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The synthesis and antimicrobial study of some azetidinone derivatives with the *para*-anisidine moiety

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Abstract: Azetidinones were synthesized from *p*-anisidine in two steps. First the Schiff's bases were prepared by reacting the hydrazide of an anisidine derivative with different aromatic aldehydes. Cyclocondensation of the Schiff's bases with chloroacetyl chloride in the presence of triethylamine resulted in the formation of the corresponding azetidinone analogues. The structures of the newly synthesized compounds were confirmed by IR, ^{1}H NMR and mass spectroscopic analysis. The antibacterial and antifungal potential of the synthesized compounds were evaluated by the agar disc method.

Keywords: azetidinones, Schiff's bases, antibacterial, antifungal.

INTRODUCTION

β-Lactam antibiotics are the most commonly used antibiotics. The 2-carbonyl dereivative of azetidine (four-membered heterocyclic ring with nitrogen as the hereroatom) is designated as 2-azetidinone or, more commonly, β-lactam.

A large number of 3-chloro monocyclic β-lactams having substitution at positions 1 and 4 possess powerful anti-bacterial, anti-microbial, sedative, anti-fungal and anti-tubercular activity.[1–11]

Azetidinones can be prepared from Schiff's bases, which are the condensation products of aldehydes and amino compounds. They are considered significant owing to their wide range of biological applications. They are also employed as intermediates in chemical synthesis.

Previously, Havaldar *et al.*[12] synthesized azetidinone analogues by treating 2-oxo-2*H*-chromen-4-yl 2-(benzylidene)hydrazinecarboxylates with chloroacetyl chloride in the presence of triethylamine and reported their antibacterial activity.

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Also, Bhat *et al.*[13] synthesized Schiff's bases by condensation of the acid hydrazide of ibuprofen with different aromatic aldehydes, which on treatment with chloroacetyl chloride in the presence of triethylamine afforded 2-azetidinones.

On consideration of the above factors, it was decided to synthesize some new substituted azetidinone derivatives from the *p*-anisidine moiety and screen 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.

RESULTS AND DISCUSSION

Synthesis of the azetidinone derivatives by the described method resulted in good yields of the products, as can be seen from Table I, which also lists the physical data of compounds **5a–h**.

TABLE I. Physical data of the synthesized azetidinone compounds

Compound No.	Physical state	Melting point/°C	Molecular formula	% yield
5a	yellow crystals	208–210	$C_{18}H_{17}O_3N_3Cl_2$	70.4
5b	brown crystals	210–212	$C_{18}H_{17}O_5N_4Cl$	69.3
5c	yellow crystals	235–237	$C_{18}H_{18}O_4N_3Cl$	60.2
5d	yellow crystals	245–248	$C_{19}H_{20}O_5N_3Cl$	68.1
5e	dark red crystals	240–242	$C_{20}H_{23}O_3N_4Cl$	68.4
5f	pale yellow crystals	252–254	$C_{18}H_{17}O_5N_4Cl$	60.3
5g	brown crystals	246–248	$C_{18}H_{18}O_3N_3Cl$	62.4
5h	brown crystals	250–252	$C_{16}H_{16}O_4N_3Cl$	60.1

The spectral data of the synthesized azetidinone compounds are as follows.

N-[*3-Chloro-2-(4-chlorophenyl)-4-oxoazetidin-1-yl*]-*2-(4-methoxyphenylamino)acetamide* (**5a**). IR (KBr, cm^{-1}): 3445 (–NH, str.), 2970 (–CH, str.), 1637 (–CONH, str.), 1733 (–C=O); ^{1}H NMR (δ, ppm): 7.4–7.8 (8H, Ar-H), 7.2 (2H, NH), 3.9 (3H, –OCH_3), 3.1 (2H, azetidinone), 1.5 (2H, CH_2); Mass (*m/z*): 393 (molecular ion peak), 102 (base peak).

N-[*3-Chloro-2-(4-nitrophenyl)-4-oxoazetidin-1-yl*]-*2-(4-methoxyphenylamino)acetamide* (**5b**). IR (KBr, cm^{-1}): 3425 (–NH, str.), 2968 (–CH, str.), 1626 (–CONH, str.), 1733 (–C=O) ^{1}H NMR (δ, ppm): 7.3–7.8 (8H, Ar-H), 7.1 (2H, NH), 3.8 (3H, –OCH_3), 3.1 (2H, azetidinone), 1.5 (2H, CH_2); Mass (*m/z*): 402 (molecular ion peak), 102 (base peak).

N-[*3-Chloro-2-(4-hydroxyphenyl)-4-oxoazetidin-yl*]-*2-(4-methoxyphenylamino)acetamide* (**5c**). IR (KBr, cm^{-1}): 3433 (–NH, str.), 2974 (–CH, str.), 1634 (–CONH, str.), 1731 (–C=O); ^{1}H NMR (δ, ppm): 7.4–7.8 (8H, Ar-H), 7.2 (2H, NH), 3.9 (3H, –OCH_3), 3.1 (2H, azetidinone), 5.3 (1H, –OH), 1.5 (2H, CH_2); Mass (*m/z*): 375 (molecular ion peak), 102 (base peak).

N-[*3-Chloro-2-(4-hydroxy-3-methoxyphenyl)-4-oxoazetidin-1-yl*]-*2-(4-methoxyphenylamino)acetamide* (**5d**). IR (KBr, cm^{-1}): 3630 (–OH, str.), 3445 (–NH, str.), 2975 (–CH, str.), 1636 (–CONH, str.), 1735 (–C=O); ^{1}H NMR (δ, ppm): 7.4–7.8 (7H, Ar-H), 7.2 (2H, NH), 3.9 (6H, 2×–OCH_3), 3.1 (2H, azetidinone), 1.5 (2H, CH_2); Mass (*m/z*): 405 (molecular ion peak), 102 (base peak).

N-*{3-Chloro-2-[4-(dimethylamino)phenyl]-4-oxoazetidin-1-yl}-2-(4-methoxyphenylamino)acetamide* (**5e**). IR (KBr, cm^{-1}): 3447 (–NH, str.), 2972 (–CH, str.), 1635 (–CONH, str.), 1737 (–C=O); ^{1}H NMR (δ, ppm): 7.4–7.8 (8H, Ar-H), 7.2 (2H, NH), 3.9 (3H, –OCH_3), 3.1 (2H, azetidinone), 1.5 (2H, CH_2), 2.4 (6H, 2×–CH_3); Mass (*m/z*): 400 (molecular ion peak), 102 (base peak).

N-[*3-Chloro-2-(2-nitrophenyl)-4-oxoazetidin-1-yl*]-*2-(4-methoxyphenylamino)acetamide* (**5f**). IR (KBr, cm^{-1}): 3448 (–NH, str.), 2971 (–CH, str.), 1639 (–CONH, str.), 1739 (–C=O); ^{1}H NMR (δ, ppm): 7.4–7.8 (8H, Ar-H), 7.2 (2H, NH), 3.9 (3H, –OCH_3), 3.1 (2H, azetidinone), 1.5 (2H, CH_2); Mass (*m/z*): 402 (molecular ion peak), 102 (base peak).

N-*(3-Chloro-2-oxo-4-phenylazetidin-1-yl)-2-(4-methoxyphenylamino)acetamide* (**5g**). IR (KBr, cm^{-1}), 3450 (–NH, str.), 2979 (–CH, str.), 1640 (–CONH, str.), 1740 (–C=O); ^{1}H NMR (δ, ppm): 7.4–7.8 (9H, Ar-H), 7.2 (2H, NH), 3.9 (3H, –OCH_3), 3.1 (2H, azetidinone), 1.5 (2H, CH_2); Mass (*m/z*): 359 (molecular ion peak), 102 (base peak).

TABLE II. Antimicrobial activity of the synthesized azetidinone derivatives

Compound No	Diameter of the inhibition zone/mm				
	B. subtilis	*S. aureus*	*E. coli*	*P. aeruginosa*	*C. albicans*
5a	13	19	20	12	10
5b	15	20	21	14	11
5c	12	17	18	–	–
5d	12	17	18	11	–
5e	14	18	19	13	9
5f	14	18	20	12	10
5g	11	15	16	10	–
5h	11	12	14	10	–
Ampicillin	16	20	21	15	–
Griseofulvin	–	–	–	–	13

N-[*3-Chloro-2-(3-furyl)-4-oxoazetidin-1-yl*]-*2-(4-methoxyphenylamino)acetamide* (**5h**). IR (KBr, cm^{-1}): 3445 (–NH, str.), 2970 (–CH, str.), 1642 (–CONH, str.), 1736 (–C=O), ^{1}H NMR (δ, ppm): 7.4–7.8 (7H, Ar-H), 7.2 (2H, NH), 3.9 (3H, –OCH_3), 3.1 (2H, azetidinone), 1.5 (2H, CH_2); Mass (*m/z*): 349 (molecular ion peak), 102 (base peak).

The results of the biological evaluation of the synthesized compounds are given in Table II.

From Table II it can be seen that compounds **5a, 5b** and **5f** showed significant antibacterial activity and compounds **5c, 5d** and **5e** showed moderate activity against the tested bacteria when compared to that of the standard drug ampicillin.

Also, compounds **5a, 5b** and **5f** showed moderate antifungal activity against *Candida albicans* when compared with that of the standard drug griseofulvin.

EXPERIMENTAL

The solvents and reagents used in the synthetic work were of laboratory grade and were purified by distillation or crystallization where necessary and their melting points were compared with the available literature values.

The azetidinone derivatives were prepared from 4-methoxyphenylamine (*p*-anisidine) according to Scheme 1. Generally, 4-methoxyphenylamine (**1**) on reaction with ethyl chloroacetate yielded ethyl 4-methoxyphenylaminoacetate (**2**), which on refluxing with hydrazine hydrate yielded 4-methoxyphenylaminoacetohydrazide (**3**). On refluxing **3** with aromatic aldehydes, the corresponding Schiff's bases **4** were obtained, which on cyclocondensation with chloroacetyl chloride in the presence of triethylamine afforded the corresponding azetidinone analogue **5** (see Scheme 1).

Preparation of ethyl 4-methoxyphenylaminoacetate (***2***)

A mixture of *p*-anisidine (0.1 mol), ethyl chloroacetate (0.1 mol) and anhydrous potassium carbonate (19.5 g, 0.15 mol) in dry acetone was refluxed on a water bath for 20 h. The resulting reaction mixture was cooled and filtered. The acetone was removed from the filtrate by distillation and the remaining filtrate was poured into well stirred, ice-cold water. The organic layer was extracted with diethyl ether. The ether layer was washed with 5 % HCl and dried over anhydrous sodium sulphate. The ether was then removed by evaporation on a water bath and the remaining liquid vacuum distilled to afford pure ethyl 4-methoxyphenylaminoacetate.

IR/cm^{-1}: 3445 (N–H), 2970 (C–H), 1730 (C=O), 1635 (C=C); ^{1}H NMR (δ, ppm): 7.9 ($–OC_2H_5$), 5.7 ($Ar–NH_2$), 7.4–7.7 (Ar–H), 1.5 ($–CH_2$), 3.9 ($–OCH_3$).

Preparation of 4-methoxyphenylaminoacetohydrazide (***3***)

A mixture of **2** (0.05 mol), hydrazine hydrate (99 %, 0.07 mol) in ethanol was refluxed for 6 h. From the resulting mixture, the excess ethanol was removed by distillation. On cooling, white, needle-like crystals of the required products separated, which were collected and recrystalized from ethanol.

IR/cm^{-1}: 3442 (N–H), 2971 (C–H), 1640 (–CONH), 1630 (C=C); ^{1}H NMR (δ, ppm): 5.6 ($ArNH_2$), 1.6 ($–CH_2$), 7.4–7.7 (Ar–H), 7.2 (NH).

Preparation of 2-(4-methoxyphenylamino)-N-*(substituted benzylidene)acetohydrazides* (***4a–h***)

A mixture of **3** (0.01 mol dissoslved in the minimum quantity of ethanol) and the required aromatic or heterocyclic aldehyde (0.01 mol dissolved in the minimum quantity of ethanol) together with about 0.01 mol sulphuric acid as catalyst was refluxed in a round bottom flask on a water bath for 6 h. The formed precipitate was filtered, washed with ice-cold water and recrystallized from ethanol.

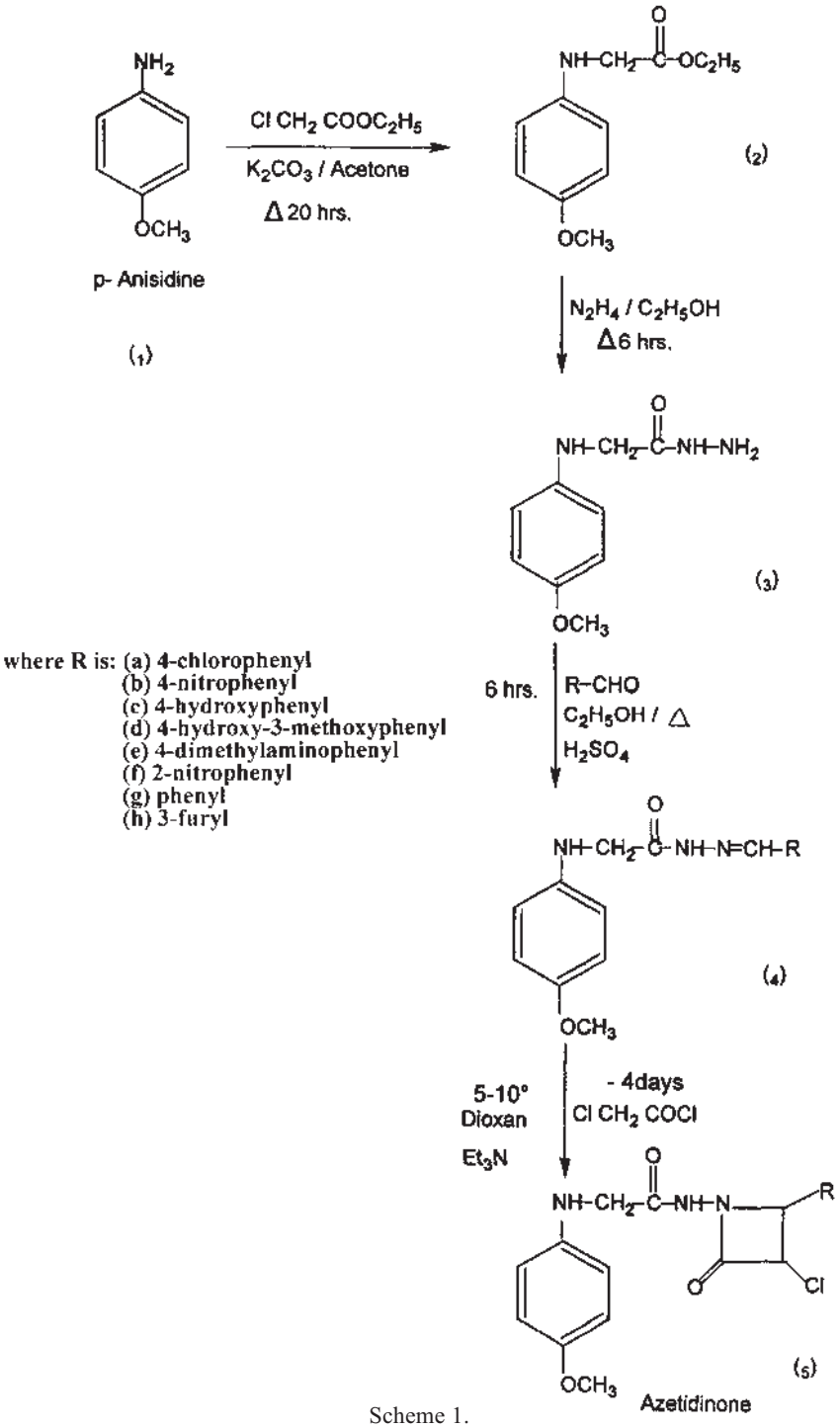
IR/cm^{-1}: 3445 (NH), 2971 (CH), 1635 (-CONH), 1628 (C=C); ^{1}H NMR (δ, ppm): 5.6 (ArNH), 1.7 (CH_2), 7.4–7.7 (Ar-H), 7.1 (NH).

Preparation of N-*(2-aryl-3-chloro-4-oxoazetidin-1-yl)-2-(4-methoxyphenylamino)acetamides* (***5a–h***)

Chloroacetyl chloride was added dropwise to the required Schiff's base **4** (0.01 mol) and triethylamine (0.02 mL) in dioxane (25 mL) at 5–10 °C. The mixture was stirred for 20 h and left at room temperature for three days. The contents were filitered, dried and recrystallized from ethanol.

The purity of the compounds were determined by their melting points and by thin layer chromatography.

Melting points were taken in an open capillary in a liquid paraffin bath and are uncorrected.


NH2
OCH3
p- Anisidine
(1)
Cl CH2 COOC2H5
K2CO3 / Acetone
Δ 20 hrs.
NH-CH2-C-OC2H5
O
(2)
N2H4 / C2H5OH
Δ 6 hrs.
NH-CH2-C-NH-NH2
(3)
where R is: (a) 4-chlorophenyl
(b) 4-nitrophenyl
(c) 4-hydroxyphenyl
(d) 4-hydroxy-3-methoxyphenyl
(e) 4-dimethylaminophenyl
(f) 2-nitrophenyl
(g) phenyl
(h) 3-furyl
6 hrs.
R-CHO
C2H5OH / Δ
H2SO4
NH-CH2-C-NH-N=CH-R
(4)
5-10°
Dioxan
Et3N
- 4days
Cl CH2 COCl
NH-CH2-C-NH-N
R
Cl
(5)
Azetidinone

Scheme 1.

The IR spectra were recorded using a Nicolet-Impact-400 FTIR spectrometer as thin films supported on KBr pellets. The FAB mass spectra were recorded on a JEOL-SX 102/DA-600 mass spectrometer. The ^{1}H NMR spectra were recorded on a JEOL-JMS D-300 instrument in $CDCl_3$ using TMS as the internal standard.

The antimicrobial activity was assayed using the cup-plate agar diffusion method by measuring the zone of inhibition in mm. All the samples were tested at a concentration of 50 μg/mL in dimethylformamide.

The bacterial strains employed were *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. The fungal strain used was *Candida albicans*. The employed standard drugs were ampicillin and griseofulvin.

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ИЗВОД

СИНТЕЗА И ПРОУЧАВАЊЕ АНТИМИКРОБНЕ АКТИВНОСТИ НЕКИХ АЗЕТИДИНОНА СА *p*-АНИЗИДИНСКОМ ГРУПОМ

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Азетидинони су синтетисани полазећи од *p*-анизидина у два корака. Најпре су добијене Shiff-ове базе у реакцијама хидразида једног анизидинског деривата са различитим ароматичним алдехидима. Циклокондензација Schiff-ових база са хлорацетил-хлоридом у присуству триетиламина довела је до настајања одговарајућих азетидинонских аналога. Структуре новосинтетизованих једињења потврђене су IR, ^{1}H NMR и масеном спектрометријском анализом. Антибактеријски и антигљивични потенцијал синтетисаних једињења процењен је методом диска на агару.

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