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Synthesis of novel *N*-(2,3-dihydro-1,4-benzodioxin-6-yl)-2-{[5-(un/substituted-phenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}acetamides as potent antibacterial agents

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Abstract: The synthetic methodology was initiated by reacting 1,4-benzodioxane-6-amine (1) with 2-bromoacetyl bromide (2) in aqueous alkaline media under dynamic pH control to get compound 2,3-dihydro-1,4-benzodioxin-6-yl-2-bromoacetamide (3). In the subsequent reactions, a variety of un/substituted-benzoic acids (4a-k), through a succession of three steps, was converted into respective oxadiazole nucleophiles, 7a-k. Finally, the targeted molecules, 8a-k, were obtained by reacting 7a-k with electrophile 3 in an aprotic polar solvent. These compounds were corroborated by spectral characterization like IR, EI-MS, ^{1}H-NMR, and CHN analysis data. These molecules were screened for their antibacterial potential and most of them exhibited a potent activity. Moreover, their cytotoxicity was profiled through hemolytic activity and it was observed that majority of them was very modest in toxicity.

Keywords: 1,4-benzodioxane, 1,3,4-oxadiazol-2-thiols, acetamides, antibacterial and hemolytic activity.

INTRODUCTION

Oxadiazole, a five membered heterocyclic furan like moiety comprising of two nitrogen atoms, one oxygen atom and two double bonds (Sanchit and Pandeya, 2011; Taha *et al*., 2015). Oxadiazoles have noteworthy biologically activities. They serve as antibacterial and fungicide. They find primary position in material sciences and pharmaceutical chemistry (Abbasi *et al*., 2013; Pouliot *et al*., 2012). They show potent inhibition of cancer and tumors (Sanchit and Pandeya, 2011; Singh *et al*., 2013). Furthermore, oxadiazole nucleus possesses diverse activity as anti-inflammatory and antiviral agents (Parikh *et al*., 2011). It also possesses properties such as analgesic and antidepressant (Rajak *et al*., 2009). Substituted-1,3,4-oxadiazoles derivatives displayed broad and valuable pharmaceutics. It is well cleared from the ground facts of progressively increasing number of manuscripts, research grants and patents on the cited subject. 2-amino-1,3,4-oxadiazole derivatives shows remarkable muscle relaxants activity. Oxadiazole derivatives also reveal parasidical and antimalarial activities (Khan *et al*., 2006). *Mycobacterium tuberculosis*, an agent of Tuberculosis which is known to be an infectious disease hits large community. The derivatives of 1,3,4-oxadiazoles demonstrated potential against *M. tuberculosis* strain H37Rv (Navarrete *et al*., 2007). Moreover, The 1,3,4-oxadiazoles are liable to electrophilic substitution (Nagaraj *et al*., 2011). Such reactivity of 1,3,4-oxadiazoles paves a way for production of series of its derivatives. 1,3,4-Oxadiazole is found to be a significant anticonvulsant agent (Dabholkar *et al*., 2011). The inclusion of nitrogen and oxygen in the heterocyclic ring along with exocyclic polarized sulfur atoms ensures the nucleophilic disposition of oxadiazoles and are susceptible to attack of electrophilic reagents. They are also important intermediates in organic synthesis.

1,4-Benzodioxane scaffold is used in many drugs (Bolchi *et al*., 2020) and its ring system shows excellent anti-hepatotoxicity (Ahmad *et al*., 2011; Jing *et al*., 2004). The natural products possessing this moiety are found potentially hepatoprotective in nature. Keeping in view the biological activities many 1,4-benzodioxane neolignans have been synthesized (Chambers *et al*., 2010). Amides have been reported as good antibacterial and antifungal agents (Soyer and Erac, 2007; Sui *et al*., 2020). In the same manner acetamide derivatives also possess vital properties of physicochemical and pharmacokinetic (Shahid *et al*., 2013; Bhandari *et al*., 2008). Similarly one of the versatile heterocyclic compound 2(3*H*)-benzoxazolone execute magnificent biological activities ranging from anticonvulsant, antimicrobial, anti-inflammatory and even profound in controlling AIDS and cancer (Clapp *et al*., 1985; Tappel, 1953 and Erdag *et al*., 2021). Leukotrienes synthesized from leukocytes (Rahman *et al*., 2020) have found as crucial and active mediators candidates in many biologically inflammations and associated diseases events

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such as bronchial asthma inflammation (Evans *et al*., 1987; Baylac and Racine, 2003).

The presented research work is focused on correlation of structurally variant molecules and their bioactivity. As depicted from the manuscript it's the continuation of our foregoing research efforts to synthesize various bioactive polyfunctional molecules. So, in the present study, we synthesized some novel bi-heterocyclic molecules by amalgamation of benzodioxane and 1,3,4-oxadiazole heterocycles to explore their antibacterial potentials.

MATERIALS AND METHODS

The chemicals of analytical grade were utilized in presented research work and were purchased from Sigma Aldrich/Fluka. All solvents were distilled properly before being used for reaction. The melting points of all the synthesized molecules were conducted on a Griffin and George apparatus; by using open capillary tube and were reported uncorrected. The reaction completion and purity was verified by thin layer chromatography (TLC). For TLC rationale the pre-coated silica gel G-25-UV254 plates were used; with gradient mobile system of ethyl acetate and *n*-hexane assuring single spot. UV region at 254 nm was employed as visualizing agent. On a Jasco-320-A spectrophotometer the IR spectra were recorded by KBr pellet method and findings (wave number υ) were reported in cm^{-1}. We make use of the Bruker spectrometer for ^{1}H-NMR spectral analysis; the samples dissolution was made in $CDCl_3/CD_3OD$ and run at 400 MHz frequency. Chemical shifts (δ) were ascertained in ppm and coupling constants are measured in Hz. Electron impact mass spectra (EIMS) were reported on a JMS-HX-110 spectrometer.

Synthesis of 2,3-dihydro-1,4-benzodioxin-6-yl-2-bromoaceatamide (3)

An equimolar mixture of 1,4-benzodioxane-6-amine (1.51 mL; 0.01mol, 1) and 2-bromoacetyl bromide (2.0mL; 0.01 mol, 2) in 20mL of distilled water contained in a round bottomed flask having 50mL capacity. The pH of the reaction mixture was maintained at 9-10 by aqueous 10% $Na_2CO_3$and reaction contents were subjected to vigorously stirring at 0^oC to RT till completion of reaction. The reaction success was scrutinized by TLC and done workup after giving single spot. The reaction mixture was further acidified by few drops of conc. HCl till pH 2. The precipitates so obtained were filtered and rinsed with distilled water; the air-drying yielded to afford 2,3-dihydro-1,4-benzodioxin-6-yl-2-bromoacetamide (3) as light purple amorphous solid in 96% yield having m.p. 57^oC, molecular mass $C_{10}H_{10}O_3NBr$ and molar mass 272 $gmol^{-1}$; IR (KBr, υ/cm^{-1}): 3347 (N-H, str.), 2976 (Ar C-H, str. of aromatic ring), 2920 (-CH_2- str.), 1661 (C=O, str.), 1573 (C=C, aromatic str.), 508 (C-Br, str.); ^{1}H-NMR (400 MHz, CD_3OD, ppm): δ7.21 (d, J = 2.1 Hz,1H, H-5), 6.96 (dd, J = 2.1, 8.7 Hz, 1H, H-7), 6.80 (d, J = 8.7 Hz, 1H, H-8), 4.19 (s, 4H, CH_2-2 & CH_2-3), 3.97 (s, 2H, CH_2-2').

General procedure for synthesis of ethyl un/substituted-benzoates (5a-k)

Different un/substituted-benzoic acids (4a-k; 2.5 g each) were made to react with 60mL of refluxing EtOH, in the presence of catalytically amount of conc. H_2SO_4 (1.25 mL) in each individual reaction. The reaction was refluxed for 4-5 hrs in 250mL of round bottomed flask. The monitoring of the reaction was accomplished on silica coated TLC plates. After completion of reaction the excess of chilled distilled water about 150mL was added progressively and pH was made little alkaline to 8-10 on adding 20% of aq. Na_2CO_3 solution. The product was collected by solvent extraction from $CHCl_3$ about 150mL volume was used. The product was acquired on distilling chloroform. In various cases, the solid products were accumulated on filtration. The esters, 5a-k, so obtained were utilized for respective hydrazide synthesis.

General procedure for synthesis of substituted-benzohydrazides (6a-k)

The un/substituted-benzohydrazides (6a-k) were obtained by refluxing 4.5mL of ethyl esters (5a-k) with 7.2mL of 80% hydrazine hydrate solution ($N_2H_4{\cdot}H_2O$) for 3-4 hrs. These reactants were refluxed in 20 mL of ethanol contained in 100mL of RB flask. The reaction progress was ascertained by TLC. On successful condensation, the un/substituted-benzohydrazides (6a-k) precipitates were achieved by pouring surplus ice cold distilled H_2O (60 mL). The respective ppts of hydrazides were filtered and rinsed with distilled water. The drying of precipitate yielded desired products in sufficient yield.

General procedure for synthesis of 5-(un/substituted-phenyl)-1,3,4-oxadiazol-2-thiols (7a-k)

Solid KOH (0.029 mol) was dissolved in 25mL EtOH on reflux in 100mL RB flask. 0.029 mol of un/substituted-benzohydrazides (6a-k) were refluxed with 0.058 mol of CS_2 in medium basic pH for 5-6 hrs. The reaction was conducted in EtOH as solvent and monitoring was accomplished by TLC. On TLC authentication the excessive ice cold distilled water (60mL) was poured and vigorous hand shaking was done to acquire desired product. On neutralization with dilute HCl and adjusting pH of 5-6; the products were precipitates out promptly. The formed ppts were filtered & washed with distilled H_2O. On drying respective precipitates of 5-(un/substituted-phenyl)-1,3,4-oxadiazol-2-thiols (7a-k) were obtained. The rectified spirit was used for re-crystallization from EtOH.

Synthesis of N-(2,3-dihydro-1,4-benzodioxin-6-yl)-2-{[5-(un/substituted-phenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}acetamides (8a-k)

2,3-Dihydro-1,4-benzodioxin-6-yl-2-bromoacetamide (0.27 g; 0.001 mol, 3) in 10 mL *N,N*-dimethyl formamide

along with catalytic amount of LiH(0.1 mmol)was taken in a round bottomed flask and was stirred for 0.5 hr at room temperature after which respective 5-(un/substituted-phenyl)-1,3,4-oxadiazol-2-thiols (0.001 mol, 7a-k) were added to the reaction mixture which was additionally stirred for 3 hrs RT. The advancement of reaction was checked with TLC till showing single spot in a gradient mobile phase bearing *n*-hexane and ethyl acetate in 80:20 %. The reaction contents were diluted with cold distilled water. Precipitates obtained were filtered. The washing of targets with cold water and air drying provided the conditions to achieve our desired bi-heterocyclic compounds, *N*-(2,3-dihydro-1,4-benzodioxin-6-yl)-2-{[5-(un/substituted-phenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}acetamides (8a-k).

Structural Characterization

N-(2,3-Dihydro-1,4-benzodioxin-6-yl)-2-[(5-phenyl-1,3,4-oxadiazol-2-yl)sulfanyl]acetamide (8a)

Light brown powder, yield: 88%;m.p. 224-225°C, molecular formula: $C_{18}H_{15}O_4N_3SO_4$, molecular mass: 369 gmol^{-1}; IR (KBr,*v*/cm^{-1}): 3355 (N-H, str.), 2985(Ar C-H, str. of aromatic ring), 2923 (-CH_2- str.), 1665 (C=O, str.), 1577 (C=C, aromatic str.), 1530 (C=N, imine str.);^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 7.92 (dist. t, *J* = 7.2 Hz, 2H, H-2''' & H-6'''), 7.48-7.53 (m, 3H, H-3'''- H-5'''), 7.08 (br.s, 1H, H-5), 6.97 (dd, *J* = 1.2, 8.8 Hz, 1H, H-7), 6.87 (d, *J* = 8.8 Hz, 1H, H-8), 4.26 (s, 4H, CH_2-2 & CH_2-3), 4.12 (s, 2H, CH_2-2'); EI-MS:*m/z* 369 $[M(C_{18}H_{15}N_3O_4S)]^+$, 250 $[C_{11}H_{10}N_2O_3S]^+$,224 $[C_{10}H_{10}NO_3S]^+$,219 $[C_{10}H_7N_2O_2S]^+$, 191 $[C_9H_7N_2OS]^+$, 178 $[C_9H_8NO_3]^+$, 177 $[C_8H_5N_2OS]^+$, 150 $[C_8H_8NO_2]^+$, 135 $[C_8H_7O_2]^+$, 122 $[C_6H_4NO_2]^+$, 119 $[C_7H_5NO]^{\cdot+}$, 107 $[C_6H_3O_2]^+$, 90 $[C_6H_4N]^+$, 81 $[C_4HO_2]^+$, 77 $[C_6H_5]^+$, 75 $[C_6H_3]^+$, 51 $[C_4H_3]^+$; Anal. Calc. for $C_{18}H_{15}N_3SO_4$ (369.08): C, 58.53; H, 4.09; N, 11.38. Found: C, 58.50; H, 4.07; N, 11.33.

2-{[5-(2-Chlorophenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}-N-(2,3-dihydro-1,4-benzodioxin-6-yl)acetamide (8b)

Brown granular solid, yield: 86%; m.p. 190-191°C, molecular formula: $C_{18}H_{14}O_4N_3SCl$ molecular mass: 403 gmol^{-1}; IR (KBr, *v*/cm^{-1}): 3345 (N-H, str.), 2980 (Ar C-H, str. of aromatic ring), 2920 (-CH_2- str.), 1668 (C=O, str.), 1583 (C=C, aromatic str.), 1534 (C=N, imine str.), 1069 (C-Cl, str.); ^{1}H-NMR (400 MHz, CD_3OD, ppm): δ 7.90 (d, *J*=7.6 Hz, 1H, H-6'''), 7.51 (d, *J* = 8.0 Hz, 1H, H-3'''), 7.44 (br.t, *J*=7.6 Hz, 1H, H-5'''), 7.37 (br.t, *J*=7.6 Hz, 1H, H-4'''), 7.11 (d, *J*=1.0 Hz, 1H, H-5),6.98 (dist. dd, *J* =1.0, 8.4 Hz, 1H, H- 7), 6.84 (d, *J*= 8.4 Hz, 1H, H-8), 4.24 (s, 4H, CH_2-2 & CH_2-3), 4.16 (s, 2H, CH_2-2'); EI-MS: *m/z* 407 $[M+4]^+$, 405 $[M+2]^+$, 403 $[M(C_{18}H_{14}ClN_3O_4S)]^+$, 250 $[C_{11}H_{10}N_2O_3S]^+$,224 $[C_{10}H_{10}NO_3S]^+$, 255 $[C_{10}H_6ClN_2O_2S+2]^+$,253 $[C_{10}H_6ClN_2O_2S]^+$,227 $[C_9H_6ClN_2OS+2]^+$,225 $[C_9H_6ClN_2OS]^+$, 213 $[C_8H_4ClN_2OS]^+$, 211 $[C_8H_4ClN_2OS]^+$,178 $[C_9H_8NO_3]^+$, 155 $[C_7H_4ClNO]^{\cdot+}$, 153 $[C_7H_4ClNO]^{\cdot+}$,150 $[C_8H_8NO_2]^+$, 135 $[C_8H_7O_2]^+$, 122 $[C_6H_4NO_2]^+$, 107 $[C_6H_3O_2]^+$, 90 $[C_6H_4N]^+$, 81 $[C_4HO_2]^+$, 113 $[C_6H_4Cl+2]^+$, 111 $[C_6H_4Cl]^+$, 75 $[C_6H_3]^+$, 51 $[C_4H_3]^+$; Anal. Calc. for $C_{18}H_{14}O_4N_3SCl$ (403.04): C, 53.53; H, 3.49; N, 10.41. Found: C, 53.50; H, 3.47; N, 10.39.

2-{[5-(3-Chlorophenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}-N-(2,3-dihydro-1,4-benzodioxin-6-yl)acetamide (8c)

Light pink solid, yield: 89%; m.p. 178-179 °C, molecular formula: $C_{18}H_{14}O_4N_3SCl$ molecular mass: 403 gmol^{-1}; IR (KBr, *v*/cm^{-1}): 3352 (N-H, str.), 3053 (Ar C-H, str. of aromatic ring), 2926 (-CH_2- str.), 1666 (C=O, str.), 1572 (C=C, aromatic str.), 1534 (C=N, imine str.), 1069 (C-Cl, str.); ^{1}H-NMR (400 MHz, CD_3OD, ppm): δ7.98 (dd, *J* = 2.1, 8.5 Hz, 1H, H-6'''), 7.97 (t, *J* = 8.5 Hz, 1H, H-5'''), 7.52 (d, *J* = 2.1 Hz, 1H, H-2'''), 7.48 (dd, *J* = 2.1, 8.5 Hz, 1H, H-4'''), 7.12 (d, *J* = 1.1 Hz, 1H, H-5), 6.95 (dd, *J* = 1.1, 8.0 Hz, 1H, H- 7), 6.82 (d, *J*= 8.0 Hz, 1H, H-8), 4.23 (s, 4H, CH_2-2 & CH_2-3), 4.13 (s, 2H, CH_2-2'); EI-MS: *m/z* 407 $[M+4]^+$, 405 $[M+2]^+$, 403 $[M(C_{18}H_{14}ClN_3O_4S)]^+$, 250 $[C_{11}H_{10}N_2O_3S]^+$,224 $[C_{10}H_{10}NO_3S]^+$, 255 $[C_{10}H_6ClN_2O_2S+2]^+$,253 $[C_{10}H_6ClN_2O_2S]^+$,227 $[C_9H_6ClN_2OS+2]^+$,225 $[C_9H_6ClN_2OS]^+$, 213 $[C_8H_4ClN_2OS]^+$, 211 $[C_8H_4ClN_2OS]^+$, 178 $[C_9H_8NO_3]^+$, 155 $[C_7H_4ClNO]^{\cdot+}$, 153 $[C_7H_4ClNO]^{\cdot+}$, 150 $[C_8H_8NO_2]^+$, 135 $[C_8H_7O_2]^+$, 122 $[C_6H_4NO_2]^+$, 107 $[C_6H_3O_2]^+$, 90 $[C_6H_4N]^+$, 81 $[C_4HO_2]^+$, 113 $[C_6H_4Cl+2]^+$, 111 $[C_6H_4Cl]^+$, 75 $[C_6H_3]^+$, 51 $[C_4H_3]^+]^+$; Anal. Calc. for $C_{18}H_{14}O_4N_3SCl$ (403.04): C, 53.53; H, 3.49; N, 10.41. Found: C, 53.48; H, 3.45; N, 10.39.

2-{[5-(3-Aminophenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}-N-(2,3-dihydro-1,4-benzodioxin-6-yl)acetamide (8d)

Brownish colored powder, Yield: 80%; m.p. 202-203°C, molecular formula: $C_{18}H_{16}O_4N_4S$ molecular mass: 384 gmol^{-1}; IR (KBr, *v*/cm^{-1}): 3417 (NH_2, str.), 3351 (N-H, str.), 3048 (Ar C-H, str. of aromatic ring), 2929 (-CH_2- str.), 1663 (C=O, str.), 1571 (C=C, aromatic str.), 1537 (C=N, imine str.); ^{1}H-NMR (400 MHz, CD_3OD, ppm): δ7.97 (dd, *J*=2.2, 8.4 Hz, 1H, H-6'''), 7.95 (t, *J*=8.4 Hz, 1H, H-5'''), 7.47 (d, *J*=2.0 Hz, 1H, H-2'''), 7.46 (dd, *J*=2.2, 8.4Hz, 1H, H-4'''), 7.09 (d, *J*=1.2 Hz, 1H, H-5),6.96 (dd, *J* =1.2, 8.2 Hz, 1H, H- 7), 6.85 (d, *J*=8.2 Hz, 1H, H-8), 4.25 (s, 4H, CH_2-2 & CH_2-3), 4.14 (s, 2H, CH_2-2'); EI-MS: *m/z* 384 $[M(C_{18}H_{16}N_4O_4S]^+$, 250 $[C_{11}H_{10}N_2O_3S]^+$, 234 $[C_{10}H_8N_3O_2S]^+$, 224 $[C_{10}H_{10}NO_3S]^+$, 206 $[C_9H_8N_3OS]^+$, 192 $[C_8H_6N_3OS]^+$, 178 $[C_9H_8NO_3]^+$, 150 $[C_8H_8NO_2]^+$, 135 $[C_8H_7O_2]^+$, 134 $[C_7H_6N_2O]^{\cdot+}$, 122 $[C_6H_4NO_2]^+$, 107 $[C_6H_3O_2]^+$, 92 $[C_6H_6N]^+$, 90 $[C_6H_4N]^+$, 81 $[C_4HO_2]^+$, 75 $[C_6H_3]^+$, 66 $[C_4H_4N]^+$; Anal. Calc. for $C_{18}H_{16}O_4N_4S$ (384.09): C, 56.24; H, 4.20; N, 14.57. Found: C, 56.20; H, 4.17; N, 14.55.

2-{[5-(4-Aminophenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}-N-(2,3-dihydro-1,4-benzodioxin-6-yl)acetamide (8e)

Off-white solid, yield: 95%; m.p. 197-198°C, molecular formula: $C_{18}H_{16}O_4N_4S$ molecular mass: 384 gmol^{-1};IR (KBr, *v*/cm^{-1}): 3411 (NH_2, str.), 3346 (N-H, str.), 3044 (Ar C-H, str. of aromatic ring), 2931 (-CH_2- str.), 1661 (C=O, str.), 1575 (C=C, aromatic str.), 1533 (C=N, imine str.); ^{1}H-NMR (400 MHz, CD_3OD, ppm): δ 7.93 (d, *J*=8.0 Hz, 2H, H-2''' & H-6'''), 7.19 (d, *J*=8.2 Hz, 2H, H-3''' &

H-5'"), 7.07 (d, *J*=1.0 Hz, 1H, H-5),6.94 (dd, *J*=1.0, 8.0 Hz, 1H, H- 7), 6.83 (d, *J*=8.0 Hz, 1H, H-8), 4.27 (s, 4H, CH_2-2 & CH_2-3), 4.12 (s, 2H, CH_2-2'); EI-MS: *m/z* 384 $[M(C_{18}H_{16}N_4O_4S]^+$, 250 $[C_{11}H_{10}N_2O_3S]^+$,234 $[C_{10}H_8N_3O_2S]^+$, 224 $[C_{10}H_{10}NO_3S]^+$, 206 $[C_9H_8N_3OS]^+$, 192 $[C_8H_6N_3OS]^+$, 178 $[C_9H_8NO_3]^+$, 150 $[C_8H_8NO_2]^+$, 135 $[C_8H_7O_2]^+$, 134 $[C_7H_6N_2O]^{\cdot+}$, 122 $[C_6H_4NO_2]^+$, 107 $[C_6H_3O_2]^+$, 92 $[C_6H_6N]^+$, 90 $[C_6H_4N]^+$, 81 $[C_4HO_2]^+$, 75 $[C_6H_3]^+$, 66 $[C_4H_4N]^+$; Anal. Calc. for $C_{18}H_{16}O_4N_4S$ (384.09): C, 56.24; H, 4.20; N, 14.57. Found: C, 56.19; H, 4.16; N, 14.56.

N-(2,3-Dihydro-1,4-benzodioxin-6-yl)-2-{[5-(3-nitrophenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}acetamide (8f)

Bright yellow powder, yield: 90%;m.p. 172-173 °C, molecular formula: $C_{18}H_{14}O_6N_4S$ molecular mass: 414 $gmol^{-1}$;IR (KBr, *v*/cm^{-1}): 3353 (N-H, str.), 3041 (Ar C-H, str. of aromatic ring), 2923 (-CH_2- str.), 1667 (C=O, str.), 1576 (C=C, aromatic str.), 1542 (NO_2, str.), 1532 (C=N, imine str.); ^{1}H-NMR (400 MHz, CD_3OD, ppm): δ 7.78 (br.s, 1H, H-2'"), 6.91-6.94 (m, 3H, H-4'" - H-6'"), 7.12 (d, *J* = 1.3 Hz, 1H, H-5),6.93 (dd, *J* = 1.3, 8.5 Hz, 1H, H- 7), 6.81 (d, *J*= 8.5 Hz, 1H, H-8), 4.26 (s, 4H, CH_2-2 & CH_2-3), 4.15 (s, 2H, CH_2-2'); EI-MS: *m/z* 414 $[M(C_{18}H_{14}N_4O_6S]^+$, 264 $[C_{10}H_6N_3O_4S]^+$, 250 $[C_{11}H_{10}N_2O_3S]^+$, 236 $[C_9H_6N_3O_3S]^+$, 224 $[C_{10}H_{10}NO_3S]^+$, 222 $[C_8H_4N_3O_3S]^+$, 178 $[C_9H_8NO_3]^+$, 164 $[C_7H_4N_2O_3]^{\cdot+}$, 150 $[C_8H_8NO_2]^+$, 135 $[C_8H_7O_2]^+$, 122 $[C_6H_4NO_2]^+$, 107 $[C_6H_3O_2]^+$, 96 $[C_4H_2NO_2]^+$, 90 $[C_6H_4N]^+$, 81 $[C_4HO_2]^+$, 75 $[C_6H_3]^+$; Anal. Calc. for $C_{18}H_{14}O_6N_4S$ (414.06): C, 52.17; H, 3.41; N, 17.52. Found: C, 52.14; H, 3.37; N, 17.48.

N-(2,3-Dihydro-1,4-benzodioxin-6-yl)-2-{[5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}acetamide (8g)

Light yellow solid, yield: 92%;m.p.232-233°C, molecular formula: $C_{18}H_{14}O_6N_4S$ molecular mass: 414 $gmol^{-1}$;IR (KBr, *v*/cm^{-1}): 3351 (N-H, str.), 3049 (Ar C-H, str. of aromatic ring), 2932 (-CH_2- str.), 1666 (C=O, str.), 1577 (C=C, aromatic str.), 1544 (NO_2, str.), 1531 (C=N, imine str.); ^{1}H-NMR (400 MHz, CD_3OD, ppm): δ 7.94 (d, *J* = 8.4 Hz, 2H, H-3'" & H-5'"), 7.19 (d, *J* = 8.5 Hz, 2H, H-2'" & H-6'"), 7.13 (d, *J* = 1.0 Hz, 1H, H-5), 6.91 (dd, *J* = 1.0, 8.2 Hz, 1H, H- 7), 6.80 (d, *J*= 8.2 Hz, 1H, H-8), 4.20 (s, 4H, CH_2-2 & CH_2-3), 4.16 (s, 2H, CH_2-2'); EI-MS: *m/z* 414 $[M(C_{18}H_{14}N_4O_6S]^+$, 264 $[C_{10}H_6N_3O_4S]^+$, 250 $[C_{11}H_{10}N_2O_3S]^+$, 236 $[C_9H_6N_3O_3S]^+$, 224 $[C_{10}H_{10}NO_3S]^+$, 222 $[C_8H_4N_3O_3S]^+$, 178 $[C_9H_8NO_3]^+$, 164 $[C_7H_4N_2O_3]^{\cdot+}$, 150 $[C_8H_8NO_2]^+$, 135 $[C_8H_7O_2]^+$, 122 $[C_6H_4NO_2]^+$, 107 $[C_6H_3O_2]^+$, 96 $[C_4H_2NO_2]^+$, 90[C_6H_4N]$^+$, 81 $[C_4HO_2]^+$, 75 $[C_6H_3]^+$; Anal. Calc. for $C_{18}H_{14}O_6N_4S$ (414.06): C, 52.17; H, 3.41; N, 17.52. Found: C, 52.14; H, 3.35; N, 17.49.

N-(2,3-Dihydro-1,4-benzodioxin-6-yl)-2-{[5-(3,5-dinitrophenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}acetamide (8h)

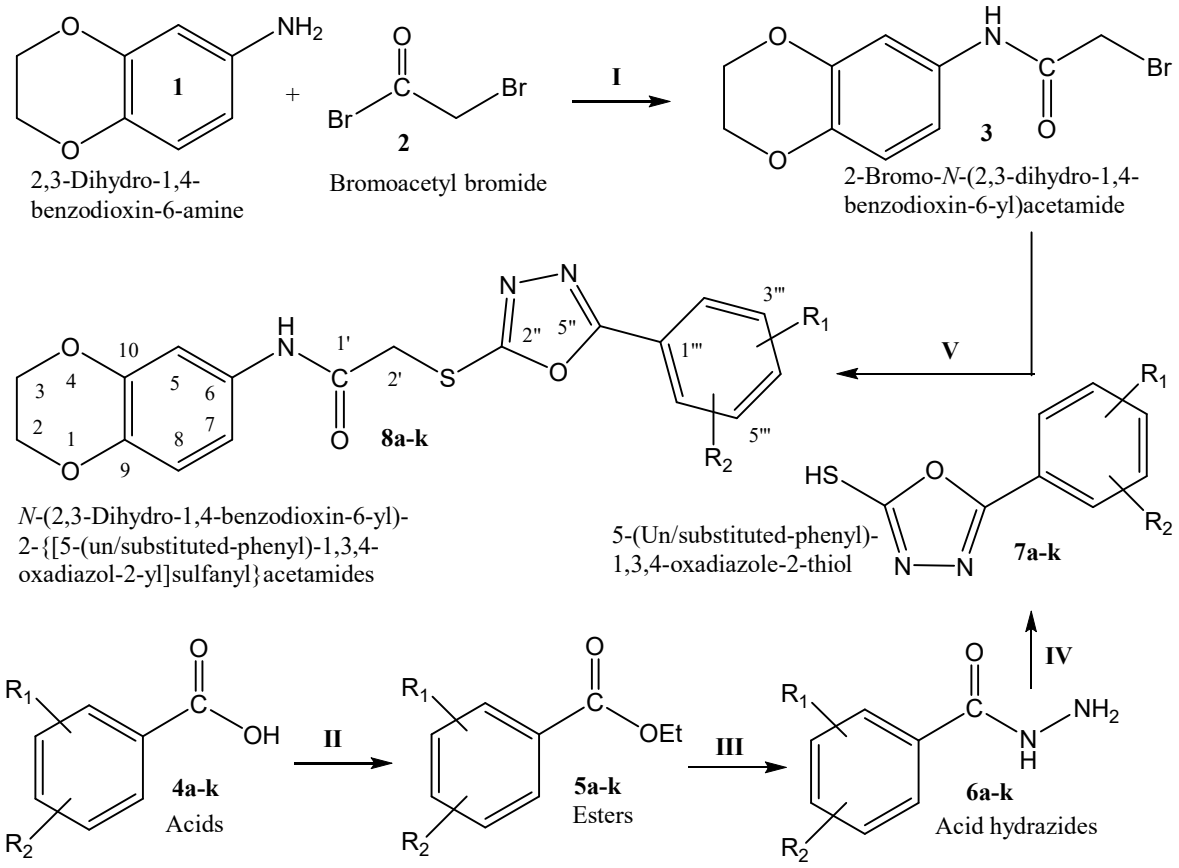
Dark brown solid, yield: 81%; m.p. 213-214°C, molecular formula: $C_{18}H_{13}O_8N_5S$ molecular mass: 459 $gmol^{-1}$; IR (KBr, *v*/cm^{-1}): 3356 (N-H, str.), 3037 (Ar C-H, str. of aromatic ring), 2926 (-CH_2- str.), 1666 (C=O, str.), 1577 (C=C, aromatic str.), 1545 (NO_2, str.), 1533 (C=N, imine str.); ^{1}H-NMR (400 MHz, CD_3OD, ppm): δ 8.30 (s, 1H, H-4'"), 8.27 (s, 2H, H-2'" & H-6'"), 7.08 (d, *J* = 1.1 Hz, 1H, H-5), 6.92 (dd, *J* = 1.1, 8.0 Hz, 1H, H- 7), 6.83 (d, *J*= 8.0 Hz, 1H, H-8), 4.21 (s, 4H, CH_2-2 & CH_2-3), 4.17 (s, 2H, CH_2-2'); EI-MS: *m/z* 459 $[M(C_{18}H_{13}N_5O_8S]^+$, 309 $[C_{10}H_5N_4O_6S]^+$, 281 $[C_9H_5N_4O_5S]^+$, 267 $[C_8H_3N_4O_5S]^+$, 250 $[C_{11}H_{10}N_2O_3S]^+$,224 $[C_{10}H_{10}NO_3S]^+$, 209 $[C_7H_3N_3O_5]^{\cdot+}$, 178 $[C_9H_8NO_3]^+$, 167 $[C_6H_3N_2O_4]^+$, 150 $[C_8H_8NO_2]^+$, 141 $[C_4HN_2O_4]^+$135 $[C_8H_7O_2]^+$, 122 $[C_6H_4NO_2]^+$, 107 $[C_6H_3O_2]^+$, 90 $[C_6H_4N]^+$, 81 $[C_4HO_2]^+$, 75 $[C_6H_3]^+$; Anal. Calc. for $C_{18}H_{13}O_8N_5S$ (459.05): C, 47.06; H, 2.85; N, 15.24. Found: C, 47.01; H, 2.82; N, 15.20.

N-(2,3-Dihydro-1,4-benzodioxin-6-yl)-2-{[5-(4-hydroxyphenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}acetamide (8i)

Brown viscous liquid, yield: 88%;molecular formula: $C_{18}H_{15}O_5N_3S$,molecular mass: 385 $gmol^{-1}$; IR (KBr, *v*/cm^{-1}): 3358 (O-H, str.), 3350 (N-H, str.), 3041 (Ar C-H, str. of aromatic ring), 2934 (-CH_2- str.), 1668 (C=O, str.), 1576 (C=C, aromatic str.), 1532 (C=N, imine str.); ^{1}H-NMR (400 MHz, CD_3OD, ppm): δ 7.87 (d, *J* = 8.2 Hz, 2H, H-2'" & H-6'"), 6.91 (d, *J* = 8.7 Hz, 2H, H-3'" & H-5'"), 7.14 (d, *J* = 1.3 Hz, 1H, H-5), 6.95 (d, *J* = 8.4 Hz, 1H, H-7), 6.86 (d, *J*= 8.4 Hz, 1H, H-8), 4.25 (s, 4H, CH_2-2 & CH_2-3), 4.14 (s, 2H, CH_2-2'); EI-MS: *m/z*385 $[M(C_{18}H_{15}N_3O_5S]^+$, 250 $[C_{11}H_{10}N_2O_3S]^+$,235 $[C_{10}H_7N_2O_3S]^+$, 224 $[C_{10}H_{10}NO_3S]^+$, 207 $[C_9H_7N_2O_2S]^+$, 193 $[C_8H_5N_2O_2S]^+$, 178 $[C_9H_8NO_3]^+$, 150 $[C_8H_8NO_2]^+$, 135 $[C_8H_7O_2]^+$, 135 $[C_7H_5NO_2]^{\cdot+}$, 122 $[C_6H_4NO_2]^+$, 107 $[C_6H_3O_2]^+$, 90 $[C_6H_4N]^+$, 81 $[C_4HO_2]^+$, 93 $[C_6H_5O]^+$, 75 $[C_6H_3]^+$, 67 $[C_4H_3O]^+$; Anal. Calc. for $C_{18}H_{15}O_5N_3S$ (385.05): C, 56.10; H, 3.92; N, 10.90. Found: C, 56.05; H, 3.84; N, 10.07.

N-(2,3-Dihydro-1,4-benzodioxin-6-yl)-2-{[5-(2-methoxyphenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}acetamide (8j)

Light brown powder, yield: 95%; m.p. 85-86 °C, molecular formula: $C_{19}H_{17}O_5N_3S$ molecular mass: 399 $gmol^{-1}$; IR (KBr, *v*/cm^{-1}): 3352 (N-H, str.), 3044 (Ar C-H, str. of aromatic ring), 2931 (-CH_2- str.), 1663 (C=O, str.), 1574 (C=C, aromatic str.), 1538 (C=N, imine str.), 1156 (C-O-C, str.); ^{1}H-NMR (400 MHz, CD_3OD, ppm): δ 7.72 (d, *J* = 8.0 Hz,1H, H-6'"), 7.35 (t, *J*= 7.6 Hz, 1H, H-4'"), 7.13 (t, *J*= 8.0 Hz, 1H, H-5'"), 7.09 (d, *J* = 1.2 Hz, 1H, H-5), 7.08 (d, *J* = 7.6 Hz, 1H, H-3'"), 6.94 (dd, *J* = 1.2, 8.4 Hz, 1H, H-7), 6.87 (d, *J*= 8.4 Hz, 1H, H-8), 4.24 (s, 4H, CH_2-2 & CH_2-3), 4.13 (s, 2H, CH_2-2'), 3.85 (s, 3H, OCH_3-1'"); EI-MS: *m/z* 399 $[M(C_{19}H_{17}N_3O_5S]^+$, 250 $[C_{11}H_{10}N_2O_3S]^+$,249 $[C_{11}H_9N_2O_3S]^+$, 224 $[C_{10}H_{10}NO_3S]^+$, 221 $[C_{10}H_9N_2O_2S]^+$, 207 $[C_9H_7N_2O_2S]^+$, 178 $[C_9H_8NO_3]^+$, 150 $[C_8H_8NO_2]^+$, 149 $[C_8H_7NO_2]^{\cdot+}$, 135 $[C_8H_7O_2]^+$, 122 $[C_6H_4NO_2]^+$, 107 $[C_6H_3O_2]^+$, 107 $[C_7H_7O]^+$, 90 $[C_6H_4N]^+$,



Scheme 1: Outline for the synthesis of *N*-(2,3-dihydro-1,4-benzodioxin-6-yl)-2-{[5-(un/substituted-phenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}acetamides. Reagents & Conditions: (I) aq. 5% Na_2CO_3 soln./manual vigorous stirring. (II) EtOH/H_2SO_4/refluxing for 3-4 hrs. (III) MeOH/$N_2H_4 \cdot H_2O$/ refluxing for 4-6 hrs. (IV) EtOH/CS_2/KOH/refluxing for 3-6 hrs. (V) DMF/LiH/stirring for 1-2 hrs.

81 $[C_4HO_2]^+$, 81 $[C_5H_5O]^+$, 75 $[C_6H_3]^+$; Anal. Calc. for $C_{19}H_{17}O_5N_3S$ (399.09): C, 57.13; H, 4.29; N, 10.52. Found: C, 57.09; H, 4.25; N, 10.47.

N-(2,3-Dihydro-1,4-benzodioxin-6-yl)-2-{[5-(4-methylphenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}acetamide (8k)

Pink amorphous solid, yield: 95%; m.p. 207-208°C, molecular formula: $C_{19}H_{17}O_4N_3S$ molecular mass: 383 $gmol^{-1}$; IR (KBr, υ/cm^{-1}): 3353 (N-H, str.), 3037 (ArC-H, str. of aromatic ring), 2929 (-CH_2- str.), 1666 (C=O, str.), 1573 (C=C, aromatic str.), 1536 (C=N, imine str.); ^{1}H-NMR (400 MHz, CD_3OD, ppm): δ7.86 (d, J = 8.0 Hz, 2H, H-2'''& H-6'''), 7.27 (d, J = 8.0 Hz, 2H, H-3'''& H-5'''), 7.12 (d, J = 1.3 Hz, 1H, H-5), 6.98 (dd, J = 1.3, 8.5Hz, 1H, H-7), 6.89 (d, J= 8.5 Hz, 1H, H-8), 4.25 (s, 4H, CH_2-2 & CH_2-3), 4.16 (s, 2H, CH_2-2'), 2.34 (s, 3H, CH_3-1'''); EI-MS:*m/z* 383 $[M(C_{19}H_{17}N_3O_4S]^+$, 250 $[C_{11}H_{10}N_2O_3S]^+$,233 $[C_{11}H_9N_2O_2S]^+$, 224 $[C_{10}H_{10}NO_3S]^+$, 205$[C_{10}H_9N_2OS]^+$, 191 $[C_9H_7N_2OS]^+$, 178 $[C_9H_8NO_3]^+$, 150 $[C_8H_8NO_2]^+$, 135 $[C_8H_7O_2]^+$, 133 $[C_8H_7NO]^+$, 122 $[C_6H_4NO_2]^+$, 107 $[C_6H_3O_2]^+$, 91$[C_7H_7]^+$, 90 $[C_6H_4N]^+$, 81 $[C_4HO_2]^+$, 75 $[C_6H_3]^+$, 65 $[C_5H_5$; Anal. Calc. for $C_{19}H_{17}O_5N_3S$ (399.09): C, 59.52; H, 4.47; N, 10.96. Found: C, 59.49; H, 4.45; N, 10.93.

Antibacterial assay

Antibacterial activity was performed in sterile 96-wells micro plate under aseptic conditions (Kaspady *et al.*, 2009). In the presented work, *Escherichia coli*, *Pseudomonas aeruginosa* & *Salmonella typhi* were among the Gram-negative bacterial strains whereas Gram-positive bacteria strains comprised of *Bacillus subtilis* & *Staphylococcus aureus*. The well plate was incubated for four days at 37°C and kept in an airtight environment so that no evaporation can take place from it. The absorbance of tested samples was measured before and after incubation with the help of microplate reader. The wavelength was fixed at 540 nm. The absorbance difference thus obtained was referred to as 'index of bacterial growth'. The % inhibition was calculated as:

$$\text{Inhibition (\%)} = \frac{X-Y}{Y} \times 100$$

Here, X and Y are variables showing absorbance of control and test sample respectively. Ciprofloxacin was used as standard.

Table 1: Different groups (-R_1& -R_2) in scheme-1.

Compounds	-R_1	-R_2
4a, 5a, 6a, 7a, 8a	-H	-H
4b, 5b, 6b, 7b, 8b	2-Cl	-H
4c, 5c, 6c, 7c, 8c	3-Cl	-H
4d, 5d, 6d, 7d, 8d	3-NH_2	-H
4e, 5e, 6e, 7e, 8e	4-NH_2	-H
4f, 5f, 6f, 7f, 8f	3-NO_2	-H
4g, 5g, 6g, 7g, 8g	4-NO_2	-H
4h, 5h, 6h, 7h, 8h	3-NO_2	5-NO_2
4i, 5i, 6i, 7i, 8i	4-OH	-H
4j, 5j, 6j, 7j, 8j	2-OCH_3	-H
4k, 5k, 6k, 7k, 8k	4-CH_3	-H

Table 2: Antibacterial activity of 3 and *N*-(2,3-dihydro-1,4-benzodioxin-6-yl)-2-{[5-(un/substituted-phenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}acetamides (8a-k).

Codes	MIC_{50}				
	Gram (+)		Gram (-)		
	S. aureus (+)	*B. subtilis (+)*	*E. coli (-)*	*S. typhi (-)*	*P. aeruginosa (-)*
3	7.52 ± 1.54	7.20 ± 0.70	8.44 ± 0.65	7.45 ± 0.80	10.28 ± 0.67
8a	7.99 ± 0.14	14.63 ± 0.42	13.89 ± 0.60	8.25 ± 0.56	11.92 ± 0.65
8b	12.98 ± 0.89	8.59 ± 0.50	8.68 ± 0.89	8.14 ± 0.92	11.23 ± 0.50
8c	10.10 ± 0.25	11.42 ± 0.70	11.23 ± 0.40	9.44 ± 0.90	14.25 ± 0.89
8d	14.06 ± 0.16	10.38 ± 0.58	10.11 ± 0.04	10.62 ± 0.59	13.93 ± 1.05
8e	8.66 ± 0.54	9.46 ± 0.90	12.66 ± 0.33	8.00 ± 0.89	9.11 ± 0.78
8g	8.58 ± 0.98	8.41 ± 0.98	14.22 ± 0.70	9.7 ± 0.45	16.01 ± 0.82
8h	14.99 ± 0.86	9.44 ± 0.68	18.38 ± 0.20	-	14.33 ± 0.87
8j	7.89 ± 0.34	8.00 ± 0.21	8.19 ± 0.98	8.01 ± 0.23	8.09 ± 0.67
8k	8.87 ± 0.73	9.43 ± 0.90	9.10 ± 0.76	8.62 ± 0.13	12.81 ± 1.00
Ciprofloxacin	7.00 ± 1.54	7.22 ± 0.67	8.01 ± 0.12	7.83 ± 0.78	7.98 ± 0.89

Note: Compounds 8f and 8i remained inactive. Each value represents mean ± Standard deviation. Ciprofloxacin was used as standard.

Table 3: Hemolytic activity of 3 and *N*-(2,3-dihydro-1,4-benzodioxin-6-yl)-2-{[5-(un/substituted-phenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}acetamides (8a-k).

Compound	% Hemolysis	Compound	% Hemolysis
3	2.77	8f	16.52
8a	27.31	8g	47.80
8b	4.40	8h	14.93
8c	3.56	8i	9.26
8d	13.22	8j	14.04
8e	15.41	8k	3.87
Triton X	89.00	PBS (% Hemolysis) = 0.54	

Most of the compounds had very low toxicity, for example the molecule 3 showed 2.77%, 8c having 3.56%, 8k possessed 3.87% and 8brendered 4.40% lysis. So, in general, it was outcome that most of the molecules have very modest cytotoxicity.

Hemolytic Assay

Hemolytic activity of the compound was conducted by already reported method (Abbasi *et al*., 2019). 0.1% v/v of Triton X-100 and phosphate buffer saline (PBS) were selected as positive and negative controls respectively. Using Quant (Biotek, USA) the absorbance was noted at 576 nm. For each sample the % of RBCs lysis was computed.

STATISTICAL ANALYSIS

After three-folds performance and statistical data analysis by Microsoft Excel 2010, results are mentioned in terms of ± SEM. EZ Fit Perrella Scientific Inc. Amherst USA software was used to find out minimum inhibitory concentration (MIC) value under different dilutions values (ranging 5-30 μg/well).

RESULTS

The synthesis of targeted molecules (8a-k), has been outlined in Scheme-1 and varying groups are listed in table 1. The elaborated procedures are given in the experimental section. The structural characterization of the designed molecules was corroborated through spectral analysis.All these molecules were evaluated for their anti-

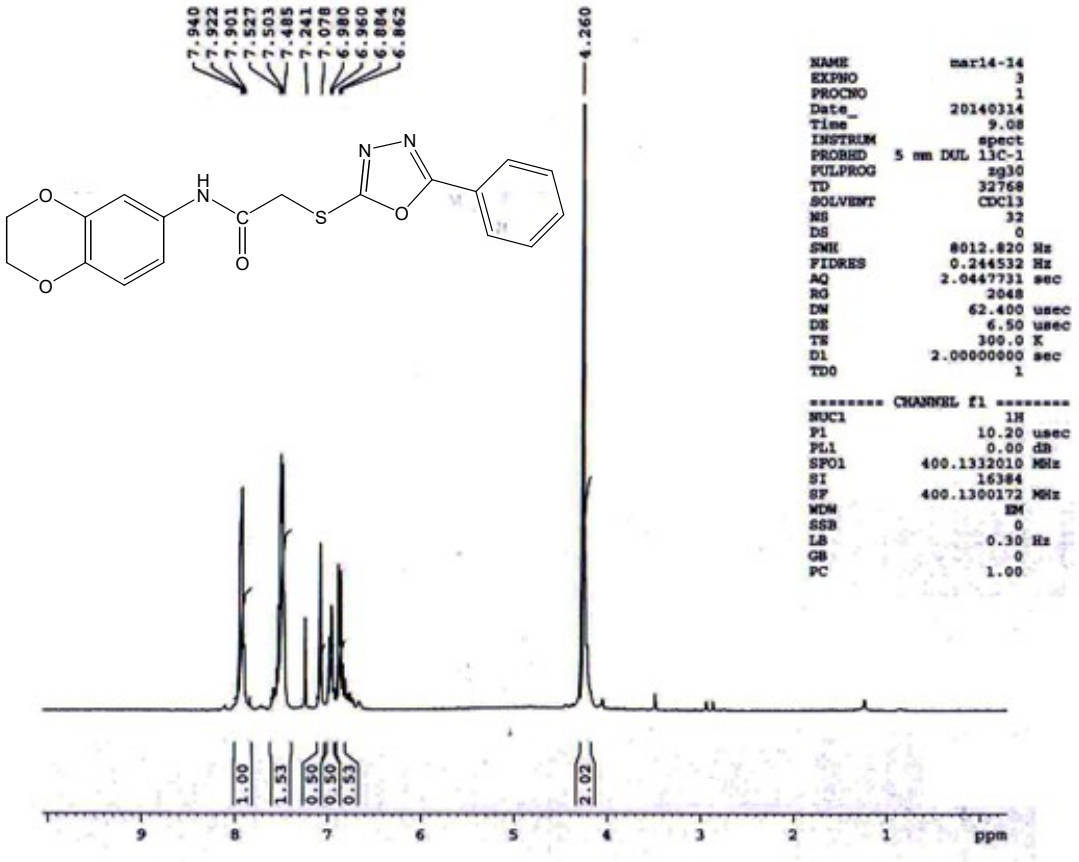


Fig. 1: ^{1}H-NMR spectrum of *N*-(2,3-dihydro-1,4-benzodioxin-6-yl)-2-[(5-phenyl-1,3,4-oxadiazol-2-yl)sulfanyl] acetamide (8a).

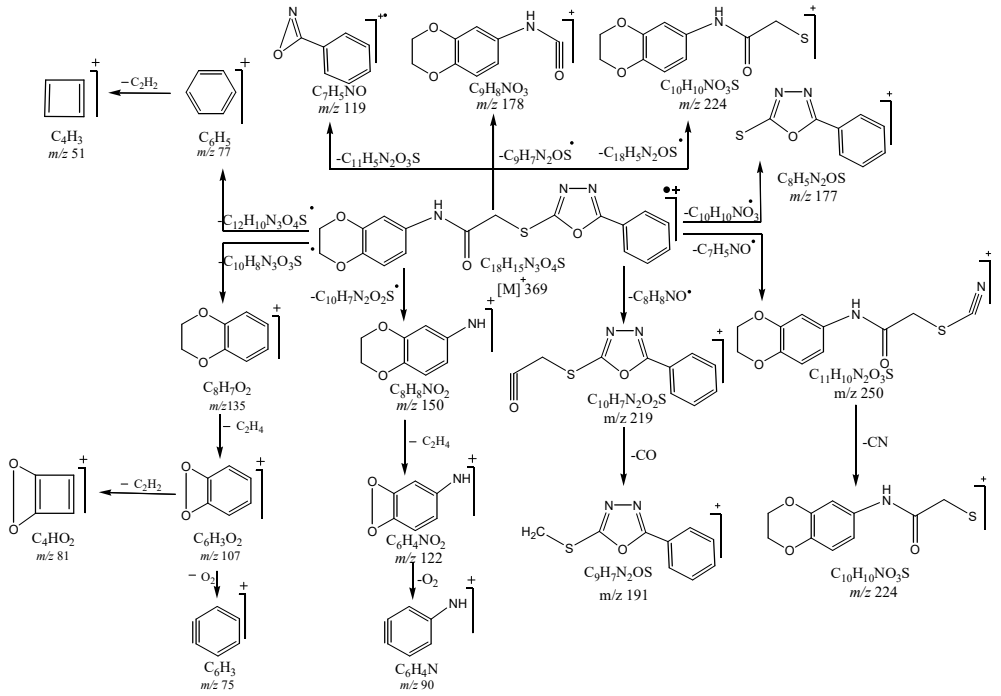


Fig. 2: Mass fragmentation pattern of *N*-(2,3-dihydro-1,4-benzodioxin-6-yl)-2-[(5-phenyl-1,3,4-oxadiazol-2-yl)sulfanyl]acetamide (8a).

bacterial potential and the consequent results are tabulated in table 2. Moreover, the cytotoxicity of these derivatives was also assessed through hemolytic assay and these results are presented in table 3.

DISCUSSION

Chemistry

The nucleophilic *N*-acylation reaction was carried out between equimolar quantities of 1,4-benzodioxane-6-amine (1) and 2-bromoacetyl bromide (2) in aqueous alkaline medium under dynamic pH control at 9-10 with 10% aqueous Na_2CO_3 (Abbasi *et al*., 2020). 2,3-Dihydro-1,4-benzodioxin-6-yl-2-bromoacetamide (3) was precipitated at pH 2 using concentrated HCl. In a parallel sequence of reactions; various nucleophiles were formulated. While starting from variety of un/substituted-benzoic acids (4a-k), by condensation reaction, respective esters were acquired (5a-k). The acid hydrazides (6a-k) were obtained from 5a-k by treating with hydrazine hydrate. The cyclization of hydrazides 6a-k yielded 5-(un/substituted-phenyl)-1,3,4-oxadiazole-2-thiols (7a-k) under refluxing for 3-6 hrs with EtOH/CS_2/KOH. These oxadiazole-2-thiols (7a-k), served as nucleophiles and finally these were coupled one by one with electrophile 3 in DMF and LiH as a base. The reaction was quenched with cold distilled water to afford *N*-(2,3-dihydro-1,4-benzodioxin-6-yl)-2-{[5-(un/substituted-phenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}acetamides (8a-k).

The structures of the synthesized derivatives were validated through IR, EI-MS and ^{1}H-NMR spectral data. The structural characterization of one of the compounds is discussed hereby in detail for the benefit of the readers. The molecular formula, $C_{18}H_{15}N_3O_4S$, of 8a was established through its EI-MS spectrum which showed molecular ion peak at *m/z* 369 and this assignment was also supported by its CHN analysis data. The IR spectrum revealed characteristic absorption bands at υ 3355 (N-H, stretching), 2985 (Ar C-H, str. of aromatic ring), 2923 (-CH_2- str.), 1665 (C=O, str.), 1577 (C=C, aromatic str.) (Abbasi *et al*., 2020) and 1530 (C=N, imine str.) cm^{-1} for speculated functionalities. In its ^{1}H-NMR spectrum (fig. 1), the phenyl ring was recognized by overall two resonances in aromatic region at δ 7.92 (dist.t, *J*=7.2 Hz, 2H, H-2''' & H-6''') and δ 7.48-7.53 (m, 3H, H-3'''- H-5'''). The 2,3-dihydro-1,4-benzodioxin-6-yl moiety in the molecule was rationalized by a typical AMX spin system in aromatic region represented by δ 7.08 (br.s, 1H, H-5), 6.97 (dd, *J*=1.2, 8.8 Hz, 1H, H-7) and δ 6.87 (d, *J*=8.8 Hz, 1H, H-8) along with a four-proton singlet at δ 4.26 for two symmetrical methylene groups (CH_2-2 & CH_2-3). Another sharp singlet at δ 4.12, integrated for two-protons, was symbolic for the interconnection of a 2,3-dihydro-1,4-benzodioxin-6-yl moiety with 5-phenyl-1,3,4-oxadiazole-2-thiol unit through an acetamidic linkage. The mass fragmentation pattern of this molecule was also in full agreement with the structural units ascribed by other spectral techniques and it has been outlined in fig. 2.

Hence, on the grounds of above mentioned summative confirmations, the structure of molecule 8a was affirmed and named as *N*-(2,3-dihydro-1,4-benzodioxin-6-yl)-2-[(5-phenyl-1,3,4-oxadiazol-2-yl)sulfanyl]acetamide.
Similarly, all other synthesized derivatives, 8b-k, were characterized by aforesaid spectral techniques.

Antibacterial activity and structure-activity relationship

The synthesized bi-heterocyclic derivatives, 8a-k, were screened for their antibacterial potential against isolated Gram positive and Gram negative bacteria using Ciprofloxacin as standard. All compounds exhibited excellent to good activity as revealed from their MIC_{50} values (table 2).

The parent molecule, 2,3-dihydro-1,4-benzodioxin-6-yl-2-bromoacetamide (3), exhibited extraordinary inhibition as evident from its IC_{50}values against all bacterial strain; 7.52±1.54, 7.20±0.70 and 8.44±0.65, 7.45±0.80 and 9.11±0.78mM and values were much closer to the standard. Similarly, the derivative 8j also showed potent antibacterial potential against all strains having IC_{50}values of 7.89±0.34, 8.00±0.21, 8.19±0.98, 8.00±0.89 and 8.09±0.67mM and these values were also comparable to standard Ciprofloxacin. This superb antibacterial potential might be attributed to the structural unification of 2,3-dihydro-1,4-benzodioxin and 2-methoxyphenyl and 1,3,4-oxadiazole-2-thiol moieties in this molecule.

Hemolytic activity

These compounds were also subjected to hemolytic activity to explore their cytotoxicity profile using negative control of phosphate-buffered saline (PBS) and positive control of Triton-X-100. Cytotoxicity was estimated as percentage lysis (table 3).

CONCLUSION

The novel bi-heterocyclic *N*-(2,3-dihydro-1,4-benzodioxin-6-yl)-2-{[5-(un/substituted-phenyl)-1,3,4-oxadiazol-2-yl]sulfanyl}acetamides (8a-k) were synthesized in good yields and their projected structures were substantiated by spectral data. Furthermore, most of the compounds exhibited tremendous antibacterial potential against all bacterial strains. These molecules revealed the cytotoxicity range which can be considered as safe in pharmaceutical preparations. Hence, on the basis of aforesaid results, these synthesized derivatives provide an overall indispensable basis to introduce new antibacterial agents which can be utilized for the treatment of infectious ailments.

ACKNOWLEDGEMENT

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