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Research Article

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Synthesis and antimicrobial activity of 2- azetidinones derived from benzimidazole

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ABSTARCT

Two series of chloro/p-chloro phenoxy substituted azetidinones were synthesized incorporating benzimidazole moiety. Phthalimide and glycine were reacted to give N-phthalyl acetic acid (1) which was further cyclized to give N-methyl phthalyl benzimidazole (2) on treatment with o-phenylene diamine. Further treatment with chloro sulphonic acid and then with hydrazine hydrate, followed by reaction with different aromatic aldehydes gave the Schiff bases(5a-d). These schiff bases formed when treated with chloro/ p-chlorophenoxy acetyl chloride underwent cyclization to give the azetidinones (7a-d).The chemical structure of the newly synthesized compounds were characterized by elemental analysis, IR , ^{1}H-NMR and Mass spectra. The title compounds were screened for their antimicrobial activity against four bacterial and two fungal strains. The bacterial strains used were Escherichia coli, Alcaligenes faecalis , Pseudomonas aeruginosa and Klebsiella pneumoniae and fungal strains used were Chaetomium globosum and Curvularia lunata . The synthesized compounds were evaluated for qualitative (zone of inhibition) antimicrobial activity by agar cup plate method at three concentrations (500,1000 and 3000ppm). Compound (6b) and (7c) showed good to excellent activity against all the tested strains while others were moderately active.

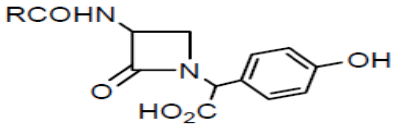
Keywords : Benzimidazole, Azetidinones, Antibacterial and Antifungal Activities.

INTRODUCTION

Even more than 70 years after the discovery of Penicillin, β-lactam antibiotics remain as one of the most important contributions of science to humanity[1]. The 2-azetidinone (β -lactam) ring system is the common structural feature of a number of broad spectrum β -lactam antibiotics, including penicillins, cephalosporins, carbapenems, nocardicins, monobactams , clavulanic acid, sulbactams and tazobactams, which have been widely used as chemotherapeutic agents to treat bacterial infections and microbial diseases [2-4]. In recent years, renewed interest has been focused on the synthesis and modification of β -lactam ring to obtain compounds with diverse pharmacological activities like cholesterol absorption inhibitory activity, human tryptase, thrombin and chymase inhibitory activity, vasopressin V1a antagonist activity, antidiabetic, anti-inflammatory, antiparkinsonian and anti-HIV activity[5-10]. They are also found to be a potent inhibitor of serine protease, human leukocyte elastase and human cytomegalovirus protease enzyme[11-14] and are effective on the central nervous system. In recent past these derivatives are also found to be moderately active against several types of cancer[15].

An interesting group of β-lactams are the monocyclic β-lactams, which are molecules that do not contain another ring fused to the β-lactam one. The discovery of the nocardicins, 1, and monobactams, 2, demonstrated for the first

time that β-lactams do not require a conformationally constrained bicyclic structure to have antibacterial properties[16], suggesting that the biological activity was strictly correlated to the presence of a suitably functionalized 2-azetidinone ring[17]. A large number of 3-chloro monocyclic β-lactams possess powerfull antibacterial, anti-inflammatory, anticonvulsant and antitubercular activity[18-21].



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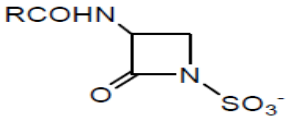

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The biological activity of the β-lactam skeleton is generally believed to be associated with the chemical reactivity of their β -lactam ring and on the substituents especially at nitrogen of the 2-azetidinone ring. The oxo group is at 2nd position i.e.2-azetidinone substituents at the N-1, C-3 and C-4 position may be varied. Keeping in view of these facts we have synthesized some 2-azetidinones incorporating the benzimidazole group at N -1 and substituted benzylidene group at C-4 and screened them for their antibacterial and antifungal activities. The structures of the synthesized compounds were assigned on the basis of their FTIR, ^{1}H NMR and mass spectral data.

EXPERIMENTAL SECTION

All the melting points were determined in open capillary tubes and were uncorrected. Spectroscopic data were recorded using following instruments, **IR:** FTIR RX1 Perkin Elmer Spectrophotometer, **^{1}HNMR**: Bruker DRX 300 MHz Spectrophotometer in DMSO-d_6 using TMS as internal standard, **Mass**: JMS-T100 LC, Accu TOF Mass Spectrophotometer (DART).

General procedures for synthesis:

Synthesis of N-phthalyl acetic acid (1) :

0.5 gm of glycine and 1 gm of phthalic anhydride were taken in a test tube and immersed in a previously heated oil bath (180-185°C) .The mixture was stirred occasionally during first 10 min. and any phthalic anhydride which sublimed was pushed down into the reaction mixture till there was complete fusion. The mixture was undisturbed for 5 min. when the liquid mass solidified. The solid so obtained was then recrysatllized from 10% ethanol.

Yield : 62%, m.p.: 140°C ,ν_{max} (KBr) : 1685 (>C=O carboxy),1710 (>C=O, phthalimido). δ_H (300 MHz, DMSO-d_6) : 2.50 (s,2H,CH_2) 7.23-7.78 (m,4H,ArH), 13.03 (s,1H,OH).*m/z :* (**M** $^+$)204. (Found: C, 58.41; H, 3.21; N, 6.64%. Calc. for $C_{10}H_7O_4N$,C, 58.53; H, 3.41; N 6.82%)

Synthesis of N-(methyl phthalyl)- benzimidazole (2) :

0.1 mol of N-phthalyl acetic acid and 0.1 mol of *o*-phenylene diamine were refluxed in 30 ml of 4 N HCl for 2 hrs. The solution on cooling gave a precipitate which was filtered , dried and recrytallized from ethanol.

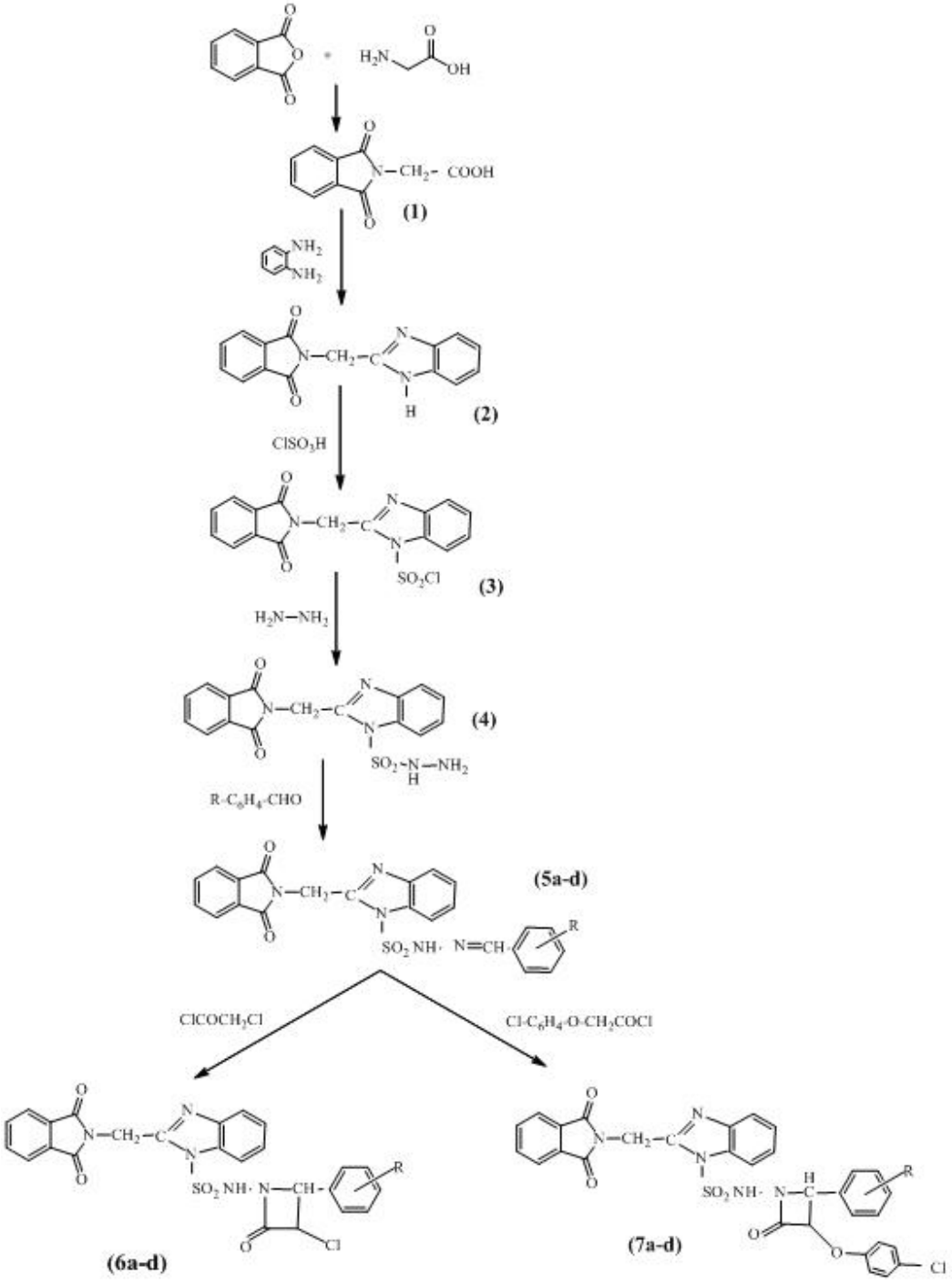
Yield : 70%, m.p.: >220°C ,ν_{max} (KBr) : 1225 (C-N),1620 (C=N), 1715(>C=O, phthalimido),3250(NH). δ_H (300 MHz, DMSO-d_6) :2.68 (s,2H,CH_2) 7.05-7.67 (m,8H,ArH), 8.98 (s,1H,NH). *m/z* : (**M**$^+$)276. (Found: C, 69.06; H, 3.70; N, 14.87%. Calc. for $C_{16}H_{11}O_2N_3$; C, 69.31; H, 3.97; N 15.16%)

Synthesis of 1-(N-chloro sulphonyl)-2- (N-methyl-phthalyl)-benzimidazole (3) :

Chloro sulphonic acid (0.01 mol) was added drop by drop to 0.01 mol of the substituted Benzimidzole **(2)**. It was shaken from time to time to ensure through mixing. The reaction mixture after complete addition of acid, was kept on a water bath for 30 minutes. The solution was cooled and gradually poured into crushed ice. The solid obtained was filtered, dried and recrystallized from ethanol.

Yield : 66%, m.p.: 180°C ν_{max} (KBr): 1100 (SO_2 symmetric), 1228 (C-N),1340 (SO_2 asymmetric), 1622 (C=N),1714 (>C=O, phthalimido).δ_H (300 MHz, DMSO-d_6) : 2.55 (s,2H,CH_2) 7.28-7.86 (m,8H,ArH). *m/z* : **(M$^+$)** 374, **(M+2)** 376. (Found: C, 49.94; H, 2.38; N, 10.94%. Calc. for $C_{16}H_{10}O_4N_3SCl$; C, 51.20; H, 2.66; N 11.20%)

The general scheme of synthesis of compounds is given in Scheme I.



Here R= 3-OCH_3-4-OH (a), 4-OH (b), 2,4-di Cl (c), 4-NO_2 (d)

Scheme 1

Synthesis of 1-(N- sulphonyl hydrazino)-2- (N-methyl-phthalyl)-benzimidazole (4) :
0.01 mol of compound **(3)** , hydrazine hydrate (99%, 0.01 mol) and absolute ethanol (10-15 ml) were refluxed for 4-6 hrs. Excess ethanol was distilled off, solid thus obtained was filtered, washed with water, dried and recrystallized from ethanol.

Yield : 58%, m.p.: 150°C ν_{max} (KBr): 1108 (SO_2 symmetric), 1227 (C-N),1342 (SO_2 asymmetric), 1630 (C=N),1714 (>C=O, phthalimido), 3220,3410(NH-NH_2). δ_H (300 MHz, DMSO-d_6) :2.45 (s,2H,CH_2), 5.20 (s,broad,2H, NH_2), 7.35-7.97 (m,8H,ArH), 8.98 (s,1H,NH). *m/z* : (**M**$^+$) 370. (Found: C, 51.37; H, 3.31; N, 18.71%. Calc. for $C_{16}H_{13}O_4N_5S$; C, 51.75; H, 3.50; N 18.86%)

Synthesis of 1-(substituted benzylidene)(N- sulphonyl hydrazino)-2- (N-methyl-phthalyl)-benzimidazole (5a-d) :
Yield : 70%, m.p.: >220°C 0.01 mol of compound **(4)** and equimolar proportion of substituted benzaldehyde and anhydrous sodium acetate (0.82 gm) was added to 10-12 ml of glacial acetic acid in a round bottom flask. It was refluxed for 4 hrs and then poured into ice cold water. The solid thus obtained was filtered, dried and recrytallized from ethanol.

1-(4'-hydroxy-3'-methoxy-benzylidene)(N-sulphonyl hydrazino)-2- (N-methyl-phthalyl)-benzimidazole (5a) :
Yield : 61%, m.p.: 178°C ν_{max} (KBr): 1105 (SO_2 symmetric), 1230 (C-N),1342 (SO_2 asymmetric), 1540 (N=CH), 1624 (C=N), 1718(>C=O, phthalimido), 3322 (NH), 3425 (OH). δ_H (300 MHz, DMSO-d_6) : 2.48 (s,2H,CH_2), 4.30 (s,3H,OCH_3), 4.92 (s,1H, N=CH), 7.41-7.99 (m,11H,ArH), 8.93 (s,1H,NH), 11.24 (s,1H,OH). *m/z* : (**M**$^+$)503. (Found: C, 56.92; H, 3.54; N, 13.63%. Calc. for $C_{24}H_{19}O_6N_5S$; C, 57.14; H, 3.76; N 13.88%)

1-(4'-hydroxy benzylidene)(N-sulphonyl hydrazino)-2- (N-methyl-phthalyl)-benzimidazole (5b) :
Yield : 55%, m.p.: 194°C ν_{max} (KBr): 1110 (SO_2 symmetric), 1222 (C-N),1344 (SO_2 asymmetric), 1543 (N=CH), 1625 (C=N), 1713(>C=O, phthalimido), 3325 (NH), 3428 (OH). δ_H (300 MHz, DMSO-d_6) : 2.51 (s,2H,CH_2), 4.89 (s,1H, N=CH), 7.40-7.95 (m,12H,ArH), 8.88 (s,1H,NH), 11.21 (s,1H,OH). *m/z* : (**M**$^+$) 474. (Found: C, 57.94; H, 3.11; N, 14.21%. Calc. for $C_{23}H_{17}O_5N_5S$; C, 58.10; H, 3.57; N 14.73%)

1-(2',4'-dichloro benzylidene)(N-sulphonyl hydrazino)-2- (N-methyl-phthalyl)-benzimidazole (5c) :
Yield : 71%, m.p.: 164°C ν_{max} (KBr): 1105 (SO_2 symmetric), 1228 (C-N),1343 (SO_2 asymmetric), 1545 (N=CH), 1625 (C=N), 1718(>C=O, phthalimido), 3322 (NH). δ_H (300 MHz, DMSO-d_6) : 2.45 (s,2H,CH_2), 4.85 (s,1H, N=CH), 7.42-7.91 (m,11H,ArH), 8.90 (s,1H,NH).*m/z* : **(M**$^+$**)** 527 **(M+4)** 531. (Found: C, 52.01; H, 2.63; N, 13.00%. Calc. for $C_{23}H_{15}O_4N_5SCl_2$; C, 52.27; H, 2.84; N 13.25%)

1-(4'-nitro benzylidene)(N-sulphonyl hydrazino)-2- (N-methyl-phthalyl)-benzimidazole (5d) :
Yield : 64%, m.p.: 202°C ν_{max} (KBr): 1100 (SO_2 symmetric), 1226 (C-N),1341 (SO_2 asymmetric), 1543 (N=CH), 1545 (NO_2), 1623 (C=N), 1716(>C=O, phthalimido), 3320 (NH). δ_H (300 MHz, DMSO-d_6) : 2.38 (s,2H,CH_2), 4.79 (s,1H, N=CH), 7.38-7.90 (m,11H,ArH), 9.21 (s,1H,NH). *m/z* : (**M**$^+$) 503. (Found: C, 54.54; H, 2.85; N, 16.34%. Calc. for $C_{23}H_{16}O_6N_6S$; C, 54.76; H, 3.17; N 16.66%)

Synthesis of 1-(N-sulphamido) –[2'- (N-methyl-phthalyl)-benzimidazoyl]-(3-chloro-4- (substituted phenyl)-azetidin-2-ones (6a-d) :
A mixture of compound (**5a/b/c/d)** in ethanol (10-12 ml) and chloroacetyl chloride (0.01 mol) was heated under reflux for 2 hrs. The solution on cooling gave a solid which was filtered, dried and recrystallized from ethanol.

1-(N- sulphamido) –[2'- (N-methyl-phthalyl)-benzimidazoyl]-3-chloro-4- (4''-hydroxy-3''-methoxy phenyl)-azetidin-2-one (6a) :
Yield : 75%, m.p.: 188°C ν_{max} (KBr): 776 (C-Cl), 1105 (SO_2 symmetric), 1222 (C-N),1342 (SO_2 asymmetric), 1625 (C=N), 1650 (C=O,β-lactam),1715(>C=O, phthalimido). δ_H (300 MHz, DMSO-d_6) : 2.53 (s,2H,CH_2), 3.32 (d,1H,C_4), 4.61(d,1H,C_3), 4.68 (s,3H,OCH_3), 7.05-7.86 (m,11H,ArH), 9.32 (s,1H,NH), 12.82 (s,1H,OH). *m/z* : **(M**$^+$**)** 580 **(M+2)** 582. (Found: C, 53.42; H, 3.02; N, 11.74%. Calc. for $C_{26}H_{20}O_7N_5SCl$; C, 53.70; H, 3.44; N 12.04%)

1-(N- sulphamido) –[2'- (N-methyl-phthalyl)-benzimidazoyl]-3-chloro-4- (4''-hydroxyphenyl)-azetidin-2-one (6b) :
Yield : 59%, m.p.: >220°C ν_{max} (KBr): 774 (C-Cl), 1108 (SO_2 symmetric), 1225 (C-N),1342 (SO_2 asymmetric), 1628 (C=N), 1652 (C=O,β-lactam),1718(>C=O, phthalimido). δ_H (300 MHz, DMSO-d_6) : 2.50 (s,2H,CH_2), 3.52 (d,1H,C_4), 4.65(d,1H,C_3), 7.41-7.99 (m,12H,ArH), 9.38 (s,1H,NH), 11.55 (s,1H,OH). *m/z* : **(M^+)** 550 **(M+2)** 552. (Found: C, 54.18; H, 2.92; N, 12.54%. Calc. for $C_{25}H_{18}O_6N_5Cl$; C, 54.44; H, 3.26; N 12.70%)

1-(N- sulphamido) –[2'- (N-methyl-phthalyl)-benzimidazoyl]-3-chloro-4- (2'',4''-dichlorophenyl)-azetidin-2-one (6c) :
Yield : 65%, m.p.: 212°C ν_{max} (KBr): 770 (C-Cl), 1106 (SO_2 symmetric), 1220 (C-N),1345 (SO_2 asymmetric), 1622 (C=N), 1655 (C=O,β-lactam),1713(>C=O, phthalimido). δ_H (300 MHz, DMSO-d_6) : 2.52 (s,2H,CH_2), 3.57 (d,1H,C_4), 4.70(d,1H,C_3),7.24-7.90 (m,11H,ArH), 9.20 (s,1H,NH). *m/z* :**(M^+)** 602 **(M+4)** 606. (Found: C, 49.51; H, 2.31; N, 11.45%. Calc. for $C_{25}H_{16}O_5N_5SCl_3$; C, 49.75; H, 2.65; N 11.60%)

1-(N- sulphamido) –[2'- (N-methyl-phthalyl)-benzimidazoyl]-3-chloro-4- (4''-nitrophenyl)-azetidin-2-one (6d):
Yield : 52%, m.p.: 200°C ν_{max} (KBr): 775(C-Cl), 1109 (SO_2 symmetric), 1228 (C-N),1342 (SO_2 asymmetric), 1545 (NO_2), 1621 (C=N), 1656 (C=O,β-lactam),1715 (>C=O, phthalimido). δ_H (300 MHz, DMSO-d_6) : 2.52 (s,2H,CH_2), 3.57 (d,1H,C_4), 4.62(d,1H,C_3),7.24-7.90 (m,12H,ArH), 9.20 (s,1H,NH). *m/z* : **(M^+)** 579 **(M+2)** 581. (Found: C, 51.53; H, 2.53; N, 14.26%. Calc. for $C_{25}H_{17}O_7N_6SCl$; C, 51.72; H, 2.93; N 14.48%)

Synthesis of 1-(N- sulphamido) –[2'- (N-methyl-phthalyl)-benzimidazoyl]-(4''- chlorophenoxy)-4-(substituted phenyl)-azetidin-2-ones (7a-d):
Equimolar proportions of compounds **(5a/b/c/d)** and *p*-chloro phenoxy acetyl chloride (0.01 mol) in ethanol (10-15 ml) was refluxed for 2 hrs and cooled. The product which separated out, was filtered, dried and recrystallized from ethanol.

1-(N-sulphamido) –[2'- (N-methyl-phthalyl)-benzimidazoyl]-(4''chlorophenoxy) - 4 - (4'''-hydroxy-3'''-methoxyphenyl)-azetidin-2-one (7a):
Yield : 68%, m.p.: 192°C ν_{max} (KBr): 772 (C-Cl), 1105 (SO_2 symmetric), 1223 (C-N),1341 (SO_2 asymmetric), 1626 (C=N), 1655 (C=O,β-lactam),1708(>C=O, phthalimido). δ_H (300 MHz, DMSO-d_6) : 1.63 (s,3H,CH_3), 2.60(s,2H,CH_2), 3.85 (d,1H,C_4), 4.62(d,1H,C_3), 7.12-7.96 (m,15H,ArH), 9.24 (s,1H,NH), 11.23 (s,1H,OH). *m/z :* **(M^+)** 672 **(M+2)** 674. (Found: C, 56.94; H, 3.39; N, 10.19%. Calc. for $C_{32}H_{24}O_8N_5SCl$; C, 57.05; H, 3.56; N 10.40%)

1-(N- sulphamido) –[2'- (N-methyl-phthalyl)-benzimidazoyl]-(4''- chlorophenoxy)-4- (4'''-hydroxyphenyl)-azetidin-2-one (7b):
Yield : 56%, m.p.: 198°C ν_{max} (KBr) : 768 (C-Cl), 1105 (SO_2 symmetric), 1228 (C-N),1343 (SO_2 asymmetric), 1626 (C=N), 1652 (C=O,β-lactam),1719(>C=O, phthalimido). δ_H (300 MHz, DMSO-d_6) :2.64 (s,2H,CH_2), 3.78 (d,1H,C_4), 4.65(d,1H,C_3), 7.02-7.93 (m,16H,ArH), 9.32 (s,1H,NH), 11.02 (s,1H,OH). *m/z :* **(M^+)** 642 **(M+2)** 644. (Found: C, 57.73; H, 3.18; N, 10.61%. Calc. for $C_{31}H_{22}O_7N_5SCl$; C, 57.85; H, 3.42; N 10.88%)

1-(N- sulphamido) –[2'- (N-methyl-phthalyl)-benzimidazoyl]-(4''- chlorophenoxy)-4- (2''',4'''-dichlorophenyl)-azetidin-2-one (7c):
Yield : 64%, m.p.: >230°C ν_{max} (KBr): 774 (C-Cl), 1110 (SO_2 symmetric), 1219 (C-N),1345 (SO_2 asymmetric), 1622 (C=N), 1654 (C=O,β-lactam),1716(>C=O, phthalimido). δ_H (300 MHz, DMSO-d_6) : 2.59 (s,2H,CH_2), 3.67 (d,1H,C_4), 4.68(d,1H,C_3), 7.09-7.87 (m,15H,ArH), 9.37 (s,1H,NH). *m/z:* **(M^+)** 694 **(M+4)** 698. (Found: C, 53.24; H, 2.58; N, 9.74%. Calc. for $C_{31}H_{20}O_6N_5SCl_3$; C, 53.52; H, 2.87; N 10.07%)

1-(N- sulphamido) –[2'- (N-methyl-phthalyl)-benzimidazoyl]-(4''- chlorophenoxy)-4- (4'''-nitrophenyl)-azetidin-2-one (7d):
Yield : 72%, m.p.: 185°C ν_{max} (KBr): 773 (C-Cl), 1105 (SO_2 symmetric), 1220 (C-N),1340 (SO_2 asymmetric), 1542 (NO_2), 1625 (C=N), 1650 (C=O,β-lactam),1711(>C=O, phthalimido). δ_H (300 MHz, DMSO-d_6) : 2.53 (s,2H,CH_2), 3.75 (d,1H,C_4), 4.65(d,1H,C_3), 7.11-7.84 (m,16H,ArH), 9.43 (s,1H,NH). *m/z:* **(M^+)** 671 **(M+2)** 673. (Found: C, 55.01; H, 2.86; N, 12.27%. Calc. for $C_{31}H_{21}O_8N_6SCl$; C, 55.35; H, 3.12; N 12.50%)

Antimicrobial activity:
The cup plate method[22] using Hi-Media agar medium was employed to study the antibacterial activity of 6a-d and 7a-d against the bacterial strains ***Escherichia coli, Alcaligenes faecalis , Pseudomonas aeruginosa*** and ***Klebsiella pneumoniae*** and fungal strains ***Chaetomium globosum*** and ***Curvularia lunata***. Preparation of nutrient broth, subculture, base layer medium, agar medium and peptone water was done as per the standard procedure. Each test compound (30 mg) was dissolved in dimethylsulphoxide (DMSO) (10 mL, 3000 μg/mL). Using a sterilized cork borer cups were scooped out of agar medium contained in a petri dish which was previously inoculated with the microorganisms. The test compound solution was added in the cups and the petri dishes were subsequently incubated at 37 °C for 24 hrs (for bacteria) and 72 hrs. (for fungus).Three concentration levels i.e. 3000,1000 and 500 ppm were taken of the ten compounds Ciprofloxacin and Flucanazole were used as reference drugs and dimethylsulphoxide as a negative control. Zones of inhibition produced by each compound was measured in mm, and the results are listed in **Table I** and **II.**

RESULTS AND DISCUSSION

The FTIR of compound **(1)** gave characteristic vibrations at 1685 and 1710 which were identified as the (C=O,carboxy) and the later one as (C=O, phthalimido). Cyclization to benzimidazole **(2)** was characterized by the appearance of new vibrations at 1225, 1620 and 3250 which were characterized as C-N, C=N and NH, hence confirming the cyclization .The chlorosulphonyl benzimidazole **(3)** showed a new vibration at 1110 and 1340 which were identified for SO_2 symmetric and asymmetric vibrations. A new vibration between 3224-3410 was identified as the hydrazino group ($NHNH_2$) in compound **(4).** Further the cyclization with chloro acetyl chloride gave a new vibration at 1650 cm^{-1} in compound **6a** which was the carbonyl group vibration part of a β-lactam ring.

The NMR spectrum of compound **(2)** showed a singlet at 8.98 which integrated for one proton , identified as the imino proton, supporting the formation of the benzimidazole nucleus. Reaction with chlorosulphonic acid gave **(3)** .Its NMR spectrum showed absence of the singlet in the downfield region.Further reaction with hydrazine hydrate gave the corresponding hydrazide **(4).** A broad singlet integrating for two protons was visible at δ 5.20.This indicated the presence of an NH_2 group. Conversion to the benzylidene derivative by reacting with 3 – methoxy – 4 - hydroxy benzaldehyde gave compound **(5a)** .The NMR spectra showed two singlets at δ 4.30 and at δ 4.90.The former integrated for three protons which were identified as the methoxy protons while a slightly downfield signal as a singlet was characterized as the azomethine proton. Subsequent reaction with chloroacetyl chloride gave the azetidinone **(6a).** Two doublets centered at δ 3.32 (C_4) and at δ 4.61 (C_3) were visible which supported the formation of β-lactam ring

The mass spectrum gave the molecular ion peak at 147 **(1),** 276 **(2),** 370 **(4),** 503 **(5a)**, 474 **(5b),** 503 **(5d)** respectively supporting the molecular weight and molecular formula of these derivatives. The isotopic peaks in **(3), (5c), (6a-d)** and **(7a-d)** confirmed the presence of halogen and substituent.

Antimicrobial activity:
The results of antimicrobial activity are shown in **Table I** and **II**. The test compounds showed significant antibacterial and antifungal activity.

The chloroacetyl derived azetidinone, (**6b)** showed a zone size between 20-25 mm against ***E coli, A. faecalis , K pneumoniae*** while (**6c)** showed a zone size of 20mm against ***P aeruginosa*** at 1000 ppm .Compounds (**6c)** showed moderate zone size of 11-20 mm against ***E coli*** and ***K pneumoniae*** while (**6a)** and (**6d)** were inactive (9-11mm) against these strains. In case of ***P aeruginosa,*** (**6a)** and (**6b)** were moderately active (12-16mm) while **(6d)** was inactive (8mm).Against fungus **(6c)** showed a zone size of 15-19mm while all the derivatives except **(6c)** were moderately active (15-19mm) against ***C globosum*** and ***C lunata*** .

In case of chlorophenoxy substituted azetidinones**,(7b)** showed the maximum inhibition of 16-25mm zone size against all the bacterial strains. **(7a)** and **(7c)** showed good inhibition zone of 23-25mm against ***P aeruginosa*** and ***K pneumoniae*** respectively while others moderately inhibited the growth of ***E coli*** .Others showed moderate to zone size of 9-21 mm against all the bacterial strains with **(7d)** being inactive . Against both the fungus ***C globosum*** and ***C lunata*** all the compounds showed moderate activity.

Table I : Antibacterial activity of compounds (6_{a-d}) and (7_{a-d})
(Zone inhibition in mm)

S.No.	*E. coli*			*A. faecalis*			*P. areuginosa*			*K. pneumonae*		
	500 ppm	1000 ppm	3000 ppm	500 Ppm	1000 ppm	3000 ppm	500 Ppm	1000 ppm	3000 ppm	500 ppm	1000 ppm	3000 ppm
6a	9	9	3	8	14	4	7	12	15	4	10	16
6b	4	20	8	10	25	6	8	16	18	14	22	22
6c	8	11	10	3	10	10	7	20	22	10	20	23
6d	5	8	2	7	13	7	4	8	8	5	11	16
7a	9	10	5	5	6	12	7	23	25	7	10	10
7b	4	16	7	8	16	11	12	25	22	17	26	20
7c	6	13	8	5	5	6	7	21	23	7	25	20
7d	5	10	5	4	12	15	3	7	8	4	12	15
Std.	14	22	32	18	27	35	13	26	38	20	28	33

Control: DMSO (negative); Reference Standard: Ciprofloxacin

Table II : Antifungal activity of compounds (6_{a-d}) and (7_{a-d})
(Zone inhibition in mm)

S.No.	*C. globosum*			*C. lunata*		
	500 ppm	1000 ppm	3000 ppm	500 ppm	1000 ppm	3000 ppm
6a	4	4	4	8	13	15
6b	5	12	6	10	11	9
6c	4	15	17	13	19	4
6d	3	7	7	8	10	7
7a	6	8	5	4	8	5
7b	3	17	7	12	15	9
7c	4	7	8	16	17	6
7d	5	14	6	9	13	4
Std.	19	24	33	20	25	32

Control: DMSO (negative); Reference Standard: Flucanazole

Structural activity relationship showed that an increase antimicrobial activity was obsaerved in *p*-chlorophenoxy substituted azetidinones (**7a-d)** than the chloro substituted azetidinones. The antimicrobial screening data clearly indicate that a hydroxyl and a chloro substituent in azetidinone moiety has enhanced the pharmacological profile of the compounds.

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