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(71) Applicant: **ALBERT EINSTEIN COLLEGE OF MEDICINE, INC.** [US/US]; 1300 Morris Park Avenue, Bronx, NY 10461 (US).

(72) Inventors: **YEH, Syun-Ru**; 251 Village Circle Drive, Fort Lee, NJ 07024 (US). **LEWIS-BALLESTER, Ariel**; 2523 Eastchester Road, BSMT, Bronz, NY 10469 (US). **PHAM, Khoa, Ngoc**; 20220 SW 48th Street, Miami, FL 33165 (US).

(74) Agent: **AMOS, Brian, J.** et al.; Amster, Rothstein & Ebenstein LLP, 90 Park Avenue, New York, NY 10016 (US).

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(54) Title: DRUG TARGETING OF HUMAN INDOLEAMINE 2,3-DIOXYGENASE

(57) Abstract: A system and a method for identifying novel hIDO inhibitors.

DRUG TARGETING OF HUMAN INDOLEAMINE 2,3-DIOXYGENASE

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit of U.S. Provisional Application No. 62/430,408, filed December 6, 2016, the contents of which are hereby incorporated by reference.

STATEMENT OF GOVERNMENT SUPPORT

[0002] This invention was made with government support under grant number GM086482 awarded by the National Institutes of Health. The government has certain rights in the invention.

MEGATABLES INCOPORATION

[0003] The ".txt" MEGATABLES 1, 2 and 3 filed by EFS in connection with the priority application, U.S. Provisional Application No. 62/430,408, filed December 6, 2016, and which are, respectively (i) IDEWTCNTRP_ref13_May24.txt, 978 kilobytes in size, created on November 23, 2016; (ii) IDES167H17_refine_37-may23.txt, 1 MB in size, created on November 23, 2016, and (iii), F270G30day10_refine_11.txt, 986 kilobytes in size created on November 23, 2016, are each hereby incorporated by reference.

BACKGROUND OF THE INVENTION

[0004] Throughout this application various publications are referred to. Full citations for the references may be found at the end of the specification. The disclosures of these publications are hereby incorporated by reference in their entirety into the subject application to more fully describe the art to which the subject invention pertains.

[0005] Human Indoleamine 2,3-dioxygenase (hIDO) is an immunosuppressive enzyme, which is induced in a wide range of cancers (1). It is critical for cancer immune escape, as well as transplant and maternal-fetal tolerance (2). Consequently, hIDO has been identified as an important therapeutic target (3). Recent advances in the development of hIDO-inhibitors as immunotherapy for cancer have trigger several million-to-billion dollar deals in pharmaceutical industry to license hIDO-inhibitors.

[0006] Owing to poor structural information, so far all the structural-based drug design of hIDO inhibitors is limited to substrate analogs that target the active site (4). The use of

the active site as the drug target poses a variety of problems. First, the active site exhibits unusual structural flexibility, hindering the utility of structure-based drug design. Second, drugs bound in the active site can potentially coordinate to or interact with the heme iron, leading to poor selectivity against other heme-containing proteins, such as P450's. In addition, the potential interaction of drugs with the heme iron causes strong sensitivity of hIDO to the electronic nature of drug candidates, making in-silico drug design difficult. Last, but not the least, the active site does not only bind Trp, but it also binds O_2 (on the heme iron), making the determination of the inhibition modes cumbersome

[0007] The present invention addresses the need for a new method and system for identifying novel hIDO inhibitors.

SUMMARY OF THE INVENTION

[0008] Provided is a non-transitory computer-readable medium coupled to the one or more data processing apparatus having instructions stored thereon which, when executed by the one or more data processing apparatus, cause the one or more data processing apparatus to perform a method comprising:

providing a crystal coordinate structure of the small molecule binding site on a human indoleamine 2,3-dioxygenase (hIDO) enzyme, wherein the small molecule binding site is not a hIDO active site;

contacting the small molecule binding site in silico with a candidate small organic molecule structure; and

determining if the candidate small organic molecule structure binds the small molecule binding site at a free energy level below a predetermined free energy level,

wherein a candidate small organic molecule structure that binds the small molecule binding site at a free energy level below the predetermined free energy level is identified and detected as a molecule which binds to a small molecule binding site on a human indoleamine 2,3-dioxygenase (hIDO) enzyme.

[0009] Also provided is a system for identifying a cognate ligand molecule for a known biological receptor comprised of amino acid residues, comprising:

one or more data processing apparatus;

a graphical user interface; and

a non-transitory computer-readable medium coupled to the one or more data processing apparatus having instructions stored thereon which, when executed by the one or more data processing apparatus, cause the one or more data processing apparatus to perform a method comprising:

providing a crystal coordinate structure of the small molecule binding site on a human indoleamine 2,3-dioxygenase (hIDO) enzyme, wherein the small molecule binding site is not a hIDO active site;

contacting the small molecule binding site in silico with a candidate small organic molecule structure; and

determining if the candidate small organic molecule structure binds the small molecule binding site at a free energy level below a predetermined free energy level,

wherein a candidate small organic molecule structure that binds the small molecule binding site at a free energy level below the predetermined free energy level is identified and detected as a molecule which binds to a small molecule binding site on a human indoleamine 2,3-dioxygenase (hIDO) enzyme.

[0010] Also provided is a method of detecting a molecule which binds to a small molecule binding site on a human indoleamine 2,3-dioxygenase (hIDO) enzyme, wherein the small molecule binding site is not a hIDO active site, comprising:

providing a crystal coordinate structure of the small molecule binding site on a human indoleamine 2,3-dioxygenase (hIDO) enzyme, wherein the small molecule binding site is not a hIDO active site;

contacting the small molecule binding site in silico with a candidate small organic molecule structure; and

determining if the candidate small organic molecule structure binds the small molecule binding site at a free energy level below a predetermined free energy level,

wherein a candidate small organic molecule structure that binds the small molecule binding site at a free energy level below the predetermined free energy level is identified and detected as a molecule which binds to a small molecule binding site on a human indoleamine 2,3-dioxygenase (hIDO) enzyme.

[0011] A method of activating or increasing activation of human indoleamine 2,3-dioxygenase (hIDO) enzyme comprising contacting the hIDO with an amount of Apigenin, Baicalein or Chrysin having the structures as set forth in Table 1, or a pharmaceutical salt of one thereof, in an amount effective to activating or increasing activation of hIDO.

[0012] A method of effecting immunosuppression in a subject comprising administering an amount of Apigenin, Baicalein or Chrysin having the structures as set forth in Table 1, or a pharmaceutical salt of one thereof, in an amount effective to activate or increasing activation of hIDO.

[0013] A method of treating an autoimmune disease or inhibiting an organ transplant rejection in a subject comprising administering an amount of Apigenin, Baicalein or Chrysin having the structures as set forth in Table 1, or a pharmaceutical salt of one thereof, in an amount effective to treat an autoimmune disease or inhibit an organ transplant rejection.

[0014] A pharmaceutical composition for activating or increasing activation of human indoleamine 2,3-dioxygenase comprising an amount of Apigenin, Baicalein or Chrysin having the structures as set forth in Table 1, or a pharmaceutical salt of one thereof, and a pharmaceutically acceptable carrier.

[0015] Additional objects of the invention will be apparent from the description which follows.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] Fig. 1A-1F. Three dimensional structure of substrate-bound hIDO showing the presence of two small molecule binding sites (Sa and Si) and solution data demonstrating the ability of the Si site in binding the substrate Trp, an effector IDE and an uncompetitive inhibitor MitoC. In (a), the heme and its two axial ligands are labeled in magenta, the Trp bound in the Sa site is labeled in green, while the indole ethanol (IDE) bound in the Si site is labeled in cyan. The bottom inset in (1a) shows the blow-up view of the specific interactions between the small molecules bound in the Sa and Si sites and their surrounding environment. (1b-1c) Activity data supporting the inhibition effect of Trp binding to the Si site, as well as a cartoon illustrating the associated mechanism. (1d) Activity data demonstrating the enhanced activity induced by hIDO binding to the Si site. (1e) Uncompetitive inhibition of hIDO induced by Mitomycin C (MitoC) binding to the Si site. (1f) Chemical structures of Trp, IDE and MitoC.

DETAILED DESCRIPTION OF THE INVENTION

[0017] Provided is a non-transitory computer-readable medium coupled to the one or more data processing apparatus having instructions stored thereon which, when executed by

the one or more data processing apparatus, cause the one or more data processing apparatus to perform a method comprising:

providing a crystal coordinate structure of the small molecule binding site on a human indoleamine 2,3-dioxygenase (hIDO) enzyme, wherein the small molecule binding site is not a hIDO active site;

contacting the small molecule binding site in silico with a candidate small organic molecule structure; and

determining if the candidate small organic molecule structure binds the small molecule binding site at a free energy level below a predetermined free energy level,

wherein a candidate small organic molecule structure that binds the small molecule binding site at a free energy level below the predetermined free energy level is identified and detected as a molecule which binds to a small molecule binding site on a human indoleamine 2,3-dioxygenase (hIDO) enzyme.

[0018] In an embodiment, the crystal coordinate structure is provided in Megatable 1, 2 or 3 as listed herein.

[0019] Also provided is a system for identifying a cognate ligand molecule for a known biological receptor comprised of amino acid residues, comprising:

one or more data processing apparatus;

a graphical user interface; and

a non-transitory computer-readable medium coupled to the one or more data processing apparatus having instructions stored thereon which, when executed by the one or more data processing apparatus, cause the one or more data processing apparatus to perform a method comprising:

providing a crystal coordinate structure of the small molecule binding site on a human indoleamine 2,3-dioxygenase (hIDO) enzyme, wherein the small molecule binding site is not a hIDO active site;

contacting the small molecule binding site in silico with a candidate small organic molecule structure; and

determining if the candidate small organic molecule structure binds the small molecule binding site at a free energy level below a predetermined free energy level,

wherein a candidate small organic molecule structure that binds the small molecule binding site at a free energy level below the predetermined free energy level is identified and

detected as a molecule which binds to a small molecule binding site on a human indoleamine 2,3-dioxygenase (hIDO) enzyme.

[0020] In an embodiment, the crystal coordinate structure is provided in Megatable 1, 2 or 3 as listed herein.

[0021] Also provided is a method of detecting a molecule which binds to a small molecule binding site on a human indoleamine 2,3-dioxygenase (hIDO) enzyme, wherein the small molecule binding site is not a hIDO active site, comprising:

providing a crystal coordinate structure of the small molecule binding site on a human indoleamine 2,3-dioxygenase (hIDO) enzyme, wherein the small molecule binding site is not a hIDO active site;

contacting the small molecule binding site in silico with a candidate small organic molecule structure; and

determining if the candidate small organic molecule structure binds the small molecule binding site at a free energy level below a predetermined free energy level,

wherein a candidate small organic molecule structure that binds the small molecule binding site at a free energy level below the predetermined free energy level is identified and detected as a molecule which binds to a small molecule binding site on a human indoleamine 2,3-dioxygenase (hIDO) enzyme.

[0022] In an embodiment, the crystal coordinate structure is provided in Megatable 1, 2 or 3 as listed herein.

[0023] In an embodiment of the inventions, the candidate small organic molecule structure is an analog of tryptophan. In an embodiment of the inventions, the analog of tryptophan comprises an indole of tryptophan.

[0024] In an embodiment of the inventions, the candidate small organic molecule structure is an analog of indole ethanol.

[0025] In an embodiment of the inventions, the candidate small organic molecule structure is an analog of mitomycin C.

[0026] In an embodiment of the methods, the method further comprises contacting an isolated human indoleamine 2,3-dioxygenase (hIDO) enzyme with an amount of the candidate agent identified as binding to the small molecule binding site on the hIDO in the presence an amount of L-tryptophan and determining whether the candidate agent increases or decreases a rate of degradation of the L-tryptophan to N-formylkynurenine relative to a predetermined control rate of degradation, and identifying the candidate agent as an agonist

of hIDO where the candidate agent increases the rate of degradation, or identifying the candidate agent as an antagonist of hIDO where the candidate agent decreases the rate of degradation.

[0027] Embodiments of the invention and all of the functional operations described in this specification can be implemented in digital electronic circuitry, or in computer software, firmware, or hardware, including the structures disclosed in this specification and their structural equivalents, or in combinations of one or more of them. Embodiments of the invention can be implemented as one or more computer program products, i.e., one or more modules of computer program instructions encoded on a non-transitory computer readable medium for execution by, or to control the operation of, data processing apparatus. The non-transitory computer readable medium can be a machine readable storage device, a machine readable storage substrate, a memory device, or a combination of one or more of them. The term "data processing apparatus" encompasses all apparatus, devices, and machines for processing data, including by way of example a programmable processor, a computer, or multiple processors or computers. The apparatus can include, in addition to hardware, code that creates an execution environment for the computer program in question, e.g., code that constitutes processor firmware, a protocol stack, a database including a database management system, an operating system, or a combination of one or more of them.

[0028] A computer program (also known as a program, software, software application, script, or code) can be written in any form of programming language, including compiled or interpreted languages, and it can be deployed in any form, including as a stand-alone program or as a module, component, subroutine, or other unit suitable for use in a computing environment. A computer program does not necessarily correspond to a file in a file system. A program can be stored in a portion of a file that holds other programs or data (e.g., one or more scripts stored in a markup language document), in a single file dedicated to the program in question, or in multiple coordinated files (e.g., files that store one or more modules, sub-programs, or portions of code). A computer program can be deployed to be executed on one computer or on multiple computers that are located at one site or distributed across multiple sites and interconnected by a communication network.

[0029] The processes and logic flows described in this specification can be performed by one or more programmable processors executing one or more computer programs to perform functions by operating on input data and generating output. The processes and

logic flows can also be performed by, and apparatus can also be implemented as, special purpose logic circuitry, e.g., an FPGA (field programmable gate array) or an ASIC (application-specific integrated circuit).

[0030] Processors suitable for the execution of a computer program include, by way of example, both general and special purpose microprocessors, and any one or more processors of any kind of digital computer. Generally, a processor will receive instructions and data from a read-only memory or a random access memory or both. The essential elements of a computer are a processor for performing instructions and one or more memory devices for storing instructions and data. Generally, a computer will also include, or be operatively coupled to receive data from or transfer data to, or both, one or more mass storage devices for storing data, e.g., magnetic, magneto-optical disks, or optical disks. However, a computer need not have such devices. Moreover, a computer can be embedded in another device, e.g., a mobile telephone, a personal digital assistant (PDA), a mobile audio player, a Global Positioning System (GPS) receiver, to name just a few. Non-transitory computer-readable media suitable for storing computer program instructions and data include all forms of non-volatile memory, media and memory devices, including by way of example semiconductor memory devices, e.g., EPROM, EEPROM, and flash memory devices; magnetic disks, e.g., internal hard disks or removable disks; magneto-optical disks; and CD-ROM and DVD-ROM disks. The processor and the memory can be supplemented by, or incorporated in, special purpose logic circuitry.

[0031] To provide for interaction with a user, embodiments of the invention can be implemented on a computer having a display device, e.g., a CRT (cathode ray tube) or LCD (liquid crystal display) monitor, for displaying information to the user and a keyboard and a pointing device, e.g., a mouse or a trackball, by which the user can provide input to the computer. Other kinds of devices can be used to provide for interaction with a user as well; for example, feedback provided to the user can be any form of sensory feedback, e.g., visual feedback, auditory feedback, or tactile feedback; and input from the user can be received in any form, including acoustic, speech, or tactile input.

[0032] Embodiments of the invention can be implemented in a computing system that includes a back-end component, e.g., as a data server, or that includes a middleware component, e.g., an application server, or that includes a front-end component, e.g., a client computer having a graphical user interface or a Web browser through which a user can interact with an implementation of the invention, or any combination of one or more such

back-end, middleware, or front-end components. The components of the system can be interconnected by any form or medium of digital data communication, e.g., a communication network. Examples of communication networks include a local area network ("LAN") and a wide area network ("WAN"), e.g., the Internet.

[0033] The computing system can include clients and servers. A client and server are generally remote from each other and typically interact through a communication network. The relationship of client and server arises by virtue of computer programs running on the respective computers and having a client-server relationship to each other.

[0034] A non-transitory computer readable medium comprising instructions stored thereon for performing the methods described herein is also provided.

[0035] A method of activating or increasing activation of human indoleamine 2,3-dioxygenase (hIDO) enzyme comprising contacting the hIDO with an amount of Apigenin, Baicalein or Chrysin having the structures as set forth in Table 1, or a pharmaceutical salt of one thereof, in an amount effective to activating or increasing activation of hIDO.

[0036] In an embodiment, the hIDO is contacted with the amount of a pharmaceutical salt of Apigenin, Baicalein or Chrysin.

[0037] A method of effecting immunosuppression in a subject comprising administering an amount of Apigenin, Baicalein or Chrysin having the structures as set forth in Table 1, or a pharmaceutical salt of one thereof, in an amount effective to activate or increasing activation of hIDO.

[0038] In an embodiment, the hIDO is contacted with the amount of a pharmaceutical salt of Apigenin, Baicalein or Chrysin.

[0039] A method of treating an autoimmune disease or inhibiting an organ transplant rejection in a subject comprising administering an amount of Apigenin, Baicalein or Chrysin having the structures as set forth in Table 1, or a pharmaceutical salt of one thereof, in an amount effective to treat an autoimmune disease or inhibit an organ transplant rejection.

[0040] In an embodiment, the hIDO is contacted with the amount of a pharmaceutical salt of Apigenin, Baicalein or Chrysin. In an embodiment, the autoimmune disease is treated. In an embodiment, the organ transplant rejection is inhibited. In an embodiment, the Apigenin, Baicalein or Chrysin is administered parenterally.

[0041] Autoimmune diseases treatable by the invention include acute disseminated encephalomyelitis (ADEM), alopecia areata, antiphospholipid syndrome, autoimmune

cardiomyopathy, autoimmune hemolytic anemia, autoimmune hepatitis, autoimmune inner ear disease, autoimmune lymphoproliferative syndrome, autoimmune peripheral neuropathy, autoimmune pancreatitis, autoimmune polyendocrine syndrome, autoimmune progesterone dermatitis, autoimmune thrombocytopenic purpura, autoimmune urticarial, autoimmune uveitis, Behçet's disease, celiac disease, Chagas disease, cold agglutinin disease, Crohn's disease, dermatomyositis, diabetes mellitus type 1, eosinophilic fasciitis, gastrointestinal pemphigoid, Goodpasture's syndrome, Grave's syndrome, Guillain-Barré syndrome, Hashimoto's encephalopathy, Hashimoto's thyroiditis, lupus erythematosus, Miller-Fisher syndrome, mixed connective tissue disease, myasthenia gravis, pemphigus vulgaris, pernicious anaemia, polymyositis, psoriasis, psoriatic arthritis, relapsing polychondritis, rheumatoid arthritis, rheumatic fever, Sjögren's syndrome, temporal arteritis, transverse myelitis, ulcerative colitis, undifferentiated connective tissue disease, vasculitis, Wegener's granulomatosis.

[0042] A pharmaceutical composition for activating or increasing activation of human indoleamine 2,3-dioxygenase comprising an amount of Apigenin, Baicalein or Chrysin having the structures as set forth in Table 1, or a pharmaceutical salt of one thereof, and a pharmaceutically acceptable carrier.

[0043] Administration can be auricular, buccal, conjunctival, cutaneous, subcutaneous, endocervical, endosinusial, endotracheal, enteral, epidural, via hemodialysis, interstitial, intrabdominal, intraamniotic, intra-arterial, intra-articular, intrabiliary, intrabronchial, intrabursal, intracardiac, intracartilaginous, intracaudal, intracavernous, intracavitary, intracerebral, intracisternal, intracorneal, intracoronary, intradermal, intradiscal, intraductal, intraepidermal, intraesophagus, intragastric, intravaginal, intragingival, intraileal, intraluminal, intralesional, intralymphatic, intramedullary, intrameningeal, intramuscular, intraocular, intraovarian, intraepicardial, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrasinal, intraspinal, intrasynovial, intratendinous, intratesticular, intrathecal, intrathoracic, intratubular, intratumor, intratympanic, intrauterine, intravascular, intravenous, intraventricular, intravesical, intravitreal, laryngeal, nasal, nasogastric, ophthalmic, oral, oropharyngeal, parenteral, percutaneous, periarticular, peridural, rectal, inhalationally, retrobulbar, subarachnoid, subconjuctival, sublingual, submucosal, topically, transdermal, transmucosal, transplacental, transtracheal, ureteral, uretheral, and vaginal.

[0044] The compounds of the instant invention may be in a salt form. As used herein, a "salt" is salt of the instant compounds which has been modified by making acid or base,

salts of the compounds. In the case of compounds used for treatment of a disease, the salt is pharmaceutically acceptable. Examples of pharmaceutically acceptable salts include, but are not limited to, mineral or organic acid salts of basic residues such as amines; alkali or organic salts of acidic residues such as phenols. The salts can be made using an organic or inorganic acid. Such acid salts are chlorides, bromides, sulfates, nitrates, phosphates, sulfonates, formates, tartrates, maleates, malates, citrates, benzoates, salicylates, ascorbates, and the like. Phenolate salts are the alkaline earth metal salts, sodium, potassium or lithium. The term "pharmaceutically acceptable salt" in this respect, refers to the relatively non-toxic, inorganic and organic acid or base addition salts of compounds of the present invention. These salts can be prepared in situ during the final isolation and purification of the compounds of the invention, or by separately reacting a purified compound of the invention in its free base or free acid form with a suitable organic or inorganic acid or base, and isolating the salt thus formed. Representative salts include the hydrobromide, hydrochloride, sulfate, bisulfate, phosphate, nitrate, acetate, valerate, oleate, palmitate, stearate, laurate, benzoate, lactate, phosphate, tosylate, citrate, maleate, fumarate, succinate, tartrate, napthylate, mesylate, glucoheptonate, lactobionate, and laurylsulphonate salts and the like. (See, e.g., Berge et al. (1977) "Pharmaceutical Salts", J. Pharm. Sci . 66:1-19).

[0045] All combinations of the various elements described herein are within the scope of the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

[0046] This invention will be better understood from the Experimental Details, which follow. However, one skilled in the art will readily appreciate that the specific methods and results discussed are merely illustrative of the invention as described more fully in the claims that follow thereafter.

Experimental Details

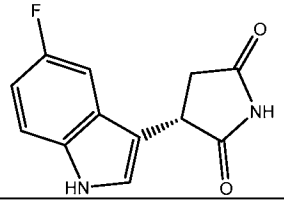
[0047] The crystal structure of hIDO was first solved in 2006, based on two substrate-free complexes (5). Subsequently, several additional inhibitor-bound structures were reported (6,7). However, until now drug design has targeted the active site. Herein the inventors have solved the crystal structures of substrate-bound complexes of hIDO for the first time. In addition to the active site (Sa), which binds the substrate tryptophan (Trp), a previously unknown small molecule binding site (Si) is identified herein which is, in the

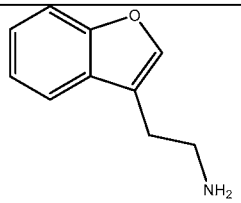
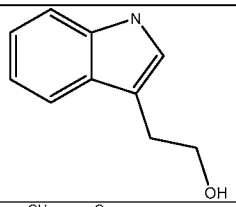
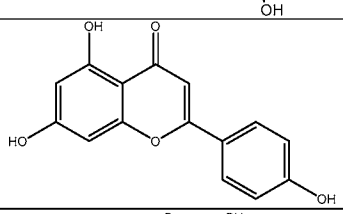
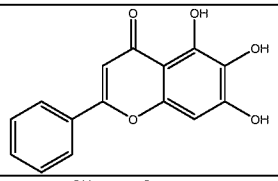
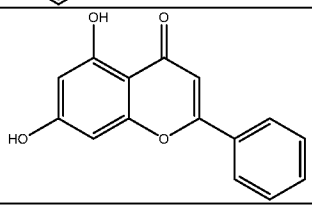
physiological context, occupied by either a second substrate, Trp, or an effector, indole ethanol (IDE) (Fig. 1a).

[0048] The existence of the Si site in hIDO was first proposed by us in 2009 based on solution studies (8). Our solution data indicate that the binding of Trp to the Si site inhibits the enzyme activity (Fig. 2b-c) (8), while the binding of the effector IDE to the Si site promotes it (Fig. 2d)8. In addition, the data suggest that the Si site can bind an uncompetitive inhibitor MitoC, thereby inhibiting the enzyme activity (with a Ki of 25 μM, Fig. 2e)8. It is notable that for decades MitoC has been widely used as a chemotherapy drug for several types of cancer (9). Our data demonstrate that hIDO is a target of MitoC that at least partially accounts for its therapeutic effect.

[0049] The possibility that an Si site in hIDO might exist was controversial due to the lack of direct structural evidence supporting it. New crystallographic data obtained herein shows that an Si site in hIDO does indeed exist, and also provides the three-dimensional structural coordinate data of the Si site that can be used to identify structural analogs of IDE, Trp and MitoC, as agonists or antagonists for hIDO, which can be used alone or in combination with Sa site inhibitors for treating cancer or suppressing organ transplantation rejection. Obtaining this data was a demanding task. Obtaining crystals of the enzyme, in which the target site is occupied, is a challenging task. Two mutants of the enzyme were designed and, in concert with use of an effector (IDE), establishing the existence and identification of the site was finally achieved. The wild type enzyme was used as well as two mutants: F270G and S167H. Megatables 1, 2 and 3, incorporated by reference herein, contain the full crystal structural data and are .txt versions of Protein Databank (PDB) files of (i) the wild type enzyme, (ii) a mutant (S167H), and (iii) a second mutant, (F270G). It was demonstrated that in the wild type enzyme, IDE bound in the novel target site, Si; in the F270G mutant, Trp bound in the Si site, and in the F270G mutant, IDE could bind the Si site also.

[0050] Table 1: Examples of compounds tested for activity at the Si site by Xtal/docking.

Activity type	Molecule name	Structure
Inhibitor	Pfizer (PF068400003)	

Inhibitor	Ark1 (2-Benzofuran-3-yl-ethylamine)	
Effector	IDE	
Effector	Apigenin	
Effector	Baicalein	
Effector	Chrysin	

[0051] A number of compounds were tested for Si activity using the methods presented herein. Pfizer (PF068400003) and Ark1 (2-Benzofuran-3-yl-ethylamine), not previously known to act on the Si site (as opposed to the Sa site) indicated inhibitory activity. IDE showed effector activity. Apigenin, Baicalein, and Chrysin, none previously known to have hIDO effects, were all determined to be effectors at the hIDO Si site.

REFERENCES

1. Uyttenhove, C. et al. Evidence for a tumoral immune resistance mechanism based on tryptophan degradation by indoleamine 2,3-dioxygenase. Nat Med 9, 1269-74 (2003).

2. Munn, D.H. & Mellor, A.L. Indoleamine 2,3 dioxygenase and metabolic control of immune responses. Trends Immunol 34, 137-43 (2013).

3. Sheridan, C. IDO inhibitors move center stage in immuno-oncology. Nat Biotechnol 33, 321-2 (2015).

4. Rohrig, U.F., Majjigapu, S.R., Vogel, P., Zoete, V. & Michielin, O. Challenges in the Discovery of Indoleamine 2,3-Dioxygenase 1 (IDO1) Inhibitors. J Med Chem 58, 9421-37 (2015).

5. Sugimoto, H. et al. Crystal structure of human indoleamine 2,3-dioxygenase: catalytic mechanism of O2 incorporation by a heme-containing dioxygenase. Proc Natl Acad Sci U S A 103, 2611-6 (2006).

6. Tojo, S. et al. Crystal Structures and Structure-Activity Relationships of Imidazothiazole Derivatives as IDO1 Inhibitors. ACS Med Chem Lett 5, 1119-23 (2014).

7. Peng, Y.H. et al. Important Hydrogen Bond Networks in Indoleamine 2,3-Dioxygenase 1 (IDO1) Inhibitor Design Revealed by Crystal Structures of Imidazoleisoindole Derivatives with IDO1. J Med Chem 59, 282-93 (2016).

8. Lu, C., Lin, Y. & Yeh, S.R. Inhibitory substrate binding site of human indoleamine 2,3-dioxygenase. J Am Chem Soc 131, 12866-7 (2009).

9. Carter, S.K. & Crooke, S.T. (eds.). Mitomycin C: current status and new developments, (Academic Press, New York, 1979).

What is claimed is:

1. A non-transitory computer-readable medium coupled to the one or more data processing apparatus having instructions stored thereon which, when executed by the one or more data processing apparatus, cause the one or more data processing apparatus to perform a method comprising:
providing a crystal coordinate structure of the small molecule binding site on a human indoleamine 2,3-dioxygenase (hIDO) enzyme, wherein the small molecule binding site is not a hIDO active site;
contacting the small molecule binding site in silico with a candidate small organic molecule structure; and
determining if the candidate small organic molecule structure binds the small molecule binding site at a free energy level below a predetermined free energy level,
wherein a candidate small organic molecule structure that binds the small molecule binding site at a free energy level below the predetermined free energy level is identified and detected as a molecule which binds to a small molecule binding site on a human indoleamine 2,3-dioxygenase (hIDO) enzyme.

2. A system for identifying a cognate ligand molecule for a known biological receptor comprised of amino acid residues, comprising:

one or more data processing apparatus;

a graphical user interface; and

a non-transitory computer-readable medium coupled to the one or more data processing apparatus having instructions stored thereon which, when executed by the one or more data processing apparatus, cause the one or more data processing apparatus to perform a method comprising:
providing a crystal coordinate structure of the small molecule binding site on a human indoleamine 2,3-dioxygenase (hIDO) enzyme, wherein the small molecule binding site is not a hIDO active site;
contacting the small molecule binding site in silico with a candidate small organic molecule structure; and
determining if the candidate small organic molecule structure binds the small molecule binding site at a free energy level below a predetermined free energy level,

wherein a candidate small organic molecule structure that binds the small molecule binding site at a free energy level below the predetermined free energy level is identified and detected as a molecule which binds to a small molecule binding site on a human indoleamine 2,3-dioxygenase (hIDO) enzyme.

3. A method of detecting a molecule which binds to a small molecule binding site on a human indoleamine 2,3-dioxygenase (hIDO) enzyme, wherein the small molecule binding site is not a hIDO active site, comprising:
providing a crystal coordinate structure of the small molecule binding site on a human indoleamine 2,3-dioxygenase (hIDO) enzyme, wherein the small molecule binding site is not a hIDO active site;
contacting the small molecule binding site in silico with a candidate small organic molecule structure; and
determining if the candidate small organic molecule structure binds the small molecule binding site at a free energy level below a predetermined free energy level,
wherein a candidate small organic molecule structure that binds the small molecule binding site at a free energy level below the predetermined free energy level is identified and detected as a molecule which binds to a small molecule binding site on a human indoleamine 2,3-dioxygenase (hIDO) enzyme.

4. The method of Claim 3, wherein the candidate small organic molecule structure is an analog of tryptophan.

5. The method of Claim 4, wherein the analog of tryptophan comprises an indole of tryptophan.

6. The method of Claim 3, wherein the candidate small organic molecule structure is an analog of indole ethanol.

7. The method of Claim 3, wherein the candidate small organic molecule structure is an analog of mitomycin C.

8. The method of any of Claims 3-7, further comprising contacting an isolated human indoleamine 2,3-dioxygenase (hIDO) enzyme with an amount of the candidate agent identified as binding to the small molecule binding site on the hIDO in the presence an amount of L-tryptophan and determining whether the candidate agent increases or decreases a rate of degradation of the L-tryptophan to N-formylkynurenine relative to a predetermined control rate of degradation, and identifying the candidate agent as an agonist of hIDO where the candidate agent increases the rate of degradation, or identifying the candidate agent as an antagonist of hIDO where the candidate agent decreases the rate of degradation.

9. A method of activating or increasing activation of human indoleamine 2,3-dioxygenase (hIDO) enzyme comprising contacting the hIDO with an amount of Apigenin, Baicalein or Chrysin having the structures as set forth in Table 1, or a pharmaceutical salt of one thereof, in an amount effective to activating or increasing activation of hIDO.

10. The method of Claim 9, wherein the hIDO is contacted with the amount of a pharmaceutical salt of Apigenin, Baicalein or Chrysin.

11. A method of effecting immunosuppression in a subject comprising administering an amount of Apigenin, Baicalein or Chrysin having the structures as set forth in Table 1, or a pharmaceutical salt of one thereof, in an amount effective to activate or increasing activation of hIDO.

12. The method of Claim 11, wherein the hIDO is contacted with the amount of a pharmaceutical salt of Apigenin, Baicalein or Chrysin.

14. A method of treating an autoimmune disease or inhibiting an organ transplant rejection in a subject comprising administering an amount of Apigenin, Baicalein or Chrysin having the structures as set forth in Table 1, or a pharmaceutical salt of one thereof, in an amount effective to treat an autoimmune disease or inhibit an organ transplant rejection.

15. The method of Claim 14, wherein the hIDO is contacted with the amount of a pharmaceutical salt of Apigenin, Baicalein or Chrysin.

16. The method of Claim 14 or 15, wherein the autoimmune disease is treated.

17. The method of Claim 14 or 15, wherein the organ transplant rejection is inhibited.

18. The method of any of Claims 14-17, wherein the Apigenin, Baicalein or Chrysin is administered parenterally.

19. A pharmaceutical composition for activating or increasing activation of human indoleamine 2,3-dioxygenase comprising an amount of Apigenin, Baicalein or Chrysin having the structures as set forth in Table 1, or a pharmaceutical salt of one thereof, and a pharmaceutically acceptable carrier.

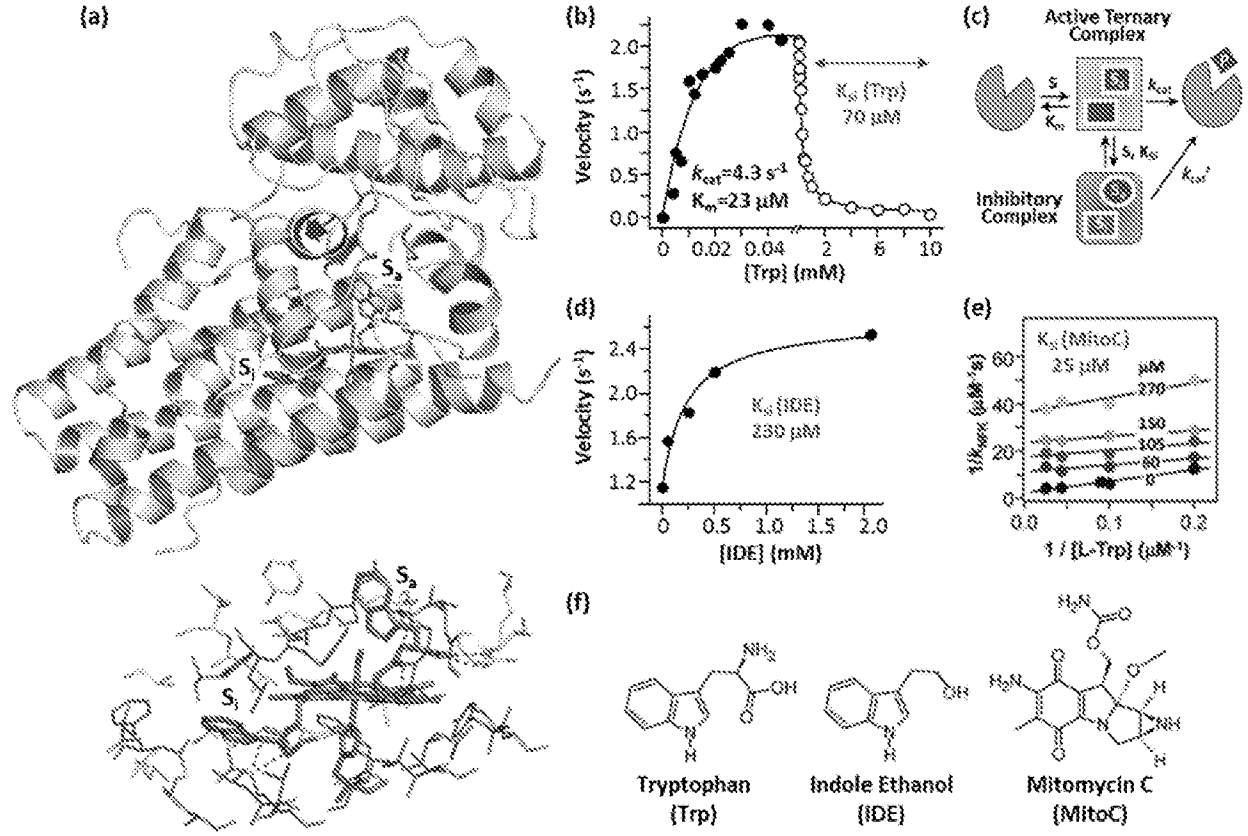

(a)
S_a
S_i
S_a
S_i
(b)
Velocity (s^{-1})
2.0
1.5
1.0
0.5
0.0
K_{cat}=4.3 s^{-1}
K_m=23 µM
K_{si} (Trp)
70 µM
0 0.02 0.04 2 6 10
[Trp] (mM)
(c)
Active Ternary Complex
S
K_m
k_{cat}
S_i K_{si}
k_{cat}'
Inhibitory Complex
(d)
Velocity (s^{-1})
2.4
2.0
1.6
1.2
K_{si} (IDE)
230 µM
0 0.5 1.0 1.5 2.0
[IDE] (mM)
(e)
K_{si} (MitoC)
25 µM
µM
270
150
105
60
0
$1/V_{app}$ ($\mu M^{-1}s$)
60
40
20
0
0.0 0.1 0.2
1 / [L-Trp] (μM^{-1})
(f)
Tryptophan
(Trp)
Indole Ethanol
(IDE)
Mitomycin C
(MitoC)

Fig. 1A-1F

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/64460

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 45/06, A61K 31/496, A61K 31/136, A61K 31/4412, C07C 211/45, A61K 31/5377 (2018.01)
CPC - A61K 31/497, C07D 487/04, A61K 31/166, A61K 31/506, C07C 2601/14, C07C 255/50, C07D 211/08, A61K 31/47, C07C 217/80, A61K 45/06, C07C 275/30, A61K 31/277, C07C 233/59, A61K 31/519, C07D 215/233, C07D 213/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Sugimoto et al. 'Crystal structure of human indoleamine 2,3-dioxygenase: Catalytic mechanism of O2 incorporation by a heme-containing dioxygenase', Proceedings of the National Academy of Sciences of the USA, 21 February 2006 (21.02.2006), Vol.103, pages2611-2616; Abstract, p2616	1-8
A	Lu et al. 'Inhibitory Substrate Binding Site of Human Indoleamine 2,3-Dioxygenase', Journal of American Chemical Society, 20 August 2009 (20.08.2009), Vol.131, pages12866-12867; p12867	1-8
A	Lu et al. 'Spectroscopic Studies of Ligand and Substrate Binding to Human Indoleamine 2,3-Dioxygenase', Biochemistry, 17 May 2010 (17.05.2010), Vol.49, pages5028-5034; Abstract, p5030, p5032	1-8
A	US 2014/0066625 A1 (NewLink Genetics Corp) 06 March 2014 (06.03.2014); entire document	1-8
A	US 2004/0234623 A1 (Munn et al.) 25 November 2004 (25.11.2004); entire document	1-8

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 January 2018	11 MAY 2018
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/64460

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 18
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See Supplemental Box

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-8

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/64460

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: Claims 1-8 drawn to a method comprising: providing a crystal coordinate structure of the small molecule binding site on a human indoleamine 2,3-dioxygenase (hIDO) enzyme, wherein the small molecule binding site is not a hIDO active site; contacting the small molecule binding site in silico with a candidate small organic molecule structure; and determining if the candidate small organic molecule structure binds the small molecule binding site at a free energy level below a predetermined free energy level

Group II: Claims 9-17 and 19 drawn to a method of activating/increasing activation of human indoleamine 2,3-dioxygenase (hIDO) enzyme, or effecting immunosuppression comprising contacting the hIDO with an amount of Apigenin, Baicalein or Chrysin having the structures as set forth in Table 1, or a pharmaceutical salt of one thereof, in an amount effective to activating/increasing activation of hIDO or treat an autoimmune disease or inhibit an organ transplant rejection

The inventions listed as Groups I-II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features

Group I requires a method comprising: providing a crystal coordinate structure of the small molecule binding site on a human indoleamine 2,3-dioxygenase (hIDO) enzyme, wherein the small molecule binding site is not a hIDO active site; contacting the small molecule binding site in silico with a candidate small organic molecule structure; and determining if the candidate small organic molecule structure binds the small molecule binding site at a free energy level below a predetermined free energy level, not required by Group II

Group II requires a method of activating/increasing activation of human indoleamine 2,3-dioxygenase (hIDO) enzyme, or effecting immunosuppression comprising contacting the hIDO with an amount of Apigenin, Baicalein or Chrysin having the structures as set forth in Table 1, or a pharmaceutical salt of one thereof, in an amount effective to activating/increasing activation of hIDO or treat an autoimmune disease or inhibit an organ transplant rejection, not required by Group I

Shared Common Features

Groups I-II share technical feature of human indoleamine 2,3-dioxygenase (hIDO) enzyme. However, theses shared technical feature does not represent a contribution over prior art, because the shared technical feature as being anticipated by WO 2015/181266 A1 to Spring Bioscience Corporation (hererinafter 'Spring'). Spring discloses human indoleamine 2,3-dioxygenase (hIDO) enzyme (Abstract "The invention provides antibodies that bind specifically to human indoleamine 2,3-dioxygenase").

As the technical features were known in the art at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the groups. Groups I-II therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.

Note Re: Item 4:
Claim 18 determined unsearchable because this is dependent claim and is not drafted in accordance with the second and third sentences of Rule 6.4(a).