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Synthesis and biological activity of 2,5-diaryl-3-methylpyrimido[4,5-*c*]quinolin-1(2*H*)-one derivatives

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Abstract—A series of 2,5-diaryl-3-methylpyrimido[4,5-*c*]quinolin-1(2*H*)-ones (**7**–**30**), variously substituted at the 2- and 5-phenyl moieties, were synthesized and evaluated for their in vitro cytotoxic activity against a PC3 cancer cell line. Cytotoxicity data revealed that the type of substituent as well as substitution pattern have variable influence on cytotoxic activity. Among the compounds tested, compounds (**9**), (**13**), (**18**), (**19**), and (**23**) demonstrated appreciable cytotoxic activity with mean IC_{50} values of 2.0, 1.4, 1.6, 2.2, and 1.9 μM, respectively. Methyl substitution at the 2-phenyl ring was found to yield the least active compounds. Two of the most potent compounds (**13**) and (**18**) were further investigated for inhibition of tubulin polymerization and found to have no activity at the concentrations used in the assay.

1. Introduction

The multi-drug resistance as well as the neurological and hematological adverse effects of the most clinically successful natural products in current use such as the taxanes and Vinca alkaloids have triggered an extensive worldwide search for new synthetic antitumor agents.[1–3] In this respect, there has been a growing interest during the past few decades in the antitumor activity of 2-phenyl-4-quinolones (A), 2-phenyl-4-anilinoquinolines (B), and their condensed heterocyclic derivatives.[4–16] As a part of an ongoing research program on the structurally relevant 5-arylpyrimido[4,5-*c*]quinolin-1(2*H*)-ones, we have previously reported the synthesis of 2-amino-5-aryl-3-substituted-pyrimido[4,5-*c*]quinolin-1(2*H*)-ones (C) as a new class of cytotoxic antimitotic agents.[17] It was concluded that peripheral substituents have a major impact on cytotoxic activity. In order to further investigate structural determinants of cytotoxic activity of this class of compounds, we were interested in exploiting the 2-position as a part of an extended SAR study. In the present work, a series of 2,5-diaryl-3-methylpyrimido[4,5-*c*]quinolin-1(2*H*)-ones having variously substituted 2- and 5-phenyl rings (D) were synthesized and tested for in vitro cytotoxic activity (Chart 1).

2. Chemistry

The starting 3-amino-2-arylquinoline-4-carboxylic acids (**3** and **4**) were synthesized according to a reported literature procedure by reacting isatin with phenacylamine hydrochlorides (**1** and **2**) under the conditions of the Pfitzinger reaction.[18,19] The key intermediate lactones (**5** and **6**) were obtained in good yields by cyclization of the starting acids (**3** and **4**) using acetic anhydride under reflux conditions as reported previously.[17] Finally, acid-catalyzed condensation of (**5** and **6**) with substituted anilines afforded the target 2,5-diaryl-3-methylpyrimido[4,5-*c*]quinolin-1(2*H*)-ones (**7**–**30**) in moderate yields as outlined in Scheme 1. Our attempts to prepare 2,3,5-triarylpyrimido[4,5-*c*]quinolin-1(2*H*)-ones from their corresponding lactones under different condensation conditions were unsuccessful. Structure of the target

Keywords: Cytotoxic agents; Tubulin polymerization; Pyrimido[4,5-*c*]quinolin-1(2*H*)-ones.
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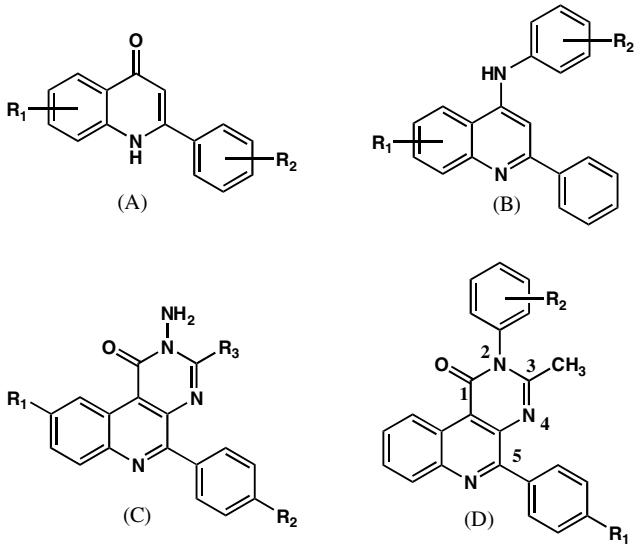
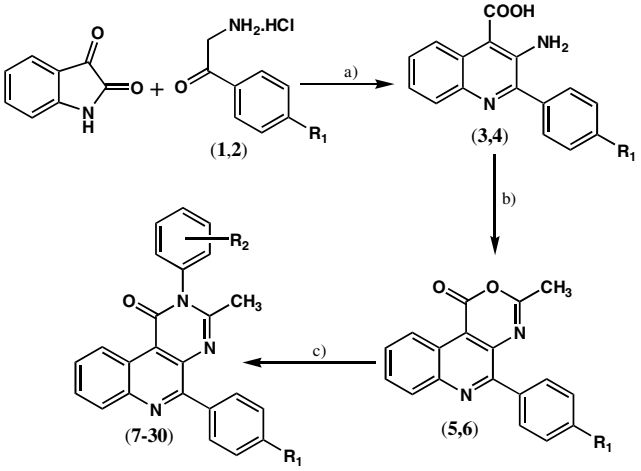


Chart 1.



Scheme 1. Reagents and conditions: (a) NaOH, EtOH, H_2O, THF, reflux, 30 min; (b) $(CH_3CO)_2O$, reflux, 5 h; (c) substituted anilines, CH_3COONa, HOAc, reflux, 24 h.

compounds (**7**–**30**) was confirmed by IR and ^1H NMR spectral data. In addition, all the new compounds were microanalyzed satisfactorily for C, H, N.

3. Results and discussion

A total of 24 compounds were tested for cytotoxicity in PC3 cells using the MTT assay. Half of these were measurably cytotoxic at low micromolar concentrations. The other compounds showed some cytotoxicity at higher concentrations. IC_{50} values for these molecules were higher than the solubility limit of these substances and therefore could not be determined with accuracy. All other molecules were found to be inactive up to the solubility limit of each (Table 1).

A clear structure–activity relationship emerged in the 5-(4-bromophenyl) series. In this group, the cytotoxicity

Table 1. In vitro cytotoxicity of the target pyrimido[4,5-*c*]quinolin-1(2*H*)-ones using the MTT assay

Compound	R_1	R_2	IC_{50}, PC3 (μM)
7	Cl	H	3.2 ± 0.2
8	Cl	4-F	4.0 ± 0.05
9	Cl	2-Cl	2.0 ± 0.55
10	Cl	3-Cl	>26
11	Cl	4-Cl	>28
12	Cl	2-Br	>42
13	Cl	3-Br	1.4 ± 0.2
14	Cl	4-Br	2.5 ± 0.47
15	Cl	2-CH_3	>38
16	Cl	4-CH_3	>32
17	Cl	2-OCH_3	10.5 ± 0.5
18	Cl	4-OCH_3	1.6 ± 0.23
19	Br	H	2.2 ± 0.58
20	Br	4-F	5.5 ± 3.5
21	Br	2-Cl	>28
22	Br	3-Cl	>25
23	Br	4-Cl	1.9 ± 0.05
24	Br	2-Br	>20
25	Br	3-Br	>10
26	Br	4-Br	3.3 ± 0.2
27	Br	2-CH_3	>30
28	Br	4-CH_3	>21.3
29	Br	2-OCH_3	6.7 ± 2.3
30	Br	4-OCH_3	2.5 ± 0.1
Colchicine			0.018 ± 0.003

was strongly influenced by the position of the substituent on the aromatic ring. A halogen at the 4-position of the 2-phenyl ring (**20**, **23** and **26**) yielded an active compound (IC_{50} values within a factor of three of the unsubstituted parent compound **19**). However, a halogen substituent at the 2- or 3-position of the 2-phenyl ring sharply decreased activity relative to the parent molecule. In general, electronic rather than steric features of the 4-substituent seem to be a more important determinant of activity, since the methyl substituted derivative was more than 10 times less active than the methoxy substituted derivative.

Interestingly, changing R_1 on the parent molecule to chlorine resulted in a different structure–activity relationship in the series. Four of the six active molecules were actually slightly more cytotoxic than the parent (compare IC_{50} values of **9**, **13**, **14**, and **18** to the IC_{50} value of **7**) and one was only slightly less cytotoxic (**8**). In this series, however, there is no clear structure–activity relationship. For example, the only active 2-phenyl chlorinated compound (R_2 = Cl) is substituted on the 2-position (**9**) of the 2-phenyl ring. In contrast, in the 2-phenyl brominated series (R_2 = Br), the 3- and 4- substituted (**13** and **14**) compounds were active and the 2- substituted compound (**12**) was found to be inactive. Clearly, additional derivatives would be necessary to discern the structure–activity relationship in this series.

There are two substitution patterns that yield consistent results in the two sets. A methoxy substituent on either 2- or 4-position of each parent molecule yielded substances that retained cytotoxicity, with the 2-substituted derivative being less active in each pair (**17** vs **18**, **29** vs **30**).

Methylation of the 2-phenyl ring (**15**, **16** and **27**, **28**) resulted in inactive molecules regardless of the halogen of the parent molecule.

Two of the most potent compounds, **13** and **18**, were tested for inhibition of in vitro microtubule assembly (Fig. 1). No inhibition of polymerization was observed, regardless of whether microtubule associated proteins were present (panel a) or absent (panel b). The compounds were tested at the limits of their solubilities under the assay conditions (27 μM for **13** and 40 μM for **18**). It is concluded that neither of the molecules significantly affected tubulin assembly under these assay conditions. It should be noted, however, that concentrations required to observe inhibition of tubulin assembly in vitro are typically one to two orders of magnitude greater than concentrations necessary to observe cytotoxicity[20]; such concentrations could not be achieved with these molecules. Further elaboration of the pyrimido[4,5-*c*]quinolin-1(2*H*)-one series will include additional polar functional groups to increase the aqueous solubility of the molecules.

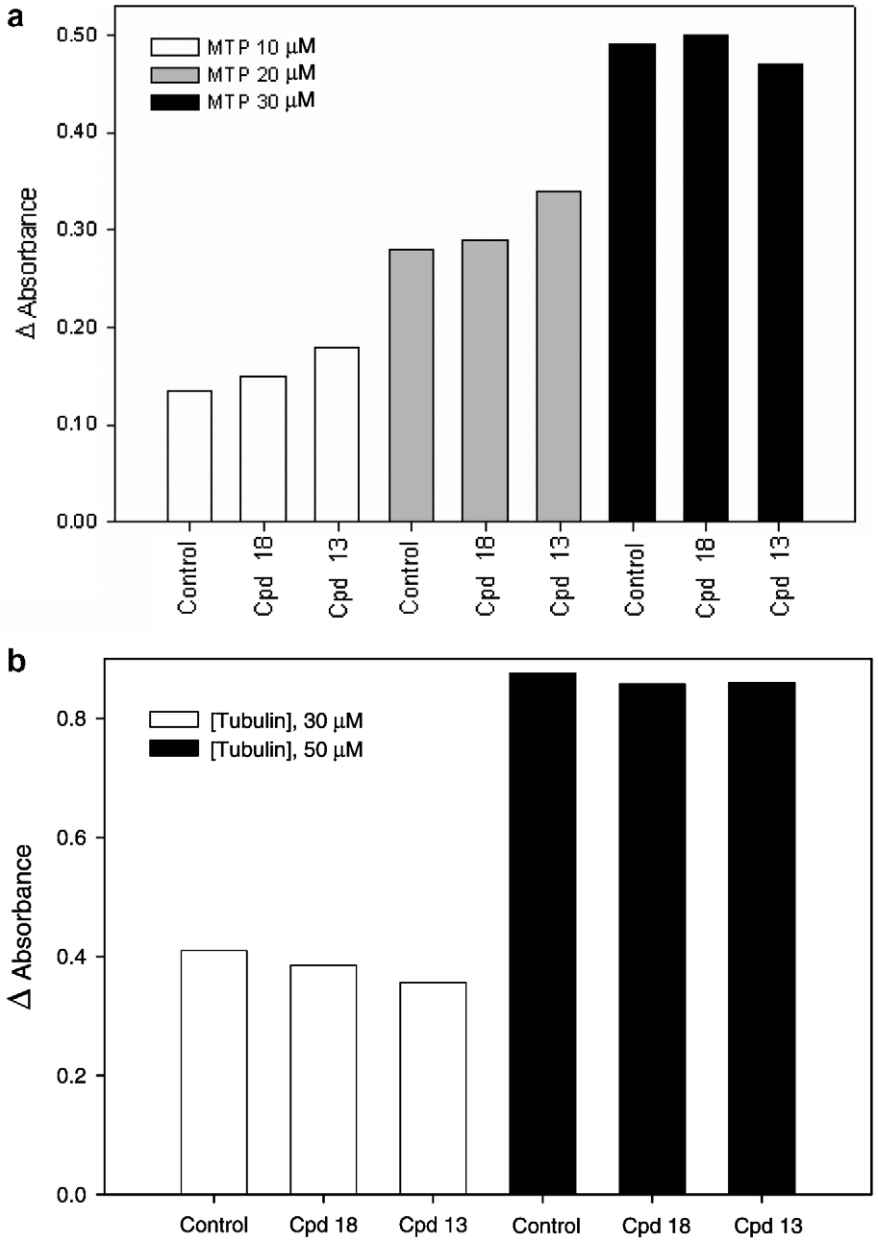


Figure 1. Varying concentrations of MTP (panel a) and tubulin (panel b) were incubated with 27 μM of compound **13** and 40 μM of compound **18** in presence of 4% DMSO at 25 °C for 40 min. Appropriate controls in absence of drugs were also subjected to the same conditions. Polymerization kinetics was monitored at elevated temperatures (37 °C) by apparent light scattering at 350 nm. Reactions were terminated when no change in the absorbance was observed. GTP induced assembly of microtubule protein (MTP, tubulin plus microtubule associated proteins) was unaffected by compound **13** or **18** within experimental error (±10% of the ΔA). (Panel b) GTP-induced assembly of purified tubulin was unaffected by compound **13** or **18** within experimental error (±10% of the ΔA).

4. Conclusion

We have synthesized and tested a series of 2,5-diaryl-3-methylpyrimido[4,5-*c*]quinolin-1(2*H*)-ones that are variously substituted at the 2- and 5-phenyl moieties. Nearly half of the derivatives retained cytotoxicities in the low micromolar range. No inhibition of tubulin assembly was observed with two of the most cytotoxic molecules. It cannot be determined whether the lack of microtubule activity is a real phenomenon or was unable to be detected under the assay conditions. Synthesis of additional molecules in this class to further elaborate the structure–activity relationships and to increase the aqueous solubilities of the molecules is underway.

5. Experimental

5.1. Synthesis

Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. IR spectra were recorded on a Brucker FT-IR spectrophotometer using KBr pellets. ^{1}H NMR spectra were recorded on a Varian-Mercury 200 MHz spectrometer in DMSO-d_6. Chemical shifts were expressed in parts per million (ppm) with tetramethylsilane (TMS) as an internal standard. Elemental analyses (C, H, and N) were performed at the National Research Center, Cairo, Egypt. All compounds were routinely checked by thin-layer chromatography (TLC) on aluminum-backed silica gel plates. All solvents were dried by standard methods. Compounds (**1**–**6**) were reported previously.[17,18]

5.1.1. General procedure for 2,5-diaryl-3-methylpyrimido[4,5-*c*]quinolin-1(2*H*)-ones (7–30). A mixture of the appropriate 1*H*-[1,3]oxazino[4,5-*c*]quinolin-1-one (**5** and **6**; 10 mmol), the appropriate substituted aniline (10 mmol), and fused sodium acetate (1.64 g, 20 mmol) in glacial acetic acid (20 mL) was heated at reflux for 24 h. After cooling, the precipitated solid was filtered, washed with cold ethanol, and dried. The crude product was purified by column chromatography on silica gel using petroleum ether/ethyl acetate as a gradient eluent followed by recrystallization from glacial acetic acid.

5.1.1.1. 5-(4-Chlorophenyl)-3-methyl-2-phenylpyrimido[4,5-*c*]quinolin-1(2*H*)-one (7). Yield 54%, mp 261–262 °C; ^{1}H NMR (DMSO-d_6) δ: 2.21 (s, 3H, CH_3), 7.51 (d, 3H, J = 8.19 Hz, Ar-H), 7.74–7.89 (m, 6H, Ar-H), 8.08 (d, 2H, J = 8.39 Hz, Ar-H), 8.18 (d, 1H, J = 7.59 Hz, Ar-H), 9.52 (d, 1H, J = 7.99 Hz, Ar-H). Anal. Calcd for $C_{24}H_{16}ClN_3O$: C, 72.45; H, 4.05; N, 10.56. Found: C, 72.25; H, 4.14; N, 10.75.

5.1.1.2. 5-(4-Chlorophenyl)-2-(4-fluorophenyl)-3-methylpyrimido[4,5-*c*]quinolin- 1(2*H*)-one (8). Yield 61%, mp 243–245 °C; ^{1}H NMR (DMSO-d_6) δ: 2.22 (s, 3H, CH_3), 7.43–7.52 (m, 2H, Ar-H), 7.61 (d, 2H, J = 7.99 Hz, Ar-H), 7.79–7.87 (m, 2H, Ar-H), 8.16–8.20 (m, 3H, Ar-H), 9.54 (d, 3H, J = 7.79 Hz, Ar-H). Anal. Calcd for $C_{24}H_{15}ClFN_3O$: C, 69.32; H, 3.64; N, 10.10. Found: C, 69.37; H, 3.71; N, 10.22.

5.1.1.3. 2-(2-Chlorophenyl)-5-(4-chlorophenyl)-3-methylpyrimido[4,5-*c*]quinolin-1(2*H*)-one (9). Yield 59%, mp 236–238 °C; ^{1}H NMR (DMSO-d_6) δ: 2.22 (s, 3H, CH_3), 7.56–7.90 (complex m, 7H, Ar-H), 8.15–8.22 (m, 3H, Ar-H), 9.51 (d, 2H, J = 7.99 Hz, Ar-H). Anal. Calcd for $C_{24}H_{15}Cl_2N_3O$: C, 66.68; H, 3.50; N, 9.72. Found: C, 66.67; H, 3.50; N, 9.92.

5.1.1.4. 2-(3-Chlorophenyl)-5-(4-chlorophenyl)-3-methylpyrimido[4,5-*c*]quinolin-1(2*H*)-one (10). Yield 63%, mp 237–240 °C; ^{1}H NMR (DMSO-d_6) δ: 2.22 (s, 3H, CH_3), 7.55–7.89 (complex m, 6H, Ar-H), 8.15–8.21 (m, 3H, Ar-H), 9.51 (d, 3H, J = 8.39 Hz, Ar-H). Anal. Calcd for $C_{24}H_{15}Cl_2N_3O$: C, 66.68; H, 3.50; N, 9.72. Found: C, 66.50; H, 3.51; N, 9.95.

5.1.1.5. 2,5-Bis(4-chlorophenyl)-3-methylpyrimido[4,5-*c*]quinolin-1(2*H*)-one (11). Yield 51%, mp 268–269 °C; IR (KBr, cm^{-1}): 1690 (C=O). ^{1}H NMR (DMSO-d_6) δ: 2.21 (s, 3H, CH_3), 7.57–7.89 (complex m, 8H, Ar-H), 8.15–8.21 (m, 3H, Ar-H), 9.51 (d, 1H, J = 7.59 Hz, Ar-H). Anal. Calcd for $C_{24}H_{15}Cl_2N_3O$: C, 66.68; H, 3.50; N, 9.72. Found: C, 66.72; H, 3.64; N, 9.82.

5.1.1.6. 2-(2-Bromophenyl)-5-(4-chlorophenyl)-3-methylpyrimido[4,5-*c*]quinolin-1(2*H*)-one (12). Yield 56%, mp 218–220 °C; ^{1}H NMR (DMSO-d_6) δ: 2.22 (s, 3H, CH_3), 7.59–7.65 (m, 3H, Ar-H), 7.75–7.88 (m, 3H, Ar-H), 8.15–8.22 (m, 3H, Ar-H), 9.52 (d, 3H, J = 7.99 Hz, Ar-H). Anal. Calcd for $C_{24}H_{15}BrClN_3O$: C, 60.46; H, 3.17; N, 8.81. Found: C, 60.24; H, 3.17; N, 8.82.

5.1.1.7. 2-(3-Bromophenyl)-5-(4-chlorophenyl)-3-methylpyrimido[4,5-*c*]quinolin-1(2*H*)-one (13). Yield 58%, mp 218–219 °C; ^{1}H NMR (DMSO-d_6) δ: 2.22 (s, 3H, CH_3), 7.59–7.64 (m, 3H, Ar-H), 7.75–7.87 (m, 4H, Ar-H), 8.15–8.21 (m, 2H, Ar-H), 9.51 (d, 3H, J = 7.99 Hz, Ar-H). Anal. Calcd for $C_{24}H_{15}BrClN_3O$: C, 60.46; H, 3.17; N, 8.81. Found: C, 60.35; H, 3.42; N, 8.74. Anal. Calcd for $C_{24}H_{15}BrClN_3O$: C, 60.46; H, 3.17; N, 8.81. Found: C, 60.35; H, 3.42; N, 8.74.

5.1.1.8. 2-(4-Bromophenyl)-5-(4-chlorophenyl)-3-methylpyrimido[4,5-*c*]quinolin-1(2*H*)-one (14). Yield 65%, mp 282–284 °C; ^{1}H NMR (DMSO-d_6) δ: 2.21 (s, 3H, CH_3), 7.50 (d, 2H, J = 7.59 Hz, Ar-H), 7.60 (d, 2H, J = 8.19 Hz, Ar-H), 7.74–7.86 (m, 3H, Ar-H), 8.15–8.19 (m, 3H, Ar-H), 9.51 (d, 2H, J = 7.99 Hz, Ar-H). Anal. Calcd for $C_{24}H_{15}BrClN_3O$: C, 60.46; H, 3.17; N, 8.81. Found: C, 60.28; H, 3.38; N, 8.81.

5.1.1.9. 5-(4-Chlorophenyl)-2-(2-methylphenyl)-3-methylpyrimido[4,5-*c*]quinolin-1(2*H*)-one (15). Yield 49%, mp 193–196 °C; ^{1}H NMR (DMSO-d_6) δ: 2.10 (s, 3H, CH_3), 2.16 (s, 3H, CH_3), 7.38–7.57 (m, 3H, Ar-H), 7.60 (d, 2H, J = 6.99 Hz, Ar-H), 7.75–7.90 (m, 2H, Ar-H), 8.16 (d, 3H, J = 6.59 Hz, Ar-H), 9.54 (d, 2H, J = 7.99 Hz, Ar-H). Anal. Calcd for $C_{25}H_{18}ClN_3O$: C, 72.90; H, 4.40; N, 10.20. Found: C, 72.93; H, 4.28; N, 10.20.

5.1.1.10. 5-(4-Chlorophenyl)-2-(4-methylphenyl)-3-methylpyrimido[4,5-*c*]quinolin-1(2*H*)-one (16). Yield 53%, mp 231–232 °C; ^{1}H NMR (DMSO-d_6) δ: 2.20 (s, 3H, CH_3), 2.43 (s, 3H, CH_3), 7.32–7.51 (m, 4H, Ar-H), 7.60 (d, 1H, J = 8.19 Hz, Ar-H), 7.78–7.87 (m, 2H, Ar-H), 8.15–8.27 (m, 3H, Ar-H), 9.55 (d, 2H, J = 8.39 Hz, Ar-H). Anal. Calcd for $C_{25}H_{18}ClN_3O$: C, 72.90; H, 4.40; N, 10.20. Found: C, 73.02; H, 3.77; N, 10.22.

5.1.1.11. 5-(4-Chlorophenyl)-2-(2-methoxyphenyl)-3-methylpyrimido[4,5-*c*]quinolin-1(2*H*)-one (17). Yield 57%, mp 227–228 °C; IR (KBr, cm^{-1}): 1685 (C=O). ^{1}H NMR (DMSO-d_6)δ: 2.19 (s, 3H, CH_3), 3.79 (s, 3H, OCH_3), 7.15–7.23 (t, 1H, Ar-H), 7.30 (d, 1H, J = 8.19 Hz, Ar-H), 7.47–7.63 (m, 2H, Ar-H), 7.75–7.90 (m, 2H, Ar-H), 8.15–8.22 (m, 3H, Ar-H), 9.53 (d, 3H, J = 8.19 Hz, Ar-H). Anal. Calcd for $C_{25}H_{18}ClN_3O_2$: C, 70.18; H, 4.24; N, 9.82. Found: C, 70.13; H, 4.77; N, 9.91.

5.1.1.12. 5-(4-Chlorophenyl)-2-(4-methoxyphenyl)-3-methylpyrimido[4,5-*c*]quinolin-1(2*H*)-one (18). Yield 62%, mp 278–280 °C; IR (KBr, cm^{-1}): 1683 (C=O). ^{1}H NMR (DMSO-d_6)δ: 2.20 (s, 3H, CH_3), 3.86 (s, 3H, OCH_3), 7.13 (d, 2H, J = 8.59 Hz, Ar-H), 7.41 (d, 1H, J = 8.19 Hz, Ar-H), 7.59 (d, 1H, J = 8.19 Hz, Ar-H), 7.73–7.88 (m, 3H, Ar-H), 8.15 (d, 3H, J = 8.19 Hz, Ar-H), 9.53 (d, 2H, J = 8.19 Hz, Ar-H). Anal. Calcd for $C_{25}H_{18}ClN_3O_2$: C, 70.18; H, 4.24; N, 9.82. Found: C, 70.18; H, 4.44; N, 9.98.

5.1.1.13. 5-(4-Bromophenyl)-3-methyl-2-phenylpyrimido[4,5-*c*]quinolin-1(2*H*)-one (19). Yield 55%, mp 264–265 °C; ^{1}H NMR (DMSO-d_6) δ: 2.22 (s, 3H, CH_3), 7.51 (d, 2H, J = 7.59 Hz, Ar-H), 7.57–7.65 (m, 2H, Ar-H), 7.73–7.86 (m, 3H, Ar-H), 8.11 (d, 2H, J = 7.39 Hz, Ar-H), 8.18 (d, 1H, J = 8.19 Hz, Ar-H), 9.54 (d, 3H, J = 7.99 Hz, Ar-H). Anal. Calcd for $C_{24}H_{16}BrN_3O$: C, 65.17; H, 3.65; N, 9.50. Found: C, 64.67; H, 3.63; N, 9.47.

5.1.1.14. 5-(4-Bromophenyl)-2-(4-fluorophenyl)-3-methylpyrimido[4,5-*c*]quinolin-1(2*H*)-one (20). Yield 69%, mp 250–251 °C; ^{1}H NMR (DMSO-d_6) δ: 2.21 (s, 3H, CH_3), 7.44–7.52 (m, 2H, Ar-H), 7.58–7.65 (m, 1H, Ar-H), 7.74–7.90 (m, 3H, Ar-H), 8.08 (d, 2H, J = 8.19 Hz, Ar-H), 8.18 (d, 1H, J = 7.59 Hz, Ar-H), 9.54 (d, 3H, J = 6.59 Hz, Ar-H). Anal. Calcd for $C_{24}H_{15}BrFN_3O$: C, 62.62; H, 3.28; N, 9.13. Found: C, 62.47; H, 3.27; N, 8.95.

5.1.1.15. 5-(4-Chlorophenyl)-2-(2-methoxyphenyl)-3-methylpyrimido[4,5-*c*]quinolin-1(2*H*)-one (17). Yield 59%, mp 235–236 °C; ^{1}H NMR (DMSO-d_6) δ: 2.20 (s, 3H, CH_3), 7.65–7.88 (complex m, 7H, Ar-H), 8.07 (d, 2H, J = 7.59 Hz, Ar-H), 8.19 (d, 1H, J = 7.99 Hz, Ar-H), 9.50 (d, 2H, J = 7.99 Hz, Ar-H). Anal. Calcd for $C_{24}H_{15}BrClN_3O$: C, 60.46; H, 3.17; N, 8.81. Found: C, 60.74; H, 2.75; N, 8.95.

5.1.1.16. 5-(4-Bromophenyl)-2-(3-chlorophenyl)-3-methylpyrimido[4,5-*c*]quinolin-1(2*H*)-one (22). Yield 52%, mp 221–223 °C; ^{1}H NMR (DMSO-d_6) δ: 2.22 (s, 3H, CH_3), 7.58–7.61 (m, 2H, Ar-H), 7.73–7.86 (m, 4H, Ar-H), 8.09 (d, 2H, J = 7.99 Hz, Ar-H), 8.17 (d, 1H, J = 7.99 Hz, Ar-H), 9.51 (d, 3H, J = 7.99 Hz, Ar-H). Anal. Calcd for $C_{24}H_{15}BrClN_3O$: C, 60.46; H, 3.17; N, 8.81. Found: C, 60.51; H, 3.57; N, 8.95.

5.1.1.17. 5-(4-Bromophenyl)-2-(4-chlorophenyl)-3-methylpyrimido[4,5-*c*]quinolin-1(2*H*)-one (23). Yield 61%, mp 293–295 °C; ^{1}H NMR (DMSO-d_6) δ: 2.20 (s, 3H, CH_3), 7.56–7.60 (m, 2H, Ar-H), 7.68–7.76 (m, 6H, Ar-H), 8.07 (d, 2H, J = 6.99 Hz, Ar-H), 8.16 (d, 1H, J = 7.59 Hz, Ar-H), 9.50 (d, 1H, J = 6.59 Hz, Ar-H). Anal. Calcd for $C_{24}H_{15}BrClN_3O$: C, 60.46; H, 3.17; N, 8.81. Found: C, 60.08; H, 3.27; N, 8.88.

5.1.1.18. 2-(2-Bromophenyl)-5-(4-bromophenyl)-3-methylpyrimido[4,5-*c*]quinolin-1(2*H*)-one (24). Yield 51%, mp 231–234 °C; ^{1}H NMR (DMSO-d_6) δ: 2.19 (s, 3H, CH_3), 7.52–7.60 (m, 2H, Ar-H), 7.65 (d, 1H, J = 7.99 Hz, Ar-H), 7.74 (d, 2H, J = 7.79 Hz, Ar-H), 7.84 (d, 1H, J = 7.99 Hz, Ar-H), 7.94 (d, 1H, J = 7.99 Hz, Ar-H), 8.08 (d, 2H, J = 7.19 Hz, Ar-H), 8.20 (d, 1H, J = 7.79 Hz, Ar-H), 9.51 (d, 2H, J = 8.19 Hz, Ar-H). Anal. Calcd for $C_{24}H_{15}Br_2N_3O$: C, 55.31; H, 2.90; N, 8.06. Found: C, 55.22; H, 2.90; N, 7.88.

5.1.1.19. 2-(3-Bromophenyl)-5-(4-bromophenyl)-3-methylpyrimido[4,5-*c*]quinolin-1(2*H*)-one (25). Yield 53%, mp 241–242 °C; ^{1}H NMR (DMSO-d_6) δ: 2.23 (s, 3H, CH_3), 7.63–7.83 (complex m, 6H, Ar-H), 8.08 (d, 2H, J = 6.99 Hz, Ar-H), 8.18 (d, 1H, J = 7.59 Hz, Ar-H), 9.51 (d, 3H, J = 7.99 Hz, Ar-H). Anal. Calcd for $C_{24}H_{15}Br_2N_3O$: C, 55.31; H, 2.90; N, 8.06. Found: C, 55.26; H, 2.86; N, 7.96.

5.1.1.20. 2,5-Bis(4-bromophenyl)-3-methylpyrimido[4,5-*c*]quinolin-1(2*H*)-one (26). Yield 62%, mp > 300 °C; ^{1}H NMR (DMSO-d_6) δ: 2.20 (s, 3H, CH_3), 7.51–7.64 (m, 6H, Ar-H), 7.74–7.89 (m, 2H, Ar-H), 8.16 (d, 3H, J = 8.19 Hz, Ar-H), 9.54 (d, 1H, J = 7.99 Hz, Ar-H). Anal. Calcd for $C_{24}H_{15}Br_2N_3O$: C, 55.31; H, 2.90; N, 8.06. Found: C, 55.16; H, 2.86; N, 8.08.

5.1.1.21. 5-(4-Bromophenyl)-2-(2-methylphenyl)-3-methylpyrimido[4,5-*c*]quinolin-1(2*H*)-one (27). Yield 51%, mp 206–207 °C; ^{1}H NMR (DMSO-d_6) δ: 2.10 (s, 3H, CH_3), 2.17 (s, 3H, CH_3), 7.47–7.51 (m, 3H, Ar-H), 7.74–7.87 (m, 4H, Ar-H), 8.09 (d, 2H, J = 8.19 Hz, Ar-H), 8.19 (d, 1H, J = 7.59 Hz, Ar-H), 9.54 (d, 2H, J = 8.39 Hz, Ar-H). Anal. Calcd for $C_{25}H_{18}BrN_3O$: C, 65.80; H, 3.98; N, 9.21. Found: C, 65.54; H, 3.63; N, 8.93.

5.1.1.22. 5-(4-Bromophenyl)-2-(4-methylphenyl)-3-methylpyrimido[4,5-*c*]quinolin-1(2*H*)-one (28). Yield 56%, mp 256–258 °C; ^{1}H NMR (DMSO-d_6) δ: 2.20 (s, 3H, CH_3), 2.43 (s, 3H, CH_3), 7.37–7.48 (m, 4H, Ar-H), 7.73–7.89 (m, 4H, Ar-H), 8.08 (d, 2H, J = 7.99 Hz, Ar-H), 8.17 (d, 1H, J = 7.99 Hz, Ar-H), 9.53 (d, 1H, J = 7.99 Hz, Ar-H). Anal. Calcd for $C_{25}H_{18}BrN_3O$: C, 65.80; H, 3.98; N, 9.21. Found: C, 65.45; H, 3.42; N, 9.04.

5.1.1.23. 5-(4-Bromophenyl)-2-(2-methoxyphenyl)-3-methylpyrimido[4,5-*c*]quinolin-1(2*H*)-one (29). Yield 47%,

mp 228–230 °C; ^{1}H NMR (DMSO-d_6) δ: 2.19 (s, 3H, CH_3), 3.79 (s, 3H, OCH_3), 7.15–7.23 (t, 1H, Ar-H), 7.30 (d, 1H, J = 7.79 Hz, Ar-H), 7.47 (d, 1H, J = 7.59 Hz, Ar-H), 7.54–7.62 (t, 1H, Ar-H), 7.73–7.89 (m, 3H, Ar-H), 8.07 (d, 2H, J = 8.59 Hz, Ar-H), 8.17 (d, 1H, J = 7.99 Hz, Ar-H), 9.53 (d, 2H, J = 7.59 Hz, Ar-H). Anal. Calcd for $C_{25}H_{18}BrN_3O_2$: C, 63.57; H, 3.84; N, 8.90. Found: C, 63.51; H, 3.72; N, 8.81.

5.1.1.24. 5-(4-Bromophenyl)-2-(4-methoxyphenyl)-3-methylpyrimido[4,5-*c*]quinolin-1(2*H*)-one (30). Yield 53%, mp 299–300 °C; ^{1}H NMR (DMSO-d_6) δ: 2.21 (s, 3H, CH_3), 3.86 (s, 3H, OCH_3), 7.13 (d, 2H, J = 8.59 Hz, Ar-H), 7.41 (d, 2H, J = 8.19 Hz, Ar-H), 7.73–7.85 (m, 4H, Ar-H), 8.08 (d, 2H, J = 8.19 Hz, Ar-H), 8.17 (d, 1H, J = 7.99 Hz, Ar-H), 9.54 (d, 1H, J = 8.59 Hz, Ar-H). Anal. Calcd for $C_{25}H_{18}BrN_3O_2$: C, 63.57; H, 3.84; N, 8.90. Found: C, 63.13; H, 3.72; N, 8.92.

5.2. Biology

5.2.1. Solubility studies. A stock solution of each compound was prepared in 100% DMSO. Four hundred microliter of DMSO was added to 5 mg of each compound and the solutions were mixed thoroughly. The undissolved fractions were removed by centrifuging the solutions in microfuge (Eppendorf centrifuge 5414) at 5600*g* for 10 min. The supernatant was collected and the concentration of each compound in DMSO was determined by absorption spectroscopy, using extinction coefficients (determined in DMF) at 375 nm. The solubility of each compound was evaluated in 4% DMSO. Twenty microliter of each stock solution was added to 480 μL of PME (0.1 M PIPES, 1 mM $MgSO_4$, and 2 mM EGTA, pH 6.90) buffer and the solutions were mixed well. The absorption spectrum of the resulting solutions was obtained. Since we do not know anything about the solubility of these molecules in 4% DMSO solution, we used a standard assay to find out the exact soluble concentration of each molecule. For this reason, the absorption of each molecule in DMSO was obtained and a wavelength range (500–600 nm) was chosen where the apparent absorption is close to zero and parallel to wavelength (*x*-) axis. Then apparent absorption of each molecule in 4% DMSO in the wavelength range of 500–600 nm was obtained. If this apparent absorption was found to be greater than same concentration of the molecules in DMSO, it was concluded that the solutions contain undissolved particles. In that case, the concentration of molecule was decreased and the apparent absorption was measured again. The experiment was repeated until the absorption spectrum of each compound is comparable with their corresponding absorption spectra in 100% DMSO. This concentration of each compound was deemed to be the maximum ligand concentration that can be used in the tubulin polymerization experiment.

5.2.2. In vitro cytotoxicity. The new compounds were evaluated in vitro for cytotoxic activity using the MTT assay as described previously.[21] Human prostate carcinoma (PC3) cells (purchased from ATCC, American Type Culture Collection) were cultured in T-75 flasks in RPMI-1640 medium at 37 °C supplemented with 10% fetal calf serum, 5 IU/mL of penicillin, and 5 μg/mL of streptomycin in a 5% CO_2 air atmosphere. When the cells became confluent, the old RPMI-1640 medium was discarded and the cells were washed twice with PBS buffer. Then the cells were subjected to trypsinization (50 mg/T-75 flask) and were incubated at 37 °C for 15 min to dislodge the cells from the flask. The cells were transferred into a centrifuge tube and RPMI-1640 medium was added to neutralize trypsin activity. The cells were centrifuged (1200*g*, 8 min) and appropriate volume of medium was added and was thoroughly triturated. Prior to plating, the cells were counted by using hemocytometer. Approximately 15,000 cells were plated to each well of a 96-well plate and were allowed to grow for two days. After two days, the cells were treated with various concentrations of the compounds to be tested and incubated for three days. Appropriate controls for each compound were included in each plate. After three days, the old medium was discarded and 50 μL of medium containing 50 μg MTT was added to each well and incubated for an additional 4 h. The reaction was quenched by adding 200 μL of isopropanol to each well. The solution in each well was thoroughly mixed so that the solution became homogeneous resulting in the development of a purple color. The absorbance was detected at 570 nm (single wavelength) using an ultra microplate reader (EL_X 808) and IC_{50} of each compound was determined by plotting absorbance at 570 nm versus ligand concentrations. For each compound the experiment was performed in duplicate and IC_{50} was determined from at least two independent experiments.

5.2.3. Microtubule assembly assays. MTP pellets were thawed and desalted in PME buffer using 1 ml Sephadex-G 50 columns. MTP (10 μM, which is the minimum concentration of MTP needed to polymerize in vitro at 37 °C in presence of 1 mM GTP) was incubated with the test compounds in PME buffer (4% DMSO) at 25 °C for 40 min. Polymerization was initiated at elevated temperature (37 °C) by adding GTP to a final concentration of 2 mM. Polymerization kinetics were monitored by apparent light scattering at 350 nm on a Hewlett–Packard 8453 absorption spectrophotometer with a thermostatic multicell holder that was maintained at 37 °C. The reaction was terminated when no change in the rate was observed. Differences in absorbance (ΔA) values were calculated from each of the absorbance versus time graphs. Appropriate control experiments containing only MTP (4% DMSO) were performed. Inhibition of polymerization was determined from the change in absorbance of sample and corresponding control.[22] The effects of compounds **13** and **18** on the assembly of purified bovine brain tubulin were also assessed in a similar fashion. Since the minimum concentration required to polymerize purified tubulin in the presence of GTP is 20 μM, the tubulin concentrations employed were 30 and 50 μM.

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