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Synthesis, anti-HIV activity, integrase enzyme inhibition and molecular modeling of catechol, hydroquinone and quinol labdane analogs

Rohan Pawar [a,†], Tiyasa Das [b,†], Sanjay Mishra [a], Nutan [b], Boskey Pancholi [b], Satish K. Gupta [b,*], Sujata V. Bhat [a,*]

[a] Laboratory for Advanced Research in Natural and Synthetic Chemistry, V.G. Vaze College, Mumbai University, Mithagar Road, Mulund (East), Mumbai 400 081, India
[b] National Institute of Immunology, Aruna Asaf Ali Marg, New Delhi 110 067, India

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ABSTRACT

Labdane analogs with *o*-quinol, catechol and hydroquinone moiety have been synthesized using Diels–Alder reaction of methyl 3,4-dioxocyclohexa-1,5-diene-carboxylate, 3,4-dioxocyclohexa-1,5-diene-carboxylic acid and 3,6-dioxocyclohexa-1,4-dienecarboxylic acid with mono terpene 1,3-dienes, namely ocimene and myrcene. The resulting molecules and their derivatives were evaluated for their anti-HIV-1 activity using TZM-bl cell based virus infectivity assay. Two molecules **13** and **18** showed anti-HIV activity with IC_{50} values 5.0 (TI = 11) and 4.6 (TI = 46) μM, respectively. The compounds **17**, **18** and **20** showed efficacy against HIV-1 integrase activity and showed inhibition with IC_{50} 13.4, 11.1 and 11.5 μM, respectively. The HIV-1 integrase inhibition activity of these synthetic molecules was comparable with integric acid, the natural fungal metabolite. Molecular modeling studies for the HIV-1 integrase inhibition of these active synthetic molecules indicated the binding to the active site residues of the enzyme.

Acquired immune deficiency syndrome (AIDS) caused by the infection with human immunodeficiency virus (HIV) represents a major health problem worldwide, especially in the developing countries.[1–3] Highly active anti-retroviral therapy (HAART), mainly consisting of HIV-1 protease and reverse transcriptase inhibitors, introduced in the late 1990s, has largely improved patients' lives in the developed countries. However, drug resistance and toxicity are the main problems during HIV treatment.[4] Although understanding of the pathogenesis and transmission dynamics of HIV infection has advanced, protective vaccine remains elusive.[5] Therefore, there is a continuous need for novel and effective drugs against HIV-1.[6]

There are several cyclic natural terpenes with *o*-quinol or catechol moiety, which exhibit interesting biological activities. For example, the eremophilane class of sesquiterpene **1** displayed phytotoxic activity.[7,8] Members of quassinoid family **2** exhibit a gamut of biological activities, which include anti-HIV, anti-tumor, anti-malarial and anti-inflammatory activities.[9] The quinonemethide, taxodione **3** from *Tadodium disticum* displayed anti-tumor and anti-microbial activities.[9] Some molecules with *o*-keto-enol functionality displayed anti-HIV activity due to HIV-1 integrase inhibition. For example, cytosporic acid **4**, a fungal metabolite produced by a *Cytospora sp.*, displayed HIV-1 integrase inhibitory activity due to inhibition of strand-transfer reaction of HIV-integrase.[10,11] Integric acid **5**,[12–14] an eremophilane sesquiterpenoid from *Xylaria sp.* inhibited 3′-end processing, strand transfer and disintegration reactions catalyzed by HIV-1 integrase enzyme (Fig. 1).[15–19]

o-Quinones are active molecules that can be readily generated in situ by oxidation of catechols.[20] They exhibit properties of both diene and dienophile. It has been shown that *o*-quinones bearing an electron withdrawing substituent at the 2nd and 4th position are sufficiently reactive as dienophiles in cycloadditions with reactive acyclic 1,3-dienes.[21] We report herein the synthesis and evaluation of anti-HIV-1 activity of the labdane analogs, Some of these molecules displayed good anti-HIV-1 and HIV-1 integrase inhibition activities.

Labdane analogs with *o*-quinol moiety were synthesized by Diels–Alder reaction of methyl 3,4-dioxocyclohexa-1,5-diene-carboxylate (generated in situ by the oxidation of methyl 3, 4-dihydroxy-benzoate using Ag_2O) with monoterpene 1,3-dienes

Abbreviations: MTT, 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide; TI, therapeutic index; RT, room temperature; IBD, iodoso benzene diacetate; ACN, acetonitrile; IN CCD, integrase catalytic core domain.

* Corresponding authors. Tel.: +91 1126741249; fax: +91 11 26742125 (S.K.G.); tel.: +91 22 21631421x113; fax: +91 022 21634262 (S.V.B.).
E-mail addresses: skgupta@nii.ac.in (S.K. Gupta), sujata8b@gmail.com (S.V. Bhat).

† Equal contribution.

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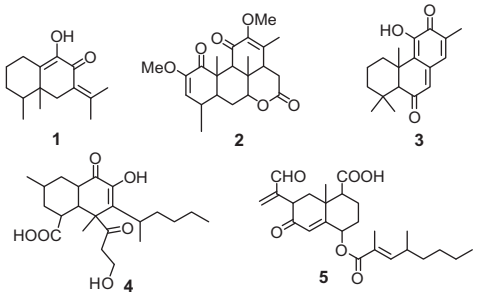
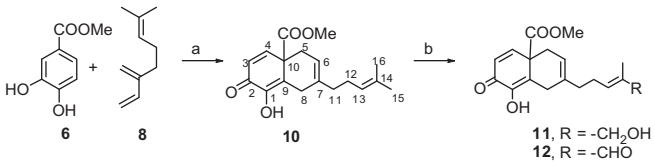


Figure 1. Bioactive terpenoids.

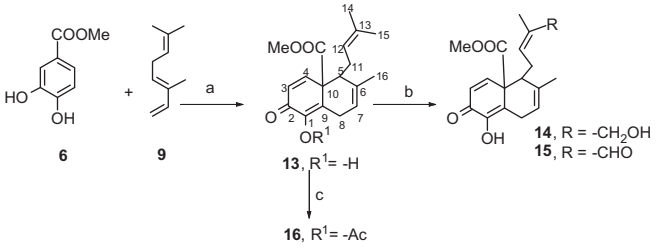
namely myrcene and ocimene. Similarly, labdane analogs with catechol and hydroquinone moiety were synthesized by Diels–Alder reaction of 3,4-dioxocyclohexa-1,5-diene-carboxylic acid and of 3,6-dioxocyclohexa-1,4-dienecarboxylic acid (generated in situ by the oxidation of 3,4-dihydroxy-benzoic acid and 2,5-dihydroxybenzoic acid using IBD) with myrcene and ocimene. *o*-Quinones being unstable were prone to rapid dimerization. To circumvent this problem and to increase yield in the cycloaddition reaction, an excess of the diene was used and the reaction was conducted at 0 °C to avoid dimerization.

Thus, a mixture of monoterpene diene myrcene or ocimene (5 mM), methyl 3,4-dihydroxy-benzoate (1 mM) and freshly prepared Ag_2O (1 mM) was stirred at 0 °C for 3 h, then at RT for 20 h and the reaction mixture was subjected to the usual work-up to yield the adducts **10** and **13**, respectively (Schemes 1 and 2). The Diels–Alder reaction of methyl 3,4-dihydroxybenzoate **6** with myrcene **8** yielded a mixture of regio-isomers (9:1) (Scheme 1), which were further separated to obtain major *para* adduct **10** (with respect to carbomethoxy group), by preparative thin layer chromatography (PTLC), the stereochemistry of the compound was confirmed by NMR ROESY experiments (Fig. 2 and Supporting information). Similarly, the stereochemistry of the compound **13** was also confirmed by NMR ROESY experiments. To obtain adduct **17** and **18** or **19** and **20** a mixture of terpenic diene myrcene or ocimene (5 mM), 3,4-dihydroxy-benzoic acid or 2,5-dihydroxybenzoic acid (1 mM) and freshly prepared IBD (1 mM) was stirred at 0 °C for 1 h, the reaction mixture was subjected to the usual work-up (Schemes 3 and 4). The Diels–Alder reaction of 3,4-dihydroxybenzoic acid **7a** and 2,5-dihydroxybenzoic acid **7b** with myrcene **8** yielded only *para* adducts **17** and **19**, respectively (Schemes 3 and 4). The excess diene recovered at the end of the reaction, has been characterized by spectral data. Similarly, reactions of acids **7a** and **7b** with ocimene **9** yielded single *ortho*-adducts **18** and **20**, respectively (Schemes 3 and 4).

Structures of adducts were supported on the basis of spectral data.[21] The IR spectra of cyclo-adducts **10** and **13** showed bands at ~3412, 1736 and 1649 cm^{-1} indicating the presence of hydroxyl, ester and conjugated carbonyl group, respectively, whereas the adducts **17**–**20** showed bands at 3400 and 1595 cm^{-1} indicating the presence of phenolic OH and aromatic C=C stretching, respectively. The absence of peak around 1700–1650 cm^{-1}, indicated that these adduct underwent decarboxylation and



Scheme 1. Diels–Alder reaction of methyl 3,4-dioxocyclohexa-1,5-diene-carboxylate with myrcene and SeO_2 oxidation of adduct. Reagents and conditions: (a) toluene:diethyl ether, Ag_2O, 0 °C for 3 h, RT for 20 h (b) SeO_2, *t*-BuOOH, RT, 15 h.



Scheme 2. Diels–Alder reaction of methyl 3,4-dioxocyclohexa-1,5-diene-carboxylate with ocimene and SeO_2 oxidation of adduct. Reagents and conditions: (a) toluene:ether, Ag_2O, 0 °C for 3 h, RT for 20 h (b) SeO_2, *t*-BuOOH, RT for 15 h (c) Ac_2O, pyridine, RT for 18 h.

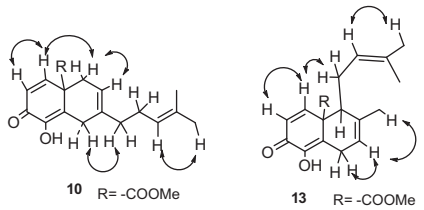
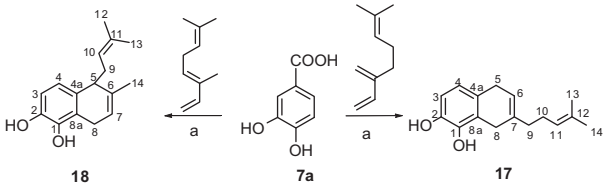
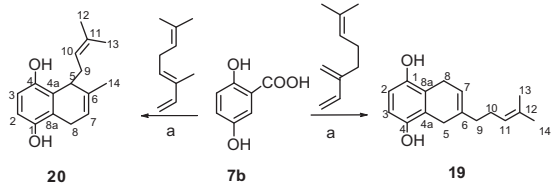


Figure 2. Correlation of protons obtained from ROESY spectrum of compounds **10** and **13**.



Scheme 3. Diels–Alder reaction of 3,4-dioxocyclohexa-1,5-diene-carboxylic acid with ocimene and myrcene. Reagents and conditions: (a) diisopropyl ether, ACN, IBD, 0 °C for 1 h.



Scheme 4. Diels–Alder reaction of 3,6-dioxocyclohexa-1,4-dienecarboxylic acid with ocimene and myrcene. Reagents and conditions: (a) diisopropyl ether, ACN, IBD, 0 °C for 1 h.

aromatization to give catechol analogs **17**, **18** and hydroquinone analogs **19** and **20**. The ^1H NMR spectra of the compounds **10** and **13** displayed a pair of doublets around δ 6.95 and 6.40 (1H each, J = 10 Hz) for C-3, C-4 protons. The ring olefinic protons (C-6 or C-7) and side-chain olefinic protons resonated at δ ~5.5 (broad triplet) and δ ~5.1 (triplet) (1H, J = 5.5 Hz), respectively. Thus, these products are formed through Diels–Alder reaction followed by enolization of the resulting *o*-diketone to the tautomeric diosphenol. This was confirmed not only by the ^1H NMR spectrum but also by making an acetate derivative of the enol. The formation of the enol acetate (**16**, Scheme 2) was supported by the ^1H NMR spectrum of the compound **16**, which showed the presence of a acetate peak at δ 2.33 (3H, singlet) and also by the appearance of additional band at 1748 cm^{-1} in the IR spectrum. Adducts **10** and **13** were subjected to oxidation with catalytic amount of selenium dioxide in the presence of *t*-butyl hydroperoxide to yield allylic