Synthesis and biological evaluation of azetidinone derivatives of 2-(3-chloro-2-(4-substituted phenyl)-4-oxoazetidin-1-yl)-5-fluorobenzonitrile

C. GOPI [*], N. SANTHI [†] and M.EMAYAVARAMBAN[†]

***E.G.S Pillay Engineering College Nagapattinam, Tamil nadu, India.**

[†]PG & Research department of chemistry, Govt. Arts College, Tamil nadu, India.

gopicm01@gmail.com

Abstract - A series of 2-(3-chloro-2-(4-substituted phenyl)-4-oxoazetidin-1-yl)-5-fluorobenzonitrile compounds (3a-e) have been synthesized from Schiff bases (2a-e). Schiff bases were synthesized by condensation reaction of 2-amino-5-fluorobenzonitrile with substituted aromatic aldehyde and characterized by chemical and spectral analyses such as IR, ^{1}H NMR, ^{13}C NMR. All the synthesized compounds 3a-e were screened for their antibacterial and antifungal activities against some selected bacteria, fungi with their zone of inhibition values and antitubercular activity screened against *M. tuberculosis.* Some compounds of the series showed good activities.

Keywords: 2-amino-5-fluorobenzonitrile, azetidinone, antimicrobial and antitubercular.

I. INTRODUCTION

2-Azetidinone or β-lactums are outstanding class of heterocyclic compounds among natural and therapeutic chemistry[1,2]. They are most endorsed anti-microbial in prescription. The β-lactums nucleus is the structural feature and the core of the biological activity of one of the most successful classes of therapeutic agents to date characterized by a broad spectrum of activity and low toxicity. Unfortunately, long-term use related to the overuse and misuse of β-lactam antibiotics have resulted in the proliferation of resistant organisms among a variety of clinically noteworthy species of bacteria becoming an important worldwide problem. Their effectiveness has been seriously compromised by the bacterial ability to develop different competitive mechanisms in order to survive Besides their antibiotic activity azetidinones are also known to exhibit some other types of biological activities, for example, antibacterial [3-5] ,antimicrobial[6], Antitubercular[7],anti-inflammatory,[8,9,] enzyme inhibitors[10], central nervous system[11-12] and anticonvulsant,[13,14] Antitumor activity,[15,16,17.]

Bacterial and fungal infection is most common problem of the world. Some serious and life treating diseases also caused by bacteria or fungal infection. In case of accident and limb transplantation or surgery microbial infection is also common problem. From the last decade, researchers made a continuous effort to fight these diseases. Several new classes of chemotherapeutic agents have been introduced in the last decade. As part of interest in heterocyclic that have been explored for developing pharmaceutically important molecules. Biocidal activities of azetidinone have been well established. These have been attributed to the toxophoric C=N linkage in them. All synthesized compounds were screened against selected bacteria and fungi for their antimicrobial activity and antitubercular activity[18] screened against *M. Tuberculosis*. Structures of all the newly synthesized compounds were confirmed by elemental analysis such as IR, ^{1}H NMR and ^{13}C NMR.

II. EXPERIMENTAL

Melting points were taken in open capillaries are uncorrected. Progress of reaction was monitored by silica gel-G coated TLC plates using MeOH: $CHCl_3$ system (2:8). The spot was visualized by exposing dry plate at iodine vapours chamber. IR spectra were recorded in KBr disc on Schimadzu 8201 PC, FTIR spectrophotometer (Vmax in cm-1) and ^{1}H NMR and ^{13}C NMR spectra were measured on a Brucker DRX-300 spectrometer in $CDCl_3$ at 300 and 75 MHz using TMS as an internal standard respectively. All chemical shifts were reported on δ scales. The analytical data of all the compounds were highly satisfactory. For column chromatographic purification of the products Merck silica Gel 60 (230-400 Mesh) was used. The analar grade chemicals were purchased from the commercial sources and further purified before use.

General procedure for the synthesis of Schiff bases (2a-e)

2-amino-5-fluorobenzonitrile (1) (1 mmol), Substituted aromatic aldehydes (1 mmol) and 95% ethanol (20 ml) were taken into a 100 ml conical flask. The mixture was irradiated by an ultrasonic generator in a water-bath for 10 min. The completion of the reaction was monitored by silica gel-G coated TLC plates. After the completion of the reaction the product was filtered with suction on a Buchner funnel. The purified product was dried under vacuum and recrystallized from ethanol at room temperature to yield compound **2a-e**.

 DOI : 10.18231/2454-9150.2018.0106

(E)-2-(benzylideneamino)-5-fluorobenzonitrile (2a) Yield: 82%; m.p. 90°C. M.F; C_{14} H_9FN_2; IR (KBr pellet,) *v*max cm^{-1}: 1510 (C=C str), 3060 (Ar C-H str), 1587 (HC=N), 2937 (C-H str) ^1H NMR ($CDCl_3$) (δ/ppm): 8.60 (S, 1H, HC=N), 6.87 - 7.38 (m, 8H, Ar-H); ^{13}C NMR (DMSOd6) (δ/ppm): 118.44-133.58 (Ar-C), 163.97 (C=N). Anal. Calcd. For: C_{14} H_9FN_2: C, 74.99; H, 4.05; N, 12.49. Found: C, 74.97; H, 4.02; N, 12.47.

(E)-2-(4-chlorobenzylideneamino)-5-fluorobenzonitrile (2b) Yield: 85%; m.p. 116 °C. M.F; $C_{14}H_8ClFN_2$; IR (KBr pellet,) *v*max cm^{-1}: 1508 cm^{-1} (C=C str) 3053 cm^{-1}(Ar C-H str), 1591 cm^{-1}(HC=N), 2931 cm^{-1} (C-H str) ;^1H NMR ($CDCl_3$) (δ/ppm): 9.87 (S, 1H, HC=N), 7.03-8.04 (m, 7H, Ar-H); ^{13}C NMR ($CDCl_3$) (δ/ppm): 118.53-134.60 (Ar-C), 165.01 (C=N). Anal. Calcd. For: $C_{14}H_8ClFN_2$: C, 65.00; H, 3.12; N, 10.83. Found: C, 65.01; H, 3.13; N, 1.82.

(E)-2-(4-fluorobenzylideneamino)-5-fluorobenzonitrile (2c) Yield: 85%; m.p. 122°C. M.F; $C_{14}H_8F_2N_2$; IR (KBr pellet,) *v*max cm^{-1}: 1506 cm^{-1} (C=C str) 3062 cm^{-1}(Ar C-H str), 1589 cm^{-1}(HC=N), 2931 cm^{-1} (C-H str) ;^1H NMR ($CDCl_3$) (δ/ppm): 8.50 (S, 1H, HC=N), 7.07-8.48 (m, 7H, Ar-H); ^{13}C NMR ($CDCl_3$) (δ/ppm): 115.09-131.98 (Ar-C), 149.66 (C=N) . Anal. Calcd. For: $C_{14}H_8F_2N_2$: C, 69.42; H, 3.33; N, 11.57. Found: C, 69.41; H, 3.33; N, 11.60.

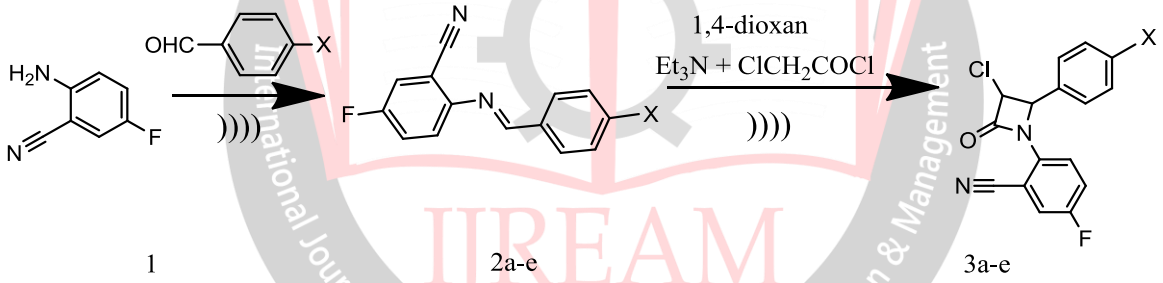
(E)-5-fluoro-2-((4-methylbenzylidene) amino)benzonitrile (2d) Yield: 82%; m.p. 85 °C. M.F; C_{15} H_{11} FN_2; IR (KBr pellet,) *v*max cm^{-1}: 1539 cm^{-1} (C=C str) 3030 cm^{-1}(Ar C-H str), 1562 cm^{-1}(HC=N), 2908 cm^{-1} (C-H str) ; ^1H NMR ($CDCl_3$) (δ/ppm): 8.17 (S, 1H, HC=N), 2.50 (S,3H,-CH_3), 6.35-7.81 (m, 6H, Ar-H); ^{13}C NMR ($CDCl_3$) (δ/ppm): 116.62-134.60 (Ar-C), 160.36 (C=N), 29.68(-CH_3). Anal. Calcd. For: $C_{15}H_{11}FN_2$: C, 75.62; H,4.65; N, 11.76. Found: C, 75.61; H, 4.64; N, 11.72.

(E)-2-(4-methoxybenzylideneamino)-5-fluorobenzonitrile (2e) Yield: 83%; m.p. 107 °C. M.F; $C_{15}H_{11}FN_2O$; IR (KBr pellet,) *v*max cm^{-1}: 1508 cm^{-1} (C=C str) 3062 cm^{-1}(Ar C-H str), 1598 cm^{-1}(HC=N), 2927 cm^{-1} (C-H str) ; ^1H NMR ($CDCl_3$) (δ/ppm): 9.40 (S, 1H, HC=N), 6.33-7.80 (m, 7H, Ar-H), 3.39 (S,3H,(-OCH_3); ^{13}C NMR ($CDCl_3$) (δ/ppm): 127.92-139.93 (Ar-C), 145.43 (C=N), 56.49 (-OCH_3). Anal. Calcd. For: C_{15} H_{11} FN_2O: C, 70.86; H, 4.36; N, 11.02. Found: C, 70.84; H, 4.32; N, 11.04.

General procedure for the synthesis of azetidin-2-ones (3a-e)

The condensation reaction of (E)-2-(4-chlorobenzylideneamino)-5-fluorobenzonitrile with Chloroacetyl chloride presence of triethylamine yields the corresponding azetidinones 3a-e .The products were characterized on the basis of their Physical properties, IR, ^1H NMR, and ^{13}C NMR Spectroscopy. The compounds were prepared by scheme (1)

Scheme (1) Synthesis of azetidinone derivatives (3a-e)



1 2a-e 3a-e

(X= H, Cl, F, -CH_3, -OCH_3)

2-(3-chloro-2-oxo-4-phenylazetidin-1-yl)-5-fluorobenzonitrile (3a) Yield: 64 %; m.p. 210-212°C. Brown Colour, M.F; $C_{16}H_{10}ClFN_2O$; IR (KBr pellet,) *v* max cm^{-1}: 3039 (Ar C-H str.) 1514 (Ar C=C str.), 1651 (C=O), 725 (C-Cl), 1213 (C-N), 1255 (C-F); ^1H NMR ($CDCl_3$) (δ/ppm): 3.024 (S, 1H, HC-Cl), 7.36-7.92 (m, 7H, Ar-H), 3.917 (S,1H,(CH); ^{13}C NMR ($CDCl_3$) (δ/ppm): 161.88 (C=O), 67.02 (C-H), 55.44 (CH-Cl), 127.44-145.28 (Ar C),114.5 (CN). Anal. Calcd. For: $C_{16}H_{10}ClFN_2O$: C, 63.90; H, 3.35; N, 9.32. Found: C, 62.40; H, 3.32; N, 9.10.

2-(3-chloro-2-(4-chlorophenyl)-4-oxoazetidin-1-yl)-5-fluorobenzonitrile (3b) Yield: 64 %; m.p. 222-223 °C. Light Brown Colour, M.F; $C_{16}H_9Cl_2FN_2O$; IR (KBr pellet,) *v* max cm^{-1}: 3030 (Ar C-H str.) 1510 (Ar C=C str.), 1653 (C=O), 813 (C-Cl), 1176 (C-N), 1217 (C-F); ^1H NMR ($CDCl_3$) (δ/ppm): 4.66 (S, 1H, HC-Cl), 6.88-7.63 (m, 7H, Ar H), 4.32 (S,1H,(CH); ^{13}C NMR ($CDCl_3$) (δ/ppm): 162.29 (C=O), 67.23 (C-H), 55.49 (CH-Cl), 118.96-149.95 (Ar C), 114.61 (CN). Anal. Calcd. For: $C_{16}H_9Cl_2FN_2O$: C, 57.34; H, 2.71; N, 8.36. Found: C, 56.41; H, 2.61; N, 8.34.

2-(3-chloro-2-(4-fluorophenyl)-4-oxoazetidin-1-yl)-5-fluorobenzonitrile (3c) Yield: 68 %; m.p. 243-244 °C, Light Brown Colour, M.F; $C_{16}H_9ClF_2N_2O$, IR (KBr pellet,) *v* max cm^{-1}: 3043 (Ar C-H str.), 1512 (Ar C=C str.), 1654 (C=O), 796 (C-Cl), 1184 (C-N), 1215 (C-F); ^1H NMR ($CDCl_3$) (δ/ppm): 3.52 (S, 1H, HC-Cl), 7.28-8.18 (m, 7H, Ar-H), 3.38 (S,1H,(CH); ^{13}C NMR ($CDCl_3$) (δ/ppm): 162.30 (C=O), 65.07 (C-H), 55.47 (CH-Cl), 123.55-147.30 (Ar C), 114.29 (CN) Anal. Calcd. For: $C_{16}H_9ClF_2N_2O$: C, 60.30; H, 2.85; N, 8.79. Found: C, 60.28; H, 2.67; N, 8.70.

2-(3-chloro-2-oxo-4-p-tolylazetidin-1-yl)-5-fluorobenzonitrile (3d) Yield: 70 %; m.p. 216-217 °C, Yellow Colour, M.F; $C_{17}H_{12}ClFN_2O$, IR (KBr pellet,) *v* max cm^{-1}: 2922 (Ar C-H str.), 3049 (Ar C=C str.), 1662 (C=O), 821 (C-Cl), 1126 (C-N), 1174 (C-F); ^1H NMR

 DOI : 10.18231/2454-9150.2018.0106

($CDCl_3$) (δ/ppm): 3.39 (S, 1H, HC-Cl), 7.28-8.18 (m, 7H, Ar-H), 3.53 (S,1H,(CH), 2.26 (S,1H,(CH_3); ^{13}C NMR ($CDCl_3$) (δ/ppm): 162.30 (C=O), 65.97 (C-H), 55.97 (CH-Cl), 121.61-139.37 (Ar C), 113.6 (CN). Anal. Calcd. For: $C_{17}H_{12}ClFN_2O$: C, 60.87; H, 2.87; N, 8.87. Found: C, 60.80; H, 2.77; N, 8.80.

2-(3-chloro-2-(4-methoxyphenyl)-4-oxoazetidin-1-yl)-5-fluorobenzonitrile (3e) Yield: 72 %; m.p. 208-210 °C, light brown Colour, M.F; $C_{17}H_{12}ClFN_2O_2$, IR (KBr pellet,) *v* max cm^{-1}: 2922 (Ali C-H str.), 3051 (Ar C-H str.), 1510 (Ar C=C str.), 1660 (C=O), 819 (C-Cl), 1124 (C-N), 1176 (C-F); ^{1}H NMR ($CDCl_3$) (δ/ppm): 4.77 (S, 2H, $COCH_2$), 6.98-8.28 (m, 7H, Ar-H), 4.56 (S,1H,(CH), 3.78 (S,3H,(CH_3O); ^{13}C NMR ($CDCl_3$) (δ/ppm): 164.63 (C=O), 65.05 (C-H), 55.63 (CH-Cl), 123.54-139.66 (ArC), 55.03 (OCH_3). Anal. Calcd. For: $C_{17}H_{12}ClFN_2O_2$: C, 61.73; H, 3.66; N,8.47. Found: C, 61.73; H, 3.66; N, 8.45.

III. Results and Discussion

The structure of the compounds was assigned on the basis of spectral (IR, ^{1}H NMR, ^{13}C-NMR) data. The IR spectra of compounds (2a-e) showed absorption bands for the characteristic azomethine group (CH=N) in the 1598-1562 cm^{-1} range But this peak disappeared in the compounds (3a-e) this clearly indicated the formation of 3a-e. The carbonyl group of the β-lactam ring appeared as a characteristic absorption band in the range of 1662-1651 cm^{-1}. The absorption peaks due to C-Cl were appeared in the range of 725-821 cm^{-1} in all the compounds. The peaks at 1174-1255 cm^{-1} are attributed to C-F. The aromatic C-H and C=C showed stretching vibration in the range of 3030-3051 cm^{-1} and 1510-1514 cm^{-1}. In ^{1}H NMR the aromatic multiplets were observed at between δ 6.88-8.18 ppm a singlet at in range of δ 3.38- 4.56 ppm due to the presence of CH proton of azetidinone. In ^{13}C-NMR spectra of the azetidinone derivatives, the characteristic signals for a β-lactam, CHCl signal is present in all the compounds in the range of δ 55.44-55.97 ppm. The aromatic carbons showed multiplet in the region of δ118.96-149.95 ppm respectively. The CH was found at δ 65.05-67.23 ppm. The delta value for the carbonyl carbon in all the compounds was seen at 162.29-168.8 ppm. The CH-Cl exhibited singlet at δ 3.02- 4.77 ppm. The spectral data lend strong support to the proposed structures of all the synthesized compounds.

Antimicrobial activity

The Zone of inhibition in diameter values of compounds 3a-e has been determined using the filter paper disc diffusion method and the concentrations have been used in mm. All the final synthesized compounds 3a-e have been screened for their antibacterial activity against Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Streptococcus Pyogenes, Pseudomonas aeruginosa and screened for their antifungal activity against Aspergillus flavus, Aspergillus niger, Fusariumoxysporum, Penicillim chryogenum and Trigoderma viride .The Zone of inhibition values of standard Streptomycin and Amphotericin-B for all bacteria and fungi were in the range of 20-27 and 17-26 mm respectively. Compound 3b and 3d were active against the Staphylococcus aureus bacteria and Aspergillus niger fungai. The Zone of inhibition values of the compounds 3a-e were presented in (Table 1).

Antitubercular activity

The synthesized compounds 3(a-e) were screened against M. tuberculosis (H37Rv strain) using L. J. Medium at 50 µg/mL and lower concentrations. The standard antitubercular drugs Isoniazid and Rifampicin (MIC range 2-4 µg/mL) were taken as standards. The results were showing in (Table 2).

Table 1. Antibacterial and antifungal activities of compounds 3(a-e).

Compound	Zone of inhibition in diameter (mm)									
	BA	SA	EC	SP	PA	AF	AN	FO	PC	TV
3a	10	15	19	12	18	15	22	24	9	20
3b	14	25	20	12	16	11	21	15	13	12
3c	7	19	21	8	16	12	22	16	10	13
3d	13	17	18	12	15	0	24	14	12	12
3e	18	20	22	17	20	9	24	22	14	16
Streptomycine	27	24	26	20	22					
Amphotericin-B						22	25	26	17	22

BA:Bacillus subtilis,SA:Staphylococcus aureus,EC:Escherichia coli,SP:Streptococcus Pyogenes,PA:Pseudomonasaeruginosa,AF:Aspergillusflavus,AN:Aspergillusniger, FO:Fusariumoxysporum,PC:Penicillimchryogenum, TV:Trigoderma viride

 DOI : 10.18231/2454-9150.2018.0106

Table 2. Antitubercular activity of compounds 3a-e.			
Compound	Concentration	Compound	Concentration
3a	5.25	3d	2.2
3b	5.5	3e	2.3
3c	5.3		

IV. Conclusion

Concluded that all compounds have been synthesized successfully and screened for antimicrobial and antitubercular activities data of compounds (shown in Table 1 and 2) revealed that the compounds shows moderate to good activities against all the strains compared with standard drugs.

Acknowledgements

The author acknowledges the SAIF for providing the spectral data.

References

[1] Margherita, De. Rosa.; Giovanni Vigliotta.; Giuseppe Palm.; Carmela Saturnino .;Annunziata Soriente. Molecule. 2015, 20, 22044.

[2] Patel,PS.; Gor, DG.; Patel, PA. International journal for pharmaceutical research scholars.2012, 1, 12.

[3] Navin, B.; Patel, Jaymin C.; Patel. Arabian J. Chemistry. 2011, 4, 403.

[4] Llarrull ,L.I.; Testero, S.A.; Fisher, J.F.; Mobashery,s. Microbial. 2010, 13,551.

[5] Kong .K.F.; Mathee,L. APMIS.2010, 1, 118.

[6] Satyanarayan, V.;Sreevani ,P.; Sivakumar.A, Arkivoc. 2008, 17.

[7] Mosaad, S.M.; Mohammed, K.I.; Ahmed, M.A.; Abdel-Hamide, S.G. J. Appl. Sci.2004, 4, 302.

[8] Kumar, A.; Rajput, C.S; Bhati, S.K. Bioorg. Med. Che. 2007, 15, 3089.

[9] Smith, E.M.; Sorota,S.; Kim, H.M.; McKittrick, B.A; Nechuta, T.L.; Bennett C, Knutson, C.; Burnett, D.A. Bio org. Med. Chem. Lett.2010, 20, 4602.

[10] Ameya, A.; Chavan .; Nandini, R.Pai. Molecules. 2007, 2, 2467.

[11] Freddy, H.; Havaldar.; Sushil Kumar, J.Mishra. Indian. J. Heterocycl.Chem. 2004, 13, 197.

[12] Patel, K.H.; Mehta, A.G. E. J. Chem.2006, 3, 267.

[13] Vijay Kumar, M.M.J.; Yogananda, R.; Snehalatha.; Shameer, H.; Jayachandra, E.; Sreenivas, G.M. J. of biomed. Sci. and res.2009, 1, 1.

[14] Gavernet, L.; Barrios, I.; Cravero, M.S.; Bruno-Blanch, L.E. Bioorg. Med.Chem.2007, 15, 5604.

[15] Aldo, Andreani.; Silvia, Burnell. Bioorg. Med. Chem.2010, 18, 3004.

[16] Fabian,E.; Olazarana, Gildardo Riverab. ACS Med. Chem. Lett. 2016, 10.

[17] Niamh, MO'Boyle.; Miriam, Carr. J. Med. Chem. 2010, 53, 8569.

[18] Oana, Maria Dragostin.; Florentina, Lupascu.; Cornelia ,Vasile.; ValentinNastasa Ramona Florina, Moraru.; DragosPieptu.; Lenuta ,Profire. Molecules. 2013, 18, 4140.

 DOI : 10.18231/2454-9150.2018.0106