

NOVEL MONOCYCLIC β -LACTAM MOLECULES: DESIGNED AND CHARACTERIZED AS POSSIBLE ANTIBACTERIAL AGENTS

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ABSTRACT

A reaction of Schiff bases (**1a-j**) with monochloro acetyl chloride afforded a series of 1-[4'-(2'',4''-dichloro-5''-fluoro phenyl)-6'-(2''-thienyl)pyrimidin-2'-yl-ureido]-4-substituted phenyl-3-chloro-2-azetidinone derivatives (**2a-j**) in moderate yields. All the monocyclic β -lactam products have been characterized on the basis of physical properties of the molecule and satisfactory spectral (IR, ¹H NMR) data. The mechanism of formation of the product is shown. The antibacterial activity of the compounds against some Gram (+) and Gram (-) bacterial strains is reported.

KEYWORDS: Cyclocondensation, Schiff base, Monocyclic β -lactam, Amide linkage, Antibacterial activity.

INTRODUCTION

β -lactam antibiotics have accumulated many lives since 1945.^[1] Apart from their clinical use, recent reports on the use of β -lactams for purpose, other than antibiotics are gaining attention. This four-membered cyclic amide has been extensively used for the synthesis of several biologically active heterocyclic compounds.^[2-3] It has been established that Taxol can be prepared by a coupling reaction between natural baccatin and suitably substituted hydroxy β -lactam.^[4] Banik and his coworkers have been engaged in the synthesis and biological evaluation of compounds in which a polycyclic aromatic ring is present.^[5] During the course

of this study, we became interested in the synthesis of cyclic amides (for example, β -lactam) also bound to a polyaromatic ring.

Compounds containing 2-azetidinone ring system possess marked biological activity^[6-10], including antibacterial activity^[11], anti-inflammatory activity^[12-13], herbicidal^[14] and anticonvulsant activity.^[15] Recent years have witnessed a great upsurge in the introduction of azetidinone derivatives for the treatment of tuberculosis.^[16] Even fluorinated organic compounds have been the subject of much attention in recent years owing to their unique physical and biological properties. In this review we highlight some of the recent development connected with the various modes of monocyclic β -lactam.

EXPERIMENTAL SECTION

The melting point of synthesized compounds was determined in open capillary tubes and so the values recorded are therefore uncorrected. The Infrared (IR) Spectra were recorded on a FTIR-8400 Shimadzu spectrometer using potassium bromide pellets. The Proton Nuclear Magnetic Resonance (¹H NMR) spectra were recorded on a Brüker Avance dpx-200 (at 200 MHz) spectrometer using tetramethylsilane (TMS) as the internal standard. Chemical shifts are expressed in part per million (ppm). Elemental analysis was performed by Central Drug Research Institute, Lucknow and results are within $\pm 0.4\%$ of the calculated values. Thin Layer Chromatography was performed on readymade silica gel plates (Merck) and were visualized with UV (254 nm) and/or Iodine to check the purity of compounds. All reagents were of the highest purity and commercially available, and they were used without further purifications.

This study contains detailed information about ten novel monocyclic 2-azetidinone derivatives. The synthesis of the starting material, 1- (substituted phenyl) - 4 - [4' - (2'', 4'' - dichloro - 5''-fluoro phenyl)- 6'-(2''-thienyl) pyrimidin -2'-yl] semicarbazides **1a-j** (Schiff Bases) is reported^[17] by us and now its 2-azetidinone compounds has been prepared to study the variation of the structure activity relationship (SAR). This various Schiff bases on cyclocondensation with monochloro acetyl chloride in basic medium at lower temperature gave 1-[4' - (2'', 4''-dichloro-5''- fluoro phenyl) -6' - (2''- thienyl) pyrimidin -2'- yl - ureido]- 4 -substituted phenyl- 3 -chloro-2-azetidinone derivatives **2a-j**.

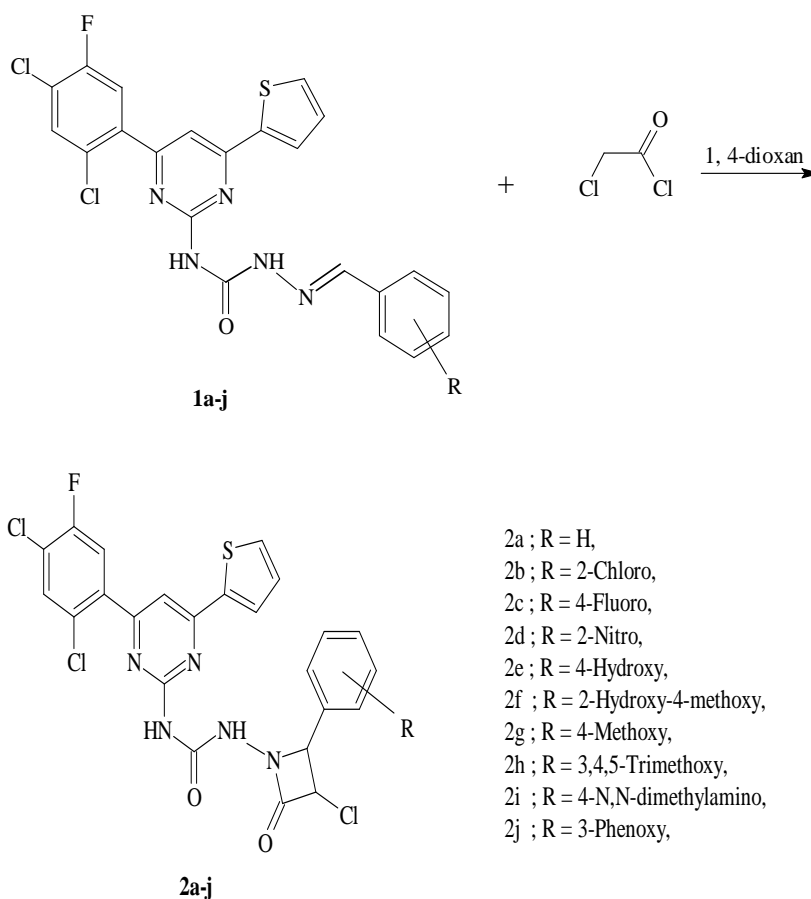
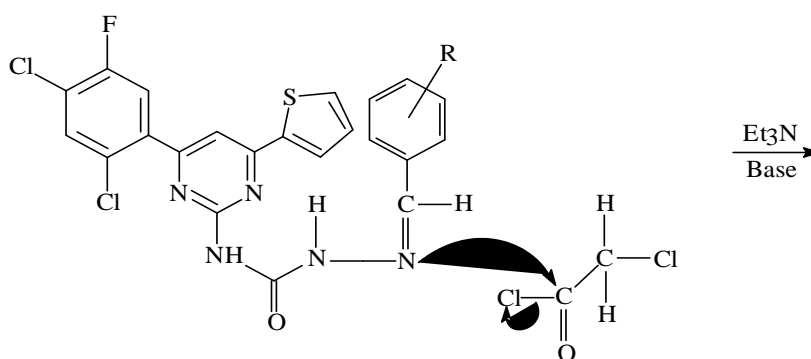


Fig.-1: Synthesis path for 2a-j

MECHANISM

Several useful syntheses of β -lactams which have been developed consist of the addition of C-N to a C-CO component to form the ring in a single operation with a stepwise mechanism. Substituted acetyl chlorides with electron-withdrawing substituents and at least one hydrogen at the α -carbon add to imines (Schiff bases) in the presence of amine bases.^[18] The mechanism is probably as depicted as follow.



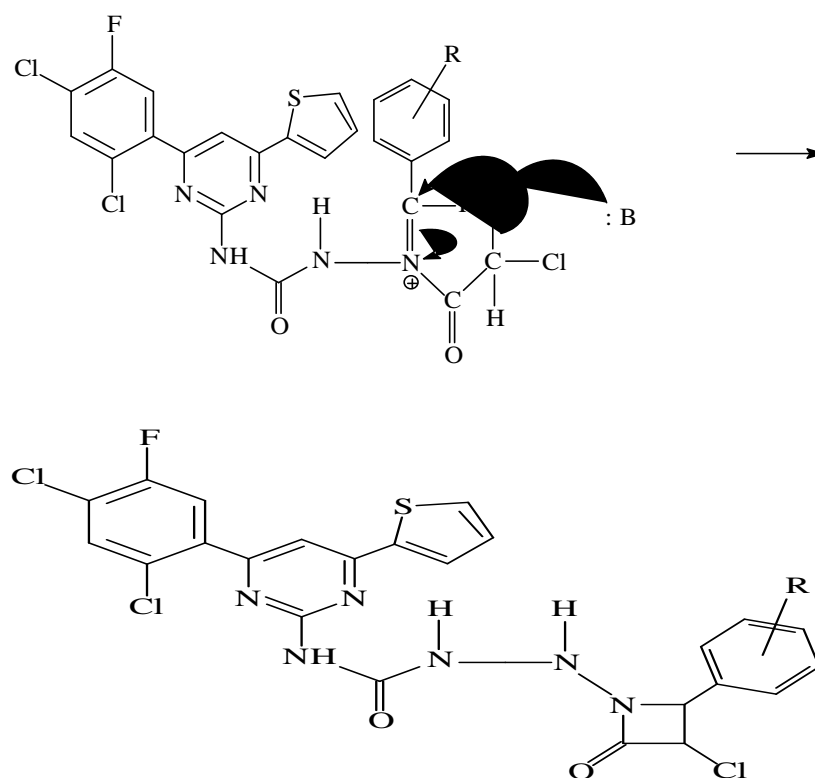


Fig.-2: Synthesis mechanism for 2a-j

General Procedure for the synthesis of 1-[4'-(2'',4''-dichloro-5''-fluoro phenyl) - 6' - (2''-thienyl) pyrimidin - 2' - yl - ureido]-4 - substituted phenyl - 3 - chloro - 2 - azetidinones.^[19]

A solution of 1-substituted phenylidene -4-[4'-(2'', 4''-dichloro-5''-fluoro phenyl)-6'-(2''-thienyl) pyrimidin -2'-yl] semicarbazides (0.01mol) **[1a-j]** in dry 1,4-dioxan (50 ml) and triethylamine (0.012 mol) was added monochloro acetyl chloride (0.012mol) drop wise with well stirring at 0°-5°C. The reaction mixture was stirred for 9 hrs and stirred 48 hours at room temperature. Then the reaction mass treated with ice-cold water. The solid product was filtered, washed with water, dried and recrystallized from chloroform.

Similarly, all the compounds **[2a-j]** were prepared by the same above general procedure and their formulas, melting points, yields and analytical data are given Table No.1.

Table No. 1 Analytical data of 2-Azetidinones 2a-j

| NO. | R | MOLECULAR FORMULA | M.P. (°C) | Y (%) | FOUND (CALCULATED %) | | |
|-----|----------------|--|-----------|-------|----------------------|----------------|------------------|
| | | | | | C | H | N |
| 2a | Phenyl | C ₂₄ H ₁₅ N ₅ O ₂ SCl ₃ F | 121 | 49 | 51.16 (51.20) | 2.57 (2.67) | 12.38 (12.44) |
| 2b | 2-Chlorophenyl | C ₂₄ H ₁₄ N ₅ O ₂ SCl ₄ F | 157 | 65 | 48.29 (48.24) | 2.29 (2.35) | 11.69 (11.73) |

| | | | | | | | |
|----|-----------------------------|---|---------|----|------------------|----------------|------------------|
| 2c | 4-Fluoro phenyl | $C_{24}H_{14}N_5O_2SCl_3F$ ₂ | 164 | 64 | 49.65 (49.61) | 2.46 (2.41) | 12.07 (12.05) |
| 2d | 2-Nitro phenyl | $C_{24}H_{15}N_5O_2SCl_3F$ | 147 | 61 | 47.38 (47.40) | 2.27 (2.30) | 13.87 (13.83) |
| 2e | 4-Hydroxy phenyl | $C_{24}H_{15}N_5O_2SCl_3F$ | 122 | 70 | 49.73 (49.78) | 2.53 (2.59) | 12.14 (12.10) |
| 2f | 2-Hydroxy-4-methoxyphenyl | $C_{24}H_{15}N_5O_2SCl_3F$ | 134 | 69 | 49.34 (49.30) | 2.81 (2.79) | 11.54 (11.50) |
| 2g | 4-Methoxy phenyl | $C_{25}H_{17}N_5O_3SCl_3F$ | 155 | 62 | 50.68 (50.63) | 2.80 (2.87) | 11.77 (11.81) |
| 2h | 3,4,5-Tri methoxyphenyl | $C_{24}H_{15}N_5O_2SCl_3F$ | 161 | 71 | 49.62 (49.66) | 3.24 (3.22) | 10.78 (10.73) |
| 2i | 4-N,N-dimethyl amino phenyl | $C_{24}H_{15}N_5O_2SCl_3F$ | 123-124 | 65 | 51.53 (51.53) | 3.26 (3.30) | 13.82 (13.87) |
| 2j | 3 - Phenoxy phenyl | $C_{24}H_{15}N_5O_2SCl_3F$ | 156 | 63 | 55.02 (55.00) | 2.95 (2.90) | 10.73 (10.70) |

RESULTS AND DISCUSSION

The IR spectra of 2-azetidinone derivatives exhibited a band due to =CH str. and C=C str. in a range of 3100-3000 cm^{-1} and 1635-1495 cm^{-1} respectively.^[20,22] Absorption band was observed due to C-H bending [1,2,4,5-substituted] near 900-860 cm^{-1} , C-H bending [1,4-substituted] near 840-800 cm^{-1} and C-H bending [1,3-substituted] near 810-750 cm^{-1} .^[21-22]

Examination of IR spectra reveals that all the compounds exhibit absorption near 1760-1730 cm^{-1} which indicates the presence of C=O of 2-azetidinone moiety.^[20,24,25] C-NO₂ showed absorption near 1370-1300 cm^{-1} (sym.).^[20,25] Absorption observed in the region of 800-700 cm^{-1} and 1100-1000 cm^{-1} indicates the presence of C-Cl and C-F stretching respectively.^[20,21] The IR spectrum showed absorption in the region between 3500-3180 cm^{-1} due to N-H str. and 1680-1630 cm^{-1} due to C=O str. which indicates the presence of amide.^[22-24] The final structures [2a-j] were established by the appearance of band at 1742 cm^{-1} due to C=O (β -lactam ring). An IR spectral features seems to assume the structure of present synthesized compounds.^[20-25]

¹H NMR spectra displayed nature of different protons of synthesized compounds respectively.^[24] The carefully study of ¹H NMR (δ , CDCl₃) spectrum, it can be concluded that appearance of the signal at δ : 3.25 due to -CH₂-Cl and δ : 3.75 due to -N-CH₂-Ar prove the presence of linkage in 2-azetidinone ring. Appearance of the signal at δ : 2.9 proves the presence of -N(CH₃)₂ in the compound.

ANTIBACTERIAL ACTIVITY

The targeted molecules were tested for antibacterial activity against *Escherichia coli* & *Pseudomonas aeruginosa* and *Staphylococcus aureus* & *Bacillus subtilis* bacteria using Cup plate agar diffusion method.^[26] Streptomycin is used a reference compound for comparison. The degree of inhibition varied with the test compound as well as with bacterium. In this series all the compounds contain heterocyclic moiety having chain (linkage). The connecting chain in this case is called amide linkage. The amide linkage contains extra nitrogen left of the -CONH- group. From the above biological data, it has been observed that all the compounds are moderate active against *S. aureus*, *B. subtilis*, and *E. coli* and *P. aeruginosa* bacterial strains.

The zone of inhibition of reference compound Streptomycin mentioned in Table No.2. The correlation between the structure and the antibacterial activity is relatively clear for the results represented in Table No. 2.

Table No. 2 Antibacterial activity of 2-Azetidinones 2a-j

| NO. | R | ANTIBACTERIAL ACTIVITY | | | |
|------|---------------------------------|--|----------------|------------|------------------|
| | | DIAMETER OF ZONE OF INHIBITION (IN MM) | | | |
| | | S. aureus | B. subtilis | E. coli | P. aeruginosa |
| 2a | Phenyl | 6 | 7 | 5 | 6 |
| 2b | 2-Chloro phenyl | 9 | 10 | 10 | 9 |
| 2c | 4-Fluoro phenyl | 10 | 9 | 9 | 8 |
| 2d | 2-Nitro phenyl | 8 | 6 | 7 | 8 |
| 2e | 4-Hydroxy phenyl | 6 | 7 | 8 | 9 |
| 2f | 2-Hydroxy-4-methoxy phenyl | 9 | 9 | 12 | 13 |
| 2g | 4-Methoxy phenyl | 12 | 12 | 13 | 12 |
| 2h | 3,4,5-Trimethoxy phenyl | 13 | 12 | 15 | 16 |
| 2i | 3-Phenoxy phenyl | 6 | 6 | 7 | 5 |
| 2j | 4 - N,N - dimethyl amino phenyl | 11 | 12 | 13 | 12 |
| STD. | STREPTOMYCIN | 25 | 28 | 26 | 32 |

STRUCTURE ACTIVITY RELATIONSHIP

It is observed that all samples tested for antibacterial screening shows some antibacterial activity. Among these compounds **2f** was found to be the more active. When comparison is made between the compounds **2c**, **2b**, **2g**, it seems that antibacterial activity is enhanced due to the presence of methoxy, fluoro and N-methyl groups as a substituents on the phenyl ring.

The comparison of inhibition value of the compound **2b** also shows that the introduction of chloro group moderately enhanced the antibacterial activity. On the contrary, the comparison of inhibition value between the compounds **2d**, **2e** and **2i** reveals that the presence of a nitro, hydroxy and phenoxy groups on phenyl ring contributes almost lowest to the antibacterial activity.

Substitution by N-methyl group at fourth position of aldehydes also improves antibacterial activity against all bacteria. In some way fluoro group slightly enhanced activity and chloro group at fourth position slightly improve activity against *S. aureus*. On the basis of the inhibition value of these compounds, it can be concluded that substituted 2-azetidinone in amino pyrimidine skeleton furnishes active antibacterial activity.

However, best antibacterial activity can be obtained with fluoro, methoxy and N-methyl substitution on the phenyl ring. Thus it comes into view that more potential antibacterial activity of compounds can be achieved with introduction substituents like fluoro and methoxy groups.

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