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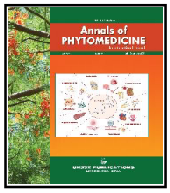

Original Article : Open Access

Cytotoxic activity evolution of bichalcones through the piperazine Mannich Base linkage analogs on a panel of 25 human cancer cell lines

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Abstract

A series of novel bichalcone analogs through the piperazine Mannich base linkage, were synthesized (3-23) previously, using a Claisen-Schmidt Condensation reaction and structurally they are confirmed by spectral analysis including 1H, 13C NMR, IR and Mass analysis. These compounds were evaluated for their cytotoxic activity against using a panel of 25 human cancer cell lines including GBM-8401, MO59K, SH-SY5Y, NT-2, NPC 039, NPC 076, Fa Du, CE146T/VGH, HSC-3, CAL-27, SAS, A549, H-460, SK1-CP1, Huh-7, HepG2, Hep3B, MDA-MB-231, MCF7, A375, HeLa, MG-63, U-2 OS, HT-29 and Colo 25. Out of the 23 bichalcone analogs, compounds 3, 4 and 20 were showed more potent cytotoxic activity against tested all cancer cell lines. Compound 3 and 20 were exhibited more cytotoxic activity against tested all cancer cell lines with IC_{50} values like GBM-8401 (IC_{50} 0.4 ± 0.02 µM), NPC039 (IC_{50} 0.6 ± 0.04 µM), NPC076 (IC_{50} 0.6 ± 0.04 µM), FaDu (IC_{50} 0.8 ± 0.12 µM), CE146/VGH (IC_{50} 1 ± 0.06 µM), HSC-3 (IC_{50} 1 ± 0.06 µM), CAL-27 (IC_{50} 0.4 ± 0.04 µM), A549 (IC_{50} 0.8 ± 0.04 µM), H460 (IC_{50} 0.6 ± 0.06 µM), SK1-CP1(IC_{50} 0.8 ± 0.02 µM), Hep G2 (IC50 0.6 ± 0.02 µM), MDA-MB-231 (IC50 0.8 ± 0.04 µM), MCF-7 (IC50 0.8 ± 0.06 µM), A375 (IC_{50} 0.6 ± 0.06 µM), HeLa (IC50 0.8 ± 0.06 µM), MG-63 (IC50 0.6 ± 0.04 µM), U-1 OS (IC50 0.6 ± 0.02 µM) and GBM-8401 (IC50 1.0 ± 0.08 µM), CE146T/VGH (IC50 6.0 ± 0.05 µM), CAL-27 (IC50 1.0 ± 0.12 µM), H460 (IC_{50} 10 µM), SK1-CP1 (IC_{50} 2.0 ± 0.12 µM), MDA-MB-231 (IC_{50} 2.0 ± 0.03 µM), MCF-7 (IC_{50} 4.0 ± 0.07 µM), A375 (IC_{50} 0.8 ± 0.02 µM), HeLa (IC_{50} 2.0 ± 0.04 µM), MG-63 (IC_{50} 1.0 ± 0.06 µM), U-2OS (IC_{50} 1.0 ± 0.08 µM) and HT-29 (IC_{50} 4.0 ± 0.08 µM), respectively. Structure activity relationships of cytotoxicity are also discussed for active compounds on cancer cell lines.

1. Introduction

Flavonoids are an important class of natural products; particularly, they belong to a class of plant secondary metabolites having a polyphenolic structure, widely found in fruits, vegetables and certain beverages (Reddy *et al.,* 2022). Flavonoids are associated with a broad spectrum of health-promoting effects and are an indispensable component in a variety of nutraceutical, pharmaceutical, medicinal and cosmetic applications. This is because of their antioxidative, anti-inflammatory, antimutagenic and anticarcinogenic properties coupled with their capacity to modulate key cellular enzyme functions (Kuo *et al.*, 1997). They are also known to be potent inhibitors for several enzymes, such as xanthine oxidase (XO), cyclooxygenase (COX), lipoxygenase and phosphoinositide-3-kinase (Metodiewa *et al*., 1997; Hayashi *et al.,* 1988; Walker *et al.* 2000). Therefore, dietary flavonoids have attracted attention as chemo-preventive agents. Chalcones are the important precursors in the biosynthesis of flavonoids, and their structure differs considerably from the others member of the flavonoid family due to they are characterized by the absence of 'ring C' of the basic flavonoid skeleton structure. Hence, they can also be referred to as open-chain flavonoids. Chalcones are characterized with diverse biological activities among anti-inflammatory, antimalaria, antiprotozoal, antibacterial, nitric oxide inhibition, tyrosinase inhibition, cytotoxic, anticancer, and anti-leishmanial activities have been reported in the past decade (Mukherjee *et al.,* 2001; Nielsen *et al.,* 2005; Goker *et al.,* 2005; Bhat *et al.*, 2005: Boeck *et al.*, 2006). The bichalcones are also well represented in the Anacardiacea family and *Cycas*, *Rhus* genus is rich source for biflavonoids and bichalcones. In general, naturally occurring bichalcones are either C-O-C or C-C linkage between the two chalcones units. Naturally occurring bichalcones, rhuschalcones (1-6) were isolated from *Rhus pyroides,* which were submitted to the U.S. National Cancer Institute for *in vitro* primary cytotoxic screening using a panel of 60 different human tumor cell lines (Boyd *et al.,* 1995). All the bichalcones manifested varying degrees of cytotoxic activity on some cell lines, but the bichalcones as a group showed more activity on colon cancer cell lines, especially the HT29 and HCT-116 cell lines (Ladislaus *et al.,* 2003).

The Mannich base reaction is a fundamentally important carbon-carbon bond forming reaction in organic synthesis, and it has been widely utilized in the synthesis of nitrogen-containing drugs, natural

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products and biologically active compounds (Kleinman *et al.,* 1991). It provides convenient access to many useful synthetic building blocks, because the amino group can be easily converted into various functionalities like metal complexes, simple salts and various quaternary ammonium salts. We reported (Reddy *et al.,* 2008) synthesis and cytotoxic evaluation of Mannich bases of heterocyclic chalcones are more potent on four human cancer cell lines including PC-3 (prostate cancer), MCF-7 (human breast cancer), KB (nasopharyngeal carcinoma) and KB-VIN (vincristine-resistant KB subline) and chalcone dimers (Reddy *et al.,* 2014; 2011; 2012) through piperazine Mannich base linkage and bichalcones. In addition to this, we reported a series of bichalcones through the piperazine Mannich base linkage with different substitution patterns in the B-ring and examined NOS-dependent NO production in microglial cells and further evaluated there *in vitro* cytotoxic activity against DU145 (prostate cancer), A549 (non-small cell lung cancer), KB (nasopharyngeal carcinoma) and HCT-8 (ileocecal) cell lines. The effect of these compounds on cytotoxicity and inhibition of NO production was taken as a biological indicator of efficacy and potency (Reddy *et al.,* 2011 and 2016).

The pharmacological importance of bichalcones and importance of Mannich base group, in the present paper previously synthesized 23 bichalcone analogs through the piperazine Mannich base linkage (1a-23) with different substitution pattern in the B-ring, were tested for cytotoxic activity against a panel of 25 human cancer cell lines including, GBM-8401, M059K (human glioblastoma cell lines), Fa Du (human pharyngeal squamous carcinoma cell line), CE146T/VGH (human esophageal carcinoma cell line), HSC-3 (human oral squamous carcinoma cell line), CAL-27 and SAS (human tongue squamous carcinoma cell lines), A549 and H-460 (human lung carcinoma cell lines), SK1-CP1, Huh7, HepG2 and Hep3B (human hepatoma cell lines), MDA-MB-231 and MCF-7 (human breast adenocarcinoma cell 4 lines), A375 (human melanoma cell line), HeLa (human cervical carcinoma cell line), MG-63 and U-2 OS (human osteosarcoma cell lines), HT-29 and Colo 25 (human colon carcinoma cell lines), NPC-039 and NPC-076 (human nasopharyngeal carcinoma cell lines), and NT-2 and SH-SY5Y (human neuroblastoma cell lines) to obtain preliminary biological profiles of this compound series that will be useful in the further design and development of bichalcones. Structure activity relationship also discussed.

2. Materials and Methods

2.1 Preparation of compounds

Synthesis of bichalcone compounds procedure was already reported previously (Reddy *et al.,* 2011) as follows. The hydroxy substituted acetophenones, 4-hydroxyacetophenone (1) and 4-hydroxy-3-methoxyacetophenone (2) were reacted with 4-piperazinoaceto-phenone and paraformaldehyde in EtOH at 120°C for 18-22 h to obtain C-5 substituted Mannich base derivatives 1a and 2a, respectively (Figure 1). These compounds (1a and 2a) are having two acetyl groups on benzene rings. The designed target compounds (3-20) (Figure 2) were obtained by the reaction between 1a and 2a with different substituted aldehydes under Claisen-Schmidt conditions using 30% KOH in methanol at room temperature. For comparison purposes, we have also prepared some 4-piperazinoacetophenone Mannich bases of monomer chalcone analogs of general structures 21, 22 and 23 (Figure 3). These compounds (21-23) were obtained by reacting 1 with pyrrole-2-carboxaldehyde, N-methylpyrrole-2-carboxaldehyde and 2 with 3-pyridinecarboxaldehyde under Claisen-Schmidt conditions and the resulting chalcones were further reacted with 4-piperazinoaceto phenone and paraformaldehyde in ethanol at 120°C for 18-22 h, to obtain 21, 22 and 23 in good yields. After 24-36 h, the solvent was removed under reduced pressure and 5% HCl (50-70 ml) were added to the residue and extraction with EtOAc, the organic layer was washed with brine, dried (Na_2CO_3), and then concentrated in *vacuo*. The residue was purified by column chromatography on silica gel, eluting with EtOAc in hexanes, to afford 3-23 compounds.

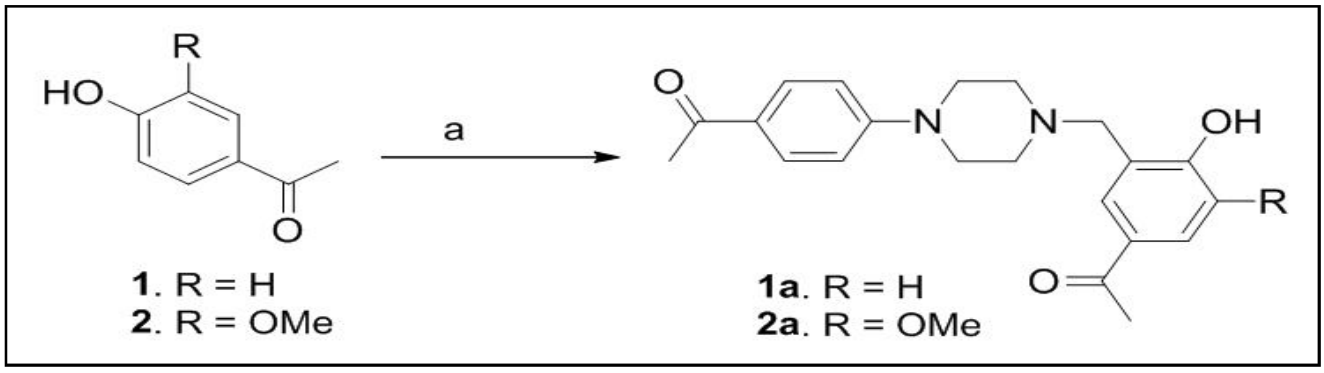
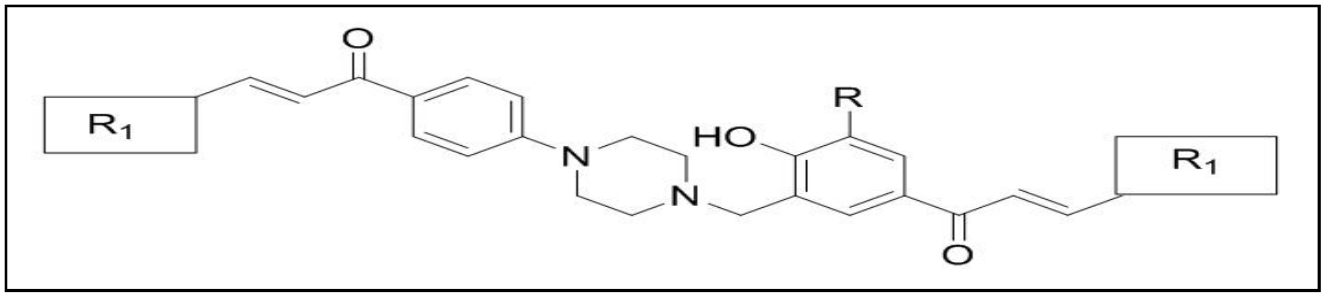


Figure 1: (a) Reagents and conditions: 4-piperiziono acetophenone, paraformal dehyde, ethanol reflux at 120°C in 18-22h



3. R = H, R_1 = 2-pyridyl
4. R = H, R_1 = 3-pyridyl
5. R = H, R_1 = 2-furan
6. R = H, R_1 = 2-thiophene
7. R= H, R_1 = 3-methyl-2-thiophene
8. R = H, R_1 = 5-methyl-2-furan
9. R = H, R_1 = N-methyl pyrrole
10. R = H, R_1 = phenyl
11. R = H, R_1 = 4-methoxybenzene
12. R = H, R_1 = 3,4-methylenedioxybenzene
13. R = H, R_1 = 2-chlorobenzene
14. R = H, R_1 = 2,4-dichlorobenzene
15. R = OMe, R_1 = 2-thiophene
16. R = OMe, R_1 = 3-methyl-2-thiophene
17. R = OMe, R_1 = phenyl
18. R = OMe, R_1 = 4-methoxybenzene
19. R = OMe, R1 = 2-chlorobenzene
20. R = OMe, R1 = 2-pyridyl

Figure 2: Bichalcone (3-23) analogs through the piperazine mannich base linkage

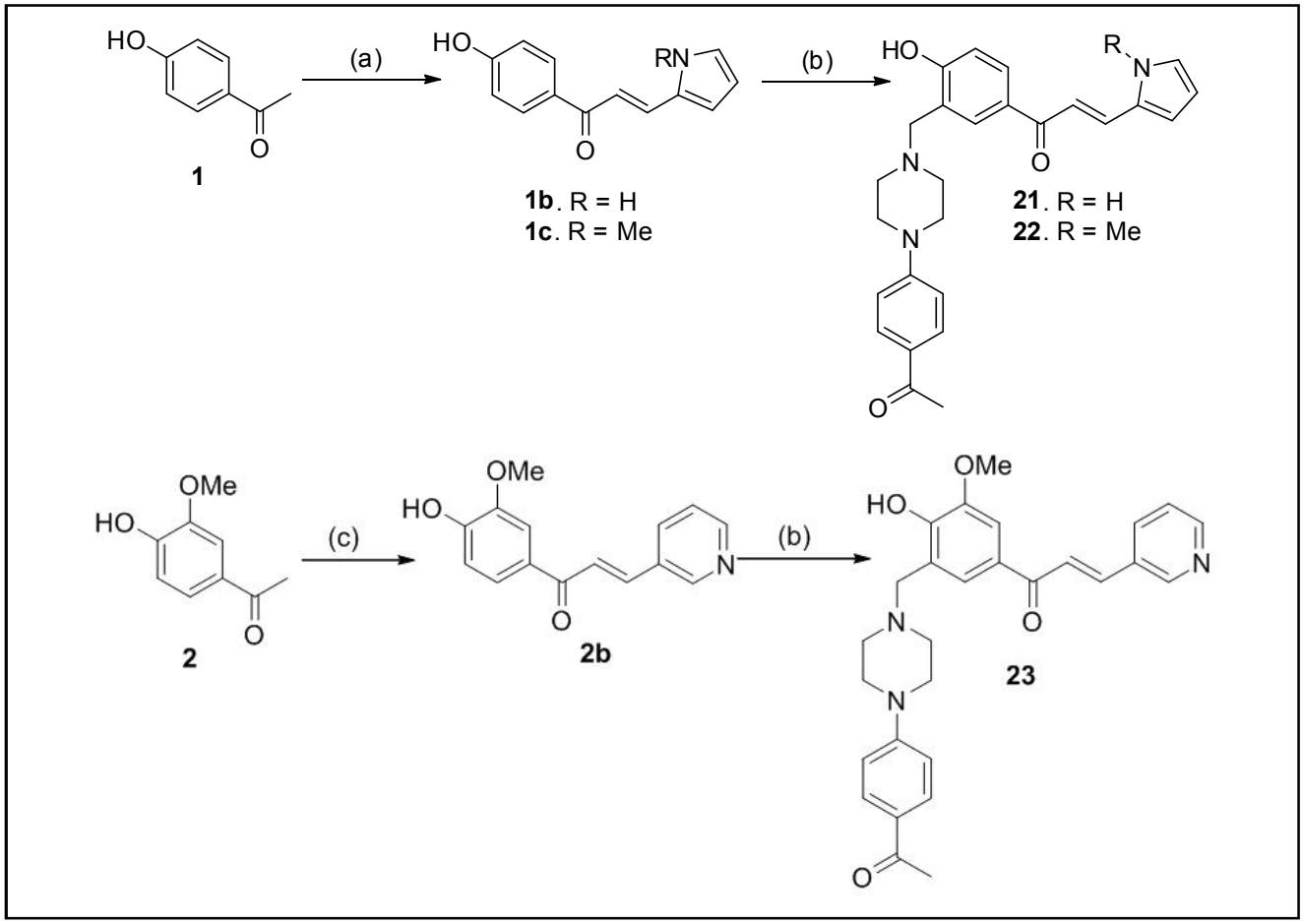


Figure 3: Reagents and conditions (a) for lb from 1, pyrrole-2-carboxaldehyde, melha: for lc from 1, N-methylpyrr ole-2-carboxaldeivc (b) 4-pipenzmo acetophenone, paraformaldehyde, e 10I, 30% KOH, room temperature, 24 h.e, methanol, 30% KOH, room temperature. 24 li. thanol reflux at 120°C m 18-22 h. (c) for 2b from 2. 3-pyndinecarboxaldehyde, methanol, 30% KOH, room temperature, 24 h.

2.2 Cell culture preparation

The human glioblastoma cell lines (GBM-8401 and M059K), human pharyngeal squamous carcinoma cell line (FaDu), human esophageal carcinoma cell line (CE146T/VGH), human oral squamous carcinoma cell line (HSC-3), human tongue squamous carcinoma cell lines (CAL-27 and SAS), human lung carcinoma cell lines (A549 and H-460), human hepatoma cell lines (SK1-CP1, Huh7, HepG2 and Hep3B), human breast adenocarcinoma cell lines (MDA-MB-231 and MCF7), human melanoma cell line (A375), human cervical carcinoma cell line (HeLa), human osteosarcoma cell lines (MG-63 and U-2 OS), and the human colon carcinoma cell lines (HT-29 and Colo 25) were obtained from the Food Industry Research and Development Institute (Hsinchu, Taiwan). The human nasopharyngeal carcinoma cell lines (NPC-TW 039 and NPC-TW 076) were provided by Dr. C. Y. Yang (Institute of Molecular Biology, National Chung Hsing University, Taichung, Taiwan). The human neuroblastoma cell line (NT-2) was kindly provided by Dr. C. L. Liao (Department of Microbiology and Immunology, National Defense Medical Center, Taichung, Taiwan). The human neuroblastoma cell line (SH-SY5Y) was provided by J. G, Chung (Department of Biological Science and Technology, China Medical University, Taichung, Taiwan). The CAL-27, FaDu, Huh7, HeLa, and MCF-7 cell lines were cultured in MEM supplemented with 5% fetal bovine serum (FBS). The SH-SY5Y, NPC-39, NPC-076, A375, A549, Colo 25, CE146T/VGH, HSC-3, HT-29, MDA-MB-231, SAS, SK1-CP1, HepG2, Hep3B, and MG-63 cell lines were cultured routinely in DENM supplemented with 5% FBS. The NT-2 cell line was cultured in OPTI-MEM supplemented with 5% FBS. The GBM-8401 and H-460 cell lines were grown in RPMI 1640 medium containing 5% FBS. The U-2 OS cell line was grown in McCoy's medium supplemented with 5% FBS. All cell lines were grown in 10-cm tissue culture dishes at 37°C in a humidified incubator containing 5% CO_2.

2.3 MTT assay

The cells were seeded at a density of 0.5~1 C 10^4 cells per well in to 96-well plates. After 16 h of incubation, cells were grown to ~60% confluence and treated with either vehicle or various concentrations of compounds at 37°C for 36 h before being harvested. The treated cells were washed once with PBS and incubated with 0.5 mg/ml MTT for 5 h. The resulting formazan precipitate was dissolved in 100 μL of DMSO and the optical density (OD) of formazan was determined using an ELISA reader (Thermo Labs stems Multi scan Spectrum, Frankin, MA, USA) at 570 nm.

3. Results

Cytotoxicity of Bichalcone analogs (3-23) were tested against 25 panels of human cancer cell lines the obtained results were expressed in Table 1 (compounds 1a-12) and 2 (compounds 13-23), more potent cytotoxic activity showed compounds are represented diagrammatically in Figure 4.

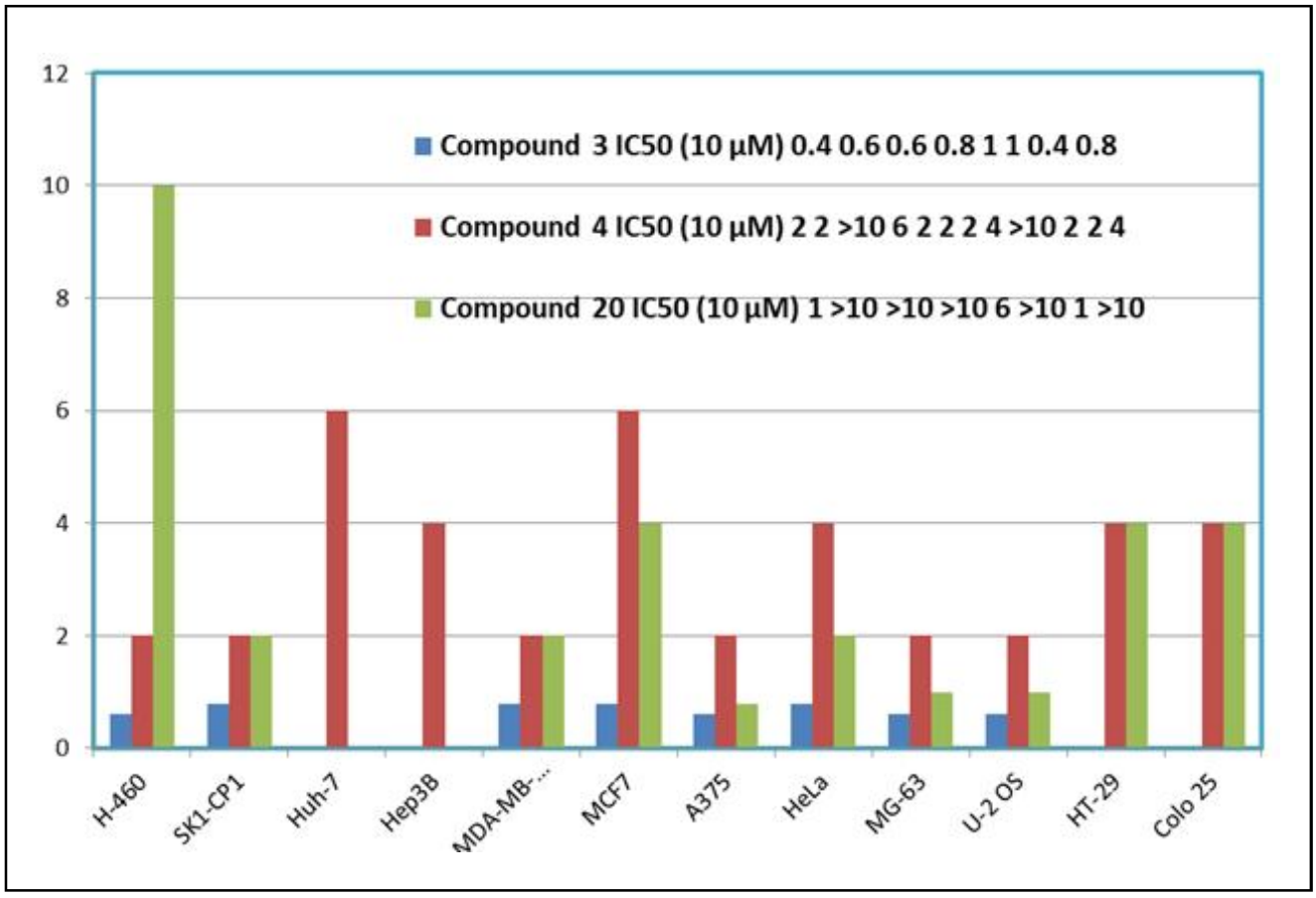


Figure 4: Compound 3, 4 and 20 cytotoxic activity data

Table 1: Anticancer activity of bichalcones on human cancer cell lines IC_{50} (μM) (1a-12)

Cell lines	1a	2a	3	4	5	6	7	8	9	10	11	12
GBM-8401	>10	>10	0.4 ± 0.02	2 ± 0.04	>10	>10	>10	>10	>10	>10	>10	>10
M059K	ND	ND	ND	2 ± 0.06	ND	ND	ND	ND	ND	ND	ND	ND
SH-SY5Y	ND	ND	ND	>10	ND	ND	ND	ND	ND	ND	ND	ND
NT-2	ND	ND	ND	6 ± 0.08	ND	ND	ND	ND	ND	ND	ND	ND
NPC039	>10	>10	0.6 ± 0.04	2 ± 0.04	>10	>10	>10	>10	>10	>10	>10	>10
NPC076	>10	>10	0.6 ± 0.04	2 ± 0.02	>10	>10	>10	>10	>10	>10	>10	>10
FaDu	>10	>10	0.8 ± 0.12	2 ± 0.12	>10	>10	>10	>10	>10	8 ± 0.02	>10	>10
CE146T/VGH	>10	>10	1 ± 0.06	4 ± 0.06	>10	>10	>10	>10	>10	>10	>10	>10
HSC-3	>10	>10	1 ± 0.06	>10	>10	>10	>10	>10	>10	>10	>10	>10
CAL-27	>10	>10	0.4 ± 0.04	2 ± 0.04	>10	>10	>10	>10	>10	>10	>10	>10
SAS	ND	ND	ND	2 ± 0.02	ND	ND	ND	ND	ND	ND	ND	ND
A549	>10	>10	0.8 ± 0.04	4 ± 0.08	>10	>10	>10	>10	>10	6 ± 0.06	>10	>10
H-460	>10	>10	0.6 ± 0.06	2 ± 0.12	>10	6 ± 0.02	>10	>10	>10	>10	8 ± 0.04	>10
SK1-CP1	>10	1 ± 0.05	0.8 ± 0.02	2 ± 0.12	8 ± 0.08	8 ± 0.06	>10	>10	>10	>10	10	>10
Huh-7	ND	ND	ND	6 ± 0.05	ND	ND	ND	ND	ND	ND	ND	ND
Hep3B	ND	ND	ND	4 ± 0.08	ND	ND	ND	ND	ND	ND	ND	ND
MDA-MB-231	>10	>10	0.8 ± 0.04	2 ± 0.02	>10	>10	>10	>10	>10	>10	>10	>10
MCF7	>10	>10	0.8 ± 0.06	6 ± 0.12	>10	>10	>10	>10	>10	>10	>10	>10
A375	>10	>10	0.6 ± 0.12	2 ± 0.04	>10	>10	>10	>10	>10	4 ± 0.04	>10	>10
HeLa	>10	>10	0.8 ± 0.06	4 ± 0.07	>10	>10	>10	>10	>10	>10	>10	>10
MG-63	>10	>10	0.6±0.04	2 ± 0.09	>10	>10	>10	>10	>10	>10	>10	>10
U-2 OS	>10	>10	0.6±0.02	2 ± 0.06	>10	>10	>10	>10	>10	>10	>10	>10
HT-29	>10	>10	ND	4 ± 0.05	>10	>10	>10	>10	>10	>10	>10	>10
Colo 25	>10	>10	ND	4 ± 0.09	>10	>10	>10	>10	>10	>10	>10	>10

Cells were incubated with various concentrations of compounds, or vehicle solvent (0.01% DMSO) for 36 h, and the cell viability was examined by MTT assay. The IC_{50} values of different cell lines were examined. Compounds giving less than 50% inhibition at this concentration are described with IC_{50}>10 *i*M. ND: Not done

Table 2: Anticancer activity of bichalcones on human cancer cell lines IC_{50} (μM) (13-23)

Cell lines	13	14	15	16	17	18	19	20	21	22	23
GBM-8401	>10	>10	>10	>10	>10	>10	>10	1 ± 0.08	>10	>10	>10
M059K	ND	ND	ND	ND	ND	ND	ND	ND	>10	>10	>10
SH-SY5Y	ND	ND	ND	ND	ND	ND	ND	ND	>10	>10	>10
NT-2	ND	ND	ND	ND	ND	ND	ND	ND	>10	>10	>10
NPC039	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10
NPC076	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10
FaDu	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10
CE146T/VGH	>10	>10	>10	>10	>10	>10	>10	6 ± 0.05	>10	>10	>10
HSC-3	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10
CAL-27	8 ± 0.06	6 ± 0.08	>10	>10	6 ± 0.05	>10	>10	1 ± 0.12	>10	>10	1 ± 0.06
SAS	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
A549	>10	>10	>10	>10	>10	>10	>10	>10	10	>10	>10

H-460	>10	>10	>10	>10	>10	>10	>10	10	6 ± 0.02	10	>10
SK1-CP1	>10	>10	>10	>10	>10	>10	>10	2 ± 0.12	>10	8 ± 0.06	8 ± 0.03
Huh-7	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Hep3B	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MDA-MB-231	>10	>10	>10	>10	>10	>10	>10	2 ± 0.03	>10	>10	>10
MCF7	>10	>10	>10	>10	>10	>10	>10	4 ± 0.07	>10	>10	>10
A375	>10	>10	>10	>10	>10	>10	>10	0.8 ± 0.02	>10	>10	>10
HeLa	>10	>10	>10	>10	>10	>10	>10	2 ± 0.04	>10	>10	>10
MG-63	>10	>10	>10	>10	>10	>10	>10	1 ± 0.06	>10	>10	10
U-2 OS	>10	>10	>10	>10	>10	>10	>10	1 ± 0.08	>10	>10	10
HT-29	>10	>10	>10	>10	>10	>10	>10	4 ± 0.12	>10	>10	>10
Colo 25	>10	>10	>10	>10	>10	>10	>10	4 ± 0.08	>10	>10	>10

Cells were incubated with various concentrations of compounds, or vehicle solvent (0.01% DMSO) for 36 h, and the cell viability was examined by MTT assay. The IC_{50} values of different cell lines were examined. Compounds giving less than 50% inhibition at this concentration are described with IC_{50}>10 *i*M. ND: Not done

4. Discussion

Compound 2a selectively exhibited potent cytotoxicity against SK-1-CP-1 cancer cell line with the IC_{50} value 1.0 ± 0.05 µM concentration. Comparison was found between the compounds 3 and 4, which have 2-pyridyl and 3-pyridyl groups in the B-ring, respectively. Concerning the B-ring, compound 3 showed more potent cytotoxicity against tested all cancer cell lines with IC_{50} values ranges from 0.4 ± 0.02 to 1 ± 0.06 µM (Table-1) and compound 4 exhibited with IC_{50} values ranges from 2 ± 0.02 to 8 ± 0.09 *i*M. These results clearly indicate that 2-pyridyl group as B-ring was responsible for more potent cytotoxic activity in 3 than 3-pyridyl group as B-ring in compound 4. When the 2-pyridyl and 3-pyridyl groups as B-ring in compounds 3 and 4 were replaced with phenyl group in compound 10 showed moderate cytotoxicity against selected cancer cell lines like Fa Du (IC_{50} 8 ± 0.02 µM), A549 (IC_{50} 6 ± 0.06 µM) and A375 (IC_{50} 4 ± 0.04 µM).These findings shows that the pyridyl group is indispensable responsible at the ring-B of compounds 3 and 4 with respect to its enhance cytotoxicity against all cancer cell lines. In compounds 5 and 6 ring-B had 2-furan and 2-thiophene groups, respectively. Compound 5 exhibited selective cytotoxicity on human hepatoma cell line (SK1-CP1) with IC_{50} value 8 ± 0.08 µM concentration and 6 showed on H-460 (IC_{50} 6 ± 0.02 µM) and SK1-CP1 (IC_{50} 8 ± 0.06 µM) cell lines. Compounds 7, 8 and 9 are having 3-methyl-2-thiophene, 5-methyl-2-furan and N-methyl pyrrole substitution pattern in ring-B, respectively, were showed lesser cytotoxicity observed in tested all cancer cell lines. A similar comparison was found between compounds 10 and 11, which have phenyl and 4-methoxybenzene groups are B-ring, respectively. Compound 10 showed significant cytotoxicity on Fa Du (IC_{50} 8 ± 0.02 µM), A549 (IC_{50} 6 ± 0.06 µM) and A375 (IC_{50} 4 ± 0.04 µM) cancer cell lines, and 11 showed on cytotoxic activity on H-460 cell line with the concentration IC_{50} 8 ± 0.04 µM. In compound 12 ring-B as 3,4-methylenedioxy benzene was not exhibited any significant cytotoxic activity against tested all cancer cell lines. These results clearly indicates that methylenedioxy functional group in 12 was responsible for decrease the cytotoxic activity compared to compound 10. Compounds 13 and 14 are having 2-chloro and 2,4-dichloro substitution pattern in ring-B, respectively, were showed significant cytotoxic activity against CAL-27 cell line with the IC_{50} values 8 ± 0.06 µM and 6 ± 0.08 *i*M concentration. Compounds 11 and 17 are having methoxy group in ring-B and ring-A, respectively. Compound 11 exhibited significant cytotoxic activity against H-460 cell line with the IC_{50} value 8 ± 0.04 µM and 17 showed on CAL-27 cell line with the IC_{50} values 6 ± 0.05 µM concentration. In both compounds 13 (CAL-27 cancer cell line with IC_{50} value 8 ± 0.06 µM) and 19 are having 2-chlorobenzene substitution pattern in ring-B, but in 19 additional methoxy group at ring-A and no cytotoxic activity observed. Compounds 3 and 20 are having 2-pyridyl substitution pattern in ring-B, but additional methoxyl group was attached at ring-A in compound 20. Compound 3 and 20 were exhibited more cytotoxic activity against tested all cancer cell lines like GBM-8401 (IC_{50} 0.4 ± 0.02 µM), NPC039 (IC_{50} 0.6 ± 0.04 µM), NPC076 (IC_{50} 0.6 ± 0.04 µM), FaDu (IC_{50} 0.8 ± 0.12 µM), CE146/VGH (IC_{50} 1 ± 0.06 µM), HSC-3 (IC_{50} 1 ± 0.06 µM), CAL-27 (IC_{50} 0.4 ± 0.04 µM), A549 (IC_{50} 0.8 ± 0.04 µM), H460 (IC_{50} 0.6 ± 0.06 µM), SK1-CP1(IC_{50} 0.8 ± 0.02 µM), Hep G2 (IC_{50} 0.6 ± 0.02 µM), MDA-MB-231 (IC_{50} 0.8 ± 0.04 µM), MCF-7 (IC_{50} 0.8 ± 0.06 µM), A375 (IC_{50} 0.6 ± 0.06 µM), HeLa (IC_{50} 0.8 ± 0.06 µM), MG-63 (IC_{50} 0.6 ± 0.04 µM), U-1 OS (IC_{50} 0.6 ± 0.02 µM) and GBM-8401 (IC_{50} 1.0 ± 0.08 µM), CE146T/VGH (IC_{50} 6.0 ± 0.05 µM), CAL-27 (IC_{50} 1.0 ± 0.12 µM), H460 (IC_{50} 10 µM), SK1-CP1 (IC_{50} 2.0 ± 0.12 µM), MDA-MB-231 (IC_{50} 2.0 ± 0.03 µM), MCF-7 (IC_{50} 4.0 ± 0.07 µM), A375 (IC_{50} 0.8 ± 0.02 µM), HeLa (IC_{50} 2.0 ± 0.04 µM), MG-63 (IC_{50} 1.0 ± 0.06 µM), U-2OS (IC_{50} 1.0 ± 0.08 µM) and HT-29 (IC_{50} 4.0 ± 0.08 µM), respectively. This result clearly indicates that pyridyl moiety was responsible for more cytotoxic activity exhibiting tested all human cancer cell lines. Compounds 21 and 22 are derivatives of pyrrole and N-methyl pyrrole showed cytotoxicity against H-460 (IC_{50} 6.0 ± 0.02 µM) and SK1-CP1 (IC_{50} 8.0 ± 0.06µM), respectively. Compound 23 showed more cytotoxic activity against CAL-27 (IC_{50} 1.0 ± 0.06 µM) and SK1-CP1 (IC_{50} 8.0 ± 0.03 µM).

5. Conclusion

Previously synthesized bichalcone analogs through the piperazine Mannich base linkage 3-23, were evaluated for their cytotoxic activity against using a panel of 25 human cancer cell lines including GBM-8401, MO59K, SH-SY5Y, NT-2, NPC 039, NPC 076, FaDu, CE146T/

VGH, HSC-3, CAL-27, SAS, A549, H-460, SK1-CP1, Huh-7, HepG2, Hep3B, MDA-MB-231, MCF7, A375, HeLa, MG-63, U-2 OS, HT-29 and Colo 25. Out of the 23 bichalcone analogs, compounds 3, 4 and 20 were showed more potent cytotoxic activity against tested all cancer cell lines. Out of the 23 bichalcone analogs, compounds 3, 4 and 20 were showed more potent cytotoxic activity against tested all cancer cell lines. Structure activity relationships are also discussed. Furthermore, detailed pharmacological evaluations are needed for more potent compounds to demonstrate the exact mechanism involve for the anticancer activity

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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