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Synthesis and Antimicrobial Activity of Benzo Thiazinen Derivatives

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ABSTRACT
In order to develop relatively small molecules as pharmacologically active molecules, a series of novel oxazolidinones having benzothiazinen and their derivatives were synthesized, and characterized by IR, ^{1}H NMR and Mass spectral studies. Various substituted oxazolidinones benzothiazinen were prepared by simple reflux in the presence of acetonitrile. Treatment of these oxazolidinones benzothiazinen deravatives with methanesulfonyl gives its sulphonates derivatives on further treatment with sodium azide and tri phenyl phosphine in acetic anhydride to give its acetamide derivatives. Further the synthesized compounds were evaluated for antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and antifungal activity against, *Candida albicans and Aspergillus niger*.

Keywords: Oxazolidinones, Benzothiazinen, Antibacterial, Antifungal, Antimicrobial.

INTRODUCTION

Oxazolidinone are well known five membered nitrogen and oxygen containing compounds. These have been reported to possess biological activities such as antibacterial activity. [1] The emergence of bacterial resistance to the antibiotics poses a serious concern for medical professionals during the last decade. [2] In particular multi-drug-resistant Gram-positive methicillin -resistant *Staphylococcus aureus* (MRSA) [3] and *Staphylococcus epidermidis* (MRSE), and vancomycin-resistant *Enterococci* (VRE) are of major concern. [4]

Oxazolidinones, a new class of synthetic antibacterial agents, exhibit activity against a large number of Gram-positive organisms. Many oxazolidinone derivatives are in clinical use such as linezolid, eperezolid as antimicrobial agent [5] Linezolid is the first oxazolidinone approved for the treatment of Gram-positive bacterial infections in humans. [6] Since Linezolid, the many attractive traits of oxazolidinone series have encouraged further work in this area, and also the literature reveals extensive chemical programs exist. [7] At present, most efforts are focused on substituted phenyl oxazolidinones. Benzothiazinen are associated with diverse biological and pharmacological activities like antimicrobial [8], anti-inflammatory. [9] By considering the above facts and their increasing importance in pharmaceutical and biological field, it was considered of interest to synthesize some new active
pharmacophores in a single molecular frame work and to evaluate their biological activities.

The incorporation of two moieties increases biological activity of both and thus it was of value to synthesize some new heterocyclic derivatives having two moieties in the same molecules. The synthesized compounds were screened for antibacterial and antifungal activities

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MATERIALS AND METHODS

All the chemicals were analytical grade; all substituted sulfonate,
Dichloromethane, Oxazolidene, Hydrochloric acid, Glacial acetic acid, Tri phenyl phosphine and Sodiumazide. General procedure to synthesis of oxazolidinones is having benzo thiazinen moieties and its derivatives.

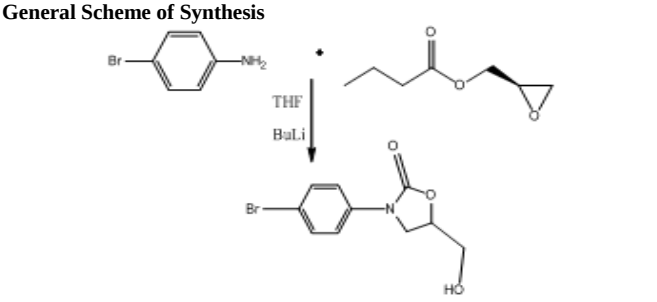
STEP 1

A mixture of 0.1mol of para bromo analine and freshly distilled tetrahydrofuran (150 mL) was taken in round-bottomed flask. The resulting solution was cooled and butyl litihium solution (0.4mol in hexanes) was added. The reaction mixture was stirred and was cooled in the ice bath and 0.1mol of glcidyl butyrate was added. Reaction mixture was further allowed to warm to room temperature and stirred for 22 h. To the resulting thick slurry was then was separated and extracted with ethyl acetate, and the combined organic layers were dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The residual solid was dried in a vacuum oven at 80°C for 72 h to provide crystalline solid of final product. [10]

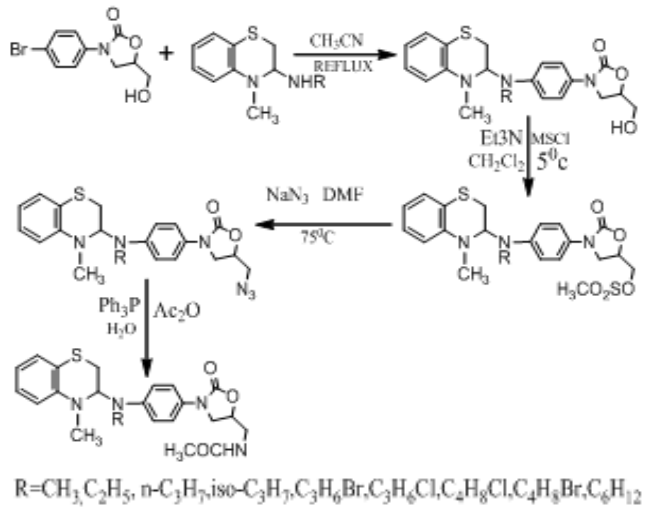
STEP 2

1. Synthesis of 5-(hydroxymethyl)-3-(4-(4-methyl-3, 4dihydro-2H-benzo[b][1,4]thiazin-3-ylamino)phenyl) oxazolidin-2-one derivatives from benthiazine amines derivatives and (3-(4-flurophenyl) methylene oxazolidine-5yl by simple reflux for three hours using acetonitrile solvent. [11]
2. Conversion of 5-(hydroxymethyl)-3-(4-(4-methyl-3,4dihydro-2H-benzo[b][1,4]thiazin- 3-ylamino) phenyl) oxazolidin-2-one derivatives to its methane sulfonate derivatives by using triethylamine in DCM later methanesulfonyl chloride added drop wise under vigorous stirring. Stirring for an additional 10–15 min completed the reaction. [12]
3. 3-(4-(3,4dihydro-2h-benzo[b] [1, 4] thiazin-3-ylamino) phenyl)-oxoxazolindin-5-yl) methyl methane sulfonate derivatives was convertated to azido derivatives by treating with sodiumazide in *N*, *N*-dimethyl formamide (DMF). [12]
4. 5-(Azidomethyl)-3-(4-(4-Methyl-3, 4-dihydro-2h-Benzo [B] [1, 4] Thiazin3ylamino) Phenyl) Oxazolidin -2-one was convertated to its acetamide derivatives by treating with tri phenyl phosphine and hydrochloric acid later extracted with AcOEt. [12]

General Scheme of Synthesis



STEP 1



R=CH_3, C_2H_5, n-C_3H_7, iso-C_3H_7, C_3H_6Br, C_3H_6Cl, C_4H_8Cl, C_4H_8Br, C_6H_{12}

STEP 2

All reactions were carried out under prescribed laboratory conditions. All the reactions requiring anhydrous conditions were conducted in flame dried apparatus. The solvents and reagents used in the synthetic work were of laboratory reagent grade and were purified by distillation and crystallization techniques wherever necessary and their melting points were checked with the available literature. Melting points of newly synthesized compounds were determined by open capillary method and were uncorrected. The final products were purified by recrystallization.

The entire synthesised compound was purified by TLC method and characterised by IR, ^{1}H NMR and mass spectral method.IR was recorded in bruker alpha model using FTIR. ^{1}H NMR data were recorded in (DMSO) on a Avance 400 MHZ spectrophotometer using TMS as an internal standard. The mass spectra were recorded using LC-MS (SHIMADZU 2010-AT) under electro spray ionisation (ESI) technique

N-((3-(4-(ethyl(4-methyl-3,4-dihydro-2H-benzo[b][1,4]thiazin-3-yl)amino)phenyl)-2-oxooxazolidin-5-yl) methyl) acetamide (PKSN2B)

IR (KBr) cm^{-1}: 3389(N-H), 3110 (aromatic C-H stretching), 2500 (S-H stretching) 2310 (aromatic C=C stretching), 1612 (aromatic C=C stretching), 1466 (C-N stretching) 824 (aromatic C-H deformation), 670 (C-Cl stretching), 1442 (C-N stretching), 1670 (C=O stretching in Oxoazolidine),

^{1}H NMR (δ) in ppm**:** 8.03 (1H, s, NH), 6.58-7.33 (9H, d, Ar-H), 6.66-7.21 (4H, d, Ar-H in Benzothiazine ring), 3.31(1H, d, N-C-H in Benzothiazine ring), 4.03 (1H, d, S-C-H in Benzothiazine), 3.33 (1H, d, Oxazolidine ring), 3.03 (1H,d,N-CH_3 in Benzothiazine ring) MS *m/z* (M^+): 441.

N-((3-(4-((3-chloropropyl)(4-methyl-3,4-dihydro-2H-benzo[b][1,4]thiazin-3-yl)amino) phenyl)-2-oxooxazolidin-5-yl) methyl)acetamide (PKSN2E)

IR (KBr) cm^{-1}:3386 (N-H stretching), 3009 (aromatic C-H stretching), 2510 (S-H stretching) 2350 (aromatic C=C stretching), 1612 (aromatic C=C stretching), 825 (aromatic C-H deformation), 2550 (S-H stretching) 1189 (C-N stretching), 1670 (C=O stretching in Oxoazolidine ring)

^{1}H NMR (δ) in ppm: 8.03 (1H, s, NH), 6.58-7.27 (8H, d, Ar-H), 6.66-7.21 (4H, d, Ar-H in Benzothiazine ring), 3.31(1H, d, N-C-H in Benzothiazine ring), 4.03 (1H, d, S-C-H in Benzothiazine), 3.33 (1H, d, Oxazolidine ring),3.03(1H,d,N-CH_3 in Benzothiazine ring) MS *m/z* (M^+)**:** 489**.**

N-((3-(4-((4-chlorobutyl)(4-methyl-3,4-dihydro-2H-benzo[b][1,4]thiazin-3-yl)amino)phenyl)-2-oxooxazolidin-5-yl)methyl)acetamide (PKSN2G)

IR (KBr) cm^{-1}:3381(N-H stretching), 3050 (aromatic C-H stretching), 2550 (S-H stretching), 1612 (aromaticC=C stretching), 824 (C-H deformation), 1189 (C-N stretching), 1671 (C=O stretching in Oxazolidine ring).

^{1}H NMR (δ) in ppm : 8.03 (1H, s, NH), 6.58-7.21 (8H, d, Ar-H), 6.66-7.21 (4H, d, Ar-H in Benzothiazine ring), 3.31(1H, d, N-C-H in Benzothiazine ring), 4.03 (1H, d, S-C-H in Benzothiazine), 3.33(1H, d, Oxaazolidine ring), 3.03(1H,d,N-CH_3 in Benzothiazine ring). M*S* m/z (M^+): 503.

Anti-microbiological Evaluation

All the synthesized compounds were evaluated for the antimicrobial activity by serial dilution method and cup plate method. The following micro organisms were used to study the antibacterial activity of synthesized compound *B. subtilis*, *S. aureus*, *E. coli*, *P. aeruginosa* whereas antifungal activities of synthesized compounds were studied against *Candida albicans* and *A. niger* .Amoxicillin and Fluconazole was taken as standard drug for the comparison of the activity of the synthesized compound for antibacterial and anti-fungal activity respectively.

RESULTS AND DISCUSSION

The effect of synthesized oxazolidinones having benzo thiazinen moieties has shown antibacterial and antifungal activity to certain extent. The results of these synthesized compounds are summarized in Table 1 & Table 2. Among the screened compounds, PKSN2C and PKSN2E have shown

Table 1: Antimicrobial data activity by minimum inhibitory concentration

Comp Code	Minimum inhibitory concentration (µg)					
	B. subtilis	***S. aureus***	***E. coli***	***P. aeruginosa***	***C. albicans***	***A. niger***
PKSN2 A	5.2	10.8	10.4	10.4	6.1	11.3
PKSN2 B	10.4	11.1	10.5	9.7	10.2	42.2
PKSN2 C	4.2	4.3	4.4	4.4	21.3	21.4
PKSN2 D	21.2	12.2	11.8	19.2	21.6	20.5
PKSN2 E	11.4	5.1	5.4	5.2	19.3	22.1
PKSN2 F	5.6	12.2	11.6	5.4	19.2	23.4
PKSN2 G	22.3	5.2	12.1	10.6	6.1	11.4
PKSN2 H	11.6	21.2	40.2	40.3	40.2	41.3
PKSN2 I	10.8	19.2	12.1	20.4	12.2	22.4
Amoxicillin	**1**	**2**	**2**	**1**		
Fluconazole					**16.6**	**8.3**

Table 2: Antimicrobial data activity by cup plate method

S. No	Compound Code	Diameter of zone of inhibition (mm)					
		B. subttlis	***S. aureus***	***E. coli***	***P. aeruginosa***	***C. albicans***	***A. niger***
1.	PKSN2 A	19	18	20	19	21	18
2.	PKSN2 B	17	19	19	20	18	16
3.	PKSN2 C	20	23	22	24	18	15
4.	PKSN2 D	14	16	21	21	16	16
5.	PKSN2 E	20	24	23	24	17	17
6	PKSN2 F	17	20	19	18	18	14
7	PKSN2 G	16	20	16	19	22	19
8	PKSN2 H	17	16	11	11	12	12
9	PKSN2 I	18	18	15	12	19	15
10	Amoxicillin	23	28	29	28	-	-
11	Fluconazole	-	-	-	-	25	21
12	Control	-	-	-	-	-	-

Table 3: Physical data of synthesized compound

S. No	Comp. Code	Mol. Formula	Mol. Wt	M.P (°C)	Rf value	Solvent system	Physical Nature	% Yield
1	PKSN2 A	$C_{22}H_{26}N_4O_3S$	426	160-163	0.26	C_2H_5COO:C_6H_6 (20:80)	White Crystal	80
2	PKSN2B	$C_{23}H_{28}N_4O_3S$	440	162-165	0.32	C_2H_5COO:C_6H_6 (20:80)	White Crystal	80
3	PKSN2C	$C_{24}H_{30}N_4O_3S$	454	170-172	0.38	C_2H_5COO:C_6H_6 (20:80)	White Crystal	75
4	PKSN2D	$C_{24}H_{30}N_4O_3S$	454	168-171	0.37	$C_6H_5CH_3$:CH_3OH (95:5)	White Crystal	71
5	PKSN2 E	$C_{24}H_{30}ClN_4O_3S$	488	180-183	0.33	$C_6H_5CH_3$:CH_3OH (95:5)	Greenish Crystal	70
6	PKSN2 F	$C_{24}H_{30}BrN_4O_3S$	533	186-189	0.38	$C_6H_5CH_3$:CH_3OH (95:5)	Brown Crystal	75
7	PKSN2 G	$C_{25}H_{31}ClN_4O_3S$	502	182-185	0.33	C_2H_5COO:C_6H_6 (20:80)	Greenish Crystal	70
8	PKSN2 H	$C_{25}H_{31}BrN_4O_3S$	547	192-195	0.42	C_2H_5COO:C_6H_6 (20:80)	Brown Crystal	75
9	PKSN2 I	$C_{27}H_{35}N_4O_3S$	495	184-187	0.31	C_2H_5COO:C_6H_6 (20:80)	White Crystal	65

good antibacterial activity against gram +ve and gram -ve bacteria compared to the standard drug amoxicillin, whereas PKSN2A and PKSN2G have shown significant antifungal activity against both *C. albicans* and *A. niger* compared to the standard drug Fluconazole

Results of present study demonstrate that a new class of different Oxazolidinones having benzothiazinen moieties were synthesized and evaluated for anti-microbial activities. Among tested compounds PKSN2C, PKSN2E, PKSN2A, & PKSN2G moiety showed better anti-microbial activity. It can be concluded that Oxazolidinones having benzothiazinen moieties class of compounds certainly holds great promise towards the good activity leads in medicinal chemistry. A further study require more information concerning pharmacological activity is in progress

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