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Synthesis and Biological Evaluation of some heterocyclic derivatives from carbonyl compounds as Anticancer agents

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Abstract
The key intermediate 3-(3-Dimethylamino- acryloyl)-4-hydroxy-6-methylpyran-2-one **1** was prepared by the condensation of dehydroacetic acid with N,N-dimethylformamide-dimethylacetal in the presence of a catalytic amount of acetic acid. Treatment of **1** with different reagents afforded the 2-pyrano derivatives **3-21**. Preliminary biological screening of the prepared compounds revealed significant anticancer activity of some of derivatives.
Keywords: 2-pyrono derivatives, N,N-dimethylformamide-dimethylacetal, Anticancer activity.

1. Introduction

The chemotherapy of neoplastic disease has become increasingly important in recent years. An indication of this importance is the establishment of a medical specialty in oncology, wherein the physicians practice various protocols of adjuvant therapy, chemotherapy and surgical operations. Consequently, the rapid spread of cancer has stimulated an unprecedented level of research activity directed towards the search for new structure leads that may be of use in designing novel antitumor drugs. In this context, compounds comprising the 2H-pyran-3(6H)-one functionality have recently attracted great attention, either as versatile synthetic intermediates[1-4] or as biologically important molecules with significant anticoccidia1,[10] antibacterial, [5,6] pesticida1,[7] or herbicidal activity.[8] Furthermore, derivatives of 2H-pyran-3(6H)-one are components of naturally occurring anthracycline antibiotics.[9] There is ongoing interest in the synthesis and pharmacology of 2H-pyran-3(6H)-ones, and many new derivatives have been synthesized with additional properties such as acaricidall[10] and fungicidal[11] activities. Moreover, Coumarins one of 2H-pyrone derivatives are nowadays an important group of organic compounds that are used as bactericides [12-14], fungicides [15], anti-inflammatory [16], anticoagulant [17] and antitumour agents[18.19]. These pharmacological properties of 2H-pyran-3(6H)-ones aroused our interest in synthesizing

several new compounds featuring different heterocyclic rings fused onto the 2H-pyrane moiety with the aim of obtaining more potent pharmacologically active compounds.

2. Results and Discussion

2.1 Chemistry:

Refluxing dehydroacetic acid (DHAA) with N,N-dimethylformamide-dimethylacetal (DMF-DMA) in dichloromethane in the presence of a catalytic amount of acetic acid directly afforded the 3-(3-Dimethylaminoacryloyl)-4-hydroxy-6-methylpyran-2-one **1** in good yield scheme 1. The IR spectra of **1** showed two carbonyl absorption in the region 1664 cm^{-1}, 1710 cm^{-1} and OH band at 3318 cm^{-1}, as well as an olefinic C=C absorption at 1614 cm^{-1}. The ^{1}H NMR spectra showed the olefinic protons, H-α and H-β as two doublets (J = 12Hz) at □ 8.05 and 6.48 respectively as well as two singlet at □ 3.00 and 3.24 for N methyl groups. The structures of the above chalcones were further confirmed from their ^{13}C NMR data which showed two carbonyl carbons at □184.86 and 184.16 as well as the expected number of aliphatic and aromatic carbons signals. Reaction of 3-(3-Dimethylaminoacryloyl)-4-hydroxy-6-methylpyran-2-one **1** with acetyl acetone in absolute ethanol afforded the corresponding bipyranyl-2'-one **2**. The IR spectra of the above bipyranyl **2** showed two carbonyl absorption in at 1674 cm^{-1} and 1708 cm^{-1} as well as OH absorption at 3327 cm^{-1}. The ^{1}H NMR spectra showed three methyl singlets at □ 2.09, 2.36 and 2.67 as well as two multiplets at□ □ 2.67 and 4.61 for the H-4 and H-3 of pyrane moiety respectively**)**. The structure was further confirmed from ^{13}C NMR data which showed two carbonyl carbons at □183.66 and 162.96 as well as the expected number of aliphatic and aromatic carbons signals. Moreover,reaction of 3-(3-Dimethylaminoacryloyl)-4-hydroxy-6-methylpyran-2-one **1** with ethyl glycinate hydrochloride in absolute ethanol yielded the ethyl 3-(4-Hydroxy-6-methyl-2-oxo-2H-pyran-3-yl)-2,5-dihydro-1H-pyrrole-2-caboxylate **3**. The IR spectra of this pyrrole ester showed three carbonyl absorption in the region 1687 cm^{-1}, 1715 $cm^{-1}$and as well as OH absorbtion at 3322 cm^{-1}. The ^{1}H NMR spectra showed beside the protons of the ester group, a double doublet of two proton intensity at δ 3.27 due to the CH_2 of pyrrole moiety. The structurewas further confirmed from their ^{13}C NMR data which showed two carbonyl carbons at 168.34

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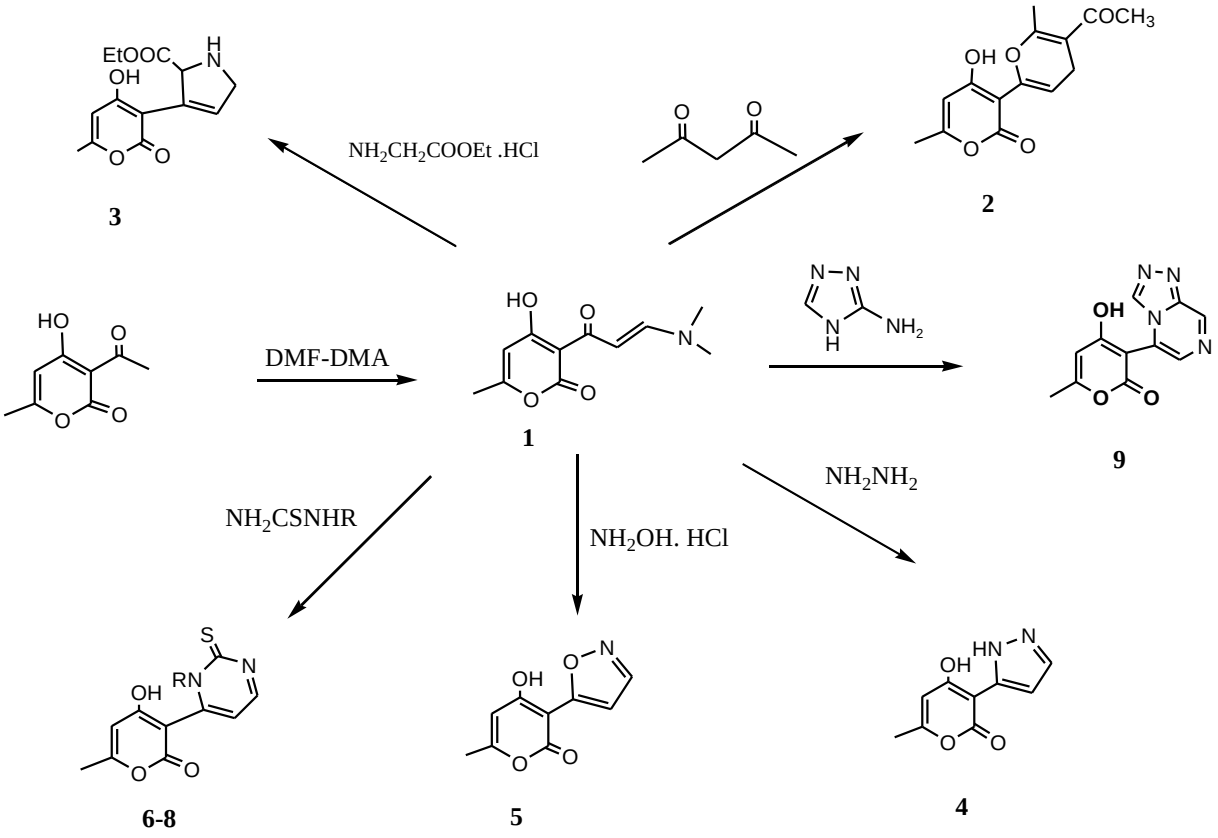
and 186.26 as well as the expected number of aliphatic and aromatic carbons signals. On the other hand, reaction of **1** with hydrazine hydrate, hydroxyl amine, thiourea and 2-amino-1H-[1,2,4]triazole afforded the corresponding pyrazole, isoxazole, pyrimidine 2-thiones and Triazolo[4,3-a]pyrazine derivatives **4**, **5** ,**6-8** and **9** respectively scheme 1. Their IR spectra showed two absorbtion bands in the regions 1705-1712 cm^{-1} and 3298-3325 cm^{-1} for the carbonyl and OH groups respectively. In addition, the IR spectra of **6-8** exhibited a CS absorbtion at 1225-1244 cm^{-1}. The strucrures of the above compounds were confirmed from their ^{1}H NMR and ^{13}C NMR data which showed the expected number of aliphatic and aromatic protons and carbons signals (experimental section).



R=H,CH_3,Ph

Scheme 1

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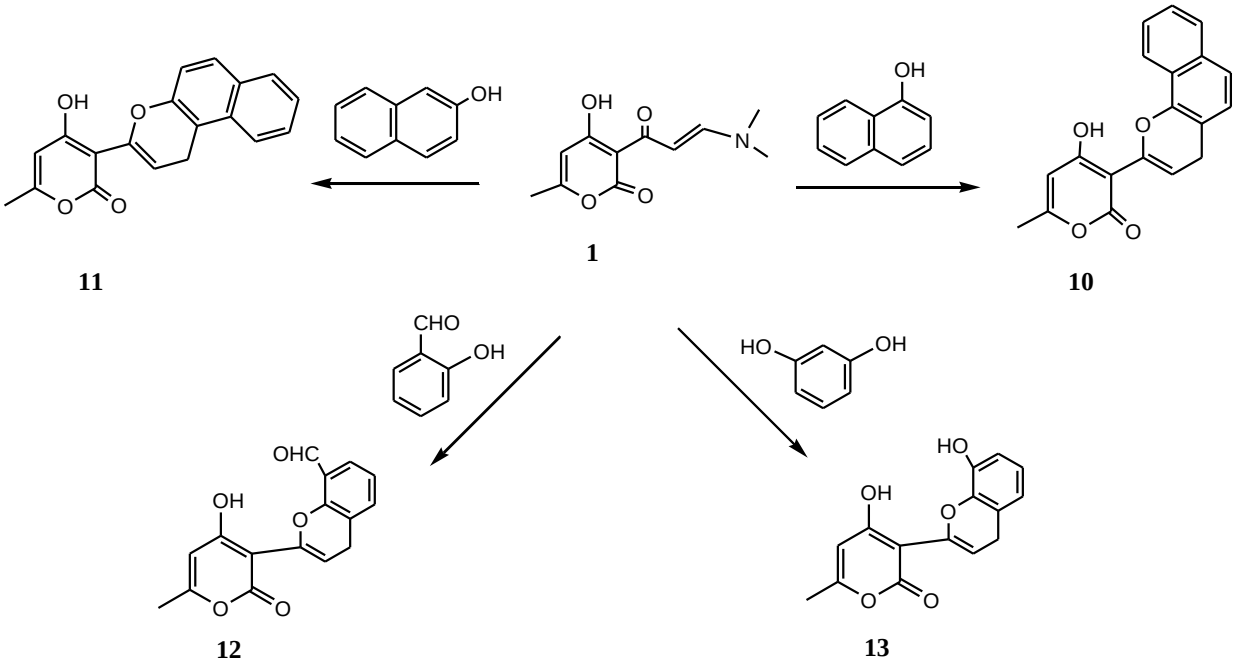
Refluxing of 3-(3-Dimethylaminoacryloyl)-4-hydroxy-6-methylpyran-2-one **1** with α- and β-naphthols in glacial acetic acid afforded the corresponding chromene derivatives 3-(4H-Benzo- [h]chromen-2-yl)-4-hydroxy-6-methyl-2H-pyran-2-one **10** and 3-(1H-Benzo[f]chromen-3-yl)-4-hydroxy-6-methyl-2H-pyran-2-on and **11** respectively in moderate yields scheme 2. In a similar manner, the reaction of enaminone **1** with resorcinol in refluxing glacial acetic acid yielded the chromene derivative **13**. Similarly, salicaldehyde reacted with the same enaminone **1** in boiling glacial acetic acid to give 2-(4-hydroxy-6-methyl-2-oxo-2H-pyran-3-yl)-4H-chromene-8-carbaldehyde **14**. Their IR spectra of the chromene derivatives **10-13** showed two absorbtion at 1711-1716 cm^{-1} and 3296 cm^{-1} for the carbonyl and OH groups respectively. Moreover, chromene derivative **12** exhibited another CO band at 1695 cm^{-1} due to the aldehydic function. The structures of the above compounds were confirmed from their ^{1}H NMR and ^{13}C NMR data which showed the expected number of aliphatic and aromatic protons and carbons signals (experimental section).



11 **1** **10**

12 **13**

Scheme 2

Treatment of DHAA with one equivalent of bromine in refluxing glacial acetic acid gave the corresponding bromo derivative[20] **14** in 70% yield**.** The IR spectrum of the above bromo derivative **14** showed two carbonyl absorptions at 1692 cm^{-1} and 1716 cm^{-1} as well as OH band at 3324 cm^{-1}. The ^{1}H NMR spectrum showed three singlet at δ2.47,4.68 and 5.99 for the CH_3, CH_2 and H-5 functions. The structure of the above bromo- derivative was further confirmed from its ^{13}C NMR data which showed the expected number of aliphatic and aromatic carbons signals as well as two carbonyl carbons at δ 170.13 and 197.28. Condensation of 3-(2-Bromoacetyl)-4-hydroxy-6-methylpyran-2-one **14** with the appropriate diamine in dry ethanol afforded the amino-imino acetylpyran-2-one derivatives **15-18**. Refluxing the amino-imino acetylpyran-2-one derivative **15** with acetic acid afforded the pyrazine-pyran-2-one derivative **19.** Other hand, condensation of the bromo derivative **14** with ethanolamine gave the corresponding 4-hydroxy-3-[2-(2-hydroxyethylamino)acetyl]-6-methylpyran-2-one **20**. Moreover, reaction of two equivalent of the 3-bromopyran2-one derivative **14** with one equivalent the 1, 2-diaminoethane yielded the corresponding bispyrane amino-imino derivative **21** (scheme 3).

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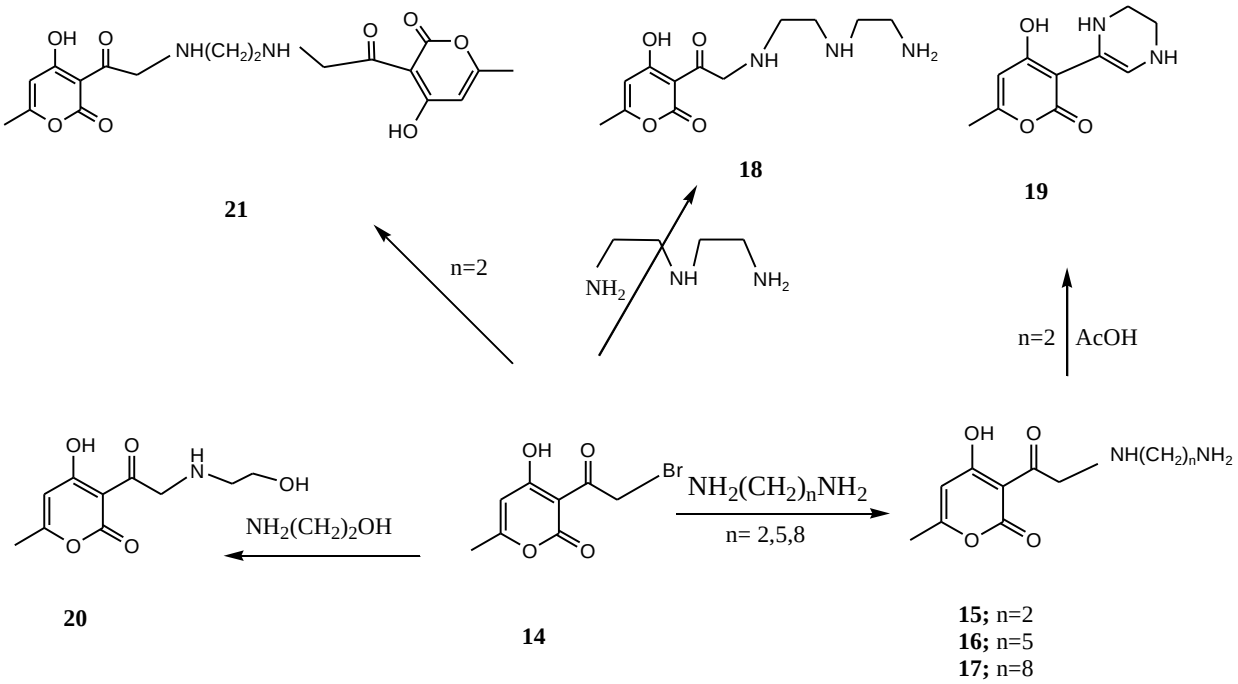

Scheme 3

The IR spectra of compounds **15-21** showed a pyrane carbonyl absorption in the region 1710-1722 cm^{-1} and an acetylamine carbonyl in the region 1662-1668 cm^{-1} as well as OH and NH bands in the region 3295-3312 cm^{-1}. The strucrures of the above compounds were further confirmed from their ^{1}H NMR and ^{13}C NMR data which showed the expected number of aliphatic and aromatic protons and carbons signals (experimental section).

.2. In vitro MTT cytotoxicity assay2

Seventeen analogise **2,4,5,7-13** and **15-21** were selected to be evaluated for their *in vitro* cytotoxic effect via the standard MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] method[21,22] against a panel of three human tumor cell lines namely; Caucasian breast adenocarcinoma MCF7, hepatocellular carcinoma HePG2 and colon carcinoma HT29. The results are presented in Table 1 as LC_{50} (µg/mL) which is the lethal concentration of the compound which cause death of 50% of the cells in 24h. The obtained data revealed that, the three tested human tumor cell lines exhibited variable degree of sensitivity profiles towards all the tested compounds. Among these, compounds **15**, **16** and **17** showed pronounced activity against the human colon

carcinoma HT29 cell line with LC_{50} values 11.5, 10.1 and 7.9 µg/mL, respectively. Moreover, a remarkable cytotoxic potential was displayed by compounds **8**, **9** and **10** against the same cell line (18.2,15.4 and 16.9 µg/mL). Compounds **20** and **21** revealed an obvious cytotoxicity profile against colon carcinoma HT29 with LC_{50} values **32.6** and **30.3**µg/mL, respectively. However, compounds **11,12**and **13** were able to exhibit moderate activity against the same cell line with LC_{50} values range of 50.4, 48.3and 54.3 µg/mL. Furthermore, the growth of the human hepatocellular carcinoma HePG2 cell line was found to be moderately inhibited by ten of the active compounds **4,5,8,9,10,11,12,15,16 and 17** with LC_{50} values range of 11.6-50.4µg/mL. Among these, the highest cytotoxic activity was displayed by compounds **10, 15, 16** and **17** which were almost equipotent (LC_{50} values 20.2, 18.6,12.4 and 11.6 µg/mL, respectively). On the other hand, human breast cancer MCF 7 was proved to be the least sensitive among the cell lines tested as it was affected by only eight of the test compounds. However, an outstanding growth inhibition potential was shown by compounds **10, 15, 16** and **17** as evidenced from their LC_{50} values (9.7, 3.8,2.8 and 2.0 µg/mL, respectively). The rest five active compounds namely **4, 9,** and **12** showed moderate to mild activity against the same cell line with LC_{50} values of 45.4, 49.2 and 40.4 µg/mL, respectively (Table **1**). Further interpretation of the results revealed that, compounds **10, 15, 16** and **17** showed considerable broad spectrum of cytotoxic activity against the three tested human tumor cell lines. In particular, compounds **15, 16** and **17** proved to be the most active members in this study with a broad spectrum of activity against the tested cell lines, with special effectiveness against the human colon carcinoma HT29 and human breast cancer MCF 7 (Table **1**).

Table 1. Cytotoxic effects LC_{50}; µg/mL[a] of the active compounds on some human

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tumor cell lines using the MTT assay.

Compd no.	Human colon carcinoma HT29	Human hepatocellular carcinoma HePG2	Human breast cancer MCF 7
2	72.5	-	-[b]
4	65.4	48.2	45.4
5	68.2	50.4	-
7	70.5	-	-
8	18.2	32.3	
9	15.4	30.2	49.2
10	16.9	20.3	9.7
11	50.4	47.7	-
12	48.3	45.99	40.4
13	54.3	-	-
15	11.5	18.6	3.8
16	10.1	12.4	2.8
17	7.9	11.6	2.0
18	64.4	-[e]	-
19	60.8	57.2	46.3
20	32.6	-	-
21	30.3	-	-
Doxorubicin [c]	21.1	1.69	2.14

[a]LC50: Lethal concentration of the compound which causes death of 50% of cells in 24h (µg/mL).
[b] Totally inactive against this cell line.
[c] positive control cytotoxic agent.

3. Experimental

3.1. Chemistry

Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. The infrared (IR) spectra were recorded on Shimadzu FT-IR 8400S infrared spectrophotometer using the KBr pellet technique. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker WM-600 FT NMR spectrometer using tetramethylsilane as the internal standard and DMSO-d_6 as a solvent (Chemical shifts in δ, ppm). Splitting patterns were designated as follows: *s*: singlet; *d*: doublet; *m*: multiplet; *q*: quartet. The impact ionization mass spectra were recorded on a Nermag R10-10C at 70ev. 1000 Ex spectrometer.Elemental analyses were performed on a 2400 Perkin Elmer Series 2 analyzer and the found values were within ±0.4% of the theoretical values. Follow up of the reactions and checking the homogeneity of the compounds were made by TLC on silica gel-protected aluminum.

3-(3-Dimethylaminoacryloyl)-4-hydroxy-6-methylpyran-2-one 1

A solution of dehydroacetic acid (DHAA) (1.68g, 10 mmol) in dichloromethane (20 mL) was refluxed with DMF-DMA (10 mmol) in presence for 3 drops of AcOH for 6h. The reaction mixture was concentrated and the separated product was filtered, washed with ethanol and recrystallized from the ethanol as yellow needles (1.7g, 75%) m.p.164-165°C. $\nu_{max.}$ (cm^{-1}, KBr):1710 and 1664(2CO), 3318(OH),1614 cm^{-1}(olefinic C=C).^{1}HNMR (δ/ppm, DMSO-d_6): 2.11 (s,3H,CH_3), 3.00,3.24(2s,each 3H,2N-CH_3),5.73(s,1H, H-5),6.48(d, J=12Hz, 1H, H-β),8.05(d,J=12Hz,1H,H-α).14.18 (s,1H,OH). ^{13}CNMR (δ/ppm,DMSO-d_6): 19.75(CH_3),37.66,45.73[$(CH_3)_2N$], 156.78(C-α),104.46(C-β),161.91(C-3),164.73(C-4), 90.82(C-5), 95.03(C-6), 184.16, 184.86(2CO).Anal.% Calcd for $C_{11}H_{13}NO_4$: C, 59.19; H, 5.87; N, 6.27. Found: C, 59.22; H,5.76; N, 6.22.

5-Acetyl-4'-hydroxy-6-6'-dimethyl-4-H-[2,3']bipyranyl-2'-one 2

A solution of 3-(3-Dimethylaminoacryloyl)-4-hydroxy-6-methylpyran-2-one **1** (2.2g,10 mmol) in absolute ethanol (20 mL) was refluxed with actylacetone (1.0g,10 mmol) for 6h. The reaction mixture was concentrated and the separated product was filtered, washed with ethanol and recrystallized from the ethanol as yellow needles (1.8g, 68%) m.p.215-217°C. $\nu_{max.}$ (cm^{-1}, KBr):1717 and 1648 (2CO), 3305(OH).^{1}HNMR (δ/ppm, DMSO-d_6): 2.09 (s,3H, CH_3), 2.23(s, 3H, CH_3), 2.67(s, 3H,$COCH_3$), 2.67 (m,2H, CH_2 pyrane), 4.61 (m, 1H, H-3 pyrane), 6.05 (s,1H,H-5 pyrone), 11.86 (s,1H,OH).^{13}CNMR(δ/ppm,DMSO-d_6) : 15.94, 20.66, 22.73 (3CH_3), 55.50(CH_2), 100.40(C-5 pyrone),93.01,114.40, 120.37, 128.96, 146.36,158.14,160.56 (ArC), 162.96, 183.66(2CO).Anal.% Calcd for $C_{14}H_{14}O_5$: C, 64.12; H, 5.38. Found: C, 64.30; H,5.42 .

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Ethyl 3-(4-Hydroxy-6-methyl-2-oxo-2H-pyran-3-yl)-2,5-dihydro-1H-pyrrole-2-caboxylate 3

A mixture of 3-(3-Dimethylaminoacryloyl)-4-hydroxy-6-methylpyran-2-one **1** (2.2g,10 mmol) and ethyl glycinate hydrochloride (1.4g, 10 mmol) in absolute ethanol (20 mL) was refluxed for 8h. The reaction mixture was concentrated and the separated product was filtered, washed with ethanol and recrystallized from the ethanol as yellow needles (2.1g, 78%) m.p.144-146°C. $\nu_{max.}$ (cm^{-1}, KBr):1735 and 1714(2CO), 3289(OH),3348(NH).^{1}HNMR (δ/ppm, DMSO-d_6): 1.23(t,3H,CH_3),2.20 (s,3H, CH_3) , 4.21 (q, 2H,CH_2), 3.27(dd, 2H, CH_2 pyrrole), 4.41(s,1H,CH pyrrole), 5.78 (s,1H, H-5 pyrone),5.66(t,1H,H-4 pyrrole), 7.10(s,1H,NH).^{13}CNMR (δ/ppm, DMSO-d_6): 14.25, 20.00 ($2CH_3$), 58.21(CH_2), 48.45(C-5 pyrrole),66.34(C-2 pyrrole), 99.23,102.69, 118.54 , 139.54, 167.42,168.24(ArC), 174.32,186.45 (2CO). Anal.% Calcd for $C_{13}H_{15}NO_5$: C, 58.86; H, 5.70;N,5.28. Found: C, 58.72; H,5.66;N,5.35 .

4-Hydroxt-6-methyl-3-(1H-pyrazol-3-yl)-2H-pyran-2-one 4

A mixture of 3-(3-Dimethylaminoacryloyl)-4-hydroxy-6-methylpyran-2-one **1** (2.2g,10 mmol) (2.2g,10 mmol) and hydrazine hydrate (0.75g, 15 mmol) in absolute ethanol (20 mL) was refluxed for 8h. The reaction mixture was concentrated and the separated product was filtered, washed with ethanol and recrystallized from the ethanol as yellow needles (1.5g, 82%) m.p.202-204°C. $\nu_{max.}$ (cm^{-1}, KBr):1700 (CO), 3279 (OH)3384(NH).^{1}HNMR (δ/ppm, DMSO-d_6): 2.16(s, 3H, CH_3) , 5.79 (s,1H, H-5 pyrone),7.23(d,1H,H-3 pyrazole),7.36(d,1H,H-3 pyrazole), 12.00 (s,1H,NH), 14.85 (s,1H,OH).^{13}CNMR (δ/ppm,DMSO-d_6): 20.18 (CH_3), 96.70, 103.95, 131.65, 136.97, 162.48,165.03,167.78 (Ar C),179.65(CO).Anal.% Calcd for $C_9H_8N_2O_3$: C, 56.25; H, 4.20;N,14.58. Found: C, 56.32; H,4.40;N,14.62 .

4-Hydroxt-3-isoxazol-5-yl-6-methyl-2H-pyran-2-one 5

A mixture of 3-(3-Dimethylaminoacryloyl)-4-hydroxy-6-methylpyran-2-one **1** (2.2g,10 mmol) and hydroxylamine hydrochloride (0.68g, 10 mmol) in absolute ethanol (20 mL) was refluxed in presence of few drops of triethylamine for 8h. The reaction mixture was concentrated and the separated product was filtered, washed with ethanol and recrystallized from the ethanol as pale yellow needles (1.6g, 84%) m.p.182-183°C. $\nu_{max.}$ (cm^{-1}, KBr):1709(CO), 3298(OH).^{1}HNMR (δ/ppm, DMSO-d_6): 2.09 (s,3H, CH_3) , 5.76 (s,1H, H-5 pyrone), 5.27(d,1H,H-4 isoxazole), 7.96(d,1H,H-3 isoxazole), 15.49(s,1H,OH).^{13}CNMR (δ/ppm,DMSO-d_6): 20.23(CH_3), 93.40, 100.48, 102.92,146.41,152.53,162.01,165.37(ArC), 182.41(CO).Anal.% Calcd for $C_9H_7NO_4$: C, 55.96; H, 3.65;N,7.25. Found: C, 56.02; H,3.75; N,7.32 .

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4-Hydroxy-6-methyl-3-(2-thioxo-2,3-dihydropyrimidin-4-yl)pyran-2-one 6 and 4-Hydroxy-6-methyl-3-(3-substituted-2-thioxo-2,3-dihydropyrimidin-4-yl)pyran-2-one 7 & 8

A mixture of 3-(3-Dimethylaminoacryloyl)-4-hydroxy-6-methylpyran-2-one **1** (2.2g,10 mmol) and the appropriate thiourea (10 mmol) in absolute ethanol (20 mL) was refluxed in presence of few drops of triethylamine for 8h. The reaction mixture was concentrated and the obtained solid material was filtered, washed with ethanol and recrystallized from the proper solvent as pale yellow needles.

6: Rrecrystallized from ethanol as needles. (2.1, 88%) m.p.180-182°C. $\nu_{max.}$ (cm^{-1}, KBr):1258 (CS),1689(CO), 3283(OH),3195(NH).^{1}HNMR (δ/ppm, DMSO-d_6): 2.23 (s,3H, CH_3) , 5.54 (s,1H, H-5 pyrone), 5.86(d,1H,H-5pyrimidine),7.16(d,1H,H-6 pyrimidine),13.42 (s,1H,NH),14.28 (s,1H,OH).^{13}CNMR (δ/ppm,DMSO-d_6): 22.10 (CH_3), 97.56,100.42 , 104.46,157.09,144.12, 167.47, 175.63(CO)182.16 (CS). Anal.% Calcd for $C_{10}H_8N_2O_3S$: C, 50.84; H, 3.41;N,11.86. Found: C, 50.92; H,3.56;N,11.68 .

7: Rrecrystallized from ethanol as needles. (2.2, 86%) m.p.160-162°C. $\nu_{max.}$ (cm^{-1}, KBr): 1249(CS), 1694(CO), 3310 (OH).^{1}HNMR (δ/ppm, DMSO-d_6): 2.20 (s,3H,CH_3) , 2.47(N-CH_3),5.62 (s,1H, H-5 pyrone), 5.34(d,1H,H-5pyrimidine),7.00(d,1H,H-6 pyrimidine),14.66 (s,1H,OH).^{13}CNMR (δ/ppm,DMSO-d_6): 22.12 (CH_3),37.72(N-CH_3), 101.48), 98.62,102.87, 156.89,144.56,164.10, 167.78, 174.42(CO) ,183.82 (CS).Anal.% Calcd for $C_{11}H_{10}N_2O_3S$: C, 52.79; H, 4.03;N,11.19. Found: C, 52.89; H,4.18;N,11.18 .

8: Rrecrystallized from ethanol as needles. (2.6, 84%) m.p.130-132°C. $\nu_{max.}$ (cm^{-1}, KBr): 1252(CS),1702(CO), 3318 (OH).^{1}HNMR (δ/ppm, DMSO-d_6): 2.16 (s,3H,CH_3) , 5.70 (s,1H, H-5 pyrone), 5.39(d,1H,H-5pyrimidine), 6.60-7.44 (m, 6H, Ph H +Pyrimidine H-6), 14.58 (s,1H, OH).^{13}CNMR (δ/ppm,DMSO-d_6): 20.25 (CH_3), 97.16,101.65, 114.49, 123.79, 130.94,138.67, 143.18,150.79,160.37,162.13,165.19 (Ar C),173.75 (CO),184.63(CS).Anal.% Calcd for $C_{11}H_{10}N_2O_3S$: C, 52.79; H, 4.03; N,11.19. Found: C, 52.89; H,4.18;N,11.18 .

3-([1,2,4]triazolo[4,3-a]pyrazin-5-yl)-4-hydroxy-6-methyl-2H-pyran-2-one 9

A solution of 3-(3-Dimethylaminoacryloyl)-4-hydroxy-6-methylpyran-2-one **1** (2.2g, 10 mmol) in glacial acetic acid (20 mL) was refluxed with 3-amino-1H-[1,2,4]triazole (0.8g, 10 mmol) for 5h. The reaction mixture was concentrated and the separated product was

filtered, washed with ethanol and recrystallized from ethanol in cream needles (1.7g, 72%) m.p.174-176°C. $\nu_{max.}$ (cm^{-1}, KBr):1711(CO), 3288(OH). ^{1}HNMR (δ/ppm, DMSO-d_6): 2.21 (s,3H, CH_3) , 6.13 (s,1H, H-5 pyrone), 8.81-9.34 (m,3H,ArH) 15.25(s,1H,OH). ^{13}CNMR (δ/ppm,DMSO-d_6): 20.74 (CH_3), 93.40, 100.48,125.02,125.79,128.96,129.83,136.20,146.37,162.41(Ar C), 183.77(CO). Anal.% Calcd for $C_{11}H_8N_4O_3$: C, 54.10; H, 3.30;N,22.94. Found: C, 54.25; H,3.25;N,22.78 .

3-(4H-Benzo[h]chromen-2-yl)-4-hydroxy-6-methyl-2H-pyran-2-one 10 and 3-(1H-Benzo[f]-chromen-3-yl)-4-hydroxy-6-methyl-2H-pyran-2-on and 11

A solution of enaminone **1**(2.2g,10 mmol) in glacial acetic acid (20 mL) was refluxed with α- or β-naphthole (1.4g,10 mmol) for 12h.The reaction mixture was concentrated and the separated product was filtered, washed with methanol and recrystallized from ethanol in dark brown needles.

10: Rrecrystallized from ethanol as needles. (2.1, 70%) m.p. 129-130°C. $\nu_{max.}$ (cm^{-1}, KBr): 1696(CO), 3319 (OH).^{1}HNMR (δ/ppm, DMSO-d_6): 2.12 (s,3H,CH_3) ,3.90(d,2H, CH_2 chromone),5.33 (t,1H,H-3 chromen),5.92 (s,1H, H-5 pyrone), 7.01-8.18 (m, 6H, Ar). ^{13}CNMR (δ/ppm,DMSO-d_6): 22.21 (CH_3), 27.24 (C-3 chromen), 97.82, 112.70, 116.46,116.60,125.89, 126.26,127.28, 127.34,128.10,128.73,129.50,136.66,140.25,148.22,150.69,161.59 (Ph-C), 179.13(CO).Anal.% Calcd for $C_{19}H_{14}O_4$: C, 74.50; H, 4.61;. Found: C, 74.47; H,4.59 .

11: Rrecrystallized from ethanol as needles. (2.1, 70%) m.p.125-126°C. $\nu_{max.}$ (cm^{-1}, KBr): 1698(CO), 3322 (OH).^{1}HNMR (δ/ppm, DMSO-d_6): 2.24 (s,3H,CH_3) ,3.86(d,2H, CH_2), 5.37(t,1H,H-3 chromen),6.05 (s,1H, H-5 pyrone), 7.15-8.12 (m, 6H, Ar H) 14.98 (s,1H, OH).^{13}CNMR (δ/ppm, DMSO-d_6): 22.22 (CH_3), 27.19 (C-3 chromen), 97.42,99.62, 103.11, 117.57,122.98, 125.43,126.12,127.36,127.72,127.89,128.33,132.83, 143.22,154.25, 166.45, 169.34(Ar-C), 191.43(CO).Anal.% Calcd for $C_{19}H_{14}O_4$: C, 74.50; H, 4.61;. Found: C, 74.52; H,4.48 .

2-(4-Hydroxy-6-methyl-2-oxo-2H-pyran-3-yl)-4H-chromene-8-carboxaldehyde 12

A solution of enaminone **1**(2.2g,10 mmol) in glacial acetic acid (20 mL) was refluxed with salicylaldehyde (1.2g,10 mmol) for 12h. The reaction mixture was concentrated and the separated product was filtered, washed with ethanol and recrystallized from ethanol in

brown needles. (2.3g, 82%) m.p.136-137°C. $\nu_{max.}$ (cm^{-1}, KBr):1699(CO), 1725(HCO), 3343(OH). 2.15 (s,3H,CH_3) ,3.88(d,2H, CH_2),5.34 (t,1H,H-3 chromen), 6.01 (s,1H, H-5 pyrone), 7.02-7.26 (m, 3H, Ar H),10.02(s,1H,CHO), 12.06(s,1H,OH). ^{13}CNMR (δ/ppm, DMSO-d_6): 20.66 (CH_3), 27.30 (C-4 chromen), 93.01, 100.40, 114.40,120.37,125.77,126.83,128.96,131.06, 146.36,158.14, 160.56,162.96 (Ar-C), 174.80 (CO),183.66 (CO). Anal.% Calcd for $C_{16}H_{12}O_5$: C, 67.60; H, 4.25. Found: C, 67.56; H,4.19.

4-Hydroxy-3-(8-hydroxy-4H-chromen-2-yl)-6-methylpyran-2-one 13

A solution of enaminone **1**(2.2g,10 mmol) in glacial acetic acid (20 mL) was refluxed with resorcinol (1.1g,10 mmol) for 12h. The reaction mixture was concentrated and the separated product was filtered, washed with ethanol and recrystallized from ethanol in brown needles. (2.1g, 76%) m.p.130-132°C. $\nu_{max.}$ (cm^{-1}, KBr):1687(CO), 3343 (broad OH). 2.19 (s,3H,CH_3) ,3.24(d,2H, CH_2),5.35(t,1H,H-3 chromen), 6.20 (s,1H, H-5 pyrone), 6.88-7.02 (m, 3H, Ar H),10.24(s,1H,OH), 15.12(s,1H,OH). ^{13}CNMR (δ/ppm, DMSO-d_6): 22.21 (CH_3), 27.40 (C-3 chromen), 96.92,99.28,103.01, 123.22,125.51, 126.29,127.33, 135.54,144.28,155.85,158.23, 167.40 (Ar-C), 190.45 (CO). Anal.% Calcd for $C_{15}H_{12}O_5$: C, 66.17; H, 4.44. Found: C, 66.20; H,4.28

3-(Bromoacetyl)-4-hydroxy-6-methyl-2H-pyran-2-one 14

A glacial acetic acid solution (10 mL) of bromine (0.80 g, 5 mmol) was added to a hot solution of the DHA (0.84, 5 mmol) in glacial acetic acid (20 mL) and refluxed for 2 h [23]. The reaction mixture was poured in a mixture of water (100 mL) and ice (50 g) and the obtained solid filtered off and recrystallized from the hexane– chloroform mixture(1:1). This compound was obtained as yellow small crystals. (1.7g, 72%) m.p. 118–119°C (*Lit.*[20]m.p. 111–114 °C). $\nu_{max.}$ (cm^{-1}, KBr): 1692,1712 (2CO), 3162(broad OH)). ^{1}HNMR (δ/ppm, $CDCl_3$) 2.47 (s,3H,CH_3), 4.68(s,2H, CH_2Br),5.99(s,1H,H-5),15.48(s,1H,OH).^{13}CNMR(δ/ppm,$CDCl_3$):18.23(CH_3),20.85(CH_3), 35.23 (CH_2Br),98.29(C-3),101.31(C-5),160.64(C-6),180.98(C-4),170.13 (CO), 196.32 (CO).Anal.% Calcd for $C_8H_7BrO_4$: C,38.89; H, 2.86. Found: C,38.77; H, 2.90.

3-[2-(2-Amino-alkylamino)acetyl]-4-hydroxy-6-methylpyran-2-ones 15-17

A solution of 3-(Bromoacetyl)-4-hydroxy-6-methyl-2H-pyran-2-one **14** (2.47 g, 10 mmol) and the appropriate diamine (10 mmol) in ethanol (5 mL) was stirred for 1h. The obtained solid was filtered and recrystallized from ethanol.

15: Recrystallized from ethanol as needles. (1.9g, 84%) m.p. 132-134°C. ν $_{max.}$ (cm^{-1}, KBr): 1672,1720(2CO), 2978 (broad OH)), 3322-3345 (NH&NH_2). ^{1}HNMR (δ/ppm, $CDCl_3$): 2.15 (s,3H,CH_3), 2.70 (m,2H,CH_2NH_2), 3.03(m,2H,CH_2NH), 4.25(d,2H,CH_2CO),5.77(s,1H,H-5),5.25(t,2H,NH_2),7.21(m,1H, NH),15.48(s,1H,OH). ^{13}CNMR(δ/ppm,$CDCl_3$):22.65(CH_3),43.32 (CH_2NH_2),53.12(CH_2NH),57.62 (CH_2CO), 99.34(C-3),103.97 (C-5),146.52(C-6), 179.71(C-4),169.45,196.25(CO). Anal.% Calcd for $C_{10}H_{14}N_2O_4$: C,53.09; H, 6.24; N, 12.38. Found: C,53.16; H, 6.32; N, 12.45.

16: Oily derivative(2.1g, 78%). $\nu_{max.}$ (cm^{-1}, KBr): 1676,1718(2CO), 2962(broad OH), 3366-3384(NH_2 &NH). ^{1}HNMR (δ/ppm, DMSO-d_6): 1.29-1.55(m,6H,3CH_2), 2.13(s,3H, CH_3), 2.54-2.68(m,4H,2CH_2), 4.26(d,2H,CH_2CO)6.01 (s,1H, H-5 pyrone), 5.12(t,2H,NH_2), 7.12(s, 1H, NH),14.97(s,1H,OH).^{13}CNMR (δ/ppm, DMSO-d_6): 21.95 (CH_3), 24.85,31.65,34.12,42.32,49.02 (5CH_2), 57.65 (CH_2CO),98.27(C-3),104.85(C-5),146.53(C-6), 175.32(C-4)177.22,193.34 (2CO).

17: Oily derivative(1.9g, 72%). $\nu_{max.}$ (cm^{-1}, KBr): 1678,1720(2CO), 2955 (broad OH), 3358-3378(NH_2 &NH). ^{1}HNMR (δ/ppm, DMSO-d_6): 1.28-1.46(m,12H,6CH_2), 2.14(s,3H, CH_3), 2.55-2.65(m,4H,2CH_2), 4.25(d,2H,CH_2CO)6.02 (s,1H, H-5 pyrone), 5.48(t,2H,NH_2), 7.16 (m, 1H, NH),14.34 (s,1H,OH).^{13}CNMR (δ/ppm, DMSO-d_6): 21.87 (CH_3), 27.43,27.90,30.02,30.12,31.93, 34.47,42.32,49.03 (8CH_2),57.93 (CH_2CO), 98.83(C-3),103.95(C-5),145.79(C-6), 176.02(C-4)178.12,192.16 (2CO).

3-{2-[2-(2-Aminoethylamino)ethylamino]acetyl}-4-hydroxy-6-methylpyran-2-one 18

A solution of 3-(Bromoacetyl)-4-hydroxy-6-methyl-2H-pyran-2-one **14** (2.47 g, 10 mmol) and diethylenetriamine (1.03g,10 mmol) in ethanol (30 mL) was stirred for 2h. The obtained solid was filtered and recrystallized from ethanol as needles. (1.9g, 84%) m.p. 184-186°C. ν $_{max.}$ (cm^{-1}, KBr): 1676,1722(2CO), 2969 (broad OH)), 3343-3358 (NH&NH_2). ^{1}HNMR (δ/ppm, $CDCl_3$): 2.14 (s,3H,CH_3),2.67-2.81 (3m,8H,4CH_2), 4.20(d,2H,CH_2CO), 6.21(s,1H,H-5),6.84(t,2H,NH_2),7.88(s,1H,NH), 8.12(s, 1H, NH),15.54(s,1H,OH).^{13}CNMR(δ/ppm, $CDCl_3$): 20.72(CH_3),35.57,40.58, 50.41, 55.23 (4CH_2), 58.89(CH_2CO),99.94(C-3),101.12 (C-5),141.98(C-

6), 180.23(C-4), 168.54,183.17(CO). Anal.% Calcd for $C_{12}H_{19}N_3O_4$: C,53.52; H, 7.11; N, 15.60. Found: C,53.46; H, 7.19; N, 15.63.

4-hydroxy-6-methyl-3-(1,4,5,6-tetrahydropyrazin-2-yl)pyran-2-one 19

A solution of 3-[2-(2-aminoethylamino)acetyl]-4-hydroxy-6-methylpyran-2-one **14** (2.4 g, 10 mmol) in glacial acetic acid (20 mL) was refluxed for 2h. The obtained solid was filtered and recrystallized from ethanol as needles. (1.9g, 84%) m.p. 236-238°C. ν $_{max.}$ (cm^{-1}, KBr): 1716(CO), 2955 (broad OH)), 3367 (NH). ^{1}HNMR (δ/ppm, $CDCl_3$): 2.154 (s,3H,CH_3), 3.09 (s,4H,2CH_2),4.01(s,2H,2NH), 5.25(s,1H,H-3Pyrazine) 6.07(s,1H,H-5)), 15.50 (s,1H,OH).^{13}CNMR(δ/ppm, $CDCl_3$): 19.96(CH_3),55.90(2CH_2 109.32(C-3 pyrazine),129.95(C-2pyrazine), 96.49(C-3),107.39, (C-5),115.80, 128.70,162.99(C-6), 184.84(C-4), 172.44(CO). Anal.% Calcd for $C_{10}H_{12}N_2O_3$: C,57.68; H, 5.81; N, 13.45. Found: C,57.49; H, 5.76; N, 13.53.

4-hydroxy-3-[2-(2-hydroxyethylamino)acetyl]-6-methyl-2H-pyran-2-one 20

A solution of 3-(Bromoacetyl)-4-hydroxy-6-methyl-2H-pyran-2-one **14** (2.47 g, 10 mmol) and ethanolamine (0.61g,10 mmol) in absolute ethanol (5 mL) was stirred for 1h. The obtained solid was filtered and recrystallized from ethanol. as needles. (1.8g, 79%) m.p. 128-130°C. ν $_{max.}$ (cm^{-1}, KBr): 1678,1720 (2CO), 2955 (broad OH)), 3362 (NH). ^{1}HNMR (δ/ppm, $CDCl_3$): 2.15 (s,3H,CH_3),2.73(m,2H,CH_2N),3.46 (m,2H,CH_2OH), 3.78(m, 1H, OH),4.22(d,2H,CH_2CO), 6.21(s,1H,H-5),5.44(m,1H,NH), 15.74(bs,1H,OH). ^{13}CNMR (δ/ppm, $CDCl_3$): 19.95(CH_3),37.42(CH_2N), 55.97(CH_2CO),67.67(CH_2OH),96.47(C-3), 107.40 (C-5),149.14(C-6), 184.81(C-4),163.96,172.61(2CO). Anal.% Calcd for $C_{10}H_{13}NO_5$: C,52.86; H,5.77; N, 6.16. Found: C,52.76; H, 5.81; N, 6.09.

1,2-Di(3-acetylamino)-4-hydroxy-6-methyl-2-oxo-pyrano)ethane 21

A solution of 3-(Bromoacetyl)-4-hydroxy-6-methyl-2H-pyran-2-one **36** (2.47 g, 10 mmol) and 1,2-diaminoethane (1.2g,20 mmol) in ethanol (5 mL) was stirred for 1h. at room temperature.The obtained solid was filtered and recrystallized from ethanol as needles. (2.9g, 70%) m.p. 185-186 °C. ν $_{max.}$ (cm^{-1}, KBr): 1674,1718(2CO), 3018 (broad OH), 3364 (NH). ^{1}HNMR (δ/ppm, $CDCl_3$): 2.18 (s,6H,2CH_3), 3.92 (s,4H,2CH_2N), 3.01(s,4H,2CH_2CO),6.20(s,2H,2H-5),7.21 (m,2H, NH),15.46(s,2H,2OH).^{13}CNMR(δ/ppm, $CDCl_3$):19.49(CH_3), 40.58(2CH_2 CO),57.17 (CH_2NH_2), 96.40(C-3),107.40 (C-5),163.69(C-6),

184.85(C-4),172.78,182.84(CO). Anal.% Calcd for $C_{20}H_{26}N_2O_8$: C,56.86; H, 6.20; N, 6.63. Found: C,56.92; H, 6.30; N, 6.56.

3.2. Methodology of the In vitro MTT cytotoxicity assay

The synthesized compounds were investigated for their *in vitro* cytotoxic effect *via* the standard [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] method (MTT)[21,22] against a panel of three human tumor cell lines namely; Caucasian breast adenocarcinoma MCF7, hepatocellular carcinoma HepG2 and colon carcinoma HT29. The procedures were done in a sterile area using a Laminar flow cabinet biosafety class II level (Baker, SG403INT, Stanford, ME, USA). Cells were batch cultured for 10 days, then seeded at concentration of $10x10^3$ cells/well in fresh complete growth medium in 96-well microtiter plastic plates at 37°C for 24h under 5% CO_2 using a water jacketed carbon dioxide incubator (Sheldon, TC2323, Cornelius, OR, USA). Media was aspirated, fresh medium (without serum) was added and cells were incubated either alone (negative control) or with different concentrations of the test compounds to give a final concentration of (100 – 50 – 25 – 12.5 – 6.25 – 3.125 – 1.56 – 0.78 µg/mL). DMSO was employed as a vehicle for dissolution of the tested compounds and its final concentration on the cells was less than 0.2%. Cells were suspended in RPMI 1640 medium (for HepG2 and HT29 cell lines) and DMEM (for MCF 7 cell line), 1% antibiotic-antimycotic mixture (10,000 IU/mL penicillin potassium, 10,000 µg/mL streptomycin sulphate and 25 µg/mL amphotericin B), and 1% L-glutamine in 96-well flat bottom microplate at 37°C under 5% CO_2. After 24h of incubation, the medium was aspirated, 40 µL of MTT salt (2.5 µg/mL) were added to each well and incubated for further 4h at 37°C under 5% CO_2. To stop the reaction and dissolve the formed crystals, 200 µL of 10% sodium dodecyl sulphate (SDS) in deionized water was added to each well and incubated overnight at 37°C. The absorbance was then measured using a microplate multi-well reader (Bio-Rad Laboratories Inc., model 3350, Hercules, California, USA) at 595 nm and a reference wavelength of 620 nm. A statistical significance was tested between samples and negative control (cells with vehicle) using independent *t*-test by SPSS 11 program. The results are presented in Tables 1&2 as LC_{50} (µg/mL) which is the lethal concentration of the compound which causes death of 50% of the cells in 24 h.

4. Conclusions

In this paper, some new 2-pyrano derivatives were synthesized and their structures were confirmed by elemental analysis, IR, ^{1}H and ^{13}C NMR spectral analysis. Preliminary biological testing of some of these compounds revealed that some derivatives exhibited significant anticancer activity.

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