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Exploring the Design, Synthesis, and Comprehensive Biological Evaluation of Novel Imidazo[1-b][1,3,4]thiadiazole Derivatives for Potential Therapeutic Applications: A Multifaceted Investigation

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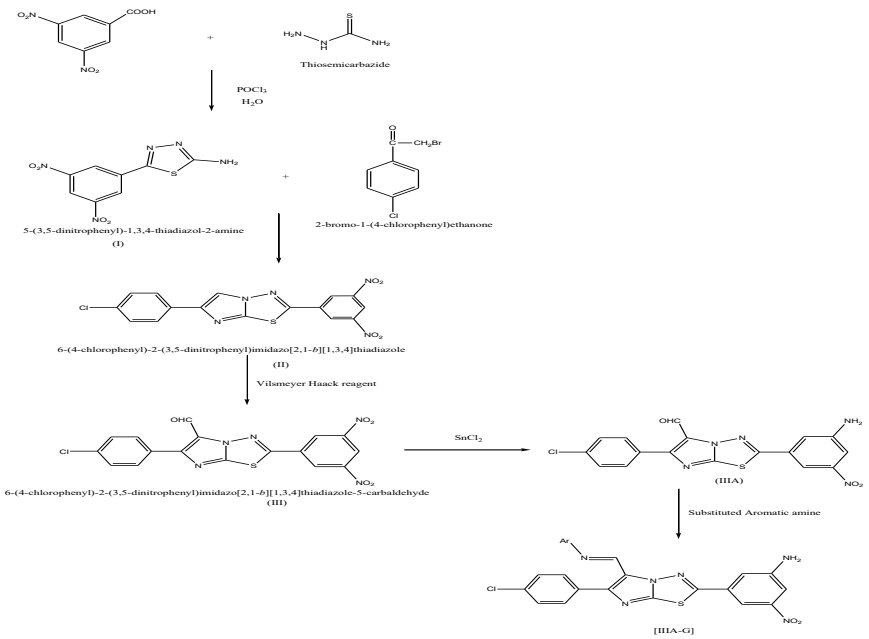
Abstract: In order to manufacture imidazo[1-b][1,3,4]thiadiazole, the beginning components that are used are the 2,4-dinitro benzoic acid and the thiosemicarbazide. The synthesis of the corresponding imidazolthadiazole derivative was brought about as a consequence of their interaction with phenacyl bromides, which are also referred to as p-substituted phenacyl bromides. The structure of these compounds was validated since the spectrum features of the compounds that were discussed before were taken into consideration. A couple of these compounds were shown to have an effect that varied from mild to moderate against the microorganisms Staphylococcus aureus, Candida albicans, Pseudomonas aeruginosa, and Escherichia coli. This was identified via the process of discovery.

Keywords: *1,3,4-thiadiazole, thiosemicarbazide, phenacyl bromide, antimicrobial activity*

Introduction

Imidazo Synthesis [2,1-b] Intricate Thiadiazoles (1) are heterocyclic systems that fall within this category. The imidazole and 1,3,4-thiadiazole rings fuse together in these systems due to the presence of a nitrogen atom at the bridgehead. It was discovered for the first time during the start of the nineteenth century's 1950s. Numerous imidazo[1-b][1,3,4]thiadiazole compounds have been synthesized, and their biological activities have been extensively documented in the scientific literature. These compounds vary in the substitutions made at the C-2, C-5, and/or C-6 positions of the basic structures. It is well known that molecules exhibiting a wide range of antibacterial action include 2-amino-1,3,4-thiadiazole derivatives [3]. These compounds are produced by using Schiff base after the corresponding aldehydes have been used [4].They seemed to be the most useful method for dealing with fused imidazo[2,1-b]-1,3,4-thiadiazole rings [13], which may have interesting applications down the road. Several of these substances have antitubercular, analgesic, antisercetory, antibacterial, antifungal, anticonvulsant, anticancer, and antiapoptotic properties. [4] Antitubercular substances have anticancer properties as well. A few compounds containing the imidazo[2,1-b]-1,3,4-thiadiazole moiety are also used in the synthesis of pigments and in the management of inflammatory, cardiotonic, diuretic, and herbicidal conditions. Having taken all of this into account, the current work concentrated on synthesizing a number of novel fused imidazo[2,1-b]-1,3,4-thiadiazole rings and evaluating their reactivity against Staphylococcus aureus, Candida albicans, Pseudomonas aeruginosa, and Escherichia coli in addition to electrophilic substitution processes.

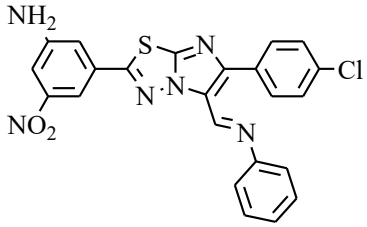
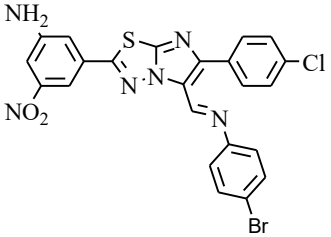
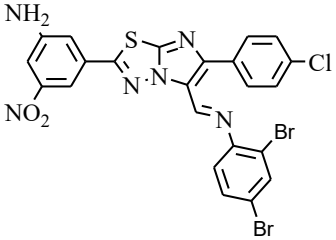
Reaction Scheme

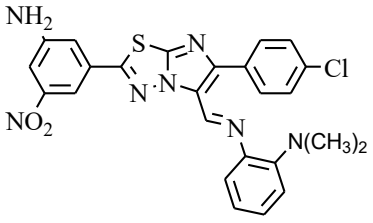
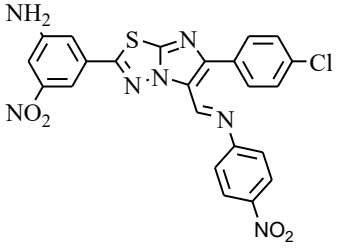
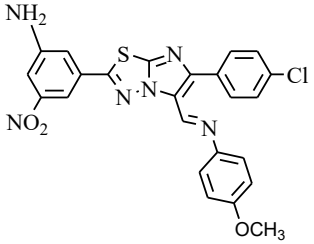
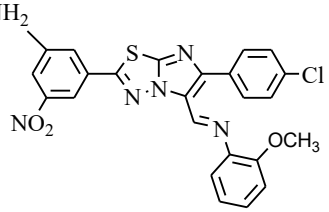


Chemistry

Synthesis of fused imidazo[2,1-b][1,3,4]thiadiazole III(A-R) is outlined in Scheme 1. 5-(3,5-dinitrophenyl)-1,3,4-thiadiazol-2-amine is prepared as per the reported method [6].Condensation of 1 with respective bromoacetyl compound in ethanol and dimethylformamide yields imidazo thiadiazole 2 and 5 in good yields. VilsmeiereHack reaction of imidazo thiadiazole 2 and 5, in DMF and $POCl_3$ provided respective 5-formyl derivatives 3 and 6 . The aldehyde functional group when treated with amines gave the corresponding imine derivatives III(A-R). The detail reaction mechanism is depicted in physical data is given in Table 1.

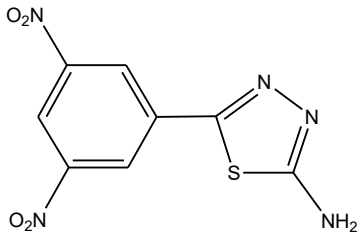
Table 1. Physical Data of synthesized compounds

Sr. No	Compound	Molecular Formula	Molecular Weight	Yield
IIIA		$C_{23}H_{15}ClN_6O_2S$	474.07	45%
IIIB		$C_{23}H_{14}BrClN_6O_2S$	553.82	72%
IIIC		$C_{23}H_{11}Br_2ClN_6O_4S$	662.697	43%

IIID		$C_{25}H_{20}ClN_7O_2S$	517.99	62%
IIIE		$C_{23}H_{14}ClN_7O_4S$	519.92	72%
IIIF		$C_{24}H_{17}ClN_6O_3S$	504.95	82%
IIIG		$C_{24}H_{17}ClN_6O_3S$	504.95	42%

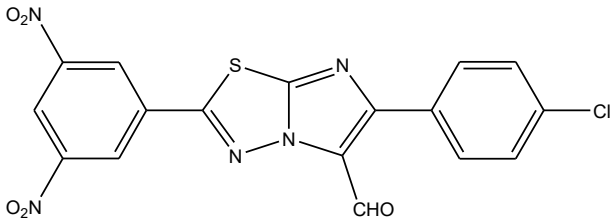
Experimental protocols

5-(3, 5-dinitrophenyl)-1, 3, 4-thiadiazol-2-amine (I)



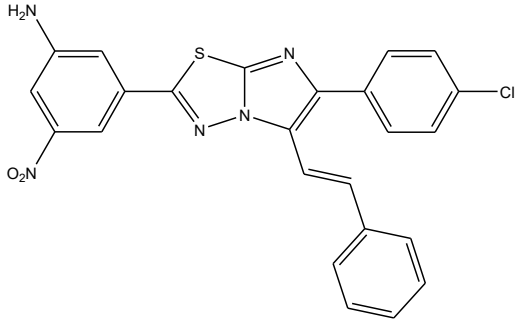
IR (KBr) ν_{max}**:** 1450.12cm^{-1} (Ar. C=C stretch), 3092.64cm^{-1} (Ar. C-H stretch), 1075.71cm^{-1} (C-N stretch), 1685.68cm^{-1} (C=N stretch), 3178.16cm^{-1} (N-H stretch), 1539.80cm^{-1} & 1345.05cm^{-1} (NO2 stretch), 723.13cm^{-1} (C-S-C stretch), 2942.51cm^{-1} (C- H stretch). $_1$H NMR (DMSO-d_6) d $_{ppm}$**:** 9.04 (s, 2H, aromatic), 8.72 (s, 1H, aromatic), 6.99 (s, 2H, aromatic C-NH).**^{13}CNMR** (DMSO-d_6) d $_{ppm}$**:** 161.6 (C1), 174.1 (C2), 149.3(C3 & C4), 135.3 (C5), 118.1 (C6), 128.9 (C7 & C8). HRMS (EI) m/z calcd for C8H5N5O4S: 267.01; found: 267.05.

6-(4-chlorophenyl)-2-(3,5-dinitrophenyl)imidazo[2,1-b][1,3,4]thiadiazole-5-carbaldehyde (III)



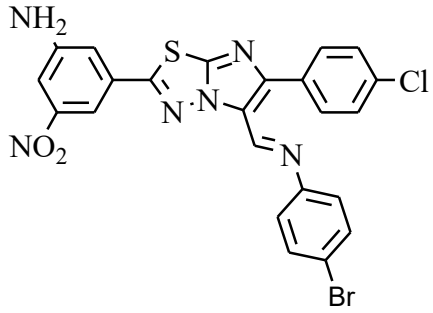
IR (KBr) ν_{max}: 1482.93cm^{-1} (Ar. C=C stretch), 3089.41cm^{-1} (Ar. C-H stretch), 1169.82cm^{-1} (C-N stretch), 1680.77cm^{-1} (C=N stretch), 662.57 cm^{-1} (C-Cl stretch), 1543.95cm^{-1} & 1344.38cm^{-1} (NO2 stretch), 729.77cm^{-1} (C-S-C stretch) $_1$H NMR (DMSO-d$_6$) d $_{ppm}$ **:** 9.04 (s, 2H, aromatic), 8.72 (s, 1H, aromatic), 9.75 (s, 1H, CHO),7.98 (d, 2H, aromatic), 7.55 (d, 2H,aromatic).^{13}CNMR (DMSO-d$_6$) d $_{ppm}$**:** 145.0 (C1), 143.3 (C2), 159.9 (C3), 136.7 (C4), 134.3 (C5),149.3 (C6 & C7), 135.3 (C8), 132.7 (C9), 129.3 (C10 & C15), 118.1 (C11), 128.9 (C12 & C13), 131.6 (C14 & C16), 188.9 (C17). HRMS (EI) m/z calcd for $C_{17}H_8ClN_5O_5S$: 428.99; found: 428.94.

(E)-N-((6-(4-chlorophenyl)-2-(3,5-dinitrophenyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl)methylene)aniline (IIIA)



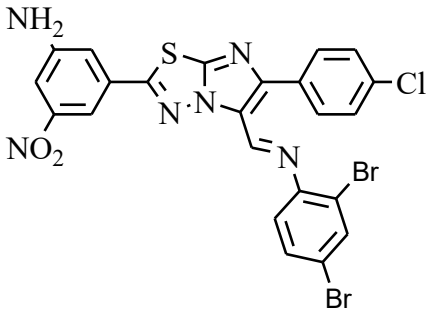
IR (ATR-FTIR, cm^{-1}): 1486.62cm^{-1} (Ar. C=C stretch), 3090.96cm^{-1} (Ar. C-H stretch), 1090.74cm^{-1} (C-N stretch), 1676.78cm^{-1} (C=N stretch), 663.47cm^{-1} (C-Cl stretch), 1544.08cm^{-1} & 1344.68cm-^{1} (NO2 stretch), 1500cm-^{1} (NH2 stretch), 729.75cm^{-1} (C-S-C stretch). $_1$H NMR (DMSO-d$_6$) d $_{ppm}$ **:** 9.04 (s, 2H, aromatic), 8.72 (s, 1H, aromatic), 7.98 (d, 2H, aromatic), 7.55 (d, 2H, aromatic), 8.40 (s, 1H, CHO), 7.47 (d,1H, aromatic), 7.45 (t, 2H, aromatic), 7.06 (t, 1H, aromatic). 4.0(aromatic C-NH) ^{13}CNMR (DMSO-d$_6$) d $_{ppm}$**:** 136 (C1), 143.3 (C2), 146.0 (C3), 116.4 (C4), 134.3 (C5), 149.3(C6 & C7), 151.2 (C8), 135.3 (C9), 132.7 (C10), 129.3 (C11 & C17), 118.1 (C12), 128.9 (C13 & C15), 122.3 (C14 & C18), 131.6 (C16 & C19), 130.0 (C20 & C21), 127.2 (C22), 151.7 (C23). HRMS (EI) m/z calcd for $C_{24}H_{14}ClN_5O_4S$: 503.05; found: 503.08.

(E)-4-Bromo-N-((6-(4-chlorophenyl)-2-(3,5dinitrophenyl)imidazo[2,1b][1,3,4]thiadiazol-yl)methylene) (IIIB)



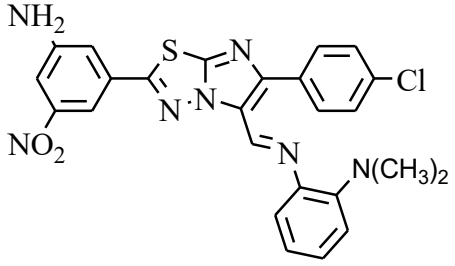
IR (ATR-FTIR, cm^{-1}): 3034cm^{-1} (CH), 1671cm^{-1} (C=N), 1551cm^{-1} (C=C), 708 cm^{-1} (C-Cl), 658 cm^{-1}(C-Br) **HNMR (DMSO, δppm):** 8.04 (s, 1H, CH=N), 6.93-7.88 (m, 11H, Ar-H), 8.05 (s, 2H, Ar-H), .^{13}C NMR (DMSO-d_6) d $_{ppm}$: 149.4, 145.6, 136.4, 128.4 (imidazo[2,1-b][1,3,4]thiadiazole) 129.4, 128.9, 118.5 2-(3,5-dinitrophenyl)150.4, 134.8, 131.5, 124.6, 114.2 (5-methylidene aniline) 135.8, 135.5, 132.8, 133.8,133.6,131.8 (6-phenyl); HRMS (EI) m/z calcd for $C_{23}H_{12}BrN_6O_4S$: 551.98; found: 551.95

2,4-dibromo-N-((6-(4-chlorophenyl)-2-(3,5-dinitrophenyl)imidazo[2,1-b][1,3,4]thiadiazolyl)methylene)benzenamine(IIIC)



IR (ATR-FTIR, cm^{-1}): 3034cm^{-1} (CH), 1671cm^{-1} (C=N), 1551cm^{-1} (C=C), 705 cm^{-1} (C-Cl), 651 cm^{-1}(C-Br) **HNMR (DMSO, δppm):** 8.04 (s, 1H, CH=N), 6.93-7.88 (m, 11H, Ar-H), 8.05 (s, 2H, Ar-H), .^{13}C NMR (DMSO-d_6) d $_{ppm}$: 149.4, 145.6, 136.4, 128.4 (imidazo[2,1-b][1,3,4]thiadiazole) 126.4, 125.6, 116.5 2-(3,5-dinitrophenyl) 150.4, 134.8, 131.5, 124.6, 114.2 (5-methylidene aniline) 135.8, 135.5, 132.8, 133.8,133.6,131.8 (6-phenyl); HRMS (EI) m/z calcd for $C_{23}H_{13}Br_2N_6O_2S$: 629.89; found: 629.85.

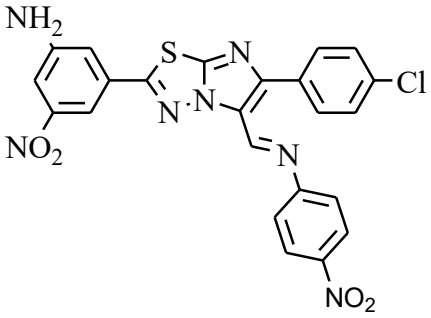
(2-(3-amino-5-nitrophenyl)-6-(4-chlorophenyl) imidazo [2,1-b][1,3,4]thiadiazol-5-yl)methylene)-N,N-dimethylbenzene-1,2-diamine(IIID)



IR (ATR-FTIR, cm^{-1}): 3036cm^{-1} (CH), 1681cm^{-1} (C=N), 1551cm^{-1} (C=C), 705 cm^{-1} (C-Cl), 651 cm^{-1}(C-Br) **HNMR (DMSO, δppm):** 8.04 (s, 1H, CH=N), 6.93-7.88 (m, 11H, Ar-H), 8.05

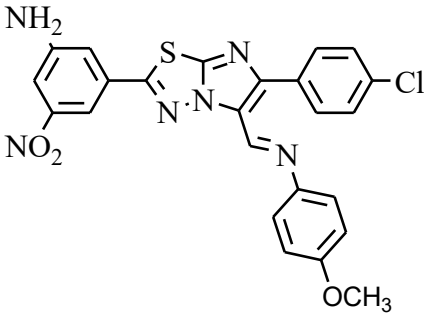
(s, 2H, Ar-H), .^{13}C NMR (DMSO-d_6) d $_{ppm}$: 147.4, 145.6, 136.4, 128.4 (imidazo[2,1-b][1,3,4]thiadiazole) 129.4, 125.6, 118.5 2-(3,5-dinitrophenyl)148.4, 134.8, 131.5, 124.6, 114.2 (5-methylidene aniline) 135.8, 135.5, 132.8, 133.8,133.6,131.8 (6-phenyl); HRMS (EI) m/z calcd for $C_{23}H_{14}BrN_6O_2S$: 517.11; found: 517.17

N-((6-(4-chlorophenyl)-2-(3,5-dinitrophenyl)imidazo[2,1-b][1,3,4]thiadiazol-yl)methylene)–4-nitroaniline(IIIE)



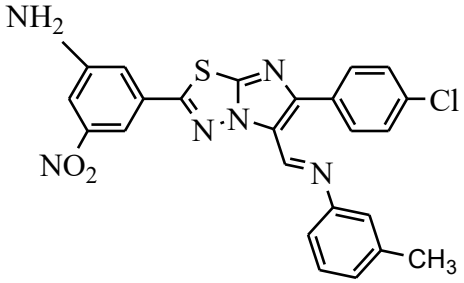
IR (ATR-FTIR, cm^{-1}): 3061cm^{-1} (CH), 1678cm^{-1} (C=N), 1581cm^{-1} (C=C), 1342 cm^{-1} (NO_2), 1249(C-O), 708(C-Cl),**HNMR (DMSO, δppm):** 8.62 (s, 1H, CH=N), 7.01-8.14 (m, 1H, Ar-H), 6.12(s, 2H, CH_2), .^{13}C NMR (DMSO-d_6) d $_{ppm}$: 148.4, 146.6, 145.4, 124.4 (imidazo[2,1-b][1,3,4]thiadiazole) 128.4, 126.6, 116.5 2-(3,5-dinitrophenyl) 158.4, 154.8, 121.5, 120.6, 114.2 (5-methylidene aniline) 137.8, 135.5, 132.8, 133.8,132.6,131.8 (6-phenyl); HRMS (EI) m/z calcd for $C_{23}H_{14}ClN_7O_4S$: 519.05; found: 518.04

N-((6-(4-chlorophenyl)-2-(3,5-dinitrophenyl)imidazo[2,1-b][1,3,4]thiadiazol-yl)methylene)–4-methoxyaniline(IIIF)



IR (ATR-FTIR, cm^{-1}): 3078cm^{-1} (CH), 1656cm^{-1} (C=N), 1511cm^{-1} (C=C), 1249(C-O), 728(C-Cl),**HNMR (DMSO, δppm):** 8.32 (s, 1H, CH=N), 7.01-8.14 (m, 11H, Ar-H), 3.19(s, 3H, OCH_3), .^{13}C NMR (DMSO-d_6) d $_{ppm}$: 158.4, 156.6, 145.4, 124.4 (imidazo[2,1-b][1,3,4]thiadiazole) 138.4, 134.6, 116.5 2-(3,5-dinitrophenyl) 153.4, 150.8, 121.5, 120.6, 114.2 (5-methylidene aniline) 137.8, 135.5, 132.8, 133.8,132.6,131.8 (6-phenyl); HRMS (EI) m/z calcd for $C_{24}H_{17}ClN_6O_3S$: 504.08; found: 508.08

(E)-N-((6-(4-chlorophenyl)-2-(3,5-dinitrophenyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl)methylene)-3-methylaniline (III G)



IR (ATR-FTIR, cm^{-1}): 1482.34cm^{-1} (Ar.C=C stretch), 3098.91cm^{-1} (Ar. C-H stretch), 2924.93cm^{-1} (Alkane C-H stretch), 1087.46cm^{-1} (C-N stretch), 1680.92cm^{-1} (C=N stretch), 619.42cm^{-1} (C-Cl stretch), 1545.19cm^{-1} & 1346.04cm^{-1} (NO_2 stretch),730.02cm^{-1} (C-S-C stretch).**^{1}HNMR (DMSO, δppm):** 9.04 (s, 1H, CH=N), 6.92-7.88 (m, 12H, Ar-H), 8.05 (s, 2H, Ar-H), 2.59 (s, 3H, Ar-H).^{13}C NMR (DMSO-d_6) d $_{ppm}$: 150.4, 145.6, 136.4, 122.4 (imidazo[2,1-b][1,3,4]thiadiazole) 129.4, 124.6, 118.5 **2-(3,5-dinitrophenyl)**148.4, 144.8, 131.5, 128.6, 114.2 (5-methylidene aniline) 135.8, 134.5, 132.8, 130.8 (6-phenyl) 24.3 (CH_3); HRMS (EI) m/z calcd for $C_{24}H_{17}ClN_6O_2S$: 488.95; found: 518.07.

Biological Activity

Antimicrobial activities test were performed on Agar plate diffusion methodon E. coli, P. aeruginosa and S. aureus. Ciprofloxacin was used as the standard antibacterial agents. The bacteria isolates were subcultured on nutrient agar plates and incubated at 37˚C for 24 h. The nutrient agar plates was incubated into a nutrient broth (50 ml) at 37˚C for 18 h with vigorous shaking. The bacterial strains were grown at 37˚C overnight and maintained on nutrient agar. Stock solution of the compounds were prepared in DMF at 50˚C to give a final concentrations; after pouring into plates and allow the agar to set, plates were inoculated with standardized inocula of the test bacteria, and further incubated at 37˚C for 24h under aseptic conditions.

The *in-vitro* antimicrobial activity of the compounds **(IIIA-G)** showed that the compounds were more active against gram positive bacteria as compared to gram negative bacteria. Compounds IIIE,IIIF and IIIG exhibited more activity against *S. aureus*. Compound IIIE showed more activity with zone of inhibition of 2.4cm and 1.4cm against *S. aureus* and *E.coli respectively.*

Compounds IIIE and IIIF contains the electronegative groups *i.e.* methoxy (-OCH3), and Nitro (-NO2) respectively which are active with zone of inhibition 1.8cm, 2.3cm, respectively. Compounds IIIA and IIIG contains electron releasing groups *i.e.* proton (-H) and methyl (-CH3)respectively which are active with zone of inhibition range 0.9cm and 1.5cm. So, the presence of electron withdrawing groups on the phenyl ring makes the derivatives more potent when compared with the derivatives containing electron releasing groups.

Table 2. *In-vitro* antimicrobial activity of synthesized compounds (IIIA-G)

Derivatives	Conc. (µg/ml)	Diameter of zone of inhibition (mm) against the bacterial Strains							
		Gram –ve bacteria *E. coli*				Gram +ve bacteria *S. aureus*			
		T1	T2	T3	Average	T1	T2	T3	Average

IIIA	100	-	-	-	-	-	-	-	-
	250	6	5	4	5	6	7	5	6
	500	7	6	7	6.666	7	10	11	9.333
IIIB	100	-	-	-	-	-	-	-	-
	250	8	9	7	8	10	9	7	8.66
	500	12	10	9	10.33	14	17	16	15.66
IIIC	100	7	6	8	7	6	6	7	6.33
	250	10	10	11	10.333	10	11	13	11.33
	500	11	13	12	12	17	20	19	18.66
IIID	100	9	7	8	8	19	17	17	17.66
	250	11	10	12	11	21	20	22	21
	500	14	12	13	13	23	22	25	23.33
IIIE	100	8.5	9	7.2	8.23	13	11	9	11
	250	9	10	12	10.33	22	19	21	20.66
	500	16	14	13.5	14.5	26	23	25	24.66
IIIF	100	9	11	11	7.333	10	12	11	11
	250	12	13	12	12.333	19	21	19	19.666
	500	16	14	12	14	22	21	23	22
IIIG	100	16	14	15	15	26	24	25	25
	250	14	15	18	15.666	14	17	16	15.666
	500	22	21	25	22.666	23	21	23	22.333
Standard	100	21	19	20	20	22	24	22	22.66
	250	24	23	26	24.33	24	26	25	25
	500	27	28	26	27	30	31	28	29.66

Conclusion

Novel designed compounds show appreciable growth-inhibitory activity against the bacteria *S. aureus* when compared with the standard compound ciprofloxacin. Maximum inhibition zone observed for compounds IIIF and IIIG as compared to standard drug at 500 µg/ml which is quite comparable. Compounds IIIE and IIIF contains the electronegative groups *i.e.* methoxy (-OCH_3), and Nitro (-NO_2)respectively which are active with zone of inhibition 1.8cm, 2.3cm, respectively. Compounds IIIA and IIIG contains electron releasing groups *i.e.* proton (-H) and methyl (-CH_3)respectively which are active with zone of inhibition range 0.9cm and 1.5cm. So, the presence of electron withdrawing groups on the phenyl ring makes the derivatives more potent when compared with the derivatives containing electron releasing groups. The novel compounds could further be optimized to improve the activity.

References

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