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X-ray Structures of Two Xanthine Inhibitors Bound to PEPCK and *N*-3 Modifications of Substituted 1,8-Dibenzylxanthines

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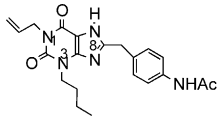
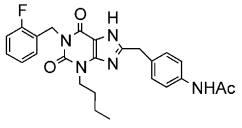
Abstract—The analysis of the X-ray structures of two xanthine inhibitors bound to PEPCK and a comparison to the X-ray structure of GTP bound to PEPCK are reported. The SAR at *N*-1, *N*-7 and developing SAR at *C*-8 are consistent with information gained from the X-ray structures of compounds **1** and **2** bound to PEPCK. Representative *N*-3 modifications of compound **2** that led to the discovery of 3-cyclopropylmethyl and its carboxy analogue as optimal *N*-3 groups are presented.

Normally gluconeogenesis maintains blood glucose levels during periods of fasting, however, in the diabetic patient it contributes to hyperglycemia. Since the cytosolic phosphoenolpyruvate carboxykinase (PEPCK) enzyme is known to catalyze the rate-limiting step in gluconeogenesis[1] its control could provide a new therapy for type 2 diabetes.

We previously reported[2] the first competitive inhibitors of PEPCK with respect to GTP. Compounds **1** and **2**, with IC_{50}s from an enzyme assay, were the most active compounds prepared in each *N*-1 class. The protocol for the enzyme assay is given in the preceding paper.[2]



1 (IC_{50} 18.8±6.2μM)



2 (IC_{50} 2.11±0.60μM)

We now present a comparison of the structure of compound **1** bound to human cytosolic PEPCK[3] (compound **1**-PEPCK) with the reported structure of a GTP analogue bound to PEPCK.[4] We also report *N*-3 modifications aimed at picking up H-bonds found in the GTP structure. This work led to the discovery of a hydrophobic *N*-3 modification of xanthine **2** that improved the in vitro activity by ~3-fold, providing a >250-fold improvement over the initial HTS hit and a sub-micromolar inhibitor of PEPCK.

The X-ray structure of compound **1**-PEPCK is shown in Figure 1.[3] Figure 2a and b provide a comparison of the guanosine portion of the previously determined X-ray structure of a non-hydrolyzable GTP analogue bound to PEPCK[4] with the structure of compound **1**-PEPCK. The X-ray structure of compound **1**-PEPCK (Fig. 1 and depicted in Fig. 2b) shows that GTP's guanosine unit and the xanthine inhibitor bind in the same cleft on the surface of PEPCK. The X-ray of compound **1**-PEPCK is in agreement with the SAR for positions *N*-1, *C*-8 and *N*-7[2] and supports a hypothesis that the xanthine inhibitors are binding in a different orientation from GTP's guanine ring. The X-ray shows the xanthine ring of **1** to be flipped and rotated relative to the guanine moiety of GTP. Rotations of other purine antagonists relative to the natural ATP ligand in adenosine receptors[5] and CDK2 have been reported.[6]

Figure 2a and b show the various interactions of GTP and compound **1** with the purine-binding site of PEPCK. Comparison of Figure 2a and b shows that the xanthine nitrogens at *N*-1 and *N*-3 overlap with GTP's *N*-7 and *N*-9, respectively. Interestingly, the *C*-6 carbonyl interaction with PEPCK is identical in both GTP

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and compound **1**. A water molecule is found to bridge xanthine's *N*-7 hydrogen and PEPCK's Trp516 and Trp527, in agreement with the decreased activity of all compounds with a substituent at *N*-7 (ref 2 and data not shown). Xanthine's *C*-2 carbonyl picks up a H-bond to PEPCK's Asn292 side chain, an interaction that has no counterpart in the binding of the GTP ligand.

The novel π interaction of PEPCK's Phe525 with the *C*-2 amino group of GTP[4] is replaced in compound **1** by an offset π–π interaction with the phenyl ring of the *C*-8 benzyl unit. This phenyl ring is also in a favorable edge-to-face interaction with Phe530. PEPCK's Phe530 and Phe517 serve the same function with GTP and the xanthine ligand, in effect sandwiching the aromatic purine cores between the two side-chain phenyl rings.

The allyl group proved to be the most potent of the early *N*-1 groups.[2] The X-ray suggests the π-system of the allyl group in **1** may act as a H-bond acceptor, with the Asn533 side-chain amide as donor, although at 3.5 Å the distance is long.

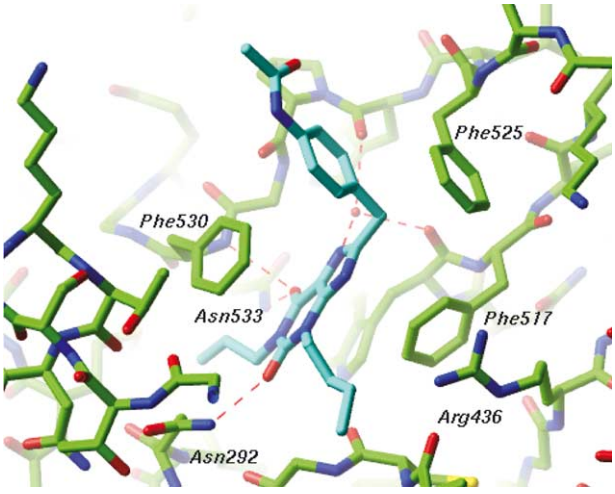


Figure 1. Xanthine **1** binding site in PEPCK. Compound **1** in light blue and the amino acids in the purine-binding site of PEPCK are shown in green. In both the xanthine and PEPCK, the atoms of oxygen and nitrogen are shown in red and dark blue, respectively. H-bonds are shown with dashed lines in orange and a single water molecule is shown in pink.

The X-ray structure of compound **2**[7] is important in that it shows that compound **2** binds to the protein in the same orientation as compound **1** with the xanthine core of each making the same interactions with the protein (a stereoview is shown in Fig. 3).

As reported,[2] replacement of the *N*-1 allyl group of compound **1** with benzyl and 2-fluorobenzyl groups improved activity by 2- and >8-fold, respectively. The X-ray of **2** suggests that part of the improvement was due to phenyl ring stacking on top of the planar peptide bond between Asn292 and Leu293. In addition to this favorable π–π stacking, the fluorine in compound **2** is optimally placed to accept a H-bond from the Asn533 side-chain amide (distance 3.1 Å).

While the X-ray structures of compounds **1** and **2** bound to PEPCK explained the reason for the improved activity of compounds with a *C*-8 benzyl group, they failed to provide an explanation for the improved activity of xanthines containing a substituent on the phenyl ring of the *C*-8 benzyl unit. Future papers will cover modifications at *C*-8 that explored this issue.

The observation that groups at *N*-3 extended into the area of the protein that bound the ribose ring of GTP (see Fig. 2a and b) resulted in many unsuccessful excursions aimed at mimicking these interactions via xanthine modifications at *N*-3.

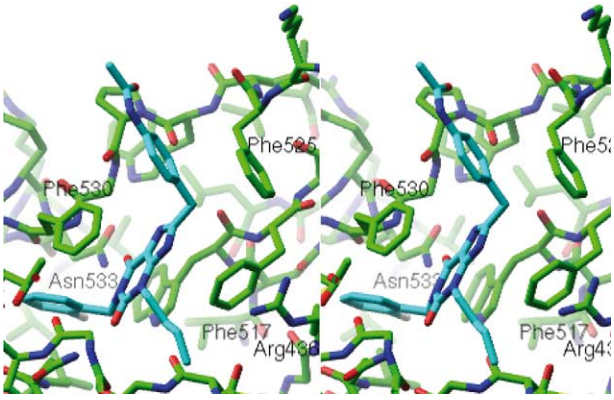


Figure 3. Stereoview of xanthine **2** bound to PEPCK. Legend same as for Figure 1.

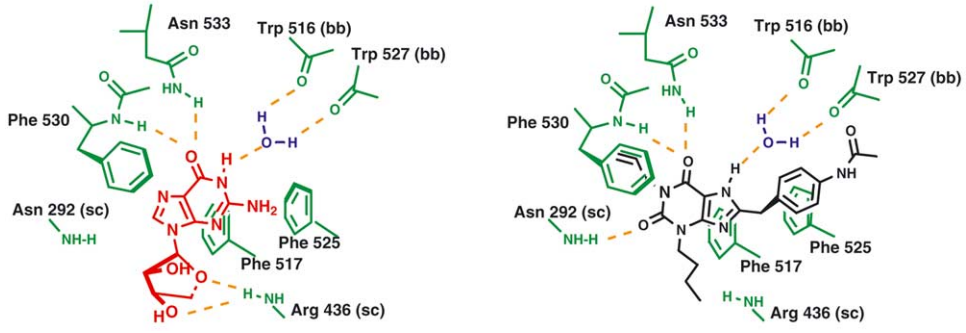
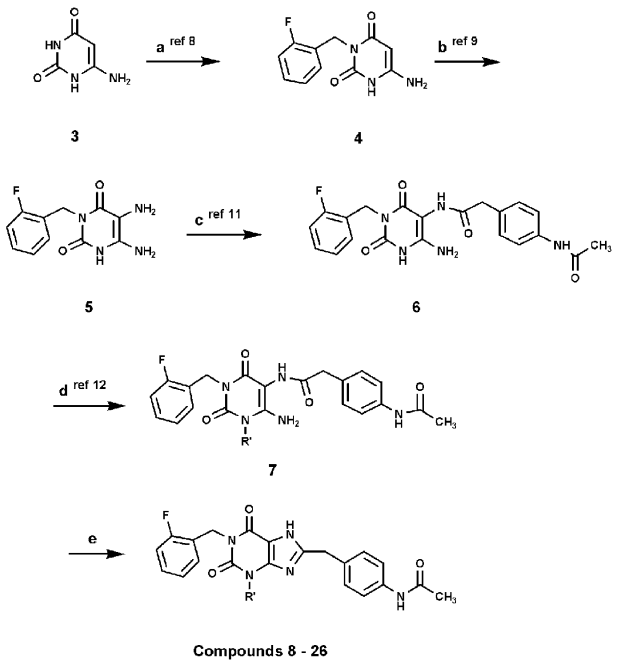


Figure 2. (a) Depiction of GTP's guanosine unit bound to PEPCK. (b) Depiction of xanthine **1** bound to PEPCK. Guanosine portion of GTP in red and PEPCK light green, H-bonds indicated in orange, water in blue, xanthine **1** in black. Notations (sc) and (bb) indicate PEPCK side chain groups and backbone groups, respectively.

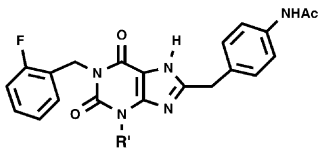


Scheme 1. Route to 3-alkyl-1-(2-fluorobenzyl)-8-(*N*-acetyl-4-aminobenzyl)xanthines. Reagents and conditions: (a) HMDS, $(NH_4)_2SO_4$ + 2-fluorobenzyl bromide, reflux; (b) (1) $NaNO_2$ in HOAc/H_2O, 85–100 °C; (2) $Na_2S_2O_4$, NH_4OH or 53 psi H_2, PtO_2 EtOH;[14] (c) EDCI, *N*-acetyl-4-amino-phenylacetic acid,[10] imidazole (cat), DMAP (cat), DMF; (d) R′-halide in DMF, K_2CO_3, 40 °C; (e) 10% NaOH in H_2O/MeOH heated in a 50 °C oil bath.

An efficient route for the preparation of *N*-3 modifications of xanthine **2** involved the use of the common intermediate **6,** as shown in Scheme 1. The 3-(2-fluorobenzyl)-6-amino uracil (**4**) was prepared from 6-aminouracil (**3**) and 2-fluorobenzyl bromide using the method of Müller.[8] The alkylated uracil **4** was converted into the diaminouracil **5** as shown in Scheme 1.[9] Acylation of the *C*-5 amino group with *N*-acetyl-4-aminophenylacetic acid[10] used the conditions of Jacobson et al.[11] to afford the acyl uracil **6**. This acylation of the *C*-5 amino group of uracil **6** allowed selective *N*-1 alkylation (uracil numbering), as noted by Müller et al.[12] Reaction of **6** with a variety of alkyl halides provided the dialkylated uracil derivative **7**. The cyclization of **7** used aqueous 10% sodium hydroxide solution in methanol with heating in a 50 °C oil bath, conditions allowing minimal loss of the *C*-8 *N*-acetyl group, to afford the xanthines **8**–**26**.

A representative sample of *N*-3 modifications prepared is shown in Table 1. In comparison with the *N*-3 butyl analogue **2**, cyclic ethers **8** and **9**, furan-3-ylmethyl **10** and longer alkyls, for example hexyl **11**, failed to improve activity. The shorter alkyls, the propyl analogue **12** and *iso*-butyl analogue **13** also provided no improvement over the butyl analogue **2**. The *N*-3 methyl derivative is not shown in Table 1 since it was prepared in the *N*-1 benzyl class, where it was 6-fold less potent when compared to the corresponding *N*-3 butyl analogue.[13] Relative to the butyl analogue **2** the *N*-3 cyclopentylmethyl analogue **14** and cyclobutylmethyl **15** provided no advantage. However the *N*-3 cyclopropylmethyl **16** was ~3-fold more active than **2** providing the first sub-micromolar xanthine inhibitor.

Table 1. IC_{50} values from the enzyme assay for representative *N*-3 modifications of xanthine **2**[a]



Compd	R′	IC_{50}, μM[b]
2	Butyl	2.11 ± 0.60 (32)[c]
8	Tetrahydropyran-2-ylmethyl	4.5
9	Tetrahydrofuran-2-ylmethyl	5.7
10	Furan-3-ylmethyl	4.5
11	Hexyl	8.9
12	Propyl	1.49 ± 0.36
13	*iso*-Butyl	2.02 ± 0.30 (6)
14	Cyclopentylmethyl	3.05 ± 0.35
15	Cyclobutylmethyl	1.18 ± 0.24 (6)
16	Cyclopropylmethyl	0.69 ± 0.29 (6)
17	3-Hydroxylpropyl	5.45 ± 0.07
18	4-Hydroxylbutyl	2.85 ± 0.07
19	$-(CH_2)_2CO_2H$	2.0
20	$-(CH_2)_3CO_2H$	1.95 ± 0.07
21	$-(CH_2)_3C(O)NH_2$	6.6

[a]^{1}H NMR and HRMS data for compound **16** is found in ref 15.
[b]IC_{50} inhibitory values from the enzyme assay. Results showing standard deviation (SD) values were assayed more than once. If multiple assays were preformed, the number of repetitions is shown in parenthesis. Assays were conducted with each compound run in duplicate. A single IC_{50}, without SD, indicates a single assay with the average of the duplicate run indicated.
[c]The IC_{50} for compound **2** run as a standard and for a direct comparison in these assays was 2.0–2.4 nM.

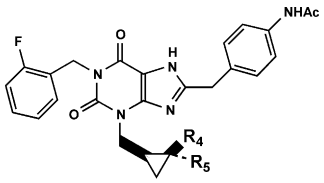
Returning to compounds with H-bonding capabilities at *N*-3, the 3-hydroxylpropyl analogue **17** and 4-hydroxylbutyl analogue **18** and the acids **19** and **20** were not as active as the *N*-3 cyclopropylmethyl analogue **16**. Amides derived from the *N*-3 acids **19** and **20**, for example compound **21**, also provided no improvement in activity.

A K_i of 0.2 μM for the butyric acid analogue **20** (IC_{50} 1.95 ± 0.07 μM) binding to PEPCK was measured via isothermal titration calorimetry (ITC).[4] This, as mentioned in the previous paper,[2] suggested the K_is for the xanthine inhibitors described here could be as much as 9-fold lower than the reported IC_{50}s.

Table 2 shows several attempts at using the *N*-3 cyclopropyl ring of xanthine **16** as a scaffold for groups that might provide H-bond acceptors, however, only the carboxyl analogue **22** retained the activity of the unsubstituted analogue **16**. Increasing the hydrophobicity of the cyclopropylmethyl group by the addition of a methyl group, compound **26**, also provided no advantage.

In summary, the first X-ray structures of two xanthine inhibitors bound to PEPCK and a comparison to GTP-PEPCK are presented. We also present a number of *N*-3 modifications of xanthine **2**. Two of these modifications, the cyclopropylmethyl and its carboxy analogue, provided GTP competitive inhibitors of PEPCK with sub-micromolar IC_{50}s.

Table 2. IC_{50} values from the enzyme assay for modifications of xanthine **16** on the cyclopropyl ring[a]



Compd	R4	R5	IC_{50}, μM[b]
16	H	H	0.69±0.29 (6)
22	Carboxyl	H	0.56±0.19 (8)
23	Methyl carboxylate	H	3.00
24	Hydroxymethyl	H	2.27±0.21 (4)
25	Hydroxymethyl	Hydroxymethyl	2.00±0.42
26	Methyl	H	1.82±0.31 (6)

[a]^{1}H NMR and HRMS for compounds **16** and **22** in ref 15.
[b]Legend same as Table 1. The IC_{50} for compound **2** run as a standard in these assays was 2.2–2.3 μM.

Acknowledgements

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References and Notes

1. Cimbala, A. N.; Lamers, W. H.; Nelson, J. E.; Monahan, J. E.; Yoo-Warren, H.; Hanson, R. W. *J. Biol. Chem.* **1982**, *257*, 7629.
2. Foley, L. H.; Wang, P.; Dunten, P.; Ramsey, G.; Gubler, M.-L.; Wertheimer, S. J. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3607.
3. PDB code: 1m51, Resolution 2.25 Å, R factor 18.1%.
4. Dunten, P.; Belunis, C.; Crowther, R.; Hollfelder, K.; Kammlott, U.; Levin, W.; Michel, H.; Ramsey, G. B.; Swain, A.; Weber, D.; Wertheimer, S. J. *J. Mol. Biol.* **2002**, *316*, 257.
5. (a) van Galen, P. J. M.; Van Vlijmen, H. W.Th.; Ijzerman, A. P.; Soudijn, W. *J. Med. Chem.* **1990**, *33*, 1708. (b) Peet, N. P.; Lentz, N. L.; Meng, E. C.; Dudley, M. W.; Ogden, A. M. L.; Demeter, D. A.; Weintraub, H. J. R.; Bey, P. *J. Med. Chem.* **1990**, *33*, 3127. (c) Grahner, B.; Winiwarter, S.; Lanzner, W.; Müller, C. E. *J. Med. Chem.* **1994**, *37*, 1526.
6. Gray, N. S.; Wodicka, L.; Thunnissen, A.-M. W. H.; Norman, T. C.; Kwon, S.; Espinoza, F. H.; Morgan, D. O.; Barnes, G.; LeClerc, S.; Meijer, L.; Kim, S.-H.; Lockhart, D. J.; Schultz, P. G. *Science* **1998**, *281*, 533.
7. PDB code: 1nhx, Resolution 2.1 Å, R factor 17.5%.
8. Müller, C. E. *Tetrahedron Lett.* **1991**, *32*, 6539.
9. Müller, C. E.; Shi, D.; Manning, M., Jr.; Daly, J. W. *J. Med. Chem.* **1993**, *36*, 3341.
10. Janda, K. D.; Ashley, J. A.; Jones, T. M.; McLoed, D. A.; Schloeder, D. M.; Weinhouse, M. I.; Lerner, R. A.; Gibbs, R. A.; Benkovic, P. A.; Hilhorst, R.; Benkovic, S. J. *J. Am. Chem. Soc.* **1991**, *113*, 291.
11. Jacobson, K. A.; Gallo-Rodriguez, C.; Melman, N.; Fischer, B.; Maillard, M.; van Bergen, A.; van Galen, P. J. M.; Karton, Y. *J. Med. Chem.* **1993**, *36*, 1333.
12. Müller, C. E.; Sandoval-Ramirez, J. *Synthesis* **1995**, 1295.
13. 1-Benzyl-3-methyl-8-(*N*-acetyl-4-aminobenzyl) xanthine (IC_{50} 50 μM) in the same assay 1-benzyl-3-butyl-8-(*N*-acetyl-4-aminobenzyl)xanthine (IC_{50} 8.2 μM).
14. Wells, J. N.; Garst, J. E.; Kramer, G. L. *J. Med. Chem.* **1981**, *24*, 954.
15. **Spectra.** General information is given in ref 18 of the previous paper.[2] All compounds described here had ^{1}H NMR and HRMS in agreement with the structure. ^{1}H NMR and HRMS are provided below for three of the most active compounds.
N-{4-[3-Cyclopropylmethyl-1-(2-fluorobenzyl)-2,6 dioxo-2,3,6,7-tetrahydro-1H-purin-8-ylmethyl]-phenyl} acetamide (**16**): ^{1}H NMR (DMSO-d_6) δ 0.42 (m, 4H), 1.24 (m, 1H), 2.02 (s, 3H), 3.85 (d, 2H), 4.00 (s, 2H), 5.12 (s, 2H), 7.04–7.27 (m, 6H), 7.50 (d, 2H), 9.89 (s, 1H ex), 13.47 (s, 1H ex); HRMS calcd for $C_{25}H_{24}N_5O_3F$ M+ 461.1863; obsd 461.1863.
2-[8-(4-Acetylamino-benzyl)-1-(2-fluoro-benzyl)-2,6-dioxo-1,2,6,7-tetrahydropurin-3-ylmethyl]-cyclopropanecarboxylic acid (**22**): ^{1}H NMR (DMSO-d_6) δ 0.99 (m, 1H), 1.08 (m, 1H), 1.58 (m, 1H), 1.69 (m, 1H), 2.02 (s, 3H), 4.01 (s, 2H), 4.11 (m, 1H), 4.26 (m, 1H), 5.12 (s, 2H), 7.05–7.40 (m, 4H), 7.24 (d, 2H), 7.50 (d, 2H), 9.90 (s, 1H ex), 12.18 (br s, 1H ex), 13.48 (s, 1H ex); HRMS calcd for $C_{26}H_{24}N_5O_5F$ M+ 506.1840; obsd 506.1826.