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An efficient synthesis, invitro and insilco evaluation of new pyrazole and isoxazole derivatives as anti-inflammatory agents

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The present study is the synthesis of new heterocyclic moieties like pyrazole, isoxazole and thiazole containing benzimidazole nucleus. The title compounds were synthesized from 4-(1*H*-benzo[*d*]imidazol-2-yl) oxazol-2-amine. The newly synthesised compounds were screened for their in vitro anti-inflammatory activity and demonstrated excellent to moderate activity and molecular docking study reports are supporting anti-inflammatory activity showed high inhibition constant and binding energy. The structures of synthesised compounds were characterized by IR, ^{1}HNMR, Mass spectroscopic methods.

Keywords: Benzimidazole, Docking study, Isoxazole, Pyrazole, Synthesis

Chemistry of heterocycles is highly essential for the present pathogenic environment to address the real-world demands[1,2]. Synthesis of heterocyclic compounds is a great challenge for higher efficiency and eco-friendly point of view[3,4]. As the quest for synthesis of new heterocycles for controlling and curing the emerging diseases continues, Scientists have paid attention to design and development of novel heterocyclic compounds from natural products and synthetic methods[5,6], Especially nitrogen-containing heterocyclic compounds are occupying unique position in the area of modern drugs and pharmaceuticals[7,8]. Majority of the pharmaceutical products are heterocycles which meet the expectations of the current industrial and biological requirements.

In recent days, benzimidazole and its derivatives have received great attention in the field of medicinal chemistry[9-11]. The current study involves the synthesis of most biodynamic compound benzimidazole containing oxazole, thiazole, pyrazole and isoxazole which exhibit various biological properties like antitumor[12], anthelmintic[13], anal gesic[14], antimicrobial[15] and antibacterial[16] and anti-inflammatory activity[17]. Hence, in this direction efforts have been undertaken to introduce most versatile and biologically active molecules containing nitrogen, oxygen and sulphur heterocyclic compounds like 6-(4-(1*H*-Benzo[*d*]imidazole-2-yl) oxazole-2-yl)-3,3a,5,6-tetrahydrothiazolo[4,5-c]isoxazole and 4-(1*H*-benzo[*d*]imidazol-2-yl)-2-(3,5-diphenyl-3,3a-dihydro-2*H*-pyrazolo[3,4-*d*] thiazol-6(5*H*)-yl)oxazole derivatives which were synthesized from 4-(1H-Benzo[*d*]imidazol-2-yl)oxazol-2-amine

Experimental Section

Materials and Methods

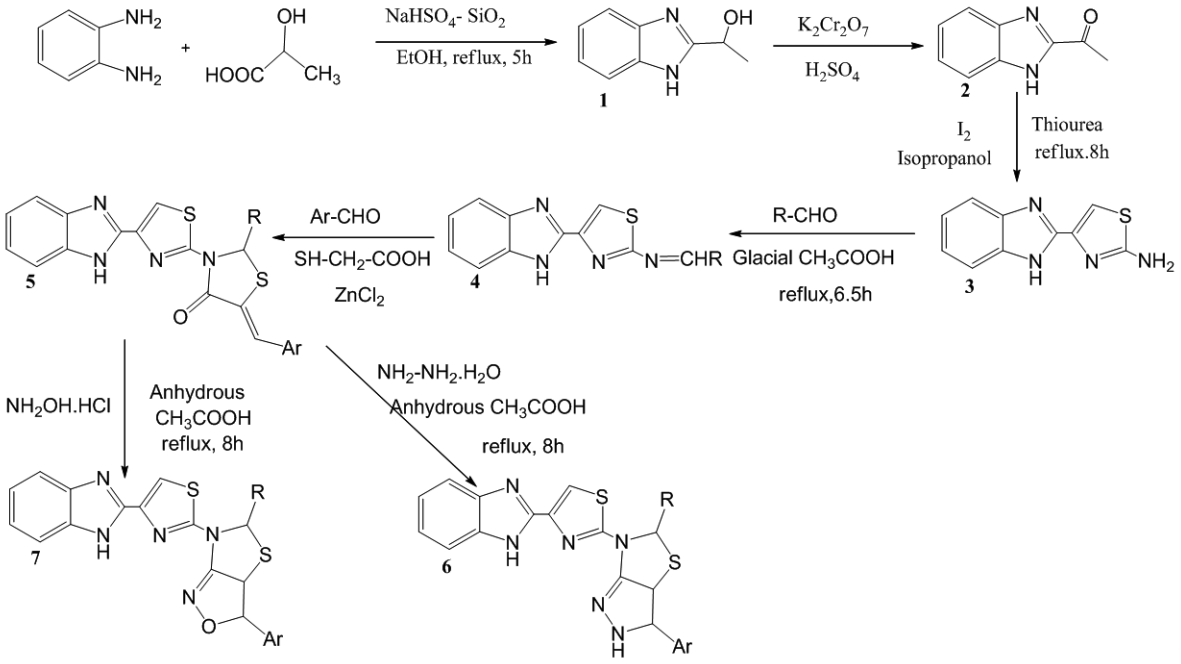
The reaction progress was monitored by TLC. IR Spectrum of compounds were recorded by KBr pellet method using Perkin Elmer BX series and HNMR spectrum was recorded by Brucker 400 MHz using DMSO used as solvent and TMS used as internal standard. Chemical shifts (δ) were expressed in ppm. Mass spectrum of compounds were measured on GC/MS-QP1000 EX (EI,70 Ev) mass spectrometer. Elemental analysis was recorded by using Perkin Elmer 240 CHN analyser (Table 1).

General reaction procedure for Compound 4

Equimolar mixture of Benzaldehyde (0.004 mol) and 4-(1*H*-benzo[*d*]imidazol-2-yl)thiazol-2-amine[18] (3) (0.004 mol) were refluxed in ethanol for about 4h with few drops of glacial acetic acid added. Progress of the reaction was monitored by TLC. After the completion of the reaction, it was cooled and the product was filtered and recrystallized with methanol to afford the compound Schiff base (2) (Scheme 1).

Table 1 — Physical data of synthesized compounds 5, 6 and 7 (a-e)

Compounds	5a	5b	5c	5d	5e
Ar	Ph	4-CH_3- Ph	4-OCH_3- Ph	4-OH- Ph	4-NO_2- Ph
M.P (°C)	250-52	255-57	256-58-26	251-53	258-60
Yield (%)	78	82	79	80	75
Compounds	6a	6b	6c	6d	6e
Ar	Ph	4-CH_3- Ph	4-OCH_3- Ph	4-OH- Ph	4-NO_2- Ph
M.P (°C)	255-57	258-60	260-62	259-61	263-65
Yield (%)	82	78	76	81	74
Compounds	7a	7b	7c	7d	7e
Ar	Ph	4-CH_3- Ph	4-OCH_3- Ph	4-OH- Ph	4-NO_2- Ph
M.P (°C)	258-60	261-63	264-66	261-63	265-67
Yield (%)	80	81	76	73	74



Scheme 1

General reaction procedure for Compound 5

Equimolar mixture of compound 4 (0.01mol), thioglycolic acid (0.01mol), Benzaldehyde (0.01mol) in 1,4 dioxane (30 mL), with a pinch of zinc chloride was refluxed for about 6.5 hours. After refluxing the solution was filtered and cooled in ice bath. Solid product was filtered, washed with 10% $NaHCO_3$ solution and it was recrystallized from alcohol. The compounds 3(b-h) were prepared by similar procedure with minor changes in reaction conditions (Scheme 1).

(Z)-3-(4-(1H-Benzo[d]imidazol-2-yl) thiazol-2-yl)-5-benzylidene-2-phenyl thiazolidin-4-one (5a)

IR (KBr,cm^{-1}): 3325(NH), 1543 (C=N), 1662 (C=O), 1230 (C=S); ^{1}H NMR (DMSO-d_6, 400MHz, δ in ppm): 5.92(s,1H,N-CH-Ar), 6.54(s,1H,CH,Ar), 7.13-7.23(m,4H,Ar-H), 7.25-7.46(m,5H,Ar-H),7.56-7.76(m,5H, Ar-H), 9.85(bs, 1H,NH). MS, m/z (%), 466 (M+); Anal.Calcd for $C_{26}H_{17}N_3OS$: C,66.93; H,3.89; N,12.01%. Found: C,66.23; H,3.72; N, 11.68%.

(Z)-3-(4-(1H-Benzo[d]imidazole-2-yl)thiazol-2-yl)-5-(4-methylbenzylidine)-2-phenyl thiazolidine -4-one (5b)

IR(KBr,cm-1): 3337(NH), 2690(C-H), 1560(C=N), 1672(C=O),1249 (C=S); ^{1}H NMR (DMSO-d_6 ,400 MHz, δ in ppm) : 2.85(s,3H,CH_3), 5.66 (s, 1H,N-CH-Ar), 6.54 (s,1H,CH,Ar), 7.15-7.24 (m, 4H,Ar-H); 7.28-7.38(m, 4H,Ar-H), 7.42-7.64 (m, 4H,Ar-H), 9.95(bs,1H,NH): MS, m/z(%), 480 (M+); Anal.Calcd

for $C_{27}H_{20}N_4OS_2$: C,67.48; H,4.19; N,11.66% Found : C,67.08; H,3.90; N,11.05%.

(Z)-3-(4-(1H-Benzo[d]imidazol-2-yl)thiazol-2-yl)-5-(4-methoxybenzylidene)-2-phenyl thiazolidin-4-one (5c)

IR (KBr,cm-1): 33340 (NH), 3045(C-H), 1560 (C=N), 1684(C=O), 1236 (C=S); ^{1}H NMR (DMSO-d_6,400 MHz, δ in ppm); 3.45 (s, 3H, OCH_3), 5.72 (s,1H,N-CH-Ar), 6.58 (s,1H,CH,Ar),7.26-7.50 (m,5H,Ar-H), 7.65-7.84 (m, 4H,Ar-H), 10.05(bs, 1H,NH); MS, m/z(%), 469 (M^+); Anal .Calcd for $C_{27}H_{20}N_4O_2S_2$: C, 65.30; H, 4.06; N,11.28 %.Found : C, 65.04; H,3.86; N, 10.85%.

(Z)-3-(4-(1H-Benzo[d]imidazol-2-yl)thiazol-2-yl)-5-(4-hydroxybenzylidene)-2-phenyl thiazolidin4one(5d)

IR(KBr,cm^1):3513(OH),3345(NH),1568(C=N),1683(C=O),1238(C=S); ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 5.88(s,1H, N-CH-Ar), 6.56(s,1H,CH Ar); 7.14-7.27(m,4H,Ar-H),7.30-7.49(m,5H,Ar-H),7.55-7.72(m, 4H, Ar-H),10.14 (bs,1H,NH),11.25 (s, 1H,OH), ;MS, m/z (%), 482 (M^+); Anal. Calcd for $C_{26}H_{18}N_4O_2S_2$: C, 64.71; H, 3.76; N, 11.61%. Found: C, 64.42; H, 3.42; N, 11.38%.

(Z)-3-(4-(1H-Benzo[d]imidazol-2-yl)thiazol-2-yl)-5-(4-nitrobenzylidene)-2-phenyl thiazolidin-4-one (5e)

IR(KBr,cm^{-1}): 3340(NH),1572(C=N),1525 (NO_2),1683(C=O), 1234 (C=S); ^{1}H NMR (DMSO-d_6,400MHz, δ in ppm): 5.84 (s,1H, N-CH-Ar),6.58 (s,1H, CH Ar); 7.13-7.26 (m,4H, Ar-H),7.25-7.48(m,5H, Ar-H), 8.16-8.35 (m,4H, Ar-H),10.20 (bs,1H,NH), MS, m/z (%), 511 (M^+); Anal.Cacld for $C_{26}H_{17}N_5O_3S_2$;C, 61.04; H, 3.35; N, 13.69%. Found: C, 60.82; H, 3.06; N, 13.22%.

General reaction procedure for compound 6

Equimolar mixture of compound 5a (0.03 moL), hydrazine hydrate (0.03 moL) and anhydrous sodium acetate (0.001 moL) in glacial acetic acid (30 mL) was heated under reflux for about 6.5 h, cooled at r.t and poured into crushed ice. The solid product was filtered, washed with water and recrystallized from Ethyl alcohol to afford the pure compound. The remaining compounds 4(b-h) were prepared by similar procedure with minor changes as per the reaction conditions (Scheme 1).

6-(4-(1H-Benzo[d]imidazol-2-yl)thiazol-2-yl)-3,5-diphenyl-3,3a, 5, 6- tetrahydro-2H-pyrazolo[3,4-d]thiazole(6a)

IR (KBr, cm^{-1}): 3348 (NH), 3078 (C-H ring), 1546(C=N), 1238 (C=S), 1042(N-N); ^{1}H NMR (DMSO-d_6, 400MHz, δ in ppm):4.85 (d, 1H, CH-N), 4.62 (d, 1H, CH-S), 5.90 (s,1H,N-CH-Ar),6.56 (s,1H, CH Ar); 7.10-7.22(m,4H, Ar-H), 7.26-7.45(m,5H,Ar-H),7.55-7.78 (m,5H, Ar-H), 9.75 (bs, 1H,NH). MS, m/z(%),480 (M^+); Anal. Calcd for $C_{26}H_{20}N_6S_2$: C, 64.98; H, 4.19; N, 17.49%. Found: C, 64.58; H, 4.02; N, 17.18%.

6-(4-(1H-Benzo[d]imidazol-2-yl)thiazol-2-yl)-5-phenyl- 3-(p-tolyl)-3,3a,5,6-tetra hydro-2H-pyrazolo[3,4-d]thiazole(6b)

IR (KBr, cm^{-1}): 3342 (NH),3084(C-H ring), 2963 (C-H), 1565 (C=N), 1249 (C=S), 1052(N-N); ^{1}H NMR (DMSO-d_6, 400MHz, δ in ppm): 2.82 (s,3H,CH_3),4.82 (d, 1H, CH-N), 4.60 (d, 1H, CH-S), 5.81 (s,1H, N-CH-Ar),6.51(s,1H, CH Ar); 7.18-7.26 (m,4H, Ar-H), 7.28-7.38(m,4H, Ar-H), 7.45-7.68 (m,4H, Ar-H), 9.98 (bs, 1H,NH).MS, m/z (%), 494 (M^+); Anal. Calcd for $C_{27}H_{22}N_6S_2$: C, 65.56; H, 4.48; N, 16.99%. Found: C, 65.34; H, 4.28; N, 16.55%.

6-(4-(1H-Benzo[d]imidazol-2-yl)thiazol-2-yl)-3-(4-methoxyphenyl) -5-phenyl-3,3a, 5,6-tetrahydro-2H-pyrazolo[3,4-d]thiazole(6c)

IR (KBr, cm^{-1}) : 3343(NH),3039(C-H),1559(C=N), 1241 (C=S)1063(NN); ^{1}H NMR (DMSO-d_6, 400MHz, δinppm):3.48(s,3H,OCH_3),4.82(d,1H,CH-N),4.60(d,1H, CH-S),5.74(s,1H,N-CH-Ar),6.58(s,1H, CH Ar); 7.21-7.28 (m,4H,Ar-H), 7.31-7.58 (m,5H,Ar-H), 7.68-7.87(m,4H,Ar-H),10.04(bs,1H,NH), MS, m/z (%), 510 (M^+); Anal. Calcd for $C_{27}H_{22}N_6OS_2$:C, 63.51; H, 4.34; N, 16.46%. Found: C, 63.31; H, 4.18; N, 16.16 %.

4-(6-(4-(1H-Benzo[d]imidazol-2-yl) thiazol-2-yl)-5-phenyl-3,3a,5,6-tetra hydro-2H-pyrazolo[3,4-d]thiazol-3-yl) phenol (6d)

IR (KBr, cm^{-1}): 3516(OH), 3352 (NH),1571 (C=N), 1242 (C=S) 1043 (N-N); ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 4.72(d,1H,CH-N), 4.61 (d,1H, CH-S),5.92(s,1H,N-CH-Ar),6.53(s,1H,CH Ar); 7.18-7.31(m,4H,Ar-H),7.28-7.52 (m,5H, Ar-H), 7.61-7.74 (m,4H,Ar-H), 10.15 (bs,1H,NH), 11.35(s, 1H,OH) ;MS, m/z (%),413(M^+);Anal.Calcd for $C_{26}H_{20}N_6OS_2$: C, 62.88; H, 4.06; N, 16.92%. Found: C, 62.62; H, 3.86; N, 16.52 %.

6-(4-(1H-Benzo[d]imidazol-2-yl) thiazol-2-yl)-3-(4-nitrophenyl)-5-phenyl-3,3a,5,6 tetrahydro-2H-pyrazolo[3,4-d] thiazole(6e)

IR (KBr, cm^{-1}): 3346 (NH), 1575 (C=N), 1528 (NO_2), 1236 (C=S), 1052(N-N); ^{1}H NMR (DMSO-d_6, 400MHz, δ in ppm): 4.91(d,1H,CH-N), 4.75 (d,1H, CH-S), 5.88 (s,1H, N-CH-Ar), 6.62(s,1H, CH Ar); 7.16-7.29 (m,4H, Ar-H), 7.28-7.52 (m,5H, Ar-H), 8.18-8.41 (m,4H, Ar-H),10.22 (bs,1H,NH),MS, m/z (%), 525 (M^+); Anal. Calcd for $C_{26}H_{19}N_7O_2S_2$: C,

59.41; H, 3.64; N, 18.65 %. Found: C, 59.12; H, 3.35; N, 18.34%.

General reaction procedure for compound 7

Equimolar mixture of compound 3a (0.03 mol), hydroxylamine hydrochloride (0.03 mol) and anhydrous sodium acetate (0.001 mol) in glacial acetic acid (30 mL) was heated under reflux for about 7h , cooled at r.t and poured in to crushed ice. The solid product was filtered, washed with water and recrystallized from ethyl alcohol to afford the pure compound. The remaining compounds 4(b-h) were prepared by similar procedure with minor changes as per reaction conditions (Scheme 1).

6-(4-(1H-Benzo[d]imidazol-2-yl)thiazol-2-yl)-3,5-diphenyl-3,3a, 5,6-tetrahydro thiazolo[4,5-c]isoxazole(7a)

IR (KBr, cm^{-1}): 3348 (NH), 3078 (C-H ring), 1546(C=N), 1238 (C=S); ^{1}H NMR (DMSO-d_6, 400MHz, δ in ppm):4.95 (d, 1H, CH-O), 4.72 (d, 1H, CH-S), 5.90 (s,1H,N-CH-Ar),6.58 (s,1H, CH Ar); 7.10-7.22(m,4H, Ar-H), 7.26-7.45(m,5H,Ar-H),7.55-7.78 (m,5H, Ar-H), 9.75 (bs, 1H,NH). MS, m/z (%), 481 (M^+); Anal. Calcd for $C_{26}H_{19}N_5OS_2$: C, 64.84; H, 3.98; N, 14.54%. Found: C, 64.25; H, 3.45; N, 14.24%.

6-(4-(1H-Benzo[d]imidazol-2-yl)thiazol-2-yl)-5-phenyl-3-(p-tolyl) -3,3a,5,6-tetra hydrothiazolo[4,5-c]isoxazole(7b)

IR (KBr, cm^{-1}): 3342 (NH),3084(C-H ring), 2963 (C-H), 1565 (C=N), 1249 (C=S) ; ^{1}H NMR (DMSO-d_6, 400MHz, δ in ppm): 2.82 (s,3H,CH_3), 4.98 (d, 1H, CH-O), 4.75 (d, 1H, CH-S), 5.81 (s,1H, N-CH-Ar),6.61 (s,1H, CH Ar); 7.18-7.26 (m,4H, Ar-H), 7.28-7.38(m,4H, Ar-H), 7.45-7.68 (m,4H, Ar-H), 9.98 (bs, 1H,NH).MS, m/z (%), 495 (M^+); Anal. Calcd for $C_{27}H_{21}N_5S_2$: C, 65.43; H, 4.27; N, 14.13%. Found: C, 65.12; H, 4.04; N, 13.89%.

6-(4-(1H-Benzo[d]imidazol-2-yl)thiazol-2-yl)-3-(4-methoxyphenyl) -5-phenyl-3,3a, 5,6-tetrahydrothiazolo[4,5-c] isoxazole(7c)

IR (KBr,cm^{-1}):3343 (NH), 3039 (C-H),1559(C=N), 1241 (C=S); ^{1}H NMR (DMSO-d_6, 400MHz, δ in ppm): 3.48 (s,3H,OCH_3), 5.12(d,1H,CH-O), 4.74 (d,1H, CH-S), 5.74(s,1H,N-CH-Ar),6.64 (s,1H, CH Ar); 7.21-7.28 (m,4H,Ar-H), 7.31-7.58(m,5H,Ar-H), 7.68-7.87(m,4H, Ar-H), 10.04 (bs,1H, NH), MS, m/z (%), 511 (M^+); Anal. Calcd for $C_{27}H_{21}N_5O_2S_2$: C, 63.38; H, 4.14; N, 13.69%. Found: C, 63.05; H, 4.05; N, 13.21%.

4-(6-(4-(1H-Benzo[d]imidazol-2-yl) thiazol-2-yl)-5-phenyl-3,3a,5,6-tetra hydrothiazolo[4,5-c]isoxazol-3-yl) phenol (7d)

IR (KBr, cm^{-1}): 3516(OH), 3352 (NH), 1571 (C=N), 1242 (C=S); ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 5.08 (d,1H,CH-O), 4.72 (d,1H, CH-S), 5.92 (s,1H, N-CH-Ar), 6.63 (s,1H, CH Ar); 7.18-7.31 (m,4H,Ar-H), 7.28-7.52 (m,5H, Ar-H), 7.61-7.74 (m,4H,Ar-H), 10.15 (bs,1H,NH), 11.35 (s, 1H,OH) ;MS, m/z (%), 497 (M^+); Anal. Calcd for $C_{26}H_{20}N_6OS_2$: C, 62.76; H, 3.85; N, 14.07%. Found: C, 62.76; H, 3.85; N, 14.07%.

6-(4-(1H-Benzo[d]imidazol-2-yl)thiazol-2-yl)-3-(4-nitrophenyl) -5-phenyl-3,3a,5,6-tetra hydrothiazolo [4,5-c]isoxazole(7e)

IR (KBr, cm^{-1}): 3346 (NH), 1575 (C=N), 1528 (NO_2), 1236 (C=S); ^{1}H NMR (DMSO-d_6,400MHz, δ in ppm):5.14 (d,1H,CH-O), 4.85 (d,1H, CH-S), 5.88 (s,1H, N-CH-Ar),6.65 (s,1H, CH Ar); 7.16-7.29 (m, 4H, Ar-H), 7.28-7.52 (m,5H, Ar-H), 8.18-8.40 (m,4H, Ar-H),10.22 (bs,1H,NH),MS, m/z (%), 526 (M^+); Anal. Calcd for $C_{26}H_{18}N_6O_3S_2$: C, 59.30; H, 3.45; N, 15.96%. Found: C, 58.90; H, 3.15; N, 15.56%.

In vitro Anti-inflammatory activity

About 5 mL of reaction mixture which contains 0.2 mL of egg albumin, 2.8 mL of phosphate buffered saline (PBS, *p*H 6.4) was added with 2 mL of synthesized compounds at 50 and 100 µg/ml and double-distilled water with equal volume was used as control. The prepared mixtures were incubated at about (37±2)°C in BOD incubator for about 15 min and followed by heating at 70°C for about 5 min. After attaining the r.t, absorbance was measured at 660 nm using vehicle as blank and viscosity was determined by Ostwald viscometer. Diclofenac sodium was used as standard drug[19] and its concentrations were maintained at 50 and 100 µg/mL (Table 2).

The inhibition (%) of protein denaturation was measured by using the following formulae

$$\% \text{ of inhibition} = 100 \times (Vt/Vc-1)$$

Where Vt = Test sample absorbance Vc= Absorbance of control

The concentration of drug for 50% inhibition (IC_{50}) was determined by plotting % of inhibition with respect to control against treatment concentration.

Each value in the table is represented as mean ± SD (n = 3). Values in the same column followed by a different letter (a-e) are significantly different ($p < 0.05$).

Inhibitions of protein denaturation of the compounds are shown in the descending order: 7e>6e>7c>7d>6c>7b>6d>7a>6b>6a>

In Silico anti-inflammatory studies

The docking studies was done by using the molecular docking server[20,21]. The cyclooxygenase enzyme was downloaded from the PDB(Protein data bank) and was docked to the title compound and the results are tabulated given below.

Docking studies of 6-(4-(1H-benzo[d]imidazol-2-yl) thiazol-2-yl)-3,5-diphenyl-3,3a,5,6-tetrahydro-2H-pyrazolo [3, 4-d] thiazole (6a):

Docking studies of6-(4-(1H-benzo[d]imidazol-2-yl) thiazol-2-yl)-3, 5-diphenyl-3, 3a, 5, 6-tetrahydrothiazolo [4, 5-c] Isoxazole (7a):

Results and Discussion

The present investigation is the synthesis of new biheterocyclic rings that are thiazole-pyrazole and thiazole-isoxazole derivatives are linked at 2nd position of benzimidazole in an appreciable yield. The compound 4 (Schiff's base) was synthesized by using 2-aminobenzimidazole with benzaldehyde under simple procedure. The compound 5 (Chalcone derivatives of thiazolidinone) was synthesized by cyclization of one pot three -component method using compound 4, thioglycolic acid and substituted aromatic aldehydes using anhydrous zinc chloride[22]. Later, the intermediate compound 5 undergoes cyclization with hydrazine hydrate affords the compound 6 with pyrazole ring. Whereas, intermediate compound 5 undergoes cyclization with hydroxyl amine hydrochloride in the presence of anhydrous acetic acid to afford the compound 7 with isoxazole ring.

The structures of newly synthesized compound were confirmed based on spectral and analytical data. The compounds showed IR absorption bands at 3348 cm^{-1} (NH), 1564 cm^{-1} (C=N), 1238 cm^{-1}(C=S), 1042 cm^{-1} (N=N), 1680 cm^{-1} (C=O) respectively. ^{1}H NMR Spectra of title compounds showed singlet signals at 5.58 for N-CH-S, singlet thiazole protein signals at 6.56, doublet signals at 4.62 CH-S, 4.82 CH-N and 9.82 singlet proton for NH and also phenylic protons as multiplet in the range of 7.10-8.41 ppm respectively. Mass spectra of synthesized compounds showed a molecular ion peak at m/z corresponding molecular weights.

In vitro anti-inflammatory activity

The synthesized derivatives were tested for their efficacy to inhibit the protein denaturation. The process is carried out by using egg albumin. All the compounds showed concentration dependent inhibition property. Compounds (7e and 6e) are found to exhibit significant inhibition percentage compared to other synthesized compounds. Nevertheless, Compounds (7c, 6c, 6e and 7e) are competed in exhibiting a notable activity with good inhibition percentage shown in Table 2. On the other hand, the compounds 6d and 7d showed moderate activity. The variations in the activity might be due to different functional groups present in derivatives. By these studies, it is concluded that benzimidazole derivatives showed preliminary anti-inflammatory activity.

The IC_{50} values represented in Table 3 are shown as mean ±SD. According to the data, the IC_{50} 24±0.1, 21±0.5 of compounds 7c and 6d are significant and are comparable with standard Diclofenac sodium standard.

In silico anti-inflammatory activity

Molecular docking studies have shown that the ligand 1 has shown a binding energy of-6.06 kcal/mol (Table 4). Figure 1 shows the docking pose between

Table 2 — In vitro anti-inflammatory activity of compounds (6a-e) and (7a-e) percentage of inhibition (%)

Compounds		6a	6b	6c	6d	6e	7a	7b	7c	7d	7e	Standard
Conc. (µg/mL)	50	40	41	44	43	45	42	43	44	43	46	48
	100	74	75	78	76	85	75	77	84	83	88	90

Table: 3 — In vitro anti-inflammatory activity of compounds (6a-e) and (7a-e) percentage of inhibition in IC_{50} (%)

Compounds	6a	6b	6c	6d	6e	Standard
IC_{50}	5.1±2.4	6.5±2.3	7.9±0.5	21±0.5	17±1.0	28 ± 0.5
Compounds	7a	7b	7c	7d	7e	Standard
IC_{50}	10.±1.5	09±0.7	24±0.1	15.±2.3	14.5±2.1	32±0.5

Table 4 — Free energy of binding between the enzyme and the ligand 1

Est. Free Energy of Binding	Est. Inhibition Constant, Ki	vdW + Hbond + desolv Energy	Electrostatic Energy	Total Intermolec. Energy	Frequency	Interact. Surface
-6.06 kcal/mol	36.07 uM	-7.15 kcal/mol	-0.04 kcal/mol	-7.19 kcal/mol	50%	689.87

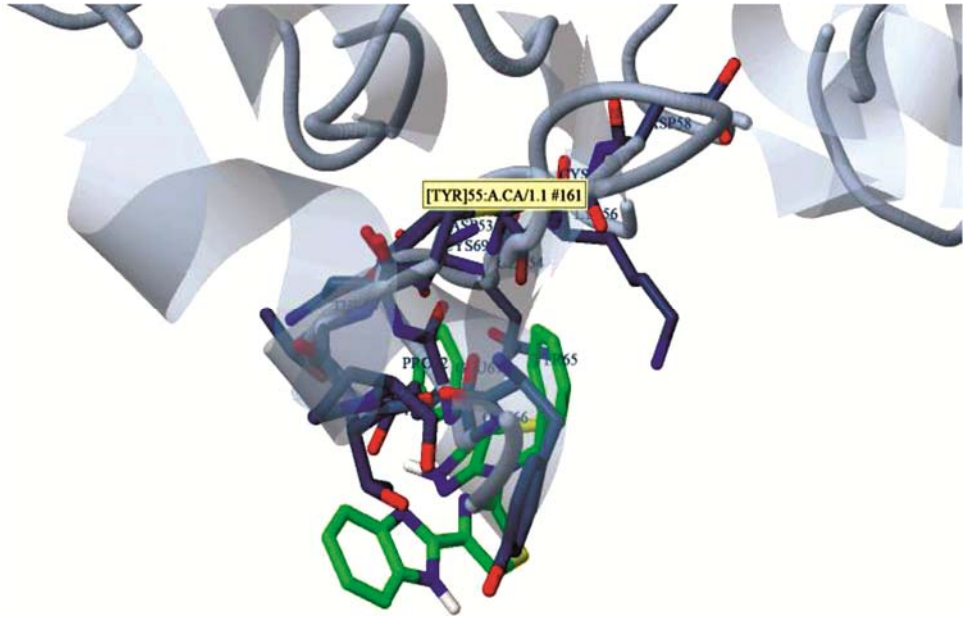


Fig. 1 — Docking pose showing the interaction between the enzyme and the ligand 1

Table 5 — Interaction table the various types of interactions between the aminoacids of the enzyme and the ligand 1

Polar			Hydrophobic			pi-pi			other		
N5 (*20*) [*2.88*]	–	GLU67 (*OE1, OE2*)	C23 (*31*) [*3.89*]	–	CYS57 (*CB*)	C18 (*26*) [*3.24*]	–	TYR55 (*CD1, CE1*)	C19 (*27*) [*3.79*]	–	GLN54 (*CB*)
N6 (*21*) [*3.22*]	–	GLU67 (*OE1, OE2*)				C19 (*27*) [*3.88*]	–	TYR55 (*CD1*)	N4 (*15*) [*3.63*]	–	GLU67 (*CB, CG*)
H9 (*43*) [*2.69*]	–	GLU67 (*OE1, OE2*)							C11 (*16*) [*3.23*]	–	GLU67 (*CB, CD, CG, OE1, OE2*)
									C9 (*12*) [*3.69*]	–	GLU67 (*CB*)
									N3 (*11*) [*2.93*]	–	GLU67 (*CB*)
									N2 (*9*) [*3.69*]	–	GLU67 (*CB*)
									C12 (*17*) [*3.38*]	–	GLU67 (*CD, CG, OE2*)
									N5 (*20*) [*3.11*]	–	GLU67 (*CD, CG*)
									C22 (*30*) [*3.77*]	–	GLU67 (*CG*)
									N6 (*21*) [*3.41*]	–	GLU67 (*CD*)
									H9 (*43*) [*3.08*]	–	GLU67 (*CD*) GLU67 (*OE2*)
									C14 (*22*) [*3.48*]	–	GLU67 (*OE2*)
									C15 (*23*) [*3.32*]	–	GLU67 (*OE2*)
									C17 (*25*) [*3.28*]	–	GLU67 (*OE2*)
									C3 (*3*) [*3.42*]	–	THR71 (*CG2*)
									C2 (*2*) [*3.19*]	–	THR71 (*CG2*)
									C1 (*1*) [*3.69*]	–	THR71 (*CG2*)

the enzyme and the ligand 1where different amino acids bind to the active site of the ligand. Interaction table the various types of interactions between the amino acids of the enzyme and the ligand 1 are shown in Table 5 where the major amino acids involved in binding are gluatamic acid, cysteine, tyrosine, glutamine and threonine at the active site. Docking studies between the ligand 2 has shown a binding energy of -6.40 kcal/mol (Table 6). Figure 2 shows the docking pose between the enzyme and the ligand 2 where various amino acids bound to the active site of the ligand. Interaction table the various types of interactions between the amino acids of the enzyme and the ligand 1 are shown in Table 7 where the major amino acids involved in binding are tyrosine, glutamic acid, glutamine, cysteine and lysine.

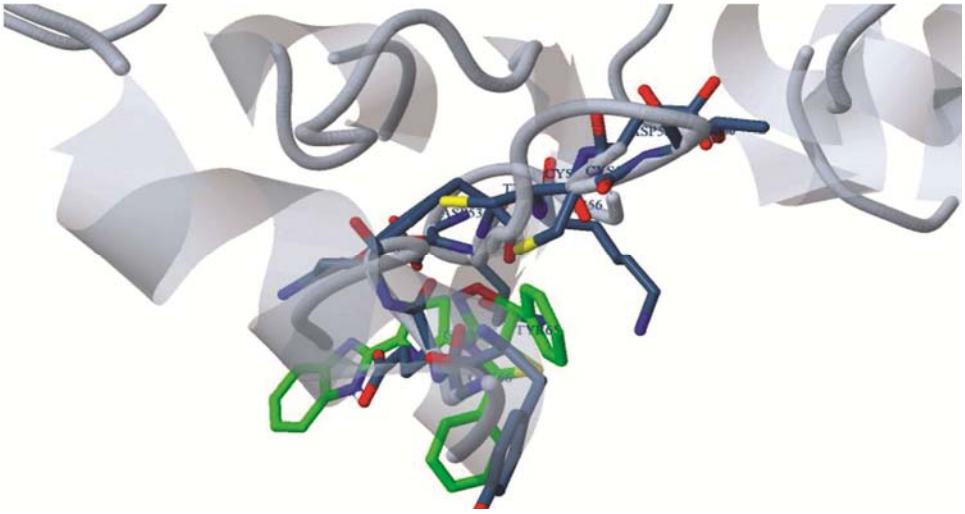


Fig. 2 — Docking pose showing the interaction between the enzyme and the ligand 2

Table 6 — Free energy of binding between the enzyme and the ligand 2

Est. Free Energy of Binding	Est. Inhibition Constant, Ki	vdW + Hbond + desolv Energy	Electrostatic Energy	Total Intermolec. Energy	Frequency	Interact. Surface
-6.40 kcal/mol	20.35 uM	-6.93 kcal/mol	-0.51 kcal/mol	-7.43 kcal/mol	50%	568.269

Table 7 — Interaction table the various types of interactions between the aminoacids of the enzyme and the ligand 2

Hydrogen bonds			Polar			Hydrophobic			pi-pi			other		
N5 (*20*) [*3.12*]	–	TYR55 (*CD2, CE1, CE2, CZ, O, OH*)	H5 (*39*) [*3.86*]	–	GLU67 (*OE2*)	C18 (*25*) [*3.25*]	–	CYS57 (*CB*)	C11 (*14*) [*3.61*]	–	TYR55 (*CD1, CE1*)	C11 (*14*) [*3.88*]	–	GLN54 (*CB*)
N4 (*15*) [*3.24*]	–	GLU67 (*CD, OE2*)				C19 (*26*) [*3.63*]	–	CYS57 (*CB*)	C10 (*13*) [*3.35*]	–	TYR55 (*CD1, CE1*)	C10 (*13*) [*3.80*]	–	GLN54 (*CB*)
N5 (*20*) [*3.35*]	–	GLU67 (*CD, CG, OE2*)				C19 (*26*) [*3.56*]	–	CYS59 (*SG*)				O1 (*21*) [*3.45*]	–	TYR55 (*CE2*)
N3 (*11*) [*3.08*]	–	GLU67 (*OE2*)										C17 (*24*) [*3.81*]	–	LYS56 (*NZ*)
												C22 (*29*)[*3.48*]	–	LYS56 (*NZ*)
												O1 (*21*) [*3.88*]	–	CYS57 (*SG*)
												C12 (*16*) [*3.28*]	–	GLU67 (*CD, CG, OE2*)
												C13 (*17*) [*3.45*]	–	GLU67 (*CG*)
												O1 (*21*) [*3.29*]	–	GLU67 (*CG*)
												C23 (*30*) [*3.39*]	–	GLU67 (*CD, OE1, OE2*)
												C24 (*31*) [*3.68*]	–	GLU67 (*OE1*)
												C9 (*12*) [*3.20*]	–	GLU67 (*OE2*)
												C8 (*10*) [*3.89*]	–	GLU67 (*OE2*)

Compared with ligand 1, ligand 2 has higher free energy of binding and could act as a better anti-inflammatory compound.

Conclusion

It has been concluded that new analogues of pyrazolo-thiazoles and isoxazole-thiazoles linked with benzimidazole nucleus is a novel method. In the present work that the title compounds are exhibiting excellent in vitro and in silico anti-inflammatory activity.

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