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RESEARCH ARTICLE

Synthesis of mono Mannich bases of 2-(4-hydroxybenzylidene)-2,3-dihydroinden-1-one and evaluation of their cytotoxicities

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Abstract

Chalcones and Mannich bases are a group of compounds known for their cytotoxicities. In this study restricted chalcone analogue, compound 2-(4-hydroxybenzylidene)-2,3-dihydroinden-1-one MT1, was used as a starting compound to synthesize new mono Mannich bases since Mannich bases may induce more cytotoxicity than chalcone analogue that they are derived from by producing additional alkylating center for cellular thiols. In this study, cyclic and acyclic amines were used to synthesize Mannich bases. All compounds were tested against Ca9–22 (gingival carcinoma), HSC-2, HSC-3 and HSC-4 (oral squamous cell carcinoma) as tumour cell lines and HGF (gingival fibroblasts), HPC (pulp cells) and HPLF (periodontal ligament fibroblasts) human normal oral cells as non tumour cell lines. Cytotoxicity, selectivity index (SI) values and potency selectivity expression (PSE) values expressed as a percentage were determined for the compounds. According to data obtained, the compound MT8 with the highest PSE value bearing *N*-methylpiperazine moiety seems to be a good candidate to develop new cytotoxic compounds and is suited for further investigation.

Keywords

Chalcone analogue, cytotoxicity, dihydroinden-1-one, 1-indanone, Mannich bases, phenol, selectivity index, MTT, synthesis

History

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Introduction

Styryl ketones which have α,β-unsaturated ketone moiety are known as potential cytotoxic and anticancer compounds[1]. These compounds showed remarkable affinity for thiol groups and did not react with amino or hydroxy groups[2,3]. Amino and hydroxy groups are available in nucleic acids, and hence α,β-unsaturated ketones may not have the genotoxic effects which are associated with various alkylating agents used in cancer chemotherapy[4]. Mannich bases are a group of compounds having various biological activities such as cytotoxic[5–15] anti-inflammatory[16,17] and anticonvulsant[18,19] activities.

α,β-Unsaturated ketone/s available in the chemical structure of Mannich bases or produced from Mannich bases by deamination process is/are responsible for their cytotoxicities. α,β-Unsaturated ketones alkylate cellular thiols to produce cytotoxicity[20]. It was reported that Mannich bases of styryl ketones have increased cytotoxicity comparing to its styryl ketone analogue[21].

The cytotoxic and anticancer properties of chalcone (1,3-diphenyl-2-propenone) and related compounds have been reported[7,8,15,22–28]. Conferring Mannich bases to phenolic chalcones increased their cytotoxicity[7,8,15,26,27]. Chalcones are conjugated molecules and sufficiently flexible to permit a wide variety of conformations. Hence, discernment of the optimal shape of molecule which is responsible for the cytotoxicity is not possible. The aim of the present study was to synthesize conformationally restricted chalcone analogue, 2-(4-hydroxybenzylidene)-2,3-dihydroinden-1-one, and its Mannich bases (MT8-MT15). Different amines having different pK_a values such as *N*-methyl piperazine (MT8), morpholine (MT9), piperidine (MT10), pyrrolidine (MT11), dimethylamine (MT12), diethylamine (MT13), dipropylamine (MT14) and dibenzylamine (MT15) were considered. Different pK_a values can direct the deamination ratio and alkylation ratio of the compounds. It was also aimed to evaluate the cytotoxicities of the compounds against human tumour cell lines [gingival carcinoma (Ca9–22), oral squamous cell carcinoma (HSC-2, HSC-3 and HSC-4)] and human normal oral cells [gingival fibroblasts (HGF), periodontal ligament fibroblasts (HPLF) and pulp cells (HPC)].

Materials and methods

Melting points were determined using an Electrothermal 9100 (IA9100; Bibby Scientific Limited, Staffordshire, UK) instrument and are uncorrected. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were obtained using a Varian Mercury Plus spectrometer (Varian Inc., Palo Alto, CA). Chemical shifts (δ) were reported in parts per million. Mass spectra were undertaken on an HPLC-TOF Waters Micromass LCT Premier XE (Waters Corporation, Milford, MA) mass spectrometer using an electrospray ion source (ESI). All reactions were carried out in CEM

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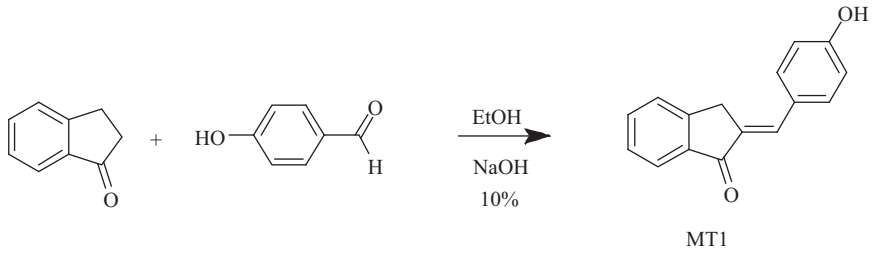
Scheme 1. Synthesis of MT1.



MT1

Discover Microwave Synthesis Systems (CEM Corporation, Matthews, NC; 908010).

Synthesis of 2-(4-hydroxybenzylidene)-2,3-dihydroinden-1-one (MT1)

A solution of the 4-hydroxybenzaldehyde (0.02 mol) in ethanol (6 ml) was added into a stirred solution of 1-indanone (0.02 mol) in aqueous solution of sodium hydroxide (10 ml, 10%). Stirring was maintained at room temperature overnight. The reaction mixture was then poured onto water (100 ml) and neutralized with hydrochloric acid (10%, *w*/*v*). The precipitated solid product was filtered, washed with water, and crystallized from water–ethanol[22,29] (Scheme 1).

General synthesis of Mannich bases (MT8–MT15)

The mixture of the compound MT1 (21 mmol), paraformaldehyde (25 mmol) and suitable secondary amine (21 mmol) [MT8 (*N*-methyl piperazine), MT9 (morpholine), MT10 (piperidine), MT11 (pyrrolidine), MT12 (dimethylamine), MT13 (diethylamine), MT14 (dipropylamine) and MT15 (dibenzylamine)] was heated in 15 ml of acetonitrile (120 °C, 200 Watt, 13.8 barr) for 15 min (MT8, MT10, MT13, MT14 and MT15) or 30 min (MT9, MT11 and MT12). Reactions were monitored by TLC. When the reaction was stopped, reaction solvent was removed under vacuum. The compounds MT8, MT15 and MT10 were purified by crystallization. It was EtOH (MT8 and MT15) or MeOH/aseton (MT10). On the other hand, the compounds MT9, MT11, MT12, MT13 and MT14 were purified by column chromatography on silica gel (SiO_2) using suitable solvent system. It was $CHCl_3$:MeOH (98:2) for MT9, $CHCl_3$:MeOH:NH_3 (98:2:2) for MT11, MT13, MT14 and $CHCl_3$:MeOH:NH_3 (90:10:2) for MT12 (Scheme 2).

2 -(4-Hydroxy-benzylidene)-indan-1-one (MT1)

Yield: 76.3%. Melting point (m.p.): 227–229 °C, 235–236 °C[29]. ^{1}H-NMR (CD_3OD) δ 4.04 (2H, s, indeno H-3), 6.88–6.91 (2H, m, phenyl H-3, H-5), 7.44–7.50 (2H, m, =CH–, indeno H-6), 7.57–7.69 (4H, m, phenyl H-2, H-6, indeno H-4, H-5), 7.80 (1H, d, indeno H-7, $J = 7.7$ Hz), 7.90 (1H, s, phenyl 4-OH); ^{13}C-NMR 195.5, 159.9, 150.5, 137.9, 134.9, 134.7, 133.1, 131.7, 127.5, 126.8, 126.5, 123.6, 115.9 and 32.15; mass spectrum: 235.08 $[M-H]^+$; HRMS (ESI-MS) Calcd: 235.0759 for $C_{16}H_{11}O_2$ $[M-H]^+$, found: 235.0758.

2-(4-Hydroxy-3-((4-methylpiperazin-1-yl)methyl) benzylidene)-2,3-dihydroinden-1-one (MT8)

Yield: 51%. m.p.: 152–154 °C. ^{1}H-NMR ($CDCl_3$) δ 2.31 (3H, s, N–CH_3), 2.32–2.60 (8H, m, CH_2-piperazin ring), 3.79 (2H, s, CH_2–N), 3.99 (2H, s, indeno H-3), 6.90 (1H, d, phenyl H-5, $J = 8.8$ Hz), 7.31 (1H, s, phenyl H-2), 7.41 (1H, t, indeno H-6, $J = 7.2$ Hz), 7.54–7.61 (4H, m, =CH–, indeno H-4, H-5, phenyl H-6) 7.89 (1H, d, indeno H-7, $J = 7.7$ Hz); ^{13}C-NMR 194.3, 159.8, 149.4, 138.3, 134.2, 134.1, 131.9, 131.8, 131.7, 127.5, 126.8, 126.0, 124.3, 121.6, 116.9, 61.3, 54.8, 52.5, 45.8 and 32.5; Mass spectrum: 349.19 ($M^+ + 1$); HRMS (ESI-MS) Calcd: 349.1916 for $C_{22}H_{25}N_2O_2$ $[M^+H^+]$, found: 349.1902.

2-(4-Hydroxy-3-(morpholinomethyl)benzylidene)-2,3-dihydroinden-1-one (MT9)

Yield: 11.3%. m.p.: 171–174 °C. ^{1}H-NMR ($CDCl_3$) δ 2.62 (4H, bs, CH_2-morpholine), 3.79 (6H, s, CH_2-morpholine, CH_2–N), 4.00 (2H, s, indeno H-3), 6.91 (1H, d, phenyl H-5, $J = 8.4$ Hz),7.31 (1H, s, phenyl H-2), 7.41 (1H, t, indeno H-6, $J = 7.3$ Hz), 7.54–7.62 (4H, m, =CH–, indeno H-4, H-5, phenyl H-6), 7.89 (1H, d, indeno H-7, $J = 7.7$ Hz); ^{13}C-NMR 194.6, 159.8, 149.6, 138.5, 134.5, 134.2, 132.4, 132.1, 132.0, 127.8, 127.3, 126.3, 124.5, 121.4, 117.2, 66.9, 62.0, 53.1 and 32.7; Mass spectrum: 336.15 ($M^+ + 1$); HRMS (ESI-MS) Calcd: 336.1600 for $C_{21}H_{22}NO_3$ $[M^+H^+]$, found: 336.1590.

2-(4-Hydroxy-3-(piperidin-1-ylmethyl)benzylidene)-2,3-dihydroinden-1-one (MT10)

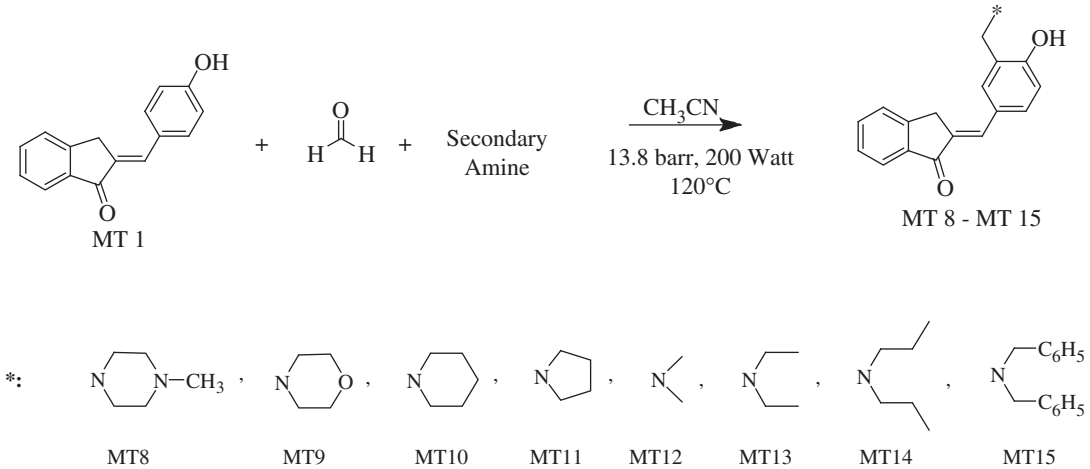
Yield: 6.1%. m.p.: 163–166 °C. ^{1}H-NMR ($CDCl_3$) δ 1.66 (10H, bs, CH_2-piperidine), 3.74 (2H, s, CH_2–N), 3.99 (2H, s, indeno H-3), 6.88 (1H, d, phenyl H-5, $J = 8.4$ Hz), 7.28 (1H, d, phenyl H-2, $J = 1.8$ Hz), 7.41 (1H, t, indeno H-6, $J = 6.8$ Hz), 7.54–7.62 (4H, m, =CH–, indeno H-4, H-5, phenyl H-6), 7.89 (1H, d, indeno H-7, $J = 7.7$ Hz); ^{13}C NMR 194.3, 160.4, 149.4, 138.4, 134.3, 134.2, 131.9, 131.5, 131.4, 127.5, 126.5, 126, 124.2, 122.1, 116.9, 62.0, 53.9, 32.5, 25.7 and 23.8; Mass spectrum: 334.18 ($M^+ + 1$); HRMS (ESI-MS) Calcd: 334.1807 for $C_{22}H_{24}NO_2$ $[M^+H^+]$, found: 334.1804.

2-(4-Hydroxy-3-(pyrrolidin-1-ylmethyl)benzylidene)-2,3-dihydroinden-1-one (MT11)

Yield: 22.2%. m.p.: 152–154 °C. ^{1}H-NMR ($CDCl_3$) δ 1.81–1.86 (4H, m, CH_2-pyrrolidine), 2.62 (4H, s, CH_2-pyrrolidine), 3.84 (2H, s, CH_2–N), 3.90 (2H, s, indeno H-3), 6.84 (1H, d, phenyl H-5, $J = 8.4$ Hz), 7.24 (1H, s, phenyl H-2), 7.34 (1H, t, indeno H-6, $J = 6.6$ Hz), 7.47–7.54 (4H, m, =CH–, indeno H-4, H-5, phenyl H-6), 7.83 (1H, d, indeno H-7, $J = 7.7$ Hz); ^{13}C-NMR 194.2, 160.4, 149.4,138.3, 134.2, 134.1, 131.5, 131.4, 131.2, 127.4, 126.3, 126.1, 124.1, 122.9, 116.7, 58.7, 53.4, 32.5 and 23.7; Mass spectrum: 318.14 ($M^+ + 1$); HRMS (ESI-MS) Calcd: 318.1494 for $C_{21}H_{20}NO_2$ $[M^+H^+]$, found: 318.1484.

2-(4-Hydroxy-3-(dimethylaminomethyl)-benzylidene) -2,3-dihydroinden-1-one (MT12)

Yield: 7.4%. m.p.: 166–169 °C. ^{1}H-NMR ($CDCl_3$) δ 2.38 (6H,s, $2 \times CH_3$), 3.74 (2H, s, CH_2–N), 4.00 (2H, s, indeno H-3), 6.91 (1H, d, phenyl H-5, $J = 8.4$ Hz), 7.30 (1H, s, phenyl H-2), 7.43 (1H, t, indeno H-6, $J = 7.0$ Hz), 7.54–7.62 (4H, m, =CH–, indeno H-4, H-5, phenyl H-6), 7.89 (1H, d, indeno H-7, $J = 7.6$ Hz); ^{13}C-NMR 194.3, 160.3, 149.4, 138.4, 134.2, 131.8, 131.7, 131.6, 127.5, 126.6, 126.1, 124.2, 122.2, 116.9, 62.7, 44.4 and 32.5;



Scheme 2. Synthesis of MT8–MT15.

Mass spectrum: 294.14 ($M^+ + 1$); HRMS (ESI-MS) Calcd: 294.1494 for $C_{19}H_{20}NO_2$ [M^+H^+], found: 294.1480.

2-(4-Hydroxy-3-(diethylaminomethyl)-benzylidene)-2,3-dihydroinden-1-one (MT13)

Yield: 23.5%. m.p.: 138–141 °C. ^{1}H-NMR ($CDCl_3$) δ 1.13 (6H, t, $2 \times CH_3$, $J = 7.1$ Hz), 2.66 (4H, q, $2 \times CH_2$, $J = 7.3$ Hz), 3.84 (2H, s, CH_2–N), 3.99 (2H, s, indeno H-3), 6.87 (1H, d, phenyl H-5, $J = 8.4$ Hz), 7.29 (1H, s, phenyl H-2), 7.41 (1H, t, indeno H-6, $J = 7.1$ Hz), 7.54–7.61 (4H, m, =CH–, indeno H-4, H-5, phenyl H-6), 7.90 (1H, d, indeno H-7, $J = 7.3$ Hz); ^{13}C-NMR 194.6, 160.9, 149.7, 138.6, 134.6, 134.4, 132.1, 131.6, 127.7, 126.7, 126.3, 124.5, 122.8, 177.2, 94.6, 57.1, 46.6, 32.8 and 11.3; Mass spectrum: 322.18 ($M^+ + 1$); HRMS (ESI-MS) Calcd: 322.1807 for $C_{21}H_{24}NO_2$ [M^+H^+], found: 322.1805.

2-(4-Hydroxy-3-(dipropylaminomethyl)-benzylidene)-2,3-dihydroinden-1-one (MT14)

Yield: 10.4%. m.p.: 113–115 °C. ^{1}H-NMR ($CDCl_3$) δ 0.90 (6H, t, $2 \times CH_3$, $J = 7.3$ Hz), 1.54–1.63 (4H, m, $2 \times CH_2$), 2.51 (4H, t, $2 \times CH_2$, $J = 7.7$ Hz), 3.83 (2H, s, CH_2–N), 4.00 (2H, s, indeno H-3), 6.88 (1H, d, phenyl H-5, $J = 8.4$ Hz), 7.28 (1H, s, phenyl H-2), 7.41 (1H, t, indeno H-6, $J = 7.1$ Hz), 7.54–7.61 (4H, m, =CH–, indeno H-4, H-5, phenyl H-6), 7.90 (1H, d, indeno H-7, $J = 7.3$ Hz); ^{13}C-NMR 194.6, 160.8, 149.7, 138.6, 134.6, 134.4, 132.2, 131.7, 127.7, 126.7, 126.3, 124.5, 122.9, 117.1, 58.4, 55.6, 32.8, 19.6 and 11.9; Mass spectrum: 350.21 ($M^+ + 1$); HRMS (ESI-MS) Calcd: 350.2120 for $C_{23}H_{28}NO_2$ [M^+H^+], found: 350.2103.

2-(4-Hydroxy-3-(dibenzylaminomethyl)-benzylidene)-2,3-dihydroinden-1-one (MT15)

Yield: 21.2%. m.p.: 188–190 °C. ^{1}H-NMR ($CDCl_3$) δ 3.66 (4H, s, $2 \times CH_2$–C_6H_5), 3.74 (2H, s, CH_2–N), 3.98 (2H, s, indeno H-3), 7.24–7.63 (17H, m, phenyl H-2, H-5, H-6, =CH–, indeno H-4, H-5, H-6, protons of dibenzylamine), 7.91 (1H, d, indeno H-7, $J = 7.3$ Hz); ^{13}C-NMR 194.7, 188.3, 182.6, 167.5, 165.5, 162.6, 158.1, 156.1, 149.8, 146.6, 145.9, 140.9, 138.7, 138.3, 136.9, 134.9, 134.4, 131.8, 131.7, 129.8, 129.3, 128.9, 128.7, 127.7, 127.6, 126.8, 126.3, 124.7, 124.5, 58.3, 54.0 and 32.9; Mass spectrum: 446.20 ($M^+ + 1$); HRMS (ESI-MS) Calcd: 446.2080 for $C_{26}H_{28}N_3O_4$ [M^+H^+], found: 446.2096.

Biological activity

Cytotoxicity evaluation

The cytotoxicity of the compounds MT1 and MT8-MT15 was assayed towards human tumour cell lines [gingival carcinoma (Ca9–22), oral squamous cell carcinoma (HSC-2, HSC-3, HSC-4)] and human normal oral cells [gingival fibroblasts (HGF), periodontal ligament fibroblasts (HPLF) and pulp cells (HPC)] as described[30] with some minor modifications. In brief, all cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS). The following concentrations of the compounds in dimethylsulfoxide (DMSO) were added to the medium and incubated at 37 °C for 48 h: MT1 and MT8-MT15 (0.32, 1, 3.2, 10, 31.6, 100, 316 and 1000 µmol/L), melphalan (3.12, 6.25, 12.5, 25. 50, 100, 200 and 400 µmol/L) and 5-FU (7.8, 15.6, 31.3, 62.5, 125, 250, 500 and 1000 µmol/L). The media that contained the same concentration of DMSO (0.0078, 0.156, 0.03125, 0.0625, 0.125, 0.25, 0.5 or 1%) were used as controls, since DMSO above 0.25% is cytotoxic. The viable cell numbers were determined by the MTT method. The CC_{50} values were determined from dose-response curves.

Results and discussion

In this study, 2-(3-((amino)methyl)-4-hydroxybenzylidene)-2,3-dihydroinden-1-one types of mono Mannich bases MT8-MT15 were designed and synthesized starting from MT1 [2-(4-hydroxybenzylidene)-indan-1-one]. *N*-methyl piperazine (MT8), morpholine (MT9), piperidine (MT10), pyrrolidine (MT11), dimethylamine (MT12), diethylamine (MT13), dipropylamine (MT14) and dibenzylamine (MT15) were used as an amine compound. All Mannich bases MT8–MT15 reported here are new. The chemical structures of the compounds were confirmed by ^{1}H-NMR, ^{13}C-NMR and HRMS. The ^{1}H NMR and ^{13}C NMR data of MT1, which is starting compound for Mannich bases and known compounds, are in accordance with literature[29].

The cytotoxicities of these synthesized compounds were tested against Ca9–22, HSC-2, HSC-3 and HSC-4 human tumour cell lines and also against HGF, HPLF and HPC human normal oral cells. The cytotoxicity results were reported at Table 1.

The first question to be answered is whether there is the antineoplastic property of the compounds or not. Except compound MT15, which is dibenzylamine derivative, CC_{50} values (the concentration of the compound that kills 50% of the cells as µmol/L) were in 6.8 to >1000 micromolar range towards Ca9–22,

J Enzyme Inhib Med Chem, Early Online: 1–6

Table 1. Cytotoxicities of the compounds and log *P* values.

	CC_{50} (μmol/L)															
	Tumour cell lines							Non-malignant cells					PSE	
Compound	Ca9-22 (A)	SI	HSC-2 (B)	SI	HSC-3 (C)	SI	HSC-4 (D)	SI	Mean ± SD (E)	SI (J)	HGF (F)	HPLF (G)	HPC (H)	Mean ± SD (I)	J/E ×100	Log *p**
MT1	304	2.28	155	4.48	262	2.65	186	3.73	227 ± 68	3.28	789	540	754	694 ± 135	1.44	3.12
MT8	48	1.13	7.1	7.6	23	2.35	6.8	7.9	21 ± 19	4.75	55	46	62	54 ± 8.0	22.62	2.81
MT9	124	>3.85	61	>7.84	52	>9.19	178	>2.69	104 ± 59	>5.89	262	173	>1000	>478	5.66	2.66
MT10	>1000	<0.8	547	1.46	77	10.39	>1000	<0.8	>656	<3.36	806	705	888	800 ± 92	<0.51	3.79
MT11	55	4.05	104	2.14	32	6.97	104	2.14	74 ± 36	3.83	227	200	241	223 ± 21	5.17	3.37
MT12	121	1.59	92	2.09	105	1.83	81	2.37	100 ± 17	1.97	139	208	228	192 ± 47	1.97	3.06
MT13	187	1.66	142	2.18	101	3.07	170	1.82	150 ± 38	2.18	453	220	257	310 ± 125	1.45	3.74
MT14	88	7.84	68	10.15	84	8.21	89	7.75	82 ± 10	8.49	570	717	784	690 ± 109	10.35	4.71
MT15	>1000	>1	>1000	>1	>1000	>1	>1000	>1	>1000	>1	>1000	>1000	>1000	>1000	>0.1	6.52
Average	>325.22	>2.70	>241.78	>4.32	>192.8	>5.07	>312.75	>3.35	>268.22	>3.86	>477.88	>507.55	>579.33	>493.44	5.47	
Melphalan	22.6	6.5	8.5	17.3	5.6	26.2	11.9	12.3	12.2 ± 7.4	15.6	140.0	179.0	123.0	147.0 ± 29.0	127.87	
5-FU	29	>34.5	13	>76.9	16	>62.5	13	>76.9	18 ± 7.6	>62.7	>1000	>1000	>1000	>1000	348.33	

In this table, the CC_{50} values refer to the concentrations of the compounds in micromoles which kill 50% of the cells. The letters SI indicate the selectivity index, i.e. the quotient of the average CC_{50} figures towards HGF, HPC and HPLF non-malignant cells divided by the CC_{50} figure of the compound towards a specific tumour cell line. The letters PSE indicate the potency selectivity expression which is the product of the reciprocal of the average CC_{50} figure towards Ca9–22, HSC-2, HSC-3 and HSC-4 cell lines and the average SI value towards these neoplasms expressed as a percentage. *Log *P* values were calculated using ChemDraw Ultra 12.0 software.

HSC-2, HSC-3 and HSC-4, which are cancer lines (Table 1). The cytotoxic potencies of MT1 and its Mannich bases MT8–MT15 towards cell lines used were compared with clinically used anticancer drugs: melphalan (alkylating agent) and 5-fluorouracil (5-FU, pyrimidine antagonist). Only compound MT8 was more cytotoxic than melphalan and 5-FU towards HSC-2 and HSC-4 cell lines.

The second aspect of these compounds to be considered is whether they are tumour-specific cytotoxins since tumours are surrounded by different types of normal cells in oral cavity. Selectivity index (SI) value, which is the quotient of the average CC_{50} value of the non-malignant cells and the CC_{50} value of a compound towards a specific malignant cell line, was generated. The compounds, which have SI values of >1, can be considered as tumour-specific antineoplastic agents (Table 1). Accordingly it can be said that the compounds MT8–MT14 have shown remarkable tumour specificity against all cancer cell lines except MT10 towards Ca9–22 and HSC-4 cell lines (Table 1). However, MT10 showed the highest SI value against HSC-3 cells (SI = 10.39).

When MT1 was converted to some Mannich bases, increased cytotoxicity was observed in MT8 (6.33 times), MT9 (2.45 times), MT11 (5.53 times), MT12 (2.51 times), MT13 (1.62 times) and MT14 (3.45 times) towards Ca9–22 cancer cell line compared with MT1. But, only compounds MT9 (>1.68 times), MT11 (1.77 times) and MT14 (3.43 times) showed increased selectivity against Ca9–22 cell line, as compared with MT1.

Increased cytotoxicities were observed at Mannich bases MT8 (21.80 times), MT9 (2.54 times), MT11 (1.49 times), MT12 (1.68 times), MT13 (1.09 times) and MT14 (2.28 times), compared with MT1 towards HSC-2 cancer cell line. When the increases in SI of the compounds compared with MT1 were considered, increased SI was observed at MT8 (1.69 times), MT9 (>1.75 times), MT14 (2.26 times) towards HSC-2 cell line. On the other hand, except MT15, the increases in cytotoxicities of all Mannich bases were observed towards HSC-3 cancer cell line compared with MT1. MT8 (11.39 times), MT9 (5.04 times), MT10 (3.40 times), MT11 (8.18 times), MT12 (2.49 times), MT13 (2.59 times) and MT14 (3.12 times) were more cytotoxic than MT1 against HSC-3. When SI of Mannich bases was compared with that of MT1 against the same cell line, MT9 (>3.46 times), MT10 (3.92 times), MT11 (2.63 times), MT13 (1.15 times) and MT14 (3.09 times) showed remarkably increased selectivity against HSC-3 cell line.

Additionally, increases in cytotoxicities of Mannich bases compared with MT1 were observed against HSC-4 cell lines. These compounds were MT8 (27.35 times), MT9 (1.04 times), MT11 (1.79 times), MT12 (2.29 times), MT13 (1.09 times) and MT14 (2.09 times). But when the increase in SI value was considered, compared with that of MT1 against HSC-4 cell lines, only MT8 (2.11 times) and MT14 (2.07 times) showed increased SI values. It is quite natural to observe different cytotoxicity levels for compounds which have different chemical structures towards different cell lines, since the mechanism of action for any compound can be different towards each cell line. That is why cytotoxicity value and selectivity index can be found different for any compound.

Lead compound/s should possess both marked cytotoxic potency and also selective toxicity for tumours. In order to identify such molecule/s, a potency selectivity expression (PSE) was devised which is the product of the reciprocal of average CC_{50} values towards Ca9–22, HSC-2, HSC-3 and HSC-4 cells (that reflects cytotoxic potential, the column E, Table 1) and the average SI values towards these cell lines (that reflects the tumour-selectivity, the column J, Table 1) and expressed as a percentage. The PSE data are also shown in Table 1. When PSE

values were considered, all compounds had lower PSE values than the reference compounds melphalan and 5-FU. It was clear that except compounds MT10 and MT15 (PSE $\leq$ 0.0051, >0.001), the other Mannich bases had higher PSE values than MT1 (PSE = 0.0144). MT8, which has the *N*-methylpiperazine moiety in its chemical structure had the highest PSE value (PSE = 0.2262) (15.7-fold increase as compared with MT1), followed by MT14 (PSE = 0.1035) (7.2-fold increase). It can be said that MT8 having *N*-methylpiperazine moiety in its chemical structure seems to be a candidate compound to develop new tumour selective cytotoxins and is suited for further investigation.

The only difference in chemical structure of Mannich bases synthesized was the difference of amine part. Amines used have different pK_a values which may govern the cytotoxicity by affecting the deamination ratio. The compound MT8 has second nitrogen atom while the others has only one nitrogen atom. When six-membered cyclic amine compounds MT8 (*N*-methylpiperazine), MT9 (morpholine), MT10 (piperidine), MT11 (pyrrolidine) and acyclic amine compounds MT12 (dimethylamine), MT13 (diethylamine), MT14 (dipropylamine) and MT15 (dibenzylamine) were compared in terms of PSE value obtained, it was noticed that PSE values was in the order of MT8 > MT9 > MT10. It means that the contribution of different atom available in the cyclic amines into PSE value was in the order of N > O > C. PSE value of MT9 (0.0566) was ~1/4 of MT8 (0.2262) while MT10 (< 0.0051) had 1/11 PSE value of MT9. When five-membered cyclic amine was used for MT11 instead of six-membered cyclic amine, the PSE value of MT11 (0.0517) was similar to that of MT9. On the other hand, when acyclic amines were used instead of cyclic amines such as dimethylamine, diethylamine and dipropylamines in MT12, MT13 and MT14, respectively, PSE values of MT12–MT14 (0.00145–0.1035) were quite lower than the compounds having six-membered cyclic amines.

When MT14, which has dipropylamine, was considered, its PSE value was more than five times of MT12 and MT13, and it was approximately two times higher than those of compounds MT9, which has six-membered cyclic amine morpholine, and MT11, which has five-membered cyclic amine pyrrolidine. It is quite natural to observe increased PSE values in MT14 compared with MT12 and MT13 because it has higher log *P* value than the other homologues MT12 with dimethylamine and MT13 with diethylamine (Table 1). Increased log *P* value may increase membrane permeability of the compounds. On the other hand, although MT13 and MT10 had similar log *P* values as 3.74 and 3.79, respectively; PSE value of MT13 was approximately three times of MT10. This suggests that log *P* value may not be the sole determinant of PSE value of the compound. In addition, when PSE values of MT15 having dibenzylamine and MT12 having dimethylamine were compared, addition of two phenyl rings increased the log *P* values 1.5 times but caused the loss of activity and selectivity, supporting that cytotoxicity and PSE value of the compounds are not directly related to their log *P* value. The activity of the compounds may be related to their chemical structure, their stability, their mechanism of action and the cell type used.

Pyrrolidine and diethylamine are non-classical bioisoster with each other. MT11 having pyrrolidine ring had 3.6 times higher PSE value than that of MT13 having diethylamine. In this case, better selectivity was observed in conformationally restricted amine compound MT11.

In conclusion, the compound MT8 with the highest PSE value bearing *N*-methylpiperazine moiety seems to be a good candidate to develop new cytotoxic compounds and is suited for further investigation.

Declaration of interest

The authors report no conflict of interest. The authors alone are responsible for the content and writing of this article.

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