Anti-Cancer Potential of Benzophenone-*Bis*-Schiff bases on Human Pancreatic Cancer Cell Line

[1]Khalid Mohammed Khan*, [2]Huma Rasheed, [1]Bibi Fatima, [1]Muhammad Hayat, [3]Fazal Rahim, [3]Hayat Ullah, [1]Abdul Hameed, [4,5]Muhammad Taha, [2]Affan Tahir and [6]Shahnaz Perveen

[1]H. E. J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi-75270, Pakistan.

[2]Dr. Panjwani Center for Molecular Medicine and Drug Research, International Center for Chemical and Biological Sciences, University of Karachi, Karachi-75270, Pakistan.

[3]Depatment of Chemistry, Hazara University, Mansehra-21300, Khyber Pakhtunkhwa, Pakistan.

[4]Atta-ur-Rahman Institute for Natural Product Discovery, Universiti Teknologi MARA (UiTM), Puncak Alam Campus, 42300 Bandar Puncak Alam, Selangor D. E. Malaysia.

[5]Faculty of Applied Science Universiti Teknologi MARA, 40450 Shah Alam, Malaysia.

[6]PCSIR Laboratories Complex, Karachi, Shahrah-e-Dr. Salimuzzaman Siddiqui, Karachi-75280, Pakistan.

Khalid.khan@iccs.edu*

(Received on 10th November 2015, accepted in revised form 17th June 2016)

Summary: Synthetic benzophenone-*bis*-Schiff bases (**1-17**) were evaluated for PC3 cell line inhibition. Most of the compounds showed cytotoxic effects but compounds **4**, **8**, **14**, **16**, and **17** were found to be potent. The growth inhibition of PC3 cells was in a concentration-dependent manner when treated with compounds **4**, **8**, **14**, **16** and **17** (Figure-2) after 24 h exposure time. The IC_{50} values of all five most active compounds were calculated as **4** ($IC_{50} = 24 \pm 1.4$ μM), **8** ($IC_{50} = 29 \pm 1.3$ μM), **14** ($IC_{50} = 66 \pm 2.8$ μM), **16** ($IC_{50} = 28 \pm 1.4$ μM) and **17** ($IC_{50} = 18 \pm 2.8$ μM) when compared with standard cisplatin ($IC_{50} = 20 \pm 1.1$ μM). This study has identified potent anticancer agents.

Keywords: Synthesis, Benzophenone-*Bis*-Schiff bases, Anti-cancer, PC3 Cell line, Structure-activity Relationship

Introduction

Cancer, the uncontrolled cell growth, is a principal cause of death throughout the world and it was found that 7.6 million deaths in 2008 occur due to cancer which is about 13% of total deaths [1, 2]. To minimize the chances of death due to this disease great efforts have been made in the area of cancer research over the past few decades but still it is a major cause of death among other diseases. The medicinal chemists are in search of developing novel compounds that will have potent anti-cancer potential with less adverse effects.

Regardless of considerable development accomplished in anticancer therapy, the control of malignancies in humans still founds a major contest for current medicine [3-6]. The chemotherapy mostly results in serious side effects that cause the destruction of normal cells. It was observed that currently used anticancer drugs result in substantial toxicity in the nervous system and is liable for neurological side effects [7-11]. It is an important need of the day to develop such anti-cancer compounds that exhibit anti-proliferative potential in tumor cells without affecting the normal cells. The major side effects of currently used anti-cancer drugs is the development of resistance [12] that reveals the crucial requisite for the discovery of novel anti-cancer agents, particularly those with unusual and discriminating mechanisms of action.

The Schiff bases or hydrazone scaffold with extensive pharmacological properties [13] exhibited activity against malaria [14]. Benzophenone hydrazones analogs also showed insecticidal activities [15]. The substituted hydrazones were reported as an exciting agent against insect pests and other lepidopteran [16-18]. The hydrazones molecules having carbamate showed potent insecticidal properties [19].

The hydrazone analogs have anti-leukemic and antitumor activity [20] and because of emergent biological importance the research in this area is quiet of great attention. The derivatives of hydrazones like 2-acetylthiophene 2-thiazolyhydrazone [21] and dexamethasone have been described as dual COX/5-LO inhibitors which show anti-inflammatory and analgesic activity [22].

The present study deals with the synthesis of various benzophenone-*bis*-Schiff bases and their cytotoxic effect on PC3 human pancreatic cancer cell line.

*To whom all correspondence should be addressed.

Experimental

General Experimental

NMR analyses were performed on Avance Bruker AM 300, 500 MHz instruments. CHN analyses were done on a Carlo Erba instrument, Strumentazione-Mod-1106, Italy. EI-MS were calculated on a Finnegan MAT-311A, Germany. TLC was performed on pre-coated silica gel aluminum plates (Kieselgel 60, 254, E. Merck, Germany). Chromatograms were visualized by UV at 254 and 365 nm or iodine vapors.

Cell line, Chemicals and Culture Reagents

Human pancreatic cancer cell line was purchased from the American Type Culture Collection (Rockville, MD, USA). Roswell Park Memorial Institute (RPMI) 1640 medium, phosphate buffer saline (PBS) and 1% penicillin/streptomycin (pen/strep) were obtained from GIBCO BRL Life Technologies (Gaithersburg, MD, USA). Cell titer blue was purchased from Promega (Madison, UI) while trypsin-EDTA and fetal bovine serum (FBS) were from Hyclone (Logan, UT, USA).

Cell Culture

Human pancreatic cells were cultured in 75 cm^2 polystyrene culture flask with canted neck and filtered caps (Corning Inc, NY, USA) in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% Fetal bovine serum (FBS) and 1% penicillin/streptomycin (100U/ml/100 μg/ml). Cells were consistently provided a warm and humidified environment of 37 °C, 5% CO_2 and 95% humidity and grown at 80% confluency before every experiment. The cancer cells were washed with phosphate buffer saline (PBS) and harvested with a solution of trypsin–EDTA and suspended cells were counted in a Casey 1 cell counter (Scharfe, Germany) for further experiments.

Cytotoxicity Assay

PC3 cells at a density of 5 x 10^3 were cultured in a black 96-well plate and after 24 hours cells were treated with different concentrations of benzophenone-*bis*-Schiff bases **1**-**17**. Next day, the cells were exposed to the fluorescent dye, cell titer blue (CTB), the indicator compound in the CTB is blue colored resazurin which is oxidized by the viable cells in to red colored resorufin compound. Following 2 and 4 h of incubation at 37 °C, the fluorescence was measured on Spectra Max M5 spectrofluorometer (Molecular Devices Corporation, CA, USA) at excitation/emission wavelengths of 490/514 nm [23].

General procedure for the synthesis of Benzophenone-Bis-Schiff bases derivatives **(1-17)**

Benzophenone-*bis*-Schiff bases **1-17** were synthesized by reacting commercially available benzophenone Schiff base and different aromatic aldehydes in anhydrous methanol in the presence of few drops of acetic acid. The resulting products were obtained in good yields.

In a typical reaction few drops of acetic acid were added in stirred mixture of benzophenone-Schiff bases (3.0 mmol) and substituted benzaldehydes (3.0 mmol) in methanol. The reaction mixture was refluxed for 2-3 h and monitored by TLC. When reaction completed, then it was allowed to cool to room temperature. Re-crystallization from methanol afforded pure benzophenone-*bis*-Schiff bases **1**-**17** in high yields (Scheme-1) [24].

Characterization of 2-Hydroxy-3-methoxybenzaldehyde-N-(diphenylmethylene)hydrazone **(1)**

Yield: 0.30g (90%); ^{1}H-NMR: (300 MHz, DMSO): δ 9.64 (s, 1H, -OH), 8.78 (s, 1H, *vin*-H), 7.60 (d, 2H, J = 7.5 Hz, H-2/6), 7.45 (m, 6H, H-3'/4'/5'/3/4/5), 7.24 (d, 2H, J = 7.5 Hz, H-2'/6'), 6.89 (d, 1H, J = 8.5 Hz, H-6''), 6.39 (d, 1H, J = 8.5 Hz, H-5''), 6.37 (d, 1H, J = 8.5 Hz, H-4''); MS: *m/z* (rel. abund. %), 330 (M^+, 100), 207 (39), 165 (25), 77 (22). Anal. calcd. for $C_{21}H_{18}N_2O_2$ (330.14); C, 76.34; H, 5.49; N, 8.48; O, 9.69, Found C, 76.32; H, 5.47; N, 8.45; O, 9.66 [24].

The data of compounds **(2-17)** has already reported in our previous publication [24].

Results and Discussion

Chemistry

Benzophenone-*bis*-Schiff bases derivatives **1-17** were synthesized by reacting commercially available benzophenone-Schiff base and different aromatic aldehydes in methanol. The resulting products were obtained in good yields.

In a typical reaction few drops of acetic acid were added in stirred mixture of benzophenone-Schiff base (3.0 mmol) and substituted benzaldehydes or different ketones (3.0 mmol) in methanol. Refluxed the reaction mixture for 2-3 h and monitored by TLC the reaction progress. When reaction completed, then it was allowed to cool to room temperature. Re-crystallization from methanol pure benzophenone-*bis*-Schiff bases **1**-**17** were obtained in high yields (Scheme-1) [24].

Table-1: Different substituted benzophenone-*bis*-Shicff bases **1-17.**

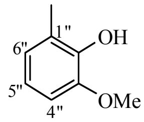
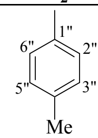
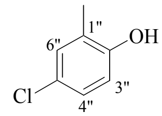
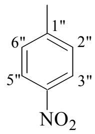
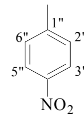
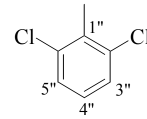
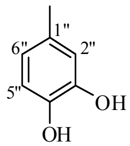
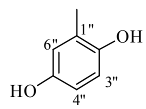
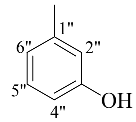
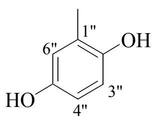
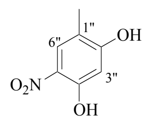
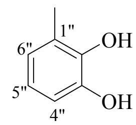
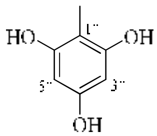
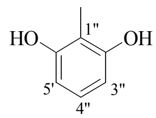
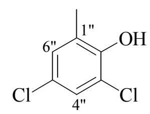
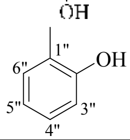
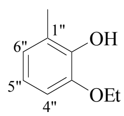
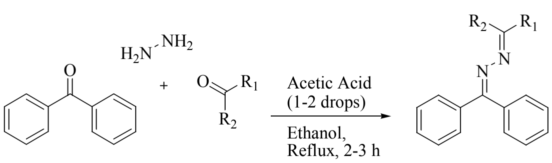
S. No.	R_1	R_2	S. No.	R_1	R_2	S. No.	R_1	R_2
1	**H**		**7**	**H**		**13**	**H**	
2	**H**		**8**	**Me**		**14**	**H**	
3	**H**		**9**	**Me**		**15**	**H**	
4	**H**		**10**	**Me**		**16**	**H**	
5	**H**		**11**	**Me**		**17**	**H**	
6	**H**		**12**	**H**		**-**	**-**	**-**

Table-2: PC-3 Cell line activity of substituted benzophenone-*bis*-Shicff bases **4, 8, 14, 16** and **17.**

S. No.	Compounds	IC_{50} (μM ± SEM[a])
1	4	24 ± 1.4
2	8	29 ± 1.3
3	14	66 ± 2.1
4	16	28 ± 1.4
5	17	18 ± 0.1
Cisplatin[std]		20 ± 1.1

SEM[a] is the standard error of the mean



Scheme-1: Synthesis of Benzophenone-*Bis*-Schiff bases **(1-17).**

Biological Activity

Synthetic benzophenone-*bis*-Schiff bases **1-17** have been evaluated for their anti-cancer potential. The compounds **4**, **8**, **14**, **16**, and **17** showed significant anti-cancer activity (Table-2).

The data of various compounds is presented in IC_{50} value in μM. To determine the cytotoxic effect of the above-mentioned compounds on human pancreatic cancer cells, a cytotoxic assay using a fluorescence dye cell-titer blue (CTB), was performed with various concentrations of compounds *i.e.* 50, 100, 200 μM. The cytotoxic action is based upon the conversion of resazurin in to resorufin, a fluorescent dye, which emits fluorescence making the assay more sensitive than the conventional MTT colorimetric assay [25]. Results indicate that all synthetic benzophenone-*bis*-Schiff bases (**1-17**) showed cytotoxic effects as shown in Figure-1, however, compounds **4**, **8**, **14**, **16**, and **17** were found to be potent. The growth inhibition of PSN-1 cells was in a concentration-dependent manner when treated with compounds **4**, **8**, **14**, **16**, and **17** (Figure-2) after a 24 h exposure time. Later the IC_{50} values of all five compounds were calculated as **4** (IC_{50} = 24 ± 1.4 μM), **8** (IC_{50} = 29 ± 1.3 μM), **14** (IC_{50} = 66 ± 2.8 μM), **16** (IC_{50} = 28 ± 1.4 μM), and **17** (IC_{50} = 18 ± 2.8 μM).

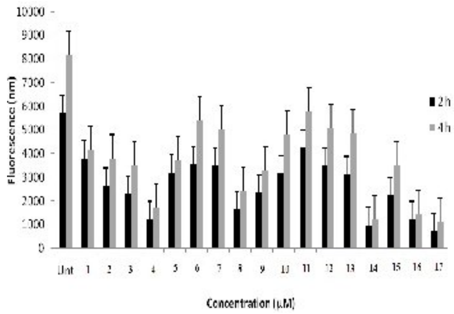
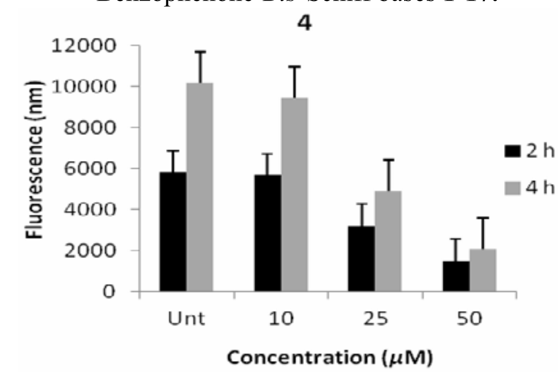
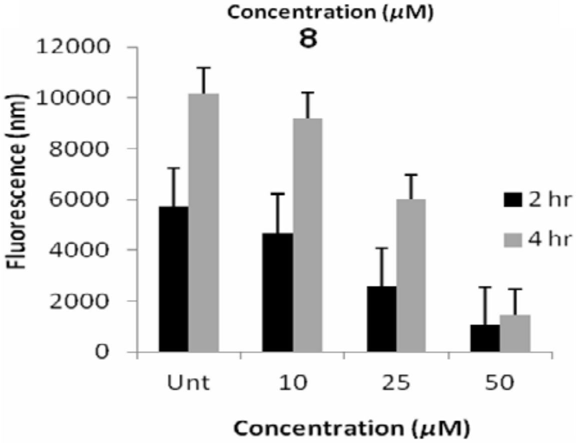
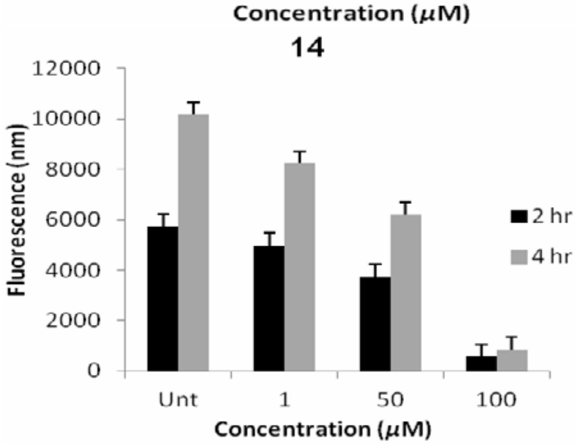
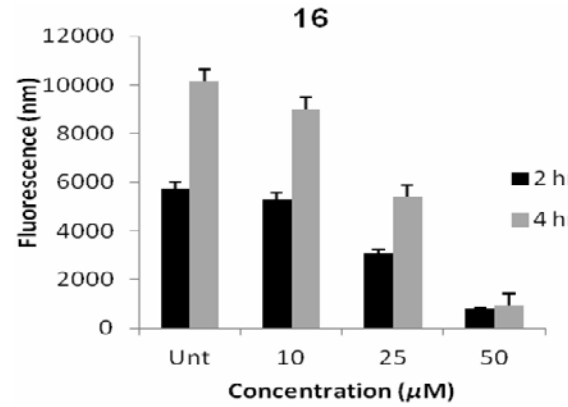


Figure-1: PC3 cell line inhibitory potential of Benzophenone-*Bis*-Schiff bases **1-17.**









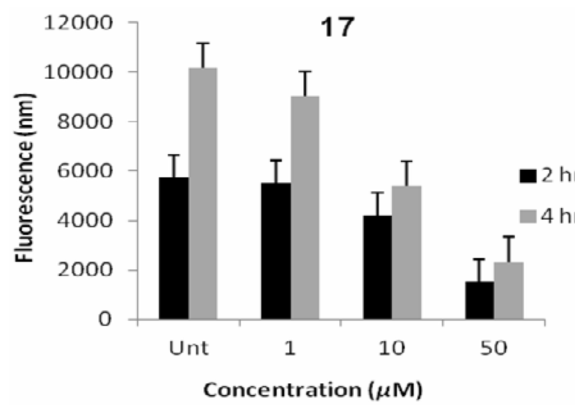


Fig. 2: PC3 cell line inhibitory potential of benzophenone-*bis*-Schiff bases **4, 8. 14, 16,** and **17.**

Conclusion

Compounds **4**, **8**, **14**, **16**, and **17** were found to be potent and identified as lead molecules for anti-cancer activities and further chemical refining of these molecules may produce potential anti-cancer agents.

Acknowledgements

The authors are thankful to the Higher Education Commission (HEC) Pakistan, for financial support to research project No. 20-2073 under National Research Program for University to Prof. Dr. Khalid Mohammed Khan.

References

1. WHO Fact sheet No. 297, Feb. (2011).
2. Cancer facts and figures, (2009) American Cancer Society.
3. M.B. Sporn, The war of cancer, *Lancet,* **347,** 1377 (1996).

4. S. Vijayakumar and S. Hellman, Advances in Irradiation Oncology, *Lancet,* **349**, S1 (1997).
5. P. Workman and S. Kaye, Translating Basic Cancer Research into New Cancer Therapeutics, *Trends Mol. Med.,* **8**, 1 (2002).
6. B. A. Chabner, T. G. Roberts, Chemotherapy and the War on Cancer, *Nat. Rev. Canc.,* **5**, 65 (2005).
7. M. Hussain, A. J. Wozniak and M. B. Edelstein, Neurotoxicity of Antineoplastic Agents, *Crit. Rev. Oncol. Hematol.,* **14**, 61 (1993).
8. P. G. Komarov, E. A. Komarova, R. V. Kondratov, K. Christov-Tselkov, J. S. Coon, M. V. Chernov and A. V. Gudkov, A Chemical Inhibitor of p53 that Protects Mice from the Side Effects of Cancer Therapy, *Science,* **285,** 1733 (1999).
9. A. T. Reddy, K. Witek, Neurologic Complications of Chemotherapy for Children with Cancer. *Curr. Neurol. Neurosci. Rep.* **3**, 137 (2003).
10. W. Rzeski, S. Pruskil, A. Macke, U. Felderhoff-Mueser, A. F. Reiher, Hoerster, C. Jansma, B. Jarosz, V. Stefovska, P. Bittigau and C. Ikonomido, Anticancer Agents are Potent Neurotoxins *in Vitro* and *in Vivo*. *Ann. Neurol.,* **56**, 351 (2004).
11. C. Pisano, G. S. Bruni, G. Facchini, C. Marchetti and S. Pignata, Treatment of Recurrent Epithelial Ovarian Cancer, *Ther Clin Risk Manag.,* **5**, 421 (2009).
12. A. L. Couffignal, M. Lapeyre-Mestre, C. Bonhomme, R. Bugat and J. L. Montastruc, Adverse Effects of Anticancer Drugs: Apropos of a Pharmacovigilance Study at a Specialized Oncology Institution, *Therapie*, **55**, 635 (2000).
13. T. Wang, S. Yang, V. A. Petrenko and V. P. Torchilin, Cytoplasmic Delivery of Liposomes into mcf-7 Breast Cancer Cells Mediated by Cell-Specific Phage Fusion Coat Protein, *Mol. Pharma.*, **7**, 1149 (2010).
14. K. Bigl, A. Schmitt, I. Meiners, G. Münch and T. Arendt, Comparison of Results of the Celltiter Blue, the Tetrazolium (3-[4,5-dimethylthioazol-2-yl]-2,5-diphenyl tetrazolium bromide), and the Lactate Dehydrogenase Assay Applied in Brain Cells After Exposure to Advanced Glycation Endproducts, *Toxicol. In Vitro*., **21**, 962 (2007).
15. P. H. Richter, I. Wunderlich, H. Schleuder, A. Keckeis, Amidinohydrazones in Drug Research. II, *Pharmazie*., **48**, 163 (1993).
16. J. R. DoAmaral, J. E. J. Blanz, F. A. French, Antimalarial Activity of Guanylhydrazone Salts of Aromatic Ketones. I. Primary Search for Active Substituent Patterns. *J. Med. Chem*., **12** 21 (1969).
17. W. J. Middleton, F. Chadds, Benzophenone Hydrazones Containing Peruoroalkyl, Peruoroalkoxy and Peruoroalkylthio Substituents. US Patent 3732307 A (1973).
18. L. G. Copping, J. C. Kerry, T. I. Watkins, R. J. Willis, B. H. Palmer, Pesticidally Active Benzophenone Hydrazone Derivatives, Methods for their Production and Pesticidal Compositions and Methods, EP: 0003913 A1 (1979).
19. D. P. Giles, J. C. Kerry, A. Kozlik, B. H. Palmer, S. W. Shutler and R. J. Willis, Substituted Benzophenone Hydrazones, Pesticidal Compositions Containing Them and Method of Combating Pests, EP: 0026040 A1 (1981).
20. L. G. Copping, J. C. Kerry, T. I. Watkins, R. J. Willis and B. H. Palmer, Pesticide Compounds, US Patent 4 344 893 (1982).
21. A. C. Hsu, H. E. Aller, R. A. Murphy, D. P. Le, Insecticidal *N′*-substituted-*N*-*N′*-disubstituted-hydrazines, US Patent, 5117057 A (1992).
22. R. W. Brockman, J. R. Thomson, M. J. Bell and H. E. Skipper, Observations on the Antileukemic Activity of pyridine-2-carboxaldehyde thiosemicarbazone and thiocarbohydrazone, *Cancer Res.,* **16**, 167 (1966).
23. S. Seitz, S. Buchholz, V. A. Schally, F. Weber, M. Klinkhammer-Schalke, E. C. Inwald, R. Perez, F. G. Rick, L. Szalontay, F. Hohla, S. Segerer, C.W. Kwok, O. Ortmann and J. B. Engel, Triple Negative Breast Cancers Express Receptors for LHRH and are Potential Therapeutic Targets for Cytotoxic LHRH-Analogs, AEZS 108 and AEZS 125, *B.M.C. Cancer*, **14**, 847 (2014).
24. K. M. Khan, F. Rahim, N. Ambreen, M. Taha, M. Khan, H. Jehan, Najeebullah, A. Sheikh, S. Iqbal, S. Perveen and M. I. Choudhary, Synthesis of Benzophenone hydrazone Schiff Bases and their *in vitro* Antiglycating Activities, *Med. Chem.,* **9**, 588 (2013).
25. A. Almasirad, R. Hosseini, H. Jalalizadeh, Z. Rahimi-Moghaddam, N. Abaeian, M. Janafrooz, M. Abbaspour, V. Ziaee, A. Dalvandi and A. Shafiee, Synthesis and Analgesic Activity of 2-phenoxybenzoic acid and *N*-phenylanthranilic Acid Hydrazides, *Biol. Pharma. Bull*., **29**, 1180 (2006)