6-Alkylamino- and 2,3-Dihydro-3′-methoxy-2-phenyl-4-quinazolinones and Related Compounds: Their Synthesis, Cytotoxicity, and Inhibition of Tubulin Polymerization

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Received June 14, 2000

As part of our continuing search for potential anticancer candidates among 2-phenyl-4-quinolones and 2-phenyl-4-quinazolinones, two series of 6,7,2′,3′,4′,5′-substituted 2-phenyl-4-quinazolinones and 6,2′,3′,4′,5′-substituted 2,3-dihydro-2-phenyl-4-quinazolinones were synthesized and evaluated for cytotoxicity and as inhibitors of tubulin polymerization. In general, a good correlation was found between the two activities. Five of the 6-substituted heterocyclic 2-phenyl-4-quinozolinones (**37**−**51**) showed significant cytotoxicity against a panel of human tumor cell lines with EC_{50} values in the low micromolar to nanomolar concentration ranges. Compound **38** was the most potent of these compounds, as well as the most potent inhibitor of tubulin polymerization in this series. The activity of **38** was in the same range as those of the antimitotic natural products, colchicine, podophyllotoxin, and combretastatin A-4. Substituted 2-phenyl-4-quinazolinones and 2,3-dihydro-2-phenyl-4-quinazolinones also displayed highly selective cytotoxicity against the ovarian cancer 1A9 and P-gp resistant KB-VIN cell lines.

Introduction

Microtubules provide an important framework supporting cellular morphology in interphase, and they are essential in cell division as the key component of the mitotic spindle. Consequently, the microtubule has become an important target for the design of new antimitotic anticancer agents. The antimitotic agents currently in clinical use include vinca alkaloids,[1] which inhibit microtubule polymerization, and taxoids,[2] which promote microtubule assembly. Colchicine[3,4] is another well-known antimitotic agent. However, it is too toxic to be used as an anticancer agent, although it has clinical utility in gout and other inflammatory diseases.

In recent years, we have designed and synthesized three types of heterocyclic ketones, 2-phenyl-4-quinolones (PQ),[4−7] 2,3-dihydro-2-phenyl-4-quinolones (DHPQ),[8] and 2-phenyl-1,8-naphthyridin-4-ones (PN)[8−10] (Chart 1) as novel antimitotic agents. Among these three types of heterocyclic ketones, the common structural feature is a biaryl system composed of A- and C-rings that are linked by an interposed B-ring. However, some minor structural differences also exist. Preliminary structure−activity relationships (SARs) for these three series are described below.

In the PQ system, when functional groups with nonbonding electrons, e.g. $-OCH_3$, $-OCH_2O-$, $-NRR'$, Cl, and F (PQ_{1-7}), were placed at the 6-position of the A-ring and the 3′-position of the C-ring, activity was very potent (Chart 1). These two functional groups are about 10−11 Å apart, and these two groups possibly may interact with the tubulin binding domain by acting as receptors in hydrogen bonding. Thus, they might contribute significantly to the potency of PQ compounds. In the DHPQ system[8], the 6- and 3′-substituents ($DHPQ_1$) also play a decisive role in activity. However, in the PN system,[9,10] when the 3′-substituent is fixed (e.g. OCH_3), the identity of the 6-substituent, e.g. H (PN-1), CH_3 (PN-2), or C1 (PN-3), does not noticeably affect activity. This finding is unique to the PN system and differentiates it from the PQ and DHPQ systems.

Phenylquinazolines (PQZ) and dihydrophenylquinazolines (DHPQZ) are additional related compound classes (Chart 1). The antitumor activities of 2,3-dihydro-2-aryl-4-quinazolinones (DHPQZ) were reported around 1970.[11,12] A more recent reevaluation of this type of compound by NCI against human tumor cell lines reconfirmed that, like colchicine, they are effective inhibitors of tubulin polymerization.[13] Somewhat earlier, 2-styrylquinazolin-4-ones (SQZ)[14,15] were also identified as potent inhibitors of tubulin polymerization, but in this series the most active compounds have a hydrocarbon bridge between the B- and C-rings. More recently, we prepared a series of 6,7-methoxy-2-aryl quinazolin-4-one derivatives (PQZ) for evaluation of their cytotoxicity and inhibitory effects on tubulin polymerization.[16] Although a few 6-methoxy derivatives demonstrated moderate activity, most of the compounds were essentially inactive.

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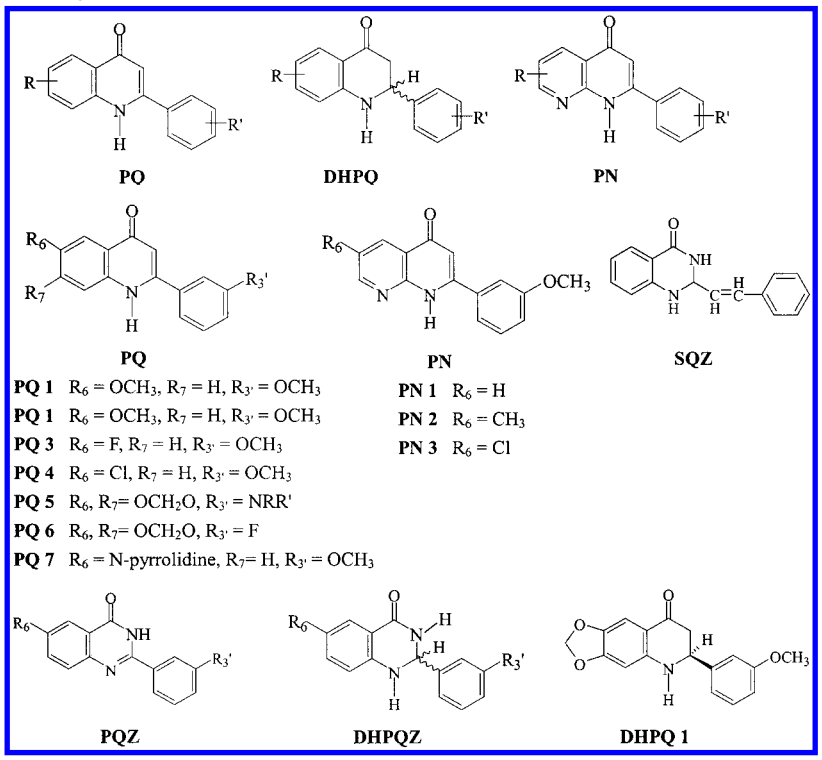
10.1021/jm000151c CCC: $19.00
Published on Web 10/28/2000

Chart 1. Antimitotic Heterocyclic Ketones

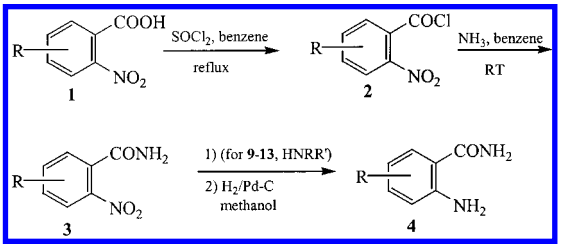


The PQ, PN, and PQZ series are related as bioisosteres; the latter two series contain a second nitrogen in the A- and B-rings, respectively, rather than a carbon as found in the PQ series. Thus, their structures, physicochemical properties, and pharmacokinetics as well as SAR results likely are similar but not identical. Definitive SAR must be first established for each class before formulating hypotheses or pharmacophores through QSAR molecular modeling. Accordingly, we chose the relatively less investigated 2-phenylquinazolin-4-one (PQZ) and 2,3-dihydro-2-phenylquinazolin-4-one (DHPQZ) as lead compounds and prepared additional derivatives, including 3′-methoxy-6-alkylamino analogues, as our targets. These PQZ and DHPQZ derivatives were evaluated for cytotoxicity and inhibition of tubulin polymerization in order to add to our understanding of microtubule binding and extend our SAR knowledge. The goal of our continuing studies is to identify novel compounds for in vivo testing and, ultimately, to aid in new antimitotic drug design.

Chemistry

Starting 4,5-substituted 2-aminobenzamides (**5**−**13**) were prepared using standard methodology[8] from substituted 2-nitrobenzoic acids by reaction with $SOCl_2$, NH_3, HNRR″ (for **9**−**13**), and H_2 as shown in the general reaction sequence in Scheme 1. The starting benzamides (**5**−**15**) were reacted with methoxybenzaldehydes (**16**−**23**) in *N*,*N*-dimethylacetamide (DMAC) in the presence of $NaHSO_3$ at 150 °C (Scheme 2). Thermal cyclodehydration/dehydrogenation gave the target substituted 2-phenyl-4-quinazolinones (**24**−**41**) in high yields. However, to obtain the 2,3-dihydro-2-phenyl-4-quinazolinones (**42**−**50**), **14** and **15** were reacted with **16**−**23** using modified reaction conditions as shown in Scheme 3 and described below for 2,3-dihydro-3′-methoxy-2-phenyl-4-quinazolinone (**43**).

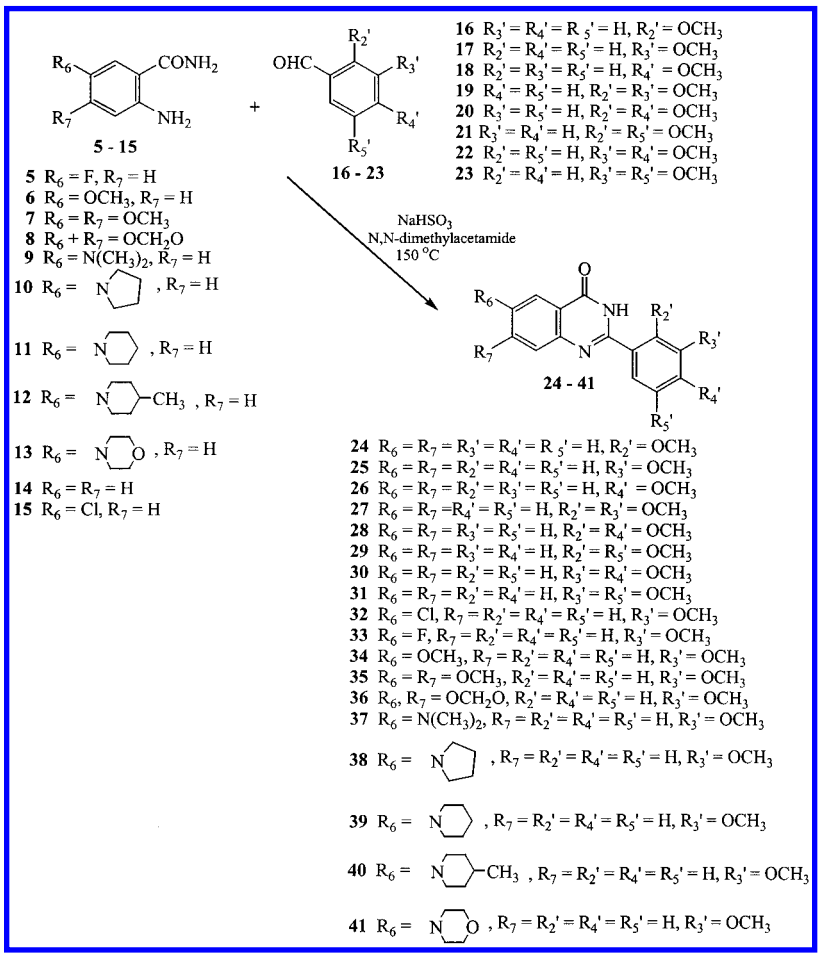
Scheme 1



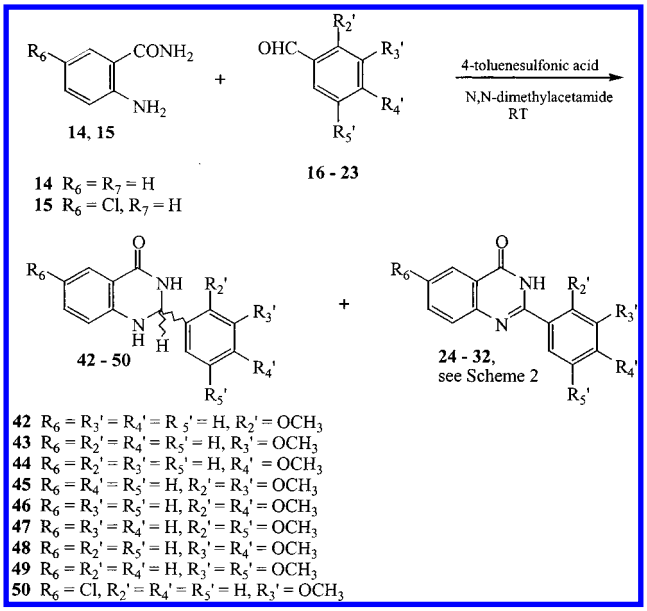
A mixture of 2-aminobenzamide (**14**) and 3-methoxybenzaldehyde (**17**) in DMAC was heated to 80 °C for 1 h. Subsequent purification with column chromatography afforded **43** (mp 148−150 °C) and **25** (mp 177−179 °C) in yields of 75% and 15%, respectively. The chemical structure of **25** was confirmed by IR, NMR, and MS spectral analysis as 3′-methoxy-2-phenyl-4-quinazolinone. For **43,** the molecular formula was determined by elemental and MS analysis (*m*/*z* 254, M^+) as $C_{15}H_{14}N_2O_2$, which matches the expected 2,3-dihydro-3′-methoxy-2-phenyl-4-quinazolinone. The ^1H NMR of **43** showed three additional proton signals not seen in the spectrum of **25**: 5.73 (H-2), 7.15 (N_1-H), and 8.34 (N_3-H). The 2D HMBC spectrum of **43** showed the expected long-range coupling between H-2 and C-4 (δ 163.77), C-8a (δ 147.98), C-2′ (δ 119.11), and C-6′ (δ 112.75). All spectral data confirmed our assignment of **43** as 2,3-dihydro-3′-methoxy-2-phenyl-4-quinazolinone.

To increase the yield of **43**, reaction conditions were adjusted to minimize the unwanted dehydrogenation of **43** and conversion to **25**. First, we lowered the reaction temperature to 20 ± 2 °C and extended the reaction time to 4 h. However, these conditions only resulted in a lower conversion as indicated by the considerable amount of starting materials (**14**, **17**) that was detected by TLC in the reaction mixture. Next, we again carried out the reaction, at 20 ± 2 °C in DMAC, but also

Scheme 2



Scheme 3



incorporated a catalytic amount of *p*-toluenesulfonic acid. The reaction was completed in 30 min, and the subsequent workup resulted in a high yield (89.0%) of **43** and a minor yield (4.0%) of **25**. Consequently, the same reaction condition was adopted in the subsequent preparation of **42** and **44**−**50** and resulted in high yields of all desired products.

When various benzamides (**6**−**15**) with electron-donating substituents (e.g. −OCH_3, −OCH_2O−, −NRR′) were used as starting materials under these conditions, TLC check analysis of the crude products did show materials resembling the expected 2,3-dihydro derivatives. However, they were readily converted to their corresponding dehydrogenated derivatives **(34**−**41)** via spontaneous dehydrogenation during the workup procedures, such as column chromatography or recrystallization. Also, although F and Cl belong to the same class of electron-withdrawing group, the fluorinated 2,3-dihydro derivative was much less stable than the Cl-containing counterpart (**50**) and readily underwent spontaneous dehydrogenation to give **33** during the final workup. Thus, 2,3-dihydro-2-phenyl-4-quinazolones containing electron-donating groups or fluorine at the 6- or 7-position were not prepared for cytotoxic evaluation, as they would likely undergo spontaneous dehydrogenation and would not be stable in the solvent required for biological testing.

All 2,3-dihydro products (**42**−**50**) were prepared as racemic mixtures. Resolution of these mixtures has not been attempted in this work but will be pursued if unusual biological activity is found in our forthcoming studies.

Results and Discussion

a. Evaluation of Cytotoxicity of PQZ and DH-PQZ Derivatives. The 6,7,2′,3′,4′,5′-substituted 2-phenyl-4-quinazolinones (**24**−**32**) and 6,2′,3′,4′,5′-substituted 2,3-dihydro-2-phenyl-4-quinazolinones (**42**−**50**) were as-

sayed for cytotoxicity in vitro against 10 human tumor cell lines, including ovarian cancer (1A9), ileocecal carcinoma (HCT-8), lung carcinoma (A-549), glioblastoma (U-87-MG), bone (HOS), epidermoid carcinoma of the nasopharynx (KB), P-gp-expressing epidermoid carcinoma of the nasopharynx (KB-VIN), prostate cancer (PC3), breast cancer (MCF-7), and melanoma (SK-MEL-2) cell lines. As shown in Table 2, the 6-alkylamino-substituted **37**−**40** showed strong cytotoxic effects toward a variety of tumor cell lines, with EC_{50} values < 1.0 μg/mL, but **37** was inactive in the U-87-MG or HOS cell lines. Notably, **37**−**40** showed highly selective effects on the ovarian cancer line (1A9). For example, the EC_{50} value of **37** in the 1A9 cell line was 0.49 μM, and growth from this more sensitive cell line was arrested at a concentration approximately 20−30 times lower than those in less sensitive cell lines (e.g. A-549, U087-MG, PC3, and SKMEL-2). Similarly, high antiovarian cell selectivity was also found with **38**−**40**. In addition, **37**−**40** displayed increased activity against the P-gp-expressing cell line KB-VIN relative to the parental cell line (KB), with **37** and **40** showing about 25 and 15 times greater activity against the drug-resistant cell line. Although the structural difference between **37** and **38** is very small, **38** was significantly more cytotoxic. However, **38** showed reduced selectivity against KB-VIN.

In terms of SAR information, PQZ derivatives without C-6 substitutions (**24**−**31**) were inactive. In comparing the effects of electron-donating or electron-withdrawing groups at the 6-position, the compound with an electron-donating methoxy (**34**) was more active than the electron-withdrawing 6-C1 (**32**) or 6-F (**33**) PQZ derivatives. The (methylenendioxy)benzene moiety occurs commonly in many antimitotic agents, such as podophyllotoxin, steganacin, and combretastatin A-2. However, the 6,7-(methylenedioxy)-substituted compound (**36**) did not show significantly increased activity compared with either an unsubstituted (**35**), 6-methoxy (**34**), or 6,7-dimethoxy (**35**) compound. Single substitution at the 6-position seemed to be beneficial for increased antitumor activity. The 6-methoxy compound (**34**) was about 5-fold more active than its corresponding 6,7-dimethoxy analogue (**35**) in virtually all cell lines. Compounds with a heterocyclic ring or $N(CH_3)_2$ at the 6-position (**37**−**41**) displayed the greatest potency, except for **41** with a morpholine substituent. Compound **38** with a 6-pyrrolidinyl ring was the most potent compound, with ED_{50} values in the nanomolar concentration range. In our previous studies, maximum activity has often been observed with a 6-pyrrolidinyl substituent in other heterocyclic ketone series.

A fairly dramatic difference was observed between the PQZ (**24**−**32**) and DHPQZ (**41**−**50**) series. In the former group, little activity was observed across all cell lines examined. In contrast, in the latter group, highly selective activities were obtained against the 1A9 and KB-Vin cell lines. Generally, DHPQZ derivatives showed greater activity than PQZ compounds with the same substitution. However, as the DHPQZ compounds tested in this study were all racemates, it is reasonable to anticipate higher activity for one of the enantiomer pair. In terms of cytotoxicity in the more sensitive cell lines, DHPQZ compounds with a methoxy at the 3′-position (**43**) were about 15 or 20 times more active than 2′-methoxy (**42**) or 4′-methoxy **(44)** derivatives. In addition, **43** was also more active than multimethoxy compounds (**45**−**49**). This result is consistent with observations in other heterocyclic ketones, such as the PQ, DHPQ, and PN derivatives. 6-Cl-3-methoxy DHPQZ (**50**) showed significantly increased cytotoxicity as compared to **43**. The EC_{50} of **50** was about 5-fold lower than that of **43**. Compound **50** was also substantially more active than its cognate PQZ derivative (**32**).

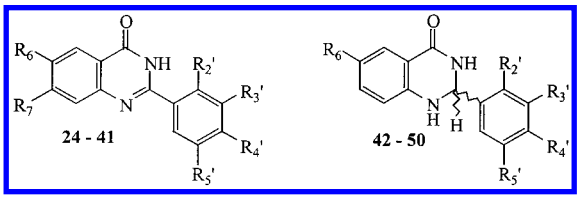
As summarized in Table 2, the cytotoxic PQZ and DHPQZ compounds showed strong sensitivity in the ovarian cancer cell line and were selective against the drug-resistant KB cell line. These interesting results make PQZ and DHPQZ unique among structurally similar heterocyclic ketones, which were generally active against most human tumor cell lines in virtually all cases. Further mechanistic studies may reveal reasons for these differences.

b. Interaction of PQZ and DHPQZ Derivatives with Tubulin. Previously, PQ, DHPQ, and PN derivatives were found to inhibit both tubulin polymerization and the binding of radiolabeled colchicine to tubulin.[5−10] The most active compound prepared so far, both in its cytotoxicity and in the strength of its interaction with tubulin, has been PQ7.[7] In most cell lines examined IC_{50} values for cell growth inhibition by PQ7 were below 1 nM, about 100−500-fold lower than the IC_{50} values obtained with compound **38.** In the previous study,[7] and in a reevaluation performed in conjunction with the current work (Table 3), PQ7 strongly inhibited tubulin polymerization and the binding of [^{3}H]colchicine to tubulin. Under the reaction conditions used, 0.32 μM PQ7 inhibited the assembly of 12 μM tubulin by 50%, and 5 μM PQ7 nearly completely inhibited the binding of 5 μM colchicine to 1 μM tubulin (Table 3). For comparison, previously we obtained IC_{50} values of 0.80, 0.46, and 0.53 μM for colchicine, podophyllotoxin, and combretastatin A-4 for inhibition of tubulin assembly, and PQ7, podophyllotoxin,[17] and combretastatin A-4 were all nearly identical as inhibitors of colchicine binding.

The chief structural difference between the PQ agents and the new PQZ series, as well as between DHPQ and the new DHPQZ series, is the additional nitrogen atom in the B-ring. The only structural difference between the two newly synthesized PQZ and DHPQZ compounds is the oxidation status of the bond between C-2 and N-3 in the B-ring. This modification results in configurational and conformational changes in the relative positions of the aromatic rings A and C. Many studies have suggested that the interaction between colchicine and tubulin is stereoselective and is highly dependent on the conformation and configuration of the biaryl system formed by the trimethoxyphenyl A-ring and tropolonic C-ring. Thus, evaluation of the new agents for interactions with tubulin should provide additional insight into the mechanism of ligand binding at the colchicine site.

In the studies reported in the previous paper,[16] selected molecules in the PQZ compounds were shown to inhibit tubulin polymerization. This finding led us to prepare additional PQZ and DHPQZ derivatives to further delineate SARs. Table 3 summarizes our findings when these compounds were evaluated as inhibi-

Table 1. Physical and Spectral Data of 6-Alkylamino-3′-methoxy-2-phenyl-4-quinazolinones, 2,3-Dihydro-3′-methoxy-2-phenyl-4-quinazolinones, and Related Compounds



compd	yield (%)	mp (°C)	UV, λ_{max} (MeOH) (log *C*)	IR, $\nu_{C=O}$ (cm^{-1})	MS (M^+) *m/z*	^{1}H NMR (DMSO-d_6) δ
24	89	198−202	211 (4.50)	1678	252	3.86 (3 H, s, OCH_3), 7.05−7.21 (2 H, m, H-3′, H-5′), 7.49−7.58 (2 H, m, H-4, H-6), 7.69−7.73 (2 H, m, H-6′, H-8), 7.83 (1 H, ddd, *J* = 1.2, 8.0, 8.0 Hz, H-7), 8.15 (1 H, dd, *J* = 1.2, 8.0 Hz, H-5), 12.13 (1 H, br s, NH)
25	95	177−179	218 (4.41)	1672	252	3.86 (3 H, s, OCH_3), 7.14 (1 H, dd, *J* = 2.2, 8.0 Hz, H-4′), 7.41−7.56 (2 H, m, H-5′, H-6), 7.74−7.88 (4 H, m, H-2′, H-6′, H-7, H-8), 8.15 (1 H, dd, *J* = 1.2, 8.0 Hz, H-5), 12.55 (1 H, br s, NH)
26	96	215−219	207 (4.38)	1678	252	3.83 (3 H, s, OCH_3), 7.08 (2 H, d, *J* = 8.0 Hz, H-3′, H-5′), 7.47 (1 H, ddd, *J* = 1.2, 8.0, 8.0 Hz, H-6), 7.67−7.85 (2 H, m, H-7, H-8), 8.10−8.20 (3 H, m, H-2′, H-6′, H-5), 12.42 (1 H, br s, NH)
27	86	179−181	223 (4.52)	1678	282	3.76 (3 H, s, 2′-OCH_3 or 3′-OCH_3), 3.87 (3 H, s, 2′-OCH_3 or 3′-OCH_3), 7.18−7.28 (3 H, m, H-4′, H-5′, H-6′), 7.54 (1 H, ddd, *J* = 1.2, 8.0, 8.0 Hz, H-6), 7.70 (1 H, dd, *J* = 1.2, 8.0 Hz, H-8), 7.84 (1 H, ddd, *J* = 1.2, 8.0, 8.0 Hz, H-7), 8.16 (1 H, dd, *J* = 1.2, 8.0 Hz, H-5), 12.23 (1 H, br s, NH)
28	84	184−186	207 (4.27)	1678	282	3.85 (3 H, s, 2′-OCH_3 or 4′-OCH_3), 3.89 (3 H, s, 2′-OCH_3 or 4′-OCH_3), 6.65−6.72 (2 H, m, H-3′, H-5′), 7.49 (1 H, ddd, *J* = 1.2, 8.0, 8.0 Hz, H-6), 7.67 (1 H, dd, *J* = 1.2, 8.0 Hz, H-8), 7.76−7.85 (2 H, m, H-6′, H-7), 8.12 (1 H, dd, *J* = 1.2, 8.0 Hz, H-5), 11.82 (1 H, br s, NH)
29	88	143−144	220 (4.70)	1698	282	3.77 (3 H, s, 2′-OCH_3 or 5′-OCH_3), 3.81 (3 H, s, 2′-OCH_3 or 5′-OCH_3), 7.10−7.12 (2 H, m, H-3′, H-4′), 7.29−7.31 (1 H, m, H-6′), 7.53 (1 H, ddd, *J* = 1.2, 8.0, 8.0 Hz, H-6), 7.71 (1 H, dd, *J* = 1.2, 8.0 Hz, H-8), 7.83 (1 H, ddd, *J* = 1.2, 8.0, 8.0 Hz, H-7), 8.14 (1 H, dd, *J* = 1.2, 8.0 Hz, H-5), 12.10 (1 H, br s, NH)
30	89	240−242	226 (4.44)	1671	282	3.84 (3 H, s, 3′-OCH_3 or 4′-OCH_3), 3.88 (3 H, s, 3′-OCH_3 or 4′-OCH_3), 7.10 (1 H, d, *J* = 8.0 Hz, H-5′), 7.48 (1 H, ddd, *J* = 1.2, 8.0, 8.0 Hz, H-6), 7.69−7.89 (4 H, m, H-2′, H-6′, H-7, H-8), 8.13 (1 H, dd, *J* = 1.2, 8.0 Hz, H-5), 12.44 (1 H, br s, NH)
31	84	200−203	221 (4.57)	1674	282	3.83 (6 H, s, 3′-OCH_3, 5′-OCH_3), 6.69 (1 H, dd, *J* = 2.2, 2.2 Hz, H-4′), 7. 38 (2 H, d, *J* = 2.2 Hz, H-2′, H-6′), 7.52 (1 H, ddd, *J* = 1.2, 8.0, 8.0 Hz, H-6), 7.74 (1 H, dd, *J* = 1.2, 8.0 Hz, H-8), 7.83 (1 H, ddd, *J* = 1.2, 8.0, 8.0 Hz, H-7), 8.15 (1 H, dd, *J* = 1.2, 8.0 Hz, H-5), 12.52 (1 H, br s, NH)
32	94	245−248	218 (4.60)	1678	286.5	3.86 (3 H, s, 3′-OCH_3), 7.16 (1 H, dd, *J* = 2.5, 8.0 Hz, H-4′), 7.46 (1 H, dd, *J* = 8.0, 8.0 Hz, H-5′), 7.73−7.80 (3 H, m, H-2′, H-6′, H-8), 7.87 (1 H, dd, *J* = 2.5, 8.0 Hz, H-7), 8.09 (1 H, d, *J* = 2.5 Hz, H-5), 12.70 (1 H, br s, NH)
33	46.8	246−247	217 (4.70)	1680	270	3.84 (3 H, s, 3′-OCH_3), 7.13 (1 H, dd, *J* = 2.5, 8.0 Hz, H-4′), 7.44 (1 H, dd, *J* = 8.0, 8.0 Hz, H-5′), 7.68−7.84 (5 H, m, H-2′, H-6′, H-5, H-7, H-8), 12.61 (1 H, br s, NH)
34	42	216−218	218 (4.69)	1659	282	3.86 (3 H, s, 3′-OCH_3 or 6-OCH_3), 3.96 (3 H, s, 3′-OCH_3 or 6-OCH_3), 7.11 (1 H, dd, *J* = 2.5, 8.0 Hz, H-4′), 7.29−7.49 (2 H, m, H-5′, H-7), 7.53 (1 H, d, *J* = 2.5 Hz, H-5), 7.67−7.82 (3 H, m, H-2′, H-6′, H-8), 12.48 (1 H, br s, NH)
35	30	266−267	253 (4.55)	1658	312	3.86 (3 H, s, 3′-OCH_3 or 6-OCH_3 or 7-OCH_3), 3.89 (3 H, s, 3′-OCH_3 or 6-OCH_3 or 7-OCH_3), 3.94 (3 H, s, 3′-OCH_3 or 6-OCH_3 or 7-OCH_3), 7.11 (1 H, dd, *J* = 1.2, 8.0 Hz, H-4′), 7.21 (1 H, s, H-8), 7.43 (1 H, dd, *J* = 8.0, 8.0 Hz, H-5′), 7.48 (1 H, s, H-5), 7.73−7.78 (2 H, m, H-2′, H-6′), 12.40 (1 H, br s, NH)
36	8.8	269−270	249 (4.48)	1665	296	3.85 (3 H, s, 3′-OCH_3), 6.20 (2 H, s, OCH_2O), 7.11 (1 H, dd, *J* = 2.2, 8.0 Hz, H-4′), 7.18 (1 H, s, H-8), 7.43 (1 H, dd, *J* = 8.0, 8.0 Hz, H-5′), 7.44 (1 H, s, H-5), 7.68−7.75 (2 H, m, H-2′, H-6′), 12.46 (1 H, br s, NH)
37	51	239−241	224 (4.79)	1659	295	3.01 (s, 6 H, $N(CH_3)_2$), 3.85 (3 H, s, 3′-OCH_3), 7.07 (1 H, dd, *J* = 2.5, 8.0 Hz, H-4′), 7. 21 (1 H, d, *J* = 2.5 Hz, H-5), 7.33 (1 H, dd, *J* = 2.5, 8.0 Hz, H-7), 7.41 (1 H, dd, *J* = 8.0, 8.0 Hz, H-5′), 7.61 (1 H, d, *J* = 8.0 Hz, H-8), 7.70−7.76 (2 H, m, H-2′, H-6′), 12.26 (1 H, br s, NH)
38	40	261−263	227 (4.54)	1653	321	1.96−2.03 (4 H, m, $CH_2CH_2NCH_2CH_2$), 3.30−3.34 (4 H, m, CH_2NCH_2), 3.85 (3 H, s, 3′-OCH_3), 7.03−7.17 (3 H, m, H-4′, H-5, H-7), 7.41 (1 H, dd, *J* = 8.0, 8.0 Hz, H-5′), 7.60 (1 H, d, *J* = 8.0 Hz, H-8), 7.68−7.75 (2 H, m, H-2′, H-6′), 12.22 (1 H, br s, NH)
39	55	222−225	223 (4.71)	1668	335	1.55−1.60 (6 H, m, $(CH_2)_2CH_2NCH_2CH_2$), 3.25−3.30 (4 H, m, CH_2NCH_2), 3.85 (3 H, s, 3′-OCH_3), 7.09 (1 H, dd, *J* = 22, 8.0 Hz, H-4′), 7.38−7.46 (2 H, m, H-5′, H-5), 7.53 (1 H, dd, *J* = 2.2, 8.0 Hz, H-7), 7.60 (1 H, d, *J* = 8.0 Hz, H-8), 7.70−7.76 (2 H, m H-2′,H-6′), 12.33 (1 H, br s, NH)
40	57	212−215	224 (4.61)	1663	349	0.93 (3 H, d, *J* = 6.2 Hz, $CH_3CH(CH_2)_2N(CH_2)_2$), 1.20−1.26, 1.68−1.74 (2 H each, both m, $(NCH_2CH_2) \times 2$), 1.48−1.58 (1 H, m, $CH_3CH(CH_2)_2N(CH_2)_2$), 2.71−2.82, 3.75−3.83 (2 H each, both m, $(NCH_2) \times 2$), 3.85 (3 H, s, 3′-OCH_3), 7.09 (1 H, dd, *J* = 2.2, 8.0 Hz, H-4′), 7.38−7.46 (2 H, m, H-5′, H-5), 7.54 (1 H, dd, *J* = 2.2, 8.0 Hz, H-7), 7.61 (1 H, d, *J* = 8.0 Hz, H-8), 7.71−7.77 (2 H, m, H-2′, H-6′), 12.34 (1 H, br s, NH)
41	48	254−257	225 (4.66)	1673	337	3.23 (4 H, t, *J* = 4.7 Hz, CH_2NCH_2), 3.75 (4 H, t, *J* = 4.7 Hz, CH_2OCH_2), 3.85 (3 H, s, 3′-OCH_3), 7.10 (1 H, dd, *J* = 2.5, 8.0 Hz, H-4′), 7.43 (1 H, dd, *J* = 8.0, 8.0 Hz, H-5′), 7.45 (1 H, d, *J* = 2.5 Hz, H-5), 7.57 (1 H, dd, *J* = 2.5, 8.0 Hz, H-7), 7.65 (1 H, d, *J* = 8.0 Hz,H-8), 7.71−7.77 (2 H, m, H-2′, H-6′), 12.39 (1 H, br s, NH)
42	90	163−165	222 (4.54)	1668	254	3.83 (3 H, s, OCH_3), 6.02 (1 H, s, H-2), 6.66 (1 H, dd, *J* = 7.5, 7.5 Hz, H-6), 6.74−6.80 (2 H, m, H-8, N_1H), 6.94 (1 H, dd, *J* = 7.5, 7.5 Hz, H-5′), 7.04 (1 H, d, *J* = 8.0 Hz, H-3′), 7.18−7.41 (3 H, m, H-4′, H-6′, H-7), 7.62 (1 H, dd, *J* = 1.0, 1.0 Hz, H-5), 8.02 (1 H, br s, N_3H)
43	89	148−105	222 (4.50)	1647	254	3.73 (3 H, s, OCH_3), 5.73 (1 H, s, H-2), 6.68 (1 H, dd, *J* = 7.5, 7.5 Hz, H-6), 6.77 (1 H, d, *J* = 8.0 Hz, H-8), 6.90 (1 H, dd, *J* = 8.0, 2.5 Hz, H-4′), 7.04−7.07 (2 H, m, H-2′, H-6′), 7.15 (1 H, br s, N_1H), 7.21−7.33 (2 H, m, H-5′, H-7), 8.15 (1 H, dd, *J* = 1.0, 7.5 Hz, H-5), 8.34 (1 H, br s, N_3H)

Table 1 (Continued)

compd	yield (%)	mp (°C)	UV, λ_{max} (MeOH) (log *C*)	IR, $\nu_{C=O}$ (cm^{-1})	MS (M^+) *m/z*	^{1}H NMR (DMSO-d_6) δ
44	93	188−190	225 (4.64)	1655	254	3.73 (3 H, s, OCH_3), 5.70 (1 H, s, H-2), 6.67−6.75 (2 H, m, H-6, H-8), 6.91−6.95 (2 H, m, H-3′, H-5′), 7.00 (1 H, br s, NIH), 7.24 (1 H, ddd, *J* = 1.5, 8.0, 8.0 Hz, H-7), 7.36−7.62 (2 H, m, H-2′, H-6′), 7.60 (1 H, dd, *J* = 1.2, 7.5 Hz, H-5), 8.19 (1 H, br s, N_3H)
45	78	232−233	224 (4.54)	1651	284	3.78 (3 H, s, 2′-OCH_3 or 3′-OCH_3), 3.81 (3 H, s, 2′-OCH_3 or 3′-OCH_3), 6.03 (1 H, s, H-2), 6.63−6.75 (2 H, m, H-6, H-8), 6.79 (1 H, br s, N_1H), 7.04−7.10 (3 H, m, H-4′, H-5′, H-6′), 7.23 (1 H, ddd, *J* = 1.0, 8.0, 8.0 Hz, H-7), 7.62 (1 H, dd, *J* = 1.0, 8.0 Hz, H-5), 8.02 (1 H, br s, N_3H)
46	86	182−183	225 (4.45)	1663	284	3.75 (3 H, s, 2′-OCH_3 or 4′-OCH_3), 3.81 (3 H, s, 2′-OCH_3 or 4′-OCH_3), 5.94 (1 H, s, H-2), 6.49−6.77 (5 H, m, H-3′, H-5′, H-6, H-8, N_1H), 7.21 (1 H, ddd, *J* = 1.0, 8.0, 8.0 Hz, H-7), 7.31 (1 H, d, *J* = 8.0, H-6′), 7.61 (1 H, dd, *J* = 1.0, 7.5 Hz, H-5), 7.94 (1 H, br s, N_3H)
47	75	163−165	223 (4.53)	1653	284	3.66 (3 H, s, 2′-OCH_3 or 5′-OCH_3), 3.78 (3 H, s, 2′-OCH_3 or 5′-OCH_3), 5.98 (1 H, s, H-2), 6.68 (1 H, dd, *J* = 7.5, 7.5 Hz, H-6), 6.77 (1 H, d, *J* = 8.0 Hz, H-8), 6.84−7.00 (4 H, m, H-3′, H-4′, H-6′, N_1H), 7.23 (1 H, ddd, *J* = 1.0, 8.0, 8.0 Hz, H-7), 7.63 (1 H, dd, *J* = 1.0, 7.5 Hz, H-5), 8.02 (1 H, br s, N_3H)
48	80	210−213	223 (4.61)	1655	284	3.73 (3 H, s, 3′-OCH_3 or 4′-OCH_3), 3.74 (3 H, s, 3′-OCH_3 or 4′-OCH_3), 5.70 (1 H, s, H-2), 6.66 (1 H, dd, *J* = 7.5, 7.5 Hz, H-6), 6.75 (1 H, d, *J* = 7.5 Hz, H-8), 6.91−7.02 (3 H, m, H-2′, H-5′, H-6′), 7.12 (1 H, br s, N_1H), 7.25 (1 H, ddd, *J* = 1.0, 7.5, 7.5 Hz, H-7), 7.61 (1 H, dd, *J* = 1.0, 7.5 Hz, H-5), 8.19 (1 H, br s, N_3H)
49	93	89−92	223 (4.54)	1658	284	3.72 (6 H, s, 3′-OCH_3, 5′-OCH_3), 5.67 (1 H, s, H-2), 6.46 (1 H, s, H-4′), 6.64−6.77 (4 H, rn, H-2′, H-6′, H-6, H-8), 7.13 (1 H, br s, N_1H), 7.24 (1 H, ddd, *J* = 1.0, 7.5, 7.5 Hz, H-7), 7.60 (1 H, dd, *J* = 1.0, 7.5 Hz, H-5), 8.30 (1 H, br s, N_3H)
50	95	193−195	223 (4.57)	1658	288.5	3.74 (3 H, s, OCH_3), 5.75 (1 H, s, H-2), 6.79 (1 H, d, *J* = 8.0 Hz, H-8), 6.92 (1 H, dd, *J* = 2.5, 8.0 Hz, H-4′), 7.02−7.05 (2 H, m, H-2′, H-6′), 7.25−7.36 (3 H, m, H-5′, H-7, NH), 7.53 (1 H, d, *J* = 2.5 Hz, H-5), 8.50 (1 H, br s, N_3H)

Table 2. In Vitro Cytotoxicity of 6,7,2′,3′,4′,5′-Substituted 2-Phenyl-4-quinazolinones (**24**−**41**) and 6,2′,3′,4′,5′-Substituted 2,3-Dihydro-2-phenyl-4-quinazolinones (**42**−**50**)

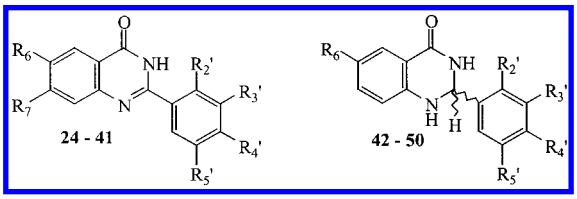
	ED_{50} (g/mL)[a]									
compd	IA9[b]	HCT-8[b]	A-549[b]	U-87-MG[b]	HOS	KB[b]	KB-VIN[b]	PC3[b]	MCF-7[b]	SKMEL-2[b]
24	>20 (8)[c]	NA	>20 (15)	NA	>20 (5)	>20 (6)	>20 (9)	NA	>20 (23)	NA
25	>20 (27)	>20 (8)	>20 (21)	NA	>20 (6)	NA	>20 (12)	>20 (16)	26	>20 (12)
26	>20 (22)	>20 (29)	>20 (42)	NA	>20 (21)	>20 (23)	>20 (40)	>20 (32)	>20 (42)	>20 (24)
27	NA	NA	>20 (19)	NA	NA	NA	>20 (11)	>20 (17)	>20 (24)	NA
28	NA	>20 (14)	>20 (31)	>20 (16)	>20 (7)	>20 (5)	>20 (8)	>20 (16)	>20 (15)	>20 (5)
29	>20 (19)	>20 (21)	>20 (33)	NA	>20 (20)	>20 (10)	>20 (21)	>20 (30)	>20 (20)	>20 (9)
30	>20 (34)	>20 (15)	>20 (29)	NA	>20 (11)	>20 (12)	>20 (22)	>20 (26)	>20 (48)	>20 (15)
31	NA	NA	>20 (29)	NA	NA	NA	>20 (12)	>20 (8)	>20 (28)	>20 (5)
32	>20 (29)	6.0	3.2	10.0	17.0	11.5	6.5	4.5	17.8	4.8
33	NA	>20 (31)	16.5	>20 (30)	NA	>20 (39)	18.0	20.0	>20 (40)	>20 (41)
34	3.4	16.5	12.5	>20 (11)	>20 (43)	17.5	3.5	>20 (37)	8.0	>20 (49)
35	16.5	>20 (37)	>20 (49)	>20 (28)	>20 (27)	>20 (45)	19.8	15.0	12.0	18.0
36	>20 (26)	NA	>20 (11)	NA	>20 (6)	>20 (16)	>20 (19)	>20 (12)	>20 (40)	>20 (14)
37	0.49.	1.05	14.0	>20 (17)	>20 (46)	10.0	0.42	15.0	0.85	19.0
38	0.09	0.06	0.50	13.8	7.0	0.23	0.10	15.0	0.22	0.09
39	0.90	4.3	2.8	9.0	15.4	10	0.60	10.0	8.5	4.5
40	0.80	4.0	6.0	15.5	14.4	10.8	2.5	12.4	10.0	7.5
41	3.8	10.4	10.9	20	12.5	8.2	3.7	13.4	4.5	4.8
42	14.7	>20 (21)	>20 (44)	NA	>20 (38)	>20 (32)	10.2	>20 (30)	>20 (48)	<20 (52)$_d$
43	1.0	1.5	3.0	>20 (35)	>20 (42)	3.0	1.20	>20 (43)	>20 (48)	>20 (49)
44	20	>20 (19)	>20 (34)	NA	>20 (13)	>20 (14)	>20 (39)	>20 (14)	>20 (44)	>20 (23)
45	11.4	>20 (39)	>20 (39)	NA	>20 (34)	>20 (41)	8.6	>20 (20)	>20 (40)	<20 (53)
46	12.5	>20 (29)	>20 (43)	NA	>20 (40)	>20 (43)	7.6	>20 (28)	>20 (44)	>20 (37)
47	10.0	>20 (32)	>20 (42)	>20 (5)	>20 (40)	>20 (40)	8.4	>20 (22)	>20 (42)	>20 (44)
48	14.6	>20 (25)	>20 (41)	NA	>20 (24)	>20 (28)	16.0	>20 (30) 20	20	>20 (24)
49	1.92	3.0	5.0	>20 (37)	>20 (41)	4.7	1.6	>20 (44)	>20 (49)	4.4
50	0.27	0.48	0.6	>20 (37)	20.0	0.95	0.42	2.5	16.0	<2.5 (56)

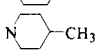
[a] ED_{50} was the concentration of compound which afforded 50% reduction in cell number after 3−4 days of incubation. [b]Human ovarian cancer (1A9), ileocecal carcinoma (HCT-8), lung carcinoma (A-549), glioblastoma (U-87-MG), bone (HOS), epidermoid carcinoma of the nasopharynx (KB), P-gp-expressing epidermoid carcinoma of the nasopharynx (KB-VIN), prostate cancer (PC3), breast cancer (MCF-7), and melanoma (SKMEL-2) cell lines. [c]Inhibition of <50% at highest test concentration (percent observed is given in parentheses). [d]Plateau dose response apparent to maximum inhibition of 50% seen at low dose but plateaus over broad dose range (range indicated with inhibition values given in parentheses).

tors of tubulin polymerization. In addition, the most potent of the new compounds (**38**) was compared to PQ7 for its effects on the binding of [^{3}H]colchicine to tubulin.

Considering first the PQZ derivatives (**24**−**41**), they differ from the previously studied PQ, DHPQ, and PN derivatives in that most of these compounds have only moderate inhibitory effects on tubulin assembly and their relative potencies varied only slightly. IC_{50} values in a relatively narrow range (7−17 μM) were obtained for compounds **24**−**36, 39**, and **40**. Only three compounds with a *m*-methoxy group in the C-ring and nitrogen-containing substituents at C-6 were more potent inhibitors of the tubulin assembly reaction. These compounds were **37**, with a dimethylamino C-6 subsituent (IC_{50} 3.5 μM), **41**, with a morpholino group at C-6 (IC_{50} 4.4 μM), and especially **38**, with a pyrrolidinyl

Table 3. Antitubulin Effects of 6,7,2′,3′,4′,5′-Substituted 2-Phenyl-4-quinazolinones **24**−**41** and 6,2′,3′,4′,5′-Substituted 2,3-Dihydro-2-phenyl-4-quinazolinones **42**−**50**



compd	R_6	R_7	$R_{2'}$	$R_{3'}$	$R_{4'}$	$R_{5'}$	ITP[a] IC_{50} (μM) ± SD	inhib of colchicine binding (%) ± SD
24	H	H	OCH_3	H	H	H	17 ± 1	
25	H	H	H	OCH_3	H	H	12 ± 1	
26	H	H	H	H	OCH_3	H	12 ± 2	
27	H	H	OCH_3	OCH_3	H	H	15 ± 0.07	
28	H	H	OCH_3	H	OCH_3	H	10 ± 0.4	
29	H	H	OCH_3	H	H	OCH_3	11 ± 0.9	
30	H	H	H	OCH_3	OCH_3	H	15 ± 2	
31	H	H	H	OCH_3	H	OCH_3	11 ± 2	
32	Cl	H	H	OCH_3	H	H	12 ± 0.9	
33	F	H	H	OCH_3	H	H	14 ± 2	
34	OCH_3	H	H	OCH_3	H	H	6.7 ± 0.08	
35	OCH_3	OCH_3	H	OCH_3	H	H	9.1 ± 0.9	
36	OCH_2O		H	OCH_3	H	H	9.6 ± 0.6	
37		H	H	OCH_3	H	H	3.5 ± 0.3	
38		H	H	OCH_3	H	H	1.1 ± 0.02	24 ± 10
39		H	H	OCH_3	H	H	9.3 ± 2.26	
40		H	H	OCH_3	H	H	12 ± 0.3	
41		H	H	OCH_3	H	H	4.4 ± 0.1	
42	H	H	OCH_3	H	H	H	>40	
43	H	H	H	OCH_3	H	H	5.6 ± 1	
44	H	H	H	H	OCH_3	H	>40	
45	H	H	OCH_3	OCH_3	H	H	>40	
46	H	H	OCH_3	H	OCH_3	H	>40	
47	H	H	OCH_3	H	H	OCH_3	32 ± 4	
48	H	H	H	OCH_3	OCH_3	H	>40	
49	H	H	H	OCH_3	H	OCH_3	12 ± 3	
50	Cl	H	H	OCH_3	H	H	1.5 ± 0.01	
PQ-7							0.32 ± 0.06	97 ± 3

[a] ITP = inhibition of tubulin polymerization.

group at C-6 (IC_{50} 1.1 μM). Thus, only a modest correlation exists between the cytotoxicity data and inhibitory effects on tubulin polymerization, with the important exception that the most active agent in both assays was compound **38**. It should also be noted that **38** is structurally identical to PQ7, except for the additional nitrogen atom in the B-ring. This modification results in a major loss in cytotoxic activity for **38** relative to PQ7 (cf. data in Table 2 with the cytotoxicity data in ref 7), a 3−4-fold loss in activity of **54** vs PQ7 as an inhibitor of tubulin polymerization, and a substantial reduction in activity of **54** vs PQ7 as an inhibitor of colchicine binding (Table 3).

Turning to DHPQZ derivatives, the data obtained show a wider range in activities depending on substituent pattern. Only two derivatives, compounds **43** and **50**, showed significant inhibitory effects on tubulin assembly, with IC_{50} values of 5.6 and 1.5 μM, respectively. The third most active analogue, compound **49**, had an IC_{50} value of 12 μM. On comparing the data in Tables 2 and 3 for the DHPQZ derivatives, good correlation was found between inhibitory effects on tubulin assembly and cytotoxicity. The most active analogue in both assays was **50**, followed in succession by **43** and **49**. For this series, these derivatives indicate that activity requires a *m*-methoxy substituent in the C-ring, with substantial enhancement by a substituent at C-6. This pattern repeats that previously observed with the PZ, DHPQ, and PN series.

However, in comparing cognate PQZ and DHPQZ derivatives, an interesting divergent pattern was observed. In most cases, reduction of the B-ring resulted in a loss of activity in inhibiting tubulin polymerization (cf. **42** with **24**, **44** with **26**, **45** with **27**, **46** with **28**, **47** with **29**, and **49** with **31**). These compounds have either no *m*-methoxy substituent in the C-ring or a second C-ring methoxy group. The opposite was observed with the two most active members of the DHPZQ series, those with a single, *m*-methoxy substituent in the C-ring. When **25** was reduced to **43**, a 2-fold increase occurred in the inhibitory effect on tubulin polymerization (if only one enantiomer of **43** is active, as is likely, then a 4-fold increase in activity occurred). The enhancement of activity by reduction is even more dramatic with compounds **32** and **50**: 8-fold for the racemic mixture and, presumably, 16-fold for the active enantiomer. These observations suggest some subtle interaction, which has not been elucidated, exists between the *meta* substituent in the C-ring and the heterocyclic B-ring.

Conclusion

This work has established preliminary SAR of PQZ compounds and provided important information for future QSAR molecular modeling study. Although **38**, the most potent compound in this study, is not as potent as the previously identified PQ7, **38** belongs to a related but different compound class and is worthwhile for future in vivo investigation.

Experimental Section

Chemistry. Melting points were determined on a Yanaco MP-500D melting point apparatus and are uncorrected. IR spectra were recorded on Shimadzu IR-440 and Nicolet Impact 400 FT-IR spectrophotometers as KBr pellets. NMR spectra were obtained on a Bruker Advance DPX-200 FT-NMR spectrometer in DMSO-d_6. The following abbreviations are used: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; and br, broad. MS spectra were measured with an HP 5995 GC-MS instrument. The UV spectra were recorded on a Shimadzu UV-160A UV−vis recording spectrophotometer as methanolic solutions. Elemental analyses (C, H, N) were performed at China Medical College, Taiwan, and the results were within ±0.4% of the calculated values.

General Procedure for Synthesis of Starting 2-Aminobenzamides. (a) Thionyl chloride (1.5 g, 12.6 mmol) was added dropwise to a suspension of a 5-chloro-2-nitrobenzoic acid (**1**) (1.0 g, 6.3 mmol) in benzene (30 mL) under reflux. The resulting mixture was stirred under reflux for 4 h and dried under vacuum. (b) The residue was dissolved in 200 mL of benzene and treated with anhydrous ammonia gas at room temperature. (c) After removing solvent, the intermediate 5-chloro-2-nitrobenzamide was dissolved in DMF (10 mL), treated with the appropriate amine at 110 °C for 3 h, and poured into ice water (300 mL). (d) The precipitate was collected, washed with water, dried in vacuo, dissolved in MeOH and hydrogenated over 10% Pd/C for 6 h. The catalyst was removed by filtration, and the solution was dried under vacuum to afford the substituted 2-aminobenzamides **9**−**13**.

Compound **5** was prepared from 2-aminofluorobenzamide by steps a and b only. Compounds **6** and **7** were prepared from 5-methoxy- or 4,5-dimethoxy-2-nitrobenzoic acid using steps a, b, and d. Compound **8** was prepared by oxidation of 4,5-methylenedioxy-2-nitrobenzaldehyde to the corresponding benzoic acid using Conforth reagent (CrO_3−pyridine−water) followed by steps a, b, and d. Compounds **14** and **15** were commercially available.

2-(2′-Methoxyphenyl)-4-quinazolinone (24). Sodium hydrogen sulfite (0.8 g, 7.5 mmol) was added to a solution of 2-aminobenzamide (**14**) (1.0 g, 7.3 mmol) and 2-methoxybenzaldehyde (**16**) (1.0 g, 7.3 mmol) in *N*,*N*-dimethylacetamide (DMAC) (20 mL). The mixture was heated with stirring at 150 °C for 2 h and poured into ice water (200 mL). The precipitate was collected, washed with water, and dried in vacuo. After recrystallization from EtOH, **24** was obtained (1.6 g) as pale yellow needles. Yield, melting point, and spectral data of **24** and all subsequent compounds are summarized in Table 1.

The method used to prepare **24** was used with the indicated substituted benzaldehyde and benzamide to afford **25**−**41**.

2-(3′-Methoxyphenyl)-4-quinazolinone (25): 1.7 g from **14** and 3-methoxybenzaldehyde (**17**) (1.0 g, 7.3 mmol), pale yellow needles.

2-(4′-Methoxyphenyl)-4-quinazolinone (26): 1.8 g from **14** and 4-methoxybenzaldehyde **(18)** (1.0 g, 7.3 mmol), pale yellow needles.

2-(2′,3′-Dimethoxyphenyl)-4-quinazolinone (27): 1.8 g from **14** and 2,3-dimethoxybenzaldehyde (**19**) (1.2 g, 7.3 mmol), pale yellow needles.

2-(2′,4′-Dimethoxyphenyl)-4-quinazolinone (28): 1.7 g from **14** and 2,4-dimethoxybenzaldehyde (**20**) (1.2 g, 7.3 mmol), pale yellow needles.

2-(2′,5′-Dimethoxyphenyl)-4-quinazolinone (29): 1.8 g from **14** and 2,5-dimethoxybenzaldehyde (**21**) (1.2 g, 7.3 mmol), pale yellow prism crystals.

2-(3′,4′-Dimethoxyphenyl)-4-quinazolinone (30): 1.8 g from **14** and 3,4-dimethoxybenzaldehyde (**22**) (1.2 g, 7.3 mmol), pale yellow prism crystals.

2-(3′,5′-Dimethoxyphenyl)-4-quinazolinone (31): 1.7 g from **14** and 3,5-dimethoxybenzaldehyde (**23**) (1.2 g, 7.3 mmol), pale yellow needles.

2-(3′-Methoxyphenyl)-6-chloro-4-quinazolinone (32): 1.6 g from 2-amino-5-chlorobenzamide (**15**) (1.0 g, 5.9 mmol) and **17** (0.8 g, 5.9 mmol), pale yellow needles.

2-(3′-Methoxyphenyl)-6-fluoro-4-quinazolinone (33): 0.8 g from 2-amino-5-fluorobenzamide (**5**) (1.0 g, 6.5 mmol) and **17** (0.9 g, 6.5 mmol), pale yellow needles.

2-(3′-Methoxyphenyl)-6-methoxy-4-quinazolinone (34): 0.7 g from 2-amino-5-methoxybenzamide (**6**) (1.0 g, 6.0 mmol) and **17** (0.8 g, 6.0 mmol), pale yellow needles.

2-(3′-Methoxyphenyl)-6,7-dimethoxy-4-quinazolinone (35): 0.5 g from 2-amino-4-methoxybenzamide (**7**) (1.0 g, 5.1 mmol) and **17** (0.7 g, 5.1 mmol), pale yellow prism crystals.

2-(3′-Methoxyphenyl)-6,7-methylenedioxy-4-quinazolinone (36): 0.1 g from 2-amino-4,5-methylenedioxybenzamide (**8**) (1.0 g, 5.5 mmol) and **17** (0.7 g, 5.5 mmol), pale yellow needles.

2-(3′-Methoxyphenyl)-6-(*N*,*N*-dimethylamino)-4-quinazolinone (37): 0.8 g from 2-amino-5-(*N*,*N*-dimethylamino)benzamide (**9**) (1.0 g, 5.6 mmol) and **17** (0.8 g, 5.6 mmol), pale yellow needles.

2-(3′-Methoxyphenyl)-6-pyrrolidinyl-4-quinazolinone (38): 0.6 g from 2-amino-5-pyrrolidinylbenzamide (**10**) (1.0 g, 4.9 mmol) and **17** (0.7 g, 4.9 mmol), pale yellow prism crystals.

2-(3′-Methoxyphenyl)-6-piperidinyl-4-quinazolinone (39): 0.8 g from 2-amino-5-piperidinylbenzamide (**11**) (1.0 g, 4.6 mmol) and **17** (0.6 g, 4.6 mmol), pale yellow prism crystals.

2-(3′-Methoxyphenyl)-6-(4-methylpiperidinyl)-4-quinazolinone (40): 0.9 g from 2-amino-5-(4-methylpiperidinyl)benzamide **12** (1.0 g, 4.3 mmol) and **17** (0.6 g, 4.3 mmol), pale yellow prism crystals.

2-(3′-Methoxyphenyl)-6-morpholinyl-4-quinazolinone (41): 0.7 g from 2-amino-5-morpholinylbenzamide **(13)** (1.0 g, 4.5 mmol) and **17** (0.6 g, 4.5 mmol), pale yellow needles.

2,3-Dihydro-2-(3′-methoxyphenyl)-4-quinazolinone (43). Method A: 2-Aminobenzamide (**14**) (1.0 g, 7.3 mmol) and **17** (1.0 g, 7.3 mmol) were heated with stirring in DMAC (20 mL) at 80 °C for 2 h and then poured into ice water (200 mL). The precipitate was collected, washed with water, dried, and purified by column chromatography (silica gel; ethyl acetate/*n*-hexane) to afford **25** (0.3 g, 15.0%) and **43** (1.4 g, 75.2%) as pale yellow powders.

Method B: *p*-Toluenesulfonic acid monohydrate (0.1 g, 0.3 mmol) was added to a solution of **14** (1.0 g, 7.3 mmol) and **17** (1.0 g, 7.3 mmol) in DMAC (20 mL). The mixture was stirred at room temperature for 2 h and poured into ice water (200 mL). The precipitate was collected, washed with water, dried, and purified by column chromatography (silica gel; ethyl acetate/*n*-hexane) to afford **25** (70 mg, 4.0%) and **43** (1.7 g, 89.0%) as pale yellow powders.

Method B used for the preparation of **43** was used with the appropriate benzaldehyde and benzamide to afford **42** and **44**−**50**, together with **24** and **44**−**32**.

2,3-Dihydro-2-(2′-methoxyphenyl)-4-quinazolinone (42): pale yellow powder, **24** (90.0 mg) and **42** (1.7 g) from **14** (1.0 g, 7.3 mmol) and **16** (1.0 g, 7.3 mmol).

2,3-Dihydro-2-(4′-methoxyphenyl)-4-quinazolinone (44): pale yellow powder, **26** (40.0 mg) and **44** (1.7 g) from **14** (1.0 g, 7.3 mmol) and **18** (1.0 g, 7.3 mmol).

2,3-Dihydro-2-(2′,3′-dimethoxyphenyl)-4-quinazolinone (45): pale yellow powder, **27** (80.1 mg) and **45** (1.6 g) from **14** (1.0 g, 7.3 mmol) and **19** (1.2 g, 7.3 mmol).

2,3-Dihydro-2-(2′,4′-dimethoxyphenyl)-4-quinazolinone (46): pale yellow powder, **28** (99.9 mg) and **46** (1.8 g) from **14** (1.0 g, 7.3 mmol) and **20** (1.2 g, 7.3 mmol).

2,3-Dihydro-2-(2′,5′-dimethoxyphenyl)-4-quinazolinone (47): pale yellow powder, **29** (80.1 mg) and **47** (1.7 g) from **14** (1.0 g, 7.3 mmol) and **21** (1.2 g, 7.3 mmol).

2,3-Dihydro-2-(3′,4′-dimethoxyphenyl)-4-quinazolinone (48): pale yellow powder, **30** (60.2 mg) and **48** (1.6 g) from **14** (1.0 g, 7.3 mmol) and **22** (1.2 g, 7.3 mmol).

2,3-Dihydro-2-(3′,5′-dimethoxyphenyl)-4-quinazolinone (49): pale yellow powder, **31** (40.2 mg) and **49** (1.9 g) from **14** (1.0 g, 7.3 mmol) and **23** (1.2 g, 7.3 mmol).

2,3-Dihydro-2-(3′-methoxyphenyl)-6-chloro-4-quinazolinone (50): colorless powder, **32** (20.0 g, 1.1%) and **50** (1.6 g, 95.0%) from **15** (1.0 g, 5.9 mmol) and **17** (0.8 g, 5.9 mmol).

Cytotoxicity Assays. Compounds **24**−**50** were assayed for in vitro cytotoxicity in a panel of human tumor cell lines at the School of Pharmacy, University of North Carolina at Chapel Hill, according to procedures described previously.[18−20] The cell lines included human ovarian cancer (1A9), ileocecal carcinoma (HCT-8), lung carcinoma (A-549), glioblastoma (U-87-MG), bone (HOS), epidermoid carcinoma of the nasopharynx (KB), P-gp-expressing epidermoid carcinoma of the nasopharynx (KB-VIN), prostate cancer (PC3), breast cancer (MCF-7), and melanoma (SKMEL-2) cell lines. The cytotoxic effects of each compound were obtained as EC_{50} values, which represent the molar drug concentrations required to cause 50% inhibition.

Antimicrotubule Assay. Electrophoretically homogeneous bovine brain tubulin was purified as described previously.[21] The tubulin polymerization[7] and colchicine binding assays[22] were performed as described previously, except that Beckman DU7400/7500 spectrophotometers equipped with "high-performance" temperature controllers were used in the former assays. Unlike the manual control possible with the previously used Gilford spectrophotometers, the polymerization assays required use of programs provided by MD Analytical Associates, South Plainfield, NJ, since the Beckman instruments are microprocessor controlled. The Beckman instruments were unable to maintain 0 °C, and the lower temperature in the assays fluctuated between 2 and 5 °C. Temperature changes were, however, more rapid than in the Gilford instruments with the jump from the lower temperature to 26 °C taking about 20 s and the reverse jump about 95 s.

Acknowledgment. This investigation was supported by research grants from the National Science Council of the Republic of China (NSC88-2314-B-039-010) awarded to S.-C. Kuo and from the National Cancer Institute (CA 17625) awarded to K.-H. Lee.

References

(1) Rowinsky, E. K.; Donehower, R. C. The clinical pharmacology and use of antimicrotubule agents in cancer chemotherapeutics. *Pharmacol. Ther.* **1992**, *52*, 35−84.

(2) Verweij, J.; Clavel, M.; Chevalier, B. Paclitaxel (Taxol) and docetaxel (Taxotere): not simply two of a kind. *Ann. Oncol.* **1994**, *5*, 495−505.

(3) Hastie, S. B. Interactions of colchicine with tubulin. *Pharmacol. Ther.* **1991**, *51*, 377−401.

(4) Brossi, A.; Yeh, H. J.; Chrzanowska, M.; Wolff, J.; Hamel, E.; Lin, C. M.; Quinn, F.; Suffness, M.; Silverton, J. Colchicine and its analogues: recent findings. *Med. Res. Rev.* **1988**, *8*, 77−94.

(5) Kuo, S. C.; Lee, H. Z.; Juang, J. P.; Lin, Y. T.; Wu, T. S.; Chang, J. J.; Lednicer, D.; Paull, K. D.; Lin, C. M.; Hamel, E.; Lee, K. H. Synthesis and cytotoxicity of 1,6,7,8- and 4′-substituted 2-phenyl-4-quinolones and related compounds: identification as antimitotic agents interacting with tubulin. *J. Med. Chem.* **1993**, *36*, 1146−1156.

(6) Li, L.; Wang, H. K.; Kuo, S. C.; Lednicer, D.; Lin, C. M.; Hamel, E.; Lee, K. H. 2′,3′,4′,5′,5,6,7-Substituted 2-phenyl-4-quinolones and related compounds: their synthesis, cytotoxicity, and inhibition of tubulin polymerization. *J. Med. Chem.* **1994**, *37*, 1126−1135.

(7) Li, L.; Wang, H. K.; Kuo, S. C.; Wu, T. S.; Mauger, A.; Lin, C. M.; Hamel, E.; Lee, K. H. Synthesis and biological evaluation of 3′,6′,7-substituted 2-phenyl-4-quinolones as antimitotic antitumor agents. *J. Med. Chem.* **1994**, *37*, 3400−3407.

(8) Xia, Y.; Yang, Z. Y.; Xia, P.; Bastow, K. F.; Tachibana, Y.; Kuo, S. C.; Hamel, E.; Hackl, T.; Lee, K. H. Synthesis and biological evaluation of 6,7,2′,3′,4′-substituted-1,2,3,4-tetrahydro-2-phenyl-4-quinolones as a new class of antimitotic agents. *J. Med. Chem.* **1998**, *41*, 1155−1162.

(9) Chen, K.; Kuo, S. C.; Hsih, M. C.; Mauger, A.; Lin, C. M.; Hamel, E.; Lee, K. H. 2′,3′,4′,5,6,7-Substituted 2-phenyl-1,8-naphthyridin-4-ones: their synthesis, cytotoxicity, and inhibition of tubulin polymerization. *J. Med. Chem.* **1996**, *40*, 2266−2275.

(10) Chen, K.; Kuo, S. C.; Hsih, M. C.; Mauger, A.; Lin, C. M.; Hamel, E.; Lee, K. H. Synthesis and biological evaluation of substituted 2-aryl-1,8-naphthyridin-4(1H)-ones as antimitotic antitumor agents that inhibit tubulin polymerization. *J. Med. Chem.* **1997**, *40*, 3049−3056.

(11) Yale, H. J.; Kalkstein, M. Substituted 2,3-dihydro-4(1H)-quinazolinones. A new class of inhibitors of cell multiplication. *J. Med. Chem.* **1967**, *10*, 334−336.

(12) Neil, G. L.; Li, L. H.; Buskirk, H. H.; Moxlcy, T. E. Antitumor effects of the antispermatogenic agent, 2,3-dihydro-2-(1-naphthyl)-4(1H)-quinazolinones. *Cancer Chemother.* **1972**, *56*, 163−173.

(13) Hamel, E.; Lin, C. M.; Plowman, J.; Wang, H. K.; Lee, K. H.; Paull, K. D. Antitumor 2,3-dihydro-2-(aryl)-4(1H)-quinazolinone derivatives. Interactions with tubulin. *Biochem. Pharmacol.* **1996**, *51*, 53−59.

(14) Jiang, J. B.; Hesson, D. P.; Dusak, B. A.; Dexter, D. L.; Kang, G. J.; Hamel, E. Synthesis and biological evaluation of 2-styrylquinazolin-4(3H)-ones, a new class of antimitotic anticancer agents which inhibit tubulin polymerization. *J. Med. Chem.* **1990**, *33*, 1721−1728.

(15) Lin, C. M.; Kang, G. J.; Roach, M. C.; Jiang, J. B.; Hesson, D. P.; Luduena, R. F.; Hamel, E. Investigation of the mechanism of the interaction of tubulin with derivatives of 2-styrylquinazolin-4(3H)-one. *Mol. Pharmacol.* **1991**, *40*, 827−832.

(16) Xia, Y.; Yang, Z. Y.; Hour, M. J.; Kuo, S. C.; Xia, P.; Bastow, K. F.; Nakanishi, Y.; Nampoothiri, P.; Hackl, T.; Hamel, E.; Lee, K. H. Synthesis and biological evaluation of substituted 2-arylquinazolinones. *Bioorg. Med. Chem. Lett.*, submitted.

(17) Xia, Y.; Wang, Z. Y.; Morris-Natschke, S. L.; Lee, K. H. Recent advances in the discovery and development of quinolones and analogues as antitumor agents. *Curr. Med. Chem.* **1999**, *6*, 179−194.

(18) Lee, K. H.; Lin, Y. M.; Wu, T. S.; Zhang, D. C.; Yamagishi, T.; Hayashi, T.; Hall, I. H.; Chang, J. J.; Wu, R. Y.; Yang, T. H. The cytotoxic principles of *Prunella vulgaris*, *Psychotria serpens*, and *Hyptis captitata:* Ursolic acid and related derivatives. *Planta Med.* **1988**, *54*, 308−312.

(19) Boyd, M. R. Status of the NCI preclinical antitumor drug discovery screen. In *Cancer: Principles and Practice of Oncology Updates;* De Vita, V. T., Hellman, S., Rosenberg, S. A., Eds; J. B. Lippincoft: Philadelphia, 1989; pp 1−12.

(20) Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Vaigro-Woiff, A.; Gray-Goodrich, M.; Campbell, H.; Mayo, J.; Boyd, M. Feasibility of a high-flux anticancer drug screen utilizing a derived panel of human tumor cell lines in culture. *J. Natl. Cancer Inst.* **1991**, *83*, 757−766.

(21) Hamel, E.; Lin, C. M. Separation of active tubulin and microtubule-associated proteins by ultracentrifugation and isolation of a component causing the formation of microtubule bundles. *Biochemistry* **1984**, *23*, 4173−4184.

(22) Verdier-Pinard, P.; Lai, J. Y.; Yoo, H. D.; Yu, J.; Marquez, B.; Nagle, D. G.; Nambu, M.; White, J. D.; Falck, J. R.; Gerwick, W. H.; Day, B. W.; Hamel, E. Structure−activity analysis of the interaction of curacin A, the potent colchicine site antitmitotic agent, with tubulin and effects of analogues on the growth of MCF-7 breast cancer cells. *Mol. Pharmacol.* **1998**, *53*, 62−76.

JM000151C