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DESIGN OF NOVEL AGENTS FOR THE THERAPY OF NON-INSULIN DEPENDENT DIABETES MELLITUS (NIDDM)

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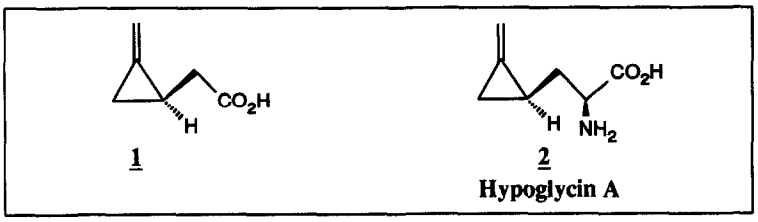
Abstract: Methylenecyclopropylacetate 1 - the hypoglycemic but toxic metabolite of hypoglycin A (2) - was chosen as a lead structure for the design of novel agents to treat NIDDM. The successful retargeting of 1 from short- and medium-chain acyl CoA β-dehydrogenase inhibition towards the selective blockade of long-chain β - dehydrogenase yielded the novel hypoglycemic compound $3d_1$, devoid of toxic aciduria associated with 1 itself.

NIDDM is characterized by abnormal insulin secretion from pancreas, increased basal glucose output by the liver and insulin resistance in peripheral tissue [1,2]. The strong genetic and environmental components and environmental risk factors include the hallmarks of a modern Western lifestyle: low physical activity and high calorie intake. While the incidence of NIDDM is ~8% in the US, an enormous public health problem may arise in developing countries; Zimmet estimates, that the "coca-colonization" [3] of China and India will create 50 million diabetics in these two nations alone by the year 2000. In spite of the significant effort directed towards the treatment of NIDDM [4], few therapeutic advances have been made in the last decade. Considering the predictions of Zimmet, the development of novel agents becomes an urgent task.

Our approach to reduce the increased basal glucose output in NIDDM is based on the observation, that rates of fat oxidation are increased in NIDDM [9] and the hypothesis, that elevated free fatty acids are the cause of increased endogenous glucose production and resultant hyperglycemia in NIDDM [10]. A rational treatment would therefore be to inhibit the abnormally high rate of fatty acid oxidation. Among the various routes of inhibiting fatty acid oxidation, the specific inhibition of long- or medium-chain acyl CoA β-dehydrogenase (LCADH or MCADH) appeared to be a reasonable approach. There are inherited disorders of each of these β-dehydrogenases; the mildest of these deficiencies involves the MCADH. Patients with this metabolic disease are prone to episodes of non-ketotic hypoglycemia, but apparently live without serious illness [20]. The design of compounds with specific inhibitory effects on LCADH or MCADH is facilitated be the large amount of work published around hypoglycin A (2) and its active metabolite 1.



1 2
Hypoglycin A

Hypoglycin A was isolated in 1954 [5] and is the causative agent of the Jamaican vomiting sickness [6].

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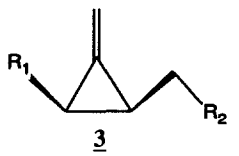
1 is an inhibitor of MCADH and short-chain acyl CoA β-dehydrogenase (SCADH), leading to severe hypoglycemia and depletion of liver glycogen and massive glutaric aciduria in humans [7]. The toxicity of hypoglycin is due to inhibition of SCADH, resulting in an accumulation of short-chain fatty acids. Investigations of the in vitro "suicide" inactivation of acyl CoA dehydrogenases by 1-CoA have shown that most or all of the inactivation involves covalent modification of the flavin adenin dinucleotide (FAD) cofactor of the dehydrogenase, but the mechanistic and structural aspects of the inactivation process still seem imperfectly understood [8].

Methylenecyclopropylacetate 1 appeared to be a useful lead-structure in the design of novel hypoglycemic agents for NIDDM. Our plan was to retarget 1 towards LCADH or MCADH, thereby generating compounds with inhibitory effects on β-oxidation and gluconeogenesis but lacking the toxicity associated with 1 itself. By adding side-chains of various length to 1, we predicted, that the length of the side-chain would specify the inhibitory action for LCADH, MCADH or SCADH. N-butyl, n-octyl and n-dodecyl groups were selected and attached to 1. The cis- and trans acids 3 and 4 [21] thus obtained were tested for their specificity and potential to inhibit gluconeogenesis.

Table I. Effect of methylenecyclopropylacetate analogs 3 and 4 on rat hepatocyte glucose production from 10mM lactate / 1mM pyruvate / 0.5mM oleate (all compounds except $3d_1$ and $3d_2$ are racemates)



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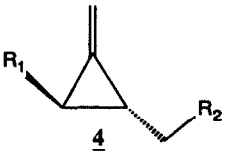

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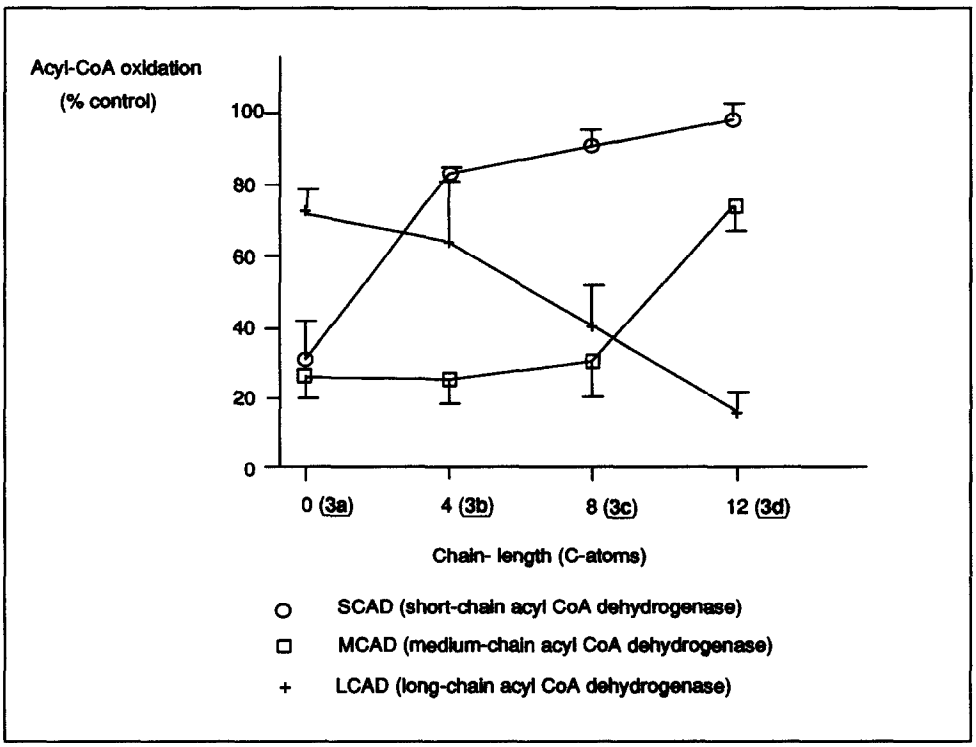
No	R_1	R_2	IC_{50} (uM)	R_1	R_2	No
3a	H	CO_2Na	60			
3a'	H	$CONH_2$	30			
3b	nC_4H_9	CO_2Na	15			
			30	nC_4H_9	CO_2Na	4b
3b'	nC_4H_9	$CONH_2$	4			
			60	nC_4H_9	$CONH_2$	4b'
3c	nC_8H_{17}	CO_2Na	5			
3c'	nC_8H_{17}	$CONH_2$	8			
3d	$nC_{12}H_{25}$	CO_2Na	25			
			200	$nC_{12}H_{25}$	CO_2Na	4d
3d'	$nC_{12}H_{25}$	$CONH_2$	32			
$3d_1$	R,R-enantiomer of 3d		13			
$3d_2$	S,S-enantiomer of 3d		160			

Table I demonstrates the effects of 3 and 4 on rat hepatocyte glucose production [11]. Figure 1 shows, how the chain-length of the more active cis isomers 3 directs their specificity.

Figure 1. Effect of analogues 3a, 3b, 3c and 3d (30uM) on acyl-CoA dehydrogenase activities of rat liver mitochondria [13]



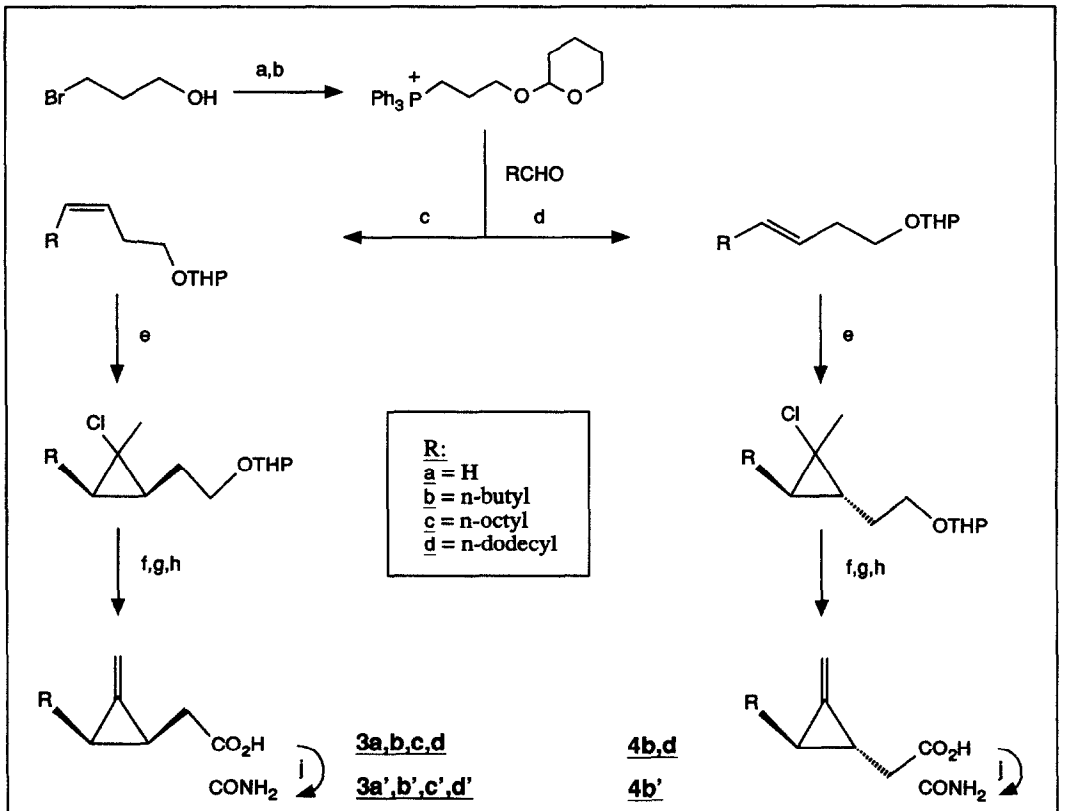
The following conclusions can be drawn from these studies: (i) all derivatives of 3 and 4 - at sufficiently high concentrations - can totally inhibit gluconeogenesis. (ii) cis acids and amids 3 are more active than the corresponding trans compounds 4. (iii) butyl und octyl acids 3b and 3c are more active than the unsubstituted 3a (= racemic 1) and the dodecyl compound 3d. (iv) specificity of all presented analogs is directed by the length of their side chain. 3d - due to its dodecyl chain - proved to be a specific inhibitor of LCADH with an IC_{50}= 1µM. 3d had very low activity against SCADH and MCADH at concentrations up to 200µM. The R,R-enantiomer $3d_1$ (its R-configuration corresponds to the ring-configuration of hypoglycin A and its metabolite 1) was considerably more active than its S,S-isomer $3d_2$ as an inhibitor of gluconeogenesis (Table I) and was therefore selected for in vivo studies. 40µmol/kg $3d_1$ given orally to 18-hour fasted rats reduced serum glucose levels to 65% of control. $3d_2$ was inactive. As expected from hypoglycin-toxicity, urine from rats treated with 200µmol/kg 3a (= racemic 1) showed massive increases in

short- and medium-chain mono- and dicarboxylic acids, while in the same experiment, 3d, with the same dose, produced almost no increase in organic acids relative to untreated controls.

In summary, our prediction was correct, that the length of the side chain will determine the specificity of the analogs. The useful profile of $3d_1$ qualifies this compound as a potential novel drug for NIDDM.

The preparation of racemic cis- and trans-methylencyclopropyl acetates 3 and 4 is described in Scheme 1, starting with the selective cis- and trans-olefination of pentanal, nonanal and tridecanal. Cyclopropanation

Scheme 1: Synthesis of methylenecyclopropylacetates 3 and 4

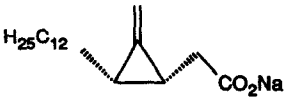


Reaction conditions:
a: DHP, Et_2O, pTsOH, 5hrs. b: acetonitrile, K_2CO_3,PPh_3,rfl.12hrs. c: tBuOK,DMF,r.t. 14hrs. d: 2 PhLi.LiBr,THF/Et_2O, -70 to -30 C. e: 1,1-dichloroethane,n-BuLi,Et_2O, -30 to -35 C. f: tBuOK,DMSO,70 C, 3.5hrs. g: MeOH, pTsOH,r.t. 14hrs. h: Jones oxidation, -20 to -5 C 6hrs. j: 1) DCC, Hydroxysuccinimide, EtOAc, r.t. 12hrs. 2) THF, NH_3-gas, 45 min. r.t.

with 1,1-dichloroethane/n-BuLi followed by dehydrochlorination with tBuOK gave the desired methylenecyclopropanes [18]. Removal of the THP-protecting group and oxidation of the primary alcohol delivered the target acids 3 and 4 in good yield. The separation of 3d into its enantiomers $3d_1$ and $3d_2$ was

achieved via chromatography of its imide with (+)-10,2-Camphorsultam followed by hydrolysis with LiOH. X-Ray analysis of the Camphorsultam-derivatives allowed the assignement of their absolute configurations. An enantioselective synthesis of $\underline{3d_1}$ has been published recently [19].

$\underline{3d_1}$



$\underline{3d_2}$

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[11] Hepatocytes were isolated from the livers of 18-hour fasted Sprague-Dawley rats (weighing 280-290 g) according to the procedure of Berry and Friend [12]. Hepatocyte glucose production from 10mM lactate/1mM pyruvate/0.5mM oleate was measured with a spectroscopic (A_{505}) automated glucose oxidase method.

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[13] Mitochondria were isolated [14] from hepatocytes and incubated, in a manner similar to [15] with the test compounds for 15 min at 37 C. The samples were then frozen and stored at -80 C. Acyl CoA dehydrogenases were assayed spectrophotometrically by a modification of the PMS-DCPIP dye reduction procedure [16]. β-dehydrogenase isozymes were determined by the activity supported by different chain

length substrates (fatty acyl CoAs). Activity supported by butyryl CoA, octanoyl CoA and oleoyl CoA represented SCADH, MCADH and LCADH, respectively.

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