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Short Note

(3,5-Dimethylpyrazol-1-yl)-[4-(1-phenyl-1*H*-pyrazolo [3,4-*d*]pyrimidin-4-ylamino)phenyl]methanone

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Abstract: In an attempt to enhance cytotoxic activity of pyrazolo[3,4-*d*]pyrimidine core, we synthesized (3,5-dimethylpyrazol-1-yl)-[4-(1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamino)phenyl]methanone (**4**) by reacting 4-(1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamino)benzohydrazide (**3**) with acetylacetone. Antiproliferative activity of this compound was screened against breast (MCF-7), colon (HCT-116), and liver (HEPG-2) cancer cell lines. The tested compound exhibited cytotoxic activity with IC_{50} = 5.00–32.52 μM. Moreover, inhibitory activity of this compound was evaluated against the epidermal growth factor receptor (EGFR), the fibroblast growth factor receptor (FGFR), the insulin receptor (IR), and the vascular endothelial growth factor receptor (VEGFR). This target compound showed potent inhibitory activity, especially against FGFR with IC_{50} = 5.18 μM.

Keywords: pyrazole; pyrazolo[3,4-*d*]pyrimidine; anticancer; protein kinases

1. Introduction

Protein kinases play an important role in cell proliferation, differentiation, migration, metabolism, and apoptosis [1,2]. Dysregulation of protein kinases occurs in a variety of diseases including cancer [3,4]. Overexpression of protein kinase in tumor cells can be blocked by selective kinase inhibitors, so these inhibitors are considered a promising approach for treatment of cancer [5–8].

Pyrazolo[3,4-*d*]pyrimidine derivatives attract great interest because of their diverse biological and pharmacological properties. Among these properties, their anticancer effects have been extensively evaluated [9–12].

The cytotoxic effect of this class of compounds is attributed to different mechanisms. They have been reported as acting as cyclin dependent kinase (CDK) inhibitors [13–15], glycogen synthase kinase (GSK) inhibitors [16,17], epidermal growth factor receptor (EGFR) inhibitors [18], and dual src/Ab1 kinase inhibitors [19].

A new series of 2-(3,6-dimethyl-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yloxy)-*N*-(4-substitutedbenzylidene)acetohydrazides, specifically compound **1** (Figure 1), displayed cytotoxic activity against breast carcinoma (MCF-7), non-small cell lung cancer (A549), and human colorectal adenocarcinoma (HT-29) cell lines, and proved to be inhibitors of EGFR protein kinase [20]. El Hamid et al. synthesized and evaluated a new set of 1-aryl-4-benzylidenehydrazinyl-3-methylsulphanyl-pyrazolo[3,4-*d*]pyrimidines as anti-breast cancer agents. Compound **2** was the most active compound in this search with an IC_{50} equal to 7.5 nM [21]. In addition, the literature survey revealed that the pyrazole moiety represents an important pharmacophore in several anticancer active

agents [22–28]. All these facts encouraged us to hybridize the pyrazolo[3,4-*d*]pyrimidine scaffold with the pyrazole nucleus in a trial to obtain a promising new anticancer active agent.

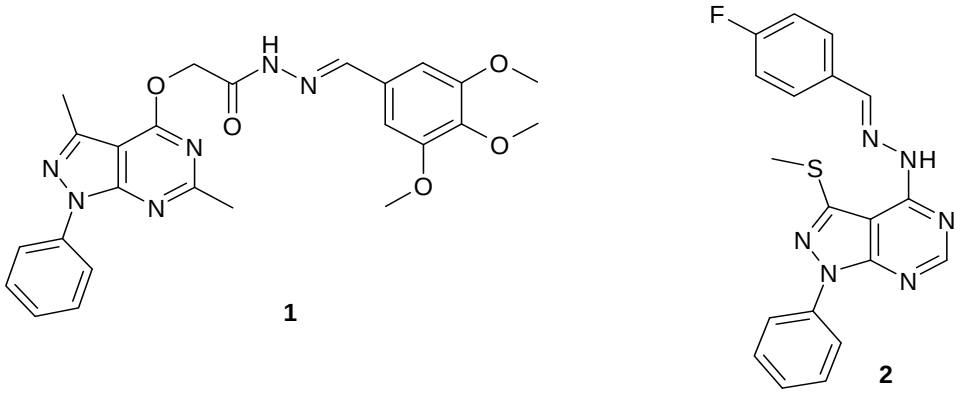
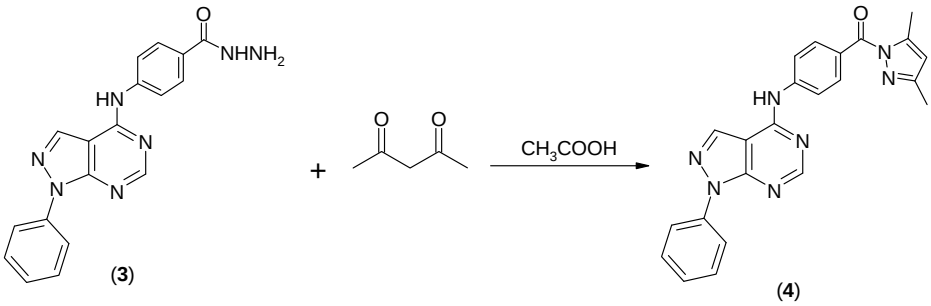


Figure 1. Chemical structures of some reported pyrazolo[3,4-*d*]pyrimidine derivatives as anticancer agents.

2. Results and Discussion

2.1. Chemistry

(3,5-Dimethylpyrazol-1-yl)-[4-(1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamino)phenyl]methanone (**4**) was prepared by refluxing 4-(1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamino)benzohydrazide (**3**) [29] with acetylacetone in acetic acid for 10 h, as shown in Scheme 1.



Scheme 1. Synthetic pathway for target compound **4**.

The structure of the target compound **4** was elucidated by IR, ^{1}H-, and ^{13}C-NMR, MS, and elemental analysis. All data are in accordance with the assumed structure. The IR spectrum of this compound revealed the presence of a NH group at 3232 cm^{-1} and a C=O group at 1697 cm^{-1}.

The ^{1}H-NMR spectrum showed two singlet signals at δ 2.20 and 2.56 ppm corresponding to the two methyl protons in addition to a singlet signal at δ 6.28 ppm, corresponding to the pyrazole H-4proton. Inspection of the ^{13}C-NMR spectrum indicated an appearance of two methyl peaks at δ 13.90 and 14.27 ppm and pyrazole C4 at δ 111.53 ppm. Finally, the mass spectrum of **4** demonstrated a molecular ion peak at *m*/*z* 409 ($M^{+\cdot}$) and a base peak at *m*/*z* 314.

2.2. Pharmacological Screening

The newly synthesized compound was screened in vitro for its anticancer activity against three cancer cell lines—breast (MCF-7), liver (HEPG-2), and colon (HCT-116) cancer cell lines. The results were expressed in terms of IC_{50} values (the concentration that resulted in a 50% inhibition of cell viability) where the well-known anticancer agents doxorubicin was used as a positive control (Table 1).

Table 1. Cytotoxic activity of compound **4** and doxorubicin on three cancer cell lines—MCF-7, HEPG-2, and HCT-116.

Compound	IC_{50} (μM)		
	MCF-7	**HEPG-2**	**HCT-116**
4	32.52 ± 0.02	5.00 ± 0.02	14.31 ± 0.05
Doxorubicin	2.60 ± 0.02	5.66 ± 0.12	8.48 ± 0.32

The tested compound showed a marked antitumor activity against all the tested cell lines with IC_{50} values of 5.00–32.52 μM. Moreover, this compound was more potent (IC_{50} = 5.00 μM) than doxorubicin (IC_{50} = 5.66 μM) against the liver cancer cell line (HEPG-2).

Furthermore, this compound was evaluated against different protein kinases such as the epidermal growth factor receptor (EGFR), the fibroblast growth factor receptor (FGFR), the insulin receptor (IR), and the vascular endothelial growth factor receptor (VEGFR) in an attempt to explore the mechanism of action of this compound. The obtained results demonstrated that this compound exhibited good inhibitory activity against all used protein kinases with IC_{50} values of 5.18–27.89 μM (Table 2).

Table 2. Kinase inhibitory activity of compound **4**.

IC_{50} (μM)			
EGFR	IR	VEGFR	FGFR
13.47	10.29	27.89	5.18

3. Materials and Methods

3.1. Chemistry

Melting points was determined on a Thomas–Hoover capillary apparatus and are uncorrected. Infrared (IR) spectrum was recorded as films on NaCl plates using a Nicolet 550 Series II Magna FT-IR spectrometer (Shimadzu, Kyoto, Japan). ^{1}H-NMR and ^{13}C-NMR spectra were measured on a BrukerAvance III 400 MHz for ^{1}H and 100 MHz for ^{13}C (Bruker AG, Fällanden, Switzerland) with a BBFO Smart Probe and a Bruker 400 AEON Nitrogen-Free Magnet, Faculty of Pharmacy, Beni-Suef University, Egypt in DMSO-d_6 with TMS as the internal standard, where *J* (coupling constant) values are estimated in Hertz (Hz), and chemical shifts were recorded in ppm δ scale. Mass spectrum (MS) was recorded on a Hewlett Packard 5988 spectrometer (Hewlett-Packard Co., Palo Alto, CA, USA). Microanalyses for C, H, and N were carried out on a Perkin–Elmer 2400 analyzer (Perkin-Elmer, Norwalk, CT, USA) at the Micro analytical unit of Cairo University, Egypt All other reagents, purchased from the Aldrich Chemical Company (Milwaukee, WI, USA), were used without further purification. 4-(1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamino)benzohydrazide (**3**) was prepared according to a literature procedure [29].

(3,5-Dimethylpyrazol-1-yl)-[4-(1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-ylamino)phenyl]methanone) (**4**)

A mixture of 4-(1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamino)benzohydrazide (**3**, 3.59 g, 10 mmol) and acetylacetone (1 g, 10 mmol) in acetic acid (10 mL) was heated under reflux for 10 h. After cooling, the reaction mixture was poured onto ice water. The colorless powder obtained was crystallized from ethanol to yield compound **4**. Mp 180–182 °C; yield: 56%; IR (cm^{-1}): 3232 (NH); 3070 (CH aromatic); 2954 (CH aliphatic); 1697 (C=O); 1608 (C=N); ^{1}H-NMR (DMSO-d_6) δ ppm: 2.20 (s, 3H, CH_3), 2.56 (s, 3H, CH_3), 6.28 (s, 1H, pyrazole H-4 7.37–7.41 (m, 1H, phenyl H-4), 7.57–7.61 (m, 2H, phenyl H-3, H-5), 8.01 (d, *J* = 8.8 Hz, 2H, aminophenyl H-2, H-6), 8.09 (d, *J* = 8.8 Hz, 2H, aminophenyl H-3, H-5), 8.21 (s, 1H, pyrazole CH), 8.23 (s, 1H, pyrimidine CH), 8.67 (d, *J* = 12 Hz, 2H, phenyl H-2, H-6), 10.55 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6) δ: 13.90, 14.27, 103.35,

111.53, 119.80, 121.45, 127.06, 127.53, 129.70, 132.74, 134.09, 138.95, 143.48, 144.85, 151.92, 153.45, 154.63, 156.34, 167.43; EIMS (*m*/*z*) 409 ($M^{+\cdot}$, 28%), 314 (100%). Anal.Calcd for $C_{23}H_{19}N_7O$: C, 67.47; H, 4.68; N, 23.95. Found: C, 67.53; H, 4.42; N, 24.01.

3.2. Pharmacological Studies

3.2.1. Cell Viability Analysis

The mammary gland breast cancer cell line (MCF-7), the human hepatocellular carcinoma cell line (HEPG-2), and the colon carcinoma cell line (HCT-116) were obtained from the American Type Culture Collection (ATCC). Doxorubicin (positive control) and all chemicals used in this study are of high analytical grade and were obtained from either Sigma-Aldrich or Biorad. The different cell lines mentioned above were used to determine the inhibitory effects of the tested compounds on cell growth using the SulphoRhodamine-B (SRB) assay using the method of Skehan et al. [30]. Cells were plated in 96 multi-well plates for 24 h before treatment with the compounds to allow attachment of the cells to the wall of the plate. Different concentrations of the tested compounds (0, 6.25, 12.5, 25, 50, and 100 μg/mL) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compounds for 48 h at 37 °C and in an atmosphere of 5% CO_2; cells were fixed, washed, and stained with Sulforhodamine B stain. Excess stain was washed with acetic acid and attached stain was recovered with a Tris EDTA buffer. Color intensity was measured in an ELISA reader and the relation between surviving fraction and drug concentration was plotted and IC_{50} (the concentration required for 50% inhibition of cell viability) was determined for each compound by Sigma plot software (Sigma Plot 11.0 software, Systat Software Inc.; San Jose, California).

3.2.2. Kinases Inhibitory Activity

Kinase activity was determined using Kinase-Glo Plus luminescence kinase assay kit according to the previously reported method [31].

Supplementary Materials: The following are available online at www.mdpi.com/1422-8599/2016/4/M915, Figure S1: spectrum of compound **4**, Figure S2: ^{13}C-NMR spectrum of compound **4**, Figure S3: IR spectrum of compound **4**.

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Author Contributions: R.B.B. conceived, designed, and performed the experiments, analyzed the data, and wrote the paper. A.B.M. performed the anticancer screening and kinase inhibitory activity.

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

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