Bioorganic & Medicinal Chemistry Letters 20 (2010) 4132–4134

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and biological activity of halophenols as potent antioxidant and cytoprotective agents

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ARTICLE INFO

Article history:
Received 11 February 2010
Revised 16 May 2010
Accepted 18 May 2010
Available online 8 June 2010

Keywords:
Bromophenols
Halophenols
DPPH radical-scavenging activity
Cytoprotective activity
HUVEC

ABSTRACT

A series of new bromophenols and chlorophenols were prepared by a practical route. The in vitro antioxidative activity of the halophenols was evaluated by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging assay, and their cytoprotective activity was also tested on hydrogen peroxide (H_2O_2)-induced injury in human umbilical vein endothelial cells (HUVEC). All halophenols tested displayed moderate to good DPPH radical-scavenging activity, and two bromophenols, 2,3′-dibromo-4,5,6′-trihydroxydiphenylmethanone (**16c**) and 2,3-dibromo-4,5-dihydroxydiphenylmethanone (**17c**) exhibited high protective activity against H_2O_2-induced injury in HUVEC with EC_{50} values of 0.4 and 0.8 μM, respectively. The preliminary structure–activity relationships of these compounds were also investigated in order to determine the essential structures required for their bioactivities.

Bromophenols frequently isolated from various marine algae, ascidians, and sponges have attracted much research interest due to their wide spectrum of bioactivities including antioxidative,[1,2] antithrombotic,[3] antimicrobial,[4,5] anti-inflammatory,[6] enzyme inhibition,[7] cytotoxic,[4,8] and feeding deterrent[9] activities. Two natural bromophenols with diphenyl skeletons, bis(2,3,6-tribromo-4,5-dihydroxyphenyl)methane (**1**)[2,7] and (+)-3-(2,3-dibromo-4,5-dihydroxyphenyl)-4-bromo-5,6-dihydroxy-1,3-dihydroisobenzofuran (**2**),[3,8] exhibited good in vitro antioxidative and in vivo antithrombotic activity, respectively (Fig. 1). However, their corresponding structure–activity relationships are still unclear.

Encouraged by these interesting pharmacological activities, we synthesized analogs of the diphenyl bromophenols. In this paper, we report on the synthesis and in vitro activities of a series of bromophenols and chlorophenols along with their primary structure–activity relationships.

The general procedure for the synthesis of the halophenols started with preparation of the methoxylated diphenyl intermediates **11**–**14** (Scheme 1). Compounds **7**–**10** were obtained by treating **3**–**6** with dry $SOCl_2$ and a catalytic amount of DMF, and Friedel–Crafts acylation of **7**–**10** with 1,2-dimethoxybenzene catalyzed by $AlCl_3$ which gave **11**–**14**,[10,11] in which methoxy groups were used as hydroxy protecting groups.

As shown in Scheme 2, the bromination of **11** with 2.2 equivalents of bromine in acetic acid at room temperature provided an excellent yield of **15a**.[12] Reduction of the benzylic ketone under standard conditions then afforded **15b**.[13,14] Demethylation of the methoxy groups in **15a** and **15b** with BBr_3 gave two bromophenols, 2,3′-dibromo-4,4′,5-trihydroxydiphenylmethanone (**15c**) and 2,3′-dibromo-4,4′,5-trihydroxydiphenylmethane (**15d**), respectively.[15]

To investigate the positional effects of hydroxyl groups in the aromatic ring on bioactivity, two analogs of **15c** and **15d**, 2,3′-dibromo-4,5,6′-trihydroxydiphenylmethanone (**16c**) and 2,3′-dibromo-4,5,6′-trihydroxydiphenylmethane (**16d**), were prepared in the same manner (Scheme 3).

In order to examine the changes in bioactivity resulting from the different numbers and positions of the bromine atoms attached to the phenol ring, two monobrominated analogs 2-bromo-4,5-dihydroxydiphenylmethanone (**18c**), 2-bromo-4,5-dihydroxydiphenylmethane (**18d**), and two dibrominated analogs 2,3-dibromo-4,5-dihydroxydiphenylmethanone (**17c**), 2,3-dibromo-4,5-dihydroxydiphenylmethane (**17d**) were synthesized by the same method described in Scheme 2. The number of brominated atoms was determined by the equivalents of **13** and bromine, reaction time and temperature (Scheme 4).

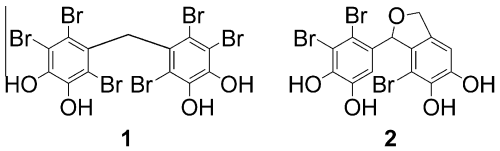

Figure 1. Bioactive bromophenols.

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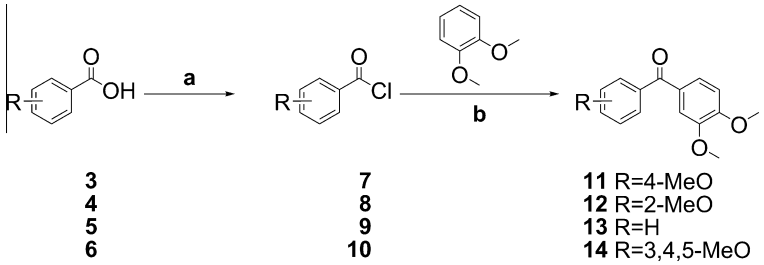
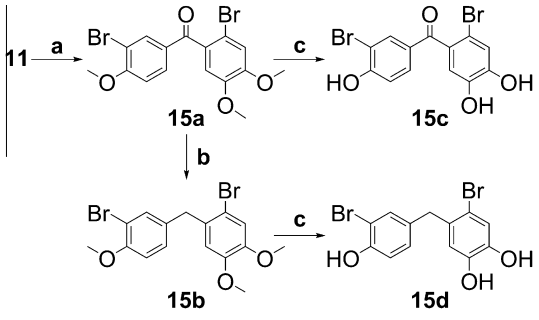
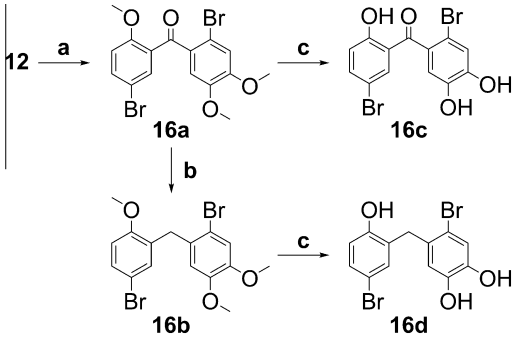
doi:10.1016/j.bmcl.2010.05.068



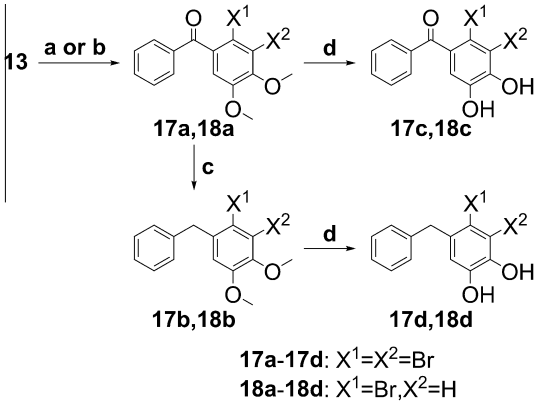
Scheme 1. Reagents and conditions: (a) $SOCl_2$, DMF, reflux, 5 h; (b) $AlCl_3$, CH_2Cl_2, rt, 2 h, 80–85%.



Scheme 2. Reagents and conditions: (a) Br_2 (2.2 equiv), AcOH, rt, 4 h, 95%; (b) $LiAlH_4$, $AlCl_3$, reflux, 2 h, 61%; (c) BBr_3 (10 equiv), CH_2Cl_2, −78 °C to rt, 4 h, 72–75%.

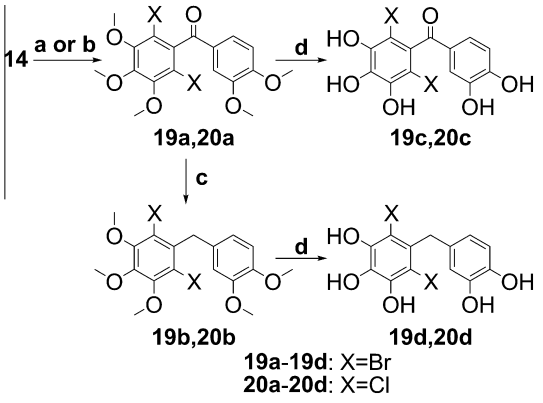


Scheme 3. Reagents and conditions: (a) Br_2 (2.2 equiv), AcOH, rt, 4 h, 84%; (b) $LiAlH_4$, $AlCl_3$, reflux, 2 h, 52%; (c) BBr_3 (10 equiv), CH_2Cl_2, −78 °C to rt, 4 h, 70–73%.



Scheme 4. Reagents and conditions: (a) Br_2 (2.2 equiv), AcOH, rt, 24 h, 86%; (b) Br_2 (1.1 equiv), AcOH, rt, 4 h, 91%; (c) $LiAlH_4$, $AlCl_3$, reflux, 2 h, 50–55%; (d) BBr_3 (10 equiv), CH_2Cl_2, –78 °C to rt, 4 h, 69–72%.

We subsequently focused on developing the SARs with respect to different halogen atoms. Halophenols with bromine or chlorine atoms on the same position were prepared according to Scheme 5.



Scheme 5. Reagents and conditions: (a) Br_2 (2.2 equiv), AcOH, rt, 4 h, 91%; (b) SO_2Cl_2 (2.5 equiv), CH_2Cl_2, reflux, 6 h, 89%; (c) $LiAlH_4$, $AlCl_3$, reflux, 2 h, 60–62%; (d) BBr_3 (10 equiv), CH_2Cl_2, −78 °C to rt, 4 h, 65–67%.

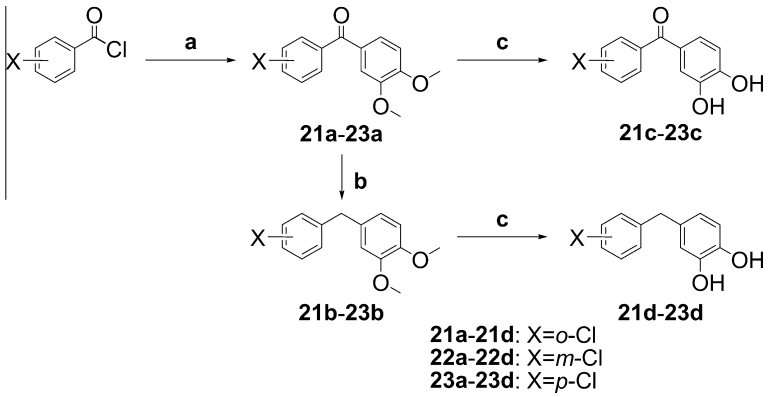
Chlorination of **14** with SO_2Cl_2 refluxing in CH_2Cl_2 provided **20a** in good yield. Two chlorophenols, 2,6-dichloro-3,3′,4,4′,5-pentahydroxydiphenylmethanone (**20c**) and 2,6-dichloro-3,3′,4,4′,5-pentahydroxydiphenylmethane (**20d**) were prepared after reduction and deprotection, and the bromophenols, 2,6-dibromo-3,3′,4,4′,5-pentahydroxydiphenylmethanone (**19c**) and 2,6-dibromo-3,3′,4,4′,5-pentahydroxydiphenylmethane (**19d**) were also obtained by the same method.

In the above reactions, regioselective halogenation of **11**, **12**, **13**, and **14** gave **15a**, **16a**, **17a**, **18a**, **19a**, and **20a**. The substitution number of halogen atoms and the substitution position were determined by analysis of ESI-MS, MS–MS, ^1H NMR, and ^{13}C NMR spectra.

Chlorination of **11**–**13** were also undertaken but gave a mixture of mono-chlorinated and poly-chlorinated phenols. In order to obtain more chlorophenols, we used *o*-, *m*-, and *p*-chlorobenzoyl chlorides as starting materials, and six chlorophenols were prepared as described above. The corresponding chlorophenols were identified as **21c**–**23c**[16] and **21d**–**23d** (Scheme 6).

As shown in Table 1, some of the halophenols were subjected to in vitro antioxidative activity testing using the DPPH radical-scavenging assay as previously reported.[17] In addition, a number of the intermediates with methoxy groups were also investigated in order to determine their SARs. All the halophenols displayed moderate to good antioxidative activity, while the intermediates showed no activity, this demonstrated that the hydroxyl groups were essential for the antioxidative activity of the halophenols.

All the halophenols and some of the intermediates were subsequently evaluated for their cytoprotective effects on H_2O_2-induced injury in HUVEC.[18] Some of the bromophenols and chlorophenols exhibited varying degrees of moderate to good activity, while the intermediates displayed no activity. Two bromophenols, 2,3′-dibromo-4,5,6′-trihydroxydiphenylmethanone (**16c**) and 2,3-dibromo-4,5-dihydroxydiphenylmethanone (**17c**) exhibited high cytoprotective activity with EC_{50} values of 0.4 and 0.8 μM, respectively, and three chlorophenols showed moderate activity.



Scheme 6. Reagents and conditions: (a) $AlCl_3$, CH_2Cl_2, rt, 2 h, 85–90%; (b) $LiAlH_4$, $AlCl_3$, reflux, 2 h, 65–70%; (c) BBr_3 (10 equiv), CH_2Cl_2, −78 °C to rt, 4 h, 75–86%.

Table 1
DPPH radical-scavenging activity and cytoprotective activity against H_2O_2-induced injury in HUVEC

Compound	DPPH scavenging activity IC_{50}[a] (μM)	Cytoprotective activity EC_{50}[b] (μM)
13	NA	NA
15a–20a	NA	NA
23a	NA	NA
15b–23b	NA	NA
15c	61.6	2.0
16c	79.6	0.4
17c	87.7	0.8
18c	84.0	11.5
19c	96.2	NA
20c	87.3	NA
15d	87.4	13.0
16d	88.5	1.1
17d	88.0	3.9
18d	163.8	12.2
19d	84.2	NA
20d	102.5	NA
21c	112.9	NA
22c	109.7	5.0
23c	102.8	8.9
21d	130.9	NA
22d	222.9	NA
23d	202.5	8.8
BHT	169.1	ND[c]
Quercetin	ND[c]	18.0

[a] Values are means of three experiments; NA, not active at 100 μg/mL.
[b] Values are means of three experiments; NA, not active at 5 μg/mL.
[c] ND, not detected.

It should be noted that compounds **19c**, **20c**, **19d**, and **20d** with more hydroxyl groups than the other halophenols displayed no activity, this showed that the cytoprotective activity of the halophenols was not directly related to the number of hydroxyl groups. The dibrominated phenolic compounds, **17c** and **17d** displayed better activity than the monobrominated phenols, **18c** and **18d**. The *o*-chlorinated phenolic compounds **21c** and **21d** showed no activity, while some of the *m*- and *p*-chlorophenols displayed moderate protective effects against H_2O_2-induced injury in HUVEC.

From these findings, we can conclude that hydroxyl groups on the diphenyl backbone are essential for the in vitro antioxidative activities of these compounds, and the presence of one or more halogen atoms on the phenol ring is also necessary. The number and position of the hydroxyl groups and halogen atoms may influence the potency of activity.

In summary, a number of new halophenols were synthesized and evaluated for their in vitro antioxidative activity using the DPPH radical-scavenging assay. All the halophenols were found to display moderate to good activity. The cytoprotective activity of the halophenols on H_2O_2-induced injury in HUVEC was also determined, and some of the bromophenols and chlorophenols exhibited promising activity. Among these, two bromophenols, 2,3′-dibromo-4,5,6′-trihydroxydiphenylmethanone (**16c**) and 2,3-dibromo-4,5-dihydroxydiphenylmethanone (**17c**) had high protective activity against H_2O_2-induced injury in HUVEC, with EC_{50} values of 0.4 and 0.8 μM, respectively. Further in vivo tests of these compounds are now under way.

Acknowledgments

The authors thank Professor Guanhua Du, Dr. Lianhua Fang, and Dr. Guorong He for the in vitro assay of the compounds. This work was financially supported by the National '863' Program of China (No. 2006AA09Z446), by the State Key Laboratory of Natural and Biomimetic Drugs, Peking University, by the Program for the Top Young and Middle-aged Innovative Talents of Higher Learning Institutions of Shanxi, and by the Shanxi Province Foundation for Oversea Returnees.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.05.068.

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