Synthesis and anticancer evaluation of novel morpholine analogues

Dr. Mohammed Al-Ghorbani

Department of Chemistry, Science College, Taibah University, Madina, Saudi Arabia

Abstract

A series of novel 4-benzyl-morpholine-2-carboxylic acid N'-[2-(4-benzoyl-phenoxy)-acetyl]-hydrazide derivatives 8a-j has been synthesized from (4-hydroxy-aryl)-aryl methanones through a multi-step reaction sequence and then evaluated for anti-proliferative activity *in vitro* against various types of neoplastic cells of mouse and human such as DLA, EAC, MCF-7 (breast) and A549 (lung) cells. From the cytotoxic studies and structural activity relationship of compounds 8a-j, it is clear that methyl group on the benzophenone is essential for antiproliferative activity and bromo at ortho position (compound 8b) and methyl at para position (compound 8f) on a ring of benzophenone are significant for extensive anti-mitogenic activity.

Introduction

Morpholine ring system is a core structure in various synthetic compounds displaying a broad spectrum of therapeutic applications [1,2]. Literature survey revealed that morpholine derivatives have been proved as an excellent class of anticancer agents against a variety of cancer cell lines such as human colorectal adenocarcinoma, metastatic human breast cancer, gastric cancer, mammalian target of rapamycin, non small cell lung cancer, prostate cancer [3].

On the other hand, the proficiency of benzophenone analogues as chemotherapeutic agents, especially as anticancer, is well documented [4]. Previously, our group has reported some benzophenone-heterocycle hybrids with good anticancer activity [5]. In continuation of our efforts toward the design of new anticancer agents, we considered it worthwhile to pursue further modifications on the benzophenone part by appending morpholine subunit at 2-position on (4-benzoyl-phenoxy)-acetic acid hydrazide for inhibition of tumour cell proliferation of mouse (DLA and EAC cells) and human (MCF-7 and A549 cells) origin.

Keywords: Benzophenone; Morpholine; Anti-mitogenicity; DLA and MCF-7.

Methodology

4-Benzyl-morpholine-2-carboxylic acid N'-[2-(4-benzoyl-phenoxy)-acetyl]-hydrazides (**8a-j**) were synthesized by coupling reaction of 4-benzyl-morpholine-2-carboxylic acid and (4-benzoyl-phenoxy)-acetic acid hydrazides (**6a-j**) using EDCI as catalyst.

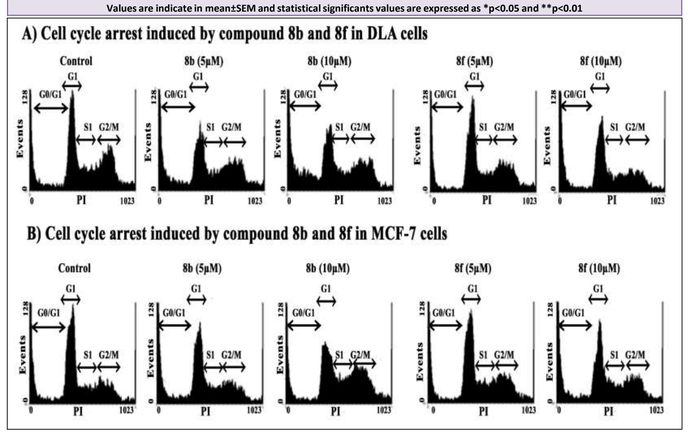
Various types of tumor cells of different origin such as DLA and EAC cells (murine) and MCF-7 and A549 cells (human) were used for determining the IC50 value of newly synthesized series **8a-j** by MTT, LDH leak and trypan blue assays. Extended anti-mitogenic efficacies of the lead compounds were evaluated by colony formation assay in selected tumor cells such as DLA and MCF-7. Also anti-proliferative effect was studied through FACS analysis.

Compounds	Cancer cells from murine origin					
	IC_{50} value (μM) against DLA cells			IC_{50} value (μM) against EAC cells		
	MTT assay	LDH leak assay	Trypan blue assay	MTT assay	LDH leak assay	Trypan blue assay
Control	--------	--------	--------	--------	--------	--------
8a	66.7±1.3	68.4±2.1	61.2±1.0	63.5±1.4	72.0±1.9	63.4±2.0
8b	7.0±1.0*	8.1±1.5	7.4±.1.2	9.5±1.1*	10.1±1.3	9.0±1.4
8c	47.3±3.4	52.0±2.4	49.4±3.0	48.6±2.4	57.0±2.1	51.6±3.2
8d	78.5±2.4	78.7±1.9	71.1±2.8	69.1±3.2	76.4±2.3	59.8±2.1
8e	67±3.8	70.0±3.2	62.6±4.3	64.3±2.8	70.8±3.0	64.4±1.9
8f	9.5±1.4	11.2±1.2	10.3±1.0*	10.2±2.1	11.6±1.3	10.6±1.6
8g	>100	>100	>100	91.9±3.2	88.5±4.3	89.8±2.9
8h	91.1±3.8	87.3±4.1	89.0±1.7	95.5±1.8	92.2±2.9	95.3±2.1
8i	>100	>100	98.8±3.2	>100	>100	95.7±2.1
8j	48.4±3.5	58.1±2.7	51.4±3.0	47.3±2.6	54.6±2.4	46.2±2.9
5-FU	12.0±1.3	13.5±2.1	10.7±1.7	11.8±2.1	12.1±1.2	11.3±1.0

Values are indicate in mean±SEM and statistical significants values are expressed as *p<0.05 and **p<0.01

Compounds	Cancer cells from human origin					
	IC_{50} value (μM) against MCF-7 cells			IC_{50} value (μM) against A549 cells		
	MTT assay	LDH leak assay	Trypan blue assay	MTT assay	LDH leak assay	Trypan blue assay
Control	--------	--------	--------	--------	--------	--------
8a	48.5±2.0	46.7±1.8	48.9±1.4	53.4±3.0	62.1±2.6	54.7±3.2
8b	7.1±0.8**	7.3±1.2	7.0±0.7**	10.1±0.6**	11.2±0.9*	9.1±1.0*
8c	47.7±1.2	54.2±1.5	45.6±2.3	56.4±2.5	58.3±2.1	52.9±3.4
8d	75.2±1.5	76.3±1.8	70.8±1.6	76.8±2.2	79.6±3.2	76.8±1.9
8e	57.0±3.2	63.2±3.8	57.7±1.2	63.3±2.8	73.8±3.3	64.7±1.7
8f	9.1±0.8**	10.3±1.2	8.6±1.8	13.1±1.1*	13.8±1.3	13.7±1.2
8g	>100	>100	95.8±3.8	>100	>100	>100
8h	87.6±3.1	93.7±4.2	79.9±3.2	89.0±3.2	94.5±2.1	90/4±1.0
8i	>100	90.2±1.8	87.8±2.4	>100	>100	92.4±3.2
8j	44.8±3.2	49.6±2.8	45.2±1.6	57.8±3.1	63.5±1.2	50.7±2.3
5-FU	14.5±1.1	14.6±1.3	13.1±2.0	13.3±1.4	14.1±1.2	12.3±2.2

Values are indicate in mean±SEM and statistical significants values are expressed as *p<0.05 and **p<0.01

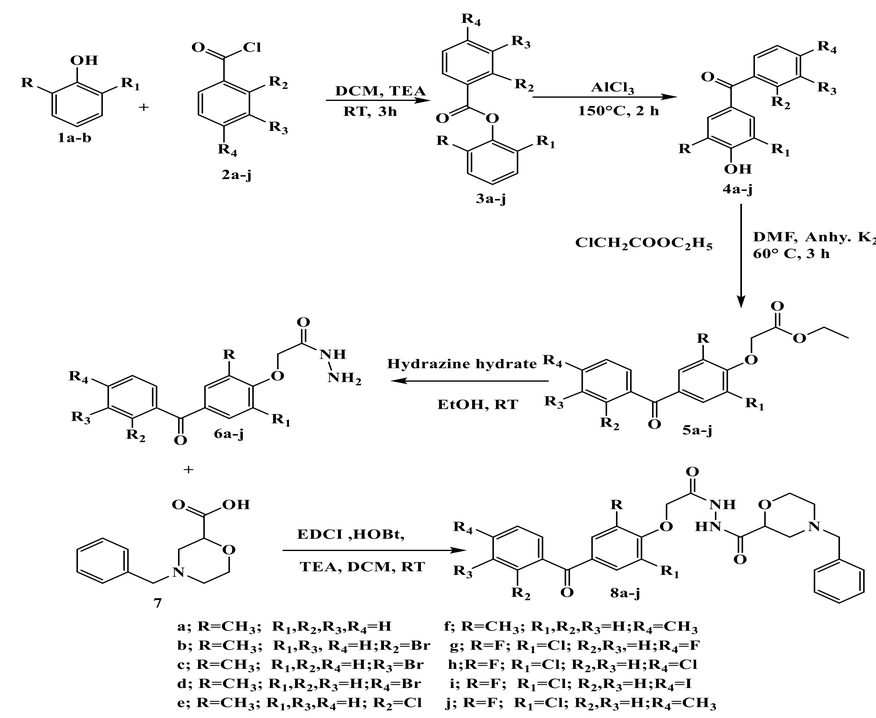


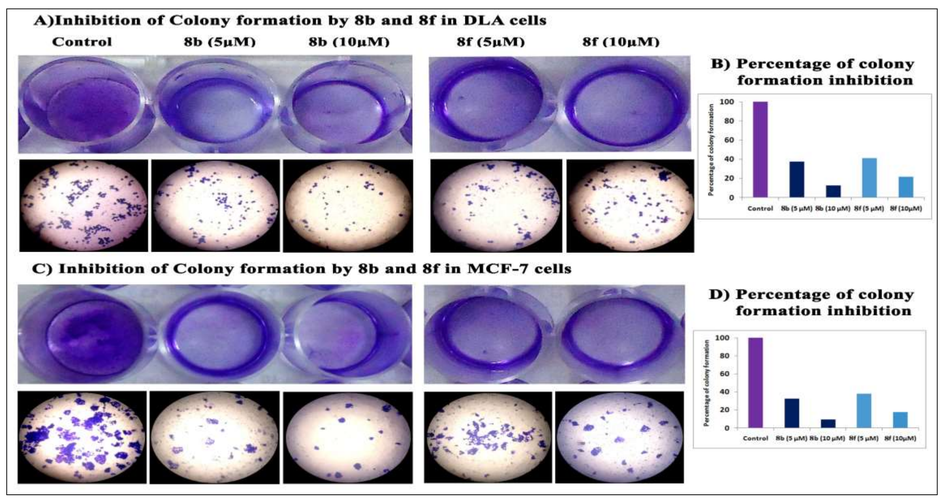
Conclusion

In summary, a series of morpholine conjugated benzophenone analogues **8a-j** were synthesized and evaluated for *in vitro* anti-proliferative activity against DLA, EAC, MCF-7 and A549 cells. Compound **8b** is fundamental for antiproliferative activity. Also in compound **8f** is significant to exhibited extensive anti-mitogenic activity. Further investigation in clonogenic assay and FACS suggests that compounds **8b** and **8f** have potency to exhibit the prolonged anti-mitogenicity against diverse number cancer cells of different origin.

Results

In the present investigation, new potent analogues were synthesized, by integrated morpholine nuclei to benzophenone moiety. Initially, antiproliferative/ anti-mitogenic efficacy of benzophenone-morpholine analogues **8a-j** were evaluated against murine cancer cells (DLA and EAC) by performing MTT, trypan blue and LDH leak assays. The average cytotoxicity of **8b** and **8f** was calculated against each cell line by cytotoxic studies. The compounds **8b** and **8f** were found to exhibit a promising anti-mitogenic effect against murine ascites lymphoma (DLA) cells with IC_{50} of ~7.5 μM and ~10.3μM respectively. The synchronized results were obtained against murine ascites carcinoma (EAC) cells with IC_{50} of ~9.5 μM and ~10.8 μM for compounds **8b** and **8f** respectively. These results prompted us to extend the studies in human cancer cells for improving efficiency of compounds **8b** and **8f** and then cytotoxicity of compounds **8a-j** evaluated against MCF-7 and A549 cells (Table 1B). The study reveals that compounds **8b** and **8f** have potency to show anti-neoplasmic property with IC_{50} of ~7.1 μM and ~9.3 μM, respectively against human breast carcinoma cells (MCF-7).





References

[1] A. Insuasty, J. Ramírez, M. Raimondi, C. Echeverry, J. Quiroga, R. Abonia, M. Nogueras, J. Cobo, M.V. Rodríguez, S.A. Zacchino, B. Insuasty, Molecules. 18 (2013) 5482-5497.

[2] Y. Li, C. Tan, C. Gao, C. Zhang, X. Luan, X. Chen, H. Liu, Y. Chen, Y. Jiang, Bioorg. Med. Chem. 19 (2011) 4529–4535.

[3] W. Zhu, C. Sun, S. Xu, C. Wu, J. Wu, M. Xu, H. Zhao, L. Chen, W. Zeng, P. Zheng, Bioorg. Med. Chem. 22 (2014) 6746-6754.

[4] G.R. Pettit, M.P. Grealish, D.L. Herald, M.R. Boyd, E. Hamel, R.K. Pettit, J. Med. Chem. 43 (2000) 2731-2737.

[5] H.D. Gurupadaswamy, P.Thirusangu, B.R. Vijay Avin, V. Vigneshwaran, M.V. Prashanth Kumar, T.S. Abhishek, V.L. Ranganatha, S.A. Khanum, B.T. Prabhakar, Biomed. Pharmacother. 68 (2014) 791-797.