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A Comprehensive Review On Structural and Biological Activity Relationship of 3-Acetoxyazetidin-2-Ones

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ABSTRACT

Azetidin-2-ones (β-lactams) have been a mainstay as therapeutics for the treatment of a wide range of bacterial infections. The interest in β-lactams stems from their highly unusual structure-activity profiles towards a genera of pathogenic bacteria. Tremendous efforts have been made in the literature towards the development of novel β-lactam compounds for a study of their biological evaluation. The present review begins with a brief introduction of β-lactam antibiotics. This is preceded by the progress made towards the biological evaluation of a variety of racemic as well as optically active *cis*- or *trans*-3-acetoxyazetidin-2-ones. Their application in medicinal field as antifungal, antibacterial, antitumor, anticancer, IMR32 cytotoxic agents and carbonic anhydrase inhibitors is discussed.

Keywords: 3-Acetoxyazetidin-2-ones, antifungal, antibacterial, antitumor, anticancer, carbonic anhydrase inhibitor

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INTRODUCTION

Microbes produce a large number of secondary metabolites that are of great therapeutic value as anticancer drugs (e.g. doxorubicin), immunosuppressors (e.g. cyclosporins) and antibiotics (such as β-lactam antibiotics and erythromycin).[1] Antibiotics are of major clinical importance to combat bacterial infections in man and animals (cattle). Since the late 1930's, bacterial infections were cured with synthetic chemical substances derived from sulfanilamide or *p*-aminobenzene sulphonamide, the so called sulfa drugs. These compounds have been replaced by natural microbial products which include aminoglycosides (e.g. streptomycin, neomycin), penicillins (e.g. penicillin G), cephalosporins (e.g. cephalosporin C), tetracyclines (e.g. chlorotetracycline, oxytetracycline), macrolides (e.g. erthyromycin).[2]

First synthesized in 1907 by Staudinger,[3] the four membered cyclic amide derivatives of 3-aminopropionic acids known as β-lactams did not come to the fore in organic chemistry until Fleming's landmark 1929 discovery of penicillin.[4] Today, thousands of chiral compounds containing β-lactam rings are known. Whether isolated from natural sources or chemically synthesized, they are marked by high efficacy and safe toxicological profiles, so more than 70 years after its initial discovery, penicillin **A** and its derivatives are still the most commonly used antibiotics.[5] Also, various β-lactam derivatives such as cephalosporins **B**, carbapenems **C**, nocardicins **D** and monobactams **E** are known for their anti-microbial activity and pharmacokinetic performance (Figure 1).[6]

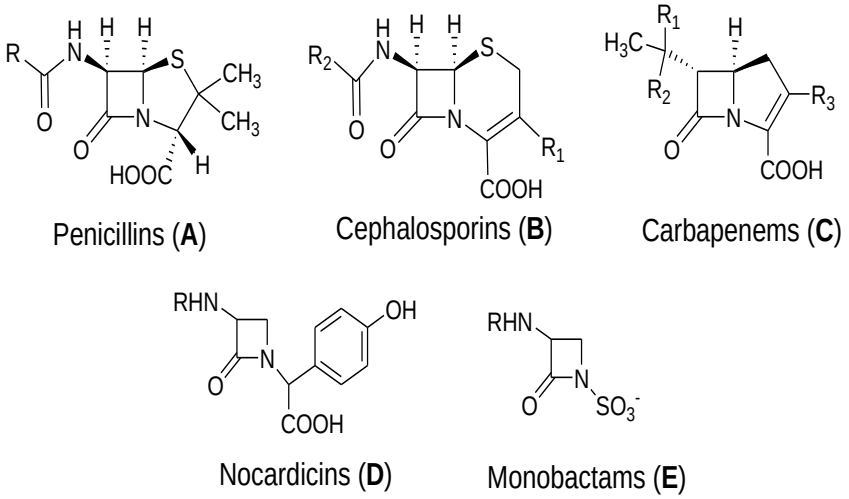


Figure 1: Clinically relevant β-lactam antibiotics

β-Lactam scaffold has also been used as a synthon in the preparation of various non-β-lactam compounds of biological significance.[7] Some of the most notable discoveries on β-lactams concern the development of potential quality-of-life applications such as human leukocyte elastase

inhibitors, human cytomegalovirus protease inhibitors, thrombin inhibitors, cholesterol acyl transferase inhibitors, matrix-metallo protease inhibitors, cysteine protease inhibitors, gene activators, apoptosis inductors, and β-turn nucleators.[8] The abilities of mono- and polycyclic β-lactams to inhibit serine-enzymes lie mainly in the β-lactam ring. Its action consists of kidnapping the Ser residue at the active site of the enzyme by a nucleophilic attack that opens the β-lactam ring and produces a stable acyl-enzyme covalent complex that turns the enzyme inactive.[9]

Also, β-lactams have been used for assembly of the C-13 side chain in the antitumor drug paclitaxel,[10] have found new pharmaceutical applications such as LHRH antagonists[11] and cholesterol absorption inhibitors.[12] The ring strain of the β-lactam skeleton facilitates ring opening reactions,[13] and this unique property has been exploited for the synthesis of a variety of medicinally active compounds.

The biological activity of a particular β-lactam ring is influenced by the type of substitution attached to the basic nucleus. To cite few examples, Ezetimibe **F** bearing a C-3 alkyl-aryl side chain, has been used clinically for the treatment of hypercholesterolemia[14] whereas 3-methoxy-*N*-thiolated monobactams **G** have been found to induce apoptosis and to inhibit tumour cell growth.[15] Spiro-β-lactam **H** acts as poliovirus and human rhinovirus 3C-proteinases inhibitors.[16] β-Lactams with acetate functionality at C-4 of the ring *viz.*, (3*R*,4*R*)-4-acetoxy-3-[(1′*R*-*tert*-butyldimethylsilyloxy)ethyl]-2-azetidinone and its derivatives **I** are well known to be highly versatile intermediates for the synthesis of thienamycin and carbapenem derivatives and other novel antibiotics that might defeat bacterial resistance (Figure 2).[17]

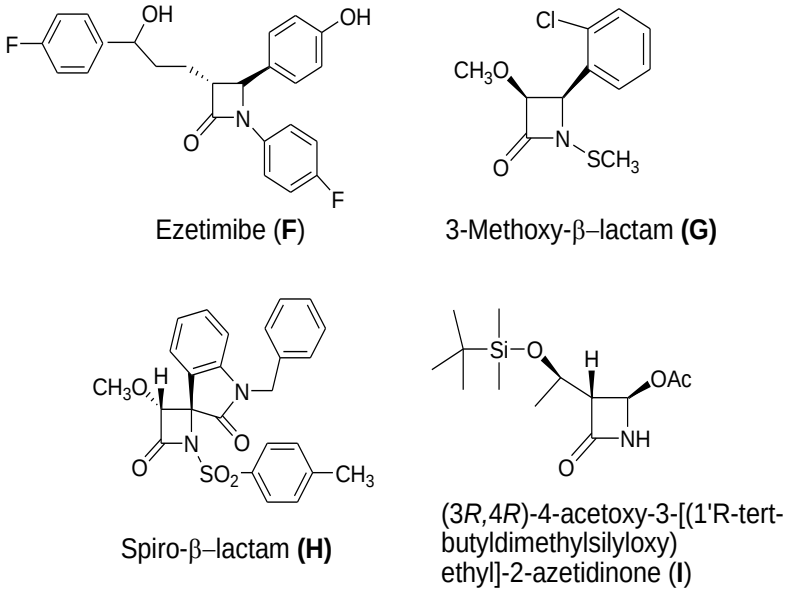


Figure 2: Biologically active β-lactam antibiotics

The unique characteristics and in vivo reactivity of the β-lactam make it a coveted pharmacophore to assemble and a valuable synthetic building block to transform. It can be chemically constructed through cyclization reactions, cycloaddition reactions, organometallic-mediated reactions, and other miscellaneous approaches such as ring contraction and radical processes. Additionally, studying the formation and destruction of the β-lactam ring has provided remarkable insight to chemical reactivity and biochemical mechanisms. From the chemist's perspective, both the synthesis of the β-lactam scaffold and the understanding of its reactivity as a synthetic building block (a synthon) to other non-β-lactam structures are pivotal in illuminating in vivo structure−activity relationships (SAR), accessing/studying novel compounds, and allotting new possibilities for drug discovery.

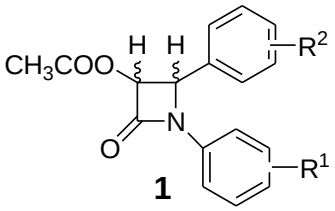
Our research group[8,18] has been actively engaged in the synthetic β-lactam chemistry for example novel β-lactam precursors, 3-thio/seleno-β-lactams and their Lewis acid mediated functionalization, stereoselective *cis*- and *trans*-3-alkoxy-β-lactams, spirocyclic-β-lactams, α-keto-β-lactams, bicyclic-β-lactams, novel 4-pyrazolyl-β-lactams, 4-pyrazolylspirocyclic-β-lactams and (*E*)- and (*Z*)-3-allylidene-β-lactams.

The present review aims to cover β-lactams with an acetate functionality at C-3 of the β-lactam ring for their biological evaluation against a variety of bacterial strains. The focus of this present study is thus, to understand the role of stereochemistry (*cis*/*trans* at C-3/C-4 stereocentres) as well as the substituents linked to C-4 and N-1 of 3-acetoxy-β-lactams in the enhancement or depletion of a particular biological activity. The review covers a range of structural variations amongst the C-4 and N-1 substituents, differing in their polarities, lipophilicities, steric requirements, hydrogen bonding capabilities, size and orientation of the aryl substituents. The biological evaluation of a variety of racemic as well as optically active 3-acetoxy-β-lactams as potent bioactive molecules is discussed, the presence of acetate functionality at C-3 being obligatory for the potent bioactive nature.

A. 3-Acetoxyazetidin-2-ones: Antifungal and antibacterial agents

Walsh and co-workers[19] have reported a series of monocyclic 3-acetoxyazetidin-2-ones **1,2** (Figures 3-5), bicyclic 7-acetoxy-6-phenylcephams **3** and their oxidized analogues **4**,**5** (Figure 6). These β-lactams were screened for antifungal activity against *Candida neoformans*, *Candida albicans*, *Candida parapsilosis*, *Candida tropicalis*, *Candida glabrata* and *Trichosporon cutaneum* (MIC evaluated by method of progressive double dilutions in liquid Casitone medium). Antibacterial activity was tested against *Staphylococcus aureus*, *Proteus vulgaris*, *Pseudomonas*

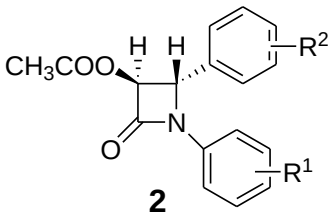
aeruginosa, *Bacillus subtilis*, *Klebsiella aerogenes*, *Escherischia coli* and *Streptococcus faecalis* (by radial growth assay procedure) using Ampicillin as the standard antibiotic.



1

R^1 = H, 4-OCH_3, 4-Br, 4-CO_2CH_3

R^2 = H, 3,4-OCH_2O, 4-Cl, 4-F, 3,4-$(OCH_3)_2$, 2,3-CH=CHCH=CH-, 4-NO_2, 4-OCH_3, 4-CH_3



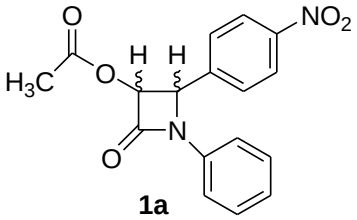
2

R^1 = 4-COOH, 3-COOH

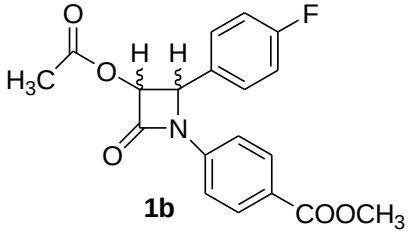
R^2 = 4-OCH_3, H, 4-OC_2H_5, 4-CH_3, 3,4-OCH_2O, 4-Br, 4-F

Figure 3: 3-Acetoxy-1,4-diarylazetidin-2-ones 1 and *trans*-3-acetoxy-4-aryl-1-carboxyphenylazetidin-2-ones 2

3-Acetoxy-4-(4′-nitrophenyl)-1-phenylazetidin-2-one **1a** has been found to be the most effective antifungal agent, with MIC < 18.4 μM against *Candida neoformans*, *Candida albicans*, *Candida parapsilosis*, *Candida tropicalis* and *Candida glabrata*. Also, 3-acetoxy-4-(4′-fluorophenyl)-azetidin-2-one **1b** exhibited MIC < 8.4 mM against *Candida tropicalis* and *Trichosporon cutaneum* (Figure 4).



1a



1b

Figure 4: 3-Acetoxy-1,4-diarylazetidin-2-ones 1a,b

Monocyclic β-lactams possessing a free carboxylic acid functionality have gained importance for antibacterial and antifungal activity evaluations.[20] Taking a clue from this, *trans*-3-acetoxy-1,4-diarylazetidin-2-ones **2** bearing a free carboxylic acid group on the *N*-aryl were synthesized by Walsh and co-workers.[19] Only *trans*-3-acetoxy-4-phenyl-1-(4′-carboxyphenyl)-azetidin-2-one **2a** was antifungal against *Candida glabrata* with MIC < 18.4 μM (Figure 5). All the newly synthesized β-lactams **2** displayed antibacterial activity against *Staphylococcus aureus*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Klebsiella aerogenes* and *Escherischia coli* with zones of inhibition < 9 mm (in diameter) at concentrations 2.48-3.08 mM. Particularly, *trans*-3-acetoxy-4-(4′-ethoxyphenyl)-1-(4′-carboxyphenyl)-azetidin-2-one **2b** and *trans*-3-acetoxy-1-(3′-carboxyphenyl)-azetidin-2-one **2c** inhibited *Staphylococcus aureus* bacteria at 0.054 mM

concentration. Further, **2a** and **2b** inhibited *Escherischia coli* at 0.062 and 0.054 mM concentrations (Figure 5).

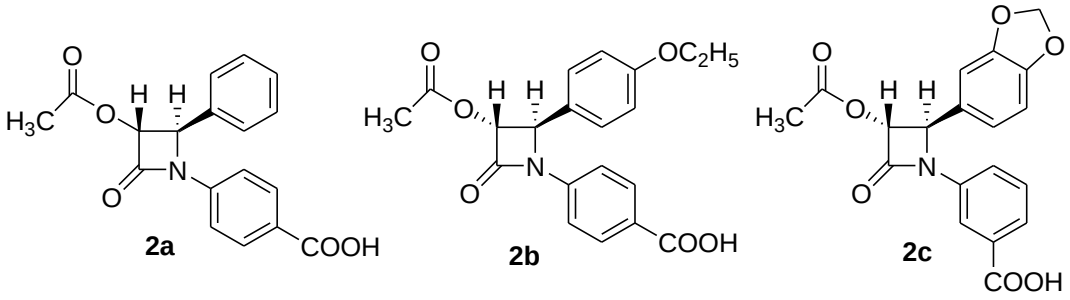


Figure 5: *trans*-3-Acetoxy-4-aryl-1-carboxyphenylazetidin-2-ones 2a-c

A free carboxylic acid group located at C-3 of penicillins, C-4 of cephalosporins and C-2 of carbapenems serves as an essential feature contributing towards the antibacterial activity of bicyclic β-lactams.[21] For the bicyclic cepham derivatives (Figure 6), the antifungal biological evaluation studies displayed results that 7-acetoxy-6-phenylcepham sulfoxide **4** (with MIC < 20.47 μM; except for *Trichosporon cutaneum*) and 7-acetoxy-6-phenylcepham sulfone **5** (with MIC < 19.41 μM) were more active than bicyclic 7-acetoxy-6-phenylcepham (7-acetoxy-6-phenyl-5-thia-1-azabicyclo[4.2.0]octan-8-one) **3** (with MIC > 45.12 μM) against all organisms.

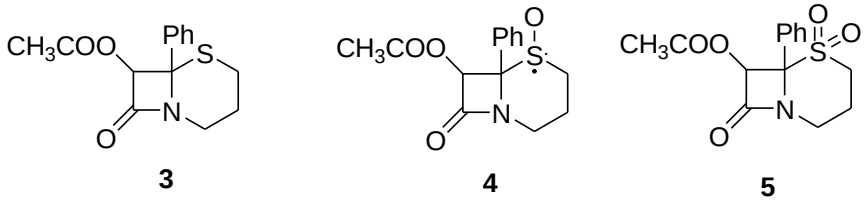


Figure 6: 7-Acetoxy-6-phenylcepham 3, 7-acetoxy-6-phenylcepham sulfoxide 4 and 7-acetoxy-6-phenylcepham sulfone 5

B. 3-Acetoxyazetidin-2-ones: IMR32 cytotoxic and antitumor agents

A potent naturally occurring antimitotic agent, combretastatin A-4 (CA4) **6** inhibits cellular tubulin polymerization by binding to the colchicine site (Figure 7).[22] It exhibits strong cytotoxicity against a variety of human cancer cells, with *Z*-geometry of the two aromatic rings linked by the alkene group being an essential feature for biological activity, however *E*-isomer being inactive. Replacement of the double bond in the CA4 structure with cyclic heterocyclic moieties tends to reduce the isomerization of biologically active *Z*-CA4 to more stable but biologically inactive *E*-CA4.[23] Also, *Z*-CA4 exerts cytotoxic activity against human neuroblastoma cells (IMR32), determined using a standard MTT assay kit.[24] Presence of 3,4,5-trimethoxy substituents in one of the aromatic rings in CA4 and its heterocyclic ring analogues has been demonstrated to be essential for its biological activity.[25] In this context, Fuselier et al.[26] have synthesized a series of

cis-3-acetoxyazetidin-2-ones **7** bearing 3,4,5-trimethoxy substituent in one of the aromatic rings of R^1/R^2, as conformationally restricted analogues of CA4 **6** because molecular modeling studies highlight close geometric similarities (Figure 7).

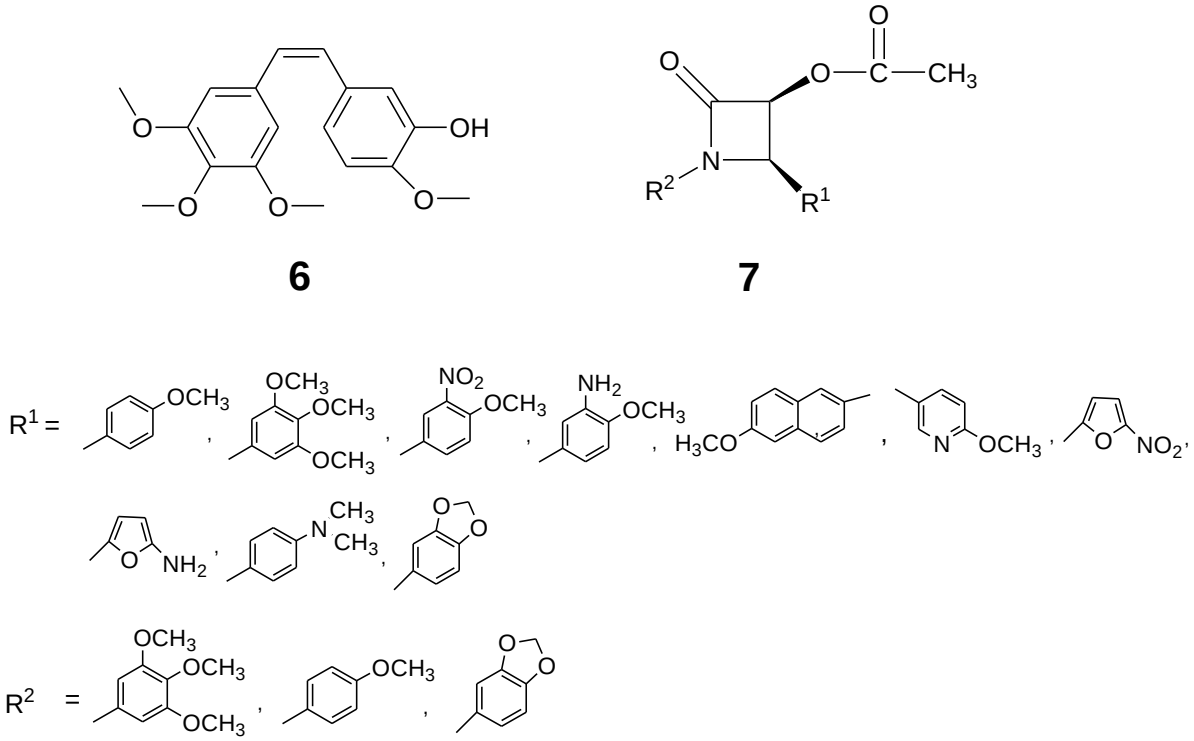


Figure 7: Combretastatin A-4 6 and *cis*-3-acetoxy-1,4-diarylazetidin-2-ones 7

These were evaluated for cytotoxic activity against human neuroblastoma cells (IMR32). *cis*-3-Acetoxy-β-lactams bearing 3,4,5-trimethoxyphenyl substituent on *N*-1 and 4-methoxyphenyl (IC_{50} 251 nM)/benzo[1,3]dioxol-5-yl (IC_{50} 910 nM) substitutent on C-4 **7a**/**7b** (Figure 8) exhibited superior cytotoxic activity than similar *cis*-3-acetoxy-β-lactams in which aromatic rings were reversed viz. 3,4,5-trimethoxyphenyl substituent on C-4 and 4-methoxyphenyl (IC_{50} > 1000 nM)/benzo[1,3]dioxol-5-yl (IC_{50} > 1000 nM) substitutent on N-1 **7c**/**7d** (Figure 8). A carbonyl group directed towards 3,4,5-trimethoxyphenyl substitutent in **7a**/**7b** accounts for the superior biogical activity, as reported for CA4 like compound podophyllotoxin.[27] For the *cis*-3-acetoxy-β-lactams bearing 3,4,5-trimethoxyphenyl substituent on N-1, best activity was observed for **7e** and **7f** bearing 4-methoxy-3-nitrophenyl (IC_{50} 123 nM) and 3-amino-4-methoxyphenyl (IC_{50} 73 nM) groups, on C-4 of β-lactam ring (Figure 8).

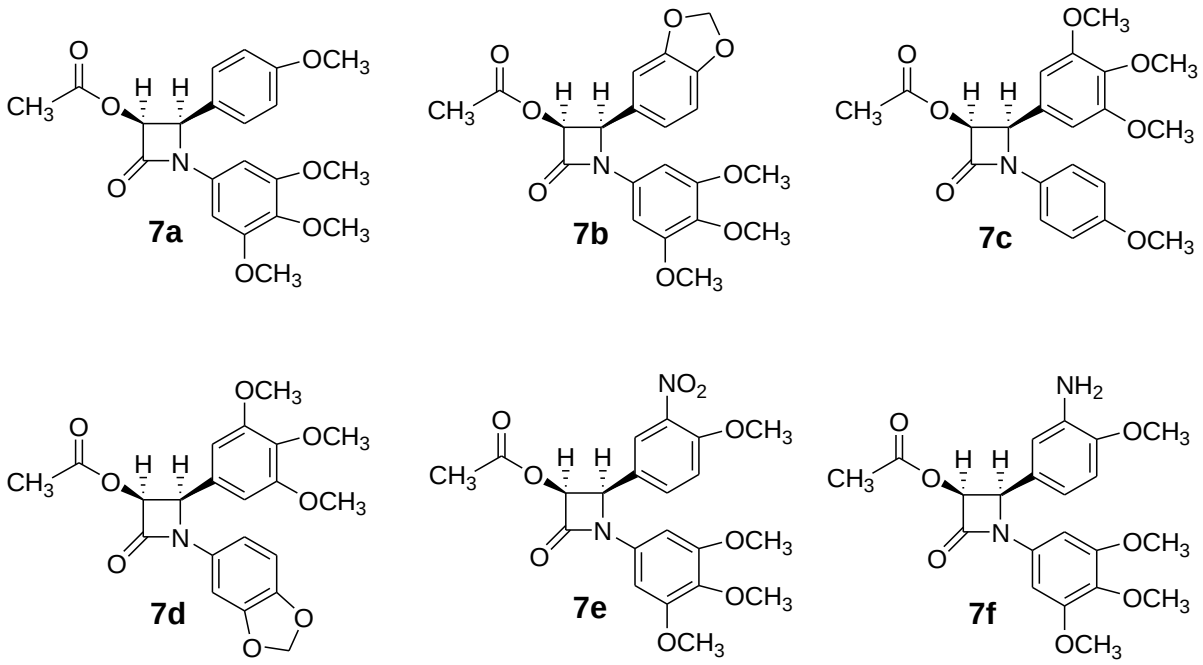


Figure 8: *cis*-3-Acetoxy-1,4-diarylazetidin-2-ones 7a-f

The synthesized *cis*-3-acetoxy-β-lactams **7e**,**f** were evaluated against twelve tumor cell lines[26] *viz.* Chinese hamster ovary, Human renal adenocarcinoma, Prostate carcinoma, Human neuroblastoma, Human lung carcinoma, Human breast carcinoma, Human colon carcinoma, Human glioblastoma, Human cervix adenocarcinoma, Human pancreatic cancer, Human colon adenocarcinoma, Rat pancreatic tumor and Human leukemia. **7e** and **7f** exhibited inhibitory concentration IC_{50} in the range 105.3-595.4 nM and 70.46-461.6 nM respectively. However, IC_{50} > 10000 nM against all the cell lines was shown by β-lactam **7d** (Figure 8).

Authors also investigated the stability of 3-acetoxy-β-lactams **7f** in rat serum and phosphate buffer. Complete stability was observed in buffer however, rapid degradation was observed in rat serum with half-lives of around 15 minutes. Shorter serum half-lives could lead to lower toxicity depending on the route of administration.

C. 3-Acetoxyazetidin-2-ones: Carbonic anhydrase inhibitors

Carbonic anhydrase (CAs, E.C. 4.2.1.1) are metalloenzymes containing Zn^{2+} ion, which reversibly catalyse hydration of CO_2 to bicarbonate ion.[28] They are involved in multiple physiologic or pathologic processes, such as respiration and transport of CO_2/bicarbonate between metabolizing tissues and lungs, pH and CO_2 homeostasis and tumorigenecity.[29] Phthalazines **8** are an important class of nitrogen containing heterocycles (Figure 9). Derivatized phthalazines have been known to exhibit anticonvulsant,[30a] antihypertensive,[30b] antiinflammatory[31a] and antimicrobial activities,[31b] thereby, serving as important therapeutic agents.

Under these considerations, Gencer and co-workers[32] have synthesized derivatized phthalazine substituted 3-acetoxy-β-lactams **9** (Figure 9) and examined their inhibitory effects on purified human carbonic anhydrase (hCA I and II). All the synthesized β-lactams **9a-e** inhibited CA isoenzyme activity, when subjected to CA inhibition assay using CO_2 as the substrate. Inhibitory concentration IC_{50} values were obtained in the range 6.97-15.11 μM and 8.48-17.48 μM for slow cytosolic isoform, hCA I and second off target isoform, hCA II respectively. **9a** with IC_{50} = 6.97 μM for hCA I and 8.48 μM for hCA II served as the best hCA I and hCA II inhibitor, amongst the synthesized 3-acetoxy-β-lactams **9a-e**.

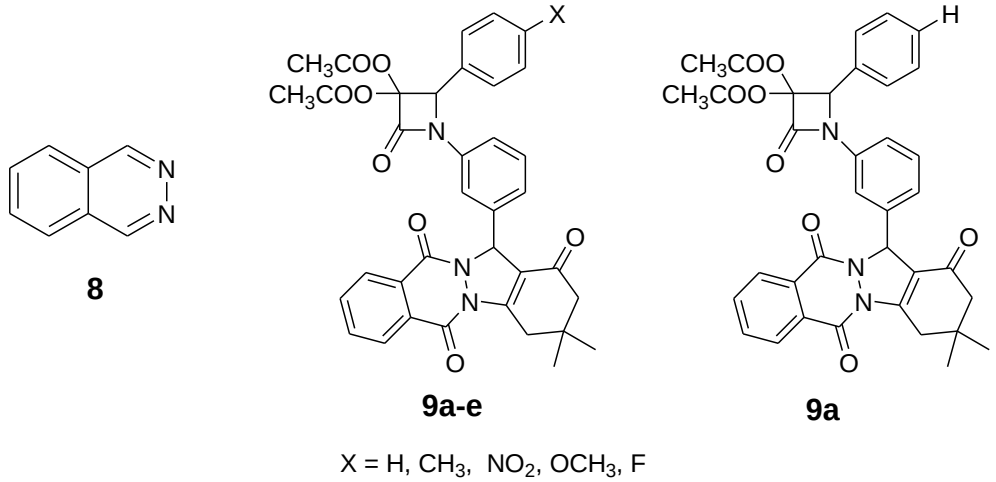


Figure 9: Phthalazine 8 and phthalazine substituted 3-acetoxyazetidin-2-ones 9a-e

Authors have correlated the enzyme inhibition to the bulkiness of the phthalazine moiety. Binding occurs similar to coumarin groups.[33] Interaction between metalloenzyme active site and β-lactam leads to a transition state, formed by the initial attack of zinc-bound hydroxide ion to β-lactam carbonyl. Further, co-ordination of adjacent Zn^{2+} ion to O^- stabilizes the generated anionic transition state. This is followed by C-N bond cleavage due to strain of the β-lactam ring (Figure 10).

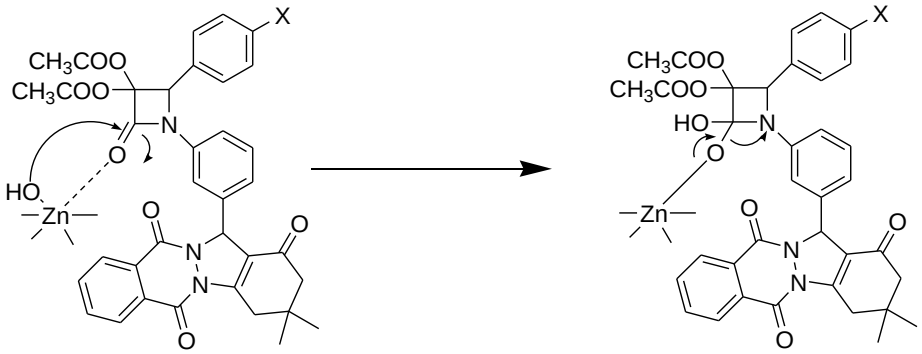


Figure 10: Proposed mechanism of the enzyme interaction with derivatized phthalazine substituted azetidin-2-ones

D. 3-Acetoxyazetidin-2-ones: Antibacterial agents

Turos and co-workers[34] have worked upon the synthesis of *N*-thiolated-β-lactams **10** that possess promising antibacterial properties directed towards *Staphylococcus* bacteria, including methicillin-resistant strains of *Staphylococcus aureus* (MRSA). Extending these studies further, Turos et al.[35] synthesized a group of 3-acetoxy-*N*-methylthio-azetidin-2-ones **11** (Figure 11) and tested them for antibacterial activity against β–lactamase producing one MSSA strain (ATCC 25923), nine MRSA strains (ATCC 43300) and eight clinal isolates from a local hospital (by Kirby-Bauer method of well diffusion). Penicillin (Pen G) and vancomycin (Van) were chosen as the standard.

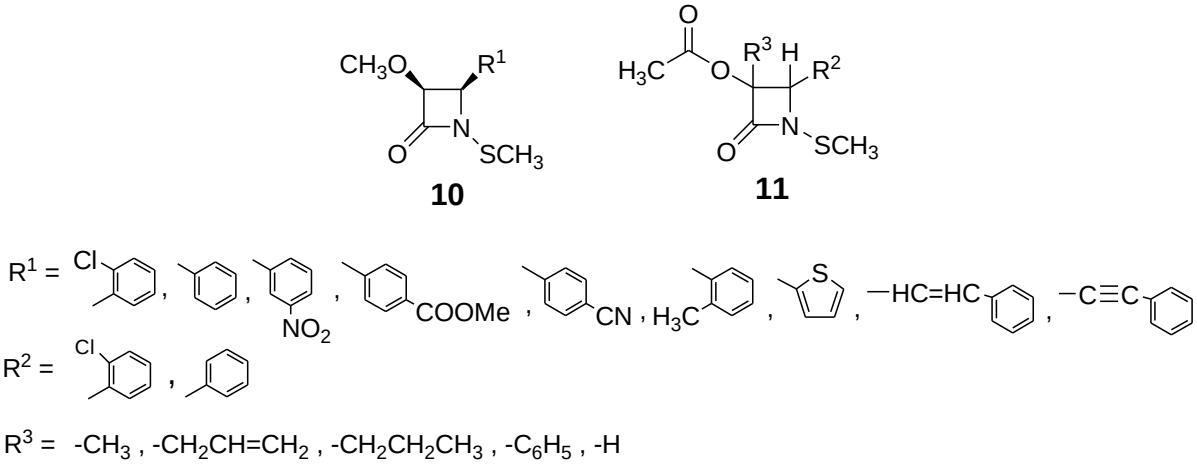


Figure 11: 3-Methoxy *N*-methylthioazetidin-2-ones 10 and 3-acetoxy-*N*-methylthioazetidin-2-ones 11

The zone inhibition data for 3-acetoxy-*N*-thiolatedazetidin-2-ones **11** indicated these to be more potent than Pen G against all the bacterial strains. 3,3-Disubstituted compounds **11b-e** (Figure 12) were more potent than the corresponding 3-monosubstituted analogue **11a** indicating that steric crowding on the ring enhances bioactivity. The saturated alkyl side chain (methyl and propyl) analogues **11b,d** were atleast 33% more potent than **11c,e** bearing allyl or phenyl groups. MIC values for these 3-acetoxy-β-lactams were observed between 16-64 μg/mL, with the order being **11d**>**11b**>**11e**>**11c**. A comparison of the *cis*- and *trans*-3-acetoxy-*N*-methylthio-β-lactams **11a** and **11′a** highlighted *trans* isomer to be about 10% more active than the *cis* isomer, but with similar zone of inhibition in the mid-20 mm range (Figure 12).

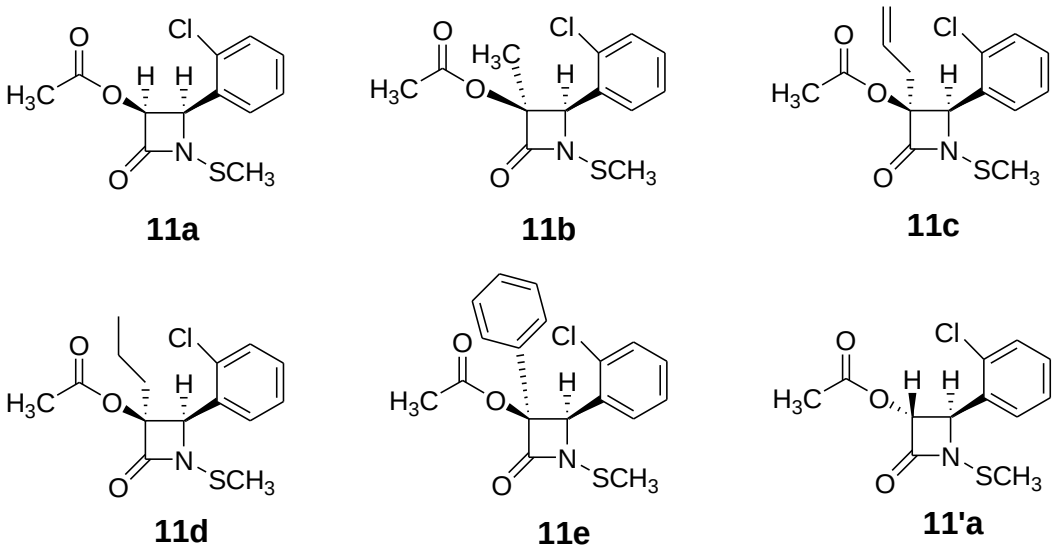


Figure 12: 3-Acetoxy-*N*-methylthioazetidin-2-ones 11a-e, 11′a

The effect of absolute stereochemistry on bioactivity was studied for antimicrobial evaluation of enantiomeric *cis*-3-acetoxy-*N*-thiolated-β-lactams **(-)-11f** and **(+)-11′f** synthesized by asymmetric synthesis (Figure 13).[35] Broth dilution experiments indicated equal anti-microbial activity against MRSA strains, equal zone of growth inhibition on agar plates as well as equivalent MIC in both antipodes of **11f**. This dictates that neither relative nor absolute stereochemistry is a factor in anti-MRSA activity.

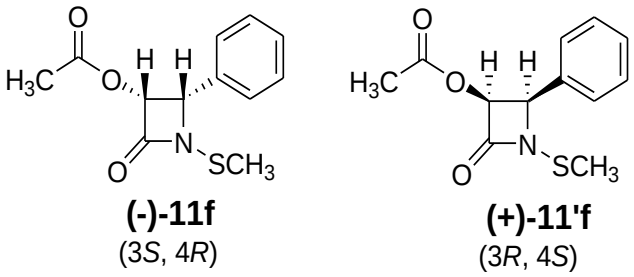


Figure 13: Optically active *cis*-3-acetoxy-*N*-thiolated-β-lactams (-)-11f and (+)-11′f

In continuation, Turos and co-workers[36] studied the effect of absolute stereochemistry within the *N*-organothio side-chain on anti-MRSA activity. The research group independently examined stereoisomeric forms of *cis*-3-acetoxy-*N*-*sec*-butylthioazetidin-2-ones **12a-d** (Figure 14). β-Lactams **12a**,**b** exhibited equivalent activity against nine clinical traits of MRSA, however, these were less potent than enantiomeric **12c,d**. It is thereby suggested that the absolute stereochemistry of the lactam ring affects the antibacterial activity with a chiral *N*-organothio functionality.

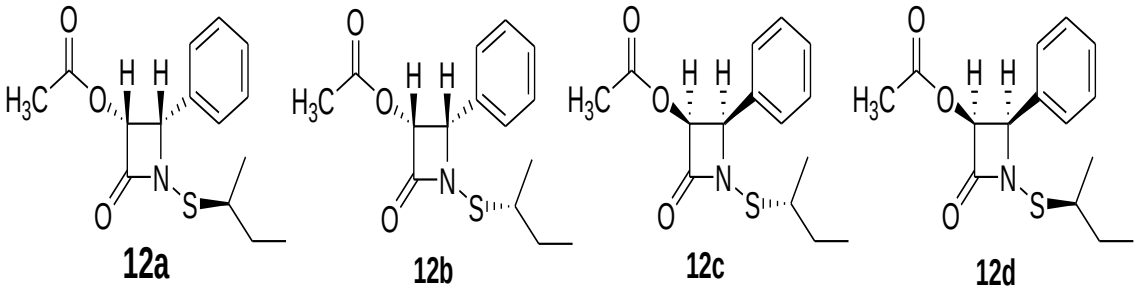


Figure 14: Optically active 3-acetoxy-*N*-*sec*-butylthioazetidin-2-ones 12a-d

Authors propose *N*-thiolated-β-lactams **11**,**12** to react covalently with their biological target (here represented as nucleophile; nucleophile attacks directly on the sulphur centre) by transfer of sulphide side-chain upon passing through the bacterial cell membrane (Figure 15).[35] Lower bacteriostatic activity is expected for compounds that are too polar to get through the membrane. On the other hand, compounds with too much lipophilicity may be sequestered in the membrane or internal organelles, thereby, lowering the effective concentration in the cytoplasm. No dependence on relative or absolute stereochemistry suggests that the β-lactam may not experience significant non-bonding interactions with its biological target prior to transferring the sulfenyl side chain.

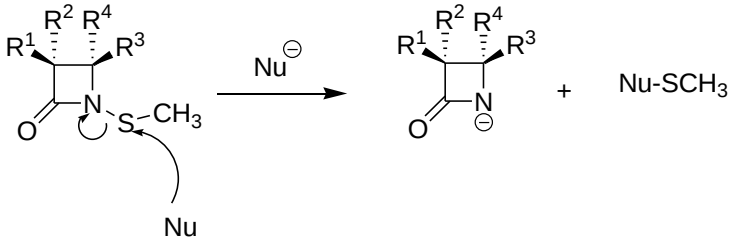


Figure 15: Proposed mechanism for interaction of *N*-thiolated-β-lactam with biological target

E. 3-Acetoxyazetidin-2-ones: Anticancer agents

Banik and co-workers[37] have been engaged in the synthesis and biological evaluation of some derivatives of polyaromatic amines, which are open-chain amides **13a,b** and **13′a,b** bounded with polycyclic residue (Figure 16). Conformationally constrained molecules often have a greater effect on biological properties than relatively flexible open-chain compounds.[38]

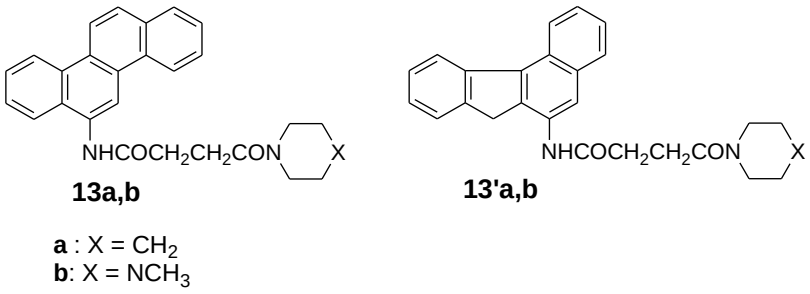


Figure 16: Open-chain amides bearing polyaromatic groups 13a,b and 13′a,b

Further, Banik research group[39] have synthesized conformationally restricted analogues of the potent compounds **13** *viz*., *trans*-3-acetoxy-β-lactams **14** bearing aromatic/polyaromatic moieties on the β-lactam nitrogen (Figure 17).

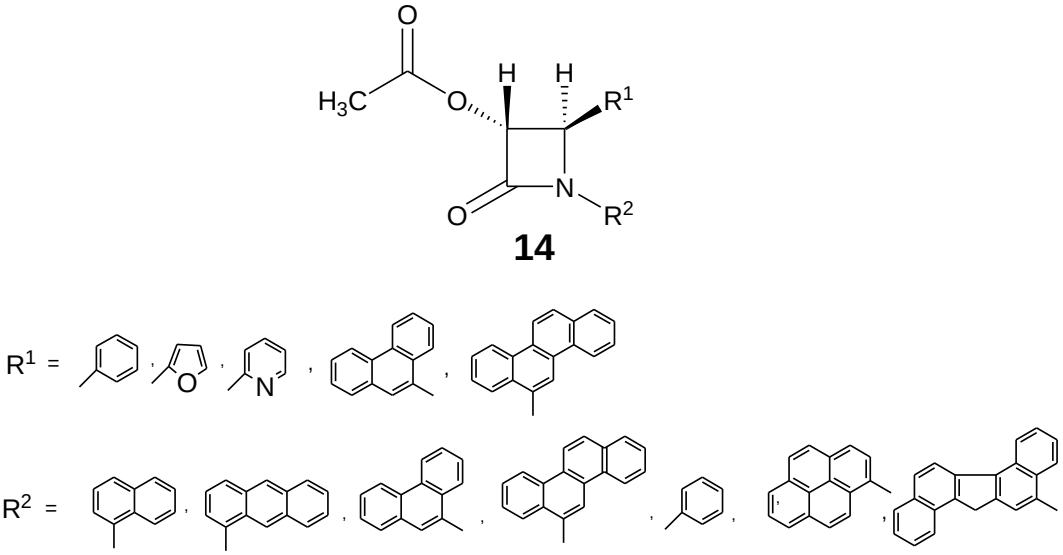


Figure 17: *trans*-3-Acetoxy-1,4-diarylazetidin-2-ones 14

As a measure of cytotoxicity, these *trans*-3-acetoxy-β-lactams **14** were assayed against nine human cancer cell lines: BRO, MCF-7, MDA-231, OVCAR, SKOV, PC-3, HL-60, K-562 and HT-29, with cisplatin and **13b** as controls. Two *trans*-*N*-chrysenyl and one *trans*-*N*-phenanthrenyl-3-acetoxy-β-lactams **14a,b** and **14c** showed significant in vitro anticancer activity (Figure 18). Almost identical anti-cancer activity against breast cancer cell line MCF-7 was exhibited by cisplatin, **14a** and **14c**. On the colon cancer cell line HT-29, **14a** was approximately three times as active as cisplatin. On the ovarian cancer cell line OVCAR, cisplatin and **14a** had almost identical activities while **14c** had little. The present study confirms **14a-c** to be more potent than **13b** against many cancer cell lines in vitro.

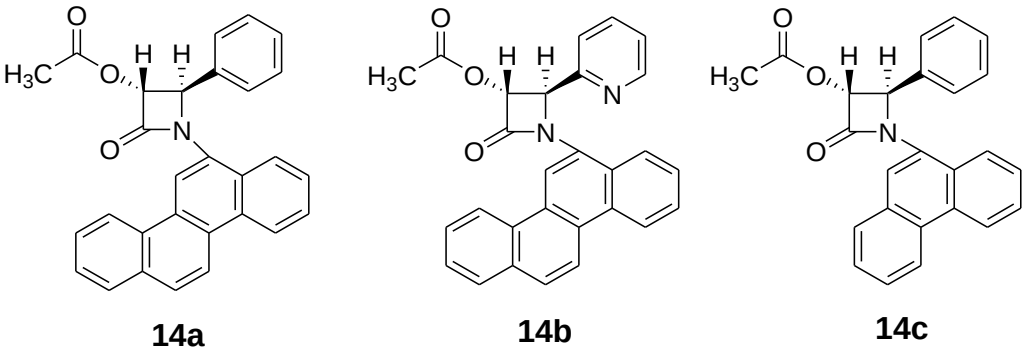


Figure 18: *trans*-*N*-Chrysenyl-4-aryl-3-acetoxyazetidin-2-one 14a,b and *trans*-*N*-phenanthrenyl-4-phenyl-3-acetoxyazetidin-2-one 14c

The comparable *trans*-3-acetoxy-*N*-anthracenyl, naphthyl, dibenzofluorenyl, and pyrenyl-β-lactams were completely inactive, thereby indicating that the minimal structural requirement for cytotoxicity was atleast three aromatic rings in an angular fashion (as in chrysenyl **14a**,**b** and phenanthrenyl **14c** products). Also, *trans*-3-phenoxy and *trans*-3-phthalimido-β-lactams bearing *N*-polyaromatic moieties were inactive, thus directing that presence of acetoxy group at C-3 of β-lactams is obligatory for their anti-cancer activity. Acetoxy group is a well-established leaving group for reactions with a wide range of nucleophiles. This suggests that an enzymatic or other alteration at this site was involved in the activation of the compound.

Also, the corresponding *cis*-3-acetoxy-β-lactams **14d,e** in which aromatic groups (chrysenyl/phenanthrenyl and phenyl) are reversed on *C*-4 and *N*-1 (Figure 19), were comparatively much less active than *trans*-**14a** and **14c**. This indicates a possible interaction of **14a-c** with specific receptor or enzyme, which has stringent stereochemical requirements.

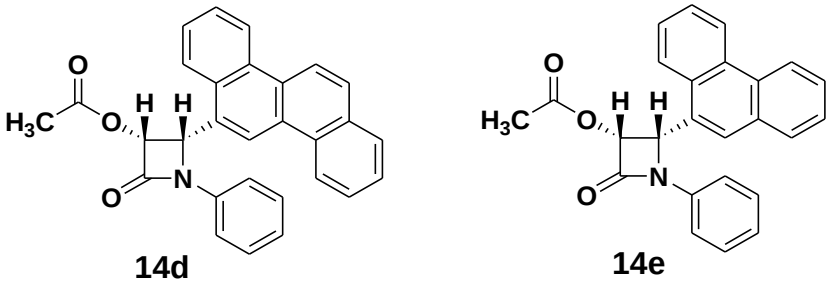


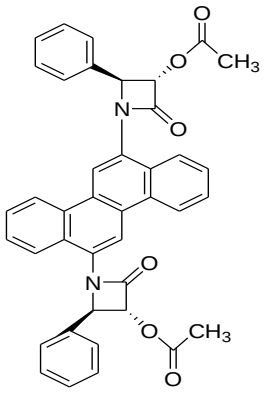
Figure 19: *cis*-1-Phenyl-4-chrysenyl-3-acetoxyazetidin-2-one 14d and *cis*-1-phenyl-4-phenanthrenyl-3-acetoxyazetidin-2-one 14e

14a was also evaluated for in vivo anticancer activity against K-562 acute leukemia cells (inoculated subcutaneously into the right shoulder of female mice), SKOV-3 ovarian carcinoma cells (inoculated subcutaneously into the male athymic [nu/nu] mice) and HT-29 colon cancer cells (injected intraperitoneally into female [nu/nu] mice). In comparison with standard antitumor drugs such as cisplatin and adriamycin, **14a** had negligible toxicity. By this method, no deaths were observed in more than 50 mice, however a maximum weight loss of 3.52 g per animal was recorded. Within 3-5 days of administration of the last dose, the mice's weight rapidly returned to that of the mice in the control group.

Banik and co-workers[40] extended these biological studies further by evaluating *trans*-3-acetoxy-β-lactams **14a** and **14c** for mutagenecity assays, apotosis, interaction with topoisomerases and studying their cell cycle activity. Mutagenecity assay results reveal that neither of the 3-acetoxy-β-lactams **14a,c** demonstrate a positive response with the tester strains (in the presence or absence of Aroclor-induced rat liver S9), thereby indicating their nonmutagenic behaviour. Using **14a,c**, no

inhibition of topoisomerase I and II was observed, thus indicating that these 3-acetoxy-β-lactams had no cytotoxic activity in sensitive cell lines through interaction with DNA or DNA-related enzyme systems. HL-60 cells treated with 10 μg of **14a** exhibited a consistent increase in the percentage of cells in the G_2 phase. The average percentage of untreated cells in G_2 was 17.2%, while that of cells treated with **14a** and **14c** was 38.4% and 23.6% respectively. Hence, 3-acetoxy-β-lactams **14a,c** invoke striking G_2 bolckade that is clearly distinct in the cell cycle analysis.

Banik and Becker[41] synthesized a novel bis β-lactam **15** with *trans* stereochemistry at the ring junctions (Figure 20). The molecule incorporates two acetoxy groups at C-3 of the β-lactam rings in **15** and was tested for anti-cancer activity against human cancer cell lines: MDA-231, BRO, PC-3, SKOV-3, HL-60, K-562 and HT-29, with cisplatin as the control. In vitro cytotoxicity results indicate bis β-lactam **15** to be completely devoid of anti-cancer activity against all cancer cell lines. This indicates that presence of two acetoxy groups in the bis β-lactam destroys the anti-cancer activity completely.



15

Figure 20: Bis β-lactam 15

Literature studies postulate that optically active isomer of a racemic compound have better selective biological activity in many cases. A carbohydrate-based approach (employing protected sugars with highly specific multiple stereogenic centres) has been utilized by Banik and co-workers[42,43] to obtain both enantiomers of the anticancer *trans*-1-*N*-chrysenyl-4-phenyl-3-acetoxyazetidin-2-ones **14a** and **14'a**.

Starting from β-glycoside **16** as the chiral synthon,[42] the synthesized optically active *trans*-1-*N*-chrysenyl-4-phenyl-3-acetoxyazetidin-2-ones **(+)-14a** and **(-)-14′a** (Figure 21) were evaluated individually for anticancer activity against seven human cancer cell lines *viz*. BRO, MDA-231, SKOV-3, PC-3, HL-60, K-562 and HT-29, with cisplatin and racemic **14a** as controls. Chiral **(-)-14′a** expressed significantly improved activity against 6/7 tumor cell lines while the activity of

other chiral isomer **(+)-14a** is reduced in 6/7 tumor lines, in comparison to racemic 3-acetoxy-β-lactam **14a**. These studies conclude that chiral β-lactam **(-)-14′a** demonstrates superior in vitro antitumor cytotoxicity in comparison to **(±)-14a** and its chiral isomer **(+)-14a**. (Figure 21).[42]

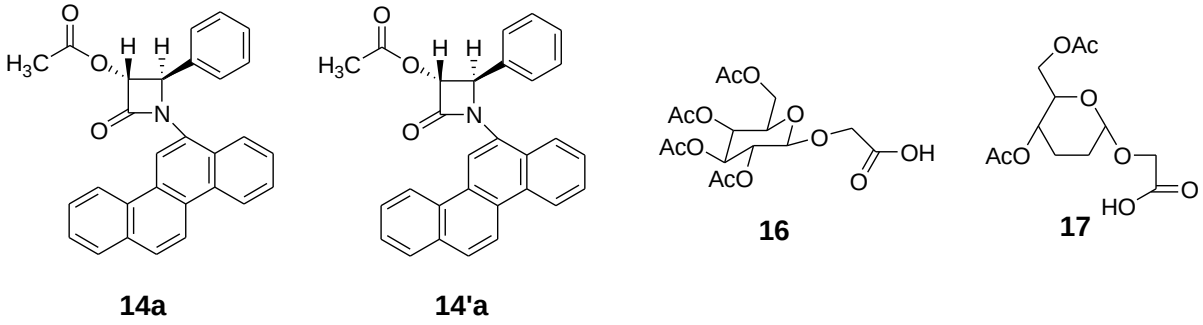


Figure 21: Optically active *trans*-*N*-chrysenyl-4-phenyl-3-acetoxyazetidin-2-one 14a, 14′a, β-Glycoside 16 and α-Glycoside 17

In continuation to these studies, they employed α-glycoside **17** as the starting substrate[43] for the synthesis optically active *trans*-1-*N*-chrysenyl-4-phenyl-3-acetoxyazetidin-2-ones **(-)-14a** and **(+)-14′a** (Figure 21). Similarly, they were tested for cytotoxicity studies against seven human cancer cell lines as in previous case. Cell growth inhibition data highlights chiral β-lactam **(+)-14′a** to be more potent than **(±)-14a** in 5/7 tumor cell lines and equally potent against 2/7 cell lines (HL-60 and K-562). However, the activity of the other optically active β-lactam **(-)-14a** is reduced compared to **(±)-14a** in 5/7 tumor cell lines, equally potent in one cell line (MDA-231) and greater in 1/7 cell lines (SKOV-3). The high degree of selectivity for specific tumor cell lines supports the concept of a high degree of specificity of the target of actions. No toxicity against normal human cancer cell lines was exhibited by racemic **14a**.

CONCLUSION

Several reports from the literature have been listed in the present review which summarizes significant developments made towards the structural and biological activity relationship of 3-acetoxyazetidin-2-ones as antifungal, antibacterial, antitumor, anticancer, IMR32 cytotoxic agents and carbonic anhydrase inhibitors (MIC values have been incorporated). Hopefully, the synthesis and biological evaluation of novel and variably substituted 3-acetoxy-β-lactams will continue to attract researchers in near future and more interesting results will be forthcoming. We apologize to the scientists whose work may not have appeared in this review either due to the limited description of the review or oversight.

REFERENCES

1. Walsh CT. The Chemical Versatility of Natural-Product Assembly Lines. Acc Chem Res 2008; 41: 4-10.
2. Walsh C. Where will the antibiotics come from? Nat Rev Microbiol 2003;1: 65-70.
3. Staudinger H. Contribution to our Knowledge of Ketenes. Liebigs Ann Chem 1908; 356: 51-123.
4. Morin RB, Gorman M. Chemistry and Biology of β-Lactam Antibiotics. Eds.; Academic Press: New York, 1982; Vols. 1-3.
5. Nathwani D, Wood MJ. Penicillins. A Current Review of their Clinical Pharmacology and Therapeutic Use. Drugs 1993; 45: 866-894.
6. Halve AK, Bhadauria D, Dubey R. N/C-4 Substituted azetidin-2-ones: Synthesis and preliminary evaluation as new class of antimicrobial agents. Bioorg Med Chem Lett 2007; 17: 341-345, and references cited therein.
7. Alcaide B, Almendros P, Aragoncillo C. β-Lactams: Versatile Building Blocks for the Stereoselective Synthesis of Non-β-Lactam Products. Chem Rev 2007; 107: 4437-4492.
8. Bhalla A, Venugopalan P, Bari SS. A new synthetic approach to novel spiro β-lactams. Eur. J. Org. Chem. 2006; 4943-4950 and references cited therein.
9. (a) Walker B, Lynas JF. Strategies for the inhibition of serine proteases. Cell Mol Life Sci 2001; 58: 596–624 (b) Hedstrom L. Serine Protease Mechanism and Specificity. Chem Rev 2002; 102: 4501–4524.
10. Brieva R, Crich JZ, Sih CJ. Chemoenzymic synthesis of the C-13 side chain of Taxol: optically active 3-hydroxy-4-phenyl-β-lactam derivatives. J Org Chem 1993; 58: 1068-1075.
11. Guillon CD, Koppel GA, Brownstein MJ, Chaney MO, Ferris CF, Lu SF, Fabio KM, Miller MJ, Heindel ND, Hunden DC, Cooper RDG, Kaldox SW, Skelton JJ, Dressman BA, Clay MP, Steinberg MI, Bruns RF. Azetidinones as vasopressin V1a antagonists. Bioorg Med Chem 2007; 15: 2054-2080.
12. Burnett DA. Beta-lactam cholesterol absorption inhibitors. Curr Med Chem 2004; 11: 1873-1887.
13. (a) Del Buttero P, Molteni G, Roncoroni M. Reductive ring opening of 2-azetidinones promoted by sodium borohydride. Tetrahedron Lett 2006; 47: 2209-2211 (b) Alcaide B, Martin-Cantalejo Y, Rodriguez-Lopez J, Sierra M. New reactivity patterns of the β-lactam ring: tandem C3-C4 bond breakage-rearrangement of 4-acyl- or 4-imino-3,3-dimethoxy-2-azetidinones promoted by stannous chloride ($SnCl_2$-$2H_2O$). J Org Chem 1993; 58: 4767-4770.

14. Ara R, Tumur I, Pandor A, Duenas A, Williams R, Wilkinson A, Paisley S, Chilcott J. Ezetimib for the treatment of hypercholesterolaemia: a systematic review and economic evaluation. Health Technol Assess 2008; 12: 1-212.
15. (a) Kuhn D, Coates C, Daniel K, Chen D, Bhuiyan M, Kazi A, Turos E, Dou QP. Beta-lactams and their potential use as novel anticancer chemotherapeutics drugs. Front. Biosci. 2004; 9: 2605-2617 (b) Kazi A, Hill R, Long TE, Kuhn DJ, Turos E, Dou QP. Novel *N*-thiolated β-lactam antibiotics selectively induce apoptosis in human tumor and transformed, but not normal or untransformed cells. Biochem Pharmacol 2004; 67: 365-374.
16. Skiles JW, McNeil D. Spiro indolinone beta-lactams: inhibitors of poliovirus and rhinovirus 3C-proteinases. Tetrahedron Lett 1990; 31: 7277–7280.
17. Zhou G, Guan Y, Tang, H, Zhao Y, Yang L. Practical synthetic approach to 4-acetoxy-2-azetidinone for the preparation of carbapenem and penem antibiotics. Res Chem Intermed 2012; 38: 251–259 and references cited therein.
18. Bhalla A, Bari SS, Vats S, Bhalla J, Sharma K, Narula D. One pot, simple, and efficient synthesis of (*E*)- and (*Z*)-3-allylidene-β-lactams from 3-allyl-3-phenylseleno-β-lactams: analogues of β-lactamase inhibitors. Tetrahedron Lett. 2016; 57: 4763-4766 and references cited therein.
19. Walsh OM, Meegan MJ, Prendergast RM, Nakib TA. Synthesis of 3-acetoxyazetidin-2-ones and 3-hydroxyazetidin-2-ones with antifungal and antibacterial activity. Eur J Med Chem 1996; 31: 989-1000.
20. (a) Just G, Zambom R. The synthesis of *cis*-*N*-(2′-carboxyphenyl)-3-*N*-phenylacetamido-4-methoxymethyl-2-azetidinone. Can. J. Chem. 1978; 56: 2720-2724 (b) Bose AK, Sharma SD, Kapur JC, Manhas MS. A convenient synthesis of β-lactams containing a free carboxy group. Synthesis 1973; 4: 216-218 (c) Arnoldi A, Grasso S, Meinardi G, Merlini L. Synthesis and anti-fungal activity of simple β-lactams. Eur J Med Chem 1988; 23: 149-154.
21. Bose AK, Manhas MS, Kapur JC, Sharma SD, Amin SG. Antibacterial activity of monocyclic beta-lactams. J Med Chem 1974; 17: 541-544.
22. Pettit GR, Singh SB, Hamel E, Lin CM, Alberts DS, Garcia-Kendal, D. Isolation and structure of the strong cell growth and tubulin inhibitor combretastatin A-4. Experientia 1989; 45: 209–211.
23. (a) Oshumi K, Hatanaka T, Fujita K, Nakagawa R, Fukuda Y, Nihei Y, Suga Y, Morinaga Y, Akiyama Y, Tsuji T. Synthesis and anti-tumor activity of *cis* restricted Combretastatins: 5 membered heterocyclic analogs. Bioorg Med Chem Lett 1998; 8: 3153-3158 (b) Shirai R,

Okabe T, Iwasaki S. Synthesis of conformationally restricted Combretastatins. Heterocycles 1997; 46: 145–148.

24. Fuselier JA, Sun L, Woltering SN, Murphy WA, Vasilevitch N, Coy DH. An adjustable release rate linking strategy for cytotoxin-Peptide conjugates. Bioorg Med Chem Lett 2003; 13: 799–803.
25. (a) Kim Y, Nam NH, You YJ, Ahn BZ. Synthesis and cytotoxicity of 3,4-Diaryl-2(5*H*)-furanones. Bioorg Med Chem Lett 2002; 12: 719–722. (b) Nam NH, Kim Y, You YJ, Hong DH, Kim HM, Ahn BZ. Synthesis and anti-tumor activity of novel combretastatins: combretocyclopentenones and related analogues. Bioorg Med Chem Lett 2002; 12: 1955–1958.
26. Sun L, Vasilevich NI, Fuselier JA, Hocart SJ, Coy DH. Examination of the 1,4-disubstituted azetidinone ring system as a template for combretastatin A-4 conformationally restricted analogue design. Bioorg Med Chem Lett 2004; 14: 2041-2046.
27. Gensler WJ, Murthy CD, Trammel MH. Nonenolizable podophyllotoxin derivatives. J Med Chem 1977; 20: 635–644.
28. Supuran CT, Scozzafava A. Carbonic anhydrase inhibitors. Curr Med Chem 2001; 1: 61–97.
29. (a) Hewett-Emmett D. in The Carbonic Anhydrase—New Horizons, Basel: BirkhauserVerlag, 2000; 29–78 (b) Akiba T, Alpern RJ, Eveloff J, Warnock DG. Electrogenic sodium/bicarbonate cotransport in rabbit renal cortical basolateral membrane vesicles. J Clin Invest 1986; 78: 1472–1478.
30. (a) Sivakumar R, Gnanasam SK, Ramachandran S, Leonard JT. Pharmacological evaluation of some new 1-substituted-4-hydroxy-phthalazines. Eur J Med Chem 2002; 37: 793–801 (b) Demirayak S, Karaburun AC, Beis R. Some pyrolle substituted aryl pyridazinone and phthalazinone derivatives and their antihypertensive activities. Eur J Med Chem 2004; 39: 1089–1095.
31. (a) Dogruer DS, Kupeli E, Yesilada E, Sahin MF. Synthesis of new 2-[1(2H)-phthalazinon-2-yl]-acetamide and 3-[1(2H)-phthalazinon-2-yl]propanamide derivatives as antinociceptive and anti-inflammatory agents. Arch Pharm 2004; 337: 303–310 (b) Soenmez M, Berber I, Akbas E. Synthesis, antibacterial and antifungal activity of some new pyridazinone metal complexes. Eur J Med Chem 2006; 41: 101–105.
32. Berber N, Arslan M, Bilen C, Sackes Z, Gençer N, Arslan O. Synthesis and Evaluation of New Phthalazine Substituted β-Lactam Derivatives as Carbonic Anhydrase Inhibitors. Russian J Bioorg Chem 2015; 41: 414–420.

33. Maresca A, Supuran CT. Coumarins incorporating hydroxy- and chloro- moieties selectively inhibit transmembrane, tumor-assisted carbonic anhydrase isoforms IX and XII over the cytosolic ones I and II. Bioorg Med Chem Lett 2010; 20: 4511–4514.
34. Turos E, Long TE, Konaklieva MI, Coates C, Shim JY, Dickey S, Lim DV, Cannons A. *N*-thiolated β-lactams: Novel Antibacterial agents for Methicillin-Resistant Staphylococcus aureus. Bioorg Med Chem Lett 2002; 12: 2229-2231.
35. Turos E, Coates C, Shim J-Y, Wang Y, Leslie JM, Long TE, Reddy GSK, Ortiz A, Culbreath M, Dickey S, Lim DV, Alonsoc E, Gonzalez J. *N*-Methylthio β-lactam antibacterials: Effects of the C3/C4 ring substituents on anti-MRSA activity. Bioorg Med Chem 2005; 13: 6289–6308.
36. Heldreth B, Long TE, Jang S, Reddy GSK, Turos E, Dickey S, Lim DV. *N*-Thiolated β-lactam antibacterials: Effects of the *N*-organothio substituent on anti-MRSA activity. Bioorg Med Chem 2006; 14: 3775-3784.
37. (a) Becker FF, Banik BK. Polycyclic Aromatic Compounds as Anticancer Agents: Synthesis and Biological Evaluation of Some Chrysene Derivatives. Bioorg Med Chem Lett 1998; 8: 2877-2880 (b) Becker FF, Mukhopadhyay C, Hackfeld L, Banik I, Banik BK. Polycyclic Aromatic Compounds as Anticancer Agents: Synthesis and Biological Evaluation of Dibenzofluorene Derivatives. Bioorg Med Chem 2000; 8: 2693-2699.
38. (a) Lin TH, Rogers TS, Hill DL, Simpson-Herren L, Farnell DR, Kochhar DM, Alam M, Brouillette WJ, Muccio DD. Murine toxicology and pharmacology of UAB-8, a conformationally controlled analog of retinoic acid. Toxicol Appl Pharmacol 1996; 139: 310–316 (b) Garratt PJ, Vonhoff S, Rowe SJ, Sugden D. Mapping the melatonin receptor. Synthesis and biological activity of indole derived melatonin analogues with restricted conformation of the C-3 amidoethane side chain. Bioorg Med Chem Lett 1994; 4: 1559–1564.
39. Banik I, Becker FF, Banik BK. Stereoselective Synthesis of β-Lactams with Polyaromatic Imines: Entry to New and Novel Anticancer Agents. J Med Chem 2003; 46: 12-15.
40. Banik BK, Becker FF, Banik, I. Synthesis of anticancer β-lactams: mechanism of action. Bioorg Med Chem 2004; 12: 2523–2528.
41. Banik BK, Becker FF. Selective anticancer activity of β-lactams derived from polyaromatic compoun**d.** Molecular Medicine REPORTS 2010, 3: 315-316.
42. Banik BK, Samajdar S, Becker FF. Asymmetric synthesis of anticancer β-lactams via Staudinger reaction. Molecular Medicine REPORTS 2010; 3: 319-321.

43. Banik BK, Banik, I, Becker FF. Asymmetric synthesis of anticancer β-lactams via Staudinger reaction: Utilization of chiral ketene from carbohydrate. Eur J Med Chem 2010; 45: 846–848.