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Design of novel tyrosine-nitrogen mustard hybrid molecules active against uterine, ovarian and breast cancer cell lines

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ABSTRACT

L-*para*-Tyrosine was linked to *ortho*-hydroxyaniline, *meta*-hydroxyaniline and *para*-hydroxyaniline giving three distinct tyrosinamide molecules. The new extended amino acid derivatives were constructed to imitate, in part, the estradiol (E_2, the natural female sex hormone) nucleus. The resulting tyrosinamides were then linked to chlorambucil either directly, or via a 5 and 10 carbon atoms spacer chain. This was done in an attempt to target cancerous cells expressing the estrogen receptor alpha (ERα) and to obtain a more specific chemotherapeutic agent. The tyrosinamide–chlorambucil molecules were designed and synthesized in good yields, according to two different approaches. The novel compounds were evaluated for their anticancer efficacy in hormone-dependent and hormone-independent (ER+; MCF-7 and ER−; MDA-MB-231) breast cancer cell lines. Interestingly, the *meta*-hydroxyphenyl-tyrosinamide-chlorambucil derivatives were more active than the *ortho*- and *para*- analogs. The molecules bearing a 5 carbon atoms spacer were selected for additional biological study using a panel of female cancerous cells; breast (ZR-75-1, MDA-MB-436, MDA-MB-468), ovarian (OVCAR-3, A2780) and uterine (Ishikawa, HEC-1A). It was discovered that for breast cancer cells, the new compounds were up to 4.2 times more active than chlorambucil itself.

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1. Introduction

Chemotherapy is a very common anticancer treatment which uses anti-proliferative molecules to kill cancerous cells. These chemotherapeutic agents are highly active drugs which also affect healthy cells, leading to severe side effects [1]. Drug targeting is a challenging research subject which can be applied to various diseases such as cancer. In recent years, many cytotoxic compounds have been designed in order to get more specific anticancer drugs and as a result, to minimize toxic side effects [2,3]. Targeting strategies have been applied to cases where characteristic physiological differences exist between diseased and healthy tissues [4].

Female organs (breast, ovary and uterus) are known to express a specific protein, the estrogen receptor alpha (ERα) [5]. The ERα is a member of the nuclear receptor superfamily which is responsible for the concentration of physiological levels of estradiol (E_2) (**1**, Fig. 1), the most active steroidal female hormone [6]. In most cases, female cancers are classified as hormone-dependent, which means that the tumor development is stimulated by the presence of the ERα. Also, when normal cells become cancerous, ERα is found to be overexpressed [7]. Hence, ERα is an attractive target which has been recognized as a useful tool for endocrine therapy.

The ERα has already been targeted in an attempt to accumulate a known chemotherapeutic agent to breast cancer cells [4]. Endogenous hormones have been used as potential carrier ligands for the ERα. These have been modified and designed to create ligand-cytotoxic molecules targeting ERα overexpressing cells [8]. Anti-proliferative drugs have been linked to the steroidal ligand. E_2-based anticancer molecules reported have shown cytocidal activity against hormone-dependent cancer cells at a higher level than the parent drug given alone [9–12]. Therefore, the use of a shuttle ligand such as E_2 has demonstrated enhanced effectiveness of known anticancer drug giving further evidence of the transport role of E_2 [13]. Moreover, such steroidal-anticancer molecules have shown to reach the ERα and then, induce E_2-like genomic effects through the targeted protein [14].

In an attempt to avoid the promotion of cancer in response to estrogenic activity, non-steroid ligands conjugated to cytotoxic compounds were also designed [8]. There are numerous reports of compounds with unusual structural skeleton binding to the ERα [15]. Among these molecules, some are selective and have high binding affinity for the ERα. Hence, many different molecules can bind to the ERα, even if their structures are quite dissimilar to the natural hormone steroidal backbone. This can also be explained by the known mobility of protein segments upon ligand binding

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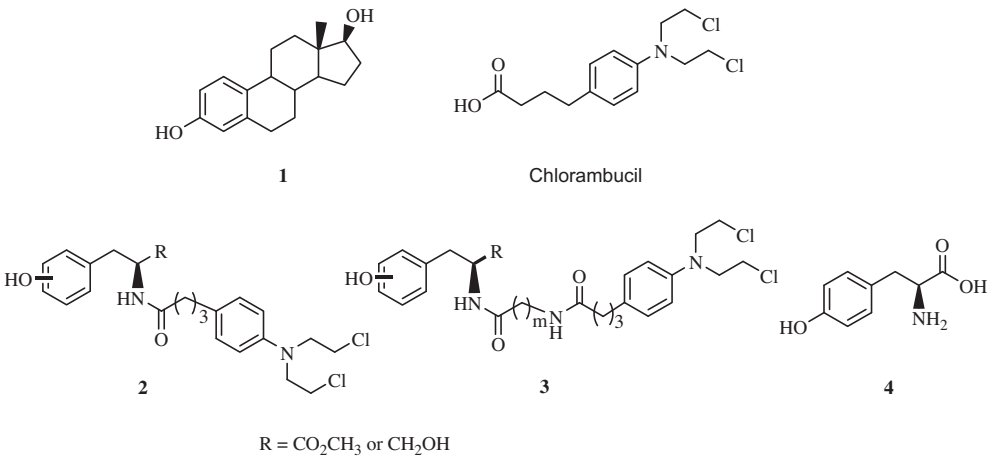

Fig. 1. Structure of 17β-estradiol (**1**), chlorambucil, tyrosine–chlorambucil hybrid molecules (**2** and **3**) and L-*para*-tyrosine (**4**).

[16]. Therefore, the binding cavity of the ERα is known to accommodate various non-steroidal synthetic ligands.

Several structural characteristics need to be considered in order to build an estrogen-like ligand. A phenol ring, similar to the A-ring of E_2, must be a part of the molecule [17]. Moreover, two polar functional groups, such as hydroxyl, must be also present. The 3-OH and 17β-OH located at the two extremities of E_2 skeleton are involved in the E_2–ER binding [18]. The interatomic distance of the hydroxyl groups in E_2 is 10.9 Å. Thus, in order to bind to the corresponding residue into the ERα, a molecule most have two polar groups far away from each other. Other molecules, with polar functional groups from 10.3 to 12.1 Å apart, have also shown interesting binding affinity for the ERα [18].

Recently, several tyrosine–chlorambucil derivatives (**2** and **3**, with R = CO_2CH_3 or CH_2OH, Fig. 1) have been reported [9,19]. Chlorambucil was linked, directly or via a tether chain, to tyrosine ester (R = CO_2CH_3) or tyrosine hydroxymethyl (R = CH_2OH) molecules. Chlorambucil is a known anticancer agent which structure is shown in Fig. 1. In this article, tyrosine (**4**, Fig. 1) was selected as a template to mimic, at least in part, the A-ring phenol of the estradiol nucleus. Tyrosine is a natural amino acid reported to have a structure similar to that of the phenol group of E_2 [18]. Molecular modeling study showed that the phenol group of tyrosine can interact into the ERα binding cavity in the same manner as the A-ring phenol of E_2 does [19]. The tyrosine–chlorambucil hybrid molecules **2** and **3** showed enhanced cytotoxicity when compared to chlorambucil itself and this can possibly indicate the role of tyrosine as a cytotoxic agent carrier.

In spite of the non negligible cytotoxicity observed, molecular docking calculations showed that the tyrosine skeleton does not fully occupy the ERα binding cavity. Tyrosine seems too small to fit correctly (and completely) into the ERα binding pocket [19]. Hence, there is a reason to believe that perhaps, tyrosine-based molecules with extended skeleton could interact more adequately into the receptor network and consequently, be more active.

The new tyrosine-based molecules were designed and synthesized keeping in mind the minimal structural requirements needed to create ERα ligands. Tyrosine was modified and coupled to a hydroxyaniline moiety in order to obtain an extended backbone bearing two phenol groups distant from each other. Then, tyrosine ester (R = CO_2CH_3) and tyrosine hydroxymethyl (R = CH_2OH) derivatives previously synthesized were replaced by a tyrosinamide entity. The two phenol groups of the tyrosinamide unit could possibly create hydrogen bonding interactions with the same residue than E_2 does. It is hypothesized that these molecules with an extended shape could fit more effectively into the receptor than the smaller tyrosine–chlorambucil analogs, previously reported [19]. In addition, adding an aromatic moiety to the ligand structure would certainly increase the lipophilicity of the final derivatives. Such molecule would be less polar than tyrosine and consequently, more comparable to the steroid backbone. Hence, tyrosine–hydroxyaniline conjugates would be more susceptible to imitate the natural steroid, estradiol.

In this particular study, *ortho*, *meta* and *para*-hydroxyaniline moieties have been linked to the L-*para*-tyrosine (**4**), in attempt to obtain ligands with different binding abilities. The position of a second hydroxyl group, as a part of the hydroxyaniline moiety, was varied. The tyrosinamide regioisomers were linked to chlorambucil, directly or via a 5 and a 10 carbon atoms spacer chain.

This manuscript gives the detailed synthesis of all the tyrosinamide–chlorambucil regioisomers made (**5** and **6**, Fig. 2). Two methodologies were used and compared; the linear and the convergent synthesis. Then, the biological activity of all the cytotoxic molecules synthesized was evaluated on hormone-dependent (MCF-7) and hormone-independent (MDA-MB-231) breast cancer cell lines. The influence of hydroxyl group location (*ortho*, *meta* and *para*) on the tyrosinamide–chlorambucil hybrids was studied. Next, the molecules bearing a 5 carbon atoms spacer were selected for a more thorough biological evaluation. These L-*para*-tyrosinamide–chlorambucil derivatives were further tested on a panel of hormone-dependent and hormone-independent female cancer cell lines; breast: ZR-75-1 (ER+), MDA-MB-468 (ER−), MDA-MB-436 (ER−); ovarian: OVCAR-3 (ER+), A2780 (ER−); uterine: Ishikawa (ER+), HEC-1A (ER−).

2. Experimental

2.1. Chemistry

All reactions were performed with ACS Fisher solvents. In some cases, solvent, as well as starting materials and reactants, were first purified and dried by standard means [20]. Anhydrous reactions required an inert atmosphere of dry nitrogen. The 6-aminohexanoic acid, 11-aminoundecanoic acid, *N*-Boc-tyrosine and all hydroxyaniline isomers (*ortho*, *meta* and *para*) were purchased from Sigma–Aldrich Canada Ltd., Oakville, Ontario, Canada. All reactions were monitored by UV fluorescence or staining with iodine on Sigma T 6145 commercial TLC plates (polyester silica gel 60 Å, 0.25 mm). Purifications were done using flash column chromatography according to the method of Still et al. [21] on Silicycle UltraPure Flash Silica Gel, 40–63 μm mesh. Hexanes and acetone

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