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Short Note

4-(3-Phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl) thieno[3,2-*d*]pyrimidine

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Abstract: A new hybrid compound, 4-(3-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)thieno[3,2-*d*] pyrimidine **3**, with promising biological activity was efficiently synthesized by the reaction of 3-phenyl-1-(thieno[3,2-*d*]pyrimidin-4-yl)-1*H*-pyrazol-5-amine with Vilsmeier–Haack reagent and subsequent treatment with ammonium carbonate. The structure of the synthesized compound was fully characterized by ^{1}H-, ^{13}C-NMR, IR spectroscopy, mass-spectrometry and elemental analysis.

Keywords: thieno[3,2-*d*]pyrimidine; pyrazolo[3,4-*d*]pyrimidine; Vilsmeier–Haack reagent

1. Introduction

The thienopyrimidines and pyrazolopyrimidines have attracted considerable interest because of their various biological properties and therapeutic potentials [1–4]. Among them, thieno[3,2-*d*]pyrimidines were known to have pharmacological significance including anticancer [5], antitumor [6], antimicrobial [7], antiviral [8], and anti-inflammatory [9] activities. Pyrazolo[3,4-*d*]pyrimidines, which structurally resemble purines, have been reported to possess antiviral [10], anticancer [11], anti-inflammatory [12], antileishmanial [13] and antimicrobial [14] activities.

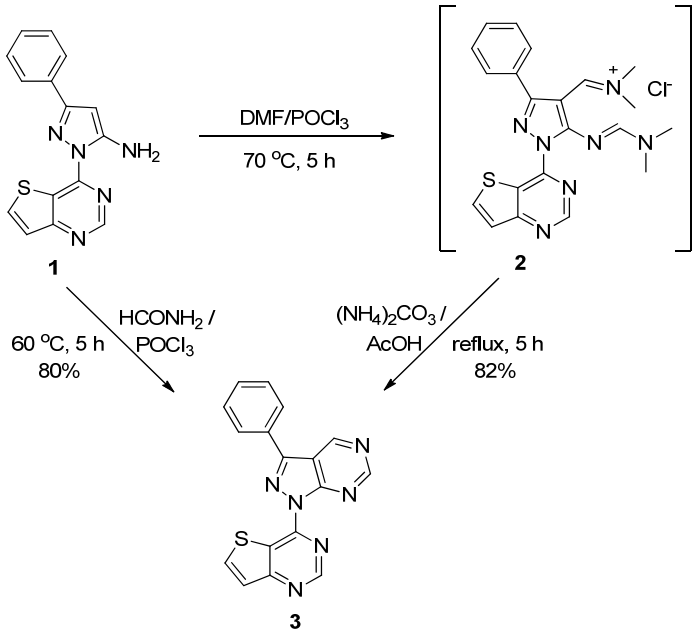
We have recently synthesized thienopyrimidine-pyrazolo[3,4-*b*]pyridine hybrids [15], and thienopyrimidines containing [1,2,4]triazolo[4,3-*a*]pyridinylpyrazole moiety as IL-6 inhibitor [16]. In the continuation of our research, we report herein the synthesis of a hybrid compound **3**, thieno[3,2-*d*]pyrimidine bearing a pyrazolo[3,4-*d*]pyrimidine.

2. Results and Discussion

The synthesis of the new hybrid compound **3** is outlined in Scheme 1. The starting material **1**, 3-phenyl-1-(thieno[3,2-*d*]pyrimidin-4-yl)-1*H*-pyrazol-5-amine, was prepared by our previously reported method [15]. Compound **1** was reacted with DMF and phosphorus oxychloride (Vilsmeier–Haack reagent) at 70 °C for 5 h to afford intermediate **2**. Subsequent refluxing of the solution of **2** and ammonium carbonate in acetic acid resulted in the formation of compound **3**, 4-(3-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)thieno[3,2-*d*]pyrimidine, in 82% yield [17].

The structure of **3** was confirmed by ^{1}H-, ^{13}C-NMR, IR spectroscopy, mass-spectrometry and elemental analysis (see Supplementary Materials). The ^{1}H-NMR spectrum of compound **3** showed three singlet signals at δ 9.95, 9.38 and 9.21 ppm due to two protons of newly formed pyrimidine ring and one proton of pyrimidine in thieno[3,2-*d*]pyrimidine ring. It presented also a pair of doublet signals at δ 8.61 and 7.74 (J = 5.8 Hz) attributed to thiophene protons of thieno[3,2-*d*]pyrimidine ring, and other multiplet signals for the phenyl ring. In the ^{13}C-NMR spectrum, compound **3** exhibited characteristic peaks at δ 154.8 and 154.2 ppm for the newly formed pyrimidine carbons, including two pyrimidine peaks at δ 163.3 and 157.0 ppm in thieno[3,2-*d*]pyrimidine ring. The infrared (IR) spectrum of compound **3** revealed a characteristic absorption band at 1620 cm^{-1} for C=N bond stretching.

The mass spectrum showed *m*/*z* = 331.21 corresponding the molecular formula, $C_{17}H_{10}N_6S$. Elemental analysis also provided satisfactory results for compound **3**.



Scheme 1. Synthesis strategy for the hybrid compound **3**.

The hybrid compound **3** was also obtained in 80% yield by the reaction of compound **1** with a modified Vilsmeier–Haack reagent, $HCONH_2$ and $POCl_3$, using the Wong's method [18]. The spectral data of the resultant product were identical in all respects (mp, ^{1}H-, ^{13}C-NMR, and mass spectra) with the data of compound **3**.

The inhibitor of IL-6 induced activation of STAT3 could be a useful candidate for the development of new anticancer and anti-inflammatory drugs [19]. The preliminary biological test of inhibitory activity on STAT3 dependent luciferase activity induced by IL-6 for compound **3** was performed, according to the reported method [16], and it exhibited an IC_{50} value of 2.55 μM. Genistein with IC_{50} value of 15.0 μM was used as positive control in this assay system [20].

3. Materials and Methods

3.1. General Information

Commercial reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA) and TCI (Tokyo, Japan). The solvents used were purified using standard techniques. Melting point was determined on Kofler apparatus and was uncorrected. The ^{1}H-, and ^{13}C-NMR spectrum were recorded in deuterated chloroform with TMS as the standard on a UNITY INOVA 400 NB FT-NMR. The UV spectrum was obtained using a Perkin-Elmer Lambda-25 UV-vis spectrophotometer. The IR spectrum was recorded on Shimazu FTIR Prestige-21 spectrometer. The mass spectrum was obtained with a Finnigan MAT INCOS50 instrument.

3.2. Synthesis of 4-(3-Phenyl-1H-Pyrazolo[3,4-d]Pyrimidin-1-yl)Thieno[3,2-d]Pyrimidine **3**

(1) Method using Vilsmeier–Haack reagent: A solution of 3-phenyl-1-(thieno[3,2-*d*]pyrimidin-4-yl)-1*H*-pyrazol-5-amine **1** (100 mg, 0.34 mmol) and $POCl_3$ (0.10 mL, 1.1 mmol, 3.0 equiv) in DMF (3 mL) was heated at 70 °C with stirring for 5 h. The mixture was cooled to room temperature and evaporated to dryness. Acetic acid (5 mL) and ammonium carbonate (105 mg, 1.1 mmol) were added to the residue, the reaction mixture was heated under reflux for 5 h. When the

reaction was completed, the mixture was added to saturated aqueous sodium bicarbonate (15 mL) and extracted with CH_2Cl_2 (2 × 15 mL). The organic extracts were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (eluent: ethyl acetate/petroleum ether (bp 40–60 °C) = 1/10) to give the title compound **3** (92 mg, 82%) and recrystallized as light yellow needles, mp 263–264 °C (EtOH); TLC *Rf* = 0.25 (ethyl acetate/*n*-hexane = 20/80); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 9.95 (s, 1H), 9.38 (s, 1H), 9.21 (s, 1H), 8.61 (d, *J* = 5.8 Hz, 1H), 8.36–8.34 (m, 2H), 7.74 (d, *J* = 5.8 Hz, 1H), 7.72–7.68 (m, 2H), 7.66–7.62 (m, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$) 163.3, 157.0, 154.8, 154.2, 153.6, 152.2, 146.4, 141.4, 130.5, 130.0, 129.4 (2 × C), 127.5 (2 × C), 124.1, 118.5, 114.6. IR (KBr) 1620 (C = N), 755, 690 cm^{-1}. UV (λmax in MeOH, log ε) 350 (4.15), 288 (3.90) nm. MS (APCI) *m/z* = 331.21 (MH^+, 96%). Anal. calcd. for $C_{17}H_{10}N_6S$, %: C, 61.80; H, 3.05; N, 25.44. Found, %: C, 61.95; H, 3.10; N, 25.51.

(2) Direct method using a modified Vilsmeier–Haack reagent: A solution of 3-phenyl-1-(thieno[3,2-*d*] pyrimidin-4-yl)-1*H*-pyrazol-5-amine **1** (100 mg, 0.34 mmol) and $POCl_3$ (0.10 mL, 1.1 mmol, 3.0 equiv) in formamide (3 mL) was heated at 60 °C with stirring for 5 h. After the completion, the reaction mixture was concentrated and worked-up in the same way as above. The residue was purified by column chromatography (eluent: ethyl acetate/petroleum ether (bp 40–60 °C) = 1/10) to give the title compound **3** (89 mg, 80%).

Supplementary Materials: The following are available online at http://www.mdpi.com/1422-8599/2020/2/M1136/s1, Figure S1: ^{1}H- and ^{13}C-NMR spectra of compound **3**, Figure S2: Mass spectrum of compound **3**.

Author Contributions: E.S.N., S.M.K. and Y.-H.S. conceived and designed the experiments; E.S.N. and S.M.K. performed the experiments; Y.-H.S. analyzed data and wrote the paper. All authors have read and agreed to the published version of the manuscript.

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

References

1. Ali, E.M.H.; Abdel-Maksoud, M.S.; Oh, C.-H. Thieno[2,3-*d*]pyrimidines as a promising scaffold in medicinal chemistry: Recent advances. *Bioorg. Med. Chem.* **2019**, *27*, 1159–1194. [CrossRef] [PubMed]
2. Ghith, A.; Ismail, N.S.M.; Youssef, K.; Abouzid, K.A.M. Medicinal attributes of thienopyrimidine based scaffold targeting tyrosine kinases and their potential anticancer activities. *Arch. Pharm.* **2017**, *350*, 1700242. [CrossRef] [PubMed]
3. Abdellatif, K.R.A.; Bakr, R.B. New advances in synthesis and clinical aspects of pyrazolo[3,4-*d*]pyrimidine scaffolds. *Bioorg. Chem.* **2018**, *78*, 341–357. [CrossRef] [PubMed]
4. Chauhan, M.; Kumar, R. Medical attributes of pyrazolo[3,4-*d*]pyrimidines: A review. *Bioorg. Med. Chem. Lett.* **2013**, *21*, 5657–5668. [CrossRef] [PubMed]
5. Romagnoli, R.; Prencipe, F.; Oliva, P.; Baraldi, S.; Baraldi, P.G.; Schiaffino Ortega, S.; Chayah, M.; Salvador, M.K.; Lopez-Cara, L.C.; Brancale, A.; et al. Design, synthesis, and biological evaluation of 6-substituted thieno[3,2-*d*]pyrimidine analogues as dual epidermal growth factor receptor kinase and microtubule inhibitors. *J. Med. Chem.* **2019**, *2*, 1274–1290. [CrossRef] [PubMed]
6. Hu, H.; Dong, Y.; Li, M.; Wang, R.; Zhang, X.; Gong, P.; Zhao, Y. Design, synthesis and biological evaluation of novel thieno[3,2-*d*]pyrimidine and quinazoline derivatives as potent antitumor agents. *Bioorg. Chem.* **2019**, *90*, 103086. [CrossRef] [PubMed]
7. Hafez, H.N.; El-Gazzar, A.-R.B.A.; Zaki, M.E.A. Simple approach to thieno[3,2-*d*]pyrimidines as new scaffolds of antimicrobial activities. *Acta Pharm.* **2016**, *66*, 331–351. [CrossRef] [PubMed]
8. Wang, Z.; Kang, D.; Chen, M.; Wu, G.; Feng, D.; Zhao, T.; Zhou, Z.; Huo, Z.; Jing, L.; Zuo, X.; et al. Design, synthesis, and antiviral evaluation of novel hydrazone-substituted thiophene[3,2-*d*]pyrimidine derivatives as potent human immunodeficiency virus-1 inhibitors. *Chem. Biol. Drug Des.* **2018**, *92*, 2009–2021. [CrossRef] [PubMed]

9. Barone, M.; Graziano, A.C.E.; Marrazzo, A.; Gemmellaro, P.; Santagati, A.; Cardile, V. Synthesis and biological evaluation of new benzo-thieno[3,2-*d*]pyrimidin-4-one sulphonamide thio-derivatives as potent selective cyclooxygenase-2 inhibitors. *Mol. Divers.* **2013**, *17*, 445–458. [CrossRef] [PubMed]
10. Wang, Y.-Y.; Xu, F.-Z.; Zhu, Y.-Y.; Song, B.; Luo, D.; Yu, G.; Chen, S.; Xue, W.; Wu, J. Pyrazolo[3,4-*d*]pyrimidine derivatives containing a Schiff base moiety as potential antiviral agents. *Bioorg. Med. Chem. Lett.* **2018**, *28*, 2979–2984. [CrossRef] [PubMed]
11. Cherukupalli, S.; Chandrasekaren, B.; Krystof, V.; Aleti, R.R.; Sayyad, N.; Merugu, S.R.; Kushwaha, N.D.; Karpoormath, R. Synthesis, anticancer evaluation, and molecular docking studies of some novel 4,6-disubstituted pyrazolo[3,4-*d*]pyrimidines as cyclin-dependent kinase 2 (CDK2) inhibitors. *Bioorg. Chem.* **2018**, *79*, 46–59. [CrossRef] [PubMed]
12. Tageldin, G.N.; Fahmy, S.M.; Ashour, H.M.; Khalil, M.A.; Nassra, R.A.; Labouta, I.M. Design, synthesis and evaluation of some pyrazolo[3,4-*d*]pyrimidines as anti-inflammatory agents. *Bioorg. Chem.* **2018**, *78*, 358–371. [CrossRef] [PubMed]
13. Feitosa, L.M.; da Silva, E.R.; Hoelz, L.V.B.; Souza, D.L.; Come, J.A.A.S.S.; Cardoso-Santos, C.; Batista, M.M.; Soeiro, M.N.C.; Boechat, N.; Pinheiro, L.C.S. New pyrazolopyrimidine derivatives as Leishmania amazonensis arginase inhibitors. *Bioorg. Med. Chem.* **2019**, *27*, 3061–3069. [CrossRef] [PubMed]
14. Hassaneen, H.D.; Saleh, F.M.; Abdallah, T.A.; Mohamed, M.F.; Mohamed, Y.S.; Awad, E.M.; Abdelhamid, I.A. Synthesis, cytotoxicity, antimicrobial and docking simulation of novel pyrazolo[3,4-*d*]pyrimidine and pyrazolo[4,3-*e*]triazolo[3,4-*c*]pyrimidine derivatives. *Mini Rev. Med. Chem.* **2019**, *19*, 657–670. [CrossRef] [PubMed]
15. Park, J.W.; Song, Y.-H. Synthesis of thienopyrimidine-pyrazolo[3,4-*b*]pyridine hybrids. *Heterocycl. Commun.* **2017**, *23*, 281–285. [CrossRef]
16. Jang, H.-J.; Kim, S.M.; Rho, M.-C.; Lee, S.W.; Song, Y.-H. Synthesis of thienopyrimidine derivatives as inhibitors of STAT3 activation induced by IL-6. *J. Microbiol. Biotechnol.* **2019**, *29*, 856–862. [CrossRef] [PubMed]
17. Simay, A.; Takacs, K.; Horvath, K.; Dvortsak, P. Vilsmeier-Haack reaction of 5-amino- and 5-acylaminopyrazoles. *Acta Chim. Acad. Sci. Hung.* **1980**, *105*, 127–140.
18. Chang, C.-H.; Tsai, H.J.; Huang, Y.-Y.; Lin, H.-Y.; Wang, L.Y.; Wu, T.S.; Wong, F.F. Selective synthesis of pyrazolo[3,4-*d*]pyrimidine, *N*-(1*H*-pyrazol-5-yl)formamide, or *N*-(1*H*-pyrazol-5-yl)formamidine derivatives from *N*-1-substituted-5-aminopyrazoles with new Vilsmeier-type reagents. *Tetrahedron* **2013**, *69*, 1378–1386. [CrossRef]
19. Zhang, M.; Zhu, W.; Li, Y. Discovery of novel inhibitors of signal transducer and activator of transcription 3 (STAT3) signaling pathway by virtual screening. *Eur. J. Med. Chem.* **2013**, *62*, 301–310. [CrossRef] [PubMed]
20. Li, H.-C.; Zhang, G.-Y. Inhibitory effect of genistein on activation of STAT3 induced by brain ischemia/reperfusion in rat hippocampus. *Acta Pharmacol. Sin.* **2003**, *24*, 1131–1136. [PubMed]