Graphical Abstract

Imidazole analogues of resveratrol: synthesis and cancer cell growth evaluation

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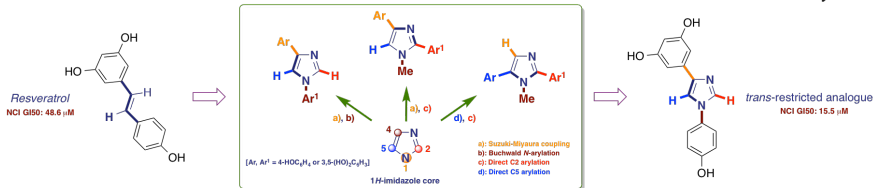

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Imidazole analogues of resveratrol: synthesis and cancer cell growth evaluation

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ABSTRACT

Novel *trans*-restricted analogues of resveratrol in which the C-C double bond of the natural derivative has been replaced by diaryl-substituted imidazole derivatives have been designed. The syntheses of 1,4-, 2,4- and 2-5-diarylimidazoles, in which the two aryl moieties are linked to the heteroaromatic core in a 1,3 fashion in order to preserve the *trans* stereochemistry, have been successfully carried out by regioselective sequential transition metal-catalyzed arylations of simple, commercially available imidazole precursors. The anticancer activity of selected analogues has been evaluated *in vitro* against the NCI 60 human tumor cell lines panel. From this screening, we were able to select a synthetic candidate that resulted more active than its natural lead.

Cross-coupling; Imidazoles, Resveratrol; Arylation; Palladium catalyst; Regioselectivity; Bioactive compounds; Anticancer compounds

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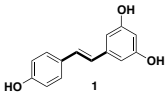
1. Introduction

Stilbenes belong to the nonflavonoid class of polyphenols.[1] They are 1,2- diarylethenes presenting a *trans*-configured double bond substituted with a phenyl group on both carbon atoms of the double bond. The most well known natural stilbene is resveratrol (**1**), firstly isolated in 1939 from roots of *Veratrum grandiflorum* (white hellebore)[2] and since then found in various edible plants, notably in *Vitis vinifera* L. (Vitaceae).[3]



Resveratrol is a natural phytoalexin; it is produced by at least 72 species of plants distributed among 31 genera and 12 families in response to stress, injury, fungal infection, and UV irradiation.[4]

Resveratrol attracted little interest until 1992, when it was postulated to explain some of the cardioprotective effects of red wine, the so-called "French Paradox".[5] Since then, a significant high number of papers have attributed to **1** antioxidant,[6] antiobesity,[7] antiviral,[8] antidiabetic,[9] and anticancer[4a,10] activities based on *in vitro* and animal models.[4b,11] In particular, several studies have shown that **1** is an inhibitor of carcinogenesis at multiple stages via its ability to inhibit cyclooxygenase,[10a,12] and is an anticancer agent with a role in antiangiogenesis.[10a] Moreover, both *in vitro* and *in vivo* studies showed that **1** induces cell cycle arrest and apoptosis in tumor cells.[10c,13]

However, the potential beneficial effects of **1** on health are compromised by its low bioavailability *in vivo*, thus hindering its arrival at target tissues. Clinical studies in humans evidenced that about 70% of administered **1** (25 mg) is rapidly (< 30 min) absorbed after oral intake, and that the low level observed in the blood stream is caused by a fast conversion by conjugation with sulphate and glucuronic acid, or reduction by the intestine microflora, into metabolites that are readily excreted from the body.[4b] The *trans*-configured double bond of **1** constitutes a critical requirement for its activity, because it may be prone to *E*/*Z* isomerization or reduction. In fact, conjugates of reduced resveratrol may account for as much as 50% of an oral dose of **1**,[14] and reduced resveratrol has a strong proliferative effect on hormone-sensitive cancer cell lines such as breast cancer cell line MCF-7.[15] As a consequence, considerable efforts have been aimed at modifying **1**, and bioavailable *trans*-restricted analogues based on the bioisosteric replacement of the olefinic double bond of the natural derivative with heteroaromatic nuclei, in analogy to the strategy used to improve the antitumor activity of the *cis*-stilbene Combretastatin A-4,[16] have been developed. Tron, Genazzani and coworkers employed click chemistry to generate a series of 1,4-diaryl-substituted triazole analogues of **1**, and evaluated their cytotoxicity against MDA-MB-431 breast cancer cell line.[17] Some of the screened compounds resulted more active than **1** as antiproliferative/cytotoxic agents, displayng IC_{50} values between 10 μM and 10 nM. In 2010 Macchia and coworkers developed new resveratrol analogues in which the *trans* double bond of **1** was embedded into benzofuran, quinoline or benzothiazole scaffolds.[18] These compounds displayed strong antiproliferative activity against MD-MBA-431 cell line; in particular, 3-(3,5-dihydroxyphenyl)-7-hydroxyquinoline exhibited the most potent antiproliferative effect (IC_{50} = 17.4 μM). Two years later, Cushman and coworkers replaced the *trans* stilbene double bond of **1** with a 1,2,4-thiadiazole scaffold, and to keep the *trans* geometry close to that of **1** the two phenyl rings were attached on the 1 and 3 positions of this five-membered heterocyclic core.[19] The 3,5-diaryl-1,2,4-thiadiazoles so obtained were evaluated against the MCF-7 breast cancer cell line but, except 3,5-bis(4-hydroxyphenyl)-1,2,4-thiadiazole (IC_{50} 4.7 μM), all of the compounds had weak anti-proliferative activities.

Recently, in continuation of our investigations into the synthesis and evaluation of the cytotoxic activity of diaryl-substituted five-membered heterocycles,[16a,20] in order to obtain derivatives with enhanced metabolic stability we devoted our synthetic efforts to the preparation of *trans*-restricted analogues of **1** in which the stilbene double bond of this derivative is embedded in an imidazole nucleus. To keep the *trans* geometry, the two aryl rings were linked to the heteroaromatic core in a 1,3 fashion (Fig. 1). It was hypothesized that this design should give derivatives with improved metabolic stability but retaining the chemo-preventive properties of the trans stilbene scaffold.[19]

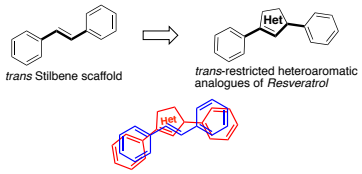
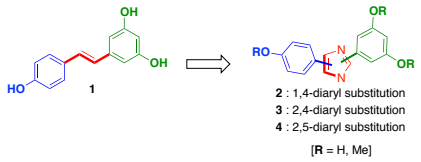


Figure 1.

There are a number of established *de novo* methods for the synthesis of substituted imidazoles where the imidazole ring is constructed via cyclo-condensation reactions.[21] Although these traditional approaches have been greatly improved over the past decade, each method has its scope and efficiency limitations. Often, condensation methods are inefficient for the assembly of series of compounds: for example, regioisomers (2,4- versus 4,5-substitution pattern) or focused analogues (different arene rings in the 4-position). In most cases, the synthesis of each analogue of the library will require the entire *de novo* synthetic sequence, which translates to parallel repetition of linear synthetic sequences.

A more general strategy for the synthesis of functionalized imidazole derivatives, also developed in recent years, involves the regioselective introduction of substituents into the preformed imidazole ring via transition metal-catalyzed reactions.[22] According to this last synthetic approach, in this paper we describe the selective preparation of O-methylated and OH-free 1,4-, 2,4- and 2,5-diaryl-1*H*-imidazoles of general structures **2**, **3** and **4**, respectively, which may be regarded as potential *trans*-restricted analogues of **1** (Scheme 1).



Scheme 1.

In fact, these structural patterns (1,4-, 2,4- and 2,5-disubstitution) are the only that allow the 1,3 relative spatial relationship between the 4-hydroxyphenyl and the 3,5-dihydroxyphenyl rings typical of **1**. The synthetic strategies to compounds **2**, **3** and **4** were conceived in order to allow ones the preparation not only of the trihydroxyphenyl derivatives, but also of their methyl ethers, because it has been reported that the replacement of hydroxyl groups with methoxy groups may

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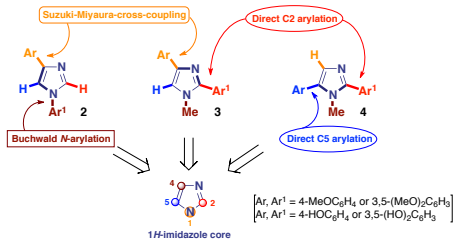
improve the cytotoxic activity and the chemopreventive properties of resveratrol's analogues.[23]

As depicted in Scheme 2, imidazoles **2**, **3** and **4** were efficiently obtained through sequential transition metal-catalyzed regioselective arylation reactions, which included palladium-catalyzed Suzuki-Miyaura cross couplings, copper-catalyzed Buchwald *N*-arylation, and palladium-copper mediated direct C-H arylation protocols.



Scheme 2. Retrosynthetic pathways to compounds **2**, **3** and **4**

In order to address their anticancer activity, some selected imidazole-based resveratrol analogues were then evaluated *in vitro* against the NCI-60 DTP human tumor cell lines panel. From this screening, we were able to select a candidate that resulted more active than its natural lead.

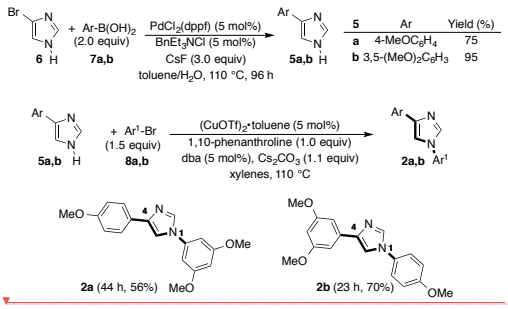
2. Results and discussion

*2.1. Synthesis of O-methylated and OH-free 1,4-diaryl-1H-imidazoles analogues of **1**: sequential Suzuki-Miyaura coupling and Buchwald N-arylation*

Despite their potential biological activity,[24] to the best of our knowledge only two general methods have been reported in the literature for the synthesis of 1,4-diaryl-1*H*-imidazoles **2**. The first one involves the construction of the imidazole ring by multistep sequences that generally starts from the condensation between phenacyl bromides and anilines,[25] while copper-promoted direct *N*-arylation of 4(5)-arylimidazoles **5** with arylboronic acids[26] or aryl halides[27] represents the key synthetic step of the second protocol. The latter methodology undoubtedly offers a number of advantages over the first procedure, including convenience, simplicity, and the use of readily available starting materials, and despite sometimes the tautomeric nature of **5** gave only moderate regioselectivities,[26] their Cu-catalyzed arylation seemed to us the best choice for the preparation of the target compounds **2**.

The synthesis of the title compounds is depicted in Scheme 2. 4(5)-Aryl-1*H*-imidazoles **5a,b** were obtained by Suzuki-Miyaura cross coupling involving commercially available 4(5)-bromo-1*H*-imidazole (**6**) and arylboronic acids **7a,b** according to the procedure recently developed by us.[28] Thus, **6** was treated with 2.0 equiv of the required boronic acid **7** in the presence of 5 mol% $PdCl_2$(dppf), 3 equiv of CsF, 5 mol% $BnEt_3NCl$ in a mixture of toluene and water (1:1) at 110 °C for 96 h, giving raise to 4(5)-(4-methoxyphenyl)-1*H*-imidazole **5a** and 4(5)-(3,5-dimethoxyphenyl)-1*H*-imidazole **5b** in 75 and 95% isolated yield, respectively (Scheme 3).

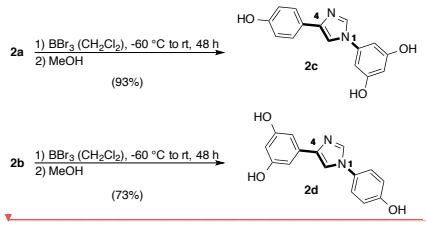
Among the several methods reported in the literature for the direct *N*-arylation of imidazoles,[26,27,22a] the simple protocol developed by Buchwald and coworkers in 1999 was adopted due to the high chemical yield and the complete regioselectivity obtained when 4-phenylimidazole was arylated with iodobenzene.[27a]



Scheme 3. Synthesis of *O*-methylated 1,4-diarylimidazole **2a,b**

According to this method, imidazoles **5a** and **5b** were reacted with 1.5 equiv of 1-bromo-3,5-dimethoxybenzene **8b** and 1-bromo-4-methoxybenzene **8a** in the presence of 5 mol% $Cu(OTf)_2$•toluene complex, 1.0 equiv of phenanthroline, 5 mol% dba, 1.1 equiv of Cs_2CO_3 in xylenes at 110 °C for 24 h, obtaining the required 1,4-diarylimidazoles **2a,b** in 56 and 70% isolated yield, respectively (Scheme 3). As expected, only the 4-substituted isomer was detected in the crude reaction mixtures, confirming that the coupling occurred preferentially at the less hindered nitrogen atom.[27e]

Finally, the OH-free 1,4-diarylated imidazole analogues of **1** were obtained by treating O-protected derivatives **2a,b** with a solution of BBr_3 in CH_2Cl_2 for 48 h. In this way 1-(3,5-dihydroxyphenyl)-4-(4-hydroxyphenyl)-1*H*-imidazole (**2c**) and 4-(3,5-dihydroxyphenyl)-1-(4-hydroxyphenyl)-1*H*-imidazole (**2d**) were isolated in 93% and 83% yield, respectively (Scheme 4).



Scheme 4. Synthesis of OH-free 1,4-diarylimidazoles **2c,d**

*2.2. Synthesis of O-methylated and OH-free 2,4-diaryl-1-methyl-1H-imidazoles analogues of **1**: sequential Suzuki-Miyaura coupling and direct C2-arylation*

Similarly to what was reported previously for the parent 1,4 diarylated imidazoles **2**, relatively few methods for the synthesis of 2,4-diaryl-1-methyl-1*H*-imidazoles **3** have been described in the literature, mainly devoted to the preparation of bioactive derivatives. The methods include the condensation of aromatic α-bromoketones with benzamidine,[29] the sequential double direct C-arylation and trans-*N*-alkylation of 1-SEM-1*H*-imidazole,[30] and the copper-catalyzed oxidative diamination of terminal alkynes by amidines.[31] However, these multi-step procedures appear of limited scope, suffer from providing in general modest yields, and require unavailable and/or expensive reagents. Thus, we thought it right to develop a concise and effective novel approach for the preparation of imidazoles **3**, and we discovered that they can be easily obtained from a Pd/Cu-mediated regioselective C2 direct arylation of 4-aryl-1-methyl-1*H*-

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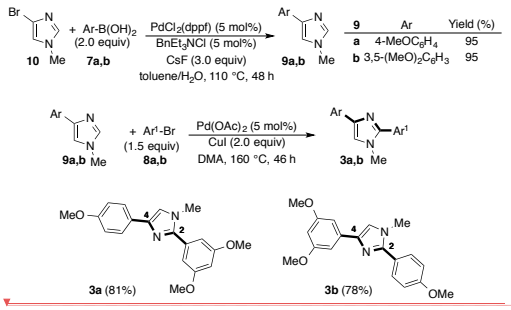
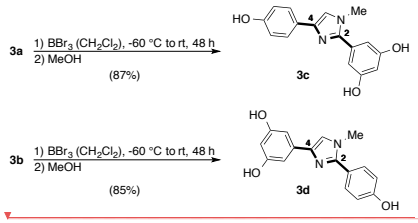
imidazole **9** with aryl bromides **8** (Scheme 4). In particular, according to our ligandless and base free protocol for the direct C2 arylation of azoles,[32a,32b,28,32c] 4-aryl-1-methylimidazoles **9a,b** were reacted with aryl bromides **8b** and **8a** in the presence of 5 mol% $Pd(OAc)_2$, 2.0 equiv of CuI in DMA at 160 °C for 46 h. After this period of time, *O*-methylated imidazoles **3a,b** were recovered in a satisfactory 81 and 78% isolated yield, respectively (Scheme 5).



Scheme 5. Synthesis of *O*-methylated 2,4-diarylimidazoles **3a,b**

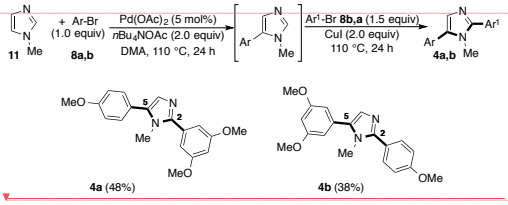
4-Arylimidazoles **9a,b**, which represent the key synthetic intermediates towards **3a,b**, have been efficiently prepared from the commercially available 4-bromo-1-methyl-1*H*-imidazole (**10**) and arylboronic acids **7a** and **7b** under the experimental conditions previously employed to convert 4(5)-bromoimidazole **6** into the corresponding 4(5)-arylimidazoles **5** (see Scheme 3). Hence, a mixture of **10** and 2 equiv of arylboronic acids **8a,b** in toluene and water (1:1) was treated with 5 mol% $PdCl_2$(dppf), 5 mol% $BnEt_3NCl$ and 3 equiv of CsF for 48 h at 110 °C (Scheme 5). The required imidazoles **3a,b** were isolated both in excellent yields (95%) and subsequently deprotected with BBr_3 in CH_2Cl_2, as summarized in Scheme 6, to give 2-(3,5-dihydroxyphenyl)-4-(4-hydroxyphenyl)-1-methyl-1*H*-imidazole (**3c**) and 4-(3,5-dihydroxyphenyl)-2-(4-hydroxyphenyl)-1-methyl-1*H*-imidazole (**3d**) in 87 and 85% isolated yield, respectively.



Scheme 6. Synthesis of OH-free 2,4-diarylimidazoles **3c,d**

*2.3. Synthesis of O-methylated and OH-free 2,5-diaryl-1-methyl-1H-imidazoles analogues of **1**: sequential one-pot C5 and C2 direct arylations*

Recently, in the course of a study aimed at developing general methods for the direct C-arylation of azoles, we reported a mild and convenient protocol for the direct C5 arylation of 1-methyl-1*H*-pyrazole, thiazole, oxazole and 1-methyl-1*H*-imidazole with aryl bromides.[33] In that study we were also able to prepare two naturally occurring 2,5-diarylated oxazoles, balsoxin and texalin, pairing in a one-pot procedure the newly described C5 direct arylation with our established protocol for the ligandless and base-free C2 direct arylation of azoles.[33] We reasoned that this protocol could be easily extended to the preparation of 2,5-diarylated imidazoles and, with our great delight, we found that methoxylated resveratrol analogues **4a,b** may be successfully obtained by the one-pot sequential C5 and C2 direct arylation reactions starting from 1-methylimidazole **11**, as illustrated in Scheme 7.

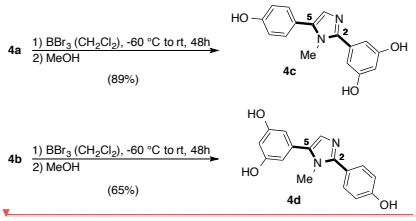


Scheme 7. Synthesis of *O*-methylated 2,5-diarylimidazole **4a,b**

In details, **11** was treated with 1 equiv of aryl bromides **8a** or **8b**, 5 mol% $Pd(OAc)_2$ and 2 equiv of nBu_4NOAc in DMA at 110 °C. After 24 h, at this mixture were sequentially added 1.5 equiv of aryl bromides **8b** or **8a**, respectively, and 2 equiv of CuI. After further 24 h at 110 °C the expected 2,5-diarylimidazoles **4a,b** were recovered in a 48% and 38% isolated yield, respectively (Scheme 7).

It should be noted that this robust and practical synthetic protocol is different from those previously reported in literature, which involve the preparation of compounds **4** via multistep sequences based on the functionalization of the imidazole core,[22b,21f,21a] or on the construction of the imidazole ring from commercially unavailable starting reagents.[21e,21f]

Finally, the methyl aryl ether subunits of compounds **4a,b** were converted into the corresponding phenol moieties by reaction with BBr_3 in CH_2Cl_2. In this way, 2-(3,5-dihydroxyphenyl)-5-(4-hydroxyphenyl)-1-methyl-1*H*-imidazole (**4c**) and 5-(3,5-dihydroxyphenyl)-2-(4-hydroxyphenyl)-1-methyl-1*H*-imidazole (**4d**) were isolated in 89 and 65% yield, respectively (Scheme 8).



Scheme 8. Synthesis of OH-free 2,5-diarylimidazoles **4c,d**

2.4. Biological results

As mentioned above, resveratrol (**1**) possesses numerous potential molecular targets resulting in multiple potential cellular outcomes. It is therefore possible that all the compounds synthesized share with the original molecule at least one of its actions. To evaluate a potential biological effect of these imidazole-based compounds, we decided to investigate their anticancer activity. Therefore, the chemical structures of compounds **2a-d**, **3a-d**, and **4a-d** were submitted to NCI (National Cancer Institute, Bethesda, Maryland, USA) in order to participate in their NCI-60 DTP Human Tumor Cell Line Screen.[34] The operation of this screen utilizes 60 different human tumor cell lines, representing leukemia, melanoma and cancers of the lung, colon, brain, ovary, breast, prostate, and kidney. The aim is to prioritize for further evaluation synthetic compounds or

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natural product samples showing selective growth inhibition or cell killing of particular tumor cell lines.

The screening is a two-stage process, beginning with the evaluation of all compounds against the 60 cell lines at a single high dose (10^{-5} M in DMSO). Then, the compound(s) that satisfy pre-determined threshold inhibition criteria in a minimum number of cell lines was/were progress to the full 5-dose assay, that consists in the measurement of the dose-response curves of each compound for each of the 60 cell lines of the NCI panel with five different drug concentrations (from 10^{-4} to 10^{-8} M in DMSO). The threshold inhibition criteria for progression to the 5-dose screen is selected to efficiently capture compounds with anti-proliferative activity based on careful analysis of historical DTP screening data (the threshold criteria is periodically updated by NCI as additional data becomes available).

Structures are generally selected or declined by NCI on the basis of the ability to add diversity to their small molecule compound collection; in our case, only the three polyphenolic imidazoles **2c**, **2d** and **4d**, and the methoxyaryl substituted precursors **2b** and **4b** passed this preliminary selection.

The results of the one-dose assay for the selected imidazoles are reported in Figure 2 as the mean graph mid-point (MGM) values, which are based on a calculation of the average of the percent of growth of the treated cells compared to the untreated control cells. Results from Figure 2 indicate that only compound **2d** was found to be significantly cytotoxic against the NCI panel, and it was further evaluated in the 5-doses assay (see below). It is interesting to observe that **2c**, the regioisomer of **2d**, scored inhibition values lower than **2d**, testifying the fact that the position of the two aryl rings on the azole core is of paramount importance for the biological activity of these derivatives. Moreover, as demonstrated by the results for compound **4d**, the presence of free hydroxy groups may also deplete the activity when compared to that displayed by the corresponding *O*-methylated analogue **4b**. Hence, the antitumor activity showed by these imidazole analogues is related to the whole three-dimensional molecular structure, and not simply to the presence of the two phenolic moieties typical of **1** or to a *trans*-like configuration.

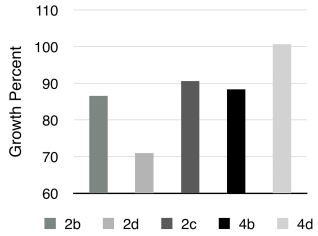

Figure 2. NCI 60 human tumor cell lines screening (one dose): mean growth (%) for all tested cell lines

In Figure 3 are reported the GI_{50} values of **2d** and of resveratrol (**1**) as the average of the GI_{50} for every single cancer line grouped by type of tumor, and also the MGM GI_{50} values for all the 60 cell lines, scored by the two molecules in the 5-dose assay of NCI. From these data it is indisputable that **2d** is more cytotoxic than the natural lead **1**, displaying the highest activity against leukemia and renal cancer cell lines (4.1 and 8.3 μM, respectively).

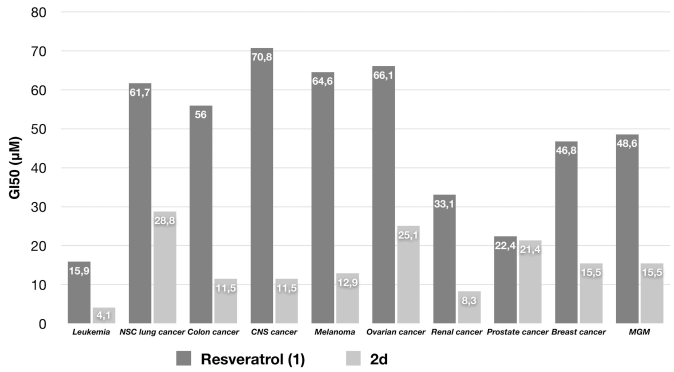

Figure 3. NCI 60 human tumor cell lines screen over a 5-log dose range: lead candidate **2d** vs. resveratrol (**1**)

3. Conclusions

In conclusion, in this work we efficiently prepared a series of 1,4-, 2,4- and 2,5-diaryl substituted imidazoles, that may be considered *trans*-restricted analogues of resveratrol, by short reaction sequences involving regioselective transition metal-catalyzed carbon-carbon and carbon-nitrogen bond-forming reactions. With the aim to preserve the *trans* stereochemistry of the natural compound, these derivatives have been designed in order to place the two aryl moieties in a 1,3 relative position onto the heteroaromatic core. We demonstrated that the bioisosteric replacement with an imidazole core of the double bond of resveratrol (**1**) provides a means to increase the anticancer activity *in vitro* against the NCI 60 human cell lines panel, but also that this improvement deeply depends on the relative position of the two aryl rings on the azole scaffold. In fact, among the analogues selected by NCI, the 1,4-diaryl substituted imidazole **2d** had higher cytotoxic activity against all the nine types of human tumor cells selected by NCI then resveratrol itself, and indeed it is significantly more potent than its regioisomer **2c**. Moreover, we also found that the presence of the 4-hydroxyphenyl and 3,5-dihydroxyphenyl moieties is, on its own, not enough to secure a good bioactivity. This is clearly evidenced by a comparison of the results scored by compounds **4d** and **4b**. In the next future, we will perform an in-depth SAR

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study and we will try to elucidate the biological mechanism of action of our derivatives and to test the efficacy *in vivo* of these interesting molecules.

4. Experimental section

Melting points were recorded on a hot-stage microscope (Reichert Thermovar). Fluka precoated 60 F254 aluminium silica gel sheets were used for TLC analyses. GLC analyses were performed using two types of capillary columns: an Alltech AT-35 bonded FSOT column (30 m x 0.25 mm i.d.) and an Alltech AT-1 bonded FSOT column (30 m x 0.25 mm i.d.). Purifications by flash-chromatography were performed using silica gel Merck 60 (particle size 0.040–0.063 mm). EI-MS spectra were measured at 70 eV by GLC/MS. The ESI spectra of compounds **2c**, **2d**, **3c**, **3d**, **4c**, and **4d** were acquired on a PE Sciex API 365 coupled with a Perkin Elmer 200 HPLC, and were obtained under the following conditions: ionspray voltage, - 4.0 kV; orifice voltage, - 60 V; scan range, *m/z* = 400–600; scan time, 4.73 s; no interscan delay; unity-mass resolution. NMR spectra were recorded at room temperature at 200 MHz (^{1}H) and 50.3 MHz (^{13}C) and were referred to TMS or to the residual protons of deuterated solvents. High- resolution mass spectra (HRMS) were obtained with an Agilent 6540 Q-TOF spectrometer by the Istituto di Fisiologia Clinica, CNR, Pisa (Italy). All reactions were performed under argon, by standard syringe, cannula and septa techniques. 4(5)-Bromo-1*H*-imidazole (**6**), 4-methoxyphenylboronic acid (**7a**), 3,5-dimethoxyphenylboronic acid (**7b**), 1-bromo-4-methoxybenzene (**8a**), 1-bromo-3,5-dimethoxybenzene (**8b**), 4-bromo-1-methyl-1*H*-imidazole (**10**), benzyltriethylammonium chloride, tetrabutylammonium acetate, copper(I) trifluomethanesulfonate toluene complex, 1,10-phenanthroline, [1,1'-bis(diphenylphosphino)ferrocene]dichloro palladium, palladium acetate, copper(I) iodide, cesium fluoride were commercially available and, unless otherwise stated, were used as received. 1-Methyl-1*H*-imidazole (**11**) was purified by vacuum distillation. Unless otherwise stated, commercially anhydrous solvents were used as received. Dichloromethane was anhydrified by distillation over CaH_2.

*4.1. General procedure for the synthesis of 4(5)-aryl-1H-imidazoles **5** by Suzuki-Miyaura cross-coupling*

A deaerated mixture of 4(5)-bromo-1*H*-imidazole (**6**) (0.735 g, 5.00 mmol), an arylboronic acid **7** (10.0 mmol), CsF (2.28 g, 15.0 mmol), $PdCl_2$(dppf) (0.183 g, 0.25 mmol), and $BnEt_3NCl$ (0.057 g, 0.25 mmol) in toluene (30 mL) and water (30 mL) was heated at reflux under argon for 96 h. The mixture was then cooled to room temperature and partitioned between water and AcOEt, and the organic extract was dried and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel to provide the desired product. This procedure was used to prepare compounds **5a** and **5b**, in 75 and 95% yield.

*4.2. 4(5)-(4-Methoxyphenyl)-1H-imidazole (**5a**).*

The crude reaction product obtained by Pd-catalyzed Suzuki-Miyaura reaction of **6** with **7a** was purified by flash chromatography on silica gel with a mixture of CH_2Cl_2 and methanol (93:7) as eluent to give **5a** (0.653 g, 75%) as a colorless solid: mp 121–123 °C (lit.[28] mp 113-115 °C); ^{1}H NMR ($CDCl_3$) δ 11.28 (br s, 1H), 7.63 (m, 3H), 7.25 (d, *J* = 2.2 Hz, 1H), 6.90 (m, 2H), 3.80 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 158.8, 138.2, 135.3, 126.3 (2C), 125.7, 114.7, 114.2 (2C), 55.3; EI-MS *m/z* 175 (11), 174 (100), 159 (61), 131 (27), 77 (11). GLC analysis showed that **5a**, which had ^{1}H and ^{13}NMR data in agreement with those previously reported,[28] was chemically pure.

*4.3. 4(5)-(3,5-Dimethoxyphenyl)-1H-imidazole (**5b**).*

The crude reaction product obtained by Pd-catalyzed Suzuki-Miyaura reaction of **6** with **7b** was purified by flash chromatography on silica gel with a mixture of CH_2Cl_2 and methanol (94:6) as eluent to give **5b** (0.970 g, 95%) as a colorless solid: mp 157–158 °C; ^{1}H NMR (CD_3OD) δ 7.63 (s, 1H), 7.29 (s, 1H), 6.88 (d, *J* = 2.0 Hz, 2H), 6.37 (t, *J* = 2.0 Hz, 1H), 3.79 (s, 3H); ^{13}C NMR (CD_3OD) δ 162.5 (2C), 139.4 136.8, 136.0, 116.9, 103.8 (2C), 100.0, 55.7 (2C). EI-MS: m/z 205 (13), 204 (100), 203 (40), 175 (16), 174 (13), 160 (13). GLC analysis showed that **5b**, which had ^{1}H and ^{13}NMR data in agreement with those previously reported,[35] was chemically pure.

*4.4. General procedure for the synthesis of 1,4-diaryl-1H-imidazoles **2** by Buchwald N-arylation*

To a flame-dried reaction vessel were added imidazole (**5**) (1.0 mmol), 1,10-phenanthroline (0.180 g, 1.00 mmol), *trans,trans*-dibenzylidene acetone (dba) (0.010 g, 0.050 mmol), Cs_2CO_3 (0.360 g, 1.1 mmol), copper(I) trifluomethanesulfonate toluene complex (0.030 g, 0.050 mmol). The reaction vessel was fitted with a silicon septum, evacuated, and back-filled with argon, and this sequence was repeated twice. Xylenes (1 mL) and an aryl bromide **8** (1.5 mmol) were then added successively under a stream of argon by syringe at room temperature. The resulting mixture was stirred under argon at 110 °C until GLC analysis showed that the reaction was complete (23-44 h). The resultant heterogeneous mixture was allowed to cool to room temperature, diluted with EtOAc, filtered through a plug of silica gel, and eluted with additional EtOAc. The filtrate was concentrated under reduced pressure, and the residue was purified by flash chromatography on silica gel to provide the desired product. This procedure allowed us to prepare compounds **2a** and **2b** in 56 and 70% yield.

*4.5. 1-(3,5-Dimethoxyphenyl)-4-(4-methoxyphenyl)-1H-imidazole (**2a**)*

The crude reaction product obtained after 44 h from the copper(I)-catalyzed reaction of **5a** with **8b** was purified by flash chromatography on silica gel with a mixture of AcOEt and toluene (60 : 40) as eluent to give **2a** (0.17 g, 56%) as a light red solid: mp 103–105 °C; ^{1}H NMR ($CDCl_3$) δ 7.84 (d, *J* = 1.0 Hz, 1H), 7.75 (m, 2H), 7.43 (d, *J* = 1Hz, 1H), 6.93 (m, 2H), 6.54 (d, *J* = 2.2 Hz, 2H), 6.43 (t, *J* = 2.2 Hz, 1H), 3.82 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 161.5 (2C), 158.8, 142.8, 138.8, 135.4, 126.5, 126.1 (2C), 114.0 (2C), 112.6, 99.8 (2C), 98.8, 55.6 (2C), 55.2; EI-MS: m/z 311 (20), 310 (100), 296 (8), 295 (42), 267 (8), 155 (6). GLC analysis showed that **2a** was chemically pure.

*4.6. 4-(3,5-Dimethoxyphenyl)-1-(4-methoxyphenyl)-1H-imidazole (**2b**)*

The crude reaction product obtained after 23 h from the copper(I)-catalyzed reaction of **5b** with **8a** was purified by flash chromatography on silica gel with a mixture of AcOEt and toluene (60 : 40) as eluent to give **2b** (0.217 g, 70%) as a light red solid: mp 90–91 °C; ^{1}H NMR ($CDCl_3$) δ 7.79 (d, *J* = 1.1 Hz, 1H); 7.48 (d, *J* = 1.1 Hz, 1H); 7.34 (m, 2H); 6.99 (m, 4H); 6.41 (t, *J* = 2.2 Hz, 1H); 3.85 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 160.9 (2C), 158.9, 142.6, 135.8, 135.7, 130.4, 122.9 (2C), 114.8 (2C), 114.7, 102.7 (2C), 99.7, 55.6, 55.4 (2C); EI-MS: m/z 311 (20), 310

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(100), 309 (63), 281 (11), 280 (16). GLC analysis showed that **2b** had chemically purity higher than 98%.

*4.7. General procedure for the synthesis of 4-aryl-1-methyl-1H-imidazoles **9** by Suzuki-Miyaura cross-coupling*

A deaerated mixture of 4-bromo-1-methyl-1*H*-imidazole (**10**) (0.800 g, 5.00 mmol), an arylboronic acid **7** (10.0 mmol), CsF (2.28 g, 15.0 mmol), $PdCl_2$(dppf) (0.041 g, 0.05 mmol), and $BnEt_3NCl$ (0.011 g, 0.05 mmol) in toluene (30 mL) and water (30 mL) was heated at reflux under argon for 48 h. The mixture was then cooled to room temperature and partitioned between water and AcOEt, and the organic extract was dried and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel to provide the desired product. This procedure was used to prepare compounds **9a** and **9b**, both in 95% yield.

*4.8. 4-(4-Methoxyphenyl)-1-methyl-1H-imidazole (**9a**).*

The crude reaction product obtained by Pd-catalyzed Suzuki-Miyaura reaction of **10** with **7a** was purified by flash chromatography on silica gel with a mixture of CH_2Cl_2 and methanol (95:5) as eluent to give **9a** (0.89 g, 95%) as a light brown solid: mp 138–140 °C (lit.[36] mp 145-147 °C); ^{1}H NMR ($CDCl_3$) δ 7.68 (m, 2H), 7.44 (s, 1H), 7.06 (s, 1H), 6.91 (m, 2H), 3.82 (s, 3H), 3.69 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 158.4, 141.9, 137.6, 126.8, 125.9 (2C), 114.9, 113.8 (2C), 55.3, 33.4; EI-MS: m/z 189 (13), 65 188 (100), 173 (67), 146 (6), 145 (38), 91 (7). GLC analysis showed that **9a**, which had ^{1}H and ^{13}NMR data in agreement with those previously reported,[36] was chemically pure.

*4.9. 4-(3,5-Dimethoxyphenyl)-1-methyl-1H-imidazole (**9b**).*

The crude reaction product obtained by Pd-catalyzed Suzuki-Miyaura reaction of **10** with **7b** was purified by flash chromatography on silica gel with a mixture of CH_2Cl_2 and methanol (98:2) as eluent to give **9b** (1.04 g, 95%) as a colorless solid: mp 127–129 °C; ^{1}H NMR ($CDCl_3$) δ 7.43 (s, 1H); 7.14 (s, 1H), 6.94 (d, *J* = 2.4 Hz, 2H), 6.37 (t, *J* = 2.4 Hz, 1H), 3.83 (s, 6H), 3.67 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 160.8 (2C), 141.9, 137.7, 136.1, 116.3, 102.5 (2C), 99.2, 55.3 (2C), 33.4; EI-MS: m/z 219 (14), 218 (100), 217 (67), 189 (14), 188 (23), 187 (15). GLC analysis showed that **9b** had chemically purity higher than 97%.

*4.10. General procedure for the synthesis of 2,4-diaryl-1-methyl-1H-imidazoles **3** by direct C2 arylation*

A compound **9** (1.00 mmol), $Pd(OAc)_2$ (11.2 mg, 0.050 mmol), CuI (0.380 g, 2.00 mmol) were placed in the reaction vessel under a stream of argon. The reaction vessel was fitted with a silicon septum, evacuated and back-filled with argon, and this sequence was repeated twice. An aryl bromide **8** (1.50 mmol) and deaerated DMA (5 mL) were then added by syringe under a stream of argon at room temperature, and the resulting mixture was stirred at 160 °C under argon. The degree of completion of the reaction was established by GLC and GLC-MS analysis of a sample of the crude reaction mixture after treatment with a saturated aqueous NH_4Cl solution and extraction with AcOEt. After being cooled to 20 °C, the reaction mixture was diluted with AcOEt and poured into a saturated aqueous NH_4Cl solution. The resulting mixture was basified with a few drops of aqueous NH_4OH, stirred in the open air for 0.5 h, and then extracted with AcOEt. The organic extract was washed with water, dried, and concentrated under reduced pressure, and the residue was purified by flash chromatography on silica gel. The chromatographic fractions containing the required compound were collected and concentrated. This procedure was employed to prepare 2,4-diaryl-1-methyl-1*H*-imidazoles **3a** and **3b** in 81 and 78% yield, respectively.

*4.11. 2-(3,5-Dimethoxyphenyl)-4-(4-methoxyphenyl)-1-methyl-1H-imidazole (**3a**)*

The crude product obtained after 46 h from the Pd-catalyzed and Cu-mediated reaction between **9a** and **8b** was purified by flash chromatography on silica gel with a mixture of toluene and AcOEt (80:20) as eluent to give **3a** (0.260 g, 81%) as colorless solid: mp 104-106 °C; ^{1}H NMR ($CDCl_3$) δ 7.75 (m, 2H), 7.14 (s, 1H), 6.92 (m, 2H), 6.81 (s, 2H), 6.52 (s, 1H), 3.84 (s, 9H), 3.74 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 160.8 (2C), 158.7, 147.8, 140.8, 132.2, 127.0, 126.3 (2C), 117.3, 114.1 (2C), 107.1 (2C), 101.2, 55.7 (2C), 55.4, 34.7; EI-MS: m/z 325 (21), 324 (100), 323 (29), 309 (27), 281 (7); Anal. Calcd for $C_{19}H_{20}N_2O_3$: C, 70.35; H, 6.21; N, 8.64. Found: C, 68.99; H, 6.15; N, 8.71. GLC analysis showed that **3a** had chemical purity higher than 98%.

*4.12. 4-(3,5-Dimethoxyphenyl)-2-(4-methoxyphenyl)-1-methyl-1H-imidazole (**3b**)*

The crude product obtained after 46 h from the Pd-catalyzed and Cu-mediated reaction between **9b** and **8a** was purified by flash chromatography on silica gel with a mixture of toluene and AcOEt (75:25) as eluent to give **3b** (0.25 g, 78%) as a yellow solid: mp 134-136 °C; ^{1}H NMR ($CDCl_3$) δ 7.60 (m, 2H), 7.20 (s, 1H), 6.98 (m, 4H), 6.37 (t, *J* = 2.2 Hz, 1H), 3.83 (s, 9H), 3.69 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 160.8 (2C), 159.9, 147.9, 145.5, 136.3, 130.2 (2C), 122.9, 118.1, 113.9 (2C), 102.7 (2C), 99.3, 55.4 (2C), 55.3, 34.4; EI-MS: m/z 325 (21), 324 (100), 323 (68), 295 (9), 294 (19); Anal. Calcd for $C_{19}H_{20}N_2O_3$: C, 70.35; H, 6.21; N, 8.64. Found: C, 69.97; H, 6.24; N, 8.53. GLC analysis showed that **3a** had chemical purity higher than 99%.

*4.13. General procedure for the synthesis of 2,4-diaryl-1-methyl-1H-imidazoles **3** by sequential one-pot C5 and C2 direct arylations*

$Pd(OAc)_2$ (11.2 mg, 0.050 mmol) and Bu_4NOAc (0.600 g, 2.00 mmol) were added to a flame-dried reaction vessel. The reaction vessel was fitted with a silicon septum, evacuated, and back-filled with argon. This sequence was repeated twice. DMA (5 mL), an aryl bromide **8a** or **8b** (1.00 mmol), and 1-methyl-1*H*-imidazole (**11**) (93 μL, 90.3 mg, 1.10 mmol) were then added successively under a stream of argon by syringe at room temperature. The resulting mixture was stirred at 110 °C under argon for 24 h. CuI (0.38 g, 2.0 mmol) and an aryl bromide **8b** or **8a** (1.5 mmol) were then sequentially added to the resulting brown solution under a stream of argon. The reaction mixture was heated to 110 °C and stirred at this temperature for 24 h. After cooling to room temperature, the reaction mixture was diluted with AcOEt and poured into a saturated aqueous NH_4Cl solution. The resulting mixture was basified with a few drops of aqueous NH_4OH, stirred in the open air for 0.5 h, and then extracted with AcOEt. The organic extract was washed with water, dried, and concentrated under reduced pressure, and the residue was purified by flash chromatography on silica gel. The chromatographic fractions containing the required compound were collected and concentrated. This procedure was employed to prepare 2,4-diaryl-1-methyl-1*H*-imidazoles **4a** and **4b** in 48 and 38% yield, respectively.

*4.14. 2-(3,5-Dimethoxyphenyl)-5-(4-methoxyphenyl)-1-methyl-1H-imidazole (**4a**).*

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The crude reaction product obtained by Pd-catalyzed and Cu-mediated one pot sequential direct arylation reactions involving **8a** in the first step and with **8b** in the second step was purified by flash chromatography on silica gel with a mixture of AcOEt and toluene (60:40) as eluent to give **4a** (0.160 g, 48%) as a light yellow solid: mp 121–123 °C; ^{1}H NMR ($CDCl_3$) δ 7.33 (m, 2H), 7.10 (s, 1H), 6.95 (m, 2H), 6.81 (d, *J* = 2.0 Hz, 2H), 6.49 (t, *J* = 2.0 Hz, 1H), 3.80 (s, 9H), 3.62 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 160.5 (2C), 159.3, 148.4, 135.2, 132.5, 129.9 (2C), 126.6, 122.3, 114.0 (2C), 106.6 (2C), 100.8, 55.4 (2C), 55.2, 33.6; EI-MS: m/z 325 (21), 324 (100), 323 (59), 309 (22), 294 (7). Anal. Calcd for $C_{19}H_{20}N_2O_3$: C, 70.35; H, 6.21; N, 8.64. Found: C, 71.98; H, 6.19; N, 8.85. GLC analysis showed that **4a** had chemical purity higher than 98%.

*4.15. 5-(3,5-Dimethoxyphenyl)-2-(4-methoxyphenyl)-1-methyl-1H-imidazole (**4b**).*

The crude reaction product obtained by Pd-catalyzed and Cu-mediated one pot sequential direct arylation reactions involving **8b** in the first step and with **8a** in the second step was purified by flash chromatography on silica gel with a mixture of AcOEt and toluene (90:10) as eluent to give **4b** (0.120 g, 38%) as a light yellow oil; ^{1}H NMR ($CDCl_3$) δ 7.61 (m, 2H), 7.17 (s, 1H), 7.00 (m, 2H), 6.58 (d, *J* = 2.2 Hz, 2H), 6.48 (t, *J* = 2.2 Hz, 1H), 3.85 (s, 3H), 3.83 (s, 6H), 3.65 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 160.8 (2C), 159.9, 149.6, 135.2, 132.0, 130.1 (2C), 127.2, 123.2, 113.9 (2C), 106.7 (2C), 106.7, 99.7, 55.4 (2C), 55.3, 33.8; EI-MS: m/z 325 (21), 324 (100), 323 (28), 309 (23), 281 (6). Anal. Calcd for $C_{19}H_{20}N_2O_3$: C, 70.35; H, 6.21; N, 8.64. Found: C, 72.46; H, 6.17; N, 8.91. GLC analysis showed that **4a** had chemical purity higher than 97%.

*4.16. General procedure for the synthesis of polyphenolic imidazole-based analogues of **1** by demethylation with BBr_3*

To a solution of *O*-methoxyphenyl imidazoles **2a**, **2b**, **3a**, **3b**, **4a**, or **4b** (0.3 mmol) in dry CH_2Cl_2 (40 ml), which was stirred at -78 °C, was added a 1 M solution of BBr_3 in CH_2Cl_2 (2.7 ml, 2.7 mmol). The reaction mixture was allowed to warm up to room temperature and stirred at this temperature for 48 h. After cooling to 0 °C, the reaction mixture was diluted with methanol (1.5 mL), and AcOEt (10 mL) and a 10% aqueous solution of NaOH (10 mL) were sequentially added. The organic phase was recovered and acidified with a 10% solution of HCl cooled at 0 °C. The formed precipitate was collected by filtration and dried in vacuo, to give the title compounds chemically pure. This procedure was employed to prepare polyphenolic imidazoles **2c**, **2d**, **3c**, **3d**, **4c** and **4d** in 93, 73, 87, 85, 89 and 65% yield, respectively.

*4.17. 1-(3,5-Dihydroxyphenyl)-4-(4-hydroxyphenyl)-1H-imidazole (**2c**)*

Pale grey solid: mp 161-163 °C; ^{1}H NMR (CD_3OD) δ 8.03 (s, 1H), 7.67 (s, 1H), 7.61 (d, *J* = 8.4 Hz, 2H), 6.82 (d, *J* = 8.4 Hz, 2H), 6.49 (d, *J* = 1.5 Hz, 2H), 6.30 (t, *J* = 1.5 Hz, 1H); ^{13}C NMR (CD_3OD) δ 160.8 (2C), 157.9, 143.7, 139.9, 136.5, 127.4 (2C), 126.3, 116.4 (2C), 114.0, 102.6, 100.6 (2C); ESI-MS: m/z $[M+H]^+$ = 269, $[M-H]^-$ = 267; HRMS (–ESI): m/z calcd for $C_{15}H_{12}N_2O_3$ [M–H]$^-$: 267.0770, found: 267.0770.

*4.18. 4-(3,5-Dihydroxyphenyl)-1-(4-hydroxyphenyl)-1H-imidazole (**2d**)*

Pale grey solid: mp 217-219 °C; ^{1}H NMR ($(CD_3)_2CO$ + CD_3OD) δ 8.02 (s, 1H), 7.75 (s, 1H), 7.46 (m, 2H), 6.95 (m, 2H), 6.81 (d, *J* = 2.2 Hz, 2H); 6.23 (t, *J* = 2.2Hz, 1H); ^{13}C NMR ($(CD_3)_2CO$ + CD_3OD) δ 159.6 (2C), 157.9, 143.1, 136.7, 136.4, 130.2, 123.6 (2C), 117.0 (2C), 115.6, 104.2 (2C), 102.2; ESI-MS: m/z $[M+H]^+$ = 269, $[M-H]^-$ = 267; HRMS (–ESI): m/z calcd for $C_{15}H_{12}N_2O_3$ [M–H]$^-$: 267.0770, found: 267.0771.

*4.19. 2-(3,5-Dihydroxyphenyl)-4-(4-hydroxyphenyl)-1-methyl-1H-imidazole (**3c**)*

Pale grey solid: mp 185-188 °C; ^{1}H NMR (CD_3OD) δ 7.76 (s, 1H), 7.58 (s, 2H), 6.91 (s, 2H), 6.64 (s, 2H), 6.56 (s, 1H), 3.90 (s, 3H); ^{13}C NMR (CD_3OD) δ 160.6 (2C), 160.0, 146.6, 135.5, 128.3 (2C), 125.6, 119.6, 119.4, 117.0 (2C), 108.8 (2C), 106.9, 36.1; ESI-MS: m/z $[M+H]^+$ = 283, $[M-H]^-$ = 281; HRMS (–ESI): m/z calcd for $C_{16}H_{14}N_2O_3$ [M–H]$^-$: 281.0926, found: 281.0928.

*4.20. 4-(3,5-Dihydroxyphenyl)-2-(4-hydroxyphenyl)-1-methyl-1H-imidazole (**3d**)*

Pale grey solid: mp 194-196 °C; ^{1}H NMR (CD_3OD) δ 7.46 (m, 2H), 7.34 (s, 1H), 6.91 (m, 2H), 6.69 (d, *J* = 2.0Hz, 2H), 6.18 (t, *J* = 2.0Hz, 1H), 3.68 (s, 3H); ^{13}C NMR (CD_3OD) δ 159.7, 159.6 (2C), 149.6, 141.2, 136.9, 131.6 (2C), 122.0, 119.4, 116.4 (2C), 104.6 (2C), 102.3, 34.7; ESI-MS: m/z $[M+H]^+$ = 283, $[M-H]^-$ = 281; HRMS (–ESI): m/z calcd for $C_{16}H_{14}N_2O_3$ [M–H]$^-$: 281.0926, found: 281.0925.

*4.21. 2-(3,5-Dihydroxyphenyl)-5-(4-hydroxyphenyl)-1-methyl-1H-imidazole (**4c**)*

Pale grey solid: mp 175-178 °C; ^{1}H NMR (CD_3OD) δ 7.30 (m, 2H), 6.96 (s, 1H), 6.89 (m, 2H), 6.56 (s, 2H), 6.37 (s, 1H), 3.60 (s, 3H); ^{13}C NMR (CD_3OD) δ 159.8 (2C), 158.8, 149.8, 136.8, 133.2, 131.3 (2C), 125.8, 122.1, 116.6 (2C), 108.5 (2C), 104.3, 34.0.; ESI-MS: m/z $[M+H]^+$ = 283, $[M-H]^-$ = 281; HRMS (–ESI): m/z calcd for $C_{16}H_{14}N_2O_3$ [M–H]$^-$: 281.0926, found: 281.0923.

*4.22. 5-(3,5-Dihydroxyphenyl)-2-(4-hydroxyphenyl)-1-methyl-1H-imidazole (**4d**)*

Pale brown solid: mp 181-184 °C; ^{1}H NMR (CD_3OD) δ 7.49 (m, 2H), 7.05 (s, 1H), 6.92 (m, 2H), 6.42 (s, 2H), 6.33 (s, 1H), 3.64 (s, 3H); ^{13}C NMR (CD_3OD) δ 159.9 (3C), 150.4, 136.7, 132.5, 131.5 (2C), 125.4, 121.9, 116.5 (2C), 108.2 (2C), 103.5, 34.3; ESI-MS: m/z $[M+H]^+$ = 283, $[M-H]^-$ = 281; HRMS (–ESI): m/z calcd for $C_{16}H_{14}N_2O_3$ [M–H]$^-$: 281.0926, found: 281.0928.

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