# Chalcones as anti-invasive agents: challenges and opportunities

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## SUMMARY

Invasion and metastasis are responsible for 90% of cancer-related mortality. An overview is presented of the issues and opportunities regarding the development of effective inhibitors of these phenomena. Our efforts in the discovery and validation (*in vitro* and *in vivo*) of synthetic chalcones as potent anti-invasive agents are summarised, taking into account various concerns regarding the suitability of the chalcone scaffold as a template for the development of bona fide pharmacological tools.


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## INTRODUCTION

Over the past decades, major advances have been made in the understanding and tackling of primary tumour growth. Unfortunately, mortality figures for many cancer types have not declined accordingly. The main reason for this discrepancy is that 90% of cancer-related deaths are not caused by tumour growth, but by the sequelae of invasion and metastasis.[1] Since half of the patients present with localised disease, a significant survival benefit can be expected from agents modulating the mechanisms contributing to invasion and metastasis.[2] Unfortunately, there are no effective drugs available in the clinic for these indications. The present paper's objective is to critically assess the opportunities offered by small molecules of the chalcone class to tackle this unmet medical need. First, the issues associated with the development of anti-invasive and antimetastatic agents, as well as the literature on antineoplastic chalcones, will be summarised.

## STATE OF THE ART

At present, no drugs have been registered for an antimetastatic indication. The majority of investigational drugs are still targeting tumour growth, although the unmet need for the former is by far larger. A variety of reasons underlie this disparity.[3-5]

Firstly, with respect to the primary tumour, metastatic cells have distinct mutations, expression profiles and stroma, and are often subject to lower drug exposure. Therefore, drugs targeting primary tumours have limited effects on metastases. Secondly, until recently, a lack of understanding of the underlying events made rational targeting of invasion and metastatic dissemination impossible. Accordingly, there was a long paucity of relevant preclinical models. Furthermore, clinical trial designs and schedules are not compatible with the endpoints and timelines relevant to antimetastatic therapy. Finally, the few antimetastatic drugs that made it into development (e.g. MMP and integrin inhibitors) suffered late-stage clinical failures. All this has led to a total deprioritization of anti-metastatic drug development by pharmaceutical companies.

Our ever increasing understanding of the cellular and molecular events that drive invasion and metastasis has led to the identification of several druggable targets. These insights have also enabled the development of superior *in vitro* and *in vivo* models, a better appreciation of their limitations, and the advent of appropriate pharmacodynamic biomarker endpoints. Moreover, developmental issues are being tackled. The NIH has presented viable clinical trial designs tailored to the particularities of metastasis-preventive compounds.[6] Together with

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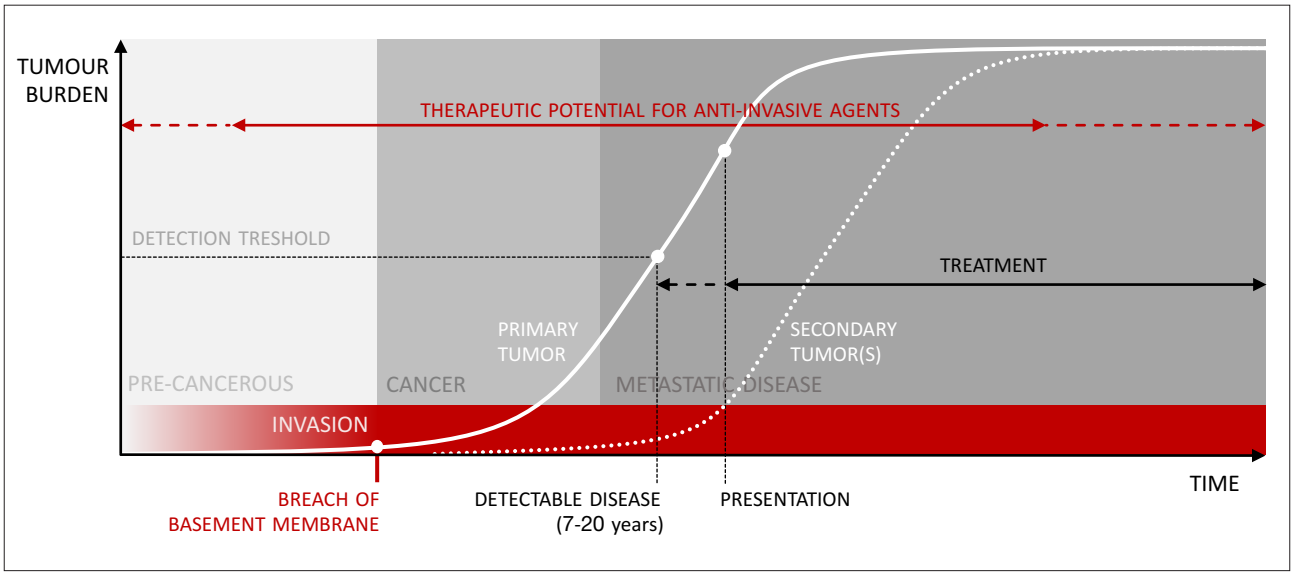


**FIGURE 1** Cancer development timeline (adapted from *Jones et al.*).[4]

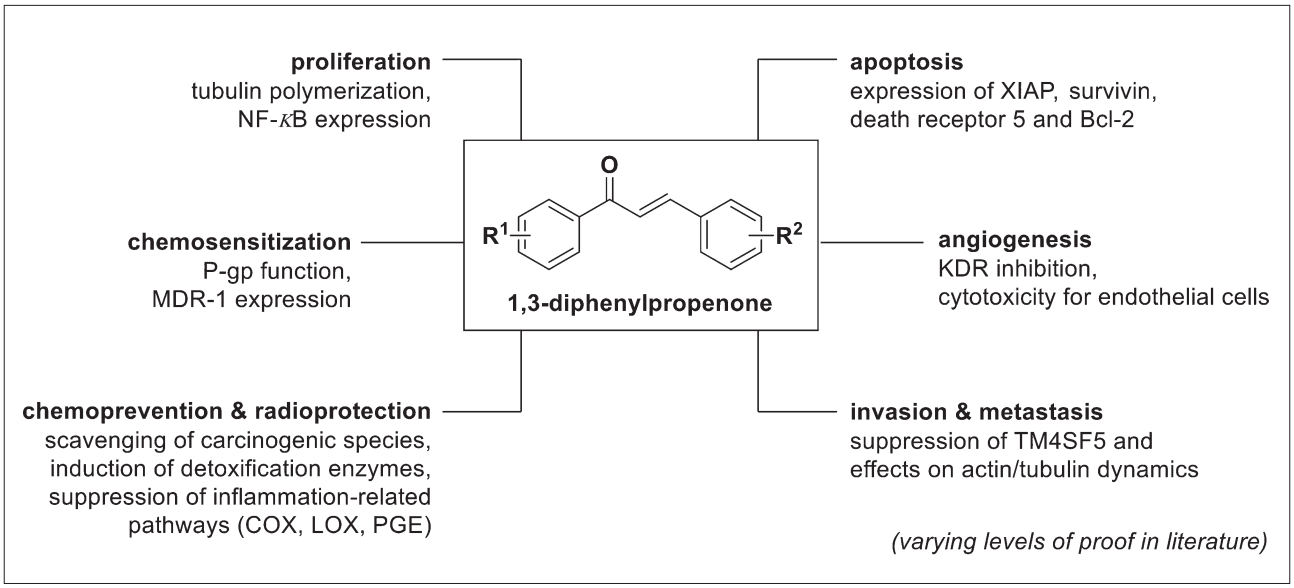


**FIGURE 2** Chalcones: structure and putative *in vivo* modes of action/molecular targets.[9]

the rise of non-profit drug discovery and development institutions, a commitment has surfaced to reduce cancer patient morbidity and mortality by tackling metastasis.[6,7] These organisations have the resources to set up a limited clinical trial themselves. Such an endeavour is needed in order to advance the field: industry will await success before committing to investments.

## POTENTIAL OF ANTI-INVASIVE THERAPY

Far from a linear cascade of events, metastasis is nowadays seen as a complex, multistep and multifunctional phenomenon. Several key steps require an invasive phenotype, i.e. the ability to detach from the primary tumour and breach the basement membrane (BM) and extracellular matrix (ECM) of tissues (primary tumour, endothelium of blood and/or lymphatic vessels, colonised tissue). This requires alterations in adhesion mechanisms, motility and proteolytic enzyme activity, as well as changes in cross-talks with and behaviour of the tumour microenvironment. The underlying pathways contain targets for anti-invasive therapy.

Invasion and metastasis may occur early or late during primary tumour formation, and may require a brief period or decades to complete. Goals of anti-invasive therapy include the reduction of morbidity and mortality due to prevention of initial metastasis in risk groups,

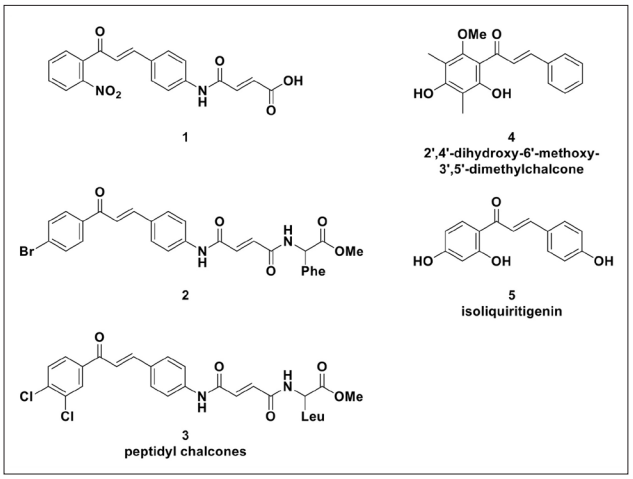


**FIGURE 3** Known promiscuous chalcones.[9]

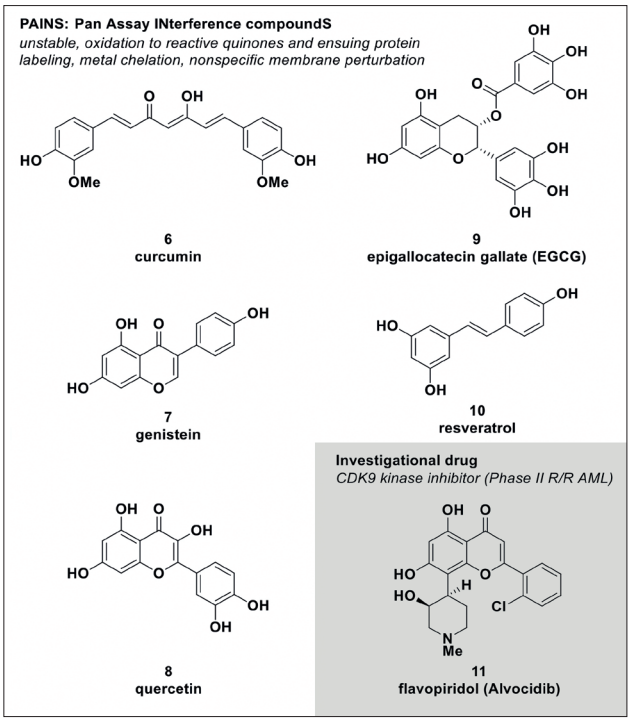


**FIGURE 4** Structural resemblance of PAINS and investigational drugs.

and additional metastases in patients with limited disease (*Figure 1*). A further aim is survival prolongation in late-stage disease by the inhibition of reactivated metastatic showers. Also, therapy-induced metastasis may be attenuated by the administration of appropriate adjuvants. Finally, anti-invasive drugs may be of benefit in the management of local invasion in malignant glioma.

## CHALCONES

Our laboratory is presently involved in the discovery and preclinical validation of novel anti-invasive pharmacological tools.[8] Besides synthetic molecules, we have scouted for active chemotypes in the natural product realm. An area of particular attention has been that of the chalcones (1,3-diphenylpropenones), a class of secondary metabolites belonging to the flavonoid family. Many reports have appeared on the putative *in vivo* antineoplastic activity of chalcones, either as single agents or as adjuvants to chemo- or radiotherapy. *Figure 2* enlists those mechanisms for which *in vivo* evidence of efficacy has been provided (albeit to a varying extent).[9] Detailed analysis of the literature used to compile *Figure 2* (for the complete list of papers, consult reference 9) raises multiple concerns. Firstly, several reports use *in vitro* data obtained at high concentrations (≥10 $\mu$M), and may thus concern artificial effects. Moreover, the target profile of the chalcone scaffold appears capricious, as small structural changes can radically alter target affinity. In reality, many of these chalcones may be promiscuous, and in none of the studies was a selectivity profile determined. Multi-targeted behaviour has been confirmed for several chalcones, e.g. compounds 1-5 (*Figure 3*).

A more fundamental problem that surfaces when a family of closely related molecules signals against a range of targets over a range of assay platforms, as for the chalcones, is non-specific interference. Pan assay interference compounds (PAINS) do not engage in drug-like interactions with protein binding sites, but interfere in chemical and cellular assay readouts through behaviour such as aggregation, membrane perturbation, redox activity, metal chelation, fluorescence, protein labelling, chemical instability or promiscuous binding.[10] Avoiding PAINS requires a comprehensive literature survey, potent and relevant cell-based activity, confirmatory assays and the presence of sharp SAR and meaningful PK/PD. Natural products are not immune to PAINS behaviour (*Figure 4*).[11] In particular polyhydroxylated secondary metabolites are notorious in this respect. Many putative antineoplastic chalcones bear strong structural resemblance to the latter compounds. Nevertheless, the PAINS issue begs for bidirectional caution: flavopiridol 11 (*Figure 4*), for example, is both a polyhydroxylated flavanone and drug undergoing phase II clinical trials. Also marketed drugs contain PAINS motifs. The discovery of these bona fide molecules went through traditional approaches, involving initial activity observations at close to therapeutically relevant concentrations in animal models. Therefore, while PAINS concerns for anti-

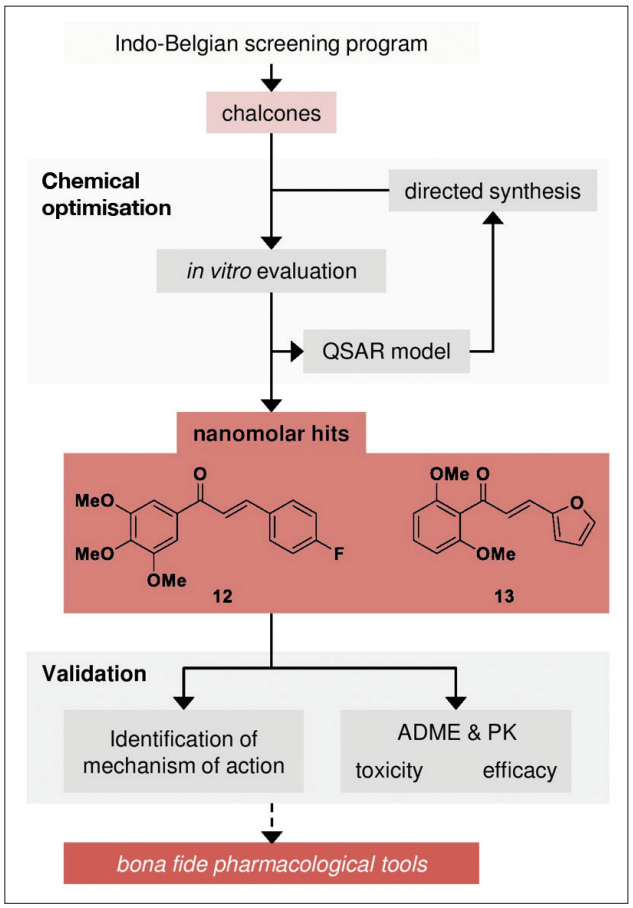


**FIGURE 5** Discovery, optimisation and validation program for anti-invasive chalcones.[8]

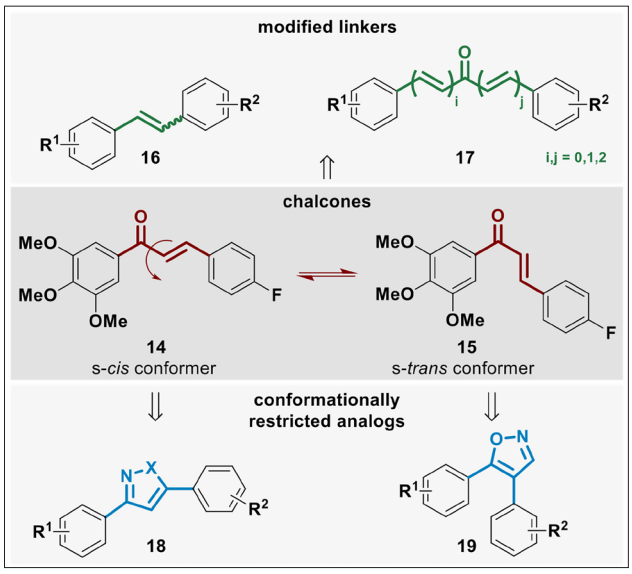


**FIGURE 6** Synthesised chalcones and analogues during our SAR investigations.[8,13,14]

cancer chalcones are certainly warranted, the activities in *Figure 2* should not be discarded as such, as they were observed in animal models.

There are several other issues with the literature on antineoplastic chalcones. Many compounds were tested in only one animal model, with oftentimes low relevance to the pathogenesis in man. In several *in vivo* experiments, irrelevant dose levels were used. Another problem is that none of the putative agents has been subjected to validation studies. Determination of their genuine potential as (leads for) pharmacological tools requires a more detailed characterisation of pharmacodynamics, pharmacokinetics and toxicity. A final hurdle in the development of chalcones is the crowded legal landscape.

Taken together, chalcone literature has been poised with reports of scattered activities, oftentimes for disreputable structures or at irrelevant concentrations. Though striking *in vivo* effects have been observed, additional evidence is needed to shift from conjecture to empirical proof. PAINS, pharmacokinetics and toxicity issues must be addressed. If no red flags emerge, investments in compound evaluation in relevant *in vivo* models are justifiable. Bona fide agents can then be optimised, taking into account patentable chemical space.

## OWN RESEARCH

Over the last years, we have been involved in the development of effective invasion inhibitors out of chalcone hits. The starting point for this effort was a data set on the anti-invasive activity of hundreds of natural compounds (polyphenols, alkaloids, steroids, etc.), stemming from the Indo-Belgian screening program.[8] This data was obtained in the chick heart invasion (CHI) assay, an organotypic model in which normal chick heart tissue is confronted with aggregates of human invasive mammary MCF-7/6 carcinoma cells.[12] In this assay, invasion is defined as the progressive occupation and destruction of the heart tissue by the confronting partner. The model incorporates host cells and matrix as well as cytokines, thus mimicking physical and biochemical cues of the tumour environment. The CHI has proved highly relevant to the situation in humans.

Looking closer at the screening results, our attention was drawn towards chalcones: five out of the eleven most potent compounds (1 $\mu$M) shared the 1,3-diarylpropenone scaffold. Accordingly, these chalcones were considered hits and taken further into an optimisation

## KEY MESSAGES FOR CLINICAL PRACTICE

1. **Non-profit drug discovery and development institutions get ready to set up much needed clinical trials of antimetastatic agents in order to break their development standstill.**
2. **Anti-invasive agents may be useful in several disease stages, and both for local invasion and distant metastasis.**
3. **Natural product research efforts should focus on bona fide inhibitors, and thoroughly scan for PAINS behaviour.**
4. **Members of the chalcone chemical class may provide an entry to potent and efficacious pharmacological tools for the in vivo modulation of metastasis, but considerable preclinical evaluation remains necessary.**

and validation program (*Figure 5*).[8] Indeed, in the light of the above concerns surrounding literature chalcones, we designed not only a chemical optimisation round, but also a thorough validation program.

The first part of the work focused on obtaining a thorough understanding of the structure-activity relationship (SAR) of the decoration pattern of the aromatic rings (*Figure 5*). To this end, a quantitative SAR (QSAR) model was constructed and validated. This computational model successfully correlates parameters that can be computed solely from molecular structure to the lowest efficacious concentration in the CHI assay. The model enabled us to screen large libraries of hypothetical chalcones and focus synthetic efforts on molecules with a favourable potency prediction. Two promising chalcones surfaced during this SAR work: 12 and 13 proved efficacious in the CHI down to 10 and 100 nM, respectively.

Next, we investigated the role of the propenone linker (*Figure 6*). Firstly, linker shortening was tested. A set of (*E*)- (*Z*)-stilbenes 16 with similar substitution patterns as 12 and 13 were prepared.[13] Four compounds proved efficacious down to 10 nM. Congeners bearing longer linkers (17) proved less potent and more toxic.[14] Within all series, fluoro and/or trimethoxy substitution resulted in increased potency, thereby confirming the earlier SAR work. Finally, *in vitro* evaluation and *in silico* geometry studies on conformationally restrained mimics (18,19) allowed us to obtain a clearer view on the active conformation of the chalcones, which is presumably s-*trans* like.[15]

Based on the above data, chalcones 12 and 13 were chosen as prototype molecules.[8] Their efficacy was confirmed in a counter screen (Matrigel invasion assay) against melanoma (BLM) and ovarian carcinoma (SK-OV-3) cells. Furthermore, both molecules were shown to have a satisfactory pharmacological profile in terms of physicochemical properties, target promiscuity and *in vivo* toxicity. Compound 12 caused significant and meaningful prolongation of survival in an artificial metastasis model in nude mice (intracardiac injection of MCF-7/6 cells). Meanwhile, we have also confirmed satisfactory oral bioavailability and systemic exposure (unpublished data). Together with the nanomolar potency and sharp SAR, this data allowed us to eliminate PAINS concerns and most other issues cited above. We are presently evaluating compound 12 in more complex *in vivo* models of invasion and metastasis.

A phenotypic model as a primary screen has the (dis) advantage that pluralities of targets are probed. Therefore, to date, the exact mechanism exploited by our anti-invasive chalcones remains elusive. Nevertheless, we have strong indications that both prototype molecules act through the same mechanism(s) of action. Importantly, the observed effects are not due to cytotoxicity: for 12 and 13, anti-invasive and cytotoxic levels are separated by a concentration factor of >100. Besides, targets commonly associated with antineoplastic chalcones have been eliminated. Thus, the compounds do not act through an evident, but more likely through a novel mechanism of action. We are currently deploying powerful techniques (e.g. next-generation sequencing and proteomics-based approaches) to identify the direct interaction target and underlying pathways.

## CONCLUSION

There is an urgent and unmet clinical need for therapies that inhibit metastasis, and a strong will to tackle this challenge. Anti-invasive agents may be of benefit in several disease stages. We have discovered molecules with

potent anti-invasive activity in disease-relevant models. These compounds belong to the chalcone chemical class, for which severe concerns exist regarding their suitability as bona fide pharmacological tools. Nevertheless, thorough validation efforts have not brought up red flags. One of our prototype molecules exhibits *in vivo* efficacy and possesses a good pharmacological profile. Present focus lies on the evaluation of this compound in more relevant *in vivo* models, and on the elucidation of its molecular mechanism of action.

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