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CONCISE ARTICLE

# Synthesis and biological activity profile of novel 2-cinnamylidene-1,3-diones related to coruscanone A: promising new antileishmanial agents†

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A series of 2-cinnamyliden-1,3-diones related to the natural product coruscanone A was prepared from simple commercially available precursors *via* Knoevenagel condensation, and evaluated for antifungal, antibacterial and antiparasitic activities. Most of the compounds tested displayed antifungal effects against opportunistic pathogen *C. neoformans* comparable to coruscanone A and its derivatives suggesting that the mechanism of action of this class of compounds would involve Michael addition of a nucleophilic site of the biological target on the styryl side chain instead of the previously proposed reaction at the cyclopentenedione ring. In addition, some showed good *in vitro* antileishmanial activity (*L. donovani*), one of the compounds being superior to pentamidine and comparable to amphotericin B.

Synthetic as well as natural 2-arylidene-cyclopentane-1,3-diones have attracted a great deal of attention due to their wide range of biological activities.[1] Examples of distinct members of this class include methyllinderone (**1**, Fig. 1)[2] isolated from *Lindera erythrocarpa* which was shown to inhibit human chymase, a serine protease associated with cardiovascular diseases and chronic inflammation following fibrosis; and coruscanone A (**2**),[3] isolated more recently from the ethanol extract of the Peruvian plant *Piper coruscans*, which displayed strong antifungal activity against two major opportunistic pathogens, *Candida albicans* and *Cryptococcus neoformans*. Also, methyllucidone (**3**), the major component of the extract of fruits of *L. erythrocarpa*, inhibits human colon tumor cells by inducing apoptosis through the caspase-3 pathway.[4] It has been suggested that both **1** and **2** exert their action by alkylation of their molecular targets acting as Michael acceptors. However, while **2** has been postulated to react at the cyclopentenedione ring,[5] **1** was proposed to suffer Michael addition on the styryl side chain based on LUMO coefficient calculations and on docking studies on the binding mode of 1-oxacephem to human chymase (Fig. 2).[2b]

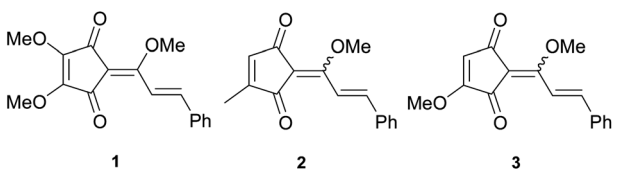


**Fig. 1** Natural 2-cinnamylidene-cyclopentene-1,3-diones.

We recently reported an efficient light-mediated protocol for the transformation of α,β,γ,δ-unsaturated dienones derived from β-ionone into bridged-1,2,4-trioxane derivatives for their further antimalarial evaluation.[6] In order to extend our study and also to validate the versatility of the photooxygenation process developed, we prepared a series of new dienonic precursors *via* Knoevenagel condensation between 1,3-dicarbonyl substrates and α,β-unsaturated aldehydes. Although these simplified coruscanone A analogues lack the cyclopentenedione ring moiety suggested as the "warhead" of the molecule for their antifungal action,[5] they may still behave as Michael acceptors and therefore, from the perspective of drug discovery, we set out to evaluate their biological activity profile on a panel of fungal, bacterial and protozoan pathogens. Herein we wish to report the synthesis of this novel series of 2-cinnamyliden-1,3-diones which displays promising *in vitro* antileishmanial activity.

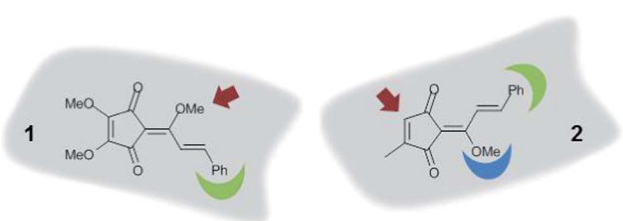


**Fig. 2** Proposed mechanism of action of compounds **1** and **2**.

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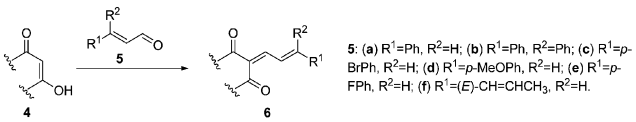
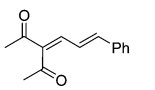
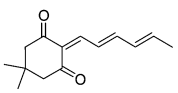
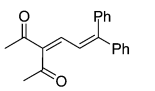
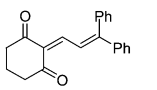
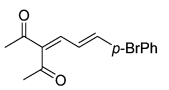
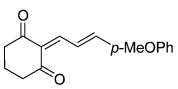
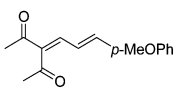
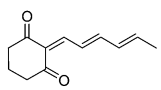
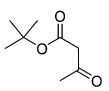
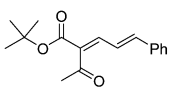
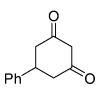
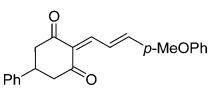
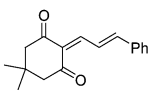
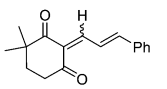
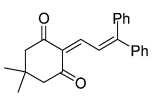
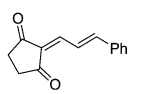
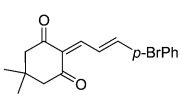
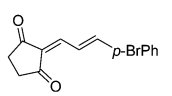
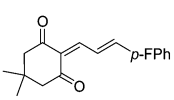
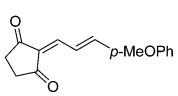
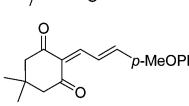
† Electronic supplementary information (ESI) available: Detailed experimental procedures. Copies of $^{1}H$ and $^{13}C$ NMR spectra of all new products. See DOI: 10.1039/c2md20143g

One of the most conventional and probably versatile methods for the synthesis of 2-allylidene-1,3-diketo compounds involves a Knoevenagel-type condensation between 1,3-dicarbonyl derivatives and α,β-unsaturated aldehydes.[7] This process has long been studied and, provided suitable substrates are carefully chosen, subsequent oxa-6π-electrocyclisation affording 2*H*-pyran valence isomers can be prevented. Generally, there is a complete equilibrium shift toward the ring-closed form when there is steric destabilisation or the absence of stabilising mesomeric effects in the conjugated dienone open-form.[8] Another side-product that makes this scenario a little more intricate may arise if former conjugated enone products suffer a subsequent Michael addition from a second molecule of dicarbonyl equivalent, which has been especially advantageous for the preparation of xanthenes and derivatives.[9]

Recently, Goswami and Das demonstrated that condensations between cinnamaldehyde (**5a**) and dicarbonyl substrates such as acetylacetone (**4a**), *tert*-butyl acetoacetate (**4b**) and dimedone (5,5-dimethyl-cyclohexane-1,3-dione, **4c**) actually proceed in good yield under solvent-free conditions *via* organocatalysis provided by L-proline.[7] This interesting protocol provided compounds **6a**, **6e** and **6f** in 90, 83 and 92% yields, respectively (Table 1). Unfortunately, all attempts to extend this procedure to other dicarbonyl and enal substrates failed and hence other catalysts and solvents were assayed. After considerable experimentation, the well-known organocatalyst "Tietze base" (ethylenediammonium diacetate, EDDA) and dichloromethane at reflux were chosen as optimum reaction conditions.[10] Aldehydes such as **5a**, β-phenyl-cinnamaldehyde (**5b**), *p*-bromocinnamaldehyde (**5c**), *p*-methoxy-cinnamaldehyde (**5d**), *p*-fluorocinnamaldehyde (**5e**) and

**Table 1** Synthesis of 2-cinnamyliden-1,3-diones **6a–s** *via* Knoevenagel condensation[a]




| Dicarbonyl substrate | Ald | Product | Yield [%][b] | Dicarbonyl substrate | Ald | Product | Yield [%][b] |
|---|---|---|---|---|---|---|---|
|  | **4a 5a** |  **6a** | 90[c] | | **4c 5f** |  **6k** | 40[a]/80[d] |
| | **4a 5b** |  **6b** | 85 |  | **4d 5b** |  **6l** | 70 |
| | **4a 5c** |  **6c** | 90 | | **4d 5d** |  **6m** | 70 |
| | **4a 5d** |  **6d** | 60 | | **4d 5f** |  **6n** | 50[a]/65[d] |
|  | **4b 5a** |  **6e** | 83[c] |  | **4e 5d** |  **6o** | 85 |
|  | **4c 5a** |  **6f** | 92[c] |  | **4f 5a** |  **6p** | 60 |
| | **4c 5b** |  **6g** | 75 |  | **4g 5a** |  **6q** | 8 |
| | **4c 5c** |  **6h** | 90 | | **4g 5c** |  **6r** | 30 |
| | **4c 5e** |  **6i** | 80 | | **4g 5d** |  **6s** | 55 |
| | **4c 5d** |  **6j** | 80 | | | | |

[a] All reactions were run under the following conditions: aldehyde **5** (1 equiv.), EDDA (0.2 equiv.), DCM (0.2 M), at reflux, 4 h, unless otherwise noted. [b] Isolated yields. [c] Solvent-free protocol using L-Pro (10 mol%), 10 min grinding, ref. 7. [d] Reactions using the catalyst MTFA (20 mol%) instead of EDDA.

sorbaldehyde (**5f**) were condensed with 1,3-dicarbonyl substrates including **4a**, **4b**, **4c**, cyclohexane-1,3-dione (**4d**), 5-phenyl-cyclohexane-1,3-dione (**4e**), 4,4-dimethyl-cyclohexane-1,3-dione (**4f**) and cyclopentane-1,3-dione (**4g**) and the results are summarised in Table 1. As shown, products **6a–s** were prepared from simple commercially available substrates in yields ranging from 8 to 90%. In some cases better results were obtained using morpholinium trifluoroacetate (MTFA), an efficient recently reported catalyst for aldol condensations.[11] In particular, reactions using cyclopentane-1,3-dione (**4g**) as a nucleophilic partner proved troublesome. Other researchers have also noted the difficulty in the preparation of such adducts and to our knowledge this is the first report on the isolation of conjugated carbonyl systems using this 1,3-dicarbonyl substrate.[12] In spite of all efforts made, we could not find improved conditions for these condensations. As expected, the stereochemistry of the aldehydes employed was conserved in the products and therefore when symmetrical 1,3-dicarbonyl substrates were used, only one stereoisomer was obtained. However, while the condensation between the unsymmetrical β-ketoester **4b** and enal **5a** gave only one stereoisomer as previously reported (**6e**; *E*,*E*),[7] reaction between **4f** and **5a** gave an inseparable mixture of *Z*,*E* and *E*,*E* diastereoisomers (**6p**, see ESI†). For all synthesised products, no isomerisation was observed over time.

Biological activities of compounds **6a–s** were then evaluated *in vitro* on a panel of fungal, bacterial and protozoan pathogens including *C. albicans*, *C. glabrata*, *C. krusei*, *A. fumigatus*, *C. neoformans*, *S. aureus*, methicillin resistant *S. aureus*, *E. coli*, *P. aeruginosa*, *M. intracellulare*, *P. falciparum* (chloroquine sensitive and resistant clones) and *L. donovani* (Table 2). Antifungal activities were compared to amphotericin B as the drug standard and coruscanone A as the parent structure. Antileishmanial activities were compared to amphotericin B and pentamidine as control drugs. The *in vitro* cytotoxicities of the prepared compounds **6a–s** were tested against Vero cells (African green monkey kidney fibroblasts).

Interestingly, an inspection of Table 2 as regards antifungal activities reveals that compound **6** displays moderate and selective activities against *C. neoformans* ($IC_{50}$s 11.69 to >60 μM), one of the major opportunistic pathogens associated with AIDS patients. No compound was found to be active against either *C. albicans* or *A. fumigatus* (see ESI†) and only few showed weak activities against *C. glabrata* and *C. krusei* with $IC_{50}$ values around 20 μM for the most active compounds. Although none of the prepared compounds displayed better fungicidal activities (*C. neoformans*) than those found previously for the natural product coruscanone A and synthetic derivatives, these are in the same order of magnitude suggesting that, considering the structural similarities, they may act *via* the same mechanism of action. If that is the case, then Michael addition to coruscanone A and derivatives from a molecular target would occur on the styryl side chain instead of hitherto suggested reaction at the cyclopentenedione ring. This is consistent with the previously reported mechanism for inhibition of human chymase inhibitors methyllinderone (**1**) and derivatives as stated above. Another interesting conclusion is that the cyclopentane ring seems not to be essential for fungicidal activity considering that **6c** is slightly less active than coruscanone A. In addition, our results are in agreement with previous observations made from SAR evaluations of other coruscanone A analogues highlighting the importance of the styryl side chain.[5] Compounds **6k** and **6n** lacking the aromatic nucleus were not active against *C. neoformans* supporting the idea that the styryl chain could be associated with binding affinity for the cellular target.

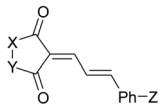
It has been established that fungi and some kinetoplastids usually display similar sensitivity to certain compounds.[13] Those similarities are based on some shared common biochemical differences with the mammalian hosts, which have been the rationale behind some recently developed drugs.[14] Following this idea, we decided to assay all the prepared compounds **6a–s** against the etiological agent of visceral leishmaniasis.[15] This parasitic disease, primarily caused by *Leishmania donovani*, is the most severe of the three existing forms, being fatal in almost 100% of the cases if left untreated. Visceral leishmaniasis has major public health implications in the Indian subcontinent, and in East Africa and Latin America. During the last few decades much effort has been made to develop new drugs to add to the arsenal of oral chemotherapeutics against leishmaniasis. Amphotericin B, pentamidine and miltefosine were some of the approved drugs but they still have some drawbacks such as high costs, difficulty of availability, or the presence of undesirable side

**Table 2** *In vitro* antifungal and antileishmanial activities and cytotoxicity of compounds **6a–s**[a]

| | CG[b] | CK[c] | CN[d] | LD[e] | LD[f] | CV[g] | | CG[b] | CK[c] | CN[d] | LD[e] | LD[f] | CV[g] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **6a** | >60 | >60 | >60 | 19.13 | 52.26 | >22.2 | **6l** | >60 | >60 | 14.45 | 10.25 | 26.12 | >15.7 |
| **6b** | >60 | >60 | 35.30 | 9.29 | 66.11 | >16.4 | **6m** | 55.95 | >60 | 44.71 | 19.51 | 91.69 | >18.6 |
| **6c** | >60 | >60 | 11.69 | 9.55 | 24.55 | >16.2 | **6n** | >60 | >60 | >60 | 56.78 | >100 | >25.0 |
| **6d** | >60 | >60 | >60 | 38.47 | >100 | >19.5 | **6o** | >60 | >60 | >60 | 7.52 | 14.74 | >14.3 |
| **6e** | >60 | >60 | >60 | 46.64 | 99.15 | >17.5 | **6p** | >60 | >60 | 19.89 | 11.79 | 20.05 | >18.7 |
| **6f** | 35.27 | >60 | 19.03 | 20.05 | 27.52 | >18.7 | **6q** | >60 | 42.60 | 11.87 | 2.16 | 3.58 | >22.4 |
| **6g** | >60 | >60 | 23.60 | 9.38 | 26.63 | >14.4 | **6r** | >60 | >60 | 26.86 | 8.58 | 14.08 | >16.3 |
| **6h** | >60 | >60 | 24.73 | 9.60 | 18.60 | >14.3 | **6s** | 34.99 | 24.06 | 13.37 | 7.01 | 19.81 | >19.6 |
| **6i** | 35.55 | >60 | 16.63 | 10.65 | 24.97 | >17.5 | **Cor A**[h] | NT[k] | NT | 8.02 | NT | NT | 19.3 |
| **6j** | >60 | >60 | 38.22 | 30.59 | 68.93 | >16.7 | **Amb**[i] | 0.28 | 0.78 | 0.35 | 0.31 | 1.09 | 7.6 |
| **6k** | >60 | >60 | >60 | 45.80 | >100 | >21.8 | **Pent**[j] | NT | NT | NT | 3.82 | 12.40 | NT |

[a] All the analogues were tested at least at six different concentrations, each in duplicate. The mean values were used to generate the growth inhibition curves and determination of $IC_{50}$ values. [b] *Candida glabrata* ATCC 90030, $IC_{50}$ (μM). [c] *C. krusei* ATCC 6258, $IC_{50}$ (μM). [d] *Cryptococcus neoformans* ATCC 90113, $IC_{50}$ (μM). [e] *L. donovani*, $IC_{50}$ (μM). [f] *L. donovani*, $IC_{90}$ (μM). [g] Cytotoxicity Vero cells (African green monkey kidney fibroblasts), $TC_{50}$ (μM). [h] Coruscanone A. [i] Amphotericin B. [j] Pentamidine. [k] Not tested.




| Z=H | | *L. donovani* |
|---|---|---|
| | X-Y | $IC_{50}$ μM |
| **6q** | $CH_2$-$CH_2$ | 2.16 |
| **6p** | $C(CH_3)_2$-$CH_2$-$CH_2$ | 11.79 |
| **6f** | $CH_2$-$C(CH_3)_2$-$CH_2$ | 20.05 |
| **6a** | -$CH_3$ $CH_3$- | 19.13 |

| Z=OMe | | *L. donovani* |
|---|---|---|
| | X-Y | $IC_{50}$ μM |
| **6s** | $CH_2$-$CH_2$ | 7.01 |
| **6m** | $CH_2$-$CH_2$-$CH_2$ | 19.51 |
| **6j** | $CH_2$-$C(CH_3)_2$-$CH_2$ | 30.59 |
| **6o** | $CH_2$-CHPh-$CH_2$ | 7.52 |
| **6d** | -$CH_3$ $CH_3$- | 38.47 |

**Fig. 3** Effects of ring size and substituents on antileishmanial activity.

effects.[16] This scenario requires a major worldwide multicenter effort to develop novel and improved candidates.

As shown in Table 2, the compounds of this new series of 2-cinnamyliden-1,3-diones exhibit *in vitro* activity against *L. donovani* with $IC_{50}$ values ranging from *ca.* 100 μM for the least active to 2.16 μM for **6q**, the most active compound. An initial conclusion that can be drawn from the analysis of Table 2 is that once again, as previously seen for antifungal activities, replacement of the phenyl aromatic nucleus by an allyl moiety exerts a negative effect on activity, compound **6k** being about 5-fold less active than **6f**.

Taking into account only $IC_{90}$ values, compounds obtained using acyclic dicarbonyl substrates were the least active regardless of the substitution pattern, suggesting that planarity of the conjugated carbonyl system would be essential for the mechanism of action of this family consistent with proposed Michael addition reaction from a cellular target.[17] As regards the ring size and substituent effects, an interesting SAR analysis can be performed by looking at the series depicted in Fig. 3 for the purpose of comparison. Cyclopentanedione derivatives were considerably more active than their cyclohexanedione counterparts. Among the latter, the presence of substituents over the ring also imparted a negative effect on activity with the exception of the phenyl-substituted analogue **6o** that is as active as **6s**. The fact that a phenyl substituent over the ring makes **6o** the most active cyclohexanedione derivative could be attributed to some binding stabilisation by π-stacking or to an increased lipophilicity. Also, the electronic nature of substituents on the aromatic nucleus of the styryl side chain did significantly affect biological activities as well. Compounds possessing a *p*-methoxy electron-donating group were less active than the corresponding unsubstituted analogues probably as a consequence of a reduced electrophilicity of the pharmacophore (*e.g.*, **6q** *vs.* **6s** or **6a** *vs.* **6d**).

We then challenged the prepared library of compounds against *P. falciparum*, the etiological agent of malaria. None of the synthesised products were active against either chloroquine sensitive (D6) or resistant (W2) strains of *P. falciparum* at the maximum concentration tested (see ESI†). The fact that the series of analogues was only active against *L. donovani* parasites validates our hypothesis that the prepared library would be interacting with a common target that is biochemically relevant for fungi and kinetoplastids. Based on the reported precedents on the mechanism of action of coruscanone A and analogues, it is highly probable that their antileishmanial activity also results from their behaviour as Michael acceptors.

Further studies on other stages of the life cycle of the parasite as well as experiments to elucidate the mechanism of action of this series are currently being performed and will be reported in the future.

## Conclusions

This study establishes a new template for a novel class of anti-leishmanial agents which are easily prepared in one step *via* Knoevenagel condensation between 1,3-dicarbonyl substrates and α,β-unsaturated aldehydes. 2-Cinnamylidene-cyclopentane-1,3-dione derivatives showed to be the most active, making the ring form a privileged, but not exclusive, scaffold. The most active compound **6q** was superior to the amidine drug pentamidine and comparable to amphotericin B without being more cytotoxic against mammalian Vero cells.

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