

# Synthesis & Antifungal Screening of Novel Azetidin-2-ones

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**Abstract:** A new series of 4-[3-chloro-2-(4-hydroxy-3-methoxybenzylidene)-4-oxoazetidin-1-yl]amino-N-(substituted) benzenesulfonamide, 4-{3-chloro-2-[5-methoxy-2-nitro-4-(prop-2-en-1-yloxy)benzylidene]-4-oxoazetidin-1-yl}amino-N-(substituted)benzenesulfonamide and 4-{3-chloro-2-[4-hydroxy-3-methoxy-5-(prop-2-en-1-yl)benzylidene]-4-oxoazetidin-1-yl}amino-N-(substituted) benzenesulfonamide were synthesized using appropriate synthetic route. The chemical structures of all the synthesized compounds were deduced on the basis of elemental analysis and spectroscopic data. The antifungal activity of the synthesized compounds was screened against several fungus. The synthesized compounds show potent antifungal activity against *Aspergillus niger* & *Aspergillus flavus* and significant structure-activity relationship (SAR) trends.

**Keywords:** *Aspergillus flavus*, Azetidin-2-ones, SAR.

## INTRODUCTION

Heterocycles constitute one of the most significant areas of research in the field of medicinal chemistry [1]. The azetidin-2-ones ( $\beta$ -lactam) heterocycles are considered as an important contribution of science to humanity [2], since they have been constituents of living organisms, natural products, drugs and many more substances useful to mankind and society in all walks of life. Their synthesis and evaluation has always drawn the attention of chemists & biologists over the years [3]. Since the discovery of penicillins [4, 5], and cephalosporins as the most successful antibiotics, azetidin-2-ones have been the subject of regular discussion and investigation. The azetidin-2-ones antibiotics endowed with unique structure and potent antibacterial activity, include, penicillins, cephalosporins, carbapenems, nocardicins, clavulanic acid, sulbactams, and tazobactams. These molecules operate by forming a covalent adduct with membrane-bound bacterial transpeptidases, which are also known as penicillin binding proteins (PBPs), involved in the biosynthesis of cell walls. These mechanism-based inhibitors prevent the construction of cell wall and eventually lead to cell lysis and death. Moreover, due to their  $\beta$ -lactamase inhibitory action, azetidin-2-ones based heterocycles represent an attractive target of contemporary organic synthesis. Azetidin-2-ones and its derivatives are important compounds due to their broad range of biological activities such as antimicrobial, anti-tubercular, anticancer, cholesterol absorption inhibition, antidiabetic, analgesic and anti-inflammatory, thrombin inhibition, antiparkinsonian, vasopressin via antagonist, anti-convulsant [6-30].

## MATERIALS AND METHODS

All chemicals and solvents, reagents used in the present study were of analytical grade and solvents were used after distillation. All the melting points of the synthesized

compounds were determined by open capillary and are uncorrected. The purity of the compounds was checked using precoated TLC plates (MERCK) using chloroform: ethyl acetate (8:2) solvent system. The developed chromatographic plates were visualized under UV at 254nm. IR spectra were recorded using KBr on Perkin Elmer spectrophotometer. <sup>1</sup>HNMR spectra in DMSO on a BRUKER FT-NMR instrument using TMS as internal standard and chemical shift values were expressed in ppm. Elemental analysis (CHN) was performed on Carlo Erba 1108.

The present work is undertaken to explore more possibilities of finding a suitable derivative, which would exceed its activity more than the already known drugs containing azetidin-2-ones ring. With the alarming trends in fungal resistance to many azetidin-2-ones antibiotics it has become necessary to synthesize some novel azetidin-2-ones for bio-assay of antifungal activity and the need for drugs with more specific antifungal activity. Therefore it was thought of interest to combine all the above-mentioned biolabile heterocyclic rings together in a molecular framework of imines in order to enhance the additive effect toward the biological activity. Keeping this in mind and in continuation of our earlier studies, we designed and synthesized 4-[3-chloro-2-(4-hydroxy-3-methoxybenzylidene)-4-oxoazetidin-1-yl]amino-N-(substituted) benzenesulfonamide, 4-{3-chloro-2-[5-methoxy-2-nitro-4-(prop-2-en-1-yloxy) benzylidene]-4-oxoazetidin-1-yl}amino-N-(substituted) benzenesulfonamide and 4-{3-chloro-2-[4-hydroxy-3-methoxy-5-(prop-2-en-1-yl)benzylidene]-4-oxoazetidin-1-yl}amino-N-(substituted) benzene sulfonamide with fascinating structural features. Moreover, in order to assess the antifungal potentiality of azetidin-2-ones nucleus and to investigate the structure-activity-relationship, the constructed molecules were screened for their antifungal activities.

The synthetic pathway includes an electrocyclisation reaction of imine derivatives to give direct access to the desired 4-[3-chloro-2-(4-hydroxy-3-methoxybenzylidene)-4-oxoazetidin-1-yl]amino-N-(substituted)benzenesulfonamide,

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4-{3-chloro-2-[5-methoxy-2-nitro-4-(prop-2-en-1-yloxy)benzylidene]-4-oxoazetidin-1-yl]amino}-N-(substituted)benzenesulfonamide and 4-{3-chloro-2-[4-hydroxy-3-methoxy-5-(prop-2-en-1-yl)benzylidene]-4-oxoazetidin-1-yl]amino}-N-(substituted)benzenesulfonamide.

The approach involves the initial preparation of precursors 4-{{(E)-(4-hydroxy-3-methoxy benzylidene) amino}-N-(substituted)benzenesulfonamide, 4-{{(E)-[5 -methoxy-2- nitro- 4- (prop -2 -en- 1 -yloxy) benzylidene] amino}-N-(substituted)benzenesulfonamide and 4-{{(E)- (4-hydroxyl-3-methoxy-5-(prop-2-en-1-yl)benzylideneamino)-N-(substituted)benzenesulfonamide. The reactive imine derivatives were accessible via the reaction of an equimolar quantity of aromatic aldehyde containing allyl and allyloxy group with different sulpha drugs in ethanol at room temperature which resulted in the formation of 4-{{(E)-(4-hydroxy-3-methoxy benzylidene) amino}-N-(substituted) benzenesulfonamide, 4-{{(E)-[5 -methoxy- 2- nitro- 4- (prop -2 -en- 1 -yloxy) benzylidene] amino}-N-(substituted) benzenesulfonamide and 4-{{(E)-(4-hydroxyl-3-methoxy-5-(prop-2-en-1-yl) benzylideneamino)-N-(substituted)benzenesulfonamide [31] in good yield.

Further, the initially formed reactive imines underwent an electrocyclic reaction of chloroacetyl chloride and triethyl amine in 1,4-dioxane afforded the target compounds 4-[3-chloro-2-(4-hydroxy-3-methoxybenzylidene)-4-oxoazetidin-1-yl]amino-N-(substituted) benzenesulfonamide, 4-[3-chloro-2-[5-methoxy-2-nitro-4-(prop-2-en-1-yloxy) benzylidene]-4-oxoazetidin-1-yl]amino}-N-(substituted) benzenesulfonamide and 4-[3-chloro-2-[4-hydroxy-3-methoxy-5-(prop-2-en-1-yl)benzylidene]-4-oxoazetidin-1-yl] amino}-N-(substituted)benzenesulfonamide [32] in good yield. Typically, ketenes are generated thermally from acid chlorides in the presence of triethylamine, which on subsequent reaction with reactive imines yield the desired azetidin-2-ones [33].

### General Procedure for the Synthesis of Azetidinones: [3A-L]

Imine (0.001mole) was added to a constantly stirred solution of 1,4 dioxane (15ml), triethylamine (0.001mole) and chloroacetylchloride (0.001mole). The reaction mixture was stirred at 50°C. The reaction vessel was then kept at room temperature for 30min and refluxed for 8hr. On cooling the precipitate was obtained, which was filtered and, thoroughly washed with water.

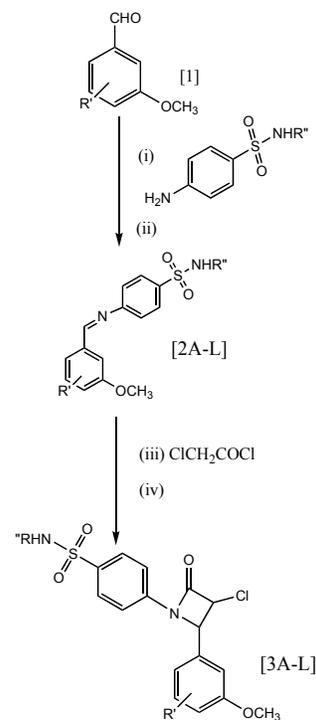
## RESULTS AND DISCUSSION

Structures of newly obtained compounds have been ascertained on the basis of their consistent IR, <sup>1</sup>H NMR and elemental analysis assignments.

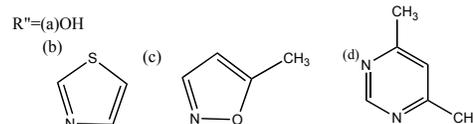
### 2(A) 4-{{(E)-(4-hydroxy-3-methoxyphenyl)methylidene} amino}benzenesulfonamide

Recrystallization from ethanol: C<sub>14</sub>H<sub>14</sub>N<sub>2</sub>O<sub>4</sub>S; Molecular wt. 306; Yield 58%; m.p.200°C; colour-whitsh yellow; IR (KBr) cm<sup>-1</sup> 3400cm<sup>-1</sup> (O-H), 1670 cm<sup>-1</sup> (CH=N), 1139 cm<sup>-1</sup> (S=O); <sup>1</sup>HNMR (DMSO d<sub>6</sub>) δ,ppm: 3.75 (s,3H,CH), 3.0 (s,1H,OH), 5.2-7.2 (m,3H,Ar-CH), 8.4 (s,1H,CH=N), 7.6 (t,2H,CH), 7.9 (t,2H,CH), 2.2 (s,2H,SO<sub>2</sub>NH<sub>2</sub>). Anal.Calcd. (%): C=54.90, H=4.60, N=9.12, O=20.91, S=10.47.

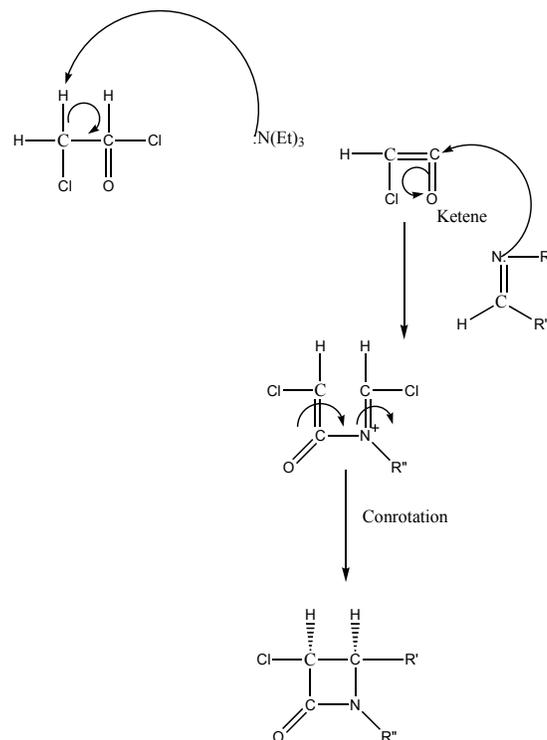
### Scheme of Reaction



Where R' = (I) p-OH, (p position with respect to aldehyde)  
(II) p-CH<sub>2</sub>=CH-OCH<sub>2</sub>, o-NO<sub>2</sub> (p and o position with respect to aldehyde)  
(III) p-OH, m-CH<sub>2</sub>=CH-CH<sub>2</sub>, (p and m position with respect to aldehyde)



Reagents and conditions: (ii) EtOH, reflux, 2h; (iv) (C<sub>2</sub>H<sub>5</sub>)<sub>3</sub>N, 1,4-dioxane, stirred 1h, reflux, 8h



### Mechanism of Reaction

**2(B) 4-[(4-hydroxy-3-methoxybenzylidene)amino]-N-(1,3thiazol-2-yl)benzenesulfonamide**

Recrystallization from ethanol: C<sub>17</sub>H<sub>15</sub>N<sub>3</sub>O<sub>4</sub>S<sub>2</sub>; Molecular wt. 389; Yield 72%; m.p.150<sup>0</sup>C; colour-yellow; IR (KBr) cm<sup>-1</sup> 3420cm<sup>-1</sup> (O-H), 1650 cm<sup>-1</sup> (CH=N), 1115 cm<sup>-1</sup> (S=O); <sup>1</sup>HNMR (DMSO d<sub>6</sub>) δ,ppm: 3.75 (s,3H,CH), 3.0 (s,1H,OH), 5.2-7.2 (m,3H,Ar-CH), 8.4 (s,1H,CH=N),7.6 (t,2H,CH), 7.9 (t,2H,CH), 4.2(s,1H,SO<sub>2</sub>NH), 6.5-7.5 (m,2H,CH). Anal.Calcd.(%): C=52.40, H=3.91, N=10.70, O=16.52, S=16.47.

**2(C)4-[(4-hydroxy-3-methoxybenzylidene)amino]-N-(5-methylisoxazol-2-yl)benzenesulfonamide**

Recrystallization from ethanol: C<sub>18</sub>H<sub>17</sub>N<sub>3</sub>O<sub>5</sub>S; Molecular wt. 387; Yield 98%; m.p.190<sup>0</sup>C; colour-pale yellow; IR (KBr) cm<sup>-1</sup> 3440cm<sup>-1</sup> (O-H), 1670 cm<sup>-1</sup> (CH=N), 1149 cm<sup>-1</sup> (S=O); <sup>1</sup>HNMR (DMSO d<sub>6</sub>) δ,ppm:3.75 (s,3H,CH), 3.0 (s,1H,OH), 5.2-7.2 (m,3H,Ar-CH), 8.4 (s,1H,CH=N), 7.6 (t,2H,CH), 7.9 (t,2H,CH), 4.2 (s,1H,SO<sub>2</sub>NH), 5.2 (s,1H,CH), 2.5 (s,3H,CH<sub>3</sub>). Anal.Calcd. (%): C=52.30, H=4.01, N=10.70, O=16.52, S=16.47.

**2(D)N-(4,6-dimethylpyrimidin-2-yl)-4-[(E)-(4-hydroxy-3-methoxyphenyl)methylidene]amino } benzene sulfonamide**

Recrystallization from ethanol: C<sub>20</sub>H<sub>20</sub>N<sub>4</sub>O<sub>4</sub>S; Molecular wt. 412; Yield 50%; m.p.110<sup>0</sup>C; colour-yellow ;IR (KBr) cm<sup>-1</sup> 3450cm<sup>-1</sup> (O-H), 1660 cm<sup>-1</sup> (CH=N), 1153 cm<sup>-1</sup> (S=O); <sup>1</sup>HNMR (DMSO d<sub>6</sub>) δ,ppm: 3.75 (s,3H,CH), 3.0 (s,1H,OH), 5.2-7.2 (m,3H,Ar-CH), 8.4 (s,1H,CH=N), 7.6 (t,2H,CH), 7.9 (t,2H,CH), 4.2 (s,1H,SO<sub>2</sub>NH), 3.8 (s,1H,CH), 2.8-3 (s,6H,CH<sub>3</sub>). Anal.Calcd. (%):C=58.20, H=4.93, N=13.50, O=15.60, S=7.77.

**2(E) 4-[(5-Methoxy-2-nitro-4-vinylloxymethyl-benzylidene)-amino]-benzenesulfonamide**

Recrystallization from ethanol: C<sub>17</sub>H<sub>17</sub>N<sub>3</sub>O<sub>6</sub>S; Molecular wt. 391; Yield 66%; m.p.120<sup>0</sup>C; colour-yellow; IR (KBr) cm<sup>-1</sup> 1617 cm<sup>-1</sup> (CH=N), 1598 cm<sup>-1</sup> (NO<sub>2</sub>), 1139 cm<sup>-1</sup> (S=O); <sup>1</sup>HNMR (DMSO d<sub>6</sub>) δ,ppm:3.75 (s,3H,CH), 5.2-7.2 (m,2H,Ar-CH), 5.4 (s,2H,CH), 6.5 (t,1H,CH), 4.2 (d,2H,CH), 8.4 (s,1H,CH=N), 7.6 (t,2H,CH), 7.9 (t,2H,CH), 4.2 (s,2H,SO<sub>2</sub>NH<sub>2</sub>). Anal.Calcd.(%): C=52, H=4.55, N=10.70, O=24.57,S=8.19.

**2(F)4-[(5-Methoxy-2-nitro-4-vinylloxymethyl-benzylidene)-amino]-N-thiazol-2-yl-benzene sulfonamide**

Recrystallization from ethanol: C<sub>20</sub>H<sub>18</sub>N<sub>4</sub>O<sub>6</sub>S<sub>2</sub>; Molecular wt. 474; Yield 70%; m.p.110<sup>0</sup>C; colour- brown yellow; IR (KBr) cm<sup>-1</sup> 1627 cm<sup>-1</sup> (CH=N), 1588cm<sup>-1</sup> (NO<sub>2</sub>), 1190 cm<sup>-1</sup> (S=O); <sup>1</sup>HNMR (DMSO d<sub>6</sub>) δ,ppm:3.75 (s,3H,CH), 5.2-7.2 (m,2H,Ar-CH), 5.4 (s,2H,CH), 6.5 (t,1H,CH), 4.2 (d,2H,CH), 8.4 (s,1H,CH=N), 7.6 (t,2H,CH), 7.9 (t,2H,CH), 4.2 (s,1H,SO<sub>2</sub>NH), 6.5-7.5 (m,2H,CH). Anal.Calcd.(%): C=50.30%, H=3.82, N=11.81, O=20.55, S=13.51.

**2(G)N-(4,6-Dimethyl-pyrimidin-2-yl)-4-[(5-methoxy-2-nitro-4-vinylloxymethyl-benzylidene)-amino]-benzenesulfonamide**

Recrystallization from ethanol: C<sub>23</sub>H<sub>23</sub>N<sub>5</sub>O<sub>6</sub>S; Molecular wt. 520; Yield 60%; m.p.130<sup>0</sup>C; colour- brown yellow; IR

(KBr) cm<sup>-1</sup> 1620 cm<sup>-1</sup> (CH=N), 1542cm<sup>-1</sup> (NO<sub>2</sub>), 1139 cm<sup>-1</sup> (S=O); <sup>1</sup>HNMR (DMSO d<sub>6</sub>) δ,ppm:3.75 (s,3H,CH), 5.2-7.2 (m,3H,Ar-CH), 5.4 (s,2H,CH), 6.5 (t,1H,CH),. 2 (d,2H,CH), 8.4 (s,1H,CH=N), 7.6 (t,2H,CH), 7.9 (t,2H,CH), 4.2 (s,1H,SO<sub>2</sub>NH), 3.8 (s,1H,CH), 2.8-3 (s, 6H, CH<sub>3</sub>). Anal. Calcd. (%):C=55.50, H=4.68, N=14, O=19.37, S=6.44.

**2(H)4-[(4-Allyloxy-5-methoxy-2-nitro-benzylidene)-amino]-N-(5-methyl-isoxazol-3-yl)-benzene sulfonamide**

Recrystallization from ethanol: C<sub>21</sub>H<sub>20</sub>N<sub>4</sub>O<sub>7</sub>S; Molecular wt. 472; Yield 70%; m.p.120<sup>0</sup>C; colour- brown yellow; IR (KBr) cm<sup>-1</sup> 1640 cm<sup>-1</sup> (CH=N), 1540cm<sup>-1</sup> (NO<sub>2</sub>), 1120 cm<sup>-1</sup> (S=O); <sup>1</sup>HNMR (DMSO d<sub>6</sub>) δ,ppm:3.75 (s,3H,CH), 5.2-7.2 (m,2H,Ar-CH), 5.4 (s,2H,CH), 6.5 (t,1H,CH), 4.2 (d,2H,CH), 8.4 (s,1H,CH=N), 7.6 (t,2H,CH), 7.9 (t,2H,CH), 4.2 (s,1H,SO<sub>2</sub>NH), 5.2 (s,1H,CH), 2.5 (s,3H,CH<sub>3</sub>). Anal.Calcd.: C=53.30, H= 4.35, N=11.80, O= 23.76, S= 6.79.

**2(I)4-[(3-Allyl-4-hydroxy-5-methoxy-benzylidene)-amino]-benzenesulfonamide**

Recrystallization from ethanol: C<sub>17</sub>H<sub>18</sub>N<sub>2</sub>O<sub>4</sub>S; Molecular wt. 346; Yield 72%; m.p.75<sup>0</sup>C; colour- yellow; IR (KBr) cm<sup>-1</sup> 3430cm<sup>-1</sup> (O-H), 1630 cm<sup>-1</sup> (CH=N); <sup>1</sup>HNMR (DMSO d<sub>6</sub>) δ,ppm: 3.75 (s,3H,CH), 3.0 (s,1H,OH), 5.2-7.2 (m,2H,Ar-CH), 4.96 (d,2H,CH), 6.4 (p,1H,CH), 3.5 (d,2H,CH), 8.4 (s,1H,CH=N), 7.6 (t,2H,CH), 7.9 (t,2H,CH), 2.2 (s,2H, SO<sub>2</sub>NH<sub>2</sub>). Anal.Calcd.(%): C=58.90, H=5.28, N=8.09, O=18.47, S=9.27.

**2(J)4-[(3-Allyl-4-hydroxy-5-methoxy-benzylidene)-amino]-N-thiazol-2-yl-benzenesulfonamide**

Recrystallization from ethanol: C<sub>20</sub>H<sub>19</sub>N<sub>3</sub>O<sub>4</sub>S; Molecular wt. 429; Yield 80%; m.p.65<sup>0</sup>C; colour- cremish yellow; IR (KBr) cm<sup>-1</sup> 3410cm<sup>-1</sup> (O-H), 1650 cm<sup>-1</sup> (CH=N); <sup>1</sup>HNMR (DMSO d<sub>6</sub>) δ,ppm: : 3.75 (s,3H,CH), 3.0 (s,1H,OH), 5.2-7.2 (m,2H,Ar-CH), 4.96 (d,2H,CH), 6.4 (p,1H,CH), 3.5 (d,2H,CH), 8.4 (s,1H,CH=N), 7.6 (t,2H,CH), 7.9 (t,2H,CH), 4.2 (s,1H,SO<sub>2</sub>NH), 6.5-7.5 (m,2H,CH). Anal.Calcd.(%): C=55.90, H=4.49, N=9.70, O=14.98,S=14.93.

**2(K)4-[(3-Allyl-4-hydroxy-5-methoxy-benzylidene)-amino]-N-(5-methyl-isoxazol-3-yl)-benzene sulfonamide**

Recrystallization from ethanol: C<sub>21</sub>H<sub>21</sub>N<sub>3</sub>O<sub>5</sub>S; Molecular wt. 427; Yield 95%; m.p.70<sup>0</sup>C; colour- cremish white; IR (KBr) cm<sup>-1</sup> 3400cm<sup>-1</sup>(O-H), 1670 cm<sup>-1</sup> (CH=N); <sup>1</sup>HNMR (DMSO d<sub>6</sub>) δ,ppm: 3.75 (s,3H,CH), 3.0 (s,1H,OH), 5.2-7.2 (m,2H,Ar-CH), 4.96 (d,2H,CH), 6.4 (p,1H,CH), 3.5 (d,2H,CH), 8.4 (s,1H,CH=N), 7.6 (t,2H,CH), 7.9 (t,2H,CH), 4.2 (s,1H,SO<sub>2</sub>NH), 5.2 (s,1H,CH), 2.5 (s,3H,CH<sub>3</sub>). Anal.Calcd.(%): C=59.00, H=4.98%, N=9.80, O=18.70, S=7.51.

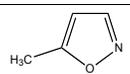
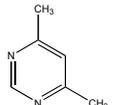
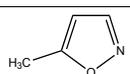
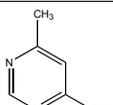
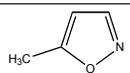
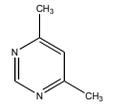
**2(L)4-[(3-Allyl-4-hydroxy-5-methoxy-benzylidene)-amino]-N-(4,6-dimethyl-pyrimidin-2-yl)-benzene sulfonamide**

Recrystallization from ethanol: C<sub>23</sub>H<sub>24</sub>N<sub>4</sub>O<sub>4</sub>S; Molecular wt. 452; Yield 80%; m.p.72<sup>0</sup>C; colour- cremish white; IR (KBr) cm<sup>-1</sup> 3450cm<sup>-1</sup>(O-H), 1640 cm<sup>-1</sup> (CH=N); <sup>1</sup>HNMR (DMSO d<sub>6</sub>) δ,ppm: 3.75 (s,3H,CH), 3.0 (s,1H,OH), 5.2-7.2 (m,2H,Ar-CH), 4.96 (d,2H,CH), 6.4 (p,1H,CH), 3.5 (d,2H,CH), 8.4 (s,1H,CH=N), 7.6 (t,2H,CH), 7.9 (t,2H,CH),

Table 1. Physical characteristics of the synthesized azetidin-2-ones.

S.no	Comp.	M.p( <sup>o</sup> c)	Yield	Colour
1	3A	200 <sup>o</sup>	35%	Black colour
2	3B	190 <sup>o</sup>	40%	Yellowish colour
3	3C	210 <sup>o</sup>	40%	Black colour
4	3D	180 <sup>o</sup>	45%	Reddish brown colour
5	3E	140 <sup>o</sup>	40%	Pale yellow colour
6	3F	130 <sup>o</sup>	35%	Brown colour
7	3G	120 <sup>o</sup>	40%	Black colour
8	3H	135 <sup>o</sup>	40%	Black colour
9	3I	120 <sup>o</sup>	45%	Black colour
10	3J	130 <sup>o</sup>	35%	Black colour
11	3K	110 <sup>o</sup>	40%	Brown colour
12	3L	110 <sup>o</sup>	42%	Black-brown colour

Table 2. Results of *in-vitro* antibacterial & Antifungal activity observed for the synthesized azetidin-2-ones compounds through disc diffusion assay.

	R	R''	Inhibition zone diameters <sup>a</sup>	
			<i>Asperillus aureus</i>	<i>Asperillus flavus</i>
3A	p-OH	H	10	11
3B	p-OH		10	14
3C	p-OH		11	14
3D	p-OH		10	13
3E	p-CH <sub>2</sub> =CH-OCH <sub>2</sub> 'o-NO <sub>2</sub>	H	12	28
3F	p-CH <sub>2</sub> =CH-OCH <sub>2</sub> 'o-NO <sub>2</sub>		10	26
3G	p-CH <sub>2</sub> =CH-OCH <sub>2</sub> 'o-NO <sub>2</sub>		11	26
3H	p-CH <sub>2</sub> =CH-OCH <sub>2</sub> 'o-NO <sub>2</sub>		-	28
3I	p-OH,m-CH <sub>2</sub> =CH-CH <sub>2</sub>	H	-	-
3J	p-OH,m-CH <sub>2</sub> =CH-CH <sub>2</sub>		11	12
3K	p-OH,m-CH <sub>2</sub> =CH-CH <sub>2</sub>		12	24
3L	p-OH,m-CH <sub>2</sub> =CH-CH <sub>2</sub>		12	20
Standard		Terbiforce	14	48

Antifungal susceptibility of compounds was measured in terms of zone of growth inhibitions. (-) means no activity. <sup>a</sup>Inhibition zone diameters in millimeters at 1000 µg/mL concentration of compounds.

4.2 (s, 1H, SO<sub>2</sub>NH), 3.8 (s, 1H, CH), 2.8-3 (s, 6H, CH<sub>3</sub>). Anal. Calcd. (%): C, C=61.10, H=5.30, N=12.38, O=14.14, S=7.09

(3A-L) mp 110-200<sup>o</sup>C, yield 35%-45%: IR KBr (m cm<sup>-1</sup>) 3512 (O-H), 1760 (C=O, fourmembered lactam), 1578 (N=N), 1498 (N=O, asym), 1349 (N=O, sym), 1602, 1599, 1464 (C-C, ring str), 1H NMR (d) ppm 4.2 (s, 1H, OH), 5.1 (d, 1H, J<sub>3,4</sub> = 4.4 Hz, C4-H), 5.8 (d, 1H, J<sub>3,4</sub> = 4.4 Hz, C3-H), 7.1-7.4 (m, 12H, ArH); Elemental analysis, found (calcd) (%) C, 61.23 (61.99); H, 3.18 (3.69); N, 13.41 (13.77) (Table 1).

In disc-diffusion assay, few colonies of organisms were inoculated in 2-5 mL nutrient broth and grown for 2.5 h. The agar plates were dried and inoculated by spreading the fungal suspension evenly over it. The sterile paper discs (6 mm) impregnated with fixed dose viz., 400 µg/mL of compound were placed on the preinoculated surface. The disc-bearing plates were incubated at 37 °C and examined at 48 h for zone of inhibition, if any, around the disc. Chloromycetin was used in assay as a standard control drug. An additional negative control disc without any sample but impregnated with equivalent amount of solvent (DMF) was also used in the assay. The diameter of inhibition zone is directly proportional to the degree of sensitivity of fungal strain and the concentration of compound under test.

All the newly synthesized azetidin-2-ones 3(A-L) were assayed *in vitro* for their growth inhibitory activity against pathogenic micro-organisms. We examine the antifungal potentialities of azetidin-2-ones against pathogenic fungi viz; *Aspergillus niger* & *Aspergillus flavus* by disc diffusion assay and compared with Terbiforce as a reference standard. It is observed that *Aspergillus aureus* show moderate to good activity while *Aspergillus flavus* show good to maximum inhibition nearly in all compounds (Table 2).

## CONCLUSION

The results of antifungal screening clearly indicate that the nature of substituents and their position on azetidin-2-ones nucleus affected the *in vitro* antifungal activity significantly. The presence of electron-withdrawing groups on the aromatic ring in general increases the antifungal activity of tested compounds. It can be concluded that the presence of sulfonyl group in the azetidin-2-ones moiety enhanced the antifungal activity of the compounds. Sulphamethoxazole substituent frequently appears in many drugs and it follows the trend here also as sulphamethoxazole substituted compounds seem to be more potent than other sulpha substituents. Antifungal activity of sulphanilimide and sulphathiazole substituted azetidin-2-ones is average. Here we are able to identify some interesting structure-activity relationship on azetidinone ring. Our results encourage the synthesis of new class substituted azetidin-2-ones analogs showing good antifungal activity.

## CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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- [31] Experimental: All melting points were measured in open capillary and are uncorrected. The chemicals and reagents used were of AR grade. The product mixtures were analyzed by thin-layer chromatography (TLC) on silica gel sheets. IR spectra (in  $\text{cm}^{-1}$ ) were recorded on PerkinElmer 337 spectrometer. <sup>1</sup>H NMR was recorded on a Varian EM-390 MHz NMR spectrometer in DMSO-*d*<sub>6</sub>, chemical shifts are given in  $\delta$  ppm using TMS as an internal standard. Mass spectral analysis was performed on Jeol SX-102 spectrometer using FAB technique.
- [32] General procedure for the synthesis of imines [2A-L]: An equimolar mixture (0.005 M) of aldehyde and appropriate amine in ethanol was refluxed for 3 h. The reaction mixture was cooled in an ice bath and a drop of sulfuric acid was added to it. The product obtained was filtered, washed and recrystallised by EtOH as shiny yellow crystals.
- [33] General procedure for the synthesis of  $\beta$ -lactam [3A-L]: In a closed vessel containing compound (0.001 M) in 20 mL of 1,4-dioxan, 0.095 mL of chloroacetyl chloride and 0.16 mL of triethylamine were added and the reaction mixture was stirred at 50 °C for 1 h. The reaction mixture was then kept at room temperature for 30 min and further refluxed for 8 h. The filtrate was concentrated under reduced pressure and poured into ice-cold water. The product so obtained was recrystallised from methanol as light brown crystals.

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