Antimicrobial Activities of Newly Synthesized Monoazaphenothiazine Derivatives.

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Abstract
The antimicrobial activity of newly synthesized linear monoazaphenothiazine derivatives; 3-benzamido-1-azaphenothiazine, 3-trifluoromethamido-1-azaphenothiazine, 3-trichloromethanamido-1-azaphenothiazine and 3-(4-nitrobenzamido)-1- azaphenothiazine have been evaluated against bacteria and fungal strains. Results showed that most of the linear monoazaphenothiazine derivatives were significantly active against the tested microorganisms. The correlation between antimicrobial activity and the chemical structure of phenothiazines was discussed.
Keywords: *phenothiazine; monoazaphenothiazine,* microorganisms, antimicrobial, structure – activity relationship.

1.0 Introduction

The chemistry of nitrogen-sulfur heteroatom containing aromatic compounds is becoming more popular as an area of research. Phenothiazines are heterocyclic molecules containing two benzene rings linked in a tricyclic system containing nitrogen and sulphur atom [1]. A slight variation in the substitution pattern on the phenothiazine nucleus often causes a marked difference in activities, this means the introduction of new substituent into the phenothiazine skeleton as well as the modification of the tricyclic ring system alters biological activities [2]. These moieties have shown diverse biological activities including tranquilizers [3], anti-inflammatory [4], antimalarial [5], antipsychotic [6], antimicrobial [7], antitubercular [8], antitumour [9] etc. It has been reported that some phenothiazine derivatives inhibit intracellular replication of viruses including human immunodeficiency viruses (HIV) [10-11]. Also, some of the derivatives of phenothiazine have been reported to exhibit significant anticancer activities [13-14]. Although, some successful preparations of the linear and angular branched system have been reported, [15-17] there are still few reports on the anti-microbial properties of

phenothiazine derivatives. The authors here report the successful screening of some synthesized derivatives of linear monoazaphenothiazines.

2. 0 Experimental

All the reagents used in this study were of Analytical Grade (AR) with the highest level of purity and were used without further purification. All the amidation reactions were carried out under a nitrogen atmosphere. 2, 3, 5-trichloropyridine, 2- aminothiophenol, trichloroacetamide, trifluroroacetamide, benzamide, nitrobenzamide, potassium trioxocarbonate(IV), *ter*-butylhydroxide, nickel(II)chloride, ethyl acetate and triphenylphosphine ligand were all purchased from Zayo-Sigma Chemicals, Germany in sure-seal bottles and were used as received. Distilled water was degassed by 30 sec sonication under vacuum. The synthesized 3-amido-derivatives were purified by column chromatography using Merck's Silica Gel (60-230 Mesh). Melting points of prepared analogues were determined with a Fisher-Johns apparatus and were uncorrected.

2.1. In vitro antimicrobial activity

2.1.1. Paper disc diffusion technique

All prepared analogues were assessed for in vitro antimicrobial activity by a paper disc diffusion method using Muellere Hinton agar and Sabouraud dextrose agar culture medium [18]. These analogue anti-microbial activities were calculated from the zone of inhibition against a panel of gram-positive, gram-negative and fungal organism such as ***S. Aureus*, *B subtitis, E. coli, P. Aeruginosa, A niger and C. albican*** etc., Different concentration of prepared analogues and standard drug in ethanol were placed in the culture medium using impregnated paper discs and incubated at 37 ^{O} C for 48 h. Here, Ciprofloxacin and ketoconazole were considered as standard

for antibacterial and antifungal activity respectively. The solvent control was kept separately in the Petri dish.

2.1.2. Minimum inhibitory concentration

Minimum inhibitory concentration (MIC) is the lowest concentration of prepared analogues found to inhibit the visible growth of a particular microorganism after a specified period of incubation in an appropriate culture medium. The MIC of prepared analogues was determined by solid dilution method using a series of petri plate containing a suitable culture medium. A highly potent analogue inhibits the detectable growth of the microorganism even at the least concentration. Whereas an antimicrobial agent called as less effective needs high concentration to kill the micro-organism. Here, the anti-microbial activities of different concentrations of the prepared analogues (compound 1-4) in ethanol were calculated against different micro-organism by incubating them for 48 h at 37 ^{O}C [19].

Table 1: Zone of inhibition of synthesized compound against different micro-organism (mm).

Compound(s)	Gram Positive Bacteria		Gram negative bacteria		Fungi	
	S. aureus	*B.subtilis*	*E. coli*	*P. aeruginosa*	*A. niger*	*C. albican*
1	**15**	**24**	**20**	**10**	**-**	**-**
2	**24**	**22**	**27**	**05**	**-**	**24**
3	**26**	**16**	**32**	**16**	**18**	**21**
4	**28**	**21**	**31**	**36**	**24**	**19**
CPFX	**30**	**27**	**32**	**34**	**-**	**-**
KTNZ	**-**	**-**	**-**	**-**	**22**	**24**
ETHANOL	**-**	**-**	**-**	**-**	**-**	**-**

Ciprofloxacin (CPFX) = reference drug for bacteria.
Ketoconazole (KTNZ) = reference drug for fungi.
Compound 1 : 3-benzamido-1-azaphenothiazine
Compound 2 : 3-trifluoromethamido-1-azaphenothiazine,
Compound 3 : 3-trichloromethanamido-1-azaphenothiazine
Compound 4 : 3-(4-nitrobenzamido)-1- azaphenothiazine

Table 2: Results of the Minimum Inhibitory Test (Summary of the antibacterial and antifungal effects of synthesized compounds (concentrations in μg/ml))

Compounds	***S. Aureus***	***B subtitis***	***E. coli***	***P. Aeruginosa***	***A niger***	***C. albican***
1	20	05	16	32	-	-
2	08	08	05	16	03	03
3	05	14	03	04	06	04
4	04	16	02	02	02	08
Ciprofloxacin	03	04	03	-	-	-
Ketoconazole	-	-	-	-	03	05

3.0 Results and Discussion

For the gram-negative bacteria, the activity against *P. aeruginosa,* were highest for compounds 3 and 4 with corresponding MIC values of 4 ug/mL, and 2 mg/mL and zone of inhibition of 34 mm and 36 mm respectively. The activity of compound 3 and 4 against *E. coli* was also quite amazing; compound 3 and 4 have MIC values of 3 ug/mL, and 2 mg/mL and zone of inhibition of 31 mm and 32 mm respectively. For the gram-negative bacteria, the activity against *S. aureus,* were highest for compounds 2, 3 and 4 with corresponding MIC values of 8 ug/mL, 5 ug/mL and 4ug/mL and zone of inhibition of 24 mm, 26 mm and 28 mm respectively. Compound 1 however was active against the bacteria strain but at a very high concentration 20 ug/mL and zone of inhibition of 15 mm respectively. For the fungal test organisms, the activity against *A.niger* was maximal for Compounds **2** and **3** with an MIC value of 3ug/mL and 6 ug/mL whereas Compound **4** showed the highest activity against A. *niger* with an MIC value of 2 ug/mL. Compounds **1** showed no activity against the fungal strain. The activity against *C. albican* was maximal for Compounds **3** and **4** with an MIC value of 4ug/mL and 8 ug/mL. Compound **4** showed the highest activity against the fungal strain with MIC and zone of inhibition of 4 2 ug/mL and 21 mm respectively. Compounds **1** and **2** showed no activity against

the *C. albican*. In vitro antimicrobial results of the entire synthesized compounds were summarized in Tables 1 and 2.

3.1 Structure activity relationship

The newly synthesized compounds were screened for in vitro antimicrobial activity against a panel of gram-positive, gram negative and fungal organism. From the result, the following structural activity relationship was derived. The tested phenothiazine derivatives were found to be active, highly potent and promising activity against different micro-organism. These compound antimicrobial activities were compared with Ciprofloxacin and Ketoconazole at the same strength. The structural activity relationship study suggests that substituted phenothiazine are needed for the significant anti-microbial activity. Compounds having an electron withdrawing group on the phenyl ring attached to thiazole are responsible for excellent anti-microbial activity compared to compounds linked with unsubstituted and electron donating group. Compounds 2, 3 and 4 were substituted with trifluro (CF_3), trichloro (CCl_3) and the nitro group at the meta position have shown the highest degree of antimicrobial activity against gram-positive, gram-negative and fungus organism. Commonly, unsubstituted or electron donating group on the phenyl ring attached to thiazole decreases the anti-microbial activity.

Conclusion

The synthesized derivatives showed varying activities against the cultured bacteria and fungi strains used. Interestingly, compound **3** and **4** were more active than the referent drug against *E. coli, P.aeruginosa.* However, some of the synthesized analogues were less active when compared with standard antibacterial (Ciprofloxacin). It is imperative to note that the standard antifungal drug (Ketoconazole) showed less activity against *A. niger* when compared to compounds **3** and **4**. Compound **2** have equal anti-fungal activity against *C.albican.* It is therefore concluded that

the compounds which possess higher activities should be recommended for further preclinical screening which could be useful in combating the bacterial and fungal infections.

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