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High-resolution α -glucosidase and radical scavenging profiling combined with HPLC-HRMS-SPE-NMR for identification of bioactive constituents in crude extract of *Pueraria lobata*

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Abstract:

This work describes the identification of active constituents in *Pueraria lobata* root extract by dual high-resolution α -glucosidase inhibition¹ and radical scavenging profiling² combined with HPLC-HRMS-SPE-NMR.³ This analytical platform enabled pinpointing of bioactive constituents in HPLC chromatograms direct from crude extracts. Bioactive constituents were cumulatively trapped on SPE cartridges and the structures identified and elucidated by spectral data obtained in the HPLC-HRMS-SPE-NMR mode. A total of 24 compounds were identified, and several of these showed radical scavenging activity while two isoflavonoids showed α -glucosidase inhibitory activity.

BACKGROUND

Pueraria lobata is a perennial leguminous vine, which is widely distributed in China where it is used as a dietary supplement and herbal medicine due to its profound pharmacological functions.⁴ *P. lobata* extract has previously shown antioxidant activity - one of the most important effects of functional food, dietary supplements and anticancer natural products - as well as α -glucosidase inhibitory activity - an important effect for managing blood glucose for type 2 diabetics.⁵



Figure 1. *Pueraria lobata*

METHOD

Methanol extract of *Pueraria lobata* was investigated by dual high-resolution α -glucosidase/radical scavenging profiling combined with HPLC-HRMS-SPE-NMR.

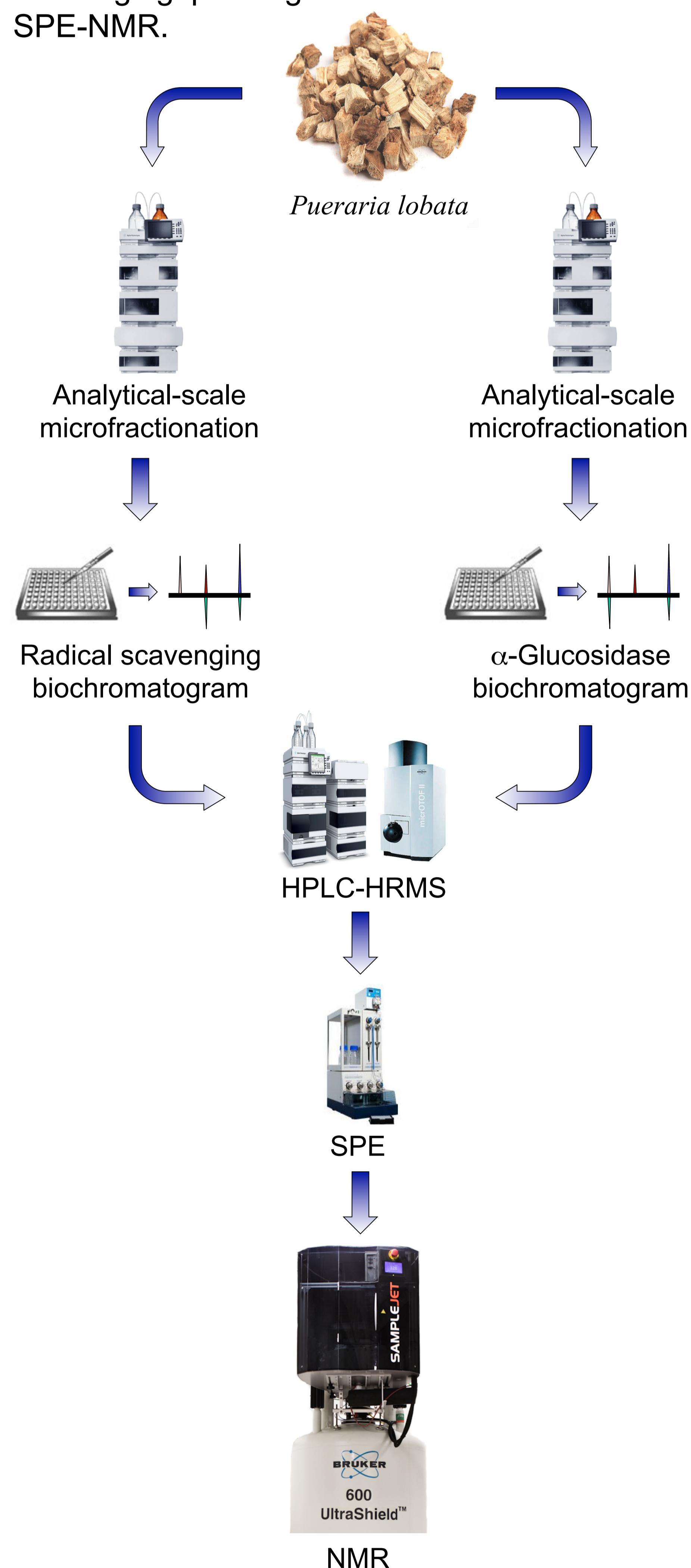


Figure 2. Principle of dual α -glucosidase/radical scavenging profiling combined with HPLC-HRMS-SPE-NMR

RESULTS

Dual high-resolution α -glucosidase/radical scavenging profiling provided an α -glucosidase inhibition profile (red trace in Figure 3) and a radical scavenging profile (blue trace in Figure 3) below the HPLC chromatogram. This showed several constituents with radical scavenging activity as well as two constituents, i.e., 18 and 23, with α -glucosidase inhibitory activity.

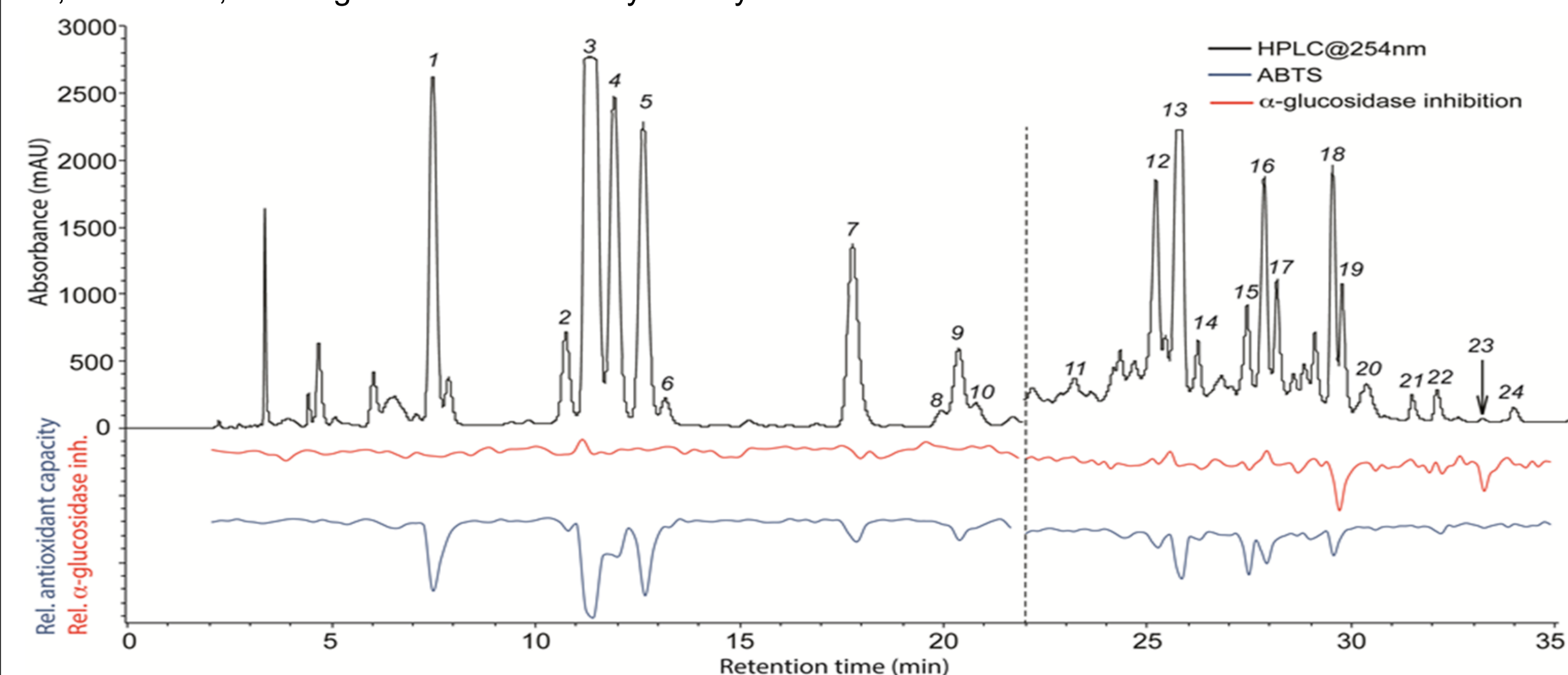


Figure 3. Dual high-resolution α -glucosidase inhibition profile and radical scavenging profile.

Analysis of HRMS and NMR data obtained in the HPLC-HRMS-SPE-NMR mode led to identification of 24 compounds (Figure 4), of which 6 and 14, were new compounds.

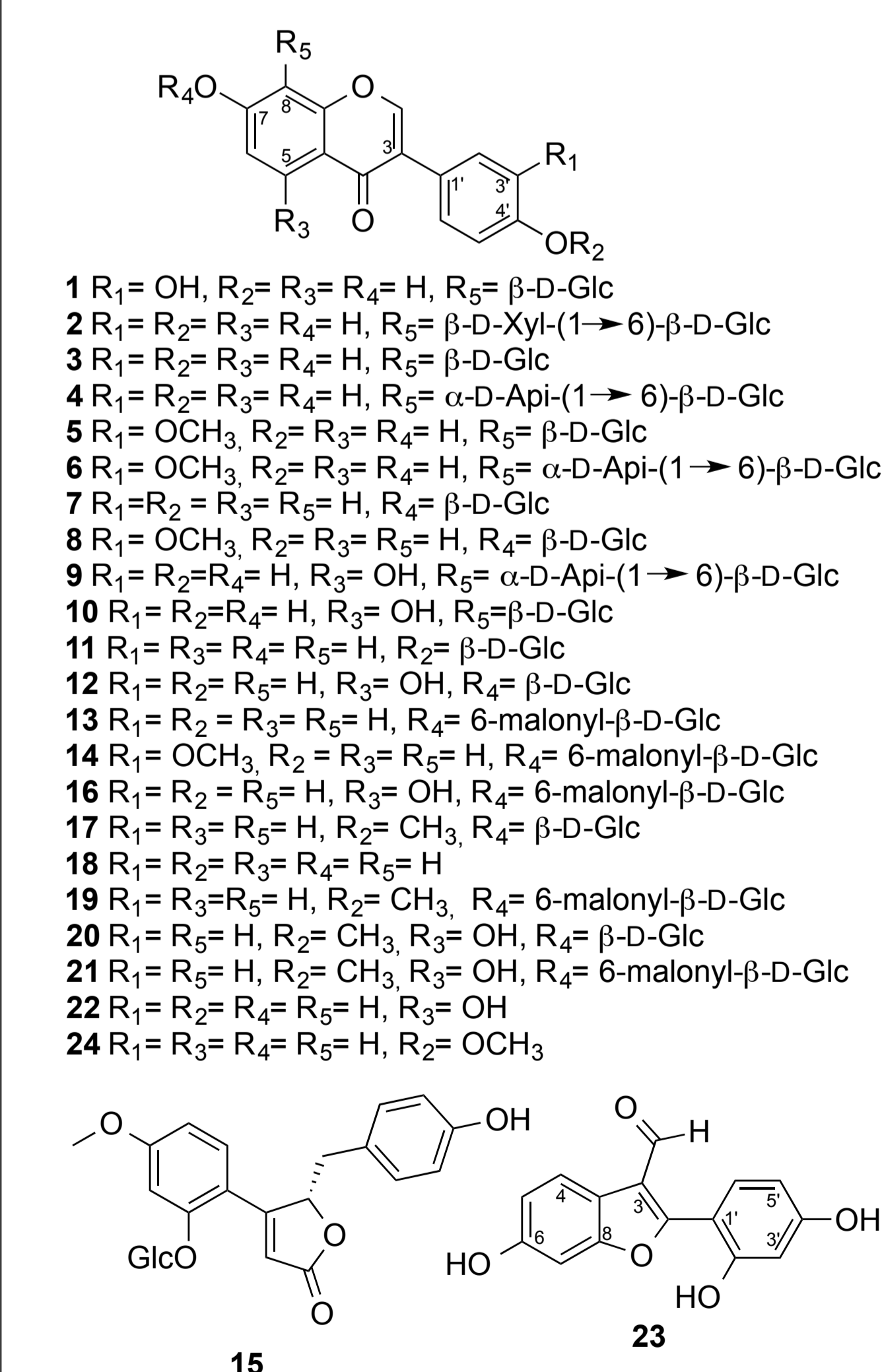


Figure 4. Structure of 1-24

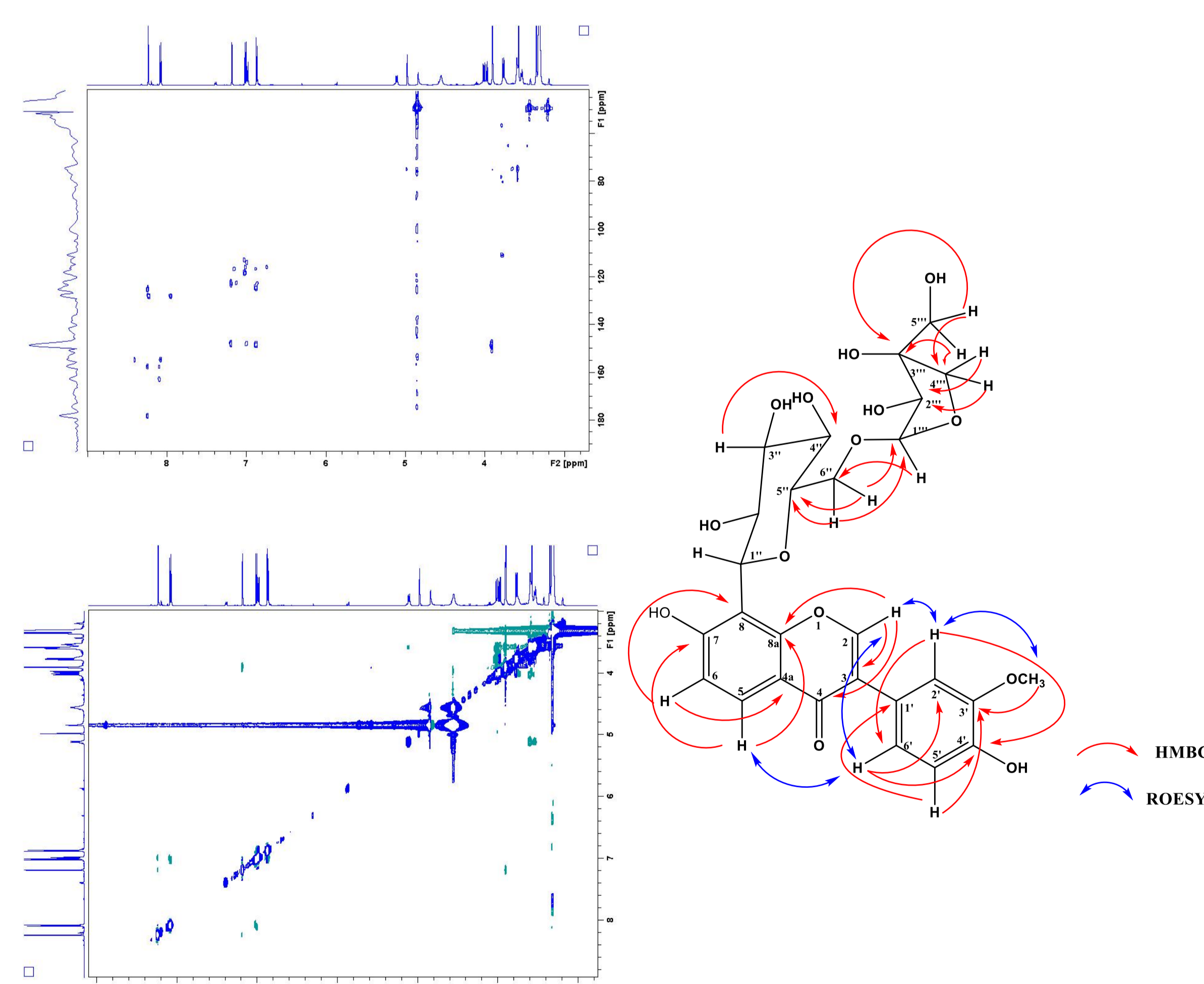


Figure 5. Key HMBC and ROESY correlations in spectra of 6

Conclusion

In this work, dual high-resolution α -glucosidase inhibition and radical scavenging profiling combined with HPLC-HRMS-SPE-NMR allowed direct analysis of α -glucosidase inhibitors and radical scavengers in crude extract of *Pueraria lobata* without prepurification. Furthermore, 24 constituents - including new compounds 6 and 14 - were identified by analysis of HRMS and NMR spectral data. This work shows the full advantage of high-resolution bioactivity profiling/HPLC-HRMS-SPE-NMR, and promise even more efficient research in functional food, dietary supplements and traditional medicine.

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