

Full Paper

Synthesis of Novel Nitro-substituted Triaryl Pyrazole Derivatives as Potential Estrogen Receptor Ligands

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Abstract: Novel tetrasubstituted pyrazole derivatives bearing a nitro substituent on their A-phenol ring were synthesized and their binding affinity towards the estrogen receptor (ER) subtypes ER α and ER β was determined. Among compounds tested, the 2-nitrophenol derivative **5c** was found to bind satisfactorily to both estrogen receptor subtypes (RBA α =5.17 and RBA β =3.27). In general, the introduction of a nitro group into the A ring of these compounds was found to benefit their ER β binding abilities.

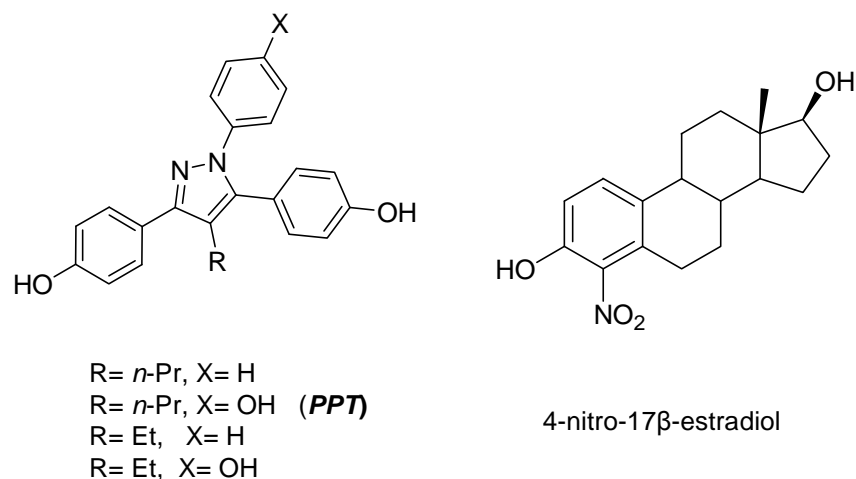
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Introduction

The estrogen receptor (ER) displays a considerable capacity for binding with a wide range of steroidal and nonsteroidal ligands. [1] Particularly, the nonsteroidal synthetic estrogens exhibit a tolerance in respect their structural motifs which can encompass, besides the phenolic function, which is a prerequisite for good binding, a large number of core building blocks. The latter may vary in length and size, including a plethora of –fused and nonfused– carbocyclic and heterocyclic ring systems [2-4].

An appreciable number of innovative **S**elective **E**strogen **R**eceptor **M**odulator compounds (SERMs) has been found to display a broad spectrum of agonist and antagonist actions at different target tissues. In addition, the further characterization of a second form of the receptor (ER β subtype) [5-7], has focused the drug development research towards the discovery of ligands capable to activate or inhibit selectively each of the ER subtypes. For the development of novel SERMs a variety of diverse ligands has been considered.

Figure 1. 1,3,5-Triaryl-4-alkyl substituted pyrazole derivatives and 4-nitro-17 β -estradiol.



Among them, the tetrasubstituted pyrazole derivatives constitute an intriguing case, since 1,3,5-triaryl-4-alkyl substituted pyrazoles, exemplified by the propylpyrazole triol **PPT** (Figure 1), have been found to possess particularly high ER α -selective binding affinity and potency [8-10]. In this context, various research findings have indicated that the substitution pattern of these pyrazole derivatives greatly affects their binding affinity to the Estrogen Receptors [11].

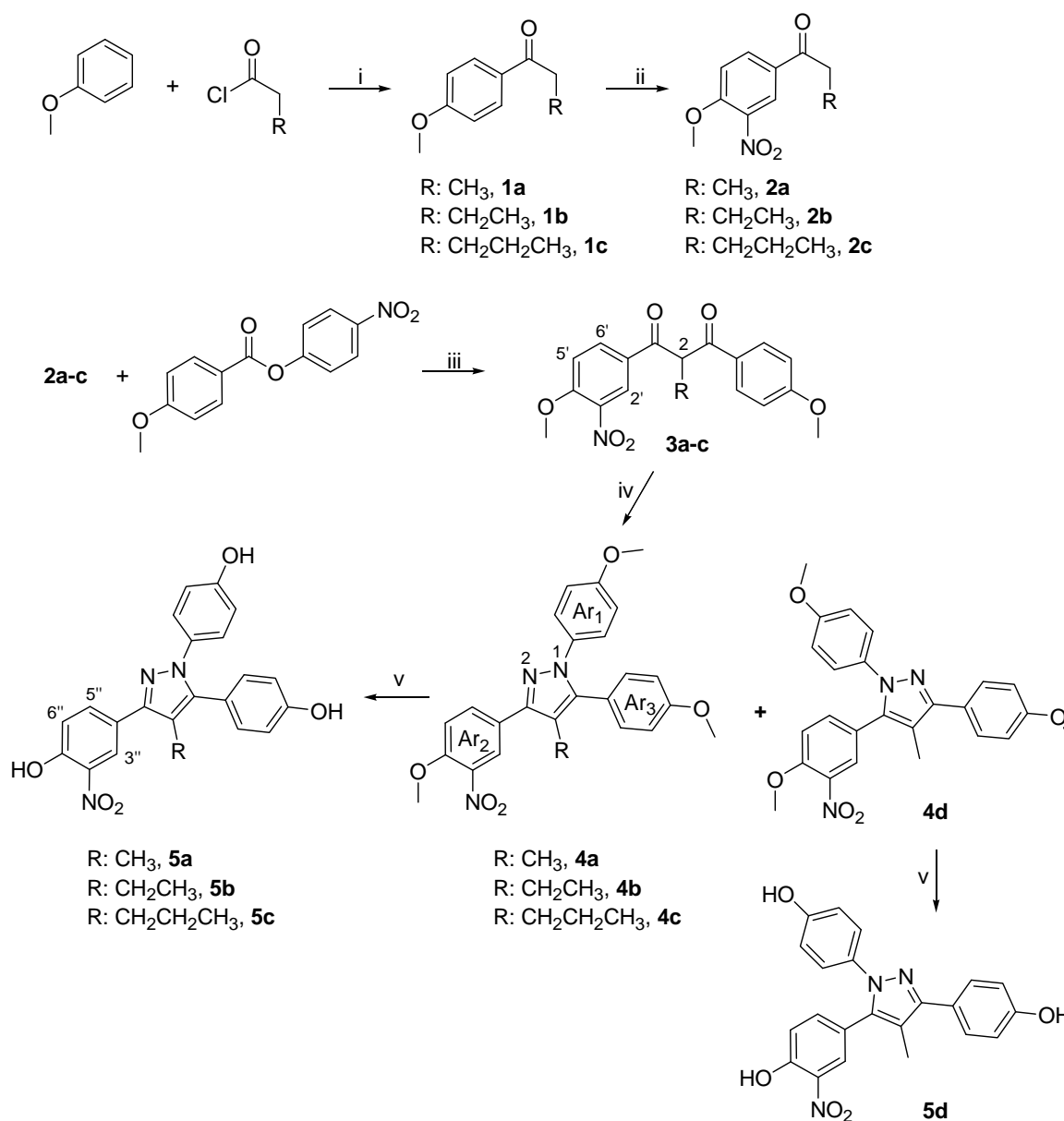
In connection to our interest in ER binding selectivity pattern studies, we envisioned the synthesis of novel tetrasubstituted pyrazole derivatives bearing an *o*-nitro substituent on their A-phenol ring and possess the – critical for binding – alkyl group on C-4 of the pyrazole ring. Recent reports have established that similar ring systems constitute valuable substrates for A-ring functionalised steroidal scaffolds [12] with potential application in asymmetric synthesis [13], supramolecular recognition [14], cancer chemotherapy [15], steroid receptor-based imaging [16] and material science [17]. In this regard, the 4-(and/or 2)-nitro-17 β -estradiol (Figure 1) have been exploited as compounds with anticancer activity and probes for ligand-receptor interaction studies [18–20]. Thus, we were interested in determining the impact of the presence of the electron-rich nitro group, as a peripheral substituent, on the ligand-receptor interaction of the propylpyrazole triol (PPT) analogue, that reduce the possible orientations of the A-phenol ring on the binding pocket of the ER. The design rational of these molecules is to combine in a single molecule the pharmacophore nitroestradiol ring with a tetra substituted pyrazole ring.

Results and Discussion

Chemistry

The synthetic route to the target compounds includes the early stage preparation of the appropriate 1,3-diketones that were further used as substrates for the construction of the desired pyrazole substituted skeleta. In this regard, the starting alkylphenones **1a-c** were prepared in good yields via the Friedel-Crafts acylation of anisole (Scheme 1).

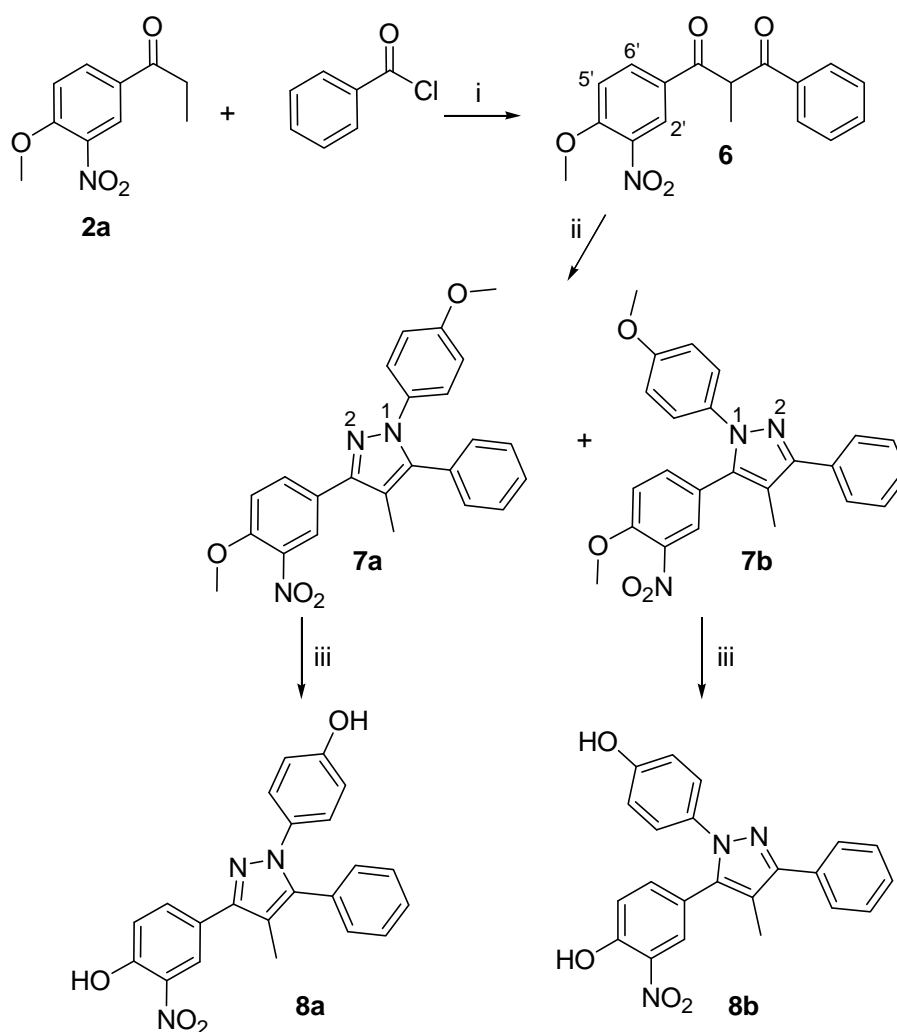
Scheme 1. Synthesis of 1,3,5-triphenols **5a-d**.



Reagents and conditions: (i) AlCl₃, CH₂Cl₂; (ii) KNO₃, H₂SO₄; (iii) LHMDS, THF; (iv) CH₃OArNHNH₂·HCl, DMF / THF (3:1), 120°C; (v) BBr₃·CH₂Cl₂, -78°C.

Nitration of compounds **1a-c** in sulfuric acid produced the nitrophenyl derivatives **2a-c**, which were further alkylated to give β -diketones **3a-c**. Double condensation of the latter with 4-methoxyphenylhydrazine hydrochloride provided the triarylpyrazole (**4a-c**) core ring system in satisfactory yields [21]. In the case of 2-methyl-propane-1,3-dione (**3a**) however, we also obtained the corresponding minor regioisomer **4d** (**4a/4d** ratio = 4:1), presumably because the attack from the opposite site is also possible in this case. This observation is partly in accordance with previously reported relative literature data [10]. The regioisomers obtained were separated by semi-preparative HPLC and their subsequent BBr_3 promoted desmethylation provided the target phenolic pyrazoles **5a-d**.

Scheme 2. Synthesis of 3,5-Diphenols **8a** and **8b**.



Reagents and conditions: (i) LHMDS, THF; (ii) $\text{CH}_3\text{OArNHNH}_2 \cdot \text{HCl}$, DMF / THF (3:1), 120°C ; (iii) BBr_3 , CH_2Cl_2 , -78°C .

The structure of the novel triaryl pyrazoles was determined by 2D-NOE spectroscopy experiments. More specifically, for compound **4d**, the observed enhancement of the deshielded aromatic protons of the phenylhydrazine moiety (Ar_1 , 7.08 ppm, doublet) with the H-6' (resonates at 8.03 ppm) is

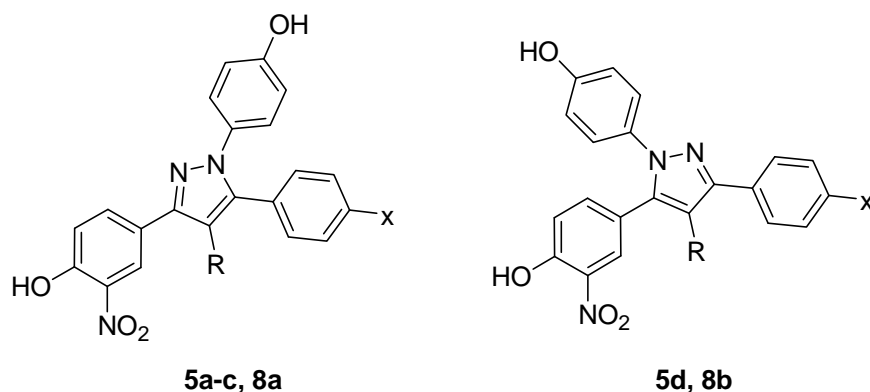
indicative of the assigned regiochemistry. These results were further confirmed by gradient inverse detected long range $^1\text{H} - ^{15}\text{N}$ correlation experiments. Thus, for compound **4d** the four bond correlation observed between the aromatic proton H-6' (8.03 ppm) and the nitrogen atom (N-1) that resonates at 197.4 ppm is an additional confirmation of the assigned structure. Furthermore, for compounds **4a-c** the absence of NOE enhancement among the phenylhydrazine aromatic protons and the H-6' of the nitro-substituted ring is indicative of the assigned regiochemistry. These results were also confirmed by gradient inverse detected long range $^1\text{H} - ^{15}\text{N}$ correlation experiments. In this regard the four bond correlation observed between the aromatic proton H-6' (8.03 ppm) and the deshielded nitrogen atom (N-2) that resonates at 301.6 ppm confirms the regiochemistry of compound **4a**.

The second pathway refers to the use of benzoyl chloride as acylating agent of 1-(4-methoxy-3-nitro-phenyl)-propan-1-one (**2a**) to provide smoothly (Scheme 2) the diketone **6**. The latter by condensation with 4-methoxyphenylhydrazine furnished an equimolar mixture of the regioisomers **7a,b**. These derivatives were identified and analyzed after separation by semi-preparative HPLC as reported in the experimental part.

The structure of these isomers was also determined, through 2D-NOE spectroscopy experiments. More specifically, for compound **7b**, the observed strong cross peak among the deshielded phenylhydrazine group protons (multiplet at 7.37-7.14 ppm) with the H-6' (resonates at 8.09 ppm) of the nitro-substituted ring is indicative of the assigned regiochemistry. These results were further verified by gradient inverse detected long range $^1\text{H} - ^{15}\text{N}$ correlation experiments. In this regard, a four bond correlation for compound **7b** of the same aromatic proton H-6' (8.09 ppm) and the shielded nitrogen atom (N-1) that resonates at 199.8 ppm, was observed. According to the assigned structure the other nitrogen atom (N-2) resonates at 295.7 ppm and correlates with the N-attached aromatic protons and the Ar_3H . Finally the desired diphenols **8a,b** were obtained after ether cleavage with BBr_3 in CH_2Cl_2 at -78°C .

ER Binding Affinity of Tetrasubstituted Pyrazoles to isolated human ER α and ER β

The RBA values of the novel nitro triaryl pyrazole derivatives have been summarized in Table 1, reflecting the consequence of the steric interaction between the C(4)-alkyl substituent and the nitro substituent of the A-ring. In this regard, compound **5c** (PPT nitro analogue) was the most efficient, since was assayed to bind reasonably well to both ER α and ER β . As compared to previously reported results concerning PPT [8], its RBA β binding ability has been increased by 27 fold, while a 9 fold decrease to ER α binding was also observed. Similar results were also obtained for the other triphenol derivatives tested (**5b** and **5d**, 7 and 16 fold respective increase), indicating that the introduction of a nitro group into the A ring of these compounds force the neighboring alkyl group to an orientation that favours the ER β binding.

Table 1. Estrogen receptors (ER α and ER β) binding affinities of Novel Triaryl Pyrazoles (5a-d, 8a,b).

Compound	X	R	RBA ER α ^a	RBA ER β ^a
1,3,5-Triphenols				
5c	OH	<i>n</i> -Pr	5.17 \pm 2.03	3.27 \pm 0.04
5b	OH	Et	0.34 \pm 0.02	0.83 \pm 0.18
5d	OH	Me	0.49 \pm 0.33	1.91 \pm 0.34
5a	OH	Me	<i>nd</i>	<i>nd</i>
3,5-Diphenols				
8a	H	Me	0.74 \pm 0.30	0.36 \pm 0.10
8b	H	Me	0.53 \pm 0.16	0.38 \pm 0.21
PPT	OH	<i>n</i> -Pr	49 \pm 12	0.12 \pm 0.04

^a The RBA values (mean \pm SEM of at least three independent experiments) for ER α (RBA α) and ER β (RBA β) were calculated by [(IC₅₀ estradiol / IC₅₀ derivative) X 100], where IC₅₀ values are estradiol and derivative concentrations capable of inhibiting binding of the fluorescent estrogen ES2 (1 nM) to ER α and ER β by 50%. IC₅₀ values of estradiol for ER α and ER β were 3.42 \pm 0.99 and 2.87 \pm 0.64, respectively. The RBA α and RBA β of estradiol were set equal to 100. *nd*: non determined

Experimental

General

All anhydrous reactions were carried out under argon atmospheres. Solvents were dried by distillation prior to use. Solvent mixtures employed in chromatography were reported as volume to volume ratios. Starting materials were purchased from Aldrich (analytical reagent grades) and used without further purification. Analytical thin-layer chromatography (TLC) was conducted on Merck glass plates coated with silica gel 60 F₂₅₄ and spots were visualized with UV light or/and an alcohol solution of anisaldehyde. Flash column chromatography was performed using Merck silica gel 60 (230-400 mesh ASTM).

Melting points were determined on a Büchi melting point apparatus and are uncorrected. ^1H and 2D NMR spectra were recorded at 400 MHz on a Bruker DRX-400 spectrometer in the indicated solvents. The coupling constants are recorded in Hertz (Hz) and the chemical shifts are reported in parts per million (δ , ppm) downfield from tetramethylsilane (TMS), which was used as an internal standard (by asterisk are indicated the overlapped peaks). For the ^1H - ^{15}N GHMQC spectrum, data were acquired as 3072 x 400 data points with a total of 290 transients accumulated/ t_1 increment. Pulse widths were 8.55 μs for ^1H and 27.7 μs for the ^{15}N at powers of 0 and -3 dB. The F1 spectral window employed was set from 100 to 400 ppm. Pulsed field gradients, gt1-gt3, had durations of 0.8 ms. Gradient pairs were optimized as 70:30:50 for ^{15}N .

Infrared spectra were obtained on a Nicolet Magna 750, series II spectrometer. HPLC separations were performed using a Hewlett Packard 1100 series instrument with a variable wavelength UV detector and coupled to HP Chem.-Station utilizing the manufacturer's 5.01 software package. A Kromasil column with pore size 100 Å, internal diameter of 5 μm , and a C-18 bond phase was used.

General Procedure for the Preparation of Alkylphenones

To an ice-cold solution of AlCl_3 (46.8 mmol) in 1,2-dichloroethane (10 mL) an acid chloride (39.0 mmol) was added dropwise over 10 min. The resulting solution was allowed to reach the room temperature and dissolve the AlCl_3 . Then, the reaction mixture was cooled to 0 °C and a solution of anisole (46.8 mmol) in 1,2-dichloroethane (20 mL) was added dropwise over a period of 30 min. Upon completion, the reaction was allowed to reach the room temperature and stirred for 8-15 h. The mixture was quenched by pouring over ice (100 g) and extracted with EtOAc (3 x 25 mL). The combined organic layers were successively washed with water, NaHCO_3 (satd), brine, dried over anhydrous MgSO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography using a 5% EtOAc/hexanes system as eluent.

1-(4-Methoxy-phenyl)-propan-1-one (1a)

Prepared according to the general procedure as outlined above affording, after flash chromatographic purification (5% EtOAc/hexanes), the title compound was obtained as a pale yellow oil (5.61 g, 73%). ^1H -NMR (CDCl_3) δ , 7.91 (dd, $J = 2.0, 7.0$ Hz, 2H, ArH), 6.89 (dd, $J = 2.0, 7.0$ Hz, 2H, ArH), 3.83 (s, 3H, OCH_3), 2.91 (q, $J = 7.4$ Hz, 2H, $\text{CH}_2\text{C}=\text{O}$), 1.18 (t, $J = 7.4$ Hz, 3H, CH_3).

1-(4-Methoxyphenyl)-butan-1-one (1b)

Anisole (46.8 mmol) was reacted with butyl chloride (39.0 mmol) according to the general procedure. Flash column chromatography (5% EtOAc/hexanes) provided the title compound as a pale yellow oil (5.41 g, 65%). ^1H -NMR (CDCl_3) δ , 8.37 (dd, $J = 2.0, 7.0$ Hz, 2H, ArH), 7.34 (dd, $J = 2.0, 7.0$ Hz, 2H, ArH), 4.28 (s, 3H, OCH_3), 3.31 (t, $J = 7.3$ Hz, 2H, $\text{CH}_2\text{C}=\text{O}$), 2.18 (m, 3H, CH_2), 1.41 (t, $J = 7.3$ Hz, 3H, CH_3).

1-(4-Methoxyphenyl)-pentan-1-one (1c)

Prepared from anisole (46.8 mmol) and valeryl chloride (39.0 mmol) following the general procedure, which afforded the title compound as a yellow oil (6.56 g, 73 %). ¹H-NMR (CDCl₃) δ, 7.91 (dd, *J* = 2.0, 7.0 Hz, 2H, ArH), 6.90 (dd, *J*₁ = 2.0, 7.0 Hz, 2H, ArH), 3.84 (s, 3H, OCH₃), 2.89 (t, *J* = 6.2 Hz, 2H, CH₂C=O), 1.68 (m, 2H, COCH₂CH₂), 1.37 (m, 2H, COCH₂CH₂CH₂), 0.92 (t, *J* = 7.4 Hz, 3H, CH₃).

General Procedure for Nitration.

To an ice-cold solution of **1a-c** (6.7 mmol) in H₂SO₄ (12.4 mL) was added KNO₃ (7.4 mmol) over a period of 30 min. The reaction was run at room temperature for 8-15 h, quenched with H₂O (15-25 mL), extracted repeatedly with EtOAc (3 x 25 mL) and the combined organic layers were washed with a satd NaHCO₃ solution (25 mL). The organic layer was separated, dried over anhydrous MgSO₄ and concentrated under reduced pressure to afford the crude product as a white solid, which was purified by flash chromatography.

1-(4-Methoxy-3-nitro-phenyl)-propan-1-one (2a).

1a (1.1 g, 6.7 mmol) was reacted with KNO₃ (0.74 g, 7.4 mmol) according to the general nitration procedure, to give a dark yellow slurry. The latter was purified by flash chromatography (20% EtOAc/hexanes) yielding a yellow solid (0.95 g, 68%), which was recrystallized from Et₂O to give pale yellow fine needles. M.p. 88-89 °C; ¹H-NMR (CDCl₃) δ, 8.41 (d, *J* = 2.0 Hz, 1H, H-2'), 8.15 (dd, *J* = 2.0, 9.0 Hz, 1H, H-6'), 7.13 (d, *J* = 9.0 Hz, 1H, H-5'), 4.01 (s, 3H, OCH₃), 2.97 (q, *J* = 7.4 Hz, 2H, CH₂CH₃), 1.20 (t, *J* = 7.4 Hz, 3H, CH₂CH₃); Elem. Anal.: C 57.41, H 5.30, N 6.70; C 57.59, H 5.42, N 6.52.

1-(4-Methoxy-3-nitro-phenyl)-butan-1-one (2b)

Prepared according to general procedure for nitration from **1b** (1.19 g, 6.7 mmol) and purified by flash chromatography (20% EtOAc/hexanes) to afford a white solid (0.91 g, 61%). M.p. 69-70 °C; ¹H-NMR (CDCl₃) δ, 8.46 (d, *J* = 2.2 Hz, 1H, H-2'), 8.19 (dd, *J* = 2.2, 8.8 Hz, H-6'), 7.18 (d, *J* = 8.8 Hz, H-5'), 4.06 (s, 3H, OCH₃), 2.94 (t, *J* = 7.3 Hz, 2H, CH₂C=O), 2.63 (m, 2H, CH₂), 1.03 (t, *J* = 7.4 Hz, 3H, CH₃); Elem. Anal.: C 59.19, H 5.87, N 6.27; C 59.39, H 5.72, N 6.41.

1-(4-Methoxy-3-nitro-phenyl)-pentan-1-one (2c)

Prepared according to general procedure for nitration as described above from **1c** and purified by flash chromatography (20% EtOAc/hexanes) to afford a white crystalline solid (1.0 g, 63%). M.p. 81-82 °C; ¹H-NMR (CDCl₃) δ, 8.40 (d, *J* = 2.5 Hz, 1H, H-2'), 8.14 (dd, *J* = 2.5, 9.4 Hz, 1H, H-6'), 7.12 (d, *J* = 9.4 Hz, 1H, H-5'), 4.01 (s, 3H, OCH₃), 2.91 (t, *J* = 7.4 Hz, 2H, CH₂CH₂CH₂CH₃), 1.68 (m, 2H, CH₂CH₂CH₂CH₃), 1.37 (m, 2H, CH₂CH₂CH₂CH₃), 0.92 (t, *J* = 7.4 Hz, 3H, CH₂CH₂CH₂CH₃); Elem. Anal.: C 60.75, H 6.37, N 5.90; C 60.61, H 6.45, N 5.82.

General Procedure for the Preparation of Diketones 3a-c

To an ice-cold solution of **2a-c** (0.97 mmol) and 4-nitrophenyl-4-methoxy benzoate (0.97 mmol) [prepared from *p*-nitrophenol and 4-methoxybenzoic acid using diisopropylcarbodiimide and 4-dimethylaminopyridine] in THF (35 mL), was added a 1.0 M solution of lithium hexamethyldisilamide (3.03 mmol) dropwise over a period of 5 min. The reaction was allowed to reach the room temperature and stirred for additional 1.5 h and quenched with H₂O (25 mL). Then, the mixture was repeatedly extracted with Et₂O and the combined organic layers were washed with H₂O, dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to afford a crude solid. The unreacted ester was removed by the addition of a solution of 40% EtOAc/ hexanes and the filtration of the insoluble ester. The filtrate was concentrated in vacuo and purified by flash chromatography using various proportions of EtOAc/hexanes as eluent system.

1-(4-Methoxy-3-nitrophenyl)-3-(4-methoxyphenyl)-2-methyl-propane-1,3-dione (3a)

Prepared according to general procedure for preparation of diketones outlined above from **2a** (0.6 g, 2.9 mmol) and purified by flash chromatography (20% EtOAc/hexanes) to yield the title product as an orange oil (0.74 g, 74%). ¹H-NMR (CDCl₃) δ, 8.39 (d, *J* = 2.2 Hz, 1H, H-2'), 8.09 (dd, *J* = 2.2, 8.4 Hz, 1H, H-6'), 7.08 (d, *J* = 8.4 Hz, 1H, H-5'), 7.92 (d, *J* = 8.9 Hz, 2H, ArH), 6.93 (d, *J* = 8.9 Hz, 2H, ArH), 5.17 (m, 1H, H-2), 3.97 (s, 3H, OCH₃), 3.84 (s, 3H, OCH₃), 1.52 (d, *J* = 7.0 Hz, 3H, CH₃); Elem. Anal.: C 62.97, H 4.99, N 4.08; C 62.71, H 4.81, N 4.19.

2-Ethyl-1-(4-methoxy-3-nitrophenyl)-3-(4-methoxyphenyl)-propane-1,3-dione (3b)

Ketone **2b** (0.4 g, 1.8 mmol) reacted with 4-nitrophenyl 4-methoxybenzoate (1.8 mmol) as above to afford after flash chromatographic purification (40% EtOAc/hexanes) compound **3b** as a dark yellow oil (0.26g, 68%), while 0.15 g of the ketone **2b** remained unreacted. ¹H-NMR (CDCl₃) δ, 8.46 (d, *J* = 2.2 Hz, 1H, H-2'), 8.19 (dd, *J* = 2.2, 8.9 Hz, 1H, H-6'), 7.99 (d, *J* = 8.9 Hz, 1H, H-5'), 7.74 (d, *J* = 8.9 Hz, 2H, Ar₂H), 6.97 (d, *J* = 8.9 Hz, 2H, Ar₂H), 4.97 (t, *J* = 6.7 Hz, 1H, H-2), 4.02 (s, 3H, OCH₃), 3.89 (s, 3H, OCH₃), 2.18 (m, 2H, CH₂), 1.05 (t, *J* = 7.4 Hz, 3H, CH₃); Elem. Anal.: C 63.86, H 5.36, N 3.92; C 63.61, H 5.45, N 3.82.

1-(4-Methoxy-3-nitrophenyl)-3-(4-methoxyphenyl)-2-propylpropane-1,3-dione (3c)

Prepared according to general procedure for preparation of 1,3-diketones outlined above from **2c** (0.71 g, 3.0 mmol) and purified by flash chromatography (50% EtOAc/hexanes) to provide a red oil (0.44 g, 67%) and 0.29 g of the unreacted ketone **2c**. ¹H NMR (CDCl₃) δ, 8.47 (d, *J* = 2.2 Hz, 1H, H-2'), 8.20 (dd, *J* = 2.2, 8.8 Hz, 1H, H-6'), 7.97 (d, *J* = 8.8 Hz, 2H, Ar₂H), 7.14 (d, *J* = 8.8 Hz, 1H, H-5'), 6.98 (d, *J* = 8.8 Hz, 2H, Ar₂H), 5.05 (t, *J* = 6.6 Hz, 1H, H-2), 4.03 (s, 3H, OCH₃), 3.89 (s, 3H, OCH₃), 2.12 (m, 2H, CH₂), 1.46 (m, 2H, CH₂), 0.99 (t, *J* = 7.3 Hz, 3H, CH₃). Elem. Anal.: C 64.68, H 5.70, N 3.77; C 64.80, H 5.65, N 3.82.

1-(4-Methoxy-3-nitrophenyl)-2-methyl-3-phenylpropane-1,3-dione (6)

To a solution of **2a** (1.21 g, 5.8 mmol) in THF (15 mL), a solution of LHMDS (5.8 mmol, 1M in THF) was added dropwise over 15 min. Upon complete addition of LHMDS, the reaction was stirred for additional 15 min. Then, benzoyl chloride (5.8 mmol, in 3 mL THF) was added dropwise and the reaction was run under stirring for 12 h. The reaction mixture was repeatedly extracted with Et₂O and the combined organic layers were washed with H₂O, dried over MgSO₄ and concentrated under reduced pressure to afford a crude solid, which was purified by flash chromatography eluting using 5% EtOAc/hexanes as solvent eluting system (1.34 g, 74%). ¹H-NMR (CDCl₃) δ, 8.41 (d, *J* = 2.0 Hz, 1H, H-2'), 8.25 (d, *J* = 9.4 Hz, 1H, H-5'), 8.11 (dd, *J* = 2.0, 9.4 Hz, 1H, H-6'), 7.93 (t, *J* = 9.0 Hz, 2H, ArH), 7.46 (m, *J* = 7.8 Hz, 1H, ArH), 7.13 (d, *J* = 9.0 Hz, 2H, ArH), 5.19 (q, *J* = 7.0 Hz, 1H, CHCH₃), 3.99 (s, 3H, OCH₃), 1.24 (d, *J* = 7.0 Hz, 3H, CH₃); Elem. Anal.: C 68.17, H 4.83, N 4.47; C 67.35, H 4.71, N 4.35.

General Procedure for Pyrazole Synthesis

To a solution of diketone (1.0 mmol) in DMF (30 mL) and THF (10 mL), 4-methoxyphenylhydrazine hydrochloride (4-5 equiv) was added. The mixture was brought to reflux and the reaction progress was monitored by TLC analysis (10-20 h). Then the reaction mixture was allowed to cool to room temperature and diluted with H₂O (30 mL). The product was repeatedly extracted with EtOAc (3 x 25 mL) and the combined organic layers and sequentially washed with a satd LiCl solution (25 mL), satd NaHSO₃ (25 mL), and brine (25 mL). The organic layer was dried over Na₂SO₄ and concentrated under reduced pressure to afford a crude oil, which was purified by flash chromatography using EtOAc/hexanes as eluting solvent system.

3-(4-Methoxy-3-nitrophenyl)-1,5-bis-(4-methoxyphenyl)-4-methyl-1H-pyrazole (4a) and 5-(4-methoxy-3-nitrophenyl)-1,3-bis-(4-methoxyphenyl)-4-methyl-1H-pyrazole (4d)

Diketone **3a** was reacted with 4-methoxyphenylhydrazine hydrochlorides as described above. Flash chromatographic purification (20% EtOAc/hexanes) provided an inseparable mixture of the title compounds (68%). The latter were separated by semipreparative HPLC separation using H₂O/CH₃CN (15:85) as eluent in a flow rate of 1.6 ml/min, furnishing the two regioisomers in a 4:1 ratio for **4a** and **4d** respectively (UV detector at 254 nm, *t*_{R4a} = 12.2 min, *t*_{R4d} = 15.4 min). **4a**: ¹H-NMR (CDCl₃) δ, 8.32 (d, *J* = 2.2 Hz, 1H, H-2'), 8.03 (dd, *J* = 2.2, 8.7 Hz, 1H, H-6'), 7.71 (d, *J* = 8.7 Hz, 1H, H-5'), 7.26 (d, *J* = 8.5 Hz, 2H, Ar₃H), 7.14 (d, *J* = 8.9 Hz, 2H, Ar₁H), 6.92 (d, *J* = 8.5 Hz, 2H, Ar₃H), 6.82 (d, *J* = 8.9 Hz, 2H, Ar₁H), 4.02 (s, 3H, OCH₃), 3.84 (s, 3H, OCH₃), 3.80 (s, 3H, OCH₃), 2.24 (s, 3H, CH₃); ¹H-¹⁵N NMR (CDCl₃) δ, N-1 205.6, N-2 301.6; Elem. Anal.: C 67.41, H 5.20, N 9.43; C 67.25, H 5.41, N 9.32; **4d**: ¹H-NMR (CDCl₃) δ, 8.34 (d, *J* = 2.1 Hz, 1H, H-2'), 8.03 (dd, *J* = 2.1, 8.8 Hz, 1H, H-6'), 7.60 (d, *J* = 8.8 Hz, 1H, H-5'), 7.22 (d, *J* = 8.5 Hz, 2H, Ar₃H), 7.08 (d, *J* = 8.7 Hz, 2H, Ar₁H), 6.92 (d, *J* = 8.5 Hz, 2H, Ar₃H), 6.80 (d, *J* = 8.7 Hz, 2H, Ar₁H), 4.04 (s, 3H, OCH₃), 3.85 (s, 3H, OCH₃), 3.80 (s, 3H, OCH₃), 2.16 (s, 3H, CH₃); ¹H-¹⁵N NMR (CDCl₃) δ, N-1 197.4, N-2 294.7; Elem. Anal.: C 67.41, H 5.20, N 9.43; C 67.54, H 5.32, N 9.30.

4-Ethyl-3-(4-methoxy-3-nitrophenyl)-1,5-bis-(4-methoxyphenyl)-1H-pyrazole (4b)

Diketone **3b** (0.23 g, 0.6 mmol) was reacted with 4-methoxyphenylhydrazine hydrochloride (0.53 g, 3.0 mmol) according to the general procedure. Upon purification by flash chromatography (40% EtOAc/hexanes) the title compound was obtained as a tan solid (0.21 g, 75%). M.p. 121-122 °C; ¹H-NMR (CDCl₃) δ, 8.32 (d, *J* = 2.2 Hz, 1H, H-2'), 8.02 (dd, *J* = 2.2, 8.7 Hz, 1H, H-6'), 7.68 (d, *J* = 8.7 Hz, 1H, H-5'), 7.21 (dd, *J* = 2.0, 8.8 Hz, 2H, Ar₃H), 7.17 (dd, *J* = 2.0, 8.7 Hz, 2H, Ar₁H), 6.92 (dd, *J* = 2.0, 8.8 Hz, 2H, Ar₃H), 6.82 (dd, *J* = 2.0, 8.7 Hz, 2H, Ar₁H), 4.03 (s, 3H, OCH₃), 3.85 (s, 3H, OCH₃), 3.80 (s, 3H, OCH₃), 2.67 (q, *J* = 7.5 Hz, 2H, CH₂), 1.09 (t, *J* = 7.5 Hz, 3H, CH₃); ¹H-¹⁵N-NMR (CDCl₃) δ, N-1 201.8, N-2 299.2; Elem. Anal.: C 67.96, H 5.48, N 9.14; C 68.11, H 5.53, N 9.22.

3-(4-Methoxy-3-nitrophenyl)-1,5-bis-(4-methoxyphenyl)-4-propyl-1H-pyrazole (4c)

Diketone **3c** (0.2 g, 0.5 mmol) was reacted with 4-methoxyphenylhydrazine hydrochloride (0.48 g, 2.5 mmol) for 20 h, according to the general procedure. The resulting dark brown residue was purified by flash column chromatography (40% EtOAc/hexanes) to provide the title product **4c** as a pale brown solid (0.1 g, 85%) and 0.1 g of the substrate **3c**. M.p. 131-133 °C; ¹H-NMR (CDCl₃) δ, 8.31 (d, *J* = 2.0 Hz, 1H, H-2'), 8.01 (dd, *J* = 2.0, 8.1 Hz, 1H, H-6'), 7.68 (d, *J* = 8.1 Hz, 1H, H-5'), 7.21 (d, *J* = 7.1 Hz, 2H, Ar₃H), 7.16 (d, *J* = 8.5 Hz, 2H, Ar₁H), 6.93 (d, *J* = 7.1 Hz, 2H, Ar₃H), 6.83 (d, *J* = 8.5 Hz, 2H, Ar₁H), 4.04 (s, 3H, OCH₃), 3.85 (s, 3H, OCH₃), 3.80 (s, 3H, OCH₃), 2.60 (t, *J* = 7.7 Hz, 2H, CH₂), 1.45 (m, 2H, CH₂), 0.84 (t, *J* = 7.2 Hz, 3H, CH₃); ¹H-¹⁵N-NMR (CDCl₃) δ, N-1 202.9, N-2 300.1; Elem. Anal.: C 68.48, H 5.75, N 8.87; C 68.61, H 5.59, N 8.75.

3-(4-Methoxy-3-nitro-phenyl)-1-(4-methoxy-phenyl)-4-methyl-5-phenyl-1H-pyrazole (7a) and 5-(4-Methoxy-3-nitro-phenyl)-1-(4-methoxy-phenyl)-4-methyl-3-phenyl-1H-pyrazole (7b)

A stirred solution of compound **6** (0.33 g, 1.05 mmol) in DMF (30 mL) and THF (10 mL), was reacted with 4-methoxyphenylhydrazine hydrochloride (0.92 g, 5.25 mmol) according to the general procedure for pyrazole synthesis. Upon purification by flash chromatography (10% EtOAc/hexanes), an inseparable mixture of the title compounds was obtained as a dark brown solid (0.16 g, 69%), while 0.15 g of the starting compound **6** was recovered. Semipreparative HPLC separation using H₂O/CH₃CN (20:80) as eluent and flow rate of 1.6 ml/min, provided equal amounts of the two regioisomers (UV detector at 254 nm, *t*_{R7a} = 11.2 min, *t*_{R7b} = 13.7 min). **7a**: M.p. 127-128 °C; ¹H-NMR (CDCl₃) δ, 8.42 (d, *J* = 2.2 Hz, 1H, H-2'), 8.11 (dd, *J* = 2.2, 8.7 Hz, H-6'), 7.45 (d, *J* = 8.7 Hz, 1H, H-5'), 7.28-7.07 (m, 4H, ArH), 7.01-6.97 (m, 5H, ArH), 4.35 (s, 3H, OCH₃), 3.80 (s, 3H, OCH₃), 2.28 (s, 3H, CH₃). ¹H-¹⁵N-NMR (CDCl₃) δ, N-1 203.7, N-2 299.6; Elem. Anal.: C 69.39, H 5.10, N 10.11; C 69.51, H 5.02, N 10.30; **7b**: M.p. 141-143 °C; ¹H-NMR (CDCl₃) δ, 8.30 (d, *J* = 2.2 Hz, 1H, H-2'), 8.09 (dd, *J* = 2.2, 8.7 Hz, 1H, H-6'), 7.40 (d, *J* = 8.7 Hz, 1H, H-5'), 7.37-7.14 (m, 4H, ArH), 7.08-6.89 (m, 5H, ArH), 4.04 (s, 3H, OCH₃), 3.80 (s, 3H, OCH₃), 2.25 (s, 3H, CH₃); ¹H-¹⁵N-NMR (CDCl₃) δ, N-1 199.8, N-2 295.7; Elem. Anal.: C 69.39, H 5.10, N 10.11; C 69.67, H 4.93, N 9.94.

General Demethylation Procedure

To a stirred solution of methyl-protected pyrazole (1 equiv) in CH₂Cl₂ at -78 °C was added dropwise a 1M BBr₃ solution in CH₂Cl₂ (3-5 equiv). Upon completion of BBr₃ addition, the reaction was stirred at -78 °C for 1 h and then allowed to reach room temperature. Stirring was continued for additional 16 h, then the mixture was cooled to 0 °C, and carefully quenched with H₂O (15-25 mL) and extracted repeatedly with EtOAc. The combined organic layers were dried over Na₂SO₄ and evaporated under reduced pressure. Flash chromatographic purification using a 5% MeOH/CH₂Cl₂ mixture as the eluting solvent system provided the desired demethylation products.

4-[1,5-Bis-(4-hydroxyphenyl)-4-methyl-1H-pyrazol-3-yl]-2-nitrophenol (**5a**)

A stirred solution of **4a** in CH₂Cl₂ was deprotected using BBr₃ according to the general demethylation procedure. Purification by flash chromatography afforded the title compound as a tan solid (87%). M.p. 155-156 °C; ¹H-NMR (acetone-D₆) δ, 8.87 (s, 3H, OH), 8.52 (d, *J* = 2.2 Hz, 1H, H-3''), 8.19 (dd, *J* = 2.2, 8.7 Hz, 1H, H-5''), 7.32 (d, *J* = 8.7 Hz, 1H, H-6''), 7.16 (d, *J* = 8.6 Hz, 2H, Ar₃H), 7.11 (d, *J* = 8.6 Hz, 2H, Ar₁H), 6.91 (d, *J* = 8.6 Hz, 2H, Ar₃H), 6.81 (d, *J* = 8.6 Hz, 2H, Ar₁H), 2.26 (s, 3H, CH₃). IR (ZnSe) ν = 3303 cm⁻¹, (OH); Elem. Anal.: C 65.50, H 4.25, N 10.42; C 65.31, H 4.11, N 10.58.

4-[2,5-Bis-(4-hydroxyphenyl)-4-methyl-1H-pyrazol-3-yl]-3-nitrophenol (**5d**)

A stirred solution of **4d** in CH₂Cl₂ was deprotected using BBr₃ according to the general demethylation procedure. Purification by flash chromatography afforded the title compound as a pale yellow solid (88%). M.p. 161-162 °C; ¹H-NMR (acetone-D₆) δ, 8.86 (s, 3H, OH), 8.05 (d, *J* = 1.9 Hz, 1H, H-3''), 7.66 (dd, *J* = 1.9, 8.9 Hz, 1H, H-5''), 7.23 (d, *J* = 8.9 Hz, 1H, H-6''), 7.19 (d, *J* = 8.8 Hz, 2H, Ar₃H), 7.02 (d, *J* = 8.8 Hz, 2H, Ar₁H), 6.95 (d, *J* = 8.8 Hz, 2H, Ar₃H), 6.83 (d, *J* = 8.8 Hz, 2H, Ar₁H), 2.25 (s, 3H, CH₃). IR (ZnSe) ν = 3313 cm⁻¹ (OH); Elem. Anal.: C 65.50, H 4.25, N 10.42; C 65.20, H 4.14, N 10.53.

4-[4-Ethyl-1,5-bis-(4-hydroxyphenyl)-1H-pyrazol-3-yl]-2-nitrophenol (**5b**)

A stirred solution of **4b** in CH₂Cl₂ was deprotected using BBr₃ according to the general demethylation procedure. Purification by flash chromatography afforded the title compound as a tan solid (74%). ¹H-NMR (acetone-D₆) δ, 10.52 (s, 1H, OH), 8.73 (s, 1H, OH), 8.64 (s, 1H, OH), 8.53 (d, *J* = 2.2 Hz, 1H, H-3''), 8.19 (dd, *J* = 2.2, 8.7 Hz, 1H, H-5''), 7.33 (d, *J* = 8.7 Hz, 1H, H-6''), 7.16 (d, *J* = 8.6 Hz, 2H, Ar₃H), 7.13 (d, *J* = 8.6 Hz, 2H, Ar₁H), 6.92 (d, *J* = 8.6 Hz, 2H, Ar₃H), 6.80 (d, *J* = 8.6 Hz, 2H, Ar₁H), 2.69 (q, *J* = 7.5, 12.5 Hz, 2H, CH₂), 1.11 (t, *J* = 7.5 Hz, 3H, CH₃). IR (ZnSe) ν = 3283 cm⁻¹, (OH); Elem. Anal. : C 66.18, H 4.59, N 10.07; C 66.33, H 4.74, N 9.88.

4-[1,5-Bis-(4-hydroxyphenyl)-4-propyl-1H-pyrazol-3-yl]-2-nitrophenol (5c)

A stirred solution of **4c** in CH₂Cl₂ was deprotected using BBr₃ according to the general demethylation procedure. Purification by flash chromatography afforded the title compound as a tan solid (60%). ¹H-NMR (acetone-D₆) δ, 10.52 (s, 1H, OH), 8.78 (s, 1H, OH), 8.68 (s, 1H, OH), 8.50 (d, *J* = 2.2 Hz, 1H, H-3''), 8.20 (dd, *J* = 2.1, 8.7 Hz, 1H, H-5''), 7.38 (d, *J* = 8.7 Hz, 1H, H-6''), 7.16 (d, *J* = 8.3 Hz, 2H, Ar₃H), 7.12 (d, *J* = 7.7 Hz, 2H, Ar₁H), 6.89 (d, *J* = 8.3 Hz, 2H, Ar₃H), 6.81 (d, *J* = 7.7 Hz, 2H, Ar₁H), 2.65 (t, *J* = 7.9 Hz, 2H, CH₂), 1.20 (m, 2H, CH₂), 0.85 (t, *J* = 7.9 Hz, 2H, CH₃); IR (ZnSe) ν = 3283 cm⁻¹ (OH); Elem. Anal.: C 66.81, H 4.91, N 9.74; C 67.02, H 5.10, N 9.88.

4-[1-(4-Hydroxyphenyl)-4-methyl-5-phenyl-1H-pyrazol-3-yl]-2-nitrophenol (8a)

A stirred solution of **7a** in CH₂Cl₂ was deprotected using BBr₃ according to the general demethylation procedure. Purification by flash chromatography afforded the title compound as a tan solid (70%). ¹H-NMR (acetone-D₆) δ, 9.30 (s, 1H, OH), 8.70 (s, 1H, OH), 8.40 (d, *J* = 2.2 Hz, 1H, H-3''), 8.07 (dd, *J* = 2.2, 8.7 Hz, H-5''), 7.35 (d, *J* = 8.7 Hz, 1H, H-6''), 7.19-7.11 (m, 5H, ArH), 7.08-6.98 (m, 4H, ArH), 4.35 (s, 3H, CH₃O), 3.80 (s, 3H, CH₃O), 2.28 (s, 3H, CH₃); IR (ZnSe) ν = 3193 cm⁻¹ (OH); Elem. Anal.: C 68.21, H 4.42, N 10.85; C 68.44, H 4.29, N 10.71.

4-[2-(4-Hydroxyphenyl)-4-methyl-5-phenyl-2H-pyrazol-3-yl]-2-nitrophenol (8b)

A stirred solution of **7b** in CH₂Cl₂ was deprotected using BBr₃ according to the general demethylation procedure. Purification by flash chromatography afforded the title compound as a tan solid (78%). ¹H-NMR (acetone-D₆) δ, 9.28 (s, 1H, OH), 8.69 (s, 1H, OH), 8.25 (d, *J* = 2.2 Hz, 1H, H-3''), 8.02 (dd, *J* = 2.2, 8.7 Hz, 1H, H-5''), 7.47-7.24 (m, 4H, ArH), 7.26 (d, *J* = 8.7 Hz, 1H, H-6''), 6.98-6.78 (m, 5H, ArH), 4.04 (s, 3H, CH₃O), 3.80 (s, 3H, CH₃O), 2.25 (s, 3H, CH₃); IR (ZnSe) ν = 3199 cm⁻¹ (OH); Elem. Anal.: C 68.21, H 4.42, N 10.85; C 68.39, H 4.49, N 10.71.

Binding to isolated human ERα and ERβ

The relative binding affinity (RBA) values were assessed as previously described [22]. Briefly, the concentrations of **5a-d**, **8a,b** that inhibited ES2 (a fluorescein-labelled estrogen from Invitrogen) binding to isolated human ERα or ERβ (Invitrogen) by 50% (IC₅₀), as assessed using a Beacon 2000 Fluorescence Polarization Reader (Invitrogen), were used to derive the RBA values of Table 1, as described in the legend to the Table.

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