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Research article

DESIGN, SYNTHESIS AND EVALUATION OF ANTIMICROBIAL ACTIVITIES OF SOME NOVEL 2-MERCAPTO-1,3,4-OXADIAZO-2-YL DERIVATIVES CLUBBED WITH 1*H*-BENZIMIDAZOLE.

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ABSTRACT: A series of *N*-(substitutedphenyl)-2-(1-((5-mercapto-1,3,4-oxadiazol-2-yl)methyl)-1*H*-benzo[*d*] imidazol-2-ylthio)acetamide**5 (A-H)**,2-(1*H*-benzo[*d*]imidazol-2-ylthio)-*N*-(4-(5-(substituted benzyl or pyridinylmethylthio)-1,3,4-oxadiazole-2-yl)phenyl)acetamide**5(J-M)**&2-(1*H*-benzo[*d*]imidazol-2-ylthio)-*N*-(4-(5-(2-(arylamino)-2-oxo-ethylthio)-1,3,4-oxadiazol-2-yl)phenyl)phenyl) acetamide**5 (N-S)** were prepared by the reaction of carbon disulphide with corresponding acid hydrazides **4 (A-H).**On the other compounds **5(J-M)** &**5 (N-S)**were prepared by the reaction of various benzyl halides or pyridinyl methyl halide and various chloroacetylated amines with 2-(1*H*-benzo[*d*]imidazol-2-ylthio)-*N*-(4-(5-mercapto-1,3,4-oxadiazo-2-yl)phenyl)acetamide **5I.** All the synthesized compounds were characterized by IR, ^{1}H NMR, and mass spectral technique and evaluated for their antimicrobial activity.

Key words: 2-MercaptoBenzimidazole, 1, 3,4-Oxadiazole, Antimicrobial Screening

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INTRODUCTION

Regardless of numerous attempts to search and develop new structural prototype as effective antimicrobials, benzimidazoles still remain as potential class of compounds. Recently, thechemistry and biological profiles of various pharmacophore having N-1 substituted and 2-substituted benzimidazole derivatives have been worked out (Ansari, K.F., Lal, C., 2008). Effect of substituent on the benzimidazole ring exhibited correlated structure–activity relationship (Powers, J.P., et.al, 2006).Integration of an imidazole nucleus, a biologically active pharmacophore, in the benzimidazole molecule has made it an adaptable heterocycle with wide spectrum of biological activity. Moreover, benzimidazole derivatives are structural isopterans of naturally occurring nucleotides, which allow them to interact easily with the biophores (Starcevic, K., et.al., 2007).Therefore, numerous biological activities of benzimidazole derivatives have been described; antimicrobial (Kus, C., et.al., 2009), anticancer (Thimmegowda, N.R., et.al., 2008), anti-inflammatory[a) Gangula, M.R., et.al., 2012, b) Mader M., et.al., 2008],antiviral(Vazquez, G.N., et.al., 2001), antiparasitic (Kazimierczuk, Z., et.al., 2002),antiprotozoal(Gomez, H.T., et.al., 2008), antihelminitics (Dahiya, R., Pathak, D., 2007), protein kinase inhibitors (Bernatowicz, A.N., et.al., 2009) and H^+/K^+ ATPase inhibitors (Cho, S.Y., et.al., 2001). 1,3,4-Oxadiazole (Figure 1) is a heterocyclic compound containing an oxygen atom and two nitrogen atoms in a five-membered ring. It can be derived from furan by substitution of two methylene groups (=CH) with two pyridine type nitrogens (-N=)[a) Bostrom, J., et.al., 2012, b) Nagaraj, et.al., 2011].

There are three known isomers: (1)1,3,4-oxadiazole (2) 1,2,5-oxadiazole (3) 1,2,3-oxadiazole (4) 1,2,4-oxadiazole (Figure 1)However, 1,3,4-oxadiazole and 1,2,4-oxadiazole are better known, and more widely studied by researchers because of their many important chemical and biological properties.

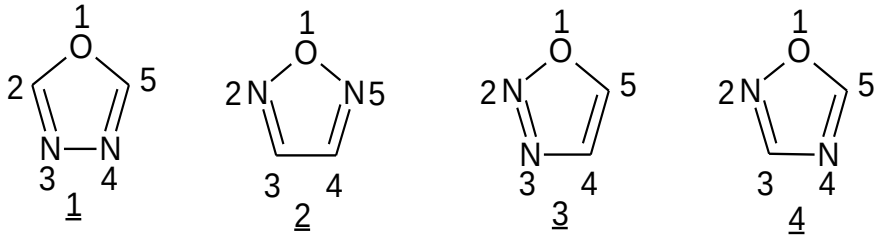


Figure-1: Isomers of Oxadiazole

Among various heterocyclic compounds, 1,3,4-oxadiazole has become an important construction motif for the development of new drugs. Compounds comprising 1,3,4-oxadiazole cores have a broad biological activity spectrum including antibacterial, antifungal, analgesic, anti-inflammatory(Dhani, R., 2012), antiviral, anticancer, antihypertensive, anticonvulsant, and anti-diabetic properties(Cledualdo S. O., et.al., 2012). They have also gain interest in medicinal chemistry in form of surrogates (bioisosteres) for carboxylic acids, esters and carboxamides. The ability of 1,3,4-oxadiazole heterocyclic compounds to undergo various chemical reactions has made them noteworthy for molecule planning because of their privileged structure, which has enormous biological potential. Two examples of compounds containing the 1,3,4-oxadiazole unit currently used in clinical medicine are: Raltegravir® , an antiretroviral drug(Savarino, A. A., 2006) and Zibotentan® an anticancer agent(James, N.D., Growcott, J.W., 2009).

MATERIAL AND METHOD

Chemistry

All chemicals and solvents were supplied by Merck, S.D. Fine Chem limited. All the solvents were distilled and dried before use. The reactions were monitored with the help of thin-layer chromatography using pre-coated aluminum sheets with GF254 silica gel, 0.2 mm layer thickness (E. Merck). Various solvent systems used for developing the chromatograms were (a) chloroform/methanol (9:1), (b) chloroform/ methanol (9.5:0.5), (c) ethyl acetate/hexane (5:5). Melting points of the synthesized compounds were recorded on the Veego (VMP-MP) melting point apparatus and not corrected.IR spectrum was acquired on a Shimadzu Infra Red Spectrometer, (model FTIR-8400S). 1H NMR spectra of the synthesized compounds were performed in DMSO with IR spectra were recorded on Shimadzu FT-IR-8400 instrument using KBr pellet method. Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using direct inlet probe technique. ^{1}H NMR and ^{13}C NMR was determined in DMSO-d6 solvent on a Bruker AC 400 MHz spectrometer.

EXPERIMENTAL

General procedure for the synthesis of compounds1 (A-I)

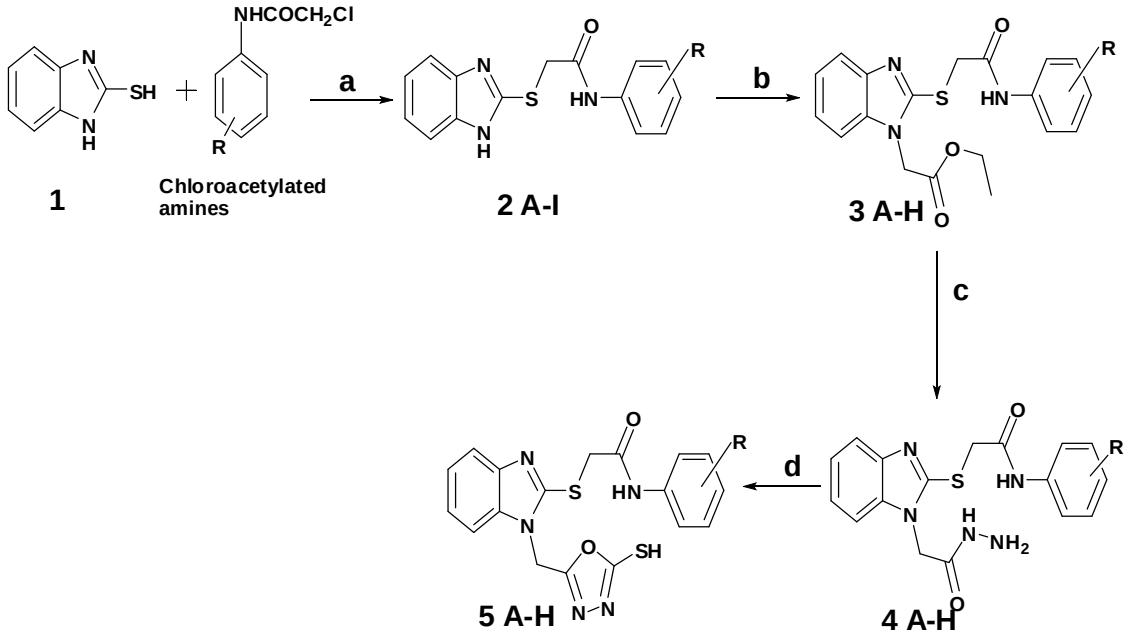
In a round bottom flask, chloro acetyl chloride (0.08mole) was added drop wise to the stirred solution of aromatic amines (5gm, 0.05mole) in equal quantity of acetic acid and saturated aq.solution of sodium acetate. Reaction mixture was stirredfor another 3h at room temperature.Reaction was monitored by TLC,and precipitated solid was collected via filtration, washed with acetic acid: water(50:50),water and dried well to get compounds **1(A-I)** in 80%yield.

General procedure for the synthesis of compounds 2 (A-I)

A mixture of 2-mercaptobenzimidazole **1**(5.0gm, 0.033 mole), chloroacetylated amines (0.033mole) and piperidine (0.066mole) in acetonitrile was heated at 60^{o}C for 3h in a RBF (monitored by TLC). The reaction mixture was cooled and poured into crushed ice and resulting solid was filtered, washed with water, dried well and purified with methanol to obtained **2 (A-I**)in 70% yield.

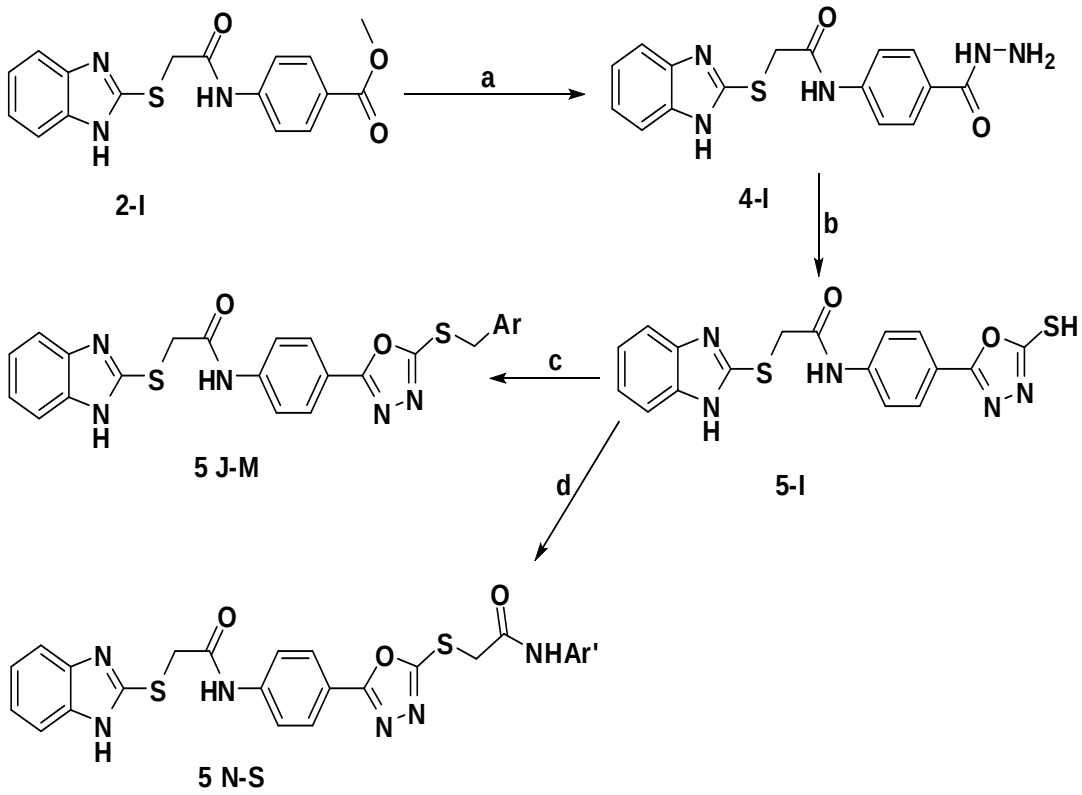
General procedure for the synthesis of compounds 3 (A-H)

A suspension of compounds **2 (A-H)** (3gm 0.02mole), bromoethylacetate (0.02mole) and sodium carbonate (0.04mole) in acetonitrile was heated at 60^{o}C for 1h. The reaction mixture was cooled and poured into crushed ice and resulting solid was filtered, washed with water, dried well and purified in methanol to get desired compounds **3 (A-H)** in 90% yield.



Where R = -F, -Cl, -CH_3, -CH_2CH_3, -OCH_3,-$COOCH_3$, -$NHCOCH_3$

Figure-2: Reaction Scheme-1: Reactants: (a) Piperidine, CH_3CN, 60°C (b) $BrCH_2COOEt$,K_2CO_3, CH_3CN, 60°C (c) Hydrazine hydrate, CH_3OH, Room temperature (d) CS_2,KOH, CH_3CH_2OH,reflux



Where **Ar = -C_6H_5, -2,3,5 $F_3C_6H_2$, -4 $OCH_3C_6H_4$, -3,4,-di$OCH_3C_5H_2N$**

Ar'= -F, -Cl, -CH_2CH_3, - 4 OCH_3, -$NHCOCH_3$

Figure-3: Reaction Scheme-2 Reactants: (a) Hydrazine hydrate, CH_3OH, 60°C (b)CS_2,KOH,CH_3CH_2OH,90°C (c) NaOH, $ArCH_2Cl$, CH_3OH, 60°C (d) NaOH, Ar'-$NHCOCH_2Cl$, CH_3OH, 60°C

General procedure for the synthesis of compounds 4 (A-H)

Hydrazine hydrate (1ml) was added to a stirred suspension of compounds **3 (A-H**)(2gm, 0.005mole) in methanol and stirred for 2h at ambient temperature. The precipitated solid was filtered and washed with methanol and dried well to get pure compound **4 (A-H)**in 80% yield.

General procedure for the synthesis of compounds 5(A-H)

A mixture of Compounds **4(A-H)** (1gm, 0025mole), potassium hydroxide (0.005mol) and carbon disulfide (3ml) in ethanol (20 ml) was stirred at 25-30 ^{o}C for 1h. The precipitated xanthate salt was filtered, washed with ethanol and again taken in ethanol (25ml).It was heated under reflux untilthe evolution of hydrogen sulfide ceased. The reaction mixturewas cooled to room temperature and poured into ice cold water(100 ml). It was neutralized with dilute hydrochloric acid. Theprecipitated solid was filtered, washed with water and the driedproduct was recrystallized from ethanol to get compounds **5(A-H).**

Procedure for the synthesis of compounds (4-I)

A mixture of compound **2-I** (10gm, 0.029mole) and excess amount of hydrazine hydrate in methanolwas refluxed for 5h.After being refluxed, reaction mixture cooled to room temperature and precipitated solid was filtered and dried well to obtain **4-I** in 60% yield.

Procedure for the synthesis of compounds (5-I)

Carbon disulphide (2ml) was added to a solution of compound **4-I** (4gm, 0.0116 mole) in ethanol (20ml) containing potassium hydroxide (0.0232mole)) and stirred for 1 hr. The precipitated xanthate salt was filtered, washed with ethanol and again taken in ethanol (20ml). It was heated under reflux untilthe evolution of hydrogen sulfide ceased.After being heated reaction mixture was allowed to cool at room temperature, poured into crushed ice and acidified it with 1N HCl. The precipitated solid was collected via filtration, dried well and purified in methanol to obtain**5-I** in 60% yield.

General procedure for the synthesis of compounds 5 (J-M)

A mixture of compound**5-I**(0.5gm, 0.0013mole), potassium hydroxide (0.0026mole) and substituted benzyl halides (0.0013mole) in methanol was refluxed for 1h. The reaction mixture was allowed to cool at room temperature, poured into crushed ice and precipitated solid was collected via filtration, dried well and purified by methanol to get **5 (J-M).**

General procedure for the synthesis of compounds*5* (N-S)

A suspension of compound **5-I**(0.5gm, 0.0013mole), potassium hydroxide (0.0026mole) and chloroacetylated amines (0.0013mole) in methanol was heated to reflux for 1h. The reaction mixture was allowed to cool at room temperature, poured into crushed ice and precipitated solid was collected via filtration, dried well and purified by methanol to get **5 (N-S).**

2.1.5.1 *N*-(3-chlorophenyl)-2-(1-((5-mercapto-1,3,4-oxadiazol-2-yl)methyl)-1*H*-benzo[*d*]imi-dazol-2-ylthio) acetamide (5A)

This compound was prepared and purified as per the above mentioned procedure. IR (KBr, cm^{-1}): 1650(–C=O stretching,–CONH–), 3076, 3057 (C–H stretching, aromatic ring), 2878 (C–H stretching, –CH_2–), 1488, 1501(C=C stretching,aromatic ring), 1445 (C–H bending, –CH_2–),1183 (C–O–C stretching, oxadiazole), 772 (C–Cl), ^{1}H NMR (400 MHz, DMSO-d_6, ppm):4.38(s,2H,),5.71(s,2H),7.11-7.26(m,3H),7.32(t,1H),7.42(d,1H),7.57(dd,2H),7.78(s,1H), 10.68(s,1H), MS**:** m/z 431.7 $(M+H)^{+}$;m.p.140-143^{o}C,Yield: 60%

2-(1-((5-mercapto-1, 3, 4-oxadiazol-2-yl) methyl)-1*H*-benzo[*d*]imidazol-2-ylthio)-*N*-m-tolylacetamide (5B)

This compound was prepared and purified as per the above mentioned procedure. IR (KBr, cm-1): 1673(–C=O stretching, –CONH–), 3064, 3048 (C–H stretching, aromatic ring), 2877 (C–H stretching, –CH_2–), 1385(C-H stretching, –CH_3)1530, 1594(C=Cstretching, aromatic ring), 1445 (C–H bending, –CH_2–), 1187 (C–O–C stretching, oxadiazole). ^{1}H NMR (400 MHz, DMSO-d6, ppm): 2.26(s,3H), 4.27 (s, 2H,), 5.42 (s, 2H), 6.86 (d,1H),7.15-7.23 (m,3H), 7.35 (bs,1H),7.37-7.56(m,3H),7.78(s,1H),10.40(s,1H),MS:m/z 412.29 $(M+H)^{+}$m.p.130-132^{o}CYield: 66%

***N*-(4-acetamidophenyl)-2-(1-((5-mercapto-1,3,4-oxadiazol-2-yl)methyl)-1*H*-benzo[*d*]imidazol-2-ylthio) acetamide (5C)**

This compound was prepared and purified as per the above mentioned procedure. IR (KBr, cm^{-1}): 1673(–C=O stretching,–CONH–), 3064, 3048 (C–H stretching, aromatic ring), 2877 (C–H stretching, –CH2–), 1385(C-H stretching –CH_3)1530, 1594(C=C stretching, aromatic ring), 1445 (C–H bending, –CH2–), 1187 (C–O–C stretching, oxadiazole)^{1}H NMR (400 MHz, DMSO-d6, ppm):2.01(s,3H), 4.34 (s, 2H,), 5.71 (s, 2H),7.24-7.29(m,2H),7.45(dd,4H),7.56-7.62(m,2H),9.91(s,1H),10.40(s,1H), MS**:** m/z 454.9 $(M+H)^{+}$; m.p.185-187^{o}C,Yield: 58%.

2-(1-((5-mercapto-1, 3, 4-oxadiazol-2-yl) methyl)-1*H*-benzo[*d*]imidazol-2-ylthio)-*N*-(4-ethoxyphenyl) acetamide (5D)

This compound was prepared and purified as per the above mentioned procedure. IR (KBr, cm^{-1}): 1677(–C=O, –CONH–), 3068, 3052 (C–H stretching, aromatic ring), 2870 (C–H stretching, $–CH_2–$),1544, 1699(C=Cstretching, aromatic ring), 1447 (C–H bending, $–CH_2–$), 1180 (C–O–C stretching, oxadiazole), ^{1}H NMR (400 MHz, DMSO-d6, ppm): 3.71(s,3H), 4.32 (s, 2H,), 5.69 (s, 2H), 6.85-6.90(m,2H), 7.20-7.28(m,2H), 7.45-7.50(m,2H), 7.57-7.59(m,2H), 10.31 (s,1H)**,** MS**:** m/z 428.19 $(M+H)^+$, m.p.150-153^{o}C;Yield: 63%

2-(1-((5-mercapto-1, 3, 4-oxadiazol-2-yl) methyl)-1*H*-benzo[*d*]midazol-2-ylthio)-*N*-o-tolylacetamide (5E)

This compound was prepared and purified as per the above mentioned procedure. IR (KBr, cm-1): 1673(–C=O stretching,–CONH–), 3064, 3048 (C–H stretching, aromatic ring), 2877 (C–H stretching, $–CH_2–$), 1385(C-H stretching $–CH_3$)1530, 1594(C=C stretching,aromatic ring), 1445 (C–H bending, –CH2–), 1187 (C–O–C stretching, oxadiazole).^{1}H NMR (400 MHz, DMSO-d6,ppm):2.25(s,3H), 4.26 (s, 2H,), 5.40 (s, 2H),6.80-7.02 (m,2H),7.14-7.28(m,5H),7.49-7.59(m,2H),10.40(s,1H),MS:m/z 412.29 $(M+H)^+$; m.p.135-137^{o}C; Yield: 60%

***N*-(4-fluorophenyl)-2-(1-((5-mercapto-1,3,4-oxadiazol-2-yl)methyl)-1*H*-benzo[*d*]imi -dazol-2-ylthio) acetamide (5F)**

This compound was prepared and purified as per the above mentioned procedure. IR (KBr, cm-1): 1670(–C=O stretching ,–CONH–), 3060, 3042 (C–H stretching, aromatic ring), 2870 (C–H stretching, $–CH_2–$),1533, 1599(C=Cstretching,aromatic ring), 1440 (C–H bending, $–CH_2–$), 1187 (C–O–C stretching, oxadiazole), 700 (C-F),^{1}H NMR (400 MHz, DMSO-d6, ppm):4.40(s,2H,),5.73(s,2H),7.12–7.26(m,4H),7.47-7.59(m,4H), 10.71 (s,1H),MS:m/z 415.2 $(M+H)^+$; m.p.132-134^{o}C;Yield: 57%

Methyl 4-(2-(1-((5-mercapto-1, 3, 4-oxadiazol-2-yl) methyl)-1*H*-benzo[*d*]imidazol-2-ylthio) acetamido) benzoate (5G)

This compound was prepared and purified as per the above mentioned procedure. IR (KBr, cm-1): 1673(–C=O stretching, –CONH–), 1720(–C=O stretching ester) 3070, 3060 (C–H stretching, aromatic ring), 2871 (C–H stretching, $–CH_2–$),1540, 1693(C=C,stretching,aromatic ring), 1440 (C–H bending, $–CH_2–$), 1187 (C–O–C stretching, oxadiazole), ^{1}H NMR (400 MHz, DMSO-d6, ppm): 3.89(s,3H), 4.16(s,2H,), 5.73(s,2H),7.13–7.25(m,2H), 7.48-7.62(m,2H), 7.69 (dd, 2H),7.82(dd,2H),10.70(s,1H),MS:m/z 457.2 $(M+H)^+$; m.p.160-163^{o}C;Yield: 62%

***N*-(2-ethylphenyl)-2-(1-((5-mercapto-1,3,4-oxadiazol-2-yl)methyl)-1*H*-benzo[*d*]imid -azol-2-ylthio) acetamide.(5H)**

This compound was prepared and purified as per the above mentioned procedure. IR (KBr, cm-1): 1673(–C=O stretching –CONH–), 3068, 3055 (C–H stretching, aromatic ring), 2877 (C–H stretching, $–CH_2–$), 1396(C-H stretching $–CH_3$)1533, 1599(C=Cstretching, aromatic ring), 1440 (C–H bending, $–CH_2–$), 1187 (C–O–C stretching, oxadiazole),^{1}H NMR (400 MHz, DMSO-d6,ppm): 1.23(t,3H), 2.55(q,2H), 4.25(s,2H,), 5.39(s, 2H), 6.82 (m,1H), 7.16-7.30(m,5H) ,7.50-7.61(m,2H),10.40(s,1H),**MS:** m/z 426.3 $(M+H)^+$; m.p.150-153^{o}C;Yield: 67%

2-(1*H*-benzo[*d*]imidazol-2-ylthio)-*N*-(4-(5-(benzylthio)-1,3,4-oxadiazol-2-yl) phenyl) acetamide (5J)

This compound was prepared and purified as per the above mentioned procedure. Yield: 58%. M.P 185-187^{o}C,IR (KBr, cm^{-1}): 1650(–C=O stretching, –CONH–), 3076, 3057 (C–H stretching, aromatic ring), 2878 (C–H stretching, $–CH_2–$), 1488, 1501(C=Cstretching, aromatic ring), 1445 (C–H bending, –CH2–),1183 (C–O–C stretching, oxadiazole), ^{1}H NMR (400MHz, DMSO-d6): 4.09(s,2H) ,4.6(s,2H), 7.24-7.42(m,7H), 7.60(dd,2H), 7.79(d,2H), 7.89(d,2H), 10.39
(s,1H),MS: m/z 474.1 $(M+H)^+$, M.P 185-187^{o}C, Yield: 58%.

2-(1*H*-benzo[*d*]imidazol-2-ylthio)-*N*-(4-(5-(2,3,5-trifluorobenzylthio)-1,3,4-oxadiazol-2-yl)phenyl)acetamide(5K)

This compound was prepared and purified as per the above mentioned procedure. IR (KBr, cm^{-1}): 1650(–C=O stretching, –CONH–), 3076, 3057 (C–H stretching, aromatic ring), 2878 (C–H stretching, $–CH_2–$), 1488, 1501(C=Cstretching, aromatic ring), 1445 (C–H bending, –CH2–),1183 (C–O–C stretching, oxadiazole), 792 (C–F), ^{1}H NMR (400 MHz, DMSO-d6): 4.09(s,2H), 4.6(s,2H), 6.51-7.57(m,2H), 7.23(dd,2H), 7.59(dd,2H), 7.77(d,2H), 7.89(d,2H), 10.41 (s,1H), MS: m/z 528.1 $(M+H)^+$ M.P190-192^{o}C,Yield: 55%.

2-(1*H*-benzo[*d*]imidazol-2-ylthio)-*N*-(4-(5-(4-methoxybenzylthio)-1,3,4-oxadiazo-l-2-yl)phenyl) acetamide (5L)

This compound was prepared and purified as per the above mentioned procedureIR (KBr, cm^{-1}): 1650(–C=O, –CONH–), 3076, 3057 (C–H stretching, aromatic ring), 2878 (C–H stretching, $–CH_2–$), 1488, 1501(C=Cstretching, aromatic ring), 1445 (C–H bending, $–CH_2–$),1183 (C–O–C stretching, oxadiazole) ^{1}H NMR (400 MHz, DMSO-d6): 3.82(s,3H), 4.07(s, 2H), 4.57(s, 2H), 6.94(s,4H), 7.22 (dd,2H), 7.59(d,2H),7.79(d,2H),7.89(d,2H),10.39(s,1H), MS: m/z 504.0 $(M+H)^+$,M.P175-178^{o}C,Yield: 50%.

2-(1*H*-benzo[*d*]imidazol-2-ylthio)-*N*-(4-(5-((3,4-dimethoxypyridin-2-yl) methyl thio)-1,3,4-oxadiazol-2-yl)phenyl)acetamide(5M)

This compound was prepared and purified as per the above mentioned procedure. IR (KBr, cm^{-1}): 1650(–C=O stretching , –CONH–), 3076, 3057 (C–H stretching, aromatic ring), 2878 (C–H stretching, $–CH_2–$), 1488, 1501(C=C, stretching, aromatic ring), 1445 (C–H bending, $–CH_2–$),1183(C–O–C stretching, oxadiazole),^{1}HNMR (400MHz,DMSO-d6):3.85(s,6H), 4.09(s,2H) ,5.25(s,2H),7.25(m,2H),7.59-7.63(m,3H),7.78(d,2H),7.90(d, 2H),8.27 (d,1H), 10.44 (s,1H), MS: m/z 535.0 $(M+H)^+$, M.P193-195^{o}C,Yield: 55%.

2-(1*H*-benzo[*d*]imidazol-2-ylthio)-*N*-(4-(5-(2-(4-fluorophenylamino)-2-oxo ethyl thio)-1,3,4-oxadiazol-2-yl)phenyl)acetamide (5N)

This compound was prepared and purified as per the above mentioned procedure. IR (KBr, cm^{-1}): 1650(–C=O stretching, –CONH–) 3076, 3057 (C–H stretching, aromatic ring), 2878 (C–H stretching, $–CH_2–$), 1488, 1501(C=C stretching,aromatic ring), 1445 (C–H bending, $–CH_2–$),1183 (C–O–C stretching, oxadiazole), ^{1}H NMR (400 MHz,DMSO-d6): 4.35(s,4H), 7.22-7.26(m,4H), 7.60-7.65 (m,4H), 7.78(d,2H), 7.89(d,2H), 10.64(s,1H), 10.89(s,1H), MS: m/z 535.7 $(M+H)^+$, M.P175-177^{o}C,Yield: 53%.

2-(1*H*-benzo[*d*]imidazol-2-ylthio)-*N*-(4-(5-(2-(3-chlorophenylamino)-2-oxoethyl thio)-1,3,4-oxadiazol-2-yl)phenyl) acetamide (5O)

This compound was prepared and purified as per the above mentioned procedure. IR (KBr, cm^{-1}): 1650(–C=O stretching –CONH–), 3076, 3057 (C–H stretching, aromatic ring), 2878 (C–H stretching, $–CH_2–$), 1488, 1501(C=Cstretching, aromatic ring), 1445 (C–H bending, $–CH_2–$),1183 (C–O–C stretching, oxadiazole), ^{1}H NMR (400 MHz,DMSO-d6): 4.32(s,4H),7.13-7.14(m,3H),7.33-7.45 (m,4H), 7.76(d,2H), 7.89(d,2H), 10.64(s,1H), 10.89(s,1H), MS: m/z 552.4 $(M+H)^+$,M.P122-124^{o}C Yield: 58%

2-(1*H*-benzo[*d*]imidazol-2-ylthio)-*N*-(4-(5-(2-(4-methoxyphenylamino)-2-oxoeth–yl thio)-1,3,4-oxadiazol-2-yl) phenyl)acetamide (5P)

This compound was prepared and purified as per the above mentioned procedure. IR (KBr, cm^{-1}): 1650(–C=O stretching –CONH–), 3076, 3057 (C–H stretching, aromatic ring), 2878 (C–H stretching, $–CH_2–$), 1488, 1501(C=Cstretching, aromatic ring), 1445 (C–H bending, $–CH_2–$), 1183(C–O–C stretching, oxadiazole), ^{1}H NMR (400MHz,DMSO-d6): 3.71 (s,3H), 4.29 (s,4H), 6.88(d,2H) ,7.13(bs,2H), 7.47-7.50(m,4H) ,7.77 (d, 2H), 7.90 (d,2H), 10.30 (s,1H), 10.89 (s,1H), MS: m/z 547.3 $(M+H)^+$.M.P205-207^{o}C Yield: 56%.

2-(1*H*-benzo[*d*]imidazol-2-ylthio)-*N*-(4-(5-(2-(2-ethylphenylamino)-2-oxoethylthio)-1 ,3,4-oxadiazol-2-yl)phenyl)acetamide (5Q)

This compound was prepared and purified as per the above mentioned procedure., IR (KBr, cm^{-1}): 1650(–C=O stretching ,–CONH–) 3076, 3057 (C–H stretching, aromatic ring), 2878 (C–H stretching, $–CH_2–$), 1488, 1501(C=C stretching, aromatic ring), 1445 (C–H bending, $–CH_2–$),1183(C–O–C stretching, oxadiazole),^{1}H NMR (400 MHz, DMSO-d6): 1.04(t,3H) ,2.50 (q,2H),4.30(s,4H),7.17-7.25 (m,5H),7.33-7.39(m,2H),7.50-7.5 2(m,1H) ,7.78(d,2H), 7.93(d,2H), 9.75(s,1H),10.88 (s,1H), MS: m/z 545.1 $(M+H)^+$.M.P185-187^{o}C, Yield: 52%.

2-(1*H*-benzo[*d*]imidazol-2-ylthio)-*N*-(4-(5-(2-(4-acetamidophenylamino)-2-oxoethy-lthio)-1,3,4-oxadiazol-2-yl)phenyl)acetamide (5R)

This compound was prepared and purified as per the above mentioned procedure. IR (KBr, cm^{-1}): 1650(–C=O stretching, ,–CONH–), 3076, 3057 (C–H stretching, aromatic ring), 2878 (C–H stretching, $–CH_2–$), 1488, 1501(C=C, stretching, aromatic ring), 1445 (C–H bending, $–CH_2–$),1183 (C–O–C stretching, oxadiazole), ^{1}H NMR (400 MHz,DMSO-d6): 2.1(s,3H) ,4.25 (s,4H) ,7.13-7.17(m,2H),7.51-7.62(m,6H),7.75(d,2H),7.87(d, 2H),10.32(s,1H),10.86(s,1H), MS: m/z 574.3 $(M+H)^+$.M.P178-180^{o}C, Yield: 50%.

Antimicrobial activity

All the synthesized compounds 5(A-H), 5(J-M) and 5(N-R) were tested in vitro for their antibacterial and antifungal activity. All the glass apparatus used were sterilized before use. The broth dilution technique was used to determine the minimum inhibitory concentration (MIC) of the synthesized compounds. Bacterial strain of*Staphylococcus aureus*(MTCC737) ,*Bacillus megaterium*(MTCC2444) as a gram positive, *Escherichia coli*(MTCC1687) *Pseudomonas aeruginosa*(MTCC3541) as a gram negative used in a present study. Fungal strains of*Aspergillusniger*(MTCC282) and *Aspergillusflavus* (MTCC418) were taken. DMSO was used as the solvent for the compounds. A blank test was carried out to check the antimicrobial activity of DMSO. ampicillin,streptomycin were used as the standard drugs for antibacterial activity and nystatin was used as the standard drug for antifungal activity. Activity results are depicted in Table 1&2.

RESULT AND DISCUSSION

In the present work, first we synthesized compounds **2(A-I)** by the reaction of 2-mercapto benzimidazole with various chloroacetylatedamines (Saxena, P., Singh, D.C.P., 2013). (Reaction Scheme-1).Literature survey revealed that–NH in the benzimidazole nucleus undergoes nucleophilic substitution with bromoethylacetate in the presence of base (Ansari, K.F., Lal, C., 2009). Thus we synthesized compounds **3(A-H)** by the reaction of compounds **2(A-I)** with bromoethylacetate in the presence of base**.** Compounds **3(A-H)** were converted to their corresponding acid hydrazides**4 (A-H).** Finally desired compounds **5 (A-H)** were synthesised via oxidative cyclisation of **4(A-H)** with carbon disulphide in alkaline ethanol. On the other hand this biologically active scaffold also constructed with compound **2-I** using same synthetic strategies and substituted with various benzyl halide and chloroacetylated amines [Reaction Scheme-2(**Figure-3)]** to give compounds **5(J-M)**and **5(N-S)**.

The spectral data of the title compounds **5 (A-H),5 (J-M) &5 (N-R)** shown IR band at 1183 cm^{-1},which confirmed the formation of 1,3,4-oxadiazol-2-yl-ring.In ^{1}H NMR two singlet between 4.0-5.7 ppm confirmed the presence of –S-CH_2– & –N-CH_2– .The formation of the title compounds further confirmed by the mass spectral data.

All the synthesized compounds were screened for their antimicrobial activities (Table-1&2). The examination of data reveals that compounds **5H, 5J, 5M, 5N, 5P, 5Q** have broad spectrum antimicrobial activities, which can inhibit the growth of Gram+Ve, Gram-Ve bacteria as well as fungi. On the other hand compounds **5A, 5D, 5F** and **5G**have good antimicrobial activity but not anti- fungal activity. Compound **5C, 5K, 5L, 5O** and **5R** have moderate antibacterial activity against Gram –Ve, Gram +Ve bacteria and fungi respectively.

Table-1 Antimicrobial activity (Minimum inhibition concentration, µg/ml) 5 A-H

Compounds	*Bacillus megaterium*	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Aspergillus niger*	*Aspergillus flavus*
Streptomycin			50	50		
Ampicillin	100	100				
Nystatin					100	100
5A	500	500	250	250	1000	1000
5B	1000	1000	1000	1000	1000	1000
5C	1000	1000	500	500	1000	1000
5D	500	500	250	1000	1000	1000
5E	1000	1000	1000	1000	1000	1000
5F	500	500	500	250	1000	1000
5G	1000	500	500	1000	1000	1000
5H	250	500	500	250	500	500

Table-2 Antimicrobial activity (Minimum inhibition concentration, µg/ml) 5 J-R

Compounds	*Bacillus megaterium*	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Aspergillus niger*	*Aspergillus flavus*
Streptomycin			50	50		
Ampicillin	100	100				
Nystatin					100	100
5J	125	125	125	500	125	500
5K	500	500	500	500	500	500
5L	500	500	500	500	500	1000
5M	500	500	250	250	250	1000
5N	500	500	250	250	250	250
5O	500	500	500	500	250	500
5P	500	1000	500	500	500	500
5Q	250	500	500	500	125	200
5R	500	1000	250	500	500	500

CONFLICT OF INTEREST

Authors have no conflict of interest for publication of present work.

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