



## Article

# Potent Quinoline-Containing Combretastatin A-4 Analogues: Design, Synthesis, Antiproliferative, and Anti-Tubulin Activity

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**Abstract:** A novel series of quinoline derivatives of combretastatin A-4 incorporating rigid hydrazone and a cyclic oxadiazole linkers were synthesized and have demonstrated potent tubulin polymerization inhibitory properties. Many of these novel derivatives have shown significant antiproliferative activities in the submicromolar range. The most potent compound, **19h**, demonstrated superior IC<sub>50</sub> values ranging from 0.02 to 0.04  $\mu$ M against four cancer cell lines while maintaining low cytotoxicity in MCF-10A non-cancer cells, thereby suggesting **19h**'s selectivity towards proliferating cancer cells. In addition to tubulin polymerization inhibition, **19h** caused cell cycle arrest in MCF-7 cells at the G2/M phase and induced apoptosis. Collectively, these findings indicate that **19h** holds potential for further investigation as a potent chemotherapeutic agent targeting tubulin.

**Keywords:** combretastatin A-4; quinoline; tubulin; apoptosis; hydrazone; oxadiazole

## 1. Introduction

The well-known protein tubulin plays a crucial role in several cellular processes involving maintenance of the cell structure, motility, division and intercellular transport, where it is responsible for spindle formation and chromosomal separation [1–4]. Tubulin polymerization inhibitors interfere with tubulin and are becoming a well-verified strategy for the development of highly efficient anticancer drugs [5]. Several natural products such as colchicine, paclitaxel, and the vinca alkaloids inhibit tubulin polymerization by binding to tubulin at their respective binding sites. In the case of tubulin polymerization inhibitors at the colchicine binding site, combretastatin A-4 and many of its derivatives, inclusive of the *cis* restricted  $\beta$ -lactam derivatives of CA-4, the downstream consequences include significant occlusion of blood supply to solid tumors as anti-angiogenic and vascular disrupting agents (VDAs), resulting in considerable necrosis for malignant cells [6–8].

Combretastatin A-4 (CA-4) is the lead antimitotic agent within the combretastatin family and is highly active against several cancer cell lines due to its potential vascular disrupting and antiangiogenic activity. This is in addition to its potency against multidrug resistance cell lines, a property which makes derivatives of CA-4 attractive for further development, specifically towards clinical use where typically used anti-cancer regimens have been exhausted or are limited. (Figure 1) [6,7,9,10]. The water-soluble phosphate and amino acid prodrugs namely, (fosbretabulin disodium and ombrabulin, Figure 1) are now under clinical trials to overcome the limitations of CA-4's poor water solubility (0.35 mM), and improve its pharmacokinetic profile [11–14].

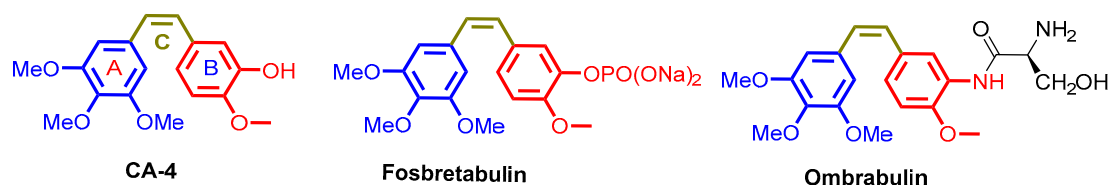


Figure 1. CA-4 and structurally related analogues.

SAR analysis of CA-4 revealed that, the main pharmacophoric features are the trimethoxyphenyl ring A, ring B with various substituent groups, contributing variant steric, electronic and lipophilic properties and finally the *cis* double bond acting as the A-B ring linker [15]. Several literature reports document that CA-4 is vulnerable towards photoisomerization whereby it is converted to the inactive *trans* isomer during both storage and administration. The *trans* isomer is largely inactive as an anti-cancer agent with minimal anti-tubulin and cytotoxic activity [16,17]. Accordingly, rigidification of the alkenyl double bond has overcome this undesirable isomerization towards the less active *trans* isomer of CA-4. Introduction of rigid scaffolds in place of the alkenyl bond induces *cis* restriction of the A and B ring and various modifications have achieved such *cis* restriction. Insertion of a heterocyclic linker such as pyrrole, oxadiazole, isoxazole and imidazole are among some examples which have resulted in compounds with potent cytotoxic activity (e.g., 1–3, Figure 2)) [18–20]. *N*-acylhydrazones 4–6 as open chain linkers of the latter compounds have also displayed promise as potent colchicine-site-targeting tubulin inhibitors [21]. In addition, 3,4,5-trimethoxyhydrazine-containing compounds with a naphthalene moiety showed antiproliferative activity as tubulin inhibitors in the nanomolar concentration range [22].

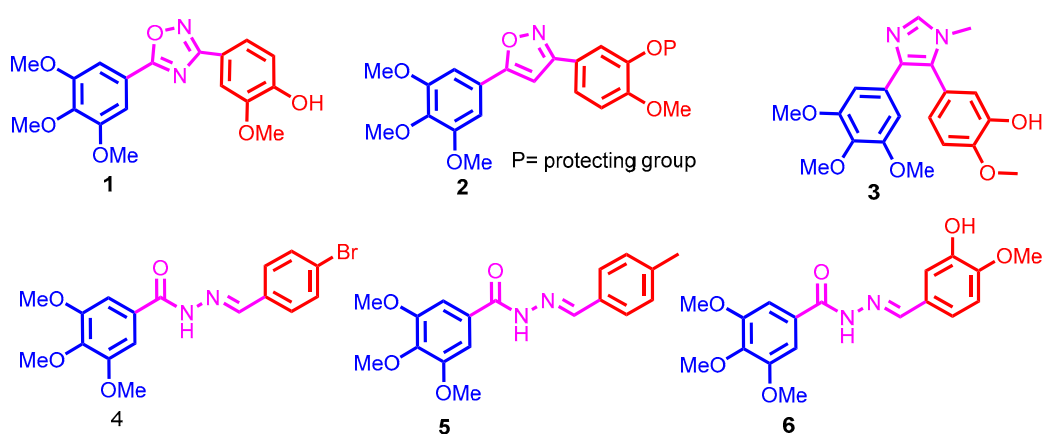
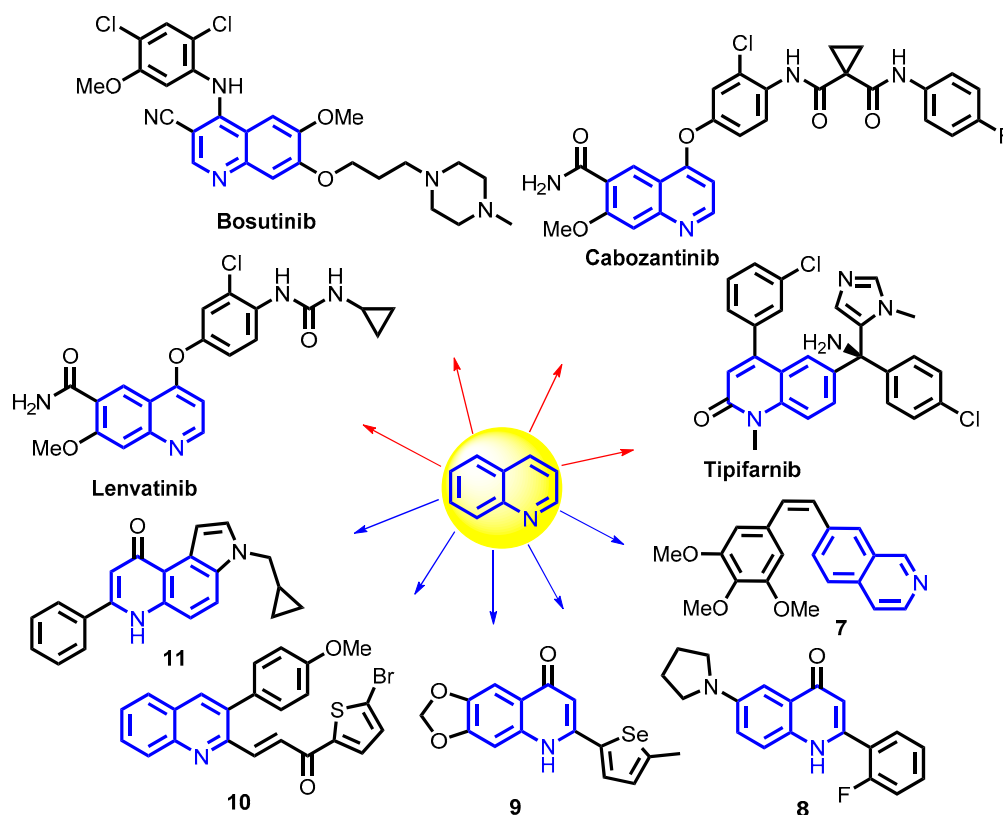


Figure 2. Structurally related CA-4 analogues.

From a biological point of view, bicyclic heteroaromatic systems are often worthy of much greater attention than their constituting monocyclic relatives. Alteration of the monocyclic fragment B in CA-4 with quinoline, i.e., compound 7, displays 10-fold more potent anti-tubulin activity in comparison to CA-4 [23]. Indeed, the quinoline scaffold is a well-known moiety utilized among bicyclic systems

in the development of anticancer drugs. Among such drugs currently in clinical use are lenvatinib, cabozantinib, tipifarnib and bosutinib (Figure 3) [24].



**Figure 3.** Examples of quinoline containing antimitotic agents and tubulin polymerization inhibitors & quinoline containing anticancer drugs.

Quinoline derivatives **8–11** (Figure 3) also display excellent anti-cancer activity over a variety of cancer cell lines through various mechanisms including apoptosis and cell cycle arrest; acting as growth inhibitors, disruption of cell migration, inhibition of angiogenesis and inhibition of tubulin polymerization,

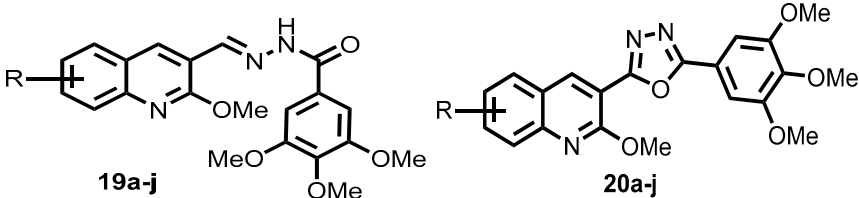
Based on the findings mentioned above, we aimed to optimize CA-4 through synthesis of a series of novel CA-4 analogues as promising potent tubulin inhibitors working through two approaches. All the synthesized analogues using both modes contain a ring A structure identical to that of CA-4. For mode 1, the target compounds were synthesized using a 3,4,5-trimethoxyphenyl moiety (**ring A**) and a quinolyl moiety as a bioisosteric replacement of CA-4's ring B with a variety of electronic substituents. For mode 2, compounds which were designed to increase the structure's rigidity *via* introduction of ring C (a hydrazone open linker and its cyclic form, the oxadiazole ring) in place of the *cis*-olefinic bond. Such introduction of the hydrazone linker/oxadiazole ring holds potential to create another novel type and desirable conformational restriction that could prevent *in vivo* isomerization of CA-4 into the inactive *trans*-isomer. Additionally, this modification may provide additional hydrogen bond donors and/or acceptors, in turn potentially increasing/modifying favorably the interaction with tubulin. These novel quinoline analogues underwent screening for their antiproliferative activity in HL-60, MCF-7, HCT-116 and HeLa cancer cells, after which further mechanistic biochemical investigations were performed for the most potent compound.

## 2. Results and Discussion

### 2.1. Chemistry

The syntheses of our proposed targeted compounds **19a–j**, and **20a–j** (Table 1) involved two core structural features: (i) a 2-methoxyquinolyl-3-carbaldehyde moiety, and (ii) a 3,4,5-trimethoxyphenyl moiety. Firstly, three steps led to the formation of 2-methoxyquinoline-3-carbaldehyde derivatives **15a–j** as shown in Scheme 1. This began with acetylation of the appropriate aniline derivatives **12a–j** under typical condition using glacial acetic acid and acetic anhydride at 0 °C to deliver the corresponding amides **13a–j** [24]. The latter compounds were subjected to Vilsmeier–Haack reactions to furnish the quinoline aldehyde derivatives **14a–j**. Installing the important methoxy substituent in the former compounds has been achieved utilizing sodium methoxide at 40 °C which then gave the substituted methoxy-aldehydes **15a–j**.

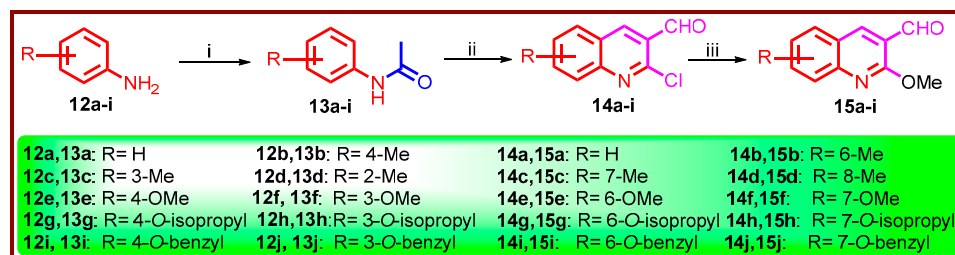
**Table 1.** Antiproliferative activity of quinolines **19a–j** and **20a–j** against human cancer cell lines.



Compound N°.	R	Antiproliferative Activities IC <sub>50</sub> μM <sup>a</sup>				Inhibition of Tubulin Polymerization <sup>b</sup> IC <sub>50</sub> μM
		HL-60	MCF-7	HCT-116	HeLa	
19a	H	0.013 ± 0.009	0.030 ± 0.001	0.308 ± 0.012	0.093 ± 0.006	4.69
19b	6-CH <sub>3</sub>	0.045 ± 0.002	0.072 ± 0.004	0.371 ± 0.002	0.028 ± 0.001	1.48
19c	7-CH <sub>3</sub>	4.210 ± 0.0062	0.703 ± 0.002	3.830 ± 0.026	1.151 ± 0.254	35.64
19d	8-CH <sub>3</sub>	1.810 ± 0.004	1.097 ± 0.049	0.450 ± 0.022	0.107 ± 0.006	2.26
19e	6-OCH <sub>3</sub>	0.088 ± 0.002	0.031 ± 0.007	0.812 ± 0.020	0.124 ± 0.019	3.647
19f	7-OCH <sub>3</sub>	0.150 ± 0.062	0.044 ± 0.002	0.366 ± 0.030	0.167 ± 0.009	8.96
19g	6-OCH(CH <sub>3</sub> ) <sub>2</sub>	0.137 ± 0.007	0.078 ± 0.003	0.233 ± 0.017	0.014 ± 0.004	3.04
19h	7-OCH(CH <sub>3</sub> ) <sub>2</sub>	0.040 ± 0.003	0.026 ± 0.002	0.022 ± 0.001	0.038 ± 0.003	1.32
19i	6-OCH <sub>2</sub> Ph	0.983 ± 0.042	0.530 ± 0.001	0.138 ± 0.004	0.152 ± 0.004	4.08
19j	7-OCH <sub>2</sub> Ph	0.585 ± 0.051	0.204 ± 0.001	0.067 ± 0.012	0.082 ± 0.001	3.34
20a	H	1.551 ± 0.032	3.480 ± 0.001	4.030 ± 0.013	1.960 ± 0.003	17.55
20b	6-CH <sub>3</sub>	0.336 ± 0.011	0.185 ± 0.007	0.378 ± 0.002	0.616 ± 0.002	8.83
20c	7-CH <sub>3</sub>	0.012 ± 0.009	0.094 ± 0.007	0.024 ± 0.009	0.210 ± 0.002	2.41
20d	8-CH <sub>3</sub>	0.147 ± 0.018	0.076 ± 0.006	0.087 ± 0.009	0.117 ± 0.014	6.62
20e	6-OCH <sub>3</sub>	0.166 ± 0.075	0.094 ± 0.008	0.179 ± 0.001	0.144 ± 0.010	6.45
20f	7-OCH <sub>3</sub>	0.055 ± 0.001	0.44 ± 0.005	0.199 ± 0.007	0.095 ± 0.001	3.25
20g	6-OCH(CH <sub>3</sub> ) <sub>2</sub>	0.270 ± 0.015	0.044 ± 0.003	0.086 ± 0.003	0.071 ± 0.003	2.31
20h	7-OCH(CH <sub>3</sub> ) <sub>2</sub>	0.109 ± 0.006	0.042 ± 0.006	0.146 ± 0.009	0.193 ± 0.002	5.80
20i	6-OCH <sub>2</sub> Ph	0.043 ± 0.002	0.039 ± 0.003	0.082 ± 0.001	0.138 ± 0.003	4.60
20j	7-OCH <sub>2</sub> Ph	0.029 ± 0.007	0.028 ± 0.003	0.129 ± 0.009	0.069 ± 0.003	2.29
CA-4	-	0.076 ± 0.004	0.019 ± 0.004	0.026 ± 0.001	0.064 ± 0.004	2.17

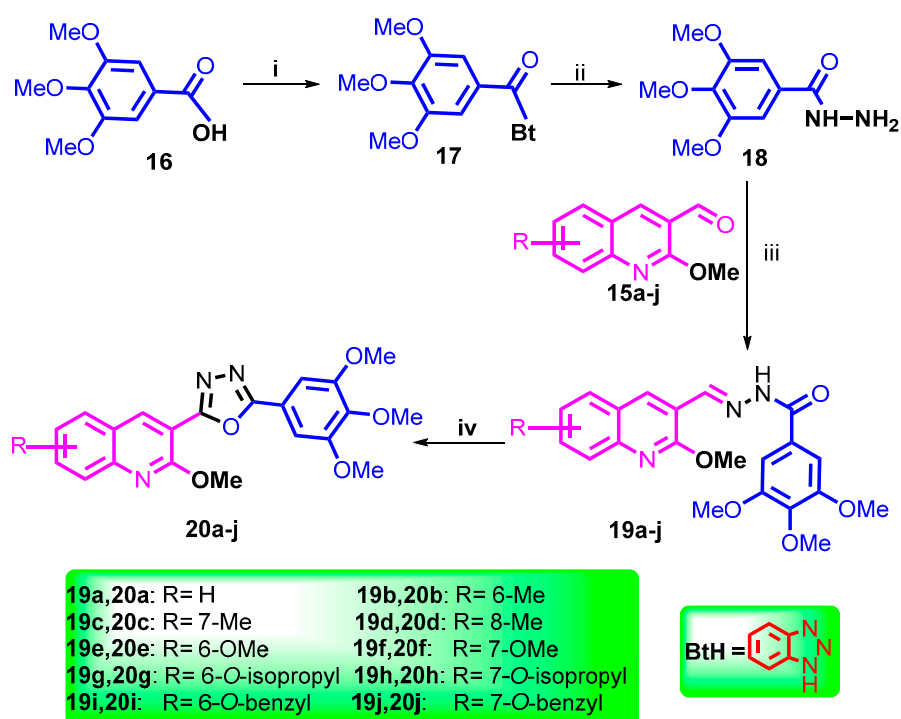
<sup>a</sup> IC<sub>50</sub> values are 50% inhibitory concentrations and mean ± SD for three experiments performed in triplicate;

<sup>b</sup> The tubulin assembly assay measured the extent of assembly of polymerization of 10 μM tubulin after 20 min at 30 °C.



**Scheme 1.** Synthetic route for preparation of aldehydes **15a–i**. Reagents and conditions: (i) Ac<sub>2</sub>O, AcOH, 0 °C, 1 h (90–95%); (ii) DMF, POCl<sub>3</sub>, 70–90 °C, 18 h (65–80%); (iii) CH<sub>3</sub>ONa, MeOH, 40 °C, 3–6 h (73–88%).

Chlorine, an excellent leaving group, was subjected to an addition-elimination reaction under the effect of the strong nucleophile, sodium methoxide. The reaction is activated by the electron withdrawing effect of the *N*-heteroatom. Similarly, another acylation has been established for the starting material, acid **16**, in acidic medium, using SOCl<sub>2</sub> to deliver the acyl benzotriazole **17** (Scheme 2). Unlike halogens, the benzotriazole group rarely leaves in the absence of a heteroatom at the the  $\alpha$ -carbon. The advantage of using benzotriazole is provision of the direct attachment to the carbonyl group that forms the *N*-acylbenzotriazole synthon. In general, this synthon is classified as an efficient *N*-acylating reagent [25]. Indeed, the carboxylic acid hydrazide **18** was the key for the preparation of the first tranche of our designed compounds (Scheme 2). This was prepared by hydrazinolysis of the acyl benzotriazole **17** through reaction with hydrazine hydrate in acetonitrile at room temperature. Use of hydrazide **18** to condensation reactions with the appropriate quinoline aldehydes **15a–j** in acetic anhydride using a catalytic amount of sodium acetate led to the formation of our rationalized hydrazones **19a–j** (Scheme 2).



**Scheme 2.** Synthetic route for the preparation of our targeted CA-4 analogs. Reagents and conditions:

(i) SOCl<sub>2</sub>, BtH, DCM, r.t (90%); (ii) NH<sub>2</sub>NH<sub>2</sub>, CH<sub>3</sub>CN, r.t, 15 m (92%); (iii) Dioxane, 80 °C, 9 h (70–89%); (iv) I<sub>2</sub>/DMSO, K<sub>2</sub>CO<sub>3</sub>, 100 °C, 4 h (70–88%).

The <sup>1</sup>H-NMR spectra of these open chain targets showed a common sharp singlet signal appeared at  $\delta$  8.20–8.80 ppm due to the azomethine proton, while the corresponding amide N–H proton appeared as a broad singlet at between  $\delta$  11.40–11.90 ppm. Similarly, the protons of the heterocyclic systems and ring A nucleus showed resonances at the expected chemical shifts between  $\delta$  7.00–8.50 ppm. Oxidative cyclization of acylhydrazones **19a–j** was performed under alkaline conditions using iodine dissolved in DMSO in the presence of K<sub>2</sub>CO<sub>3</sub> at 100 °C to afford the corresponding oxadiazoles **20a–j** at 70–88% yields. The IR spectra of the oxadiazoles showed a common disappearance of two bands at 3320 and 1645 cm<sup>−1</sup> due to the exchangeable NH and (C=O) in the starting materials, respectively. Moreover, <sup>1</sup>H-NMR revealed a common absence of the singlet signals due to the azomethine (CH=N) and (NH) protons at  $\delta$  8.68 and 11.91 ppm, respectively. (Figure S1–S40, Supplementary Materials).

## 2.2. Biological Results and Discussion

### 2.2.1. In Vitro Antiproliferative Activities

The quinoline compounds are mainly arranged into two distinct structural series with modifications to the ethylene bridge of CA-4 by either a hydrazone linker or an oxadiazole moiety. Each of the synthesized compounds were screened for their antiproliferative activity against four representative cancer cell lines, including MCF-7 breast, HL-60 leukemia, HCT-116 colon and HeLa cervical cancer cells and compared with CA-4 as reference compound using the MTT assay. As shown in Table 1, most of the target compounds displayed potent antiproliferative activity in nanomolar range in these cell lines (if not specified, the  $IC_{50}$  value for each analogue is expressed as the average of all four cancer cell lines).

The first series of analogues **19a–j** examined contained the hydrazone linker with different substituents on the quinoline ring. The 7-*tert*-butyl substituted quinoline **19h** was the most potent of all the hydrazone derivatives ( $IC_{50}$  values in HL-60, MCF-7, HCT-116 and HeLa cancer cells were 0.040, 0.026, 0.022 and 0.038  $\mu$ M, respectively). Replacement of the 7-*tert*-butyl substituent with the bulkier 7-benzyloxy group (compound **19j**) slightly reduced the activity by 2- to 14- fold in four cancer cell lines. Moving the 7-*tert*-butyl and 7-benzyloxy groups to the 6-position of the quinoline ring yielding 6-*tert*-butyl and 6-benzyloxy derivatives **19g** and **19i** impacted the antiproliferative activity;  $IC_{50}$  values for **19g** and **19i** ranged from 0.014 to 0.233 and from 0.138 to 0.983  $\mu$ M which were less potent than their corresponding analogues **19h** ( $IC_{50}$ : 0.022–0.040  $\mu$ M) and **19j** ( $IC_{50}$ : 0.067–0.585  $\mu$ M). Following the same pattern, a 7-methoxy group (compound **19f**) was more potent ( $IC_{50}$ : 0.044–0.366  $\mu$ M) compared to its corresponding 6-methoxy group analogue **19e** ( $IC_{50}$ : 0.03–0.812  $\mu$ M). This indicates that these substituents are more favourable at the 7-position of the quinoline ring compared to the 6-position, which enhances the antiproliferative activity. Introduction of the methyl substituent on different positions on the quinoline ring affected the antiproliferative activity; compound **19b** (containing a methyl group at the 6-position of the quinoline ring) displayed impressive potency comparable with CA-4 against all four cell lines ( $IC_{50}$ : 0.028–0.371  $\mu$ M) compared to 7-methyl and 8-methyl analogues **19c** and **19d**, with  $IC_{50}$  values of 0.703–4.210 and 0.107–1.810  $\mu$ M, respectively. In general, the nature of the substituents on the quinoline ring of the hydrazone significantly influenced the biological activity. Interestingly, unsubstituted quinoline **19a** maintained good antiproliferative activity with  $IC_{50}$  values of 0.013–0.308  $\mu$ M which could reflect the flexibility of the hydrazone moiety and its potential ability to adopt an appropriate orientation inside the colchicine binding domain of tubulin.

A further series of quinoline compounds containing a rigid oxadiazole core was synthesized and evaluated for antiproliferative activity (Table 1). The unsubstituted quinoline analogue **20a** was the least potent in this series, with activity in micromolar range ( $IC_{50}$ : 1.551–4.030  $\mu$ M) in four cell lines, in contrast to the results obtained for the unsubstituted hydrazone derivative **19a**. In a similar manner to the hydrazone analogues, a methyl substituent at different positions on the quinoline heterocycle significantly influenced the antiproliferative activity against the four selected cancer cell lines. For example, **20c** (7-methyl) displayed superior antiproliferative activity ( $IC_{50}$ : 0.012–0.210  $\mu$ M) compared to other positions like 6-methyl (compound **20b**) or 8-methyl (compound **20d**), with  $IC_{50}$  values of 0.185–0.616 and 0.076–0.147  $\mu$ M, respectively. Replacement of the methyl group with the stronger electron-releasing methoxy group (c.f. compounds **20b** and **20c** with **20e** and **20f**, respectively) impacted the antiproliferative activity; methoxy-containing compound **20e** was 2.6-fold more active than methyl-containing **20b**, while **20f** was 2.3-fold less active than **20c**. Larger substituents at different positions on the quinoline ring as in **20i** (6-benzyloxy) and **20j** (7-benzyloxy) exhibited potent antiproliferative activity similar to CA-4 and an increase in activity compared to their corresponding analogues **20g** (6-*tert*-butyl) and **20h** (7-*tert*-butyl) by 1.5- and 1.9-fold, respectively.

It could be hypothesized that varying both the substituents and positions on the quinoline ring may affect the interaction with surrounding tubulin residues. Remarkably, the hydrazone linker was more flexible compared to the rigid oxadiazole which displayed potent antiproliferative effects. Due to



the impressive antiproliferative potency of hydrazone analogue **19h** (containing a *tert*-butyl group in the 7-position of the quinoline ring), it was selected for further molecular biochemical investigations in MCF-7 breast cancer cells.

## 2.2.2. In Vitro Inhibition of Tubulin Polymerization and Competitive Colchicine-Binding Assays

Studies demonstrate that trimethoxyphenyl (TMP)-containing stilbenoid derived tubulin inhibitors that bind to tubulin at the colchicine site, such as colchicine and CA-4, result in microtubule depolymerization [26,27]. To further investigate the molecular mechanism of action of the quinoline compounds, a tubulin polymerization assay was performed on all the quinoline compounds including hydrazone analogues **19a–j** and oxadiazole analogues **20a–j**, compared with the reference compound CA-4 (Table 1). Amongst the hydrazone derivatives, 7-*tert*-butyl substituted compound **19h** exhibited strong tubulin polymerization inhibitory activity ( $IC_{50}$ : 1.32  $\mu$ M) which agrees with the excellent cell growth inhibitory activity ( $IC_{50}$ : 0.022–0.040  $\mu$ M) against the cancer cells. 6-Methyl analogue **19b** also potently inhibited the tubulin polymerization followed by the 8-methyl analogue **19d** ( $IC_{50}$ : 1.48 and 2.26  $\mu$ M), respectively, similar to that of CA-4 ( $IC_{50}$ : 2.17  $\mu$ M), while the 7-methyl analogue **19c** had poor inhibitory activity ( $IC_{50}$ : 35.64  $\mu$ M). Among the oxadiazole derivatives, the 7-methyl analogue **20c**, 7-O-isopropyl analogue **20g** and 7-benzyloxy derivative **20j** strongly inhibited tubulin assembly with  $IC_{50}$  values of 2.41, 2.31 and 2.29  $\mu$ M, respectively, comparable to CA-4 ( $IC_{50}$ : 2.17  $\mu$ M). The unsubstituted analogue **20a** was inactive ( $IC_{50}$ : 17.55  $\mu$ M). This assay revealed that the potent quinoline compounds with low  $IC_{50}$  values in the antiproliferative activity showed approximately the same ability to inhibit tubulin polymerization when compared to CA-4.

To confirm whether quinoline CA-4 analogues directly bind to the colchicine binding site, a [ $^3$ H] colchicine binding assay was performed. Hence, representative quinoline compounds including two hydrazone analogues (compounds **19b** and **19h**) and two oxadiazole analogues (compounds **20c** and **20j**) were examined for their ability to compete with colchicine for binding to tubulin at two different concentrations (1 and 5  $\mu$ M) using CA-4 as a positive control. The binding potency of hydrazone analogues **19b** and **19h** to colchicine was 83% and 86% at 5  $\mu$ M, respectively (Table 2), indicating that they displace colchicine from its binding site on tubulin and hence are colchicine-binding site inhibitors. Oxadiazole analogues **20c** and **20j** also inhibited [ $^3$ H]-colchicine binding to tubulin, with 73 and 80% inhibition at 5  $\mu$ M, respectively; this is less than the positive control to CA-4 (97% inhibition at 5  $\mu$ M) and also hydrazones **20b** and **20h**. Therefore, considering the excellent activities of compound **20h** in the in vitro antiproliferative assay, the tubulin polymerization inhibition experiment, and the colchicine-displacement assay, it was selected for further mechanism of action studies.

**Table 2.** Inhibition of [ $^3$ H] colchicine binding by selective quinoline compounds and CA-4.

Compound	Colchicine Binding <sup>a</sup> (% $\pm$ SD)	
	1 $\mu$ M Drug	5 $\mu$ M Drug
<b>19b</b>	79 $\pm$ 1.6	83 $\pm$ 0.54
<b>19h</b>	80 $\pm$ 0.4	86 $\pm$ 0.13
<b>20c</b>	69 $\pm$ 0.8	73 $\pm$ 0.27
<b>20j</b>	75 $\pm$ 1.5	80 $\pm$ 0.26
<b>CA-4</b>	86 $\pm$ 0.9	97 $\pm$ 0.2

<sup>a</sup> Inhibition of [ $^3$ H] colchicine binding. Tubulin, 1  $\mu$ M; [ $^3$ H] colchicine, 5  $\mu$ M; and inhibitors, 1 or 5  $\mu$ M. Incubation was performed for 10 min at 37 °C. Values represent the mean for two experiments and expressed as the mean  $\pm$  SD.

## 2.2.3. Cell Cycle Analysis in MCF-7 Cells

G<sub>2</sub>/M phase cell cycle arrest is strongly associated with tubulin polymerization inhibition and is well established that CA-4 causes cell cycle arrest at G<sub>2</sub>/M phase [28–30]. The excellent potency of compound **19h** with respect to inhibition of MCF-7 cell proliferation prompted further investigation us on whether the activity of **19h** was due to cell cycle arrest. The effect of hydrazone analogue **19h** was

investigated in breast cancer MCF-7 cells by flow cytometry at two concentrations (50 nM and 250 nM) for different time times (0, 24, 48 and 72 h). As shown in Figure 4A, it was clearly demonstrated that **19h** caused a significant G<sub>2</sub>/M arrest and apoptosis in a time and concentration dependent manner. The percentage of cells in G<sub>2</sub>/M phase increased to 23.7 and 35.6% at a concentration of 50 nM and 250 nM respectively after 48 h compared to the control (7.6%) (Figure 4B). A similar trend was found for concentrations after 72 h, suggesting that the release of cells induced mitotic block. This finding is comparable with CA-4 (50 nM) which caused a significant increase in the percentage of cells in G<sub>2</sub>/M arrest at 24, 48 and 72 h with 40.3, 43.8 and 47.7% of MCF-7 cells respectively with a concomitant decrease of cells in the cell cycle G<sub>0</sub> phase (Figure 4B,C). Moreover, **19h** induced a gradual increase in apoptosis as the population in the sub-G<sub>1</sub> phase was increased at 24, 48 and 72 h time point with 12.2, 20.85 and 33.9%, respectively, at 250 nM compared to 1.7% for untreated cells (Figure 4D). These findings are in agreement with the previously observed for antimetabolic derivatives in the series of related quinoline analogues which significantly induce G<sub>2</sub>/M cycle arrest and apoptosis in MCF-7 cells [7,31–34].

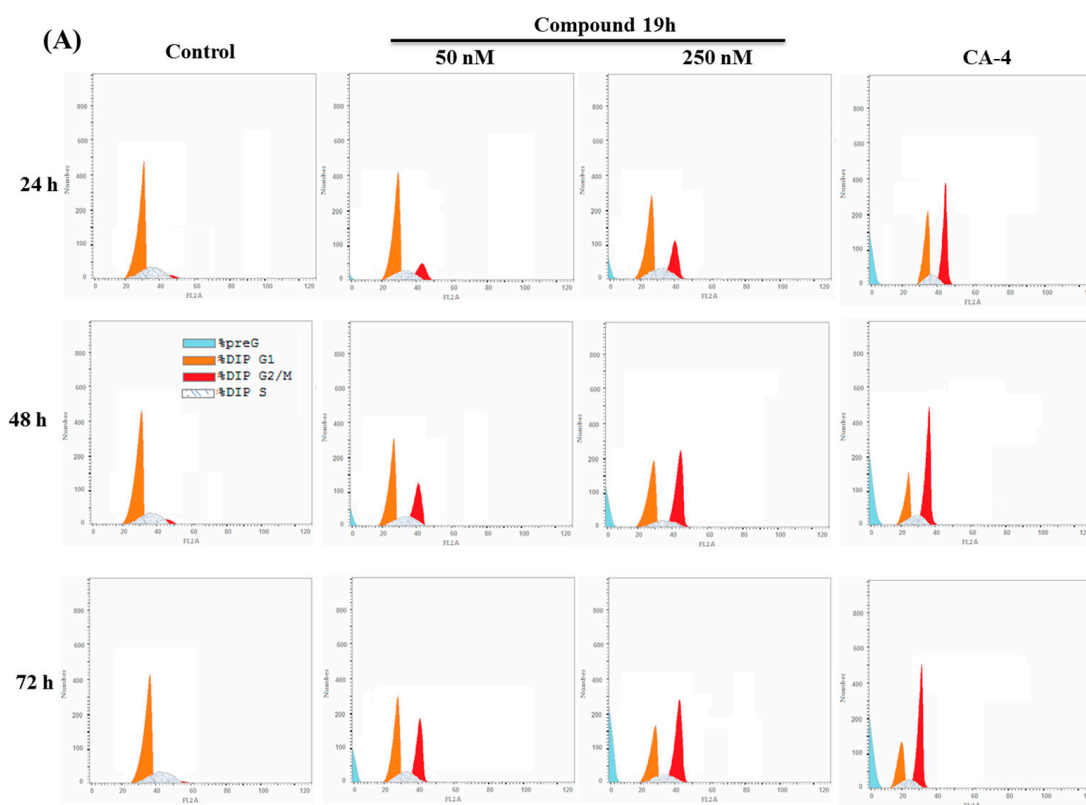
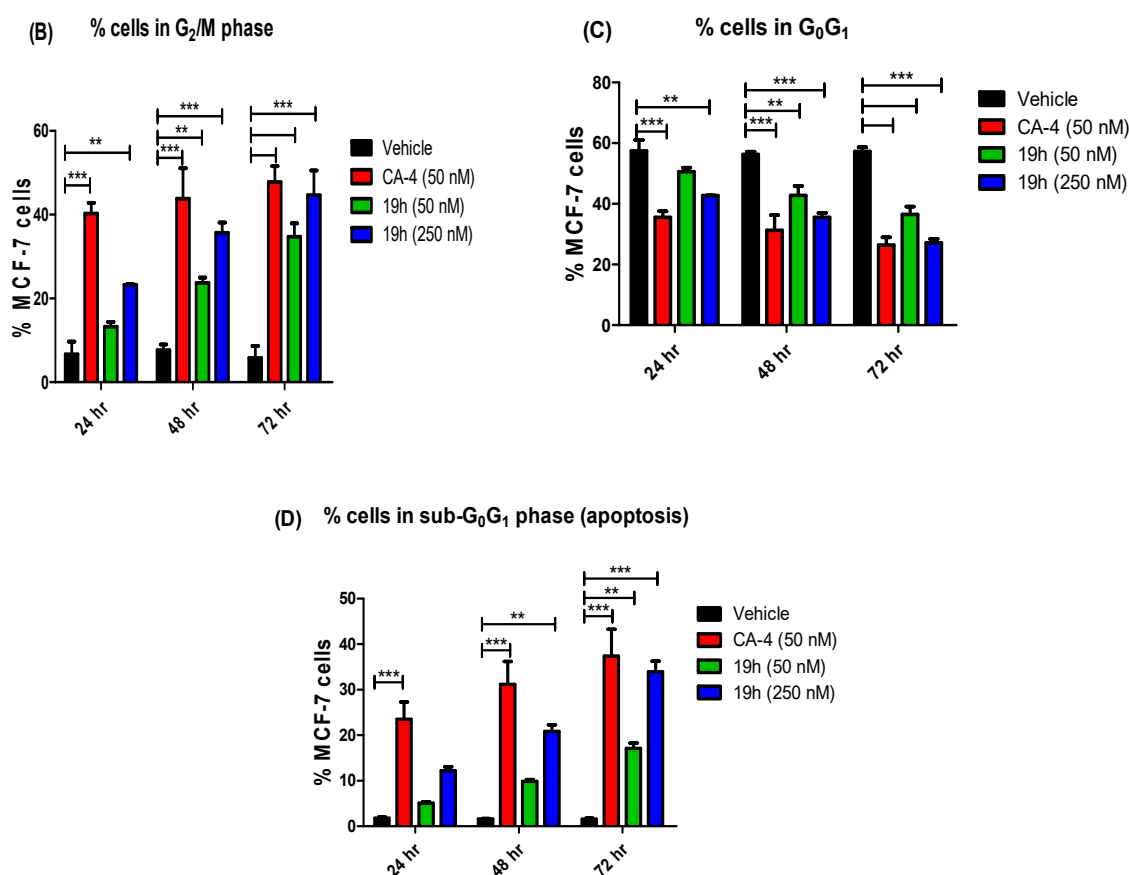


Figure 4. Cont.

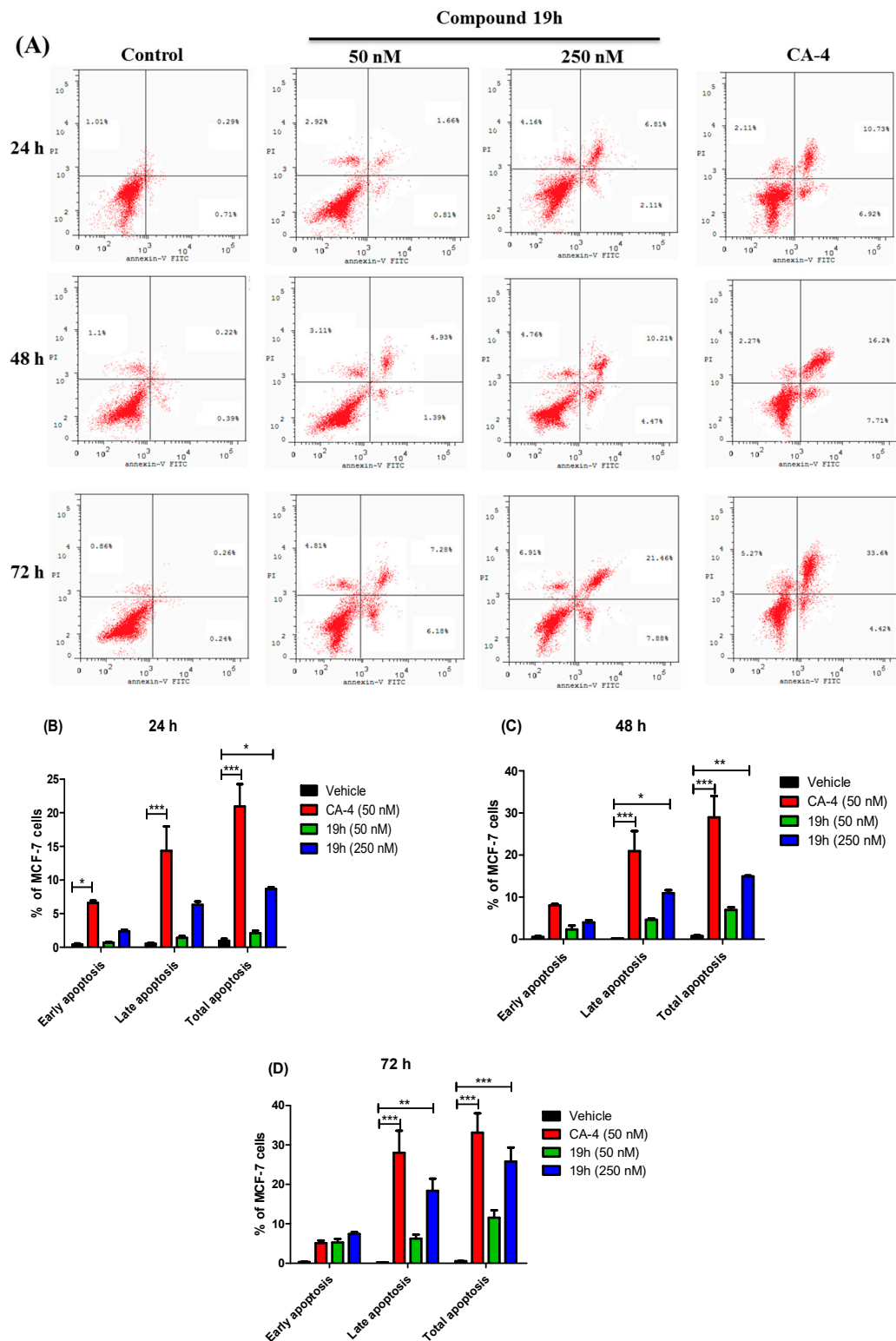




**Figure 4.** (A) Effect of compound **19h** on the cell cycle distribution of MCF-7 cells. MCF-7 cells were treated with either vehicle [0.1% ethanol (*v/v*)], CA-4 (50 nM), **19h** (50 nM and 250 nM) for 24 h, 48 h and 72 h. Cells were then fixed, stained with PI, and analyzed by flow cytometry with CellQuest software. Cell cycle analysis was performed on histograms of gated counts per DNA area (FL2-A). The number of cells with (B) 4N (G<sub>2</sub>/M), (C) 2N(G<sub>0</sub>G<sub>1</sub>), and (D) <2N (sub-G<sub>1</sub>). All values represent the mean  $\pm$  SEM for three independent experiments. Statistical analysis was performed using two-way ANOVA (\*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ ).

#### 2.2.4. Apoptosis Quantification in MCF-7 Cells

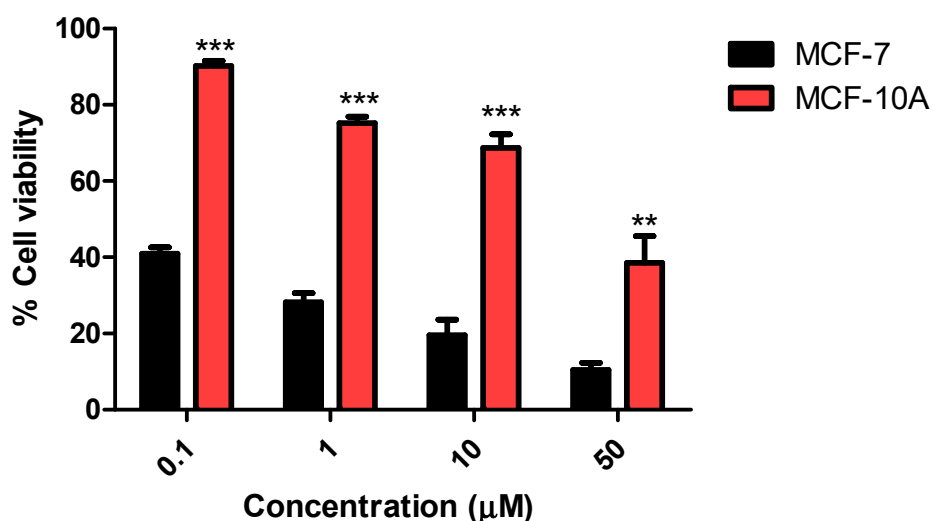
Numerous studies demonstrate that tubulin polymerization inhibitors are capable of inducing cellular apoptosis [35,36]. To evaluate the mode of cell death induced by **19h**, Annexin-V/PI assay was used. MCF-7 cells were treated with two different concentrations (50 and 250 nM) of **19h** at different time points (24, 48, 72 h). **19h** caused significant accumulation of annexin-V positive cells, inducing both early and late apoptosis in a concentration and time dependent manner as compared to the untreated control cells. As shown in Figure 5A, when the cells were treated with **19h** at 50 and 250 nM and CA-4 (50 nM), at the 48 h time point the average proportion of Annexin V-staining positive cells (total apoptotic cells) significantly increased from 0.8 % in control cells to 7%, 15% and 29%, respectively. The percentage of early and late apoptotic cells together for **19h** increased significantly after 72 h to 12% and 26% at 50 and 250 nM, respectively when compared to the control cells (1.8%). As supported from cell cycle arrest and apoptosis findings above (Figure 5B–D), these results suggested that compound **19h** could efficiently induce apoptosis of MCF-7 cells in a dose and time dependent manner.



**Figure 5.** (A) Flow cytometric analysis of apoptotic cells after treatment of MCF-7 cells with **19h** at different time points after double staining of the cells with Annexin-V-FITC and PI. MCF-7 cells treated with 50 and 250 nM of compound **19h** and 50 nM of CA-4 for 24 h, 48 h and 72 h. Quantitative analysis of apoptosis for (B) 24 h, (C) 48 h, (D) 72 h. All values represent the mean  $\pm$  SEM for three independent experiments. Statistical analysis was performed using two-way ANOVA (\*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ ).

### 2.2.5. Antiproliferative Activity in Non-Cancer Cells

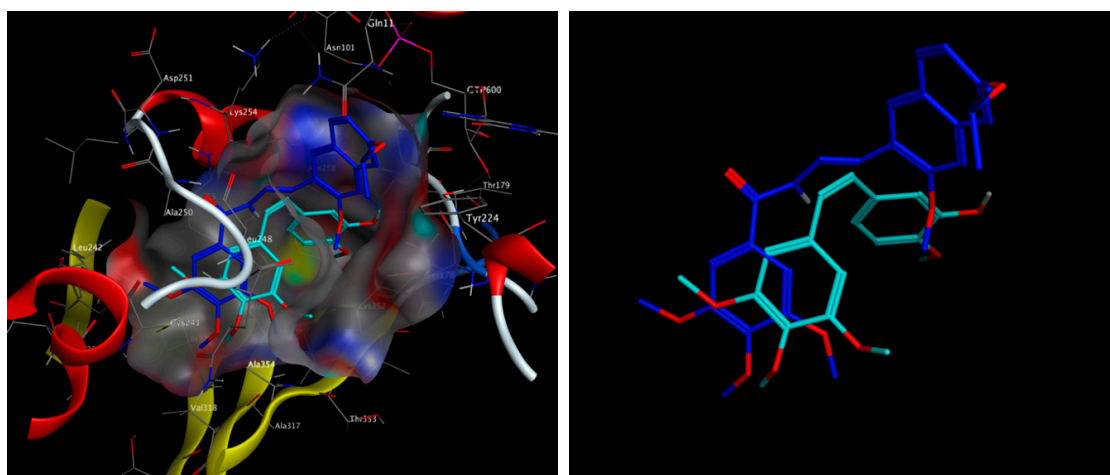
The non-tumorigenic cell line MCF-10A (normal epithelial breast) was selected to investigate the toxicity and selectivity of quinoline derivative **19h** towards cancer cells. As demonstrated in Figure 6, the IC<sub>50</sub> value of **19h** was more than 35  $\mu$ M in MCF-10A cells which was significantly higher than that observed in cancer cell lines including MCF-7, HL-60, HCT-116 and HeLa (IC<sub>50</sub> = 0.040, 0.026, 0.022 and 0.038  $\mu$ M, respectively) (Figure 6). These results demonstrated that **19h** has better cell selectivity towards breast carcinogenic human cells with less toxicity to normal non-cancerous human breast cells.



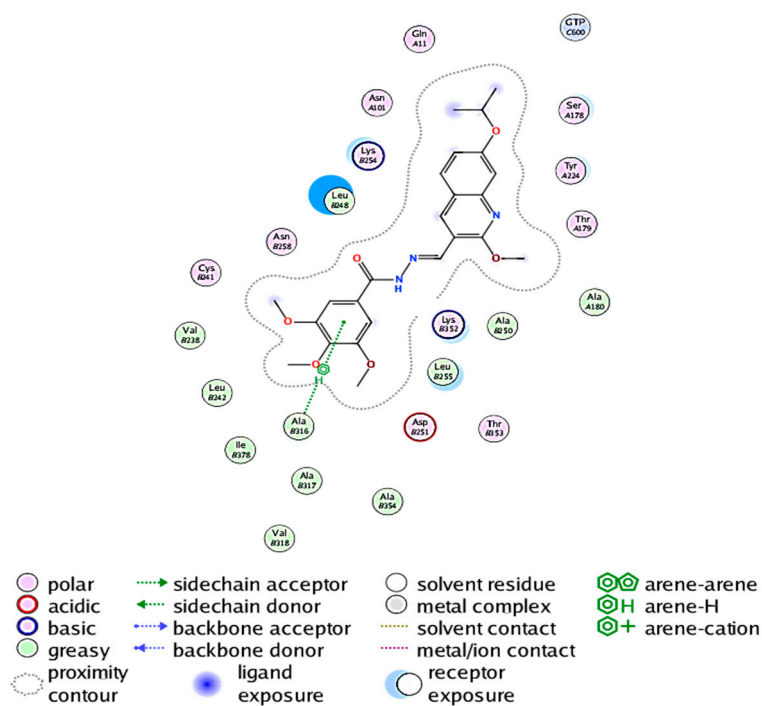
**Figure 6.** Dose response curve for compound **19h** on the proliferation of breast cancer MCF-7 and normal breast MCF-10A cells. Cells were treated at the indicated concentrations for 72 h. Cell viability was expressed as percentage of vehicle control [ethanol 1% (v/v)] treated cells and was measured by MTT assay (average of three independent experiments). Statistical analysis was performed using one-way ANOVA-Bonferroni post-hoc test (\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ ).

### 2.2.6. Molecular Modelling for Quinoline Analogues in the Colchicine Binding Site of Tubulin

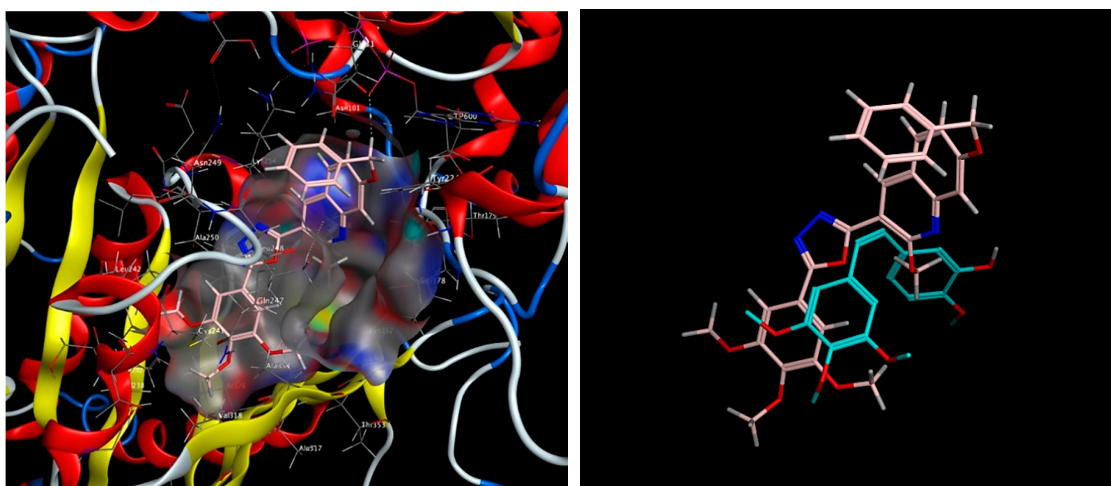
Five of the quinolines with the best in vitro biochemical activity were studied in silico to predict the binding modes in the colchicine-binding site using the 1SAO co-crystal structure of tubulin in complex with DAMA-colchicine [37]. Quinolines **19h** and **20j** were the most potent, followed by **18b**, **20c** and **20g**. Quinoline **18h** aligns well with CA-4 at the colchicine-binding site (Figure 7) and maintains interactions with the most important amino acid residue interactions noted for CA-4, including Cys $\beta$ 241 and Val $\beta$ 318 interactions with the trimethoxyphenyl moiety (Figure 8). Its scoring function is high at  $-9.5738$  indicating a favourable binding orientation. Quinoline **20j** binds in a similar orientation (Figure 9), maintaining key interactions with the colchicine-binding site (Figure 10). Molecular docking for **19b** and **20g** indicates similar docked orientations (Figure S41A-C, Supporting Information). The highest scoring function for **19b** is  $-8.772$  and for **20g** is  $-9.341$ . As **20g** is not the most potent of the five docked quinoline analogues, scoring function cannot be taken as a surrogate of potency at the colchicine-binding site as it does not consider biological parameters and metabolism. However, it may be taken as an estimate of the predicted affinity for a particular receptor site. Quinoline **20c** appears to bind in a reverse orientation at the colchicine-binding site (Figures S41B and S44). Only the seventh-ranked scoring orientation indicated overlap of the trimethoxyphenyl rings of CA-4 and DAMA-colchicine for this quinoline.



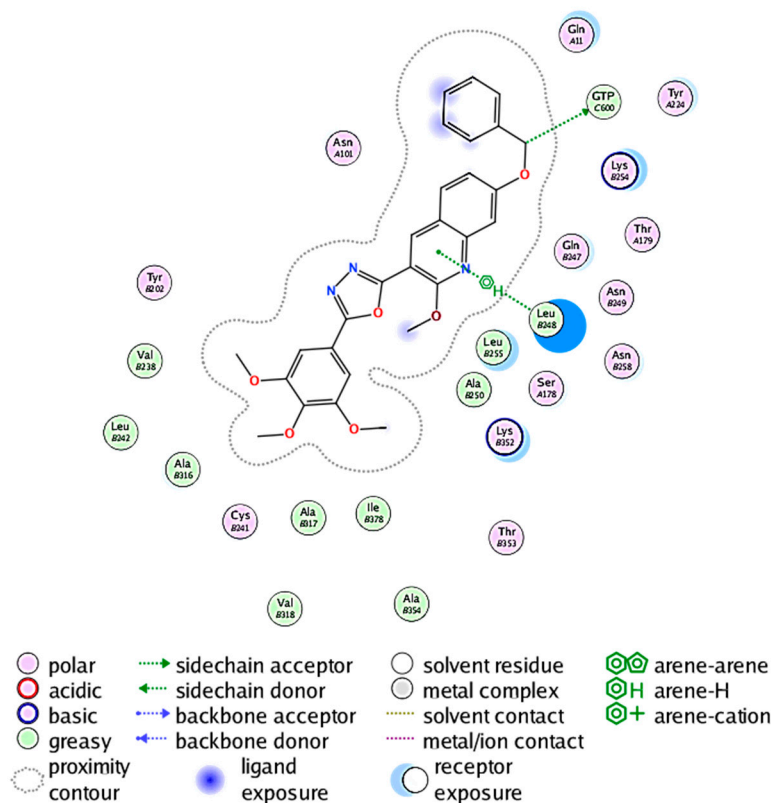
**Figure 7.** Quinoline **19h** (blue) and CA-4 (cyan/light blue) docked at the colchicine-binding site of tubulin in co-crystal 1SAO. Protein residues removed on right for clarity. (red = oxygen, grey = hydrogen).



**Figure 8.** 2D Ligand Interaction Schematic for **19h** as generated by MOE.



**Figure 9.** Quinoline 20j (pink) and CA-4 (cyan/light blue) docked at the colchicine-binding site of tubulin in co-crystal 1SAO. Protein residues removed on right for clarity. (red = oxygen, grey = hydrogen).



**Figure 10.** 2D Ligand Interaction Schematic for 20j as generated by MOE.

### 3. Materials and Methods

#### 3.1. General Information

Melting points were determined with a Gallenkamp melting point apparatus (London, UK) and are uncorrected. IR spectra ( $\text{KBr}$ ,  $\text{cm}^{-1}$ ) were recorded on Vector 22FT-IR Fourier transform infrared (FTIR) spectrometer (Bruker, Ettlingen, Germany). Unless otherwise specified, proton ( $^1\text{H}$ ) and carbon ( $^{13}\text{C}$ ) NMR spectra were recorded at room temperature in base filtered  $\text{CDCl}_3$  on a spectrometer operating at 400 or 300 MHz for protons and 100 or 75 MHz for carbon nuclei. The signal due to residual  $\text{CHCl}_3$  appearing at  $\delta$  H 7.26 and  $(\text{CH}_3)_2\text{SO}$  appearing at  $\delta$  H 2.5 and the central resonance of the  $\text{CDCl}_3$

triplet appearing at  $\delta$  C 77.0 and for the  $(\text{CD}_3)_2\text{SO}$  multiplet appearing at  $\delta$  C 39.0 were used to reference the  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectra, respectively.  $^1\text{H}$ -NMR data are described as follows: chemical shift ( $\delta$ ) [multiplicity, coupling constant(s)  $J$  (Hz), relative integral] where multiplicity is defined as s = singlet; d = doublet; t = triplet; q = quartet; and m = multiplet or combinations of the above. Elemental analyses were determined using a Manual Elemental Analyzer (Heraeus, Hanau, Germany) or an Automatic Elemental Analyzer CHN Model 2400 (Perkin Elmer, Waltham, MA, USA) located at the Microanalytical Center, Faculty of Science, Cairo University (Cairo, Egypt). All the elemental analyses results corresponded to the calculated values within experimental error. Progress of the reaction was monitored by thin-layer chromatography (TLC) using TLC sheets precoated with ultraviolet (UV) fluorescent silica gel (60F254, Merck, Cairo, Egypt) and spots were visualized by iodine vapours or irradiation with UV light (254 nm). All the chemicals were purchased from Sigma-Aldrich (Cairo, Egypt) or Lancaster Synthesis Corporation (London, UK). Intermediates **12–15a–j** [38–40] and **17** [41–43], **18** [44,45] were prepared as previously reported.

### 3.2. Chemistry

#### 3.2.1. General Procedure for Preparation of Quinoline Analogues **19a–i**

An equimolar amount of 2-methoxy-3-formylquinoline derivatives **15a–j** (1 mmol) and 3,4,5-trimethoxybenzohydrazide (**18**, 0.23 g, 1 mmol) in dioxane (5 mL) was stirred at 80 °C for 9 h. The reaction mixture was monitored by TLC. After reaction completion the mixture was cooled, concentrated under reduced pressure on a rotary evaporator, the residue was washed with petroleum ether and then recrystallized from ethanol.

##### N'-[(2-Methoxyquinolin-3-yl)methylene]-3,4,5-trimethoxybenzohydrazide (**19a**)

Yellowish white solid, Yield (85%); m.p. 191–193 °C. IR (KBr)  $\nu$  = 3222 (NH), 1640 (C=O), 1621 (C=N), 1607, 1584 (C=C)  $\text{cm}^{-1}$ .  $^1\text{H}$ -NMR (300 MHz,  $\text{DMSO}-d_6$ )  $\delta$ : 11.87 (s, 1H, exch., NH), 8.81 (s, 1H, Ar-H), 8.74 (s, 1H, N=CH), 8.03–7.47 (m, 4H, Ar-H), 7.29 (s, 2H, Ar-H), 4.10 (s, 3H,  $\text{OCH}_3$ ), 3.88 (s, 6H,  $2\text{OCH}_3$ ), 3.74 (s, 3H,  $\text{OCH}_3$ ) ppm.  $^{13}\text{C}$ -NMR (75 MHz,  $\text{DMSO}-d_6$ )  $\delta$ : 159.3, 152.6, 146.1, 134.3, 130.5, 128.6, 128.1, 126.4, 124.7, 120.8, 118.5, 107.1, 105.4, 60.0, 56.1, 53.5 ppm. MS (70 eV):  $m/z$  (%): 395 (8.90)  $[\text{M}^+]$ ; Anal. Calcd for  $\text{C}_{21}\text{H}_{21}\text{N}_3\text{O}_5$ : C, 63.79; H, 5.35; N, 10.63. Found: C, 63.75; H, 5.30; N, 10.70.

##### N'-[(2-Methoxy-6-methylquinolin-3-yl)methylene]-3,4,5-trimethoxybenzohydrazide (**19b**)

Yellowish white solid, Yield (89%); m.p. 199–201 °C.  $^1\text{H}$ -NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$ : 11.87 (s, 1H, exch., NH), 8.77 (s, 1H, Ar-H), 8.59 (s, 1H, N=CH), 7.77 (s, 1H, Ar-H), 7.66 (d,  $J$  = 8.0 Hz 1H, Ar-H), 7.50 (d,  $J$  = 8.0 Hz, 1H, Ar-H), 7.26 (s, 2H, Ar-H), 4.03 (s, 3H,  $\text{OCH}_3$ ), 3.85 (s, 6H,  $2\text{OCH}_3$ ), 3.71 (s, 3H,  $\text{OCH}_3$ ), 2.42 (s, 3H,  $\text{CH}_3$ ) ppm.  $^{13}\text{C}$ -NMR (75 MHz,  $\text{DMSO}-d_6$ )  $\delta$ : 162.8, 159.3, 153.2, 145.0, 142.21, 141.0, 134.4, 134.2, 133.1, 128.6, 128.0, 126.8, 125.4, 118.9, 109.9, 105.7, 60.6, 56.5, 54.0, 21.3 ppm. Anal. MS (70 eV):  $m/z$  (%): 409 (6.75)  $[\text{M}^+]$ ; Calcd for  $\text{C}_{22}\text{H}_{23}\text{N}_3\text{O}_5$ : C, 64.54; H, 5.66; N, 10.26. Found: C, 64.51; H, 5.61; N, 10.30.

##### N'-[(2-Methoxy-7-methylquinolin-3-yl)methylene]-3,4,5-trimethoxybenzohydrazide (**19c**)

Yellowish white solid, Yield (83%); m.p. 210–212 °C.  $^1\text{H}$ -NMR (300 MHz,  $\text{DMSO}-d_6$ )  $\delta$ : 11.83 (s, 1H, exch., NH), 8.79 (s, 1H, Ar-H), 8.67 (s, 1H, N=CH), 7.92 (d,  $J$  = 9.0 Hz, 1H, Ar-H), 7.60 (s, 1H, Ar-H), 7.32 (d,  $J$  = 6.0 Hz, 1H, Ar-H), 7.28 (s, 2H, Ar-H), 4.07 (s, 3H,  $\text{OCH}_3$ ), 3.88 (s, 6H,  $2\text{OCH}_3$ ), 3.75 (s, 3H,  $\text{OCH}_3$ ), 2.49 (s, 3H,  $\text{CH}_3$ ) ppm.  $^{13}\text{C}$ -NMR (75 MHz,  $\text{DMSO}-d_6$ )  $\delta$ : 159.4, 152.6, 146.3, 140.8, 134.1, 128.3, 128.2, 126.7, 125.8, 122.6, 120.9, 117.6, 107.9, 105.0, 60.0, 56.1, 53.4, 21.3 ppm. MS (70 eV):  $m/z$  (%): 409 (10.80)  $[\text{M}^+]$ ; Anal. Calcd for  $\text{C}_{22}\text{H}_{23}\text{N}_3\text{O}_5$ : C, 64.54; H, 5.66; N, 10.26. Found: C, 64.49; H, 5.63; N, 10.31.



N'-[(2-Methoxy-8-methylquinolin-3-yl)methylene]-3,4,5-trimethoxybenzohydrazide (**19d**)

Yellowish white solid, Yield (86%); m.p. 216–218 °C. <sup>1</sup>H-NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ: 11.89 (s, 1H, exch., NH), 8.82 (s, 1H, Ar-H), 8.70 (s, 1H, N=CH), 7.87 (d, *J* = 9 Hz, 1H, Ar-H), 7.57 (d, *J* = 9.0 Hz, 1H, Ar-H), 7.36–7.29 (m, 3H, Ar-H), 4.10 (s, 3H, OCH<sub>3</sub>), 3.88 (s, 6H, 2OCH<sub>3</sub>), 3.75 (s, 3H, OCH<sub>3</sub>), 2.64 (s, 3H, CH<sub>3</sub>) ppm. <sup>13</sup>C-NMR (75 MHz, DMSO-*d*<sub>6</sub>) δ: 158.4, 152.7, 144.9, 141.7, 134.6, 134.1, 130.7, 128.2, 126.5, 124.5, 124.4, 118.1, 105.3, 60.1, 56.1, 53.3, 17.2 ppm. MS (70 eV): *m/z* (%): 409 (11.30) [M<sup>+</sup>]; Anal. Calcd for C<sub>22</sub>H<sub>23</sub>N<sub>3</sub>O<sub>5</sub>: C, 64.54; H, 5.66; N, 10.26. Found: C, 64.48; H, 5.60; N, 10.33.

N'-[(2,6-Dimethoxyquinolin-3-yl)methylene]-3,4,5-trimethoxybenzohydrazide (**19e**)

Yellowish white solid, Yield (85%); m.p. 225–227 °C. <sup>1</sup>H-NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ: 11.93 (s, 1H, exch., NH), 8.81 (s, 1H, Ar-H), 8.70 (s, 1H, N=CH), 7.71 (d, *J* = 9.0 Hz, 1H, Ar-H), 7.54 (s, 1H, Ar-H), 7.37–7.31 (m, 3H, Ar-H), 4.06 (s, 3H, OCH<sub>3</sub>), 3.89 (s, 6H, 2OCH<sub>3</sub>), 3.87 (s, 3H, OCH<sub>3</sub>), 3.75 (s, 3H, OCH<sub>3</sub>) ppm. <sup>13</sup>C-NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ: 162.9, 158.5, 156.4, 153.2, 142.2, 141.0, 133.9, 128.7, 128.3, 125.9, 122.9, 118.8, 107.5, 105.7, 60.6, 56.6, 56.0, 54.0 ppm. MS (70 eV): *m/z* (%): 425 (5.85) [M<sup>+</sup>]; Anal. Calcd for C<sub>22</sub>H<sub>23</sub>N<sub>3</sub>O<sub>6</sub>: C, 62.11; H, 5.45; N, 9.88. Found: C, 62.08; H, 5.41; N, 9.94.

N'-[(2,7-Dimethoxyquinolin-3-yl)methylene]-3,4,5-trimethoxybenzohydrazide (**19f**)

Yellowish white solid, Yield (87%); m.p. 194–196 °C. <sup>1</sup>H-NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ: 11.82 (s, 1H, exch., NH), 8.78 (s, 1H, Ar-H), 8.67 (s, 1H, N=CH), 7.96 (d, *J* = 9.0 Hz, 1H, Ar-H), 7.28–7.07 (m, 4H, Ar-H), 4.08 (s, 3H, OCH<sub>3</sub>), 3.92 (s, 6H, 2OCH<sub>3</sub>), 3.88 (s, 3H, OCH<sub>3</sub>), 3.74 (s, 3H, OCH<sub>3</sub>) ppm. <sup>13</sup>C-NMR (75 MHz, DMSO-*d*<sub>6</sub>) δ: 161.5, 159.8, 152.7, 148.3, 142.0, 134.2, 129.9, 128.3, 120.7, 119.6, 116.8, 115.7, 106.2, 105.3, 60.1, 56.1, 55.5, 53.5 ppm. MS (70 eV): *m/z* (%): 425 (10.85) [M<sup>+</sup>]; Anal. Calcd for C<sub>22</sub>H<sub>23</sub>N<sub>3</sub>O<sub>6</sub>: C, 62.11; H, 5.45; N, 9.88. Found: C, 62.02; H, 5.39; N, 9.91.

N'-[(6-Isopropoxy-2-methoxyquinolin-3-yl)methylene]-3,4,5-trimethoxybenzohydrazide (**19g**)

Yellowish white solid, Yield (78%); m.p. 181–193 °C. <sup>1</sup>H-NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ: 11.78 (s, 1H, exch., NH), 8.78 (s, 1H, Ar-H), 8.63 (s, 1H, N=CH), 7.92 (d, *J* = 9.0 Hz, 1H, Ar-H), 7.28 (s, 2H, Ar-H), 7.17 (s, 1H, Ar-H), 7.05 (d, *J* = 9.0 Hz, 1H, Ar-H), 4.85–4.81 (m, 1H, OCH-), 4.07 (s, 3H, OCH<sub>3</sub>), 3.87 (s, 6H, 2OCH<sub>3</sub>), 3.74 (s, 3H, OCH<sub>3</sub>), 1.34 (d, *J* = 6.0 Hz, 6H, 2CH<sub>3</sub>) ppm. <sup>13</sup>C-NMR (75 MHz, DMSO-*d*<sub>6</sub>) δ: 154.1, 152.6, 141.37, 133.4, 128.1, 127.8, 125.5, 123.2, 120.6, 120.5, 118.4, 112.2, 109.4, 105.4, 69.6, 60.0, 56.1, 53.3, 21.7 ppm. MS (70 eV): *m/z* (%): 453 (11.80) [M<sup>+</sup>]; Anal. Calcd for C<sub>24</sub>H<sub>27</sub>N<sub>3</sub>O<sub>6</sub>: C, 63.56; H, 6.00; N, 9.27. Found: C, 63.51; H, 5.96; N, 9.31.

N'-[(7-Isopropoxy-2-methoxyquinolin-3-yl)methylene]-3,4,5-trimethoxybenzohydrazide (**19h**)

Yellowish white solid, Yield (86%); m.p. 203–205 °C. <sup>1</sup>H-NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ: 11.79 (s, 1H, exch., NH), 8.78 (s, 1H, Ar-H), 8.64 (s, 1H, N=CH), 7.92 (d, *J* = 9.0 Hz, 1H, Ar-H), 7.29–7.04 (m, 4H, Ar-H), 4.85–4.71 (m, 1H, OCH-), 4.08 (s, 3H, OCH<sub>3</sub>), 3.88 (s, 6H, 2OCH<sub>3</sub>), 3.75 (s, 3H, OCH<sub>3</sub>), 1.33 (d, *J* = 6.0 Hz, 6H, 2CH<sub>3</sub>) ppm. <sup>13</sup>C-NMR (75 MHz, DMSO-*d*<sub>6</sub>) δ: 159.8, 159.7, 152.7, 148.2, 142.1, 134.1, 129.1, 128.3, 120.6, 120.6, 119.4, 117.6, 115.7, 107.7, 105.4, 69.7, 60.1, 56.2, 53.5, 21.7 ppm. MS (70 eV): *m/z* (%): 453 (9.30) [M<sup>+</sup>]; Anal. Calcd for C<sub>24</sub>H<sub>27</sub>N<sub>3</sub>O<sub>6</sub>: C, 63.56; H, 6.00; N, 9.27. Found: C, 63.52; H, 5.94; N, 9.33.

N'-[(6-(Benzyloxy)-2-methoxyquinolin-3-yl)methylene]-3,4,5-trimethoxybenzohydrazide (**19i**)

Yellowish white solid, Yield (82%); m.p. 187–189 °C. IR (KBr) ν: 3212 (NH), 1644 (C=O), 1612 (C=N), 1581, 1539 (C=C) cm<sup>-1</sup>. <sup>1</sup>H-NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ: 11.92 (s, 1H, exch., NH), 8.81 (s, 1H, Ar-H), 8.68 (s, 1H, N=CH), 7.75–7.67 (m, 2H, Ar-H), 7.54 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.44–7.34 (m, 4H, Ar-H), 7.30 (s, 2H, Ar-H), 5.22 (s, 2H, OCH<sub>2</sub>-), 4.07 (s, 3H, OCH<sub>3</sub>), 3.89 (s, 6H, 2OCH<sub>3</sub>), 3.75 (s, 3H, OCH<sub>3</sub>) ppm. <sup>13</sup>C-NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ: 162.9, 160.9, 158.6, 155.5, 153.2, 142.2, 141.0, 137.3, 133.9, 129.0, 128.8, 128.7, 128.4, 126.0, 123.1, 119.1, 109.2, 107.3, 105.8, 70.1, 60.6, 56.6, 54.0 ppm.

MS (70 eV):  $m/z$  (%): 501 (13.50) [ $M^+$ ]; Anal. Calcd for  $C_{28}H_{27}N_3O_6$ : C, 67.06; H, 5.43; N, 8.38. Found: C, 67.01; H, 5.40; N, 8.41.

**N'-((7-(Benzyloxy)-2-methoxyquinolin-3-yl)methylene)-3,4,5-trimethoxybenzohydrazide (19j)**

Yellowish white solid, Yield (83%); m.p. 221–223 °C.  $^1H$ -NMR (300 MHz, DMSO- $d_6$ )  $\delta$ : 11.81 (s, 1H, exch., NH), 8.79 (s, 1H, Ar-H), 8.65 (s, 1H, N=CH), 7.87 (d,  $J$  = 9.0 Hz 1H, Ar-H), 7.49–7.19 (m, 9H, Ar-H), 5.29 (s, 2H, OCH<sub>2</sub>-), 4.07 (s, 3H, OCH<sub>3</sub>), 3.88 (s, 6H, 2OCH<sub>3</sub>), 3.74 (s, 3H, OCH<sub>3</sub>) ppm.  $^{13}C$ -NMR (75 MHz, DMSO- $d_6$ )  $\delta$ : 160.5, 159.8, 152.7, 148.1, 136.6, 134.1, 129.9, 128.4, 128.2, 127.8, 127.6, 119.7, 117.1, 115.9, 107.4, 105.4, 69.5, 60.1, 56.1, 53.5 ppm. MS (70 eV):  $m/z$  (%): 501 (9.50) [ $M^+$ ]; Anal. Calcd for  $C_{28}H_{27}N_3O_6$ : C, 67.06; H, 5.43; N, 8.38. Found: C, 67.03; H, 5.39; N, 8.42.

**3.2.2. General Procedure for Preparation of Quinoline Analogues 20a–i**

To solution of DMSO (5 mL) containing potassium carbonate (0.42 g, 3 mmol) and iodine (0.30 g, 1.2 mmol), the appropriate hydrazine **19a–j** (1 mmol) was added to the reaction mixture while stirring at 100 °C. The reaction mixture was monitored by TLC and after 4 h the reaction was cooled down to room temperature then treated with 5%  $Na_2S_2O_3$  (20 mL). The products were obtained by filtration, dried and recrystallized from ethanol.

**2-(2-Methoxyquinolin-3-yl)-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazole (20a)**

Yellowish white solid, Yield (89%); m.p. 221–223 °C. 1619 (C=N), 1603 (C=C)  $cm^{-1}$ .  $^1H$ -NMR (400 MHz, DMSO- $d_6$ )  $\delta$ : 9.11 (s, 1H, Ar-H), 8.13 (s, 1H, Ar-H), 7.86 (d,  $J$  = 13.0 Hz, 2H, Ar-H), 7.57 (s, 1H, Ar-H), 7.40 (s, 2H, Ar-H), 4.16 (s, 3H, OCH<sub>3</sub>), 3.94 (s, 6H, 2OCH<sub>3</sub>), 3.79 (s, 3H, OCH<sub>3</sub>) ppm.  $^{13}C$ -NMR (100 MHz, DMSO- $d_6$ )  $\delta$ : 187.4, 165.0, 164.2, 161.3, 157.9, 153.5, 146.7, 141.0, 132.2, 128.9, 126.7, 125.2, 123.9, 118.4, 108.7, 104.2, 60.3, 56.3, 54.1 ppm. MS (70 eV):  $m/z$  (%): 393 (13.50) [ $M^+$ ]; Anal. Calcd for  $C_{21}H_{19}N_3O_5$ : C, 64.12; H, 4.87; N, 10.68. Found: C, 64.03; H, 4.89; N, 10.74.

**2-(2-Methoxy-6-methylquinolin-3-yl)-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazole (20b)**

Yellowish white solid, Yield (88%); m.p. 228–230 °C.  $^1H$ -NMR (400 MHz, DMSO- $d_6$ )  $\delta$ : 8.92 (s, 1H, Ar-H), 7.82 (s, 1H, Ar-H), 7.75 (d,  $J$  = 8.0 Hz, 1H, Ar-H), 7.63 (d,  $J$  = 8.0 Hz, 1H, Ar-H), 7.35 (s, 2H, Ar-H), 4.11 (s, 3H, OCH<sub>3</sub>), 3.92 (s, 6H, 2OCH<sub>3</sub>), 3.78 (s, 3H, OCH<sub>3</sub>), 2.48 (s, 3H, CH<sub>3</sub>) ppm.  $^{13}C$ -NMR (100 MHz, DMSO- $d_6$ )  $\delta$ : 164.6, 161.8, 158.1, 154.1, 145.7, 141.4, 140.8, 135.2, 134.6, 128.1, 126.8, 124.0, 118.8, 115.4, 109.2, 104.7, 60.8, 56.7, 54.5, 21.4 ppm. MS (70 eV):  $m/z$  (%): 407 (9.30) [ $M^+$ ]; Anal. Calcd for  $C_{22}H_{21}N_3O_5$ : C, 64.86; H, 5.20; N, 10.31. Found: C, 64.77; H, 5.11; N, 10.39.

**2-(2-Methoxy-7-methylquinolin-3-yl)-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazole (20c)**

Yellowish white solid, Yield (84%); m.p. 240–242 °C.  $^1H$ -NMR (300 MHz, DMSO- $d_6$ )  $\delta$ : 8.91 (s, 1H, Ar-H), 7.92 (d,  $J$  = 9.0 Hz, 1H, Ar-H), 7.61 (s, 1H, Ar-H), 7.33 (s, 3H, Ar-H), 4.11 (s, 3H, OCH<sub>3</sub>), 3.81 (s, 6H, 2OCH<sub>3</sub>), 3.79 (s, 3H, OCH<sub>3</sub>), 2.49 (s, 3H, CH<sub>3</sub>) ppm.  $^{13}C$ -NMR (75 MHz, DMSO- $d_6$ )  $\delta$ : 163.9, 161.3, 157.8, 153.4, 146.8, 142.4, 140.8, 140.2, 128.3, 127.0, 125.8, 121.7, 120.7, 118.3, 107.5, 107.0, 104.2, 60.2, 56.2, 53.8, 21.4 ppm. MS (70 eV):  $m/z$  (%): 407 (15.50) [ $M^+$ ]; Anal. Calcd for  $C_{22}H_{21}N_3O_5$ : C, 64.86; H, 5.20; N, 10.31. Found: C, 64.81; H, 5.14; N, 10.34.

**2-(2-Methoxy-8-methylquinolin-3-yl)-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazole (20d)**

Yellowish white solid, Yield (86%); m.p. 246–248 °C.  $^1H$ -NMR (400 MHz, DMSO- $d_6$ )  $\delta$ : 9.04 (s, 1H, Ar-H), 7.94 (s, 1H, Ar-H), 7.69 (s, 1H, Ar-H), 7.45–7.38 (m, 3H, Ar-H), 4.17 (s, 3H, OCH<sub>3</sub>), 3.94 (s, 6H, 2OCH<sub>3</sub>), 3.79 (s, 3H, OCH<sub>3</sub>), 2.68 (s, 3H, CH<sub>3</sub>) ppm.  $^{13}C$ -NMR (100 MHz, DMSO- $d_6$ )  $\delta$ : 168.4, 161.5, 157.0, 154.2, 153.5, 145.3, 141.3, 140.9, 134.5, 132.3, 126.7, 125.2, 118.4, 108.2, 104.2, 60.3, 56.3, 54.1, 17.2 ppm. MS (70 eV):  $m/z$  (%): 407 (17.20) [ $M^+$ ]; Anal. Calcd for  $C_{22}H_{21}N_3O_5$ : C, 64.86; H, 5.20; N, 10.31. Found: C, 64.77; H, 5.15; N, 10.32.

2-(2,6-Dimethoxyquinolin-3-yl)-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazole (**20e**)

Yellowish white solid, Yield (83%); m.p. 227–229 °C. <sup>1</sup>H-NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ: 9.01 (s, 1H, Ar-H), 7.81 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.56 (s, 1H, Ar-H), 7.50–7.47 (m, 1H, Ar-H), 7.40 (s, 2H, Ar-H), 4.12 (s, 3H, OCH<sub>3</sub>), 3.94 (s, 6H, 2OCH<sub>3</sub>), 3.91 (s, 3H, OCH<sub>3</sub>), 3.79 (s, 3H, OCH<sub>3</sub>) ppm. <sup>13</sup>C-NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ: 172.3, 164.6, 156.8, 154.4, 141.0, 140.2, 128.5, 124.3, 120.3, 119.1, 108.0, 104.8, 61.1, 56.9, 56.2, 54.3 ppm. MS (70 eV): *m/z* (%): 423 (10.10) [M<sup>+</sup>]; Anal. Calcd for C<sub>22</sub>H<sub>21</sub>N<sub>3</sub>O<sub>6</sub>: C, 62.41; H, 5.00; N, 9.92. Found: C, 64.33; H, 4.94; N, 9.99.

2-(2,7-Dimethoxyquinolin-3-yl)-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazole (**20f**)

Yellowish crystalline solid, Yield (87%); m.p. 225–227 °C. <sup>1</sup>H-NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ: 8.96 (s, 1H, Ar-H), 7.99 (d, *J* = 8.9 Hz, 1H, Ar-H), 7.36 (s, 2H, Ar-H), 7.25 (s, 1H, Ar-H), 7.19–7.16 (m, 1H, Ar-H), 4.13 (s, 3H, OCH<sub>3</sub>), 3.94 (s, 6H, 2OCH<sub>3</sub>), 3.92 (s, 3H, OCH<sub>3</sub>), 3.77 (s, 3H, OCH<sub>3</sub>) ppm. <sup>13</sup>C-NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ: 163.9, 162.6, 161.5, 158.3, 153.3, 149.0, 140.7, 140.3, 129.9, 118.7, 118.2, 117.4, 106.3, 105.7, 104.1, 60.1, 56.3, 55.7, 54.1 ppm. MS (70 eV): *m/z* (%): 423 (18.50) [M<sup>+</sup>]; Anal. Calcd for C<sub>22</sub>H<sub>21</sub>N<sub>3</sub>O<sub>6</sub>: C, 62.41; H, 5.00; N, 9.92. Found: C, 62.35; H, 4.96; N, 10.01

2-(6-Isopropoxy-2-methoxyquinolin-3-yl)-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazole (**20g**)

Yellowish white solid, Yield (79%); m.p. 230–232 °C. <sup>1</sup>H-NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ: 8.98 (s, 1H, Ar-H), 7.78 (d, *J* = 9.1 Hz, 1H, Ar-H), 7.55 (d, *J* = 2.7 Hz, 1H, Ar-H), 7.44–7.39 (m, 3H, Ar-H), 4.76–4.73 (m, 1H, OCH<sub>2</sub>-), 4.11 (s, 3H, OCH<sub>3</sub>), 3.93 (s, 6H, 2OCH<sub>3</sub>), 3.78 (s, 3H, OCH<sub>3</sub>), 1.36 (d, *J* = 6.0 Hz, 6H, 2OCH<sub>3</sub>) ppm. <sup>13</sup>C-NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ: 164.3, 156.7, 154.4, 153.6, 141.9, 139.8, 138.1, 137.8, 128.1, 124.7, 124.5, 118.5, 109.3, 108.6, 104.2, 69.8, 60.3, 56.3, 53.9, 21.7 ppm. MS (70 eV): *m/z* (%): 451 (16.00) [M<sup>+</sup>]; Anal. Calcd for C<sub>24</sub>H<sub>25</sub>N<sub>3</sub>O<sub>6</sub>: C, 63.85; H, 5.58; N, 9.31. Found: C, 63.81; H, 5.52; N, 9.37.

2-(7-Isopropoxy-2-methoxyquinolin-3-yl)-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazole (**20h**)

Yellowish white solid, Yield (82%); m.p. 239–241 °C. <sup>1</sup>H-NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ: 8.96 (s, 1H, Ar-H), 7.99 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.38 (s, 2H, Ar-H), 7.23 (s, 1H, Ar-H), 7.14 (d, *J* = 8.8 Hz, 1H, Ar-H), 4.94–4.85 (m, 1H, OCH<sub>2</sub>-), 4.14 (s, 3H, OCH<sub>3</sub>), 3.93 (s, 6H, 2OCH<sub>3</sub>), 3.78 (s, 3H, OCH<sub>3</sub>), 1.37 (d, *J* = 5.9 Hz, 6H, 2CH<sub>3</sub>) ppm. <sup>13</sup>C-NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ: 164.2, 161.3, 156.6, 154.2, 153.5, 142.0, 140.9, 139.7, 128.1, 124.8, 122.4, 118.6, 109.2, 108.5, 104.3, 69.8, 60.3, 56.3, 53.9, 21.7 ppm. MS (70 eV): *m/z* (%): 451 (12.50) [M<sup>+</sup>]; Anal. Calcd for C<sub>24</sub>H<sub>25</sub>N<sub>3</sub>O<sub>6</sub>: C, 63.85; H, 5.58; N, 9.31. Found: C, 63.78; H, 5.51; N, 9.38.

2-[6-(Benzyloxy)-2-methoxyquinolin-3-yl]-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazole (**20i**)

Yellowish white solid, Yield (85%); m.p. 243–245 °C. <sup>1</sup>H-NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ: 8.99 (s, 1H, Ar-H), 7.83 (d, *J* = 9.0 Hz, 1H, Ar-H), 7.67 (s, 1H, Ar-H), 7.55–7.53 (m, 3H, Ar-H), 7.46–7.35 (m, 5H, Ar-H), 5.25 (s, 2H, OCH<sub>2</sub>-), 4.13 (s, 3H, OCH<sub>3</sub>), 3.94 (s, 6H, 2OCH<sub>3</sub>), 3.79 (s, 3H, OCH<sub>3</sub>) ppm. <sup>13</sup>C-NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ: 163.9, 161.6, 158.5, 153.5, 148.8, 140.7, 140.3, 136.6, 130.2, 128.5, 128.0, 127.8, 118.9, 118.5, 117.8, 107.2, 105.9, 104.1, 69.7, 60.4, 56.3, 54.0 ppm. MS (70 eV): *m/z* (%): 499 (13.40) [M<sup>+</sup>]; Anal. Calcd for C<sub>28</sub>H<sub>25</sub>N<sub>3</sub>O<sub>6</sub>: C, 67.33; H, 5.04; N, 8.41. Found: C, 67.39; H, 4.97; N, 8.47.

2-[7-(Benzyloxy)-2-methoxyquinolin-3-yl]-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazole (**20j**)

Yellowish white solid, Yield (70%); m.p. 255–257 °C. <sup>1</sup>H-NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ: 8.94 (s, 1H, Ar-H), 8.00 (d, *J* = 9.0 Hz, 1H, Ar-H), 7.51–7.23 (m, 9H, Ar-H), 5.31 (s, 2H, OCH<sub>2</sub>-), 4.13 (s, 3H, OCH<sub>3</sub>), 3.92 (s, 6H, 2OCH<sub>3</sub>), 3.78 (s, 3H, OCH<sub>3</sub>) ppm. <sup>13</sup>C-NMR (75 MHz, DMSO-*d*<sub>6</sub>) δ: 163.9, 161.6, 158.4, 153.5, 148.8, 140.2, 136.5, 130.1, 128.4, 127.9, 127.6, 120.6, 120.5, 118.8, 118.4, 117.5, 107.3, 105.8, 104.3, 69.7, 60.2, 56.2, 53.9 ppm. MS (70 eV): *m/z* (%): 499 (9.80) [M<sup>+</sup>]; Anal. Calcd for C<sub>28</sub>H<sub>25</sub>N<sub>3</sub>O<sub>6</sub>: C, 67.33; H, 5.04; N, 8.41. Found: C, 67.41; H, 4.98; N, 8.46.

### 3.3. Biochemical Evaluation of Activity

All biochemical assays were performed in triplicate on at least three independent occasions for the determination of mean values reported.

#### 3.3.1. Cell Culture

The four human tumour cell lines MCF-7, HCT-116, HL-60 and HeLa were obtained from the VACSERA (Giza, Egypt). All these cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) with 10% fetal bovine serum, 2 mM L-glutamine and 100 g/mL penicillin/streptomycin. Cells were maintained at 37 °C in 5% CO<sub>2</sub> in a humidified incubator. All cells were sub-cultured 3 times/week by trypsinization using TrypLE Express (1×).

#### 3.3.2. Cell Viability Assay

The quinoline compounds **19a–j** and **20a–j** were evaluated for antiproliferative effect using the MTT viability assay of four cancer cell lines (MCF-7, HCT-116, HL-60 and HeLa) and normal breast cells MCF-10A to calculate the relative IC<sub>50</sub> values for each compound. Cells were seeded in 96-well plates at a density of  $10 \times 10^3$  cells/mL in a total volume of 200 µL per well. 0.1% of DMSO was used as a vehicle control. After 24 h, the cells were treated with 2 µL test compound which had been pre-prepared as stock solutions to furnish the concentration range of study, 0.001 µM to 50 µM, and re-incubated for 72 h. The culture medium was then removed, and the cells washed with phosphate buffered saline (PBS) and 100 µL MTT was added (final concentration of 5 mg/mL MTT). Cells were incubated for 3 h in darkness at 37 °C. 200 µL DMSO was then added to each well and the cells maintained at room temperature in darkness for 30 min. Absorbance was detected with a microplate reader at 570 nm. Results were expressed as percentage viability relative to vehicle control (100%). Dose response curves were plotted and IC<sub>50</sub> values were obtained using Prism software (GraphPad Software, Inc., La Jolla, CA, USA). All the experiments were repeated in three independent experiments.

#### 3.3.3. Tubulin Polymerization Assay

The assembly of purified bovine tubulin was monitored using a kit, BK006, purchased from Cytoskeleton Inc., (Denver, CO, USA). The assay was carried out in accordance with the manufacturer's instructions using the standard assay conditions [46]. Briefly, purified (>99%) bovine brain tubulin (3 mg/mL) in a buffer consisting of 80 mM PIPES (pH 6.9), 0.5 mM EGTA, 2 mM MgCl<sub>2</sub>, 1 mM GTP and 10% glycerol was incubated at 37 °C in the presence of either vehicle (2% (*v/v*) DMSO), CA-4, varying quinoline compounds concentration. Light is scattered proportionally to the concentration of polymerized microtubules in the assay. Therefore, tubulin assembly was monitored turbidimetrically at 340 nm in a Spectramax 340 PC spectrophotometer (Molecular Devices, Sunnyvale, CA, USA). The concentration that inhibits tubulin polymerization by 50% (IC<sub>50</sub>) was determined using area under the polymerization curve (AUC). The IC<sub>50</sub> value for each compound was computed using GraphPad Prism Software.

#### 3.3.4. Colchicine Site Competitive Binding Assay

The affinity of selective quinoline compounds **19b**, **19h**, **20c** and **20j** as well as CA-4 to colchicine binding site was determined using Colchicine Site Competitive Assay kit CytoDYNAMIX Screen15 (Cytoskeleton, Inc., Denver, CO, USA) using the standard protocol of the manufacturer to determine Ki values (µM) [47]. Briefly, selected concentrations of each compound were added to 96-well plate. Tritiated colchicine (100 mL; Perkin-Elmer; specific activity 70–80 Ci mmol<sup>−1</sup>) was added to 300 mL of tubulin-binding buffer. From this, 10 µL was added in each well. Premix tubulin-biotine-streptavidin scintillation proximity assay beads (180 mL; 4.4 mg streptavidin yttrium silicate beads (Amersham Bioscience, London, UK) are mixed into 15 mL buffer and incubated on a slow 10 r.p.m. rotator at 4 °C

for 30 min, and were added to each well for 2 h at 37 °C. The plates were then read on a scintillation counter (Topcount Microplate Reader, Packard Instruments, Winooski, VT, USA) [48,49].

### 3.3.5. Cell Cycle Analysis

MCF-7 cells were seeded at a density of  $1 \times 10^5$  cells/well in 6-well plates and treated with CA-4 (50 nM) and compound **19h** (50 and 250 nM) for 24, 48 and 72 hr. After trypsinization, the cells were collected by and centrifuged at  $800\times g$  for 15 min. Cells were fixed in ice-cold 70% ethanol overnight at  $-20\text{ }^{\circ}\text{C}$ . Fixed cells were centrifuged at  $800\times g$  for 15 min and stained with 50  $\mu\text{g/mL}$  of PI, containing 50  $\mu\text{g/mL}$  of DNase-free RNase A, at 37 °C for 30 min. The DNA content of cells (10,000 cells) was analyzed by flow cytometer at 488 nm using a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA, USA).

### 3.3.6. Annexin V/PI Apoptotic Assay

Flow cytometry using Annexin V and propidium iodide (PI) was performed to detect apoptotic cell death. MCF-7 cells were seeded in 6 well plates at density of  $1 \times 10^5$  cells/well and treated with vehicle (0.1 % (v/v) EtOH), positive control (50 nM of CA-4) or compound **19h** (50 and 250 nM) for 24, 48 and 72 hr. Cells were then harvested and prepared for flow cytometric analysis. Cells were washed in 1X binding buffer and incubated in the dark for 30 min on ice in Annexin V-containing binding buffer [1:100]. Then, cells were re-suspended in PI-containing binding buffer [1:1000]. Samples were analyzed immediately using the BD Accuri flow cytometer and GraphPad Prism software version 5.01 for analysis the data. Four populations are produced during the assay Annexin V and PI negative (Q4, healthy cells), Annexin V positive and PI negative (Q3, early apoptosis), Annexin V and PI positive (Q2, late apoptosis) and Annexin V negative and PI positive (Q1, necrosis).

### 3.4. Computational Procedure

The MOE 2019.0102 software package [50] was used alongside XQuartz 2.7.11 [51]. ChemDraw version 16.0.1.4 was used to construct .sdf files of IUPAC structures prior to importation to MOE. MOE was then used to generate energy minimized structures using the Merck Molecular force field MMFF94s [52] which is tailored for energy minimization purposes. The chosen forcefield was MMFF94 which is superior to MMFF94s for docking simulations [53,54]. PDB structure 1SAO [37] was prepared for docking by using the ‘add hydrogens’, ‘correct’ and ‘type and fix potential’ options. DAMA-colchicine was selected as the ligand and the preparation was finalized using QuickPrep in MOE.

Ligands for docking were loaded as an .mdb file containing colchicine and CA-4. The number of placements and refinements was decided upon by docking CA-4 and resulting in optimal alignment of key functional motifs. This was confirmed by presence of known hydrogen bonding interaction between CA-4’s B ring hydroxy substituent with Thr $\alpha$ 179. In order to ensure that the compounds continued to dock at the CBS, the docking site was chosen as the ligand atoms i.e., DAMA colchicine and the ‘wall constraint’ functionality was selected to restrict docking to the CBS only at the  $\alpha\beta$  tubulin interface. Values for the number of placements and refinements for each compound were found optimal at 200 and 200 respectively. Each conformation was subsequently docked and scored using the London dG score. The top binding poses defined by scoring function were refined using the GBVI/WSA dG method. The chosen forcefield was MMFF94 which is superior to MMFF94s for docking simulations [53,54].

## 4. Conclusions

In this study, we designed, synthesized and evaluated a series of novel quinolines as potential inhibitors of tubulin polymerization. Structurally, these compounds represent non-isomerizable analogues of CA-4 with two linker configurations: a hydrazone open linker and its cyclic form the oxadiazole ring. The most potent compound **19h** with a hydrazone linker was identified as a novel

potent anticancer agent exhibiting activity against HL-60, MCF-7, HCT-116 and HeLa cancer cell lines with IC<sub>50</sub> values of 0.040, 0.026, 0.022 and 0.038  $\mu$ M, respectively. It demonstrated low cytotoxicity to MCF-10A non-cancer cells, indicating selectivity for cancer cells. The microtubule polymerization inhibitory action of **19h** was investigated during an in vitro tubulin polymerization assay, colchicine inhibition assay and molecular docking studies. These studies confirmed tubulin as the molecular target of **19h** and the colchicine-binding site as the location of interaction with tubulin. Additionally, **19h** was demonstrated to effectively arrest the cell cycle in the G<sub>2</sub>/M phase and induce apoptosis in MCF-7 cells. In conclusion, these results highlighted the quinoline **19h** as promising anti-tubulin agent for progression for treatment of breast cancer.

**Supplementary Materials:** The following are available online at <http://www.mdpi.com/1424-8247/13/11/393/s1>. Figure S1: <sup>1</sup>H NMR for compound **19a**, Figure S2: <sup>13</sup>C NMR for compound **19a**, Figure S3: <sup>1</sup>H NMR for compound **19b**, Figure S4: <sup>13</sup>C NMR for compound **19b**, Figure S5: <sup>1</sup>H NMR for compound **19c**, Figure S6: <sup>13</sup>C NMR for compound **19c**, Figure S7: <sup>1</sup>H NMR for compound **19d**, Figure S8: <sup>13</sup>C NMR for compound **19d**, Figure S9: <sup>1</sup>H NMR for compound **19e**, Figure S10: <sup>13</sup>C NMR for compound **19e**, Figure S11: <sup>1</sup>H NMR for compound **19f**, Figure S12: <sup>13</sup>C NMR for compound **19f**, Figure S13: <sup>1</sup>H NMR for compound **19g**, Figure S14: <sup>13</sup>C NMR for compound **19g**, Figure S15: <sup>1</sup>H NMR for compound **19h**, Figure S16: <sup>13</sup>C NMR for compound **19h**, Figure S17: <sup>1</sup>H NMR for compound **19i**, Figure S18: <sup>13</sup>C NMR for compound **19i**, Figure S19: <sup>1</sup>H NMR for compound **19j**, Figure S20: <sup>13</sup>C NMR for compound **19j**, Figure S21: <sup>1</sup>H NMR for compound **20a**, Figure S22: <sup>13</sup>C NMR for compound **20a**, Figure S23: <sup>1</sup>H NMR for compound **20b**, Figure S24: <sup>13</sup>C NMR for compound **20b**, Figure S25: <sup>1</sup>H NMR for compound **20c**, Figure S26: <sup>13</sup>C NMR for compound **20c**, Figure S27: <sup>1</sup>H NMR for compound **20d**, Figure S28: <sup>13</sup>C NMR for compound **20d**, Figure S29: <sup>1</sup>H NMR for compound **20e**, Figure S30: <sup>13</sup>C NMR for compound **20e**, Figure S31: <sup>1</sup>H NMR for compound **20f**, Figure S32: <sup>13</sup>C NMR for compound **20f**, Figure S33: <sup>1</sup>H NMR for compound **20g**, Figure S34: <sup>13</sup>C NMR for compound **20g**, Figure S35: <sup>1</sup>H NMR for compound **20h**, Figure S36: <sup>13</sup>C NMR for compound **20h**, Figure S37: <sup>1</sup>H NMR for compound **20i**, Figure S38: <sup>13</sup>C NMR for compound **20i**, Figure S39: <sup>1</sup>H NMR for compound **20j**, Figure S40: <sup>13</sup>C NMR for compound **20j**, Figure S41A–C: A. Quinoline **19b** (yellow) and CA-4 (cyan) docked at the colchicine-binding site of tubulin in co-crystal 1SAO. B. Quinoline **20c** (pink) and DAMA-colchicine (green) docked at the colchicine-binding site of tubulin in co-crystal 1SAO. C. Quinoline **9=20g** (red) and CA-4 (cyan) docked at the colchicine-binding site of tubulin in co-crystal 1SAO. Protein residues removed on right for clarity. (red = oxygen, grey = hydrogen), Figure S42: 2D Ligand Interaction Schematic for **19b** as generated by MOE, Figure S43: 2D Ligand Interaction Schematic for **20c** as generated by MOE, Figure S44: 2D Ligand Interaction Schematic for **20g** as generated by MOE.

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## References

- Desai, A.; Mitchison, T.J. Microtubule Polymerization Dynamics. *Annu. Rev. Cell Dev. Biol.* **1997**, *13*, 83–117. [CrossRef] [PubMed]
- Nogales, E.; Wolf, S.G.; Downing, K.H. Erratum: Structure of the  $\alpha\beta$  Tubulin Dimer by Electron Crystallography. *Nature* **1998**, *393*, 191. [CrossRef]
- Downing, K.H.; Nogales, E. Tubulin Structure: Insights into Microtubule Properties and Functions. *Curr. Opin. Struct. Biol.* **1998**, *8*, 785–791. [CrossRef]
- Kline-Smith, S.L.; Walczak, C.E. Mitotic Spindle Assembly and Chromosome Segregation. *Mol. Cell* **2004**, *15*, 317–327. [CrossRef] [PubMed]
- Vindya, N.; Sharma, N.; Yadav, M.; Ethiraj, K. Tubulins—the target for anticancer therapy. *Curr. Top. Med. Chem.* **2015**, *15*, 73–82. [CrossRef]
- Kamath, P.R.; Sunil, D.; Ajees, A.A. Synthesis of Indole–Quinoline–Oxadiazoles: Their Anticancer Potential and Computational Tubulin Binding Studies. *Res. Chem. Intermed.* **2016**, *42*, 5899–5914. [CrossRef]



7. Khelifi, I.; Naret, T.; Renko, D.; Hamze, A.; Bernadat, G.; Bignon, J.; Lenoir, C.; Dubois, J.; Brion, J.-D.; Provot, O.; et al. Design, Synthesis and Anticancer Properties of Iso Combretaquinolines as Potent Tubulin Assembly Inhibitors. *Eur. J. Med. Chem.* **2017**, *127*, 1025–1034. [CrossRef]
8. Zweifel, M.; Jayson, G.C.; Reed, N.S.; Osborne, R.; Hassan, B.; Ledermann, J.; Shreeves, G.; Poupard, L.; Lu, S.-P.; Balkissoon, J.; et al. Phase II Trial of Combretastatin A4 Phosphate, Carboplatin, and Paclitaxel in Patients With Platinum-Resistant Ovarian Cancer. *Ann. Oncol.* **2011**, *22*, 2036–2041. [CrossRef]
9. Lu, Y.; Chen, J.; Xiao, M.; Li, W.; Miller, D.D. An Overview of Tubulin Inhibitors That Interact with the Colchicine Binding Site. *Pharm. Res.* **2012**, *29*, 2943–2971. [CrossRef]
10. Lee, M.; Brockway, O.; Dandavati, A.; Tzou, S.; Sjöholm, R.; Nickols, A.; Babu, B.; Chavda, S.; Satam, V.; Hartley, R.M.; et al. Design and Synthesis of Novel Enhanced Water Soluble Hydroxyethyl Analogs of Combretastatin A-4. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 2087–2091. [CrossRef]
11. Cooney, M.M.; Ortiz, J.; Bukowski, R.M.; Remick, S.C. Novel Vascular Targeting/Disrupting Agents: Combretastatin A4 Phosphate and Related Compounds. *Curr. Oncol. Rep.* **2005**, *7*, 90–95. [CrossRef] [PubMed]
12. U.S. National Library of Medicine. Available online: <https://clinicaltrials.gov/ct2/show/NCT00060242?term=NCT00060242&rank=1> (accessed on 16 October 2020).
13. Ohsumi, K.; Hatanaka, T.; Nakagawa, R.; Fukuda, Y.; Morinaga, Y.; Suga, Y.; Nihei, Y.; Ohishi, K.; Akiyama, Y.; Tsuji, T. Synthesis and Antitumor Activities of Amino Acid Prodrugs of Amino-Combretastatins. *Anti-Cancer Drug Des.* **1999**, *14*, 539–548.
14. Tron, G.C.; Pirali, T.; Sorba, G.; Pagliai, F.; Busacca, A.S.; Genazzani, A.A. Medicinal Chemistry of Combretastatin A4: Present and Future Directions. *J. Med. Chem.* **2006**, *49*, 3033–3044. [CrossRef] [PubMed]
15. Gaspari, R.; Prota, A.E.; Bargsten, K.; Cavalli, A.; Steinmetz, M.O. Structural Basis of Cis-and Trans-Combretastatin Binding to Tubulin. *Chem* **2017**, *2*, 102–113. [CrossRef]
16. Tarade, D.; Ma, D.; Pignatelli, C.; Mansour, F.; Simard, D.; Berg, S.V.D.; Gauld, J.; McNulty, J.; Pandey, S. Structurally Simplified Biphenyl Combretastatin a4 Derivatives Retain In Vitro Anti-Cancer Activity Dependent on Mitotic Arrest. *PLoS ONE* **2017**, *12*, e0171806. [CrossRef]
17. Das, B.C.; Tang, X.-Y.; Rogler, P.; Evans, T. Design and Synthesis of 3,5-Disubstituted Boron-Containing 1,2,4-Oxadiazoles as Potential Combretastatin A-4 (CA-4) Analogs. *Tetrahedron Lett.* **2012**, *53*, 3947–3950. [CrossRef]
18. Kaffy, J.; Monneret, C.; Mailliet, P.; Commerçon, A.; Pontikis, R. 1,3-Dipolar Cycloaddition Route to Novel Isoxazole-Type Derivatives Related to Combretastatin A-4. *Tetrahedron Lett.* **2004**, *45*, 3359–3362. [CrossRef]
19. Mahal, K.; Biersack, B.; Schrufer, S.; Resch, M.; Ficner, R.; Schobert, R.; Mueller, T. Combretastatin A-4 Derived 5-(1-Methyl-4-Phenyl-Imidazol-5-Yl)Indoles With Superior Cytotoxic and Anti-Vascular Effects on Chemoresistant Cancer Cells and Tumors. *Eur. J. Med. Chem.* **2016**, *118*, 9–20. [CrossRef]
20. Cury, N.M.; Mühlethaler, T.; Laranjeira, A.B.A.; Canevarolo, R.R.; Zenatti, P.P.; Lucena-Agell, D.; Barasoain, I.; Song, C.; Sun, D.; Dovat, S.; et al. Structural Basis of Colchicine-Site targeting Acylhydrazones active against Multidrug-Resistant Acute Lymphoblastic Leukemia. *iScience* **2019**, *21*, 95–109. [CrossRef]
21. Medarde, M.; Maya, A.B.; Pérez-Melero, C. Review Article Naphthalene Combretastatin Analogues: Synthesis, Cytotoxicity and Antitubulin Activity. *J. Enzym. Inhib. Med. Chem.* **2004**, *19*, 521–540. [CrossRef]
22. Salum, L.B.; Mascarello, A.; Canevarolo, R.R.; Altei, W.F.; Laranjeira, A.B.; Neuenfeldt, P.D.; Stumpf, T.R.; Chiaradia-Delatorre, L.D.; Vollmer, L.L.; Daghestani, H.N.; et al. N-(1'-naphthyl)-3,4,5-Trimethoxybenzohydrazide as Microtubule Destabilizer: Synthesis, Cytotoxicity, Inhibition of Cell Migration and in Vivo Activity Against Acute Lymphoblastic Leukemia. *Eur. J. Med. Chem.* **2015**, *96*, 504–518. [CrossRef] [PubMed]
23. Afzal, O.; Kumar, S.; Haider, R.; Ali, R.; Kumar, R.; Jaggi, M.; Bawa, S. A Review on Anticancer Potential of Bioactive Heterocycle Quinoline. *Eur. J. Med. Chem.* **2015**, *97*, 871–910. [CrossRef] [PubMed]
24. Penthala, N.R.; Janganati, V.; Bommagani, S.; Crooks, P.A. Synthesis and Evaluation of a Series of Quinoliny Trans-Cyanostilbene Analogs as Anticancer Agents. *MedChemComm* **2014**, *5*, 886–890. [CrossRef]
25. Zhou, Y.; Yan, W.; Cao, D.; Shao, M.; Li, D.; Wang, F.; Yang, Z.; Chen, Y.; He, L.; Wang, T.; et al. Design, Synthesis and Biological Evaluation of 4-Anilinoquinoline Derivatives as Novel Potent Tubulin Depolymerization Agents. *Eur. J. Med. Chem.* **2017**, *138*, 1114–1125. [CrossRef] [PubMed]

26. Hadfield, J.A.; Ducki, S.; Hirst, N.; McGown, A.T. Tubulin and Microtubules as Targets for Anticancer Drugs. *Prog. Cell Cycle Res.* **2003**, *5*, 309–326.
27. Ansari, M.; Shokrzadeh, M.; Karima, S.; Rajaei, S.; Fallah, M.; Ghassemi-Barghi, N.; Ghasemian, M.; Emami, S. New Thiazole-2(3H)-Thiones Containing 4-(3,4,5-Trimethoxyphenyl) Moiety as Anticancer Agents. *Eur. J. Med. Chem.* **2020**, *185*, 111784. [\[CrossRef\]](#)
28. Greene, L.M.; Meegan, M.J.; Zisterer, D.M. Combretastatins: More Than Just Vascular Targeting Agents? *J. Pharmacol. Exp. Ther.* **2015**, *355*, 212–227. [\[CrossRef\]](#)
29. Agut, R.; Falomir, E.; Murga, J.; Martín-Beltrán, C.; Gil-Edo, R.; Pla, A.; Carda, M.; Marco, J.A. Synthesis of Combretastatin A-4 and 3'-Aminocombretastatin A-4 derivatives with Aminoacid Containing Pendants and Study of their Interaction with Tubulin and as Downregulators of the VEGF, hTERT and c-Myc Gene Expression. *Molecules* **2020**, *25*, 660. [\[CrossRef\]](#)
30. Pérez-Pérez, M.-J.; Priego, E.-M.; Bueno, O.; Martins, M.S.; Canela, M.-D.; Liekens, S.; Liekens, S. Blocking Blood Flow to Solid Tumors by Destabilizing Tubulin: An Approach to Targeting Tumor Growth. *J. Med. Chem.* **2016**, *59*, 8685–8711. [\[CrossRef\]](#)
31. Mirzaei, S.; Hadizadeh, F.; Eisvand, F.; Mosaffa, F.; Ghodsi, R. Synthesis, Structure-Activity Relationship and Molecular Docking Studies of Novel Quinoline-Chalcone Hybrids as Potential Anticancer Agents and Tubulin Inhibitors. *J. Mol. Struct.* **2020**, *1202*, 127310. [\[CrossRef\]](#)
32. Chaudhary, V.; Venghateri, J.B.; Dhaked, H.P.S.; Bhoyar, A.S.; Guchhait, S.K.; Panda, D. Novel Combretastatin-2-aminoimidazole Analogues as Potent Tubulin Assembly Inhibitors: Exploration of Unique Pharmacophoric Impact of Bridging Skeleton and Aryl Moiety. *J. Med. Chem.* **2016**, *59*, 3439–3451. [\[CrossRef\]](#) [\[PubMed\]](#)
33. Shobeiri, N.; Rashedi, M.; Mosaffa, F.; Zarghi, A.; Ghandadi, M.; Ghasemi, A.; Ghodsi, R. Synthesis and Biological Evaluation of Quinoline Analogues of Flavones as Potential Anticancer Agents and Tubulin Polymerization Inhibitors. *Eur. J. Med. Chem.* **2016**, *114*, 14–23. [\[CrossRef\]](#) [\[PubMed\]](#)
34. Li, W.; Xu, F.; Shuai, W.; Sun, H.; Yao, H.; Ma, C.; Xu, S.; Yao, H.; Zhu, Z.; Yang, D.-H.; et al. Discovery of Novel Quinoline-Chalcone Derivatives as Potent Antitumor Agents with Microtubule Polymerization Inhibitory Activity. *J. Med. Chem.* **2019**, *62*, 993–1013. [\[CrossRef\]](#) [\[PubMed\]](#)
35. Shaheen, M.A.; El-Emam, A.A.; El-Gohary, N.S. 1,4,5,6,7,8-Hexahydroquinolines and 5,6,7,8-Tetrahydronaphthalenes: A New Class of Antitumor Agents Targeting the Colchicine Binding Site of Tubulin. *Bioorg. Chem.* **2020**, *99*, 103831. [\[CrossRef\]](#) [\[PubMed\]](#)
36. Gao, F.; Liang, Y.; Zhou, P.; Cheng, J.; Ding, K.; Wang, Y. Design, Synthesis, Antitumor Activities and Biological Studies of Novel Diaryl Substituted Fused Heterocycles as Dual Ligands Targeting Tubulin and Katanin. *Eur. J. Med. Chem.* **2019**, *178*, 177–194. [\[CrossRef\]](#) [\[PubMed\]](#)
37. Ravelli, R.B.; Gigant, B.; Curmi, P.A.; Jourdain, I.; Lachkar, S.; Sobel, A.; Knossow, M. Insight Into Tubulin Regulation From a Complex With Colchicine and a Stathmin-Like Domain. *Nat. Cell Biol.* **2004**, *428*, 198–202. [\[CrossRef\]](#) [\[PubMed\]](#)
38. Ibrahim, T.S.; Bokhtia, R.M.; Al-Mahmoudy, A.M.; Taher, E.S.; Alawadh, M.A.; Elagawany, M.; Abdel-Aal, E.H.; Panda, S.; Gouda, A.M.; Asfour, H.Z.; et al. Design, Synthesis and Biological Evaluation of Novel 5-((Substituted Quinolin-3-Yl/1-Naphthyl) Methylene)-3-Substituted Imidazolidin-2,4-Dione as HIV-1 Fusion Inhibitors. *Bioorganic Chem.* **2020**, *99*, 103782. [\[CrossRef\]](#)
39. Karkara, B.B.; Mishra, S.S.; Singh, B.N.; Panda, G. Synthesis of 2-Methoxy-3-(Thiophen-2-Ylmethyl)Quinoline Containing Amino Carbinols as Antitubercular Agents. *Bioorg. Chem.* **2020**, *99*, 103775. [\[CrossRef\]](#)
40. Insuasty, D.; Abonia, R.; Insuasty, B.; Quiroga, J.; Laali, K.K.; Nogueras, M.; Cobo, J. Microwave-Assisted Synthesis of Diversely Substituted Quinoline-Based Dihydropyridopyrimidine and Dihydropyrazolopyridine Hybrids. *ACS Comb. Sci.* **2017**, *19*, 555–563. [\[CrossRef\]](#)
41. Ibrahim, T.S.; Seliem, I.A.; Ibrahim, T.S.; Al-Mahmoudy, A.M.M.; Abdel-Samii, Z.K.M.; Alhakamy, N.A.; Asfour, H.Z.; Elagawany, M. An Efficient Greener Approach for N-acylation of Amines in Water Using Benzotriazole Chemistry. *Molecules* **2020**, *25*, 2501. [\[CrossRef\]](#)
42. Agha, K.A.; Abo-Dya, N.E.; Ibrahim, T.S.; Abdel-Aal, E.H.; Abdel-Samii, Z.K. N-Acylbenzotriazole: Convenient Approach for Protecting Group-Free Monoacylation of Symmetric Diamines. *Mon. Chem.* **2020**, *151*, 589–598. [\[CrossRef\]](#)
43. Agha, K.A.; Abo-Dya, N.E.; Ibrahim, T.S.; Abdel-Aal, E.H. Efficient Synthesis of N-Acylbenzotriazoles Using Tosyl Chloride: En Route to Suberoylanilide Hydroxamic Acid (SAHA). *ARKIVOC* **2016**, *3*, 161–170. [\[CrossRef\]](#)

44. Wang, Z.; Zhang, H.; Killian, B.J.; Jabeen, F.; Pillai, G.G.; Berman, H.M.; Mathelier, M.; Sibble, A.J.; Yeung, J.; Zhou, W.; et al. Synthesis, Characterization and Energetic Properties of 1,3,4-Oxadiazoles. *Eur. J. Org. Chem.* **2015**, *2015*, 5183–5188. [CrossRef]
45. Wang, X.; Yu, H.; Xu, P.; Zheng, R. A Facile Synthesis of Acylhydrazines from Acylbenzotriazoles. *J. Chem. Res.* **2005**, *2005*, 595–597. [CrossRef]
46. Wienecke, A.; Bacher, G. Indibulin, a Novel Microtubule Inhibitor, Discriminates between Mature Neuronal and Nonneuronal Tubulin. *Cancer Res.* **2009**, *69*, 171–177. [CrossRef]
47. Mustafa, M.; Anwar, S.; Elgamal, F.; Ahmed, E.R.; Aly, O.M. Potent Combretastatin A-4 Analogs Containing 1,2,4-Triazole: Synthesis, Antiproliferative, Anti-Tubulin Activity, and Docking Study. *Eur. J. Med. Chem.* **2019**, *183*, 111697. [CrossRef]
48. Tahir, S.K.; Kovar, P.; Rosenberg, S.H.; Ng, S.-C. Rapid Colchicine Competition-Binding Scintillation Proximity Assay Using Biotin-Labeled Tubulin. *Biotechniques* **2000**, *29*, 156–160. [CrossRef]
49. Moussa, S.A.; Osman, E.E.A.; Eid, N.M.; Abou-Seri, S.M.; El Moghazy, S.M. Design and Synthesis of Novel 5-(4-Chlorophenyl)Furan Derivatives With Inhibitory Activity on Tubulin Polymerization. *Future Med. Chem.* **2018**, *10*, 1907–1924. [CrossRef]
50. *Molecular Operating Environment*; Version 2019.0102; Chemical Computing Group: Montreal, ON, Canada, 2019.
51. XQuartz 2.7.11; X.org Foundation: Portland, OR, USA, 2016.
52. MMFF94 Force Field (mmff94). Available online: <https://open-babel.readthedocs.io/en/latest/Forcefields/mmff94.html> (accessed on 28 April 2020).
53. Halgren, T.A. Merck Molecular Force Field. V. Extension of MMFF94 Using Experimental Data, Additional Computational Data, and Empirical Rules. *J. Comput. Chem.* **1996**, *17*, 616–641. [CrossRef]
54. Halgren, T.A.; Nachbar, R.B. Merck Molecular Force Field. IV. Conformational Energies and Geometries for MMFF94. *J. Comput. Chem.* **1996**, *17*, 587–615. [CrossRef]

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