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Synthesis and Biological Evaluation of New Substituted 2-Mercaptobenzimidazole Derivatives as Anticancer and Anti-Inflammatory Agents

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A series of fourteen 2-mercaptobenzimidazole derivatives (**B1-B14**) bearing a 6-chloro-5-(2,4-dichlorophenoxy) substituent was synthesized through S-alkylation of the corresponding benzimidazole-2-thiol. The derivatives were obtained in 75-85% yields and characterized by FTIR, ^{1}H NMR, ^{13}C NMR and mass spectrometry. Their biological potential was investigated through *in vitro* protein-denaturation and MTT assays. The protein-denaturation assay revealed concentration-dependent anti-inflammatory activity across the tested derivatives. Compounds **B7** and **B12** exhibited the strongest inhibition, followed by compounds **B11** and **B14**, while **B4**, **B6**, **B10** and **B13** displayed moderate activity. At 100 µg/mL, all evaluated derivatives showed appreciable inhibition of protein denaturation, although the reference drug remained more active. In the MTT assay against MCF-7 breast cancer cells, compounds **B6** and **B14** exhibited the strongest concentration-dependent cytotoxic responses among the synthesized derivatives over 20-100 µg/mL. The findings identify compounds **B7** and **B12** as promising anti-inflammatory candidates and compounds **B6** and **B14** as potential antiproliferative leads, providing a basis for further mechanistic and pharmacological investigation.

Keywords: 2-Mercaptobenzimidazole, Anti-inflammatory, Anticancer, Alkyl halides.

INTRODUCTION

2-Mercaptobenzimidazole scaffold has long been recognized as a versatile building block in medicinal chemistry [1]. Over the years, investigators have extensively explored its derivatives, uncovering a remarkable diverse range of biological activity including antibacterial [2], anti-inflammatory [3], antifungal [4], and analgesic [5] actions. Its reach extends further into neurological research, showing promise as anxiolytic [6], antimicrobial [7], anticonvulsant [8] and neurotropic [9] agents.

One of the most effective ways to modify this core is through the alkylation of 2-mercaptobenzimidazole with various alkyl or aryl halides to yield thioether compounds [10]. This approach has already been validated by the clinical success of anti-ulcer drugs like omeprazole, lansoprazole, rabeprazole, ilaprazole and esomeprazole [11], which are essential for suppressing gastric acid secretion. Beyond gastric health, recent efforts have focused on both S- and N-alkyl derivatives for their -glucosidase inhibitory effects [11]. The therapeutic potential of this class continues to grow, with derivatives being screened for antidiabetic and anticonvulsant activities, as well as their role in DNA cleavage studies [12]. Furthermore, benzimidazole-based compounds, particularly 2-mercapto-benzimidazolium salts, have shown significant potential as inhibitory and cytotoxic agents [13]. Significant work by Khalifa [14] recently described the synthesis of new 2-mercaptomethylbenzimidazole scaffolds as potential antibacterial, antioxidant and cytotoxic agents. Inspired by these diverse applications, we have synthesized a novel series of substituted mercaptobenzimidazoles. This work began with the synthesis of the key intermediate, 6-chloro-5-(2,4-dichlorophenoxy)-1*H*-benzo[*d*]imidazole-2-thiol, which was synthesized from 4-chloro-5-(2,4-dichlorophenoxy)benzene-1,2-diamine using carbon disulfide in methanolic NaOH.

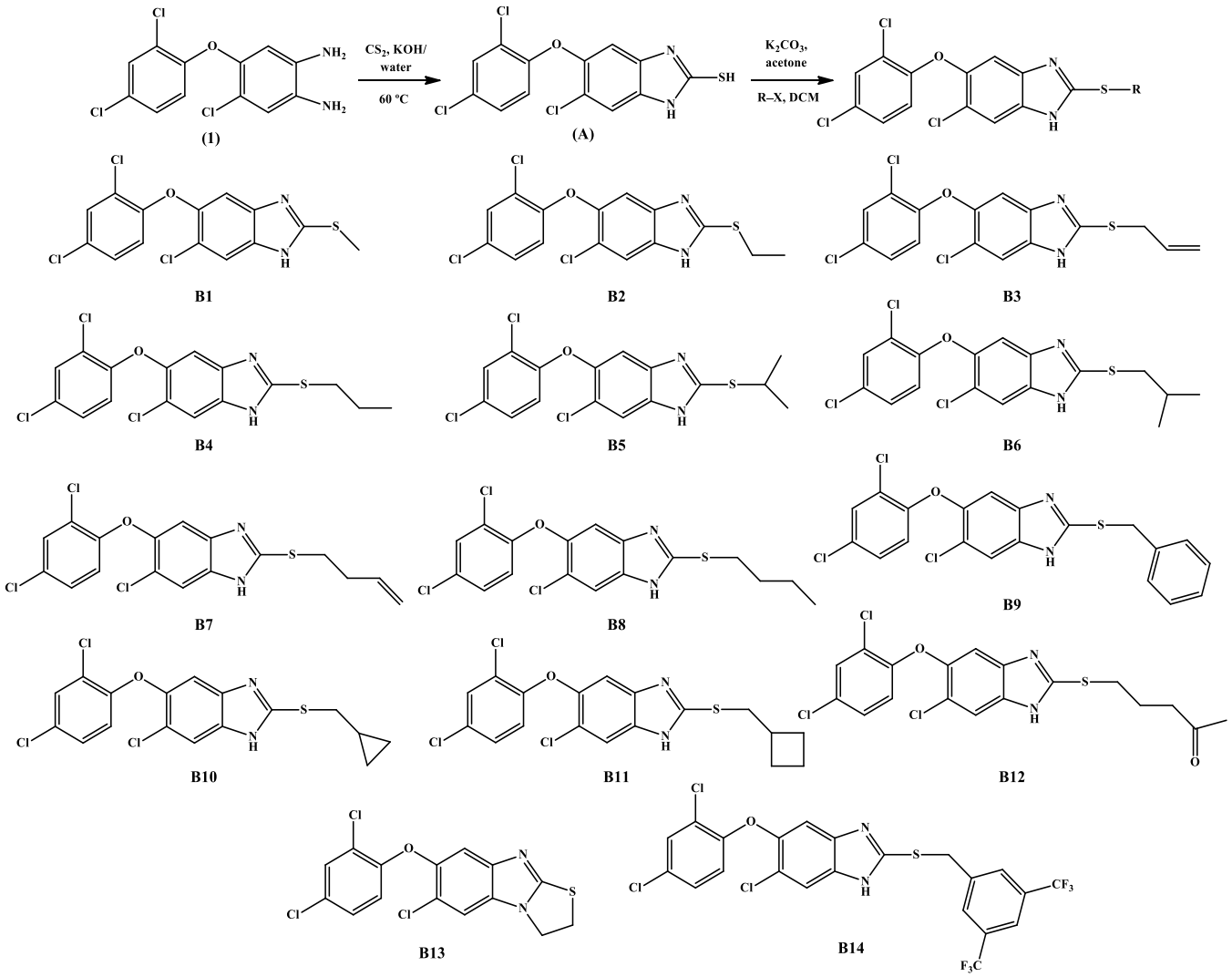
EXPERIMENTAL

All the reagents and solvents used for the synthesis were obtained from Molychem, TCI (India) and Spectrochem and

were used as such without further purification. The melting point of all compounds was determined on the Campbell Model PMB-2 apparatus and uncorrected. IR spectra were measured on FT/IR-4700 type A spectrophotometer with ATR-PRO-ONE. ^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 using the TMS as an internal standard on a Bruker 400 MHz FT-NMR spectrophotometer, respectively. Mass spectra of representative compounds were recorded on a Waters Acquity TQ-XS spectrometer. Thin-layer chromatography was performed on pre-coated gel 60 F_{254} aluminium sheets (E. Merck, Germany) using various solvent systems and spots were identified by UV light, $KMnO_4$ and iodine stains.

Synthesis of 6-chloro-5-(2,4-dichlorophenoxy)-2-(alkylthio)-1*H*-benzo[*d*]imidazole (B1-B14): A solution of 2-chloro-4,5-diamino-1-(2,4-dichlorophenoxy)benzene (**1**, 50 g, 0.165 mol) in 250 mL of MeOH:H_2O (80:20), KOH (14.77g, 0.268 mol) and CS_2 (15.00 mL, 18.84 g, 0.248 mol) was stirred at 60 ºC for 3 h. After completion of reaction (by TLC), was cooled. The cold reaction mixture was poured into 600 mL of H_2O and treated with 20% AcOH solution to a pH 5-6. The precipitate was separated, washed with water and dried in an oven at 60 ºC (45.5 g, 80%). Isolated product **A** was sufficiently pure and used without purification as white solid (m.p. 310-312 ºC). A solution of **A** (4.3 mmol) in acetone (20 mL) was treated with a powdered K_2CO_3 (8.6 mmol) at room temperature. The yellow solution formed was slowly treated with appropriate alkyl halide (4.3 mmol) at 25-30 ºC till completion of reaction by TLC. The solvent was evaporated *in vacuo*, followed by the addition of dichloromethane and water. Dichloromethane layer washed with saturated $NaHCO_3$ solution, concentrated to residue and purified by using column chromatography afford to obtain 1.2 g of the title compound off white solid/liquid in pure state (**Scheme-I**).

6-Chloro-5-(2,4-dichlorophenoxy)-2-(methylthio)-1*H*-benzo[*d*]imidazole (B1): Off white crystals; yield: 82%; m.p.: 182-184 ºC, IR (ATR, ν_{max}, cm^{-1}): 3464 (-NH), 3022 (Ar-CH), 2927 (aliphatic-CH), 1622 (C=N), 1578 (C=C), 1253 (O-Ar), 1234 (-C-S), 1174 (dichloro), 800-600 (mono chloro-); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.502 (s, 3H, -S-CH_3), 6.706-7.721 (m, 5H-Ar, 2.8, 8.8 Hz), 12.847 (s, 1H, -NH benzimidazole); ^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 154.640, 152.734, 145.2, 130.389-118.510, 14.272; Mass (ESI$^+$): *m/z* 359.36 $[M+H]^+$, 361.36 $[M+H+2]^+$, 363.35 $[M+H+4]^+$.



Scheme-I: Synthesis of substituted 2-mercaptobenzimidazole derivatives (B1-B14)

6-Chloro-5-(2,4-dichlorophenoxy)-2-(ethylthio)-1*H*-benzo[*d*]imidazole (B2): Off white crystals; yield: 80%; m.p.: 146-148 ºC, IR (ATR, ν_{max}, cm^{-1}): 3459 (-NH), 3092 (Ar-CH), 2924 (aliphatic-CH), 1622 (C=N), 1580 (C=C), 1257 (O-Ar), 1221 (-C-S), 1149 (dichloro), 800-600 (mono chloro-); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 1.187-1.385 (t, 3H, -CH_3, 7.6 Hz), 3.245-3.301 (q, 2H, -CH_2, 7.6 Hz), 6.709-7.764 (m, 5H-Ar, 1.2, 8 Hz), 12.777-12.824 (2s, 1H, -NH benzimidazole); ^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 153.830, 153.380, 152.697, 145.317-103.958, 26.025, 15.578; Mass (ESI$^+$): *m/z* 373.38 $[M+H]^+$, 375.37 $[M+H+2]^+$, 377.36 $[M+H+4]^+$.

2-(Allylthio)-6-chloro-5-(2,4-dichlorophenoxy)-1*H*-benzo[*d*]imidazole (B3): Off white crystals; yield: 80%; m.p.: 152-154 ºC, IR (ATR, ν_{max}, cm^{-1}): 3459 (-NH), 3087 (Ar-CH), 2927 (aliphatic-CH), 1627 (C=N), 1580 (C=C), 1250 (O-Ar), 1219 (-C-S), 1149 (dichloro), 800-600 (mono chloro-); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 3.956-3.973 (d, 2H, -CH_2, 6.8 Hz), 5.107-5.349 (m, 2H, =CH_2, 10, 16 Hz), 5.948-6.050 (m, 1H, =CH-, 6.8, 7.2 Hz), 6.725-7.726 (m, 5H Ar, 2.4, 8.8 Hz), 12.844 (s, 1H, -NH benzimidazole); ^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 152.853, 152.684, 145.370-118.659, 34.252; Mass (ESI$^+$): *m/z* 385.38 $[M+H]^+$, 387.38, $[M+H+2]^+$, 389.38, $[M+H+4]^+$.

6-Chloro-5-(2,4-dichlorophenoxy)-2-(propylthio)-1*H*-benzo[*d*]imidazole (B4): Off white crystals; yield: 83%; m.p.: 138-140 ºC, IR (ATR, ν_{max}, cm^{-1}): 3435 (-NH), 3090 (Ar-CH), 2927 (aliphatic-CH), 1625 (C=N), 1578 (C=C), 1252 (O-Ar), 1232 (-C-S), 1149 (dichloro), 800-600 (mono chloro-); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 0.980-0.999 (t, 3H, -CH_3, 7.2 Hz), 1.699-1.790 (m, 2H, -CH_2, 7.2 Hz), 3.244-3.280 (t, 2H, -CH_2-, 7.2 Hz), 6.732-7.743 (m, 5H-Ar, 2.8, 8.8 Hz), 12.796 (s, 1H, -NH benzimidazole); ^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 153.71, 152.71, 145.27, 130.40, 118.65, 33.48, 23.11, 13.50; ESI-MS (*m/z*): 387.38 $[M+H]^+$, 389.41 $[M+H+2]^+$.

6-Chloro-5-(2,4-dichlorophenoxy)-2-(isopropylthio)-1*H*-benzo[*d*]imidazole (B5): White crystals; yield: 80%; m.p.: 168-170 ºC, IR (ATR, ν_{max}, cm^{-1}): 3099 (Ar-CH), 2964 (aliphatic-CH), 1626 (C=N), 1580 (C=C), 1248 (O-Ar), 1229 (-C-S), 1150 (dichloro), 800-600 (mono chloro-); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 1.399-1.416 (d, 6H, 2-CH_3, 6.8 Hz), 3.952-4.020 (m, 1H, -CH, 6.8 Hz, 6.727-7.729 (m, 5H-Ar, 2.4, 8.8 Hz); ^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 152.701, 145.344, 130.393-118.660, 37.784, 23.697; ESI-MS (*m/z*): 385.39 $[M-H]^-$.

6-Chloro-5-(2,4-dichlorophenoxy)-2-(isobutylthio)-1*H*-benzo[*d*]imidazole (B6): White crystals; yield: 85%; m.p.: 148-150 ºC, IR (ATR, ν_{max}, cm^{-1}): 3390 (-NH), 3031 (Ar-CH), 2924 (aliphatic-CH), 1624 (C=N), 1578 (C=C), 1251 (O-Ar), 1227 (-C-S), 1137 (dichloro), 800-600 (mono chloro-); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 0.998-1.014 (d, 6H, 2-CH_3, 6.4 Hz), 1.922 -2.022 (m, 1H, -CH, 6.8 Hz), 3.198-3.215 (t, 2H, -CH_2-, 6.8 Hz), 6.729-7.747 (m, 5H-Ar, 2.4, 8.8 Hz); ^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 153.88, 152.70, 145.29, 130.41, 118.69, 40.60, 28.59, 21.94; MS ESI-MS (*m/z*): 401.42 $[M+H]^+$, 403.42 $[M+H+2]^+$.

2-(But-3-en-1-ylthio)-6-chloro-5-(2,4-dichlorophenoxy)-1*H*-benzo[*d*]imidazole (B7): Off white crystals; yield: 78%; m.p.: 144-146 ºC, IR (ATR, ν_{max}, cm^{-1}): 3433 (-NH), 3087 (Ar-CH), 2927 (aliphatic-CH), 1627 (C=N), 1578 (C=C), 1469, 1412 (-NH bending), 1251 (O-Ar), 1221 (-C-S), 1143 (dichloro), 800-600 (mono chloro-); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 2.476-2.493 (m, 2H, -CH_2, 6.8 Hz), 3.349-3.377 (t, 2H, -CH_2, 7.2 Hz), 5.060-5.150 (m, 2H, -CH_2-terminal-, 10.4, 15.6 Hz), 5.819-5.920 (m, 1H-CH-6.4 Hz), 6.746-7.771 (m, 5H-Ar, 6.4 Hz), 12.806 (s, -NH, benzimidazole); ^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 153.38, 152.70, 145.33, 118.55, 117.12, 112, 110.63, 103.96 40.60, 33.71, 30.77; ESI-MS (*m/z*): 399.39 $[M+H]^+$, 401.39 $[M+H+2]^+$.

2-(Butylthio)-6-chloro-5-(2,4-dichlorophenoxy)-1*H*-benzo[*d*]imidazole (B8): Off white crystals 78%; m.p.: 12-128 ºC, IR (ATR, ν_{max}, cm^{-1}): 3460 (-NH), 3084 (Ar-CH), 2925 (aliphatic-CH), 1624 (C=N), 1577 (C=C), 1250 (O-Ar), 1220 (-C-S), 1140 (dichloro), 800-600 (mono chloro-); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 0.868-0.905 (t, 3H, -CH_3, 7.6 Hz), 1.367-1.456 (m, 2H, -CH_2, 7.2, 7.6 Hz), 1.650-1.724 (m, 2H, -CH_2, 7.2, 7.6 Hz), 3.254-3.290 (t, 2H, -S-CH_2-, 7.2 Hz), 6.719-7.718 (m, 5H-Ar, 2.4, 7.2 Hz), 12.782 (s, 1H, -NH benzimidazole); ^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 153.635, 152.720, 145.267, 130.381-103.915, 31.734, 31.292, 26.799, 21.713, 13.912; ESI-MS (*m/z*): 401.42 $[M+H]^+$, 403.42 $[M+H+2]^+$, 405.39 $[M+H+4]^+$.

2-(Benzylthio)-6-chloro-5-(2,4-dichlorophenoxy)-1*H*-benzo[*d*]imidazole (B9): Off white crystals; yield: 75%; m.p.: 76-78 ºC, IR (ATR, ν_{max}, cm^{-1}): 3406 (-NH), 3026 (Ar-CH), 2923 (aliphatic-CH), 1623 (C=N), 1578 (C=C), 1251 (O-Ar), 1227 (-C-S), 1137 (dichloro) and 800-600 (mono chloro-); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 4.579 (s, 2H, -CH_2-Ph), 6.732-7.745 (m, 10H-Ar, 2.4, 8.8 Hz), 12.686 (s, 1H, -NH benzimidazole), ^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 152.686, 145.399, 137.846, 118.707, 35.53; ESI-MS (*m/z*): 435.40 $[M+H]^+$, 437.40 $[M+H+2]^+$, 439.38, $[M+H+4]^+$.

6-Chloro-2-((cyclopropylmethyl)thio)-5-(2,4-dichlorophenoxy)-1*H*-benzo[*d*]imidazole (B10): Off white crystals; yield: 85%; m.p.: 138-140 ºC, IR (ATR, ν_{max}, cm^{-1}): 3456 (-NH), 1469, 1412 (-NH bending), 3087 (Ar-CH), 2924 (aliphatic-CH), 1624 (C=N), 1578 (C=C), 1250 (O-Ar), 1221 (-C-S), 1142 (dichloro), 800-600 (mono chloro-); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 0.552-0.572 (m, 4H, -CH_2-CH_2), 0576-0.586 (m, 1H, -CH), 3.242-3.384 (d, 2H, -S-CH_2), 6.726-7.743 (m, 5H-Ar) ^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 153.57, 152.52, 145.30-117.13, 37.28, 26.80, 18.94, 11.49, 6.17; ESI-MS (*m/z*): 399.39 $[M+H]^+$, 401.41 $[M+H+2]^+$, 403.39, $[M+H+4]^+$.

6-Chloro-2-((cyclobutylmethyl)thio)-5-(2,4-dichlorophenoxy)-1*H*-benzo[*d*]imidazole (B11): White crystals; yield: 84%; m.p.: 140-142 ºC, IR (ATR, ν_{max}, cm^{-1}): 1470 and 1412 (-NH bending), 3021 (Ar-CH), 2924 (aliphatic-CH), 1618 (C=N), 1578 (C=C), 1257 (O-Ar), 1224 (-C-S), 1135 (dichloro), 800-600 (mono chloro-); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 1.177-2.059 (m, 7H, -CH_2-CH_2, 3.6, 5.2, 6.8, 10.4 Hz, cyclobutyl), 3.377 (d, 2H, -S-CH_2), 6.730-7.741 (m, 5H-Ar, 2.4, 8.8 Hz), 12.791 (s, -NH, benzimidazole); ^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 153.66, 152.71, 145.29, 104.2, 37.58, 34.98, 27.40, 17.76; ESI-MS (*m/z*): 413.40 $[M+H]^+$, 415.39 $[M+H+2]^+$, 417.40, $[M+H+4]^+$.

5-((6-Chloro-5-(2,4-dichlorophenoxy)-1*H*-benzo[*d*]-imidazol-2-yl)thio)pentan-2-one (B12): Yellow oil; yield: 81%; IR (ATR, ν_{max}, cm^{-1}): 3088 (Ar-CH), 2923 (aliphatic-CH), 1704 (-C=O), 1624 (C=N), 1578 (C=C), 1470, 1413 (-NH bending), 1252 (O-Ar), 1227 (-C-S), 1138 (dichloro), 800-600 (mono chloro-); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 1.871 -1.942 (m, 2H, -CH_2-, 6.8 and 7.2 Hz, central), 2.10 (s, 2H, -CH_3-attached to carbonyl), 2.590-2.625 (t, 2H, -CH_2-, next to carbonyl), 3.354-3.372 (t, 2H, -S-CH_2, 7.2 Hz), 6.702-7.747 (m, 5H-Ar, 2.4 and 8.8 Hz), 12.791 (s, -NH, benzimidazole); ^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 208.22, 152.69, 145.34, 130.41-103.96, 41.76-40.18, 35.82-23.91; ESI-MS (*m/z*): 429.40 $[M+H]^+$, 431.40 $[M+H+2]^+$, 433.40 $[M+H+4]^+$.

6-Chloro-7-(2,4-dichlorophenoxy)-2,3-dihydrobenzo-[4,5]imidazo[2,1-*b*]thiazole (B13): White solid; yield: 86%; m.p.: 95-97 ºC, IR (ATR, ν_{max}, cm^{-1}): 3399 (-NH), 3060 (Ar-CH), 2928 (aliphatic-CH), 1620 (C=N), 1575 (C=C), 1255 (O-Ar), 1237 (-C-S), 1130 (dichloro) and 800-600 (mono chloro-); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 4.012-4.081 (m, 2H, -CH_2-, 6.8 and 7.2 Hz), 6.683-7.796 (m, 5H-Ar, 2.4 and 8 Hz) ^{13}C NMR (400 MHz, DMSO-d_6): δ 161.66, 161.30, 152.74, 148.67, 145.79-103.74, 44.65-40.19, 35.69-22.57; ESI-MS (*m/z*): 371.35 $[M+H]^+$, 373.34 $[M+H+2]^+$, 375.32, $[M+H+4]^+$.

2-((3,5-*Bis*(trifluoromethyl)benzyl)thio)-6-chloro-5-(2,4-dichlorophenoxy)-1*H*-benzo[*d*]imidazole (B14): White crystals; yield: 75%; m.p.: 130-132 ºC, IR (ATR, ν_{max}, cm^{-1}): 3092 (Ar-CH), 2926 (aliphatic-CH), 1622 (C=N), 1575 (C=C), 1473, 1417 (-NH bending), 1273 (O-Ar), 1223 (-C-S), 1124 (dichloro), 800-600 (mono chloro-); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 4.733 (s, 2H, -CH_2-S-), 6.673-8.222 (m, 8H-Ar, 2 and 8 Hz) ^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 161.66, 161.30, 152.74, 148.67, 145.79, 103.74, 44.65, 40.19, 35.69, 22.57; ESI-MS (*m/z*): 571.31 $[M+H]^+$, 573.32 $[M+H+2]^+$, 575.33, $[M+H+4]^+$.

***In vitro* anti-inflammatory activity by protein denaturation method:** The reaction mixture (10 mL) consisted of 0.4 mL of egg albumin (fresh hen's egg), 5.6 mL of phosphate buffered saline (PBS, pH 6.4) and 100 µL of different concentration sample [15]. Similar volume of double-distilled water served as control. Then the mixtures were incubated at (37 ºC ± 2) in an incubator for 15 min and then heated at 70 ºC for 5 min. After cooling, their absorbance was measured at 660 nm by using vehicle as blank. Diclofenac sodium at the concentration was used as reference drug and treated similarly for determination of absorbance. The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\text{Inhibition (\%)} = \frac{C - T}{C}$$

where, T = absorbance of test sample C = absorbance of control.

MTT assay

Experimental procedure: MCF-7 cells were incubated at a concentration of 1×10^4 cells/mL in culture medium for 24 h at 37 ºC and 5% CO_2 and were seeded at a concentration (100 µL) 10^4 cells/well) in 100 µL culture medium and 20, 40, 60, 80, 100 µg/mL of samples into microplates, respectively (tissue culture grade and 96 wells). Control wells were incubated with DMSO (0.2% in PBS) and cell line [16]. All samples were incubated in triplicate. Controls were maintained to determine the control cell survival and the percentage of live cells after culture. Cell cultures were incubated for 24 h at 37 ºC and 5% CO_2 in CO_2 incubator. After incubation, the medium was completely removed, added 20 µL of MTT reagent (5 mg/mL PBS). After addition of MTT, cells incubated for 4 h at 37 ºC in CO_2 incubator. Observed the wells for formazan crystal formation under microscope. The yellowish MTT was reduced to dark coloured formazan by viable cells only. After removing the medium completely. Added 200 µL of DMSO (kept for 10 min) and incubate at 37 ºC (wrapped with aluminum foil). Triplicate samples were analyzed by measuring the absorbance of each sample by a micro plate reader at a wavelength of 550 nm.

RESULTS AND DISCUSSION

2-Mercaptobenzimidazole derivatives (**B1-B14**) were synthesized by reacting 6-chloro-5-(2,4-dichlorophenoxy)-1*H*-benzo[*d*]imidazole-2-thiol with various alkyl halides in acetone under basic conditions at room temperature. The target compounds were obtained in moderate to good yields of 75-85%. IR spectra displayed characteristic Ar–O–Ar stretching near 1250 cm^{-1}, N–H stretching bands in the 3400-3200 cm^{-1} region and C–S absorption around 1230 cm^{-1}. In the ^{1}H NMR spectra, the imidazole N–H proton resonated at δ 12.4-12.8 ppm, while the S–CH_2 protons appeared at δ 3.7-3.8 ppm. The aromatic protons occurred as multiplets within δ 6.5-8.5 ppm. The ^{13}C NMR spectra contained resonances near δ 153 and 152 ppm attributable to the quaternary carbon attached to sulfur and the Ar–O–Ar carbon, respectively, with terminal –CH_3 carbon signals near δ 13 ppm. Mass spectra displayed molecular ion peaks consistent with the expected trichloro isotope pattern, supporting the assigned structures.

Anti-inflammatory activity: The protein-denaturation assay was used to assess the anti-inflammatory potential of **B1-B14**. The synthesized derivatives exhibited concentration dependent inhibition of albumin denaturation across the tested concentration range. Compounds **B7** and **B12** occupied the upper activity range, followed by compounds **B11** and **B14**. Compounds **B4**, **B6**, **B10** and **B13** displayed intermediate inhibition, whereas the remaining derivatives showed comparatively weaker responses (Table-1). At 100 µg/mL, all tested compounds exerted appreciable inhibition of protein denaturation. Diclofenac sodium maintained the highest inhibitory response across the concentration range and served as the more potent reference under the experimental conditions. The activity profile of the synthesized derivatives points to their ability to stabilize protein structures against denaturation, a property commonly associated with anti-inflammatory action.

Anticancer activity: The antiproliferative potential of synthesized compounds **B1-B14** was examined against MCF-7 breast cancer cells using the MTT assay at concentrations of 20-100 µg/mL. Cell viability decreased progressively with increasing compound concentration for the tested derivatives

TABLE-1
ANTI-INFLAMMATORY ACTIVITY DATA OF THE TESTED COMPOUNDS BY PROTEIN DENATURATION ASSAY

Compound	Conc. (µg/mL)	Protein denaturation assay					
		Absorbance at 660 nm				Inhibition (%)	IC_{50} (µg/mL)
		Test 1	Test 2	Test 3	Mean		
Control		1.54	1.54	1.54	1.54	–	–
Standard (Diclofenac Sodium)	20	1.20	1.20	1.22	1.20	22.08	52.21
	40	0.91	0.89	0.93	0.91	40.91	
	60	0.67	0.65	0.63	0.65	57.79	
	80	0.40	0.37	0.44	0.40	74.03	
	100	0.21	0.18	0.23	0.20	87.01	
B4	20	1.39	1.35	1.42	1.39	9.74	NE
	40	1.25	1.20	1.28	1.24	19.48	
	60	1.13	1.10	1.17	1.13	26.62	
	80	0.96	0.92	0.99	0.96	37.66	
	100	0.82	0.81	0.85	0.83	46.10	
B6	20	1.45	1.40	1.48	1.44	6.49	NE
	40	1.30	1.35	1.27	1.31	14.94	
	60	1.17	1.15	1.13	1.15	25.32	
	80	1.03	1.09	1.12	1.08	29.87	
	100	0.87	0.84	0.88	0.86	44.16	
B7	20	1.22	1.15	1.18	1.18	23.38	78.70
	40	1.09	1.05	1.03	1.06	31.17	
	60	0.92	0.84	0.95	0.90	41.56	
	80	0.77	0.77	0.72	0.75	51.30	
	100	0.64	0.61	0.58	0.61	60.39	
B10	20	1.34	1.31	1.37	1.34	12.99	98.05
	40	1.20	1.25	1.18	1.21	21.43	
	60	1.03	1.02	1.08	1.04	32.47	
	80	0.87	0.84	0.82	0.84	45.45	
	100	0.73	0.71	0.77	0.74	51.95	
B11	20	1.25	1.20	1.27	1.24	19.48	72.21
	40	0.96	0.92	0.90	0.93	39.61	
	60	0.80	0.83	0.87	0.83	46.10	
	80	0.67	0.63	0.66	0.65	57.79	
	100	0.53	0.48	0.45	0.49	68.18	
B12	20	1.14	1.10	1.17	1.14	25.97	58.70
	40	0.88	0.84	0.92	0.88	42.86	
	60	0.75	0.73	0.77	0.75	51.30	
	80	0.61	0.67	0.57	0.62	59.74	
	100	0.48	0.45	0.41	0.45	70.78	
B13	20	1.27	1.25	1.32	1.28	16.88	78.05
	40	1.05	1.02	1.09	1.05	31.82	
	60	0.91	0.93	0.97	0.94	38.96	
	80	0.74	0.71	0.78	0.74	51.95	
	100	0.59	0.53	0.55	0.56	63.64	
B14	20	1.35	1.41	1.33	1.36	11.69	74.81
	40	1.12	1.15	1.11	1.13	26.62	
	60	0.93	0.97	0.98	0.96	37.66	
	80	0.70	0.73	0.64	0.69	55.19	
	100	0.54	0.59	0.51	0.55	64.29	

(Table-2). The reference drug exhibited the strongest cytotoxic response, reaching approximately 85-88% inhibition at 100 µg/mL. Among the synthesized compounds, **B6** and **B14** exhibited the most pronounced inhibition across the concentration range. Their responses remained below that of the reference drug but are sufficiently distinct from the less active derivatives to merit further evaluation. The activity of **B6** and **B14** may reflect the influence of their respective thioether substituents on cellular interactions, although a definitive structure–activity relationship cannot be established from the present dataset alone.

Conclusion

Fourteen 2-mercaptobenzimidazole derivatives (**B1-B14**) were synthesized in good yields and structurally characterized by spectroscopic techniques. Biological screening estab-

TABLE-2
CYTOTOXIC EFFECTS OF THE TESTED COMPOUNDS AGAINST MCF-7 BREAST CANCER CELLS.

Compd.	Conc. (µg/mL)	Optical density			Mean	Inhibition (%)	Viability (%)	IC_{50} (µg/mL)
Control			1.482		–	–	–	–
Standard	20	0.916	0.915	0.916	0.915	38.25	61.75	
5-Flurouracil	40	0.732	0.729	0.734	0.731	50.67	49.33	39.33
	60	0.507	0.504	0.502	0.504	65.99	34.01	
	80	0.315	0.312	0.314	0.313	78.87	21.13	
	100	0.208	0.205	0.207	0.206	86.09	13.91	
B4	20	1.469	1.461	1.463	1.464	1.21	98.79	NE
	40	1.365	1.362	1.369	1.365	7.89	92.11	
	60	1.263	1.266	1.259	1.262	14.84	85.16	
	80	1.119	1.121	1.127	1.122	24.29	75.71	
	100	0.888	0.882	0.892	0.887	40.15	59.85	
B6	20	1.229	1.232	1.227	1.229	17.07	82.93	78.65
	40	0.972	0.978	0.981	0.977	34.08	65.92	
	60	0.841	0.844	0.839	0.841	43.25	56.75	
	80	0.716	0.721	0.728	0.721	51.35	48.65	
	100	0.554	0.559	0.551	0.554	62.62	37.38	
B7	20	1.402	1.406	1.409	1.405	5.20	94.80	99.39
	40	1.281	1.289	1.278	1.282	13.50	86.50	
	60	1.167	1.169	1.162	1.166	21.32	78.68	
	80	0.912	0.909	0.905	0.908	38.73	61.27	
	100	0.728	0.732	0.738	0.732	50.61	49.39	
B10	20	1.412	1.409	1.402	1.407	5.06	94.94	NE
	40	1.386	1.391	1.396	1.391	6.14	93.86	
	60	1.236	1.239	1.233	1.236	16.60	83.40	
	80	1.101	1.106	1.109	1.105	25.44	74.56	
	100	0.839	0.833	0.841	0.837	43.52	56.48	
B11	20	1.246	1.252	1.242	1.246	15.92	84.08	94.87
	40	1.196	1.192	1.197	1.195	19.37	80.63	
	60	0.859	0.852	0.861	0.857	42.17	57.83	
	80	0.748	0.741	0.744	0.744	49.80	50.20	
	100	0.662	0.669	0.666	0.665	55.13	44.87	
B12	20	1.277	1.273	1.279	1.276	13.90	86.10	NE
	40	1.156	1.153	1.1545	1.154	22.13	77.87	
	60	0.952	0.948	0.954	0.951	35.83	64.17	
	80	0.792	0.796	0.788	0.792	46.56	53.44	
	100	0.537	0.539	0.533	0.536	63.83	36.17	
B13	20	1.372	1.379	1.375	1.375	7.22	92.78	NE
	40	1.293	1.294	1.287	1.291	12.89	87.11	
	60	1.195	1.199	1.192	1.195	19.37	80.63	
	80	1.129	1.123	1.127	1.126	24.02	75.98	
	100	0.911	0.916	0.918	0.915	38.26	61.74	
B14	20	0.940	0.943	0.949	0.944	36.30	63.70	51.77
	40	0.775	0.779	0.771	0.775	47.71	52.29	
	60	0.617	0.619	0.621	0.619	58.23	41.77	
	80	0.563	0.569	0.566	0.566	61.81	38.19	
	100	0.464	0.462	0.469	0.465	68.62	31.38	

lished distinct activity profiles among the derivatives. Compounds **B7** and **B12** were the most active compounds in the protein-denaturation assay, with **B11** and **B14** occupying the next activity range. **B6** and **B14** exhibited the strongest antiproliferative responses against MCF-7 cells, although their activity remained lower than that of the reference drug. The results identify **B7** and **B12** as promising anti-inflammatory leads and **B6** and **B14** as candidates for further anticancer investigation.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

DECLARATION OF AI-ASSISTED TECHNOLOGIES

During the preparation of this manuscript, the authors used an AI-assisted tool(s) to improve the language and no

data, results or scientific conclusions were generated by AI. All analyses, interpretations and conclusions are the authors' own. The authors reviewed and edited the content and take full responsibility for the published work.

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