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Novel Highly Potent and Selective Nonsteroidal Aromatase Inhibitors: Synthesis, Biological Evaluation and Structure−Activity Relationships Investigation

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In further pursuing our search for potent and selective aromatase inhibitors, a new series of molecules was designed and synthesized, exploring possible structural modifications of a previously identified xanthone scaffold. Among them, highly potent compounds, with inhibitory activity in the low nanomolar range, were found. In particular, substitution of the heterocyclic oxygen atom in the xanthone core by a sulfur atom and/or increase in structure flexibility seemed to be favorable for the interaction with the enzyme.

Introduction

Breast cancer is one of the leading causes of cancer-related mortality among women worldwide.[1] In a high percentage of cases, it proves to be hormone-dependent because tumor progression is dependent on high levels of circulating estrogens, which play a critical role in cancer cell proliferation. Moreover, in postmenopausal women, biologically active estrogens are locally produced from circulating inactive steroids in an intracrine mechanism in breast cancer tissues and confer estrogenic activities to carcinoma cells.[2] A series of enzymes are involved in this intratumoral or in situ production of estrogens in breast carcinoma tissues, but aromatase (CYP19[a]), a member of the cytochrome P450 family, is the key enzyme involved in their synthesis, promoting the aromatization of the A ring of androgen precursors.[3] Different strategies have been devised to control or block the progression of hormone-dependent breast cancer, and one of the main approaches involves reduction of estrogen levels by inhibition of CYP19. Although third generation aromatase inhibitors (AIs), such as letrozole, anastrozole, and exemestane (Chart 1), are now considered a valid alternative to tamoxifen as first line treatment of advanced breast cancer,[4,5] the search for potent and selective AIs still remains an attractive subject.[6−9] Moreover, alternative strategies such as development of multipotent compounds[10,11] are being evaluated from different research groups to deal with the complexity and multiplicity of factors involved in the development of hormone-dependent breast cancer.[12]

In the first paper of this series, we reported on some molecules featuring different oxygenated heterocycles such as flavone, chromone, and xanthone[13] as aromatase inhibitors. Then,

Chart 1. Most Representative Aromatase Inhibitors

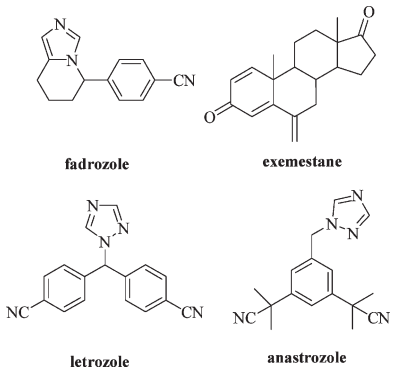
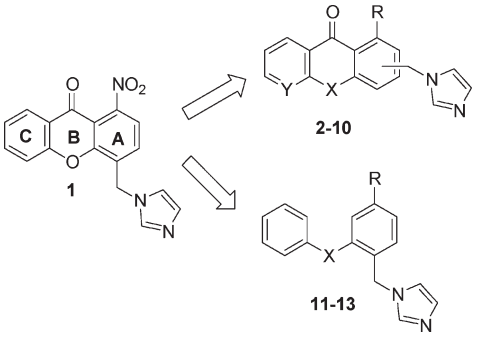


Chart 2. Design Strategy for the New Compounds



with the aim to increase potency and selectivity versus other CYP enzymes, mainly 17α-hydroxylase/17,20-lyase (CYP17), a cytochrome P450 involved in the synthesis of androgens, several concerted structural modifications on these scaffolds were introduced and very potent and selective AIs were synthesized.[14−16] Among early synthesized molecules,[13] high inhibitory activity was shown by appropriately substituted xanthones such as **1** (Chart 2), confirming the importance of

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[a] Abbreviations: CYP19, aromatase; AIs, aromatase inhibitors; CYP17, 17α-hydroxylase/17,20-lyase; PPA, polyphosphoric acid; SAR, structure−activity relationships.

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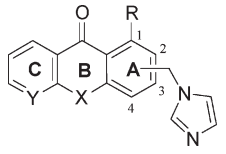
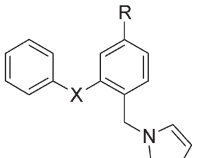
Table 1. Structures and Biological Profile of the New Compounds



1-10



11-13

compd	CH_2-imid position	R	X	Y	CYP19[a] IC_{50} nM[c] or % inhib (10 μM)	CYP17[b] IC_{50} nM[c] or % inhib (2.5 μM)
1	4	1-NO_2	O	CH	40	4%
2	4	2-NO_2	O	CH	53	NA
3	4	3-NO_2	O	CH	1900	9%
4	4	1-NO_2	S	CH	16.5	5%
5	4	1-NO_2	O	N	101	NA
6	4	H	O	CH	17	17%
7	4	H	S	CH	3.98	28%
8	3	H	O	CH	390	33%
9	2	H	O	CH	7900	11%
10	1	H	O	CH	150	880
11		NO_2	O		11.45	NA
12		NO_2	S		5.59	17%
13		H	O		389.2	8%
fadrozole					52	NA
ketoconazole					17.7%	2780

[a] Human aromatase, placental microsomes, substrate 1β[^{3}H]androstenedione 500 nM. [b] Human CYP17 expressed in *E. coli*, substrate progesterone 25 μM. [c] The given values are mean values of at least three experiments. The deviations were within ±5%. "NA" = no activity detected.

this class of heterocycles in several medicinal chemistry research fields.[17,18]

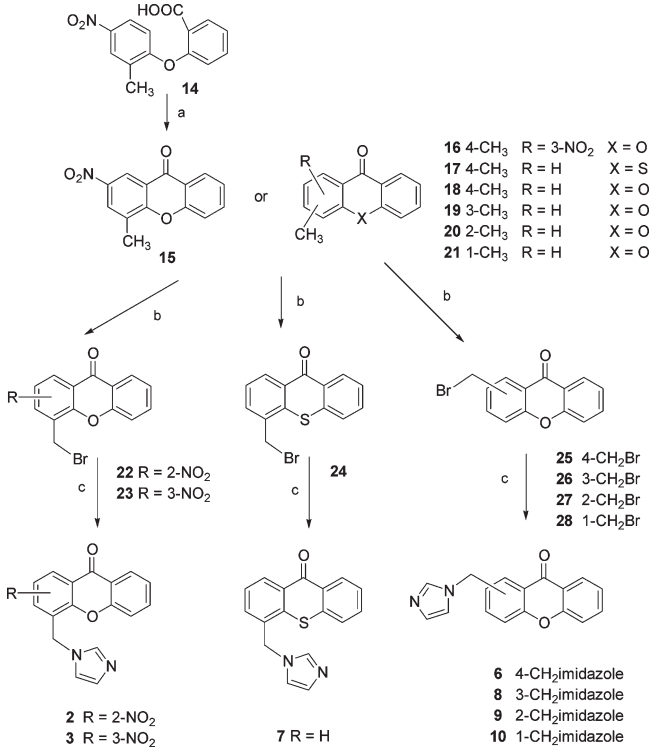
Thus, from further exploring possible structural modifications on the scaffold of **1** in order to investigate the effect on aromatase inhibition and to confirm the hypothesis of an H-bond network of these ligands with the enzyme,[13,16] we here report on xanthones, aza- and thioxanthones, and phenoxy- and phenylsulfanylbenzylimidazoles, lacking the ketone moiety, which can be regarded as bioisosteres and "open" analogues of the parent structure **1**, respectively, with or without an H-bond accepting substituent (Chart 2). In particular, the appropriate distances between the imidazole nitrogen, the nitro and the carbonyl moieties were explored shifting the position of the nitro group on the xanthone nucleus (compounds **2** and **3**). Thioxanthone and azaxanthone bioisosteres (**4** and **5**, respectively) were also synthesized, and finally, the roles of the putative H-bond accepting groups on the different scaffolds were evaluated by removing the nitro group (**6** and **7**), varying the position of the imidazole with respect to the carbonyl (**8**−**10**) or removing the carbonyl itself (**11**−**13**). The structures of the new compounds are shown in Table 1.

Chemistry

The xanthen- and thioxanthen-9-one derivatives **2**−**3** and **6**−**10** (Scheme 1) were obtained by reaction of bromo derivatives **22**−**28** with imidazole. While compounds **23**−**28** were previously described[19−21] and synthesized according to literature procedures, compound **22** was obtained by bromination of the corresponding methyl derivative **15**. This intermediate was synthesized by cyclization in PPA of **14**, obtained via the Ullmann reaction, heating *o*-chlorobenzoic acid and 2-methyl-4-nitrophenol with potassium carbonate, Cu, and CuI. 3-Imidazol-1-ylmethylxanthen-9-one **8**, described in a previous paper,[13] was also included in this series.

In Scheme 2, the synthesis of compounds **4**, **11**, and **12** is depicted: compound **4** was obtained by cyclization of the 2-(2-methyl-5-nitrophenylsulfanyl)benzoic acid **29**, prepared via the Ullmann reaction from 2-mercaptobenzoic acid and 2-bromo-1-methyl-4-nitrobenzene, followed by bromination and reaction with imidazole. The same intermediate was decarboxylated by heating at 220 °C with a catalytic amount of Cu and then treated as above to obtain compound **12**.

Scheme 1[a]



[a] Reagents and conditions: (a) PPA, 120 °C; (b) NBS, CCl_4, benzoyl peroxide, reflux; (c) imidazole, N_2, CH_3CN, reflux.

Compound **11** was prepared as described for **12**, starting from 2-(2-methyl-5-nitrophenoxy)benzoic acid **30**, prepared via the Ullmann reaction from salicylic acid. The same procedure was applied to prepare compound **13** (Scheme 3), starting from 2-*o*-tolyloxybenzoic acid.[22]

Finally, compound **5** (Scheme 4) was synthesized starting from 8-methyl-9-oxa-1-aza-anthracen-10-one **39**,[23] which was nitrated, brominated, and then reacted with imidazole. All the final compounds were characterized by ^{1}H and ^{13}C NMR, mass spectra, and HPLC.

Biological Evaluation

The new compounds were tested for inhibition of aromatase using human placental microsomes[24] incubated with 1β[^{3}H]-androstenedione and measuring the tritiated water formed during the aromatization of the substrate, as previously described.[25] The inhibitory activity of the compounds toward 17α-hydroxylase/17,20-lyase (CYP17) was also assessed in order to evaluate their selectivity toward a related enzyme. For the CYP17 inhibition tests human CYP17 expressed in *Escherichia coli* and P450 reductase were used.[25] In these experiments, fadrozole[26] (Chart 1) was used as a positive control for aromatase and ketoconazole[27] as a positive control for 17α-hydroxylase/17,20-lyase.

Results and Discussion

Xanthones are well-known natural and synthetic chemical entities that were found to be endowed with several important biological activities. Their interaction with different biological targets appears to be dependent on the substitution pattern on the heterocyclic scaffold because the nature and position of different substituents could direct the biological effect of the molecule.[17,18] Recently, some natural occurring prenylated xanthones, derived from the pericarp of *Garcinia mangostana*, have shown aromatase inhibition properties.[28] As regards synthetic derivatives, a xanthone scaffold bearing an imidazole ring able to contact the heme iron of the enzyme and a group capable of establishing H-bond interactions with the enzyme, in particular the nitro group, have proved to be critical features for antiaromatase activity.[13] In this study, the role of each element in the assessment of the inhibitory potency of the compounds was evaluated. Table 1 shows that some of the new compounds were highly potent AIs, with an inhibitory activity in the low nanomolar range. Moreover, all the compounds showed high selectivity for aromatase with respect to lyase. In particular, only very low percentages of inhibition of CYP17 were seen at the highest dose tested (2.5 μM) for most of the studied compounds, while for some of them no activity could be detected. Interestingly, the only compound for which an IC_{50} for inhibition of lyase could be determined (**10**) is the only one that carries the imidazole substituent in position 1, confirming that this pattern of substitution is related to a higher affinity for this enzyme, as seen with a previous series of molecules.[13]

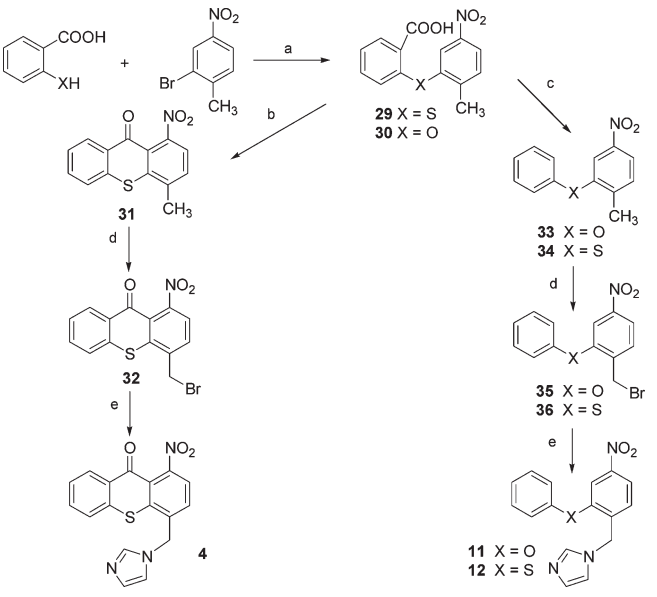
Hereafter, we report on the results of the structure−activity relationships (SAR) analysis.

Role of the Heteroatom on the Potency of the Compounds. The insertion of different heteroatoms (X, Y in Table 1) on the central core of the molecule, maintaining the nitro group in the same position as for the parent compound **1**, strongly influenced the inhibitory potency. While the introduction of a nitrogen, to give the azaxanthone **5**, caused a significant drop in the activity, the thioxanthone **4**, in which the oxygen was substituted by a sulfur leading to a more lipophilic derivative, showed an increase in potency with respect to **1**.

Role of the Nitro Group on the A-Ring. Taking into account the relative distances between the imidazole nitrogen and the nitro group, it can be noticed that moving the latter from position 1 to position 2 on the xanthone nucleus (compound **2**) did not seem to modify the potency of the compounds, while a further repositioning closer to the imidazole (**3**) led to a significant decrease in activity. Here, the occurrence of steric hindrance between these key features of the molecules could be responsible for this drop in activity, leading to a less favorable interaction with the heme iron of aromatase.

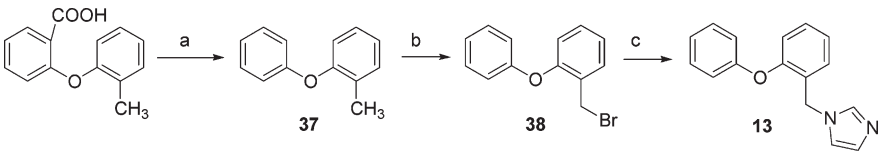
Successively, the nitro group was removed from both the xanthone and the thioxanthone scaffolds (compounds **6** and **7**, respectively) in order to explore the possibility for the ketone to act as H-bond acceptor, mimicking the NO_2 of **1** and **4**, as seen for previous series of AIs.[16] Both compounds showed an increase in activity, confirming the role of the ketone that proved to be in the proper position to interact with the enzyme as postulated above. Again, the thioxanthone

Scheme 2[a]



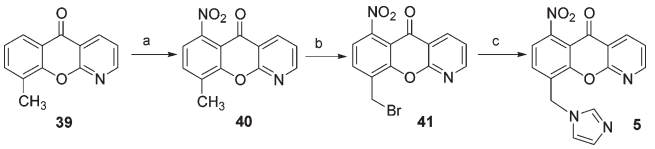
[a] Reagents and conditions: (a) Cu, CuI, K_2CO_3, nitrobenzene, 180 °C; (b) PPA, 120 °C; (c) Cu, 220 °C; (d) NBS, CCl_4, benzoyl peroxide, reflux; (e) imidazole, N_2, CH_3CN, reflux.

Scheme 3[a]



[a] Reagents and conditions: (a) Cu, 220 °C; (b) NBS, CCl_4, benzoyl peroxide, reflux; (c) imidazole, N_2, CH_3CN, reflux.

Scheme 4[a]



[a] Reagents and conditions: (a) HNO_3, H_2SO_4, 0−5 °C; (b) NBS, CCl_4, benzoyl peroxide, reflux; (c) imidazole, N_2, CH_3CN, reflux.

showed higher activity with respect to the oxygen analogue. Considering compounds **1**, **4**, **5**, **6**, and **7**, a close relationship between decrease in lipophilicity and loss of potency could be suggested, pointing out the important role of this physicochemical property for high aromatase inhibition. Moreover, the increase in activity for **6** and **7** could also be a consequence of reduced overall steric hindrance due to the absence of the nitro group competing with the ketone for the H-bond with aromatase.

Spatial Relationship between Ketone and Imidazole. To further evaluate the spatial relationship between the ketone and the imidazole ring, the position of the imidazolylmethylene on the xanthone scaffold was varied. While a decrease of a log unit in activity was noticed moving the chain from position 4 to position 3 and again moving it to position 2 (compounds **8** and **9**, respectively), some potency was regained when the chain was placed in position 1 on the xanthone (compound **10**). Still, none of these modifications could increase the activity, leading to the identification of position 4 as the most favorable for an optimal positioning of the side chain with respect to the ketone.

Influence of Conformational Flexibility and the Role of the Nitro Group on the Diphenyl-Ether and -Thioether. Finally, the ketone itself was removed, keeping the nitro group from both the xanthone and the thioxanthone leading to the corresponding more flexible diphenyl-ether **11** and -thioether **12**, respectively. This modification allowed us to obtain derivatives for which a closer similarity to clinically available compounds, such as letrozole and anastrozole, could be observed. Both compounds proved to be very potent aromatase inhibitors, showing activity in the low nanomolar range. When compared to the corresponding cyclic derivatives **1** and **4**, it clearly appeared that the increase in flexibility led to an increase in activity for both molecules. On the other hand, these flexible derivatives did not show any significant improvement with respect to the most potent compound of the series (**7**). Again, the substitution of the oxygen by a sulfur atom proved to be beneficial for the inhibition and compound **12** resulted one of the most potent AIs synthesized so far. The importance of the nitro group as H-bond acceptor in this series of molecules, due to the absence of the ketone moiety, was confirmed by the synthesis of compound **13**, in which the NO_2 was removed, which showed very low activity.

Conclusions

Xanthones have long been recognized as an important class of natural and synthetic compounds, endowed with a wide range of interesting biological activities. In particular, they proved to be useful molecular scaffolds that could be functionalized in order to meet the structural requirements of different biological systems. In pursuing our search for novel improved aromatase inhibitors, we introduced some modifications on a previously reported xanthone (**1**) that led to a significant increase in activity. In particular, substitution of the oxygen by a sulfur atom always enhanced the potency, likely due to an increase in lipophilicity. The nitro group proved not to be essential in xanthone derivatives, as the ketone itself was in the proper position to form H-bonds with the enzyme when no nitro group was present. On the other hand, when the ketone was removed, the NO_2 became essential for H-bond interaction, stabilizing the more flexible diphenyl-ether derivatives and leading to a slight increase in potency. Among the new compounds, **7** and **12** (IC_{50} 3.98 and 5.59 nM, respectively) resulted the most potent AIs synthesized in this project. Considering that the high activity of these compounds was achieved in in vitro testing, further development strategies for the present molecules, together with previous obtained potent AIs, could involve in depth investigations, further biological evaluation, and assessment of their selectivity toward other significant P450 enzymes.

Experimental Section

Chemistry. General Methods. See Supporting Information.

General Method for Preparation of Imidazol-1-yl Derivatives 2−13. A mixture of the selected bromomethyl derivative (0.001 mol) and imidazole (0.003 mol) in 50 mL of acetonitrile was refluxed for 7 h under nitrogen. The solvent was removed under reduced pressure, and the residue was purified by flash chromatography.

4-Imidazol-1-ylmethyl-2-nitroxanthen-9-one (2). Starting from **22**, 0.22 g of **2** (71%) were obtained (toluene/acetone 3:2), mp 179 °C (dec). ^{1}H NMR: δ 5.70 (s, 2H, *CH$_2$-imi*), 7.00−8.40 (m, 9H, *Ar+imi*). ^{13}C NMR: δ 44.85, 117.12, 121.32, 121.86, 122.48, 125.78, 126.54, 127.03, 127.43, 127.69, 129.76, 132.01, 140.03, 141.27, 157.89, 164.45, 186.98. ES-MS *m/s*: 322 (MH^+).

4-Imidazol-1-ylmethyl-3-nitroxanthen-9-one (3). Starting from **23**,[19] 0.20 g of **3** (63%) were obtained (toluene/acetone 3:2), mp 153−155 °C. ^{1}H NMR: δ 5.80 (s, 2H, *CH$_2$-imi*), 7.20−8.40 (m, 9H, *Ar+imi*). ^{13}C NMR: δ 37.03, 116.11, 116.98, 121.36, 122.58, 125.88, 126.50, 127.68, 129.84, 131.87, 132.67, 139.78, 152.43, 157.93, 159.35, 187.01. ES-MS *m/s*: 322 (MH^+).

4-Imidazol-1-ylmethyl-1-nitrothioxanthen-9-one (4). Starting from **32**, 0.22 g of **4** (66%) were obtained (toluene/acetone 3:2), mp 189−191 °C. ^{1}H NMR: δ 5.45 (s, 2H, *CH$_2$-imi*), 6.90−8.45 (m, 9H, *Ar+imi*). ^{13}C NMR: δ 48.35, 121.55, 122.54, 126.03, 126.67, 130.64, 133.08, 133.35, 134.33, 134.97, 135.29, 139.66, 140.54, 146.11, 147.80, 186.95. ES-MS *m/s*: 338 (MH^+).

8-Imidazol-1-ylmethyl-5-nitro-9-oxa-1-azaanthracen-10-one (5). Starting from **41**, 0.12 g (38%) of **5** were obtained (toluene/acetone 9.5:0.5), mp 202−207 °C. ^{1}H NMR: δ 5.40 (s, 2H, *CH$_2$-imi*), 7.00−8.55 (m, 8H, *Ar+imi*). ^{13}C NMR: δ 45.72, 117.98, 119.46, 119.97, 120.76, 122.48, 126.03, 135.21 136.55, 139.76, 140.39, 147.44, 151.66, 157.00, 164.76, 187.21. ES-MS *m/s*: 323 (MH^+).

4-Imidazol-1-ylmethylxanthen-9-one (6). Starting from **25**,[20] 0.14 g (50%) of **6** were obtained (toluene/acetone 3:2), mp 195−196 °C. ^{1}H NMR: δ 5.55 (s, 2H, *CH$_2$-imi*), 6.60−8.30 (m, 10H, *Ar+imi*). ^{13}C NMR: δ 45.53, 116.89, 121.11, 121.43, 122.51, 125.80, 126.21, 126.44, 126.66, 126.90, 129.87, 132.09, 132.58, 139.79, 157.59, 158.33, 187.00. ES-MS *m/s*: 277 (MH^+).

4-Imidazol-1-ylmethylthioxanthen-9-one (7). Starting from **24**,[21] 0.17 g (58%) of **7** were obtained (toluene/acetone 3:2), mp 112−115 °C. ^{1}H NMR: δ 5.38 (s, 2H, *CH$_2$-imi*), 6.98−8.30 (m, 10H, *Ar+imi*). ^{13}C NMR: δ 48.17, 121.98, 126.05, 126.45, 126.87, 128.04, 129.89, 132.23, 132.67, 133.04, 134.12, 139.78, 139.89, 140.23, 140.43, 186.96. ES-MS *m/s*: 293 (MH^+).

2-Imidazol-1-ylmethylxanthen-9-one (9). Starting from **27**,[20] 0.16 g (62%) of **9** were obtained (toluene/acetone 3:2), mp 175−176 °C. ^{1}H NMR: δ 5.60 (s, 2H, *CH$_2$-imi*), 6.80−8.10 (m, 10H, *Ar+imi*). ^{13}C NMR: δ 55.23, 116.85, 117.03, 121.23, 122.56, 126.01, 126.55, 126.78, 129.80, 130.67, 131.98, 132.54, 139.78, 154.54, 157.56, 187.11. ES-MS *m/s*: 277 (MH^+).

1-Imidazol-1-ylmethylxanthen-9-one (10). Starting from **28**,[20] 0.11 g (42%) of **10** were obtained (toluene/acetone 3:2), mp 169−170 °C. ^{1}H NMR: δ 5.45 (s, 2H, *CH$_2$-imi*), 6.85−8.15 (m, 10H, *Ar+imi*). ^{13}C NMR: δ 49.13, 114.13, 117.36, 121.21, 121.97, 122.56, 125.87, 126.60, 127.45, 129.89, 131.76, 131.98, 138.89, 139.76, 157.34, 157.65, 186.69. ES-MS *m*/*s*: 277 (MH^+).

1-(4-nitro-2-phenoxybenzyl)-1*H*-imidazole (11). Starting from **35**, 0.18 g (62%) of **11** were obtained (toluene/acetone 4:1), mp 107−110 °C. ^{1}H NMR: δ 5.35 (s, 2H, *CH$_2$-imi*), 7.00−7.90 (m, 11H, *Ar+imi*). ^{13}C NMR: δ 45.85, 112.45, 116.53, 117.11, 121.34, 122.65, 126.02, 128.11, 129.78, 132.56, 139.91, 144.89, 155.98, 157.87. ES-MS *m*/*s*: 296 (MH^+).

1-(4-Nitro-2-phenylsulfanylbenzyl)-1*H*-imidazole (12). Starting from **36**, 0.21 g (71%) of **12** were obtained (toluene/acetone 3:2), mp 115−116 °C. ^{1}H NMR: δ 5.30 (s, 2H, *CH$_2$-imi*), 6.90−8.00 (m, 11H, *Ar+imi*). ^{13}C NMR: δ 48.21, 120.98, 122.65, 126.02, 126.12, 127.04, 129.21, 130.70, 130.98, 131.76, 133.54, 139.76, 145.89, 146.43. ES-MS *m*/*s*: 312 (MH^+).

1-(2-phenoxybenzyl)-1H-imidazole (13). Starting from **38**, 0.15 g (63%) of **13** were obtained (toluene/acetone 3:2) as an oil (lit.[29] mp hydrochloric salt 144−145 °C). ^{1}H NMR: δ 5.15 (s, 2H, *CH$_2$-imi*), 6.98−7.60 (m, 12H, *Ar+imi*). ^{13}C NMR: δ 45.67, 117.21, 117.39, 121.76, 121.90, 122.87, 125.76, 126.13, 126.54, 128.43, 128.98, 139.53, 156.23, 156.78. ES-MS *m*/*s*: 251 (MH^+).

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Supporting Information Available: Experimental and spectroscopic details of intermediate compounds **14**, **15**, **22**, **29**−**38**, **40**, **41**. This material is available free of charge via the Internet at http://pubs.acs.org.

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