benzhydryl group in 3f form multiplet at 7.27 and 7.35 ppm. The methylene proton from the benzhydryl residue forms a singlet with weak to medium intensity at 4.36 ppm. The integral curves correspond to the exact number of the protons. The values of the chemical shifts of the protons registered by ^{1}H NMR spectra were compared with simulated values.[18−21] Due to an impossibility to render an account of influence of the solvent, we observed only small deviations of the computed values from the experimental values. Regardless, the simulated ^{1}H NMR spectra are in good correlation with experimental ones.

Antioxidant assays

In this paper, we apply four common methods [14−17] for the determination of free radicals scavenging activity, total antioxidant capacity and antioxidant activity against lipid peroxidation to both lipophilic and hydrophilic antioxidants in an attempt to establish the most appropriate one for evaluation of possible antioxidant effects, demonstrated from the newly synthesized arylpiperazine derivatives. The described methods were chosen for their user-friendly mechanisms for antioxidant activity determination since they require a simple machine like a spectrophotometer, which is commonly available in most laboratories.

The free radicals scavenging activity was measured by using DPPH and ABTS methods with slight modifications.[20] The inhibitory effect of different concentrations of compounds (6.25−1000 μmol/L^2) on DPPH (Figure 1) and ABTS (Figure 2) was determined by recording the absorbance of the reaction mixture at 517 and 734 nm, respectively. The IC_{50} values (concentration of sample where the absorbance of DPPH and ABTS decreases 50% with respect to the absorbance of blank) of the samples were determined.

The applied FRAP assay was done according to the method described previously.[14] The obtained results are

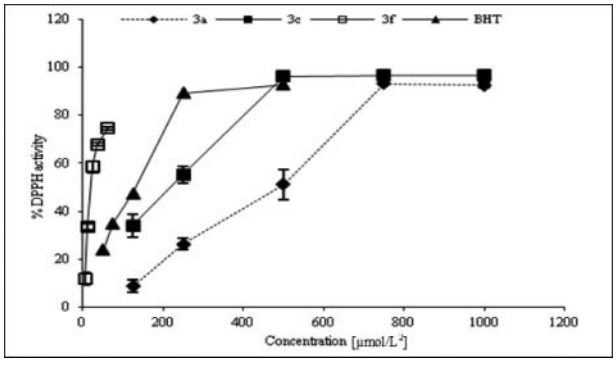

Figure 1. DPPH radical scavenging activity of studied compounds 3a, 3c, 3f and BHT.

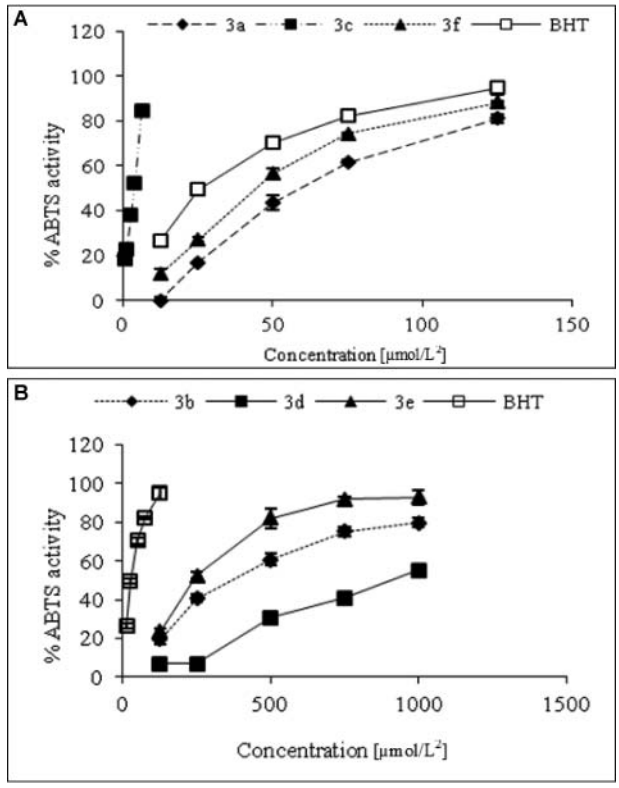

Figure 2. ABTS-radical scavenging activity of studied compounds (a) 3a, 3c, 3f and BHT and (b) 3b, 3e, 3d and BHT.

expressed in μmol/L Trolox equivalent for mmol/L compound (μmol/L TE/[μmol/L TE/mmol/L]) for 1000 μmol/L^2 solutions of piperazines.

The corresponding radical scavenging activities against DPPH, ABTS and FRAP of the compounds were compared with those of BHT, used as positive control. All determinations are performed in triplicate ($n = 3$), and the results are expressed as IC_{50} μmol L^{-2} of inhibition in DPPH and ABTS determinations and as mmol/L TE mM^{-1} in FRAP determination. The obtained values are presented in Table 1.

Among the analysed structures, DPPH radical scavenging activity was shown by compounds 3a (IC_{50} 371.97 μmol/L), 3c (IC_{50} 189.42 μmol/L) and 3f (IC_{50} 420.57 μmol/L), with the highest antioxidant activity demonstrated by compound 3c. However, the obtained IC_{50} values of all piperazines were lower than that of BHT (IC_{50} 113.17 μmol/L).

All studied compounds demonstrated ABTS-radical scavenging decreasing in order: 3c (IC_{50} 3.45 μmol/L) > BHT (IC_{50} 26.29 μmol/L) > 3f (IC_{50} 41.04 μmol/L) > 3a (IC_{50} 55.87 μmol/L) > 3e (IC_{50} 242.48 μmol/L) > 3b (IC_{50} 345.25 μmol/L) > 3d (IC_{50} 1028.99 μmol/L).

In the performed FRAP assay, the reduction of ferric tripyridyl triazine (Fe III TPTZ) complex to ferrous form (which has an intense blue colour) at low pH was monitored by measuring the change in the absorption at