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SYNTHESIS, CHARACTERIZATION & BIOLOGICAL EVALUATION OF SOME AZETIDINE DERIVATIVES

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ABSTRACT: New series of N-(3-chloro-2-oxo-4-substituted-azetidine-1-yl) 2-methyl-1*H*-indole-3-carboxamide derivatives (VIa-d) were prepared and tested for their antibacterial and antifungal activity. The synthesis is based on the condensation of phenylhydrazine and ethyl acetoacetate in the presence of acetic acid to ethyl (2-methyl-1H- indol-3-yl)-2-oxoacetate (III) which on reaction with hydrazine hydrate gives 2-(2-methyl-1H-indol-3-yl)-2-oxoacetohydrazide (IV). Further condensation of oxoacetohydrazide and substituted benzaldehyde gave carbohydrazide derivatives (Va-d). Finally addition of triethylamine in dry 1, 4-dioxane and chloroacetyl chloride gave N-(3-chloro-2-oxo-4-substituted-azetidine-1-yl)2-methyl-1*H*-indole-3-carboxamide derivatives (VIa-d). The structure of newly synthesized 4-Oxoazetidin Substituted derivatives has been established by spectral (IR, ^{1}HNMR) data. These compounds were screened for antibacterial and antifungal activity against various gram-positive and gram-negative strains. All the compounds show significant antibacterial and antifungal activity.

INTRODUCTION: Synthetic and semi-synthetic antimicrobial agents have been used for a long time against the life-threatening infectious diseases [1]. Deaths from bacterial and fungal infection have dropped currently, but still, those are the major cause of death in the world. The treatment of infectious diseases remains an important and challenging problem because of a combination of factors including emerging infectious diseases and the increasing number of multi-drug resistant microbial pathogens with particular relevance for Gram-positive bacteria [2, 3].

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Bacterial resistance has currently become a grave concern for physicians [4]. Azetidine is a 4 member heterocyclic ring system with nitrogen as heteroatom. 2-Azetidinones are also known as β-lactams, and it is one of the most common heterocyclic rings found in antibiotics. 2-Azetidinones consists of a carbonyl group on the second position [5]. The 2-azetidinone (β-lactam) ring system is the common structural feature of some broad-spectrum β-lactam antibiotics, including penicillins, cephalosporins, carbapenems, nocardiosis, monobactams, clavulanic acid, sulbactam, and tazobactam; which have been widely used as chemotherapeutic agents to treat bacterial infections and microbial diseases [6]. Literature survey reveals that azetidine has shown various biological activities along with antimicrobial activity [7-15]. Given these findings, some azetidine derivatives have been synthesized and evaluated for antibacterial, antifungal activity.

MATERIAL AND METHODS:

Chemistry: In the present study titled compounds (VIa-e) were prepared by the condensation of phenylhydrazine and ethyl acetoacetate in the presence of acetic acid to ethyl (2-methyl-1H-indol-3-yl)-2-oxoacetate (III) which on reaction with hydrazine hydrate gives 2-(2-methyl-1H-indol-3-yl)-2-oxoacetohydrazide (IV). Further condensation of oxoacetohydrazide and substituted benzaldehyde gave carbohydrazide derivatives (Va-d) finally the addition of triethylamine in dry 1, 4-dioxane and chloroacetyl chloride gave N-(3-chloro-2-oxo -4-substituted- azetidine-1-yl) 2-methyl-1*H*-indole-3-carboxamide derivatives (VIa-d).

Synthesis of ethyl (2-methyl-1H- indol-3-yl)-2-oxoacetate (III): In a 500 ml three-necked flask fitted with a dropping funnel, a sealed stirrer unit, and reflux condenser, place a mixture of 0.1 mol of ethyl acetoacetate (II) and 0.1 mol of acetic acid heat under reflux with stirring and add 0.1 mol of phenylhydrazine (I) during 1 h. Continue the stirring for another one hour. Pour the reaction mixture into a 1-liter beaker and stir vigorously while it solidifies. Cool to 5 ºC and filter at the vacuum pump through Buchner funnel; cool the filtrate in ice and refilter through the same Buchner funnel wash the solid on the filter with 50 ml of water, suck almost dry and then wash with 50 ml of ethanol then keep overnight in room temperature recrystallized from ethanol.

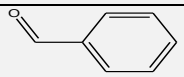
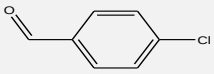
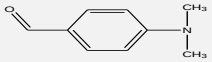
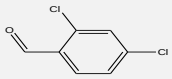
Synthesis of 2-(2-methyl-1*H*- indol-3-yl)-2-oxoacetohydrazide (IV): A mixture of ethyl (2-methyl-1*H*- indol-3-yl)-2-oxoacetate (III) and hydrazine hydrate in equimolar portion and 15 ml of ethanol were taken in a round bottom flask and refluxed for 4-6 h. Excess of ethanol was removed by distillation. On cooling crude product was separated. It was filtered, collected and recrystallized from ethanol to obtain silky white crystals.

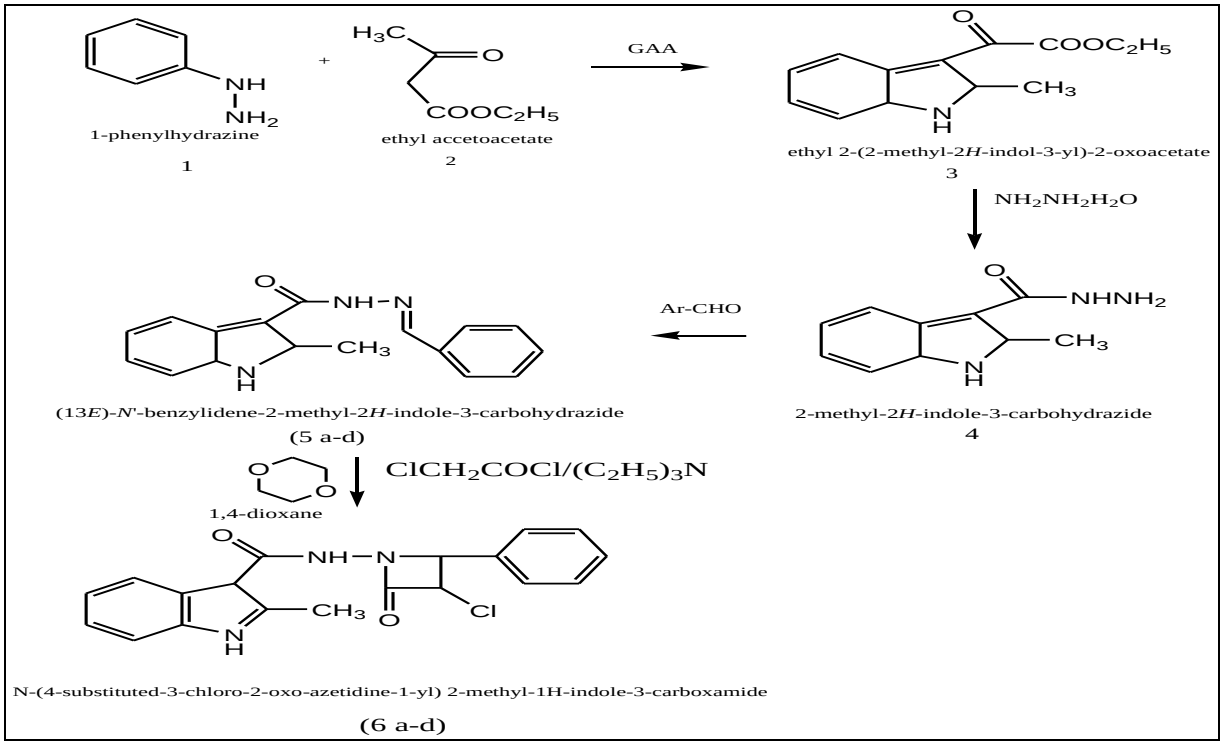
General Procedure for the Synthesis of substituted 2-methyl- 1*H*- indole- 3-carbohydrazide (Va-d): A mixture of 2-(2-methyl-1*H*- indol-3-yl)-2-oxoacetohydrazide (IV) (0.01 mol) and substituted benzaldehyde (0.01 mol) was refluxed in ethanol (30 ml) in the presence of catalytic amount of glacial acetic acid for 3 h. The reaction mixture was concentrated, cooled; the solid separated was filtered and recrystallized from aqueous DMF.

General Procedure for the Synthesis of 2-oxo-azetidine derivatives of 2-methyl-1*H*-indole-3-carboxamide (VIa-d): Substituted 2-methyl-1*H*-indole-3-carbohydrazide (0.01mol) (Va-f) were dissolved in 1:4 Dioxane (20ml) with constant stirring, triethylamine (0.01 mol) was added followed by dropwise addition of 2-chloro acetyl chloride (0.01 mol). The content was stirred vigorously for 15 minutes and refluxed for 5 h. The mixture was cooled at room temperature, filtered, washed with ice-cooled water, dried and recrystallized from DMF.

Spectral Studies: All melting points were recorded in Digital melting point apparatus and are uncorrected. The IR spectra were recorded on Perkin Elmer FTIR spectrometer using KBr pellets ^{1}HNMR spectra were recorded on Bruker Avance II 400MHz NMR (d in ppm) relative to TMS as an internal standard. The purity of compounds were checked by TLC using precoated silica gel G plate method using ethyl acetate: glacial acetic acid: water and the spots were examined by I_2 Vapor or under a UV lamp and R_f value has been reported in **Table 1.**

TABLE 1: CHARACTERIZATION DATA OF COMPOUNDS VI a-d

Compound Code	R	Mol. Formula	Mol. Weight	M.P.(^{0}C)	% Yield	R_f Value
VI a		$C_{19}H_{16}ClN_3O_2$	353.80	146-148	65	.61
VI b		$C_{19}H_{15}Cl_2N_3O_2$	388.24	148-150	66	.63
VI c		$C_{21}H_{21}Cl_1N_4O_2$	396.87	152-154	67	.71
VI d		$C_{19}H_{14}Cl_3N_3O_2$	422.69	167-169	65	.61



Synthesis of N-(4-phenyl- 3-chloro-2-oxo-azetidine-1-yl) 2-methyl -1 *H* – indole - 3-carboxamide (VIa):

I.R (KBr, cm-1) 3427 (NH), 2923 (-C-H-), 1762 (C=O), 1630 (C=O, CONH), 1354 (C-N), 866 (C-Cl).

^{1}HNMR (Acetone) 6-8.5 (9H, Ar-H), 2.2 (3H, CH_3), 0.9 (2H, N-H), 2.4 (1H, CH-Cl), 3 (1H, N-CH).

Synthesis of N-(4-chlorophenyl- 3-chloro-2-oxo-azetidine-1-yl) 2-methyl – 1 *H* - indole-3-carboxamide (VIb):

I.R (KBr, cm-1) 3427 (NH), 2923 (-C-H-), 1758 (C=O), 1727(C=O, CONH), 1307 C-N), 732 (C-Cl):

^{1}HNMR (Acetone) 6-8.5 (8H, Ar-H), 2.2 (3H, CH_3), 0.9 (2H, N-H), 2.4 (1H, CH-Cl), 3(1H, N-CH)

Synthesis of N-(4-(dimethylamino) phenyl - 3-chloro-2-oxo-azetidine-1-yl) 2-methyl-1*H*-indole-3-carboxamide (VIc):

I.R (KBr, cm-1) 3427 (NH), 2923 (-C-H-), 1756 (C=O), 1638(C=O, CONH), 1313 (C-N), 1065 (C-N 3^o):

^{1}HNMR (Acetone) 6-8.5 (7H, Ar-H), 2.2 (3H, CH_3), 0.9 (2H, N-H), 2.4 (1H, CH-Cl), 3 (1H, N-CH)

Synthesis of N-(4-(2, 4-dichlorophenyl) - 3-chloro-2-oxo-azetidine-1-yl) 2-methyl-1*H*-indole-3-carboxamide (VId):

I.R (KBr, cm^{-1}) 3427 (NH), 2923 (-C-H-), 1762 (C=O), 1630 (C=O, CONH), 1354 (C-N), 866 (C-Cl):

^{1}HNMR (Acetone) 6-8.5 (8H, Ar-H), 2.2 (9H, $3CH_3$), 0.9 (2H, N-H), 2.4 (1H, CH-Cl), 3 (1H, N-CH).

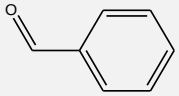
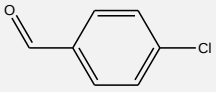
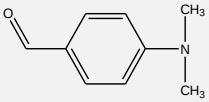
Antimicrobial Activity:

Antibacterial Activity: A novel prepared series of carboxamide derivatives were screened for their antibacterial activity *in-vitro* in comparison with amoxicillin as a reference drug using the standard agar disc diffusion method against four bacterial species: *Staphylococcus aureus* (AUMC B71), *Bacillus cereus* (AUMC B70) represented by *Escherichia coli* (AUMC B69) and *Pseudomonas aeruginosa* (AUMC B72).

Nutrient agar medium of the requisite composition *viz.*, peptone (2.5g), beef extract (0.5g), agar-agar (10g) and distilled water (500ml) was prepared, and pH of the medium was adjusted to 6.6.

For the preparation of media, all the above ingredients (except agar-agar) were weighed and dissolved in distilled water (250 ml) by application of gentle heating. After dissolving the ingredients completely, more distilled water and weighed agar-agar added. Then, it was filtered through cotton to obtain a clear solution. The mixture was autoclaved for 30 min at a pressure of 1.5 kg/cm^2. All the glass wares were cleaned with chromic acid and then sterilized by keeping in the oven. The medium was cooled to 37.1 ℃ and the homogenous suspension was prepared by transferring aseptically a loopful of all the corresponding microorganism from a fresh subculture into agar medium followed by vigorous shaking 20 ml of this medium was poured into each sterilized Petri-dish under aseptic conditions and allowed to set. Sterile 5-mm filter paper disk was saturated with 10 µl of the solution of test compound and Ampicillin as a reference drug. Also another disc was impregnated with the solvent DMSO and served as a negative control. The discs were then dried for 1h and placed in each plate. The seeded plates were incubated at 35 ± 2 ℃ for 24-48 h. The radii of inhibition zones (in mm) of triplicate sets were measured and results are given in **Table 2**.

TABLE 2: *IN-VITRO* ANTI-BACTERIAL AND ANTI-FUNGAL ACTIVITY OF NEWLY SYNTHESIZED COMPOUNDS VIa-d

		Inhibition Zone(in mm)					
		Gram-Positive		Gram-Negative		Pathogens	
S. no.	R	*S. aureus*	*B. cereus*	*P. aeruginosa*	*E. coli*	*C. albicans*	*A.niger*
VI a	R	9	8	12	15	10	9
VI b		19	16	16	17	8	6
VI c		22	18	15	17	7	8
VI d		10	12	10	24	12	13
	Ampicillin	24	24	25	25	-	-
	fluconazole					16	16

— indicates no activity as representative of gram-positive strains while gram-negative strains were

Antifungal Activity: The synthesized compounds VI a-d were tested for their antifungal activity *in-vitro* in comparison with fluconazole as a reference drug using the standard agar disc diffusion method against two pathogens namely *Candida albicans* and *Aspergillus niger*.

Spore suspension in sterile distilled water was prepared from a 2-5 days old culture of the test fungi grown on Sabourarud agar media. The final spore concentration was nearly 5×10^4 spore mL^{-1}. About 15 mL of growth medium was added to sterilized Petri dishes of 9 cm diameter and inoculated with 1 mL of spore suspension. Plates were shaken gently to homogenize the inocula. Sterile 5-mm filter paper disc was saturated with 10 µL of test compound solution and fluconazole (40 µ mol mL^{-1} in DMSO). Also, another disc was impregnated with DMSO and served as a negative control. The discs were dried for 1 h and placed in the center of each plate. The seeded plates were incubated at 28 ± 2 ℃ for 7 days. The radii of inhibition zones (in mm) of triplicate sets were measured at successive intervals during the incubation period and the results are given in **Table 2.**

RESULT AND CONLCUSION: Some derivatives of 2-methyl-1*H*-indole-3-carboxamide (VIa-d) were prepared and tested for their antibacterial and antifungal activity. The synthesis is based on the condensation of phenylhydrazine and ethyl acetoacetate in the presence of acetic acid to ethyl (2-methyl-1H- indol-3-yl)-2-oxoacetate (III) which on reaction with hydrazine hydrate gives 2- (2- methyl- 1H- indol- 3- yl)- 2-oxoacetohydrazide (IV). Further, condensation of oxoacetohydrazide and substituted benzaldehyde gave carbohydrazide derivatives (Va-d) finally the addition of triethylamine in dry 1, 4-dioxane and chloroacetyl chloride gave carboxamide derivatives

(VIa-d). All the above reactions are observed summarized in the scheme.

All synthesized compound (VIa-d) was recrystallized from DMF and identified by TLC using ethyl acetate: glacial acetic acid: water. Spots were visualized through the iodine chamber or under a UV lamp and the R_f value was calculated and found to be in the range of 0.61-0.71 cm. The structures of various compounds were assigned on the basis of their melting points, R_f values, IR, ^{1}HNMR spectral data. The compounds were they are evaluated for antibacterial activity using the standard agar disc diffusion method. They showed moderate activity against most of the tested bacterial strains. Among them, compound VI b and VIc showed maximum activity than the other compounds against both Gram-positive and Gram-negative bacteria compared with standard drug Ampicillin. The compounds also evaluated for antifungal activity standard agar disc diffusion method against two pathogens namely *Candida albicans* and *Aspergillus niger*. The compound VId shows highly significant activity compared with standard drug fluconazole.

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CONFLICT OF INTEREST: Nil

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