Accepted Manuscript

Benzimidazoles as new scaffold of sirtuin inhibitors: green synthesis, *in vitro* studies, molecular docking analysis and evaluation of their anti-cancer properties

Yeong Keng Yoon, Mohamed Ashraf Ali, Ang Chee Wei, Amir Nasrolahi Shirazi, Keykavous Parang, Tan Soo Choon

PII: S0223-5234(14)00589-3

DOI: 10.1016/j.ejmech.2014.06.060

Reference: EJMECH 7103

To appear in: *European Journal of Medicinal Chemistry*

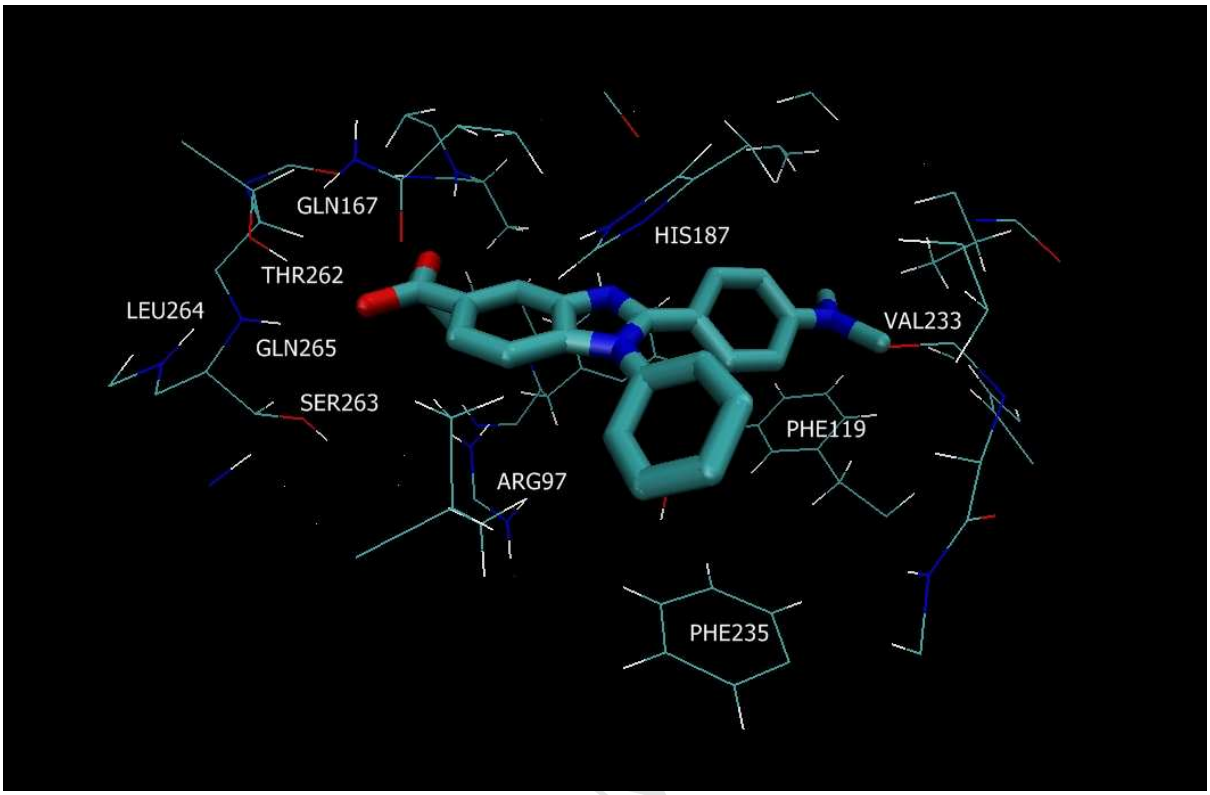
Received Date: 14 March 2014

Revised Date: 23 June 2014

Accepted Date: 26 June 2014

Please cite this article as: Y.K. Yoon, M.A. Ali, A.C. Wei, A.N. Shirazi, K. Parang, T.S. Choon, Benzimidazoles as new scaffold of sirtuin inhibitors: green synthesis, *in vitro* studies, molecular docking analysis and evaluation of their anti-cancer properties, *European Journal of Medicinal Chemistry* (2014), doi: 10.1016/j.ejmech.2014.06.060.

GRAPHICAL ABSTRACT



The most active compound (**4j**) showed potent SIRT1 and SIRT2 inhibition with IC_{50} of 54.21 µM and 26.85 µM respectively. It possessed good anti-proliferative activity against three cancer cell lines tested.

Benzimidazoles as new scaffold of sirtuin inhibitors: green synthesis, *in vitro* studies, molecular docking analysis and evaluation of their anti-cancer properties

Yeong Keng Yoon[a*], Mohamed Ashraf Ali[a,b,c], Ang Chee Wei[a], Amir Nasrolahi Shirazi[d], Keykavous Parang[d], Tan Soo Choon[a]

[a]*Institute for Research in Molecular Medicine, Universiti Sains Malaysia, Minden 11800, Penang, Malaysia.*

[b]*New Drug Discovery Research, Department of Medicinal Chemistry, Alwar Pharmacy College, Alwar, Rajasthan-301030, India.*

[c]*New Drug Discovery Research, Department of Medicinal Chemistry, Sunrise University, Alwar, Rajasthan-301030, India.*

[d]*Department of Biomedical and Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island, Kingston, RI, 02881, United States.*

ABSTRACT

Two series of novel benzimidazole derivatives were designed, synthesized and evaluated for their SIRT1 and SIRT2 inhibitory activity. Among the newly synthesized compounds, compound **4j** displayed the best inhibitory activity for SIRT1 (IC_{50} = 54.21 µM) as well as for SIRT2 (IC_{50} = 26.85 µM). Cell proliferation assay showed that compound **4j** possessed good antitumor activity against three different types of cancer cells derived from colon (HCT-116), breast (MDA-MB-468) and blood-leukemia (CCRF-CEM) with cell viability of 40.0%, 53.2% and 27.2% respectively at 50 µM. Docking analysis of representative compound **4j** into SIRT2 indicated that the interaction with receptor was primarily due to hydrogen bonding and π-π stacking interactions.

Keywords: Sirtuin, Benzimidazole, Anti-proliferative, Green chemistry synthesis

* Corresponding author:

Yeong Keng Yoon

Institute for Research in Molecular Medicine, Universiti Sains Malaysia, Minden, 11800, Penang, Malaysia.

Phone: +604 6593140

Fax: +604 6569796

Email: kyyeong@gmail.com

1.Introduction

Sirtuins are a family of NAD^+ -dependent deacetylases and/or ADP-ribosyl transferases that modify a broad range of protein substrates [1-3]. The mammalian sirtuin family consists of seven family members (SIRT1-7), characterized by a conserved 275 amino acid catalytic core and unique additional N-terminal and/or C-terminal sequences of variable length [4]. Of the seven human sirtuin isoforms that have been discovered, SIRT1 [5] and SIRT2 [6] are the most studied. Between them, SIRT1 & SIRT2 have been shown to deacetylate more than 35 different substrates such as H4K16, p53, forkhead box class O (FOXO), nuclear factor-κb (NK-κB), and many others [7-9]. Since SIRT1 & SIRT2 are involved in so many downstream enzyme activities, it is not surprising that both SIRT1 and SIRT2 have been implicated with numerous disorders such as cancer [10,11] and neurological diseases [12,13]. Therefore, potent SIRT1 and SIRT2 modulators could be used as valuable tools to gain insight into the specific cellular functions of their effector proteins.

In view of the importance of these enzymes, sirtuin biology has been advancing at a tremendous pace in recent times. Many new discoveries were made at the genetic level for studying these important enzymes [14-16]. Several classes of small molecule sirtuin inhibitors have been identified so far including sirtinol [17], splitomycin analogs [18] and tenovins [19]. Recently, a large high-throughput screening effort led to the discovery of a potent indole-based SIRT1 inhibitor (EX-527) [20]. Since many benzimidazole-containing compounds exhibit important biological properties [21,22], and the fact that indole and benzimidazole share some structure similarities the potential of benzimidazole and its derivatives exhibiting sirtuin inhibitory activities is an interesting proposition.

As benzimidazole is a very important class of heterocyclic compounds in the area of drug design [23], the synthesis of benzimidazoles has received considerable attention in the past few decades [24]. However, the diversified synthesis of regiodefined 1,2-disubstituted

benzimidazoles remains complicated. The commonly used approach of condensation between 4-substituted-phenylenediamine and aldehyde often leads to mixture of 1,2-disubstituted, 2-substituted benzimidazoles and bis dihydrobenzimidazoles [25].

Herein, we would like to report a new green method where the synthetic regiodefined ethyl-(1,2-disubstituted)-5-carboxylate reaction of benzimidazole is totally carried out at ambient temperature utilizing water/ethanol as solvent. Sirtuin inhibitory activities of the newly synthesized benzimidazoles were reported. Docking studies for **4b** and **4j** (the most potent compound for 3-aminopropyl-2-pyrrolidinone and phenyl substituted series respectively) with SIRT2 were also performed. In addition, anti proliferation activity of the benzimidazole derivatives against three different tumor cell lines belonging to colon (HCT-116), breast (MDA-MB-468) and leukemia (CCRF-CEM) were also reported.

2. Results and Discussion

2.1 Chemistry

The synthetic scheme is a four-step pathway leading to the formation of a variety of benzimidazole derivatives (**Scheme 1**). This methodology was applied to synthesize both series of ethyl-(1,2-disubstituted)-5-carboxylate benzimidazole derivatives.

The electronic effects of the different substituted aldehydes have also been investigated. It has been found that both electron withdrawing groups (-nitro, -trifluoromethyl) as well as electron donating groups (-methyl, -hydroxy, -dimethylamino) do not affect the efficiency of the transformation. Halogen substituents are also well tolerated. This showed the versatility of the protocol over a broad range of substituents from weak to strong electron withdrawing/donating groups. Excellent yields for the synthesized ethyl-(1,2-disubstituted)-

5-carboxylate benzimidazoles were obtained and products were easily isolated as they tend to precipitate out in water and require minimum purification.

2.2 Enzymatic Assays

The *in vitro* enzymatic screening assay for SIRT1 and SIRT2 inhibitory activity were performed using Sensolyte® fluorimetric drug discovery kits (AnaSpec, Fremont, CA) according to the manufacturer's protocol. Cambinol and Tenovin-6, two of the few sirtuin inhibitors which showed antitumor activities in mouse xenograft models, were used as standard control for both the SIRT1 and SIRT2 assays while DMSO was used as a vehicle control. IC_{50} values were determined for all compounds which showed over 50% inhibition for either SIRT1 or SIRT2 at 50 µM. Preliminary structure-activity relationship (SAR) was established when *in vitro* screening on the compounds showed that compounds with strong electron donating group (dimethylamino) at $\mathbf{R^2}$ possessed the best sirtuin inhibitory activities. The importance of strong electron donating group is highlighted as it is instrumental in providing good sirtuin inhibition. Removal of the dimethylamino group led to a drastic drop in sirtuin inhibitory activities. Generally, the novel benzimidazole derivatives showed better inhibition on SIRT2 as compared to SIRT1 (**Table 1**)**.** Experiments were performed in triplicates. Standard deviation obtained for all experiments are less than 20%.

The most potent inhibitor for SIRT1 as well as SIRT2 was found to be **4j** (SIRT1 IC_{50} = 54.21 µM; SIRT2 IC_{50} = 26.85 µM). Compound **4j** showed better SIRT2 inhibitory activity compared to the standard controls used (cambinol and Tenovin-6). It is also equipotent compared with cambinol and Tenovin-6 in terms of SIRT1 inhibitory activities.

2.3 Molecular Docking Analysis

In an attempt to predict the binding mode of this novel chemical series, docking study of representative compound **4b** (the most potent for 3-aminopropyl-2-pyrrolidinone substituted series) and **4j** (the most potent for phenyl substituted series) into the active site of human SIRT2 (PDB entry code: 3ZGV, x-ray resolution = 2.30 Å) was performed [26]. The receptor and the drug candidate were optimized before actual docking in Autodock 4.2 using standard procedure of the software.

Analysis of the top-ranked pose of compound **4j** docked within the SIRT2-ADPr cofactor binding site demonstrated several plausible molecular interactions between **4j** and the receptor. The docking analysis reveals that the compound **4j** interact with receptor primarily due to hydrogen bonding as well as π-π stacking interactions. The N-H group of SER263 is hydrogen bonded strongly to the oxygen from the ester chain of compound **4j** .Other hydrogen bonds which could be observed include interactions with Thr262, Leu264, Gln265, Arg97, and Gln167 (**Figure 1**). This is relatively consistent with the hydrogen bonds observed between SIRT2 and ADPr complex [26]. Other SIRT2-inhibitor predictions including salermide and NF-675 also reported similar bonding results [27].

The benzene ring on the position-2 of the benzimidazole core was stabilized through π-π stacking interactions between the imidazole group of His187 and benzene ring of Phe235. The importance of the diaminobenzene substituent was highlighted through its interactions with Val233 and Phe119. Lone pair oxygen-π interactions could also be observed through Val233 and the phenyl ring of dimethylaminobenzene substituent as well as the N from the dimethylamino group with benzene ring from Phe119.

As for compound **4b,** the mode of interaction with receptor was deemed to be different as compared to compound **4j**. The ketone oxygen from the pyrrolidinone moeity of compound **4b** was hydrogen bonded strongly to Lys287. Another strong hydrogen bond observed was

between the oxygen from the ethyl ester group of compound **4b** with Ala85. Other hydrogen bond interactions which could be observe included interaction of **4b** with Asn286, Ser98, Arg97, Ser263 and Gln167. However, as shown in **Figure 2**, the benzene ring at position-2 was situated out from the receptor cavity. Therefore, substituents on the benzene ring would deem not have a significant effect on the sirtuin inhibitory activity on SIRT2 enzyme as shown by compounds **4a-h** in **Table 1**.

2.4 Cellular Assays

The antitumor activity of various known sirtuin inhibitors has been previously demonstrated in the literature [28,29]. We measure the entire library of newly synthesized compounds' cytotoxicity in a panel of human cancer cell lines to probe the relationship between SIRT1/SIRT2 inhibition and cancer cell anti-proliferative activity. The three representative compounds which possessed good sirtuin inhibitory activities (**4b**, **4j** and **4l**) were then screened for their anti-proliferative effect against three different cancer cell lines derived from colon (HCT-116), breast (MDA-MB-468) and blood-leukemia (CCRF-CEM) at 50 μM concentration. Interestingly as shown in **Figure 3**, the most potent compound (**4j**) gave the best cytotoxic activity against all three cancer cells while less pronounced effect was observed for the other two compounds with inferior SIRT2 inhibition. Compound **4j** possessed anti-proliferative activity against all three different types of cancer cells tested (cell viability 40.0% for HCT-116, 53.2% for MDA-MB-468, 27.2% for CCRF-CEM, after 72 hrs treatment), which showed its broad spectrum ability in inhibiting cancer cell growth. Standard deviation obtained for all experiments are less than 20%.

From the results obtained, although it seems SIRT2 could potentially play a more important role in inhibiting cancer cells, we cannot rule out the possibility that combinational inhibitory

effects of SIRT1 and SIRT2 activity may contribute to the observed cytotoxicity as has been reported previously by Peck et al [30].

As literature highlights that benzimidazole and its derivatives might also be toxic to normal cells [31,32], the cytotoxocity of the newly synthesized compounds on human fibroblast cells GM00637 were also evaluated. The tolerable toxicity of the compounds **4a-p** was confirmed by the cytotoxicity test at concentrations up to 50 µM. After 72 hours 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.

3.Conclusion

In conclusion, we have discovered three novel benzimidazole derivatives which showed good SIRT1/SIRT2 inhibition activity with micromolar IC_{50} values. Moreover, they possessed good antitumor activity against three cancer cell lines evaluated in this study. The most potent compound discovered in this study, **4j,** showed better SIRT2 inhibitory activity compared to the standard controls used. More importantly, the correlation between *in vitro* SIRT2 (and to a lesser extent SIRT1) inhibition and cancer cell cytotoxicity using small molecule sirtuin inhibitors was established. Further studies to explore the mechanism of action of these potent small molecule sirtuin inhibitors on cancer cells are currently underway in our laboratory. Compounds with potent SIRT2 inhibition and which demonstrates cyototoxic effect such as **4j** is prime candidates for modifications to further improve their activities.

4. Experimental

4.1. Chemistry

All general chemicals were supplied by Sigma-Aldrich (U.S.A) and Merck Chemicals (Germany). Standard control cambinol and Tenovin-6 were obtained from Cayman Chemicals (U.S.A). 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 (254nm)/long (365nm) UV light. Elemental analyses were performed on Perkin Elmer 2400 Series II CHN Elemental Analyzer and were within ± 0.4% of the calculated values. ^{1}H and ^{13}C NMR were performed on Bruker Avance 500 spectrometer in $CDCl_3$ using TMS as internal standard. Mass spectra were recorded on Varian 320-MS TQ LC/MS using ESI mode.

4.1.1 ***General procedure for synthesis of ethyl-(1,2-disubstituted)-5-carboxylate*****:** The starting material, 4-fluoro-3-nitro benzoic acid was esterified in the presence of catalytic sulfuric acid in ethanol by refluxing for 6 hours to afford **1** in 70% yield. Ethyl-4-fluoro-3-nitrobenzoate, **1** (0.5 g, 2.34 mmol) and amine (2.58 mmol) [**a-h**: 1-(3-aminopropyl)-2-pyrrolidinone; **i-p**: aniline] were mixed in ethanol (5 mL) and stirred at room temperature for 1 hour. The solvent was evaporated under reduced pressure and resuspended in ethyl acetate. The organic layer was then washed with water (10 mL x 3), dried over Na_2SO_4 and evaporated to dryness to yield **2** (90%). The nitroethyl ester, **2** (1 mmol), ammonium formate (3 mmol) and 10% Pd/C (100 mg) were mixed in ethanol (10 mL). The reaction mixture was stirred at room temperature until completion (solution turned colourless). The reaction mixture was then filtered through Celite 545. The filtrate was evaporated under reduced pressure. It was resuspended in ethyl acetate and washed with water, dried over Na_2SO_4 and evaporated to dryness to yield **3** (71%). The aminoethyl ester, **3** (1 mmol) and various

benzaldehydes (1 mmol) and sodium metabisulfite (1 mmol) were mixed in water (5 mL). The reaction mixture was stirred at room temperature for 2 hours. Precipitate formed were then filtered, washed with cold water and dried under reduced pressure to yield final compounds **4a-p** (76-90%).

4.1.2 ***Ethyl 1-(3-(2-oxopyrrolidin-1-yl)propyl)-2-phenyl-1H-benzo[d]imidazole-5-carboxylate*** **(4a)** Yield: 89%; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.45 (3H, t, *J* = 6.9 Hz), 1.90-2.10 (4H, m), 2.35 (2H, t, *J* = 6.0 Hz), 3.34 (2H, t, *J* = 5.0 Hz), 3.50 (2H, t, *J* = 6.0 Hz), 4.31 (2H, t, *J* = 6.5 Hz), 4.48 (2H, q, *J* = 6.9 Hz), 6.90-7.10 (5H, m), 7.48 (1H, d, *J* = 9.0 Hz), 8.10 (1H, dd, *J* = 1.5 Hz, *J* = 9.0 Hz), 8.55 (1H, s). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.78, 18.24, 28.07, 31.15, 40.22, 42.93, 47.28, 61.28, 109.80, 122.78, 124.86, 125.53, 129.29, 129.63, 130.43, 130.59, 138.97, 143.13, 155.51, 167.44, 175.71. ESI-MS: m/z 392.3. $[M+H]^+$. Anal. Calc for $C_{23}H_{25}N_3O_3$: C, 70.56%; H, 6.46%; N, 10.71%. Found: C, 70.53%; H, 6.42%; N, 10.80%.

4.1.3 ***Ethyl 2-(4-(dimethylamino)phenyl)-1-(3-(2-oxopyrrolidin-1-yl)propyl)-1H-benzo[d]imidazole-5-carboxylate*** **(4b)** Yield: 85%; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.43 (3H, t, *J* = 6.9 Hz), 1.90-2.10 (4H, m), 2.35 (2H, t, *J* = 6.0 Hz), 3.05 (6H, s), 3.22 (2H, t, *J* = 5.0 Hz), 3.32 (2H, t, *J* = 6.0 Hz), 4.30 (2H, t, *J* = 6.5 Hz), 4.42 (2H, q, *J* = 6.9 Hz), 6.80 (2H, d, *J* = 9.0 Hz), 7.37 (1H, d, *J* = 9.0 Hz), 7.61 (2H, d, *J* = 9.0 Hz), 8.01 (1H, dd, *J* = 1.5 Hz, *J* = 9.0 Hz), 8.51 (1H, s). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.39, 17.85, 27.56, 30.82, 39.92, 40.17, 42.64, 46.96, 60.81, 109.14, 111.83, 116.40, 121.57, 123.98, 124.87, 130.22, 138.70, 142.43, 151.46, 155.92, 167.15, 175.29. ESI-MS: m/z 435.3. $[M+H]^+$. Anal. Calc for $C_{25}H_{30}N_4O_3$: C, 69.12%; H, 7.01%; N, 12.90%. Found: C, 69.12%; H, 7.03%; N, 12.87%.

4.1.4 ***Ethyl 2-(4-hydroxyphenyl)-1-(3-(2-oxopyrrolidin-1-yl)propyl)-1H-benzo[d]imidazole-5-carboxylate*** **(4c)** Yield: 80%; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.43 (3H, t, *J* = 6.9 Hz), 1.90-

2.10 (4H, m), 2.46 (2H, t, *J* = 6.0 Hz), 3.30 (2H, t, *J* = 5.0 Hz), 3.45 (2H, t, *J* = 6.0 Hz), 4.30 (2H, t, *J* = 6.5 Hz), 4.47 (2H, q, *J* = 6.9 Hz), 6.83 (2H, d, *J* = 9.0 Hz), 7.47 (1H, d, *J* = 9.0 Hz), 7.60 (2H, d, *J* = 9.0 Hz), 8.01 (1H, dd, *J* = 1.5 Hz, *J* = 9.0 Hz), 8.52 (1H, s). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.62, 18.29, 28.75, 32.01, 41.23, 43.43, 48.28, 62.02, 107.83, 109.87, 110.10, 111.04, 123.05, 126.59, 153.40, 157.70, 167.99, 175.16. ESI-MS: m/z 408.2. $[M+H]^+$. Anal. Calc for $C_{25}H_{30}N_4O_3$: C, 67.81%; H, 6.21%; N, 10.34%. Found: C, 67.92%; H, 6.14%; N, 10.41%.

4.1.5 ***Ethyl 1-(3-(2-oxopyrrolidin-1-yl)propyl)-2-p-tolyl-1H-benzo[d]imidazole-5-carboxylate*** (**4d**) Yield: 82%; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.44 (3H, t, *J* = 6.9 Hz), 2.10-2.20 (4H, m), 2.44 (2H, t, *J* = 6.0 Hz), 2.62 (3H, s), 3.30 (2H, t, *J* = 5.0 Hz), 3.43 (2H, t, *J* = 6.0 Hz), 4.30 (2H, t, *J* = 6.5 Hz), 4.45 (2H, q, *J* = 6.9 Hz), 7.01 (2H, d, *J* = 9.0 Hz), 7.40 (1H, d, *J* = 9.0 Hz), 7.65 (2H, d, *J* = 9.0 Hz), 7.97 (1H, dd, *J* = 1.5 Hz, *J* = 9.0 Hz), 8.52 (1H, s). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.60, 18.17, 24.50, 28.80, 32.01, 41.15, 43.25, 48.33, 62.51, 107.83, 109.87, 110.10, 111.04, 123.05, 126.59, 129.88, 153.42, 167.67, 175.10. ESI-MS: m/z 406.2. $[M+H]^+$. Anal. Calc for $C_{24}H_{27}N_3O_3$: C, 71.09%; H, 6.71%; N, 10.36%. Found: C, 71.22%; H, 6.64%; N, 10.40%.

4.1.6 ***Ethyl 2-(4-(trifluoromethyl)phenyl)-1-(3-(2-oxopyrrolidin-1-yl)propyl)-1H-benzo[d]imidazole-5-carboxylate*** **(4e)** Yield: 90%; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.45 (3H, t, *J* = 6.9 Hz), 2.20-2.30 (4H, m), 2.64 (2H, t, *J* = 6.0 Hz), 3.45 (2H, t, *J* = 5.0 Hz), 3.60 (2H, t, *J* = 6.0 Hz), 4.33 (2H, t, *J* = 6.5 Hz), 4.44 (2H, q, *J* = 6.9 Hz), 7.45 (1H, d, *J* = 9.0 Hz), 7.85 (2H, d, *J* = 9.0 Hz), 7.90 (2H, d, *J* = 9.0 Hz), 8.12 (1H, dd, *J* = 1.5 Hz, *J* = 9.0 Hz), 8.70 (1H, s). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.78, 18.01, 28.12, 31.00, 40.01, 42.76, 46.15, 61.27, 110.17, 121.54, 122.80, 125.00, 125.73, 127.89, 129.33, 131.64, 139.05, 143.02, 150.81, 167.36, 175.10. ESI-MS: m/z 460.2. $[M+H]^+$. Anal. Calc for $C_{24}H_{24}N_3O_3F_3$: C, 62.72%; H, 5.33%; N, 9.24%. Found: C, 62.70%; H, 5.33%; N, 9.19%.

4.1.7 ***Ethyl 2-(4-nitrophenyl)-1-(3-(2-oxopyrrolidin-1-yl)propyl)-1H-benzo[d]imidazole-5-carboxylate*** **(4f)** Yield: 80%; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.45 (3H, t, *J* = 6.9 Hz), 2.00-2.20 (4H, m), 2.39 (2H, t, *J* = 6.0 Hz), 3.35 (2H, t, *J* = 5.0 Hz), 3.46 (2H, t, *J* = 6.0 Hz), 4.40 (2H, t, *J* = 6.5 Hz), 4.52 (2H, q, *J* = 6.9 Hz), 7.43 (1H, d, *J* = 9.0 Hz), 7.68 (2H, d, *J* = 9.0 Hz), 7.88 (1H, d, *J* = 9.0 Hz), 8.20 (1H, dd, *J* = 1.5 Hz, *J* = 9.0 Hz), 8.59 (1H, s). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.58, 18.23, 28.56, 28.68, 31.01, 38.55, 41.37, 46.79, 61.50, 109.96, 122.50, 125.56, 125.88, 126.18, 129.72, 129.99, 138.68, 142.51, 154.25, 167.03, 175.01. ESI-MS: m/z 437.2. $[M+H]^+$. Anal. Calc for $C_{23}H_{24}N_4O_5$: C, 63.32%; H, 5.54%; N, 12.80%. Found: C, 63.45%; H, 5.59%; N, 12.72%.

4.1.8 ***Ethyl 2-(4-chlorophenyl)-1-(3-(2-oxopyrrolidin-1-yl)propyl)-1H-benzo[d]imidazole-5-carboxylate*** **(4g)** Yield: 88%; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.45 (3H, t, *J* = 6.9 Hz), 1.90-2.10 (4H, m), 2.48 (2H, t, *J* = 6.0 Hz), 3.43 (2H, t, *J* = 5.0 Hz), 3.55 (2H, t, *J* = 6.0 Hz), 4.30 (2H, t, *J* = 6.5 Hz), 4.48 (2H, q, *J* = 6.9 Hz), 7.46 (3H, m), 7.80 (2H, d, *J* = 9.0 Hz), 8.11 (1H, dd, *J* = 1.5 Hz, *J* = 9.0 Hz), 8.49 (1H, s). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.40, 18.13, 27.97, 30.75, 39.96, 42.03, 46.88, 61.01, 109.93, 122.44, 124.67, 125.92, 126.13, 127.45, 137.97, 142.72, 154.53, 167.50, 174.99. ESI-MS: m/z 426.2. $[M+H]^+$. Anal. Calc for $C_{23}H_{24}N_3O_3Cl$: C, 64.89%; H, 5.72%; N, 9.91%. Found: C, 65.05%; H, 5.83%; N, 9.82%.

4.1.9 ***Ethyl 2-(4-bromophenyl)-1-(3-(2-oxopyrrolidin-1-yl)propyl)-1H-benzo[d]imidazole-5-carboxylate*** **(4h**) Yield: 84%; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.45 (3H, t, *J* = 6.9 Hz), 2.00-2.10 (4H, m), 2.46 (2H, t, *J* = 6.0 Hz), 3.37 (2H, t, *J* = 5.0 Hz), 3.47 (2H, t, *J* = 6.0 Hz), 4.32 (2H, t, *J* = 6.5 Hz), 4.45 (2H, q, *J* = 6.9 Hz), 7.46 (2H, d, *J* = 9.0 Hz), 7.60 (1H, d, *J* = 9.0 Hz), 8.14 (1H, dd, *J* = 1.5 Hz, *J* = 9.0 Hz), 8.60 (1H, s). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.58, 18.17, 28.04, 30.16, 40.23, 41.79, 46.4, 61.03, 108.10, 122.65, 124.78, 125.40, 126.19, 128.77, 129.21, 129.82, 130.56, 139.11, 143.13, 156.25, 167.57, 174.99. ESI-MS: m/z 470.1

$[M+H]^+$. Anal. Calc for $C_{23}H_{24}N_3O_3Br$: C, 58.67%; H, 5.13%; N, 8.91%. Found: C, 58.62%; H, 5.12%; N, 8.81%.

4.1.10 ***Ethyl 1,2-diphenyl-1H-benzo[d]imidazole-5-carboxylate*** (**4i**) Yield: 76%; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.45 (3H, t, *J* = 7.1 Hz), 4.43 (2H, q, *J* = 7.1 Hz), 7.10 (1H, d, *J* = 9 Hz), 7.30-7.70 (10H, m), 7.87 (1H, dd, *J* = 1.5 Hz, 9 Hz), 8.55 (1H, s). ^{13}C NMR (125 MHz, $CDCl_3$): 14.51, 61.42, 110.34, 111.80, 111.99, 112.56, 113.14, 113.91 115.67, 118.82, 119.05, 130.24, 131.00, 136.56, 140.28, 142.44, 152.16, 167.88. ESI-MS: m/z 343.1 $[M+H]^+$. Anal. Calc for $C_{22}H_{18}N_2O_2$: C, 77.17%; H, 5.30%; N, 8.18%. Found: C, 77.06%; H, 5.35%; N, 8.32%.

4.1.11 ***Ethyl 2-(4-(dimethylamino)phenyl)-1-phenyl-1H-benzo[d]imidazole-5-carboxylate*** (**4j**) Yield: 87%; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.43 (3H, t, *J* = 7.1 Hz), 2.97 (6H, s), 4.41 (2H, q, *J* = 7.1 Hz), 6.58 (2H, d, *J* = 9 Hz), 7.18 (1H, d, *J* = 9 Hz), 7.35-7.60 (5H, m), 7.46 (2H, d, *J* = 9 Hz), 7.92 (1H, dd, *J* = 1.5 Hz, 9 Hz), 8.56 (1H, s). ^{13}C NMR (125 MHz, $CDCl_3$): 14.79, 40.43, 61.22, 110.04, 111.74, 116.39, 121.56, 124.65, 125.67, 127.91, 129.21, 130.42, 130.99, 137.49, 140.76, 142.71, 151.55, 155.13, 167.57. ESI-MS: m/z 386.1 $[M+H]^+$. Anal. Calc for $C_{24}H_{23}N_3O_2$: C, 74.78%; H, 6.01%; N, 10.90%. Found: C, 74.76%; H, 6.02%; N, 10.90%.

4.1.12 ***Ethyl 2-(4-hydroxyphenyl)-1-phenyl-1H-benzo[d]imidazole-5-carboxylate*** (**4k**) Yield: 77%; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.43 (3H, t, *J* = 7.1 Hz), 4.42 (2H, q, *J* = 7.1 Hz), 7.19 (2H, d, *J* = 9 Hz), 7.28 (1H, d, *J* = 9 Hz), 7.35-7.60 (5H, m), 7.47 (2H, d, *J* = 9 Hz), 7.99 (1H, dd, *J* = 1.5 Hz, 9 Hz), 8.56 (1H, s). ^{13}C NMR (125 MHz, $CDCl_3$): 14.26, 61.05, 118.95, 120.87, 122.54, 124.79, 126.95, 127.26, 128.08, 129.17, 130.34, 132.96, 136.15, 140.28, 143.03, 158.69, 167.00. ESI-MS: m/z 359.1 $[M+H]^+$. Anal. Calc for $C_{22}H_{18}N_2O_3$: C, 73.73%; H, 5.06%; N, 7.82%. Found: C, 73.56%; H, 5.16%; N, 7.88%.

4.1.13 ***Ethyl 1-phenyl-2-p-tolyl-1H-benzo[d]imidazole-5-carboxylate*** (**4l**) Yield: 84%; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.41 (3H, t, *J* = 7.1 Hz), 2.34 (3H, s), 4.40 (2H, q, *J* = 7.1 Hz), 7.26 (2H, d, *J* = 9 Hz), 7.31 (1H, d, *J* = 9 Hz), 7.35-7.60 (5H, m), 7.53 (2H, d, *J* = 9 Hz), 7.99 (1H, dd, *J* = 1.5 Hz, 9 Hz), 8.55 (1H, s). ^{13}C NMR (125 MHz, $CDCl_3$): 14.24, 24.36, 60.96, 118.92, 120.75, 122.53, 124.82, 126.95, 127.23, 128.00, 129.17, 130.33, 132.96, 136.15, 140.28, 143.03, 151.95, 166.97. ESI-MS: m/z 357.1 $[M+H]^+$. Anal. Calc for $C_{23}H_{20}N_2O_2$: C, 77.51%; H, 5.66%; N, 7.86%. Found: C, 77.62%; H, 5.62%; N, 8.78%.

4.1.14 ***Ethyl 2-(4-(trifluoromethyl)phenyl)-1-phenyl-1H-benzo[d]imidazole-5-carboxylate*** (**4m**) Yield: 89%; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.44 (3H, t, *J* = 7.1 Hz), 4.44 (2H, q, *J* = 7.1 Hz), 7.27 (1H, d, *J* = 9 Hz), 7.33-7.60 (5H, m), 7.58 (2H, d, *J* = 9 Hz), 7.70 (2H, d, *J* = 9 Hz), 8.03 (1H, dd, *J* = 1.5 Hz, 9 Hz), 8.63 (1H, s). ^{13}C NMR (125 MHz, $CDCl_3$): 14.38, 61.03, 110.32, 122.45, 122.67, 124.84, 125.33, 125.36, 125.41, 125.97, 127.29, 130.30, 132.89, 136.15, 140.28, 142.43, 152.26, 166.89. ESI-MS: m/z 410.1 $[M+H]^+$. Anal. Calc for $C_{24}H_{23}N_3O_2$: C, 67.31%; H, 4.18%; N, 6.83%. Found: C, 67.30%; H, 4.12%; N, 6.88%.

4.1.15 ***Ethyl 2-(4-nitrophenyl)-1-phenyl-1H-benzo[d]imidazole-5-carboxylate*** (**4n**) Yield: 90%; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.45 (3H, t, *J* = 7.1 Hz), 4.47 (2H, q, *J* = 7.1 Hz), 7.15 (1H, d, *J* = 9 Hz), 7.35-7.60 (5H, m), 7.50 (2H, d, *J* = 9 Hz), 7.63 (2H, d, *J* = 9 Hz), 8.01 (1H, dd, *J* = 1.5 Hz, 9 Hz), 8.62 (1H, s). ^{13}C NMR (125 MHz, $CDCl_3$): 14.38, 61.11, 111.06, 122.59, 124.84, 125.33, 125.36, 125.41, 125.97, 127.37, 130.33, 132.96, 136.15, 140.27, 142.42, 150.49, 151.99, 167.10. ESI-MS: m/z 388.1 $[M+H]^+$. Anal. Calc for $C_{22}H_{17}N_3O_4$: C, 68.21%; H, 4.42%; N, 10.85%. Found: C, 68.20%; H, 4.42%; N, 10.87%.

4.1.16 ***Ethyl 2-(4-chlorophenyl)-1-phenyl-1H-benzo[d]imidazole-5-carboxylate*** (**4o**) Yield: 81%; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.43 (3H, t, *J* = 7.1 Hz), 4.42 (2H, q, *J* = 7.1 Hz), 7.25 (1H, d, *J* = 9 Hz), 7.28 (2H, d, *J* = 9 Hz), 7.40-7.60 (5H, m), 7.54 (2H, d, *J* = 9 Hz), 8.00 (1H, dd, *J* = 1.5 Hz, 9 Hz), 8.52 (1H, s). ^{13}C NMR (125 MHz, $CDCl_3$): 14.40, 61.64, 110.28,

111.80, 116.26, 122.70, 122.97, 124.65, 125.67, 127.91, 129.20, 130.35, 131.18, 137.50, 140.76, 142.71, 152.68, 167.48. ESI-MS: m/z 377.1 $[M+H]^+$. Anal. Calc for $C_{22}H_{17}N_2O_2Cl$: C, 70.12%; H, 4.55%; N, 7.43%. Found: C, 70.10%; H, 4.52%; N, 7.54%.

4.1.17 ***Ethyl 2-(4-bromophenyl)-1-phenyl-1H-benzo[d]imidazole-5-carboxylate*** **(4p**) Yield: 79%; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.44 (3H, t, *J* = 7.1 Hz), 4.44 (2H, q, *J* = 7.1 Hz), 7.24 (1H, d, *J* = 9 Hz), 7.32 (2H, d, *J* = 9 Hz), 7.40-7.60 (5H, m), 7.58 (2H, d, *J* = 9 Hz), 8.00 (1H, dd, *J* = 1.5 Hz, 9 Hz), 8.60 (1H, s). ^{13}C NMR (125 MHz, $CDCl_3$): 14.39, 61.65, 110.30, 111.82, 116.37, 122.75, 123.89, 124.65, 125.67, 127.91, 129.20, 130.32, 131.08, 137.50, 140.76, 142.71, 152.69, 167.50. ESI-MS: m/z 421.1 $[M+H]^+$. Anal. Calc for $C_{22}H_{17}N_2O_2Br$: C, 62.72%; H, 4.07%; N, 6.65%. Found: C, 62.76%; H, 4.02%; N, 6.73%.

4.2 Biology

Fluorescent optical density for *in vitro* assay was measured on Tecan Infinite M200. Optical density for cell proliferative assay was measured with Thermo Scientific MultiSkan FC microplate reader.

4.2.1. SIRT1 *in vitro* Assay

3.3 µM of SIRT1 substrate derived from human p53 sequences, 66.7 µM NAD^+, 50 µM of interested compounds (all final concentration) and 0.5 µg of SIRT1 human recombinant (GenBank Accession #: NM_012238) with 193- 741 amino acids and GST tag at its N-terminal, were incubated for 45 minutes at 37^{o}C. 50 µL of stop solution consisting nicotinamide and SIRT1 developer was then added and the mixture was incubated for a further 10 minutes at 37^{o}C. Fluorescence was measured at 490 nm (excitation) and 520 nm (emission) and the inhibition was calculated as the ratio of absorbance under each experimental condition to that of the control.

4.2.2. SIRT2 *in vitro* Assay

6.7 µM of SIRT2 substrate derived from human p53 sequences, 333 µM NAD^+, 50 µM of interested compounds (all final concentration) and 0.5 µg of SIRT2 human recombinant (GenBank Accession #: NM_030593) with 13- 319 amino acids and His tag at its C-terminal, were incubated for 45 minutes at 37°C. 50 µL of stop solution consisting nicotinamide and SIRT1 developer was then added and the mixture was incubated for a further 10 minutes at 37°C. Fluorescence was measured at 490 nm (excitation) and 520 nm (emission) and the inhibition was calculated as the ratio of absorbance under each experimental condition to that of the control.

4.2.3. Cell Proliferation Assay

All cell lines were obtained from the American Type Culture Collection (Rockville, MD). Cells were seeded in 96-well plates at a density of 5×10^3 per well (for MDA-MB-468 and HCT-116) or 4 x 10^4 per well (for CCRF-CEM). The cells were treated with 50 µM of interested compounds and allowed to adhere for 72 hours. Then, the proliferative activity was determined by 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt assay (CellTiter 96 Non-Radioactive Cell Proliferation Assay; Promega, Madison, WI) to monitor the number of viable cells according to the manufacturer's instructions. Briefly, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt solution was added at 20 µL/well, and after 1 hour of incubation at 37°C in a humidified 5% CO_2 atmosphere, the conversion of 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt to formazan was measured in a plate reader at 490 nm. All experiments were done in triplicate, and the proliferation rate was calculated as the ratio of absorbance under each experimental condition to that of the control nontransfectant.

4.2.4. Autofluorescence

Compounds in DMSO at 50 µM concentration (100 µL) were pipetted into Nunc Microwell 96-wells plate. DMSO was used as control well. Excitation wavelength was set at 490 nm and emission wavelength at 520 nm. The criteria for a compound being considered autofluorescent was defined as having >50% fluorescence of the control wells. None of the analyzed compounds were found to be autofluorescent.

Acknowledgements

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 work. This work was funded through FRGS Grant No (203/C1PPM/6711335) and HiCoE research Grant No (311.CIPPM.4401005).

The authors hereby declare there is no conflict of interests.

References

[1] S. Michan, D. Sinclair, Sirtuins in mammals: insights into their biological function, Biochem. J., 404 (2007) 1-13.

[2] J.M. Denu, The Sir2 family of protein deacetylase, Curr. Opin. Chem. Biol., 9 (2005) 431-440.

[3] D.M. Taylor, M.M. Maxwell, R. Luthi-Carter, A.G. Kazantsev, Biological and potential therapeutic roles of sirtuin deacetylases, Cell. Mol. Life Sci., 65 (2008) 4000-4018.

[4] R.A. Fyre, Phylogenetic classification of prokaryotic and eukaryotic Sir2-like proteins, Biochem. Biophys. Res. Commun., 273 (2000) 793-798.

[5] J. Milner, Cellular regulation of SIRT1, Curr. Pharm. Des., 15 (2009) 39-44.

[6] T. Inoue, M. Hiratsuka, M. Osaki, M. Oshimura, The molecular biology of mammalian SIRT proteins: SIRT2 in cell cycle regulation, Cell Cycle, 6 (2007) 1011-1018.

[7] B. Zschoernig, U. Mahlknecht, SIRTUIN 1: regulating the regulator, Biochem. Biophys. Res. Commun., 376 (2008) 251-255.

[8] A. Mai, D. Rotili, S. Valente, A.G. Kazantsev, Histone deacetylase inhibitors and neurodegenerative disorders: holding the promise, Curr. Pharm. Des., 15 (2009) 3940-3957.

[9] A.L. Garske, B.C. Smith, J.M. Denu, Linking SIRT2 to Parkinson's disease, ACS Chem. Biol., 2 (2007) 529-532.

[10] L.R. Saunders, E. Verdin, Sirtuins: critical regulators at the crossroads between cancer and aging, Oncogene, 26 (2007) 5489-5504.

[11] L. Bosch-Presegue, A. Vaquero, The dual role of sirtuins in cancer, Genes Cancer, 2 (2011) 648-662.

[12] J. Chen, Y. Zhou, S. Mueller-Steiner, L.F. Chen, H. Kwon, S. Yi, L. Mucke, L. Gan, SIRT1 protects against Microglia-dependent Amyloid-β toxicity through inhibiting NF-κB signaling, J. Biol. Chem., 280 (2005) 40364-40374.

[13] A.Z. Herskovits, L. Guarente, Sirtuin deacetylases in neurodegenerative diseases of aging, Cell Research, 23 (2013) 746-758.

[14] F. Yeung, J.E. Hoberg, C.S. Ramsey, M.D. Keller, D.R. Jones, R.A. Frye, M.W. Mayo, Modulation of NF-κb-dependent transcription and cell survival by the SIRT1 deacetylase, EMBO J., 23 (2004) 2369-2380.

[15] A. Berdichevsky, L. Guarente, A stress response pathway involving sirtuins, forkheads and 14-3-3 proteins, Cell Cycle, 5 (2006) 2588-2591.

[16] M. Lakshminarasimhan, D. Rauh, M. Schutkowski, C. Steegborn, Sirt1 activation by resveratrol is substrate sequence-selective, Aging, 5 (2013) 151-154.

[17] C.M. Grozinger, E.D. Chao, H.E. Blackwell, D. Moazed, S.L. Schreiber, Identification of a class of small molecule inhibitors of the sirtuin family of NAD-dependent deacetylases by phenotype screening, J. Biol. Chem., 276 (2001) 38837-38843.

[18] J. Posakony, M. Hirao, S. Stevens, J.A. Simon, A. Bedalov, Inhibitors of Sir2: evaluation of splitomicin analogues, J. Med. Chem., 47 (2004) 2635-2644.

[19] S. Lain, J.J. Hollick, J. Campbell, O.D. Staples, M. Higgins, M. Aoubala, A. McCarthy, V. Appleyard, K.E. Murray, L. Baker, A. Thompson, J. Mathers, S.J. Holland, M.J.R. Stark, G. Pass, J. Woods, D.P. Lane, N.J. Westwood, Discovery, in-vivo activity, and mechanism of action of a small molecule p53 activator, Cancer Cell, 13 (2008) 454-463.

[20] A.D. Napper, J. Hixon, T. McDonagh, K. Keavey, J.F. Pons, J. Barker, W.T. Yau, P. Amouzegh, A. Flegg, E. Hamelin, R.J. Thomas, M. Kates, S. Jones, M.A. Navia, J.O.

Saunders, P.S. DiStefano, R. Curtis, Discovery of indoles as potent and selective inhibitors of the deacetylase SIRT1, J. Med. Chem., 48 (2005) 8045-8054.

[21] M. Mader, A. de Dios, C. Shih, B.D. Anderson, Imidazolyl benzimidazoles and imidazo[4,5-*b*]pyridines as potent p38α MAP kinase inhibitors with excellent in vivo antiinflammatory properties, Bioorg. Med. Chem. Lett., 18 (2008) 179-183.

[22] T. Roth, M.L. Morningstar, P.L. Boyer, S.H. Hughes, R.W. Buckheit Jr., C.J. Michejda, Synthesis and biological activity of novel nonnucleoside inhibitors of HIV-1 reverse transcriptase. 2-aryl-substituted benzimidazoles, J. Med. Chem., 40 (1997) 4199.

[23] Horton, D.A.; Bourne G.T.; Smythe, M.L. (2003) The combinatorial synthesis of bicyclic privileged structures or priviledged substructures. Chem. Rev., 103: 893-930.

[24] For some selected examples in recent years, see: (a) Kim, Y.; Kumar, M.R.; Park, N.; Heo, Y.; Lee, S. (2011) Copper-catalyzed, one-pot, three-component synthesis of benzimidazoles by condensation and C-N bond formation. J. Org. Chem., 76: 9577-9583; (b) Shen, M.; Driver, T.G. (2008) Iron(II) bromide-catalyzed synthesis of benzimidazoles from aryl azides. Org. Lett., 10: 3367-3370; (c) Tandon, V.K.; Kumar, M. (2004) $BF_3 \cdot Et_2O$ promoted one-pot expeditious and convenient synthesis of 2-substituted benzimidazoles and 3,1,5-benzoxadiazepines. Tetrahedron Lett., 45: 4185-4187; (d) Dudd, L.M.; Venardou, E.; Garcia-Verdugo, E.; Licence, P.; Blake, A.J.; Wilson, C.; Poliakoff, M. (2003) Synthesis of benzimidazoles in high-temeperature water. Green Chem., 5: 187-192.

[25] K. Nagata, T. Itoh, H. Ishikawa, A. Ohsawa, Synthesis of 2-Substituted Benzimidazoles by Reaction of o-Phenylenediamine with Aldehydes in the Presence of Sc(OTf)~3 Heterocycles, 61 (2003) 93-96.

[26] S. Moniot, M. Schutkowski, C. Steegborn, Crystal structure analysis of human Sirt2 and its ADP-ribose complex, J. Struct. Biol. 182 (2013) 136-143.

[27] S. Sakkiah, M. Arooj, M.R. Kumar, S.H. Eom, K.W. Lee, Identification of inhibitor binding site in human Sirtuin 2 using molecular docking and dynamics simulations, PLoS ONE, 8 (2013) e51429.

[28] B. Heltweg, T. Gatbonton, A.D. Schuler, J. Posakony, H. Li, S. Goehle, R. Kollipara, R.A. Depinho, Y. Gu, J.A. Simon, A. Bedalov, Antitumor activity of a small-molecule inhibitor of human silent information regulator 2 enzymes, Cancer Res., 66 (2006) 4368-4377.

[29] S. Lain, J.J. Hollick, J. Campbell, O.D. Staples, M. Higgins, M. Aoubala, A. McCarthy, V. Appleyard, K.E. Murray, L. Baker, A. Thompson, J. Mathers, S.J. Holland, M.J.R. Stark, G. Pass, J. Woods, D.P. Lane, N.J. Westwood, Discovery, in-vivo activity, and mechanism of action of a small molecule p53 activator, Cancer Cell, 13 (2008) 454-463.

[30] B. Peck, C.Y. Chen, K.K. Ho, P. Di Fruscia, S.S. Myatt, R.C. Coombes, M.J. Fuchter, C.D. Hsiao, W.F. Eric Lam, SIRT1 inhibitors induce cell death and p53 acetylation through targeting both SIRT1 and SIRT2, Mol. Cancer Ther., 9 (2010) 844-855.

[31] K. Fahey, L. O'Donovan, M. Carr, M.P. Carty, F. Aldabbagh, The influence of aziridinyl substituent of benzimidazoles and benzimidazolequinones on toxicity towards normal and Fanconi anaemia cells, Eur. J. Med. Chem., 45 (2010) 1873-1879.

[32] E. Moriarty, M. Carr, S. Bonham, M.P. Carty, F. Aldabbagh, Synthesis and toxicity towards normal and cancer cell lines of benzimidazolequinones containing fused aromatic rings and 2-aromatic ring substituents, Eur. J. Med. Chem., 45 (2010) 3762-3769.

Tables, Figures and Scheme:

1. **Table 1.** SIRT1 and SIRT2 inhibitory activities of novel benzimidazole derivatives.
2. **Figure 1.** Molecular interactions between **4j** and SIRT2.
3. **Figure 2.** Molecular interactions between **4b** and SIRT2.
4. **Figure 3.** Inhibitory activity of representative compounds **4b**, **4j** and **4l** against HCT-116, MDA-MB-468 and CCRF-CEM cell line.
5. **Scheme 1.** Synthesis protocol of titled compounds **4a-p**

Table 1. SIRT1 and SIRT2 inhibitory activities of synthesized benzimidazole derivatives

Entry	Compound	SIRT1 inhibition at 50 µM ± S.D. (%)	IC_{50} SIRT1 inhibition (µM)	SIRT2 inhibition at 50 µM ± S.D. (%)	IC_{50} SIRT2 inhibition (µM)
1	**4a**	30.03 ± 9.08	N.D.	43.91 ± 4.45	N.D.
2	**4b**	49.15 ±10.12	60.31 ± 4.22	57.90 ± 6.80	52.78 ± 5.07
3	**4c**	30.93 ± 5.13	N.D.	43.81 ± 6.77	N.D.
4	**4d**	33.56 ± 11.24	N.D.	45.40 ± 13.31	N.D.
5	**4e**	32.84 ± 7.09	N.D.	33.17 ± 2.21	N.D.
6	**4f**	37.36 ± 3.45	N.D.	42.98 ± 3.18	N.D.
7	**4g**	27.16 ± 8.73	N.D.	34.50 ± 5.68	N.D.
8	**4h**	27.30 ± 4.94	N.D.	40.06 ± 8.20	N.D.
9	**4i**	21.03 ± 3.69	N.D.	34.31 ± 4.91	N.D.
10	**4j**	60.59 ± 8.26	49.12 ± 3.33	71.69 ± 6.74	26.85 ± 1.92
11	**4k**	11.01 ± 10.29	N.D.	22.94 ± 8.40	N.D.
12	**4l**	43.93 ± 5.96	79.87 ± 8.10	50.79 ± 11.94	60.60 ± 2.42
13	**4m**	33.76 ± 2.43	N.D.	45.52 ± 8.47	N.D.
14	**4n**	34.20 ± 10.87	N.D.	48.03 ± 9.93	N.D.
15	**4o**	19.82 ± 3.49	N.D.	32.52 ± 3.54	N.D.
16	**4p**	26.70 ± 7.33	N.D.	41.31 ± 15.83	N.D.
17	**Cambinol**	67.59 ± 4.85	47.90 ± 4.55	62.04 ± 9.80	45.86 ± 3.31
18	**Tenovin-6**	44.00 ± 7.84	58.10 ± 3.67	66.20 ± 5.98	29.73 ± 1.84

N.D. = Not determined

Figure 1. Molecular interactions between **4j** and SIRT2.

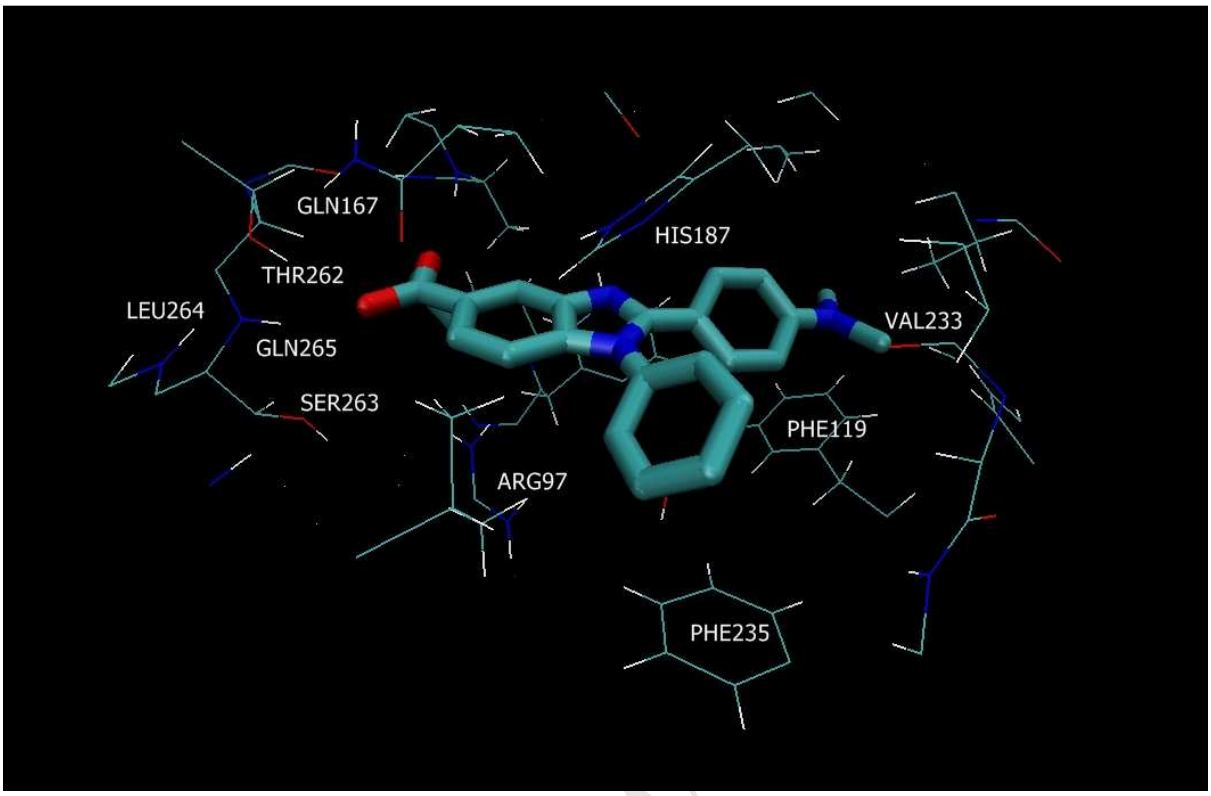


Figure 2. Molecular interactions between **4b** and SIRT2

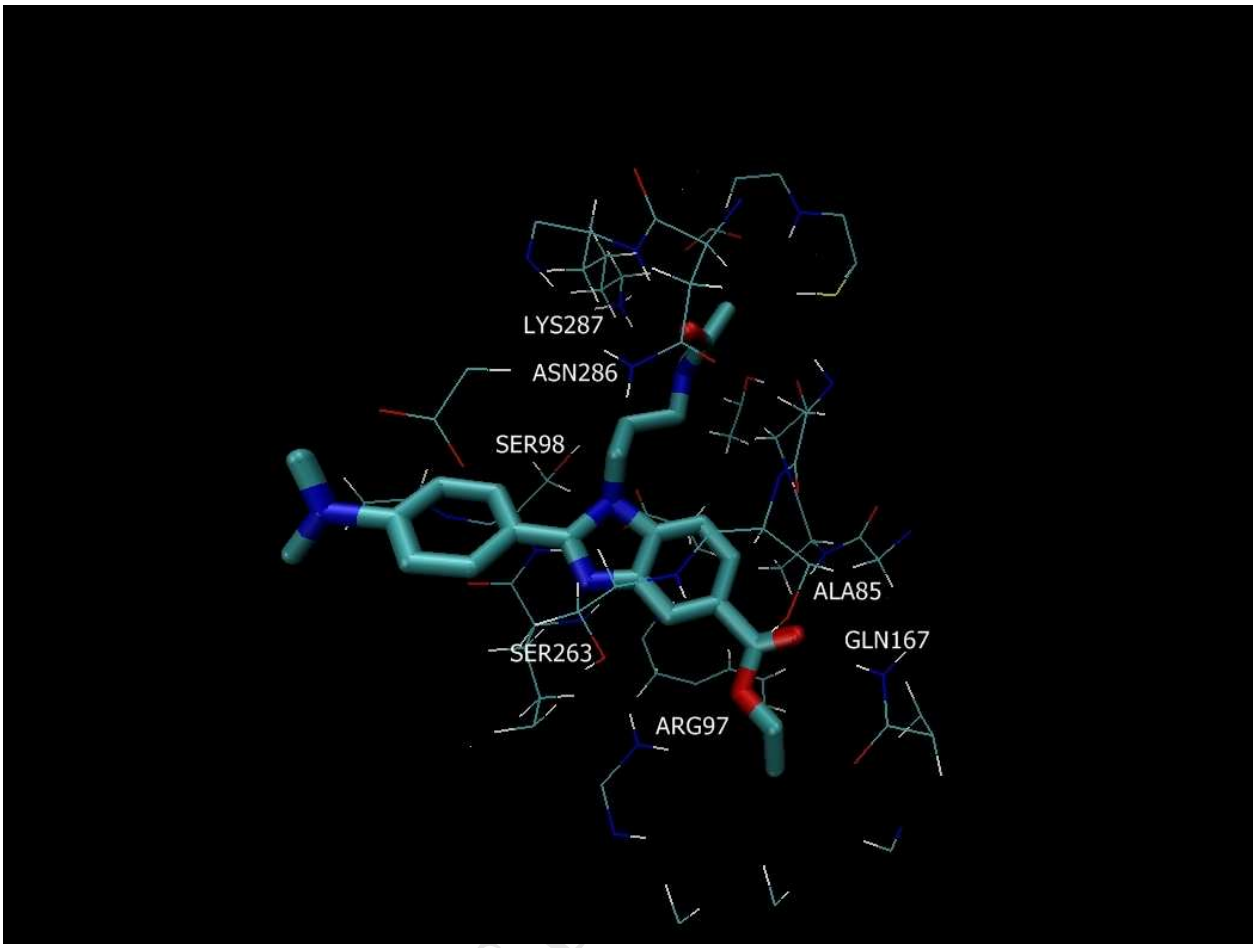
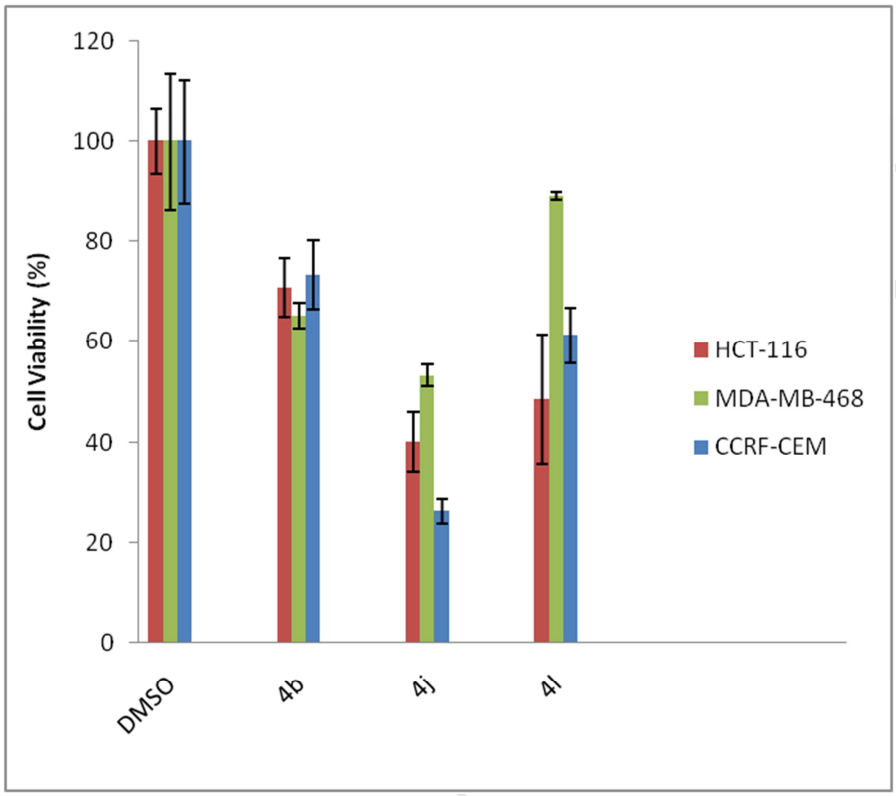
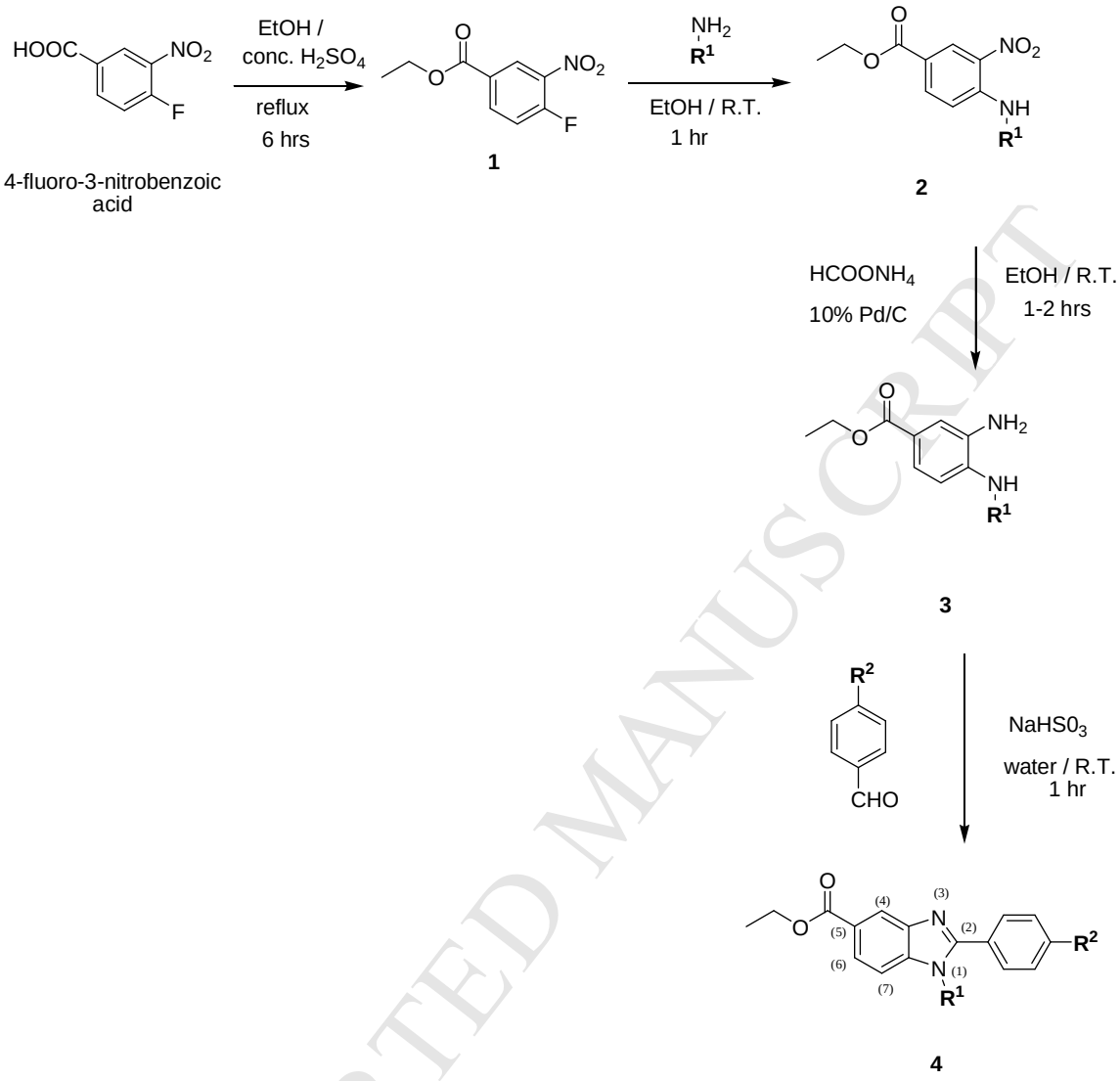


Figure 3. Inhibitory activity of representative compounds **4b**, **4j** and **4l** against HCT-116, MDA-MB-468 and CCRF-CEM cell line.



Scheme 1. Synthesis protocol of titled compounds **4a-p**



4a	R^1 = 3-(2-oxopyrrolidin-1-yl)propyl	R^2 = -H
4b	R^1 = 3-(2-oxopyrrolidin-1-yl)propyl	R^2 = -N(CH_3)$_2$
4c	R^1 = 3-(2-oxopyrrolidin-1-yl)propyl	R^2 = -OH
4d	R^1 = 3-(2-oxopyrrolidin-1-yl)propyl	R^2 = -CH_3
4e	R^1 = 3-(2-oxopyrrolidin-1-yl)propyl	R^2 = -CF_3
4f	R^1 = 3-(2-oxopyrrolidin-1-yl)propyl	R^2 = -NO_2
4g	R^1 = 3-(2-oxopyrrolidin-1-yl)propyl	R^2 = -Cl
4h	R^1 = 3-(2-oxopyrrolidin-1-yl)propyl	R^2 = -Br
4i	R^1 = phenyl	R^2 = -H
4j	R^1 = phenyl	R^2 = -N(CH_3)$_2$
4k	R^1 = phenyl	R^2 = -OH
4l	R^1 = phenyl	R^2 = -CH_3
4m	R^1 = phenyl	R^2 = -CF_3
4n	R^1 = phenyl	R^2 = -NO_2
4o	R^1 = phenyl	R^2 = -Cl
4p	R^1 = phenyl	R^2 = -Br

HIGHLIGHTS:

- Compounds with better *in vitro* SIRT2 inhibition than cambinol and Tenovin-6
- Good anti proliferative effect against three different cancer cell lines
- Binding mode of potent compounds with SIRT2 was established
- Novel green chemistry method in benzimidazole synthesis
- Benzimidazoles as potential anti-cancer agent

Benzimidazoles as new scaffold of sirtuin inhibitors: green synthesis, *in vitro* studies, molecular docking analysis and evaluation of their anti-cancer properties

Yeong Keng Yoon[a*], Mohamed Ashraf Ali[a,b,c], Ang Chee Wei[a], Amir Nasrolahi Shirazi[d], Keykavous Parang[d], Tan Soo Choon[a]

[a]*Institute for Research in Molecular Medicine, Universiti Sains Malaysia, Minden 11800, Penang, Malaysia.*

[b]*New Drug Discovery Research, Department of Medicinal Chemistry, Alwar Pharmacy College, Alwar, Rajasthan-301030, India.*

[c]*New Drug Discovery Research, Department of Medicinal Chemistry, Sunrise University, Alwar, Rajasthan-301030, India.*

[d]*Department of Biomedical and Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island, Kingston, RI, 02881, United States.*

SUPPLEMENTARY DATA CONTENTS:

1. **Figure S1**. ^{1}H NMR for **4j.**
2. **Figure S2**. ^{13}C NMR for **4j.**
3. **Figure S3**. Direct Infusion LC-MS for **4j.**
4. **Figure S4**. ORTEP diagram for **4j.**
5. **Figure S5**. ^{1}H NMR for **4b.**
6. **Figure S6**. ^{13}C NMR for **4b.**
7. **Figure S7**. Direct Infusion LC-MS for **4b.**

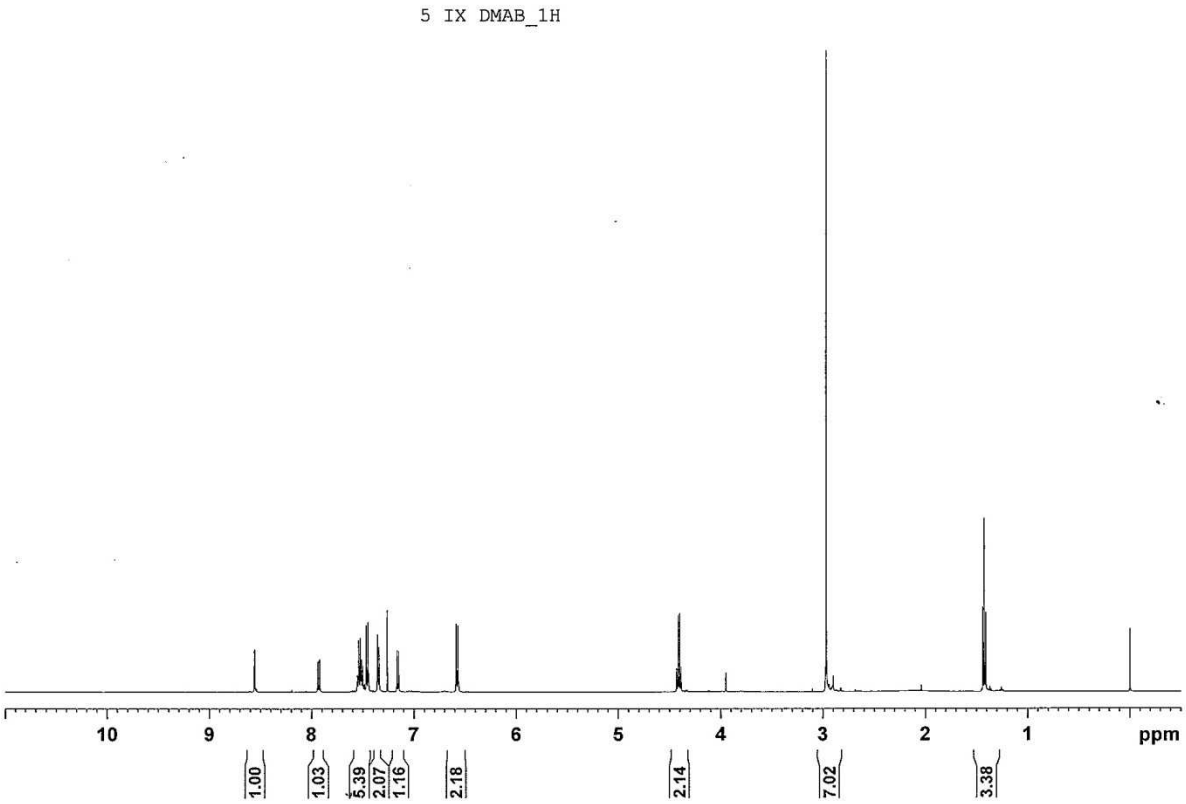


Figure S1. ^{1}H NMR for **4j.**

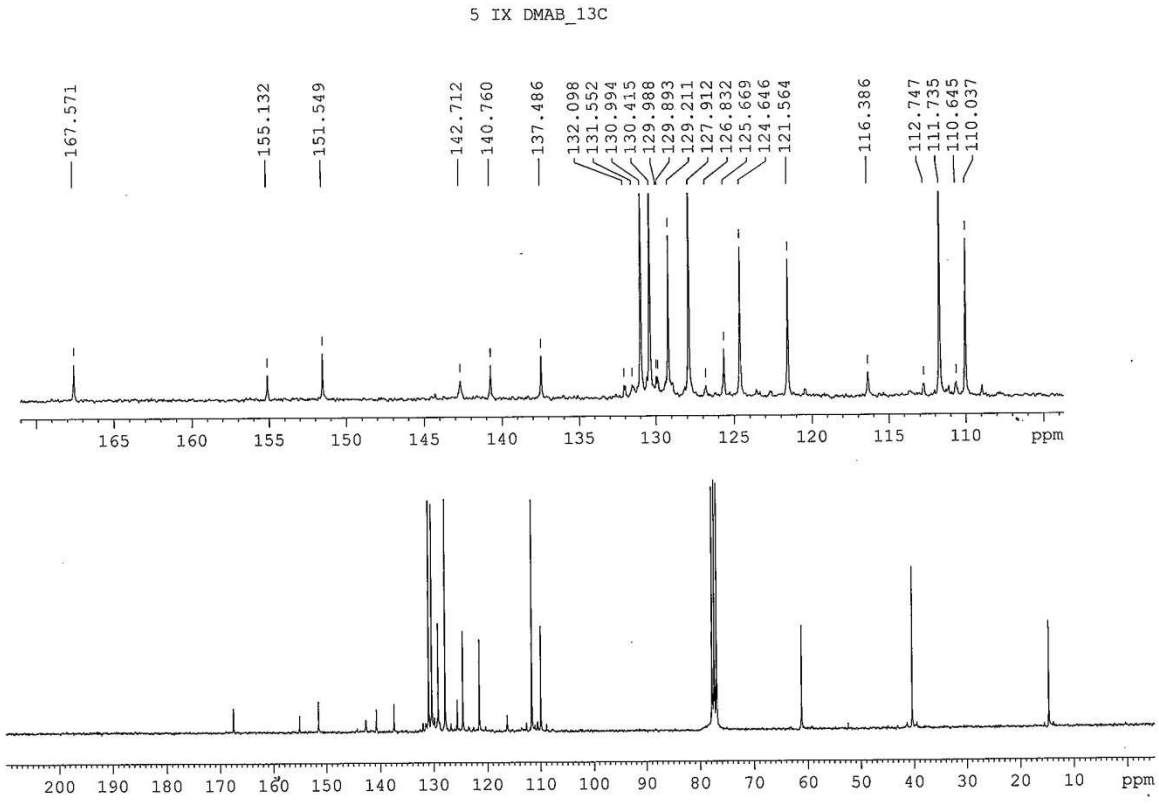


Figure S2. ^{13}C NMR for **4j.**

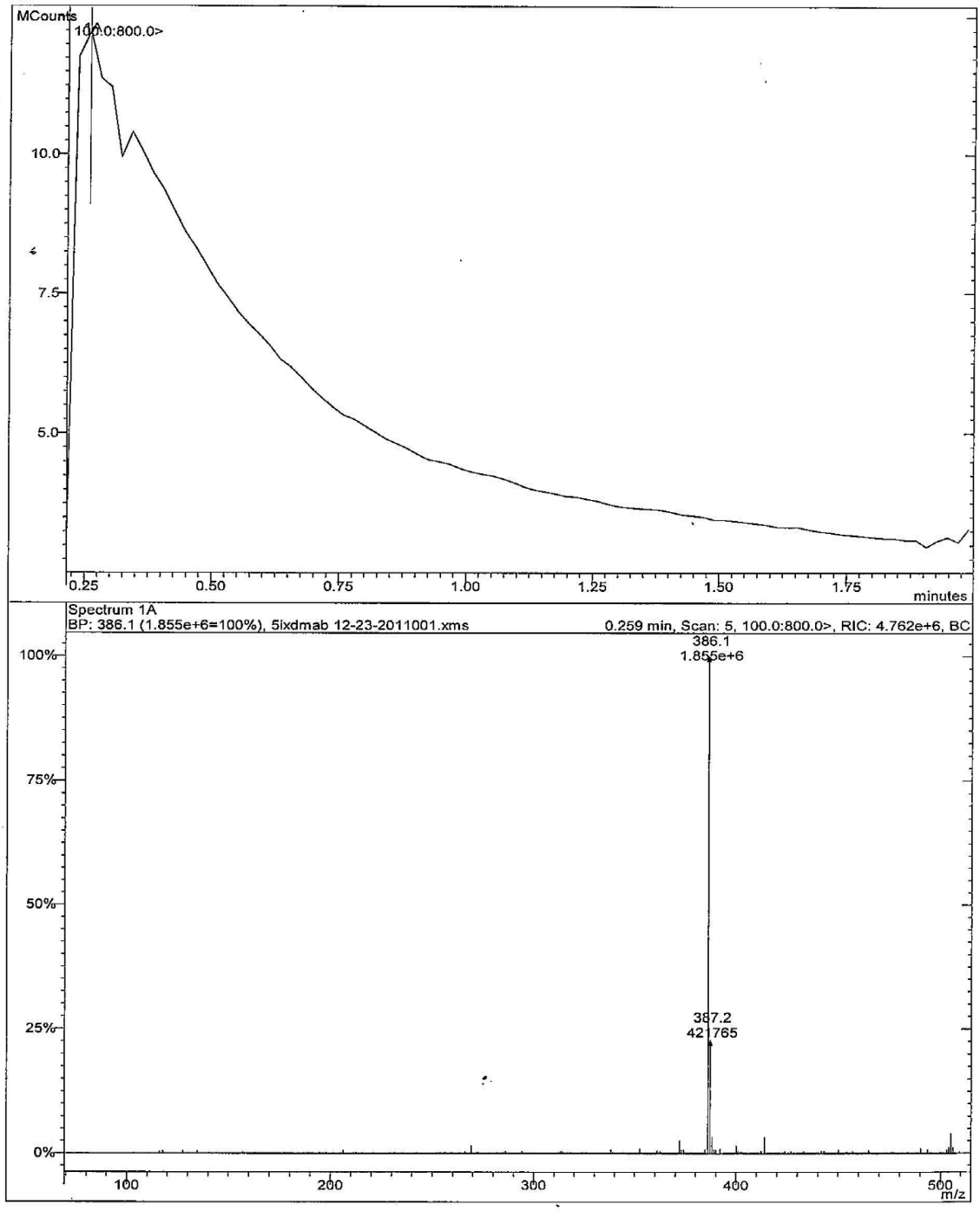


Figure S3. Direct Infusion LC-MS for **4j.**

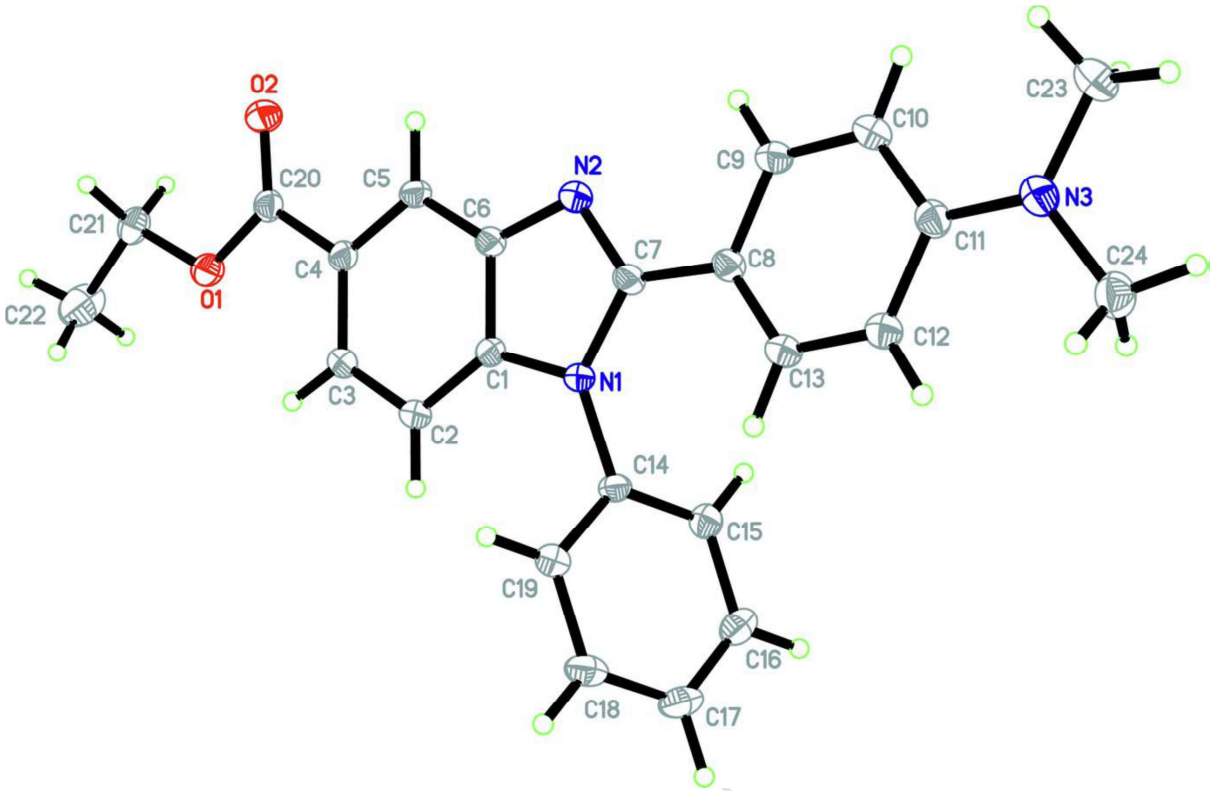


Figure S4. ORTEP diagram for **4j.**

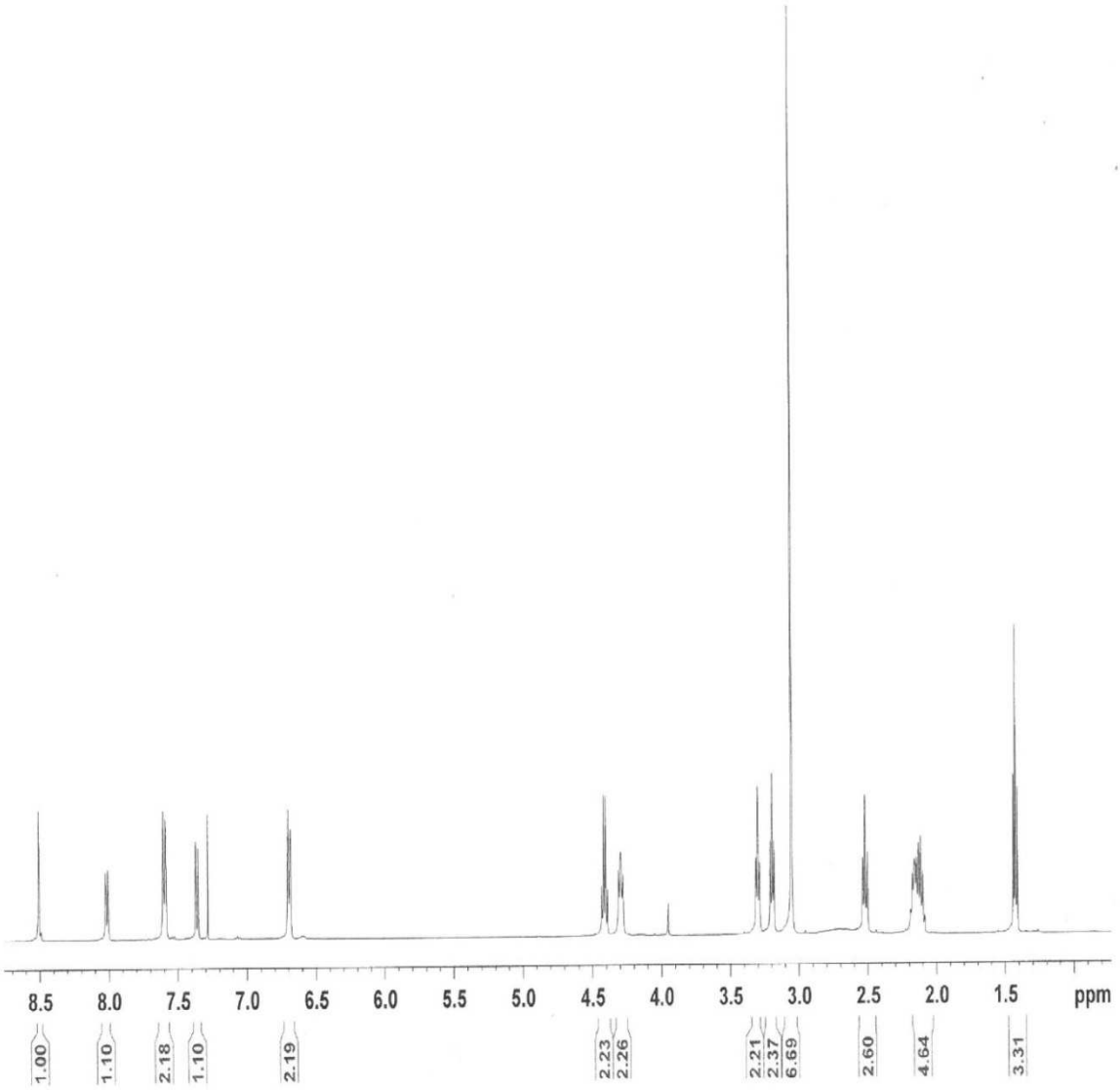


Figure S5. ^{1}H NMR for **4b.**

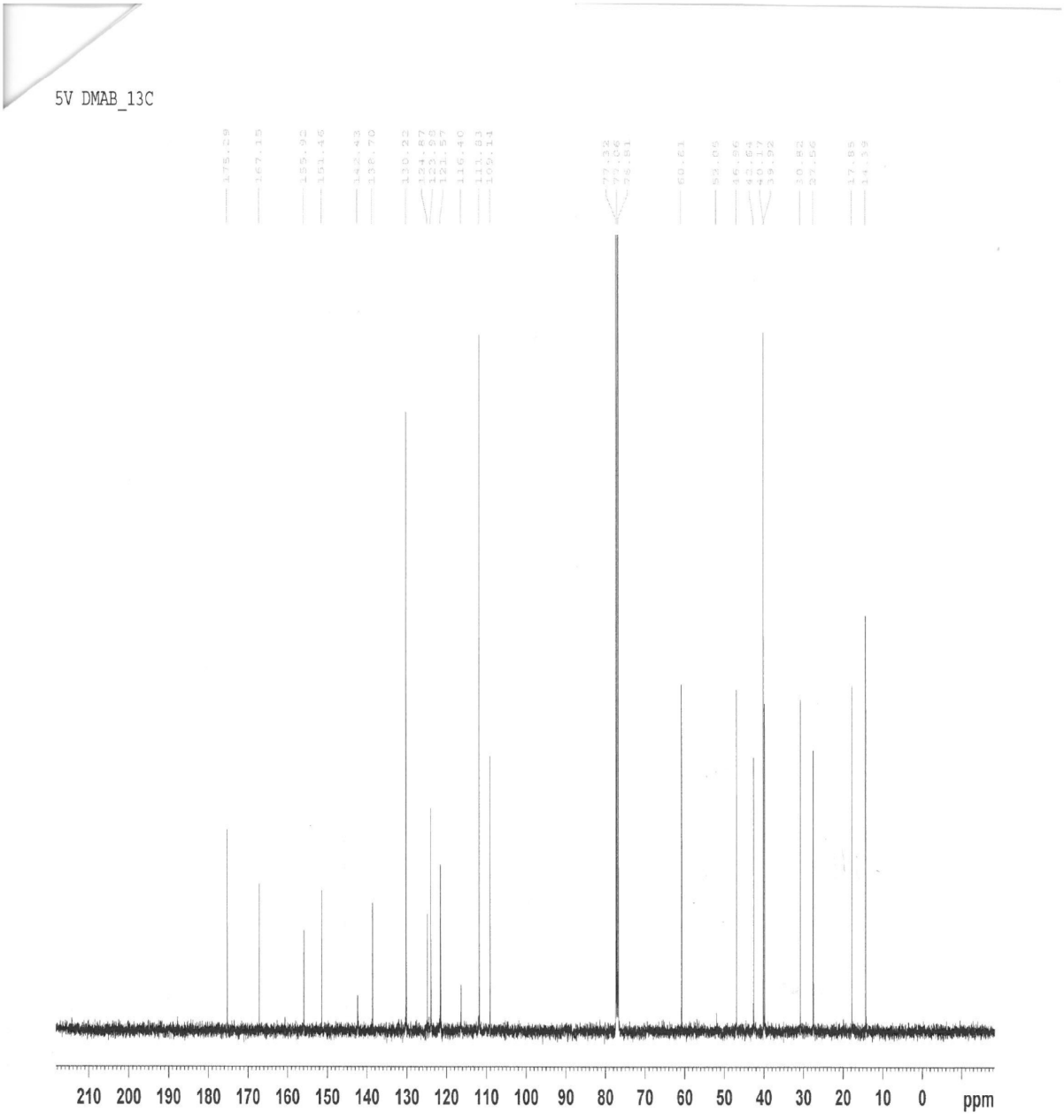


Figure S6. ^{13}C NMR for **4b**

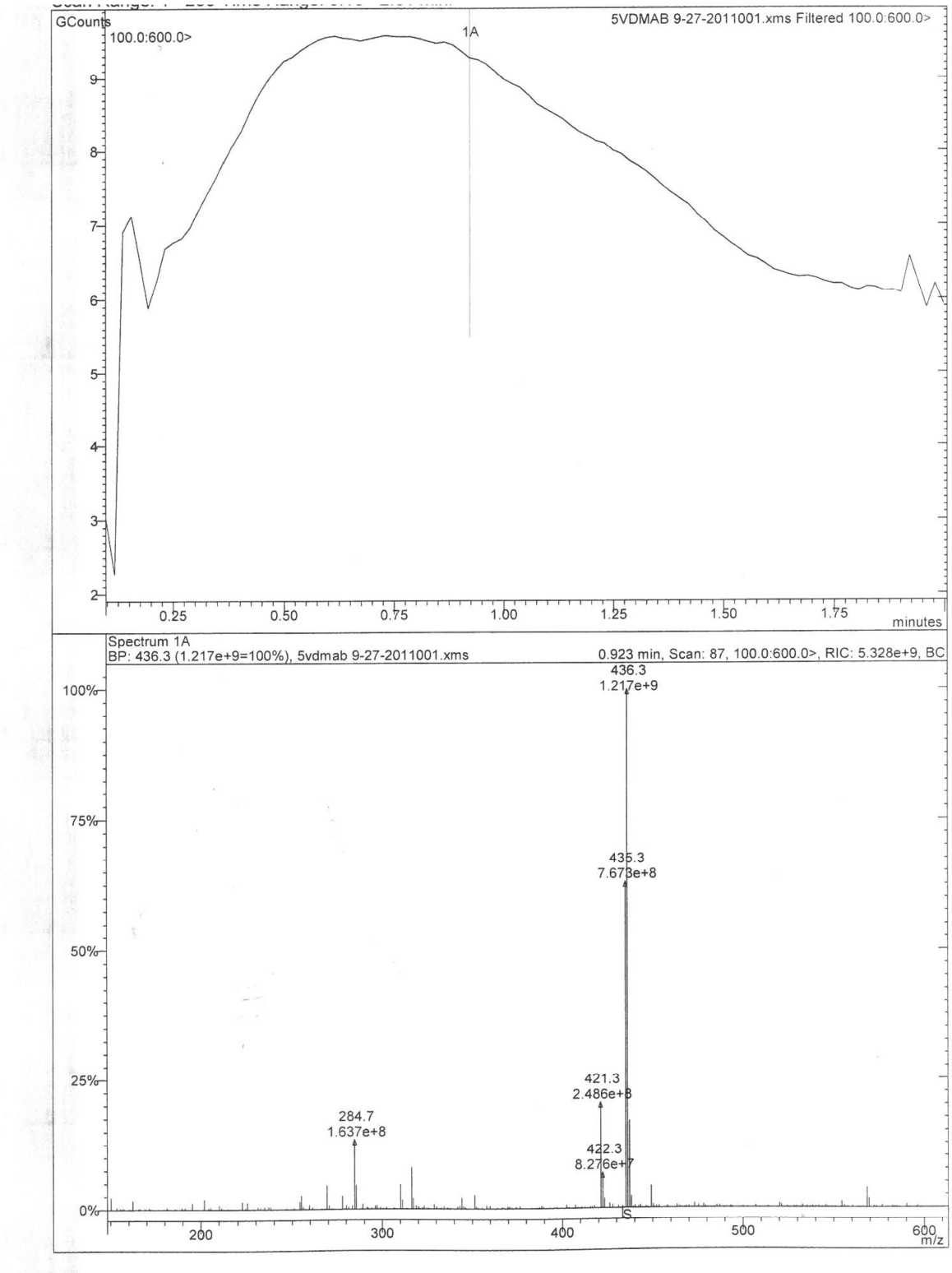


Figure S7. Direct Infusion LC-MS for **4b.**