

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 ¹H-, ¹³C-NMR, IR spectroscopy, mass-spectrometry and elemental analysis.

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

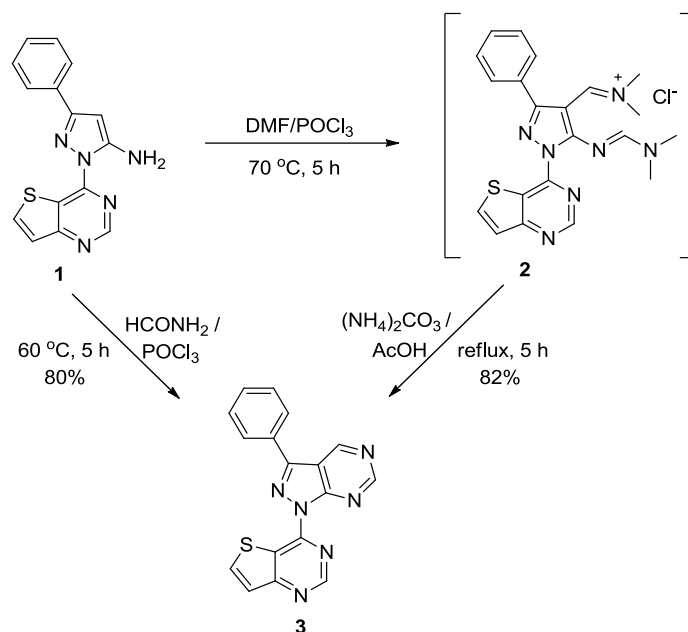
1. Introduction

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].



Scheme 1. Synthesis strategy for the hybrid compound 3.

The structure of **3** was confirmed by ^1H -, ^{13}C -NMR, IR spectroscopy, mass-spectrometry and elemental analysis. The ^1H -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, $\text{C}_{17}\text{H}_{10}\text{N}_6\text{S}$. Elemental analysis also provided satisfactory results for 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, ^1H -, ^{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 $2.55\text{ }\mu\text{M}$. Genistein with IC_{50} value of $15.0\text{ }\mu\text{M}$ 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 ^1H -, 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 Shimadzu 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)-1H-pyrazol-5-amine **1** (100 mg, 0.34 mmol) and POCl₃ (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 CH₂Cl₂ (2 × 15 mL). The organic extracts were dried over MgSO₄, 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 R_f = 0.25 (ethyl acetate/*n*-hexane = 20/80); ¹H-NMR (400 MHz, CDCl₃) 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). ¹³C-NMR (100 MHz, CDCl₃) 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 (λ_{max} in MeOH, log ε) 350 (4.15), 288 (3.90) nm. MS (APCI) *m/z* = 331.21 (MH⁺, 96%). Anal. calcd. for C₁₇H₁₀N₆S, %: 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)-1H-pyrazol-5-amine **1** (100 mg, 0.34 mmol) and POCl₃ (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, Figure S1: ¹H and ¹³C 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.

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