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Synthesis and Antimicrobial Activity of Some Novel Pyrrolidine Derivatives

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Abstract : A novel synthesis of N-(substituted benzyl imino) 4-(2″,5″-dioxo pyrrolidin-1-yl) benzamides and their antimicrobial activity is described. A four step synthesis is described in which first step was the reaction of benzocaine with succinic anhydride and then cyclization in the presence of sodium acetate and further treatment of the cyclized product with hydrazine hydrate. Finally the formed hydrazides were treated with different substituted aldehydes.
Keywords: pyrrolidine; pyrrolidine2,5-dione derivatives; Antibacterial activity, Antifungal activity.

Introduction

The discipline of medicinal chemistry is devoted to the discovery and development of new agents for treating diseases. Most of this activity is directed to new natural or synthetic organic compound[1].
The pyrrolidine ring system is found in a vast variety of compounds displaying an impressive range of biological activities. Thus, the incorporation of different substitution patterns and motives into the basic heterocyclic substance common to such activities has potential in the discovery of new substances with useful pharmacological properties[2].
Review of literature revealed that pyrrolidines are well known for their versatile pharmacological activities such as antimicrobial[3,4,5], antitumor[6], anti-HIV-1[7], anticonvulsant[8,9], sphingosine-1-phosphate (S1P) receptor agonists[10,11], malic enzyme inhibitors[12], ketoamide-based cathepsin K inhibitors[13], human melanocortin-4 receptor agonists[14], etc. In view of these observations, we would like to report synthesis of new pyrrolidine derivatives as potential antimicrobial agents.

Experimental

The chemicals and reagents used in the present project were of AR and LR grade, procured from Aldrich, Hi-media, Merck, Ranbaxy, sigma and S.D-Fine Chem Ltd. Melting points of the synthesized compounds were determined by open capillary method and are uncorrected.
The IR spectra of the synthesized compounds were recorded on a Fourier Transform IR spectrometer (8400S, Shimadzu) at M.S. Ramaiah College of pharmacy, Bangalore. ^{1}H NMR spectra were recorded on NMR spectrometer (AMX-400, Bruker) at Indian Institute of Science Bangalore using $CDCl_3$ and chemical shifts (δ) are reported in parts per million downfield from internal reference Tetramethylsilane (TMS). Mass spectra were provided at by Uwin Global Services, Bangalore,

which were recorded on Mass spectrometer (LCMS-2010 A, Shimadzu). Antimicrobial activity data were provided in table-IA.

(a) Synthesis of 4-{[4-(ethoxy carbonyl) phenyl amino]}-4-oxo butanoic acid [01][15].

A solution of benzocaine, (0.02mol) and succinic anhydride, (0.022mol) in the presence of anhydrous tetrahydrofuran (60ml), pyridine (50µl) and an excess of acetic anhydride (60ml) was stirred at 0°c for 5 hr. The volume of the solution was reduced to about 25ml. and this concentrated solution was added to crushed ice. The residue was washed for three times with 0.001M HCl and the desired white colour product was obtained. The yield of the purified product was 66%.

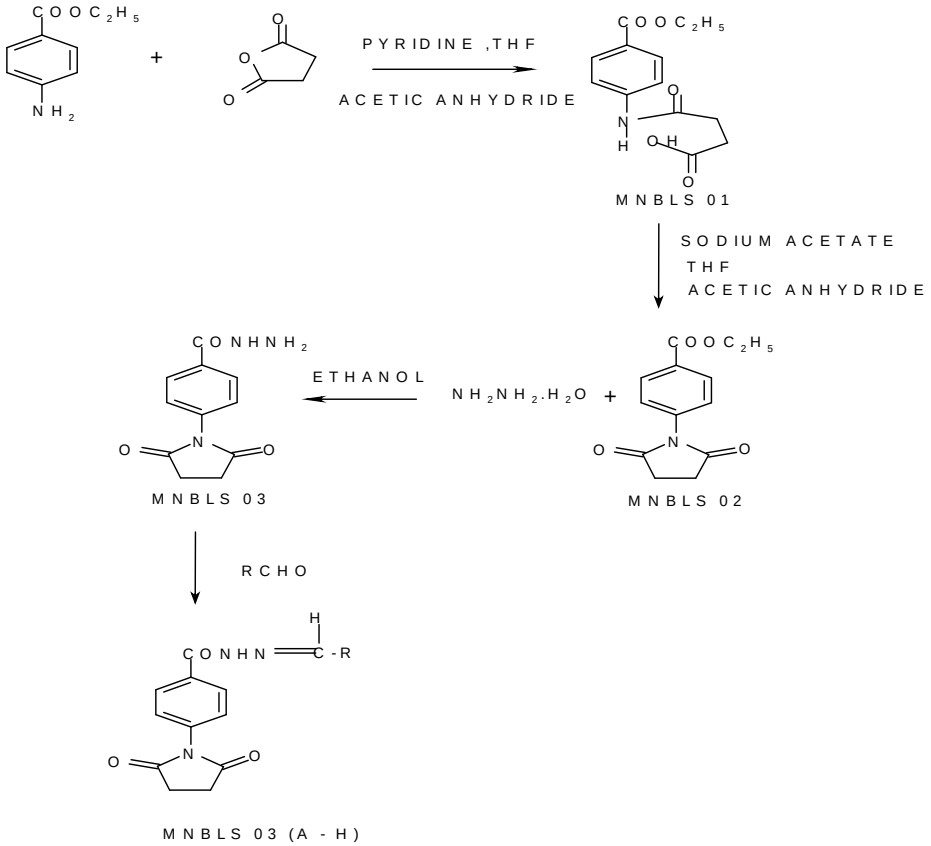
4-{[4-(ethoxy carbonyl)phenyl amino]}-4-oxo butanoic acid [01]:-white coloured solid; m.p. 79 ^{o}C; %yield 66%; Rf 0.75 (Benzene: Ethylacetate: :9:1); IR (KBr) ν 3043.46 & 3076.25(Ar.CH), 3336.62(NHstr.), 3201.61(OHstr.), 1280.65(C-Nstr.),2844.81-2995.25(CHstr.), 1706.88 (C=Ostr.), 1012.56(C-Ostr),1645.17(C=Ostr. Amide) 1685.67 (C=Ostr.). ^{1}H NMR (400 MHz, $CDCl_3$) δ10.01(1H,NH),10.4(1H,OH), 7.5-8.2(4H, Ar.), 4.30(2H,OCH_2), 2.83(2H), 2.55(2H)aliphatic CH_2 ,1.3(3H, CH_3). MS (APCI +) *m/z* 265 (M^+).

(b) Synthesis of ethyl 4-(2',5'-dioxo pyrrolidin-1-yl) benzoate [02][15].

A solution of 4-{[4-(ethoxy carbonyl) phenyl amino]}-4-oxo butanoic acid (0.01mol) in the presence of anhydrous tetrahydrofuran (60ml) and eqimolecular amount of sodium acetate (0.01mol) and acetic anhydride (1.5ml) was stirred at 0°c for 8 hr, followed by the evaporation of the solvent under vacuum. The residue was washed for three times with 0.001M HCl and the desired white crystals were obtained. The yield of the purified product was 60%.

Ethyl 4-(2',5'-dioxo pyrrolidin-1-yl) benzoate [02]:-white crystals; m.p. 72 ^{o}C; %yield 60%; Rf 0.65 (Benzene: Ethylacetate::9:1); IR (KBr) ν 3018.39(Ar.CH),2989.46(CHstr.)1280.36 (C-Nstr.), 1654.81(C=Ostr), 1714.60(C=Ostr.),1020.27(C-Ostr). ^{1}H NMR (400 MHz, Acetone) δ7.3-8.2(4H,Ar.), 4.30(2H,OCH_2), 2.92(2H,CH_2), 1.29 (3H,CH_3). MS (APCI +) *m/z* 249 (M^+).

SCHEME FOR SYNTHETIC METHODLOGY:



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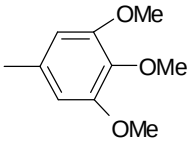
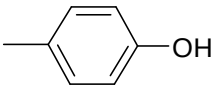
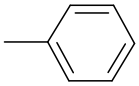
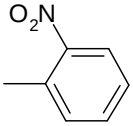
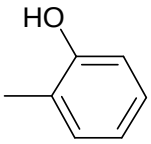
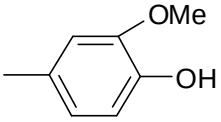
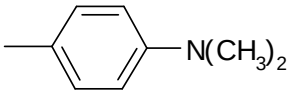
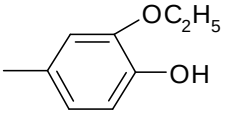
1	MNBLS03(A)	
2	MNBLS03(B)	
3	MNBLS03(C)	
4	MNBLS03(D)	
5	MNBLS03(E)	
6	MNBLS03(F)	
7	MNBLS03(G)	
8	MNBLS03(H)	

(c) Synthesis of 4-(2',5'-dioxo pyrrolidin-1-yl) benzoic acid hydrazide [03][16].

Ethyl 4-(2',5'-dioxo pyrrolidin-1-yl) benzoate (0.01mol) was dissolved in ethanol and this solution was added to the solution of hydrazine hydrate (0.04mol) in ethanol. The solution was then stirred at room temperature for 24 hr. The solution was evaporated to dryness and the residue was washed with diethyl ether (50ml), filtered and recrystallized from ethanol to give the desired white crystalline product. The yield of the purified product was 55%.

4-(2',5'-dioxo pyrrolidin-1-yl) benzoic acid hydrazide [03]:-white crystals;m.p.84-86 ^{o}C; %yield 55%; Rf 0.54(Benzene: Ethylacetate::9:1); IR (KBr) ν 3334.69 & 3296.12(NHstr.pri.), 3110.97(NHstr.sec.), 1683.74(C=Ostr.amide), 3043.46(Ar.CH), 2908.45,2983.67, 2995.25(CHstr.), 1704.96(C=Ostr), 1280.65(C-Nstr.). ^{1}H NMR (400 MHz,$CDCl_3$) δ 9.81(1H,NH), 7.5-8.1(4H,Ar.), 4.53(2H,NH_2), 2.92(2H,CH_2). MS (APCI +) *m/z* 233 (M^+).

(d) General procedure for the synthesis of N-(substituted benzyl imino) 4-(2″,5″-dioxo pyrrolidin-1-yl)benzamide [03(A-H)][17].

(0.002mol) of 4-(2',5'-dioxopyrrolidin-1-yl) benzoic acid hydrazide and (0.0022mol) of substituted benzaldehyde were dissolved in methanol and taken into evaporating dish and heated on a water bath for appropriate time. The mixture was frequently stirred with a glass rod, globules of water soon appeared on the surface of the oil. The mixture was then cooled and stirred well, the solid obtained was recrystallized from methylated spirit. The yields of the products ranged from 58-69%.

N-(3',4',5' trimethoxy benzyl imino) 4-(2″,5″-dioxo pyrrolidin-1-yl) benzamide(03A):-
Cream powder; m.p.55-58 ^{o}C; %yield 62%; Rf 0.51(Benzene: Ethylacetate::9:1); IR (KBr) ν 3334.69(NHstr.), 1280.65(C-Nstr.),1704.96 (C=Ostr), 1683.74(C=Ostr. amide), 1504.37 (C=Nstr.imines), 1234.36(C-Ostr). ^{1}H NMR (400 MHz,$CDCl_3$) δ 9.88(1H,NH), 8.36(1H,CH), 7.1-8.2(6H,Ar.), 3.83(4H,OCH_3), 2.92 (2H,CH_2). MS (APCI +) *m/z* 411 (M^+). Other peaks observed at 325, 239.

N-(4'-hydroxy benzyl imino) 4-(2″,5″-dioxo pyrrolidin-1-yl) benzamide (03B):-brown powder; m.p.63-65 ^{o}C; %yield 69%; Rf 0.58(Benzene: Ethylacetate::9:1); IR (KBr) ν 3334.69(NHstr.), 3043.46(Ar CH), 1600.81(C=Ostr amide),1286.43 (C-Nstr.), 1679.88 (C=Ostr), 1525.59 (C=Nstr.imines), 3168.83(OHstr.). ^{1}H NMR (400 MHz,$CDCl_3$) δ 9.88(1H,NH), 9.43(1H,OH), 8.36(1H,CH), 7.1-8.2(8H,Ar.), 2.92(2H,CH_2). MS (APCI +) *m/z* 337 (M^+).

N-(benzyl imino) 4-(2″,5″-dioxo pyrrolidin-1-yl) benzamide (03C):-cream powder; m.p.60-62 ^{o}C; % yield 66%; Rf 0.62(Benzene: Ethylacetate::9:1); IR (KBr) ν 3334.69(NHstr.),3070.04(Ar.CH), 1683.74 (C=Ostr amide),1288.36 (C-Nstr.), 1703.03 (C=Ostr), 1525.59(C=Nstr. Imines). ^{1}H NMR (400 MHz,$CDCl_3$) δ 9.88(1H,NH), 8.36(1H,CH), 7.1-8.2(9H,Ar.), 2.92(2H,CH_2). MS (APCI +) *m/z* 320(M^+).

N-(2' nitro benzyl imino)4-(2″,5″-dioxo pyrrolidin-1-yl)benzamide (03D):-pale yellow powder; m.p.72-75 ^{o}C; %yield 64%; Rf 0.68 (Benzene: Ethylacetate::9:1); IR (KBr) ν 3263.33(NHstr),1672.17(C=Ostramide),1704.96(C=Ostr)1272.93(CNstr.),1535.23(C=Nstrimines),1371.29($ArNO_2$). ^{1}H NMR (400MHz, $CDCl_3$) δ 9.88(1H,NH), 9.10(1H,CH) 7.1-8.3(8H,Ar.), 2.92(2H,CH_2). MS (APCI +) *m/z* 367(M^+). Other peaks observed at 326, 227, 211, 195.

N-(2' hydroxy benzyl imino) 4-(2″,5″-dioxo pyrrolidin-1-yl) benzamide (03E):-cream powder; m.p.72-75 ^{o}C; %yield 64%; Rf 0.68(Benzene: Ethylacetate::9:1); IR (KBr) ν 3334.69(NHstr.), 3043.46(Ar CH), 1600.81(C=Ostr amide),1286.43 (C-Nstr.), 1679.88(C=Ostr), 1525.59 (C=Nstr.imines), 3168.83(OHstr.). ^{1}H NMR (400 MHz,$CDCl_3$) δ 11.26(1H,OH), 9.88(1H,NH), 8.78(1H,CH), 7.3-8.2(8H,Ar.), 2.92(2H,CH_2). MS (APCI +) *m/z* 337(M^+).

N-(4' hydoxy 3'methoxy benzyl imino) 4-(2″,5″-dioxo pyrrolidin-1-yl) benzamide (03F):-pale yellow powder; m.p.72-75 ^{o}C; %yield 64%; Rf 0.68(Benzene: Ethylacetate ::9:1); IR (KBr) ν3263.33(NHstr.),3191.97(OHstr.),1172.64(COstr.ether), 1670.24 (C=Ostramide), 1706.88(C=Ostr.), 1260.07(CNstr.), 1539.09 (C=Nstr.imines). ^{1}H NMR (400 MHz,$CDCl_3$) δ 9.88(1H,NH), 9.83(1H,OH), 8.36(1H,CH), 7.1-8.3(7H,Ar.) 3.83(3H,OCH_3), 2.92(2H,CH_2). MS (APCI +) *m/z* 368(M^+).Other peaks observed at 327, 305.

N-(4' dimethyl amino benzyl imino) 4-(2″,5″-dioxo pyrrolidin-1-yl) benzamide (03G):-
Yellow powder; m.p.53-55 ^{o}C; %yield 64%; Rf 0.68(Benzene: Ethylacetate::9:1); IR (KBr) ν 1315.36 (C-Nstr),1272.93(C-Nstr),1541.02(C=Nstr imines)1672.17,(C=Ostr amide, 1706.88(C=Ostr). ^{1}H NMR (400 MHz,$CDCl_3$) δ 9.88(1H,NH), 8.36(1H,CH), 7.4-8.2(8H Ar.), 3.8(3H, NCH_3), 2.92(2H, CH_2). MS (APCI +) *m/z* 365(M^+). Other peaks observed at 210, 209.

N-(3' ethoxy 4' hydroxy benzyl imino) 4-(2″,5″-dioxo pyrrolidin-1-yl) benzamide (03H):-cream powder; m.p.53-55 ^{o}C; %yield 64%; Rf 0.68(Benzene: Ethylacetate::9:1); IR (KBr) ν 3305.76(OHstr),3363.62(NHstr.), 1172.64(C-Ostr), 1280.65(C-Nstr), 1517.87(C=N str.imines). ^{1}H NMR (400 MHz,$CDCl_3$) δ10.4(1H,OH), 9.88 (1H,NH), 8.36(1H,CH), 7.2-8.1(7H,Ar.), 2.92 (2H,CH_2), 2.3(2H,OCH_2), 1.1 (3H,CH_3). MS (APCI +) *m/z* 381(M^+). Other peaks observed at 303, 289, 83.

Results and Discussion

i) Antibacterial activity

The antibacterial activity of newly synthesized pyrrolidine derivatives was carried out by agar diffusion method against *Staphylococcus aureus and Enterococci (*gram-positive) and *Pseudomonas aerugenosa and Escherichia coli (gram-negative)* using Norfloxacin and Gatifloxacin as standard reference drugs.

***Staphylococcus aureus and Enterococci (*gram-positive)**

The zone of inhibition exhibited by 100µg of the compound N-(2' nitro benzyl imino) 4-(2″,5″-dioxo pyrrolidin-1-yl)benzamide [3D] against *Staphylococcus aureus and Enterococci* is 14.4mm and 13.6mm respectively, which is significant but less than the inhibition shown by 10µg of standard Norfloxacin (19.1mm and 15mm respectively) and 5µg of Gatifloxacin (23.6mm and 16.6 mm respectively). All compounds have shown antibacterial activity against gram-positive bacteria namely *Staphylococcus aureus and Enterococci.*

The order of the antibacterial activity for the synthesized compounds against gram-positive organisms is as follows.

a) For *Staphylococcus aureus*

3D (14.4mm) > 3A (13.6mm) > 3B (12mm) > 3H (11mm) > 3E (10mm) > 3C (9.6mm) > 3F (9.3mm) > 3G (8.mm).

b) For *Enterococci*

3D (13.6mm) > 3A (12mm) > 3H (10.6mm) > 3B, 3F (9.6mm) > 3E (08mm) > 3G (7.6mm) > 3C (7.3mm).

Pseudomonas aeruginosa and Escherichia coli (gram-negative)

The zone of inhibition exhibited by 100µg of the compound N-(2' nitro benzyl imino) 4-(2″,5″-dioxo pyrrolidin-1-yl)benzamide [3D] against *Pseudomonas aerugenosa* and *Escherichia coli* is 13mm and 18mm respectively, which is significant but less than the inhibition shown by 10µg of standard Norfloxacin (14mm and 27mm respectively) and 5µg of Gatifloxacin (15mm and 26mm respectively).

All compounds have shown antibacterial activity against gram-negative bacteria namely *Pseudomonas aeruginosa and Escherichia coli.*

The order of the antibacterial activity for the synthesized compounds against gram-negative organisms is as follows.

a) For *Pseudomonas aeruginosa*

3D (13mm) > 3A (11.3mm) > 3F (9.6mm) > 3H (9.3mm) > 3E (8.6mm) > 3B (08mm) > 3C, 3G (06mm).

b) For *Escherichia coli*

3D (18mm) > 3A (14mm) > 3F (12mm) > 3B, 3H (10.3mm) > 3G (9.6mm) > 3E (09mm) > 3C (7.6mm).

ii) Antifungal activity

Aspergillus niger and Aspergillus flavus

The antifungal activity of newly synthesized pyrrolidine derivatives have been evaluated against *Aspergillus niger* and *Aspergillus flavus* by agar diffusion method. The standards used are Clotrimazole and Amphotericin B.

The zone of inhibition exhibited by 100µg of the compound N-(2' nitro benzyl imino) 4-(2″,5″-dioxo pyrrolidin-1-yl) benzamide [3D] against *Aspergillus niger* and *Aspergillus flavus* is 12.3mm and 13.3mm respectively and the corresponding zones of inhibition of compound N-(3',4',5' trimethoxy benzyl imino) 4-(2″,5″-dioxo pyrrolidin-1-yl) benzamide [3A] is 10.6mm and 12mm respectively.

All compounds have shown antifungal activity against *Aspergillus niger* and *Aspergillus flavus.*

The order of the antifungal activity for the synthesized compounds is-

a) For Aspergillus niger

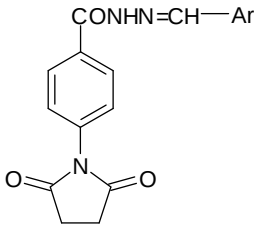
3D (12.3mm) > 3A (10.6mm) > 3H (10.3mm) > 3F(09mm) > 3E (08mm) > 3B (7.3mm)> 3G (07mm) > 3C (5.3mm).

b) For Aspergillus flavus

3D(13.3mm) > 3A (12mm) > 3E (10mm) > 3H (9.6mm) > 3F (9.3mm) > 3B, 3G (09mm) > 3C (6.3mm).

ANTIMICROBILOGICAL DATA

TABLE-I(A): RESULTS OF ANTIMICROBIAL ACTIVITY



Compounds	Ar	ANTIBACTERIAL ACTIVITY Zone of Inhibition (mm)				ANTIFUNGAL ACTIVITY Zone of Inhibition (mm)	
		S.aureus	*P.aeru*	*E.coli*	*E.cocci*	*A. niger*	*A. flavus*
[3A]	3,4,5 trimethoxyPhenyl	13.6	11.3	14	12	10.6	12
[3B]	P-hydroxy phenyl	12	08	10.3	9.6	7.3	09
[3C]	phenyl	9.6	06	7.6	7.3	5.3	6.3
[3D]	2 nitro phenyl	14.4	13	18	13.6	12..3	13.3
[3E]	o-hydroxy phenyl	10	8.6	09	08	08	10
[3F]	4-hydroxy 3- methoxy phenyl	9.3	9.6	12	9.6	09	9.3
[3G]	4-dimethyl amino phenyl	8.6	06	9.6	7.6	07	09
[3H]	3-ethoxy 4-hydroxy phenyl	11	9.3	10.3	10.6	10.3	9.6
Norfloxacin	---------	19.1	14	27	15	--------	---------
Gatifloxacin	---------	23.6	15	26	16.6	---------	---------
Amphotericin B	---------	----------	-------	-------	---------	18.6	14.6
Clotrimazole	----------	----------	--------	--------	--------	17	16.3
Control(DMF)		NI	NI	NI	NI	NI	NI

NOTE: - Average zone diameter in mm of triplicates
NI: - No inhibition

Conclusion

The objective of the present work was to synthesize, purify, characterize and evaluate the antimicrobial activity of the newly synthesized pyrrolidine and its derivatives.

The yield of the products ranged from 55-70%. The purity was checked by TLC. The structures of the newly synthesized compounds [3a-3h] are characterized and confirmed by spectral data viz. IR, ^{1}HNMR and Mass spectra and all the synthesized compounds [3a-3h] were screened for antimicrobial activity.

From this study it may be concluded that some of the derivatives of pyrrolidine have shown moderate to good antimicrobial activity.

Here the nitro and 3,4,5 trimethoxy derivatives have shown better activity against all the organisms (gram negative, gram positive bacteria and fungal strains) as compared to other derivatives in the series of compounds synthesized.
With these encouraging results, all the synthesized compounds can be further explored for structural modification and detailed microbiological investigations.

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