Available online at www.sciencedirect.com
ScienceDirect
Current Opinion in Chemical Biology

Metabolomics for phytomedicine research and drug development

Lie-Fen Shyur and Ning-Sun Yang

Metabolomics, including both targeted and global metabolite profiling strategies, is fast becoming the approach of choice across a broad range of sciences including systems biology, drug discovery, molecular and cell biology, and other medical and agricultural sciences. New analytical and bioinformatics technologies and techniques are continually being created or optimized, significantly increasing the crossdisciplinary capabilities of this new biology. The metabolomes of medicinal plants are particularly a valuable natural resource for the evidence-based development of new phytotherapeutics and nutraceuticals. Comparative metabolomics platforms are evolving into novel technologies for monitoring disease development, drug metabolism, and chemical toxicology. An efficient multidisciplinary marriage of these emerging metabolomics techniques with agricultural biotechnology will greatly benefit both basic and applied medical research.

Addresses
Agricultural Biotechnology Research Center, Academia Sinica, No. 128, Sec. 2, Academia Road, Nankang, Taipei 115, Taiwan, ROC

Corresponding author: Shyur, Lie-Fen (lfshyur@ccvax.sinica.edu.tw) and Yang, Ning-Sun (nsyang@gate.sinica.edu.tw)

Current Opinion in Chemical Biology 2008, **12**:66–71

This review comes from a themed issue on
Proteomics and Genomics
Edited by Natalie Ahn and Andrew H.-J. Wang

Available online 21st February 2008

1367-5931/$ – see front matter

DOI 10.1016/j.cbpa.2008.01.032

Introduction

Metabolomics is an emerging and rapidly evolving science and technology system of comprehensive experimental analysis of metabolite profiles, either as a targeted subset of related chemicals or more globally, for diverse applications in diagnosis, toxicology, disease development and animal disease models, genetic modification of specific organisms, drug discovery and development, and phytomedicines [1••,2,3•,4,5••,6–8]. Metabolomics is also a vital component of the systems biology approach, in which it at once reflects and connects the genotype with the diverse and yet specific phenotypes of cells, tissues, or organs [9]. Pioneering research in the metabolomics approach involved the quantitative metabolite profiling of urine or urinary drug metabolites of humans [10,11]. In the plant research field, metabolite profiling was first used as a diagnostic technique to determine the mode of action of herbicides on barley seedlings [12]. The concept of the 'metabolome' was first reported in 1998 as a way to quantitatively and qualitatively measure specific or defined phenotypes to assess gene function in yeast [13] and to discuss the interplay between the global metabolite pool and specific environmental conditions in *Escherichia coli* [14].

In the decade since these early beginnings, the use of metabolomics technologies in biological research expanded exponentially. If one just looks quickly at PubMed literature search results, metabolomics-related research articles have increased from some 40 published articles in 2002 to 100, 170, 200 and >250 articles in the years 2004, 2005, 2006 and 2007, respectively. Owing to its great utility in a variety of basic and applied research fields, metabolomics has quickly become a universal tool and a key component for systems biology studies in medical research.

Systems biology research using genomics, proteomics, and metabolomics approaches is investigating characteristic molecular signatures for disease diagnosis, prognosis, and therapeutics [15]. Recent developments in technology platforms and experimental approaches for metabolomics studies in drug development are outlined in this review. We also urge the greater use of metabolomics in the development of active secondary metabolites from medicinal plants as novel or improved phytotherapeutic agents.

Technology development and experimental approaches

Metabolomics is defined as a comprehensive quantitative and qualitative analysis of all metabolites present in a specific cell, tissue, or organism. The term 'metabolomics' is sometimes used synonymously with metabolite profiling, mainly because at present, unlike in genomics or proteomics, the one-step analysis and exhibition of all metabolites in a metabolome is not possible, because of the enormous complexity of chemicals in biological systems especially in plants [16]. Metabonomics, commonly substituted by the term metabolomics, is the term more properly used for the measurement of metabolite profiles, activities, and reactions toward the environment, medication, or disease, of a given tissue or biological fluid [17]. Metabolic fingerprinting, like metabonomics, usually refers to high-throughput global analysis of metabolites with minimal sample preparation. The two major approaches in metabolomics are the targeted and the global (or un-biased) metabolite analyses. Targeted

metabolite analysis or metabolite profiling, as the name implies, targets a subset of metabolites in a sample, instead of a complete metabolome analysis, using a particular set of analytic technique(s) such as gas chromatography–mass spectrometry (GC–MS) and liquid chromatography–mass spectrometry (LC–MS), together with an estimate of quantity. Various other techniques including thin layer chromatography (TLC), Fourier transform infrared spectroscopy (FT-IR), Raman spectroscopy, and nuclear magnetic resonance (NMR) are also part of the metabolite analysis arsenal.

Remarkable recent developments in analytical chemistry for small molecular mass compound detection and characterization, such as MS and high-field NMR, coupled with user-friendly multivariate statistics, have led to a highly efficient system for comprehensive analysis of the metabolite data matrices generated by metabolomics experiments [2]. NMR-based metabolite profiling/metabolomics was first used in a pioneering study for the rapid multicomponent analysis of urine [18]. This approach has since been successfully used to examine the structure of metabolic pathways and network modules in yeast metabolism [19], and in understanding the pathways involved in adaptation to hypoxia in *Drosophila* flight muscle [20]. One-dimensional (1D) NMR spectrometry has shown its utility for high-throughput analysis and classification of similar chemical groups of samples; however, the large numbers of overlapping peaks generated by this method may hinder the accurate identification of specific metabolites. Recently, replacing the 1D ^{1}H NMR spectroscopic technology, a two-dimensional (2D) ^{1}H–^{13}C NMR strategy (fast metabolite quantification, FMQ, by NMR), for the analysis of metabolites as multivariate statistical objects has been developed [21]. The new 'hyphenated' techniques, coupling various forms of liquid chromatography with NMR, such as HPLC–SPE-NMR, have improved the sensitivity of NMR analyses and are capable of characterizing both the high and low abundance metabolites in complex crude plant extracts [22,23].

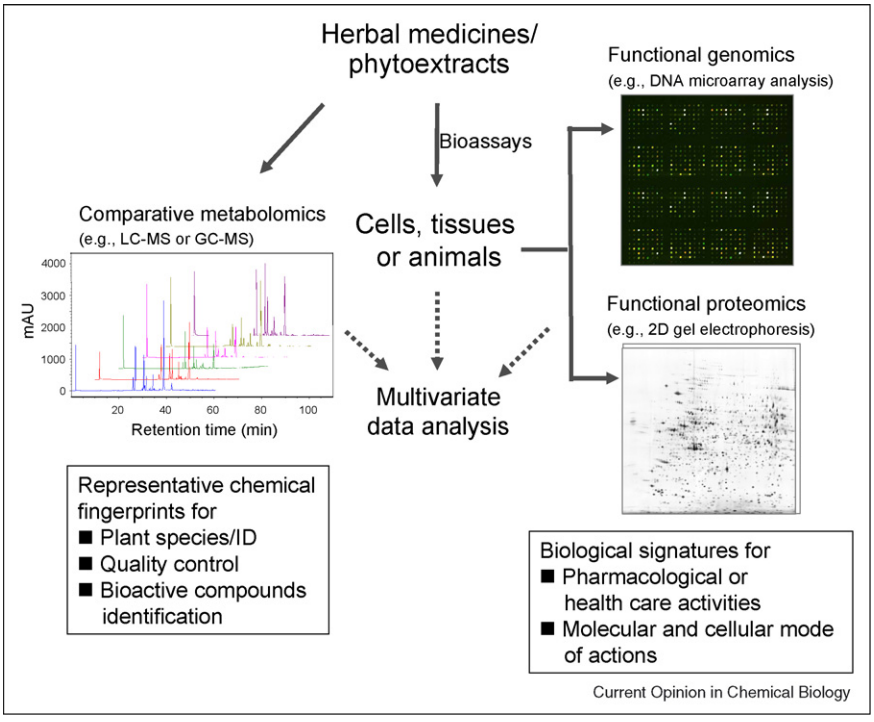
MS is currently the most widely applied technology in metabolomics. Among a variety of MS techniques, GC–MS has long been used in metabolite profiling of human body fluids or plant extracts [10–12,24]. Rapid and high-resolution 2D GC × GC–TOF MS was applied in the phenotyping of natural rice variants [25] and for efficient quality control of herbal medicines [26]. Recently, capillary electrophoresis–MS has also been developed as a metabolomics tool, capable of simultaneously analyzing over 1000 charged chemical species, a technique with obvious utility in biological samples [27]. A shotgun approach using MALDI-TOF/TOF MS has been used for the rapid analysis of negatively charged metabolites in mammalian tissues to facilitate the detection of low abundance metabolites (e.g. cAMP, cGMP, and IP3) and discriminate isomeric molecular species [28].

To efficiently link the flood of experimental data and metabolite information to biology and metabolism, traditional bioinformatics is being combined with cheminformatics to generate a basic computational infrastructure of metabolomics [29••,30]. A number of metabolomics databases, some covering only chemical spectral data, some based on both chemical and biological/biochemical data, have recently been made publicly available [30]. The Human Metabolome Database (HMDB) is currently the largest and most complete database in breadth and depth, offering spectral, physico-chemical, clinical, biochemical and genomic, and metabolism information for a library of >2500 known human metabolites [31]. Other databases include the BioMagResBank (BMRB) with an emphasis on NMR data (>270 pure compounds), the Madison Metabolomics Consortium Database (MMCD) which presents MS and/or NMR data of more than 10 000 metabolites [32], MassBank.jp for high-field mass spectral data, the Golm Metabolome Database (GMD) specific for plants and focusing on GC–MS data [33], and BiGG for human, yeast and bacterial metabolites, pathways and reactions. Algorithmic development and informatics innovations in data reduction, normalization, and alignment that offer sufficient biological insight into metabolic profiles are the future of development in computational metabolomics, as recently reviewed by Wishart [29••].

Metabolomics in phytomedicine research

Plant metabolomes are expected to be much more complex than their mammalian counterparts: by some estimates, over 200 000 molecules will eventually be detected [34]. The diversity of plant secondary metabolites evolved through the continuous interaction with challenging and predominantly hostile environments, coupled with characteristic species and agronomic differences, and these metabolites generally confer a specific bioactivity related to their biochemical structures [35]. A whole range of well-known cancer chemotherapeutic drugs are derived from plant secondary metabolites, such as paclitaxel (taxol), camptothecin (irinotecan, topotecan), and podophyllotoxins (etoposide, teniposide). The great potential of plant secondary metabolites or natural products to serve as health care products or lead compounds for new drug development have renewed interest in pharmaceutical and nutraceutical research. Only a few cases of successful development of novel drugs have so far stemmed from *de novo* combinational chemistry, and natural products or their derivatives are still considered by many scientists as the most productive source of leads for drug development [36,37••]. The use of whole plants or extracts as medicines gave way to the isolation of active compounds, beginning in the early 19th century with the isolation of morphine from opium; however, in the reductionistic approach, single active phytocompounds are sometimes barely able to be identified because of their low abundance in plants, or a spectrum of pharmacological efficacy traditionally

Figure 1



Key features of the technologies used in metabolomics for herbal medicine research.

observed arises only as a synergistic action of multiple ingredients in a single plant or from a multiple medicinal plant formulation, as in traditional Chinese medicine (TCM) [38].

The application of systems biology technologies and approaches, that is, genomics, proteomics, and metabolomics, to phytomedicine research may greatly assist evidence-based phytotherapeutics, and such research may also lead to a change of paradigm in the development and application of complex plant/phytocompound mixtures in modern medicine [5••]. Wang *et al.* [39] pointed out that metabolomics could provide the needed links between the complex chemical mixtures used in TCM and molecular pharmacology. Phytocompound-specific signatures in gene and/or protein expression profiles can also be highly useful in pharmacological standardization, such as their use in 'biological fingerprinting' of medicinal plant extracts (i.e. bioactivity spectra of phytoextracts or phyto-compounds versus their medicinal efficacy in test animal or human systems) [5••,40,41]. Metabolomics approaches using GC–MS, LC–MS, or 2D NMR are an effective tool for quality control of medicinal plants or herb medicinal products [26,42,43]. In addition, they may provide proof of the toxicology/safety measures of specific phytopreparations after metabolism in test animals/humans [5••]. Some key aspects of the technology used in a metabolomics approach to herbal medicine research are outlined in Figure 1.

Recently, we have employed a comparative metabolomics approach for characterizing candidate phytomedicines from a traditional Western herbal remedy, by integrating special plant extract preparation, on-line GC–MS or LC–ESI/MS/MS, and metabolite clustering systems to analyze extracts of the three medicinal *Echinacea* species that are frequently mislabeled in commercial products, that is, *E. purpurea*, *E. pallida*, and *E. angustifolia* [44]. The metabolomics strategy was quite useful in profiling unique groups of plant secondary metabolites that can be used for improved classification of the three *Echinacea* species, and for better quality control of medicinal *Echinacea* extracts under various pharmacological considerations (Shyur *et al.*, unpublished). Hyphenated NMR techniques are also a highly valuable tool for the elucidation of the chemical composition of a complex, bioactive fraction of natural origin [45]. As an alternative to the reductionist approach, plant metabolomics strategies are providing new and important insights for medicinal herb research, linking putative bioactivity with the constituent phytocompounds of herbal medicines [37••,45].

Metabolomics, metabolic diseases, and drug metabolism

Much current metabolomics research in medical science concerns the comparison of small-metabolite portraits to distinguish health and disease states [46]. MS and NMR technology systems are being increasingly used in tumor

metabolomics, *in vitro*, in animal models and clinically, for tumor diagnosis and assessment of prognosis and response to treatment [7]. For instance, an NMR-based metabolomics study of the drug rosiglitazone (RSG) in Type 2 diabetes mellitus (T2DM) patients demonstrated that a broad array of early-responding biomarkers (fingerprints) can readily be detected, and this information can then expedite the clinical development of novel drugs (e.g. thiazolidinediones) for T2DM [47]. Focused or targeted metabolomics platforms, for example, lipid profiling or lipidomics, are being applied in the drug development process, especially for lipid-related metabolic disorders and inflammatory diseases [48,49].

Recently, metabolomics has been used as a high-throughput tool to characterize and monitor the disease process in mouse models [8]. In oncological metabolomics, NMR was used to target biomarkers for prostate cancer by analyzing the global variation of metabolites involved in the development and progression of this cancer for better future management [50,51]. Metabolomics approaches to monitor tumor metabolism have also been used in animal models of human brain tumors, lymphomas, liver tumors, and colon cancers [7]. Currently, the grouping of molecularly distinct diseases into different clinical classes is mainly based on morphology; however, cases with the same clinical and morphological diagnoses are known to result in markedly different clinical outcomes [52]. ^{1}H NMR spectrometry has been successfully applied to distinguish benign and malignant breast tumors, and identify the pathological features of nodal involvement and vascular invasion [52]. By providing metabolic tumor fingerprints [4] with advanced metabolomics technologies, we may expect greatly improved taxonomic classification of cancers.

Comparative metabolomics approaches using LC–MS-based techniques are a useful technique in drug or xenobiotic metabolism and chemical toxicology research. For instance, the comparison of metabolite profiles in mouse urine following vehicle and xenobiotic treatment, or in unlabeled versus stable isotope-labeled xenobiotic treatment have led to the identification of novel xenobiotic metabolites [53]. The combination of genetically modified mouse models with LC–MS-based metabolomics is a powerful new tool for mechanistic studies of drug metabolism, especially genotype-dependent metabolism [3•,53].

Conclusion, future challenges, and perspectives

Unlike other 'omics' technologies, there is currently not a single preferred or generic method for metabolome identification at the global level, despite the introduction of more and more sophisticated technologies for targeted or focused metabolomics, and this is a key hurdle in current metabolomics research [1••,17]. To overcome the problems of metabolite complexity and allow a deeper mining of the metabolome, the development of combinations of multidimensional chromatographic separations with multidimensional MS or NMR spectrometric analysis is urgently needed to achieve maximal output of existing technologies. Further optimization of chromatographic separations and better sensitivity of NMR or MS instruments, with higher resolution capability and greater mass accuracy, will be essential for future improvements in metabolomics [54]. However, metabolomics studies using hyphenated separation and analytical methods already face the problem of lack of reproducibility of results, because of batch variability or when performed in different laboratories, especially with regard to the use of metabolomics in herbal medicine and clinical studies [5••]. It is therefore essential to establish Standard Operating Protocols (SOPs) to minimize and limit technical variations in extraction procedures across a broad base of metabolomics data sets. A continuous effort to develop computational metabolomics to allow it to simulate biologically meaningful metabolic networking is crucial to the routine adoption of metabolomics in medical, agricultural, and pharmaceutical research, as classical bioinformatics has already turned genomics and proteomics into routine and reliable methods in modern biology.

Herbal medicines, in addition to their traditional values, also hold great public and medical interest worldwide as sources of nutraceuticals or novel lead compounds for drug development. A thorough integration of information from genomics, proteomics, and metabolomics is expected to provide solid evidence-based scientific rationales for the development of modern phytomedicines. The search for active phytocompounds will be greatly advanced by the combination of various metabolomics approaches with an array of bioactivity assays in mammalian systems to differentiate between plant species, tissues, or phytopreparations, and to identify novel lead compound candidates for future development. In a complementary development, the use of metabolome-refined herbal extracts with other biochemical components in combination, rather than as isolated single compound(s), may prove to be very useful as broader and holistic therapeutical or pharmacological agents for a variety of human health care applications.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Ellis DI, Dunn WB, Griffin JL, Allwood JW, Goodacre R: **Metabolic •• fingerprinting as a diagnostic tool**. *Pharmacogenomics* 2007, **8**:1243-1266.

This article suggests that metabolomics offers some unique advantages over the other 'omics' disciplines and that metabolic fingerprinting is the core approach of metabolomics for disease diagnostics. It also made an overview of the main metabolic fingerprinting approaches considered as useful for disease diagnostics.

2. Lindon JC, Holmes E, Nicholson JK: **Metabonomics in pharmaceutical R&D**. *FEBS J* 2007, **274**:1140-1151.

3. • Chen C, Gonzalez FJ, Idle JR: **LC–MS-based metabolomics in drug metabolism**. *Drug Metab Rev* 2007, **39**:581-597.
This review article described that LC–MS-based metabolomic techniques are useful tools for drug or xenobiotic metabolism research. The novel approaches and technological platforms of LC–MS-based metabolomics in xenobiotic metabolism are summarized.

4. Claudino WM, Quattrone A, Biganzoli L, Pestrin M, Bertini I, Di Leo A: **Metabolomics: available results, current research projects in breast cancer, and future applications**. *J Clin Oncol* 2007, **25**:2840-2846.

5. •• Ulrich-Merzenich G, Zeitler H, Jobst D, Panek D, Vetter H, Wagner H: **Application of the ''-omic-'' technologies in phytomedicine**. *Phytomedicine* 2007, **14**:70-82.
In this report, the authors suggest that the application of the '-omic-' technologies may lead to a change of paradigms toward the application of complex mixtures in medicine and open the new fields of phytogenomics, proteomics, and metabolomics. These technologies may be useful for the chemical and pharmacological standardization and the proof of the toxicological potential of a plant extract.

6. Kell DB: **Systems biology, metabolic modelling and metabolomics in drug discovery and development**. *Drug Discov Today* 2006, **11**:1085-1092.

7. Griffin JL, Kauppinen RA: **Tumour metabolomics in animal models of human cancer**. *J Proteome Res* 2007, **6**:498-505.

8. Griffin JL: **Understanding mouse models of disease through metabolomics**. *Curr Opin Chem Biol* 2006, **10**:309-315.

9. Fiehn O, Kopka J, Dormann P, Altmann T, Trethewey RN, Willmitzer L: **Metabolite profiling for plant functional genomics**. *Nat Biotechnol* 2000, **18**:1157-1161.

10. Pauling L, Robinson AB, Teranishi R, Cary P: **Quantitative analysis of urine vapor and breath by gas–liquid partition chromatography**. *Proc Natl Acad Sci U S A* 1971, **68**: 2374-2376.

11. Horning EC, Horning MG: **Metabolic profiles: gas-phase methods for analysis of metabolites**. *Clin Chem* 1971, **17**:802-809.

12. Sauter H, Lauer M, Fritsch H: **Metabolic profiling of plant: a new diagnostic technique**. In *Synthesis and Chemistry of Agrochemicals II.* Edited by Baker DR, Moberg WK, Fenyes JG. American Chemical Society Press; 1991:288-299.

13. Oliver SG, Winson MK, Kell DB, Baganz F: **Systematic functional analysis of the yeast genome**. *Trends Biotechnol* 1998, **16**:373-378.

14. Tweeddale H, Notley-McRobb L, Ferenci T: **Effect of slow growth on metabolism of *Escherichia coli*, as revealed by global metabolite pool (''metabolome'') analysis**. *J Bacteriol* 1998, **180**:5109-5116.

15. Malyankar UM: **Tumor-associated antigens and biomarkers in cancer and immune therapy**. *Int Rev Immunol* 2007, **26**: 223-247.

16. Sumner LW, Mendes P, Dixon R: **Plant metabolomics: large-scale phytochemistry in the functional genomics era**. *Phytochemistry* 2003, **62**:817-836.

17. Dunn WB, Ellis DI: **Metabolomics: current analytical platforms and methodologies**. *Trend Anal Chem* 2005, **24**:285-294.

18. Bales JR, Higham DP, Howe I, Nicholson JK, Sadler PJ: **Use of high-resolution proton nuclear magnetic resonance spectroscopy for rapid multi-component analysis of urine**. *Clin Chem* 1984, **30**:426-432.

19. Bundy JG, Papp B, Harmston R, Browne RA, Clayson EM, Burton N, Reece RJ, Oliver SG, Brindle KM: **Evaluation of predicted network modules in yeast metabolism using NMR-based metabolite profiling**. *Genome Res* 2007, **17**:510-519.

20. Feala JD, Coquin L, McCulloch AD, Paternostro G: **Flexibility in energy metabolism supports hypoxia tolerance in Drosophila flight muscle: metabolomic and computational systems analysis**. *Mol Syst Biol* 2007, **3**:99.

21. Lewis IA, Schommer SC, Hodis B, Robb KA, Tonelli M, Westler WM, Sussman MR, Markley JL: **Method for determining molar concentrations of metabolites in complex solutions from two-dimensional (1)H–(13)C NMR spectra**. *Anal Chem* 2007 [Epub ahead of print].

22. Lambert M, Wolfender JL, Staerk D, Christensen SB, Hostettmann K, Jaroszewski JW: **Identification of natural products using HPLC-SPE combined with CapNMR**. *Anal Chem* 2007, **79**:727-735.

23. Clarkson C, Staerk D, Hansen SH, Smith PJ, Jaroszewski JW: **Discovering new natural products directly from crude extracts by HPLC–SPE-NMR: chinane diterpenes in *Harpagophytum procumbens***. *J Nat Prod* 2006, **69**:527-530.

24. Sandberg DH, Sjoevall J, Sjoevall K, Turner DA: **Measurement of human serum and bile acids by gas–liquid chromatography**. *J Lipid Res* 1965, **6**:182-192.

25. Kusano M, Fukushima A, Kobayashi M, Hayashi N, Jonsson P, Moritz T, Ebana K, Saito K: **Application of a metabolomic method combining one-dimensional and two-dimensional gas chromatography–time-of-flight/mass spectrometry to metabolic phenotyping of natural variants in rice**. *J Chromatogr B Analyt Technol Biomed Life Sci* 2007, **855**:71-79.

26. Zeng ZD, Liang YZ, Chau FT, Chen S, Daniel MK, Chan CO: **Mass spectral profiling: an effective tool for quality control of herbal medicines**. *Anal Chim Acta* 2007, **604**:89-98.

27. Soga T: **Capillary electrophoresis–mass spectrometry for metabolomics**. *Methods Mol Biol* 2007, **358**:129-137.

28. Sun G, Yang K, Zhao Z, Guan S, Han X, Gross RW: **Shotgun metabolomics approach for the analysis of negatively charged water-soluble cellular metabolites from mouse heart tissue**. *Anal Chem* 2007, **79**:6629-6640.

29. •• Wishart DS: **Current progress in computational metabolomics**. *Brief Bioinform* 2007, **8**:279-293.
This review was intended to familiarize readers with the field of metabolomics and it outlined the needs, the challenges, and the recent progress made in four fields of computational metabolomics: first, metabolomics databases; second, metabolomics LIMS; third, spectral analysis tools for metabolomics; and fourth, metabolic modeling.

30. Shulaev V: **Metabolomics technology and bioinformatics**. *Brief Bioinform* 2006, **7**:128-139.

31. Wishart DS, Tzur D, Knox C, Eisner R, Guo AC, Young N, Cheng D, Jewell K, Arndt D, Sawhney S *et al.*: **HMDB: the Human Metabolome Database**. *Nucleic Acids Res* 2007, **35**:D521-D526.

32. Markley JL, Anderson ME, Cui Q, Eghbalnia HR, Lewis IA, Hegeman AD, Li J, Schulte CF, Sussman MR, Westler WM *et al.*: **New bioinformatics resources for metabolomics**. In *Pacific Symposium on Biocomputing.* Edited by Altman RB, Dunker AK, Hunter L, Murray T, Klein TE. World Scientific Press; 2007:157-168.

33. Kopka J, Schauer N, Krueger S, Birkemeyer C, Usadel B, Bergmuller E, Dormann P, Weckwerth W, Gibon Y, Stitt M *et al.*: **GMD@CSB.DB: the Golm Metabolome Database**. *Bioinformatics* 2005, **21**:1635-1638.

34. Fiehn O: **Metabolomics — the link between genotypes and phenotypes**. *Plant Mol Biol* 2002, **48**:155-171.

35. Schauer N, Fernie AR: **Plant metabolomics: towards biological function and mechanism**. *Trends Plant Sci* 2006, **11**:508-516.

36. Harvey AL: **Natural products as screening resource**. *Curr Opin Chem Biol* 2007, **11**:480-484.

37. •• Newman DJ, Cragg GM: **Natural products as sources of new drugs over the last 25 years**. *J Nat Prod* 2007, **70**:461-477.
This article makes a good review for all approved agents in the time frame from January 1981 to June 2006 for all diseases worldwide and from 1950 to June 2006 for all approved antitumor drugs worldwide. From their data analyses, the utility of natural products as sources of novel structures, though not necessarily as the final drug entity, is still in good demand and progress. Notably, in the area of cancer, approximately 50% small molecule agents were either natural products or their direct derivatives.

38. Williamson EM: **Synergy and other interactions in phytomedicines**. *Phytomedicine* 2001, **8**:401-409.

39. Wang M, Lamers RJ, Korthout HA, van Nesselrooij JH, Witkamp RF, van der Heijden R, Voshol PJ, Havekes LM, Verpoorte R, van der Greef J: **Metabolomics in the context of systems biology: bridging traditional Chinese medicine and molecular pharmacology**. *Phytother Res* 2005, **19**:173-182.

40. Wang CY, Chiao MT, Yen PJ, Huang WC, Hou CJ, Chung SC, Yeh KC, Yang WC, Shyur LF, Yang NS: **Modulatory effects of *Echinacea purpurea* extracts on human dendritic cells: a cell- and gene-based study**. *Genomics* 2006, **88**:9801-9808.

41. Yang NS, Shyur LF, Chen CH, Wang SY, Tzeng CM: **Medicinal herb extract and a single-compound drug confer similar complex pharmacogenomic activities in MCF-7 cells**. *J Biomed Sci* 2004, **11**:418-422.

42. Ye M, Liu SH, Jiang Z, Lee Y, Tilton R, Cheng YC: **Liquid chromatography/mass spectrometry analysis of PHY906, a Chinese medicine formulation for cancer therapy**. *Rapid Commun Mass Spectrom* 2007, **21**:3593-3607.

43. Yang SY, Kim HK, Lefeber AWM, Erkelens C, Angelova N, Choi YH, Verpoorte R: **Application of two-dimensional nuclear magnetic resonance spectroscopy to quality control of Ginseng commercial products**. *Planta Med* 2006, **72**:364-369.

44. Gilroy CM, Steiner JF, Byers T, Shapiro H, Georgian W: ***Echinacea* and truth in labeling**. *Arch Internal Med* 2003, **163**:699-704.

45. Clarkson C, Madikane EV, Hansen SH, Smith PJ, Jaroszewski JW: **HPLC–SPE-NMR characterization of sesquiterpenes in an antimycobacterial fraction from *Warburgia salutaris***. *Planta Med* 2007, **73**:578-584.

46. Yang C, Richardson AD, Smith JW, Osterman A: **Comparative metabolomics of breast cancer**. *Pac Symp Biocomput* 2007, **12**:181-192.

47. van Doorn M, Vogels J, Tas A, van Hoogdalem EJ, Burggraaf J, Cohen A, van der Greef J: **Evaluation of metabolite profiles as biomarkers for the pharmacological effects of thiazolidinediones in Type 2 diabetes mellitus patients and healthy volunteers**. *Br J Clin Pharmacol* 2007, **63**:562-574.

48. Morris M, Watkins SM: **Focused metabolomic profiling in the drug development process: advance from lipid profiling**. *Curr Opin Chem Biol* 2005, **9**:407-412.

49. Meer GV, Leeflang BR, Liebisch G, Schmitz G, Goñi FM: **The European lipidomics initiative: enabling technologies**. In *Methods in Enzymology*, vol 432. Edited by Alex Brown H. Elsevier Inc.; 2007:213-232.

50. Jordan KW, Cheng LL: **NMR-based metabolomics approach to target biomarkers for human prostate cancer**. *Expert Rev Proteomics* 2007, **4**:389-400.

51. Cheng LL, Burns MA, Taylor JL, He W, Halpern EF, McDougal WS, Wu CL: **Metabolic characterization of human prostate cancer with tissue magnetic resonance spectroscopy**. *Cancer Res* 2005, **65**:3030-3034.

52. Brenton JD, Carey LA, Ahmed AA, Caldas C: **Molecular classification and molecular forecasting of breast cancer: ready for clinical application?** *J Clin Oncol* 2005, **23**:7350-7360.

53. Chen C, Ma X, Malfatti MA, Krausz KW, Kimura S, Felton JS, Idle JR, Gonzalez FJ: **A comprehensive investigation of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) metabolism in the mouse using a multivariate data analysis approach**. *Chem Res Toxicol* 2007, **20**:531-542.

54. Griffiths WJ, Karu K, Hornshaw M, Woffendin G, Wang Y: **Metabolomics and metabolite profiling: past heroes and future developments**. *Eur J Mass Spectrom* 2007, **13**:45-50.