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STRUCTURE–ACTIVITY RELATIONSHIPS (SAR) OF SOME TETRACYCLIC HETEROCYCLES RELATED TO THE IMMUNOSUPPRESSIVE AGENT BREQUINAR SODIUM

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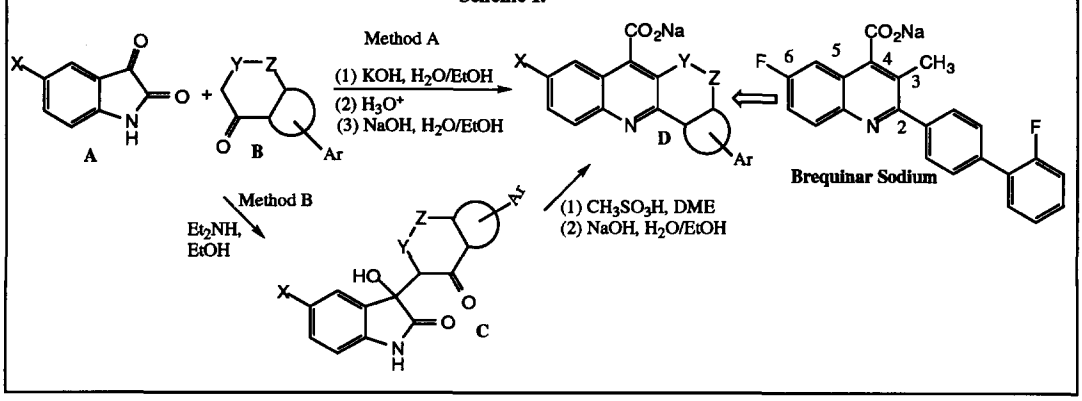
Abstract. The structure–activity relationships of some tetracyclic heterocycles related to Brequinar were explored. Activities as inhibitors of dihydroorotate dehydrogenase and the mixed lymphocyte reaction are related to ring system, heteroatom placement, and pendant ring substitution.

Introduction. Brequinar Sodium (Brequinar, BQR)[1] is a potent inhibitor of the enzyme dihydroorotate dehydrogenase (DHODase), which is required for the *de novo* biosynthesis of pyrimidine nucleotides. The potential importance of BQR in organ transplantation, as well as in diseases which have an autoimmune component such as rheumatoid arthritis and psoriasis, prompted a chemistry effort aimed at finding other molecules which would possess both *in vitro* and *in vivo* activity comparable to BQR. Such molecules would be good candidates to enter clinical trials. In a previous paper,[2] we discussed the structure–activity relationships of compounds with various substituents at the 2-, 3-, 4-, and 6-position of the quinoline ring system as well as changes to the quinoline core. These structural changes had a pronounced effect on both inhibition of DHODase and a measure of cellular immune response, the mixed lymphocyte reaction (MLR). This paper reports the SAR of tetracyclic heterocycles related to BQR, one of which (compd **32**) possesses subnanomolar cellular activity.

Scheme 1.



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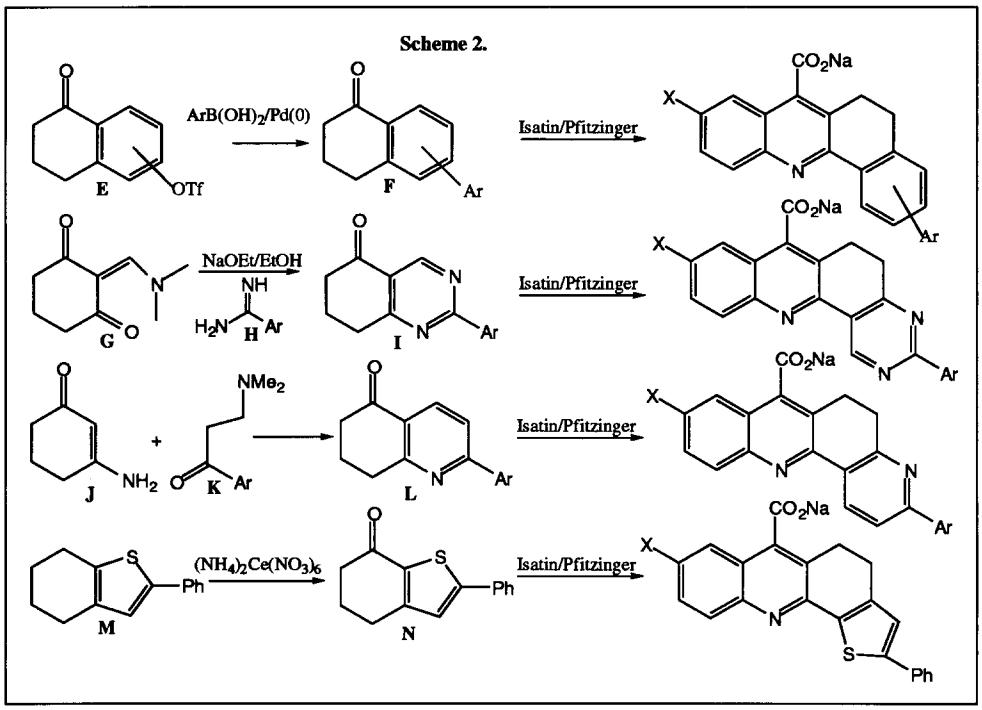
Chemistry. The BQR analogs in this paper were prepared by Pfitzinger condensation of a substituted isatin **A** with a cyclic ketone **B**. In most cases condensation with alkaline potassium hydroxide provided the product **D** in yields greater than 60%. In a few cases (YZ= CH_2O and OCH_2) it was more effective to isolate the aldol product **C** and subject the intermediate to acid mediated closure (Scheme 1).[3]

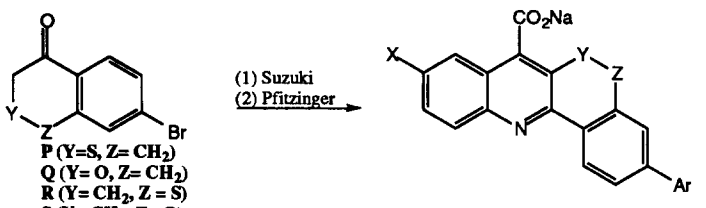
The synthesis of the ketones required for the Pfitzinger condensation could be achieved by a variety of methods. In some cases the pendant aryl group was incorporated via Suzuki coupling of either the aryl triflate[4] or the aryl bromide,[5] which allowed for rapid development of SAR in this region of the molecule. For ease of purification of the final product, the Suzuki coupling was usually performed prior to the Pfitzinger condensation.

The synthesis of the cyclohexanone derivatives are shown in Scheme 2. The tetralones **F** were synthesized from the readily available aryl triflate **E**.[6] The quinazoline **I** was prepared by condensation of the



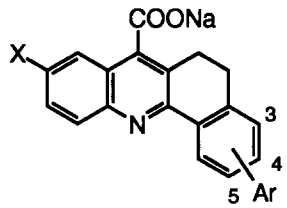
2-dimethylaminomethylene-1,3-cyclohexanedione **G** with a benzamidine **H**.[7] The tetrahydroquinoline **L** was prepared by condensation of an enaminoketone **J** with an acetophenone **K**, the intermediate dihydropyridine spontaneously disproportionating to give the desired product.[8] The synthesis of the tetrahydrobenzothiophene **N** was accomplished by a regiospecific cerium oxidation of compound **M**.[9] All of these ketones underwent the Pfitzinger condensation via method A to provide the desired tetracyclic compounds.

Scheme 3.



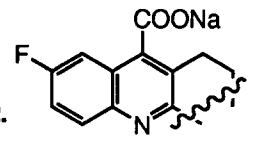
The synthesis of tetracyclic BQR analogs with variation in the bridging ring represented by the atom variations YZ required a variety of approaches, the experimental details of which have been reported.[3] In brief, ketones **P** and **R** were prepared by a Friedel–Crafts ring closure. Compound **Q** was prepared by a Dieckman ring synthesis, and ketone **S** was prepared by a chromanone synthesis from the *o*-hydroxy-acetophenone.

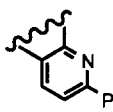
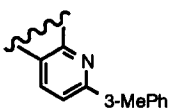
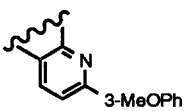
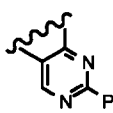
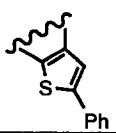
Table 1.



Compd	X	Ar	DHOD K_i, nM	MLR IC_{50}, nM
1	F	4-Ph	8.3	30
2	CF_3	4-Ph	31	52
3	F	3-Ph	300	>5000
4	F	3-PhO	962	>5000
5	F	5-Ph	>1000	>5000
6	F	4-(4-CF_3Ph)	370	850
7	F	4-(4-MePh)	40	28
8	F	4-(4-MeOPh)	55	60
9	F	4-(3CF_3Ph)	43	18
10	F	4-(3-MePh)	59	60
11	F	4-(3-MeOPh)	25	10
12	F	4-(2-CF_3Ph)	40	50
13	F	4-(2-MePh)	10	30
14	F	4-(2-MeOPh)	20	50
15	F	4-(2-FPh)	34	40
16	F	4-(3-thienyl)	52	170
17	F	4-(3-furyl)	190	280
18	F	4-(2-thiazolyl)	>180	>5000

Table 2.



Compd	Ar	DHOD K_i, nM	MLR IC_{50},nM
19		409	100
20		81	180
21		160	300
22		380	600
23		>182	>10000

The oxygen containing tetracyclic BQR analogs required the use of Pfitzinger Method B. Use of HCl as the acid in the Pfitzinger synthesis resulted in isolation of the ring opened benzylchlorides. This difficulty

could be overcome by ring closure with methanesulfonic acid, which provided the desired heterocycles in ca. 30% yield. The remaining heterocyles were synthesized in at least 60% yield using Pfitzinger Method A. The sulfur bridged compounds could be oxidized to the sulfoxide by m-chloroperbenzoic acid and to the sulfone with oxone.

Biology. Two primary assays were utilized to evaluate the analogs of Brequinar. The first, an isolated enzyme assay (DHOD)[10] measured the inhibition of formation of orotate from radiolabeled dihydroorotate. K_i values (nM) are reported in Tables 1–3. The second test used was the human mixed lymphocyte reaction (MLR), a standard model of cell-based immunity.[1] Tables 1–3 report the IC_{50} values (nM) required to inhibit proliferation in a mixture of human lymphocytes from two unrelated donors.

Discussion and Conclusions. Brequinar Sodium has been examined in clinical trials as an immunosuppressive agent to inhibit the rejection of transplanted organs,[1] and has possible application to other diseases with an autoimmune component such as rheumatoid arthritis and psoriasis. Brequinar's action is believed to result from its potent inhibition of the enzyme DHODase[12] (K_i = 12 nM). Interference with pyrimidine biosynthesis in T-cells results in immunosuppression. This immunosuppression is reflected in Brequinar's potency in the mixed lymphocyte reaction (MLR IC_{50} = 15 nM). Recently, Leflunomide (N-(4-trifluoromethylphenyl)-5-methylisoxazol-4-carboxamide), a drug in clinical trials for rheumatoid arthritis, has been shown to inhibit pyrimidine biosynthesis.[13] Studies on a metabolite of leflunomide, A771726 (2-cyano-3-hydroxy-N-(4-trifluoromethylphenyl)crotonamide), has shown it to be a potent inhibitor of DHODase (K_i = 104 nM).[14] Thus, DHODase inhibition by a variety of chemical structures may ultimately result in clinically useful agents.

We examined several different tetracyclic analogs of Brequinar. Table 1 reports the results of some ethylene bridged BQR derivatives. Compounds **1** and **2**, which possess a 6-F or 6-CF_3 substituent, were similar to BQR in activity.[15] As a result this series was examined in more detail. Due to the constraint of the tetracyclic system we examined the substituents at the 3- and 5- position of the ring in compounds **3–5**. These compounds were weakly active in DHODase and showed poor activity in the cell based assay. Subtituents with diverse electronic character at the 2 or 3-positions of the 4-phenyl ring, as in compounds **9–14**, appear to have good activity, while substituent at the 4-position as in compounds **6–8** showed more variation. In particular compound **6** with an electron withdrawing -CF_3 group showed a significant loss in potency. In compound **16**, a thiophene is substituted for the 4-phenyl substituent with little change in potency. The furyl group in compound **17** was tolerated, but substitution for the 4-phenyl substituent by the 2-thiazolyl group, in compound **18**, resulted in a dramatic decrease in potency.

We next examined other heterocycles with an ethylene bridge, as shown in Table 2. Fused pyridyl and pyrimidyl ring systems are exemplified in compounds **19–22**, and were significantly less active than phenyl fused system. This might be due to changes in lipophilicity and basicity. Some variation in activity with substituents on the pendant phenyl ring was observed suggesting these ring systems may be amenable to further optimization. The fused thiophene **23** was less potent than expected. We had observed in thiophene compound **16** and phenyl compound **1** that the two groups are essentially isosteric. The tetracyclic analogs of Brequinar possess a relatively small amount of conformational flexability. A 13–15° angle was obtained using molecular modeling to measure the angle described by the pendant phenyl ring in thiophene **23** (yellow), the

centroid (purple), and the pendant phenyl ring in compound **1** (white). This difference in projection angle of the pendant phenyl ring in thiophene **23** may result in a steric interaction in the enzyme active site which is responsible for the loss in potency.[16] Thus, it appears a fused phenyl ring in this position is preferable to the fused heterocycles examined.

Overlap of compound 1 and compound 23

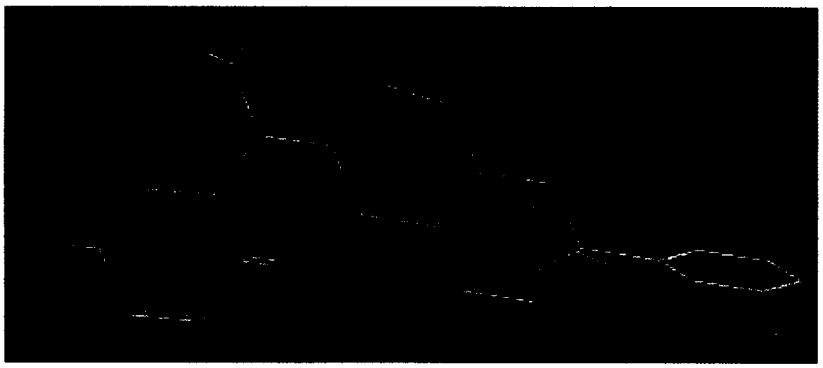
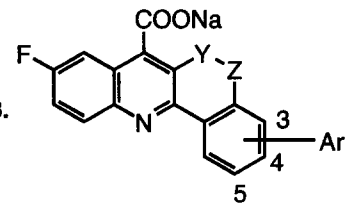


Table 3.



Cpd	Y	Z	Ar	DHOD	MLR
24	S	CH_2	4-Ph	170	310
25	S	CH_2	4-(4-MePh)	218	350
26	S	CH_2	4-(3-MePh)	135	170
27	S	CH_2	4-(2-MePh)	25	65
28	O	CH_2	4-Ph	>204	380
29	CH_2	S	4-Ph	17	16
30	CH_2	S	4-(4-MePh)	13	50
31	CH_2	S	4-(3-MePh)	37	14
32	CH_2	S	4-(2-MePh)	27	0.6
33	CH_2	O	4-Ph	48	220
34	CH_2	SO	4-Ph	206	240
35	CH_2	SO_2	4-Ph	139	8,500

As reported in reference 2, unfused BQR analogs where the 3-methyl group has been substituted with either a -OMe or a -SMe group had been prepared. The DHODase activity of both compounds was 13 nM. It was logical to close the ring and prepare the analogous tetracyclic compounds **24–28** (See Figure 1). It was found that the tetracyclic compounds **24–28** were less active than the ring open analogs. Examination of Table **3** shows that compound **27** which contained a 2-methyl substituent on the pendant phenyl ring was

significantly more potent than the unsubstituted compound **24**. A change in the position of the heteroatom from Y to Z as in compounds **29–33** generally resulted in compounds with greater activity in both DHODase and MLR than their corresponding isomers. It is not clear why compounds **24–28** are less potent than either the ring opened analogs or the isomeric compounds **29–33**. Of particular note was compound **32** which showed a dramatic increase in activity in the MLR assay compared to the simple phenyl ring. Since compound **32** did not show a parallel increase in DHODase inhibition the excellent MLR potency could be due to differences in cell penetration. The sulfur in compound **29** was oxidized to provide potential metabolites. Both sulfoxide **34** and sulfone **35** were significantly less active than the parent compound **29**.

In summary, a variety of tetracyclic analogs of BQR were prepared and evaluated in both the DHODase and MLR assays in an effort to better understand the requirements for activity. Use of the Suzuki coupling methodology allowed wide variation of the pendant aryl group, which has expanded the SAR and allowed the observation of a general trend of improved potency with 2-substitution. In most cases a correlation between DHODase and MLR were observed. Compounds with an ethylene bridge or compounds where Y=CH_2 and Z = S represent the best of the tetracyclic compounds discussed.[17] Molecular modeling showed the topology of all the tetracyclic ring systems to be similar. The differences in activity observed with these topologically similar compounds can in some cases be attributed to changes in lipophilicity or basicity, or projection angle of the pendant aryl ring. Several of the compounds have activity in DHODase and MLR comparable to Brequinar warranting further investigation as potential clinically useful immunosuppressive agents.

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