Taylor & Francis
Taylor & Francis Group

Xenobiotica
the fate of foreign compounds in biological systems

ISSN: 0049-8254 (Print) 1366-5928 (Online) Journal homepage: https://www.tandfonline.com/loi/ixen20

Aromatase inhibition by 2-methyl indole hydrazone derivatives evaluated via molecular docking and *in vitro* activity studies

Senem Ozcan-Sezer, Elif Ince, Atilla Akdemir, Özlem Öztürk Ceylan, Sibel Suzen & Hande Gurer-Orhan

To cite this article: Senem Ozcan-Sezer, Elif Ince, Atilla Akdemir, Özlem Öztürk Ceylan, Sibel Suzen & Hande Gurer-Orhan (2019) Aromatase inhibition by 2-methyl indole hydrazone derivatives evaluated via molecular docking and *in vitro* activity studies, Xenobiotica, 49:5, 549-556, DOI: 10.1080/00498254.2018.1482029

To link to this article: https://doi.org/10.1080/00498254.2018.1482029

XENOBIOTICA
2019, VOL. 49, NO. 5, 549–556
https://doi.org/10.1080/00498254.2018.1482029

Taylor & Francis
Taylor & Francis Group

RESEARCH ARTICLE

Aromatase inhibition by 2-methyl indole hydrazone derivatives evaluated via molecular docking and *in vitro* activity studies

Senem Ozcan-Sezer[a]*, Elif Ince[a]*, Atilla Akdemir[b], Özlem Öztürk Ceylan[c], Sibel Suzen[c] and Hande Gurer-Orhan[a]

[a]Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Ege University, Izmir, Turkey; [b]Computer-Aided Drug Discovery Laboratory, Faculty of Pharmacy, Department of Pharmacology, Bezmialem Vakif University, Istanbul, Turkey; [c]Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Ankara University, Ankara, Turkey

ABSTRACT

1. A causal association is reported between prolonged exposures to elevated levels of estrogen and breast cancer. Therefore inhibiting aromatase (CYP19A), which catalyses the conversion of androgens to estrogens, is an important approach in prevention and treatment of estrogen receptor positive (ER+) breast cancer.
2. Melatonin, a natural indolic hormone, is reported to prevent free radical induced carcinogenesis and block local estrogen synthesis in breast tissue via aromatase inhibition. However several features of melatonin limit its therapeutic use.
3. In the present study aromatase inhibiting potential of 2-methyl indole hydrazones are investigated, and compared with melatonin, by two *in vitro* models; a cell-free assay using a fluorescence substrate and a cell-based assay where cell proliferation was determined in ER + human breast cancer cells (MCF-7 BUS) in the absence of estrogen and the presence of testosterone. Aromatase inhibitory effect is also explored by molecular modelling studies.
4. In biological activity assays *monochloro* substituted indole hydrazones were found to have stronger aromatase inhibitory activity among all tested derivatives and were more active than melatonin. This finding is further confirmed by molecular modelling.
5. These results may be useful in the design and synthesis of novel melatonin analogues with higher inhibitory potency against aromatase.

ARTICLE HISTORY
Received 3 April 2018
Revised 23 May 2018
Accepted 24 May 2018

KEYWORDS
Aromatase inhibition; melatonin analogues; indole derivatives; molecular modelling; breast cancer

Introduction

Melatonin (MLT, *N*-acetyl-5-methoxytryptamine) is an indole hormone which is mainly synthesised in pineal gland and reach peak levels in plasma at night. The main physiological role of melatonin is to regulate circadian rhythm and sleep. In addition, several other beneficial effects are suggested for melatonin on hormone-dependent tumours especially breast cancer (González-González et al., 2018). Among many protectors, melatonin has been proven to be particularly efficient (Gurer-Orhan et al., 2016) with well-known potent scavenging effect against reactive oxygen and nitrogen species (ROS and RNS) (Gurer-Orhan & Suzen, 2015; Suzen et al., 2013) as one of the suggested mechanisms for its oncostatic effect. It was reported that physiological concentrations (1 nM) of melatonin inhibited proliferation of ER positive (ER+) human breast cancer cells (Cos & Sánchez-Barceló, 1995; Hill & Blask, 1988). Furthermore, increased incidence of breast cancer is reported in night shift workers, mainly nurses (Schernhammer et al., 2001), which supports the importance of melatonin in breast cancer prevention. Different mechanisms of actions have been revealed to explain oncostatic actions of melatonin; act as a selective estrogen receptor modulator (SERM) by reducing estrogen binding to ERα (Chottanapund et al., 2014) and act as a selective estrogen enzyme modulator (SEEM) by inhibiting enzymes which are involved in the biosynthesis of estrogens in peripheral tissues (Cos et al., 2005; Martínez-Campa et al., 2009).

Aromatase is a cytochrome P450 (CYP) enzyme complex which catalyses the conversion of androgens into estrogens and it catalyses the rate limiting step of endogen estrogen biosynthesis (Ahmad, 2015; Di Nardo & Gilardi, 2013; Furr, 2006; Ghosh et al., 2016; Santen et al., 2009). Aromatase is a source of local estrogen production in breast cancer tissue and increased aromatase expression is reported in postmenopausal women with ER + breast cancer (Brueggemeier et al., 2005). Therefore, inhibition of aromatase is a promising target for hormone-dependent breast cancer therapy (Briest & Davidson,

CONTACT Hande Gurer-Orhan hande.gurer.orhan@ege.edu.tr; hgurer@gmail.com Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Ege University, 35100 Izmir, Turkey
*These authors contributed equally to the present work.

2007; Ghosh et al., 2009). Aromatase inhibitors can be classified into two major groups as steroidal and non-steroidal derivatives. Among the non-steroidal inhibitors, the most studied compounds were indoles. (Brueggemeier et al., 2005; Furr, 2006). Furthermore, many indole-based compounds have been shown to inhibit aromatase activity in several studies (Kang et al., 2018; Pingaew et al., 2018; Wang et al., 2013).

Melatonin is reported to modulate aromatase activity and expression (Alvarez-García et al., 2013; Cos et al., 2005, 2006; Martínez-Campa et al., 2009). However, short half-life of melatonin as a result of its rapid metabolic inactivation limits its therapeutic use (Sanchez-Barcelo et al., 2007). Therefore, designing and synthesising new melatonin analogues with longer half-lives might overcome this limitation. Our groups have been working on the synthesis of new melatonin analogue indole derivatives and one of our important findings is that many indole derivatives have antioxidant potential (Suzen 2015). Cytotoxic activity of the newly synthesised indole derivatives was also evaluated by various *in vitro* assays (Gurer-Orhan et al., 2016; Suzen et al., 2013).

In the present study, possible effects of the novel indole-based melatonin derivatives, namely 2-methyl indole hydrazones (Figure 1), on aromatase activity were investigated by two *in vitro* assays. Direct measurement of aromatase activity was evaluated by a cell-free *in vitro* assay using a fluorescence substrate, 7-methoxy-4-trifluoromethyl coumarin. Effects of the synthesised compounds on indirect measurement activity assay were also investigated in MCF-7 BUS cell line, an estrogen-dependent human breast cancer cell having aromatase activity (Cos et al., 2005). Docking studies of active melatonin analogues into the aromatase active site was also employed to detect interaction with enzyme.

Materials and methods

Materials

MCF-7 BUS cells were kindly provided by Prof. Ana Soto and maintained at 37 °C in 5% CO_2 atmosphere in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% foetal bovine serum (FBS). All chemical reagents used in the syntheses and biological activity studies were purchased from Sigma-Aldrich (St. Louis, MO, USA). 2-methylindole-3-carboxaldehyde hydrazones were synthesised by using 2-methylindole-3-carboxaldehyde as the starting material, and their purity was determined by ^{1}H and ^{13}C NMR (Varian 400 MHz Instrument, Palo Alto, CA, USA), ESI mass spectrometry (Waters micromass ZQ) and FT-IR spectrometer (Waters, Milford, MA, USA). Synthesis and chemical characterization of the compounds **1a–y** were described in details in our earlier study (Ozturk et al submitted to CJC 2017).

Aromatase activity assays

Direct measurement of aromatase activity

Potential inhibitory activity of the compounds was determined according to an established procedure using a commercially available CYP19/MFC high-throughput screening kit from BD Gentest. Substrate for the reaction is 7-methoxy-4-trifluoromethyl coumarin (MFC) and human recombinant CYP19 enzyme is used as enzyme sources. In this assay, fluorescence substrate MFC is converted to its fluorescent metabolite 7-hydroxytrifluoromethyl coumarin (HFC) by aromatase

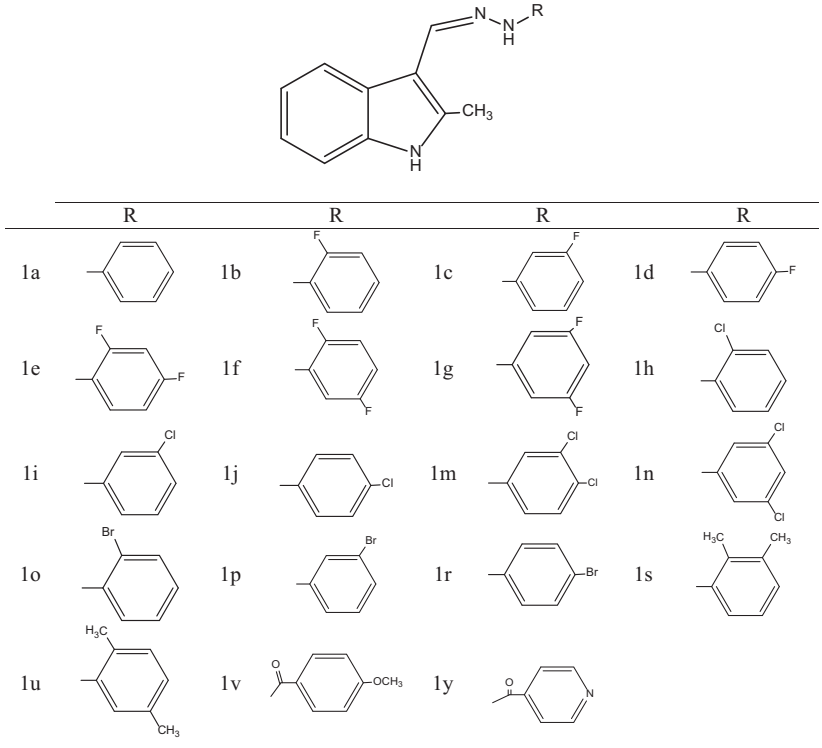


Figure 1. Chemical structures of the tested 2-methyl indole hydrazone derivatives.

in the presence of NADPH-generating system, therefore reduction of the fluorescence intensity refers to aromatase inhibitory activity. Enzyme reactions were performed according to the manufacturer's protocol, using acetonitrile solutions of the compounds, in black 96-well plates. Briefly, 100 μL sample solutions containing serial dilutions of test compounds and NADPH-Cofactor Mix (16.25 μM NADP+, 825.14 μM $MgCl_2$, 825.14 μM glucose-6-phosphate and 0.4 Units/mL glucose-6-phosphate dehydrogenase) were added to plates and pre-warmed for 10 min at 37 °C. After that reaction was initiated by adding 100 μL enzyme-substrate mix (recombinant human aromatase; 50 μM MFC; 20 mM phosphate buffer, pH 7.4) and incubated for 30 min at 37 °C. All the reactions were stopped by adding 0.1 M tris base dissolved in acetonitrile (75 μL). Fluorescence of the formed HFC was recorded at 409 nm excitation and 530 nm emission wavelength. Percent inhibition of each inhibitor concentration relative to the wells without inhibitor is calculated. IC_{50} values of the each compound were obtained by using the GraphPad Prism5 software.

Indirect measurement of aromatase activity

Indirect aromatase activity was measured in a cell-based assay by using human breast cancer cells (MCF-7 BUS). In estrogen-free media, cell proliferation depends on aromatisation of androgens to estrogen in estrogen-dependent cells like MCF-7 BUS. Therefore, aromatase activity can be measured indirectly in MCF-7 BUS cells according to previously described method (Cos et al., 2005). Briefly, MCF-7 BUS cells were plated in 96-well plates at a density of 6000 cells/well, in DMEM supplemented with FBS 10% and incubated at 37°C in a humid atmosphere containing 5% CO_2. After 48-h incubation for attachment, medium was replaced to DMEM without phenol red supplemented with 10% charcoal stripped FBS, 1% sodium pyruvate and 1% non-essential amino acid solution containing either testosterone (10 μM) alone or testosterone and tested compounds (10 μM) together. A control group is also included in which the cells are grown in estrogen-depleted media without any testosterone or test molecule. Following the five days incubation period cell viability was assessed by MTT assay. The medium was removed; cells were washed with phosphate-buffered saline (PBS) and then incubated with MTT (1 mg/ml) for 4 h at 37 °C. MTT solution was removed and formazan crystals were dissolved in DMSO. The absorbance was recorded at 490 nm on a microplate reader. The ratio of the absorbance of treated samples to the absorbance of testosterone control (taken as 100%) was expressed as % cell viability.

Molecular modelling studies

Preparation of human CYP19A1 crystal structure for docking studies

The crystal structure of human placental CYP19A1 (hCYP19A1) in complex with the aromatase inhibitor 4-androstene-3-17-dione (ASD) was obtained from the Protein Data Bank (pdb entry: 3s79, 2.75 Å). Hydrogens atoms were added using the protonate 3D tool (Labute, 2009) of the MOE software package (v2015.10, Chemical Computing Group Inc., Montreal, Canada). The side chain of Asp309 has been assigned a neutral charge, because it has been suggested to be unprotonated at physiological pH values (Di Nardo et al., 2015). All water and buffer molecules and the ligand were deleted and the protein was saved as a mol2-file.

Preparation of ligands for docking studies

Three-dimensional molecular structures were constructed for all ligands using the MOE software package (v2015.10, Chemical Computing Group, Inc., Montreal, Canada). All strong bases were protonated and all strong acids were deprotonated. Subsequently, a steepest-descent energy minimization protocol was applied using the MMFF94x forcefield. The ligands were saved as mol2-files.

Docking studies

All ligands were docked into the hCYP19A1 structure (pdb entry: 3s79) using the P450 enzyme settings and the GoldScore scoring function in the GOLD suite software package (v5.6, CCDC, Cambridge, UK). For each ligand 50 dockings were performed with default settings. The binding pocket was defined as all residues within 12 Å of atom C10 of the cocrystallised inhibitor ASD (coordinates *X*: 86.009; *Y*: 52.398 and *Z*: 46.001).

Statistical analysis

Data were expressed as means ± SD. Statistical analysis was performed by using Student's *t*-test. Differences were considered significant $p < .05$. *p* Values are given in figure legends.

Results

Effects of the compounds on aromatase activity

Direct measurement of aromatase activity

Potential inhibitory activities of the synthesized melatonin analogues were evaluated by a cell-free *in vitro* assay using a fluorescence substrate and human CYP19 aromatase. Almost all 2-methyl indole hydrazone derivatives showed moderate to high inhibitory activity at 100 μM concentration (Figure 2).

Compounds that have high inhibitory effect at 100 μM were tested at eight different concentrations – ranging between 0.137 and 300 μM and enzyme inhibition curves were obtained. All experiments were performed at least triplicate to determine the IC_{50} values (Figure 3). As indicated in Figure 3, compounds **1c**, **1g**, **1i**, **1j** and **1y** showed reproducible and statistically significant inhibitory activity on aromatase enzyme.

Indirect measurement of aromatase activity

Effects of the melatonin analogues that have inhibitory activity in direct measurement assay were also investigated in a cell-based *in vitro* assay. MCF-7 BUS cells are estrogen

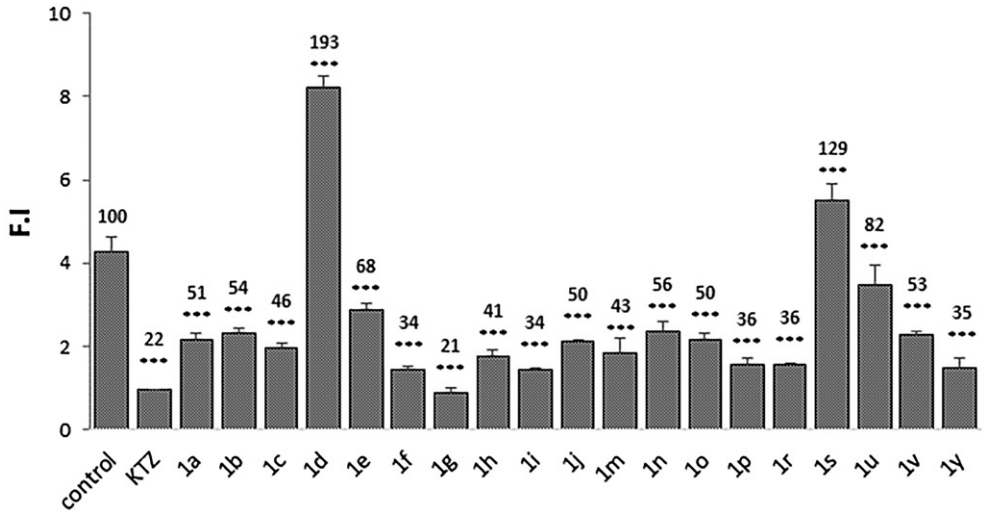
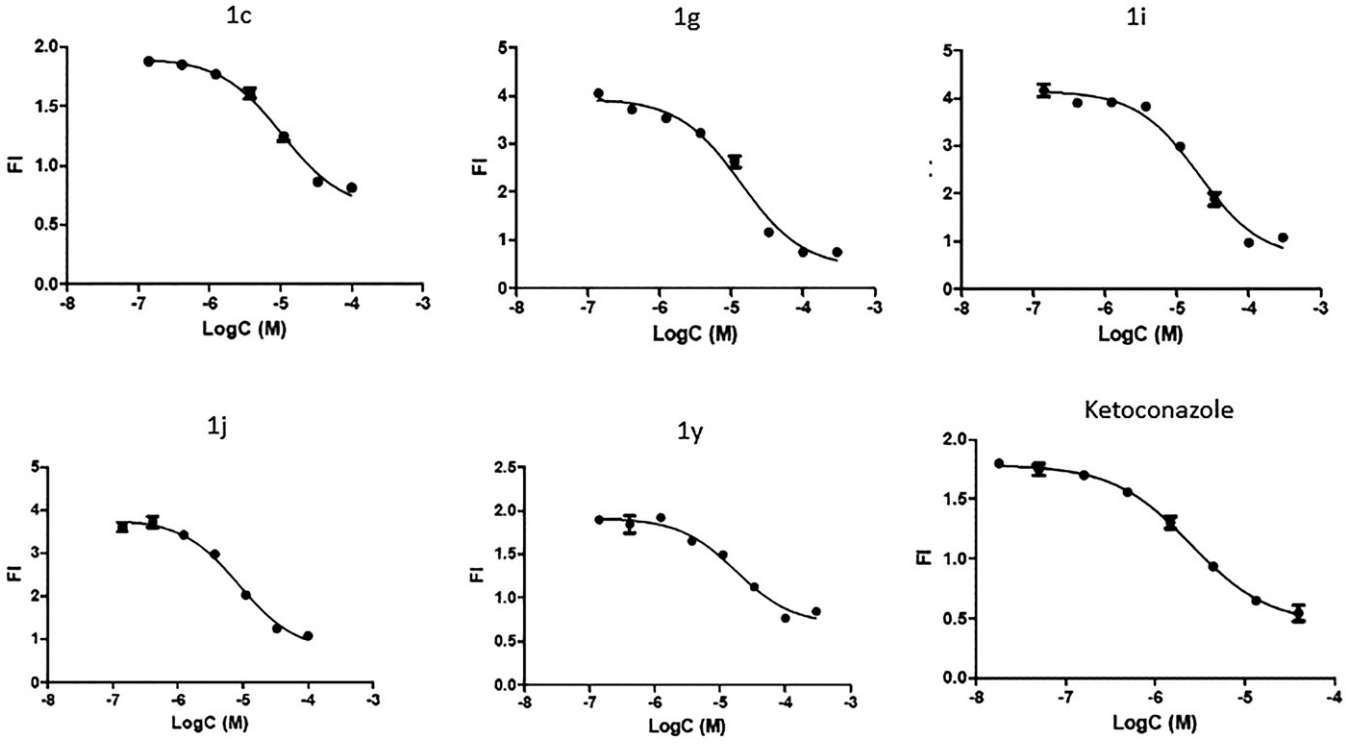


Figure 2. Effects of the 2-methyl indole hydrazone derivatives (100 μM) on aromatase activity in direct measurement assay. Bars represent percentage values compared to control group from four different experiments. FI: Fluorescence intensity. $^{***}p < 0.001$.



	IC50 (μM ± SD)		IC50 (μM ± SD)
1c	11.5± 1.58	1j	8.67±0.07
1g	13.01± 0.83	1y	16.37±1.95
1i	19.15±1.47	KTZ	2.39 ±0.18

Figure 3. Aromatase inhibition curves obtained with five potent derivatives in the direct measurement assay.

receptor positive human breast cancer cells which depends on estrogen for proliferation. Therefore, cell proliferation depends on biotransformation of testosterone to estrogens via aromatase activity in estrogen-free media. Aromatase inhibitory effect of the compounds was evaluated by detecting cell proliferation of MCF-7 BUS cells in the presence of testosterone and the compounds within the estrogen-free media. It was found that all tested compounds inhibited aromatase enzyme in MCF-7 BUS cells where the effect of 1 g was statistically insignificant (Figure 4).

Compounds **1c,1g, 1i, 1j** and **1 y** -which showed significant and reproducible aromatase inhibitory effect in direct measurement assay – were also tested at their various concentrations ranging between 0.5 and 32 μM to evaluate their aromatase inhibitory effect in indirect measurement assay. While compounds **1i** and **1j** statistically significantly inhibited aromatase activity at 8 and 16 μM, respectively Figure 5B,C; compounds **1c, 1 g** and **1 y** have inhibited aromatase enzyme only at 32 μM Figure 5A,D and E.

Molecular modelling studies

Compound **1j** shows the lowest IC50 for hCYP19A1 (Figure 3). Several similar docked poses have been obtained of compound **1j** in the active site of hCYP19A1 (Figure 6). In poses 1 and 2, the phenyl group with its para-chlorine substituent points towards the entrance of the active site, where the electronegative chlorine atom is close enough to the side chain of Arg192 for electrostatic interactions (highest distance <4.8 Å). Hydrophobic interactions are formed with the side chain of Phe221. The indole moiety shows flexibility and forms either hydrophobic interactions with the side chain of Leu477 or with the haem group (Figure 6A). In pose 3, the ligand has moved more towards the entrance of the active site and further away from the haem group (Figure 6B). The side chains of Arg192 and Gln218 form cation-π and hydrogen-π interactions with the ligand's phenyl group, respectively. The indole group of the ligand forms hydrophobic with the side chain of Phe221.

All the docked ligands can adopt at least one of these three poses in the active site of hCYP19A1.

Discussion

Earlier studies have shown that melatonin has a potential to inhibit human aromatase activity as well as downregulating the expression of aromatase enzyme both in human breast cancer cells, MCF-7(Cos et al., 2005) and in human umbilical vein endothelial cells (Alvarez-García et al., 2013). Direct aromatase inhibitory activity of melatonin is confirmed in the present study (data not shown) in a cell-free *in vitro* assay by using a fluorescence substrate, MFC and human CYP19 aromatase. Furthermore, almost all tested 2-methyl indole hydrazones inhibited aromatase activity in the direct measurement assay (Figure 2) with varying potencies. Five out of 19 compounds showed rather strong inhibitory activities towards CYP19 A as indicated by IC_{50} values lower than 20 μM (Figure 3). Those five compounds are shown to be stronger aromatase inhibitors than melatonin, their parent compound, which was found to have 114 μM IC_{50} value. They have approximately 10 times less activity than a well-known aromatase inhibitor, ketoconazole, which was found to have IC_{50} value of 2.5 μM in our assay.

Compounds which have strong inhibitory activity in the direct measurement assay were further evaluated in a cell-based indirect measurement assay and their inhibitory activities were confirmed (Figure 5). Compound **1j** which has *p*-chloro substitution on the phenyl ring was found to be the most potent aromatase inhibitor with an IC_{50} value of 8.72 μM in the direct inhibition among all novel indole derivatives. Possible binding poses for this compound have been suggested with docking studies (Figure 6). Present data suggest a beneficial role for 2-methyl indole hydrazones, especially **1i** and **1j**, in prevention and treatment of ER + breast cancer via aromatase inhibition.

On the other hand, aromatase is responsible for local synthesis of estrogen which has many physiological roles such as being responsible for neurite growth and migration and

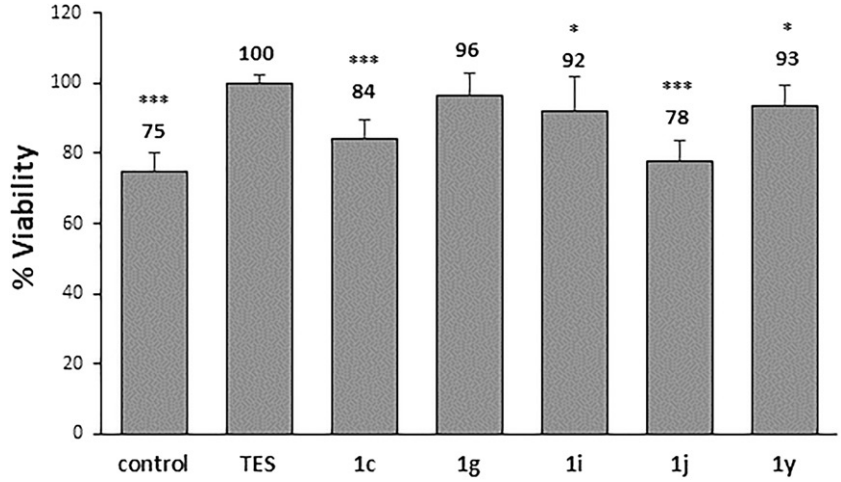


Figure 4. Effects of the compounds (10 μM) on indirect measurement of aromatase activity. Bars show percentage viability values compared to testosterone group from four different experiments. Control group is grown in estrogen-depleted media without any testosterone addition. Statistical comparisons of samples were compared to testosterone group. $*p < 0.05$,$**p < 0.005$ and $***p < 0.001$.

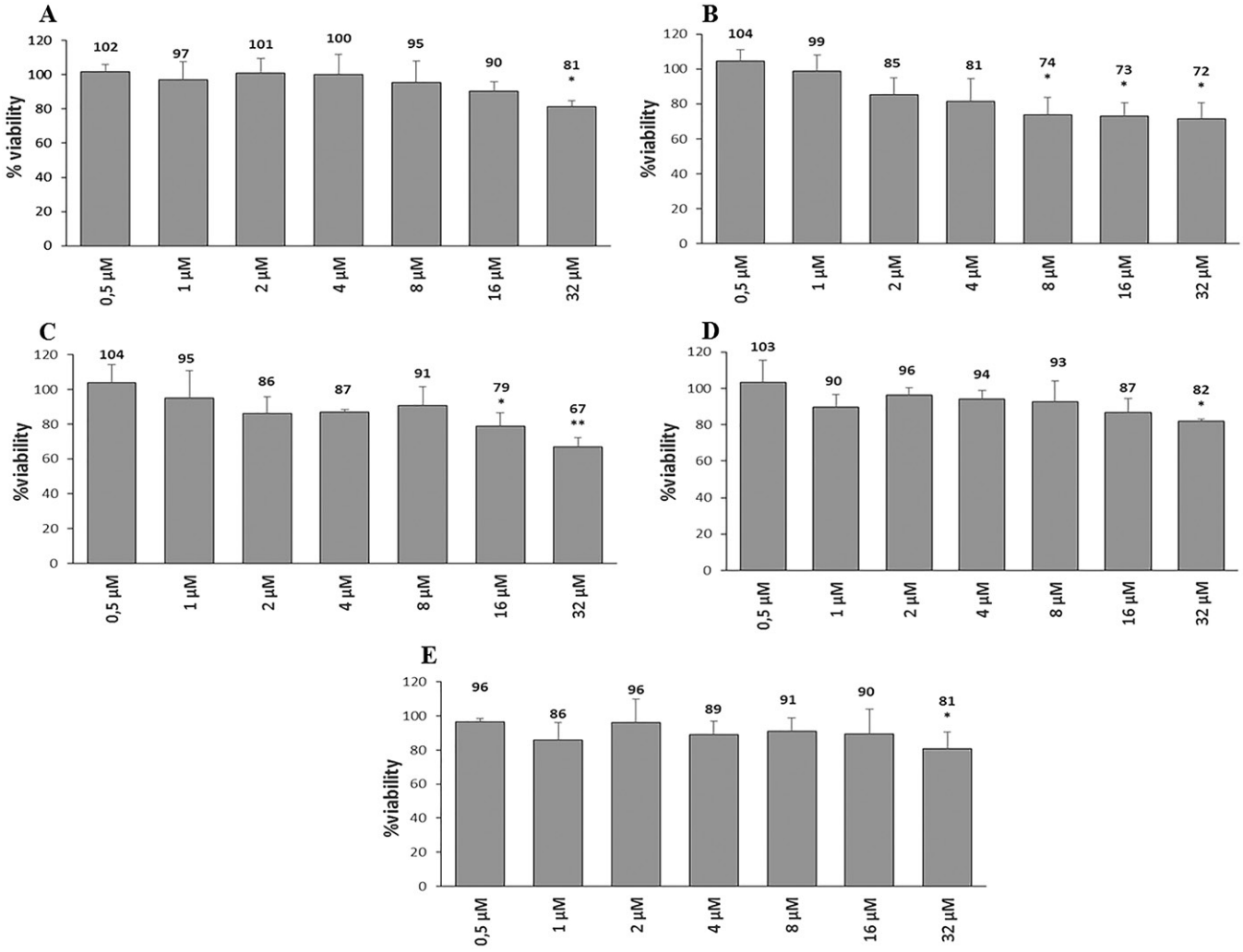


Figure 5. Dose-dependent effects of the compounds on aromatase activity in the indirect measurement assay. Bars show mean percentage viability values compared to testosterone control group from four different experiments. Statistical analysis was performed by comparing samples with the testosterone control group. $^{*}p < 0.05$, $^{**}p < 0.005$. (A) **1c**, (B) **1i**, (C) **1j**, (D) **1g** and (E) **1y**.

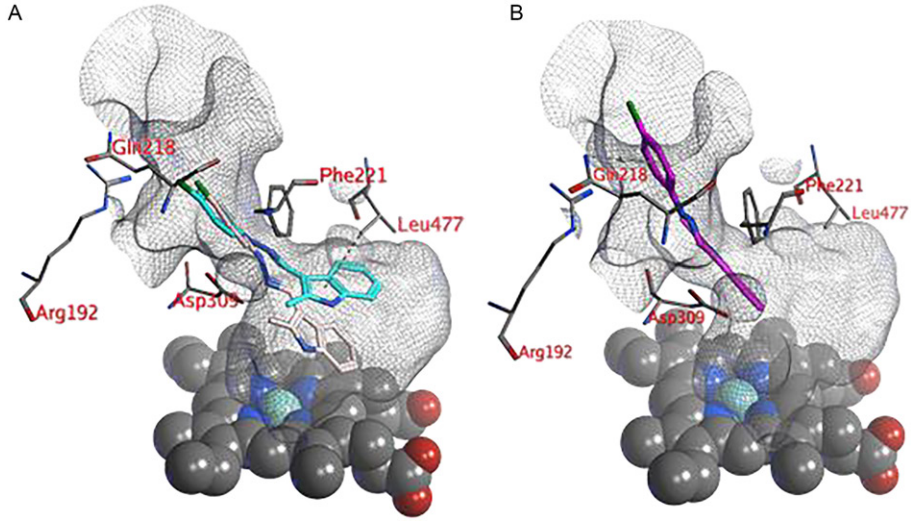


Figure 6. The docked poses of compound **1j** (A, pose 1 and 2; B, pose 3) in the active site of hCYP19A1. Hydrophobic and hydrogen-π interactions are indicated with dashed lines. Docked pose 1 is indicated in turquoise, docked pose 2 is indicated in light brown, and docked pose 3 is indicated in purple. The haem group is shown in CPK representation. For clarity, only the amino acids that participate in ligand–protein interactions are shown.

protection against Alzheimer's and Parkinson's diseases in brain (Davis et al., 1996; Saldanha et al., 2009) and responsible for sexual and reproductive function in ovaries (Sanderson, 2006). Therefore, inhibition of aromatase may have toxicological consequences as well as its therapeutical benefits. Endocrine disruption is an increasing concern for xenobiotics since a huge variety of chemicals such as pharmaceuticals, pesticides, plasticizers and natural phytoestrogens are found to effect endocrine system (Sanderson, 2006). Many known endocrine disruptors are reported to act via aromatase inhibition (Baravalle et al., 2018; Cheshenko et al., 2008; Sanderson, 2006; Whitehead & Rice, 2006). Therefore, the present tested compounds that are found to have aromatase inhibitory effects might cause an adverse effect, endocrine disruption.

Oxidative stress modulating effect of 2-methylindole hydrazone derivative melatonin analogues was evaluated in our previous study and almost all derivatives were found to have reducing potential against H_2O_2-induced oxidation in Chinese Hamster Ovary cells (unpublished data). The role of reactive oxygen species and oxidative stress in carcinogenesis is already reviewed (Gurer-Orhan et al., 2017). Reactive oxygen species, by attacking DNA, can induce DNA damage which in turn can either inhibit or induce transcription, signal transduction pathways, replication errors and genomic instability. All these effects are known to be associated with carcinogenesis. Therefore, oxidative stress modulating effect of 2-methylindole hydrazone derivatives, found in our previous study, suggests a protective role in breast carcinogenesis as well as many other oxidative stress-related diseases.

In conclusion, our compounds, especially *m*- and *p*-chlorinated 2-methyl indole hydrazone derivatives **(1i** and **1j**, respectively), seem to be promising candidates for prevention and treatment of ER + breast cancer because of their dual benefits as aromatase inhibitors and oxidative stress modulators. On the other hand, their endocrine disrupting potential should be considered while suggesting them solely as an oxidative stress modulator in diseases related to oxidative stress, like neurodegenerative diseases and diabetes. The present results might be useful in designing and synthesising novel indole hydrazone derivatives with either more or less inhibitory activities towards aromatase in the consideration of these compounds from a toxicological or a therapeutic point of view.

Acknowledgements

Ege University FABAL facilities are used for biological activity assays.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by The Scientific and Technological Research Council of Turkey (TÜBİTAK) [grant number 112S375], [109S099] and Ege University Research Fund [13BIL009], [13ECZ008].

ORCID

Atilla Akdemir http://orcid.org/0000-0001-8416-0471
Hande Gurer-Orhan http://orcid.org/0000-0002-4890-299X

References

Ahmad IS. (2015). Recent developments in steroidal and nonsteroidal aromatase inhibitors for the chemoprevention of estrogen-dependent breast cancer. Eur J Med Chem 102:375–86.

Alvarez-García V, González A, Martínez-Campa C, et al. (2013). Melatonin modulates aromatase activity and expression in endothelial cells. Oncol Rep 29:2058–64.

Baravalle R, Ciaramella A, Baj F, et al. (2018). Identification of endocrine disrupting chemicals acting on human aromatase. Biochim Biophys Acta (BBA) – Proteins Proteom 1866:88–96.

Briest S, Davidson NE. (2007). Aromatase inhibitors for breast cancer. Rev Endocr Metab Disord 8:215–28.

Brueggemeier RW, Hackett JC, Diaz-Cruz ES. (2005). Aromatase inhibitors in the treatment of breast cancer. Endocr Rev 26:331–45.

Cheshenko K, Pakdel F, Segner H, et al. (2008). Interference of endocrine disrupting chemicals with aromatase CYP19 expression or activity, and consequences for reproduction of teleost fish. General Comp Endocrinol 155:31–62.

Chottanapund S, Van Duursen MBM, Navasumrit P, et al. (2014). Anti-aromatase effect of resveratrol and melatonin on hormonal positive breast cancer cells co-cultured with breast adipose fibroblasts. Toxicol In vitro Int J pub assoc BIBRA 28:1215–21.

Cos S, González A, Güezmes A, et al. (2006). Melatonin inhibits the growth of DMBA-induced mammary tumors by decreasing the local biosynthesis of estrogens through the modulation of aromatase activity. Int J Cancer 118:274–8.

Cos S, Martinez-Campa C, Mediavilla MD, Sanchez-Barcelo EJ. (2005). Melatonin modulates aromatase activity in MCF-7 human breast cancer cells. J Pineal Res 38:136–42.

Cos S, Sánchez-Barceló EJ. (1995). Melatonin inhibition of MCF-7 human breast-cancer cells growth: influence of cell proliferation rate. Cancer Lett 93:207–12.

Davis EC, Popper P, Gorski RA. (1996). The role of apoptosis in sexual differentiation of the rat sexually dimorphic nucleus of the preoptic area. Brain Res 734:10–8.

Furr BJA. (2006). Milestones in drug therapy, aromatase inhibitors. Basel, Switzerland: Birkhäuser, p.182

Ghosh D, Lo J, Egbuta C. (2016). Recent progress in the discovery of next generation inhibitors of aromatase from the structure-function perspective. J Med Chem 59:5131–48.

Ghosh D, Griswold J, Erman M, Pangborn W. (2009). Structural basis for androgen specificity and oestrogen synthesis in human aromatase. Nature 457:219–23.

González-González A, Mediavilla MD, Sánchez BEJ. (2018). Melatonin: a molecule for reducing breast cancer risk. Molecules (Basel, Switzerland) 23: 336.

Gurer-Orhan H, Ince E, Konyar D, et al. (2017). The role of oxidative stress modulators in breast cancer. Curr Med Chem 24.

Gurer-Orhan H, Karaaslan C, Ozcan S, et al. (2016). Novel indole-based melatonin analogues: Evaluation of antioxidant activity and protective effect against amyloid β-induced damage. Bioorganic Med Chem 24:1658–64.

Gurer-Orhan H, Suzen S. (2015). Melatonin, its metabolites and its synthetic analogs as multi-faceted compounds: antioxidant, prooxidant and inhibitor of bioactivation reactions. Curr Med Chem 22:490–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25515518. [last accessed: 27 Feb 2017].

Hill SM, Blask DE. (1988). Effects of the pineal hormone melatonin on the proliferation and morphological characteristics of human breast cancer cells (MCF-7) in culture. Cancer Res 48: 6121–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/3167858 [last accessed 20 Feb 2018].

Kang H, Xiao X, Huang C, et al. (2018). Potent aromatase inhibitors and molecular mechanism of inhibitory action. Eur J Med Chem 143:426–37.

Labute P. (2009). Protonate3D: assignment of ionization states and hydrogen coordinates to macromolecular structures. Proteins 75:187–205.

Martínez-Campa C, González A, Mediavilla MD, et al. (2009). Melatonin inhibits aromatase promoter expression by regulating cyclooxygenases expression and activity in breast cancer cells. Brit J Cancer 101:1613–9.

Di Nardo G, Breitner M, Bandino A, et al. (2015). Evidence for an elevated aspartates pK(a) in the active site of human aromatase. J Biol Chem 290:1186–96.

Di Nardo G, Gilardi G. (2013). Human aromatase: perspectives in biochemistry and biotechnology. Biotechnol Appl Biochem 60:92–101.

Pingaew R, Mandi P, Prachayasittikul V, et al. (2018). Synthesis, molecular docking, and QSAR study of sulfonamide-based indoles as aromatase inhibitors. Eur J Med Chem 143:1604–15.

Saldanha CJ, Duncan KA, Walters BJ. (2009). Neuroprotective actions of brain aromatase. Front Neuroendocrinol 30:106–18.

Sanchez-Barcelo EJ, M-C, CM., Mediavilla MD, Gonzalez A, et al. (2007). Melatonin and melatoninergic drugs as therapeutic agents: ramelteon and agomelatine, the two most promising melatonin receptor agonists. Recent Pat Endocr Metab Immune Drug Discov, 142–51.

Sanderson JT. (2006). The steroid hormone biosynthesis pathway as a target for endocrine-disrupting chemicals. Toxicol Sci 94:3–21.

Santen RJ, Brodie H, Simpson ER, et al. (2009). History of aromatase: saga of an important biological mediator and therapeutic target. Endocr Rev 30:343–75.

Schernhammer ES, Laden F, Speizer FE, et al. (2001). Rotating night shifts and risk of breast cancer in women participating in the nurses' health study. J Nat Cancer Instit 93:1563–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11604480 [last accessed: 20 Feb 2018].

Suzen S. (2015). Evaluation of synthetic melatonin analogue antioxidant compounds. In: Srinivasan V, Gobbi G, Shillcutt S.D, Suzen S, eds. Melatonin: therapeutic value and neuroprotection. Boca Raton, FL: Taylor & Francis, Chapter 21, 259–69. ISBN: 9781482220100 1482220105

Suzen S, Tekiner-Gulbas B, Shirinzadeh H, et al. (2013). Antioxidant activity of indole-based melatonin analogues in erythrocytes and their voltammetric characterization. J Enzyme Inhib Med Chem 28:1143–55.

Wang R, Shi H-F, Zhao J-F, et al. (2013). Design, synthesis and aromatase inhibitory activities of novel indole-imidazole derivatives. Bioorganic Med Chem Lett 23:1760–2.

Whitehead SA, Rice S. (2006). Endocrine-disrupting chemicals as modulators of sex steroid synthesis. Best Pract Res Clin Endocrinol Metabol 20:45–61.