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SYNTHESIS OF BENZAMIDE FRAGMENT OF POLO-LIKE KINASE 1 INHIBITOR BI 2536: NOVEL APPROACHES IN ADDRESSING NEGLECTED TROPICAL DISEASES

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ABSTRACT:

Neglected Tropical Diseases (NTDs) remain a significant global health challenge, disproportionately affecting impoverished populations in developing countries. These diseases often result in severe disabilities, socio-economic consequences, and a cycle of poverty. Recent advances in medicinal chemistry have introduced innovative treatment strategies, including targeting cellular pathways critical to parasite survival. Polo-like Kinases (PLKs) have emerged as promising targets due to their essential roles in cell-cycle regulation. This study explores the synthesis and evaluation of BI 2536, a potent PLK1 inhibitor, as a potential Alveolar Echinococcosis (AE) therapeutic agent. By employing fragment-based drug design and leveraging advanced synthetic methodologies, this research contributes to the growing arsenal of targeted therapies against NTDs, offering insights into novel chemotherapeutic pathways. Synthesis of benzamide fragment of polo-like kinase 1 inhibitor bi 2536 was achieved by employing the fragment-based synthetic methodologies.

Keywords: Neglected Tropical Diseases, Polo-like Kinases, BI 2536, Benzamide fragment, Synthesis

INTRODUCTION:

Neglected Tropical Diseases (NTDs) are a group of infectious diseases that predominantly affect rural and underserved communities in low-income countries. Despite their profound socio-economic and health impacts, NTDs have historically received limited attention compared to diseases such as tuberculosis and HIV/AIDS (Molyneux et al., 2013). Alveolar Echinococcosis (AE), caused by the parasitic cestode *Echinococcus multilocularis*, is one such NTDs. Characterized by tumor-like infiltrative growth primarily in the liver, AE presents significant treatment challenges due to its life-threatening nature and the limited efficacy of existing therapies (Hotez, P. J., et al, 2013).

Polo-like Kinases (PLKs) are serine/threonine kinases crucial for eukaryotic cell-cycle progression. Of the five human PLKs, PLK1 is the most extensively studied due to its role in mitotic entry and centrosome maturation (Schubert, T., et al, 2017). High expression of PLK1 in proliferating cells, including cancer cells, underscores its potential as a therapeutic target (Chen. Q., et al, 2018). Similarly, PLKs in parasitic flatworms, such as *E. multilocularis*, have shown promise as drug targets. BI 2536, initially developed for cancer therapy, has demonstrated potent PLK1 inhibition and selective activity, making it a compelling candidate for AE treatment (Long, T., et al, 2016).

This study aims to synthesize and evaluate BI 2536 analogues using fragment-based drug design and advanced synthetic methodologies. The work focuses on addressing key challenges in NTD drug development, including selectivity, efficacy, and structural optimization.

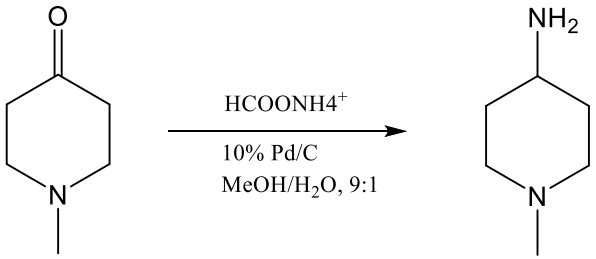
MATERIALS AND METHODS:

General Experimental Procedures: All reactions were performed in oven-dried glassware under an inert nitrogen (N_2) atmosphere. Anhydrous solvents were used as supplied without further purification. Nuclear Magnetic Resonance (NMR) spectra were recorded on a Bruker Ascend™ 500 MHz

spectrometer. Chemical shifts are reported in parts per million (ppm) and referenced to residual solvent peaks (CHCl3: δH 7.26, δC 77.2; DMSO: δH 2.50, δC 39.5). Thin-layer chromatography (TLC) was conducted on Merck silica gel plates (60 F254), visualized under Ultraviolet (UV) light. Purification of compounds was performed using column chromatography on silica gel (40-63 μm).

Synthesis of 1-Methylpiperidin-4-amine (2):

1-Methyl-4-piperidone (5 g, 44.2 mmol) was dissolved in methanol (24 mL), followed by the addition of ammonium formate (2.58 g, 83.66 mmol). Water (2.7 mL) was then introduced, and the reaction mixture was stirred vigorously for 15 minutes. After complete dissolution, 10% palladium on carbon (Pd/C; 1.08 g) was added, and the reaction was allowed to proceed overnight at room temperature. Thin layer chromatography (TLC) (eluent: EtOH/NH4OH, 8:2) confirmed reaction completion. The catalyst was removed by filtration through Celite™, and the filtrate was concentrated under reduced pressure to yield a dark yellow oil (8.3 g, 72.3%).

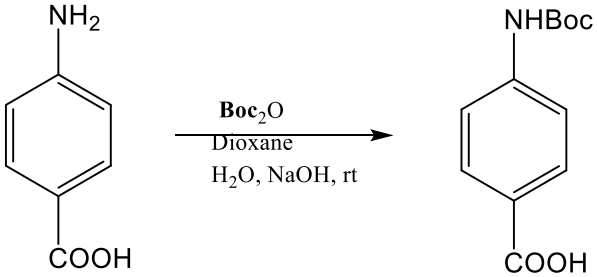


1 **2**

1H NMR (500 MHz, CDCl3): δ 2.82 (t, J = 11.2 Hz, 2H), 2.66 (d, J = 11.8 Hz, 1H), 2.27 (s, 1H), 2.17 (s, 2H), 1.87-1.79 (m, 2H, NH2), 1.46-1.36 (m, 2H).
13C NMR (126 MHz, CDCl3): δ 54.72, 46.15, 41.05, 33.12.

Synthesis of 4-[(tert-butoxycarbonyl) amino] benzoic acid (4):

To a solution of 4-aminobenzoic acid (5 g, 36.5 mmol) in dioxane/water (2:1, 100 mL), NaOH (1.45 g, 36.5 mmol) was added. Di-tert-butyl dicarbonate (Boc2O, 12 g, 55 mmol) was introduced, and the mixture was stirred at room temperature for 24 hours. The solvent was removed under reduced pressure, and the residue was dissolved in water (200 mL). The solution was acidified to pH 3 with 1M HCl, and the precipitate was filtered and dried under vacuum at 50 °C. Excess Boc2O was removed by trituration with dichloromethane, yielding a white solid (8.5 g, 92%).



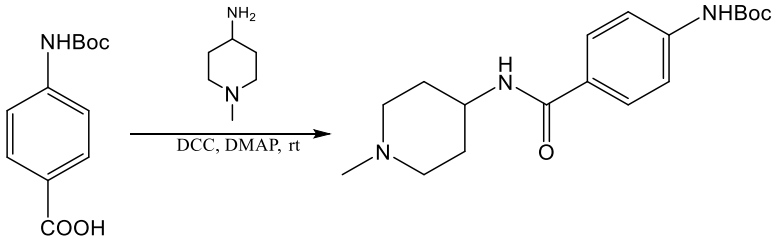
3 **4**

1H NMR (500 MHz, DMSO): δ 9.74 (s, 1H), 7.86 (d, J = 8.7 Hz, 2H), 7.58 (d, J = 8.8 Hz, 2H), 1.50 (s, 9H).
13C NMR (126 MHz, DMSO): δ 207.37, 153.17, 130.47, 117.37, 39.86, 28.46.

Synthesis of 4-[N-(tert-butoxycarbonyl) amino]-N-(4-N-methylpiperazinyl) benzamide (6):

4-[(Tert-butoxycarbonyl) amino] benzoic acid (2.0 g, 8.4 mmol) was dissolved in dichloromethane (50 mL). Dicyclohexylcarbodiimide (DCC, 1.9 g, 9.2 mmol) and dimethylaminopyridine (DMAP, 0.2 g, 1.6 mmol) were added, and the mixture was stirred for 15 minutes. 1-Methylpiperidine-4-

amine (1.0 g, 8.7 mmol) was then introduced, stirring the reaction overnight. TLC (eluent: MeOH/DCM, 9:1) confirmed reaction completion. The catalyst was filtered, and the solvent was removed using a rotary evaporator, yielding a solid compound (1.21 g, 79%).



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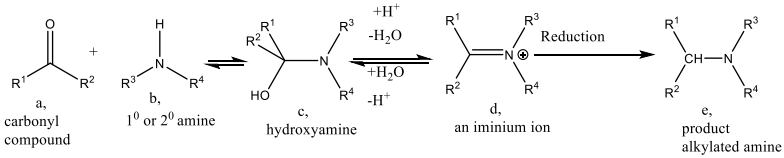
1H NMR (500 MHz, CDCl3): δ 8.37 (s, NH), 7.73 (d, J = 8.6 Hz, 2H), 7.55 (d, J = 8.6 Hz, 2H), 4.17 (m, J = 15.0, 7.2 Hz, 1H), 2.24-2.13 (m, 4H), 1.56 (s, 9H).
13C NMR (126 MHz, CDCl3): δ 156.84, 130.85, 53.44, 49.20, 33.95, 28.33, 25.63.

RESULTS AND DISCUSSION:

Reductive Amination

Reactions involving ketones or aldehydes with ammonia, primary amines, or secondary amines in the presence of reducing agents to yield primary, secondary, or tertiary amines, respectively, are known as reductive aminations or reductive alkylations. These reactions are among the most useful tools for synthesizing various amines. Initially, an intermediate called the carbinol amine forms and dehydrates to create an imine. The reaction usually occurs under weakly acidic to neutral conditions, resulting in the protonation of the imine to form an iminium ion.

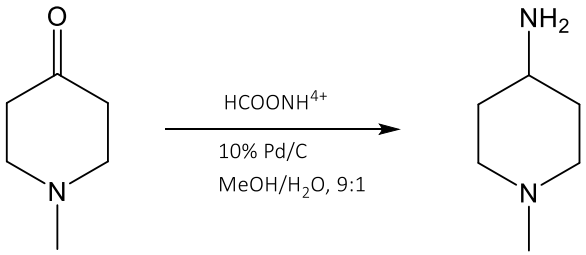
Thus, the reduction of the iminium ion produces the desired alkylated amine. However, some studies suggest a direct reduction of the carbinol amine as a potential pathway. The choice of reducing agent is crucial, as it must selectively reduce amines or iminium ions over ketones or aldehydes.



Scheme 1: Proposed general mechanism for reductive amination.

Synthesis of 1-methylpiperidine-4-amine

Reductive amination is a significant method for preparing 1-methylpiperidine-4-amine, using readily available 1-methylpiperidone as the starting material. Numerous studies have outlined procedures for synthesizing 1-methylpiperidine-4-amine through reductive amination of a ketone, resulting in high yields. The reaction is straightforward, utilizing ammonium formate, methanol, and a palladium catalyst (10% Pd/C). This catalyst was chosen to avoid the high temperatures associated with the Leuckart reaction. The advantages of using 10% Pd/C include its affordability, availability, and ease of filtration.



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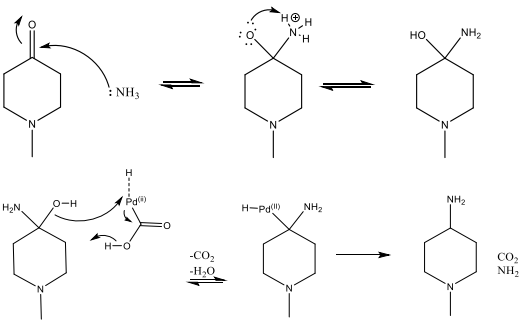


Scheme 2: Proposed catalytic hydrogenation mechanism for reductive amination using Palladium.

The reductive amination was performed by dissolving 1-methyl-4-piperidone and ammonium formate in methanol. After stirring with Pd/C overnight, filtration and solvent removal yielded the product, identified by the chemical shifts of the methylene groups, signals E and F, in figure 3, coupled to a new signal for the CHN methine.

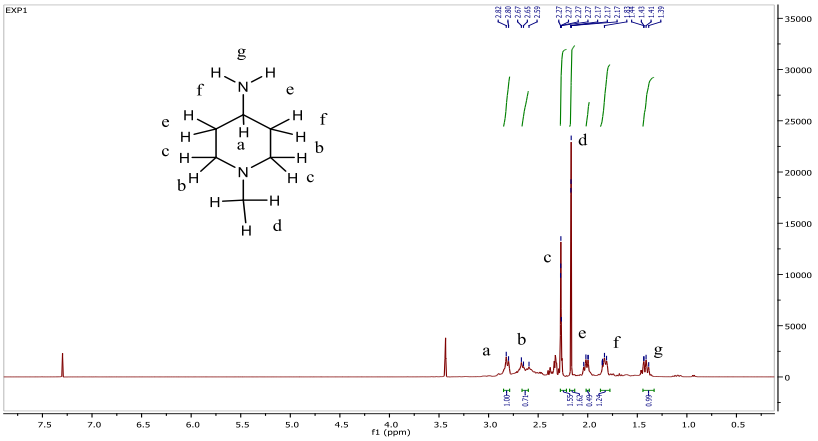


Figure 1: HNMR of 1-methylpiperidine-4-amine.

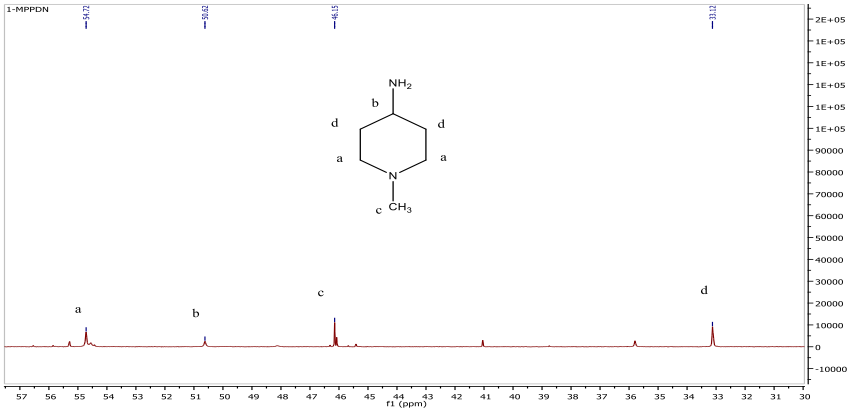


Figure 2: CNMR of 1-methylpiperidine-4-amine.

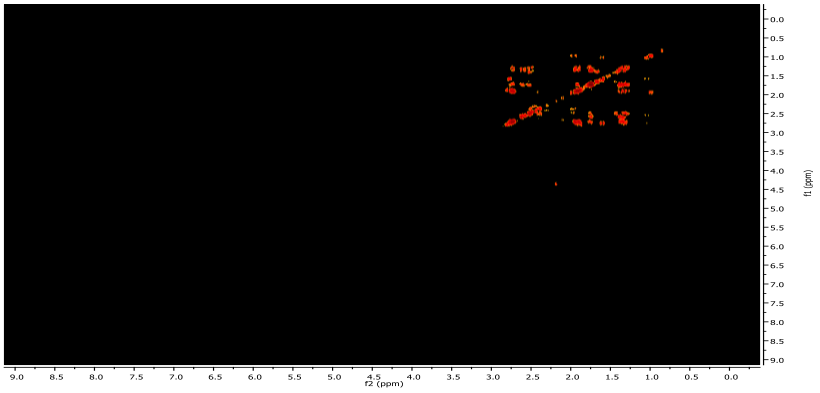
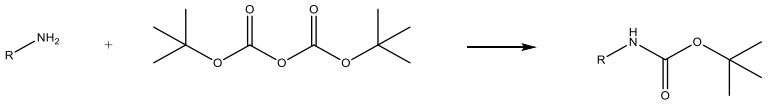


Figure 3: Cosy spectrs of 1-methylpiperidin-4-amine

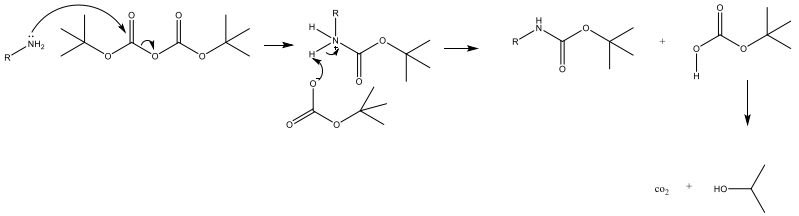
Boc Protection

The formation of Boc-protected amines and amino acids can occur under aqueous or anhydrous conditions, by reacting with a base and the anhydride Boc2O. Active esters and other derivatives like Boc-ONH2 and Boc-N3 can also be used. The Boc group is stable against most nucleophiles and bases,

allowing for an orthogonal protection strategy using a base-labile protection group like Fmoc. Tert-Butyl carbamates are cleaved under anhydrous acidic conditions, producing tert-butyl cations. Scavengers such as thiophenol may prevent alkylation of nucleophilic substrates.



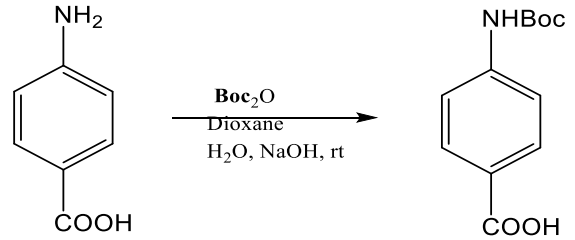
Reaction for Boc-protection



Scheme 3: Proposed mechanism of Boc protection.

Synthesis of 4-[(tert-butoxycarbonyl) amino] benzoic acid

The base-catalyzed procedure, as employed by (Qin, X., et al., 2014) was chosen for synthesizing building block 4. The reaction was performed by stirring overnight in an aqueous dioxane mixed solvent system, with sodium hydroxide as the base. The product was precipitated by acidification, and excess Boc anhydride was removed by trituration, yielding a near-quantitative 92% yield. A change in the HNMR spectrum was observed, with signals shifting from 7.68 ppm and 6.67 ppm to 7.84 ppm and 7.56 ppm compared to the starting material.



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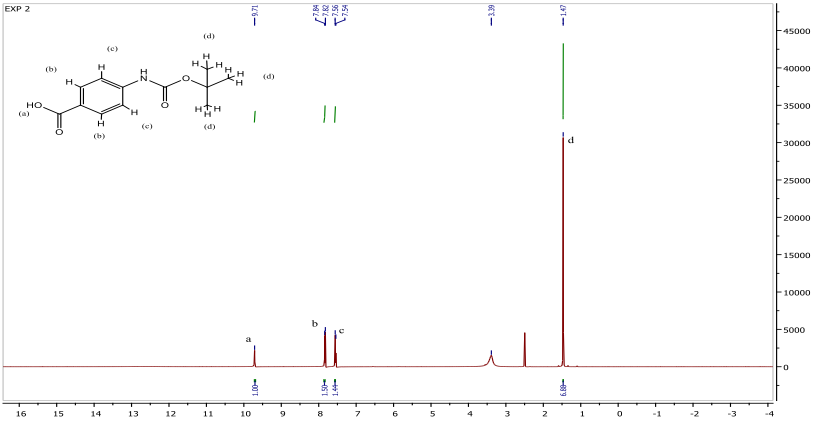


Figure 4: HNMR of 4-[(tert-butoxycarbonyl) amino] benzoic acid.

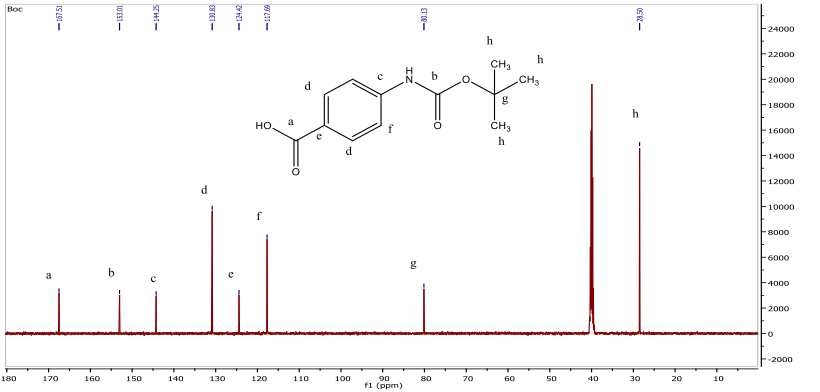
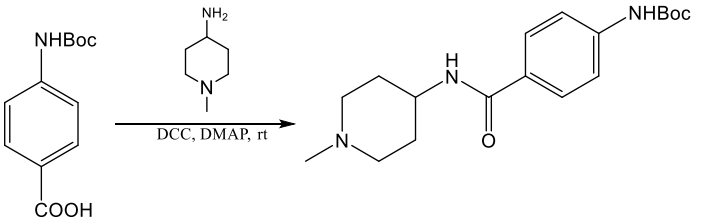


Figure 5: CNMR of 4-[(tert-butoxycarbonyl) amino] benzoic acid.

Synthesis of 4-[N-(tert-butoxycarbonyl) amino]-N-(4-N methylpiperazinyl) benzamide

The synthesis of 4-[N-(tert-butoxycarbonyl) amino]-N-(4-N methylpiperazinyl) benzamide involves coupling the two building blocks 2 and 4 to form an amide. Due to the importance of amide (peptide) bonds in protein synthesis, various coupling reagents are available for joining amines and carboxylic acids. DCC is a commonly used reagent. The reaction, performed initially for BI 2536, afforded a 79% yield of the product. Compared to the precursor carboxylic acid 4, the carbonyl CO group of the amide shifted to 1711.78 cm-1, distinct from the Boc CO at 1163.25 cm-1.



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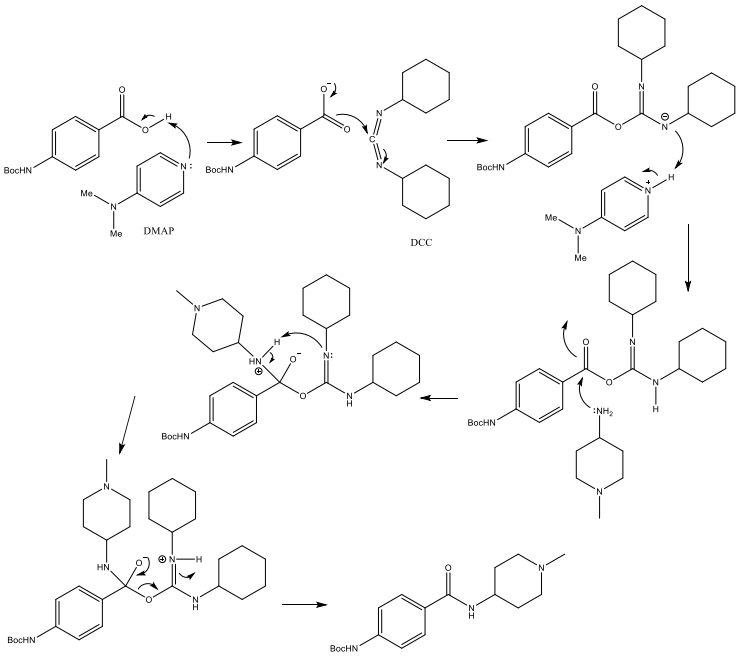

Scheme 4: Proposed mechanism of reductive amination with DCC as a catalyst.

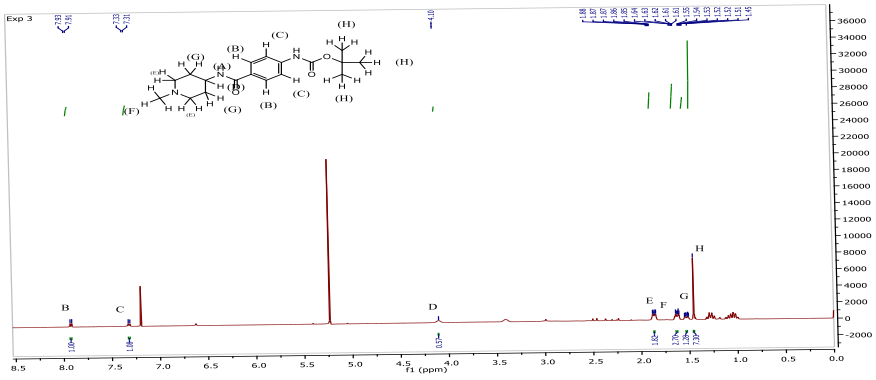

Figure 6: HNMR of 4-[N-(tert-butoxycarbonyl) amino]-N-(4-N methylpiperazinyl) benzamide.

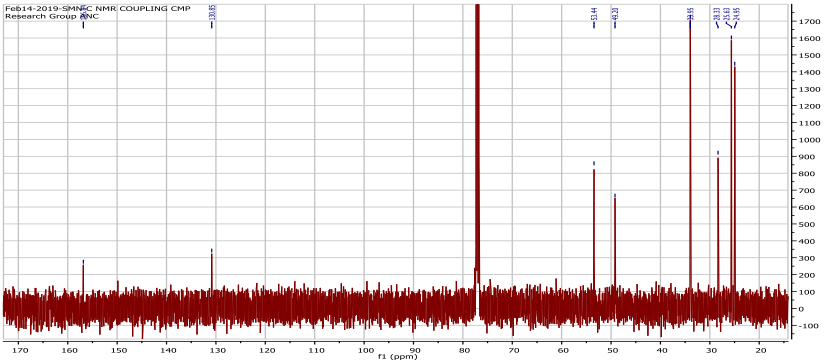

Figure 7: CNMR of 4-[N-(tert-butoxycarbonyl) amino]-N-(4-N methylpiperazinyl) benzamide.

Following the confirmation of the N,N'-dicyclohexylcarbodiimide (DCC) procedure with the preparation of compound 5, the reaction will be repeated with various commercially available amines (cyclopentylamine, allyl amine, benzylamine, 3-bromobenzylamine, 4-methoxybenzylamine, and 2-aminopyridine) to create a library of amides corresponding to fragment A.

CONCLUSION

The synthesis of 4-[N-(tert-butoxycarbonyl)amino]-N-(4-N methylpiperazinyl) benzamide was successfully achieved through a series of well-defined reactions, including reductive amination and Boc protection. Reductive amination was efficiently employed to produce 1-methylpiperidine-4-amine, with the use of ammonium formate and a palladium catalyst proving to be effective and convenient. The subsequent Boc protection reaction was also performed successfully, yielding 4-[(tert-butoxycarbonyl)amino]benzoic acid with high purity and efficiency. Finally, the coupling of the two building blocks using N,N'-dicyclohexylcarbodiimide DCC as a coupling reagent resulted in the desired amide with a good yield.

All the reactions are confirmed to be correct from the analysis obtained from figure 1-7above. The overall process demonstrated the viability and practicality of the chosen synthetic route for producing the target compound, hence paving a way to a total synthesis of Polo-like kinase 1 inhibitor BI 2536.

Limitations

Choice of Reducing Agent: The choice of reducing agent is crucial in reductive amination to ensure selective reduction of amines or iminium ions over ketones or aldehydes. Any deviation in this selectivity could lead to unwanted by-products.

Reaction Conditions: The reductive amination and Boc protection reactions require specific reaction conditions, such as controlled temperature, pH, and solvent systems. Any variations in these conditions could affect the reaction yield and purity of the products.

Catalyst Efficiency: The efficiency of the palladium catalyst (10% Pd/C) used in the reductive amination reaction could be affected by factors such as catalyst poisoning or degradation over time. Consistent catalyst performance is essential for obtaining high yields.

Product Purity: Achieving high purity of the final products is critical for accurate characterization and application. Impurities or incomplete reactions could complicate the isolation and analysis of the target compounds.

Scalability: While the reactions were successful on a laboratory scale, scaling up the synthesis for industrial production might pose challenges related to reaction control, equipment, and cost-effectiveness.

Environmental and Safety Concerns: The use of certain reagents and solvents in the synthesis poses environmental and safety concerns. Proper waste management and adherence to safety protocols are essential to minimize the environmental impact and ensure the safety of laboratory personnel.

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