Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 3835–3839
Bioorganic & Medicinal Chemistry Letters

C-8 Modifications of 3-alkyl-1,8-dibenzylxanthines as inhibitors of human cytosolic phosphoenolpyruvate carboxykinase

Sherrie L. Pietranico,[a] Louise H. Foley,[a,*] Nicholas Huby,[a] Weiya Yun,[a] Pete Dunten,[a,†] John Vermeulen,[a] Ping Wang,[a] Katherine Toth,[a] Gwendolyn Ramsey,[b] Mary-Lou Gubler[b] and Stanley J. Wertheimer[b]

[a]*Department of Discovery Chemistry, Roche Research Center, Hoffmann-La Roche Inc., Nutley, NJ 07110, USA*
[b]*Department of Metabolic Diseases, Roche Research Center, Hoffmann-La Roche Inc., Nutley, NJ 07110, USA*

Received 9 March 2007; revised 4 May 2007; accepted 7 May 2007
Available online 22 May 2007

Abstract—New modifications on the *C*-8 4-aminobenzyl unit of the previously reported 3-alkyl-1,8-dibenzylxanthine inhibitors of cPEPCK are presented. The most active compound reported here is the 5-chloro-1,3-dimethyl-1*H*-pyrazole-4-sulfonic acid amide derivative **2** with an IC_{50} of 0.29 ± 0.08 μM. An X-ray analysis of a heteroaromatic sulfonamide is presented showing a new π–π interaction.

Previously we reported[1,2] the first GTP-competitive inhibitors of cytosolic phosphoenolpyruvate carboxykinase (cPEPCK). The PEPCK enzyme catalyzes the rate-limiting step in gluconeogenesis[3] and a number of papers show that its overexpression in transgenic animals leads to fasting hyperglycemia and impaired glucose tolerance.[4] Reduction of cPEPCK protein levels using a RNAi has recently been shown to lower blood glucose levels in diabetic mice[5] providing further support for cPEPCK as a therapeutic target in diabetes.

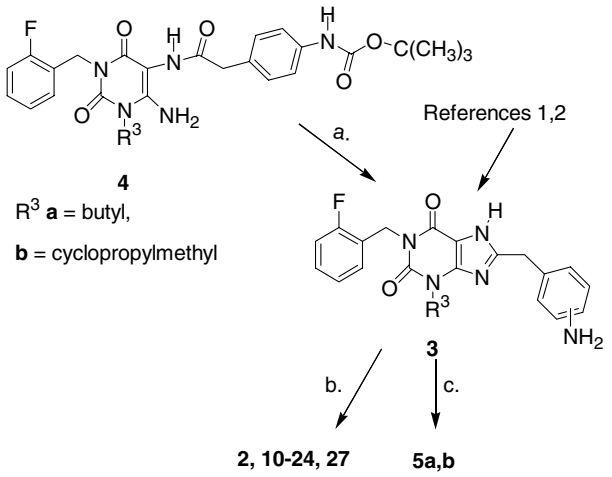
Our earlier reports[1,2] covered the discovery, X-ray studies, and modifications at *N*-1, *N*-3 as well as limited modifications at *C*-8 of a class of xanthines that led to xanthine **1** as the first submicromolar inhibitor of the cPEPCK enzyme. We now describe use of the previously described enzyme assay[1] for the study of new modifications at *C*-8 of 3-alkyl-1,8-dibenzylxanthines. Results of a cellular assay,[6] evaluating a compound's ability to inhibit PEPCK-dependent gluconeogenesis in rat hepatoma cells, are reported for some of the more potent compounds. The new modifications at *C*-8 improved the in vitro activity by >2-fold over that of xanthine **1** and provided the most active inhibitor reported here, 5-chloro-1,3-dimethyl-1*H*-pyrazole-4-sulfonic acid {4-[3-cyclopropylmethyl-1-(2-fluorobenzyl)-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-purin-8-ylmethyl]phenyl}amide (**2**), with an IC_{50} in the enzyme assay of 0.29 ± 0.08 μM[7] and EC_{50} in the cellular assay of 2.17 ± 0.57 μM.

The required *C*-8 3- or 4-aminobenzyl xanthines **3** (R^3 = butyl or cyclopropylmethyl) were prepared from *N*-trifluoro-3- or 4-aminophenyl acetic acid using the methods described in earlier papers.[1,2] A second route to **3** employed the *N*-Boc protected uracil **4** as shown in Scheme 1. The *N*-Boc protected uracils **4** were prepared from *N*-Boc-4-amino phenyl acetic acid[8] using the methods described in Refs. 1 and 2. The 3- or 4-aminobenzyl xanthines **3** were converted into the sulfonamides **2**, **10**–**24**, **27** or the trifluoroacetamides **5a**,**b** as shown in Scheme 1.

Initial SAR development at xanthine's *C*-8 position had suggested that the benzyl unit was critical and that an acetamido substituent on the 4-position of the phenyl group improved activity.[1] However the X-ray studies of early xanthines[2] failed to provide an explanation for the improved activity when the *C*-8 benzyl group contained the 4-acetamido unit. The lack of improved

Keywords: cPEPCK inhibitors; Xanthine class; X-ray; Enzyme and cellular assays.
* Corresponding author at present address: 1757 Augusta Drive, Fort Myers, FL 33907, USA. Tel.: +1 315 369 3769; e-mail address: louf1203@aol.com
† Corresponding author concerning the X-ray data: Stanford Linear Accelerator Center, 2575 Sand Hill Rd., Mailstop 99, Menlo Park, CA 94025, USA; e-mail address: pete@slac.standford.edu

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.05.013



Scheme 1. Routes to 3-alkyl-1,8-dibenzylxanthine sulfonamides and *N*-trifluoroacetamides. Reagents and conditions: (a) (1) 4 M HCl, dioxane, rt; (2) 10% NaOH, MeOH/H_2O, 50 °C oil bath; (b) $R^{8''}$–$S(O_2)$–Cl in pyridine, rt; (c) *N*-(trifluoroacetoxy)succinimide.[9]

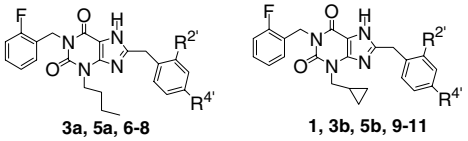
activity of the trifluoroacetyl analogs **5a** and **b** (Table 1), with their highly electronegative trifluoromethyl group, over the corresponding acetamide analogs (compounds **6** and **1**) made a H-bonding explanation unlikely.

A sampling of other *C*-8 modifications of compounds in the *N*-3 butyl and *N*-3 cyclopropylmethyl classes is given in Table 1. The decreased activity shown by the 2,4-disubstitution on the *C*-8 benzyl group, compounds **7** and **8**, when compared to the 4-substituted analogs **6** and **3a** suggested that the presence of an additional 2-substituent decreased activity relative to compounds containing only a 4-substituent. Extending the acetyl unit on *C*-8 by attaching a dimethylamino group, compound **9**, increased solubility but lowered the activity relative to the corresponding amide analog **1**. The activities observed with the sulfonamides **10** and **11** suggested further investigation of this compound class might be warranted.

The benzyl sulfonamide **12** and phenyl sulfonamides whether unsubstituted **13** (Table 2) or substituted with electron rich or electron poor groups (data not shown), however, provided no improvement over the sulfonamides **10** and **11**. However, changing the aromatic group on the sulfonamide to a 5-membered nitrogen containing aromatic heterocycle led to improved activity in the enzyme assay; compare the thiophenes **14**, **15** with imidazole **16** and the pyrazole **17** and also note the activities of the thiazole **18** and oxazole **19** in the *N*-3 cyclopropylmethyl class. Although the imidazoles **16** and **20** showed good activity against the enzyme (relative to **6** and **1**, respectively) they were inactive in cells. The pyrazoles **17**, **2**, **21**, **22** all showed improved activity in the enzyme assay and compound **2** showed improved activity in the cellular assay when compared to the corresponding *N*-acetyl derivative **1**. Changing the *C*-8 aminobenzyl unit in **2** to a 5-amino-2-pyridyl unit resulted in a compound with slightly decreased activity against the enzyme and a 2-fold decrease in activity in cells, data not shown. Interestingly, replacing the pyrazole unit of **2** with a pyridine, compound **23**, gave an analog less active in the enzyme assay than even the corresponding *N*-acetyl derivative **1**. The 8-quinolyl **24** showed improved activity against the enzyme relative to the pyridyl analog **23** but showed no activity in the cellular assay.

Explanation of some of the increase in activity due to an aromatic substituent's attachment to the sulfur of the sulfonamide was found in the X-ray study of sulfonamide **16**,[10] (Fig. 1). This X-ray showed that the sulfonamide linkage allowed a turn that placed the imidazole ring on top of Phe530; thus Phe530 was now sandwiched

Table 1. IC_{50} values from the enzyme assay for representative *C*-8 benzyl modifications of xanthines **6** and **1**

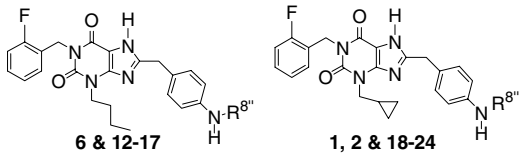


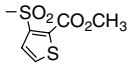
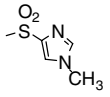
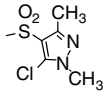
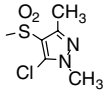
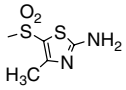
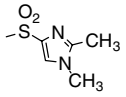
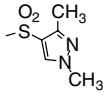
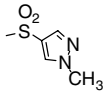
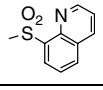
Compound	$R^{4'}$	$R^{2'}$	IC_{50}[a,b] (μM)
3a	Amino	H	2.56 ± 0.05 (5)
5a	*N*-Trifluoroacetylamino	H	2.30
6	*N*-Acetylamino	H	2.11 ± 0.60(32)
7	*N*-Acetylamino	*N*-Acetylamino	8.70 ± 0.85 (2)
8	Amino	Amino	5.65 ± 0.07 (2)
1	*N*-Acetylamino	H	0.69 ± 0.29 (6)
3b	Amino	H	1.56 ± 0.53 (6)
5b	*N*-Trifluoroacetylamino	H	1.80
9	NH–CO–CH_2–$N(CH_3)_2$	H	2.07 ± 0.31 (6)
10	NH–SO_2–CH_3	H	1.25 ± 0.30 (6)
11	NH–SO_2–$N(CH_3)_2$	H	0.96 ± 0.12 (3)

[a] The assay protocol is given in Ref. 1.

[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 parentheses. 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.

Table 2. IC_{50} and EC_{50} values from the enzyme and cellular assays for representative sulfonamide modifications of the *C*-8 benzylamino unit of xanthines **6** and **1**



Compound	$R^{8''}$	IC_{50} (μM)[a,b] [ ] [c]	EC_{50} (μM) [e,f,g] [ ][c]
6	–C(O)CH_3	2.11 ± 0.60 (32)	8.6
12	–S(O)$_2$–Bn	31% at 1[d]	ND
13	–S(O)$_2$–Ph	22% at 1[d]	ND
14		19% at 1[d]	ND
15		30% at 1[d]	ND
16		0.63 ± 0.14 (16)	IA
17		0.54 ± 0.07 (9)	3.97 ± 1.5 (5)
1	–C(O)CH_3	0.69 ± 0.29 (6)	4.70 ± 1.6 (7)
2		0.29 ± 0.08 (38)	2.17 ± 0.57 (9)
18		0.23 [0.23, **2** = 0.25]	8.20 [8.20, **2** = 1.90, **21** = 4.70]
19		0.79 ± 0.26 (6)	3.40 [3.40, **21** = 4.00]
20		0.34 ± 0.04 (3) [0.30, **2** = 0.25]	IA
21		0.24 ± 0.05 (44)	5.57 ± 0.97 (8)
22		0.39 ± 0.05 (4) [0.43, **2** = 0.32, **21** = 0.23]	8.75 ± 0.25 (2) [9.00, **2** = 2.40, **21** = 6.40]
23		1.95 ± 0.07 (2) [1.90, **2** = 0.35]	5.50 [5.50, **21** = 6.50]
24		0.45 ± 0.02 (2)	IA

[a] Legends same as Table 1.
[b] Legends same as Table 1.
[c] The numbers in brackets are the average of a duplicate assay on the compound indicated and, for comparison, compounds **2** and/or **21** assayed at the same time.
[d] Percent inhibition at the μM concentration indicated.
[e] EC_{50} values from the cellular assay[6] represent the results of duplicate assays.
[f] ND, not determined.
[g] IA, inactive up to 10 μM.

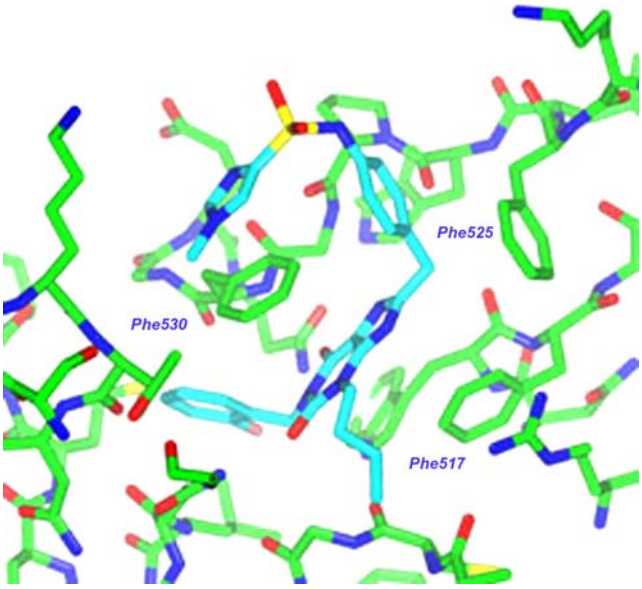
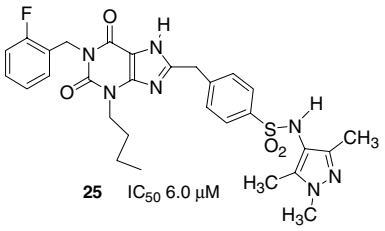


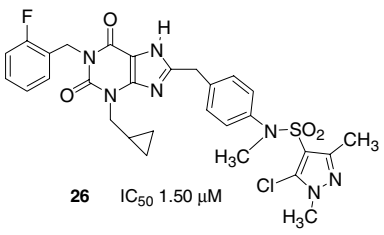
Figure 1. Xanthine **16** bound in the purine-binding site of cPEPCK. Compound **16** is in light blue and the amino acids in the purine-binding site of cPEPCK are shown in green. In both the xanthine and cPEPCK the atoms of oxygen, nitrogen, and sulfur are shown in red, dark blue, and yellow, respectively.

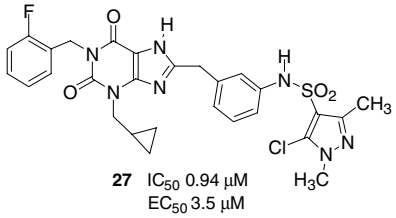
between the imidazole ring and the xanthine core of the ligand. The lower activities observed for the methylsulfonamide **10** and its dimethylamino derivative **11** are in agreement with the X-ray results. The fact that the upper face of the xanthine-binding cleft, the area above the imidazole and sulfonamide moieties, is exposed to solvent may explain the need for more hydrophilic aromatic groups. The other interactions of the protein with the xanthine inhibitor are identical to those already described.[2] However, the sulfonamide **16** has trapped a slightly different conformation of the enzyme in the crystal than reported earlier.[2] The C-terminal domain (residues 435–622) is rotated 4.8° with respect to the N-terminal domain, in a slightly more open conformation than seen in the X-ray structure of GTP bound to PEPCK[11] and the previously reported xanthines.

Several *N*-heteroaromatic benzylsulfonamides (hereafter called reverse sulfonamides) were prepared[12] and all were less active than the *N*-benzyl sulfonamides. For example the direct comparison of the reverse sulfonamide **25** with **17** showed at least a 9-fold decrease in activity in the enzyme assay. While this compares a chloro-di-methyl substituted pyrazole with a trimethyl pyrazole, in other xanthine classes these two pyrazole modifications were found to have nearly identical activities against the enzyme. Comparison of the *N*-methyl analog of sulfonamide **2**, the *N*-methyl-*N*-chloro-dimethylpyrazole sulfonamide **26**, with **2** showed a 6-fold decrease in activity in the enzyme assay. The loss of activity observed with the reverse sulfonamide **25** may be due to steric interactions between the sulfone oxygens and the methyl groups on the pyrazole ring and in the *N*-methyl analog **26** the *N*-methyl of the *N*-methyl sulfonamide with the methyl and halogen on the pyrazole ring. These interactions, not seen in the parent compounds, would increase the energy of any conformational changes required for binding to the protein. This analysis assumes that the preferred conformation for sulfonamides is where nitrogen's lone pair bisects the sulfone oxygens[13] as observed in the X-ray presented here.

The *N*-benzyl sulfonamides where the amine attachment was moved to the 3-position of the phenyl unit, for example, compound **27**, showed a decreased activity in both the enzyme (>3-fold) and cellular (>2-fold) assays relative to the corresponding 4-substituted analog **2**. In the parent *C*-8 benzyl class the 3-amino and 3-acetamido analogs showed a similar loss in activity when compared to the corresponding 4-substituted analogs (data not shown). These results suggested that while there may be space for groups at the 3-position, neither the *N*-benzyl 3-amine, -acetamide nor -sulfonamide groups were making any new interactions with the protein.







In summary, we present modifications at xanthine's *C*-8 aminobenzyl moiety that show 5-membered heteroaromatic *N*-benzylsulfonamides provide a new π–π interaction and further increase the inhibitory activity of the xanthine class of cPEPCK inhibitors. In the sulfonamide class the 5-chloro-1,3-dimethylpyrazole analog **2** provides the most active inhibitor in both the enzyme and cellular assays.

Acknowledgments

We thank Dr. David Fry (Roche) for making us aware of an analysis by Prof. Françios. Diederich (ETH-Zürich) and Dr. Klaus Mueller (Roche) of small molecule X-ray structures showing the conformational preference for sulfonamides noted here.[13]

References and notes

1. Foley, L. H.; Wang, P.; Dunten, P.; Ramsey, G.; Gubler, M.-L.; Wertheimer, S. J. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3607.
2. Foley, L. H.; Wang, P.; Dunten, P.; Ramsey, G.; Gubler, M.-L.; Wertheimer, S. J. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3871.
3. Cimbala, A. N.; Lamers, W. H.; Nelson, K.; Monahan, J. E.; Yoo-Warren, H.; Hanson, R. W. *J. Biol. Chem.* **1982**, *257*, 7629.
4. (a) Rosella, G.; Zajac, J. D.; Baker, L.; Kaczmarczyk, S. J.; Andrikopoulos, S.; Adams, T. E.; Proietto, J. *Mol. Endocrinol.* **1995**, *9*, 1396; (b) Valera, A.; Pujol, A.; Pelegrin, M.; Bosch, F. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 9151; (c) Sun, Y.; Liu, S.; Ferguson, S.; Wang, L.; Klepcyk, P.; Yun, J. S.; Freidman, J. E. *J. Biol. Chem.* **2002**, *277*, 23301.
5. Gómez-Valadés, A. G.; Vidal-Alabró, A.; Molas, M.; Boada, J.; Bermúdez, J.; Bartrons, R.; Perales, J. C. *Mol. Ther.* **2006**, *13*, 401.
6. The PEPCK cellular assay measured the test compounds inhibition of glucose production in H4IIE rat hepatoma cells. To maximally activate PEPCK, cells were starved overnight to deplete their endogenous stores of glycogen. The H4IIE cells were plated in a 12-well dish at a density of $\sim$2–3 $\times$ 10^5 cells/well. Cells were incubated overnight at 37 °C in RPMI medium with 10% fetal calf serum (FCS). The following day, medium was removed and cells were washed twice in 1 mL phosphate-buffered saline +calcium and magnesium (PBS +Ca/Mg). To each well was then added 1 mL RPMI medium (−glucose, −FCS, +1 mg/mL BSA, +20 mM Na Pyruvate) and incubated overnight at 37 °C. Prior to addition, dilutions of the test compounds were prepared in DMSO and then added to the experimental medium such that the final DMSO content was 0.5%. Experimental medium containing the test compound (500 μL) was added to each well and assays were preformed in duplicate. After incubating the cells for 6 h at 37 °C, the medium was collected and stored frozen at −20 °C. The amount of glucose in the medium was determined using a fluorometric assay (AMPLEX RED/ Molecular Probes) and measured using a plate reader.
7. As noted earlier [2] the IC_{50}s reported here might be 9-fold higher than the true binding constants.
8. Yu, C.; Taylor, J. W. *Bioorg. Med. Chem.* **1999**, *7*, 161.
9. Bergeron, R. J.; McManis, J. S. *J. Org. Chem.* **1988**, *53*, 3108.
10. PDB code: 2 GMV, Resolution 2.30 Å, *R* factor 21.7%.
11. 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*, 215.
12. Prepared from ethyl 4-(chlorosulfonyl)phenyl acetate Kawashima, Y.; Sato, M.; Yamamoto, S.; Shimazaki, Y.; Chiba, Y.; Satake, M.; Iwata, C.; Hatayama, K. *Chem. Pharm. Bull.* **1995**, *43*, 1132, The sulfonyl chloride was reacted with the desired heterocyclic amine and then the ester hydrolyzed to give the needed phenyl acetic acid derivative for reaction with the uracil according to Ref. 1.
13. Other X-rays showing this phenomenon may be found in the following references: Yamada, K.; Fujihara, H.; Yamamoto, Y.; Miwa, Y.; Taga, T.; Timioka, K. *Org. Lett.* **2002**, *4*, 3509; (a) Aggarwal, V. K.; Charmant, J. P. H.; Ciampi, C.; Hornby, J. M.; O'Brien, C. J.; Hynd, G.; Parsons, R. *J. Chem. Soc. Perkin Trans. 1* **2001**, 3159; (b) Hendi, M. S.; Davis, R. E.; Wheeler, K. A. *Cryst. Eng.* **2000**, *3*, 209.