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Brief Article

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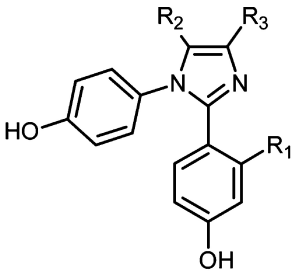

7a, R_1: H, R_2: H, R_3: 4-HO-Ph
7b, R_1: H, R_2: H, R_3: 2-Cl, 4-HO-Ph
7c, R_1: Cl, R_2: H, R_3: 2-Cl, 4-HO-Ph
7d, R_1: H, R_2: Et, R_3: 4-HO-Ph
7e, R_1: H, R_2: H, R_3: CF_3

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Brief Articles

Synthesis and Pharmacological Evaluation of 1*H*-Imidazoles as Ligands for the Estrogen Receptor and Cytotoxic Inhibitors of the Cyclooxygenase

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The 1*H*-imidazoles **7a**−**e** were synthesized and tested for biological activity in vitro. The results pointed to a clear structure−activity relationship. The introduction of an ethyl chain at C5 of the 1,2,4-tris(4-hydroxyphenyl)-1*H*-imidazole **7a** caused hormonal activity in estrogen receptor positive MCF-7-2a cells. An *o*-chlorine substituent in the phenolic rings at C2 and C4 as realized in **7b** and **7c** increased the antiproliferative effects against human breast cancer cell lines MCF-7 and MDA-MB 231. Additionally, both compounds showed strong inhibitory effects on cyclooxygenase enzymes. Therefore, a mode of action including the interference in the arachidonic acid cascade might be possible.

Introduction

Estradiol (E2) is one of the native ligands of the estrogen receptor (ER) and plays an essential role in regulating normal physiological processes such as the development and function of the reproductive and cardiovascular system or bone density.[1−3] Nonsteroidal estrogenic (e.g. diethylstilbestrol) and antiestrogenic ligands (e.g. raloxifene) have been developed to regulate these processes or their pathological dysfunction, including breast cancer, osteoporosis, and infertility.

Selective estrogen receptor modulators (SERMs) are the most recently approved class of antiresorptive drugs. In some tissues, such as bone, SERMs imitate the effects of estrogens, while they act as antiestrogens on uterine and breast tissue and block unwanted estrogenic effects.

Most potent SERMs consist of a heterocyclic core (e.g. pyrazoles,[4] furans[5] or 2-imidazolines,[6] and piperazines[7]) substituted with aromatic rings or alkyl chains and resemble a large number of nonsteroidal antiinflammatory drugs (NSAIDs) which exert their activity mainly through inhibition of cyclooxygenases (COX) the key enzymes of the prostaglandin (PG) biosynthesis.

During the past few years, cyclooxygenases (COX-1 and COX-2) were discussed as new targets for anticancer drugs, since abnormally high levels of COX-2 protein were observed in several types of cancer and precancerous tissue, including breast cancer. COX-2 proteins increase new blood vessel formation and proliferation. A tumor growth inhibition can thus be achieved by blocking COX-2 as demonstrated for indomethacin and the COX-2 selective inhibitor 4-[5-(4-chlorophenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl]benzenesulfonamide (SC-236).[8] Furthermore, the induction of tumor cell apoptosis was verified for both compounds as consequence of a COX inhibition.[9]

These results and the high structural analogy of our already described trisubstituted 1*H*-imidazoles to celecoxib and SC-236, but especially to the COX-inhibitors 1-[4-(methylsulfonyl)phenyl]-2-phenyl-4-(trifluoromethyl)-1*H*-imidazole (**Im-I**) and 2-(4-chlorophenyl)-1-[4-(methylsulfonyl)phenyl]-4-phenyl-1*H*-imidazole (**Im-II**), induced us to use 1,2,4-tris(4-hydroxyphenyl)-1*H*-imidazole **7a** as a lead structure. We studied the influence of an ethyl chain at C5, the 4-hydroxyphenyl−trifluoromethyl exchange at C4 and the introduction of *o*-chlorine substituents in the C2/C4 standing phenolic rings on hormonal, cytotoxic, and COX-inhibiting properties.

Chemistry. The 1,2-diaryl-, 1,2,4-triaryl-, and 1,2,4-triaryl-5-alkyl-1*H*-imidazoles were prepared as depicted in Scheme 1. *N*-Arylbenzamidines **3a** and **3b** were synthesized as key intermediates from aryl nitriles (**1**, **2**) and anisidine according to Gautier[10] or Daoust[11] using sodium amide as condensation agent.

The subsequent reaction with the α-bromoketones[12,13] (**4**, **5a**−**c**) at room temperature in a mixture of $CHCl_3$/H_2O (6/1) utilizing K_2CO_3 as base resulted in 1*H*-imidazoles. This cyclization took place with high regioselectivity and quantity because neither regioisomers of **6a**−**e** nor 4-hydroxy-2-imidazolines as intermediates[14,15] could be separated from the reaction mixture. The necessary ether cleavage to afford the phenols **7a**−**e** was performed with boron tribromide.

The structural characterization of the 1*H*-imidazoles was exemplarily performed for compound **7a** by double resonance nuclear Overhauser enhancement experiment

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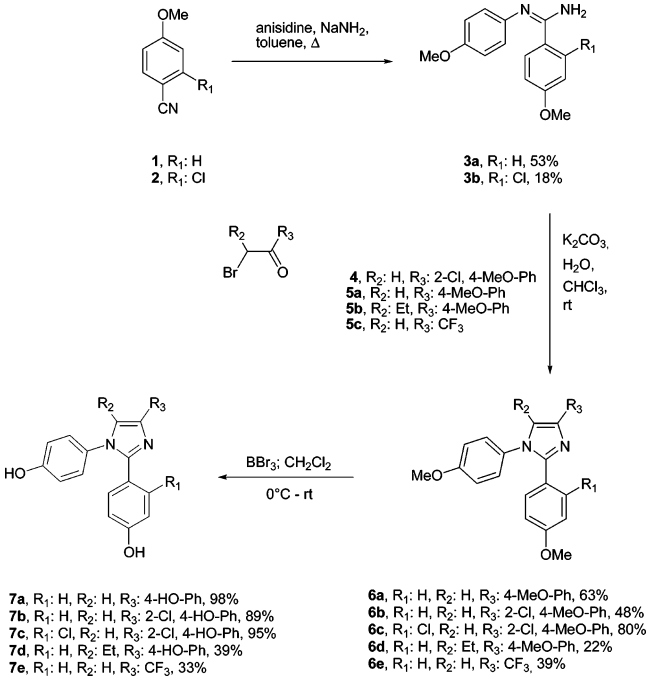
Scheme 1



(NOESY) and confirmed the 1,2,4-tris(4-hydroxyphenyl)-1*H*-imidazole formation.

Biological Activity. A luciferase assay[16] using ER positive MCF-7 breast cancer cells stably transfected with the plasmid $ERE_{wtc}luc$ (MCF-7 2a cells) was used to study the ER interaction of the 1*H*-imidazoles **7a**−**e** on molecular level. The binding of dimeric ER/drug conjugates to the estrogen response element (ERE) of the plasmid leads to the expression of luciferase, which correlates well with the estrogenic potency of the drug.[17]

As depicted in Figure 1A, the C(5)-ethyl derivative **7d** activated the luciferase expression with an $EC_{50} = 7.7 \times 10^{-8}$ M (E2 = 8.0×10^{-11} M). All other compounds were inactive. Interestingly, the concentration activation curves indicated for the 2-Cl substituted derivatives **7b** and **7c** distinctly negative relative activations of −37% at a concentration of 10 μM. This effect was the consequence of a strong reduction of the cell density and not of antiestrogenic properties because the compounds were not able to antagonize the effect of estradiol (data not shown) but indicated cytotoxic properties as verified in time-dependent in vitro assays (Figure 2).[18,19]

Both compounds reduced the growth of hormone-dependent MCF-7 breast cancer cells whereby **7b** (T/C ≈ 50% at 10 μM, Figure 2A) proved to be less cytotoxic than **7c** (T/C ≈ 0% at 10 μM; see Figure 2B). Hormone independent MDA-MB 231 cells treated with **7b** and **7c** (Figure 2C, D) showed a growth characteristic at lower concentrations (1 and 5 μM) comparable to MCF-7 cells with the restriction that in the case of **7c** the cells recuperated after 90 h and showed an onset of proliferation. At 10 μM, however, the effects of **7b** and **7c** differ from the one observed in the MCF-7 cell line: **7b** was more active than **7c** and caused cytocidal effects (τ = −20%) already after 80 h of incubation while **7c** reached its maximal effect at the end of the test (142 h). It should be noted that the other compounds (**7a**, **7d**, **7e**) were not able to exert an influence on the growth of MCF-7 and MDA-MB 231 cells (data not shown).

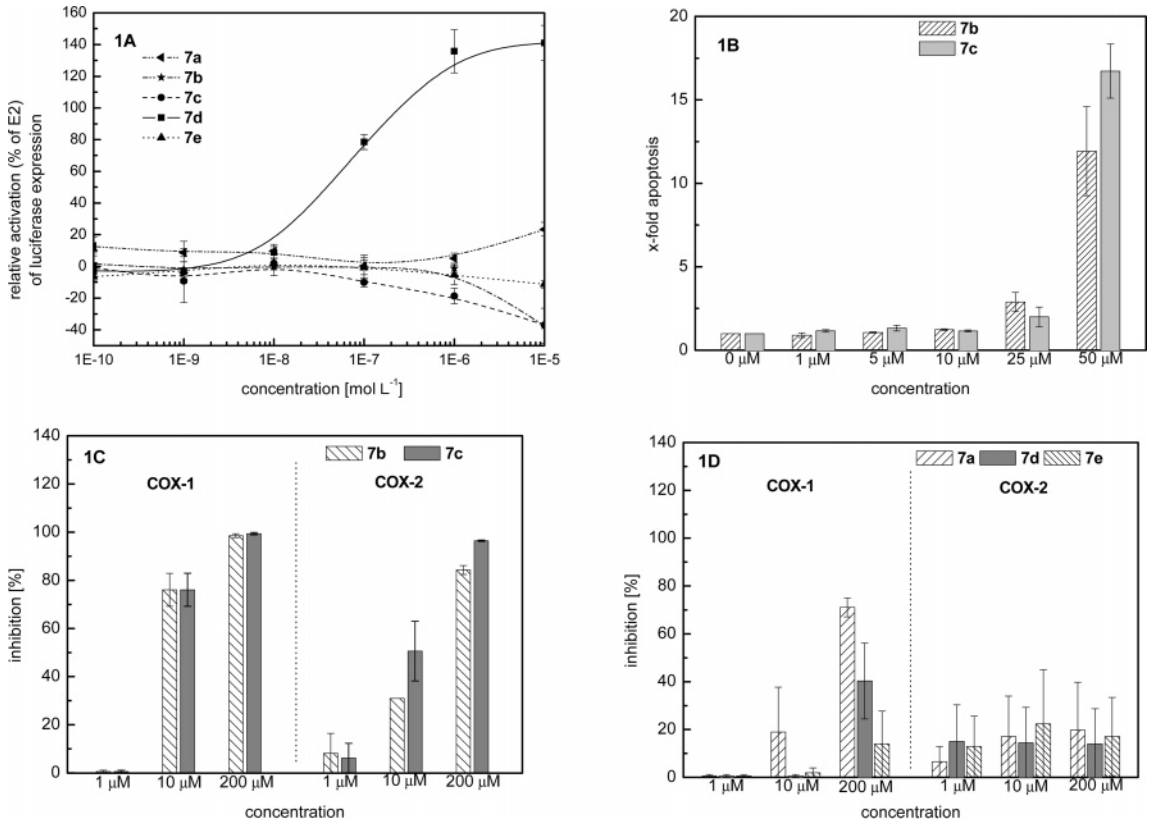


Figure 1. Activation (%) of luciferase gene expression in MCF-7 2a cells by the 1*H*-imidazoles **7a**−**e**. Values expressed are the means ± SE of 3-fold determination in a single experiment (A). Induction of apoptosis in MDA-MB 231 cells during the incubation with imidazoles **7b** and **7c**. The mean apoptosis of three experiments is shown (B). Inhibition of COX-1 and COX-2 enzymatic activity by the 1*H*-imidazoles **7a**−**e** in concentrations of 1, 10, and 200 μM (C, D).

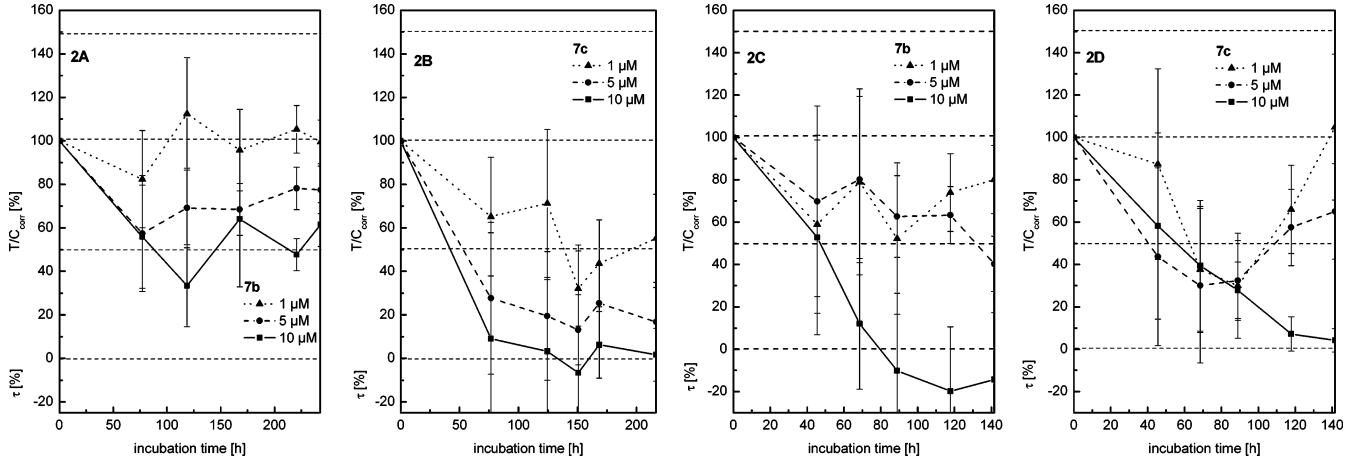


Figure 2. Growth curves of MCF-7 (A, B) and MDA-MB 231 (C, D) breast cancer cells in the presence of the 1,2,4-triaryl-1*H*-imidazoles **7b** and **7c**. Cell density was determined by crystal violet staining expressed as $T/C_{corr} = [(T - C_0)/(C - C_0)] \times 100$ [%] with T (test), C (control), and C_0 (cell extract immediately before compound treatment). Values expressed are the means ± SE of 16-fold determination in a single experiment.

To get more insight into the mode of action, we quantified the ssDNA (single stranded DNA) in MDA-MB 231 cells as an indicator of apoptosis.[20] As expected, neither the short time incubation (2 h) nor incubation for 120 h of MDA-MB 231 cells (data not shown) with **7b** nor **7c** in lower concentrations induced apoptosis. Only in concentrations of 25 and 50 μM ssDNA values significantly increased (see Figure 1B). These concentrations correspond very well with those used in the cytotoxicity assay because a significantly higher amount of tumor cells was used in the apoptosis assay.

The knowledge of the relevance of cyclooxygenase enzymes (COX-1 and COX-2) for the proliferation of MCF-7 and MDA-MB 231 cells as well as the findings that COX-inhibitors caused tumor cell apoptosis induced us to evaluate the interaction of the 1*H*-imidazoles **7a**−**e** with isolated ovine COX-1 and human recombinant COX-2 in an enzyme linked immunsorbant assay (ELISA, see Figure 1C,D).

In these experiments the reference substance aspirin showed only a weak inhibition of COX-1 (80% at 200 μM) and was inactive at COX-2 while the second reference indomethacin was much more active (COX-1 inhibition: 56%; COX-2 inhibition: 100%; concentration: 10 μM).

The cytotoxic 1*H*-imidazoles **7b** and **7c** showed concentration-dependent effects and reduced both the activity of COX-1 and COX-2. The IC_{50} value for COX-1 inhibition amounted to 8.4 μM for both compounds. At COX-2 **7c** was slightly more active with $IC_{50} = 9.4$ μM (**7b**: $IC_{50} = 11.5$ μM). Among the "noncytotoxic" compounds, **7a** ($IC_{50} = 12.2$ μM) and **7d** ($IC_{50} > 200$ μM) were able to reduce the COX-1 activity slightly. At COX-2, **7a**, **7d**, and **7e** were inactive in the used concentrations (see Figure 1C,D).

Discussion

This SAR study throws light on a widely examined but unresolved aspect about the connection of antiproliferative effects on tumor cell lines and COX inhibition. We synthesized 1,2,4-triaryl-1*H*-imidazoles in order to get effective ligands for the ER. Although the number of tested compounds is limited, some structure−activity relationships can be worked out.

Three phenolic OH-groups (the respective methoxy derivatives were inactive; data not shown) and especially a C(5)-ethyl chain (compare **7a** and **7d**) were necessary for estrogenic activity. In contrast to the results obtained with compounds out of the 2,3-diarylpiperazine[7,21] and 4,5-diaryl-2-imidazoline[6,21] series, the enhanced lipophilicity due to chlorination of the C(2)- and C(4)-standing phenolic ring did not result in estrogenically active compounds but led to derivatives with enhanced antiproliferative properties. The 1*H*-imidazoles **7b** und **7c** reduced the growth of MCF-7 as well as MDA-MB 231 cells. The high activity at the hormone-independent MDA-MB 231 cell line and the lack of ability to antagonize the effects of estradiol made an antiestrogenic mode of action unlikely. More likely was an interference of **7b** and **7c** in the prostaglandin biosynthesis, as previous examinations[22] showed that hormone-dependent MCF-7 cells exhibited a relatively high expression of COX-1. COX-2 was barely detectable but was transiently induced by treatment with tetradecanoyl phorbol acetate. The hormone-independent MDA-MB 231 cell line showed a low COX-1 expression but a high constitutive level of COX-2. **7b** and **7c** are potent inhibitors of COX-1 and COX-2 and inhibited the growth of both cell lines.

The COX inhibitory effects showed a clear structural dependence. The C(4)-phenol ring was necessary for high activity. Ortho-chlorination of the C(4)-phenol ring at **7a** increased the activity at both COX-1 and COX-2. The attempt to further enhance the potency through the introduction of an additional 2-Cl substituent in the C(2)-phenol ring failed. The exchange of the C(4)-phenol ring by a CF_3 group, which is characteristic for a lot of selective COX-2 inhibitors, led to a complete loss of activity (see **7e**). These findings (unselective COX inhibition) are in accordance with an extensive number of investigations which emphasized on the importance of a p-SO_2NH_2- or p-SO_2CH_3-substituted phenyl for the COX-2 selectivity as shown for celecoxib and SC-236 or the 1*H*-imidazoles **Im-I** and **Im-II**.[14,15] Additionally, the studies with these compounds confirmed our findings about the importance of the C(4)-aryl ring for high COX inhibition. The 1,2,4-triaryl-1*H*-imidazole **Im-II** was much more active than the C(4)-CF_3 derivative **Im-I**.[14]

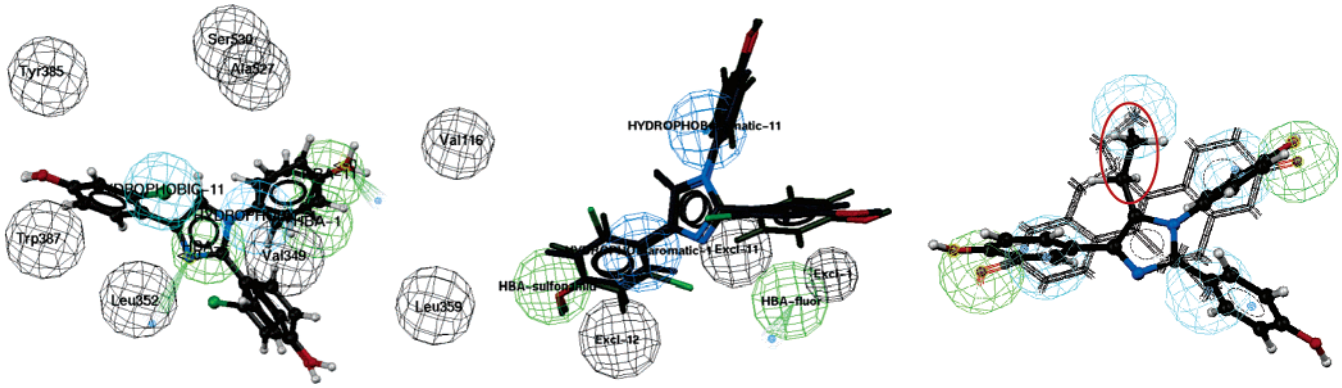


Figure 3. The active compounds **7b** and **7c** superimposed on the COX-1 model (left) and the COX-2 model (middle). The colored spheres represent chemical features of the ligand: hydrophobic (cyan), hydrophobic aromatic (blue), hydrogen bond acceptors (green). Amino acid residues flanking the binding pocket are represented by exclusion volume spheres depicted as black spheres. Compound **7d** (ball-and-stick style) and *R*,*R*-5,11-*cis*-diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol (mesh style) fitted into the model for ER activators (right). The highlighted ethyl residue of **7d** maps a hydrophobic feature of the model.

To get an insight into the possible mechanism of interaction with the cyclooxygenase enzymes, we fitted the compounds **7a**−**e** into structure-based pharmacophore models of COX-1 and COX-2.

In crystallization experiments, it was observed that all COX-1 inhibitors interacted with the putative catalytic amino acid residue Tyr385 and formed hydrogen bonds with Arg120. Many COX-1 inhibitors additionally interact with Tyr355 via a second hydrogen bond.[23] When mapping the active compounds **7b** and **7c** into the COX-1 model (Figure 3 left), it could be observed that the HYDROPHOBIC-11 (HBA-11) feature in close vicinity to Tyr385 was well mapped by the chlorinated phenol ring. The 4-hydroxyphenyl ring might hypothetically bind via a hydrogen bond and charge-transfer interaction to Tyr385. The HBA-11 feature representing the hydrogen bond acceptor partner for Arg120 was also well mapped by **7b** and **7c**. Because of the close vicinity of Arg120 and Tyr355 to each other, a bifurcated hydrogen bond between the phenolic hydroxyl group and these residues could be possible.

For COX-2, three distinct anchoring sites that contributed to substrate and inhibitor binding have been identified in crystallization and site-directed mutagenesis experiments. One major anchor point lies at the junction of Arg120 and Tyr355. The second anchor point is a side pocket defined by the residues Tyr355, Val523, His90, Gln192, and Arg513. At the top of the active site, additional interactions with Tyr385 and Ser530 contribute to inhibitor binding.[24] In the COX-2 model, the HYDROBHOB aromatic-11 feature represents an aromatic ligand substructure in the range of Tyr385. This feature is well mapped by the highly active compounds **7b** and **7c** (Figure 3 middle). The HBA-fluor feature localizes hydrogen bond acceptor structures that are in an ideal position for forming bifurcated hydrogen bonds with Arg120 and Tyr355. However, this feature is not mapped by the active compounds and seems therefore not to be essential for COX-2 inhibition. In the pharmacophore model, interactions with the side pocket anchor point are represented by the HYDROPHOB aromatic-1 feature (hydrophobic interaction with Val523) and the HBA-sulfonamide feature (hydrogen bond with Arg513) which are well mapped by **7b** and **7c**.

To rationalize the potent ER activation observed with **7d**, the compound was fitted into a structure-based model of ER based on the PDB crystal structure 1l2i.[25] The fitting conformation was then compared with the bioactive conformation of the cocrystallized ER activator *R*,*R*-5,11-*cis*-diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol (Figure 3 right).

7d maps well into the model for ER activators. The ethyl group of **7d** which is not present in all other compounds from this series fits into a hydrophobic feature and might thus constitute an essential interaction site with the ER receptor.

Conclusion

1,2-Diaryl- and 1,2,4-triaryl-1*H*-imidazoles were synthesized and investigated for pharmacological activity. While a (C5)-ethyl chain and the presence of three phenolic rings led to a compound with hormonal activity, *o*-chlorine substituents in the aromatic rings increased the cytotoxicity but not the estrogenicity. A mode of action might be possible in which inhibition of COX enzymes plays a major role.

Experimental Section

General Procedure for the Ether Cleavage with BBr_3. A solution of the methyl ether (1.00 mmol) in 20 mL of dry CH_2Cl_2 was cooled to −60 °C. BBr_3 (4.5 mmol) in 5 mL of dry CH_2Cl_2 was added at this temperature under N_2 atmosphere. Then the reaction mixture was allowed to warm to room temperature and was stirred for further 18 h. After the reaction mixture was cooled with an ice bath, the surplus of BBr_3 was hydrolyzed three times with methanol and the phenolic product was dissolved in 10% $NaHCO_3$ (50 mL). The solution was extracted with CH_2Cl_2 (3 × 20 mL), and the organic layers were combined and dried over Na_2SO_4. The solvent was removed under reduced pressure, and the resulting crude product was purified by chromatography on silica gel.

1,2,4-Tris(4-hydroxyphenyl)-*1H*-imidazole (7a). From **6a** (429 mg, 1.11 mmol) and BBr_3 (420 μL, 4.44 mmol); column chromatography with gradual elution, CH_2Cl_2/methanol: 95/5, 9/1, 3/1. Yield: 375 mg (98%); colorless solid (mp: 147−152 °C). TLC Si_2O (CH_2Cl_2/MeOH: 9/1): R_f = 0.3. ^{1}H NMR (DMSO-d_6): δ = 9.80 (s, 1H, O*H*), 9.64 (s, 1H, O*H*), 9.34 (s, 1H, O*H*), 7.65 (d, 2H, *J* = 8.5 Hz, Ar*H*), 7.58 (s, 1H, 5-*H*), 7.18

(d, 2H, J = 8.7 Hz, Ar*H*), 7.12 (d, 2H, J = 8.7 Hz, Ar*H*), 6.82 (d, 2H, J = 8.7 Hz, Ar*H*), 6.77 (d, 2H, J = 8.6 Hz, Ar*H*), 6.67 (d, 2H, J = 8.7 Hz, Ar*H*). MS (EI, 60°C): m/z (%) = 344 $[M]^{+\bullet}$ (100), 225 (79). IR (KBr): $\bar{\nu}$ = 3389 (m), 1612 (m), 1514 (s), 1444 (m), 1246 (s), 1170 (m), 836 (s). Anal. ($C_{21}H_{16}N_2O_3$) C, H, N.

4-(2-Chloro-4-hydroxyphenyl)-1,2-bis(4-hydroxyphenyl)-*1H*-imidazole (7b). From **6b** (125 mg, 0.30 mmol) and BBr_3 (127 μL, 1.34 mmol); column chromatography with CH_2Cl_2/methanol: 9/1. Yield: 112.4 mg (89%); colorless solid (mp: 262 °C). TLC Si_2O (CH_2Cl_2/methanol: 9/1): R_f = 0.6. ^{1}H NMR (DMSO-d_6): δ = 12.14 (s, 1H, O*H*), 9.90 (s, 1H, O*H*), 9.78 (s, 1H, O*H*), 7.90 (s, 1H, 5-*H*), 7.82 (d, 1H, J = 8.3 Hz, Ar*H*), 7.22−7.18 (m, 4H, Ar*H*), 6.94 (d, 1H, J = 2.1 Hz, Ar*H*), 6.90 (dd, 1H, J = 2.1 Hz, J = 8.3 Hz, Ar*H*), 6.86 (d, 2H, J = 8.7 Hz, Ar*H*), 6.72 (d, 2H, J = 8.7 Hz, Ar*H*). MS (EI, 340 °C): m/z (%) = 378 $[M]^{+\bullet}$ (100), 212 (57), 44 (36). IR (KBr): $\bar{\nu}$ = 3427 (m), 1612 (m), 1518 (s), 1439 (m), 1245 (m), 838 (m). Anal. ($C_{21}H_{15}ClN_2O_3$) C, H, N.

2,4-Bis(2-chloro-4-hydroxyphenyl)-1-(4-hydroxyphenyl)-*1H*-imidazole (7c). From **6c** (233 mg, 0.51 mmol) and BBr_3 (218 μL, 2.30 mmol); column chromatography with CH_2Cl_2/methanol: 9/1. Yield: 206 mg (95%); colorless solid (mp: 257 °C). TLC Si_2O (CH_2Cl_2/methanol: 9/1): R_f = 0.5. ^{1}H NMR (DMSO-d_6): δ = 11.84 (s, 1H, O*H*), 10.26 (s, 1H, O*H*), 9.77 (s, 1H, O*H*), 8.02 (s, 1H, 5-*H*), 7.83 (d, 1H, J = 8.3 Hz, Ar*H*), 7.31 (d, 1H, J = 8.4 Hz, Ar*H*), 7.07 (d, 2H, J = 8.6 Hz, Ar*H*), 6.94−6.90 (m, 2H, Ar*H*), 6.82 (d, 1H, J = 2.1 Hz, Ar*H*), 6.78−6.74 (m, 3H, Ar*H*). MS (EI, 280 °C): m/z (%) = 412 $[M]^{+\bullet}$ (100), 378 (26), 246 (77). IR (KBr): $\bar{\nu}$ = 3409 (m), 1610 (m), 1581 (m), 1518 (s), 1431 (m), 1275 (m), 1232 (m), 1202 (m), 899 (m), 836 (m), 810 (m). Anal. ($C_{21}H_{14}Cl_2N_2O_3$) C, H, N.

5-Ethyl-1,2,4-tris(4-hydroxyphenyl)-*1H*-imidazole (7d). From **6d** (97 mg, 0.26 mmol) and BBr_3 (99 μL, 1.05 mmol); column chromatography with CH_2Cl_2/methanol: 9/1. Yield: 34 mg (39%); colorless solid (mp: 163 °C). TLC Si_2O (CH_2Cl_2/methanol: 9/1): R_f = 0.1. ^{1}H NMR (DMSO-d_6): δ = 9.92 (s, 1H, O*H*), 9.59 (s, 1H, O*H*), 9.39 (s, 1H, O*H*), 7.50 (d, 2H, J = 8.4 Hz, Ar*H*), 7.12−7.18 (m, 4H, Ar*H*), 6.87 (d, 2H, J = 8.6 Hz, Ar*H*), 6.82 (d, 2H, J = 8.4 Hz, Ar*H*), 6.62 (d, 2H, J = 8.6 Hz, Ar*H*), 2.53 (q, 2H, J = 7.7 Hz, C*H*$_2$CH$_3$), 0.92 (t, 3H, J = 7.4 Hz, CH$_2$C*H*$_3$). MS (EI, 80 °C): m/z (%) = 372 $[M]^{+\bullet}$ (100), 357 (84), 119 (26). IR (KBr): $\bar{\nu}$ = 3401 (m), 2971 (w), 2934 (w), 1611 (m), 1512 (s), 1457 (m), 1382 (w), 1247 (m), 1172 (m), 837 (m). Anal. ($C_{23}H_{20}N_2O_3$) C, H, N.

1,2-Bis(4-hydroxyphenyl)-4-(trifluoromethyl)-*1H*-imidazole (7e). From **6e** (286 mg, 0.82 mmol) and BBr_3 (235 μL, 2.49 mmol); column chromatography with gradual elution, CH_2Cl_2/methanol: 98/2, 95/5, 9/1. Yield: 83 mg (32%); colorless solid (mp: 163 °C). TLC Si_2O (CH_2Cl_2/methanol: 9/1): R_f = 0.2. ^{1}H NMR (DMSO-d_6): δ = 9.94 (s, 1H, O*H*), 9.45 (s, 1H, O*H*), 7.29 (d, 2H, J = 8.7 Hz, Ar*H*), 7.01 (s, 1H, 5-*H*), 6.82 (d, 2H, J = 8.8 Hz, Ar*H*), 6.67 (d, 2H, J = 9.2 Hz, Ar*H*), 6.65 (d, 2H, J = 9.0 Hz, Ar*H*). MS (EI, 80 °C): m/z (%) = 320 $[M]^{+\bullet}$ (100). IR (film): $\bar{\nu}$ = 3383 (w), 3072 (w), 3007 (w), 2840 (w), 1611 (s), 1511 (s), 1253 (s), 1181 (s), 1031 (m), 838 (m). Anal. ($C_{16}H_{11}F_3N_2O_2$) C, H, N.

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Supporting Information Available: Elemental analyses of the target compounds **7a**−**e**. Synthesis of the compounds **3a**,**b**, **5a**−**c**, and **6a**−**e**. Biological methods: transcriptional binding assay; in vitro chemosensitivity assay, COX-inhibitor screening assay; ssDNA apoptosis ELISA. Molecular modeling. This material is available free of charge via the Internet at http://pubs.acs.org.

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