Bioorganic & Medicinal Chemistry Letters 18 (2008) 4736–4740

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and antiviral properties of some polyphenols related to Salvia genus

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ARTICLE INFO

Article history:
Received 23 May 2008
Revised 17 June 2008
Accepted 18 June 2008
Available online 21 June 2008

Keywords:
Natural polyphenols
Salvianolic acids
Boron tribromide
Integrase inhibitors
Antivirals

ABSTRACT

An efficient synthesis of the acid part of salvianolic acid E **2** is described. Compound **2** was obtained from vanillin in 10 steps and 21% overall yield. During the synthesis of **2** an unexpected 5-oxo-4*b*,9*b*-dihydro-indano[1,2-*b*]benzofuran **rac-12** was isolated. Both compounds together with the acid part of salvianolic acid D were active as HIV-1 integrase inhibitors at the submicromolar level. But they did not inhibit the replication of the virus on MT-4 cells.

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Currently, there are three distinct mechanistic classes of antiretrovirals: inhibitors of the HIV-1 reverse transcriptase and protease enzymes and inhibitors of HIV entry, including receptor and co-receptor binding and cell fusion. The integration of viral cDNA into the host genome is an essential step in the HIV-1 life cycle and is mediated by the virally encoded enzyme, integrase (IN). IN is an attractive drug target because it is essential for a stable and productive HIV-1 infection, and there is no mammalian homologue of IN. Inhibitors of integrase enzyme (INI) block the integration of viral double-stranded DNA into the host cell's chromosomal DNA. HIV-1 integration has several potential steps that can be inhibited, and many new compounds that target specific integration steps have been identified. A great number of HIV-1 integrase inhibitors have been described in the last decade, and numerous reviews have been published.[1–7] Two INIs, GS-9137 (Elvitegravir)[8] and MK-0518 (Raltegravir),[9,10] demonstrated promising early clinical trial results and have been advanced into later stage trials, the latter being the first US FDA-approved drug targeting IN under the brand name Isentress®. Despite the enormous progresses made in this new class of antiretrovirals, we need to find new IN inhibitors to overcome the resistance to INIs.[11] Today, many research groups are exploring the biodiversity of the plant kingdom to find new and better anti-IN drugs with novel mechanisms of action. Natural caffeic acid derivatives which present anti-HIV-1 activities were recently reviewed.[12] Rosmarinic and lithospermic acids for example (Fig. 1) may serve as lead structures for the development of new anti-HIV agents from natural source.[13–15]

For a long time, our laboratory has been implicated in the synthesis of natural polyphenols and the design of new HIV-1 integrase inhibitors.[16–19] We focused our attention on the caffeic moiety of salvianolic acids since the ester function is expected to be easily cleaved by esterases. Herein we present the total synthesis of the acid part of salvianolic acid E, the anti-integrase activities and the antiviral properties of the acid part of salvianolic acid D, **1**[20] and E, **2** (Fig. 1) and of a side-product **rac-12** (Scheme 2) obtained during the synthesis of compound **2**.

The entry into the backbone of **7** was first found in the aldol condensation of 3,4-dimethoxybenzaldehyde and benzopyranone **4**[21] (Scheme 1). This aldol condensation performed in piperidine/pyridine mixture gave **5**[22] in 49% yield as a mixture of E and Z isomers. Opening of the lactone ring gave **6** as the E isomer exclusively. Unfortunately, oxidation using the Swern reaction did not allow us to isolate **7** in a satisfactory yield (only 15%) in contradiction with a previous report,[22] and then we turned to a more convenient route. The designed common precursor **7** was more efficiently obtained starting from the intermediate **8**, previously described in the preparation of the acid part of salvianolic acid D.[20] Compound **8** was converted into the acetal **9** with trimethyl orthoformate according to Detterbeck et al.[23] procedure in 91% yield (Scheme 1). For the aldol-type reaction of **9** with veratraldehyde, we found of the greatest importance to carry out the whole reaction, including the quenching process at low temperature (−78 °C). Deprotonation of **9** was best done by a freshly prepared

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doi:10.1016/j.bmcl.2008.06.063

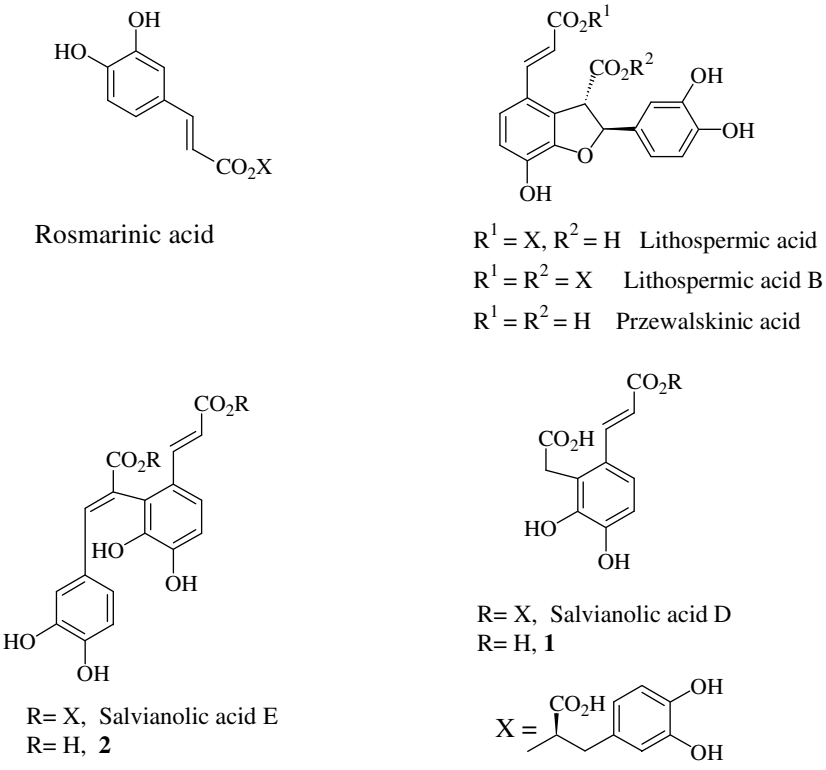
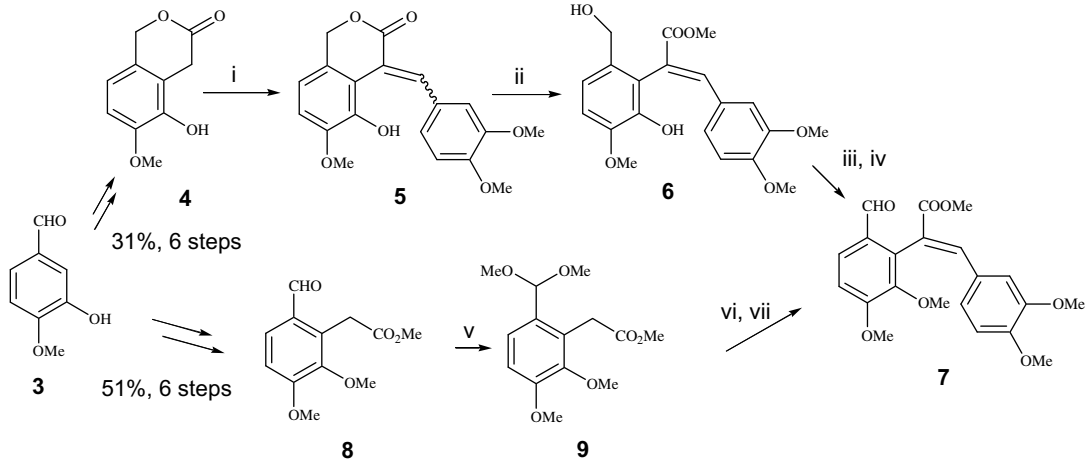


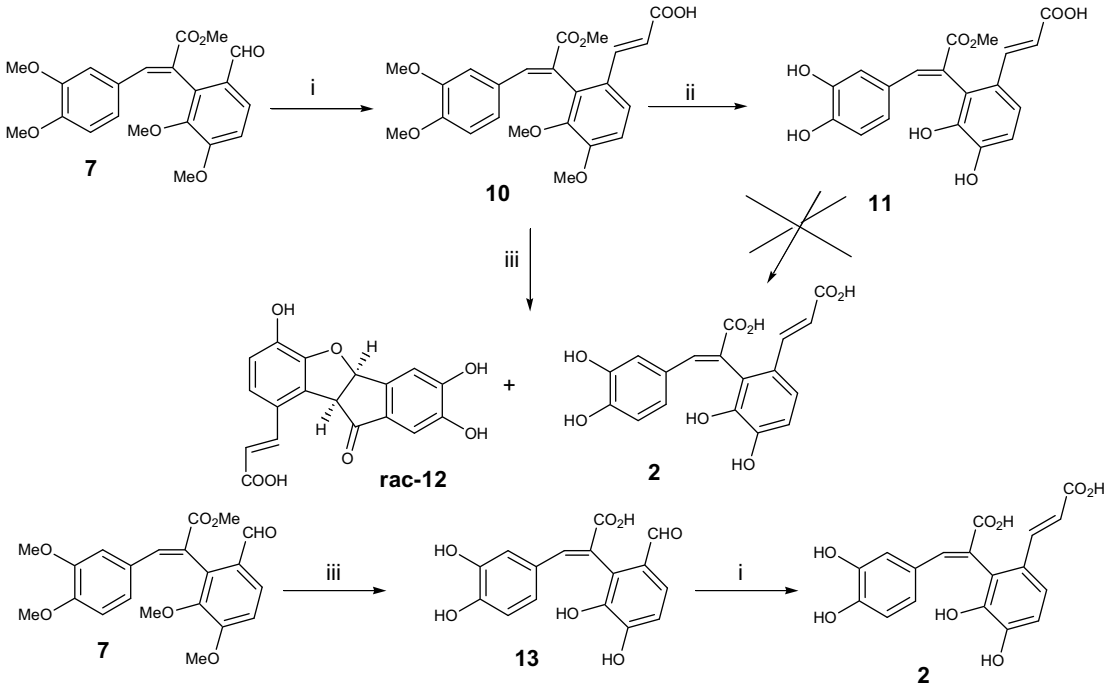
Figure 1. Structures of some caffeic acid natural derivatives. Rosmarinic acid, lithospermic acids, przewalskinic acid and salvianolic acids.



Scheme 1. Reagents and conditions: (i) 3,4-dimethoxybenzaldehyde, pyridine, piperidine, 49%; (ii) MeONa, MeOH, reflux, 91%; (iii) $(COCl)_2$, DMSO, 15%; (iv) K_2CO_3, MeI, 95%; (v) $HC(OMe)_3$, MeOH, NH_4Cl, reflux, 2 h, 91%; (vi) 3,4-dimethoxybenzaldehyde, LDA, THF, −78 °C, 1 h; (vii) 3 N HCl, reflux, 12 h, 63%.

solution of lithium diisopropylamide (LDA) in dry THF followed by the addition of 3,4-dimethoxybenzaldehyde. Classical work up under acidic conditions afforded the product **7** in very low yield after purification. Conversely, when the reaction mixture was refluxed for 12 h under acidic conditions (3 N HCl), **7** was easily obtained in good yield after column chromatography purification (63%). This procedure afforded a substantial improvement in terms of yield and simplification of the process versus the reaction described by Detterbeck et al.[23]

The construction of the α,β-unsaturated carboxylic acid chain, via a Knoevenagel condensation, was easily performed to give **10** in 90% yield (Scheme 2). Our standard conditions of deprotection (1 h reaction time, room temperature, 7 equivalents of BBr_3, quenching by water at room temperature and 1 h stirring) were first applied. Under these conditions, the crude ester **11** polluted by the target molecule was obtained in 60% yield. Attempts to isolate compound **2** by saponification of this crude product failed. At this stage, no intramolecular cyclization leading to przewalskinic acid was observed. In order to improve the yield in **2** or to obtain przewalskinic acid, reaction time in presence of boron tribromide was prolonged to 12 h at room temperature. A new major product **rac-12** (75%) was isolated with **2** (24%). From the ^1H and ^{13}C NMR data, the structure of **rac-12** was proposed and its formula was confirmed by mass spectrometry. In the ^1H NMR spectrum, the classical 1,2,4-trisubstituted set of splitting due to the aromatic ring was replaced by two singlets characteristic of a 1,2,4,5-tetrasubstituted aromatic ring whilst two doublets appeared at 4.72 ppm and 6.21 ppm indicating the formation of a dihydroben-



Scheme 2. Reagents and conditions: (i) $CH_2(COOH)_2$, pyridine, piperidine, reflux, 7 → 10, 90%, 13 → 2, 95%; (ii) BBr_3, 7 equiv, CH_2Cl_2, rt, 1 h then water, rt, 1 h, 60%; (iii) BBr_3, 7 equiv, CH_2Cl_2, rt, 12 h then water, rt, 1 h, 10 → rac-12 (75%) + 2 (24%), 7 → 13, 75%.

zofuran ring. The presence of a carbonyl function was confirmed by its ^{13}C chemical shift at 199.3 ppm. These data are in accordance with a 5-oxo-4*b*,9*b*-dihydroindano[1,2-*b*]benzofuran, a new tetracyclic system.[24] According to the literature, the ^3J value suggests that the stereochemistry of the junction bond is expected to be cis.[25,26]

Attempts to limit the cyclization process to the furan ring by modifying reaction time (from 1 h to 15 h) and quenching process (pouring the reaction mixture into cold water or NaH_2PO_4 saturated solution) failed, leading to variable mixtures of **2** and **rac-12**. Whatever the quenching conditions, **rac-12** was always obtained in the same amount suggesting that the Friedel–Crafts cyclization occurred during the reaction process with BBr_3. Since Detterbeck et al. reported that aldehyde **7** cyclized when treated by BBr_3, the same reaction conditions (12 h, room temperature) were used. Surprisingly, the aldehyde **7** did not cyclize and led to **13** in 75% yield. Knoevenagel reaction of **13** gave **2** in 95% yield. This alternative pathway furnished a substantial yield improvement (**7**–**2**: 21% yield via **10** and 71% yield via **13**). Attempts to promote the cyclization of **11** or **13** using HBr[22] also failed. Compound **13** was almost quantitatively recovered, whereas **11** was totally degraded.

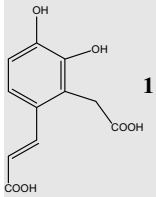
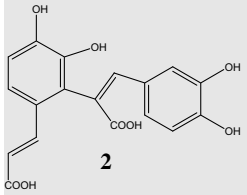
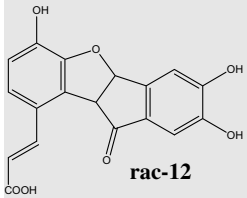
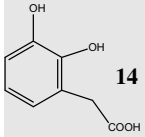
1, **2**, **rac-12** and 2,3-dihydroxyphenylacetic acid **14**[27] were tested in IN inhibition assays which have been recently reviewed[28,29] and data are summarized in Table 1. The overall IN enzymatic activity has been evaluated in two independent laboratories using two different protocols (see Supporting Information) with two different preparations of the enzyme and magnesium ions as co-factor (protocol 1, Table 1, column 4 and protocol 2, Table 1, column 6). The strand transfer reaction inhibition was evaluated using protocol 1 (Table 1, column 5). The overall HIV-1 IN inhibition assays show that higher IC_{50} values were obtained with protocol 1 when compared to protocol 2. Similar discrepancies were already observed for rosmarinic acid derivatives.[30] But the same general hierarchy between **1**, **2** and **rac-12** was observed in both assays.

1, **2** and **rac-12** were found to be very active against wild-type IN in the presence of Mg^{2+}. Caffeic acid had been previously evaluated as IN inhibitor in the presence of Mn^{2+}, and presented IC_{50} of 2.8 and 24 μM on the overall reactions and on strand transfer, respectively.[31] The difference between the two assays indicated that caffeic acid is more likely to be active at either the viral DNA binding or 3′ processing steps. The correlation observed between relative drug potency in the presence of Mg^{2+} and relative potency in the wild-type enzyme suggests that assays performed using wild-type enzyme and Mg^{2+} represent the most stringent conditions with respect to drug inhibition.[32] Since the caffeic acid assays were conducted using Mn^{2+}, caffeic acid would be expected to show lower activity using Mg^{2+} and greater IC_{50} values. Our results showed that **1** has higher anti-IN activities than **14** and caffeic acid. The presence of the two acidic functions has a synergistic effect on the anti-IN activity of **1** and **2**, and a second catechol function slightly improves the anti-IN activities (**2** and **rac-12**) comparable to those of lithospermic acids.[13] The best results were obtained with the unexpectedly obtained rigid and folded structure of **rac-12** and strand transfer inhibition assays also indicate that these compounds are active inhibitors at that point in the viral replication cycle.

The antiviral activity of our compounds on HIV-1-induced CPE in human lymphocyte MT-4 cell culture was determined by the MT-4/MTT-assay[33] and cytotoxicity was also evaluated in MT-4 cells (Table 1). The three tested molecules were found to be toxic in cell cultures, and it was not possible to determine their antiviral activities.

Numerous polyhydroxylated aromatic compounds had been previously identified as HIV-1 IN. The main disadvantages of these compounds are the lack of significant activity against replicating virus, cell toxicity, lack of selectivity or a combination of these factors. But few have met the criterion for "lead" compounds. This was the case for L-chicoric acid, lithospermic acid, lithospermic acid B and 3,5-dicaffeoyl quinic acid.[6,12] Herein the high cell toxicity displayed by our compounds renders them unsuitable as poten-

Table 1
Inhibition of HIV-1 IN catalytic activities, antiviral activity, and cytotoxicity of test compounds

Compound	MT-4 cells		HIV-1 integrase inhibition		
	EC_{50}[a] (μM)	CC_{50}[b] (μM)	Overall IC_{50}[c] (μM)	ST IC_{50}[c] (μM)	overall IC_{50}[d] (μM)
 **1**	>44 >77.9	44 77.9	1.34	1.09	0.32
 **2**	>6.4 >4.5	6.4 4.5	0.56	0.73	0.22
 **rac-12**	>23.3 >27.4	23.3 27.4	0.15	0.30	0.05
 **14**	NT[e]	NT[e]	NT[e]	NT[e]	>100

[a] Effective concentration required to reduce HIV-1-induced cytopathic effect by 50% in MT-4 cells.
[b] Cytotoxic concentration to reduce MT-4 cell viability by 50%.
[c] Concentration required to inhibit by 50% the in vitro integrase activity using protocol 1.
[d] Concentration required to inhibit by 50% the in vitro integrase activity using protocol 2.
[e] Non-tested.

tial therapeutic drugs. This shortcoming may be overcome by introducing a tartaric acid moiety on the caffeoyl carboxylic acids of our compounds. Indeed L-chicoric acid (L-CA) represents to date the most potent and selective anti-HIV agent amongst the family of polyhydroxylated aromatic compounds. L-CA is a dicaffeoyl derivative in which two caffeoyl moieties are bridged by a tartaric acid. It is active against HIV-1 IN at submicromolar range and against HIV-1 replication at micromolar range.[34,35] The results concerning its cellular toxicity are controversial. Whereas Robinson et al. revealed the lack of cell toxicity for L-CA (LD_{50} = 700 μM),[35] further studies were in favour of selectivity indexes around 10.[34,36–38] Based upon its structure, several dicaffeoyltartaric acid analogues were elaborated and inhibited HIV-1 IN and HIV-1 replication at non-toxic concentrations.[34] So the bridging of two salvianolic acid fragments by a tartaric acid may lead to compounds with increased anti-HIV activity. These derivatives would of course not be devoid of cell toxicity since the potential toxicity is generally associated with the presence of catechol groups. But the latter ones are necessary for HIV-1 IN inhibitory and anti-HIV properties, which were in most cases correlated to the presence of at least one catechol group. However these new compounds are expected to present largely more favourable selectivity indexes than their parent ones. An alternative solution may consist in a same manner in bridging two molecules by a quinic acid since 3,5-dicaffeoylquinic acid was found to be very active against HIV-1 IN catalyzed reactions (IC_{50} = 640 nM) and HIV replication (ED_{50} = 1.9 μM; LD_{50} = 300 μM) with a favourable selectivity index.[35]

In summary, the substituted caffeic acid moieties of natural salvianolic acids D and E revealed very strong activities against IN, amongst the best reported in this family of IN inhibitors. But these compounds, like many other polyhydroxylated ones, were devoid of any anti-HIV activity due to a high cell toxicity. Their structures need to be modified as previously described in order to improve their antiviral activities and to lower their cytotoxicities. The dihydrobenzofuran ring of **rac-12** could represent a potential new hit in the field of HIV-1 IN inhibitors and structure–activity relationships are worth being investigated for this compound.

Acknowledgments

This work was financially supported by grants from le Centre National de la Recherche Scientifique (CNRS), l'Agence Nationale de la Recherche contre le Sida (ANRS) and the European Commission (Grant LSHB-CT-2003-503480). The European TRIoH Consortium is gratefully acknowledged. The mass spectrometry facility used in this study was funded by the European Community (FEDER), the Région Nord-Pas de Calais (France), the CNRS and the Université des Sciences et Technologies de Lille. We are grateful to Martine Michiels, Nam Joo Vanderveken and Barbara Van Remoortel for excellent technical assistance.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.06.063.

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