molbank

MDPI

Communication

2-[2-Methyl-5-phenyl-1-(3,4,5-trimethoxyphenyl)-1*H*-pyrrol-3-yl]-2-oxo-*N*-(pyridin-4-yl) acetamide

Ebrahim Saeedian Moghadam and Mohsen Amini *

Department of Medicinal Chemistry, Faculty of Pharmacy and Drug Design & Development Research Center, The Institute of Pharmaceutical Sciences (TIPS), Tehran University of Medical Sciences, Tehran 1417614411, Iran; e-saeedian@razi.tums.ac.ir

* Correspondence: moamini@tums.ac.ir; Tel.: +98-21-6695-9062

Received: 16 June 2018; Accepted: 26 June 2018; Published: 28 June 2018

Abstract: We synthesized 2-[2-methyl-5-phenyl-1-(3,4,5-trimethoxyphenyl)-1*H*-pyrrol-3-yl]-2-oxo-*N*-(pyridin-4-yl) acetamide **4** as a novel compound derived from the indibulin and combretastatin scaffolds, which are known anti-mitotic agents, using a multistep reaction. We tested its cytotoxic activity against three breast cancer cell lines, namely, MCF-7, T47-D, and MDA-MB 231 as well as normal cell line NIH-3T3, by 3-(4,5-dimethylthiazoyl-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. The biological activity results showed good cytotoxicity on cancerous cell lines (IC_{50} value 27.7–39.2 μM) and low toxicity on normal cell line (NIH-3T3, IC_{50} value > 100 μM).

Keywords: multistep synthesis; stetter reaction; Paal-Knorr pyrrole synthesis; drug design; anti-cancer activity; MTT

1. Introduction

In a word, cancer has an unpleasant meaning for everyone: it is the second leading cause of death in Europe and North America after cardiovascular disease. Despite huge efforts in diagnosis, prevention, and treatment, cancer has remained a significant challenge for the medical community. An important molecular target inside the body for both the design and development of anticancer agents is tubulin protein. Recently, laboratory research and clinical investigations have focused on anti-tubulin agents to apply a series of compounds in the treatment of several types of cancer [1–9].

Combretastatin A-4 (CA-4) (Figure 1A) is one of the most important lead compounds in designing anti-tubulin agents. According to the structure activity relationship (SAR) of CA-4 analogues, the cis configuration of rings A and B is important in the exertion of biological activity. The presence of the 3,4,5-trimethoxy substitution pattern on one ring is critical for anti-tubulin activity. One of the problems of CA-4 is its chemical instability. Isomerization to the less-active trans-form of CA-4 has encouraged researchers to prepare 1,2-di-aryl heterocyclic compounds to lock the aryl rings into a cis conformation. Therefore, medicinal chemists have designed various 1,2-diaryl heterocyclic compounds, which contain a 3,4,5-trimethoxy substituted phenyl ring and a second aryl group with different types of substitutions (Figure 1B) [10–13].

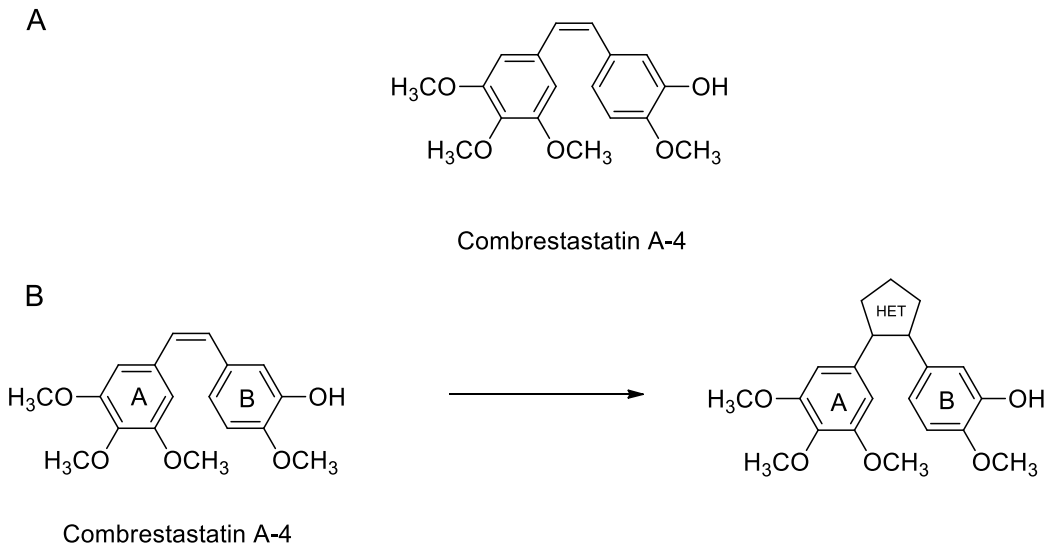


Figure 1. (**A**) Combretastatin A-4 structure; (**B**) Modified structural of combretastatin A-4.

Indibulin (Figure 2) is a new generation of anticancer compound with anti-mitotic properties. Unlike many anti-mitotic agents, it does not exert peripheral neuropathy; this benefit distinguishes indibulin from other similar acting compounds [14]. Significant efforts have been made to find new anti-tubulin derivatives, inspired by the structure of indibulin [15–17] (Figure 2).

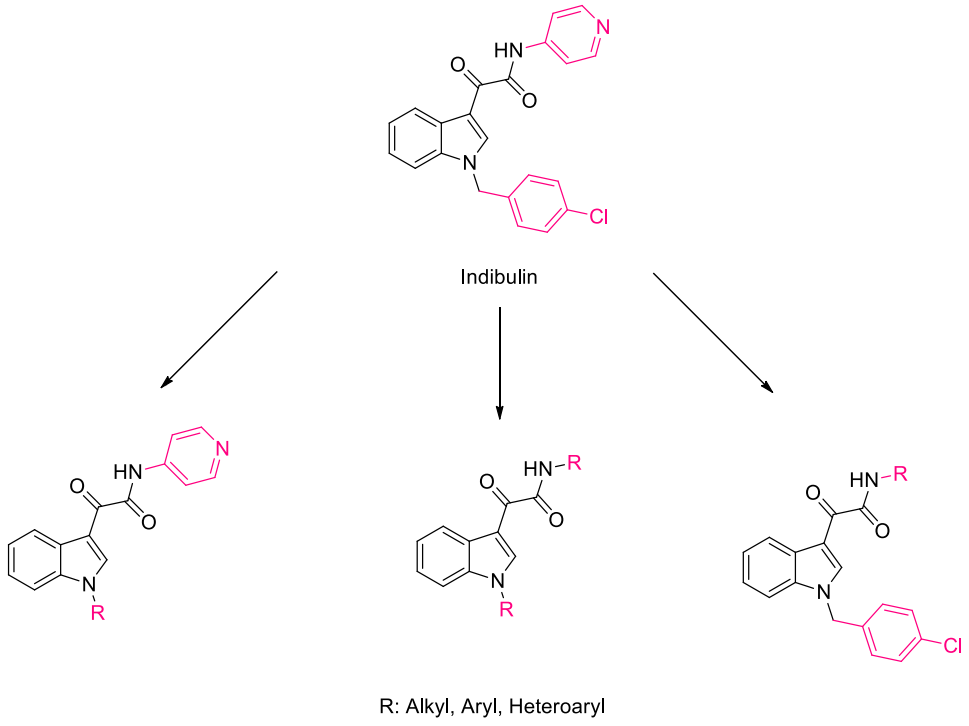


Figure 2. Structure of indibulin and modification of indibulin reported in the literature.

Continuing our earlier efforts to find new, potent, and safe anticancer agents [18–23], we synthesized a novel molecule based on the combretastatin-indibulin structures (Figure 3) and evaluated its anti-cancer activity.

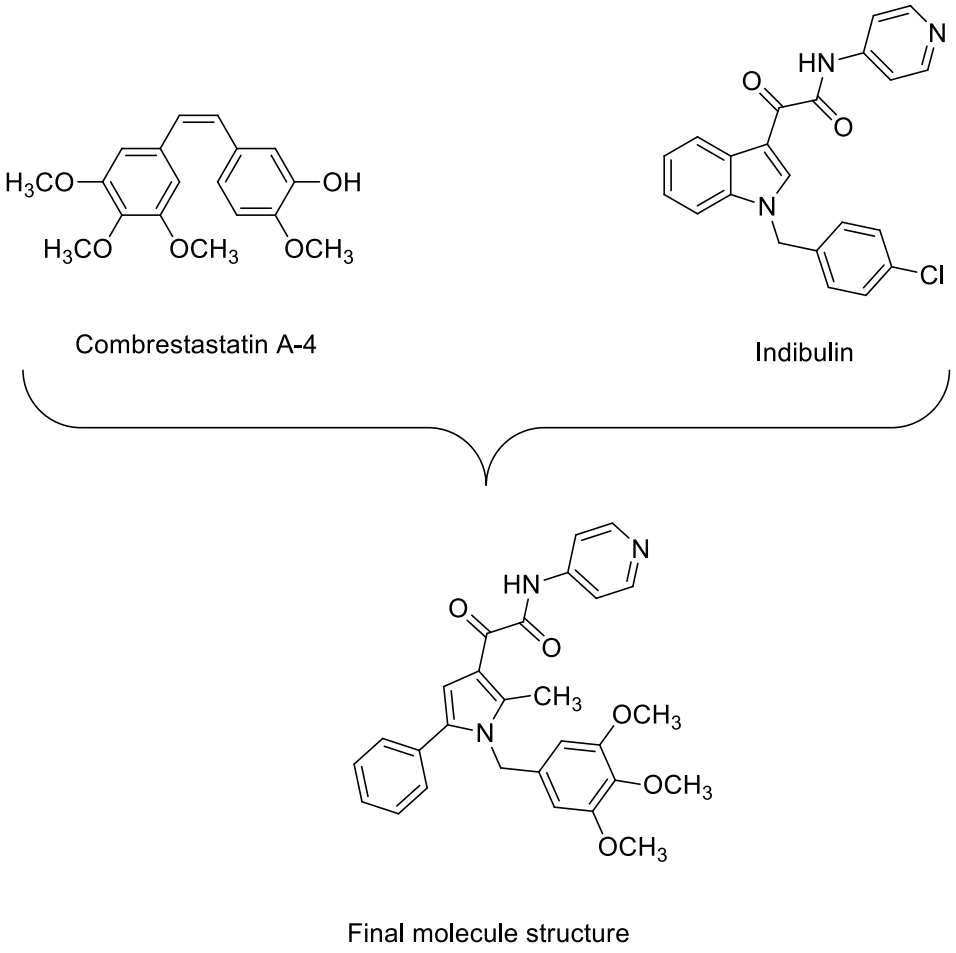
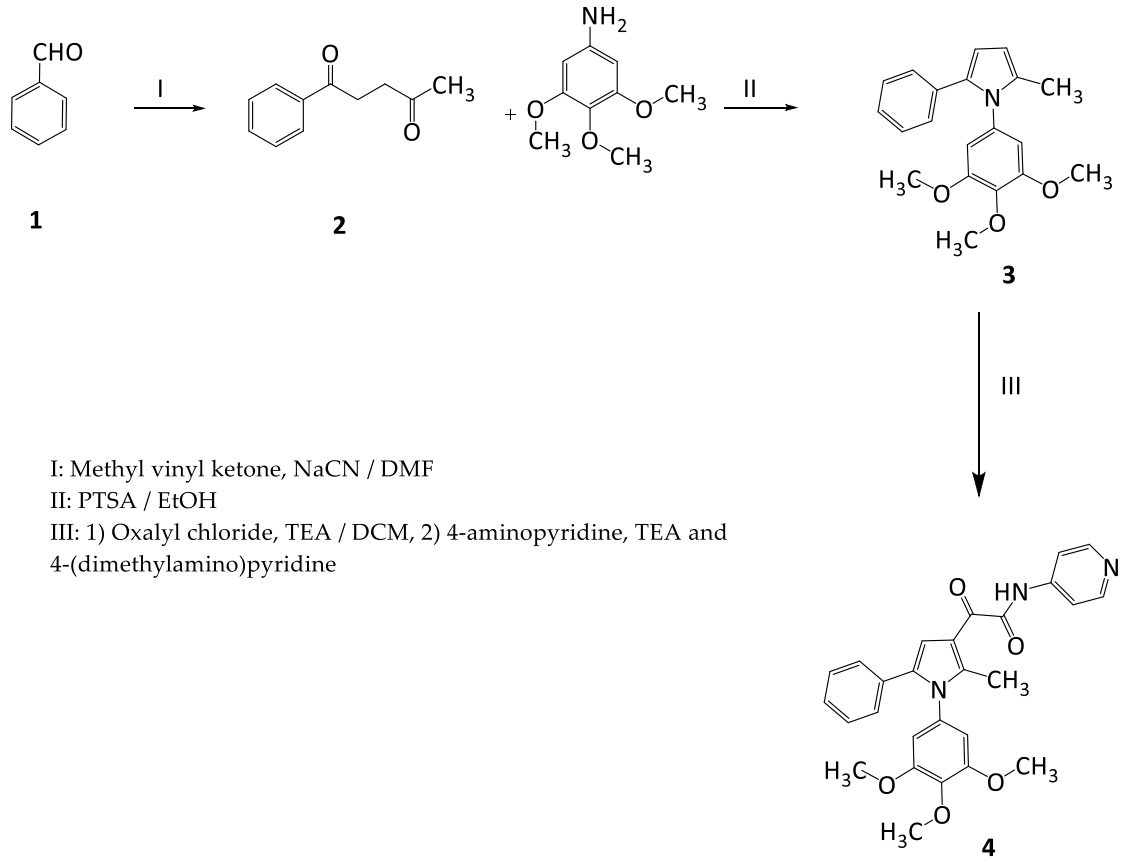


Figure 3. Structure of the designed and synthesized molecule in the current work.

2. Results and Discussion

2.1. Chemistry

The synthetic pathway to prepare compound **4** is outlined in Scheme 1. Firstly, using Stetter reaction conditions, benzaldehyde **1** was treated with methyl vinyl ketone in the presence of catalytic amounts of sodium cyanide in DMF to yield 1-phenyl-1,4-pentanedione (**2**). Secondly, using the Paal-Knorr pyrrole synthesis method, dione **2** was refluxed with trimethoxyaniline using a catalytic amount of *p*-toluenesulfonic acid (PTSA) in ethanol for 6 h to yield 2-methyl-5-phenyl-1-(3,4,5-trimethoxyphenyl)-1*H*-pyrrole (**3**). Target compound **4** was prepared in two steps. In the first step, reaction of oxalyl chloride with pyrrole **3** in the presence of dry triethylamine (TEA) in dichloromethane (DCM) at room temperature (RT) installed the α-oxo acid chloride at C3, following the removal of excess oxalyl chloride. The residue was dissolved in dry DCM and TEA, and a catalytic amount of 4-(dimethylamino)pyridine and 4-aminopyridine was added. The reaction mixture was stirred at RT for 12 h. The mixture was concentrated under reduced pressure and the final product **4** was purified by column chromatography (hexane/ethyl acetate 3:1, as eluent). The chemical structure of the final compound was confirmed by ^{1}H-NMR, ^{13}C-NMR, MS, IR, and elemental analysis.



Scheme 1. Synthetic pathway of final compound **4**.

2.2. Anticancer Activity

The cytotoxic effect of final compound **4** against three breast cancer cell lines (MCF-7, T47-D, and MDA-MB231) and mouse embryonic fibroblast cell line (NIH-3T3) as a representative normal cell line was checked by the 3-(4,5-dimethylthiazoyl-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay method. Results show that compound **4** has cytotoxicity on cancerous cell lines and low toxicity on normal cell line. (Table 1).

Table 1. In vitro cytotoxic activities (IC_{50}) [a,b] of synthesized compound **4**.

Compound	NIH-3T3	MCF-7	T47-D	MDA-MB231
4	>100	29.1 ± 1.8	39.2 ± 3.2	27.75 ± 2.6
paclitaxel	10.2 ± 1.1	0.5 ± 0.3	1.0 ± 0.7	1.5 ± 0.5

[a] IC_{50} values are in μM; [b] IC_{50}, compound concentration required to inhibit cell proliferation by 50%.

A comparison of the cytotoxicity of compound **4** with the reference anti-cancer drug paclitaxel on NIH-3T3 cell line showed low toxicity of **4** for normal cell.

3. Materials and Methods

3.1. General

All chemicals compounds and reagents were purchased from Merck (Darmstadt, Germany). Melting points were taken on a Kofler hot-stage apparatus (Reichert, Vienna, Austria) and were uncorrected. The ^{1}H-NMR spectra were recorded on a Bruker FT-500 MHz spectrometer (Bruker, Darmstadt, Germany) using $CDCl_3$ as solvent. The instrument was set at 125 MHz for acquiring

^{13}C-NMR spectra. Coupling constant (*J*) values are presented in Hertz (Hz), and spin multiplicities are given as s (singlet), d (doublet), t (triplet), and m (multiplet). Elemental analyses were carried out with a Perkin Elmer Model 240-C apparatus (PerkinElmer, Hopkinton, MA, USA). Elemental analyses were within ±0.4% of theoretical values for C, H, and N. IR spectra were recorded on a Nicolet Magna 550-FT spectrometer (Nicolet Instrument Corporation, Madison, WI, USA). Mass spectra were recorded on an Agilent 5975B (Agilent Technologies, Santa Clara, CA, USA) with triple-axis detector.

3.2. Chemistry

1-Phenyl-1,4-pentanedione (**2**) was prepared as described in the literature [24]. Viscous light yellow oil. Yield: 81%. ^{1}H-NMR (500 MHz, $CDCl_3$): 2.20 (s, 3H, CH_3), 2.83 (t, *J* = 6 Hz, 2H, CH_2), 3.21 (t, *J* = 6 Hz, 2H, CH_2), 7.41 (t, *J* = 7.5 Hz, 2H, H-Ar), 7.51 (t, *J* = 7.5 Hz, 1H, H-Ar), 7.94 (d, *J* = 7.5 Hz, 2H, H-Ar).

3.2.1. Procedure for the Preparation of 2-Methyl-5-phenyl-1-(3,4,5-trimethoxyphenyl)-1*H*-pyrrole (**3**)

A mixture of dione **2** (1 mmol), 3,4,5-trimethoxyaniline (1.1 mmol), and a catalytic amount of PTSA in ethanol (10 mL) was refluxed for 6 h. After completion of the reaction (TLC), the reaction mixture was concentrated under reduced pressure and the residue was purified by flash chromatography (hexane: ethyl acetate 10:1 as diluent) to obtain the pure pyrrole derivative **3**.

White solid. Yield 77%. m.p. 109–110 °C. ^{1}H-NMR (500 MHz, $CDCl_3$): 2.19(s, 3H, CH_3), 3.69 (s, 6H, di-OCH_3), 3.87 (s, 3H, OCH_3), 6.08 (s, 1H, H-Pyrrole), 6.34 (d, *J* = 3.5 Hz, 1H, H- Pyrrole), 6.37 (s, 2H, H-Ar), 7.01–7.16 (m, 5H, H-Ar). ^{13}C-NMR (125 MHz, $CDCl_3$): 13.4, 56.3, 61.1, 106.3, 107.6, 108.5, 125.8, 127.7, 128.0, 131.6, 133.6, 134.3, 135.0, 137.5, 153.3. Mass, *m*/*z* (%): 323 (100), 308 (50), 267 (14), 145 (15), 115 (11). Anal. Calcd for $C_{20}H_{21}NO_3$: C, 74.28; H, 6.55; N, 4.33. Found: C, 74.16; H, 6.52; N, 4.35.

3.2.2. Procedure for the Preparation of 2-[2-Methyl-5-phenyl-1-(3,4,5-trimethoxyphenyl) 1*H*-pyrrol-3-yl]-2-oxo-*N*-(pyridin-4-yl) acetamide (**4**)

To a mixture of pyrrole **3** (1 mmol) and triethylamine (TEA) (1.2 mmol) in dichloromethane (DCM) (10 mL), oxalyl chloride (1.1 mmol) was added dropwise at 0 °C, and the mixture was stirred at room temperature (RT). After 4 h, the mixture was concentrated under reduced pressure to remove residual oxalyl chloride. The residue was dissolved in DCM. TEA (1.2 mmol), 4-aminopyridine, and a catalytic amount of 4-(dimethylamino)pyridine were added to the mixture and the reaction was stirred at RT for 12 h. After completion of the reaction, the mixture was concentrated, and the residue was purified by column chromatography (hexane/ethyl acetate 3:1, as eluent) to obtain target compound **4** as pure crystals.

Yellow solid. Yield 68%. m.p. 148–150 °C. IR (KBr, cm^{-1}): 3339, 3020, 2939, 2828, 1704, 1641, 1596, 1502, 1412, 1326, 1128, 823, 760, 696. ^{1}H-NMR (500 MHz, $CDCl_3$): 2.55 (s, 3H, CH_3), 3.72 (s, 6H, OCH_3), 3.88 (s, 3H, OCH_3), 6.34 (s, 2H, H-Ar), 7.14 (d, *J* = 7.5 Hz, 2H, H-Ar), 7.18–7.22 (m, 3H, H-Ar), 7.62 (s, 1H, *H*-Pyrrole), 7.65 (d, *J* = 6 Hz, 2H, *H*-Pyridine), 8.58 (d, *J* = 6 Hz, 2H, *H*-Pyridine), 9.36 (s, 1H, NH). ^{13}C-NMR (125 MHz, $CDCl_3$): 13.9, 56.2, 61.0, 105.6, 112.1, 113.7, 116.5, 127.1, 128.0, 128.1, 131.5, 132.5, 135.2, 138.1, 144.0, 144.6, 150.0, 153.4, 161.0, 180.3. Mass, *m*/*z* (%): 471 (17), 350 (100), 320 (7), 175 (5), 78 (6). Anal. Calcd for $C_{27}H_{25}N_3O_5$: C, 68.78; H, 5.34; N, 8.91. Found: C, 68.90; H, 5.32; N, 8.94.

3.3. Biological Evaluation

3.3.1. Cell Culture

Four cell lines, namely, T47-D, MCF-7, MDA-MB231 (breast carcinoma cell lines), and NIH-3T3 (mouse fibroblast cell line), were obtained from the Pasteur Institute (Tehran, Iran). The cells were cultured in RPMI-1640 medium (Sigma-Aldrich, St. Louis, MO, USA), supplemented with 10% heat-inactivated fetal bovine serum (Gibco, Grand Island, NY, USA), penicillin (100 U/mL), and

(100 μg/mL) streptomycin (Roche, Mannheim, Germany) at 37 °C in a humidified incubator with 5% CO_2.

3.3.2. Cytotoxicity Evaluation by MTT Assay

Compound **4** was tested for cytotoxic activity at a range concentration of 0.01–100 μM. Briefly, cells were seeded in 96-well plates at a density of 10,000 viable cells per well and incubated for 24 h before treatment to allow cell attachment. The stock of compounds in dimethyl sulfoxide (DMSO) was diluted with media and added into each well of the plate. Cells were then incubated for another 48 h. The response of the cells to compounds was evaluated by determining cell survival using MTT. For this purpose, cells were washed in phosphate-buffered saline (PBS), and 20 μL of MTT reagent (5 mg/mL) in phosphate-buffered saline (PBS) was added to each well. After 4 h incubation at 37 °C, the medium was discarded and DMSO (100 μL) was added to each well. The solution was vigorously mixed to dissolve the purple tetrazolium crystals. The absorbance of each well was measured by plate reader (Anthous 2020, Biochrom, Cambridge, UK) at a test wavelength of 550 nm against a standard reference solution at 690 nm. Assays were performed in triplicate in three independent experiments, and the IC_{50} values were determined by a nonlinear regression analysis and expressed in mean ± SD [25].

4. Conclusions

In the current work, we have designed and synthesized a new molecular structure **4** based on indibuin and combretastatin A-4 scaffolds. The synthetic pathway involved four steps and the final yield was 68% after purification procedures. The structure of compound **4** and intermediate **3** were confirmed by spectroscopic methods. The purity of final compounds was checked by TLC and elemental analysis before evaluation of the biological activity. Compound **4** was screened for anti-proliferative activity against three breast cancer cell lines and the results of biological investigation showed remarkable growth inhibitory activity. However, compound **4** presented cytotoxicity on the three cancerous cell lines lower than paclitaxel as positive reference anti-cancer drug; its effect on normal cell line (NIH-3T3) was nil. This suggests that compound **4** can serve as anti-cancer agent with low undesired side effects. The resulting data encourages the synthesis of additional compounds analogous to **4**.

Supplementary Materials: The following data are available online, Figure S1: ^{1}H-NMR Spectrum of compound **4**, Figure S2: ^{1}H-NMR Spectrum of compound **4**—Expanded, Figure S3: ^{13}C-NMR Spectrum of compound **4**, Figure S4: ^{13}C-NMR Spectrum of compound **4**—Expanded, Figure S5: IR Spectrum of compound **4**, Figure S6: Mass Spectrum of compound **4**, Figure S7: ^{1}H-NMR Spectrum of compound **3**, Figure S8: ^{1}H-NMR Spectrum of compound **3**—Expanded, Figure S9: ^{13}C-NMR Spectrum of compound **3**, Figure S10: ^{13}C-NMR Spectrum of compound **3**—Expanded, Figure.S11: Mass Spectrum of compound **3**.

Author Contributions: M.A. designed the experiment and analyzed the data. E.S.M. performed the experiments.

Funding: This work was financially supported by the Research Council of Tehran University of Medical Sciences.

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

References

1. Prakasham, A.P.; Saxena, A.K.; Luqman, S.; Chanda, D.; Kaur, T.; Gupta, A.; Yadav, D.K.; Chanotiya, C.S.; Shanker, K.; Khan, F.; et al. Synthesis and anticancer activity of 2-benzylidene indanones through inhibiting tubulin polymerization. *Bioorgan. Med. Chem.* **2012**, *20*, 3049–3057. [CrossRef] [PubMed]
2. Qu, S.; Mulamoottil, V.A.; Nayak, A.; Ryu, S.; Hou, X.; Song, J.; Yu, J.; Sahu, P.K.; Zhao, L.X.; Choi, S.; et al. Design, Synthesis, and Anticancer Activity of C8-Substituted-4′-Thionucleosides as Potential HSP 90 Inhibitors. *Bioorgan. Med. Chem.* **2016**, *24*, 3418–3428. [CrossRef] [PubMed]

3. Vilanova, C.; Díaz-Oltra, S.; Murga, J.; Falomir, E.; Carda, M.; Redondo-Horcajo, M.; Díaz, J.F.; Barasoain, I.; Marco, J.A. Design and Synthesis of Pironetin Analogue/Colchicine Hybrids and Study of Their Cytotoxic Activity and Mechanisms of Interaction with Tubulin. *J. Med. Chem.* **2014**, *57*, 10391–10403. [CrossRef] [PubMed]
4. Baytas, S.N.; Inceler, N.; Yilmaz, A.; Olgac, A.; Menevse, S.; Banoglu, E.; Hamel, E.; Bortolozzi, R.; Viola, G. Synthesis, biological evaluation and molecular docking studies of trans-indole-3-acrylamide derivatives, a new class of tubulin polymerization inhibitors. *Bioorgan. Med. Chem.* **2014**, *22*, 3096–3104. [CrossRef] [PubMed]
5. Xie, M.; Lapidus, R.G.; Sadowska, M.; Edelman, M.J.; Hosmane, R.S. Synthesis, anticancer activity, and SAR analyses of compounds containing the 5:7-fused 4,6,8-triaminoimidazo[4,5-*e*] [1,3]diazepine ring system. *Bioorgan. Med. Chem.* **2016**, *24*, 2595–602. [CrossRef] [PubMed]
6. Siegel, R.L.; Miller, K.D.; Jemal, A. Cancer Statistics, 2017. *CA Cancer J. Clin.* **2017**, *67*, 7–30. [CrossRef] [PubMed]
7. Perreault, M.; Maltais, R.; Roy, J.; Dutour, R.; Poirier, D. Design of a Mestranol 2-*N*-Piperazino-Substituted Derivative Showing Potent and Selective in vitro and in vivo Activities in MCF-7 Breast Cancer Models. *ChemMedChem* **2017**, *12*, 177–182. [CrossRef] [PubMed]
8. Rahmani-Nezhad, S.; Safavi, M.; Pordeli, M.; Ardestani, S.K.; Khosravani, L.; Pourshojaei, Y.; Mahdavi, M.; Emami, S.; Foroumadi, A.; Shafiee, A. Synthesis, in vitro cytotoxicity and apoptosis inducing study of 2-aryl-3-nitro-2Hchromene derivatives as potent anti-breast cancer agents. *Eur. J. Med. Chem.* **2014**, *86*, 562–569. [CrossRef] [PubMed]
9. Xiao, M.; Ahn, S.; Wang, J.; Chen, J.; Miller, D.D.; Dalton, J.T.; Li, W. Discovery of 4-Aryl-2-benzoyl-imidazoles as Tubulin Polymerization Inhibitor with Potent Antiproliferative Properties. *J. Med. Chem.* **2013**, *56*, 3318–3329. [CrossRef] [PubMed]
10. Zhang, Q.; Peng, Y.; Wang, X.I.; Keenan, S.M.; Arora, S.; Welsh, W.J. Highly Potent Triazole-Based Tubulin Polymerization Inhibitors. *J. Med. Chem.* **2007**, *50*, 749–754. [CrossRef] [PubMed]
11. Romagnoli, R.; Baraldi, P.G.; Cruz-Lopez, O.; Lopez Cara, C.; Carrion, M.D.; Brancale, A.; Hamel, E.; Chen, L.; Bortolozzi, R.; Basso, G.; et al. Synthesis and Antitumor Activity of 1,5-Disubstituted 1,2,4-Triazoles as Cis-Restricted Combretastatin Analogues. *J. Med. Chem.* **2010**, *53*, 4248–4258. [CrossRef] [PubMed]
12. Romagnoli, R.; Baraldi, P.G.; Salvador, M.K.; Preti, D.; Aghazadeh Tabrizi, M.; Brancale, A.; Fu, X.H.; Li, J.; Zhang, S.Z.; Hamel, E.; et al. Synthesis and Evaluation of 1,5-Disubstituted Tetrazoles as Rigid Analogues of Combretastatin A-4 with Potent Antiproliferative and Antitumor Activity. *J. Med. Chem.* **2012**, *55*, 475–488. [CrossRef] [PubMed]
13. Galli, U.; Travelli, C.; Aprile, S.; Arrigoni, E.; Torretta, S.; Grosa, G.; Massarotti, A.; Sorba, G.; Canonico, P.L.; Genazzani, A.A.; et al. Design, synthesis, and biological evaluation of combretabenzodiazepines: A novel class of anti-tubulin agents. *J. Med. Chem.* **2015**, *58*, 1345–1357. [CrossRef] [PubMed]
14. Wienecke, A.; Bacher, G. Indibulin, a novel microtubule inhibitor, discriminates between mature neuronal and nonneuronal tubulin. *Cancer Res.* **2009**, *69*, 171–177. [CrossRef] [PubMed]
15. Colley, H.E.; Muthana, M.; Danson, S.J.; Jackson, L.V.; Brett, M.L.; Harrison, J.; Coole, S.F.; Mason, D.P.; Jennings, L.R.; Wong, M.; et al. An orally bioavailable, indole-3-glyoxylamide based series of tubulin polymerization inhibitors showing tumor growth inhibition in a mouse Xenograft model of head and neck cancer. *J. Med. Chem.* **2015**, *58*, 9309–9333. [CrossRef] [PubMed]
16. Marchand, P.; Antoine, M.; Le Baut, G.; Czech, M.; Baasner, S.; Günther, E. Synthesis and structure–activity relationships of *N*-aryl(indol-3-yl)glyoxamides as antitumor agents. *Bioorgan. Med. Chem.* **2009**, *17*, 6715–6727. [CrossRef] [PubMed]
17. Li, W.T.; Hwang, D.R.; Chen, C.P.; Shen, C.W.; Huang, C.L.; Chen, T.W.; Lin, C.H.; Chang, Y.L.; Chang, Y.Y.; Lo, Y.K.; et al. Synthesis and Biological Evaluation of *N*-Heterocyclic Indolyl Glyoxylamides as Orally Active Anticancer Agents. *J. Med. Chem.* **2003**, *46*, 1706–1715. [CrossRef] [PubMed]
18. Ghasemi, M.; Ghadbeighi, S.; Amirhamzeh, A.; Tabatabai, S.A.; Ostad, S.N.; Shafiee, A.; Amini, M. Synthesis, Molecular Docking Study, and Cytotoxic Activity of 1,3,5-triaryl Pyrazole Derivatives. *Lett. Drug Des. Discov.* **2016**, *13*, 121–128. [CrossRef]
19. Ghadbegi, S.; Ostad, S.N.; Shafiee, A.; Amini, M. Synthesis and Anticancer Activity of 1,3,5-triaryl-1*H*-pyrazole. *Lett. Drug Des. Discov.* **2015**, *12*, 754–759. [CrossRef]

20. Miralinaghi, P.; Salimi, M.; Amirhamzeh, A.; Norouzi, M.; Kandelousi, H.M.; Shafiee, A.; Amini, M. Synthesis, molecular docking study, and anticancer activity of triaryl-1,2,4-oxadiazole. *Med. Chem. Res.* **2013**, *22*, 4253–4262. [CrossRef]
21. Salehi, M.; Ostad, S.N.; Riazi, G.H.; Assadieskandar, A.; Shavi, T.C.; Shafiee, A.; Amini, M. Synthesis, cytotoxic evaluation, and molecular docking study of 4,5-diaryl-thiazole-2-thione analogs of combretastatin A-4 as microtubule-binding agents. *Med. Chem. Res.* **2014**, *23*, 1465–1473. [CrossRef]
22. Zareian, B.; Ghadbeighi, S.; Amirhamzeh, A.; Ostad, S.N.; Shafiee, A.; Amini, M. Synthesis, Molecular Docking Study, and Cytotoxic Activity of 3,4-diaryl-5-(4-pyridinyl)-1,2,4-oxadiazole. *Med. Chem.* **2016**, *12*, 394–401. [CrossRef] [PubMed]
23. Elahian, F.; Akbari, M.; Ghasemi, M.; Behtooee, N.; Taheri, M.; Amini, M. Synthesis and Anticancer Activity of 2,4,5-triaryl Imidazole Derivatives. *Lett. Drug Des. Discov.* **2014**, *11*, 840–843. [CrossRef]
24. Peloquin, A.J.; Stone, R.L.; Avila, S.E.; Rudico, E.R.; Horn, C.B.; Gardner, K.A.; Ball, D.W.; Johnson, J.E.B.; Iacono, S.T.; Balaich, G.J. Synthesis of 1,3-Diphenyl-6-alkyl/aryl-Substituted Fulvene Chromophores: Observation of π-π Interactions in a 6-Pyrene-Substituted 1,3-Diphenylfulvene. *J. Org. Chem.* **2012**, *77*, 6371–6376. [CrossRef] [PubMed]
25. Assadieskandar, A.; Amini, M.; Ostad, S.N.; Riazi, G.H.; Shavi, T.C.; Shafiei, B.; Shafiee, A. Design, synthesis, cytotoxic evaluation and tubulin inhibitory activity of 4-aryl-5-(3,4,5-trimethoxyphenyl)-2-alkylthio-1*H*-imidazole derivatives. *Bioorgan. Med. Chem.* **2013**, *21*, 2703–2709. [CrossRef] [PubMed]