RESEARCH ARTICLE PHARMACOLOGIA

DOI: 10.5567/pharmacologia.2013.617.622

Facile Synthesis and Evaluation of Sirtuin Inhibitory Activity of Novel Benzimidazole Derivatives

[1]Yeong Keng Yoon, [1,2,3]Mohamed Ashraf Ali, [1]Ang Chee Wei and [1]Tan Soo Choon

[1]Institute for Research in Molecular Medicine, Universiti Sains Malaysia, Minden, Penang, 11800, Malaysia

[2]Department of Medicinal Chemistry, New Drug Discovery Research, Alwar Pharmacy College, Alwar, Rajasthan, 301030, India

[3]Department of Medicinal Chemistry, New Drug Discovery Research, Sunrise University, Alwar, Rajasthan, 301030, India

ABSTRACT

Background: Sirtuins are a family of NAD^+-dependent deacetylases and/or ADP-ribosyl transferases that modify a broad range of protein substrates. Inhibitors of sirtuins have also been touted as potential anti-cancer agents. This study was done in view to develop potent sirtuin inhibitors. **Materials and Methods:** A series of novel polar benzimidazoles were synthesized in a 4-step reaction starting from basic compound 4-fluoro-3-nitrobenzoic acid. They were subsequently evaluated for their Sirtuin 1 (SIRT1) and Sirtuin 2 (SIRT2) inhibitory activity using fluorimetric drug discovery kits. Preliminary cytotoxicity test was also carried out. **Results:** Two of the novel benzimidazoles synthesized showed good SIRT1 and SIRT2 inhibitory activity. Compound 5 g was found to be the most active with IC_{50} of 18.62 and 45.01 μM against SIRT1 and SIRT2, respectively. All the compounds were found to be non-toxic up to 50 μM during cytotoxicity test with VERO cells. **Conclusion:** The findings of this study would prove valuable in the quest to develop potent sirtuin inhibitors. Compounds with potent sirtuin inhibitory activities such as 5 g are prime candidates for modifications to further improve their activities.

Key words: Sirtuin, anti-cancer, benzimidazole, piperazine, ammonium formate reduction

Pharmacologia 4 (12): 617-622, 2013

INTRODUCTION

Sirtuins are a family of NAD^+-dependent deacetylases and/or ADP-ribosyl transferases that modify a broad range of protein substrates (Sebastian *et al*., 2012). The mammalian sirtuin family consists of seven family members (SIRT1-7) (Frye, 2000). Of the seven human sirtuin isoforms that have been discovered, SIRT1 and SIRT2 are the most studied because they have been implicated in influencing many cellular processes including aging and metabolic disorders (Blum *et al*., 2011; Longo and Kennedy, 2006; Pillarisetti, 2008). They are also believed to be involved in diseases such as cancer (Brooks and Gu, 2008). Inhibition of situins by small molecules has recently been proposed as a promising anti-cancer strategy (Heltweg *et al*., 2006).

Recently, a series of indole compounds has been discovered as interesting inhibitors of SIRT1, including one of the most potent compounds known so far, EX-527 (Napper *et al*., 2005). Since indole and benzimidazole share some structure similarities, we embark to synthesize and evaluate the potential of utilizing benzimidazoles as sirtuin inhibitors. The pharmacokinetics of benzimidazoles were also well studied, therefore they are a good starting point in developing new drugs. Here we would like to report the synthesis, sirtuin inhibitory activities as well as their limited structure-activity-relationship.

MATERIALS AND METHODS

All chemicals were supplied by Sigma-Aldrich (U.S.A) and Merck Chemicals (Germany). Purity of the compounds was checked on Thin Layer Chromatography (TLC) plates (silica gel G) in the solvent system chloroform-methanol (9:1). The spots were located under short (254 nm)/long (365 nm) UV light. Elemental analyses were performed on Perkin Elmer 2400 Series II CHN Elemental Analyzer and were within±0.4% of the calculated values. ^1H and ^{13}C NMR were performed on Bruker Avance 300 (^1H: 300 MHz, ^{13}C: 75 MHz) spectrometer in $CDCl_3$

Corresponding Author: Yeong Keng Yoon, Institute for Research in Molecular Medicine, Universiti Sains Malaysia, Minden, Penang, 11800, Malaysia Tel: +604 6593140 Fax: +604 6569796

using TMS as internal standard. Direct-infusion mass spectra were recorded on Varian 320-MS TQ LC/MS using ESI mode.

Preparation of Ethyl-4-fluoro-3-nitrobenzoate (1): 4-Fluoro-3-nitrobenzoic acid (5 g, 27 mmol) was refluxed in ethanol (50 mL) and concentrated H_2SO_4 (2 mL) for 8 h. After completion of reaction (as evident from TLC), the solvent was evaporated under reduced pressure. The aqueous layer was extracted with ethyl acetate (25 mL×3). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to yield 1 as cream-coloured powder (75%).

Preparation of Ethyl 4-(2-(piperazin-1-yl)ethylamino)-3-nitrobenzoate (2): N-(2-aminoethyl)piperazine (1.30 mL, 9.90 mmol) and N,N-Diisopropylethylamine, DIPEA (0.49 mL, 2.78 mmol) were mixed in dichloromethane (10 mL). Ethyl-4-fluoro-3-nitrobenzoate, 1 (0.5 g, 2.34 mmol) was added very slowly over 5 min. The reaction mixture was stirred overnight at room temperature. The reaction mixture was then washed with water (10 mL×2) followed by 10% Na_2CO_3 solution (10 mL). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to afford 2 as yellow solid (92%).

Preparation of Ethyl 4-(2-(piperazin-1-yl) ethylamino)-3-aminobenzoate (3): N-(3-aminopropyl)imidazole, 2 (0.322 g, 1 mmol), ammonium formate (0.189 g, 3 mmol) and Pd/C (50 mg) were mixed in ethanol (10 mL). The reaction mixture was refluxed until completion (solution turned colourless). The reaction mixture was then filtered through Celite 545. The filtrate was evaporated under reduced pressure. It was re-suspended in ethyl acetate and washed with water, dried over Na_2SO_4 and evaporated to dryness to yield 3 (85%) which was used without further purification.

General procedure for the preparation of sodium bisulfite addcuts of 4-substituted benzaldehyde (4a-g): Appropriate benzaldehyde (10 mmol) was dissolved in ethanol (20 mL). Sodium metabisulfite (15 mmol) in 5 mL water was added in portion over 5 min. The reaction mixture was stirred at room temparature for 1 h and subsequently stirred at 4°C overnight. The precipitate formed was filtered and dried to afford sodium bisulfite adducts (96%).

General procedure for the preparation of 2-substituted benzimidazole derivatives (5a-g): Ethyl 4-(2-(piperazin-1-yl)ethylamino)-3-aminobenzoate, 3 (1 mmol) and various sodium bisulfite adducts, 4a-g (1.5 mmol) were dissolved in DMF (5 mL). The reaction mixture was stirred at 90°C under N_2 atmosphere for 24-48 h. After completion of reaction (evident by TLC), the reaction mixture was diluted in ethyl acetate (25 mL) and washed with water (10 mL×3). The organic layer was collected, dried over Na_2SO_4 and evaporated under reduced pressure to afford compounds 5a-g in 75-89% yields.

Ethyl 2-phenyl-1-(2-(piperazin-1-yl)ethyl)-1H-benzo[d]imidazole-5-carboxylate (5a): This compound was obtained as yellow oil. Yield: 87%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ_H = 1.43 (t, 3H, J = 7.2 Hz); 2.24 (t, 4H, J = 4.8 aHz); 2.78 (t, 2H, J = 6.9 Hz); 3.11 (t, 4H, J = 4.8 Hz); 3.50 (t, 2H, J = 6.9 Hz); 4.35 (q, 2H, J = 7.2 Hz); 7.20-7.80 (m, 6H); 8.05 (dd, 1H, J_1 = 8.4 Hz, J_2 = 1.5 Hz); 8.55 (s, 1H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ_C = 14.38, 42.79, 53.90, 55.49, 60.93, 62.01, 109.72, 122.44, 124.57, 124.73, 125.36, 129.15, 131.00, 132.08, 138.77, 142.73, 154.62, 167.00 ppm. LC-MS ESI-MS: m/z 380.3 $[M+H]^+$. Anal. Calc for $C_{22}H_{25}N_3O_3$: C, 69.82%; H, 6.92%; N, 14.80%. Found : C, 69.62%; H, 6.97%; N, 14.95%.

Ethyl 2-(4-hydroxyphenyl)-1-(2-(piperazin-1-yl)ethyl)-1H-benzo[d]imidazole-5-carboxylate (5b): This compound was obtained as light brown powder. Yield: 89%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ_H = 1.44 (t, 3H, J = 7.2 Hz); 2.21 (t, 4H, J = 4.8 Hz); 2.76 (t, 2H, J = 6.9 Hz); 3.10 (t, 4H, J = 4.8 Hz); 3.48 (t, 2H, J = 6.9 Hz); 4.34 (q, 2H, J = 7.2 Hz); 6.85 (d, 2H, J = 8.4 Hz); 7.39 (d, 2H, J = 8.4 Hz); 7.87 (d, 1H, J = 8.4 Hz); 8.01 (dd, IH, J_1 = 8.4 Hz, J_2 = 1.5 Hz); 8.54 (s, 1H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ_C = 14.38, 24.54, 42.79, 51.76, 53.85, 57.50, 61.12, 109.97, 122.49, 125.57, 125.82, 127.35, 127.76, 129.63, 130.03, 138.48, 142.50, 154.19, 167.05 ppm. LC-MS ESI-MS: m/z 396.3 $[M+H]^+$. Anal. Calc for $C_{22}H_{25}N_3O_3$: C, 66.99%; H, 6.64%; N, 14.20%. Found : C, 66.75%; H, 6.84%; N, 14.33%.

Ethyl 1-(2-(piperazin-1-yl)ethyl)-2-p-tolyl-1H-benzo[d]imidazole-5-carboxylate (5c): This compound was obtained as brown oil. Yield: 77%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ_H = 1.44 (t, 3H, J = 7.2 Hz); 2.23 (t, 4H, J = 4.8 Hz); 2.45 (s, 3H); 2.77 (t, 2H, J = 6.9 Hz); 3.11 (t, 4H, J = 4.8 Hz); 3.49 (t, 2H, J = 6.9 Hz); 4.36 (q, 2H, J = 7.2 Hz); 6.87 (d, 2H, J = 8.4 Hz); 7.39 (d, 2H, J = 8.4 Hz); 7.88 (d, 1H, J = 8.4 Hz); 8.01 (dd, 1H, J_1 = 8.4 Hz, J_2 = 1.5 Hz); 8.55 (s, 1H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ_C = 14.38, 42.80, 51.75, 53.90, 57.54, 61.12, 109.97, 116.49, 118.73, 122.49, 124.30, 126.41, 128.50, 129.63, 130.06, 138.49, 142.48, 154.17, 159.07, 167.00 ppm. LC-MS ESI-MS: m/z 394.3

$[M+H]^+$. Anal. Calc for $C_{22}H_{25}N_3O_3$: C, 70.38%; H, 7.19%; N, 14.27%. Found : C, 70.08%; H, 7.40%; N, 14.26%.

Ethyl 2-(4-methoxyphenyl)-1-(2-(piperazin-1-yl)ethyl)-1H-benzo[d]imidazole-5-carboxylate (5d): This compound was obtained as light brown powder. Yield: 86%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ_H = 1.43 (t, 3H, J = 7.2 Hz); 2.21 (t, 4H, J = 4.8 Hz); 2.75 (t, 2H, J = 6.9 Hz); 3.09 (t, 4H, J = 4.8 Hz); 3.48 (t, 2H, J = 6.9 Hz); 3.87 (s, 3H); 4.36 (q, 2H, J = 7.2 Hz); 6.86 (d, 2H, J = 8.4 Hz); 7.37 (d, 2H, J = 8.4 Hz); 7.80 (d, 1H, J = 8.4 Hz); 8.00 (dd, 1H, J_1 = 8.4 Hz, J_2 = 1.5 Hz); 8.53 (s, 1H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ_C = 14.39, 42.81, 51.75, 53.90, 56.19, 57.54, 61.12, 110.04, 116.52, 118.73, 122.49, 124.30, 126.41, 128.50, 129.65, 130.06, 138.49, 142.48, 154.16, 159.33, 167.02 ppm. LC-MS ESI-MS: m/z 410.3 $[M+H]^+$. Anal. Calc for $C_{22}H_{25}N_3O_3$: C, 67.63%; H, 6.91%; N, 13.72%. Found : C, 67.50%; H, 7.02%; N, 13.86%.

Ethyl 2-(4-(trifluoromethyl)phenyl)-1-(2-(piperazin-1-yl)ethyl)-1H-benzo[d]imidazole-5-carboxylate (5e): This compound was obtained as yellow oil. Yield: 85%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ_H = 1.43 (t, 3H, J = 7.2 Hz); 2.31 (t, 4H, J = 4.8 Hz); 2.73 (t, 2H, J = 6.9 Hz); 2.95 (t, 4H, J = 4.8 Hz); 4.40 (q, 2H, J = 7.2 Hz); 4.41 (t, 2H, J = 6.9 Hz); 7.48 (d, 1H, J = 8.4 Hz); 7.82 (d, 2H, J = 8.4 Hz), 7.97 (d, 2H, J = 8.4 Hz), 8.09 (dd, 1H, J_1 = 8.4 Hz, J_2 = 1.5 Hz); 8.55 (s, 1H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): ä$_C$ = 14.39, 42.76, 51.75, 53.87, 57.48, 61.10, 109.97, 122.49, 124.91, 125.57, 125.82, 125.85, 125.88, 129.73, 130.00, 138.68, 142.50, 154.16, 167.03 ppm. LC-MS ESI-MS: m/z 448.2 $[M+H]^+$. Anal. Calc for $C_{23}H_{25}N_4O_2F_3$: C, 61.87%; H, 5.64%; N, 12.55%. Found : C, 61.75%; H, 5.38%; N, 12.79%.

Ethyl 2-(4-nitrophenyl)-1-(2-(piperazin-1-yl)ethyl)-1H-benzo[d]imidazole-5-carboxylate (5f): This compound was obtained as orange solid. Yield: 88%. ^{1}H NMR ($CDCl_3$, 300 MHz): ä$_H$ = 1.45 (t, 3H, J = 7.2 Hz); 2.33 (t, 4H, J = 4.8 Hz); 2.75 (t, 2H, J = 6.9 Hz); 2.95 (t, 4H, J = 4.8 Hz); 4.25 (t, 2H, J = 6.9 Hz); 4.40 (q, 2H, J = 7.2 Hz); 7.47 (d, 1H, J = 8.4 Hz); 7.80 (d, 2H, J = 8.4 Hz), 7.88 (d, 2H, J = 8.4 Hz), 8.08 (dd, 1H, J_1 = 8.4 Hz, J_2 = 1.5 Hz); 8.54 (s, 1H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): ä$_C$ = 14.42, 42.76, 51.75, 53.87, 57.48, 61.10, 109.97, 122.49, 125.36, 125.88, 125.87, 125.80, 129.73, 130.08, 138.59, 142.50, 149.70, 154.50, 167.00 ppm. LC-MS ESI-MS: m/z 424.2 $[M+H]^+$. Anal. Calc for $C_{22}H_{25}N_5O_4$: C, 62.40%; H, 5.95%; N, 16.54%. Found : C, 62.51%; H, 5.90%; N, 16.45%.

Ethyl 2-(4-(dimethylamino)phenyl)-1-(2-(piperazin-1-yl)ethyl)-1H-benzo[d]imidazole-5-carboxylate (5g): This compound was obtained as brown oil. Yield: 75%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ_H = 1.43 (t, 3H, J = 7.2 Hz); 2.20 (t, 4H, J = 4.8 Hz); 2.75 (t, 2H, J = 6.9 Hz); 2.84 (s, 6H); 3.10 (t, 4H, J = 4.8 Hz); 3.48 (t, 2H, J = 6.9 Hz); 4.34 (q, 2H, J = 7.2 Hz); 6.85 (d, 2H, J = 8.4 Hz); 7.39 (d, 2H, J = 8.4 Hz); 7.87 (d, 1H, J = 8.4 Hz); 8.04 (dd, IH, J_1 = 8.4 Hz, J_2 = 1.5 Hz); 8.55 (s, 1H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ_C = 14.30, 39.40, 42.79, 51.72, 53.85, 57.50, 61.11, 109.95, 122.46, 125.57, 125.82, 127.35, 127.76, 128.59, 129.33, 138.48, 149.45, 154.02, 167.01 ppm. LC-MS ESI-MS: m/z 422.3 $[M+H]^+$. Anal. Calc for $C_{24}H_{31}N_5O_2$: C, 68.38%; H, 7.41%; N, 16.61%. Found: C, 68.26%; H, 7.49%; N, 16.72%.

RESULTS AND DISCUSSION

Chemistry: The mechanism for the formation of the novel benzimidazole derivatives is proposed and summarized as in Fig. 1. The synthetic study into polar benzimidazoles started with 4-fluoro-3-nitro benzoic acid which was esterified in the presence of catalytic sulfuric acid in ethanol by refluxing for 8 hours to afford the ethyl-4-fluoro-3-nitrobenzoate 1 in 75% yield. The ethylbenzoate 1 was then treated with N-(3-aminopropyl) piperazine and DIPEA in dry dichloromethane at room temperature to yield ethyl 4-(2-(piperazin-1-yl)ethylamino)-3-nitrobenzoate 2. Compound 2 was reduced to ethyl 4-(2-(piperazin-1-yl)ethylamino-3-aminobenzoate 3 using ammonium formate and 10% Pd/C for 1 hour to give 85% yield. Reduction reaction using sodium borohydride was also tested. However, comparatively, the yield obtained from $NaBH_4$ was far lower (57%). This proved that palladium-catalysed transfer hydrogenation is an excellent method in reducing nitrobenzene to aminobenzene. This method is convenient, economical and uses a stable nonpyrophobic catalyst. The phenylenediamine 3 was then refluxed with various substituted bisulfite adduct of aromatic aldehydes 4a-g in DMF overnight to afford benzimidazole derivatives 5a-g in good to excellent yields. Among the literature reports available for the synthesis of benzimidazoles by the reaction of phenylenediamine with acid chloride (Zeller *et al*., 2010), aldehyde (Gill *et al*., 2008) and acid (Thimmegowda *et al*., 2008), access into benzimidazole derivatives *via* this metabisulfite route was found to be efficient, environmental friendly and afforded good yield of the benzimidazoles.

Sirtuin inhibition assay: Initial screening of similar but less polar benzimidazoles found poor solubility in

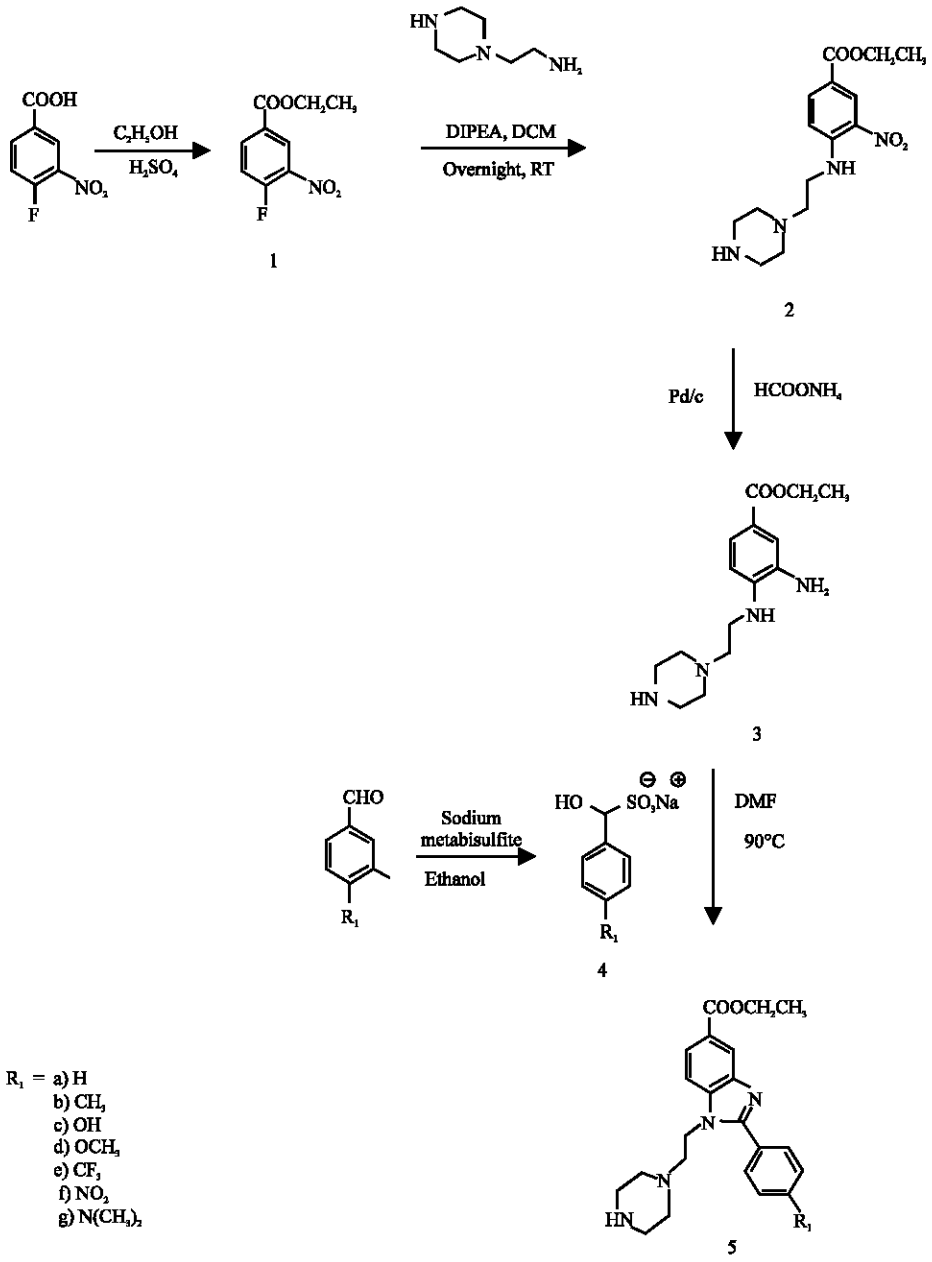

Fig. 1: Synthesis of novel benzimidazole derivatives 5a-g from 4-fluoro-3-nitrobenzoic acid

biological assay. However, the problem could be overcome by introducing polar group to improve on the solubility of the compounds. Polar benzimidazoles are more water soluble and potentially less toxic to cells. To increase the water solubility, ethyl piperidine group was incorporated at position-2 of the benzimidazole core.

The *in vitro* enzymatic screening assay for SIRT1 and SIRT2 inhibitory activity were performed using fluorimetric drug discovery kits (AnaSpec, Fremont, CA) according to the manufacturer's protocol. Sirtinol was used as standard control for both the SIRT1 and SIRT2 assays while DMSO was used as a vehicle control.

Table 1: Sirtuin inhibitory activity (IC_{50}) values of synthesized compound 5a-g

	IC_{50} (μM)	
Compound	Sirt1	Sirt2
5a	>100	>100
5b	>100	>100
5c	68.29	40.50
5d	>100	>100
5e	>100	>100
5f	>100	>100
5g	45.01	18.62
Sirtinol	39.46	90.65

A series of 7 novel compounds bearing the piperazinyl ethylbenzimidazole core were synthesized. Compounds with different substitution comprising electron-donating as well as electron-withdrawing groups at position-4 in the phenyl ring were synthesized. Among the tested compounds, it was noted that compounds with electron donating groups at R_1 gave rise to better sirtuin inhibitory activities. The most potent SIRT1 inhibitor was found to be 5 g (SIRT1 IC_{50} = 18.62 μM, SIRT2 IC_{50} = 45.01 μM). SIRT2 inhibitory activity for compound 5b and 5 g were found to be more potent than Sirtinol. As for SIRT1, both compound 5b and 5g gave comparable potency as Sirtinol as shown in Table 1.

The potent sirtuin inhibitory activity of compound 5g could be well due to the dimethylamino substituent group capable of hydrogen bonding intermolecularly to the enzymes. The strong electron donating effect of the substituent could also potentially give rise to increase potency since the imidazole ring of relatively similar compounds have been known to have interactions with the enzymes (Yoon *et al*., 2013).

Cytotoxicity: The tolerable toxicity of the compounds 5a-g was confirmed by the cytotoxicity test (IC_{50}) in VERO cells at concentrations up to 50 μM. After 72 h of exposure, viability was assessed on the basis of cellular conversion of MTS into a formazan product using the Promega Cell Titer 96 Non-radioactive Cell proliferation method according to manufacturer's protocol. All the compounds were found to be non-toxic up to 50 μM.

CONCLUSION

In summary, a series of novel polar benzimidazoles was successfully synthesized under mild reaction condition in good yield. Piperazinyl ethylbenzimidazole derivatives were derived from ethyl 4-(2-(piperazin-1-yl) ethylamino-3-aminobenzoate with various substituted bisulfite adducts of benzaldehyde under reflux conditions. The synthesizednovel polar benzimidazoles have potential biological applications especially as anti-cancer agents in view of their good sirtuin inhibitory activities. Compounds with potent sirtuin inhibitory activities such as 5 g are prime candidates for modifications to further improve their activities.

ACKNOWLEDGMENTS

The authors wish to express their gratitude and appreciation to Pharmacogenetics and Novel Therapeutics Research Cluster, Institute for Research in Molecular Medicine, Universiti Sains Malaysia, Penang for supporting this study. This study was funded through Research Grant No. RUC 1001/PSK/8620012 and HiCoE research Grant No. 311. CIPPM.4401005.

REFERENCES

Blum, C.A., J.L. Ellis, C. Loh, P.Y. Ng, R.B. Perni and R.L. Stein, 2011. SIRT1 modulation as a novel approach to the treatment of disease of aging. J. Med. Chem., 54: 417-432.

Brooks, C.L. and W. Gu, 2008. p53 activation: A case against Sir. Cancer Cell., 13: 377-378.

Frye, R.A., 2000. Phylogenetic classification of prokaryotic and Eukaryotic Sir2-like proteins. Biochem. Biophys. Res. Commun., 273: 793-798.

Gill, C., G. Jadhav, M. Shaikh, R. Kale and A. Ghawalkar *et al*., 2008. Clubbed [1,2,3] Triazoles by Fluorine Benzimidazole: A Novel Approach to H37Rv Inhibitors As A Potential Treatment for Tuberculosis. Bioorg. Med. Chem. Lett., 18: 6244-6247.

Heltweg, B., T. Gatbonton, A.D. Schuler, J. Posakony and H. Li *et al*., 2006. Antitumor activity of a small-molecule inhibitor of human silent information regulator 2 enzymes. Cancer Res., 66: 4368-4377.

Longo, V.D. and B.K. Kennedy, 2006. Sirtuins in aging and age-related disease. Cell, 126: 257-268.

Napper, A.D., J. Hixon, T. McDonagh, K. Keavey and J.F. Pons *et al*., 2005. Discovery of indoles as potent and selective inhibitors of the deacetylase SIRT1. J. Med. Chem., 48: 8045-8054.

Pillarisetti, S., 2008. A review of Sirt1 and Sirt1 modulators in cardiovascular and metabolic diseases. Recent Patents Cardiovascular Drug Discovery, 3: 156-164.

Sebastian, C., F.K. Satterstrom, M.C. Haigis and R. Mostoslavsky, 2012. From Sirtuin biology to human disease: An update. J. Biol. Chem., 287: 42444-42452.

Thimmegowda, N.R., S. Nanjunda Swamy, C.S. Ananda Kumar, Y.C. Sunil Kumar, S. Chandrappa, G.W. Yipb and K.S. Rangappaa, 2008. Synthesis, characterization and evaluation of benzimidazole derivative and its precursors as inhibitors of MDA-MB-231 human breast cancer cell proliferation. Bioorg. Med. Chem. Lett., 18: 432-435.

Yoon, Y.K., M.A. Ali, A.C. Wei, T.S. Choon, H. Osman and K. Parang, 2013. Synthesis and evaluation of novel benzimidazole derivatives as sirtuin inhibitors with antitumor activities. Bioorg. Med. Chem. (In Press). 10.1016/j.bmc.2013.12.029

Zeller, W., A.S. Kiselyov and J. Singh, 2010. Regiospecific synthesis of 3-alkyl-4-hydroxybenzimidazoles as intermediates for an expedient approach to potent EP3 receptor antagonists. Tetrahedron Lett., 51: 1380-1382.