Indian Journal of Chemistry
Vol. 62, March 2023, pp. 244-250
DOI: 10.56042/ijc.v62i3.71995

New quinazolinone-based Mannich bases: Synthesis and *in vitro* cytotoxic evaluation

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Received 15 August 2021; accepted (revised) 24 November 2022

This research presents the synthesis of new quinazolinone-based Mannich bases in good yields *via* a three-step procedure. The first step is the reaction of 6-hydroxyanthranilic acid **1** with an excess of acetic anhydride at 150°C for 2 h to afford benzoxazinone **2** in 87% yield. Compound **2** is then reacted with 4-aminophenol in DMSO at reflux for 7 h to give compound **3** in 75% yield. Finally, the reaction of **3** with paraformadehyde and secondary amines in ethanol affords new quinazolinone-based Mannich bases **4a-c** and **5a-e** in 55-70% yields. The structure of Mannich bases have been characterized by NMR and MS spectra. The bio-assay results show that some new Mannich bases exhibited weak to moderate cytotoxic activity against SKLu-1 and MCF-7 cell lines.

Keywords: Cancer, Mannich, Cytotoxicity, Quinazolinone

Cancer is a term that refers to a group of diseases involving the abnormal growth of cells with potential to invade or spread to other parts of the body. According to Globocan 2020, the global number of cancer cases is 19.3 million cases and 10 million cancer deaths in 2020. Therefore, cancer research has been receiving intense attention hoping to make significant advances in the field of its diagnosis and treatment[1]. Despite great advances in cancer research and treatment, the continuous search for new anti-cancer molecules remains crucial due to problems related to selectivity, potency and drug resistance. For discovery of new anti-cancer moieties, the search for small molecules with a specific target is very important and is requested frequently. One of the approaches for developing anti-cancer agents is the introduction of heterocyclic moieties into a molecule to make new molecules with new effects.

Quinazolinone is a class of nitrogen-containing heterocycles, and its derivatives exist in the form of natural or synthetic biomolecules with pharmacological properties such as antitumor, antifungal, antibacterial, anticonvulsant, antiviral, antihypertensive, anti-inflammatory, and analgesic activities[2,3].

Mannich reaction is the reaction between aldehydes, amines, or heterocyclic acidic proton-containing compounds[4,5]. It is considered as the one of the basic C-C bond-forming reactions in organic chemistry, and has been studied extensively[6]. Mannich reactions have been employed in the synthesis of natural products as well as in medicinal chemistry, especially for the synthesis of nitrogenous heterocyclic molecules[7–9]. However, great contribution has been made in the field of medicinal chemistry[10] where the Mannich reaction provides a wide range of biological activities such as anti-cancer[11], antibacterial[12], anti-inflammatory[13], anthelmintic[14], analgesic[15], and a anticonvulsant[16]. In fact, many examples of clinically approved drugs consist of aminoalkyl chains, such as ranitidine, amodiaquine, procyclidine, *etc*. (Fig. 1) [17,18].

Given anti-cancer property of quinazolinone moiety and Mannich bases, it envisaged that the combined effect of all entities would result in increased anti-cancer activity. Accordingly, in this

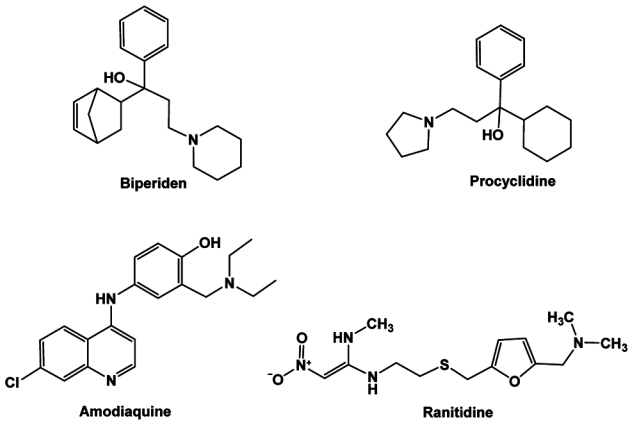


Fig. 1 — Several Mannich bases containing aminoalkyl chains

report, we present a synthesis of new quinazolinone-based Mannich bases and evaluate cytotoxic activity against several cancer cell lines.

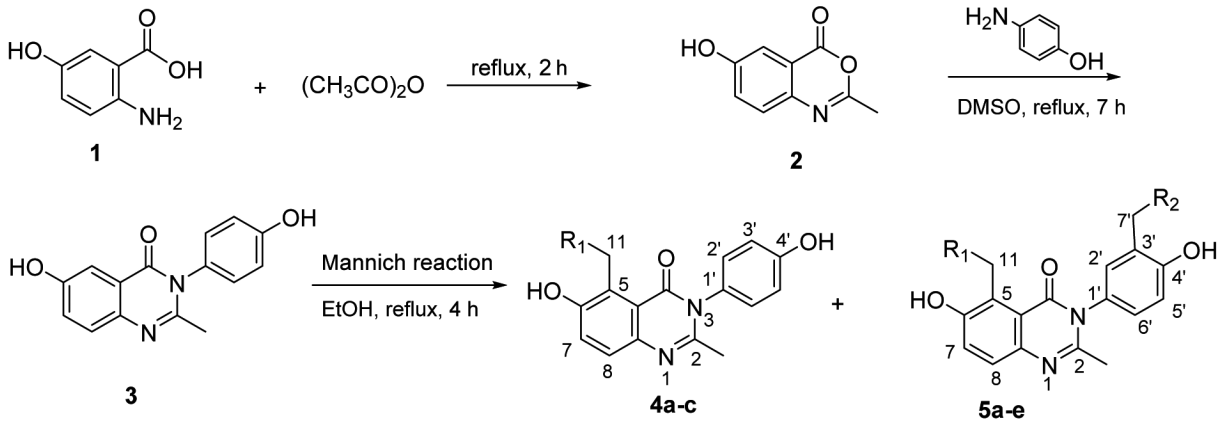
Results and Discussion

Chemistry

New quinazolinone-based Mannich bases were synthesized in good yields *via* a three-step procedure (Scheme 1). The first step is the reaction of 6-hydroxyanthranilic acid **1** with the excess of acetic anhydride at 150°C for 2 h to afford benzoxazinone **2** in 87% yield[19,20]. Compound **2** was next reacted with 4-aminophenol in DMSO at reflux for 7 h, instead of heating to give compound **3** in 75% yield. At first, we also tried to prepare the important intermediate compound **3** as reported[21], but compound **3** was obtained with low yield. It is possible that high temperature and solvent-free conditions led to the decomposition of the product. Finally, the reaction of **3** with the excess of paraformadehyde and secondary amines in ethanol afforded new quinazolinone-based mono Mannich bases **4a-c** and **5a-e**. In order to obtain mono Mannich and bis Mannich bases for biological evaluation, at the beginning, one equivalent of all secondary amines was used. However, only three mono Mannich bases **4a-c** were obtained. The structure of **4a-c** were characterized by ^{1}H and ^{13}C NMR, and MS spectra. Due to the structural similarity of target compounds, compound **4a** was used as an example to elucidate the structure.

The ^{1}H NMR spectrum of **4a** shows the presence of all protons in the molecule, in which 6 aromatic protons of the quinazolinone and the phenyl nucleus in the characteristic aromatic field were observed. Two equivalent proton pairs of the phenyl nucleus resonate as two doublet signals at δ 7.04 and 6.91 (J = 9.0 Hz) showing that Mannich reaction occurred at quinazolinone moiety, and the Mannich group connected to the position 5 of the quinazolinone skeleton was confirmed *via* two resonance signals of H-7 and H-8 as doublets at δ 7.56 and 7.25 (J = 8.5 Hz). In addition, this is further confirmed by a singlet signal at δ 4.72 of the Mannich methylene and 8 protons of piperazine groups were observed at δ 2.71-2.65. The singlet signals at δ 2.36 and 2.30 are attributed to the CH_3 groups of the quinazolinone and the piperazine nucleus.

The ^{13}C NMR spectrum of **4a** also shows all signals of the carbon in the molecule, in which the signal at the lowest field is attributed to C-4, and C-2 resonates at δ 151.6. Besides, two carbon connected to the OH of the quinazolinone and phenyl



Reagents and conditions: (i) $(CH_3CO)_2O$, 150°C, 2 h, 87%; (ii) 4-aminophenol, DMSO, reflux, 7 h, 75%; (iii) secondary amines (1 – 3 eq), ethanol, reflux, 4 h, 55 – 70%.

Scheme 1 — Synthesis of compounds **4** & **5**

nucleus resonate at δ 158.3 and 157.3, and the Mannich methylene group resonates at δ 57.47. The signals at δ 54.6, 52.1 and 45.6 are attributed to 4 carbons of the piperazine and methyl group.

Bis Mannich bases **5a-e** were synthesized in the same manner, but using the excess of secondary amines as shown in Scheme 1. However, only **5a-e** were obtained in good yields. The presence of bis Mannich groups in the structure was confirmed using ^{1}H, ^{13}C NMR and HMBC spectra. Compound **5a** was used as an example to elucidate the structure of the series. The ^{1}H NMR spectrum of **5a** showed 5 protons in the low-field aromatic region, in which three doublet signals at δ 7.51, 7.22 (J = 9.0 Hz) and 7.94 (J = 8.5 Hz) are attributed to the H-8, H-7 and H-5′, respectively. Another doublet signal at δ 6.82 (J = 2.5 Hz) is attributed to H-2′. The last aromatic proton appeared as a doublet of doublet signal at δ 6.99 (J = 2.5 Hz, 8.5 Hz) belongs to H-6′. In the high field region, two Mannich methylene groups appeared as two couples of doublet signals at δ 4.82, 4.72 and 3.86, 3.79 (J = 16.0 Hz). In addition, eight methylene protons of of the Mannich groups resonate as a multiplet signal at δ 2.66 and two methyl groups appear as a triplet at δ 1.14 (J = 4.0 Hz) (Fig. 2).

The ^{13}C NMR spectrum of **5a** showed the presence of 14 carbon signals in the high field, in which the amide carbon (C-4) appears at δ 163.4. Two carbons attached to the OH group of the quinazolinone ring (C-6) and the phenyl ring (C-4′) resonate at δ 159.5 and 159.0. The signal at δ 151.1 is attributed to C-2. Besides, the HMBC spectrum (Fig. 3) helps to determine others chemical shifts at δ 141.9 (C-9), 128.9 (C-1′), 127.9 (C-8), 127.54 (C-6′), 127.5 (C-2′), 125.1 (C-3′), 123.3 (C-5), 119.7 (C-7), 119.2 (C-10), and 117.5 (C-5′). In addition, two resonance signals at δ 56.9 and 54.1 are attributed to Mannich methylene and other four methylene groups resonate at δ 47.1 and 46.6. The chemical shifts of two methyl groups are observed at δ 23.9 and 11.3.

Bioassay

Eight Mannich bases were evaluated for their *in vitro* cytotoxicity against SKLU-1 and MCF-7 using SRB method[22]. All compounds were initially screened at a fixed concentration of 100 µg/mL. If the compounds are active, they will be further screened at smaller concentrations (*e.g.*, 20 µg/mL, 4 µg/mL, 0.8 µg/mL and 0.16 µg/mL), and IC_{50} values. The bio-assay result was illustrated in Table 1.

As can be seen in the Table 1 that three mono Mannich bases **4a-c** were inactive against two cancer cell lines tested. In the mean time, bis Mannich bases **5a-e** except the compound **5b** displayed cytotoxic activity on the two human cancer cell lines. However, these compounds showed weak cytotoxic activity against both cell lines. It was also observed that bis Mannich bases showed better cytotoxic activity than mono Mannich bases. Though no compounds can be comparable to ellipticin in terms of cytotoxicity, the research results suggest that the presence of the Mannich group in the phenyl ring has more beneficial effect on the cytotoxic activity than in the quinazolinone nucleus.

Experimental Details

Chemistry

All products were examined by thin-layer chromatography (TLC), performed on Whatman® 250 µm Silica Gel GF Uniplates and visualized under

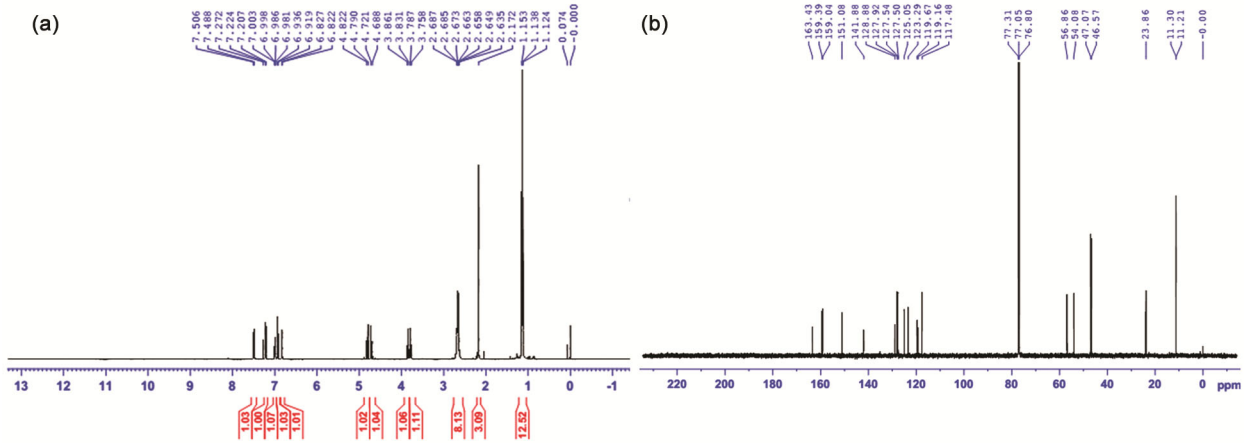


Fig. 2 — ^{1}H and ^{13}C NMR spectra of compound **5a**

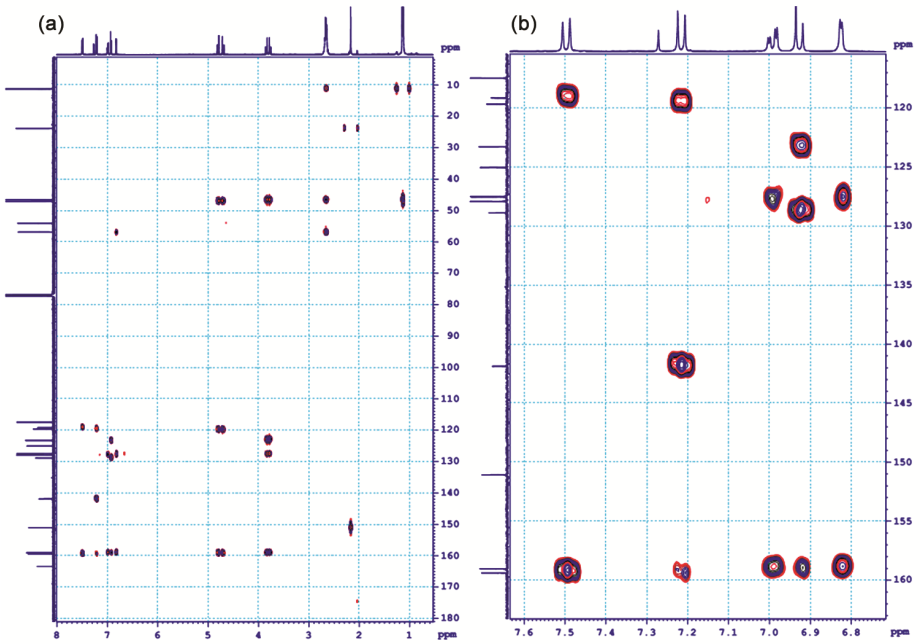
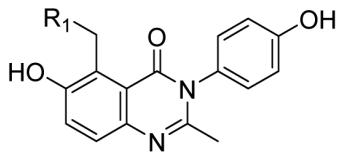
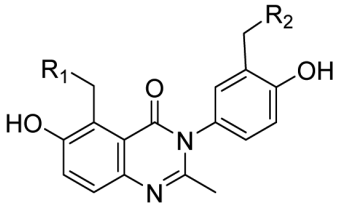


Fig. 3 — HMBC spectra of compound **5a**

Table 1 — *In vitro* cytotoxic activity of **4a-c** and **5a-e**



4a-c



5a-e

S. No.	Compd	R_1, R_2	IC_{50}, µg/mL [a] / Cell lines [b]	
			SKLu-1	MCF-7
1	**4a**	R_1 = 4-methylpiperazine	>100	>100
2	**4b**	R_1 = piperidine	>100	>100
3	**4c**	R_1 = 2-methylpiperidine	>100	>100
4	**5a**	$R_1 = R_2$ = diethylamine	66.82±6.66	97.77± 5.51
5	**5b**	$R_1 = R_2$ = pyrrolidine	>100	>100
6	**5c**	$R_1 = R_2$ = 4-methylpiperazine	52.09±6.53	68.84±3.25
7	**5d**	$R_1 = R_2$ = 2-methylpiperidine	59.20± 3.30	97.23 ± 8.61
8	**5e**	$R_1 = R_2$ = piperidine	42.71 ± 2.02	83.03 ± 2.15
	Ellipticine	–	0.41 ± 0.04	0.38 ± 0.05

[a] Concentration (µg/mL) that produces a 50% reduction in cell growth, the numbers represent the averaged results from triplicate experiments with deviation of less than 10%. [b] Cell lines: SKLU-1, lung cancer; MCF-7, breast cancer

UV light at 254 nm. Melting points were determined in open capillaries on Electrothermal IA 9200 Shimazu apparatus and are uncorrected. Purification was done by crystallization and the open flash silica gel column chromatography using Merck silica gel 60 (240 to 400 mesh). Nuclear magnetic resonance spectra (^{1}H, ^{13}C NMR and HMBC) were recorded using tetramethylsilane (TMS) as an internal standard

on a Bruker 500 MHz spectrometer with $CDCl_3$, CD_3OD and DMSO-d_6 as solvents. Chemical shifts are reported in parts per million (ppm) downfield from TMS as internal standard, and coupling constants (*J*) are expressed in Hertz (Hz). Multiplicities are shown as the abbreviations: s (singlet), brs (broad singlet), d (doublet), t (triplet), m (multiplet). Mass spectra were recorded on FTICR MS Varian. Reagents and solvents were purchased from Aldrich or Fluka Chemical Corp or Merck unless noted otherwise. Solvents were distilled and dried before use.

Bioassay

All media, sera and other reagents used for cell cultures were obtained from GIBCO Co. Ltd. (Grand Island, New York, USA) and two human cancer cell lines for testing including SKLU-1 (lung cancer) and MCF-7 (breast cancer) were provided by Institute of Biotechnology, Vietnam Academy of Science and Technology. The cytotoxicity of synthesized compounds was determined by a method of the American National Cancer Institute (NCI) as described in literature. Briefly, these cancer cell lines were grown as monolayers in 2 mM of L-glutamine, 10 mM of HEPES, 1.0 mM of sodium pyruvate, and supplemented with 10% fetal bovine serum- FBS (GIBCO). Cells were cultured for 3-5 days after transfer, and maintained at 37°C in a humidified atmosphere containing 5% CO_2. Assay samples were initially dissolved in DMSO and serially diluted to appropriate concentrations with a culture medium right before the assay. Then the cells in each well, incubated for 24 h as described above, were treated with 20 µL of samples at 20 µg/mL; 0.8 µg/mL; 0.16 µg/mL. The plates were further incubated for 48 h. The medium was removed and the cells were fixed by 10% solution of trifluoroacetic acid. The fixed cells were stained for 30 min by a staining solution (RSB method). Protein-bound dye was dissolved in a 10 mM tri-base solution and the OD_s were measured at 510 nm using an Elisa reader. The IC_{50} values were then calculated using Probits method. Ellipticin (Sigma) was used as a positive control and the values reported for the compounds are presented as average of three determinations.

Synthesis of 6-hydroxy-2methyl-4*H*-benzo[d] [1,3] ozazin-4-one, 2

A mixture of 5-hydroxy anthranilic acid **1** (5.0 g, 32.67 mmol) in acetic anhydride (15 mL) was refluxed at 150°C for 2 h. The mixture was then poured in ice-water. The resulting precipitate was filtered, washed with distilled water and dried in vacuum to afford **2** (5.03 g, 87%) which was used for the next step.

6-Hydroxy-3-(4-hydroxyphenyl)-2-methylquinaz olin-4(3*H*)-one, 3

A mixture of **2** (1 g, 5.64 mmol, 1eq), 4-aminophenol (737 mg, 1.2 eq) in DMSO (5 mL) was refluxed for 7 h. The reaction was monitored by thin layer chromatography using CH_2Cl_2 : MeOH (100 : 5) as a developing system. The reaction mixture was diluted by a mixture of CH_2Cl_2/H_2O (20 mL). The resulting crystal was filtered and washed with *n*-hexane to obtain **3**. Light brown solid. Yield 75%. R_f = 0.58 (*n*-hexane : ethyl acetate = 6 : 4); ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.98 (s, 1H, OH), 9.78 (s, 1H, OH), 7.51 (d, *J* = 8.5 Hz, 1H), 7.38 (d, *J* = 3.0 Hz, 1H), 7.26 (dd, *J* = 3.0 Hz, 8.5 Hz, 1H), 7.15 (d, *J* = 9.0 Hz, 2H), 6.89 (d, *J* = 9.0 Hz, 2H), 2.08 (s, 3H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6): δ 161.3 (C=O), 157.5, 155.8, 151.6 (C=N), 140.5, 129.3, 129.0, 128.2, 123.8, 121.4, 115.9, 109.1, 23.6 (CH_3).

General procedure for the synthesis of mono Mannich bases, 4a-c

A mixture of **3** (300 mg, 1.12 mmol), paraformaldehyde (3eq) and secondary amine (1eq) in ethanol (10 mL) was refluxed for 4 h. The reaction was monitored by TLC using CH_2Cl_2 : MeOH (100 : 5) as a developing system. The mixture was diluted with CH_2Cl_2 (20 mL) and extracted with H_2O (20 mL × 3). The organic phase was separated and dried on anhydrous Na_2SO_4. The solvent was removed by vacuum rotary evaporator. The residues was subjected to silica gel column chromatography using CH_2Cl_2 : MeOH (100 : 2) as a solvent system to give desired products.

6-Hydroxy-3-(4-hydroxyphenyl)-2-methyl-5-((4-methylpiper-azin-1-yl)methyl)quinazolin-4(3*H*)-one, 4a: White solid. Yield 65%. R_f = 0.52 (dichloromethane : methnol = 9 : 1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.56 (d, *J* = 8.5 Hz, 1H), 7.25 (d, *J* = 8.5 Hz, 1H), 7.04 (d, *J* = 9.0 Hz, 2H), 6.91 (d, *J* = 9.0 Hz, 2H), 4.72 (s, 2H, CH_2), 2.71-2.65 (m, 8H), 2.36 (s, 3H, CH_3), 2.30 (s, 3H, N-CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 163.2 (C=O), 158.3, 157.3, 151.6, 142.2, 129.7, 129.1, 127.7, 124.8, 119.3, 118.9, 116.9, 57.5, 54.6, 52.1, 45.6 (N-CH_3), 23.7 (CH_3); ESI-MS: *m/z* 381.4 $[M+H]^+$.

6-Hydroxy-3-(4-hydroxyphenyl)-2-methyl-5-(piperidin-1-yl- methyl)quinazolin-4(3*H*)-one, 4b: White solid. Yield 70%. R_f = 0.62 (dichloromethane : methnol = 9 : 1).^{1}H NMR (500 MHz, $CDCl_3$): δ 7.50 (d, *J* = 9.0 Hz, 1H), 7.22 (d, *J* = 9.0 Hz, 1H), 7.02 (d, *J* =9.0 Hz, 2H), 6.98 (d, *J* = 9.0 Hz, 2H), 4.66 (s, 2H), 2.56 (brs, 4H), 2.17 (s, 3H, C*H*$_3$), 1.66 (brs, 4H), 1.50 (brs, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 163.1 (C=O), 158.6, 157.8, 151.1, 142.0, 129.4, 128.7, 127.5, 124.5, 119.2, 118.9, 116.6, 57.9, 53.6, 25.4, 24.5, 23.5 (CH_3); ESI-MS: *m/z* 366.2 $[M+H]^+$.

6-Hydroxy-3-(4-hydroxyphenyl)-2-methyl-5-((2-methylpiperidin-1-yl)methyl)quinazolin-4(3*H*)-one, 4c: White solid. Yield 68%. R_f = 0.65 (dichloromethane : methnol = 9 : 1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.45 (d, *J* = 8.5 Hz, 1H), 7.17 (d, *J* = 8.5 Hz, 1H), 7.02 (d, *J* = 9.0 Hz, 2H), 6.97 (d, *J* = 9.0 Hz, 2H), 4.82 (d, *J* = 15.5 Hz, 1H), 4.69 (d,*J* = 15.5 Hz, 1H), 2.56 (s, 2H), 2.34 (br, 1H), 2.16 (s, 3H, C*H*$_3$), 1.76-1.73 (m, 2H), 1.70-1.60 (brs, 2H), 1.46-1.40 (br, 2H), 1.19 (d, *J* = 6.5 Hz, 3H, C*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 163.2 (C=O), 158.9, 158.0, 151.1, 142.1, 129.4, 128.9, 127.4, 124.6, 119.1, 116.7, 116.6, 60.4, 55.4, 50.8, 34.9, 26.7, 23.7, 23.2, 20.2; ESI-MS: *m/z* 380.1 $[M+H]^+$.

General procedure for the synthesis of Bis-Mannich bases, 5a-e

A mixture of **3** (270 mg, 1 mmol), paraformaldehyde (3eq) and corresponding secondary amines (3 eq) including diethylamine, pyrrolidine, 4-methylpiperazine, 2-methylpiperidine and piperidin in ethanol (10 mL) was refluxed for 4 h. The reaction was monitored by TLC using CH_2Cl_2 : MeOH (100 : 5) as a developing system. The mixture was diluted with CH_2Cl_2 (20 mL) and extracted with H_2O (20 mL × 3). The organic phase was separated and dried on anhydrous Na_2SO_4. The solvent was removed by vacuum rotary evaporator. The residues was subjected to silica gel column chromatography using CH_2Cl_2 : MeOH (100 : 2) as a solvent system to give desired products.

5-((Diethylamino)methyl)-3-(3-((diethylamino) methyl)-4-hydroxyphenyl)-6-hydroxy-2-methylquinazolin-4 (3*H*)-one, 5a: White solid. Yield 61%. R_f = 0.45 (dichloromethane : methnol = 9 : 1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.51 (d, *J* = 9.0 Hz, 1H), 7.22 (d, *J* = 9.0 Hz, 1H), 6.99 (dd, *J* = 2.5 Hz, 8.5 Hz, 1H), 7.94 (d, *J* = 8.5 Hz, 1H), 6.83 (d, *J* = 2.5 Hz, 1H), 4.82 (d, *J* = 16.0 Hz, 1H), 4.72 (d, *J* = 16.0 Hz, 1H), 3.86 (d, *J* = 16.0 Hz, 1H), 3.79 (d, *J* = 16.0 Hz, 1H), 2.68-2.63 (m, 8H, 4CH_2), 2.17 (s, 3H, CH_3), 1.14 (t, *J* = 8.5 Hz, 12H, 4CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 163.4 (C=O), 159.4, 159.0, 151.1 (C=N), 141.9, 128.9, 127.9, 127.54, 127.5, 125.1, 123.3, 119.7, 119.2, 117.5, 56.9, 54.1, 47.1, 46.6, 23.9 (CH_3), 11.3(CH_3); ESI-MS: *m/z* 439.1 $[M+H]^+$.

6-Hydroxy-3-(4-hydroxy-3-(pyrrolidin-1-ylmet hyl)phenyl)-2-methyl-5-(pyrrolidin-1-ylmethyl)quinazolin-4-(3*H*)-one, 5b: White solid. Yield 65%. R_f = 0.44 (dichloromethane : methnol = 9 : 1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.51 (d, *J* = 9.0 Hz, 1H), 7.22 (d, *J* = 9.0 Hz, 1H), 6.99 (dd, *J* = 2.5 Hz, 8.5 Hz, 1H), 6.94 (d, *J* = 8.5 Hz, 1H), 6.83 (d, *J* = 2.5Hz, 1H), 4.82 (d, *J* = 16.0 Hz, 1H), 4.72 (d, *J* = 16.0 Hz, 1H), 3.86 (d, *J* = 16.0 Hz, 1H), 4.79 (d, *J* = 16.0 Hz, 1H), 2.68-2.64 (m, 8H), 2.17 (s, 3H, CH_3), 1.14 (m, 8H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 161.9 (C=O), 157.8, 156.8, 151.0 (C=N), 141.4, 128.8, 128.2, 127.7, 127.2, 124.1, 123.7, 119.7, 118.6, 115.7, 55.7, 54.3, 52.0, 23.3, 23.2; ESI-MS: *m/z* 435.3 $[M+H]^+$.

6-Hydroxy-3-(4-hydroxy-((4-methylpiperazin-1-yl)methyl)-phenyl)-2-methyl-5-((4- methylpiperazin-1-yl)methyl)quinazolin-4-(3*H*)-one, 5c: White solid. Yield 55%. R_f = 0.41 (dichloromethane : methnol = 9 : 1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.44 (d, *J* = 8.5 Hz, 1H), 7.17 (d, *J* = 8.5 Hz, 1H), 7.08-7.06 (m, 2H), 6.86 (d, *J* = 8.5 Hz, 1H), 4.58 (d, *J* =16.0 Hz, 1H), 4.54 (d, *J* =16.0 Hz, 1H), 3,68 (d, *J* =16.0 Hz, 1H), 3.62 (d, *J* =16.0 Hz, 1H), 2.53-2.36 (brs, 16H, 8CH_2), 2.16 (s, 6H, 2CH_3), 2.04 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 161.9 (C=O), 157.3, 156.9, 151.2 (C=N), 141.8, 128.9, 128.7, 127.9, 127.3, 123.8, 123.2, 119.1, 118.9, 115.9, 58.1, 56.5, 54.3, 52.0, 45.5 (N-CH_3), 23.4 (CH_3); ESI-MS: *m/z* 493.3 $[M+H]^+$.

6-Hydroxy-3-(4-hydroxy-3((2-methylpiperidin-1-yl)methyl)-phenyl)-2-methyl-5-((2- methylpiperidin-1-yl)methyl)quinazolin-4-(3*H*)-one, 5d: White solid. Yield 59%. R_f = 0.48 (dichloromethane : methnol = 9 : 1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.49 (d, *J* = 8.5 Hz, 1H), 7.21 (d, *J* = 8.5 Hz, 1H), 6.97 (dd, *J* = 2.5 Hz, 8.5 Hz), 6.92 (d, *J* = 8.5 Hz, 1H), 6.81 (d, *J* = 2.5 Hz), 4.81 (d, *J* = 16.0 Hz, 1H), 4.73 (d, *J* = 16.0 Hz, 1H), 3.42 (d, *J* = 16.0 Hz, 1H), 3.33 (d, *J* = 16.0 Hz, 1H), 2.61-2.53 (m, 4H), 2.39-2.36 (m, 2H), 2.17 (s, 3H, CH_3), 1.75-1.72 (m, 4H), 1.63-1.59 (m, 4H), 1.48-1.33 (m, 4H), 1.20 (d, *J* = 6.0 Hz, 2CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 163.4 (C=O), 158.8, 158.5, 151.0 (C=N), 141.8, 128.9, 128.7, 127.7, 127.4, 125.1, 123.6, 118.9, 117.5,

117.4, 58.5, 57.3, 54.2, 41.0, 34.6, 25.7, 24.2, 23.9, 20.7; ESI-MS: *m/z* 491.5 $[M+H]^+$.

6-Hydroxy-3-(4-hydroxy-3((piperidin-1-yl) methyl)phenyl)-2-methyl-5-((piperidin-1-yl) methyl)quinazolin-4-(3*H*)-one, 5e: White solid. Yield 60%. R_f = 0.47 (dichloromethane : methnol = 9 : 1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.51 (d, *J* = 9.0 Hz, 1H), 7.22 (d, *J* = 9.0 Hz, 1H), 7.00 (dd, *J* = 2.5 Hz, 8.5 Hz), 6.94 (d, *J* = 8.5 Hz, 1H), 6.82 (d, *J* = 2.5 Hz, 1H), 4.73 (d, *J* = 16.0 Hz, 1H), 4.62 (d, *J* = 16.0 Hz, 1H), 3.76 (d, *J* = 16.0 Hz, 1H), 3.68 (d, *J* = 16.0 Hz, 1H), 2.55 (brs, 8H), 2.16 (s, 3H, CH_3), 1.67-1.65 (brs, 8H), 1.53-1.50 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 163.4 (C=O), 158.9, 158.8, 151.2 (C=N), 142.0, 128.9, 128.0, 127.7, 127.5, 125.0, 123.8, 119.3, 118.9, 117.4, 61.5, 58.4, 54.0, 53.8, 25.8, 23.7; ESI-MS: *m/z* 463.2 $[M+H]^+$.

Supplementary Information

Supplementary information is available in the website http://nopr.niscpr.res.in/handle/123456789/58776.

Conclusion

A series of new quinazolinone-based Mannich bases have been designed and synthesised. These compounds have been evaluated for their *in vitro* cytotoxicity against two human cancer cell lines, including SKLu-1 and MCF-7. The biological evaluation result showed that some new bis Mannich bases **5a** and **5c-e** exhibited weak to moderate cytotoxic activity against both cell lines.

Acknowledgements

This work was financially supported by the Hanoi University of Science and Technology (HUST) under project number T2020-TĐ-201.

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