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Synthesis, cytotoxic activity and structure–activity relationships of hedychenone analogues

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ABSTRACT

Hedychenone, a plant-derived labdane diterpenoid, showed potent in vitro cytotoxic activity against cancerous cells. In the present study, a series of analogues have been synthesized by modification of the furanoid ring, double bond and the vinylic methyl functionality of this natural product lead and evaluated for their cytotoxic activities against human cancer cell lines. The structures of the target compounds were established by IR, ^{1}H NMR and mass spectral analysis. Majority of the analogues displayed potent activity than the parent compound, hedychenone. Preliminary structure–activity relationship studies indicated that furanoid ring has a greater impact on cytotoxicity than that of the decalone nucleus. However, dimerization through C-8 significantly enhanced the cytotoxic activity of the hedychenone.

The search for novel chemotherapeutic agents and approaches to cancer treatment is still an active research field stimulated by the discovery of new biological targets and by the possibility of obtaining new drugs without undesirable side effects. The past decade has witnessed an increasing interest in search of plant based lead compounds for development of new pharmaceuticals, along with the increase of deadly illness such as AIDS, cancer, hepatitis, etc.[1] In this regard, labdane diterpenes have attracted the scientific interest because of their wide range of biological activities such as antialgal, antibacterial, antifungal, antiprotozoal, enzyme inducing, anti-inflammatory, modulation of immune cell functions, as well as cytotoxic against human tumor cell lines.[2–4] Plants of zingiberaceae have been proven to be good source of labdane diterpenes, featuring diverse array of carbon skeletons.[5] Most of these diterpenoids are characterised by possessing a furanoid or lactone fragment usually attached to a decalone core through double bond.[6]

In connection with our recent investigations of *Hedychium spicatum* rhizomes for value added products,[7] we have isolated large quantities of hedychenone (**1**) (0.23%),[8] which prompted us to synthesize derivatives and screen for the anticancer activity. Medicinally, essential oils of *H. spicatum* rhizomes is used for the treatment of skin diseases, stomach ailments,[9] analgesic,[10] anti-inflammatory,[11] antimicrobial,[12] In vitro pediculicidal,[13] and cytotoxic activity.[14] Hedychenone (**1**), a hydrophobic labdane diterpenoid has been shown wide spectrum of biological and pharmacological activities.[6a,7b,12] Despite the prominent potential of hedychenone, very little effort has been devoted into the synthesis of hedychenone derivatives.[15] Therefore, this small molecule natural product is an ideal structural template for the synthesis of a series of analogues in order to explore their structure–activity relationships (SARs), thus affording the information for further lead optimization of this class of compounds as potential cytotoxic agents.

To develop the robust pharmacophoric model and understand the basis for the cytotoxicity, its structure can be subdivided into three key structural moieties: furanoid ring (A), double bond (B) and vinylic methyl moiety (C) (Fig. 1). Each structural moiety can be modified independently in order to facilitate the systematic refinement of the search for increasingly effective hedychenone

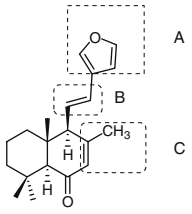

Figure 1. Key structural moieties of hedychenone (**1**).

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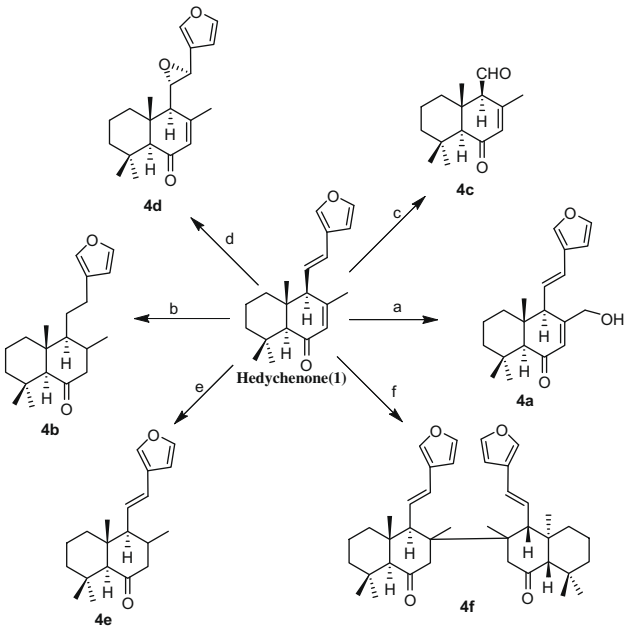
analogues. We, herein report the synthesis of hedychenone derivatives along with their cytotoxic activities against the cancer cell lines.

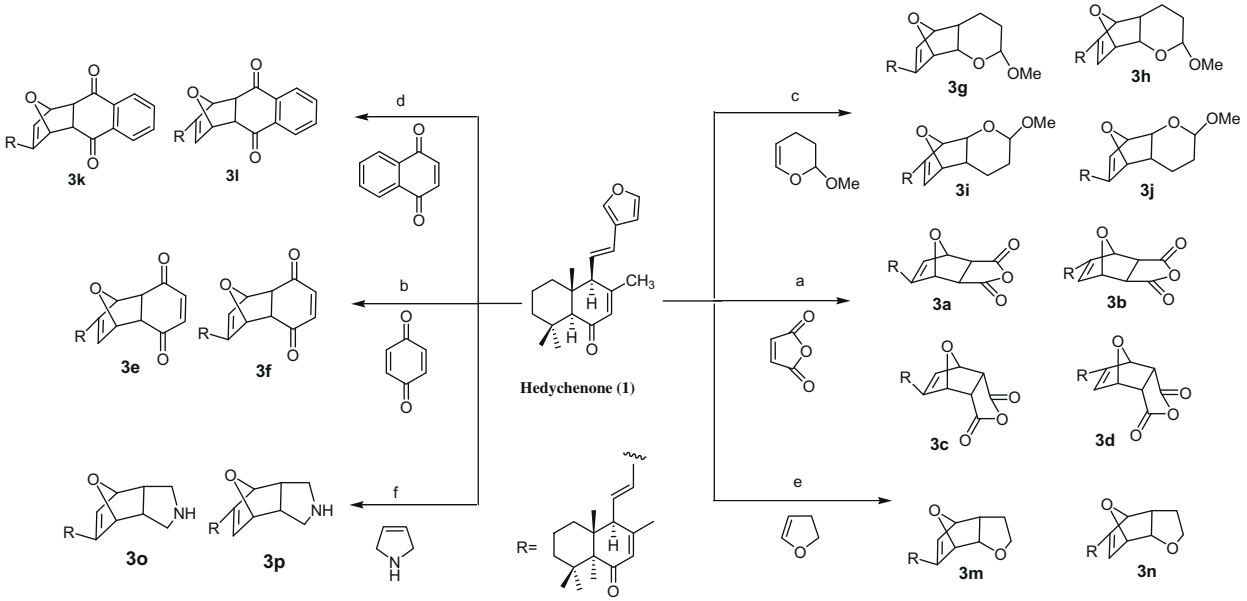
Our first modification involved in the modification of furanoid ring of hedychenone, as this group was most amenable to the Diels–Alder reactions. It is well known that quinones and pyran structural units occur in various molecules exhibiting diverse biological activities especially anticancer properties. Based on this core reason, we have focused to prepare the Diels–Alder adducts (**3a–3p**) from dienophiles like maleic anhydride, benzoquinone, 3,4-dihydro-2methoxy-2*H*-pyran, napthaquinone, 4,5-dihydro furan and 3-pyrroline (Scheme 1). Thus, reaction of **1** with excess of maleic anhydride at 0 °C in chloroform gave two thermodynamically dominated *exo* adducts **3a** and **3b**, together with a mixture of two kinetically dominated *endo* adducts **3c** and **3d.** However, the same reaction was conducted at 110 °C in toluene solvent, we have observed predominately **3a** and **3b,** without any traces of **3c** and **3d**.[16a] From this observation, it is evident that high temperatures are favorable for the formation of *exo* adducts rather than *endo* adducts.

The structure of **3a, 3b** as well as **3c** and **3d** were determined by spectral methods, *exo* and *endo* products were deduced from the coupling constants. In a similar manner, the reaction of **1** with benzoquinone at 0 °C in $CHCl_3$, we did not observe any change in the chemical reaction, whereas using toluene as a solvent under reflux conditions, adducts exclusively **3e** and **3f** (*exo* products) were obtained. Similarly, reaction of hedychenone (**1**) with 3,4-dihydro-2-methoxy-2*H*-pyran, naphtha quinone, 4,5-dihydro furan and 3-pyrroline yielded Diels–Alder adducts (**3g–3p)** (Scheme 1).

To estimate the impact of allylic methyl group at C-17, double bonds at $\Delta^{7(8)}$ and $\Delta^{11(12)}$ six analogues (**4a**–**4f**) were synthesized by keeping the furanoid ring (structural moiety A) intact. As illustrated in Scheme 2, allylic oxidation of hedychenone with SeO_2 in dry dioxane under reflux conditions afforded yunnacoronarin D **(4a)** in 94% yield.[16] Hydrogenation of **1** in the presence of Pd/C (10%) in EtOH afforded 6,7-dihydro hedychenone **(4e)** in 70% yield,[17] which was further subjected to the reduction with $LiAlH_4$ in THF at 0 °C afforded 6,7,11,12-tetrahydro hedychenone (**4b)** in 50% yield.[17] However, reduction of hedychenone with aluminum-mercury alloy[9b] yielded dimerized product (**4f**). Ozonolysis of **1** in presence of O_3 in DCM at −10 °C afforded compound **4c** in 55% yield.[17] Epoxidation of hedychenone (**1**) with *m*-CPBA in DCM at room temperature afforded compound **4d** in 70% yield[5b] (Scheme 2). The chemical structures of the synthesized compounds were confirmed by their spectral data (^{1}H NMR, mass and FTIR). Representative characterizations of compounds are described in Supplementary data associated with this Letter.



Scheme 2. Synthesis of compounds from **4a**–**4f**. Reagents and conditions: (a) Seo_2, dry dioxane/reflux, 70 °C, 1 h, 94%; (b) (1) Pd/C (10%), EtOH, 1 h; (2) $LiAlH_4$, THF, 0 °C, 4 h, 50%; (c) O_3, CH_2Cl_2, −10 °C, 4 h, 55%; (d) *m*-CPBA, CH_2Cl_2, rt, 2 h, 70%; (e) Pd/C (10%), EtOH, 1 h, 70%; (f) Al-Hg alloy, HCl–MeOH/reflux, 3 h, 40%.



Scheme 1. Synthesis of Diels–Alder adducts of hedychenone (**3a**–**3p**). Reagents and conditions: (a) maleic anhydride, $CHCl_3$, $BF_3{\cdot}OEt_2$ (catalytic amount), 0 °C, 3 h; (b) benzoquinone, toluene/reflux, 110 °C, $BF_3{\cdot}OEt_2$ (catalytic amount), 4 h; (c) 3,4-dihydro-2-methoxy-2*H*-pyran, toluene/reflux, 115 °C, $BF_3{\cdot}OEt_2$ (catalytic amount), 4 h; (d) napthaquinone, toluene/reflux, 110 °C, $BF_3{\cdot}OEt_2$ (catalytic amount), 3 h; (e) 4,5-dihydro furan, toluene/reflux, 110 °C, $BF_3{\cdot}OEt_2$ (catalytic amount), 4 h; (f) 3-pyrroline, toluene/reflux, 110 °C, $BF_3{\cdot}OEt_2$ (catalytic amount), 4 h.

Table 1
Cytotoxic effects of hedychenone analogues

Compound	Cell lines (IC_{50} μg/ml)				
	MCF-7	HL-60	CHO	A-375	A-549
Hedychenone	19.41 ± 1.15	17.68 ± 0.13	8.20 ± 1.07	21.13 ± 1.19	33.12 ± 1.18
3a	18.61 ± 0.07	17.02 ± 1.18	10.41 ± 1.18	20.39 ± 0.07	39.87 ± 1.06
3b	17.02 ± 1.86	16.94 ± 1.53	9.11 ± 1.64	20.04 ± 1.21	36.42 ± 1.18
3c	23.18 ± 2.12	21.73 ± 1.97	11.08 ± 1.18	27.84 ± 1.76	NT
3d	24.09 ± 3.83	28.12 ± 1.34	12.13 ± 1.64	30.41 ± 1.81	NT
3e	15.73 ± 1.22	14.08 ± 0.09	8.19 ± 1.11	19.03 ± 1.18	35.89 ± 1.23
3f	15.41 ± 2.23	14.73 ± 1.18	8.28 ± 2.13	20.11 ± 0.06	NT
3g	28.08 ± 1.14	27.68 ± 0.18	13.07 ± 1.81	30.18 ± 0.03	43.18 ± 3.21
3h	27.99 ± 0.07	27.13 ± 0.02	14.15 ± 1.18	32.13 ± 1.09	42.96 ± 1.18
3i	29.30 ± 1.13	28.41 ± 0.18	13.91 ± 1.08	31.12 ± 0.06	47.71 ± 0.09
3j	NT	NT	14.03 ± 1.14	NT	NT
3k	14.98 ± 1.22	13.69 ± 1.10	7.94 ± 0.06	18.73 ± 0.09	NT
3l	14.72 ± 0.05	13.88 ± 0.08	7.91 ± 0.41	18.64 ± 0.51	34.48 ± 1.09
3m	30.14 ± 0.86	38.37 ± 1.18	NT	NT	48.41 ± 0.87
3n	33.87 ± 0.92	37.99 ± 1.93	NT	43.09 ± 1.14	NT
3o	36.72 ± 0.79	39.87 ± 0.18	19.18 ± 0.18	NT	NT
3p	31.98 ± 0.87	43.18 ± 1.18	NT	NT	NT
4a	34.28 ± 0.96	26.18 ± 4.13	39.03 ± 0.96	40.01 ± 0.94	50.13 ± 1.97
4b	>100	86.98 ± 3.18	NT	59.97 ± 1.14	NT
4c	38.15 ± 0.87	39.69 ± 1.13	34.91 ± 0.06	35.13	NT
4d	20.10 ± 0.48	19.43 ± 0.55	9.61 ± 0.85	26.14 ± 0.77	31.15 ± 1.14
4e	>100	>100	NT	>100	NT
4f	11.19 ± 0.93	13.86 ± 0.18	6.91 ± 0.84	16.81 ± 0.87	23.01 ± 0.99

NT = Not tested, each value represents the mean ± standard deviation.

Biological activity and SAR studies. Hedychenone (**1**) and its analogues (**3a**–**3p** and **4a**–**4f**) were evaluated for their in vitro cytotoxic activity against MCF-7 (breast cancer), HL-60 (human promyleocytic leukemia), CHO (Chinese hamster ovary), A-375 (human malignant melanoma) and A-549 (human lung carcinoma) cell lines.[18,19] The results of the cytotoxicity studies were indicated in Table 1 (IC_{50} value, defined as the concentration corresponding to 50% growth inhibition). Preliminary SAR studies were developed in this series in order to further explore its full potential. As demonstrated in Table 1, it can be stated that most of analogues displayed better cytotoxic activity compared to parent compound, hedychenone (**1**) and modification of the furanoid ring (moiety A) has greater impact than that of the decalone nucleus. Among Diels–Alder adducts tested, **3a, 3b, 3e, 3f, 3k,** and **3l** compounds shown significant activity on CHO (Chinese hamster ovary) cell line. The cytotoxic orders of these compounds are **3l** > **3k** > **3e** > **3f** > **3b** > **3a**. Recently, it has been demonstrated that Diels–Alder adduct (cantharidine analogue) showed potent antitumour properties against hepatoma and esophageal carcinoma, indicating the therapeutic potential of this scaffold.[20] It was interesting to note that all adducts (**3a–3p**) were less active than hedychenone on A-549 cell line. Mild activity of compounds **3g, 3h, 3i, 3m, 3n, 3o**, and **3p** suggesting the nature and participation of dienophile that could influence the cytotoxic activity. It is important to mention that potent activity was observed with **3l** and **3k**, which were prepared using the dienophile, napthaquinone.

After this preliminary exploration, decalone ring was considered as an optimum substitution pattern. Taking this into consideration, a thorough analysis of the SAR around the decalone moiety was thus carried out with the aim of finding suitable substituents that would provide more potent analogues. As shown in Table 1, drastic decrease or lack in cytotoxicity of compounds **4b, 4e** against all tested cell lines, implying that double bonds ($\Delta^{7(8)}$ and $\Delta^{11(12)}$) are necessary for the activity. However, epoxidation of $\Delta^{11(12)}$ -double bond (compound **4d**) enhanced the activity against CHO cell line. Further, compounds **4a** and **4c,** which were derived by allylic oxidation and ozonolysis of **1**, showed marginal activity against the tested cell lines. Interestingly, dimerization of the hedychenone through C-8, as in derivative **4f**, exhibited potent cytotoxic activity against all cell lines. It became clear from these results that the terminal furanoid moiety in compound **1** is an essential feature for activity, and that the modification of this moiety with quinones through Diels–Alder reaction led to significant enhancement in the activity. To the best of our knowledge, this is first report on the cytotoxic activity of the hedychenone analogues.

In summary, we have prepared a series of hedychenone derivatives by a rationally designed classical medicinal chemistry approach and evaluated for their in vitro cytotoxic activity. This study has demonstrated that the furan moiety and *trans* double bond seems to be better situation to achieve potential cytotoxic activity of hedychenone analogues. However, dimerization of hedychenone through C-8 significantly enhanced the activity. This has laid a solid foundation for further lead optimization of this class of compounds by a systematic refinement or modification including the synthesis of water-soluble compounds to improve their overall pharmaceutical properties.

Acknowledgements

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.02.101.

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19. *In vitro cytotoxicity evaluation*. All the derivatives were tested for in vitro cytotoxicity on different cancer cell lines. The cell lines used in this study were HL-60 (human promyelocytic leukemia), A-375 (human malignant melanoma), MCF-7 (breast cancer), CHO (Chinese hamster ovary) and A-549 (human lung carcinoma) cancerous cell lines. All the cell lines were obtained from National Center for cellular Sciences (NCCS), Pune, India. Cell lines were maintained in DMEM supplemented with 10% fetal bovine serum, 2 mM glutamine, 100U/mL penicillin, 100 μg/mL streptomycin, at 37 °C with 5% CO_2. The cells were seeded at 1×10^4 cells/well. After 24 h, cells were treated with the test compound and IC_{50} values were calculated in μg/mL.
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