-3-thiol* ***11b***

White crystals, m.p. 236-238 ºC, yield 40.3%, IR (KBr) ύ (cm^{-1}) 3419 (NH); 1592, 1561, 1505, 1479, 1463 (C=N & C=C); ^{1}H-NMR (60 MHz, $CDCl_3$/DMSO-d_6 4:1): 2.73 (s; 3H; SC$\underline{H}_3$), 5.50 (s; 2H; NC$\underline{H}_2$), 6.90 - 8.00 (m; 8H; 2$C_6\underline{H}_4$), 10.60 (s; 1H; C$\underline{H}$=N), 14.18 (br s, 1H, N$\underline{H}$). Anal. Calc. (%) for $C_{18}H_{15}ClN_6S_2$: C, 52.10; H, 3.64; N, 20.25; Found: C, 52.41; H, 3.78; N, 20.47.

2.1.5.3. *4-(4-methylbenzylideneamino)-5-((2-(methylthio)-1H-benzo[d]imidazol-1-yl)methyl)-4H-1,2,4-triazole-3-thiol* ***11c***

White crystals, m.p. 222-224 ºC, yield 68.1%, IR (KBr) ύ (cm^{-1}) 3430 (NH); 1603, 1498, 1480, 1465, 1447 (C=N & C=C); ^{1}H-NMR (60 MHz, $CDCl_3$/DMSO-d_6 4:1): 2.50 (s; 3H; *p*-$C_6H_4C\underline{H}_3$), 2.80 (s; 3H; SC$\underline{H}_3$), 5.56 (s; 2H; NC$\underline{H}_2$), 7.00 - 8.00 (m; 8H; 2$C_6\underline{H}_4$), 10.25 (s; 1H; C$\underline{H}$=N), 13.73 - 14.23 (br s, 1H, N$\underline{H}$). Anal. Calc. (%) for $C_{19}H_{18}N_6S_2$: C, 57.84; H, 4.60; N, 21.30; Found: C, 58.09; H, 4.82; N, 21.48.

2.1.5.4. *4-(4-methoxybenzylideneamino)-5-((2-(methylthio)-1H-benzo[d]imidazol-1-yl)methyl)-4H-1,2,4-triazole-3-thiol* ***11d***

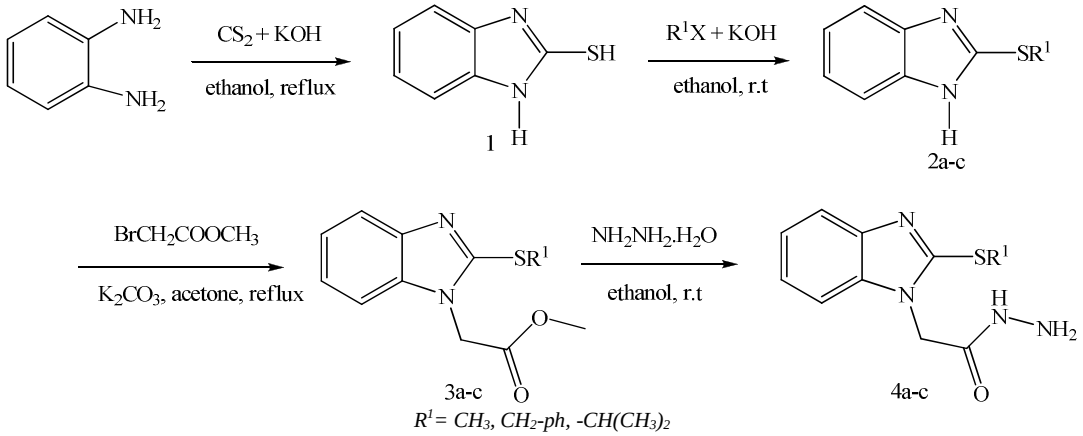
White crystals, m.p. 231-233 ºC, yield 66.0%, IR (KBr) ύ (cm^{-1}) 3415 (NH); 1604, 1567, 1514, 1480, 1465 (C=N & C=C); ^{1}H-NMR (60 MHz, $CDCl_3$/DMSO-d_6 1:1): 2.70 (s; 3H; SC$\underline{H}_3$), 3.86 (s; 3H; OC$\underline{H}_3$), 5.45 (s; 2H; NC$\underline{H}_2$), 6.70 - 7.90 (m; 9H; 2C$_6\underline{H}_4$ & N$\underline{H}$), 9.96 (s; 1H; C$\underline{H}$=N). Anal. Calc. (%) for $C_{19}H_{18}N_6OS_2$: C, 55.59; H, 4.42; N, 20.47; Found: C, 55.73; H, 4.49; N, 20.65.

2.1.5.5. *5-((2-(methylthio)-1H-benzo[d]imidazol-1-yl)methyl)-4-(4-nitrobenzylideneamino)-4H-1,2,4-triazole-3-thiol* ***11e***

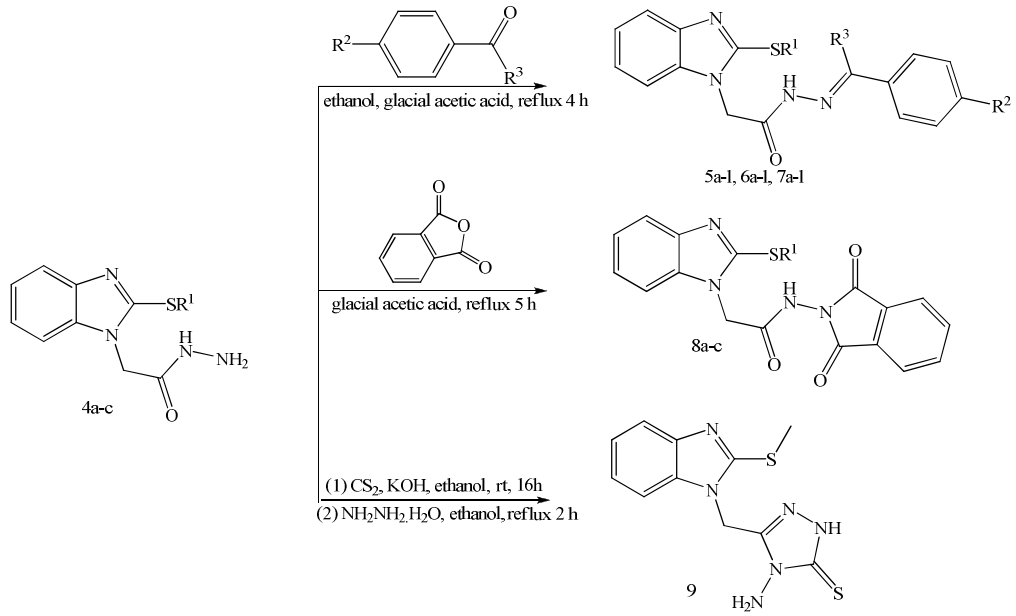
Yellow crystals, m.p. 234-236 ºC, yield 62.2%, IR (KBr) ύ (cm^{-1}) 3435 (NH); 1612, 1579, 1478, 1462 (C=N & C=C); 1522, 1348 (NO_2); ^{1}H-NMR (60 MHz, $CDCl_3$/DMSO-d_6 1:1): 2.71 (s; 3H; SC$\underline{H}_3$), 5.60 (s; 2H; NC$\underline{H}_2$), 7.00 - 8.53 (m; 9H; 2C$_6\underline{H}_4$& NH), 10.80 (s; 1H; C$\underline{H}$=N). EI-MS: *m/z* (%): 427.16 ($M^{\cdot+}$+2, 2.78%), 426.15 ($M^{\cdot+}$+1, 5.48%), 425.14 ($M^{\cdot+}$,23.26%), 163.09 (100.00%). Anal. Calc. (%) for $C_{18}H_{15}N_7O_2S_2$: C, 50.81; H, 3.55; N, 23.04; Found: C, 51.13; H, 3.66; N, 23.19.

2.1.5.6. *4-((3-mercapto-5-((2-(methylthio)-1H-benzo[d]imidazol-1-yl)methyl)-4H-1,2,4-triazol-4-ylimino) methyl) phenol* ***11f***

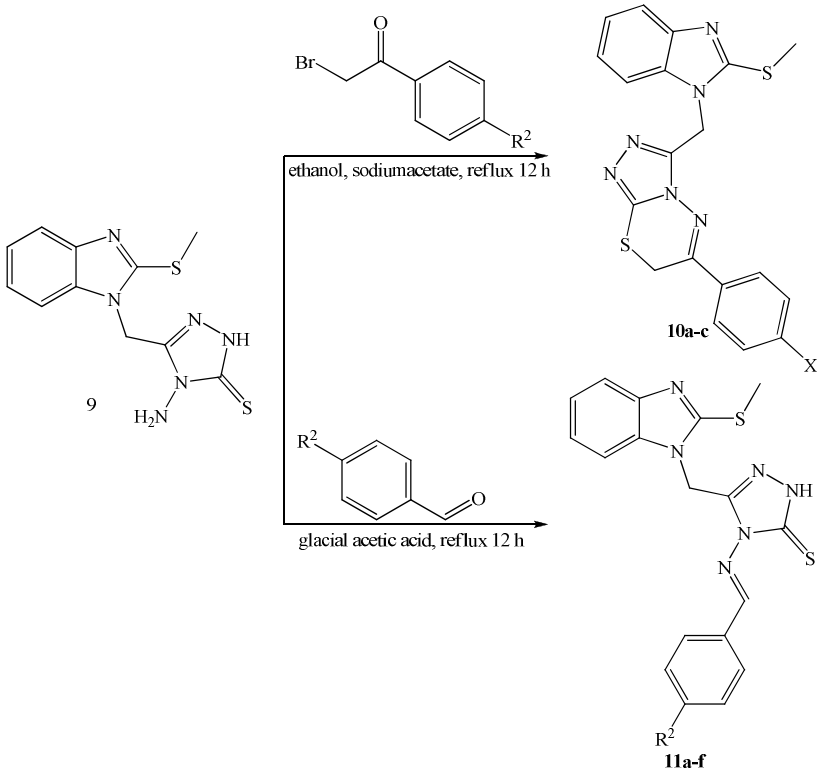
White crystals, m.p. 206-207 ºC, yield 40.0%, IR (KBr) ύ (cm^{-1}) 3408 (OH); 3153 (NH); 1595, 1513, 1479, 1446 (C=N & C=C); ^{1}H-NMR (60 MHz, $CDCl_3$/DMSO-d_6 3:1): 2.73 (s; 3H; SC$\underline{H}_3$), 5.46 (s; 2H; NC$\underline{H}_2$), 6.66 - 7.90 (m; 10H; 2C$_6\underline{H}_4$, O$\underline{H}$ & N$\underline{H}$), 9.83 (s; 1H; C$\underline{H}$=N). Anal. Calc. (%) for $C_{18}H_{16}N_6OS_2$: C, 54.53; H, 4.07; N, 21.20; Found: C, 54.80; H, 4.11; N, 21.46.



Scheme 1: Synthetic route of compounds 1, 2a-c, 3a-c and 4a-c



Scheme 2: Synthetic route of compounds 5a-l, 6a-l, 7a-l, 8a-c and 9



R^2= -H, -Cl, -NO_2, -OH, -CH_3, -OCH_3; X= -H, -Cl, -CH_3

Scheme 3: Synthetic route of compounds 10a-c and 11a-f

2.2. Anti-inflammatory activity:

2.2.1. *In vivo* anti-inflammatory activity:

The rat paw thickness was measured with a digital Vernier calliper (SMIEC, Shangahai, China). Male adult albino rats were obtained from the animal house, EGY Vaccine Company, Helwan, Egypt. Indomethacin (INM) (Liometacin® vial, Nile Company, Egypt), carrageenan (Sigma, USA), sodium carboxymethylcellulose (NaCMC) (El Nasr Pharm. Company, Egypt) and normal saline (Almottahedoon Pharma Company, Egypt) were obtained from the local market. Animals were housed in separate cages, 3 animals each, in temperature-controlled rooms at 25˚C. Animals were allowed free access to food and water and maintained at a 12 h light/dark cycle. Work was conducted in accordance with the internationally accepted principles for laboratory animals' use and care as found in the European Community Guidelines [28] and Institutional Ethical Committee Approval was obtained. The anti-inflammatory activity of all newly synthesized compounds (**5a-l, 6a-l, 7a-l, 8a-c, 9, 10a-c** and **11a-f**) was determined according to paw induced edema method [29, 30] in comparison to INM as a reference drug. The test is based on the pedal inflammation in rat paws induced by subplantar injection of carrageenan suspension (0.2 ml of 1% solution in normal saline) into the right hind paw of the rats. Male adult albino rats (120-150 g) were divided into groups, each of four animals, they were fed *ad libitum* with rodent's chow and allowed free access to drinking water. The thickness of rat paw was measured before and 1h after carrageenan injection to detect the carrageenan induced inflammation. Test compounds (**5a-l, 6a-l, 7a-l, 8a-c, 9, 10a-c** and **11a-f**) and INM at a dose of 0.02 mmol/Kg were suspended in 1% NaCMC in normal saline. Suspensions were injected intraperitoneal (i.p.) to different groups of rats. Control group received a vehicle (1% NaCMC solution in normal saline), while reference group received INM i.p. at 0.02 mmol/Kg. The difference between the thicknesses of the two paws was taken as a measure of edema. The measurement was carried out at 0.5, 1.0, 2.0, 3.0 and 4.0 h after injection of the test compounds, reference drug, and control. The percentages of edema inhibition; **Table 1** were calculated according to the following equation [29,30]:

$$\%\,\text{Edema inhibition} = \frac{(V_R - V_L)\,\text{control} - (V_R - V_L)\,\text{treated}}{(V_R - V_L)\,\text{control}} \times 100$$

Where, V_R: Average right paw thickness, V_L: Average left paw thickness.

Table 1: Percentage of edema inhibition of compounds (5a-l, 6a-l, 7a-l, 8a-c, 9, 10a-c and 11a-f) and indomethacin on carrageenan induced paw edema in rats

Compd. No.	Edema inhibition (%)				
	0.5 h	1 h	2 h	3 h	4 h
Negative Control	--	--	--	--	--
Indomethacin	41.32	45.77	65.71	75.42	77.23
5a	13.10	19.10	15.89	16.32	16.38
5b	7.71	20.14	24.77	48.17	22.28
5c	10.78	17.76	23.76	52.27	45.41
5d	9.06	15.09	23.90	40.48	53.24
5e	2.33	18.36	31.70	37.03	42.10
5f	7.94	19.25	40.80	43.28	45.19
5g	4.72	20.73	27.37	23.59	23.71
5h	16.77	22.81	17.98	25.74	15.45
5i	15.57	19.92	40.87	41.77	38.44
5j	16.70	25.93	29.82	32.00	33.98
5k	17.89	27.05	44.70	50.69	56.54
5l	6.22	32.62	47.15	59.46	46.92
6a	14.68	40.94	33.87	33.79	13.01
6b	6.82	22.44	25.85	31.35	39.16
6c	9.21	32.17	14.23	13.45	10.71
6d	17.74	19.10	42.97	63.05	43.11
6e	11.68	37.60	19.79	19.56	14.59
6f	17.82	37.30	46.29	33.15	11.79
6g	21.64	43.10	45.85	53.56	50.87
6h	19.17	42.20	60.29	60.68	63.15
6i	10.11	29.28	40.15	43.71	39.16
6j	18.94	46.96	50.55	32.86	23.86
6k	9.29	25.27	29.25	26.60	13.65
6l	20.74	37.15	31.77	23.15	21.13
7a	-3.82	16.35	47.51	56.37	57.12
7b	15.95	42.06	49.68	68.88	60.35
7c	11.23	18.58	33.65	57.95	70.77
7d	17.15	29.28	38.63	56.65	50.00
7e	13.70	31.95	37.19	43.86	50.65
7f	23.81	25.64	51.99	65.07	79.67
7g	5.69	32.99	43.76	58.02	68.61
7h	17.89	25.41	37.41	47.74	64.66
7i	5.84	23.48	21.81	25.81	32.69
7j	13.78	24.89	30.55	20.78	18.18
7k	13.78	15.31	26.00	49.25	63.80
7l	0.23	-0.53	5.28	2.74	2.09
8a	9.51	29.57	29.17	20.35	5.97
8b	25.00	46.07	35.17	25.31	12.50
8c	14.68	35.37	28.45	17.83	11.14
9	24.03	20.66	20.65	20.42	19.98
10a	20.66	30.17	36.90	24.02	13.08
10b	35.71	67.17	39.50	35.88	29.03
10c	39.53	56.02	46.21	45.37	35.92
11a	6.59	56.77	46.94	26.46	19.40
11b	22.24	41.09	40.15	33.94	29.96
11c	24.78	22.74	22.03	21.43	12.65
11d	11.46	42.87	42.24	22.72	13.51
11e	17.15	52.01	49.90	32.93	28.67
11f	30.17	70.58	39.14	27.90	26.51

2.2.2. Cyclooxygenase Inhibitory Studies:

Cyclooxygenase inhibition studies were carried out at the department of biochemistry, faculty of medicine, Cairo University, Cairo, Egypt. Synthesized compounds that showed highest *in vivo* anti-inflammatory activity were tested for their ability to inhibit ovine COX-1 and human recombinant COX-2; **Table 2**; using a COX inhibitor screening assay kit (Catalog No. 560131, Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's instructions. Cyclooxygenase catalyzes the first step in the biosynthesis of arachidonic acid (AA) to PGH_2. $PGF_{2\alpha}$, produced from PGH_2 by reduction with stannous chloride, is measured by enzyme immunoassay (ACE competitive EIA). Stock solutions of test compounds were dissolved in a minimum volume of DMSO. Briefly, to a series of

supplied reaction buffer solutions (960 µL, 0.1 M Tris-HCl pH 8.0 containing 5 mmole EDTA and 2 mmole phenol) with either COX-1 or COX-2 (10 µL) enzyme in the presence of heme (10 µL); 10 µL of various concentrations of test drug solutions (0.001, 0.01, 0.1, 1, 10, 100 and 500 µmole in a final volume of 1 ml) was added. These solution were incubated for a period of 2 min at 37 ^{0}C after which 10 µL of AA (100 µM) was added, and the COX reaction was stopped by the addition of 50 µL of 1 M HCl after 2 min. $PGF_{2\alpha}$, produced from PGH_2 by reduction with stannous chloride, was measured by enzyme immunoassay This assay is based on the competition between PGs and a PG-acetylcholinesterase conjugate (PG tracer) for a limited amount of PG antiserum. The amount of PG tracer that is able to bind to the PG antiserum is inversely proportional to the concentration of PGs in the wells since the concentration of the PG tracer is held constant while the concentration of PGs varies. This antibody-PG complex binds to a mouse antirabbit monoclonal antibody that had been previously attached to the well. The plate is washed to remove any unbound reagents and then Ellman's Reagent, which contains the substrate to acetylcholinesterase, is added to the well. The product of this enzymatic reaction produces a distinct yellow color that absorbs at 405 nm. The intensity of this color, determined spectrophotometrically, is proportional to the amount of PG tracer bound to the well, which is inversely proportional to the amount of PGs present in the well during the incubation: absorbance α [bound PG tracer] α 1/PGs. Percent inhibition was calculated by comparison of compound treated to various control incubations. The concentration of the test compound causing 50% inhibition (IC_{50}, µmole) was calculated from the concentration-inhibition response curve (duplicate determinations).

Table 2: *In vitro* ovine COX-1 and human recombinant COX-2 enzymes inhibitory activities of tested compounds

Compound number	COX-1 IC_{50} (µmole)	COX-2 IC_{50} (µmole)	COX-2 SI *
Celecoxib	14.80	0.05	296.00
diclofenac sodium	3.90	0.80	4.87
Indomethacin	0.039	0.49	0.08
6h	8.22	0.51	16.11
7a	3.41	0.64	5.33
7c	7.91	0.33	23.96
7f	5.41	0.67	8.07
7g	2.98	0.39	7.64
7h	4.11	0.81	5.07
7k	2.51	0.49	5.12
10b	6.74	0.93	7.24
10c	8.22	0.28	29.35
11f	5.33	0.32	16.65

** In vitro COX-2 selectivity index (COX-1 IC_{50}/COX-2 IC_{50}). The in vitro test compound concentration required to produce 50% inhibition of ovine COX-1 or human recombinant COX-2. The result (IC_{50}, µmole) is the mean of two determinations acquired using the enzyme immunoassay kit (Catalog No. 560131, Cayman Chemicals Inc., Ann Arbor, MI), and the deviation from the mean is <10% of the mean value.*

2.3. Molecular docking

Molecular modelling and docking simulation studies were carried out at the Department of Pharmaceutical Medicinal Chemistry, Faculty of Pharmacy, Assiut University; on a Processor Intel(R) Core(TM) i7-2675QM CPU @ 2.20GHz, 8 GB Memory with Microsoft Windows 8.1 pro (64 Bit) operating system using Molecular Operating Environment [31] (MOE 2014.0901, 2014) as the computational software. All energy minimization were performed with MOE until a RMS gradient of 0.00001 Kcal/mol/Å and RMS distance of 0.05 Å with MMFF94x force-field [32] were attained and the partial charges were automatically calculated. The X-ray crystallographic structure of murine COX-2 complexed with INM (PDB ID: 4COX) was obtained from protein data bank. The enzyme was prepared for docking studies where: i) Protein structures were repaired to ascertain the health of protein using structure preparation command and appropriately protonated in the presence of ligands using the Protonate 3D process in MOE. ii) Ligand molecules were removed from the enzyme active site and the receptor (first chain) was kept. iii) MOE Alpha Site Finder was used for the active sites search in the enzyme structure and dummy atoms were created from the obtained alpha spheres. iv) The obtained model was then used in predicting the ligand-enzyme interactions at the active site. v) The best pose for each ligand was explored using LigX tool.

RESULTS AND DISCUSSION

3.1. Chemistry:

The synthetic procedures adopted to obtain the target compounds are outlined in **Schemes 1, 2** and **3**. The key precursors 2-(2-(substitutedthio)-1H-benzo[d]imidazol-1-yl)acetohydrazides **4a-c** were synthesized according to reported method [25]. The target compounds acetohydrazones; **(5a-l, 6a-l, 7a-l)** were synthesized by condensation of compounds **(4a-c)** respectively with one equivalent (un)substituted benzaldehydes or acetophenones as illustrated in **Scheme 2.** The structures of compounds **(5a-l, 6a-l, 7a-l)** were elucidated by IR and ^{1}H-NMR as well as elemental analyses. The IR spectra of compounds **(5a-l, 6a-l, 7a-l)** showed the disappearance of (-NH_2) bands and displayed strong stretching absorption bands at 3216–3180 cm^{-1} and 1698 -1674 cm^{-1} for the NH and amidic C=O

groups, respectively. ^{1}H-NMR spectra of compounds **(5a-l, 6a-l, 7a-l)** revealed disappearance of NH_2 signal of hydrazide structure and ensured an increase in the integration of aromatic protons due to the additional benzaldehyde or acetophenone moieties. Some of them were further confirmed by mass spectral analysis, for example, mass spectrum of compound **5f** revealed the molecular ion peak at 340.00 (21.79%) corresponding to it's relative molecular mass (340.40) and a base peak at 77.00 (100.00%) for Phenyl cation. In addition, it showed $M^{\cdot+}$+2 at 342.00 (8.84%). Acetohydrazides **4a-c** were reacted with equimolar amount of phthalic anhydride in acidic media to give 1,3-dioxoisoindolines derivatives, compounds **8a-c**, as illustrated in **Scheme 2**. The structures of compounds **8a-c** were confirmed by spectral data and elemental methods of analysis. IR spectra of compounds **8a-c** showed the absence of (-NH_2) band; instead they showed characteristic stretching bands for (-NH) group at 3147 - 3100 cm^{-1}; in addition to presence of two to three characteristic stretching bands for (-C=O) groups at 1796 - 1698 cm^{-1}. ^{1}H-NMR spectrum showed the disappearance of (-NH_2) signal and ensured an increase in the integration of aromatic protons due to the additional phthalic anhydride moiety. One of them was further elucidated by mass spectral analysis. Mass spectrum of compound **8a** revealed the molecular ion peak at 365.95 (63.13%) corresponding to it's relative molecular mass (366.08) and a base peak at 204.95 (100.00%) for ($M^{\cdot+}$ - $C_{10}H_9N_2O_2$). In addition, it showed $M^{\cdot+}$+2 at 367.95 (5.13%). Compound **(9)**, 1,2,4-triazole derivative was prepared by condensation of acetohydrazide **(4a)** with carbon disulfide in ethanolic potassium hydroxide to yield potassium dithiocarbazate salt which was cyclized by heating under reflux with hydrazine hydrate to afford the triazole compound **(9)**; as shown in **Scheme 2**. The structure of compound **(9)** was verified by spectral data as well as elemental analysis. IR spectrum of compound **(9)** showed two bands at 3435 and 3319 cm^{-1} due to (-NH) and (-NH_2) stretching absorption band and absence of absorption bands around 1662 cm^{-1} due to carbonyl group of hydrazide. Also the spectrum exhibited a band at 2795 cm^{-1} for (-SH) group and a strong stretching band at 1244 cm^{-1} (-C=S) (thiol-thione tautomers). ^{1}H-NMR spectrum of compound **(9)** exhibited a new singlet signal appeared at δ 5.06 ppm (exchangeable with D_2O) due to (-NH_2) of triazole ring integrating for two protons and a broad signal at δ 13.43 - 14.06 ppm due to exchanged (-NH) proton of triazole ring. Mass spectrum of compound **(9)** displayed the molecular ion peak at 292.05 (83.88%) corresponding to it's relative molecular mass (292.38) and a base peak at 64 (100.00%) for S_2. In addition, it showed $M^{\cdot+}$+2 at 294.05 (10.21%). For further verification of the structure of compound **(9)**, ^{13}C-APT spectrum was then recorded to differentiate among methyl, methylene, methine, and quaternary carbons. In the APT spectrum; methyl and methine carbons are positive (up; in the other side), and methylene and quaternary carbons are negative (down; in the solvent side). ^{13}C-APT spectrum in DMSO-d_6 showed 15.12 (S<u>C</u>H$_3$), 38.65 (N<u>C</u>H$_2$), 110.25 – 122.22 (CH of benzimidazole), 136.87, 143.35 (C of benzene ring of benzimidazole), 148.04 (<u>C</u>-S of imidazole), 153.35, 167.35 (C of triazole). Cyclization of compound **(9)** with phenacyl bromides in ethanol in presence of catalytic amount of sodium acetate to afford [1,2,4]triazolo[3,4-b][1,3,4]thiadiazine derivatives, compounds **(10a-c)**; as presented in **Scheme 3**. Structures of compounds **(10a-c)** were elucidated by spectral and elemental methods of analysis. IR spectra of compounds **(10a-c)** showed disappearance of (-NH_2) and (-NH) bands of triazole ring. ^{1}H-NMR spectra was devoid of broad signals of (-NH_2) and (-NH) of triazole; instead; a new singlet signal appeared at δ 3.83 - 4.10 ppm of (-CH_2) of the formed thiadiazine ring integrating for two protons. Also the spectra showed an increase in the integration of aromatic protons due to the additional phenacyl bromides moieties. Mass spectrum of compound **(10c)** revealed the molecular ion peak at 406.22 (6.85%) corresponding to it's relative molecular mass (406.10) and a base peak at 91.10 (100.00%) for tropyllium cation. ^{13}C-APT spectrum in $CDCl_3$ of compound **(10c)** displayed 15.28 (S-CH_3), 21.58 (*p*-CH_3), 23.34 (S-CH_2), 38.53 (N-CH_2), 109.87 – 129.72 (aromatic CH), 130.11 – 153.96 (aromatic quaternary C). Compounds **(11a-f)**, hydrazones derivatives were prepared by reaction of compound **(9)** with equimolar amount of (un)substituted benzaldehydes; as illustrated in **Scheme 3**. Structures of compounds **(11a-f)** were verified by spectral and elemental analysis. IR spectra showed the disappearance of (-NH_2) band of compound **(9)**. Also the spectra exhibited a band at 2725 - 2691 cm^{-1} for (-SH) group and a strong stretching band at 1282 - 1247 cm^{-1} (-C=S) (thiol-thione tautomers). ^{1}H-NMR spectra showed the absence of (-NH_2) band of compound **(9)**; instead; it showed a new singlet signal of the azomethine (-N=CH-) group at δ 9.83 - 10.80 ppm integrating for one proton. Mass spectrum of compound **(11e)** exhibited the molecular ion peak at 425.14 (23.26%) corresponding to it's relative molecular mass (425.07) and a base peak at 163.09 (100.00%) for ($M^{\cdot+}$ - $C_{10}H_8N_5O_2S$).

3.2. Biology:

3.2.1. *In vivo* anti-inflammatory activity:

In the present work, the newly synthesized compounds (**5a-l, 6a-l, 7a-l, 8a-c, 9, 10a-c** and **11a-f**) were evaluated for their *in vivo* anti-inflammatory activity by using carrageenan induced paw edema bioassay in male rats using INM as a reference drug [29,30]. Results were presented as percentage of edema inhibition at a dose of 28 µmole/Kg at time intervals 0.5, 1.0, 2.0, 3.0 and 4.0 h; **Tables 1**. The obtained results of the anti-inflammatory activity of the tested compounds could be related to their structural modifications and variations, taking percentages activity relative to INM after 1-4 h intervals as a criterion for assessment of the tested compounds relative to INM; **Figures 1**. The data mentioned below is about the maximum inhibitory activity reached by different classes of the tested compounds. The anti-inflammatory activity results revealed that all the tested compounds showed a gradual increase of the anti-inflammatory activity up to its maximum after 1 h except compounds (**5b-f**, **5i**, **5k-l**, **6b**, **6d**, **7a-d**, **7f-h** and **7k-l**).

Compounds (**5f**, **5i** and **7l**) exhibited their highest effect at 2 h, while compounds (**5b-c**, **5l**, **6d**, **7a-b** and **7d**) revealed their maximum inhibition of edema at 3 h. on the other hand, compounds (**5d-e**, **5k**, **6b**, **7c**, **7f-h** and **7k**) displayed their maximum inhibition after 4 h. Results of the anti-inflammatory activity of hydrazone derivatives **5a-l** ($R^1 = CH_3$) showed that compounds **5c-f** (R^2 = H) and **5i-l** ($R^2 = CH_3$) ($R^3 = CH_3$, OCH_3, NO_2, OH respectively) had moderate anti-inflammatory activity relative to INM (44.00 - 73.21%) after 4 h intervals; **Figure 1**. Compound **5k** ($R^2 = CH_3$, $R^3 = NO_2$) was the most potent hydrazone derivative in this series that exhibited 73.21% anti-inflammatory activity of INM at the same time interval. Also, it's noteworthy to mention that compound **5l** ($R^2 = CH_3$, R^3 = OH) showed 78.84% relative to INM after 3 h interval. Moreover, Most of hydrazone series **6a-f** (R^1 = isopropyl, R^2 = H) revealed significant anti-inflammatory activity compounds (**6a**, **6c**, **6e** and **6f**) (R^3 = H, CH_3, NO_2, OH respectively) exhibited 89.45, 70.29, 82.15 and 81.49% respectively anti-inflammatory activity relative to INM at 1 h interval; **Figure 1**. It is important to mention that the highest anti-inflammatory activity for compound **6d** is reached after 3 h since it displayed 83.59% anti-inflammatory activity relative to INM at the same time interval. On the other hand, condensation of acetohydrazide moiety with (un)substituted acetophenone; compounds **6g-l** ($R^2 = CH_3$, R^3 = H, Cl, CH_3, OCH_3, NO_2, OH respectively) resulted in more active compounds than those containing an azomethine –N = CH- group; compounds **6a-f**. The most potent compounds were (**6g**, **6h** and **6l**) (R^3 = H, Cl and OH respectively) that exhibited % anti-inflammatory activity (94.17, 92.20 and 81.17% respectively) that was comparable to that of INM after 1 h interval, while compound **6j** ($R^3 = OCH_3$) surpassed the activity of INM giving 102.60% anti-inflammatory activity relative to INM at the same time interval; **Figure 1**.

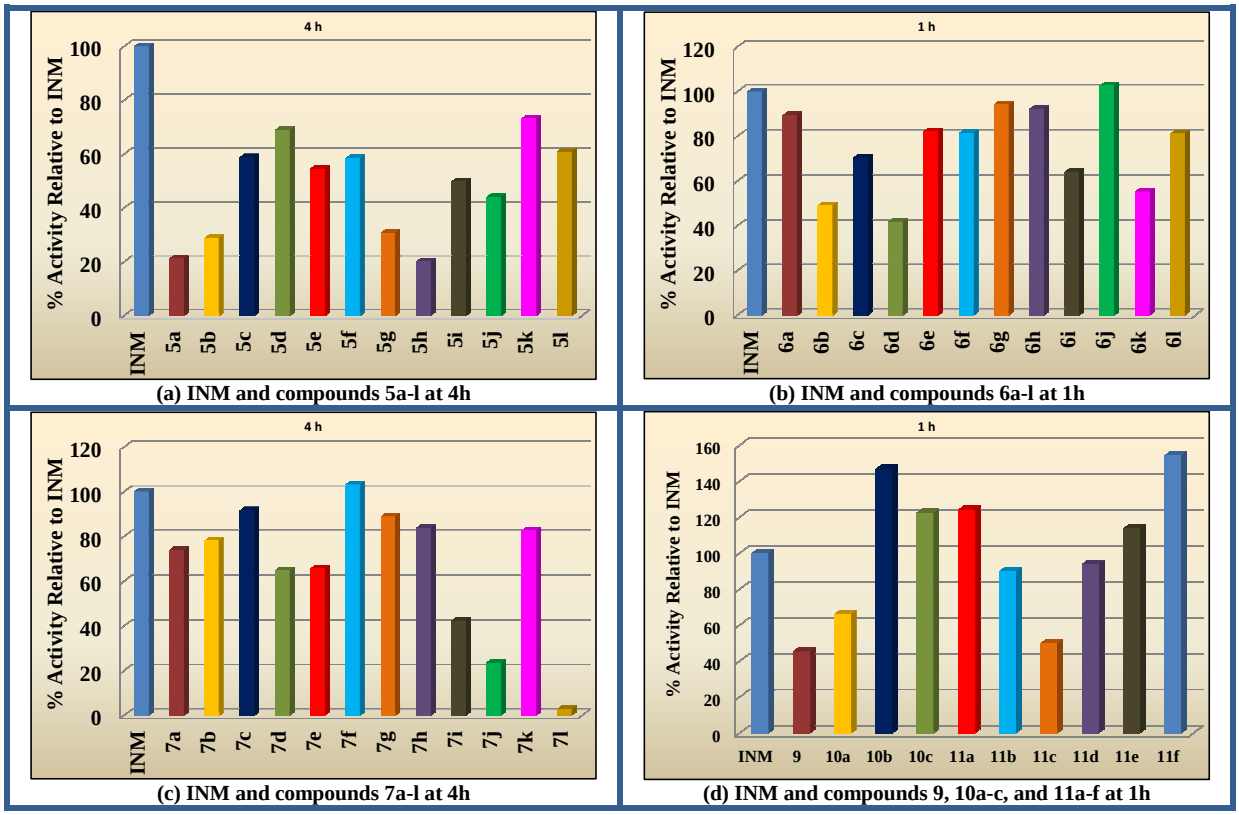


Figure 1: % Anti-inflammatory activity relative to INM of compounds (5a-l, 6a-l, 7a-l, 9, 10a-c, 11a-f and INM) on induced paw edema.

Furthermore, the anti-inflammatory activity enhanced markedly in case of hydrazone derivatives **7a-f** (R^1 = benzyl, R^2 = H), as they exhibited anti-inflammatory activity ranging from (64.00 - 103.16%) after 4 h relative to INM; **Figure 1**. The most potent compounds were **7c** ($R^3 = CH_3$) and **7f** (R^3 = OH) that exhibited 91.64% and 103.16% anti-inflammatory activity respectively relative to that of INM at the same time interval. However, replacement of H with CH_3 group in the azomethine moiety as shown in compounds **7g-l**, decreased the activity markedly except for compounds **7g**, **7h** and **7k** (R^3 = H, Cl, NO_2 respectively) that displayed 88.84%, 83.72% and 82.61% respectively anti-inflammatory activity relative to INM after 4 h; **Figure 1**. Regarding the above observations, it could be concluded that the best results for hydrazone series; compounds (**5a-l**, **6a-l** and **7a-l**) were found when S-aralkylation of benzimidazole by benzyl group was performed. This indicates the importance of the presence of a bulky lipophilic aryl moieties at 2-position of benzimidazole to enhance the anti-inflammatory activity in this class of compounds. Condensation of acetohydrazide moiety with phthalic anhydride; compounds (**8a-c**) resulted in moderate to high anti-inflammatory activity (64.61-100.66%) relative to INM after 1 h interval. The most potent one

was compound **8b** (R^1 = isopropyl-) that displayed 100.66% of INM at the same time interval; **Figure 1**. Similarly, cyclization of acetohydrazide moiety to 1,2,4-triazole **9** had non-significant activity (45.14%) after 1 h relative to INM; **Figure 1**. On the other hand, condensation of 1,2,4-triazole with phenacyl bromides to afford 1,3,4-thiadiazine derivatives **10a-c** gave promising anti-inflammatory activity compared to INM after 1 h (65.91 - 146.75%) especially compounds **10b** and **10c** (R^3 = Cl, CH_3 respectively) that showed anti-inflammatory activity surpassing the activity of INM to a large extent (146.75% and 122.39% respectively) anti-inflammatory activity relative to INM at the same time interval; **Figure 1**. So; this means that these compounds are considered to have rapid onset of anti-inflammatory activity. likewise, introduction of (un)substituted arylmethylidene moieties to 1,2,4-triazole to afford compounds (**11a-f**) (R^3 = H, Cl, CH_3, OCH_3, NO_2, OH respectively) exhibited potent anti-inflammatory activity after 1 h relative to INM (89.77 - 154.21%) except for compound **11c** (R^3 = CH_3) that showed moderate (49.68%) anti-inflammatory activity relative to INM at the same time interval. The most promising compounds were **11a**, **11e** and **11f** (R^3 = H, NO_2, OH respectively) that exhibited 124.03%, 113.63% and 154.21% respectively anti-inflammatory activity after 1 h relative to INM; **Figure 1**. In, general, declining of the activity of compounds **10a-c** and **11a-f** after 1 h may be due to pharmacokinetic effects inside the rat model.

3.2.2. *In vitro* COX-1 and COX-2 inhibitory activity:

Synthesized compounds that showed highest *in-vivo* anti-inflammatory activity in each class of the tested compounds were tested for their ability to inhibit ovine COX-1 and human recombinant COX-2 enzymes *in vitro* using a COX inhibitor screening assay kit (Catalog No. 560131, Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's instructions. The chosen compounds were (4-(un)substituted (arylmethylidene or α-arylethylidene)acetohydrazide derivatives (**6h**, **7a**, **7c**, **7f-h**, and **7k**), [1,2,4]triazolo[3,4-b][1,3,4]thiadiazine derivatives (**10b** and **10c**); and arylmethylidene[1,2,4]triazolo derivative (**11f**)). The efficacies of the tested compounds were estimated as the concentration causing 50% enzyme inhibition (IC_{50}, µmole) compared to the reference drugs; INM, diclofenac sodium and celecoxib (**Table 2**). The results indicated that all tested compounds exhibited moderate inhibitory activity against ovine COX-1 enzyme, however they were less active than INM (IC_{50} = 2.51 - 8.22 µmole vs. 0.049 µmole respectively) except 4-(un) substituted (arylmethylidene or α-arylethylidene)acetohydrazide derivatives (**7a**, **7g** and **7k**) that exhibited more activity to COX-1 than diclofenac sodium (IC_{50} = 3.41 µmole, 2.98 µmole, 2.51 µmole vs. 3.90 µmole respectively), **Table 2**. However, they all showed better inhibitory profiles (IC_{50} = 0.28 - 0.93 µmole) against human recombinant COX-2 enzyme than INM and diclofenac sodium (IC_{50} = 0.49 and 0.80 µmole respectively). Specially compounds **7c**, **7g**, **7k**, **8a**, **10c** and **11f** that revealed superior inhibitory profiles against human recombinant COX-2 as evidenced by their IC_{50} values (0.33, 0.39, 0.49, 0.28 and 0.32 µmole respectively), when compared with INM and diclofenac sodium. To further assess the COX-2 inhibitory activity and selectivity profiles of the tested compounds, their activity and selectivity indices were compared to that of INM, diclofenac sodium and the standard COX-2 selective inhibitor, celecoxib, **Figure 2**. The study proved that all the tested compounds were selective inhibitors for COX-2 (SI = 5.07 - 29.35) exceeding INM and diclofenac sodium (0.08 and 4.87 respectively). Compounds (**6h**, **7c**, **10c**, **11f**) showed the highest selectivity to COX-2 (COX-2 SI = 16.11 - 29.35). In conclusion; the [1,2,4]triazolo[3,4-b][1,3,4]thiadiazine derivative, compound **10c** (R^3 = CH_3), was the most potent COX-2 inhibitor (IC_{50} = 0.28 µmole) among the tested compounds with approximate selectivity ratio of 29.35, that was higher than INM and diclofenac sodium (COX-2 SI = 0.08 and 4.87 respectively). Conversely, the [1,2,4]triazolo[3,4-b][1,3,4]thiadiazine derivative, compound **10b** (R^3 = Cl), displayed lower inhibitory activity and selectivity towards COX-2 than it's above mentioned analogue (IC_{50} = 0.93 µmole; COX-2 SI = 7.24). Additionally, the arylmethylidene acetohydrazide derivative, compound **7c** (R^1 = benzyl-, R^2 = H, R^3 = CH_3), demonstrated both remarkable COX-2 potency and selectivity (IC_{50} = 0.33 µmole, COX-2 SI = 23.96). Furthermore, both the arylmethylidene 1,2,4-triazolo derivative, compound **11f** (R^3 = OCH_3), and the arylethylidene acetohydrazide derivative, compound **6h** (R^2 = CH_3, R^3 = Cl) showed a considerable equipotent selectivity to COX-2 (IC_{50} = 0.32 µmole, 0.51 µmole, COX-2 SI = 16.65, 16.11 respectively). Moreover, in parallel to the results of the *in vivo* anti-inflammatory assay, the arylmethylidene acetohydrazide derivatives, compound **7f** (R^1 = benzyl, R^2 = H, R^3 = OH), and arylethylidene acetohydrazide derivative, compound **7g** (R^1 = benzyl, R^2 = CH_3, R^3 = H), displayed higher COX-2 inhibition properties compared to that of diclofenac sodium (IC_{50} = 0.67 µmole, 0.39 µmole vs. 0.80 µmole, COX-2 SI= 8.07, 7.64 vs. 4.87 respectively). Also, results showed that the hydrazone derivatives (R^1 = benzyl) compounds **7a** (R^2 = H, R^3 = H), **7h** (R^2 = CH_3, R^3 = Cl), and **7k** (R^2 = CH_3, R^3 = NO_2) revealed approximately equipotent selectivity towards COX-2 enzyme (COX-2 SI = 5.33, 5.07 and 5.12 respectively) but taking into consideration that compound **7k** had higher inhibitory activity towards COX-2 (IC_{50} = 0.49).

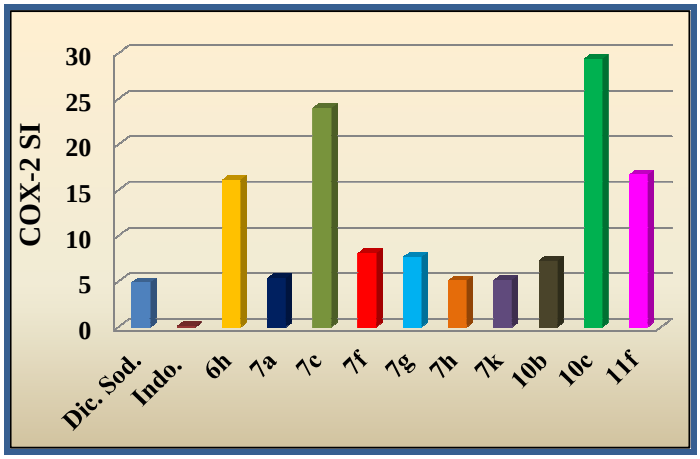


Figure 2: COX-2 selectivity index of the tested compounds and the reference drugs (INM and diclofenac sodium).

3.3. Molecular docking

Docking of the newly synthesized compounds (**5a-l**, **6a-l**, **7a-l**, **8a-c**, **9**, **10a-c**, **11a-c**) to COX-2 were made to rationalize the obtained biological results. The binding affinity of the docked molecules was evaluated by (S) kcal/mol. It was found that most of the newly synthesized compounds showed good binding to COX-2 active site. Amino acid Arg120 was found to have an important role in the hydrogen bond interaction of the most active synthesized compounds towards COX-2 active site which is in agreement with the reported data [33], in addition to the presence of hydrophobic interactions with the hydrophobic cleft of COX-2 enzyme. Acetohydrazones compounds (**5a-l**, **6a-l**, **7a-l**) showed complete alignment into COX-2 active site with binding scores ranging from -12.00 to -14.80. The best binding scores were for compounds **7a-l** (-13.51 to -14.80) compared to other analogs (**5a-l** and **6a-l**) which augments with their high anti-inflammatory activity relative to INM. All compounds exhibited hydrogen bond interaction of C=O group with guanidinium moiety of Arg 120 (2.28 Å – 3.35 Å) which means that this type of binding is essential to give anti-inflammatory activity in this class of compounds except compounds **5a**, **5d** and **5f** that showed binding of C=O group with hydroxyl side chain of Ser 530 (2.47 Å and 2.60 Å) and hydroxyl side chain of Tyr 355 (2.45 Å) respectively. Moreover, Ligand sulfur atom displayed hydrogen bond interaction with either Met 522 (3.72 Å) in compound **5a**, Glu 524 (3.75 Å, 3.66 Å, 3.82 Å, 3.76 Å) in compounds **5b**, **5i**, **6f** and **7k**; **Figure 3**, respectively, Arg 120 (3.51 Å and 3.09 Å) in compounds **5f** and **5h** respectively; or with Val 349 (4.30 Å) in compound **7j**. Furthermore, acetohydrazide linkage NH-**N** showed hydrogen bond interaction with either hydroxyl side chain of Tyr 355 (2.75 Å, 2.61 Å, 2.74 Å, 2.75 Å) in compounds **5g**, **7c**, **7d** and **7i** or guanidinium side chain of Arg 120 (2.67 Å, 2.68 Å) in compounds **6l** and **7j** respectively. In addition, Imidazole nitrogen N^b was bounded to hydroxyl side chain of Ser 530 (2.66 Å) in compound **5l**; **Figure 3**, or guanidinium side chain of Arg 513 (2.78 Å) in compound **7b**. Also, there were other hydrogen bond interactions between the docked compounds and the enzyme active site that varied according to the type and position of substituent on the aromatic moiety: i) NO_2 group in compounds **5e**, **5k**, **6e**, **6k**, **7e** and **7k**; **Figure 3**, formed hydrogen bond interaction with nitrogen atom in the indole ring of Trp 387 (3.09 Å, 3.04 Å, 3.12 Å, 3.12 Å, 3.10 Å, 3.09 Å respectively). ii) OH group in compounds **5f**, **7f** and **7l** displayed hydrogen bond interaction to either Phe 518 (2.81 Å, 2.43 Å, 2.56 Å) respectively or with His 90 (2.53 Å) in compound **5l**. On the other hand, Interactions other than hydrogen bonding were also observed including: i) arene-H binding of: 1) Ligand imidazole ring with Tyr 355 (3.52 Å) in compounds **5e**, **5k** and **7e** ($R^3 = NO_2$). 2) Benzene ring of binzimidazole with either Leu 531 (4.44 Å, 4.20 Å) in compounds **5b and 6e** or Ser 353 (3.64 Å, 3.62 Å) in compounds **7a** and **7g** respectively. 3) *p*-$CH_3(C_6H_4)$ with Gly 526 (4.35 Å, 4.26 Å, 4, 4.24 Å) in compounds **5c**, **6c** and **6i** ($R^3 = CH_3$). 4) Benzyl moiety with Trp 387 (3.82 Å) in compound **7g** or with Gly 526 (4.29 Å and 4.02 Å) in compounds **7d** and **7j** ($R^3 = OCH_3$). ii) Arene-cation binding of *p*-$CH_3(C_6H_4)$ with Arg 513 (4.85 Å) in compound **7c**. It's noteworthy to mention that, most compounds showed van der Waals' and hydrophobic interactions with different amino acid residues such as Leu 352, Val 349, Ala 527, Phe 381, Phe 518, Val 523, Leu 93, Val 89 and Leu 531.

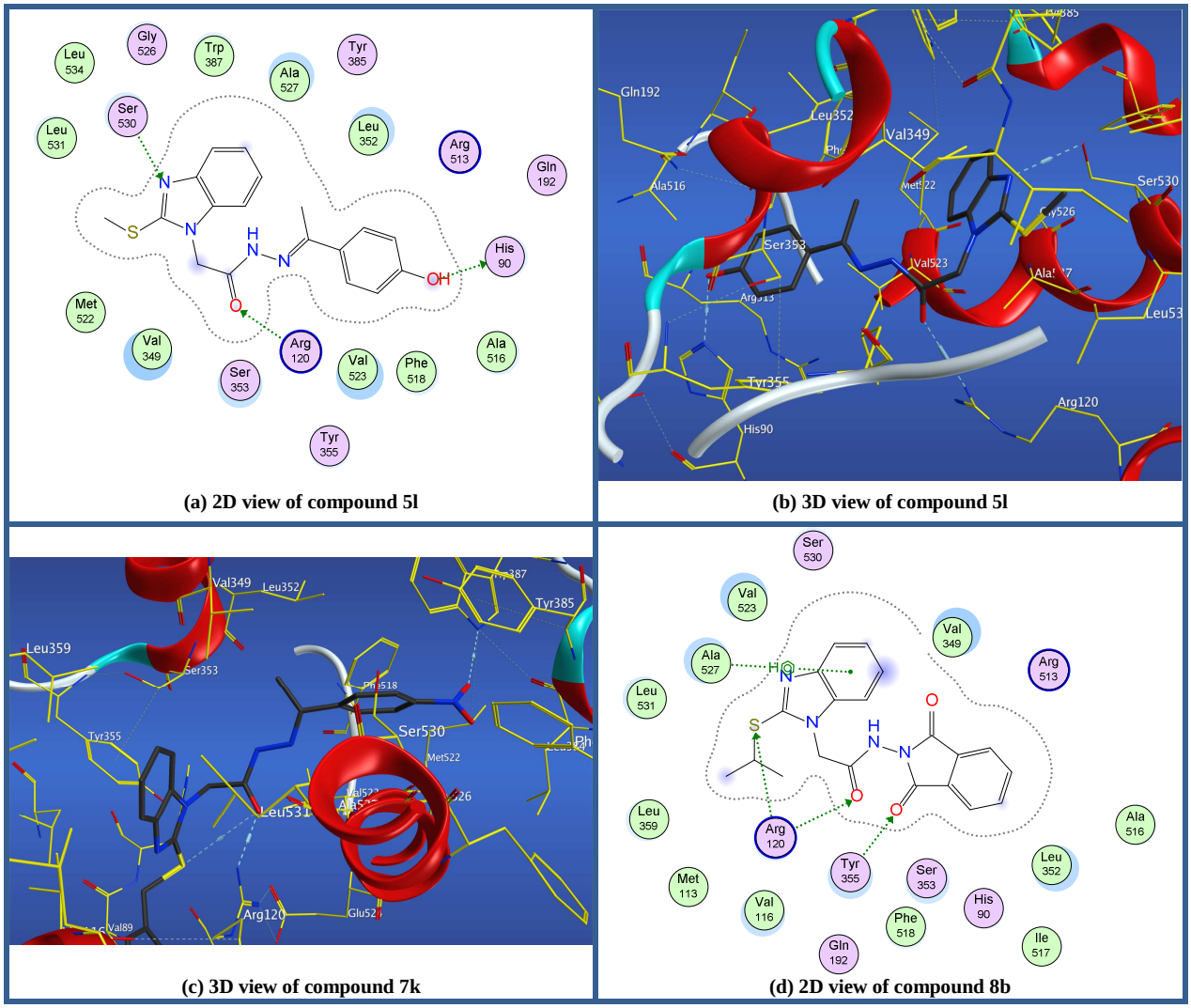


Figure 3: A molecular modelling representation of the predicted binding poses of compounds 5l, 7k and 8b (sticks) within the binding pocket of COX-2 (PDB: 4COX). Hydrogen bond interactions are shown as dashed lines

(1,3-dioxoisoindolin-2-yl)acetamide derivatives, compounds **8a-c**, showed high binding scores ranging from -13.08 to -14.99 that were in consistent with their *in vivo* anti-inflammatory activity. All docked ligands **8a-c** exhibited hydrogen bond interaction of C=O groups of 1,3-dioxoisoindoline with guanidinium side chain of Arg 120 (3.08 Å and 3.12 Å) in compounds **8a** and **8c** respectively and hydroxyl side chain of Tyr 355 (2.51 Å, 2.47 Å and 2.61 Å) in compounds **8a**, **8b** and **8c** respectively; **Figure 3**, which may enhance the binding of these compounds with COX-2 active site. In addition, ligand Sulfur atom showed hydrogen bond interaction with Val 349 (3.74 Å) and guanidinium side chain of Arg 120 (3.32 Å) in compounds **8a** and **8b** respectively. Moreover, compound **8b** displayed hydrogen bond interaction of C=O group of acetohydrazide moiety with guanidinium side chain of Arg 120 (2.47 Å) and arene-H binding of benzene ring of benzimidazole with Ala 527 (3.92 Å). Furthermore, most compounds showed hydrophobic interactions with different amino acid residues at the hydrophobic cleft of the enzyme active site like Ser 353, Leu 531, Ala 527, Leu 352, Val 523, Phe 518, Val 116, Met 113, Ser 530 and Leu 359.

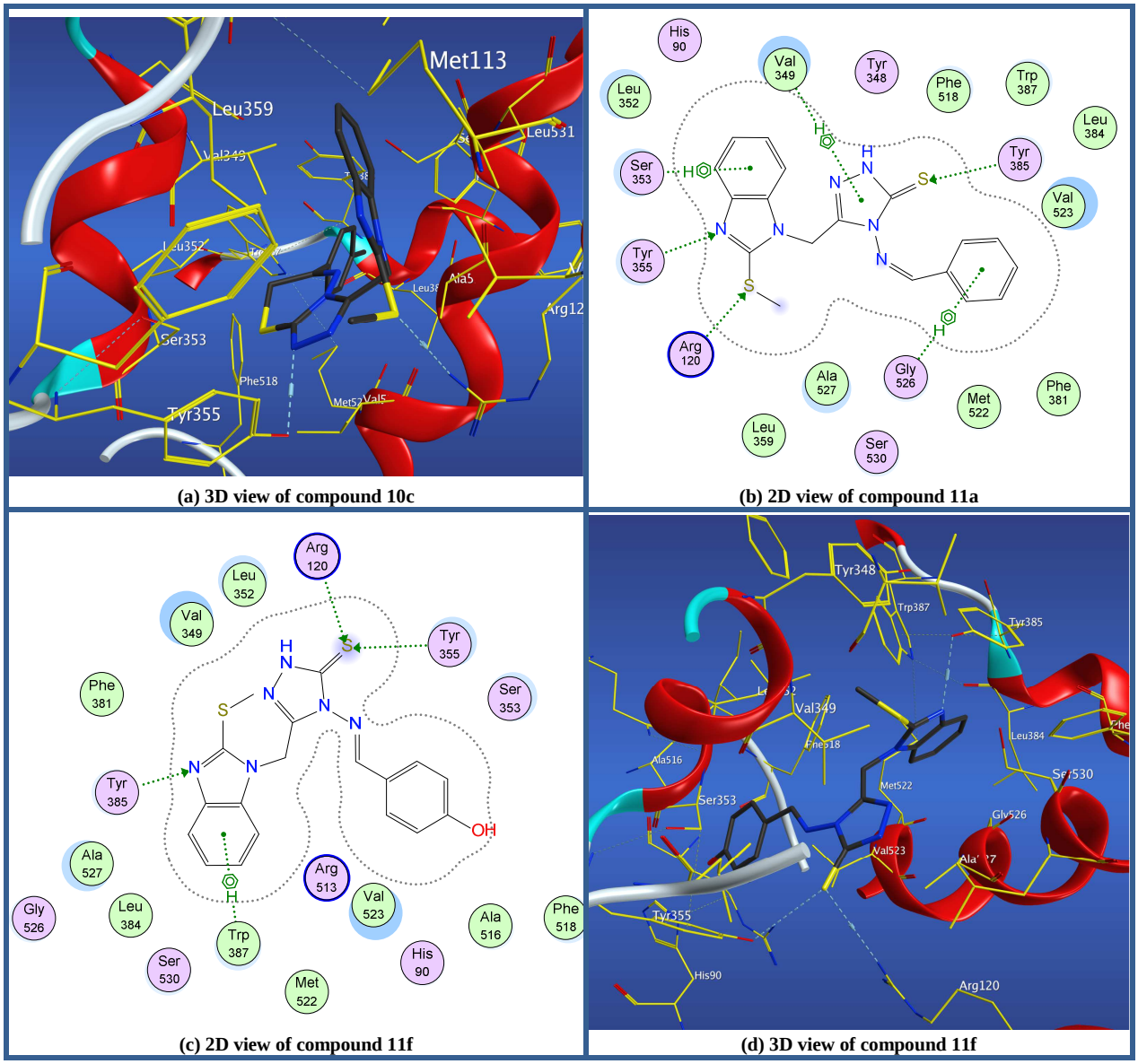


Figure 4: A molecular modelling representation of the predicted binding poses of compounds 11c, 12a and 12f (sticks) within the binding pocket of COX-2 (PDB: 4COX). Hydrogen bond interactions are shown as dashed lines

Docking of 1,2,4-triazole derivative, compound **9**, into COX-2 active site showed low binding score -12.35 that was in consistent with it's low anti-inflammatory activity. It exhibited hydrogen bond interaction of NH_2 group with guanidinium side chain of Arg 120 (2.64 Å) and hydroxyl side chain of Tyr 355 (2.69 Å). Interactions other than hydrogen bonding include hydrophobic and van der Waals' were also observed with different amino acid residues such as Val 349, Phe 518, Leu 359 and Ala 527. Thiadiazine compounds **10a-c** exhibited very good affinity to COX-2 enzyme active site with high binding scores ranging from -14.25 to -14.49 which were in agreement with their high anti-inflammatory activity relative to INM. In addition, they all exhibited hydrogen bond interaction of ligand sulfur atom $S^{b'}$ with guanidinium moiety of Arg 120 (3.36 Å – 3.50 Å) which may be essential to give anti-inflammatory activity. Moreover, compounds **10a** and **10b** showed hydrogen bond interaction of imidazole nitrogen N^{b} with hydroxyl side chain of Tyr 355 (2.46 Å and 2.47 Å respectively). Also triazole Nitrogen N^{a} in compounds **10a** and **10c** displayed hydrogen bond interaction with Val 349 (3.12 Å) and hydroxyl side chain of Tyr 355 (2.75 Å) respectively; **Figure 4**. Compound **10b** showed hydrogen bond interaction of Cl atom with Leu 384 (2.88 Å). Furthermore, interactions other than hydrogen bonding include hydrophobic and van der Waals' were also observed with different amino acid residues at the hydrophobic cleft of the enzyme active site such as Met 522, Val 523, Phe 381, Trp 387, Gly 526, Phe 518, Leu 359 and Ala 527. Moreover, docked compounds **11a-f** exhibited very good affinity to COX-2 enzyme active site with high binding scores ranging from -13.43 to -14.18 which were in consistent with their high anti-inflammatory activity relative to INM. It was observed that, binding of triazole thione atom $S^{a'}$ with guanidinium side chain of Arg 120 (3.53 Å and 3.60 Å) in compounds **11e** and **11f** respectively;

Figure 4, or with hydroxyl side chain of Ser 530 (4.15 Å and 2.80 Å) in compounds **11b** and **11d** respectively; or with hydroxyl side chain of Tyr 355 (3.60 Å and 3.78 Å) in compounds **11e** and **11f** respectively; or with hydroxyl side chain of Tyr 385 (3.48 Å) in compound **11a**; **Figure 5**, is essential for anti-inflammatory activity because compound **11c** that exhibited 59.9% anti-inflammatory activity relative to INM didn't show such interaction. Moreover, Imidazole nitrogen N^b showed hydrogen bond interaction with hydroxyl side chain of Tyr 355 (2.44 Å and 2.45 Å) in compounds **11a** and **11b** respectively; and to Val 523 (3.21 Å) in compound **11d**; and to hydroxyl side chain of Tyr 385 (2.90 Å and 2.87 Å) in compounds **11e** and **11f** respectively; **Figure 4**. Again this interaction also didn't appear in compounds **11c**. On the other hand, Compound **11c** showed hydrogen bond interaction of triazole nitrogen N^a with Tyr 355 (2.37 Å) and interaction of hydrazide nitrogen N-**NH** with His 90 (3.58 Å). Furthermore, ligand sulfur atom $S^{b'}$ showed hydrogen bond interaction with Arg 120 (3.35 Å) in compound **11a**; **Figure 4**. In addition, other hydrogen bond interactions between the docked compounds and the enzyme active site that varied according to the type of substituent on the aromatic moiety: i) Cl atom in compound **11b** showed hydrogen bond interaction with Leu 384 (3.25 Å). ii) Compound **11d** displayed hydrogen bond interaction of OCH_3 group with Arg 120 (2.89 Å). Moreover, interactions other than hydrogen bonding were also observed including arene-H binding of: i) Benzene ring of benzimidazole with either Trp 387 (3.61 Å and 3.59 Å) in compounds **11e** and **11f** respectively or with Ser 353 (3.60 Å) in compound **11a**. ii) Triazole with either Val 349 (3.77 Å) in compound **11a** or with Ser 353 (3.85 Å) in compound **11c**. iii) Phenyl (C_6H_5) with Gly 526 (3.82 Å) in compound **11a**. Similarly, interactions other than hydrogen bonding include hydrophobic and van der waals were also observed. It exhibited hydrophobic interactions with different amino acid residues at the hydrophobic cleft of the enzyme active site such as Met 522, Val 523, Phe 381, Trp 387, Val 349, Gly 526, Phe 518, Leu 352 and Ala 527. It's noteworthy to mention that the designed chemical modification in the structure of the parent triazole **9** improved the anti-inflammatory activity as well as the binding to COX-2 active site.

CONCLUSION

The present study reported the synthesis and structure elucidation of novel derivatives of new 2-alkyl/ aryl substituted mercapto-benzimidazoles that incorporate acylhydrazones, or hetertocyclic ring system; 1,3-dioxoisoindoline, 1,2,4-triazole, and [1,2,4]triazolo[3,4-b][1,3,4]thiadiazine. All compounds were assessed for their anti-inflammatory activity *in vivo* compared to INM. Most of the tested compounds exhibited moderate to high anti-inflammatory activity which in some derivatives surpasses that of INM to a large extent, especially thiadiazine derivatives **10b** and **10c** that showed anti-inflammatory activity (146.75% and 122.39% relative to INM respectively) at the same time interval. likewise, arylmethylidene 1,2,4-triazole derivatives; **11a**, **11e** and **11f** that exhibited anti-inflammatory activity 124.03%, 113.63% and 154.21% respectively after 1 h relative to INM. The most effective derivatives *in vivo* were evaluated for their COX-1/COX-2 inhibitory activity *in vitro*. All tested compounds were effective as COX-1 and COX-2 inhibitors. The [1,2,4]triazolo[3,4-b][1,3,4]thiadiazine derivative; compound **10c** was the most potent COX-2 inhibitor (IC_{50} = 0.28 µmole) among the tested compounds with approximate selectivity ratio of 29.35, that was higher than INM and diclofenac sodium (COX-2 SI = 0.08 and 4.87 respectively), which makes it a good lead-candidate for further optimization. A molecular docking study revealed a direct correlation between the docking affinity score and the biological activity.

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