Research Article

Synthesis and Antioxidant Evaluation of New Heterocyclic Compounds on Benzimidazole Nucleus

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ABSTRACT

A series of new thiazolo-pyrazoles and thiazolo-isoxazoles were efficiently synthesized on the benzimidazole nucleus. All the synthesized compounds were screened for their antioxidant activity with the determination of DPPH radical scavenging and nitric oxide methods. The compounds 6c, 7c, 6d and 7d have shown excellent activity as compared to standard drug. The structures of newly synthesized compounds were established on the basis of spectroscopic analysis.

Keywords: Synthesis, Pyrazoles, isoxazoles, Benzimidazole, Antioxidant activity.

INTRODUCTION

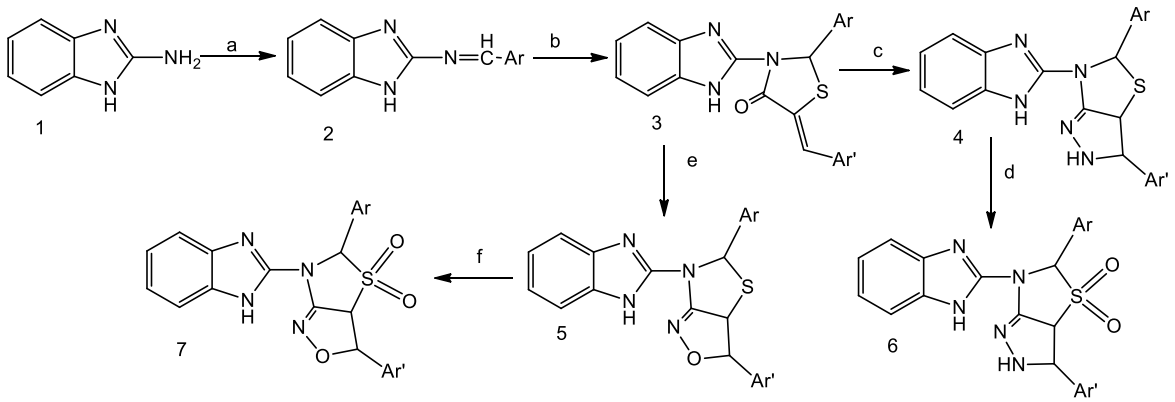
The heterocyclic compounds are widely distributed in nature and are essential to living organisms to carry-out metabolic reactions in the living cells. Among large number of heterocycles found in nature, particularly nitrogen heterocycles containing oxygen or sulphur are often immense importance due to their wide distribution in nucleic acids, vitamins, proteins and their involvement in physiological process of plants and animals.

Benzimidazole and derivatives are very important due to their enormous pharmacological and biological properties[1-2]. Benzimidazole is one of the privileged heterocyclic structure, which serves as an axial ligand for cobalt in vitamin B12 with an excellent biological activity[3-4]. There are many possibilities to synthesize the other heterocycles like thiazole, pyrimidine, oxazole, triazole, triazinanes etc on 2nd position of benzimidazole to expand the bio applicability and which fulfil urgent requirements of the biological, industrial and medicinal chemistry [5-6].

The five membered heterocyclic compounds like thiazole, pyrazole and isoxazole also exhibit wide variety of biological applications like antitumor[7], fungicidal[8], antimicrobial [9-10], anti-inflamatory[11-12], antibacterial [13-14] and antiparasitic activity[15]. In view of the pharmacological importance of pyrazole and isoxazole, it has been planned to incorporate these five membered heterocyclic moieties on benzimidazole nucleus. The synthesis and antioxidant evaluation of new 6-(1H-benzo[d]imidazol-2-yl)-5-phenyl-3-(p-substituted phenyl)-3,3a,5,6-tetrahydro-2H-pyrazolo [3,4-d] thiazole 4,4-dioxide and 6-(1H-benzo[d]imidazol-2-yl)-3-(p-substituted phenyl)-5-phenyl-3,3a,5,6-tetrahydro thiazolo [4,5-c]isoxazole 4,4-dioxide derivatives were taken up in the present study.

MATERIALS AND METHODS

The Progress of the reaction was monitored by TLC plates. Infra red Spectrum of compounds were recorded by Perkin Elmer BX series and proton NMR spectra were recorded by Bruker 400 MHz instrument using DMSO as solvent and Tetramethylsilane used as an internal standard. Chemical shifts (δ) were expressed in ppm. Mass spectrums of the compounds were measured on a GC/MS-QP1000EX (EI, 70 eV) mass spectrometer. Elemental analysis was performed on PerkinElmer 240 CHN analyzer.



(**a**)=Benzaldehyde, Glacial acetic acid, EtOH, reflux, 4h; (**b**) =aromatic aldehydes, HS-CH_3COOH, 1, 4 Dioxane, $ZnCl_2$, reflux, 6h; (**c**)=NH_2-NH_2.H_2O, anhydrous sodium acetate, Glacial acetic acid, ethanol, reflux,6.5h; (**d**) = H_2O_2,Glacial acetic acid, 3h; (**e**) =NH_2-OH.HCl, anhydrous sodium acetate, Glacial acetic acid, ethanol, reflux,6 h; (**f**)= H_2O_2, Glacial acetic acid,3.5h:

Table 1: Physical data of Synthesized compounds 4, 5, 6 & 7 (a-e)

Ar'	Ph	4-OCH_3- Ph	4- NO_2 Ph	4-Cl - Ph	4- OH - Ph
Compounds	**4a**	**4b**	**4c**	**4d**	**4e**
M.P (^{0}C)	212-14	218-20	221-23	219-21	218-20
Yield (%)	75	72	76	69	65
Compounds	**5a**	**5b**	**5c**	**5d**	**5e**
M.P (^{0}C)	215-17	220-22	222-24	221-23	222-24
Yield (%)	78	72	73	70	68
Compounds	**6a**	**6b**	**6c**	**6d**	**6e**
M.P (^{0}C)	228-30	231-33	234-36	232-34	233-35
Yield (%)	76	71	76	67	63
Compounds	**7a**	**7b**	**7c**	**7d**	**7e**
M.P (^{0}C)	231-33	234-36	235-37	236-38	235-38
Yield (%)	74	70	68	71	64

General reaction procedure for compound 4a:

Equimolar mixture of 3 (ref) (0.03 mol), hydrazine hydrate (0.03mol) and anhydrous CH_3COONa (0.001 mol) in glacial acetic acid (30ml) were heated under reflux for about 6.5 hours, the resulting compound was cooled at room temperature and poured in to crushed ice. The product was filtered, washed with water and recrystallized with ethanol to afford the pure compound. The remaining compounds (4b-4h) were prepared by similar procedure with minor changes as per the reaction conditions.

6-(1H-benzo[d]imidazole-2-yl)-3,5-diphenyl-3,3a,5,6-tetrahydro-2H-pyrazolo[3,4-d] thiazole (4a):

IR (KBr, cm-1): 3345 (N-H) , 3072(C-H), 1563(C=N), 1674 (C=O), 1235(C=S), 1045 (N-N); ^{1}HNMR (DMSO-d_6 ,400MHz, δ in ppm) :4.41(d,1H, CH-S), 4.84 (d, 1H, CH-N), 5.92 (s,1H, N-CH-Ar), 7.12-7.24 (m,4H,Ar-H),7.28-7.48 (m,5H,Ar-H),7.56-7.79 (m,5H,Ar-H), 9.76 (br, 1H,NH); MS, m/z (%) 397 (M^+) ; Anal. Calcd for C_{23} $H_{19}N_5S$: C, 69.92; H, 4.87; N, 17.97%. Found: C, 69.50; H, 4.52; N, 17.62%.

6-(1H-benzo[d]imidazole-2-yl)-3-(4-methoxyphenyl)-5-phenyl-3,3a,5,6-tetrahydro-2H-pyrazolo [3,4-d] thiazole (4b):

IR (KBr, cm-1): 3348 (N-H) , 3075 (C-H), 1562 (C=N), 1678 (C=O), 1240 (C=S), 1052 (N-N); ^{1}HNMR (DMSO-d_6 ,400MHz, δ in ppm): 3.45 (s, 3H,OCH_3), 4.66 (d, 1H, CH-S), 4.92 (d,1H, N-CH-Ar),5.75(s,1H,N-CH-Ar), 7.22-7.28 (m,4H,Ar-H) ,7.33-7.59 (m,4H,Ar-H),7.65-7.88 (m,4H,Ar-H), 10.08 (br, 1H,NH); MS, m/z (%) 427 (M^+) ; Anal. Calcd for C_{24} $H_{21}N_5OS$: C, 67.45; H, 4.95; N, 16.64 %. Found: C, 67.02; H, 4.26; N, 16.02%.

6-(1H-benzo[d]imidazol-2-yl)-3-(4-nitrophenyl)-5-phenyl-3,3a,5,6-tetrahydro-2H-pyrazolo [3,4-d] thiazole (4c):

IR (KBr, cm-1): 3349 (N-H), 1578 (C=N), 1526(NO_2) 1681 (C=O), 1236 (C=S), 1055 (N-N); ^{1}HNMR (DMSO-d_6,400MHz, δ in ppm): 4.72 (d, 1H, CH-S), 4.95 (d,1H, N-CH-Ar), 5.95 (s,1H,N-CH-Ar), 7.18-7.32 (m,4H,Ar-H) ,7.29-7.55 (m,5H,Ar-H), 8.13-8.41 (m,4H,Ar-H), 10.25 (br, 1H,NH) ; MS, m/z (%) 442 (M^+) ; Anal. Calcd for C_{23} $H_{18}N_6O_2S$: C, 62.45; H, 4.19; N, 18.52 %. Found: C, 68.45; H, 4.03; N, 10. 42%.

6-(1H-benzo[d]imidazol-2-yl)-3-(4-chlorophenyl)-5-phenyl-3,3a,5,6-tetrahydro-2H-pyrazolo [3, 4-d] thiazole (4d):

IR (KBr, cm-1): 3352 (N-H) , 1568 (C=N) ,1685 (C=O), 1234 (C=S), 875(C-Cl), 1056 (N-N); ^{1}HNMR (DMSO-d_6 ,400MHz, δ in ppm): 4.71 (d, 1H, CH-S), 4.95 (d,1H, N-CH-Ar), 5.82 (s,1H,N-CH-Ar), 7.15-7.34 (m,4H,Ar-H) ,7.35-7.54 (m,4H,Ar-H),7.61-7.86 (m,4H,Ar-H),10.18 (br, 1H,NH) ; MS, m/z (%) 431 (M^+) ; Anal. Calcd for $C_{23}H_{26}N_5$ S: C, 66.96; H, 4.26; N, 16.25 %. Found: C, 66.24; H, 4.03; N, 15.82%.

4-(6-(1H-benzo[d]imidazole-2-yl)-5-phenyl-3,3a,5,6-tetrahydro-2H-pyrazolo[3,4-d]thiazol-3-yl) phenol(4e):

IR (KBr, cm-1): 3518 (OH), 3351(N-H), 3035(C-H) , 1574 (C=N), 1681 (C=O), 1240 (C=S), 1045 (N-N); ^{1}HNMR (DMSO-d_6 ,400MHz, δ in ppm): 4.65 (d, 1H, CH-S), 4.72 (d,1H, N-CH-Ar), 5.92(s,1H,N-CH-Ar), 7.16-7.27 (m,4H,Ar-H) ,7.30-7.55 (m,5H,Ar-H),7.62-7.78 (m,4H,Ar-H), 10.15 (br, 1H,NH), 11.32 (s,1H, OH) ; MS, m/z (%) 413 (M^+) ; Anal. Calcd for C_{24} $H_{19}N_5$ OS: C, 66.81; H, 4.66; N, 16.88 %. Found: C, 67.23; H, 3.96; N, 16.42%.

General reaction procedure for compound 5:

A mixture of compound 3a (0.03 mol), hydroxylamine hydrochloride (0.03mol) and anhydrous sodium acetate (0.001 mol) in glacial acetic acid (25 ml) was heated under reflux for about 6 hours , the resulting product was cooled at room temperature and poured in to crushed ice. The resulting solid was filtered, washed with water and recrystallized from ethanol to afford the pure compound. The remaining compounds 5(b-e) were prepared by similar procedure with minor changes as per the reaction conditions.

6-(1H-benzo[d]imidazol-2-yl)-3,5-diphenyl-3,3a,5,6-tetrahydrothiazolo[4,5-c]isoxazole (5a)

IR (KBr, cm^{-1}): 3345 (N-H),3079 (C-H),1565 (C=N), 1674(C=O), 1235 (C=S); ^{1}HNMR (DMSO-d_6 ,400MHz, δ in ppm) :4.46 (d,1H, CH-S), 4.88 (d, 1H, CH-N), 5.92 (s,1H, N-CH-Ar),7.10-7.25 (m,4H,Ar-H), 7.25-7.48 (m,5H,Ar-H),7.56-7.80 (m,5H,Ar-H),9.78(br,1H, NH) ; MS, m/z (%) 398 (M^+) ;

Anal. Calcd for C_{23} $H_{18}N_4OS$: C, 69.32; H, 4.55; N, 14.06 %. Found: C, 69.40; H, 4.42; N, 13.62%.

6-(1H-benzo[d]imidazol-2-yl)-3-(4-methoxyphenyl)-5-phenyl-3,3a,5,6-tetrahydrothiazolo [4,5-c] isoxazole (5b):

IR (KBr, cm-1): 3345 (N-H) , 3033 (C-H), 1563 (C=N), 1688 (C=O), 1244 (C=S); ^{1}HNMR (DMSO-d_6 ,400MHz, δ in ppm): 3.45 (s, 3H,OCH_3), 4.65 (d, 1H, CH-S), 4.89 (d,1H, N-CH-Ar),5.74(s,1H,N-CH-Ar), 7.21-7.28 (m,4H,Ar-H) ,7.31-7.58 (m,4H,Ar-H),7.65-7.87 (m,4H,Ar-H), 10.08 (br, 1H,NH); MS, m/z (%) 428 (M^+) ; Anal. Calcd for C_{24} $H_{20}N_4O_2S$: C, 67.27; H, 4.70; N, 13.07 %. Found: C, 67.02; H, 4.26; N, 12.82%.

6-(1H-benzo[d]imidazol-2-yl)-3-(4-nitrophenyl)-5-phenyl-3,3a,5,6-tetrahydrothiazolo[4,5-c]isoxazole (5c):

IR (KBr, cm^{-1}): 3346 (N-H) , 1574 (C=N), 1528(NO_2) ,1682 (C=O), 1235 (C=S); ^{1}HNMR (DMSO-d_6 ,400MHz, δ in ppm): 4.78 (d, 1H, CH-S), 4.98 (d,1H, N-CH-Ar), 5.97 (s,1H,N-CH-Ar), 7.18-7.26 (m,4H,Ar-H) ,7.29-7.52 (m,5H,Ar-H),8.19-8.44 (m,4H,Ar-H), 10.21 (br, 1H,NH) ; MS, m/z (%) 443 (M^+); Anal. Calcd for C_{23} $H_{17}N_5O_3S$: C, 62.29; H, 3.86; N, 15.79 %. Found: C, 61.95; H, 3.83; N, 14.92%.

6-(1H-benzo[d]imidazol-2-yl)-3-(4-chlorophenyl)-5-phenyl-3,3a,5,6-tetrahydrothiazolo[4,5-c]isoxazole (5d):

IR (KBr, cm-1): 3365 (N-H), 1563 (C=N) ,1686 (C=O), 1233 (C=S), 875 (C-Cl); ^{1}HNMR (DMSO-d_6, 400MHz, δ in ppm): 4.78 (d, 1H, CH-S), 4.96 (d,1H, N-CH-Ar), 5.84 (s,1H,N-CH-Ar), 7.18-7.32 (m,4H,Ar-H) ,7.36-7.52 (m,4H,Ar-H),7.60-7.85 (m,4H,Ar-H),10.16 (br, 1H,NH) ; MS, m/z (%) 432 (M^+), 434($M+^1$) ; Anal. Calcd for $C_{23}H_{17}ClN_4OS$: C, 63.81; H, 3.96; N, 12.94 %. Found: C, 63.24; H, 3.55; N, 12.12%.

4-(6-(1H-benzo[d]imidazol-2-yl)-5-phenyl-3,3a,5,6-tetrahydrothiazolo[4,5-c]isoxazol-3-yl)phenol (5e):

IR (KBr, cm-1): 3510 (OH), 3355 (N-H), 3034 (C-H), 1574 (C=N), 1682 (C=O), 1243 (C=S), ^{1}HNMR (DMSO-d_6 ,400MHz, δ in ppm): 4.64 (d, 1H, CH-S), 4.82 (d,1H, N-CH-Ar), 5.98 (s,1H,N-CH-Ar), 7.19-7.26 (m,4H,Ar-H), 7.29-7.58 (m,5H,Ar-H),7.64-7.76 (m,4H,Ar-H), 10.18 (br, 1H,NH), 11.32 (s,1H, OH) ; MS, m/z (%) 414 (M^+) ; Anal. Calcd for C_{23} $H_{18}N_4O_2S$: C, 66.65; H, 4.38; N, 13.52 %. Found: C, 66.13; H, 3.96; N, 14.92%.

General reaction procedure for compound 6:

An ice cold solution of the compound 4 (1mmol) in glacial acetic acid (30 ml) was treated with 30% H_2O_2 (20 ml) in portions. The contents were allowed to attain laboratory temperature and then refluxed for 3.5 h. The reaction mixture was cooled and acetic acid was removed in vacuo. The residual portion was cooled by filtration and was further purified by recrystallisation using water. The remaining compounds (6b-e) were prepared by similar procedure with minor change in reaction conditions.

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

IR (KBr, cm-1): 3344 (N-H) , 3072 (C-H), 1560 (C=N), 1675 (C=O), 1234 (C=S), 1168 (SO_2), 1047 (N-N); ^{1}HNMR (DMSO-d_6 ,400MHz, δ in ppm) :4.4 5(d,1H, CH-S), 4.80 (d, 1H, CH-N), 5.93 (s,1H, N-CH-Ar), 7.12-7.24 (m,4H,Ar-H),7.28-7.48 (m,5H, Ar-H),7.57-7.79 (m,5H,Ar-H), 9.78 (br, 1H,NH); MS, m/z (%) 429 (M^+) ; Anal. Calcd for C_{23} $H_{19}N_5O_2S$: C, 64.32; H, 4.46; N, 16.31 %. Found: C, 64.04; H, 4.12; N, 15.82%.

6-(1H-benzo[d]imidazol-2-yl)-5-phenyl-3-(p-methoxy)-3,3a,5,6-tetrahydro-2H-pyrazolo [3,4-d] thiazole 4,4-dioxide(6b):

IR (KBr, cm-1): 3351 (N-H) , 3035 (C-H), 1566 (C=N), 1688 (C=O), 1244 (C=S),1165 (SO_2), 1065 (N-N); ^{1}HNMR (DMSO-d_6 ,400MHz, δ in ppm): 3.45 (s, 3H,OCH_3), 4.66 (d, 1H, CH-S), 4.85(d,1H, N-CH-Ar),5.76 (s,1H,N-CH-Ar), 7.26-7.31 (m,4H,Ar-H) ,7.35-7.59 (m,4H,Ar-H),7.65-7.88 (m,4H,Ar-H), 10.08 (br, 1H,NH); MS, m/z (%) 459 (M^+) ; Anal. Calcd for C_{24} $H_{21}N_5O_3S$: C, 62.73; H, 4.61; N, 15.24 %. Found: C, 62.42; H, 4.26; N, 14.82%.

6-(1H-benzo[d]imidazol-2-yl)-3-(4-nitrophenyl)-5-phenyl-3,3a,5,6-tetrahydro-2H-pyrazolo [3,4-d]thiazole 4,4-dioxide (6c):

IR (KBr, cm-1): 3348 (N-H) , 1572 (C=N), 1526 (NO_2) ,1683 (C=O), 1234 (C=S), 1168 (SO_2), 1052 (N-N); ^{1}HNMR (DMSO-d_6 ,400MHz, δ in ppm): 4.78 (d, 1H, CH-S), 4.94 (d,1H, N-CH-Ar), 5.96 (s,1H,N-CH-Ar), 7.18-7.25 (m,4H,Ar-H) ,7.29-7.52 (m,5H,Ar-H),8.16-8.44 (m,4H,Ar-H), 10.25 (br, 1H,NH) ; MS, m/z (%) 474 (M^+) ; Anal. Calcd for C_{23} $H_{18}N_6O_4S$: C, 58.22; H, 3.82; N, 17.71 %. Found: C, 58.05; H, 3.53; N, 17.52%.

6-(1H-benzo[d]imidazol-2-yl)-3-(4-chlorophenyl)-5-phenyl-3,3a,5,6-tetrahydro-2H-pyrazolo [3,4-d]thiazole 4,4-dioxide (6d):

IR (KBr, cm-1): 3354 (N-H) , 1562 (C=N) ,1694 (C=O), 1234 (C=S),1164(SO_2), 875 (C-Cl), 1054 (N-N); ^{1}HNMR (DMSO-d_6 ,400MHz, δ in ppm): 4.72 (d, 1H, CH-S), 4.94 (d,1H, N-CH-Ar), 5.86 (s,1H,N-CH-Ar), 7.19-7.34 (m,4H,Ar-H) ,7.38-7.54 (m,4H,Ar-H),7.62-7.88 (m,4H,Ar-H),10.18 (br, 1H,NH) ; MS, m/z (%) 463 (M^+), 465($M+^2$) ; Anal. Calcd for $C_{23}H_{18}ClN_5O_2S$: C, 59.54; H, 3.91; N, 15.10 %. Found: C, 59.24; H, 3.73; N, 14.82%.

6-(1H-benzo[d]imidazol-2-yl)-3-(4-hydroxyphenyl)-5-phenyl-3,3a,5,6-tetrahydro-2H-pyrazolo [3,4-d]thiazole 4,4-dioxide (6e):

IR (KBr, cm-1): 3518 (OH), 3352 (N-H) ,3034 (C-H) , 1574 (C=N), 1682 (C=O), 1248 (C=S),1161(SO_2), 1042 (N-N); ^{1}HNMR (DMSO-d_6 ,400MHz, δ in ppm): 4.63 (d, 1H, CH-S), 4.79 (d,1H, N-CH-Ar), 5.97(s,1H,N-CH-Ar), 7.14-7.24 (m,4H,Ar-H), 7.31-7.54 (m,5H,Ar-H), 7.63-7.76 (m,4H,Ar-H), 10.18 (br, 1H,NH), 11.42 (s,1H, OH) ; MS, m/z (%) 445 (M^+); Anal. Calcd for C_{23} $H_{19}N_5O_2S$: C, 64.32; H, 4.46; N, 16.31%. Found: C, 64.03; H, 3.96; N, 15.92%.

General reaction procedure for compound 7:

An ice cold solution of the compound 5 (1mmol) in glacial acetic acid (30 ml) was treated with 30% H_2O_2 (20 ml) in portions. The contents were allowed to attain laboratory temperature and then refluxed for 3h. The reaction mixture was cooled and acetic acid was removed in vacuo. The

residual portion was cooled by filtration and was further purified by recrystallisation using water. The remaining compounds were prepared by similar procedure with minor change in reaction conditions.

6-(1H-benzo[d]imidazol-2-yl)-3,5-diphenyl-3,3a,5,6-tetrahydrothiazolo[4,5-c]isoxazole 4,4-dioxide(7a):

IR (KBr, cm-1): 3345 (N-H) , 3068 (C-H), 1562 (C=N), 1674 (C=O), 1235 (C=S); ^{1}HNMR (DMSO-d_6 ,400MHz, δ in ppm) :4.45 (d,1H, CH-S), 4.88 (d, 1H, CH-N), 5.93 (s,1H, N-CH-Ar),7.12-7.28(m,4H,Ar-H),7.32-7.45(m,5H,Ar-H),7.58-7.81 (m,5H,Ar-H), 9.68 (br, 1H,NH); MS, m/z (%) 430 (M^+) ; Anal. Calcd for C_{23} $H_{18}N_4O_3S$: C, 64.17; H, 4.21; N, 13.01 %. Found: C, 63.90; H, 4.02; N, 12.62%.

6-(1H-benzo[d]imidazol-2-yl)-3-(4-methoxyphenyl)-5-phenyl-3,3a,5,6-tetrahydro thiazolo [4,5-c]isoxazole 4,4-dioxide(7b):

IR (KBr, cm-1): 3343 (N-H) , 3038 (C-H), 1560 (C=N), 1685 (C=O), 1241 (C=S), 1174(SO_2),1HNMR (DMSO-d_6 ,400MHz, δ in ppm): 3.48 (s, 3H,OCH_3), 4.62 (d, 1H, CH-S), 4.83 (d,1H, N-CH-Ar),5.74(s,1H,N-CH-Ar), 7.21-7.28 (m,4H,Ar-H) ,7.31-7.58 (m,4H,Ar-H),7.68-7.87 (m,4H,Ar-H), 10.06 (br, 1H,NH); MS, m/z (%) 460 (M^+) ; Anal. Calcd for C_{24} $H_{20}N_4O_4S$: C, 62.60; H, 4.38; N, 12.17 %. Found: C, 62.22; H, 4.26; N, 12.02%.

6-(1H-benzo[d]imidazol-2-yl)-3-(4-Nitro phenyl)-5-phenyl-3,3a,5,6-tetrahydro thiazolo [4,5-c] isoxazole 4,4-dioxide (7c):

IR (KBr, cm-1): 3348 (N-H) , 1576 (C=N), 1523 (NO_2) ,1688 (C=O), 1232 (C=S), 1172(SO_2), ^{1}HNMR (DMSO-d_6 ,400MHz, δ in ppm): 4.73 (d, 1H, CH-S), 4.98 (d,1H, N-CH-Ar), 5.95 (s,1H,N-CH-Ar), 7.18-7.34 (m,4H,Ar-H) ,7.27-7.54 (m,5H,Ar-H),8.15-8.45 (m,4H,Ar-H), 10.25 (br, 1H,NH) ; MS, m/z (%) 475 (M^+) ; Anal. Calcd for C_{23} $H_{17}N_5O_5S$: C, 58.10; H, 3.60; N, 10.42 %. Found: C, 57.85; H, 3.03; N, 10. 12%.

7d: 6-(1H-benzo[d]imidazol-2-yl)-3-(4-chlorophenyl)-5-phenyl-3,3a,5,6-tetrahydro thiazolo [4,5-c]isoxazole 4,4-dioxide(7d):

IR (KBr, cm-1): 3348 (N-H), 1563 (C=N), 1691 (C=O), 1233 (C=S),1164(SO_2), 872(C-Cl); ^{1}HNMR (DMSO-d_6 ,400MHz, δ in ppm): 4.73 (d, 1H, CH-S), 4.95 (d,1H, N-CH-Ar), 5.88 (s,1H,N-CH-Ar),7.15-7.36(m,4H,Ar-H),7.368-7.58(m,4H,Ar-H),7.64-7.89(m,4H,Ar-H), 10.18 (br, 1H,NH) ; MS, m/z (%) 464 (M^+), 466($M+^1$) ; Anal. Calcd for $C_{23}H_{17}ClN_4O_3S$: C, 59.42; H, 3.69; N, 12.05 %. Found: C, 59.14; H, 3.03; N, 11.82%.

7e: 6-(1H-benzo[d]imidazol-2-yl)-3-(4-hydroxyphenyl)-5-phenyl-3,3a,5,6-tetrahydro thiazolo [4,5-c]isoxazole 4,4-dioxide(7e):

IR (KBr, cm-1): 3518 (OH), 3356 (N-H), 3034 (C-H) , 1574 (C=N), 1680 (C=O), 1243 (C=S), 1163(SO_2), ^{1}HNMR (DMSO-d_6 ,400MHz, δ in ppm): 4.65 (d, 1H, CH-S), 4.76 (d,1H, N-CH-Ar), 5.98 (s,1H,N-CH-Ar), 7.19-7.26 (m,4H,Ar-H) ,7.32-7.58 (m,5H,Ar-H),7.64-7.78 (m,4H,Ar-H), 10.18 (br, 1H,NH), 11.32 (s,1H, OH) ; MS, m/z (%) 446 (M^+) ; Anal. Calcd for C_{23} $H_{18}N_4O_4S$: C, 61.87; H, 4.06; N, 13.55 %. Found: C, 61.23; H, 3.96; N, 13.02%.

Antioxidant Activity

DPPH free radical scavenging assay

The antioxidant activity of compounds was measured in terms of hydrogen donation or radical scavenging ability using the stable free radical according to DPPH method[16]. 200 ml aliquots of sample at different concentrations were mixed with 1.8 ml of the DPPH solution (0.5 mM). The reaction mixture was allowed to stand at room temperature for 30 minutes and absorbance was measured at 517nm using UV-VIS spectrophotometer. Control solution consisting of DPPH and DMSO without compounds was used as blank. Ascorbic acid was used as standard. The percentage inhibition of DPPH radical was calculated by comparing the results of the test with those of the control.

Nitric oxide scavenging activity

Briefly, 0.005mmol, sodium nitroprusside was prepared in phosphate buffered saline and mixed with different concentrations of compounds (100 µg/ml) followed by incubation at 25°C for 30 min. A control without the samples but with equivalent amounts of methanol was taken. After 30 min, 1.5 ml of incubated solution was removed and diluted with 1.5 ml of Griess reagent. The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with N-1- Napthyl ethylenediamine dihydrochloride was measured at 546 nm and percentage scavenging activity was measured with reference standard[17].

RESULTS AND DISCUSSION

Present investigation is the continuation of earlier research of authors in the synthesis and evaluation of antitubercular activity of pyrazole derivatives. Hence, in this direction, efforts have been undertaken to develop a versatile triheterocyclic moieties of the thiazolo-pyrazoles and thiazolo-isoxazoles on benzimidazole nucleus. The research work has been extended by adopting most biologically active sulfonyl group on the thiazole ring. The research report revealed that the antioxidant activity of sulfonyl group showed excellent activity than the unsulfonized thiazole which were tabulated in table 2. The compound **3** (Chalcone derivatives of thiazolidinone) is synthesised by one pot three-component cyclization using compound **2**, mercaptoacetic acid and benzaldehyde and anhydrous $ZnCl_2$[18]. Later, the Chalcone derivative of thiazolidinone **(3)** undergoes cyclization with hydrazine hydrate in presence of anhydrous acetic acid to afford the compound **4,** and the compound **5** was synthesized by cyclization of compound **3** and hydroxyl amine hydrochloride in anhydrous sodium acetate. The compounds **6** & **7** were synthesized by the oxidation of compound 4 and 5 with hydrogen peroxide in glacial acetic acid to afford the title compounds containing sulfonyl group. The structures of synthesised compounds were confirmed based on spectral and analytical data. The compounds showed IR absorption bands at 3345 cm^{-1} (NH), 1563 cm^{-1} (C=N), 1240 cm^{-1} (C=S), 1674 cm^{-1},(C=O) respectively. ^{1}HNMR spectra of test compounds displayed

singlet signals at 5.76 for N-CH-Ar, doublet signals at 4.66 CH-S, 4.85 CH-N, and 10.08 ppm for NH protons and also phenylic protons as multiplet in the range of 7.15-8.44 ppm. Mass spectra of synthesised compounds showed molecular ion peak at m/z with respect to their molecular weights.

All the synthesized compounds 4-7 (a-e) were screened for their antioxidant activity using free radical scavenging assay using 2,2-diphenyl-1-picrylhydrazyl(DPPH) and Nitric oxide scavenging method. Ascorbic acid is used as standard drug. The compounds were dissolved in DMSO with concentration of 100 µg/ml. The examination results of free radical scavenging ability of the newly synthesized compounds are summarized in table 2.

Table 2: Antioxidant activity of compounds 4, 5, 6 & 7 (a-e):

Compound	DPPH	Nitric oxide	Compound	DPPH	Nitric oxide
4a	2.54±0.1	2.66±0.5	**6a**	2.55±0.2	2.86±0.3
4b	1.25±0.1	1.80±0.2	**6b**	2.19±0.3	1.78±0.2
4c	0.76±0.3	0.85 ±0.2	**6c**	0.59 ±0.4	0.72±0.3
4d	1.12±0.4	1.25 ±0.4	**6d**	1.02±0.5	1.09±0.4
4e	1.44±0.5	1.98±0.5	**6e**	1.75±0.3	1.84 ±0.5
5a	2.24±0.1	2.66±0.5	**7a**	2.55±0.2	2.86±0.3
5b	2.08±0.1	2.18±0.2	**7b**	2.19±0.3	2.78±0.2
5c	1.26±0.3	1.32 ±0.2	**7c**	0.88±0.4	0.95±0.3
5d	1.23±0.4	1.15 ±0.4	**7d**	0.92±0.5	0.85±0.4
5e	1.54±0.5	1.98±0.5	**7e**	1.65±0.3	1.24 ±0.5
Ascorbic acid	0.55 ±0.5	0.63±0.3	*	*	*

Results are shown in standard error of the mean and standard deviation (SEM ± SD). $P < 0.5$

The test results of free radical scavenging ability of synthesized compounds 4-7(a-e) showed that the compound 6c and 7c are exhibiting excellent activity with DPPH & Nitric oxide (0.59 ±0.4, 0.72±0.3, 0.88±0.4 and 0.95±0.3) respectively due to more electron withdrawing NO_2 group, while compounds 6d and 7d also showed very good activity with DPPH & Nitric oxide (1.02±0.5, 1.09±0.4, 0.92±0.5 and 0.85±0.4) due to the presence of More electronegative chlorine atom. It was observed that the compounds 6c and 7c are showing the activity nearer to that of ascorbic acid (0.55 ±0.5 with DPPH, and 0.63±0.3 with Nitric oxide). The compounds 4c,5c ,4d and 5d are also exhibiting significant activity but lesser than 6c,7c,6d and 7d because, compounds 6 and 7 are containing sulfonyl group, which is more electron withdrawing and biologically more active than compounds 4 and 5. The remaining compounds also have good to moderate scavenging activity with IC_{50} values ranging from 1.12 ± 0.4 to 2.86 ± 0.3.

CONCLUSION

The present study reports the synthesis of new triheterocyclic compounds containing highly challenging benzimidazole, thiazole, pyrazole and isoxazole moieties in an appreciable yield. Based on the experimental results, it has been observed that the compounds scavenged DPPH, Nitric oxide with a significant activity as campared to the standard drug ascorbic acid.

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