Potential Nec2/Nek1 inhibitor and INH1 analog N-[4-(2,4-Dimethylphenyl)-2-thiazolyl]-4-pyridinecarboxamide via Hantzsch Thiazol Condensation

Isabella G. McDonald[†]; Lukas D. Shelton[†]; Robert E. Lee[†*]; Paige E. Heiple[‡]

[†] *Department of Chemistry and Physics, Bob Jones University, Greenville, SC 29614, USA*

[‡] *Cambrex High Point, High Point, NC 27265*

The potential active pharmaceutical ingredient (API), **N-[4-(2,4-Dimethylphenyl)-2-thiazolyl]-4-pyridinecarboxamide** (6) is a cancer cell metabolism mitosis inhibitor via the Nek2 and Hec1 enzymes. The three-step synthesis starts with the bromination of 1-(2,4-dimethylphenyl)ethan-1-one (1) using $CuBr_2$ to produce 2-bromo-1-(2,4-dimethylphenyl)ethan-1-one (2) with a yield of 90%.[1,2,3] Followed by Hantzsch thiazol ring synthesis as (2) reacts with thiourea (3) resulting in 4-(2,4-dimethylphenyl)thiazol-2-amine (4)[4] in 80% yield. Finally, isonicotinoyl chloride hydrochloride (5) and (4) reacted to form the amide (6).[5,6,7,8,9,10] Characterization of the final product was determined by HPLC, GCMS, ^{1}H-NMR, FTIR, and mp with a crude yield of 60%.

Key Terms: Nec2/Nek1 inhibitor, INH1 analog CAS# 560103-80-2, bromination, Hantzsch thiazol condensation, amidation

Introduction

This research target was enzyme inhibitor (6) as shown in Scheme 1. To support the intermediate and final reaction product characterization, Spartan '20[11] molecular modelling program was used to model ^{1}H NMR and FTIR spectra with good results. The chemical transformations that occurred are described below.

Bromination.[1,2,3] The bromination of the alpha position of the ketone (1) to form (2) was accomplished using $CuBr_2$ with its characteristic green solution color that dissipated to yield a white precipitant assumed to be CuBr.

Hantzsch Thiazole Synthesis.[4] The Hantzsch thiazole synthesis was performed using (2) and thiourea (3), which allowed for the formation of (4) with its thiazole ring and primary amine. Literature indicated the thiazole ring heavily contributes to the function of this inhibitor.[12] The nucleophilic displacement of bromine by thiourea's sulfur followed by amine attack of the carbonyl group and dehydration and rearrangement led to the thiazole ring in (4).

Amidation. Nucleophilic acyl addition of the primary amine of (4) reacted with an acid chloride derivative (5) followed by loss of HCl yielded (6).

Methods and Results

(2) Procedure

0.3893 g (2.6 mmol) of (1) were added to a 50-mL round-bottom flask along with 1.0847 g (4.9 mmol) of $CuBr_2$, 25 mL of EtOAc, and a stir bar. This dark green mixture was allowed to reflux under nitrogen at 80°C for 24 hours. White solid precipitated out almost immediately, and the green reaction mixture turned yellow. The flask was removed from the heat and allowed to cool. The contents of the flask were then filtered using a glass funnel. The solution was transferred to a separatory funnel and washed with deionized water three times. Decolorization occurred with the addition of water. The organic layer was dried using $MgSO_4$. The solvent was removed under vacuum (60°C, 155 rpm, ≈ 15 mbar). The dry product was removed from the flask using a few mL of EtOAc to dissolve it before pouring it onto a watch glass to recrystallize.

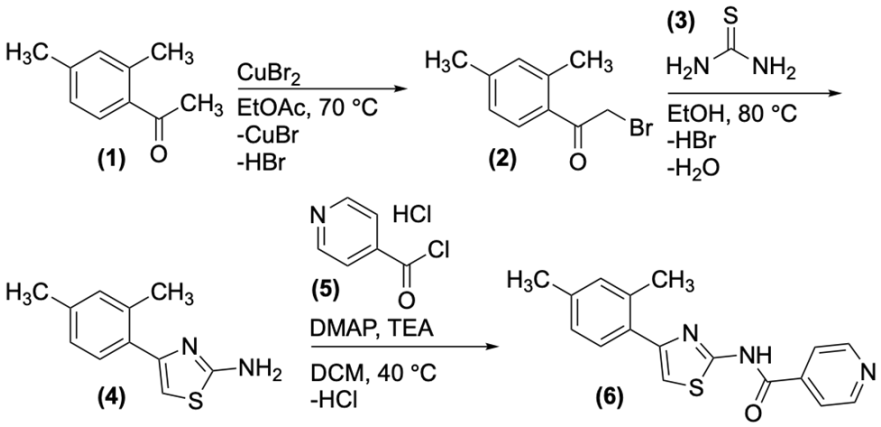
(2) Characterization

TLC using 3:1 DCM/n-heptane gave the Rf value for (1) as 0.82 and the Rf value for (2) as 0.70. The percent yield of the bromination step averaged over 90%, which is comparable to the anticipated 88%.[2]

The IR spectrum for (2) was calculated using Spartan '20 software[11] and compared to the experimental FTIR spectrum of (2) using a Nicolette 3800 IR Spectrometer, (Table 1). ^{1}H NMR of (2) in $CDCl_3$ using the Bruker F80 was compared to the Spartan '20 calculated spectra as shown in Table 2.

(4) Procedure

0.40 g (1.8 mmol) of (2) and 0.14 g (1.8 mmol) of (3) were added to a round-bottom flask and dissolved in 6 mL EtOH. The reaction mixture was refluxed at 80°C for 30 mins. Solvent was removed under vacuum (60°C, 155 rpm, ≈ 15 mbar). A saturated aqueous solution of sodium bicarbonate was added, and the mixture was transferred to a separatory funnel. The mixture was then washed with 30 mL of DCM, the phases were separated, and the organic layer was dried with $MgSO_4$. Solvent was removed under vacuum (40°C, 155 rpm, 15 mbar). DCM (3 mL) was added to the flask to solvate the product before transferring onto a watch glass to crystallize.



Scheme 1: Overall synthesis of (6).

Table 1: Key experimental and calculated IR Spectra peaks for (2)

Feature	Experimental Wavenumber, cm^{-1}	Calculated Wavenumber, cm^{-1}
Sp^3 C-H	3009	3022
Sp^3 C-H	2968	2977
Sp^3 C-H	2922	2910
C=O	1687	1735
Benzene ring	1609	1604

Table 2: ^{1}H NMR Spectra (2) in $CDCl_3$

Calculated Chemical Shift	Experimental Chemical Shift	Integration
2.34	2.28	s, 3 H
2.72	2.43	s, 3 H
4.60	4.32	s, 2 H
7.09	7.03	d, 2 H
7.54	7.48	d, 1 H

(4) Characterization

The melting point was determined to be 94-95°C, (85°C ref). TLC 1:1:1 EtOAc/DCM/n-heptane gave Rf of (2) at 0.91 and the Rf of (4) at 0.65. The IR spectrum for (4) was calculated[11] and compared to the FTIR spectrum (4) in Table 3. Table 4 summarizes the ^{1}H NMR results for (4) and compares the experimental spectrum and calculated model.

(6) Procedure

To prepare (6), (4) (0.33 g) (1.76 mmol), DMAP (0.49 g) (4.01 mmol), were mixed in 20 mL DCM at room temperature. (5) (0.38 g) (2.13 mmol) and TEA (0.98 g) (9.69 mmol) was added after 10 minutes of mixing. The round-bottom flask was then placed under reflux at 40°C for 72 hours. The reaction mixture was washed with a saturated aqueous solution of sodium bicarbonate, and then the organic layer was dried with $MgSO_4$. The final product was isolated under vacuum and then purified via silica gel chromatography 1:1:1 EtOAc/DCM/n-heptane.

(6) Characterization

TLC using 1:1:1 EtOAc/DCM/n-heptane gave Rf (4) 0.65 and the Rf (6) 0.34. The melting point of (6) was determined to be 139-140°C (150°C ref.)[5] The IR spectrum for (6) was calculated and compared to the experimental FTIR spectrum in Table 5. The NMR spectrum of (6) was calculated and compared to the experimental spectrum in Table 6. (6) was evaluated by Agilent GCMS (4.0-minute 40°C, 40°C/minute ramp to 300ºC, 10-minute hold) retention time 13.727-minutes with parent ion m/z 309.20 consistent with (6). Under the same conditions (4) had retention time 10.156-minutes with parent ion m/z 204.10 consistent with expectations.

Discussion

FTIR, ^{1}H NMR, TLC, and melting point were used to characterize the product of the bromination step, which matched the calculated and literature values of (2) and (4). Unlike previous research on the synthesis of (6), synthesis took several days under a reflux environment, not 20 minutes.[3] GCMS, FTIR, ^{1}H NMR, TLC, and melting point were used to characterize the product of the amidation step, which matched the calculated and literature values of (6). This indicates that (6) was successfully synthesized. Purification by column chromatography remains to be optimized. Several batches were combined and run together on a column and determined to be 99% pure by HPLC. Crude yields were on the order of 60% by weight.

Table 3: Calculated and experimental IR Spectra of (4).

Feature	Calculated Wavenumber, cm^{-1}	Experimental Wavenumber, cm^{-1}
Aromatic amine	1279	1294
Aromatic ring	1524	1559
N-H	1596	1673
sp^2 C-H	2890	2855
sp^3 C-H	2973	2924

Table 4: ^{1}H NMR Spectra (4) in $CDCl_3$

Ref	Calculated Chemical Shift	Experimental Chemical Shift	Integration
2.35	2.39	2.32	s, 3 H
2.42	3.55	2.39	s, 3 H
5.29	6.15	5.36	br, 2 H
6.42	6.99	6.36	s, 1 H
7.06-7.03	7.10	7.04	m, 2 H
7.43	NA	7.46	d, 1H

Table 5: Experimental and calculated IR Spectra (6)

Feature	Experimental Wavenumber, cm^{-1}	Calculated Wavenumber, cm^{-1}
sp^2 C-H	3028	3032
sp^3 C-H	2924	2950
C=N	1573	1541
Aromatic C-H	1559	1481

Table 6: ^{1}H NMR Spectra (6) in $CDCl_3$[13]

Reference	Experimental Chemical Shift	Integration
2.21	2.21	s, 6 H
6.79-6.76	6.81	m, 2 H
6.94	6.94	s, 1 H
7.08	7.05	d, 1 H
7.38	7.36	d, 2 H
8.53	8.57	d, 2 H
12.96	unobserved	s, 1 H, br

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*Corresponding author email: rlee@bju.edu

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