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$ZnCl_2$ CATALYSED ONE POT SYNTHESIS OF BENZOXAZOLE LINKED PYRIDINE DERIVATIVES, BIOLOGICAL EVALUATION AND DFT STUDIES

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ABSTRACT

Multicomponent reactions (MCRs) have drawn high efforts in recent years owing to exceptional synthetic efficiency, intrinsic atom economy, high selectivity, and procedural simplicity. The novel $ZnCl_2$-catalyzed one-pot multicomponent synthesis of 2-amino-4-(2-chloroquinolin-3-yl)-6-(2,7a-dihydro-1,3-benzoxazol-2-ylsulfanyl) pyridine-3,5-dicarbonitrile using conventional heating method. Reaction of 2-mercapto benzoxazole with different aromatic aldehydes in presence of malano nitrile in ethanol media using zinc chloride as a catalyst under reflux condition afforded 2-amino-4-(2-chloroquinolin-3-yl)-6-(2,7a-dihydro-1,3-benzoxazol-2-ylsulfanyl) pyridine-3,5-dicarbonitrile in a good yield. The obtained solid purified using column chromatography technique and the derivatives are characterized by IR, NMR, Mass spectral and frontier molecular orbital studies. Further the synthesized compounds were screened for antibacterial, antioxidant activities

KEY WORDS

Benzoxazole, quinolone, pyridine and HOMO-LUMO.

1 INTRODUCTION

Benzoxazoles are privileged organic compounds of medicinal significance due to their recognized biological chemotherapeutic activities.[1,2] Benzoxazole derivatives exhibit antimicrobial, [3-5] antiviral, [6,7] multi-drug resistance cancer cell activities,[8] with inhibitory activity on eukaryotic topoisomerase II enzyme in cell-free system.[9-11] Recently Anusha and Rao [12] reported the synthesis and biological evaluation of benzoxazole derivatives as new antimicrobial agents. Mary et al. reported the vibrational spectroscopic and SERS studies of some benzoxazole derivatives.[13,14] Fighting against bacterial infections has resulted in the development of a wide variety of antibiotics. After years of misuse of antibiotics, bacteria have become antibiotic-resistant, resulting in a potential global health crisis. Infectious diseases due to Gram-positive bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin- resistant *Enterococcus faecium* (VREF), and penicillin resistant *Streptococcus pneumoniae* (PRSP) are the leading causes of morbidity and mortality today.[15] Besides, during the past 20 years an increase in invasive fungal infections, particularly in immunosuppressed patients, has been observed which are now considered to be the causes of morbidity and mortality as well. Therefore, there is still need for new antifungal and antibacterial agents.[16]

Quinolines and pyridine are important class of organic compounds which possess a variety of pharmacological activities such as antimalarial, antifungal, hypotensive and antidepressant activity.[17-20] As a part of our research programme on quinolone derivatives.[21]

In the present study synthesis and characterization of Benzoxazole fused with quinoline moiety and screened for antibacterial, antioxidant activity and HOMO LUMO study are also reported.

2 EXPERIMENTAL SECTIONS

(i) **Synthesis of 1, 3-benzoxazole-2-thiol 1a**

To the solution of methanol (50 ml) and KOH (1.1eq), carbon disulphide (1.1eq) was added slowly at room temperature. To the reaction mass, derivatives of chloro substituted 2-aminophenol (1. eq) was added with stirring. The reaction mass was refluxed for 6 hrs on water bath. Completion of the reaction was monitored by TLC. The reaction mixture was poured on to ice cold water and acidified with glacial acetic acid (pH6). The obtained solid was filtered, dried and recrystallized using ethanol to get the compound **1a.**

Yield: 92%; M.P : 193^0 C; color: cream; IR (KBr, cm^{-1}): 3494cm^{-1} (SH); the ^{1}H-NMR (DMSO-d6, δ ppm): 7.26(s, 2H, Ar-H), 7.38(s, 1H Ar-H), 13-14(s,1H SH); ^{13}C-NMR (DMSO-d6, δ ppm): 112-155(7C, Ar-C), elemental analysis: calculated (%) for C_7H_4ClNOS: C, 45.29; H, 2.17; N, 7.55; observed ; C, 45.21; H, 2.12; N, 7.53; M^+ 185.6, M^{+2} 187.6.

(ii)**Synthesis of 2-amino-6-[(5-chloro-2,7a-dihydro-1,3-benzoxazol-2-yl) sulfanyl]-4-(2,6-dichloroquinolin-3-yl) pyridine-3,5-dicarbonitrile 3a**

The compound 5-chloro-1,3-benzoxazole-2-thiol (0.5 gm) **1a** were treated with 2,6-dichloroquinoline-3-carbaldehyde **2a** in presence of malano nitrile in ethanol media using zinc chloride as a catalyst under reflux condition afforded 2-amino-6-[(6-chloro-2,7a-dihydro-1,3-benzoxazol-2-yl)sulfanyl]-4-(2,6-dichloroquinolin-3-yl)pyridine-3,5-dicarbonitrile **3a,** The reaction mixture was poured onto crushed ice. Solid product thus obtained was filtered, dried and recrystalized from ethanol to get compound **3a.**

The compounds **3(b-h)** can be prepared by following similar procedure.

Yield: 0.3821 (70 %), m.p. 253-255^0 C; IR (KBr v_{max} cm^{-1}): 2265.16, (Ar CN str), 3314.35 (Ar NH_2 str), 732.16, (Ar Ar-Cl str). ^{1}H NMR (DMSO-d_6, 400MHz) δ. 6.80-7.88(m, 7H, Ar-H), 3.86 (s, 2H, NH_2) (D_2O exchangeable).^{13}C NMR (DMSO-d_6, 100 MHz) δ. 116-152(21C Ar-C), 126 (2H, CN) elemental analysis: calculated (%) for $C_{23}H_{11}Cl_3N_6OS$; C,52.54; H,2.11; N,15.98; observed C,52.48; H,2.08; N,15.95; M^+, 526; M^{+2},528; M^{+4},530.

(iii)**Synthesis of 2-amino-6-[(5-chloro-2,7a-dihydro-1,3-benzoxazol-2-yl) sulfanyl]-4-(2-chloro-6-fluoroquinolin-3-yl) pyridine-3,5-dicarbonitrile 3b**

Yield: 0.3518 (66 %), m.p. 288-290^0 C; IR (KBr v_{max} cm^{-1}): 2270.16, (Ar CN str), 3420.35 (Ar NH_2 str), 738.45, (Ar Ar-Cl str). ^{1}H NMR (DMSO-d_6, 400MHz) δ. 7.12-7.95(m, 7H, Ar-H), 3.92 (s, 2H, NH_2) (D_2O exchangeable).^{13}C NMR (DMSO-d_6, 100 MHz) δ. 121-148(21C Ar-C), 132 (2H, CN) elemental analysis: calculated (%) for $C_{23}H_{11}Cl_2FN_6OS$; C,54.24; H,2.18; N,16.50; observed C,54.21; H,2.11; N,16.46; M^+, 509; M^{+2},511.

(iv)**Synthesis of 2-amino-4-(6-bromo-2-chloroquinolin-3-yl)-6-[(5-chloro-2,7a-dihydro-1,3-benzoxazol-2-yl) sulfanyl] pyridine-3,5-dicarbonitrile 3c**

Yield: 0.3249 (72 %), m.p. 276-278^0 C; IR (KBr v_{max} cm^{-1}): 2257.36, (Ar CN str), 3316.98 (Ar NH_2 str), 768.57, (Ar Ar-Cl str). ^{1}H NMR (DMSO-d_6, 400MHz) δ. 6.96-7.67(m, 7H, Ar-H), 3.92 (s, 2H, NH_2) (D_2O exchangeable).^{13}C NMR (DMSO-d_6, 100 MHz) δ. 122-152(21C Ar-C), 138 (2H, CN) elemental analysis: calculated (%) for $C_{23}H_{11}BrCl_2N_6OS$; C,45.44; H,1.94; N,14.74; observed C,45.41; H,1.90; N,14.70; M^+, 570; M^{+2},572.

(v)**Synthesis of 2-amino-6-[(5-chloro-2,7a-dihydro-1,3-benzoxazol-2-yl) sulfanyl]-4-(2-chloro-6-nitroquinolin-3-yl) pyridine-3,5-dicarbonitrile 3d**

Yield: 0.4360 (87 %), m.p. 325-327^0 C; IR (KBr v_{max} cm^{-1}): 23.16, (Ar CN str), 3285.14 (Ar NH_2 str), 717.60, (Ar Ar-Cl str). ^{1}H NMR (DMSO-d_6, 400MHz) δ. 7.10-8.15(m, 7H, Ar-H), 4.12 (s, 2H, NH_2) (D_2O exchangeable).^{13}C NMR (DMSO-d_6, 100 MHz) δ. 125-162(21C Ar-C), 142 (2H, CN) elemental analysis: calculated (%) for $C_{23}H_{11}Cl_2N_7O_3S$; C,51.50; H,2.07; N,18.28; observed C,51.48; H,2.04; N,18.22; M^+, 536; M^{+2},538.

(vi)**Synthesis of 2-amino-4-(2,6-dichloroquinolin-3-yl)-6-[(6-nitro-2,7a-dihydro-1,3-benzoxazol-2-yl) sulfanyl] pyridine-3,5-dicarbonitrile 3e**

Yield: 0.4126 (78 %), m.p. 286-288^0 C; IR (KBr v_{max} cm^{-1}): 2286.13, (Ar CN str), 3375.47 (Ar NH_2 str), 756.63 (Ar Ar-Cl str). ^{1}H NMR (DMSO-d_6, 400MHz) δ. 7.05-8.15(m, 7H, Ar-H), 3.96 (s, 2H, NH_2) (D_2O exchangeable).^{13}C NMR (DMSO-d_6, 100 MHz) δ. 112-158(21C Ar-C), 135 (2H, CN) elemental analysis: calculated (%) for $C_{23}H_{11}Cl_2N_7O_3S$; C,51.50; H,2.07; N,18.28; observed C,51.45; H,2.02; N,18.25; M^+, 536; M^{+2},538.

(vi)**Synthesis of 2-amino-4-(2-chloro-6-fluoroquinolin-3-yl)-6-[(6-nitro-2,7a-dihydro-1,3-benzoxazol-2-yl) sulfanyl] pyridine-3,5-dicarbonitrile 3f**

Yield: 0.3617 (71 %), m.p. 242-244^0 C; IR (KBr v_{max} cm^{-1}): 2290.38, (Ar CN str), 3385.57 (Ar NH_2 str), 733.74, (Ar Ar-Cl str). ^{1}H NMR (DMSO-d_6, 400MHz) δ. 7.16-8.54(m, 7H, Ar-H), 4.18 (s, 2H, NH_2) (D_2O exchangeable).^{13}C NMR (DMSO-d_6, 100 MHz) δ. 122-162(21C Ar-C), 138 (2H, CN) elemental analysis: calculated (%) for $C_{23}H_{11}ClFN_7O_3S$;

C,53.13; H,2.13; N,18.86; observed C,53.09; H,2.09; N,18.82; M^+, 520; M^{+2},522.

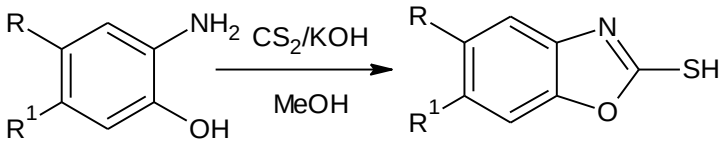
***(vii)*Synthesis of 2-amino-4-(6-bromo-2-chloroquinolin-3-yl)-6-[(6-nitro-2,7a-dihydro-1,3-benzoxazol-2-yl) sulfanyl] pyridine-3,5-dicarbonitrile 3g**

Yield: 0.3872 (78 %), m.p. 253-255^0 C; IR (KBr ν_{max} cm^{-1}): 2268.48, (Ar CN str), 3367.50 (Ar NH_2 str), 748.60, (Ar Ar-Cl str). ^{1}H NMR (DMSO-d_6, 400MHz) δ. 7.10-8.52(m, 7H, Ar-H), 3.96 (s, 2H, NH_2) (D_2O exchangeable).^{13}C NMR (DMSO-d_6, 100 MHz) δ. 118-155(21C Ar-C), 130 (2H, CN) elemental analysis: calculated (%) for $C_{23}H_{11}BrClN_7O_3S$; C,47.56; H,1.91; N,16.88; observed C,47.51; H,1.88; N,16.81; M^+, 581; M^{+2},583.

***(vii)*Synthesis of 2-amino-4-(2-chloro-6-nitroquinolin-3-yl)-6-[(6-nitro-2,7a-dihydro-1,3-benzoxazol-2-yl) sulfanyl] pyridine-3,5-dicarbonitrile 3h**

Yield: 0.4528 (78 %), m.p. 325-327^0 C; IR (KBr ν_{max} cm^{-1}): 2283.18, (Ar CN str), 3320.73 (Ar NH_2 str), 740.52, (Ar Ar-Cl str). ^{1}H NMR (DMSO-d_6, 400MHz) δ. 7.15-7.95(m, 7H, Ar-H), 4.06 (s, 2H, NH_2) (D_2O exchangeable).^{13}C NMR (DMSO-d_6, 100 MHz) δ. 123-163(21C Ar-C), 1238 (2H, CN) elemental analysis: calculated (%) for $C_{23}H_{11}ClN_8O_5S$; C,50.51; H,2.03; N,20.49; observed C,50.48; H,2.00; N,20.46; M^+, 547.

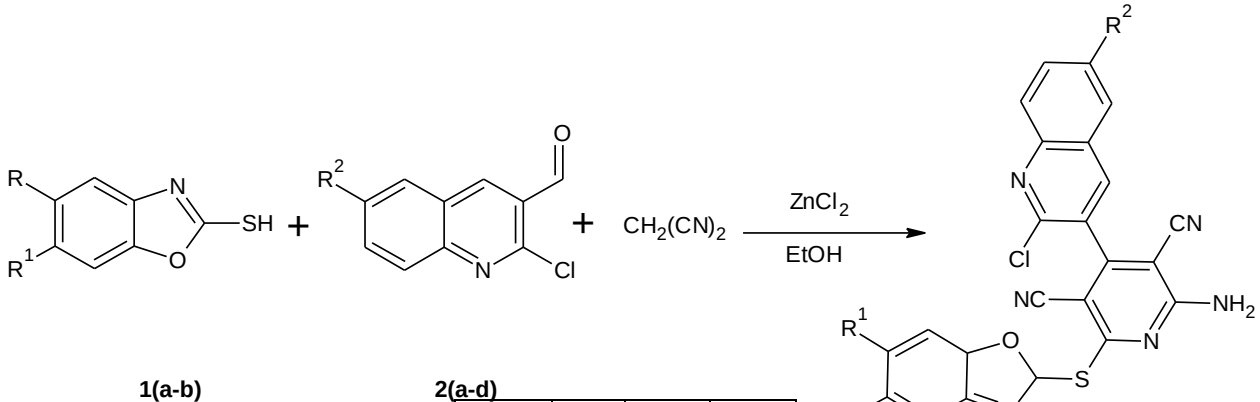
3 RESULT AND DISCUSSION:



1

R	**Cl**
R^1	**NO_2**

Scheme 1: Synthetic route for the preparation of compound 1



1(a-b) **2(a-d)** **3(a-h)**

Code	R	R^1	R^2
3a	Cl	H	Cl
3b	Cl	H	F
3c	Cl	H	Br
3d	Cl	H	NO_2
3e	H	NO_2	Cl
3f	H	NO_2	F
3g	H	NO_2	Br
3h	H	NO_2	NO_2

Scheme 2: Synthetic route for the preparation of compound 3(a-h)

Chemistry

The Cholro and nitro substituted compound of aminophenol **were** treated with carbon disulphide and potassium hydroxide in the presence of ethanol as solvent to give intermediate compound **1**, 3-benzoxazole-2-thiol **2 (Scheme 1)**. The compound **1** was characterized by ^{1}H NMR, which exhibited one singlet at δ 12-14, for –SH (D_2O exchangeable). Which is used as intermediate compound to synthesis of targeted molecule **3(a–h)**.

The chloro and nitro substituted mercapto benzoxazole reacted with different 2-chloroquinoline-3-carbaldehyde and malano nitrile in presence of zinc chloride as a catalyst in ethanol was refluxed afforded substituted 2-amino-4-(2-chloroquinolin-3-yl)-6-(2,7a-dihydro-1,3-benzoxazol-2-ylsulfanyl) pyridine-3,5-dicarbonitrile. Which can be confirmed by IR, ^{1}H NMR, mass, and elemental analysis. IR spectrum shows 2270 cm^{-1} for (CN), 3390 cm^{-1} for (NH_2) and ^{1}H NMR shows δ 6.83-8.2 (m, 7H, Ar-H), 4.2 (s, 2H NH_2) (D_2O exchangeable), Which confirms the disappearance for –SH one singlet in compound **1a** at δ (12-14) and for –CHO one singlet in compound **2a** at δ (9.9) by reacting with 2-chloroquinoline-3-carbaldehyde forms cyclized benzoxazol-2-ylsulfanyl)pyridine-3,5-dicarbonitrile ring. The Mass spectra are concurrence with Molecular weights of the target compounds. **(Scheme 2).**

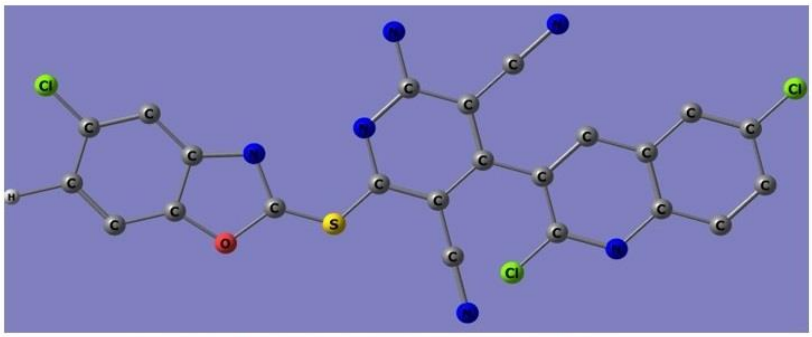
The derivatives **3(a–h)** were screened for antibacterial, antioxidant activity, cytotoxic and with frontier molecular orbital studies. In the antibacterial study, the compounds have shown inhibition of test bacteria. Among synthesized compounds, marked inhibition of test bacteria was observed to compounds 3**b, 3c, 3f, 3g** and **3h**. The compounds **3c, 3d, 3f** and **3h** exhibited potent free radical scavenging activity. The compounds **3c, 3f** and **3h** displayed more non-cell viability as compared to other compounds. In case of DFT studies the C-C bond length C19-C21 (1.540 Å) is greater than that of C1-C3 (1.386 Å) because of the delocalization of electron density of C19-C21 with the oxazole fused phenyl ring. Also, in C1-C3 (1.386 Å) is less bond length due to one NH_2 and two CN group directly bonded to the pyridine ring and C19-S16-C6 (1.820 Å) showed highest bond length due to S16 is bonded with two phenyl rings. Bond angles N6-C6-C5 (119.80 ^{0}) are less than 120 ^{0} indicates the presence of nitrogen in pyridine ring. At C6 position, the bond angles N6-C6-C5 is reduced by 0.2 ^{0} due to one NH_2 and two CN group directly bonded to the pyridine ring and delocalization electron. The pyridine moiety is tilted from phenyl ring as is evident from the torsion angles C1-C2-N4=117.06, C1-C2-N15=121.46, and C2-N4-C6=122.093 the pyridine ring of the title compound is somewhat irregular and the spread of C-C bond distance is 1.386-1.540, It is important that ionization potential (I), electron affinity (A), electrophilicity index (ω), chemical potential (μ), electronegativity (χ) and hardness (η) to be put into a molecular orbital frame work. Based on density functional descriptors, global chemical reactivity descriptors of compounds such as hardness, chemical potential, softness, electro negativity and electrophilicity index as well as local reactivity has been defined. Using Koopman's theorem for closed shell components η, μ and χ can be defined as η=(I-A)/2; μ = -(I +A)/2; χ =(I+A)/2; where I and A are the ionization potential and electron affinity, respectively. The ionization energy(I) and electron affinity (A) can be expressed through HOMO and LUMO orbital energies as I = E_{HOMO} = -2.935 eV and A = E_{LUMO} = -2.735 eV for the compound **3a** and I = E_{HOMO} = -3.766 eV and A = E_{LUMO} = -2.945 eV for the compound **3e**. Electron affinity refers to the capability of ligand to accept precisely one electron from a donor. However, in many kinds of bonding viz. covalent hydrogen bonding, partial charge transfer takes place. Considering the chemical hardness (η), large HOMO-LUMO energy gap means a hard molecule and small HOMO-LUMO gap means a soft molecule. One can also relate the stability of the molecule to hardness, which means that the molecule with smaller HOMO-LUMO gap is more reactive. For the title compound, the energy gap is 0.202 eV and 0.821 respectively. Parr et al. have defined a new descriptor to quantity the global electrophilic power of the compound as electrophilicity index (ω) which defines a quantitative classification of global electrophilic nature of a compound. Parr et al. have proposed electrophilicity index (ω) as a measure of energy lowering due to maximal electron flow between donor and acceptor. They defined electrophilicity index as follows: ω= $\mu^2/2\eta$. The usefulness of this new reactivity measure has been recently demonstrated in understanding the toxicity of various pollutants in terms of their reactivity and site selectivity [41]. The calculated values, of ω, μ, χ and η are 39.7601, 2.834, -2.834 and

0.101 eV for compound **3a** and 13.7101, 2.834, -3.355 and 0.4105 eV respectively for the compound **3e**. The calculated value of electrophilicity index describes the biological activity of the title compound. The atomic orbital components of the frontier molecular orbital are shown in Fig. 5.

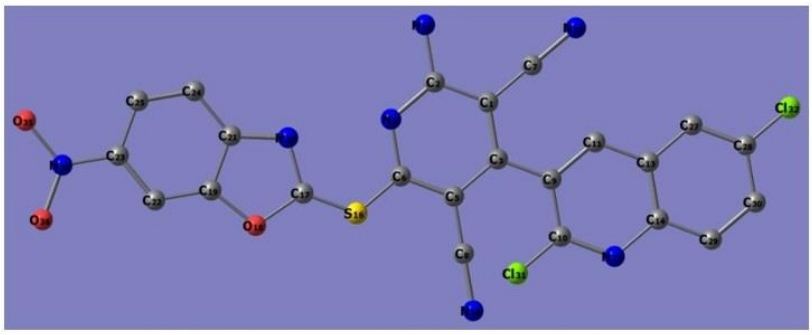
Geometrical optimization

Calculations of the title compound were carried out with Gaussian 09 software using B3LYP/6-31G basis set to predict the molecular structure and vibrational wavenumbers. This basis set was chosen particularly because of its advantage of doing faster calculations with relatively better accuracy and structures and it contains both soft and polarization functions and it has proven to yield reliable descriptions of the molecular structure. As the DFT hybrid B3LYP functional tends to overestimate the wavenumbers of the fundamental modes, a scaling factor of 0.9613 has been uniformly applied to the calculated wavenumbers. The assignments of the calculated wavenumbers are aided by the animation option of GAUSSVIEW program and potential energy distribution by GAR2PED software package. The theoretically optimized geometrical parameters are given in Table 1 and Table 2. The compounds **3a** and **3e** derivatives are optimized at B3LYP/6-31G and B3LYP/LANL2DZ level in gas phase to get the stable geometry. Geometrical parameters of the **3a** and **3e** compounds (Figure 1) are collected from the stable geometry (Table 1 and 2).

Figure 1. Optimised geometry of the compound 3a and 3e



Optimised geometry of the compound 3a



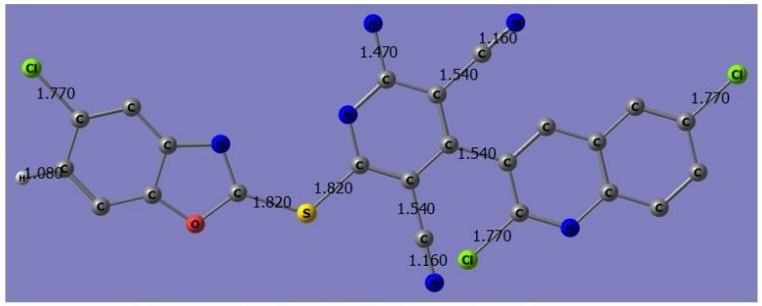
Optimised geometry of the compound 3e

Chemical reactivity study

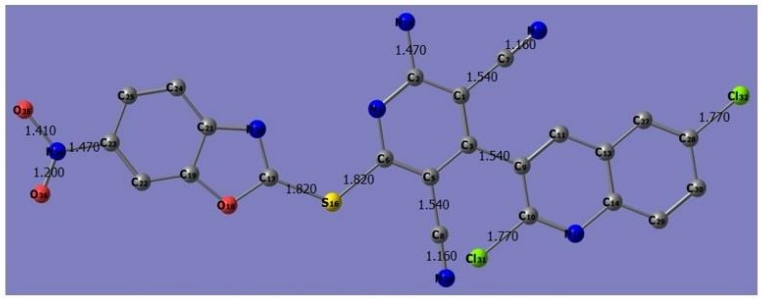
The E_{HOMO} and E_{LUMO} energy of the compounds **3a** and **3e** are calculated to evaluate the global reactivity descriptors as chemical hardness, electrochemical potential, electrophilicity and chemical hardness. Calculation of molecular orbital coefficients indicates that the possible covalent bond sites of the compound are –N-O-, -S-C-, -N-C-, -C-Cl- and –CN- group, that binds the carbon atom, Since the E_{HOMO} and E_{LUMO} values are found negative that indicate the compounds are stable[24, 25]. The decrease of EHOMO values of the compounds with the value of the compound **3a** and **3e** that confirms the weakening of –S-C, -N-C- new bond formation sites. The HOMO level for the compound is localized on the S16, C6, N2, N4, N3 and CN (Figure 4 and 5) indicates that these are the preferred bonding

sites for the neuclephilic attack. The energy gap between E_{HOMO} and E_{LUMO} is an important parameter to determine the chemical reactivity and chemical stability of the molecule[26]. The energy gap [E_{HOMO} - E_{LUMO}] of the ligand is found to be less than the energy gap of the complexes that indicates greater reactivity of the compounds and hence more stability of the compounds (Figures 4 and 5).

Figure 2. Selected bond length (Å) of compound 3a and 3e

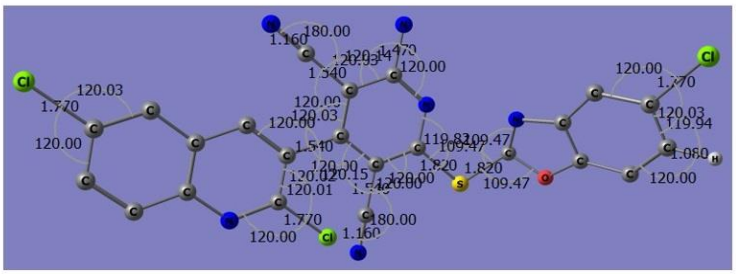


Selected bond length (Å) of compound 3a

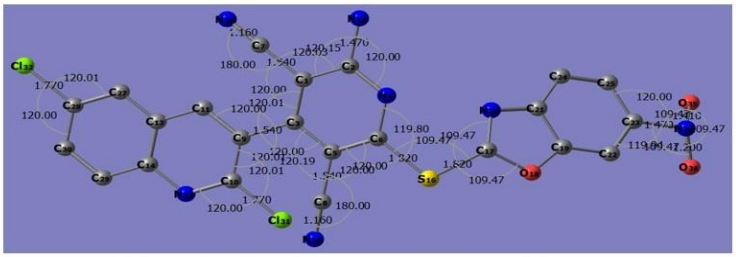


Selected bond length (Å) of compound 3e

Figure 3. Selected bond angle (degree) of compound 3a and 3e



Selected bond angle (degree) of compound 3a



Selected bond angle (degree) of compound 3e

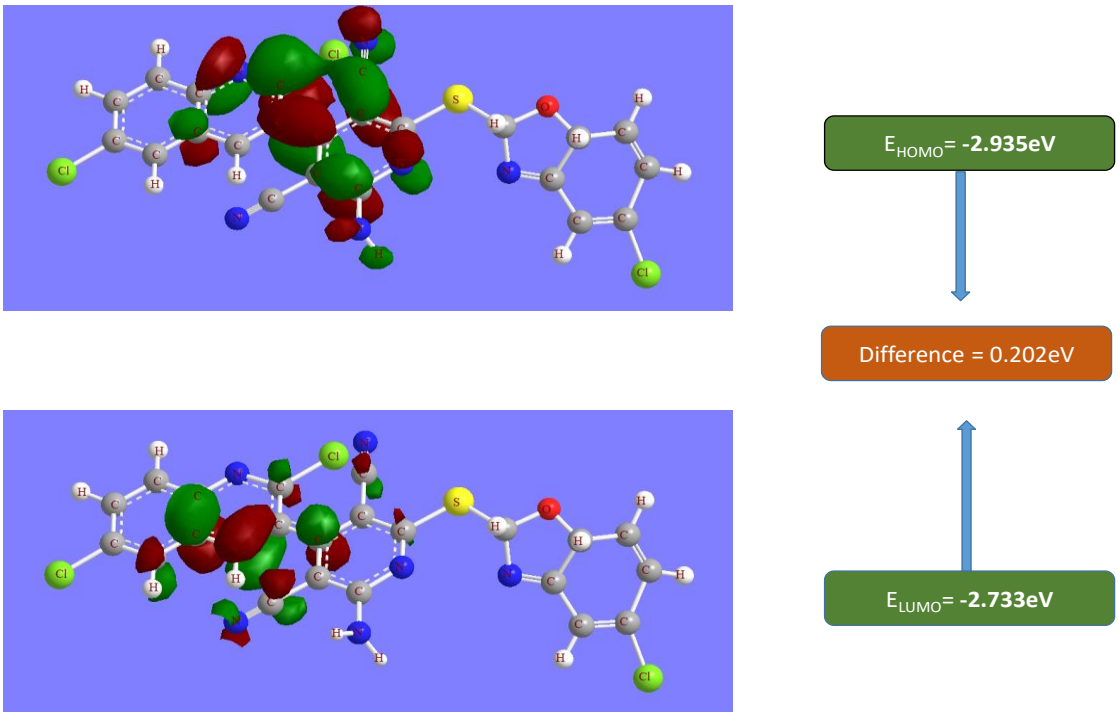
Table 1. Selected bond length (Å) and bond angle (degree) of compound 3a

Bond	Bond length (Å)	Angle	(°)	Dihedral angle	(°)
C (1)-C (3)	1.386	C(21)-C(24)-H (39)	119.999	C(2)-C (1)-C (3)	120.000
C (1)-C (7)	1.540	C(25)-C(24)-H (39)	119.999	C(2)-C (1)-C (7)	119.999
C(2)-N (4)	1.430	C(23)-C(25)-C(24)	114.911	C(3)-C(1)-C (7)	119.999
N(15)-H(36)	1.028	C(23)-C(25)-Cl(26)	137.495	C(1)-C(2)-N(4)	117.064
N(15)-H(37)	1.028	C(24)-C(25)-Cl(26)	107.590	C(1)-C(2)-N(15)	121.466
S(16)-C(17)	1.820	C(13)-C(28)-C(29)	120.000	N(4)-C(2)-N(15)	121.466
C(17)-O(18)	1.410	C(13)-C(28)-H(43)	119.999	C(1)-C(3)-C(5)	119.999
C(17)-N(20)	1.446	C(29)-C(28)-H(43)	119.999	C(1)-C(3)-C(9)	120.000
C(17)-H(45)	1.122	C(28)-C(29)-C(31)	119.963	C(5)-C(3)-C(9)	119.999
O(18)-C(19)	1.343	C(28)-C(29)-Cl(33)	120.018	C(2)-N(4)-C(6)	122.937
C(19)-C(21)	1.540			C(3)-C(5)-C(6)	120.000

Table 2. Selected bond length (Å) and bond angle (degree) of compound 3e

Bond	Bond length (Å)	Angle	(°)	Dihedral angle	(°)
N(4)-C(6)	1.301	C(2)-N(4)	1.430	N(4)-C(6)	1.301
C(8)-N(34)	1.128	N(4)-C(6)	1.301	C(5)-C(6)	1.386
C(9)-C(10)	1.386	C(5)-C(6)	1.386	N(12)-C(14)	1.301
C(9)-C(11)	1.386	C(5)-C(8)	1.540	N(15)-H(37)	1.028
S(16)-C(17)	1.790	C(6)-S(16)	1.790	N(15)-H(38)	1.028
C(17)-O(18)	1.410	C(7)-N(33)	1.128	S(16)-C(17)	1.790
C(17)-N(20)	1.446	C(8)-N(34)	1.128	C(17)-O(18)	1.410
C(17)-H(47)	1.122	C(9)-C(10)	1.386	N(20)-C(21)	1.244
O(18)-C(19)	1.343	N(12)-C(14)	1.301	C(21)-C(24)	1.540
C(19)-C(21)	1.540	N(15)-H(37)	1.028	C(24)-H(41)	1.122
C(19)-C(22)	1.540	N(15)-H(38)	1.028	C(25)-H(39)	1.122

Figure 4. Molecular orbital diagram with HOMO and LUMO orbital of Compound 3a



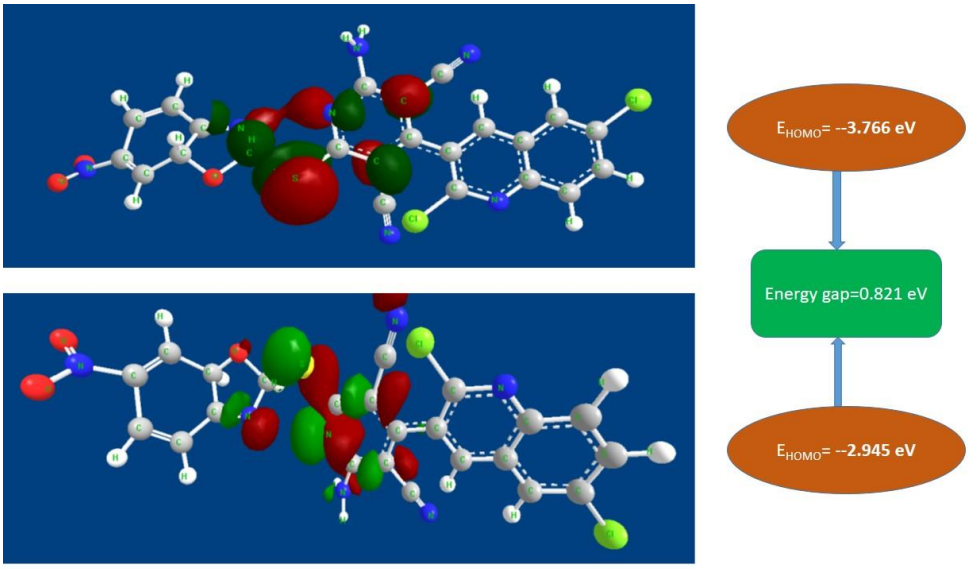


Figure 5. Molecular orbital diagram with HOMO and LUMO orbital of Compound 3e

The DFT/B3LYP method indicates the chemical reactivity and the selection of active sites of the molecular system. The energy of FMOs and the energy band gap explain the charge transfer interaction within the molecule. The chemical reactivity values as electronegativity (χ), chemical potential (μ), global hardness (η), global softness (S) and global electrophilicity index (ω) [27, 28] are listed in (Table 3).

$\chi = (E_{LUMO} + E_{HOMO})/2$
$\mu = -\chi = (E_{LUMO} + E_{HOMO})/2$
$\eta = (E_{LUMO} + E_{HOMO})/2$
$S = 1/2\eta$
$\omega = \mu^2/2\eta$
$\sigma = 1/\eta$

Table 3. Calculated quantum chemical parameters for compound 3a

Compound	HOMO in eV	LUMO in eV	ΔE in eV	χ Pauling	η in eV	σ	μ in eV	S	ω eV
3a	-2.935	-2.733	0.202	-2.834	0.101	9.9009	2.834	4.9504	39.7601
3e	-3.766	-2.945	0.821	-3.355	0.4105	2.4360	3.355	1.2180	13.7101

3. Biological activity of the synthesized compounds 3(a-h)

3.1 Antibacterial Activity of the compound:

The newly synthesized benzoxazol-2-ylsulfanyl)pyridine-3,5-dicarbonitrile derivatives **3(a-h)** was tested for their antibacterial activity against *Staphylococcus aureus* (ATTC-6538), *Bacillus cereus* (ATTC-11778), *Bacillus subtilis* (ATTC-6633), *Staphylococcus epidermidis* (ATTC-12228), *Pseudomonas aeruginosa* (ATTC-9027), *Salmonella typhimurium* (ATTC-23564) and *Escherichia coli* (ATTC-8739) bacterial strains was using agar well diffusion method[29]. The 24 hr old Mueller-Hinton broth cultures of test bacteria were swab bed on sterile Mueller-Hinton agar plates using sterile cotton swab followed by punching wells of 6mm with the help of sterile cork borer. The standard drug (chloramphenicol, 1mg/ml of sterile distilled water), compounds **3(a–h)** (20mg/mL of 10% DMSO) and control (10% DMSO) were added to the respectively labeled wells. The plates were allowed to stand for 30 minutes and were incubated at 37°C for 24 hr in upright position, and the zone of inhibition was recorded and tabulated in Table 4.

Table 4. Antibacterial activity of compounds 3(a-h)

Compounds	Zone of inhibition in mm					
	Gram positive bacteria			*Gram negative bacteria*		
	S.aureus	***B.subtilis***	***S.epidermis***	***P.aeruginosa***	***S.typhi***	***E.coli***
3a	21.00±1.01	21.04±0.31	20.07±0.25	24.01±0.65	24.09±0.63	19.90±0.32
3b	**25.39±0.77**	23.01±0.0	24.99±0.90	23.02±0.75	**25.00±0.98**	21.03±0.33
3c	21.00±0.98	20.00±0.98	22.00±0.54	23.07±0.64	22.99±0.22	**23.05±0.88**
3d	19.00±0.85	18.98±0.69	21.47±0.49	19.06±0.55	22.68±0.9	18.54±0.23
3e	24.00±0.48	21.09±1.20	23.29±0.22	24.02±0.77	23.19±9.90	20.28±09
3f	20.99±0.66	22.08±0.43	**25.06±0.0**	24.09±0.76	24.08±0.22	19.00±0.78
3g	24.57±0.45	24.32±0.85	23.02±0.66	**25.02±0.00**	**26.00±0.99**	22.02±0.12
3h	24.77±0.26	23.92±0.37	22.09±0.75	**26.99±0.24**	**25.98±0.56**	20.98±0.29
Std	**28.55±98**	**29.29±0.00**	**28. 09±0.33**	**28.00±0.88**	**30.38±0.44**	**26.09±0.36**

Std: Chloramphenicol

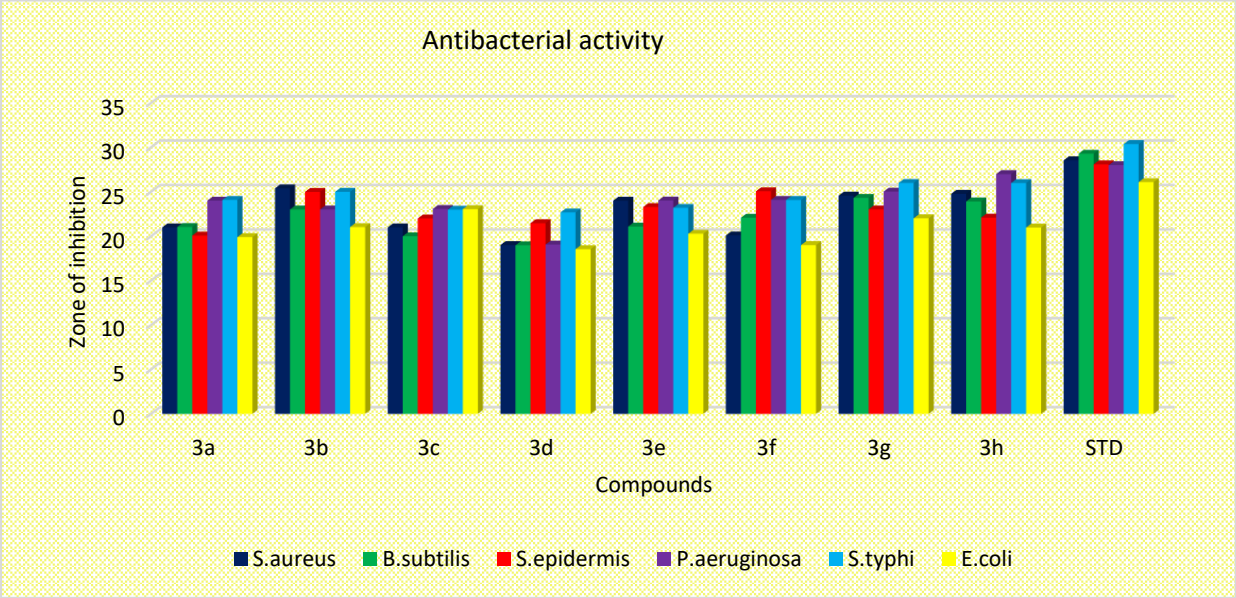


Figure 6. Antibacterial activity of compounds 3(a-h)

3.2 Antioxidant activity synthesized compounds 3(a-h)

DPPH Assay.

The radical scavenging ability of synthesized compounds and the ascorbic acid (standard) was tested on the basis of radical scavenging effect on a DPPH free radical. Different concentrations (25, 50, 75 and 100μg/mL) of compounds and standard were prepared in methanol. In clean and labeled test tubes, 3 mL of DPPH solution (0.002% in methanol) was mixed with **05, 10, 15, 20 and 25** μg/mL concentrations of compounds and standard separately and make up the solution up to 4 mL by adding methanol. The tubes were incubated at room temperature in dark for 30 minutes, and the optical density was measured at 517 nm using UV-Visible Spectrophotometer. The absorbance of the DPPH control was also noted. The scavenging activity was calculated using the formula. Scavenging activity (%) = $A - B/A \times 100$, where A is the absorbance of DPPH and B is the absorbance of DPPH in standard combination[30].

Table 5. Antioxidant activity of synthesized compound 3(a-h)

Compounds	Scavenging activity of different concentration (Mg/mL) in %				
	5	10	15	20	25
3a	53.67	58.30	61.71	64.38	69.09
3b	50.92	56.18	61.73	63.12	68.15
3c	**82.16**	**85.10**	**86.63**	**88.78**	**89.48**
3d	**72.02**	**75.36**	**78.41**	**80.63**	**82.25**
3e	50.81	53.00	54.40	58.78	62.38
3f	**78.25**	**80.43**	**83.71**	**86.45**	**88.53**
3g	48.18	50.56	53.74	58.46	61.89
3h	**76.56**	**79.67**	**81.12**	**83.63**	**85.10**
Ascorbic acid	**86.71**	**88.57**	**90.04**	**93.62**	**96.33**

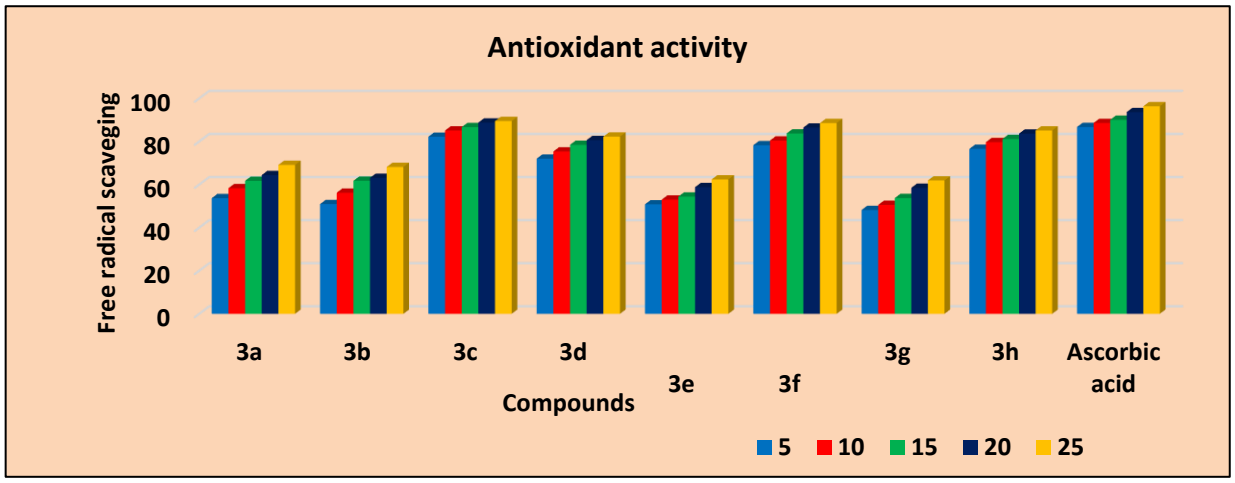


Figure 7. Antioxidant activity of synthesized compound 3(a-h)

3.3 Cytotoxic activity

Preparation of Peripheral Blood Mononuclear Cells (PBMCs) or Buffy Coat

Blood samples from healthy volunteers were collected by venipuncture and transferred into 2 ml heparin coated vacutainers. It was diluted to 1:1 ratio with PBS (Phosphate buffer solution, pH 7.0) layered onto 4 mL Ficol without getting mixed up. It was further separated by centrifuging at 1,000 rpm for 30 min at room temperature. During the centrifugation the PBMCs move from plasma and suspend as the density gradient. Plasma was removed down to 1 cm above buffy coat and discarded the white layer lying on top of the red cells. The buffy coat layer was washed twice with PBS. Roswell Park Memorial Institute (Gibco, Life Technologies) medium was prepared by mixing 10 mL of Fetal bovine serum (Invitrogen) and 200µL antimycotic [Antibiotic antimycotic solution with Streptomycin (10mg/20mL), 10,000 U Penicillin, Amphotericin B and 0.9% normal saline]. This mixture (4mL) was dispensed into falcon tubes, 30µL of Phytohemagglutin (Invitrogen) and 200µL of PBMCs were incubated at the atmosphere of 95% air and 5% CO_2 at 37°C for 4 hr[31].

About **10 µg/mL**, **50 µg/mL** and **100 µg/mL** of the selected compounds (1mg/mL) were added to the respectively labeled PBMCs tubes and incubated for 72 hr at the earlier mentioned conditions. After 72 hr, cell viability was determined by the trypan-blue dye exclusion method[32].

Trypan blue exclusion test cells were clarified by centrifuging at 1000 rpm for 30 min at room temperature. The supernatant liquid was discarded and to the solution 10µL of PBMCs, 10µL of tryphan blue was added and incubated for 10 min at room temperature. About 10µL of incubated sample was loaded on previously cleaned Haemo cytometer and counted the number of live cells, total cells and dead cells at four corners under Trinucular microscope, Nikon Eclipse E200. The percentage of cell viability and non-viability was tabulated in Table-6.

Table 6. Cytotoxic activity of newly synthesized derivatives against PBMCs.

Sample	Total cells	Live cells	Dead cells	% of Cells viability	%of cells non-viability
3c-10µg/mL	82	32	50	39.02	60.97
3c-50µg/mL	212	60	142	28.3	66.98
3c-100µg/mL	**136**	**38**	**98**	**27.94**	**72.05**
3f-10µg/mL	90	42	48	46.66	53.33
3f-50µg/mL	**125**	**27**	**98**	**21.60**	**78.40**
3f-100µg/mL	92	68	24	73.91	26.08
3h-10µg/mL	78	29	49	37.1	62.8
3h-50µg/mL	82	38	44	46.3	53.7
3h-100µg/mL	**186**	**46**	**140**	**24.73**	**75.26**
Control	118	17	101	14.4	**85.5**

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