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RESEARCH ARTICLE

Diversity oriented synthesis for novel anti-malarials

Chandramohan Bathula[1] · Shailja Singh[2] · Subhabrata Sen[1]

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Abstract Malaria a global pandemic has engulfed nearly 0.63 million people globally. It is high time that a cure for malaria is required to stop its ever increasing menace. Our commentary discusses the advent and contribution of diversity oriented synthesis (DOS) in the drug discovery efforts towards developing cure for malaria. DOS based on chemical genetics focusses on design and synthesis of molecular libraries which covers large tracts of biologically relevant chemical space. Herein we will discuss the applications, advantages, disadvantages and future directions of DOS with respect to malaria.

Keywords Diversity oriented synthesis · Phenotypic screening · Michael addition · Chemical genetics

Introduction

Malaria as we all know is a mosquito borne infectious disease from the species *Plasmodium* causing a pandemic worldwide (World Health Organization 2013). In 2010, the atrocities caused by malaria affected 216 million people causing death to ~0.65 million people (majority in Africa) (Murray et al. 1980). About half the world's population is at a risk, where it is more prevalent in underdeveloped countries. Hence the challenge (viz. technical, economic and financial) to eradicate is almost insurmountable. Vaccination (the most potent remedy) is not yet available and drug resistant parasites have rendered a conservative treatment failure (Ballou et al. 1999).

The modulation of protein functions is the basis of medicinal chemistry and chemical genetics and such modulators can be obtained by screening collections of small molecules (O'Connor et al. 2011; Lehar et al. 2008). Due to these reasons small molecules have always been the centre of attraction in the discovery of malaria drug. However it is worth mentioning that like any other drugs discovery programs malaria drug discovery's decade-spanning successes is gradually dwindling. The reason being reduced sensitivity to artemisinin therapy, in accordance with the emergence of parasite resistance to all partner drugs, and also due to its inherent paucity in further identifying appropriate small molecules for therapeutic purposes. Hence newer novel strategies are required to improve this process which then can generate effective antimalarial small molecules. In this respect one of the key areas targeted by the scientific community are library designing techniques. Diversity oriented synthesis (DOS), biology oriented synthesis (BIOS), scaffold hopping etc. have been applied as a part of proof of concept studies. Some of them have been successful to some extent with promises of further improvement and some of them failed. Herein we discuss the effectiveness of DOS in the library design of antimalarial small molecules.

Since early 90 s the libraries of compounds were carefully evaluated and it became evident that it was not the library size but the library diversity and its complexity in terms of molecular structure and function that was crucial (Kennedy et al. 2008). All these years the flat molecules modulated effectively the so called simple biological

✉ Subhabrata Sen
subhabrata.sen@snu.edu.in

[1] Department of Chemistry, School of Natural Science, Shiv Nadar University, Village Chithera, Tehsil Dadri, District Gautam Buddha Nagar 201314, UP, India

[2] Department of Life Science, School of Natural Science, Shiv Nadar University, Village Chithera, Tehsil Dadri, District Gautam Buddha Nagar 201314, UP, India

responses, which can be compared to plucking low lying easily accessible fruits from a tree, compared to the ones which are higher up. As the biological targets get complicated the effectiveness of these sp^2 predominant flat compounds reduces mainly due to poor binding affinity to the targets. Additionally the structure of any small molecule dictates its biological activity hence the functional diversity of any small molecule collection is expected to be directly proportional to its overall structural diversity. Therefore structural diversity plays an important role in discovering the quality molecules that can generate favorable biological response for complex targets. As now it is quite clear that compounds with simple structures will not modulate complex biological responses. To address this problem DOS is an efficient tool which has been utilized to generate structurally diverse libraries with exciting biological properties (Haggarty 2005; Rottmann et al. 2010). It is a forward synthetic approach where single intermediate containing orthogonal groups can be transformed into compounds with various architectures that can interrogate broader areas of chemical space with the potency to also access unchartered territories of the chemical space. Traditional combinatorial chemistry is typically dependent on diversifying a common template. Libraries from DOS are architecturally more varied. However the boundary between them is fuzzy, as many of the techniques from combinatorial chemistry are also applied in DOS. We can consider DOS as a more matured manifestation of combinatorial chemistry which creates molecules that imbricate wider area in chemical space (Fig. 1).

Molecules' functional diversity, linked to structural diversity evolved from the surface interactions between small molecules and biomacromolecules. The structural diversity is the essence of a DOS library. The four principle categories of structural diversity are: (1) appendage (building block) diversity, (2) functional group diversity, (3) stereochemical diversity, and (4) skeletal diversity. The first and second of these can be imbibed combinatorially by several structural moieties or functional groups around a common scaffold. Stereochemical diversity is incorporated by use of stereo controlled reactions. Introducing skeletal diversity is the most critical aspect of DOS. A DOS library is complete when the molecules are diverse in stereochemistry and architectural framework, rich in sp^3 carbons and finally the most important can modulate different cellular functions. At the end of the day, it is not the architectural complexity that determines the success of a library but to what extent it is biologically relevant.

In the post-genomic era with the discovery of several novel biological targets the field of epigenetics became very promising. However the key challenge remained the paucity of limited number of proteins with known specific ligands. A recent study by Overington et al. intimates that the current portfolio of small molecule drugs address only 207 protein targets encoded in the human genome. DOS can potentially solve the challenge of creating libraries of structurally disparate, highly potent bioactive small molecules that could dramatically expedite critical biochemical discoveries. Consequently a large number of diseases especially like malaria where substantial drug resistance has been developed, can be managed, and finally be eradicated .

Such DOS directed efforts towards the discovery of antimalarial molecules will be discussed in this commentary (Scheme 1).

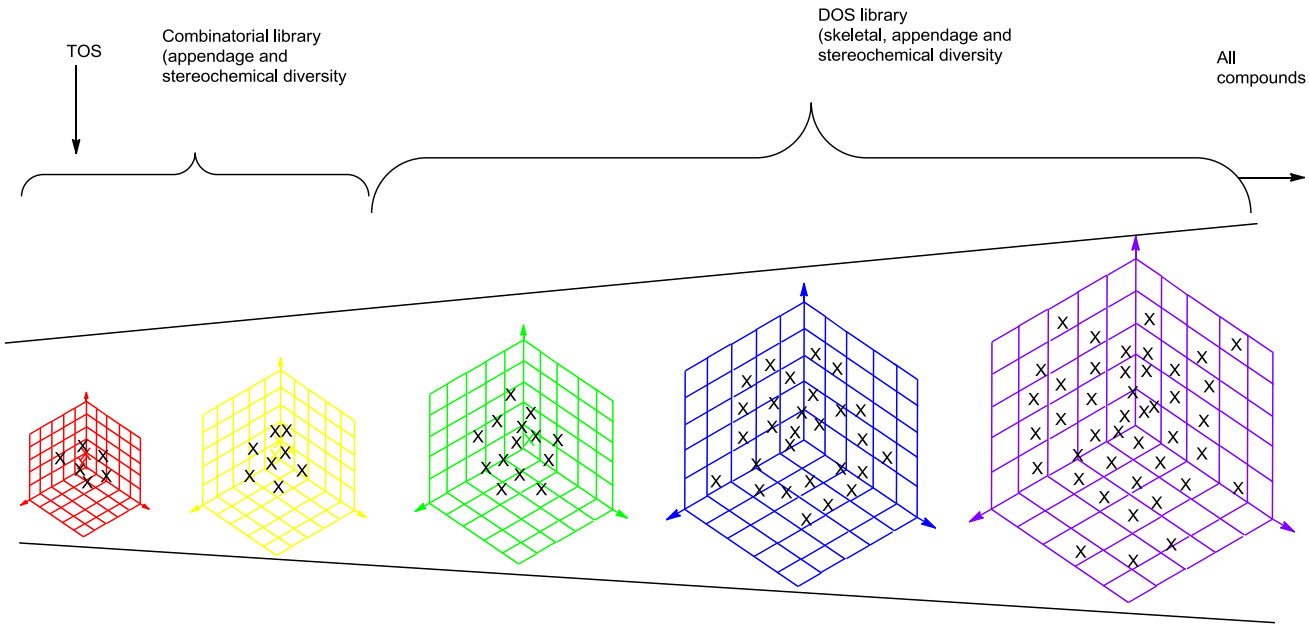
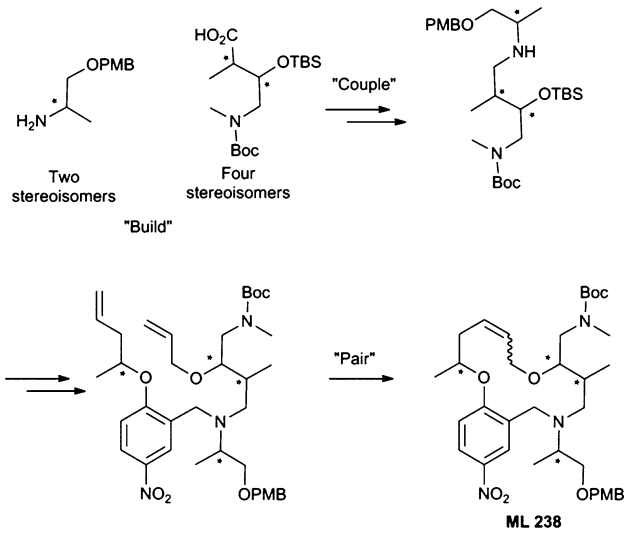
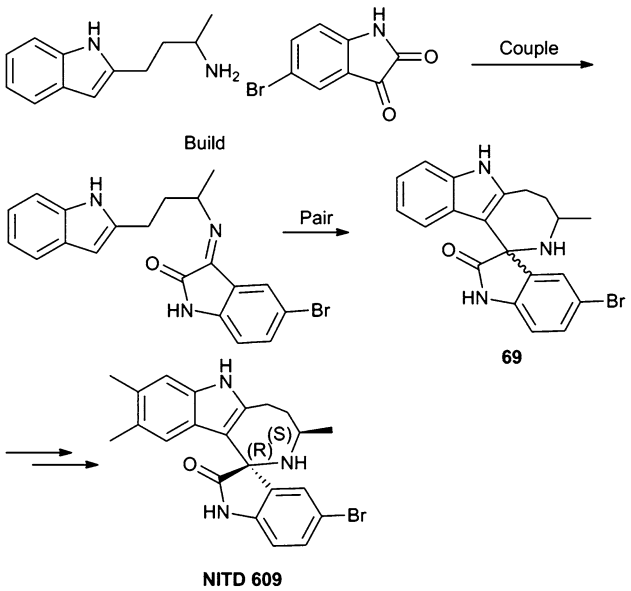


Fig. 1 From target oriented synthesis to diversity oriented synthesis



Scheme 1 Synthesis of **ML238**

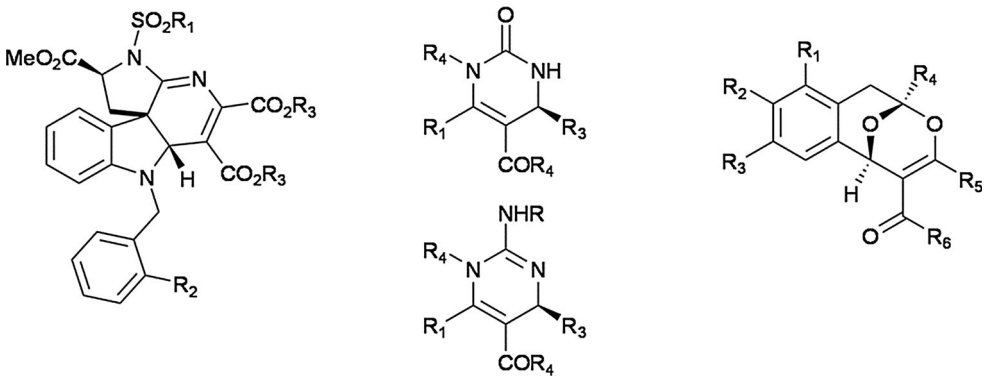


Scheme 2 Synthesis of **NITD609**

To begin with we discuss here few of the case studies involving DOS towards the discovery of a novel antimalarial agent. In 2012, Schreiber et al. discovered an antimalarial macrocyclic lactam **ML238** using diversity oriented synthesis and phenotypic screening of *Plasmodium falciparum* asexual blood-stage parasites (Heidebrecht et al. 2012). A list of 8000 molecules were screened against multidrug-resistant Dd2 *P. falciparum* and depending on the activity in growth inhibition assay was trimmed to a library of 20 compounds, which were synthesized via Build (B)/Couple (C)/Pair (P) approach as depicted in the scheme above. A B/C/P approach involves building (B) blocks via asymmetric syntheses followed by coupling them via intermolecular coupling reactions that join the building blocks and provides the basis for stereochemical diversity finally pairing involves intramolecular coupling reactions that join pairwise combinations of functional groups incorporated during the 'build' phase this process provides the basis for skeletal diversity. Finally the desired malaria inhibitor **ML238** was identified which exhibiteda subnanomolar potency (L/D = 0.54 nM) and good 2010 by a consortium aqueous solubility. This is one of the most effective applications of DOS in tandem with phenotypic screening. In a similar endeavor, **NITD 609** was discovered in led by Novartis Institute of Tropical Disease (NITD). Rottmann et al. (2010) screening a compound collection of 10,000 synthetic compounds and 2000 natural products in a phenotypic growth inhibition assay against the blood-stage parasite of malaria led to the generation of 275 compounds with submicromolar activity. A toxicological screening followed by physical and pharmacokinetic property analysis generated a lead compound **1** for further development. **1**, its regiomers and diastereomers were synthesized under a build/couple/pair approach of DOS. And after a rigorous screening and streamline phase, **NITD 609** was discovered with low nanomolar potency with no cross resistance against several drug-resistant strains (Scheme 2).

A collaborative effort by the Center for Chemical Methodology and Library Development at Boston University (CMLD-BU) and the NIH Chemical Genomics Center (NCGC) led to identifying several lead antimalarial candidates. CMLD-BU aims at harnessing newer organic strategies to develop small molecule libraries for high throughput screening (HTS). The compound collection in CMLD-BU represents structural diversity rich in sp^3 content and stereogenic centers. Additionally chemical shape space analysis (plotting principle moment of inertia and defining the architectural framework of compounds, thereby categorizing them as rods, spheres and discs) are also used to classify the library molecules in 2-D. NCGC focusses on translating the information from human genome into therapeutics by developing biological screening assays and high throughput screens. To identify novel antimalarials, NCGC applied SYBR green I dye DNA staining assay to evaluate the proliferation of *P. falciparum* in human erythrocytes. The compounds were screened against five demographically disparate parasites viz. CP250, Dd2, HB3, 7G8 and GB4. The initial screening involved a proprietary qHTS (quantitative high throughput screening) technique of NCGC. This complementary effort generated three potent antimalarial scaffolds. An inverse demand Diels–Alder based indoline scaffold, an asymmetric Mannich reaction based dihydropyrimidones and guanidines and finally a polycyclic ketal scaffold through multidimensional reaction screening. All these scaffolds

Fig. 2 Diverse scaffolds active against malaria



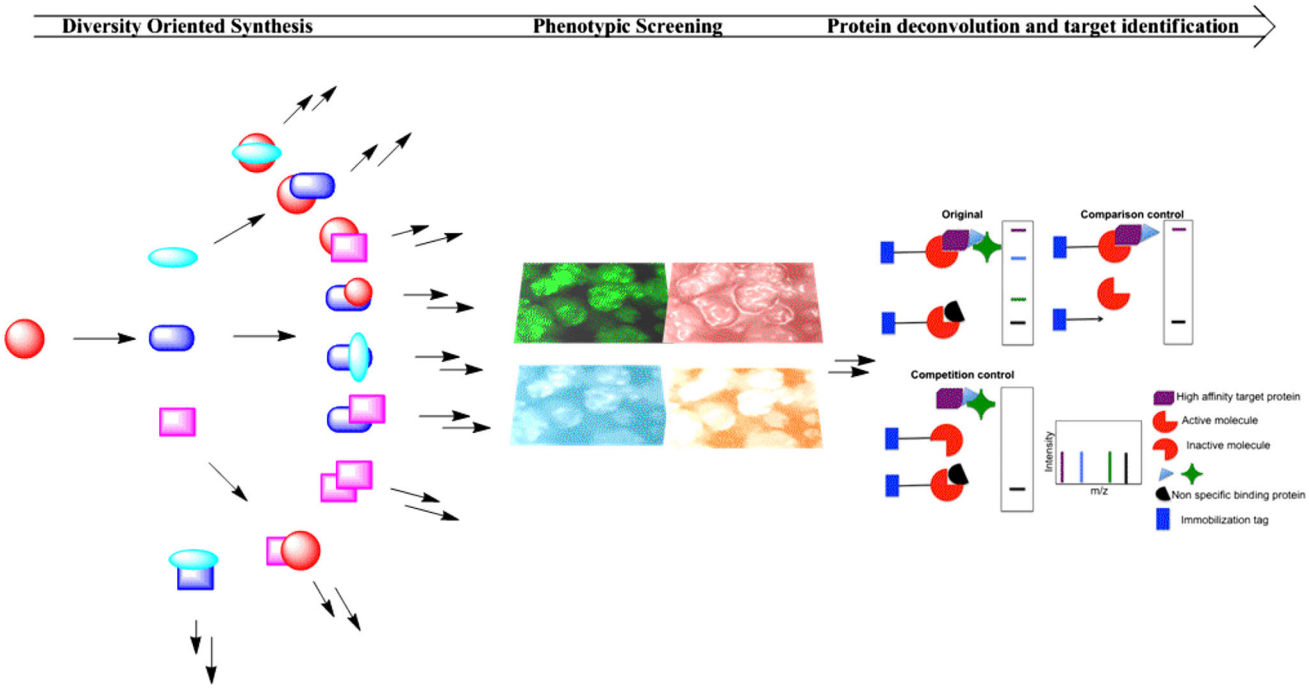


Fig 3 A comprehensive technique involving DOS-Phenotypic screening and protein deconvolution for target identification

exhibit inhibitory activity in the range of 0.02–46 μM among all the parasites, thereby categorizing them as highly potent to moderate leads as antimalarial compounds (Fig. 2).

From phenotypic screening, effective antimalarial compounds with sub Nano molar IC_{50} can be chosen for identification of target protein by protein de-convolutions technology Fig. 3. Whole genome sequence of the human malaria parasite *P. falciparum* and and four other human malaria strains of same species are available (http://plasmodb.org). From the total gene sequence and proteins, non-human homologs genes and their encoding protein can categorize and used for target screening. After getting a target protein, further validation can be done using the active site prediction tools, under the optimized conditions. One can design a set of other derivatives and by docking with different derivatized ligand the best fit can be chosen which might be the better compound that binds to the active site of target protein and inhibits their activities. Therefore, combination of DOS synthesis, phenotypic screening and target identification and in silico analysis provides rapid and potential approach for identification of drug target and designing of drug against malaria.. Furthermore, these examples of DOS indicate opportunities to alter and improve the process of malaria drug discovery to generate cure via augmenting the existing methods. It is still in its nascent stage and requires substantial improvement to reach to the level where they can be used for the good of mankind.

These studies are successful proof of concepts and can be taken forward to the next step. Mankind has already been cornered in his battle with malaria. Conventional

techniques have failed, hence out of the box strategies like DOS are our hope to turn the tides in our favor.

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