# Combining Ethnopharmacology and Virtual Screening for Lead Structure Discovery: COX-Inhibitors as Application Example

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Virtual screening of large libraries of organic compounds combined with pharmacological high throughput screening is widely used for drug discovery in the pharmaceutical industry. Our aim was to explore the efficiency of using a biased 3D database comprising secondary metabolites from antiinflammatory medicinal plants as a source for the virtual screening. For this study pharmacophore models of cyclooxygenase I and II (COX-1, COX-2), key enzymes in the inflammation process, were generated with structure-based as well as common feature based modeling, resulting in three COX hypotheses. Four different multiconfomational 3D databases limited in molecular weight between 300 and 700 Da were applied to the screening in order to compare and analyze the obtained hit rates. Two of them were created in-house (DIOS, NPD). The database DIOS consists of 2752 compounds from phytochemical reports of antiinflammatory medicinal plants described by the ethnopharmacological source '*De material medica*' of Pedanius Dioscorides, whereas NPD contains almost 80 000 compounds gathered arbitrarily from natural sources. In addition, two available multiconformational 3D libraries comprising marketed and development drug substances (DWI and NCI), mainly originating from synthesis, were used for comparison. As a test of the pharmacophore models' capability in natural sources, the models were used to search for known COX inhibitory natural products. This was achieved with some exceptions, which are discussed in the paper. Depending on the hypothesis used, DWI and NCI library searches produced hit rates in the range of 6.6% to 13.7%. A slight increase of the number of molecules assessed for binding was achieved with the database of natural products (NPD). Using the biased 3D database DIOS, however, the average increase of efficiency reached 77% to 133% compared to the hit rates resulting from WDI and NCI. The statistical benefit of a combination of an ethnopharmacological approach with the potential of computer aided drug discovery by in silico screening was demonstrated exemplified on the applied targets COX-1 and COX-2.

## INTRODUCTION

As clearly reported over the last few decades, natural products (NPs) represent a successful and profitable source in drug discovery.[1,2] Approximately one-third of the top-selling drugs in the world are derived from compounds of natural sources. First, their diversity in chemical space is highly superior to that exhibited by compounds originating from combinatorial chemistry. Second, they show a wide range of pharmacological activities, because the expression of secondary metabolites has an evolutionary reason and background. Although in most cases the multiple functions of secondary plant constituents are unknown, they have obviously evolved from balanced interactions between plants and microorganisms as well as plants and herbivores.[3] They are expressed as a result of stimuli and are targeted to interact with receptors.[4]

The pharmacological investigation of crude extracts and prefractionated extracts of medicinal plants, which are empirically well-known for a specific activity, represents a profound and promising proceeding for discovering new biologically active compounds. An additional bioguided fractionation enables the disclosure of the bioactive principle/s, as is described in a wealth of successful searches that have been published.[5−11] Since this procedure is well established but very time consuming, the benefits of a pure compound screening based on compound libraries were recently discussed by Bindseil and colleagues, who concluded that this method was much faster as well as significantly less expensive than using plant extracts.[12]

Computer aided screening technologies such as pharmacophore modeling together with virtual screening (VS) are important approaches in modern drug discovery. In particular, the potential of in silico screening of compound libraries for lead discovery is widely accepted and have been proved not only in the academic field but also in the pharmaceutical industry.[13] However, this method is only efficient, if enough structural information about the target, or compounds that bind to the target, is available to create a reliable pharma-

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cophore model. Ethnopharmacological screening and virtual screening are considered distinct approaches, since the first is an empirical one and the other a theoretical. Both strategies are well established, but they are hardly ever integrated, mainly because only a few 3D libraries of NPs are available. A successful example, reported by Bernard and co-workers,[14] who used a combined approach to rationalize a phytochemical lead discovery, was the disclosure of betulin and betulinic acid as phospholipase $A_2$-inhibiting constituents of plant material with traditionally known antiinflammatory properties (i.e. in bark and sap of *Leopoldinia imperialis*).

In the present study we focus on the statistical benefit by conducting this integrated concept of virtual and ethnopharmacological screening using medicinal plants of '*De materia medica'* of Pedanius Dioscorides as an ethnobotanical source. This work, written in five books around the years 65 to 75 A.D., deals with approximately 1000 simple drugs which were used in medical practice until modern times.[15−17] It served as the primary text of pharmacology until the end of the 15th century in the European and Arabian world.

The foremost aims were first to create an extensive 3D database of NPs containing structures that had been published in the scientific world, called NPD (Natural Product Database). Second, we generated a 3D database (DIOS) of plant constituents out of those medicinal plants described by Dioscorides to possess antiinflammatory activities. For the virtual screening we elaborated chemical feature-based pharmacophore models for cyclooxygenase-1 and -2 (COX-1, COX-2), since both isoforms represent key enzymes in the complex inflammation process. Phospholipases, especially cytosolic phospholipase $A_2$, liberate the phospholipid-bound arachidonate, and then the cyclooxygenase-complex transforms the liberated arachidonate by a sequential introduction of two moles of oxygen (lipoxygenase reaction), followed by a COX reaction. This transformation results in a 15-hydroperoxide prostaglandin ($PGG_2$) that is reduced to the corresponding hydroxy product ($PGH_2$).[18] Then, a battery of enzymes is leading to different inflammation mediators such as prostaglandins. Cyclooxygenases exist in at least two different isoforms. COX-1 is a constitutively expressed gene, whereas COX-2 is mainly induced by various inflammatory stimuli. Conceptually, COX-2 selective inhibitors should be expected to retain antiinflammatory efficacy by inhibition of the prostaglandins, while reducing or eliminating the gastric, renal, and haemostatic side effects commonly associated with nonsteroidal antiinflammatory drugs (NSAIDs) use.[19] However, this simple idea of focusing on the selective inhibition of COX-2 to profit exclusively from the desired effects is only valid with caveats. The family of prostaglandins induced by COX-2 may replace that by COX-1 in some situations and vice versa. Both COX isoenzymes contribute to mucosal defense, and the inhibition of the two isoforms contributes to the pathogenesis of NSAID-induced gastric damage.[20] Fiorucci and colleagues could demonstrate that the administration of aspirin leads to a rapid biosynthesis of lipoxin $A_4$ via COX-2 up-regulation, which in turn diminishes the aspirin-induced damage in the rat stomach.[21]

Within the past decade COX inhibitors have also been described as useful for treating Alzheimer's disease.[22,23] Several epidemiological studies have further demonstrated that NSAIDs have cancer-preventative and tumor regressive effects in the human colon.[24,25] Thus, the regulation of the COX pathway provides an excellent approach for the discovery of cancer chemo-preventative agents.[26−28] There has been a real explosion of interest in COX pharmacology, whereby the search of potent COX inhibiting components by VS of 3D databases may elucidate interesting structural features derived from natural sources. In this work, an evaluation of hit lists resulting from the VS of our in-house databases NPD and DIOS with regard to the results obtained from the available databases of *The National Cancer Institute* (NCI) and the *Derwent World Drug Index* (WDI) was performed, and the results are discussed.

## METHODS

***De materia medica* as an Ethnobotanical Source.** Of the 1000 or so drugs comprised in Dioscorides' ΠΕΡΙ ΥΛΗΣ ΙΑΤΡΙΚΗΣ (the common Latin title is *De materia medica*) the author described about 800 plants—the rest are derived from mineral and animal origins. In the first book the treatise deals with spices, oils, and trees, whereas the second book mainly describes animal products (milk, honey, etc.). Books three and four deal with herbs and roots, and book five describes different sorts of wine and minerals. Due to our priority of investigating plant constituents we employed books one, three, and four for the ethnobotanical source, using Julius Berendes' edition from 1902[15] as well as Max Aufmesser's edition from 2002.[16] All plants were selected from those which Dioscorides claimed to possess activities against inflammatory disorders such as rheumatic or arthritic pains, joint swelling, pus, snake bites, and fever. Although Dioscorides provided his treatise with partly profound descriptions and comparisons of plant species, there is still an indisputable problem of the assignment of the plant material. In case of doubt we considered more proposals of plant species and followed the comments and recommendations of J. Berendes and his co-worker, the physician and botanist C. Fraas.[15] At present, two online databases of chemicals including NPs are available to us and were used to search for literature dealing with the isolation of secondary metabolites corresponding to the selected plant species: SciFinder Scholar (American Chemical Society, http://www.cas.org/SCIFINDER/SCHOLAR/) and Beilstein Crossfire (MDL Information Systems, http://www.Beilstein.com/). Structural information of plant constituents was then retrieved from the original literature, and this revealed a variable number of structures associated with each plant.

**Creating the 3D Databases DIOS and NPD.** Based on the analysis of a prescreening with our elaborated pharmacophore models, the range of molecular weight was found to be between 300 and 700 Da. Therefore, molecules within these weight limits were gathered for creating the 3D databases. Three-dimensional models of the NPs were built using the structure editor within the Catalyst Software Package (ref. Catalyst Version 4.7 Accelrys, San Diego, CA). Compounds were minimized using the default parameters of the CHARMM force field in order to reduce internal strain energy. Multiconformational models were generated by randomized conformational analysis using the Poling algorithm[29,30] and stored in a binary database using the CatDB procedure within Catalyst (FAST algorithm for conformer generation; maximum of conformers: 100). The final

databases NPD and DIOS contained 79 483 and 2752 compounds, respectively.

**Available 3D Databases.** The *Derwent World Drug Index* is an authoritative index for drug substances. The version released in 1996 and used by us contains chemical, biomedical, and synonym data for over 48 000 marketed and development drugs worldwide. The database of *The National Cancer Institute* is a multiconformational drug library containing more than 123 000 compounds which have been tested for potential anticancer activity. The library originates both from synthetic chemistry and natural sources and was provided with Catalyst 4.7 by Accelrys, San Diego, CA. In analogy to our in-house databases NPD and DIOS, the libraries provided by *Derwent* and *The National Cancer Institute* were reduced to compounds with a molecular weight between 300 and 700 Da, resulting in 27 914 and 38 645 compounds, respectively. These databases limited by their molecular weight are in the following named WDI and NCI.

**Generation of Feature-Based Pharmacophore Models for COX-1 and COX-2 Inhibitors.** Chemical feature-based pharmacophore models have proven to be highly useful tools for rationalizing ligand−target interaction and for making this information available to virtual screening (VS) techniques. The utility of feature-based pharmacophore as queries for successful 3D database search has recently been reviewed.[13] Such pharmacophore models consist of a number of so-called 'features' that are located relative to each other in coordinate space as points surrounded by a sphere of tolerance. Each sphere represents the region in space that should be occupied by a certain chemical functionality capable of the kind of interaction specified by the feature type. In organic molecules, different structural motifs can express the similar chemical behavior and therefore the same biological effect. The concept of feature-based pharmacophores is implemented within the Catalyst software environment representing a widely used platform for VS.

Feature-based pharmacophore models may be obtained either starting from 3D structural information available from protein−ligand complexes or purely from ligand information using algorithms aimed at the definition of common features within a set of bioactive molecules. For this study, both approaches were applied using the Catalyst software package.[31]

The 3D structure of cyclooxygenase has been known since the first X-ray crystal structure described by Picot and coworkers of the isoenzyme COX-1 complexed with the nonselective NSAID flurbiprofen (**1**) was solved.[32] The structure of the human COX-2 enzyme, obtained from X-ray crystal data[33] or by homology modeling[34] has also been published. Kurumbail and coauthors[35] have recently deposited structures of the COX-2 in the Brookhaven Protein Databank, both in free form as well as complexed with the selective inhibitor SC-558 (**2**) or the nonselective inhibitors indomethacin (**3**) and flurbiprofen (**1**). The three-dimensional structures of both complexes were used as the starting point for pharmacophore model generation in the present study.

A first pharmacophore was manually built based upon the atomic coordinates published in the PDB entry 1CQE, representing the COX-1/flurbiprofen complex.[32] This model (COX-1-A) contains two hydrogen bond acceptor (HBA) features, representing the H-bonds formed by amino acids ARG120 and TYR355 that point to the two oxygen atoms

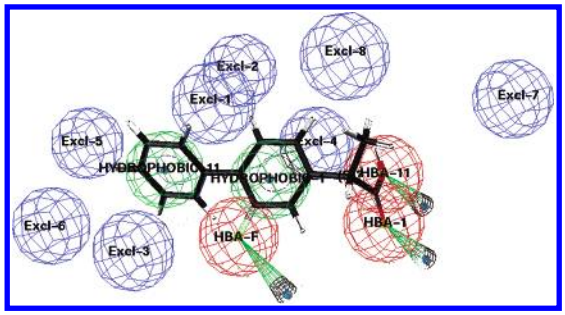
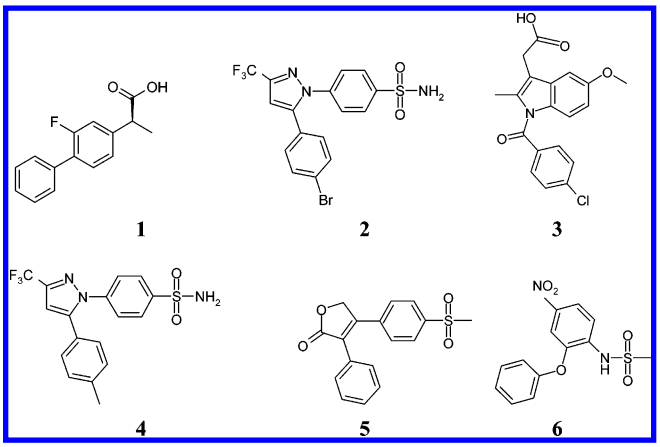



**Figure 1.** Flurbiprofen (**1**) fitted on COX-1 pharmacophore model.




of the carboxyl group of **1**. Moreover, the fluorine atom of **1** was defined as additional interaction center with ARG120 (HBA-F), since in other complexes a further hydrogen bond can be found originating from this amino acid pointing to an H-bond acceptor atom located at a comparable position within the ligand. Two general hydrophobic features representing the phenyl and the methyl moieties of **1** were added as well as one aromatic hydrophobic feature encoding for the flourophenyl ring. Exclusion volume spheres of 1.5 Å were set on the side chains of Val349, Ser530, Leu352, Ala527, Tyr385, Trp387, Val116, and Leu359 in order to mimic the boundary of the binding site while still taking into consideration flexibility of the side chain. A second model was obtained from the first one simply by omitting one hydrophobic feature located on the methyl substituent of the ligand. This model (COX-1-B) is represented in Figure 1, together with flurbiprofen (**1**), fitted on the pharmacophore features. The Cartesian coordinate triples for all the features of this pharmacophore model are listed in Table 1.

For COX-2, two different pharmacophore modeling approaches were used: First, a structure-based model (COX-2-A) was manually generated starting from the atomic coordinates published in the PDB entry 1CX2, representing the COX-2/SC-558 complex,[35] and, second, an automatically built ligand-based pharmacophore (COX-2-B, starting from four different COX-2 inhibitors: SC-558 (**2**), Celecoxib (**4**), Rofecoxib (**5**), and Nimesulide (**6**)).

Model COX-2-A was built based on the following interactions; a hydrogen bond pointing from amino acid HIS90 to the nitrogen atom of the sulfonamide moiety, a hydrogen bond acceptor mimicking function located on the trifluoromethyl group, an aromatic hydrophobic feature located on the phenyl moiety bearing the sulfonamide group, and a hydrophobic function placed on the bromophenyl ring. Additionally, three exclusion volume spheres of 1.0 and

**Table 1.** Cartesian Coordinates of Pharmacophore Models Described

| feature | size (Å) | Cartesian coordinates (Å) X | Y | Z |
|---|---|---|---|---|
| COX-1 Pharmacophore Model | | | | |
| hydrophobic | 1.5 | −42.4340 | 2.1456 | −17.6030 |
| hydrophobic | 1.5 | −40.8540 | −0.4410 | −21.0120 |
| HBA | 1.5 | −44.8660 | 5.5040 | −16.0730 |
| HBA | 1.5 | −46.1050 | 3.7120 | −16.0400 |
| HBA-F | 1.5 | −41.1780 | 3.4340 | −19.4210 |
| exclusion volume | 1.5 | −49.3950 | 3.6860 | −11.5270 |
| exclusion volume | 1.5 | −40.5940 | 1.2530 | −10.0370 |
| exclusion volume | 1.5 | −39.7210 | −0.4960 | −15.7430 |
| exclusion volume | 1.5 | −37.3700 | −0.5520 | −24.3200 |
| exclusion volume | 1.5 | −37.5930 | 2.3780 | −20.8740 |
| exclusion volume | 1.5 | −41.9840 | −4.1390 | −25.7910 |
| exclusion volume | 1.5 | −46.0780 | −3.4580 | −20.1170 |
| exclusion volume | 1.5 | −47.7220 | 0.4650 | −19.8750 |
| COX-2-A Pharmacophore Model | | | | |
| hydrophobic aromatic | 1.5 | 0.5920 | 1.8870 | −6.0220 |
| hydrophobic aromatic | 1.5 | −2.6090 | 0.1120 | −2.2670 |
| HBA-F | 1.5 | 2.5520 | −2.0870 | 0.1370 |
| HBA-$SO_2H$ | 1.5 | −6.3730 | −1.5110 | −1.8430 |
| exclusion volume | 1.5 | −3.6660 | 2.0000 | 1.0920 |
| exclusion volume | 1.5 | 4.1910 | 1.4280 | −0.0220 |
| exclusion volume | 1.5 | 0.7260 | −4.3630 | −2.5700 |
| COX-2-B Pharmacophore Model | | | | |
| hydrophobic aromatic | 1.5 | −0.6050 | 0.3940 | 8.2100 |
| hydrophobic aromatic | 1.5 | −2.8130 | 3.3590 | 4.4040 |
| HBA | 1.5 | −3.3240 | 7.0830 | 3.2600 |
| HBA projected point | 2.0 | −2.0610 | 5.0780 | 1.4270 |
| HBA | 1.5 | −0.2360 | −2.7830 | 2.0320 |
| HBA projected point | 2.0 | 1.3460 | −0.4390 | 0.9930 |
| exclusion volume | 1.8 | 3.5760 | 0.8210 | 8.0000 |
| exclusion volume | 1.8 | −5.0530 | 2.0340 | 7.5000 |
| exclusion volume | 1.8 | 1.1780 | 5.4650 | 4.0000 |

1.5 Å were set on the side chains of amino acids VAL523, LEU352, and SER353.

Multiconformational structural models of the four COX-2 inhibitors **2** and **4**−**6** were used for building a ligand-based common feature pharmacophore model (COX-2-B) of these compounds. The *HipHop* algorithm[36] was used within the Catalyst software with a maximal omitted feature value of 0 defined for all training set members. The model obtained by applying this procedure contained two hydrogen-acceptor functions and two aromatic hydrophobic features. This was refined manually by insertion of three exclusion volume spheres of 1.8 Å set on the positions occupied by the side chains of amino acids VAL523, LEU352, and SER353. The Cartesian coordinate triples for all the features of both pharmacophore models are listed in Table 1.

All models were validated by testing whether they were able to correctly retrieve active COX-1 and COX-2 inhibitors from our self-compiled database containing COX inhibitors known from literature.

## RESULTS AND DISCUSSION

**Evaluation of the Hit Rates.** VS experiments using four different sources (3D databases WDI, NCI, NPD, DIOS) were conducted within Catalyst with the elaborated COX models as search queries and by using the Fast Flexible Search option. Compounds present in the database are stored as three-dimensional structure models in multiconformational data format, and an index is built according to the presence or absence of chemical features in the molecules. In the VS procedure, first the index is searched for compounds fulfilling the requirements defined by the query pharmacophore. All molecules remaining are then fitted in each of their stored conformations by rigid rotation and translation in order to determine if the chemical features of the structures are able to map the requested functions of the pharmacophore model. A compound is considered to be a hit only if all functions of the pharmacophore model are mapped by defined functions of the respective molecule. The quality of the fit (fitting value) depends on two parameters: the weights assigned to the hypothesis features and how close the features in the molecule are to the centers of the corresponding location constraints in the pharmacophore hypothesis. In the present study, weights were set to a value of one for all features.

The major aims were (a) to critically analyze the obtained hits according to their cyclooxygenase inhibitory activity that are already evident from literature, (b) to interpret the obtained hit rates of our biased 3D database DIOS with respect to the application of the different COX hypotheses, and (c) to compare the hit rates relative to the screened databases.

(a) A first evaluation of the hit lists disclosed some well-known compound families already described as potent COX inhibiting substances, e.g. flavonoids or stilbenoid derivatives. The findings are in accordance with structural features experimentally proved to be required for COX inhibition.

Within the chemical family of stilbenoids our in-house databases could not retrieve the low molecular weighted stilbens such as resveratrol or oxyresveratrol,[37] 4,3′,5′-trihydroxystilbene, 4,3′-dihydroxy-5′-methoxystilbene, 4-hydroxy-3′,5′-dimethoxystilbene,[38] or isorhapontigenin,[39] although they clearly inhibit COX-1 and COX-2 in the in vitro enzyme assay. Obviously their structures are too small to fulfill the requirements of an interaction for all of the set features in the pharmacophore models without omitting one. Another possible reason may be aggregate formation, which may explain the activity of many nonspecific inhibitors. The mechanism of these so-called promiscuous hits was recently discussed and demonstrated by Susan McGovern and coworkers for some widespread molecules of databases used for pharmacological screenings.[40] As soon as the stilbenoid skeleton grows in size and simultaneously loses its planarity, e.g. by dimerization or insertion of a 3-(2,3-dihydroxy-3-methylbutyl) group, the retrieval of significant COX inhibitors could be achieved, e.g. compound **7**[37] or compound **8**.[41]

A potentially useful lead structure which was not captured by our VS is the secondary plant metabolite tryptanthrin (**9**). This indoloquinazolinone, isolated as one of the active principles from *Isatis tinctoria*, is described as a potent COX-2 and 5-lipoxygenase inhibitory agent by Danz and coauthors.[8,42] Since this molecule is totally planar the H-bond acceptor features together with the hydrophobic zones cannot map the three-dimensional arrangement of the pharmacophore model used. This may be a hint for an alternative binding mode of this compound within the active site of the enzyme (see Chart 1). Concerning the flavonoid derivatives, a virtual activity congruent with a described COX inhibitory activity from literature was found for structural features belonging to prenylated flavanones and prenylated flavonols (e.g. compounds **10**,[43] **11**,[44] **12**[45]), for numerous flavan derivatives (e.g. **13**,[37] **14**,[46] **15**[47]) as well as for the flavon-dimer amentoflavon **16**[48] or the myricetin-3-glucuronide **17**.[49]

**Chart 1**

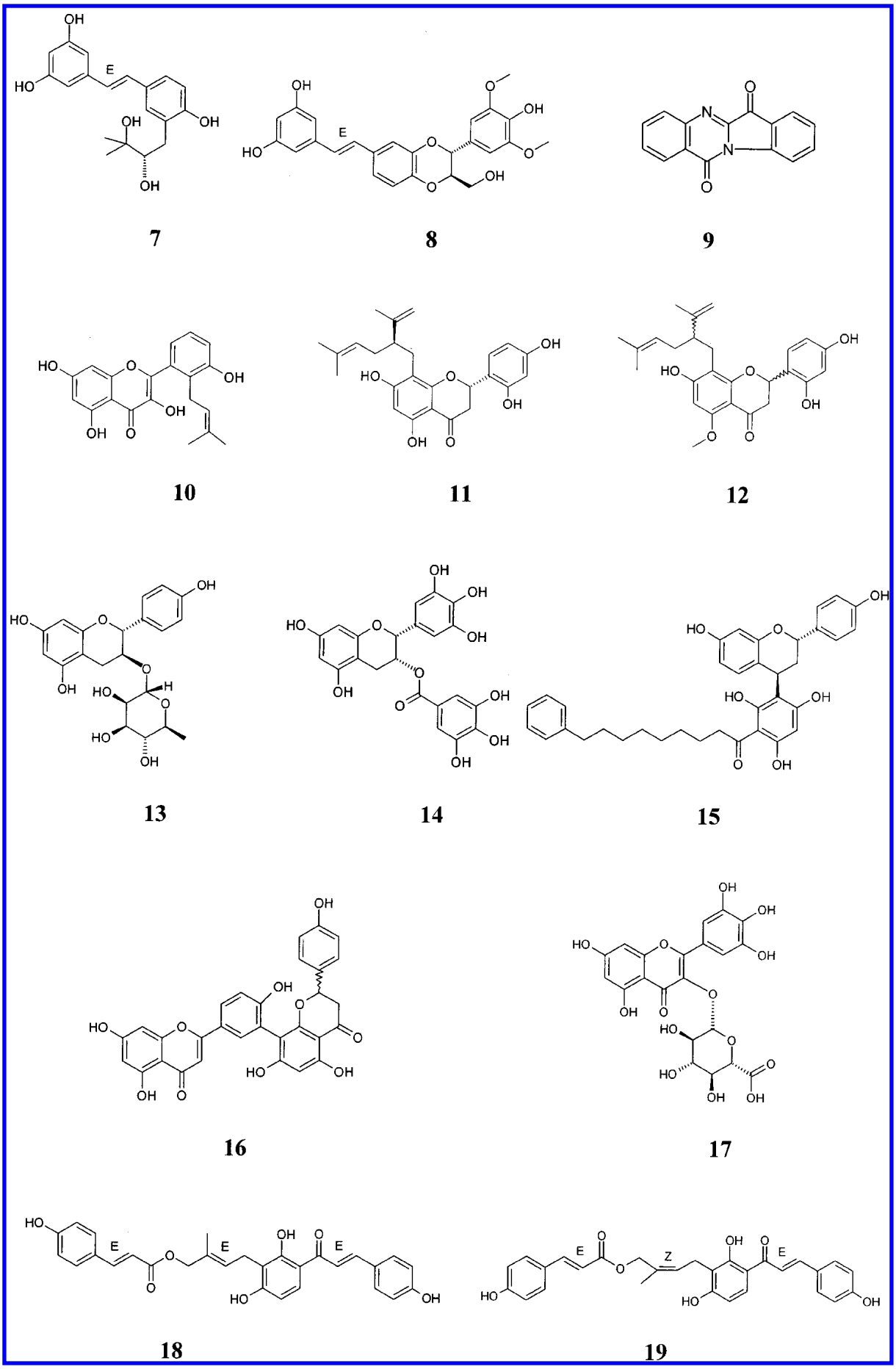
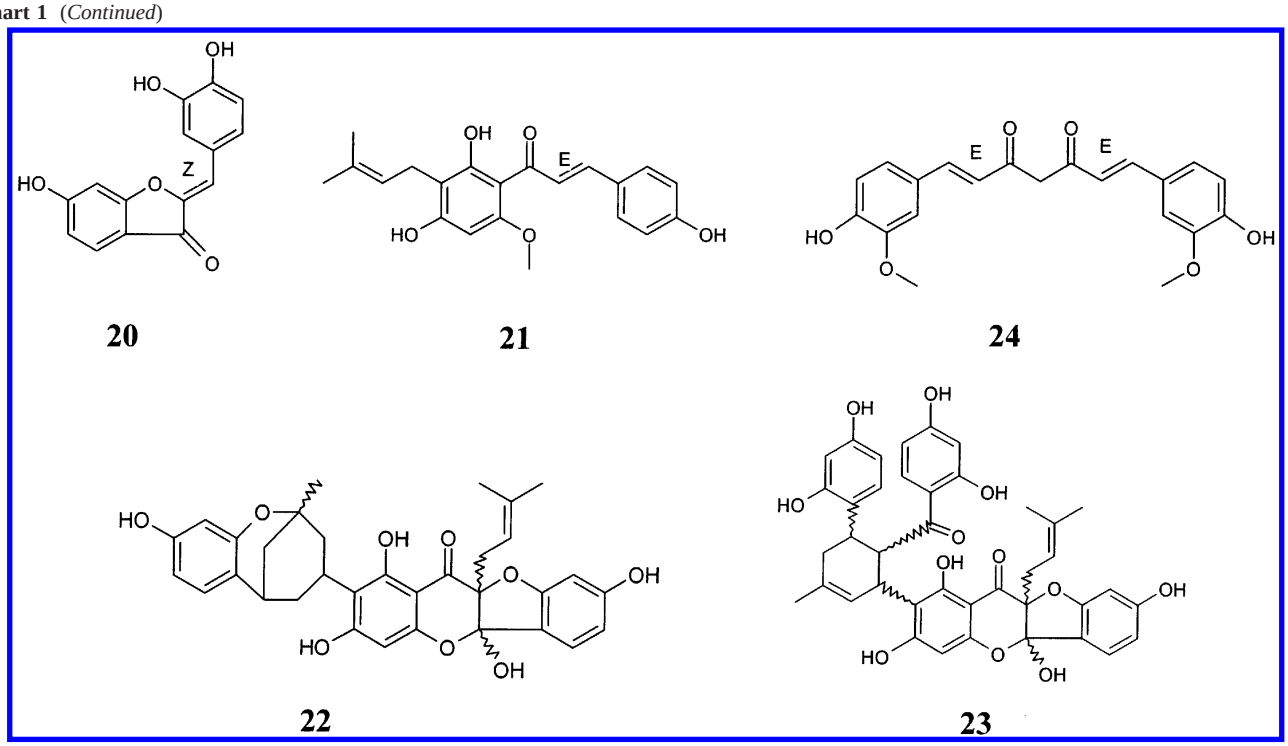


**Chart 1** (*Continued*)



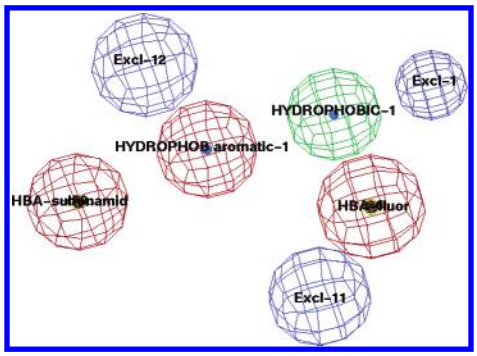




**Figure 2.** Pharmacophore model COX-2-A (structure based).

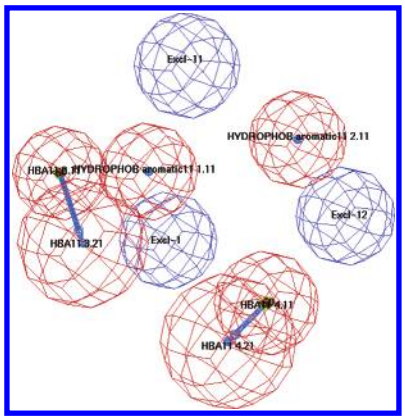



**Figure 3.** Pharmacophore model COX-2-B (ligand based).

In the flavonoid subclass of chalcons, the retrieval of structural features derived from simple and dimeric chalcons was achieved (e.g. compounds **18**, **19**,[37] **20**,[50] and **21**[51]) as well as some of their Diels−Alder-adducts, e.g. compounds **22** and **23**.[45] In addition the whole set of curcumines (e.g. **24**) described as potent COX inhibitors by Ramsewak and co-workers[52] could be found by our models.

(b) From our in-house 3D database DIOS the number of compounds that were assessed for target binding was analyzed in terms of the application of the elaborated pharmacophore models, COX-1-B, COX-2-A, COX-2-B. Additionally, the common hits of COX-2-A and B as well as the common hits of all three models (COX-1-B 2-AB) were analyzed. The data are listed in Figure 4 whereby the numbers of hits are depicted in relation to the total amount of compounds present in the DIOS database (2752). Three hundred twenty-seven compounds (11.9%) were assessed to fit to the pharmacophore model COX-1-B, 657 (23.9%) on COX-2-A, and 501 (18.2%) on COX-2-B. An interesting discovery was the high number of common hits (i.e. 490 compounds or 17.8%) with a hypothetic COX-2 inhibitory activity resulting from the VS with the structure based model COX-2-A as well as with the ligand based model COX-2-B. The latter proved to be the more selective one, however;

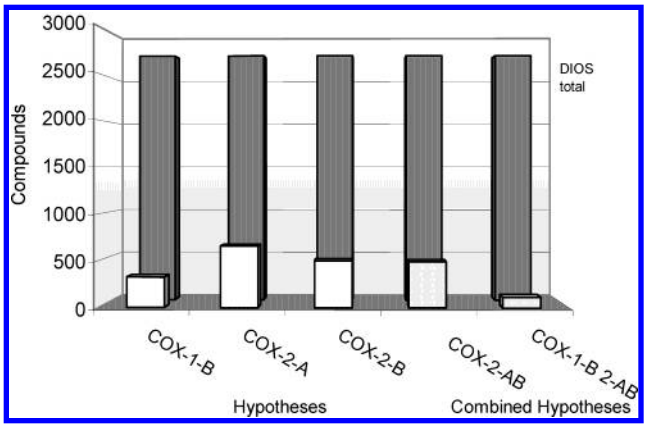



**Figure 4.** DIOS: Number of hits obtained from different pharmacophore models.

98% of the COX-2-B hits are included in the number of the common COX-2 hits (Figure 4, COX-2-AB). In the last row of Figure 4, the common hits of all three pharmacophore models (113 compounds or 4.1%) are depicted, representing compounds which interact (but not selectively) with the elaborated models of both cyclooxygenase isoenzymes.

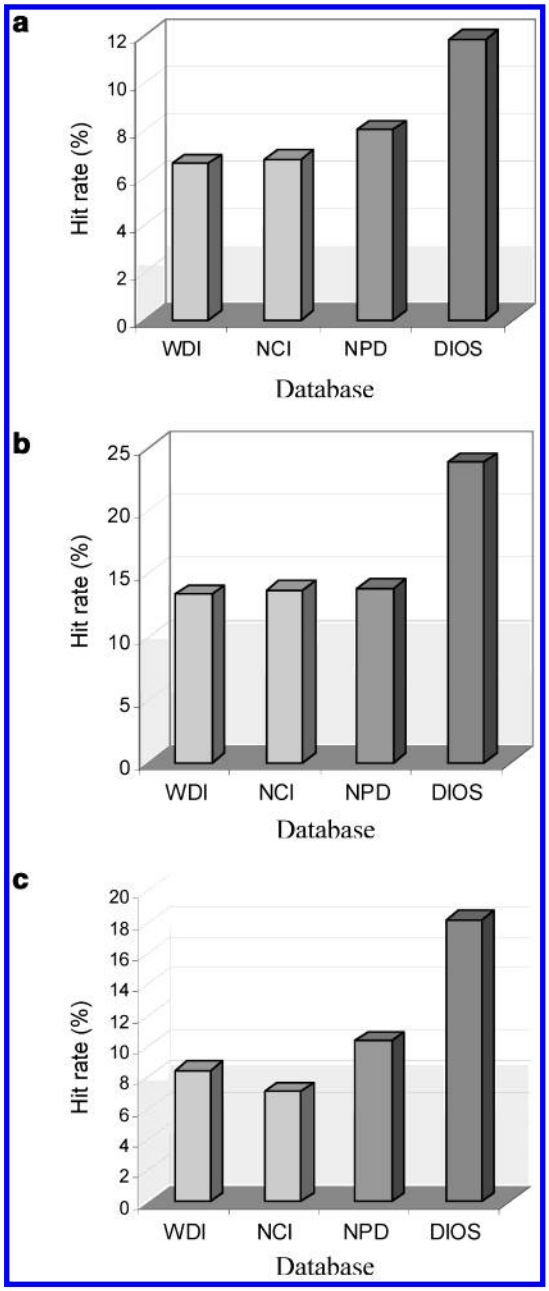



**Figure 5.** a: COX-1-B: Pharmacophore hypothesis based on Flurbiprofen/COX-1 complex X-ray structure. b: COX-2-A: Pharmacophore hypothesis based on SC558-4/COX-2 complex X-ray structure. c: COX-2-B: Pharmacophore hypothesis based on Common Features of selective COX-2 inhibitors.

(c) A comparison of the hit rates obtained from the VS using four different 3D compound databases helps to interpret the efficiency of the compounds' sources. The results are graphically shown in Figure 5 a, b, and c for all three elaborated COX hypotheses, respectively. The numbers of compounds to be assessed for binding from the libraries of the WDI and the NCI are situated in the same range (deviations between 0.7% and 1.4%). Relating to these hit rates, the 3D database NPD, which exclusively consists of NPs, shows an average increase of hits of 20.1% for the hypotheses COX-1-B and of 33.4% for the COX-2-B (Figure 5a and c). The VS against the COX-2-A hypothesis resulted in an almost stagnation of hit rates compared to the libraries of WDI and NCI (Figure 5 b). However, using the DIOS database as a library source for the VSs, the average increase of efficiency with respect to the unbiased database of NPs, i.e., NPD, amounted to 47.8% (COX-1-B), 72.2% (COX-2-A), and 75.2% (COX-2-B), and reached between a 77% and 133% increase compared to the hit rates resulting from the drug libraries WDI and NCI.

Although it must be assumed that in our result lists still a considerable number of compounds may be false positive hits, these results underline the high potential of NPs as stated and statistically proved by numerous authors, e.g. by Newman and coauthors.[1] NPs are often suitable for use as medicines since they show the desired metabolism-distribution with low toxicity,[2] because their structures have evolved to provide the plant with a reservoir of signals conceptually targeted toward receptors. In this way they do have a particularly useful role in this area and stand comparison with libraries containing pharmacokinetically experienced synthetic or semisynthetic drug substances. In addition, the discovery of most plant drugs of natural origin was as a result of ethnopharmacological observations.[53−55] The impact of medicinal plants, the knowledge of their pharmacological activities, and their benefit toward suffering people found expression in ethnopharmacological sources exemplified by the '*De materia medica*' of Pedanius Dioscorides. By considering the plants that Dioscorides claimed had an antiinflammatory activity, our 3D database DIOS gained biased information toward one or more receptors involved in the complex and multifarious inflammation mechanism. This information is reflected in the superior hit rates obtained using the DIOS database as a library source for the VSs against all three hypotheses (Figure 5a-c, last row respectively).

## CONCLUSIONS AND PERSPECTIVES

There is a continuing need for novel drug like lead compounds against an increasing number of targets provided by the exploration of the mechanisms of human disorders. Elucidation of the targets' functions and X-ray structures and the knowledge about selective agonists and antagonists enable the elaboration and application of pharmacophore models. These act as versatile tools to aid in the discovery and development of new lead compounds by the VS of large libraries of organic compounds. Furthermore, NPs are an especially rich source of functionally active leads. The discovery of most of the currently used drugs of natural origin was through ethnopharmacological hints, which provided bioinformatic data, allowing a rationalization of a phytochemical lead discovery. But instead of a pharmacological high throughput screening in this stage of the art, arbitrarily conducted with extracts and isolated compounds of medicinal plants and further ethnopharmacologically associated natural sources, a predictive assessment of compound binding affinity to the desired receptor (e.g. COX-1, COX-2) seems to be a rational method to reduce the number of compounds to be tested in a pharmacological screening.

In our study we could statistically demonstrate the efficiency of a combination of an ethnopharmacological approach by creating a biased 3D database out of NPs showing a traditional usage toward inflammation with the potential of computer aided drug discovery. We intend to use this method to discover novel potential COX-1 and COX-2 inhibitors within the inexhaustible pool of secondary metabolites provided by medicinal plants.

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