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Synthesis and biological evaluation of 2,6-di-*tert*-butylphenol hydrazones as 5-lipoxygenase inhibitors

Ana M. Cuadro[a], Jesús Valenciano[a], Juan J. Vaquero[a], Julio Alvarez-Builla[a]*, Carlos Sunkel[b], Miguel Fau de Casa-Juana[b], M. Pilar Ortega[b]

[a]*Departamento de Química Orgánica, Universidad de Alcalá, 28871 Alcalá de Henares, Madrid, Spain*
[b]*Departamento de Investigación Laboratorios Alter, S.A, Madrid, Spain*

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Abstract

The preparation of hydrazone derivatives of 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde and the inhibition of 5-lipoxygenase (5-LO) by these compounds is discussed.

1. Introduction

Leukotrienes, identified as 5-lipoxygenase (5-LO) metabolites of arachidonic acid (AA), have been implicated as mediators in a diversity of diseases, including asthma and a number of other inflammatory pathologies, such as rheumathoid arthritis, inflammatory bowel disease, psoriasis and glomerulonephritis [1a–d]. Although leukotrienes may not be involved in the initiating events, they appear to play important roles in the propagation of the disease states, exacerbating the local events, and ultimately leading to tissue damage.

Through the action of 5-LO, AA is initially oxygenated to give 5-hydroperoxyeicosa- tetraenoic acid (5-HPETE), which is then transformed by the same enzyme to leukotriene A_4 (LTA_4). LTA_4 is then converted to LTB_4, a potent chemotactic agent which enhances the infiltration of leukocytes and their subsequent degranulation [2]. Alternatively, LTA_4 can couple to glutathione to produce peptidoleukotrienes (PLTs, LTC_4, LTD_4 and LTE_4) which have profound effects on bronchial and vascular smooth muscle contractility, and promote extensive plasma extravasation by increasing the permeability of the postcapillary venules [3a–d].

Since the elucidation of the 5-LO biosynthetic pathway, there has been an ongoing debate in drug development as to whether it would be better to inhibit the 5-LO enzyme or to antagonize peptido- or non-peptido-leukotriene receptors. Recent evidence suggests that 5-LO inhibitors may be superior to LT-receptor antagonists, since 5-LO inhibitors block the action of the full spectrum of 5-LO products [4a,b] whereas LT-antagonists would produce narrower effects. In addition, LT-antagonists appear to prolong the half-lives of LTs by hindering their metabolism [5].

Numerous attempts have been made in the last decade to identify and develop 5-LO inhibitors as therapeutic agents. Many of the products in development are characterised by the presence of a 2,6-di-*tert*-butylphenol moiety, and have been studied as both cyclooxygenase (CO) and 5-LO inhibitors. A variety of compounds have been reported, in which the di-*tert*-butylphenol fragment is either directly bonded [6a,b] as in **1**, or linked through a vinyl bridge to various heterocyclic systems [6c–f] as in **2** or **3** (Fig. 1).

This paper describes the synthesis and biological activity of a series of compounds **4** in which the phenolic portion of the molecule forms part of a heteroaryl hydrazone (Fig. 1). The activity of the products is expressed as IC_{50}, calculated as the concentration of test compound required to cause 50% inhibition of LTB_4 (5-LO) formation, as measured on rat peritoneal neutrophils (PMNL). Investigations into this new series have resulted in the identification of several compounds, such as **4k**, as potent inhibitors of 5-LO activity.

*Corresponding author.

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2. Results and discussion

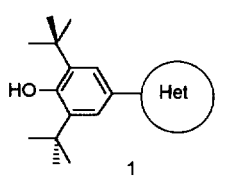
The compounds initially synthesized for testing as 5-LO inhibitors were the hydrazones **4a–e**, in which various heterocycles were linked to a phenolic moiety through a hydrazone spacer (Scheme 1). All the hydrazones were prepared by condensation of 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde **5** with the corresponding hydrazine derivatives **3a–l** [8].

The results of the in vitro 5-LO inhibition tests, indicated in Table 1, showed the azine derivatives, pyridine- (**4c**), pyrazine- (**4d**) and pyrimidine- (**4e**) to be active inhibitors. Although there were only small differences in activity between the models, the more stable pyrimidine derivative **4e** was chosen to explore substituent variations in the heterocyclic ring, in an attempt to maximize the activity. Furthemore, the same product was taken as a model to test the in vivo efficacy using AA-induced ear inflammation in mice.

The synthesis of compounds **4f–l** should start by the preparation of the corresponding chloropyrimidines as arylhydrazine precursors. However, although the preparation of 2-chloropyrimidines is normally achieved by reacting the corresponding 2-pyrimidinones with phosphorus oxychloride or related reagents [7a,b], we decided, after the initial tests, to prepare these compounds using a process based on an addition/dehydrogenation aproach [7c,d]. Thus, as indicated in Scheme 2, 2-chloropyrimidine was reacted with a lithium reagent R_3Li, to give the corresponding dihydropyrimidine intermediate, which was then dehydrogenated, without isolation, by treatment with DDQ to furnish the corresponding 2-chloro-4-(alkyl/aryl)-pyrimidine. As these compounds still have one unsubstituted C=N bond, they can be subjected to a second addition with lithium reagents, to give, after oxidation, the corresponding 4,6-disubstituted pyrimidines with higher yields than those obtained by previously described methods. The final 4,6-disubstituted-2-chloropyrimidines were readily converted into the corresponding hydrazines using conventional methodology [8a–d]. Finally, the hydrazone derivatives **4f,l** were prepared by condensation of 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde **5** with the hydrazine derivatives **3f-l** (Scheme 3).

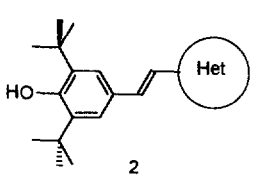
Introduction of either one or two phenyl groups (**4f** and **4g**) or one or two methyl groups (**4h** and **4i**) in the 4- and 6-positions of the pyrimidine moiety, produced compounds with much lower potency than the unsubstituted derivative **4e**. The introduction of the more lipophilic *n*-butyl group in the 4-position of the pyrimidine ring afforded the more active **4j**. Moreover, insertion of a second butyl group in the 6-position, increasing the lipophilicity of pyrimidine ring, resulted in the most potent compound **4k**. A combination of methyl and phenyl substituents produced the inactive compound **4l**. The in vivo efficacy of **4e** was evaluated in AA-induced ear inflamation in mice (AAE). The degree of inflammation is reflected in the difference in weigh between the AA and contralateral acetone-treated ears (as indicated in Table 2). Drugs were dissolved in acetone and applied to ear 30 min before the appliction of AA (see Experimental section) or 15–20 min per oral administration. The pyrimidine derivative **4e** inhibited edema formation by −29% and −38% at 500 μ/ear and

General Structures



1

Het: **1a**, 1,3,4-thiadiazole (CI-986)
1b, 1,2,4-oxadiazole
1c, 1,2,4-thiadiazole



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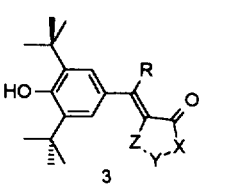
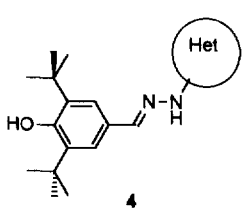
Het: **2a**, 5-isoxazole
2b, 5-isothiazole
2c, 3-pyrazole
2d, 3-pyridine



3

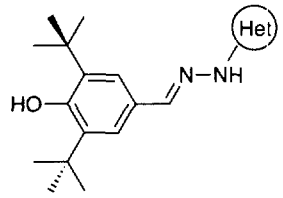
3a, R= H; X= O; Z-Y= -CH_2-CH_2- (KME-4)
3b, R= H; X= NOMe; Z-Y= -CH_2-CH_2- (E-5110)
3c, R= H, Me; Y-X= -CR=N-; Z= O, S, NMe



4

Fig. 1.

Table 1
Inhibition of 5-LO by hydrazones **4**



Compound	Heterocycle	$IC_{50}(\mu M)$[a]
4a	benzoxazol-2-yl	N[b]
4b	benzothiazol-2-yl	1.93 ± 0.072
4c	pyridin-2-yl	0.143 ± 0.006
4d	pyrazin-2-yl	0.100 ± 0.011
4e	pyrimidin-2-yl	0.229 ± 0.008
4f	4-phenylpyrimidin-2-yl	2.313 ± 0.004
4g	4,6-diphenylpyrimidin-2-yl	2.601 ± 0.803
4h	4-methylpyrimidin-2-yl	5.485 ± 0.645
4i	4,6-dimethylpyrimidin-2-yl	4.097 ± 0.015
4j	4-butylpyrimidin-2-yl	0.383 ± 0.015
4k	4,6-dibutylpyrimidin-2-yl	0.084 ± 0.028
4l	4-methyl-6-phenylpyrimidin-2-yl	N[b]

[a]Data reported as $IC_{50}(\mu M)$, calculated as the concentration of test compound required to cause 50% inhibition of LTB_4 formation, measured on rabbit peritoneal neutrophils (PMNL). Data are indicated as the mean ± s.d. of three different experiments performed in duplicate.
[b]Inactive (N) is defined as < 30% inhibition at a concentration of 10.0 μM.

250 μ/ear, respectively. Comparison of **4e** with standard antiinflammatory compound indicates that **4e** had a good profile of action and these results are summarized in Table 2.

In conclusion, the 2,6-di-*tert*-butyl-4-(2-azinylhydrazonomethyl)phenol derivatives showed interesting potential as 5-LO inhibitors. The activity, in the pyrimidine derivatives, seems to be highly susceptible to the introduction of different substitution patterns on the pyrimidine ring, and a similar behaviour is expected in the other azine derivatives.

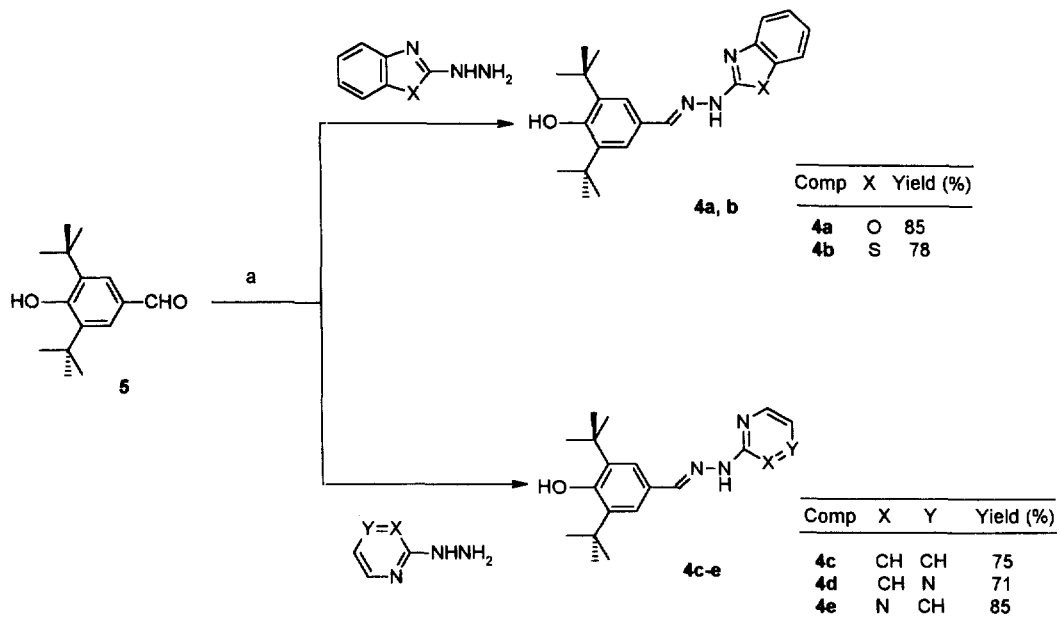
3. Experimental

3.1 Biology

The 5-LO enzyme, which is found primarly in cells of myeloid origin such as polymorpho- nuclear leukocytes, eosinophiles, and macrophages, catalyses the first step of the biochemical pathway in which AA is converted into LTA_4. Few cells actually possess the 5-LO enzyme, whereas virtually all cells possess LTA_4-hydrolase, which transforms LTA_4 into all other classes of LTs [9a,b]. Therefore, our studies on 5-LO activity were carried out on rabbit peritoneal neutrophils (PMNL).

3.2 Preparation of rabbit peritoneal polymorphonuclear leukocytes (PMNL)

Glycogen-elicited rabbit peritoneal PMNL were obtained by the method of Klotz [10]. An ip injection of



Scheme 1. Reagents and conditions: (a) Hydrazinoheterocycle, absolute EtOH, room temperature.

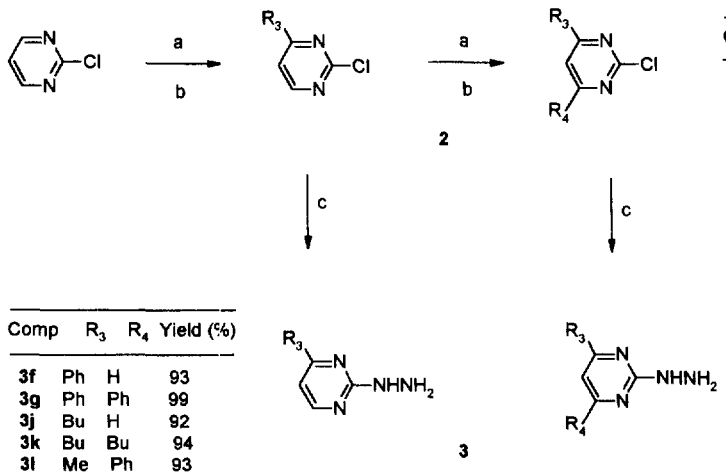
300 ml of 0.1% (w/v) glycogen in sterile saline was administered 6 h before the rabbits were sacrificed by iv Nembutal. Peritoneal exudates were collected in 5 mM EGTA, and residual red cells were removed by lysis in ammonium chloride. These cell preparations were greater than 96% PMNL, as asessed by Wright-Giemsa staining and greater than 95% viable as assessed by Trypan blue exclusion.

3.3 Radioisotopic assay of 5-LO activity

Cells in suspension were be pre-incubated for 20 min at room temperature with ^{14}C-labelled AA and diluted to 3000 cells μl^{-1}. Thereafter, calcium ionophore A23187 (1 $\mu g\,ml^{-1}$) was added and incubation followed for a further 15 min under stirring at 37°C. After incubation, the aqueous sample was successively shaken with a mixture of chloroform/methanol/formic acid (12:12:1) for 30 s and with chloroform for 10 s. Organic phases containing LTB_4, 5-HPETE, diHPETEs, and prostaglandins were pooled, evaporated to dryness under nitrogen and dissolved into a small amount of chloroform:MeOH (1:1). Separation of the 5-LO metabolites was performed by thin-layer chromatography (TLC) on silicagel G plates developed in diethyl ether:-hexane:acetic acid (60:40:1, v/v/v). ^{14}C-labelled compounds were detected by radiochromatogram scanning.

3.4 Incubation with the products

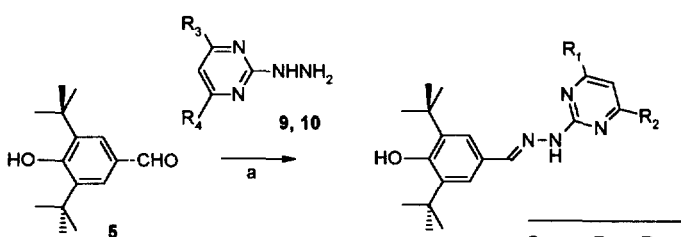
For the inhibition study, several concentrations, namely 10.0, 1.0, 0.1, and 0.01 μM, of the test compounds dissolved in dimethylsulfoxide were added to cells 15 min before the addition of calcium ionophore A23187. All incubations including controls were carried out in the presence of DMSO (0.1%).



Comp	R_3	R_4	Yield (%)
2f	Ph	H	84
2g	Ph	Ph	82
2j	Bu	H	84
2k	Bu	Bu	64
2l	Me	Ph	51

Comp	R_3	R_4	Yield (%)
3f	Ph	H	93
3g	Ph	Ph	99
3j	Bu	H	92
3k	Bu	Bu	94
3l	Me	Ph	93

Scheme 2. Reagents and conditions: (a) Alkyl or aryllithium, THF, −30°C, quenching with acetic acid/water, (b) DDQ, THF, room temperature, (c) hydrazine monohydrate, EtOH.



Comp	R_1	R_2	Yield (%)
4f	H	Ph	91
4g	Ph	Ph	67
4h	H	Me	89
4i	Me	Me	69
4j	H	Bu	85
4k	Bu	Bu	85
4l	Me	Ph	90

Scheme 3. Reagents and conditions: (a) Hydrazinoheterocycle, absolute EtOH, room temperature.

Table 2
Inhibitory effects of **4e** on the arachidonic acid-induced ear inflammation in mice

	Mean inhibition ± s.d. at dose in μg/ear[a]
	250
4e	−37.83 ± 5.7
MK-886[b]	−25.75 ± 8.1

[a]Percent inhibition of AA-induced ear inflammation in mice; $n = 10$ animals per experimental group.
[b]Ref 12.

3.5 Pharmacology

While various types of skin inflammation have been applied to 5-LO inhibitor screening, our study was carried out by Arachidonic acid-induced ear inflammation in mice (AAE) [11a–c] with either topical or oral dosing of drug.

3.6 Arachidonic acid-induced ear inflammation in mice (AAE)

Male OF1 mice 26–30 g body weight were used. The mice were divided into groups of ten. Irritative dermatitis was induced by topical application to both surfaces of mice left ears with 20 μl (10 μl each surface) of 10% AA (Sigma) in acetone. The right ear (control) received acetone (10 μl each surface). Either AA solution or acetone were applied with an automatic pipette of 10 μl.

3.6.1 Treatment

For topical application, the drugs were dissolved in a mixture of DMSO (50 μl) and acetone (350 μl). The compounds to study were applied in both surfaces of mice left ear (10 μl each surface), 15 min before the AA solution. The right ear (control) received the solvent (10 μl each surface). The control animals received only the vehicle in both right and left ears in the same experimental conditions.

After 1 h postadministration of phlogogen, the animals were anesthetized with an ip injection of pentobarbital (40 mg/Kg) and a 5 mm punch biopsy was performed on each ear. The swelling induced by AA was assessed as the difference on the weight between both left and right ears.

3.7 Chemistry

Melting points were recorded on an Electrothermal IA6304 and are uncorrected. ^{1}H NMR spectra were obtained on a Varian Unity 300 spectrometer at 300 MHz. IR spectra were recorded on a Perkin-Elmer 1310 spectrophotometer using KBr pellets. Mass spectra were determined on a Hewlett-Packard 5988A (70 eV). Elemental Analyses were performed by the Unidad Central de Servicios Analiticos of the University. Flash Chromatography was carried out using Merck Kieselgel 60 Merck (230–400 mesh). Solvents and reagents were of commercial origin, and were purified by standard procedures. 2-Chloropyrimidines were prepared as reported [7c,d], and 2-hydrazinoheterocycles were prepared according to known procedures [8a–d], and only the new pyrimidines **3j–l** are described.

3.8 Synthesis of hydrazinoheterocycles

3.8.1 (4-Butyl-pyrimidin-2-yl)-hydrazine (3j)

Hydrazine monohydrate (1 ml, 20 mmol) was added dropwise to a stirred solution of 4-butyl-2-chloropyrimidine (0.68 g, 4 mmol) in absolute EtOH (20 ml). The mixture was kept with stirring at room temperature for 16 h, the solvent was removed under reduced pressure, distilled water was added (20 ml) and the precipitate was collected by filtration, affording 0.66 g (92%) of **3j** as white prisms: mp 85–86°C (hexane); IR (KBr): 3257, 3193, 3054, 2961, 2934, 1599, 1561, 1461, 1334 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.23–8.20 (*m*, 1H), 6.50 (d, 1H, $J = 4.8$ Hz), 6.44 (s, 1H), 3.50 (s, 2H), 2.59 (t, 2H, $J = 7.9$ Hz), 1.72–1.60 (m, 2H), 1.44–1.30 (m, 2H), 0.93 (*t*, 3H, $J = 7.3$ Hz) ppm. Anal. calcd for $C_8H_{14}N_4$: C, 57.81; H, 8.49; N, 33.71. Found: C, 57.47; H, 8.39; N, 34.25.

3.8.2 (4,6-Dibutyl-pyrimidin-2-yl)-hydrazine (3k)

To a solution of 4,6-dibutyl-2-chloropyrimidine (0.91 g, 4 mmol) in absolute EtOH (20 ml) hydrazine monohydrate was added dropwise (1.9 ml, 40 mmol). The mixture was refluxed for 24 h, then concentrated and the resulting residue was dissolved in 20 ml CH_2Cl_2. The organic solution was dried over $MgSO_4$ and evaporated to yield **3k** as a yellow oil (0.83 g, 94%), which slowly crystallized on standing: mp 48–49°C; IR (KBr): 3265, 2956, 2932, 2867, 1563, 1354 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.37 (s, 1H), 6.26 (s, 1H), 3.96 (s, 2H), 2.56 (t, 4H, $J = 7.9$ Hz), 1.71–1.59 (m, 4H), 1.44–1.30 (m, 4H), 0.92 (t, 6H, $J = 7.3$ Hz) ppm. Anal. calcd for $C_{12}H_{22}N_4$: C, 64.83; H, 9.97; N, 25.20. Found: C, 65.25; H, 10.10; N, 24.86.

3.8.3 (4-Methyl-6-phenyl-pyrimidin-2-yl)-hydrazine (3l)

Hydrazine monohydrate (1.9 ml, 40 mmol) was added dropwise to a stirred solution of 2-chloro-4-methyl-6-phenylpyrimidine (0.82 g, 4 mmol) in absolute EtOH (20 ml). The mixture was refluxed for 16 h, then concentrated and the resulting residue was dissolved in 20 ml CH_2Cl_2. The organic solution was dried over $MgSO_4$ and evaporated to yield 0.76 g (93%) of **3l** as yellow prisms: mp 118–119°C (EtOH); IR (KBr): 3218, 1558, 1462, 1437, 1342, 1221 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 8.14–8.09 (m, 2H), 8.06 (s, 1H), 7.50–7.45 (m, 3H),

7.08 (s, 1H), 4.22 (s, 2H), 2.32 (s, 3H) ppm. Anal. calcd for $C_{11}H_{12}N_4$: C, 65.98; H, 6.04; N, 27.98. Found: C, 65.89; H, 6.18; N, 28.14.

3.9 Synthesis of hydrazones: general procedure A

The corresponding hydrazine (4.4 mmol) was added in small portions to a stirred suspension of 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde (0.94 g, 4 mmol) in absolute EtOH (20 ml). After stirring at room temperature for 2 h, the resulting precipitate was collected and crystallized.

3.9.1 4-(Benzoxazol-2-yl-hydrazonomethyl)-2,6-di-tert-*butyl-phenol (**4a**)*

Following the general procedure, 2-hydrazinobenzoxazole (0.66 g, 4.4 mmol), furnished 0.24 g (85%) of **4a** as white prisms: mp 234–235°C (EtOH); IR (KBr): 2957, 1663, 1590, 1479, 1457, 1240. 1214, 1151 cm^{-1}; ^1H NMR (DMSO-d_6) δ 11.85 (s, 1H), 8.11 (s, 1H), 7.50 (d, 1H, $J = 7.9$ Hz), 7.45 (s, 2H), 7.40–7.35 (m, 2H), 7.20 (t, 1H, $J = 7.6$ Hz), 7.09 (t, 1H, $J = 7.6$ Hz), 1.41 (s, 18H) ppm. Anal. calcd for $C_{22}H_{27}N_3O_2$: C, 72.30; H, 7.45; N, 11.50 Found: C, 72.62; H, 7.45; N, 11.56.

3.9.2 4-(Benzothiazol-2-yl-hydrazonomethyl)-2,6-di-tert-*butyl-phenol (**4b**)*

The general procedure applied to 2-hydrazinobenzothiazole (0.73 g, 4.4 mmol) gave **4b** (1.19 g, 78%) as white prisms: mp 225–226°C (EtOH); IR (KBr): 3624, 2958, 1607, 1554, 1440, 1211, 1122 cm^{-1}; ^1H NMR (DMSO-d_6) δ 12.10 (s, 1H), 8.04 (s, 1H), 7.78 (d, 1H, $J = 7.3$ Hz), 7.48 (s, 2H), 7.40–7.35 (m, 2H), 7.27 (t, 1H, $J = 7.6$ Hz), 7.08 (t, 1H, $J = 7.6$ Hz), 1.41 (s, 18H) ppm. Anal. calcd for $C_{22}H_{27}N_3OS$: C, 69.26; H, 7.13; N, 11.01. Found: C, 69.44; H, 7.03; N, 11.05.

3.9.3 2,6-Di-tert-*butyl-4-(pyridin-2-yl-hydrazonomethyl)-phenol (**4c**)*

The general procedure applied to 2-hydrazinopyridine (0.48 g, 4.4 mmol) gave 0.98 g (75%) of the title compound as colourless prisms: mp 214–215°C (EtOH); IR (KBr): 2953, 1600, 1582, 1458, 1441, 1317, 1230, 1211, 1146, 1129 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.39 (s, 1H), 8.11 (d, 1H, $J = 4.0$ Hz), 7.71 (s, 1H), 7.64–7.56 (m, 1H), 7.50 (s, 2H), 7.33 (d, 1H, $J = 8.4$ Hz), 6.77–6.71 (m, 1H), 5.40 (s, 1H), 1.48 (s, 18H) ppm; MS (EI) *m/z* 325 (M^+, 24); 120 (13); 94 (100). Anal. calcd for $C_{20}H_{27}N_3O$: C, 73.81; H, 8.36; N, 12.91. Found: C, 73.53; H, 8.06; N, 12.59.

3.9.4 2,6-Di-tert-*butyl-4-(pyrazin-2-yl-hydrazonomethyl)-phenol (**4d**)*

From 2-hydrazinopyrazine (0.49 g, 4.4 mmol), and following the general procedure, **4d** (0.93 g ,71%) was obtained as pale-yellow needles: mp 245–246°C (EtOH); IR (KBr): 2953, 1582, 1426, 1237, 1212, 1096 cm^{-1}; ^1H NMR (DMSO-d_6) δ 10.96 (s, 1H), 8.52 (d, 1H, $J = 0.6$ Hz), 8.09 (dd, 1H, $J = 1.8$, 0.9 Hz), 7.98 (s, 1H), 7.95 (d, 1H, $J = 1.5$ Hz), 7.41 (s, 2H), 7.29 (s, 1H), 1.41 (s, 18H) ppm; MS (EI) *m/z* 326 (M^+, 24); 216 (88); 95 (100). Anal. calcd for $C_{19}H_{26}N_4O$: C, 69.91; H, 8.03; N, 17.16 Found: C, 69,99; H, 8.03; N, 17.48.

3.9.5 2,6-Di-tert-*butyl-4-(pyrimidin-2-yl-hydrazonomethyl)phenol (**4e**)*

The general procedure, applied to 2-hydrazinopyrimidine (0.49 g, 4.4 mmol), provided **4e** as white needles (1.11 g; 85%): mp 236–237°C (EtOAc); IR (KBr): 3633, 2955, 1585, 1541, 1444, 1408, 1233, 1153 cm^{-1}; ^1H NMR (DMSO-d_6) δ 10.95 (s, 1H), 8.41–8.38 (m, 2H), 8.02 (s, 1H), 7.36 (s, 2H), 7.25 (s, 1H), 6.70 (m, 1H), 1.38 (s, 18H) ppm; MS (EI) *m/z* 326 (M^+, 16); 216 (60); 95 (100). Anal. calcd for $C_{19}H_{26}N_4O$: C, 69.91; H, 8.03; N, 17.16. Found: C, 70.35; H, 8.13; N, 17.21.

3.9.6 2,6-Di-tert-*butyl-4-[(4-phenyl-pyrimidin-2-yl)-hydrazonomethyl]phenol (**4f**)*

The general procedure applied to 2-hydrazino-4-phenylpyrimidine (0.82 g, 4.4 mmol), after stirring at room temperature for 6 h, yielded 1.47 g (91%) of **4f** as pale-yellow prisms: mp 222–223°C (EtOH); IR (KBr): 2956, 1566, 1428, 1276, 1232, 1157 cm^{-1}; ^1H NMR (DMSO-d_6) δ 11.09 (s, 1H), 8.49 (d, 1H, $J = 5.1$ Hz), 8.19–8.16 (m, 2H), 8.07 (s, 1H), 7.52–7.49 (m, 3H), 7.45 (s, 2H), 7.35 (d, 1H, $J = 5.1$ Hz), 7.24 (s, 1H), 1.41 (s, 18H) ppm. Anal. calcd for $C_{25}H_{30}N_4O$: C, 74.60; H, 7.51; N, 13.92. Found: C, 74.34; H, 7.31; N, 13.83.

3.9.7 2,6-Di-tert-*butyl-4-[(4,6-diphenyl-pyrimidin-2-yl)-hydrazonomethyl]phenol (**4g**)*

The general procedure applied to 2-hydrazino-4,6-diphenylpyrimidine (1.15 g, 4.4 mmol), after stirring at room temperature for 16 h, gave **4g** (1.28 g, 67%) as yellow prisms: mp 260–261°C (EtOH); IR (KBr): 3625, 2956, 1536, 1492, 1435, 1355, 1233, 1154 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.62 (s, 1H), 8.21–8.17 (m, 4H), 7.91 (s, 1H), 7.63 (s, 3H), 7.53–7.50 (m, 6H), 5.41 (s, 1H), 1.50 (s, 18H) ppm. Anal. calcd for $C_{31}H_{34}N_4O$: C, 77.79; H, 7.16; N, 11.71. Found: C, 77.68; H, 7.47; N, 11.28.

3.10 General procedure B

The corresponding hydrazine (4.4 mmol) was added in small portions to a stirred suspension of 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde (0.94 g, 4 mmol) in 20 ml of absolute EtOH. After stirring at room temperature for 16–24 h, the solvent was removed and the residue purified by flash chromatography (hexane:EtOAc, 1:1 as eluent).

*3.10.1 2,6-Di-*tert*-butyl-4-[(4-methyl-pyrimidin-2-yl)-hydrazonomethyl]phenol (**4h**)*

The procedure B was applied to 2-hydrazino-4-methylpyrimidine (0.55 g, 4.4 mmol), and after stirring the mixture for 48 h, 1.21 g (89%) of **4h** were obtained as colourless prisms: mp 194–195°C (hexane:EtAcO); IR (KBr): 2956, 1590, 1566, 1460, 1431, 1362, 1322, 1211 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.34 (s, 1H), 8.30 (d, 1H, $J = 5.1$ Hz), 7.83 (s, 1H), 7.53 (s, 2H), 6.61 (d, 1H, $J = 5.1$ Hz), 5.42 (s, 1H), 2.44 (s, 3H), 1.47 (s, 18H) ppm. Anal. calcd for $C_{20}H_{28}N_4O$: C, 70.56; H, 8.29; N, 16.46. Found: C, 70.58; H, 8.52; N, 16.68.

*3.10.2 2,6-Di-*tert*-butyl-4-[(4,6-dimethyl-pyrimidin-2-yl)-hydrazonomethyl]phenol (**4i**)*

Following the procedure B with 2-hydrazino-4,6-dimethylpyrimidine (0.61 g, 4.4 mmol), and after stirring the mixture at room temperature for 16 h, 0.98 g (69%) of **4i** were obtained as colourless prisms: mp 181–182°C (hexane:EtOAc); IR (KBr): 2957, 1592, 1559, 1456, 1434, 1370, 1336 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.34 (s, 1H), 7.79 (s, 1H), 7.51 (s, 2H), 6.49 (s, 1H), 5.41 (s, 1H), 2.40 (s, 6H), 1.45 (s, 18H) ppm. Anal. calcd for $C_{21}H_{30}N_4O$: C, 71.15; H, 8.53; N, 15.80. Found: C, 71.42; H, 8.55; N, 15.79.

*3.10.3 2,6-Di-*tert*-butyl-4-[(4-butyl-pyrimidin-2-yl)-hydrazonomethyl]phenol (**4j**)*

Following the procedure B with 4-butyl-2-hydrazinopyrimidine (0.73 g, 4.4 mmol) and after stirring the mixture for 3 h and isolating the product by column chromatography (hexane: EtOAc 8:2), **4j** (1.30 g, 85%) was obtained as colourless prisms: mp 134–135°C (hexane); IR (KBr): 3625, 2956, 2871, 1570, 1409, 1231, 1157, 1124 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.35 (s, 1H), 8.33 (d, 1H, $J = 5.1$ Hz), 7.83 (s, 1H), 7.53 (s, 2H), 6.60 (d, 1H, $J = 5.1$ Hz), 5.42 (s, 1H), 2.66 (t. 2H, $J = 7.7$ Hz), 1.79–1.63 (m, 2H), 1.50–1.35 (m, 20H, 18H), 0.95 (t, 3H, $J = 7.3$ Hz) ppm. Anal. calcd for $C_{23}H_{34}N_4O$: C, 72.21; H, 8.96; N, 14.65. Found: C, 71.91; H, 9.04; N, 14.62.

*3.10.4 2,6-Di-*tert*-butyl-4-[(4,6-dibutyl-pyrimidin-2-yl)-hydrazonomethyl]phenol (**4k**)*

Following the procedure B, with 4,6-dibutyl-2-hydrazinopyrimidine (0.98 g, 4.4 mmol), the mixture was stirred at room temperature for 1 h. Purification by chromatography (hexane: EtOAc, 9:1) gave **4k** (1.49 g, 85%) as pale-green prisms: mp 126–127°C (hexane); IR (KBr): 2957, 2869, 1556, 1433, 1368, 1216, 1110 cm^{-1}; ^1H NMR ($CDCl_3$) −8.28 (s, 1H), 7.80 (s, 1H), 7.51 (s, 2H), 6.48 (s, 1H), 5.38 (s, 1H), 2.64 (t, 4H, $J = 7.8$ Hz), 1.76–1.64 (m, 4H), 1.50–1.34 (m, 22H, 18H terbut + 4H-CH_2-), 0.94 (t, 6H, $J = 7.3$ Hz) ppm. Anal. calcd for $C_{27}H_{42}N_4O$: C, 73.93; H, 9.65; N, 12.77. Found: C, 73.67; H, 9.90; N, 12.82.

*3.10.5 2,6-Di-*tert*-butyl-4-[(4-methyl-6-phenyl-pyrimidin-2-yl)-hydrazonomethyl]phenol (**4l**)*

Following the procedure B, with 2-hydrazino-4-methyl-6-phenylpyrimidine (0.80 g, 4 mmol), **4l** (1.49 g, 90%) was obtained as pale-green prisms: mp 200–201°C (hexane:EtOAc); IR (KBr): 2956, 1585, 1550, 1459, 1438, 1367, 1345, 1232 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.42 (s, 1H), 8.05–8.01 (m, 2H), 7.79 (s, 1H), 7.51 (s, 2H), 7.43–7.41 (m, 3H), 7.01 (s, 1H), 5.36 (s, 1H), 2.48 (s, 3H), 1.43 (s, 18H) ppm. Anal. calcd for $C_{26}H_{32}N_4O$: C, 74.97; H, 7.74; N, 13.45. Found: C, 75.05; H, 8.00; N, 13.50.

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