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URIDINE PHOSPHORYLASE INHIBITORS: CHEMICAL MODIFICATION OF BENZYLOXYBENZYL-BARBITURIC ACID AND ITS EFFECTS ON URDPASE INHIBITION

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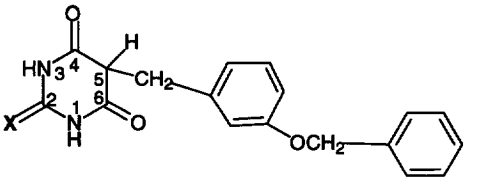
Abstract: 5-(*o*-Benzyloxy)benzylbarbituric acid (**6**) and 5-(*p*-benzyloxy)benzylbarbituric acid (**7**) were prepared and their inhibitory activities compared to 5-(*m*-benzyloxy)-benzylbarbituric acid (BBB) a known, potent inhibitor of uridine phosphorylase (UrdPase). Compounds **6** and **7** were 18-fold and 51-fold less active, respectively, than BBB in inhibiting UrdPase. These data provide solid evidence that the 5-benzylbarbituric acids possessing *meta* substituents are the most active inhibitors. In addition, 2-*thio*BBB (**11**) was synthesized and it was shown to be as active an inhibitor as BBB.

A continuous effort is maintained in our laboratories for the development of cost-efficient, potent inhibitors of human uridine phosphorylase (UrdPase). Recently, we prepared and identified certain barbituric acid analogues, [5-(*m*-benzyloxy)benzylbar-bituric acid (BBB)] as specific inhibitors of this enzyme.[1,2] In an effort to enhance the activity of BBB, we first explored what effect the placement of the benzyloxy group on the 5-benzylbarbituric acid ring (*ortho* and *para* versus *meta*) might have on it and then selected the best candidate of the three for modification of the *C*2 position (replacement of oxygen with sulfur).



X = O, BBB
X = S, 2-*thio* BBB

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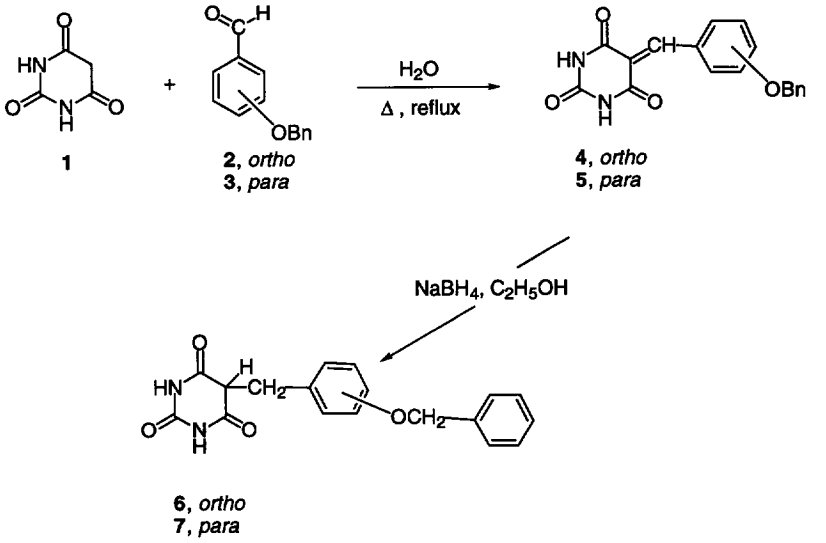
Preparation of 5-(*o*-benzyloxy)benzylbarbituric acid (**6**) and 5-(*p*-benzyloxy)benzyl-barbituric acid (**7**) followed the known[1] procedure depicted in Scheme 1. An aqueous solution of barbituric acid (**1**) was heated at reflux for approximately 1 h with a slight excess of aldehyde **2/3**. The 5-benzylidenebarbituric acids **4** and **5** were obtained in good yield and could be used without further purification. Reduction of the exocyclic double bond at *C*5 was accomplished with sodium borohydride in ethanol to furnish **6** and **7**.

Scheme 1



1

2, *ortho*
3, *para*

4, *ortho*
5, *para*

6, *ortho*
7, *para*

Compared to BBB, barbiturates **6** and **7** were 18-fold and 51-fold less active, respectively, toward inhibiting UrdPase (Table 1). These data now provide concrete evidence that the hydrophobic pocket of the enzyme[2] accommodates *meta* substituted 5-benzylbarbituric acids more easily, thus leading to greater inhibition of UrdPase. The 5-benzylidine derivatives **4** and **5** did not exhibit activity and this expected result is identical to our earlier report.[2] The rigidity caused by double bond at *C*5 restricts the proper orientation of the 5-benzyloxybenzyl moiety to adequately fit and bind in the enzyme's active site.

Table 1. Apparent K_i values for inhibition of human liver UrdPase.[a,b]

Compound	Apparent K_i (μM)
5-(*m*-Benzyloxy)benzylbarbituric acid (**BBB**)	2.3 ± 0.4
5-(*o*-Benzyloxy)benzylbarbituric acid (**6**)[c]	41.8 ± 5.5
5-(*p*-Benzyloxy)benzylbarbituric acid (**7**)[c]	118.2 ± 11.7
5-(*m*-Benzyloxy)benzyl-2-*thio*barbituric acid (**11**)[c]	3.7 ± 0.6

[a]Concentration range 0.0–0.09 mM. Substrate concentration (Uridine) is 0.125 mM. [b]Assay conditions and procedure as reported in reference 2. [c]All new compounds had satisfactory elemental analyses (±0.4%) and ^1H and ^{13}C NMR spectra.

Next, we turned our attention to the modification of the *C*2 position. It has been suggested that ionization, enolization, or both of barbituric acid analogues may enhancing binding of such inhibitors to UrdPase.[2] Niedzwicki[3] have demonstrated that the addition of an electron-withdrawing group at *C*5 of uracil enhances binding of these derivatives to UrdPase. They reported that such groups increase the acidity of these analogues, thus promoting ionization during the enzymatic assay. They surmised that the anionic forms of these inhibitors bind more strongly to UrdPase than their neutral counterparts. Under the assay conditions (pH 8),[2] BBB, **6**, and **7** are most likely present as monoanions.[4] We felt that it would be of interest to see what influence a 2-*thio* substituent would have on enolization/ionization[5] and ultimately binding to and inhibiting of UrdPase.

Since BBB remained the best candidate of the three benzyloxy derivatives, 2-*thio*BBB (**11**) was synthesized and evaluated as an inhibitor of UrdPase. The synthesis followed the same route as described for **6** and **7**. 2-*Thio*barbituric acid (**8**) was reacted with 3-benzyloxybenzaldehyde (**9**) to furnish the 2-*thio*benzylidene **10** which on reduction provided 5-(*m*-benzyloxy)benzyl-2-*thio*-barbituric acid (**11**, 2-*thio*BBB). 2-*Thio*BBB (**11**) is nearly as potent as BBB in inhibiting UrdPase (Table 1). Earlier, we suggested[2] that binding of barbiturates to UrdPase could be influenced by an enolic form of barbituric acid or ionization at either *C*4 or *C*6. Biehl and coworkers,[6] demonstrtated that the enolic form of 5-phenylbarbituric acid could be observed in its

^{13}C NMR spectrum (93 ppm) under basic conditions. On the other hand, 2-*thio*barbituric acid existed in DMSO-d_6 as the keto (40.0 ppm, 35%) and enol (82.2 ppm, 65%)[5] tautomers. The ^{13}C NMR spectra of our target barbiturates **6** and **7** in DMSO-d_6 both exhibited a *C*5 signal at 50 ppm indicating the keto form whereas 2-*thio*BBB (**11**) only displayed a resonance at 94 ppm (enol form). As mentioned above, under assay conditions, **11** most likely exists as the *C*4/*C*6 anion. The testing data suggests that regardless of the function group on *C*2 (oxo versus thione) as long as it can promote enolization/ionization at *C*4/*C*6, binding and inhibition of UrdPase should fall into the same range.

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