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A Selective Inhibitor of *Escherichia coli* Prephenate Dehydratase

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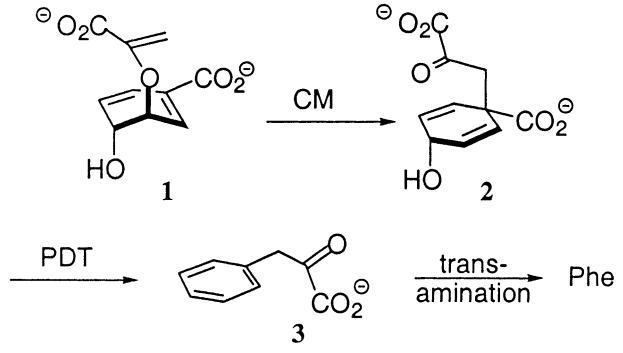
Abstract—To identify selective prephenate dehydratase (PDT) inhibitors, a series of substituted biphenic acid derivatives was synthesized using the Ullmann reaction. Screening experiments identified **18** as a promising new PDT inhibitor.

Most aromatic compounds in bacteria, fungi, and higher plants arise via the shikimic acid metabolic pathway.[1] In *Escherichia coli*, the bifunctional P-protein plays a central role in *E. coli* phenylalanine (Phe) biosynthesis by transforming chorismate **1** via prephenate **2** to phenylpyruvate **3** (Scheme 1). The 386 residue P-protein (MW 43 kDa) contains two catalytic domains, chorismate mutase (CM) and prephenate dehydratase (PDT), as well as a regulatory (R) domain that is responsible for feedback inhibition by Phe.[2] Subdomain cloning and expression studies have identified discrete domains for CM, PDT, and regulatory functions.[3]

Initial attempts to crystallize PDT for X-ray structural analysis utilized the 22 kDa fragment of the P-protein corresponding to residues 101–300, hereafter designated PDT22.[2] However, that effort was hampered by the absence of a suitable, active-site directed PDT inhibitor. Using a library screening approach, we identified the *S*-enantiomer of 6,6′-dinitrobiphenic acid (*S*-DNBA, Fig. 1) as an active-site directed, competitive inhibitor of both PDT22 and CM.[4] It was therefore of interest to explore further other members of this compound class in search of inhibitors with a higher affinity for the PDT active site. Here, we report the synthesis of a family of DNBA analogues and derivatives, and their evaluation as PDT22 inhibitors. Parallel screening for inhibition of CM activity led to the identification of a new, achiral isomer of DNBA that displays significant selectivity as a PDT inhibitor.



Scheme 1.

Prior to our work, the best known PDT inhibitors were sulfoxides **4** and **5** (Fig. 1).[5] Unfortunately, these compounds displayed only weak to moderate levels of inhibition. DNBA, previously synthesized by the known Ullmann coupling of methyl 2-iodo-3-nitrobenzoate,[6] exists as separable enantiomeric atropisomers, and was resolved following a published method.[6] Both *S*-(−)-DNBA and *R*-(+)-DNBA inhibited PDT22, displaying IC_{50} values of 600 and 900 μM, respectively. Under

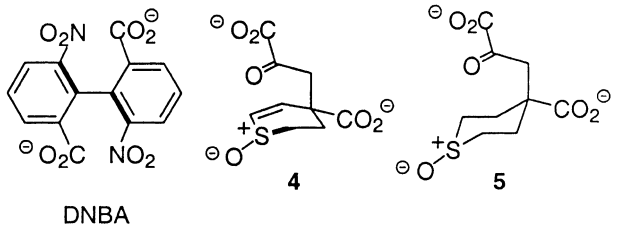


Figure 1.

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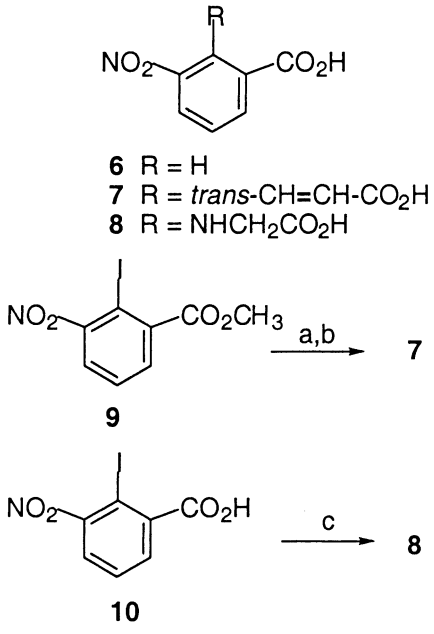
steady-state conditions, inhibition of PDT22 by *S*-DNBA was competitive, with $K_i = 300\,\mu M$ (**2** as substrate, $K_m = 710\,\mu M$, pH 7.8, 37 °C). Thus, *S*-DNBA was more potent than **4**, which was the better of the two sulfoxide-based PDT inhibitors (Fig. 1). Compared to **4** ($IC_{50}/K_m = 16$), *S*-DNBA **6** displayed $IC_{50}/K_m = 0.4$.

S-(−)-DNBA also competitively inhibited EcCM, with an $IC_{50} = 50\,\mu M$ and K_i of $13\,\mu M$ (**2** as substrate, $K_m = 290\,\mu M$, pH 7.8, 37 °C). A comparative kinetic analysis with prephenate suggested that the inhibitor acted as a prephenate mimic.[4] Because the molecular volume of DNBA (368 Å^3) was much larger than that of chorismate or prephenate (260–280 Å^3), it was of interest to determine whether smaller structures might be designed based on the biphenyl scaffold that not only preserved the important PDT22 binding determinants, but also enhanced the activity, thus leading to a more selective PDT inhibitor. Initially, the series of substituted benzoic acids **6**–**8** (Scheme 2) was assayed against PDT22. Compound **6** was commercially available. Diacid **7** was synthesized by the Heck reaction of iodobenzoate **9** with methyl acrylate, followed by saponification of the product diester. Diacid **8** was prepared by the reaction of glycine with 2-iodo-3-nitrobenzoic acid **10**. Unfortunately, no inhibition of PDT22 was observed when the enzyme was assayed in the presence of **6**–**8** at concentrations up to 500 μM. These findings, while disappointing, were consistent with previous studies in which benzoic and phenylacetic acids were found to inhibit prephenate dehydrogenase only at millimolar concentrations.[7]

As another approach to optimizing the activity of *S*-DNBA, a focused subset of biphenic acids was synthesized, which included a family of 2-aryl-3-nitrobenzoic acids **11** (Fig. 2) that preserved on one aromatic ring the substitution pattern found in DNBA while systematically varying the 2-aryl substituent with various electron-donating and electron-withdrawing groups at the *ortho, meta*, and *para* positions. For that objective, compounds **12**–**20** were synthesized as prospective PDT22 inhibitors. A second subset included more highly substituted biphenic acids not restricted to the nitrobenzoate template **11**. For this purpose, compounds **21**–**25** were prepared as candidate inhibitors. Also included for testing was 2,2′-biphenic acid (not shown), which is commercially available.

Most of the compounds shown in Figure 2 were synthesized by the standard, copper-promoted Ullmann coupling reaction using readily available *ortho*-iodobenzoic acids. Triacid **24** was prepared by permanganate oxidation of the known (*o*-nitrophenyl)mesitylene.[8] Biphenic acids **12**,[9] **16**, **19**, and **22** were prepared by the template-directed, intramolecular Ullmann coupling of salicyl diesters developed by Takahashi et al.[10–12] That protocol involves sequential acylation of salicyl alcohol with two different iodobenzoyl halides to afford a bis-iodoester that undergoes intramolecular Ullmann coupling. Hydrolysis from the template affords the desired biaryl. Because of its intramolecularity, the method is particularly effective in promoting sterically crowded couplings. Subsequent chemical modification of the initially-prepared Ullman coupling products led to additional members of the library shown in Figure 2.



Scheme 2. (a) $Pd(OAc)_2$, methyl acrylate (3 equiv), DMF, $NaHCO_3$, Bu_4NBr, 75%; (b) KOH, THF–H_2O, 85%; (c) H_2O, glycine (5 equiv), reflux, 2 days, 30%.

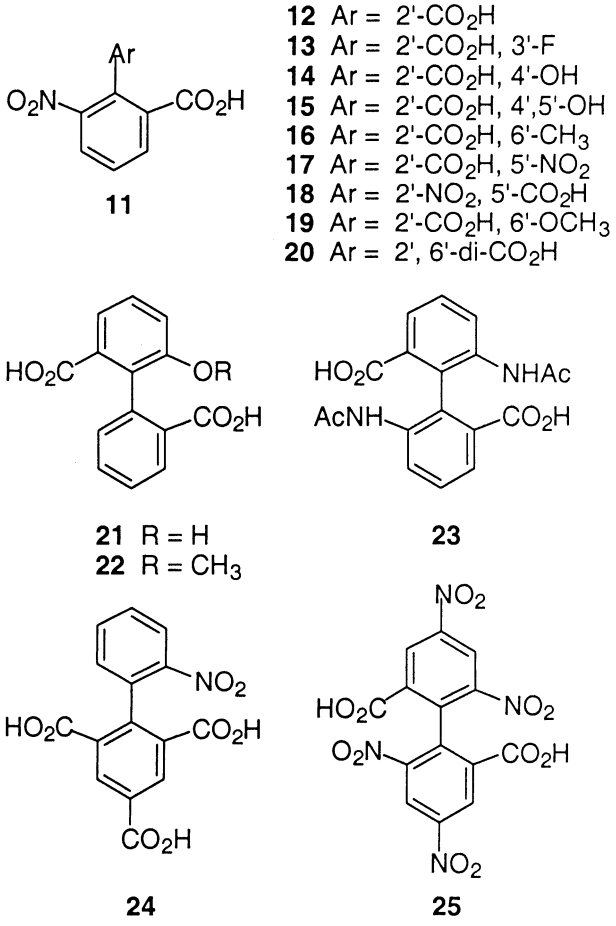
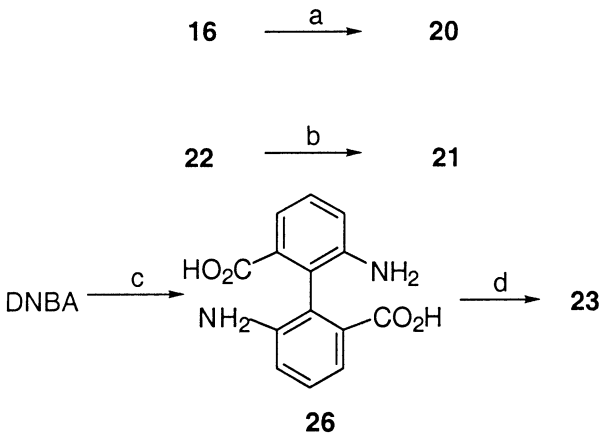


Figure 2.



Scheme 3. (a) $KMnO_4$–H_2O; (b) BBr_3, CH_2Cl_2; (c) Cu bronze, DMF, reflux; (d) AcCl, pyridine.

Table 1. Screening of biphenic acid derivatives as inhibitors of PDT22 and CM

Compound	IC_{50}-PDT (μM)	IC_{50}-CM (μM)
S-DNBA	600	50
12	1900	280
13	2250	600
14	950	350
15	—[a]	1000
16	1300	730
17	750	1150
18	550	1350
19	3000	800
20	1700	450
21	2900	1000
22	4000	—[b]
23	—[c]	—[c]
24	1850	3000
25	—[d]	—[d]
2,2′-Biphenic acid	—[c]	—[c]

[a] < 25% inhibition at [I] = 500 μM.
[b] Not determined.
[c] No inhibition at [I] = 500 μM.
[d] Strong UV absorbance interfered with assay.

Triacid **20** was prepared by permanganate oxidation of **16**. Diacid **21** was made from the known[13] methyl ether **22** by BBr_3 treatment. Compound **23** was prepared by reduction of the two nitro groups in DNBA and acetylation of the resulting diamine **26** (Scheme 3). The known tetranitrobiphenic acid **25** was prepared by bisnitration of DNBA.[6]

As an initial screen of biological activity, compounds **12**–**25** (Fig. 2) were assayed to determine IC_{50} values against both PDT22 and CM, following published procedures.[5,14] Data summarized in Table 1 indicate the importance of the nitro substituents in both PDT22 and CM inhibition. Removing one nitro group in *S*-DNBA, as in **12**, or replacing a nitro group with different substituents, as in compounds **13**–**16**, **19**, and **20**, resulted in weaker levels of both PDT22 and CM inhibition. Replacing both nitro groups with hydrogens (as in 2,2′-biphenic acid) or with other electron donating groups (as in **21**–**23**) further diminished CM and PDT22 inhibition.

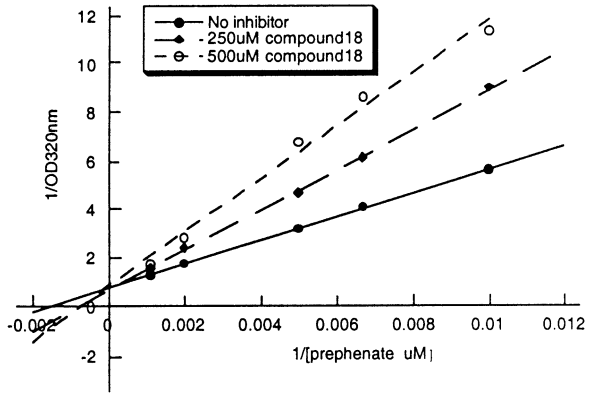


Figure 3. Reciprocal plot of the velocity of the PDT22 reaction versus the concentration of prephenate **2** in the presence and absence of diacid **18** (conditions: 50 mM Tris buffer, 0.5 mM EDTA, 10 mM mercaptoethanol, pH 7.5, 37 °C).

Several of the substances tested displayed little or no activity against either CM or PDT22 (e.g., **15**, **21**, **22**, and **24**). Moreover, none of the compounds proved to be more potent than DNBA as a CM inhibitor. However, two compounds, **17** and **18**, displayed better inhibitory activity against PDT22 than against CM. The selective inhibition of PDT22 was more pronounced with 2-(2′-nitro-5′-carboxyphenyl)-3-nitrobenzoic acid **18**, a previously unknown, achiral regioisomer of DNBA.[15] The activity of **18** was comparable to *S*-DNBA against PDT22 ($IC_{50} = 550$ μM), but markedly weaker against CM. Under steady-state conditions, **18** exhibited competitive inhibition of PDT22 with a K_i of 449 ± 35 μM (Fig. 3).

Diacid **18** represents a promising new lead in the search for selective, active site-directed PDT inhibitors. It belongs to a family of little-known substituted 2,3′-biphenyldicarboxylic acids. The parent diacid has been described,[16] and several related tetracarboxybiphenyls have been isolated from coal.[17] Syntheses of additional representatives of this family are planned.

Acknowledgements

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