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Design, Synthesis and *In Vitro* Cytotoxic Activity Evaluation of New Mannich Bases

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A series of Novel Mannich bases has been synthesized and evaluated *in vitro* cytotoxic activity against the human hepatocellular carcinoma (HepG2), human lung carcinoma (SK-LU-1), and human breast cancer (MCF-7). Compound **9f** was found to be most potent against three cell lines with IC_{50} values of 1.57, 1.16 and 1.21 μg/mL, respectively. In addition, compounds **9g**, **10f** exhibited very significant activity against MCF-7 cell line with IC_{50} values of 2.0 μg/mL.

Key Words : Anticancer, Cytotoxicity, Chalcone, Mannich, Paraformaldehyde

Introduction

Chalcones, which are 1,3-diphenyl-2-propene-1-one, are synthesized by the condensation of aldehyde and ketone via base catalyzed or acid catalyzed dehydration. This process is known as Claisen-Schmidt reaction.[1] Chalcones are reported to be abundant in edible plants and precursors of flavonoids and isoflavonoids. They are also reported to possess a wide spectrum of biological activities such as anti-leishmanial,[2] anti-tuberculosis,[3] anti-fungal,[4] anti-malarial,[5] immunosuppressive, and anti-tumor.[6]

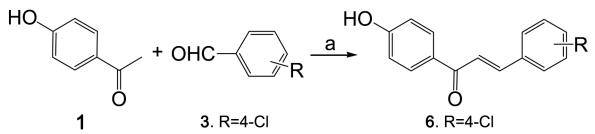
Mannich bases are beta-amino ketones, which are generally formed by the reaction between formaldehyde and a secondary amine. Recently, Mannich bases have received the increasing consideration due to physiologically reactivity of the basic function rendering the molecule soluble in aqueous solvents when it is transformed into ammonium salt. Mannich bases display varied biological activities, including antitubercular,[7] anti-malarial,[8] anti-cancer,[9] anti-inflammatory.[10,11]

Some Mannich bases synthesized from 4-hydroxyacetophenone or its derivatives containing aromatic or heterocyclic rings in the B ring have proved their effectiveness as cytotoxic,[12-15] antitumor agents.[16,17] 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.[18,19] Some Mannich bases, which have recently synthesized based on heterocylic chalcones, exhibited very potent activity against some tumor cell lines.[14,20] 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. To our best knowledge, there are no studies reporting the cytotoxic activity of Mannich bases, in which the A ring possesses a series of different Mannich bases.

Being intrigued by this observation, and in our continuous program in the search for new candidates to cytotoxic agents, we turned our attention to the synthesis of a small library of new Mannich base derivatives based on the chalcones with different substitution groups in the A-ring and *in vitro* cytotoxic activity evaluation of Mannich bases. All new analogs were tested for cytotoxic activity against HepG2 (Hepatocellular carcinoma), SK-LU-1 (Human lung carcinoma) and MCF-7 (Human breast cancer).

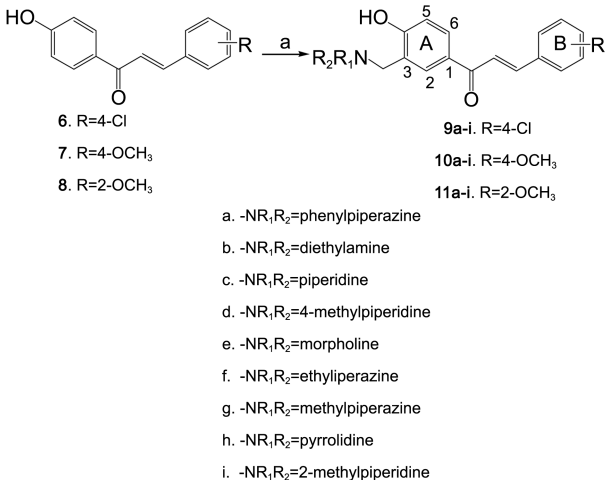
Experimental Section

Chemistry. All chemicals and reaction solvents were purchased from Merck and Aldrich. Melting points were determined in open capillaries on Electrothermal IA 9200 Shimazu apparatus and uncorrected. IR spectra were recorded on FT-IR IMPACT-410 using KBr discs. ^{1}H NMR spectra were recorded on Brucker AVANCE 500 MHz spectrometer in $CDCl_3$, CD_3OD and DMSO-d_6. Chemical shifts (δ) are in ppm relative to TMS, and coupling constants (J) are expressed in hertz (Hz). ESI-HRMS Mass spectra were recorded on FTICR MS Varian. Progress of the reaction was monitored by thin-layer chromatography (TLC) using precoated TLC sheets with Ultraviolet (UV) fluorescent silica gel (Merck 60F254) and spots were visualized by Dragendoff reagent. Multiplicities are shown as the abbreviations: s



1 | 3. R=4-Cl | 4. R=4-OCH_3 | 5. R=2-OCH_3 | 6. R=4-Cl | 7. R=4-OCH_3 | 8. R=2-OCH_3

Scheme 1. Reagents and conditions: (a) NaOH, EtOH, rt, 18-24 h, 86-88%.



Scheme 2. Reagents and conditions: (a) EtOH, paraformaldehyde (1eq), amine (1eq): a. phenylpiperazine; b. diethylamine; c. piperidine; d. 4-methylpiperidine; e. morpholine; f. ethylpiperazine; g. methylpiperazine; h. pyrrolidine; i. 2-methylpiperidine; reflux, 24-36 h, 35-72%.

(singlet), brs (broad singlet), d (doublet), brd (broad doublet) t (triplet), m (multiplet). Column chromatography was carried out using silica gel 40-230 mesh. Solvents were commercially available materials of reagent grade.

General Procedure for the Synthesis of Mannich Bases. Amine (3.88 mmol) was treated with paraformaldehyde (3.88 mmol) in ethanol (20 mL) at reflux for 1 h. To the reaction mixture, chalcone (3.88 mmol) was then added in one portion. The reaction mixture was refluxed for 24-36 h. Ethanol was then removed under vacuum. The residue was diluted with water and extracted with dichloromethane. Organic phase was separated, dried on anhydrous Na_2SO_4, and evaporated under reduced pressure. Pure target compounds were obtained by crystallization of precipitated solid or column chromatography on silica gel using various solvent mixtures.

(*E*)-1-(4-Chlorophenyl)-3-[4-hydroxy-3-(4-phenyl-piperazin-1-ylmethyl)-phenyl]-propenone (9a): Yield 72% (white solid); mp 184-185 °C. IR (KBr, ν (cm^{-1})): 3393, 2870, 1655, 1600, 1523, 1445, 1350, 1226, 1022, 802, 732, 617. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.95 (dd, *J* = 2.5 Hz, 8.5 Hz, 1H, H-6), 7.81 (d, *J* = 2.5 Hz, 1H, H-2), 7.76 (d, *J* = 15,5 Hz, 1H, H-β), 7.59 (d, *J* = 8 Hz, 2H), 7.54 (d, *J* = 15.5 Hz, 1H, H-α), 7.40 (d, *J* = 8.5 Hz, 2H), 7.30-7.26 (t, *J* = 8.5 Hz, 2H), 6.94-6.88 (m, 4H), 3.87 (s, 2H, CH_2), 3.26 (brs, 4H, H-piperazine), 2.78 (brs, 4H, H-piperazine). ^{13}C NMR ($CDCl_3$ 125 MHz) *δ* 188.2, 162.8, 150.8, 142.3, 136.2, 133.7, 130.4, 129.9, 129,7, 129.5, 129.3, 129.2, 122,3, 121.2, 120.4, 116.5, 116.2, 61.2, 52.6, 49.2. HR-ESIMS *m/z* calcd for $C_{26}H_{25}ClN_2O_2$, 432.16045; found, 433.16040 $[M+H]^+$.

(*E*)-1-(4-Chloro-phenyl)-3-(3-diethylaminomethyl-4-hydroxy-phenyl)-propenone (9b): Yield 37% (yellow oil); IR (Neat, ν (cm^{-1})): 3419, 2831, 1653, 1527, 1493, 1447, 1349, 1270, 1033, 825, 767, 736, 694. ^{1}H NMR (CD_3OD, 500 MHz) δ 8.0 (dd, *J* = 2.5 Hz, 8.5 Hz, 1H, H-6), 7.91 (d, *J* = 2.5 Hz, 1H, H-2), 7.81 (d, *J* = 15.5 Hz, 1H, H-β), 7.74 (d, *J* = 8.5 Hz, 2H, B-ring), 7.70 (d, *J* = 15.5 Hz, 1H, H-α), 7.44 (d, *J* = 8.5 Hz, 2H, B-ring), 6.77 (d, *J* = 8.5 Hz, 1H, H-5), 4.06 (s, 2H, CH_2), 2.89 (q, *J* = 7 Hz, 4H, $2CH_2$), 1.24 (t, *J* = 7 Hz, 6H, $2CH_3$). ^{13}C NMR (CD_3OD, 125 MHz) *δ* 189.8, 163.5, 142.8, 137.0, 135.4, 132.4, 132.0, 130.9, 130.2, 130.0, 129.7, 123.8, 118.4, 57.3, 47.5, 10.7. HR-ESIMS *m/z* calcd for $C_{20}H_{22}ClNO_2$, 343.13391; found, 344.14175 $[M+H]^+$.

(*E*)-1-(4-Chloro-phenyl)-3-[4-hydroxy-3-(4-methyl-piperidin-1-ylmethyl)-phenyl]-propenone (9d): Yield 54% (yellow oil); IR (Neat, ν (cm^{-1})): 2887, 1649, 1599, 1529, 1482, 1416, 1384, 1333, 1279, 1111, 1059, 1037, 998, 927, 825, 744, 627. ^{1}H NMR (CD_3OD, 500 MHz) δ 8.02 (dd, *J* = 2.5 Hz, 8.5 Hz, 1H, H-6), 7.92 (d, *J* = 2.5 Hz, 1H, H-2), 7.83 (d, *J* = 16 Hz, 1H, H-β), 7.78 (d, *J* = 8.5 Hz, 2H, B-ring), 7.73 (d, *J* = 16 Hz, 1H, H-α), 7.47 (d, *J* = 8.5 Hz, 2H, B-ring), 6.82 (d, *J* = 8.5 Hz, 1H, H-5), 3.95 (s, 2H, CH_2), 3.02 (d, *J* = 10 Hz, 2H), 2.18 (brs, 2H), 1.73 (d, *J* = 12.5 Hz, 2H), 1.47 (m, 1H), 1.37-1.29 (m, 2H), 1.01 (d, *J* = 6.5 Hz, 3H, CH_3). ^{13}C NMR (CD_3OD, 125 MHz) δ 188.6, 162.8, 143.0, 137.0, 135.5, 131.7, 131.5, 130.9, 130.2, 129.7, 124.3, 122.8, 117.5, 61.6, 53.9, 34.8, 31.3, 21.7. HR-ESIMS *m/z* calcd for $C_{22}H_{24}ClNO_2$, 369.14956; found, 370.15747 $[M+H]^+$.

(*E*)-1-(4-Chloro-phenyl)-3-(4-hydroxy-3-morpholin-4-yl-methyl-phenyl)-propenone (9e): Yield 46% (white solid); mp 161-162 °C. IR (KBr, ν (cm^{-1})): 2948, 2856, 1654, 1594, 1495, 1276, 1119, 983, 819. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.94 (dd, *J* = 2 Hz, 8.5 Hz, 1H, H-6), 7.80 (d, *J* = 2 Hz, H-2), 7.75 (d, *J* = 15.5 Hz, 1H, H-β), 7.58 (d, *J* = 8.5 Hz, 2H, B-ring), 7.53 (d, *J* = 15.5 Hz, 1H, H-α), 7.39 (d, *J* = 8.5 Hz, 2H, B-ring), 6.92 (d, *J* = 8.5 Hz, 1H, H-5), 3.82 (s, 2H, CH_2), 3.78 (br, 4H), 2.62 (br, 4H). ^{13}C NMR ($CDCl_3$, 125 MHz) *δ* 188.1, 162.6, 142.3, 136.1, 133.6, 130.5, 130.0, 129.8, 129.5, 129.2, 122.2, 120.7, 116.2, 66.6, 61.5, 52.8. HR-ESIMS *m/z* calcd for $C_{20}H_{20}ClNO_3$, 357.11317; found, 358.11447 $[M+H]^+$.

(*E*)-1-(4-Chloro-phenyl)-3-[3-(4-ethyl-piperazin-1-yl-methyl)-4-hydroxy-phenyl]-propenone (9f): Yield 49% (bright yellow solid); mp 143-144 °C. IR (KBr, ν (cm^{-1})): 3019, 2837, 1654, 1599, 1445, 1389, 1311, 1276, 1222, 1199, 1122, 1029, 997, 925, 864, 825, 756. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.93 (d, *J* = 2 Hz, 8.5 Hz, 1H, H-6), 7.77 (d, *J* = 2Hz, 1H, H-2), 7.75 (d, *J* = 16 Hz, 1H, H-β), 7.58 (d, *J* = 8 Hz, 2H, B-ring), 7.53 (d, *J* = 16 Hz, 1H, H-α), 7.39 (d, *J* = 8.5 Hz, 2H, B-ring), 6.89 (d, *J* = 8.5 Hz, 1H, H-5), 3.82 (s, 2H, CH_2), 2.95-2.74 (brs, 8H, H-piperazine), 2.49 (q, *J* = 7.5 Hz, 2H, CH_2), 1.11 (t, *J* = 7 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz) *δ* 188.1, 162.9, 142.2, 136.2, 133.7, 130.3, 129.8, 129.6, 129.5, 129.2, 122.3, 121.2, 116.2, 61.1, 52.4, 52.3, 52.2, 11.8. HR-ESIMS *m/z* calcd for $C_{22}H_{25}ClN_2O_2$, 384.16045; found, 385.16067 $[M+H]^+$.

(*E*)-1-(4-Chloro-phenyl)-3-[3-(4-methyl-piperazin-1-yl-methyl)-4-hydroxy-phenyl]-propenone (9g): Yield 48% (yellow solid); mp 130-131 °C. IR (KBr, ν (cm^{-1})): 3057, 2929, 1656, 1584, 1484, 1324, 1277, 1205, 1119, 989, 812. ^{1}H NMR (CD_3OD, 500 MHz) *δ* 8.02 (dd, *J* = 2.5 Hz, 8.5 Hz, 1H, H-6), 7.96 (d, *J* = 2H, 1H, H-2), 7.83 (d, *J* = 16 Hz, 1H, H-β), 7.77 (d, *J* = 8.5 Hz, 2H, B-ring), 7.74 (d, *J* = 16 Hz, 1H, H-α), 7.47 (d, *J* = 8.5 Hz, 2H, B-ring), 6.90 (d, *J* = 8.5

Hz, 1H, H-5), 3.85 (s, 2H, CH_2), 2.65 (br, 8H, H-piperazine), 2.33 (s, 3H, CH_3). ^{13}C NMR (CD_3OD, 125 MHz) δ 188.1, 164.3, 143.5, 135.1, 132.9, 131.6, 131.0, 130.2, 129.7, 129.3, 123.7, 122.2, 117.0, 60.6, 55.8, 53.1, 45.9. HR-ESIMS *m/z* calcd for $C_{21}H_{23}ClN_2O_2$, 370.14480; found, 371.15266 $[M+H]^+$.

(*E*)-1-(4-Chloro-phenyl)-3-(4-hydroxy-3-pyrrolidin-1-ylmethyl-phenyl)-propenone (9h): Yield 47% (yellow solid); mp 122-123 ºC. IR (KBr, ν (cm^{-1})): 3390, 2897, 1562, 1530, 1355, 1280, 675. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.92 (dd, *J* = 2.5 Hz, 8.5 Hz, 1H, H-6), 7.77 (d, *J* = 2 Hz, 1H, H-2), 7.74 (d, *J* = 15.5 Hz, 1H, H-β), 7.58 (d, *J* = 8.5 Hz, 2H, B-ring), 7.54 (d, *J* = 15.5 Hz, 1H, H-α), 7.38 (d, *J* = 8.5 Hz, 2H, B-ring), 6.88 (d, *J* = 8 Hz, 1H, H-5), 3.92 (s, 2H, CH_2), 2.68 (s, 4H), 1.88 (m, 4H). ^{13}C NMR ($CDCl_3$, 125 MHz) *δ* 188.2, 163.5, 142.0; 136.0, 133.6, 130.2, 129.4, 129.2, 129.1, 129.0, 122.4, 122.3, 117.2, 58.5, 53.4, 23.6. HR-ESIMS *m/z* calcd for $C_{20}H_{20}ClNO_2$, 341.11826; found, 342.12618 $[M+H]^+$.

(*E*)-1-(4-Chloro-phenyl)-3-[4-hydroxy-3-(2-methyl-piperidin-1-ylmethyl)-phenyl]-propenone (9i): Yield 44% (yellow solid); mp 120-121 ºC. IR (KBr, ν (cm^{-1})): 3019, 2857, 1659, 1589, 1444, 1389 1321, 1266, 997, 925, 864. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.89 (dd, *J* = 2 Hz, 8.5 Hz, 1H, H-6), 7.76 (d, *J* = 2 Hz, 1H, H-2), 7.74 (d, *J* = 15.5 Hz, 1H, H-β), 7.58 (d, *J* = 8.5 Hz, 2H, B-ring), 7.54 (d, *J* = 15.5 Hz, 1H, H-α), 7.38 (d, *J* = 8.5 Hz, 2H, B-ring), 6.86 (d, *J* = 8.5 Hz, 1H, H-5), 1.77 (br, 2H), 1.62 (br, 2H), 1.42 (br, 2H), 1.21 (m, 4H), 1.01 (d, *J* = 6.5 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 188.2, 141.9, 136.5, 133.7, 130.6, 130.0, 129.8, 129.4, 129.2, 128.4, 127.9, 122.4, 116.2, 57.3, 25.5. HR-ESIMS *m/z* calcd for $C_{22}H_{24}ClNO_2$, 369.14956; found, 370.15747 $[M+H]^+$.

(*E*)-3-[4-Hydroxy-3(4-phenyl-piperazin-1-ylmethyl)-phenyl]-1-(4-methoxy-phenyl)-propenone (10a): Yield 68% (whity solid); mp 165-166 ºC. IR (KBr, ν (cm^{-1})): 3391, 2877, 1599, 1535, 1355, 1288, 1103, 925, 806, 675. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.94 (dd, *J* = 2.5 Hz, 8.5 Hz, 1H, H-6), 7.81 (d, *J* = 2.5 Hz, 1H, H-2), 7.80 (d, *J* = 16 Hz, 1H, H-β), 7.61 (d, *J* = 7.5 Hz, 2H), 7.45 (d, *J* = 16 Hz, 1H, H-α), 7.27 (d, *J* = 7.5 Hz, 2H), 6.94-6.88 (m, 6H), 3.86 (s, 2H, CH_2), 3.85 (s, 3H, OCH_3), 3.25 (brs, 4H, H-piperazine), 2.77 (brs, 4H, H-piperazine). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 188.5, 162.5, 161.5, 150.8, 143.6, 130.3, 130.1, 129.8, 129.2, 127.9, 121.0, 120.4, 119.5, 116.4, 116.1, 114.4, 113.6, 61.2, 55.4, 52.6, 49.2. HR-ESIMS *m/z* calcd for $C_{27}H_{28}N_2O_3$, 428.20999; found, 429.21782 $[M+H]^+$.

(*E*)-3-(3-Diethylaminomethyl-4-hydroxy-phenyl)-1-(4-methoxy-phenyl)-propenone (10b): Yield 39% (yellow oil); IR (Neat, ν (cm^{-1})): 2871, 1661, 1569, 1529, 1492, 1433, 1330, 1195, 1168, 995, 783. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.91 (dd, *J* = 2 Hz, 8.5 Hz, 1H, H-6), 7.78 (d, *J* = 16 Hz, 1H, H-β), 7.77 (d, *J* = 2 Hz, 1H, H-2 (overlapped with H-β), 7.61 (d, *J* = 8.5, 2H, B-ring), 7.45 (d, *J* = 16 Hz, 1H, H-α), 6.94 (d, *J* = 9 Hz, 2H, B-ring), 6.86 (d, *J* = 8.5 Hz, 1H, H-5), 3.86 (s, 2H, CH_2), 3.85 (s, 3H, OCH_3), 2.67 (q, *J* = 7 Hz, 4H), 1.13 (t, *J* = 7 Hz, 6H, $2CH_3$). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 188.6, 163.5, 161.4, 143.3, 130.0, 129.9, 129.6, 129.4, 128.0, 122.1, 119.7, 115.9, 114.4, 56.8, 55.4, 46.4, 11.1. HR-ESIMS *m/z* calcd for $C_{21}H_{25}NO_3$, 339.18344; found, 340.19128 $[M+H]^+$.

(*E*)-3-[4-Hydroxy-3-(4-methyl-piperidin-1-ylmethyl)-phenyl]-1-(4-methoxy-phenyl)-propenone (10d): Yield 52% (yellow solid); mp 104-106 ºC. IR (KBr, ν (cm^{-1})): 3363, 2878, 1659, 1608, 1523, 1449, 1359, 1229, 1020, 802. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.91 (dd, *J* = 2 Hz, 8.5 Hz, 1H, H-6), 7.78 (d, *J* = 15.5 Hz, 1H, H-β), 7.56 (d, *J* = 2H, 1H, H-2), 7.61 (d, *J* = 8.5 Hz, 2H, B-ring), 7.45 (d, *J* = 15.5 Hz, 1H, H-α), 6.94 (d, *J* = 8.5 Hz, 2H, B-ring), 6.87 (d, *J* = 8.5 Hz, 1H, H-5), 3.85 (s, 3H), 3.78 (s, 2H), 2.98 (d, *J* = 10.5 Hz, 2H), 3.85 (s, 3H, OCH_3), 3.77 (s, 2H, CH_2), 2.16 (br, 2H), 1.72 (d, *J* = 13 Hz, 2H), 1.46 (br, 1H), 1.33-1.25 (m, 2H), 0.96 (d, *J* = 6.5 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 188.6, 163.2, 161.4, 143.4, 130.0, 129.9, 129.7, 129.5, 127.9, 121.6, 119.7, 115.9, 114.4, 61.5, 55.4, 53.3, 34.0, 30.4, 21.6. HR-ESIMS *m/z* calcd for $C_{23}H_{27}NO_3$, 365.19909; found, 366.20652 $[M+H]^+$.

(*E*)-3-(4-Hydroxy-3-morpholin-4-ylmethyl-phenyl)-1-(4-methoxy-phenyl)-propenone (10e): Yield 43% (white solid); mp 122-123 ºC. IR (KBr, ν (cm^{-1})): 2879, 1655, 1609, 1533, 1444, 1351, 1226, 1022, 802, 732. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.94 (dd, *J* = 2 Hz, 8.5 Hz, 1H, H-6), 7.79 (s, 1H, H-2), 7.76 (d, *J* = 15 Hz, 1H, H-β), 7.61 (d, *J* = 9 Hz, 2H, B-ring), 7.44 (d, *J* = 15 Hz, 1H, H-α), 6.94 (d, *J* = 9 Hz, 2H, B-ring), 6.90 (d, *J* = 8.5 Hz, 1H, H-5), 3.85 (s, 3H, OCH_3), 3.81 (s, 2H, CH_2), 3.70-3.77 (br, 4H), 2.61 (s, 4H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 188.5, 162.3, 161.5, 143.6, 130.3, 130.2, 130.1, 129.9, 127.9, 120.7, 129.5, 116.0, 114.4, 66.7, 61.7, 55.4, 52.9. HR-ESIMS *m/z* calcd for $C_{21}H_{23}NO_4$, 353.16271; found, 354.16052 $[M+H]^+$.

(*E*)-3-[3-(4-Ethyl-piperazin-1-ylmethyl)-4-hydroxy-phenyl]-1-(methoxy-phenyl)-propenone (10f): Yield 51% (pale yellow oil); IR (Neat, ν (cm^{-1})): 2841, 1660, 1569, 1525, 1492, 1433, 1330, 1192, 1165, 995, 783, 748. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.93 (dd, *J* = 2.5 Hz, 8.5 Hz, 1H, H-6), 7.78 (d, *J* = 15.5 Hz, 1H, H-β), 7.77 (d, *J* = 2.5 Hz, 1H, H-2), 7.61 (d, *J* = 8.5 Hz, 2H, B-ring), 7.44 (d, *J* = 15.5 Hz, 1H, H-α), 6.94 (d, *J* = 8.5 Hz, 2H, B-ring), 6.89 (d, *J* = 8.5 Hz, 1H, H-5), 3.85 (s, 3H, OCH_3), 3.81 (s, 2H, CH_2), 2.65-2.57 (brs, 8H, H-piperazine), 2.45 (q, *J* = 7.5 Hz, 2H, CH_2), 1.09 (t, *J* = 7.5 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 188.5, 162.6, 161.4, 143.5, 130.1, 130.0, 129.9, 129.6, 127.9, 121.1, 119.5, 115.9, 114.3, 61.1, 55.3, 52.4, 52.3, 52.0, 11.9. HR-ESIMS *m/z* calcd for $C_{23}H_{28}N_2O_3$, 380.20999; found, 381.20655 $[M+H]^+$.

(*E*)-3-[3-(4-Methyl-piperazin-1-ylmethyl)-4-hydroxy-phenyl]-1-(methoxy-phenyl)-propenone (10g): Yield 53% (yellow oil); IR (Neat, ν (cm^{-1})): 2879, 1648, 1606, 1519, 1450, 1422, 1365, 1289, 1190, 657. ^{1}H NMR (CD_3OD, 500 MHz) δ 7.99 (dd, *J* = 2 Hz, 8.5 Hz, 1H, H-6), 7.92 (d, *J* = 2 Hz, 1H, H-2), 7.75 (d, *J* = 15.5 Hz, 1H, H-β), 7.71 (d, *J* = 8.5 Hz, 2H, B-ring), 7.65 (d, *J* = 15.5 Hz, 1H, H-α), 6.99 (d, *J* = 8.5 Hz, 2H, B-ring), 6.89 (d, *J* = 8.5 Hz, 1H, H-5), 3.86 (s, 3H, OCH_3), 3.82 (s, 2H, CH_2), 2.63-2.53 (brs, 8H, H-pipera-

zine), 2.32 (s, 3H, CH_3). ^{13}C NMR (CD_3OD, 125 MHz) δ 190.7, 164.0, 163.3, 145.2, 131.8, 131.5, 131.4, 131.0, 129.1, 123.0, 120.4, 116.9, 115.5, 60.7, 55.8, 55.7, 53.0, 45.8. HR-ESIMS *m/z* calcd for $C_{22}H_{26}N_2O_3$, 366.19434; found, 367.20218 $[M+H]^+$.

(*E*)-3-(4-Hydroxy-3-pyrrolidin-1-ylmethyl-phenyl)-1-(4-methoxy-phenyl)-propenone (10h): Yield 49% (pale yellow oil); IR (Neat, ν (cm^{-1})): 2875, 1652, 1601, 1516, 1493, 1288, 1120, 1036, 928. ^1H NMR (CD_3OD, 500 MHz) δ 7.99 (dd, *J* = 2 Hz, 8.5 Hz, 1H, H-6), 7.94 (d, *J* = 2 Hz, 1H, H-2), 7.73 (d, *J* = 15.5 Hz, 1H, H-β), 7.69 (d, *J* = 8.5 Hz, 2H, B-ring), 7.67 (d, *J* = 15.5 Hz, 1H, H-α), 6.99 (d, *J* = 8.5 Hz, 2H, B-ring), 6.79 (d, *J* = 8.5 Hz, 1H, H-5), 4.08 (s, 2H, CH_2), 3.85 (s, 3H, OCH_3), 2.97 (m, 4H, H-pyrrolidine), 1.97 (m, 4H, H-pyrrolidine). ^{13}C NMR (CD_3OD, 125 MHz) δ 190.3, 163.2, 162.7, 144.6, 132.4, 132.1, 131.3, 129.2, 128.1, 122.6, 120.6, 118.3, 115.5, 58.5, 55.9, 54.3, 24.4. HR-ESIMS *m/z* calcd for $C_{21}H_{23}NO_3$, 337.16779; found, 338.17566 $[M+H]^+$.

(*E*)-3-[4-Hydroxy-3-(2-methyl-piperidin-1-ylmethyl)-phenyl]-1-(4-methoxy-phenyl)-propenone (10i): Yield 46% (yellow oil). IR (Neat, ν (cm^{-1})): 3388, 2879, 1659, 1609, 1511, 1495, 1288, 1120, 1036, 928. ^1H NMR ($CDCl_3$, 500 MHz) δ 7.89 (dd, *J* = 2 Hz, 8.5 Hz, 1H, H-6), 7.77 (d, *J* = 15.5 Hz, 1H, H-β), 7.75 (d, *J* = 2 Hz, 1H, H-2), 7.60 (d, *J* = 8.5 Hz, 2H, B-ring), 7.45 (d, *J* = 15.5 Hz, 1H, H-α), 6.93 (d, *J* = 8.5 Hz, 2H, B-ring), 6.85 (d, *J* = 8.5 Hz, 1H, H-5), 3.85 (s, 3H, OCH_3), 3.81 (s, 2H, CH_2), 2.88 (br, 1H), 1.78-1.72 (m, 1H), 1.70-1.59 (m, 2H), 1.58-1.34 (m, 2H), 1.19-1.17 (d, *J* = 6.5 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 188.5, 161.4, 160.7, 143.3, 129.9, 129.7, 129.3, 127.9, 122.5, 119.6, 115.9, 114.3, 113.7, 57.3, 55.3, 25.5. HR-ESIMS *m/z* calcd for $C_{23}H_{27}NO_3$, 365.19909; found, 366.20693 $[M+H]^+$.

(*E*)-3-[4-Hydroxy-3(4-phenyl-piperazin-1-ylmethyl)-phenyl]-1-(2-methoxy-phenyl)-propenone (11a): Yield 64% (white solid); mp 148-149 °C. IR (KBr, ν (cm^{-1})): 3424, 2841, 1655, 1607, 1444, 1221, 1119, 802. ^1H NMR ($CDCl_3$, 500 MHz) δ 8.12 (d, *J* = 15.5 Hz, 1H, H-β), 7.95 (dd, *J* = 2 Hz, 8.5 Hz, 1H, H-6), 7.81 (d, *J* = 2Hz, 1H, H-2), 7.65 (d, *J* = 1.5 Hz, 1H), 7.64 (d, *J* = 15.5 Hz, 1H, H-α), 7.37 (dt, 1.5 Hz, 8.5 Hz, 1H), 7.28 (t, *J* = 7.5 Hz, 2H), 6.99 (t, *J* = 7.5 Hz, 1H), 6.95-6.88 (m, 5H), 3.94 (s, 3H, OCH_3), 3.87 (s, 2H, CH_2), 3.28 (br, 4H, H-piperazine), 2.78 (br, 4H, H-piperazine). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 189.2, 162.5, 161.5, 150.8, 143.7, 134.2, 131.4, 130.9, 129.9, 129.1, 127.1, 124.9, 124.2, 122.7, 120.9, 120.5 116.5, 116.1, 111.3, 61.2, 55.6, 52.6, 49.1. HR-ESIMS *m/z* calcd for $C_{27}H_{28}N_2O_3$, 428.20999; found, 429.21784 $[M+H]^+$.

(*E*)-3-(3-Diethylaminomethyl-4-hydroxy-phenyl)-1-(2-methoxy-phenyl)-propenone (11b): Yield 35% (yellow oil); IR (Neat, ν (cm^{-1})): 3370, 2879, 1655, 1600, 1521, 1444, 1353, 1221, 1119, 802. ^1H NMR ($CDCl_3$, 500 MHz) δ 8.11 (d, *J* = 15.5 Hz, 1H-β), 7.91 (dd, *J* = 2Hz, 8.5 Hz, 1H, H-6), 7.77 (d, *J* = 1.5 Hz, 1H, H-2), 7.65 (dd, *J* = 1.5 Hz, 7.5 Hz, 1H, overlapped with H-α), 7.64 (d, *J* = 15.5 Hz, 1H-α), 7.36 (dt, *J* = 1.5 Hz, 8.5 Hz, 1H), 6.98 (t, *J* = 7.5 Hz, 1H), 6.94 (d, *J* = 8.5 Hz, 1H), 6.86 (d, *J* = 8.5 Hz, 1H), 3.91 (s, 3H, OCH_3), 3.76 (s, 2H, CH_2), 2.66 (q, *J* = 7 Hz, 4H, $2CH_2$), 1.13 (t, *J* = 7.5 Hz, 6H, $2CH_3$). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 189.2, 163.5, 158.7, 139.0, 131.3, 130.1, 129.7, 129.5, 128.9, 124.3, 122.8, 121.9, 120.7, 115.9, 111.2, 56.8, 55.5, 46.4, 11.1. HR-ESIMS *m/z* calcd for $C_{21}H_{25}NO_3$, 339.18344; found, 340.19124 $[M+H]^+$.

(*E*)-3-(4-Hydroxy-3-piperidin-1-ylmethyl-phenyl)-1-(2-methoxy-phenyl)-propenone (11c): Yield 49% (yellow oil); IR (Neat, ν (cm^{-1})): 2880, 1622, 1598, 1528. ^1H NMR ($CDCl_3$, 500 MHz) δ 8.11 (d, *J* = 15.5 Hz, 1H-β), 7.91 (dd, *J* = 2 Hz, 8.5 Hz, 1H, H-6), 7.76 (d, *J* = 2 Hz, 1H, H-2), 7.64 (dd, *J* = 1.5 Hz, 7.5 Hz, 1H, overlapped with H-α), 7.63 (d, *J* = 15.5 Hz, 1H-α), 7.36 (dt, *J* = 1.5 Hz, 7.5 Hz, 1H), 6.98 (t, *J* = 7.5 Hz, 1H), 6.94 (d, *J* = 8Hz, 1H), 6.87 (d, *J* = 8.5 Hz, 1H), 3.91 (s, 3H, OCH_3), 3.77 (s, 2H, CH_2), 2.99-2.00 (m, 4H, H-piperidine), 1.83-1.20 (m, 6H, H-piperidine). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 189.2, 163.2, 158.7, 139.1, 131.4, 130.1, 129.7, 129.6, 128.9, 124.3, 122.8, 121.6, 120.7, 115.9; 111.2; 61.5, 55.5, 53.3, 34.0, 21.6. HR-ESIMS *m/z* calcd for $C_{22}H_{25}NO_3$, 351.18344; found, 352.18124 $[M+H]^+$.

(*E*)-[4-Hydroxy-3-(4-methyl-piperidin-1-ylmethyl)-phenyl]-1-(2-methoxy-phenyl)-propenone (11d): Yield 51% (yellow solid); mp 108-110 °C. IR (KBr, ν (cm^{-1})): 3234, 2838, 1659, 1599, 1501, 1455, 1175, 1029, 983. ^1H NMR ($CDCl_3$, 500 MHz) δ 8.10 (d, *J* = 15.5 Hz, 1H-β), 7.91 (dd, *J* = 2 Hz, 8.5 Hz, 1H, H-6), 7.76 (d, *J* = 2 Hz, 1H, H-2), 7.64 (dd, *J* = 1.5 Hz, 7.5 Hz, 1H, overlapped with H-α), 7.63 (d, *J* = 15.5 Hz, 1H-α), 7.36 (dt, *J* = 1.5 Hz, 7.5 Hz, 1H), 6.98 (t, *J* = 7.5 Hz, 1H), 6.94 (d, *J* = 8 Hz, 1H), 6.87 (d, *J* = 8.5 Hz, 1H), 3.91 (s, 3H, OCH_3), 3.77 (s, 2H, CH_2), 2.99-2.90 (m, 4H, H-piperidine), 1.69-166 (m, 1H), 1.58-1.55 (m, 4H, H-piperidine), 0.98 (d, *J* = 6.5 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 189.2, 163.2, 158.7, 139.1, 131.4, 130.1, 129.7, 129.6, 128.9, 124.3, 122.8, 121.6, 120.7, 115.9; 111.2; 61.5, 55.5, 53.3, 34.0, 21.6. HR-ESIMS *m/z* calcd for $C_{23}H_{27}NO_3$, 365.19909; found, 366.20693 $[M+H]^+$.

(*E*)-3-(4-Hydroxy-3-morpholin-4-ylmethyl-phenyl)-1-(2-methoxy-phenyl)-propenone (11e): Yield 40% (whity solid). IR (KBr, ν (cm^{-1})): 3404, 2821, 1656, 1600, 1444. ^1H NMR ($CDCl_3$, 500 MHz) δ 8.14 (d, *J* = 15.5 Hz, 1H, H-β), 7.94 (dd, *J* = 2.5 Hz, 8.5 Hz, 1H, H-6), 7.79 (d, *J* = 2.5 Hz, 1H, H-2), 7.65 (d, *J* = 1.5 Hz, 8 Hz, 1H), 7.62 (d, *J* = 15.5 Hz, 1H-α), 7.37 (dt, *J* = 1.5 Hz, 8 Hz, 1H), 6.99 (t, *J* = 7.5 Hz, 1H), 6.95 (d, *J* = 8.5 Hz, 1H), 6.90 (d, *J* = 8.5 Hz, 1H), 3.92 (s, 3H, OCH_3), 3.78 (s, 2H, CH_2), 3.77 (br, 4H, H-morpholine), 2.61 (br, 4H, H-morpholine). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 189.2, 162.2, 158.7, 139.3, 134.3, 131.5, 130.6, 130.4, 129.9, 124.2, 122.6, 120.7, 120.2, 116.0, 111.3, 66.7, 61.6, 55.5, 52.8. HR-ESIMS *m/z* calcd for $C_{21}H_{23}NO_4$, 353.16271; found, 354.17055 $[M+H]^+$.

(*E*)-3-[3-(4-Ethyl-piperazin-1-ylmethyl)-4-hydroxy-phenyl]-1-(2-methoxy-phenyl)-propenone (11f): Yield 42% (light yellow solid); mp 95-96 °C. IR (KBr, ν (cm^{-1})): 3334, 2831, 1657, 1599, 1508, 1451, 1172, 1029, 983. ^1H NMR ($CDCl_3$, 500 MHz) δ 8.11 (d, *J* = 15.5 Hz, 1H-β), 7.93 (dd, *J* = 2 Hz, 8.5 Hz, 1H, H-6), 7.77 (d, *J* = 2 Hz, 1H, H-2), 7.65 (d, *J* = 1.5 Hz, 1H), 7.63 (d, *J* = 15.5 Hz, 1H, H-α), 7.36 (dt,

J = 1.5 Hz, 8.5 Hz, 1H), 6.98 (t, *J* = 7.5 Hz, 1H), 6.94 (d, *J* = 8.5 Hz, 1H), 6.88 (d, *J* = 8.5 Hz, 1H), 3.91 (s, 3H, OCH_3), 3.76 (s, 2H, CH_2), 2.65 (brs, 8H, H-piperazine), 2.45 (q, *J* = 7 Hz, 2H, CH_2), 1.09 (t, *J* = 7 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 189.1, 162.5, 158.6, 139.1, 131.4, 130.2, 129.9, 129.7, 128.9, 124.2, 122.6, 121.1, 120.6, 115.9, 111.2; 61.1, 55.5, 52.4, 52.3, 52.0, 11.9. HR-ESIMS *m/z* calcd for $C_{23}H_{28}N_2O_3$, 380.20999; found, 381.20787 $[M+H]^+$.

(*E*)-3-[3-(4-Methyl-piperazin-1-ylmethyl)-4-hydroxy-phenyl]-1-(2-methoxy-phenyl)-propenone (11g): Yield 41% (yellow solid); mp 124-126 °C. IR (KBr, ν (cm^{-1})): 3335, 2899, 1658, 1597, 1492, 1446, 1037, 991, 929. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.11 (d, *J* = 15.5 Hz, 1H-β), 7.93 (dd, *J* = 2 Hz, 8.5 Hz, 1H, H-6), 7.78 (d, *J* = 2 Hz, 1H, H-2), 7.65 (dd, *J* = 1.5 Hz, 7.5 Hz, 1H), 7.62 (d, *J* = 15.5 Hz, 1H, H-α), 7.36 (dt, *J* = 2 Hz, 7.5 Hz, 1H), 6.99 (t, *J* = 7.5 Hz, 1H), 6.94 (d, *J* = 8.5 Hz, 1H), 6.89 (d, *J* = 8.5 Hz, 1H), 3.91 (s, 3H, OCH_3), 3.81 (s, 2H, CH_2), 2.80-2.40 (brs, 8H, H-piperazine), 2.31 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 189.1, 162.5, 158.7, 139.2, 131.4, 130.6, 130.1, 129.8, 128.9, 124.2, 122.6, 121.1, 120.7, 115.9, 111.2, 61.1, 55.5, 54.7, 52.4, 45.8. HR-ESIMS *m/z* calcd for $C_{22}H_{26}N_2O_3$, 366.19434; found, 367.20116 $[M+H]^+$.

(*E*)-3-(4-Hydroxy-3-pyrrolidin-1-ylmethyl-phenyl)-1-(2-methoxy-phenyl)-propenone (11h): Yield 46% (yellow oil); IR (Neat, ν (cm^{-1})): 2836, 1649, 1587, 1523, 1450, 1389, 1346, 1188, 1032, 825. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8,11 (d, *J* = 15.5 Hz, 1H, H-β), 7.92 (dd, *J* = 2 Hz, 8.5 Hz, 1H, H-6), 7.78 (d, *J* = 2 Hz, 1H, H-2), 7.65 (dd, *J* = 1.5 Hz, 7.5 Hz, 1H, overlapped with H-α), 7.64 (d, *J* = 15.5 Hz, 1H, H-α), 7.36 (dt, *J* = 1.5 Hz, 8.5 Hz, 1H), 6.99 (t, *J* = 7.5 Hz, 1H), 6.95 (d, *J* = 8 Hz, 1H), 6.88 (d, *J* = 8.5 Hz, 1H), 3.92 (s, 3H, OCH_3), 3.79 (s, 2H, CH_2), 2.68 (brs, 4H, H-pyrrolidine), 1.88 (m, 4H, H-pyrrolidine). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 189.2, 163.1, 158.7, 139.1, 131.4, 130.1, 129.7, 129.0, 124.3, 124.3, 122.8, 122.4, 120.7, 115.8, 111.2, 58.6, 55.6, 53.5, 23.7. HR-ESIMS *m/z* calcd for $C_{21}H_{23}NO_3$, 337.16779; found, 338.17554 $[M+H]^+$.

(*E*)-3-[4-Hydroxy-3-(2-methyl-piperidin-1-ylmethyl)-phenyl]-1-(2-methoxy-phenyl)-propenone (11i): Yield 47% (yellow solid); mp 97-98 °C. IR (KBr, ν (cm^{-1})): 3339, 2849, 1637, 1590, 1538, 1441, 1182, 1026, 988. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.1(d, *J* = 15,5 Hz, 1H, H-β), 7.89 (dd, *J* = 2 Hz, 8.5 Hz, 1H, H-6), 7.76 (d, *J* = 2 Hz, 1H, H-2), 7.65 (dd, *J* = 1.5 Hz, 7.5 Hz, 1H), 7.64 (d, *J* = 15.5 Hz, 1H, H-α), 7.36 (dt, *J* = 1.5 Hz, 7.5 Hz, 1H), 6.98 (t, *J* = 7 Hz, 1H), 6.94 (d, *J* = 8 Hz, 1H), 6.85 (d, *J* = 8.5 Hz, 1H), 3.91 (s, 3H, OCH_3), 3.76 (s, 2H, CH_2), 1.78-1.69 (m, 2H), 1.62-1.59 (br, 2H), 1.43 (br, 2H), 1.20 (d, *J* = 5 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 189.2, 163.3, 158.7, 138.9, 131.3, 129.9, 129.6, 129.3, 128.9, 124.3, 122.8, 122.2, 120.7, 115.9, 111.2, 55.4, 55.5, 25.6. HR-ESIMS *m/z* calcd for $C_{23}H_{27}NO_3$, 365.19909; found, 366.19692 $[M+H]^+$.

Pharmacology. The bio-assay of Mannich bases was carried out at the Institute of Chemistry, Vietnam Academy of Science and Technology. The cytotoxic activity evaluation were undertaken according to the described protocol.[22] The IC_{50} of the assay was carried out using three human cancer cell lines: HepG2 (*ATCC-HB-8065)*, SK-LU-1 (*ATCC-HTB-57)* and MCF-7 (*ATCC-HTB-22).* Ellipticine was used as a standard substance.

Results and Discussion

The synthesis of new Mannich bases **9a-i**, **10a-i** and **11a-i** was outlined in Scheme 1 and 2. Firstly, chalcones **6**, **7** and **8** were synthesized by Claisen-Schmidt condensation between 4-hydroxyacetophenone (**1**) and different aldehydes: 4-chlorobenzaldehyde (**3**), 4-methoxybenzaldehyde (**4**) and 2-methoxybenzaldehyde (**5**) in ethanol for 18-24 h in the presence of NaOH as a catalyst at room temperature (Scheme 1). After a work-up by dilution with water, acidification with 1 M HCl to pH 2, the resulting solid was obtained and recrystallized from methanol - water mixture to yield the desired products. The structure of synthesized chalcones **6**, **7** and **8** was elucidated based on spectroscopic methods and compared with the known documents.[15,21]

The designed target compounds **9a-i**, **10a-i** and **11a-i** were prepared by the reaction of **6**, **7** and **8** with various secondary amines and paraformaldehyde[15] in EtOH at reflux for 24-36 h (Scheme 2). In almost cases, compounds after a work-up with water and dichloromethane were purified by column chromatography. For compounds **9c-e**; **9h**,**i**; **10c-e**; **10h**,**i**; **11c-e** and **11h**,**i**, column chromatography was carried out on silica gel using *n*-hexane and ethyl acetate as an eluent solvent system. Compounds **9b**, **9f**,**g**; **10b**, **10f**,**g**; **11b**, and **11f**,**g** were obtained by column chromatography on silica gel using dichloromethane and methanol as an eluent solvent system. Compounds **9a**, **10a** and **11a** were obtained by crystallization in methanol. The structure of compounds was confirmed by the ^{1}H-NMR, ^{13}C-NMR, ESI-HRMS and IR spectra.

The presence of mono Mannich base group was confirmed by the spectra data. The case of compound **9a** was an example. The presence of more five prontons of the phenyl group was observed in the ^{1}H NMR spectra. The appearance of ABC splitting pattern instead of AA'BB' pattern in A ring of chalcone can be observed with H-6 at downfield: δ 7.95 ppm as a doublet of doublet (*J* = 2.5 Hz and 8.5 Hz), H-2 as doublet (*J* = 2.5 Hz) at 7.81 ppm and H-5 that is overlapped with protons of phenyl group at higher field. The methylene protons appeared as a singlet at δ 3.87 ppm, and 8 protons of piperazine nucleus were observed as two broad singlets at 3.26 and 2.77 ppm. Protons of α,β-unsaturated ketone of Mannich chalcone **9a** were observed as doublets with *J* = 15.5 Hz at 7.76 ppm for H_β and 7.54 ppm for H_α. The *J* characteristic values were the evidence of *E* isomers. In the ^{13}C NMR spectra, besides the appearance of four more carbons of the phenyl group were observed, the carbon peaks of olefinic group in **9a** were observed at δ 142.3 and 122.3 ppm. In addition, there are two strong absorption peaks observed at 1655 and 1600 cm^{-1} in the IR spectra that support the α,β-unsaturated ketone structure.

Table 1. Cytotoxic activity data for Mannich bases: **9a-i**, **10a-i** and **11a-i**

Compound	IC_{50} (μg/mL)		
	HepG2	SK- LU-1	MCF-7
9a	81.29	27.65	32.0
9b	5.23	2.0	3.47
9c	3.73	3.95	2.0
9d	>128	128	>128
9e	4.56	6.49	4.28
9f	1.57	1.16	1.21
9g	3.16	3.72	2.0
9h	2.90	3,09	1.58
9i	8.25	2.8	4.52
10a	>128	>128	>128
10b	10.85	16.64	6.84
10c	21.08	12.80	17.37
10d	32.0	13.68	26.85
10e	21.81	15.66	21.16
10f	5.16	8.0	2.0
10g	12.80	5.49	6.01
10h	16.06	6.56	7.34
10i	10.96	4.93	8.0
11a	56.87	108.38	47.2
11b	7.31	10.74	6.04
11c	6.27	7.29	4.92
11d	7.37	15.9	5.28
11e	2.58	5.13	4.70
11f	3.76	4.44	3.60
11g	2.61	4.45	2.69
11h	7.08	6.76	5.95
11i	7.21	19.05	7.24
ellipticine	0.99	0.78	0.82

Note: The reference substance, ellipticine, exhibited cytotoxic activity against HepG2 (*ATCC-HB-8065*), SK-LU-1 (*ATCC-HTB-57*) and MCF-7 (*ATCC-HTB-22*) cells with IC_{50} values of 0.99, 0.78, and 0.82 μg/mL, respectively. The values shown for these compounds are the average of three determinations.

Although the compounds **9c** and **10c** were synthesized and tested for cytotoxic activity against PC-3 cell line,[15] in this communication, three series of Mannich bases **9a-i**, **10a-i** and **11a-i** were evaluated for cytotoxic activity against three human cancer cell lines: (HepG2, SK-LU-1, and MCF-7). The results are shown in Table 1. In series **9a-i**, the initial structure-activity observations showed that the phenyl piperazine Mannich group at the C-3 position in the A-ring of compound **9a** resulted in weaker cytotoxic activity against three cell lines than other substituent groups. Compounds **9b**, **9e** and **9i** exhibited moderate cytotoxic activities with IC_{50} values of 5.23 (HepG2), 4.56 (HepG2), 6.49 (SK-LU-1), 4.28 (MCF-7), and 4.52 (MCF-7) μg/mL. Compound **9b** showed significant activity against only MCF-7 cell line with IC_{50} value of 3.47 μg/mL. In addition, the compounds **9b** and **9i** exhibited potent activity against SK-LU-1 with IC_{50} values of 2.0 and 2.8 μg/mL, respectively. For compounds **9c**, **9g** and **9h**, they also showed significant activities against two cell lines with IC_{50} values of 3.73 (HepG2), 3.95 (SK-LU-1), 3.16 (HepG2), 3.72 (SK-LU-1), 2.90 (HepG2), 3,09 (SK-LU-1) μg/mL. Notably, these compounds exhibited strong activity against MCF-7 with IC_{50} values of 2.0, 2.0 and 1.58 μg/mL, respectively. Unexpectedly, compound **9d** was no active, although it has only one structural difference with compound **9c**. Among the compounds in series **9a-i**, compound **9f**, which contains ethylpiperazine group was the most active against all three cell lines with IC_{50} values of 1.57, 1.16 and 1.21 μg/mL, respectively and exhibited about 1.5 times lower potency than control, ellipticine.

In series **10a-i**, it was found that the phenyl piperazine Mannich group at the C-3 position in the A-ring of compound **10a** resulted in no cytotoxic activity against three cell lines. Compounds **10c**, **10d** and **10e** showed weak activity against three cell lines. Compound **10b** was weak active against HepG2 and SK-LU-1 cell lines, but exhibited moderate activity against MCF-7 with IC_{50} value of 6.84 μg/mL. Compounds **10g**, **10h** and **10i** exhibited weak activity against HepG2 cell line. However, these compounds showed moderate activity against SK-LU-1 cell line with IC_{50} values of 5.49, 6.56 and 4.93 μg/mL, respectively. In this series, compound **10f** containing ethylpiperazine group showed the highest activity against MCF-7 cell line with IC_{50} value of 2.0 μg/mL while exhibited moderate activity against HepG2 cell line.

In series **11a-i**, compounds **11b-d**, and **11h**,**i** exhibited from weak to moderate activity against HepG2 and SK-LU-1 cell lines, but **11c** and **11d** showed improved activity against MCF-7 cell line with IC_{50} values of 4.92 and 5.28 μg/mL, respectively. Compounds **11e-g** showed significant activity against three cell lines, in which compound **11g** containing methylpiperazine group exhibited more significant activity against HepG2 and MCF-7 cell lines with IC_{50} values of 2.61 and 2.69 μg/mL, respectively. From an SAR viewpoint, it was observed in this research that the phenylpiperazine group seems to be responsible for reducing cytotoxic activity in **9a**, **10a**, and **11a**, whereas the more basic and polar groups may be beneficial for enhancing cytotoxic activity in **9f**,**g**; **10f**,**g** and **11f**,**g**. Interestingly, 4-chloro group in the B ring is beneficial for enhancing cytotoxic activity against three cell lines in almost cases compared with methoxy group.

Conclusion

In summary, we have synthesized a series of new Mannich bases through a simple procedure, and screened for cytotoxic activity. The results showed that compound **9f** exhibited most potent cytotoxic activity against three cell lines with IC_{50} values of 1.57, 1.16 and 1.21 μg/mL, respectively. In addition, compounds **9g**, **10f** exhibited very significant activity against MCF-7 cell line with IC_{50} value of 2.0 μg/mL. The biological evaluation study indicated that some Mannich bases derivatives could be identified as potential *in vitro* cytotoxic agents, and could serve as a foundation for further biological investigation.

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