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**SYNTHESIS OF CHLORO, FLUORO, PHENYL SUBSTITUTED AZETIDIN-2-ONE  
DERIVATIVES BY MICROWAVE METHOD AND SCREENING FOR  
ANTIMICROBIAL ACTIVITIES**

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**Abstract**

As azetidines and their derivatives such as penicillins and cephalosporins are best known for their antimicrobial activity against a wide range of microorganisms, we were interested in synthesizing azetidinone derivatives with different substituents specially halogens, which are known for their activity. Hence we have synthesized a series of Chloro, Fluoro substituted azetidinone derivatives and screened them against different bacteria and fungi. We have found that these derivatives have shown promising activities as antibacterial, antifungal and anti-tubercular agents.

We have employed microwave technique in our research work as it will give good yield in short span of time and it is eco friendly too. We have synthesized our compounds by microwave technique and later compared them with the same synthesized by conventional technique. We found that the results were promising.

Series of Chloro, Fluoro, Phenyl Substituted Azetidin-2-ones with different substituent's were synthesized both by microwave technique and conventional method. Their structures were characterized by different spectral techniques such as IR, <sup>1</sup>H NMR, LC-MS. The synthesized compounds were screened for antimicrobial activities.

**Key words:** Azetidinones, antibacterial, antifungal, antitubercular, microwave, Schiff's bases.

## Introduction

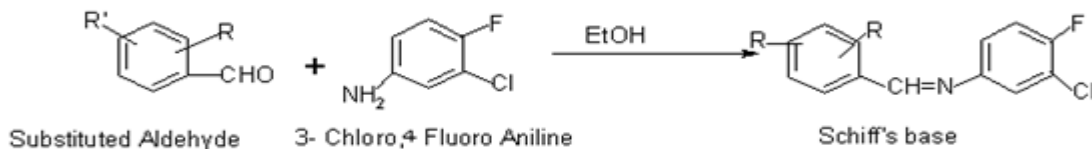
Azetidinone derivatives are known to possess wide range of pharmacological activities like antimicrobial<sup>1-5</sup>, antiviral<sup>6</sup>, antitubercular<sup>7</sup>, antidepressant<sup>8</sup> and anti-inflammatory<sup>9</sup> are widely used today. We have synthesized Chloro, Fluoro, Phenyl substituted Azetidin-2-one derivatives in order to obtain more potent antimicrobial (including anti-tubercular) agents.

Because of its synthetic value and wide range of pharmacological activities, Azetidinone is an important heterocyclic nucleus and has gained wide acceptance across the world.

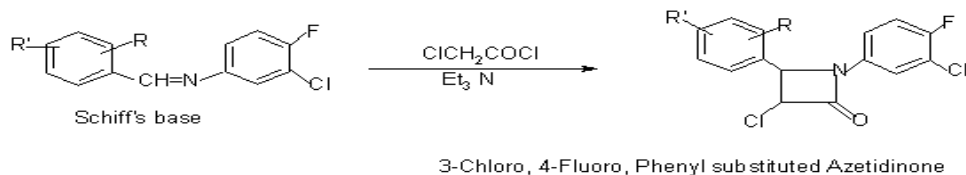
As conventional methods of synthesis is time consuming process and also requires patience, using microwave as an alternative to the conventional method would be more useful as it requires short time and fast yielding technique. It is also proven to be eco friendly unlike conventional methods. As synthesizing a new molecule from conventional way and developing it into a finished product requires years together, we can think of replacing them by using such techniques such as microwave to save lot of time in this process.

In a nutshell, as bacteria continue to challenge the society and scientific community with their mortality, keeping this in mind we aim to synthesize new and substituted Azetidinone derivatives as antimicrobial agents to contribute to the society.

### Step-1



### Step-2



R = H, OH, Cl, NO<sub>2</sub>, CH<sub>3</sub>, OCH<sub>3</sub>,

a) **SCHEME** : Synthesis of 3- Cl, 4- F, Phenyl Substituted Azetidinones.

## Materials and Methods

For the synthesis of Chloro, Fluoro Phenyl substituted Azetidinone derivatives different substituted aromatic aldehydes and Chloro, Fluoro Aniline used in this process were purchased from Aldrich chemical Co. N-(substituted benzylidene)3-Chloro, 4-Fluoro benzanamine and different Cl, F Phenyl substituted azetidinone were synthesized using BPL 2300 ET domestic microwave according to literatures.

Synthesis of N-(substituted benzylidene) 3-Chloro, 4-Fluoro benzanamine using microwave irradiation<sup>10</sup>:

To a solution of substituted aromatic aldehydes (1) (0.01 mol) in Ethanol, 3-Cl, 4-F Aniline (2) (0.01 mol) was added slowly with constant stirring. The reaction mixture was irradiated under the microwave for 4-5 minutes. The resulting reaction mixture was poured into ice cold water. A solid mass obtained, which was filtered, dried and recrystallised from aqueous ethanol, gave us 85% yield (3a-j).

Melting points were determined using Thiel's tube. The compound (3a) has shown IR (KBr) bands at  $3572\text{ cm}^{-1}$  (OH),  $1618\text{ cm}^{-1}$  (N=CH),  $1272\text{ cm}^{-1}$  (C-N),  $1225\text{ cm}^{-1}$  (Ar C-F) and  $802\text{ cm}^{-1}$  (Ar C-Cl). <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$ -12.8 (1H-OH), 8.6-7.4 (4H, Ar C-H), 7.2-6.9 (3H, Ar C-H), 1.5 (1H, C-H). TLC was performed on silica gel 60 coated plates to confirm the completion of reaction using Petroleum ether and Ethyl acetate as the mobile phase.

Synthesis of different Cl, F Phenyl substituted azetidin-2-ones using microwave irradiation<sup>10</sup>:

To the solution of above formed Schiff's base, N-(substituted benzylidene) 3- Cl, 4-F, benzanamine (0.01 mol) in DMF, added chloroacetylchloride (0.01 mol) and triethylamine (0.01 mol) slowly in cold condition. This mixture was irradiated under microwave for 5-6 minutes. The resulting reaction mixture was filtered, dried and recrystallised from aqueous ethanol. The compounds 4(a-j) were obtained were characterized by IR, <sup>1</sup>H NMR, LC-MS.

Synthesis of 3- Cl, 4- F, Phenyl Substituted Azetidinones<sup>11,12</sup>

Chloro Fluoro, Phenyl substituted Azetidine derivatives (4a-j) were synthesized here by reacting different Schiff's bases with chloroacetylchloride in presence of triethylamine under microwave irradiation technique. We have also synthesized compounds 4a-j by conventional method. We found that microwave technique gives more yield in short span of time when compared with the conventional method.

## Results

All the synthesized compounds AZ1-AZ10, were initially subjected to M.P determination and TLC for monitoring of the reactions. Later, among all the synthesized compounds, some representative compounds were subjected to IR, HNMR and LCMS for characterization and confirmation of their molecular structures. The different absorption bands of the derivatives observed in the spectra (IR, HNMR and LCMS) are in conformity with the data shown in the tables and hence our discussion is valid.

**Table-1: Physical parameters of the synthesized compounds.**

Code	M.P	R <sub>f</sub>	TLC-Mobile . Phase
AZ1	135-137 <sup>0</sup> C	0.78	Petroleum Ether:Ethyl acetate(9:1)
AZ2	74-75	0.79	
AZ3	68-70	0.81	
AZ4	84-86	0.78	
AZ5	108-110	0.81	
AZ6	110-112	0.78	
AZ7	88-90	0.80	
AZ8	72-74	0.78	
AZ9	95-97	0.79	
AZ10	123-125	0.80	

The IR spectra (KBr) of compounds AZ1- AZ4 have shown characteristic vibrational bands for Ar C-H, C=O and amide (NH<sub>2</sub>-CO) groups of these derivatives in table 2. The <sup>1</sup>H NMR (CDCl<sub>3</sub>) of compounds AZ1, AZ2 and AZ3 have shown characteristic aromatic C-H and aliphatic C-H peaks along with the functional group OH of AZ1 in table-3. LC-MS data of compounds AZ2 and AZ4 has shown molecular ion peak (M<sup>+</sup>), isotope peak (M+2) and some of the identifiable fragment ions in table 3. So the assigned structures of the synthesized compounds were substantiated by IR (KBr), <sup>1</sup>H NMR (CDCl<sub>3</sub>) and LC-MS data.

**Table-2: IR –Spectral data of the synthesized compounds.**

Code	Spectral vibrations(in cm-1)	Molecular nature
AZ 1	3389 3092 1746 1691 1264 757	-OH str -CH C=O (Amide)NHCO -C-F(Ar) C-Cl(Ar)
AZ 2	3090 1754 1667 1404 817	-CH str C=O str Amide -C-F(Ar) C-Cl(Ar)
AZ 3	3100 1762 1692 814	-CH str C=O str Amide C-Cl(Ar)
AZ 4	3060 1764 1672 1492 1225 753	-CH str C=O str Amide Conjugated -C-F(Ar) C-Cl(Ar)

**Table-3: Spectral data (<sup>1</sup>H NMR & LC-MS) of the synthesized compounds.**

Code	MOL FORMULA	MOL WEIGHT	<sup>1</sup> H NMR (In δ ppm) AND PROTON NATURE	MASS DATA
AZ1	C <sub>15</sub> H <sub>10</sub> NO <sub>2</sub> Cl <sub>2</sub> F	325	8.7 - 6.9, 7H(Ar-H) 5.9 - 5.6, 1H(OH) 3.8 - 2.4, 2H(C-H)	324.62 (M+) 327(M+2)
AZ2	C <sub>15</sub> H <sub>9</sub> NOCl <sub>3</sub> F	343	8.2 - 4.5, 7H(Ar-H) 3.5 - 2.4, 2H(C-H)	343.52 (M+) 345(M+2) 234, 171,91
AZ3	C <sub>15</sub> H <sub>8</sub> NOFCl <sub>4</sub>	377	8.4 - 4.1, 7H(C-H) 3.6 - 1.5, 2H(C-H)	376.7 (M+) 379(M+2)
AZ4	C <sub>15</sub> H <sub>9</sub> N <sub>2</sub> O <sub>3</sub> FCl <sub>2</sub>	354	8.0 - 4.2, 7H (C-H) 3.5 - 1.8, 2H (C-H)	353.35 (M+) 355(M+2) 234, 182,171

Details of the representative compound-AZ4

N-(3-Chloro, 4-Fluoro Phenyl) 4-(4-Nitro phenyl),3-Chloro, Azetidin-2-one.

Molecular Formula: C<sub>15</sub>H<sub>9</sub>N<sub>2</sub>O<sub>3</sub>FCl<sub>2</sub>

Molecular Weight : 354

Melting Point : 84 -86 °C

Percentage Yield: 62%

The synthesized compounds were screened for their antibacterial, antifungal(cup method) and antitubercular activities.

The standard subculture strains of micro-organisms used were *S. aureus* (ATCC 2091) and *E. coli* (ATCC 8190) were used for evaluation for antibacterial activity as against Ciprofloxacin by cup plate method.

The results obtained were noteworthy because some derivatives have shown promising results as derivative A shown in table-4. AZ3 has shown moderate antibacterial activity against *S. aureus*, mild activity against *E. coli* and AZ4 has shown mild to moderate activity against both the strains when Compared to standard drug Ciprofloxacin.

**Table-4: Antibacterial activity of the synthesized compounds.**

Code	Dose µg/ml	ZONE OF INHIBITION	
		<i>S. aureus</i>	<i>E. coli</i>
4a	75	12	12
4c		22	10
4d		14	18
4e		10	8
4h		12	10
4j		8	6
<b>Std Ciprofloxacin</b>	<b>10</b>	<b>32</b>	<b>30</b>

4a	<b>50</b>	10	12
4c		14	14
4d		14	12
4g		12	10
4h		10	8
<b>StdCiprofloxacin</b>	<b>10</b>	<b>30</b>	<b>27</b>

Standard subcultures of *C. albicans* (ATCC 2091) and *A. fumigatus* (ATCC 8199) were used for screening of antifungal activities. We found that many of the synthesized derivatives have shown moderate to good activity as compared to the standard as shown in table 5. Compound AZ3 has shown good antifungal activity against *C. albicans* and *A. fumigatus*, AZ4 has shown good antifungal activity against *C. albicans*, moderate activity against *A. fumigatus*, AZ8 has shown moderate antifungal activity against both the strains, when compared to standard drug Fluconazole<sup>11,12</sup>. Results are giving us a valuable piece of information regarding the presence of substituents on phenyl rings.

**Table-5: Antifungal activity of synthesized compounds.**

Code	Dose µg/ml	ZONE OF INHIBITION	
		<i>C. albicans</i>	<i>A.fumigatus</i>
4a	<b>75</b>	18	14
4c		<b>34</b>	<b>20</b>
4d		<b>36</b>	<b>14</b>
4g		18	14
4h		<b>20</b>	<b>16</b>
4j		16	14
<b>*Fluconazole</b>	<b>10</b>	<b>36</b>	<b>24</b>

4a	<b>50</b>	14	12
4c		<b>30</b>	<b>17</b>
4d		<b>30</b>	<b>12</b>
4g		14	12
4h		<b>16</b>	<b>12</b>
4j		14	12
<b>*Fluconazole- 6 µg/ml</b>		<b>30</b>	<b>19</b>

Later the synthesized compounds were screened for their antitubercular activities. H<sub>37</sub>RV strain of *M. tuberculosis* was used and the results were compared with the standard drug streptomycin<sup>13</sup>. The derivatives AZ1, AZ3, AZ4, AZ5, AZ6, AZ8 and AZ10 have shown significant activity as there was sensitivity produced to these compounds by the microorganism. Other derivatives AZ2, AZ7 and AZ9 have shown weak activity as there was resistance observed to these compounds by as shown in table- 6.

**Table- 6: Anti tubercular activity of the synthesized compounds.**

<b>Compound</b>	<b>25 µg/ml</b>	<b>50 µg/ml</b>
4a	--	--
4b	++	++
4c	--	--
4d	--	--
4e	--	--
4f	--	--
4g	++	++
4h	--	--
4i	++	++
4j	--	--
<b>Std Streptomycin</b>	--	--



+ +: (Resistance) Denotes growth      - - : (Sensitivity) Denotes no growth

The results are inclined towards the contribution of the substituents towards overall activities. Surprisingly the electronegative groups on the phenyl rings have dominated over the other groups in their potency.

### Conclusion

A series of Chloro, Fluoro Phenyl substituted Azetidin-2-one derivatives were prepared both by microwave and conventional methods and characterized by IR, H NMR and LC-MS data. The synthesized compounds were screened for their antibacterial, antifungal and antitubercular activities. We have found that the synthesized compounds have shown significant activity as antifungal and antitubercular agents, where as moderate activity as antibacterial agents compared with the standard. The change in their activity is mainly due to the substituent/s especially electronegative on the phenyl rings.

We presume that these compounds would show more promising activities as antimicrobial agents if more research work is carried out in this direction.

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