Send Orders for Reprints to reprints@benthamscience.ae

Anti-Cancer Agents in Medicinal Chemistry, 2015, *15*, 933-946

Anti-cancer Glycosidase Inhibitors from Natural Products: A Computational and Molecular Modelling Perspective

Ashona Singh[a], Ndumiso Mhlongo[a] and Mahmoud E.S. Soliman[a,b,*]

[a]*School of Health Sciences, University of KwaZulu-Natal, Westville, Durban 4001, South Africa;* [b]*Department of Pharmaceutical Organic Chemistry, Faculty of Pharmacy, Zagazig University, Zagazig 44519, Egypt*

M.E.S. Soliman

Abstract: The implementation of computational tools in pharmaceutics has proven an effectual strategy in creating harmony between the physical and chemical aspects of proteins and potential inhibitors. This is achieved by bringing to life the three dimensional retrospect of biological systems, which takes into consideration computational approaches such as quantum mechanics and molecular dynamics to facilitate drug design and discovery. In this work, we aim to provide a summary of the computational aspects of naturally derived anti-cancer inhibitors targeting the enzyme family of glycosidases. Our study offers insight into the evolution of drug discovery, molecular modelling and molecular binding modes of natural product inhibitors associated with glycosidase enzymes.

Keywords: Anti-cancer inhibitors, glycosidase, molecular modelling, natural product.

INTRODUCTION

Natural Products in Drug Discovery

Since the beginning of time man has exploited the use of plant and animal based products for their medicinal application. Ancient Eastern traditional healers have documented the healing properties of roots, bulbs, flowers and other herbs like ginseng, for at least 4,500 years [1]. European healers dating back to the 10th century used foxglove (*Digitalis purpurea* L.), which was found to contain the active ingredient digitoxin which is effective in the management of congestive heart failure, along with its many structure based analogues [2]. The pharmacological use of natural products was, however, only established in Western culture by the 19th century the most famous being the synthesis of the anti-inflammatory, aspirin, derived from salicin, isolated from the bark of the willow tree (*Salix alba* L.). By 1803 the alkaloid morphine was isolated from opium poppy (*Papaver somniferum* L.) which had been used by the Sumerians and Ancient Greeks, and dubbed by the Arabs as being addictive. Later during the 1870's crude morphine treated with acetic anhydride yielded heroin, which was found to readily convert to codeine, a painkiller [2, 3]. The track record of nature lending itself as a curative, preventative or symptomatic treatment has been our only saving grace in eradicating infection and alleviating disease and other syndromes. The current status of pharmaceutics worldwide indicates that approximately 40% of all dispensable medicines are either natural or semi-synthetic analogues of natural derivatives [4]. We are only at the precipice in drug discovery in terms of natural products and have yet to uncover and explore hidden secrets in different species genome using genome sequencing or single molecule real-time methods. Such discovery would allow new introspection in our management of disease, how some organisms remain immune to certain infections, as well as it would provide a new mechanism by which alternate biocatalysts and natural products may be identified.

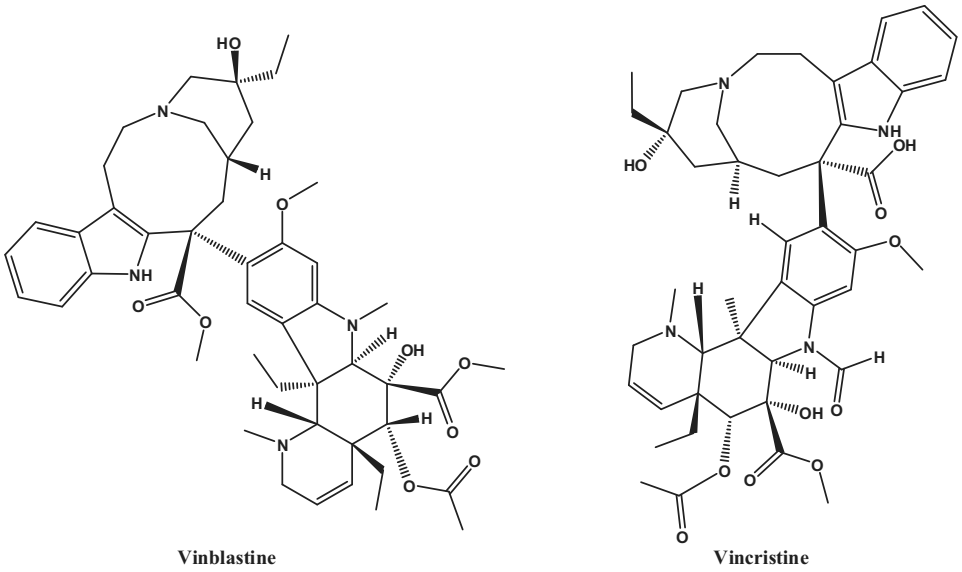
The bulk of natural product derivatives currently in clinical study are dedicated to the treatment of cancer [5]. There are a multitude of natural products sanctioned as leads in anticancer, there exists four main plant based drug classes, these include: 1) vinca alkaloids, 2) epipodophyllotoxins, 3) taxanes, and 4) camptothecines. Each class targets different stages or pathways of the cell cycle of cancerous cells. The alkaloids vinblastine and vincristine (Fig. **1**), block mitosis with metaphase arrest by binding to tubulin resulting in its depolymerisation. In combination with other cancer chemotherapeutic drugs the alkaloids can be used in the treatment of a variety of cancers including leukemias, lymphomas, advanced testicular cancer, breast and lung cancer, and Kaposi's sarcoma. Podophyllotoxin derivatives etoposide and teniposide (Fig. **2**), bind to tubulin, leading to DNA strand degradation irreversibly inhibiting topoisomerase II. These two derivatives are used in the treatment of lymphomas, as well as bronchial and testicular cancers. The class of taxane, which include paclitaxel (taxol®) and other like derivatives act by disrupting the assembly of tubulin, they show specific activity in patients diagnosed with breast, ovarian and non-small cell lung cancer. Camptothecin analogues selectively inhibit topoisomerase I. The more effective and safer analogues being topotecan, an ovarian and small cell lung cancer chemotherapy and irinotecan, used in patients with colorectal cancer (Fig. **3**). There exists many other natural products implicated in the treatment of different cancers, which only amplifies the cause for continued research efforts toward investigating natural products and their derivatives for their medicinal activity [6-8].

Cancer and the Role of Glycosylation

Cancer has been deemed one of the world's major causes of mortality. Characterised by the "out-of-growth" cell growth, prohibiting normal bodily functions depending on the type of cell infected. Cancer is a disease instigated by the function or dysfunction of catalytic pathways or cellular proteins. Being a heterogeneous disease there are a number of biological therapies available to patients, which target specific genetic markers or enzymes relevant to the development and growth of the condition. Through high through-put screening/dock it has been established that carbohydrates play an important role in cancer; and circulating or cell surface tumour-associated carbohydrate antigens serve as diagnostic markers [9-11]. Cell surface glycosylation is universal to all living cells reflecting their physiological state, and are perfectly positioned to mediate adhesion and motility. A shift from the normal glycosylation pathway leads to altered glycan expression due to one or more of the following changes: (1) under- or overexpression of glycosyltransferases deregulated at the level of

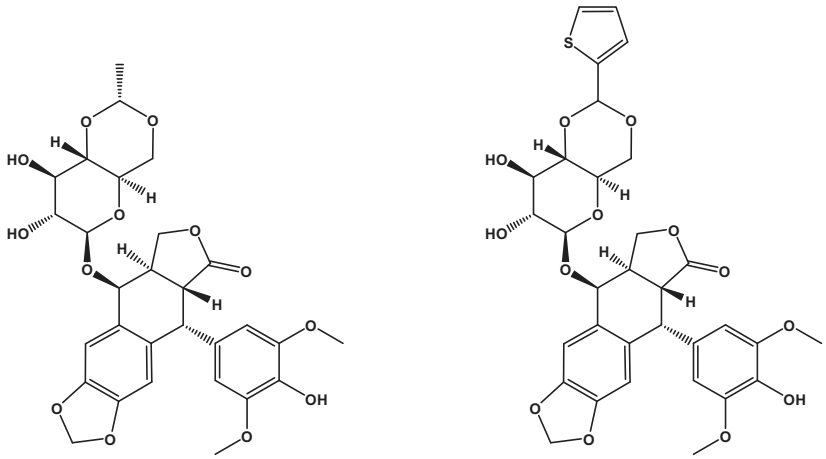
*Address correspondence to this author at the School of Health Sciences, University of KwaZulu-Natal, Westville, Durban 4001, South Africa;
Tel: +27 31 260 7413; Fax: +27 31 260 779; E-mail: soliman@ukzn.ac.za

1875-5992/15 $58.00+.00

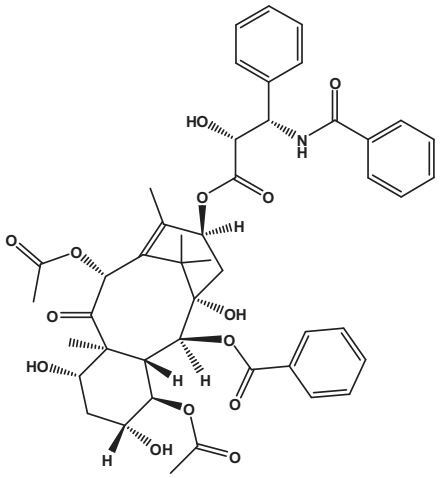


Vinblastine **Vincristine**

Fig. (1). Alkaloid anti-cancer derivatives vinblastine and vincristine [5].



Etoposide **Teniposide**



Taxol

Fig. (2). Podophylltoxins (etoposide and teniposide) and taxane (taxol®) anti-cancer agents [5].

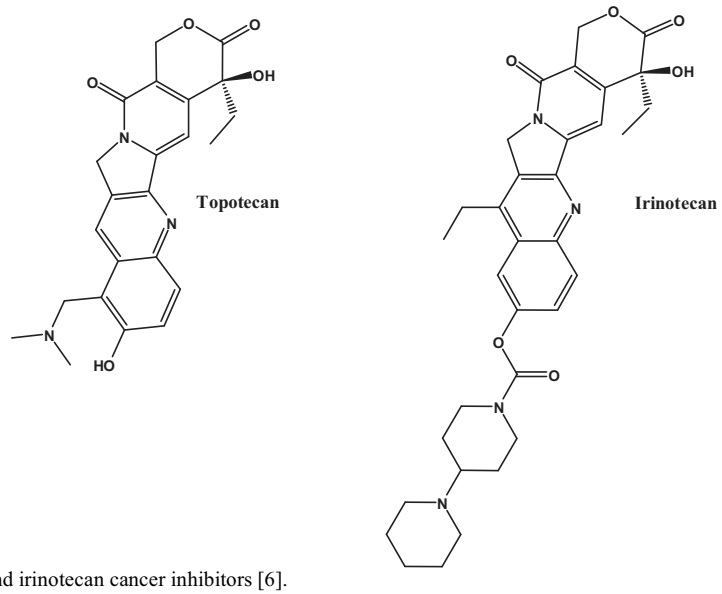


Fig. (3). Camptothecins; topotecan and irinotecan cancer inhibitors [6].

epigenetics [12, 13], transcription [14-16], post-transcription [17], and/or chaperone [18]; (2) altered glycosidase activity [19-21]; (3) altered expression of glycoconjugate acceptor together with availability and abundance of the sugar nucleotide donor [22]; (4) modified sugar nucleotide transporter activity [23]; and (5) malfunction of the Golgi structure, the warehouse of glycosyltransferases [9, 24, 25]. Abundant literature has suggested aberrant glycosylation contributes to various aspects of cancer development and progression, including proliferation, invasion, angiogenesis, metastasis and immunity [9, 26, 27].

Fig. **4**, depicts the *N*-glycosylation pathway on the surface of the Golgi apparatus. Overall the biosynthesis of *N*-glycans have been deemed an expensive process in terms of the number of actively participating enzymes in the synthesis and trimming of *N*-glycans. Structural diversity of *N*-glycans in mature proteins in the cell surface are introduced by glycosyltransferases in the Golgi complex as a terminal step. The core glycans in the endoplasmic reticulum are universal from yeast to mammal and are considered intermediates [28].

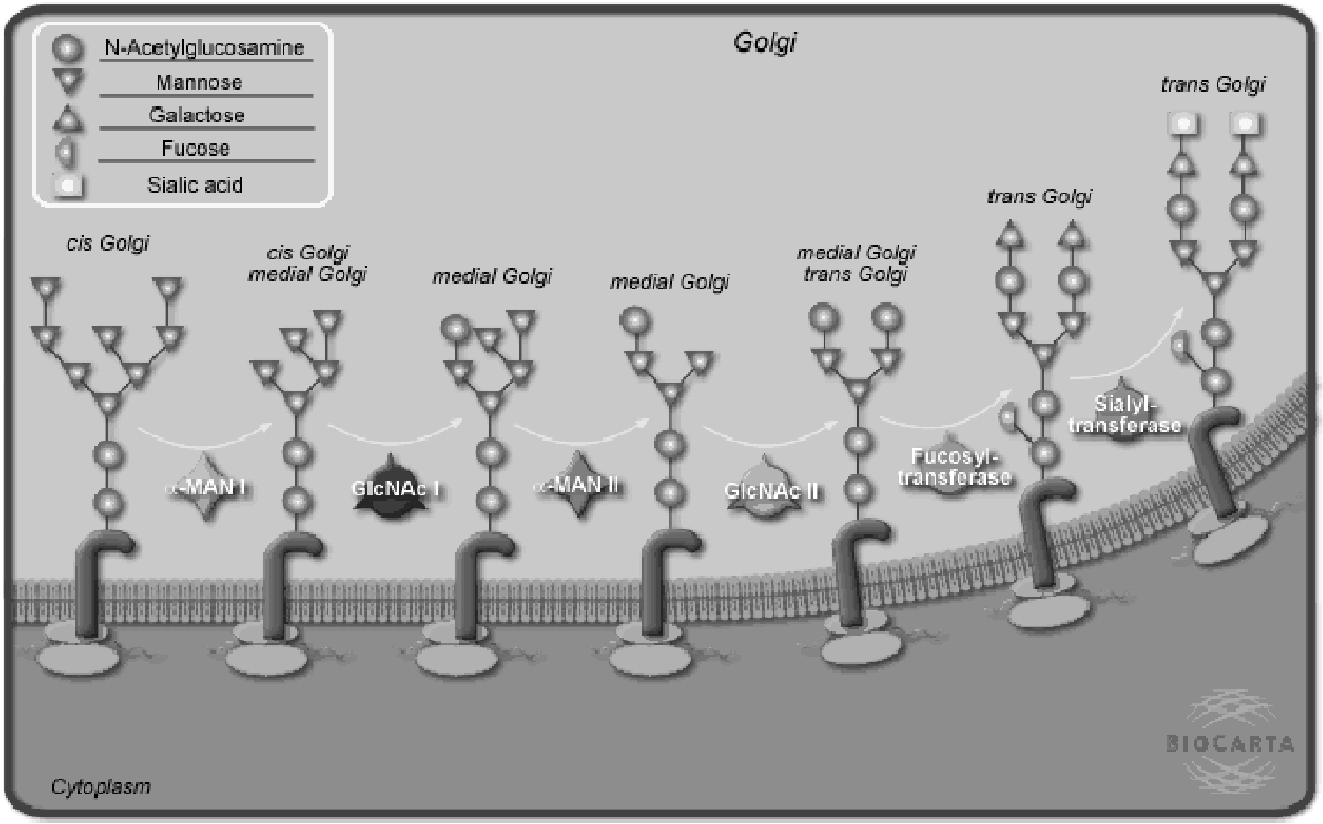


Fig. (4). Diagram of *N*-glycosylation pathway in human Golgi complex [28].

Glycosidases are a vast class of enzymes designated to cleave glycosidic bonds of carbohydrates or polysaccharides to essentially assist in biochemical processes such as protein folding in living cells [27, 29]. Vocadlo and Davies provided insight into the mechanistic behaviour of glycosidase enzymes by understanding the enzyme reaction co-ordinates, through collective works in computational and structural studies [27]. The fundamental function of these enzymes is to hydrolyse glucose residues from glucosides. Their specificity originates at a structural level, in terms of their α- and/or β- configurations as well as the configuration of its particular substrate. The inability of a glycosidase to function correctly or expressions of the enzyme have been implicated as a cause of cancer. Related cancers include: breast cancer [30], brain tumours [31], prostate [32], lung, gastric, bone and ovarian [33]. The glycosylation pathway is an imperative target in the prevention of development of cancer or further spread of the disease [34].

There are an estimated 16 enzymes steam rolling the glycosylation pathway within the cellular framework. These sites include the endoplasmic reticulum, golgi apparatus, cytosol and nucleus. Each of the glycosidase enzymes, based on tissue loci and organism, exists in either an α- and/or β-configuration and are governed by the structural feature of the bond on which they exert themselves [35]. A prime example of the uniqueness of this class of enzyme is glucosidase. The α-glucosidase depicted in Fig. **5**, is derived from sugar beet and classified as an endoplasmic reticulum inner membrane bound protein, which is responsible for the cleavage of α-(1,2)- linked glucose units from amylose to extended carbohydrate chains [36].

Fig. **6**, highlights the covalent intermediate of human cytosolic β-glucosidase. This enzyme has been associated with essential organs of the human metabolism such as the small intestine, kidneys and liver. It is thought to play a role in the detoxification of xenobiotics (foreign chemical compounds which pose a toxic threat to the healthy cells), by hydrolysing β-glucoside moiety providing a site for conjugation which would lead to rapid excretion in the bile and urine [38, 39].

A vast array of natural product glycosidase inhibitors have been explored due to their extensive ability in the treatment of cancer. These include but are not limited to: castanospermine and deoxynojirimycin (glucosidase I & II and lysosomal α-glucosidase inhibitors), swainsonine (lysosomal and aryl-mannosidases inhibitor, and a potent mannosidase II inhibitor), kifunensine (potent mannosidase I inhibitor), allosamidin, salacinol, nojirimycin, mannostatin A & B, isogaomine, kojibiose, sophorose, nigerose, trehalose and xanthone derivatives.

Evolution of Computational Techniques to Present Day

By the 1950's much improvement had been made in the way of instrumental techniques developed to analyse chemical reactions and molecular structures. However, the lack of understanding in terms of kinetic models, visualisation of dynamic interactions as well as how and what the conformations, transition states and reaction intermediates look like and existence in their natural systems, left many an unanswered question. It was not until 1957 when researchers Alder and Wainwright, reported the first successful molecular dynamic (MD) simulation. Even in its infancy the pair brought to life phase transition. Their investigation was based on a solid-fluid system evolution comprised of rigid spheres colliding instantaneously. With limited computing, a 500-particle system was designed, emulating collisions between particles with a duration of one hour on an IBM 704 computer [41].

This sparked a ripple effect where in 1960, Gibson and colleagues implemented the first continuous repulsive Born-Mayer interaction potential in MD simulation. This attempt may have been the first recorded MD method in materials science. Their study was based on the radiation damage in a copper (Cu) target. This was performed by applying a constant force towards each atom on the boundary of the crystallite to account for the attractive part of the interatomic interaction [42]. In 1964 Aneesur Rahman described the attractive and repulsive forces in an 864 argon atom system using the Lennard-Jones potential [43]. The computational methods applied in his study, such as pair correlation function, velocity

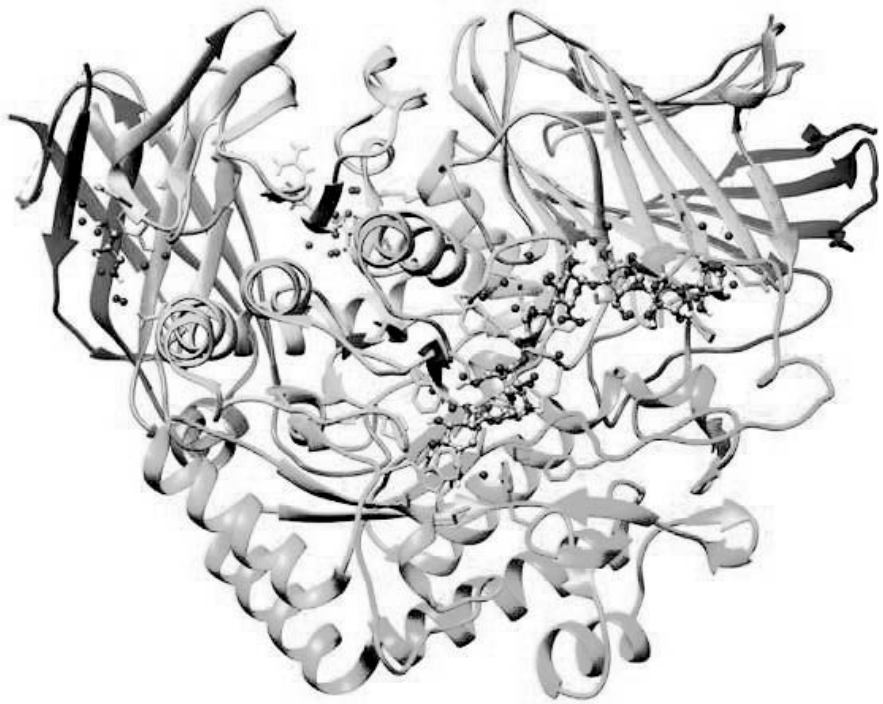


Fig. (5). Crystal structure of sugar beet α-glucosidase (PDB code: 3WEO) [37].

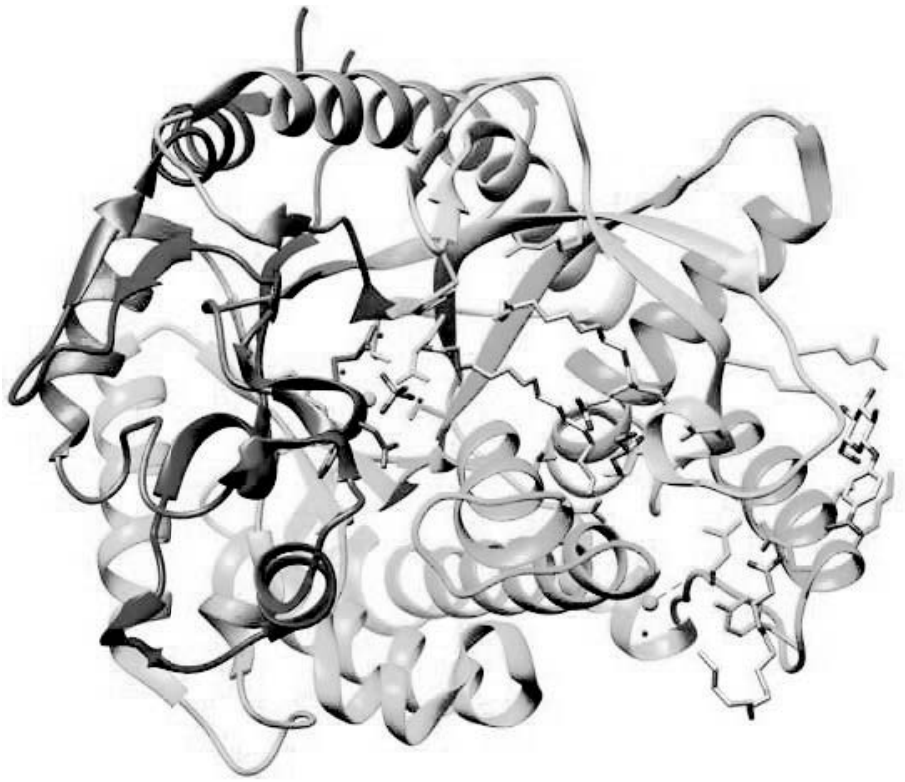


Fig. (6). Crystal structure of the covalent intermediate of human cytosolic *β*-glucosidase (PDB code: 2ZOX) [40].

autocorrelation function and mean square displacement calculated for liquid Argon, are still relevant in current studies.

Current studies have observed the evolution of MD simulation and the vast approaches and information that can be derived from such analysis. MD studies have offered a remarkable platform in the field of pharmaceutics; with regards to design and discovery of new and effective drug therapies for a multitude of diseases [44]. Nobel prize winner Richard Feynman described the motion of atoms being governed by probability functions, where chemical bonds are not formed mechanically but rather by the shifting of electron clouds which act as both waves and particles [45]. Thus the standard of the 'lock and key' theory has been set aside to accommodate the new age of binding models which accounts for conformational changes as well as the random motions of receptors and ligands. This seemingly minor difference allows molecular dynamic simulations to stand apart from other static two dimensional models, thus playing an important role in drug discovery.

With the ability to analyse a three dimensional (3D) crystal structure of protein or target, we may gain perspective into the key features that enable such structures to function and possibly enhance or inhibit the expression of the protein species. Such techniques which exploit these attributes are molecular dynamics and quantum mechanics, homology modelling and virtual screening. Each of which offers different introspection into the specific aim of study [46].

COMPUTATIONAL APPROACHES ON NATURAL AGENTS AGAINST CANCER

Homology Modelling

Homology modelling is a technique applied in the prediction of protein structure based on the fact that proteins of similar sequences bear similar structures. The technique has promoted the identification of protein function and mechanism, drug binding to specific sites and rationalisation of select amino acids in the discovery of pertinent biological function ascribable to mutagenesis. Due to computational methods being economically viable high throughput docking is an ideal method to select promissory compounds of appropriate chemical nature from extensive virtual chemical libraries, since they incur minor errors. Lead optimisation is a monotonous process of modifying the chemical structure of a known hit by modifying its physico-chemical and pharmacological properties to improve bioavailability, minimize unwanted toxicity and obtain the admissible drug profile appropriate for animal model studies and clinical trials [47].

Protein-ligand interaction comprehension is crucial in homology-based lead optimization, since the character of the structure obtained can steer ligand optimization towards enhanced pharmacological profiles. Homology modelling has a significant contribution to structure-based drug discovery through prediction and generation of rational 3D models for drug targets. It generates plausible protein structures by presenting a 3D model from a protein template sequence subject to previously reported homologous protein structures. When building a homology model for proteins, the following typical procedure should be adhered to: 1) 3D protein structures with ~30% primary structure similarity over the protein of unknown structure should be used [46, 48]; 2) the alignment of template and target protein sequences; 3) identification of variable and structurally conserved regions; 4) generation of structurally conserved residues of the unknown structure from template structure(s); 5) generation of loop conformations for the unknown structure; 6) generation of side chain conformations for the modelled protein; 7) refinement and evaluation of the generated unknown structure. The RMSD (root of mean square deviation distance between C_α atoms) of the homology model is compared to that of the experimental structure, which should be ~ 1-2 Å, to verify the accuracy of the homology model [49]. The accuracy of a homology model is defined by the template

of choice, alignment accuracy and application of efficient refinement methods [47]. Homology models generated with over 50% sequence identity have satisfactory accuracy for drug discovery applications; whereas models of sequence identities of 25% to 50% can be applied in target druggability assessment and design mutagenesis. Homology models have proven invaluable as a rationalising tool of SAR data and the prediction of binding modes of experimental compounds [50, 51].

Heparanase is an endo-β-D-glucuronidase, which has been reported as a target for antimetastatic agents. Its unique function in degrading heparin sulphate glycosaminoglycans in mammalian tissue has highlighted the enzyme. Over expression would result in invasive normal and malignant cells such as; immune cells, lymphoma, melanoma, and carcinoma cells as well as human head and neck tumours. Ishida *et al.*, had proposed the design of selective inhibitors of heparanase by using a homology model of the enzyme. The homology modelled enzyme was based on the sequence alignment of human heparanase and 1,4-β-xylanase from *Penicillium simplicissimum*. Based on this model it was identified that the interaction between inhibitor and heparanase enzyme was stabilised by arylalkylation [52].

Glucosidases are last stage carbohydrate digestive enzymes. They are responsible for hydrolysing the glycosidic bond of oligosachharides. Park *et al.*, wished to design new inhibitors derived from structure based virtual screening. In order to accomplish this they were required to obtain a high quality amino acid sequence of the enzyme. This was achieved by the alignment of sequence from baker's yeast α-glucosidase and oligo-1,6-glucosidase from *Bacillus cereus*. Such that these sequences shared an amino acid identity and similarity of 38.5% and 58.4% respectively. The homology model was sufficient to perform docking studies. It was observed that the target and the template displayed highly similar folding structures, whereby the catalytic residues were conserved in terms of position in the active site when compared to the x-ray crystal structure of oligo-1,6-glucosidase (Figs. **7** & **8**) [53].

Researchers Moorthy, Ramos and Fernandes conducted homology modelling of an α-glucosidase enzyme which was later used in a quantitative structure activity relationship study of xanthone derivatives. They had deduced that the active site of the model retained residues aspartic acid, histidine and glutamic acid, which contributed to the specific structural features of potential inhibitors [54].

Human glycans which include *N*-linked glycans, ABO blood group and Lewis antigens have prominent structural moieties of α-L-fucose a residue by-product of reaction catalysed by α-L-fucosidase [55, 56]. Many cancers are associated with the high levels of fucosylation which increases with expression of the enzyme [57, 58]. Bueren *et al.*, analysed the reaction co-ordinate of α-L-fucosidases by combining structural and quantum mechanical approaches. They were able to interpret inhibitor binding by deriving a homology model of enzyme from *B. thetaiotaomicron* as the sequence identity was in high correlation with that of the human enzyme [59].

Qualitative Structure Activity Relationship (QSAR)

QSAR is a technique that has infiltrated the scene of drug design and discovery as an invaluable tool. It has been implemented to identify ligands with high affinities for specific macromolecular

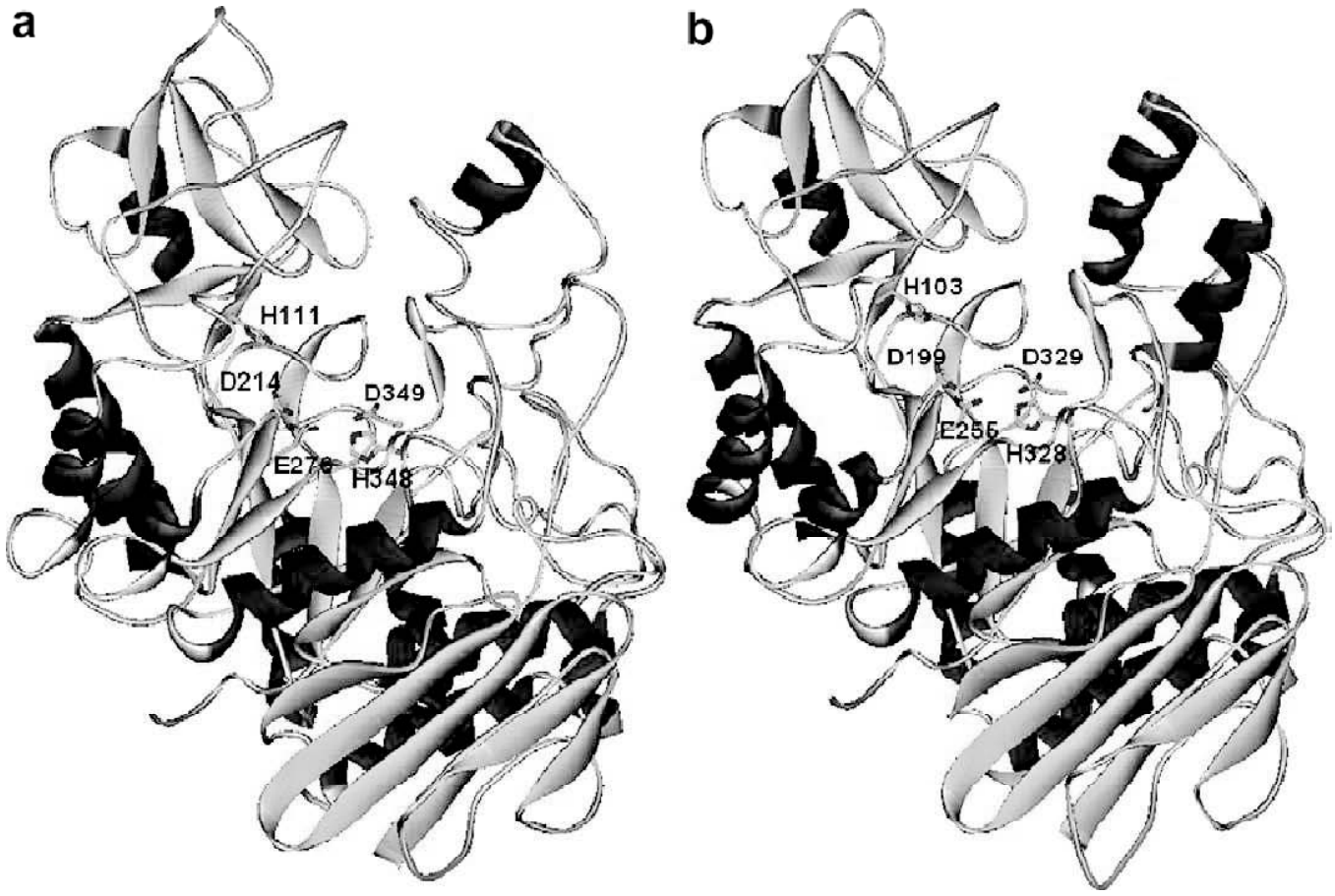


Fig. (7). Comparative view of (**a**) homology modelled structure of α-glucosidase and (**b**) the x-ray crystal structure of oligo-1,6-glucosidase [53].

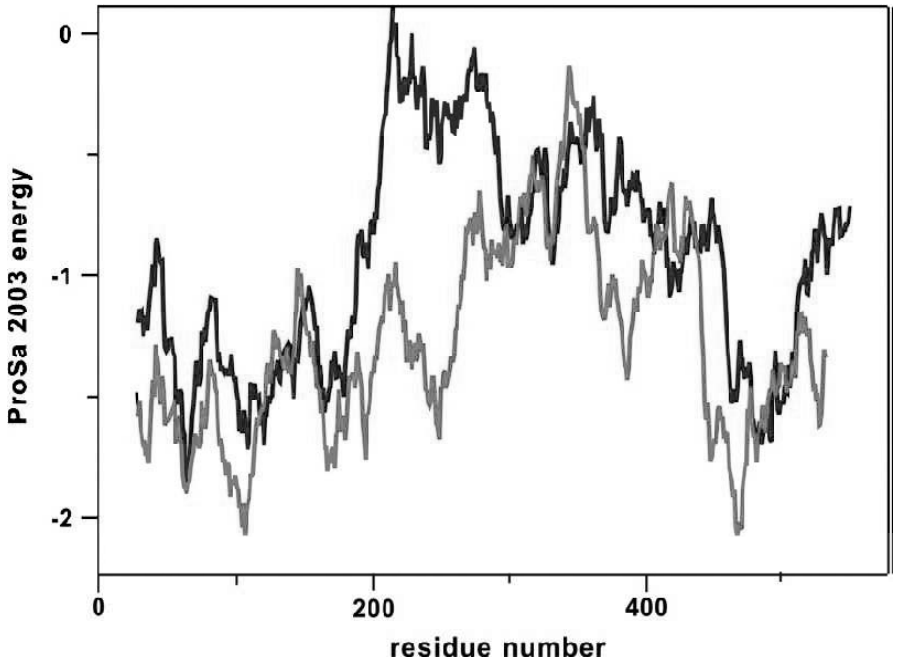


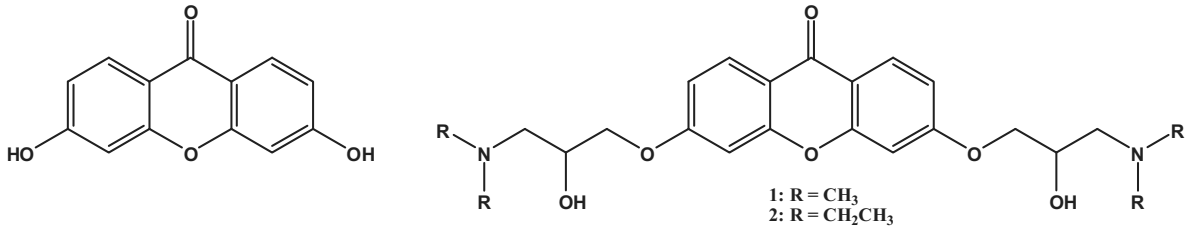
Fig. (8). Comparison of the ProSa energy profiles of the homology modelled structure of α-glucosidase (red) and the x-ray crystal structure of oligo-1,6-glucosidase (green) [53].

targets, by screening drugs and providing potential outcomes of synthesis and testing. It has also been applied in extended research in predicting adsorption, distribution, metabolism, elimination, toxicity properties as well as bioavailability of compounds. Such studies can be undertaken on different dimensions ranging from 1D-QSAR, where only a single property of the ligand is exploited correlating to its affinity; to 6D-QSAR which involves the ligand being represented as an ensemble of configurations with the explicit representation of different induced fit models and the representation of different solvation scenarios [60].

Glycosidases have evolved finely tuned active site configurations for the specific hydrolysis of glycosidic bonds. The inhibition of glucosidases has offered a noteworthy effect on the glycon structure ultimately affecting the maturation, transport, secretion and function of glycoproteins. Thus this could potentially have an altered recognition of cell-cell processes. Moorthy *et al.*, attempted QSAR studies on associated xanthone derivatives based on the data set published by Yan *et al.*, as inhibitors of α-glucosidase. Xanthones are natural derivatives extracted from different medicinal plant material (Fig. **9**) [61, 62]. Due to the limited information presented from current literature, they decided to delve into the link of biological activity data and physiochemical descriptors of molecules. By exploring structural features which have a determining effect on the binding affinity, mechanism of inhibition, topology and hydrophobicity. It was established that heteroatoms such as; oxygen bonded to carbon, are favourable features for enzyme inhibition. Using an E-state count descriptor, the most favourable framework for inhibition of enzyme activity is a carbon atom linked with three aromatic bonds and hydrogen or other atoms [54, 63, 64]. Moorthy and colleagues performed additional studies on the α-glucosidase enzyme of *B. Stearothermophilus* and *S. cerevisiae* and their interaction with chlorogenic acid inhibitors [65].

Virtual Screening

Virtual screening has offered a cost effective method by which we may search for lead structures for further development as therapeutic agents in different biological systems and against a multitude of targets. An advantage of this technique is the resulting computational prediction of binding affinity, which contributes to bottlenecking and reduces the number of compounds required for testing. In terms of drug design, a range of computational methods are employed at distinctive stages of the procedure. As previously mentioned, high through-put screening of large compound libraries focuses on decreasing the number of potential ligands. Post lead-optimization, reduction of experimental costs and time is emphasized. Molecular dynamic simulations have improved docking procedures in terms of Molecular Mechanics Poison-Boltzman/ Generalised Born Surface Area (MM/PBSA or MM/GBSA), Free Energy Perturbation (FEP), Thermodynamic Integration (TI) and Linear Interaction Energy (LIE) approaches [66-69]. They add invaluable insight into the dynamic behaviour of



Xanthone **Xanthonoxypropanolamines**

Fig. (9). Xanthone and subsequent xanthone derivatives.

proteins at various time-frames, from speedy internal motions to steady conformational changes or even protein folding processes. Studying of the explicit solvent molecule effect [70] on protein structure and stability to characterise a biomolecular system is plausible. Such system characteristics include density, conductivity, dipolar moment and thermodynamic parameters inclusive of interaction energies as well as entropies [71, 72].

Several MD-based in silico methods for binding energy predictions have been extensively applied in drug design as they provide statistically meaningful conformational ensembles for thermodynamic calculations at a reasonable computational cost. MM/PBSA and MM/GBSA are considered more computationally efficient calculations as opposed to FEP/TI. This is attributed to the former free binding energy calculations having fewer constraint rules to obey, as well as their capability to dissect total binding free energy into different interaction terms [73]. MM-PBSA/GBSA are, however, theoretically more rigorous as such calculations take into consideration the conformation of free ligand, receptor and ligand-receptor complex. The FEP theory was initially introduced by Zwanzig in 1954, where he correlated the free energy distinction between reference and target state of a system to its average function of energy estimated by sampling initial states [74]. Since then much improvement has been made by way of calculating free energy differences using this technique. These include coupling the mathematical algorithm with advanced molecular dynamics and Monte Carlo sampling to elucidate respective solvation free energies, pK_a values, medium-effects on conformational equilibria, host-guest binding affinities, organic and biochemical reactions free energy surfaces [75]. Alternatively, binding energies can be calculated by scoring functions as they compute results faster than the MM-GB/PBSA models. However, scoring functions lack precision, with an average error of ~2.5 kcal/mol [69].

In 2008 Park and colleagues suggested discovering novel inhibitors of *α*-glucosidase through virtual screening encouraged by a homology-modelled protein structure [53]. The technique allowed the screening of a library estimating 85,000 compounds, resulting in the reduction of sample size to 200 of the highest scoring compounds. It was later established that only 13 of these suggested agents presented a 50% inhibition at a concentration range between 0 μM and 50 μM [53]. Figs. **10**, **11**, are representative inhibitors,

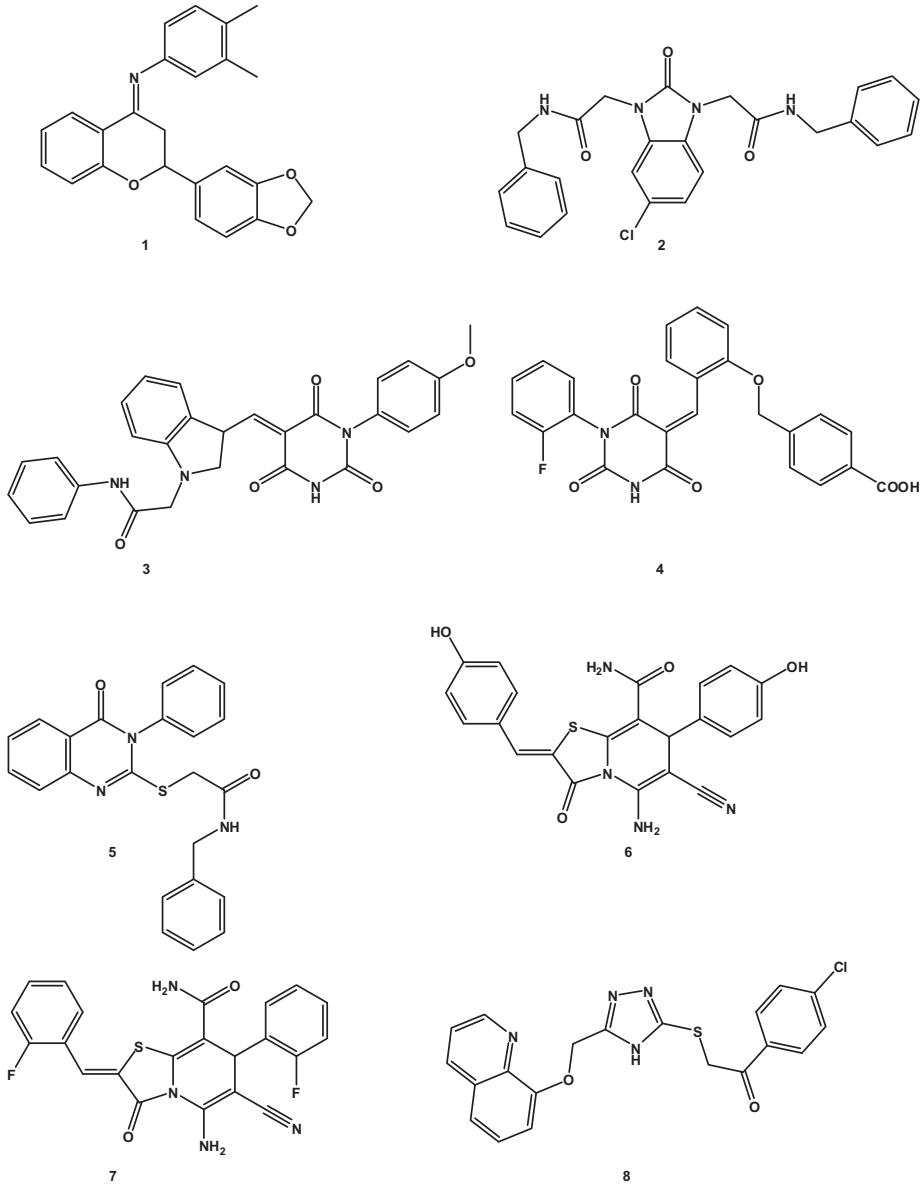


Fig. (10). Compounds 1 - 8 of newly identified α-glucosidase inhibitors [53].

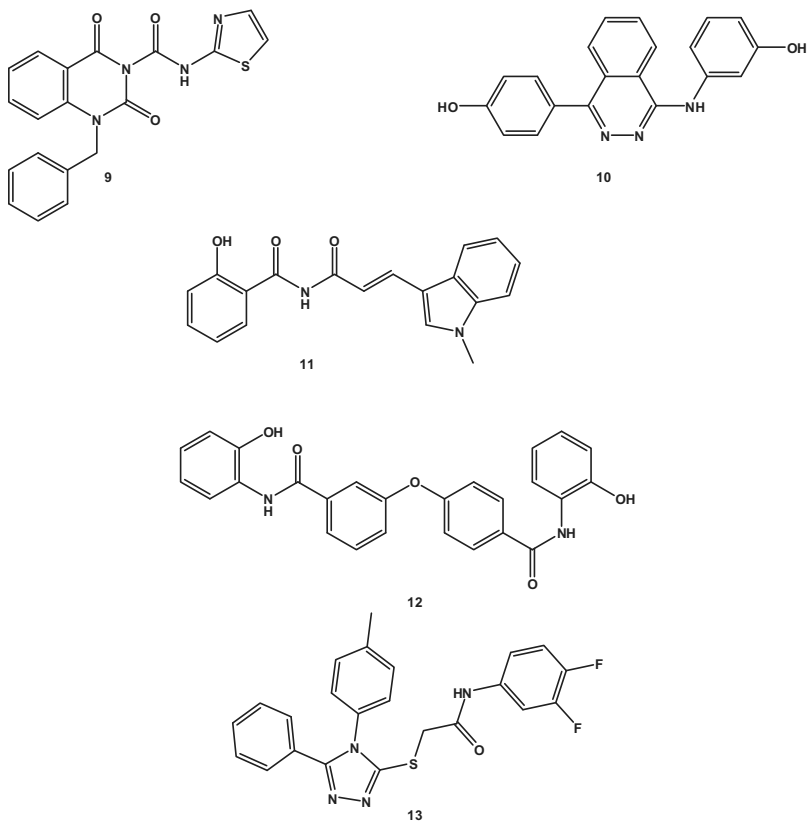


Fig. (11). Chemical structures 9 - 13 of newly identified α-glucosidase inhibitors [53].

their scaffolds now form the templates for further development and investigation of structure based *de novo* synthetic inhibitors.

Molecular Modelling and Binding Modes

Molecular modelling is a fusion of structure and function of molecules in the form of proteins, ligands and cellular entities. The manipulation of chemistry and laws of protein folding allows scientists to seek understanding of physiology of disease. The essence of the technology utilises molecular biology, x-ray crystallography and quantum mechanics [71, 76, 77]. The allure of molecular modelling techniques lies in the atomistic level description of molecular systems. There are three common stages involved in molecular modelling studies. The first stage is a selection process whereby a model is chosen to best describe the intra- and inter- molecular interactions in a system. The two most frequently used models are quantum mechanics and molecular mechanics, both follow the fundamentals of the energy associated with the arrangement of atoms and molecules within the system. The second stage encompasses the actual mathematical calculation of the system, this includes energy minimisation, molecular dynamic or Monte Carlo simulations or a conformational search. This leads directly into the final stage of post-analysis of the calculations carried out in stage two. It is no small wonder that from these stages computational chemistry or molecular modelling is regarded as a classical example of scientific art [78]. From the post-analysis of calculations performed a multitude of information may be generated with respect to molecular geometry, energies, electronic properties, spectroscopic properties and bulk properties [78, 79].

Allosamidin has been classified as a pseudo-trisaccharide (Fig. **12**), isolated from the mycelia of *Streptomyces* sp. [80]. Germer and colleagues performed molecular modelling studies on the allosamidin and six additional analogues based on the allosamidin framework in an effort to validate conformational information extracted from 2D NMR studies. Of which it was concluded that the results of each method were aligned. Proving once again that computational approaches to scientific investigations can act both as a tool of diagnosis and validation [81]. Kara *et al*., proposed a mechanism of action of allosamidin in chitinase, which is regarded as a type of glycohydrolase enzyme. With modelling, Kara and colleagues suggested that mechanism of action of the enzyme involves the bending of acetalamido group to oxygen of the substrate ring which subsequently neutralises the charge. Allosamidin mimics the transition state of the catalytic reaction and thus perpetuates competitive and selective inhibition of the enzyme [82].

α-Mannosidase is a class II enzyme partaking in the *N*-glycosylation reaction, with a function to link oligosaccharides to distinct asparagine amino acids in incipient proteins. Thus subsequent inhibitors show radical antitumour and antimetastatic activity. Such inhibitors include kifunensine an alkaloid derived from actinomycete *Kitasatosporia kifunense,* salacinol extracted from *Salacia reticulata* and swainsonine [83-85].

Shah *et al*., compared the binding of kifunensine and 1-deoxymannojirimycin to *α*-mannosidase (Fig. **13**). 1-Deoxymannojirimycin, once thought only a derivative of the natural inhibitor nojirimycin a piperidine alkaloid isolated from *Streptomyces* sp., was found naturally occurring from the extract of mulberry leaves, *Bacillus* and *Streptomyces* sp. as well as Micronesian marine sponge [5, 86, 87]. However, both entities proved viable inhibitors of glycosidases and thus exerted an effective activity as antitumor and anticancer agents. Shah and colleagues performed energy directed studies which proposed kifunensine inferring moderate inhibition to class II mannosidase as compared to being more potent against class I mannosidase. With kifunensine maintaining a $^{1}C_{4}$ conformation in each of the different

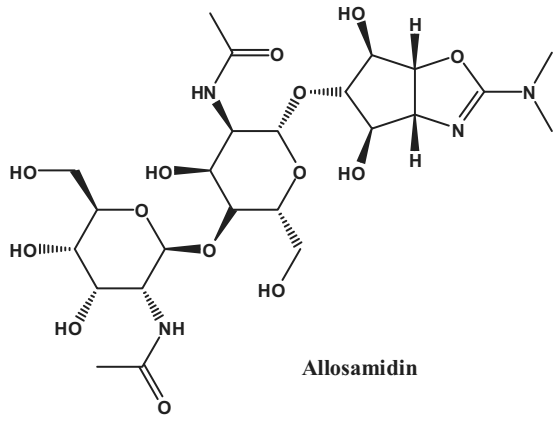


Fig. (12). Structure of allosamidin.

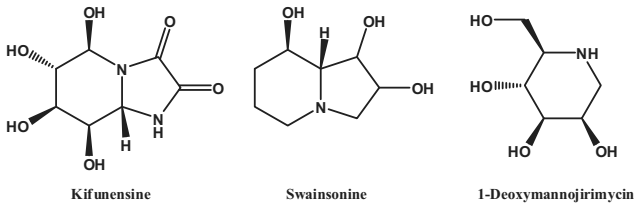


Fig. (13). Structure of α-mannosidase inhibitors kifunensine, swainsonine and 1-deoxymannojirimycin [88].

active sites. 1-Deoxymannojirimycin being a smaller inhibitor observed a $^4C_1$ conformation in the class I mannosidase active site but a $^1C_4$ conformation in the class II mannosidase active site [88].

Wen *et al.*, investigated the binding mode off the previously mentioned inhibitors docked in the glycosidase enzyme. The study revealed that the five membered ring of kifunensine is planar. There exists three distinct binding modes differing by the distance between zinc and hydroxyl polar groups. In mode I the distance was estimated to 2.3 Å, mode II 3.31 Å, whereas with mode III the ligand appeared to turn completely within the active site and thus the distance was of polar group to zinc was too far. With respect to salacinol the inflexibility of its five membered ring offered pseudo-rotation (Fig. **14**). The three distinct binding modes of this system reasoned that the relative conformations are independent of the interaction between the polar species of the ligand and active site interactive moieties. In conclusion these findings satisfied those observed from x-ray crystallography [89].

Mannostatin A and B (Fig. **15**), were discovered by Aoyagi and collaborators in 1989 from isolates of *Streptoverticilliu verticillus* variation *quantum*. Classed as novel aminocylcopentitol structures they were found to be active inhibitors of *α*-mannosidase and *α*-glucosidase [90]. Kawatkar *et al.*, explored the ligand-protein

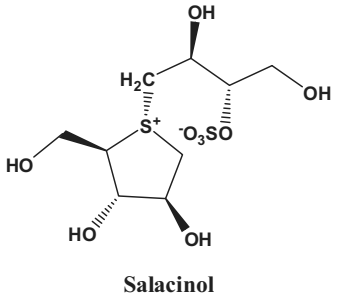


Fig. (14). Structure of salacinol.

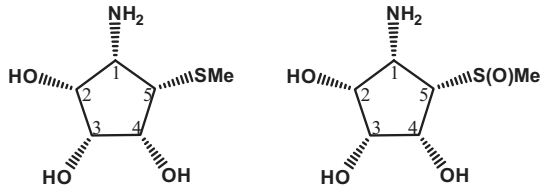


Fig. (15). Structure of mannostatin A and B with correponding inhibition values [92].

interactions of mannostatin A and *α*-mannosidase II. Their research team had elucidated that mannostatin A mimicked covalently linked mannosyl intermediate which adopts a $^1S_5$ skew boat conformation. It was established that the thiomethyl group is required for high affinity binding, which was reported to have good overlay with the C-6 hydroxyl of the covalently linked intermediate [91]. Kuntz *et al*., had performed binding mode studies on a similar system. Their findings were *in cognito* with corresponding literature, that inhibition sees crucial contribution of zinc interactions, interactions with Asp341 and Asp472, as well as hydrophobic interactions with Phe206 of the enzyme [92].

Cardona *et al.*, conducted molecular dynamic simulations on glucoamylase II from *Aspergillus awamori* with natural inhibitors 1-deoxynojirimycin and lentiginosine (Fig. **16**) in an attempt to understand binding for further investigation in the design of new inhibitors in human systems as antitumour agents. It was then determined that lentiginosine observed optimal conformation in the enzyme cavity when hydrogen bonding to residues Arg54 and Arg55. As well as inhibition was dominated by the interaction of hydroxyl groups of the substrate with key enzyme residues [93]. Zhou *et al.*, performed similar simulations, however, the system involved comprised of 1-deoxynojirimycin and isogaomine. They showed that 1-deoxynojirimycin bound to glucosidase in a protonated chair conformation with a binding energy value of -46.76 kJ/mol. Major thermodynamic contributions implying favourable enthalpy of binding were attributed to strong hydrogen bonds and electrostatic interactions between enzyme and ligand [94].

Pereira *et al.,* performed a conformational and dynamical study on disaccharides in water using explicit-solvent molecular dynamic simulations [95, 96]. They analysed eight reducing disaccharides of *β*-anomeric configuration isolated from honey: kojibiose, sophorose and nigerose (Fig **17**), as well as laminarabiose, maltose, cellobiose, isomaltose and gentiobiose. In current literature most of the disaccharides have been characterised and theoretically investigated using MD simulations, molecular mechanics calculations or quantum mechanics calculations. In the study a continuous 50 ns run time was performed, during which time it was concluded that the preference of dihedral angles which dictates that polar substituents are to be orientated away from the ring [97].

Kräutler *et al.*, explored conformation, dynamic, solvation and respective stabilities of specific *β*-hexopyranoses in water using a molecular dynamic platform. Four *β*-D-aldohexopyranoses monosaccharides were studied, namely: *β*-D-glucose, *β*-D-mannose, *β*-D-galactose and *β*-D-talose; which were simulated in a series of 200 ns timescale. During the simulation the latter three substrates

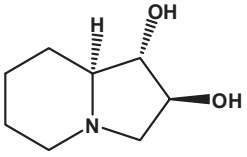


Fig. (16). Structure if lentiginosine.

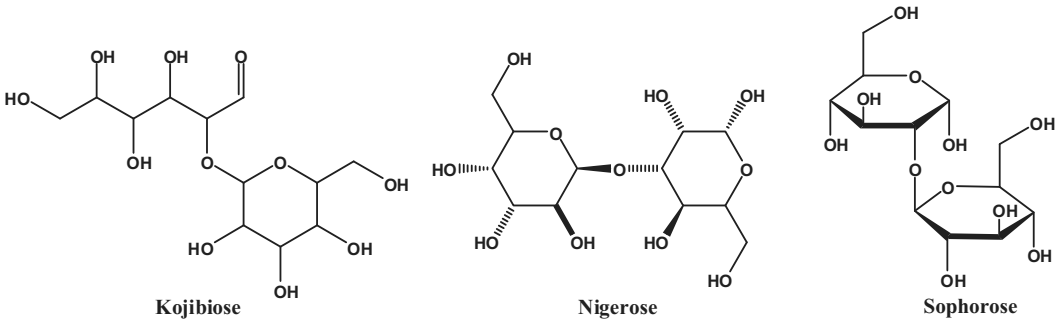


Fig. (17). Structure of kojibiose, nigerose and sophorose disaccharides.

maintained a $^4C_1$ chair conformation, unlike glucose which has evolving boat and twisted configurations. Kräutler *et al.*, described the intramolecular hydrogen-bonding pattern with each substrate, and were deemed opportunistic contributors to the relative conformation and stability of the structure. They had ranked the estimated epimerisation energies in order of decreasing stability, with talose exhibiting the least stability superseded by galactose and mannose in turn, with glucose registered as most stable. This confirmed results obtained from intramolecular effects and hydrophilicity investigations [98].

Trehalose (Fig. **18**), a sugar extract from yeast, exists as a naturally occurring disaccharide in mushrooms and other fungi. This unique combination of α-D-glucopyranosyl-α-D-glucopyranoside has been implicated as prominent inhibitor of the glucosidase enzyme [99]. The dysfunction of the enzyme has proven a target in the prevention of growth and development of metastatic cancer. Trehalose has been shown to form direct hydrogen bonds to proteins which endure the ligand requiring occupation of specific active site in a specific orientation [100]. There have been a number of reported simulations investigating the structural dynamics of binary sugar/water solution in an attempt to elucidate physical properties [101-111]. Sum *et al.* [112] and Pereira *et al.* [113, 114] had conducted a simulation of a lipid membrane in the presence of sugars. They discovered that specific hydrogen bonding governed the interaction between sugar and lipid molecules. Lerbret *et al.* had decided to simulate a lysozyme in aqueous solution of trehalose, sucrose and maltose. It was suggested that trehalose was the most hydrated of all the three sugars as it maintained a greater number of hydrogen bonds with water [115].

Park and collaborators, took their study of novel α-glucosidase inhibitors a step further by exploiting the binding modes of the 13, then, newly identified molecules in the active site of the enzyme during which they noticed that the phenolic oxygen of the select inhibitor was a key feature in the migration of a hydrogen bond from His348 to Asp349, essentially translating to the phenol moiety playing a pivotal role for binding to the active site. It was also established that the stabilisation of docked ligands can be attained by inducing hydrophobic interactions at across from the active site [53].

Pistarà *et al.*, had provided *in silico* characterisation of cyclitols, inosose the ketone structure of inositols found in mammalian tissues and *Streptomyces griseus*. The inosose molecules were docked in human maltase-glucoamylase which has been crystallised with miglitol (Fig. **19**). It was determined that nitrogen that engages in a hydrogen bond with Asp443 in the catalytic residue is an essential entity for inhibitory activity. Additional structural components such as benzyl have the ability of filling the active site [116]. The binding free energy of compound 1 was calculated to be -6.0 kcal/mol and was found to possess the greatest inhibitory effect with an inhibition constant of 36.9 μM.

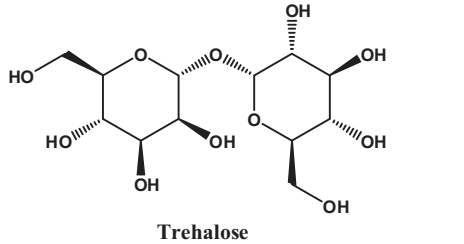


Fig. (18). Structure of trehalose.

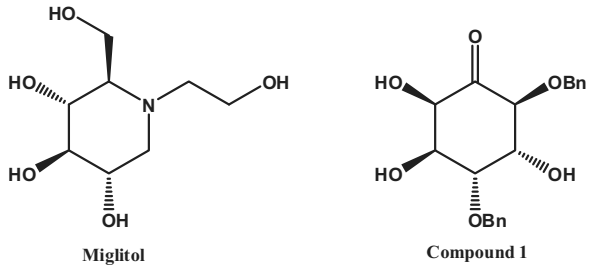


Fig. (19). (left) structure of miglitol and (right) structure of compound 1 [116].

CONCLUSION

From the topics described above, it is evident that there exist considerable gaps in the way of computational studies performed on the natural element inhibitors of glycosidase enzymes. These include the understanding of kinetic mechanisms of inhibitor-enzyme interaction, conformational support within active site, effects of mutagenesis, selective and competitive inhibition of enzyme species; and many more. Thus, there is great opportunity for continued research toward answering many of these essential components by way of a computational approach.

Additional research toward understanding the reaction kinetics and mechanism of glycosidase enzymes in their natural biological system, would be imperative in our quest to discover and design innovative anticancer inhibitors. Despite the large array of high resolution crystal structures available within the protein databank, much effort is required in acquiring crystal structures of human glycosidase. Such that accurate study may be performed to investigate possible inhibitors. Intensive molecular dynamic study encompassing molecular biology techniques would aid research in determining critical enzyme reactions that perpetuate cancerous species. Thus by isolating specific drug targets, it would allow us to build into the inhibitor, selective drug delivery systems exerting a specialised response. An attractive feature of obtaining refined crystal structures of human glycosidase enzymes, offers insight into the active site residues. Thus accurate binding modes may be established as well as chemical structuring of potential inhibitors may be designed according to supported conformational and steric properties.

Despite limitations of the crystal library with the aid of computational tools we may continue studies in this field by accurately constructing best-fit homology models of associated enzymes. Extensive studies based on QSAR and QM/MM of biological systems will dramatically improve our understanding of the influence of glycosidase enzymes in cancer development. As well as it could bridge the gap between theoretical and experimental studies, by economising on time, cost of study and prioritising higher possibility enzyme structure and mechanistic predictions.

The above mentioned attributes contribute to the design and development of new drugs as anticancer agents as well as provides pivotal information with regards to the specific enzyme pathways implicated in cancer. Molecular dynamic studies present an opportunistic methodology whereby we may unlock and unveil the mystery underlying the disease thus unleashing great strides in our fight against cancer.

Computational techniques boast an arsenal to unravel and offer a broad introspection into the molecular dynamics of glycosidase enzymes. Thus the study of this family is not exclusive or restricted to its effect on cancer but rather a multi-dimensional study on other congenital disorders of glycosylation may be investigated. Armed with computational tools the study of glycosidase enzymes remains limitless, and with the limited literature available within this topic much research is yet to be done in this field.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

Declared none.

REFERENCES

[1] Wong, K.C., Wang, C.; Wu, L. History of chinese medicine: Being a chronicle of medical happenings in china from ancient times to the present period1936: Tientsin Press.

[2] Dias, D.A.; Urban, S.; Roessner, U. A historical overview of natural products in drug discovery. *Metabolites,* **2012**, *2*(2), 303-336.

[3] Balunas, M.J. Kinghorn, A.D. Drug discovery from medicinal plants. *Life Sci.,* 2005. **78**(5), 431-441.

[4] Li, J.W.; Vederas, J.C. Drug discovery and natural products: End of an era or an endless frontier? *Science,* **2009**, *325*(5937), 161-165.

[5] Cragg, G.M.; Newman, D.J. Plants as a source of anti-cancer agents. *J. Ethnopharmacol.,* **2005**, *100*(1-2), 72-79.

[6] Srivastava, V. Plant-based anticancer molecules: A chemical and biological profile of some important leads. *Bioorg. Med. Chem.,* **2005**, *13*(21), 5892-5908.

[7] Newman, D.J.; Giddings, L.A. Natural products as leads to antitumor drugs. *Phytochem. Rev.,* **2013**, *13*(1), 123-137.

[8] Gordaliza, M. Natural products as leads to anticancer drugs. *Clin. Trans. Oncol.,* **2008**, *9*(12), 767-776.

[9] Padler-Karavani, V. Aiming at the sweet side of cancer: Aberrant glycosylation as possible target for personalized-medicine. *Cancer Lett.*, **2013.**

[10] Dube, D.H.; Bertozzi, C.R. Glycans in cancer and inflammation [mdash] potential for therapeutics and diagnostics. *Nat. Rev. Drug Discov.,* **2005**, *4*(6), 477-488.

[11] Ludwig, J.A.; Weinstein, J.N. Biomarkers in cancer staging, prognosis and treatment selection. *Nat. Rev. Cancer,* **2005**, *5*(11), 845-856.

[12] Kannagi, R.;Yin, J.; Miyazaki, K.; Izawa, M. Current relevance of incomplete synthesis and neo-synthesis for cancer-associated alteration of carbohydrate determinants—Hakomori's concepts revisited. *Biochimica. Biophysica. Acta,* **2008**, *1780*(3), 525-531.

[13] Miyazaki, K.; Ohmori, K.; Izawa, M.; Koike, T.; Kumamoto, K.; Furukawa, K.; Ando, T.; Kiso, M.; Yamaji, T.; Hashimoto, Y.; Suzuki, A.; Yoshida, A.; Takeuchi, M.; Kannagi, R. Loss of disialyl lewisa, the ligand for lymphocyte inhibitory receptor sialic acid-binding immunoglobulin-like lectin-7 (siglec-7) associated with increased sialyl lewisa expression on human colon cancers. *Cancer Res.,* **2004**, *64*(13), 4498-4505.

[14] Buckhaults, P.; Chen, L.; Fregien, N.; Pierce, M.Transcriptional regulation ofn-acetylglucosaminyltransferase v by the srconcogene. *J. Biol. Chem.,* **1997**, *272*(31), 19575-19581.

[15] Le Marer, N.; Laudet, V.; Svensson, E.C.; Cazlaris, H.; Van Hille, B.; Lagrou, C.; Stehelin, D.; Montreuil, J.; Verbert, A.; Delannoy, P. The c-Ha-ras oncogene induces increased expression of β -galactoside α -2,6-sialyltransferase in rat fibroblast (FR3T3) cells. *Glycobiology,* **1992**, *2*(1), 49-56.

[16] Hatano, K.; Miyamoto, Y.; Mori, M.; Nimura, K.; Nakai, Y.; Nonomura, N.; Kaneda, Y. Androgen-regulated transcriptional control of sialyltransferases in prostate cancer cells. *PLoS One,* **2012**, *7*(2), e31234.

[17] Kim, J.K.; Noh, J.H.; Jung, K.H.; Eun, J.W.; Bae, H.J.; Kim, M.G.; Chang, Y.G.; Shen, Q.; Park, W.S.; Lee, J.Y.; Borlak, J.; Nam, S.W. Sirtuin7 Oncogenic Potential in HUman Hepatocellular Carcinoma and its regulation by the tumor suppressors MiR-125a-5p nd MiR-125b. *Hepatology,* **2013**, *57*(3), 1055-1067.

[18] Schietinger, A.; Philip, M.; Yoshida, B.A.; Azadi, P.; Liu, H.; Meredith, S.C.; Schreiber, H. A mutant chaperone converts a wild-type protein into a tumor-specific antigen. *Science,* **2006**, *314*(5797), 304-308.

[19] Miyagi, T. Aberrant expression of sialidase and cancer progression. *Proc. JPN. Acad. Ser. B. Phys. Biol. Sci.,* **2008**, *84*(10), 407-418.

[20] Miyagi, T.; Wada, T.; Yamaguchi, K.; Hata, K. Sialidase and malignancy: A minireview. *Glycoconj. J.,* **2003**, *20*(3), 189-198.

[21] Kakugawa, Y.; Wada, T.; Yamaguchi, K.; Yamanami, H.; Ouchi, K.; Sato, I.; Miyagi, T. Up-regulation of plasma membrane-associated ganglioside sialidase (Neu3) in human colon cancer and its involvement in apoptosis suppression. *Proc. Nat. Acad. Sci.,* **2002**, *99*(16), 10718-10723.

[22] Dennis, J.W.; Laferté, S.; Waghorne, C.; Breitman, M.L.; Kerbel, R.S. Beta 1-6 branching of Asn-linked oligosaccharides is directly associated with metastasis. *Science,* **1987**, *236(*4801), 582-585.

[23] Kumamoto, K.; Goto, Y.; Sekikawa, K.; Takenoshita, S.; Ishida, N.; Kawakita, M.; Kannagi, R. Increased expression of udp-galactose transporter messenger rna in human colon cancer tissues and its implication in synthesis of thomsen-friedenreich antigen and sialyl lewis a/x determinants. *Cancer Res.,* **2001**, *61*(11), 4620-4627.

[24] Kellokumpu, S.; Sormunen, R.; Kellokumpu, I. Abnormal glycosylation and altered Golgi structure in colorectal cancer: Dependence on intra-Golgi pH. *FEBS Lett.*, **2002**, *516*(1-3), 217-224.

[25] Moremen, K.W.; Tiemeyer, M.; Nairn, A.V. Vertebrate protein glycosylation: Diversity, synthesis and function. *Nat. Rev. Mol. Cell Biol.,* **2012**, *13*(7), 448-462.

[26] Meany, D.L.; Chan, D.W. Aberrant glycosylation associated with enzymes as cancer biomarkers. *Clin. Proteomics,* **2011**, *8*(1), 7.

[27] Vocadlo, D.J.; Davies, G.J. Mechanistic insights into glycosidase chemistry. *Curr. Opin. Chem. Biol.,* **2008**, *12*(5), 539-555.

[28] Gramatikoff, K. Steps in the glycosylation of mammalian n-linked oligosaccarides. Pathways [cited 2014 02 September]; Available at: http://www.biocarta.com/pathfiles/h_aMANPathway.asp#contributors.

[29] Divakar, S.; Glycosidases, *Enzymatic Transforation,* **2013**, 5-21.

[30] Kushen Ramessur, P.G.A.M.D. Glycosidases and breast cancer cell migration through the extracellular matrix. *The Biomed. Scient.,* **2010**.

[31] Prabha, M.N.R.S.; Ravi, V. Specific activity of glycosidases in brain tumors and their expression in primary explants culture. *J. Biochem. Technol.,* **2013**, 5(1), 654-665.

[32] Bellezza, I.; Aisa, M.C.; Palazzo, R.; Costanzi, E.; Mearini, E.; Minelli, A. Extracellular marix degrading enzymes at the prostasome surface. *Prostate Cancer Prost. Dis,* **2005**, 8, 344-348.

[33] Niedbala, M.J.; Madiyalakan, R.; Matta, K.; Crickard, K.; Sharma, M.; Bernacki, R.J. Role of glycosidases in human ovarian carcinoma cell mediated degradation of subendothelial extracellular matrix. *Cancer Res.,* **1987**, *47*, 4634-4641.

[34] Yarema, K.J., Bertozzi, C.R. Characterizing glycosylation pathways. *Genome Biol,* **2001**, 2(5).

[35] Gerber-Lemaire, S.; Juillerat-Jeanneret, L. Glycosylation pathways as drug targets for cancer: Glycosidase Inhibitors. *Mini-Rev. Med. Chem.,* **2006**, *6*(9), 1043-1052.

[36] Yamasaki, Y.; Konno, H. Purification and properties of alpha-glucosidase from suspension-cultures sugar-beet cells. *Phytochemistry,* **1991**, *30*(9). 2861-2863.

[37] Tagami, T.; Yamashita, K.; Okuyama, M.; Mori, H.; Yao, M.; Kimura, A. Sugar b*eet al*pha-glucosidase with acarviosyl-maltohexaose. [cited 2014 02 September]; Available at: http://www.rcsb.org/pdb/explore/explore.do?structureId=3weo.

[38] Day, A.J.; DuPont, M.S.; Ridley, S.; Rhodes, M.; Rhodes, M.J.; Morgan, M.R.; Williamson, G. Deglycosylation of flavonoid and isoflavonoid glycosides by human small intestine and liver β -glucosidase activity. *FEBS Lett.,* 1998. *436*(1), 71-75.

[39] Pelkonen, O.; Jorma, T.A.; Jukka, H.; Miia, T. Consideration of metabolism in *in vitro* cellular systems, in In Vitro toxicology Systems, *Meth. Pharmacol. Toxicol.,* **2014**, 501-519.

[40] Noguchi, J.; Hayashi, Y.; Baba, Y.; Okino, N.; Kimura, M.; Ito, M.; Kakuta, Y. Crystal structure of the covalent intermediate of human cytosolic beta-glucosidase. *Biochem. Biophys. Res. Commun.,* **2008**, *374*(3), 549-552.

[41] Alder, B.J.; Wainwright, T.E. Studies in molecular dynamics. I. general method. *J. Chem. Phys.,* **1959**, *31*(2), 459-466.

[42] Gibson, J.B.; Dynamics of radiation damage. *Phys. Rev.,* **1960**, *120*(4), 1229-1253.

[43] Rahman, A. Normalization of diffraction data from liquids. *J. Chem. Phys*., **1965**, *42*(10), 3540-3542.

[44] Karplus, M.; McCammon, J.A. Molecular dynamics simulations of biomolecules. *Nat. Struct. Biol.,* **2002**, *9*(9), 646-652.

[45] Durrant, J.; McCammon, J.A. Molecular dynamics simulations and drug discovery. *BMC Biol.,* **2011**, *9*(1), 71.

[46] Honarparvar, B.; Govender, T.; Maguire, G.E.; Soliman, M.E.; Kruger, H.G. Integrated approach to structure-based enzymatic drug design: Molecular modeling, spectroscopy, and experimental bioactivity. *Chem. Rev.,* **2013**, *114*(1), 493-537.

[47] Cavasotto, C.N.; Phatak, S.S. Homology modeling in drug discovery: Current trends and applications. *Drug Disc. Today,* **2009**, *14*(13), 676-683.

[48] Power, T.D.; Ivanciuc, O.; Schein, C.H.; Braun, W.Assessment of 3D models for allergen research. *Proteins,* **2013**, *81*(4), 545-554.

[49] Rodrigues, J.P.; Melquiond, A.S.; Karaca, E.; Trellet, M.; Van Dijk M.; Van Zundert G.C.; Schmitz, C.; De Vries S.J.; Bordogna, A.; Bonati, L.; Kastritis, P.L.; Bonvin, A.M. Defining the limits of homology modeling in information-driven protein docking. *Proteins*, **2013**, *81*(12), 2119-2128.

[50] Schmidt, T.; Bergner, A.; Schwede, T. Modelling three-dimensional protein structures for applications in drug design. *Drug Discov. Today*, **2014**, *19*(7), 890-897.

[51] Nakamura, S. Homology modeling of human alpha-glucosidase catalytic domains and sar study of salacinol derivatives. *Open J. Med. Chem.*; **2012**, *2*(03), 50-60.

[52] Ishida, K.; Hirai, G.; Murakami, K.; Teruya, T.; Simizu, S.; Sodeoka, M.; Osada, H. Structure-based design of a selective heparanase inhibitor as an antimetastatic agent. *Mol. Cancer Therapeut.,* **2004**, 3(9).

[53] Park, H.; Hwang, K.Y.; Oh, K.H.; Kim, Y.H.; Lee, J.Y.; Kim, K. Discovery of novel α -glucosidase inhibitors based on the virtual screening with the homology-modeled protein structure. *Bioorg. Med. Chem.,* **2008**, *16*(1) 284-292.

[54] Moorthy, N.S.; Ramos, M.J.; Fernandes, P.A. Topological, hydrophobicity, and other descriptors on alpha-glucosidase inhibition: A QSAR study on xanthone derivatives. *J. Enzyme Inhib. Med. Chem.,* **2011**, 26(6), 755-766.

[55] Liu, T.W.; Ho, C.W.; Huang, H.H.; Chang, S.M.; Popat, S.D.; Wang, Y.T.; Wu, M.S, Chen, Y.J.; Lin, C.H. Role for alpha-L-fucosidase in the control of Helicobacter pylori-infected gastric cancer cells. *Proc. Natl. Acad. Sci. USA,* **2009**, *106*(34), 14581-14586.

[56] Liu, T.W.; Ho, C.W.; Huang, H.H.; Chang, S.M.; Popat, S.D.; Wang, Y.T.; Wu, M.S.; Chen, Y.J.; Lin, C.H. Identification of essential residues of human α -l-fucosidase and tests of its mechanism. *Biochemistry*, **2008**, *48*(1), 110-120.

[57] Patrick, J.; Willems, H.C.S.; Paul, C. Rossana tonlorenzi and john s o'brien, spectrum of mutations in fucosidosis. *Eur. J. Human Genet.,* **1999**, *7*(1), 60-67.

[58] Yuan, K.; Kucik, D.; Singh, R.K.; Listinsky, C.M.; Listinsky, J.J.; Siegal, G.P. Alterations in human breast cancer adhesion-motility in response to changes in cell surface glycoproteins displaying alpha-L-fucose moieties. *Int. J. Oncol,* **2008**, *32*(4), 797-807.

[59] Lammerts van Bueren, A.; Ardèvol, A.; Fayers-Kerr, J.; Luo, B.; Zhang, Y.; Sollogoub, M.; Blériot, Y.; Rovira, C.; Davies, G.J. Analysis of the reaction coordinate of α-l-fucosidases: A combined structural and quantum mechanical approach. *J. Am. Chem. Soc.,* **2010**, *132*(6), 1804-1806.

[60] Lill, M.A. Multi-dimensional QSAR in drug discovery. *Drug Discov. Today*, **2007**, *12*(23-24), 1013-1017.

[61] Pinto, M.M.; Sousa, M.E.; Nascimento, M.S. Xanthone Derivatives: New insights in biological activities. *Curr. Med. Chem.,* **2005**, 12, 2517-2538.

[62] Negi, J.S.; Singh, V.K.B.P.; Rawat, M.S.M.; Joshi, G.P. Naturally occurring xanthones: Chemistry and biology. *J. App. Chem.,* **2013**.

[63] Liu, Y.; Ke, Z.; Cui, J.; Chen, W.H.; Ma, L.; Wang, B. Synthesis, inhibitory activities, and QSAR study of xanthone derivatives as α-glucosidase inhibitors. *Bioorg. Med. Chem.,* **2008**, *16*(15) 7185-7192.

[64] Liu, Y.; Zou, L.; Ma, L.; Chen, W.H.; Wang, B.; Xu, Z.L. Synthesis and pharmacological activities of xanthone derivatives as α-glucosidase inhibitors. *Bioorg. Med. Chem.,* **2006**, *14*(16), 5683-5690.

[65] Narayana Moorthy N.S.; Ramos, M.J.; Fernandes, P.A. Comparative structural analysis of alpha-glucosidase inhibitors on difference species: A computational study. *Arch. Pharm. Chem. Life Sci.,* **2012**, *345*, 265-274.

[66] Yang, Y.; Qin, J.; Liu, H.; Yao, X. Molecular dynamics simulation, free energy calculation and structure-based 3d-qsar studies of b-raf kinase inhibitors. *J. Chem. Inform. Mod.,* **2011**, *51*(3), 680-692.

[67] Xue, Q.; Zhang, J.L.; Zheng, Q.C.; Cui, Y.L.; Chen, L.; Chu, W.T.; Zhang, H.X. Exploring the molecular basis of dsRNA recognition by Mss116p using molecular dynamics simulations and free-energy calculations. *Langmuir*, **2013**, 29(35), 11135-11144.

[68] Cui, Y.L.; Zheng, Q.C.; Zhang, J.L.; Xue, Q.; Wang, Y.; Zhang H.X. Molecular dynamic investigations of the mutational effects on structural characteristics and tunnel geometry in CYP17A1. *J. Chem. Inform. Mod*, **2013**, *53*(12), 3308-3317.

[69] Xu, L.; Sun, H.; Li, Y.; Wang, J.; Hou, T. Assessing the performance of MM/PBSA and MM/GBSA methods. 3. The impact of force fields and ligand charge models. *J. Phys. Chem. B,* **2013**, *117*(28), 8408-8421.

[70] Pang, X.; Zhou, H.X. Poisson-Boltzmann Calculations: Van der waals or molecular surface? *Commun. Comput. Phys.,* **2013**, *13*(1), 1-12.

[71] Alonso, H.; Bliznyuk, A.A.; Gready, J.E. Combining docking and molecular dynamic simulations in drug design. *Med. Res. Rev.,* **2006**, **26**(5), 531-568.

[72] McInnes, C. Virtual screening strategies in drug discovery. *Curr. Opin. Chem. Biol.,* **2007**, *11*(5), 494-502.

[73] Sun, H.; Li, Y.; Tian, S.; Xu, L.; Hou, T. Assessing the performance of MM/PBSA and MM/GBSA methods. 4. accuracies of MM/PBSA and MM/GBSA methodologies evaluated by various simulation protocols using PDBbind data set. *Phys. Chem. Chem. Phys.,* **2014**, *16*(31) 16719-16729.

[74] Jorgensen, W.L.; Thomas, L.L. Perspective on free-energy perturbation calculations for chemical equilibria. *J. Chem. Theory Comput*., **2008**, *4*(6), 869-876.

[75] Chipot, C. Frontiers in free-energy calculations of biological systems. Wiley Interdisciplinary Reviews: *Comput. Mol. Sci.,* **2014**, *4*(1), 71-89.

[76] Meyer, E.F.; Swanson, S.M.; Williams, J.A. Molecular modelling and drug design. Pharmacology & Therapeutics, **2000**, *85*(3), 113-121.

[77] Caflisch, A. Focal Point: Medicinal Chemistry. *Chimia*, 1999. *50*, 35-36.

[78] Redhu, S.; Jindal, A. Molecular modelling: A new scaffolding for drug design. *Int. J. Pharm. Pharmaceut. Sci*., **2013**, 5(1).

[79] Friedman, R.; Boye, K.; Flatmark, K. Molecular modelling and simulations in cancer research. *Biochim. Biophys. Acta,* **2013**, 1836(1), 1-14.

[80] Berecibar, A.; Grandjean, C.; Siriwardena, A. Synthesis and biological activity of natural aminocyclopentitol glycosidase inhibitors: Mannostatins, trehazolin, allosamidins, and their analogues. *Chem. Rev.,* 1999. *99*, 779-844.

[81] Germer, A.; Peter, M.G.; Kleinpeter, E. Solution-State conformational study of the hevamine inhibitor allosamidin and six potential inhibitor analogues by nmr spectroscopy and molecular modeling. *J. Org. Chem.,* **2002**, *67*(18), 6328-6338.

[82] Bortone, K.; Monzingo, A.F.; Ernst, S.; Robertus, J.D. The Structure of an allosamidin complex with the coccidioides immitis chitinase defines a role for a second acid residue in substrate-assisted mechanism. *J. Mol. Biol.,* **2002**, *320*(2), 293-302.

[83] Kawatkar, S.P. Molecular modeling insights into the inhibition and catalytic mechanism of golgi alpha-mannosidase II, **2006**, University of Georgia Athens, Georgia.

[84] Elbein, A.D.; Tropea, J.E.; Mitchell, M.; Kaushal, G.P. Kifunensine, a potent inhibitor of the glycoprotein processing mannosidase I. *J. Biol. Chem*., **1990**, *265*(26), 15599-15605.

[85] Michael, J.P. Indolizidine and quinolizidine alkaloids. *Nat. Prod. Rep.,* **2004**, *21*(5), 625-649.

[86] Borges de Melo, E.; Da Silveira Gomes, A.; Carvalho, I. α- and β-Glucosidase inhibitors: Chemical structure and biological activity. *Tetrahedron,* **2006**, *62*(44), 10277-10302.

[87] Da Rocha A.B.; Lopes, R.M.; Schwartsmann, G. Natural products in anticancer therapy. *Curr. Opin. Pharmacol.,* **2001**, *1*, 364-369.

[88] Shah, N.; Kuntz, D.A.; Rose, D.R. Comparison of kifunensine and 1-deoxymannojirimycin binding to class I and II alpha-mannosidases demonstrates different saccharide distortions in inverting and retaining catalytic mechanisms. *Biochemistry*, **2003**, *42*(47), 13812-13816.

[89] Wen, X.; Yuan, Y.; Kuntz, D.A.; Rose, D.R.; Pinto, B.M. A combined STD-NMR/molecular modeling protocol for predicting the binding modes of the glycosidase inhibitors kifunensine and salacinol to Golgi alpha-mannosidase II. *Biochemistry*, **2005**, *44*(18), 6729-6737.

[90] Nobili, S.; Lippi, D.; Witort, E.; Donnini, M.; Bausi, L.; Mini, E.; Capaccioli, S. Natural compounds for cancer treatment and prevention. *Pharmacol. Res*., **2009**, 59(6), 365-378.

[91] Kawatkar, S.P.; Kuntz, D.A.; Woods, R.J.; Rose, D.R.; Boons GJ. Structural basis of the inhibiton of golgi alpha-mannosidase II by Mannostatin A and the role of the thiomethyl moiety in ligand-protein interactions. **2006**.

[92] Kuntz, D.A.; Zhong, W.; Guo, J.; Rose, D.R.; Boons, G.J. The molecular basis of inhibition of Golgi alpha-mannosidase II by mannostatin A. *Chem.Bio.Chem.,* **2009**, *10*(2) 268-277.

[93] Francesca, C.; Andrea, G.; Alberto, B.; Maria, S.; Neri, N.; Stefano, M. Molecular dynamics simulations on the complexes of Glucoamylase II (471) from Aspergillus awamori var. X100 with 1-Deoxynojirimycin and lentiginosine. *Mol. Mod. Ann.,* **1997**, *3*(7), 249-260.

[94] Jin-Ming, Z.; Jun-Hong, Z.; Yi, M.; Min-Bo, C. Molecular dynamics simulation of iminosugar inhibitor−glycosidase complex: insight into the binding mechanism of 1-deoxynojirimycin and isofagomine toward β-Glucosidase. *J. Chem. Theor. Comput.,* **2005**, *2*(1), 157-165.

[95] Karplus, M.; Kushick, J.N. Method for estimating the configurational entropy of macromolecules. *Macromolecules,* **1981**, *14*(2) 325-332.

[96] Ilario, G.T.; René, S.; Paul, E.S.; Wilfred, F.; Van, G. A generalized reaction field method for molecular dynamics simulations. *J. Chemi. Phys.,* **1995**, *102*(13), 5451-5459.

[97] Pereira, C.S.; Kony, D.; Baron, R.; Müller, M.; Van Gunsteren, W.F.; Hünenberger, PH.Conformational and dynamical properties of disaccharides in water: A molecular dynamics study. *Biophys. J.,* **2006**, *90*(12), 4337-4344.

[98] Kräutler, V.M.; Müller, M.; Hünenberger, P.H. Conformation, dynamics, solvation and relative stabilities of selected β-hexopyranoses in water: a molecular dynamics study with the gromos 45A4 force field. *Carbohydrate Res.,* **2007**, *342*(14), 2097-2124.

[99] Lerbret, A.; Bordat, P.; Affouard, F.; Descamps, M.; Migliardo, F. How homogeneous are the trehalose, maltose, and sucrose water solutions? An insight from molecular dynamics simulations. *J. Phys. Chem. B,* **2005**, *109*(21), 11046-11057.

[100] Sastry, G.M.; Agmon, N. Trehalose prevents myoglobin collapse and preserves its internal mobility. *Biochemistry*, 1997. *36*(23), 7097-7108.

[101] Brady, J.W.; Schmidt, R.K. The role of hydrogen bonding in carbohydrates: Molecular dynamics simulations of maltose in aqueous solution. *J. Phys. Chem.,* **1993**, *97*(4), 958-966.

[102] Qiang, L.; Schmidt, R.K.; Teo, B.; Karplus, P.A.; Brady, J.W. Molecular dynamics studies of the hydration of α,α-Trehalose. *J. Am. Chem. Soc.,* **1997**, *119*(33), 7851-7862.

[103] Minoru, S.; Masashi, M.; Yoshio, I.; Akihiro, H.; Syouichi, K. Molecular-dynamics study of aqueous solution of trehalose and maltose: Implication for the biological function of trehalose. *Bull. Chem. Society Japan,* **1997**, *70*(4), 847-858.

[104] Roberts, C.J.; Debenedetti, P.G. Structure and dynamics in concentrated, amorphous carbohydrate−water systems by molecular dynamics simulation. *J. Physical Chem. B,* **1999**, *103*(34), 7308-7318.

[105] Conrad, P.B.; De Pablo, J.J. Computer simulation of the cryoprotectant disaccharide α,α-Trehalose in aqueous solution. *J. Physical Chem.* A, **1999**, *103*(20), 4049-4055.

[106] Bonanno, G.; Noto, R.; Fornili, S.L. Water interaction with [small alpha],[small alpha]-trehalose: Molecular dynamics simulation. *J. Chem. Soc, Faraday Trans.,* **1998**, *94*(18), 2755-2762.

[107] Naidoo, K.J.; Kuttel, M. Water structure about the dimer and hexamer repeat units of amylose from molecular dynamics computer simulations. *J. Comput. Chem.,* **2001**, *22*(4), 445-456.

[108] Engelsen, S.B.; Monteiro, C.; Hervé de Penhoat, C.; Pérez, S. The diluted aqueous solvation of carbohydrates as inferred from molecular dynamics simulations and NMR spectroscopy. *Biophys. Chem.,* **2001**, 93(2-3),103-127.

[109] Ekdawi-Sever, N. Diffusion of Sucrose and α,α-Trehalose in Aqueous Solutions. *J. Phy. Chem. A,* **2003**, *107*(6), 936-943.

[110] Molinero, V.; Çağın, T.; Goddard Iii, W.A. Sugar, water and free volume networks in concentrated sucrose solutions. *Chem. Phy. Lett*., **2003**, *377*(3-4), 469-474.

[111] Bordat, P.; Lerbret, A.; Demaret, J.P.; Affouard, F.; Descamps, M. Comparative study of trehalose, sucrose and maltose in water solutions by molecular modelling. *Euro.Phys. Lett.,* **2004**, *65*(47).

[112] Sum, A.K.; Faller, R.; Pablo, J.J.D. Molecular simulation study of phospholipid bilayers and insights of the interactions with disaccharides. *Biophys. J.*; **2003**, *85*(5), 2830-2844.

[113] Pereira, C.S.; Lins, R.D.; Chandrasekhar, I.; Freitas, L.C Hünenberger, P.H. Interaction of the disaccharide trehalose with a phospholipid bilayer: A molecular dynamics study. *Biophys. J.,* 2004. *86*(4), 2273-2285.

[114] Pereira, C.S.; Hünenberger, P.H. Interaction of the Sugars trehalose, Maltose and glucose with a phospholipid bilayer: A comparative molecular dynamics study. *J. Phys. Chem. B,* **2006**, *110*(31), 15572-15581.

[115] Lerbret, A. Molecular dynamics simulations of lysozyme in water/sugar solutions. *Chem. Phys.,* **2008**, *345*(2-3), 267-274.

[116] Venerando, P.; Giuseppe, M.L.; Antonio, R.; Alessia, B.; Felicia D', A.; Francesco, P. Experimental and in silico characterization of a biologically active inosose. *Struct. Chem*., **2013**, *24*(3) 955-965.

Received: August 15, 2014 Revised: September 16, 2014 Accepted: December 10, 2014