SYNTHESIS AND BIOLOGICAL ACTIVITY OF IMIDAZO[2,1-B][1,3,4]THIADIAZOLE DERIVATIVES

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Article History: Received: 02/09/22 Revised: 25/10/22 Accepted: 20/11/22

Abstract:
Imidazo[2,1-b][1,3,4]thiadiazoles are synthesized from the 2,4-dinitro benzoic acid and thiosemicarbazide. Their reaction with phenacyl (p-substituted phenacyl) bromides led to formation of the respective imidazolthadiazole derivative. . The structure of the above compounds was confirmed from their spectral characteristics. Some of these compounds were found to possess slight to moderate activity against the microorganisms Staphylococcus aureus, Candida albicans, Pseudomonas aeruginosa, and Escherichia coli.

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

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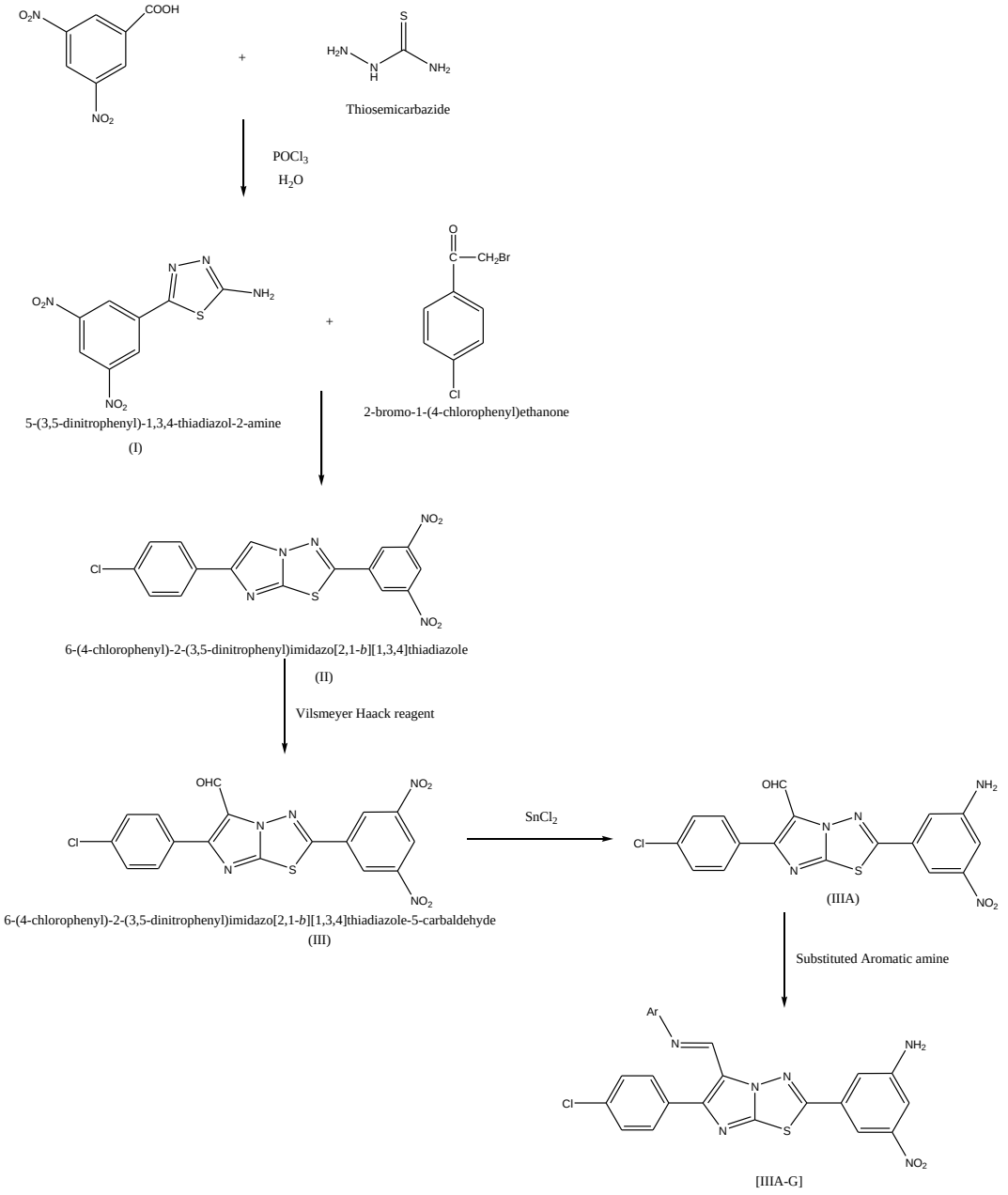
DOI: 10.53555/ecb/2022.11.12.281

Introduction

Synthesis of Imidazo[2,1-b][1,3,4]thiadiazoles(1) are heterocyclic systems in which imidazole and 1,3,4-thiadiazole rings are fused to each other via a bridgehead nitrogen atom. It was first discovered in the early fifties of nineteenth century. Numerous imidazo[2,1-b][1,3,4]thiadiazoles with various substitutions at C-2, C-5, and/or C-6 positions of the general structures have been synthesized and their biological activities have been reported extensively in the literature. 2-Amino-1,3,4-thiadiazole derivatives are well known as compounds of a wide range of antimicrobial activity [3] that have been prepared from their respective aldehydes [4] and then Schiff base .They appeared to be the most feasible route to fused imidazo[2,1-*b*]-1,3,4-thiadiazole rings [13] of interesting potential applications . Some of these compounds show anticancer antitubercular [4], antibacterial antifungal ,anticonvulsant analgesic , antisercetory and antiapoptotic properties. Also, certain compounds incorporating the imidazo[2,1-*b*]-1,3,4-thiadiazole moiety are used as anti-inflammatory , cardiotonic , diuretic and herbicidal agents and are used in the manufacture of dyes . With all this in mind, the present paper focused on the synthesis of some new fused imidazo[2,1-*b*]-1,3,4-thiadiazole rings and testing their reactivity towards electrophilic substitution reactions and against Staphylococcus aureus, Candida albicans, Pseudomonas aeruginosa, and Escherichia coli.

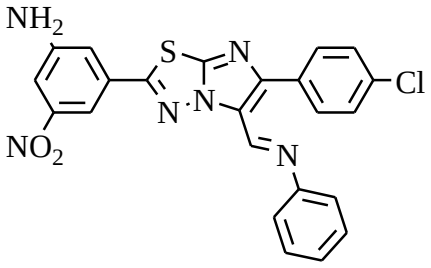
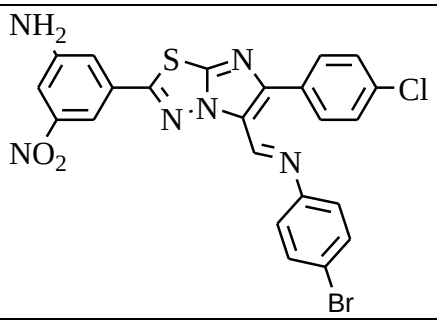
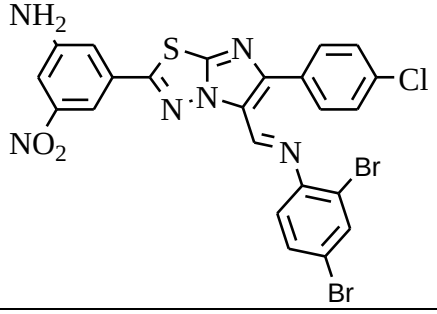
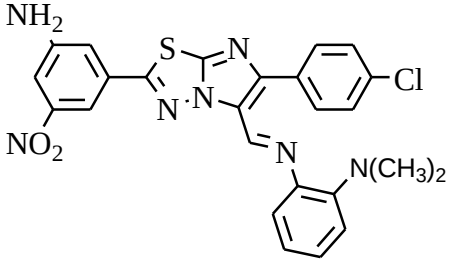
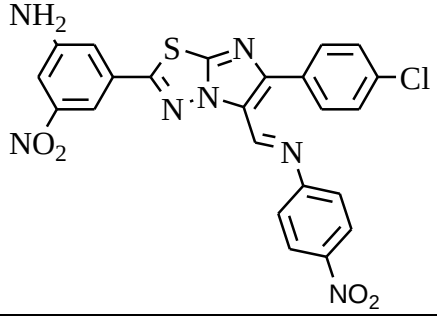
Reaction Scheme

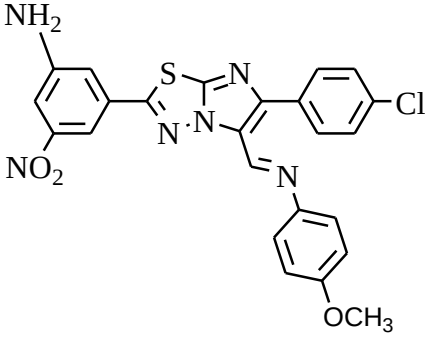
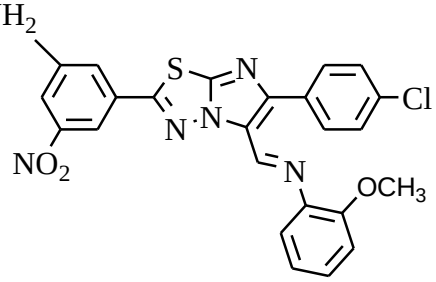


Chemistry

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.

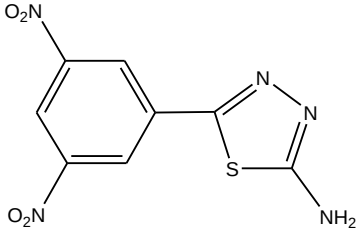
Table 1. Physical Data of synthesized compounds

Sr.No A	Compound	Molecular Formula	Molecular Weight	Yield
IIIA		$C_{23}H_{15}ClN_6O_2S$	474.07	44%
IIIB		$C_{23}H_{14}BrClN_6O_2S$	553.82	75%
IIIC		$C_{23}H_{11}Br_2ClN_6O_4S$	662.697	40
IIID		$C_{25}H_{20}ClN_7O_2S$	517.99	65
IIIE		$C_{23}H_{14}ClN_7O_4S$	519.92	73

IIIF		$C_{24}H_{17}ClN_6O_3S$	504.95	88
IIIG		$C_{24}H_{17}ClN_6O_3S$	504.95	45

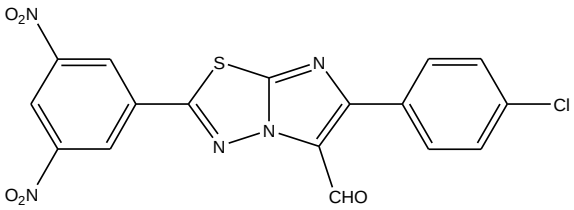
Experimental protocols

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



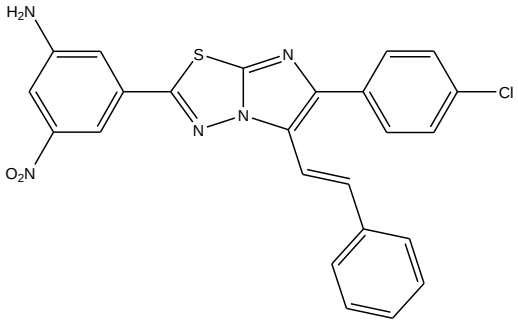
IR (KBr) ν_{max}**:** 1450.12cm^{-1} (Ar. C=C stretch), 3092.64cm^{-1} (Ar. C-H stretch), 1075.71cm^{-1} (C-N stretch), 1685.68cm^{-1} (C=N stretch), 3178.16cm^{-1} (N-H stretch), 1539.80cm^{-1} & 1345.05cm^{-1} (NO_2 stretch), 723.13cm^{-1} (C-S-C stretch), 2942.51cm^{-1} (C- H stretch). $_1$H NMR (DMSO-d_6) 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} (NO_2 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.

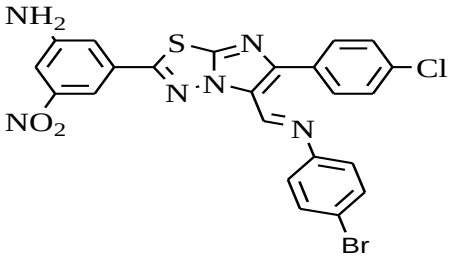
(E)-N-((6-(4-chlorophenyl)-2-(3,5-dinitrophenyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl)methylene)aniline (IIIA)



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} (NO_2 stretch), 1500cm-^{1} (NH_2 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,

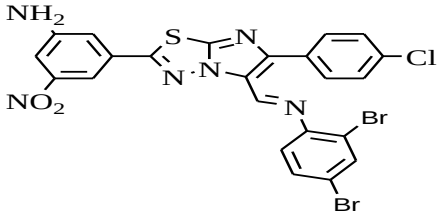
aromatic), 7.45 (t, 2H, aromatic), 7.06 (t, 1H, aromatic). 4.0(aromatic C-NH) ^{13}CNMR (DMSO-d_6) d $_{ppm}$**:** 136 (C1), 143.3 (C2), 146.0 (C3), 116.4 (C4), 134.3 (C5), 149.3(C6 & C7), 151.2 (C8), 135.3 (C9), 132.7 (C10), 129.3 (C11 & C17), 118.1 (C12), 128.9 (C13 & C15), 122.3 (C14 & C18), 131.6 (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)



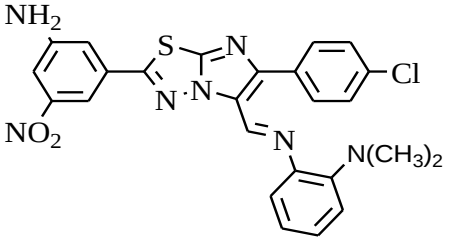
IR (ATR-FTIR, cm^{-1}): 3034cm^{-1} (CH), 1671cm^{-1} (C=N), 1551cm^{-1} (C=C), 708 cm^{-1} (C-Cl), 658 cm^{-1}(C-Br) **HNMR (DMSO, δppm):** 8.04 (s, 1H, CH=N), 6.93-7.88 (m, 11H, Ar-H), 8.05 (s, 2H, Ar-H), .^{13}C NMR (DMSO-d_6) d $_{ppm}$: 149.4, 145.6, 136.4, 128.4 (imidazo[2,1-b][1,3,4]thiadiazole) 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]thiadiazol yl)methylene)benzenamine(IIIC)



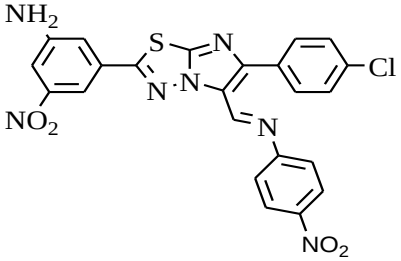
IR (ATR-FTIR, cm^{-1}): 3034cm^{-1} (CH), 1671cm^{-1} (C=N), 1551cm^{-1} (C=C), 705 cm^{-1} (C-Cl), 651 cm^{-1}(C-Br) **HNMR (DMSO, δppm):** 8.04 (s, 1H, CH=N), 6.93-7.88 (m, 11H, Ar-H), 8.05 (s, 2H, Ar-H), .^{13}C NMR (DMSO-d_6) d $_{ppm}$: 149.4, 145.6, 136.4, 128.4 (imidazo[2,1-b][1,3,4]thiadiazole) 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)



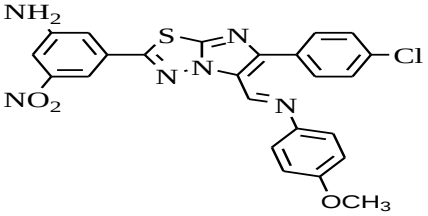
IR (ATR-FTIR, cm^{-1}): 3036cm^{-1} (CH), 1681cm^{-1} (C=N), 1551cm^{-1} (C=C), 705 cm^{-1} (C-Cl), 651 cm^{-1}(C-Br) **HNMR (DMSO, δppm):** 8.04 (s, 1H, CH=N), 6.93-7.88 (m, 11H, Ar-H), 8.05 (s, 2H, Ar-H), .^{13}C NMR (DMSO-d_6) d $_{ppm}$: 147.4, 145.6, 136.4, 128.4 (imidazo[2,1-b][1,3,4]thiadiazole) 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)



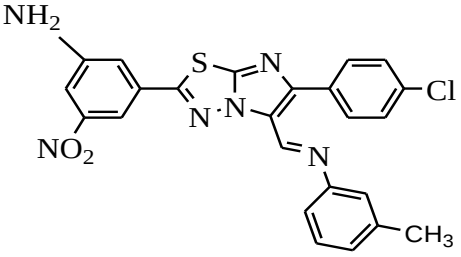
IR (ATR-FTIR, cm^{-1}): 3061cm^{-1} (CH), 1678cm^{-1} (C=N), 1581cm^{-1} (C=C), 1342 cm^{-1} (NO_2), 1249(C-O), 708(C-Cl),**HNMR (DMSO, δppm):** 8.62 (s, 1H, CH=N), 7.01-8.14 (m, 1H, Ar-H), 6.12(s, 2H, CH_2), .^{13}C NMR (DMSO-d_6) d $_{ppm}$: 148.4, 146.6, 145.4, 124.4 (imidazo[2,1-b][1,3,4]thiadiazole) 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 method on 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.

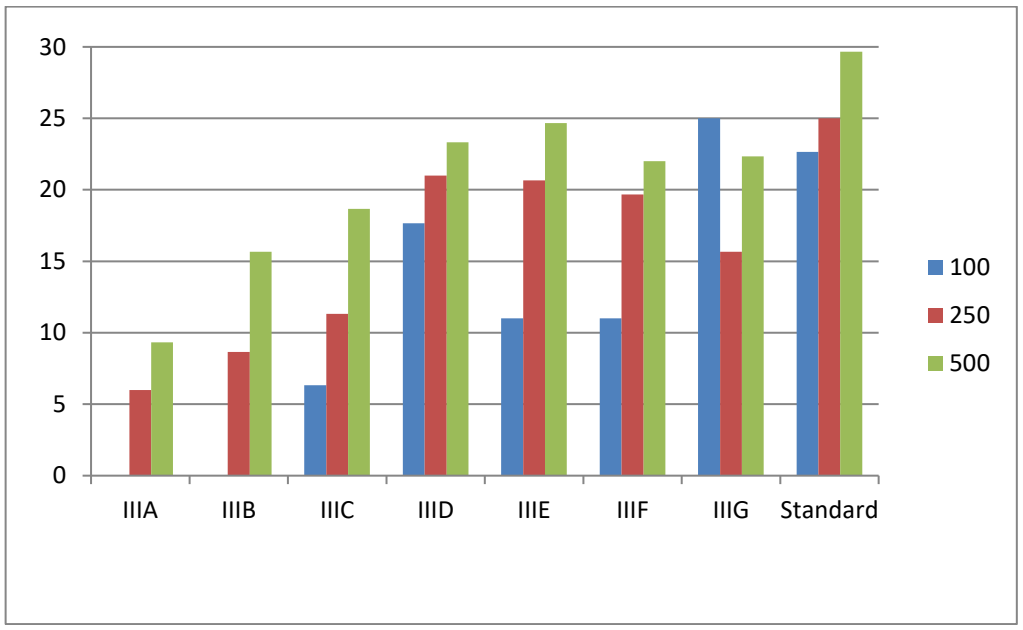
The *in-vitro* antimicrobial activity of the compounds **(IIIA-G)** showed that the compounds were more active against gram positive bacteria as compared to gram negative bacteria. Compounds IIIE,IIIF and IIIG exhibited more activity against *S. aureus*. Compound IIIE showed more activity with zone of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

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

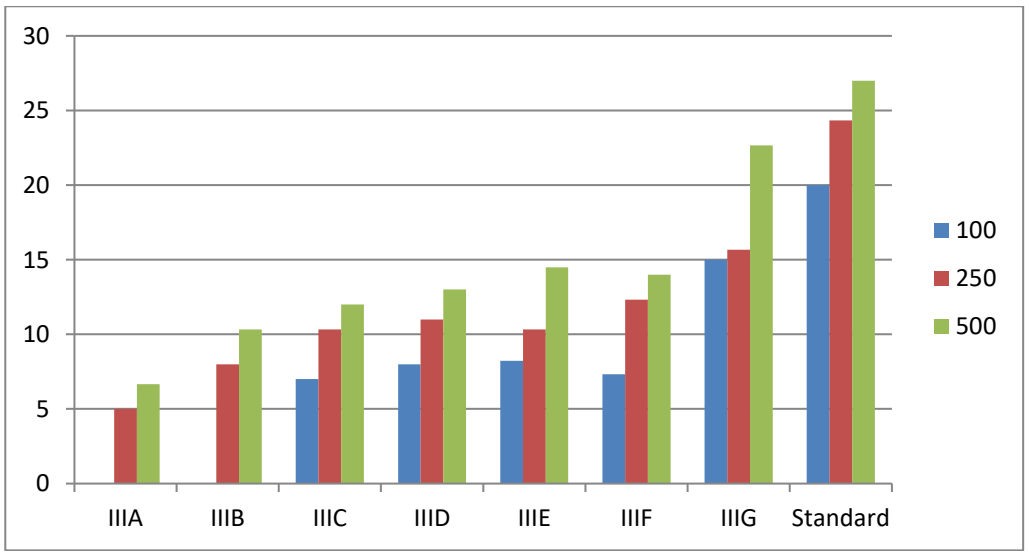
Derivatives	**Conc. (**μg/ml)	**Diameter of zone of inhibition (mm) against the bacterial Strains**							
		Gram –ve bacteria *E. coli*				**Gram +ve bacteria *S. aureus***			
		T1	T2	T3	Average	T1	T2	T3	Average
IIIA	100	-	-	-	-	-	-	-	-
	250	6	5	4	5	6	7	5	6
	500	7	6	7	6.666	7	10	11	9.333
IIIB	100	-	-	-	-	-	-	-	-
	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



S. aureus

Fig. 2.1Antibacterial activity of compounds (IIIA-IIIG) against gram positive bacteria



E. coli

Fig. 2.2Antibacterial activity of compounds (IIIA-IIIG) against gram negative bacteria

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.

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