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SIMPLE SYNTHESIS, SPECTRAL CHARACTERIZATION AND BIOLOGICAL EVALUATION OF DIHYDROXY-XANTHONE DERIVEDMANNICH BASE DERIVATIVES AS POTENTIAL ANTICANCER COMPOUNDS

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Abstract:

*Natural and synthetic xanthone derivatives exhibit a broad spectrum of biological activities, such as antioxidant activity, inhibition of cholinesterase activity, and α-glucosidase inhibitory activity. Mannich bases of 1, 3-dihydroxyxanthone derivatives were prepared by optimized synthetic route. 1, 3-dihydroxyxanthone was firstly obtained through the one-pot reaction using salicylic acid and phloroglucinol as the raw materials. The corresponding Mannich bases of 1, 3- dihydroxy-xanthone derivatives were found to be novel compounds and were characterized by MS, NMR and IR spectra. All the prepared compounds were evaluated for their cytotoxicity invitro against six cancer cell lines by MTT assay [28], with 5-flurouracil (5-Fu) as the positive control. Their structures were confirmed by IR, MS, NMR spectra and their cytotoxicity against six cancer cell lines was tested. The results showed that most of the compounds showed moderate to potent cytotoxic activity. The most active compound was **4c** with IC50 value of 1.47 μM against TCA. Further extensive research is necessary to establish the activity of above specified mannich base derivatives of dihydroxy xanthone derivatives*

Key words: *Mannich Base, Xanthone derivatives, Cytotoxicity study, spectral analysis.*

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INTRODUCTION:

The xanthone nucleus or *9H*-xanthen-9-one (dibenzo-γ pirone**1**, Fig. (**1**)) comprises an important class of theoxygenated heterocycles [1]. Natural xanthones can be subdivided, depending on the nature of the substituents in the dibenzo-γ -pirone scaffold, into: simple oxygenated xanthones, glycosylated xanthones, prenylated xanthones and their derivatives, xanthone dimers, xanthonolignoids and miscellaneous [1-4]. On the other hand, the xanthones of a synthetic origin can have simple groups such as hydroxyl, methoxyl, methyl, carboxyl, as well as more complex substituents such as epoxide, azole, methylidenebutyrolactone, aminoalcohol, sulfamoyl, methylthiocarboxylic acid, and dihydropyridine in their scaffold. Since the growing interest in this class of compounds has been associated with the pharmacological properties demonstrated by both natural and synthetic derivatives, this aspect will be emphasized in this review. Xanthone (9*H*-xanthen-9-one or dibenzo-γ-pyrone) is an important pharmacophore with simple three-membered ring at the linear plane (Fig. **1**). Xanthones are mainly found as secondary metabolites in higher plants and microorganisms.

Natural and synthetic xanthone derivatives exhibit a broad spectrum of biological activities, such as antioxidant activity, inhibition of cholinesterase activity, and α-glucosidase inhibitory activity [1-3].At present, xanthones are of documented relevance to human diseases. The most remarkable example is dimethylxanthenone-4-acetic acid or DMXAA (This compound is currently undergoing clinical trials as an antitumor agent. Based on the different substituents and positionson the core ring, xanthones could interact with variouspharmacological targets. Large numbers of natural and syntheticxanthone compounds show potential antitumor activities[4-10].

Synthesis: Mannich bases of 1, 3-dihydroxyxanthone derivatives (**1a~1e**, **2a~2e**, **3a~3e**, **4a~4e**) were repaired as shown in Scheme **1**. 1, 3-dihydroxyxanthone was firstly obtained through the one-pot reaction using salicylic acid and phloroglucinol as the raw materials [23, 24]. Then, etherification of the hydroxyl at the position 3 of 1, 3-dihydroxyxanthone was carried out under alkaline condition and intermediate compound**4** with different substituents were thus obtained [14]. Finally, compounds **1 and 4** were reacted with formaldehyde and various secondary amines in the methanol or acidic solution by the Mannich reaction, respectively [15, 25], yielding the corresponding Mannich bases of 1, 3- dihydroxy-xanthone derivatives. All the new compounds (**1a-1e and 4a-4e**) were characterized by MS, NMR and IR spectra.

EXPERIMENTAL:

All the reagents and chemicals were obtained from commercialsuppliers and used without further purification unlessstated. All reactions were monitored by thin layer chromatography(TLC) and spots were located by UV lamp or iniodine chamber. Melting points were measured in X-4 micro-melting point instrument and were uncorrected. IR spectrawere taken on Nicolet ESP 360 FI-IR using KBr pellets.Direct MS spectra were performed on ESQUIRE HTC instrumentin positive mode. 1H NMR and 13C NMR spectrawere recorded in CDCl3 or Acetone on Bruker AVANCEAV 500/125 MHz instruments. Chemical shifts were reportedas δ ppm using tetra-methylsilane (TMS) as the internalstandard and couplings expressed in Hertz. Spin multiplicitieswere given as follows: s (singlet), d (doublet), t(triplet), m (multiplet), or br (broad). Column chromatographywas accomplished on Qingdao silica gel (100-200, 200-300 or 300-400 mesh).

PROCEDURES FOR THE SYNTHESIS OF 1, 3-DIHYDROXYXANTHONE (1):

To a mixture of salicylic acid (13.8 g, 0.1 mol), phloroglucinol(12.6 g, 0.1 mol), and freshly fused zinc chloride(30 g), phosphoryl chloride (80 mL) was added. The reactionmixture was stirred at 65~70 °C for 4~8 h, then slowlypoured into crushed ice, and allowed to stand overnight. Thesolid was collected by filtration, washed with saturatedaqueous NaHCO3 and water, dried. The crude products werepurified by column chromatography (PE: EtOAc = 4: 1) toafford **1** as yellow solids.

SPECTRAL ANALYSIS OF 1, 3-DIHYDROXYXANTHONE (1):

*1,3-dihydroxyxanthone(**1**)* 11.4 g 1,3-dihydroxyxanthoneas obtained as light yellow solid. Yield: 50 %; m.p.260~262 °C; (reference: 256~258 °C); IR (KBr), *v*(cm–1):3327, 1654, 1610, 1570, 1491, 1470, 1445, 1222, 1163,1078, 827, 762; 1H NMR (500 MHz, DMSO-*d*6), δ: 12.80(s, 1 H, O**H**-1), 11.07 (s, 1 H, 3-O**H**), 8.10 (dd, *J* = 8.0, 1 H,1.5 Hz, H-8), 7.80~7.83 (m, 1 H, HG-6), 7.55 (d, *J* = 8.0 Hz, 1H, H-5), 7.42~7.45 (m, 1 H, H-7), 6.37 (d, *J* =2.1 Hz, 1 H,H-4), 6.20 (d, *J* = 2.1 Hz, 1 H, H-2).

GENERAL PROCEDURE FOR THE SYNTHESIS OF COMPOUND-4:

To a solution of **1** (4.50 g, 19.7 mmol) in acetone (200mL) was added freshly ignited K2CO3 (3.40 g, 24.6 mmol)followed by methyl iodide, allyl bromide, or prenyl bromideat room temperature. Each reaction mixture was separatelystirred at room temperature or refluxed for 4~8 h. The progressof the reaction was monitored by TLC. On

completionof the reaction, the solvent was removed by filtration, thefiltrate was evaporated under reduced pressure and the residuewas purified by column chromatography to yield the compound-**4.**

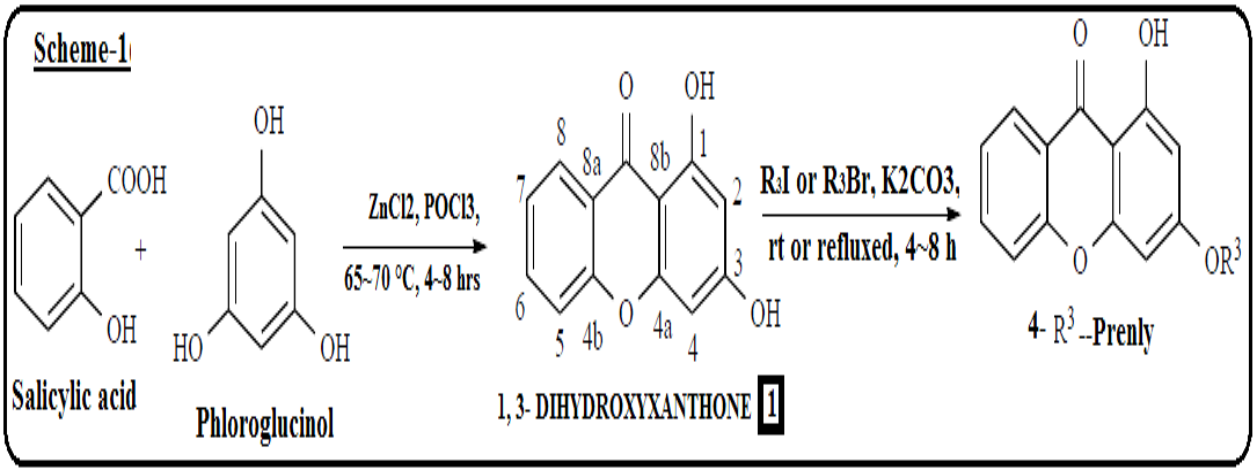

Figure-1: General synthesis of compound-4

1-hydroxy-3-(3-methylbut-2-enyloxy)-9H-xanthen-9-one (4):

Compound **1** and prenyl bromide (2.95 mL, 24 mmol)were reacted according to the general procedure at reflux.The crude product was purified by column chromatographyeluting with PE: EtOAc = 16:1 to yield **4** as a yellow-whitesolid. Yield: 83.6%; m.p. 137~138 °C; 1H NMR (CDCl3, 500MHz) *δ*: 12.88 (s, 1H, OH), 8.27 (dd, *J*=1.4, 8.0 Hz, 1H, H-8), 7.73 (td, *J*=1.6, 8.6 Hz, 1H, H-6), 7.45 (d, *J*=8.5 Hz, 1H,H-5), 7.40 (t, *J*=7.5 Hz, 1H, H-7), 6.46 (d, *J*=2.1 Hz, 1H, H-4), 6.38 (d, *J*=2.2 Hz, 1H, H-2), 5.53 (t, *J*=6.7 Hz, 1H, C**H**=),4.63 (d, *J*=6.8 Hz, 2H, OC$\mathbf{H}_2$), 1.85 (s, 3H, =C-C$\mathbf{H}_3$), 1.80(s, 3H, =C-C$\mathbf{H}_3$).

Structure Elucidation: For the 1, 3-dihydroxyxanthone and the oxygenated derivative(**4**), 2- and 4-position are active sites and theMannich reaction can be processed in these two position. Inorder to establish the exact position of substituents of thetitle compounds, we hypothesized that if the reaction wascarried out at the position 2, the H of CH2 inducted by MannichReaction would correlate with C-1 of xanthone ring inHMBC, if not the H of CH2 correlated with C-4a. To validatethe hypothesis, **2c** was subjected to HSQC and HMBC spectralanalysis, which were shown in Table **1**. IR spectrashowed absorption band at 3425 cm–1, indicating the presenceof a hydroxyl group. The ketone C=O stretching bandwas observed at about 1625 cm–1. The absorption bands at1622, 1492, 1468, 1443 cm–1 indicated the presence ofphenyl groups. It was not difficult to confirm the 1H NMRspectral assignments of **2c** by analyzing its coupling relationand comparing its 1H NMR data with that of 1, 3-dihydroxyxanthone [26]. In 1H NMR spectrum, the consecutivearomatic protons of A ring were observed at 8.24 (d,*J*=7.9 Hz, 1H), 7.35 (t, *J*=7.5 Hz, 1H), 7.68 (t, *J*=7.9 Hz,1H), 7.40 (d, *J*=8.4 Hz, 1H); the only reserved proton of Cring was observed at 6.43 (s, 1H) and the inducted CH_2Nwas observed as singlet at 3.76 ppm, which indicated thatone of the aromatic protons of C ring (2- or 4-position) wasreplaced by the Mannich reaction. Through the HSQC,HMBC spectra, and comparing the **13**C NMR data with thatof 1, 3-dihydroxyxanthone [27], its 13C NMR assignmentswere easily accomplished, in which C-1 and C-4a in xanthonecore were observed at 161.70, 157.54, respectively. Inthe HMBC spectrum, the proton of CH**2**N was observed tocorrelate with C-1 of xanthone ring, not with C-4a, whichconfirmed that the Mannich reaction was carried out at theposition 2. The main connectives found in the HMBC weredepicted in Fig. (**2**). Other target compounds' spectral datawas similar to that of **2c** and all the new compounds werecharacterized by MS, NMR and IR spectra.

GENERAL PROCEDURE FOR THE PREPARATION OF COMPOUNDS 1A~1E:

Paraformaldehyde (60 mg, 2 mmol) was dissolved in methanol (15 mL) at 65 °C, treated with the corresponding secondary amines (2.5 mmol), stirred for 1 h. Then a solution of 1, 3-dihydroxyxanthone (0.456 g, 2 mmol) in methanol (10 ml) was added to the reaction mixture. After stirring at reflux for 4~12 h, the progress of the reaction was monitored by TLC. On completion, the reaction mixture was cooled, concentrated under vacuum and then subjected tocolumn chromatography on silica gel to offer compounds**1a~1e** respectively.

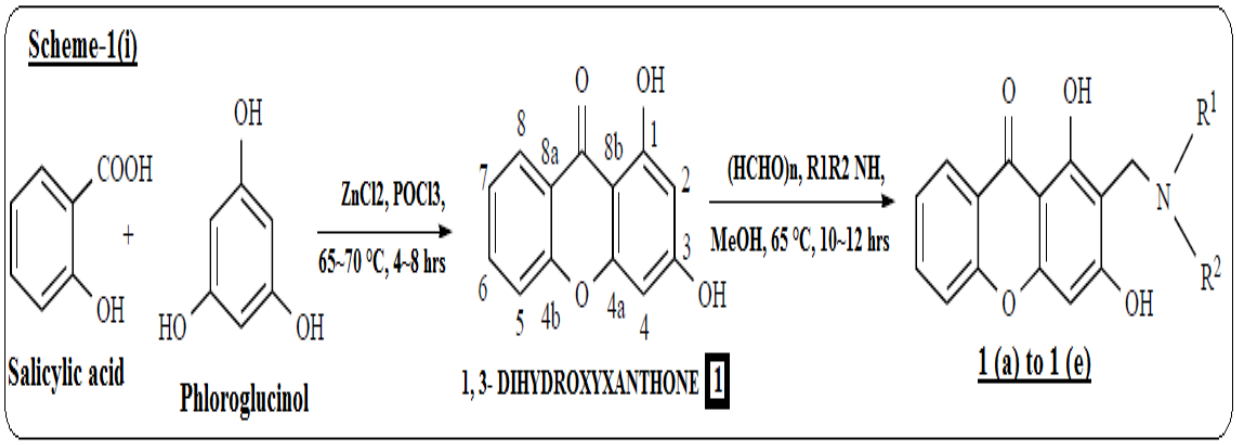

Figure-2: General synthesis of Compund-1and derivatives of 1a-1e:

2-((dimethylamino)methyl)-1,3-dihydroxy-9H-xanthen-9- one (1a):

Compound **1** and a solution of dimethylamine (0.63 mL,33%, 3 mmol) were reacted were reacted according to thegeneral procedure. The crude product was purified by columnchromatography eluting with chloroform: methanol =20: 1 to yield **1a** as a yellow solid. Yield: 31.5%; m.p.148~149 °C; IR (KBr) *v*: 3810, 3450, 2846, 2368, 1651,1615, 1507, 1467, 1384, 1359, 1322, 1303, 1224, 1173,1133, 1108, 1078, 1057, 838, 751, 606 cm–1; 1H NMR ($CDCl_3$, 500 MHz) *δ*: 8.23 (dd, *J*=1.6, 7.8 Hz, 1H, H-8), 7.68 (td,*J*=1.6, 7.3 Hz, 1H, H-6), 7.40 (d, *J*=8.4 Hz, 1H, H-5), 7.34 (t,*J*=7.45 Hz, 1H, H-7), 6.36 (s, 1H, H-4), 3.89 (s, 2H, C**H**$_2$N),2.45 (s, 6H, 2C**H**$_3$); 13C NMR (CDCl3, 125 MHz) *δ*: 180.53(C-9), 168.39 (C-3), 160.42 (C-1), 157.39 (C-4a), 155.98 (C-4b), 134.66 (C-6), 125.69 (C-8), 123.72 (C-7), 120.62 (C-8a), 117.54 (C-5), 102.66 (C-8b), 102.34 (C-2), 94.83(C-4),54.58 (**C**H3), 44.19 (**C**H2N); APCI-MS *m/z*: 285.95[M+H]+.Anal. calcd for C16H15NO4: C 67.36, H 5.30, N 4.91; found C67.20, H 5.73, N 4.92.

2-((diethylamino)methyl)-1,3-dihydroxy-9H-xanthen-9-one (1b):

Compound **1** and a solution of diethylamine (0.3 mL, 3mmol) were reacted according to the general procedure. Thecrude product was purified by column chromatographyeluting with chloroform: methanol = 40: 1 to yield **1b** as ayellow solid. Yield: 24.0%; m.p. 99~100 °C; IR (KBr) *v*:3840, 3741, 3439, 2950, 1648, 1613, 1539, 1465, 1358,1322, 1226, 1173, 1078, 828, 757, 603 cm–1; 1H NMR($CDCl_3$, 500 MHz) *δ*: 8.22 (d, *J*=8.0 Hz, 1H, H-8), 7.67 (t,*J*=7.2 Hz, 1H, H-6), 7.40 (d, *J*=8.5 Hz, 1H, H-5), 7.34 (t,*J*=7.3 Hz, 1H, H-7), 6.31 (s, 1H, H-4), 3.98 (s, 2H, C**H**$_2$N),2.73~2.81 (m, 4H, 2C**H**$_2$), 1.19 (t, *J*=7.3 Hz, 6H, 2C**H**$_3$); 13CNMR ($CDCl_3$, 125 MHz) *δ*: 179.16 (C-9), 168.35 (C-3),159.13 (C-1), 156.17 (C-4a), 154.81 (C-4b), 133.32 (C-6),124.48 (C-8), 122.43 (C-7), 119.53 (C-8a), 116.32 (C-5),101.35 (C-8b), 100.91 (C-2), 93.79 (C-4), 48.29 (2**C**H$_2$CH$_3$),45.37 (**C**H2N), 9.14 (2**C**H3); APCI-MS *m/z*: 314.05[M+H]+.Anal. calcd for C18H19NO4: C 68.99, H 6.11, N 4.47; found C68.89, H 6.12, N 4.48.

1,3-dihydroxy-2-(pyrrolidin-1-ylmethyl)-9H-xanthen-9-one (1c):

Compound **1** and a solution of pyrrolidine (0.25 mL, 3mmol) were reacted according to the general procedure. Thecrude product was purified by column chromatographyeluting with chloroform: methanol = 40: 1 to yield **1c** as ayellow solid. Yield: 19.3%; m.p. 174~176 °C; IR (KBr) *v*:3427, 2102, 1640, 1596, 1539, 1463, 1356, 1333, 1288,1223, 1169, 1146, 1100, 989, 844, 745, 590 cm–1; 1H NMR(CDCl3, 500 MHz) *δ*: 8.24 (d, *J*=7.9 Hz, 1H, H-8), 7.68 (td,*J*=1.61, 9.2 Hz, 1H, H-6), 7.41 (d, *J*=8.4 Hz, 1H, H-5), 7.35(td, *J*=0.51, 7.5 Hz, 1H, H-7), 6.33 (s, 1H, H-4), 4.06 (s, 2H,C**H**2N), 2.83 (brs, 4H, H-1' and 4'), 1.91~1.96 (m, 4H, H-2'and 3'); 13C NMR (CDCl3, 125 MHz) *δ*: 180.34 (C-9),169.12 (C-3), 160.01 (C-1), 157.36 (C-4a), 155.97 (C-4b),134.50 (C-6), 125.65 (C-8), 123.61 (C-7), 120.67 (C-8a),117.48 (C-5), 103.19 (C-8b), 102.08 (C-2), 94.87 (C-4),53.48 (C-1' and 4'), 51.05 (**C**H2N), 23.73 (C-2' and 3');APCI-MS *m/z*: 312.01[M+H]+. Anal. calcd for C18H17NO4: C69.44, H 5.50, N 4.50; found C 69.34, H 5.51, N 4.49 .

1,3-dihydroxy-2-(piperidin-1-ylmethyl)-9H-xanthen-9-one (1d):

Compound **1** and piperidine (0.3 mL, 3 mmol) were reactedaccording to the general procedure. The crude productwas purified by column chromatography eluting with chloroform:methanol = 40: 1 to yield **1d** as a yellow solid. Yield:27.7%; m.p. 147~149 °C; IR (KBr) *v*: 3436, 2937, 1650,1614, 1464, 1396, 1352, 1225, 1152, 828, 760, 613 cm–1; 1HNMR (CDCl3, 500 MHz) *δ*: 8.24 (d, *J*=7.9 Hz, 1H, H-8),7.69 (td, *J*=1.09, 8.34 Hz, 1H, H-6), 7.42 (d, *J*=8.4 Hz, 1H,H-5), 7.36 (t, *J*=7.7 Hz, 1H, H-7), 6.34 (s, 1H, H-4), 3.90 (s,2H, C**H**2N), 2.27 (brs, 4H, H-1' and 5'), 1.72 (brs, 6H,

H-2',3'and 4'); 13C NMR (CDCl3, 125 MHz) *δ*: 180.41 (C-9),168.81 (C-3), 160.43 (C-1), 157.28 (C-4a), 155.98 (C-4b),134.58 (C-6), 125.69 (C-8), 123.66 (C-7), 120.67 (C-8a),117.53 (C-5), 102.25 (C-8b), 101.99 (C-2), 94.88 (C-4),54.13 (C-1' and 5'), 53.70 (**C**H2N), 25.54 (C-2' and 4'), 23.67(C-3'); APCI-MS *m/z*: 326.03[M+H]+. Anal. calcd forC19H19NO4: C 70.14, H 5.89, N 4.31; found C 70.10, H 5.90,N 4.32 .

1,3-dihydroxy-2-(morpholinomethyl)-9H-xanthen-9-one (1e):

Compound **1** and morpholine (0.26 mL, 3 mmol) werereacted according to the general procedure. The crude productwas purified by column chromatography eluting with PE:EtOAc = 16: 1 to yield **1e** as a yellow solid. Yield: 10.0 %;m.p. 172~175 °C; IR (KBr) *v*: 3436, 2850, 2363, 1643, 1615,1573, 1442, 1419, 1345, 1330, 1289, 1229, 1173, 1145,1117, 1074, 986, 830, 760 cm–1; 1H NMR (CDCl3, 500MHz) *δ*: 8.24 (d, *J*=7.9 Hz, 1H, H-8), 7.72 (t, *J*=7.2 Hz, 1H,H-6), 7.44 (d, *J*=7.7 Hz, 1H, H-5), 7.39 (t, *J*=7.8 Hz, 1H, H-7), 6.29 (s, 1H, H-4), 4.03 (s, 2H, C**H**2N), 3.82 (brs, 4H, H-2'and 4'), 2.72 (brs, 4H, H-1' and 5'); 13C NMR (CDCl3, 125MHz) *δ*: 180.56 (C-9), 166.80 (C-3), 163.15 (C-1), 155.61(C-4a), 154.72 (C-4b), 134.77 (C-6), 125.89 (C-8), 124.12(C-7), 120.55 (C-8a), 117.37 (C-5), 103.12 (C-8b), 99.24 (C-2), 97.79 (C-4), 66.79 (C-2' and 4'), 54.01 (C-1' and 5'),52.96 (**C**H2N); APCI-MS *m/z*: 327.99[M+H]+. Anal. Calcdfor C18H17NO5: C 66.05, H 5.23, N 4.28; found C 66.21, H5.24, N 4.27.

***GENERAL PROCEDURE FOR THE PREPARATION OF COMPOUNDS- 4A~4E*:**

The selected secondary amine cooled in a ice-water bathfor about 5 min was followed by drop-wise adding with formaldehyde formaldehydesolution (1 ml, 0.0133 moles, 37~40%), glacial

Acetic acid (10 ml), then the reaction mixture was stirred forabout 1 h at room temperature, treated with **compound-4**. After stirring at room temperature for about 1~6 d orstirring at 65 °C for about 1~4 h, treated with water (50 ml),and continued stirring for about 4~6 h, and filtered. The filtratewas stirred and treated with NaOH (10%) until the pH was9~10, the precipitate was filtered, washed with water, driedand then subjected to column chromatography on silica gelto yield the compounds **4a**~**4e** respectively.

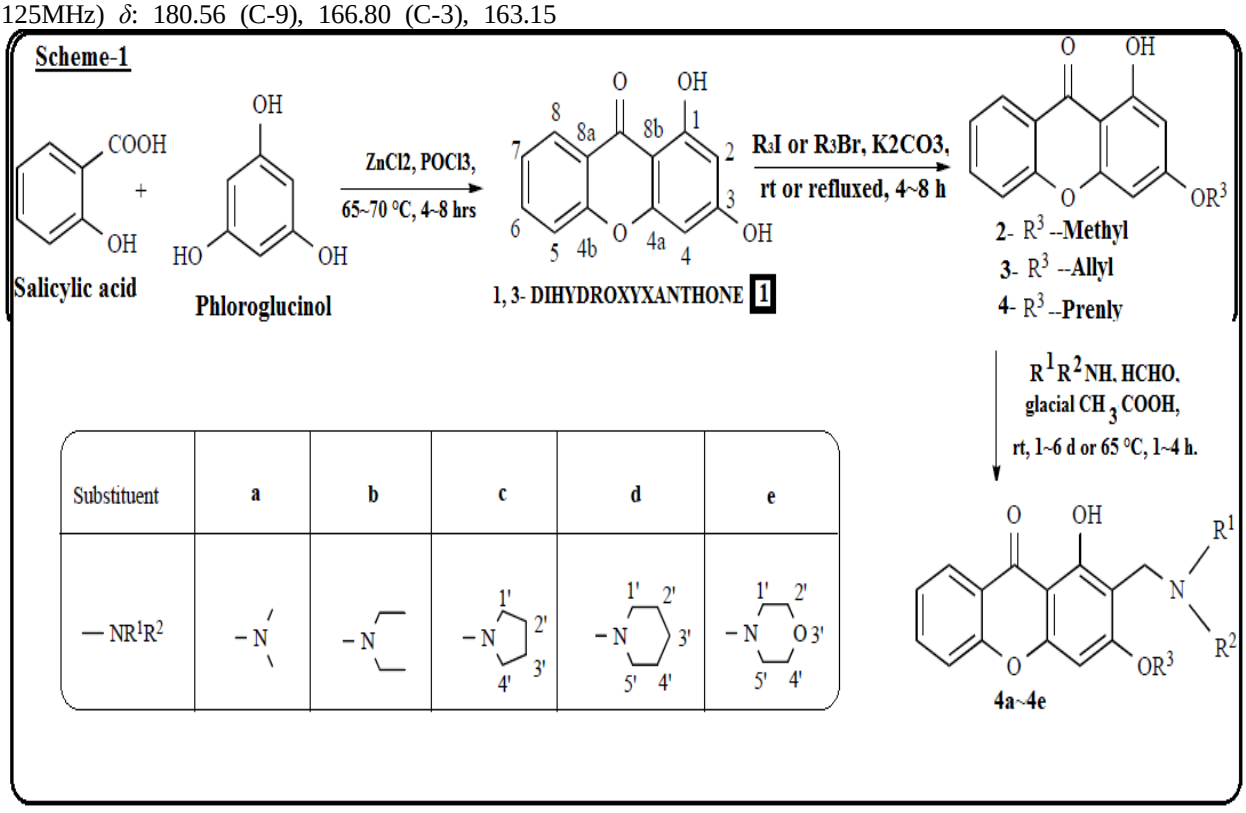

Figure-3: General Synthesis of Compounds 4a-4e:

2-((dimethyl amino) methyl)-1-hydroxy-3-(3-methylbut-2-enyloxy)-9H-xanthen-9-one (4a):

Compound **4** (0.296 g, 1 mmol) and a solution of dimethylamine (2 mL, 33%, 8.7 mmol) were reacted according to the general procedure. The crude product was purified by column chromatography eluting with chloroform: methanol: ammonia = 40: 1: 0.5 to yield **4a** as a yellow solid. Yield: 32.0%; m.p. 122~124 °C; IR (KBr) *v*: 3429, 3089, 2962, 2936, 2855, 2815, 2770, 2756, 1644, 1607, 1568, 1480,1461, 1439, 1397, 1383, 1369, 1341, 1317, 1275, 1259,1229, 1206, 1182, 1159, 1133, 1093, 1036, 1019, 1002, 975,877, 846, 819, 776, 761, 617 cm–1; 1H NMR (CDCl3, 500MHz) *δ*: 8.29 (dd, *J*=1.4, 7.9 Hz, 1H, H-8), 7.72 (td, *J*=1.5,8.5 Hz, 1H, H-6), 7.44 (d, *J*=8.4 Hz, 1H, H-5), 7.39 (t, *J*=7.7Hz, 1H, H-7), 6.47 (s, 1H, H-4), 5.53 (t, *J*=6.5 Hz, 1H,C**H**=), 4.68 (d, *J*=6.8 Hz, 2H, OC**H**$_2$), 3.59 (s, 2H, C**H**$_2$N),2.34 (s, 6H, 2NC**H**$_3$), 1.84 (s, 3H, =C-C**H**$_3$), 1.79 (s, 3H, =CC**H**$_3$);13C NMR (CDCl$_3$, 125 MHz) *δ*: 180.60 (C-9), 164.71(C-3), 161.49 (C-1), 157.09 (C-4a), 155.85 (C-4b), 138.31(C-6), 134.76 (CH$_2$**C**H), 125.91 (C-8), 123.93 (C-7), 120.80(C-8a), 118.95 (**C**H$_2$=CH), 117.46 (C-5), 109.00 (C-8b),103.64 (C-2), 90.67 (C-4), 65.85 (O**C**H$_2$), 49.71 (N**C**H$_3$),45.52 (**C**H$_2$N), 25.82 (**C**H$_3$), 18.41 (**C**H$_3$); APCI-MS *m/z*:354.06[M+H]+. Anal. calcd for C21H23NO4: C 71.37, H 6.56,N 3.96; found C 71.40, H 6.57, N 3.97.

2-((diethylamino) methyl)-1-hydroxy-3-(3-methylbut-2-enyloxy)-9H-xanthen-9-one (4b):

Compound **4** (0.296 g, 1 mmol) and diethylamine (1 mL,9.70mmol) were reacted according to the general procedure.The crude product was purified by column chromatographyeluting with chloroform: methanol: ammonia = 40: 1: 0.5 toyield **4b** as a yellow solid. Yield: 35.5%; m.p. 105~107 °C;IR (KBr) *v*: 3425, 2963, 2344, 1653, 1608, 1569, 1483, 1467,1398, 1384, 1313, 1268, 1233, 1192, 1165, 1146, 1120,1091, 1048, 982, 814, 782, 757, 612 cm–1; 1H NMR (CDCl3,500 MHz) *δ*: 8.29 (dd, *J*=1.4, 7.9 Hz, 1H, H-8), 7.71 (td,*J*=1.7, 8.6 Hz, 1H, H-6), 7.43 (d, *J*=8.3 Hz, 1H, H-5), 7.38(td, *J*=0.7, 7.9 Hz, 1H, H-7), 6.46 (s, 1H, H-4), 5.53 (t, *J*=6.6Hz, 1H, C**H**=), 4.66 (d, *J*=6.6 Hz, 2H, OC**H**2), 3.72 (s, 2H,C**H**2N), 2.66 (q, 4H, 2C**H**2CH3), 1.84 (s, 3H, =C-C**H**3), 1.79(s, 3H, =C-C**H**3), 1.13 (t, 6H, *J*=7.1 Hz, 2C**H**3) ; 13C NMR(CDCl3, 125 MHz) *δ*: 180.16 (C-9), 164.60 (C-3), 161.60(C-1), 157.05 (C-4a), 155.76 (C-4b), 138.41 (C-6), 134.54(CH2**C**H=), 125.96 (C-8), 123.70 (C-7), 121.00 (C-8a),118.92 (**C**H2=CH), 117.36 (C-5), 109.54 (C-8b), 103.87 (C-2), 90.60 (C-4), 65.70 (O**C**H2), 46.81 (**C**H2CH3), 43.67(**C**H2N), 25.75 (=CH2**C**H3), 18.31 (=CH2**C**H3), 11.28 (**C**H3);APCI-MS *m/z*: 382.09[M+H]+. Anal. calcd for C23H27NO4: C72.42, H 7.13, N 3.67; found C 72.36, H 7.13, N 3.68.

1-hydroxy-3-(3-methylbut-2-enyloxy)-2-(pyrrolidin-1-ylmethyl)-9H-xanthen-9-one (4c):

Compound **4** (0.296 g, 1 mmol) and pyrrolidine (1 mL,12.1 mmol) were reacted according to the general procedure.The crude product was purified by column chromatographyeluting with chloroform: methanol: ammonia = 40: 1: 0.5 toyield **4c** as a yellow solid. Yield: 42.7%, m.p. 151~153 °C;IR (KBr) *v*: 3791, 3431, 3069, 2967, 2947, 2873, 2814, 2783,2238, 1643, 1610, 1496, 1485, 1463, 1443, 1404, 1375,1316, 1262, 1228, 1213, 1139, 1123, 1088, 1046, 1032,1009, 975, 952, 928, 876, 838, 800, 787, 750, 611, 557 cm–1;1H NMR (CDCl3, 500 MHz) *δ*: 8.29 (dd, *J*=1.5, 8.0 Hz, 1H,H-8), 7.72 (td, *J*=1.6, 8.6 Hz, 1H, H-6), 7.44 (d, *J*=8.4 Hz,1H, H-5), 7.39 (t, *J*=7.3 Hz, 1H, H-7), 6.47 (s, 1H, H-4),5.53 (t, *J*=6.5 Hz, 1H, C**H**=), 4.67 (d, *J*=6.5 Hz, 2H, OC**H**2),3.82 (s, 2H, C**H**2N), 2.66 (s, 4H, H-1' and 4'), 1.84 (s, 3H,=C-C**H**3), 1.76~1.80 (m, 7H, =C-C**H**3, H-2' and 3'); 13CNMR (CDCl3, 125 MHz) *δ*: 180.50 (C-9), 164.57 (C-3),161.37 (C-1), 156.99 (C-4a), 155.83 (C-4b), 138.33 (C-6)134.68 (CH2**C**H), 125.90 (C-8), 123.87 (C-7), 120.82 (C-8a),118.92 (**C**H2=CH), 117.42 (C-5), 109.04 (C-8b), 103.68 (C-2), 90.58 (C-4), 65.74 (O**C**H2), 53.88 (C-1' and 4'), 45.47(**C**H2N), 25.77 (=CH2**C**H3), 23.49 (C-2' and 3'), 18.35(=CH2**C**H3); APCI-MS *m/z*: 380.09[M+H]+. Anal. calcd forC23H25NO4: C 72.80, H 6.64, N 3.69; found C 72.77, H 6.64,N 3.70.

1-hydroxy-3-(3-methylbut-2-enyloxy)-2-(piperidin-1-ylmethyl)-9H-xanthen-9-one (4d):

Compound **4** (0.296 g, 1 mmol) and piperidine (1 mL, 10.1 mmol) were reacted according to the general procedure. The crude product was purified by column chromatography eluting with chloroform: methanol: ammonia = 40: 1: 0.5 to yield **4d** as a yellow solid. Yield: 45.5%, m.p. 166~168 °C; IR (KBr) *v*: 3424, 3071, 2965, 2937, 2927, 2852, 2818, 2758, 1646, 1619, 1607, 1484, 1468, 1401, 1387, 1317, 1295, 1271, 1249, 1226, 1209, 1149, 1132, 1110, 1092, 1035,1010, 987, 970, 875, 845, 809, 756, 613 cm–1; 1H NMR (CDCl3, 500 MHz) *δ*: 8.29 (dd, *J*=1.5, 8.0 Hz, 1H, H-8), 7.72 (td, *J*=1.6, 8.6 Hz, 1H, H-6), 7.44 (d, *J*=8.4 Hz, 1H, H- 5), 7.39 (t, *J*=7.6 Hz, 1H, H-7), 6.46 (s, 1H, H-4), 5.52 (t, *J*=6.5 Hz, 1H, C**H**=), 4.65 (d, *J*=6.5 Hz, 2H, OC**H**$_2$), 3.71 (s, 2H, C**H**$_2$N), 2.55 (brs, 4H, H-1' and 5'), 1.85 (s, 3H, =CC**H**$_3$), 1.80 (s, 3H, =C-C**H**$_3$), 1.57~1.62 (m, 4H, H-2' and 4'), 1.41 (brs, 2H, H-3'); 13C NMR (CDCl$_3$, 125 MHz) *δ*: 180.34

(C-9), 164.76 (C-3), 161.63 (C-1), 157.06 (C-4a), 155.80 (C-4b), 138.51 (C-6), 134.63 (CH$_2$**C**H), 125.94 (C-8), 123.85 (C-7), 120.92 (C-8a), 118.88 (**C**H$_2$=CH), 117.39 (C-5),108.31 (C-8b), 103.77 (C-2), 90.57 (C-4), 65.68 (O**C**H2), 54.20 (C-1' and 5'), 49.52 (**C**H$_2$N), 26.02 (C-2' and 4'), 25.77 (=CH$_2$**C**H$_3$), 24.25 (C-3'), 18.34 (=CH$_2$**C**H$_3$);

APCI-MS *m/z*:394.08[M+H]+. Anal. calcd for C24H27NO4: C 73.26, H 6.92, N 3.56; found C 73.35, H 6.93, N 3.57.

1-hydroxy-3-(3-methylbut-2-enyloxy)-2-morpholinomethyl)-9H-xanthen-9-one (4e):

Compound **4**(0.296 g, 1 mmol) and morpholine(1 mL, 17.2 mmol) were reacted according to the general procedure. The crude product was purified by column chromatography eluting with chloroform: methanol: ammonia = 40: 1: 0.5 to yield **4e** as a yellow solid. Yield: 16.6%, m.p. 135~138 °C; IR (KBr) *v*: 3440, 2925, 2856, 1651, 1608, 1482, 1466, 1404, 1385, 1314, 1287, 1229, 1205, 1162, 1138, 1115, 1092, 1033, 1001, 863, 828, 788, 757, 610, 490 cm–1; 1H NMR (CDCl3, 500 MHz) *δ*: 8.29 (dd, *J*=1.5, 8.0 Hz, 1H, H-8), 7.74 (td, *J*=1.7, 8.6 Hz, 1H, H-6), 7.45 (d, *J*=8.3 Hz, 1H, H- 5), 7.40 (td, *J*=0.7, 7.9 Hz, 1H, H-7), 6.48 (s, 1H, H-4), 5.52 (t, *J*=6.6 Hz, 1H, C**H**=), 4.66 (d, *J*=6.5 Hz, 2H, OC**H**2), 3.71~3.74 (m, 6H, C**H**2N, H-2' and 4'), 2.60 (t, *J*=4.5 Hz, 4H, H-1' and 5'), 1.85 (s, 3H, =C-C**H**3), 1.80 (s, 3H, =C-C**H**3); 13C NMR (CDCl3, 125 MHz) *δ*: 180.67 (C-9), 164.87 (C-3), 161.62 (C-1), 157.18 (C-4a), 155.87 (C-4b), 138.78 (C-6),134.85 (CH2**C**H), 125.93 (C-8), 124.02 (C-7), 120.77 (C-8a), 118.71 (**C**H2=CH), 117.49 (C-5), 107.74 (C-8b), 103.63 (C- 2), 90.66 (C-4), 67.16 (O**C**H2), 65.75 (C-2' and 4'); 53.39 (C-1' and 5'), 48.96 (**C**H2N), 25.82 (=CH2**C**H3), 18.39 (=CH2**C**H3); APCI-MS *m/z*: 396.05 [M+H]+. Anal. calcd for C23H25NO5: C 69.86, H 6.37, N 3.54; found C 69.90, H 6.38,N 3.55.

Biological Activity:

Except for the insoluble intermediates of**1** and **4**, all the preparedcompounds were evaluated for their cytotoxicity *invitro* against six cancer cell lines by MTT assay [28], with 5-flurouracil (5-Fu) as the positive control. The results wereshown as IC50 values in the Table **2**.The data in Table **2** indicated that most of the testedcompounds exhibited mild to good cytotoxic activity againstthe tested cancer cells, some preliminary structure-activityrelationships were summarized as follows:(1) Effect of the substitutents at the position 3 of the 1, 3-dihydroxyxanthone. In the case of Mannich bases of 1, 3-dihydroxylxanthone(**1a~1e**) without modification at the 3 position, no obviouscytotoxic activities were observed. Most of the prenylatedxanthones and the allylatedxanthonesshowed better activity [7], the IC50 values reached micromolelevel and the most active compound was **4c** with IC50value of 1.47 μM against TCA. These results suggested thatthe hydrophobic environment and electron donating of substitutedgroups might be essential for improvement of itsanticancer activity. The alkylation of hydroxyl at the position3 of 1, 3-dihydroxyxanthone was required for the activity.(2) Effect of the different amino substituents at the position2 of the 1, 3-dihydroxyxanthoneAs for compounds with different amino substituted at theposition 2 of 1,3-dihydroxylxanthone, in most case diethylaminemethyl substituted compounds hold the best inhibitoryactivity on the cancer cell lines, while no notable activitieswere observed for the morpholino methyl substitutedcompounds with a concentration of 100 μM. Interestingly, compound-**4c** with pyrrolidinyl methyl substituted at the position 2showed potential activity against HepG2 with IC50 value of3.35 μM and TCA with IC50 value of 1.47 μM, respectively. Hence, different amino substitutions at the position 2 also haveobvious impact on its anticancer activity.

Table-1: The NMR Data of Compound 4-C ($CDCl_3$; 1H NMR: 500 MHz; 13C NMR: 125 MHz).

Assigned #	13-C-NMR	1H-NMR	HSQC	HMBC
1	161.70			
4	90.09	6.43 (s, 1H)	90.09	157.54, 104.15, 109.21
4-a	157.54			
4-b	156.21			
5	117.76	7.40 (d, *J*=8.4 Hz, 1H)	117.74	121.19, 124.27
6	135.07	7.68 (t, *J*=7.9 Hz, 1H)	135.07	126.27, 156.21
7	124.27	7.35 (t, *J*=7.5 Hz, 1H)	124.27	117.76, 121.19
8	126.27	8.24 (d, *J*=7.9 Hz, 1H)	126.27	180.96, 135.07, 156.21
8-a	121.19			
9	180.96			
8-b	109.21			
OCH_3	56.37	3.92 (s, 3H)	56.37	165.67
CH_2N	45.96	3.76 (s, 2H)	45.96	165.67,161.70, 109.21, 54.24
1' and 4'	54.24	2.63 (brs, 4H)	54.24	23.81

Note: NCI-H460 (lung cancer), TCA-8113 (tongue squamous cell carcinomas), BEL-7402 (liver cancer), HepG2 (hepato-carcinoma), SGC-7901 (gastric carcinoma) and T24 (urinary bladder carcinoma)

Table-2: Cytotoxic Activity of Compounds against Six Tested Cancer Cells:

Compound	NCI-H460	TCA	BEL-7402	HepG2	SGC-7901	T24
1-a	--	-	135.36	84.93	–	–
1-b	86.49	-	–	–	–	–
1-c	85.09	-	106.8	–	–	–
1-d	32.4	-	136.04	–	–	94.66
1-e	97.04	-	–	–	–	–
4-a	13.67	22.99	31.33	3.71	73.76	5.91
4-b	13.67	7.32	9.53	13.41	36.73	42.99
4-c	14.57	1.47	58.55	14.33	63.5	13.46
4-d	------	–	–	–	–	–
4-e	97.86	–	–	–	–	–
5-FU	9.75	7.63	30.64	41.82	14.85	42.61

Note: – represent that no obvious inhibitory activity was observed at the test concentration of 100 μM

CONCLUSION:

In summary, a novel series of Mannich bases of 1, 3- dihydroxyxanthone derivatives were designed and synthesized. Their structures were confirmed by IR, MS, NMR spectra and their cytotoxicity against six cancer cell lines was tested. The results showed that most of the compounds showed moderate to potent cytotoxic activity. The most active compound was **4c** with IC50 value of 1.47 μM against TCA. Further studies of the mechanism of action for **4c** are underway.

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