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# New Clicked Full Agonists of the Estrogen Receptor β

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A click chemistry approach was used to synthesize a series of 1,4-diaryl-substituted 1,2,3-triazoles designed to behave as estrogen receptor ligands. We studied their affinities for both receptors α and β, their agonist activities in a cell-based luciferase reporter assay and their effect on the proliferation of the hormone dependent MCF-7 cell line. We found two compounds (**3a** and **3c**) that behave as selective full agonists for ERβ at a 20 μM concentration, and one of them (**3c**) deprived of proliferative effect on MCF-7 cells.

## Introduction

The estrogen receptor (ER) is a member of the nuclear receptor gene family binding the steroid hormone estradiol.[1] Two subtypes are known of ER, designated ERα and ERβ. Both ER subtypes have overlapping but also unique roles in estrogen-dependent action and are important targets in pharmaceutical industry. Additionally, ERα and ERβ have different transcriptional activities in certain ligand, cell-type, and promoter contexts.

The low expression level of ERβ in reproductive tissues such as uterus, suggests that a selective ERβ agonist may maintain the beneficial effects of estrogen, without the increased risk of breast and endometrial cancer. A number of selective ligands have been already identified.[2] ERβ selective agonist ERB-041 (226-fold selective for β) has been used to demonstrate that this receptor may be a useful target for certain inflammatory processes.[3] Other nonsteroidal scaffolds which have been developed as ERβ ligands are diarylpropanenitriles (DPN),[4] 2-phenylnaphthalenes (WAY-202196),[2, 5] and aryll-2H-indazoles.[6] We have described a series of benzonaphthofuran and naphthothiophene based ligands which behave as ERβ agonists and ERα antagonists,[7] and present an interesting antitumor activity against two pancreatic cell lines.[8] Introduction of a basic side chain in these scaffolds has led to full antagonists of ERβ, with potency in the low micromolar concentration in a cell-based luciferase reporter assay, and completely devoid of activity against the ERα at the same concentration range.[9]

Click chemistry has had a profound effect on the design and development of novel compounds for therapeutic applications.[10] In particular, the Copper-Catalyzed reaction between an Azide and an Alkyne (CuAAC) has been widely used in fragment-based drug design, and target-guided synthesis (*in situ* click chemistry).[11]

Tron and coworkers[12] used CuAAC click chemistry to obtain a series of 1,4-diaryl-substituted 1,2,3-triazoles **1** (Fig. 1) and evaluated their effect on the proliferation of the hormone dependent MCF-7 cell line. The only active compound promoted proliferation at a 100 pM concentration and possessed the two hydroxy groups in *meta* position. This compound was capable of promoting transcriptional activation in HeLa cells expressing higher levels of ERβ than ERα at low concentrations. These data suggest ER-β selectivity, but further studies on the affinity and transcriptional response on both receptors are required to establish the subtype selectivity of this type of compounds.

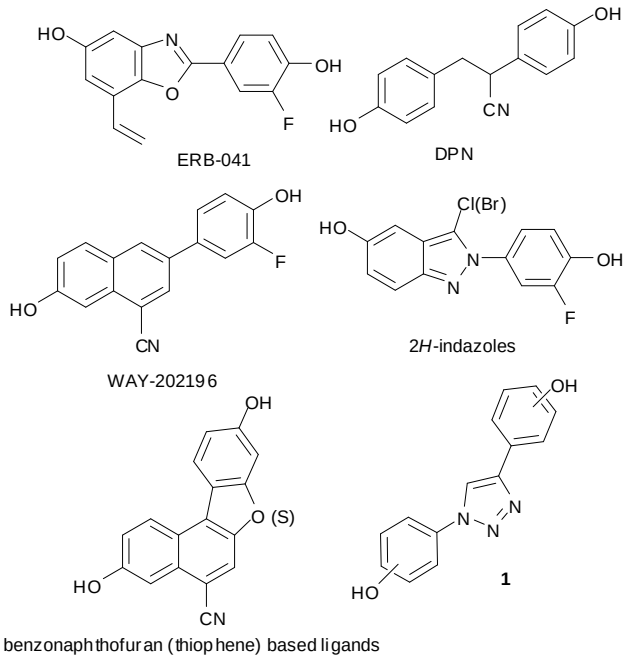



**Fig. 1** Chemical structures of some known ERβ-selective ligands

In the search of new and selective ligands of both estrogen receptor isoforms, we were interested in the observation of the authors that the introduction of a triazole ring is compatible with binding to the estrogen receptor, and the possibility to apply this efficient synthetic procedure to obtain analogues with additional substituents on the aromatic rings that could favor the interactions with one of the receptor subtypes.

Thus, we have synthesized two series of 1,4-bis(hydroxyphenyl)-1*H*-1,2,3-triazoles **2a-f** and **3a-f** (Fig. 2), where methyl, trifluoromethyl, fluoro, carboxy and methoxycarbonyl groups have been introduced in *orto* and *meta* positions of one of the aromatic rings. We have evaluated the ERα and ERβ binding affinities of the synthesized compounds and studied the transcriptional activation and proliferative activity in MCF-7 breast cancer line of the most interesting of these.

The best binders were compounds **2a** and **3a**, showing $IC_{50}$ of 8.4 and 8.00 μM, respectively in ERα and lower binding affinity to ERβ. Compound **3a** emerged as a full ERβ agonist at 20 μM, while its effect on ERα was negligible at the same concentration. This compound stimulated proliferation of MCF-7 cells with maximal proliferation at 10 μM. Interestingly, analogue **3c** behaved as well as a full ERβ agonist at 20 μM, but was deprived of proliferative activity.

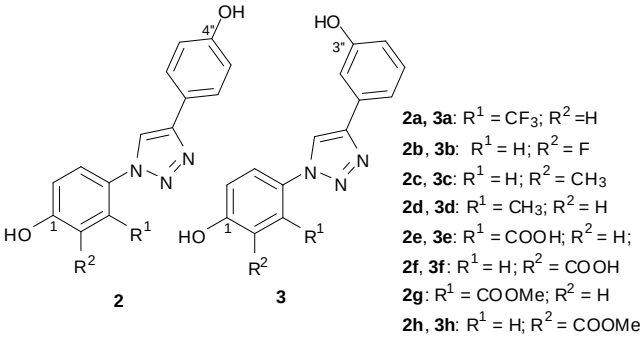



**Fig.2** Structure of the synthesized 1,4-diaryl-substituted 1,2,3-triazoles

## Results and discussion

### Molecular Modelling

Preliminary docking studies were carried out in order to assess the capability of the designed compounds to interact with the ligand binding domain (LBD) of both ERs. Several crystallographic structures of the two receptor subtypes in complex with several ligands have been considered: ERα in complex with estradiol (PDB 1A52), raloxifene (PDB 1ERR), genistein (PDB 1X7R), WAY-244 (PDB 1X7E), and 4-hydroxytamoxifen (PDB 3ERT), and ERβ in complex with genistein (PDB 1X7J), THC (PDB 1L2J), WAY-202196 (PDB 1YYE), and 4-hydroxytamoxifen (PDB 2FSZ).

All the candidates to be synthesized (**2** and **3**) were able to bind ERs, at least theoretically.

Regarding predicted binding energies, these were more favourable in the case of ERβ-ligand complexes, as most of the ligands led to values within the highest energy range (from - 7.3 to -9.5 kcal/mol) together with estradiol-like poses. However, in the case of ERα, only two compounds, **2a** and **3a**, led to the best values in ERα (-7.9 kcal/mol), although with different docked poses: **2a** presents a pose different to that for estradiol, while **3a** establishes interactions similar to those found for estradiol, as will be discussed below.

Regarding the binding mode, the analysis of the docking studies showed that all compounds could adopt estradiol-like poses in ERβ, while in ERα no estradiol-like poses were found or they did not show proper binding. Compounds **3a** and **3d**, were an exception, because they were the only ligands exhibiting estradiol-like poses when docked inside ERα (predicted binding energies of -7.9 and -6.8 kcal/mol, respectively).

Overall, these results may point to a preference for ERβ binding, with the exception of compounds **2a** and **3a**, which may present binding abilities for both receptors.

When studying ligand-receptor interactions at atomic detail, it can be observed that compound **3a** establishes hydrogen bonds between the OH-1 group and Glu305-Arg346, and the OH-3'' group with His475 (ERβ numbering), in an estradiol-like fashion in both ER subtypes. Additionally, the $CF_3$ group is hosted in a hydrophobic cavity delimited by Leu339, Met340, Leu343, and Leu380 (in ERα: Leu387, Met388, Leu391, and Leu428). In the case of **3d**, which also establishes estradiol-like binding modes in both receptors, the presence of the $CH_3$ group in this position led to a decrease of 1.1 kcal/mol in the docked binding energy, indicating the role of the $CF_3$ group in the binding (see ESI). The $CF_3$ group is also present in **2a**. In ERβ, docking studies for this compound led to an estradiol-like binding pose with the opposite orientation to that found for **3a**. So, hydrogen bonds were identified between OH-1 and His-475; and between OH-4'' and Glu305-Arg346. The $CF_3$ is accommodated in the vicinity to the previously mentioned hydrophobic cavity, contacting Leu380, Ile376, and Phe377 side chains (Fig. 3). In the case of ERα, this compound is predicted to bind in a non estradiol-like pose. These results suggest that the presence of a lipophilic $R^1$ group could favour the binding by the establishment of hydrophobic interactions with the cavity delimited by Leu339, Met340, Leu343, Ile376, Leu380 and Phe377 side chains (ERβ numbering), and that scaffold **3** is more suitable for anchoring the receptor (specially ERβ).

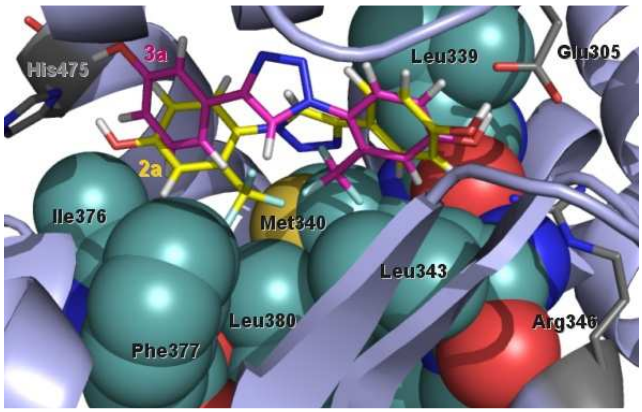
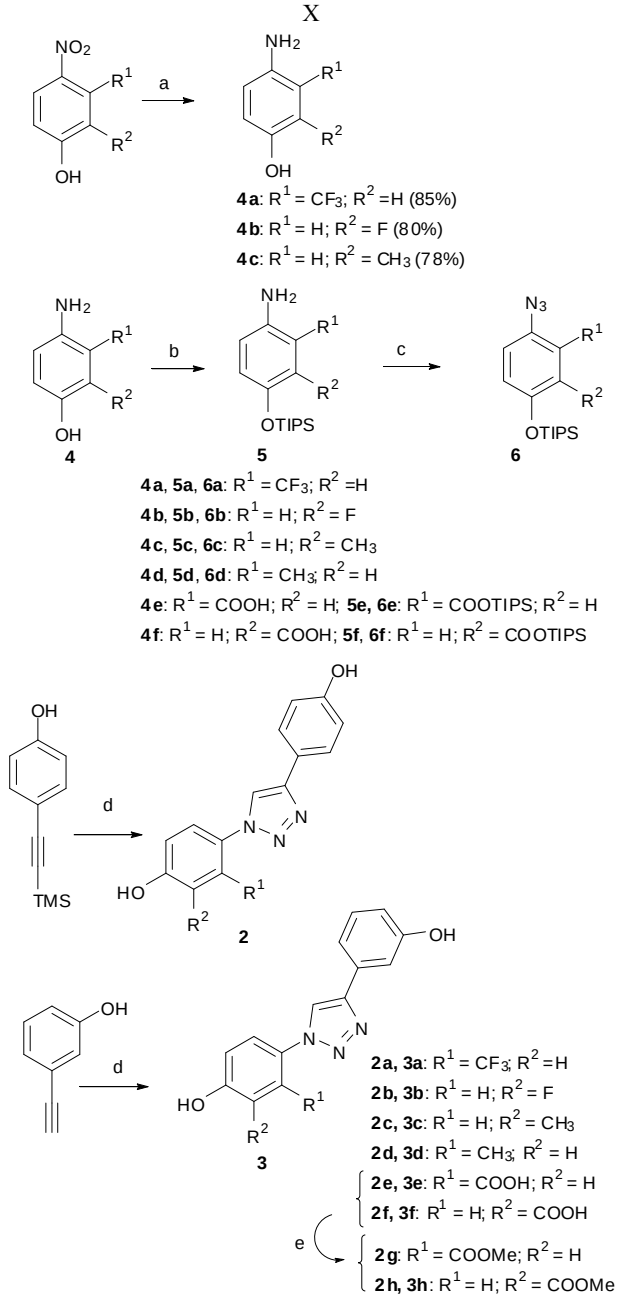



**Fig. 3** Docked binding modes obtained for compounds **2a** and **3a** in ERβ.

### Chemistry

As a result of the previous theoretical study, we decided to synthesize compounds **2** and **3**. The detailed synthesis of the azide fragment is shown in Scheme 1. Some of the amines used




**Scheme 1.** Synthesis of ligands **2** and **3**. (a) $H_2$, Pd/C for **4a**, Sn/HCl for **4b** and **4c**; b) 1 eq. TIPSCl/$Et_3N$ for **5a-d**, 2 eq. TIPSCl/$Et_3N$ for **5e-f**; (c) t-BuONO/$TMSN_3$; (d) TBFA, $CuSO_4.5H_2O$, sodium ascorbate, DMF; (f) MeOH, $H_2SO_4$.

(**4d-f**) are commercially available, and some had to be synthesized (**4a-c**) by Sn/HCl reduction of the corresponding nitrocompounds. After protection of the hydroxyl group (together with the carboxylic groups in the case of **4e** and **4f**), the TIPS-protected derivatives were transformed into azides **6** by reaction with *tert*-butyl nitrite and azidotrimethylsilane.

These azides were coupled to 3-ethynylphenol and 4-((trimethylsilyl)ethynyl)phenol. The former is commercially available, and the latter was obtained by Sonogashira coupling between the commercially available 4-iodophenol and trimethylsilyl acetylene.

For the CuAAC reaction, we used $CuSO_4$ (0.1 eq.) and ascorbic acid (0.2 eq) in the presence of tetrabutylammonium fluoride to bring about the deprotection of 4-((trimethylsilyl)ethynyl)phenol in the reaction mixture and the deprotection of the final triazoles.

We have chosen this synthetic pathway, involving the use of protective groups, because, in our first attempt to carry out the click reaction starting from unprotected azides, we observed a low solubility of the reactants in the reaction medium, and the formation of a complex mixture of compounds by TLC and $^1$H-NMR analysis of the crude reaction.

Finally, acids **2e**,**f** and **3f** were transformed into the corresponding methyl esters **2g**,**h** and **3h** by reaction with MeOH in the presence of $H_2SO_4$.

**Estrogen Receptor Binding Assays.**

The affinity for ERα and ERβ of compounds **2** and **3** were determined in an in vitro competitive binding assay, following a reported method,[13] with some modifications. All compounds were tested at 10 μM and only compounds **2a**, **2e**, **3a** and **3d** presented enough affinity at this concentration to determine the $IC_{50}$ at least in one of the receptors. Table 1 shows a summary of the results.

**Table 1** Affinities[a] of compounds **2** and **3** ($IC_{50}$) and % of [$^3$H]-estradiol binding to ERα and β at 10μM.

| ligand | $IC_{50}$ ERα (μM) | $IC_{50}$ ERβ (μM) | β/α ratio | 10μM ERα | 10μM ERβ |
|---|---|---|---|---|---|
| **2a** | 8.4 | 22.6 | 2.6 | 47 | 75.3 |
| **2b** | NA | NA | - | 98.5 | 100 |
| **2c** | NA | NA | - | 100 | 100 |
| **2d** | NA | NA | - | 76.9 | 87.3 |
| **2e** | 14.5 | 130.0 | 8.9 | 60.9 | 79.7 |
| **2f** | NA | NA | - | 94.8 | 100 |
| **2g** | NA | NA | - | 83.9 | 94.4 |
| **2h** | NA | NA | - | 98.6 | 100 |
| **3a** | 8.00 | >100 | | 50.7 | 100 |
| **3b** | NA | NA | -. | 100 | 100 |
| **3c** | NA | NA | - | 100 | 100 |
| **3d** | 61.4 | >100 | - | 72.5 | 100 |
| **3e** | NA | NA | - | 100 | 100 |
| **3f** | NA | NA | - | 97.8 | 100 |
| **3h** | NA | NA | - | 78.3 | 100 |

[a] Values are an average of at least 2 experiments with typical standard errors below 15%. NA -not achieved

[b] The percentage of specific binding of [2,4,6,7,16,17-3H]-estradiol to ER.

**Transcription Assays.**

To characterize the agonist/antagonist profile we first submitted three compounds (**2a, 2e**, and **3a**) with the highest binding affinity.

They were assayed for transcriptional activity through both receptor subtypes, with estradiol as a reference. Reporter gene assays were conducted in mammalian cells containing either ERα or ERβ-responsive luciferase reporter gene. The luciferase gene expression appears when ligand-bound ER undergoes nuclear translocation, DNA binding, assembling of the co-activators and other factors which are necessary to obtain a functional

transcription complex, in which target gene is expressed. The readout from those reporter cells fulfils similar interaction demands to those which can be observed in vivo. In the reporter gene assays on cells treated with saturating concentrations of estradiol, ERβ showed a maximal activity which was 62% lower than the maximal activity observed on ERα. This observation is in agreement with previous published results.[14]

Compounds **2a** and **3a** showed agonistic effects in ERβ (Fig. 4). Thus, compound **2a** induced a significant increase in luciferase activity (414±8,46 % of control) at 10 μM, and compound **3a** showed a very high efficacy at 20 μM (633±67,8 of control), which is comparable to the maximum response of estradiol (694,38±6,21 of control at 4 nM). Compound **2e** induced some increase in transcriptional activity but it was not concentration-dependent.

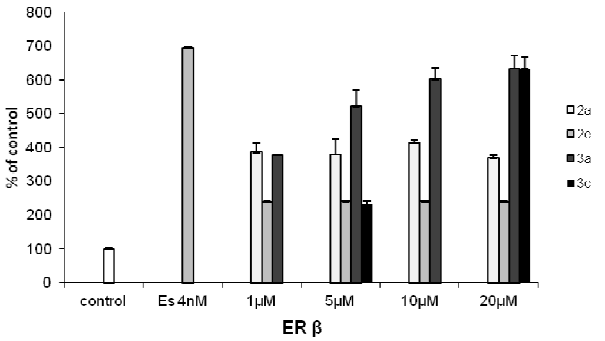



**Fig. 4** Transcriptional activation of ERβ by estradiol (4 nM) and compounds: **2a**, **2e**, **3a** and **3c.**

In the case of ERα (Fig. 5), compound **2a** was able to activate the receptor with the maximum activity at 20 μM (859,56% of control) in comparison to the maximum response of estradiol in this receptor (1689,06±74,17 at 100 nM). These results suggest that **2a** behaves as an agonist for both receptors with similar efficacy.

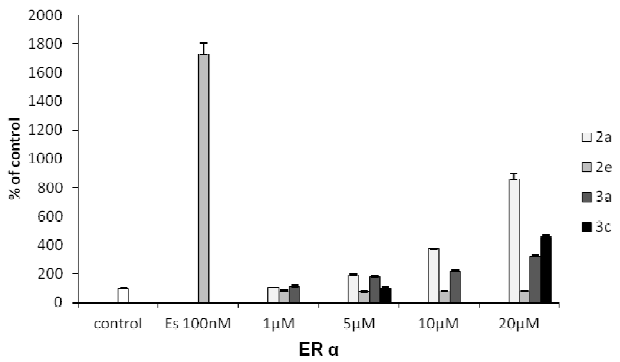



**Fig. 5** Transcriptional activation of ER α by estradiol (100nM) and compounds **2a**, **2e**, **3a** and **3c**

More interestingly, only a slight activity on ERα was observed in the case of compound **3a** at 20 μM (320,42±14,74 of control in comparison to 1689,06±74,17 for 100 nM of estradiol. This result demonstrates that **3a** is selective in its transcriptional activity, behaving as a full agonist for ERβ at 20 μM, while being a weak agonist for ERα at the same concentration. It is of great interest to detect non-steroidal molecules that display agonistic or antagonistic properties on the ERs in a specific manner.

It should be noted that there is no correlation between binding affinity and transcriptional potency for this compound. This lack of correlation has been observed in other cases,[15] and could be explained by differences in the mode of binding between the ligands, the ERs and various cellular coregulators, which could modulate the final ligand response in the transcriptional assay.

Finally, no transcriptional effect was present on ERα at the tested concentrations in the case of **2e**. This compound could be classified as a selective partial agonist of ERβ. No antagonistic activity was observed for all tested compounds.

**Effect on the proliferation of a MCF-7 breast cancer cell line.**

We determined the proliferative activity of all the synthesized compounds on the hormone dependent MCF-7 breast cancer line (Fig. 6 and Fig. 7).

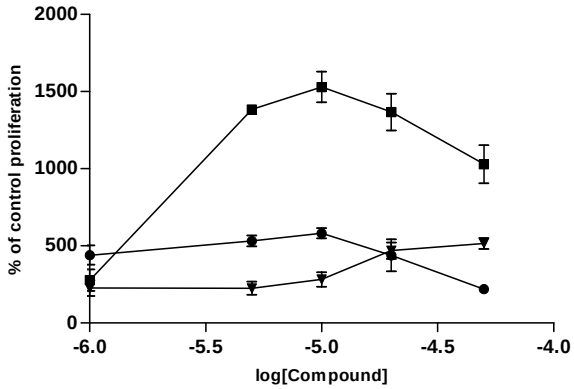



**Fig.6** Concentration response curves of proliferation of MCF-7 treated with the following compounds: **2a**(●), **2e**(▼), and **3a**(■),evaluated with crystal violet assay. Data are mean ±SD of at least 5 determinants in 2 independent experiments.

Compound **3a** induced an increase in proliferation, displaying a bell-shape dose response curve, with maximal proliferation at 10 μM (1529,15%±99,6) of solvent control in comparison to 446,17%±45,4 obtained for 1 pM estradiol. The other two ER binders **2a** and **2e** also increased proliferation but with lower efficacy with maximum at 10 μM (581,59±33,12) and 50 μM (483,35±39,81) for **2a** and **2e**, respectively.

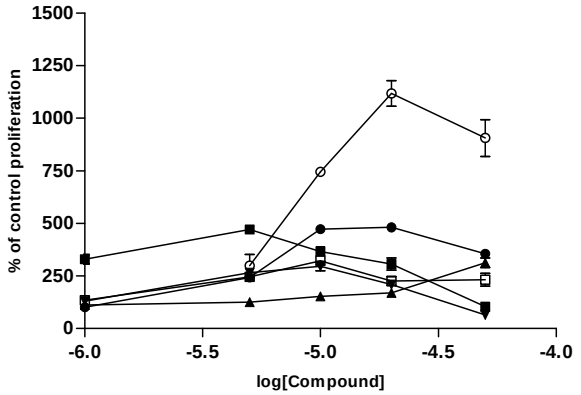
This proliferative activity could be explained by its effect on the ER pathway, as we proved that these compounds behave as ER agonists.

The rest of compounds presented different profiles that did not correlate with their binding affinities (Fig. 7). Interestingly compound **3c** did not induce proliferation of MCF7 cells, what is in agreement with its lack of affinity for both receptors in the binding assay. However, taking into account the observed lack of correlation between the binding affinity and the functional

activity of the previously studied compounds (**2a**, **2e** and **3a**), we decided to study its transcriptional activation capability (Fig. 4 and Fig. 5). We found that **3c** behaved as a full agonist at 20 μM, increasing luciferase activity (634,1±30,8 of control) in a similar extent than estradiol at 4 nM (694,38±6,21), while its activation of ERα (455,2±14,2 of control) was only 27% of control activity of estradiol (1689,06±74,17 at 100 nM) (Fig. 4 and Fig. 5)

Thus, **3c** is a full ERβ agonist, without promoting the proliferation of MCF-7 cells, a biological profile which holds promise for the treatment of inflammatory diseases including rheumatoid arthritis, and cardiovascular and CNS conditions.[14]

a)




b)

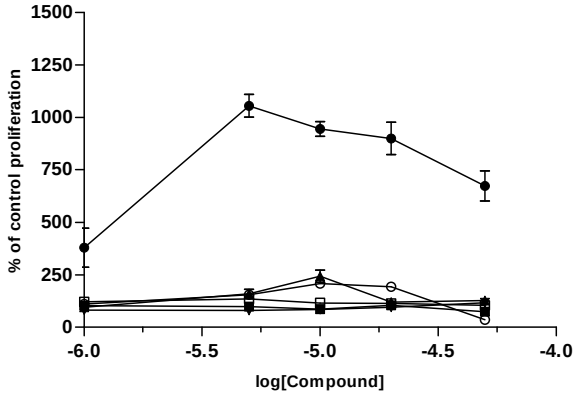



**Fig.7** Concentration response curves of proliferation of MCF-7 treated with the following compounds: a) **2b**(●), **2c** (▼), **2d** (■), **2f** (▲), **2g**(○), **2h** (□) and b) **3b**(○), **3c** (■), **3d**(●), **3e** (▼), **3f** (▲), **3h** (□). Data are mean ±SD of at least 5 determinants in 2 independent experiments.

## Conclusions

A series of *clicked* 1*H*-1.2.3-triazoles have been synthesized, and their affinity and transcriptional activation of both ERα and ERβ have been determined by means of in vitro assays. This study has allowed us to detect two compounds (**3a** and **3c**) that behave as selective full agonists for ERβ at 20 μM, while its effect on ERα is considerably lower at the same concentration. These compounds could be useful to gain knowledge on the physiological role of ERβ. While **3a** induced the proliferation of MCF-7 cells, its analogue **3c** was completely inactive in this breast cancer cell line. This compound constitutes therefore, a new and promising candidate for the development of ER binding agents useful for the treatment of inflammatory, cardiovascular and CNS diseases, without promoting undesired proliferative effects on breast.

## Experimental

**General Methods.** Melting points (uncorrected) were determined on a Stuart Scientific SMP3 apparatus. Infrared (IR) spectra were recorded with a Perkin-Elmer 1330 infrared spectrophotometer. $^{1}$H and $^{13}$C NMR were recorded on a Bruker 300-AC instrument. Chemical shifts (δ) are expressed in parts per million; coupling constants (*J*) are in Hertz. Mass spectra were run on a Bruker Esquire 3000 spectrometer. Thin-layer chromatography (TLC) was run on Merck silica gel 60 F-254 plates. Unless stated otherwise, starting materials used were high-grade commercial products. For all tested compounds > 95% purity was confirmed by HPLC/MS using a Esquire 3000 ion-trap mass spectrometer (Bruker-Daltronics, Bremen, Germany) coupled to an Agilent HPLC system type HP 1100. A LiChroCART Supersphere100 RP-18 column (125 x 2 mm; 4 μm particle size) was used, eluting with $H_2O$ + 0.1% TFA / MeOH + 0.1% TFA or Acetonitrile + 0.1% TFA. Acid **2f** was obtained in a complex mixture with other unknown compounds, and was not possible to isolate it pure. However it could be transformed into the corresponding methyl ester **2h**.

**General method for the synthesis of amines 4a-c**:

To a 100 ml round bottomed flask, containing a stirrer bar and a reflux condenser, tin (1.65 g, 14 mmol) and the corresponding phenol (10 mmol) were added. Then water (24 mL) followed by concentrated hydrochloric acid (9 mL) were added, and the solution was heated under reflux for 1.5 h. Then, the reaction mixture was cooled in an ice bath, a 5 M NaOH solution (20 mL) was added, and the aqueous solution was extracted with AcOEt (3 × 50 cm$^3$) and the combined organic extracts were washed with saturated aqueous $NaHCO_3$ and brine, dried ($MgSO_4$) and concentrated to dryness to give the desired amine **4**

**4-Amino-3-trifluoromethylphenol 4a.** Yield 88%; $^{1}$H NMR $\delta_H$ (300 MHz; DMSO) 4.88 (2H, s, $NH_2$), 6.68-6.80 (3H, m, ArH), 8.92 (1H, s, OH).

**4-Amino-2-fluorophenol 4b.** Yield 93%**;** $^{1}$H NMR $\delta_H$ (300 MHz; $CD_3OD$) 6.25-6.32 (1H, m, ArH), 6.40 (1H, dd, *J* 12.1, 2.4), 6.59 (1H, dd, *J* 9.6 and 8.6); $^{13}$C NMR $\delta_c$ (75.4 MHz, $CD_3OD$) 105.3 ($J_{CF}$ 21Hz), 112.9 ($J_{CF}$ 3Hz), 119.3 ($J_{CF}$ 4Hz), 138.0 ($J_{CF}$ 13Hz), 141.6 ($J_{CF}$ 9Hz) and 153.4 ($J_{CF}$ 238Hz)

**4-Amino-2-methylphenol 4c.** Yield 90%; $^{1}$H NMR $\delta_H$ (300 MHz; $CD_3OD$) 2.01 (3H, s, $CH_3$), 6.34 (1H, dd, *J* 8.1 and 2.3, ArH), 6.41-6.48 (2H, m, ArH); $^{13}$C NMR $\delta_c$ (75.4 MHz, CD3OD) 16.76, 116.20, 116.77, 120.68, 126.64, 140.40 and 149.90.

**General method for the synthesis of the TIPS-protected compounds 5a-d**:

To a solution of the corresponding phenol (2.83 mmol) in dry DMF (2 mL) was added a solution of triisopropylsilyl chloride (0.655 g, 3.40 mmol) and triethylamine (0.315 g, 3.11 mmol) in dry DMF (1 mL) under $N_2$, and the mixture was stirred at RT for 24 hours. The solution was filtered and the solvent was evaporated. The resulting residue was purified by column chromatography using hexane/AcOEt (15:1) as eluent to give the desired product.

**2-(Trifluoromethyl)-4-((triisopropylsilyl)oxy)aniline 5a.** Yield 87%, oil; $^1$H NMR $\delta_H$ (300 MHz; $CDCl_3$) 1.08 (9H, d, *J* 7.3, $CH_3$), 1.12 (9H, d, *J* 7.3, $2CH_3$), 1.12-1.27 (3H, m, 3CH), 1.28-1.45 (3H, m, 3CH), 2.80-3.70 (2H, brs, $NH_2$), 6.54 (1H, d, *J* 8.7, ArH), 6.77 (1H, dd, *J* 8.7 and 2.7, ArH), 6.88 (1H, d, *J* 2.7, ArH).

**3-Fluoro-4-((triisopropylsilyl)oxy)aniline 5b.** Yield 80%, oil**;** $^1$H NMR $\delta_H$ (300 MHz; $CDCl_3$) 1.00 (18H, d, *J* 6.9, $6CH_3$), 1.12-1.35 (3H, m, 3CH), 3.37 (2H, brs, $NH_2$), 6.20-6.25 (1H, m, ArH), 6.32 (1H, dd, *J* 12.1, 2.8, ArH), 6.66 (1H, t, *J* 8.9, ArH).

**3-Methyl-4-((triisopropylsilyl)oxy)aniline 5c.** Yield 82%, oil; $^1$H NMR $\delta_H$ (300 MHz; $CDCl_3$) 1.01 (18H, d, *J* 7.0, $6CH_3$), 1.10-1.21 (3H, m, 3CH), 2.09 (3H, s, $CH_3$Ar), 3.10-3.40 (2H, brs, $NH_2$), 6.29 (1H, dd, *J* 8.3, 2.9, ArH), 6.42 (1H, d, *J* 2.9, ArH), 6.50 (1H, d, *J* 8.3, ArH).

**2-Methyl-4-((triisopropylsilyl)oxy)aniline 5d.** Yield 84%, oil; $^1$H NMR $\delta_H$ (300 MHz; $CDCl_3$) 1.08 (9H, d, *J* 7.3, $3CH_3$), 1.12 (9H, d, *J* 7.3, $3CH_3$), 1.12-1.27 (3H, m, 3CH), 1.28-1.45 (3H, m, 3CH), 2.05 (3H, s, $CH_3$), 2.60-3.40 (2H, brs, $NH_2$), 6.46 (1H, d, *J* 8.3, ArH), 6.49 (1H, dd, *J* 8.3, 2.6, ArH), 6.55 (1H, d, *J* 2.6, ArH).

**General method for the synthesis of the TIPS-protected compounds 5e and 5f**:

To a solution of the corresponding acid (2.35 mmol) in dry DMF (2 mL) was added a solution of triisoprophylsilyl chloride (1 g, 5.19 mmol) and triethylamine (0.500 g, 4.95 mmol) in dry DMF (1 mL) under $N_2$, and the mixture was stirred at RT for 24 hours. The solution was filtered and the solvent was evaporated. The resulting residue was purified by column chromatography using hexane/AcOEt (15:1) as eluent to give the desired product.

**Triisopropylsilyl 2-amino-5-((triisopropylsilyl)oxy)benzoate 5e.** Yield 94%, oil; $^1$H NMR $\delta_H$ (300 MHz; $CDCl_3$) 1.08 (18H, d, *J* 7.3, $3CH_3$), 1.12 (18H, d, *J* 7.3, $3CH_3$), 1.12-1.27 (3H, m, 3CH), 1.28-1.45 (3H, m, 3CH), 5.00-5.85 (2H, brs, $NH_2$), 6.56 (1H, d, *J* 8.7, ArH), 6.89 (1H, dd, *J* 8.8 and 2.9, ArH), 7.43 (1H, d, *J* 2.9, ArH).

**Triisopropylsilyl 5-amino-2-((triisopropylsilyl)oxy)benzoate 5f.** Yield 86%, oil; $^1$H NMR $\delta_H$ (300 MHz; $CDCl_3$) 1.00 (18H, d, *J* 7.3, $6CH_3$), 1.03 (18H, d, *J* 7.3, $3CH_3$), 1.12-1.38 (6H, m, 6CH), 2.55-3.00 (2H, br s, $NH_2$), 6.56-6.67 (2H, m, ArH), 7.02 (1H, d, *J* 2.1, ArH).

**General method for the synthesis of azides.**

To an ice cooled solution the corresponding compound **6a-f** (1 g, 2.15 mmol) in $CH_3CN$ (5mL), was added dropwise *t*-BuONO (0.332 g, 381 µL, 3.22 mmol) followed by $TMSN_3$ (0.301 g, 341 µL, 2.57 mmol) and the solution was stirred at room temperature for 4 h. After concentrating under vacuum, the crude product was purified by silica gel chromatography (hexane) to give the desired product.

**(4-Azido-3-(trifluoromethyl)phenoxy)triisopropylsilane 6a.** Yield 67%, oil; $^1$H NMR $\delta_H$ (300 MHz; $CDCl_3$) 1.01 (18H, d, *J* 7.3, $CH_3$ iPr), 1.10-1.21 (1H, m, CH iPr), 7.00 (1H, dd, *J* 8.6, 2.6, ArH), 7.05-7.09 (2H, m, ArH).

**(4-Azido-2-fluorophenoxy)triisopropylsilane 6b.** Yield 71%, oil

$^1$H NMR $\delta_H$ (300 MHz; $CDCl_3$) 1.00 (18H, d, *J* 6.9, $6CH_3$), 1.09-1.28 (3H, m, 3CH), 6.55-6.63 (1H, m, ArH), 6.67 (1H, dd, *J* 11.1 and 2.6, ArH), 6.84 (1H, t, *J* 8.9, ArH); $^{13}$C NMR $\delta_c$ (75.4 MHz, $CDCl_3$) 11.6, 16.7, 106.6 ($J_{CF}$ 22Hz), 113.5 ($J_{CF}$ 3Hz), 121.4 ($J_{CF}$ 3Hz), 132.1 ($J_{CF}$ 9Hz), 140.1 ($J_{CF}$ 12Hz) 153.2 ($J_{CF}$ 246Hz).

**(4-Azido-2-methylphenoxy)triisopropylsilane 6c.** Yield 64%, oil; $^1$H NMR $\delta_H$ (300 MHz; $CDCl_3$) 1.01 (18H, d, *J* 7.0, $3CH_3$), 1.10-1.21 (3H, m, CH), 2.15 (3H, s, $CH_3$), 6.62 (1H, dd, *J* 8.5, 2.8, ArH), 6.67 (1H, dd, *J* 8.5, 6.7, ArH), 6.72 (1H, m, ArH); $^{13}$C NMR $\delta_c$ (75.4 MHz, $CDCl_3$) 12.99, 17.08, 17.99, 116.96, 118.82, 121.33, 130.27, 131.92, 151.73.

**(4-Azido-3-methylphenoxy)triisopropylsilane 6d.** Yield 70%, oil; $^1$H NMR $\delta_H$ (300 MHz; $CDCl_3$) 1.01 (18H, d, *J* 7.3, $3CH_3$), 1.10-1.21 (3H, m, 3CH), 2.08 (3H, s, $CH_3$), 6.61 (1H, d, *J* 2.6, ArH), 6.65 (1H, dd, *J* 8.5 and 2.6, ArH), 6.86 (1H, d, *J* 8.5, ArH); $^{13}$C NMR $\delta_c$ (75.4 MHz, $CDCl_3$) 17.95, 18.31, 19.31, 119.74, 121.84, 124.70, 134.20, 134.79, 154.80.

**Triisopropylsilyl 2-azido-5-((triisopropylsilyl)oxy)benzoate 6e.** Yield 85%, oil**;** $^1$H NMR $\delta_H$ (300 MHz; $CDCl_3$) 1.01 (18H, d, *J* 7.3, $3CH_3$), 1.05 (18H, d, *J* 7.3, $3CH_3$), 1.10-1.21 (3H, m, 3CH), 1.23-1.38 (3H, m, 3CH), 6.99 (1H, dd, *J* 8.6, 2.6, ArH), 7.04 (1H, d, *J* 8.6, ArH), 7.42 (1H, d, *J* 2.6, ArH); $^{13}$C NMR $\delta_c$ (75.4 MHz, $CDCl_3$) 12.0, 12.6, 17.80, 17.82, 121.2, 123.11, 124.1, 125.2, 133.5, 152.7, 164.1.

**Triisopropylsilyl 5-azido-2-((triisopropylsilyl)oxy)benzoate 6f.** Yield 70%, oil; $^1$H NMR $\delta_H$ (300 MHz; $CDCl_3$) 1.03 (18H, d, *J* 7.3, $CH_3$ iPr), 1.06 (18H, d, *J* 7.3, $6CH_3$), 1.12-1.38 (6H, m, 6CH), 6.79 (1H, d, *J* 8.7, ArH), 6.90 (1H, dd, *J* 8.7 and 2.9, ArH), 7.38 (1H, d, J 2.9, ArH); $^{13}$C NMR $\delta_c$ (75.4 MHz, $CDCl_3$) 12.04, 13.13, 17.88, 17.98, 121.76, 122.06, 123.24, 124.62, 131.86, 153.83, 163.39.

**4-((Trimethylsilyl)ethynyl)phenol.** A solution of 4-iodophenol (0.880 g, 4 mmol), trimethylsilylacetylene (0.855 mL, 6 mmol), $PdCl_2$ (35.8 mg, 0.20 mmol, 5 mol%), $Ph_3P$ (0.106 g, 0.40 mmol, 10 mol%), CuI (19 mg, 0.10 mmol, 2.5 mol%), and triethylamine (3.94 mL, 28.2 mmol) in of dry acetonitrile (20 mL) was heated at reflux under an argon atmosphere. After 3 h of reflux, the reaction mixture was filtered through a celite pad. The filtrate was concentrated under reduced pressure and chromatographed on silica gel using a AcOEt/hexane (1:4) as eluent to afford the desired compound (0.533 g, 70%) as a brown solid; $^1$H NMR $\delta_H$ (300 MHz; $CDCl_3$) 0.16 (9H, s, $3CH_3$), 4.60-5.50 (1H, brs, OH), 6.65 (2H, d, *J* 8.7, ArH), 7.27 (2H, d, *J* 8.7, ArH)**;** $^{13}$C NMR $\delta_c$ (75.4 MHz, $CDCl_3$) 0.15, 103.68, 106.71, 114.22, 116.85, 132.78, 158.43.

**General method for the click reactions.**

A solution of 4-((trimethylsilyl)lethynyl)phenol (0.1 g, 0.53 mmol) or 3-ethynylphenol (0.063 g, 0.53 mmol) and the corresponding azide (0.53 mmol) in DMF (10 mL) was treated with 1M solution of tetrabuthylamonium fluoride in THF **(**see the

amount below for each example) under argon. The reaction mixture was stirred at 0 ºC for 30 min. Then, a freshly prepared 1 M aqueous solution of sodium ascorbate (0.2 eq) was added, followed by the addition of copper (II) sulfate pentahydrate (0.1 eq). After stirring for 24 h under argon atmosphere, the reaction mixture was concentrated under vacuum. Then AcOEt (50 mL) was added and the solution was washed with 0.1 M HCl. The solvent was evaporated and the resulting residue was purified by column chromatography on silica using AcOEt/hexane as eluent, to yield the desired product.

**4-(4-(4-Hydroxyphenyl)-1*H*-1,2,3-triazol-1-yl)-3-(trifluoromethyl)phenol 2a.** From tetrabuthylamonium fluoride (1.11 mL, 1M solution in THF); chromatography eluent: AcOEt/Hexane 3:2; yield 78%; mp 183-184 ºC; $\nu_{max}$ (KBr)/cm$^{-1}$ 3500, 3037, 2917, 1619; $^1$H NMR $\delta_H$ (300 MHz; $CD_3OD$) 5.08 (2H, brs, 2OH), 6.90 (2H, d, *J* 8.6, ArH), 7.18 (1H, dd, *J* 8.6, 2.6, ArH), 7.29 (1H, d, *J* 2.6, ArH), 7.43 (1H, d, *J* 8.6, ArH), 7.72 (2H, d, *J* 8.6, ArH), 8.34 (1H, s, Triazole-H); $^{13}$C NMR $\delta_c$ (75.4 MHz, $CD_3OD$) 115.0 ($J_{CF}$ 5Hz), 116.9, 120.5, 122.6, 124.1 ($J_{CF}$ 273Hz), 124.2, 127.06, 128.0, 128.4, 128.7 ($J_{CF}$ 32Hz), 131.9, 135.1, 149.0, 159.2, 161.0. MS (ESI) *m/z*: 344.00 [M+Na]$^+$, 322.04 [M+H]$^+$

**2-Fluoro-4-(4-(4-hydroxyphenyl)-1*H*-1,2,3-triazol-1-yl)phenol 2b.** From tetrabuthylamonium fluoride (1.11 mL, 1M solution in THF); chromatography eluent: AcOEt/Hexane 3:2; yield 76%, mp 258-259 °C**;** $\nu_{max}$ (KBr)/cm$^{-1}$ 3268, 3156, 2917, 1615; $^1$H NMR $\delta_H$ (300 MHz; $CD_3OD$) 4.83 (2H, br s, 2OH), 6.86 (2H, d, *J* 8.6, ArH), 7.09 (1H, t, *J* 8.9, ArH), 7.47-7.53 (1H, m, ArH), 7.62 (1H, dd, *J* 11.5 and 2.5, ArH), 7.69 (2H, d, *J* 8.6, ArH), 8.59 (1H, s, Triazole-H); $^{13}$C NMR $\delta_c$ (75.4 MHz, $CD_3OD$) 110.3 ($J_{CF}$ 23Hz), 116.8, 117.9 ($J_{CF}$ 3Hz), 119.3 ($J_{CF}$ 13Hz), 119.44, 122.8, 128.3, 130.6, 147.1 ($J_{CF}$ 13Hz), 152.8 ($J_{CF}$ 243Hz), 159.2. MS (ESI) *m/z*: 293.98 [M+Na]$^+$, 271.99 [M+H]$^+$

**4-(4-(4-Hydroxyphenyl)-1*H*-1,2,3-triazol-1-yl)-2-methylphenol 2c.** From tetrabuthylamonium fluoride (1.11 mL, 1M solution in THF); chromatography eluent: AcOEt/Hexane 3:2; yield 83%, mp 242-243 °C**;** $\nu_{max}$ (KBr)/cm$^{-1}$ 3335, 3149, 2917, 1612; $^1$H NMR $\delta_H$ (300 MHz; DMSO) 2.23 (3H, s, $CH_3$), 6.86 (2H, d, *J* 8.0, ArH), 6.95 (1H, d, *J* 8.5, ArH), 7.52 (1H, d, *J* 7.9, ArH), 7.62 (1H, s, ArH), 7.73 (2H, d, *J* 7.9, ArH), 8.89 (1H, s, Triazole-H), 9.60 (1H, s, OH), 9.82 (1H, s, OH); $^{13}$C NMR $\delta_c$ (75.4 MHz, DMSO) 15.9, 115.0, 115.6, 117.9, 118.7, 121.4, 122.5, 125.2, 126.6, 128.6, 147.2, 155.6, 157.3. MS (ESI) *m/z*: 268.00 [M+H]$^+$

**4-[4-(4-Hydroxyphenyl)-1*H*-1,2,3-triazol-1-yl]-3-methylphenol 2d.** From tetrabuthylamonium fluoride (1.11 mL, 1M solution in THF); chromatography eluent: AcOEt/Hexane 3:2; yield 70%, mp 203 ºC; $\nu_{max}$ (KBr)/cm$^{-1}$ 3425, 3186, 2537, 1612; $^1$H NMR $\delta_H$ (300 MHz; $CD_3OD$) 2.10 (3H, s, $CH_3$), 5.00 (2H, br s, 2OH), 6.77-6.83 (2H, m, ArH), 6.89 (2H, d, *J* 8.5, ArH), 7.20 (1H, d, *J* 8.5, ArH), 7.71 (2H, d, *J* 8.5, ArH), 8.25 (1H, s, Triazole-H); $^{13}$C NMR $\delta_c$ (75.4 MHz, $CD_3OD$) 17.9, 114.6, 116.9, 118.6, 122.9, 123.2, 128.4, 129.8, 136.7, 149.0, 159.1, 160.1. MS (ESI) *m/z*: 267.98 [M+H]$^+$

**5-Hydroxy-2-(4-(4-hydroxyphenyl)-1*H*-1,2,3-triazol-1-yl)benzoic acid 2e.** From tetrabuthylamonium fluoride (1.65 mL, 1M solution in THF); chromatography eluent: AcOEt/Hexane 5:1; yield 63%, 122-128 ºC; $\nu_{max}$ (KBr)/cm$^{-1}$ 3261, 2925, 1612.08; $^1$H NMR $\delta_H$ (300 MHz; $CD_3OD$) 5.00 (2H, brs, OH), 6.87 (2H, d, *J* 8.4, ArH), 7.08 (1H, dd, *J* 8.7 and 3.0, ArH), 7.38 (2H, dd *J* 8.7 and 3.0, ArH), 7.69 (2H, d, *J* 8.4, ArH), 8.34 (1H, s, Triazole-H); $^{13}$C NMR $\delta_c$ (75.4 MHz, $CD_3OD$) 116.8, 118.2, 119.6, 123.0, 123.5, 128.3, 128.9, 129.4, 148.8, 159.0, 160.2, 169.4, 175.6. MS (ESI) *m/z*: 297.98 [M+H]$^+$

**2-Hydroxy-5-(4-(4-hydroxyphenyl)-1*H*-1,2,3-triazol-1-yl)benzoic acid 2f.** From tetrabuthylamonium fluoride (1.65 mL, 1M solution in THF); chromatography eluent: AcOEt/Hexane 5:1; yield 71%, mp 272-273 ºC (dec.); $\nu_{max}$ (KBr)/cm$^{-1}$ 3395, 3141, 2917, 2850, 1675, 1612; $^1$H NMR $\delta_H$ (300 MHz; $CD_3OD$) 4.86 (2H, brs, OH), 6.89 (2H, d, *J* 8.6, ArH), 6.99 (1H, d, *J* 8.7, ArH), 7.71-7.79 (3H, m, ArH), 8.30 (1H, d, *J* 2.1, ArH), 8.58 (1H, s, Triazole-H); $^{13}$C NMR $\delta_c$ (75.4 MHz, $CD_3OD$) 117.0, 118.7, 119.2, 121.1, 123.0, 124.0, 126.2, 128.3, 129.4, 149.7, 159.3, 163.63. MS (ESI) *m/z*: 297.99 [M+H]$^+$

**4-(4-(3-Hydroxyphenyl)-1*H*-1,2,3-triazol-1-yl)-3-(trifluoromethyl)phenol 3a.** From tetrabuthylamonium fluoride (0.55 mL, 1M solution in THF); chromatography eluent: AcOEt/Hexane 3:2; yield 82%, mp 182-183 ºC ; $\nu_{max}$ (KBr)/cm$^{-1}$ 3261, 3194, 2925, 2589, 1612; $^1$H NMR $\delta_H$ (300 MHz; $CD_3OD$) 4.94 (2H, br s, 2OH), 6.80-6.84 (1H, m, ArH), 7.16 (1H, dd, *J* 8.6, 2.8, ArH), 7.24 (1H, d, *J* 7.8, ArH), 7.29-7.37 (3H, m, ArH), 7.44 (1H, d, *J* 8.6, ArH), 8.41 (1H, s, Triazole-H); $^{13}$C NMR $\delta_c$ (75.4 MHz, $CD_3OD$) 113.7, 114.9, 116.7, 118.2, 120.5, 124.1 ($J_{CF}$ 273Hz), 125.3, 127.0, 128.7 ($J_{CF}$ 37Hz), 131.2, 131.9, 132.5, 148.8, 159.1, 161.0. MS (ESI) *m/z*: 344.01 [M+Na]$^+$, 322.05 [M+H]$^+$

**2-Fluoro-4-(4-(3-hydroxyphenyl)-1*H*-1,2,3-triazol-1-yl)phenol 3b.** From tetrabuthylamonium fluoride (0.55 mL, 1M solution in THF); chromatography eluent: AcOEt/Hexane 3:2; yield 81%, mp 225-226 °C; $\nu_{max}$ (KBr)/cm$^{-1}$ 3305, 2925, 2850, 1615; $^1$H NMR $\delta_H$ (300 MHz; $CD_3OD$) 4.85 (2H, br s, 2OH), 6.79-6.82 (1H, m, ArH), 7.09 (1H, t, *J* 8.9, ArH), 7.24 (1H, t, *J* 7.9, ArH), 7.31-7.35 (2H, m, ArH), 7.48-7.52 (1H, m, ArH), 7.64 (1H, dd, *J* 11.4 and 2.5, ArH), 8.64 (1H, s, Triazole-H); $^{13}$C NMR $\delta_c$ (75.4 MHz, $CD_3OD$) 110.2 ($J_{CF}$ 23Hz), 113.6, 116.6. 118.0 ($J_{CF}$ 4Hz), 118.2, 119.4 ($J_{CF}$ 4Hz), 120.3, 130.4 ($J_{CF}$ 8Hz), 131.1, 132.6, 147.2 ($J_{CF}$ 13Hz), 149.5, 152.8 ($J_{CF}$ 243Hz), 159.1. MS (ESI) *m/z*: 293.97 [M+Na]$^+$, 271.97 [M+H]$^+$

**4-(4-(3-Hydroxyphenyl)-1*H*-1,2,3-triazol-1-yl)-2-methylphenol 3c.** From tetrabuthylamonium fluoride (0.55 mL, 1M solution in THF); chromatography eluent: AcOEt/Hexane 3:2; yield 84%, mp 196-197 °C; $\nu_{max}$ (KBr)/cm$^{-1}$ 3318, 3141, 2925, 2850, 1615; $^1$H NMR $\delta_H$ (300 MHz; $CD_3OD$) 2.28 (3H, s, $CH_3$), 4.83 (2H, brs, OH), 6.78-6.82 (1H, m, ArH), 6.90 (1H, d, *J* 8.6, ArH), 7.25 (1H, t, *J* 7.9), 7.32-7.35 (3H, m, ArH), 7.47 (1H, dd, *J* 8.6 and 2.6), 7.55 (1H, d, *J* 2.5, ArH), 8.59 (1H, s, Triazole-H); $^{13}$C NMR $\delta_c$ (75.4 MHz, $CD_3OD$) 16.3, 113.6, 116.1, 116.5, 118.2, 120.4, 120.5, 124.3, 127.4, 130.5, 131.1, 132.8, 149.3, 157.6, 159.1. MS (ESI) *m/z*: 268.00 [M+H]$^+$

**4-(4-(3-Hydroxyphenyl)-1*H*-1,2,3-triazol-1-yl)-3-methylphenol 3d.** From tetrabuthylamonium fluoride (0.55 mL, 1M solution in THF); chromatography eluent: AcOEt/Hexane 3:2; yield 80%, mp 210-211 ºC; $\nu_{max}$ (KBr)/cm$^{-1}$ 3291, 2440, 1615; $^1$H NMR $\delta_H$ (300 MHz; $CD_3OD$) 2.12 (3H, s, $CH_3$), 4.83 (2H, br s, 2OH), 6.76-6.83 (3H, m, ArH), 7.21-7.35 (4H, m, ArH), 8.35 (1H, s, Triazole-H); $^{13}$C NMR $\delta_c$ (75.4 MHz, $CD_3OD$)

17.8, 113.6, 114.6, 116.5, 118.2, 118.6, 124.3, 128.5, 129.8, 131.2, 132.8, 136.7, 148.8, 159.2, 160.2. MS (ESI) *m/z*: 289.98 $[M+Na]^+$, 267.98 $[M+H]^+$

**5-Hydroxy-2-(4-(3-hydroxyphenyl)-1*H*-1,2,3-triazol-1-yl)benzoic acid 3e.** From tetrabuthylamonium fluoride (1.11 mL, 1M solution in THF); chromatography eluent: AcOEt/Hexane 5:1; yield 71%, mp 165-166 °C; $\nu_{max}$ (KBr)/cm$^{-1}$ 3313, 2917, 1705, 1589; $^1$H NMR $\delta_H$ (300 MHz; DMSO) 6.73-6.76 (1H, m, ArH), 7.10 (1H, dd, *J* 8.6 and 2.8), 7.22-7.35 (4H, m, ArH), 7.44 (1H, d, *J* 8.5, ArH), 8.77 (1H, s, Triazole-H), 9.54 (1H, brs, OH), 10.38 (1H, brs, OH), 12.92 (1H, brs, COOH); $^{13}$C NMR $\delta_c$ (75.4 MHz, DMSO) 111.8, 114.8, 116.0, 116.6, 118.6, 123.1, 127.1, 128.3, 129.7, 129.8, 131.8, 146.0, 157.7, 158.3, 166.1. MS (ESI) *m/z*: 297.98 $[M+H]^+$

**2-Hydroxy-5-(4-(3-hydroxyphenyl)-1*H*-1,2,3-triazol-1-yl)benzoic acid 3f.** From tetrabuthylamonium fluoride (1.11 mL, 1M solution in THF); chromatography eluent: AcOEt/Hexane 5:1; yield 68%, mp 243-244 ºC with decomposition; $\nu_{max}$ (KBr)/cm$^{-1}$ 3134, 2917, 1675, 1619; $^1$H NMR $\delta_H$ (300 MHz; DMSO) 6.78 (1H, d, *J* 7.7, ArH), 7.20 (1H, d, *J* 8.9, ArH), 7.27 (1H, t, *J* 7.9, ArH), 7.34-7.39 (2H, m, ArH), 8.07 (1H, dd, *J* 8.9 and 2.6, ArH), 8.29 (1H, d, *J* 2.6, ArH), 9.23 (1H, s, Triazole-H), 9.57 (1H, brs, OH); $^{13}$C NMR $\delta_c$ (75.4 MHz, DMSO) 112.0, 113.9, 115.1, 116.2, 118.5, 119.5, 121.5, 127.2, 128.5, 129.9, 131.4, 147.2, 157.7, 160.7, 170.8. MS (ESI) *m/z*: 297.99 $[M+H]^+$

**General Method for the synthesis of methyl esters 2g,h and 3h.**

The corresponding acid (**2e**, **2f**, or **3f)** (0.17 mmol) was dissolved in MeOH (10 mL) and few drops of sulfuric acid were added. The reaction mixture was heated under reflux for 6 hours in argon atmosphere. Then the reaction mixture was concentrated under vacuum and the resulting residue was purified by column chromatography on silica eluting using AcOEt/DCM (3:1) as eluent to yield the desired product.

**Methyl 5-hydroxy-2-(4-(4-hydroxyphenyl)-1*H*-1,2,3-triazol-1-yl)benzoate 2g.** Yield 73%, mp 224-225 ºC; $\nu_{max}$ (KBr)/cm$^{-1}$ 3373, 3059, 2925, 1723, 1686, 1619; $^1$H NMR $\delta_H$ (300 MHz; $CD_3OD$) 3.74 (3H, s, $OCH_3$), 6.00 (2H, brs, OH), 6.97 (2H, d, *J* 8.7, ArH), 7.21 (1H, dd, *J* 8.7 and 2.7, ArH), 7.51 (1H, d, *J* 2.6 ArH), 7.62 (1H, d, *J* 8.7, ArH),7.74 (2H, d, *J* 8.7, ArH), 9.01 (1H, s, Triazole-H); $^{13}$C NMR $\delta_c$ (75.4 MHz, $CD_3OD$) 53.4, 115.8, 117.7, 119.3, 121.0, 127.4, 127.6, 129.2, 129.7, 130.3, 144.8, 161.7, 161.9, 165.6. MS (ESI) *m/z*: 312.03 $[M+H]^+$

**Methyl 2-hydroxy-5-(4-(4-hydroxyphenyl)-1*H*-1,2,3-triazol-1-yl)benzoate 2h** Yield 86%, mp 211-213 °C; $\nu_{max}$ (KBr)/cm$^{-1}$ 3261, 3141, 2955, 2925, 1679, 1619; $^1$H NMR $\delta_H$ (300 MHz; DMSO) 3.95 (3H, s, $CH_3$), 6.88 (2H, d, *J* 8.4, ArH), 7.23 (1H, d, *J* 8.4, ArH), 7.75 (2H, d, *J* 8.6, ArH), 8.05 (1H, dd, *J* 8.9 and 2.8, ArH), 8.25 (1H, d, J 2.8, ArH), 9.08 (1H, s, Triazole-H), 9.68 (1H, br s, OH), 10.60 (1H, br s, OH); $^{13}$C NMR $\delta_c$ (75.4 MHz, DMSO) 52.6, 114.3, 115.6, 118.8, 121.2, 121.3, 126.7, 126.9, 128.8, 147.5, 157.5, 159.2, 167.8. MS (ESI) *m/z*: 312.03 $[M+H]^+$

**Methyl 2-hydroxy-5-(4-(3-hydroxyphenyl)-1*H*-1,2,3-triazol-1-yl)benzoate 3h.** Yield 72%, mp 202-203 °C; $\nu_{max}$ (KBr)/cm$^{-1}$ 3141, 2955, 1671, 1612; $^1$H NMR $\delta_H$ (300 MHz; DMSO) 3.95 (3H, s, $CH_3$), 6.76-6.80 (1H, m, ArH), 7.25 (2H, t, *J* 8.9, ArH), 7.30-7.39 (2H, m, ArH), 8.07 (1H, dd, *J* 8.9, 2.8, ArH), 8.27 (1H, d, *J* 2.8, ArH), 9.21 (1H, s, Triazole-H), 9.59 (1H, br s, OH), 10.69 (1H, br s, OH); $^{13}$C NMR $\delta_c$ (75.4 MHz, DMSO) 52.6, 112.0, 114.3, 115.1, 116.2, 118.8, 119.5, 121.4, 127.0, 128.7, 129.9, 131.4, 147.3, 157.7, 159.2, 167.7. MS (ESI) *m/z*: 312.02 $[M+H]^+$

**Computational methods**.

To perform the theoretical study of the binding mode both subtypes of ER α and ERβ, have been taken into account. As macromolecules, several crystallographic structures of the two receptor subtypes in complex with several ligands have been considered: ERα in complex with estradiol (PDB 1A52), raloxifene (PDB 1ERR), genistein (PDB 1X7R), WAY-244 (PDB 1X7E), and 4-hydroxytamoxifen (PDB 3ERT), and ERβ in complex with genistein (PDB 1X7J), THC (PDB 1L2J), WAY-202196 (PDB 1YYE), and 4-hydroxytamoxifen (PDB 2FSZ). Water molecules close to the amino acids Arg394 (ERβ Arg346) and Glu353 (Glu305 ERβ) were kept for the docking procedures. Eleven ligands (compounds **2a-2g** and **3a-3f**) were built using Maestro LigPrep module (www.schrodinger.com). Gaussian 03[16] at B3LYP/3-21G* level was used to optimize the geometries and to calculate point charges. Atom types and bond types were assigned, and mol2 files were generated. Macromolecule geometries were refined by using Protein Preparation module in Maestro. The Glide module was used to perform the docking calculations.[17, 18] The center of the box was positioned on the center of the bound ligand present in the crystallographic structure, and box size was set up to enclose the ligand binding domain. The docking procedure was performed with XP (extra precision) mode, and a van der Waals radii scale factor of 1.0/0.8 for receptor and ligand, respectively. Induced Fit Docking was also used, and contained constrained minimization of the receptor with an RMSD cutoff of 0.18 Å, and Prime-side-chain prediction on residues within a 5 Å of any ligand pose. Glide redocking was performed into structures within 30 kcal mol$^{-1}$ of the lowest energy structure with van der Waals scaling of 1.0/0.8 for receptor and ligand, respectively. The best obtained result for each ligand was considered for analysis of the ligand-receptor interactions.

**Biological Assays**

**Chemicals**. 17-β-estradiol, crystal violet, dextran coated charcoal, PSB, Tween-20, BSA, insulin were purchased from Aldrich. Estradiol [2,4,6,7,16,17-3H(N)], scintillation counting liquid (Optifase HiSafe2) were obtained from Perkin-Elmer, Salem, MA). Estrogen receptors α and β produced in insect cells and sodium pyruvate were purchased from Invitrogen. Cell culture medium DMEM, FBS, antibiotics, trypsin-EDTA, amino acids, L-glutamine were purchased from Lonza. DCC-FBS was obtained from Hyclone (Erembodegem, Aalst, Belgium) and DMEM without phenol red from Gibco.

**Receptor Binding Studies**. The ability of the compounds to bind to ERs has been determined by competition assay according to method of Arcaro with some modifications (1). Purified full-length human ERα and ERβ have been incubated for 4 hr at 23°C with different concentrations of compounds in the presence of 5 nM [2,4,6,7,16,17-3H]-estradiol in total volume 150 µl. The stock

of tested compounds has been prepared in DMSO. All these compounds, including [2,4,6,7,16,17-3H]-estradiol and receptors were diluted in Tween\PBS buffer (99,85:0,15 w/v). A vehicle control contained 0,1% of DMSO. After incubation the non-bound [2,4,6,7,16,17-3H]-estradiol has been removed by adding mixture of 10% DCC and 2% albumin bovin serum, incubating 15 min at 4°C, followed by centrifugation at 6000g for 5 min at 4°C. 150 µl of supernatant was added to 4 mL of scintillation liquid and the bound estradiol was measured in liquid scintillation counter Beckman LS 6500 (Beckman Coulter, Inc.). Two independent experiments with three repetitions for each compound were performed. Results were expressed s a the percentage of specific binding of [2,4,6,7,16,17-3H]-estradiol to ER versus log of competitor concentration. Graph Pad Prism software (non-linear regression analysis) was used to calculate the concentration needed to displace 50% of [2,4,6,7,16,17-3H]-estradiol ($IC_{50}$). The values of $IC_{50}$ for estradiol were 8.98 and 6.87 nM for ERα and ERβ, respectively.

**Proliferation assay**. MCF-7 cells were seeded in 96-well plates at $5*10^3$cells/well in DMEM containing 10% FBS, 0,01mg/ml insulin solution, 0,1 mM nonessential amino acids. After 24 hours medium was changed for DMEM without phenol red, containing 5% dextran-coated charcoal stripped FBS (DCC-FBS), 0,1 mM nonessential amino acids, 1mM sodium pyruvate and 2mM L-glutamine and preincubated 3 days prior to treatment. Afterwards different concentrations of assayed compounds (1-50 µM) were added to cells with/without 1pM estradiol in order to test the capacity to induce or prevent the proliferation of MCF-7 cells. The final vehicle concentration maximally 0,1% of DMSO (and 0,1% of ethanol in case of treatment with estradiol) served as a solvent control. On day 4 medium with compounds was refreshed. On day 8 the media were removed and cells were fixed and stained with a solution containing 1% ethanol and 0.5% crystal violet. After rinsing and drying, the dye was solubilized with 1% SDS and the absorbance was read at 570 nm (Biotec). The viability was calculated considering controls without test substance as 100% viable.

**Agonist Profile**. The agonistic and antagonistic activities of compounds were evaluated using a commercially available cell-based assay (INDIGO Bioscience's ER Reporter Assay), which allows to quantify functional activities of the test compounds, against ERα and ERβ. The system utilizes non-human mammalian cells engineered to provide constitutive high-level expression of ERα and ERβ. Additionally, these cells contain either ERα or ERβ-responsive luciferase reporter gene. Thus, quantification of luciferase activity provides a surrogate measure of ERα and ERβ activity in the treated reporter cells. Reporter cells were dispensed in 96-well plate and then immediately dosed with the test compounds. Following overnight incubation, the treatment media were discarded and luciferase detection reagent was added. The intensity of light emission from the ensuing luciferase reaction provides a measure that is directly proportional to the level of ER activation in the reporter cells. The assays were configured to perform agonist and antagonist dose-response curves. In order to obtain agonist dose-response curves ER reporter cells were treated with media alone. Estradiol was used as a positive control agonist. The $EC_{50}$ values of estradiol for ERα and ERβ were 330 and 77 pM, respectively. The highest concentrations of estradiol used in order to ensure the saturating conditions were 100nM (ERα) and 4nM (ERβ).The final solvent control didn't exceed 0,1% of DMSO. All measurements were performed in triplicate.

## Abbreviations

| | |
|---|---|
| DCM | dichloromethane |
| THF | tetrahydrofuran |
| DMF | dimethylformamide |
| RT | room temperature |
| ER | estrogen receptor |
| CuACC | copper-catalyzed reaction between an azide and an alkyne |
| TLC | thin layer chromatography |
| TIPSCl | triisopropylsilyl chloride |
| $TMSN_3$ | azidotrimethylsilane |
| CNS | central nervous system |
| TFA | trifluoroacetic acid |
| EDTA | ethylenediaminetetraacetic acid |
| DCC-FBS | dextran-coated charcoal-stripped fetal bovine serum |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## Notes


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† Electronic Supplementary Information (ESI) available: NMR spectra of compounds **2a-2h, 3a-3f** and **3h**. Predicted binding energies from the docking studies

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