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Short Note

2-((5-(3-(2-Fluorophenyl)acryloyl)-4-methylthiazol-2-yl)amino) isoindoline-1,3-dione

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Abstract: In this work, the title compound was synthesized via the Claisen–Schmidt condensation of a 2-((5-acetyl-4-methylthiazol-2-yl)amino)isoindoline-1,3-dione with 2-fluorobenzaldehyde. The structure of the synthesized compound (yield 62%) was confirmed by ^{1}H, ^{13}C NMR, and LC–MS spectra. According to US NCI protocols, the compound displayed a high level of antimitotic activity against tested human tumor cells, with mean GI_{50}/TGI values of 15.72/50.68 μM. The drug-like properties of the synthesized compound were evaluated using SwissAdme, revealing satisfactory drug-like parameters, and it presents interest for the design of new synthetic agents with biological activity.

Keywords: thiazole; Claisen–Schmidt condensation; anticancer activity; pharmacokinetics prediction

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1. Introduction

Thiazole derivatives are the structural basis of many lead compounds and essential components of numerous drugs, biologically active natural compounds, and cofactors [1,2]. An important argument in favor of the choice of thiazoles as objects of scientific research in the construction of drug-like molecules is the possibility of their extensive chemical modification to obtain various classes of condensed and non-condensed derivatives [2]. In addition, the use of thiazole/thiazolidinone derivatives in the context of the "hybrid-pharmacophore" approach made it possible to obtain many existing drugs and compounds in the preclinical research stages [3].

In particular, among the functional thiazole derivatives, there is also a large number of synthetic compounds that are at the stages of preclinical research [3], in particular, clomethiazole as an allosteric modulator of $GABA_A$ receptors for the treatment of withdrawal syndrome in alcoholism; niridazole as an antischistosomiasis agent; aldose reductase inhibitor zopolrestat, which is used in the treatment of diabetic complications; teneligliptin, balaglitazone, mitoglitazone, and halicin as agents for the treatment of type 2 diabetes; hypolipidemic agent netoglitazone; podotimod as an immunomodulatory agent; the A2 adenosine receptor agonist tozadenant as a drug of treating cocaine addiction; efatutazone, quizartinib, pidnarulex, and epalrestat as antitumor agents; talarozole as a drug in the treatment of psoriasis and other skin diseases; hypouricemic agent dotinurad; ebopiprant as a treatment for preterm labor; and JNK kinase inhibitor bentamipod for the treatment of endometriosis (Figure 1).

It is worth noting that the combination of thiazole with other biophore fragments (indole, isoindole, pyrazole, oxadiazole, and fragments of existing drugs) in one molecule allows obtaining derivatives that have enhanced biological activity, the presence of polypharmacological properties, or a low toxicity profile [4]. Therefore, in-depth studies of polyfunctional thiazole derivatives, their synthesis, and the study of their physico-chemical and

biological properties with the subsequent establishment of the structure-activity relationship are of great interest both from the point of view of theoretical research and in terms of the directed synthesis of potential drug-like molecules.

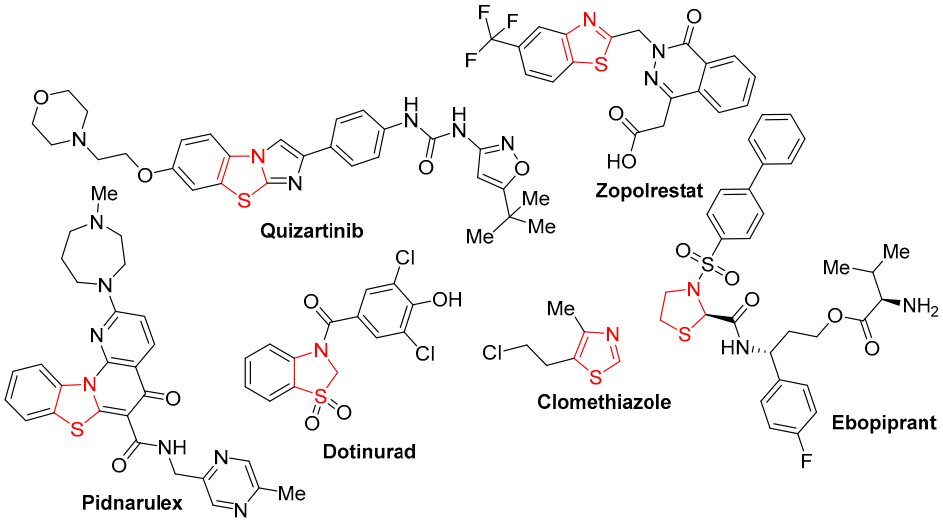
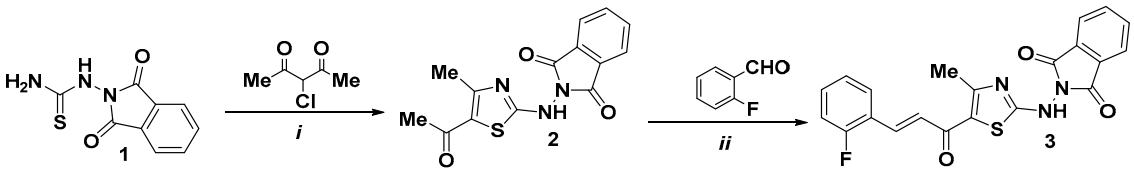


Figure 1. Structures of thiazole-bearing drugs.

2. Results and Discussion

2.1. Synthesis of the Title Compound **3**

Compound **2**, obtained from a [2+3]-cyclocondensation reaction with 1-(1,3-dioxoisoindolin-2-yl)thiourea **1** as the *S*,*N*-binucleophile [5] and 2-chloroacetylacetone as the dielectrophilic synthon, was employed as the starting reagent in the synthesis of the targeted derivative **3**, as depicted in Scheme 1. Subsequently, compound **3**, (*E*)-2-((5-(3-(2-fluorophenyl)acryloyl)-4-methylthiazol-2-yl)amino)isoindoline-1,3-dione, was synthesized via a Claisen–Schmidt condensation between compound **2** and 2-fluorobenzaldehyde. Notably, this reaction utilized potassium *tert*-butoxide as a catalyst in an ethanol solvent medium [6].



Scheme 1. Synthesis of hybrid isoindole-thiazole derivatives. Reagents and conditions: (*i*) 1-(1,3-dioxoisoindolin-2-yl)thiourea **1** (0.01 mol), 3-chloropentane-2,4-dione (0.011 mol), AcONa (0.01 mol), AcOH (10 mL), reflux, 5 h, 74%; (*ii*) compound **2** (0.01 mol), 2-fluorobenzaldehyde (0.02 mol), *t*-BuOK (0.015 mol), EtOH (15 mL), reflux; 5 h; AcOH to pH = 7.0, 62%.

The identity and purity of the isoindole-thiazole derivatives acquired were confirmed through analysis of ^{1}H, ^{13}C NMR, and LC–MS spectral data. The signals for the C-4 methyl groups in **2** and **3** each appear as a three-proton singlet at 2.36 and 2.71 ppm, respectively. Meanwhile, the enone fragment of 2-((5-(3-(2-fluorophenyl)acryloyl)-4-methylthiazol-2-yl)amino)isoindoline-1,3-dione manifested as two doublets at 7.73 and 7.95 ppm, each with spin-spin coupling constants of 16.1 Hz, indicating a *trans*-configuration of these protons. Furthermore, in the ^{13}C NMR spectrum of compound **3**, the signals of carbon atoms within the carbonyl (C=O) groups were observed at 182.8 and 177.9 ppm.

The molecular ion peak observed at a *m*/*z* value of 408.0 $[M + H]^+$ in positive ionization mode in the mass spectrum confirmed the formation of the title compound **3**.

2.2. In Vitro Evaluation of the Anticancer Activity of Compound **3**

Compound **3** underwent assessment by the National Cancer Institute (NCI) through the Developmental Therapeutic Program (DTP), employing a single-dose assay (10^{-5} M) across a panel of approximately sixty cancer cell lines, following the established NCI protocol as described previously [7–10]. The synthesized 2-((5-(3-(2-fluorophenyl)acryloyl)-4-methylthiazol-2-yl)amino)isoindoline-1,3-dione (**3**) exhibited significant efficacy, displaying an average cell growth inhibition rate (GP_{mean}) of 12.53%. Notably, compound **3** demonstrated its most potent cytotoxic effects against the non-small cell lung cancer cell line HOP-62 (GP = −17.47%), CNS cancer SF-539 (GP = −49.97%), melanoma MDA-MB-435 (GP = −22.59%), ovarian cancer OVCAR-8 (GP = −27.71%), prostate cancer DU-145 (GP = −44.35%), and breast cancer MDA-MB-468 (GP = −15.65%) (Table 1, Figure S7).

Table 1. Anticancer screening data in concentration 10^{-5} M.

Compound	60 Cell Lines Assay in One Dose, 10 μM		
	Mean Growth, %	Range of Growth, %	Most Sensitive Cell Line(s) Growth Inhibition Percent [1]/Line/Panel [2]
3	12.53	−49.97 to 62.87	−3.50/RPMI-8226/L −17.47/HOP-62/NSCLC 3.87/KM12/CC −49.97/SF-539/CNSC 4.88/SNB-19/CNSC 3.98/U251/CNSC −22.59/MDA-MB-435/M 4.94/SK-MEL-5/M 0.65/UACC-62/M −5.19/OVCAR-3/OC −27.71/OVCAR-8/OC −3.97/NCI/ADR-RES/OC 1.87/RXF 393/RC −2.72/SN12C/RC −44.35/DU-145/PC −10.35/T-47D/BC −15.65/MDA-MB-468/BC

[1] Percent growth (GP%) ≤ 5%. [2] Abbreviations: L—leukemia; NSCLC—non-small cell lung cancer; CC—colon cancer; CNSC—CNS cancer; M—melanoma; OC—ovarian cancer; RC—renal cancer; PC—prostate cancer; BC—breast cancer.

Compound **3** was also selected for in-depth screening at concentrations ranging from 10^{-4} to 10^{-8} M toward 59 cell lines using the SRB protein assay to estimate cell viability or growth. Three antitumor activity dose-response parameters were calculated for each cell line: GI_{50}, TGI, and LC_{50}. A mean graph midpoint (MG_MID) was calculated for each parameter. Compound **3** showed inhibition activity (GI_{50} < 10 μM) against all 59 human tumor cell lines with average GI_{50}/TGI/LC_{50} values of 15.72/50.68/91.85 μM. The most sensitive cell lines to compound **3** at a concentration of $GI_{50} \leq 10$ μM are shown in Table 2. All the results of the effect of compound **3** on the growth of individual tumor cell lines are shown in Table S1.

Table 2. Selective influence of compound **3** on the growth of individual tumor cell lines.

Disease	Cell Line	GI_{50}, μM [1]	SI (GI_{50})	TGI, μM	SI (TGI)	LC_{50}, μM
NSC lung cancer	HOP-62	3.18	4.94	>100.0	-	>100.0
	NCI-H460	6.25	2.51	28.1	1.80	>100.0
Colon cancer	COLO 205	1.70	9.24	No data	No data	No data
Ovarian cancer	SK-OV-3	3.28	4.79	>100.0	-	>100.0
Breast cancer	T-47D	2.78	5.65	>100.0	-	>100.0

[1] $GI_{50} \leq 10$ μM.

The selectivity index (SI) [11] was determined for compound **3** for each cell line (μM). Compound **3** exhibited non-selectivity in this study at the GI_{50} and TGI levels (Table S1).

Nevertheless, the mentioned derivative displayed a certain selectivity profile toward specific cell lines at the TGI level, with a selectivity index of 9.24 for COLO 205 (colon cancer) (Table 2), and a moderate level of selectivity was observed for breast cancer T-47D (SI = 5.65), non-small cell lung cancer cell line HOP-62 (SI = 4.94), and ovarian SK-OV-3 (SI = 4.79) cell lines.

2.3. Molecular and Pharmacokinetic Properties

The ADME prediction for the evaluated compound was performed using the SwissAdme online server [12]. The findings indicate favorable gastrointestinal absorption and a lack of ability to penetrate the blood-brain barrier. The predicted lipophilicity, denoted by log Po/w, suggests advantageous permeability across the cell membrane and oral absorption for the investigated derivative. Notably, the compound is not expected to serve as a substrate for P-glycoprotein. The negative skin permeability of the compound indicates limited penetration through the skin's cellular barrier. SwissAdme predicts potential pharmacokinetic interactions with specific cytochrome P450 enzymes (CYP450), including CYP1A2, CYP2C19, CYP2C9, and CYP3A4. Despite these observations, the cumulative predictive data endorse compound **3** as a promising candidate for further in-depth investigations (Table 3).

Table 3. Physicochemical and pharmacokinetic properties of the studied compound **3**.

	Physicochemical Properties	
1	Molecular weight	407.42
2	Num. of heavy atoms	29
3	Num. of arom. heavy atoms	17
4	Num. of rotatable bonds	5
5	Num. of H-bond acceptors	5
6	Num. of H-bond donors	1
7	Molar refractivity	111.14
8	TPSA Å^2	107.61
9	Consensus log Po/w	3.82
10	Lipinski Rule	Yes
	Pharmacokinetics	
11	GI absorption	High
12	BBB permeant	No
13	P-gp substrate	No
14	CYP1A2 inhibitor	Yes
15	CYP2C19 inhibitor	Yes
16	CYP2C9 inhibitor	Yes
17	CYP2D6 inhibitor	No
18	CYP3A4 inhibitor	Yes
19	Log Kp (SP) (cm/s) (skin permeation)	−5.42
20	Bioavailability score	0.55

3. Materials and Methods

3.1. General Information and Compound **3** *Synthesis*

Melting points were measured in open capillary tubes on an IA 9200 electrothermal melting point apparatus (Bibby Scientific Limited, Stone, UK) and are uncorrected. The elemental analyses (C, H, and N) were performed using the FlashSmart CHNS/O analyzer (Thermo Scientific, Waltham, MA, USA) and were within ±0.4% of the theoretical values. The 400 MHz-^{1}H and 126 MHz-^{13}C spectra were recorded on a Varian Unity Plus 400 (400 MHz) spectrometer (Varian Inc., Paulo Alto, CA, USA). Chemical shifts (δ) are quoted in ppm, and coupling constants (*J*) are reported in Hz. LC–MS spectra were obtained on the Agilent 1260 Infinity II with the single-quadrupole mass-selective detector Agilent 6125 (Agilent Technologies, Santa Clara, CA, USA). The reaction mixture was monitored

by thin-layer chromatography (TLC) using commercial glass-backed TLC plates (Merck Kieselgel 60 F_{254}). Solvents and reagents (3-chloro-2,4-pentanedione, CAS number: 1694-29-7; sodium acetate, CAS number: 127-09-3; 2-fluorobenzaldehyde, CAS number: 446-52-6; potassium *tert*-butoxide, CAS number: 865-47-4) that are commercially available were used without further purification. The 1-(1,3-dioxoisoindolin-2-yl)thiourea **1** was prepared according to the method described in [5].

3.1.1. 2-((5-Acetyl-4-methylthiazol-2-yl)amino)isoindoline-1,3-dione (**2**)

A mixture of 1-(1,3-dioxoisoindolin-2-yl)thiourea **1** (10 mmol) with 3-chloropentane-2,4-dione (11 mmol) and anhydrous sodium acetate (10 mmol) was refluxed for 5 h in glacial acetic acid (10 mL) (monitored by TLC). The obtained solid product was collected after cooling by filtration and recrystallized from the mixture of DMF and ethanol (in a ratio of 1:2).

Yellow crystals, yield: 71%, Rf = 0.67 (ethyl acetate/benzene: 1/2), mp 304–306 °C (DMF:EtOH). ^{1}H NMR (400 MHz, DMSO-*d6*): δ (ppm) 2.36 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 7.94 (m, 4H, arom.), 11.13 (s, 1H, NH). ^{13}C NMR (126 MHz, DMSO-d_6, δ): 18.1, 29.8, 124.2, 129.8, 135.7, 165.2, 169.7, 189.6. LCMS (ESI): m/z 302.0 (100.00%, $[M + H]^+$). Anal. Calc. for $C_{14}H_{11}N_3O_3S$: C 55.81%; H 3.68%; N 13.95%. Found: C 55.70%; H 3.77%; N 13.80%.

3.1.2. (*E*)-2-((5-(3-(2-Fluorophenyl)acryloyl)-4-methylthiazol-2-yl)amino)isoindoline-1,3-dione (**3**)

A mixture of compound **2** (10 mmol) with 2-fluorobenzaldehyde (20 mmol) and potassium *tert*-butoxide (15 mmol) in ethanol (15 mL) was heated under reflux for 2 h (monitored by TLC). After completion, the reaction mixture was cooled to room temperature and acidified to pH 7 with acetic acid. The resulting yellow solid was collected by filtration, washed with ethanol (5–10 mL), and recrystallized from acetic acid.

Brown crystals yield 62%, Rf = 0.67 (ethyl acetate/benzene: 1/2), mp 231–234 °C (AcOH). ^{1}H NMR (400 MHz, DMSO-d_6, δ): 2.71 (s, 3H, CH_3), 7.31 (m, 2H, arom.), 7.51 (m, 2H, arom.), 7.73 (d, 1H, *J* = 16.1 Hz, =CH), 7.77 (m, 1H, arom.), 7.86 (t, 1H, *J* = 7.4 Hz, arom.), 7.95 (d, 1H, *J* = 16.1 Hz, =CH), 8.07 (d, 1H, *J* = 7.8 Hz, arom.), 8.23 (d, 1H, *J* = 7.7 Hz, arom.).^{13}C NMR (126 MHz, DMSO-d_6): δ 19.1, 111.2, 121.3, 125.5, 126.9, 127.9, 129.5, 130.2, 133.6, 134.7, 136.1, 141.0, 158.6, 166.3, 169.1, 177.9, 182.8. LCMS (ESI+) *m/z* 408.0 (100%, $[M + H]^+$). Anal. calc. for $C_{21}H_{14}FN_3O_3S$: C, 61.91%; H, 3.46%; N, 10.31%. Found: C, 61.70%; H, 3.30%; N, 10.40%.

3.2. In Vitro Anticancer Assay

A primary anticancer assay was conducted on approximately sixty human tumor cell lines originating from nine neoplastic diseases, following the protocol of the Drug Evaluation Branch at the National Cancer Institute, Bethesda [7–10]. The tested compounds were introduced to the culture at a single concentration (10^{-5} M), and the cultures were incubated for 48 h. Endpoint determinations were made using the protein-binding dye Sulphorhodamine B (SRB). Results for each tested compound were expressed as the percentage of growth in treated cells compared to untreated control cells and evaluated spectrophotometrically against controls not exposed to test agents.

The cytotoxic and growth inhibitory effects of the most active selected compounds were further examined in vitro against the entire panel of human tumor cell lines, with concentrations ranging from 10^{-4} to 10^{-8} M. A continuous 48-h drug exposure protocol was followed, and an SRB protein assay was employed to estimate cell viability or growth.

Using absorbance measurements (i.e., time zero (Tz), control growth in the absence of drug (C), and test growth in the presence of drug (Ti)), the percentage growth was calculated for each drug concentration. Percentage growth inhibition was calculated as follows:

$$[(\mathrm{Ti} - \mathrm{Tz})/(\mathrm{C} - \mathrm{Tz})] \times 100 \text{ for concentrations for which } \mathrm{Ti} \geq \mathrm{Tz},$$

$$[(\mathrm{Ti} - \mathrm{Tz})/\mathrm{Tz}] \times 100 \text{ for concentrations for which } \mathrm{Ti} < \mathrm{Tz}.$$

Dose-response parameters (GI_{50}, TGI, and LC_{50}) were computed for each compound. The growth inhibition of 50% (GI_{50}) was determined using the formula $[(Ti - Tz)/(C - Tz)] \times 100 = 50$, representing the drug concentration resulting in a 50% lower net protein increase in treated cells (measured by SRB staining) compared to the net protein increase in control cells. The concentration leading to total growth inhibition (TGI) was calculated as Ti = Tz. The LC_{50}, denoting the drug concentration causing a 50% reduction in measured protein at the end of treatment compared to the beginning, indicating a net loss of cells, was computed from $[(Ti - Tz)/Tz] \times 100 = -50$.

Values for each parameter were calculated if the desired activity level was achieved; however, if the effect was not attained or was excessive, the parameter value was expressed as more or less than the maximum or minimum concentration tested. The most sensitive cell lines yielded the lowest values. Compounds with GI_{50} values ≤ 100 μM were categorized as active.

3.3. Molecular and Pharmacokinetic Properties

The physical properties and adsorption, distribution, metabolism, elimination, and toxicity (ADMET) parameters of (*E*)-2-((5-(3-(2-fluorophenyl)acryloyl)-4-methylthiazol-2-yl)amino)isoindoline-1,3-dione were calculated using the SwissAdme online server of the Swiss Institute of Bioinformatics (http://www.swissadme.ch/index.php (accessed on 20 July 2023)).

4. Conclusions

As a result of this study, we obtained the target hybrid isoindole-thiazole derivative **3**, whose synthesis was validated, and the compound was fully characterized by spectral analysis methods. The preliminary anticancer results of compound **3** displayed mean GI_{50}/TGI_{50} values of 15.72/50.68 μM in the NCI 60 cell-line assay with a certain sensitivity profile towards the colon cancer COLO 205 cell line (SI = 9.24).

Supplementary Materials: Figures S1–S6: copies of NMR and LC–MS spectra of compounds **2** and **3**; Figures S7 and S8a–g: copies of NCI-60 cell lines screening protocols; Table S1: anticancer activity of compound **3** on the growth of individual tumor cell lines.

Author Contributions: Conceptualization was carried out by R.L.; methodology and experimental works were carried out by O.-M.F., A.L. and M.S.; data analysis was carried out by A.L.; writing, reviewing, and editing the paper were carried out by A.L. and R.L.; project administration and supervision were carried out by R.L. All authors have read and agreed to the published version of the manuscript.

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

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