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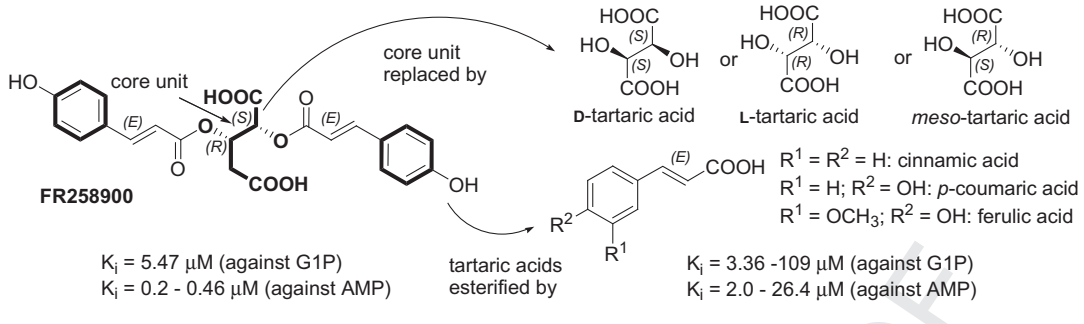
Synthesis of tartaric acid analogues of FR258900 and their evaluation as glycogen phosphorylase inhibitors **pp xxx–xxx**

Gergely Varga, Tibor Docsa, Pál Gergely, László Juhász *, László Somsák *



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Synthesis of tartaric acid analogues of FR258900 and their evaluation as glycogen phosphorylase inhibitors

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ABSTRACT

Di-*O*-cinnamoylated, -*p*-coumaroylated, and -feruloylated D-, L- and *meso*-tartaric acids were synthesized as analogues of the natural product FR258900, a glycogen phosphorylase (GP) inhibitor with in vivo anti-hyperglycaemic activity. The new compounds inhibited rabbit muscle GP in the low micromolar range, and bound to the allosteric site of the enzyme. The best inhibitor was 2,3-di-*O*-feruloyl *meso*-tartaric acid and had K_i values of 2.0 μM against AMP (competitive) and 3.36 μM against glucose-1-phosphate (non-competitive).

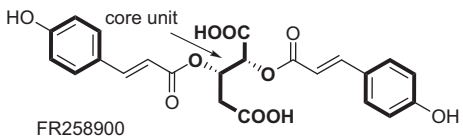
The number of patients suffering from diabetes mellitus (DM) is dramatically increasing. In 2011 the international diabetes federation project (IDF) indicated that the number of diabetic patients was more than 360 million worldwide.[1] In 2001 this number was predicted to be reached in 2030 only.[2] More than 90% of the diagnosed cases belong to type 2 or non-insulin dependent diabetes mellitus (T2DM or NIDDM) characterized by peripheral insulin resistance, elevated hepatic glucose production, and defects in pancreatic insulin secretion.[3] Although several drugs are in clinical use for symptomatic treatment of T2DM,[4,5] these therapies are inadequate for 30–40% of the patients.[6]

Among several investigational fields to diminish hepatic glucose output in T2DM, glycogen phosphorylase (GP) as a main regulatory enzyme of glycogen metabolism has become a validated target.[2]

Protein crystallographic studies have shown that endogenous and synthetic modulators can bind to six major sites in GP: the catalytic, the inhibitor, the allosteric (or AMP-binding), the glycogen storage, and the new allosteric sites,[7] as well as the newly discovered benzimidazole site.[8] A number of GP inhibitors for the different binding sites have been disclosed and several of them have considerable in vivo effects towards normalizing blood glucose and liver glycogen levels.[9,10]

In recent years, the interaction of GP and glycogen targeting subunit (G_L) of protein phosphatase 1 (PP1) has been identified as a novel molecular target for the treatment of T2DM.[11–13] It was demonstrated in an in vivo mouse model that disruption of G_L–GP interaction resulted in an increased glycogen synthase activity and the mice had improved glucose tolerance.[14] X-ray crystallography showed that the G_L–GP interaction took place by binding the C-terminal region of G_L to the allosteric site of GP.[15] Thus, occupation of this site may prevent G_L–GP interaction, thereby enhancing glycogen synthesis which, on the other hand, diminishes hepatic glucose production. This effect could be achieved by modulators that bind to the allosteric site of GP.

Several molecules of high structural diversity were reported to bind to the allosteric site of GP, such as derivatives of acyl urea, dihydropyridine dicarboxylic acid, pentanedioic acid, phthalic acid, *N*,*N*′-diaryl-urea, and pentacyclic triterpenoids.[9,16]



FR258900

1

IC_{50} = 2.5 μM (human liver GP)[17]
K_i = 0.46 μM (rabbit muscle GP)[18]

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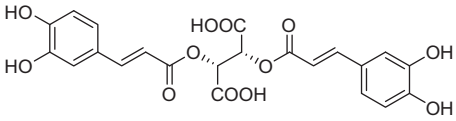
FR258900, a bis-*O*-(*p*-coumaroylated)2,3-dihydroxypentanedioic acid derivative **1**, was isolated from the fermentation broth of fungi No. 138354.[17] The compound was shown to inhibit glycogen phosphorylases and to bind to the allosteric site of GP.[18] Compound **1** stimulated glycogen synthesis in primary rat hepatocytes, and investigations on glucagon-induced hyperglycemia in C57BL/6 mice suggested that **1** could suppress hepatic glucose output in vivo.[19]

On the basis of the above information and as a continuation of our research on the design and synthesis of small molecule inhibitors of GP we envisaged the preparation of structural analogues of **1**. Since the synthesis of 2,3-dihydroxy-pentanedioic acid is unknown in the literature, the core unit was planned to be replaced by easily available tartaric acid, also allowing to study the influence of configurational isomerism on the biological activity. Furthermore, the substitution pattern of the aromatic rings was also modified.

A similar derivative to our target compounds is chicoric acid[20] (**2**) with immunostimulator and HIV-1 integrase inhibitor activities.[21]

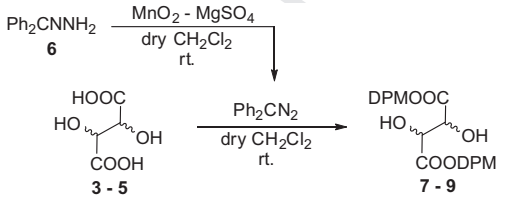


2

Crucial points for both the syntheses of chicoric acid[20–23] and the preparation of our target compounds are the protections of phenolic OH and COOH groups. Acylation of unprotected tartaric acids with carbonylcaffeoyl chloride is feasible, but this carbonate type protection can be used only for caffeic acid.[20] Benzylic protection of both functionalities seemed very attractive, however, for the removal an equimolar amount of $Pd(OAc)_2$ for each protective group was necessary rendering this method extremely expensive.[23] Orthogonal ester type protection (acetyl for OH and *tert*-butyl for COOH[22] or methoxycarbonyl for OH and diphenylmethyl for COOH[21]) were also applied in the syntheses of **2** and their analogues. Our comparative preliminary experiments showed that introduction of the latter pair of protecting groups was more efficient and easier to reproduce than that of the benzylic protection.

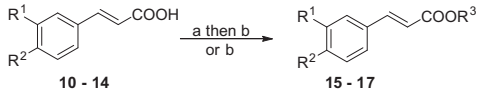
Thus, the COOH groups of D-, L- and *meso*-tartaric acids (**3–5**) were transformed into diphenylmethyl (DPM) esters (**7–9**) by diphenyldiazomethane (DPDAM) generated in situ from benzophenone-hydrazone (**6**) by oxidation with activated MnO_2 in CH_2Cl_2[24] (Scheme 1). DPM esters **7–9** were isolated in excellent yields as white crystals and used further without any purification.

Next, acid-chlorides **15–17** were prepared from commercially available cinnamic (**10**), *p*-coumaric (**11**), and ferulic acids (**12**), respectively (Scheme 2), whereby phenolic OH groups of **11** and **12** were protected as methyl-carbonates **13** and **14**, respectively.



Starting compound	Configuration	Product	Yield (%)
3	D or (2*S*,3*S*)	**7**	85
4	L or (2*R*,3*R*)	**8**	83
5	*meso* or (2*R*,3*S*)	**9**	82

Scheme 1. Preparation of DPM esters **7–9**.



a: $ClCOOCH_3$ in 50% aq. NaOH at 0 °C; b: $SOCl_2$, reflux, 6 h.

	R^1	R^2	R^3	Yield (%)	Abbreviation
10	H	H			Cinn-OH
11	H	OH			Coum-OH
12	OCH_3	OH			Feru-OH
13	H	$OCOOCH_3$	OH	84	4-MC-Coum-OH
14	OCH_3	$OCOOCH_3$	OH	88	4-MC-Feru-OH
15	H	H	Cl	100*	4-MC-Cinn-Cl
16	H	$OCOOCH_3$	Cl	100*	4-MC-Coum-Cl
17	OCH_3	$OCOOCH_3$	Cl	100*	4-MC-Feru-Cl

* Conversion of the starting material.

Scheme 2. Preparation of acid chlorides **15–17**.

Thionyl-chloride treatment of carboxylic acids **10**, **13**, and **14** gave acid-chlorides **15–17** which were used for acylations without further purification.

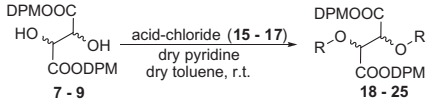
Acylations of **7–9** were carried out in dry toluene using 2.2 equiv of acid-chlorides **15–17** and 2.2 equiv dry pyridine as base (Scheme 3). The fully protected **18–25** were isolated by column chromatography in acceptable yields.

Subsequent deprotections following the suggested protocol[21] (removal of methoxycarbonyl groups with Na_2CO_3/aq THF and cleavage of DPM esters with 70% aq AcOH) caused in our hands total decomposition of the molecules, irrespective of the order of the deprotection steps.

Therefore, a new protocol for the cleavage of protecting groups in **18–25** was developed. The DPM esters could be cleaved by using dry anisole–TFA reagent in dry CH_2Cl_2 at room temperature.[25] Purification of the crude products by column chromatography ($PhCH_3$:AcOH 3:1) gave **26–33** in good to excellent yields (Scheme 4). Hydrolysis of the methoxycarbonyl esters was achieved by using an aq solution of NH_3 in MeOH and the products **34–39** were isolated in good to excellent yields. Reversing of the sequence resulted in decomposition of the molecules in the first step. The structure of the molecules was identified by NMR and MS measurements.

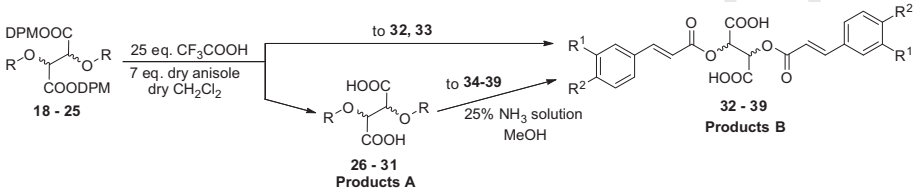
The synthesized derivatives were evaluated as inhibitors of rabbit muscle glycogen phosphorylase b (rmGPb), and the results are summarized in Table 1. For comparison FR258900 (**1**) was also tested under our conditions. Compound **1** proved a competitive inhibitor against AMP and the obtained K_i of 0.2 μM showed a good agreement with the literature value. When tested against G1P, **1** appeared as a non-competitive inhibitor with a K_i of 5.47 μM (please, see Fig. 1 in the Supplementary data for details of the measurements and plots of the data). Similar conclusions could be drawn from the kinetic studies of compounds **32–39**, as well, thereby indicating that in general the tartaric acid derivatives bound to the same site as FR258900 (**1**).

As non-competitive inhibitors against G1P, the cinnamoyl derivatives **32** and **33**, lacking the 4-OH substituents characteristic of the natural product **1**, proved practically inefficient. In the *p*-coumaroyl (**34–36**) and feruloyl (**37–39**) series the *meso*-configured compounds **36** and **39** proved most efficient. The latter demonstrated that introduction of an additional substituent in the



Starting compound	R	Product (Configuration)	Yield (%)
7	Cinn	**18** (D)	63
8		**19** (L)	52
7	4-MC-Coum	**20** (D)	54
8		**21** (L)	48
9		**22** (*meso*)	83
7	4-MC-Feru	**23** (D)	48
8		**24** (L)	50
9		**25** (*meso*)	43

Scheme 3. Acylations with acid chlorides **15**–**17**.



R	Starting compound (Configuration)	**Products A**	Yield (%)	**Products B**	R^1	R^2	Yield (%)
Cinn	**18** (D)	-	-	**32**	H	H	83
	19 (L)	-	-	**33**	H	H	86
4-MC-Coum	**20** (D)	**26**	88	**34**	H	OH	85
	21 (L)	**27**	82	**35**	H	OH	89
	22 (*meso*)	**28**	82	**36**	H	OH	30
4-MC-Feru	**23** (D)	**29**	85	**37**	CH_3O	OH	81
	24 (L)	**30**	79	**38**	CH_3O	OH	69
	25 (*meso*)	**31**	92	**39**	CH_3O	OH	88

Scheme 4. Cleavage of the protective groups.

Table 1
Inhibition (K_i [μM]) of rabbit muscle glycogen phosphorylase b by the synthetic compounds

Compound (configuration)	G1P dependence	AMP dependence
1 (L[a])	5.47	0.20 0.46[18]
32 (D)	800[b]	–
33 (L)	No inh.	–
34 (D)	109	26.4
35 (L)	300[b]	–
36 (*meso*)	71.4	5.68
37 (D)	29.1	19.0
38 (L)	28.5	2.68
39 (*meso*)	3.36	2.0

[a] Configuration of the natural product corresponds to that of L-tartaric acid.
[b] Calculated from the IC_{50} value by using a web-based tool.[26]

aromatic rings (3-CH_3O) was very advantageous and **39** proved equipotent with **1**.

As competitive inhibitors against AMP, beside the *meso*-configured **36** and **39**, the L-configured **38** proved most efficient. Nevertheless, the efficiency of the inhibitors lagged behind that of **1**. The presence of the 3-CH_3O moieties in **39** rendered this compound the best inhibitor under these test conditions, as well.

In order to determine the molecular basis of the efficiency of these synthetic compounds, preparation of further analogues as well as molecular dockings and X-ray crystallographic studies are in progress, and will be reported in due course.

In conclusion, three series of D-, L- and *meso*-tartaric acids di-*O*-acylated by cinnamic, *p*-coumaric, and ferulic acids were prepared as synthetic analogues of FR258900, a natural product with glycogen phosphorylase inhibitory and in vivo antihyperglycemic activities. The syntheses were characterized by using methoxycarbonyl (MC) and diphenylmethyl (DPM) esters to protect phenolic OH and COOH groups, respectively. New methods were applied for the removal of these protective groups (anisole–TFA for the cleavage of DPM, aq NH_3–MeOH to hydrolyse MC). Some of the new compounds proved inhibitors of rabbit muscle glycogen phosphorylase b (competitive inhibition against AMP, non-competitive against G1P) in the low micromolar range. It was demonstrated by this work that simple synthetic compounds can bind to rmGPb similarly to the natural product, thereby opening up a new way for further studies of allosteric site inhibitors. The synthetic availability of these analogs offers obvious advantages over FR258900.

Acknowledgments

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Supplementary data

Supplementary data (kinetics of FR258900 inhibition of rmGPb) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.01.042.

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