AZETIDINONE DERIVED DRUGS: BIOLOGICAL SIGNIFICANCE AND THEIR FUTURE SCOPE IN CHEMICAL SYNTHESIS

Abstract

Drug discovery and chemical synthesis have been globalizing the pharmaceutical market. The process of drug discovery takes along many stages of drug development viz., targeting a disease, repurposing an existing drug, conjugating obsolete drugs with natural compounds, searching for different receptors for specificity and rationality, and finally testing the toxicity before exposing it tothe clinical trial. This long path from targeting to clinical trial takes many efforts of scientists and chemists. When entering into the drug development core, the heterocyclic compounds were ruling the world by their swiftness in reaction mechanism and displaying good pharmacological activity. Focusing on the heterocyclic compounds, β-lactam is a well-known pharmacophore being accepted for many existing drugs viz., Penicillin, cefixime, cefalixine, and other derivatives of penicillin. The congeners of 2-azetidine were also known to possess numerous biological actions viz., antitubercular, anti-inflammatory, anticancer, antidiabetic, antipsychotic, anti-inflammatory,and vasopressin via antagonist activities.

Keywords: 2-Azetidinone; β-lactam; Antibacterial activity; Aztreonam

Authors

Monalisa Mahapatra
Department of Medicinal Chemistry
School of Pharmaceutical Sciences
Siksha 'O' Anusandhan Deemed to be University
Bhubaneswar, Odisha, India.

Kakarla Pakeeraiah
Department of Medicinal Chemistry
School of Pharmaceutical Sciences
Siksha 'O' Anusandhan Deemed to be University
Bhubaneswar, Odisha, India.

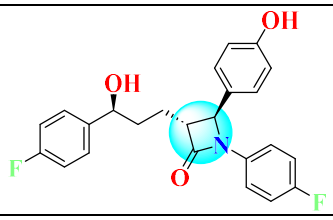
Suman Kumar Mekap*
School of Pharmacy and Life Sciences
Centurion University of Technology and Management
Jatani, Odisha, India.

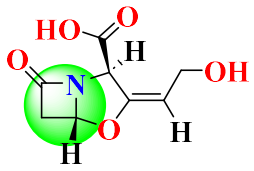
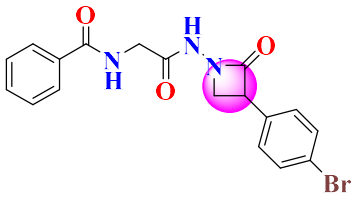
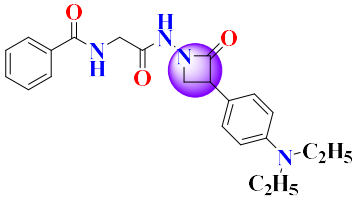
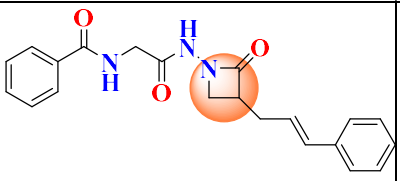
Sudhir Kumar Paidesetty*
Department of Medicinal Chemistry
School of Pharmaceutical Sciences
Siksha 'O' Anusandhan Deemed to be University
Bhubaneswar, Odisha, India.

I. INTRODUCTION

Antibiotics apprehended the position of a miraculous agent due to bearing all possible remedies for numerous infectious diseases.[1] The 2-Azetidinone (β-lactam), the core ring of the age-old antibiotics viz., penicillins, cephalosporins, carbapenems, monobactams (aztreonam), clavulanic acid and tazobactam, etc., known to serve the human era from deadly infectious diseases, however, the resistance effect has suppressed the popularity. Therefore, medicinal chemistry has been advanced by conjugating heterocyclic rings with the secondary metabolites of plants or inexorable use of β-lactam ring enclosing antibiotics leading to the development of hybrid congeners.[2] The conjugation with isoniazid has been proved to be of great biological value as the modification in the β-lactam ring is convinced for decreasing the resistance effect. Azetidinone derivatives have been explored to date for their synthesis and antibacterial activity, since the demand for antibacterial activity with less resistance effect raised, its conjugation with other scaffolds came into existence.[1] β-lactam ring has been accepted largely as animportant entity for various synthetic reactions due to the advantage of manipulating the strain energy linked with it. The 2-azetidine synthesis procedure was extensively carried out by the Staudinger reaction, which is a ketene-imine cycloaddition reaction, this versatile method has been used irrespective of various new schemes being developed.[3]Singh 2003 has developed various other new methods for the synthesis of derivatives of 2-azetidinones among which enolate-imine condensations and cyclization were involved in the reaction. Medicinal chemistry approach has been always in demand due to the exploitation of these various scaffolds with versatile hybrids, ensuing this, the β-lactam ring was conjugated with various substracts*viz*., peptides, polyamino ethers, polyamine alcohols have been developed. [4] This article explored the various derivatives of 2-azetidinones with their pharmacological actions.

β-lactam is a four-membered nitrogen-containing heterocyclic ring which is an essential structural part of penicillin and cephalosporin drugs. 2-Azetidinone is chemically a four-membered cyclic amide β-lactam. These scaffold derivatives have been reported for several enzyme inhibitors *viz*., a serine protease, tryptase, elastase, *etc*. Aztreonam, a monobactam class of β-lactam antibiotics bearing 2-azetidinone moiety which acts as an inhibitor of cephalosporins. These derivatives have been known best for their antibacterial and antifungal properties. Moreover, the azactam groups were extremely active against Gram-negative bacteria at minimum concentration, especially it acts as an alternate to aminoglycoside or third-generation antibiotics, cephalosporins for serious gram-negative infections. The gram-negative includes pseudomonal infection has been significantly monitored by utilizing the 2-azetidinone congeners. The extensive use of antibiotics leads to antibiotic resistance; this leads to the rationale approach for the innovation of hybrid compounds.

S. No	Name of the Drug	Structure	Mechanism of action	Uses
1	Ezetimibe		Reduce the cholesterol level by inhibiting the synthesis of LDL	Atherosclerosis

2	Calvulanic acid		Inactivates some beta-lactamase enzymes that are produced by bacteria	Antibiotic
3	Azetidinone hippuric acid derivatives		Fungicidal	Fungicidal
4	Azetidinone hippuric acid derivatives		Fungicidaal	Fungicidal
5	Azetidinone hippuric acid derivatives		Fungicidaal	Fungicidal

1. **Azetidinone& its Biological Activity:** The 2-azetidinone is a novel scaffold possessing versatile biological properties and clinically value-added scaffold used in various formulations and drug development processes. The pharmacological activities of the azetidinone nucleus have been categorized as follows:

2. **Antimicrobial and antifungal:**The 2-azetidinone and its derivative were reported to be possessing a more promising effect upon gram-negative strain as compared to Gram-positive bacteria, however, analysis of substitutions at different positions of 2-azetidinone has increased the activity of the derivatives when the electron-withdrawing group is present in the aromatic ring. Other substituents containing bromo, chloro, and fluoro at the para position of the aromatic ring among which the p-bromo substituentwas found to be the most favorable than other substituents for the enhancement of the antibacterial and antifungal activity. [5]

3. **Antitubercular:**Kagthara*et al*. 2013 have designed some of the new compounds bearing azetidinone moiety which possess antitubercular activity and compared to the standard Rifampin, with inhibition of >97%.The chlorophenyl (ortho-substituted) and 2,4-di-chlorophenyl compounds showed the highest MIC, which may have been due to the electronegativity of chlorine and the ability to bond with hydrogen.[6]

4. **Cholesterol absorption inhibitor:**Salisbury *et al.* 1995 synthesized different analogsof 2-azetidinone by the usual conventional method. The compound (3R,4S)-l,4-bis-(4-

methoxyphenyl)-3-(3-phenylpropyl)-2-azetidinone was an effective cholesterol absorption inhibitor at a minimum concentration of ED_{50S} of 0.2mg/kg per day. The SAR of the compound was further analyzed by Clader*et al*[7] interpreted that the azetidinone nucleus bearing 4-methoxyphenyl substituent at the C-4 position and phenylalkyl substituent at the C-3 position has been effective for cholesterol inhibition activity. 2-azetidinone nucleus was also found to play an important role in inhibiting protease activity, anti-HIV activity, a potent inhibitor of tryptase and chymase leads to anti-inflammatory activity. It also exhibits antivirus activity by procrastinating the activity of human cytomegalovirus protease (HCMV). These activities are evident to declare the scaffold to be a novel moiety.[8]

5. **Chymase and Tryptase Inhibition:** Tryptase - a serine-protease and the most popular protein of the mast cells. Degranulation ofmast cells makes themactivated;as a result, the mediators*viz*., tryptase, and histamine are released alongwith causing allergic and inflammatory diseases. Tryptase is one of the reasons causing allergiesand inflammatory and autoimmune illnesses, and it is thought to play an essential part in the inflammatory, broncho-constructive, and remodeling processes associated with asthma[9].Ayoma et al. synthesized a series of 3-benzylazetidine-2-one compounds and tested them for chymase inhibitor activity. The inhibitory action of the azetidinone derivatives revealed which had shown the chymase inhibition activity towards 3-benzyl-4-(4-methylpiperazincarbonyl)phenoxy-1-[1-(phenylethyl)aminocarbonyl]. Azetidinone was highly effective against human chymase enzyme and it shows the IC_{50} was 0.46 nM[10].

6. **Anti-diabetic or Hyperglycemic Activity:** A member of the nuclear hormone receptor superfamily - Peroxisome proliferator-activated receptors (PPARs) has received a lot of interest in the last decade astherapeutic targets for diabetes and dyslipidemia[11].Goel et al. produced a unique derivative of 2-azetidinone compounds by combining imines with ketenes in a 2,2-cycloaddition (Staudinger) process[12]. The antihyperglycemic activity of the produced compounds was investigated. High glucose levels and a decrease in hepatic glycogen contents were reported in alloxan-induced diabetic rats, this attributes to the decreased availability of the active form of the enzyme glycogen synthetase. The anti-hyperglycemic activity of the test compounds was demonstrated by considerably lowering serum glucose levels. This effect of test substances could be attributable to enhanced glucose utilisation, as seen by lower serum glucose levels and an increase in insulin levels[13].

7. **Anti-tumor Activity:** Azetidinone derivatives can prevent the proliferation of several types of tumor cells when treated *in vitro*. The reaction of heterocyclic thiols with sulfoxides of 6,6-dihydro and 6a-chloropenicillanates resulted in the synthesis of novel derivatives of 4-heteryldithio-substituted azetidinones[13]. Sun et al. synthesized a series of novel 1,4-diaryl-2-azetidinones[14] through Staudinger reaction. The selected compounds were evaluated *in vitro* on human and rat cancer cell lines, as well as normal cells. The best compounds exhibited IC_{50} values of 25-74 nM against human neuroblastoma IMR32 cell growth and a range of different cell lines.[15]

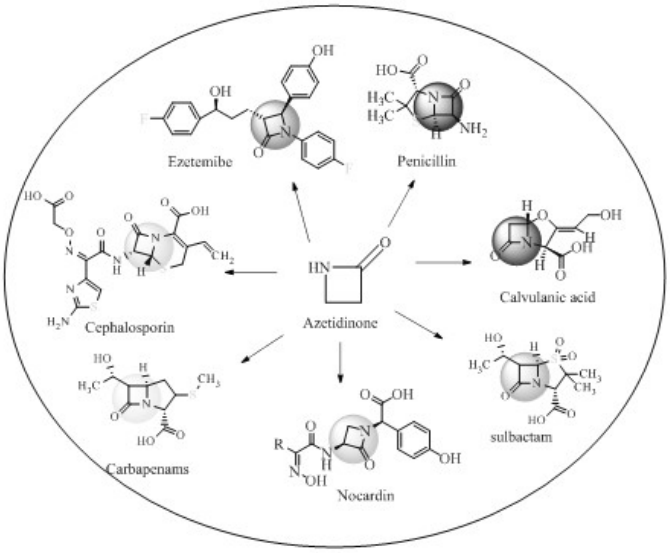


Figure 1: Drugs containing Azetidinone Scaffold

II. STRUCTURE-ACTIVITY RELATIONSHIP OF AZETIDINONES

The proteins found to have an affinity towards penicillin viz., transpeptidase (bacterial cell wall membrane-bound protein) were the main target for the drugs binding to the β-lactam ring, this β-lactamase gene interferes with azetidinone nucleus to hydrolyze the β-lactam ring and leads to diminishing the antibacterial action. The introduction of 2-azetidinone scaffold/ derivatives has been reported to reduce the resistance effect by tumbling the bacterial defense mechanism. An enzyme-containing serine as a nucleophile in the active site binds to the azetidinonering of penicillin. An overall SAR of the azetidinone derivatives has shown more inhibitory activity in gram-negative bacteria than the gram-positive bacteria, and the activity is enhanced when any electron-withdrawing group is attached to the phenyl ring at the C4position of azetidinone. The drugs containing azetidinone nucleus have always remained the best acting for bacterial infection and the broad spectrum antibiotic viz., clavulanic acid, cephalosporins, penicillin, and some other FDA-approved drugs have been depicted in **Fig.1.** Azetidinone-bearing drug Ezetimibe has been popularly used as a cholesterol inhibitor and its synthetic derivatives compounds (3R,4S)-l,4-bis-(4-methoxyphenyl)- 3-(3-phenylpropyl)-2-azetidinone and ezetimibe were potent hypocholesterolemic agents, later one being 400 times more potent than the former. The 2-azetidinone has also proved its efficiency by providing promising results as an antitubercular and antifungal agents. Various other activities have also been reported for the 2-azetidinones via a leukocyte elastase inhibitor (4-[(4-carboxyphenyl)oxyl-3,3-dialkyl-1-[[(1-phenylalkyl)-amino]carbonyl]azetidin-2-ones), derivatives of 3-(3-guanidine isopropyl)-azetidine-2-one were found to be effective against thrombin.[16] Some of the 2-azetidinone derivatives were found to be equally potent against cancer and HIV.

III. LIMITATIONS TOWARDS AZETIDINONE SCAFFOLD CONTAINING DRUGS

Modern chemistry revolves around the position of heteroatoms in a molecule and their contribution to medicinal chemistry. The nitrogen-containing lone pair has always opened the

gateway for multiple reactions viz., polarity, solubility lipophilicity, and ability to make hydrogen bonds which qualifies a drug to be a lead candidate by increasing its ADMET properties [18, 19]. The derivatives of 2-azetidinone and their nucleus were found to possess extremely versatile pharmacological activities which were described in this article. As the β-lactam ring containing compound Aztreonam is more effective and selective against gram-negative bacteria; the 2-azetidinone-derived drugs are restricted to only a few strains, hence, the spectrum activity with a wide range of bacteria could be enhanced by either alteration or conjugation with their antibacterial agents. The nucleus is attracting more attention from medicinal chemists due to its property as an intermediate synthon for different reactions. [17]However, the scaffold 2-azetridinone is limited to gram-negative bacteria irrespective of its substituent[20].

IV. CONCLUSION AND FUTURE SCOPE

Clinically used drugs flow in the realm of continuous success and fall due to resistance patterns, guarding the scaffold to be constant in the case of the β-lactam ring, however, nowadays drugs have been developed lacking the four-membered ring. The ultimate aim of the article focuses on inhibitory effects on microorganisms, cholesterol absorption, and enzyme inhibition whereas many potential targets could not have been reached in the current research. The clinically used drugs pertaining to containing 2-azetidinone substitutions at the positions N-1, C-3, and C-4 have been explored. In this review, it can be concluded that the azetidinone moiety is an essential pharmacophore for antibacterial action in terms of inhibiting the transpeptidase enzyme of the bacterial cell wall and also inhibits cholesterol absorption. Though the azetidinone derivatives have been known to exhibit potent action againsta few strains of gram-negative bacteria they are limited to showing action against gram-positive bacteria. As the moiety is an attractive scaffold for several molecular targets and possesses a wide range of applications, hence any future research and developments could be resulting in various novel outputs in the field of

REFERENCES

[1] SinghG.S., “β-Lactams in the New Millennium. Part-II: Cephems, Oxacephems, Penams, and Sulbactam.” Mini-Reviews in Med. Chem., 4, 93-109, 2004.

[2] ThomasA.B., NandaR.K., KothapalliL.P., HamaneS.C. “Synthesis and biological evaluation of Schiff’s bases and 2-azetidinones of isonocotinylhydrazone as a potential antidepressant and nootropic agents.” *Arab. J. Chem.*9, S79–S90, 2016. Doi: http://dx.doi.org/10.1016/j.arabjc.2011.02.015.

[3] MehtaP. D., SengarN.P.S., PathakA.K., “2-Azetidinone: A new profile of various pharmacological activities.” *Eur. J. Med. Chem.* 45, 5541-5560, 2010, DOI:10.1016/j.ejmech.2010.09.035

[4] Rex N. Brogden and Rennie C. Heel, “Aztreonam: A Review of its Antibacterial Activity, Pharmacokinetic Properties, and Therapeutic Use.” *Drugs* 31: 96-130 (1986) DOI: 0012-6667/86/0002-0096/$17.50/0.

[5] DeepA., KumarP., NarasimhanB., limS.M., RamasamyK.,.MishraR.K and ManiV., “ 2-azetidinone derivatives: synthesis, antimicrobial, Anticancer evaluation and QSAR studies.”*Acta Pol. Pharm*., Vol. 73 No. 1 pp. 65-78, 2016.

[6] K. Elumalai, M.A.Ali, M. Elumalai, K. Eluri, S.Srinivasan, S.K.Mohanti, A. Thota, “Design, synthesis and biological evaluation of some novel isoniazid cyclocondensedazetidinones.” *Drug Invention Today* 5, 100-104, 2013 doi: http://dx.doi.org/10.1016/j.dit.2013.05.007

[7] D.A. Burnett, M.A.Caplen, H.R. Davis, Jr., R. E. Burrier, and J. W. Clader, “2-Azetidinones Absorption as Inhibitors of Cholesterol.” *J. Med. Chem.* 37,12, 1994. Doi: 0022-2623/94/1837-1733$04.5QJ

[8] J. Tözsér1, and S. Oroszlan, “Proteolytic Events of HIV-1 Replication as Targets for Therapeutic Intervention.” *Curr. Pharma. Design*., 9, 1803-1815,2003.

[9] Brian G. Salisbury *et al*, " Hypocholesterolemic activity of a novel inhibitor of cholesterol absorption, SCH 48461." Atherosclerosis 1995, 115, 45-63.
[10] Louis S. Hegedus *et al*, "A Contribution to the Confusion Surrounding the Reaction of Ketenes with Imines To Produce P-Lactams. A Comparison of Stereoselectivity Dependence on the Method of Ketene Generation: Acid Chloride/Triethylamine vs Photolysis of Chromium-Carbene Complexes." *J. Americ. Chem. Soci.* 1991, 113, 5784-5791.
[11] Abraham WM. Tryptase: potential role in airway inflammation and remodeling. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2002 282(2) L193-6.
[12] Mehta PD, Sengar NP, Pathak AK. 2-Azetidinone–a new profile of various pharmacological activities. *Eur. J. Med. Chem.* 2010 Dec 1;45(12):5541-60.
[13] Elrod KC, Numberof RP. Emerging therapeutic targets in asthma: the rationale for mast cell tryptase inhibition. *Emerg. Ther. Targets.*1999 3(2) 203-12.
[14] Aoyama Y, Uenaka M, Kii M, Tanaka M, Konoike T, Hayasaki-Kajiwara Y, Naya N, Nakajima M. Design, synthesis and pharmacological evaluation of 3-benzylazetidine-2-one-based human chymase inhibitors.*Bioorg. Med.Chem. Lett.* 2001 9(11) 3065-75.
[15] Veinberg G, Bokaldere R, Dikovskaya K, Vorona M, Mucel' D, Kazhoka K, Turovskis I, Shestakova I, Kanepe I, Domracheva I, Lukevics E. Synthesis of penicillin derivatives and study of their cytotoxic properties. *Chem.Heterocyc.* 1998 34(11)1266-75.
[16] 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(9) 2041-6.
[17] Govindarao K., Srinivasan N., Suresh R., Raheja R.K., Annadurai S., Bhandare R. R., Shaik, A. B. Quinoline conjugated 2-azetidinone derivatives as prospective anti-breast cancer agents: In vitro antiproliferative and anti-EGFR activities, molecular docking and in-silico drug likeliness studies. *J. Saudi Chem. Soc.*2022 26(3) 101471.
[18] Bagherwal A, Baldi A, Nagar RK, Patidar DK. Synthesis and antimicrobial studies of azetidinone derivatives from naphthylamine moiety. Intern. J. Chem. Tech. Res. 2011 3(1) 274-9.
[19] Joyeau R., Molines H., Labia R., Wakselman M. "N-Aryl 3-halogenated azetidin-2-ones and benzocarbacephems, inhibitors of. beta.-lactamases." *J. Med. Chem*. 1988 (2) 370-4.
[20] Om. D. Tyagi, DnyandeoRagho Rane, Sanjay Mahajan, Yuvraj Atmaram Chavan, US patent no US 8,008,478 B2.
[21] J.Jampilek, Heterocycles in medicinal chemistry, Molecules 24 (2019) 3839.
[22] A.B. Kasetti, I. Singhvi, R. Nagasuri, R.R. Bhandare, A.B. Shaik, Thiazole-chalcone hybrids as prospective antitubercular and antiproliferative agents: design, synthesis, biological, molecular docking studies and in silico ADME evaluation, Molecules 26 (2021) 2847.

