Article

Synthesis of New 1-Aryl-2-(3,5-dimethylpyrazol-1-yl)ethanone Oxime Ether Derivatives and Investigation of Their Cytotoxic Effects

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Abstract: In this study, 12 new 1-aryl-2-(3,5-dimethylpyrazol-1-yl)ethanone oxime ether derivatives were designed and synthesized to investigate their cytotoxic effects. The in vitro cytotoxic activities of the compounds were evaluated against cervix, colon, breast, glioma, neuroblastoma, and lung cancer cell lines, as well as a healthy cell line using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays with 5-fluorouracil (5-FU) as the reference compound. Compound **5f** (IC$_{50}$ = 5.13 μM) was found to be more effective than 5-FU (IC$_{50}$ = 8.34 μM) in the C6 cancer cell line, and it had no cytotoxic effect on the L929 healthy cell line. Flow cytometry was used to investigate the mechanism of action of compound **5f** on the cell cycle of the C6 cell line. The analysis showed that cell death was significantly due to apoptosis. These results indicate that compound **5f** induces cell cycle arrest, and may be effective in treating glioma.

Keywords: 3,5-dimethylpyrazole; oxime ether; cytotoxic activity; glioma; neuroblastoma

1. Introduction

Cancer is one of the most significant health problems worldwide. In addition, it is the second-leading cause of death in the world after cardiovascular diseases. According to the World Health Organization (WHO), approximately 9.6 million deaths worldwide were due to cancer in 2018 [1]. Surgery and radiotherapy are generally the primary treatments for early-stage local and non-metastatic cancers. The use of anticancer drugs (e.g., chemotherapy and hormone or biological treatments) is especially preferred in metastatic cancers. Chemotherapy is generally based on preventing cell division as a result of targeting rapidly growing cancer cells [2,3].

However, cancer treatment is still a challenging area due to low selectivity, side effects, the development of resistance in various strains, and a lack of efficacy. Therefore, intensive

studies are consistently carried out in this field to develop safe, more effective, and selective anticancer agents [4].

Oxime ethers are a functional group frequently used for chemical modifications in medicinal chemistry and are included in the structure of active molecules. Specifically, the ant-Alzheimer's agents, anticancer, anticonvulsant, antimicrobial, and anti-inflammatory activities of oxime derivatives are well known [5–7]. Moreover, they are found in the structures of antidepressant, antifungal, and antibacterial drugs, such as fluvoxamine (**1**), oxiconazole (**2**), cefuroxime (**3**), and aztreonam (**4**) (Figure 1).
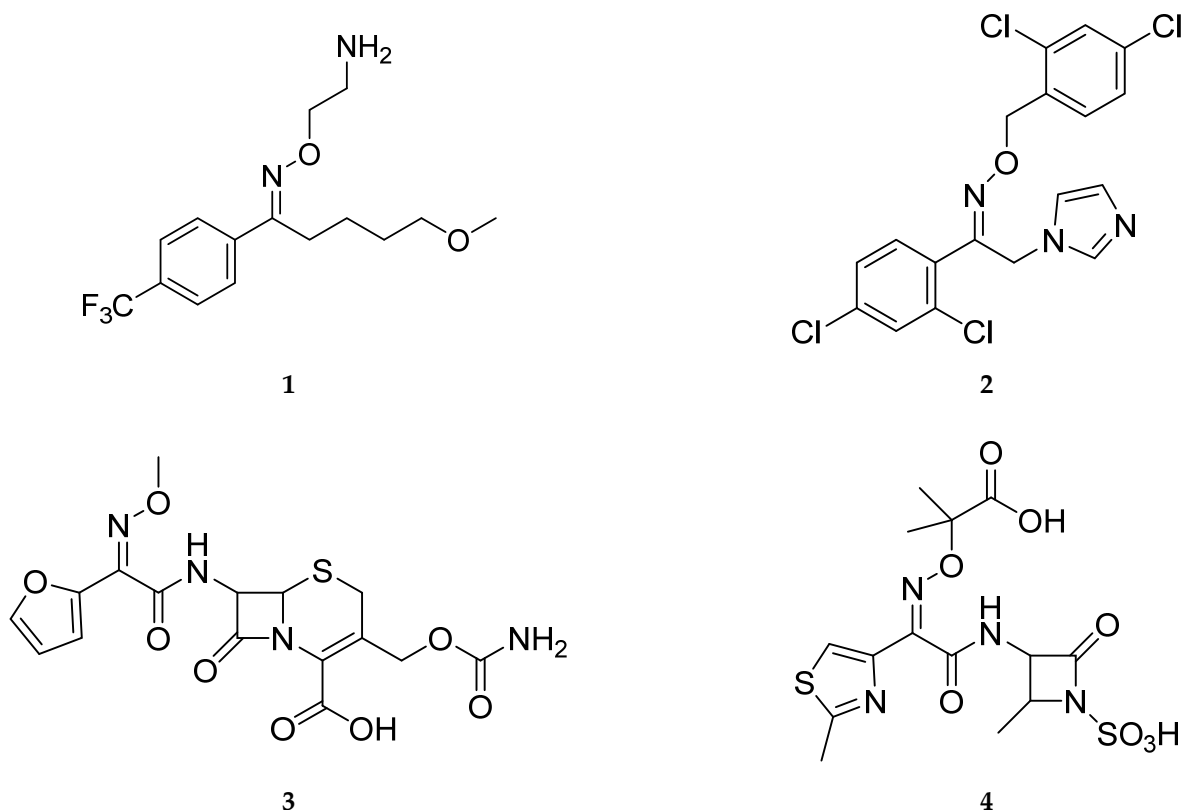

Figure 1. Structure of drugs with an oxime ether structure: fluvoxamine (**1**), oxiconazole (**2**), cefuroxime (**3**), and aztreonam (**4**).

Pyrazoles are heterocyclic aromatic compounds characterized by a five-membered ring (1,2-diazole) structure consisting of two nitrogen atoms and three carbon atoms adjacent to each other. Many biologically active compounds that carry pyrazole or substituted pyrazole derivatives, which exhibit interesting biological activities, such as antitumor, analgesic, anti-inflammatory, anticonvulsant, antipsychotic, antidepressant, anti-Alzheimer, anti-Parkinson, antihyperglycemic, antiobesity, antihypertensive, antibacterial, fungicidal, antiviral, and antitubercular activities, have been reported in the literature [8–16].

Azole compounds have gained their importance in cancer treatment. In addition, letrozole (**5**), ruxolitinib (**6**), fadrozole (**7**), crizotinib (**8**), and pralsetinib (**9**) are among the anticancer drugs with an azole moiety. Moreover, crizotinib (Figure 2) is currently undergoing clinical trials against neuroblastoma and other advanced solid tumors in both adults and children [4,17–19]. In the recent literature, some compounds with a dimethylpyrazole ring were reported to be potent anticancer agents (**10**–**13**) (Figure 3) [20–24].
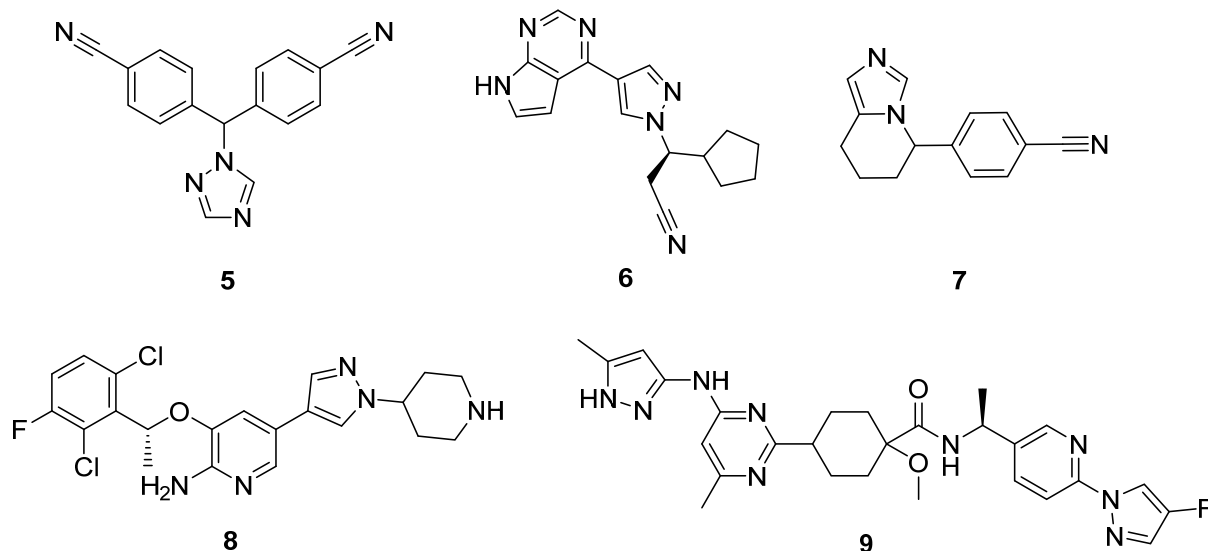
Figure 2. Chemical structures of anticancer drugs with an azole moiety: letrozole (**5**), ruxolitinib (**6**), fadrozole (**7**), crizotinib (**8**), and pralsetinib (**9**).
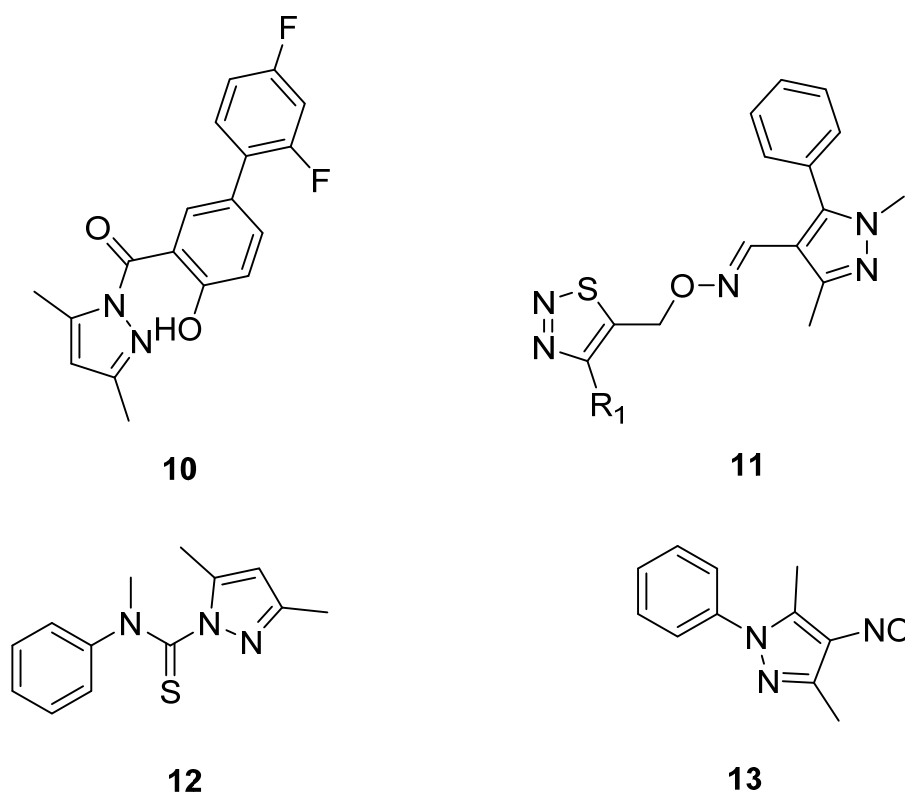
Figure 3. Chemical structures of cytotoxic compounds with a dimethylpyrazole ring. (2′,4′-difluoro-4-hydroxy-[1,1′-biphenyl]-3-yl)(3,5-dimethyl-1H-pyrazol-1-yl)methanone (**10**), (E)-1,3-dimethyl-5-phenyl-1H-pyrazole-4-carbaldehyde O-((1,2,3-thiadiazol-5-yl)methyl) oxime derivatives (**11**), N,3,5-trimethyl-N-phenyl-1H-pyrazole-1-carbothioamide (**12**), 3,5-dimethyl-4-nitroso-1-phenyl-1H-pyrazole (**13**).

Previously, the cytotoxic activities of some 2-(3,5-dimethyl-1*H*-pyrazol-1-yl)-1-arylethanone derivatives (**14**) (Figure 4) have been evaluated, and they were found to be active against human colon cancer and lung adenocarcinoma cell lines [11].
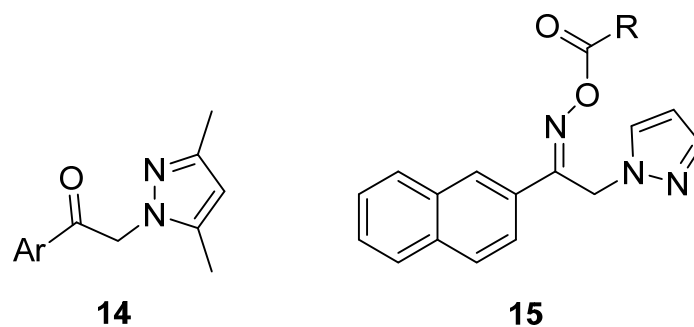
Figure 4. The 2-(3,5-dimethyl-1*H*-pyrazol-1-yl)-1-arylethanone derivatives (**14**) and oxime ester derivatives carrying a pyrazole ring with cytotoxic activity (**15**).

In our previous study, some oxime ester derivatives carrying a pyrazole ring were determined to exhibit cytotoxic activity against the SH-SY5Y (e.g., human neuroblast cells) cell line (**15**). These aryl oxime esters bearing the naphthalene and pyrazole ring did not show a better activity than the standard compounds. In addition, they did not affect healthy mouse fibroblast cells (Figure 4) [25].

In the present study, 12 new 1-aryl-2-(3,5-dimethylpyrazol-1-yl)ethanone derivatives were synthesized (Figure 5). This study is based on the ketone compounds prepared by Kumar et al., which were cytotoxic [11]. For this reason, the 3,5-dimethylpyrazole group was protected, which was thought to be effective in the activity. In addition, the structure-activity relationship (SAR) was established by synthesizing oxime derivatives with different aryl groups. Then, methyl and isobutyl oxime ethers were prepared to investigate the effect of lipophilicity on the activity. Since the naphthalene and aryl oxime esters with a pyrazole ring in our previous study did not have significant cytotoxic activity, we decided to prepare oxime ether compounds containing a limited number of alkyl halides.
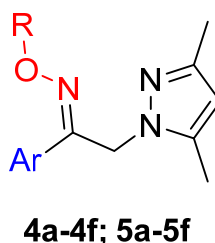
4a-4f; 5a-5f

Ar = 2-Naphtyl, phenyl and substitue phenyl (-F, -Cl, -CH$_3$, -OCH$_3$)
R= -CH$_3$, -CH$_2$-CH(CH$_3$)$_2$

Figure 5. Chemical structure of the designed compounds (**4a-4f; 5a-5f**).

The series include 2-naphthyl, phenyl, and substituted phenyl groups as the aryl groups. Moreover, this study investigated the effect of various substituents (e.g., -F, -Cl, -CH$_3$, and -OCH$_3$) bound to the phenyl group on the cytotoxic activity. Ketone and oxime derivatives were used as the starting material in the synthesis of the new oxime ether derivatives. Furthermore, in the present study, the in vitro cytotoxic activities of the compounds were evaluated against A549, HCT 116 (i.e., human colon cancer cells), HeLa (i.e., human cervical cancer cells), MCF7 (i.e., human breast adenocarcinoma cells), C6 (i.e., rattus norvegicus brain glioma), SH-SY5Y, and L929 (i.e., mouse fibroblast) cell lines.

These 12 new oxime ether compounds (**4a-4f; 5a-5f**) were synthesized from the appropriate oxime compounds (**3a-3f**). Since the -OH group of the oxime compound did not react with the alkyl halide, the -OH group was treated with Na^0 and -ONa was obtained. This reaction is the step with the lowest yield. The synthesis of **3a-3f** began from the appropriate ketone compounds (**2a-2f**). The compounds 3a-3f were synthesized as a result of an easy reaction of **2a-2f** with hydroxylamine hydrochloride in ethanol at pH = 11.

Compounds **2a-2f** were obtained by the substitution of 1-(aryl)-2-bromoethanone (**1a-1f**) with 3,5-dimethylpyrazole.

2. Results and Discussion

2.1. Chemistry

The structures and general synthetic routes of the compounds are shown in Scheme 1.
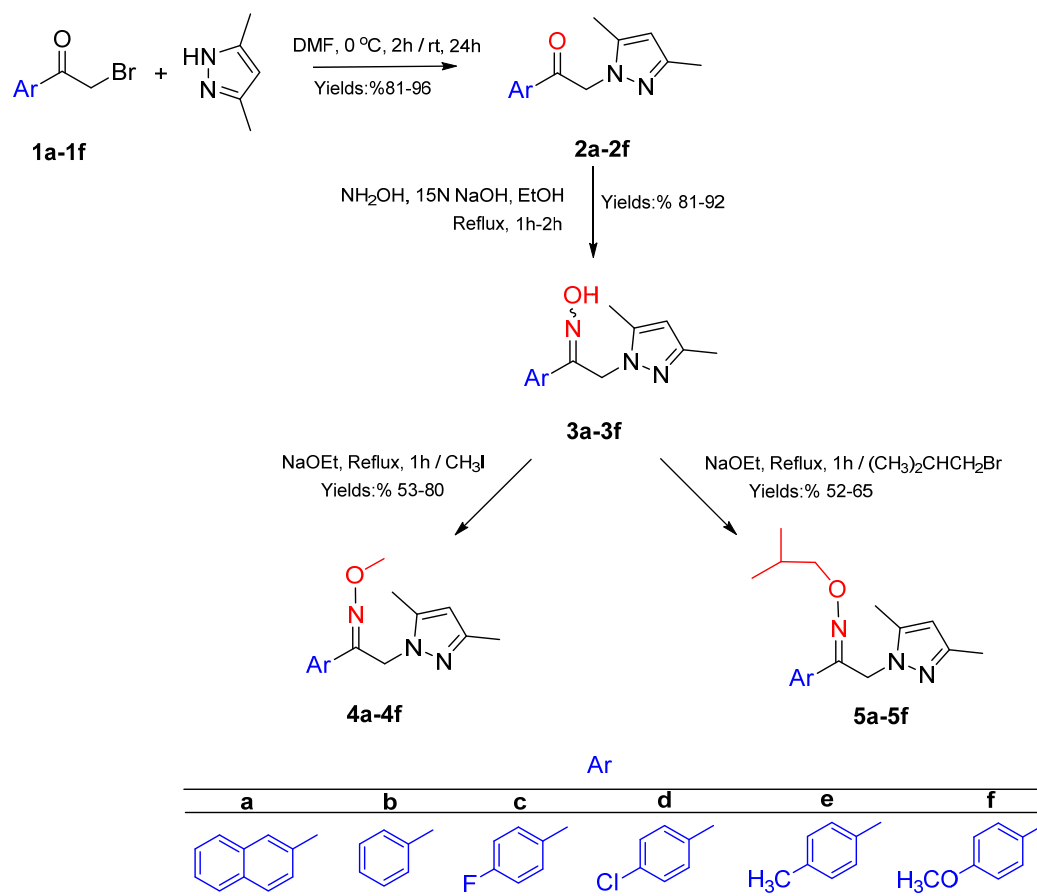
Scheme 1. Synthetic route.

First, we attempted the ring closure reaction presented in Kumar et al. to obtain the 1-aryl-2-(1*H*-3,5-dimethylpyrazol-1-yl)ethanone derivatives [11]. Although the reaction time was extended, the reaction did not end completely, and the reaction yield was very low. Therefore, the synthesis of these compounds was carried out via a *N*-alkylation reaction of 1-aryl-2-bromoethanone with 3,5-dimethylpyrazole. The spectral data of the compounds (**2a-2f**) were compatible with the results reported in the literature [11]. The oxime derivatives (**3a-3f**) were prepared via the reaction of 1-aryl-2-(3,5-dimethylpyrazol-1-yl)ethanone and hydroxylamine hydrochloride [26]. The compounds were obtained in high yields ranging from 81 to 93%. We adjusted our synthesis method, which used NaOH rather than $NaHCO_3$, to a pH of 11, and the reaction time was extended to 90 min. Therefore, we obtained the same compounds with a much higher yield than the reaction yields (32–80%) of Sharma et al. [27]. Two of these oximes (**3a** and **3f**) are novel compounds, and their spectral data are provided in the experimental section. The spectral data of the other oxime derivatives were in accordance with the results reported in the literature [27]. The oxime compounds were obtained as *E*/*Z* isomer mixtures.

The synthesis of the 12 novel oxime ether derivatives (**4a-4f–5a-5f**) was carried out via O-alkylation of the respective oximes with the appropriate alkyl halides. They were purified through column chromatography, and their yields ranged between 45–80%. A

double spot was observed in the TLC analysis of the reaction medium. However, a single isomer was obtained for each oxime ether derivative as a result of purification. This was probably due to the solvent effect, which converted one isomer into another.

In the IR spectra of the oxime and oxime ether derivatives, in accordance with the data reported in the literature, the peaks of C-O were observed at 1009–1072 cm^{-1}, C=N at 1513–1612 cm^{-1}, and N-O at 851–982 cm^{-1}. The disappearance of the flat peak of the -OH group at 3000–3400 cm^{-1} and the formation of a new peak belonging to the C-O group at 1100–1000 cm^{-1} showed the transformation of the oxime structures into their respective oxime ether derivatives.

The C=N-OH protons in the oxime derivatives were observed as a singlet in the range of 10.6–11.9 ppm. The aromatic protons were observed as multiples between 6.8–8.1 ppm. The protons of the -CH$_2$ attached to the pyrazole ring resonated as a singlet between 4.9–5.5 ppm. The =CH proton of the pyrazole was observed as a singlet between 5.67–5.72 ppm. Since the chemical environment of the protons of the -CH$_3$ attached to the pyrazole was different, they were observed as two separate singlets between 1.98–2.21 ppm.

The oximes (**3a-f**) were obtained as mixtures of the *E* and *Z* isomers. Therefore, the peaks of both isomers were seen together in the ^{1}H NMR spectra. The percentages of isomers were calculated using the ratios of the integral values of both isomer peaks for specific protons. According to the ^{1}H NMR spectrum analysis of compound **3b** (see Supplementary Data), the -OH proton was observed as two singlets at 10.68 and 11.11 ppm, with a relative integration of 23 and 77%, respectively. Likewise, the -CH proton of the pyrazole produced two singlets at 5.67 and 5.72 ppm.

In the ^{13}C NMR spectra of the oxime derivatives, the -CH$_3$ groups attached to the pyrazole were observed at 10–20 ppm, and the -CH at the 4th position of the pyrazole was within 100–110 ppm. The aromatic carbons of the benzene were seen at 120–160 ppm. The C=N of the pyrazole resonated at a higher chemical shift value than the =CH due to the nitrogen atom. The C=N-O carbon was observed between 140–165 ppm. The -O-C of the oxime ether derivatives were in the range of 60–65 ppm, and the aliphatic carbon peaks were in the range of 10–40 ppm. Since the two isomers were together in the oxime compounds, two isomer peaks with different intensities for each carbon could also be seen in the ^{13}C NMR spectra. In the ^{13}C NMR spectra of the compounds carrying fluorine atoms, the peaks of some carbon atoms in the phenyl ring were split in two due to coupling with the fluorine.

The HRMS spectra of the compounds were recorded using the positive ion (ESI+) electrospray ionization technique. In this method, only the [M + H]$^+$ (molecular ion + H) peaks and isotope peaks can be seen. The calculated [M + H]$^+$ values were compatible with the analysis results. In the HRMS spectra of **5a-5e**, a [M + H]$^+$ peak at 100% was observed, while [M + 2 + H]$^+$ peaks at 33% confirmed the presence of one chlorine atom in the structure (see Supplementary Data).

2.2. Cytotoxic Activity Assay

In this study, the cytotoxic activity of the compounds was studied on six different cancer cell lines (MCF-7, A549, HCT116, HeLa, C6, SH-SY5Y) and one healthy cell line (L929). The cell lines were treated with five different concentrations of the compounds (5, 10, 25, 50, and 100 µM) for 24 h, and 5-fluorouracil (5-FU) was used as a positive control. Although 14 of the 30 compounds were original, they were all included in the cytotoxic activity screening tests.

The cytotoxic activity of the 12 compounds were screened, among which nine compounds were found to be more active against the C6 cell line and three compounds against the SH-SY5Y cell line, compared with the standard 5-FU. No activity was seen against the MCF-7, A549, HCT116 or HeLa cell lines, as presented in Table 1. According to the results, the compounds were significantly effective against the SH-SY5Y and C6 cell lines in comparison with the other cell lines and positive control 5-FU. Moreover, it was observed

that the compounds did not exhibit cytotoxic effects on the L929 healthy cell line. The IC_{50} values of compounds **4a–5f** on the C6 and SH-SY5Y cell lines are presented in Table 2.

Table 1. Inhibitions of **4a–5f** against MCF-7, A549, HCT116, and HeLa cancer cell lines after 24 h.

Compounds	IC_{50} (μM)			
	MCF-7	A549	HCT116	Hela
4a	218.80 ± 4.87	117.20 ± 7.98	185.70 ± 10.65	126.30 ± 1.08
4b	212.20 ± 3.89	122.30 ± 6.54	-	190.10 ± 0.76
4c	545.40 ± 6.98	392.70 ± 7.08	-	211.00 ± 10.56
4d	-	302.20 ± 7.09	-	90.33 ± 9.06
4e	438.10 ± 1.76	369.90 ± 9.06	404.30 ± 8.79	90.14 ± 7.98
4f	-	-	-	-
5a	149.10 ± 10.08	157.90 ± 9.08	84.25 ± 7.48	72.20 ± 12.67
5b	-	-	75.64 ± 4.98	97.70 ± 15.78
5c	456.70 ± 16.97	185.30 ± 9.75	58.99 ± 3.98	137.50 ± 17.76
5d	173.30 ± 2.98	151.80 ± 6.98	112.10 ± 10.89	77.29 ± 9.65
5e	250.00 ± 3.96	145.50 ± 3.20	99.10 ± 9.07	113.10 ± 2.35
5f	305.50 ± 3.97	66.08 ± 2.89	328.30 ± 13.31	-
5-FU	11.75 ± 0.34	8.34 ± 1.09	7.86 ± 0.54	35.75 ± 2.98

"-": Not determined.

Table 2. Inhibitions of **4a–5f** against C6 and SH-SY5Y cancer cell lines after 24 h.

Compounds	IC_{50} (μM)		Compounds	IC_{50} (μM)	
	C6	SH-SY5Y		C6	SH-SY5Y
4a	7.93 ± 0.85	5.52 ± 0.23	**5a**	19.01 ± 0.48	5.00 ± 0.37
4b	24.31 ± 0.94	9.69 ± 0.87	**5b**	6.02 ± 0.85	10.08 ± 0.34
4c	7.16 ± 0.84	8.71 ± 0.95	**5c**	6.56 ± 0.92	8.55 ± 0.45
4d	7.42 ± 0.39	9.52 ± 0.34	**5d**	5.45 ± 0.19	9.02 ± 0.78
4e	6.91 ± 0.09	29.85 ± 1.23	**5e**	14.15 ± 0.13	20.28 ± 1.09
4f	6.21 ± 0.94	10.03 ± 0.54	**5f**	5.13 ± 0.34	8.56 ± 0.78
5-FU	8.34 ± 0.37	8.53 ± 0.43			

There was no clear structure activity relationship established between the changes in the aromatic structure on the compound for the activity against the C6 cell line. However, a significant potential could be seen in the naphthyl and nonsubstituted phenyl derivatives against the SH-SY5Y cell line, which was reversed for the methyl and methoxyphenyl compounds.

The IC_{50} values of compounds **4a–5f** ranged from 5.00–29.85 μM in the SH-SY5Y cell line and 5.13–24.31 μM in the C6 cell line. However, the IC_{50} values of 5-FU were calculated as 8.34 and 8.53 μM in the C6 and SH-SY5Y cell lines, respectively.

Further studies were performed on the C6 cells, as these compounds have a better cytotoxic activity against C6, compared to the other cells. Compound **5f** is the best cytotoxic compound, with 5.13 μM on the C6 cell line, and 5a is the best cytotoxic compound, with 5.00 μM on the SH-SY5Y cell line.

In the L929 cell line, the % viability values ranged between 71.28 and 115.74 μM with a 10 μM-concentration (Table S1). The fact that all of the compounds exhibited activity against glioblastoma and neuroblastoma with close IC_{50} values while not being active against the other cell lines shows that the effects of the compounds are selective.

2.2.1. Flow Cytometry Analysis

The flow cytometry analysis was used to investigate the mechanical effect of compound **5f** (at a concentration of IC_{50} value), which had the lowest IC_{50} value in the C6 cell line, on the cell cycle. With this method, it is possible to determine the number of cells in each division phase. In the analysis performed on the C6 cell line, it was found that compound **5f** killed 79.02% of the cancer cells. Cell line deaths were mostly caused

by apoptosis. In this case, 4.88% of these deaths were early apoptotic, 69.27% were late apoptotic, and 4.88% were necrosis (Figure 6). The apoptosis-necrosis ratio was calculated as 15:19. These results show that compound **5f** had a significant cytotoxic effect on the C6 cell line.
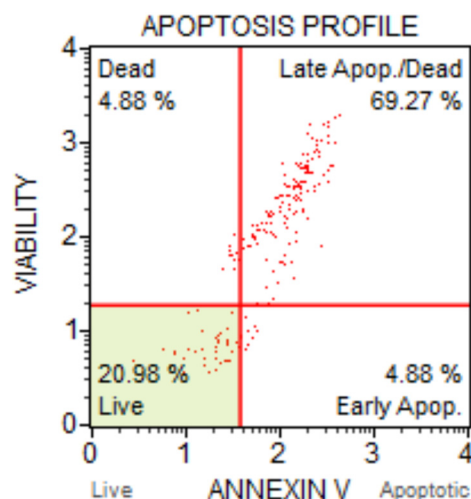

Figure 6. Treatment of the C6 cell line with compound **5f** for 24 h via quantification of apoptotic cells via the flow cytometric analysis.

2.2.2. Cell Cycle Analysis

The cell cycle is the process in which biochemical activities and morphological changes are observed in the cell. It begins with the division of one cell, continuing with the division of the other cells. The cell cycle consists of two parts: Interphase (G1, S, and G2) and mitosis (M). Until the cells receive a division signal, they remain in the resting phase of the cell cycle, the G0 (Gap) phase. When the division signal is received, the cell divides by passing through the G1, S, G2, and M phases, respectively.

In the cell cycle analysis performed on the C6 cell line, it was determined that compound **5f** inhibited the cell cycle by 45.1% in the G0/G1 phase, 32.9% in the S phase, and 19.5% in the G2/M phase (Figure 7). These results indicate that compound **5f** induces cell cycle arrest, and might be effective in the glioma treatment.
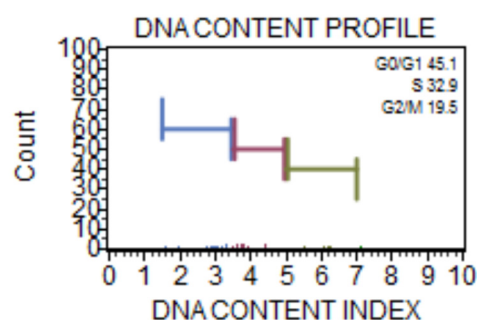
Figure 7. Cell cycle analysis of C6 cells treated with compound **5f**.

3. Materials and Methods

3.1. Chemistry

All of the chemicals used in this study were purchased from Merck and Sigma-Aldrich. Dimethylformamide was dried with a 4A molecular sieve. All of the reactions were carried out in atmospheric conditions. The reactions were monitored by thin layer chromatography (TLC) with Merck Kieselgel 60 F254 aluminum plates. Preparative TLC for the purification of the compounds was performed on Kieselgel 60 plates (Merck). The melting points were

determined with a Stuart SMP30 capillary melting point apparatus and were uncorrected. The infrared (IR) spectra were recorded in KBr disks with a Perkin Elmer Spectrum One FTIR Spectrometer. ^{1}H- and ^{13}C-nuclear magnetic resonance (NMR) spectra were recorded on an Agilent WB Ultrashield^{TM} NMR Spectrometer in DMSO-d_6 or CDCl$_3$ at 400 MHz. All of the chemical shifts were expressed in δ (ppm). The splitting patterns were designated as follows—s: Singlet; d: Doublet; dd: Doublet of doublets; t: Triplet; q: Quartet; and m: Multiplet. The high-resolution mass spectrometry (HRMS) spectra of the solutions of the compounds in methanol were recorded via positive ion (ESI +) electrospray ionization techniques using the Waters LCT Premier XE UPLC/MS TOFF system and MassLynx 4.1 software.

General Methods for the Preparation of the Compounds

Synthesis of 1-(Aryl)-2-(1*H*-3,5-dimethylpyrazol-1-yl)ethanone (2a-2f)

A solution of 3,5-dimethylpyrazole (0.03 mol, 3.0 equiv., 288.39 mg) in dry dimethylformamide (2.5 mL) was cooled down to 0–5 °C in an ice bath. A solution of 1-(aryl)-2-bromoethanone (0.01 mol, 1.0 equiv., 233.10 mg 1a, 181.97 mg 1b, 201.03 mg 1c, 217.49 mg 1d, 195.98 mg 1e, 211.93 mg 1f) in dry dimethylformamide (2.5 mL) was then slowly added and stirred. After 2 h, the reaction was stirred at room temperature for 24 h. Thereafter, the reaction medium was poured into ice water in a dropwise manner. The formed precipitates were filtered, washed with distilled water, and dried [28].

1-(Naphthalen-2-yl)-2-(1*H*-3,5-dimethylpyrazol-1-yl)ethanone (2a)

Yield: 81%; mp: 100–2 °C, white powder. ^{1}H NMR (400 MHz, CDCl$_3$-d): δ 2.25 (s, 3H, -CH$_3$), 2.41 (s, 3H, -CH$_3$), 6.15 (s, 2H, -CH$_2$), 6.29, (s, H, pyrazole), 7.42–8.05 (m, 6H, naphthalene), 8.67 (s, H, naphthalene). ^{13}C NMR (100 MHz, CDCl$_3$-d) δ 189.44, 146.42, 145.16, 136.05, 132.25, 131.15, 130.56, 129.94, 128.91, 127.71, 123.17, 107.39, 54.33, 11.34, 10.97. HRMS (ESI): *m/z* calcd for C$_{17}$H$_{16}$N$_2$O [M+H]$^+$ 265.1296; found: 265.2000.

1-Phenyl-2-(1*H*-3,5-dimethylpyrazol-1-yl)ethanone (2b)

Yield: 90%; mp: 95–8 °C, white powder. ^{1}H NMR (400 MHz, CDCl$_3$-d): δ 2.17 (s, 3H, -CH$_3$), 2.29 (s, 3H, -CH$_3$), 5.46 (s, 2H, -CH$_2$), 5.92, (s, H, pyrazole), 7.39–7.70 (m, 3H, phenyl) 7.89–8.04 (m, 2H, phenyl). ^{13}C NMR (100 MHz, CDCl$_3$-d) δ 192.74, 148.31, 140.51, 134.61, 133.97, 128.92, 128.09, 105.84, 55.31, 13.56, 11.03. HRMS (ESI): *m/z* calcd for C$_{13}$H$_{14}$N$_2$O [M+H]$^+$ 215.1140; found: 215.0000.

1-(4-Fluorophenyl)-2-(1*H*-3,5-dimethylpyrazol-1-yl)ethanone (2c)

Yield: 85%; mp: 110–11 °C, white powder. ^{1}H NMR (400 MHz, CDCl$_3$-d): δ 2.16 (s, 3H, -CH$_3$), 2.24 (s, 3H, -CH$_3$), 5.42 (s, 2H, -CH$_2$), 5.91 (s, H, pyrazole), 7.09–7.28 (m, 2H, phenyl), 7.95–8.07 (m, 2H, phenyl). ^{13}C NMR (100 MHz, CDCl$_3$-d) δ 191.31, 167.86, 164.47, 148.41, 140.51, 131.03, 116.28, 115.98, 105.91, 55.22, 13.52, 11.01. HRMS (ESI): *m/z* calcd for C$_{13}$H$_{14}$FN$_2$O [M+H]$^+$ 233.1045; found: 233.1000.

1-(4-Chlorophenyl)-2-(1*H*-3,5-dimethylpyrazol-1-yl)ethanone (2d)

Yield: 96%; mp: 108–109 °C, white powder. ^{1}H NMR (400 MHz, CDCl$_3$-d): δ 2.17 (s, 3H, -CH$_3$), 2.25 (s, 3H, -CH$_3$), 5.42 (s, 2H, -CH$_2$), 5.92 (s, H, pyrazole), 7.43–7.53 (m, 2H, phenyl), 7.98–7.86 (m, 2H, phenyl). ^{13}C NMR (100 MHz, CDCl$_3$-d) δ 191.77, 148.49, 140.52, 140.50, 132.90, 129.57, 129.28 105.96, 55.30, 13.53, 11.03. HRMS (ESI): *m/z* calcd for C$_{13}$H$_{13}$ClN$_2$O [M+H]$^+$ 249.0750; found: 249.0000.

1-(4-Methylphenyl)-2-(1*H*-3,5-dimethylpyrazol-1-yl)ethanone (2e)

Yield: 89%; mp: 83–85 °C, white powder. ^{1}H NMR (400 MHz, CDCl$_3$-d): δ 2.29 (s, 3H, -CH$_3$), 2.44 (s, 3H, -CH$_3$), 2.48 (s, CH$_3$-C$_6$H$_4$), 6.10 (s, 2H, -CH$_2$), 6.21, (s, H, pyrazole), 7.24–7.39 (m, 2H, phenyl), 7.98–7.87 (m, 2H, phenyl). ^{13}C NMR (100 MHz, CDCl$_3$-d) δ 188.80, 146.21. HRMS (ESI): *m/z* calcd for C$_{14}$H$_{16}$N$_2$O [M+H]$^+$ 229.1296; found: 228.0000.

1-(4-Methoxyphenyl)-2-(1*H*-3,5-dimethylpyrazol-1-yl)ethanone (2f)

Yield: 87%; mp: 174–6 °C, white powder. ^{1}H NMR (400 MHz, CDCl$_3$-d): δ 2.27 (s, 3H, -CH$_3$), 2.45 (s, 3H, -CH$_3$), 3.85 (s, 3H, O-CH$_3$), 6.10 (s, 2H, -CH$_2$), 6.20 (s, H, pyrazole), 6.89–7.04 (m, 2H, phenyl), 8.06–7.93 (m, 2H, phenyl). ^{13}C NMR (100 MHz, CDCl$_3$-d) δ 187.47, 164.82, 146.62, 145.04, 131.01, 126.27, 114.40, 107.41, 55.67, 53.67, 11.28, 10.98. HRMS (ESI): *m/z* calcd for C$_{14}$H$_{16}$N$_2$O$_2$ [M+H]$^+$ 245.1245; found: 245.1000.

Synthesis of 1-(Aryl)-2-(3,5-dimethyl-1*H*-pyrazol-1-yl)ethanone oxime (3a-f)

1-(Aryl)-2-(3,5-dimethyl-1*H*-pyrazol-1-yl))ethanone (0.015 mol, 1.0 equiv., 265.12 mg 2a, 215.14 mg 2b, 233.10 mg 2c, 249.07 mg 2d, 229.12 mg 2e, 245.12 mg 2f) was dissolved in absolute ethanol (75 mL). The reaction was heated under reflux and hydroxylamine hydrochloride (0.03 mol, 3.0 equiv., 208.47 mg) was added. The medium was alkalized to pH = 11 with 3 mL 15 N sodium hydroxide solution and was stirred under reflux for 1–2 h. The ethanol was evaporated to dryness. The resulting solid was treated with ice water and filtered [26].

1-(2-Naphthyl)-2-(1*H*-3,5-dimetylpyrazol-1-yl)ethanone oxime (3a)

Yield: 84%; mp: 174–6 °C, white powder; IR (KBr) cm^{-1}; 3135, 3051, 2939, 2845, 1600, 1552, 107, 897. ^{1}H NMR (400 MHz, DMSO-d6): δ 1.99 (s, 3H, -CH$_3$; 83%), 2.00 (s, 3H, -CH$_3$, 17%), 2.08 (s, 3H, -CH$_3$, 83%), 2.16 (s, 3H, -CH$_3$, 17%), 5.10 (s, 2H, -CH$_2$, 17%), 5.36 (s, 2H, -CH$_2$, 83%), 5.67 (s, 1H, =CH, 83%), 5.68 (s, 1H, =CH, 17%), 7.43–7.91 (m, 6H, naphthyl), 8.08 (s, H, naphthyl), 11.88 (s, 1H, =N-OH). ^{13}C NMR (100 MHz, DMSO-d6) δ 152.78, 151.94, 146.41, 146.32, 139.74, 133.29, 133.05, 132.73, 132.60, 139.70, 128.69, 128.59, 127.94, 127.90, 127.81, 127.52, 127.18, 127.02, 126.84, 126.74, 126.71, 126.37, 124.44, 105.37, 105.17, 52.37, 42.86, 13.73, 11.11, 11.01. HRMS (ESI): *m/z* calcd for C$_{17}$H$_{17}$N$_3$O [M+H]$^+$ 280.1450; found: 280.1444.

1-Phenyl-2-(1*H*-3,5-dimethylpyrazol-1-yl)ethanone oxime (3b) [27]

Yield: 81%; mp: 132–5 °C, white powder; IR (KBr) cm^{-1}; 3150, 3054, 2939, 1553, 1071, 897. ^{1}H-NMR (CDCl$_3$-d, 400 MHz): δ ppm 2.05 (3H, s, -CH$_3$, 23%), 2.08 (3H, s, -CH$_3$, 77%), 2.18 (3H, s, -CH$_3$, 77%), 2.21 (3H, s, -CH$_3$, 23%), 5.05 (2H, s, -CH$_2$-, 23%), 5.47 (2H, s, -CH$_2$-, 77%), 5.67 (H, s, =CH-, 77%, pyrazole), 5.72 (H, s, =CH-, 23%, pyrazole), 7.16–7.50 (5H, m, 23%, phenyl), 7.43–7.49 (5H, m, 77%, phenyl), 10.68 (H, s, =N-OH, 23%), 11.11 (H, s, =N-OH, 23%). ^{13}C-NMR (CDCl$_3$-d, 100 MHz), 154.24, 152.52, 148.09, 147.60, 140.13, 139.97, 133.91, 131.70, 129.00, 128.98, 128.23, 128.13, 127.80, 127.15, 105.49, 52.29, 43.90, 13.29, 10.94, 10.87. HRMS (ESI): *m/z* calcd for C$_{13}$H$_{15}$N$_3$O [M+H]$^+$ 230.1293; found: 230.1286.

(4-Fluorophenyl)-2-(1*H*-3,5-dimethylpyrazol-1-yl)ethanone oxime (3c) [27]

Yield: 91%; mp: 118–20 °C, white powder; IR (KBr) cm^{-1}; 3142, 3021, 2870, 1509, 1060, 917. ^{1}H-NMR (CDCl$_3$-d, 400 MHz): δ ppm 2.05 (3H, s, -CH$_3$, 17%), 2.07 (3H, s, -CH$_3$, 83%), 2.18 (3H, s, -CH$_3$, 83%), 2.20 (3H, s, -CH$_3$, 17%), 5.02 (2H, s, -CH$_2$-, 17%), 5.44 (2H, s, -CH$_2$-, 83%), 5.69 (H, s, =CH-, 83%, pyrazole), 5.72 (H, s, =CH-, 17%, pyrazole), 6.85–7.04 (2H, m, phenyl), 7.25–7.46 (2H, m, phenyl), 11.21 (H, s, =N-OH). ^{13}C-NMR (CDCl$_3$-d, 100 MHz), 164.42, 161.95, 153.38, 151.37, 148.20, 147.76, 140.18, 140.04, 130.05, 130.00, 129.96, 129.12, 129.04, 127.39, 115.41, 115.25, 115.20, 115.04, 105.64, 105.60, 52.22, 43.93, 13.24, 10.96, 10.83. HRMS (ESI): *m/z* calcd for C$_{13}$H$_{14}$FN$_3$O [M+H]$^+$: 248.1199; found: 248.1177.

1-(4-Chlorophenyl)-2-(1*H*-3,5-dimethylpyrazol-1-yl)ethanone oxime (3d) [27]

Yield: 92%; mp: 140–2 °C, white powder; IR (KBr) cm^{-1}; ^{1}H-NMR (DMSO-d6, 400 MHz) δ ppm: 1.98 (3H, s, -CH$_3$, 77%), 1.99 (3H, s, -CH$_3$, 23%), 2.06 (3H, s, -CH$_3$, 23%), 2.12 (3H, s, -CH$_3$, 77%), 4.99 (2H, s, -CH$_2$-, 23%), 5.21 (2H, s, -CH$_2$-, 77%), 5.69 (H, s, =CH-, 77%, pyrazole), 5.70 (H, s, =CH-, 23%, pyrazole), 7.30–7.62 (4H, m, phenyl), 11.28 (H, s, =N-OH, 23%), 11.88 (H, s, =N-OH, 77%). ^{13}C-NMR (DMSO-d6, 100 MHz), 152.09, 150.97, 146.50, 146.42, 139.76, 139.70, 134.05, 133.88, 133.68, 131.06, 130.51, 128.82, 128.58,

128.39, 105.44, 105.21, 52.05, 42.80, 13.72, 13.70, 11.06, 10.93. HRMS (ESI): *m/z* calcd for $C_{13}H_{14}ClN_3O$ [M+H]$^+$: 264.0904; found: 264.0786.

1-(4-Methylphenyl)-2-(1*H*-3,5-dimethylpyrazol-1-yl)ethanone oxime (3e) [27]

Yield: 87%; mp: 138–40 °C, white powder; IR (KBr) cm^{-1}; 3128, 2991, 1512, 1030, 890. ^{1}H-NMR (DMSO-d6, 400 MHz) δ ppm: 1.99 (3H, s, -CH$_3$, 77%), 2.00 (3H, s, -CH$_3$, 23%), 2.06 (3H, s, -CH$_3$, 23%), 2.10 (3H, s, -CH$_3$, 77%), 2.25 (3H, s, CH$_3$-C$_6$H$_4$, 77%), 2.26 (3H, s, CH$_3$-C$_6$H$_4$, 23%), 4.96 (2H, s, -CH$_2$-, 23%), 5.20 (2H, s, -CH$_2$-, 77%), 5.67 (H, s, =CH-, 77%, pyrazole), 5.69 (H, s, =CH-, 23%, pyrazole), 7.07–7.47 (4H, m, phenyl), 11.06 (H, s, =N-OH, 23%), 11.64 (H, s, =N-OH, 77%). ^{13}C-NMR (DMSO-d6, 100 MHz), 152.74, 151.60, 146.25, 146.17, 139.64, 139.56, 138.58, 138.51, 132.36, 129.40, 129.12, 128.84, 128.53, 126.94, 105.31, 105.13, 52.30, 42.97, 21.33, 21.21, 13.76, 11.11, 10.96. HRMS (ESI): *m/z* calcd for $C_{14}H_{17}N_3O$ [M+H]$^+$: 244.1450; found: 244.1442.

1-(4-Metoxyphenyl)-2-(1*H*-3,5-dimetylpyrazol-1-yl)ethanone oxime (3f)

Yield: 88%; mp: 150–2 °C, white powder; IR (KBr) cm^{-1}, 3128, 3028, 2992, 1556, 1029, 890. ^{1}H NMR (400 MHz, DMSO-d6): δ 2.00 (s, 3H, -CH$_3$), 2.09 (s, 3H, -CH$_3$, 20%), 2.11 (s, 3H, -CH$_3$, 80%), 3.72 (s, 3H, -C$_6$H$_4$-OCH$_3$, 80%), 3.73 (s, 3H, -C$_6$H$_4$-OCH$_3$, 20%), 4.98 (s, 2H,-CH$_2$, 20%), 5.19 (s, 2H, -CH$_2$, 80%), 5.68 (s, 1H, =CH), 6.82–6.90 (m, 2H, phenyl), 7.40–7.55 (m, 2H, phenyl), 11.07 (s, 1H, =N-OH, 20%), 11.55 (s, 1H, =N-OH, 80%). ^{13}C NMR (100 MHz, DMSO-d6) δ 160.08, 159.68, 152.35, 150.93, 146.19, 139.64, 139.59, 130.36, 128.39, 127.57, 124.28, 113.94, 113.62, 105.34, 105.13, 55.51, 55.49, 52.30, 42.96, 13.77, 13.75, 11.16, 10.96. HRMS (ESI): *m/z* calcd for $C_{14}H_{17}N_3O_2$ [M+H]$^+$ 260.1399; found: 260.1390.

Synthesis of 1-(aryl)-2-(3,5-dimethyl-1*H*-pyrazol-1-yl)ethanone oxime ethers (4a-4f; 5a-5f)

Specifically, 0.011 mol NaOH was dissolved in absolute ethanol (10 mL), and 1-(aryl)-2-(3,5-dimethyl-1*H*-pyrazol-1-yl)ethanone oxime (0.01 mol, 1.0 equiv., 280.14 mg 3a, 230.12 mg 3b, 248.11 mg 3c, 264.09 mg 3d, 244.14 mg 3e, 260.13 mg 3f) was added. The mixture was stirred under reflux for 1 h. The ethanol in the flask was evaporated to dryness. The remaining solid was dissolved via the addition of dry dimethylformamide (10 mL). The appropriate alkyl halide (0.02 mol, 2.0 equiv., 283.88 mg Iodomethane, 271.96 mg 1-bromo-2-metylpropane) was added to the solution and stirred at room temperature for 3–4 h [28,29]. The reaction medium was poured into ice water, and the precipitate was filtered off and dried. The compounds were purified via column chromatography using ethyl acetate/n-hexane (1:3).

1-(2-Naphthyl)-2-(1*H*-3,5-dimetylpyrazol-1-yl)ethanone O-methyl oxime (4a)

Yield: 74%; mp: 69–72 °C, white powder; IR (KBr) cm^{-1}: 3058, 2967, 1553, 1039, 907. ^{1}H NMR (400 MHz, DMSO-*d6*): δ 2.05 (s, 3H, -CH$_3$), 2.25 (s, 3H, -CH$_3$), 4.05 (s, 3H, -OCH$_3$), 5.35 (s, 2H, -CH$_2$), 5.70 (s, 1H, =CH), 7.60–7.90 (m, 6H, naphthyl), 8.15 (s, 1H, naphthyl). ^{13}C NMR (100 MHz, CDCl$_3$-d): δ 143.75, 141.42, 139.30, 136.65, 134.50, 134.01, 129.50, 128.66, 126.89, 126.42, 123.38, 121.19, 60.86, 47.02, 13.67, 12.03. HRMS (ESI): *m/z* calcd for $C_{18}H_{19}N_3O$ [M+H]$^+$: 294.1562; found: 294.1604.

1-Phenyl-2-(1*H*-3,5-dimetylpyrazol-1-yl)ethanone O-methyl oxime (4b)

Yield: 53%, white viscous liquid, IR (KBr) cm^{-1}: 3010, 2930, 1513, 1026, 891. ^{1}H NMR (400 MHz, CDCl$_3$-d): δ 2.05 (s, 3H, -CH$_3$), 2.16 (s, 3H, -CH$_3$), 4.04 (s, 3H, -OCH$_3$), 5.31 (s, 2H, -CH$_2$), 5.66 (s, 1H, =CH), 7.23–7.31 (m, 3H, phenyl), 7.46–7.51 (m, 2H, phenyl). ^{13}C NMR (100 MHz, CDCl$_3$-d): δ 154.01, 147.48, 139.61, 133.40, 129.21, 128.16, 127.18, 105.48, 62.42, 44.63, 13.44, 10.80. HRMS (ESI): *m/z* calcd for $C_{14}H_{17}N_3O$ [M+H]$^+$: 244.1450; found: 244.1447.

1-(4-Fluorophenyl)-2-(1*H*-3,5-dimetylpyrazol-1-yl)ethanone O-methyl oxime (4c)

Yield: 69%, mp: 55–57 °C, white powder, IR (KBr) cm^{-1}: 3011, 2983, 1612, 1026, 891. ^{1}H NMR (400 MHz, CDCl$_3$-d): δ 2.05 (s, 3H, -CH$_3$), 2.16 (s, 3H, -CH$_3$), 4.03 (s, 3H, -OCH$_3$), 5.29 (s, 2H, -CH$_2$), 5.68 (s, 1H, =CH), 6.89–6.98 (m, 2H, phenyl), 7.44–7.52 (m, 2H, phenyl). ^{13}C NMR (100 MHz, CDCl$_3$-d): δ 163.36 (d, $^{1}J_{F-Ar}$ = 249.17 Hz), 153.08, 147.61, 139.61, 129.50, 129.47, 129.07 (d, $^{2}J_{F-Ar}$ = 8.36 Hz), 115.30, 115.08, 105.59, 62.46, 44.59, 13.42, 10.77. HRMS (ESI): *m/z* calcd for C$_{14}$H$_{16}$FN$_3$O [M+H]$^{+}$: 262.1356; found: 262.1350.

1-(4-Chlorophenyl)-2-(1*H*-3,5-dimetylpyrazol-1-yl)ethanone O-methyl oxime (4d)

Yield: 80%; mp: 64–66 °C, white powder; IR (KBr) cm^{-1}: 3005, 2995, 1552, 1026, 895. ^{1}H NMR (400 MHz, CDCl$_3$-d): δ 2.05 (s, 3H, -CH$_3$), 2.16 (s, 3H, -CH$_3$), 4.03 (s, 3H, -OCH$_3$), 5.29 (s, 2H, -CH$_2$), 5.68 (s, 1H, =CH), 7.21–7.25 (m, 2H, phenyl), 7.43–7.47 (m, 2H, phenyl). ^{13}C NMR (100 MHz, CDCl$_3$-d): δ 152.91, 147.65, 139.61, 135.30, 131.84, 129.42, 128.49, 128.40, 105.66, 62.56, 44.40, 13.44, 10.79. HRMS (ESI): *m/z* calcd for C$_{14}$H$_{16}$ClN$_3$O [M+H]$^{+}$: 278.1060; found: 278.1052.

1-(4-Methylphenyl)-2-(1*H*-3,5-dimetylpyrazol-1-yl)ethanone O-methyl oxime (4e)

Yield: 58%; yellow viscous liquid; IR (KBr) cm^{-1}: 3008, 2962, 1555, 1035, 890. ^{1}H NMR (400 MHz, CDCl$_3$-d): δ 2.05 (s, 3H, -CH$_3$), 2.17 (s, 3H, -CH$_3$), 2.29 (s, 3H, C$_6$H$_4$-CH$_3$), 4.02 (s, 3H, -OCH$_3$), 5.30 (s, 2H, -CH$_2$), 5.67 (s, 1H, =CH), 7.04–7.08 (m, 2H, phenyl), 7.36–7.41 (m, 2H, phenyl). ^{13}C NMR (100 MHz, CDCl$_3$-d): δ 153.84, 147.39, 139.61, 139.22, 130.52, 128.91, 127.00, 105.46, 62.33, 44.57, 21.28, 13.46, 10.81. HRMS (ESI): *m/z* calcd for C$_{15}$H$_{19}$N$_3$O [M+H]$^{+}$: 258.1606; found: 258.1603.

1-(4-Methoxyphenyl)-2-(1*H*-3,5-dimetylpyrazol-1-yl)ethanone O-methyl oxime (4f)

Yield: 49%; mp: 70–72 °C, white powder; IR (KBr) cm^{-1}: 3133, 2995, 1555, 1028, 982. ^{1}H NMR (400 MHz, CDCl$_3$-d): δ 2.05 (s, 3H, -CH$_3$), 2.17 (s, 3H, -CH$_3$), 3.77 (s, 3H, C$_6$H$_4$-OCH$_3$), 4.01 (s, 3H, -OCH$_3$), 5.29 (s, 2H, -CH$_2$), 5.67 (s, 1H, =CH), 6.75–6.80 (m, 2H, phenyl), 7.42–7.46 (m, 2H, phenyl). ^{13}C NMR (100 MHz, CDCl$_3$-d): δ 160.39, 153.48, 147.40, 139.66, 128.55, 125.87, 113.59, 105.50, 62.28, 55.19, 44.59, 13.46, 10.80. HRMS (ESI): *m/z* calcd for C$_{15}$H$_{19}$N$_3$O$_2$ [M+H]$^{+}$: 274.1556; found: 274.1552.

1-(2-Naphthyl)-2-(1*H*-3,5-dimetylpyrazol-1-yl)ethanone O-2-methylpropyl oxime (5a)

Yield: 65%; mp: 67–69 °C, white powder; IR (KBr) cm^{-1}: 3039, 2939, 1554, 1072, 897. ^{1}H NMR (400 MHz, CDCl$_3$-d): δ 0.99 (d, *J* = 6.72 Hz, 6H, -CH(CH$_3$)$_2$, 77%), 0.88 (d, *J* = 6.72 Hz, 6H, -CH(CH$_3$)$_2$, 23%), 2.00 (m, 1H, -CH-), 2.07 (s, 3H, -CH$_3$, 77%), 2.14 (s, 3H, -CH$_3$, 23%), 2.15 (s, 3H, -CH$_3$, 23%), 2.19 (s, 3H, -CH$_3$, 77%), 4.07 (d, 2H, -CH-CH$_2$-, 77%), 3.90 (d, 2H, -CH-CH$_2$-, 23%), 5.47 (s, 2H, -N-CH$_2$-, 77%), 5.12 (s, 2H, -N-CH$_2$-, 23%), 5.65 (s, 1H, =CH, 77%), 5.72 (s, 1H, =CH, 23%), 7.40–7.50 (m, 4H, naphthyl), 7.7–7.8 (m, 2H, naphthyl), 7.97 (s, 1H, naphthyl). ^{13}C NMR (100 MHz, CDCl$_3$-d): δ 153.14, 147.44, 139.68, 133.49, 132.99, 130.95, 128.64, 128.50, 127.73, 127.58, 127.49, 127.46, 127.21, 126.64, 126.56, 126.08, 125.60, 124.24, 105.74, 105.60, 81.15, 81.19, 53.01, 44.80, 28.13, 27.98, 19.23, 19.16, 13.45, 11.19. HRMS (ESI): *m/z* calcd for C$_{21}$H$_{25}$N$_3$O [M+H]$^{+}$: 336.2076; found: 336.2065.

1-Phenyl-2-(1*H*-3,5-dimetylpyrazol-1-yl)ethanone O-2-methylpropyl oxime (5b)

Yield: 63%; white viscous liquid; IR (KBr) cm^{-1}: 3013, 2959, 1557, 1026, 926. ^{1}H NMR (400 MHz, CDCl$_3$-d): δ 0.95 (d, *J* = 6.72 Hz, 6H, -CH(CH$_3$)$_2$), 2.04–2.10 (m, 4H, -CH, CH$_3$), 2.16 (s, 3H, -CH$_3$), 4.02 (d, *J* = 6.72, 2H, -CH-CH$_2$-), 5.34 (s, 2H, -CH$_2$-N), 5.66 (s, 1H, =CH), 7.22–7.30 (m, 3H, phenyl), 7.47–7.51 (m, 2H, phenyl). ^{13}C NMR (100 MHz, CDCl$_3$-d): δ 153.19, 147.32, 133.68, 133.45, 129.08, 128.14, 127.15, 105.45, 81.40, 44.85, 28.08, 19.17, 13.43, 10.83. HRMS (ESI): *m/z* calcd for C$_{17}$H$_{23}$N$_3$O [M+H]$^{+}$: 286.1919; found: 286.1910.

1-(4-Fluorophenyl)-2-(1*H*-3,5-dimetylpyrazol-1-yl)ethanoneO-2-methylpropyl oxime (5c)

Yield: 56%; white viscous liquid; IR (KBr) cm^{-1}: 3014, 2960, 1604, 1050, 926. ^{1}H NMR (400 MHz, CDCl$_3$-d): δ 0.97 (d, *J* = 6.72 Hz, 6H, -CH(CH$_3$)$_2$), 2.06 (s, 3H, -CH$_3$-), 2.01-2.11 (m, 4H, -CH, CH$_3$), 4.01 (d, *J* = 6.78, 2H, -CH-CH$_2$-), 5.32 (s, 2H,-N-CH$_2$), 5.68 (s, 1H, =CH), 6.90–6.97 (m, 2H, phenyl), 7.45–7.52 (m, 2H, phenyl). ^{13}C NMR (100 MHz, CDCl$_3$-d): δ 163.30 (d, $^{1}J_{F-Ar}$ = 248.83 Hz), 152.55, 147.55, 139.58, 129.74, 129.71, 129.17, 129.08, 115.27, 115.06, 105.56, 81.45, 44.81, 28.07, 19.16, 13.42, 10.80. HRMS (ESI): *m/z* calcd for C$_{17}$H$_{22}$FN$_3$O [M+H]$^{+}$: 304.1825; found: 304.18.

1-(4-Chlorophenyl)-2-(1*H*-3,5-dimetylpyrazol-1-yl)ethanoneO-2-methylpropyl oxime (5d)

Yield: 63%; mp: 55–58 °C, white powder; IR (KBr) cm^{-1}: 3010, 2960, 1551, 1009, 851. ^{1}H NMR (400 MHz, CDCl$_3$-d): δ 0.96 (d, *J* = 6.72 Hz, 6H, -CH(CH$_3$)$_2$), 2.01-2.11 (m, 4H, -CH, CH$_3$), 4.02 (d, *J* = 6.76, 2H, -CH-CH$_2$), 5.31 (s, 2H, N-CH$_2$), 5.68 (s, 1H, =CH), 7.20–7.24 (m, 2H, phenyl), 7.44–7.48 (m, 2H, phenyl). ^{13}C NMR (100 MHz, CDCl$_3$-d): δ 157.37, 147.58, 139.56, 135.16, 132.10, 128.46, 128.37, 105.62, 81.55, 44.60, 28.07, 19.15, 13.43, 10.81. HRMS (ESI): *m/z* calcd for C$_{17}$H$_{22}$ClN$_3$O [M+H]$^{+}$: 320.1530; found: 320.1521.

1-(4-Methylphenyl)-2-(1*H*-3,5-dimetylpyrazol-1-yl)ethanoneO-2-methylpropyl oxime (5e)

Yield: 52%; yellow viscous liquid; IR (KBr) cm^{-1}: 3005, 2922, 1555, 1030, 874. ^{1}H NMR (400 MHz, CDCl$_3$-d): δ 0.97 (d, *J* = 6.74 Hz, 6H, -CH(CH$_3$)$_2$), 2.03–2.10 (m, 4H, -CH, CH$_3$), 2.17 (s, 3H, -CH$_3$), 2.29 (s, 3H, C$_6$H$_4$-CH$_3$), 4.01 (d, *J* = 6.78, 2H, -CH-CH$_2$), 5.33 (s, 2H, -N-CH$_2$), 5.67 (s, 1H, =CH), 7.03–7.08 (m, 2H, phenyl), 7.37–7.42 (m, 2H, phenyl). ^{13}C NMR (100 MHz, CDCl$_3$-d): δ 153.28, 147.30, 139.56, 139.07, 130.78, 128.88, 126.98, 105.43, 81.30, 44.80, 28.07, 21.27, 19.19, 13.45, 10.83. HRMS (ESI): *m/z* calcd for C$_{18}$H$_{25}$N$_3$O [M+H]$^{+}$: 300.2076; found: 300.2078.

1-(4-Methoxyphenyl)-2-(1*H*-3,5-dimetylpyrazol-1-yl)ethanone   O-2-methylpropyl oxime (5f)

Yield: 45%; yellow viscous liquid; IR (KBr) cm^{-1}: 3004, 2957, 1608, 1040, 874. ^{1}H NMR (400 MHz, CDCl$_3$-d): δ 0.96 (d, *J* = 6.72 Hz, 6H, -CH(CH$_3$)$_2$), 2.02–2.09 (m, 4H, -CH, CH$_3$), 2.17 (s, 3H, -CH$_3$), 3.76 (s, 3H, C$_6$H$_4$-OCH$_3$), 3.98 (d, *J* = 6.76, 2H, -CH-CH$_2$), 5.32 (s, 2H, -N-CH$_2$), 5.67 (s, 1H, =CH), 6.74–6.80 (m, 2H, phenyl), 7.42–7.47 (m, 2H, phenyl). ^{13}C NMR (100 MHz, CDCl$_3$-d): δ 160.30, 152.93, 147.32, 139.60, 128.52, 126.14, 113.57, 105.46, 81.24, 55.18, 44.82, 28.06, 19.20, 13.46, 10.82. HRMS (ESI): *m/z* calcd for C$_{18}$H$_{25}$N$_3$O$_2$ [M+H]$^{+}$: 216.2025; found: 216.2020.

3.2. Activity

3.2.1. MTT Assay

All of the cell lines were purchased from the American Type Cell Culture (ATCC). With this assay, it is possible to determine the ability of a cell line to grow and live in vitro. It is principally based on the staining of living cells with MTT. Additionally, it is taken up by living cells and metabolized to formazan crystals. These formed crystals are dissolved with acidic 2-propanol, which helps photometrically determine the number of viable cells after the treatment. This method was used to study the A549, HCT 116, HeLa, MCF7, C6, and SH-SY5Y cancer and L929 healthy cell lines [30]. Towards that end, 10 μL of sterile MTT solution (prepared in 5 mg/mL PBS) was pipetted into a 96-well plate (well) and remained in an incubator for 4 h. Then, the media in all of the wells were removed (adherent cells), and 110 μL (prepared with 0.04 N HCl and isopropanol) was pipetted into all of the wells. The formazan crystals were dissolved by removing the solution in the wells. It was measured at 540 nm (or 570 nm) (against the 690 nm reference) in an ELISA Plate Reader. Cells in the untreated wells were used as the reference [31].

3.2.2. Flow Cytometry

The flow cytometric analysis was performed using Annexin-V/PI commercial kits. Compound **5f** (at a concentration of IC_{50} value) was treated on the C6 cell lines for 24 h. At the end of this period, the content of the plate was removed, and the cells were washed with phosphate-buffered saline (PBS), treated with trypsin-EDTA, and centrifuged at 800 rpm for 8 min. The obtained cells were counted with trypan blue, and then 5 μL of Annexin V and 5 μL of propidium iodide (PI) were added. They were kept in the dark at room temperature for 15 min. After incubation, 400 μL of the binding buffer was added over ice, and flow cytometry measurements were performed (emission: 530 nm, excitation: 488 nm) [32–34].

3.2.3. Cell Cycle Analysis

The C6 cells were seeded in a 6-well plate at a concentration of 10^6 cells per well and incubated for 24 h. Then, the IC_{50} values of the samples were applied and the cells were left to incubate again for 24 h. After incubation, the medium and sample were removed, the cells were washed three times with 5 mL of PBS, and 1 mL of trypsin-EDTA solution was added to the plates. Thereafter, the cells were collected in falcon tubes with 4 mL of Dulbecco's Modified Eagle Medium (DMEM) in each tube, and the cell suspensions were centrifuged at 300 g for 5 min. Following this step, each 10 μL of the cell suspensions were mixed with 10 μL of trypan blue, and the cell counts were made in Neubauerlam. The number of cells in each tube was set to be 10^6. The cells were maintained at $-20\ ^\circ$C for 3 h and all of the supernatants were carefully removed. After the supernatant was dissolved in 1 mL of PBS, it was placed in a 200 μL muse cell cycle kit, incubated for 30 min at room temperature, and read using the flow cytometry. The results were given as % cells [35,36].

4. Conclusions

In this study, 12 new 1-aryl-2-(3,5-dimethylpyrazol-yl)ethanone oxime ether derivatives were synthesized. Their cytotoxic effects were evaluated against the A549, HCT 116, HeLa, MCF7, C6, and SH-SY5Y cell lines. The methyl and isobutyl ether derivatives of the oxime groups were synthesized to obtain more lipophilic compounds. Moreover, it was determined that, in general, the isobutyl oxime ether derivatives had higher cytotoxic activities in the C6 cell line. This finding is interesting in terms of the development of new anticancer compounds with better properties stemming from improving the properties of the active molecules. In the L929 healthy cell line, it was observed that the compounds did not show cytotoxic effects. These results show that 1-aryl-2-(3,5-dimethylpyrazol-1-yl)ethanone derivatives may have anticancer effects. In particular, compound **5f** induces cell cycle arrest and may be effective in the treatment of glioma.

Based on the literature, we thought that ethanone derivatives containing 3,5-dimethylpyrazole could have an anticancer activity. In this study, we first evaluated the effect of aryl groups on the activity. In future studies, the effect of methyl substitutions on the pyrazole ring on the activity will be evaluated by adding another substituted pyrazole ring rather than 3,5-dimethylpyrazole. Furthermore, the study is aimed at developing more effective compounds by synthesizing oxime ether derivatives containing various aryl and alkyl groups.

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