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TRITERPENE AND DITERPENE INHIBITORS OF PYRUVATE DEHYDROGENASE KINASE (PDK)

Thomas D. Aicher,* Robert E. Damon, Judit Koletar, Christine C. Vinluan, Leonard J. Brand, Jiaping Gao, Suraj S. Shetty, Emma L. Kaplan, and William R. Mann

Metabolic & Cardiovascular Diseases Research, Novartis Institute for Biomedical Research, 556 Morris Avenue, Summit, NJ 07901, U.S.A.

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Abstract: Several oximes of triterpenes with a 17-β hydroxyl and abietane derivatives are inhibitors of pyruvate dehydrogenase kinase (PDK) activity. The oxime **12** and dehydroabietyl amine **2** exhibit a blood glucose lowering effect in the diabetic *ob/ob* mouse after a single oral dose of 100 μmol/kg. However, the mechanism of the blood glucose lowering effect is likely unrelated to PDK inhibition.

Introduction: The pyruvate dehydrogenase complex (PDC) catalyses the first irreversible step in the oxidation of glucose, the decarboxylation of pyruvate, through the highly regulated and coordinated action of multiple enzyme subunits. The complex is regulated via reversible phosphorylation, catalyzed by several specific kinase and phosphatase isozymes. It is well documented that the activity of the pyruvate dehydrogenase (PDH) complex is reduced during conditions of reduced oxidative glucose metabolism such as occur during obesity, starvation, and diabetes.[1-6] Activation of PDC via inhibition of PDK would be expected to result in increased oxidation of pyruvate in muscle and fat, and a concomitant decrease in the conversion of pyruvate to the gluconeogenic substrates lactate and alanine. Indeed, in early clinical studies, dichloroacetate (DCA, a known inhibitor of PDK) reduced blood lactate, alanine, and glucose levels in Type 2 diabetic patients.[7-9] Based on these data, orally active PDK inhibitors should have utility as a therapy for diabetes and possibly also other conditions in which PDC activity is reduced (ischemia,[10] lactic acidosis,[11] and cardiac insufficiency[12]).

Unfortunately, the toxicity of DCA precludes its use as a therapeutic agent. Long-term use has been reported to cause neuropathic effects, cataract formation, and testicular degeneration.[13-16] These toxicities have been attributed in part to the accumulation of the principle metabolite of DCA, oxalic acid. However, a significant portion of the toxicity may also be due to the parent compound, since α-halocarbonyl compounds also exhibit similar toxicities.[17,18] No compounds other than α,α-dihalogenated carbonyl compounds have previously been reported inhibit PDK activity.[19] Herein, we shall describe the SAR of a new series of PDK inhibitors, tri- and diterpenes (e.g., nortestosterone **1**, dehydroabietyl amine **2**, and **3**).

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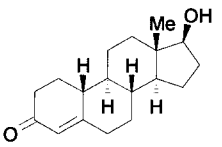
Our initial discovery that diterpenes and triterpene derivatives inhibit PDK activity led us to explore the structural characteristics of the molecules that influence this effect. Our initial four approaches were to study the effect of (1) replacement of the ketone of **1** and amine moiety of **2** with different functionalities, (2) removal of the C, and D rings of **1**, (3) different substituents at the C17 center of **1**, and (4) different functionalities on the abietane ring system of **2**.



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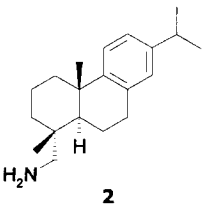

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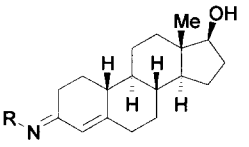
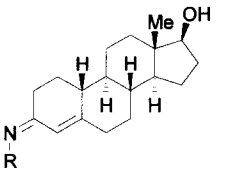
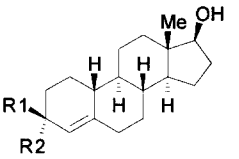
Chemistry: Molecules **3–10** were either purchased or targeted for synthesis to study the replacement of the ketone moiety of **1** with different chemical functionality. The majority of the oximes of this report are known,



3 (*anti*) R = OH
4 (*anti*) R = OMe
5 (*anti*) R = OBn
6 (*anti*) R = CH2COOH
7 R = NH_2
8 R = $NHCONH_2$
9 R = NH(=NH)NH_2



3 (*syn*) R = OH
4 (*syn*) R = OMe
5 (*syn*) R = OBn



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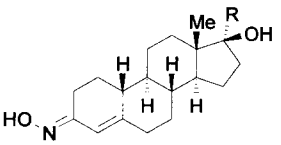
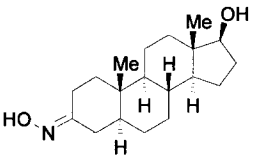


11 R = CCH
12 R = CH=C=CH_2



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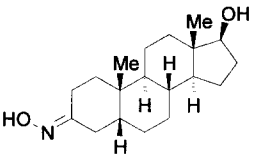

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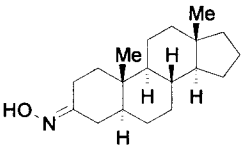

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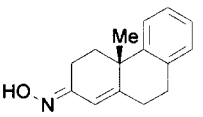

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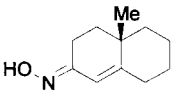
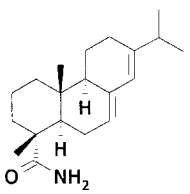


17

and all were synthesized via a modification of the method of Delgado.[20] Briefly, the oxime **3** was synthesized via the condensation of nortestosterone with hydroxylamine hydrochloride in an ethanolic suspension of sodium acetate. Alkylated oximes **4** and **5** result from the analogous condensation with the appropriate alkoxyamine.

The oximes (**11**, **12**, **16**, and **17**) were prepared similarly from readily available ketones.[21] All of these condensations result typically in a 50–80% total yield.[22] These reactions result in a mixture of the (*anti*) and (*syn*) stereoisomers. The (*anti*) oxime isomers are easily separated from the (*syn*) isomers via either chromatography or crystallization when the ketone is unsaturated.[23] The oximes (**13**–**15**) of the saturated ketones were tested as a mixture of isomers. Attempts to synthesize the hydrazone **7** from **1** via a condensation with hydrazine were unsuccessful and afforded a 2:1 adduct.[24] Compounds **8** and **9** were prepared from **1** via condensation with semicarbazide and aminoguanidine utilizing the general procedure for the oximes. The alcohol **10** was prepared via reduction of **1** with $NaBH_4$ in methanol.



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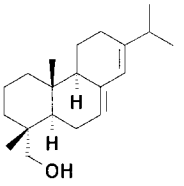

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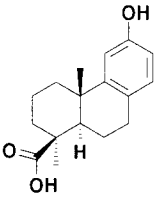

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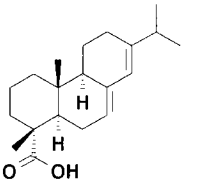

21

Abietic acid **21** was converted to its acyl chloride via treatment with oxalyl chloride and a catalytic amount of DMF in methylene chloride. Treatment of the crude acid chloride with ammonia afforded **18**. Treatment of abietic acid with $LiAlH_4$ afforded **19**.

Results and Discussion

The reported compounds were tested for their ability to reduce PDK catalyzed PDC inactivation in a primary enzyme assay[25,26] At concentrations up to 100 μM, many of the compounds exhibited marked PDK inhibition. However, several diterpenes and triterpenes exhibited biphasic characteristics: at concentrations equal to or greater than 100 μM, they inhibited the PDC in absence of ATP (Table 1) (i.e., they inhibited PDC directly). Potent inhibitors were evaluated for their ability to increase the conversion of [^{14}C]-lactate into $^{14}CO_2$ in a modification of Ofenstein's assay (see Table 1).[27]

Replacement of the ketone moiety of **1** with a hydroxylamine moiety afforded more potent inhibitors (compare **3** (***anti***), **11**, **12**, and **13** to **1**) in the PDK enzyme assay (see Table 1). The approximate tenfold increase in potency could be due to a favorable hydrogen bond from the oximes' hydrogen to the binding site(s) on the kinase, since the methylated analog **4** did not inhibit PDK activity (compare **4** to **3**). However, the semicarbazone derivatives **8** and **9**, which could also contribute a putative favorable hydrogen bond, were inactive.

Our attempts to replace the triterpene nucleus of the oximes (**3** (***anti***), **11**, **12**, and **13**) with analogues with fewer rings were unsuccessful. The bicyclic oxime **17** and the tricyclic oxime **16**, which is similar to the diterpene

Table 1. Physical Characteristics and In Vitro Data of **1–21**.

Entry	Formula[a]	mp (°C)	% Yield	IC_{50}[b]	EC_{50}[c]
DCA			[d]	> 2000	100?
1			[d]	120 ± 20[e]	
2			[d]	9.5 ± 1.1	inactive
3 (*anti*)	$C_{18}H_{27}NO_2$	180–182	44	5.4 ± 0.91	
4(*anti*)	$C_{19}H_{29}NO_2$	129	41	Inactive	
4(*syn*)	$C_{19}H_{29}NO_2$	113	25	Inactive	
5(*anti*)	$C_{25}H_{33}NO_2$	96–98	35	Inactive	
5(*syn*)	$C_{25}H_{33}NO_2$	114	26	Inactive	
6			[d]	56 ± 25	
8	$C_{19}H_{29}N_3O_2$	243.5–244.5	64	Inactive	
9	$C_{19}H_{31}N_4OCl$	291–293	27	Inactive	
10	$C_{18}H_{28}O_2$	163–165	65	Inactive	
11	$C_{20}H_{27}NO_2$	80	51	5.8 ± 1.3	
12	$C_{21}H_{29}NO_2$	90–92	64	0.840 ± 0.034[e]	1.7 ± 0.15[e]
12 (*syn*)	$C_{21}H_{29}NO_2$	95–97	32	Inactive	inactive
13	$C_{19}H_{31}NO_2$	210–212	74	51 ± 7.6	
14	$C_{19}H_{31}NO_2$		81	36 ± 15	
15	$C_{27}H_{47}NO$	200–201	93	Inactive	
16	$C_{15}H_{17}NO$	60–65	53	Inactive	
17	$C_{11}H_{17}NO$	121–122	59	Inactive	
18	$C_{20}H_{31}NO$	164–166	40	6.7 ± 1.0	inactive
19	$C_{20}H_{32}O$	74–75	37	~10[e]	inactive
20			[d]	Inactive	
21			[d]	20 ± 3.4	inactive

[a]Analytical results (C, H, N) were within ± 0.4% of the theoretical value.
[b]IC_{50} (μM ± standard error.) in primary enzymatic assay of PDK inhibition (ref 25). Inactive compounds were tested up to 1 mM.
[c]EC_{50} (μM ± standard error) in normal human dermal fibroblasts, modification of ref 27.
[d]Purchased from the Sigma-Aldrich Corporation.
[e]Displays a biphasic dose response at concentrations greater than or equal to 100 μM.

Table 2. In Vivo Activity of Selected Compounds in a Model of Diabetes, the *ob/ob* Mouse.[a]

Entry	Dose (μmol/kg/day)	Day 1 2 h % Efficacy	Day 1 4 h % Efficacy	Day 1 6 h % Efficacy	Day 3 2 h % Efficacy	Day 3 4 hr % Efficacy	Day 3 6 h % Efficacy
DCA	1000	59*	39*	29	52*	50*	56*
2	30	18	-1	11	-4	2	19
2	100	30	28	22	26	36	47*
2	300	68*	69*	65*	85*	86*	90*
12	30	-6	-3	2	38	43	-6
12	100	3	4	26	73*	70*	47*
12	300	-2	19	51*	92*	83*	75*
18	200	4	-2	16	13	9	16
19	100	7	-8	19	7	10	24
21	200	45*	25	42*	24*	22	29*

[a](Ref 28). % efficacy = $\{1-([glucose]_{compound}-100)/([glucose]_{vehicle}-100)\}*100$.
* $p < 0.05$

2, did not inhibit PDK activity at concentrations ≤300 μM. These data suggest that a second interaction of the active triterpenes may result from functionality on the D-ring. This putative interaction could be a second hydrogen bond effected by the 17-β hydroxyl group of the active oximes, since the des-hydroxy analog **15** did not inhibit PDK activity (compare **13** to **15**).

Oximes **11**, **12**, and **12** (***syn***) and the diterpenes **2**, **18**, **19**, and **21** were profiled in a cellular PDC activation assay (see Table 1) and in an animal model of Type 2 diabetes, the *ob/ob* mouse[28] (see Table 2). The diterpenes **2** and **21** lowered blood glucose levels in the *ob/ob* mouse model. However, they did not increase the conversion of lactate to CO_2 in the cellular assay (see Table 1) and exhibited no effect on PDC activity ex vivo in the heart of *ob/ob* mice. For these reasons, the hypoglycemic activity of **2** and **21** are likely unrelated to their ability to inhibit PDK activity.

The triterpene **12** inhibited PDK activity with an IC_{50} of 840 ± 0.034 nM, and increased the conversion of lactate to CO_2 in the cellular assay with an EC_{50} of 1.7 ± 0.15 μM. The maximal increase of lactate conversion was approximately threefold above control. It displayed a biphasic dose response; at concentrations greater than 30 μM lactate conversion to CO_2 declined, reaching levels below control incubations at a concentration of 300 μM. The triterpene **12** acutely lowered blood glucose levels in the *ob/ob* mouse model following oral administration (100 or 300 μmol/kg). When **12** was administered orally to 24 h fasted normal (Sprague–Dawley) rats (300 μmol/kg), it lowered blood lactate levels (–18%, $p < 0.05$, two and four hours post dose, Table 3) (blood lactate lowering is an

Table 3. Effect of **12** on Blood Glucose and Blood Lactate Levels After Oral Dosing in Sprague–Dawley Rats.

	Blood Glucose (mg/dl)			**Blood Lactate (mM)**		
Treatment	**0 h**	**2 h**	**4 h**	**0 h**	**2 h**	**4 h**
Vehicle	49.6 ± 1.0	49.1 ± 1.6	49.8 ± 1.4	1.01 ± 0.09	0.98 ± 0.07	0.96 ± 0.02
12 (300 μmol/kg)	49.5 ± 1.7	49.1 ± 1.1	49.3 ± 2.0	1.01 ± 0.09	0.80 ± 0.03	0.79 ± 0.04
% of Control	100	101	99	100	82*	82*

*$p < 0.05$

early in vivo result of PDK inhibition[9]). However, treated animals unexpectedly exhibited reduced tibialis anterior skeletal muscle PDC activity (determined in an arylamine acetyltransferase-coupled spectrophotometric enzyme assay[29]) by approximately 60% (7 versus 18 milli units PDC activity/unit citrate synthase activity for compound- and vehicle-treated animals, respectively).

In summary, oximes of triterpenes such as **12** and diterpenes such as **2** are the first reported inhibitors of PDK without halogens alpha to a carbonyl. Compound **12** enhanced the conversion of lactate to CO_2, and upon oral adminstration lowers blood lactate levels following acute oral dosing (300 μmol/kg) in Sprague–Dawley rats. Compounds **2**, **12** and **21** lowered blood glucose levels after acute oral dosing of 300 μmol/kg. That PDK inhibition is the cause of this hypoglycemic effect is questionable. Compounds **2** and **21** do not have activity in the cellular assay, and **12** is dosed at concentrations much greater than is necessary for its antiprogestational effects of **12** and similar oximes.[30]

References and Notes

1. Caterson, I. D.; Kerby, A. L.; Cooney, G. J.; Frankland, R.; Denyer, G. S.; Nicks, J.; Williams, P. F. *Biochem. J.* **1988**, *253*, 291.
2. Denyer, G. S.; Lam, D.; Cooney, G. J.; Caterson, I. D. *FEBS Lett* **1989**, *250*, 464.
3. Mondon, C. E.; Jones, I. R.; Azhar, S.; Hollenbeck, C. B.; Reaven, G. M. *Diabetes* **1992**, *41*, 1547.
4. Mandarino, L. J.; Consoli, A.; Kelly, D. E.; Reilly, J. J.; Nurjhan, N. *J. Clin. Endocrinol. Metab.* **1990**, *71*, 1544.
5. Del Prato, S.; Bonadonna, R. C.; Bonora, E.; Gulli, G.; Solini, A.; Shank, M.; DeFronzo, R. A. *J. Clin. Invest.* **1993**, *91*, 484.
6. Henry, R. R.; Thorburn, A. W.; Beerdson, P.; Gumbiner, B. *Am. J. Physiol.* **1991**, *261*, E132.
7. Pratt, M. L.; Roche, T. E. *J. Biol. Chem.* **1979**, *254*, 7191.
8. Whitehouse, S.; Cooper, R. H.; Randle, P. J. *Biochem J.* **1974**, *141*, 761.
9. Stacpoole, P. W.; Moore, G. W.; Kornhauser, D. M. *N. Engl. J. Med.* **1978**, *298*, 526. Glucose lowering in normal animals (i.e., Sprague–Dawley rats) was not expected, as DCA only lowered blood glucose in diabetic human subjects.
10. Bersin, R. M.; Stacpoole, P. W. *Am. Heart J.* **1997**, *134*, 841.
11. Henderson, G. N.; Curry, S. H.; Derendorf, H.; Wright, E. C.; Stacpoole, P. W. *J. Clin. Pharmacol.* **1997**, *37*, 416.
12. Dichloroacetic acid showed efficacy in these indications. For a review of the pharmacology of DCA, see: Stacpoole, P. W. *Metabolism* **1989**, *38*, 1124.
13. Stacpoole, P. W.; Moore, G. W.; Kornhauser, D. M. *N. Engl. J. Med.* **1979**, *300*, 372.
14. Yount, E. A.; Felten, S. Y.; O'Connor, B. L.; Peterson, R. G.; Powell, R. S.; Yum, M. N.; Harris, R. A. *J. Pharmacol. Exp. Ther.* **1982**, *222*, 501.
15. Katz, R.; Tai, C. N.; Diener, R. M.; McConnell, R. F.; Semonick, D. E. *Toxicol. Appl. Pharmacol.* **1987**, *57*, 273.
16. Toth, G. P.; Kelty, K. C.; George, E. L.; Read, E. J.; Smith, M. K. *Fundam. Appl. Toxicol.* **1992**, *19*, 57.
17. Halpert, J. R.; Balfour, C.; Miller, N. E.; Kaminsky, L. S. *Mol. Pharmacol.* **1986**, *30*, 19.
18. Bookstaff, R. C.; Moore, R. W.; Ingall, G. B.; Peterson, R. E. *Toxicol. Appl. Pharmacol.* **1990**, *104*, 322.
19. Espinal, J.; Leesnitzer, T.; Hassman, A.; Beggs, M.; Cobb, J. *Drug Development Research* **1995**, *35*, 130.
20. Khouri, K.; Usubillaga, A.; Cedillo Vaz, S.; Delgado, J. N. *J. Pharm. Sci.* **1991**, *80*, 661.
21. Volpe, J.; Revial, G.; Pfau, M.; D'Angelo, J. *Tetrahedron Lett.* **1987**, *28*, 2367.
22. Typical experimental procedure: Nortestosterone (1.25 g, 4.55 mmol) was added to a mixture of methoxylamine hydrochloride (1.18 g, 14.1 mmol) and sodium acetate (3.08 g, 37.6 mmol) in ethanol (20 mL). The mixture was stirred for 18 h at room temperature. Water (50 mL) and methylene chloride (40 mL) were added. The organic layer was washed with water, dried ($MgSO_4$), and conc. For all compounds in this paper, the (*anti*) isomer of unsaturated oximes elutes first with either a mixture of hexane/ethyl acetate or with a mixture of CH_2Cl_2/MeOH. In such a manner, eluting with a 3:1 mixture of hexane: ethyl acetate, **4(*anti*)** eluted first and was concentrated to afford white crystals (570 mg, 41%): mp. 129 °C. **4(*syn*)** followed and was concentrated to afford white crystals (346 mg, 25%): mp. 113 °C.
23. The (*anti*) isomers can be distinguished from the (*syn*) isomers via NMR (The A-ring olefinic proton always has a higher ppm for the *anti* isomer) or via CNMR (A ring C4 about 8 ppm higher for *anti* vs. *syn*). Both methods agree in their stereochemical assignment. See: Oka, K.; Hara, S. *Chem. Ind.* **1968**, *27*, 911.
24. Structure not shown. The 2:1 adduct did not inhibit PDK.
25. Jackson, J. C.; Vinluan, C. C.; Dragland, C. J.; Sundararajan, V.; Yan, B.; Gounarides, J. S.; Nirmala, N. R.; Topiol, S.; Ramage, P.; Blume, J. E.; Aicher, T. D.; Bell, P. A.; Mann, W. R. *Biochem. J.* **1998**, *334*, 703.
26. Available from Sigma Aldrich Corporation, Milwaukee, Wisconsin (Sigma cat.# P-7032).
27. Ofenstein, J. P.; Kiechle, F. L.; Dandurand, D. M.; Belknap, W. M.; Moore, K. H.; Holmes, R. D. *Anal. Biochem.* **1993**, *210*, 332.
28. The in vivo assay was conducted as described previously with the modification of when blood samples (6 h timepoint) were taken, see: Aicher, T. D.; Balkan, B.; Bell, P. A.; Brand, L. J.; Cheon, S. H.; Deems, R. O.; Fell, J. B.; Fillers, W. S.; Fraser, J. D.; Gao, J.; Knorr, D. C.; Kahle, G. G.; Leone, C.; Nadelson, J.; Simpson, R.; Smith, H. C. *J. Med. Chem.* **1998**, *41*, 4556.
29. Coore, H. G.; Denton, R. M.; Martin, B. R.; Randel, P. J. *Biochem. J.* **1971**, *125*, 115.
30. The ED_{50} for the oxime of norethisterone acetate oxime is 0.95 mg/kg.