

Communication

In Silico Evaluation of a Promising Key Intermediate Thieno [2,3-d] Pyrimidine Derivative with Expected JAK2 Kinase Inhibitory Activity

Elshaymaa I. Elmongy^{1,2,*} and Hanan Ali Henidi³

¹ Department of Pharmaceutical Sciences, College of Pharmacy, Princess Nourah bint Abdulrahman University, Riyadh P.O. Box 84428, Saudi Arabia

² Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Helwan University, Ain Helwan, Cairo P.O. Box 11795, Egypt

³ Research Department, Health Sciences Research Center, Princess Nourah bint Abdulrahman University, Riyadh P.O. Box 84428, Saudi Arabia; hahenidi@pnu.edu.sa

* Correspondence: eielmongy@pnu.edu.sa

Abstract: This work describes the synthesis and the cytotoxic evaluation of thiophene and thienopyrimidine derivatives. The investigated compound was subjected to target prediction that indicated its high affinity to kinases and to Janus kinase 2 (JAK2) specifically. Molecular docking screening was performed on three different JAK2 proteins downloaded from the Protein Data Bank (PDB: 5AEP, 4C62 and 3ZMM). In vitro kinase inhibitory activity was evaluated and then compound cytotoxicity was performed on three different cancerous cell lines (HT-29, HepG-2, and MCF-7). Marked cytotoxic activity of the thienopyrimidine derivative against the HepG-2 cell line was demonstrated, reflected by its IC₅₀ value of 8.001 ± 0.0445 μM, which is better than that of the reference standard (IC₅₀ 13.91 ± 2.170 μM). Pharmacokinetic studies revealed good well permeability and GI absorption with no violations against Lipinski's rule.



Citation: Elmongy, E.I.; Henidi, H.A. In Silico Evaluation of a Promising Key Intermediate Thieno [2,3-d] Pyrimidine Derivative with Expected JAK2 Kinase Inhibitory Activity. *Molbank* **2022**, *2022*, M1352. <https://doi.org/10.3390/M1352>

Academic Editor: Ian R. Baxendale

Received: 15 February 2022

Accepted: 7 March 2022

Published: 9 March 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Keywords: pyrimidines; thienopyrimidines; kinase enzyme; anticancer; JAK2

1. Introduction

Cancer is the global second leading cause of disease-related mortality and morbidity [1]. Resistance to the currently accessible medicines is quickly becoming a serious international issue. The necessity to develop new substances to combat this resistance has emerged as one of today's most pressing issues [2,3].

Thienopyrimidines, with a heterocyclic core derivatized from pyrimidine and resembling purines, are of unique importance as scaffolds for the preparation of a variety of biologically active compounds as enzyme inhibitors, including kinases [4,5], poly (ADP-ribose) polymerase (PARP) inhibitors [6], and as anticancer agents against some tumor cell lines [7–11], in addition to their antioxidant activity [12–14]. Meanwhile they are an interesting target for protein kinases (PKs), which are enzymes known to regulate protein-biological activity, as they are responsible for protein phosphorylation that is involved in cell cycle proliferation, in addition to regulating cell progression and division [15–17]. Janus kinase 2 (JAK2) is a member of the JK family, that are non-receptor protein tyrosine kinases implicated in catalytic signaling by cytokine receptors members [18]. Mutations in JAK2 have been implicated in many myeloproliferative and myelofibrosis disorders [19]. Various therapeutic applications are related to the inhibition of JAK2; these applications include the treatment of cancer, inflammatory diseases [20,21], rheumatoid arthritis [22] and various other autoimmune diseases [23]. This might be due to the binding of cytokine receptors such as the erythropoietin receptor and the growth hormone receptor, which are exclusive to JAK2.

The FDA approved medication for myelofibrosis, known as fedratinib [24], which was approved for use in 2019, is a reported JAK2 enzyme inhibitor that is semi selective (Figure 1). Another reported Janus kinase inhibitor for both JAK1 and JAK2, also used against myelofibrosis, is ruxolitinib, which was approved in 2021 and is applied for the treatment of atopic dermatitis. It is worth mentioning that it is an orally active applied medication (Figure 1) [25]. Baricitinib is a drug that is used for rheumatoid arthritis, and it is a Janus kinase inhibitor for both subtypes 1 and 2 (Figure 1). It is worth mentioning that baricitinib is in a current clinical trial for use against the COVID-19 viral infection [26].

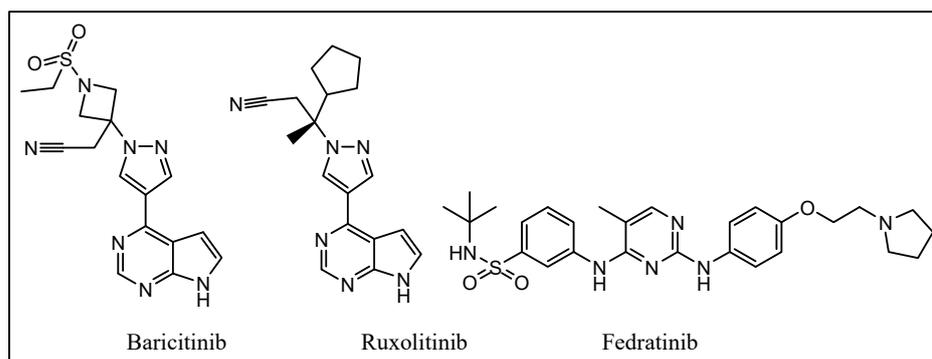


Figure 1. Some JAK inhibitors that are FDA approved and/or in clinical trials.

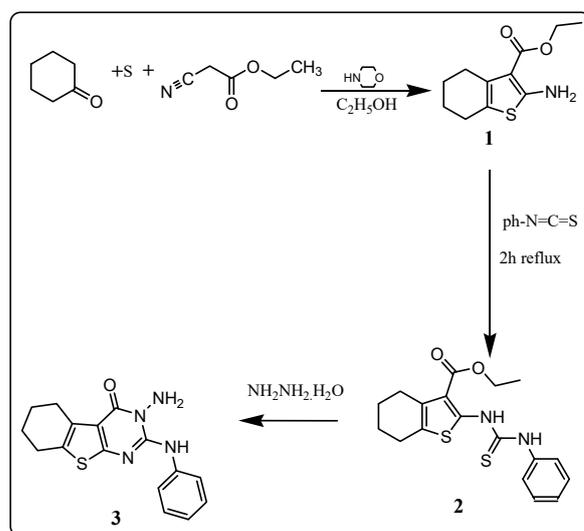
Unfortunately, a continuously reported adverse effect due to the use of JAK inhibitors is the evolution of bacterial, fungal, or viral microbial infections [27].

Learning information is being transformed into concrete advancements in diagnosis, prevention, and therapy. The foregoing facts on the importance of JAK2 inhibitors piqued our interest to evaluate the synthesized thienopyrimidine derivative for its cytotoxic activity, keeping in mind that it probably targets Janus kinase 2 enzyme.

2. Results and Discussion

2.1. Chemistry

The reaction between aminothiophene carboxylate ester **1** and phenyl isothiocyanate in ethanol resulted in thioureido thiophene **2**, which was then condensed with hydrazine hydrate using ethyl alcohol as a solvent to yield the targeted compound, thienopyrimidine derivative **3** (Scheme 1).



Scheme 1. Scheme for synthesis of the target compound.

2.2. In Silico Target Prediction

Scanning for target prediction using Swiss Target was performed, which showed promising affinity for kinases, in particular JAK2 enzyme. The percentages are illustrated in Figure 2, where kinase represents the highest percentage as a target with 66.7% and JAK2 represents the highest probability within the kinases.

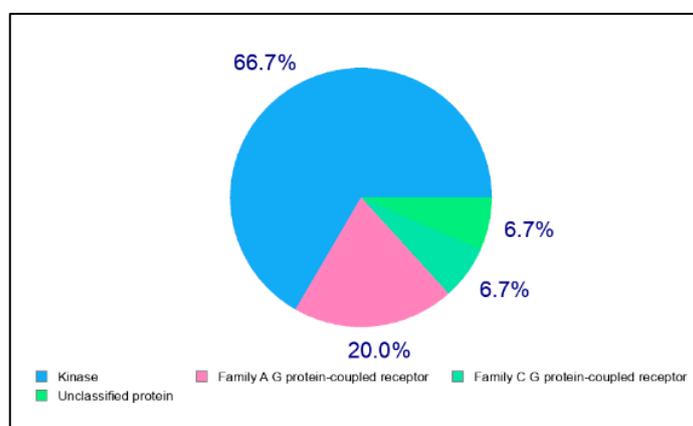


Figure 2. Percentage affinity of the designed compounds for kinases rather than other targets.

2.3. Molecular Docking

Molecular docking studies were performed on three crystal structures of JAK2 proteins. The crystal structures of the required proteins bound to their ligands were downloaded from the Protein Data Bank (PDB: 5AEP, 4C62 and 3ZMM). To investigate the affinity of the prepared compound 3 to JAK2 kinase enzyme, docking simulation of the synthesized compound at the active site was carried out in comparison with the co-crystallized ligand of the target protein downloaded from the Protein Data Bank. The results of docking on PDB: 5AEP showed binding affinity of -6.3902 , while that for protein PDB: 4C62 was -5.239 , and that for PDB: 3ZMM recorded -6.0183 . Leu 932 was found to be the main amino acid involved in the interaction between all the three co-crystallized ligands and their chains.

The results regarding binding affinity (E), the amino acids involved in the interactions, in addition to the root mean square deviation of RMSD (A) for the ligand with the three downloaded proteins are tabulated below (Table 1). These interactions were either hydrogen bonding or hydrophobic interactions. Interestingly, the key amino acid Leu 932 was involved in the interactions between the prepared compound and the receptor in both 4C62 and 3ZMM (Figures 3 and 4a,b).

Table 1. Binding affinities and RMSD scores in addition to the amino acid interactions of compound 3 at three target JAK2 kinase proteins (PDB: 5AEP, 4C62, and 3ZMM).

Protein PDB Code	E	RMSD (A)	Co-Crystallized Ligand Amino Acid Interactions	Amino Acids Involved in Interactions by the Prepared Compound
5AEP	-6.3902	1.1599	Arg 980, Glu 930, Leu 932, Leu 855	Arg 980, Glu 930, Leu 855
4C62	-5.239	1.9175	Leu 932, Leu 855	Leu 932
3ZMM	-6.0183	1.7528	Glu 930, Leu 932	Leu 932, Glu 930, Leu 855, Gly 935

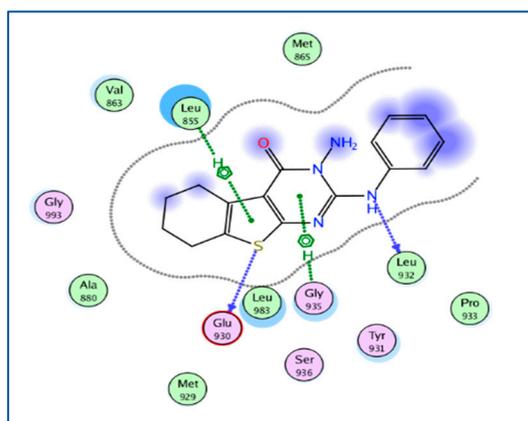
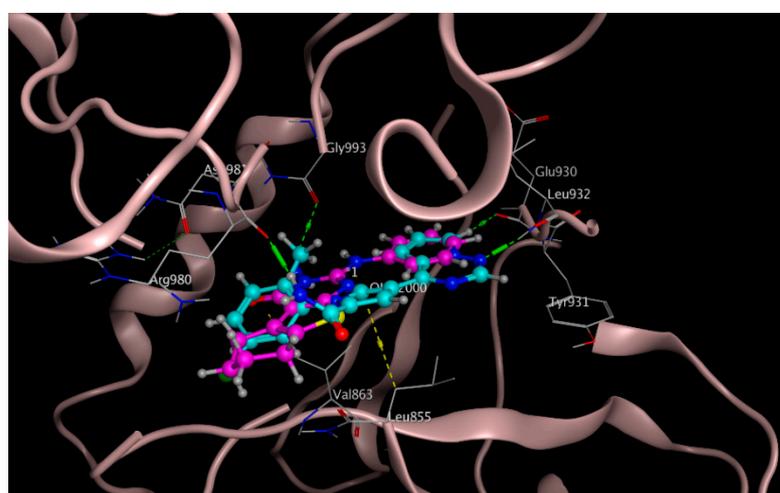
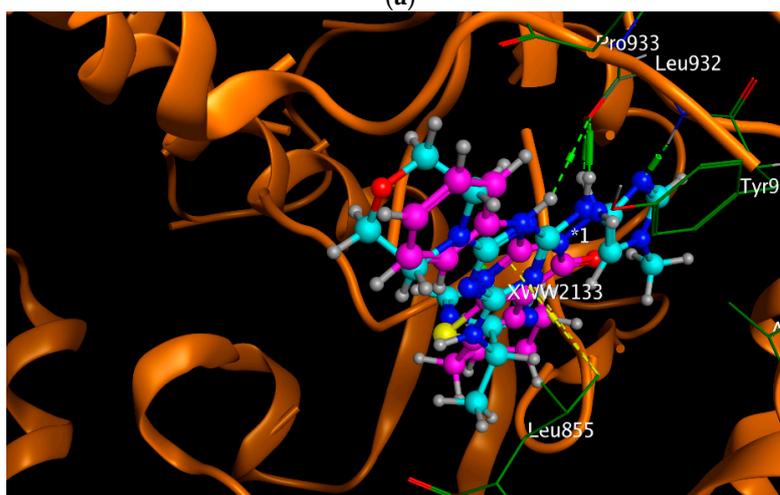


Figure 3. Prepared compound 3 2D interaction at PDB: 3ZMM with H-bond at Glu 930 and 932, and H- π interaction at Glu 935 and 855.



(a)



(b)

Figure 4. Compound 3 (magenta ball and stick) aligned with the co-crystallized ligand (cyan ball and stick) at the target protein: (a) PDB: 5AEP, (b) PDB: 4C62. Interactions with H-bonds are in green, and with H- π are in yellow.

2.4. In Vitro Kinase Screening

Kinase screening was performed to determine the percentage kinase inhibition effect of the synthesized compound. The screened compound showed inhibition at the testing dose (20 μM) of 49.02%. Data are expressed as mean \pm SD, $n = 3$. Significant difference from the control group (p value < 0.0001).

2.5. In Vitro Cytotoxic Evaluation against Three Cancerous Cell Lines

Compound **3** was assessed for its cytotoxicity and its IC_{50} value against three cell lines (HT-29, HepG-2, and MCF-7), calculated using doxorubicin as a positive reference. The results are tabulated as IC_{50} values in the μM (Table 2). The prepared compound showed marked promising cytotoxic activity against the HepG-2 cell line, reflected by its IC_{50} value of $8.001 \pm 0.0445 \mu\text{M}$, which is better than that of the reference standard (IC_{50} $13.91 \pm 2.170 \mu\text{M}$). However, it showed moderate activity against both HT-29 and MCF-7, as expressed by IC_{50} values of $4.526 \pm 0.130 \mu\text{M}$ and $15.055 \pm 0.785 \mu\text{M}$, respectively, in comparison to doxorubicin that demonstrated IC_{50} values of 1.358 ± 1.156 and $8.434 \pm 0.522 \mu\text{M}$, respectively. In addition, in reference to our previously reported work [11], the compound under investigation shows a comparable antiproliferative effect.

Table 2. Antiproliferative activities in different cancer cell lines (IC_{50} (μM)).

	HT-29	HepG-2	MCF-7
Compound 3	4.526 ± 0.130	8.001 ± 0.0445	15.055 ± 0.785
Doxorubicin	1.358 ± 1.156	13.91 ± 2.170	8.434 ± 0.522

2.6. Pharmacokinetics Study

The tested compound was subjected to pharmacokinetics assessment using Molsoft and Swiss pharmacokinetics (Figure 5). The investigated compound had good well-permeability and GI absorption [16] as tabulated below (Table 3). It had two hydrogen-bond donors (HBD) and two hydrogen-bond acceptors (HBA), in addition to a strong toleration by cell membranes, as reflected by its $\log P$ (2.63) < 5 and its molecular weight which is less than 500, as tabulated below. The tested compound showed a positive drug likeness score of 0.57 (Table 3). The blood brain barrier (BBB) score is between 0 and 6, as reported [28,29] recording 4.01. Oral bioavailability probability is illustrated in Figure 4, where the red net represents the optimal zone for oral bioavailability, and as shown, the compound under investigation presents promising data records. All the represented data indicate no violations against Lipiniski's rule.

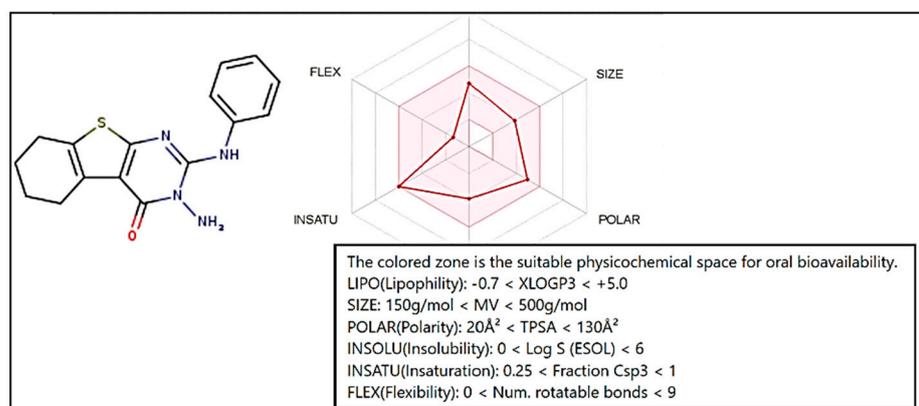


Figure 5. Oral bioavailability represented in the red zone for the compound under investigation.

Table 3. Pharmacokinetics data and drug likeness value for the tested compound.

Compound	Molecular Weight	Number of HBA	Number of HBD	Log <i>P</i>	MR	TPSA	BBB Score	GI Absorption	Drug Likeness Score	Compounds in Relation to Drug and Non-Drug Scores
3	312.39	2	2	2.63	91	101.18	4.01	High	0.57	<p>Drug-likeness model score: 0.57</p>

3. Materials and Methods

3.1. Molecular Modeling Study

Three different crystal structures of JAK2 kinase with their co-crystallized ligands were downloaded from the Protein Data Bank (PDB: 5AEP, 4C62 and 3ZMM). Protein optimization was performed by calculating partial charges, protonation, and energy minimization.

The prepared ligand was optimized using MOE.2014, the bond order was fixed, and energy was minimized after the addition of partial charges and 3D protonation. The compound under investigation was then added to database in mol2 format.

The selected docking protocol was induced fit. The active site was selected at ligand atoms where alpha spheres were used to guide the placement with 5 Å. Pharmacophore annotations were excluded. The MDB file was set to be the ligand for docking. It was browsed as the database of the investigated compound in mdb format. The gradient was set to 0.05 for energy minimization, and MMFF94X was the selected force field.

3.2. Chemistry

3.2.1. Ethyl-2-amino-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate: **1**

According to the published procedures [11], the starting aminothiophene ester **1** was prepared using a mixture of cyclohexanone (~5 mL, 50 mmol), ethyl cyanoacetate (6.35 mL, 50 mmol), elemental sulfur (1.6 g, 50 mmol), and morpholine (4.35 mL, 50 mmol), that was heated at 60 °C, while stirring in absolute ethanol (20 mL) for 6 h (Gewald reaction). The mixture was left at room temperature overnight. The formed solid was collected by filtration, washed with ethanol (2 × 10 mL), dried and crystallized from absolute ethanol. The formed precipitate was filtered, left to dry, and was then recrystallized using absolute alcohol, affording yellow crystals. Yield: 93%, mp 110 °C; IR (KBr disc) (cm⁻¹): 3522–3376 forked band (NH₂), 1737 (C = O); ¹H NMR (DMSO-d₆): 1.23 (t, 3H, CH₃ of ester), 1.34–2.97 (m, 8H cyclohexyl), 4.19 (q, 2H, -CH₂-CH₃), 7.01 (s, 2H, D₂O replaceable, NH₂); ¹³C NMR (DMSO-d₆): 14.7, 22.9, 23.3, 24.4, 26.6, 59.0, 103.2, 115.5, 131.6, 163.2, 165.4. CHN calcd. for: C₁₁H₁₅NO₂S (225): C, 58.64; H, 6.71; N, 6.22; S, 14.23; found: C, 58.68; H, 6.70; N, 6.27.

3.2.2. Ethyl-2-(3-phenylthioureido)-5,6,7,8-tetrahydrocyclohexa[b]thiophene-3-carboxylate: **2**

Reaction under reflux where thiophene derivative (10 mmol) **1** was dissolved in 10 mL of ethyl alcohol and then 15 mmol of phenyl isothiocyanate was added dropwise. The reaction proceeded on water bath for 2 h and then was left to cool overnight. The formed precipitate was collected by filtration under vacuum, yielding yellow crystals upon crystallization from ethyl alcohol. Yield of 92%, mp 170–172 °C; IR (KBr disc) (cm⁻¹): 3323 (NH), 2371 (-SCN-), 1702 (C = O). ¹H-NMR (DMSO) δ (ppm) 1.22 (t, 3H, CH₃ at C2-pyrimidine), 1.34–2.29 (m, 8H cyclohexyl) 4.25 (q, 2H, CH₂-CH₃), 7.10–7.45 (m, aromatic-5H), 10.80 (s, 1H, NH, D₂O replaceable), 11.50 (s, 1H, NH, D₂O exchangeable). ¹³C NMR (DMSO-d₆) δ ppm: 14.5, 22.9, 23.0, 24.1, 26.3, 39.5, 40.8 60.7, 112.4, 124.7, 126.1, 126.3, 129.4, 130.5, 138.7, 150.0, 166.0, 176.4. Analysis for: C₁₈H₂₀N₂O₂S₂ (360): CHN calcd. C, 59.97; H, 5.59; N, 7.77; found: C, 60.11; H, 6.04; N, 7.79.

3.2.3. 3-Amino-2-phenylamino-5,6,7,8-tetrahydrocyclohexa[4,5]thieno[2,3-d]pyrimidin-4(3H)-one: **3**

Refluxing a mixture of the thioureido thiophene structure **2** (10 mmol) and hydrazine hydrate (20 mmol) for 8 h in 10 mL of ethanol afforded the titled compound **3**. The reaction was left to cool; the formed precipitate was collected by filtration under vacuum, dried and then recrystallized from ethanol, yielding a yellow powder product. Yield: 71%, mp 198–200 °C; IR (KBr) (cm⁻¹): 3521(NH), 3336–3277 (forked NH₂), 3105 (CH-aromatic), 1654 (C = O). ¹H-NMR (DMSO) δ (ppm): 1.50–3.33 (m, 8H cyclohexyl), 2.76 (s, 2H, NH₂, D₂O exchangeable), 7.50–7.99 (m, aromatic-5H), 12.55 (s, 1H, NH, D₂O exchangeable). ¹³C NMR

(DMSO-d6) ppm: 20.4, 23.5, 23.5, 25.0, 114.7, 114.7, 116.8, 117.8, 122.5, 126.9, 127.5, 139.2, 145.1, 152.4, 159.2, 162.8. Analysis for: C₁₆H₁₆N₄OS (312): CHN calcd. C, 61.52; H, 5.16; N, 17.90; found: C, 61.66; H, 5.18; N, 18.01.

3.3. In Vitro Kinase Inhibition Assessment

To assess the effect of compound **3** on kinase activity, universal kinase assay was used by monitoring ADP formation, which is directly proportional to enzyme phosphotransferase activity. The kinase assay was performed using a mixture of kinases extracted from the HT-29 cell line and the enzymatic inhibition was measured by using ADP-Glo assay (Promega, Madison, WI, USA). The screening assay was undertaken in a single dose concentration (20 μ M). Kinases were incubated with the synthesized compound at the tested dose (20 μ M) in 96-well plates. After 45 min of incubation, the kinases' activity was determined using luminescent luciferase–luciferin reaction. Luminescence signals were measured by a Varioskan™ LUX multimode microplate reader (Thermo Scientific, Waltham, MA, USA). Kinase inhibition was expressed as the percentage of remaining kinase activity relative to the vehicle kinase reaction.

3.4. Cell Culture

A breast cancer cell line (MCF-7), human hepatoma cell line (HepG-2), and colorectal cell line (HT-29) were used to evaluate the anticancer activity of compound **3**. All the cell lines were cultured in their optimum media (RPMI-1640 or DMEM) containing 100 μ g/mL of streptomycin, 100 units/mL of penicillin, and 10% heat-inactivated fetal bovine serum in humidified air with 5% CO₂ at 37 °C.

Cell Viability Assay:

The MTT assay was used to determine the viability of the cancer cells (HepG-2, MCF-7, and HT-29) after exposure to the synthesized compound. All tested cell lines were plated in 96-well plates (5000 cells/well) and allowed to attach for 24 h before treatment. Then, the cells were exposed for 72 h to serial concentration (0.1–100 μ L) of the compound **3** and doxorubicin was used as a positive control. After the exposure, the MTT assay protocol was followed as described [30]. The absorbance of the viable cells was measured at 540 nm with a Varioskan™ LUX multimode microplate reader (Thermo Scientific, Waltham, MA, USA).

4. Conclusions

This work describes the cytotoxic evaluation of a thienopyrimidine derivative along with its in silico modeling studies and pharmacokinetics assessment. Target prediction revealed the high affinity to kinases, specifically Janus kinase 2 (JAK2). Molecular docking screening was performed on three different JAK2 proteins downloaded from the Protein Data Bank (PDB: 5AEP, 4C62 and 3ZMM). In vitro kinase inhibitory activity was evaluated with 49% kinase inhibition, and then compound cytotoxicity was performed on three different cancerous cell lines (HT-29, HepG-2 and MCF-7). Marked cytotoxic activity of the thienopyrimidine derivative against the HepG-2 cell line was demonstrated, reflected by its IC₅₀ value of 8.001 \pm 0.0445 μ M, which is better than that of the reference standard (IC₅₀ 13.91 \pm 2.170 μ M), while it showed moderate cytotoxicity against both HT-29 and MCF-7. Pharmacokinetic studies revealed a good drug likeness score, well permeability, and GI absorption, with no violations against Lipinski's rule. The prepared compound is a promising key intermediate for the synthesis of a new series of thienopyrimidines due to its amino group that can react with a variety of chemical groups.

Author Contributions: Conceptualization, E.I.E.; methodology, E.I.E. and H.A.H.; software, E.I.E.; validation, E.I.E. and H.A.H.; formal analysis, E.I.E. and H.A.H.; investigation, E.I.E. and H.A.H.; resources, E.I.E. and H.A.H.; data curation, E.I.E. and H.A.H.; writing—original draft preparation, E.I.E. and H.A.H.; writing—review and editing, E.I.E.; visualization, E.I.E. and H.A.H.; supervision, E.I.E.; project administration, E.I.E. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Kim, C.; Kim, B. Anti-cancer natural products and their bioactive compounds inducing ER stress-mediated apoptosis: A review. *Nutrients* **2018**, *10*, 1021. [[CrossRef](#)] [[PubMed](#)]
2. Ferlay, J.; Colombet, M.; Soerjomataram, I.; Mathers, C.; Parkin, D.M.; Piñeros, M.; Znaor, A.; Bray, F. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. *Int. J. Cancer* **2019**, *144*, 1941–1953. [[CrossRef](#)] [[PubMed](#)]
3. Baudino, T. Targeted cancer therapy: The next generation of cancer treatment. *Curr. Drug Discov. Technol.* **2015**, *12*, 3–20. [[CrossRef](#)] [[PubMed](#)]
4. Gryshchenko, A.A.; Bdzhola, V.G.; Balanda, A.O.; Briukhovetska, N.V.; Kotey, I.M.; Golub, A.G.; Ruban, T.P.; Lukash, L.L.; Yarmoluk, S.M. Design, synthesis, and biological evaluation of N-phenylthieno[2,3-d]pyrimidin-4-amines as inhibitors of FGFR1. *Bioorg. Med. Chem.* **2015**, *23*, 2287–2293. [[CrossRef](#)] [[PubMed](#)]
5. Elmongy, E.I. Thieno[2,3-d]pyrimidine derivatives: Synthetic approaches and their FLT3 kinase inhibition. *J. Heterocycl. Chem.* **2020**, *57*, 2067–2078. [[CrossRef](#)]
6. Elmongy, E.I.; Khedr, M.A.; Taleb, N.A.; Awad, H.M.; Abbas, S.E.S. Design, synthesis, and biological evaluation of some cyclohepta[b]thiophene and substituted pentahydrocycloheptathieno[2,3-d]pyrimidine derivatives. *J. Heterocycl. Chem.* **2017**, *54*, 1084–1093. [[CrossRef](#)]
7. Kumar, K.S.; Chamakuri, S.; Vishweshwar, P.; Iqbal, J.; Pal, M. AlCl₃-induced (hetero)arylation of thienopyrimidine ring: A new synthesis of 4-substituted thieno[2,3-d]pyrimidines. *Tetrahedron Lett.* **2010**, *51*, 3269–3273. [[CrossRef](#)]
8. Gorja, D.R.; Kumar, K.S.; Mukkanti, K.; Pal, M. C–C (alkynylation) vs C–O (ether) bond formation under Pd/Cu catalysis: Synthesis and pharmacological evaluation of 4-alkynylthieno[2,3-d]pyrimidines. *Beilstein J. Org. Chem.* **2011**, *7*, 338–345. [[CrossRef](#)]
9. Gad, E.M.; Nafie, M.S.; Eltamany, E.H.; Hammad, M.S.A.G.; Barakat, A.; Boraie, A.T.A. Discovery of New Apoptosis-Inducing Agents for Breast Cancer Based on Ethyl 2-Amino-4,5,6,7-Tetra Hydrobenzo[b]Thiophene-3-Carboxylate: Synthesis, In Vitro, and In Vivo Activity Evaluation. *Molecules* **2020**, *25*, 2523. [[CrossRef](#)]
10. Abuelhassan, S.; Bakhite, E.A.G.; Abdel-Rahman, A.E.; El-Mahdy, A.F. Synthesis, characterization, and biological activities of some novel thienylpyrido[3',2':4,5]thieno[3,2-d]pyrimidines and related heterocycles. *J. Heterocycl. Chem.* **2021**, *58*, 1784–1801. [[CrossRef](#)]
11. Elmongy, E.I.; Attallah, N.G.M.; Altwaijry, N.; AlKahtani, M.M.; Henidi, H.A. Design and synthesis of new thiophene/thieno[2,3-d]pyrimidines along with their cytotoxic biological evaluation as tyrosine kinase inhibitors in addition to their apoptotic and autophagic induction. *Molecules* **2021**, *27*, 123. [[CrossRef](#)] [[PubMed](#)]
12. El-Mekabaty, A.; Fouda, A.E.-A.S.; Shaaban, I.E.I. Convenient synthesis of functionalized thienopyrimidine-4-ones and thienopyridine-4-ones bearing a pyridine moiety with anticipated antioxidant activity. *J. Heterocycl. Chem.* **2020**, *57*, 2928–2935. [[CrossRef](#)]
13. Nagaraju, K.; Bhaskaruni, V.H.; Kishore, R.; Maddila, S.; Singh, P.; Jonnalagadda, S.B. Synthesis and antioxidant evaluation of a new class of thienopyrimidinerhodanine hybrids. *Lett. Drug Des. Discov.* **2018**, *15*, 118–126. [[CrossRef](#)]
14. Elmongy, E.I.; Khedr, M.A.; Taleb, N.A.; Abbas, S.E. Design and synthesis of new thienopyrimidine derivatives along with their antioxidant activity. *Egypt. J. Chem.* **2021**, *64*, 6857–6867. [[CrossRef](#)]
15. Sharaky, M.; Kamel, M.; Aziz, M.A.; Omran, M.; Rageh, M.M.; Abouzid, K.A.; Shouman, S.A. Design, synthesis and biological evaluation of a new thieno[2,3-d]pyrimidine-based urea derivative with potential antitumor activity against tamoxifen sensitive and resistant breast cancer cell lines. *J. Enzym. Inhib. Med. Chem.* **2020**, *35*, 1641–1656. [[CrossRef](#)] [[PubMed](#)]
16. Wu, C.H.; Coumar, M.S.; Chu, C.Y.; Lin, W.H.; Chen, Y.R.; Chen, C.T.; Shiao, H.Y.; Rafi, S.; Wang, S.Y.; Hsu, H.; et al. Design and synthesis of tetrahydropyridothieno[2,3-d]pyrimidine scaffold based epidermal growth factor receptor (EGFR) kinase inhibitors: The role of side chain chirality and michael acceptor group for maximal potency. *J. Med. Chem.* **2010**, *53*, 7316–7326. [[CrossRef](#)]
17. Heng, H.; Zhi, Y.; Yuan, H.; Wang, Z.; Li, H.; Wang, S.; Tian, J.; Liu, H.; Chen, Y.; Lu, T.; et al. Discovery of a highly selective FLT3 inhibitor with specific proliferation inhibition against AML cells harboring FLT3-ITD mutation. *Eur. J. Med. Chem.* **2019**, *163*, 195–206. [[CrossRef](#)]
18. O'Shea, J.J.; Holland, S.M.; Staudt, L.M. Mechanisms of disease: JAKs and STATs in immunity, immunodeficiency, and cancer. *N. Engl. J. Med.* **2013**, *368*, 161–170. [[CrossRef](#)]
19. Kralovics, R.; Passamonti, F.; Buser, A.S.; Teo, S.S.; Tiedt, R.; Passweg, J.R.; Tichelli, A.; Cazzola, M.; Skoda, R.C. A gain-of-function mutation of JAK2 in myeloproliferative disorders. *N. Engl. J. Med.* **2005**, *352*, 1779–1790. [[CrossRef](#)]
20. Hart, S.; Goh, K.C.; Novotny-Diermayr, V.; Hu, C.Y.; Hentze, H.; Tan, Y.C.; Madan, B.; Amalini, C.; Loh, Y.K.; Ong, L.C.; et al. SB1518, a novel macrocyclic pyrimidine-based JAK2 inhibitor for the treatment of myeloid and lymphoid malignancies. *Leukemia* **2011**, *25*, 1751–1759. [[CrossRef](#)]
21. Pesu, M.; Laurence, A.; Kishore, N.; Zwillich, S.H.; Chan, G.; O'Shea, J.J. Therapeutic targeting of janus kinases. *Immunol. Rev.* **2008**, *223*, 132–142. [[CrossRef](#)]

22. Norman, P. Selective JAK inhibitors in development for rheumatoid arthritis. *Expert Opin. Investig. Drugs* **2014**, *23*, 1067–1077. [[CrossRef](#)] [[PubMed](#)]
23. Kontzias, A.; Kotlyar, A.; Laurence, A.; Changelian, P.; O’Shea, J.J. Jakinibs: A new class of kinase inhibitors in cancer and autoimmune disease. *Curr. Opin. Pharmacol.* **2012**, *12*, 464–470. [[CrossRef](#)] [[PubMed](#)]
24. Talpaz, M.; Kiladjian, J. Fedratinib, a newly approved treatment for patients with myeloproliferative neoplasm-associated myelofibrosis. *Leukemia* **2021**, *35*, 1–17. [[CrossRef](#)]
25. Mesa, R.A. Ruxolitinib, a selective JAK1 and JAK2 inhibitor for the treatment of myeloproliferative neoplasms and psoriasis. *IDrugs Investig. Drugs J.* **2010**, *13*, 394–403.
26. Cingolani, A.; Tummolo, A.M.; Montemurro, G.; Gremese, E.; Larosa, L.; Cipriani, M.C.; Pasciuto, G.; Liperoti, R.; Murri, R.; Pirroni, T.; et al. Baricitinib as rescue therapy in a patient with COVID-19 with no complete response to sarilumab. *Infection* **2020**, *48*, 767–771. [[CrossRef](#)]
27. O’Shea, J.J.; Kontzias, A.; Yamaoka, K.; Tanaka, Y.; Laurence, A. Janus kinase inhibitors in autoimmune diseases. *Ann. Rheum. Dis.* **2013**, *72*, ii111–ii115. [[CrossRef](#)]
28. Gupta, M.; Lee, H.J.; Barden, C.J.; Weaver, D.F. The Blood–Brain barrier (BBB) score. *J. Med. Chem.* **2019**, *62*, 9824–9836. [[CrossRef](#)]
29. Khan, O.A.; Gore, M.; Lorigan, P.; Stone, J.; Greystoke, A.; Burke, W.; Carmichael, J.; Watson, A.J.; McGown, G.; Thorncroft, M.; et al. A phase I study of the safety and tolerability of olaparib (AZD2281, KU0059436) and dacarbazine in patients with advanced solid tumours. *Br. J. Cancer* **2011**, *104*, 750–755. [[CrossRef](#)]
30. Elmongy, E.I.; Altwajry, N.; Attallah, N.G.M.; AlKahtani, M.M.; Henidi, H.A. In-Silico Screening of Novel Synthesized Thienopyrimidines Targeting Fms Related Receptor Tyrosine Kinase-3 and Their In-Vitro Biological Evaluation. *Pharmaceuticals* **2022**, *15*, 170. [[CrossRef](#)]