

Synthesis and Characterization of 5-Bromo-6-(4-Chlorophenyl)-2-Propylimidazo[2,1-b][1,3,4]Thiadiazole A Potential Anti-Cancer Drug

Jouella Camille Q. Mercado^{1,*} Charlene Q. Ocampo¹, Glenn G. Oyong¹, Glenn V. Alea¹, and Faith Marie G. Laguna¹

¹Department of Chemistry, De La Salle University-Manila

*Corresponding Author: jouella_10@yahoo.com

Abstract: An imidazo[2,1-b][1,3,4]thiadiazole derivative, 5-bromo-6-(4-chlorophenyl)-2-propylimidazo[2,1-b][1,3,4]thiadiazole (**5**) was synthesized and characterized. The synthesis involved the preparation of an acylated thiosemicarbazide derivative (**3**) followed by dehydrocyclization with 2-Bromo-4'-chloroacetophenone to generate the imidazo[2,1-b][1,3,4]thiadiazole ring. This was then brominated to afford the target compound (**5**) in 76% yield. The identity of the synthesized compounds was confirmed via Mass Spectrometry (ESI-MS). The compound's potential anticancer activity was assessed with a PrestoBlue™ assay which showed a close cytotoxicity index with the standard anticancer drug (Bleomycin) on human colon adenocarcinoma grade II (HT-29) and human breast adenocarcinoma (MCF-7) cancer cell lines. It was also observed to possess lower toxicity towards normal cells. These results may be used to assess the structure activity relationship (SAR) of other derivatives with different alkyl chain lengths with respect to its anticancer activity.

Key Words: Imidazothiadiazole derivatives; Breast Cancer; Colon Cancer; Nucleophilic acyl substitution; Dehydrocyclization

1. INTRODUCTION

Cancer therapy has been a foremost concern in the past decade due to the prevalence of the disease, which has caused around 8.2 million deaths in 2012. (Stewart and Wild, 2014)

One of the goals of cancer research nowadays is to find treatment, which can both prevent the proliferation and metastasis of tumor cells, and at the same time have lesser harmful side effects on the patient's immune system.

The discovery of Levamisole as an effective drug in treating patients with small tumor burdens, has lead research to explore the potential of its structural analogs, imidazo[2,1-b][1,3,4]thiadiazole derivatives, to have similar biological activities with Levamisole, which is structurally similar to it.

Imidazo[2,1-b][1,3,4]thiadiazole derivatives have been reported to exhibit different pharmacological properties, which includes antibacterial (Alagawadi and Alegaon, 2011) antifungal (Dhepe et.al., 2011), antitubercular

(Alegaon et.al, 2012) and anti-inflammatory (Jadhay et.al, 2008) activities, among others. Past researches also presented the anticancer activity of imidazo[2,1-b][1,3,4]thiadiazole derivatives. One of the previous studies reported the cytotoxic activity of 2-aralkyl-5-substituted-6-(4'-fluorophenyl)-imidazo[2,1-b][1,3,4]thiadiazole on human T-cell leukemia cell line (Karki et.al., 2011). This study reported the cytotoxic activity of the compound manifested by apoptosis.

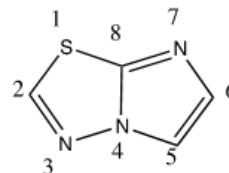


Fig 1. Imidazo[2,1-b][1,3,4]thiadiazole ring

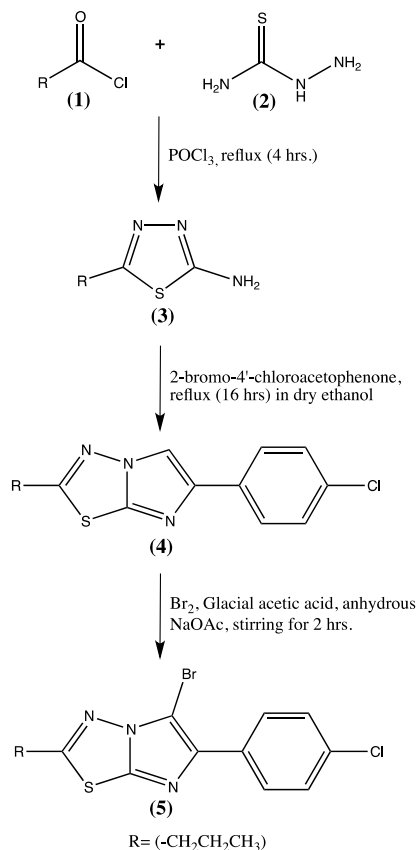
The aim of this research is to synthesize an imidazo[2,1-b][1,3,4]thiadiazole derivative (5) with an alkyl, bromine, and 4-chlorobenzene substituent at positions 2, 5, and 6 of the imidazo[2,1-b][1,3,4]thiadiazole ring (Figure 1) in the hopes that this compound will have potential anticancer activity.

This compound may also be used as part of a set of compounds for SAR studies to explore the effect of changing the substituent at the position 2 of the imidazo[2,1-b][1,3,4]thiadiazole ring to contain alkyl chains of varying lengths. This modification was based on a previous study that has shown that an imidazo[2,1-b][1,3,4]thiadiazole ring having a cyclopropyl group at position 2, a bromine atom at position 5 and a 4-chlorobenzene group at position 6 has the highest among a set of compounds towards a leukemic cancer cell line. (Noolvi, et.al., 2011)

2. RESULTS AND DISCUSSION

The variety of the biological activities discovered from the ring structure of imidazothiadiazoles has attracted research on further investigations on the role of several types of moieties which may be placed on the ring structure to improve the bioactivity of the molecule. In this study an imidazothiadiazole ring substituted with an alkyl, bromine and 4-chlorobenzene at positions 2,5, and 6 respectively was synthesized, characterized and tested for its cytotoxicity against normal cells, and a few cancer cell lines.

The synthesis of 5-bromo-6-(4-chlorophenyl)-2-propylimidazo[2,1-b][1,3,4]thiadiazole (5) was done in three steps as shown in Scheme 1. To synthesize compound (5), butanoyl chloride (1) was reacted with thiosemicarbazide (2) via Nucleophilic acyl substitution. The precursor compound, 5-propyl-1,3,4-thiadiazol-2-amine (3) generated was reacted with 2-Bromo-4-chloroacetophenone to allow the formation of the imidazo[2,1-b][1,3,4]thiadiazole ring with the 4-chlorobenzene substituent at position 6 of the ring. Bromination of 6-(4-chlorophenyl)-2-propylimidazo[2,1-b][1,3,4]thiadiazole (4) via electrophilic substitution produced the target compound (5) as shown in Scheme 1.



Scheme 1: Synthesis of 5-bromo-6-(4-chlorophenyl)-2-propylimidazo[2,1-b][1,3,4]thiadiazole (5).

2.1 Synthesis of 5-bromo-6-(4-chlorophenyl)-2-propylimidazo[2,1-b][1,3,4]thiadiazole (5)

The reaction produced an orange clay-like solid with a yield of 76.41%. The melting point range was 108-112°C. This shows the relative purity of the compound synthesized. The TLC of the compound showed a single spot with an R_f value of 0.364 (1Hexane:1CH₂Cl₂) which is less polar than its precursor compound (4). The mass obtained from mass spectrometry was three pseudomolecular ion peaks at m/z 356.0 [M+H]⁺, 358 [M+3]⁺, and 360 [M+5]⁺. These three pseudomolecular ion peaks show specific masses with the different isotopes of chlorine and bromine in different combinations in the molecule. The m/z 356.0 [M+H]⁺ is the combination of ³⁵Cl and ⁷⁹Br. The combination ³⁵Cl and ⁸¹Br or ³⁷Cl and ⁷⁹Br is for the m/z 358.0 [M+H]⁺. Lastly, m/z 360.0 [M+H]⁺ is for ³⁷Cl and ⁸¹Br. Since the

pseudomolecular peaks obtained coincide with the expected value of the calculated molecular weight: MF: $C_{13}H_{11}BrClN_3S$ [356.69 g/mole]., this confirms the identity of the target compound (5).

2.2 Determination of cell viability and cytotoxicity on normal and cancer cell lines

The cytotoxicity assay of the target compound (5) was carried out using PrestoBlue™ assay to evaluate the effect of 5-bromo-6-(4-chlorophenyl)-2-propylimidazo[2,1-b][1,3,4]-thiadiazole (5) on normal and cancer cell lines. Human dermal fibroblast (HDFn), human colon adenocarcinoma grade II (HT-29), and human breast adenocarcinoma (MCF-7) cell lines were treated with the target compound (5) in varying concentrations. Bleomycin, a known antitumor drug, was used as the positive control and the untreated wells as the negative control. The results were evaluated using BioTek ELx800 Absorbance Microplate Reader and by measuring the absorbance at 570 nm. The cytotoxicity index and IC_{50} were calculated by plotting the average amount of cells that were killed against the treatment concentration of the sample compound (5) in $\mu\text{g/mL}$.

On human dermal fibroblast cells, Bleomycin attained a cytotoxicity index higher than 50%, signifying a high percentage of death of normal cells. On the other hand, the target compound (5) only attained a cytotoxicity index much lower than 40%, which is indicative of a lower cytotoxicity of the target compound (5) towards normal cells compared to Bleomycin, a standard antitumor drug, as shown in Figure 2.

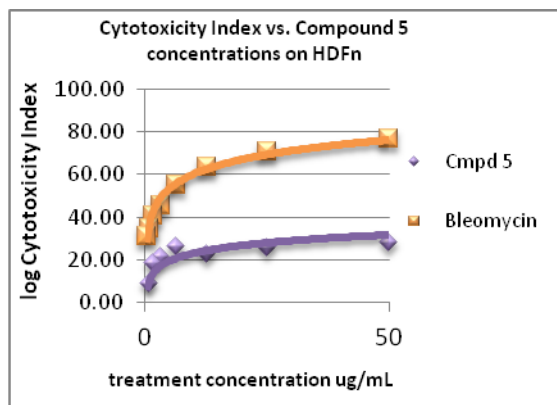


Fig 2. Cytotoxicity Profile of Compound (5) on human dermal fibroblast (HDFn) cells

On colon cancer cells, Bleomycin attained a cytotoxicity index higher than 70%. This indicates high effectiveness of the drug towards colon cancer cells. The target compound (5) showed a close cytotoxicity index to that of Bleomycin. The relative closeness of the values indicates a similar effectivity of the synthesized compound (5) with that of Bleomycin towards colon cancer cells. The IC_{50} was calculated to indicate the concentration required to inhibit/kill 50% of the cells' population. The IC_{50} for compound 5 is 12.029 while that of Bleomycin was 11.076. This shows a relatively higher concentration of compound 5 needed to inhibit the colon cancer cells as compared to Bleomycin. The cytotoxicity profile of the target compound (5) on colon cancer cells is presented in Figure 3.

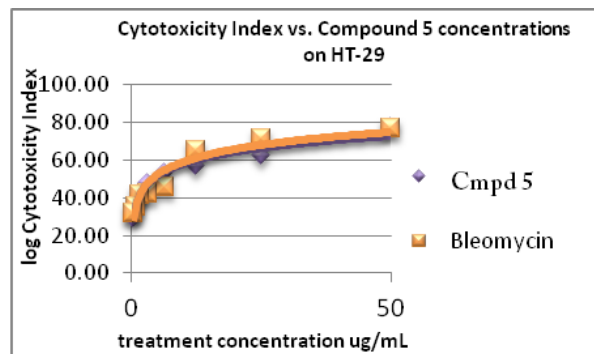


Fig 3. Cytotoxicity Profile of Compound (5) on colon cancer (HT-29) cells

On breast cancer cells, Bleomycin still has a higher cytotoxicity index than the target compound (5). However, the cytotoxicity index values of each are relatively close to one another. This signifies a relatively similar activity of the target compound (5) and Bleomycin against breast cancer cells. The IC_{50} calculated for compound (5) on breast cancer cells was 15.835 while Bleomycin's IC_{50} was 13.489. This indicates a higher concentration needed for compound 5 to inhibit/kill 50% of the breast cancer cells. The cytotoxicity profile of the target compound (5) on breast cancer cells is presented in Figure 4.

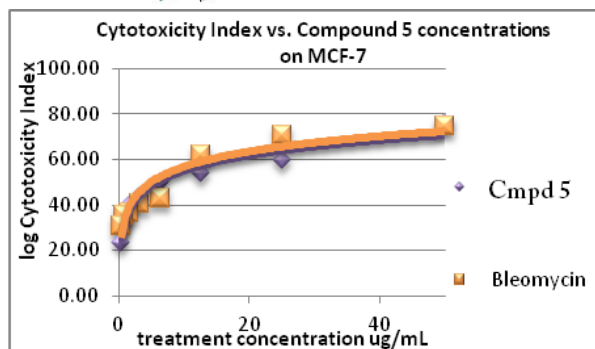


Fig 4. Cytotoxicity Profile of Compound (5) on breast cancer (MCF-7) cells

The results of the PrestoBlue™ assay confirms the potential of 5-bromo-6-(4-chlorophenyl)-2-propylimidazo[2,1-b][1,3,4]-thiadiazole (5) as a chemotherapeutic drug. The target compound (5) has relatively close cytotoxic activity on colon, and breast cancer cells compared to Bleomycin. The advantage of compound 5 over Bleomycin is its low cytotoxic effect on normal human cells. This result may pose the likelihood of 5-bromo-6-(4-chlorophenyl)-2-propylimidazo[2,1-b][1,3,4]-thiadiazole (5) as a better chemotherapeutic drug than Bleomycin. However, further tests must be carried out to further support this claim.

3. METHODOLOGY

The synthesis of 5-bromo-6-(4-chlorophenyl)-2-propylimidazo[2,1-b][1,3,4]thiadiazole (5) was done in three steps. For the first step, a 1,3,4-thiadiazole derivative was synthesized via nucleophilic acyl substitution followed by cyclization. This was proceeded with dehydrocyclization reaction to yield the heterocycle system, imidazo[2,1-b][1,3,4]-thiadiazole, with 4-chlorophenyl and propyl groups at 6th and 2nd position respectively. Electrophilic substitution was then done where bromine was attached to the 5th position of the heterocycle ring.

3.1 Synthesis of 5-propyl-1,3,4-thiadiazol-2-amine (3)

In a round bottom flask, thiosemicarbazide (0.914 g, 0.100 mmol) and butanoyl chloride (1.05 mL, 10.0 mmol) were mixed in the presence of phosphorus chloride (5 mL, 54.8 mmol) in nitrogen gas environment. The solution was refluxed for 4 hours with a constant temperature of 90-100°C. This resulted to a pale yellow solution with white precipitate (unreacted thiosemicarbazide). It was

stirred overnight for further completion of the reaction. Distilled water (14.5 mL) was added drop wise which formed a cloudy pale yellow colored solution and fumes. The solution was filtered via gravity filtration. The filtrate was neutralized with 0.558M potassium hydroxide (120 mL), producing a cloudy yellow solution with precipitate. The precipitate was filtered off via vacuum filtration, recrystallized with ethanol, and washed with cold petroleum ether. The product generated beige needle-like crystals (0.7392 g, 10.32%); R_f value: 0.517 (25EtOAc:1EtOH:1CH₃COOH); MP: 150-152°C; IR (KBr Disk): 3099.89 cm⁻¹ and 3271.30 cm⁻¹ (primary amine, stretch), 1644.50 cm⁻¹ (C=N); MS (m/z): 144.1 [M+H]⁺; MF: C₅H₉N₃S [MM: 143 g/mole]

3.2 Synthesis 6-(4-chlorophenyl)-2-propylimidazo[2,1-b][1,3,4]thiadiazole (4)

In a round bottom flask, compound 3 (0.500 g, 3.49 mmol), was reacted with 2-bromo-4-chloroacetophenone (0.818 g, 3.49 mmol) in the presence of dry ethanol (46 mL) under reflux for 16 hours with constant stirring at 90-95°C. This resulted to red orange solution with white precipitate. The amount of the solvent was reduced and the solution was further refluxed for 4 hours. The excess solvent was evaporated to produce the solid hydrobromide, which was obtained, filtered and suspended in water. Cold saturated sodium carbonate (10 mL) was used to get the expected free base, yielding to a dirty yellow precipitate. The volume of the cold sodium carbonate exceeded the required amount to neutralize the solution. This resulted to a basic solution with a pH of 11. The generated precipitate was filtered, washed with water, dried and recrystallized with cold ethanol to generate dirty brown crystals (0.5018 g, 51.76%); R_f value: 0.236 (1C₆H₁₄:1CH₂Cl₂); MP: 123-125°C; MS (m/z): 278.1 (³⁵Cl isotope) [M+H]⁺, 279.1 [M+2]⁺, 280.0 (³⁷Cl isotope) [M+H]⁺, 281.1 [M+4]⁺, 282.0 [M+5]⁺; MF: C₁₃H₁₂ClN₃S [MM: 277.8 g/mole]

3.3 Synthesis of 5-bromo-6-(4-chlorophenyl)-2-propylimidazo[2,1-b][1,3,4]thiadiazole (5)

In a round bottom flask, glacial acetic acid (0.180 mL, 0.360 mmol), anhydrous sodium acetate (0.0620 g, 0.756 mmol), and compound 4 (0.1007 g, 0.362 mmol) were mixed. The bromine (0.1 mL, 1.95 mmol) was added to the well-stirred mixture with constant stirring at room temperature. After which, stirring was continued for 2 hours. The mixture was then

poured into ice-cold water and was basified with cold sodium carbonate (4 mL, 96 mmol). An orange precipitate was formed. The precipitate was filtered, washed with cold ice water, and dried. The product generated an orange clay-like solid (0.0978 g, 76.41%); R_f value: 0.364($C_6H_{14}:1CH_2Cl_2$); MP: 108-112°C; MS (m/z): 356.0 ($^{35}Cl + ^{79}Br$) $[M+H]^+$, 358.0 ($^{35}Cl + ^{81}Br$) or ($^{37}Cl + ^{79}Br$) $[M+3]^+$, 360.0 ($^{37}Cl + ^{81}Br$) $[M+H]^+$, 281.1 $[M+4]^+$, 355.69 $[M]^+$; MF: $C_{13}H_{11}BrClN_3S$ [356.69 g/mole].

3.4 Determination of cell viability and cytotoxicity on normal and cancer cell lines

The effect of 5-bromo-6-(4-chlorophenyl)-2-propylimidazo[2,1-b][1,3,4]thiadiazole (5) was evaluated using PrestoBlue™ assay. The cell lines used in the determination of cell viability were human dermal fibroblasts (HDFn), human colon adenocarcinoma grade II (HT-29), and human breast adenocarcinoma (MCF-7). The concentrations of the extracts used were varied from 0.39 $\mu\text{g/mL}$ – 50 $\mu\text{g/mL}$.

The cells used were washed with phosphate-buffered saline (PBS) at pH 7.2. The cells were then resuspended with 100 μL culture media. Two-part dilution was carried out by pipetting first a 100 μL portion of extract to the first well followed by serial dilution. Three trials were performed per extract. Bleomycin was used as the positive control while the untreated wells were used as the negative control. The cells were incubated for 3 days at 37°C in a humidified atmosphere of 5% CO_2 prior to assay.

Each well containing the treated cells was added with 10 μL of PrestoBlue™ dye. The mixture was then incubated for 30 minutes to allow the conversion of resazurin to resorufin. BioTek ELx800 Absorbance Microplate Reader was used to measure the absorbance at 570 nm. The cytotoxicity index was calculated using the equation:

$$\% \text{ Cytotoxicity Index} = \{100 - [(A_{us} - A_m) / A_m]\} \times 100 \text{ (Eq 1)}$$

where: % Cytotoxicity Index = number of dead cells;
 A_{us} = absorbance of the untreated sample;
 A_m = absorbance measured per well

IC_{50} values were calculated by using the generated equation of the line from the cytotoxicity index plot. The value of x in the equation of the line was taken as the minimum concentration (in $\mu\text{g/mL}$)

of the sample compound (5), which can inhibit/kill 50% of the cells.

4. CONCLUSIONS

An imidazo[2,1-b][1,3,4]thiadiazole derivative, 5-bromo-6-(4-chlorophenyl)-2-butylimidazo[2,1-b][1,3,4]thiadiazole (5) was synthesized and characterized. The target compound was obtained in 76.41% yield. The synthesis involved the generation of an acylated thiosemicarbazide precursor, 5-propyl-1,3,4-thiadiazol-2-amine (3) which was generated in 10.32% yield. This was followed by dehydrocyclization with 2-Bromo-4'-chloroacetophenone to generate the precursor compound, 6-(4-chlorophenyl)-2-propylimidazo[2,1-b][1,3,4]thiadiazole (4) in 51.76% yield. Subsequent bromination of compound (4), allowed for the formation of the target compound (5). The synthesis had an overall yield of 4.1%.

This imidazo[2,1-b][1,3,4]thiadiazole derivative has potential anticancer activity as shown in the relatively close cytotoxicity index values with the standard antitumor drug used, Bleomycin. The lower cytotoxicity towards normal cells compared to that of Bleomycin is also an advantage of the synthesized compound (5). This derivative may also be used as part of SAR studies on the effect of various alkyl chains lengths as a substituent in position 2 of the imidazo[2,1-b][1,3,4]thiadiazole ring. The compound may also be tested for other biological activities such as antibacterial, anti-inflammatory, antitubercular, antifungal, activities which are similar to previously synthesized imidazo[2,1-b][1,3,4]thiadiazole derivatives.

The structure of the target compound may further be confirmed by determining its 1H -NMR and ^{13}C -NMR spectra.

5. ACKNOWLEDGMENTS

The group would like to acknowledge the University Research Coordinating Office (URCO) of De La Salle University (Manila, Philippines) for funding this research.

6. REFERENCES

Alagawadi, K. R.; Alegaon, S. G. (2011) Synthesis, characterization and antimicrobial activity evaluation of new imidazo[2,1-b][1,3,4]thiadiazole derivatives. *European Journal Medicinal Chemistry*. 2, 94-99.



- Alegaon, S. G.; Alagawadi, K. R.; Sonkusare, P. V.; Chaudhary, S. M.; Dadwe, D. H.; Shah, A. S. (2012). Novel imidazo[2,1-b][1,3,4]thiadiazole carrying rhodanine-3-acetic acid as potential antitubercular agents. *Bioorganic and Medicinal Chemistry Letters*, 22. 1917-1921.
- Dhepe, S.; Kumar, S.; Vinaykumar, R.; Ramareddy, S. A.; Karki, S.S. (2011) Microwave-assisted synthesis and antimicrobial activity of some imidazo[2,1-b][1,3,4]thiadiazole derivatives. *Medicinal Chemistry Research*, 21, 1550-1556.
- Jadhav, V. B.; Kulkarni, M. V.; Rasal, V. P.; Biradar, S. S.; Vinay, M. D. (2008) Synthesis and anti-inflammatory evaluation of methylene bridged benzofuranyl imidazo[2,1-b][1,3,4]thiadiazoles. *European Journal Medicinal Chemistry*, 43. 1721-1729.
- Karki, S. S.; Panjamurthy, K.; Kumar, S.; Nambiar, M.; Ramareddy, S. A.; Chiruvella, K. K.; Raghavan, S. C. (2011). Synthesis and biological evaluation of novel 2-aryl-5-substituted-6-(4'-fluorophenyl)-imidazo[2,1-b][1,3,4]thiadiazole derivatives as potent anti-cancer agents. *European Journal Medicinal Chemistry*, 46, 2109-2116.
- Noolvi, M. N.; Patel, H. M.; Singh, N.; Gadad, A. K.; Cameotra, S. S.; Badiger, A. (2011) Synthesis and anticancer evaluation of novel 2-cyclopropylimidazo[2,1-b][1,3,4]thiadiazole derivative. *European Journal Medicinal Chemistry*, 46. 4411-4418.
- Stewart, B. and Wild, C. (2014) Cancer. *World Health Organization*. Retrieved July 15 2014, from:
<http://www.who.int/mediacentre/factsheets/fs297/en/>.