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Synthesis and *In-Vitro* Anti-Microbial Activity of Some New N-Phenyl Acetamide Derivatives

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ABSTRACT
The purpose of the research work is to synthesised a new potent antimicrobial derivatives of N-phenyl acetamide. A series of new methyl 4-oxo-4-(Piperidine phenylamino) butanoate **4(a)** and methyl 4-oxo-4-(Imidazole phenylamino) butanoate **4(b)** were synthesized by treating N -Phenyl acetamide with methyl choloroacetate in the presence of anhydrous potassium carbonate to give an intermediate compound (**3)** which on further treatrment with piperidine and imidazole at 70°C temperature gives the new compounds **4(a)** and **4(b)** respectively. The structures of newly synthesised derivatives were confirmed on the basis of Melting Point, TLC, IR, NMR and spectral data. The anti microbial activity of the synthesized compounds was evaluated by Disc diffusion test.

Keywords: N-phenyl acetamide, methyl chloroacetate, Amines, Antimicrobial activity.

INTRODUCTION

An antimicrobial is a substance that kills or inhibits the growth of microorganisms such as bacteria, fungi, or protozoans, as well as destroying viruses. Antimicrobial drugs either kill microbes (microbicidal) or prevent the growth of microbes (microbistatic). Disinfectants are antimicrobial substances used on non-living objects. [1-4]

Some N-phenyl acetamide derivatives of *N, N*-substituted acetamides have been found to possess significant antimicrobial activity when compared with Ofloxacine. [5-8] Based on the structural data some common features can be deduced in all the pharmacological divergent classes. [9] A basic nitrogen which may be part of heteroaromatic ring or cyclic/acyclic system intended to interact electrostatically with the appropriate target. [10-12]

EXPERIMENTAL

Equipment

Melting points of all the synthesized compounds were determined by Thieles's tube method and uncorrected by melting point determing apparatus (SISCO). The intermediate compounds synthesized were confirmed on the basis of their melting reported in the literature and the functional group tests. All the solvents were used after purification [13-17], distillation and dried. Purity of all compounds was checked by Silica gel GF254 plates TLC (e-Merck and Co.) with Ultra Violet spectroscopy detection method.

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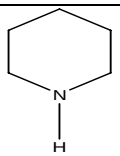
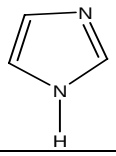
The FT-IR spectra (KBr cm^{-1}) were recorded on the Shimadzu Fourier transformed infrared (FT-IR) spectrophotometer (spectrum 8400).The physical data of the synthesized compounds were presented in Table 1.

Table 1: Characterization of the compounds

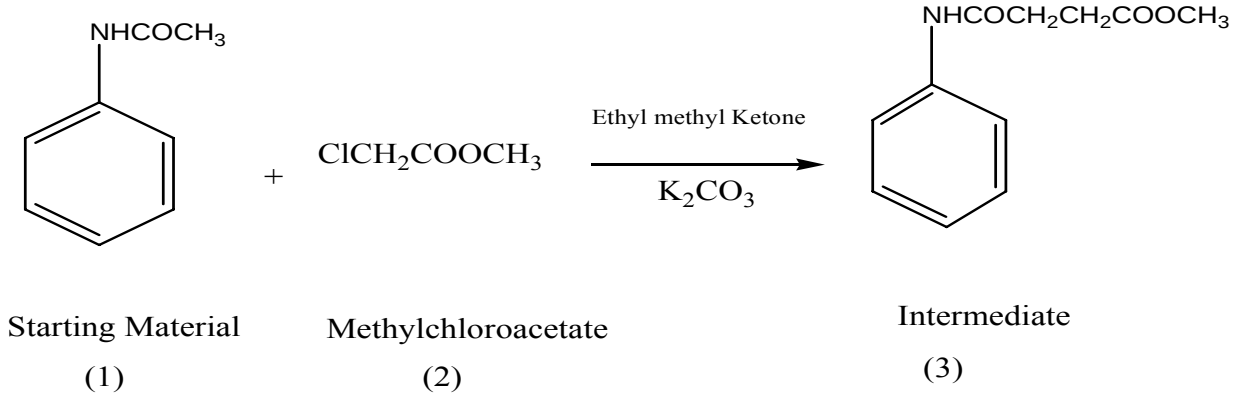
Compound	R	M.P.(°C)	Yield (%)	M. Weight	Molecular formula
4(a)		128-132	47.6%	298	$C_{16}H_{30}N_2O_3$
4(b)		150-155	40.2%	300	$C_{15}H_{29}N_3O_3$

Preparation of Derivatives

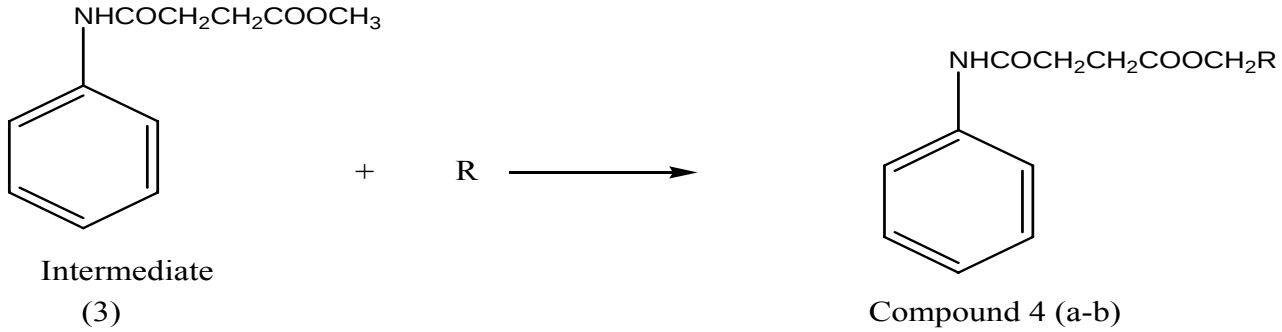
Preparation of Intermediate methyl 4-oxo-4-(phenylamino) butanoate

Methyl 4-oxo-4-(phenylamino) butanoate {(**1**) (500 mg} was dissolved in ethyl methyl ketone (70 ml) and anhydrous potassium carbonate (2.0 g) was added to the solution. The reaction mixture was refluxed for 2 h. Then, methyl chloroacetate (3 ml) was added, continued refluxing for 6 h and reaction was monitored with the help of TLC. [15-16] The slurry was filtered and the solvent was removed under reduced pressure. The solid was collected & re-crystallized from ethanol to yield (M. p. 121-125°C). IR: 3529.49, 1234.36, 1741.69, 1161.07, 3080.90, 1396.37 cm-1. 1H-

NMR: δ 7.79 (d, *J* = 7.5, 1H), 7.52 (t, *J* = 1.5, 1H), 7.06 (d, *J* = 1.5, 1H), 6.94 (dd, *J* = 1.6, 7.5, 1H), 6.66 (t, *J* = 1.4, 1H), 4.92 (s, 2H), 4.70 (s, 1H), 3.78 (s, 3H), 2.15 (s, 2H) (Scheme 1)



Scheme 1



Scheme 2

Starting material= N-phenyl acetamide
Intermediate= methyl 4-oxo-4-(phenylamino) butanoate
R= Amines (Piperidine, imidazole)

Table 2: Antimicrobial activities of the compounds

S. No.	Compound	Zone of Inhibition(in mm)				
		Eschericia coli	*Pseudomonos aeruginosa*	*Bacillus subtilus*	*Staphylococcus aurens*	*Candida albicans*
01	4a	12	15	18	14	15
02	4b	13	16	20	16	18
03	Ofloxacin	25	22	27	24	25

Table 3: Antimicrobial activities of compounds

S. No.	Microorganism	Activity Index		% Activity	
		4a	4b	4a	4b
01	*Escherichia coli*	0.48	0.52	48%	52 %
02	*Pseudomonos aeruginosa*	0.68	0.72	68%	72 %
03	*Bacillus subtilus*	0.66	0.74	66%	74 %
04	*Staphylococcus aureus*	0.58	0.66	58%	66 %
05	*Candida albicans*	0.59	0.70	59%	70 %

Preparation of methyl 4-oxo-4-(Piperidine phenylamino) butanoate

Methyl 4-oxo-4-(Piperidine phenylamino) butanoate) (**3**) (100 mg) and Piperidine (2 ml) were stirred magnetically at 70°C temperature for 3 h. Crushed ice was added to the contents and the solid obtained was crystallized from a mixture of acetone and petroleum ether to obtain the target compound **4a**. (M. P. 128-132°C). IR: 3681.86, 3120.61, 1261.36, 1666.38, 2852.52, 1165.35 cm-1. 1H-NMR: δ 8.11 (dd, *J* = 1.5, 7.5, 1H), 7.89 (dd, *J* = 1.5, 7.5, 1H), 7.50 (td, *J* = 1.5, 7.5, 1H), 7.42 (s, 1H), 7.31 (td, *J* = 1.5, 7.5, 1H), 5.26 (s, 2H), 4.92 (s, 2H), 3.78 (ddt, *J* = 1.0, 2.2, 12.1, 2H), 3.78 (ddt, *J* = 1.0, 2.2, 12.1, 2H), 3.70 – 3.65 (m, 3H), 3.70 – 3.65 (m, 3H), 3.68 – 3.61 (m, 4H), 3.68 – 3.61 (m, 4H), 3.61 – 3.53 (m, 2H), 3.61 – 3.53 (m, 2H), 2.45 (s, 2H). (Scheme 2)

Preparation of methyl 4-oxo-4-(Imidazole phenylamino) butanoate

Methyl 4-oxo-4-(Imidazole phenylamino) butanoate) (**3**) (100 mg) and Imidazole (2 gm) were magnetically stirred at 70°C temperature for 3 h. Crushed ice was added to the contents and the solid obtained was crystallized from a mixture of acetone and petroleum ether to obtain the target compound **4b.** (M. p. 150-155°C). IR: 3616.28, 2856.38, 2997.54, 1276.79, 1662.52, 1155.38, 1365.51 cm-1. 1H-NMR: δ 7.77 (d, *J* = 7.5, 1H), 7.76 (t, *J* = 1.5, 1H), 7.30 (d, *J* = 1.5, 1H), 6.98 (dd, *J* = 1.5, 7.5, 1H), 6.70 (t, *J* = 1.5, 1H), 4.91 (s, 1H), 4.70 (s, 1H), 3.52 (q, *J* = 6.0, 3H), 3.52 (q, *J* = 6.0, 3H), 2.15 (s, 1H), 1.27 (t, *J* = 6.0, 4H), 1.27 (t, *J* = 6.0, 4H). (Scheme 2)

Antimicrobial Activity

In-vitro antimicrobial activity was carried using Muller Hinton agar (Hi-media) paltes at 37°C agar diffusion cup plate methods. [18-20] All the compounds were screened for the antimicrobial actitvity at 1000 µg/ml (1 mg/ml) concentration against the following bacterial stains: *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans*. Ofloxacin dissolved in N, N – dimethylformide (DMF) was taken as the reference standard for comaprision of antimicrobial activity under similar conditions. Tested compounds were dissolved in N, N -dimethylformide (DMF) to get the a solution of 1mg/ml. Inhibition zones were measured in millimeters at the end of incubation period of 24 h at 37°C. Paper discs, (3 mm diameter), were saturated with the dilutions of and placed on the surface of the seeded agar (each disc absorbs approximately 0.08 ml of solution). Two discs saturated with the reference standard were placed on assay plate opposite each other, and other discs of samples were placed in the quadrants. All plates were incubated for 24-48 h at 37°C. The diameter of zone of inhibition of the reference standard discs was measured by the use of millimeter scale. [21-24]

RESULT AND DISCUSSION

The aim of the present research work to find out a new synthetic potential derivative of New N – Phenyl acetamide derivatives with antimicrobial activity. Series of new compounds were synthesized and assessed for antimicrobial activity. The data reported in Table 2 & 3 shows that effect of variation in basic chemical structure of parent compound on antimicrobial activity. Substitution of piperdine and imidazole to the N-Phenyl acetamide leads to the synthesis of 4(a) and 4(b) compounds with potent antimicrobial activity.

CONCLUSION

Screening and evaluation establised that the new compound were Methyl 4-oxo-4-(Piperidine phenylamino)butanoate),**4(a)** and Methyl 4-oxo-4-(Imidazole phenylamino) butanoate **4(b)** showed a potent antimicrobial activity against various Gram positive and Gram negative bacteria. Results show that antimicrobial activity is attributed due to the substituent piperidine and imidazole ring in the parent compound. The data reported in this research article may be beneficial as a reference for the medicinal and pharmaceutical chemist who is doing research in this field.

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