Heterocyclic Letters
Vol. 13| No.2|375-390|February-April|2023
ISSN : (print) 2231–3087 / (online) 2230-9632
CODEN: HLEEAI
http://heteroletters.org

SYNTHESIS OF DIFLUOROMETHOXYBENZIMIDAZOLE CLUBBED CHALCONE DERIVATIVES: A STRATEGIC APPROACH FOR DERIVING IMPROVED CLASS OF IN-VITRO ANTIMICROBIAL AGENTS

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ABSTRACT: The present study reports the synthesis of novel benzimidazole clubbed-chalcone derivatives possessing substituted *difluoromethoxy* (-$OCHF_2$) functional group. Formation of the titled derivatives was confirmed using IR, ^{1}H NMR, ^{13}C NMR, mass spectra, and elemental analysis. These were screened for therapeutic potential against a broad spectrum of bacteria and fungi and their minimum inhibitory concentration (MIC) values were recorded. Molecular docking analysis against critical microbial target *DNA gyrase* was performed to aid in understanding the possible binding modes and interactions between the synthesized compounds and protein target. Structure-Activity Relationship (SAR) study could provide insights into the structural fragments (functional group of an atom) responsible for the *in-vitro* antimicrobial activity.

KEYWORDS: fluorine, molecular docking, SAR study, antibacterial, antifungal, *DNA gyrase*

INTRODUCTION: With a continuous effort to improve the antibacterial and antifungal potential of our previously synthesized benzimidazole clubbed chalcone derivatives[i-ii], a series bearing *difluoromethoxy* fragment is reported here. It is observed that the incorporation of fluorine atoms enhances the biological activity of a chemical entity due to its property of being highly electronegative and highly polarized[iii]. Thus fluorine atom(s) are found to be lucrative substituents in the optimization of therapeutic properties of drug molecules.
Chalcone is a very versatile moiety as it acts as a pivotal scaffold for physiologically active compounds and substrates for the evaluation of various organic syntheses. Presence of reactive α, β-unsaturated keto group in chalcones is found to be responsible for their biological activity.

Some of the substituted vinyl chalcones have proved to be TNF-α and IL-6 inhibitors[iv]. Chalcones act as potent hydrophobic modulators of P-glycoprotein-mediated multidrug resistance[v]. In view of biological evaluation, these motifs have been reported to possess an array of important therapeutic properties, such as analgesic[vi], cardiovascular[vii], cytotoxic[viii-xi], antimalarial[xii-xiii], antileishmanial[xiv-xv], anti-inflammatory[xvi-xvii], anti-HIV[xviii], antifungal[xix], antitubercular[xx-xxi], antioxidant[xxii-xxiii] and as tyrosine kinase inhibitors[xiv]. Being such a biologically privileged scaffold, chalcones have always been the center of attraction for medicinal chemists all over the globe, and therefore to synthesize them, several strategies have been developed.

In recent times, benzimidazole-bearing chalcones have emerged as good antimicrobials[i,ii,xxv]. Benzimidazole derivatives when combined with chalcones have produced compounds possessing antiviral properties[xxvi]. The reason for numerous biological activities and functions of benzimidazole derivatives lies in the fact that they are structural isosteres of naturally occurring nucleotides that interact easily with the biopolymers of the living system. The incorporation of such biologically accepted pharmacophore has made it a versatile heterocyclic moiety possessing a wide spectrum of biological activities such as antimicrobial [xxvii], antihistamine[xxviii], neurotropic[xxix] and anticonvulsant[xxx] activities.

Several methods for the synthesis of chalcone have been well documented in the literature, among them, the most widely used is base-catalyzed condensation[xxxi] whereas methodologies for the synthesis of chalcone using catalysts like Zeolites[xxxii], K_3PO_4[xxxiii] and $BF_3–Et_2O$[xxxiv] as condensing agents have also been reported. In the present work, authors have adapted acid-catalyzed aldol condensation by using thionyl chloride ($SOCl_2$) as condensing agent[xxxv] for the synthesis of the title compounds.

Molecular docking is an important *in silico* technique in structural biology and computer-aided drug design to gauze the feasible binding modes of a putative ligand with a target enzyme of a known three-dimensional structure. Therefore, to understand the interaction of all the synthesized fluoro-benzimidazole clubbed chalcone derivatives with a critical microbial target DNA gyrase, a molecular docking study was performed which could provide insight into the binding mode and interactions within guiding the biological activity.

EXPERIMENTAL

Methods, materials, and physical measurements

Melting points were determined using DBK PROGRESS scientific melting point apparatus and are uncorrected. IR spectra of the synthesized compounds were recorded on a Bruker FTIR spectrometer in the range of 400-4000cm^{-1} using KBr pellets. ^1H NMR and ^{13}C NMR spectra were recorded on Bruker NMR spectrometer - 400MHz or 100 MHz using DMSO-d_6 as a solvent; chemical shifts are expressed as δ values relative to Me_4Si as standard. The mass spectra were recorded on an Applied Biosystem mass spectrometer using the electro-spray ionization method. Elemental analyses were carried out on a Perkin Elmer-2400 CHN analyzer; the results of elemental analyses were in agreement with calculated values. Thin-layer chromatography (TLC) was performed on Merck silica gel 60F254 plates. The spots resolved were visualized under UV radiation.

Synthesis and physical data

Synthesis of *N-(4-acetylphenyl)-2-chloroacetamide*

The compound was obtained by reacting *4-aminoacetophenone* (0.01 mol, 135gm/mol, 1.35gm) with *chloroacetylchloride* (0.015 mol, 113gm/mol, 1.19ml) and *triethylamine* (3-4drops) using toluene (25 ml) as solvent by refluxing the mixture for 4 hrs. The completion of the reaction was monitored using TLC with mobile phase Toluene: Acetone (7:3).

Solid light brown crystals; Yield: 87%; M.P.: 154°C; IR (ATR, cm^{-1}): 740 (C-Cl str.), 1413 (C=C str. aromatic ring), 1640 (C=O str.), 2750 (CH_2str. methylene), 3049 (CH str. aromatic ring), 3232 (NH str.); ^{1}H NMR (400 MHz, DMSOd_6, δ, ppm): 2.56 (3H, s, -C<u>H</u>$_3$), 4.89 (2H, s, -C<u>H</u>$_2$), 7.77 – 8.10 (4H, d, Ar-<u>H</u>), 9.97 (1H, s, -N<u>H</u>); ^{13}C NMR (400 MHz, DMSO-d_6, δ, ppm): 27.2 (q), 43.2 (h), 121.0 (k), 121.0 (o), 129.2 (l), 129.2 (n), 137.2 (m), 142.2 (j), 166.0 (i), 198.2 (p); (MS (m/z): 212 (M^+).

Synthesis of *2-mercapto-5-difluoromethoxy-1H-benzimidazole*

2-mercapto-5-difluoromethoxy-1H-benzimidazole required for the preparation of the precursor of the titles compound was synthesized by the reported linear multistep procedure. [35]

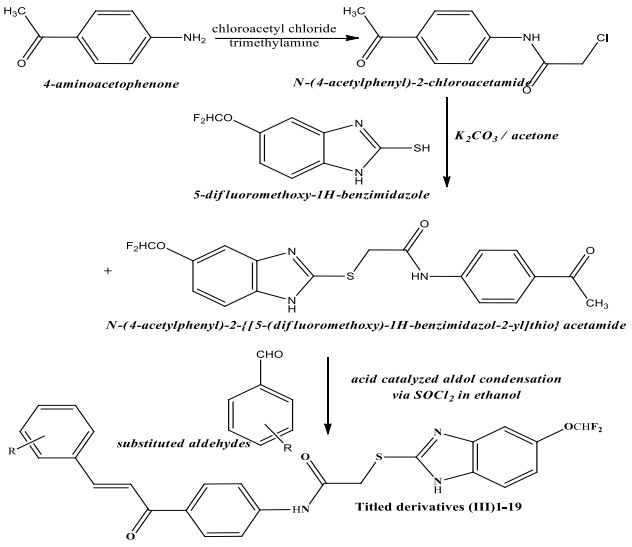
Synthesis of *N-(4-acetylphenyl)-2-{[5-(difluoromethoxy)-1H-benzimidazol-2-yl]thio} acetamide*

To a solution of *2-mercapto-5-difluoromethoxy-1H-benzimidazole* (10 mmol, 2.16 g) and ***N-(4-acetylphenyl)-2-chloroacetamide*** (10 mmol, 2.11 g) in acetone (30 cm^3), K_2CO_3 (15 mmol, 2.07 g) was added. The mixture was stirred at room temperature for 4 hrs. After the completion of the reaction (monitored by TLC), the resultant mixture was poured into ice-cooled water and stirred for half-hour. The white precipitates obtained were filtered, washed with water, and dried. Recrystallization was performed using methanol. The completion of the reaction was monitored using toluene: ethylacetate (4:6).

General procedure for the synthesis of titles compounds

Acid-catalyzed aldol condensation was implemented for the synthesis of final chalcone derivatives **(III)$_{1\text{-}19}$**. To a stirred mixture of compound ***N-(4-acetylphenyl)-2-{[5-(difluoromethoxy)-1H-benzimidazol-2-yl]thio} acetamide*** (0.01mol) and substituted aryl aldehydes (0.01mol) in ethanol (20ml), a catalytic amount of $SOCl_2$ was added drop-wise, and stirring was continued for 2 hrs at room temperature. The reaction mixture was allowed to stand for 12 hrs. After the completion of the reaction (monitored by TLC), the reaction mixture was precipitated by the addition of ice-cold water. The product obtained was filtered, washed with water, and dried. The final products thus obtained were crystallized from alcohol and melting points of each synthesized chalcones were measured.

Scheme-1: Synthesis of titled derivatives (III)1-19



(E)-N-(4-cinnamoylphenyl)-2-(5-(difluoromethoxy)-1H-benzo[d]imidazol-2-ylthio) acetamide (III)$_1$

Yield: 73.77%; M.P.:218°C; IR (ATR, cm^{-1}): 646 (C-S str.), 833 (C-H bend. aromatic ring), 1036 (C-O-C symmetric. Str.), 1135 (-CF_2 str.), 1282 (C-O-C asymmetric. Str.), 1445 (C=C str. aromatic ring), 1487 (C-H bend. methylene group), 1544 (N-H bend. Sec. amine), 1591 (C=C str. conjugated with C=O), 1649 (C=O str. α,β-unsaturated ketone), 2845 (C-H str. methylene group), 3018 (C-H str. aromatic ring); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 4.41 (2H, s, -C<u>H</u>$_2$), 7.19 (1H, s, -OC<u>H</u>F$_2$), 7.39–8.17 (9H, m, Ar-<u>H</u>), 7.73 (1H, d, CO-<u>H</u>C=CH, J=15.6Hz), 7.82 (1H, d, CO-HC=C<u>H</u>, J=15.6Hz), 10.93 (1H, s, -N<u>H</u>), 12.81 (1H, s, benzimidazole-N<u>H</u>); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 38.9 (8), 101.1 (5), 116.4 (2), 121.6 (11), 121.6 (15), 121.5 (17), 112.0 (3), 156.5 (4), 127.8 (22), 128.3 (20), 128.3 (24)128.9 (21), 128.5 (23), 131.1 (14), 131.0 (12), 135.0 (19), 131.0 (1), 140.3 (6), 144.0 (10), 145.5 (18), 147.8 (7), 168.0 (9), 190.0 (16); MS (m/z): 480 (M^+). For $C_{25}H_{19}F_2N_3O_3S$: C, 62.63%; H, 3.96%; N, 8.76%. Found: C, 62.57%; H, 3.91%; N, 8.69%.

(E)-N-(4-(3-(2-chlorophenyl)acryloyl)phenyl)-2-(5-(difluoromethoxy)-1H-benzo[d]imidazol-2-ylthio)acetamide (III)$_2$

Yield: 67.55%; M.P.:217°C; IR (ATR, cm^{-1}): 641 (C-S str.), 831 (C-H bend. aromatic ring), 1040 (C-O-C symmetric. Str.), 1136 (-CF_2 str.), 1279 (C-O-C asymmetric. Str.), 1441 (C=C str. aromatic ring), 1490 (C-H bend. methylene group), 1540 (N-H bend. Sec. amine), 1598 (C=C str. conjugated with C=O), 1659 (C=O str. α,β-unsaturated ketone), 2849 (C-H str. methylene group), 3012 (C-H str. aromatic ring); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 4.42 (2H, s, -C<u>H</u>$_2$), 7.13 (1H, s, -OC<u>H</u>F$_2$), 7.31–8.12 (8H, m, Ar-<u>H</u>), 7.76 (1H, d, CO-<u>H</u>C=CH, J=15.6Hz), 7.88 (1H, d, CO-HC=C<u>H</u>, J=15.6Hz), 10.89 (1H, s, -N<u>H</u>), 12.74 (1H, s, benzimidazole-N<u>H</u>); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 38.9 (8), 101.1 (5), 116.4 (2), 121.6 (11), 121.6 (15), 121.5 (17), 112.0 (3), 156.5 (4), 129.0 (22), 128.3 (20), 134.3 (24), 127.0 (21), 129.5 (23), 131.1 (14), 131.0 (12), 133.4 (19), 131.0 (1), 140.3 (6), 144.0 (10), 145.5 (18), 147.8 (7), 168.0 (9), 190.0 (16); MS (m/z): 514 (M^+).. For $C_{25}H_{18}ClF_2N_3O_3S$: C, 58.42%; H, 3.50%; N, 8.18%. Found: C, 58.35%; H, 3.44%; N, 8.11%.

(E)-N-(4-(3-(3-chlorophenyl)acryloyl)phenyl)-2-(5-(difluoromethoxy)-1H-benzo[d]imidazol-2-ylthio)acetamide (III)$_3$

Yield: 68.70%; M.P.:212°C; IR (ATR, cm^{-1}): 633 (C-S str.), 836 (C-H bend. aromatic ring), 1032 (C-O-C symmetric. Str.), 1132 (-CF_2 str.), 1284 (C-O-C asymmetric. Str.), 1445 (C=C str. aromatic ring), 1481 (C-H bend. methylene group), 1538 (N-H bend. Sec. amine), 1592 (C=C str. conjugated with C=O), 1654 (C=O str. α,β-unsaturated ketone), 2856 (C-H str. methylene group), 3017 (C-H str. aromatic ring); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 4.46 (2H, s, -C<u>H</u>$_2$), 7.08 (1H, s, -OC<u>H</u>F_2), 7.26–8.10 (8H, m, Ar-<u>H</u>), 7.78 (1H, d, CO-<u>H</u>C=CH, J=15.6Hz), 7.82 (1H, d, CO-HC=C<u>H</u>, J=15.6Hz), 10.85 (1H, s, -N<u>H</u>), 12.76 (1H, s, benzimidazole-N<u>H</u>); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 38.9 (8), 101.1 (5), 116.4 (2), 121.6 (11), 121.6 (15), 121.5 (17), 112.0 (3), 156.5 (4), 128.2 (22), 126.3 (20), 128.0 (24), 129.8 (21), 134.5 (23), 131.1 (14), 131.0 (12), 136.4 (19),131.0 (1), 140.3 (6), 144.0 (10), 145.5 (18), 147.8 (7), 168.0 (9), 190.0 (16); MS (m/z): 514 (M^+). For $C_{25}H_{18}ClF_2N_3O_3S$: C, 58.42%; H, 3.50%; N, 8.18%. Found: C, 58.33%; H, 3.41%; N, 8.09%.

(E)-N-(4-(3-(4-chlorophenyl)acryloyl)phenyl)-2-(5-(difluoromethoxy)-1H-benzo[d]imidazol-2-ylthio)acetamide (III)$_4$

Yield: 65.64%; M.P.:215°C; IR (ATR, cm^{-1}): 629 (C-S str.), 840 (C-H bend. aromatic ring), 1028 (C-O-C symmetric. Str.), 1138 (-CF_2 str.), 1276 (C-O-C asymmetric. Str.), 1439 (C=C str. aromatic ring), 1474 (C-H bend. methylene group), 1542 (N-H bend. Sec. amine), 1586 (C=C str. conjugated with C=O), 1645 (C=O str. α,β-unsaturated ketone), 2843 (C-H str. methylene group), 3010 (C-H str. aromatic ring); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 4.42 (2H, s, -C<u>H</u>$_2$), 7.18 (1H, s, -OC<u>H</u>F_2), 7.16–8.20 (8H, m, Ar-<u>H</u>), 7.81 (1H, d, CO-<u>H</u>C=CH, J=15.6Hz), 7.87 (1H, d, CO-HC=C<u>H</u>, J=15.6Hz), 10.79 (1H, s, -N<u>H</u>), 12.71 (1H, s, benzimidazole-N<u>H</u>); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 38.9 (8), 101.1 (5), 116.4 (2), 121.6 (11), 121.6 (15), 121.5 (17), 112.0 (3), 129.0 (24), 128.7 (21), 128.5 (23), 129.3 (20), 131.0 (12), 131.0 (1), 131.1 (14), 133.2 (22), 133.4 (19), 140.3 (6), 144.0 (10), 145.5 (18), 147.8 (7), 156.5 (4), 168.0 (9), 190.0 (16); 514 (M^+). For $C_{25}H_{18}ClF_2N_3O_3S$: C, 58.42%; H, 3.50%; N, 8.18%. Found: C, 58.37%; H, 3.43%; N, 8.13%.

(E)-N-(4-(3-(3-bromophenyl)acryloyl)phenyl)-2-(5-(difluoromethoxy)-1H-benzo[d]imidazol-2-ylthio)acetamide (III)$_5$

Yield: 63.38%; M.P.:148°C; IR (ATR, cm^{-1}): 642 (C-S str.), 831 (C-H bend. aromatic ring), 1040 (C-O-C symmetric. Str.), 1148 (-CF_2 str.), 1266 (C-O-C asymmetric. Str.), 1449 (C=C str. aromatic ring), 1464 (C-H bend. methylene group), 1552 (N-H bend. Sec. amine), 1573 (C=C str. conjugated with C=O), 1652 (C=O str. α,β-unsaturated ketone), 2845 (C-H str. methylene group), 3006 (C-H str. aromatic ring); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 4.35 (2H, s, -C<u>H</u>$_2$), 7.20 (1H, s, -OC<u>H</u>F_2), 7.26–8.27 (8H, m, Ar-<u>H</u>), 7.76 (1H, d, CO-<u>H</u>C=CH, J=15.6Hz), 7.86 (1H, d, CO-HC=C<u>H</u>, J=15.6Hz), 10.69 (1H, s, -N<u>H</u>), 12.69 (1H, s, benzimidazole-N<u>H</u>); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 38.9 (8), 101.1 (5), 116.4 (2), 121.6 (11), 121.6 (15), 121.5 (17), 112.0 (3), 156.5 (4), 130.8 (22), 127.3 (20), 133.0 (24), 129.7 (21), 123.0 (23), 131.1 (14), 131.0 (12), 137.4 (19), 131.0 (1), 140.3 (6), 144.0 (10), 145.5 (18), 147.8 (7), 168.0 (9), 190.0 (16); MS (m/z): 558 (M^+). For $C_{25}H_{18}BrF_2N_3O_3S$: C, 53.76%; H, 3.22%; N, 7.52%. Found: C, 53.70%; H, 3.14%; N, 7.45%.

(E)-N-(4-(3-(4-bromophenyl)acryloyl)phenyl)-2-(5-(difluoromethoxy)-1H-benzo[d]imidazol-2-ylthio)acetamide (III)$_6$

Yield: 70.42%; M.P.:210°C; IR (ATR, cm^{-1}): 639 (C-S str.), 839 (C-H bend. aromatic ring), 1047 (C-O-C symmetric. Str.), 1156 (-CF_2 str.), 1266 (C-O-C asymmetric. Str.), 1453 (C=C str. aromatic ring), 1467 (C-H bend. methylene group), 1543 (N-H bend. Sec. amine), 1580 (C=C str. conjugated with C=O), 1642 (C=O str. α,β-unsaturated ketone), 2851 (C-H str. methylene group), 3010 (C-H str. aromatic ring); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 4.41

(2H, s, -C<u>H</u>$_2$), 7.26 (1H, s, -OC<u>H</u>F$_2$), 7.31–8.31 (8H, m, Ar-<u>H</u>), 7.79 (1H, d, CO-<u>H</u>C=CH, J=15.6Hz), 7.85 (1H, d, CO-HC=C<u>H</u>, J=15.6Hz), 10.79 (1H, s, -N<u>H</u>), 12.70 (1H, s, benzimidazole-N<u>H</u>); ^{13}C NMR (100 MHz, DMSO-d$_6$, δ, ppm): 38.9 (8), 101.1 (5), 112.0 (3), 116.4 (2), 121.5 (17), 121.6 (11), 121.6 (15), 122.5 (22), 128.3 (20), 128.8 (24), 131.0 (12), 131.0 (1), 131.1 (14), 131.7 (21), 131.7 (23), 134.4 (19), 140.3 (6), 144.0 (10), 145.5 (18), 147.8 (7), 156.5 (4), 168.0 (9), 190.0 (16); MS (m/z): 558 (M^+). For $C_{25}H_{18}BrF_2N_3O_3S$: C, 53.76%; H, 3.22%; N, 7.52%. Found: C, 53.68%; H, 3.17%; N, 7.46%.

E)-2-(5-(difluoromethoxy)-1H-benzo[d]imidazol-2-ylthio)-N-(4-(3-(4-fluorophenyl)acryloyl)phenyl)acetamide (III)$_7$ Yield: 65.35%; M.P.:220°C; IR (ATR, cm^{-1}): 641 (C-S str.), 853 (C-H bend. aromatic ring), 1049 (C-O-C symmetric. Str.), 1139 (-CF$_2$ str.), 1251 (C-O-C asymmetric. Str.), 1441 (C=C str. aromatic ring), 1453 (C-H bend. methylene group), 1553 (N-H bend. Sec. amine), 1568 (C=C str. conjugated with C=O), 1644 (C=O str. α,β-unsaturated ketone), 2853 (C-H str. methylene group), 3012 (C-H str. aromatic ring); ^{1}H NMR (400 MHz, DMSO-d$_6$, δ, ppm): 4.32 (2H, s, -C<u>H</u>$_2$), 7.22 (1H, s, -OC<u>H</u>F$_2$), 7.27–8.35 (8H, m, Ar-<u>H</u>), 7.65 (1H, d, CO-<u>H</u>C=CH, J=15.6Hz), 7.82 (1H, d, CO-HC=C<u>H</u>, J=15.6Hz), 10.62 (1H, s, -N<u>H</u>), 12.63 (1H, s, benzimidazole-N<u>H</u>); ^{13}C NMR (100 MHz, DMSO-d$_6$, δ, ppm): 38.9 (8), 101.1 (5), 112.0 (3), 115.2 (21), 115.2 (23), 116.4 (2), 121.5 (17), 121.6 (11), 121.6 (15), 130.2 (20), 130.2 (24), 130.8 (19), 131.0 (12), 131.0 (1), 131.1 (14), 140.3 (6), 144.0 (10), 145.5 (18), 147.8 (7), 156.5 (4), 162.5 (22), 168.0 (9), 190.0 (16); MS (m/z): 480 (M^+). For $C_{25}H_{18}F_3N_3O_3S$: C, 60.36%; H, 3.62%; N, 8.45%. Found: C, 60.28%; H, 3.70%; N, 8.40%

***(E)-2-(5-(difluoromethoxy)-1H-benzo[d]imidazol-2-ylthio)-N-(4-(3-(4-methoxyphenyl)acryloyl)phenyl)acetamide* (III)$_8$** Yield: 61.53%; M.P.:152°C; IR (ATR, cm^{-1}): 634 (C-S str.), 846 (C-H bend. aromatic ring), 1042 (C-O-C symmetric. Str.), 1149 (-CF$_2$str.), 1260(C-O-C asymmetric. Str.), 1443 (C=C str. aromatic ring), 1462 (C-H bend. methylene group), 1547 (N-H bend. Sec. amine), 1570 (C=C str. conjugated with C=O), 1639 (C=O str. α,β-unsaturated ketone), 2849 (C-H str. methylene group), 3000 (C-H str. aromatic ring); ^{1}H NMR (400 MHz, DMSO-d$_6$, δ, ppm): 4.39 (2H, s, -C<u>H</u>$_2$), 7.29 (1H, s, -OC<u>H</u>F$_2$), 7.31–8.39 (8H, m, Ar-<u>H</u>), 7.68 (1H, d, CO-<u>H</u>C=CH, J=15.6Hz), 7.81 (1H, d, CO-HC=C<u>H</u>, J=15.6Hz), 10.70 (1H, s, -N<u>H</u>), 12.67 (1H, s, benzimidazole-N<u>H</u>); ^{13}C NMR (100 MHz, DMSO-d$_6$, δ, ppm): 38.9 (8), 101.1 (5), 112.0 (3), 116.4 (2), 121.5 (17), 121.6 (11), 121.6 (15), 130.3 (20), 130.2 (24), 114.7 (21), 114.7 (23), 127.4 (19), 131.0 (12), 131.0 (1), 131.1 (14), 140.3 (6), 144.0 (10), 145.5 (18), 147.8 (7), 156.5 (4), 159.5 (22), 168.0 (9), 190.0 (16); 510 (M^+). For $C_{26}H_{21}F_2N_3O_4S$: C, 61.29%; H, 4.12%; N, 8.25%. Found: C, 61.22%; H, 4.16%; N, 8.18%

(E)-2-(5-(difluoromethoxy)-1H-benzo[d]imidazol-2-ylthio)-N-(4-(3-(3,4-dimethoxyphenyl)acryloyl)phenyl)acetamide (III)$_9$

Yield: 72.97%; M.P.:154°C; IR (ATR, cm^{-1}): 640 (C-S str.), 831 (C-H bend. aromatic ring), 1041 (C-O-C symmetric. Str.), 1128 (-CF$_2$ str.), 1276 (C-O-C asymmetric. Str.), 1439 (C=C str. aromatic ring), 1496 (C-H bend. methylene group), 1537 (N-H bend. Sec. amine), 1598 (C=C str. conjugated with C=O), 1643 (C=O str. α,β-unsaturated ketone), 2839 (C-H str. methylene group), 3008 (C-H str. aromatic ring); ^{1}H NMR (400 MHz, DMSO-d$_6$, δ, ppm): 4.33 (2H, s, -C<u>H</u>$_2$), 7.15 (1H, s, -OC<u>H</u>F$_2$), 7.35–8.17 (7H, m, Ar-<u>H</u>), 7.69(1H, d, CO-<u>H</u>C=CH, J=15.6Hz), 7.84 (1H, d, CO-HC=C<u>H</u>, J=15.6Hz), 10.82 (1H, s, -N<u>H</u>), 12.85 (1H, s, benzimidazole-N<u>H</u>); ^{13}C NMR (100 MHz, DMSO-d$_6$, δ, ppm): 38.9 (8), 101.1 (5), 112.0 (3), 116.4 (2), 121.5 (17), 121.6 (11), 121.6 (15), 128.3 (20), 128.3 (24), 128.5 (23), 131.0 (12), 131.1 (14), 135.0 (19), 131.0 (1), 140.3 (6), 144.0 (10), 145.5 (18), 147.8 (7), 150.0 (22), 150.7 (21), 156.5 (4), 168.0 (9), 190.0 (16); MS (m/z): 540 (M^+). For $C_{27}H_{23}F_2N_3O_5S$: C, 60.11%; H, 4.26%; N, 7.71%. Found: C, 60.01%; H, 4.20%; N, 7.79%

E)-2-(5-(difluoromethoxy)-1H-benzo[d]imidazol-2-ylthio)-N-(4-(3-(2,5-dimethoxyphenyl)acryloyl)phenyl)acetamide (III)**10***

Yield: 73.10%; M.P.:140°C; IR (ATR, cm^{-1}): 635 (C-S str.), 827 (C-H bend. aromatic ring), 1036 (C-O-C symmetric. Str.), 1141 (-CF_2 str.), 1271 (C-O-C asymmetric. Str.), 1444 (C=C str. aromatic ring), 1493 (C-H bend. methylene group), 1541 (N-H bend. Sec. amine), 1592 (C=C str. conjugated with C=O), 1644 (C=O str. α,β-unsaturated ketone), 2842 (C-H str. methylene group), 3014 (C-H str. aromatic ring); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 4.29 (2H, s, -C<u>H</u>$_2$), 7.19 (1H, s, -OC<u>H</u>F_2), 7.29–8.12 (7H, m, Ar-<u>H</u>), 7.71(1H, d, CO-<u>H</u>C=CH, J=15.6Hz), 7.80 (1H, d, CO-HC=C<u>H</u>, J=15.6Hz), 10.79 (1H, s, -N<u>H</u>), 12.82 (1H, s, benzimidazole-N<u>H</u>); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 38.9 (8), 101.1 (5), 111.3 (20), 112.0 (3), 114.5 (22), 116.4 (2), 121.5 (17), 121.6 (11), 121.6 (15), 115.2 (23), 131.1 (14), 131.0 (12), 116.0 (19), 131.0 (1), 140.3 (6), 144.0 (10), 145.5 (18), 147.8 (7), 151.3 (24), 152.8 (21), 156.5 (4), 168.0 (9), 190.0 (16); MS (m/z): 540 (M^+). For $C_{27}H_{23}F_2N_3O_5S$: C, 60.11%; H, 4.26%; N, 7.71%. Found: C, 60.06%; H, 4.17%; N, 7.79%

(E)-2-(5-(difluoromethoxy)-1H-benzo[d]imidazol-2-ylthio)-N-(4-(3-(4-hydroxy-3-methoxyphenyl)acryloyl)phenyl)acetamide (III)11

Yield: 63.43%; M.P.:210°C; IR (ATR, cm^{-1}): 642 (C-S str.), 830 (C-H bend. aromatic ring), 1041 (C-O-C symmetric. Str.), 1139 (-CF_2 str.), 1266 (C-O-C asymmetric. Str.), 1439 (C=C str. aromatic ring), 1495 (C-H bend. methylene group), 1544 (N-H bend. Sec. amine), 1599 (C=C str. conjugated with C=O), 1651 (C=O str. α,β-unsaturated ketone), 2849 (C-H str. methylene group), 3019 (C-H str. aromatic ring); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 4.36 (2H, s, -C<u>H</u>$_2$), 7.22 (1H, s, -OC<u>H</u>F_2), 7.19–8.15 (7H, m, Ar-<u>H</u>), 7.73(1H, d, CO-<u>H</u>C=CH, J=15.6Hz), 7.85 (1H, d, CO-HC=C<u>H</u>, J=15.6Hz), 10.74 (1H, s, -N<u>H</u>), 12.80 (1H, s, benzimidazole-N<u>H</u>); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 38.9 (8), 101.1 (5), 112.0 (3), 112.0 (24), 116.4 (2), 117.0 (21), 121.5 (17), 121.6 (11), 121.6 (15), 123.0 (20), 127.8 (19), 131.1 (14), 131.0 (12), 131.0 (1), 140.3 (6), 144.0 (10), 145.5 (18), 147.8 (7), 148.0 (22), 149.2 (23), 156.5 (4), 168.0 (9), 190.0 (16); MS (m/z): 526 (M^+). For $C_{26}H_{21}F_2N_3O_5S$: C, 59.43%; H, 4.00%; N, 8.00%. Found: C, 59.49%; H, 4.07%; N, 7.92%

(E)-2-(5-(difluoromethoxy)-1H-benzo[d]imidazol-2-ylthio)-N-(4-(3-(2-hydroxyphenyl)acryloyl)phenyl)acetamide (III)12

Yield: 79.36%; M.P.:154°C; IR (ATR, cm^{-1}): 638 (C-S str.), 826 (C-H bend. aromatic ring), 1045 (C-O-C symmetric. Str.), 1142 (-CF_2 str.), 1259 (C-O-C asymmetric. Str.), 1441 (C=C str. aromatic ring), 1489 (C-H bend. methylene group), 1548 (N-H bend. Sec. amine), 1595 (C=C str. conjugated with C=O), 1648 (C=O str. α,β-unsaturated ketone), 2842 (C-H str. methylene group), 3006 (C-H str. aromatic ring); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 4.32 (2H, s, -C<u>H</u>$_2$), 7.27 (1H, s, -OC<u>H</u>F_2), 7.16–8.17 (7H, m, Ar-<u>H</u>), 7.76(1H, d, CO-<u>H</u>C=CH, J=15.6Hz), 7.84 (1H, d, CO-HC=C<u>H</u>, J=15.6Hz), 10.72 (1H, s, -N<u>H</u>), 12.79 (1H, s, benzimidazole-N<u>H</u>); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 38.9 (8), 101.1 (5), 112.0 (3), 116.4 (2), 117.2 (23), 121.0 (21), 121.5 (17), 121.6 (11), 121.6 (15), 122.8 (19), 129.0 (22), 129.0 (20), 131.0 (12), 131.0 (1), 131.1 (14), 140.3 (6), 144.0 (10), 145.5 (18), 147.8 (7), 156.5 (4), 157.0 (24), 168.0 (9), 190.0 (16); MS (m/z): 496 (M^+). For $C_{25}H_{19}F_2N_3O_4S$: C, 60.60%; H, 3.83%; N, 8.48%. Found: C, 60.51%; H, 3.78%; N, 8.41%

(E)-2-(5-(difluoromethoxy)-1H-benzo[d]imidazol-2-ylthio)-N-(4-(3-(4-hydroxyphenyl)acryloyl)phenyl)acetamide (III)**13**

Yield: 66.67%; M.P.:152°C; IR (ATR, cm^{-1}): 635 (C-S str.), 830 (C-H bend. aromatic ring), 1041 (C-O-C symmetric. Str.), 1135 (-CF_2 str.), 1257 (C-O-C asymmetric. Str.), 1447 (C=C str. aromatic ring), 1492 (C-H bend. methylene group), 1555 (N-H bend. Sec. amine), 1598 (C=C str. conjugated with C=O), 1653 (C=O str. α,β-unsaturated ketone), 2846 (C-H str. methylene group), 3016 (C-H str. aromatic ring); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 4.43

(2H, s, -C<u>H</u>$_2$), 7.31 (1H, s, -OC<u>H</u>F$_2$), 7.22–8.18 (7H, m, Ar-<u>H</u>), 7.78 (1H, d, CO-<u>H</u>C=CH, J=15.6Hz), 7.85 (1H, d, CO-HC=C<u>H</u>, J=15.6Hz), 10.76 (1H, s, -N<u>H</u>), 12.85 (1H, s, benzimidazole-N<u>H</u>); ^{13}C NMR (100 MHz, DMSO-d$_6$, δ, ppm): 38.9 (8), 101.1 (5), 112.0 (3), 116.0 (21), 116.0 (23), 116.4 (2), 121.5 (17), 121.6 (11), 121.6 (15), 128.0 (19), 130.9 (20), 130.9 (24), 131.0 (12), 131.0 (1), 131.1 (14), 140.3 (6), 144.0 (10), 145.5 (18), 147.8 (7), 156.5 (4), 158.0 (22), 168.0 (9), 190.0 (16); MS (m/z): 496 (M^+). For $C_{25}H_{19}F_2N_3O_4S$: C, 60.60%; H, 3.83%; N, 8.48%. Found: C, 60.54%; H, 3.75%; N, 8.43%

(E)-2-(5-(difluoromethoxy)-1H-benzo[d]imidazol-2-ylthio)-N-(4-(3-(2,5-dihydroxyphenyl)acryloyl)phenyl)acetamide (III)$_{14}$

Yield: 62.30%; M.P.:226°C; IR (ATR, cm^{-1}): 642 (C-S str.), 837 (C-H bend. aromatic ring), 1036 (C-O-C symmetric. Str.), 1143 (-CF$_2$ str.), 1252 (C-O-C asymmetric. Str.), 1451 (C=C str. aromatic ring), 1488 (C-H bend. methylene group), 1549 (N-H bend. Sec. amine), 1602 (C=C str. conjugated with C=O), 1659 (C=O str. α,β-unsaturated ketone), 2838 (C-H str. methylene group), 3009 (C-H str. aromatic ring); ^{1}H NMR (400 MHz, DMSO-d$_6$, δ, ppm): 4.38 (2H, s, -C<u>H</u>$_2$), 7.29 (1H, s, -OC<u>H</u>F$_2$), 7.19–8.16 (7H, m, Ar-<u>H</u>), 7.77 (1H, d, CO-<u>H</u>C=CH, J=15.6Hz), 7.83 (1H, d, CO-HC=C<u>H</u>, J=15.6Hz), 10.71 (1H, s, -N<u>H</u>), 12.83 (1H, s, benzimidazole-N<u>H</u>); ^{13}C NMR (100 MHz, DMSO-d$_6$, δ, ppm): 38.9 (8), 101.1 (5), 112.0 (3), 113.0 (20), 116.4 (2), 116.5 (22), 117.0 (23), 118.0 (19), 121.5 (17), 121.6 (11), 121.6 (15), 131.0 (12), 131.0 (1), 131.1 (14), 140.3 (6), 144.0 (10), 145.5 (18), 147.8 (7), 149.9 (24), 151.0 (21), 156.5 (4), 168.0 (9), 190.0 (16); MS (m/z): 512 (M^+). For $C_{25}H_{19}F_2N_3O_5S$: C, 58.70%; H, 3.71%; N, 8.21%. Found: C, 58.62%; H, 3.65%; N, 8.16%

(E)-2-(5-(difluoromethoxy)-1H-benzo[d]imidazol-2-ylthio)-N-(4-(3-(2,4,5-trimethoxyphenyl)acryloyl)phenyl)acetamide (III)$_{15}$

Yield: 77.93%; M.P.:192°C; IR (ATR, cm^{-1}): 637 (C-S str.), 845 (C-H bend. aromatic ring), 1039 (C-O-C symmetric. Str.), 1142 (-CF$_2$ str.), 1255 (C-O-C asymmetric. Str.), 1456 (C=C str. aromatic ring), 1492 (C-H bend. methylene group), 1552 (N-H bend. Sec. amine), 1597 (C=C str. conjugated with C=O), 1652 (C=O str. α,β-unsaturated ketone), 2831 (C-H str. methylene group), 3012 (C-H str. aromatic ring); ^{1}H NMR (400 MHz, DMSO-d$_6$, δ, ppm): 4.47 (2H, s, -C<u>H</u>$_2$), 7.31 (1H, s, -OC<u>H</u>F$_2$), 7.09–8.16 (7H, m, Ar-<u>H</u>), 7.75 (1H, d, CO-<u>H</u>C=CH, J=15.6Hz), 7.83 (1H, d, CO-HC=C<u>H</u>, J=15.6Hz), 10.76 (1H, s, -N<u>H</u>), 12.86 (1H, s, benzimidazole-N<u>H</u>); ^{13}C NMR (100 MHz, DMSO-d$_6$, δ, ppm): 38.9 (8), 99.0 (23), 101.1 (5), 108.0 (19), 110.5 (20), 112.0 (3), 116.4 (2), 121.5 (17), 121.6 (11), 121.6 (15), 131.0 (12), 131.0 (1), 131.1 (14), 135.9 (24), 140.3 (6), 142.0 (21), 144.0 (10), 145.5 (18), 147.8 (7), 150.5 (22), 156.5 (4), 168.0 (9), 190.0 (16); MS (m/z): 414 (M^+). For $C_{28}H_{25}F_2N_3O_6S$: C, 59.05%; H, 4.39%; N, 7.38%. Found: C, 58.95%; H, 4.30%; N, 7.32%

(E)-2-(5-(difluoromethoxy)-1H-benzo[d]imidazol-2-ylthio)-N-(4-(3-(3,4,5-trimethoxyphenyl)acryloyl)phenyl)acetamide (III)$_{16}$

Yield: 55.17%; M.P.:222°C; IR (ATR, cm^{-1}): 635 (C-S str.), 850 (C-H bend. aromatic ring), 1041 (C-O-C symmetric. Str.), 1136 (-CF$_2$ str.), 1251 (C-O-C asymmetric. Str.), 1457 (C=C str. aromatic ring), 1487 (C-H bend. methylene group), 1549 (N-H bend. Sec. amine), 1592 (C=C str. conjugated with C=O), 1648 (C=O str. α,β-unsaturated ketone), 2839 (C-H str. methylene group), 3008 (C-H str. aromatic ring); ^{1}H NMR (400 MHz, DMSO-d$_6$, δ, ppm): 4.52 (2H, s, -C<u>H</u>$_2$), 7.28 (1H, s, -OC<u>H</u>F$_2$), 7.12–8.12 (7H, m, Ar-<u>H</u>), 7.79 (1H, d, CO-<u>H</u>C=CH, J=15.6Hz), 7.81 (1H, d, CO-HC=C<u>H</u>, J=15.6Hz), 10.82 (1H, s, -N<u>H</u>), 12.80 (1H, s, benzimidazole-N<u>H</u>); ^{13}C NMR (100 MHz, DMSO-d$_6$, δ, ppm): 38.9 (8), 101.1 (5), 103.9 (20), 103.9 (24), 112.0 (3), 116.4 (2), 121.5 (17), 121.6 (11), 121.6 (15), 126.5 (19), 131.0 (12), 131.0 (1), 131.1 (14), 138.5 (22), 140.3 (6), 144.0 (10), 145.5 (18), 147.8 (7), 153.0 (21), 153.0 (23), 156.5 (4), 168.0 (9), 190.0 (16); MS (m/z): 414 (M^+). For $C_{28}H_{25}F_2N_3O_6S$: C, 59.05%; H, 4.39%; N, 7.38%. Found: C, 58.98%; H, 4.33%; N, 7.34%

(E)-2-((5-(difluoromethoxy)-1H-benzo[d]imidazol-2-yl)thio)-N-(4-(3-(2-nitrophenyl)acryloyl)phenyl)acetamide (III)17

Yield: 77.60%; M.P.:200°C; IR (ATR, cm^{-1}): 1359 (C-N str. sec. amine), 1413 (C=C str. aromatic ring), 1596 (C=C str. conjugated to carbonyl group), 1660 (C=O str. α,β-unsaturation), 2750 (CH_2 str. methylene), 3049 (CH str. aromatic ring), 3236 (NH str. sec. amine); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 4.65 (2H, s, -C<u>H</u>$_2$), 6.62–8.10 (13H, d, Ar-<u>H</u>), 7.67(1H, d, <u>H</u>C=C<u>H</u>, J=15.7Hz), 7.82 (1H, d, <u>H</u>C=C<u>H</u>, J=15.5Hz), 9.97 (1H, s, -N<u>H</u>), 11.17 (1H, s, benzimidazole-N<u>H</u>); ^{13}C NMR (400 MHz, DMSO-d_6, δ, ppm): 39.2 (8), 116.2 (2), 116.2 (5), 121.0 (11), 121.0 (15), 121.9 (17), 124.0 (3), 124.0 (4), 125.9 (21), 128.0 (19), 128.8 (24), 129.2 (14), 129.2 (12), 129.6 (22), 134.9 (23), 148.8 (20), 140.0 (1), 140.0 (6), 144.2 (10), 146.0 (18), 148.0 (7), 166.0 (9), 191.1 (16); MS (m/z): 525 (M^+). For $C_{25}H_{18}F_2N_4O_5S$: C, 57.25%; H, 3.43%; N, 10.68%. Found: C, 57.19%; H, 3.34%; N, 10.61%

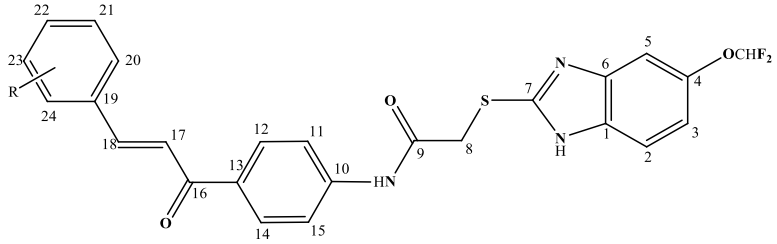
(E)-2-((5-(difluoromethoxy)-1H-benzo[d]imidazol-2-yl)thio)-N-(4-(3-(4-nitrophenyl)acryloyl)phenyl)acetamide (III)18

Yield: 65.67%; M.P.:218°C; IR (ATR, cm^{-1}): 1359 (C-N str. sec. amine), 1413 (C=C str. aromatic ring), 1596 (C=C str. conjugated to carbonyl group), 1660 (C=O str. α,β-unsaturation), 2750 (CH_2 str. methylene), 3049 (CH str. aromatic ring), 3236 (NH str. sec. amine); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 4.65 (2H, s, -C<u>H</u>$_2$), 6.62–8.10 (13H, d, Ar-<u>H</u>), 7.67(1H, d, <u>H</u>C=C<u>H</u>, J=15.7Hz), 7.82 (1H, d, <u>H</u>C=C<u>H</u>, J=15.5Hz), 9.97 (1H, s, -N<u>H</u>), 11.17 (1H, s, benzimidazole-N<u>H</u>); ^{13}C NMR (400 MHz, DMSO-d_6, δ, ppm): 39.2 (8), 116.2 (2), 116.2 (5), 121.0 (11), 121.0 (15), 121.9 (17), 124.0 (3), 124.0 (4), 127.6 (22), 128.8 (20), 128.8 (24), 128.9 (21), 128.9 (23), 129.2 (14), 129.2 (12), 136.0 (19), 140.0 (1), 140.0 (6), 142.2 (10), 146.0 (18), 148.0 (7), 166.0 (9), 191.1 (16); MS (m/z): 414 (M^+). For $C_{28}H_{25}F_2N_4O_5S$: C, 57.25%; H, 3.43%; N, 10.68%. Found: C, 57.16%; H, 3.37%; N, 10.64%

(E)-2-((5-(difluoromethoxy)-1H-benzo[d]imidazol-2-yl)thio)-N-(4-(3-(4-(dimethylamino)phenyl)acryloyl)phenyl)acetamide (III)$_{19}$

Yield: 60.90%; M.P.:220°C; IR (ATR, cm^{-1}): 1359 (C-N str. sec. amine), 1413 (C=C str. aromatic ring), 1596 (C=C str. conjugated to carbonyl group), 1660 (C=O str. α,β-unsaturation), 2750 (CH_2 str. methylene), 3049 (CH str. aromatic ring), 3236 (NH str. sec. amine); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 4.65 (2H, s, -C<u>H</u>$_2$), 6.62–8.10 (13H, d, Ar-<u>H</u>), 7.67(1H, d, <u>H</u>C=C<u>H</u>, J=15.7Hz), 7.82 (1H, d, <u>H</u>C=C<u>H</u>, J=15.5Hz), 9.97 (1H, s, -N<u>H</u>), 11.17 (1H, s, benzimidazole-N<u>H</u>); ^{13}C NMR (400 MHz, DMSO-d_6, δ, ppm): 39.2 (8), 116.2 (2), 116.2 (5), 121.0 (11), 121.0 (15), 121.9 (17), 124.0 (3), 124.0 (4), 127.6 (22), 128.8 (20), 128.8 (24), 128.9 (21), 128.9 (23), 129.2 (14), 129.2 (12), 136.0 (19), 140.0 (1), 140.0 (6), 142.2 (10), 146.0 (18), 148.0 (7), 166.0 (9), 191.1 (16); MS (m/z): 523 (M^+). For $C_{27}H_{24}F_2N_4O_3S$: C, 61.83%; H, 4.59%; N, 10.72%. Found: C, 61.90%; H, 4.54%; N, 10.66%

Figure-2: Carbon numbering for the titled derivatives (III)1-19



RESULTS & DISCUSSION

Chemistry

Synthesis of the target compounds was accomplished according to the reaction sequence outlined in Scheme 1. Formation of intermediate *N-(4-acetyl phenyl)-2-chloroacetamide* (I)

was made possible by reacting *4-aminoacetophenone* with *chloroacetyl chloride*. On the other hand, ketone-bearing benzimidazole was synthesized by a reported linear multi-step procedure[35]. In this process, fluorination of *4-hydroxyacetanilide* gave the intermediate N-[4-(difluoromethoxy)phenyl]acetamide which on subsequent reactions like nitration followed by hydrolysis, reduction and cyclization yielded *2-mercapto-5-difluoromethoxy-1H-benzimidazole* (II). The reaction of intermediate I with intermediate II resulted in the formation of N-(4-acetyl phenyl)-2-{[5-(difluoromethoxy)-1H-benzimidazol-2-yl]thio}acetamide (III). Thus product (III) when further reacted with various aryl aldehydes via acid-catalyzed aldol condensation, resulting in the formation of title compounds $(IV)_{1-19}$ in a good yield. The minimum inhibitory concentration (MIC) values of synthesized compounds are presented in Table 1.

Characterization

IR data

IR spectra obtained at 1439 and 3008 cm^{-1} proved the presence of C=C double bond and C-H stretching in aromatic rings respectively. Vibration bands observed at 3339 and 1180 cm^{-1} confirmed the presence of N-H and C-N stretching respectively of secondary amine adjacent to the carbonyl group. A sharp band at 1643 cm^{-1} confirmed the presence of the carbonyl group in the final motif while an intense band at 1594 cm^{-1} proved the presence of the C=C double bond of α, β-unsaturated ketone. Weak absorptions observed at 2839 and 640 cm^{-1} confirmed the presence of methylene group and C-S stretching in the structure.

^{1}H NMR data

^{1}H NMR spectrum observed for the compound under investigation exhibited several absorption peaks corresponding to desired protons. Proton on the nitrogen of benzimidazole nucleus appeared at δ = 12.85 whereas absorption peak at δ = 10.82 indicated the presence of proton of secondary amine adjacent to the carbonyl group. A finely resolved singlet at a 4.33δ value corresponds to protons of the methylene group in the final motif. The absorption peak for aromatic protons was found in the range of δ = 6.95-8.17. The absorption peak for α, β-unsaturated protons (O=C-CH=CH) of chalcone confirms their position at δ = 7.69 and δ = 7.84. The coupling constant value of these two protons was found to be 15.6Hz which indicated that protons attached with C=C double bond of chalcone are having trans (E) geometry.

^{13}C NMR data

The ^{13}C NMR spectral data helped to confirm the formation of the desired structure. The δ values for the spectral study were seen to vary between δ = 33.4 to δ = 189.8. Two quaternary carbonyl carbon atoms ‘‘i’’ and ‘‘p’’ appeared far downfield on the scale, nearly at 163.3 and 189.8, among both, the carbon near to α, β-unsaturation appeared most downfield. The quaternary carbon indicated as ‘‘g’’ present in benzimidazole nucleus adjacent to sulfur was observed at δ = 150. The two carbons of chalcone linkage, i.e., carbon-q appeared at δ = 122.8 and carbon-r appeared at δ = 145.8, of which, the carbon atom a bit up-field (122.8 δ) was the one near to carbonyl group. The carbon atom appearing in the most up-field region of scale (δ = 33.47) was methylene carbon. Aromatic carbon atoms were observed in the range of δ = 99 to 137. The carbon numbering to the structure is given in Figure 2.

Antimicrobial activity

The compounds $(IV)_{1–19}$were screened for their antimicrobial activities against a broad panel of bacterial and fungal strains. The antimicrobial activity in the present investigation was assessed by the Broth dilution method**.** Screening for antimicrobial activity was carried out by diluting the solution and preparing the sets consecutively from 1000, 500, 250, 125, 62.5, 31.25, 15.62 up to 7.8 µg/ml. The resulting MIC (µg/ml) values are recorded in Table 1. Ciprofloxacin and fluconazole were used as reference drugs for antibacterial and antifungal assays respectively. The MIC value for fluconazole against *C. albicans* was recorded to be 125

µg/ml whereas against *A. niger* was 62.5 µg/ml. Similarly, the MIC values for ciprofloxacin against gram-positive bacteria *S. aureus* and *E. faecalis* were 62.5 µg/ml and 125 µg/ml, respectively against gram-negative bacterial strains *E. coli* and *P. aeruginosa*; MIC value for Ciprofloxacin was observed to be 125 µg/ml. Compounds showing significant activity against particular bacteria and fungi are discussed specifically in the antibacterial and antifungal section below.

Antibacterial activity

The minimum inhibitory concentration (MIC) for antibacterial activity was determined for newly synthesized compounds $(IV)_{1\text{-}19}$ against gram-positive bacteria *S. aureus* (ATCC No. 25923) and *E. faecalis* (ATCC No. 29212) and against gram-negative bacteria *E. Coli* (ATCC No. 25922) and *P. aeruginosa* (ATCC No. 27853) by following standard protocols. The MIC values of chalcone analogs against tested microorganisms displayed significant activity with a degree of variation. The most susceptible microorganism was *S. aureus* followed by two-gram negative bacteria (*E. Coli* and *P. aeruginosa*) while the lowest inhibitory effect was encountered in the case of *E. faecalis*. Compounds $(IV)_8$, $(IV)_9$, $(IV)_{10}$ with substitution like 4-OCH_3, 3,4-$(OCH_3)_2$ and 2,5-$(OCH_3)_2$ respectively, exhibited the highest degree of inhibition against three bacterial strain (*E. Coli*, *P. aeruginosa* and *S. aureus*) while compound $(IV)_{18}$ also exhibited the highest degree of inhibition against both gram-negative bacteria. Similarly, compounds $(IV)_1$, $(IV)_3$-$(IV)_5$, $(IV)_{11}$, $(IV)_{13}$-$_{15}$, $(IV)_{17,}$ and $(IV)_{18}$ with substitutions like H, 3-Cl, 4-Cl, 3-Br, 3-OCH_3-4-OH, 4-OH, 2,5-di(OH), 2,4,5-$(OCH)_3$, 2-NO_2 and 4-NO_2 also exhibited excellent activity against selected microorganisms (Table 1). The lowest degree of inhibition was recorded for the halogen-substituted chalcones especially against *E. faecalis*. The results were compared to ciprofloxacin as a positive control.

Antifungal activity

The synthesized motifs were tested for their antifungal activity against two different fungal strains, *C. albicans* (ATCC No. 10231) and *A. niger* (ATCC NO. 16404). In the case of *C. albicans*, compound $(IV)_8$ with substitution 4-OCH_3 exhibited the best MIC value up to 31.25 µg/ml while compounds $(IV)_3$, $(IV)_5$, $(IV)_9$, $(IV)_{14}$ and $(IV)_{15}$ exhibited higher activity than reference drug but less than compound $(IV)_8$. All the target compounds displayed the lowest degree of inhibition against *A. niger* in comparison to the standard drug, fluconazole.

Structure-Activity Relationship study (SAR study)

The antibacterial and antifungal results determined, indicated based on the MIC values, that the derivatives with electron-donating group viz. -OCH_3 exhibited excellent results. The MIC data very clearly indicates that the increment in the number of electron-donating groups viz. -OCH_3 boosted the MIC by two folds.

Table 1: Minimum Inhibitory Concentration values of synthesized derivatives (III)1-19

Sr. No.	Entry	Gram-positive bacteria		Gram-negative bacteria		Fungi	
		S. aureus ATCC 25923	*E. faecalis* ATCC 29212	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27853	*C. albicans* ATCC 10231	*A. niger* ATCC 16404
1	R:H	31.25	250	125	62.5	125	250
2	R:2-Cl	125	250	125	62.5	62.5	125
3	R:3-Cl	31.25	125	250	125	125	250
4	R:4-Cl	31.25	62.5	125	62.5	125	250
5	R:3-Br	31.25	250	125	62.5	62.5	125
6	R:4-Br	125	250	125	62.5	125	250
7	R:4-F	125	250	125	125	250	250

8	R:4-OCH_3	31.25	125	31.25	31.25	31.25	125
9	R:3,4-diOCH_3	31.25	62.5	31.25	31.25	62.5	125
10	R:2,5-diOCH_3	31.25	62.5	31.25	31.25	125	250
11	R:3-OCH_3-4-OH	31.25	62.5	31.25	62.5	125	250
12	R:2-OH	62.5	62.5	62.5	62.5	125	250
13	R:4-OH	125	62.5	31.25	62.5	125	250
14	R:2,5-diOH	125	125	62.5	31.25	62.5	125
15	R:2,4,5-triOCH_3	31.25	31.25	62.5	62.5	62.5	125
16	R:3,4,5-triOCH_3	125	62.5	62.5	62.5	125	250
17	R:2-NO_2	62.5	125	31.25	62.5	125	125
18	R:4-NO_2	62.5	125	31.25	31.25	125	250
19	R:N,N-diCH_3	62.5	62.5	62.5	31.25	125	250
	Standard Drug	62.5	62.5	62.5	62.5	125	125

Standard drug: For Antibacterial activity – Chloramphenicol; For Antifungal activity – Fluconazole
The data in the table are Minimum Inhibitory Concentration values in microgram/ml

Molecular Docking

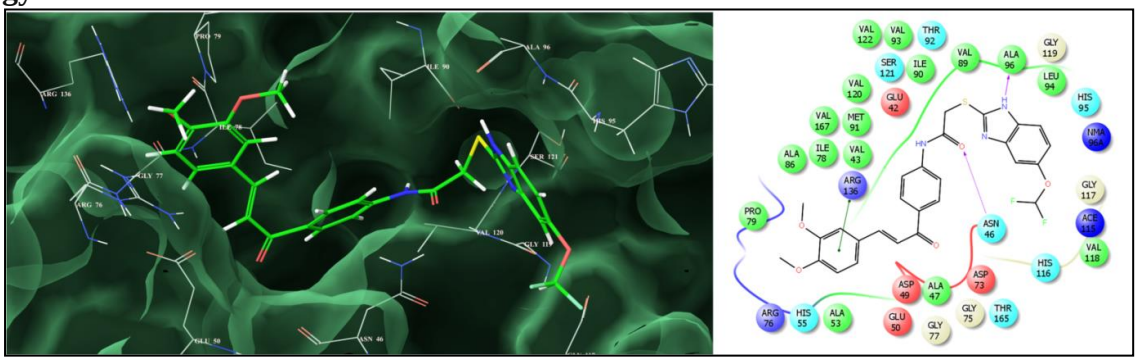
Molecular docking has evolved as an important computational technique in structural biology and computer-aided drug design to evaluate the binding modes of a putative ligand with a target protein of known three-dimensional structure. Thus to understand the interaction of the synthesized *difluorobenzimidazole* clubbed chalcone derivatives (III_{01}-III_{19}) with microbial *DNA gyrase* (PDB code: 1KZN), the molecular docking study was performed using the GLIDE (Grid-Based Ligand Docking with Energetics) program of the Small Drug Discovery Suite of Schrödinger molecular modeling software (Schrödinger, LLC, New York, NY), [37-39] Microbial DNA *gyrase*, a type II topoisomerase, catalyze changes in the topology of DNA by introducing negative supercoils into DNA by ATP consumption making it essential for cell survival. Also, it is observed in all bacteria but is absent from higher eukaryotes, making it an attractive target for antibacterial drug discovery. [40-41]

All the molecules were confined within the active site of the target protein exhibiting promising binding affinity towards DNA gyrase occupying its active site (docking score: -7.995 to -7.045) (Table 2) at coordinates close to the co-crystallized ligand by the formation of several bonded and non-bonded interactions. Detailed per-residue interaction analysis for one of the most active analogs III_{09} could identify the key interacting residues and the type of thermodynamic interaction influencing the binding affinity.

The best-docked conformation of III_{09} (Figure 1) into the active site of DNA gyrase showed that the compound snuggly fits into the protein engaging in energetically favorable interactions (Glide docking score: -7.995, Glide binding energy of -47.959Kcal/mol). Analysis of per-residue interactions showed that the compound is buried into the active site engaging in a series of favorable van der Waals interactions with Ala47(-1.343 Kcal/mol), Asp73(-1.275 Kcal/mol), Val89(-2.176 Kcal/mol), Ile90(-2.081 Kcal/mol), Ala96(-2.838 Kcal/mol), Gly119(-1.353 Kcal/mol) and Val120(-1.131 Kcal/mol) residues through the 2-(5-(difluoromethoxy)-1H-benzo[d]imidazol-2-yl component while the other section of the molecule i.e. thio-N-(4-(3-(3,4-dimethoxyphenyl)acryloyl)phenyl)acetamide engaged in similar interactions with Val43(-1.109 Kcal/mol), Asn46(-3.368 Kcal/mol), Glu50(-3.842 Kcal/mol), Arg76(-3.217 Kcal/mol), Gly77(-1.698 Kcal/mol), Ile78(-3.746 Kcal/mol), Pro79(-3.939 Kcal/mol), Ile82(-1.417 Kcal/mol), Ala86(-3.514 Kcal/mol) and Thr165(-2.409 Kcal/mol) residues. Furthermore, very significant electrostatic interactions observed with Glu42(-1.07 Kcal/mol), Asp45(-1.786 Kcal/mol), Asn46(-2.893 Kcal/mol), Glu50(-1.622 Kcal/mol), Asp73(-2.832 Kcal/mol), Arg76(-1.395 Kcal/mol) and Arg136(-2.189 Kcal/mol)

residues of active site also contributed to the enhanced binding affinity. The compound also exhibited very close hydrogen bonding interaction with Ala96(1.988Å) and Asn46(2.089Å) residues through acetamide (=O) and imidazole (-NH-) groups and also a very prominent pi-pi stacking interaction with Arg136(2.450Å) through dimethoxy phenyl ring. Such hydrogen bonding and pi-stacking interactions anchor the molecule to the active site to guide its 3D orientation and facilitate the non-bonded (steric and electrostatic) interactions with active site residues. A similar network of favorable bonded and non-bonded interactions could guide the binding of other *difluorobenzimidazole* clubbed chalcone derivatives qualifying this scaffold as a potential starting point for structure-based optimization to arrive at molecules with improved binding affinity and selectivity.

Figure-1: Binding mode and interactions of III_{09} with the active site of microbial DNA gyrase subunit B



(on the right side: most significantly interacting residues, the green line indicates pi-pi stacking interaction while pink lines correspond to H-bonding interactions)

Table 2: Docking Score of the titled derivatives (III)1-19

Sr. No.	Entry	Glide score	Hydrogen bonding (Å)	Pi-pi stacking (Å)
III_1	**R: H**	-7.381	Arg136(1.865), Asn46(2.334)	--
III_2	**R:2-Cl**	-7.155	Val120(2.218), Ala96(1.903), Asn46(2.487)	--
III_3	**R:3-Cl**	-7.243	Val120(2.563), Ala96(2.110), Asn46(2.518)	--
III_4	**R:4-Cl**	-7.327	Asn46(1.960), Glu42(2.208)	--
III_5	**R:3-Br**	-7.323	Ala96(2.005)	--
III_6	**R:4-Br**	-7.163	Val120(2.592)	--
III_7	**R:4-F**	-7.045	Asp49(1.697,1.941)	--
III_8	**R:4-OCH_3**	-7.642	Arg136(2.000)	Arg136(2.001)
III_9	**R:3,4-diOCH_3**	-7.995	Ala96(1.988), Asn46(2.089)	Arg136(2.450)
III_{10}	**R:2,5-diOCH_3**	-7.953	Arg136(2.036), Arg76(1.958)	Arg136(2.037)
III_{11}	**R:3-OCH_3-4-OH**	-7.426	Arg136(1.651), Asp73(1.909)	--

III_{12}	**R:2-OH**	-7.312	Val120(2.050), Ala96(2.107), Asp73(1.501)	---
III_{13}	**R:4-OH**	-7.361	Arg136(2.100)	Arg136(2.101)
III_{14}	**R:2,5-diOH**	-7.356	Ala96(1.780), Asp73(1.609), Asn46(2.120)	--
III_{15}	**R:2,4,5-triOCH$_3$**	-7.429	Arg76(1.177), Asn46(2.020)	Arg136(1.822), Arg76(2.178)
III_{16}	**R:3,4,5-triOCH$_3$**	-7.298	Arg136(2.506), Asn46(2.626)	Arg136(2.879)
III_{17}	**R:2-NO$_2$**	-7.385	Arg136(1.654), Asn46(2.282)	Arg136(2.879)
III_{18}	**R:4-NO$_2$**	-7.438	Arg136(1.986)	Arg136(1.987)
III_{19}	**R:N,N-diCH$_3$**	-7.322	Arg136(2.151)	Arg136(2.152)

CONCLUSION: The results presented herein help in concluding the importance of fluorine as a notable substituent on benzimidazole clubbed chalcones for enhancing the biological potential against a wide spectrum of bacterial and fungal strains. Also, the Structure-Activity Relationship helped in depicting that combination of electron-donating substituents increased the antimicrobial activity. Good docking scores observed for these molecules reveal that the titled compounds are well accommodated in the active site of the enzyme and their binding pattern demonstrated their strong interaction within the active site of the DNA gyrase enzyme. The best docking score was observed for the derivative with the electron-donating group -OCH_3, which helps in concluding the importance of electron-donating groups in the titled derivatives against bacterial as well as fungal strains for the in-vitro experiment as well as an in-silico study against the *DNA gyrase*.

ACKNOWLEDGMENT: Authors are thankful to the Department of Chemistry, HNGU, Patan, and Sheth M. N. Science College, Patan for providing infrastructural, laboratory, and library facilities. The authors also thank Schrödinger Inc. for providing the Small-Molecule Drug Discovery Suite (2018) to perform molecular docking studies.

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Received on January 30, 2023