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SYNTHESIS, CHARACTERIZATION AND BIOCHEMICAL STUDIES OF SOME NOVEL GLUTAMIC ACID DERIVATIVES OF RNA & DNA BASES

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ABSTRACT

Amino acid derivatives of RNA & DNA bases constituent an important class of heterocyclic compounds. These organic syntheses organized in solvent phase with the help of microwave irradiation. Nucleobases having considerable heterocyclic and amino acids are also having high physiological activity. With this multi-valued objective, the newly synthesized compound highly intensified. Many of them observed antifungal, antibacterial, anti-cancer, anti-inflammatory activities. The structures of newly synthesized compounds have been determined with the help of elemental analysis like IR, ^{13}C NMR, and Mass spectral data.

Keywords:RNA & DNA base, Glutamic Acid, Antibacterial activity, Antifungal activity.

1. Introduction

In human body, proteins are very important and very big complex molecules. From which type of units is made up, it is called amino acid. In heterocyclic compound, amino acids and its derivatives of RNA & DNA bases are very important. It gives many biological activities like antimicrobial (antibacterial & antifungal), anti-inflammatory, anticancer. It supports the central nervous system and act as a

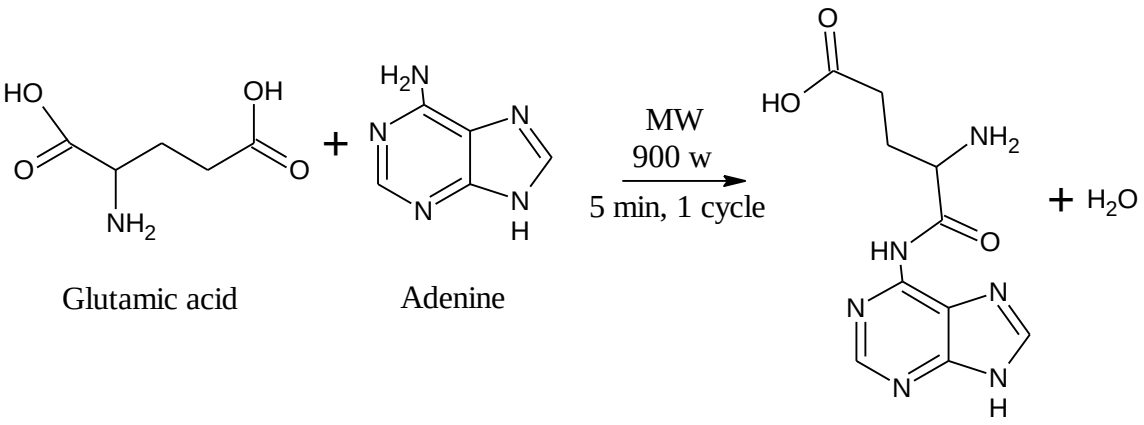
neurotransmitter and play a very important role in the treatment of depression, schizophrenia, anxiety and mood connected problems and also gives protection from heart attacks.Necleobase are also having very much biological importance. So it attract to synthesized Glutamic Acid derivatives of RNA & DNA base.

2. Materials and Method

The derivatives of amino acid have been made by reacting with different RNA & DNA base. All the chemicals are pure and granted so we used without further purification. We used Glutamic Acid, Adenine, Guanine, Thymine, Uracil, Ethanol, and Distilledwater.

General procedure for synthesis of various RNA &DNA base& amino acid derivative Glutamic Acid and RNA & DNA base were weighed equally in respect to the moles. The properly weighed compounds were thoroughly mixed well using distilled water. Then mixture is transferred into RBF (250 ml). Put the RBF into microwave oven. Set the microwave at full microwave radiation (900 W) as per reaction time and start the microwave oven. After the completion of reaction the RBF is taken from the oven very carefully. Then the reaction mixture is transferred into evaporating dish and evaporates the mixture and collects the dry product.Recrystallized from hot water. When we were used guanine, the reaction is take place in ethanol on behalf of water.

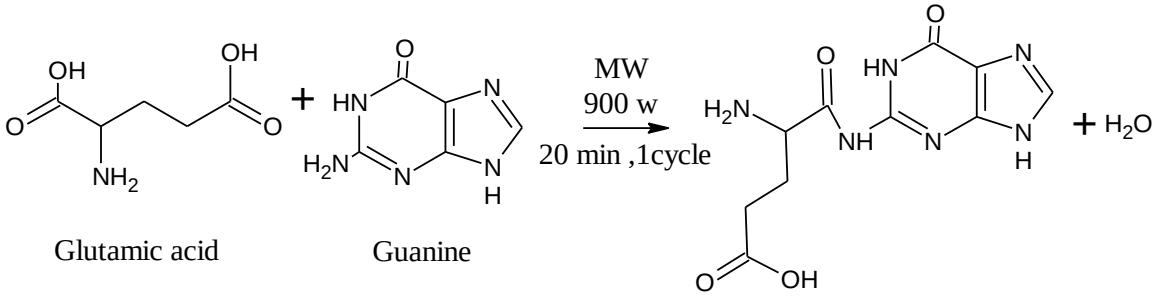
(1)Product M_4A (Glutamic Acid + Adenine)



Glutamic acid Adenine

M_4A

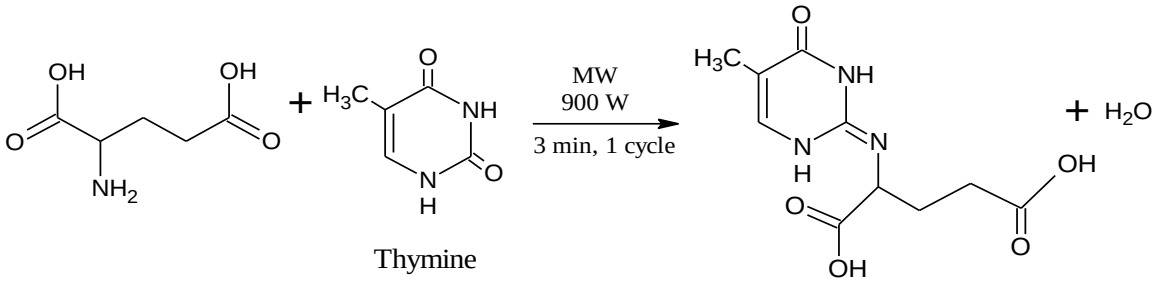
(2) Product M_4C (Glutamic Acid + Guanine)



Glutamic acid Guanine

M_4C

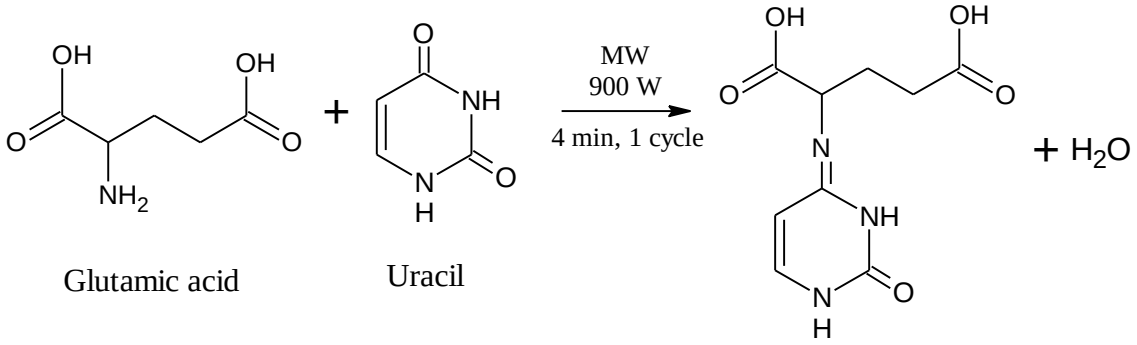
(3) Product M_4D (Glutamic Acid + Thymine)



Thymine

M_4D

(4) **Product M_4E (Glutamic Acid + Uracil)**



Glutamic acid Uracil

M_4E

3. Spectra Characterization

(1)Compound M_4A:

Infrared Spectra Feature (cm^{-1}):

1594.45: -NH_2 bend

1149.05, 1173.50, 1212.76, 1256.37: -C-N Stretch

1669.37:- C=N Stretch

3049.11:- OH (Carboxylic Group)

1750.58:- C=O Stretch (Ketone)

1558.71:-C=C Aromatic Stretch

701.19, 778.91: -C-H Aromatic out of plane bends

1413.59, 1450.25:-CH_3,CH_2

^{13}C spectral Feature (ppm):

40.09, 39.88, 39.60, 39.47, 39.26, 39.05, 38.84:- R_2-CH_2, R_3-CH, C-N

155.65:- C=O

152.36:-C=C

Mass spectral Features (ppm):

112.1:- Peak is observed due to $C_5H_7NO_2$. This is Glutamic Acid peak

69.1:-Base peak is observed due to C_4H_8N. This is Glutamic Acid peak

(2) Compound M_4C:

Infrared Spectra Features (cm^{-1}):

1558.71, 1508.21: -NH_2 bend

[11149.05, 1173.50, 1212.76, 1256.37]:C-N Stretch

1670.46, 1697.25:- C=N Stretch

3115.27, 3315.58:- OH(Carboxylic Group)

1770.69:-C=O Stretch (Ketone)

1558.71:-C=C Aromatic Stretch

701.19, 778.91:-C-H Aromatic out of plane bends

1370.37, 1416.45, 1458.26,1473.85: - CH_3, CH_2

^{13}C spectral Features (ppm):

[40.09,39.88, 39.68, 39.47, 39.26, 39.05, 38.84]:- R_2-CH_2, R_3-CH, - C-N

Mass spectral Features (ppm):

135.1:-Peak is observed due to $C_5H_3N_4O$.This is Guanine peak.

112.1:- Base peak is observed due to $C_5H_7NO_2$.This is Glutamic acid Peak

69.1:- Base peak is observed due to C_4H_8N.This is Glutamic acid Peak

(3) Compound M_4D:

Infrared Spectra Features (cm^{-1}):

1506.46, 1556.11: -NHbends

2760.65, 3030.73: -OH (Carboxylic Group)

1748:-C=O Stretch (Ketone)

1589.58:-C=C Aromatic Stretch

709.82, 756.77, 805.77, 862.62, 941.59: C-H Aromatic out of plane bends

1350.92, 1416.22:- CH_3,CH_2

[1050.60, 1086.46, 1125.22, 1150.31, 1206.30, 1350.92]:- C-N Stretch

1642.74, 1668.37: -C=N Stretch

^{13}C spectral Features (ppm):

11.17:- R-CH_3

40.07, 39.07, 39.66, 39.05, 39.24, 38.03, 38.02:- R_2-CH_2, R_3-CH, C-N

164.89:-R-COOH, C=O

137.69, 107.65:-C=C

151.46:- C in Aromatic ring

Mass spectral Features (ppm):

197.2:- Peak is observed due to $C_8H_{10}N_3O_3$

126.1:-Peak is observed due to $C_5H_5N_3O$. This is Thymine peak

69.1:-Base peak is observed due to $C_4H_8N_3$. This is Glutamic Acid peak

112.1:- Peak is observed due to $C_5H_6N_2O$.

(4) Compound M_4E:

Infrared Spectra Features (cm^{-1}):

1558.33: -NH_2 bend

3649.13:-OH(Carboxylic Group)

1746:-C=O Stretch (Ketone)

1506.66:-C=C Aromatic Stretch

711.86, 806.99, 846.74, 910.87, 944.49:C-H Aromatic out of plane bend

1418.99, 1457.45:- CH_3, CH_2

[1051.73, 1083.24, 1149.71, 1256.95, 1312.62]:- C-N Stretch

1650.53, 1683.38:- C=N Stretch

^{13}C spectral Features (ppm):

40.04, 39.83, 39.62, 39.42, 39.21, 38.00, 38.79:- R_2-CH_2, R_3-CH, C-N

164.32:-R-COOH,C=O

100.18:- C=C

151.49, 147.17:- C in Aromatic ring

Mass spectral Features (ppm):

149.1:- Peak is observed due to $C_6H_5N_3O_2$.

112.1:-Base peak is observed due to$C_4H_4N_3O$. This is Uracil peak

84.1:-Peak is observed due to C_4H_5NO. This is Glutamic Acid peak

Table 1: Various Derivatives of Glutamic acid

Sr. No	Compound Name	M.P	Nitrogen Rule	Rule Of 13		Compound Formula	Base Formula C_nH_{n+r}	Unsaturation Index (U)
				n	r			
1	M_4A	182°C	Yes	20	4	$C_{10}H_{12}N_6O_3$	$C_{20}H_{24}$	15
2	M_4C	210°C	Yes	21	7	$C_{10}H_{12}N_6O_4$	$C_{21}H_{28}$	15
3	M_4D	218°C	Yes	19	8	$C_{10}H_{13}N_3O_5$	$C_{19}H_{27}$	13
4	M_4E	290°C	Yes	26	3	$C_9H_{11}N_3O_5$	$C_{26}H_{29}$	19

4. Antimicrobial Activity

In antimicrobial activity, antibacterial activity is pulls down the growth of bacteria and gives protection against bacterial infection. Many drugs like antibiotics and other chemicals performed antibacterial activity. The radical of antibiotics is diffused by the agar, when we added antibiotics in agar cup.If the organism is having minimum concentration, we observed clear zone of retardation. If the zone is large then the MIC is vast. The size of zone is depends on the concentration of compound.

In conical flask culture is melted and cooled at 50 ^{0}C. Add nutrient agar and put for solidify. In sterile agar plate add different antibiotics. Put plate in in the incubate at 37 ^{0}C for 24 hours. After 24 hours zone is observed in the plate. Largest zones are considered the smallest minimum inhibitory

concentration (MIC) of antibiotic for that bacteria or fungi.

In antifungal activity different pathogenic organisms are used. The agar cup method was used for antifungal activity. This activity was performed sameas antibacterial activity.

We have used the **Broth Dilution Method** to evaluate the antibacterial activity.

The main advantage of the **'Broth Dilution Method'** for MIC determination lies in the fact that it can readily be converted to determine the MIC as well.

1. Serial dilutions were prepared in primary and secondary screening.
2. The control tube containing no antibiotic is immediately sub cultured [before inoculation] by spreading a loopful evenly over a quarter of plate of medium suitable for the growth of the test organism and put for incubation at 37 ^{0}C OVERNIGHT. The tubes are then incubated overnight.
3. In antimicrobial the MIC is lowest concentration (mg/L) that inhibits the growth of the microorganisms.
4. In the ICU, infection is caused by the pathogens with higher MIC.
5. In critically ill patients, regular MIC is required to decrease with the using antibiotics.

Table 2: Antibacterial Activity of Standarddrug

Bacteria	**Zone inhibition**				
	Gentamycin	**Ampicillin**	**Chloramphenlcol**	**Ciprofloxacin**	**Norfloxacin**
E coli	0.05	100	50	25	10
P.Areuginosa	1	0	50	25	10
S.Aureus	0.25	250	50	50	10
S.Pyogenus	0.5	100	50	50	10

Table 3: Activity Bacterial of Compounds

Bacteria	**Zone inhibition**		
	M_4A	**M_4D**	**M_4E**
E coli	500	100	100
P.Areuginosa	50	62.5	200
S.Aureus	25	250	500
S.Pyogenus	62.5	250	500

Table 4: Antifungal Activity of Standarddrug

Fungi	**Zone inhibition**	
	Nystatin	Greseofulvin
C.Albicans	100	500
A.Niger	100	100
A.Clavatus	100	100

Table 5: Antifungal Activity of Compounds

Fungi	**Zone inhibition**		
	M_4A	M_4D	M_4E
C.Albicans	>1000	>1000	>1000
A.Niger	>1000	200	200
A.Clavatus	>1000	200	500

Result and Discussion

According to observation Table3 Sample contain MIC range 0.001 ml to 0.005 ml constitute 0.01 mg in 10 ml solvent. The activity of M_4A, M_4D, and M_4E extract is observed between 25 mm to 500 mm. against respective strain. At each strain lowest MIC activity observed in standard drugs minimum 0.05mm andmaximum 250 mm. This activity indicate zone

of inhibition against various bacterial strain such as *E.coli, P.Areusinasa, S.Aureus and S.Pyagenls* of same dilution. The activity of standard drug was given in Table 2.

Antibacterial activity of compound M_4A, M_4D, and M_4E are excellent as compare to the standard drug at same concentration.

According to observation Table 5 Sample contain MIC range 0.001 ml to 0.005 ml constitute 0.01 mg in 10 ml solvent. The activity of M_4A, M_4D, and M4E extract is observed between 125 mm to 250 mm. against respective strain. At each strain lowest MIC activity observed in standard drugs minimum 100 mm andmaximum 500 mm. This activity indicate zone of inhibition against various fungal strain such as *C.Albicans, A.Niger, A.Clavatus* of same dilution. The activity of standard drug was given in Table 4.

Antifungal activity of compoundsM_4A, M_4D, and M_4E are excellent as compare to the standard drug at same concentration.

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