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Synthesis and antimicrobial evaluation of fused heterocyclic compound [1,2,4] triazolo [4,3-*b*] [1,2,4,5] tetra zine

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ABSTRACT

Nitrogen-containing, heterocycles, have special importance and vital, role in the discovery of effective bioactive, agents in the pharmaceutical, industry. The present article reports the synthesis of new fused, heterocycles triazolo-tetrazine by cyclo condensation, reaction as shown in scheme (1). The structures formula of synthesized compounds newly was evaluated by Ft-IR,1H-NMR spectrum, and C, H, N elemental analysis. Antimicrobial activity of triazolotetrazine studied against some pathogenic bacterial strains isolated from patients like Acinetobacter, Aeromonas, E. coli, Klebsiella, Staphylococcus, Streptococcus. Eventually, antibacterial of the fused heterocyclic compound was exhibited significant growth inhibition against some pathogenic bacteria which consider an important source of new antimicrobial compounds. The results, of such studies, are discussed in this paper.

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INTRODUCTION

In modern years, fused heterocycles rings have received considerable attention due to their great importance in agricultural and pharmacological fields.

The chemistry of triazolestetrazine has received significant attention owing to their synthetic and effective biological importance. with diverse industrial, agricultural and biological activities (Gümüs *et al*., 1989; Vinícius, R.C. *et al*., 2009; Prasad, A.; 1989) including, anticancer (A.Y. Hasssan *et al*.,2015) anti-microbial (El-Masry, A.H *et al;* 2000, Orabi, A.S *et al*; 2000) sedative, anti-convulsant (Almasirad, A *et al.*, 2004) and anti-inflammatory properties (George T *et al;* 1971)

Tetrazine is a benzene-like molecule in which the four CH units are replaced by four electronegative nitrogen atoms. There are three possible isomers, 1,2,3,5-tetrazine, 1,2,3,4-tetrazine and 1,2,4,5-tetrazine as shown in figure (No.1). The latter two play vital role and have been studied synthetically. (Pushpa Bhardwaj *et al*.; 2016).

For this reason, much attention has been directed in our laboratory for the synthesis of new five-member ring [1,2,4] triazole nucleus fused with a six-member heterocyclic ring [1,2,4,5] tetrazine to produced triazolotetrazine in order to study their antimicrobial activity.

MATERIAL AND METHODS

Chemical Materials

Benzo hydrazide, Hydrazine, Carbon disulfide, Potassium Hydroxide, Ethanol, 4-Hydroxybenzaldehyde, Lead acetate, Chloroform, hydrochloric acid, Hexane, Methanol, Ethyl acetate, Glacial acetic acid, Iodine.

METHODS

Synthesis of 4-amino-5-phenyl-4*H*-1,2,4-triazole-3-thiol(I): A mixture of, 0.2 moles (27.2 gm) of, benzo hydrazide with, KOH (5.6 gm, 0.1mole),

CS2(12 ml, 0.2 moles) and 0.4 moles of(20ml) Hydrazine, hydrate, was refluxed, in 100 ml of absolute ethanol for 7hr. The resultant, mixture was concentrated, a crystallised precipitate of 4-amino-5-phenyl-4H-1,2,4-triazole-3-thiol (I). The purity of the compound was checked by TLC technique, RF Value 0.61 of Eluent Ratio 7:3(ethanol: hexane) (Bassam A. Hasan *et al.;* 2014). And The spot pigment was detected visually in an Iodine chamber, the yield: (66.8%) and melting point M.P. 217-219 °C.

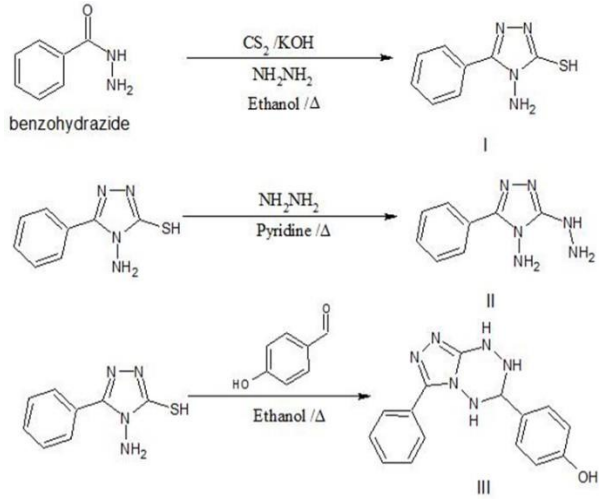

Figure 1: Synthesis of triazoles tetrazine

Synthesis of 3-hydrazino-5-phenyl-4*H*-1,2,4-triazol-4-amine(II): A mixture of (4g, 0.0208 moles) of synthesized compound (I) and hydrazine, hydrate (6 mL, 0.124 moles) in pyridine (50) were refluxed 5h in a 250 mL flask, fitted with a condenser and a guard tube. After the completion of the reaction, the solvent and excesses of hydrazine hydrate were removed under reduced pressure using rotary evaporator. The residue was washed with diethyl ether and recrystallised pyridine, ethanol. The white precipitate which separated was filtered and recrystallised from ethanol to give a pink precipitate of 4-amino-5-phenyl-4H-1,2,4-triazole-3-thiol, the purity of the compound was checked by TLC technique, RF Value 0.82 of Eluent Ratio 7:3(ethanol: hexane). And The spot pigment was detected visually in an Iodine chamber (Bassam Abdul Hussein Hasan Alsafee *et al.;* 2014), Yield (85.8%) and melting point M.P. 223-224 °C.

Synthesis of [1,2,4] triazolo [4,3-*b*] [1,2,4,5] tetrazine (III): An equimolar mixture of synthesized compound(III) (2.31g, 10mmol) and 4-hydroxybenzaldehyde (1.4g, 10mmol) in alcoholic KOH [(0.56 g, 10mmol) KOH in 30 ml ethanol] was heated under reflux for 8 h. The reaction mixture was allowed to cool and then poured on to crushed ice with scratching (A.Y. Hasssan; *et al.;* 2014). The resulting solid was filtered off and recrystallised from acetone. Shiny yellowish crystal obtained; yield (63%); m.p 189-191°C. RF Value 0.76 of Eluent Ratio 7:3(ethanol: hexane)

RESULTS AND DISCUSSION

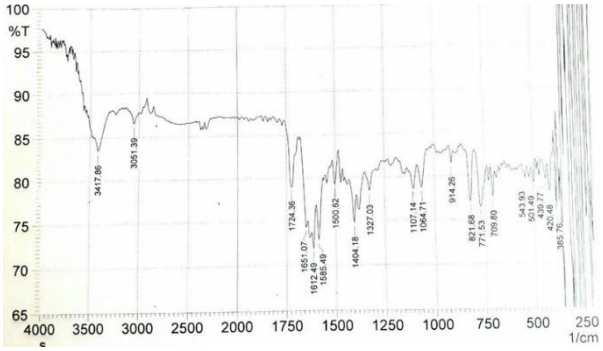

Figure 2: Ft-IR spectrum of 1,2,4-triazol-4-amine (II)

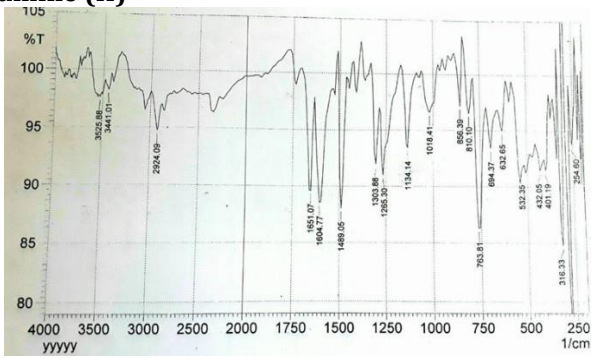

Figure 3: Ft-IR spectrum of triazolotetrazine (III)

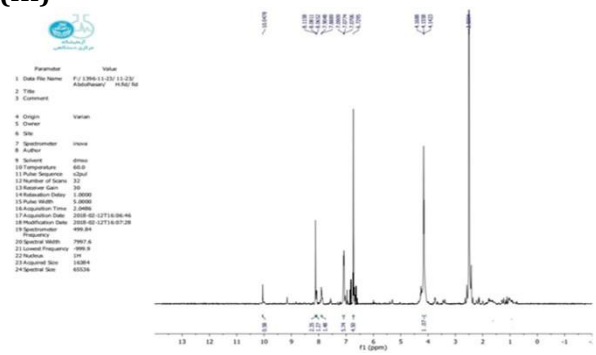

Figure 4: 1H- NMR spectrum of triazolo-tetrazine (III)

In this article, we have synthesized derivatives of 1,2,4-triazole-3-thiol(I), 3-hydrazinyl-1,2,4-triazol-4-amine(II) and triazolo triazine. The structures of the prepared compounds have been supported by elemental analyses CHN, IR and 1H NMR studies. The physical properties which included molecular formula and melting points and are shown in tables No (1,2). The IR spectrum of(II) compound due to NH_2 group it showed the absorption band at 3417 cm-1 and showed 3051 cm-1 corresponding to CH cm-1 of aromatic ring (Bassam Abdul Hussein Hasan Alsafee *et al;*2015 (Najim *et al;* Bassam Abdul Hussein Hasan Alsafee: *et al;* And Maitham Mohamed Abdulridha: *et al;* 2016).

Table 1: physical properties, Yield, Rf, and Molecular weight data of the compound

Sr.NO	Formula	M.Wt	Color	M.P °C	Yield %	Rf Value
I	$C_8H_8N_4S$	192	Light pink	217-219 °C	66.8 %	0.61
II	$C_8H_{10}N_6$	190	Pink	223-224 °C	85.8 %	0.82
III	$C_{15}H_{14}ON_6$	312	Shiny yellow	189-191 °C	63 %	0.76

Table 2: Elemental analysis of triazolestetrazine

Sr.No	Formula	C	H	N
I	$C_8H_8N_4S$	%Theoretical data		
		49.98%	4.19%	29.14%
		% Practical data		
		49.83%	4.24%	29.28%
II	$C_8H_{10}N_6$	%Theoretical data		
		50.52%	5.30%	44.18%
		% Practical data		
		50.44%	5.42%	44.25%
III	$C_{15}H_{14}ON_6$	%Theoretical data		
		61.21%	4.79%	26.58%
		% Practical data		
		61.76%	4.73%	27.5%

Table 3: FT-Ir spectra data of II, III

Comp. Sym.	O-H cm-1	N-H cm-1	C-H Aromatic cm-1	C-H aliphatic cm-1	C=N cm-1
II	-	3417	3051	-	1612
III	3525	3441	3015	2924	1604

Table 4: The effect of II, III and Cefuroxime on the growth of bacteria in vitro against some pathogenic bacterial strains isolated from patients

Types of the bacteria	Diameter zone of inhibition (mm)		
	Antimicrobial Agent		
	Traiazole (II) mm	Traiazolotetrazine (III) mm	Cefuroxime mm
Streptococcus	14	29	9
Acinetobacter	16	35	12
E.coli	11	23	2
Klebsiella	22	32	7
Staphylococcus	28	37	18
Aeromonas	26	31	11

The IR spectrum of target compound triazolotetrazine showed absorption band at 3441 cm-1 and 3015 cm-1 corresponding to NH and CH of aromatic ring consequently and showed new absorption band at 3525 cm-1 and 2924cm-1 corresponding to OH cm-1 of aromatic ring and CH of aliphatic six member heterocyclic ring (tetrazine) (Bassam Abdul Hussein Hasan Alsafee, *et al;* 2017, Maitham M. Abdulridh, *et al;* 2017); shown in Figures (2,3) that is good evidence traizole fused with tetrazine as showed in table No 3. The 1H NMR spectrum of The target compound triazolotetrazine are shown in Figures (4) showed singlet signal at δ 10.0 ppm due to OH proton. The aromatic region appears as multiples signal within the range (7.08– 8.21) ppm attributed to the aromatic ring of phenol molecule and phenyl group of the triazole compound. The NH groups of tetrazine ring appeared at 6.5-6.8, and finally, CH-aliphatic of six heterocyclic ring tetrazine appeared at 4.1(B. N. Berad *et al.;* 2017). The integration numbers of the ^1H – NMR of spectral(III) showed a good identification with the structural formula of 1,2,4 triazolotetrazine.

Minimum Inhibition concentration solution preparation: The MIC solution of synthesis 1,2,4-triazol- 4-amine derivatives(II) and triazolotetrazine (III) it was done by dissolving 0.5 gm in 10 ml of ethanol to get a 50 mg/ml which was the minimum inhibition, concentration tested as shown in the table (No4). Sterilization was done by, filtration wares through a Millipore 0.45 mm and 0.22 mm.

Antibacterial activity: In the vitro antibacterial activity of (II) and (III) against some pathogenic bacteria, strains isolated from, patients using agar cup method. (Streptococcus, Staphylococcus, Acinetobacter, Aeromonas, E. coli, and Klebsiella) (Bassam A. Hassan *et al.;* 2018, Uday Abdul- Reda Hussein, *et al.;* 2017 and Firas f. Alyaseen *et al.;* 2018). The results are summarized in table No4

and figure No 2 — types of the bacteria Diameter zone of inhibition(mm)Antimicrobial.

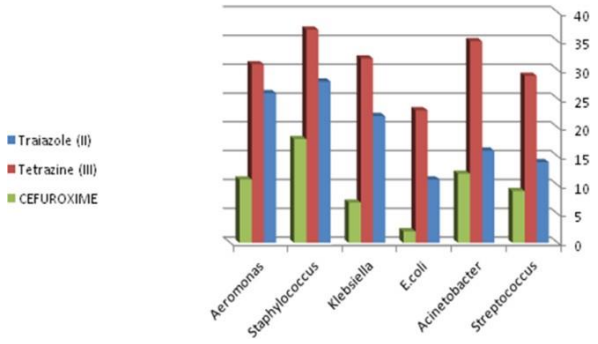

Figure 5: Traiazolotetrazine (III) Graphical representation between of Triazole derivative (II) and Cefuroxime on the growth of bacteria in a vitro against some pathogenic bacterial strains isolated from a patient

CONCLUSION

In the present work, triazolotetrazine have been synthesized and characterized based on FTIR,1HNMR and elemental analyses. According to all and physiochemical measurements as the prepared compound, we can suggest the chemical structure of the synthesized compound (I, II, III) were successfully synthesized. Antimicrobial activity of synthesized compounds Triazole derivatives and **-**triazolotetrazine tested against some pathogenic bacterial strains isolated from patients like Streptococcus, Acinetobacter, E.coli, Klebsiella, Staphylococcus, Aeromonas and compared with Cefuroxime drugs Eventually, Antimicrobial activity test found the compound containing fused heterocyclic molecule triazolotetrazine exhibited enhanced in a pharmacological activity significant than non-fused heterocyclic compound triazole derivative(II) and Cefuroxime as summarized in table No.4 and figure No.4 which indicate the fused between two heterocyclic rings will increase the pharmacological activity.

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