Bioorganic & Medicinal Chemistry Letters 25 (2015) 1027–1031

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Further studies on anti-invasive chemotypes: An excursion from chalcones to curcuminoids

Bart I. Roman [a,†], Tine De Ryck [b], Sigrid Verhasselt [a], Marc E. Bracke [b], Christian V. Stevens [a,*]

[a] Research Group SynBioC, Department of Sustainable Organic Chemistry and Technology, Faculty of Bioscience Engineering, Coupure Links 653, B-9000 Ghent, Belgium
[b] Laboratory of Experimental Cancer Research, Department of Radiation Oncology and Experimental Cancer Research, Ghent University, De Pintelaan 185, B-9000 Ghent, Belgium

ARTICLE INFO

Article history:
Received 23 December 2014
Revised 13 January 2015
Accepted 15 January 2015
Available online 22 January 2015

Keywords:
Invasion
Cancer
SAR
Chalcone
Curcuminoids

ABSTRACT

In our ongoing search for new anti-invasive chemotypes, we have made an excursion from previously reported potent 1,3-diarylpropenones (chalcones) to congeners bearing longer linkers between the aromatic moieties. Nine 1,ω-diarylalkenones, including curcumin and bisdemethoxycurcumin, were evaluated in the chick heart invasion assay. Unfortunately, these compounds proved less potent and more toxic than earlier evaluated chemotypes. In the 1,3-diarylpenta-2,4-dien-1-one series, fluoro and/or trimethoxy substitution caused an increase in potency. This agrees with observations made earlier for the chalcone class.

The hallmark that differentiates malignant neoplasms from benign tumors is the ability of a subpopulation of cells to invade neighboring tissues and cause metastases.[1] These phenomena account for 90% of all cancer deaths and currently present the most significant hurdles in the management of malignant disease.[2,3]

Over the last years, our laboratories have been searching for novel chemotypes that can suppress the invasive phenotype of malignant cells (Chart 1).[4] Starting from natural 1,3-diphenyl-prop-2-en-1-ones,[5] the main hits in our Indo-Belgian anti-invasive screening program,[6] we have developed synthetic analogues belonging to the diarylchalcone (**1b**),[7] stilbene[8] (**2**,**3**) and diarylisoxazole/pyrazole (**4**,**5**) classes,[9] and reported on the structure–activity relationship of these compounds. These studies were conducted using the phenotypic chick heart invasion (CHI) assay as an in vitro model of invasion. The most promising molecule identified yet is chalcone **1c**, which displays strong anti-invasive efficacy at 0.01 μmol·L^{-1} in the CHI and comparable behavior in the matrigel invasion assay.

The present work intends to complement the above SAR studies by evaluating longer unsaturated spacers between the two aromatic moieties. Hence, we have prepared and evaluated an array of 1,ω-diarylalkenones **6** (Chart 1). For completeness, also curcumin and bisdemethoxycurcumin (**7**) were included in this study.

With respect to the latter compounds, ambiguous health claims have been put forward in both scientific and alternative literature.

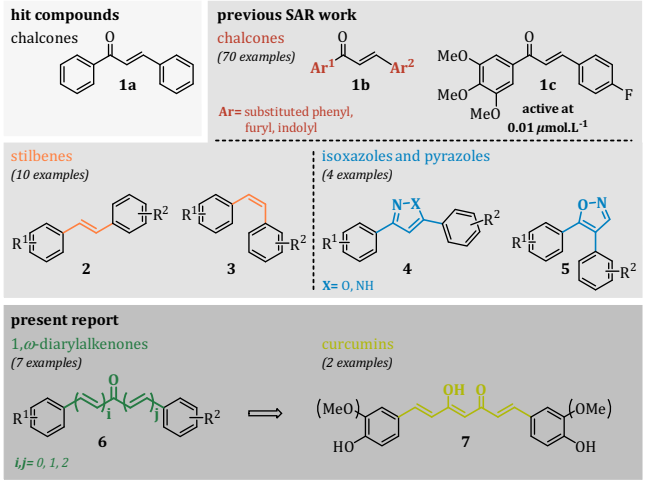

Chart 1. Overview of previously and presently investigated chemotypes in search of anti-invasive lead molecules.

* Corresponding author. Tel.: +32 9 264 5957; fax: +32 9 264 6221.
E-mail addresses: Bart1.Roman@UGent.be (B.I. Roman), Tine.DeRyck@UGent.be (T. De Ryck), Marc1.Bracke@UGent.be (M.E. Bracke), Chris.Stevens@UGent.be (C.V. Stevens).
URL: http://www.ugent.be/bw/doct/en (C.V. Stevens).
† B.I.R. is a postdoctoral fellow of the Research Foundation—Flanders (FWO—Vlaanderen).

http://dx.doi.org/10.1016/j.bmcl.2015.01.027
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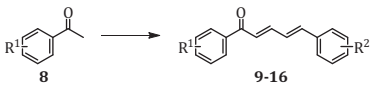
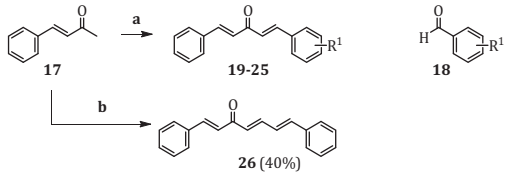


Scheme 1. Reagents and conditions: (1) 20 mol % $LiOH{\cdot}H_2O$, abs EtOH, rt or 40 °C, 10 min; (2) 1 equiv cinnamaldehyde, rt or 40 °C, 72 h, see Table 1.

Table 1
Preparation of 1,5-diarylpenta-2,4-dien-1-ones **9**–**16** (see also Scheme 1)

	R^1	R^2	Temp (°C)	Yield[a] (%)
9	H	H	rt	89
10	4′-Me	H	rt	67
11	4′-OMe	H	rt	70
12	4′-Cl	H	rt	64
13	4′-F	H	rt	67
14	4′-NO_2	H	rt	90
15	3′,4′,5′-OMe	H	40	65
16	3′,4′,5′-OMe	F	40	37

[a] Determined upon recrystallization in absolute EtOH.



Scheme 2. Reagents and conditions: (a) (1) 20 mol % $LiOH{\cdot}H_2O$, abs EtOH, rt, 10 min; (2) 1 equiv substituted benzaldehyde **18**, rt, 72 h, see Table 2; (b) (1) 20 mol % $LiOH{\cdot}H_2O$, abs EtOH, rt, 10 min; (2) 1 equiv cinnamaldehyde, Δ, 2 h.

Table 2
Preparation of 1,5-diarylpenta-1,4-dien-3-ones **19**–**25** (see also Scheme 2)

	R^1	Yield[a] (%)
19	H	77
20	4-Me	68
21	4-OMe	88
22	4-Cl	87
23	4-F	89
24	4-NO_2	90
25	2,4,6-OMe	87

[a] Determined upon recrystallization in absolute EtOH.

However, evidence suggests that, far from being a *panaceum*, curcumin possesses liabilities such as the induction of herb–drug interactions.[10,11] A more critical attitude with respect to this putative drug is desirable, since a clear view on the risk/benefit-ratio of curcumin and its synthetic analogues[12] is yet to be established. In the light of the above, it should be stressed that focus in the present research was not on the supposed merits of curcuminoids as such, but on a better grasping of the SAR of anti-invasive chalcones through evaluation of their curcuminoid congeners.

Our preparation of a curcuminoid library started with the selection and synthesis of 1,5-diarylpenta-2,4-dien-1-ones and 1,5-diarylpenta-1,4-dien-3-ones. Since the physicochemical parameters influencing distribution (e.g., *c*Log*P* and PSA) are very similar for identically substituted C_6–C_5–C_6 alkenones and chalcones, a difference in activity in the CHI assay can be attributed mainly to altered target affinity or stability. Comparison between the two selected C_6–C_5–C_6 frameworks would also provide information on the influence of the location of the carbonyl group on activity.

An array of 1,5-diarylpenta-2,4-dien-1-ones **9**–**16** was synthesized in good yields out of suitably decorated acetophenones and cinnamaldehydes via a LiOH-catalyzed Claisen–Schmidt condensation (Scheme 1, Table 1).[7,13] This is the first dedicated report on the use of $LiOH{\cdot}H_2O$ for the preparation of 1,5-diarylpenta-2,4-dien-1-ones. Only one related report has been published, in which compound **9** was obtained as a side product in the asymmetric $LiOH{\cdot}H_2O$-catalyzed Michael addition of acetophenone to cinnamaldehyde, conducted in the presence of the trimethylsilyl ether of (*R*)-diphenylprolinol.[14]

In an identical way, seven diversely substituted 1,5-diarylpenta-1,4-dien-3-ones **19**–**25** were prepared from 4-phenylbut-3-en-2-one **17** and a suitably decorated benzaldehyde **18**. All substances were obtained in very good yield and high purity (Scheme 2, Table 2).

In an excursion to C_6–C_7–C_6 curcuminoids, 1,7-diarylhepta-1,4,6-trien-3-one **26** was prepared (Scheme 2), so as to evaluate the behavior of molecules with a 7-atom linker. For completeness, curcumin **27** and bisdemethoxycurcumin **28** were also included in the library.

Having an array of 1,ω-diarylalkenones in hands, we proceeded with the evaluation of their in vitro anti-invasive activity. An optimal in vitro screening strategy for anti-invasive molecules has to discern:

(i) *Anti-invasive effects from mere toxicity.* We ultimately seek anti-invasive agents with in vivo efficacy. The main task of such molecules is to suppress invasion, not primary tumor growth. Hence, these molecules need not *per se* be cytotoxic towards neoplastic cells.

A

Grade	Observation
0	Only PHF is present; no confronting cells are observed.
I	Confronting cells are attached to the PHF, but do not occupy the heart tissue, even not the outermost fibroblastic cell layers
II	Occupation is limited to the outer fibroblast-like and myoblast cell layers; confronting cells may have surrounded the cell aggregate.
III	Confronting cells have occupied less than half of the original amount of heart tissue.
IV	Confronting cells have occupied more than half of the original volume of the PHF.

B

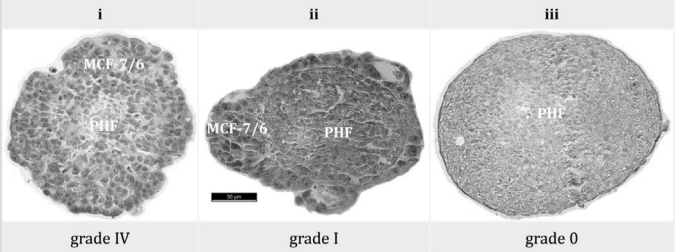
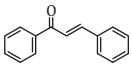
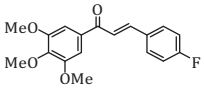
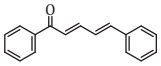
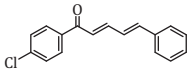
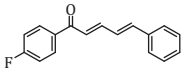
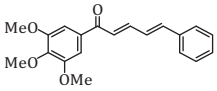
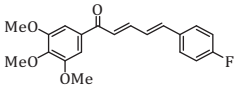
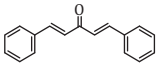
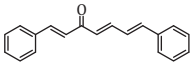
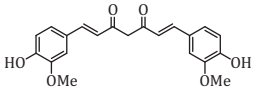
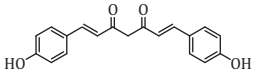


Chart 2. This illustration of the chick heart invasion assay displays 7 µm thick sections from confronting cultures between invasive human mammary carcinoma cells (MCF-7/6) and precultured heart fragments (PHF). Panel **A**: scoring criteria. Panel **B**: from left to right: (i) results from an untreated culture, in which the confronting cells have occupied more than half or the PHF, invasion grade IV: (ii) addition of an anti-invasive agent resulted in a clear histological separation between the confronting partners; the invasion grade is denoted as I; (iii) outcome for a product with selective toxicity for MCF-7/6 cells with respect to the PHF. No judgment of the anti-invasive potency can be made; this pattern is coined grade 0.

Table 3
In vitro anti-invasive activity data against MCF7/6 cells, obtained for nine 1,ω-diarylalkenones in the CHI-assay (I and II = no invasion, the compound is coined active; III and IV: invasion, the compound is inactive)

	Structure	Invasion grade at different concentrations (μmol·L^{-1})					Anti-invasive activity class[a]
		100	10	1	0.1	0.01	
1a		n.d.	**II**	III	n.d.	n.d.	**1**[g]
1c		n.d.	0[b]/I[e]	II/I	I/II	**II/II–III/I**	–**2**[g]
9		T/**II**[b]	III–IV[h]/III	III	IV	n.d.	**2**
12		III/II	III	II/III	III/IV	n.d.	**3**
13		I[d]	T	**II**	III	III	**0**
15		T/II[b]	**I**[d]/**II**[d]	III/III/IV	III	III	**1**
16		n.d.	**II**/**II**[h]	III/III[f]	III/III	n.d.	**1**
19		T/I[d]	II/I[e]	**II**	III	IV/III	**0**
26		II[d]/II[c]	I/II[c]	**II**	IV	IV/III	**0**
27		n.d.	**II**	III/IV	IV/II[f]	n.d.	**1**
28		n.d.	**II**[d]/**II**[d]	III	III	n.d.	**1**

In all runs, solvent (negative) control gave grade III/IV. All results were obtained at least in duplo; for identical outcomes, only one number is shown.
T: toxic for PHF and MCF-7/6, to a degree that renders judgment impossible; n.d.: not determined.
Scores at the lowest active concentration are highlighted in bold.

[a] Log c_{min}.
[b] Toxic for MCF-7/6.
[c] Toxic for PHF.
[d] Toxic for PHF and MCF-7/6.
[e] Very few MCF-7/6 cells left.
[f] PHF dedifferentiated.
[g] See Ref. 7.
[h] See photograph in Chart 3.

(ii) *Effects on tumor cells from effects on stroma.* The inherent complexity of tumor spread mandates that the involvement of the host tissue in the micro-ecosystem that governs tumor behavior should not be overseen.[4,6]

In such a case, a phenotypic, tissue-based assay constitutes the most informatory test setup. The chick heart invasion (CHI) model,[15] developed at the Laboratory of Experimental Cancer Research (Ghent University, Belgium), possesses all of these properties, and was accordingly used to evaluate the anti-invasive potency of our diarylalkenones. The behavior of numerous antiproliferative agents (e.g., vinblastine, vincristine, taxol, nocodazole, podophyllotoxin)[16] and of Hsp90 inhibitor AUY922[17] in this assay is well-documented.

In the model, precultured heart tissue fragments (PHFs), dissected from 9-day-old chick embryos, are confronted with aggregates of human invasive MCF-7/6 mammary carcinoma cells in the presence of a certain concentration of a test substance. After

eight days, the interaction between the partners is evaluated histologically and classified along a 5-grades scale (Chart 2).

Compounds are designated 'active' or 'anti-invasive' when the invasion grade is I or II, and 'inactive' when the grade is III or IV. For substances that exhibit selective toxicity versus MCF-7/6 cells, a judgment of the anti-invasive potency cannot be made (invasion grade 0).

Because the CHI assay is not high throughput, nine 1,ω-diarylalkenones were chosen for evaluation. A test set was compiled that would enable an assessment of the influence of the different linkers (keeping the substitution pattern unchanged), as well as the role of the aromatic substituents (keeping the linker unchanged). For these nine compounds, the invasion grade in the CHI assay was determined at concentrations ranging from 100 down to 0.01 μmol·L^{-1} (Table 3). The assay data was translated into one score per compound (Eq. 1): 'Log c_{min}', being the decadic logarithm of the lowest concentration (expressed in μmol·L^{-1}) at which a substance exhibits an anti-invasive behavior (invasion grades I or II). Six activity levels exist, ranging from class −2 for the most active compounds (invasion grade I or II at the 0.01 μmol·L^{-1} level) to class 3 (compounds with no apparent effect at a concentration of 100 μmol·L^{-1}):

$$\text{Anti-invasive activity class} = \log\ c_{min} \quad (1)$$

From the results displayed in Table 3, it is clear that alkenones **9**, **12**, **15**, **16**, **27** and **28** display a very low anti-invasive potency. Molecules **13**, **19** and **26** proved active down to 1 μmol·L^{-1}. Representative images of these results are presented in Chart 3.

The disappointing results obtained for curcumin (**27**) and bisdemethoxycurcumin (**28**) in the CHI model do not contradict the wealth of reports on the anti-invasive activity of these compounds. The efficacy documented in literature is mostly observed at high concentrations. For example, in one of the most relevant recent reports, using MCF-7 cells in the matrigel assay, curcumin was used at a concentration of 30 μmol·L^{-1}.[18] We also observe efficacy at 10 μmol·L^{-1} for curcumin, but not at lower concentrations. Therefore, we must conclude that the potential of curcumin is inferior to that of some of our earlier investigated compound classes (i.e., chalcones and stilbenes).

Overall, the evaluated 1,ω-diarylalkenones also seem to possess a low safety margin: the majority of the evaluated molecules display cytotoxic effects against the heart tissue at the lowest anti-invasive concentration or one decade above. In all, the most attractive profile is exhibited by compound **19**: this molecule is toxic for both PHF and MCF-7/6 cells at 100 μmol·L^{-1}, selectively toxic for the malignant cells at a tenfold lower concentration, and still mediating anti-invasive effects another decade lower.

Although no exceptional anti-invasive behavior was observed for the assessed molecules, the obtained results bear value from a SAR point of view. The main goal of this investigation was to gain insight into the influence of the type of spacer between the aromatic moieties on activity. Such an evaluation is best conducted for the unsubstituted scaffolds (series 1, Chart 4) and for the analogs of the most promising molecule in our anti-invasive library, 4-fluoro-3′,4′,5′-trimethoxychalcone **1c** (series 2). An overview of the potency and a number of relevant properties of these two series is presented in Chart 4.

Judged by activity class alone (Log c_{min}), our data indicate that, within both series, linker modifications are not well-tolerated.

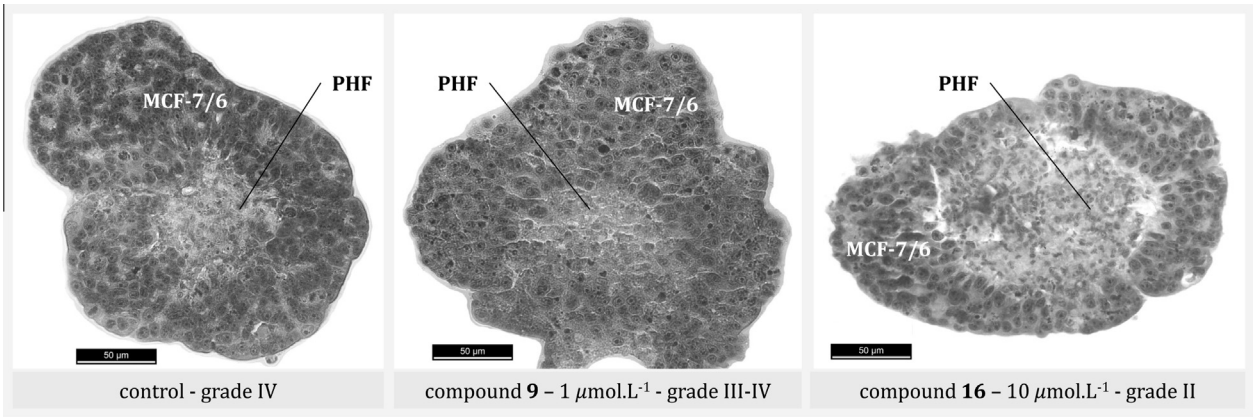


Chart 3. Representative images of notable results from the CHI assay (see also Table 3).

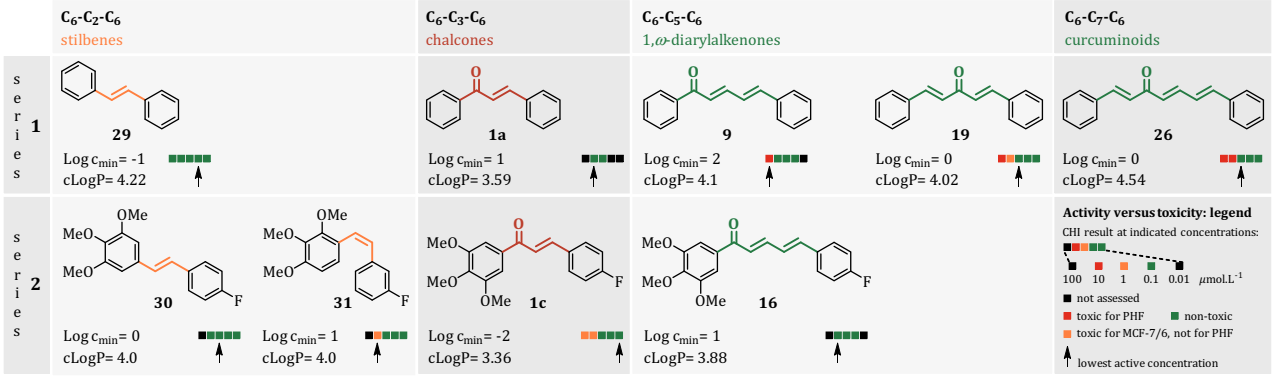


Chart 4. Comparison of the potency and toxicity of homologous stilbenes, chalcones and 1,ω-diarylalkenones in the CHI assay.

Besides, potency comparison across the two series within a given compound class (stilbene, chalcone, curcuminoid) shows that optimal linker size and geometry are dependent on the decoration pattern of the aromatic moieties. From a potency point of view, optimization of the aromatic substitution pattern and the linker should thus not be considered separate exercises.

When taking into account other properties (lipophilic efficiency, toxicity), it is evident that the curcuminoid class performs poorer than the stilbene and chalcone classes. Overall, chalcone **1c** remains the molecule with the most attractive profile: reasonable Log*P*, high anti-invasive potency but no cytotoxicity at nanomolar concentrations, and selective toxicity towards MCF-7/6 cells at 10 $\mu mol \cdot L^{-1}$.

For completeness, a short analysis can be made of the influence of the substitution pattern of 1,5-diarylpenta-2,4-dien-1-ones on activity and toxicity. Compared to the unsubstituted scaffold (**9**), introduction of a chlorine atom at the 4′-position (**12**) lowers toxicity but does not strongly influence activity. Similar to what has been observed for the chalcone class, trimethoxy- and/or fluoro substitution is attractive from a potency point of view. However, the gain in potency observed for the 3′,4′,5′-trimethoxy pattern (**15** and **16**) comes with an increase in undesired effects (toxicity, dedifferentiation). Similarly, while the presence of a fluorine atom at the 4′-position in curcuminoid **13** has a positive effect on activity, resulting in anti-invasive efficacy down to 1 $\mu mol \cdot L^{-1}$ (class 0), this molecule is particularly toxic at 10 $\mu mol \cdot L^{-1}$. Remarkably, none of our earlier evaluated fluoro chalcones proved toxic for the PHF.[7] Taken together, the aromatic substitution pattern of the 1,5-diarylpenta-2,4-dien-1-ones has a strong influence on both toxicity and potency.

In conclusion, the evaluated 1,ω-diarylalkenones, including curcumin and bisdemethoxycurcumin, lack nanomolar potency and display higher toxicity than their chalcone and stilbene analogs. Still, 1,5-diphenylpenta-1,4-dien-3-one **19** exhibits an interesting cytotoxicity profile. Analogous to what was observed earlier for the chalcone class, trimethoxy and/or fluoro substitution yields more potent compounds (**13**, **15** and **16**). However, in the case of the diarylpentenones and -heptenones, this modification entails undesired effects (toxicity, dedifferentiation).

Overall, compared to the curcuminoid and stilbene series, the chalcone class remains the most interesting source of anti-invasive compounds in this research program, with 4-fluoro-3′,4′,5′-trimethoxychalcone as the exponent.

Acknowledgements

The authors thank the Research Foundation—Flanders (Fonds voor Wetenschappelijk Onderzoek—Vlaanderen) for financial support for this research, and would like to express their sincere appreciation to Marleen De Meulemeester for meticulously conducting the CHI-assay on all compounds investigated in this study.

Supplementary data

Supplementary data (experimental details, compound characterization and ^{1}H, ^{13}C and ^{19}F NMR spectra of selected compounds) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.01.027.

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