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Synthesis, Characterization & Biological evaluation of carbazide and semicarbazide analogue of Pyrrolizine

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Abstract: A novel derivatives of 1-(5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carbonyl)-5-(substituted benzyl) carbazide and 1-(5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carbonyl)-4-alkyl/aryl semicarbazide have been synthesized and evaluated of their antimicrobial activity. The structure of this novel compounds have been confirmed by using IR, ^{1}H NMR, Mass spectrometric technique. All the synthesized compounds were screened for their in vitro antimicrobial activity.

Keywords: Pyrrolizine, Carbazide, Semicarbazide, 5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carbonyl

INTRODUCTION:

Heterocyclic compounds are one of the main groups of organic compounds possessing wide range of applications in various areas of science and high technologies. Many heterocyclic compounds are natural compounds that can also be formed by biosynthesis [1].

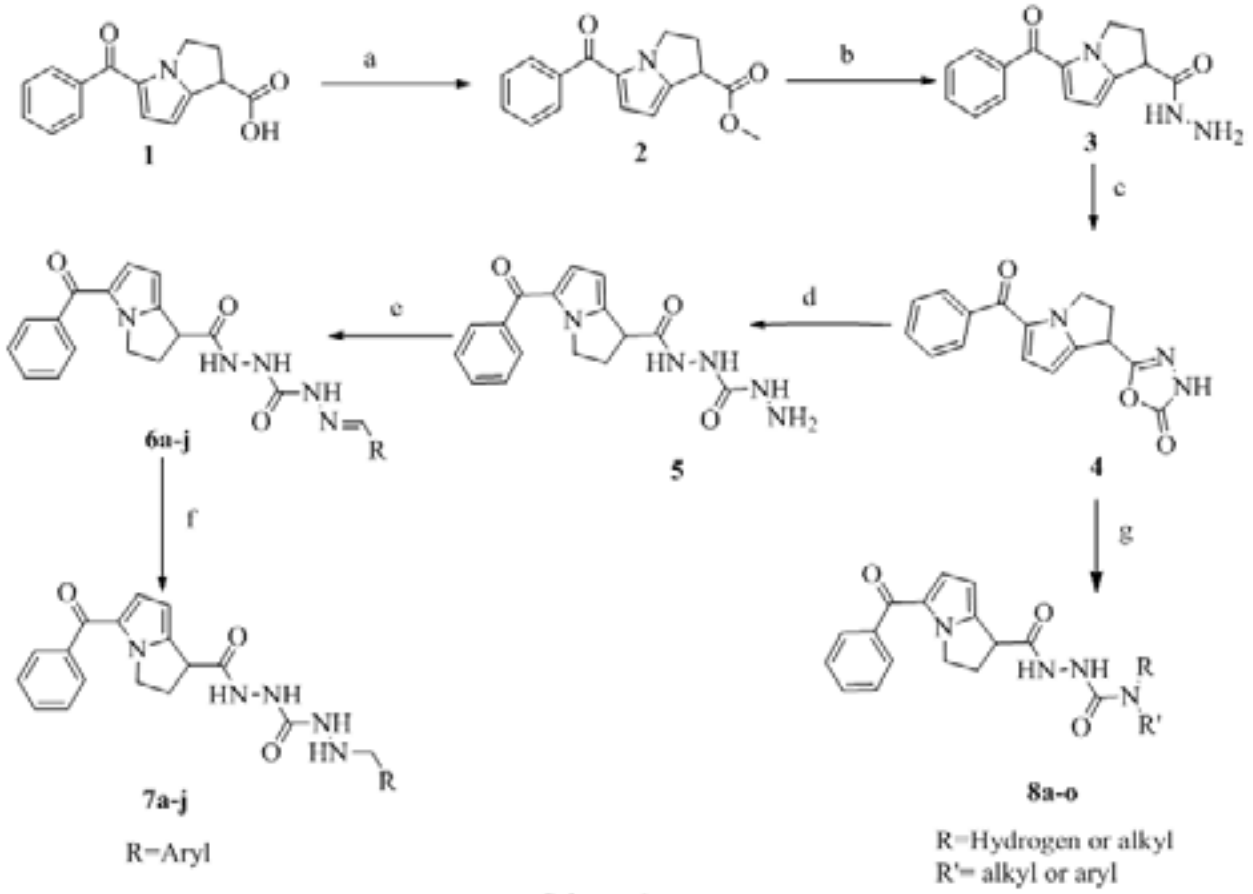
Pyrrolizine structure containing an azabicyclo[3.3.0]octane ring system[2]. Pyrrolizines are heterocyclic systems containing of two fused five member rings with one nitrogen atom at the ring junction and the rings formally contain two double bonds [3,4,5]. Many of pyrrolizine derivatives have been isolated from plants [6] and animals [7]. Pyrrolizidine constitutes the main skeleton of over 660 alkaloids identified in 6000 plants worldwide [8]. Pyrrolizidine alkaloid-containing plants are widespread in the world and probably the most common poisonous plants affecting livestock, wildlife, and humans [9]. These alkaloids are biosynthesized by plants as secondary metabolites against herbivores [10]. Pyrrolizines and Pyrrolizidine alkaloids are difficult to synthesize as active pharmacological targets. Pyrrolizines and Pyrrolizidine derivatives have exclusive structural features and remarkable biological activity [11-13]. The pyrrolizine contains a many compounds with different biological roles as antitumor [14-16], anti-inflammatory agents [17-19], antimalarial

agents [20], anticonvulsant [21], antimicrobial [22], antibiotics [23], antiproliferative [24] and anti-cancer agents [25,26]. Pyrrolizine alkaloids also used as acetylcholinesterase inhibitor agents [27] and analgesic and anti pyretic agents [28],

MATERIALS AND METHODS:

Laboratory grade chemicals were used without further purification. The progress of the reaction was monitored by analytical TLC on pre-coated plates (silica gel 60 F254) and visualized with UV light. Melting points were determined using Lab India V10 apparatus and are uncorrected. NMR spectra (^{1}H at 400 MHz) were recorded using DMSO-d^6 as a solvent and chemical shifts are expressed in parts per million (ppm) related to internal reference TMS by Varian 400 Hz. Infrared spectra were determined on a Shimadzu FT-IR. The Mass spectra were recorded using specifications of the LC/MS are as follows:

GENERAL SYNTHETIC SCHEME:



Scheme-1

Reagents:(a) Conc.H_2SO_4, CH_3OH, reflux; (b) $NH_2NH_2.H_2O$, EtOH, RT; (c) CDI, THF, TEA, RT; (d $NH_2NH_2.H_2O$, EtOH, 60-70°C; (e) RCHO, Conc.H_2SO_4, CH_3OH, reflux ; (f) Pd/[C], Ammonium formate, EtOH, water; (g) RR'NH, IPA, 70-80°C.

electrospray (+) ionization, mass range 100-800 Da, 20-V cone voltage, and X terra MS C18 column (2.1 mm x 50 mm x 3.5 μm).

EXPERIMENTAL PROCEDURE:

Preparation of Methyl 5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylate (2)

To a stirred solution of 5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid **1**(40.0g, 156.7 mmol) in dry methanol (400 ml,5T), concentrated sulfuric acid(7.68 g, 78.35 mmol) was added drop wise after 5 minutes. The resultant solution was stirred for two hours at reflux temperature. The progress of reaction was monitored by TLC and then solvent was evaporated under vacuum. The product was dissolved in water and extracted with dichloromethane (200 ml× 3). The combined organic layers were washed with 5% sodium bicarbonate solution (400 ml) followed by water, brine and dried over anhydrous sodium sulfate. The solvent was removed under vacuum to give analytical pure product **2** (38.5 g), Yield: 91 %.

Preparation of 5-Benzoyl-2,3-dihydro-1H-pyrrolizine-1-carbohydrazide (3)

To a stirred cooled (ice bath) solution of Methyl 5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylate **2**(38.0g, 141.1 mmol) in isopropyl alcohol(380ml), hydrazine hydride(14.12ml,282.2 mmol) was added drop wise in solution. The obtained solution was stirred at 20-25°C for 1-2 hours. White thick solid product was precipitate after some time. The product was isolated by filtration and washed with isopropyl alcohol (38 ml X 2) to give pure product **3** (34.2 g) as white solid, Yield; 90 %, mp. 140-145°C

Preparation of5 -(5-benzoyl-2,3-dihydro-1H-pyrrolizin-1-yl)-1,3,4 oxadiazol-2(3H)-one(4)

To a mixture of triethyl amine (19.16 g, 189.3 mmol) and 5-Benzoyl-2,3-dihydro-1H-pyrrolizine-1-carbohydrazide **3** (34.0 g, 126.2 mmol) in dichloromethane (175 ml), carbon diimidazole (26.6 g, 164.0 mmol) was added at 25-35°C. This mixture was stirred for two hours, than poured into water (340 ml) and extracted with dichloromethane (175 ml). Combine dichloromethane layers and washed with 2N hydrochloric acid solution (175 ml). The organic layer dried over sodium sulfate and solvent was removed under vacuum to give pure product **4** (30.0 g) as gray solid, yield: 80%, mp. 180-185°C.

Preparation of 1-(5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carbonyl)-carbazide (5)

To a solution of 5-(5-benzoyl-2,3-dihydro-1H-pyrrolizin-1-yl)-1,3,4 oxadiazol-2(3H)-one **4** (12.0 g, 40.63 mmol) in ethanol (60 ml), hydrazine hydrate (3.0 g, 60.95 mmol) was added. This mixture was stirred for two hours at reflux temperature. The progress of reaction was monitored by TLC. Cool the reaction mass at room temperature, thick solid precipitated which was then filtered and washed with ethanol (6 ml X 2) and dried at 40-50°C to give title scaffold compound **5** (12.0 g) as white solid, Yield:82.7%.

General preparation of 1-(5-benzoyl-2,3-dihydro-1H-pyrrolizine -1-carbonyl)-5-substituted benzylidene) carbazide (6a-j)

To a solution of 1-(5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carbonyl)-carbazide **5** (1.0 g, 3.05 mmol) and different substituted aryl aldehyde (3.36 mmol) in ethanol (10 ml), catalytic amount of conc. sulfuric acid (0.15 mmol) was added. This mixture was stirred for 4h at reflux temperature. After some time thick solid precipitated which was then filtered and washed with ethanol (5ml X 2) and dried to give title compound **6a-j**, Yield:70-90%.

General preparation of 1-(5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carbonyl)-5-

(substituted benzyl) carbazide (7a-j)

To a solution of 1-(5-benzoyl-2,3-dihydro-1H-pyrrolizine -1-carbonyl)-5-(substituted benzylidene) carbazide (24.04 mmol) and 10 % palladium charcoal (10 % loading) in ethanol (20 ml), solution of ammonium formate (48.08 mmol) in water (2 ml) was added. This mixture was stirred for 6-8 h at 50-60 °C. The progress of reaction was monitored by TLC. After completion of reaction filter the reaction mass through hi flow bed and wash the bad with ethanol. Remove the solvent under vacuum to give crude product. This crude product was purified in hot water to give pure title compound **7a-j**. Yield 60-90%. The yield and physical properties are reported in **Table-1.1.**

General preparationof 1-(5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carbonyl)-4-alkyl/aryl semicarbazide (8a-o)

To a solution of 5-(5-benzoyl-2,3-dihydro-1H-pyrrolizin-1-yl)-1,3,4 oxadiazol-2(3H)-one (1.0 g, 3.38 mmol) in isopropyl alcohol (5 ml), primary amine or secondary aliphatic amine (3.72 mmol) was added. This mixture was stirred for 4-6 h at 50-60°C. The progress of reaction was monitored by TLC. After completion of reaction cool the reaction mass at room temperature, thick solid precipitated which was then filtered and washed with isopropyl alcohol. The obtain produce was recrystallised in ethanol and dried at 40-50°C to give title compound **8a-o**, Yield: 56-76%. The yield time and physical properties are reported in **Table-1.2.**

SPECTRAL & PHYSICAL DATA:

1-(5-benzoyl-2, 3-dihydro-1H-pyrrolizine-1-carbonyl)-5-(4-methoxy benzyl) carbazide (7d) Yield: 65 %.**^{1}H NMR (400 MHz, DMSO-d^6):** δ=2.690-2.749 (q, 2H), 3.729 (s, 3H), 3.753(s, 2H), 3.960-3.995 (t, 1H), 4.284-4.333 (m, 1H), 4.385-4.434 (m, 1H), 4.913 (s, 1H), 6.054-6.064 (d, 1H), 6.751-6.761 (d, 1H), 6.857-6.878 (d, 2H), 7.174-7.295 (m, 2H), 7.496-7.532 (t, 2H), 7.577-7.614 (t, 2H), 7.734-7.754 (t, 2H), 8.187 (s, 1H), 9.899 (s, 1H), ppm; **MS:** m/z 446.2 $(M-1)^+$;**IR Cm^{-1}:**3336.85, 3273.20, 3030.17, 2945.30, 1734.01, 1716.65, 1653.00, 1456.26, 1269.16, 719.45, 669.30

1-(5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carbonyl)-5-(4-hydroxy benzyl) carbazide (7f)

Yield: 89 %.**^{1}H NMR (400 MHz, DMSO-d^6):** δ=2.603-2.816 (m, 2H), 3.700 (s, 2H), 3.963-3.999(t, 1H), 4.268-4.334 (m, 1H), 4.386-4.449 (m, 1H), 4.861 (s, 1H), 6.059-6.069 (d, 1H), 6.608-6.738 (m, 2H), 6.753-6.763 (d, 1H), 7.143-7.163 (d, 2H), 7.495-7.532 (t, 2H), 7.577-7.614 (t, 2H), 7.739-7.756 (d, 2H), 8.201 (s, 1H), 8.432 (s, 1H), ppm; **MS:** m/z432.2 $(M-1)^+$;**IR Cm^{-1}:**3336.85, 3273.20, 3030.17, 2945.30, 1734.01, 1716.65, 1653.00, 1456.26, 1271.09, 719.45, 669.30

1-(5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carbonyl)-5-(4-fluoro benzyl) carbazide (7h)

Yield: 79 %.**^{1}H NMR (400 MHz, DMSO-d^6):** δ=2.672-2.746 (q, 2H), 3.815-3.846 (d, 2H), 3.956-3.992(t, 1H), 4.283-4.447 (m, 2H), 5.056 (s, 1H), 6.051-6.061 (d, 1H), 6.750-6.760 (d, 1H), 7.102-7.146 (t, 2H), 7.397-7.417 (t, 2H), 7.432-7.512 (t, 2H), 7.532-7.733 (m, 2H),7.736-7.753 (d, 2H), 8.209 (s, 1H), 9.992 (s, 1H), ppm; **MS:** m/z 434.2 $(M-1)^+$; **IR Cm^{-1}:**3342.64, 3273.20, 3030.17, 2970.38, 1734.01, 1716.65, 1653.00, 1458.11, 1271.09, 719.45, 669.30

1-(5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carbonyl)-4-((thiophen-2-yl)ethyl) semicarbazide(8a)

Yield: 67 %.**^{1}H NMR (400 MHz, DMSO-d^6):** δ=2.700-2.758 (m, 2H), 2.891-2.926 (t, 2H), 3.242-3.276(q, 2H), 3.959-3.994 (t, 1H), 4.277-4.343 (m, 1H), 4.379-4.444 (m, 1H), 6.067-6.077 (d, 1H), 6.460-6.488 (t, 1H), 6.753-6.763 (d, 1H), 6.882-6.888 (s, 1H), 6.945-6.966 (m, 1H), 7.328-7.343 (q, 1H), 7.495-7.532 (t, 2H), 7.577-7.614 (t, 1H), 7.733-7.753 (t, 2H), 7.968

(s, 1H), 9.929 (s, 1H), ppm; **MS:** m/z 423.1 $(M+1)^+$, 445.2 $(M+Na)^+$, 461.1 $(M+K)^+$; **IR Cm^{-1}:** 3342.64, 3273.20, 2943.37, 1734.01, 1716.65, 1558.48, 1431.18, 1269.16, 1043.35, 717.52, 688.59

1-(5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carbonyl)-4-(cyclopropyl)semicarbazide (8b)
Yield: 70 %.**^{1}H NMR (400 MHz, DMSO-d^6):** δ=0.345-0.383 (m, 2H), 0.557-0.603 (m, 2H), 2.329-2.465 (m, 1H), 2.672-2.751 (m, 2H), 3.954-3.990(t, 1H), 4.273-4.439 (m, 2H), 6.066-6.076 (d, 1H), 6.546-6.551 (d, 1H), 6.754-6.764 (d, 1H), 7.496-7.533 (t, 2H), 7.578-7.615 (t, 1H), 7.72-7.753 (t, 2H), 7.857 (s, 1H), 9.885 (s, 1H), ppm; **MS:** m/z 353.2 $(M+1)^+$, 375.1 $(M+Na)^+$, 491.1 $(M+K)^+$; **IR Cm^{-1}:**3334.92, 3282.84, 2987.74, 1734.01, 1716.65, 1558.48, 1429.25, 1267.13, 1047.35, 721.38, 696.30

1-(5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carbonyl)-4- (methyl) semicarbazide (8e)
Yield: 73 %.**^{1}H NMR (400 MHz, DMSO-d^6):** δ=2.564-2.576 (d, 3H), 2.698-2.759 (m, 2H), 3.956-3.991(t, 1H), 4.264-4.340 (m, 1H), 4.376-4.440 (m, 1H), 6.074-6.084 (d, 1H), 6.289-6.300 (d, 1H), 6.753-6.763 (d, 1H), 7.732-7.753 (m, 2H), 7.882-7.884 (d, 1H), 9.883-9.887 (d, 1H), ppm; **MS:** m/z 327.2 $(M+H)^+$, 349.2 $(M+Na)^+$, 365.1 $(M+K)^+$; **IR Cm^{-1}:**3356.14, 3213.41, 2943.37, 1734.01, 1716.65, 1558.48, 1429.25, 1269.16, 1047.35, 719.45, 694.37

Table 1.1: Characteristics physical data of 7**a-j**.

Sr. No.	R	Color	M.P. (°C)	Yield (%)
7a	Thiophene	Gray	186	62
7b	2,4,5-trifluoro phenyl	Pale yellow	205	74
7c	2-bromo phenyl	Gray	196	85
7d	4-methoxy phenyl	Off white	193	65
7e	3-chloro phenyl	Off white	189	72
7f	4-hydroxy phenyl	Cream	210	89
7g	Phenyl	Off white	184	83
7h	4-fluoro phenyl	Off white	192	79
7i	4-hydroxy, 3-methoxy phenyl	Gray	240	82
7j	Pyridine	Off white	214	65

Table 1.2: Characteristics physical data of **8a-o**.

Sr. No.	R and R’	Color	M.P. (°C)	Yield (%)
8a	R=H, R’=thiophene-2-ethyl	Off white	185	67
8b	R=H, R’=cyclopropyl	Off white	176	70
8c	R=H, R’=n-butyl	White	195	69
8d	R=H, R’= (3,4-difluorophenyl) cyclopropyl	White	210	56
8e	R=H, R’=methyl	Off white	178	73
8f	R=H, R’=4-fluorophenyl ethyl	Off white	179	65
8g	R=H, R’=benzyl	Gray	186	76
8h	R=H, R’=pyridine	Off white	204	58
8i	R=H, R’=2-chloro phenyl	Off white	220	57
8j	R=H, R’=4-methoxy phenyl	Gray	204	56
8k	R=R’= piperidine	Off white	194	65
8l	R= R’= morpholene	Gray	188	68
8m	R=ethyl, R’= ethyl	Gray	180	71
8n	R=methyl, R’=methyl	Off white	175	70
8o	R=cyclohexyl, R’= cyclohexyl	Gray	195	58

BIOLOGICAL ACTIVITIES:

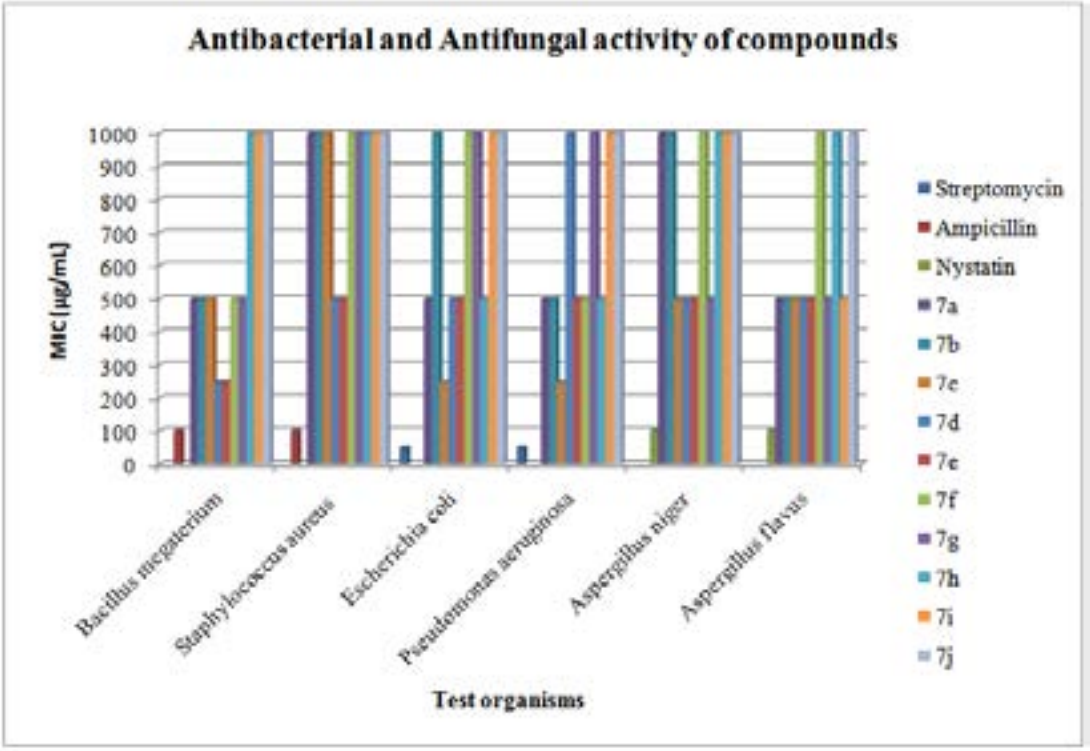
Antibacterial and antifungal activities:

The newly synthesized derivatives were evaluated for their in vitro antibacterial activity against gram negative *Escherichia coli* and *Pseudomonas aeruginosa*, gram positive *Staphylococcus aureus* and *Bacillus megaterium* and antifungal activity against *Aspergillus niger* and *Aspergillus flavus* by micro broth dilution method. The standard strains used for screening antibacterial and antifungal activities were procured from Atmiya Institute of Pharmacy in-vitro testing Laboratory, Rajkot, Gujarat, India. The MIC values are given in **Table-1.3**. The standard drugs used for antibacterial activity were Streptomycin, Ampicillin and Nystatin for antifungal activity. 1000 µg/mL, 500 µg/mL, 250 µg/mL, 125 µg/mL and 62.5 µg/mL, concentrations of the synthesized drugs were taken.

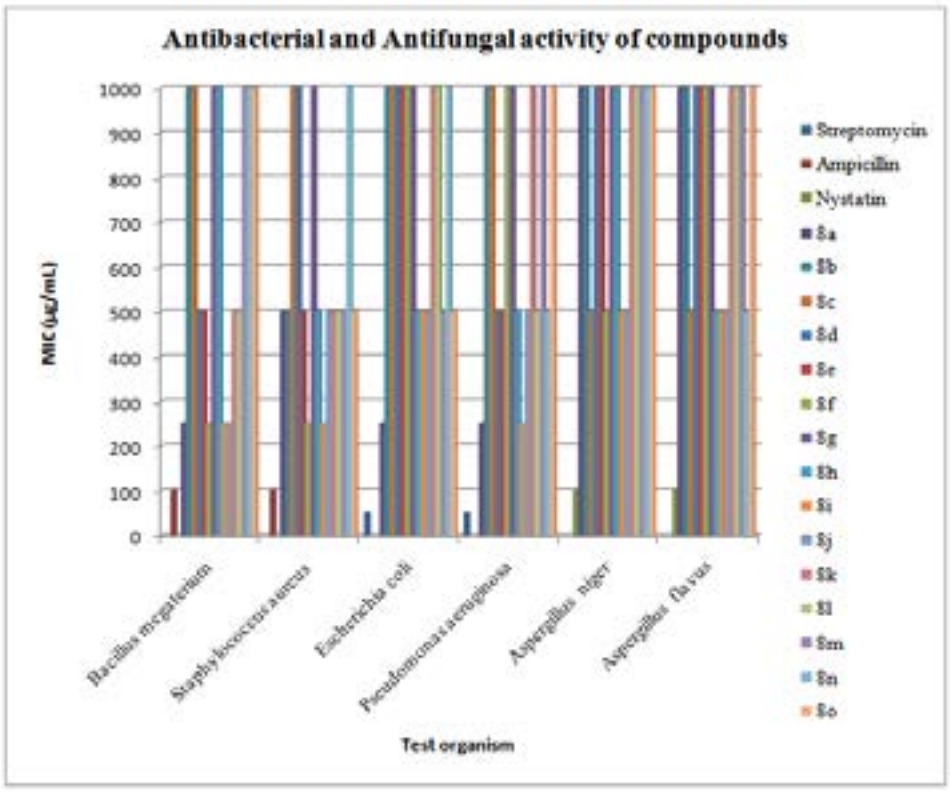
Table 1.3: Antibacterial and antifungal activity of **7a-j and 8a-o.**

	Antibacterial MIC (µg/mL)				Antifungal MIC (µg/mL)	
Compound Codes	***B.megaterium* MTCC2444**	***S.aureus* MTCC737**	***E.coli* MTCC1687**	***P. aeruginosa* MTCC3541**	***A. niger* MTCC282**	***A. flavus* MTCC418**
Streptomycin	-	-	50	50	-	-
Ampicillin	100	100	-	-	-	-
Nystatin	-	-	-	-	100	100
7a	500	1000	500	500	1000	500
7b	500	1000	1000	500	1000	500
7c	500	1000	250	250	500	500
7d	250	500	500	1000	500	500
7e	250	500	500	500	500	500
7f	500	1000	1000	500	1000	1000
7g	500	1000	1000	1000	500	500
7h	1000	1000	500	500	1000	1000
7i	1000	1000	1000	1000	1000	500
7j	1000	1000	1000	1000	1000	1000
8a	250	500	250	250	1000	1000
8b	1000	500	1000	1000	1000	1000
8c	1000	1000	1000	1000	500	500
8d	500	1000	1000	500	1000	1000
8e	500	500	1000	500	1000	1000
8f	250	250	1000	1000	500	1000
8g	1000	1000	1000	1000	1000	1000
8h	1000	500	500	500	1000	500
8i	250	250	500	250	500	500
8j	250	500	500	500	500	500
8k	500	500	1000	1000	1000	1000
8l	500	500	1000	500	1000	1000
8m	1000	500	500	1000	1000	1000
8n	1000	1000	1000	500	1000	500
8o	1000	500	500	1000	1000	1000

Graph 1.1: Antibacterial Activity, Minimum Inhibition Concentration (Graphical form) of **7a-j**



Graph 1.2: Antibacterial Activity, Minimum Inhibition Concentration (Graphical form) of **8a-o**



RESULT AND DISCUSSION:

Chemistry: As describe in synthetic scheme 1,5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid **1** was reacted with methanol in the presence of catalytic conc. sulfuric acid at reflux temperature to give methyl 5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylate **2** which was converted into its cabohydrazide (5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carbohydrazide) **3** by reaction with hydrazine hydride in isopropyl alcohol at 25-35 °C. The carbohydrazide **3** cyclized with carbon dimidazole in the presence of triethyl amine in dichloromethane at 25-35°C to give oxadiazole, 5-(5-benzoyl-2,3-dihydro-1H-pyrrolizin-1-yl)-1,3,4 oxadiazol-2(3H)-one **4.** This oxadiazole ring was then reopened with hydrazine hydrate in ethanol to give 1-(5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carbonyl)-carbazide scaffold **5**. The carbazide scaffold then converted into Schiff's base by the reaction with different substitutes aromatic aldehyde in the presence of conc. sulfuric acid in ethanol to give different novel 1-(5-benzoyl-2,3-dihydro-1H-pyrrolizine -1-carbonyl)-5-(substituted benzylidene) carbazide **6.** Schiff's base **6** was reduce in the presence of ammonium formate and palladium charcoal in ethanol at reflux temperature to give novel 1-(5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carbonyl)-5-(substituted benzyl) carbazide **7a-j**. Schiff's base **6** was reduced with different reducing agent like sodium borohydrate, lithium borohydrate, zinc and ammonium chloride, zinc and ammonium formate, iron and acetic acid but the best result was getting with yield in palladium and ammonium formate.

The oxadiazole ring **4** was reopened with different primary or secondary amine in isopropyl alcohol at 60-70°C to give novel 1-(5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carbonyl)-4-alkyl/aryl semicarbazide **8a-o**.

CONCLUSION:

These new compounds were purified and crystallized in appropriate solvent. All the reactions were smooth, and provided the products in the good yield.

The structure of all newly synthesized compound **7a-j** and **8a-o** was established on the basis of spectral analysis like IR, ^{1}H NMR, and mass data. The physical characterization data are listed in table 1.1 and 1.2

The MIC values revealed that some of the newly synthesized compounds (**table 1.3**) showed moderate to good inhibition. Compounds **7a, 7b, 7c, 7d, 7e, 8h, 8i** and **8j** exhibited good activities against bacterial strains. Whereas rest of the compounds is also potent drug, gives narrow spectrum action against pathogenic microbes.

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