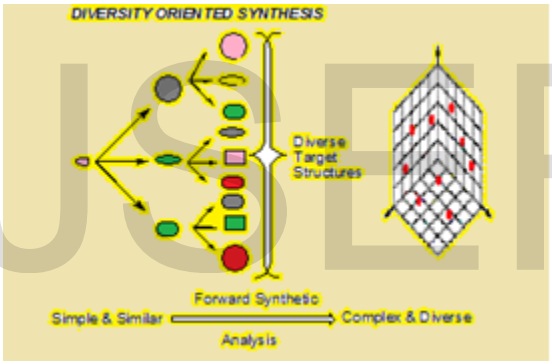
An Overview on Diversity Oriented Synthesis, Such as an Enormous Implications for the Discovery of Small Molecules.

Ashutosh Kumar Dash, Debaraj Mukherjee

ABSTRACT: The deliberate synthesis of a collections of a number of molecules or, library, that can entrap a spacious area is described in a single word Diversity-oriented synthesis (DOS), which is an emerging field involving the synthesis of combinatorial libraries of diverse small molecules for biological screening all these days. Instead of directing toward a single biological target, DOS libraries are especially used to identify new ligands for a variety of targets. Several different strategies for library design have been developed to target the biologically relevant regions of chemical structure space. DOS has provided powerful probes to investigate biological mechanisms and also served as a new driving force for advancing synthetic organic chemistry.

Key Words: DOS, Structural Diversity, Chemical and Biological space, Privileged structure.



INTRODUCTION:

The roles of small molecules are quite precious as powerful tools for studying biological systems. They allow rapid and conditional modulation of biological functions, often in a

reversible, dose-dependent manner. Moreover, they can modulate individual functions of multifunctional targets and distinguish different post-translational modification and conformational states of proteins. By dint of these features only, the chemical genetic or pharmacological approach has been awarded a valuable complement to genetic and RNA interference−based methods, particularly for dissecting complex, dynamic biological processes[1-6] .These small molecules can also be used to illuminate new potential therapeutic targets and provide a very direct means of validating these targets in model systems.

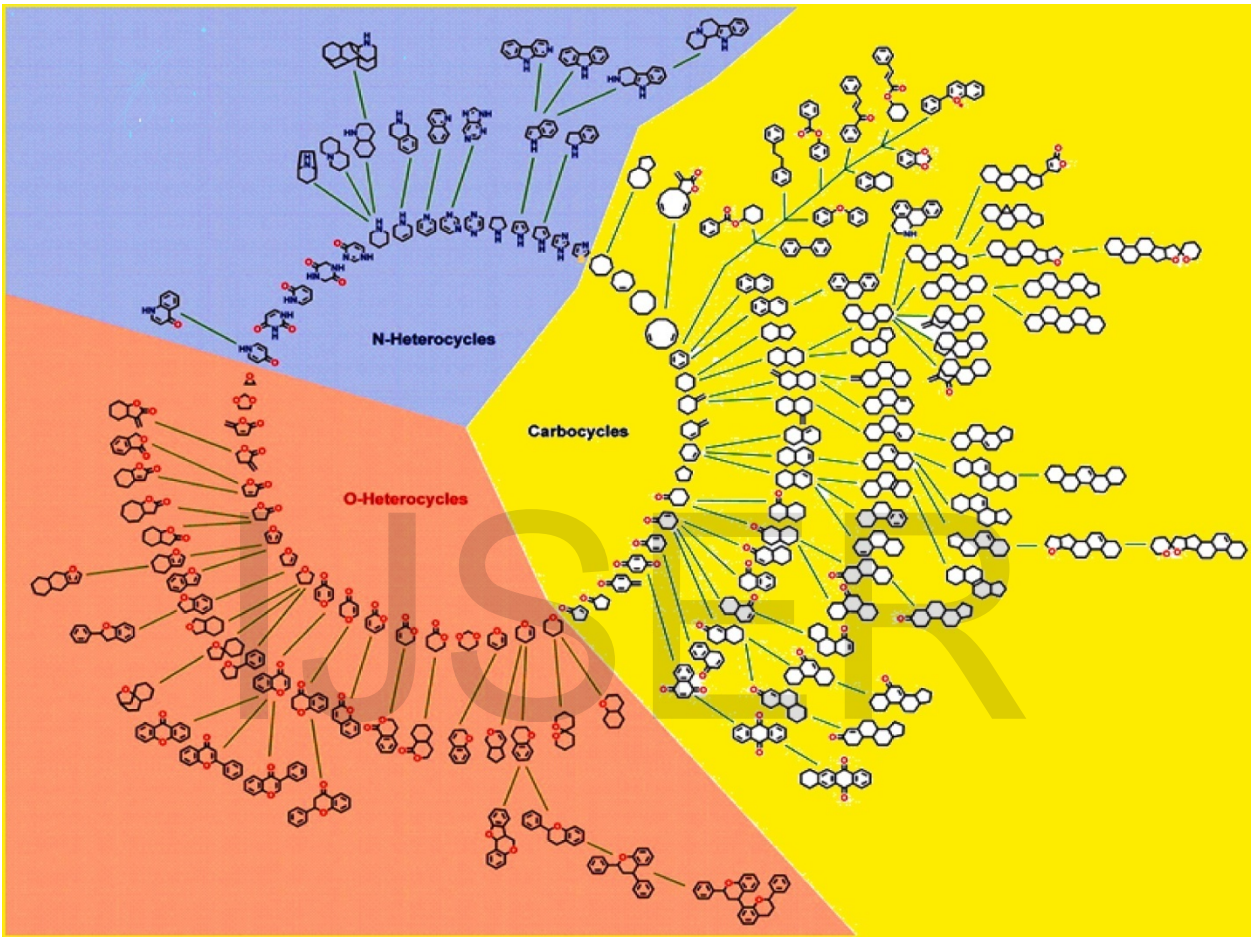


Fig-1: Diversity Oriented Synthesis a common diagram

Although the identification of new, highly specific small-molecule probes remains intact, this is a real challenge in chemical biology. Structure- or mechanism-based rational design is occasionally feasible when a single protein target and native ligands have their identity. Conversely, high-throughput screening (HTS) [7] of small-molecule libraries has emerged as a practical and effective solution for individual targets that may be less well-characterized and for systems that involve multiple targets. However various chemical libraries are now available commercially, these remain focused primarily on so-called 'drug-like' compounds[8]. Because these libraries are concentrated in a relatively narrow region of chemical structure space, it is quite conspicuous that, they will provide useful probes for all biological targets of interest.

That is why an important need, of diversity-oriented synthesis (DOS) has emerged as a valuable approach to generating libraries that explore untapped or under-represented regions of chemical structure space[9]. Efforts in DOS have produced powerful new biological probes and also spurred continuing advances in synthetic organic chemistry which, leads to therapeutically effective molecules.

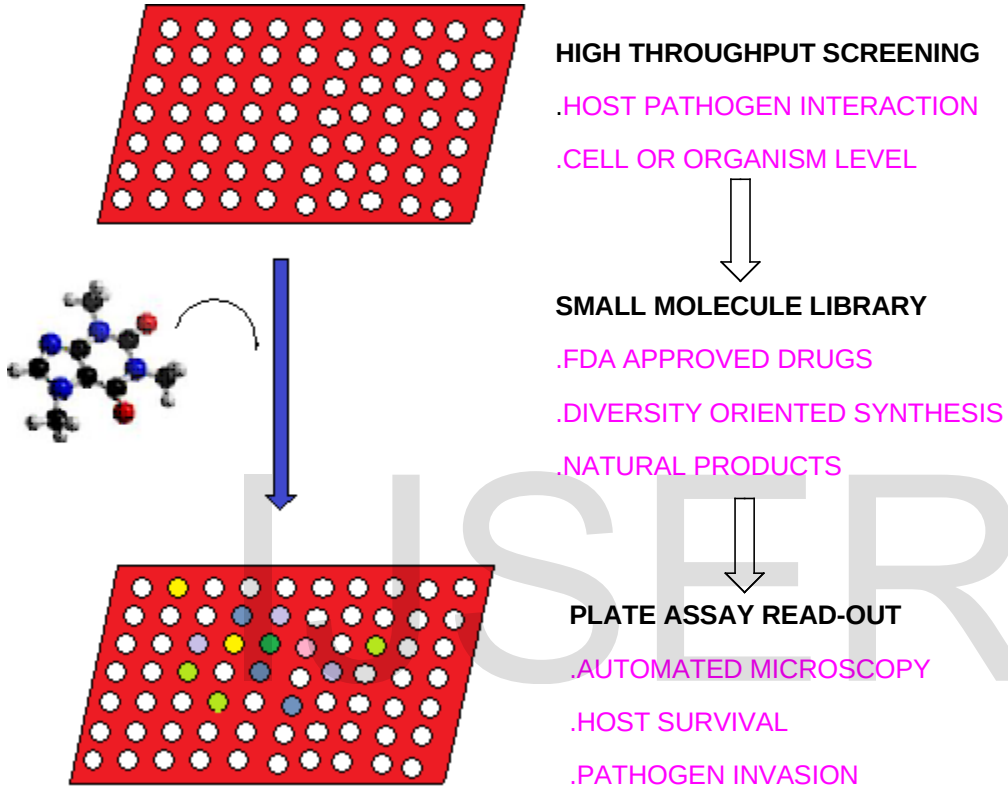


Fig-1: Schematic diagram showing about the process of High-throughput screening.

The Birth of Diversity Oriented Synthesis:

Synthetic Engineering. A number of synthetic technologies underpin the foundations of DOS. But the real fact is foremost among these is Merrifield's development of solid-phase peptide synthesis in the early 1960s[10]. Which provides a rapid and convenient means to separate reagents and byproducts from solid support−bound reaction products, and that is simply by rinsing the solid supports with various solvents. This circumvents the need for tedious purifications of synthetic intermediates during multistep syntheses. In the concluding step of the synthesis, the final products are cleaved from the solid supports and subjected to an isolated purification as required. Solid-phase techniques have been given extension to the synthesis of non-biopolymer

small molecules, and those are basically, natural products and synthetic drugs. Moreover, several related strategies have been developed to facilitate the recovery and handling of synthetic intermediates[11]

The synthetic technology can be bifurcated as well; (a) Reaction substrates can be attached to solid supports, precipitation tags or fluorous tags to facilitate separation of excess reagents and reaction byproducts from the desired reaction products. (b) All of these separation platforms facilitate parallel synthesis, in which each individual library member is synthesized in a separate reaction vessel.

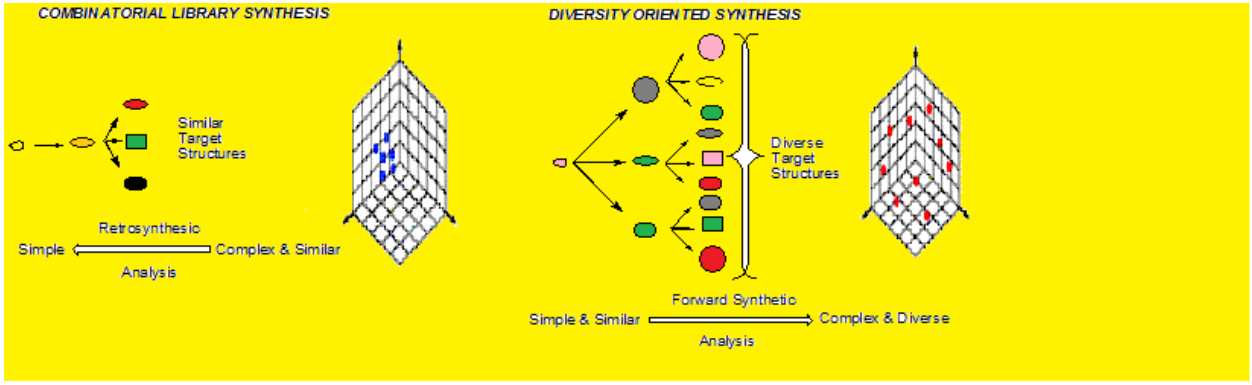
Solid-phase synthesis allows split-pool protocols to be used, in which support-bound synthetic intermediates are mixed and redistributed between each chemical transformation. As a result, very large libraries can be synthesized rapidly with each bead carrying only a single library member. However, recursive deconvolution or encoding strategies must be used to determine the identity of a given library member.

One more technology is combinatorial synthesis from where the idea of diversity oriented synthesis is generated, which involves involves systematic mixing and matching of various chemical building blocks to generate libraries of small molecules. In shortly, solid-phase synthesis allows convenient handling and distribution of synthetic intermediates to facilitate this combinatorialization process. This feature was leveraged by the Furka and Lam groups separately in the early 1990s to synthesize peptide libraries using a technique called split-pool synthesis[12, 13]. Subsequently, as with solid-phase synthesis, combinatorial chemistry has been extended to the synthesis of non-biopolymer small-molecule libraries.

But, solid-phase combinatorial synthesis also poses new challenges for organic chemists. Because the synthetic intermediates cannot be purified using standard chromatographic techniques, every reaction in the synthetic sequence must proceed at high efficiency, the final products be so impure as to make purification impossible. Further, each reaction must be compatible with hundreds or even thousands of different substrates generated by the preceding combinatorial steps. So, the same ideals that have driven reaction development in traditional organic synthesis—high yield; selectivity and generality—apply to DOS to a large extent.

When these conditions can be fulfilled, a key advantage of screening synthetic combinatorial libraries, as opposed to collections of individually archived compounds, becomes evident. Once a flexible synthetic route is in hand, a 'primary' library of diverse molecules can be screened to identify early 'hit' molecules and to provide information on structure-activity relationships (SAR). Using the same synthetic route, the initial hits can then be readily optimized through the synthesis and testing of 'secondary' or 'tuning' libraries and individual analogs to identify compounds with improved potency, specificity and pharmacological properties.

Moreover, this information can be used to assist in target identification and verification, which is often a particularly challenging problem when broad phenotype- or pathway-directed screens are used.[14, 15] In fact, reactive functional groups have recently been incorporated directly into 'tagged' DOS libraries for this purpose[16].



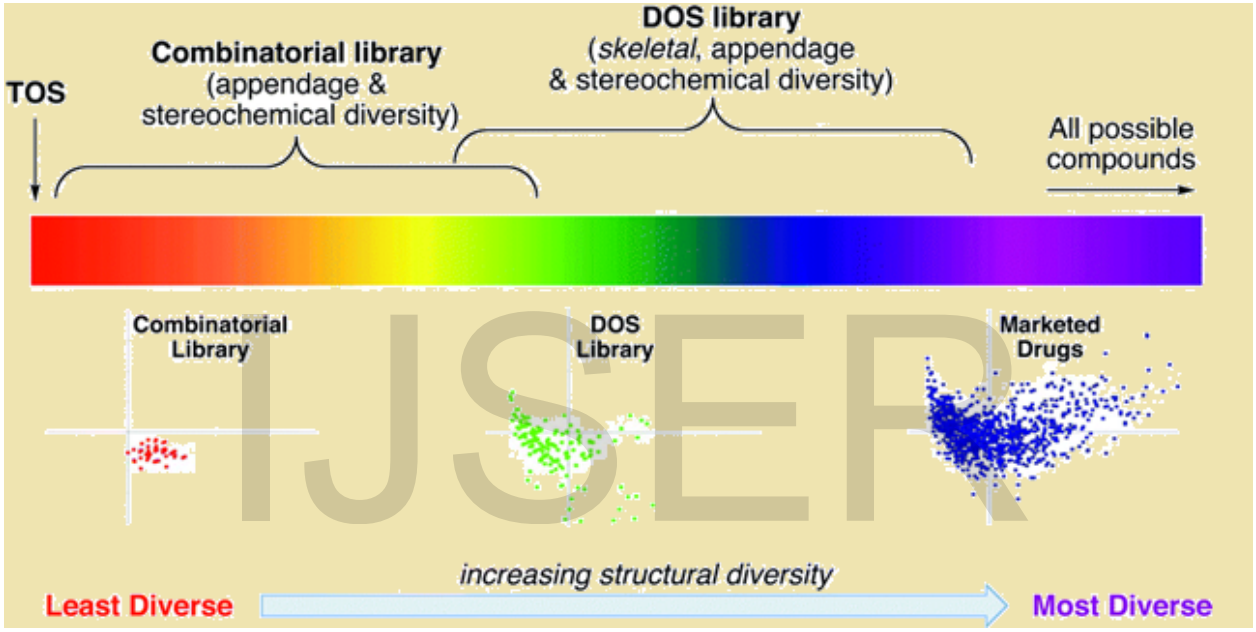


Fig-2: figure showing the differentiation of combinatorial and diversity oriented synthesis.

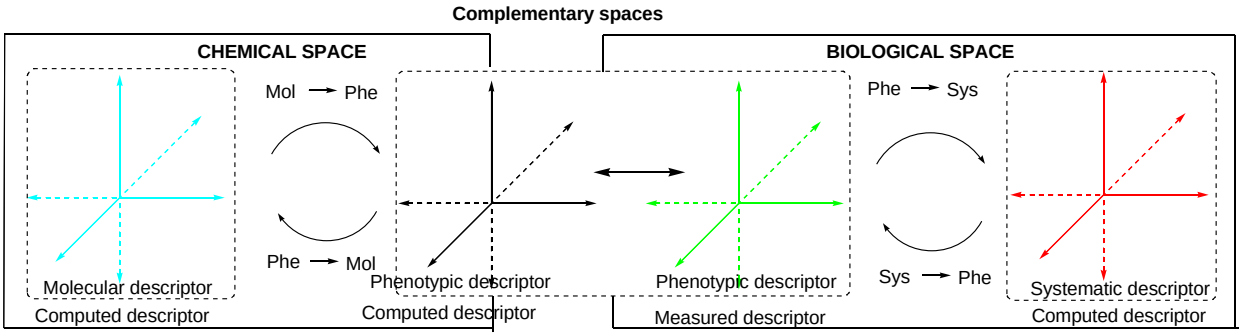
Alternative strategies. A group of related approaches have been developed that are complementary to the synthesis and screening of combinatorial libraries. Many of these can be grouped under the broad heading of fragment-based ligands discovery[17]. This involves identification of two or more low-molecular-weight 'fragments', which may bind to an individual protein target of interest. Effectively, the individual fragments can bind with very low affinities such as micromolar to millimolar or even less than it, but once they are covalently linked, either through mere laboratory synthesis or *in situ* target-directed coupling, ligands with high affinity such as , nanomolar) can be obtained. These fragment-based approaches have proven an effective means to identify new ligands, but the problem is they require selection of an individual biological target and are currently limited to screening methods biochemically.

In contrary to traditional 'wet' screening, *in silico* 'virtual' screening has also been used to identify new ligands and ligand fragments.[18, 19] Use of computational algorithms to 'dock' potential binders to an experimentally determined protein structure or to a homology model based on a similar protein is a modern trend now a day. The virtual hits are then purchased or synthesized and binding is confirmed in the same traditional wet experiments. This approach can be more cost-effective than wet screening and has successfully produced a number of new ligands. Although, it, too, requires selection of an individual biological target and it is also dependent on the availability of structural information on that target.

Use of Chemical Space and biological space in DOS:

A complete set of all possible small molecules has been variously calculated to contain 10^{30}–10^{200} structures, depending on the algorithms used and the upper limits placed on molecule size is assigned as chemical structure space.[20, 21] But the point is, is it really possible to synthesize all of the possible small molecules? Then the answer is definitely no. So what is the limit, which is moreover, even the largest screening campaigns are limited to aproximately10^6 compounds, a practically infinitesimal fraction of the total possibilities. Fortunately, however, only a small portion of that space can be expected to comprise molecules that are stable and soluble in aqueous media, which have appropriate functional groups to interact with biological targets such as proteins and nucleic acids, and have sufficient structural complexity[22] to do so with useful levels of specificity. This is even before one takes into account the additional structural constraints imposed when cell permeability or bioavailability in whole organisms are taken into account.

Now the mind first query about, DOS is how to design combinatorial libraries that target the biologically relevant regions of chemical structure space[9]. To answer this prospect, most DOS library design strategies, which gain information about existing biologically active small molecules to generate compounds that similarly target these regions. These can be based on synthetic drugs, molecules of the sort made by medicinal chemists, or on natural products, molecules derived mainly from microbes, plants or marine organisms. Although, despite the tremendous impact that natural products have historically had on drug discovery[23], there are substantial differences between the structures of synthetic drugs and natural products which cannot be neglected[8]. Thus, both classes natural as well synthetic are attractive complementary starting points for DOS library design.



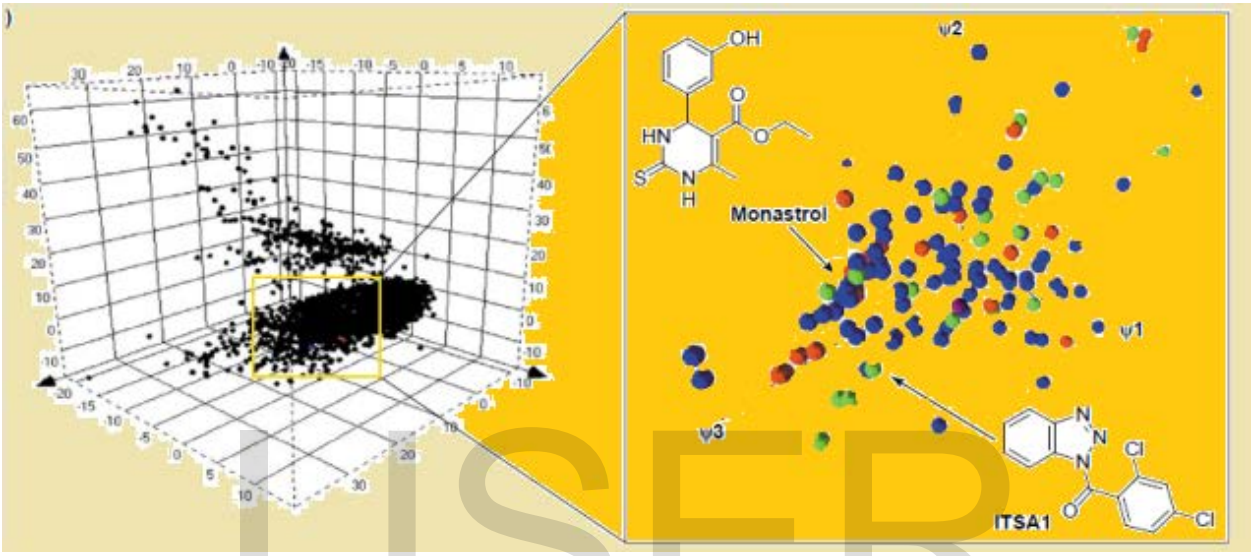


Fig-3: Overview of chemical and biological space. (a) Chemical and biological space are represented as complementary spaces composed of a total of four quadrants (I–IV) in which chemicals and biological systems, respectively, can be described quantitatively using multiple computed or measured descriptors. In quadrant I, chemicals are positioned in space using computed molecular descriptors. In quadrant II, chemicals are positioned in space using measured phenotypic descriptors of biological activity. In quadrant III, biological systems are positioned in space using measured descriptors of chemical activity. In quadrant IV, biological systems are positioned in space using computed descriptors based upon a computable model of the system. (b) 3-Dimensional representation of chemical space showing the position of 15 120 small molecules (colored balls) in a molecular descriptor space derived from the first three principal components (PC) axes (C1–C3) obtained from the analysis of the corresponding structural and physiochemical descriptors .

Libraries with reference to Privileged structures:

Synthetic drugs are often based on heteroaromatic scaffolds basically Nitrogen, that have appropriate size and hydrogen-bonding capacity to bind in the active site pockets of biological targets, which may be of enzymes and G protein−coupled receptors. They tend to have few or no stereogenic centers, which greatly simplifies their synthesis and quite conspicuous, why they are

chosen. Some of these scaffolds have been identified as 'privileged' structures in that they have an empirically demonstrated ability to bind multiple classes of protein targets.[24, 25] We can have an classical example of benzodiazepine scaffold used in antidepressants. Although the underlying basis for this privileged standing is usually not well understood, it has been suggested that conservation of protein folds may contribute.[25, 26]

This privileged structure can be better illustrated as drug scaffolds often serve as the basis for DOS of 'drug-like' libraries.[27]. Furthermore, because synthetic drugs are most useful when orally bioavailable, numerous studies have aimed at identifying physicochemical properties that correlate with this characteristic.[28-30] These properties can then be used to guide the selection of appropriate building blocks to be coupled to the scaffold. It is magically noted that, to date, many of the commercially available drug-like libraries fail to recapitulate these physicochemical parameters[8]. Hence, there remains a significant need for the development of drug-like libraries that more closely match the properties of known synthetic drugs and can make the dream fruitful.

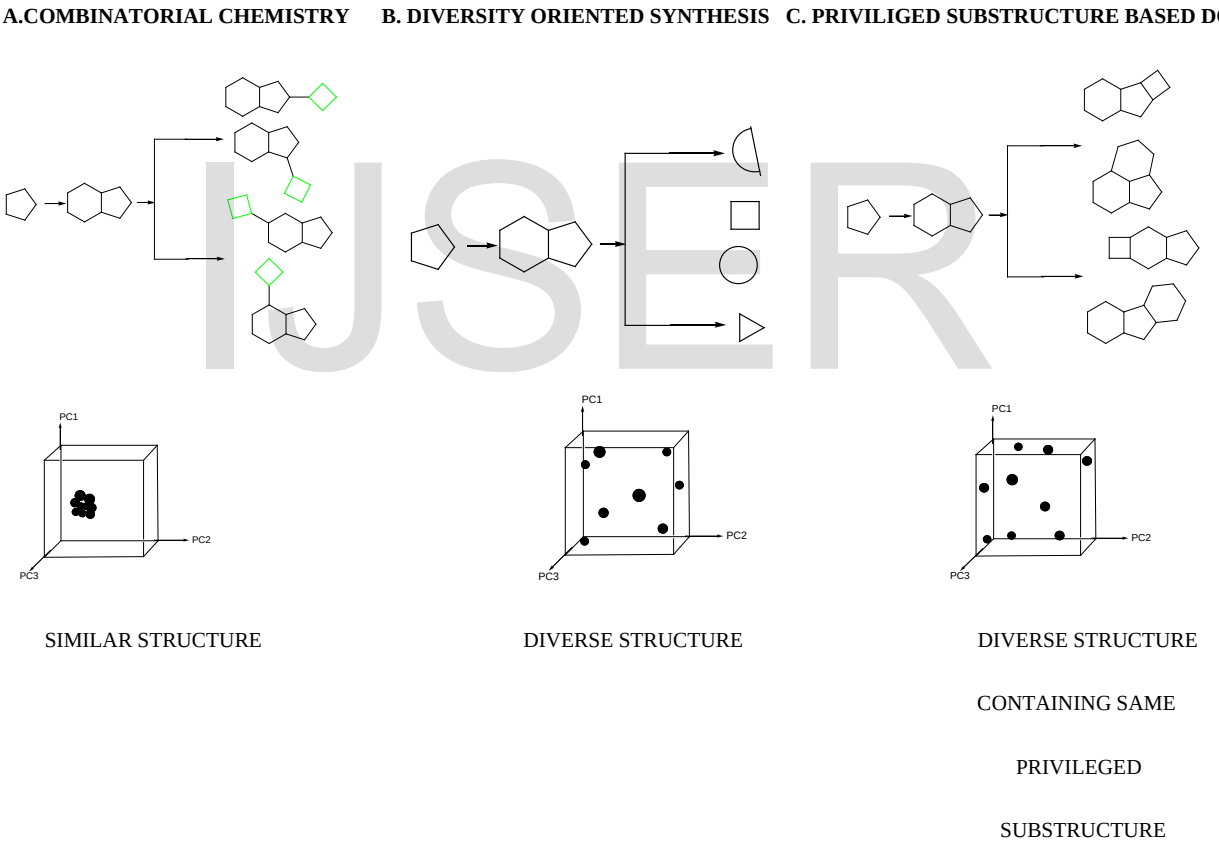


Fig-4: A schematic illustration that compares the synthetic strategies in (A) traditional combinatorial chemistry for the construction of a focused library, (B) a DOS pathway driven by pure complexity-generating reactions, and (C) the pDOS pathway.

Libraries with reference to Natural products:

Nature has given us tremendous products which show much greater structural diversity and complexity than synthetic drugs that is why more research is facing toward natural moieties. They often contain a greater proportion of oxygen than nitrogen heteroatom and a significant number of stereogenic centers[8]. But clinically used natural products are sometimes not orally bioavailable, they provide a valuable complement to synthetic drugs with respect to the spectrum of biological targets they address.[27] For example, rather than acting as ligands that bind in a protein pocket, glycopeptides antibiotics e.g., vancomycin act as receptors for the C-terminal D-Ala-D-Ala motif of bacterial peptidoglycan precursors[31]. Moreover, protein-protein interactions, which have historically been very difficult targets for synthetic drugs, [32, 33] can often be modulated with natural products, which is a real advantage of picking natural products.[34]

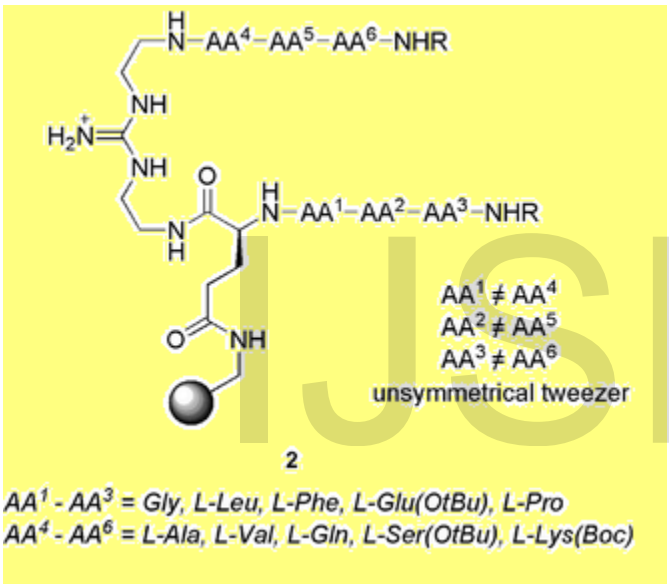


Fig-4: Vancomycin as a receptor for the C-terminal D-Ala-D-Ala motif of bacterial peptidoglycan Two-armed tweezer receptor library with a guanidinium head group as a carboxylate binding site for the binding of D-Ala-D-Ala–OH in aqueous solution. The arms are synthesized sequentially to give a structurally more diverse library of unsymmetrical receptors.

'Natural product−like' libraries are a major area of current interest especially, DOS of natural products. Library design strategies have been diversified into three broad categories, according to the degree of similarity with natural products proper, [35, 36] Firstly libraries based on the core scaffold of an individual natural product, secondly libraries based on specific structural motifs that are found across a class of natural products and finally libraries that emulate the structural characteristics of natural products in a more general sense. Each strategy balances the degree of connection to natural-product structure space against the accessibility of structural diversity that is likely required to address multiple different biological targets. Actually, some structures

originally identified in natural products have subsequently been identified as privileged structures and used in synthetic drugs now a day very frequently examples include purines, indoles and benzopyrans, etc.[25].

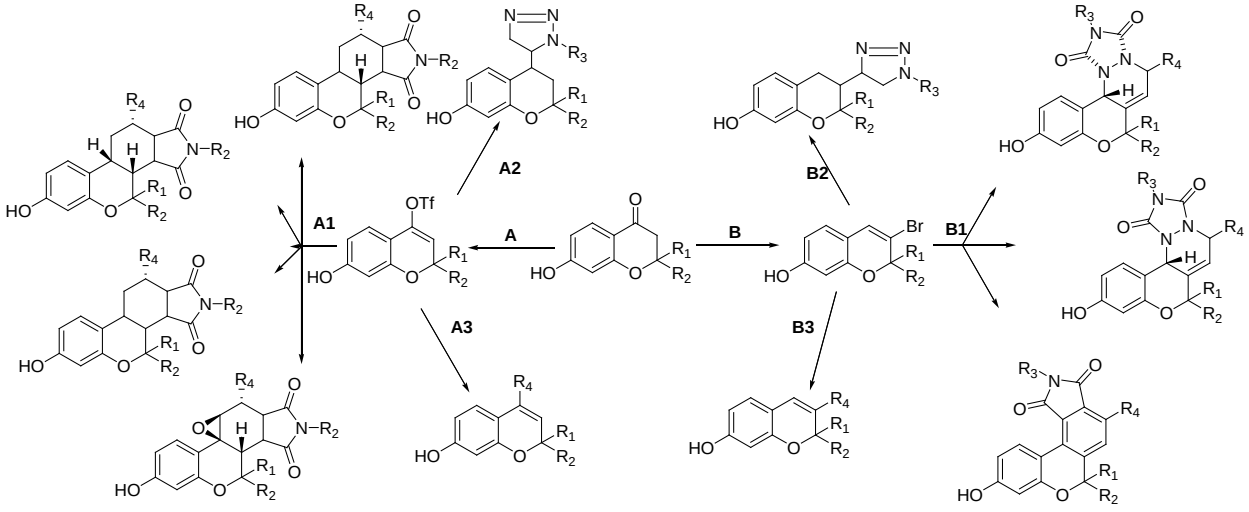


Fig: Diversity of synthesis of Benzopyran

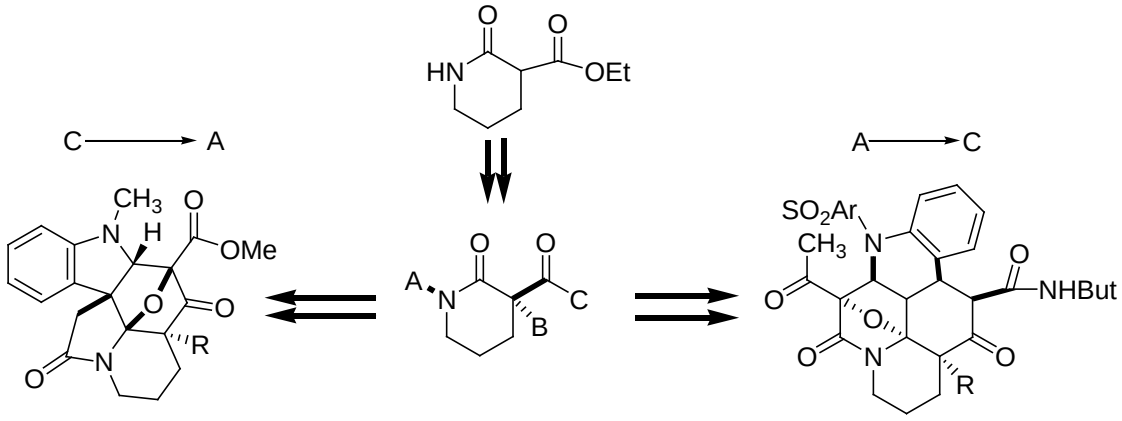


Fig: Skeletal diversity of naturally occurring indole alkaloids and the rich potential of chemistry developed by Padwa and coworkers, They conceived a pathway entailing six modes of intramolecular reactions leading to indole alkaloid-like skeletons. In this context, an efficient folding pathway via a rhodium-catalyzed tandem cyclization−cycloaddition involving three of the modes has been developed stereocontrolled manner.

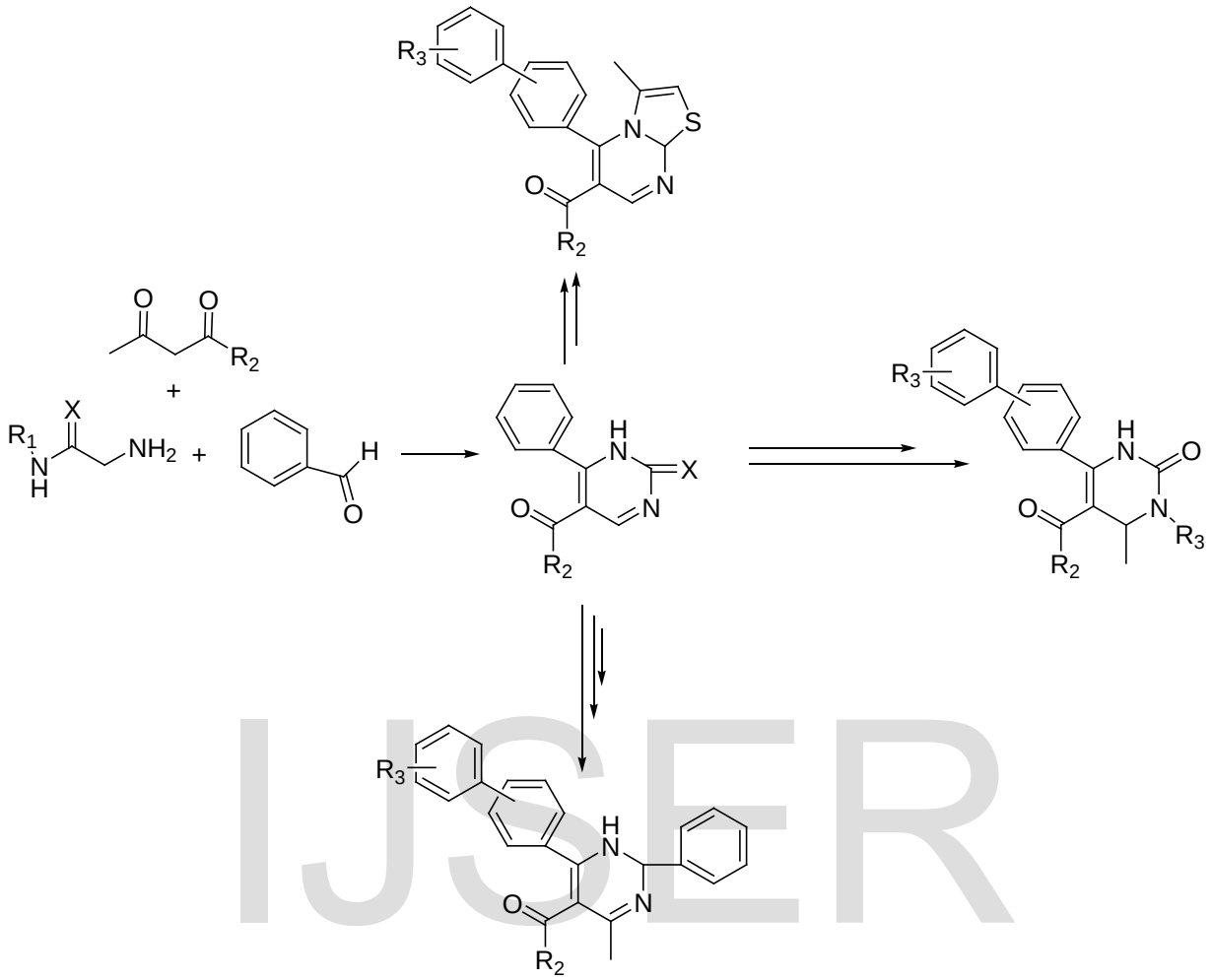
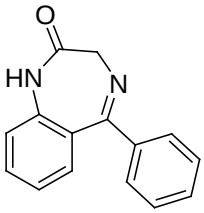
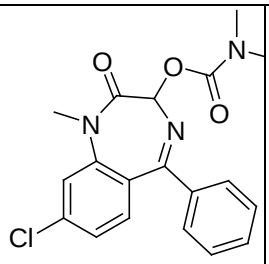
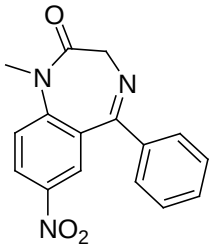
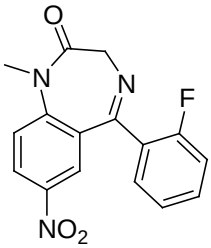


Fig: An illustration showing Dos of purines.

Sl.No.	Privileged scaffold	Structure		
1. Structure.				
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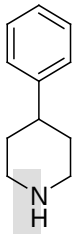
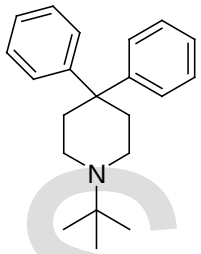
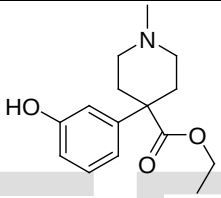
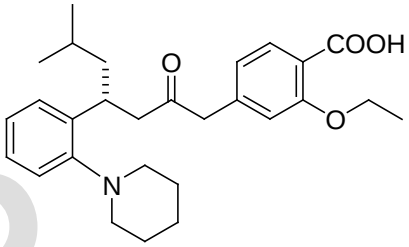
2. Structure.				
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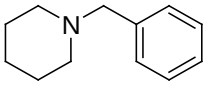
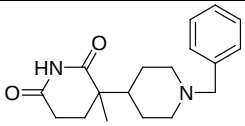
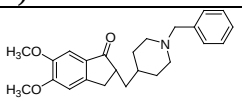
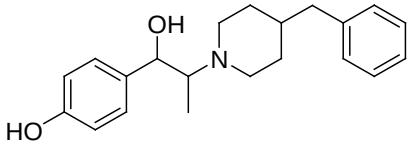
3. Structure.				
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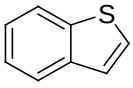
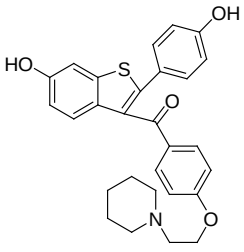
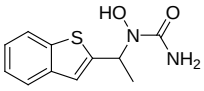
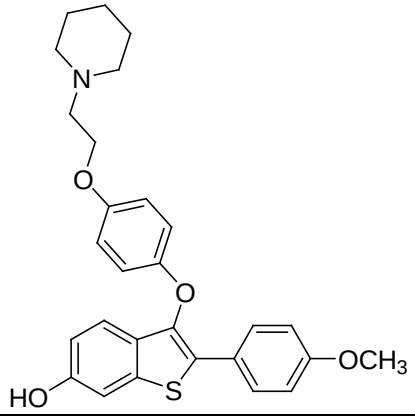
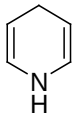
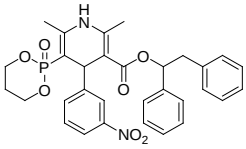
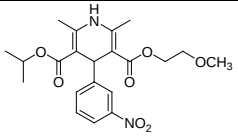
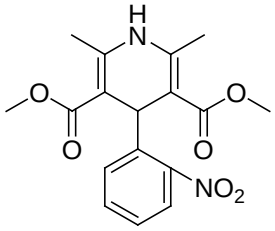
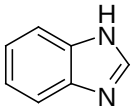
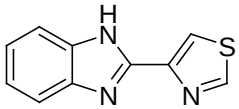
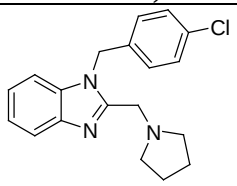
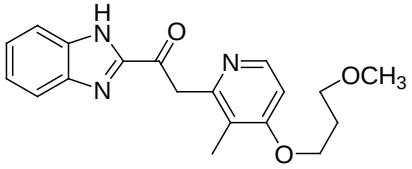
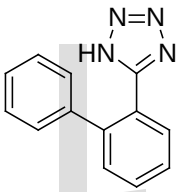
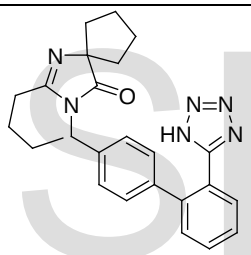
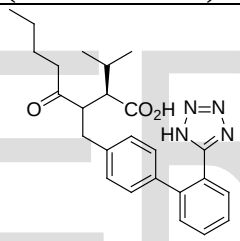
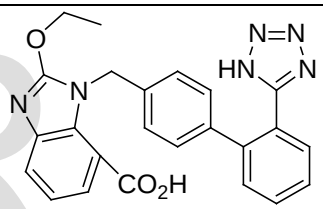
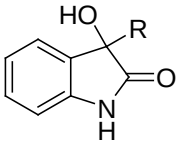
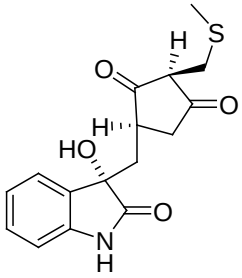
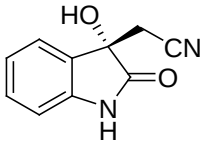
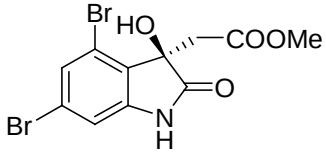
4. Structure.				
4. Name	Benzothiophene	Raloxephine	Zileuton	Arzoxifene (Antineoplastic)

(Therapeutic category)		(Antiosteoporotic)	(Antiasthmatic)	
5. Structure.				
5. Name (Therapeutic category)	Dhydropyridines	Efonidipine (Antihypertensive)	Nimodipine (Cerebral Vasolidator)	Nifedipine (Antianginal, Antihypertensive)
6. Structure.				
6. Name (Therapeutic category)	Benzimidazole	Thiobendazole (Antihelminthic)	Clebizole (Antihistaminic)	Rabeprazole (Antiulcerative)
7. Structure.				
7. Name (Therapeutic category)	Biphenyltetrazole	Irbesartan(Antihypertensive)	Valsartan (Antihypertensive)	Candesrtan (Antihypertensive in Congestive heart failure)
8. Structure.				
8. Name (Therapeutic category)	3-substituted-3hydroxy-2-oxindole	3-(R)-maremycinB Terestrial streptomycin (Cytotoxic)	(R)-(+)-cyanomethyl-3-hydroxindole(Plays in the role of Cytokines)	(R)-ConvolutamidinA (Inhibits promylocytic lekemic cell HL-62)

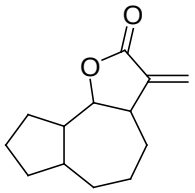
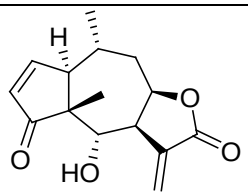
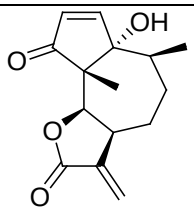
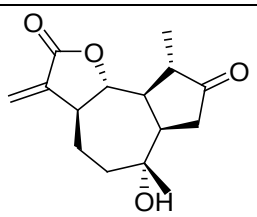
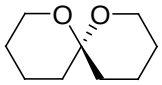
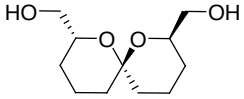
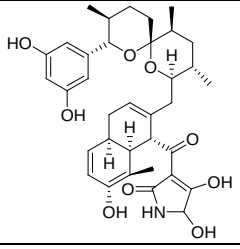
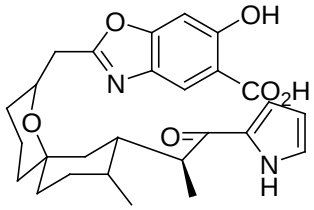
9. Structure.				
9. Name (Therapeutic category)	5-7-5 Lactone ring system	Helinalin (Antiinflammatory inhibits the transcription factor NF-kB)	Parthenin (Anti leishmanial and trypanosomal activity)	ChinensiolideB
10. Structure.				
10. Name (Therapeutic category)	6,6-spirpacetal	Spiket-P(Antimiotic)	Integramycin (HIV-1 integrase inhibitor)	Routiennocin (in vitro activity against gram positive and anaerobic bacteria)

Fig-: Example of some privileged scaffold found primarily in drugs naturally

Multiple biological target library diversity:

Actually DOS libraries are not directed toward a single biological target, thus, their utility is based on their ability to provide selective probes for multiple different biological targets. This 'functional diversity' can only be accessed through biological screening. 'Structural diversity' is often used as an intermediate metric, because it is more readily accessible and likely to correlate, at least to some extent, with functional diversity[37]. In both cases, a key tool for analyzing diversity (and similarity) is a statistical method called principal component analysis (PCA), by which different targets can be analyzed for diversity.[38]

PCA, or principal component analysis, which has a set of *n* descriptors, is defined for each compound in the library. These can be structural descriptors, such as molecular weight; physicochemical descriptors, such as experimentally determined artificial membrane permeability; or biological descriptors, such as binding constants. Each compound can then be represented as a vector in *n*-dimensional space. Of course, for $n > 3$, such vectors are difficult to visualize. Thus, PCA is used to analyze the entire data set and to define new unitless axes, called principal components or eigenvectors. Each new axis is a linear combination of the original descriptors, calculated to represent as much of the variance in the dataset as possible in each successive principal component, based on correlations between the original descriptors. The new axes are orthogonal and uncorrelated. Each compound is replotted as a vector in readily

visualized one-, two- or three-dimensional space using its coordinates, or eigenvalues, on these new axes.

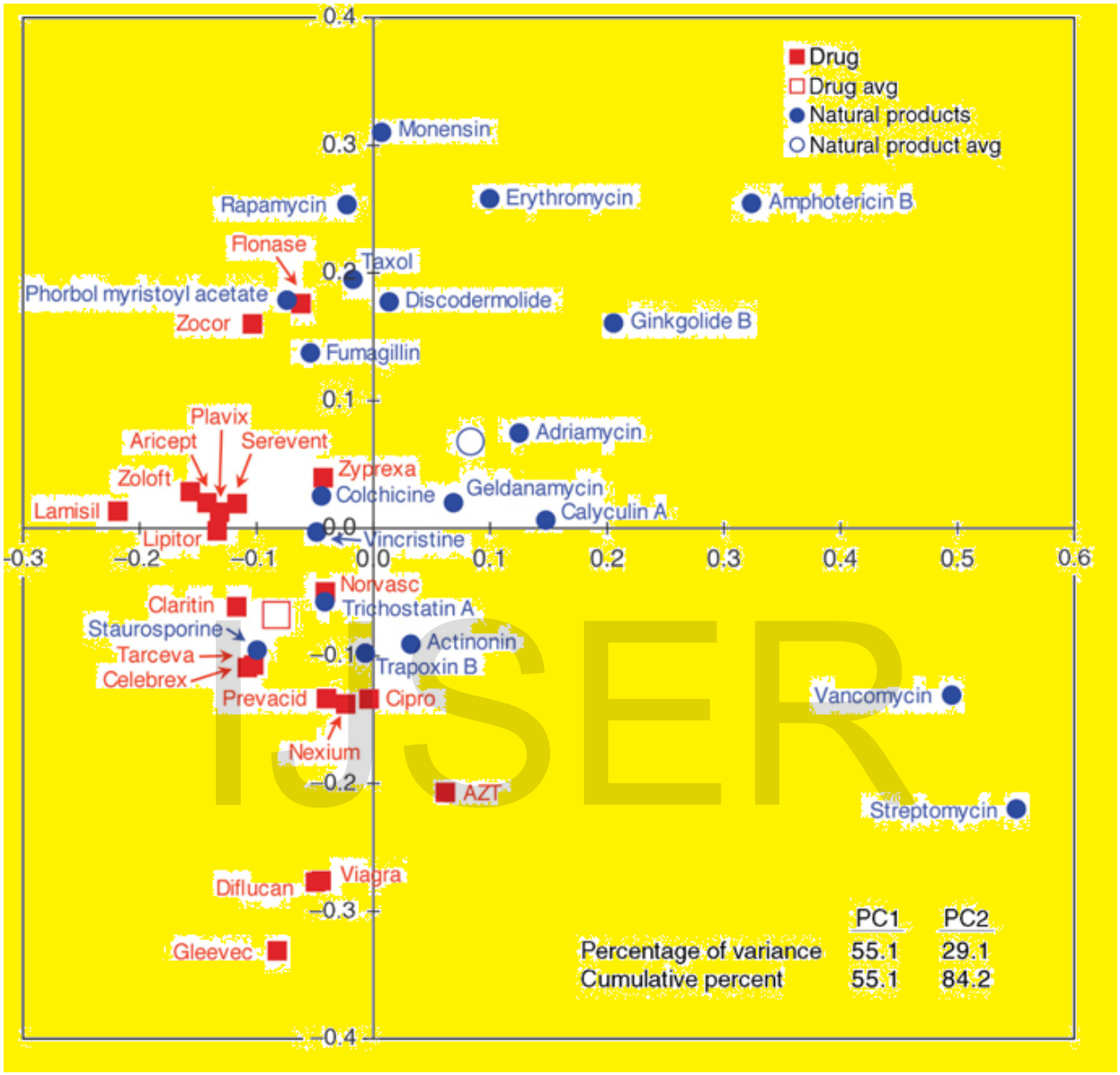


Fig: An illustration depicting the principal component analysis of natural drugs as well as other drugs.

Molecular descriptors were obtained from PubChem (http://pubchem.ncbi.nlm.nih.gov/) and ChemBank (http://chembank.broad.harvard.edu/) or calculated using ChemDraw/Biobyte and Molinspiration (http://www.molinspiration.com). PCA can be performed with R version 1.01 (http://cran.r-project.org/).

Diversity-oriented synthesis, a real challenge to chemistry:

DOS plays a real challenge for synthetic organic chemists all these days. However synthetic techniques such as solid-phase synthesis facilitate the separation of synthetic intermediates from excess reagents and soluble reaction byproducts, they do not allow separation of support-bound impurities that may arise from undesired side reactions, which is the greatest lacuna of this technique. With traditional chromatographic purification of synthetic intermediates precluded, extraordinarily high requirements are placed on reaction efficiency and selectivity. But interestingly general, DOS routes require reactions that provide greater than 90% yield and stereo-selectivity, less the synthetic sequence produce such a complex mixture as to make purification of the final product impossible. As a point of view, DOS has been an emerging engine for new advances in synthetic organic chemistry as well in medicinal chemistry.[35].

Stereoselective reactions in DOS project:

Various stereoselective reactions have been developed in the subject of DOS projects. These reactions may be found broader applications in numerous areas of synthesis chemistry. e.g., Wipf and coworkers have developed a transition metal−mediated cascade reaction that yields dicyclopropylmethylamines[39] (Scheme-1). These products can be converted stereoselectively into a variety of azaspirocyclic products[40].

Itami, Yoshida and coworkers have developed stereoselective routes to tetrasubstituted olefins in which each of the substituents can be introduced independently through cross-coupling reactions[41,42] (Scheme-2). Application to these compounds is a real big challenge in organic synthesis. The resulting products are analogs of the antiestrogen drug tamoxifen and may also have interesting electronic properties in synthetic world in chemistry.

Some interesting stereoselective reactions developed for diversity-oriented synthesis. (**a**) A cascade reaction produces dicyclopropylmethylamines, converted to spirocyclic amines.(Scheme-1a) (**b**) Palladium-catalyzed cross-coupling reactions allow stereoselective synthesis of tetrasubstituted olefins.(Sceme-1b) (**c**) A three-component coupling reaction proceeds through nitrone formation and homo-[3+2]-cycloaddition to produce densely functionalized tetrahydro-1, 2-oxazines.(Sceme-1c) (**d**) A three-component coupling reaction involving initial aza-[4+2]-cycloaddition to a bicyclic product followed by aldehyde allylboration affords bicyclic products with high diastereoselectivity. (Scheme-1d

)

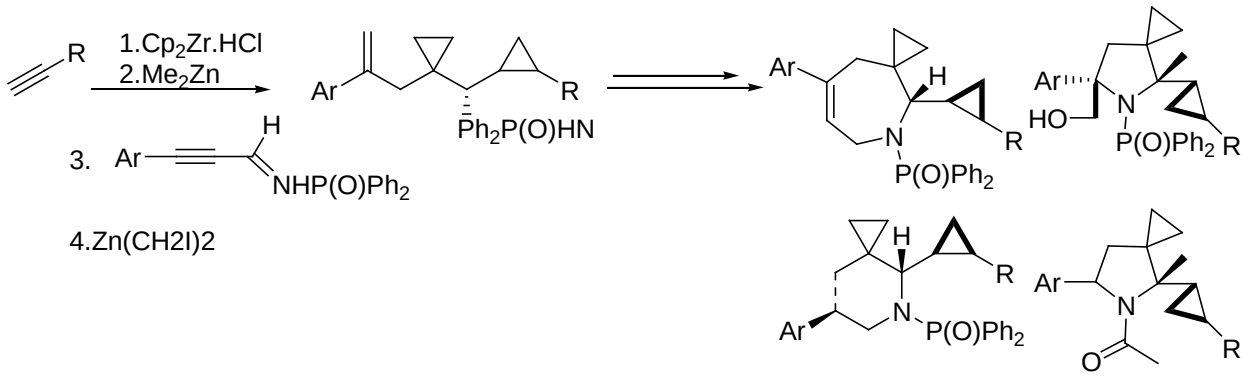
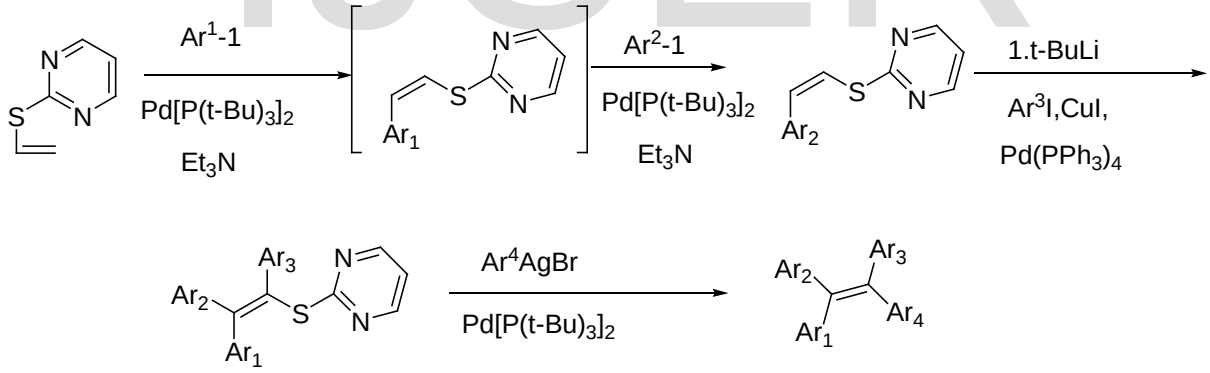
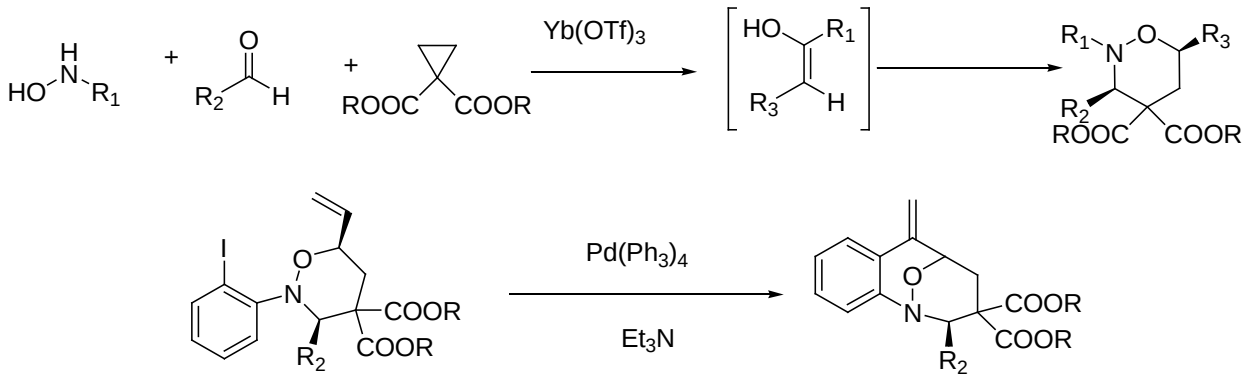


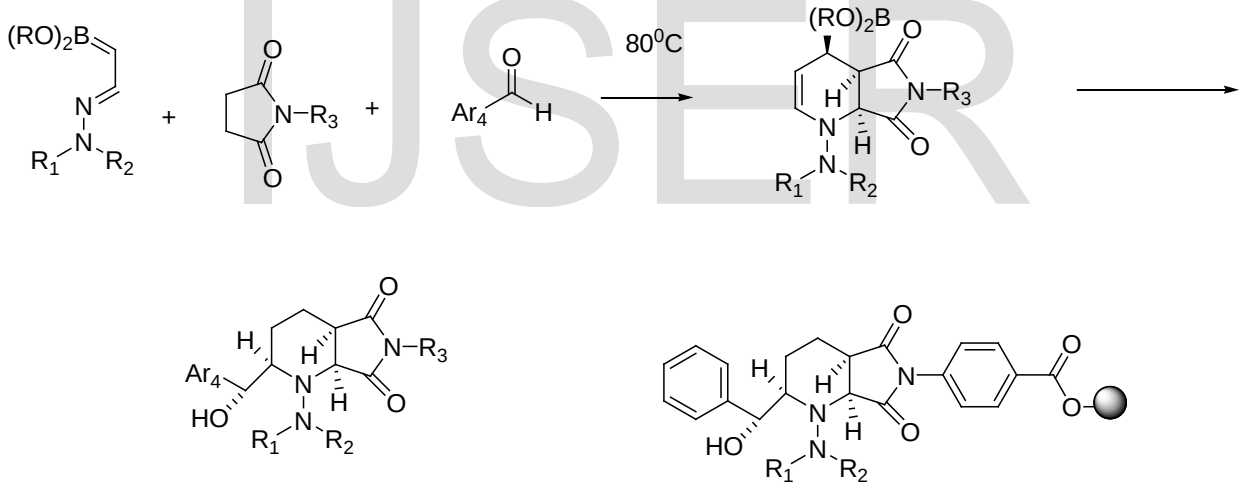
Fig: Scheme-1a



Scheme-1b



Scheme-1c



Scheme-1d

In Multicomponent Reactions:

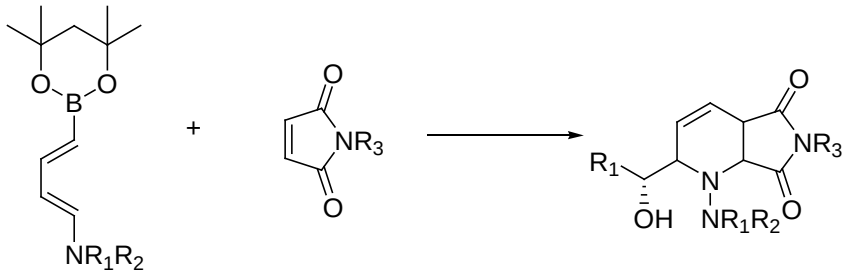
In multicomponent reactions, DOS has also spurred a resurgence of great interest, in which three or more building blocks are coupled in a single reaction.[43]

Young and Kerr developed a homo-[3+2] cycloaddition reaction of nitrones and cyclopropanes that provides densely functionalized tetrahydro-1,2-oxazines[44] (Scheme-2). The reaction can be

carried out as a multicomponent reaction in which the nitrone is formed *in situ* from a hydroxylamine and aldehyde.[45] The diastereoselective process has been used to generate a variety of products, a natural product with antitumor and antibiotic activity.

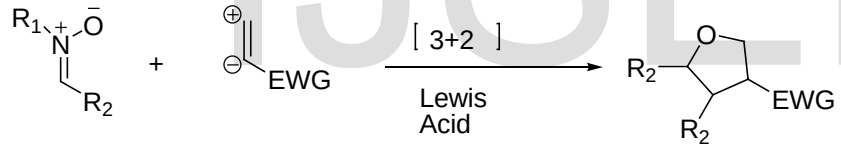
Hall and coworkers have developed a tandem aza-[4+2] cycloaddition and allylboration reaction that produces densely functionalized bicyclic products[46,47] (Scheme-3) . Piperidine core is a privileged drug scaffold under chiral auxiliary and the reaction can be performed diastereoselectively and also on solid support.

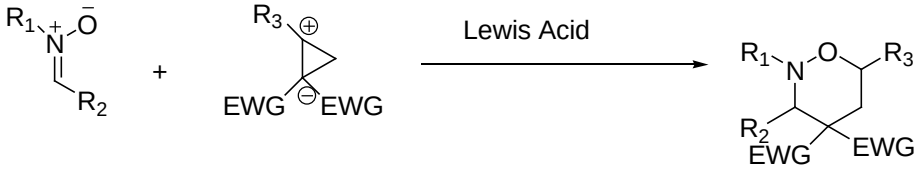
Scheme-2



Scheme-3

Fig Hall and





EWG- Electron Withdrawing Group

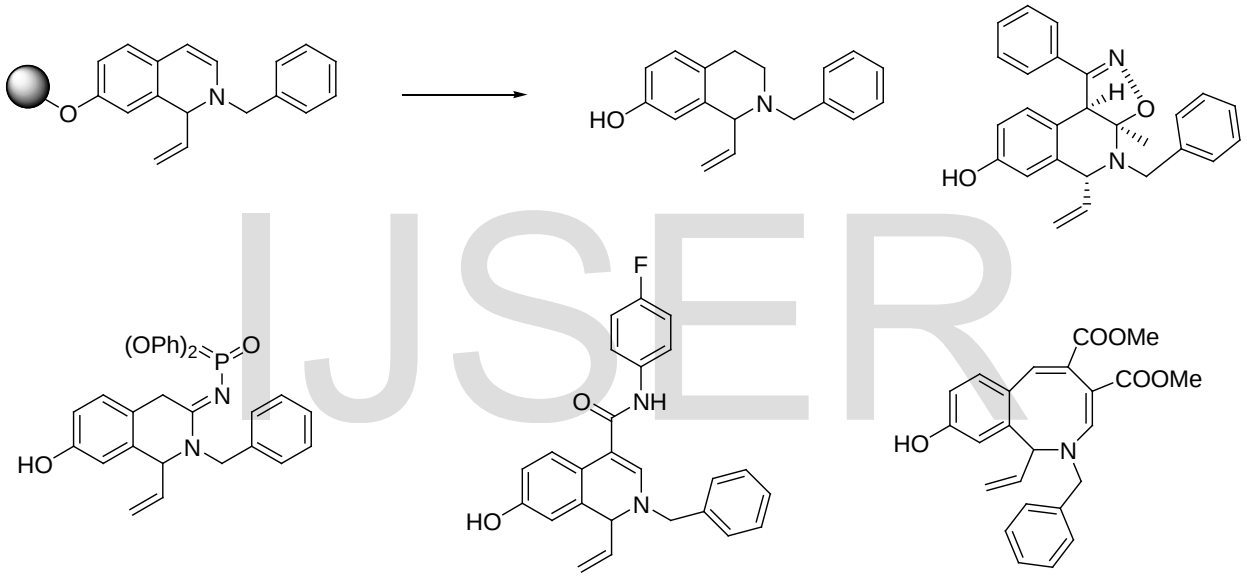
Scheme-2

Strategies towards a new synthetic world:

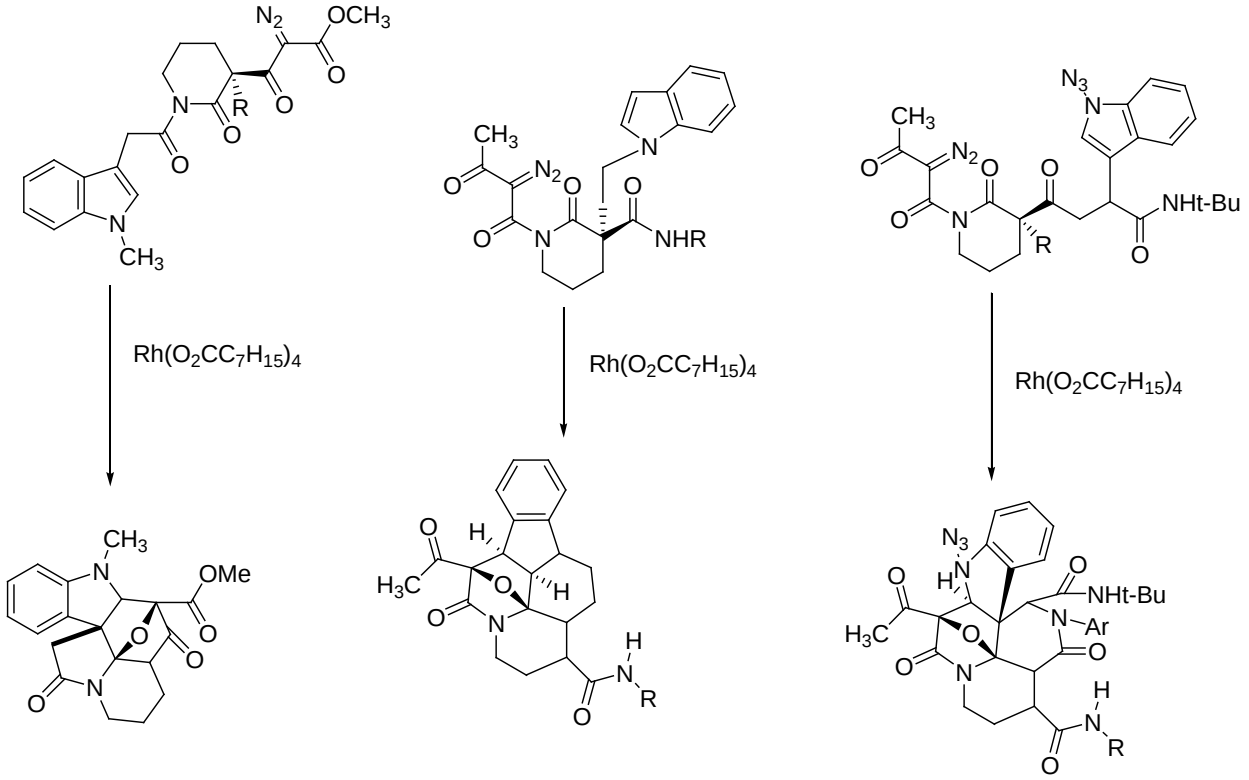
Synthetic planning strategies in DOS had developed in completely new way, which is quite interesting.[48]. Synthetic routes that provide complex, diverse structures are most desirable concisely. The availability of building blocks, either commercially or by synthesis, is an

important consideration. As a complement to building block−based diversity, the use of controlled reactions stereoselectively to generate stereochemical diversity can significantly enhance three-dimensional structural diversity.[49-51] Finally, synthetic routes that provide multiple core scaffold or backbone structures within a single library are of particular current interest. Two approaches to this problem can be visualized. In one, substrates bearing a common functional group are exposed to different reaction conditions that generate different products, and the other is, substrates bearing different functional groups can be exposed to a single set of reaction conditions that leads to different products depending on the 'programming' provided by the functional groups.

Schreiber and coworkers have exposed a reactive enamine intermediate to various reaction conditions to produce multiple alkaloid scaffolds[52] (Scheme-4). They have also used β-lactams functionalized at different positions with iodole and α-diazoketone substituents to program carbonyl ylide cycloaddition reactions to give rise distinct hexacyclic alkaloid scaffolds[53](Sceme-5).



Scheme-4



Scheme-5

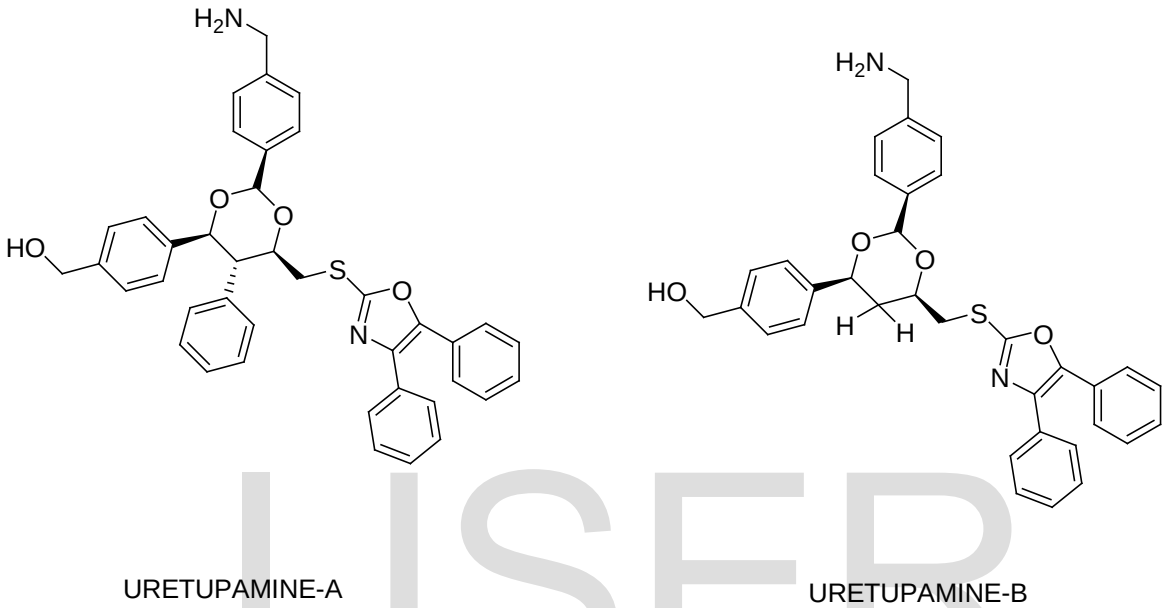
Both drug like as well as natural probes evolved biologically from DOS libraries:

DOS library screening has evolved really vital biological probes[1]. Some examples are presented below, with a particular focus on studies that have provided new biological insights. These probes have been identified from both drug-like and natural product−like libraries, using a variety of screening techniques[7] ranging from cell-free protein binding, enzyme-linked immunosorbent assays (ELISA) and fluorescence resonance energy transfer (FRET) assays to cell-based reporter gene, cytoblot[54] and phenotypic assays which are produced biologically.

Uretupamines, Ure2p and glucose signaling:

Function-selective suppressors of the yeast signaling protein Ure2p, known as uretupamines that were discovered from a DOS library.[55] Ure2p regulates cellular responses to the quality of both carbon and nitrogen nutrients (for example, glucose versus acetate and ammonium versus proline). Ure2p represses the transcription factors Nil1p and Gln3p, and differential regulation is thought to distinguish carbon- and nitrogen-nutrient-responsive signaling. Thus, these two effects cannot be separated using Ure2p knockouts (*ure2*Δ), whereas a function-selective small-

molecule inhibitor would be ideally suited to this task. Though the functional binding sites of Ure2p have not been identified, structure-based rational design is quite difficult to identify such an inhibitor. Thus, Schreiber and coworkers screened a DOS library of 1,890 natural product−like compounds[56] in an Ure2p binding assay on a small-molecule microarray[57]. The initial hits were retested in a secondary cell-based reporter gene assay, leading to the identification of uretupamine A as a functional Ure2p suppressor. SAR analysis and facile access to analogs using the established synthetic route allowed rapid development of a more potent analog, uretupamine B.



URETUPAMINE-A URETUPAMINE-B

Figure: Uretupamines, function-selective suppressors of the yeast signaling protein Ure2p.

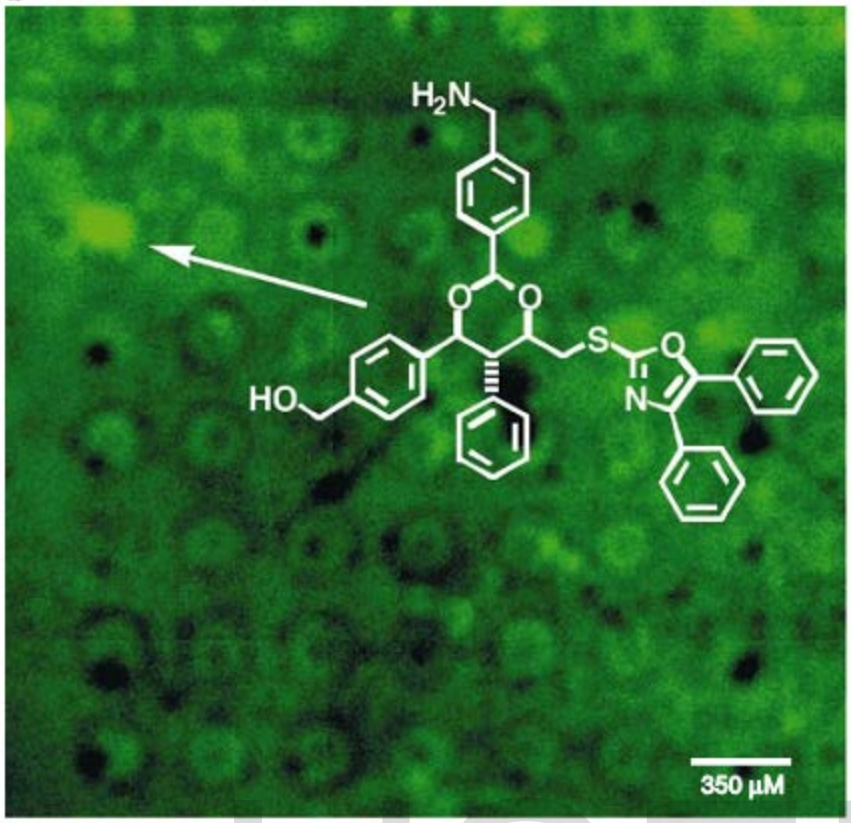


Figure: An expanded view of 64 compound spots on the 3,780-member small-molecule microarray (, 800 spots per centimeter square). Cy5-labelled Ure2p was passed over a microarray of the 1, 3-dioxane small-molecule library, and the resulting slide was washed three times and scanned forfluorescence. The spot corresponding to uretupamine A is shown.

(**a**) By the help of High Throughput Screening uretupamine A was discovered of a library of natural product−like compounds. Analysis of SAR led to the development of an improved analog, uretupamine B. **(b)** Microarray of library members for small molecules, was probed with Cy5-labeled Ure2p. The resulting fluorescent spot corresponding to Ure2p-bound uretupamine A (**c**) Transcriptional profiling of wild-type (PM38) and *ure2*Δ yeast treated with uretupamine A versus vehicle control (*N*,*N*-dimethylformamide).

Ure2p in the role of Glucose Signaling:

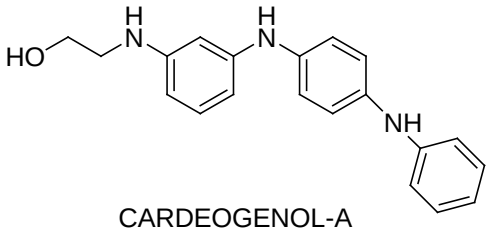
From the screening of transcriptional profiling data it was revealed that the uretupamines upregulated a subset of genes that are induced in response to carbon nutrient quality, including Nil1p. However Ure2p is usually considered a nitrogen-nutrient-responsive signaling protein, this gave an account that it might also be a direct target of carbon-nutrient-responsive pathways in opposing to pathways bypassing Ure2p and acting directly on Nil1p. Some other evidence for this model was provided by transcriptional profiling experiments with the uretupamines in *nil1*Δ and *gln3*Δ strains. It was also found to be selectively dephosphorylated in response to changes in carbon, but not nitrogen, nutrient quality. Hence, these studies with a function-selective small-molecule probe from a DOS library illuminated on the role of Ure2p in glucose signaling, which was a greatest gift in glucose signaling as well.

Modulators for Stem cell differentiation:

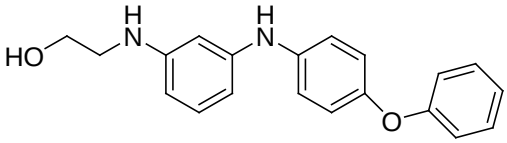
Some molecules that modulate stem cell differentiation have been identified recently from DOS libraries[58]. The ability to control stem cell fate has major potential therapeutic implications in areas such as cancer, neurodegenerative disease and tissue regeneration. Differentiations (or dedifferentiation) are valuable tools for studying these processes in the small molecules and the underlying signaling pathways that regulate them. Schultz and coworkers have identified several such molecules by screening a DOS library of 45,140 drug-like molecules built around multiple kinase-targeted heterocyclic scaffolds[59]. Cell-based phenotypic assays have been useful for identifying molecules that may act by previously unknown mechanisms to elucidate new signaling pathways that control differentiation.

Several molecules have been identified that induce differentiation of pluripotent mouse embryonic stem cells to particular tissue-specific adult stem cells. Adult stem cells have exciting therapeutic potential, but have gene-rally been difficult to obtain by direct isolation and expansion. High Throughput Screening was accomplished using pluripotent mouse carcinoma cell lines transfected with reporter genes driven by lineage-specific markers. SAR analysis and the ease of secondary tuning library synthesis again proved useful for optimizing the initial hits. Differentiation-inducing activity was further confirmed by immunostaining for additional neuronal or cardiac muscle markers in the carcinoma cell line as well as mouse embryonic stem cell lines.

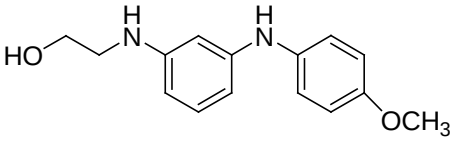
A series of compounds, the cardiogenols, induce cardiomyogenesis (EC_{50} = 0.1−1.0 μM)[60]. Affinity chromatography experiments identified GSK-3β (glycogen synthase kinase-3β) as a target of TWS119 (K_d = 126 nM, IC_{50} = 30 nM), supporting a role for this protein in neuronal differentiation.



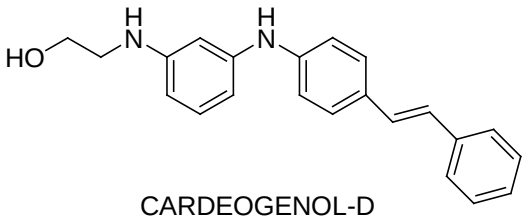
CARDEOGENOL-A



CARDEOGENOL-B

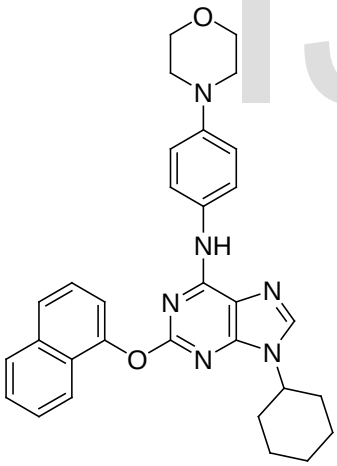


CADEOGENOL-C



CARDEOGENOL-D

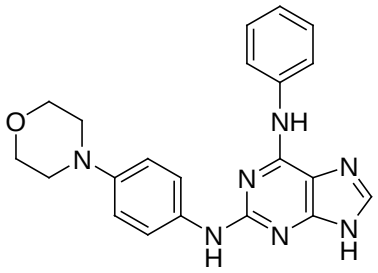
A molecule, named as purmorphamine, was chosen and screened for molecules that induce differentiation of multipotent mouse mesenchymal stem cells into osteoblasts (EC_{50} = 1 μM)[61]. High Throughput Screening was accomplished using a fluorescence-based enzymatic assay for the bone-specific marker alkaline phosphatase. With reference to its osteogenic activity, purmorphamine also upregulated Cbfa1 (or Runx2), a master regulator of bone development, and other bone-specific markers. Subsequent transcriptional profiling experiments revealed that purmorphamine follows the Hedgehog signaling pathway.[58]



PURMORPHAMINE

Reversine a molecule which, has been identified as a compound that induces dedifferentiation of mouse myoblasts to multipotent mesenchymal progenitor cells (complete at 5 μM)[62]. High Throughput Screening was accomplished using a two-stage assay involving initial treatment of myoblasts with the compound to induce dedifferentiation, followed by exchange into osteogenesis or generation of completely new bone cells-inducing medium and assaying for alkaline phosphatase expression as above to detect osteoblast formation. The dedifferentiating capacity of reversine was further confirmed by dedifferentiation of myoblasts followed by

redifferentiation to adipocytes, or fat cells and by the inability of reversine to induce direct transdifferentiation of myoblasts to osteoblasts.



REVERSINE

DOS as valuable tool in generating Protein-protein interaction antagonists:

Inhibitors of protein-protein and protein-DNA interactions, from DOS libraries, which are small molecules, have been identified.[63] Previously, these have been impossible targets to address with synthetic drug-like molecules, owing in part to the large, flat, discontinuous binding surfaces often involved and to the lack of endogenous small-molecule ligands to use as starting points for rational design[32].To resolve this important problem, Boger and coworkers have synthesized a variety of natural product−like libraries that are based loosely on peptides or other oligomeric natural products. The point is, efficient solution-phase syntheses and mixture deconvolution protocols were also developed to synthesize and screen all these DOS libraries.[64] This approach has yielded a lucrative collection of molecules that inhibit both extracellular and intracellular protein-protein interactions, as well as protein-DNA interactions[63].

A series of isoindoline-based compounds were identified by Vogt, Boger and coworkers as inhibitors of the protein-protein interaction between the Myc and Max transcription factors.[65] Myc is aberrantly activated in a number of human cancers and acts by heterodimerization with Max via their helix-loop-helix leucine zipper domains, Myc target genes by the help of transcription.

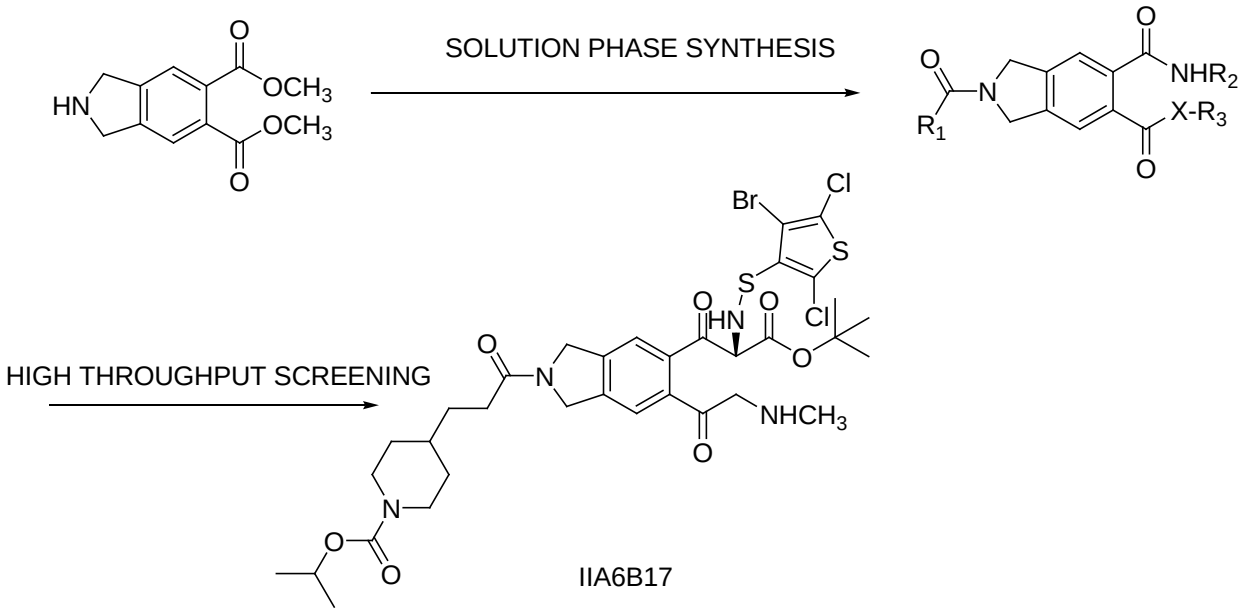


Fig: Series of isoindoline-based compounds were identified by Vogt, Boger and coworkers

A finger pointing towards Future:

In the future, the ideas that underpin DOS, such as maximal chemical space coverage and efficient complex structure generation, will remain. However, we may also see DOS being used in a more focused way, directed toward the synthesis of novel or unusual chemical structures and architectures, and used more in the field of fragment-based drug discovery. Whatever directionsDOStakes in the future, it is likely that solid-phase synthesis will remain integral to the field.

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interested explorers may be in the recent years, those who are really interested in diversity oriented synthesis.

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