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Identification of novel indole based heterocycles as selective estrogen receptor modulator

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ABSTRACT

In the present study, we have designed and synthesized indole derivatives by coalescing the indole nucleus with chromene carbonitrile and dihydropyridine nucleus. Two compounds **5c** and **6d** were selected from series **I** and **II** after sequential combinatorial library generation, docking, absorption, distribution, metabolism and excretion (ADME) filtering, anti-proliferative activity, cytotoxicity, and ER-α competitor assay kit by utilizing estrogen receptor-α (ER-α) dominant T47D BC cells line and PBMCs (Peripheral Blood Mononuclear Cells). Cell imaging experiment suggested that both the compounds successfully cross cellular biomembrane and accumulate in nuclear, cytoplasmic and plasma membrane region. Semiquantitative RT-PCR and Western blotting experiments further supported that both compounds reduced the expression of mRNA and receptor protein of ER-α, thereby preventing downstream transactivation and signaling pathway in T47D cells line. Current findings imply that **5c** and **6d** represent novel ER-α antagonists and may be used in the development of chemotherapy for the management of BC.

1. Introduction

Breast cancer (BC) is the second most widespread cancer and one of the leading causes of mortality among women globally. In clinical practice, a variety of molecular factors are considered for predicting the prognosis and response to therapy of BC patients. Abnormal expression of estrogen receptor (ER) is a salient pathological phenotype in the majority of BC cases. Preeminent levels of estrogens are thought to be associated with the incidence and proliferation of BC. ER-α is a critical growth regulator in BC, and its expression in BC cells is essential for tumor progression [1]. Selective estrogen receptor modulators (SERMs) are the compounds used for thwarting the effect of estrogens in breast tissues. These compounds bind to the ER similar to natural estrogens. However, their effects range from anti-estrogenic in some cases to estrogenic in others depending upon the target site tissues [2–4]. Since the introduction of tamoxifen as first generation SERM, subsequently second and third generation SERMs have been developed with higher efficacy and safety. Currently available SERMs include raloxifene, lasofoxifene, and bazedoxifene which are useful pharmacological agents for the prevention and treatment of osteoporosis and BC [5,6]. Bazedoxifene is a third generation indole-based SERM, in phase II clinical trial for the treatment of BC [7].

The search for a new and safer SERMs with a simple structure and high efficiency remains desideratum for a better treatment of BC. It is widely accepted that compounds bearing heteroatoms increase the stability of complex by forming hydrogen bonds with the cancer-related protein targets [8]. It has been observed that nitrogen-containing heterocyclic ring imparts a polarized character and helps in establishing an efficient interaction with ER-α [9], and thus have a potential for providing a new paradigm for maintaining the health of women [8,10]. Later, a fusion of two or more heterocyclic rings in a single molecular frame has become an appealing method for drug design. The outcome being a combined pharmacophore having structural features of two or more active substances leading to synergism, enhancement or modulation of the desired characteristics of individual components [11,12]. Another exciting feature of this approach is a generation of a diverse array of pharmacophore which has an extensive application in the field of medicinal chemistry. These molecules can also be modulated for combating the incidence of drug resistance as a single molecule can have different modes of action at the same time thereby reducing the chances of drug resistance [13–15]. In recent years, there has been an arising interest in an indole-fused heterocyclic ring structure in cancer drug discovery [16–19].

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It has been reported that 2-arylindole derivative is selective towards the ER-α and antagonizes the action of estradiol in BC cells and uterus tissues.

Owing to the importance of N-heterocycles in the present study we have designed and synthesized Series **I** (indole-chromene carbonitrile derivative) and **II** (indole-dihydropyridine derivative)), as putative SERM for targeting ER-α for the management of hormone-dependent BC (HDBC). The synthesized molecules were characterized by NMR and HRMS. Compounds were analyzed for the anticancer activity using ER-α expressing T47D cell lines, ER-α binding assay, semiquantitative RT-PCR, Western blotting and confocal microscopy. Molecular docking was used to identify the possible binding modes of indole derivatives with ER-α.

2. Results and discussion

2.1. Design of indole derivatives

Indole based compounds have a tremendous potential for the development of chemotherapeutic agents [20,21]. Structural analysis of the available literature suggests that 1-benzyl-indole-3-carbinol, an analog of indole-3-carbinol was found to be thousand times more potent in comparison to indole-3-carbinol in the suppression of both estrogen-dependent and independent BC cell lines [22]. Hence, N-benzylation significantly improves the antiproliferative activity as compared to its counterpart. It has been also reported that 2-aryl indole derivative exerts its antiestrogenic effect by targeting ER-α [19,23]. Similarly, ERA923 is another indole derivative having an anti-estrogenic activity which targets ER-α and is equally effective as tamoxifen and can overcome the resistance associated with tamoxifen [24] (Fig. 1). Bazedoxifene is another indole-based SERM which is known to bind ER-α with a higher affinity as compared to ER-β. In recent years, chromene and acridine derivatives have attracted considerable interest in the development of drug candidates for cancer chemotherapeutics. SAR studies have demonstrated that the aryl substitution at the 4th position of chromenes and acridine compound further potentiates the activity [25]. Among the wide range of biological properties of 1,4-dihydropyridines (DHP) as an anticancer moiety, they have also been recognized as multidrug resistance reversing agents in cancer therapy [26,27]. Several reports are published for different derivatives of DHPs including dexniguldipine, niguldipine, nicardipine, and nitrendipine regarding their potent activity in the management of BC by breast cancer receptor protein (BCRP) inhibition [28]. It is well established that slight structural modifications of the DHP ring may result in remarkable changes in pharmacological effects, thereby DHP core has revitalized the interest of the synthetic community. Among different derivatives of 1,4-DHPs, 1,8-acridinedione is a known scaffold with a broad spectrum of biological effects such as DNA intercalating, anti-tumor, and cytotoxic activity [28–31]. Recently, a 1,8-acridinedione derivative (3,3,6,6-Tetramethyl-9-[1-(4-fluorobenzyl)-2-(methylthio)-5-imidazolyl]-2,3,4,5,6,7,9,10-octahydro-1,8-acridinedione) have been reported to be effective at as low as 1 nm in MDR reversal and tamoxifen resistant T47D cell lines [30]. A chromene based derivative, 12-(4-hydroxyphenyl)-9,9-dimethyl-7a,8,9,10,11a,12-hexahydro-11H-benzo[a]xanthen-11-one (6.7 μg/mL) was found to possess more effective anti-proliferative activity than tamoxifen in MCF-7 cells line [32]. In another example, phenyl substituted dioxooctahydroxanthenes derivatives have been found to be active in MCF-7 cell line with an IC_{50} value of 0.02 μmol/l [33]. Recently, EM-652 which is an active chromene metabolite of EM-800 has been shown to exhibit antiestrogen profile and as in clinical trials for the treatment and prevention of BC. Another chromene derivative, 2-amino-3-cyano-7-dimethylamino-4-(3-methoxy-4,5-methylenedioxyphenyl)-4H-chromene was recognized as a potent apoptosis inducer and has shown the EC_{50} value of 73 nM in T47D cells [25,34]. α-Mangostin isolated from *Garcinia mangostana* has been reported to be effective in BC by the mechanism of arresting the cell cycle, elevating the caspases and aromatase inhibition [35]. α-Mangostin has been found to reduce the expression of ER-α in MCF-7 cell lines [36]. Hence these derivatives could be used as a template for the development of pharmacophore targeting BC. Hence, by rationally combining the structural features of Indole core with chromene and DHP we have designed indole-chromene carbonitrile and indole-dihydropyridine derivatives for targeting BC (Fig. 1).

2.2. Selection of indole derivatives

Molecular simulation techniques supported the selection of compounds for synthesis. Initially, a combinatorial library for the lead structure of Series **I** and **II** was generated. The combination of the database of reagents substituting R^1 and R^2 group (Fig. 1) generated 4086 and 4,618,200 compounds for Series **I** and **II** respectively.

The generated set of compounds were then evaluated in the two-step procedure. In the first step, ADME was applied for filtering of compounds with the most promising drug-like properties (Table 1) and next step docking to the active site of ER-α for a final selection of compounds with the highest potential activity (Table 2). With the help of qikprop module used during ADME analysis only top-ranked 500 molecules were retained. Calculation of molecular descriptors and ADME analysis minimizes the chances of failure of compounds in early pre-clinical/phase I clinical trials during the drug development process.

The acceptable limits of QP logPo/w (octanol/water partition coefficient) value were taken in the range of −2.0 to 6.5 and with maximum possible oral bioavailability**,** thus possessing desirable characteristic absorption parameters for a lead drug molecule. Higher than 6.5 value indicates that compounds will tend to have higher lipophilicity and will not be able to solubilize in the aqueous phase for systemic distribution. Log Po/w value lower than −2.5 indicates higher hydrophilicity which in result will hinder the compound from crossing the cellular biomembrane and acting on the target receptor [37]. During absorption, the molecules must cross the epithelial and endothelial cell barriers to reach the target cells. Caco and MDCK prediction assays have been successfully utilized in the study of drug transport. Caco cells have comparable permeability with transport *in vivo* human jejunum, hence establishes a correlation between bioavailability and absorption. But Caco has suffered a disadvantage of variable expression of transporters like P-gp (a P-glycoprotein, a well-recognized efflux transporter in many tissues including brain, kidney, and intestine). Therefore, MDCK (Madin-Darby Canine Kidney) cells are used to study drug efflux and active transport. Numerous studies have demonstrated that human oral drug absorption and permeability coefficient have good correlations. QPP Caco and QPP MDCK have a recommended acceptable value greater than 500 nM/s. Larger the calculated value better the compound permeability through the gut membrane and higher the oral absorption [38]. Thereby, could be absorbed effectively for next phase of blood plasma distribution. Upon absorption, drugs exist in equilibrium binding with plasma albumin for the distribution. The unbound drug is active and reaches the site of action and gets metabolized and/or excreted. QP logKhsa predicts the binding of the compound to human serum albumin and the acceptable limit was taken as −1.5 to 1.5. Predicted value more than 1.5 signifies higher albumin binding thus lower the fraction of available drug reaching the site of action. On the contrary value less than -1.5 indicates lower binding and hence reduced plasma half-life of the drug. Hence,