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Synthesis, characterization and biological activities of some azetidin-2-one as potential antifungal agents

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ABSTRACT

A new series of 2-azetidinone compounds 5(a-g) were synthesized. The structures of all the synthesized compounds were confirmed by chemical and spectral analyses such as IR, ^{1}H NMR and ^{13}C NMR. The compounds were screened for their antimicrobial activity against *Aspergillus flavus, Aspergillus niger, Penicillim chryogenum, Trigoderma veride & Fusarium oxysporum* and the zone of inhibition was determined by disc diffusion technique. All the synthesized compounds exhibited promising antidungal activity against the studied set of microorganisms. However, the activity was less than that of standard drugs used in this study.

***Keywords*:** Hydrazone, 2-azetidinone, spectral studies, Antifungal activity, disc diffusion

1. INTRODUCTION

The treatment of bacterial infections still remains a challenge for scientists because of factors that include rising infectious diseases and the increasing number of multidrugresistant microbial pathogens. So the discovery of novel and potent antibacterial as well as antifungal agent are the critical need of nowadays [1]. In recent years, there has been an increasing interest in the chemistry of 2-azetidinones because of their biological significance. Many of

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them have been widely investigated for several therapeutic uses. 2-azetidinones commonly known as β-lactams are still the most prescribed antibiotic agents used in medicine to treat bacterial infections and microbial diseases [2]. A comparative study of current antibiotics with those from previous decades shows an alarming increase in bacterial resistance to β-lactam antibiotics [3]. Azetidinones, which are part of antibiotics, are known to exhibit interesting biological activities [4]. A large number of 3-chloro monocyclic β-lactams possess powerful antibacterial, antimicrobial, anti-inflammatory, anticonvulsant and antitubercular activities [5]. Azetidin-2- ones and its derivatives possess various pharmacological properties such as antihypertensive, anti-inflammatory, anticancer, antihyperlipidemic [6-11]. Due to this, the investigation of chemistry and biology of these compounds continue to appeal the synthetic and medicinal organic chemists [12-14]. The present work is undertaken to explore more possibilities of finding a suitable derivatives, which would exceed its activity more than the already known drugs containing β- lactam ring By considering the above factors, it was thought to synthesize some substituted azetidinone derivatives for biological activities.

2. MATERIALS AND METHODS

All the chemicals and solvents used were of AR grade obtained from Sigma Aldrich, Lobachemie (India). Melting points of the synthesized compounds were determined in open-glass on a Staurt-SMP10 melting point apparatus and recorded in 0 °C without correction. The purity of the compounds was ascertained by thin layer chromatography on silica gel coated aluminum plates (Merck) as adsorbent and UV light as visualizing agent. Synthesized compounds were recrystallised using ethanol as solvent. IR spectra were recorded on SHIMADZU FT-IR spectrometer using KBr pellet technique. ^{1}H-NMR spectra were recorded on BRUKER-400 spectrometer operating at 400 MH_Z using TMS as internal standard in DMSO (chemical shifts in ppm).

3. RESULTS AND DISCUSSIONS

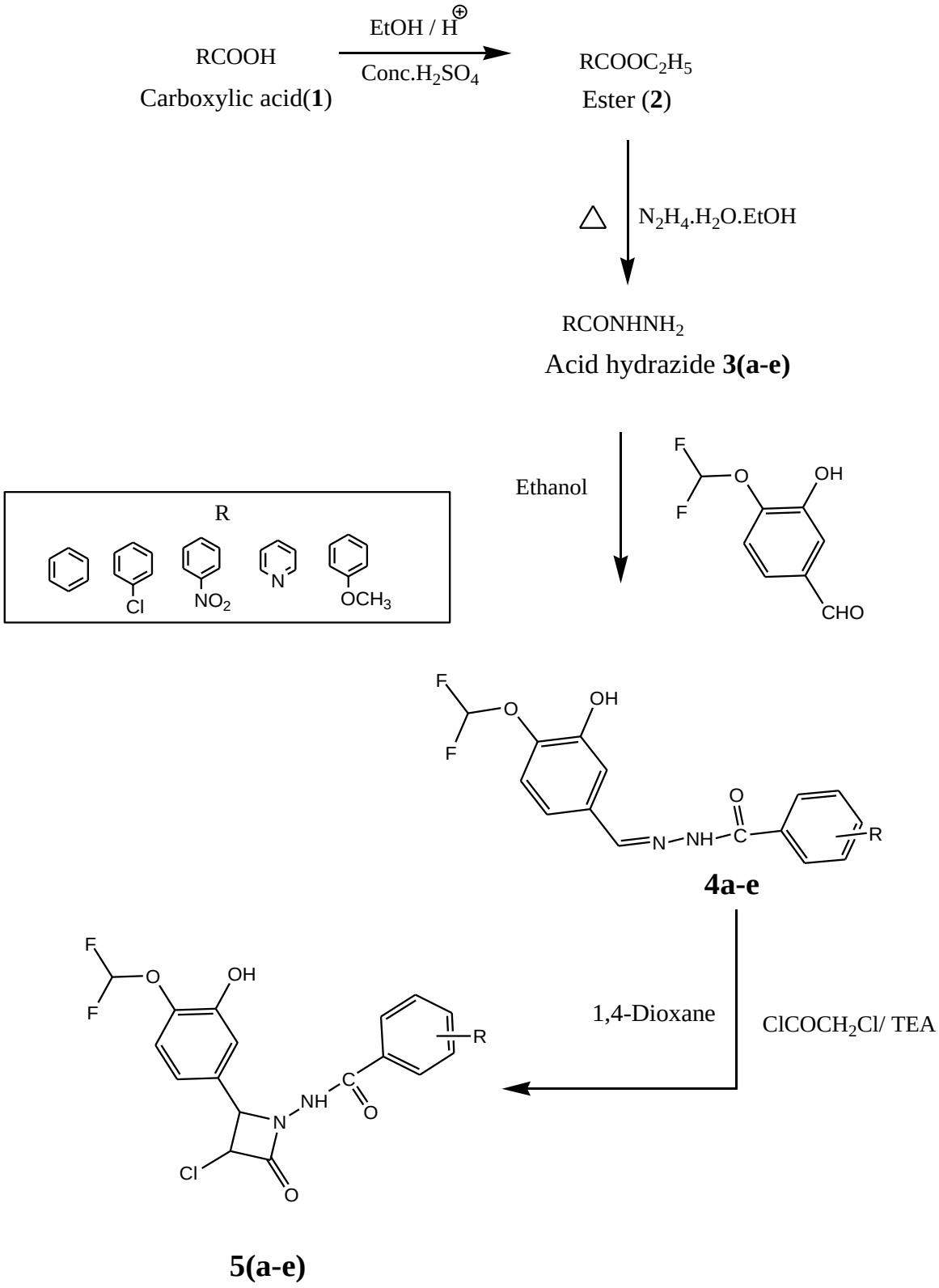
3. 1 Chemistry

3. 1. 1. General procedure for the synthesis of compounds 3a-e

In a 250 ml round bottom flask, a mixture of substituted carboxylic acid (0.1 mol), ethanol (60 ml) and conc. H_2SO_4 (1.4 ml) were refluxed for 10 hours on a water bath. The solution was cooled and poured slowly with stirring on to 200 g of crushed ice. Sufficient ammonia solution was added to render the resulting solution alkaline, generally some ester separates as oil but most of it remains dissolved in the alkaline solution. The solution was extracted five times with ether (25 ml) the combined ethereal extract was dried with anhydrous $MgSO_4$. Ether was removed by evaporation on a water bath and the residue was collected. Physical data of ester was noted. The Synthetic procedure is shown in Scheme 1.

3. 1. 2. General procedure for the synthesis of compounds 4a-e[27]

A mixture of ester and hydrazine hydrate in 1:1 portion and ethanol (30 ml) were taken in a round bottom flask and refluxed for 4-6 hrs. Excess of ethanol was removed by distillation.



Scheme I. Synthesis of the 2-Azetidinone derivatives 5(a-e)

On cooling the product, acid hydrazide separates out. It was filtered and collected. Recrystallization was carried out with ethanol and physical data was noted. The physical properties of synthesized derivatives 3(a-e) are shown in Table 1.

Table 1. Physical properties and IR Spectral Data of Synthesized Hydrazides 3(a-e)

Compound	R	Molecular formula	M. Pt (°C)	M.Formula	Physical state	IR Frequency
3a	C_6H_5	$C_7H_8N_2O$	110	136	White powder	3032 cm^{-1} (C-H Ar str); 1622 cm^{-1}(C=O str); 3296, 3215 ($NHNH_2$ str); 985 cm^{-1} (N-N str).
3b	C_6H_5Cl	$C_7H_7ClN_2O$	162	170	White powder	3016 cm^{-1} (C-H Ar str); 1645 cm^{-1} (C=O str); 3302, 3209 cm^{-1} ($NHNH_2$ str); 727 cm^{-1} (C-Cl str); 987 cm^{-1} (N-N str).
3c	$C_6H_5NO_2$	$C_7H_7N_3O_3$	215	181	Pale Yellow powder	3070 cm^{-1} (C-H Ar str); 1662 cm^{-1} (C=O str); 3296, 3180 cm^{-1} ($NHNH_2$ str); 1525 cm^{-1} (NO_2 str); 1055 cm^{-1} (N-N str).
3d	C_5H_5N	$C_6H_7N_3O$	170	137	White powder	3064 cm^{-1} (C-H Ar str); 1649 cm^{-1} (C=O str); 3305, 3178 cm^{-1} ($NHNH_2$ str); 1045 cm^{-1} (N-N str).
3e	$C_6H_5OCH_3$	$C_8H_{10}N_2O_2$	135	166	White powder	3043 cm^{-1} (C-H Ar str); 2933 cm^{-1} (C-H Aliphatic str); 1612 cm^{-1} (C=O str); 3381, 3253 cm^{-1} ($NHNH_2$ str); 1031 cm^{-1} (N-N str).

3. 1. 3. Synthesis of N'-(4-(difluoromethoxy)-3-hydroxybenzylidene)benzohydrazide (4a)

This was prepared and purified as per the above mentioned procedure: M.F: $C_{15}H_{12}F_2N_2O_3$; M.Wt: 306; yield 67%, mp 142-146 °C; IR (KBr, m, cm^{-1}): 2908 (-Ali CH), 3030 (-Aro CH), 1562 (-CH=N), 1687 (-C=O), 3197 (-OH) and 1172 (-C-F); ^{1}HNMR:

(DMSO-d6, 400 MHz), δ 8.37 (s, 1H, CH=N), δ 7.12 (s, 1H, -NH), δ 6.93 (s, 1H, -OH), δ 7.30 (s, 1H, $-CHF_2$) and δ 7.12-7.92 (m, 8H, Aromatic protons)

3. 1. 4. Synthesis of N'-(4-(difluoromethoxy)-3-hydroxybenzylidene)-4-chlorobenzohydrazide (4b)

This was prepared and purified as per the above mentioned procedure: M.F: $C_{15}H_{11}ClF_2N_2O_3$; M.Wt: 340; yield 72%, mp 115-117 °C; IR (KBr, m, cm^{-1}): 2960 (-Ali CH), 3134 (-Aro CH), 1598 (-CH=N), 1637 (-C=O), 3317 (-OH), 1109 (-C-F) and 750 (-C-Cl); ^{1}HNMR: (DMSO-d6, 400 MHz), δ 8.30 (s, 1H, CH=N), δ 7.00 (s, 1H, -NH), δ 6.89 (s, 1H, -OH), δ 7.19 (s, 1H, $-CHF_2$) and δ 7.07-7.90 (m, 7H, Aromatic protons)

3. 1. 5. Synthesis of N'-(4-(difluoromethoxy)-3-hydroxybenzylidene)-4-nitrobenzohydrazide (4c)

This was prepared and purified as per the above mentioned procedure: M.F: $C_{15}H_{11}F_2N_3O_5$; M.Wt: 351; yield 65%, mp 110-112 °C; IR (KBr, m, cm^{-1}): 2931 (-Ali CH), 3062 (-Aro CH), 1589 (-CH=N), 1687 (-C=O), 3304 (-OH) and 1128 (-C-F; ^{1}HNMR: (DMSO-d6, 400 MHz), δ 8.36 (s, 1H, CH=N), δ 7.04 (s, 1H, -NH), δ 6.94 (s, 1H, -OH), δ 7.23 (s, 1H, $-CHF_2$) and δ 7.04-8.15 (m, 7H, Aromatic protons)

3. 1. 6. Synthesis of N'-(4-(difluoromethoxy)-3-hydroxybenzylidene)isonicotinohydrazide (4d)

This was prepared and purified as per the above mentioned procedure: M.F: $C_{14}H_{11}F_2N_3O_3$; M.Wt: 307; yield 68%, mp 124-126 °C; IR (KBr, m, cm^{-1}): 2912 (-Ali CH), 3035 (-Aro CH), 1568 (-CH=N), 1645 (-C=O), 3288 (-OH) and 1170 (-C-F); ^{1}HNMR: (DMSO-d6, 400 MHz), δ 8.34 (s, 1H, CH=N), δ 7.11 (s, 1H, -NH), δ 6.93 (s, 1H, -OH), δ 7.30 (s, 1H, $-CHF_2$) and δ 7.14-8.77 (m, 7H, Aromatic protons)

3. 1. 7. Synthesis of N'-(4-(difluoromethoxy)-3-hydroxybenzylidene)-4-methoxybenzohydrazide (4e)

This was prepared and purified as per the above mentioned procedure: M.F: $C_{16}H_{14}F_2N_2O_4$; M.Wt: 336; yield 75%, mp 135-137 °C; IR (KBr, m, cm^{-1}): 2931 (-Ali CH), 3053 (-Aro CH), 1591 (-CH=N), 1687 (-C=O), 3329 (-OH) and 1130 (-C-F); ^{1}HNMR: (DMSO-d6, 400 MHz), δ 8.33 (s, 1H, CH=N), δ 7.05 (s, 1H, -NH), δ 6.91 (s, 1H, -OH), δ 7.42 (s, 1H, $-CHF_2$), δ 3.83 (s, 3H, $-OCH_3$) and δ 7.04-7.91 (m, 7H, Aromatic protons)

3. 2. General procedure for the synthesis of compounds (5a–e)

Hydrazones 3a-f (0.04 mol) and triethylamine (0.02 mol) in dioxan (20 mL) at 0-5 °C mixture was stirred for 5 h. During stirring, chloroacetyl chloride (0.01 mol) in dioxan (10 mL) was added dropwise. The mixture was refluxed for 2 h and kept for two days on room temperature. The resulting mixture was poured in the water and the solid was separated out. Recrystallization was done with ethanol–water or chloroform– water to give the azetidine-2-one, 4a-f compounds.

3. 2. 1. Synthesis of N-(3-chloro-2-(4-(difluoromethoxy)-3-hydroxyphenyl)-4-oxoazetidin-1-yl)benzamide (5a)

This was prepared and purified as per the above mentioned procedure: M.F: $C_{17}H_{13}ClF_2N_2O_4$; M.Wt: 382; yield 64%, mp 208-210 °C; IR (KBr, m, cm^{-1}): 2926 (-Ali CH), 3051 (-Aro CH), 1681 (-C=O), 3425(-OH); ^{1}HNMR (400 MHz, DMSO-d6) (ppm): 4.83 (d, 1H, –CH-N), 9.73 (d, 1H, OH), 4.53 (d, 1H, –CH-Cl) 7.09-8.04 (m, 4H, Ar-H)

3. 2. 2. Synthesis of 4-chloro-N-(3-chloro-2-(4-(difluoromethoxy)-3-hydroxyphenyl)-4-oxoazetidin-1-yl)benzamide (5b)

This was prepared and purified as per the above mentioned procedure: M.F: $C_{17}H_{12}C_{l2}F_2N_2O_4$; M.Wt: 417; yield: 60%, mp 235-237 °C; IR (KBr, m, cm^{-1}): 2920 (-Ali CH), 3047 (-Aro CH), 1664 (-C=O), 3435 (-OH); ^{1}HNMR (400 MHz, DMSO-d6) (ppm): 4.62 (d, 1H, –CH-N), 9.89 (s, 1H, OH), 4.48 (d, 1H, –CH-Cl) 6.86-7.82 (m, 4H, Ar-H)

3. 2. 3. Synthesis of N-(3-chloro-2-(4-(difluoromethoxy)-3-hydroxyphenyl)-4-oxoazetidin-1-yl)-4-nitrobenzamide (5c)

This was prepared and purified as per the above mentioned procedure: M.F: $C_{17}H_{12}ClF_2N_3O_6$; M.Wt: 427; yield: 56%, mp 240-242 °C; IR (KBr, m, cm^{-1}): 2926 (-Ali CH), 3051 (-Aro CH), 1664 (-C=O), 3431 (-OH); ^{1}HNMR (400 MHz, DMSO-d6) (ppm): 4.37(d, 1H, –CH-N), 11.89 (s, 1H, OH), 4.14 (d, 1H, –CH-Cl) 7.16-8.35 (m, 4H, Ar-H)

3. 2. 4. Synthesis of N-(3-chloro-2-(4-(difluoromethoxy)-3-hydroxyphenyl)-4-oxoazetidin-1-yl)isonicotinamide (5d)

This was prepared and purified as per the above mentioned procedure: M.F: $C_{16}H_{12}ClF_2N_3O_4$; M.Wt: 383; yield: 65%, mp 210-212 °C; IR (KBr, m, cm^{-1}): 2924 (-Ali CH), 3045 (-Aro CH), 1674 (-C=O), 3425 (-OH); ^{1}HNMR (400 MHz, DMSO-d6) (ppm): 4.77 (d, 1H, –CH-N), 11.84 (s, 1H, OH), 4.72 (d, 1H, –CH-Cl) 6.56-8.35 (m, 4H, Ar-H)

3. 2. 5. Synthesis of N-(3-chloro-2-(4-(difluoromethoxy)-3-hydroxyphenyl)-4-oxoazetidin-1-yl)-4-methoxybenzamide (5e)

This was prepared and purified as per the above mentioned procedure: M.F: $C_{18}H_{15}ClF_2N_2O_5$; M.Wt: 412; yield: 60%, mp 182-184 °C; IR (KBr, m, cm^{-1}): 2927 (-Ali CH), 3043 (-Aro CH), 1672 (-C=O), 3425 (-OH); ^{1}HNMR (400 MHz, DMSO-d6) (ppm): 4.78 (d, 1H, –CH-N), 11.69 (s, 1H, OH), 4.69 (d, 1H, –CH-Cl) 6.91-7.91 (m, 4H, Ar-H)

4. ANTIFUNGAL ACTIVITY

All the synthesized compounds were screened for antifungal activity by the disc diffusion method [15-18]. For the prepared compounds were subjected to preliminary antifungal screening by disc diffusion method against *fungal strains namely Aspergillus flavus (A. flavus), Aspergillus niger (A. niger), Penicillim chryogenum (P. chryogenum), Trigoderma veride (T. veride) & Fusarium oxysporum (F. oxysporum)* using *Amphotericin-B* as references.

4. 1. Assay of Antifungal Activity

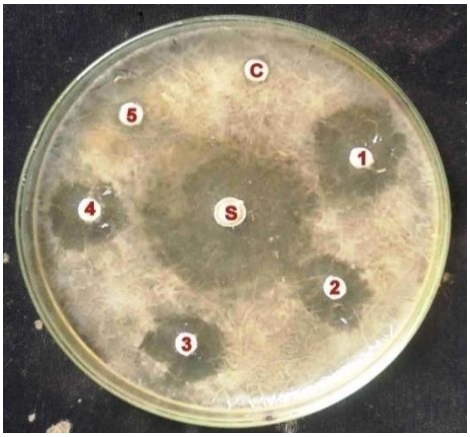
Potato Dextrose Agar was prepared and autoclaved at 15 lbs pressure for 20 minutes and cooled to 45 °C. The cooled media was added 10 ml/l tartaric acid (10%) act as antibacterial agents and poured on to sterile petriplates and allowed for solidification. The plates with media were seeded with the respective microbial suspension using sterile swab. The various solvents extract prepared discs individually were placed on the each petriplates and also placed control and standard discs. The plates were incubated at 28 °C for 72 hrs. After incubation period, the diameter of the zone formed around the paper disc were measured and expressed in mm

4. 2. Antifungal activity of compounds 4a-4e

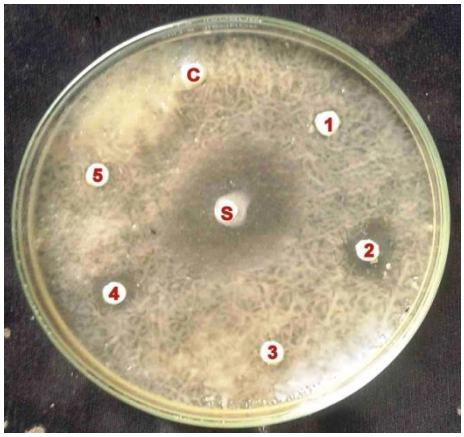
The *in vitro* antifungal activity (**Table 2**) of the synthesized novel heterocyclic compounds **4a-4e** was studied against the fungal strains viz., *Aspergillus flavus, Aspergillus niger, Penicillim chryogenum, Trigoderma veride* and *Fusarium oxysporum. Amphotericin-B* was used as a standard drug. The zone of inhibition (mm) values for **4a-4e** in the range 06-15 mm against all the tested fungi. Compound **4a** showed activity against *Aspergillus flavus, Penicillim chryogenum* and *Trigoderma veride* with activity range 09-15 mm. It fails to inhibit in the case of *Aspergillus niger* and *Fusarium oxysporum* fungal strains. The chloro substituted compound **4b** inhibit almost all the fungal strains expect *Trigoderma veride*. The compound **4c** showed good to poor activity against *Aspergillus flavus, Trigoderma veride* and *Fusarium oxysporum*. The zone of inhibition in the range 06-12 mm observed for compound **4d** and it fails to inhibit fungal strains *Trigoderma veride* and *Fusarium oxysporum*. Compound **4e** showed good activity with *Penicillim chryogenum* and *Trigoderma veride* with zone of inhibition 07 and 11 mm, respectively. The above results are reproduced in **Figs. 1**.

Table 2. Antifungal activity of **4a-4e**

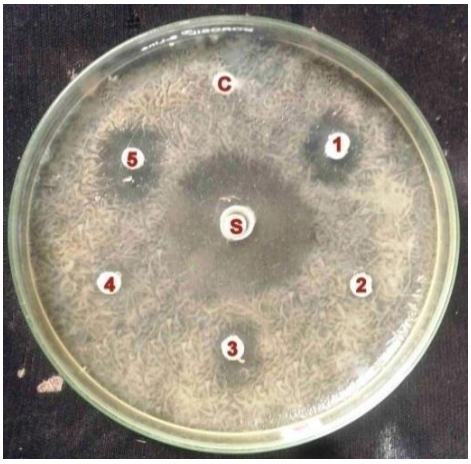
Fungal strain	Amphotericin-B	**Zone of inhibition (mm)**					
		1	**2**	**3**	**4**	**5**	
		4a (-H)	**4b (-Cl)**	**4c ($-NO_2$)**	**4d (ISN)**	**4e ($-OCH_3$)**	**Control (DMSO)**
Aspergillus flavus	30	15	12	13	12	-	-
Aspergillus niger	18	-	08	-	06	-	-
Penicillim chryogenum	28	09	11	-	09	07	-
Trigoderma veride	22	10	-	06	-	11	-
Fusarium oxysporum	24	-	12	06	-	-	



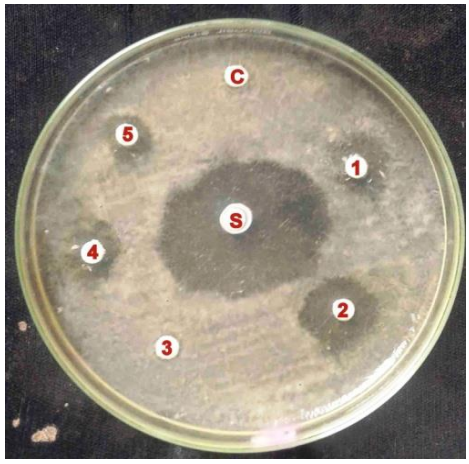
Zone of inhibition of **4a-4e** against *A. flavus*



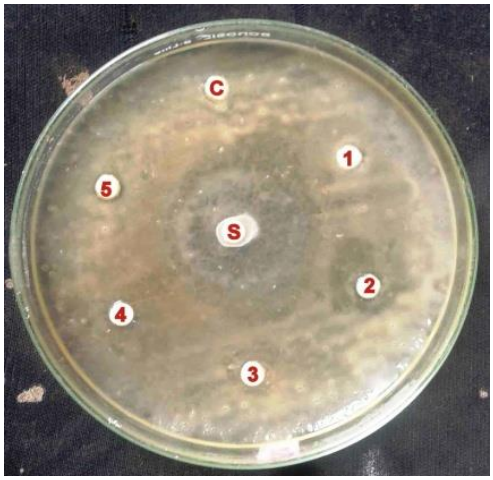
Zone of inhibition of **4a-4e** against *A. niger*



Zone of inhibition of **4a-4e** against *T. veride*



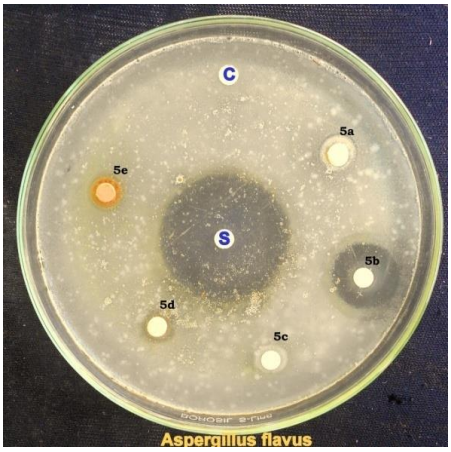
Zone of inhibition of **4a-4e** against *P. chryogenum*



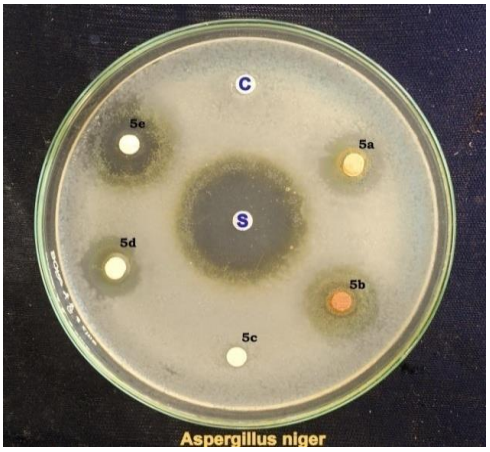
Zone of inhibition of **4a-4e** against *F.oxysporum*

Fig. 1. Antifungal activity of synthesized azetidinone derivatives (4a-e)

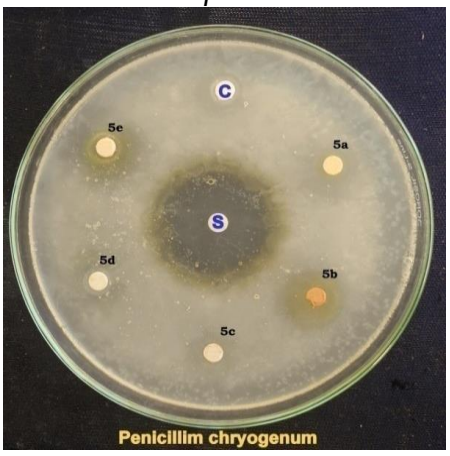
4. 3. Antifungal evaluation of compounds (5a-5e)



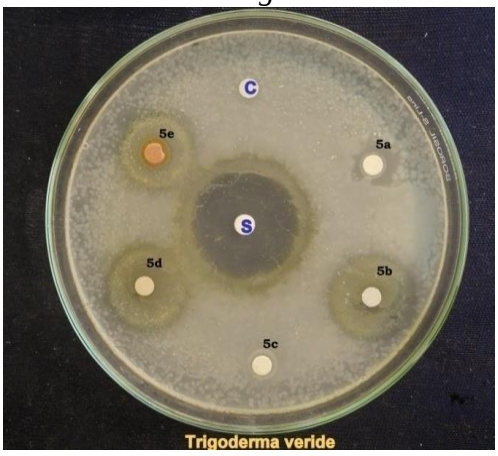
Zone of inhibition of **5a-5e** against *A. flavus*



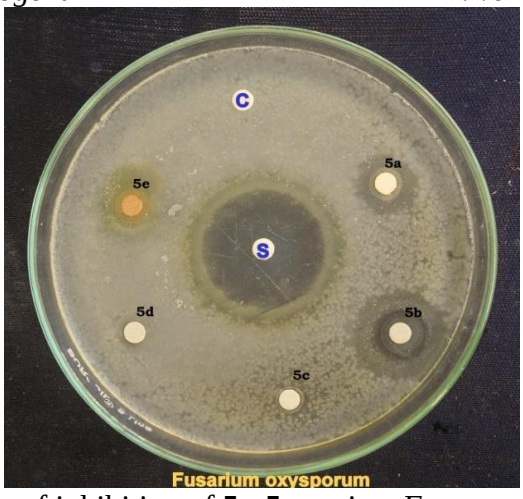
Zone of inhibition of **5a-5e** against *A. niger*



Zone of inhibition of **5a-5e** against *P. chryogenum*



Zone of inhibition of **5a-5e** against *T. veride*



Zone of inhibition of **5a-5e** against *F. oxysporum*

Fig. 2. Antifungal activity of synthesized azetidinone derivatives (5a-e)

In this discussion, compounds **5a-5e** were selected for evaluation of their structure activity relationship against standard fungal strain. Compounds **5b & 5e** were found to have better antifungal activities than those of other compounds. The higher activity of **5b & 5e** were influenced by the presence of electron with drawing and group electron donating group such as chloro and methoxy on phenyl ring. Compound **5e** having methoxy group on para position of phenyl ring showed increased activity (14 mm) and similarly the compound **5b** having chloro group on para position of phenyl ring showed 15 mm against *T.veride.* The order of activity of compounds **5a-5e** against *A. niger* was **5e** > **5b** > **5a** > **5d**. These compounds showed very good to moderate profile against the *A. niger* strain.

Among these compounds, compound **5b** has strong selectivity towards all the fungal strains, but compound **5e** has strong activity against *A. niger*. Thus, **Table 3** revealed that compound **5b** is more active towards almost all the fungal strains. Similarly in **Table 3**, compound **5d** has moderate activity against P.chryogenum, *T. veride, A. flavus,* and *A. niger* while against *F. oxysporum* poor activity is recorded.

Table 3. Antifungal activity of **5a-5e**

Fungal strain	Amphotericin-B	Zone of inhibition mm in diameter					
		5a (-H)	**5b (-Cl)**	**5c (-NO_2)**	**5d (ISN)**	**5e (-OCH_3)**	**Control**
Aspergillus flavus	24	-	14	-	08	08	-
Aspergillus niger	21	12	14	-	10	15	-
Penicillim chryogenum	22	-	10	08	08	14	-
Trigoderma veride	23	06	15	-	14	14	-
Fusarium oxysporum	23	10	14	06	-	09	-

Compounds substituted with chloro, 2-nitro, and methoxy groups are found to be more active against the selected bacterial strains. Unsubstituted phenyl ring showed comparatively lesser activity, which proved that substitution at azetidinone moiety affects the activity.

The scrutiny of the results revealed that compounds bearing a chloro group in addition to a nitro group in the phenyl ring at position - 4 of 2-azetidinone nucleus are more active against chosen bacterial strains. Significant activity is shown by compounds **5b** and **5c** with electron withdrawing groups like chloro and nitro whereas moderate activity was shown by compounds with electron donating methoxy group **5e**.

Reason for these results of compound **5e** may be due to increase of the electron density which makes compounds active against microorganisms and enhance the antimicrobial potency.

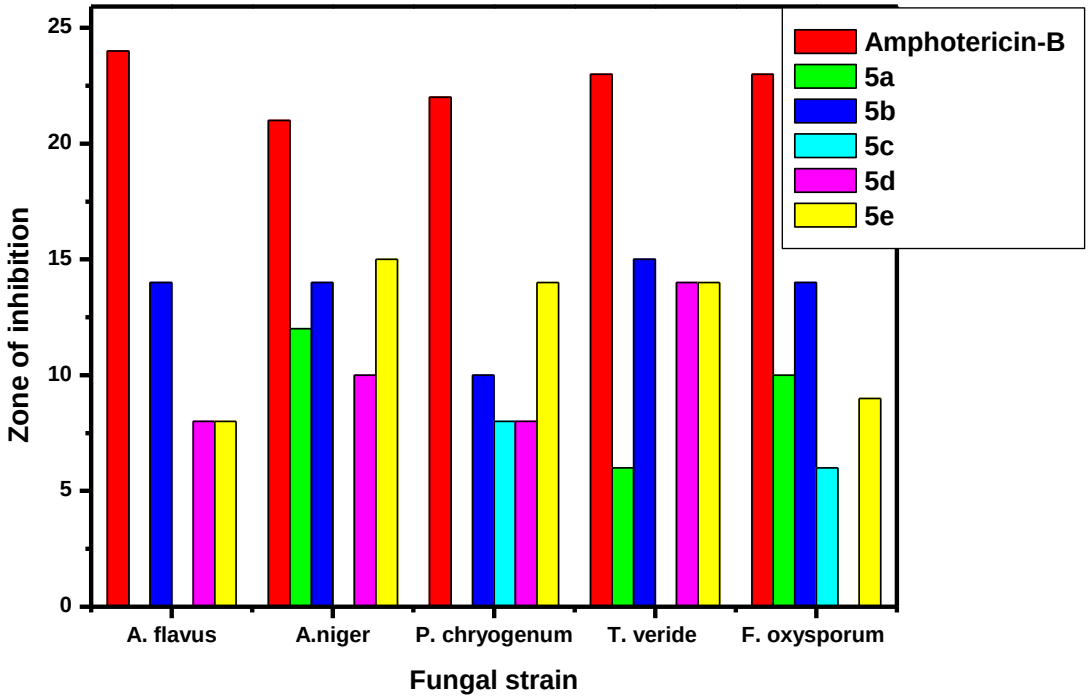


Fig. 3. Protective effect of **5a-5e** against Amphotericin-B

Reason for these results of compound **5b** and **5c** may be due to the presence of strong electron withdrawing groups Cl and NO_2 in the molecule may lead to higher polarizability of the molecule and give rise to new potential for binding the molecule to the receptor which finally enhances the van der waals force of attraction of the molecule and in turn its diffusion rate in the cell on the whole. This effect is pronounced when in addition to Cl, NO_2 was present on para position of phenyl group. The lesser activity of the unsubstituted molecule may be due to very less van der waals force of attraction or nil binding potential of the molecule to the receptor. In particular, the positions of electron withdrawing substituents on phenyl ring appreciably influenced the antimicrobial activity. Placement of electron withdrawing group in a strategic position of a molecule has materialized as a very influential and flexible tool for the development of the compounds capable with biological activities [17]. From the **Table 3** and **Fig. 2**, it can be concluded that Compound **5b** & **5e** may serve as novel template for development of potential and selective agents in the antifungal drug.

5. CONCLUSION

This paper deals with the biological activities of *N*-(3-chloro-2-(4-difluoromethoxy)-3-hydroxyphenyl)-4-oxoazetidin-1yl) aryl amide and to examine the structure activity relationship (SAR) of the newly synthesized compounds were evaluated for their antifungal activities against five fungal strains and were compared with standard drugs. Hence, in order to achieve better affinity and further potency of the new candidates, the *para* position in phenyl was subjected to a variety of new different electron donating and deactivating

functional groups including moieties of chloro (**5b**), nitro (**5c**) and methoxy (**5e**) and in compound **5d** the phenyl group incorporated with pyridine ring. Results from fungal data, show that Compounds **5b & 5e** were found to have better antifungal activities than those of other compounds. The fungal activity of **5a-5e** against *A. Niger* is in the order of **5e** > **5b** > **5a** > **5d**. All synthesized compounds showed moderate to good biological activities compared with standard drugs.

References

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