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Original article

Design, synthesis and biological evaluation of some isatin-linked chalcones as novel anti-breast cancer agents: A molecular hybridization approach

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ABSTRACT

Isatins are endogenous molecules present in human and mammals which exhibits diverse pharmacological profiles including anticancer activity. Similarly, chalcones, which are common substructures in numerous natural products belonging to the flavonoid family, show potent anticancer properties. A novel series of 3-(2-oxo-2-phenylethylidene)indolin-2-ones incorporating pharmacophoric elements of isatins and chalcones were designed and synthesized. The compounds were evaluated for anticancer activity against three breast cancer cell lines. Most of the compounds showed promising anticancer activity (<20 μM) against the studied cell lines. Compound **2c**, bearing a 5-chloro substituent in the benzo ring of the isatin moiety and 3,4-dimethoxy substitutions in the phenyl ring, was found to be the most active in the series with GI_{50} values of 8.54, 4.76 and 3.59 against MDA-MB231, MDA-MB468 and MCF7 cells, respectively. Overall, the findings of the study highlight 3-(2-oxo-2-phenylethylidene)indolin-2-one as a potential new lead in the search of drugs for the treatment of breast cancer.

1. Introduction

Breast cancer is one of the most commonly diagnosed cancers among women in both developed and developing countries [1]. Breast cancer is the second leading cause of cancer deaths in women worldwide today [1]. The currently available breast cancer therapies achieve meaningful clinical results in only 30–40% of the patients [2]. The efficacy of current chemotherapeutics is low and undesirable side effects are still unacceptably high [3]. Hence, the development of novel, efficient, and less toxic anti-breast cancer agents remains an important and challenging goal of medicinal chemists worldwide.

"Molecular hybridization" is one of the many strategies, which have been successfully applied for the design, and development of new and efficient chemotherapeutic agents [4]. Molecular hybridization involves the combination of two distinct pharmacophores or chemical entities by either linking or fusing each other to form new hybrid moieties [4,5]. The selection of the pharmacophores or chemical moieties is based upon their known bio profiles and it is expected that the hybrid molecules might exhibit synergistic or additive pharmacological activities [6,7].

Isatin (1*H*-indole-2,3-dione) is a privileged scaffold endowed with broad spectrum biological properties [8], including antitumor [9], antiangiogenic [9], antiviral [10–12], antibacterial [13], antitubercular [12], antifungal [13], anticonvulsant [14] and antimalarial activities [15]. The FDA approval of the oxindole-based sunitinib maleate (Sutent®) for the treatment of advanced renal carcinoma [16] and gastrointestinal stromal tumours [17] have established isatin as a potentially useful lead for anticancer drug development. The available scientific literature is abundant with reports on the development of isatin-based molecules against a variety of cancers (Fig. 1) [9]. Studies on the anticancer activity exerted by isatins have indicated that they act through multiple molecular mechanisms. Prominent among them are tyrosine kinase inhibition (TKIs), inhibition of cyclin-dependent kinases (CDKs) by binding to the ATP pocket and/or caspase inhibition [17–20]. On the other hand, chalcones have proved to be a useful structural motif in medicinal chemistry, having applications in the development of drugs for the treatment of malaria, cancer, protozoal infections, inflammation, etc [21,22]. The significance of the chalcone scaffold in the biological system can be realized being a common substructures in numerous natural products belonging to the flavonoid family [3]. Numerous reports have emerged on interesting anti-breast cancer activity exhibited by chalcones [23–25]. A recent revelation from our lab has shown the potent anti-breast cancer activity of a series of coumanrinyl chalcones [26].

In light of these findings, we postulated that combining the pharmacophoric elements of isatin and chalcones in a single chemical framework as isatin-linked chalcones (Fig. 1) and investigation of its anti-breast cancer activity will be worthwhile. Hence, the

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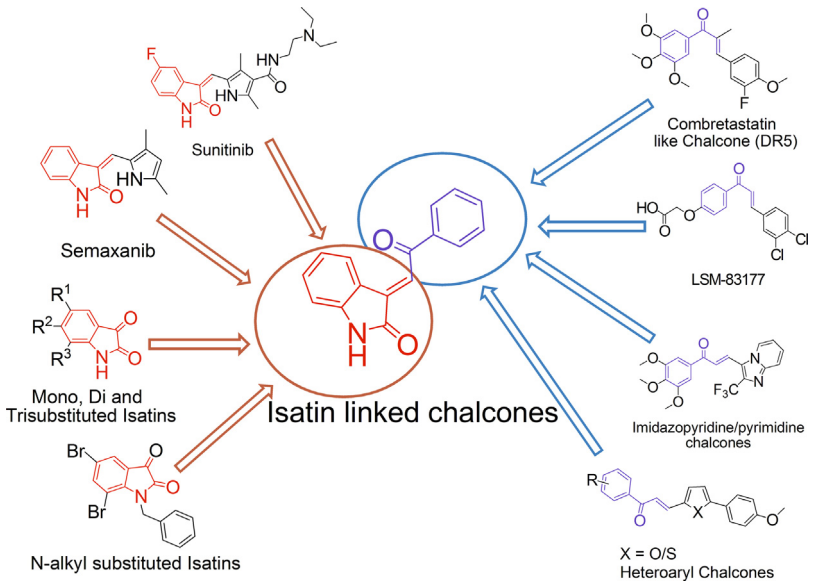


Fig. 1. Design of isatin-linked chalcones from pharmacophoric elements of known isatin and chalcones for their anticancer activity.

present study aims to synthesize a series of isatin-linked chalcones and evaluate their potential as anti-breast cancer agents.

2. Results and discussion

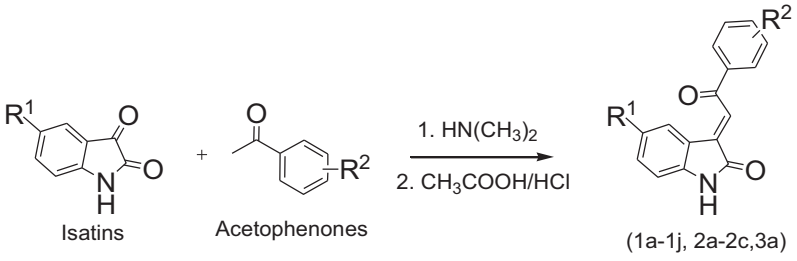
2.1. Chemistry

The synthesis of 3-(2-oxo-2-phenylethylidene)indolin-2-ones was accomplished through a one pot protocol involving the sequential addition of piperidine and concentrated HCl to a reaction mixture containing isatin and the desired acetophenones (Scheme 1) [27]. The structures of all the synthesized compounds were confirmed by ^{1}H NMR, IR and mass spectral studies. The composition of the synthesized compounds was confirmed by elemental analysis.

2.2. Biological evaluation

The synthesized compounds were tested for growth inhibitory activity against a three breast cancer cell line panel consisting of MDA-MB468 (a PTEN defective, intermediately differentiated, EGFR positive breast adenocarcinoma cell line), MDA-MB231 (estrogen receptor negative, basal-like breast cancer) and MCF7 (p53+/-, differentiated, invasive ductal breast carcinoma). Each compound, stored at 20 mM (stock), was diluted from 100 μM to 0.0064 μM by five-fold serial dilutions. Cells were treated with each compound for 48 h, followed by measuring cell growth rates by SRB-based spectrophotometry as described previously [28–30]. The reading of SRB staining is known to accurately reflect the levels of total cellular macromolecules/cell growth/proliferation [29]. The 50% growth inhibition (GI_{50}) concentration for each compound was calculated with reference to a control sample, which represents the concentration that results in a 50% decrease in cell growth after 48 h incubation in the presence of the drug. For each compound, GI_{50} was calculated from sigmoidal dose–response curves that were generated with data obtained from two independent experiments carried out each in triplicate and presented in Table 1.

As evident from Table 1, almost all the compounds exhibited greater growth inhibitory potency than the reference standard cisplatin against the three breast cancer cell lines with an exception of compound **1h**, which showed two-fold decreased potency than cisplatin against MDA-MB231 cell line. The unsubstituted compound **1a** showed five-fold higher growth inhibitory activity than cisplatin against MDA-MB468 and MCF7 cells while showing a two-fold greater growth inhibitory activity than cisplatin against MDA-MB231 cells. Encouraged by this, we sought to optimize the substitution on the phenyl ring linked to the carbonyl atom of 3-(2-oxo-2-phenylethylidene)indolin-2-one (**1a**). Fluoro substitution on the *para* position of the phenyl ring (**1b**) resulted in modest decrease in the growth inhibitory potency against MDA-MB468 and MCF7 cancer cell lines with no change in MDA-MB231 cell growth inhibition. Replacement of fluorine atom with either chlorine (**1c**) or bromine (**1d**) plunged the growth inhibitory potency against MDA-MB468 and MCF7 cancer cell lines. However, bromo substitution (**1d**) led to two-fold reductions in GI_{50} values against MDA-MB468 and MCF7 cell lines. The introduction of electron-withdrawing nitro group either at the *para* position (**1e**) or *meta* position of phenyl ring (**1f**) or electron-releasing methoxy group at *para* (**1g**) or dimethoxy substitution at *para* and *meta* position of



Scheme 1. One pot synthesis of 3-(2-oxo-2-phenylethylidene)indolin-2-ones (**1a–1j**, **2a–2c**, **3a**).