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Chemically engineered extracts: Bioactivity alteration through sulfonylation

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ABSTRACT

The chemical composition and the biomolecular properties of a series of crude plant extracts were altered without previous knowledge of their detailed chemical composition.

Natural products have been invaluable as platforms for developing front-line drugs.[1] Because the identification of new chemotypes for drug development remains an urgent need in many therapeutic areas, innovative strategies are being developed for natural products to contribute their full range of chemical diversity to the drug discovery process. Such strategies include the preparation of natural product-like libraries,[2] and the diversification of natural product mixtures by combinatorial biosynthesis and related techniques.[3]

Recently we introduced a strategy to generate bioactive compounds through chemical diversification of inactive natural product mixtures (i.e., natural extracts).[4] The approach relies on the introduction of changes in a significant proportion of the molecules present in a natural extract without previous knowledge of the exact composition of the starting material. Our approach to modify as many compounds as possible in complex natural mixtures (i.e., plant extracts) of mostly unknown composition is to focus into the transformation of chemical functionalities which are very common in natural products and thus expected to be present in a substantial proportion of the members of the mixture. Here we report that the chemical composition of crude herbal extracts can be altered in a significant way using this approach. We also illustrate how such chemical alteration can affect in a positive way the biomolecular properties of the mixtures.

Two chemical functionalities commonly found in natural products are the hydroxyl group and the amine group. According to the Dictionary of Natural Products,[5] around 84% of the structures within the database contain at least one of those groups in their structure. To gain insight into the distribution of those structures within the database, the number of molecules containing –OH, and the number of molecules containing $-NH_iR_j$ ($i + j = 3$) were analyzed in four sets of 17 groups of compounds or 'virtual extracts' selected from the database through the use of four different filters.

In set one, each group of compounds contained every molecule within the database found in one particular plant species. Despite the plant species selected were those with higher number of compounds described in the database, the number of compounds described for each plant species is still rather low. Therefore we decided to complement the data with the set two where each group of compounds contained every molecule within the database found in one particular plant genus. Again, the plant genus selected were those with higher number of compounds described in the database. In the third set, each of the 17 'virtual extracts' included only one type of secondary metabolite (i.e., flavonoids, alkaloids, etc.). This filter was used to analyze how was the distribution of the analyzed groups within the different types of metabolites. In set four each virtual extract included all the molecules in the database with molecular weights starting with arbitrarily defined values (i.e., 10, 20, etc.). This filter generates virtual extracts by grouping molecules using a characteristic that is unrelated to the functional groups that they contain. The average frequency of molecules containing at least one –OH group was between 0.74 and 0.80 for all the sets of virtual extracts (Fig. 1a) and, perhaps more important, the standard deviation values are below 0.15 regardless of the filter used.

This suggests that chemical transformation of the –OH group could be an interesting entry point to the chemical transformation

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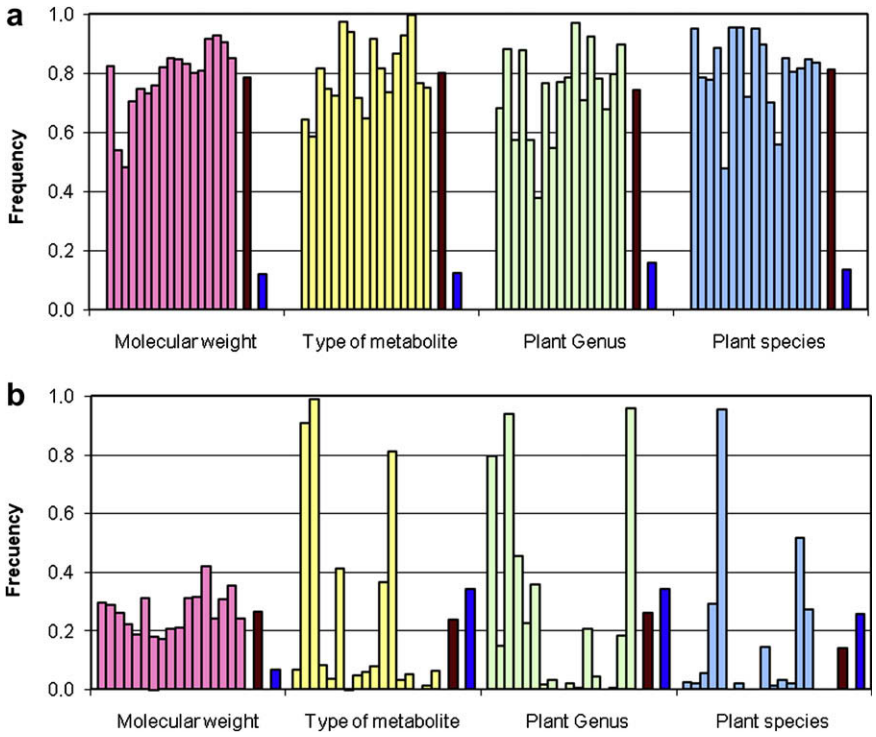

Figure 1. (a) Frequencies of structures containing the group –OH in four sets of 'virtual extracts'. (b) frequencies of structures containing the group $-NH_iR_j$ ($i + j = 3$) in four sets of 'virtual extracts'. Brown and blue bars represent the mean value and standard deviation, respectively, for each set. Selected plant species: *Withania somnifera* (L.) Dunal, *Tripterygium wilfordii* Hook. f., *Taxus mairei* (Lemée & H. Lév.) S. Y. Hu ex T. S. Liu, *Taxus baccata* L., *Buxus sempervirens* L., *Foeniculum vulgare* Mill., *Glycyrrhiza uralensis* Fisch. ex DC., *Halimium viscosum* (Willk.) P. Silva, *Glycyrrhiza glabra* L., *Cannabis sativa* L., *Azadirachta indica* A. Juss., *Helianthus annuus* L., *Panax ginseng* C. A. Mey., *Lupinus albus* L., *Clausena excavata Burm. f.*, *Melia azedarach* L., and *Cryptomeria japonica* (Thunb. ex L. f.) D. Don. Selected genus are as follows: *Thalictrum*, *Taxus*, *Strychnos*, *Solanum*, *Senecio*, *Piper*, *Pinus*, *Laurencia*, *Juniperus*, *Euphorbia*, *Glycyrrhiza*, *Citrus*, *Cassia*, *Baccharis*, *Artemisia*, *Annona*, and *Aconitum*. Selected types of metabolites are as follows: aliphatics, alkaloids, amino acids and peptides, benzofuranoids, benzopyranoids, carbohydrates, flavonoids, lignans, oxygen heterocycles, polycyclic aromatics, polyketides, polypyrroles, simple aromatics, steroids, tannins, terpenoids, and monoterpenoids. Two initial numbers molecular weight are as follows: 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, and 90.

of a significant number of the component molecules of natural extracts. A different situation was observed for nitrogen compounds (Fig. 1b). The average frequency of molecules containing at least once the group $-NH_iR_j$ ($i + j = 3$) was between 0.15 and 0.30, and the standard deviation observed for nitrogen containing groups can be relatively low or very high depending on the filter used. Thus chemical transformation of these nitrogen containing groups does not result very attractive to alter in a significant way the composition of natural extracts. However, the use of reactions that transform both chemical characters, –OH and $-NH_iR_j$, could be interesting.

Aiming at the diversification of the components of natural extracts, we tested the reaction with *p*-toluene sulfonyl chloride. The reaction results in the introduction of one or more relatively big groups into the molecule through the exchange of one hydrogen atom for an arylsulfonyl moiety. One could expect that such changes will affect considerably the recognition properties of a given molecule.

Eleven crude plant extracts were treated with *p*-toluene sulfonyl chloride and potassium carbonate in refluxing acetone for 24 h. All extracts were prepared from plants species regarded as weeds (see Supplementary data). The excess of reagent was removed from the mixture by reaction with the resin tris (2-aminoethyl)-amino polystyrene, and subsequent filtration to produce eleven chemically engineered extracts (CEEs).[6] The changes produced in the chemical composition of the mixtures were evaluated by GC–MS, HPLC-UV and ^{1}H NMR. GC–MS chromatograms of the CEEs were qualitatively and quantitatively different to the chromatograms of the natural extracts (NEs). At least 80% of the peaks observed in the chromatogram of each NE disappeared after the

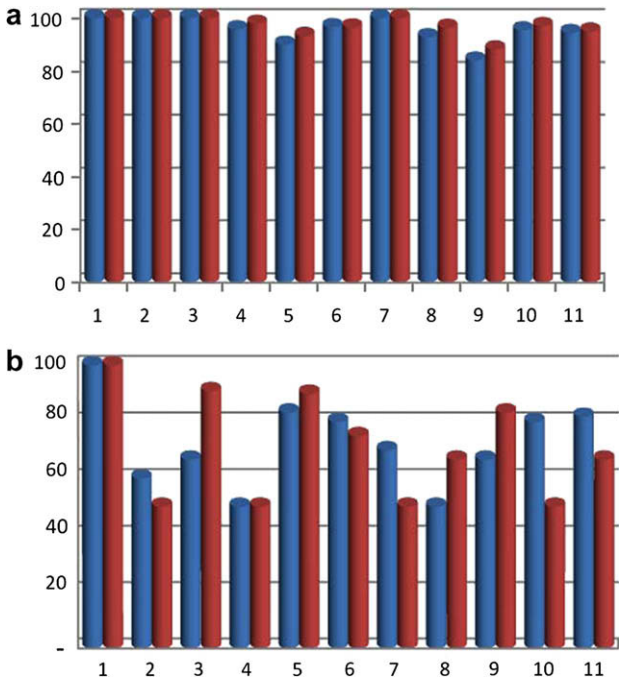

Figure 2. Comparison of GC–MS (a) and HPLC-UV, (b) chromatograms of NEs and CEEs. Blue bars represent the percentage of peaks that are present in each NE and absent in the corresponding CEE. Red bars represent the percentage of peaks that are present in each CEE and absent in the corresponding NE.

reaction (i.e., they are not present in the chromatogram of the resultant CEE). In addition, at least 87% of the peaks present in the chromatogram of each CEE are absent in the corresponding NE chromatogram (Fig. 2a).[7] Similarly, at least half of the peaks present in the HPLC-UV chromatograms of the original NEs disappear after the reaction (blue bars), and at least half of the peaks detected in the chromatograms of the CEEs are absent in the respective NEs (red bars) (Fig. 2b).

Changes in composition of the mixtures were also evident from ^{1}H NMR coupled to Principal Component Analysis (PCA). The score plot showed discrimination between two groups by principal component (PC) 1 and 2 (Fig. 3a). CEEs showed a positive PC2 value, while the NEs showed a negative PC2 value. The loading plot of PC2 shows that the signals between 7.20–7.80 ppm and 2.20–2.50 ppm corresponding to the aromatic and to the methyl group of the *p*-toluene moiety, respectively, have a positive effect in PC2 (Fig. 3b).

Comparison of areas for the signals located in those regions of the spectra of CEEs and NEs showed a significant difference for all the samples (Wilcoxon test, p <0.001). On average, areas of the signals found in the range δ = 2.20–2.50 ppm and δ = 7.20–7.80 ppm account for a 26% of the total area of the spectrum in CE extracts (SE = 4.0) whereas the integration for the signals in the same range in the spectra of natural extracts represents

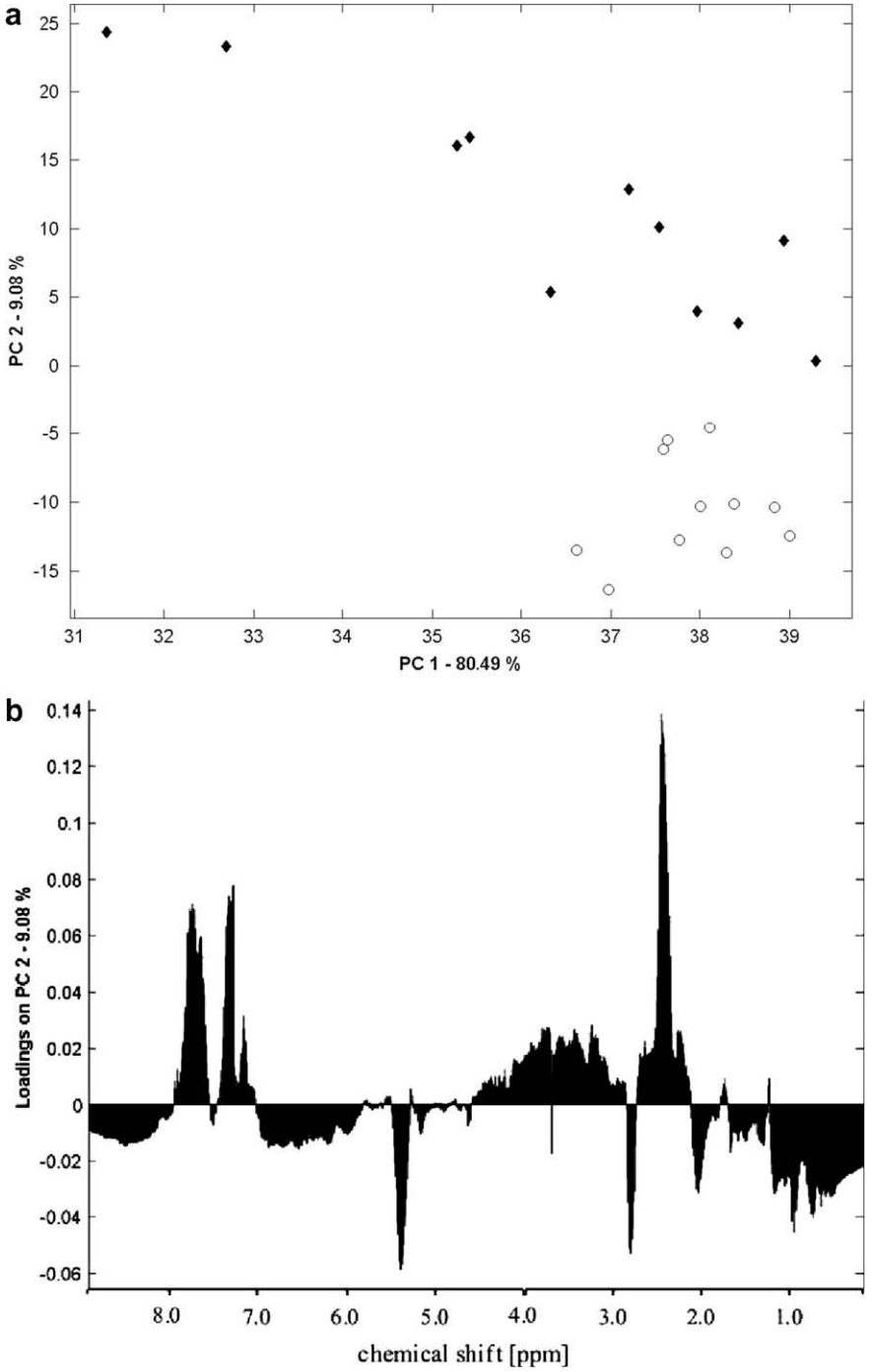

Figure 3. Score plot of PCA of NMR data: natural extracts (○) and CE extracts (♦) (a), and loading plot of PC2 (b).

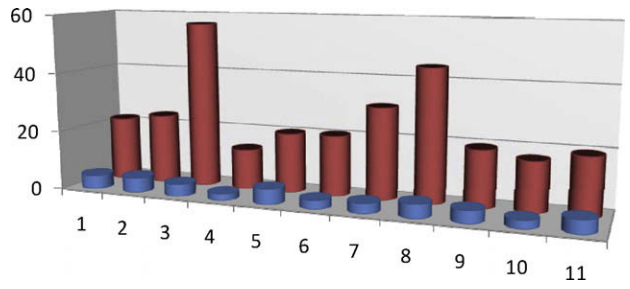

Figure 4. Percentage of the total integration of the ^{1}H NMR spectra of extracts corresponding to the chemical shift range 2.20–2.50 ppm and 7.20–7.80 ppm. Red bars correspond to CEEs and blue bars to NEs.

on average only 4% of the total area of the spectrum (SE = 0.3) (Fig. 4).

Considering the differences in composition observed between natural extracts and the CEEs, it can be expected that the interaction of those mixtures with biomolecules will also be affected.

Glycosidase inhibitors have been the subject of extensive interest[8] because of their potential as drugs for the treatment of diabetes,[9] cancer,[10] viral infection[11] and hereditary lysosomal storage diseases.[12] Preliminarily we analyzed the changes in the β-glucosidase inhibitory properties using TLC bioautography, a technique particularly suited for the analysis of complex mixtures.[13] This methodology allows the qualitative evaluation of inhibitory properties of a sample spotted onto a TLC plate covered with a gel that contains enzyme, substrate and a revealing reagent for the product.[14] Using this method, inhibition of β-glucosidase activity by NEs 2 and 6 and by CEEs 1, 3–7, 9 and 11 was observed. All positive samples were then subjected to TLC separation and re-analyzed by on-plate enzyme autography. Under these conditions, one of the natural extracts (2) and five of the CEEs (1, 4, 5, 9 and 11) did not produce inhibition, suggesting that the previously observed activity had resulted from the added individual activities of a series of compounds in the mixture. On the contrary the activity of the NE 6 and the CEEs 3, 6 and 7 was still present after the TLC was developed. Differences in the R_f values of the bioactive spots indicate that the main compound or compounds responsible for the inhibitory properties in these four mixtures are different.

In summary, we have demonstrated that chemical composition and biomolecular properties of natural mixtures can be altered considerably by reaction with *p*-toluene sulfonyl chloride. Chemical alteration of high frequency functional groups seems to be a suitable entry point to the significant chemical alteration of the composition of natural product extracts without previous detailed knowledge of their constituents. These results illustrate the potential of chemically engineered extracts as alternative sources of molecules with biomolecular properties.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.07.038.

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6. The amount of arylsulfonyl chloride present after treatment with this resin was below 0.03% w/w as determined by GC–MS.
7. This difference is evident when the chromatograms are recorded based on the ions of $m/z = 155$ or 91 corresponding to moieties present in the reagent. In those cases no peaks are observed in natural extracts whereas most of the peaks in the chromatograms of chemically engineered extracts include those ions.
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