N-(4-(2,4-dimethylphenyl)thiazol-2-yl)isonicotinamide via Hantzsch Thiazol Condensation

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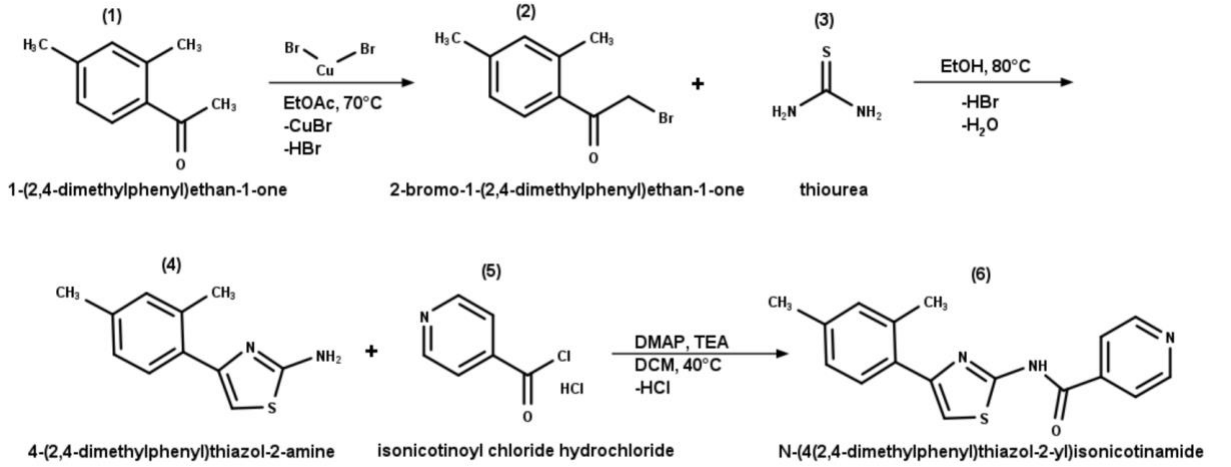
Abstract

The potential active pharmaceutical ingredient (API), N-(4-(2,4-dimethylphenyl)thiazol-2-yl)isonicotinamide (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) and, 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: CAS# 560103-80-2, bromination, Hantzsch thiazol condensation, amidation,



Scheme 1: overall synthesis of (6).

INTRODUCTION

This research target was enzyme inhibitor (6) as shown in Scheme 1 above. 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 first step of this synthesis is the bromination of the alpha position of the ketone (1) to form (2), as shown in the reaction scheme. This is accomplished with $CuBr_2$ with a characteristic green solution that dissipates as the reaction proceeds to yields a white precipitant -assumed to be CuBr.

Hantzsch Thiazole Synthesis.[4] The second step involves a Hantzsch thiazole synthesis using thiourea (3) allowing for the formation of (4) with its thiazole ring and primary amine. The thiazole ring heavily contributes to the function of this inhibitor.[12] It is presumably formed by the nucleophilic displacement of bromine by thiourea's sulfur

followed by an amine attacking the carbonyl group and dehydration followed by rearrangement to form a thiazole ring in (4).

Amidation. The primary amine of (4) combined with an acid chloride derivative (5) to form (6). It is assumed to be by nucleophilic acyl addition followed by loss of HCl.

METHOD AND RESULTS

(2) Preparation

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 mixture was dark green and allowed to reflux under nitrogen at 80 °C for 24 hours. White solid began forming in the flask almost immediately, as the green solution 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 was used in the characterization of the product of the bromination step. The Rf value for (1) was found to be 0.82 and the Rf value for (2) was found to be 0.70 in a 3:1 DCM/n-heptane.

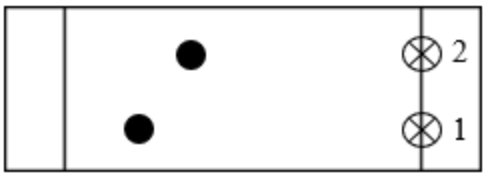


Figure 1: TLC of (1) and (2) using a 3:1 DCM/n-heptane blend-top to the left.

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 as shown in figure 1 using Spartan 20 software[11]. The FTIR spectrum of the product was taken as shown in figure 2 using the Nicolette 3800 IR Spectrometer, and the major peaks of these figures were included in table 1 with the corresponding features. ^1H NMR of (2) in $CDCl_3$ using the Bruker F80 was compared to the Spartan 20 calculated spectra and had similar peaks as shown in the figures 4&5 and table 1.

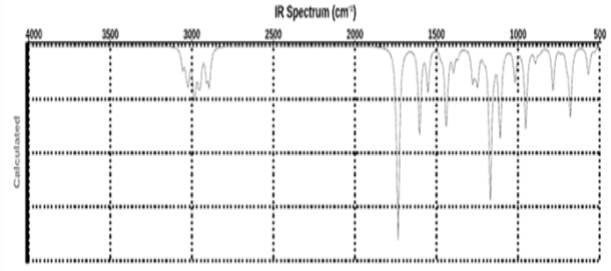


Figure 2: (2) Calculated IR Spectrum using Spartan 20[13]

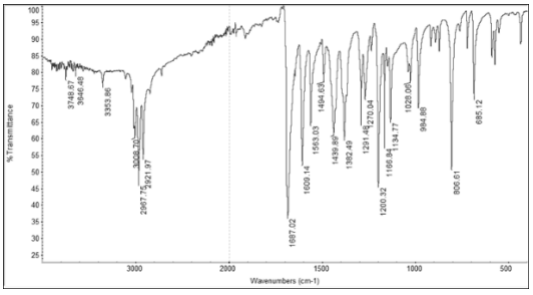


Figure 3: (2) Experimental FTIR Spectrum[14]

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

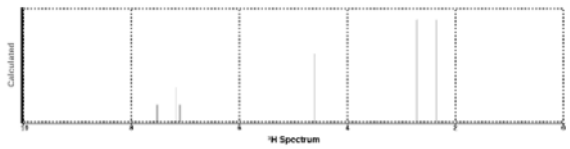


Figure 4: (2) Calculated [1]H NMR Spectrum[12]

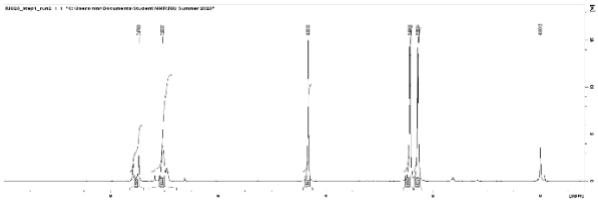


Figure 5: (2) Experimental [1]H NMR Spectrum[15]

Table 2 summarizes the chemical shifts and the peaks they represent, comparing the experimental spectrum to the calculated model.

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

Table 2: [1]H NMR Spectra (2) in $CDCl_3$

(4) Preparation

The second step of the synthesis is a variation on a Hantzsch thiazole ring. 0.40 g (1.8 mmol) of (2) and 0.14 g (1.8 mmol) of (3) were placed in 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 bar). A saturated aqueous solution of sodium bicarbonate was added to make the reaction mixture basic. The mixture was then washed with 30 mL of DCM and the organic layer was dried with $MgSO_4$. Solvent was removed under vacuum (40°C, 155 rpm, 0 mbar). 3 mL of DCM were added to the flask to solvate the product before pouring the solution onto a watch glass to crystallize.

(4) Characterization

The melting point was determined to be 94-95 °C, in comparison to the reference value of 85 °C.[16]

TLC 1:1:1 EtOAc/DCM:n-heptane gave Rf (2) 0.91 and the Rf (4) 0.65.

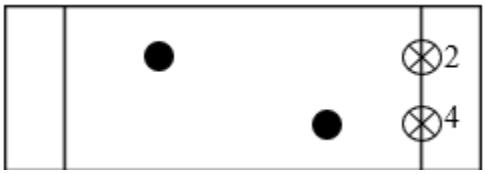


Figure 6: TLC of (2) and (4) -top to the left.

The IR spectrum for (4) was calculated[12] as shown in figure 8, the FTIR spectrum of the product was taken as shown in figure 9, and the major peaks of these figures were compared in table 3 with their corresponding features.

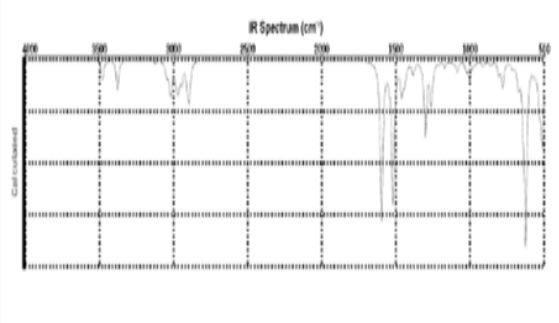


Figure 7: Calculated Spectrum IR[7]

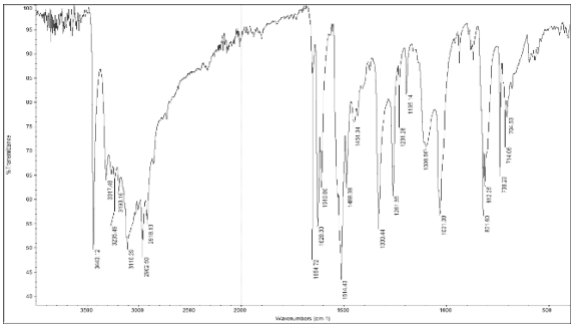


Figure 8: (4) Experimental Spectrum FTIR[8]

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

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

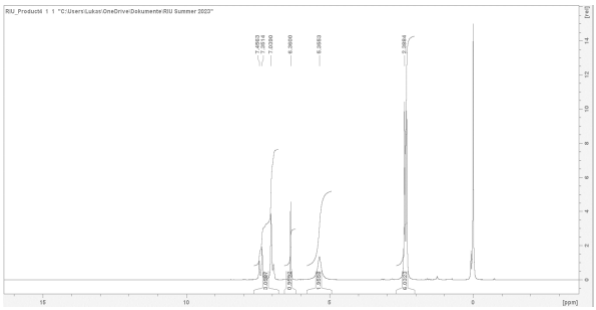


Figure 9: ^1H NMR in $CDCl_3$ Spectrum (4)[9]

Table 4 summarizes the chemical shifts and the peaks they represent, comparing the experimental spectrum to the calculated model.

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

(6) Preparation

For this step, 12 different methods were applied and are summarized in table 5. For most of these runs, (4) (0.33 g) (1.76 mmol), DMAP (0.49 g) (4.01 mmol), (5) (0.38 g) (2.13 mmol) and TEA (0.98 g) (9.69 mmol) were mixed in 20 mL DCM at room temperature, (5) being added after 10 minutes of mixing. The 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 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.

Run/Type	Reagents	Solvent/Run Time	Purification	Yield
1/Reflux	(4), (5), DMAP	DCM/20 mins	NA incomplete	0%
2/Reflux	(4), (5), DMAP	DCM/72 hrs.	Column 1	>
3/Reflux	(4), (5), DMAP, TEA	EtOAc, $CHCl_3$, DCM/ 72+ hrs.	Column 2	<4%
4/Reflux	(4), (5), DMAP, TEA	DCM/72+ hrs.	Column 2	<4%
5/Reflux	(4), (5), DMAP, TEA	DCM/72 hrs.	Column 2	<4%
6/Reflux	(4), (5), DMAP, TEA	THF/72 hrs.	NA	0%
7/Reflux	(4), (5), DMAP, TEA	DCM/72 hrs.	NA	0%
8/Reflux	(4), (5), DMAP, TEA	DCM/72 hrs.	$NaHCO_3$ wash/Acid Wash	60%
9/Reflux	(4), (5), DMAP, TEA	DCM/72 hrs.	Acid Wash	60%
10/Reflux	(4), (5), DMAP, TEA	DCM/72 hrs.	Acid Wash	60%
11/Microwave	(4), (5), TEA	DCM/5mins	NA	Positive by TLC
12/Microwave	(4), (5), TEA	DCM/5mins	NA	Positive by TLC

Table 5: Amidation Runs and Yield

(6) Characterization

TLC 1:1:1 EtOAc/DCM/n-heptane gave Rf (4) 0.65 and the Rf (6) 0.34.

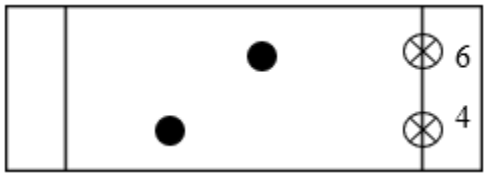


Figure 10: TLC of (4) and (6) -top to the left.

The melting point of (6) was determined to be 139-140°C (150°C ref).[5]

The IR spectrum for (6) was calculated as shown in figure 11, the FTIR spectrum of the product was taken as shown in figure 12, and the major peaks of these figures were included in table 5 with the corresponding features.

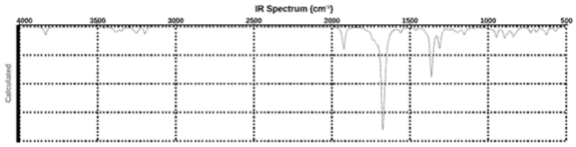


Figure 11: (6) Calculated IR Spectrum[7]

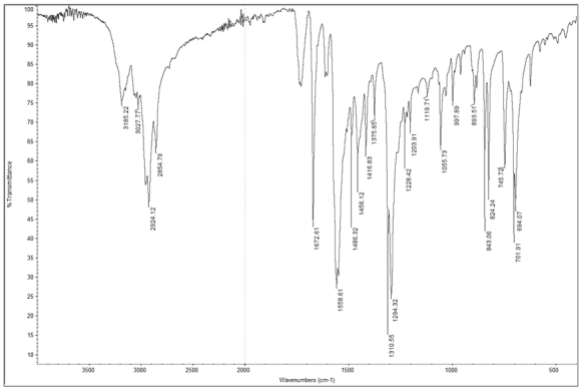


Figure 12: (6) Experimental IR Spectrum[8]

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: Experimental and calculated IR Spectra (6)

The NMR spectrum of (6) was calculated (Figure 13) and can be compared to the experimentally obtained spectrum below (Figure 14).

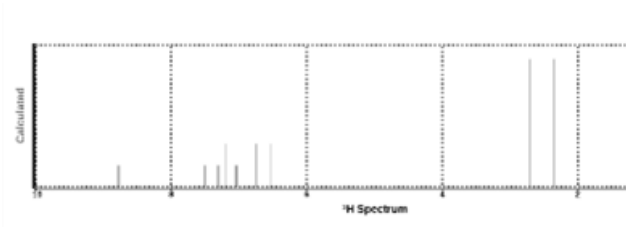


Figure 13: (6) Calculated ^1H NMR in $CDCl_3$ Spectrum[7]

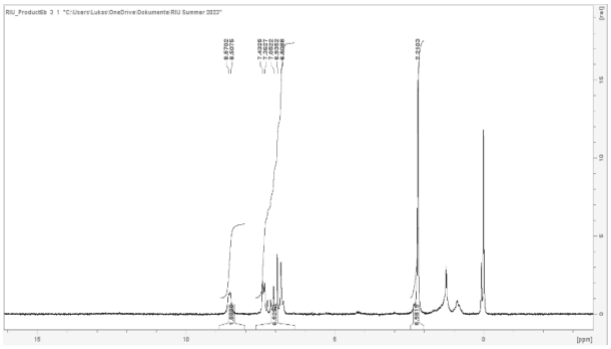


Figure 14: (6) Experimental ^1H NMR in $CDCl_3$ Spectrum[11]

Table 6 summarizes the chemical shifts and the peaks they represent, comparing the experimental spectrum to the calculated model.

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***

Table 7: ^1H NMR Spectra (6) in $CDCl_3$[15]

Run 8 from table 5 was evaluated by Agilent GCMS to yield the results in figure 15. The oven conditions 4.0-minute hold at 40C, 40C/minute ramp to 300C followed by 10-minute hold. Retention time 13.727-minutes with parent ion m/z 309.20 consistent with (6) was present with a peak with retention time 10.156-minutes parent ion m/z 204.10 consistent with (4).

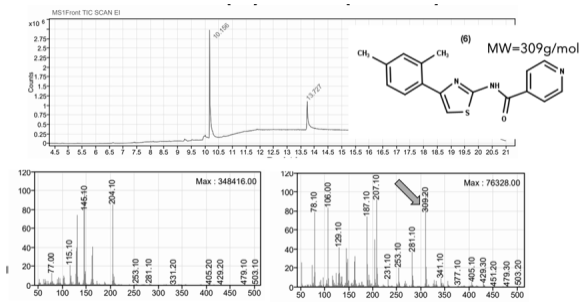


Figure 15. GCMS results for (6) run 8 showing the molecular ion at m/z 309.20.

DISCUSSION

FTIR, ^1H 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), it was found that the synthesis takes several days under a reflux environment, not 20 minutes.[3] GCMS, FTIR, ^1H 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. Several batches were combined on a column. The yield (4%) was determined to be 99% pure by HPLC. Crude yields were on the order of 60% by weight. Purification by column chromatography remains to be optimized. Early indications are that the low pH washes left the ionized product on the column.

ACKNOWLEDGEMENTS

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[14] Fourier Transformer Infrared Spectroscopy was taken using the Nicolette 3800 IR Spectrometer.

[15] ^{1}H NMR was taken using the Bruker F80 FT-NMR Spectrometer.

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