International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol.5, No.1, pp 401-408, Jan-Mar 2013

Synthesis and In Vitro Antitumor Activity of Some New Mannich Bases

Manal M. Kandeel[1], Nadia A. Abdou[2], Hanan H. Kadry[1]*, Rana M. El-Masry[2]

[1]Pharmaceutical Organic Chemistry Department, Faculty of Pharmacy, Cairo University, Cairo, 11562, Egypt.
[2]Organic Chemistry Department, October University for Modern Sciences and Arts,6 October City, Egypt.

***Corres. author : hanankadry2005@yahoo.com**
Tel.: +0020101960964, Fax: 002 02 23635140

Abstract: Two series of Novel Mannich bases has been synthesized from chalcones 3 and 7 and evaluated for their *in vitro* cytotoxic activity. Out of the newly synthesized compounds, four derivatives **4a**, **4b**, **4e**, **4f** were selected by the National Cancer Institute (NCI) to be evaluated for their *in-vitro* antitumor activity by *in-vitro* disease-oriented human cells screening panel assay. All the tested compounds exhibited a broad spectrum of antitumor activity against renal cancer UO-31.
Keywords: Antitumor activity /cytotoxicity/ Chalcones /Mannich bases / Synthesis .

Introduction

The principal aim of our work is the discovery of novel cytotoxic and anticancer agents. Several publications were reported on Mannich ketones as potential cytotoxins[1-4]. Some Mannich bases synthesized from 4-hydroxyacetophenone or its derivatives containing aromatic or heterocyclic rings in the B ring proved their effectiveness as cytotoxic [1, 2, 5]and antitumor agents.[6, 7] Also Mannich bases, which have been recently synthesized based on heterocylic chalcones, exhibited very potent activity against some tumor cell lines.[5, 8] These studies showed that besides the importance of 4-hydroxy group in the A ring, heterocyclic rings in the B ring made significant contribtution to the bioactivity of Mannich bases. The bioactivity of Mannich bases have been attributed to the deamination of the Mannich base group in chalcones into the corresponding cyclohexadienones that may generate a further site for nucleophilic attack by cellular thiols, and to chemical structure of α,β-unsaturated ketone that can alkylate nucleophiles, especially toward thiols rather than hydroxyl and amino groups present in the nucleic acids[9, 10] (fig 1).

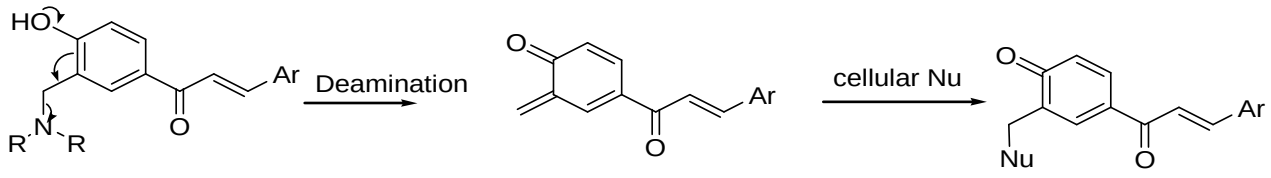


Fig 1: Formation of cyclohexadienone from mannich bases on deamination, which generates an additional alkylating site for cellular sulfahydryl nucleophiles.

Literature survey demonstrated that there are low studies concerning the cytotoxic activity of Mannich bases, in which the A ring possesses a series of different Mannich bases. Encouraged by these observations and in our continuous search for new candidates as cytotoxic agents, we turned our interest to the synthesis of new Mannich base derivatives based on the 4-hydroxychalcones with different substitution groups in the A-ring and *in vitro* cytotoxic activity evaluation of Mannich bases according to the current one-dose protocol of the National Cancer Institute (NCI) *in vitro* disease-oriented human cells screening panel assay.

Experimental

Chemistry

Melting points are uncorrected and determined in one end open capillary tubes using Gallen Kamp melting point apparatus MFB-595-010M (Gallen Kamp, London, England). Microanalysis was carried out at Micro-analytical Unit, Faculty of Science, Cairo University and the regional center for microbiology and biotechnology, Al-Azhar University. Analyses indicated were within ± 0.4 % of the theoretical values. Infrared Spectra were recorded on Schimadzu FT-IR 8400S spectrophotometer (Shimadzu, Kyoto, Japan) and expressed in wave number (cm^{-1}) using potassium bromide discs. The ^{1}HNMR spectra were recorded on a Varian Gemini 200 MHz and Varian Mercury VX-300 NMR spectrometer, in chloroform ($CDCl_3$). Chemical shifts were quoted in δ and related to that of the solvents. Mass spectra were recorded using Hewlett Packard Varian (Varian, Polo, USA) and Shimadzu Gas Chromatograph Mass spectrometer-QP 1000 EX (Shimadzu, Kyoto, Japan). TLC was carried out using Art.DC-Plastikfolien, Kieselgel 60 F254 sheets (Merck, Darmstadt, Germany). The developing solvents were benzene/acetone (4:1) and the spots were visualized at 366, 254 nm by UV Vilber Lourmat 77202 (Vilber, Marne La Vallee, France). Compounds **3**[11] ,**7 [12]**were obtained according to the reported procedures.

General procedure for the synthesis of 4a-f

Secondary amine (0.0012 mol) and para-formaldehyde (0.045 g; 0.0015 mol) were dissolved in absolute ethanol (10 mL) and refluxed for 1 h. To this reaction mixture, 1-(4-hydroxyphenyl)-3-(1H-indol-3-yl)prop-2-en-1-one (**3**) (0.26 g; 0.001 mol) was added. The mixture was refluxed for 10-24 hours with stirring then left overnight at room temperature. The formed crystals were then crystallized from aqueous ethanol.

1-(4-hydroxy-3-(morpholinomethyl)phenyl)-3-(1H-indol-3-yl)prop-2-en-1-one (4a)

solid, Yield: 97%; mp: 130-131 ^oC; IR ν_{max}/cm^{-1}: 3744.12 (OH phenolic), 3435.56 (NH), 3045.05 (CH aromatic), 2928.38, 2819.42 (CH aliphatic), 1638.23 (C=O); ^{1}H NMR (DMSO-d_6) δ ppm: 2.681-3.782 (m, 8H, $4CH_2$ of morpholine), 4.941 (s, 2H, CH_2), 7.271-8.349 (m, 7H, Ar-H and Hs of indole), 7.289 (d, 1H, *J*=8.1 Hz, olefinic H), 7.450 (d, 1H, *J*=8.7 Hz, olefinic H), 8.100 (s, 1H, OH, D_2O exch.), 9.780 (s, 1H, NH, D_2O exch.), 10.071 (s, 1H, H-2 of indole); Anal. Calcd for $C_{22}H_{22}N_2O_3$: C, 72.91; H, 6.12; N, 7.73. Found: C, 72.97; H, 6.15; N, 7.91.

1-(4-hydroxy-3-((4-methylpiperazin-1-yl)methyl) phenyl)-3-(1H-indol-3-yl)prop-2-en-1-one (4b)

solid, Yield: 49%; mp: 125-126 ^oC; IR ν_{max}/cm^{-1}: 3620.00-3380.00 (OH, NH), 3044.09 (CH aromatic), 2933.20, 2818.45 (CH aliphatic), 1645.95 (C=O); ^{1}H NMR (DMSO-d_6) δ ppm: 2.461 (s, 3H, CH_3), 2.715-2.800 (m, 8H, $4CH_2$ of piperazine), 4.823 (s, 2H, CH_2), 7.318-8.306 (m, 7H, Ar-H and Hs of indole), 7.452 (d, 1H, *J*=7.5 Hz, olefinic H), 8.323 (d, 1H, *J*=7.5 Hz, olefinic H), 9.137 (s, 1H, NH, D_2O exch.), 10.080 (s, 1H, H-2 of indole); Anal. Calcd for $C_{23}H_{25}N_3O_2$: C, 73.57; H, 6.71; N, 11.19. Found: C, 73.62; H, 6.68; N, 11.28.

1-(3-((4-ethylpiperazin-1-yl)methyl)-4-hydroxy phenyl)-3-(1H-indol-3-yl)prop-2-en-1-one (4c)

solid, Yield: 42%; mp: 139-140 ^oC; IR ν_{max}/cm^{-1}: 3760.00 (OH), 3400.00 (NH), 3043.67 (CH aromatic), 2931.80, 2889.37 (CH aliphatic), 1635.64 (C=O), 1612.49 (C=N); ^{1}H NMR (DMSO-d_6) δ ppm: 1.476 (t, 3H, *J*=7.2 Hz, $CH_2\underline{CH_3}$), 1.544-1.620 (m, 8H, $4CH_2$ of piperazine), 3.102 (q, 2H, *J*= 7.2 Hz, $\underline{CH_2}CH_3$), 4.766 (s, 2H, CH_2), 7.323-8.336 (m, 7H, Ar-H and Hs of indole), 7.465 (d, 1H, *J*=7.5 Hz, olefinic H), 7.696 (s, 1H, OH, D_2O exch.), 8.359 (d, 1H, *J*=7.5 Hz, olefinic H), 8.985 (s, 1H, NH, D_2O exch.), 10.087 (s, 1H, H-2 of indole); Anal. Calcd for $C_{24}H_{27}N_3O_2$: C, 74.01; H, 6.99; N, 10.79. Found: C, 73.98; H, 7.02; N, 10.87.

1-(4-hydroxy-3-((4-phenylpiperazin-1-yl)methyl) phenyl)-3-(1H-indol-3-yl)prop-2-en-1-one (4d)

solid, Yield: 88%; mp: 110-111 ^oC; IR ν_{max}/cm^{-1}: 3515.00-3293.00 (OH, NH), 3105.39 (CH aromatic), 2943.37, 2823.79 (CH aliphatic), 1658.78 (C=O), 1600.92 (C=N); ^{1}H NMR (DMSO-d_6) δ ppm: 1.605-3.282 (m, 8H, $4CH_2$ of piperazine), 4.971 (s, 2H, CH_2), 6.945-7.366 (m, 12 H, Ar-H and Hs of indole), 7.521 (d, 1H, *J*=6.8 Hz, olefinic H), 7.879 (s, 1H, OH, D_2O exch.), 8.320 (d, 1H, *J*=6.8 Hz, olefinic H), 9.753 (s, 1H, NH, D_2O exch.), 10.053 (s,

1H, H-2 of indole); Anal. Calcd for $C_{28}H_{27}N_3O_2$: C, 76.86; H, 6.22; N, 9.60. Found: C, 76.92; H, 6.25; N, 9.71.

1-(3-((4-(4-fluorophenyl)piperazin-1-yl)methyl)-4-hydroxyphenyl)-3-(1H-indol-3-yl)prop-2-en-1-one (4e)

solid, Yield: 51%; mp: 130-131 ^oC; IR ν_{max}/cm^{-1}: 3610.00-3348.00 (OH, NH), 3045.05 (CH aromatic), 2934.16, 2820.38 (CH aliphatic), 1638.23 (C=O); ^1H NMR (DMSO-d_6) δ ppm: 2.788-4.747 (m, 8H, $4CH_2$ of piperazine), 4.949 (s, 2H, CH_2), 6.890-7.862 (m, 11H, Ar-H and Hs of indole), 7.340 (d, 1H, *J*=6.6 Hz, olefinic H), 8.329 (d, 1H, *J*=6.9 Hz, olefinic H), 9.765 (s, 1H, NH, D_2O exch.), 10.080 (s, 1H, H-2 of indole); Anal. Calcd for $C_{28}H_{26}FN_3O_2$: C, 73.83; H, 5.75; N, 9.22. Found: C, 73.86; H, 5.77; N, 9.28.

1-(4-hydroxy-3-((4-(4-methoxyphenyl)piperazin-1-yl)methyl)phenyl)-3-(1H-indol-3-yl)prop-2-en-1-one (4f)

solid, Yield: 78%; mp: 129-130 ^oC; IR ν_{max}/cm^{-1}: 3570.00-3280.00 (OH, NH), 2930.31, 2822.31 (CH aliphatic), 1652.70 (C=O); ^1H NMR (DMSO-d_6) δ ppm: 2.848-3.164 (m, 8H, $4CH_2$ of piperazine), 3.788 (s, 3H, OCH_3), 4.933 (s, 2H, CH_2), 6.851 (d, 1H, *J*=8.7 Hz, olefinic H), 6.879-7.516 (m, 7H, Ar-H and Hs of indole), 7.330 (d, 2H, *J*=6.6 Hz, H-2, H-6 of 4-OCH_3 C_6H_4), 7.846 (d, 2H, *J*=6.9 Hz, H-3, H-5 of 4-OCH_3 C_6H_4), 8.336 (d, 1H, *J*=9.0 Hz, olefinic H), 9.000 (s, 1H, OH, D_2O exch.), 9.752 (s, 1H, NH, D_2O exch.), 10.084 (s, 1H, H-2 of indole); MS m/s 467.30 (M^+, 1.92%); Anal. Calcd for $C_{29}H_{29}N_3O_3$: C, 74.50; H, 6.25; N, 8.99. Found: C, 74.48; H, 6.29; N, 9.12.

General procedure for the synthesis of 8a-g

Secondary amine (0.0012mol) and paraformaldehyde (0.045g; 0.0015mol) were dissolved in absolute ethanol (10 mL) and refluxed for 1 h. To this reaction mixture, 1-(4-bromophenyl)-3-(4-hydroxy-3-methoxyphenyl) prop-2-en-1-one (**7**) (0.33 g; 0.001 mol) was added. The mixture was refluxed for 14-30 hours with stirring then left overnight at room temperature. The formed crystals were then crystallized from aqueous ethanol.

1-(4-bromophenyl)-3-(4-hydroxy-3-methoxy-5(morpholinomethyl)phenyl)prop-2-en-1-one (8a)

solid, Yield: 86%; mp: 165-166 ^oC; IR ν_{max}/cm^{-1}: 3502.73 (OH phenolic), 3025.00 (CH aromatic), 2935.66, 2846.93 (CH aliphatic), 1651.07 (C=O); ^1H NMR (DMSO-d_6) δ ppm: 2.824 (m, 4H, $2CH_2$ of morpholine), 3.760 (s, 1H, OH, D_2O exch.), 3.846-3.967 (m, 4H, $2CH_2$ of morpholine), 3.976 (s, 3H, OCH_3), 4.019 (s, 2H, CH_2), 7.101 (s, 1H, H-6 of 3-OCH_3-4-OH C_6H_3), 7.214 (s, 1H, H-2 of 3-OCH_3-4-OH C_6H_3), 7.238 (d, 1H, *J*=16.8 Hz, olefinic H), 7.668 (d, 1H, *J*=17.7 Hz, olefinic H), 7.771 (d, 2H, *J*=8.1 Hz, H-3, H-5 of 4-Br C_6H_4), 7.944 (d, 2H, *J*=8.4 Hz, H-2, H-6 of 4-Br C_6H_4); MS m/s 430.95 (M^+, 75.84%), 432.95 (M+2, 74.14%); Anal. Calcd for $C_{21}H_{22}BrNO_4$: C, 58.34; H, 5.13; N, 3.24. Found: C, 58.39; H, 5.18; N, 3.30.

1-(4-bromophenyl)-3-(4-hydroxy-3-methoxy-5-((4-methylpiperazin-1-yl)methyl)phenyl)prop-2-en-1-one (8b)

solid, Yield: 81%; mp: 202-203 ^oC; IR ν_{max}/cm^{-1}: 3444.87 (OH phenolic), 3032.10 (CH aromatic), 2939.52, 2804.50 (CH aliphatic), 1654.92 (C=O); ^1H NMR (DMSO-d_6) δ ppm: 2.392 (s, 3H, CH_3), 2.736-2.892 (m, 8H, $4CH_2$ of piperazine), 3.801 (s, 2H, CH_2), 3.963 (s, 3H, OCH_3), 6.978 (s, 1H, H-6 of 3-OCH_3-4-OH C_6H_3), 7.089 (s, 1H, H-2 of 3-OCH_3-4-OH C_6H_3), 7.297 (d, 1H, *J*=15.3 Hz, olefinic H), 7.642 (d, 2H, *J*=8.4 Hz, H-3, H-5 of 4-Br C_6H_4), 7.722 (d, 1H, *J*=15.6 Hz, olefinic H), 7.881 (d, 2H, *J*=8.4 Hz, H-2, H-6 of 4-Br C_6H_4); MS m/s 443.95 (M^+, 61.01%), 445.95 (M+2, 59.13%); Anal. Calcd for $C_{22}H_{25}BrN_2O_3$: C, 59.33; H, 5.66; N, 6.29. Found: C, 59.32; H, 5.69; N, 6.36.

1-(4-bromophenyl)-3-(3-((4-ethylpiperazin-1-yl)methyl)-4-hydroxy-5-methoxyphenyl)prop-2-en-1-one (8c)

solid, Yield: 92%; mp: 182-183 ^oC; IR ν_{max}/cm^{-1}: 3444.87 (OH phenolic), 3024.00 (CH aromatic), 2939.52, 2819.93 (CH aliphatic), 1658.78 (C=O); ^1H NMR (DMSO-d_6) δ ppm: 1.139 (t, 3H, *J*=7.2 Hz, $\underline{CH_2}CH_3$), 2.509 (q, 2H, *J*=7.2 Hz, $CH_2\underline{CH_3}$), 2.699-2.732 (m, 8H, $4CH_2$ of piperazine), 3.752 (s, 1H, OH, D_2O exch.), 3.799 (s, 2H, CH_2), 3.950 (s, 3H, OCH_3), 6.971 (s, 1H, H-6 of 3-OCH_3-4-OH C_6H_3), 7.089 (s, 1H, H-2 of 3-OCH_3-4-OH C_6H_3), 7.294 (d, 1H, *J*=15.3 Hz, olefinic H), 7.643 (d, 2H, *J*=8.4 Hz, H-3, H-5 of 4-Br C_6H_4), 7.726 (d, 1H, *J*=15.6 Hz, olefinic H), 7.881 (d, 2H, *J*=8.7 Hz, H-2, H-6 of 4-Br C_6H_4); Anal. Calcd for $C_{23}H_{27}BrN_2O_3$: C, 60.14; H, 5.92; N, 6.10. Found: C, 60.22; H, 5.98; N, 6.14.

1-(4-bromophenyl)-3-(4-hydroxy-3-methoxy-5-((4-phenylpiperazin-1-yl)methyl)phenyl)prop-2-en-1-one (8d)

solid, Yield: 60%; mp: 208-209 ^oC; IR ν_{max}/cm^{-1}: 3420.00 (OH phenolic), 3040.00 (CH aromatic), 2956.87, 2814.14 (CH aliphatic), 1651.07 (C=O); ^1H NMR (DMSO-d_6) δ ppm: 2.838-2.984 (m, 4H, $2CH_2$ of piprezaine), 3.308 (m, 4H, $2CH_2$ of piperazine), 3.885 (s, 2H, CH_2), 3.963 (s, 3H, OCH_3), 6.916 (s,

1H, H-6 of 3-OCH_3-4-OH C_6H_3), 6.936 (s, 1H, H-2 of 3-OCH_3-4-OH C_6H_3), 6.943-7.311 (m, 5H, Ar-H), 7.347 (d, 1H, *J*=15.6 Hz, olefinic H), 7.647 (d, 2H, *J*=7.8 Hz, H-3, H-5 of 4-Br C_6H_4), 7.743 (d, 1H, *J*=15.6, olefinic H), 7.902 (d, 2H, *J*=7.8, H-2, H-6 of 4-Br C_6H_4); MS m/s 506.00 (M^+, 13.46%), 508.00 (M+2, 12.04%); Anal. Calcd for $C_{27}H_{27}BrN_2O_3$: C, 63.91; H, 5.36; N, 5.52. Found: C, 63.98; H, 5.41; N, 5.63.

1-(4-bromophenyl)-3-(3-((4-(4-fluorophenyl) piperazin-1-yl)methyl)-4-hydroxy-5-methoxy phenyl)prop-2-en-1-one (8e)

solid, Yield: 43%; mp: 143-144 ^oC; IR ν_{max}/cm^{-1}: 3441.01 (OH phenolic), 3060.00 (CH aromatic), 2962.66, 2812.21 (CH aliphatic), 1651.07 (C=O); ^1H NMR (DMSO-d_6) δ ppm: 2.877-2.983 (m, 4H, $2CH_2$ of piprezaine), 3.248 (m, 4H, $2CH_2$ of piperazine), 3.381 (s, 1H, OH, D_2O exch.), 3.922 (s, 2H, CH_2), 3.966 (s, 3H, OCH_3), 6.861 (s, 1H, H-6 of 3-OCH_3-4-OH C_6H_3), 6.890 (d, 2H, *J*=8.1 Hz, H-3, H-5 of 4-Br C_6H_4), 6.892 (s, 1H, H-2 of 3-OCH_3-4-OH C_6H_3), 6.968 (d, 2H, *J*=8.4 Hz, H-2, H-6 of 4-F C_6H_4), 7.377 (d, 1H, *J*=15.6 Hz, olefinic H), 7.650 (d, 2H, *J*=8.1 Hz, H-3, H-5 of 4-Br C_6H_4), 7.742 (d, 1H, *J*=15.3 Hz, olefinic H), 7.913 (d, 2H, *J*=8.4 Hz, H-2, H-6 of 4-F C_6H_4); Anal. Calcd for $C_{27}H_{26}BrFN_2O_3$: C, 61.72; H, 4.99; N, 5.33. Found: C, 61.79; H, 5.05; N, 5.38.

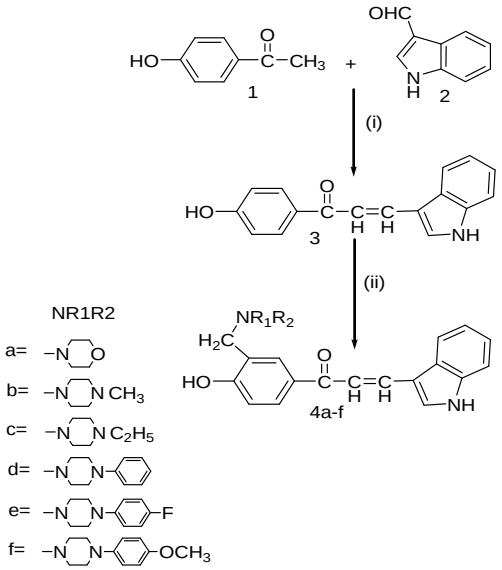
1-(4-bromophenyl)-3-(4-hydroxy-3-methoxy-5-((4-(4-methoxyphenyl)piperazin-1-yl)methyl)phenyl)prop-2-en-1-one (8f)

solid, Yield: 37%; mp: 190-191 ^oC; IR ν_{max}/cm^{-1}: 3583.74 (OH phenolic), 3055.24 (CH aromatic), 2943.37, 2812.21 (CH aliphatic), 1658.78 (C=O); ^1H NMR (DMSO-d_6) δ ppm: 2.826 (m, 4H, $2CH_2$ of piprezaine), 3.183 (m, 4H, $2CH_2$ of piperazine), 3.301 (s, 1H, OH, D_2O exch.), 3.775 (s, 3H, OCH_3), 3.871 (s, 2H, CH_2), 3.957 (s, 3H, OCH_3), 6.847 (d, 2H, *J*=9.3 Hz, H-2, H-6 of 4-OCH_3 C_6H_4), 6.904 (d, 2H, *J*=9.3 Hz, H-3, H-5 of 4-OCH_3 C_6H_4), 7.108 (s, 1H, H-6 of 3-OCH_3-4-OH C_6H_3), 7.269 (s, 1H, H-2 of 3-OCH_3-4-OH C_6H_3), 7.335 (d, 1H, *J*=15.4 Hz, olefinic H), 7.642 (d, 2H, *J*=8.4 Hz, H-3, H-5 of 4-Br C_6H_4), 7.739 (d, 1H, *J*=15.4 Hz, olefinic H), 7.896 (d, 2H, *J*=8.7 Hz, H-2, H-6 of 4-Br C_6H_4); Anal. Calcd for $C_{28}H_{29}BrN_2O_4$: C, 62.57; H, 5.44; N, 5.21. Found: C, 62.53; H, 5.46; N, 5.30.

1-(4-bromophenyl)-3-(3-((4-(2-ethoxyphenyl) piperazin-1-yl)methyl)-4-hydroxy-5-methoxy phenyl)prop-2-en-1-one (8g)

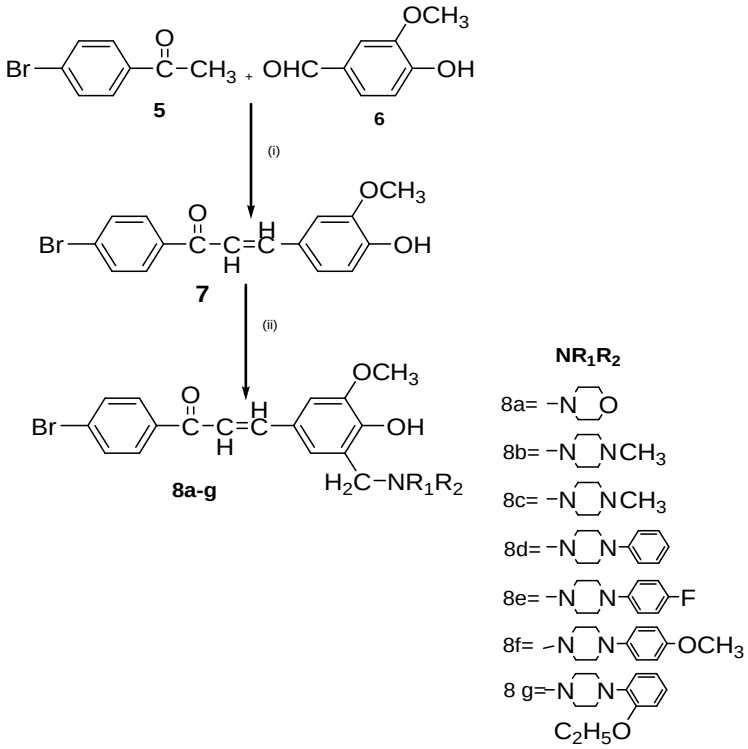
solid, Yield: 54%; mp: 170-171 ^oC; IR ν_{max}/cm^{-1}: 3433.29 (OH phenolic), 3020.00 (CH aromatic), 2903.32, 2819.93 (CH aliphatic), 1681.23 (C=O); ^1H NMR (DMSO-d_6) δ ppm: 1.431 (t, 3H, *J*=6.9 Hz, $CH_2\underline{CH_3}$), 2.949-3.276 (m, 8H, $4CH_2$ of piperazine), 3.459 (s, 1H, OH, D_2O exch.), 3.969 (s, 3H, OCH_3), 4.004 (s, 2H, CH_2), 4.070 (q, 2H, *J*=6.9 Hz, $\underline{CH_2}CH_3$), 6.867 (d, 2H, *J*=7.8 Hz, H-3, H-5 of 4-Br C_6H_4), 6.911-7.010 (m, 4H, Hs of 2-ethoxyphenyl), 7.023 (s, 1H, H-6 of 3-OCH_3-4-OH C_6H_3), 7.186 (s, 1H, H-2 of 3-OCH_3-4-OH C_6H_3), 7.664 (d, 1H, *J*=15.6 Hz, olefinic H), 7.746 (d, 1H, *J*=15.3 Hz, olefinic H), 7.930 (d, 2H, *J*=8.4 Hz, H-2, H-6 of 4-Br C_6H_4); Anal. Calcd for $C_{29}H_{31}BrN_2O_4$: C, 63.16; H, 5.67; N, 5.08. Found: C, 63.22; H, 5.72; N, 5.17.

Scheme I:



Scheme 1:Reagents and conditions: (i) EtOH, 40% KOH, stir, rt, 24 h; (ii) Secondary amine, Paraformaldehyde, EtOH, reflux, 10-17 h.

Scheme II:



Scheme 2: Reagents and conditions: (i) EtOH, 20% Alcoholic KOH, reflux, 6 h; (ii) Secondary amine, Paraformaldehyde, EtOH, reflux, 14-30 h.

Antitumor screening

Under sterile conditions, cell lines were grown in RPMI 1640 media (Gibco, NY, USA) supplemented with 10% fetal bovine serum (Biocell, CA, USA), $5X10^5$ cell/ml was used to test the growth inhibition activity of the synthesized compounds. The concentrations of the compounds ranging from 0.01 to 100 µM were prepared in phosphate buffer saline. Each compound was initially solubilized in dimethyl sulfoxide (DMSO), however, each final dilution contained less than 1% DMSO. Solutions of different concentrations (0.2 ml) were pipetted into separate well of a microtiter tray in duplicate. Cell culture (1.8 ml) containing a cell population of 6 X 10^4 cells/ml was pippeted into each well. Controls, containing only phosphate buffer saline and DMSO at identical dilutions, were also prepared in the same manner. These cultures were incubated in a humidified incubator at 37^oC. The incubator was supplied with 5% CO_2 atmosphere. After 48 h, cells in each well were diluted 10 times with saline and counted by using a coulter counter. The counts were corrected for the dilution [13-16].

Result and Discussion

The synthesis of the target compounds was accomplished according to the reaction sequences illustrated in Schemes **1** and **2**. Chalcones **3** [11] **7** [12] were synthesized by reacting 4-substituted acetophenone with indolyl-3-carboxaldehyde or vaniline respectively in the presence of potassium hydroxide by conventional Claisen-Schmidt condensation. Mannich bases **4a-f** were prepared in 42-97% yield by refluxing a mixture of chalcone **3**, paraformaldehyde and different secondary amines dissolved in ethanol according to the previously described procedure for the preparation of analogue compounds.[3, 4] The synthesized compounds were characterized by their physical and spectral data (IR, ^{1}H-NMR, MS) that confirmed the structures of the novel compounds. The case of compound **4a** was an example, the ^{1}H NMR spectrum showed multiplet signal at δ 2.681-3.782 ppm for 8 protons of $4CH_2$ groups of morpholine ring. While the spectra of compounds **4b-f** demonstrate multiplet signals in range of δ 1.605-3.282 ppm for 8 protons of $4CH_2$ groups of piperazine rings. However, the spectra of all compounds showed the presence of singlet signals at δ 7.696-9.000 ppm for the phenolic OH protons, they also showed the presence of singlet signals at δ 4.933-4.971 ppm for the 2 protons of the methylene groups. Protons of α,β-unsaturated ketone of Mannich bases derived from indolylchalcone **4a-f** were observed as doublets with J =6.8-8.1 Hz at δ 6.8-7.3 ppm for Hβ and δ 8.3 ppm for Hα. The J characteristic values were the evidence that Mannich bases derived from indolylchalcone **4a-f** appeared as *z* isomers. Further elucidation of the structure of

compound **4f** came from MS spectrum which gave molecular ion peak at 467.30. On the other hand, Bamnela and Shrivastava[12] prepared 1-(4-bromophenyl)-3-(4-hydroxy-3-methoxyphenyl)prop-2-en-1-one **7**. Unfortunately, no physical or spectral data were reported for the compound. So, we elucidate their structure by spectral data. The IR spectrum showed the presence of the characteristic band for conjugated C=O of chalcones at 1639 cm^{-1}. The structure was further supported by its ^1H NMR spectrum which showed the presence of two characteristic doublet signals for the olefinic protons at δ 7.316 and 7.760 ppm with a coupling constant J=15.6 Hz confirming the *E* configuration. It also showed a singlet signal at δ 5.940 ppm for the phenolic OH proton, where the 3 protons of the methoxy group were seen as a singlet signal at δ 3.976 ppm.

The designed target compounds **8a-g** were prepared by the reaction of **7** with various secondary amines and paraformaldehyde in ethanol under reflux for 14-30 h, [4] (Scheme 2) to give Mannich bases **8a-g** in 37-92% yield. The structure conformations of compounds **8a-g** were based on the spectral data. The ^1H NMR spectrum of compounds **8b-f** showed methylene protons as a singlet signal at δ 3.799-4.019 ppm, and protons of piperazine nucleus were showed as multiplet signal at δ 2.77 -3.26 ppm. Protons of α,β-unsaturated ketone of Mannich bases derived from vaniline chalcone **8a-g** were observed as doublets with J =15.3-16.8 Hz at 7.7 ppm for Hβ and 7.2 ppm for Hα. The J characteristic values were the evidence that mannich derivatives of 4-hydroxy-3-methoxyphenylchalcone appeared as *E* isomers. Further evidence of the structures of compounds **8a**, **8b**, and **8d** came from MS spectra which showed the molecular ion peak and M+2 in ratio 1:1.

Preliminary in-vitro anticancer screening

Table 1: Percentage growth inhibition (GI %) of in-vitro subpanel tumor cell lines at 10 µM concentration of tested compounds .

Cell Line	Compound			
	4a	**4b**	**4e**	**4f**
Leukemia				
HL-60(TB)	15.52	**L**	-	-
K-562	12.84	**L**	**L**	**L**
MOLT-4	15.39	11.86	-	-
RPMI-8226	-	-	-	-
SR	Nt	Nt	nt	Nt
Non-Small Cell Lung Cancer				
A549/ATCC	**L**	**L**	-	**L**
HOP-62	12.04	-	-	11.04
HOP-92	15.45	-	-	10.13
NCI-H226	-	**L**	**L**	**L**
NCI-H23	-	**L**	-	**L**
NCI-H322M	-	**L**	**L**	**L**
NCI-H460	**L**	**L**	**L**	**L**
NCI-H522	-	-	**L**	-
Colon Cancer				
COLO 205	**L**	**L**	**L**	**L**
HCC-2998	**30.43**	Nt	nt	Nt
HCT-116	**L**	**L**	-	**L**
HCT-15	**L**	-	-	
HT29	-	**L**	-	**L**
KM12	-	-	-	**L**
SW-620	**L**	**L**	**L**	**L**
CNS Cancer				
SF-268	**L**	**L**	-	**L**
SF-295	nt	Nt	nt	Nt
SNB-19	**L**	**L**	-	**L**
SNB-75	nt	Nt	nt	Nt

Melanoma				
LOX IMVI	-	**L**	-	-
MALME-3M	-	-	**L**	-
M14	**L**	-	-	**L**
MDA-MB-435	**L**	**L**	**L**	**L**
SK-MEL-2	**L**	**L**	**L**	**L**
SK-MEL-28	**L**	**L**	**L**	**L**
SK-MEL-5	-	-	**L**	-
UACC-257	11.71	**L**	**L**	**L**
UACC-62	**L**	**L**	**L**	**L**
Ovarian Cancer				
IGROV1	-	-	**L**	**L**
OVCAR-3	**L**	**L**	**L**	**L**
OVCAR-4	**L**	-	-	-
OVCAR-5	**L**	**L**	-	**L**
NCI/ADR-RES	**L**	**L**	**L**	**L**
SK-OV-3	**L**	**L**	**L**	-
Renal Cancer				
786-0	**L**	**L**	**L**	**L**
A498	**L**	**L**	-	**L**
ACHN	**L**	-	-	-
RXF 393	**L**	**L**	**L**	**L**
TK-10	**L**	**L**	**L**	**L**
UO-31	**40.88**	**39.48**	**40.57**	**44.13**
Prostate Cancer				
PC-3	18.37	18.55	15.29	11.69
DU-145	**L**	**L**	**L**	**L**
Breast Cancer				
MCF7	**L**	**L**	-	-
MDA-MB-231/ATCC	**L**	**L**	-	**L**
HS 578T	**L**	**L**	**L**	**L**
BT-549	**L**	**L**	-	**L**
MDA-MB-468	**L**	**L**	-	-

a -, GI <10%; nt , not tested; L, compound proved lethal to the intact cell .

All of the newly synthesized compounds were sent to the National Cancer Institute (NCI) USA to be tested for their anticancer activity. Unfortunately four derivatives **4a**, **4b**, **4e**, **4f** only were selected to be tested by *in-vitro* disease-oriented human cells screening panel assay to be evaluated for their *in-vitro* antitumor activity. A single dose (10 µM) of the test compounds were used in the full NCI 60 cell lines panel assay which includes nine tumor subpanels namely; leukemia, non-small cell lung, colon, CNS, melanoma, ovarian, renal, prostate, and breast cancer cells [13-16]. The data were reported as mean-graph of the percent growth of the treated cells, and presented as percentage growth inhibition (GI %). The obtained data revealed that all of the tested compounds exhibit sensitivity profiles against the renal cancer UO-31cell line and do not show any inhibition activity against the other cell lines except **4a** ,which demonstrates moderate inhibition activity against colon cancer HCC-2998 cell line.

Acknowledgements

We are grateful to the NCI, Bethesda, MD, USA for performing the antitumor testing of the synthesized compounds.

References

[1] Dimmock J.R., Kandepu N.M., Hetherington M., Quail J. W., Pugazhenthi U., Sudom A. M., Chamankhah M., Rose P., Pass E., Allen T. M., Halleran S., Szydlowski J., Mutus B., Tannous M., Manavathu E. K., Myers T. G., Clercq E. D., Balzarini J., Cytotoxic Activities of Mannich Bases of Chalcones and Related Compounds, J. Med. Chem., 1998, 41, 1014-1026.

[2] Gul H. I., Yerdelen K. O., Gul M., Das U., Pandit B., Li P. K., Secen H., Sahin F., Synthesis of 4`-Hydroxy-3`-piperidinomethylchalcone derivatives and their cytotoxicity against PC-3 cell lines, Arch. Pharm. Chem. Life Sci., 2007, 340, 195 – 201.

[3] GUL H. I., Yerdelen K. O., DAS U., GUL M., Pandit B., LI P. K., Dimmock J. R., Synthesis and Cytotoxicity of Novel 3-Aryl-1-(3`-dibenzylaminomethyl-4`-hydroxyphenyl)-propenones and Related Compounds, Chem. Pharm. Bull., 2008, 56, 1675—1681.

[4] Hieu B.T., Thuy L.T., Thuy V.T., Tien H.X., Chinh L.V., Hoang V.D., Vu T.K., Design, Synthesis and In Vitro Cytotoxic Activity Evaluation of New Mannich Bases, Bull Korean Chem Soc ., 2012, 33, 1586-1592.

[5] Reddy M.V.B., Sua C.R., Chioub W.F., Liuc Y.N., Chenc R.Y.H., Bastowc K.F., Leec K.H., Wua T.S., Design, synthesis, and biological evaluation of Mannich bases of heterocyclic chalcone analogs as cytotoxic agents, Bioorg & Med. Chem., 2008,16, 7358-7370.

[6] Hua G., Wang G., Duana N., Wena X., Caoa T., Xiea S., Huangb W., Design, synthesis and antitumor activities of fluoroquinolone C-3 heterocycles (IV): s-triazole Schiff–Mannich bases derived from ofloxacin, Acta Pharmaceutica Sinica B, 2012, 2, 312-317

[7] Shawa A.Y., Changa C.Y., Hsua M.Y., Lub P.J., Yangc C.N., Chenb H.L., Loc C.W., Shiaud C.W., Cherna M.K., Synthesis and structure-activity relationship study of 8-hydroxyquinoline-derived Mannich bases as anticancer agents, Eur. J. Med. Chem., 2010, 45, 2860-2867.

[8] Reddy M.V.B., Shen Y.C., Yang J.S., Hwang T.L., Bastow K.F., Qian K., Lee K.H., Wua T.S., New bichalcone analogs as NF-jB inhibitors and as cytotoxic agents inducing Fas/CD95-dependent apoptosis, Bioorg & Med. Chem., 2011, 19, 1895–1906.

[9] Dimmock J.R., Advikolanu K.M., Scott H.E., Duffy M.J., Reid R.S., Quail J.W., Jia Z., Hickie R.A., Allen T.M., Rutledge J.M., Evaluation of cytotoxicity of some Mannich bases of various aryl and arylidene ketones and their corresponding arylhydrazones, J. pharm. sci., 1992, 81, 1147-1152.

[10] Dimmock J.R. and Kumar P., Anticancer and Cytotoxic Properties of Mannich Bases., Curr. Med. Chem., 1997, 4, 1-22.

[11] Kumar D., Kumar N.M., Akamatsu K., Kusaka E., Harada H., Ito T., Synthesis and biological evaluation of indolyl chalcones as antitumor agents., Bioorg & Med. Chem. Lett., 2010, 20, 3916–3919.

[12] Bamnela R. and Shrivastava S.P., Synthesis and Biological Activity of 4, 6-Substituted aryl-1-acetyl pyrimidine-2-ols, Asian J. Chem., 2010, 22, 6553-6558.

[13] Grever M.R., Schepartz S.A., Chabner B.A., The National Cancer Institute: cancer drug discovery and development program, Semin. Oncol., 1992, 19, 622-638.

[14] Monks A., Scudiero D., Skehan P., Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumor cell lines, J. Natl. Cancer Inst., 1991, 83,757-766.

[15] Boyd M.R. and Paull K.D., Some practical considerations and applications of the national cancer institute in vitro anticancer drug discovery screen, Drug Dev. Res., 1995, 34, 91-109.

[16] Skehan P., Storeng R., Scudiero D., Monks A., McMahon J., Vistica D., Warren J.R., Bokesch H., Kenney S., Boyd M.R., New colorimetric cytotoxic assay for anticancer drug screening, J. Natl. Cancer Inst., 1990, 82, 1107-1112.

*****