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Selective active site inhibitors of human lactate dehydrogenases A_4, B_4, and C_4

Yue Yu[a], Jason A. Deck[b], Lucy A. Hunsaker[a], Lorraine M. Deck[b], Robert E. Royer[a], Erwin Goldberg[c], David L. Vander Jagt[a,*]

[a]*Department of Biochemistry and Molecular Biology, University of New Mexico School of Medicine, Albuquerque, NM, USA 87131*
[b]*Department of Chemistry, University of New Mexico, Albuquerque, NM 87131, USA*
[c]*Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, IL 60208, USA*

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Abstract

Human lactate dehydrogenases (LDH-A_4, -B_4, and -C_4) are highly homologous with 84–89% sequence similarities and 69–75% amino acid identities. Active site residues are especially conserved. Gossypol, a natural product from cotton seed, is a non-selective competitive inhibitor of NADH binding to LDH, with K_i values of 1.9, 1.4, and 4.2 μM for LDH-A_4, -B_4, and -C_4, respectively. However, derivatives of gossypol and structural analogs of gossypol in the substituted 2,3-dihydroxy-1-naphthoic acid family exhibited markedly greater selectivity and, in many cases, greater potency. For gossypol derivatives, greater than 35-fold selectivity was observed. For dihydroxynaphthoic acids with substituents at the 4- and 7-positions, greater than 200-fold selectivity was observed. Inhibition was consistently competitive with the binding of NADH, with dissociation constants as low as 30 nM. By comparison, a series of *N*-substituted oxamic acids, which are competitive inhibitors of the binding of pyruvate to LDH, exhibited very modest selectivity. These results suggest that substituted dihydroxynaphthoic acids are good lead compounds for the development of selective LDH inhibitors. Selective inhibitors of LDH-C_4 targeted to the dinucleotide fold may hold promise as male antifertility drugs. Selective inhibitors of LDH-A_4 and -B_4 may be useful for studies of lactic acidemia associated with ischemic events. More broadly, the results raise the question of the general utility of drug design targeted at the dinucleotide binding sites of dehydrogenases/reductases. © 2001 Elsevier Science Inc. All rights reserved.

Keywords: Lactate dehydrogenase; Gossypol; Dihydroxynaphthoic acids; *N*-substituted oxamic acids

1. Introduction

Mammalian LDHs [(l)-lactate:NAD^+ oxidoreductase, EC 1.1.1.27) are tetrameric NAD^+-specific dehydrogenases that catalyze the interconversion of lactate and pyruvate and participate in both glucose catabolism and gluconeogenesis from lactate. Three genes, designated *ldh-a*, *ldh-b*, and *ldh-c*, code for structurally similar proteins. Somatic cells differentially express the five combinations of tetramers derived from *ldh-a* and *ldh-b*, which are named LDH-1 (or LDH-B_4), LDH-2 (LDH-A_1B_3), LDH-3 (LDH-A_2B_2), LDH-4 (LDH-A_3B_1), and LDH-5 (LDH-A_4). LDH-A_4 and -B_4 are especially abundant in skeletal and cardiac muscle, respectively [1]. By comparison, LDH-C_4, the product of the *ldh-c* gene, is expressed only in testes and sperm [2].

The cellular distribution of LDH-C_4 in sperm is complex; this LDH is found in the cytosol, in the mitochondrial matrix, between the inner and outer mitochondrial membranes, and in the plasma membrane [3]. The mitochondrial location of LDH-C_4 has been suggested to reflect a lactate–pyruvate transport system that enables lactate to deliver reducing equivalents into sperm mitochondria [4,5]. The plasma membrane location of LDH-C_4 has been exploited to develop immunocontraceptives. Active immunization with LDH-C_4 suppresses fertility in a variety of mammalian species [6] and is the most effective contraceptive immunogen identified to date [7].

The traditional treatment of the role of LDH in somatic cells emphasizes cytoplasmic reactions. However, LDH has been observed in mitochondria from somatic cells [8,9] and recently has been reported to function in an intracellular lactate shuttle in muscle and liver [10,11], similar to the

* Corresponding author. Tel.: +1–505-272–5788; fax: +1–505-272–6587.

E-mail address: dlvanderjagt@salud.unm.edu (D.L. Vander Jagt).

Abbreviations: LDH, lactate dehydrogenase; and LDH-A_4, -B_4, and -C_4, human lactate dehydrogenases A_4, B_4, and C_4.

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proposed shuttle for lactate metabolism by sperm mitochondria. The emerging concept is that lactate may be the major source of pyruvate for the pyruvate dehydrogenase reaction in most cells, even under conditions where oxygen is abundant, and that the intracellular lactate–pyruvate shuttle is used widely [12]. The isoenzyme distribution of LDH in cytosol differs from that in mitochondria in muscle and liver cells [10]. It is unclear how LDH is transported into mitochondria; there are no apparent mitochondrial targeting sequences in LDH.

The unique distribution of LDH-C_4 in sperm and testes suggests that LDH-C_4 would be a good candidate for the development of antifertility drugs, in addition to being a promising immunocontraceptive. The antifertility properties of the natural product gossypol were initially suggested to result from inhibition of LDH-C_4 [13]. Gossypol, a polyphenolic binaphthyl disesquiterpene isolated from cotton seeds, has been studied extensively in the People's Republic of China as a potential male antifertility agent [14]. Gossypol also exhibits numerous other biological activities [15, 16]. In previous studies designed to test whether structural analogs of gossypol retain biological activity [17–19], it was observed that some compounds were selective inhibitors of LDH-A_4 or -B_4 in spite of the high sequence and structural homologies of these isoenzymes [20–22]. In the present study, we have compared gossypol derivatives and analogs as selective inhibitors of human LDH-A_4, -B_4, and -C_4 along with a series of *N*-substituted oxamic acids. Compounds related to gossypol are competitive inhibitors of NADH binding to LDH, while oxamic acids are competitive inhibitors of pyruvate binding. The goal was to determine whether there are structural features of LDH-A_4, -B_4, and -C_4 that can be exploited to design selective inhibitors. Selective inhibitors of LDH-C_4 would represent new lead compounds for the development of male antifertility drugs. Selective inhibitors of LDH-A_4 and -B_4 may be useful in experimental studies of energy metabolism involving the lactate–pyruvate shuttle. Whether or not selective inhibitors of LDH-A_4 or -B_4 may have potential therapeutic use, such as in the treatment of lactic acidemia associated with stroke, is problematic. There is ongoing debate whether elevated lactic acid in the infarcted area or in the surrounding penumbra following ischemic stroke promotes neuronal death or is protective [23].

2. Materials and methods

2.1. Materials

Human LDH-A_4 and -B_4 were from the Sigma Chemical Co. Recombinant human LDH-C_4 was purified by Blue-Sepharose affinity chromatography, chromatofocusing, and hydroxylapatite chromatography similar to the procedures described for purification of other LDHs [20]. The clone for human LDH-C_4 in expression vector pKK223–3 was grown in *Escherichia coli* strain W3110 *lacI*^{q} as described [24]. The purified LDH-C_4 was homogeneous on SDS–PAGE with a specific activity of 285 μmol/min/mg.

Gossypol (**1**) was obtained from the Southern Regional Research Center, USDA, as the acetic acid complex. Gossylic nitrile-1,1′-diacetate (**2**), gossylic iminolactone (**3**), and gossylic lactone (**4**) were synthesized as described previously [17,18]. Gossypol analogs in the dihydroxynaphthoic acid family (**5** to **17,** Fig. 1) were also synthesized as described previously [19,21]. Compounds **9, 10, 11,** and **12** are new.

7-[*o*-(Methyl)benzyl]-2,3-dihydroxy-6-methyl-4-(1-methylethyl)-1-naphthoic acid (**9**); ^{1}H NMR ($CDCl_3$) δ (relative to TMS) 1.51 (d, 6H), 2.31 (s, 3H), 2.38 (s, 3H), 3.90 (m, 1H), 4.05 (s, 2H), 6.23 (s, 1H), 6.86 (d, 1H), 7.05–7.21 (m, 3H), 7.90 (s, 1H), 8.40 (s, 1H).

7-[*m*-(Methyl)benzyl]-2,3-dihydroxy-6-methyl-4-(1-methylethyl)-1-naphthoic acid (**10**); ^{1}H NMR 1.52 (d, 6H), 2.30 (s, 3H), 2.36 (s, 3H), 3.90 (m, 1H), 4.11 (s, 2H), 6.25 (s, 1H), 6.94- 7.02 (m, 3H), 7.16 (t, 1H), 7.89 (s, 1H), 8.67 (s, 1H).

7-[*p*-(Methyl)benzyl]-2,3-dihydroxy-6-methyl-4-(1-methylethyl)-1-naphthoic acid (**11**); ^{1}H NMR 1.47 (dd, 6H), 2.25 (s, 3H), 2.31 (s, 3H), 3.86 (m, 1H), 4.13 (s, 2H), 6.22 (s, 1H), 7.02 (s, 4H), 7.85 (s, 1H), 8.99 (s, 1H).

7-[*p*-(Chloro)benzyl]-2,3-dihydroxy-6-methyl-4-(1-methylethyl)-1-naphthoic acid (**12**); ^{1}H NMR 1.50 (d, 6H), 2.34 (s, 3H), 3.88 (m, 1H), 4.14 (s, 2H), 6.23 (s, 1H), 7.45 (d, 2H), 7.87 (s, 1H), 8.04 (d, 2H), 8.64 (s, 1H).

N-Substituted oxamic acids (**18** to **27,** Fig. 2) were synthesized by literature procedures [25]. Compounds **21, 23, 24,** and **25** are new.

N-4-Methylbenzyloxamic acid (**21**); m.p. 148–149$^{o;}$ ^{1}H NMR 2.35 (s, 3H), 4.48 (d, 2H), 7.17 (s, 4ArH), 7.48 (br s, 1H, NH).

N-β-Methylphenethyloxamic acid (**23**); m.p. 102–103$^{o;}$ ^{1}H NMR 1.32 (d, 3H), 3.00 (s, 1H), 3.53 (dm, 2H), 7.27 (m, 5ArH), 8.72 (br s, 1H, NH).

N-1-Methyl-3-phenylpropyloxamic acid (24); m.p. 101–102$^{o;}$ ^{1}H NMR 1.29 (d, 3H), 1.86 (q, 2H), 2.65 (t, 2H), 4.00 (hept, 1H), 7.22 (m, 6H, 5Ar, 1NH)

N-3-Phenylpropyloxamic acid (25); m.p. 124–125$^{o;}$ ^{1}H NMR 1.93 (p, 2H), 2.68 (t, 2H), 3.38 (q, 2H), 7.25 (m, 6H, 5ArH, 1NH)

2.2. Enzyme assays and kinetics

LDH activity was measured in the direction of reduction of pyruvate to lactate by monitoring the changes in the absorbance of NADH at 340 nm, 25^{o,} in 25 mM Tris buffer, pH 7.5. Kinetic constants (K_m) for pyruvate and NADH and k_{cat} values were determined by nonlinear regression analysis (ENZFITTER, Elsevier-Biosoft). Dissociation constants (K_i) of the inhibitors were determined from double-reciprocal plots by linear regression analysis.

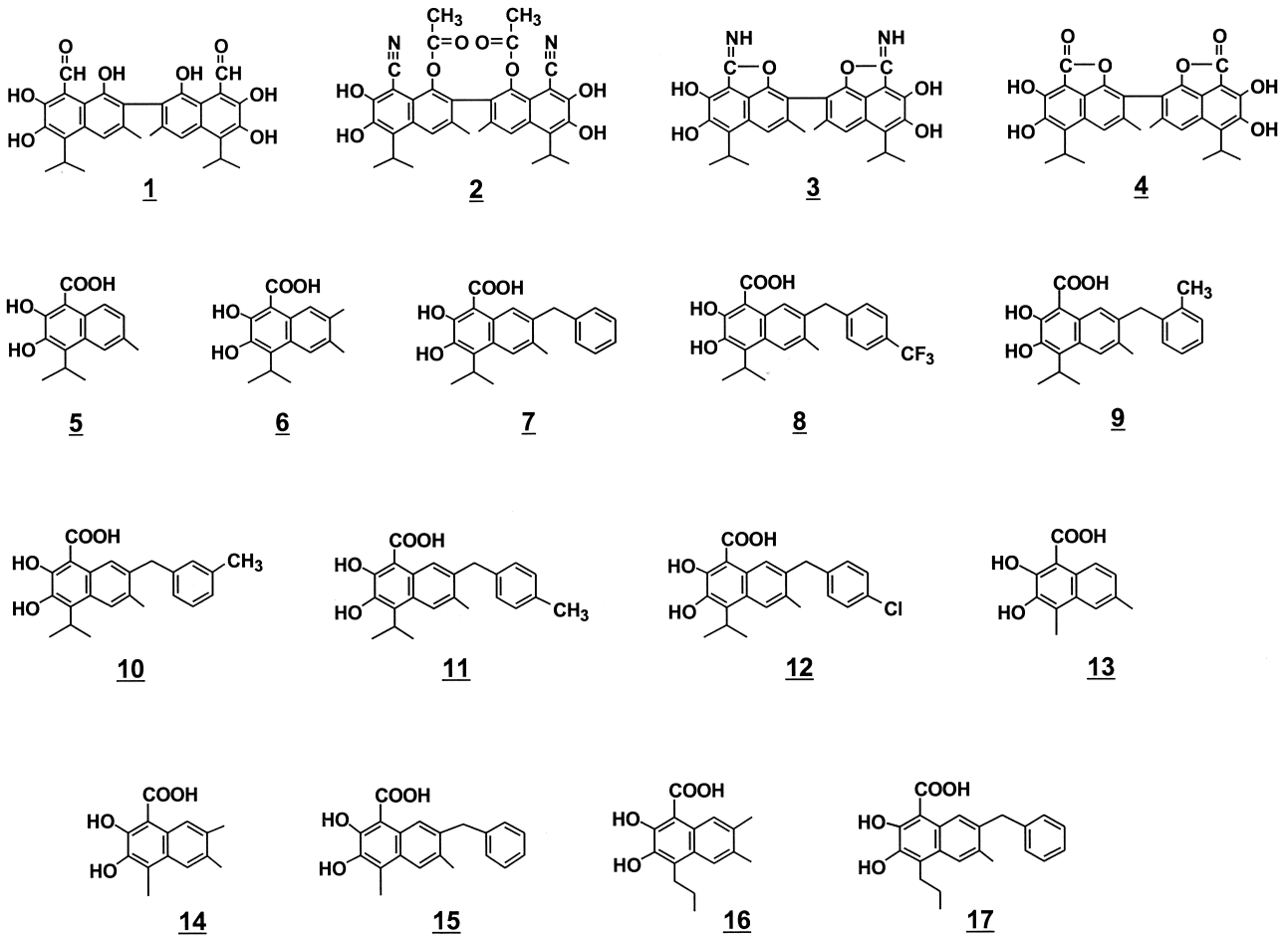

Fig. 1. Structures of gossypol (**1**), gossypol derivatives (**2**-**4**) and gossypol analogs in the 2,3- dihydroxy-1-naphthoic acid family (**5**-**17**).

3. Results

3.1. Inhibition of human LDHs by gossypol

Inhibition of human LDH-A_4, -B_4, and -C_4 by gossypol (**1**) was non-selective, as summarized in Table 1. Dissociation constants (K_i) were 1.9, 1.4, and 4.2 μM for LDH-A_4, -B_4, and -C_4, respectively. In all cases, inhibition was competitive with the binding of NADH, as shown in Fig. 3A for inhibition of LDH-C_4. The lack of selectivity by gossypol and the pattern of competitive inhibition of the binding of NADH are consistent with previous studies of the three isoenzyme forms of LDH [26] but are in disagreement with several studies in which gossypol exhibited noncompetitive or mixed inhibition with respect to the binding of NADH [27,28].

3.2. Inactivation of human LDHs by gossypol

To determine whether the inconsistencies in the patterns of inhibition of LDH by gossypol may reflect complications introduced by competing inactivation of LDH by gossypol, the time dependence of inhibition of LDH-A_4, -B_4, and -C_4 was determined. Time-dependent loss of LDH activity in the presence of gossypol has been reported previously with the isoenzymes of mouse LDH [12]. For human LDH-A_4, -B_4, and -C_4, incubation of enzyme with 10 μM gossypol for 30 min had no effect on enzyme activity measured when the samples were diluted. Thus, the inconsistencies in the literature concerning the kinetic behavior of gossypol may reflect differences in stability of the various LDHs compared with human LDH.

3.3. Inhibition of human LDHs by derivatives of gossypol

Inhibition of LDH-A_4, -B_4, and -C_4 by derivatives of gossypol (structures **2, 3,** and **4** in Fig. 1) reflects some enhancement in potency and/or increased selectivity compared with gossypol. Inhibition by gossylic lactone (**4**) was consistently about 3-fold more potent with all three isoenzymes, but was non-selective (Table 1). The structure of lactone **4** most closely resembles gossypol in its cyclic lactol tautomer (see **1c** in Fig. 4) except that the aldehyde carbon in the lactol tautomer of gossypol is sp^3 hybridization, while the lactone carbonyl carbons in **4** are sp^2 hybridization. Gossylic nitrile-1,1′-diacetate (**2**) was a less potent

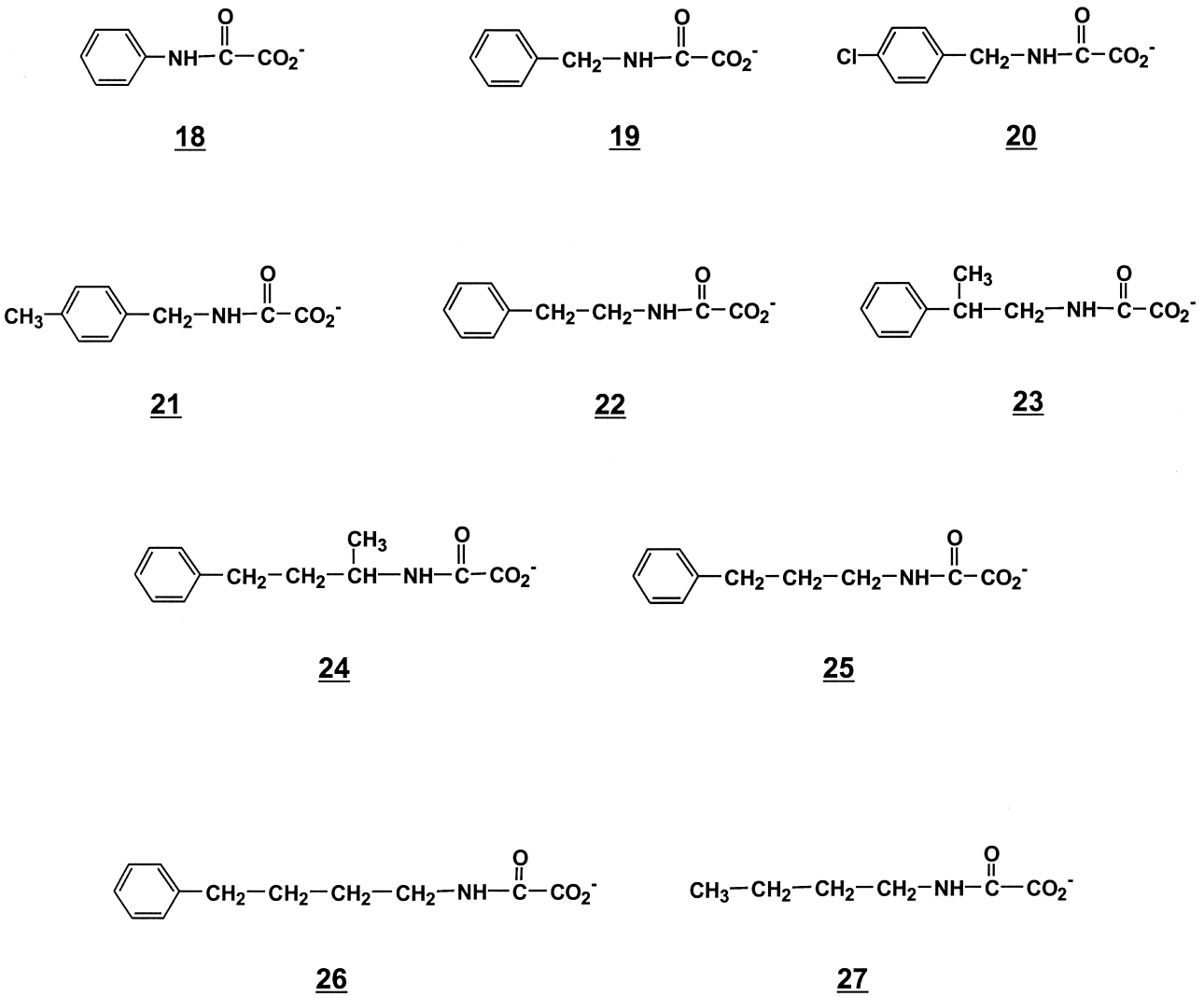

Fig. 2. Structures of *N*-substituted oxamic acids (**18**-**27**).

inhibitor than lactone **4** of all three LDHs but was more selective. LDH-A_4 and -C_4 were more sensitive than LDH-B_4 to inhibition by nitrile **2.** This same pattern was observed with gossylic iminolactone (**3**) where selectivity for LDH-A_4 and -C_4 over LDH-B_4 was greater than 35-fold. Thus, iminolactone **3** was similar to gossypol as an inhibitor of LDH-A_4 and -C_4 but was 66-fold weaker than gossypol against LDH-B_4, whereas lactone **4** was 230-fold more potent than iminolactone **3** against LDH-B_4 but only 2- to 4-fold more potent than **3** against LDH-A_4 and -C_4. As with gossypol, inhibition was competitive with the binding of NADH, as shown in Fig. 3B, for inhibition of LDH-C_4 by lactone **4.**

Table 1
Inhibition of human LDHs by gossypol and derivatives

Structure no.	K_i (μM)		
	LDH-A_4[a]	LDH-B_4	LDH-C_4
1	1.9	1.4	4.2
2	9.1	39	7.7
3	2.5	92	3.1
4	0.6	0.4	1.6

[a] Data for LDH-A_4 are from Ref. [20].

3.4. Inhibition of human LDHs by substituted dihydroxynaphthoic acids

2,3-Dihydroxy-1-naphthoic acid with substituents in the 4 and 7 positions (Fig. 1) can be considered to be analogs of gossypol, although only remotely related structurally. This series of dihydroxynaphthoic acids was analyzed as inhibitors of LDH-A_4, -B_4, and -C_4. Results are summarized in Table 2. Substituted dihydroxynaphthoic acids were competitive inhibitors of the binding of NADH, as shown in Fig. 3C for the inhibition of LDH-C_4 by **16.** The range of potency and selectivity for inhibition of the three LDHs by dihydroxynaphthoic acids was broad compared with gossy-

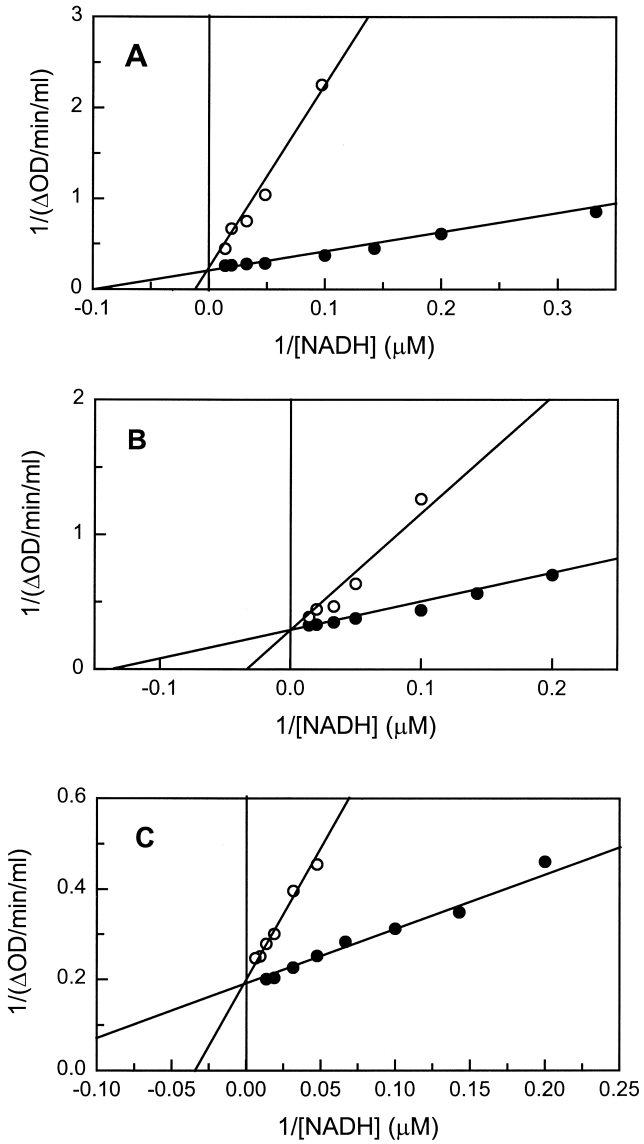

Fig. 3. Inhibition of human LDH-C_4 by gossypol (**1**) (panel A), by gossylic lactone (**4**) (panel B), and by gossypol analog **16** (panel C) with respect to NADH. Key: (○) in the presence of inhibitor; and (●) in the absence of inhibitor.

pol. Substituents at both the 4- and 7-position are important in determining potency and selectivity. For example, compound **11** was 430 times more potent than **8** as an inhibitor of LDH-A_4 but only 8- to 10-fold more potent against -B_4 and -C_4. Compound **11** differs from **8** by having a *p*-methyl rather than a *p*-trifluoromethyl group on the benzyl substituent at the 7-position. Dissociation constants as low as 0.03 μM were observed, with selectivity as high as several hundred fold. For example, compound **11** was 267 times more potent against LDH-A_4 than -B_4; compound **16** was 211 times more potent against LDH-C_4 than -B_4; compound **10** was 170 times more potent against LDH-A_4 and -C_4 than -B_4. Generally, LDH-A_4 and -C_4 were more sensitive than LDH-B_4 to inhibition by dihydroxynaphthoic acids, consistent with the results obtained with gossypol derivatives.

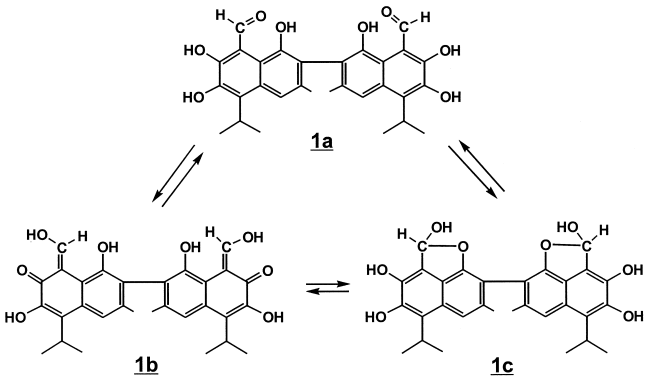

Fig. 4. Tautomeric structures of gossypol.

However, there are cases where there was significant selectivity between LDH-A_4 and -C_4, such as compound **17** which showed a 40-fold selectivity for LDH-A_4.

3.5. Inhibition of human LDHs by N-substituted oxamic acids

Oxamic acid, a structural analog of pyruvic acid, is known to inhibit LDH [29]. Therefore, a series of *N*-substituted oxamic acids was compared for inhibition of LDH-A_4, -B_4, and -C_4. Results are in Table 3. *N*-Substituted oxamic acids were not potent inhibitors of the three LDHs; dissociation constants generally were in the millimolar range. Inhibition was competitive with the binding of pyruvate, as shown in Fig. 5 for the inhibition of LDH-C_4 by *N*-3-phenylpropyloxamic acid (**25**). Selectivity was modest for the *N*-substituted oxamic acids. For example, **26** exhibited 3- to 5-fold selectivity for LDH-C_4.

Table 2
Inhibition of human LDH by substituted 2,3-dihydroxy-1-naphthoic acids

Structure No.	K_i (μM)		
	LDH-A_4[a]	LDH-B_4	LDH-C_4
5	3	91	10
6	2	78	3
7	0.2	7	0.2
8	13	81	4
9	3	> 125	2
10	0.2	34	0.2
11	0.03	8	0.5
12	1	8	0.5
13	34	> 250	40
14	4	190	3
15	0.5	39	3
16	0.1	19	0.09
17	0.05	1	2

[a] Data for compound 5–8, 13 and 14 for LDH-A_4 and B_4 are from Ref. [21].

Table 3
Inhibition of human lactate dehydrogenase by *N*-substituted oxamic acids

Structure No.	K_i (*mM*)		
	LDH-A_4	LDH-B_4	LDH-C_4
18	> 10	6	> 10
19	0.4	0.4	2
20	1	0.5	0.3
21	2	0.5	0.6
22	7	3	5
23	> 10	2	> 10
24	0.8	9	> 10
25	0.9	0.7	0.7
26	2	3	0.6
27	0.9	5	3

4. Discussion

The lack of selectivity of gossypol as an inhibitor of human LDH-A_4, -B_4, and -C_4 (Table 1) is in agreement with previous reports that gossypol effectively inhibits numerous dehydrogenases by competing with the dinucleotide cofactor [26]. Many of the inconsistencies in the literature concerning the patterns of inhibition may reflect complexities introduced by the ability of gossypol to inactivate some dehydrogenases. With human LDH, however, gossypol did not produce any inactivation. The general ability of gossypol to inhibit dehydrogenases, that is, its lack of selectivity, may contribute to the side-effects of gossypol *in vivo*. The lack of selectivity of gossypol contrasts with the selectivity observed with derivatives of gossypol and especially with substituted dihydroxynaphthoic acids, raising the question of the origin of this selectivity. Consistently, the compounds in this study were competitive inhibitors of the binding of NADH (Fig. 3).

It is not generally considered that the dinucleotide binding sites of dehydrogenases are attractive drug targets because the structures of the cofactor binding sites of many dehydrogenases, as determined by crystallography, are similar. The classic crystallography studies of Rossmann *et al.* [29] that identified the structural homology that defines the dinucleotide fold (Rossmann fold) included LDH. The dinucleotide fold of classic dehydrogenases can be identified by two $\beta\alpha\beta\alpha\beta$ units related by roughly a 2-fold axis of symmetry that includes a fingerprint region. Within this region there is a consensus sequence GXGXXG that defines a phosphate binding sequence; a conserved Asp or Glu residue in the second β strand; a conserved Arg or Lys residue in the first β strand; and six positions occupied by hydrophobic residues [30,31]. For human LDH-A_4, -B_4, and -C_4, the fingerprint regions (residues 22–52**,** Fig. 6) are almost identical. Nevertheless, gossypol derivatives and substituted dihydroxynaphthoic acids bound to the dinucleotide folds with selectivity (Tables 1 and 2), despite this structural and sequence homology. Competition fluorescence quenching studies of the binding of dihydroxynaphthoic acids to dehydrogenases suggest that these inhibitors primarily complex at the nicotinamide end of the dinucleotide fold [35]. Gossypol can be viewed as a prototype of inhibitors targeted to dinucleotide folds [22], and compounds structurally related to gossypol, such as substituted dihydroxynaphthoic acids, may hold promise as selective inhibitors of dehydrogenases.

The three human LDHs exhibit 84–89% sequence similarities and 69–75% amino acid identities. This high sequence homology extends to the substrate binding site as well as the cofactor binding site. Molecular modeling studies are in agreement with this conclusion that dihydroxynaphthoic acids bind to the nicotinamide end of the dinucleotide fold, but they also suggest that the binding of these inhibitors to dehydrogenase may extend into the substrate site [36]. The reduction of pyruvate by NADH, catalyzed by LDH, involves the ordered formation of the LDH–

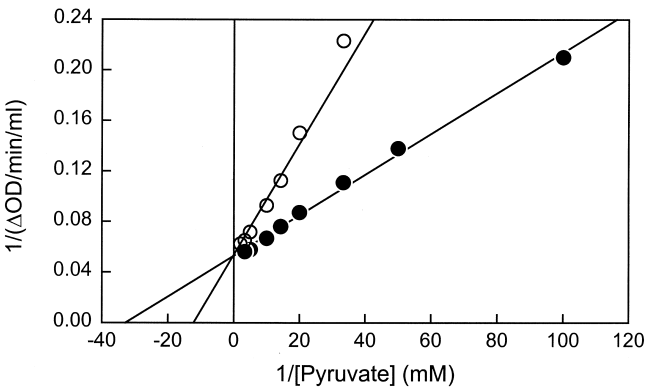

Fig. 5. Inhibition of human LDH-C_4 by *N*-3-phenylpropyloxamic acid (**25**) with respect to pyruvate. Key: (○) in the presence of inhibitor; and (●) in the absence of inhibitor.

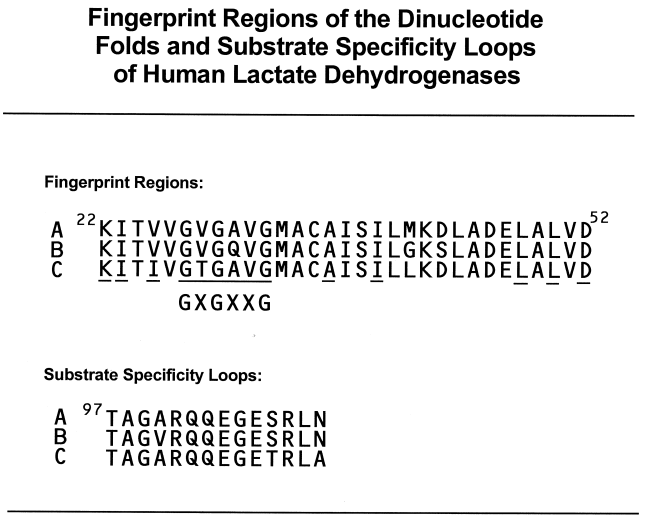

Fig. 6. Amino acid sequences of the fingerprint regions and substrate specificity loops of human LDH-A_4, -B_4, and -C_4 [32–34]. The consensus GXGXXG, D and K residues, and six conserved hydrophobic residues in the fingerprint region are underlined.

NADH binary complex and then the LDH–NADH–pyruvate ternary complex, followed by rate-determining closure of a substrate specificity loop to form a desolvated ternary complex in which hydride transfer occurs from the pro-R (H_A) C_4-hydrogen of the nicotinamide ring to the ketone functional group of pyruvate [30,31]. The D168/H195 proton donor dyad transfers a proton to the ketone functional group to facilitate hydride transfer, a process that is aided by polarization of the ketone by R109. In addition, R171 anchors the pyruvate through electrostatic interactions with the carboxylate group. D168, H195, R109, and R171 are considered to be the main catalytic residues and are conserved in all LDHs. In all mammalian LDHs, the nitrogen of the carboxamide group of the nicotinamide moiety of NADH is H-bonded to the oxygen of S163. In addition, I250 and T246 interact with bound NADH and are conserved in mammalian LDH [37]. The substrate specificity loops of the three human LDHs (Fig. 6) are nearly identical. From the sequences of the substrate specificity loops and the fingerprint regions, there are few obvious differences that might explain the selectivity of the inhibitors or explain the observation that LDH-A_4 and -C_4 exhibit patterns of inhibition that are quite similar compared with LDH-B_4. Clearly, substituted dihydroxynaphthoic acids are able to discriminate among subtle differences that differentiate the active sites of LDH-A_4, -B_4, and -C_4.

Although the antifertility activity of gossypol was suggested to involve selective inhibition of LDH-C_4 [12], this explanation has been questioned. Gossypol exhibits restricted rotation about the binaphthyl bond, resulting in the formation of isomers (atropisomerism). LDH-C_4 is inhibited by both isomers of gossypol but only the (-) isomer exhibits antifertility activity [38]. However, this observation by itself does not disprove a role for LDH-C_4 in the antispermatogenic activity of gossypol. The biological half-lives of the two isomers differ significantly [13]. The selectivity for the (-) isomer could be related to bio-availability, either reflecting different metabolic rates of elimination or reflecting differences between the two isomers in crossing the blood–testes barrier. Gossypol binds tightly to the high-affinity bilirubin binding site on serum albumin [39], with similar affinity for either isomer [40]. This high affinity of albumin for gossypol masks the effects of gossypol in many cellular studies [41], but it does not extend to the gossypol derivatives that are devoid of aldehyde functional groups [42]. Numerous gossypol derivatives have been examined for antifertility activity. The only ones with activity contain aldehyde groups, either as free groups or as Schiff base adducts. This lack of activity of derivatives of gossypol that are devoid of aldehyde functional groups also has been used to argue against LDH-C_4 as the target of gossypol. The selective toxicity of (-) gossypol against sperm development may be due to selective delivery of (-) gossypol across the blood–testes barrier by an unknown mechanism that involves the albumin–gossypol complex. Within the testes, LDH-C_4 may still be the target. It is noteworthy that treatment of isolated spermatids with albumin–gossypol complexes results in ATP depletion only by the albumin–(-) gossypol complex, consistent with the concept of selective uptake of (-) gossypol [43]. This study did not point to LDH-C_4 as the target of gossypol, however. Nevertheless, LDH-C_4 remains an attractive target for the development of antifertility drugs due to its unique location, regardless of the antispermatogenic mechanism of gossypol.

The observation that some dihydroxynaphthoic acids are much more potent inhibitors of LDH than is gossypol improves the prospect of developing potent and selective inhibitors of LDH-C_4 as potential antifertility drugs. The synthetic schemes that were used to synthesize compounds **5** to **17** (Fig. 1) are versatile and amenable to the synthesis of libraries of compounds for screening, further improving the prospect of developing selective inhibitors.

Availability of selective inhibitors of LDH-A_4 and -B_4 would provide useful experimental drugs for studies of the lactate–pyruvate shuttle in somatic cells. The studies of Brooks *et al.* [10–12] suggest that intracellular transport of lactate into mitochondria may be the normal mode of entry of glucose-derived carbon. The abundance of cytosolic LDH in many cells and the energetically favorable reduction of pyruvate to lactate indicate that the LDH reaction is likely near equilibrium at all times. This means that lactate accumulation does not necessarily indicate lack of oxygen. This equilibrium also suggests that fully oxygenated cells will still convert pyruvate to lactate in the cytosol and then transport lactate into mitochondria for conversion into pyruvate, bringing reducing equivalents into mitochondria. For this shuttle to function, there must be a major pool of LDH in mitochondria, which appears to be the case. There must also be transporters. At least nine putative mammalian H^+/monocarboxylate transporters (MCT) have been identified [44]. The best studied are MCT-1 and MCT-2 located in the plasma membrane. Less is known about MCT in mitochondria. However, MTC-1 has been shown recently to be in the mitochondria and sarcolemma of human skeletal muscle [12,45].

It has long been recognized that the lactic acidosis associated with hemorrhagic shock results more from lack of clearance than from increased lactate production [46]. Extracellular lactate also increases following ischemic stroke. Whether this is helpful or harmful is still unclear [23]. It also has been suggested that astrocytes supply lactate to neurons, both under normal conditions and especially during ischemia [47]. However, extracellular lactate can impair glucose utilization by glial cells [48]. Much remains to be learned concerning the inter- and intracellular movement of lactate under normal and pathological conditions, including the role(s) of the LDH isoenzymes. Therefore, the potential usefulness of selective inhibitors of somatic LDH as therapeutics remains unclear. Inhibition of somatic LDH may be undesirable. If this is the case, then inhibitors of LDH-C_4 as potential antifertility drugs must be highly selective for the sperm-specific isoenzyme. However, in view of the many

unanswered questions regarding lactic acidemia in various pathological states, the availability of potent and selective inhibitors of somatic LDH would offer useful experimental drugs. The compounds described in this study have not been studied extensively for their toxicities. It is noteworthy, however, that the gossypol derivatives in Table 1 are considerably less toxic than the parent compound gossypol to cells in culture, suggesting that these compounds are not highly toxic [19,49].

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