European Cooperation in the field of Scientific and Technical Research - COST -

Secretariat

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COST 252/08

MOMERANDUM OF UNDERSTANDING

Subject : Memorandum of Understanding for the implementation of a European Concerted Research Action designated as COST Action CM0804: Chemical biology with natural products

Delegations will find attached the Memorandum of Understanding for COST Action CM0804 as approved by the COST Committee of Senior Officials (CSO) at its 172nd meeting on 24-25 November 2008.

MEMORANDUM OF UNDERSTANDING

For the implementation of a European Concerted Research Action designated as

COST Action CM0804

CHEMICAL BIOLOGY WITH NATURAL PRODUCTS

The Parties to this Memorandum of Understanding, declaring their common intention to participate in the concerted Action referred to above and described in the technical Annex to the Memorandum, have reached the following understanding:

1. The Action will be carried out in accordance with the provisions of document COST 270/07 "Rules and Procedures for Implementing COST Actions", or in any new document amending or replacing it, the contents of which the Parties are fully aware of.

2. The main objective of the Action is to advance the use of natural products as tools for chemical biology.

3. The economic dimension of the activities carried out under the Action has been estimated, on the basis of information available during the planning of the Action, at EUR 44 million in 2008 prices.

4. The Memorandum of Understanding will take effect on being accepted by at least five Parties.

5. The Memorandum of Understanding will remain in force for a period of 4 years, calculated from the date of the first meeting of the Management Committee, unless the duration of the Action is modified according to the provisions of Chapter V of the document referred to in Point 1 above.

A. ABSTRACT AND KEYWORDS

The field of chemical biology has emerged since many biological questions can only be addressed using small molecules that interact with biological systems in a defined manner. In this regard natural products are unique tools to probe the functions of important proteins. The aim of this Action is to advance the use of natural products and their analogues in chemical biology. In order to obtain valuable collections of natural product analogues for use in chemical genetics studies, novel methods and strategies for preparing compound collections having a broad structural diversity will be required. For example, chemical modifications of natural products using collections of enzymes will be studied. Moreover, the development of novel catalytic processes and domino strategies aiming at the efficient construction of alkaloid-like molecules will be a key topic of the Action. Finally, novel tools that allow for target fishing using natural products are urgently needed. In this regard labeling strategies or novel linker systems will be explored. The Action will consist of three Working Groups in order to tackle the above-mentioned challenges. Furthermore, the training of the scientists in the field of chemical biology will be a prime goal of the Action.

Keywords: Chemical biology, natural products, bioinformatics, enantioselective processes, biomimetic synthesis

B. BACKGROUND

B.1 General background

Natural products have a major impact on the health and welfare sectors, especially through drug discovery and chemical biology. Natural product based drugs are being used for the treatment of cancer, cardiovascular diseases as well as bacterial and fungal infections. Epothilone, a drug which is currently on the market, provides a good recent example: It was discovered through European activities, and quickly the efforts of excellent European academic and industrial groups led to its implementation in the treatment of various forms of cancer. A distinctively large portion of the currently approved pharmaceutical agents are natural products or compounds based on natural products. In addition, the treatment of neglected diseases benefits from natural products. In order to advance and to keep the high level of natural products chemistry within Europe, the aim of this Action is to combine chemistry, biology and bioinformatics.

One of the goals is to promote the use of natural products as tools in biology. This should help to reveal new biological pathways, some of which might be of relevance for the treatment of diseases. The problem at the moment is that with regard to teaching, chemistry and biology are not integrated well enough at the university level. Thus, a further aim of the Action is to make young chemists familiar with key biology and bioinformatics topics, for example through workshops and crash courses. The advantage of using natural products for chemical biology is that they are biologically validated. It seems that a correlation between the biosynthetic enzymes and the target of a natural product exists. Accordingly, the binding of enzymes to natural products during their biosynthesis is translated to recognition of drug targets having a corresponding protein fold topology. The Action will highlight the important role of chemical synthesis. Thus, the development of new methods, which are of utmost importance to efficient syntheses of natural products, will be advanced. These include radical based reactions, organometallic transformations, organocatalysis (green chemistry, sustainable chemistry), chemistry in microreactors, and the use of solid-phase reagents. The broad application of natural products in chemical biology will require new strategies for the rational modification of natural products. In addition, reliable methods for linking natural products to solid supports or labels will be necessary. Furthermore, smart modifications that enable the use of natural products in affinity-based profiling have to be developed. These undertakings will be supported by groups with experience in cell biology and bioinformatics. It is expected that this Action will foster multilateral long-term collaborations across several disciplines. At the moment there is still a lack of communication between chemists and biologists. Networking is going to help in this matter. In all, the Action aims at a more efficient integration of European experts that will promote chemistry (structure elucidation, synthesis), biology (biosynthesis, screening), and medical applications (testing, evaluation) of natural products.

B.2 Current state of knowledge

Drug discovery is a costly and lengthy process and only a few compounds make it to the market. Despite large investments, the number of drugs approved by the US Food and Drug Administration (FDA) has dropped from 24 in 1998 to 13 in 2006. In addition, many diseases are still difficult to treat. Moreover, in the case of anti-infectives, drug resistance has become a serious problem. One disadvantage is that medicinal chemists still need to synthesise too many molecules in order to identify those with the desired activity profile and properties.

Furthermore, only the functions of a small number of molecular targets of these compounds are known. A great advance has been the human genome project, which led to the field of proteomics. The task is now the investigation of the function of proteins and deciphering complex biological systems. This should help us to understand the molecular basis of diseases and to find new strategies to treat diseases. In this regard the field of chemical biology holds great promise. Chemical biology is concerned with research at the interface of chemistry and biology. In this context chemical biology is largely focused on small molecules, including natural products, as research tools and potential therapeutics. The idea is to probe biology with small molecule tools and chemical methods. It is thus in many ways complementary to classical genetics where the focus is also on finding targets and probing signalling pathways. As is the case with classical genetics, there are two approaches in chemical genetics, which can be distinguished. In forward chemical genetics (FCG) the classical random mutagenesis approach is replaced by screening cells with a collection of different compounds where some are expected to regulate protein function. On the other hand, reverse chemical genetics (RCG) begins with an assay system on a cellular target, which is screened against a library of compounds in order to identify ligands that interact with the target protein. Once a specific ligand is identified, its function is then probed with cells or organisms. Compared to classical genetics, chemical genetics offers some advantages. For example, genes essential for survival can only be probed with chemical biology. Moreover, unlike genetic mutations, creating chemical mutations is fast and reversible. The field of chemical biology is quite popular in the US. In fact, many journals are devoted to the field of chemical biology. Also, many departments offer courses related to chemical biology. In addition, in the US many centres and initiatives related to the topic were established.

A recent success story involves the elucidation of the mode of action of the natural products leucascandrolide A and neopeltolide. Both macrolides were isolated from marine sponges and turned out to be highly potent inhibitors of cell proliferation. An active, simpler analogue of leucascandrolide A was screened against a library of 4,900 yeast strains each of which only contained one copy (a heterozygous deletion strain) instead of two copies like in the normal diploid strain. With the lower gene load, the strains become more sensitive to compounds that interact directly with the corresponding protein. This then mimics a complete gene deletion. In the case at hand, one of the supersensitive strains carried a deletion of the SNF4 gene.

This gene codes for a kinase, which is responsible for sensing the cellular AMP/ATP ratio. It is activated by inhibition of ATP production taking place in the mitochondria. Further studies based on this observation revealed the cytochrome bc1 complex as the molecular target of these compounds. Clearly, this discovery would have been more difficult to achieve via classical phenotype analysis. This study highlights the value of simpler analogues of natural products and special engineered model organisms in chemical biology. One advantage of using the fully sequenced yeast is that this organism shares 50 percent of its genetic material with humans.

On the contrary, the systematic analysis of the structures of natural products led to the concept of biology-oriented synthesis (BIOS) which builds on biologically prevalidated structures and uses them as scaffolds for the synthesis of compound collections. This way, for example, novel inhibitors for phosphatases were discovered.

B.3 Reasons for the Action

The Action is to improve the collaboration between groups engaged in natural product synthesis and biology. The application of natural products and their analogues in biology should lead to new insights into biological processes, and molecular recognition. It will help to better integrate chemistry with biology. The Action might lead to the discovery of new targets to treat diseases, to new tools in chemical biology, and new technological advances. Considering the potential benefits, the Action is aimed at both the European economic/societal needs and scientific/technological advance.

Goals:

- to promote interactions between European researchers involved in natural product chemistry (isolation, synthesis), cell biology and bioinformatics
- to put the concept of chemical biology with natural products to a higher level
- to promote the education of young chemists in modern organic synthesis, key biology issues and bioinformatics
- to transfer promising results (new natural product analogues, new protein targets) to SMEs (pharmaceutical or start-up companies)
- to facilitate the assembly of teams that are strong enough for submitting collaborative research proposals within the EU

Expected results:

– new strategies for the efficient synthesis of natural-product like molecules
– new targets for the treatment of diseases
– new insights into the biosynthesis of natural products
– new tools for screening (cells or proteomes against natural products)
– new tools for discovering the protein target of an active molecule
– new methods for the virtual screening of macrocyclic natural product-like compounds
– improving the public awareness for modern organic synthesis
– incorporation of important topics of chemical biology into advanced academic programs

Means:

– original papers in international high-impact peer-reviewed journals
– presentations at conferences, workshops, and Working Group (WG) meetings
– invitation of experts to workshops and WG meetings
– exchange of information within the network through a website
– Short Term Scientific Missions (STSMs) giving young scientists the opportunity to learn methods not available at their home university
– Training Schools (TSs)
– active participation of early stage scientists in WG meetings

B.4 Complementarity with other research programmes

To the best of knowledge available, this COST Action does not overlap with other ongoing initiatives in Europe.

There are however efforts to foster chemical biology within Europe on a broader basis. So far there a few good centres in Europe. Unlike in the US where central public databases and compound pools were established, there exists nothing comparable in Europe.

The Action may be seen partly as a further development of the previous COST Action D28, which was oriented towards the medical use of natural products.

C. OBJECTIVES AND BENEFITS

C.1 Main/primary objectives

The main objective of the Action is to advance the use of natural products as tools for chemical biology. Applying modern techniques and advancing them, natural products will prove to be instrumental in discovering target proteins and biological pathways that are of relevance to diseases. This in turn, should facilitate and speed up subsequent drug discovery efforts in the pharmaceutical industry.

C.2 Secondary objectives

Other objectives of this Action are:

– to increase the active knowledge in the field of biology for chemists within Europe
– to promote organic chemistry and synthesis to a new level

C.3 How will the objectives be achieved?

The main resources (manpower, equipment, infrastructure) needed to achieve the goals of the Action will be provided by the participating researchers.

The proven mechanisms of COST will guarantee the full exploitation of the potential of the member research teams. Interaction among the members will be steered by and discussed at Management Committee (MC) meetings (1 or 2 per year), WG meetings (1 per year), and two TSs, STSMs, and two Workshops. In addition, the Action will feature a high level kick-off Workshop, promoted by an internationally renowned chairman and a Final Workshop.

Whenever possible, some of the events will be organised in conjunction with related European scientific symposia (for example European BioPerspectives or the European Chemical Biology Symposium), to enable a wider dissemination of the activity of the Action.

C.4 Benefits of the Action

The Action will allow a more efficient exchange of know-how and methods developed by the members. This ultimately should enhance the efficiency of all member teams and provide new spirit to the field of natural product chemistry.

Furthermore, it is expected that the Action will provide additional means to disseminate the results to a broad audience, including the pharmaceutical industry, and government representatives. The teaching of young graduate students is also a very important benefit.

C.5 Target groups/end users

The expected results are likely to be of interest for the following end users:

- chemists, biologists, microbiologists, and interdisciplinary oriented scientists working in the area of drug discovery
- University professors and lecturers in chemistry, biochemistry and biology
- European and international research community
- Funding agencies and representatives thereof
- Possibly biotechnology and pharmaceutical companies

D. SCIENTIFIC PROGRAMME

D.1 Scientific focus

The chemistry of natural products has a long history and is an important part of our scientific history and culture. Early medicines were essentially based on natural products through isolation, mainly from plant sources. Later, bacteria, fungi, and marine organisms contributed many novel natural products. Some well-known examples include erythromycin, an antibiotic, rapamycin, that is used as immunosuppressant, and lovastatin, a block-buster drug that is used as cholesterol-lowering medicine. The recent book by K. C. Nicolaou and T. Montagnon entitled ‘Molecules that Changed the World’ contains a collection of natural products important to society. A lot of knowledge in biology could only have been gathered with the help of natural products. Even now roughly 50% of all drugs are based on natural products. Nevertheless, the field sometimes is considered to be losing its importance. Despite the failure of combinatorial chemistry to deliver useful drugs, some companies shut down their natural product departments. The reason for this seems to be due to several shortcomings or bottlenecks associated with natural products chemistry. First, the discovery of very novel natural products is a painstaking and slow undertaking.

Furthermore, isolation and structural elucidation are still complicated. Second, the synthesis of natural products in larger amounts is considered to be too complicated and costly. However, one should mention that advances in synthetic methodology and strategy during the last 20-30 years have improved the situation significantly. For example, the great progress that has been made in aldol technology, organometallic transformations, enantioselective catalysis, organocatalysis, and enzymatic reactions allows even small research groups to achieve the synthesis of a natural product of medium complexity (500-600 Da, up to 10 stereocentres) in relatively short time. The main issue at the university level now is rather which compounds a chemist should make. The field of chemical biology extends natural product chemistry into biology. Since scientists now have faster access to natural products and their analogues, probing biology using these synthetic compounds offers new opportunities for chemistry. Even though this is usually done in collaboration with biologists, this kind of interdisciplinary research requires advanced knowledge in biology that is not yet part of the curriculum in many universities offering degrees in chemistry. However, if a university has some active groups in chemical biology this situation will change automatically. In this regard, this Action is of utmost importance. Challenges and opportunities for natural products chemistry are related to the following topics:

a) Screening for natural products: The main problem is certainly to remove known compounds from an extract. Nevertheless, applying screening to genetically modified organisms that are sensitised for a special signal, should improve the situation. In this context the yeast deletion strains hold great promise.

b) Investigations related to an interesting natural product: With a biologically active compound available, the following challenges arise: elucidation of the mode of action, discovery of the binding site of the molecular target, structure activity studies, design and preparation of analogues, studies related to the biosynthesis.

c) Using a collection of natural products or natural-product analogues: In this regard one might use the core structure of a polyfunctional natural product as a scaffold for decoration with various groups or pharmacophores. However, this might not lead to analogues with novel modes of action. On the other hand, organic synthesis allows for more deep-seated structural variations by channelling different building blocks into the construction of the skeleton akin to the so-called mutasynthesis in biology.

d) Having an interesting protein target or assay: In this regard, collections of natural product analogues that cover a broad chemical space and at the same time are biologically validated should guarantee a high hit rate. Combined with reliable virtual screening of potential analogues, one would have to prepare only the most promising candidates.

e) Logistics, informatics: The question arises whether a central European compound depositary would make sense. Most likely, the associated costs for running such a depositary might be too high. It seems better that groups or institutes that focus on screening campaigns keep their own compound collections. Of course, exchange of compounds between such groups should be possible.

Research will involve projects from the above topics. Teams with complementary expertise and the proven COST schemes can only fulfil such multidisciplinary tasks. WG meetings and STSMs are ideally suited to support existing and new collaborations in the chemical biology field.

D.2 Scientific work plan – methods and means

The total synthesis of natural products offers perspectives for broadening knowledge since new structures and associated biological properties of novel molecules isolated from the apparently unlimited chemical diversity in nature open up a series of research opportunities. Natural product synthesis has always been closely related to the development and application of new synthetic methods. On the one hand, new synthetic solutions are needed to meet complex molecular challenges, and on the other, natural product synthesis is an excellent validation test of the scope and limitations of synthetic methods when applied in highly functionalised and crowded molecules.

The required chemo- and stereoselectivity can inspire or trigger the development of new synthetic methods. Moreover, access to new structural motifs can be essential for the design of therapeutic products with enhanced activity and pharmacokinetic properties. Finally, in spite of advances in spectroscopic techniques, the first total synthesis of a natural product is still the definitive proof of its structure, especially with regard to configurational aspects.

a) Natural product-like compound collections: A group from Italy has expertise in developing chemical libraries of novel bioactive compounds (flavonoids, benzophenones, xanthones, anthraquinones, alkaloids, steroids, terpenoids, containing different substituents), many identified from natural extracts obtained from medicinal plants. This unit provides expertise with a unique library of bioactive natural compounds for in vitro and in vivo screenings and activity. Moreover, chemical modifications of lead compounds will be addressed by means of a unique collection of enzymes in order to perform regioselective prenylation (aromatic prenyltransferases from Streptomyces), amine oxidation (amino-oxidases, methylamino oxidases and aminoacid oxidases), and phenol oxidation (bacterial peroxidases). In silico screening will also be performed on available targets and on the novel structural data of hedgehog (Hh) and Notch signalling molecules obtained in the course of the project. The library will be assembled in ready-to-use screening plates and screened for biological activity towards Hh and Notch signalling. The use of environmentally friendly $^1O_2$ in the synthesis of biologically active natural products from furan-containing substrates and the development of new synthetic methodologies is another opportunity for generating natural product-like compounds. In general, the furan substrates are readily accessible. These reactions are highly advantageous because they frequently deliver a rapid and dramatic increase in molecular complexity in high yield. Furthermore, an unusually wide structural diversity is exhibited by the molecular motifs obtained from these reaction sequences. For example, relatively minor modifications to the starting substrate and to the reaction conditions may lead to products as variable as spiroketal lactones, 3-keto-tetrahydrofurans, various types of bis-spiroketals, 4-hydroxy-cyclopentenones, or spiroperoxylactones.

b) Hsp90 inhibitors: Heat shock protein 90 (Hsp90), one of the most abundant proteins in eukaryotic cells (1-2%), is an ATP-dependent chaperone that plays a central role in regulating stabilisation, activation, and degradation of a series of proteins. A chaperone is a protein that assists the non-covalent folding/unfolding of other proteins. Hsp90 client proteins include a number of key proteins involved in cell cycle regulation and signal transduction. As expected from its key physiological role, pharmacological inhibition of this single target has been shown to simultaneously destabilise many of the substrates known to be critical for the process of multistep carcinogenesis.

Accordingly, inhibitors of Hsp90 would constitute promising anticancer compounds. Moreover, such inhibitors would facilitate further chemical biology studies. Hsp90 features a conserved ATP-binding pocket in the N-terminal domain and a coumarin binding site in the C terminus.

The interfering with the ATP-binding results in proteasomal degradation of client proteins. Typical inhibitors include ATP-like heterocycles but interestingly also some structurally unrelated natural products, namely the benzolactone radicicol and the macrolide geldanamycin were found to bind to the ATP site. Therefore, the preparation and screening of benzolactone collection, prepared by organic synthesis seems very promising. Due to the availability of efficient methods for the preparation of such macrolactones of medium complexity, the preparation of relevant compound collections is a realistic undertaking.

A biology-inspired strategy for preparation of modified macrolactones is based on the replacement of an acetate by a propionate subunit via chemical synthesis. This should help to probe for available space in the receptor. For example, most of the 14-membered benzolactones of the zearalenone type just contain acetates. Analogues will be prepared and tested where a propionate will be incorporated at some positions. An initial propionate analogue of zearalenone turned out to be a potent (IC_{50} = 80 nM) inhibitor of the carbonyl reductase CBR1, even though it did not bind to Hsp90. Based on this proof of concept study, it can be expected that other propionate analogues will provide the desired Hsp90-binding compounds. Preliminary virtual docking experiments indicated that the curvularin scaffold is promising in this regard. The screening for Hsp90 inhibitors is possible either with a fluorescence polarisation assay or by differential scanning fluorometry (DSF).

c) Nitrogen containing molecules : Organic synthesis is concerned with the rational construction of complex organic compounds from readily available starting materials. Often such endeavour requires several reaction steps and thus careful planning. Of central importance are all aspects of efficiency, related to both individual steps (tactical level) as well as to complete reaction sequences (strategy level). To develop the field it is necessary to investigate new transformations and techniques for bond formation and to evaluate their performance in the context of total synthesis of relevant and interesting compounds.

Of equal significance is the growing awareness regarding the necessity of using enantiomerically pure chiral substances whenever they are expected to interact with a chiral environment. Typically, such compounds, and also striking examples of enantiomers different activities in their intended application, can be found among pharmaceuticals, agrochemicals, cosmetics and food additives. Enantiomerically pure compounds have traditionally been obtained by resolution or by using enantiopure naturally occurring starting materials. During the last two decades, however, the main efforts in this area have been directed toward asymmetric synthesis (i. e. processes in which the required stereogenic centers are created from an achiral subunit in the starting material using an inter- or intramolecular chiral controller) and asymmetric catalysis.

The aim is the development and implementation of new synthetic methods in the synthesis of carbo- and azapolycyclic molecular structures that allow advanced polyfunctionalised intermediates to be achieved, preferably in the enantiopure form. This would allow approaching the total synthesis of complex and valuable natural products. An important naturally occurring compound class comprises the alkaloids particularly indole and piperidine alkaloids and related bioactive nitrogen compounds, which due to their structural diversity display a broad variety of biological activities. Within each compound class there are examples in which seemingly subtle changes in the periphery of the skeleton give rise to entirely different biological profiles. A striking example comes from the class of morphinoids, where slight changes in functional groups give rise to either useful or undesired activity.

Accordingly, the Action aims at the development and implementation of the following catalytic synthetic methods in the total synthesis of natural products with high structural complexity: a) cyclisation of trichloroacetamides upon alkenes catalysed by Cu(I) under atom transfer processes; b) C-C bond formation by coupling of aryl and vinyl halides with activated methylene compounds catalysed by Cu(I) in a basic medium; c) intramolecular aldol processes using organocatalysts to synthesise Wieland-Miescher ketone analogues and 2-azabicyclo[3.3.1]nonanes; d) isomerisation of 4-alkyl-3-cyclohexenones obtained by Birch reduction to the corresponding unsaturated ketones using organocatalysts.

Additionally, aminocyclisation processes will be studied either from compounds obtained by Birch reduction of omega-aminoalkylanisole derivatives or from omega-amino alkenes using N-iodosuccinimide. The building of carbo- and azabicyclic units through the aforementioned synthetic methods will be the point of departure towards the first total synthesis of the following natural products: the insecticide nominine, the antimalarial agents lepadin F and G, the anti-cancer compounds madangamine, aspernomine and fasicularin and the anti-asthmatic daphniyunnine A.

Applying the above methods, the synthesis and biological evaluation of alkaloid compound libraries inspired by natural products will be investigated. This aim can be subdivided in the following objectives:

- Synthesis of compound libraries using naturally occurring alkaloids as scaffolds
- Synthesis of compound libraries using alkaloid inspired scaffolds
- Diversity oriented synthesis of skeletally diverse compound libraries inspired by naturally occurring alkaloids
- Screening for biological activity in a large variety of assays
- In the case of identification of an interesting lead compound, further optimisation through iterative synthesis and biological evaluation rounds

These nitrogen derivatives are widespread, occurring not only in plants but also in insects and amphibians, and constitute important sources of inspiration in pharmaceutical research, with thousands of them mentioned as drug candidates in clinical and preclinical studies.

d) Synthesis and Biological Evaluation of Xenia Diterpenoids and Synthetic Analogues: Diterpenoids derived from soft corals of the genus Xenia exhibit a wide range of biological activities, including antiproliferative, antiangiogenic, or antibacterial effects. The common structural denominator of these compounds is a nine-membered carbocyclic ring, which is generally fused to a dihydropyran, a delta-lactone, or a cyclobutane moiety, thus leading to three major classes of Xenia diterpenoids that have been termed xenicins (or xenicans), xeniolides, and xeniaphyllans, respectively.

Interestingly, xeniaphyllans are structurally related to the common plant constituent beta-caryophyllene, which was recently shown to be a functional agonist at the CB2-receptor. Total syntheses so far have been reported for two different Xenia diterpenoids, coraxeniolide A and antheliolide A, but generally synthetic efforts in this area have been sparse. Likewise, the biology of Xenia diterpenoids has not been explored in detail and their potential as lead structures for drug discovery remains largely unknown. In particular, no efforts have been reported in the literature that would be directed at the identification of the molecular target(s) underlying the biological effects of individual Xenia diterpenoids.

One of the participating groups in this COST Action has recently completed the total synthesis of another Xenia diterpenoid, namely blumiolide C, through construction of the nine-membered ring by ring-closing olefin metathesis. The biological evaluation of the compound is currently ongoing. The chemistry developed in the course of the synthesis of blumiolide C provides a solid basis for the future synthesis of other Xenia diterpenoids and their biological evaluation, including experiments aiming at the identification of the molecular target(s) of these unique natural products. As is the case for most natural products of marine origin, the availability of Xenia diterpenoids from natural sources is highly limited, thus making total synthesis the only (or at least most efficient and reliable) means for the provision of material for detailed biological studies. In addition to different naturally occurring Xenia diterpenoids, synthetic analogues (i. e. compounds that could not be formed through variations in biosynthetic pathways) will be prepared for general structure-activity relationship (SAR) studies and in order to improve the biopharmaceutical properties of the natural products.

e) Tools for identifying the target of a natural product: The classical route to new natural products involves cell-based, phenotypic screens of extracts from natural sources (bio-guided isolation). While proven in many instances, this procedure requires effective methods for identifying the key cellular targets. Sometimes, experienced cell biologists are able to confine or guess the target from the observed phenotype. Otherwise, the technique of affinity chromatography has proven to be an effective way to identify an appropriate molecular target. While being a classical approach, this kind of target fishing, also known as cellular pull-down experiment, is often troublesome.

First of all, it requires covalent attachment of the small molecule to an affinity matrix, for example agarose beads. This necessitates a suitable functional group in the region of the molecule which does not interfere with the binding process. Finding a suitable attachment point is generally a cumbersome trial and error process. Other severe problems that one can encounter are non-specific binding of proteins to the linker connecting the matrix with the natural product. Furthermore, the fishing of low abundance proteins or membrane-bound proteins is particularly challenging.

Some solutions in this regard are cleavable linkers, which allow one to get rid of the non-specific proteins. Another option is to incorporate a short linker, containing a suitable functional group at the terminus, already during the synthesis of a natural product. This way, target fishing would be facilitated by ligation reactions such as click chemistry or Staudinger ligation. Other promising strategies include the synthesis of unique labels that would facilitate target identification with the help of mass spectrometry.

One of the members of this COST Action has extensive experience with the synthesis of epothilone analogues and associated SAR studies. While the potent cellular effects of epothilones are generally assumed to be mediated through their interactions with microtubules, certain discrepancies between the tubulin SAR and the SAR for cell growth inhibition indicate that targets other than tubulin may be involved in the epothilones mode of action. Based on the analysis of the extensive SAR data collection that has been accumulated for epothilone-type structures, analogues have been designed that are expected to allow the attachment of a biotin moiety or a fluorescent tag without affecting a dramatic reduction in cellular potency. These probe molecules should then allow searching for epothilone-binding proteins in cell lysates other than tubulin. While the hypothesis of an additional epothilone target is rather speculative, based on the existing SAR data it is still plausible. At the same time, the discovery of a non-tubulin target of epothilones might open completely new avenues for anticancer drug discovery.

E. ORGANISATION

E.1 Coordination and organisation

Research carried out by the members will be financed by the respective national budgets of the participating countries. The Action will provide the means to promote the activities required for the effective and concerted collaboration of the scientists involved. The Action will improve the efficacy of the research by supporting the activities necessary for the concerted collaboration of chemists, biologists, and pharmacologists.

Organisation and the management of the Action will strictly follow the rules and procedures of COST and its common organisational structures, namely:

The Action will be managed by the MC that will be established according to the COST official rules. The Action Chair, vice-Chair, WG coordinators and all other functions will be elected at the first meeting of the MC.

Three WGs will be created according to COST rules based on the preliminary groups participating in the proposal preparation. The composition and arrangement of the WGs will be discussed, approved and/or modified at the first meeting of the MC. Further modifications, proposed in the frame of the COST rules, will be considered and, if necessary, approved during the subsequent MC meetings.

The MC will be based on the nominations by the national COST coordinators. The members of the MC will elect the Action Chair, vice-Chair, STSM coordinator, TS coordinator, editorial coordinator and web coordinator. A person responsible for monitoring the Action (e.g. scientific audits by independent evaluators, STSM and budget monitoring) will be elected from the MC members. Ad hoc committees to assist those responsible will be established (e.g. organisational board of TS or programme committee of the Action Workshops). An Action Steering Committee (ASC) composed of the Chair, vice-Chair and WG coordinators may meet occasionally (if required) between the regular MC meetings.

The MC will be responsible mainly for:

– securing a constant high scientific level of the Action
– fulfilling the tasks and milestones
– supervising and assisting the implementation of COST instruments, such as STSMs, Workshops and meetings, TSs, and publications
– budget planning
– preparation and approval of the reports
– communication with the COST structure
– communication with other scientific structures and handling PR matters

MC meetings will be organised at least once per year, preferably together with some other activity (WG meetings, workshops, TSs) to reduce the travel costs and to improve communication within the Action. The MC will organise workshops or strategic meetings once per year. The MC will make a use of existing structures, e.g. European conferences devoted to natural products and chemical biology.

The workshops at these bigger meetings will ensure:

– extensive communication with the experts in the field
– higher efficiency in the use of travel funds
– possibility of presentation of the COST results at the conferences

Website:

There will be an Action website that will help to disclose the main achievements both to specialists and to the general public. It also will function as a news board. For internal affairs, to exchange data or ideas inside the Action it is planned to set up a Google group. A person appointed by the MC will be responsible for coordinating this activity (webmaster, editorial coordinator).

Evaluation:

All respective instruments e.g. STSMs, WG meetings, Workshops, TSs have to be monitored and evaluated once they are finished. Written reports from all the activities following COST procedures will contain scientific results and also clear costs breakdown. All reports will be submitted via the coordinators to the MC for evaluation and final approval, followed by publication of key topics on the web page.

Milestones of the Action will be carefully followed and evaluated.

E.2 Working Groups

Three WGs will be created according to the topical priorities, potential of application in industry and complementarity of the groups involved in the proposal. The Action will comprise the following WGs, whose activities will overlap in many bordering areas, thus individual research groups may work under one or more of these sub-headings:

WG 1) Natural product chemistry: natural product like compound collections, isolation and structure elucidation, biological testing

WG 2) Synthesis: Hsp90 inhibitors and nitrogen containing molecules, synthetic methodologies, total synthesis, (biological) function and target oriented synthesis (SAR studies)

WG 3) Chemical biology: tools for identifying the target of a natural product, biosynthesis, mutasynthesis, and mechanism of action

The WGs will be organised in a multidisciplinary manner, ideally involving also partner(s) from industry. Gender balance will be considered. Close collaboration within each WG will be pursued but also with intense collaboration across the whole Action and will be stimulated by various instruments, e. g. joint WG meetings, STSMs between different WGs, exchange of information via the Action webpage and all-Action workshops. Each WG should meet once a year, but also informal WG meetings out of the COST activities will be stimulated.

Meetings and Workshops: Each WG will organise one meeting per year, preferably together with another WG to ensure better communication across the Action. To these meetings external experts from industry and academia will be invited. The meetings will be organised as open workshops (except when dealing with organisational and financial matters) to ensure participation of students and scientists from the organising institution. This will also improve public awareness of COST activities.

Additional instruments that - according to COST rules - will be available to the WG participants, particularly to the students, are:

Short Term Scientific Missions: STSMs will be a fundamental instrument of the collaboration within the Action and promote the involvement of younger scientists. They will be organised mostly for the exchange of methodology and experimental techniques. Inter-WG STSMs will also be promoted. STSMs will be thoroughly prepared and approved by the STSM coordinator and the MC. Ethical rules and gender balance will be considered.

Training Schools: Since the training in new disciplines will be essential during the Action, TSs will be organised. Two TSs, each ca. 4-5 days duration, will be offered. The first one should be held in the first year of the Action. The second one will be held at the beginning of the last year of the Action.

E.3 Liaison and interaction with other research programmes

Some of the proponents of the Action have former extensive experience from other COST Actions, e.g. D28: Natural Products as a Source for Discovery, Synthesis, and Application of New Pharmaceuticals.

E.4 Gender balance and involvement of early-stage researchers

This COST Action will respect an appropriate gender balance in all its activities and the Management Committee will place this as a standard item on all its MC agendas. The Action will also be committed to involve early-stage researchers. This will also be as a key item on all MC agendas.

All participating partners belong to equal opportunities employers and many universities have set up measures to increase the number of women in science.

In addition, involvement of early-stage researchers in various activities of the Action will be promoted. Thus, they will not only actively participate in STSMs and TSs, but they will also be encouraged to give oral presentations at all Action meetings. Young scientists might also act as session chairs at the meetings.

F. TIMETABLE

Duration of the Action: 4 years

The proposed milestones and deliverables are:

Training Schools: 1 and 2

Workshops: 4 (Kick-off, Midterm conference and strategic planning, Workshop 3, Final Workshop)

The timetable of the Action is:

Year 1

March-April	MC first meeting
April-December	Working Group Meetings (WGMs); one per each WG
August-September	MC meeting; Preparation of Action Workshop 1
November-December	Kick-off Workshop 1; MC meeting, preparation of TS
April to December	STSMs

Year 2

January-April	TS 1, MC meeting, preparation of the mid term and strategic Workshop 2
August-December	Mid-term and strategic Workshop 2 - MC meeting
January-December	WG meetings
January to December	STSMs

Year 3

January-April	MC meeting, preparation of the Action Workshop 3
June-November	Action Workshop 3; WG meetings
September-December	MC meeting, preparation of TS 2
January-December	STSMs

Year 4

March-April	TS 2, MC meeting: preparation of the Final Action Workshop
May-August	WG meetings
October-December	Closing Conference (Workshop 4) of the Action, MC meeting - Final Report approval, publications (special issue on Action activities) - finalisation (due April year 5)
January-December	STSMs

G. ECONOMIC DIMENSION

The following COST countries have actively participated in the preparation of the Action or otherwise indicated their interest: CH, DE, ES, FR, FI, GR, IT, LT, NL, PT, UK. On the basis of national estimates, the economic dimension of the activities to be carried out under the Action has been estimated at 44 Million € for the total duration of the Action. This estimate is valid under the assumption that all the countries mentioned above but no other countries will participate in the Action. Any departure from this will change the total cost accordingly.

H. DISSEMINATION PLAN

H.1 Who?

There will be at least six target groups for the dissemination of the results of the Action:

– First of all, the participating scientists and their co-workers
– University students (mainly PhD students, but not exclusively, with interest in chemical biology)
– Industrial research scientists, both as directly involved in the Action and as external potential end users
– National and European societies active in the area of chemical biology, organic synthesis and molecular physiology
– National and European funding agencies for the promotion of chemistry, biology, and drug discovery
– The general public, for example high school students, by promoting interest in the area of applied chemistry

H.2 What?

The dissemination of the Action activities will be made possible by exploiting the following instruments:

– Action website, maintained by a webmaster and editorial coordinator
– Meetings and workshops
– Publications, review articles, description of the meetings in journals.
– TSs and teaching manuals

H.3 How?

a) Website:
The website will have two sections:

– Intranet (password protected) for exchange of data/ideas inside the Action and the respective WGs. In place of the Intranet a Google-group could be used.

– Extranet (of public domain) that will publish regular reports of Action activities (previously approved by the MC) and the main scientific achievements for both specialists and the general public.

The webpage should be:

– User-friendly allowing easy input (via password) of respective information by members without the necessity of detailed knowledge of web building, to ensure continuous update from the whole Action.
– There will be regular updates covering recent hot topics in the field (e.g. conferences, novel insights, patent reports) and important articles prepared by each WG. Information from similar consortia and centres and links to other important pages (e.g. national and international chemical biology) will be provided.
– In addition, this COST Action will publish tutorials on topics related to the Action (software usage, running assays etc.).

b) Meetings and Workshops:

WG Meetings and general Action workshops will be organised, when possible, as satellite meetings of some relevant symposia or conferences to facilitate the dissemination of Action activities towards a wider scientific audience. External experts from academia and industry will be invited to these meetings. The meetings will be organised as open workshops to ensure access of the students and scientists from the organising institution. Respective proceedings and web documents will be published from these meetings, whenever indicated.

c) Publications:

A fundamental task of each scientist is to publish high-quality papers in high-ranked and specialised peer-reviewed journals. The MC will stress the production of high quality multi-group papers within the Action. Additionally, reports to the relevant journals providing general information to scientific community but also to the economics- and policy-making organs targeting non-specialist will be published.