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# Preparation, chemical and biological study of acyclovir analogue starting from 6-aminouracil

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**ABSTRACT**

6-aminouracil[14], dibenzosulphonyl ethyl amine[11] have been prepared, as starting materials to be introduced as an alkylating reagent with sodium carbonate as catalyst. Acyclovir analogue[15] was prepared for the first time, the expected structure of the final newly acyclovir analogue was determined on the basis of NMR, IR, and mass spectroscopy, with low cost, high selectivity, safe and mild reaction conditions.

**Key words:** Chemical and biological study, acyclovir analogue, 6-aminouracil.

## INTRODUCTION

Acyclovir (ACV) (3) is acyclonucleoside consists of nitrogen base (guanine[1] connected by acyclic chain (2-hydroxy ethyl hydroxy methyl ether)[2] through carbon and nitrogen atoms, as shown in fig. 1[1-8].

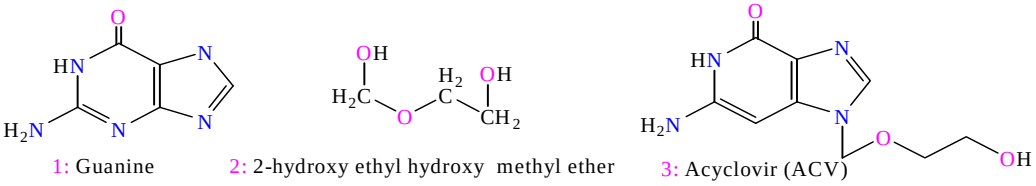



**Fig. 1: Parts of Acyclovir**

Since the discovery of modified acyclonucleoside as antiviral agents, substantial efforts have been devoted to the synthesis and biological evaluation of such compounds. For the synthesis of acyclovir and their acyclonucleosides analogues, the acetoxy ethylacetoxy methyl ether is often used as alkylating agent with some catalysts like $SnCl_4$ , $Hg(CN)_2$, $(CH_3)_3SiClO_4$ and $(CH_3)_3SiSO_3C_4F_9$. as shown in fig - 2 .[9-11].

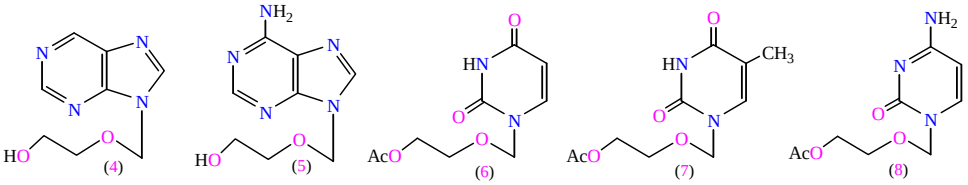


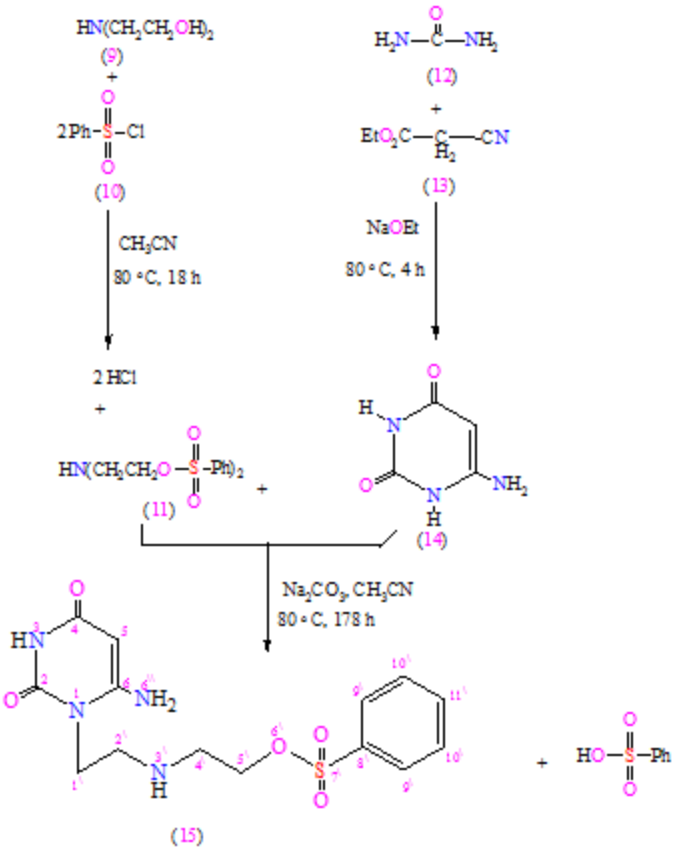

**Fig. 2: Some preparative ACV and Acyclovir analogues**

Our strategy was to develop a simple and convenient method for obtaining a series of acyclovir analogues with various side chains, and different heterocycls that replace guanine moiety.

We develop a useful carbon-nitrogen bond forming reaction with high selectivity, low cost. safe and mild reaction conditions.

It has been recognized that catalysis of organic reactions by inorganic materials such as sodium carbonate instead of $SnCl_4$ , $Hg(CN)_2$, $(CH_3)_3SiClO_4$ , $(CH_3)_3SiSO_3C_4F_9$, HMDS and $Bu_4NF$, can be a potential and efficient methodology to achieve our goals, comparing with Johnson-Hilbert method[5].

N-alkylation of nucleobases with diethanol amine derivative[9] instead of acetoxy ethyl acetoxy methyl ether under mild reaction conditions using sodium carbonate was carried out for the first time to get acyclovir analogue[15], as shown in scheme-1.




**Scheme 1: Preparation of acyclovir analogue (15)**

$N^1$-sulphonylbenzenediethylamine-6-aminouracil(15), depicted in scheme-1, was prepared by one step in 4.66% yield, starting from 6-aminouracil(14) and dibenzenesulphonyl diethyl amine(11) as starting materials using acetonitril as solvent and sodium carbonate base under refluxing at 80 °C for 178 h. (15) was obtained regioselectivity, but in low yield which is fair in such reactions[10,12,13].

**1-The IR spectroscopy of compound (15)**

IR spectrum of (15) shows the presence of broad band at 3420 $cm^{-1}$ for –NH- , -$NH_2$ groups, 3054 $cm^{-1}$ for =C-H bond, 1628 $cm^{-1}$ for C=C bond, It was noted that the band for C=O was not appeared due to resonance of amide functional group . (–N=C–OH) to give enol form ( –N–C(=O)–H )

**2-NMR spectra of compound (15)**

The NMR spectra have been recorded by different technical methods as follows :

**2-1- $^{1}$H, $^{13}$C-NMR spectra of compound (15)**

$^{1}$H-NMR spectrum shows six signals as well as two signals for deuterated DMSO.d6 at 2.5, 3.5 ppm while $^{13}$C-NMR spectrum shows five signals as shown in Table (1) and Fig. (3 & 4):

**Table 1: Chemical shift of $^{1}$H-NMR and $^{13}$C-NMR spectrum of comp. (15)**

| Kind of Carbon | Kind of Hydrogen | J (Hz) | Hydrogen's number | Kind of signals | $^{13}$C Chemical shift δ (ppm) | $^{1}$H-Chemical shift δ (ppm) | Number of atom |
|---|---|---|---|---|---|---|---|
| - | DMSO | - | - | m | - | 2.50 | - |
| -$CH_2$ | 4', 2'-$CH_2$ | 4.85 | 4 | t | 49.38 | 3.012 | 4', 2' |
| - | - | - | - | s | - | 3.506 | DMSO + $H_2O$ |
| -$CH_2$ | 5', 1'-$CH_2$ | 4.90 | 4 | t | 56.81 | 3.654 | 5', 1' |
| - | 6''-$NH_2$ | - | 2 | br | - | 5.218 | 6'' |
| =CH | 11',5=CH | - | 2 | m for 11' and s for 5 | 126.04 | 7.628 | 116,5 |
| =CH | 10', 9', 6=CH (aromatic=CH-$NH_2$) | - | 4 | m | 128.28, | 7.342 | 9', 6 |
| - | 3', 3-NH | - | 2 | br | - | 8.449 | 3', 3 |
| C=O | - | - | - | - | 148.57 | - | 8', 4, 2 |

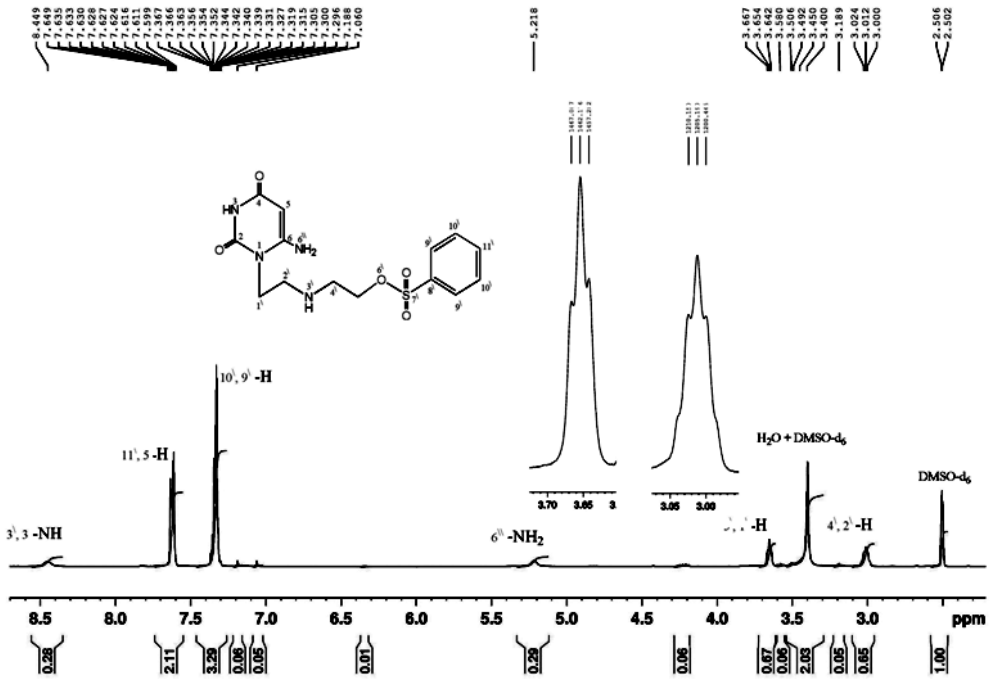



**Fig. 3: $^{1}$H-NMR spectrum of Compound (15)**

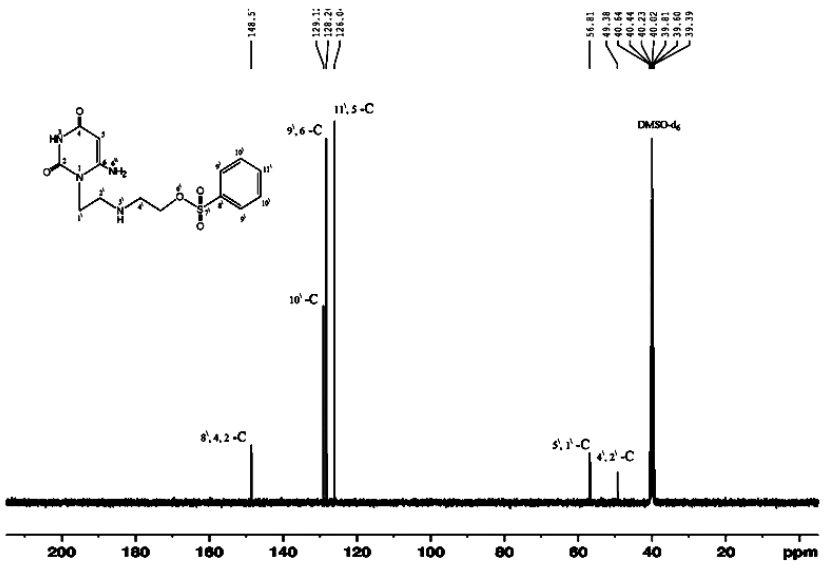



**Fig. 4: $^{13}$C-NMR spectrum of Compound (15)**

**2-2-DEPT-135 spectrum of compound (15)**

DEPT-135 spectrum of comp.(15) shows the appearance of two signals directed down of chemical shift 49.35-8 ppm and 56.81 ppm belong to secondary carbon (C-2', C-4') and (C-1', C-5') respectively, and three signals directed up of chemical shift (126.04, 128.28, and 129.12 ppm) belong to tertiary carbon (C-5, C-11') and (C-6, C-9') and (C-10') respectively, while the signal at chemical shift (148.57 ppm) belongs to quaternary Carbon atoms (C-2, C-4, and C-8') as shown in Fig. (5).

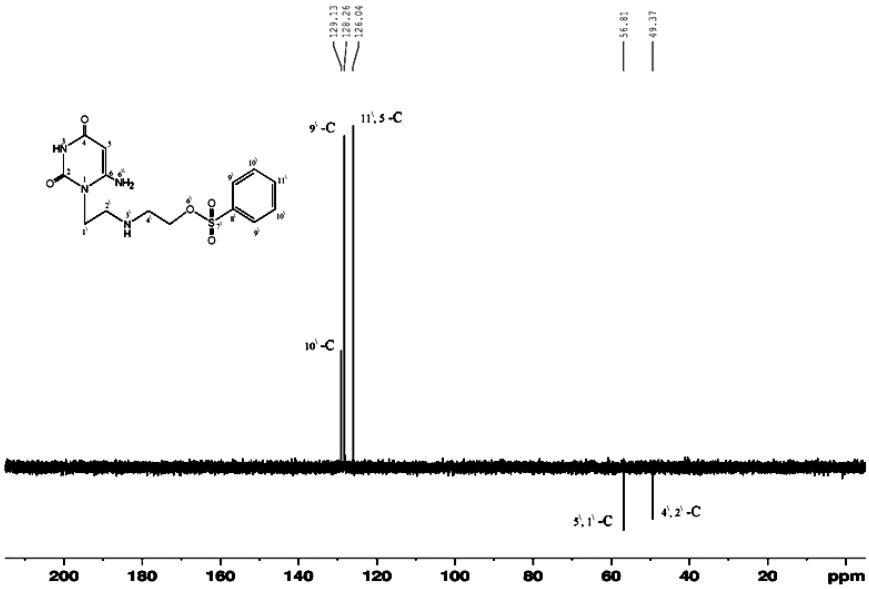



**Fig. 5: DEPT-135-NMR spectrum of Compound (15)**

**2-3-COSY-NMR spectrum of compound(15)**

It was noted in this spectrum that there are four signals for hydrogen atoms of chemical shift 3.012 ppm bonded with carbon atoms C-2' and C-4', coupled with hydrogen atoms of chemical shift 3.654 ppm bonded with carbon C-1' and C-5'. It was also noted that protons of chemical shift of 3.012 ppm bonded by carbon atoms C-2' and C-4', coupled with hydrogen atoms at chemical shift of 8.449 ppm, joined by two nitrogen atoms N-3 and N-3' respectively.

Hydrogen atoms of chemical shift 3.634 ppm bonded to carbon atom C-1' and C-5', coupled with two hydrogen atomss bonded to nitrogen atom N-6'' of chemical shift 5.248 ppm.

Two hydrogen atoms of chemical shift 7.342 ppm bonded to C-9' and C-10' were coupled with two aromatic protons of chemical shift 7.628 ppm joined by C-5 and C-11' as shown in Table (2) and Fig. (6)

**Table 2: Chemical shift of coupled hydrogen atoms bonded by carbon atom of $^1$H-$^1$H-COSY for compound (15)**

| Hydrogen chemical (δ) ppm | Coupled hydrogen atoms | Hydrogen chemical (δ) ppm | Hydrogen atoms atoms |
|---|---|---|---|
| 3.012 | 5', 1'-$CH_2$ | 3.654 | 4', 2'-$CH_2$ |
| 8.449 | 3', 3-NH | 3.012 | 4', 2'-$CH_2$ |
| 5.218 | 6''-$NH_2$ | 3.654 | 5', 1'-$CH_2$ |
| 7.342 | 10', 9' -CH | 7.628 | 11', 5-CH |

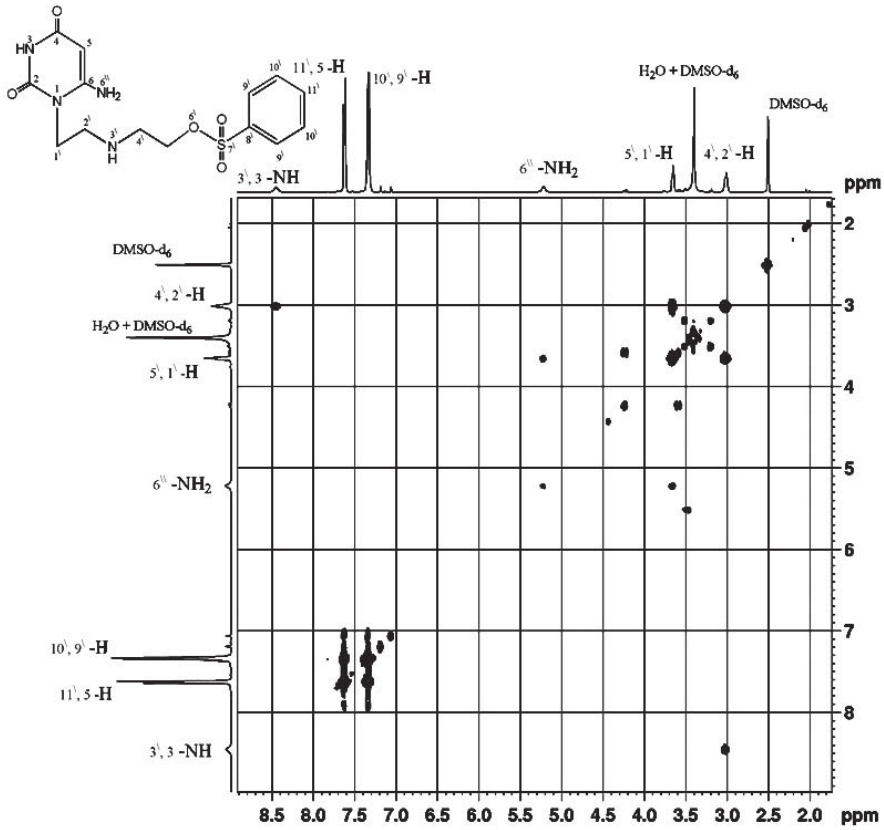



**Fig. 6: $^1$H, $^1$H-COSY NMR spectrum of Compound (15)**

**2-4- HMQC-NMR spectrum of compound(15)**

It shows that the two H-2' and H-4' of chemical shift 3.012 ppm joined to C-2' and C-4' of chemical shift 49.38 ppm while hydrogen atoms of chemical shift 3.654 ppm joined by two carbon atoms C-1' and C-5' of chemical shift 56.81 ppm.

Two hydrogen atoms of chemical shift 7.628 ppm joined to C-5 and C-11' of chemical shift 126.04 ppm and two hydrogen atoms of chemical shift 7.342 ppm joined to C-9' and C-10' of chemical shift 128.28 ppm and 129.12 ppm, as shown in Table (3) and Fig. (7) .

**Table 3: Bonded of hydrogen atoms with carbon atoms in compound (15)**

| Carbon chemical shift (δ) ppm | Carbon atom | Hydrogen atom chemical shift (δ) ppm | Hydrogen atom |
|---|---|---|---|
| 49.38 | 4‘, 2‘ | 3.012 | 4‘, 2‘-$CH_2$ |
| 56.81 | 5‘, 1‘ | 3.654 | 5‘, 1‘-$CH_2$ |
| 126.04 | 11‘, 5 | 7.628 | 11‘, 5-CH |
| 129.12, 128.28 | 10‘, 9‘ | 7.342 | 10‘, 9‘ -CH |

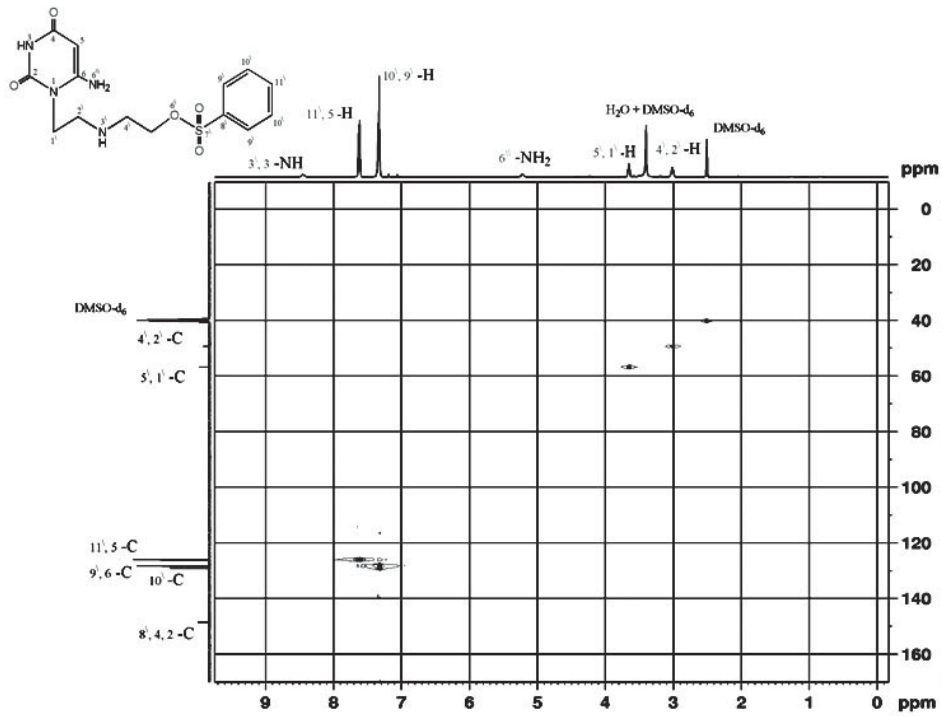



**Fig. 7: HMQC spectrum of Compound (15)**

**2-5-HMBC-NMR spectrum of compound (15)**

It shows the coupling of hydrogen atoms bonded to C-5 and C-11', with C-2 , C-4, C-8' and C-10' and correlated with C-5 and C-11'.

This indicates that H-5 coupled with C-2 and C-4' and correlated with C-5 while H-11' coupled with C-8' and C-10' and correlated with C-11' and also hydrogen atoms bonded to C-9' and C-10' were coupled with C-11', as shown in Table (4) and Fig. (8) .

**Table 4: Coupling between hydrogen and carbon atom in HMBC spectrum of compound (15)**

| Carbon chemical shift ppm (δ) | Carbon atom | Hydrogen chemical shift ppm (δ) | Hydrogen atom |
|---|---|---|---|
| 126.04 | 11‘,5 | 7.628 | 11‘, 5-CH |
| 128.28 | 10‘ | | |
| 148.57 | 8‘, 4, 2 | | |
| 126.04 | 11‘ | 7.342 | 10‘, 9‘ -CH |
| 128.28 | 10‘ | | |
| 129.12 | 9‘ | | |

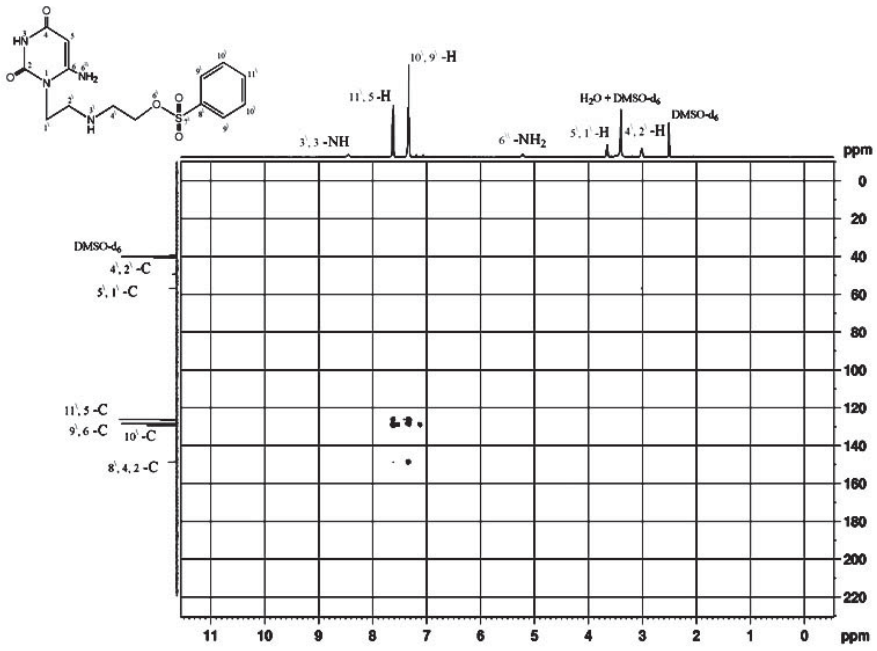



**Fig. 8: HMBC spectrum of compound (15)**

**2-6-NOESY-NMR spectrum of compound (15)**

It shows the coupling of two hydrogen atoms of chemical shift 5.128 ppm joined by nitrogen atom ($CH_2$N-6'') with hydrogen atoms of chemical shift 3.506 ppm belongs to DMSO-d6 which contain moisture.

Two hydrogen atoms of chemical shift 7.6 ppm joined by carbon atoms C-5 and C-11' are coupled with two aromatic hydrogen atoms of chemical shift 7.342 ppm joined by C-9' and C-10', as shown in Table (5) and Fig. (9) .

**Table 5: Coupling between hydrogen atoms of NOESY NMR of compound (15)**

| Hydrogen chemical shift (δ) ppm | Coupled Hydrogen atoms | Hydrogen chemical shift (δ) ppm | Hydrogen atoms |
|---|---|---|---|
| 3.506 | DMSO + $H_2O$ | 5.218 | 6“-$NH_2$ |
| 7.342 | 10‘, 9‘ -CH | 7.628 | 11‘, 5-CH |

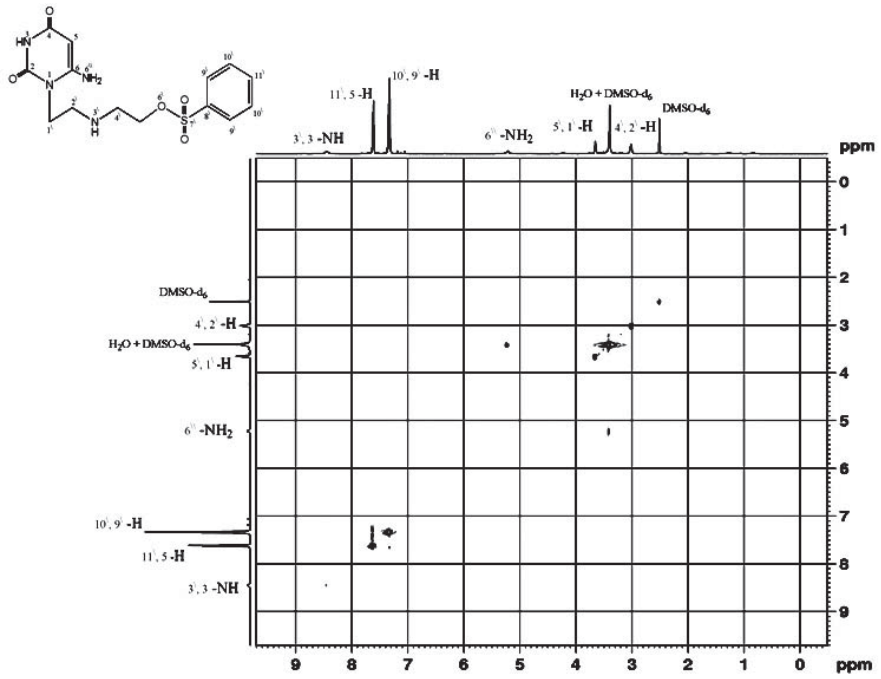



**Fig. 9: NOSEY spectrum of Compound (15)**

**3-Mass spectra of compound (15)**

Mass spectra C-I, E-I/Ms of compound (15) (Fig. 10, 11) were shown below.

The following fragments of CI, EI/Ms spectra were shown in Table (6).

**Table 6: Fragments of mass spectrum of compound (15)**

| CI/Msm/z | 42 | 55 | 57 | 86 | 87 | 99 | 100 | 124 | 130 | 158 | 160 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| % | 28.3 | 11.6 | 17 | 36 | 100 | 13.3 | 79 | 10.4 | 8 | 12 | 86 |
| EI/Msm/z | 41 | 42 | 43 | 44 | 45 | 48 | 50 | 51 | 55 | 56 | 57 |
| % | 12 | 23 | 32 | 20 | 20 | 10 | 18 | 35 | 14 | 63 | 15 |
| EI/Msm/z | 65 | 66 | 74 | 77 | 81 | 94 | 97 | 118 | 127 | 158 | - |
| % | 28 | 11 | 100 | 46.4 | 10 | 37 | 6 | 15 | 9 | 26 | - |

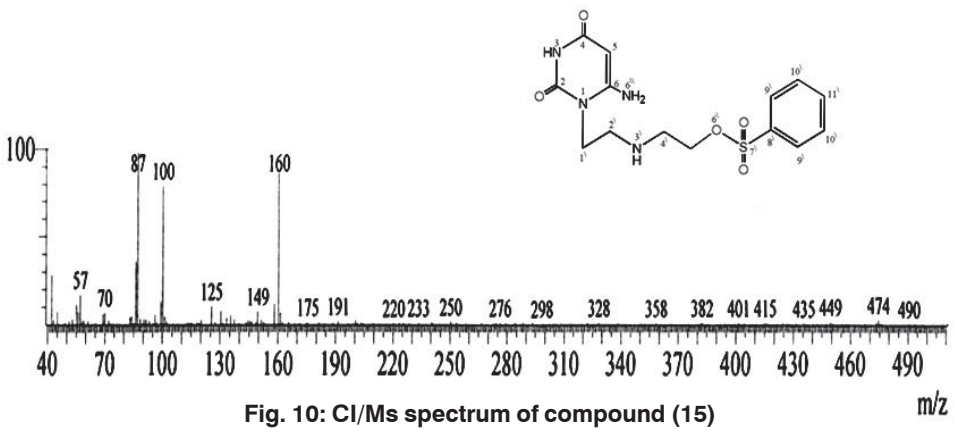



**Fig. 10: CI/Ms spectrum of compound (15)**

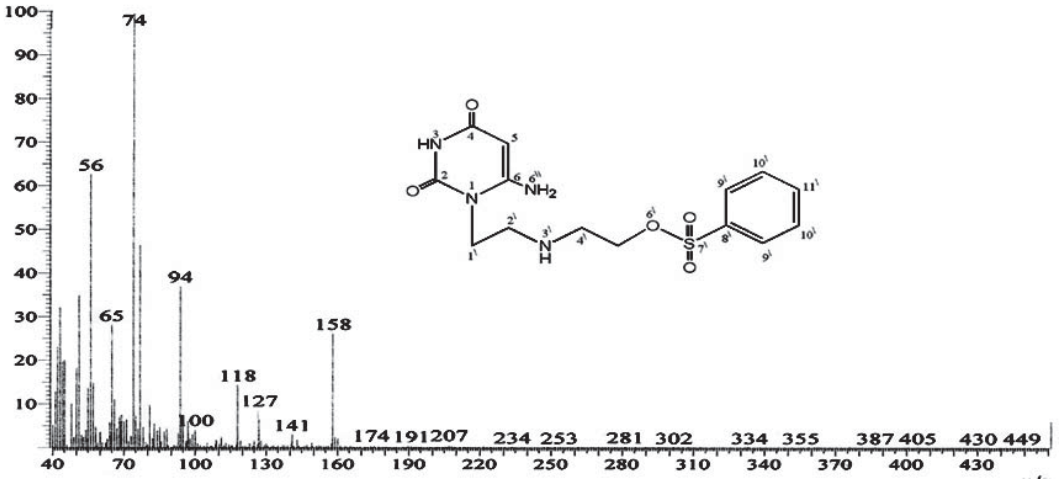



**Fig. 11: EI/Ms spectrum of compound (15)**

According to data of mass, IR and NMR spectroscopy, It was suggested the diagram (1) for fragments of compound (15). Most suggested fragments were stable by resonance and it may convent the intensity of the corresponding bands.

## EXPERIMENTAL

Melting points were determined on a Scientific Pmp-dm apparatus and are uncorrected.

IR spectra were taken as KBr disk on IR model Jasco FT/IR 410 spectrometer, NMR spectra of all compounds were recorded on Bruker AC 400 spectrometer at 400 MHz . Chemical shifts are reported in (δ) ppm down field from TMS which was an internal reference.

Mass spectra were recorded on Gc-Ms-QP5050A, Gc-17A, Shimadzu spectrometer.

TLC was carried out on 0.25 mm layers gel plates containing a fluorescent indicator; spots were detected under UV light (254nm) and Iodine. $R_f$ value was taken in Toluene : MeOH (1 : 1).

Sodium sulphate was used as the drying agent in all cases. Evaporations were performed under vacuum (rotating evaporate).

**Preparation of 6-aminouracil (14)**

[14] Absolute ethanol (500ml) was added to small pieces of sodium (19.79g, 0.86 mol), then ethyl cyano acetate (45.75g, 0.43 mol), and urea (25.7g , 0.43 mol) were added.

The solution was stirred with reflux at 80 °C for 4h. After 2h, reaction mixture would start to solidify, and at the end of reaction time, hot distill water (500 ml) (80 °C) was added to reaction mixture to dissolve the solid material with stirring for 15 min.

The reaction mixture was neutralized by glacial acetic acid (37.5 ml) , left at room temperature till white precipitate would form, Collected by filtration, 30.43g, Yield 56%, m.p.> 275 °C , $R_f$ = 0.51.

**Preparation of disulphonyl benzenediethylamine (11)**

Diethanol amine (2.5g, 204 mmol)(9), and benzene sulphonyl chloride (52.41 ml, 409 mmol) (10) dissolved in acetonitril (40ml), were stirred at 80 °C for 18h. The reaction mixture was followed by TLC, cooled and precipitate was removed by filtration , the filtrate was extracted by chloroform(50 ml x 4) and water (50 ml), drying organic layer by sodium sulfate, filtration and evaporation to get viscous reddish material (43.2g, 55% yield). TLC $R_f$ = 0.595, IR(KBr disk, $cm^{-1}$) : 3411, 3066, 2976, 2857, 1739, 1447, 1348, 757. $^1$H-NMR ($CDCl_3$) : $d_H$ 2.99 (4H, t, J=11) NH-$CH_2$- , 3.742 (4H, t, J=10) -$CH_2$-O-5.93 (1H, bs, -NH-) , 7.42-7.91, 8.03-8.06 (10H, m, -Ph).

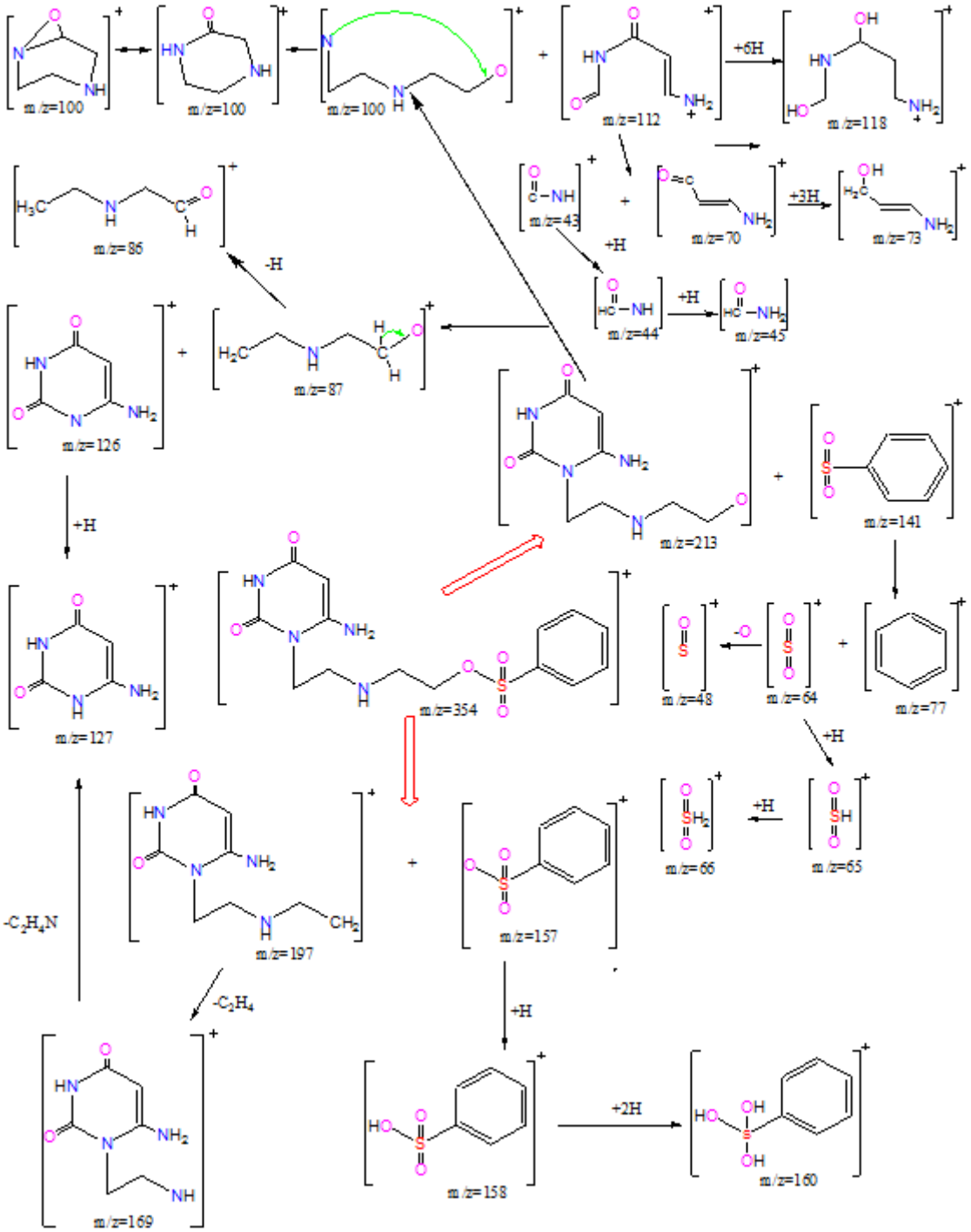



**Diagram 1: Suggested fragments for compound (15)**

**Preparation of $N^1$-sulphonyl benzenediethyl amine-6-amino uracil (15)**

6-aminouracil (2.5g, 20mmol) (14) was dissolved in acetonitreal (60ml), sodium carbonate (1.66g), and disulphonylbenzenediethylamine (6.7g, 20mmol) (11) were added, reaction mixture was stirred at 80 °C for 178 h. Reaction mixture was followed by TLC, cooled and precipitate removed by filtration. reaction.

Filtrate was evaporated under vacuum to get viscous liquid (0.79g) and acetone (1 ml) was added to get white to yellow precipitate which was washed with hot acetone (3x3ml), then filtration and recrystallization from acetone: ethanol (1 : 10) to get pure substance (0.33g, 4.66% yield), m.p. dec. 252 °C, $R_f$ = 0.673 , IR (KBr disk, $cm^{-1}$) : 3420, 3054, 1628, 1445, 1400, 1237. $^1$H-NMR 400MHz (DMSO-d6) : $\delta$ 3.012(4H, t, J = 4.85 Hz), 3.654(4H, t, J = 4.90 Hz), 5.218 (2H, br), 7.628 (2H, m), 7.342 (4H, m), 8.449(2H, br). $^{13}$C-NMR 400MHz (DMSO-d6) : $\delta$ 49.38, 56.81, 126.04, 128.28, 129.12, 148.57.

**Study of biological activity**

**Antifungal agents**

A large number of antibacterial agents are available to treat bacterial infections, only few of these agents exist for therapy of systemic fungal infections. Several reasons can be given for these differences. Less work has been noted for the development of antifungal agents because systemic fungal infections are less common than bacterial infections. Furthermore, fungal cells, like mammalian cells, are eukaryotic, and compounds which are highly specific for fungal cells where nontoxic to the parasitized host cells, difficult to developed.

**Antibacterial agents**

They are naturally occurring microbial products, chemical compounds such as sulphonamides, quinolones, nitrofuranas and imidazoles should strictly be referred to as chemotherapeutic agents. However some antibacterial agents can be manufactured synthetically while others are products of chemical manipulations of naturally occurring compounds (semi-synthetic) the distinction is now ill defined. The antibacterial agents classified according to their site of infection into:

a) Inhibitors of bacterial cell wall synthesis.
b) Inhibitors of bacterial protein synthesis.
c) Inhibitors of nucleic acid synthesis.
d) Miscellaneous antibacterial agents.

## EXPERIMENTAL

Microorganisms, which used in antimicrobial tests (like *Escherichia coli* (Gram negative bacteria), *Staphylococcus aureus* (Gram positive bacteria) and *Candida* (yeast), were isolated from human patients.

The isolated bacteria were identified by gram stain and compounds discs test, while isolated yeast was distinguish by microscopic identification and Gram staining.

Each isolate was prepared in the following procedures :

1. The isolated bacteria and yeast were cultured on nutrient agar medium in sterile plastic Petri-dishes (9cm).
2. The culture was incubated at 37°C for 24 hours.
3. A disc of 0.5cm in diameter was transferred from the growth colonies to sterile test tube, which contain 6ml of $H_2O$ and nutrient agar, then test tubes were shacked thoroughly.
4. Suspension was distributed into six test tubes (1 ml/t.t).
5. To each test tube 0.5ml (10mg/1ml) of saturated tested compounds was added, all test tubes were incubated at 37°C for 24 hours for isolated bacteria and 72 hours for isolated *Candida*.
6. The optical density (turbidity) for bacterial treatments was detected by spectrophotometer (at wave length, 8 = 515/ transmission).
7. The net wait weight of *Candida* was determined after filtration.
8. The used control is contain of studded microorganisms and nutrient agar medium.

All manipulations were done under conditions avoiding contamination. The results shown in Table (7) indicated different levels of inhibition to microbial growth which is related to the nature of microorganism and the properties of chemical agents.

**Table 7: Antimicrobial screening results of the tested compounds**

| No | Compd | *S. aureus* 8 = 515 nm | *E. coli* 8 =515 nm | *Candida* W=? mg |
|---|---|---|---|---|
| 1 | Control | 290 | 456 | 1.6 |
| 2 | (15) | 216 | 352 | 1.9 |

W = Weight

**Recommendations**

1. The results obtained from this work, may give a new acyclovir analogue due to replacement of acyclic chain 2-hydroxy ethyl hydroxy methyl ether with ethanol ethyl amine.
2. Newly acyclovir analogue give more soluble salt on treatment with 0.5% hydrochloric acid due to the presence of (-NH-) group, therefore it will be more active comparing with acyclovir.
3. It was developed useful Carbon-nitrogen bond forming reaction with high selectivity, low cost, safe and mild reaction conditions.

## ACKNOWLEDGMENTS

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