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SYNTHESIS AND CHARACTERIZATION OF SUBSTITUTED 5-([1,2,4] TRIAZOLO [3,4-B][1,3,4] THIADIAZOL-3-YL)-1, 3-BENZOXAZOLE DERIVATIVES AS ANTIMICROBIAL AGENTS

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ABSTRACT

In this study, a series of novel substituted 5-([1,2,4] triazolo [3,4-b][1,3,4] thiadiazol-3-yl)-1,3-benzoxazole derivatives have been synthesized and their structures were confirmed by IR, ^{1}H NMR, and Mass spectral data. These compounds were prepared by a mixture of substituted 4-amino-5-(1,3-benzoxazol-5-yl)-4*H*-1,2,4-triazole-3-thiols with various carboxylic acids and phosphorus oxychloride. All synthesized compounds **VII a-o** were tested by using the method of cup-plate technique against certain strains of Gram-positive, Gram-negative bacteria as well as the yeasts *Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporum* and *Curvularia lunata* in comparison with standard drugs. Microbiological results showed that the newly synthesized compounds possessed a broad spectrum of activity, compound **VII d** showed good antibacterial and **VII j** showed good antifungal activity. Rest of the compounds showed mild to moderate antifungal activity against testing organism.

Key words: Benzoxazoles, Antibacterial activity, Antifungal activity, Gram-Positive, Gram-negative.

INTRODUCTION

The rapidly increasing incidence of multiple drug-resistant Gram-positive bacteria requires an urgent discovery of novel active agents against these pathogenes[1,2]. The benzoxazoles have various biological activities such as antibacterial, antifungal[3-12], antimycobacterial[13], antitumoral[14-19], HIV-1 reverse transcriptase[20-26], and topoisomerase I inhibitory activities[27]. A benzoxazole derivative (Fig. 1) is significantly more potent as inhibitors of topoisomerase I than camptothecin[28]. UK-1 (Fig. 2) is a unique natural bisbenzoxazole product, isolated from a strain of *Streptomyces*, is a magnesium ion dependent DNA binding agent and inhibitor of human topoisomerase II. It displays a wide spectrum of potent anticancer activities in leukemia, lymphoma, and certain solid tumorderived cell lines with IC50 values as low as 20 nM[16,17,29]. Routiennocin (Fig. 3), which is a spiroketal ionophore antibiotic, isolated from a strain of *Streptomyces chartreusis* possessing a benzoxazole ring in its molecular structure**,** was found to be very active especially against some Gram-positive bacteria by acting as a good ionophore[4,30]. Moreover, 5- and/or 6- amidinobenzox azoles as well as benzimidazoles were found as inhibitors of the bacterial KinA/Spo0F.

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Many of these inhibitors exhibited good *in vitro* antibacterial activity against a variety of susceptible and resistant Gram-positive organisms[25]. Previously N-(2-(4- substitutedphenyl) benzoxazole-5yl)-2-(dialkylamino) acetamide derivatives has been synthesized as the target compounds in order to examine their microbiological activity against various Gram-positive and Gram-negative bacteria[31].

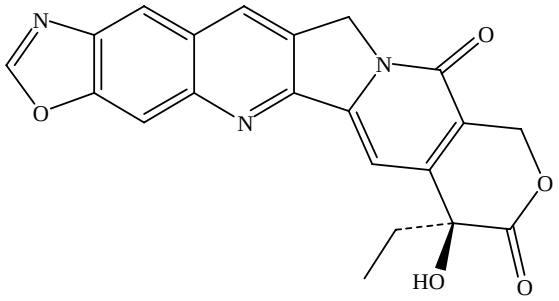


Fig. 1: A benzoxazole derivative

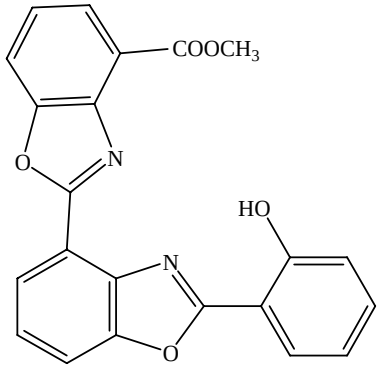


Fig. 2: UK-1

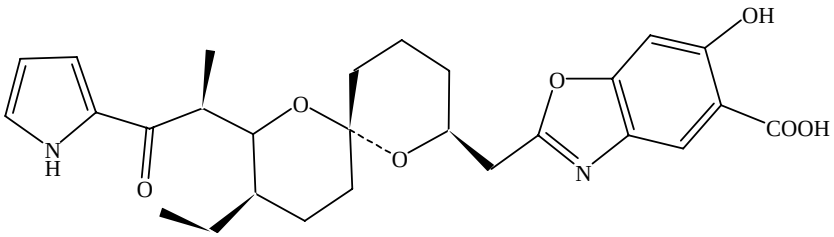


Fig. 3: Routiennocin

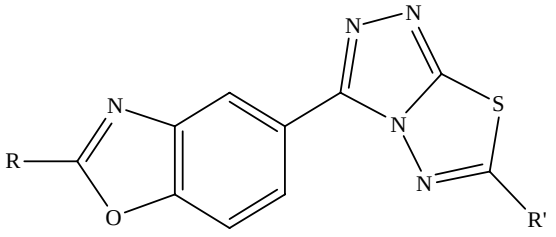
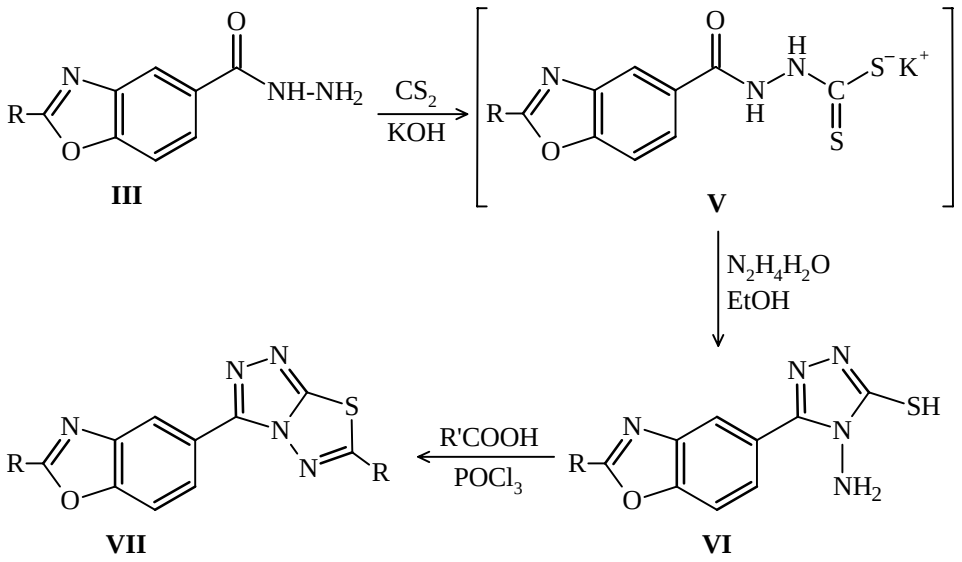


Fig. 4: Targetted Compounds (VIIa-VIIo)

EXPERIMENTAL



Scheme

Synthesis of potassium-2-(1, 3-benzoxazol-5-yl carbonyl) hydrazine carbodithioates (V)

Benzoxazole-5-carbohydrazide (III, 0.01 mol) treated with carbon disulphide in alcoholic potassium hydroxide, the reaction mixture was stirred at room temperature for 14 h. After completion of the reaction, the resulted potassium-2-(1,3-benzoxazol-5-ylcarbon yl) hydrazine carbodithioate (V) was purified by recrystallization and by coloum chromatography.

Synthesis of substituted 4-amino-5-(benzoxazol-5-yl)-4H-1,2,4-triazole-3-thiol (VI)

For instance a suspension of potassium 2-(benzoxazole-5-carbonyl) hydrazine carbodithioate (0.1 mL) was treated with added hydrazine hydrate (0.2 mL) and the reaction mixture was refluxed for 2 h. After completion of reaction, the reaction mixture was acidified with conc. HCl and the resulted compound separated was filtered and recrystallized from ethanol.

IR Spectrum data of Compound VI

The IR Spectrum (KBr) of the compound exhibited characteristic absorption bands (cm^{-1}) at: 3412 (NH), 3016 (C-H, Ar), 2610 (S-H), 1614 (C = N), 1556 (C = C, Ar), 1269 (C-O), 602 (C-S).

^{1}H NMR Spectrum data of Compound VI

PMR spectrum (DMSO-d_6) of the compound has been found to exhibit proton signals (δ ppm) at: 13.2 (s, 1H, SH), 8.1 (s, 1H, Ar-H), 7.6 (d, 1H, Ar-H), 7.4 (d, 1H, ArH), 7.2 (s, 1H, ArH), 5.6 (s, 2H, NH_2).

Synthesis of 5-([1,2,4] triazolo [3,4-b][1,3,4]thiadiazol-3-yl) benzoxazole (VII)

For instance a mixture of 4-amino-5-(benzoxazol-5-yl)-4H-1,2,4-triazole-3-thiol (0.01 mol), Formic acid (0.01 mol) and phosphorus oxychloride (10 mL) was refluxed for 6 h. The solid thus separated was washed with 2% Na_2CO_3 solution followed by washing with water and purified by recrystallization from ethanol.

IR Spectrum data of Compound VII

The IR Spectrum (KBr) of the compound exhibited characteristic absorption bands (cm^{-1}) at: 3024 (C-H, Ar), 1645 (C = N), 1542 (C = C, Ar), 1270 (C-O), 565 (C-S).

^{1}H NMR Spectrum data of Compound VII

PMR spectrum (DMSO-d_6) of the compound has been found to exhibit proton signals (δ ppm) at: 9.2 (s, 1H, ArH (thiadiazole ring)), 8.0 (s, 1H, Ar-H), 7.8 (s, 1H, Ar-H), 7.6 (d, 1H, ArH), 7.4 (d, 1H, ArH).

Antibacterial activity by cup plate method[32]

The antibacterial activity of synthesized compounds was conducted against two gram-positive bacteia viz., *Bacillus subtilis* and *Staphylococcus aureus* and two gram-negative bacteia viz., *Escherichia coli* and *Salmonella typhi* by using cup plate method. Ampicillin sodium was employed as standard to compare the results. The test organisms were subcultured using nutrient agar medium. The tubes containing sterilized medium were inoculated with respective bacterial strain. After incubation at 37°C ± 1°C for 24 hours, they were stored in refrigerator. The stock cultures were maintained. Bacteria inoculum was prepared by transferring a loopful of stock culture to nutrient broth (100 mL) in conical flasks (250 mL). The flasks were incubated at 37°C ± 1°C for 48 hours before the experimentation. Solution of the test compounds were prepared by dissolving 10 mg each in dimethylformamide (10 mL, AR grade). A reference standard for both gram-positive and gram-negative bacteria was made by dissolving accurately weighed quantity of ampicillin sodium in sterile distilled water, separately. The nutrient agar medium was sterilized by autoclaving at 121°C (15 lb/sq. inch) for 15 min. The petriplates, tube and flasks plugged with cotton were sterilized in hot-air oven at 160°, for an hour. Into each sterilized petriplate (10 cm diameter), about 27 mL of molten nutrient agar medium was poured and inoculated with the respective strain of bacteria (6 mL of inoculum to 300 mL of nutrient agar medium) was transferred asceptically. The plates were left at room temperature to allow the solidification. In each plate, three cups of 6 mm diameter were made with sterile borer. Then 0.1 mL of the test solution was added to the respective cups asceptically and labeled, accordingly. The plates were kept undisturbed for atleast 2 hours in refrigerator to allow diffusion of the solution properly into nutrient agar medium. After incubation of the plates at 37° ± 1°C for 24 hours, the diameter of zone of inhibition surrounding each of the cups was measured with the help of an antibiotic zone reader. All the experiments were carried out in triplicate. Simultaneously, controls were maintained employing 0.1 mL of dimethyl formamide to observe the solvent effects.

Antifungal activity[33]

All those compounds screened for antibacterial activity were also tested for their antifungal activity. The fungi employed for screening were: *Candida albicans* and *Aspergillus niger*. The test organisms were sub-cultured using potato-dextrose-agar medium. The tubes containing sterilized medium were inoculated with test fungi and after incubation at 25°C for 48 hours, they were stored at 4°C in refrigerator. The inoculum was prepared by taking a loopful of stock culture to about 100 mL of nutrient broth, in 250 mL conical flasks. The flasks were incubated at 25°C for 24 hours before use. The solutions of test compounds were prepared by a similar procedure described under the antibacterial activity. A reference standard (1 mg/mL conc.) was prepared by dissolving 10 mg of Clotrimazole in 10 mL of dimethylformamide (AR grade). Further, the dilution was made with dimethylformamide itself to obtain a solution of 100 μg/mL concentration. The potato-dextrose-agar medium was sterilized by autoclaving at 121°C (15 lb/sq. inch) for 15 minutes. The petriplates, tubes and flasks with cotton plugs were sterilized in hot-air oven at 150°, for an hour. In each sterilized petriplate, about 27 mL of molten potato-dextrose-agar medium inoculated with respective fungus (6 mL of inoculum in 300 mL of potato-dextrose medium) was added, aseptically. After

solidification of the medium at room temperature three discs of 6 mm diameter were made in each plate with a sterile borer. Accurately 0.1 mL (100 µg/disc) of test solution was transferred to the discs aseptically and labelled, accordingly. The reference standard 0.1 mL (10 mg/cup) was also added to the discs in each plate. The plates were kept undisturbed at room temperature for 2 hours, at least to allow the solution to diffuse properly into the potato-dextrose-agar medium. Then the plates were incubated at 25°C for 48 hours. The diameter of the zone of inhibition was read with the help of an antibiotic zone reader. The experiments were performed in triplicate in order to minimize the errors.

RESULTS AND DISCUSSION

Among the compounds substituted 5-([1,2,4] triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl)-1,3-benzoxazoles (VII) (Table 2) compound **VII d** with zone of inhibition of 16 mm, 21 mm, 25 mm, and 30 mm, respectively. Compound **VII j** with zone of inhibition of 20 mm, 24 mm, 26 mm and 22 mm, respectively. Compound **VII h** with zone of inhibition of 12 mm, 13 mm, 11 mm and 10 mm respectively were comparatively more active compounds against both Gram positive and Gram negative organisms. Compound **VII b** and Compound **VII m** were next in the order of antibacterial activity against Gram positive and Gram negative organism. Compounds **VIIa**, **VIIc**, **VIIg**, **VII**l and **VIIn** are active against Gram positive bacteria i.e. *B.subtilis* and *S.aureau*s where as compounds **VIIe**, **VIIi**, **VIIk**, **VIIl**, and **VIIo** were active against Gram negative bacteria i.e. *E.coli* and *P.vulgaris.*

Among the new series of compounds 5-([1,2,4] triazolo[3,4-*b*][1,3,4] thiadiazol-3-yl)-1,3-benzoxazoles (VII, Table 3), compounds showed mild to moderate activity against the test organism *A.niger, A. flavus, F. oxysporum* and *C. albicans.* Compound VII j showed good antifungal activity with the zone of inhibition of 18 mm, 17 mm, 20 mm and 12 mm, respectively. This was followed by compounds **VIIk**, **VIIg**, **VIIf** and **VIIa**. Rest of the compounds showed mild to moderate antifungal activity against testing organism.

Table 1: Physical data of Substituted 5-([1,2,4] triazolo [3,4-*b*][1,3,4] thiadiazol-3-yl)-1,3-benzoxazoles (VIIa-o)

S. No.	Compound	R	R'	Chemical Formula	Melting Point (^{0}C)	Yield (%)
1	VIIa	H	H	$C_{10}H_5N_5OS$	124	76
2	VIIb	H	CH_3	$C_{11}H_7N_5OS$	116	66
3	VIIc	H	CH_3CH_2	$C_{12}H_9N_5OS$	168	73
4	VIId	H	C_6H_5	$C_{16}H_9N_5OS$	210	77
5	VIIe	H	4-Cl C_6H_4	$C_{16}H_8ClN_5OS$	169	82
6	VIIf	CH_3	H	$C_{11}H_7N_5OS$	187	78
7	VIIg	CH_3	CH_3	$C_{12}H_9N_5OS$	208	69
8	VIIh	CH_3	CH_3CH_2	$C_{13}H_{11}N_5OS$	185	82
9	VIIi	CH_3	C_6H_5	$C_{17}H_{11}N_5OS$	204	72
10	VIIj	CH_3	4-Cl C_6H_4	$C_{17}H_{10}ClN_5OS$	191	69
11	VIIk	CH_3CH_2	H	$C_{12}H_9N_5OS$	202	75
12	VIIl	CH_3CH_2	CH_3	$C_{13}H_{11}N_5OS$	208	82
13	VIIm	CH_3CH_2	CH_3CH_2	$C_{14}H_{13}N_5OS$	192	82
14	VIIn	CH_3CH_2	C_6H_5	$C_{18}H_{13}N_5OS$	178	80
15	VIIo	CH_3CH_2	4-Cl C_6H_4	$C_{18}H_{12}ClN_5OS$	201	75

Table 2: Antibacterial activity of 5-([1,2,4] triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl)-1,3-benzoxazoles VII(a-o)

S. No.	Compd.	R	R'	Zone of inhibition (mm)			
				Gram positive bacteria		Gram negative bacteria	
				Bacillus subtilis	***Staphylococcus aureus***	***Escherichia coli***	***Proteusvulgaris***
1	VIIa	H	H	10	13	09	---
2	VIIb	H	CH_3	09	06	08	10
3	VIIc	H	CH_2CH_3	12	14	---	11
4	VIId	H	C_6H_5	16	21	25	30
5	VIIe	H	4-ClC_6H_4	12	---	06	09
6	VIIf	CH_3	H	---	11	08	08
7	VIIg	CH_3	CH_3	14	14	---	10
8	VIIh	CH_3	CH_2CH_3	12	13	11	12
9	VIIi	CH_3	C_6H_5	---	---	12	14
10	VIIj	CH_3	4-ClC_6H_4	20	24	26	22
11	VIIk	CH_2CH_3	H	13	---	10	08
12	VIIl	CH_2CH_3	CH_3	10	08	14	---
13	VIIm	CH_2CH_3	CH_2CH_3	11	11	16	08
14	VIIn	CH_2CH_3	C_6H_5	14	12	---	20
15	VIIo	CH_2CH_3	4-ClC_6H_4	---	---	11	21
Standard	Streptomycin			22	26	26	32

Solvent: Dimethylformamide
Concentration: 0.1 mg/mL

Table 3: Antifungal activity of 5-([1,2,4] triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl)-1,3-benzoxazoles VII(a-o)

S. No.	Compd.	R	R'	Zone of inhibition (mm)			
				Aspergillus niger	***Aspergillus flavus***	***Fusarium oxysporum***	***Curvularia lunata***
1	VIIa	H	H	09	08	12	05
2	VIIb	H	CH_3	---	06	14	07
3	VIIc	H	CH_2CH_3	10	07	---	08
4	VIId	H	C_6H_5	11	---	10	10
5	VIIe	H	4-ClC_6H_4	---	10	09	---
6	VIIf	CH_3	H	08	12	05	06
7	VIIg	CH_3	CH_3	09	08	06	09
8	VIIh	CH_3	CH_2CH_3	11	06	---	05

Cont…

S. No.	Compd.	R	R'	Zone of inhibition (mm)			
				Aspergillus niger	*Aspergillus flavus*	*Fusarium oxysporum*	*Curvularia lunata*
9	VIIi	CH_3	C_6H_5	---	04	12	06
10	VIIj	CH_3	4-ClC_6H_4	15	17	20	12
11	VIIk	CH_2CH_3	H	08	10	10	14
12	VIIl	CH_2CH_3	CH_3	10	---	08	05
13	VIIm	CH_2CH_3	CH_2CH_3	12	10	---	08
14	VIIn	CH_2CH_3	C_6H_5	---	12	11	07
15	VIIo	CH_2CH_3	4-ClC_6H_4	13	09	12	---
Standard	**Clotrimazole**			**18**	**19**	**21**	**14**

Solvent: Dimethylformamide
Concentration: 0.1 mg/mL

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