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Synthesis and Biological Evaluation of a Mutual Prodrug of Mafenide and Nalidixic Acid by Amino Acid Spacer

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Abstract: ***The aim of this study has been to synthesize a useful drug, which may act with effectiveness both on the gram-positive and gram-negative bacteria (broad-spectrum). An amide-based mutual prodrugs were synthesized by coupling Mafenideacetate withNalidixic acid by aminoacid spacer (glycine or phenylalanine), and evaluated for in-vitro antibacterial activity with significant results.***

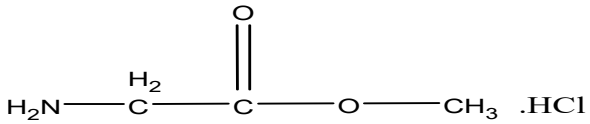
Keywords: Nalidixicacid, Mafenide acetate , Prodrug, Antibacterial, glycine and phenylalanine**.**

1. Introduction

The incidence of bacterial infection is increasing dramatically due to different factors including an increase in the number of immuno-compromised hosts (1). The increasing incidence of resistance to a large number of antibacterial agents is becoming major concern (2). These observations clearly indicate the need of as well as search for new and more effective antimicrobial agents with a broad spectrum of activity (3). Nalidixic acid is effective against infections with gram-negative bacteria, but it is less effective against most of the gram-positive bacteria (4) whereas Mafenide acetateis a broad-spectrum antibacterial agent effective against *Escherichia coli, Klebsiella species, Streptococcus species, and Staphylococcusaureus*(5). A prodrug is defined as a biologicallyinactive derivative of a drug candidate that requires a chemical or enzymatic transformation within the body to release the active drug, and has improved delivery properties over the parent molecule. Generally, in a prodrug, the carrier group or promoiety used is inert or non-toxic (6, 7). However, in certain cases the prodrug consists of two pharmacologically active agents coupled together or by spacer in the form of a single molecule. Such derivatives have been termed as mutual prodrugs (8, 9). It was considered worthwhile to synthesize a mutual prodrug of Nalidixic acid with Mafenide acetate by aminoacid spacer , with an objective of getting a compound which may act with effectiveness both on the gram-positive and gram-negative bacteria .In addition, amino acids as spacer have advantage in healing processes of burn in which Mafenide acetate is widely used.

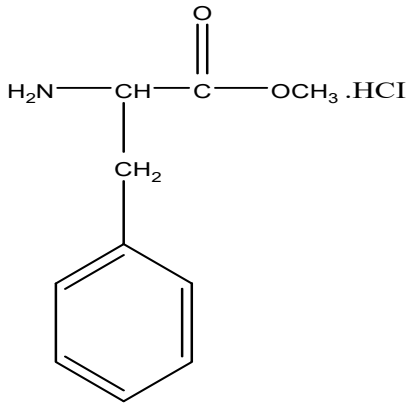
2. Experimental

Synthesis of amino acetic acid methyl ester. HCl IA(10)



A suspension of L-Glycine (20mmol, 1.5g) in (25ml) of absolute methanol, was cooled down to -15°C then thionyl chloride was added drop wise (20mmol, 1.51ml), (the temperature should be keep below 10°C), the reaction mixture was left at 40°C for 3hr, then refluxed for 3hr and left at room temperature overnight, the excess solvent was evaporated to dryness under vacuum, re-dissolved in methanol and evaporated, this process was repeated several times and re-crystallize the product from methanol-diethyl ether (3:1).The percent yield and physical data were given in table -1.

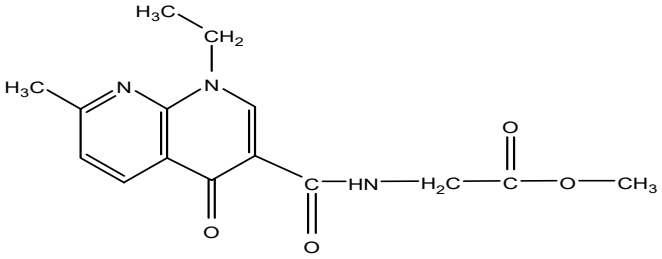
Synthesis of 2-amino- 3- phenyl propionoic acid methyl IIA(10).



A suspension of Phenylalanine (9mmol, 1.5g) dissolved in (15ml) of absolute methanol and (9mmol, 0.68ml) of thionyl chloride, then complete the procedure as mentioned in the synthesis of. IA

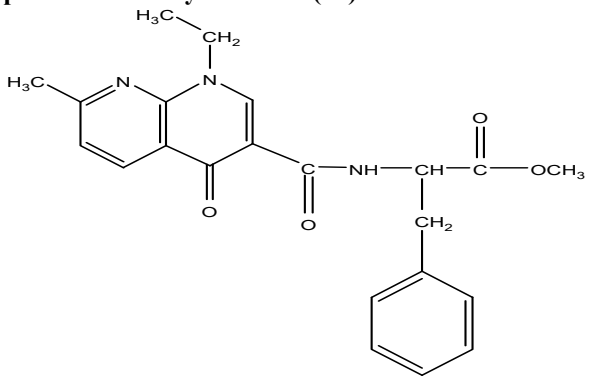
The percent yield and physical datawere given in table-1.

Synthesis of [(1-Ethyl-7-methyl-4-oxo-1,4-dihydro-[1,8]naphyridin-3-ylmethyl)-amino]-acetic acid methyl ester IB(11)



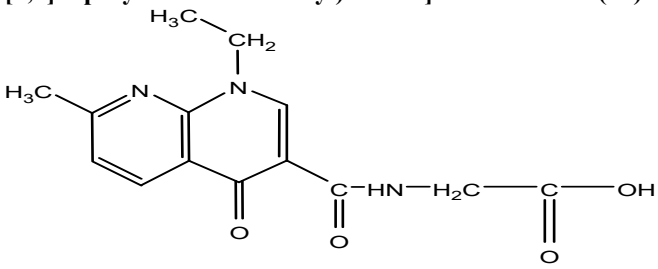
Ethylchloroformate (0.007mole,0.55ml)was added drop wise to an ice cooled stirred suspension.Nalidixic acid (0.005mole,1.16gm)and triethylamine (0.007mole,1ml)in dry chloroform (20ml),stirring was continued for 30 min at 5-10 0C. The compound IA (0.005mole,0.62gm) was then added together with an equivalent amount of triethylamine and the mixture was stirred overnight at ambient temperature. The solvent was evaporated to dryness under reduced pressure and the residue was washed with (10ml)of 5%sodium bicarbonate and then with water to exclude un reacted material and then recrystallized from ethanol The percent yield and physical data were given in table -1.

Synthesis of 2[(1-Ethyl-7-methyl-4-oxo-1,4-dihydro-[1,8]naphthyridine-3-carbonyl)-amino]-3-phenyl-propionicacid methyl ester IIB(11)



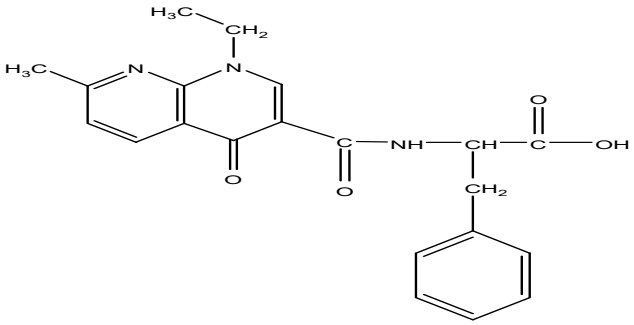
Ethylchloroformate (0.014mole,1.1ml)was added drop wise to anice cooled stirred suspension Nalidixic acid (0.01mole,2.32gm)and triethylamine (0.014mole,2ml) in dry chloroform (20ml).Stirring was continued for 30 min at 5-10. The compound IIA (0.01mole,2.157gm) was then added together with an equivalent amount of triethylamine , then complete the procedure as mentioned in the synthesis ofIB. The percent yield and physical data were given in table-1.

Synthesisof[(1-Ethyl-7-methyl-4-oxo-1,4-dihydro-[1,8]napthyridine-3-carbonyl)-amino]-acetic acid IC(12)



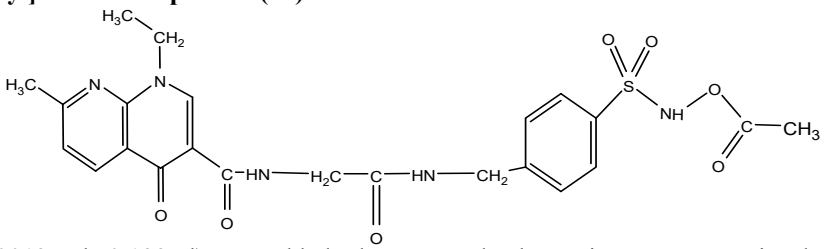
Compound IB (0.0029mole, 0.89gm), was dissolved in minimum volume of dioxane: water (5:1) mixture and the solution was cooled to 18 0 C. Then NaOH in 10ml of water (0.005mole, 0,2gm) was added drop wise, with continuous stirring over a period of 30 min. Stirring was continued at 18 0 C for additional six hours. The reaction mixture was acidified with HCl (0.005mole, 0.5ml), then excess of cold water was added. The precipitated compound was filtered, dried and crystallized from methanol: chloroform (9:1).The percent yield and physical data were given in table -1.

Synthesis of 2-(1-Ethyl-7-methyl-4-oxo-1,4-dihydro-[1,8]naphthyridin-3-ylamino)-3-phenyl-propionic acid IIC(12)



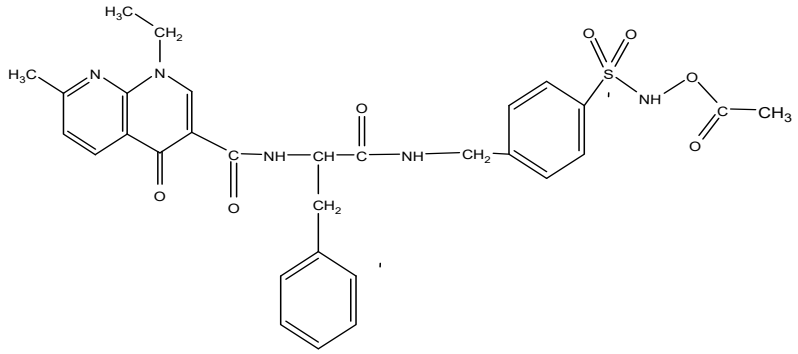
Compound IIB (0.00688mole, 2.82gm) was dissolved in minimum volume of dioxane: water (5:1) mixture and the solution was cooled to 18o C. Then NaOH in 10mlof water (0.0117mole, 0.47gm) was added drop wise, with continuous stirring over a period of 30 min., and then complete the procedure as mentioned in the synthesis of IC.The percent yield and physical data were given in table -1.

Synthesis1-Ethyl-7-methyl-4-oxo-1,4-dihydro-[1,8]naphthyridine-3-carboxylicacid[(4-acetoxysulfamoyl-benzylcarbamoyl)-methyl]-amide compound I(11)



Ethylchloroformate (0.0013mole,0.123ml)was added drop wise to an ice cooled stirred compound IC(0.0013mole,0.5gm)and triethylamine (0.0013mole,0.18ml) in dry DMF (20ml).Stirring was continued for 30 min at 5-10 0C .The Mafenide acetate(0.0013mole,0.32gm)was then added together with an equivalent amount of triethylamine and the mixture was stirred overnight at ambient temperature .The solvent was evaporated to dryness under reduced pressure and the residue was washed with (10ml) of 5%sodium bicarbonate and then with water dried and then recrystallized from absolute ethanol.The percent yield and physical data, were given in table -1.

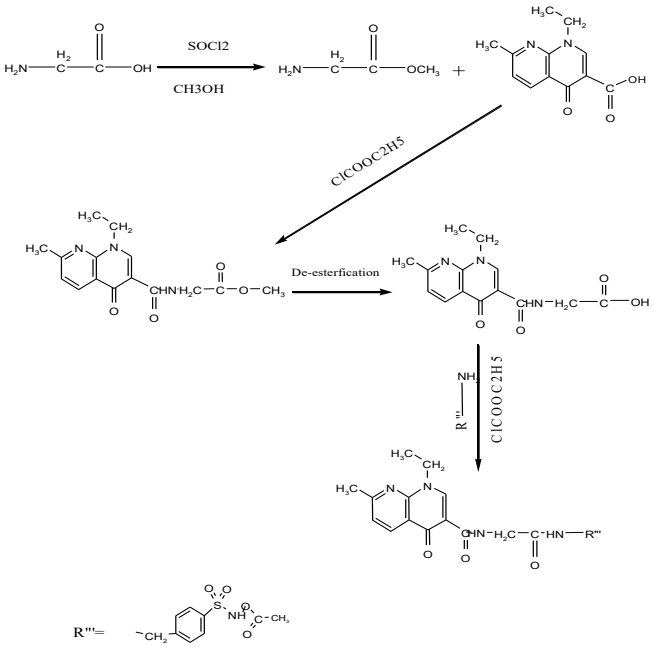
Synthesis of 1-Ethyl-7-methyl-4-oxo-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid (2-oxo-ethyl)-amide compound 2(11)



Ethylchloroformate (0.001mole0.133ml,)was added drop wise to an ice cooled stirred compound IIC (0.001mole,0.3)and triethylamine (0.001mole,0.19ml) in dry DMF (20ml).Stirring was continued for 1hr min at 5-100 C.Mafenide acetate (0.001mole,0.25gm)was then added together with an equivalent amount of triethylaminethen completed the procedure as mentioned in the synthesis of compound I.The percent yield and physical data were given in table -1.

3. Results and Discussion

Synthesis: Nalidixic acid was linked with Mafenideacetate through amino acids spacer (glycine or phenylalanine) by using ethylchloroformate in presence of triethylamine (**Scheme 1**).



Usual work up of the reaction mixture followed by washing with sodium bicarbonate5%, followed by water to exclude un reacted material dried and re-crystallization with absolute ethanol furnished the desired compound. Physical characterization and analytical data of mutual prodrug are list in table -1.

Table 1: Physical characterization and analytical data of mutual prodrug

Compound	M.Wt	M.P	Color	Yield	C%	H%	N%	S%
Mafenide-glycine-Nalidixic acid	515.54	260-262	Pale yellow Powder	73%	54.79 53.58	4.956 4.89	13.98 13.58	6.31 6.22
Mafenide-phenylalanine-Nalidixic acid	665.29	195-197	Off white powder	75%	61.81 61.33	6.72 6.51	10.67 10.52	4.91 4.82

Table 2: Infrared spectroscopy characterization

Compound	N-HStretching	Aromatic C-H stretching	C-H stretching of CH_3,CH_2	C=O of amide	Aromatic C=C stretching
Mafenide-glycine-Nalidixic acid	3278	3072	2981,2931	1649	1603,1493
Mafenide-phenylalanine-Nalidixic acid	3304,3289	3071	2934	1659	1603,1576

In-vitro antibacterial activity

The inhibition zones of the two concentrations of the final synthesized compounds were investigated in comparison with Mafenide acetate and Nalidixic acid which were used as a reference antibacterial activity against gram-positive bacteria (*Staphylococcus aureus* and *Stresptococcusspp*) and gram-negative bacteria (*Escherichia coli* and*Klebsiella pneumonia*). Antibacterial activities of each compound were evaluated by well diffusion method using Mueller–Hinton agar as culture media. The synthesized compounds

were dissolved in dimethylsulfoxide to prepare the stock solution (10mg/10ml) and the solution was diluted with dimethylsulfoxideto obtain the required concentrations of125 and 250 µg/ml. The petri dishes were inoculated with (30 µl) separately of each concentration of the synthesized compounds for each well and incubated at 37 °C for 24 h. To ensure that the solvent had no effect on the bacterial growth, a control was performed with the test medium supplemented with DMSO at the same dilutions as used with the tested compounds. At the end of the period the inhibition zones formed on media of the standards and synthesized compounds were measured with a zone reader in millimeters (13, 14).The inhibition zone values are summarized in table -3.

Table 3: Antibacterial activity of mutual prodrug

Compound	*Escherichia coli*	*Klebsiella pneumonia*	*Staphylococcus aureus*	*Stresptococcus spp*	Conc. (µg/ml)
Mafenide-glycine-Nalidixic acid	19 22	19 13	20 22	20 25	125 250
Mafenide-phenylalanine-Nalidixic acid	Nil Nil	18 Nil	Nil 15	16 19	125 250
Mafenide	9 13	9 10	10 15	18 20	125 250
Nalidixic acid	10 12	9 Nil	Nil Nil	Nil 9	125 250

4. Conclusion

Nalidixic acid and Mafenide acetate were successfully coupling together by amino acids through an amide-linkage to get a new mutual prodrug. In-*vitro* antibacterial activity of the compound against some selected bacteria showed significant antibacterial activities especially when Nalidixic acids and Mafenide acetate are coupled by glycine. The present work sheds the light on the pharmaceutical potential of mutual prodrugs comprising of classical agents.

5. Acknowledgements

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