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Synthesis of Some Novel Oxazolidinones Having Benzo Thiazinen Derivatives as Antimicrobial and Anti-Inflammatory Agents

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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 derivatives 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*. The synthesized compounds were screened for their anti-inflammatory activity by carrageenan induced paw-edema method.

Keywords: Oxazolidinones, Benzothiazinen, Antibacterial, Antifungal, Antimicrobial, Anti-inflammatory.

INTRODUCTION

Oxazolidinones 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 bacteria including 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 chemical entities incorporating the two 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 anti-inflammatory, antibacterial and antifungal activities

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MATERIAL AND METHODS

All the chemicals were analytical grade; all substituted Bezothiazine, Triethyamine, Methane sulfonate, Dichloromethane, Oxazolidene, Hydrochloric acid, Glacial acetic acid, Tri phenyl phosphine and Sodiumazide.

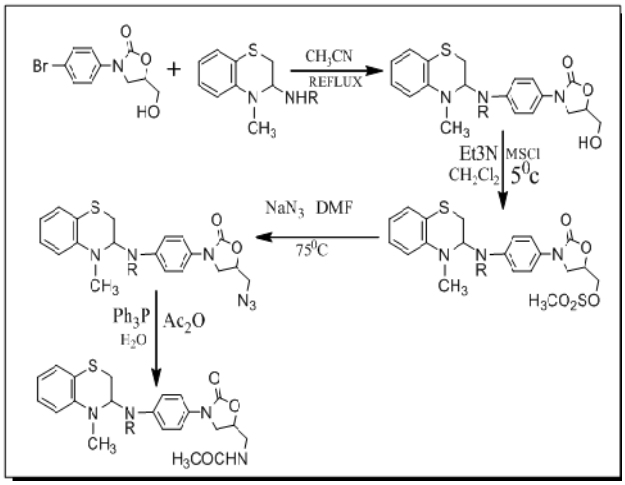
General procedure to synthesis of oxazolidinones having benzo thiazinen moieties and its derivatives

The synthesis consists of the four major steps which are as follows:

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. [10]

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 [11]
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). [11]
4. 5-(Azidomethyl)-3-(4-(4-Methyl-3,4dihydro-2h-Benzo[B][1,4]Thiazin3-ylamino)Phenyl)Oxazolidin-2-one was convertated to its acetamide derivatives by treating with tri phenyl phosphine and hydrochloric acid later extracted with AcOEt. [11]

General Scheme of synthesis



R= p-BrC$_6$H$_5$,p-ClC$_6$H$_5$, p-FC$_6$H$_5$, p-OCH$_3$C$_6$H$_5$, p-CH$_3$C$_6$H$_5$, C$_6$H$_5$, o-BrC$_6$H$_5$,o- Cl C$_6$H$_5$, p-FC$_6$H$_5$

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.

All the 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 400MHZ 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-((4-chlorophenyl) (4-methyl-3, 4-dihydro-2H-benzo[b][1,4]thiazin 3yl) amino) phenyl)-2-Oxooxazolidin-5-yl) methyl) acetamide (PKSN1B)

IR (KBr) cm^{-1}: 3389(N-H), 3050 (aromatic C-H stretching), 1612 (aromatic C=C 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^+)523.

N-((3-(4-((4-methoxyphenyl)(4-methyl-3,4-dihydro-2H-benzo[b][1,4]thiazin-3-yl)amino)phenyl)2-Oxooxazolidin-5-yl) methyl) acetamide (PKSN1E)

IR (KBr) cm^{-1}:3386 (N-H stretching), 3002 (aromatic C-H 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^+)519.

N-((3- (4-((4- methyl- 3 ,4- dihydro- 2H-benzo [b] [1,4]thiazin-3-yl)(phenyl)amino)phenyl -2-Oxooxazolidin-5-yl) methyl) acetamide (PKSN1F)

IR (KBr) cm^{-1}:3381(N-H stretching), 3050 (aromatic C-H stretching), 2550 (S-H stretching) 1612 (aromatic C=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), 3.83 (1H, s, O-CH_3), 3.03(1H,d,N-CH_3 in Benzothiazine ring).

M*S m/z* (M^+) 489.

Anti-microbiological Evaluation

Antibacterial Activity and Antifungal Activity Studies

All the synthesized compounds were evaluated for the antimicrobial activity by 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.

Pharmacological Screening

Acute toxicity studies

The preliminary pharmacological studies were conducted to assess the acute pharmacological effects and LD_{50} of the drug. The acute toxicity study was carried out in adult female albino rats by "up and down" method (OECD guidelines 425). [12]

Selection of doses

For the assessment of anti-inflammatory activity, three dose levels were chosen in such a way that, middle dose was approximately one tenth of the maximum dose during acute toxicity studies, and a low dose, which was 50% of the one tenth dose, and a high dose, which was twice that of one tenth dose (100 mg/kg, 200 mg/kg, 400 mg/kg).

Anti-inflammatory Activity

All the synthesized compounds were screened by Carrageenan induced rat paw edema model (acute-inflammatory model) for the screening of anti-inflammatory activity. Diclofenac was taken as standard drug.

Table 1: Physical Data of Synthesized Compounds

S. No	Comp. Code	Mol. Formula	Mol. Wt	M.P (°C)	Rf value (solvent system)	Physical Nature	% Yield
1	PKSN1 A	$C_{27}H_{27}BrN_4O_3S$	566	197-200	0.32 $C_2H_5COO:C_6H_6$ 20:80	Brown Crystal	65
2	PKSN1 B	$C_{27}H_{27}ClN_4O_3S$	522	190-193	0.36 $C_2H_5COO:C_6H_6$ 20:80	Yellow Crystal	67
3	PKSN1 C	$C_{27}H_{27}FN_4O_3S$	506	172-175	0.42 $C_2H_5COO:C_6H_6$ 20:80	White Crystal	65
4	PKSN1 D	$C_{28}H_{30}N_4O_4S$	518	178-181	0.21 $C_6H_5CH_3:CH_3OH$ (95:5)	Violet Crystal	71
5	PKSN1 E	$C_{28}H_{30}N_4O_3S$	502	170-173	0.31 $C_6H_5CH_3:CH_3OH$ (95:5)	Pale Yellow Crystal	74
6	PKSN1 F	$C_{27}H_{28}N_4O_3S$	488	160-163	0.27 $C_6H_5CH_3:CH_3OH$ (95:5)	Pale Yellow Crystal	62
7	PKSN1 G	$C_{27}H_{27}BrN_4O_3S$	566	198-201	0.44 $C_2H_5COO:C_6H_6$ 20:80	Brown Crystal	71
8	PKSN1 H	$C_{27}H_{27}ClN_4O_3S$	522	191-194	0.46 $C_2H_5COO:C_6H_6$ 20:80	Yellow Crystal	77
9	PKSN1 I	$C_{27}H_{27}FN_4O_3S$	506	190-193	0.48 $C_2H_5COO:C_6H_6$ 20:80	White Crystal	61

Table 2: Antimicrobial Data Activity of Oxazolidinones having benzo thiazinen moieties

S. No.	Compound Number	Diameter of zone of inhibition (mm)					
		B. subtilis	***S. aureus***	***E. coli***	***P. aeruginosa***	***C. albicans***	***A. niger***
1.	PKSN1 A	21	19	22	18	18	12
2.	PKSN1 B	22	23	23	26	15	16
3.	PKSN1 C	18	21	15	14	22	21
4.	PKSN1 D	14	15	23	23	16	16
5.	PKSN1 E	15	20	16	19	17	17
6	PKSN1 F	21	23	24	24	19	14
7	PKSN1 G	18	20	16	25	16	16
8	PKSN1 H	15	12	11	10	12	12
9	PKSN1 I	18	13	16	13	18	15
10	**Amoxicillin**	23	28	29	28	-	-
11	**Fluconazole**	-	-	-	-	25	21
12	Control	-	-	-	-	-	

Table 3: Anti-inflammatory effect of Oxazolidinones having benzothiazinen using Carrageenan-induced paw edema in rats.

Treatment	Dose mg/kg	Increase in paw volume (in ml)			
		1h	**2h**	**3h**	**4h**
Control	_	0.36±0.06	0.68±0.05	0.77±0.03	0.81±0.03
Diclofenac sodium.	13.5	0.18±0.03* (50)	0.35±0.05* (48.52)	0.41±0.04* (46.75)	0.45±0.05* (44.44)
PKSN1A	200	0.26±0.04 (27.77)	0.43±0.04 (36.76)	0.50±0.04 (35.06)	0.56±0.03 (30.86)
PKSN1B	200	0.23±0.04 (36.11)	0.41±0.04 (39.70)	0.47±0.04 (38.96)	0.52±0.04 (35.80)
PKSN1C	200	0.24±0.03 (33.33)	0.42±0.02 (38.23)	0.46±0.03 (40.25)	0.52±0.03 (35.80)
PKSN1D	200	0.19±0.04* (47.22)	0.37±0.03* (45.58)	0.42±0.03* (45.45)	0.46±0.04* (43.20)
PKSN1E	200	0.21±0.02 (41.66)	0.38±0.03* (44.11)	0.43±0.04* (44.15)	0.48±0.02* (40.74)
PKSN1F	200	0.20±0.03* (44.44)	0.37±0.03* (45.58)	0.42±0.03* (45.45)	0.46±0.03* (43.20)
PKSN1G	200	0.19±0.04* (47.22)	0.36±0.03* (47.05)	0.41±0.03* (46.75)	0.48±0.03* (40.74)
PKSN1H	200	0.21±0.04* (41.66)	0.38±0.03 (44.11)	0.43±0.03* (44.15)	0.48±0.03* (40.74)
PKSN1I	200	0.23±0.03 (36.11)	0.40±0.03 (41.17)	0.47±0.03 (38.96)	0.51±0.04 (37.03)

*P<0.05 significant compared to control

RESULTS AND DISCUSSION

In our study, new series of compounds namely Oxazolidinones having benzothiazinen moieties (PKSN1A-PKSN1I) showed significant anti-inflammatory activity ($p<0.05$) when compared with respective control groups.

The effect of synthesized Oxazolidinones having benzothiazinen moieties has shown antibacterial and

antifungal activity to certain extent. The results of these synthesized compounds are summarized in table 2. Among the screened compounds, PKSN1B and PKSN1F have shown good antibacterial activity against gram +ve and gram -ve bacteria compared to the standard drug amoxicillin **.**Whereas PKSN1C and PKSN1E have shown significant antifungal activity against both *C. albicans* and *A. niger* compared to the standard drug Fluconazole. PKSN1A PKSN1F and PKSN1I have shown significant antifungal activity against *C. albicans.*

The anti-inflammatory activity studies of synthesized compounds by carrageenan induced paw edema in rats are summarized in table3. Compound PKSN1D, PKSN1G showed good anti-inflammatory activity whereas PKSN1E and PKSN1F showed moderate anti-inflammatory activity.

Results of present study demonstrate that a new class of different Oxazolidinones having benzothiazinen moieties were synthesized and evaluated for anti-inflammatory and anti-microbial activities. Among tested compounds PKSN1D and PKSN1G moiety showed better anti-inflammatory activity, While PKSN1B, PKSN1F, PKSN1A, & PKSN1I 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 requires more information concerning pharmacological activity is in progress.

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REFERENCES

1 Park C, Brittelli D, Wang CL, Marsh F, Gregory WA, Wuonola MA. Antibacterials synthesis and structure-activity studies of 3-aryl-2-oxooxazolidinones-4- Multiply-substituted aryl derivatives. J Med Chem.1992; 35:1156-1165

2 Shinabarger D. Mechanism of action of the Oxazolidinone antibacterial agents. Expert Opin Invest Drugs. 1999; 8:1195-1202

3 Tomasz. Antibiotic Resistance in *Streptococcus pneumonia*. A N Engl J Med. 1994; 330:1247

4 Cui Y, Dang Y. Synthesis of novel oxazolidinone derivatives for antibacterial investigation. Curr Sci. 2005; 89:531-534

5 Brickner SJ, Hutchinson DK, Barbachyn MR, Manninen PR, Synthesis and antibacterial activity of U-100592andU100766, two oxazolidinone antibacterial agents for the potential treatment of multidrug-resistant gram-positive bacterial infections. J Med Chem. 1996; 31:673-679.

6 D'Andrea S, Zheng ZB, Den BK, Fung-Tomc JC. Synthesis and antibacterial activity of dihydro-1,2-oxazine and 2-pyrazoline oxazolidinones a novel analogs of linezolid. Bio Org Med Chem Lett. 2005; 15: 2834-2839.

7 Griera R, Llopart C, Amat M, Bosch J, Synthesis of a new series of oxazolidinones having spiro[2,4] heptane moieties. Bio Org Med Chem Lett. 2005; 89:2115-2118.

8 Kuo, Howard C , Michelle B, Prasun K. Aryl-link-aryl substituted thiazolidine-dione and oxazolidine-dione as anti-inflammatory agents Merck & Co Inc PCT/US03/12910 March 25, 2004.

9 Joseph GL, Edward HW. Synthesis and anti-inflammatory activity of some 3-carboxamides of 2-alkyl-4-hydroxy-2H-1,2-benzothiazine 1,1-dioxide. J Med Chem.1971; 14: 1171-1175.

10 Sharma S, Miller M, Payne j. An excellent procedure for the synthesis of oxazolidin- 2-ones and its derivatives. J Med Chem.1989; 32: 357.

11 Seoung J, Myung H, Kyung H, Jung H, Chang H. Synthesis and antibacterial activities of novel oxazolidinones having cyclic sulfonamide moieties. Bio Org Med Chem Lett. 2008; 40: 5815-5818.

12 New OCED425 guidelines, (2001) OCED guidelines for testing of animal Dec.2001; 1-26.