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Synthesis of some Amide derivatives and their Biological activity

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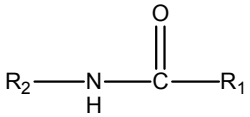
Abstract: A series of amide derivatives were synthesized. The structures of these compounds were established by means of IR, ^{1}H-NMR and Elemental analysis. All the compounds were evaluated for antimicrobial activities. Most of the compounds have shown significant antimicrobial activities when compared with standard drug.
Key words*:* Amide derivatives, amino acid, antimicrobial activity.

INTRODUCTION:

Amide derivatives were associated with broad spectrum of biological activities including antituberculosis[1], anticonvulsant[2], analgesic-antiinflammatory[3], insecticidal[4], antifungal[5], and antitumor[6] properties. Morpholine derivatives find their wide spectrum of antimicrobial activity and exhibit anthelmintic, bactericidal and insecticidal activity[7]. They are also involved as an intermediate product in the synthesis of therapeutic agents. Amide derivatives also show anti-platelet activity[8].

When amides are conjugates with other aliphatic, aromatic and heterocyclic ring produces various type of biological activity.

General structure of amide is given below:



R_1 & R_2 may be

1. H
2. Aliphatic group
3. Aromatic group
4. Heterocyclic group
5. Cyclic group like piperidine, morpholine etc.

Amide derivatives are synthesize very easily by the reaction of substituted acid group (-COOH) with different substituted amines.

A number of aromatic amides of aromatic and heterocyclic acids have been synthesized in search for new antagonists of excitatory amino acids receptors with anticonvulsant activity. Generally, benzylamides were found to be more active than other amides. On the other hand, the most effective appeared amides of acids: picolinic, nicotinic, isonicotinic, nipecotic and isonipecotic. The most effective anticonvulsants came out to be picolinic acid benzylamide (Pic-BZA, PI against MES > 28.0) and nicotinic acid benzylamide (Na-BZA, PI against MES = 4.70).

Some of derivatives of those compounds substituted in both rings were designed, prepared and pharmacologically evaluated. The best were: picolinic acid 2-fluorobenzylamide (Pic-2-F-BZA, PI against MES = 3.40) and nicotinic acid benzylamide Noxide (Nic-O-BZA, PI against MES < 5.6)[9].

MATERIALS AND METHOD:

All the chemicals used during the practical work were obtained from the Merck India (Pvt.) ltd, CDH, Sdfine limited and Himedia. The chemicals and solvent used are of synthetic and AR grade respectively.

The compound synthesized were identified and characterized by following methods such as:

Melting Point Determination: The melting point of the organic compound was determined by Thiele's melting point tube using liquid paraffin by open

capillary method. The melting point of all derivative taken are remains uncorrected.

Thin Layer Chromatography: TLC of the compound was taken by using silica gel G as a spreading agent. The solvent system used was ETHANOL: WATER (7:3).

Infra Red Spectroscopy: All the IR- spectra were carried out from the IIT Delhi. The IR spectrum was recorded using the KBr pellets. The instrument used was PERKIN ELMER.

Nuclear Magnetic Resonance Spectroscopy (^{1}HNMR): The NMR spectra of the compounds were carried out using Bruker Advanced II-400 spectrometer at IIT Delhi. The solvent used was $CDCl_3$ and DMSO.

Elemental Analysis: Elemental Analysis was carried out from the CDRI Lucknow.

EXPERIMENTAL:

There are two steps involved in the synthesis of final product:

Step-1: Esterification of Amino acid

Step-2: Synthesis of amide from substituted aniline

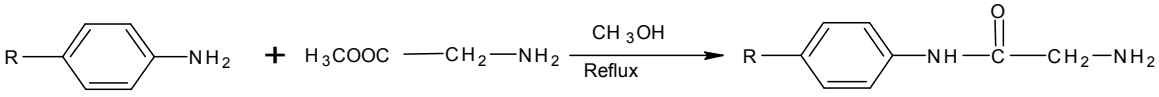
Esterification of Amino acid:

Esterifies amino acid was synthesized by Fischer-Speier method. In this method a mixture of methanol and organic acid was boiled under reflux whilst a steam of dry hydrogen chloride gas is passed, a high yield of the ester being obtained.

The formation of hydrogen chloride is protonating and catalytic, since Fischer found that 5% hydrogen chloride in the reaction mixture gave efficient esterification [10].

Synthesis of amide from substituted aniline:

Synthesis of different amide derivatives from substituted aniline is a one step reaction in which equimolar (0.1 mol) quantity of different substituted aniline with amino acid ester taken in a round bottom flask and dissolved in methanol and then reaction mixture was refluxed for 3 hr. After completion of reaction, solid crystal was obtained. The synthesized compound was analyzed by TLC with using solvent system Ethanol: water (7:3) ratio. Then the solid crystal was recrystallised from ethanol (95%).



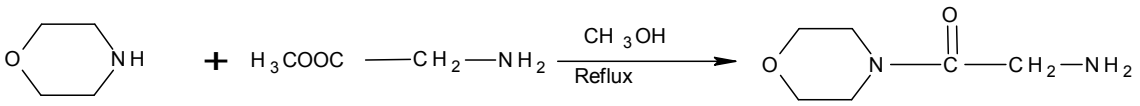
Substituted Aniline Glycine ester Substituted Amide derivative

1 (a-c) **2(a-c)**

R= a: -H

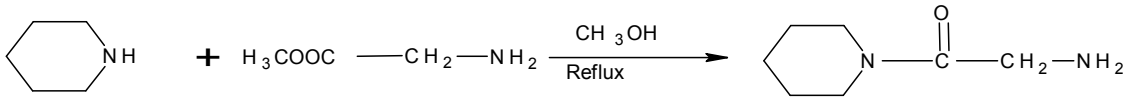
b: -Cl

c: $-NO_2$



Morpholine Glycine ester 2-amino-1-(morpholin-4-yl)ethanone

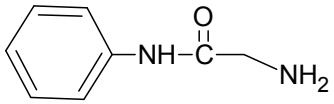
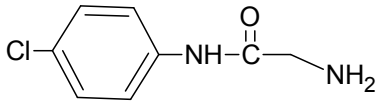
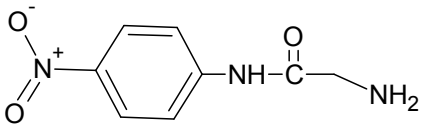
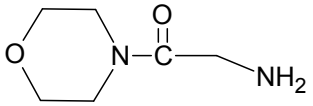
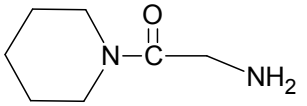
1(d) **2 (d)**



Piperidine Glycine ester 2-amino-1-(piperidin-1-yl)ethanone

1 (e) **2 (e)**

Table 1: Amide derivatives of substituted aniline

Code	Structure (IUPAC)	Mol. Formula & Mol. Wt.	m.p. (^{0}C)	Rf. Value	% Yeild
2a	 2-amino-*N*-phenylacetamide	$C_8H_{10}N_2O$ 150.18	145	0.30	69.07
2b	 2-amino-*N*-(4-clorophenyl)acetamide	$C_8H_9ClN_2O$ 184.62	160	0.41	79.86
2c	 2-amino-*N*-(4-nitrophenyl)acetamide	$C_8H_9N_3O_3$ 195.18	167	0.43	65.09
2d	 2-amino-1-(morpholin-4-yl)ethanone	$C_6H_{12}N_2O_2$ 144.17	130	0.82	70.83
2e	 2-amino-1-(piperidin-1-yl)ethanone	$C_7H_{14}N_2O$ 142.19	210	0.58	68.09

Characterization of compounds by following methods:

a) TLC:

This is used extensively for qualitative analysis, for it is a rapid process and simple apparatus. The adsorbent is usually a layer, about 0.25mm thick, of silica gel with an inactive binder, e.g. calcium sulphate, to increase the strength of the layer. Slurry is uniformly spread on the glass plate. Then TLC plate was activated by drying at 110^0 for 30 minutes; the plates can then be stored in a desiccator.

The mixture to be separated is dissolved in a suitable solvent and spotted at the bottom of the TLC plate with help of thin capillary tube. When the solvent around the spot has evaporated, the plate is placed vertically in a glass developing tank, which contains a small quantity of solvent system. The solvent raises though the adsorbent layer and the components of the mixture ascend at different rates depending on their affinities for the adsorbent.

After evaporating the solvent from the TLC plate the component of the mixture was visualize with the help of visualizing agent, then R_f value was calculated

TLC System for Amide derivative:

Stationary phase: Silica Gel G
Mobile phase: Ethanol: Water (7:3)
Visualizing agent: Ninhydrin solution

b) Column Chromatography:

The synthesized compounds were purified by column chromatography. In this method, the mixture to be separated is dissolved in a suitable solvent and allowed to pass through a tube containing the adsorbent. The component which has greater adsorbing power is adsorbed in the upper part of the column. The initial separation of the various bands can be improved by passing suitable solvent system. The various zones are cut with a knife at boundaries and the substances present in zones extracted with a suitable solvent. This process of recovery of constituents from the chromatogram is known as elution.

Column System for Amide derivative:

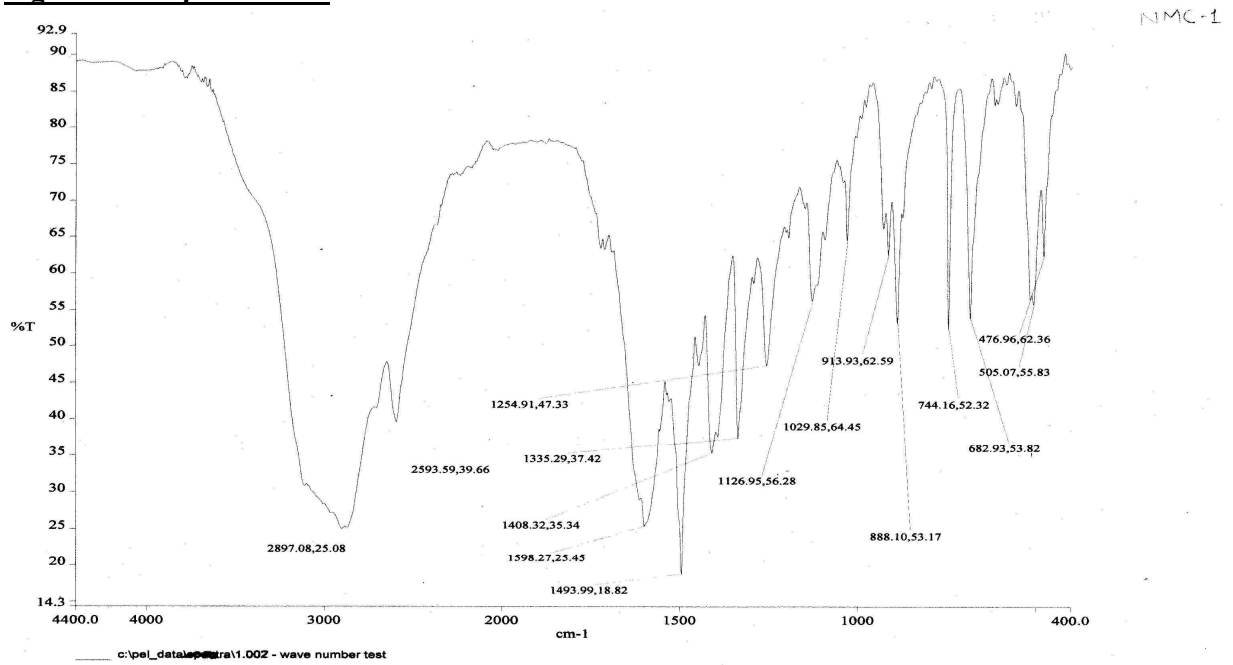
Stationary phase: Activated alumina

Mobile phase: Ethanol: Water (7:3)

Analytical data:

IR-spectra:

Figure-1: IR-spectra of 2a

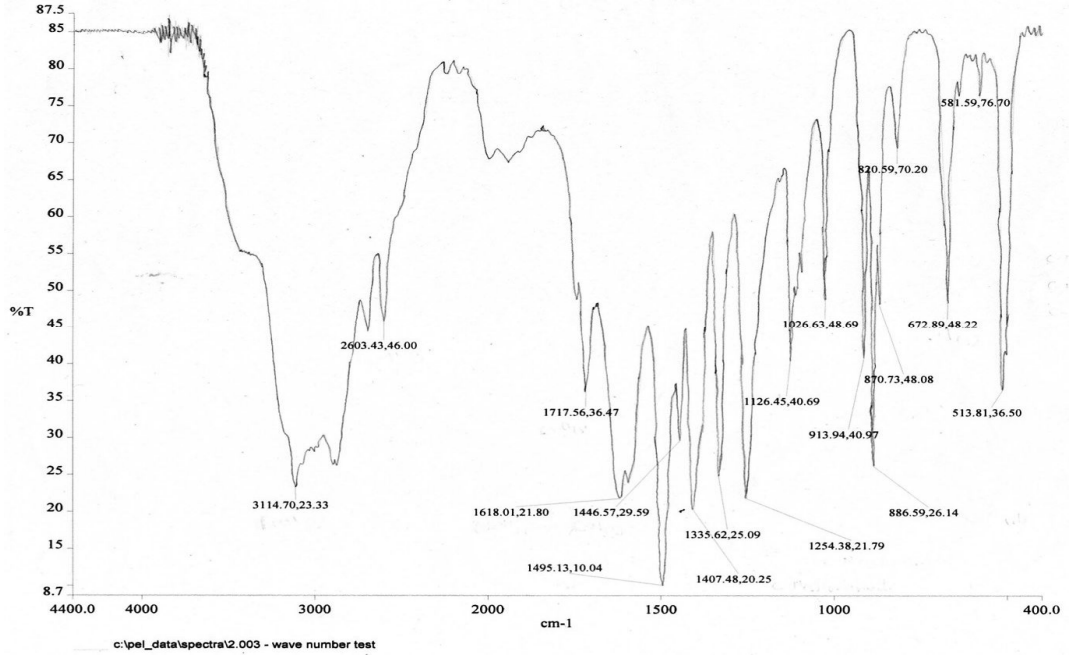


$N\text{-}H_{(d)}$ = 682.93 $C\text{-}H_{(d)}$ = 744.16 $C\text{-}C_{(s)}$ = 888.10

$C\text{-}N_{(s)}$ = 1493.99 $C{=}O_{(s)}$ = 1598.27 $C{=}C_{(s)}$ = 1408.32

$C\text{-}H_{(d)}$ = 1335.29 $C\text{-}H_{(s)}$ = 2593.59 Ar $C\text{-}H_{(s)}$ = 2897.08

Figure-2: IR-spectra of 2b



$C\text{-}Cl_{(s)}$ = 672.89 $C\text{-}H_{(d)}$ = 886.59 $C\text{-}N_{(s)}$ for amine = 1254.38

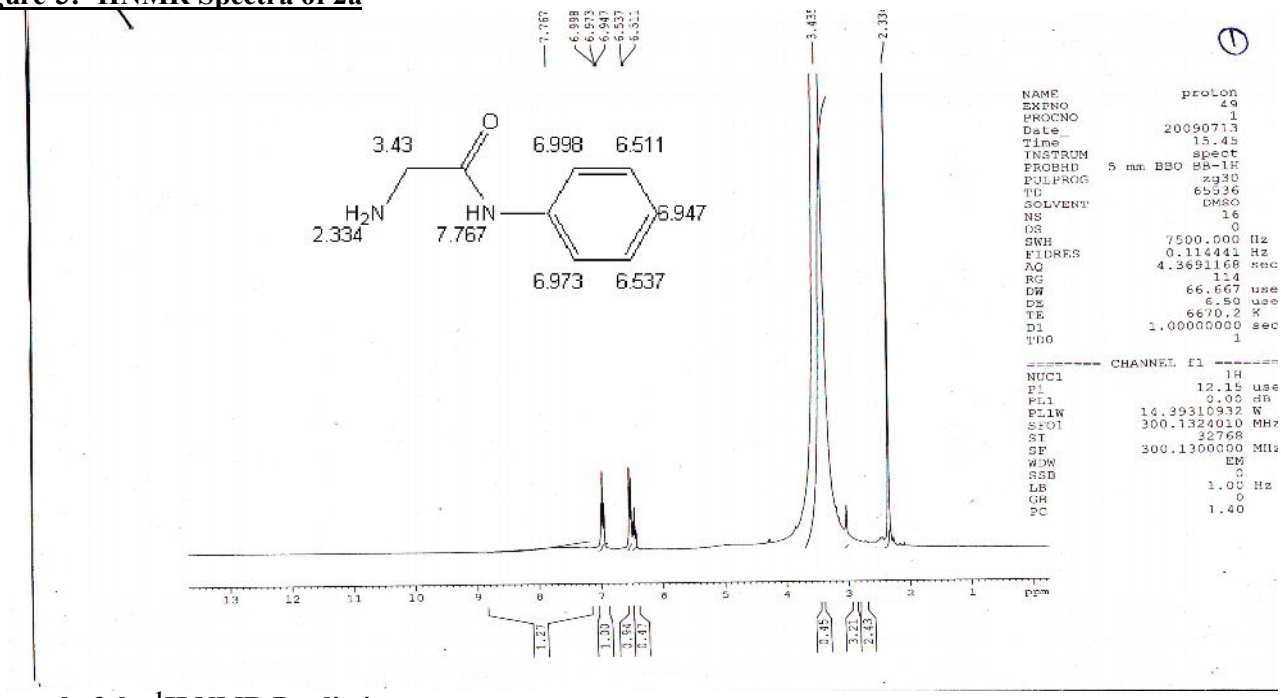
$C\text{-}C_{(s)}$ = 1335.62 $C\text{-}N_{(s)}$ for amide = 1407.48 $C{=}C_{(s)}$ = 1495.13

$C\text{-}N_{(s)}$ = 1446.57 $N\text{-}H_{(d)}$ = 1618.01 $C{=}O_{(s)}$ = 1717.56

$C\text{-}H_{(s)}$ = 2603.43 Ar-$H_{(s)}$ = 3114.70

[1]HNMR Spectra:

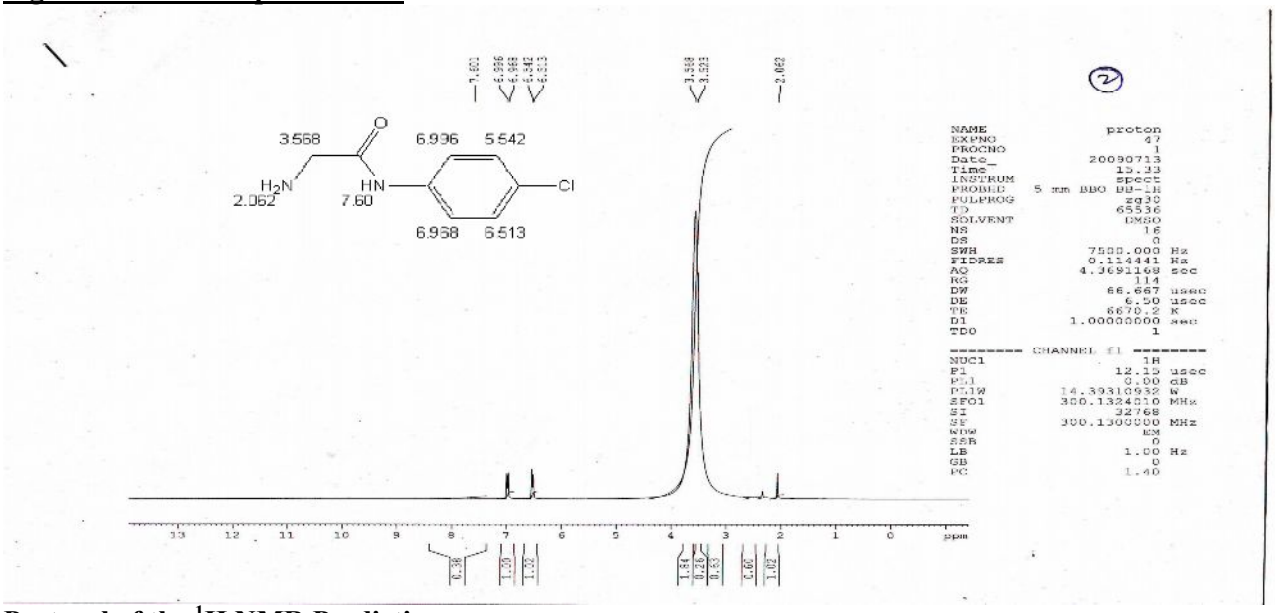
Figure-3: [1]HNMR Spectra of 2a



Protocol of the [1]H NMR Prediction:

Node	**Shift**	**Comment** (ppm rel. to TMS)
CH_2	3.43	methylene, 2H, Triplet
NH	7.76	sec. amide, 1H, Singlet
CH	6.998	1-benzene, 1H (ortho), Multiplet
CH	6.973	1-benzene, 1H (ortho), Multiplet
CH	6.947	1-benzene, 1H (para), Triplet
CH	6.537	1-benzene, 1H (meta), Multiplet
CH	6.511	1-benzene, 1H (meta), Multiplet
NH_2	2.334	amine, 2H, Triplet

Figure-4: [1]HNMR Spectra of 2b



Protocol of the [1]H NMR Prediction:

Node	**Shift**	**Comment** (ppm rel. to TMS)
CH_2	3.568	Methylene, 2H, Triplet

NH	7.601	sec. amide, 1H, Singlet
CH	6.996	1- benzene, 1H (ortho to amide group), Doublet
CH	6.968	1- benzene, 1H (ortho to amide group), Doublet
CH	6.542	1- benzene, 1H (ortho to chloro group), Doublet
CH	6.513	1- benzene, 1H (ortho to chloro group), Doublet
NH_2	2.062	amine, 2H, Triplet

Elemental Analysis:

Table-2:

Element (%) / Code	C	N	H	S	O	Cl
2b	37.99	13.23	7.94	Nil	8.67	17.23

Table-3: Anti-microbial activity:
Zone of inhibition in mm

Compound code	*Bacillus substilis*	*Escherichia coli*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Corynebacterium diphtheriae*	*Bacillus megaterium*
2a	6.5 mm	No	No	No	6.5 mm	No
2b	7.5 mm	7.0 mm	6.5 mm	7.5 mm	No	6.5 mm
2c	8.0 mm	8.5 mm	7.0 mm	6.5 mm	7.5 mm	8.0 mm
2d	6.5mm	7.5mm	7.0mm	7.5mm	No	6.5mm
2e	8.5mm	7.5mm	6.5mm	8.5mm	7.5mm	No
Ampicillin	25.0 mm	14.0 mm	26.0 mm	21.0 mm	25.0 mm	22.0 mm
DMSO	No	No	No	No	No	No

ANTIMICROBIAL STUDIES OF THE COMPOUNDS:
The synthesized compounds have to be screened for following activity.
Paper-disc method
Whatmann filter paper disc = 6.0 mm diameter
Concentration of Ampicillin (standard drug) = 250 µg/ml,
Concentration of sample = 1.0 mg/ml,
Sample injected = 3 µl on each disc
Paper-discs with a diameter of 6.0 mm are impregnated with the antimicrobial solution and placed on the culture medium. Antimicrobial can also be applied to the disc after it has been placed on the medium. Plates containing a single layer of medium with 2 mm thickness may be used for these tests. Then inhibition zone was noted.

RESULTS AND DISCUSSION:
Amide derivatives were prepared according to method reported in the synthetic scheme. These compounds are synthesized by the reaction between different substituted aryl anilines and ester of amino acid. These amide derivatives were then characterized by the elemental analysis, IR spectral studies and ^{1}H-NMR studies. The entire synthesized compounds were subjected to antimicrobial activity.

General IR spectra studies of compounds:
C-$Cl_{(s)}$ (672.89 cm^{-1}), C-H $_{(d)}$ (886.59 cm^{-1}), C-$N_{(s)}$ for amine (1254.38 cm^{-1}), C-$C_{(s)}$ (1335.62 cm^{-1}), C-$N_{(s)}$ for amide (1407.48 cm^{-1}), C=$C_{(s)}$ (1495.13 cm^{-1}), C-$N_{(s)}$ (1446.57 cm^{-1}), N-H $_{(d)}$ (1618.01 cm^{-1}), C=$O_{(s)}$ (1717.56 cm^{-1}), C-$H_{(s)}$ (2603.43 cm^{-1}), Ar-$H_{(s)}$ (3114.70 cm^{-1}), N=$O_{(s)}$(1327.18 cm^{-1}), C-N(s) for NO_2(842.16 cm^{-1}).

General ^{1}H-NMR studies of compounds: (Solvent $CDCl_3$ + DMSO)
CH_2 (δ=3.568 ppm, 2H, Triplet), NH (δ=7.601 ppm, sec. amide, 1H, Singlet), CH (δ=6.996 ppm,1H, *o*-Ar-H to amide group, Doublet), CH (δ=6.968 ppm, 1H, *o*-Ar-H to amide group, Doublet), CH (δ=6.542 ppm, 1H, *o*-Cl-Ar-H , Doublet), CH (δ=6.513 ppm, 1H, *o*-Cl-Ar-H , Doublet), NH_2 (δ=2.062 ppm, amine, 2H, Triplet).

Elemental Analysis:
Compound **2a** has (C 37.99%, N 13.23%, H 7.94%, S Nil, O 8.67%, Cl 17.23%).

All the newly synthesized compounds were initially screened for their *in vitro* antimicrobial activities against the Gram-positive (*S. aureus, C. diphtheriae*) and the Gram-negative (*E. coli* and *P. aeruginosa*), *Bacillus substilis* and *Bacillus megaterium* bacteria by disc diffusion. The inhibitory effect of these compounds against these micro-organisms is given in table 3.

The screening results indicate that some of the compounds exhibit the antimicrobial activity. Compounds **2a**, **2b**, **2c**, **2d** & **2e** showed significant activity against strains used.

ACKNOWLEDGEMENT:
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