molbank

MDPI

Short Note

Ethyl 7-Methyl-1-(4-nitrophenyl)-5-phenyl-3-(thiophen-2-yl)-1,5-dihydro-[1,2,4]triazolo [4,3-*a*]pyrimidine-6-carboxylate

Sobhi M. Gomha [1,*], Zeinab A. Muhammad [2] and Mastoura M. Edrees [2,3]

[1] Department of Chemistry, Faculty of Science, Cairo University, Giza 12613, Egypt
[2] Department of Organic Chemistry, National Organization for Drug Control and Research (NODCAR), Giza 12311, Egypt; zeinab.a.muhammad@gmail.com (Z.A.M.); mmohamededrees@yahoo.com (M.M.E.)
[3] Department of Chemistry, Faculty of Science, King Khalid University, Abha 61413, Saudi Arabia
* Correspondence: s.m.gomha@gmail.com; Tel.: +20-237-400-304; Fax: +2-025-685-799

Academic Editor: Norbert Haider
Received: 13 March 2017; Accepted: 8 May 2017; Published: 19 May 2017

Abstract: A novel compound, ethyl 7-methyl-1-(4-nitrophenyl)-5-phenyl-3-(thiophen-2-yl)-1,5-dihydro[1,2,4]triazolo[4,3-*a*]pyrimidine-6-carboxylate (**7**) was synthesized by reaction of *N*-(4-nitrophenyl)thiophene-2-carbohydrazonoyl chloride (**1**) with ethyl 6-methyl-4-phenyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**2**). The mechanism of the studied reaction is discussed and the assigned structure was confirmed by elemental analysis and spectral data. Moreover, the in vitro antitumor activities against human lung (A-549) and human hepatocellular carcinoma (HepG-2) cell lines were determined by the MTT method, and the results indicated a high potency compared with the employed standard antitumor drug (Cisplatin).

Keywords: hydrazonoyl chlorides; pyrimidinethiones; triaolopyrimidines; antitumor activity

1. Introduction

It has been reported in the literature that functionalized thiophene derivatives have received considerable attention over the last two decades because they are endowed with a wide range of anti-tumor activities [1–5]. On the other hand, the synthesis of triazolopyrimidine derivatives has attracted a great deal of attention in view of their potent biological and pharmacological activities, especially their antitumor properties [6–16]. This was exemplified by a series of triazolo[4,3-*a*]pyrimidin-6-sulfonamide derivatives **I** that demonstrated potent inhibitory effects on the growth of a wide range of cancer cell lines including leukemia and prostate and lung cancer at low dose levels [15]. Moreover, pyrido[2,3-*d*][1,2,4]triazolo[4,3-*a*]pyrimidinone derivative **II** showed relatively potent antitumor activity against PC-3 and A-549 cell lines [16]. In particular, Compound **II** was able to cause cell cycle arrest at least partly through enhancing the expression level of the cell cycle inhibitor p21 and induced cancer cell apoptosis via a caspase-3 dependent pathway. Additionally, Compound **II** exhibited pro-apoptotic activity as evidenced by its ability to induce nuclear fragmentation, in addition to augmenting caspase-3 activation (Figure 1).

Encouraged by the above observations and in continuation of our previous work on the synthesis of antitumor agents [17–21], the synthesis of a 1,2,4-triazolopyrimidine, incorporating the thiophene moiety as a promising antitumor agent, was planned, using *N*-(4-nitrophenyl)thiophene-2-carbohydrazonoyl chloride as a versatile building block.

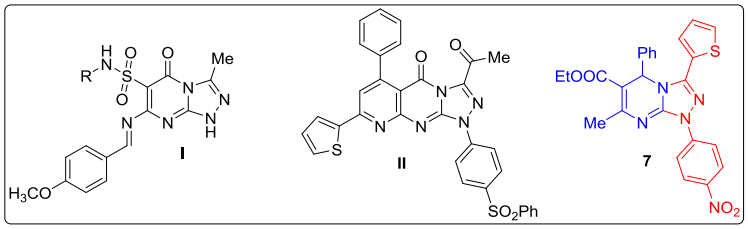
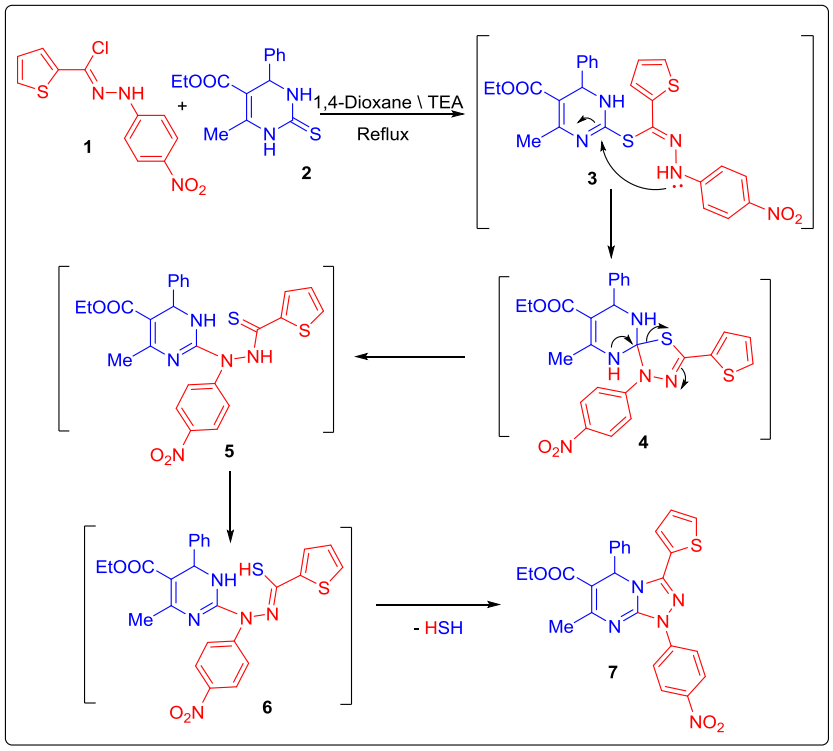


Figure 1. Structures of some anticancer 1,2,4-triazolo[4,3-*a*]pyrimidines and the target compound **7**.

2. Results and Discussion

2.1. Chemistry

N-(4-Nitrophenyl)thiophene-2-carbohydrazonoyl chloride (**1**) [22] was reacted with ethyl 1,2,3,4-tetrahydro-6-methyl-4-phenyl-2-thioxopyrimidine-5-carboxylate (**2**) [23] in 1,4-dioxane solution in the presence of triethylamine under reflux to give a single product consistent with Structure **7** based on spectroscopic data (IR, ^{1}H-NMR, and MS) and elemental analyses (Scheme 1). The mass spectrum of the isolated product **7** showed the molecular ion peak at the expected *m/z* value. Their IR spectrum showed the disappearance of the two NH groups, and revealed a carbonyl band at 1733 cm^{-1}. Additionally, the ^{1}H-NMR spectrum showed the characteristic signals of the ester protons at δ 1.07 and 4.27 ppm, and one singlet signal at δ = 2.76 ppm assigned to the CH_3 protons, in addition to the expected signals assigned to the aromatic protons. The ^{13}C-NMR spectrum provided the expected number and types of carbons. It showed five characteristic signals at δ = 13.8 ($\underline{CH_3}CH_2$), 23.2 (pyrimidine-CH_3), 56.3 (pyrimidine-CH), 60.8 ($CH_3\underline{CH_2}$), and 167.7 ($\underline{CO}CH_2CH_3$), in addition to 16 signals due to the aromatic carbons.



Scheme 1. Synthesis of the 1,5-dihydro-[1,2,4]triazolo[4,3-*a*]pyrimidine derivative **7**.

As depicted in Scheme 1, the reaction proceeds through S-alkylation to yield the non-isolated S-alkylated intermediate **3** followed by Smiles rearrangement to afford the thiohydrazide

intermediate **5**, which is consumed in situ via the elimination of hydrogen sulfide gas to yield the final product of **7**.

2.2. Cytotoxic Activity

The in vitro growth inhibitory activity of the synthesized compound against human lung (A-549) and hepatocellular carcinoma (HepG2) cell lines was investigated in comparison with the well-known anticancer standard drug cisplatin using the MTT assay. Data generated were used to plot a dose–response curve of which the concentration of the test compounds required to kill 50% of cell population (IC_{50}) was determined. The results reveal that the tested compound shows activity that is inhibitory to the tumor cell lines in a concentration-dependent manner. Interestingly, the results represented in Table 1 show that Compound **7** exhibits higher activity than the tested cisplatin reference drug under screening conditions. The in vitro growth inhibitory activity of Compound **7** against human lung (A-549) and hepatocellular carcinoma (HepG2) cell lines show promising activities with IC_{50} equal to 3.46 ± 0.5 and 5.54 ± 0.7 μM, respectively, while the IC_{50} of cisplatin against A-549 and HepG2 cell lines was found to be 19.3 ± 0.9 and 18.4 ± 1.1 μM, respectively.

Table 1. The in vitro inhibitory activity of Compound **7** against tumor cell lines expressed as IC_{50} values (μg/mL) ± standard deviation from three replicates.

Tested Compounds	Tumor Cell Lines	
	A-549	**HepG2**
7	3.46 ± 0.5	5.54 ± 0.7
Cisplatin	19.3 ± 0.9	18.4 ± 1.1

3. Experimental

3.1. Chemistry

Melting points were measured on an Electrothermal IA 9000 series digital melting point apparatus (Bibby Sci. Lim. Stone, Staffordshire, UK). IR spectra were recorded in potassium bromide discs on Shimadzu FTIR 8101 PC infrared spectrophotometer (Shimadzu, Tokyo, Japan). ^{1}H and ^{13}C-NMR spectra were recorded in DMSO-d_6 using a Varian Gemini 300 NMR spectrometer (Varian, Inc., Karlsruhe, Germany) (300 MHz for ^{1}H-NMR and 75 MHz for ^{13}C-NMR), and the chemical shifts were related to that of the solvent [24]. Mass spectra were recorded on a Shimadzu GCMS-QP1000 EX mass spectrometer (Tokyo, Japan) at 70 eV. Elemental analysis was carried out at the Microanalytical Centre of Cairo University, Giza, Egypt. All reactions were followed by TLC (silica gel coated aluminum sheets 60 F254, Merck, Merck & Co., Inc., Kenilworth, NJ, USA). The biological evaluation of the products was carried out at the Regional Center for Mycology and Biotechnology at Al-Azhar University, Cairo, Egypt.

*3.2. Synthesis of Ethyl 7-Methyl-1-(4-nitrophenyl)-5-phenyl-3-(thiophen-2-yl)-1,5-dihydro-[1,2,4]triazolo[4,3-a]pyrimidine-6-carboxylate (**8**)*

Triethylamine (0.15 mL, 1 mmol) was added to a boiling solution containing a mixture of equimolar amounts of *N*-(4-nitrophenyl)thiophene-2-carbohydrazonoyl chloride (**1**) (0.281 g, 1 mmol) and ethyl 6-methyl-4-phenyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**2**) (0.276 g, 1 mmol) in 1,4-dioxane (15 mL), and reflux continued until all of the starting materials had disappeared (20 h, monitored by TLC). The solvent was evaporated under reduced pressure, and the solid residue was crystallized from acetic acid to yield the pure 1,2,4-triazolo[4,3-*a*]pyrimidine derivative **7** in 77% yield. Yellow solid, mp 180–182 °C; IR (KBr) ν_{max} 1036, 1244 (stretch C–O), 1513, 1535, 1552 (stretch C=C), 1586 (C=N), 1733 (C=O), 2927, 3060 (C-H) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 1.07 (t, J = 7.2 Hz, 3H, $\underline{CH_3}CH_2$), 2.76 (s, 3H, CH_3), 4.27 (q, J = 7.2 Hz, 2H, $CH_3\underline{CH_2}$), 5.06 (s, 1H, pyrimidine-H6), 7.03–7.40 (m, 8H,

Ar-H), 7.72 (d, 2H, Ar-H), 8.40 (d, 2H, Ar-H); ^{13}C-NMR (DMSO-d_6): δ 13.8, 23.2 (CH_3), 56.3 (CH), 60.8 (CH_2), 114.2, 114.4, 125.6, 127.5, 128.3, 128.4, 129.0, 129.3, 129.6, 138.6, 142.8, 143.4, 147.9, 150.7, 158.0, 159.8 (Ar-C), 167.7 (C=O); MS (70 eV, EI, %), *m/z* = 487 (M^+, 19), 461 (51), 329 (18), 272 (22), 180 (28), 113 (25), 87 (36), 59 (100). Anal. Calcd. for $C_{25}H_{21}N_5O_4S$ (487.53): C, 61.59; H, 4.34; N, 14.36. Found: C, 61.74; H, 4.23; N, 14.22%.

3.3. Biological Evaluation

Antitumor activity assay:

Mammalian cell lines: A-549 cells (human Lung cancer cell line), HepG-2 cells (human Hepatocellular carcinoma) were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA).

Chemicals used: Dimethyl sulfoxide (DMSO), crystal violet, and trypan blue dye were purchased from Sigma (St. Louis, MO, USA). Fetal Bovine serum, DMEM, RPMI-1640, HEPES buffer solution, L-glutamine, gentamycin, and 0.25% Trypsin-EDTA were purchased from Lonza (BioWhittaker®, Lonza, Belgium).

Crystal violet stain (1%) was composed of 0.5% (*w*/*v*) crystal violet and 50% methanol, then made up to volume with ddH_2O and filtered through Whatmann No.1 filter paper.

Cell line propagation: The cells were propagated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 1% L-glutamine, HEPES buffer, and 50 µg/mL gentamycin. All cells were maintained at 37 °C in a humidified atmosphere with 5% CO_2 and were subcultured twice a week.

Cytotoxicity evaluation using a viability assay: For cytotoxicity assay, the cells were seeded in 96-well plate at a cell concentration of 1×10^4 cells per well in 100 µL of growth medium. Fresh medium containing different concentrations of the test sample was added after 24 h of seeding. Serial two-fold dilutions of the tested chemical compound were added to confluent cell monolayers dispensed into 96-well, flat-bottomed microtiter plates (Falcon, NJ, USA) using a multichannel pipette. The microtiter plates were incubated at 37 °C in a humidified incubator with 5% CO_2 for a period of 48 h. Three wells were used for each concentration of the test sample. Control cells were incubated without the test sample and with and without DMSO. The small percentage of DMSO present in the wells (maximally 0.1%) was found not to affect the experiment. After incubation of the cells for 24 h at 37 °C, various concentrations of sample (50, 25, 12.5, 6.25, 3.125 & 1.56 µg) were added, and the incubation was continued for 48 h and viable cell yield was determined by a colorimetric method.

In brief, after the end of the incubation period, media were aspirated, and the crystal violet solution (1%) was added to each well for at least 30 min. The stain was removed and the plates were rinsed using tap water until all excess stain was removed. Glacial acetic acid (30%) was then added to all wells and mixed thoroughly, and the absorbance of the plates were then measured after gentle shaking on a microplate reader (SunRise, TECAN, Inc., San Diego, CA, USA), using a test wavelength of 490 nm. All results were corrected for background absorbance detected in wells without added stain. Treated samples were compared with the cell control in the absence of the tested compounds. All experiments were carried out in triplicate. The cell cytotoxic effect of each tested compound was calculated. The optical density was measured with the microplate reader (SunRise, TECAN, Inc., San Diego, CA, USA) to determine the number of viable cells and the percentage of viability was calculated as $[1 - (ODt / ODc)] \times 100\%$, where ODt is the mean optical density of wells treated with the tested sample and ODc is the mean optical density of untreated cells. The relation between surviving cells and drug concentration is plotted to get the survival curve of each tumor cell line after treatment with the specified compound. The 50% inhibitory concentration (IC_{50}), the concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots of the dose–response curve for each conc. using Graphpad Prism software (San Diego, CA, USA) [25,26].

4. Conclusion

Ethyl 7-methyl-1-(4-nitrophenyl)-5-phenyl-3-(thiophen-2-yl)-1,5-dihydro-[1,2,4]triazolo[4,3-*a*] pyri-midine-6-carboxylate was synthesized and tested for its in vitro antitumor activity against two human cancer cell lines (A-549 and HepG-2).

Supplementary Materials: The following are available online: Figure S1: ^{1}H-NMR spectra, Figure S2: ^{13}C-NMR spectra and Figure S3: Mass spectra.

Acknowledgments: The support from Chemistry Department, Faculty of Science, Cairo University, is gratefully acknowledged.

Author Contributions: S.M.G. designed research; S.M.G., Z.A.M. and M.M.E. performed research and analyzed the data; Z.A.M. and S.M.G. analyzed the data and wrote the paper. All authors read and approved the final manuscript.

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

References

1. Ilies, A.; Vullo, D.; Pastorek, J.; Scozzofava, A.; Ilies, M.; Caproiu, M.T.; Pastorekova, S.; Supuran, C.T. Carbonic anhydrase inhibitors of tumor associated isozyme IX by dihalogenosulfanilamide and halogenophenylamino benzolamide derivatives. *Farmaco* **2003**, *46*, 487–496.
2. Terzioglu, N.; Gursoy, A. Synthesis and anticancer evaluation of some new hydrazone derivatives of 2,6-dimethylimidazo[2,1]-*b*]-[1,3,4]thiadiazole-5-carbohydrazide. *Eur. J. Med. Chem.* **1988**, *23*, 385–389. [CrossRef]
3. Alagarsamy, V.; Pathak, S.U.; Meena, S.; Rameshu, V.K.; Reyathi, R. Anticancer, antibacterial and antifungal activities of soidc 2-substituted (1,3,4)-thiadiazolothieno(2,3-*c*)-pyrimidin-5-(4*H*)-ones. *Ind. J. Hetercycl. Chem.* **2003**, *12*, 335–338.
4. Gomha, S.M.; Edrees, M.M.; Altalbawy, F.M.A. Synthesis and characterization of some new *bis*-pyrazolyl-thiazoles incorporating the thiophene moiety as potent anti-tumor agents. *Int. J. Mol. Sci.* **2016**, *17*, 1499. [CrossRef] [PubMed]
5. Forsch, R.A.; Wright, J.E.; Rosowsky, A. Synthesis and in vitro antitumor activity of thiophene analogues of 5-chloro-5,8-dideazafolic acid and 2-methyl-2-desamino-5-chloro-5,8-dideazafolic acid. *Bioorg. Med. Chem.* **2002**, *10*, 2067–2076. [CrossRef]
6. Shawali, A.S. Tandem in situ generation and 1,5-electrocyclization of *N*-hetaryl nitrilimines. A facile methodology for synthesis of annulated 1,2,4-triazoles and their acyclo *C*-nucleosides. *Arkivoc* **2010**, *1*, 33–97. [CrossRef]
7. Zhang, N.; Ayral-Kaloustian, S.; Nguyen, T.; Afragola, J.; Hernandez, R.; Lucas, J. Synthesis and SAR of [1,2,4]triazolo[1,5-*a*]pyrimidines, a class of anticancer agents with a unique mechanism of tubulin inhibition. *J. Med. Chem.* **2007**, *50*, 319–327. [CrossRef] [PubMed]
8. Zhao, X.L.; Zhao, Y.F.; Guo, S.C.; Song, H.S.; Wang, D.; Gong, P. Synthesis and anti-tumor activities of novel [1,2,4]triazolo[1,5-*a*]pyrimidines. *Molecules* **2007**, *12*, 1136–1146. [CrossRef] [PubMed]
9. Gomha, S.M.; Abdelrazek, F.M.; Abdulla, M.M. Synthesis of new functionalised derivatives of [1,2,4]triazolo[4,3-*a*]pyrimidine and pyrimido[2,1-*b*][1,3,5]thiadiazine as aromatase inhibitors. *J. Chem. Res.* **2015**, *39*, 425–429.
10. Baraldi, P.G.; Pavani, M.G.; Nunez, M.D.; Brigidi, P.; Vitali, B.; Gambari, R.; Romagnoli, R. Antimicrobial and antitumor activity of *N*-heteroimmine-1,2,3-dithiazoles and their transformation in triazolo-, imidazo-, and pyrazolopirimidines. *Bioorg. Med. Chem.* **2002**, *10*, 449–456. [CrossRef]
11. Farghaly, T.A.; Gomha, S.M.; Abbas, E.M.H.; Abdalla, M.M. Hydrazonoyl halides as precursors for new fused heterocycles of 5α-reductase inhibitors. *Arch. Pharm. Chem. Life Sci.* **2012**, *345*, 117–122. [CrossRef] [PubMed]
12. Gomha, S.M.; Abbas, I.M.; Elneairy, M.A.A.; Elaasser, M.M.; Mabrouk, B.K.A. Synthesis and biological evaluation of novel fused triazolo[4,3-*a*]pyrimidinones. *Turk. J. Chem.* **2015**, *39*, 510–531.
13. Gomha, S.M.; Ahmed, S.A.; Abdelhamid, A.O. Synthesis and cytotoxicity evaluation of some novel thiazoles, thiadiazoles, and pyrido[2,3-*d*][1,2,4]triazolo[4,3-*a*]pyrimidin-5(1*H*)-one incorporating triazole moiety. *Molecules* **2015**, *20*, 1357–1376. [CrossRef] [PubMed]

14. Mohamed, A.M.; Al-Qalawi, H.R.M.; El-Sayed, W.A.; Arafa, W.A.A.; Alhumaimess, M.S.; Hassan, A.K. Anticancer activity of newly synthesized triazolopyrimidine derivatives and their nucleoside analogs. *Acta Pol. Pharm. Drug Res.* **2015**, *72*, 307–318.
15. Hafez, H.N.; El-Gazzar, A.R. Synthesis and antitumor activity of substituted triazolo[4,3-*a*] pyrimidin-6-sulfonamide with an incorporated thiazolidinone moiety. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4143–4147. [CrossRef] [PubMed]
16. Fares, M.; Abou-Seri, S.M.; Abdel-Aziz, H.A.; Abbas, S.E.S.; Youssef, M.M.; Eladwy, R.A. Synthesis and antitumor activity of pyrido [2,3-*d*]pyrimidine and pyrido[2,3-*d*] [1,2,4]triazolo[4,3-*a*]pyrimidine derivatives that induce apoptosis through G1 cell-cycle arrest. *Eur. J. Med. Chem.* **2014**, *83*, 155–166. [CrossRef] [PubMed]
17. Gomha, S.M.; Salah, T.A.; Abdelhamid, A.O. Synthesis, characterization, and pharmacological evaluation of some novel thiadiazoles and thiazoles incorporating pyrazole moiety as anticancer agents. *Monatsh. Chem.* **2015**, *146*, 149–158. [CrossRef]
18. Dawood, K.M.; Gomha, S.M. Synthesis and anti-cancer activity of 1,3,4-thiadiazole and 1,3-thiazole derivatives having 1,3,4-oxadiazole moiety. *J. Heterocycl. Chem.* **2015**, *52*, 1400–1405. [CrossRef]
19. Gomha, S.M.; Riyadh, S.M.; Abbas, I.M.; Bauomi, M.A. Synthetic utility of ethylidene-thiosemicarbazide: Synthesis and and anti-cancer activity of 1,3-thiazines and thiazoles with imidazole moiety. *Heterocycles* **2013**, *87*, 341–356.
20. Gomha, S.M.; Zaki, Y.H.; Abdelhamid, A.O. Utility of 3-acetyl-6-bromo-2*H*-chromen-2-one for synthesis of new heterocycles as potential anticancer agents. *Molecules* **2015**, *20*, 21826–21839. [CrossRef] [PubMed]
21. Gomha, S.M.; Salah, T.A.; Hassaneen, H.M.E.; Abdel-Aziz, H.; Khedr, M.A. Synthesis, characterization and molecular docking of novel bioactive thiazolyl-thiazole derivatives as promising cytotoxic antitumor drug. *Molecules* **2016**, *21*, 3. [CrossRef] [PubMed]
22. Hassaneen, H.M.; Mousa, A.H.; Abeed, N.M.; Shawali, A.S. Chemistry of C-Heteroarylhydrazidoyl Halides. Synthesis and Reactions of *N*-(*p*-Nitrophenyl)-C-(2-thienyl)-formohydrazidoyl Halides. *Heterocycles* **1988**, *27*, 695–706. [CrossRef]
23. Vijay, K.; Ganapaty, S.; Rao, A.S. Synthesis, characterization and biological evaluation of some dihydropyrimidinones. *Asian J. Chem.* **2010**, *22*, 2518–2528.
24. Fulmer, G.R.; Miller, A.J.M.; Sherden, N.H.; Gottlieb, H.E.; Nudelman, A.; Stoltz, B.M.; Bercaw, J.E.; Goldberg, K.I. NMR Chemical shifts of trace impurities: Common laboratory solvents, organics, and gases in deuterated solvents relevant to the organometallic chemist. *Organometallics* **2010**, *29*, 2176–2179. [CrossRef]
25. Mosmann, T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immunol. Methods* **1983**, *65*, 55–63. [CrossRef]
26. Gomha, S.M.; Riyadh, S.M.; Mahmmoud, E.A.; Elasser, M.M. Synthesis and anticancer activities of thiazoles, 1,3-thiazines, and thiazolidine using chitosan-grafted-poly(vinylpyridine) as basic catalyst. *Heterocycles* **2015**, *91*, 1227–1243.