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SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF B LACTAMS: ANTIBACTERIAL ACTIVITIES AND ANTIFUNGAL ACTIVITIES.

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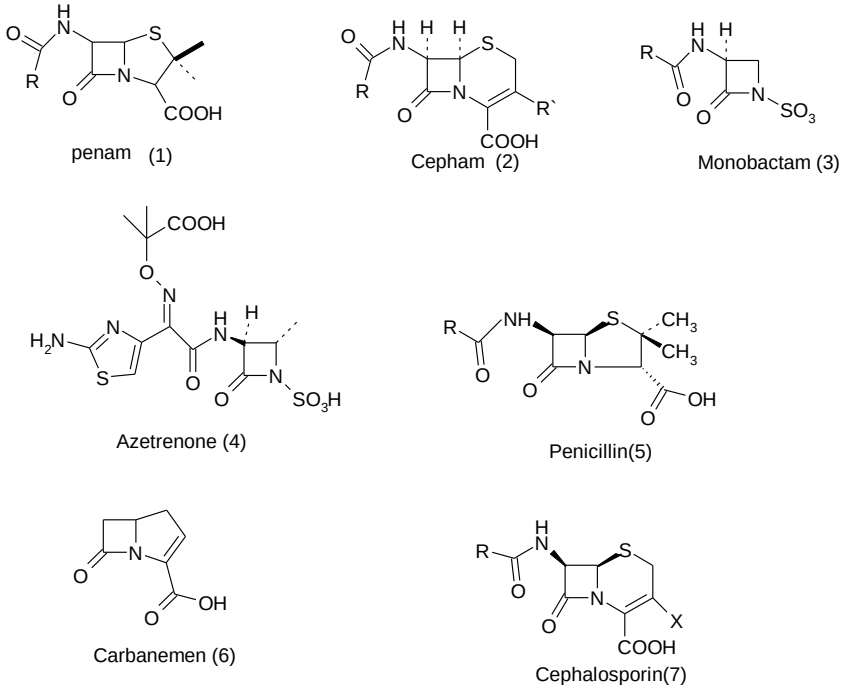
Abstract: This review deals with the preparation of new derivatives of pyrimidine-1-acetic acid hydrazide containing an azetidinone moiety was shown in the synthesis of 3-chloro-1-(4-pyrimidine methylcarbonylamino)-4-phenyl-azetidin-2-one ***(11a-h)***and their biological activity by the reaction of a mixture of pyrimidine -1-acetic acid hydrazide (**10a-h**). A series of novel β -lactams derivatives were designed and synthesized by reacting functionalized acylhydrazones with 2-chloroacetyl chloride[25]. The general method for the preparation of β –lactams derivatives containing natural Gallic acid moiety **15a-h** are outlined in (Scheme2). The pharmacological importance of β-lactams and their utility as building blocks in organic synthesis have directed considerable research activity toward the synthesis of suitably substituted 2-azetidinone rings.

Keywords: Drug activity, Biological activity, Development, chromatography, Antimicrobial activity.

Introduction

Since the discovery of penicillin, β-lactam antibiotics have been the most important family of antibacterial agents. [I] The β-lactam skeleton is still the essential structural backbone of the widely employed family of natural and unnatural antimicrobial agents to date. [II - V] The most widely used antibiotics such as the penicillin, [VI] aztreonam, cephalosporins, [VII] carumonam, monobactams , nocardicins and cabapenem [VIII] (Fig. 1) all contain the azetidine-2-one heterocyclic, which is the core structural feature in a number of broad spectrum β-lactams derivatives. [IX, X],It is also associated with a variety of therapeutic activities. [XI - XV] (Fig 1)

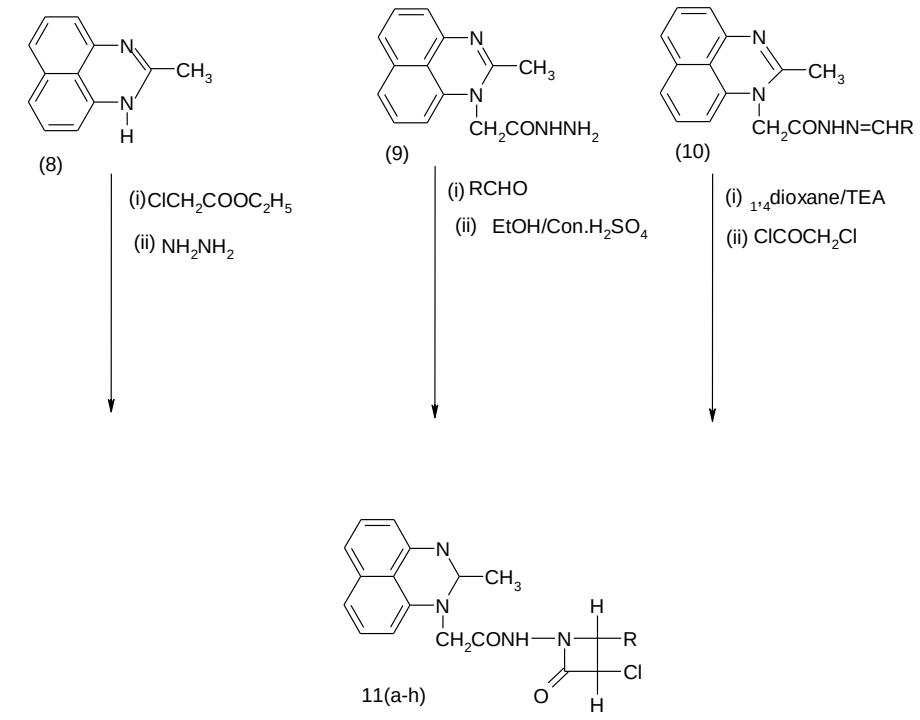


(Fig. 1)Antibiotics containing β-lactam ring

A large number of azetidinones containing β-lactam rings [XVI - XIX] are known to exhibit various biological activities like antibacterial, antifungal [XX] and antibiotic [XXI] activities. More particularly and recently these types of compounds have been found in the treatment of T.B. and other chemotherapeutic diseases. Hence, it was thought of interest in merging of both azetidinone and perimidine-1-acetic acid hydrazide moieties may enhance the drug activity of compounds up to some extent or might possess some of the above mentioned biological activities. [XXII]

Synthesis of β lactams

2.1:Preparation of new derivatives of pyrimidine-1-acetic acid hydrazide containing an azetidinone moiety was shown in the synthesis of 3-chloro-1-(4- pyrimidine methylcarbonylamino)-4-phenyl-azetidin-2-one ***(11a-h)***and their biological activity by the reaction of a mixture of pyrimidine-1-acetic acid hydrazide (**10a-h**) (0.001 mole) and triethylamine (TEA)(0.003 mole) was dissolved in 1,4-dioxane (25 mL) cooled and stirred. To this well stirred cooled solution chloroacetyl chloride (0.0012 mole) was added drop wise. The reaction mixture was stirred for 14 h at room temperature. Excess of solvent was removed by distillation. The residue was poured over crushed ice and then air dried. The product thus obtained was purified by column chromatography over silica gel using 30% ethyl acetate: 70% benzene as eluent. Recrystalization from ether/n-hexane gave white powdered 3-chloro-1-(4-perimidine methylcarbonylamino)-4-phenyl-azetidin-2-one (**11a-h**) which was obtained in 45-67% yield. (Scheme 1)



R,a=C6H5 , b, 4-OHC6H4, c,2-OHC6H4
d, 4O-CH3-C6H4
e,4-OH-3-OCH3C6H3
f,4-ClC6H4
g,2-NO2-C6H4
h,5Br-2-OH-C6H3

Biological screening

Antibacterial activities

Antibacterial activities of all the compounds were studied against gram-positive bacteria (*Bacillus subtilis and staphylococcus aureus*) and gram-negative bacteria (*E coli, Salmonellatyphi* and *Klebsiella promioe*).

The area of inhibition of zone measured in cm. Compound **11d**, **11f** and **11g** were found more active against the above microbes. Other compounds found to be less or moderate active than tetracycline.(see table 1)

Antifungal activities

The fungicidal activity of all the compounds was studied *in vitro* at 1000 ppm concentration. Plant pathogenic organisms used were *aspergillus, Nigrospora sp.***,** *Fusarium oxysporium***,** *Botrydepladia thiobromine* and *Albicans*.(see table 2).

The percentage inhibition for fungi was calculated after five days using the formula given below:

Percentage of inhibition = 100(X-Y) / X

Where, X = Area of colony in control plate

Y = Area of colony in test plate

Table 1 Antibacterial activity of compounds (11a-h)

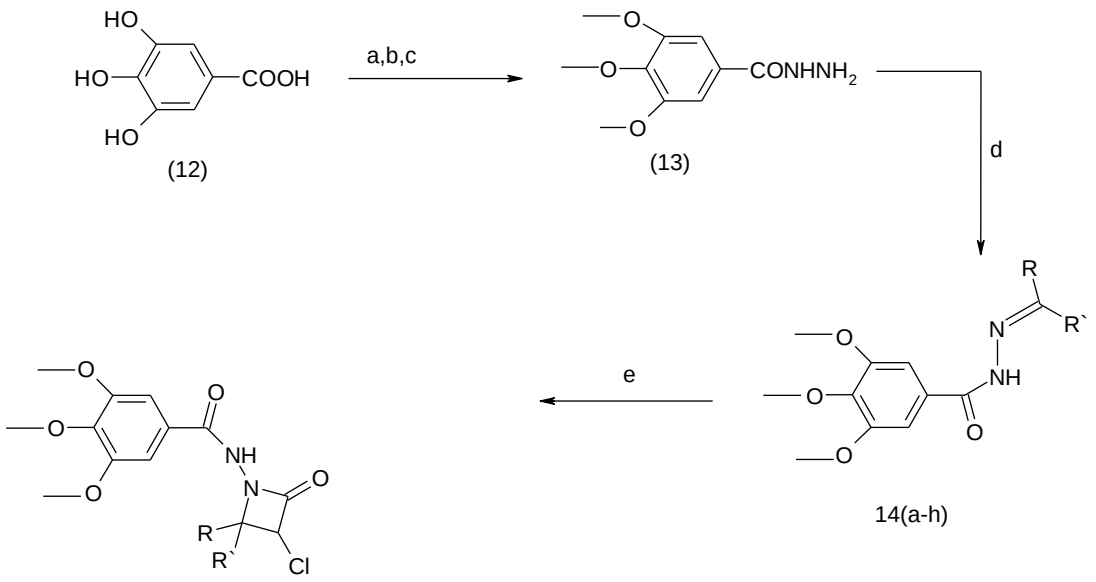
Zone of Inhibition					
	Gram +ve		Gram - ve		
Comp	*Bacillus subtitles'*	*Staphylococcus aureus*	*Klebsiella prmioe*	*Salmonella typhi*	*E.coli*
11a	57	53	48	44	67
11b	55	58	62	57	56
11c	60	62	60	54	59
11d	71	75	80	77	82
11e	50	54	42	55	58
11f	83	80	70	78	84
11g	74	82	79	72	70
11h	60	67	69	62	58
Tetracycline	80	57	83	74	72

Table 2 Antifungal activity of compounds (11a-h)

Zone of Inhibition at 1000 ppm, %					
Comp	*Aspergillus niger*	*Nigrospora Sp*	*Fusarium Oxyporium*	*Botrydepladia Thiobromine*	*Albicans*
11a	70	59	66	58	51
11b	62	68	65	50	68
11c	73	71	78	72	61
11d	72	67	64	79	69
11e	60	62	66	62	75
11f	50	63	68	71	62
11g	65	74	70	60	66
11h	69	78	69	74	73

2-Synthesis of N-substituted β-lactams derived from natural Gallic acid and their biological activity:

A series of novel β -lactams derivatives were designed and synthesized by reacting functionalized acylhydrazones with 2-chloroacetyl chloride [XXV]. The general method for the preparation of β –lactams derivatives containing natural Gallic acid moiety **15a-h** [XXIV] are outlined in (Scheme2).

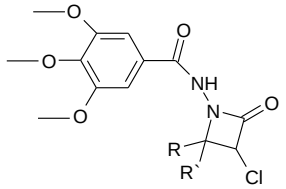


14,15	substituent	
a	R=H,R`=Ph	R=H,R`=4-Cl-Ph
	R=H,R`=2,4-Cl2Ph	R=H,R`=Pr
	R=H,R`=4-CF3-Ph	R=H,R`=Furan
h	R=H,R`=4-F-Ph	R=H,R`= Cyclohexyle

(Scheme2)

Synthetic route for azetidinones derivatives. Reagents and conditions: a. Me_2SO_4, NaOH, then HCl; b. EtOH, Conc. H_2SO_4; c. 5 equiv. $NH_2NH_2 \cdot H_2O$, reflux for 5-7 h; d. 1.1 equiv. Ketone/aldehyde, EtOH, reflux for 6-8 h; e. 1.2 equiv. ClCH2COCl, $CHCl_3$, Et_3N, r.t. to 40 ^oC for 2-5 h.

Table 3. Insecticidal activities of target compounds **15a-h** against*Heliothis armigera*



Entry	Compd. No.	Substituent		Insecticidal activity different Concentration (% µg /mL)					
		R	R\`	200	100	50	25	12.5	6.25
1	15a	H	Ph	80	50	40	30	30	0
2	15b	H	4-Cl-Ph	0	0	0	0	0	0
3	15c	H	2,4-Cl_2-Ph	0	0	0	0	0	0
4	15d	H	4-F-Ph	0	0	0	0	0	0
5	15e	H	4-Cf_3-Ph	0	0	0	0	0	0
6	15f	H	i- Propyl	90	70	30	30	0	0
7	15g	H	Furan-2-yl	50	50	30	0	0	0
8	15h		Cyclohexyl	100	50	30	0	0	0
9			Spirodiclofen	30	30	0	0	0	0

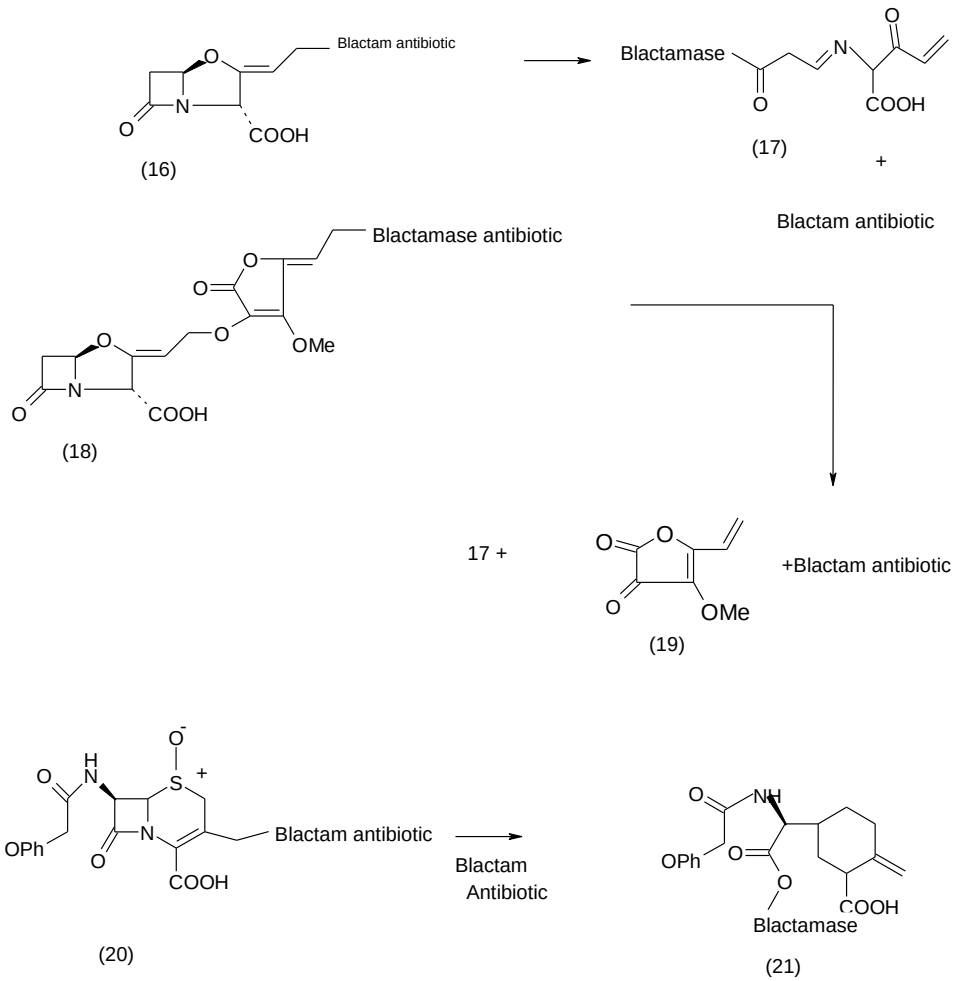
The results listed in Table 3, indicated that some of target molecules (such as compounds **15a**, **15f**, **15g**, and **15h**) displayed obviously selective insecticidal activity against*Heliothis armigera* at the dosage of 200 µg/mL. Especially, compound **15a** and **15f** bearing phenyl and isopropyl, respectively, which indicate moderate activity at the relative low concentration of 100 µ g/mL. In addition, the introduction of electron-withdrawing groups (such as halogen and trifluoromethyl)lead to the striking contrast, compounds containing these groups almost lost activities at the same concentration level (Entry 2-5, Table 3).
The rapid development and spread of mechanisms of bacterial resistance, however, are making virtually all b-lactam antibiotics obsolete. [XXVI]

The main cause of bacterial resistance to β -lactam antibiotics is the β-lactamases (β Ls), which are related in evolutionary
terms to transpeptidases. [XXVII] There are three ways to overcome the destructive action of a β L. The first into alter the structure of the β -lactam, rendering it insensitive to hydrolysis by the β L while maintaining its potency as an antibiotic. [XXVIII] It was often found that molecules more resistant to the β L were also less good as antibiotics, since at least some of the enzymes of cellwallbiosynthesis are acylated by b-lactam antibiotics at a unique serine residue in a peptide that shows convincing homology [XXIX] with the serine residue involved in acylenzymeformation by the β L. [XXX]. The second approach includes dual actions cephems, which can kill bacteria by two different mechanisms, for example release of Quinolones acting on bacterial DNA gyrase. [XXXI].

Synthesis of β - Lactam a prodrugs and their biological activity

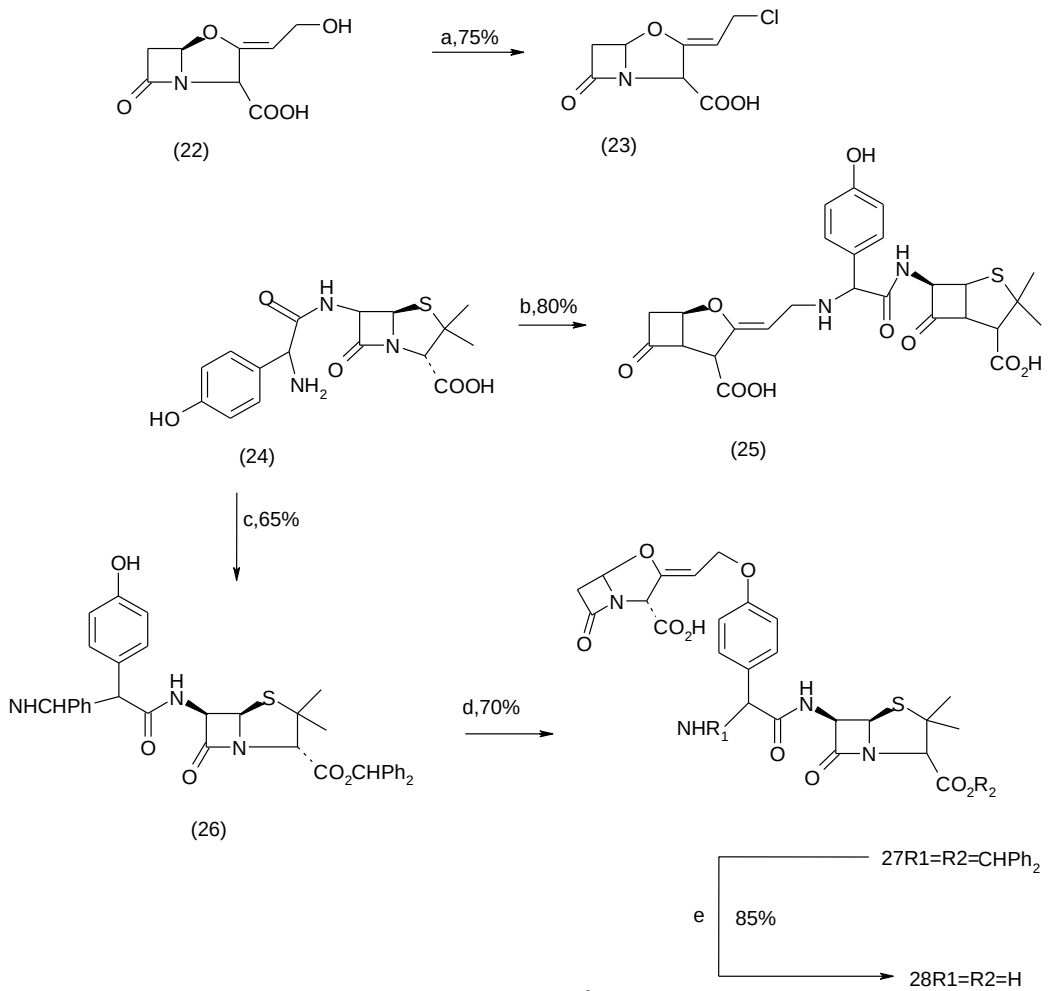
A ring opening of the β–lactam nucleus would occur when clavulanates **16** and **18** or Cephalosporin20 react with a β L. Consequently, the substituent attached at the C-9 position of **16** and **18** or at the C-3\` position of **20** is eliminated depending on the nature of the substituent. [XIV – XXIII] These β L acylation reactions by a serine residue release the β lactam antibiotic, which inhibits the trans peptidation reactions catalyzed by penicillin binding proteins (PBPs). As such,

compounds**16**, **18**, and **20**would act as a targeted prod rug for the antibacterial agent. [XXXII] (Scheme 3)



Scheme 3A novel counter attack strategy against resistant strains of pathogenic bacteria.

Synthesis of penicillin derivatives of clavaminic acid 25 and clavulanic acid 28(Scheme 4)
Reaction of clavulanic acid **(22)** with methanesulfonylchloride and pyridine in CH3CN at 25 _C afforded 9-chloro-9-deoxyclavulanic acid **(23)** in 75% yield.24a,24bAmoxicillin **(24)** was silylated with trimethylsilyl chloride and then condensed with the trimethylsilyl ester of 8 in the presence of Et3N at 25^0C to give the desired conjugate**25** in 80% yield. For the synthesis of prod rug **28**, amoxicillin **24** was first converted to its protected derivative**26** (65%) with diphenylmethyl chloride. Then, condensation of **26** with the trimethylsilyl ester of 8 in the presence of K_2CO_3 in CH_3CN at 25^0C led to the desired intermediate **27** in 70% yield. Removal of the diphenylmethylgroup from **27** by use of CF_3CO_2H–anisole inCH_2Cl_2 gave the bi functional target compound **28** in85% yield.



Reagents and conditions: (a) MeSO2Cl, pyridine, CH3CN, 25 ^{0}C, 24 h; (b) (1) Me_3SiCl, Et_3N, CH_3CN, 25 ^{0}C, 1 h; (2) trimethylsilyl ester of 8, Et_3N, 25 ^{0}C, 6 h; (c) Ph_2CHCl, Et_3N, CH_3CN, 25^{0}C, 3 h; (d) K_2CO_3, trimethylsilyl ester of 8, CH3CN, 25 ^{0}C, 13 h; (e) CF3CO2H–anisole,
CH_2Cl_2, 25 ^{0}C, 30 min.

Synthesis of penicillin-containing butenolide derivative of clavulanic acid 19 (Scheme 5)

Compound **34** was synthesized in five steps. We treated butenolide **29**[xxxii, xxxiii] with trimethylsilyl ester of**23** in the presence of K_2CO_3 in CH_3CN at25 ^{0}C to produce clavulanate derivative **30** in 90% yield. Reaction of **30** with methane sulfonyl chloride and pyridine in CH_3CN at25 ^{0}C afforded the corresponding chloro compound **31**in 76% yield. Silylation of **31** with trimethylsilyl chloride in the presence of Et_3N at 25^{0}C produced trimethylsilylester derivative **32**. Without isolation, **32** was subsequently reacted with amoxicillin derivative **26** to give the desired intermediate**33** in 73% overall yield.

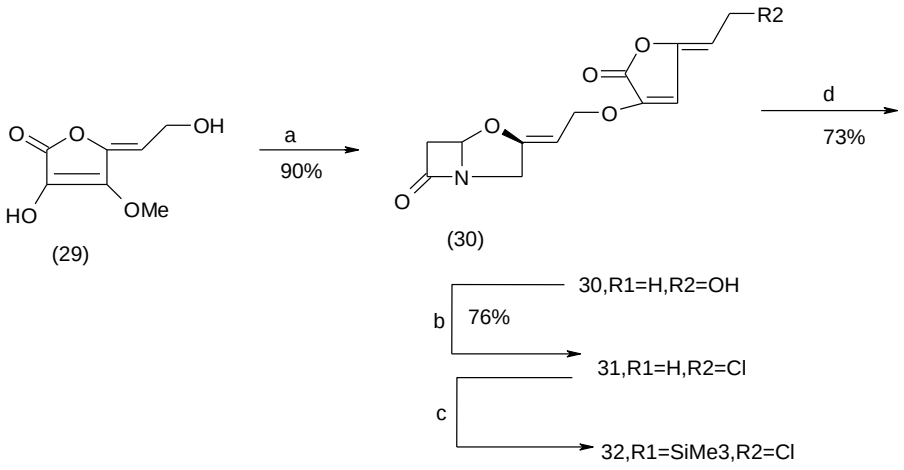
Treatment of **33** with CF_3CO_2H–anisole in CH_2Cl_2afforded the prod rug **34** in 80% yield.

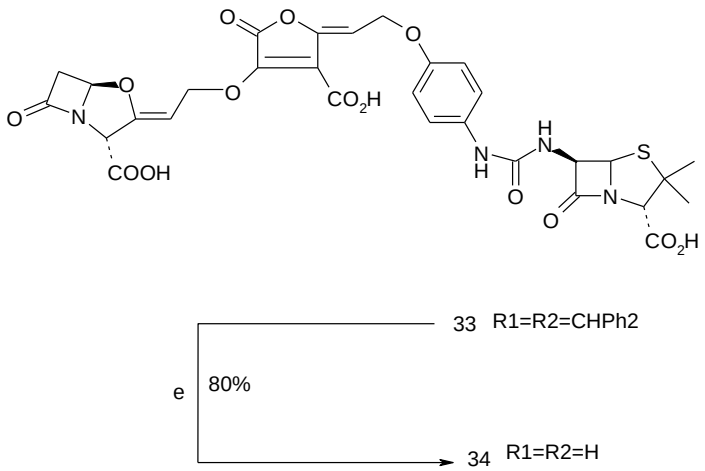
Synthesis of cephalosporin 30-amoxicillin ether 38 (Scheme 6)

Alkylation of amoxicillin derivative **26**with 3\-iodocephalosporin
35 [xxxi, xxxiv] in the presence of K_2CO_3 in CH_3CN at25 ^{0}C produced the conjugate **37** in 75%yield. Conversion of **37** to pro-dual-drug **38** (87% yield) was accomplished by use of CF_3CO_2H–anisole in CH_2Cl_2 at 25 ^{0}C.

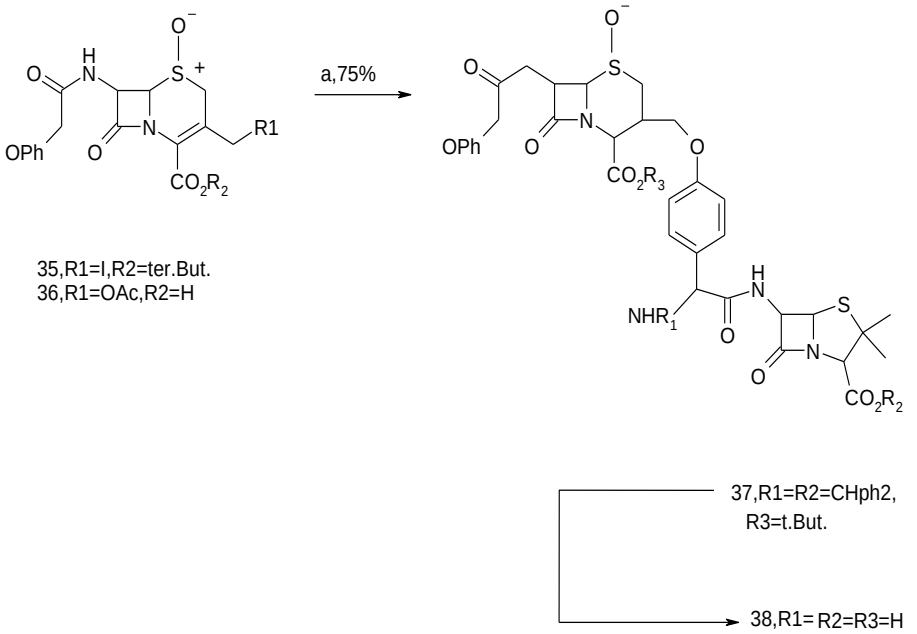
Lipophilicity, solubility, and stability studies

Lipophilicity and water solubility were determined by the distribution between 1-octanol and water according to the methods reported by Baker et al. [xxxv, xxxvi] Conjugates **25**,**28**, **30**, **34**, and**38** were observed to exhibit much higher lipophilicity than that exhibited by clavulanic acid **(22)**, amoxicillin **(24)**, and 3-[acetyloxymethyl]-7-(phenoxyacetamido)-(1-oxo)-3-cephem-4-carboxylic acid**(36)**. The solubility of **25, 28, 30,34**, and **38** in water was





Scheme 5.Reagents and conditions: (a) trimethylsilyl ester of 8, K_2CO_3, CH_3CN, 25 _C, 24 h; (b) MeSO2Cl, pyridine, CHeCN, 25^0C, 20 h; (c) Me_3SiCl, Et_3N, CH_3CN, 25^0C, 1 h; (d) K_2CO_3, 11, CH_3CN, 25^0C, 15 h; (e) CF_3CO_2H-anisole, CH_2Cl_2, 25^0C, 20 min.



Scheme 6. Reagents and conditions: (a) K2CO3, 11, CH3CN, 250C, 13 h; (b) CF3CO2H–anisole, CH2Cl2, 250C, 1.5 h.

Also found to be more compare to that of the parent molecules**22, 24**, or cephalosporin-1-oxide **(36)** (see Table 4). Unlike clavulanate-containing chlorobutenolide **31**that was converted to its hydroxylated derivative **30**(15.0 min), compounds**25, 28, 30, 34**, and **38**were found to be stable at physiological pH for >2 days as judged by HPLC and ^{1}H NMR studies. At pH=9.5, however, the b-lactam ring of clavulanate moiety in **25**,
28, 30, and **34**, as well as the β-lactam ring of cephalosporin component in 38decomposed within 7.0 min. After neutralization of the basic solution, amoxicillin **24**
Was isolated in about 60% yield. In the case of **30** or **34**, (Z)-4-(2-hydroxyethylidenyl)-2-hydroxy-3-methoxy-a,b-butenolide **29**was also isolated in about 55% yield.

Biological Results

Enzymatic hydrolysis study of clavulanic acid **22**, amoxicillin **24**, penicillin–clavaminic acid conjugate **25**, penicillin–clavulanic acid conjugate **28**, clavulanate containing butenolide **30**, clavulanate-containing amoxicillin derivative **34**, and cephalosporin–amoxicillin conjugate **38** by ^{1}H NMR:

Phosphate buffer solution (p D=7.2) was used for 1HNMR study of bLs catalyzed hydrolysis. [xxxv, xxxvii] Minimum amount of bLs necessary for hydrolysis of clavulanic acid **(22)** was used in all cases. In the presence of bLs from Staphylococcus aureus 95, S. aureus A9606,Escherichia coli A9675, E. coli 27C7 Pseudomonas aeruginosa18S-H, and Klebsiella pneumonia A20634 TEM, the 1H NMR spectra of clavulanic acid **(22)**, amoxicillin**(24)**, and cephalosporin-1-oxide **(36)** showed the b-lactam ring opening, the spectra of conjugates **25**, **28**, and**38** exhibited the appearance of the free amoxicillin **(24)**,

Table 4. Solubility in water and lipophilicity of b-lactams

Compd.	Solubility in water (mg/mL	Solubility in 1-octanol (mg/mL)	Log P (1-octanol/water)a
7	4.62	0.27	_1.23
9	3.96	0.008	_2.69
10	4.96	1.88	_0.42
13	4.72	1.79	_0.42
15	11.31	0.71	_1.20
19	4.68	3.46	_0.13
21	4.31	0.17	_1.40
23	5.04	2.13	_0.37

[Partition] coefficients were calculated as $P=[substrate]_{1\text{-octanol}}/[substrate]_{H2O}$.

The spectrum of clavulanate-containing butenolide **30**showed the liberation of butenolide **29**, and the spectrum of prod rug **34** changed rapidly to that of thee laminated compounds**24** and **29**. In the control experiments, in the absence of βLs, **22**, **24**, **25**, **28**, **30**, **34**, **36**, and**38** were stable to hydrolysis for >2 days.

Antibacterial activity

We carried out the screening experiments for antibacterial activities of the penicillin–clavaminic acid conjugate**25**; penicillin–clavulanic acid conjugate**28**, clavulanate-containing amoxicillin derivative **34**, and cephalosporin–amoxicillin conjugate 23. Amoxicillin **(24)**, [XXXVIII] a mixture of 9and clavulanic acid **(22)** (1:1 W/W), [XXXI b, XXXVIII] ampicillin, [XXXVIII] and penicillin [G XXXVIII, XXXIX] were used as the reference compounds. The experiments were performed in vitro [XL, XLI] against different strains of five pathogenic microorganisms up to 128 μg/mL The results are summarized in Table 5.

Table 5.Minimum inhibitory concentrations of novel b-lactams **25**, **28**, **34**, **38**, and the reference compounds penicillin G (pen G), ampicillin (ampn), clavulanic acid **(22)**, amoxicillin **(24),** as well as a 1:1 (W/W) mixture of **22**and**24**against pathogenic microorganisms in vitro

Microorganism	pen G	ampn	22	24	22+24	25	28	34	38
S. aureus FDA 209P	0.67	o.56	>128	0.93	0.58	1.81	1.02	2.99	1.79
S. aureus A9606b	>128	>128	>128	>128	2.27	0.75	0.34	0.82	0.25
S. aureus A15091b	>128	>128	>128	>128	3.01	0.87	0.25	1.07	0.19
S. aureus A20309b	>128	>128	>128	>128	1.98	0.55	0.07	0.89	0.10
S. aureus 95b,c	>128	>128	>128	>128	3.09	1.07	0.87	1.23	0.69
E. coli ATCC 39188	3.65	3.42	>128	4.76	2.98	4.01	3.02	3.53	2.14
E. coli A9675b	128	97.0	>128	72.1	1.79	0.68	0.03	0.50	0.08
E. coli A21223b	>128	>128	>128	93.4	1.35	0.16	0.06	0.34	0.07

E. coli 27C7b	>128	>128	>128	>128	2.48	0.75	0.07	0.90	0.10
P. aeruginosa 1101–75	>128	>128	>128	>128	8.74	7.32	6.24	5.65	4.85
P. aeruginosa 18S-Hb	>128	>128	>128	>128	6.05	1.01	0.42	1.30	0.06
S. typhi O-901	>128	>128	>128	>128	5.93	8.53	4.37	6.34	3.71
K. pneumoniae NCTC 418	>128	>128	>128	>128	2.45	3.10	2.15	5.21	1.79
K. pneumoniae A20634 TEMb	>128	>128	>128	>128	1.98	0.47	0.09	0.71	0.12

[a]The values of minimum inhibitory concentrations (μg mL^{-1}), obtained as the average of duplicate determinations, represent the lowest concentrations of antibiotics required to prevent visible growth of microorganisms. These values were obtained by use of an agar dilution method whereby organisms were deposited onto medicated agar plates by the replication device of Steers et al. [XLI]
[b]β-Lactamase-producing organism.
[c]Methicillin resistant organism

B-Lactamase inhibitory property

We tested the bLs inhibitory [XLII] properties of the penicillin–clavaminic acid conjugate **25**, penicillin–clavulanic acid conjugate **28**, clavulanate-containing amoxicillin derivative **34**, and cephalosporin–amoxicillin conjugate **38**.Clavulanic acid **(22)** and cephalosporin-1-oxide **(36)** were used in vitro as the reference compounds.

The results are shown in Table 6.

Table 6. Minimum protective concentrations of novel β-lactams **25**, **28**, **34**, **38**, as well as the reference compounds clavulanic acid **(22)** and cephalosporin-1-oxide **(36)** against bacterial βLs

β L from	22	25	28	34	36	38
*S aureus*A9606	0.53	1.29	0.76	1.87	0.97	1.05
*S. aureus*95	0.71	2.05	1.04	2.24	1.10	1.27
*E. coli*A9675	4.08	6.31	4.72	3.08	2.08	2.99
*E. coli*27C7	1.40	2.87	1.57	1.75	0.97	1.20
*P. aeruginosa*18S-H	3.08	5.20	4.02	6.21	1.68	2.11
K. pneumoniae A20634 TEM	0.20	1.45	0.39	2.03	0.94	1.03

[a]The values of minimum protective concentrations (μg mL^{-1}), obtained as the average of duplicate determinations, represent the lowest concentration of compounds required to protect an indicator, 3-[E-(2,4-dinitro)styryl]-(6R,7R)-7-(2-thienylacetamido)-3-cephem-4-carboxylic acid, from hydrolysis by bLs under standard test conditions [XLIII] within 35 min. The hydrolysis of indicator was evidenced by the appearance of a distinct red color.

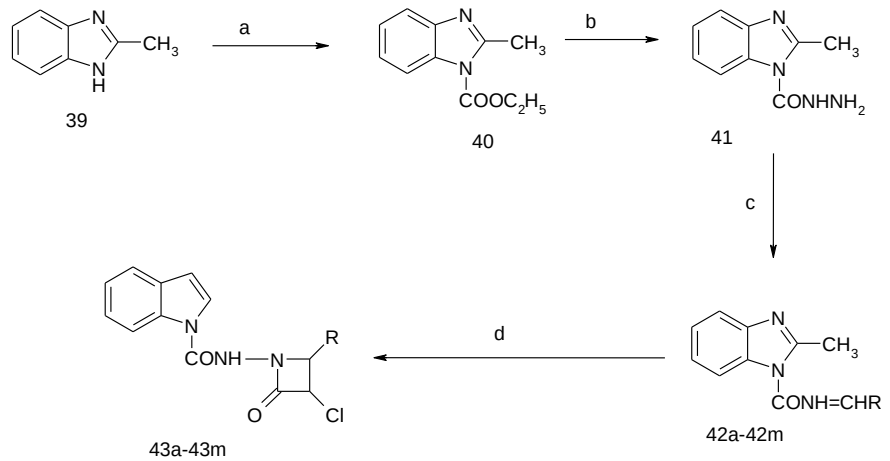
Parable to that of the parent molecule, clavulanic acid **(22)** or cephalosporin-1-oxide **(36)** . They underwent hydrolysis by βLs to liberate their amoxicillin component,

As evidenced by their notable values of the minimum protective concentrations **(MPC)** against the b-lactamases of *S. aureus* A9606, S. *aureus* 95, E. coli A9675,*E. coli* 27C7,

P. aeruginosa 18S-H, and *K. PneumoniaeA20634* **TEM**. Therefore, conjugates **25**, **28**, **34**, and **38** exhibited 'augment in-like' activity against resistant strains of pathogenic
Microorganisms, To combat resistant strains of pathogenic microorganisms, clavulanic acid **(22)** was attached to amoxicillin **(24)**at either the a-amino or the phenolic hydroxy group to
Afford the corresponding conjugates **25** and **28**, respectively. Similarly, attachment of amoxicillin **(24)** to thecephalosporin-1-oxide **(36)** at the C-30 position afforded
antibiotic**38**. Clavulanic acid **(22)** was also conjugated with amoxicillin **(24)** through a butenolide linker to produce antibacterial agent **34**. These compounds exhibited
Not able **MPC** values against the βLs of different bacterial species. Their antibacterial activity was found to be better than amoxicillin/clavulanic acid, 'Augmentin',against βL producing microorganisms, *S. aureus* A9606,*S. aureus* A15091, *S. aureus* A20309, *S. aureus* 95, *E. coli* A9675, *E. coli* A21223, E. coli 27C7, P. *aeruginosa* 18S-H, and *K. pneumonia* A20634 **TEM**. Therefore, conjugates **25**, **28**, **34**, and **38** exhibited 'augment in-like' activity against resistant strains of pathogenic
Microorganisms (see Table 5).

Synthesis and biological activity of some heterocyclic compounds containing benzamidazole and beta lactam moiety and their biological activity:

The reaction sequenced for different title compounds is outlined in scheme 1. 2-Methyl-1H-benzimidazole**39** and ethyl 2-methyl-1H-benzimidazole-1-carboxylate **40**were prepared according to the literature procedure. [XLIV] Compound **40** on treatment with hydrazine hydrate in ethanol yielded 2-methyl-1Hbenzimidazole-1-carbohydrazide**41**. Compound **41** on condensation with various aldehydes furnished2-methyl-N′-[(substituted) alkyl/aryl methylidene]-1-carbohydrazido-1H-benzimidazoles **(42a–42m)**. The four-membered β-lactam ring was introduced in compounds **(42a–42m)** at the azomethine group by the cycloaddition of chloroacetyl chloride in the presence of triethylamine, according to literature, [XLV] to yield N-[3-chloro-2-(substituted) alkyl/aryl-4-oxoazetidin-1-yl]-1-carboxamido-2-methyl-1H-benzimidazoles **(43a–m)**. Scheme 7



Reagents: (a) ClCOOC2H5/K2CO3,acetone,(b)NH2NH2.H2O/ethanol,(c);Corresponding aldehyde/ethanol, (d)ClCOCH2Cl/Et3N

Scheme 7. (Reagents: (a) ClCOOC2H5/K2CO3, acetone; (b) NH2NH2 ·H2O/ethanol; (c) corresponding aldehyde/ethanol (d) ClCOCH2Cl/Et3N).

Antimicrobial activity test:
The compounds (43a–43m) were tested for their in vitro growth inhibitory activity against different microbes. The bacterial strains used were *Staphylococcus aureus* ATCC 29213, *Streptococcus mutants* MTCC 890 and *Bacillus subtilis* MTCC 741 (all
Gram positive) and *Ecsherichia coli* ATCC 25922, *Salmonella typhi* MTCC 733 and *Pseudomonas aeruginosa* MTCC 741 (all Gram negative). For testing the antifungal activity of the synthesized compounds the fungal strains *Candida albicans*MTCC 1637, *Aspergillus flavors* AIIMS and *Aspergillus Niger* AIIMS were used.
The inhibition zones of synthesized compounds were determined using disc diffusion method. [43] In this method, paper disks (6 mm) containing specific amounts of an antimicrobial agent (300 μg for the synthesized compounds) were placed on the surface of an agar plate inoculated with a standardized suspension of the microorganisms tested. The plates were incubated at 35° C for 24 and 48 h, respectively for bacteria and fungi. Ampicillin (10 μg) for Gram positive bacteria, Nalidixic acid (30 μg) for Gram negative bacteria and Amphotericin B (30 μg) for fungi, were used as standard drugs. Paper disks with only DMSO were utilized as negative control. All experiments were carried out three times. The inhibition zones produced by the various synthesized compounds on the microbial growth were measured (diameter in mm).

Antimicrobial evaluation:
The in vitro antimicrobial activity was performed using the disc diffusion method with different strains of bacteria and fungi. Ampicillin and nalidixic acid were used as positive control for bacteria and amphotericin B was used against fungi.

The results of the final compounds for preliminary antibacterial testing are shown in table 7. The results revealed that the majority of the synthesized compounds showed varying degree of inhibition against the tested microorganisms. In general, the inhibitory activity against the Gram-positive bacteria was higher than that of the Gram-negative bacteria. The alkyl, phenyl and hydroxyphenyl substitutions at the 4-position of azetidin-2-one subunit has the best overall antibacterial profile. The methyl, chloro and methoxy

Substituent's on phenyl ring at azetidin-2-one moiety of final compounds displayed least activity.
As can be seen in table 7, although all the compounds are not as active as standard AmphotericinB, compounds **43d** and **43i** were found to be more active against *Candida albicans* and *Aspergillus flavus*. Again in antifungal activity compounds **43**e,**43**f, **43**g, **43**h, **43**l and **43**m showed less or negligible activity than the other derivatives of the same series. Although the rest of the compounds showed varying degree of inhibition, none were as effective as AmphotericinB.

Table 7. Antibacterial activity of compounds **(43a–43m).**

	Mean Zone Inhibition (in mm)[a]					
	Gram +ve			Gram - ve		
Compounds.	*S. aureus*	*S. mutans*	*B. subtilis*	*E.coli*	*S.typhi*	*P.aeruginosa*
43a	38	20	28	18	18	14
43b	37	18	28	-	16	-
43c	32	18	22	-	-	12
43d	36	16	27	-	13	12
43e	30	15	20	-	-	-
43f	30	13	20	-	-	-
43g	31	13	18	-	-	-
43h	31	10	20	-	-	-
43I	37	16	27	-	10	10
43J	36	16	26	-	10	-
43K	36	15	26	-	-	-
43L	25	14	22	-	-	-
43m	22	16	18	-	-	-
Ampicillin[b]	38	22	28	20	-	-
Nalidixic acid[b]	-	-	-	28	20	18

[a]Values are mean (n = 3)

[b]Ampicillin (10 µg/disc) and Nalidixic acid (30 µg/disc) used as positive reference; synthesized compounds (300 µg/disc)

'–' indicates no sensitivity or mean inhibition zone diameter lower than 7 mm

Table 8. Antifungal activity of compounds **(43a–43m)**

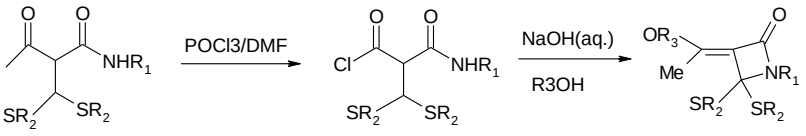
	Mean Zone Inhibition (in mm)[a]		
.	*Candida albicans*	*Aspergillus niger*	*Aspergillus flavus*
43a	26	24	22
43b	24	25	24
43c	24	24	20
43d	27	26	27
43e	22	18	14
43f	20	18	13
43g	20	16	15
43h	18	14	14
43I	27	28	26
43J	15	20	13
43K	15	14	18
43L	16	16	16
43m	18	19	16
[b]Amphotericin B	28	> 28	28

[a]Values are mean (n = 3)

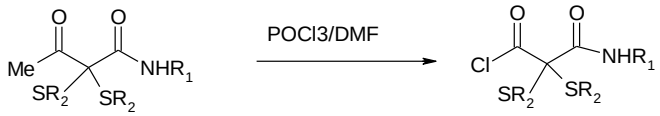
[b]Amphotericin B (30 µg/disc) used as positive reference; synthesized compounds (300 µg/disc)

The results of the final compounds for preliminary antibacterial testing are shown in table 7. The results revealed that the majority of the synthesized compounds showed varying degree of inhibition against the tested microorganisms. In general, the inhibitory activity against the Gram-positive bacteria was higher than that of the Gram-negative bacteria. The alkyl, phenyl and hydroxyphenyl substitution s at the 4-position of azetidin-2-one subunit has the best overall antibacterial profile. The methyl, chloroand methoxy
Substituents on phenyl ring at azetidin-2-one moiety of final compounds displayed least activity.
As can be seen in table 7, although all the compounds are not as active as standard AmphotericinB, compounds **43d** and **43i** were found to be more active against *Candida albicans* and *Aspergillus flavus*. Again in antifungal activity compounds **43**e,**43**f, **43**g, **43**h, **43**l and **43**m showed less or negligible activity than the other derivatives of the same series. Although the rest of the compounds showed varying degree of inhibition, none were as effective as AmphotericinB.

Synthesis of a-alkylidene-b-Lactams:
The recent discoveries of some natural monocyclic b-lactams (monobactams) displaying high anti-bacterial activity indicate that the 2-azetidinone ring is the key unit and the minimum requirement for biological activity. [XLVI, XLVII] The pharmacological importance of b-lactams and their utility as building blocks in organic synthesis have directed
Considerable research activity toward the synthesis of suitably substituted 2-azetidinone rings. [XLVIII, XLIX] Intensive research has generated numerous synthetic approaches, involving
ketene–imine cycloadditions (the Staudinger reaction), [L] ester enolate–imine condensations (the Gilman–Speeter reaction), [LI] cyclization reactions of β-amino acids or esters, [LII] coupling reactions of alkynes with nitrones, [LI] photo induced rearrangements, [LIII] carbene insertions, [LIV] and radical cyclizations, [LV] among others. Nevertheless, to match the increasing scientific and practical demand for β-lactams, new and efficient methodologies for the construction of suitable substituted 2-azetidinone skeletons are still desirable.
Over the past few decades, the utility of a-oxo ketene-S,S-acetals as versatile intermediates in organic synthesis has been recognized [LVI] .During the course of our studies on the reaction of acyl ketene-S,S-acetals under Vilsmeier conditions, [LVII] we noted that the
Readily synthesized a-(1-chlorovinyl) ketene-S,S-acetals showed promising structural
Characteristics that could be exploited in further organic transformations. Inspired by these findings and our continuing interest in the utilization of b-oxo amide derivatives in the synthesis of carob- and heterocyclic, [LVIII] we synthesized a-carbamoyl, a-(1-chlorovinyl) ketene-S,S-acetals **45**from a-acyl, a-carbamoyl ketene-S,S-acetals **44** and explored their
Synthetic potential. As a result, an efficient one-pot synthesis of highly substituted a-alkylidene-β-lactams**46** was developed from readily available **45** in aqueous media [LIX] (Scheme 8).



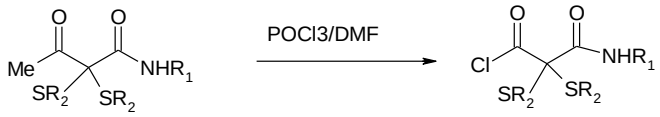
Scheme 8. Synthesis of a-alkylidene-b-lactams 46 from a-acyl, a-carbamoyl ketene-S, Sacetals 44



(44) (45)

Synthesis of compounds **45** from substrates **44** under Vilsmeier conditions

Table 9.Synthesis of compounds **45** from substrates **44** under Vilsmeier conditions

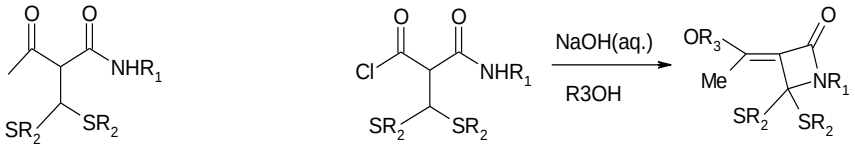


(44) (45)

Entry	44	R^1	R^2	45	Yield[a] [%]
1	44a	C_6H_5	Me	45a	85
2	44b	C_6H_5	Et	45b	82
3	44c	4-MeC_6H_4	Me	45c	86
4	44d	4-MeC_6H_4	Et	45d	87
5	44e	4-MeC_6H_4	Me	45e	86
6	44f	4-MeC_6H_4	Et	45f	84
7	44g	4-ClC_6H_4	Me	45g	91
8	44h	4-ClC_6H_4	Et	45h	90
9	44i	2,4-$Me_2C_6H_3$	Me	45i	86
10	44j	2,4-$Me_2C_6H_3$	Et	45j	88
11	44k	Me	Me	45k	87
12	44l	Me	Et	45l	83

Synthesis of substituted α-alkylidene-β-lactams 46

With compounds **45a–l** in hand, we selected 2-[bis (methylthio) methylene]-3-chloro-N-phenylbut-3-enamide **45a** as the model compound to examine its behavior under different basic conditions. Thus, the reaction of **45a**was performed in aqueous NaOH (2.0 equiv, 1.0 N)/ethanol at ambient temperature (20^{o}C) for 15 h. Work up and purification by column chromatography of the resulting mixture furnished two main products, which were characterized as (E)- and (Z)-3-(1-ethoxyethylidene)-4,4-bis(methylthio)-1-phenylazetidin-2-ones, i.e., **(E)-46a** and **(Z)-46a**, on the basis of their spectral and analytical data (Scheme 8). The structure and stereochemistry of **(E)-46a** were established by the X-ray single-crystal analysis (Fig. 1). [LX].The results suggest that ethanol plays dual roles as a co-solvent and a nucleophilic species in the cyclization reaction. It is of interest to note that the obtained compounds **(E)-** and **(Z)-46a** have remarkable structural and functional complexity since they contain the key skeleton of β -lactams. Certainly, the substituted α-alkylidene-β-lactams, as an important subset of azetidinones, have attracted significant interest among synthetic and medicinal chemists over the years mainly because of their biologically activities and their utilities as useful building blocks in organic synthesis. [LXI — LXIII].



(44) (45) (46)

Scheme 9. The reaction of 45a in NaOH (aq)/EtOH

Chemists over the years mainly because of their biologically activities and their utilities as useful building blocks in organic synthesis. [LXI — LXIII].The above findings encouraged us to investigate the reactions of**45a** for the construction of the 2-azetidinone skeletons under varied conditions. Thus, a series experiments were carried out with the aim at optimizing the reaction conditions, including the base, solvents, and reaction temperature. It was found that in the presence of K_2CO_3 (aq) in ethanol, the reaction proceeded sluggishly to afford both isomers of **46a** in much lower yields. Even no desired product**46a** was obtained when **45a** was treated with NaOH, NaH or NaOEt in absolute ethanol. We assumed that in the above cases, water might merely act as a better co-solvent for the employed base thus promoting the target cyclization. The accelerating properties of water when used as a convenient additive or co-solvent in other organic reactions have been reported elsewhere and therefore other roles should be considered. [LXIV] Using ethanol as a reactant, we performed the reaction of **45a** with aqueous NaOH in other solvents such asCH_2Cl_2, DMF, THF, and water. By comparison, the optimal conditions were obtained when **45a** (2.0 mmol) was subjected to NaOH (aq, 1.0 N, 3.0 equiv) in ethanol (25 mL) at 30^{o}C for 12 h, where by the yields of **(E)-and (Z)-46a** could reach 69 and 23%, respectively.

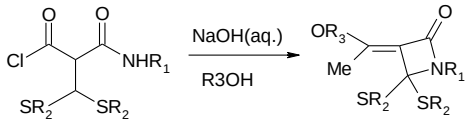
Having established the optimal conditions for the cyclization, we intended to

Determine its scope with respect to the amide and sulfanyl functionalities, and the nucleophilic species involved. Thus, substrates **45b–l** bearing varied amide groups were treated with aqueous NaOH in ethanol under the optimized reaction conditions as for 3a. The effectiveness of the cyclization proved to be suitable for N-aryl amides **45b–j** affording the corresponding compounds of type 3 in moderate to good yields (Table 10, entries 2–10). It is worth noting that in all the cases of **45b–j**, only isomer **(E)-46** was obtained. For N-alkyl amides **45k** and 2l, the reactions proceeded smoothly to furnish the corresponding **46k** and 3l, in which both (E)- and (Z)-isomers were obtained with the (E)-isomer as the predominant one(Table 10, entries 11 and 12). All the results revealed that the cyclization reaction exhibited high stereo selectivity.

As an extension of the above cyclization, a series of reactions was performed on substrates**45** in methanol under the otherwise identical conditions. Thus, the corresponding substituted 2-azetidinonesof type **(E)-46** were synthesized stereo selectively from

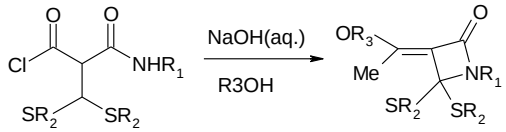
Amides**45a–l** in moderate to good yields. It was observed that, only in the case of N-alkylamide **45k**, both isomers **(E)-,** and **(Z)-46w** were obtained with the ratio of 5:2 (Table 10, entry 23). The validity of this 2-azetidinone synthesis was further evaluated in isopropanol by using **45a**. However, when **45a** was subjected to aqueous NaOH in tert-butanol, the cyclization to the corresponding 2-azetidinone was unsuccessful, and only the intact substrate was recovered. This may be due to the weak nucleophilicity and the hindered stereo effect of the bulky tert-butyl group of the alkoxide nucleophile.

Synthesis of α-alkylidene-β-lactams **46** from **45** in aqueous media



(45) (46)

Table 10.Synthesis of α-alkylidene-β-lactams **46** from **45** in aqueous media



(45) (46)

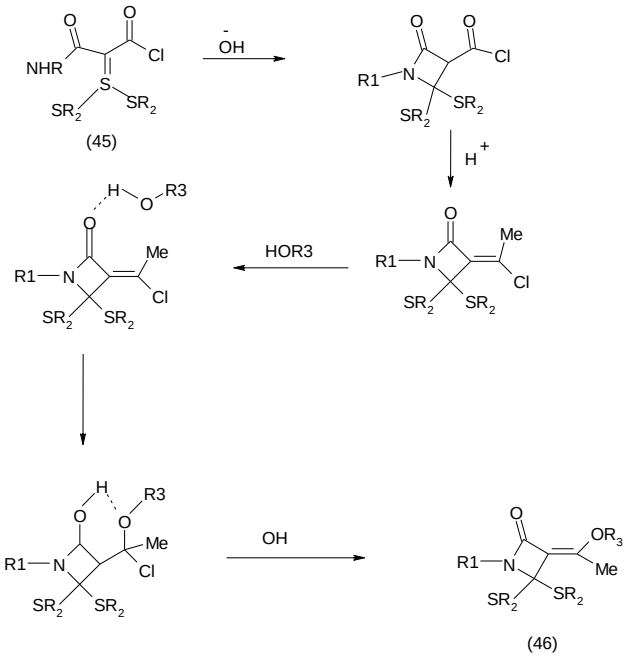
Entry	45	R^1	R^2	R^3	Time [h]	46	Yield[a] [%]
1	45a	C_6H_5	Me	Et	12	46a	69(23)
2	45b	C_6H_5	Et	Et	21	46b	61
3	45c	4-MeC_6H_4	Me	Et	15	46c	80
4	45d	4-MeC_6H_4	Et	Et	18	46d	76
5	45e	4-$MeOC_6H_4$	Me	Et	19	46e	88
6	45f	4-$MeOC_6H_4$	Et	Et	26	46f	68
7	45g	4-ClC_6H_4	Me	Et	11	46g	94
8	45h	4-ClC_6H_4	Et	Et	48	46h	67
9	45i	2,4-$Me_2C_6H_3$	Me	Et	44	46i	64
10	45j	2,4-$Me_2C_6H_3$	Et	Et	42	46j	77
11	45k	Me	Me	Et	56	46k	43(18)
12	45l	Me	Et	Et	50	46l	52(21)
13	45a	C_6H_5	Me	Me	36	46m	78
14	45b	C_6H_5	Et	Me	42	46n	56
15	45c	4-MeC_6H_4	Me	Me	38	46o	57
16	45d	4-MeC_6H_4	Et	Me	48	46p	59
17	45e	4-$MeOC_6H_4$	Me	Me	40	46q	84
18	45f	4-$MeOC_6H_4$	Et	Me	58	46r	60
19	45g	4-ClC_6H_4	Me	Me	34	46s	87
20	45h	4-ClC_6H_4	Et	Me	48	46t	63
21	45i	2,4-$Me_2C_6H_3$	Me	Me	56	46u	60
22	45j	2,4-$Me_2C_6H_3$	Et	Me	32	46v	54
23	45k	Me	Me	Me	56	46w	41(16)
24	45l	Me	Et	Me	60	46x	47
25	45a	C_6H_5	Me	i-Pr	32	46y	45
26	45a	C_6H_5	Me	t-Bu	48	46z	No reaction

[a] Isolated yields for **(E)-46** and the data in parentheses for **(Z)-46**.

Nevertheless, we provided an alternative access to α-alkylidene-β-lactams **46** via aqueous base induced cyclization of a-carbamoyl ketene-S,S-acetals **45** in the presence of alcoholic co-solvent. It should be noted that the richness of the functionality of substituted α-alkylidene-β-lactams **46**may render them versatile as synthons in further synthetic transformations, for example, upon hydrogenation or Michael addition reaction to produce the corresponding substituted β-lactams analogues bearing two chiral carbon atoms. Pak and co-workers had ever reported the synthesis of 2-quinolinonesthrough thermal cyclization of α-carbamoyl ketene-S,Sacetals**44** in NaH/DMF [LXV]. Recently, they corrected the structure of the product as substituted 4-quinolinone, and proposed an aza-Michael addition mechanism and trapped the unstable 2-azetidinoneintermediates by the use of alkyl halides. [LXVI] On the basis of our results obtained together with the reported literatures, [LXVI, LXVII] a plausible mechanism for the cyclization reaction of amides **45** is presented in

Scheme 10. The transformation commences from an intramolecular aza-Michael addition of the nitrogen atom to the unsaturated β-carbon of **45** under basic conditions, generating a carboanionic intermediate **A**. Clearly, anionic **A** is stabilized via delocalization of Negative charge to the adjacent vinyl and amide groups, and can be regarded as a 1,3-nucleophilic 3-carbon species, which subsequently undergoes protonation reaction in alcoholic aqueous media to afford intermediate B. Finally, the displacement of chloride of **B** by alkoxide via a nucleophilic vinylic substitution (S_NV) [LXVIII] reaction gives rise to 2-azetidinone **46**. During the S_NV reaction, the hydrogen bonding interaction between intermediate **B** and alcohol leads to the formation of intermediates **C** and **D**, and hence results in the high stereo selectivity of products **46**. On the other hand, alkyl thio groups might be distorted by bulkier substituents on the nitrogen atom, and this protects the attack

Scheme 10.Plausible mechanism for the cyclization of **45** in aqueous media.



Of alkoxide from the side of alkyl thio groups. Thus, when methyl group is attached to the nitrogen atom, alkoxide may attack from the side of alkyl thio groups. Actually, substituent's on the sulfur of **45**and the nucleophilicity of alkoxide also affect the
Stereochemistry.

Some Structures of b-Lactams and their Biological activity:

Antifungal activity

Modest activity against some pathogenic yeasts and filamentous fungi was reported for a cephalosporin whose side chain was an acid (*N*-benzyldithiocarbamate) having intrinsic antifungal activity. [LXIX] Much more intriguing was the finding by the same authors that the aldehyde of penicillin V showed an antifungal action. Another interesting observation that also does not seem to have been studied further, is that unidentified degradation products from aqueous solutions of some first-generation cephalosporins inhibit the growth of certain dermatophytes. [LXX]

Compounds with a modified -lactam ring

The familiar antimicrobial agents in clinical use either have an unmodified penam or cephem ring, or an alteration has been made in the larger of the two rings (e.g. clavulanate, carbapenems, latamoxef, loracarbef). The four-member β-lactam ring (azetidinone) common to both families remains unmodified. Indeed, it has become almost an article of faith that the -lactam ring is sacrosanct. How-ever, there have been other preconceptions about this group of antibiotics that have later proved on investigation to be false, e.g. that a monocyclic -lactam could never approach the activity of a bicyclic system, [LXXI] or that a free carboxyl group is essential. [LXXII] Thus this belief must be examined carefully. Changing the chemical constituents of the -lactam ring, [LXXIII] or inserting a 5–6 double bond in the penam structure (creating a 'dehydropenicillin' (**54**), [LXXIII, LXXIV] not to

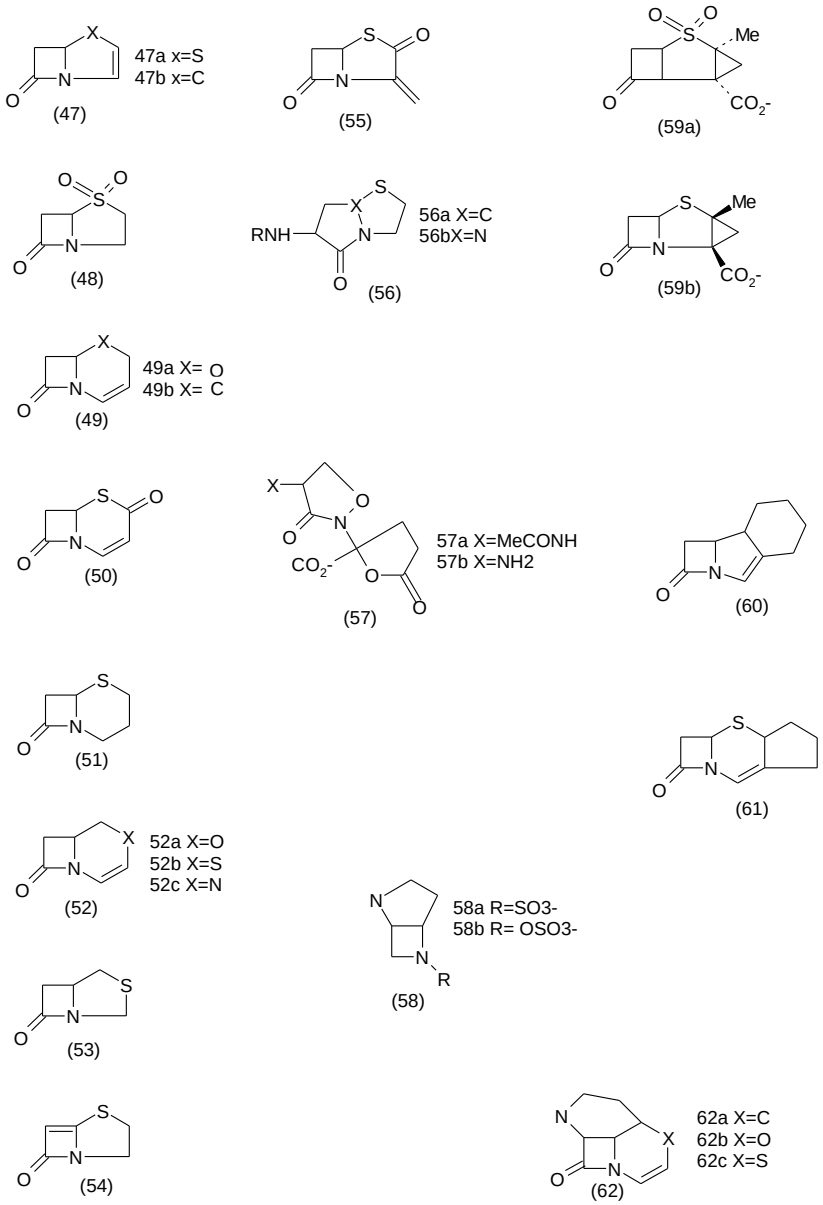


Figure2. Structures of -lactam antibiotics

be confused with an 'anhydro penicillin' (**55**) [LXXV] in which the elements of water have been removed from the thiazolidine ring) was not a successful strategy to improve antibacterial activity or to inhibit -lactamases. On the other hand, increasing the size of the ring from four-member to five-members had interesting results.

(i) Expanding the ring by a $\cdot CH_2\cdot$ moiety gives a γ–lactam (**56a**); while the compound derived from the penam nucleus was not active, its penem and carbapenem analogues did show antibacterial activity. Baldwin *et al*. [LXXIII] have reported the synthesis but not the biological activity of the -lactam analogue of ceftizoxime.

(ii) Replacing C5 in **56a**with a N atom gives aza-γ-lactam(pyrazolidinone) analogues (**56b**). Certain of these in the carbapenem series showed good activity against *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Escherichia coli*, *Enterobacter aerogenes* and *Serratiamarcescens*; however, the carbacephem analogues were devoid of activity [LXXVI, LXXVII].

(iii) It can be seen from structure **56** (in which the γ–lactam ring is fused to its five-membered neighbour), that it is not possible, for valency reasons, to substitute C5 with an oxygen atom. However, nature has provided are mark ably analogous un fused -lactam system in the form of lactivicin (**57a**). [LXXVIII] Here a -lactam ring, anisoxazolidinone closely related to cycloserine, is linked to a furan moiety. Surprisingly, lactivicin acts in many ways exactly like a 'classical' β -lactam in that it binds to penicillin binding proteins (PBPs), is a substrate for and an inducer of β-lactamases, shows increasing activity against mutants that are hypersensitive to β -lactams, and is inactive against microorganisms that do not contain mucopeptide.

These interesting properties suggested that analogues of lactivicin might be valuable antibiotics. As a result, the 4-aminolactivicinic acid (4ALA) nucleus (**XIb**), was synthesized and different side chains were added, following methods perfected for the syntheses of derivatives from the nuclei of the penicillin (6APA), cephalosporins (7ACA) and monobactams (3AMA). The 4ALA analogues of cefotaxime and cephalothin showed high activity against, respectively, Enterobacteriaceae and staphylococci, and orally available prȏd rugs were also made. [LXXIX] However, this series was not proceeded with.
An even more radical departure from conventional thinking was made by Imming, [LXXX] who synthesized penam analogues in which the -ring had been enlarged to seven or 13 members (the latter size—a -lactam—was con-side red optimal). Chemical but not biological findings are reported. [LXXX].

Compounds with extra rings

'Bridged' β -lactams have an extra ring—created by cyclization of groups outside the main ring structure(s). These fall into three categories.

(i) *Bridged monocyclic compounds*. Bridged monobactams (**58a**), sulfactams (**58b**) and other azetidinones, that contain two rings, have been reported to be good inhibitors of class C and, in some cases, class A β -lactamases. [LXXXI - LXXXIII].

(ii) *Bridged penams and carbapenems*. The tricyclic 2,3-methylene penams exist in two stereo isomeric forms ,α (**59a**) and β (**59b**). The sulphones of these compounds show interesting differential properties, the former having good antibacterial activity and inhibiting class C β -lactamases, while the latter are poor antibacterial agents but are inhibitors of class A enzymes (penicillinase type). [LXXXIV, LXXXV] Thus it seems that the isomer is recognized by β -lactamases as a penem, and the isomer as a cephemBridged carbapenems **(60)**, in which C1 and C2 are joined through a four-carbon linkage, make up the family of antibiotics given the trivial name 'tribactams', [LXXXVI] later changed to trinems. Sanfetrinem showed broad-spectrum activity [LXXXVII] and oral availability as a prod rug, but the future of this series is uncertain.

(iii)*Bridged cephems and analogues*. The first tricyclic cephems, bridged between C2 and C3 **(61)**, were reported
More than 25 years ago. [LXXXVIII] They had less antibacterial activity than their corresponding unabridged analogues.
Tricyclic carbacephems (**62a**), with a bridge between C1 and N7\ , were at first thought to have no useful activity, [LXXXIX] but later derivatives both showed significant anti-bacterial activity and inhibited class C -lactamases. [XC, XCI]

Bridged iso-oxa (**64**) and isocephems (**62c**) were found to be better inhibitors of class C -lactamases than the bridged monobactams, and furthermore, unlike the monobactams, some (especially the isocephem derivatives) had broad-spectrum antibacterial activity as well. [XCII]

Interactions with -amino butyric acid (GABA)

Examination of the three-dimensional structures of 3AMA, 6APA, 7ACA and the nocardicin nucleus (3-aminonocardicinic acid) show them to be conformation ally rigid analogues of GABA, and as such they act as com-petitive inhibitors of GABA aminotransferase. [XCIII] Inhibition of this enzyme has an anti-convulsive effect.

This relationship is fascinating because -lactam anti-biotics in current use are known to be capable of having precisely the opposite effect—i.e. to cause convulsions. It is suggested that this epileptogenic activity may be due to inhibition of binding of GABA to its receptors. [XCIV]

Inhibition of human and viral serine proteases

PBPs and many -lactamases have a serine motif at their active centers, a property they share with a large class of enzymes known as serine proteases. Several of the latter have been found to be inhibited by certain β -lactams.

(i) *Human leucocyte elastase* is inhibited by cephalosporin sulphones. [XCV] The most active compounds had IC_{50} 1 mg/L. Inappropriate activity of this enzyme has been implicated in the tissue damage observed in certain chronic conditions, such as cystic fibrosis, rheumatoid arthritis and emphysema. Further studies have identified inhibitors among cephems, penams, penems, monobactams and other related structures. [XCVI]

(ii) *Chymotrypsin* and to a lesser extent *thrombin* were inhibited by some of the sulphone analogues synthesized by Doherty *et al*. [XCV] (IC_{50} 1–10 mg/L).

(iii) *Protease from cytomegalovirus* (assemblin) is inhibited by monocyclic β-lactams. [XCVII] serine proteases (including β-lactamases), covalent inactivation of the active site serine in assemblin has been reported. [XCVIII] This enzyme is important in capsid assembly, so inhibitors may point the way to antiviral agents.

Inhibition of HIV protease

Benzylpenicillin was the starting point for the synthesis of a series of compounds with great inhibitory activity against HIV protease (an aspartate protease). The most active member had an ED_{50} in a syncytium formation assay of50 nM. Unfortunately, none of the

Compounds had satisfactory pharmacokinetic properties, and this line of research has been terminated. [XCIX]

Delivery of anticancer drugs

Advantage can be taken of the unique way in which many cephalosporins fragment when their β-lactam ring is broken, namely ejection of their substituent at C3. Attention has been drawn previously [C] to the exploitation of this mechanism in 'dual action' antibiotics: here, a new antibacterial compound is produced if the original is attacked

By a β-lactamase. Examples are cephalosporin MCO (which releases pyrothione, an antiseptic) and the cephalosporin/fluoro quinolone hybrids synthesized by Roche, which act as cephalosporins until hydrolyzed, when a fluoro quinolone is released. This process has now been

taken a stage further in the design of targeted anticancer prod rugs. [CI] The strategy is as follows: a conjugate of -lactamase with a monoclonal antibody specific for tumor-associated antigens binds to malignant cells. Then a prod rug consisting of an adduct of doxorubicin, a vinca alkaloid or a nitrogen mustard with a cephalosporin—a covalent bond having been made at the C3 position—is administered. The prod rug is activated only at the surface of the tumour, where the -lactamase is bound: breaking the -lactam bond causes ejection of the free cytotoxic drug. The advantage of this procedure is that the anticancer agent is much less toxic as a prodrug, so systemic toxicity is reduced.
Reduction in overall toxicity of an anticancer agent has also been reported by the
Reaction of a retinoid with an isocephem, via an amide linkage at the C4 position. [CII] It is of interest that the isocephem, which had the same side chain as benzyl penicillin, was highly microbiologically active, although its retinoid conjugate was not.

Conclusions

Synthesized series of N-[3-chloro-2-(substituted) alkyl/aryl-4-oxoazetidin-1-yl]-1-carboxamido-2-methyl-1H-benzimidazoles. Among the synthesized benzimidazoles, compounds with alkyl, phenyl and hydroxyphenyl at 4-position of azetidin-2-one were found to increase the antibacterial activity. Compounds with phenyl and hydroxyl phenyl
Substituent at azetidin-2-one sub-unit showed good antibacterial and antifungal activities. More extensive study is needed to confirm the preliminary results and mode of action
Studies are required to be able to optimize the effectiveness of this series of compounds.

We have described a facile and efficient synthesis of substituted α-alkylidene-β-lactams of type **46** via NaOH-promoted intermolecular cyclization reaction of α -carbamoyl, α-(1-chlorovinyl) ketene-S,S-acetals **45** in alcoholic aqueous media. The key cyclization involves intermolecular aza-Michael addition of **45**and subsequent S_NV reaction with alcohol under basic conditions. The simplicity of execution, ready availability of substrates, and important synthetic potential of products make this synthetic
Strategy attractive and practical. Further studies on the expansion of the scope and synthetic utility of this protocol are in progress.

Penicillin have been in clinical use for 55 years, and cephalosporins for about 20 years less. Their evolution as antibacterial agents has slowed from the breakneck pace of the 1960s and 1970s, and it is now not easy to discern in which directions much further advance can be made. However, as outlined above, the ingenuity of medicinal chemists combined with the extreme versatility of these molecules means that their development can by no means yet be regarded as at the end of the line. Surely we will soon see a useful inhibitor of class C β-lactamases and, as for other pharmacological applications, perhaps we should be prepared for the unexpected. [CIII]
Also we synthesized series of N-[3-chloro-2-(substituted) alkyl/aryl-4-oxoazetidin-1-yl]-1-carboxamido-2-methyl-1H-benzimidazoles. Among the synthesized benzimidazoles, compounds with alkyl, phenyl and hydroxyphenyl at 4-position of azetidin-2-one were found to increase the antibacterial activity. Compounds with phenyl and hydroxyphenyl
Substituent at azetidin-2-one sub-unit showed good antibacterial and antifungal activities. More extensive study is needed to confirm the preliminary results and mode of action
Studies are required to be able to optimize the effectiveness of this series of compounds.

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Transparency declarations
All other authors: none to declare.

Contributions
N.A.A. Elkanzi Draw structure, table,revise and wrote the manuscript, Nesrin M. Share in wrote the manuscript and table.

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