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SYNTHESIS AND BIOLOGICAL ACTIVITIES OF OXAZOLIDINONES HAVING N-METHYL 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. Oxazolidinones were prepared from R-glycidylbutarate and Para bromo aniline. Various substituted oxazolidinones benzothiazinen were prepared by simple reflux in the presence of acetonitrile. Treatment of these oxazolidinones benzo thiazinen deravatives with methanesulfonyl gives its sulphonates derivatives on further treatment wih 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

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

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.

MATERIAL AND METHODS

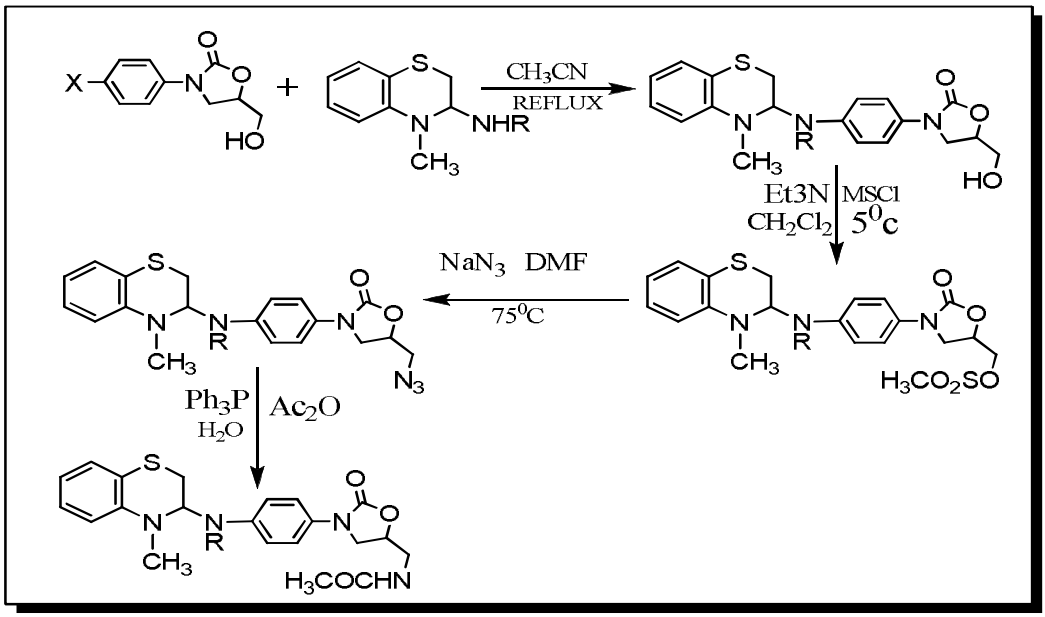
All the chemicals were analytical grade; all substituted Bezo thiazine ,Triethyamine,Methane sulfonate,Dichloro methane, Oxazolidene , Hydrochloric acid, Glacial acetic acid ,Tri phenyl phosphine. Sodiumazide General procedure to synthesis of oxazolidinones having benzo thiazinen moieties and its derivatives 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]Thiazin3ylamino)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=n-C_5H_{11},iso-C_5H_{11},neo-C_5H_{11},
nC_6H_{13},C_8H_{17},C_7H_{15},C_3H_5,C_4H_7,C_5H_9,C_6H_{17},C_7H_{13}

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 ATR .^{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-methyl-3,4-dihydro-2H-benzo[b][1,4]thiazin-3-yl)(pentyl)amino)phenyl)-2-oxooxazolidin - 5-yl) methyl)acetamide (PKSN3A)

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^+)483.

N-((3-(4-((3-ethylhexyl)(4-methyl-3,4- dihydro-2H -benzo [b] [1,4] thiazin-3-yl) amino) phenyl) -2- oxooxazolidin-5-yl)methyl)acetamide (PKSN3E)

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^+)525**.**

N-((3-(4-(cyclobutyl(4-methyl-3,4-dihydro-2H-benzo[b][1,4]thiazin-3-yl)amino)phenyl)-oxooxazolidin -5-yl) methyl)acetamide (PKSN3H)

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). MS m/z (M^+) 467.

N-((3-(4-((4-methyl-3,4-dihydro-2H-benzo[b][1,4]thiazin-3-yl)(2-methylcyclohexyl) amino) phenyl) -2- oxooxazolidin-5-yl)methyl)acetamide (PKSN3K)

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) ^1H NMR (δ) in PPM 8.01 (1H, s, NH), 6.54-7.21 (8H, d, Ar-H), 6.63-7.21 (4H, d, Ar-H in Benzothiazine ring), 3.31(1H, d, N-C-H in Benzothiazine ring), 4.01 (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). MS m/z (M^+) 509.

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* where as 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 analgesic and 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. (200 mg/kg, 400 mg/kg, 100 mg/kg).

Anti-Inflammatory Activity

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

RESULTS AND DISCUSSION

In our study, new series of compounds namely Oxazolidinones having benzo thiazinen moieties (PKSN3A-PKSN3K) showed significant anti-inflammatory activity ($p<0.05$) when compared with respective control groups. 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 2. Among the screened compounds, PKSN3B ,PKSN3F and PKSN3I have shown good antibacterial activity against gram +ve and gram -ve bacteria compared to the standard drug amoxicillin .Whereas PKSN3G and PKSN3J have shown significant antifungal activity against both *C.albicans* and *A.niger* compared to the standard drug Fluconazole.

The anti-inflammatory activity studies of synthesized compounds by carrageenan induced paw edema in rats are summarized in table3. Compound PKSN3B, PKSN3E showed good anti-inflammatory activity whereas PKSN3F and PKSN3H showed moderate anti-inflammatory activity.

Table 1: Physical Data of Synthesized Compounds

S.No	Comp. Code	Mol. Formula	Mol.Wt	M.P^{0}C	Rf value (solvent system)	Physical Nature	% Yield
1	PKSN3 A	$C_{26}H_{34}N_4O_3S$	482	170-172	0.41 C_2H_5COO:C_6H_6 20:80	White Crystal	61
2	PKSN3 B	$C_{26}H_{34}N_4O_3S$	482	171-173	0.42 C_2H_5COO:C_6H_6 20:80	White Crystal	61
3	PKSN3 C	$C_{26}H_{34}N_4O_3S$	482	170-172	0.41 C_2H_5COO:C_6H_6 20:80	White Crystal	62
4	PKSN3 D	$C_{27}H_{36}N_4O_3S$	496	190-192	0.32 $C_6H_5CH_3$:CH_3OH (95:5)	Pale Yellow Crystal	71
5	PKSN3 E	$C_{29}H_{40}N_4O_3S$	524	197-200	0.26 $C_6H_5CH_3$:CH_3OH (95:5)	Pale Yellow Crystal	72
6	PKSN3 F	$C_{28}H_{38}N_4O_3S$	510	192-194	0.29 $C_6H_5CH_3$:CH_3OH (95:5)	Pale Yellow Crystal	62
7	PKSN3 G	$C_{24}H_{28}N_4O_3S$	452	160-162	0.41 C_2H_5COO:C_6H_6 20:80	Yellow Crystal	71

8	PKSN3 H	$C_{25}H_{30}N_4O_3S$	466	164-166	0.43 $C_2H_5COO:C_6H_6$ 20:80	Yellow Crystal	72
9	PKSN3 I	$C_{26}H_{32}N_4O_3S$	480	170-172	0.48 $C_2H_5COO:C_6H_6$ 20:80	White Crystal	60
10	PKSN3 J	$C_{27}H_{34}N_4O_3S$	494	176-178	0.48 $C_2H_5COO:C_6H_6$ 20:80	White Crystal	62
11	PKSN3 K	$C_{28}H_{36}N_4O_3S$	508	195-197	0.48 $C_2H_5COO:C_6H_6$ 20:80	Yellow Crystal	64

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

Sl. No.	Compound Number	Diameter of zone of inhibition (mm)					
		B. subttlis	***S. aureus***	***E. coli***	***P. aeruginosa***	***C. albicans***	***A.niger***
1.	PKSN3 A	20	19	21	19	18	12
2.	PKSN3 B	22	25	26	25	15	16
3.	PKSN3 C	18	20	15	14	20	17
4.	PKSN3D	14	15	21	22	16	16
5.	PKSN3 E	15	20	16	19	17	17
6	PKSN3 F	21	26	26	25	19	14
7	PKSN3 G	18	20	16	25	20	16
8	PKSN3 H	15	13	12	10	12	12
9	PKSN3 I	21	24	25	25	18	15
10	PKSN3 J	16	17	20	15	22	20
11	PKSN3 K	14	18	18	17	13	16
12	**Amoxicillin**	23	28	29	28	-	-
14	**Fluconazole**	-	-	-	-	25	21
15	CONTROL	-	-	-	-	-	-

Table 3: Anti-inflammatory effect of Oxazolidinones having benzo thiazinen using Carrageenin 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
DiclofenacSodium.	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)
PKSN3A	200	0.25±0.04 (30.55)	0.42±0.04 (38.23)	0.49±0.04 (36.36)	0.55±0.03 (32.09)
PKSN3B	200	0.20±0.03* (44.44)	0.37±0.02* (45.58)	0.43±0.03* (44.15)	0.48±0.01* (40.74)
PKSN3C	200	0.25±0.03 (30.55)	0.43±0.02 (33.82)	0.47±0.03 (38.25)	0.53±0.03 (34.56)
PKSN3D	200	0.22±0.04* (38.88)	0.40±0.03* (41.17)	0.44±0.03* (42.45)	0.49±0.04* (39.50)
PKSN3E	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)
PKSN3F	200	0.21±0.03* (39.12)	0.37±0.03* (45.58)	0.42±0.03* (45.45)	0.46±0.03* (43.20)
PKSN3G	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)
PKSN3H	200	0.21±0.009* (39.12)	0.36±0.01* (46.84)	0.42±0.009* (45.45)	0.47±0.007* (39.13)
PKSN3I	200	0.22±0.009* (38.12)	0.39±0.01* (42.14)	0.43±0.007* (44.14)	0.52±0.008* (35.80)
PKSN3J	200	0.24±0.01* (33.13)	0.42±0.01* (38.14)	0.46±0.006* (40.26)	0.47±0.006* (41.10)
PKSN3K	200	0.26±0.01* (27)	0.41±0.01* (38.45)	0.47±0.01* (38.96)	0.54±0.01* (33.33)

***P<0.05 significant compared to control.**

CONCLUSION

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 PKSN3B and PKSN3E moiety showed better anti-inflammatory activity, While PKSN3B, PKSN3F& PKSN3I moiety showed better antibacterial activitiy where as PKSN3G, & PKSN3J moiety showed better anti-fungal

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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