JPP Journal of Pharmacy and Pharmacology

JPP 2007, 59: 829–835

Received November 20, 2006
Accepted February 5, 2007
DOI 10.1211/jpp.59.6.0008
ISSN 0022-3573

Heteroaryl-susbstituted phenols as potential antioxidants

Serena Ferrini, Stefania Fusi, Fabio Ponticelli and Massimo Valoti

Abstract

A series of *O*-heteroaryl phenols have been synthesised and structurally characterised. Photo-Fries rearrangement of these compounds represents a useful way to access the corresponding *C*-heteroaryl derivatives. The activity of the new phenolic compounds as radical scavengers towards the 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonate) ($ABTS^{+\bullet}$) has been evaluated. 2-*tert*-Butyl-4-(4-phenyl-isoxazol-3-ylmethoxy)-phenol (compound 3c) showed the highest scavenger activity (IC50 value (i.e. the concentration that scavenged 50% of the radicals) 3.17×10^{-6} M), which was one order of magnitude greater than that of the corresponding lead compound *tert*-butylhydroxy-anisole (BHA) (IC50 1.04×10^{-5} M). In further experiments, compound 3c showed dose-dependent inhibition of the oxidation of linoleic acid, as well as methaemoglobin formation, promoted by the presence of the radical generator 2,2′-azobis(amidino-propane) hydrochloride (AAPH) and it was markedly more potent than BHA in these assays.

Introduction

Synthetic phenolic antioxidants have been added to food and pharmaceutical preparations for decades to retard the autoxidation of lipid that leads to rancidity. The major antioxidants, butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), are used in foods world wide. BHA is generally recognised as safe by the US Food and Drug Administration and at low concentrations exhibits little toxicity in mammals when administered orally (World Health Organization, 1989). Furthermore, BHA has been show to be protective against carcinogenesis and toxicity, since it effectively inhibits cancer formation induced by a variety of carcinogens (Prochaska et al 1985; Wattenberg 1986) and it protects animals against the acute toxic effects of several chemicals (Ito and Hirose, 1989). However, Ito et al (1985) reported that very high dietary levels of BHA induced hyperplasia and forestomach carcinoma in male and female rats and papilloma in Syrian golden hamsters, although the relevance of this in terms of human health is questionable.

Recent evidence suggests that BHA and BHT are more than radical scavengers. In fact, both compounds decrease the levels of reactive oxygen species and at the same time protect against cell necrosis induced by tumour necrosis factor (Matthews et al 1987; Goossens et al 1995). This seems to be related to interaction with mitochondrial functions (Fusi et al 1992; Festjens et al 2006). We have previously demonstrated the dual action of 15 phenol derivatives: they had an antispasmogenic action on longitudinal ileum musculature and antioxidant activity in microsomal and linoleate systems (Sgaragli et al 1993). The same study showed that compounds with a phenol moiety sterically hindered by a lipophilic group, such as the antioxidants BHA and 2,6-di-*t*-butyl-4-methoxyphenol, exert their spasmolytic action via inhibition of calcium ion influx into cells through L-type calcium ion channels.

With these investigations in mind, we wanted to develop new substances with antioxidant activity. Some promising results on radical-scavenging properties of several new *O*- and *C*-glycosides of hindered phenols that we have prepared and characterised (Ponticelli et al 2001; Giorgi et al 2003) prompted us to investigate further the chemical diversity in the substituents. Here we report the preparation and properties of a series of compounds obtained by coupling of phenols with heterocycles, mainly belonging to the isoxazole group.

Dipartimento di Chimica, Università di Siena, via A. Moro 2, Siena, I-53100 Italy

Serena Ferrini, Stefania Fusi, Fabio Ponticelli

Dipartimento di Scienze Anatomiche e Biomediche, Università di Siena, via A. Moro 2, Siena, I-53100 Italy

Massimo Valoti

Correspondence: Stefania Fusi, Dipartimento di Chimica, Università di Siena, via A. Moro 2, Siena, I-53100 Italy. E-mail: fusiste@unisi.it

Acknowledgements: The authors wish to thank the University of Siena (quota Progetti 2002–2003) for funding and the Centro di Analisi e Determinazioni Strutturali of the University of Siena for recording MS spectra.

Materials and Methods

Chemistry

Melting points (MP) were determined on a Kofler hot stage and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ solutions on a Bruker AC 200 spectrometer at 200.13 MHz and 50.33 MHz, respectively (Bruker Biospin GmbH, Rheinstetten, Germany). Chemical shifts (δ) are reported in parts per million (ppm) relative to tetramethylsilane as an internal standard. Coupling constants (J) are given in Hz. All reagents were reagent grade and were used without purification. The halo-heteroaryl derivatives **1a, 1b** (Donati et al 1991) and **1d** (Argibas et al 1992), **1e** (Adembri et al 1981), **1f, 1g** (Adembri et al 1975), **1h** (Camparini et al 1977), were known compounds and were prepared and purified as reported previously. Compound **1c** (oil) was prepared from 4-phenyl-3-methylisoxazole following the procedure reported for the synthesis of compounds **1a** and **1b**. Compound **1i** was purchased from Avocado (Rho, Milan, Italy). The electron impact mass spectrometry (EI-MS) spectra were recorded on a VG 70 250S instrument (VG Analytical, Manchester, UK); electrospray ionisation mass spectrometry (ESI-MS) spectra were recorded with a LCQ-DECA instrument (Thermo Finnigan, San Jose, CA, USA). Thin-layer chromatography was performed on pre-coated 4 × 6.7 cm silica gel 60 F254 plates on aluminium (Aldrich, Milan, Italy) with detection by ultraviolet light. Column chromatography was carried out on Silica gel 230–400 mm mesh (E. Merck, Milan, Italy).

General procedure for the preparation of the phenol-ethers 3a–i, 4e, 4i, 6f and 6h

The phenols (compound 2 or 5; (3 mmol) in anhydrous Me_2CO (30 mL), anhydrous K_2CO_3 (3 mmol) and a catalytic amount of KI were refluxed under nitrogen for 30 min. The appropriate halo-heteroaryl derivative 1a–i (1 mmol) was added and the reflux continued for 20 h. The inorganic materials were filtered off and the solvent was removed in vacuo. The residue was dissolved in a minimum amount of CH_2Cl_2, and purified by column chromatography with PE: EtOAc (gradient from 9:1 to 2:1, v/v) as eluents. After a small amount of starting material 1a–i, the phenol-ethers 3a–i, 6f or 6h were eluted from the column. Afterwards, in the case of chloro derivatives 1e and 1i, the hindered phenol-ethers 4e and 4i were also obtained. All new compounds were purified by crystallisation from PE:Et_2O 1:1, v/v.

General procedure for the photochemical reactions

A nitrogen-bubbled solution of the phenol-ethers 3f, 3h, 6f and 6h (1 mmol) in EtOH (100 mL) was irradiated with a low-pressure immersion mercury lamp (254 nm, 17 W) until the starting material disappeared or was reduced to a small amount for thin-layer chromatography. The solvent was removed in vacuo and the residue was subject to column chromatography with PE:EtOAc (gradient from 15:1 to 2:1, v/v). Compounds 7a–d and 8 were crystallised from ethanol.

2-tert-*Butyl-4-(3-phenyl-isoxazol-4-ylmethoxy)-phenol (**3a**)*

MP 129–132°C. ^{1}H-NMR (200 MHz, 27°C, $CDCl_3$) δ: 1.43 (s, 9H, CMe_3), 4.70 (bs, 1H, OH), 4.88 (s, 2H, CH_2), 6.58 (d, 1H, 6-H, J = 8.3 Hz), 6.70 (dd, 5-H, J = 8.3, 2.3 Hz), 6.89 (d, 1H, 3-H, J = 2.3 Hz), 7.47–7.53, 7.79–7.86 (m, 5H, phenyl protons). ^{13}C-NMR (50 MHz, 27°C, $CDCl_3$) δ: (29.47, 34.73, 60.25, 112.41, 114.88, 115.58, 116.91, 128.32, 128.95, 129.91, 137.87, 139.23, 140.68, 149.08, 151.85, 158.66. EI-MS m/z (%): 323 (M^+, 31), 158 (100), 77 (61). *Analysis*: Calculated for $C_{20}H_{21}NO_3$: C, 74.28; H, 6.55; N, 4.33. Found: C, 74.46; H, 6.28; N, 4.71.

2-tert-*Butyl-4-(3,5-diphenyl-isoxazol-4-ylmethoxy)-phenol (**3b**)*

MP 180–182°C. ^{1}H-NMR (200 MHz, 27°C, $CDCl_3$ δ: (1.43 (s, 9H, CMe_3), 4.70 (bs, 1H, OH), 4.88 (s, 2H, CH_2), 6.67 (d, 1H, 6-H, J = 5.5 Hz), 6.70 (dd, 1H, 5-H, J = 5.5, 2.7 Hz), 7.00 (d, 1H, 3-H, J = 2.7 Hz), 7.47–7.53, 7.79–7.86 (m, 10H, phenyl protons). ^{13}C-NMR (50 MHz, 27°C, $CDCl_3$) δ: 29.47, 34.74, 60.68, 109.37, 112.43, 115.43, 116.94, 127.39, 127.59, 128.54, 128.69, 128.96, 129.10, 129.88, 130.51, 137.88, 146.62, 149.08, 151.79, 151.94, 164.01, 170.04. ESI-MS: 400 ($M\text{-}H^+$). *Analysis*: Calculated for $C_{26}H_{25}NO_3$: C, 78.17; H, 6.31; N, 3.51. Found: C, 77.96; H, 6.44; N, 3.69.

2-tert-*Butyl-4-(4-phenyl-isoxazol-3-ylmethoxy)-phenol (**3c**)*

MP 80–85°C. ^{1}H-NMR (200 MHz, 27°C, $CDCl_3$) δ: 1.37 (s, 9H, CMe_3), 4.70 (bs, 1H, OH), 5.13 (s, 2H, CH_2), 6.58 (d, 1H, 6-H, J = 8.5 Hz), 6.66 (dd, 1H, 5-H, J = 8.5, 2.8 Hz), 6.88 (d, 1H, 3-H, J = 2.8 Hz), 8.53 (s, 1H, $H_{isox.}$), 7.37–7.41, 7.50–7.55 (m, 10H, phenyl protons). ^{13}C-NMR (50 MHz, 27°C, $CDCl_3$) δ: 23.37, 34.64, 61.26, 112.46, 115.52, 116.76, 128.11, 128.17, 128.89, 121.56, 137.64, 149.34, 151.53, 152.20, 156.69, 157.16. ESI-MS: 644 [2(M-H$^-$)], 322 (M-H$^-$). *Analysis*: Calculated for $C_{20}H_{21}NO_3$: C, 74.28; H, 6.55; N, 4.33. Found: C, 74.02; H, 6.81; N, 4.17.

2-tert-*Butyl-4-(3-phenyl-[1,2,4]oxadiazol-5-ylmethoxy)-phenol (**3d**)*

MP 110–112°C. ^{1}H-NMR (200 MHz, 27°C, $CDCl_3$) δ: 1.38 (s, 9H, CMe_3), 4.70 (bs, 1H, OH), 5.25 (s, 2H, CH_2), 6.58 (d, 1H, 6-H, J = 8.5 Hz), 6.67 (dd, 1H, 5-H, J = 8.5, 2.8 Hz), 7.00 (d, 1H, 3-H, J = 2.8 Hz), 7.41–7.49, 8.06–8.11 (m, 5H, phenyl protons). ^{13}C-NMR (50 MHz, 27°C, $CDCl_3$) δ: 29.41, 34.75, 62.19, 112.32, 115.65, 116.88, 126.27, 127.56, 128.89, 131.40, 137.96, 149.78, 151.36, 168.48, 175.35. ESI-MS: 646 [2(M-H$^-$)], 323 (M-H$^-$). *Analysis*: Calculated for $C_{19}H_{20}N_2O_3$: C, 70.35; H, 6.21; N, 8.64. Found: C, 70.57; H, 5.98; N, 8.53.

2-tert-*Butyl-4-(3-phenyl-[1,2,4]oxadiazol-5-yloxy)-phenol (**3e**)*

MP 111–113°C. ^{1}H-NMR (200 MHz, 27°C, $CDCl_3$) δ: 1.43 (s, 9H, CMe_3), 5.44 (bs, 1H, OH), 6.70 (d, 1H, 6-H,

J = 8.6 Hz), 7.14 (dd, 1H, 5-H, J = 8.6, 2.9 Hz), 7.29 (d, 1H, 3-H. J = 2.9 Hz), 7.44–7.48, 7.98–8.03 (m, 5H, phenyl protons). ^{13}C-NMR (50 MHz, 27°C, $CDCl_3$) δ: 29.38, 34.84, 116.97, 117.72, 118.73, 126.81, 127.19, 128.72, 131.35, 138.07, 146.33, 152.80, 169.26, 172.87. ESI-MS: 311 ($M\text{-}H^+$). *Analysis*: Calculated for $C_{18}H_{18}N_2O_3$: C, 69.66; H, 5.85; N, 9.03. Found: C, 70.01; H, 5.74; N, 9.36.

3-tert-*Butyl-4-(3-phenyl-[1,2,4]oxadiazol-5-yloxy)-phenol (**4e**)*

MP 103–105°C. ^{1}H-NMR (200 MHz, 27°C, $CDCl_3$) δ: 1.37 (s, 9H, CMe_3), 6.03 (bs, 1H, OH), 6.69 (dd, 1H, 5-H, J = 8.7, 2.9 Hz), 6.90, (d, 1H, 3-H, J = 2.9 Hz), 7.15 (d, 1H, 6-H, J = 8.7 Hz), 7.39–7.51, 7.97–8.04 (m, 5H, phenyl protons). ^{13}C-NMR (50 MHz, 27°C, $CDCl_3$) δ: 30.10, 34.70, 113.85, 114.68, 122.73, 126.62, 127.16, 128.75, 131.43, 142.21, 145.50, 154.04, 169.31, 172.87. ESI-MS: 311 ($M\text{-}H^+$). *Analysis*: Calculated for $C_{18}H_{18}N_2O_3$: C, 69.66; H, 5.85; N, 9.03. Found: C, 69.53; H, 5.90; N, 9.21.

2-tert-*Butyl-4-(6-chloro-3-methyl-isoxazolo[4,5-c]pyridin-4-yloxy)-phenol (**3f**)*

MP 159–161°C. ^{1}H-NMR (200 MHz, 27°C, $CDCl_3$) δ: 1.40 (s, 9H, CMe_3), 2.70 (s, 3H, Me), 4.95 (bs, 1H, OH), 6.68 (d, 1H, 6-H, J = 8.5 Hz), 6.93 (dd, 1H, 5-H, J = 8.5, 2.6 Hz), 7.10 (d, 1H, 3-H, J = 2.6 Hz), 7.18 (s, 1H, H_{pyr}). ^{13}C-NMR (50 MHz, 27°C, $CDCl_3$) δ: 11.39, 29.38, 34.72, 101.03, 107.44, 116.94, 119.33, 120.11, 137.50, 145.44, 149.05, 151.85, 154.33, 157.31, 171.46. EI-MS m/z (%): 332 (M^+,100), 317 (65). *Analysis*: Calculated for $C_{17}H_{17}ClN_2O_3$: C, 61.36; H, 5.15; N, 8.42. Found: C, 61.49; H, 5.12; N, 8.37.

2-tert-*Butyl-4-(6-methoxy-3-methyl-isoxazolo[4,5-c]pyridin-4-yloxy)-phenol (**3g**)*

MP 138–142°C. ^{1}H-NMR (200 MHz, 27°C, $CDCl_3$) δ: 1.40 (s, 9H, CMe_3), 2.65 (s, 3H, Me), 3.68 (s, 3H, OMe), 5.27 (bs, 1H, OH), 6.38 (s, 1H, $H_{pyr.}$), 6.69 (d, 1H, 6-H, J = 8.5 Hz), 6.89 (dd, 1H, 5-H, J = 8.5, 2.6 Hz), 7.11 (d, 1H, 3-H, J = 2.6 Hz). ^{13}C-NMR (50 MHz, 27°C, $CDCl_3$) δ: 11.39, 29.44, 34.69, 54.50, 83.47, 102.82, 116.65, 119.51, 120.63, 137.21, 145.76, 151.53, 154.07, 163.66, 164.32, 172.76. EI-MS m/z (%): 328 (M^+,100), 313 (69). *Analysis*: Calculated for $C_{18}H_{20}N_2O_4$: C, 65.84; H, 6.14; N, 8.53. Found: C, 65.66; H, 6.31 N, 8.69.

2-tert-*Butyl-4-(6-chloro-3-methyl-isoxazolo[5,4-b]pyridin-4-yloxy)-phenol (**3h**)*

MP 255–258°C. ^{1}H-NMR (200 MHz, 27°C, $CDCl_3$) δ: 1.45 (s, 9H, CMe_3), 2.70 (s, 3H, Me), 5.15 (bs, 1H, OH), 6.42 (s, 1H, $H_{pyr.}$), 6.80 (d, 1H, 6-H, J = 8.8 Hz), 6.90 (dd, 1H, 5-H, J = 8.8, 2.6 Hz), 7.05 (d, 1H, 3-H, J = 2.6 Hz). ^{13}C-NMR (50 MHz, 27°C, $CDCl_3$) δ: 12.41, 29.32, 30.33, 104.37, 104.96, 114.18, 115.25, 117.71, 118.83, 119.81, 123.23, 139.08, 145.81, 152.73, 154.35. ESI-MS: 333 ($M\text{-}H^+$). *Analysis*: Calculated for $C_{17}H_{17}ClN_2O_3$: C, 61.36; H, 5.15; N, 8.42. Found: C, 61.20; H, 5.31; N, 8.69.

2-tert-*Butyl-4-(5-nitro-thiophen-2-yloxy)-phenol (**3i**)*

MP 150–153°C. ^{1}H-NMR (200 MHz, 27°C, $CDCl_3$): δ 1.39 (s, 9H, CMe_3), 5.03 (bs, 1H, OH), 6.31 (d, 1H, 3′-H, J = 4.9 Hz), 6.68 (d, 1H, 6-H, J = 8.7 Hz), 6.90 (dd, 1H, 5-H, J = 8.7, 2.8 Hz), 7.10 (d, 1H, 3-H, J = 2.8 Hz), 7.72 (d, 1H, 4'-H, J = 4.9 Hz). ^{13}C-NMR(50 MHz, 27°C, $CDCl_3$): δ 29.30 34.90, 108.27, 117.49, 117.80, 118.99, 128.67, 129.35, 138.83, 149.89, 152.69, 190.97. EI-MS m/z (%): 293 (M^+,100), 278 (57). *Analysis*: Calculated for $C_{14}H_{15}NO_4S$: C, 57.33; H, 5.15; N, 4.77. Found: C, 57.06; H, 5.28; N, 4.59.

3-tert-*Butyl-4-(5-nitro-thiophen-2-yloxy)-phenol (**4i**)*

Brown oil. ^{1}H-NMR (200 MHz, 27°C, $CDCl_3$): δ:1.33 (s, 9H, CMe_3), 5.70 (bs, 1H, OH), 6.37 (d, 1H, 3'-H, J = 4.9 Hz), 6.74 (dd, 1H, 6-H, J = 8.5,2.9 Hz), 6.95 (d, 1H, 2-H, J = 2.9 Hz), 7.01 (d, 1H, 5-H, J = 8.5 Hz), 7.79 (d,1H, 4'-H, J = 4.9 Hz). ^{13}C-NMR (50 MHz, 27°C, $CDCl_3$): δ 30.13, 34.73, 108.68, 115.01, 117.69, 122.14, 129.75, 139.08, 143.06, 148.92, 153.80, 171.86. EI-MS m/z (%): 293 (M^+,100), 232 (62). *Analysis*: calculated for $C_{14}H_{15}NO_4S$: C, 57.33; H, 5.15; N, 4.77. Found: C, 57.59; H, 5.06; N, 4.83.

*6-Chloro-4-(4-methoxy-phenoxy)-3-methyl-isoxazolo[4,5-c]pyridine (**6f**)*

MP 118–120°C. ^{1}H-NMR (200 MHz, 27°C, $CDCl_3$): δ 2.69 (s, 3H, Me), 3.82 (s, 3H, OMe), 6.93, 7.13 (2d, 4H, 2,6-H and 3,5-H, J = 8.9 Hz), 7.15 (s, 1H, $H_{pyr.}$). ^{13}C-NMR (50 MHz, 27°C, $CDCl_3$): δ 13.56, 56.01, 104.17, 116.45, 118.44, 119.02, 119.94, 148.85, 150.59, 152.57, 154.13, 155.35, 183.43. ESI-MS: 291 ($M\text{-}H^+$). *Analysis*: calculated for $C_{14}H_{11}ClN_2O_3$: C, 57.84; H, 3.81; N, 9.64. Found: C, 57.58; H, 3.98; N, 9.46.

*6-Chloro-4-(4-methoxy-phenoxy)-3-methyl-isoxazolo[5,4-b]pyridine (**6h**)*

MP 150–152°C.^{1}H-NMR (200 MHz, 27°C, $CDCl_3$): δ 2.61 (s, 3H, Me), 3.81 (s, 3H, OMe), 6.31 (s,1H, $H_{pyr.}$), 6.96, 7.09 (2d, 4H, 2,6-H and 3,5-H, J = 9.2 Hz). ^{13}C-NMR (50 MHz, 27°C, $CDCl_3$): δ 12.20, 55.63, 102.99, 104.26, 115.46, 121.82, 145.84, 154.13, 154.77, 158.00, 163.11, 170.85. ESI-MS: 291 ($M\text{-}H^+$). *Analysis*: calculated for $C_{14}H_{11}ClN_2O_3$: C,57.84; H,3.81; N,9.64. Found: C, 58.06; H, 3.69; N, 9.73.

2-tert-*Butyl-5-(6-chloro-3-methyl-isoxazolo[4,5-c]pyridin-4-yl)-benzene-1,4-diol (**7a**)*

MP 202–205°C. ^{1}H-NMR (200 MHz, 27°C, $CDCl_3$): δ 1.42 (s, 9H, CMe_3), 2.57 (s, 3H, Me), 4.97 (bs, 1H, OH), 6.74, 7.08 (2s, 2H, 3-H and 6-H), 7.42 (s, 1H, H_{pyr}). ^{13}C-NMR (200 MHz, 27°C, $CDCl_3$): δ 13.65, 29.50, 34.99, 103.83, 116.65, 117.11, 118.09, 134.55, 142.52, 147.20, 148.79, 150.46, 154.13, 154.88, 170.28. ESI-MS: 333 ($M\text{-}H^+$). *Analysis*: calculated for $C_{17}H_{17}ClN_2O_3$: C, 61.36; H, 5.15; N, 8.42. Found: C, 61.16; H, 5.39; N, 8.25.

*2-*tert*-Butyl-5-(6-chloro-3-methyl-isoxazolo[5,4-b]pyridin-4-yl)-benzene-1,4-diol (**7b**)*
MP 281–286°C. ^{1}H-NMR (200 MHz, 27°C, $CDCl_3$) δ: 1.42 (s, 9H, CMe_3), 2.57 (s, 3H, Me), 4.97 (bs, 1H, OH), 6.74, 7.02 (2s, 2H, 3-H and 6-H), 7.42 (s, 1H, $H_{pyr.}$). ^{13}C-NMR (50 MHz, 27°C, $CDCl_3$) δ: 13.23, 28.51, 33.94, 114.09, 116.16, 117.96, 120.12, 130.48, 138.86, 140.36, 145.61, 147.20, 148.10, 150.84, 155.89. ESI-MS: 333 ($M\text{-}H^+$). *Analysis*: Calculated for $C_{17}H_{17}ClN_2O_3$: C, 61.36; H, 5.15; N, 8.42. Found: C, 61.65; H, 5.27; N, 8.58.

*2-(6-Chloro-3-methyl-isoxazolo[4,5-c]pyridin-4-yl)-4-methoxy-phenol (**7c**)*
MP 138–141°C. ^{1}H-NMR (200 MHz, 27°C, $CDCl_3$) δ: 2.58 (s, 3H, Me), 3.82 (s, 3H, OMe), 6.94 (d, 1H, 3-H, J=2.6 Hz), 7.00 (dd, 1H, 5-H, J=8.9, 2.6 Hz), 7.07 (d, 1H, 6-H, J=8.9 Hz), 7.49 (s, 1H, H_{pyr}), 9.63 (bs, 1H, OH). ^{13}C-NMR (50 MHz, 27°C, $CDCl_3$) δ: 13.47, 55.93, 104.09, 116.36, 118.35, 118.79, 118.93, 119.83, 148.73, 150.49, 152.49, 154.05, 155.14, 170.25. ESI-MS: 291 ($M\text{-}H^+$). *Analysis*: Calculated for $C_{14}H_{11}ClN_2O_3$: C, 57.84; H, 3.81; N, 9.64. Found: C, 58.07; H, 3.62; N, 9.47.

*2-(6-Chloro-3-methyl-isoxazolo[5,4-b]pyridin-4-yl)-4-methoxy-phenol (**7d**)*
MP 121–123°C. ^{1}H-NMR (200 MHz, 27°C, $CDCl_3$) δ: 2.26 (s, 3H, Me), 3.78 (s, 3H, OMe), 6.73 (t, 1H, 3-H, J=1.8 Hz), 6.93 (d, 2H, 5-H e 6-H, J=1.8 Hz), 7.25 (s, 1H, $H_{pyr.}$). ^{13}C-NMR (50 MHz, 27°C, $CDCl_3$) δ: 11.59, 55.86, 111.45, 115.15, 116.91, 117.26, 121.07, 122.37, 146.27, 146.48, 152.61, 153.67, 156.16, 168.86. ESI-MS: 578 [2(M-H)], 289 (M-H). *Analysis*: Calculated for $C_{14}H_{11}ClN_2O_3$: C, 57.84; H, 3.81; N, 9.64. Found: C, 57.71; H, 3.90; N, 9.53.

*2-(6-Chloro-3-methyl-oxazolo[4,5-c]pyridin-4-yl)-4-methoxy-phenol (**8c**)*
MP 193–198°C. ^{1}H-NMR (200 MHz, 27°C, $CDCl_3$) δ: 2.71 (s, 3H, Me), 3.86 (s, 3H, OMe), 6.97 (d, 2H, 5-H e 6-H, J=1.56 Hz), 7.38 (s, 1H, H_{pyr}), 8.73 (t, 1H, 3-H, J=1.56 Hz). ^{13}C-NMR (50 MHz, 27°C, $CDCl_3$) δ: 14.57, 55.98, 100.07, 104.87, 114.71, 117.35, 118.87, 119.65, 120.46, 152.22, 154.02, 158.43, 164.73, 171.22. ESI-MS: 291 ($M\text{-}H^+$). *Analysis*: Calculated for $C_{14}H_{11}ClN_2O_3$: C, 57.84; H, 3.81; N, 9.64. Found: C, 58.07; H, 3.62; N, 9.47.

Evaluation of antioxidant activity

The antioxidant activity of the compounds was measured on the basis of their ability to scavenge the radical cation 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonate) ($ABTS^{+\bullet}$) by spectrophotometric analysis (Re et al 1999). The $ABTS^{+\bullet}$ cation radical was produced by the reaction between ABTS (7 mM in water) and potassium persulfate (2.45 mM), stored in the dark at room temperature for 12 h. The $ABTS^{+\bullet}$ solution was then diluted with ethanol to an absorbance of 0.70 at 734 nm and equilibrated at 30°C. Samples were diluted with ethanol to produce stock solutions at different concentrations. The reaction was initiated by the addition of 1 mL diluted ABTS to 10 μL sample solution. Measurements were repeated three times for each concentration. The percentage inhibition of absorbance at 734 nm was calculated for each concentration relative to a blank absorbance and was plotted as a function of concentration of compound. The antioxidant activity of each compound is reported as the IC50 value (the concentration that scavenged 50% of the $ABTS^{+\bullet}$).

In another set of experiments, we measured the antioxidant activity in the oxidation of linoleic acid promoted by 2,2′-azobis(amidino-propane) hydrochloride (AAPH), as previously reported (Sgaragli et al 1993), and the oxidation of haemoglobin by AAPH. Oxyhaemoglobin solutions in 0.1 M sodium phosphate buffer (pH 7.4) were incubated in the presence of 50 mM AAPH at 37°C. Oxidation was monitored from absorption spectra of haemoglobin solutions by measuring the change in absorbance at 421–401 nm for 30 min (Bartozs et al 1997).

Statistical analysis

Non-parametric tests were used to compare the effects of BHA and novel compounds in each study group. The Kruskal–Wallis test following by Dunn's post-hoc test were used to analyse the effects of concentration and compound type. Difference between two data groups were analysed using the Mann–Whitney test, using GraphPad InStat version 3.0 GraphPad Software (San Diego, CA, USA).

Results and Discussion

Chemistry

A series of heterocyclic phenol ethers 3a–i were prepared (Figure 1, Table 1) ; by the reaction of activated halo-derivatives (compounds 1a–i) with *tert*-butyl-hydroquinone (compound 2). As expected, attack on the less-hindered phenolic group was found with all compounds except the strongly activated compounds 1e and 1i, which also gave a significant

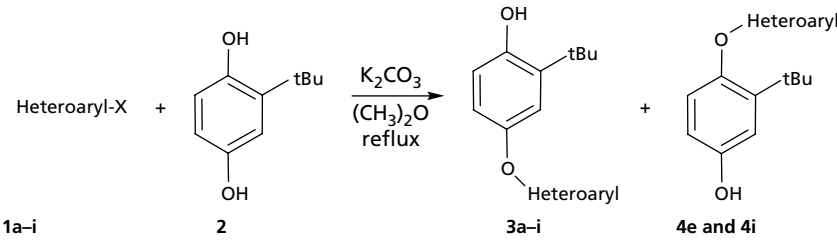
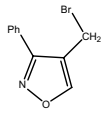
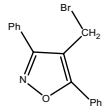
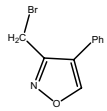
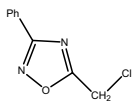
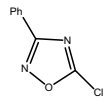
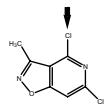
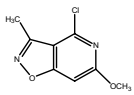
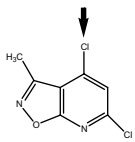
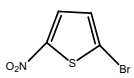


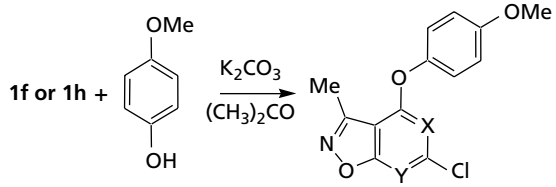
Figure 1 Scheme of the general reactions to obtain heterocyclic phenol ethers 3a–i.

Table 1 Molecular structure of the heteroaryl derivatives used for synthesis of 3a–i and 4a–i, and yield of the reaction products

Entry	Heteroaryl-X	Yield 3a–i	Yield 4a–i
1a		50%	–
1b		58%	–
1c		30%	–
1d		60%	–
1e		55%	35%
1f		63%	–
1g		65%	–
1h		57%	–
1i		52%	35%

amount of the hindered ethers 4e and 4i. Following an analogous procedure, compounds 6f and 6h were prepared from the dichloroisoxazolopyridines 1f and 1h, by reaction with 4-methoxyphenol (compound 5) (Figure 2). The *C*-substituted derivatives were also of interest to consider the effect of the heterocyclic system more directly bonded to the phenol. Initially we tried electrophilic attack of the halo heterocycles on the aromatic phenolic ring in the presence of different catalysts, but this procedure gave poor results. Photo-Fries rearrangement of compounds 3f and 3h and 6f and 6h, as shown in Figure 3, offer a good synthetic protocol, giving 7a–d in moderate yield. On further prolonged irradiation, the isoxazolopyridine 7c slowly rearranged to the corresponding oxazolopyridine 8c.

All the newly prepared compounds (3, 4, 6, 7 and 8c) gave satisfactory elemental analysis. Spectral data (NMR



5 6f and 6h

6	X	Y	Yield
f	N	CH	80%
h	CH	N	74%

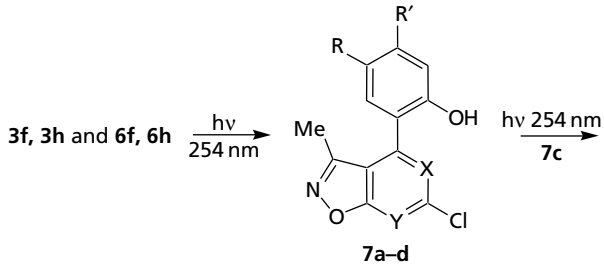
Figure 2 Pathway and yield of the general reaction to obtain isoxazolopyridine derivatives 6f and 6h.

and MS) are in agreement with the assigned structures. Nuclear Overhauser effect experiments confirmed the substitution pattern of these compounds. (Irradiation of the OH group in compounds 3a–i or 4e and 4i increases the signals of one or two aromatic protons, respectively.) The spectrum of compound 8c showed a strongly deshielded signal (8.73 ppm), attributable to the H-3. This unexpected chemical shift can be explained by supposing a hydrogen bond between the OH and the pyridine nitrogen, to give almost complete coplanarity between the omo- and heterocyclic rings. This effect is absent in the isoxazolopyridines 7a–d because the hindrance of the 3-methyl group prevents a similar conformation.

Antioxidant properties

A preliminary screening of the antioxidant properties of the new derivatives was carried out (Table 2). The statistical comparison between the calculated IC50 values gave a highly significant P value from the chi-squared distribution ($P < 0.001$). The IC50 value for compound 3c was significantly different from the values for compounds 3e and 3h ($P < 0.05$ and $P < 0.01$, respectively). Compound 3c shows the highest scavenger activity towards the $ABTS^{+\bullet}$ (see experimental section), which was one order of magnitude greater than the corresponding lead compound BHA. Compounds 3i, 3d, 3f and 3g had lower IC50 values than BHA, but these were 2–3 fold higher than that observed for 3c. Furthermore, compounds 3a and 3b, which are structurally related to 3c, had IC50 values that were similar to BHA. In compounds 3a and 3b, the phenol moiety is linked to the isoxazole ring through a methylene carbon in the β-position to nitrogen, whereas it is in the α-position in compound 3c.

In a second series of experiments, the antioxidant activity of the most potent compound, 3c, was tested in different models. The high antioxidant activity of compound 3c was confirmed when it was incubated in the presence of AAPH and linoleic acid. The IC50 value for 3c was almost 5-fold lower than that observed with BHA ($8.0 \pm 0.9 \times 10^{-6}$ M vs $3.9 \pm 0.5 \times 10^{-5}$ M, respectively; $P < 0.05$; Figure 4).



7	R	R′	X	Y	Yield
a	OH	*t*-but	N	CH	20%
b	OH	*t*-but	CH	N	20%
c	OMe	H	N	CH	32%
d	OMe	H	CH	N	30%

Figure 3 Photochemistry parameters to obtain compounds 7a–d and 8c.

Table 2 Quenching of the $ABTS^{+\bullet}$ radical promoted by different heterocyclic phenol ethers, calculated as the concentration that scavenged 50% the $ABTS^{+\bullet}$ radical (IC50). Values are mean ± s.d. of three experiments

Compound	IC50 (M)
3c	$3.17 \pm 1.26 \times 10^{-6}$
3i	$6.92 \pm 0.57 \times 10^{-6}$
3d	$7.01 \pm 0.69 \times 10^{-6}$
3f	$9.08 \pm 1.15 \times 10^{-6}$
3g	$9.72 \pm 0.86 \times 10^{-6}$
tert-butylhydroxy-anisole	$1.04 \pm 0.17 \times 10^{-5}$
7d	$1.07 \pm 1.04 \times 10^{-5}$
3a	$1.12 \pm 0.51 \times 10^{-5}$
7c	$1.14 \pm 0.34 \times 10^{-5}$
3b	$1.20 \pm 0.86 \times 10^{-5}$
3e	$1.55 \pm 0.51 \times 10^{-5}$*
3h	$3.50 \pm 0.69 \times 10^{-5}$**

*$P < 0.05$; **$P < 0001$ vs compound 3c.

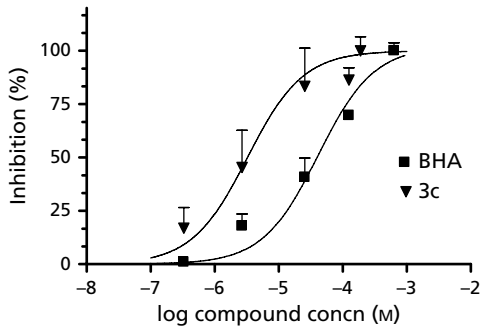


Figure 4 Inhibition of linoleic acid oxidation promoted by the radical generator 2,2′-azobis(amidino-propane) hydrochloride (AAPH). 3.3 mM linoleic acid was incubated in the presence of various concentration of compound 3c or *tert*-butylhydroxy-anisole (BHA). Oxidation was promoted by the addition of 40 mM AAPH. IC50 values were $8.0 \pm 0.9 \times 10^{-6}$ M and $3.9 \pm 0.5 \times 10^{-5}$ M, for 3c and BHA, respectively. Data are mean ± s.d. of four experiments.

A similar result was also observed in the haemoglobin oxidation study. BHA at the highest concentration used (3.3×10^{-3} M) inhibited haemoglobin oxidation promoted by

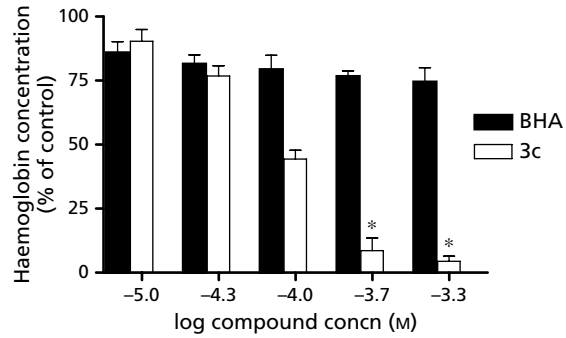


Figure 5 Inhibition of haemoglobin oxidation promoted by the radical generator 2,2′-azobis(amidino-propane) hydrochloride (AAPH). Haemoglobin (5 μM) was incubated in the presence of compound 3c or or *tert*-butylhydroxy-anisole (BHA) and the oxidation was promoted by the addition of 10 mM AAPH. Data are mean ± s.d. of four experiments. *$P < 0.05$ vs same concentration of BHA.

AAPH by 26% whereas the same concentration of compound 3c inhibited methaemoglobin formation by 95% ($P < 0.05$). The observed IC50 value for 3c was 9.5×10^{-5} M (Figure 5). Statistical analysis showed that values obtained with compound 3c at both 2×10^{-4} M and 5×10^{-4} M were significantly different from the corresponding values observed with BHA at the same concentrations ($P < 0.05$, Kruskal–Wallis test following by Dunn's post-hoc test).

These results suggests that the α-substitution, responsible for a greater electron-attraction from the phenolic system, gave rise to lower bond dissociation enthalpy of OH and, as a consequence, to better radical-scavenging activity. This was confirmed by the fact that this effect remains when the isoxazole system is replaced by other electron-attracting heterocycles (i.e. nitrothiophene or 1,2,4-oxadiazole). In fact, these derivatives (compounds 3i and 3d) also have better antioxidant activity than BHA. On the other hand, replacement of the *tert*-butyl group in the BHA molecule with a heterocyclic system (compounds 7c and 7d) did not significantly modify the antioxidant activity.

Conclusion

The heteroaryl-*O*- and *C*-substituted phenols, prepared directly or by rearrangement, show significant radical-scavenging and antioxidant activities, which are at least comparable

to or higher than those of the reference compound BHA. This result was mainly found in compounds where the heterocyclic ring is not directly in conjugation with the phenolic system, although the heterocycle probably influences the antioxidant activity. Among the phenols tested, those substituted with an electron-withdrawing group were the most efficient.

It cannot be discounted that the studied compounds, which are structurally similar to BHA, could interact with oxidative enzymes involved in free radical formation, such as cyclooxygenase. Further studies are needed to test the toxicity, safety and the interaction with other compounds, such as drugs, of these compounds. In fact, It cannot be discounted that the studied compounds, structurally similarly to BHA, might interact with cellular components such as oxidative enzymes involved in free radical formation, as cyclooxygenase.

References

Adembri, G., Camparini, A., Ponticelli, F., Tedeschi, P. (1975) Synthesis and reactivity of 3-methylisoxazolo[4,5-*c*]pyridines. *J. Chem. Soc. Perkin Trans*. 2190–2194

Adembri, G., Camparini, A., Ponticelli, F., Tedeschi P. (1981) Thermal behaviour of 3-phenol-1,2,4-oxadiazol-5-ylhydrazines. *J. Chem. Soc. Perkin Trans*. 1703–1706

Agirbas, H., Sumengen, D., Durust, Y. Durust, N. (1992) The reaction of amidoximes with chloroacetyl chloride. *Synthetic Commun*. **22**: 209–217

Bartosz, M., Kedziora, J., Bartosz, G., (1997) Antioxidant and prooxidant properties of captopril and enalapril. *Free Radic. Biol. Med*. **23**: 729–735

Camparini, A., Ponticelli, F., Tedeschi, P. (1977) New derivatives of 3-methylisoxazolo[5,4-*b*]pyridine. *J. Heterocyclic Chem*. **14**: 435–438

Donati, D., Fusi, S., Ponticelli, F. (1991) Photoreactivity of 4-nucleophile substituted isoxazoles. *Gazz. Chim. Ital*. **121**: 329–334

Festjens, N., Kalai, M., Smet, J., Meeus, A., Van Coster, R., Saelens, X., Vandenabeele, P. (2006) Butylated hydroxyanisole is more than a reactive oxygen species scavenger. *Cell Death Differ*. **13**: 166–169

Fusi, F., Sgaragli, G., Murphy, M. P. (1992) Interaction of butylated hydroxyanisole with mitochondrial oxidative phosphorylation. *Biochem. Pharmacol*. **43**: 1203–1208

Giorgi, G., Ponticelli, F., Salvini, L., Trendafilova, A., Valoti, M., Pessina, F. (2003) Synthetic approach, regio- and stereochemical characterization and differentiation of new potential antioxidant *C*- and *O*-arylglycosides. *Eur. J. Org. Chem*. **1**: 106–115

Goossens, V., Grooten, J., De Vos, K., Fiers, W. (1995) Direct evidence for tumor necrosis factor-induced mitochondrial reactive oxygen intermediates and their involvement in cytotoxicity. *Proc Natl Acad Sci USA* **92**: 8115–8119

Ito, N., Fukushima, S., Tsuda, H. (1985) Carcinogenicity and modification of the carcinogenic response by BHA, BHT, and other antioxidants. *Crit. Rev. Toxicol*. **15**: 109–150

Ito, N., Hirose, M. (1989) Antioxidants – carcinogenic and chemopreventive properties. *Adv Cancer Res*. **53**: 247–302

Matthews, N., Neale, M., L., Jackson, S. K., Stark, J. M. (1987) Tumour cell killing by tumour necrosis factor: inhibition by anaerobic conditions, free-radical scavengers and inhibitors of arachidonate metabolism. *Immunology* **62**: 153–155

Ponticelli, F., Trendafilova, A., Valoti, M., Saponara, S., Sgaragli, G. P. (2001) Synthesis and antiperoxidant activity of new phenolic *O*-glicosides. *Carbohydrate Res*. **330**: 459–468

Pratt, D. A., Di Labio, G. A., Brigati, G., Pedulli, G. F., Ingold, K. U. (2002) Substituent effects on the bond dissociation enthalpies of aromatic amines. *J. Am. Chem. Soc*. **124**: 11085–11092

Prochaska, H. J., Bregman, H. S., De Long, M. J., Talalay, P. (1985) Specificity of induction of cancer protective enzymes by analogues of tert-butyl-4-(BHA). *Biochem. Pharmacol*. **34**: 3909–3914

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C. (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med*. **26**: 1231–1237

Sgaragli, G. P., Valoti, M., Gorelli, B., Fusi, F., Palmi, M., Mantovani, P. (1993) Calcium antagonist and antiperoxidant properties of some hindered phenols. *Br. J. Pharmacol*. **110**: 369–377

Wattenberg, L. W. (1986) Protective effects of 2(3)-tert-butyl-4-hydroxyanisole on chemical carcinogenesis. *Food Chem. Toxicol*. **24**: 1099–1102

World Health Organization (1989) WHO Food Additives Series **24**: 3–22