European Journal of Chemistry 3 (3) (2012) 356-358

European Journal of Chemistry

Journal homepage: www.eurjchem.com

# Anticancer activity studies of some cyclic benzimidazole derivatives

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ARTICLE INFORMATION

Received: 23 April 2012
Received in revised form: 01 June 2012
Accepted: 17 June 2012
Online: 30 September 2012

KEYWORDS

PC3 cells
Hela cells
Cytotoxicity
Anti-cancer activity
Benzimidazole derivatives
4-Amino-2-(3,4,5-trimethoxyphenyl)benzo[4,5]imidazo[1,2-a]pyrimidine-3-carbonitrile

ABSTRACT

New benzimidazole derivatives, namely, (*N*-(4-methoxyphenyl)methylene]-1H-benzimidazol-2-amine (2a), (*N*-(3,4-dimethoxyphenyl)methylene]-1*H*-benzimidazol-2-amine (2b), and (*N*-(3,4,5-trimethoxyphenyl)methylene]-1*H*-benzimidazol-2-amine (2c) were synthesized by reaction of a Schiff base with malononitrile in absolute ethanol. Structures of compounds have been confirmed by IR, $^1$H NMR and elemental analysis. Compounds 2a-c were screened for their *in vitro* anticancer potential using HeLa and PC3 cells. All compounds showed limited cytotoxicity except compound 2a that showed a moderate cytotoxic effect towards HeLa cells.

## 1. Introduction

Cancer is the biggest health issue in the world. Cancer developed resistance against many existing anticancer drugs. This keeps a research window open in search for newer anticancer molecules. However, it is rather difficult to come up with a molecule that can selectively inhibit the proliferation of abnormal cells only with least or no effect on normal cells. Many authors worldwide have studied benzimidazole. A potential of antitumor, anti-proliferative or anticancer has been reported [1-4].

Benzimidazole nucleus has been confirmed as an important pharmacophore in drug discovery [5]. Its derivatives have been reported to exhibit antitumor [6], antibacterial [7], anti-inflammatory [8] and promising anticancer activities [9-10]. Moreover, benzimidazoles have been used as biomimetic of guanine residues [11] and they selectivity inhibit endothelial cell growth and suppress angiogenesis *in vivo* and *in vitro* [12].

Due to a broad spectrum of activities noted so far and in continuation of our research interest on the synthesis of bioactive heterocycles, we describe here the synthesis of cyclic benzimidazole derivatives and study their anticancer effect.

## 2. Experimental

### 2.1. Chemicals

2-Aminobenzimidazole, 4-methoxybenzaldehyde, 3,4,5-trimethoxybenzaldehyde, 3,4-dimethoxybenzaldehyde, piperidine and malononitrile were obtained commercially from Aldrich and Fluka Chemicals. Solvents were reagent grade and were used as received: dry absolute ethanol, petroleum ether, *n*-hexane and ethyl acetate. Progress of reactions was monitored by thin layer chromatography (TLC) using precoated aluminum sheet silica gel; Merck 60 $F_{254}$ and was visualized by UV lamp.

### 2.2. Instrumentations

Melting points were measured on an electrothermal melting point apparatus and were not corrected. Fourier-transform infrared spectra were recorded using the KBr disc technique on a JASCO 410 FTIR spectrophotometer. Elemental (CHN) analysis was performed using an Exeter CE-440 elemental analyzer. $^1$H NMR spectra of the compounds were recorded on a Varian Gemini-200 spectrometer (200 MHz) using DMSO-$d_6$ as solvent and TMS as internal reference. Anticancer activity was evaluated at the International Center for Chemical Sciences and Dr. Panjwani Center for Molecular Medicine and Drug Research, University of Karachi, Pakistan.

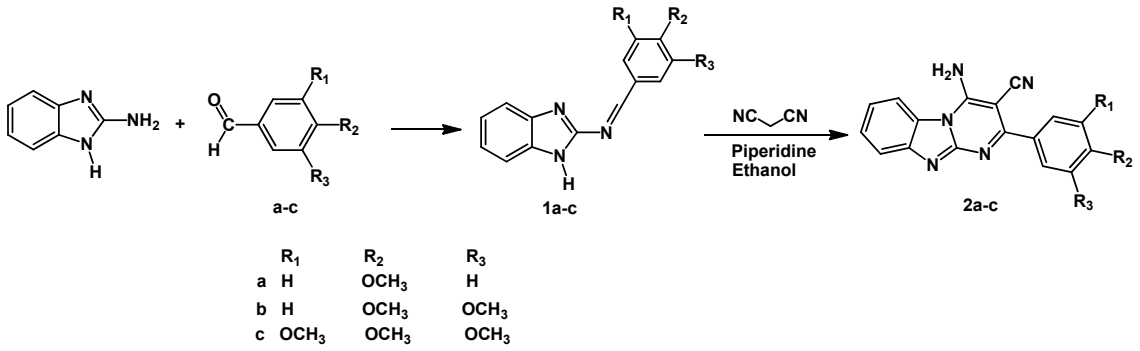
### 2.3. Synthesis of Schiff base ligands

Syntheses of Schiff base compounds **1a-c** were carried out through the method described by Nawrocka *et al.* [13] involving the condensation of an equimolar mixture of 2-aminobenzimidazole (0.01 mol) and the aldehyde as 3,4,5-trimethoxybenzaldehyde (0.01 mol), 3,4-dimethoxybenzaldehyde (0.01 mol) and 4-methoxybenzaldehyde (0.01 mol), respectively, in ethanol (25 mL). The mixture was refluxed for 24 hours under nitrogen atmosphere. Schiff base compounds formed were isolated by crystallization from a suitable solvent.

### 2.4. Synthesis of cyclic benzimidazole

Schiff base compounds (0.01 mol) were reacted with malanonitrile (0.01 mol) in ethanol with a catalytic amount of piperidine (0.2 mL). The reaction mixture was heated under reflux for 4 hrs.


ISSN 2153-2249 (Print) / ISSN 2153-2257 (Online) 
DOI:10.5155/eurjchem.3.3.356-358.621




**Scheme 1**

Then the reaction mixture was poured onto crushed ice and neutralized by hydrochloric acid to give a crude product which was filtered off, washed several times with cold water, dried and crystallized (Scheme 1).

*4-Amino-2-(4-methoxyphynyl)benzo[4,5]imidazo[1,2-a] pyrimidine-3-carbonitrile* (**2a**): Yellow crystals, recrystallized from ethanol. Yield: 35%. M.p.: 103-105 °C. FT-IR (KBr, ν, $cm^{-1}$): 3367 ($NH_2$) (amine), 2222 (CN) (cyano), 1645, 1605 (C=N) (pyrimidine and imidazole), 1571 (C=C) (aromatic). $^1H$ NMR (200 MHz, DMSO $d_6$, δ, ppm): 3.90 (s, 3H, $OCH_3$,), 6.90-8.40 (m, 8H, Ar-H, $NH_2$). Anal. calcd. for $C_{18}H_{13}N_5O$: C, 68.6; H, 4.2; N, 22.2. Found: C, 68.9; H, 3.9; N, 21.9%.

*4-Amino-2-(3,4-dimethoxyphenyl)benzo[4,5]imidazo[1,2-a] pyrimidine-3-carbonitrile* (**2b**): Dark Yellow crystals, recrystallized from ethanol. Yield: 30%. M.p.: 143-144 °C. FT-IR (KBr, ν, $cm^{-1}$): 3372 ($NH_2$) (amine), 2222 (CN) (cyano), 1642, 1600 (C=N) (pyrimidine and imidazole), 1581 (C=C) (aromatic). $^1H$ NMR (200 MHz, DMSO $d_6$, δ, ppm): 3.80 (s, 3H, $OCH_3$), 3.89 (s, 3H, $OCH_3$), 7.15-8.16 (m, 9H, Ar-H, $NH_2$). Anal. calcd. for $C_{19}H_{15}N_5O_2$: C, 66.1; H, 4.4; N, 20.3. Found: C, 62.9; H, 4.3; N, 19.8%.

*4-Amino-2-(3,4,5-trimethoxyphenyl)benzo[4,5]imidazo[1,2-a] pyrimidine-3-carbonitrile* (**2c**): Yellow crystals, recrystallized from *n*-hexane-ethyl acetate. Yield: 35%. M.p.: 117-120 °C. FT-IR (KBr, ν, $cm^{-1}$): 3397 ($NH_2$) (amine), 2224 (CN) (cyano), 1647, 1600 (C=N) (pyrimidine and imidazole), 1582 (C=C) (aromatic). $^1H$ NMR (200 MHz, DMSO- $d_6$, δ, ppm): 3.99 (s, 6H, $OCH_3$), 3.82 (s, 3H, $OCH_3$,) 6.87-8.43 (m, 8H, Ar-H, $NH_2$). Anal. calcd. for $C_{20}H_{17}N_5O_3$: C, 64.0; H, 4.6; N; 18.7. Found: C, 62.4; H, 4.5; N, 19.5%.

### *2.5. Cytotoxicity*

Cytotoxic activity of compounds was evaluated in 96-well flat-bottomed microplates by using the standard MTT (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyl-tetrazolium bromide) colorimetric assay [14]. For this purpose, PC-3 cells (Prostate Cancer) and HeLa cells were cultured in Dulbecco's Modified Eagle's Medium, and Minimal Essential Medium (MEM), supplemented with 5% of fetal bovine serum (FBS), 100 IU/mL of penicillin and 100 µg/mL of streptomycin in 25 $cm^3$ flask, and kept in 5% $CO_2$ incubator at 37 °C. Exponentially growing cells were harvested, counted with haemocytometer and diluted with a particular medium. Cell culture with the concentration of $1x10^5$ cells/mL was prepared and introduced (100 µL/well) into 96-well plates. After overnight incubation, medium was removed and 200 µL of fresh medium was added with different concentrations of compounds (1-100 µM). After 48 h, 50 µL MTT (2 mg/mL) was added to each well and incubated further for 4 hours. Subsequently, 100 µL of DMSO was added to each well. The extent of MTT reduction to formazan within cells was calculated by measuring the absorbance at 570 nm, using a micro plate reader (Spectra Max plus, Molecular Devices, Ca, USA). The cytotoxicity was recorded as concentration causing 50% growth inhibition ($IC_{50}$).

## 3. Results and discussion

### *3.1. Synthesis and characterization*

Schiff base compounds (**1a-c**) was prepared by reaction of 2-aminobenzimidazole with 4-methoxybenzaldehyde, 3,4-dimethoxybenzaldehyde and 3,4,5-trimethoxybenzaldehyde, respectively, in refluxing solvent (Scheme 1).

Schiff base compounds **1a-c** (0.01 mol) were reacted with malononitrile (0.01 mole) to give **2a-c** compounds (Scheme 1). Structures of compounds were confirmed on the basis of their elemental analysis and spectral data.

IR spectra of compounds **2a-c** showed absorption bands characteristic for $NH_2$ at 3367, 3372 and 3397 $cm^{-1}$ and CN at 2222, 2222 and 2224 $cm^{-1}$, respectively. $^1H$ NMR spectra showed multiple signals around 7.00-8.40, 7.18-8.16, and 6.80-8.40 ppm, ascribed to aromatic and $NH_2$ protons.

### *3.2. Anticancer activity*

Results of in vitro anticancer activity of the tested compounds **2a-c** were evaluated for cytotoxicity against PC3 cells and Hela cells of humans in comparison with doxorubicin as a positive control. All tested compounds showed a limited cytotoxic activity except compound **2a** that showed moderate cytotoxic effect towards Hela cells. Table 1 represents the cytotoxic activity of the tested compounds.

**Table 1.** Cytotoxic activity of compounds **2a-c** against HeLa Cells and PC3 Cells.

| Compound | PC3 | HeLa |
|---|---|---|
| | $IC_{50}$ ± SD* (µM) | $IC_{50}$ ± SD (µM) |
| 2a | >50 | 22.57 ± 0.16 |
| 2b | >50 | >50 |
| 2c | >50 | >50 |
| Doxorubicin (as control) | 0.912 ± 0.120 | 3.10 ± 0.20 |

* SD: Standard deviation.

### *Acknowledgements*

The authors would like to acknowledge the continuous support of Sana'a University and especially the Faculty of Science and the Chemistry Department. Thanks are due to The University of Science and Technology, Sana'a, as represented by the Rector, Prof. Dr. Hameed Aqlan for supporting Sama Al-Aghabari in her study for Ph.D. The authors would like also to thank Mr. Mohammed Abdelqawi Ha-el from Ha-el Saeed Corporation for financial support.

## 4. Conclusion

The present work describes the synthesis and *in vitro* anticancer evaluation of some cyclic benzimidazole derivatives **2a-c**. Most of the tested compounds showed limited cytotoxic activity except compound **2a** that showed a moderate cytotoxic effect towards Hela cells.

## References

[1]. Ramla, M. M.; Omer, M. A.; El-Khamry, A. M. M.; El-Diwani, H. I. *Bioorg. Med. Chem.* **2006**, *14*, 7324-7332.
[2]. Yang, Y. H.; Cheng, M. S.; Wang, Q. H.; Nie, H.; Liao, N.; Wang, J.; Chen, H.; Design. *Eur. J. Med. Chem.* **2009**, *44*, 1808-1812.
[3]. Penning, T. D.; Zhu, G. D.; Gandhi, V. B.; Gong Thomas, S. J.; Lubisch, W.; Gradel, R.; Wernet, W.; Park, C. H.; Fry, E. H.; Liu X.; Shi, Y.; Klinghofer, V.; Johnson, E. F.; Donawho, C. K.; Frost, D. J.; Diaz, V. B.; Bouska, J. J.; Olson, A. M.; Marsh, K. C. *Bioorg. Med. Chem.* **2008**, *16*, 6965-6975.
[4]. Garuti, L.; Roberti, M.; Pizzirani, D.; Pession A.; Leoncini, E.; Cenci, V.; Hrelia, S. *Il Farmaco* **2004**, *59*, 663-668.
[5]. Spasov, A. R.; Iezhitsa, I. N.; Bugaeva, L. I.; Anisimova, V. A. *Khim. Farm. Zh.* **1999**, *33*, 6-12.
[6]. Parshanth, A.; David, W. W. *J. Am. Chem. Soc.* **2009**, *131*, 7618-7624.
[7]. Klimesova, V.; Koci, J.; Pour, M.; Stachel, J.; Waisser, K.; Kaustova, J. *Eur. J. Med. Chem.* **2002**, *37*, 409-420.
[8]. Sondhi, S. M.; Singhal, N.; Johar, M.; Reddy, B. S.; Lown, J. W. *Curr. Med. Chem.* **2002**, *9*, 1045-1074.
[9]. Demirayak, S.; Mohsen, U. A.; Karaburun, A. C. *Eur. J. Med. Chem.* **2002**, *37*, 225-260.
[10]. Huang, S. T.; Hsei, I. J.; Chen, C. *Bioorg. Med. Chem.* **2006**, *14*, 6106-6119.
[11]. Boiani, M.; Gonzalez, M. *Mini-Rev. Med. Chem.* **2005**, *5*, 409-424.
[12]. Hori, A.; Imaeda, Y.; Kubo, K.; Kusaka, M. *Cancer lett.* **2002**, *183*, 53-62.
[13]. Nawrocka, W; Sztuba, B.; Kowalska, M. W.; Liszkiewicz, H.; Wietrzyk, J.; Nasulewicz, A.; Pelczynska, M.; Opolski, A. *Il Farmaco* **2004**, *59(2)*, 83-91.
[14]. Mosmann, T. *J. Immunol. Methods* **1983**, *65(1-2)*, 55-63.