SYNTHESIS, CHARACTERIZATION AND BIOACTIVITIES STUDIES OF 2-SUBSTITUTED-6-ARYL-IMIDAZO [2, 1-b] [1 ,3,4]-THIADIAZOLES

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ABSTRACT ***In this investigation our aim is to synthesized a number of new 2-substituted-6-aryl-imidazo [2, 1-b] [1 ,3,4]-thiadiazoles by the condensation of 2-Amino-5-isobutyl-l, 3, 4-thiadiazole and phenacyl halide. The synthesized compounds displayed different degrees of antimicrobial activity against bacterial strains viz. Bacillus spizinenii, Staphylococcus aureus, Escherichia coli, Salmonella typhi and fungal strains Aspergillus niger, Candida albicans.compound 3a, compound 3b and compound 3c exhibited potency against Bacillus spizinenii at value of 1000, 500 and 250 µg/ml respectively. Staphylococcus aureus is found to be susceptible for compound 3a, compound 3b and compound 3c at 125 µg/ml. Only compound 3b exhibited activity against Salmonella typhi at 250 µg/ml. Inversely, Escherichia coli demonstrated resistance against all respective compounds. Similarly, fungal cultures Aspergillus niger and yeast Candida albicans were highly resistant for all the tested compounds.***

Key words: Thieno-[2,3-d] pyrimidine, Antibacterial activity, Antifungal activity, Streptomycin, Fluconazole.

I.INTRODUCTION

Due to the rapid development of bacterial resistance to antibacterial agents, it is vital to discover novels scaffold for the design and synthesis of the new antibacterial agents to help in the battle against pathogenic microorganisms. Much research has been carried out with the aim to discover the therapeutic values of 1,3,4-thiadiazole derivatives.The development of 1,3,4-thiadiazole chemistry is linked to the discovery of phenyl hydrazines and hydrazine in the late nineteenth century. The first 1,3,4-thiadiazole was described by Fischer in 1882 but the true nature of the ring system was demonstrated first in 1890 by Freund and Kuh.

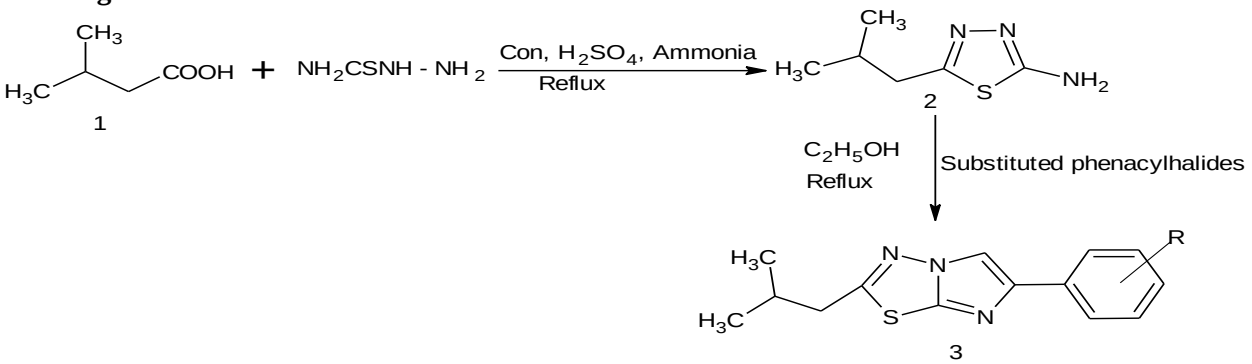
The imidazo[2,l-b][l,3,4]thiadiazole ring system is pseudoaromatic in behavior containing imidazole moiety as electron rich center. Chlprine^Bf 'bromine does not add to the double bond at 2,3-position. On the other hand, electrophilic substitution reactions like bromination, nitration etc. take place at 5-position.

It is well documented that 1, 2, 3-thiadiazoles undergo multiple transformations into a wide variety of products like, antimicrobial, anti-inflammatory,[1] anticonvulsant,[2] antituberculosis,[3] antifungal, antimycobacterial,[4] antiviral[5,6], fungicidal,[7] antibacterial,[8] antinociceptive,[9] antidepressant and anxiolytic,[10] etc.

Imidazo[2,1 -b] [1,3,4]thiadiazole. 2,5-disubstituted -1,3,4-thiadiazole derivatives arefound to possess diverse biological activities such as anti-inflammatory,[11,12] antimicrobial[13,14], antitubercular[15,16], anticonvulsant activities[17,18].

II.MATERIALS AND METHODS

Present working scheme



Experimental Section

Melting points were taken in open capillary tubes and are uncorrected. The purity of the compounds was confirmed by thin layer chromatography using Merk silica gel 60 F254 coated alumina plates. 1R spectra were recorded on a SHIMADZU-FTIR Infrared spectrometer in KBr (v max in cm1). 'H-NMR spectra were recorded in DMSO on an (400 MHz) spectrometer using TMS as internal standard.

General method of preparation of titled compounds

2-Amino-5-isobutvl-l, 3, 4-thiadiazole was prepared according to the literatur^ method (2)

Isovaleric acid (1 mmole) and thiosemicarbohydrazide (1 mmole) were taken in a round bottom flask. Cone. H2SO4 (50mL) was added. The reaction mixture was refluxed for three hours. Later the reaction mixture was cooled to room temperature and neutralized with ammonia carefully. The solid product so obtained was filtered, dried and recrystalised from ethanol and DMF mixture. Its melting point was in agreement with the literature value [m. p: 208-212].

Procedure for the preparation of 2-IsobutyI-6-aryl Imidazo(2, 1-6][1, 3, 4]thiadiazole (3a-3e)

2-Amino-5-isobutyl-l, 3, 4-thiadiazole (1) (lmmol) and phenacyl halide (lmmol) were taken in the round bottom flask. Ethanol (50 mL) was added and the reaction mixture was refluxed for three hours. Then the reaction mixture was cooled and poured in to ice cold water. The solid compound so obtained was filtered, dried and recrystallized form suitable solvent.

Table No. 1: Physical characterization of synthesized compound

Compound	R	Molecular Formila	Molecular Weight	% of Yield	Melting point (°C)
3a	4-Br	$C_{14}H_{14}N_3SBr$	335	71	136-141
3b	4-OCH_3	$C_{15}H_{17}N_3OS$	287	76	118-122
3c	4-C1	$C_{14}H_{14}N_3SC1$	291.453	67	121-126
3d	4-CH_3	$C_{15}H_{17}N_3S$	271	89	114-118
3e	H	$C_{14}H_{15}N_3S$	257	78	102-107

Characterization of synthesized compounds

Compound 3a (6-(4-bromophenyl)-2-(2-methylpropyl) imidazo[2,1-b] [1,3,4] thiadiazole)

Melting point 136-141°C; % of Yield: 71; **IR (cm $^{-1}$):** 3014 cm $^{-1}$(aromatic C-H stretch), 2953 and 2870 cm $^{-1}$(alkyl side cham), 1530,1462,1368 cm $^{-1}$ (C=N and C=C), 739 and 731 cm $^{-1}$ (C-Br).; **^{1}H NMR (DMSO):** 5 8.67 (s, 1H, imidazole), 7.79 (d, 2 H, ***J*= 8.4Hz**, pbromophenyl), 7.58 (d, 2H, ***J*= 8.4Hz**, p- bromophenyl), 2.92 (d, 2H, ***J*= 6.8Hz**, methylene protons of Isobutyl), 0.98 (d, 6H, ***J*= 6.8Hz**, 2 methylene protons of isobutyl), 2.01 to 2.11 (m, 1 H, isobutyl).; **Mass (**m/z)**:**335 96

Compound 3b (6-(4-methoxyphenyl)-2-(2-methylpropyl) imidazo[2,1- b] [1,3,4] thiadiazole)

Melting point (°C): 118-122; % of Yield: 76; IR (cm $^{-1}$): 2952 cm $^{-1}$ (C-H stretch), 1547, 1493, 1462 and 1450 cm $^{-1}$ (C=N and C=C); ^{1}H NMR(DMSO): δ 8.67 (s, 1 H, imidazole), 7.76 (d, 2H, *J*= 8.8 Hz, 4-methoxy phenyl), 6.96 (d, 2H, *J*= 8.8Hz, 4-methoxy phenyl), 2.90 (d, 2H, *J*= 7.2 Hz, methylene protons of Isobutyl), 0.98 (d, 6H, *J*= 6.8 Hz, 2 methylene protons of isobutyl), 2.01 to 2.11 (m, 1H, isobutyl), 3.78 (s, 3 H, -OCH_3).; Mass (m/z): 287

Compound 3c: 6-(4-chlorophenyl)-2-(2-methylpropyl) imidazo[2,1-b][1,3,4]thiadiazole

Melting point (°C): 121-126; % of Yield: 67; ^{1}H NMR(DMSO): δ 8.66 (s, 1H, imidazole), 7.86 (d, 2H, *J*= 9.2 Hz, 4-chlorophenyl), 7.45 (d, 2H, *J*= 9.2 Hz, 4-chlorophenyl), 2.923 (d, 2H, *J*= 7.2 Hz methylene protons of Isobutyl), 0.98 (d, 6H, two methyl groups), 2.03 to 2.10 (m, 1H, isobutyl).; Mass: m/z 291.453

Compound: 3d: 6-(4-methylphenyl)-2-(2-methylpropyl) imidazo[2,1-b][1,3,4]thiadiazole

Melting point (°C): 114-118; % of Yield: 89; ^{1}H-NMRfDMSO): δ 8.53 (s, 1H, imidazole), 7.72 (d, 2H, /= 8Hz, 4-methyl phenyl), 7.19 (d, 2H, J= 8 Hz, 4-methyl phenyl), 2.91 (d, 2H, methylene protons of Isobutyl), 0.98 (d, 6H, two methyl groups), 2.01 to 2.11 (m, 1H, isobutyl), 2.30 (d, 6H, - two methyl groups).Mass**:** m/z 271

Compound: 3e: 2-(2-methylpropyl)-6-phenylimidazo[2,1-b][1,3,4]thiadiazole

Melting point (°C): 102-107; % of Yield: 78;^{1}HNMRfDMSO): δ 8.60 (s, 1H, imidazole), 7.40 (t, 2H, phenyl), 7.84 (d, 2H, J= 9.6 Hz, phenyl), 7.27 (t, 1H, phenyl), 2.91 (d, 2H, J= 7.2 Hz methylene protons of Isobutyl), 0.98 (d, 6H, J= 6.4 Hz two methyl groups), 2.01 to 2.11 (m, 1H, isobutyl); Mass**:** m/z 257

Antimicrobial activity

Determination of the minimum inhibitory concentration (MIC):

The MIC was measured for evaluation of antimicrobial activity by disc diffusion method. All petri dishes (diameter 86 mm and 76 mm) were dry heat sterilized in a canister at 420 °C for four hours and micropipettes were calibrated with standard procedure. Media were steam sterilized at 121 °C (15 psi) for twenty minutes in an

autoclave. Bacterial and fungal cultures were grown at 37°C and 28°C, respectively on Mueller Hinton agar (MHA) and potato dextrose agar (PDA).[19] Candida albicans was grown on malt-extract and glucose-yeast extract-peptone broth (MGYP)[20] at 37°C. All the compounds were dissolved in DMSO of stock 1000 µg/ml. Standard solutions of antibacterial agent (Sreptomycin, 1000 µg/ml) and antifungal agent (Flucanazole, 1000 µg/ml) were taken as positive control and DMSO as negative control and the disc diffusion method was performed.[21] Dilution scheme as 500, 250, 125, 62.5, 31.25, 15, 7.5 µg/ml was followed. MIC values were interpreted by measuring zone of inhibition after incubation at 37 °C and 28 °C for bacterial, yeast and fungal strains, respectively.

Table No. 2: Antimicrobial activities (MIC µg/ml) of synthesized compounds(3a-c)

Compound	Minimum Inhibitory Concentration (µg/ml)					
	Anti-bacterial activity				Anti-fungal activity	
3a	125	1000	-----	-----	-----	-----
3b	125	500	-----	250	-----	-----
3c	125	250	-----	-----	-----	-----
3d	125	500		200		
3e	125	250		250		
Streptomycin	-----	250	250	-----	-----	-----
Flucanazole	-----	-----	-----	-----	-----	-----

III.RESULT AND DISCUSSION

The formation of lmidazothiadiazole (3a-c) was confirmed by IR, ^{1}H-NMR andDART- Mass spectral studies. The IR spectrum of the compound 3a showed the absence of absorption bands corresponding to NH_2group. This confirms the cyclization of the lmidazothiadiazole. The band appeared at 3014 cm $^{-1}$ was due to the aromatic C-H stretch. The absorption bands of alkyl side cham appeared at 2953 and 2870 cm $^{-1}$. The bands appeared at 1530,1462,1368 cm"1 were due to C=N and C=C groups. The band appeared at 739 and 731 was due to C-Br.

The 400 MHz ^{1}H-NMR spectrum showed a doublet at δ 0.98 with coupling constant J= 6.8 Hz integratmg for six protons of the two methyl groups. One proton of the isobutyl side chain appeared as a septet between δ 2.01 to2.11 The two protons of the methylene group appeared as a doublet at δ 2.92 with a coupling constant *J*= 7. 2 Hz and a singlet appeared at δ 8. 67 integrating for one proton of imidazole ring system. The four protons of 4-bromophenyl group appeared as two doublets at δ 7. 79 and 7. 58 each with a coupling constant *J*= 8.4 Hz respectively

The DART- Mass spectra of the compound 3a showed a molecular ion peak at m/z 335. 96 which is consistent with its molecular formula $C_{14}H_{14}N_3SBr$. It showed the isotopic peak at m/z 337

Similarly, the IR spectrum of compound 3b showed the absence of absorption bands corresponding to NH_2group and the carbonyl group of 4-methoxyphenacylbromide. This confirms the formation of imidazothiadiazole compound. The band appeared at 2952 cm $^{-1}$ was due to the aliphatic C-H stretch The bands appeared at 1547, 1493, 1462 and 1450 cm $^{-1}$ were due to C=N and C=C groups The 400 MHz ^{1}H-NMR spectrum showed a doublet at δ 0.98 with couplmg constant *J*= 6.8 Hz integrating for six protons of the two methyl groups One proton of the isobutyl side chain appeared as a septet between δ 2. 01 to 2.11 The two protons of the methylene group appeared as doublet at δ 2. 90 with a coupling constant J= 7 2 Hz and a singlet appeared at δ 8. 67 integrating for one proton of imidazole ring system. The four protons of 4-methoxyphenyl group appeared as two doublets at δ 7.76 and 6.96 each with a couplmgconstant *J*= 8. 8 Hz respectively

The DART- Mass spectrum of compound 36b showed a molecular ion peak at m/z 287 which is consistent with its molecular formula $C_{15}H_{17}N_3OS$.

The minimum inhibitory concentration (MIC) values of the screened, compound 3a, compound 3b and compound 3c exhibited potency against Bacillus spizineniiat value of 1000, 500 and 250 µg/ml respectively. Staphylococcus aureus is found to be susceptible for compound 3a, compound 3b and compound 3c at 125 µg/ml. Only compound 3b exhibited activity against Salmonella typhi at 250 µg/ml. Inversely, Escherichia coli demonstrated resistance against all respective compounds. Similarly, fungal cultures Aspergillus niger and yeast Candida albicans were highly resistant for all the tested compounds, despite the tested and standard compounds. The two bacterial cultures under study were found to be resistant to Sreptomycin while Aspergillus niger and Candida albicans did not show susceptibility to Flucanazole which is known antifungal agent (Results not observed). In general compound 3awas found to be potent against Staphylococcus aureus and Bacillus spizinenii.

IV.CONCLUSION

During an effort to discover new 2-substituted-6-aryl-imidazo [2, 1-b] [1 ,3,4]-thiadiazoles. analogues (3a-3e) with antimicrobial activity. The final synthesized compounds were characterized by spectral data (IR, ^{1}H-NMR, ^{13}C-NMR, HRMS). Both the synthesis and antimicrobial activity of the final compounds 3a–3e are for the first time. The compounds 3a–e were found to have reasonable activity against S. aureus. The most active compound was 3a. Further investigations like SAR and mechanism of action are in progress.

REFERENCES

1. Adnan, A. K., Nasser, R. E, Omar, A. A., Elsayed, E. H., Tarek, M. I. Ali, A. E. "Synthesis, antimicrobial, and anti-inflammatory activities of novel2-(1- adamantyl)-5-substituted-1,3,4-oxadiazoles and 2-(1-adamantylamino)-5- substituted-1,3,4-thiadiazoles". Eur. J. Med. Chem. 2007, 42, 235-242.
2. Chapleo, C. B., Myers, P. L., Smith, A. C. B., Stillings, M. R., Tulloch, I. F. Walter, D. S. "Substituted 1,3,4-thiadiazoles with anticonvulsant activity. 4. Amidines" J. Med. Chem. 1998, 31: 7-11.
3. Forumadi, A., Soltani, F., Jabini, R., Moshafi, M., Rasnani, M. F. "Syntesis and evaluation of *in vitro* antituberculosis activity of 2-(5-nitro-2-furyl) - and 2-(1- methyl-5-nitro-1*H*-imidazol-2-yl)-1,3,4-thiadizole derivatives" Arch. Pharm. Res. 2004, 27, 502-506.
4. Gulerman, N. N., Rollas, S., Erdeniz, H., Kiraz, M. "Antibacterial, antifungal and antimycobacterial activities of some substituted thiosemicarbazide and 2, 5- disubstituted 1,3,4-thiadiazoles" J. Pharm. Sci. 2001, 26: 1- 5.
5. Holla, B. S., Akberali, P. M. and Shivananda, M. K. "Studies on nitro phenyl furan derivatives: part XII. Synthesis, characterization, antibacterial and antiviral activities of some nitrophenyl furfurylidene- 1,2,4-triazolo [3, 4-*b*]-1,3,4- thiadiazines" Farmaco. 2001, 56: 919-927.
6. Marina, K., Anastssia, M., Panagiotis, M., Nicole, P., Spyroula, P. G., Christophe, P. Myriam, W., Erik, D. C. "Synthesis and antiviral activity evaluation of some new 6-substituted 3-(1-adamantyl) - 1,2,4- triazolo [3, 4-*b*][1,3,4] thiadiazoles" Farmaco. 2002, 57, 253-257.
7. Niewiadomy, A., Matysiak, J. "Fungicidal evaluation of substituted 4-(1,3,4-thiadiazol-2-yl) benzene-1, 3-diols" Pestycydy/Pesticides, 2010, 1-4, 5-14.
8. Qandil, A. H., Tumah, H. N., Hassan, M. A. "Synthesis and Antibacterial Activity of N-Benzoyl-N-dihydroxy-benzoylthiosemicarbazides and Their Cyclic 1,3,4-thiadiazole Derivatives" Acta PharmaceuticaSciencia. 2006, 48: 95-107.
9. Onkol, T., Cakir, B., Sahin, M.F. "Synthesis and antinociceptive activity of 2- [(2-oxabenzothiazolin-3-yl)-methyl]-5-aminoalkyl/aryl-1,3,4-thiadiazole" Turk. J. Chem. 2004, 28, 461-466.
10. Clerici, F., Pocar, D., Guido, M., Loche, A., Perlini, V.; Brufoni, M. "Synthesis of 2-amino-5-sulphonyl-1,3,4-thiadiazole derivatives and evaluation of their antidepressant and anxiolytic activity" J. Med. Chem. 2001, 44, 931-936.
11. Mohammad Asif and Chhavi Asthana, I. *J. Chem.Tech. Res.*, 1,4, 1200, 2009.
12. Plaska. E, Shahin. G, Kelicen. P, Duslu. T. N, Altino. G, *II Farmaco*, 57,101, **2002**.
13. Thomasco. M. L, Godwood. C. R, Weaver. A. V, Ochoada. M. J, Ford. W. C, *Bioogg. Med. Chem. Lett*, 13,4193, 2003.
14. Wadoker. N. K and Dhiman. M. A, *Ind. J. Chem*, 40B, 636,2001.
15. Joshi. S. H and Thaker. M. K, *Ind. J. Chem*, 44B, 410,2005.
16. Foroumadi. A, Firzaei. M, Shafiee. A, *II Farmaco*, 56,621,2001.
17. Dogan. N. H, Duran. A, Rollas. S, Sener. G, Vysal. K. M, Gulen. D, *Bioorg Med Chem.*, 10,2893, 2002.
18. Foroumadi. A, Sheibani, V, Sakhteman. A, Ramesh. K. M, Abbasi. M, Farazifard. R, Tabatabai. S. A, Shafiee A, *Darn*, 15,2,2007.
19. European committee on antimicrobial susceptibility testing (EUCAST). Disk Diffusion Method for Antimicrobial Susceptibility Testing-Version 4.0, 2014.
20. Murray P. R., Baron E.J., Jorgensen J.H., Landry M.L. and Pfaller M. A., 2, 1972- 1986, 2007.
21. Murray P. R., Baron E. J., Jorgensen J. H., Landry M. L. and Pfaller M. A., 2, 1152- 1173, 2007.