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HETEROATOM- AND CARBON-LINKED BIPHENYL ANALOGS OF BREQUINAR AS IMMUNOSUPPRESSIVE AGENTS

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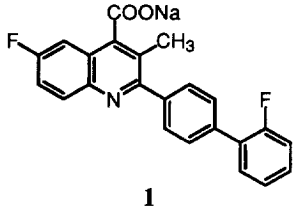
Abstract. Structure–activity relationships were explored for some analogs of Brequinar having a linking atom between the 2-biphenyl substituent and the quinoline ring. Activities as inhibitors of dihydroorotate dehydrogenase and the mixed lymphocyte reaction were related to the overall shape and lipophilicity of the 2-substituent.

Introduction. Brequinar sodium (**1**) is a potent, selective immunosuppressive agent that acts by inhibiting dihydroorotate dehydrogenase (DHODase), the controlling step in de novo pyrimidine nucleotide biosynthesis.[1] This activity is manifested in the selective inhibition of activated lymphocyte function, leading to suppression of the cellular immune response. Prevention of organ transplant rejection, rheumatoid arthritis, and psoriasis are some of the potential applications for drugs like Brequinar. Structure–activity relationships for Brequinar analogs[2] and for related tetracyclic compounds such as **2**[3] have been described. In all cases, the presence of the biphenyl 2-substituent on the quinoline ring, or an equivalent substituent with similar steric or lipophilic properties, was required for activity. Herein we describe the effect of inserting a linking atom between the biphenyl moiety and the quinoline ring, providing structures related to **3**.



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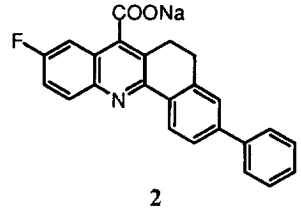

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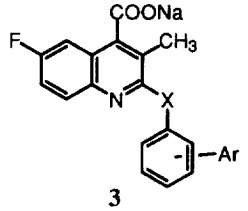

3

Chemistry. The synthetic approach to analogs of **1** with a heteroatom linker is shown in Scheme 1. The quinolone **4**, prepared in 25% yield from 5-fluoroisatin by a literature method,[4,5] was esterified and converted to the bromoquinoline **5** in 76% overall yield. This was treated with the appropriate aniline derivative in boiling ethanol, or with the sodium salt of the appropriate phenol or alcohol, followed by saponification of the ester to

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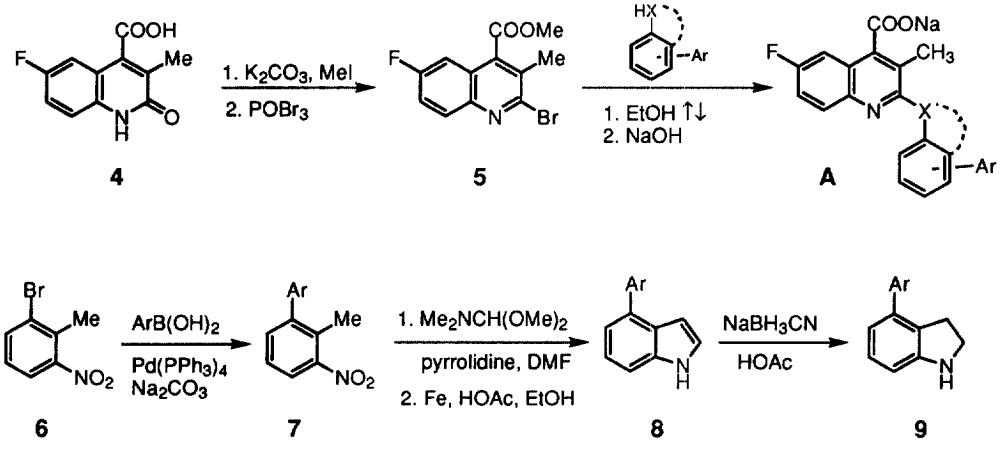
provide the products **A** in 50–80% yields. Since the parent carboxylic acids generally were very poorly soluble in water and organic solvents, the sodium salts were prepared and submitted for biological evaluation. The indolines used for synthesizing compounds **25** and **28–34** by this method were prepared from **6**. The distal aromatic ring was introduced by Suzuki coupling with the appropriate boronic acid to give **7** in 65–85% yield,[6] followed by formation of the indole **8** in 30–60% yield using the method of Batcho and Leimgruber.[7] Sodium cyanoborohydride in acetic acid reduced the indole ring, providing **9** in 78–82% yield. Indoles **8** could not be made to react with **5**, so the appropriate indoline product **A** (from **5** and **9**) was oxidized with palladium and cyclohexene in ethanol to provide the indole derivative **26**. The benzimidazole product **27** was obtained by reacting the appropriate phenylbenzimidazole directly with **5** in boiling n-butanol, but in very low yield.

Scheme 1

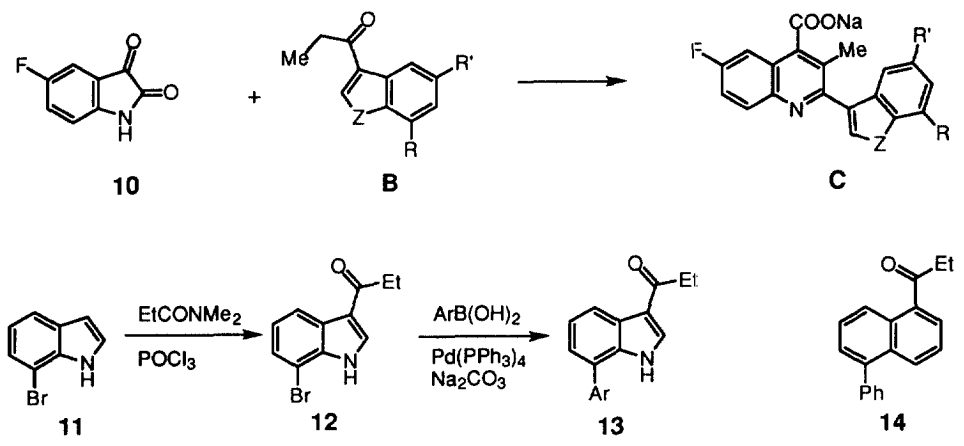


Analogs in which the linking atom is carbon were prepared using the Pfitzinger condensation, a classical approach to 2-arylcinchoninic acids, as shown in Scheme 2. Thus, an aryl ethyl ketone **B** was combined with 5-fluoroisatin **10** in the presence of potassium hydroxide,[8] or alternatively under acidic conditions,[9] to provide the product **C** in 70–85% yield. The indole ketones **13** used to prepare **35–39** were synthesized by acylation of 7-bromoindole **11**[10] in 65% yield using the method of Anthony.[11] The distal aryl group was again attached to **12** by Suzuki coupling, optionally followed by N-methylation of the indole. The naphthyl ketone **14**, used to prepare **40** via Pfitzinger condensation, was prepared in 15% overall yield from 5-bromonaphthoic acid[12] by Suzuki coupling followed by treatment of the lithium carboxylate with ethylmagnesium bromide. Experimental details for the synthetic routes outlined have been published.[13]

Biology. Two primary assays were utilized to evaluate the immunosuppressive effects of the compounds described. The first, an isolated enzyme assay (DHOD) using partially purified DHODase from human liver,[14] provided K_i values (nM) for inhibition of the formation of orotate from radiolabelled dihydroorotate. The second test used was the human mixed lymphocyte reaction (MLR), a standard model of cell-based immunity.[15] Here,

compounds were evaluated for their ability to inhibit proliferation in a mixture of human lymphocytes from two unrelated donors, providing IC_{50} values (nM). Biological results are shown in Tables 1 and 2.

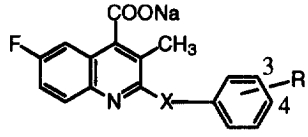
Scheme 2



Discussion and Conclusions. Structure–activity investigations on Brequinar itself[2] and tetracyclic analogs[3] demonstrated that the 2-biphenyl substituent plays a major role in the immunosuppressive activity of these compounds. The effect of extension of this group was investigated by inserting a heteroatom linker, as shown in Table 1. The simple aniline and phenol derivatives (**15** and **16**) were inactive as expected, since the distal ring or another large lipophilic substituent has been shown to be necessary for activity.[2] Analogs bearing *para*-biphenyloxy or biphenylamino substituents (**17**, **18**) were also totally inactive. However, the *meta* biphenyloxy and biphenylamino variants (**19** and **20**) showed promising activity, with the DHOD potency of **19** about the same as that for **1**. Substitution on the nitrogen atom of **20** (**21** and **22**) reduced activity significantly, as did further extension of the biphenyl substituent by an additional atom (**23** and **24**).

Examination of computer-minimized models of **1** and **19** (Figure 1) suggested that the *meta*-heteroatom-linked biphenyls can mimic the *para*-biphenyl substituent of **1**. (In Figures 1–4, **1** is shown in yellow, with the fluorine atom on the biphenyl removed for clarity.) Although greater flexibility is available in the 2-substituent of **19** relative to **1**, reasonable conformations of **19** place the end of the distal ring in a very similar position relative to the quinoline ring. The inactive *para*-linked biphenyl **17** cannot achieve this orientation (Figure 2), suggesting that the location of the distal ring relative to the quinoline is critical to activity.

Table 1.



Compd	X	R	DHOD K_i, nM	MLR IC_{50}, nM
1	bond	4-(2-F-Phenyl)	12	15
15	O	H	>1000	>10000
16	NH	H	>200	>10000
17	O	4-Phenyl	>1000	>10000
18	NH	4-Phenyl	>430	>10000
19	O	3-Phenyl	21	210
20	NH	3-Phenyl	83	340
21	NMe	3-Phenyl	240	>10000
22	NAc	3-Phenyl	>390	>10000
23	OCH_2	4-Phenyl	>200	>10000
24	OCH_2	3-Phenyl	330	>10000

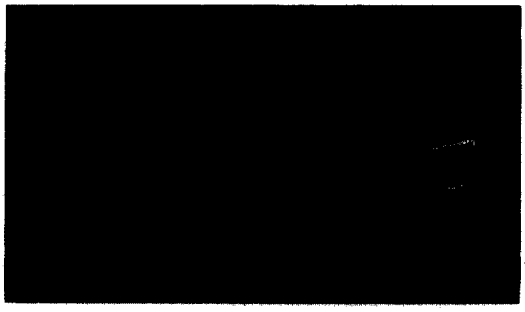


Figure 1. **1** and **19**

Figure 2. **1** and **17**

Figure 3. **1** and **19**: unfavorable rotamer

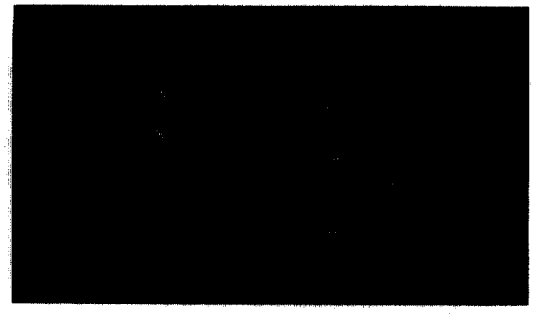
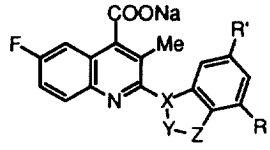


Figure 4. **1** and **25**

Although the *meta*-biphenyloxy substituent can attain the desired conformation, rotation of the biphenyl-oxygen bond of **19** would project the distal phenyl into other regions of space (for example, as shown in Figure 3), presumably disallowed by the enzyme active site. Bridging a nitrogen atom linker to the proximal ring of the biphenyl, forming an indoline or indole, would eliminate the undesirable conformations by removing the rotatable biphenyl-nitrogen bond, forcing the desired conformation for **25** as shown in Figure 4. Rotation about the nitrogen-quinoline bond of **25** causes the 2-substituent to sweep out a conical area of space, with the end of the distal ring always overlapping with that of **1**.

Indeed, the indoline **25** showed activity comparable to that of **1** in both the enzyme and cellular assays (Table 2). Oxidation to the indole **26** gave even more potent activity in the DHOD assay, but MLR activity had decreased. Incorporating a second ring nitrogen to give the benzimidazole **27** further decreased activity, especially in MLR. This suggests that decreasing lipophilicity is detrimental, especially for the cell-based assay.

Table 2.



Compd	XYZ	R	R'	DHOD K_i, nM	MLR IC_{50}, nM
1				12	15
25	NCH_2CH_2	Phenyl	H	15	26
26	NCH=CH	Phenyl	H	4.5	380
27	NCH=N	Phenyl	H	71	4700
28	NCH_2CH_2	2-F-Phenyl	H	24	2.8
29	NCH_2CH_2	2-Me-Phenyl	H	25	1.6
30	NCH_2CH_2	2-MeO-Phenyl	H	30	4.1
31	NCH_2CH_2	3-CF_3-Phenyl	H	24	0.41
32	NCH_2CH_2	3-MeO-Phenyl	H	34	4.0
33	NCH_2CH_2	Phenyl	F	30	41
34	NCH_2CH_2	Phenyl	MeO	22	35
35	CH=CHNH	Phenyl	H	>130	1600
36	CH=CHNMe	Phenyl	H	27	140
37	CH=CHNMe	2-MeO-Phenyl	H	40	650
38	CH=CHNMe	3-MeO-Phenyl	H	32	190
39	CH=CHNMe	3-CF_3-Phenyl	H	23	7
40	CH=CHCH=CH	Phenyl	H	26	150

Lipophilic substitution at the ortho position on the distal phenyl group (**28–30**) had no significant effect on DHOD, but enhanced MLR by a factor of 6–15, possibly by improving cell penetration. Meta substitution by CF_3 (**31**) had an even more dramatic effect on the MLR potency, while the *meta*-methoxy derivative **32** was similar to the ortho isomer. Substitution on the benzo ring of the indoline (**33** and **34**) had little effect on potency.

Reversing the points of attachment on the indole ring of **25** from 1,4 to 3,7 gave **35**, but activity was lost. Methylation of this analog on nitrogen (**36**) restored much of the activity, but the MLR potency was not as attractive as that seen with the indolines such as **25**. Substitution effects in **36** were similar to those seen for **25**; again, trifluoromethyl (**39**) dramatically improved the MLR potency. The naphthyl derivative **40** caused little change in activity relative to **36**. This finding was surprising since the distal phenyl ring of **40**, while parallel to that of **1**, is displaced laterally, occupying a different area of space relative to the quinoline ring.

To summarize, a variety of Brequinar analogs were synthesized, bearing 2-substituents differing in shape from the biphenyl moiety of **1**. Those analogs that positioned the distal phenyl group in an orientation similar to that seen in **1** showed similar activity, while those which could not attain this conformation are inactive, suggesting that the lipophilic binding site for the 2-substituent has fairly specific shape limitations. Restricting the flexibility of the extended sidechain by incorporating an indoline or indole ring gave compounds with better potency than **1**. Increased lipophilicity in this region of the molecule seems desirable (**19** vs. **20**; **27** vs. **26**). Lipophilic substitution of the distal ring seems to enhance MLR activity without changing DHOD activity, suggesting improved cell penetration. This class of compounds provides an interesting new series of immunosuppressive agents.

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