ACS OMEGA

http://pubs.acs.org/journal/acsodf

Article

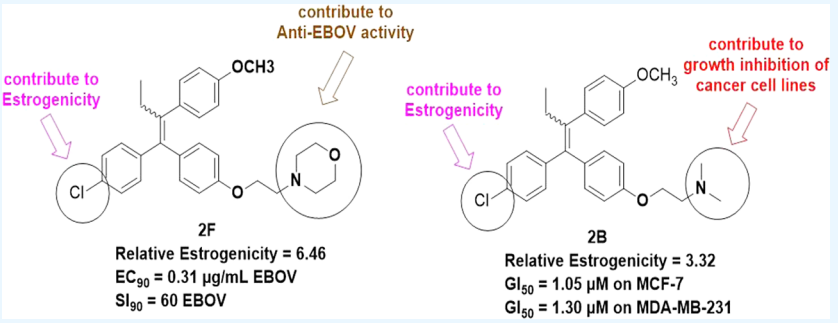
# Structure–Activity Relationships of Triphenylethylene Derivatives and Their Evaluation as Anticancer and Antiviral Agents

Nermin S. Ahmed, Heba E. El-Nakib, Marian M. Ramsis, Nouran O. Albably, Jannette Wober, Jan J. Weigand, Kai Schwedtmann, Oliver Zierau, and Ashraf H. Abadi*

Cite This: *ACS Omega* 2023, 8, 25903–25923

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information




**ABSTRACT:** Tamoxifen (TAM) is a selective estrogen receptor modulator (SERM) that is used in the treatment of breast cancer, yet with the risk of developing uterine cancer. A perfect SERM would act as an estrogen activator on bones, the cardiovascular system, and the central nervous system while providing neutral or estrogen blocking effects on the breast and the uterus. Herein, we report on the design, synthesis, and evaluation of new rigid and flexible TAM analogues. Mainly, a chloro substituent is introduced at the para position of the TAM ring **C** blocking the CYP2D6 hydroxylation site. Most compounds showed estrogenic activity higher than TAM using the yeast estrogen screen assays, indicating the determinant role of the chloro substituent upon functional activity. Despite being estrogenic, compound **2B** showed potent antiproliferative activity in the NCI 60 cell lines with mean $GI_{50}$ = 3.67 $\mu$M, $GI_{50}$ = 1.05 $\mu$M on MCF-7 cell lines, and $GI_{50}$ = 1.30 $\mu$M on MDA-MB-231. The estrogenic activity of compound **2B** was further confirmed by stimulating alkaline phosphatase in Ishikawa cells, and it showed no increase in relative uterine wet weight in ovariectomized rats. Compound **2F** showed $EC_{90}$ = 0.31 $\mu$g/mL and $SI_{90}$ = 60 against Ebola virus; this is 200-fold more potent than the positive control favipiravir. This is the first time to report estrogenic triphenylethylenes as anti-EBOV agents. The anti-EBOV activity reported is a function of the substitution pattern of the scaffold rather than the functional activity. Moreover, compound **3D** showed excellent PO pharmacokinetic properties in mice. In conclusion, for this class of TAM-like compounds, the blockage of the *p*-position of ring **C** is decisive for the functional activity; meanwhile, the triarylethylene substitution pattern is detrimental for the antiviral activity.

## 1. INTRODUCTION

Estrogen receptors (ERs) are members of the superfamily of nuclear receptors that bind to estrogen response elements (EREs) on their target genes to regulate gene expression and consequently control various cellular processes.[1] ERs represent two different genes, namely, ESR1 and ESR2 (ER$\alpha$ and ER$\beta$).[2] ER ligands with mixed agonist and antagonist activity are referred to as selective ER modulators (SERMs). These compounds have shown mixed agonist/antagonist properties depending on the target tissue.[3] SERMs are therapeutic agents used for the prevention and treatment of diseases such as osteoporosis and uterine and breast cancers, as well as alleviating postmenopausal symptoms.[4] Each SERM has a distinctive clinical profile. The ideal SERM would provide the effects of an estrogen activator on the bone to prevent bone loss and on the brain to treat hot flashes while providing neutral or estrogen blocking effects on the breast and on the lining of the uterus to reduce the risks of breast and uterine cancers. To date, the ideal SERM has not been discovered.[5] The SERM dual activity was attributed to tissue specificity, the difference in the expression of co-activators and co-repressors within a cell, and the differential expression of ER$\alpha$ and ER$\beta$ in various types of tissues.[6] The activity of ERs relies on the recruitment of co-regulatory proteins (co-activators or co-repressors).[7] The conformation of the ligand-activated ERs

Received: March 13, 2023
Accepted: June 27, 2023
Published: July 11, 2023





https://doi.org/10.1021/acsomega.3c01682
*ACS Omega* 2023, 8, 25903–25923

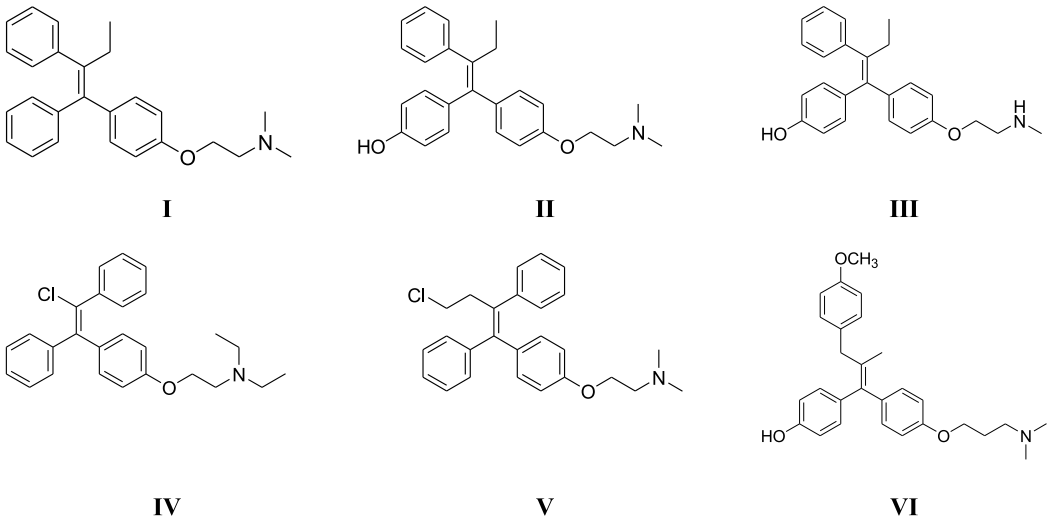



**Figure 1.** Triphenylethylene-based SERMs.

further contributes to their dual nature.[8] The position of mobile helix 12 (H12) of the ER−ligand-binding domain (LBD) dictates whether a ligand adopts an agonistic or antagonistic conformation.[9]

Tamoxifen (TAM) **I** was initially developed as a contraceptive; after failing its initial indication, it thus was repurposed for the treatment of breast cancer. Since then, it is known to be one of the most effective drugs in treating ER-positive breast cancer.[10] TAM is perceived as a prodrug that is metabolized into two clinically more active metabolites, 4-OH-TAM **II** and endoxifen **III**, Figure 1. Both metabolites possess higher affinity toward ER$\alpha$ and are up to 100 times more potent as antiestrogens in breast cancer cells than TAM itself.[11] Cytochrome P450 (CYP) enzymes, especially CYP2D6, are the principal metabolizing enzymes of TAM. The genes that encode the enzyme CYP2D6 are polymorphic with more or less active enzymatic function. As a result of this genetic polymorphism, patients may gain unequal clinical outcomes from TAM treatment due to the unequal hydroxylation on the phenyl ring and unequal formation of 4-OHTAM.[12]

4-OH-TAM, the active metabolite of TAM, adopts antagonistic conformation inside the ER LBD. It retains essential hydrophobic interactions observed with E2, and it forms a H-bond with Glu 353. 4-OH-TAM is locked in a conformation that its bulky aminoalkoxy side chain shields and neutralizes anionic Asp351, thus preventing H12 from positioning over the pocket and thereby inhibiting the recruitment of co-activators (PDB ID 3ERT).[12] It was hypothesized that the main driving force for differential agonistic or antagonistic activity is the relative positions of the aminoalkoxy side chains on ring **B**.[13] Therefore, manipulating the aminoalkoxy side chain length, involving the basic nitrogen in alicyclic structures to alter its p$K_a$ while retaining the TAM skeleton, was the most commonly adopted approach to modulate the estrogenic/antiestrogenic properties of TAM analogues.[14]

A screen of approved drugs identifies some SERMS mainly: clomiphene **IV** and toremifene **V** as active compounds that inhibit EBOV replication in vitro, Figure 1. The use of both agents caused a significant increase in survival in the murine EBOV infection model.[15] The ability of the two compounds to block EBOV is hypothesized to be independent of their ER activity. Both compounds are believed to be cationic amphiphilic drugs (CADs) that interfere with a delayed stage of EBOV entry into target cells.[16] A co-crystallized toremifene and EBOV glycoprotein (GP) (PDB 5JQ7) has been recently reported, where toremifene can bind the pocket between GP1 and GP2 and thus reduce the complex's stability, preventing viral fusion. It was suggested that TAM and clomiphene make weaker interactions with the binding sites in Ebola GP compared to toremifene.[17]

Although there are only two recent FDA-approved EBOV treatments, both Inmazeb and Ebanga are monoclonal-based treatments.[18] No small organic molecules were approved yet for the treatment of Ebola infection. Thus, developing TAM analogues as anti-EBOV agents is a major research opportunity.

Herein, we designed novel analogues to retain the triarylethylene skeleton essential for major ER hydrophobic interactions, keeping the basic aminoalkoxy side chain reported as a major determinant of compound antagonistic activity. The unique substitution at position 4 of ring **C** with a chloro group was hypothesized to cause a significant effect on ER$\alpha$ binding and receptor conformation and to block *p*-hydroxylation by CYP2D6. Moreover, different aminoalkoxy side chains of different lengths and basicity were introduced to ring **B**. Some compounds bear an element of flexibility on the rigid TPE where phenyl ring **A** is replaced by a benzyl group. In addition, different substitutions on ring **A** were introduced including a *p*-chloro and a *p*-methoxy substituent. The novel compounds were investigated for their estrogenic/antiestrogenic activity using the yeast estrogen screen (YES) assay; their estrogenic activity was further assessed using alkaline phosphatase (AlkP) activity in Ishikawa cell lines. The compounds' antiproliferative activity on 60 different cell lines was determined; compound **2B** was further tested using an in vivo ovariectomized rat model. To validate the antiviral effects of TPE, compounds were tested for their anti-influenza and anti-EBOV activity.

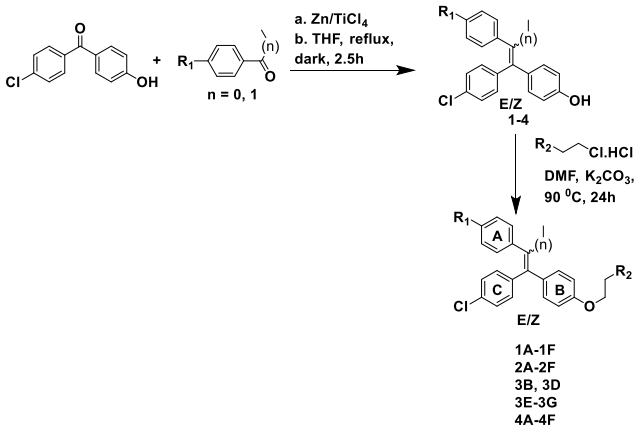
## 2. RESULTS AND DISCUSSION

**2.1. Chemistry.**[19] Two series of compounds are depicted in Schemes 1 and 2. Compounds (**1**−**6**) were synthesized using the standard McMurry coupling reaction of 4-chloro-4-hydroxybenzophenone with different commercially available aromatic ketones, namely, 4′-chloroacetophenone, propiophe-



https://doi.org/10.1021/acsomega.3c01682
ACS Omega 2023, 8, 25903−25923

**Scheme 1. Preparation of Rigid TPE Analogues (Series 1)**




| Code | n | $R_1$ | $R_2$ |
|---|---|---|---|
| 1 | | | --- |
| 1A | | | -N($CH_3$)$_2$ |
| 1B | 1 | Cl | -$CH_2$-N($CH_3$)$_2$ |
| 1C | | | -N($CH_2$-$CH_3$)$_2$ |
| 1D | | | -N-$CH_2$-$CH_2$-$CH_2$-$CH_2$- |
| 1E | | | -N-$CH_2$-$CH_2$-$CH_2$-$CH_2$-$CH_2$- |
| 1F | | | -N-$CH_2$-$CH_2$-O-$CH_2$-$CH_2$- |
| 2 | | | --- |
| 2A | | | -N($CH_3$)$_2$ |
| 2B | | | -$CH_2$-N($CH_3$)$_2$ |
| 2C | 1 | $OCH_3$ | -N($CH_2$-$CH_3$)$_2$ |
| 2D | | | -N-$CH_2$-$CH_2$-$CH_2$-$CH_2$- |
| 2E | | | -N-$CH_2$-$CH_2$-$CH_2$-$CH_2$-$CH_2$- |
| 2F | | | -N-$CH_2$-$CH_2$-O-$CH_2$-$CH_2$- |
| 3 | | | --- |
| 3B | | | -$CH_2$-N($CH_3$)$_2$ |
| 3D | | H | -N-$CH_2$-$CH_2$-$CH_2$-$CH_2$- |
| 3E | 1 | | -N-$CH_2$-$CH_2$-$CH_2$-$CH_2$-$CH_2$- |
| 3F | | | -N-$CH_2$-$CH_2$-O-$CH_2$-$CH_2$- |
| 3G | | | -N-$CH_2$-$CH_2$-$CH_2$-$CH_2$-$CH_2$-$CH_2$- |
| 4 | | | --- |
| 4A | | | -N($CH_3$)$_2$ |
| 4B | | | -$CH_2$-N($CH_3$)$_2$ |
| 4C | 0 | Cl | -N($CH_2$-$CH_3$)$_2$ |
| 4D | | | -N-$CH_2$-$CH_2$-$CH_2$-$CH_2$- |
| 4E | | | -N-$CH_2$-$CH_2$-$CH_2$-$CH_2$-$CH_2$- |
| 4F | | | -N-$CH_2$-$CH_2$-O-$CH_2$-$CH_2$- |

none, 4′-chloropropiophenone, 4′-methoxypropiophenone, 4-methoxyphenylacetone, and 4′-chlorophenylacetone using titanium tetrachloride/zinc as a catalyst to afford the six condensation products with 45−58% yield of *E*/*Z* mixtures as outlined in Schemes 1 and 2. The condensation products (**1**−**6**) were then treated with appropriate base hydrochloride salts in the presence of potassium carbonate to form ethers with approximate yields of 45−50% as mixtures of *E* and *Z* isomers. $^{13}$C NMR showed duplication of most signals; $^{1}$H NMR showed peaks integrating for double the number of protons, indicating the presence of *E*/*Z* isomers in the mixture as well. Such duplication of signal has been previously reported by Bedford and Richardson.[20] Attempts to isolate the *E*/*Z* isomers using column chromatography as well as preparative HPLC were not successful; compounds were therefore biologically assessed as mixture as previously implemented in the literature.[20]

**2.2. In Vitro Assays.** *2.2.1. YES Assay.* All the synthesized compounds were tested for their relative *β*-galactosidase activity in the YES assay at a concentration of 1 *μ*M. Compounds were tested for their relative estrogenic activity compared to DMSO (set to 1). The YES assay is a gene reporter assay; the yeast genome carries the DNA sequence of human ER*α* expression plasmid, carrying EREs in the promoter controlling the expression of the reporter gene *lacZ* (encoding the enzyme *β*-galactosidase). In the presence of estrogenic compounds, *β*-galactosidase is synthesized and secreted into the medium, where it converts the chromogenic substrate chlorophenol red-*β*-D-galactopyranoside from a yellow to a red product, whose absorbance is measured. The agonistic activity is measured directly.[21] Despite the ability of the YES assay to differentiate between agonists and antagonists, results obtained from this assay have to consider that compounds exhibit an organ-selective mode of action.[22]

Compounds **4** and **5** were screened as representatives of the phenolic intermediates (**1**−**6**); they both bear a phenolic OH at the *p*-position of ring **B**. Compound **4** is a rigid analogue that bears a chloro group at the *p*-position of ring **A** and a terminal methyl on the ethylene bond, whereas compound **5** is its flexible congener. Compound **4** showed a relative *β* galactosidase agonistic activity of 12.84, whereas compound **5** did not show significant activity. It is reported that ER binders tend to have larger log *P* values, so we compared clog *p* of diethylstilbestrol DES (a synthetic estrogen), compounds **4** and **5**. DES was less lipophilic (clog *p* = 4.63) compared to compound **4** (clog *p* = 5.67) and compound **5** (clog *p* = 6.92). The strong OH proton donor of DES is replaced by a chlorine atom; this structural modification did not abolish the agonistic activity of compound **4**. Replacing the diethyl groups of DES VI with other hydrophobic substituents still supports essential hydrophobic interactions. The methyl group of compound **4** introduces further flexibility compared to the ethyl group of DES.

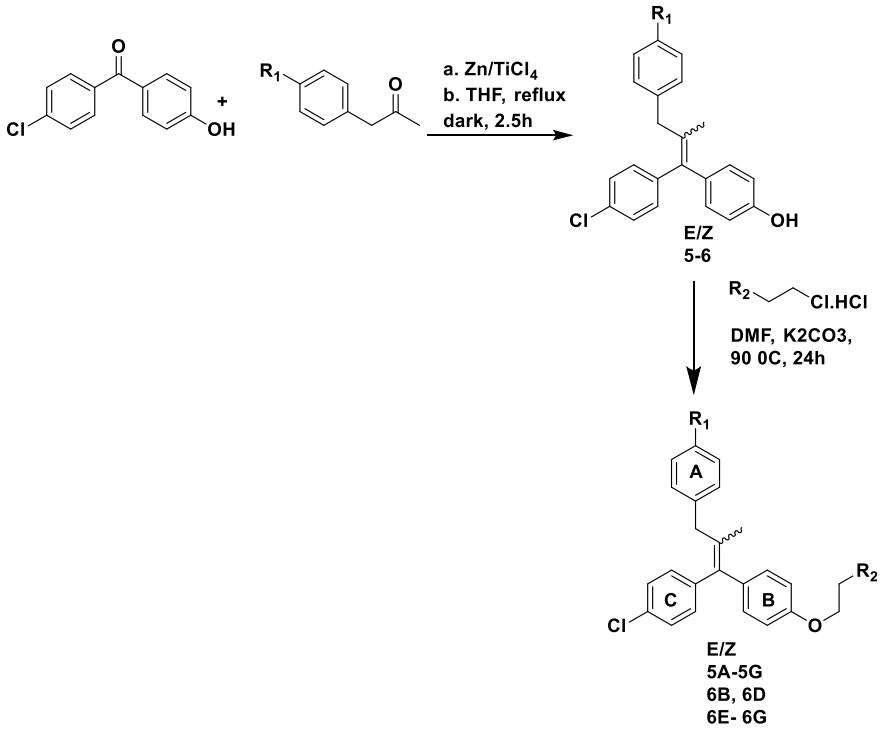
Compounds **4** and **5** only differ in flexibility of their skeletons. Compound **5** lacks agonistic activity despite its higher lipophilicity. Ring **A** must have been displaced from inside LBD. Converting the OH group of compound **4** to aminoethoxy groups led to complete loss of estrogenic activity. To further investigate the possible interactions of compound **4** inside ER*α* LBD, compound **4** was included in a simple in silico model.

All compounds bearing a *p*-chloro substituent on ring **A** and an ethyl or methyl substituent on the ethylene backbone (**1A**−**1F** and **4A**−**4F**) showed no estrogenic activity compared to DMSO. The mild electron-withdrawing effect of chlorine seems to be deleterious to the estrogenic activity.

Compounds **2A**, **2B**, **2C**, **2F**, **3B**, **3F**, and **3G** showed relative estrogenic activity compared to DMSO (5.49, 3.32, 3.27, 6.46, 2.20, 4.19, and 2.98, respectively). Compounds **2A**, **2B**, **2C**, and **2F** bear a *p*-methoxy substituent on the TPE rigid backbone. Among all estrogenic aminoalkoxy derivatives, compound **2F** showed the highest relative estrogenic activity of 6.46. We thus hypothesized that blocking the *p*-


https://doi.org/10.1021/acsomega.3c01682
ACS Omega 2023, 8, 25903−25923

**Scheme 2. Preparation of Flexible TPE Analogues (Series 2)**




| Code | $R_1$ | $R_2$ |
|---|---|---|
| **5** | Cl | --- |
| **5A** | | $-N(CH_3)_2$ |
| **5B** | | $-CH_2-N(CH_3)_2$ |
| **5C** | | $-N(CH_2-CH_3)_2$ |
| **5D** | | -N-$CH_2$-$CH_2$-$CH_2$-$CH_2$- |
| **5E** | | -N-$CH_2$-$CH_2$-$CH_2$-$CH_2$-$CH_2$- |
| **5F** | | -N-$CH_2$-$CH_2$-O-$CH_2$-$CH_2$- |
| **5G** | | -N-$CH_2$-$CH_2$-$CH_2$-$CH_2$-$CH_2$-$CH_2$- |
| **6** | $OCH_3$ | --- |
| **6B** | | $-CH_2-N(CH_3)_2$ |
| **6D** | | -N-$CH_2$-$CH_2$-$CH_2$-$CH_2$- |
| **6E** | | -N-$CH_2$-$CH_2$-$CH_2$-$CH_2$-$CH_2$- |
| **6F** | | -N-$CH_2$-$CH_2$-O-$CH_2$-$CH_2$- |
| **6G** | | -N-$CH_2$-$CH_2$-$CH_2$-$CH_2$-$CH_2$-$CH_2$- |

hydroxylation site at ring **C**, along with having a hydrophilic electron-donating methoxy substituent at ring **A** and a less basic and less hydrophobic morpholinylethoxy substituent on ring **B**, significantly enhances the agonistic activity of the compounds. Compounds **2B** and **2C** bearing dimethylamino-propoxy and diethylaminoethoxy substituents on ring **B**, respectively, were equipotent agonists; yet, both compounds **2B** and **2C** are less estrogenic than compound **2A**.

Compounds **3B**, **3F**, and **3G** are rigid analogues that bear no substituents on ring **A** in a manner like DES and TAM. Compounds **3B** and **3F** were less agonistic compared to their congeners **2B** and **2F**, indicating the positive effect of the *p*-methoxy substitution of ring **A** on agonistic activity. They were more agonistic compared to their congeners **1B** and **1F**, indicating the negative effect of the *p*-chloro substitution of ring **A**. Compound **3F**, the more active analogue, bears a



https://doi.org/10.1021/acsomega.3c01682
*ACS Omega* 2023, 8, 25903−25923

morpholinylethoxy substituent on ring **B** as well. The less basic nitrogen of the morpholine ring seems to fail to neutralize Asp351, which is an essential feature for SERM to induce antagonistic conformation of the ER; our findings prove that it additionally induces an agonistic conformation.

The main structural difference between the analogues of series 1 is the *p*-substituent on ring **A**; a mild electron-withdrawing substituent like chlorine seems to abolish any estrogenic activity, whereas a methoxy group seems to enhance estrogenicity. The morpholinylethoxy substituent on ring **B** enhances the estrogenic activity in compounds **2F** and **3F**; it seems that less lipophilic analogues show higher estrogenic activity. Among series 1, it is obvious that substitution on ring **A** is the main determinant factor of the estrogenic profile of the analogue. Table 1 shows compounds bearing a flexible

**Table 1. Relative β-Galacto Activity Estrogenic YES Assay**

| code | estrogenic activity fold ± S.D.[a] | code | estrogenic activity fold ± S.D.[a] |
|---|---|---|---|
| **1A** | 1.17 ± 0.07 | **4A** | 0.80 ± 0.35 |
| **1B** | 1.21 ± 0.03 | **4B** | 1.95 ± 0.19 |
| **1C** | 1.17 ± 0.02 | **4C** | 1.80 ± 1.05 |
| **1D** | 1.16 ± 0.09 | **4D** | 1.57 ± 0.14 |
| **1E** | 1.14 ± 0.00 | **4E** | 1.09 ± 0.20 |
| **1F** | 1.21 ± 0.07 | **4F** | 1.56 ± 0.09 |
| **2A** | 5.49 ± 0.71 | **5** | 1.10 ± 0.08 |
| **2B** | 3.32 ± 0.29 | **5A** | 1.09 ± 0.05 |
| **2C** | 3.27 ± 0.31 | **5B** | 1.14 ± 0.01 |
| **2D** | 1.77 ± 0.16 | **5C** | 1.05 ± 0.10 |
| **2E** | 1.27 ± 0.04 | **5D** | 1.05 ± 0.11 |
| **2F** | 6.46 ± 0.69 | **5E** | 1.10 ± 0.12 |
| **3B** | 2.20 ± 0.14 | **5F** | 1.07 ± 0.16 |
| **3D** | 1.13 ± 0.03 | **5G** | 1.05 ± 0.16 |
| **3E** | 1.99 ± 0.23 | **6B** | n.d.[b] |
| **3F** | 4.19 ± 0.44 | **6D** | n.d. |
| **3G** | 2.98 ± 0.44 | **6E** | n.d. |
| **4** | 12.84 ± 1.90 | **6F** | 1.07 ± 0.09 |
| | | **6G** | n.d. |

[a]Compounds screened at a dose of 1 μM. Estrogenic activity is compared to DMSO (set as 1). All compounds were tested in technical quadruplicates and biological triplicates. [b]n.d. not determined.

skeleton, and an aminoalkoxy side chain on ring **B** (**5A**−**5G** and **6B**−**6G**) showed non-significant estrogenic activity. Flexibility seems to be deleterious to agonistic activity.

*2.2.2. Yeast Antiestrogenic Screening Assay.* Compounds were tested at 1 μM concentration in the presence of 0.5 or 1 nM E2 according to the calculated $EC_{50}$ value of E2 for the experimental series. None of the tested compounds showed relative β galactosidase activity ≤0.5.

Compound **2D** showed a relative β galactosidase activity of 0.63, which is nearly 2-fold less active than that of TAM. The flexible analogues bearing the *p*-chloro substituent on ring **A** (**6B**−**6G**) showed moderate antiestrogenic activity (0.66, 0.62, 0.66, 0.87, and 0.62, respectively); compound **6F** was the least antiestrogenic due to the relative hydrophilic nature of the morpholinylethoxy substituent and the less basic characteristic of the nitrogen atom (Table 2).

We previously reported flexible TAM analogues that bear an OH group rather than a *p*-chloro group on ring **B** and the *p*-methoxy substituent on ring **A**; these analogues were more

**Table 2. Relative β-Galactosidase Activity Antiestrogenic YES Assay**

| code | antiestrogenic activity fold ± S.D.[aThe] | code | antiestrogenic activity fold ± S.D.[aThe] |
|---|---|---|---|
| **1A** | 1.15 ± 0.07 | **4C** | 1.09 ± 0.20 |
| **1B** | 1.00 ± 0.03 | **4D** | 1.11 ± 0.30 |
| **1C** | 1.15 ± 0.04 | **4E** | 0.95 ± 0.11 |
| **1D** | 1.12 ± 0.23 | **4F** | 1.28 ± 0.22 |
| **1E** | 1.10 ± 0.16 | **5A** | 0.75 ± 0.13 |
| **1F** | 1.19 ± 0.07 | **5B** | 1.14 ± 0.01 |
| **2A** | 1.93 ± 0.23 | **5C** | 1.05 ± 0.10 |
| **2B** | 1.38 ± 0.12 | **5D** | 1.05 ± 0.06 |
| **2C** | 1.06 ± 0.06 | **5E** | 1.10 ± 0.12 |
| **2D** | 0.63 ± 0.09 | **5F** | 1.07 ± 0.16 |
| **2E** | 1.19 ± 0.06 | **5G** | 1.05 ± 0.16 |
| **2F** | 2.32 ± 0.28 | **6B** | 0.66 ± 0.37 |
| **3B** | 1.71 ± 0.11 | **6D** | 0.62 ± 0.30 |
| **3D** | 1.38 ± 0.15 | **6E** | 0.66 ± 0.36 |
| **3E** | 1.53 ± 0.28 | **6F** | 0.87 ± 0.12 |
| **3F** | 2.27 ± 0.26 | **6G** | 0.62 ± 0.35 |
| **3G** | 2.01 ± 0.23 | **TAM** | 0.30 ± 0.08 |
| **4A** | 0.98 ± 0.33 | **4-OH-TAM** | 0.21 ± 0.00 |
| **4B** | 0.72 ± 0.21 | **VI**[b] | 0.18 ± 0.06 |

[aThe]antiestrogenic activity is compared to 0.5 nM E2 or 1 nM E2 (set as 1); compounds were screened at a dose of 1 μM in the presence of 0.5 nM E2. All compounds were tested in technical quadruplicates and biological triplicates. [b]Previously reported compound.[23]

potent than their chlorinated congeners.[23] The diminished antiestrogenic activity compared to that of TAM and OH-TAM and previously reported flexible congeners can be attributed to the absence of an OH group at position 4 of ring **B**; this OH group is responsible for the higher antiestrogenic activity of OH-TAM and endoxifen compared to TAM as well.

Several studies have highlighted that the antiestrogenic property of TPE SERMs depends mainly on the aminoalkoxy side chain on ring **B**.[24] However, our results showed that the presence of an aminoalkoxy side chain of different size and basicity did not induce antiestrogenic activity in all cases; on the contrary, compounds can induce estrogenic activity even in the presence of these amino groups. This led us to a conclusion that both an OH group and a basic amino group are essential for locking the ER in an antagonistic conformation. Another determinant factor for differential activity is the state of flexibility of the TPE backbone; the flexibility deteriorated the agonistic activity (compounds **4** versus **5** and **2F** versus **6F**), whereas flexibility enhanced the antagonistic activity (compounds **6B**, **6E**, **6F**, and **6G** versus **2B**, **2E**, **2F**, and **2G**). Since most of the approved TAM analogues (clomiphene and toremifene) bear an unsubstituted ring **A**, it was assumed that this substitution is of no significant effect neither on binding affinity nor on functional activity; yet, our results showed a pronounced effect of ring **A** substituents. This encourages testing different structural isomers of ring **A**.

The functional activity of triphenylethylene analogues can thus be manipulated by fine-tuning the substituent on the *p*-position of ring **B** and ring **A** and by tackling the rigidity of the molecules; a complete flip from antiestrogenic to estrogenic compounds has been achieved with proper maneuvers.

*2.2.3. NCI In Vitro Anticancer Screening.* The novel compounds were tested for their growth inhibitory effects by the Developmental Therapeutics Program (DTP) of the National Cancer Institute (NCI). A mean growth inhibition


https://doi.org/10.1021/acsomega.3c01682
ACS Omega 2023, 8, 25903−25923

on the nine subpanels is determined at 10 $\mu$M concentration of the test compounds (Table 3). If the compounds satisfy certain

**Table 3. Mean Growth Inhibition Percent on 60 NCI Tumor Cell Lines and Percent Growth Inhibition on MCF-7 at 10 $\mu$M**

| code | mean growth inhibition (%)* | growth inhibition on MCF-7 (%) | code | mean growth inhibition (%)* | growth inhibition on MCF-7 (%) |
|---|---|---|---|---|---|
| **1A** | 35.16 | 79.52 | **4B** | 16.26 | 65.72 |
| **1B** | 5.92 | 60.72 | **4C** | 34.08 | 65.17 |
| **1C** | 29.47 | 75.83 | **4D** | 21.6 | 65.90 |
| **1D** | 5.44 | 46.05 | **4E** | no inhibition | 19.53 |
| **1E** | 26.61 | 73.36 | **4F** | 1.51 | 19.33 |
| **1F** | 10.99 | 24.75 | **5** | 7 | no inhibition |
| **2A** | 8.37 | 3.34 | **5A** | 51.94 | 89 |
| **2B** | 44.30 | 90.87 | **5B** | 4.88 | 1.65 |
| **2C** | 48.05 | 86.30 | **5C** | 17.08 | 30.88 |
| **2D** | 33.90 | 81.53 | **5D** | 26.67 | 68.95 |
| **2E** | 43.26 | 84.70 | **5E** | 10.9 | 36.69 |
| **2F** | 6.90 | 2.30 | **5F** | 0.12 | 0.50 |
| **3B** | 5.02 | 38.78 | **5G** | 17.01 | 31.83 |
| **3D** | 43.96 | 76.86 | **6B** | 31.53 | 83.81 |
| **3E** | 22.84 | 63.13 | **6D** | 24.07 | 64.30 |
| **3F** | 5.16 | 13.79 | **6E** | 5.84 | 18.29 |
| **3G** | 22.74 | 23.67 | **6F** | 5.43 | 14.08 |
| **4** | ≥100 | ≥100 | **6G** | 22.39 | 37.33 |
| **4A** | no inhibition | 6.63 | **TAM** | ≥100 | ≥100 |

*Data obtained from NCI in vitro disease-oriented human tumor cell screen. Compounds tested at a concentration of 10 $\mu$M in triplicates.

threshold inhibition criteria (the mean growth inhibition on all tested cell lines is ≥50%), the assay is progressed to the five-dose level. Test compounds are assessed for their relative potency through concentration−response testing on each tumor cell line; results are expressed as $GI_{50}$ (the concentration of a compound that causes 50% growth inhibition, relative to the no drug control), total growth inhibition (TGI), and half-maximal lethal concentration ($LC_{50}$). TAM (NSC 727681) is used for comparison as shown in Table 4.

Compounds **4** and **5** were tested as examples of phenolic derivatives; compound **4** showed a broad-spectrum growth inhibitory activity over all the tested cell lines; it showed growth inhibition ≥100 on all the 60 tested cell lines. Its flexible congener compound **5** was nearly inactive in all the tested cell lines; this confirmed the importance of rigidity for phenolic analogues' cytotoxic effects. Compound **4**, the most potent estrogenic analogue, may mediate its cytotoxic activities via the ER mechanism; yet, since it showed excellent inhibitory activity versus a non-ER expressing cell line, in particular, the ER-negative breast cancer cell line (see the Supporting Information), it was assumed that other mechanisms are involved.

Compounds **1A**, **1B**, **1C**, and **1E** showed no significant percent mean growth inhibition, indicating that none of the tested compounds have broad anticancer activity. Compounds **1A**, **1B**, **1C**, and **1E** showed percent mean growth inhibition of 79.52, 60.72, 75.83, and 73.36, respectively, on MCF-7.

Compounds **2B**, **2C**, **2D**, and **2E** showed percent mean growth inhibition of 90.87, 86.30, 81.35, and 84.70, respectively, on MCF-7. It is worth mentioning that compounds **2B** and **2C** were of moderate estrogenic activity, whereas compound **2D** was rather antiestrogenic. Compounds **2A** and **2F** which are nearly 2-fold more estrogenic than **2B** and **2C** showed neither broad-spectrum inhibition nor MCF-7 inhibition.

Compounds **3D** and **3E** elicited percent growth inhibition of 76.86 and 63.13, respectively, on MCF-7, whereas compounds **3F** and **3G** with higher estrogenicity showed neither broad-spectrum inhibition nor MCF-7 inhibition. Compounds **4B**, **4C**, and **4D** showed percent mean growth inhibition of 65.72, 65. 17, and 65.90, respectively, and compound **4B** was moderately antiestrogenic. Compounds **5A** and **5D** showed percent mean growth inhibition of 65.72, 65. 17, and 65.90.

Compounds **6B** and **6D** showed percent mean growth inhibition of 83.81 and 64.30, respectively; both compounds were among the most potent antiestrogenic analogues.

It was noticed that a pyridinylethoxy substituent on ring **B** is common among all active analogues. Additionally, all compounds bearing a morpholinylethoxy side chain on ring **B** showed no appreciable growth inhibition on all cancer cell lines or on MCF. This could be attributed to the partial hydrophilicity of the morpholine ring that might have prevented its uptake by the cell lines.

Among the most potent analogues that inhibited the growth of MCF-7 were compounds **4**, **2B**, and **2C**; the three compounds showed moderate to high relative estrogenic activity (12.84, 3.32, and 3.27, respectively). Thus, these compounds can serve as anticancer agents that possess positive estrogenic effects; they can overcome the antiestrogenic effects accompanying TAM therapy like osteoporosis, venous embolism, and hot flashes.[25]

Compounds **4**, **2B**, and **5A** were selected by NCI for five-dose screening assays. $GI_{50}$, TGI, and $LC_{50}$ values were reported on 60 different cell lines and compared to TAM (NSC: 180973). Compound **4** was the most potent analogue on all the tested cell lines with a mean $GI_{50}$ = 2.51 and a median $GI_{50}$ = 0.75; its mean $GI_{50}$ is 4-fold less than that of TAM. It is 2-fold more active than that of TAM on MCF-7 and showed sub-micromolar activity on all ER-negative breast cancer cell lines (BT-549, MDA-MB-231/ATCC, MDA-MB-468, and Hs578T).

Compound **2B** was slightly more potent than TAM on MCF-7; it was 4-fold more potent on BT-459 and MDA-MB-231/ATCC. It showed lower potency on HS 578T and T-47D. It is worth noting that MCF-7 and T-47D are ER-positive hormone-dependent cell lines, whereas MDA-MB-231/ATCC, MDA-MB-468, BT-459, and HS 578T are triple negative breast cancer cell lines (TNBCs). This may indicate a partially hormonal and non-hormonal mechanism of action.

TNBC represents approximately 10−15% of all breast cancers; patients have poor clinical outcomes compared to the other subtypes of breast cancer. Interestingly, the incidence of TNBC in African-American women is 2−3 times higher than that in other ethnic groups.[26] Given the lack of validated molecular targets and the poor outcome in patients with TNBC, compound **2B** may serve as a lead for developing a clinical candidate for TNBC patients.

Compound **5A** was equipotent to TAM on MCF-7, and it was more potent on MDA-MB-231/ATCC and BT-459. The endorsement of flexibility to the rigid TPE backbone still elicited cytotoxic effects on both ER+ and ER− breast cancer cell lines (Table 4).


https://doi.org/10.1021/acsomega.3c01682
ACS Omega 2023, 8, 25903−25923

Table 4. $GI_{50}$ ($\mu$M) of Compounds 4, 2B, 5A, and TAM on 60 Different Cell Lines

| | cell line | 4 | 2B | 5A | TAM |
|---|---|---|---|---|---|
| leukemia | CCRF-CEM | 0.79 | 1.7 | 4.17 | 3.16 |
| | HL-60 (TB) | 0.64 | 1.15 | 3.89 | 2.51 |
| | K-562 | 0.38 | 1.06 | 0.98 | 1.99 |
| | MOLT-4 | 0.65 | 1.05 | 4.01 | 2.51 |
| | RPMI-8226 | 0.58 | 1.17 | 1.07 | 2.51 |
| | SR | 0.74 | 0.98 | 0.99 | 1.26 |
| non-small-cell lung cancer | A549/ATCC | 0.78 | 3.88 | 4.52 | 3.98 |
| | EKVX | 0.82 | 5.76 | 4.75 | 6.31 |
| | HOP-62 | 0.59 | 7.76 | 6.07 | 10.0 |
| | HOP-92 | 0.46 | 0.83 | 1.62 | 2.52 |
| | NCI-H226 | 2.75 | 7.7 | 4.99 | 10.0 |
| | NCI-H23 | 0.76 | 7.43 | 4.54 | 5.01 |
| | NCI-H322M | 0.91 | 6.43 | 5.03 | 7.94 |
| | NCI-H460 | 0.48 | 1.10 | 1.22 | 3.16 |
| | NCI-H522 | 0.85 | 6.56 | 5.31 | 6.31 |
| colon cancer | COLO 205 | 2.44 | 0.92 | 1.00 | 2.15 |
| | HCC-2998 | 0.75 | 8.74 | 2.03 | 3.16 |
| | HCT-116 | 0.62 | 0.86 | 3.03 | 3.98 |
| | HCT-15 | 0.74 | 1.44 | 1.19 | 3.16 |
| | HT29 | 0.85 | 1.06 | 1.36 | 2.51 |
| | KM12 | 0.58 | 2.55 | 4.16 | 3.16 |
| | SW-620 | 0.82 | 1.16 | 1.67 | 3.16 |
| CNS cancer | SF-268 | 0.74 | 7.6 | 5.01 | 6.31 |
| | SF-295 | 0.69 | 0.92 | 5.50 | 1.96 |
| | SF-539 | 0.60 | 4.08 | 4.77 | 5.01 |
| | SNB-19 | 0.96 | 1.88 | 5.04 | 6.31 |
| | SNB-75 | 0.78 | 6.02 | 3.47 | 5.01 |
| | U251 | 0.57 | 1.66 | 3.72 | 3.16 |
| melanoma | LOX IMVI | 0.42 | 0.87 | 3.02 | 2.51 |
| | MALME-3M | 0.54 | 1.20 | 3.68 | 3.16 |
| | M14 | 0.70 | 0.97 | 5.19 | 2.51 |
| | MDA-MB-435 | 0.60 | 3.28 | 4.52 | 3.16 |
| | SK-MEL-2 | 0.80 | 8.01 | 5.69 | 5.01 |
| | SK-MEL-28 | 0.81 | 1.33 | 4.85 | 3.98 |
| | SK-MEL-5 | 0.47 | n.d. | 4.40 | 2.51 |
| | UACC-257 | 0.94 | 7.93 | 4.24 | 3.16 |
| | UACC-62 | 0.50 | 1.14 | 5.19 | 6.31 |
| ovarian cancer | IGROV1 | 0.84 | 3.09 | 3.73 | 5.01 |
| | OVCAR-3 | 0.67 | 3.27 | 4.53 | 3.98 |
| | OVCAR-4 | 0.77 | 5.25 | 3.52 | 6.31 |
| | OVCAR-5 | 2.90 | 6.54 | 4.79 | 6.31 |
| | OVCAR-8 | 0.99 | 8.05 | 5.75 | 7.94 |
| | NCI/ADR-RES | 0.69 | 7.27 | 4.80 | 5.01 |
| | SK-OV-3 | 1.04 | 10.02 | 5.42 | 10.0 |
| renal cancer | 786−0 | 0.66 | 0.77 | 4.55 | 3.98 |
| | A498 | 3.12 | 5.5 | ND | 6.31 |
| | ACHN | 3.71 | 5.61 | 5.12 | 6.31 |
| | CAKI-1 | 1.01 | 5.55 | 4.63 | 3.98 |
| | RXF 393 | 0.50 | 1.16 | 3.45 | 2.51 |
| | SN12C | 0.51 | 5.05 | 4.18 | 3.98 |
| | TK-10 | 2.75 | 6.23 | 5.10 | 3.98 |
| | UO-31 | 0.65 | 5.30 | 4.27 | 6.31 |
| prostate cancer | PC-3 | 0.77 | 1.12 | 4.34 | 3.16 |
| | DU-145 | 1.10 | 3.48 | 4.74 | 6.31 |
| breast cancer | MCF7 | 0.82 | 1.05 | 1.70 | 1.58 |
| | MDA-MB-231/ATCC | 0.97 | 1.30 | 4.27 | 6.31 |
| | HS 578T | 0.60 | 7.08 | 4.72 | 3.98 |
| | BT-549 | 0.50 | 1.13 | 4.95 | 6.31 |
| | T-47D | 0.89 | 3.75 | 3.83 | 2.51 |
| | MDA-MB-468 | 0.67 | 1.06 | 2.97 | 1.96 |
| | **mean** | 2.51 | 3.67 | 3.92 | 4.41 |



https://doi.org/10.1021/acsomega.3c01682
ACS Omega 2023, 8, 25903−25923

Table 4. continued

| cell line | 4 | 2B | 5A | TAM |
|---|---|---|---|---|
| **median** | 0.75 | 3.09 | 4.34 | 3.98 |

Table 5. AlkP Activity after an Incubation of 72 h in Ishikawa Cells

| | 1 nM | 10 nM | 100 nM | 1 $\mu$M | 10 $\mu$M |
|---|---|---|---|---|---|
| E2 | n.d.[b] | 6.86 ± 0.69[a] | n.d. | n.d. | n.d. |
| Tam | n.d. | n.d. | n.d. | 1.60 ± 0.55 | 1.02 ± 0.37 |
| OH-Tam | n.d. | n.d. | n.d. | 1.73 ± 0.46 | 0.60 ± 0.20 |
| **2B** | 1.36 ± 0.97 | 1.47 ± 0.45 | 1.86 ± 0.73 | 2.01 ± 0.55[a] | 0.04 ± 0.07 |
| **2C** | 1.18 ± 0.67 | 1.32 ± 0.25 | 1.73 ± 0.71 | 1.89 ± 0.45[a] | 0.00 ± 0.00 |
| **4** | n.d. | n.d. | 3.91 ± 1.40 | 3.76 ± 1.41 | n.d. |

[a]Solvent control (DMSO) was set to 1; $p < 0.05$ (Tukey's test). [b]n.d. = not determined.

*2.2.4. AlkP Activity in the Ishikawa Cell Line.* The results of the YES assay showed the estrogenic potential of some of the novel compounds; to further validate the results, compounds were tested in an in vitro model using the human Ishikawa endometrial adenocarcinoma cell line. AlkP activity mediated by ER$\alpha$ is significantly stimulated by natural and synthetic estrogens. This assay considers the human metabolic pathways as Ishikawa cells have the capacity to metabolize the tested compounds and therefore reflects their true estrogenic activity in a more profound way than the YES assay.[27]

Compounds **4**, **2B**, and **2C** showed moderate to high relative $\beta$ galactosidase estrogenic activity (12.84, 3.32, and 3.27, respectively); additionally, they showed high percent inhibition growth on MCF at 10 $\mu$M (79.87, 86.30, and ≥100 at 10 $\mu$M). Compounds **4** and **2** showed $GI_{50}$ ($\mu$M) on MCF-7 (0.82 and 1.05, respectively) and on MDA-MB-231 (0.97 and 1.30, respectively). The three candidates seem to be promising ideal SERMs. To investigate their potential safety on endometrial tissues, compounds were tested in five different concentrations to investigate their potential estrogenic activity on the human Ishikawa endometrial adenocarcinoma cell line. The agonistic effect was compared to the vehicle control DMSO (set to 1). Estradiol at 10 nM was used as a positive control, and TAM and 4-OH-TAM at 1 $\mu$M were used as comparative controls (see the Supporting Information).

Compounds **2B** and **2C** showed no significant increase in AlkP activity after a 72 h treatment. Both compounds were able to increase the AlkP activity in a dose-pendent manner with significant effects at a concentration of 100 nM and 1 $\mu$M. The decreased activities at a concentration of 10 $\mu$M are caused by a negative influence of the treatment on the cell growth, observed with light microscopy. Compounds **2B** and **2C** showed nearly equipotent activity when compared to TAM and 4-OH-TAM, despite its higher relative $\beta$ galactosidase estrogenic activity in the YES assay.

Compound **4** was nearly 2-fold more estrogenic in AlkP assays, reflecting the less safe profile and the potential to induce endometrial carcinoma (Table 5).

*2.2.5. In Vivo Testing Using an Ovariectomized Rat Model.* A commonly adopted facile in vivo assay for estrogenicity/antiestrogenicity is the uterotrophic assay, suitable for screening ER$\alpha$ ligands. The relative Uterine Wet Weight (UWW) is the initial end point of the assay. An increase in UWW indicates the estrogenic activity of the test compounds. The previous assays deemed compound **2B** as a promising ideal SERM with a moderate relative estrogenic activity of 3.32 and no significant AlkP activity in the Ishikawa cell line; it further showed growth inhibitory activity against MCF-7 cell lines and was slightly more potent than TAM, whereas it was 6-fold more potent on MDA-MB-231/ATCC, a TNBC cell line. Thus, the compound was further selected for the in vivo uterotrophic assay. It showed less increase in UWW compared to E2 and was nearly equipotent to TAM. Compound **2B** thus potentially has low tendency to induce endometrial carcinoma (Table 6).

Table 6. Relative UWW of Ovariectomized Rats

| code | mean ± SD (g/kg BW[a]) | dose |
|---|---|---|
| vehicle | 0.61 ± 0.07 | |
| E2 | 3.85 ± 0.71 | 10 $\mu$g/kg BW/day |
| TAM | 1.42 ± 0.30 | 10 mg/kg BW/day |
| 2B | 1.46 ± 0.07 | 10 mg/kg BW/day |

[a]Average of six mice.

*2.2.6. Influenza A Virus $H_1N_1$ Screening Assay.* The potential antiviral activity of the novel compounds against influenza A $H_1N_1$ and EBOV was investigated. Twenty-eight compounds were screened for their activity against the influenza A virus $H_1N_1$ at Southern Research Institute (SRI), MD, USA. Ribavirin was used as a positive control. The median effective concentration ($EC_{50}$) and the median cytotoxic concentration ($CC_{50}$) were determined, and selectivity index ($SI_{50}$ = $CC_{50}/EC_{50}$) was calculated. The selectivity index (SI) measures the window between cytotoxicity and antiviral activity. The higher the SI, the higher the safety profile of the antiviral drug; thus, the SI of a compound is a widely accepted parameter used to express the in vitro efficacy in the inhibition of virus replication.[28]

The concentration of the tested compounds ranged from 0.1 to 100 $\mu$g/mL, while the concentration of the control drug ranged from 0.32 to 320 $\mu$g/mL. The assays use visual determination of viral cytopathic effect (CPE) inhibition and confirm the data obtained by neutral red dye uptake (Table 7).

Compounds **1A**, **1B**, **1D**, **3B**, **3D**, **4B**, and **4D** showed activity against the influenza A $H_1N_1$ virus with $EC_{50}$ values of 0.72, 0.32, 5.60, 0.29, 0.29, 3.20, and 3.20 $\mu$g/mL, respectively.

Compounds **3B** and **3D** showed $EC_{50}$ values less than ribavirin; yet, they showed low $SI_{50}$, which indicates a lower safety profile. The structural activity relationship suggests that antiviral activity improves with the dimethylaminopropoxy side chain (**1B**, **3B**, and **4B**) and a pyrrolidinethoxy side chain (**1D**, **3D**, and **4D**) on ring **B**. The highest antiviral activity was


https://doi.org/10.1021/acsomega.3c01682
ACS Omega 2023, 8, 25903−25923

Table 7. Activity of the Compounds against the Influenza A Virus

| | visual CPE | | | | visual CPE | | |
|---|---|---|---|---|---|---|---|
| code | $EC_{50}$ ($\mu$g/mL) | $CC_{50}$ ($\mu$g/mL) | $SI_{50}$[a] ($\mu$g/mL) | code | $EC_{50}$ ($\mu$g/mL) | $CC_{50}$ ($\mu$g/mL) | $SI_{50}$[a] ($\mu$g/mL) |
| **1A** | 0.72 | 1.2 | 1.7 | **3F** | >32 | 32 | <1 |
| **1B** | 0.32 | 2.4 | 7.5 | **3G** | >2.4 | 2.4 | <1 |
| **1C** | >12 | 12 | <1 | **4A** | >0.56 | 0.56 | <1 |
| **1D** | 5.6 | 32 | 5.7 | **4B** | 3.2 | 12 | 3.8 |
| **1E** | >3.2 | 3.2 | <1 | **4C** | >0.71 | 0.71 | <1 |
| **1F** | >8.4 | 8.4 | <1 | **4D** | 3.2 | 10 | 3.1 |
| **2A** | >4.2 | 4.2 | <1 | **4E** | >63 | 63 | <1 |
| **2B** | >3.2 | 3.2 | <1 | **4F** | >7.1 | 7.1 | <1 |
| **2C** | >3.2 | >320 | >100 | **6B** | >3.2 | 3.2 | <1 |
| **2D** | >3.2 | 3.2 | <1 | **6D** | >3.2 | 3.2 | <1 |
| **2E** | >3.2 | 3.2 | <1 | **6E** | >3.2 | 3.2 | <1 |
| **2F** | >7.5 | 7.5 | <1 | **6G** | >3.7 | 3.7 | <1 |
| **3B** | 0.29 | 1.5 | 5.2 | **6F** | >2.2 | 2.2 | <1 |
| **3D** | 0.29 | 1.5 | 5.2 | **ribavirin** | 1 | >320 | >320 |
| **3E** | >2.8 | 2.8 | <1 | | | | |

[a]$CC_{50}/EC_{50}$.

observed when ring **A** was left unsubstituted (**3B** and **3D**) as in TAM; yet, the highest SI was observed in compound **2C**.

Compounds **3B** and **3D** with a rigid backbone and unsubstituted ring **A** showed the highest activity against the influenza A virus with the lowest $EC_{50}$ value of 0.29 $\mu$g/mL and a selectivity index ($SI_{50}$) of 5.2. Comparing compounds **1D** and **1B**, compound **1D** with a *p*-chloro substituent on ring **A** has an $EC_{50}$ value of 5.6 $\mu$g/mL and a $SI_{50}$ value of 7.5. Thus, a chlorine substituent on ring **A** decreased the antiviral activity but increases the safety profile of the compound. Moreover, the flexible analogues having a benzylic methylene spacer and a methoxy group on ring **A** (**6B** and **6D**−**6G**) failed to show activity against the influenza A $H_1N_1$ virus. The presence of a methoxy group on ring **A** seems to abolish the activity whether on a rigid or flexible TPE backbone. Four of the tested compounds showed higher potency than ribavirin ($EC_{50}$ = 1 $\mu$g/mL).

Previous reports indicated the ability of estrogenic compounds and SERMs to reduce the replication of the influenza A virus in female but not in male patients. The mechanism involved genomic ER$\beta$ signaling, in addition to downregulation of several cell metabolic processes, including genes that encode for zinc finger proteins, many of which contain EREs in their promoters.[28]

Our results showed that the antiviral activity does not seem to correlate with the estrogenic mode of action of the tested compounds; yet, it is worth mentioning that the YES assay-adopted yeast stably transfected with human ER$\alpha$ but not with ER$\beta$.

*2.2.7. Ebola Virus Screening Assay.* Compounds **2F** and **3F** were selected for assay against EBOV. Compound **2F** showed excellent activity against EBOV infection with an $EC_{90}$ value of 0.31 $\mu$g/mL and a $SI_{90}$ value of 60. Compound **3F** showed activity against Ebola virus with an $EC_{90}$ value of 1.1 $\mu$g/mL and a $SI_{90}$ value of 13.7. Comparing compound **2F** having a methoxy group on ring **A** with its congener compound **3F** which lacks the methoxy group on ring **A**, compound **2F** displays higher anti-EBOV activity (Table 8). The Ebola virus activity of the novel analogues could be attributed to the fact that it has the properties of CAD, where the morpholine moiety serves as the polar portion of the molecule. Moreover, the presence of a methoxy group on ring **A**, along with an ethyl

Table 8. Activity of Compounds 2F and 3F against EBOV

| code | crystal violet (viral yield reduction)/neutral red (toxicity) | | |
|---|---|---|---|
| | $EC_{90}$ ($\mu$g/mL) | $CC_{50}$ ($\mu$g/mL) | $SI_{90}$[a] |
| **2F** | 0.31 | 18.62 | 60 |
| **3F** | 1.1 | 15.1 | 13.7 |
| **favipiravir** | 69 | >1000 | >14 |

[a]$SI_{90}$: selectivity index = $CC_{90}/EC_{50}$.

spacer, seems to greatly contribute to the anti-Ebola activity. To further investigate the interactions of compound **2F** and its preferred binding conformation, the compound was overlaid on co-crystallized toremifene inside the EBOV GP.[29] Unlike the ER antagonist toremifene, compounds **2F** and **3F** are ER agonists and devoid of cancer cell lines' growth inhibition effect. This indicates that the EBOV growth inhibition is mainly a function of the nature of the substituents of the triphenyl scaffold rather than the functional activity of the molecule.

*2.2.8. Pharmacokinetic Assessment of Compound 2F.* To check the in vivo safety and pharmacokinetics profile of compound **2F**, the maximum tolerated dose (MTD), pharmacokinetics profiling was performed in male and female C57BL/6 mice (Charles River Laboratories, Frederick, MD, USA). For the MTD, three male and three female C57BL/6 mice were administered compound **2F** as a single-dose escalation or de-escalation by per oral (PO) by gavage at a dose of 50, 100, 200, or 400 mg/kg. All male and female mice were administered PO (50, 100, 200, and 400 mg/kg). No acute toxicity was noticed; none of the animals showed any physiologic anomalies up to their 24 h postdose scheduled sacrifice.

The pharmacokinetic profiles of compound **2F** were studied; thus, naïve animals (12 male and 9 female C57BL/6 mice) were given a single dose of 400 mg/kg compound **2F** (PO), and blood was collected at 0.25, 0.5, 4, 6, 8, and 24 h after dose administration.

A bioanalytical method was developed, and the lower limit of quantitation (LOQ) was 10 ng/mL. After PO administration of compound **2F** (400 mg/kg), $T_{max}$, $C_{max}$, and AUC values were determined. $T_{max}$ = 4 h (males) and 0.5 h (females), and $C_{max}$ = 9400 ± 2680 ng/mL (males) and 3540


https://doi.org/10.1021/acsomega.3c01682
ACS Omega 2023, 8, 25903−25923

Table 9. Pharmacokinetics of Compound 2F in Male and Female C57BL/6 Mice

| | | | | | $C_{max}$ (ng/mL) | | $AUC_{last}$ (h ng/mL) | | $AUC_{inf}$ |
|---|---|---|---|---|---|---|---|---|---|
| route | dose mg/kg | sex | $t_{1/2}$ (hr) | $T_{max}$ (hr) | Mean | SE | Mean | SE | (h ng/mL) |
| PO | 400 | M | 8.5 | 4 | 9400 | 2680 | 10,300 | 2030 | 119,000 |
| PO | 400 | F | 8.2 | 0.5 | 3540 | 794 | 47,800 | 6850 | 55,400 |

± 794 ng/mL (females). AUC = 10,300 ± 2030 h ng/mL and 47,800 ± 6850 h ng/mL for males and females, respectively. $AUC_{inf}$ (area under the plasma concentration−time curve from time 0 to infinity) = 119,000 h ng/mL for male mice and 55,400 h ng/mL for female mice. $t_{1/2}$ = 8.5 h (males) and 8.2 h (females). No adverse effects were observed in any of the animals given compound **2F** by the PO route. The initial plasma concentrations were higher in male mice than in female mice based on $C_{max}$; exposure values based on $AUC_{last}$ (area under the plasma concentration−time curve from time zero to the time of last measurable concentration) were also 2.2-fold higher in males compared to females; the $t_{1/2}$ value was about 8.3 h (Table 9).

This indicates that compound **3F** can attain oral concentrations much higher than the concentration needed to inhibit the virus replication.

*2.2.9. In Silico Model.* The mode of binding of the most potent estrogenic compound **4** (relative $\beta$ galactosidase activity = 12.84) was investigated through a simple computational docking study. Compound **4** was docked into the ER$\alpha$ LBD co-crystallized with DES, a synthetic estrogen with full agonistic activity (PDB: 3ERD).[30] To validate the docking protocol used, the co-crystallized ligand *E*-DES was re-docked into the ER$\alpha$ LBD; all the resulting poses converged to a comparable binding mode as the co-crystallized DES, with the best pose having an rmsd value = 0.54 and an affinity value (*S* score) = −12.28. Both *E* and *Z* isomers of compound **4** and *E* DES were built using a MOE builder. Conformational search was adopted for the three compounds. The database obtained was saved as.mdb and used as docking ligands. The *E* isomer of compound **4** showed the highest affinity (*S* score = −11.92 and rmsd value = 0.53 Å), whereas the *Z* isomer showed much lower affinity (*S* score = −6.96 and rmsd value = 1.04 Å). The *E* isomer was overlaid on *E*-DES, and the 2D ligand interactions were displayed. Rings **A** and **C** of the *E* isomer of compound **4** both bearing *p*-chloro substituents adopted a similar conformation as the bisphenolic rings of DES; ring **B** bearing an −OH group overlaid one of the ethyl groups of DES (Figure 2).

DES establishes two essential interactions, namely, two H-bonds with Glu353 and His524; the *E* isomer of compound **4** lost one of the essential hydrogen bond interaction with Glu353; yet, the chloro substituent on ring **A** maintained the hydrogen bond interaction with His524. The side chain of His524 acts as a hydrogen bond donor, whereas the chloro group on ring **C** acts as a weak hydrogen bond acceptor, such interaction was previously reported by Muzangwa et al.[31]

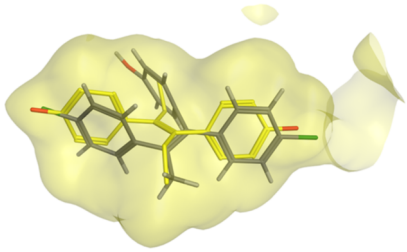


**Figure 2.** *E*-isomer of compound **4** (grey) overlaid with DES (yellow) inside ER$\alpha$ LBD.

The three aromatic rings of compound **4** were embedded in cavities lined with hydrophobic residues such as Met388 and Leu346, affording extra hydrophobic interactions. Moreover, the methyl group on the ethylene backbone was able to form hydrophobic interactions with Leu428 and Phe404. Ring **B** was able to form hydrophobic interactions with Leu540, Trp383, Leu384, and Leu387 as well (Figure 3).

*2.2.10. Molecular Docking with the EBOV GP Binding Pocket.* *Z*-toremifene (TOR) was co-crystallized with the EBOV GP; results showed that TOR was able to halt EBOV entry to the host cells. The membrane envelope of EBOV contains trimers of a GP, formed of GP1 and GP2 subunits, with a large tunnel between the two monomers. The EBOV GP fuses with the endosomal membrane to facilitate its entry into the host cell.[32] The co-crystallized TOR binds at the entrance of the large tunnel, thus lowering the stability of the viral GP and inhibiting the viral fusion. The main interactions between TOR and the binding site are hydrophobic, where ring **A** maintains hydrophobic interactions with Tyr517, Leu558, Leu68, and Val66. Ring **B** maintains hydrophobic interactions with Leu186 and Tyr517. Ring **C** maintains a hydrophobic interaction with Val66. The ethyl chloride group maintains hydrophobic interactions with Leu515, Leu558, and Leu184. The dimethylaminoethoxy group is placed toward the tunnel and is surrounded by polar residues such as Glu100, Asp522, Thr519, Thr520, and Arg64.[29]

To validate the docking method adopted, TOR was re-docked into the binding pocket. All the resulting poses converged to a comparable binding mode as the co-crystallized TOR, with the best pose having an affinity (*S* score = −14.06 and rmsd = 0.62 Å). Both *E* and *Z* isomers of compound **2F** and *Z*-TOR were built using a MOE builder. Conformational search was adopted for the three compounds. The database obtained was saved as .mdb and used as the docking ligands. Results revealed that the best pose was for the *E* isomer, which partially overlaid with the co-crystallized *Z*- TOR (*S* score = −13.78 and rmsd = 1.63 Å), Figure 4.

The 2D interactions of the *E* isomer of compound **2F** showed that it maintained most of the hydrophobic interactions as TOR. The morpholinethoxy group was positioned toward the same polar amino acids as *Z*-TOR; the polar residues Asp522, Glu100, and Arg64 are lining the cavity occupied by the *E* conformer, Figure 5.

## 3. MATERIALS AND METHODS

**3.1. Chemistry.** Solvents and reagents were obtained from commercial suppliers and were used without further purification. All organic solvents used were of pure analytical grade. Purification of intermediates and products was done using column chromatography using silica gel 70−230 $\mu$M mesh. Reaction progress was monitored by TLC using fluorescent pre-coated silica gel plates, and detection of the



https://doi.org/10.1021/acsomega.3c01682
ACS Omega 2023, 8, 25903−25923

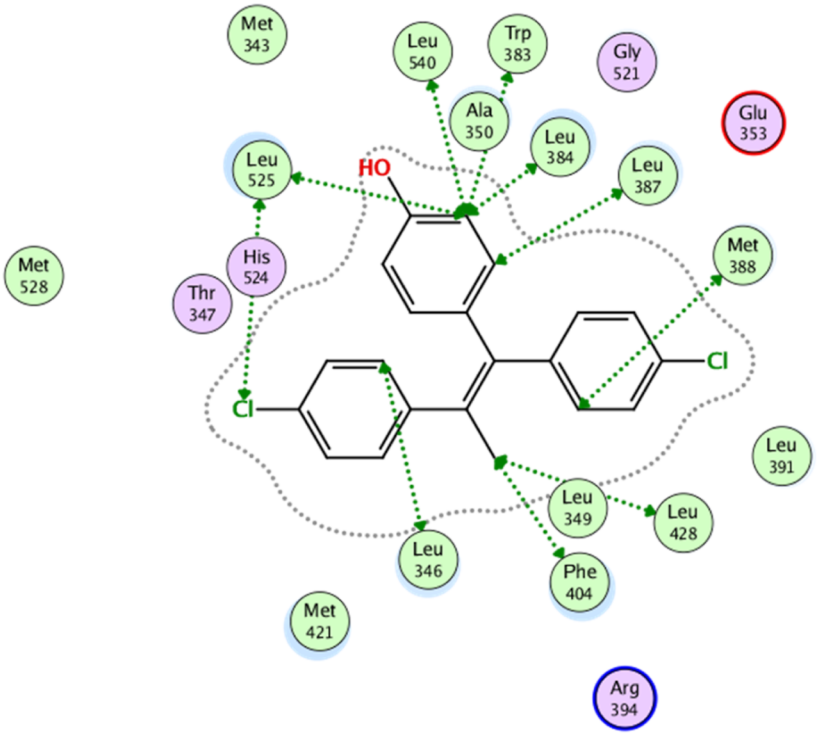



**Figure 3.** Ligand interactions of the *E* isomer of compound **4** with ER$\alpha$ LBD.

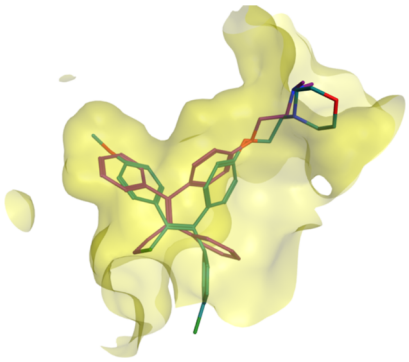


**Figure 4.** Overlay of the *E* isomer of **2F** (dark cyan) with co-crystallized TOR (purple).

components was made by a short UV light ($\lambda$ = 254 nm). $^1$H NMR spectra were run on a 400 MHz Bruker or a 500 MHz Ascend spectrophotometer, and $^{13}$C spectra were run at 101 or at 126 MHz in deuterated chloroform ($CDCl_3$). Chemical shifts ($\delta$) were reported in parts per million (ppm) downfield from TMS; all coupling constants ($J$) are given in Hz. Multiplicities are abbreviated as s: singlet; d: doublet; t: triplet; q: quartet; m: multiplet; dd: doublet of doublet; dt: doublet of triplet; and br s: broad singlet. The purities of the tested compounds were determined by HPLC coupled with a mass spectrometer. Mass spectrometric analysis (UPLC-ESI-MS) was performed using the Waters ACQUITY Xevo TQD system, which consisted of an ACQUITY UPLC H-Class system and a Xevo TQD triple-quadrupole tandem mass spectrometer with an electrospray ionization (ESI) interface (Waters Corp., Milford, MA, USA). An Acquity BEH $C_{18}$ 100 mm × 2.1 mm column (particle size, 1.7 $\mu$m) was used to separate the analytes (Waters, Ireland). The solvent system consisted of water containing 0.1% TFA (A) and 0.1% TFA in acetonitrile (B). HPLC method: flow rate 200 $\mu$L/min. The percentage of B was initially 5%, maintained for 1 min, then increased up to 100% for 10 min, kept at 100% for 2 min, and flushed back to 5% in 3 min. The MS scan was carried out at the following conditions: capillary voltage 3.5 kV, cone voltage 20 V, radio frequency lens voltage 2.5 V, source temperature 150 °C, and desolvation gas temperature 500 °C. Nitrogen was used as the desolvation and cone gas at a flow rate of 1000 and 20 L/h, respectively. System operation and data acquisition were controlled using Mass Lynx 4.1 software (Waters). LCMS, $^1$H NMR, and $^{13}$C NMR analyses were performed at German University in Cairo and at Technische Universität, Dresden.

*3.1.1. Synthesis of Compounds* ***1*****–****6*****.*** Zinc powder (10.11 g, 154 mmol) was suspended in dry THF (100 mL), and the mixture was cooled to 0 °C. $TiCl_4$ (7.5 mL, 70 mmol) was added dropwise. When the addition was complete, the mixture was warmed to room temperature and heated to reflux for 2 h. After cooling down, a solution of 4-chloro-4-hydroxybenzophenone (2.86 g, 12.3 mmol) and appropriate ketone (38.4 mmol) in dry THF (100 mL) was added at 0 °C, and the mixture was heated at reflux in the dark for 2.5−8 h. After being cooled to room temperature, THF was evaporated. The residue was dissolved and extracted with methylene chloride (100 mL × 6). The organic layers were combined and dried over anhydrous $Na_2SO_4$ and further purified by silica gel column chromatography (100% methylene chloride) to yield compounds (**1**−**6**) as oily products.

*3.1.1.1. E/Z-4-[1,2-Bis-(4-chlorophenyl)but-1-enyl]-phenol* ***(1).*** $C_{22}H_{18}Cl_2O$. Yield: 55%. Yellow oil. Purity: 95%. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.32 (d, $J$ = 1.9 Hz, 1H), 7.31 (d, $J$ = 2.0 Hz, 1H), 7.24 (d, $J$ = 1.4 Hz, 2H), 7.22 (s, 2H), 7.16 (s, 2H), 7.13 (s, 2H), 7.08 (s, 1H), 7.06 (s, 1H), 7.02 (d, $J$ = 1.9 Hz, 2H), 7.01 (d, $J$ = 2.9 Hz, 2H), 6.99 (s, 1H), 6.82 (d, $J$ = 1.9 Hz, 1H), 6.80 (d, $J$ = 1.0 Hz, 1H), 6.77 (d, $J$ = 2.0 Hz, 1H),


https://doi.org/10.1021/acsomega.3c01682
ACS Omega 2023, 8, 25903−25923

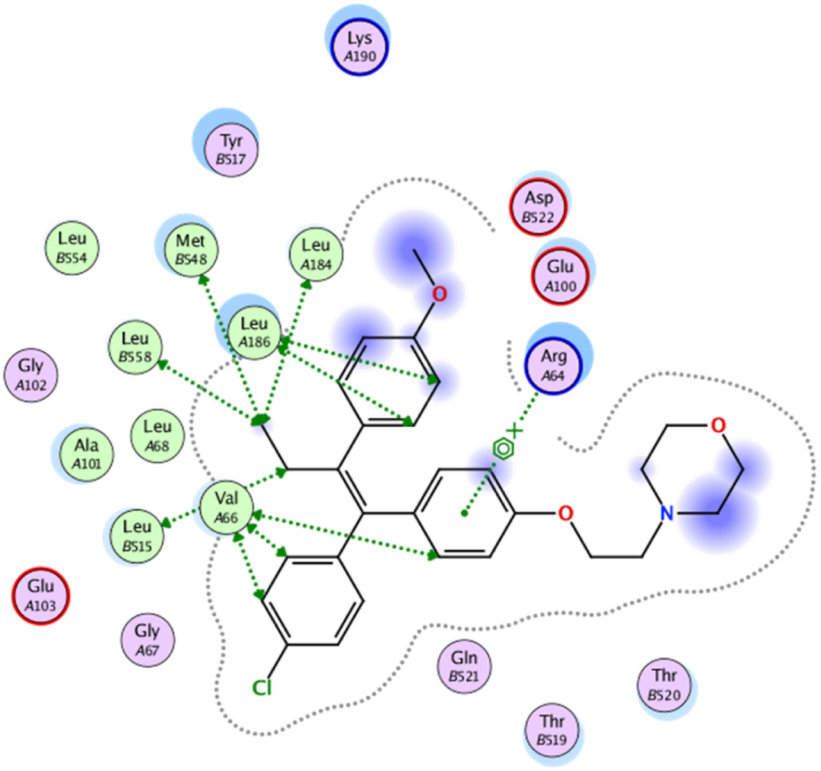



**Figure 5.** 2D interactions of the *E* isomer of compound **2F** inside EBOV GP.

6.71 (m, 1H), 6.69 (m, 1H), 6.52 (m, 1H), 6.49 (m, 1H), 5.00(br s, 1H), 4.83 (br s, 1H), 2.09 (dt, $J$ = 18.2, 7.3 Hz, 4H), 1.59 (m, 6H). $^{13}$C NMR (101 MHz, $CDCl_3$): $\delta$ 154.54, 153.79, 141.82, 141.33, 140.71, 140.52, 139.70, 138.64, 137.79, 137.73, 135.34, 134.83, 133.01, 132.76, 130.93, 130.71, 129.68, 129.17, 128.37, 128.23, 128.16, 127.73, 127.50, 127.36, 115.11, 114.57, 27.99, 27.45, 13.49, 13.45. MS (ESI): $m/z$ 369.07 $[M]^+$, $m/z$ 371.07 $[M + 2]^+$, $m/z$ 373.07 $[M + 4]^+$. $R_f$: 0.53 (100% methylene chloride).

*3.1.1.2. E/Z-4-[1-(4-Chlorophenyl)-2-(4-methoxyphenyl)-but-1-enyl]-phenol (**2**).* $C_{23}H_{21}ClO_2$. Yield: 54%. Yellow oil. Purity: 94%. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.32 (m, 1H), 7.29 (m, 1H), 7.17 (m, 1H), 7.14 (q, $J$ = 2.2 Hz, 1H), 7.90 (m, 1H), 7.06 (m, 1H), 7.03 (m, 1H), 7.01 (m, 2H), 6.97 (d, $J$ = 3.0 Hz, 2H), 6.82 (m, 1H), 6.82 (m, 1H), 6.80 (t, $J$ = 2.5 Hz, 2H), 6.78 (m, 1H), 6.78 (m, 3H), 6.73 (m, 3H), 6.51 (m, 1H), 6.48 (m, 1H), 3.79 (s, 6H), 2.43 (dq, $J$ = 17.4, 7.4 Hz, 4H), 1.26 (qd, $J$ = 6.8, 2.0 Hz, 6H). $^{13}$C NMR (101 MHz, $CDCl_3$): $\delta$ 157.96, 157.89, 154.35, 153.48, 142.17, 142.04, 141.47, 136.56, 136.47, 135.87, 135.47, 134.16, 134.07, 132.12, 132.07, 130.83, 130.77, 130.65, 130.64, 128.28, 127.54, 115.04, 114.41, 113.38, 113.31, 55.09, 28.96, 28.85, 13.60, 13.56. MS (ESI): $m/z$ 365.12 $[M]^+$, $m/z$ 367.12 $[M + 2]^+$, $m/z$ 369.12 $[M + 4]^+$. $R_f$: 0.37 (100% methylene chloride).

*3.1.1.3. E/Z-4-[1-(4-Chlorophenyl)-2-phenyl-but-1-enyl]-phenol (**3**).* $C_{22}H_{19}ClO$. Yield: 46%. Light brown oil. Purity: 94%. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.14 (m, 16H), 6.96 (m, 2H), 6.77 (m, 6H), 6.49 (d, $J$ = 2.1 Hz, 1H), 6.47 (d, $J$ = 2.0 Hz, 1H), 4.94 (br s, 1H), 4.68 (br s, 1H), 2.42 (m, 4H), 0.93 (m, 6H). $^{13}$C NMR (101 MHz, $CDCl_3$): $\delta$: 154.41, 153.56, 142.70, 142.14, 142.06, 142.03, 141.97, 141.76, 141.22, 137.06, 136.98, 135.66, 135.22, 132.39, 132.07, 132.04, 131.41, 131.18, 131.10, 130.82, 130.77, 129.57, 128.56, 128.31, 127.98, 127.96, 127.89, 127.62, 127.50, 127.36, 126.65, 126.28, 126.18, 115.08, 114.77, 114.38, 22.53, 22.48, 13.52, 13.49. MS (ESI): $m/z$ 335.08 $[M]^+$, $m/z$ 337.08 $[M + 2]^+$. $R_f$: 0.44 (100% methylene chloride).

*3.1.1.4. E/Z-4-[1,2-Bis-(4-chlorophenyl)-propenyl]-phenol (**4**).* $C_{21}H_{16}Cl_2O$. Yield: 56%. Brown oil. Purity: 100%. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.33 (m, 1H), 7.31 (m, 1H), 7.19 (m, 1H), 7.15 (q, $J$ = 4.9 Hz, 3H), 7.12 (m, 2H), 7.10 (m, 1H), 7.06 (dt, $J$ = 6.4, 2.0 Hz, 4H), 7.03 (t, $J$ = 1.6 Hz, 2H), 7.02−6.99 (m, 1H), 6.84 (m, 1H), 6.81 (t, $J$ = 2.3 Hz, 2H), 6.79 (dd, $J$ = 4.9, 2.4 Hz, 1H), 6.73 (m, 1H), 6. 70 (m, 1H), 6.54 (m, 1H), 6.52 (m, 1H), 5.07 (br s, 1H), 4.88 (br s, 1H), 2.15 (s, 3H), 2.05 (s, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$): $\delta$ 154.53, 154.42, 153.84, 142.31, 142.22, 141.81, 141.44, 138.26, 135.33, 134.95, 134.75, 134.16, 132.50, 132.13, 132.02, 131.89, 131.82, 131.30, 130.58, 128.35, 128.23, 128.14, 127.79, 115.08, 114.64, 23.32, 23.10. MS (ESI): $m/z$ 355.06 $[M]^+$, $m/z$ 357.06 $[M + 2]^+$, $m/z$ 359.06 $[M + 4]^+$. $R_f$: 0.48 (100% methylene chloride).

*3.1.1.5. E/Z-4-[1,3-Bis-(4-chlorophenyl)-2-methylpropenyl]-phenol (**5**).* $C_{22}H_{18}Cl_2O$. Yield: 45%. Yellow oil. Purity: 100%. $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 7.27 (m, 8H), 7.07 (m, 12H), 6.77 (m, 4H), 5.32 (s, 2H), 3.49 (t, $J$ = 17.5 Hz, 4H), 1.69 (d, $J$ = 16.2 Hz, 6H). $^{13}$C NMR (126 MHz, $CDCl_3$): $\delta$154.43, 154.31, 141.62, 141.58, 138.98, 138.91, 138.08, 138.06, 135.11, 135.07, 133.15, 133.01, 132.48, 132.30, 131.88, 131.85, 131.14, 130.90, 130.84, 130.04, 129.99, 128.67, 128.65, 128.52, 128.32, 115.28, 115.06, 53.57, 40.95, 20.03, 19.96. MS (ESI): $m/z$ 369.28 $[M]^+$, $m/z$ 371.28 $[M + 2]^+$, $m/z$ 373.28 $[M + 4]^+$. $R_f$: 0.49 (100% methylene chloride).

*3.1.1.6. E/Z-4-[1-(4-Chlorophenyl)-3-(4-methoxyphenyl)-2-methylpropenyl]-phenol (**6**).* $C_{23}H_{21}ClO_2$. Yield: 57%. Dark yellow oil. Purity: 95%. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.26 (m, 4H), 7.08 (m, 12H), 6.86 (d, $J$ = 1.9 Hz, 2H), 6.84 (d, $J$ = 1.9 Hz, 2H), 6.76 (m, 4H), 4.99 (s, 2H), 3.81 (s, 6H),


https://doi.org/10.1021/acsomega.3c01682
ACS Omega 2023, 8, 25903−25923

3.45 (s, 2H), 3.42 (s, 2H), 1.70 (s, 3H), 1.67 (s, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$): $\delta$: 157.83, 157.81, 154.16, 154.05, 141.74, 141.69, 137.17, 135.21, 135.16, 133.93, 133.82, 132.45, 132.36, 132.13, 131.97, 131.06, 131.03, 130.85, 130.77, 129.49, 129.44, 128.26, 128.10, 115.05, 114.87, 113.85, 113.84, 55.28, 53.42, 40.52, 19.88, 19.80. MS (ESI): $m/z$ 365.12 $[M]^+$, $m/z$ 367.12 $[M + 2]^+$.$R_f$: 0.35 (100% methylene chloride).

*3.1.1.7. Synthesis of Compounds* ***1(A−F), 2(A−F), 3B, 3(D−G), 4(A−F), 5(A−G), 6B, and 6(D−G).*** A solution of **1−6** (8.9 mmol) in DMF (100 mL) was treated with $K_2CO_3$ (3.62 g, 26.2 mmol) and heated in an oil bath at 90 °C. The resulting suspension was treated with the appropriate commercially available base hydrochloride salt (9.48 mmol) portionwise over a 2 h period and stirred for 24 h. The reaction mixture was cooled down to room temperature. The final product was further purified using column chromatography (95:5 methylene chloride/methanol) to afford the compounds as oily products.

*3.1.1.8. E/Z-(2-{4-[1,2-Bis-(4-chlorophenyl)-but-1-enyl]-phenoxy}-ethyl)-dimethyl-amine (**1A**).* $C_{26}H_{27}Cl_2NO$. Yield: 47%. Yellow oil. Purity: 100%. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.31 (m, 1H), 7.29 (m, 1H), 7.15 (m, 3H), 7.12 (d, $J$ = 2.0 Hz, 3H), 7.10 (d, $J$ = 2.1 Hz, 1H), 7.08 (m, 1H), 7.04−7.03 (m, 2H), 7.02 (d, $J$ = 2.1 Hz, 2H), 6.99 (d, $J$ = 2.4 Hz, 1H), 6.99−6.97 (m, 1H), 6.90 (d, $J$ = 2.1 Hz, 1H), 6.89−6.87 (m, 1H), 6.80−6.78 (m, 1H), 6.77 (q, $J$ = 2.1 Hz, 1H), 6.73 (d, $J$ = 2.1 Hz, 1H), 6.71 (m, 1H), 6.59 (s, 1H), 6.57 (d, $J$ = 1.9 Hz, 1H), 4.09 (t, $J$ = 5.8 Hz, 2H), 3.94 (t, $J$ = 5.8 Hz, 2H), 2.75 (t, $J$ = 5.5 Hz, 2H), 2.66 (t, $J$ = 5.8 Hz, 2H), 2.37 (s, 6H), 2.28 (m, 10H), 0.91 (m, 6H). $^{13}$C NMR (101 MHz, $CDCl_3$): $\delta$ 157.79, 157.06, 141.88, 141.46, 141.26, 140.60, 140.48, 137.90, 137.81, 135.25, 134.70, 132.52, 132.04, 131.95, 131.84, 131.78, 131.69, 130.92, 130.71, 130.45, 128.34, 128.21, 128.15, 127.70, 114.22, 113.64, 65.88, 65.66, 58.27, 58.21, 45.85, 45.83, 28.91, 28.78, 13.48, 13.45. MS (ESI): $m/z$ 440.15 $[M]^+$, $m/z$ 442.15 $[M + 2]^+$, $m/z$ 444.15 $[M + 4]^+$. $R_f$: 0.4 (9:1 methylene chloride/methanol).

*3.1.1.9. E/Z-(3-{4-[1,2-Bis-(4-chlorophenyl)-but-1-enyl]-phenoxy}-propyl)-dimethyl-amine (**1B**).* $C_{27}H_{29}Cl_2NO$. Yield: 44%. Brown oil. Purity: 95%. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.31 (m, 2H), 7.14 (t, $J$ = 2.0 Hz, 3H), 7.12 (m, 4H), 7.09 (m, 1H), 7.03 (m, 1H), 7.01 (d, $J$ = 2.1 Hz, 2H), 6.99 (d, $J$ = 2.0 Hz, 2H), 6.97 (m, 1H), 6.84 (m, 2H), 6.78 (m, 1H), 6.76 (d, $J$ = 1.9 Hz, 1H), 6.74 (q, $J$ = 1.9 Hz, 1H), 6.71 (q, $J$ = 1.8 Hz, 1H), 6.54 (m, 2H), 4.07 (t, $J$ = 5.9 Hz, 2H), 3.93 (t, $J$ = 5.8 Hz, 2H), 3.02−2.88 (m, 4H), 2.69−2.54 (m, 12H), 2.43 (dq, $J$ = 14.8, 7.4 Hz, 4H), 2.19 (m, 4H), 0.90 (m, 6H). $^{13}$C NMR (101 MHz, $CDCl_3$): $\delta$ 157.43, 156.65, 141.77, 141.40, 140.82, 140.52, 140.38, 137.75, 137.69, 135.61, 135.10, 132.58, 132.04, 131.88, 131.73, 130.92, 130.89, 130.68, 130.55, 128.37, 128.23, 128.16, 127.72, 114.10, 113.50, 65.14, 64.88, 55.93, 55.88, 43.76, 43.66, 28.90, 28.79, 25.53, 25.36, 13.45, 13.43. MS (ESI): $m/z$ 454.16 $[M]^+$, $m/z$ 456.16 $[M + 2]^+$, $m/z$ 458.16 $[M + 4]^+$. $R_f$: 0.35 (9:1 methylene chloride/methanol).

*3.1.1.10. E/Z-(2-{4-[1,2-Bis-(4-chlorophenyl)-but-1-enyl]-phenoxy}-ethyl)-diethyl-amine (**1C**).* $C_{28}H_{31}Cl_2NO$. Yield: 43%. Yellow oil. Purity: 100%. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.31 (m, 2H), 7.15 (d, $J$ = 1.1 Hz, 3H), 7.13 (d, $J$ = 1.0 Hz, 3H), 7.11 (d, $J$ = 2.0 Hz, 1H), 7.09 (d, $J$ = 1.9 Hz, 1H), 7.03 (d, $J$ = 2.1 Hz, 1H), 7.02 (d, $J$ = 1.8 Hz, 2H), 7.00 (s, 2H), 6.98 (d, $J$ = 1.9 Hz, 1H), 6.87 (m, 2H), 6.79−6.77 (m, 2H), 6.73 (m, 2H), 6.56 (m, 2H), 4.13 (t, $J$ = 5.8 Hz, 2H), 4.00 (t, $J$ = 6.1 Hz, 2H), 2.96 (t, $J$ = 6.0 Hz, 2H), 2.89 (t, $J$ = 6.1 Hz, 2H), 2.76 (m, 4H), 2.69 (m, 4H), 2.45 (m, 4H), 1.11 (dd, $J$ = 9.3, 7.2 Hz, 12H), 0.90 (dd, $J$ = 6.5, 4.6 Hz, 6H). $^{13}$C NMR (101 MHz, $CDCl_3$): $\delta$ 157.52, 156.74, 141.84, 141.41, 141.32, 140.71, 140.52, 140.44, 137.84, 137.76, 135.43, 134.91, 132.55, 132.02, 131.84, 131.72, 130.91, 130.71, 130.51, 128.35, 128.22, 128.16, 127.71, 114.20, 113.62, 65.77, 65.47, 51.55, 51.38, 47.70, 47.67, 28.91, 28.78, 13.47, 13.44, 11.19, 11.10. MS (ESI): $m/z$ 468.18 $[M]^+$, $m/z$ 470.18 $[M + 2]^+$, $m/z$ 472.18 $[M + 4]^+$. $R_f$: 0.43 (93:7 methylene chloride/methanol).

*3.1.1.11. E/Z-1-(2-{4-[1,2-Bis-(4-chlorophenyl)-but-1-enyl]-phenoxy}-ethyl)-pyrrolidine (**1D**).* $C_{28}H_{29}Cl_2NO$. Yield: 47%. Yellow oil. Purity: 99%. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.32 (m, 2H), 7.16 (m, 2H), 7.14 (m, 4H), 7.11 (m, 1H), 7.09 (m, 1H), 7.04 (m, 3H), 7.01 (t, $J$ = 2.4 Hz, 2H), 6.98 (m, 1H), 6.89 (m, 2H), 6.78 (m, 2H), 6.74 (d, $J$ = 2.1 Hz, 1H), 6.72 (m, 1H), 6.58 (m, 2H), 4.21 (t, $J$ = 5.7 Hz, 2H), 4.08 (t, $J$ = 5.7 Hz, 2H), 3.04 (t, $J$ = 5.6 Hz, 2H), 2.96 (t, $J$ = 5.6 Hz, 2H), 2.79 (d, $J$ = 18.2 Hz, 8H), 2.44 (dt, $J$ = 22.4, 7.4 Hz, 4H), 1.89 (d, $J$ = 5.2 Hz, 4H), 1.85 (d, $J$ = 6.7 Hz, 4H), 0.92 (m, 6H). $^{13}$C NMR (101 MHz, $CDCl_3$): $\delta$ 157.50, 146.12, 141.84, 141.41, 141.33, 140.70, 140.51, 140.44, 137.83, 137.75, 135.48, 134.95, 132.55, 132.03, 131.88, 131.84, 130.91, 130.71, 130.51, 128.36, 128.22, 128.17, 127.71, 114.25, 113.66, 66.27, 66.02, 54.88, 54.80, 54.66, 54.63, 28.91, 28.79, 23.42, 23.38, 13.47, 13.44. MS (ESI): $m/z$ 466.16 $[M]^+$, $m/z$ 468.16 $[M + 2]^+$, $m/z$ 470.16 $[M + 4]^+$. $R_f$: 0.5 (93:7 methylene chloride/methanol).

*3.1.1.12. E/Z-1-(2-{4-[1,2-Bis-(4-chlorophenyl)-but-1-enyl]-phenoxy}-ethyl)-piperidine (**1E**).* $C_{29}H_{31}Cl_2NO$. Yield: 45%. Yellow oil. Purity: 100%. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.31 (m, 1H), 7.30 (s, 1H), 7.15 (t, $J$ = 1.7 Hz, 3H), 7.13 (d, $J$ = 2.0 Hz, 3H), 7.10 (d, $J$ = 2.1 Hz, 1H), 7.08 (d, $J$ = 2.1 Hz, 1H), 7.03 (d, $J$ = 2.1 Hz, 2H), 7.02 (d, $J$ = 1.3 Hz, 2H), 7.00 (d, $J$ = 1.6 Hz, 1H), 6.98 (s, 1H), 6.88 (d, $J$ = 2.1 Hz, 1H), 6.85 (m, 1H), 6.78 (m, 1H), 6.76 (m, 1H), 6.73 (d, $J$ = 2.1 Hz, 1H), 6.71 (s, 1H), 6.58 (s, 1H), 6.56 (d, $J$ = 2.1 Hz, 1H), 4.13 (t, $J$ = 6.0 Hz, 2H), 4.00 (t, $J$ = 5.8 Hz, 2H), 2.81 (t, $J$ = 6.0 Hz, 2H), 2.73 (t, $J$ = 6.0 Hz, 2H), 2.46 (m, 8H), 2.41 (m, 4H), 1.61 (dq, $J$ = 11.1, 5.7 Hz, 12H), 0.90 (m, 6H). $^{13}$C NMR (101 MHz, $CDCl_3$): $\delta$ 157.75, 157.02, 141.89, 141.45, 141.26, 140.60, 140.55, 140.47, 137.90, 137.81, 135.24, 134.68, 132.53, 132.03, 131.99, 131.79, 131.70, 130.92, 130.71, 130.46, 128.34, 128.21, 128.15, 127.70, 114.24, 113.67, 65.75, 65.56, 57.88, 57.81, 55.00, 54.97, 28.91, 28.79, 25.77, 25.72, 24.07, 24.03, 13.48, 13.44. MS (ESI): $m/z$ 480.18 $[M]^+$, $m/z$ 482.18 $[M + 2]^+$, $m/z$ 484.18 $[M + 4]^+$. $R_f$: 0.57 (93:7 methylene chloride/methanol).

*3.1.1.13. E/Z-4-(2-{4-[1, 2-Bis-(4-chlorophenyl)-but-1-enyl]-phenoxy}-ethyl)-morpholine (**1F**).* $C_{28}H_{29}Cl_2NO_2$. Yield: 44%. Yellow oil. Purity: 98%. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.32 (q, $J$ = 1.5 Hz, 1H), 7.30 (d, $J$ = 2.1 Hz, 1H), 7.16 (m, 3H), 7.13 (q, $J$ = 1.7 Hz, 3H), 7.11 (d, $J$ = 2.1 Hz, 1H), 7.09 (d, $J$ = 2.2 Hz, 1H), 7.04 (m, 1H), 7.02 (q, $J$ = 0.9 Hz, 2H), 7.00 (d, $J$ = 2.4 Hz, 2H), 6.99 (d, $J$ = 2.1 Hz, 1H), 6.89 (m, 1H), 6.87 (d, $J$ = 2.1 Hz, 1H), 6.79 (m, 1H), 6.77 (d, $J$ = 2.1 Hz, 1H), 6.74 (m, 1H), 6.72 (d, $J$ = 2.2 Hz, 1H), 6.58 (m, 1H), 6.56 (m, 1H), 4.13 (t, $J$ = 5.7 Hz, 2H), 4.00 (t, $J$ = 5.7 Hz, 2H), 3.72 (m, 8H), 2.82 (t, $J$ = 5.7 Hz, 2H), 2.74 (t, $J$ = 5.7 Hz, 2H), 2.58 (m, 4H), 2.54 (m, 4H), 2.47 (m, 2H), 2.43 (m, 2H), 0.92 (m, 6H). $^{13}$C NMR (101 MHz, $CDCl_3$): $\delta$ 157.67, 156.94, 141.86, 141.42, 141.31, 140.68, 140.55, 140.43, 137.85, 137.76, 135.38, 134.82, 132.56, 132.02, 131.86, 131.82, 131.73, 130.92, 130.70, 130.49, 128.36, 128.23, 128.16, 127.72,


https://doi.org/10.1021/acsomega.3c01682
ACS Omega 2023, 8, 25903−25923

114.24, 113.67, 66.88, 66.85, 65.71, 65.51, 57.66, 57.61, 54.07, 54.04, 28.91, 28.80, 13.47, 13.44. MS (ESI): $m/z$ 482.16 $[M]^+$, $m/z$ 484.16 $[M + 2]^+$, $m/z$ 486.16 $[M + 4]^+$. $R_f$: 0.64 (95:5 methylene chloride/methanol).

*3.1.1.14. E/Z-(2-{4-[1-(4-Chlorophenyl)-2-(4-methoxyphenyl)-but-1-enyl]-phenoxy}-ethyl)-dimethyl-amine (**2A**).* $C_{27}H_{30}ClNO_2$ .Yield: 45%. Yellow oil. Purity: 95%. $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ 7.31 (d, $J$ = 8.2 Hz, 2H), 7.14 (d, $J$ = 8.1 Hz, 5H), 6.98 (m, 6H), 6.88 (d, $J$ = 7.5 Hz, 2H), 6.77 (d, $J$ = 8.0 Hz, 3H), 6.72 (m, 4H), 6.55 (d, $J$ = 8.4 Hz, 2H), 4.53 (br s, 2H), 4.38 (br s, 2H), 3.76 (s, 6H), 3.47 (br s, 2H), 3.38 (br s, 2H), 2.94 (s, 6H), 2.88 (s, 6H), 2.42 (m, 4H), 0.92 (br s, 6H). $^{13}C$ NMR (101 MHz, $CDCl_3$): $\delta$ 158.06, 157.98, 155.85, 154.96, 142.59, 142.15, 142.08, 141.78, 137.28, 136.19, 136.11, 133.93, 133.83, 132.49, 132.13, 132.08, 131.33, 131.18, 130.87, 130.82, 130.61, 128.62, 128.34, 127.60, 114.27, 113.52, 113.43, 113.41, 62.89, 62.62, 56.69, 56.58, 55.09, 43.80, 43.73, 28.96, 28.92, 13.54. MS (ESI): $m/z$ 436.20 $[M]^+$, $m/z$ 438.20 $[M + 2]^+$. $R_f$: 0.56 (85:15 methylene chloride/methanol).

*3.1.1.15. E/Z-(3-{4-[1-(4-Chlorophenyl)-2-(4-methoxyphenyl)-but-1-enyl]-phenoxy}-propyl)-dimethyl-amine (**2B**).* $C_{28}H_{32}ClNO_2$. Yield: 43%. Yellow oil. Purity: 98%. $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ 7.31 (t, $J$ = 2.2 Hz, 1H), 7.28 (m, 1H), 7.15 (t, $J$ = 2.2 Hz, 1H), 7.13 (t, $J$ = 2.2 Hz, 1H), 7.11 (t, $J$ = 2.4 Hz, 1H), 7.09 (q, $J$ = 1.9 Hz, 1H), 7.03 (d, $J$ = 6.0 Hz, 1H), 7.00 (m, 3H), 6.98 (t, $J$ = 1.9 Hz, 2H), 6.95 (m, 1H), 6.87 (m, 1H), 6.85 (m, 1H), 6.80 (m, 1H), 6.77 (t, $J$ = 2.3 Hz, 1H), 6.75 (d, $J$ = 2.1 Hz, 1H), 6.73 (m, 3H), 6.69 (m, 1H), 6.55 (m, 1H), 6.53 (m, 1H), 4.04 (t, $J$ = 5.9 Hz, 2H), 3.94 (t, $J$ = 5.9 Hz, 2H), 3.77 (s, 6H), 2.74 (m, 4H), 2.45 (m, 16H), 2.07 (m, 4H), 0.91 (tt, $J$ = 9.3, 4.6 Hz, 6H). $^{13}C$ NMR (101 MHz, $CDCl_3$): $\delta$ 157.98, 157.88, 157.47, 156.58, 142.41, 142.18, 142.05, 141.49, 136.59, 136.47, 135.96, 135.53, 134.17, 134.05, 132.12, 131.90, 131.21, 130.83, 130.64, 130.61, 128.28, 127.53, 114.08, 113.40, 113.38, 113.34, 65.54, 65.26, 56.31, 56.26, 55.08, 44.69, 44.58, 28.97, 28.89, 26.52, 26.35, 13.59, 13.56. MS (ESI): $m/z$ 450.21 $[M]^+$, $m/z$ 452.21 $[M + 2]^+$. $R_f$: 0.53 (85:15 methylene chloride/methanol).

*3.1.1.16. E/Z-(2-{4-[1-(4-Chlorophenyl)-2-(4-methoxyphenyl)-but-1-enyl]-phenoxy}-ethyl)-diethylamine (**2C**).* $C_{29}H_{34}ClNO_2$.Yield: 42%. Yellow oil. Purity: 100%. $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ 7.30 (d, $J$ = 8.2 Hz, 2H), 7.13 (dd, $J$ = 16.5, 8.2 Hz, 4H), 7.02 (d, $J$ = 4.0 Hz, 1H), 7.00 (m, 6H), 6.87 (d, $J$ = 8.5 Hz, 2H), 6.79 (m, 3H), 6.73 (d, $J$ = 5.3 Hz, 3H), 6.69 (d, $J$ = 10.7 Hz, 1H), 6.55 (t, $J$ = 8.6 Hz, 2H), 4.13 (t, $J$ = 6.0 Hz, 2H), 3.99 (t, $J$ = 6.0 Hz, 2H), 3.76 (s, 6H), 2.99 (t, $J$ = 6.0 Hz, 2H), 2.91 (t, $J$ = 5.9 Hz, 2H), 2.73 (dt, $J$ = 16.6, 5.9 Hz, 8H), 2.43 (dt, $J$ = 14.8, 7.4 Hz, 4H), 1.12 (dt, $J$ = 13.8, 6.7 Hz, 12H), 0.92 (dd, $J$ = 12.9, 7.2 Hz, 6H). $^{13}C$ NMR (101 MHz, $CDCl_3$): $\delta$ 157.98, 157.90, 157.39, 156.50, 142.43, 142.17, 142.06, 141.47, 136.62, 136.49, 135.95, 135.53, 134.15, 134.08, 132.24, 132.14, 131.89, 131.21, 130.85, 130.65, 130.60, 128.28, 127.54, 114.14, 113.48, 113.38, 113.35, 65.87, 65.57, 55.08, 51.63, 51.48, 47.78, 47.75, 28.98, 28.87, 13.61, 13.57, 11.34, 11.24. MS (ESI): $m/z$ 464.23 $[M]^+$, $m/z$ 466.23 $[M + 2]^+$. $R_f$: 0.4 (9:1 methylene chloride/methanol).

*3.1.1.17. E/Z-1-(2-{4-[1-(4-Chlorophenyl)-2-(4-methoxyphenyl)-but-1-enyl]-phenoxy}-ethyl)-pyrrolidine (**2D**).* $C_{29}H_{32}ClNO_2$. Yield: 44%. Yellow oil. Purity: 100%. $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ 7.31 (t, $J$ = 2.2 Hz, 1H), 7.29 (m, 1H), 7.16 (d, $J$ = 2.3 Hz, 1H), 7.13 (t, $J$ = 1.8 Hz, 1H), 7.12 (d, $J$ = 2.1 Hz, 1H), 7.10 (d, $J$ = 2.1 Hz, 1H), 7.02 (t, $J$ = 2.5 Hz, 1H), 7.00 (s, 2H), 6.98 (d, $J$ = 1.0 Hz, 2H), 6.96 (m, 1H), 6.89 (m, 1H), 6.87 (d, $J$ = 2.0 Hz, 1H), 6.80 (m, 2H), 6.78 (m, 1H), 6.76 (m, 1H), 6.73 (t, $J$ = 2.0 Hz, 1H), 6.72 (t, $J$ = 2.1 Hz, 2H), 6.69 (d, $J$ = 3.7 Hz, 1H), 6.58 (m, 1H), 6.57 (m, 1H), 4.21 (t, $J$ = 5.6 Hz, 2H), 4.08 (t, $J$ = 5.6 Hz, 2H), 3.76 (s, 6H), 3.09 (t, $J$ = 5.6 Hz, 2H), 3.02 (t, $J$ = 5.6 Hz, 2H), 2.85 (m, 8H), 2.42 (dt, $J$ = 13.8, 6.9 Hz, 4H), 1.90 (dt, $J$ = 8.2, 3.3 Hz, 8H), 0.91 (dt, $J$ = 6.7, 3.4 Hz, 6H). $^{13}C$ NMR (101 MHz, $CDCl_3$): $\delta$ 157.99, 157.91, 157.18, 156.29, 142.40, 142.23, 142.03, 141.55, 136.56, 136.43, 136.15, 135.73, 134.11, 134.05, 132.24, 132.13, 131.92, 131.22, 130.85, 130.64, 130.63, 128.28, 127.54, 114.20, 113.53, 113.38, 113.36, 65.99, 65.70, 55.08, 54.84, 54.77, 54.70, 54.67, 28.97, 28.88, 23.38, 23.33, 13.59, 13.57. MS (ESI): $m/z$ 462.21 $[M]^+$, $m/z$ 464.21 $[M + 2]^+$. $R_f$: 0.34 (9:1 methylene chloride/methanol).

*3.1.1.18. E/Z-(1-(2-{4-[1-(4-Chlorophenyl)-2-(4-methoxyphenyl)-but-1-enyl]-phenoxy}-ethyl)-piperidine (**2E**).* $C_{30}H_{34}ClNO_2$. Yield: 46%. Yellow oil. Purity: 100%. $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ 7.30 (m, 1H), 7.29 (d, $J$ = 1.9 Hz, 1H), 7.16 (d, $J$ = 2.3 Hz, 1H), 7.14 (d, $J$ = 1.8 Hz, 1H), 7.11 (d, $J$ = 1.7 Hz, 1H), 7.10 (d, $J$ = 1.9 Hz, 1H), 7.02 (d, $J$ = 1.6 Hz, 1H), 7.00 (m, 2H), 6.98 (d, $J$ = 1.2 Hz, 2H), 6.97 (d, $J$ = 1.9 Hz, 1H), 6.88 (d, $J$ = 2.0 Hz, 1H), 6.86 (d, $J$ = 1.9 Hz, 1H), 6.80 (d, $J$ = 2.0 Hz, 1H), 6.78 (m, 1H), 6.75 (m, 1H), 6.73 (m, 3H), 6.70 (d, $J$ = 1.2 Hz, 2H), 6.58 (m, 1H), 6.54 (q, $J$ = 1.8 Hz, 1H), 4.15 (t, $J$ = 5.8 Hz, 2H), 4.02 (t, $J$ = 5.8 Hz, 2H), 3.77 (s, 6H), 2.85 (t, $J$ = 5.8 Hz, 2H), 2.77 (t, $J$ = 5.9 Hz, 2H), 2.58 (m, 8H), 2.45 (m, 4H), 1.64 (m, 12H), 0.91 (dd, $J$ = 7.3, 6.0 Hz, 6H). $^{13}C$ NMR (101 MHz, $CDCl_3$): $\delta$ 157.97, 157.89, 157.46, 156.60, 142.44, 142.14, 142.07, 141.43, 136.64, 136.50, 135.89, 135.45, 134.16, 134.08, 132.23, 132.13, 131.86, 131.21, 130.85, 130.65, 130.63, 130.57, 128.28, 127.53, 114.19, 113.54, 113.38, 113.34, 65.59, 65.36, 57.84, 57.78, 55.08, 54.98, 54.94, 28.97, 28.88, 25.63, 25.55, 23.95, 23.89, 13.60, 13.57. MS (ESI): $m/z$ 476.23 $[M]^+$, $m/z$ 478.23 $[M + 2]^+$. $R_f$: 0.3 (92:8 methylene chloride/methanol).

*3.1.1.19. E/Z-4-(2-{4-[1-(4-Chlorophenyl)-2-(4-methoxyphenyl)-but-1-enyl]-phenoxy}-ethyl)-morpholine (**2F**).* $C_{29}H_{32}ClNO_3$. Yield: 48%. Yellow oil. Purity: 95%. $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ 7.31 (d, $J$ = 1.7 Hz, 1H), 7.29 (m, 1H), 7.16 (d, $J$ = 2.1 Hz, 1H), 7.14 (m, 1H), 7.12 (d, $J$ = 2.1 Hz, 1H), 7.10 (d, $J$ = 2.1 Hz, 1H), 7.03 (m, 1H), 7.00 (q, $J$ = 2.4 Hz, 3H), 6.98 (d, $J$ = 1.7 Hz, 2H), 6.98 (m, 2H), 6.88 (d, $J$ = 2.2 Hz, 1H), 6.87 (d, $J$ = 2.1 Hz, 1H), 6.80 (d, $J$ = 2.1 Hz, 1H), 6.78 (d, $J$ = 2.0 Hz, 1H), 6.75 (d, $J$ = 2.1 Hz, 1H), 6.74 (d, $J$ = 2.2 Hz, 1H), 6.72 (s, 1H), 6.70 (d, $J$ = 0.5 Hz, 1H), 6.58 (m, 2H), 4.13 (t, $J$ = 5.6 Hz, 2H), 4.00 (t, $J$ = 5.7 Hz, 2H), 3.76 (m, 14H), 2.83 (t, $J$ = 5.7 Hz, 2H), 2.73 (t, $J$ = 6.0 Hz, 2H), 2.61 (m, 4H), 2.55 (m, 4H), 2.44 (m, 4H), 0.92 (m, 6H). $^{13}C$ NMR (101 MHz, $CDCl_3$): $\delta$ 157.99, 157.89, 157.46, 156.61, 142.43, 142.17, 142.06, 141.47, 136.60, 136.48, 135.96, 135.50, 134.17, 134.05, 132.24, 132.12, 131.87, 131.22, 130.84, 130.64, 130.59, 128.28, 127.54, 114.19, 113.54, 113.38, 113.33, 66.86, 66.81, 65.66, 65.46, 57.67, 57.64, 55.08, 54.06, 54.03, 28.97, 28.89, 13.60, 13.57. MS (ESI): $m/z$ 478.21 $[M]^+$, $m/z$ 480.21 $[M + 2]^+$. $R_f$: 0.47 (95:5 methylene chloride/methanol).

*3.1.1.20. E/Z-(3-{4-[1-(4-Chlorophenyl)-2-phenyl-but-1-enyl]-phenoxy}-propyl)-dimethyl-amine (**3B**).* $C_{27}H_{30}ClNO$. Yield: 46%. Faint yellow oil. Purity: 96%. $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$: 7.32 (d, $J$ = 2.1 Hz, 1H), 7.30 (d, $J$ = 1.9 Hz, 1H), 7.13 (m, 14H), 6.97 (d, $J$ = 2.1 Hz, 1H), 6.95 (d, $J$ = 2.0 Hz, 1H), 6.88 (d, $J$ = 2.2 Hz, 1H), 6.87 (d, $J$ = 2.0 Hz, 1H), 6.79 (d, $J$ = 2.0 Hz, 1H), 6.78 (d, $J$ = 1.9 Hz, 1H), 6.74 (d, $J$ = 2.2 Hz, 1H), 6.72 (d, $J$ = 2.1 Hz, 1H), 6.54 (d, $J$ = 2.2 Hz, 1H),


https://doi.org/10.1021/acsomega.3c01682
ACS Omega 2023, 8, 25903−25923

6.53 (d, $J$ = 2.1 Hz, 1H), 4.03 (t, $J$ = 6.3, Hz, 2H), 3.88 (t, $J$ = 7.5, 5.2 Hz, 2H), 2.50 (m, 8H), 2.33 (s, 6H), 2.29 (s, 6H), 2.03 (m, 2H), 1.94 (m, 2H), 0.92 (m, 6H).$^{13}$C NMR (101 MHz, $CDCl_3$): $\delta$:157.77, 156.93, 142.61, 142.23, 142.09, 142.00, 141.89, 141.80, 137.16, 137.04, 135.50, 135.00, 132.34, 132.08, 131.84, 131.37, 130.83, 130.55, 129.58, 128.29, 127.94, 127.89, 127.48, 126.25, 126.14, 114.12, 113.84, 113.39, 65.95, 65.69, 56.37, 56.32, 45.20, 45.11, 29.08, 28.99, 27.23, 27.08, 13.52, 13.49. MS (ESI): $m/z$ 420.3 $[M]^+$, $m/z$ 422.4 $[M + 2]^+$. $R_f$: 0.48 (9:1 methylene chloride/methanol).

*3.1.1.21. E/Z-1-(2-{4-[1-(4-Chlorophenyl)-2-phenyl-but-1-enyl]-phenoxy}-ethyl)-pyrrolidine (**3D**).* $C_{28}H_{30}ClNO$. Yield: 43%. Yellow oil. Purity: 95%. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$: 7.32 (d, $J$ = 2.0 Hz, 1H), 7.31 (d, $J$ = 2.0 Hz, 1H), 7.13 (m, 14H), 6.96 (d, $J$ = 2.2 Hz, 1H), 6.95 (d, $J$ = 1.8 Hz, 1H), 6.90 (d, $J$ = 2.1 Hz, 1H), 6.89 (d, $J$ = 2.1 Hz, 1H), 6.79 (d, $J$ = 2.1 Hz, 1H), 6.78 (d, $J$ = 2.0 Hz, 1H), 6.74 (d, $J$ = 2.0 Hz, 1H), 6.72 (d, 1H), 6.56 (d, $J$ = 2.2 Hz, 1H), 6.55 (d, $J$ = 2.1 Hz, 1H), 4.17 (t, $J$ = 5.9 Hz, 2H), 4.02 (t, $J$ = 5.9 Hz, 2H), 2.99 (t, $J$ = 5.9 Hz, 2H), 2.89 (t, $J$ = 5.9 Hz, 2H), 2.73 (t, $J$ = 5.9 Hz, 4H), 2.67 (t, $J$ = 6 Hz, 4H), 2.49 (q, $J$ = 12.8 Hz, 2H), 2.44 (q, $J$ = 12.8, 5.4 Hz, 2H), 1.86 (m, 4H), 1.80 (m, 4H), 0.94 (d, $J$ = 7.4 Hz, 3H), 0.91 (d, $J$ = 7.4 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$): $\delta$: 157.52, 156.69, 142.64, 142.20, 142.03, 141.98, 141.94, 141.79, 137.09, 136.98, 135.66, 135.17, 132.34, 132.11, 131.86, 131.37, 130.93, 130.85, 130.58, 129.58, 129.57, 128.30, 127.96, 127.92, 127.50, 126.27, 126.18, 114.18, 113.45, 66.56, 66.29, 55.00, 54.92, 54.70, 54.66, 29.10, 29.00, 23.44, 23.40, 13.56, 13.53. MS (ESI): $m/z$ 432.20 $[M]^+$, $m/z$ 434.20 $[M + 2]^+$. $R_f$: 0.32 (93:7 methylene chloride/methanol).

*3.1.1.22. E/Z-1-(2-{4-[1-(4-Chlorophenyl)-2-phenyl-but-1-enyl]-phenoxy}-ethyl)-piperidine (**3E**).* $C_{29}H_{32}ClNO$. Yield: 46%. Faint yellow oil. Purity: 95%. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$: 7.32 (d, $J$ = 2.1 Hz, 1H), 7.30 (d, $J$ = 1.9 Hz, 1H), 7.12 (m, 14H), 6.96 (d, $J$ = 2.1 Hz, 1H), 6.95 (d, $J$ = 2.0 Hz, 1H), 6.89 (d, $J$ = 2.2 Hz, 1H), 6.87 (d, $J$ = 2.1 Hz, 1H), 6.79 (d, $J$ = 2.1 Hz, 1H), 6.77 (d, $J$ = 2.0 Hz, 1H), 6.74 (d, $J$ = 2.2 Hz, 1H), 6.72 (d, $J$ = 2.1 Hz, 1H), 6.55 (d, $J$ = 2.2 Hz, 1H), 6.53 (d, $J$ = 2.1 Hz, 1H), 4.13 (t, $J$ = 6.0 Hz, 2H), 3.98 (t, $J$ = 6.1 Hz, 2H), 2.81 (t, $J$ = 6.0 Hz, 2H), 2.70 (t, $J$ = 6.1 Hz, 2H), 2.47 (m, 12H), 1.61 (m, $J$ = 17.2, 11.2, 5.6 Hz, 8H), 1.46 (m, 4H), 0.92 (q, $J$ = 7.4 Hz, 6H). $^{13}$C NMR (101 MHz, $CDCl_3$): $\delta$: 142.00, 132.08, 131.81, 131.36, 130.82, 130.54, 129.57, 128.28, 127.93, 127.89, 127.47, 126.24, 126.14, 114.19, 113.48, 65.74, 57.91, 57.84, 55.00, 54.96, 50.78, 30.89, 29.07, 28.98, 25.78, 25.72, 24.08, 13.51, 13.48. MS (ESI): $m/z$ 446.30 $[M]^+$, $m/z$ 448.30 $[M + 2]^+$. $R_f$: 0.41 (93:7 methylene chloride/methanol).

*3.1.1.23. E/Z-4-(2-{4-[1-(4-Chlorophenyl)-2-phenyl-but-1-enyl]-phenoxy}-ethyl)-morpholine (**3F**).* $C_{28}H_{30}ClNO_2$. Yield: 44%. Faint yellow oil. Purity: 95%. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$: 7.32 (d, $J$ = 2.0 Hz, 1H), 7.30 (s, 1H), 7.19−7.06 (m, 14H), 6.96 (d, $J$ = 2.0 Hz, 1H), 6.95 (d, $J$ = 2.1 Hz, 1H), 6.89 (d, $J$ = 2.1 Hz, 1H), 6.87 (d, $J$ = 2.1 Hz, 1H), 6.79 (d, $J$ = 2.1 Hz, 1H), 6.77 (d, $J$ = 2.1 Hz, 1H), 6.74 (d, $J$ = 2.1 Hz, 1H), 6.72 (d, $J$ = 2.2 Hz, 1H), 6.55 (d, $J$ = 2.2 Hz, 1H), 6.53 (d, $J$ = 2.2 Hz, 1H), 4.12 (t, $J$ = 5.7 Hz, 2H), 3.97 (t, $J$ = 5.7 Hz, 2H), 3.74 (d, $J$ = 4.8 Hz, 4H), 3.69 (d, $J$ = 4.7 Hz, 4H), 2.81 (t, $J$ = 4.1 Hz, 2H), 2.72 (t, 2H), 2.59 (t, 4H), 2.52 (t, 4H), 2.47−2.40 (m, 4H), 0.95−0.88 (m, 6H). $^{13}$C NMR (101 MHz, $CDCl_3$): $\delta$: 157.56, 156.75, 142.67, 142.06, 141.98, 141.95, 141.77, 137.10, 135.67, 132.07, 131.83, 131.38, 131.17, 130.82, 130.57, 129.56, 128.30, 127.95, 127.89, 127.49, 126.27, 126.15, 114.20, 113.48, 66.96, 66.92, 66.87, 66.81, 65.72, 65.50, 57.68, 57.63, 54.08, 54.05, 53.54, 53.32, 29.07, 28.99, 13.51, 13.49. MS (ESI): $m/z$ 448.20 $[M]^+$, $m/z$ 450.20 $[M + 2]^+$. $R_f$: 0.57 (95:5 methylene chloride/methanol).

*3.1.1.24. E/Z-1-(2-{4-[1-(4-Chlorophenyl)-2-phenyl-but-1-enyl]-phenoxy}-ethyl)-azepane (**3G**).* $C_{30}H_{34}ClNO$. Yield: 52%. Orange oil. Purity: 96%. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$: 7.31 (d, $J$ = 2.0 Hz, 1H), 7.30 (d, $J$ = 2.0 Hz, 1H), 7.12 (m, 14H), 6.96 (d, $J$ = 2.0 Hz, 1H), 6.94 (d, $J$ = 2.1 Hz, 1H), 6.89 (d, $J$ = 2.1 Hz, 1H), 6.87 (d, $J$ = 2.1 Hz, 1H), 6.79 (d, $J$ = 2.0 Hz, 1H), 6.77 (d, $J$ = 2.0 Hz, 1H), 6.74 (d, $J$ = 2.1 Hz, 1H), 6.72 (d, $J$ = 2.2 Hz, 1H), 6.55 (d, $J$ = 2.2 Hz, 1H), 6.53 (d, $J$ = 2.1 Hz, 1H), 4.12 (t, $J$ = 6.1 Hz, 2H), 3.96 (t, $J$ = 6.1 Hz, 2H), 3.01 (t, $J$ = 6.1 Hz, 2H), 2.91 (t, $J$ = 6.1 Hz, 2H), 2.84 (t, 4H), 2.77 (t, 4H), 2.48 (m, 2H), 2.43 (m, 2H), 1.61 (m, 16H), 0.91 (q, $J$ = 7.4 Hz, 6H). $^{13}$C NMR (101 MHz, $CDCl_3$): $\delta$: 157.61, 142.63, 142.20, 142.06, 141.99, 141.93, 141.79, 137.13, 135.59, 132.33, 132.07, 131.82, 131.36, 130.82, 130.55, 129.57, 128.28, 127.93, 127.88, 127.47, 126.24, 126.14, 114.20, 113.47, 66.04, 56.39, 56.26, 55.83, 55.76, 50.76, 30.89, 29.06, 28.97, 27.40, 27.29, 26.99, 26.96, 13.51, 13.47. MS (ESI): $m/z$ 460.34 $[M]^+$, $m/z$ 462.34 $[M + 2]^+$. $R_f$: 0.34 (93:7 methylene chloride/methanol).

*3.1.1.25. E/Z-(2-{4-[1,2-Bis-(4-chlorophenyl)-propenyl]-phenoxy}-ethyl)-dimethyl-amine (**4A**).* $C_{25}H_{25}Cl_2NO$. Yield: 46%. Brown oil. Purity: 95%. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.32 (m, 1H), 7.29 (q, $J$ = 2.3 Hz, 1H), 7.17 (m, 1H), 7.14 (m, 3H), 7.11 (m, 3H), 7.09 (m, 1H), 7.05 (dt, $J$ = 6.6, 2.7 Hz, 3H), 7.02 (d, $J$ = 1.9 Hz, 2H), 6.99 (m, 1H), 6.90 (m, 1H), 6.88 (m, 1H), 6.80 (m, 1H), 6.78 (m, 1H), 6.75 (m, 1H), 6.72 (m, 1H), 6.61 (m, 1H), 6.59 (q, $J$ = 2.1 Hz, 1H), 4.14 (t, $J$ = 7.3 2H), 3.98 (t, $J$ = 7.3, 2H), 2.79 (t, $J$ = 7.3, 4.2 Hz, 2H), 2.71 (t, $J$ = 5.7 Hz, 2H), 2.44 (s, 6H), 2.38 (s, 6H), 2.13 (s, 3H), 2.09 (s, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$): $\delta$ 157.65, 157.07, 142.32, 142.26, 141.86, 141.47, 138.36, 138.32, 135.27, 134.84, 134.67, 134.05, 132.47, 132.11, 131.85, 131.79, 131.28, 131.07, 130.56, 128.32, 128.20, 128.12, 127.76, 114.18, 113.70, 65.76, 65.55, 58.21, 58.15, 45.78, 45.74, 23.30, 23.08. MS (ESI): $m/z$ 426.15 $[M]^+$, $m/z$ 428.15 $[M + 2]^+$, $m/z$ 430.15 $[M + 4]^+$. $R_f$: 0.6 (93:7 methylene chloride/methanol).

*3.1.1.26. E/Z-(3-{4-[1,2-Bis-(4-chlorophenyl)-propenyl]-phenoxy}-propyl)-dimethyl-amine (**4B**).* $C_{26}H_{27}Cl_2NO$. Yield: 47%. Brown oil. Purity: 96%. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.30 (d, $J$ = 8.3 Hz, 2H), 7.13 (d, $J$ = 6.9 Hz, 7H), 7.08 (s, 1H), 7.05 (d, $J$ = 7.1 Hz, 3H), 7.02 (d, $J$ = 1.9 Hz, 2H), 6.99 (s, 1H), 6.86 (d, $J$ = 8.5 Hz, 2H), 6.78 (d, $J$ = 8.4 Hz, 2H), 6.73 (d, $J$ = 8.5 Hz, 2H), 6.57 (d, $J$ = 8.5 Hz, 2H), 4.04 (t, $J$ = 6.2 Hz, 2H), 3.91 (t, $J$ = 6.2 Hz, 2H), 3.57 (d, $J$ = 16.8 Hz, 2H), 3.46 (d, $J$ = 16.0 Hz, 2H), 2.62 (dt, $J$ = 19.0, 7.4 Hz, 4H), 2.39 (s, 6H), 2.37 (s, 6H), 2.11 (s, 3H), 2.07 (s, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$): $\delta$ 157.73, 157.12, 142.35, 142.25, 141.86, 141.48, 138.36, 138.32, 134.79, 134.66, 134.05, 132.46, 132.11, 131.99, 131.88, 131.84, 131.27, 131.09, 130.56, 128.37, 128.32, 128.20, 128.12, 127.75, 114.10, 113.61, 65.81, 65.59, 56.29, 56.24, 44.96, 44.88, 26.91, 26.77, 23.29, 23.09. MS (ESI): $m/z$ 440.15 $[M]^+$, $m/z$ 442.15 $[M + 2]^+$, $m/z$ 444.15 $[M + 4]^+$. $R_f$: 0.42 (93:7 methylene chloride/methanol).

*3.1.1.27. E/Z-(2-{4-[1,2-Bis-(4-chlorophenyl)-propenyl]-phenoxy}-ethyl)-diethyl-amine (**4C**).* $C_{27}H_{29}Cl_2NO$. Yield: 47%. Brown oil. Purity: 95%. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.32 (m, 1H), 7.30 (m, 1H), 7.15 (m, 7H), 7.09 (m, 1H), 7.06 (m, 1H), 7.05 (t, $J$ = 2.3 Hz, 2H), 7.02 (m, 2H), 7.00 (m,


https://doi.org/10.1021/acsomega.3c01682
ACS Omega 2023, 8, 25903−25923

1H), 6.89 (m, 1H), 6.87 (m, 1H), 6.80 (m, 1H), 6.78 (m, 1H), 6.75 (m, 1H), 6.72 (m, 1H), 6.60 (q, $J$ = 2.3 Hz, 1H), 6.58 (m, 1H), 4.09 (t, $J$ = 6.3 Hz, 2H), 3.96 (t, $J$ = 6.3 Hz, 2H), 2.92 (t, $J$ = 6.3 Hz, 2H), 2.84 (t, $J$ = 6.3 Hz, 2H), 2.66 (dq, $J$ = 18.7, 7.1 Hz, 8H), 2.13 (s, 3H), 2.09 (s, 3H), 1.08 (m, 12H). $^{13}$C NMR (101 MHz, $CDCl_3$): $\delta$ 157.70, 157.11, 142.34, 142.27, 141.88, 141.49, 138.39, 138.35, 135.16, 134.74, 134.64, 134.01, 132.46, 132.11, 131.99, 131.86, 131.79, 131.28, 131.07, 130.57, 128.32, 128.20, 128.12, 127.75, 114.14, 113.67, 66.33, 66.09, 51.71, 51.57, 47.82, 47.80, 23.30, 23.07, 11.68, 11.64. MS (ESI): $m/z$ 454.16 $[M]^+$, $m/z$ 456.16 $[M + 2]^+$, $m/z$ 458.16 $[M + 4]^+$. $R_f$: 0.52 (93:7 methylene chloride/methanol).

*3.1.1.28. E/Z-1-(2-{4-[1,2-Bis-(4-chlorophenyl)-propenyl]-phenoxy}-ethyl)-pyrrolidine (**4D**).* $C_{27}H_{27}Cl_2NO$. Yield: 45%. Brown oil. Purity: 95%. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$7.32 (d, $J$ = 2.0 Hz, 1H), 7.30 (m, 1H), 7.15 (m, 1H), 7.14 (d, $J$ = 2.0 Hz, 2H), 7.12 (m, 3H), 7.09 (m, 1H), 7.06 (d, $J$ = 2.1 Hz, 2H), 7.04 (d, $J$ = 2.4 Hz, 2H), 7.02 (t, $J$ = 1.8 Hz, 2H), 6.99 (m, 1H), 6.88 (m, 2H), 6.78 (m, 2H), 6.75 (d, $J$ = 2.2 Hz, 1H), 6.73 (m, 1H), 6.60 (m, 1H), 6.58 (m, 1H), 4.20 (t, $J$ = 5.7 Hz, 2H), 4.08 (t, $J$ = 5.7 Hz, 2H), 3.03 (t, $J$ = 5.7 Hz, 2H), 2.96 (t, $J$ = 5.6 Hz, 2H), 2.78 (d, $J$ = 14.4 Hz, 8H), 2.11 (s, 3H), 2.08 (s, 3H), 1.87 (dd, $J$ = 6.8, 3.6 Hz, 8H). $^{13}$C NMR (101 MHz, $CDCl_3$): $\delta$ 157.45, 142.30, 142.23, 141.84, 141.44, 138.26, 134.11, 132.49, 132.11, 131.90, 131.28, 131.11, 130.56, 128.33, 128.21, 128.14, 127.76, 114.20, 113.72, 66.84, 66.64, 54.87, 54.81, 54.66, 54.63, 23.43, 23.39, 23.29, 23.09. MS (ESI): $m/z$ 452.15 $[M]^+$, $m/z$ 454.15 $[M + 2]^+$, $m/z$ 456.15 $[M + 4]^+$. $R_f$: 0.47 (93:7 methylene chloride/methanol).

*3.1.1.29. E/Z-1-(2-{4-[1,2-Bis-(4-chlorophenyl)-propenyl]-phenoxy}-ethyl)-piperidine (**4E**).* $C_{28}H_{29}Cl_2NO$. Yield: 46%. Brown oil. Purity: 95%. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.32 (d, $J$ = 2.1 Hz, 1H), 7.31 (t, $J$ = 2.3 Hz, 1H), 7.14 (ddd, $J$ = 8.7, 5.5, 3.2 Hz, 8H), 7.06 (s, 1H), 7.04 (dd, $J$ = 5.0, 3.1 Hz, 4H), 7.00 (t, $J$ = 3.1 Hz, 1H), 6.89 (s, 1H), 6.86 (s, 1H), 6.78 (dd, $J$ = 5.5, 3.2 Hz, 3H), 6.75 (s, 1H), 6.59 (s, 1H), 6.56 (s, 1H), 4.59 (t, $J$ = 5.8 Hz, 2H), 4.45 (t, $J$ = 5.9 Hz, 2H), 3.62 (m, 4H), 3.39 (m, 4H), 3.30 (m, 4H), 2.10 (s, 3H), 2.08 (s, 3H), 1.25 (br s, 12H). $^{13}$C NMR (101 MHz, $CDCl_3$): $\delta$ 157.51, 157.04, 142.36, 142.29, 141.81, 141.42, 138.33, 135.32, 134.90, 134.72, 134.11, 132.42, 132.10, 132.02, 131.88, 131.81, 131.23, 131.10, 130.52, 128.33, 128.27, 128.13, 127.71, 114.21, 113.64, 65.78, 65.54, 57.87, 57.82, 55.01, 54.81, 28.93, 28.81, 25.67, 25.61, 23.42, 23.09. MS (ESI): $m/z$ 466.16 $[M]^+$, $m/z$ 468.16 $[M + 2]^+$, $m/z$ 470.16 $[M + 4]^+$. $R_f$: 0.6 (93:7 methylene chloride/methanol).

*3.1.1.30. E/Z-4-(2-{4-[1,2-Bis-(4-chlorophenyl)-propenyl]-phenoxy}-ethyl)-morpholine (**4F**).* $C_{27}H_{27}Cl_2NO_2$. Yield: 43%. Yellow oil. Purity: 100%. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.32 (m, 1H), 7.30 (m, 1H), 7.16 (m, 8H), 7.06 (m, 1H), 7.04 (m, 2H), 7.02 (p, $J$ = 1.9 Hz, 2H), 7.00 (m, 1H), 6.89 (m, 1H), 6.87 (m, 1H), 6.80 (m, 1H), 6.78 (m, 1H), 6.75 (m, 1H), 6.73 (m, 1H), 6.61 (m, 1H), 6.58 (m, 1H), 4.13 (t, $J$ = 7.3 Hz, 2H), 4.03 (t, $J$ = 7.3, 2H), 3.73 (m, 8H), 2.86 (t, $J$ = 5.6 Hz, 2H), 2.78 (t, $J$ = 5.7 Hz, 2H), 2.58 (m, 8H), 2.13 (s, 3H), 2.03 (s, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$): $\delta$ 157.59, 157.02, 142.34, 142.22, 141.85, 141.46, 138.33, 138.29, 135.34, 134.90, 134.71, 134.11, 132.49, 132.10, 132.02, 131.88, 131.81, 131.27, 131.10, 130.56, 128.33, 128.21, 128.13, 127.77, 114.20, 113.73, 66.87, 66.84, 65.70, 65.52, 57.65, 57.61, 54.07, 54.04, 23.29, 23.10. MS (ESI): $m/z$ 468.14 $[M]^+$, $m/z$ 470.14 $[M + 2]^+$, $m/z$ 472.14 $[M + 4]^+$. $R_f$: 0.73 (95:5 methylene chloride/methanol).

*3.1.1.31. E/Z-(2-{4-[1,3-Bis-(4-chlorophenyl)-2-methylpropenyl]-phenoxy}-ethyl)-dimethyl-amine (**5A**).* $C_{26}H_{27}Cl_2NO$. Yield: 45%. Orange oil. Purity: 95%. $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 7.28 (m, 8H), 7.09 (m, 12H), 6.85 (m, 4H), 4.10 (dd, $J$ = 12.4, 6.0 Hz, 4H), 3.47 (d, $J$ = 18.5 Hz, 4H), 2.79 (m, 4H), 2.39 (m, 12H), 1.69 (m, 6H). $^{13}$C NMR (126 MHz, $CDCl_3$): $\delta$ 157.46, 157.35, 141.51, 141.47, 138.87, 138.78, 138.01, 134.95, 134.91, 132.95, 132.13, 131.69, 131.00, 130.77, 130.48, 129.91, 129.85, 128.52, 128.49, 128.36, 128.16, 114.29, 114.07, 65.67, 58.17, 58.16, 45.71, 40.82, 19.89, 19.81, 18.46. MS (ESI): $m/z$ 440.40 $[M]^+$, $m/z$ 442.40 $[M + 2]^+$, $m/z$ 444.40 $[M + 4]^+$. $R_f$: 0.31 (95:5 methylene chloride/methanol).

*3.1.1.32. E/Z-(3-{4-[1,3-Bis-(4-chlorophenyl)-2-methylpropenyl]-phenoxy}-propyl)-dimethyl-amine (**5B**).* $C_{27}H_{29}Cl_2NO$. Yield: 36%. Yellow oil. Purity: 96%. $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 7.27 (d, $J$ = 10.5 Hz, 10H), 7.09 (s, 10H), 6.80 (s, 4H), 4.07 (s, 4H), 3.50 (m, 2H), 3.46 (m, 2H), 3.24 (s, 4H), 2.86 (d, $J$ = 3.6 Hz, 12H), 2.39 (s, 4H), 1.68 (s, 3H), 1.65 (s, 3H). $^{13}$C NMR (126 MHz, $CDCl_3$): $\delta$ 157.51, 141.94, 138.16, 137.89, 136.78, 133.68, 133.03, 131.76, 129.54, 129.50, 129.29, 129.19, 128.80, 128.69, 128.65, 128.54, 128.45, 128.31, 115.15, 115.12, 64.55, 43.23, 40.80, 31.93, 29.70, 29.36, 24.65, 22.69, 14.12. MS (ESI): $m/z$ 454.43 $[M]^+$, $m/z$ 456.43 $[M + 2]^+$, $m/z$ 458.43 $[M + 4]^+$. $R_f$: 0.31 (9:1 methylene chloride/methanol).

*3.1.1.33. E/Z-(2-{4-[1,3-Bis-(4-chlorophenyl)-2-methylpropenyl]-phenoxy}-ethyl)-diethyl-amine (**5C**).* $C_{28}H_{31}Cl_2NO$. Yield: 39%. Yellow oil. Purity: 100%. $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 7.24 (t, $J$ = 23.3 Hz, 8H), 7.07 (m, 12H), 6.81 (m, 4H), 4.03 (m, 4H), 3.42 (dd, $J$ = 46.4, 18.6 Hz, 4H), 2.87 (d, $J$ = 41.0 Hz, 4H), 2.64 (d, $J$ = 34.2 Hz, 8H), 1.64 (d, $J$ = 46.4, 16.5 Hz, 6H), 1.07 (m, 12H). $^{13}$C NMR (126 MHz, $CDCl_3$): $\delta$ 157.67, 157.55, 141.67, 141.62, 139.02, 138.92, 138.16, 138.15, 134.96, 134.92, 133.05, 132.90, 132.44, 132.26, 131.85, 131.82, 131.14, 130.90, 130.61, 130.04, 129.98, 128.65, 128.62, 128.54, 128.49, 128.28, 114.46, 114.38, 114.16, 66.38, 51.83, 51.82, 47.93, 47.92, 40.96, 29.84, 20.03, 19.94, 11.78. MS (ESI): $m/z$ 468.46 $[M]^+$, $m/z$ 470.46 $[M + 2]^+$, $m/z$ 472.46 $[M + 4]^+$. $R_f$: 0.35 (95:5 methylene chloride/methanol).

*3.1.1.34. E/Z-1-(2-{4-[1,3-Bis-(4-chlorophenyl)-2-methylpropenyl]-phenoxy}-ethyl)-pyrrolidine (**5D**).* $C_{28}H_{29}Cl_2NO$. Yield: 37%. Yellow oil. Purity: 100%. $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 7.28 (dd, $J$ = 3.0, 1.8 Hz, 4H), 7.26 (s, 2H), 7.26 (s, 2H), 7.07 (m, 12H), 6.86 (ddd, $J$ = 9.3, 4.4, 2.4 Hz, 4H), 4.15 (dd, $J$ = 12.6, 6.1 Hz, 4H), 3.49 (d, $J$ = 7.6 Hz, 4H), 2.98 (d, q, $J$ = 5.7 Hz, 4H), 2.73 (s, 8H), 1.69 (d, $J$ = 16.4 Hz, 6H), 1.27 (m, 8H). $^{13}$C NMR (126 MHz, $CDCl_3$): $\delta$ 157.43, 157.31, 141.51, 141.47, 138.87, 138.78, 138.01, 134.96, 134.91, 132.95, 132.81, 132.31, 132.13, 131.72, 131.68, 131.00, 130.78, 130.49, 129.91, 129.85, 128.49, 128.36, 128.15, 114.31, 114.09, 66.56, 58.44, 54.95, 54.67, 50.84, 40.82, 30.93, 29.70, 23.45, 22.70, 19.89, 19.81, 18.45, 14.13. MS (ESI): $m/z$ 466.30 $[M]^+$, $m/z$ 468.30 $[M + 2]^+$, $m/z$ 470.30 $[M + 4]^+$. $R_f$: 0.37 (92:8 methylene chloride/methanol).

*3.1.1.35. E/Z-1-(2-{4-[1,3-Bis-(4-chlorophenyl)-2-methylpropenyl]-phenoxy}-ethyl)-piperidine (**5E**).* $C_{29}H_{31}Cl_2NO$. Yield: 39%. Yellow oil. Purity: 99%. $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 7.27 (d, $J$ = 8.5 Hz, 10H), 7.10 (m, 10H), 6.84 (dd, $J$ = 8.4, 3.5 Hz, 4H), 4.20 (s, 4H), 3.48 (t, $J$ = 14.6 Hz, 4H), 2.86 (d, $J$ = 45.2 Hz, 4H), 2.67 (s, 8H), 1.69 (m, 12H), 1.51 (s, 3H), 1.31 (s, 3H). $^{13}$C NMR (126 MHz, $CDCl_3$): $\delta$ 157.28, 141.49, 141.45, 138.86, 138.77, 137.98, 135.09, 133.01, 132.89,


https://doi.org/10.1021/acsomega.3c01682
ACS Omega 2023, 8, 25903−25923

132.34, 132.16, 131.75, 131.71, 131.01, 130.82, 130.77, 130.53, 129.91, 129.86, 128.54, 128.51, 128.38, 128.18, 114.32, 114.10, 65.34, 57.61, 54.84, 40.82, 29.71, 25.35, 25.32, 23.72, 23.70, 19.89, 19.81. MS (ESI): $m/z$ 480.47 $[M]^+$, $m/z$ 482.47 $[M + 2]^+$, $m/z$ 484.47 $[M + 4]^+$. $R_f$: 0.46 (97:3 methylene chloride/methanol).

*3.1.1.36. E/Z-4-(2-{4-[1,3-Bis-(4-chlorophenyl)-2-methylpropenyl]-phenoxy}-ethyl)-morpholine (**5F**).* $C_{28}H_{29}Cl_2NO_2$. Yield: 71%. Yellow oil. Purity: 96%. $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 7.27 (d, $J$ = 7.6 Hz, 10H), 7.10 (m, 12H), 6.85 (m, 2H), 4.12 (dd, $J$ = 11.8, 5.8 Hz, 4H), 3.76 (m, 8H), 3.47 (m, 4H), 2.83 (dd, $J$ = 10.2, 4.9 Hz, 4H), 2.62 (s, 8H), 1.72 (s, 3H), 1.68 (s, 3H). $^{13}$C NMR (126 MHz, $CDCl_3$): $\delta$ 157.53, 157.42, 141.62, 141.57, 138.97, 138.89, 138.11, 138.08, 135.14, 135.11, 133.12, 132.99, 132.28, 131.86, 131.83, 131.12, 130.92, 130.68, 130.02, 129.98, 128.65, 128.50, 128.30, 116.24, 114.43, 114.22, 66.93, 65.73, 57.74, 54.17, 54.15, 53.47, 40.95, 29.83, 20.02, 19.93. MS (ESI): $m/z$ 482.44 $[M]^+$, $m/z$ 484.44 $[M + 2]^+$, $m/z$ 486.44 $[M + 4]^+$. $R_f$: 0.64 (95:5 methylene chloride/methanol).

*3.1.1.37. E/Z-1-(2-{4-[1,3-Bis-(4-chlorophenyl)-2-methylpropenyl]-phenoxy}-ethyl)-azepane (**5G**).* $C_{30}H_{33}Cl_2NO$. Yield: 45%. Brown oil. Purity: 97%. $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 7.28 (dd, $J$ = 3.8, 1.9 Hz, 4H), 7.26 (m, 4H), 7.13 (d, $J$ = 1.9 Hz, 1H), 7.10 (m, 10H), 7.05 (d, $J$ = 2.8 Hz, 1H), 6.85 (m, 4H), 4.11 (dd, $J$ = 12.9, 6.4 Hz, 4H), 3.47 (m, 4H), 3.01 (m, 4H), 2.85 (dd, $J$ = 10.6, 5.3 Hz, 8H), 1.69 (d, $J$ = 17.2 Hz, 12H), 1.63 (dd, $J$ = 7.5, 4.1 Hz, 8H), 1.26 (t, $J$ = 7.0 Hz, 2H). $^{13}$C NMR (126 MHz, CDCl3): $\delta$ 157.52, 157.40, 141.53, 141.48, 138.88, 138.79, 138.03, 138.01, 134.88, 134.84, 132.93, 132.79, 132.44, 132.31, 132.13, 131.72, 131.69, 131.21, 131.00, 130.92, 130.77, 130.76, 130.48, 129.91, 129.85, 128.52, 128.49, 128.36, 128.16, 127.99, 114.32, 114.10, 113.97, 66.07, 56.37, 55.78, 40.82, 30.93, 29.71, 27.46, 27.03, 22.57, 19.90, 19.81, 18.46. MS (ESI): $m/z$ 494.50 $[M]^+$, $m/z$ 496.50 $[M + 2]^+$, $m/z$ 498.50 $[M + 4]^+$. $R_f$: 0.35 (95:5 methylene chloride/methanol).

*3.1.1.38. E/Z-(3-{4-[1-(4-Chlorophenyl)-3-(4-methoxyphenyl)-2-methylpropenyl]-phenoxy}-propyl)-dimethylamine (**6B**).* $C_{28}H_{32}ClNO_2$. Yield: 47%. Yellow oil. Purity: 100%. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.26 (s, 1H), 7.25 (s, 1H), 7.23 (s, 1H), 7.22 (s, 1H), 7.10−7.03 (m, 14H), 6.84−6.79 (m, 6H), 4.00 (q, $J$ = 6.0 Hz, 4H), 3.79 (s, 6H), 3.44−3.39 (m, 4H), 2.67−2.61 (m, 4H), 2.39 (s, 12H), 2.08−1.99 (m, 4H), 1.68 (s, 3H), 1.65 (s, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$): $\delta$: 157.84, 157.40, 141.75, 137.19, 135.11, 133.88, 132.36, 131.92, 131.06, 130.84, 130.83, 130.57, 129.46, 129.41, 128.23, 128.07, 114.09, 113.90, 113.80, 113.77, 65.69, 56.27, 55.23, 44.85, 40.51, 26.77, 19.87, 19.78. MS (ESI): $m/z$ 450.26 $[M]^+$, $m/z$ 452.26 $[M + 2]^+$. $R_f$: 0.38 (9:1 methylene chloride/methanol).

*3.1.1.39. E/Z-1-(2-{4-[1-(4-Chlorophenyl)-3-(4-methoxyphenyl)-2-methylpropenyl]-phenoxy}-ethyl)-pyrrolidine (**6D**).* $C_{29}H_{32}ClNO_2$. Yield: 43%. Dark yellow oil. Purity: 99%. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.25 (m, 6H), 7.08 (m, 12H), 6.83 (m, 6H), 4.16 (q, $J$ = 5.5 Hz, 4H), 3.79 (s, 6H), 3.42 (d, $J$ = 13.4 Hz, 4H), 3.02 (m, 4H), 2.81 (s, 8H), 1.88 (m, 8H), 1.69 (s, 3H), 1.66 (s, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$): $\delta$: 157.84, 157.06, 141.71, 137.15, 135.34, 133.94, 133.83, 132.35, 132.26, 131.94, 131.07, 130.85, 130.61, 129.46, 129.41, 128.24, 128.08, 114.19, 114.00, 113.80, 113.77, 66.04, 55.23, 54.77, 54.62, 54.59, 40.51, 23.37, 19.88, 19.79. MS (ESI): $m/z$ 462.21 $[M]^+$, $m/z$ 464.21 $[M + 2]^+$. $R_f$: 0.43 (9:1 methylene chloride/methanol).

*3.1.1.40. E/Z-1-(2-{4-[1-(4-Chlorophenyl)-3-(4-methoxyphenyl)-2-methylpropenyl]-phenoxy}-ethyl)-piperidine (**6E**).* $C_{30}H_{34}ClNO_2$.Yield: 53%. Brown oil. Purity: 99%. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.69 (m, 3H), 7.52 (m, 3H), 7.25 (m, 4H), 7.09 (m, 8H), 6.82 (m, 6H), 4.18 (m, 7H), 3.79 (s, 3H), 3.42 (d, $J$ = 12.6 Hz, 2H), 2.91 (d, $J$ = 14.1 Hz, 2H), 2.69 (d, 4H), 2.28 (m, 4H), 1.68 (m, 10H), 1.42 (m, 12H). $^{13}$C NMR (101 MHz, $CDCl_3$): $\delta$: 167.75, 132.41, 131.06, 130.87, 130.62, 129.45, 129.40, 128.78, 128.08, 114.18, 113.77, 68.13, 55.23, 54.62, 38.70, 30.33, 28.91, 23.72, 22.98, 19.78, 14.05, 10.95. MS (ESI): $m/z$ 476.23 $[M]^+$, $m/z$ 478.23 $[M + 2]^+$. $R_f$: 0.37 (93:7 methylene chloride/methanol).

*3.1.1.41. E/Z-4-(2-{4-[1-(4-Chlorophenyl)-3-(4-methoxyphenyl)-2-methylpropenyl]-phenoxy}-ethyl)-morpholine (**6F**).* $C_{29}H_{32}ClNO_3$. Yield: 47%. Yellow oil. Purity: 100%. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.24 (m, 4H), 7.08 (m, 12H), 6.83 (m, 8H), 4.09 (t, $J$ = 5.7 Hz, 4H), 3.79 (s, 6H), 3.73 (m, 8H), 3.42 (d, $J$ = 14.1 Hz, 4H), 2.80 (t, $J$ = 7.7, 3.5 Hz, 4H), 2.61−2.57 (m, 8H), 1.69 (s, 3H), 1.66 (s, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$): $\delta$: 157.86, 157.27, 141.73, 137.18, 135.22, 133.91, 132.35, 132.26, 131.95, 131.05, 130.83, 130.57, 129.45, 129.41, 128.25, 128.08, 114.19, 114.01, 113.80, 113.78, 66.82, 65.57, 57.60, 55.23, 54.03, 54.00, 40.52, 19.87, 19.78. MS (ESI): $m/z$ 478.21 $[M]^+$, $m/z$ 480.21 $[M + 2]^+$. $R_f$: 0.64 (95:5 methylene chloride/methanol).

*3.1.1.42. E/Z-1-(2-{4-[1-(4-Chlorophenyl)-3-(4-methoxyphenyl)-2-methylpropenyl]-phenoxy}-ethyl)-azepane (**6G**).* $C_{31}H_{36}ClNO_2$. Yield: 15%. Brown oil. Purity: 95%. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.26 (d, $J$ = 1.9 Hz, 1H), 7.23 (d, $J$ = 3.0 Hz, 1H), 7.08− (m, 14H), 6.80 (m, 8H), 4.26 (t, $J$ = 5.2 Hz, 4H), 3.79 (s, 6H), 3.41 (d, $J$ = 9.8 Hz, 4H), 3.28 (t, $J$ = 5.1 Hz, 4H), 3.15 (m, 8H), 1.84 (s, 8H), 1.66 (m, 14H). $^{13}$C NMR (101 MHz, $CDCl_3$): $\delta$: 157.87, 156.44, 141.59, 137.01, 135.86, 134.14, 132.25, 132.00, 131.04, 130.96, 130.82, 130.72, 129.44, 129.40, 128.27, 128.11, 114.21, 114.02, 113.80, 77.34, 77.02, 76.70, 64.22, 56.34, 55.60, 55.57, 55.24, 40.50, 26.75, 25.13, 19.85, 19.78. MS (ESI): $m/z$ 490.24 $[M]^+$, $m/z$ 492.24 $[M + 2]^+$. $R_f$: 0.32 (93:7 methylene chloride/methanol).

**3.2. Biology.** *3.2.1. Yeast ER Assay.*[21] The yeast ER assay was supplied by Dr. J.P. Sumpter (Brunel University, Uxbridge, UK) and was used to determine the relative transactivation activity of human ER$\alpha$ as formerly described (Routledge & Sumpter, 1996).

Briefly, *Saccharomyces cerevisiae* stably transfected with a human ER$\alpha$ and an estrogen-responsive element fused to the reporter gene lacZ encoding for $\beta$-galactosidase was treated with the test substances for about 48 h. The $\beta$-galactosidase enzymatic activity was measured in a colorimetric assay using a microplate photometer by hydrolysis of the substrate chlorophenol red $\beta$-D-galactopyranoside (Roche Diagnostics, Mannheim Germany), which leads to the formation of chlorophenol red. This can be measured as an increased absorption at 540 nm. All compounds were diluted in DMSO. 17$\beta$-estradiol (E2) (Sigma, Diessenhofen, Germany) 10 nM was used as a positive control, and DMSO was used as a vehicle control. All compounds, including TAM (Biotrend, Cologne, Germany) and 4-hydroxytamoxifen (4-OHTAM) (Sigma, Diessenhofen, Germany), were screened for agonistic and antiestrogenic activity in a concentration of 1 $\mu$M; antiestrogenic assays were performed in combination with 0.5


https://doi.org/10.1021/acsomega.3c01682
ACS Omega 2023, 8, 25903−25923

nM/1 nM E2 depending on the $EC_{50}$ value in each experimental series.

All compounds were tested in technical quadruplicates and biological triplicates. Statistical analysis was performed by analysis of variance (ANOVA) and Tukey's post hoc test with the significance level of $p < 0.05$.

*3.2.2. NCI Anticancer Screening.*[33] All compounds were subjected to the NCI in vitro disease-oriented human cell screening panel assay. The human tumor cell lines of the cancer-screening panel are grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells are inoculated into 96-well microtiter plates in 100 $\mu$L at plating densities ranging from 5000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates are incubated at 37 °C, 5% $CO_2$, 95% air, and 100% relative humidity for 24 h prior to the addition of experimental drugs. After 24 h, two plates of each cell line are fixed in situ with TCA to represent a measurement of the cell population for each cell line at the time of drug addition. Experimental drugs are solubilized in dimethyl sulfoxide at 400-fold of the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate is thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 $\mu$g/mL gentamicin. Additional four, 10-fold, or 1/2 log serial dilutions are made to provide a total of five drug concentrations plus a control. Aliquots of 100 $\mu$L of these different drug dilutions are added to the appropriate microtiter wells already containing 100 $\mu$L of medium, resulting in the required final drug concentrations. Following drug addition, the plates are incubated for an additional 48 h at 37 °C, 5% $CO_2$, 95% air, and 100% relative humidity. For adherent cells, the assay is terminated by the addition of cold TCA. Cells are fixed in situ by the gentle addition of 50 $\mu$L of cold 50% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 min at 4 °C. The supernatant is discarded, and the plates are washed five times with tap water and air-dried. Sulforhodamine B (SRB) solution (100 $\mu$L) at 0.4% (w/v) in 1% acetic acid is added to each well, and the plates are incubated for 10 min at room temperature. After staining, the unbound dye is removed by washing five times with 1% acetic acid, and the plates are air-dried. The bound stain is subsequently solubilized with a 10 mM Trizma base, and the absorbance is read on an automated plate reader at a wavelength of 515 nm. For suspension cells, the methodology is the same except that the assay is terminated by fixing settled cells at the bottom of the wells by gently adding 50 $\mu$L of 80% TCA (final concentration, 16% TCA). Compounds are screened at a dose of 10 $\mu$M; hits showing mean growth inhibition over 60 cell lines >50% are escalated for the five-dose screening assay. To construct a dose−response curve, about 60 cell lines of nine tumor subpanels were incubated with five concentrations (0.01−100 $\mu$M) for each compound. Three response parameters ($GI_{50}$, TGI, and $LC_{50}$) were calculated for each cell line. The $GI_{50}$ value corresponds to the compound's concentration causing a 50% decrease in net cell growth; the TGI value is the compound's concentration resulting in TGI, and the $LC_{50}$ value is the compound's concentration causing a net 50% loss of initial cells at the end of the incubation period (48 h).

*3.2.3. AlkP Activity in Ishikawa Cells.*[34,35] Estrogens stimulate the activity of AlkP in Ishikawa cells (human endometrial adenocarcinoma cells). This enzyme activity is estimated by using the chromogen substrate (4-nitrophenyl phosphate). These cells are very sensitive to estrogens; estradiol already induces the AlkP activity at a concentration of $10^{-12}$ M. The procedure was modified by Littlefield et al., 1990.[34]

Briefly, cells were cultured in DMEM/F12 medium without phenol red containing 5% dextran-coated charcoal-treated FCS (DCC, BioWest, Germany) and insulin−transferrin−selenium A (Invitrogen, Karlsruhe). Cells were kept in plastic culture flasks at 5% $CO_2$ and 37 °C and harvested by a brief exposure to trypsin (0.05%) EDTA at 37 °C. For experiments, the cells were seeded in 96-well plates at the required density of 11,000 cells per well. Compounds, diluted in DMSO (Carl Roth GmbH, Germany), were tested in a concentration of 1 $\mu$M. DMSO was used as a negative control and 1 nM 17$\beta$-estradiol (Sigma, Germany) as a positive control. After 72 h of incubation, cells were harvested, washed twice with PBS, and incubated at −80 °C for about 30 min to lyse the cells. After thawing, the lysates were resuspended in the reaction buffer (274 mM mannitol, 100 mM CAPS, 4 mM $MgCl_2$, and pH 10.4) containing 4 mM *p*-nitrophenyl phosphate (NPP). After incubation for 1 h in the dark, the AlkP activity was assayed by using the hydrolysis of *p*-nitrophenyl phosphate to *p*-nitrophenol at pH 10.4 and the spectrometric determination of the kinetic of the product formation at 405 nm.

All compounds were tested in technical triplicates and biological triplicates. Statistical analysis was performed by analysis of variance (ANOVA) and Tukey's post hoc test with the significance level of $p < 0.05$.

*3.2.4. Uterotrophic Assay.*[36] The most common short-term in vivo assay for (anti)-estrogenicity is the uterine growth test, suitable for screening ER$\alpha$ agonists and antagonists. The primary end point is the uterine wet weight (UWW). An increase in UWW indicates the estrogenic activity of the test compound. Sprague-Dawley female rats (170−200 g) were obtained from the animal colony of the National Institute of Research (Cairo, Egypt). The rats were housed in a temperature-controlled room (23−24 °C) with a 12 h light/dark cycle and with free access to food and water. They were allowed to acclimatize to the animal house of German University in Cairo for at least 1 week before initiating the experiments. All efforts were made to minimize animal discomfort and suffering. Animals were ovariectomized. After 14 days of endogenous hormonal decline, the animals were subcutaneously treated for 3 days with respective compounds. The animals were randomly allocated to treatment and vehicle groups ($n = 6$). 17$\beta$-Estradiol was administered s.c. at a dose of 10 $\mu$g/kg/d BW; all test compounds were administered at a dose of 10 mg/kg/d BW daily for a period of 3 days. Animals were sacrificed by $CO_2$ inhalation after light anesthesia by inhaling an $O_2/CO_2$ mixture around 24 h after the third administration. The UWW was determined.

*3.2.4.1. Institutional Review Board Statement.* This study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of German University in Cairo (March 2019).

*3.2.5. Anti-influenza Screening.*[37] In vitro antiviral assays using cell-based systems frequently use the viral CPE with inhibition of virus yield for follow-up evaluations. Madin Darby canine kidney cells are primarily used. The virus strain used is California/07/2009. To improve the viral CPE, trypsin and ethylene diamine tetraacetate are put in the medium. The influenza virus may effectively replicate when added to cell


https://doi.org/10.1021/acsomega.3c01682
ACS Omega 2023, 8, 25903−25923

cultures, creating new infectious virus, or the virus may not complete its replicative cycle producing non-infectious virus. Whether the virus replicates effectively or not depends on the virus, with new clinical isolates frequently necessitating passage in the amniotic cavity of eggs, and multiple cell passages before effective replication will take place. The 96-well microplate is used. The assays use visual determination of viral CPE inhibition, confirming the data obtained by neutral red dye uptake. Several one-half log 10 concentrations of test compounds were utilized in each test; in initial screening, the compound is added nearly 5 min before virus exposure and stays on the cells until the test is read. An inoculum is chosen that will trigger near-maximal (4+) CPE in 72−96 h because the antiviral activity of most compounds depends on the viral multiplicity of infection. 50 cell culture 50% infectious doses (CCID50) per microplate well are used, which is equal to a multiplicity of infection of 0.001 infectious particles per cell. Cytotoxicity controls in uninfected cells are included with each concentration of test compound. Other controls include normal controls (uninfected cells with test medium only) and virus controls (cells with virus and drug diluent). A known positive control drug, ribavirin, is screened in parallel in the antiviral screening test. Regression analysis of the CPE inhibition data determines the 50% effective (virus inhibitory) concentration ($EC_{50}$), which is used as a measure of the antiviral activity of the tested compounds. Plotting the percentage of cytotoxicity versus test compound concentration determines the 50% cytotoxic concentration ($CC_{50}$), which is a measure of the cytotoxicity of the tested compounds. A selective index (SI) is then calculated as $CC_{50}/EC_{50}$.

*3.2.6. Anti-EBOV Screening.*[38] Culture medium (Huh7 cells) containing 2% fetal bovine serum was used to dilute viruses into a cell culture 50% infectious dose ($CCID_{50}$) that formed the maximal CPE by visual excel amination in initial virus titration experiments. Half-log dilutions of favipiravir (control drug) and compounds **2F** and **3F** were added to test wells at the time of infection. For the determination of cytotoxicity, drugs were added in the absence of viral infection. Plates were incubated at 37 °C and 5% $CO_2$ until the virus-infected control wells were found to have the maximum viral CPE, at which time the plates were recorded visually for the CPE and toxicity. The average effective concentration ($EC_{90}$) and the concentration that decreased the cell viability by 90% ($CC_{50}$) were identified by regression analysis. The SI values were calculated as SI = $CC_{90}/EC_{50}$. The cell viability was measured using the neutral red dye uptake method. After the visual analysis of the CPE and toxicity, infected cells and controls were incubated with a 0.034% neutral red dye solution for 2 h at 37 °C and 5% $CO_2$. Then, the neutral red dye was removed, and the wells were washed two times with phosphate-buffered saline. The plates were left to dry totally before the vital dye was extracted with absolute ethanol buffered with Sorenson's citrate buffer for 30 min. The samples were measured at 540 nm, and the absorbance values were calculated as percentages of untreated, uninfected controls, which took up maximum dye.

*3.2.7. Pharmacokinetics Study.* As for the in vivo PK assessment, mice were housed in an animal facility [accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)]. All animal procedures were approved by the Animal Care and Use Committee of the National Institute of Allergy and Infectious Diseases, Division of Clinical Research, in compliance with the Animal Welfare Act regulations, Public Health Service policy, and the Guide for the Care and Use of Laboratory Animals recommendations. Compound **3F** was dissolved in 3% *N*-methyl-2-pyrrolidone (NMP)/80% polyethylene glycol 300 (PEG 300)/17% sterile water for injection. This vehicle was used for the remainder of the study. To determine the MTD, all PO group males and females were given compound **2F** at (50, 100, 200, and 400 mg/kg) and appeared normal up to their 24 h postdose scheduled sacrifice. The pharmacokinetic profile of compound **2F** was studied; thus, naïve animals (12 male and 9 female C57BL/6 mice) were given at a single 400 mg/kg of compound **2F** (PO), and blood was collected at 0.25, 0.5, 4, 6, 8, and 24 h after dose administration. A bioanalytical method was developed, and the lower LOQ was 10 ng/mL.

## 4. CONCLUSIONS

In conclusion, the results obtained validate our hypothesis that the estrogenic/antiestrogenic activity of TPE can be attenuated via subtle structural modifications on ring **A** and ring **C** not only via modification on ring **B**. These simple substitutions demonstrate the potential that lies within the SAR of TPEs and the possibility to develop novel ideal SERMs by revisiting this class of compounds. The success of some of the novel compounds as antivirals for EBOV and influenza encourages future modifications to augment the antiviral effects on the expense of the hormonal modulation effects and to test against other viruses.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c01682.

Characterization of selected compounds using LC−MS and NMR; DTP ($GI_{50}$, TGI, and $LC_{50}$) of compounds **4**, **2B**, and **5A**; relative AlkP activity after an incubation of 72 h in Ishikawa cells; and relative UWW following treatment of ovariectomized rats (PDF)

## ■ AUTHOR INFORMATION


### Corresponding Author

**Ashraf H. Abadi** − *Faculty of Pharmacy and Biotechnology, Department of Pharmaceutical Chemistry, German University in Cairo, 11835 Cairo, Egypt;* orcid.org/0000-0002-7433-261X; Email: ashraf.abadi@guc.edu.eg

### Authors

**Nermin S. Ahmed** − *Faculty of Pharmacy and Biotechnology, Department of Pharmaceutical Chemistry, German University in Cairo, 11835 Cairo, Egypt;* orcid.org/0000-0002-8869-3387

**Heba E. El-Nakib** − *Faculty of Pharmacy and Biotechnology, Department of Pharmaceutical Chemistry, German University in Cairo, 11835 Cairo, Egypt*

**Marian M. Ramsis** − *Faculty of Pharmacy and Biotechnology, Department of Pharmaceutical Chemistry, German University in Cairo, 11835 Cairo, Egypt*

**Nouran O. Albably** − *Faculty of Pharmacy and Biotechnology, Department of Pharmaceutical Chemistry, German University in Cairo, 11835 Cairo, Egypt*

**Jannette Wober** − *Faculty of Biology, Institute of Zoology, Technische Universität Dresden, 01062 Dresden, Germany*




https://doi.org/10.1021/acsomega.3c01682
*ACS Omega* 2023, 8, 25903−25923

**Jan J. Weigand** − *Faculty of Chemistry and Food Chemistry, Institute of Inorganic Molecular Chemistry, Technische Universität Dresden, 01062 Dresden, Germany;* orcid.org/0000-0001-7323-7816

**Kai Schwedtmann** − *Faculty of Chemistry and Food Chemistry, Institute of Inorganic Molecular Chemistry, Technische Universität Dresden, 01062 Dresden, Germany;* orcid.org/0000-0002-7965-2647

**Oliver Zierau** − *Faculty of Biology, Institute of Zoology, Technische Universität Dresden, 01062 Dresden, Germany*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.3c01682

### Author Contributions

H.E.E., M.M.R., and N.O.A. contributed equally to this work. A.H.A. and N.S.A. contributed to conceptualization; H.E.E., M.M.R., N.O.A., M.A.V., O.Z., and N.S.A. contributed to methodology; A.H.A., J.J.W., K.S., J.W., and N.S.A. contributed to supervision; J.W. and N.S.A. contributed to writing—original draft; and J.W., G.V., A.H.A., and N.S.A. contributed to writing—review and editing; N.S.A. is the PI of the projects that partially financed the work, STDF grant nos. 30298 and 5386. N.S.A. is the coordinator of the ERA+ staff mobility grant. All authors have read and agreed to the published version of the manuscript.

### Funding

This paper is based on the work supported financially by the Science and Technology Development Funding authority (STDF) under grant nos. 5386 and 30298 to N.S.A.

### Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

The authors are deeply grateful to the authority of the National Cancer Institute, USA, for the antitumor screening. The authors are deeply grateful to the Institute of Allergic and Infectious Diseases (NIAID), USA, for the antiviral testing and the in mice pharmacokinetics study. Nouran O. Albably thank the DAAD (German Academic Exchange Service) for an Erasmus+ stipend (code: 999897729).

## ■ REFERENCES

(1) Peh, W. Y. X.; Reimhult, E.; Teh, H. F.; Thomsen, J. S.; Su, X. Understanding Ligand Binding Effects on the Conformation of Estrogen Receptor Alpha-DNA Complexes: A Combinational Quartz Crystal Microbalance with Dissipation and Surface Plasmon Resonance Study. *Biophys. J.* **2007**, *92*, 4415−4423.

(2) Heldring, N.; Pike, A.; Andersson, S.; Matthews, J.; Cheng, G.; Hartman, J.; Tujague, M.; Ström, A.; Treuter, E.; Warner, M.; Gustafsson, J. Å. Estrogen Receptors: How Do They Signal and What Are Their Targets. *Physiol. Rev.* **2007**, *87*, 905−931.

(3) Farooq, A. Structural and Functional Diversity of Estrogen Receptor Ligands. *Curr. Top. Med. Chem.* **2015**, *15*, 1372−1384.

(4) An, K.-C. Selective Estrogen Receptor Modulators. *Asian Spine J* **2016**, *10*, 787−791.

(5) Taylor, H. S. Designing the Ideal Selective Estrogen Receptor Modulator–an Achievable Goal? *Menopause* **2009**, *16*, 609−615.

(6) Mirkin, S.; Pickar, J. H. Selective Estrogen Receptor Modulators (SERMs): A Review of Clinical Data. *Maturitas* **2015**, *80*, 52−57.

(7) Yaffe, K.; Krueger, K.; Sarkar, S.; Grady, D.; Barrett-Connor, E.; Cox, D. A.; Nickelsen, T. Cognitive Function in Postmenopausal Women Treated with Raloxifene. *N. Engl. J. Med.* **2001**, *56*, 554−555.

(8) Paterni, I.; Granchi, C.; Katzenellenbogen, J. A.; Minutolo, F. Estrogen receptors alpha (ER$\alpha$) and beta (ER$\beta$): Subtype-selective ligands and clinical potential. *Steroids* **2014**, *90*, 13−29.

(9) Yaşar, P.; Ayaz, G.; User, S. D.; Güpür, G.; Muyan, M. Molecular Mechanism of Estrogen-Estrogen Receptor Signaling. *Reprod. Med. Biol.* **2016**, *16*, 4−20.

(10) Ahmed, N. S.; Samec, M.; Liskova, A.; Kubatka, P.; Saso, L. Tamoxifen and Oxidative Stress: An Overlooked Connection. *Discover Oncol.* **2021**, *12*, 17.

(11) Kumar, R.; Zakharov, M. N.; Khan, S. H.; Miki, R.; Jang, H.; Toraldo, G.; Singh, R.; Bhasin, S.; Jasuja, R. The Dynamic Structure of the Estrogen Receptor. *J. Amino Acids* **2011**, *2011*, 812540.

(12) Rastinejad, F.; Huang, P.; Chandra, V.; Khorasanizadeh, S. Understanding Nuclear Receptor Form and Function Using Structural Biology. *J. Mol. Endocrinol.* **2013**, *51*, T1−T21.

(13) Ahmed, N. S.; Elghazawy, N. H.; ElHady, A. K.; Engel, M.; Hartmann, R. W.; Abadi, A. H. Design and Synthesis of Novel Tamoxifen Analogues That Avoid CYP2D6 Metabolism. *Eur. J. Med. Chem.* **2016**, *112*, 171−179.

(14) MacLeod, A. K.; McLaughlin, L. A.; Henderson, C. J.; Wolf, C. R. Application of Mice Humanized for CYP2D6 to the Study of Tamoxifen Metabolism and Drug-Drug Interaction with Antidepressants. *Drug Metab. Dispos.* **2017**, *45*, 17−22.

(15) de Souza, J. A.; Olopade, O. I. CYP2D6 Genotyping and Tamoxifen: An Unfinished Story in the Quest for Personalized Medicine. *Semin. Oncol.* **2011**, *38*, 263−273.

(16) Hansten, P. D. The Underrated Risks of Tamoxifen Drug Interactions. *Eur. J. Drug Metab. Pharmacokinet.* **2018**, *43*, 495−508.

(17) Fang, H.; Tong, W.; Shi, L. M.; Blair, R.; Perkins, R.; Branham, W.; Hass, B. S.; Xie, Q.; Dial, S. L.; Moland, C. L.; Sheehan, D. M. Structure-Activity Relationships for a Large Diverse Set of Natural, Synthetic, and Environmental Estrogens. *Chem. Res. Toxicol.* **2001**, *14*, 280−294.

(18) Kouznetsova, J.; Sun, W.; Martinez-Romero, C.; Tawa, G.; Shinn, P.; Chen, C. Z.; Schimmer, A.; Sanderson, P.; McKew, J. C.; Zheng, W.; Garcia-Sastre, A. Identification of 53 Compounds That Block Ebola Virus-like Particle Entry via a Repurposing Screen of Approved Drugs. *Emerging Microbes Infect.* **2014**, *3*, 1−7.

(19) Elnakib, H. E.; Ramsis, M. M.; Albably, N. O.; Vector, M. A.; Weigand, J. J.; Schwedtmann, K.; Wober, J.; Zierau, O.; Vollmer, G.; Abadi, A. H.; Ahmed, N. S. Manipulating Estrogenic/Anti-Estrogenic Activity of Triphenylethylenes towards Development of Novel Anti-Neoplastic SERMs. *Int. J. Mol. Sci.* **2021**, *22*, 12575.

(20) Bedford, G. R.; Richardson, D. N. Preparation and Identification of Cis and Trans Isomers of a Substituted Triarylethylene. *Nature* **1966**, *212*, 733−734.

(21) Routledge, E. J.; Sumpter, J. P. Estrogenic Activity of Surfactants and Some of Their Degradation Products Assessed Using a Recombinant Yeast Screen. *Environ. Toxicol. Chem.* **1996**, *15*, 241−248.

(22) Nilsson, S.; Gustafsson, J. A. Estrogen Receptor Transcription and Transactivation: Basic Aspects of Estrogen Action. *Breast Cancer Res.* **2000**, *2*, 360−366.

(23) Ahmed, N. S.; Wober, J. Synthesis of Novel Flexible Tamoxifen Analogues to Overcome CYP2D6 Polymorphism and Their Biological Evaluation on MCF-7 Cell Line. *Drug Dev. Res.* **2020**, *81*, 444−455.

(24) Lu, Y.; Liu, W. Selective Estrogen Receptor Degraders (SERDs): A Promising Strategy for Estrogen Receptor Positive Endocrine-Resistant Breast Cancer. *J. Med. Chem* **2020**, *63*, 15094−15114.

(25) Catherino, W. H.; Jordan, V. C. A Risk-Benefit Assessment of Tamoxifen Therapy. *Drug Saf.* **1993**, *8*, 381−397.

(26) Chavez, K. J.; Garimella, S. V.; Lipkowitz, S. Triple Negative Breast Cancer Cell Lines: One Tool in the Search for Better Treatment of Triple Negative Breast Cancer. *Breast Dis.* **2011**, *32*, 35−48.

(27) Nishida, M.; Kasahara, K.; Kaneko, M.; Iwasaki, H.; Hayashi, K. [Establishment of a new human endometrial adenocarcinoma cell

line, Ishikawa cells, containing estrogen and progesterone receptors]. *Nihon Sanka Fujinka Gakkai Zasshi* **1985**, *37*, 1103−1111.
(28) Peretz, J.; Pekosz, A.; Lane, A. P.; Klein, S. L. Estrogenic Compounds Reduce Influenza A Virus Replication in Primary Human Nasal Epithelial Cells Derived from Female, but Not Male, Donors. *Am. J. Physiol.: Lung Cell. Mol. Physiol.* **2016**, *310*, L415−L425.
(29) Zhao, Y.; Ren, J.; Harlos, K.; Jones, D. M.; Zeltina, A.; Bowden, T. A.; Padilla-Parra, S.; Fry, E. E.; Stuart, D. I. Toremifene Interacts with and Destabilizes the Ebola Virus Glycoprotein. *Nature* **2016**, *535*, 169−172.
(30) Shiau, A. K.; Barstad, D.; Loria, P. M.; Cheng, L.; Kushner, P. J.; Agard, D. A.; Greene, G. L. The Structural Basis of Estrogen Receptor/coactivator Recognition and the Antagonism of This Interaction by Tamoxifen. *Cell* **1998**, *95*, 927−937.
(31) Reid, S. A.; Nyambo, S.; Muzangwa, L.; Uhler, B. $\pi$-Stacking, C−H/$\pi$, and Halogen Bonding Interactions in Bromobenzene and Mixed Bromobenzene−Benzene Clusters. *J. Phys. Chem. A* **2013**, *117*, 13556−13563.
(32) Weissenhorn, W.; Carfí, A.; Lee, K.-H.; Skehel, J. J.; Wiley, D. C. Crystal Structure of the Ebola Virus Membrane Fusion Subunit, GP2, from the Envelope Glycoprotein Ectodomain. *Mol. Cell* **1998**, *2*, 605−616.
(33) Shoemaker, R. H. The NCI60 Human Tumour Cell Line Anticancer Drug Screen. *Nat. Rev. Cancer* **2006**, *6*, 813−823.
(34) Littlefield, B. A.; Gurpide, E.; Markiewicz, L.; McKinley, B.; Hochberg, R. B. A Simple and Sensitive Microtiter Plate Estrogen Bioassay Based on Stimulation of Alkaline Phosphatase in Ishikawa Cells: Estrogenic Action of Delta 5 Adrenal Steroids. *Endocrinology* **1990**, *127*, 2757−2762.
(35) Holinka, C. F.; Hata, H.; Kuramoto, H.; Gurpide, E. Effects of Steroid Hormones and Antisteroids on Alkaline Phosphatase Activity in Human Endometrial Cancer Cells (Ishikawa Line). *Cancer Res.* **1986**, *46*, 2771−2774.
(36) Saarinen, N. M.; Bingham, C.; Lorenzetti, S.; Mortensen, A.; Mäkelä, S.; Penttinen, P.; Sørensen, I. K.; Valsta, L. M.; Virgili, F.; Vollmer, G.; Wärri, A.; Zierau, O. Tools to Evaluate Estrogenic Potency of Dietary phytoestrogens:A Consensus Paper from the EU Thematic Network "Phytohealth" (QLKI-2002-2453). *Genes Nutr.* **2006**, *1*, 143−158.
(37) Sidwell, R. W.; Smee, D. F. In vitro and in vivo assay systems for study of influenza virus inhibitors. *Antiviral Res.* **2000**, *48*, 1−16.
(38) Westover, J. B.; Sefing, E. J.; Bailey, K. W.; Van Wettere, A. J.; Jung, K.-H.; Dagley, A.; Wandersee, L.; Downs, B.; Smee, D. F.; Furuta, Y.; Bray, M.; Gowen, B. B. Low-Dose Ribavirin Potentiates the Antiviral Activity of Favipiravir against Hemorrhagic Fever Viruses. *Antiviral Res.* **2016**, *126*, 62−68.


https://doi.org/10.1021/acsomega.3c01682
*ACS Omega* 2023, 8, 25903−25923