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Antitumor Agents. Part 204:[1] Synthesis and Biological Evaluation of Substituted 2-Aryl Quinazolinones

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Abstract—A series of 2′,3′,4′,6,7-substituted 2-aryl quinazolinones were synthesized and evaluated for biological activity. Among them, **17** displayed significant growth inhibitory action against a panel of tumor cell lines. Compound **17** was also a potent inhibitor of tubulin polymerization. Compounds **8**–**10** displayed selective activity against P-gp-expressing epidermoid carcinoma of the nasopharynx.

As a continuation of our structure–activity relationship study of substituted 2-phenyl-4-quinolones (**PQ**) (Fig. 1) and flavonoids as antimitotic antitumor agents, we have developed several types of related compounds and evaluated their cytotoxicity and interactions with tubulin. Many **PQ** derivatives displayed potent activity in these assays, with effects similar to those observed with the antimitotic natural products colchicine, podophyllotoxin, and combretastatin 4-A.[2–5] Most notably, **PQ1** totally inhibited the growth of about half of the NCI's drug screen tumor cell lines at subnanomolar concentrations ($\log \mathrm{TGI} < -9.00$). **PQ1** was also a potent inhibitor of tubulin polymerization with an IC_{50} value of 0.44 μM, and it was a highly effective inhibitor of the binding of [^{3}H]colchicine to tubulin. 2-Phenyl-1,8-naphthyridin-4-ones (**PN**)[6,7] have also been synthesized, and potent cytotoxic antimitotic agents of the **PN** series were identified. In addition, in our early studies, certain flavonoids, for example, **F1**, showed cytotoxic activity with GI_{50} values in the high nanomolar to low micromolar concentration range.[8] **F1** also inhibited tubulin polymerization with an IC_{50} value of 0.83 μM and was the first example of a flavonol displaying such bioactivity.

From a comparison of the general structures of active **PQ** and **PN** derivatives and flavonoids, it is clear that these compounds share a similar skeleton except for different heteroatoms. Interestingly, we found that activities changed dramatically with respect to the heteroatom position and number. For example, **PN** derivatives with an additional nitrogen atom in the A ring often showed more potent cytotoxic and antitubulin activities than the corresponding **PQ** compounds, whereas 2-phenylpyrido[1,2-*a*]pyrimidin-4-ones (**PP**) with a nitrogen at the 4a-position showed no activity in either assay.[6] Also, when a nitrogen atom was introduced into the C ring, that is, 2′-pyridyl replaced the 2-phenyl ring in 2-phenyl-4-quinolones, the antitubulin activity was minimal.[3]

Bioisosteric transformation is a frequently used concept in drug design and development, and interesting biological results have been obtained by studying bioisosteric

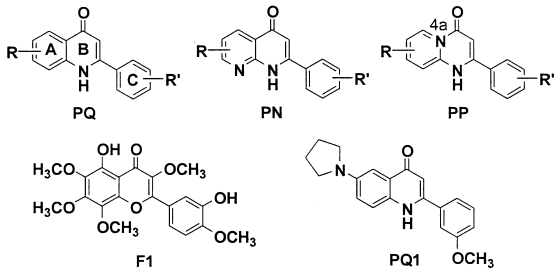

Figure 1. Structures of 2-phenyl-4-quinolones and analogues.

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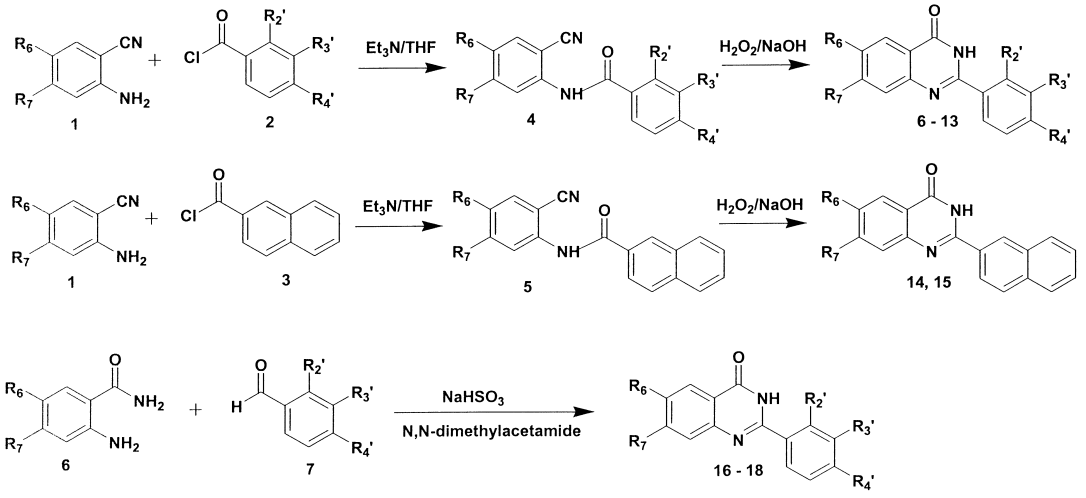


Scheme 1. General synthetic routes to 2-aryl-quinolones.

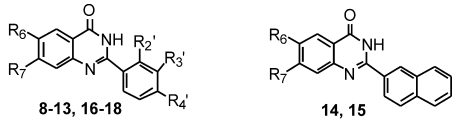
compounds.[9] Therefore, based on this general principle and the above results, it was of interest to synthesize a series of 2-phenyl-quinazolinones by introducing the nitrogen into the B-ring, and examine the structure–activity relationships.

As shown in Scheme 1, 2-aryl-quinazolinones were synthesized according to two general methods.[10] Amidation of anthranilonitrile (**1**) with benzoyl chloride (**2**) or naphthoyl chloride (**3**) gave rise to the respective diaryl amide intermediates **4** and **5**. Oxidative ring closure of **4** and **5** under basic conditions afforded quinazolinones **8**–**15**. Compounds **16**–**18** were synthesized by condensation of a substituted aminobenzamide (**6**) with benzaldehyde (**7**) at 150 °C.[11] The structures of the newly synthesized compounds are summarized in Table 1.

Table 1 also summarizes the effects of the quinazolinone derivatives as inhibitors of tubulin polymerization. Compounds **8**–**15** had no inhibitory effect on the reaction (IC_{50} > 40 μM), while as with the **PQ** derivatives, compounds with a methoxy group at the 3′-position (**16**–**18**) were active as inhibitors. In comparing compounds with different substituents at the A ring, **17** with a methoxy at the 6-position was more active than **18** with methoxy groups at both 6- and 7-positions or than unsubstituted **16**. These findings are consistent with the view that the quinazolinone biaryl system, which is composed of the fused A/B rings and the C ring, is critical for tubulin binding at the colchicine site, by analogy to the **PQ** and **PN** derivatives.

The substituted 2-aryl-quinazolinone derivatives (**8**–**18**) were assayed for their cytotoxicity in vitro against seven human tumor cell lines, including epidermoid carcinoma of the nasopharynx (KB), P-gp-expressing epidermoid carcinoma of the nasopharynx (KB-VIN), melanoma (SKMEL-2), ileocecal carcinoma (HCT-8),

Table 1. Antimicrotubule activity of substituted 2-aryl-4-quinazolinone analogues[12]



Compound	R_6	R_7	$R_{2'}$	$R_{3'}$	$R_{4'}$	ITP[a] IC_{50} (mM) ± SD
8	H	H	F	H	H	> 40
9	H	H	H	F	H	> 40
10	H	H	H	H	F	> 40
11	OCH_3	OCH_3	F	H	H	> 40
12	OCH_3	OCH_3	H	F	H	> 40
13	OCH_3	OCH_3	H	H	F	> 40
14	H	H				> 40
15	OCH_3	OCH_3				> 40
16	H	H	H	OCH_3	H	8.6 ± 0.7
17	OCH_3	H	H	OCH_3	H	4.9 ± 1
18	OCH_3	OCH_3	H	OCH_3	H	6.5 ± 0.9
Colchicine						0.80 ± 0.07[b]
Podophyllotoxin						0.46 ± 0.02[b]
Combretastatin A-4						0.53 ± 0.05[b]

[a]ITB, inhibition of tubulin polymerization.
[b]Data from ref 3.

Table 2. In vitro cytotoxic activities of 2′,3′,4′,6,7-substituted 2-aryl-4-quinazolinones[12]

Compound	ED_{50} (μg/mL)[a]						
	KB[b]	KB-VIN[b]	SKMEL-2[b]	HCT-8[b]	MCF-7[b]	A-549[b]	1A9[b]
8	> 20 (26)[c]	17.5	> 20 (31)	> 20 (11)	> 20 (21)	> 20 (35)	17.10
9	> 20 (19)	8.50	> 20 (41)	NA	> 20 (22)	9.80	> 20 (19)
10	> 20 (41)	6.50	> 20 (47)	NA	> 20 (41)	7.50	> 20 (24)
11	< 5 (60)	< 5 (67)	< 5 (65)	17.00	13.10	< 5 (62)	> 20 (44)
12	NA[d]	> 20 (21)	> 20 (14)	> 20 (6)	NA[d]	> 20 (18)	> 20 (27)
13	> 20 (36)	> 20 (49)	> 20 (39)	> 20 (10)	19.80	> 20 (41)	16.00
14	12.50	6.00	13.00	19.10	10.30	14.40	8.00
15	7.30	1.60	8.50	> 10 (49)	6.60	8.00	7.80
16	NA[d]	> 20 (12)	> 20 (12)	> 20 (8)	> 20 (26)	> 20 (21)	NA[d]
17	1.98	NA[d]	> 40(21)	> 40(45)	37.5	28.1	4.70
18	> 20 (45)	19.0	NA[d]	> 20 (37)	14.5	> 20 (49)	17.5

[a]ED_{50} was the concentration of compound which affords 50% reduction in cell number after a 3-day incubation.
[b]Human epidermoid carcinoma of the nasopharynx (KB), P-gp-expressing human epidermoid carcinoma of the nasopharynx (KB-VIN), human melanoma cancer (SKMEL-2), human ileocecal carcinoma (HCT-8), human breast cancer (MCF-7), human lung carcinoma (A-549), and human ovarian cancer (1A9).
[c]Inhibition < 50% at highest test concentration (percent inhibition observed is given in parentheses).
[d]NA, not available.

Table 3. Anti-HIV activity of quinazolinones in acutely infected H9 lymphocytes[13]

Compound	IC_{50} (μM)[a]	EC_{50} (μM)[b]	TI[c]
8			Did not dissolve
9	30.7	9.53	3.22
10	38.8	9.18	4.22
11	100	10.9	9.17
12			Did not dissolve
13			Did not dissolve
14			Did not dissolve
15	100	7.59	13.2

[a]Concentration that inhibits uninfected H9 cell growth by 50%.
[b]Concentration that inhibits viral replication by 50%.
[c]TI, therapeutic index IC_{50}/EC_{50}.

breast cancer (MCF-7), lung carcinoma (A-549), and ovarian cancer (1A9) cell lines.

As shown in Table 2, compounds **8**–**15**, which did not inhibit tubulin polymerization, also did not show significant cytotoxicity against the human tumor cell lines. Compound **17**, which was the most active inhibitor in the tubulin based assay, showed significant potency especially against epidermoid carcinoma and human ovarian cancer with ED_{50} values less than 5 μg/mL.

Despite their low cytotoxicity, fluorinated compounds interestingly showed excellent selectivity. Compounds **8**–**10** displayed increased activity against the P-gp-expressing cell line KB-VIN relative to the parental cell line. Among them, the 4′-F compound (**10**) was the most selective. In contrast, minimal selective activity against this multi-drug resistance cell line was observed with **11**–**14**, which have dimethoxy substitution in the A-ring.

These interesting quinazolinone compounds also have been screened for anti-HIV activity as shown in Table 3. Due to insolubility at the testing concentration, results of **8** and **12**–**14** were not obtained. Compounds **11** and **15** displayed selective antiviral activity with therapeutic indexes greater than 9 in acutely infected H9 lymphocytes. The HIV virus might be a new target for quinazolinones bearing methoxy groups on both the 6- and 7-positions.

In summary, cytotoxicity and antitubulin activities of quinazolinones are highly dependent on the substitutions on the skeleton, especially at the 6-position, as was observed with the **PQ** and **PN** derivatives. The anti-HIV activity observed with dimethoxy-quinazolinones and the selectivity for P-gp-expression in cells noted with fluorinated quinazolinones were both unexpected. Further SAR studies will be undertaken to elucidate the mechanisms involved.

Acknowledgements

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11. All new compounds gave satisfactory analytical and spectroscopic data. Selected spectroscopic data for 2-(3′-methoxyphenyl)-6-methoxy-4-quinazolinone (**17**): Yield: 42%; mp: 216–218 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.86 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3), 7.11 (dd, $J=2.5$, 8.0 Hz, 1H, H-4′), 7.29–7.49 (m, 2H, H-5′, H-7), 7.53 (d, $J=2.5$, 1H, H-5), 7.67–7.82 (m, 3H, H-2′, H-6′, H-8), 12.48 (br s, 1H, NH); MS (M^+) 282.
12. Cytotoxic and tubulin polymerization assays were performed as described previously.[5]
13. HIV growth inhibition assay was performed by Biotech Research Laboratories, Gaithersburg, MD. HIV growth inhibition assay was performed as described previously in Yang, Z. Y.; Xia, Y.; Xia, P.; Cosentino, L. M.; Lee, K. H. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1483.