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S4.1

Study on integrity and timing characteristics of Shaoyao and Gancao decoction on anti-inflammatory effects by BP neural network

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Aim: To study integrity and timing characteristics of traditional Chinese medicine, we used the non-linear fitting ability of BP neural network to establish the relationship mathematical model for evaluation anti-inflammatory effects of Shaoyao and Gancao decoction. **Methods:** Carrageenan into the foot of rats induced an acute inflammatory response with swelling foot and infiltration of neutrophils. The time-effect relationship of the indicators was studied after Shaoyao and Gancao decoction treatment. BP neural network was used to fit curves of the time-effect relationship of the paw edema , MPO, COX-2, and PGE_2. **Results:** The result showed that the change of each inflammation indicator (foot volume, MPO, COX-2, and PGE_2) was different at different time points after Shaoyao and Gancao decoction treatment. So it is necessary to fit the time-effect curves by BP neural network. According the fitting curve, the time window, E_{max}, and T_{max} of different inflammation indicator were calculated to evaluate integrity and timing characteristics of anti-inflammatory effects. **Conclusion:** It demonstrated that various pharmacodynamic indicators have the timing characteristics. The efficacy data is more reliable on neural network nonlinear fitting. It has the significant advantage in the study of the characteristics of integrity and timing characteristics of traditional Chinese medicine.

Keywords: Shaoyao and Gancao decoction; anti-inflammatory effects; BP neural network; time-effect curve

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S4.2

Development status of fixed-dose combination finished pharmaceutical products

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Fixed-dose combination finished pharmaceutical products (FDC-FPPs, FDCs) are fixed dose preparations with at least two active components. The objective of FDCs is to improve and enhance the whole effects *in vivo* using the beneficial factors of drug interactions. Development of FDCs has become one of the breakthrough strategies for removing the bottlenecks in drug discovery. The principle of formulating prescription is taking full advantage of the drugs' effects with a same remedy goal, then proceeding a demonstration of the prescription and multi-factor and multi-level analyses, verifying of clinical combinations, confirming of the goal prescription, finally accessing the normalize research and trials of new drug development. The whole process of FDCs research and development (pharmacy, pharmacodynamics, pharmacokinetics, toxicology and clinical trials) is as strict as such of the complete innovative drugs, and need to optimize the formula and the ratio of prescription. There are currently no unified FDCs registration laws and regulations in the world. According to the characteristics and technical content of FDCs, WHO issued the guidelines for registration of fixed-dose combination medicinal products, registered classification of FDCs could be divided into four categories. Governments and scientists in the world pay more and more attention to the development of FDCs. Grasping independent innovation research and the patents application is the only way for the development of FDCs in China.

Keywords: FDCs; drug interactions; registered classification of FDCs; development status

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S4.3

Safety of Chinese meteria medica injection improved by removing macromolecular substances

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Aim: The main aim is to observe the changes in major pharmacological effect and safety of Chinese Meteria Medica injection (CMMI) when the macromolecular substances in them are removed. **Methods:** Two kinds of CMMI (Qingkailing Zhusheye and Shuanghuanglian Zhusheye) were bought from market. The macromolecular substances were removed by molecular sieves, and two kinds of CMMI free of macromolecular substances and two kinds of CMMI rich in macromolecular substances were obtained. The fingerprinting maps of CMMI free of macromolecular substances were detected by HPLC, the antipyretic effect of them was assayed in rabbit fever model caused by lipopolysaccharide, and the normal toxicity or allergic toxicity were detected according to Chinese Pharmacopoeia (2010 edition, Book One). **Results:** Comparing with the fingerprinting map of original CMMI, the change of that from CMMI free of macromolecular substances was less than 5%, their antipyretic effect was almost not changed or even stronger, and the CMMI rich in macromolecular substances showed normal toxicity or allergic toxicity. **Conclusion:** The safety of CMMI can be improved by removing some macromolecular substances without its pharmacological effect weaken or lost.

Keywords: Chinese meteria medica injection; security; macromolecule

S4.4

Establishment of a cell based assay for screening human interleukin-6 receptor antagonists

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Aim: To set up the cell based assay for screening human interleukin-6 receptor antagonists. **Methods:** Full length of human interleukin-6 receptor α chain gene was reconstructed in plasmid pTaglite-SNAP (purchased from Cisbio Bioassays) which is a commercialized eukaryotic expression vector can express the receptor interested at the cell surface. Recombinant plasmid was analyzed and identified by restriction enzyme and sequencing. The correct expression vector was incubated with Lipofectamine® 2000 Reagent (invitrogen), and then transfected into HEK293A cell. Immunofluorescence and western blot analysis were used to detect the expression level of membrane receptor after 36 h. Cellular ELISA experimentation was analyzed through interaction between interleukin-6 and membrane receptor. **Results:** The results of sequence analysis demonstrated that interleukin-6 receptor gene had been restructured correctly in expression vector pTaglite-SNAP. Immunofluorescence and western blot analysis indicated that recombinant plasmid could be transfected into HEK293A cell successfully and the transfected cell could express highly membrane interleukin-6 receptor compared with normal control group. Cellular ELISA test showed that interleukin-6 (0.56–36 ng/μL) could bind with membrane receptor in a dose-dependent manner. **Conclusion:** Combining assay and cellular ELISA method can be used to identify interleukin-6 receptor antagonists.

Keywords: interleukin-6 receptor; antagonist; eukaryotic expression; cellular ELISA

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S4.5

Serotonin (5-HT) receptors: pharmacology, function and structure.

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5-HT is unique among monoamines as its effects are subserved by at least 13 G-protein-coupled receptors (GPCRs) and one (of a family of 5 gene products) ligand-gated ion channel(s), the $5\text{-}HT_3$ receptor. 5-HT receptors form 7 distinct classes ($5\text{-}HT_{1\text{-}7}$) based on structural/operational/signalling features. A great degree of diversity shows multiple pathways to be activated by a single receptor, allowing compounds to induce functional selectivity/biased signalling. Coupled with the actions of an avid and efficient reuptake system, this array of receptor subtypes provides almost limitless signalling capabilities. Further, posttranslational modifications *eg* alternate splicing and RNA editing, increase the number of receptor variants, and oligomerisation/heteromerisation increase the number of potential receptor complexes. Whether all these possibilities are used in vivo under physiological or pathological conditions remains to be seen. We will attempt to summarize 5-HT receptor diversity and its impact for disease and drug design. Structural biology and therefore structure based-drug design may become reality,

now that 5-HT receptor crystals/structures are becoming available, if only to explain more precisely how some of the known 5-HT ligands and clinically effective compounds interact with their targets.

S4.6

Novel PPARγ binding activity modulator discovery based on the PPARγ-Gal4 chimera receptor and its mutant derivatives

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Aim: To discover novel PPARγ modulator, PPARγ-Gal4 chimera receptor and its mutant derivatives were applied to evaluate the binding activity of ligands and their molecular basis of interaction. **Methods:** To construct a series of chimera receptors, the plasmids of pcDNA3.1 Gal4 were inserted with PPARγ ligand binding domain fragment or the amino acid site mutant derivatives, such as Ser 273, Ser289, His 323, His 449, and Tyr473, and were cotransfected with a Gal4-promoter regulated luciferase reporter gene plasmid in 293E cell. After being incubated with potential PPARγ ligands for several hours, the luciferase activity in 293E cells could be detected by luciferase-substrates reaction assay kit. **Results:** PPARγ agonists, represented by Rosiglitazone, Pioglitazone, could bind to PPARγ-Gal4 chimera receptor and induced luciferase activity more than 5 folds, while animo acid mutant with Ser 273 or Tyr473 resulted in almost 80% binding activity loss. And different binding characters were founded when this method was used to evaluate the binding activity of novel PPARγ ligand. **Conclusion:** PPARγ-Gal4 chimera receptor can be used to evaluate the binding activity of receptor modulator. Meanwhile, the loss-function of mutant derivatives suggests key sites of ligand binding and molecular interaction basis.

Keywords: PPARγ modulator; chimera receptor; binding activity.

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S4.7

Application of uniform design-high throughput screening in research of eleven bioactive monomer components of Radix Salviae Miltiorrhizae

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Aim: To evaluate the bioactive monomer components of Radix Salviae Miltiorrhizae (RXM) by uniform design-high throughput screening (UD-HTS) method. **Methods:** Eleven of bioactive monomer components of RXM (four of liposolubility and seven of water-solubility) were recombined to compatible groups through uniform design, the monomer concentration levels were 1×10^{-3}, 0.5×10^{-3}, 1×10^{-4}, 0.5×10^{-4}, 1×10^{-5}, and 0.5×10^{-5} mol/L. To obtain the better samples, the high throughput screening was taken to detect the capability of anti-DPPH oxidation and reduction ability of the compatible combination samples. The data were analyzed by the WUST package of Uniform Design and parameter optimization to obtain the optimal combination samples and then repeated the experiment (screening). The injury model of hippocampal neuronal cells established by hydrogen peroxide (H_2O_2) was used as cell target to investigate the neuroprotection of the samples (rescreening). Cell survival ratio of neuron was assayed by MTT. **Results:** After screening and rescreening, the best combination samples were obtained (cell survival ratio >90%). **Conclusion:** UD-HT screening technology is a reliable and high efficiency technology to evaluate the bioactive of monomer components of RXM .

Keywords: Radix Salviae Miltiorrhizae; Uniform Design-High Throughput Screening; H_2O_2; Hippocampal cells; Neuroprotection

S4.8

Explore the molecular-descriptor-drug-action relationship inside ligand cellular system of nuclear receptor

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Aim: Molecular descriptor is context-dependent, that is, the various interpretations of a specific molecular descriptor depend on the varying biological contexts. For example, molecular dipole can stand for its binding affinity or drug membrane transport. To clearly understand the descriptor-action relationship, our aim is to elaborate systematically the relationship between molecular descriptor and subcellular drug action, herein, limited in the ligand cellular system of nuclear receptors. **Methods:** To determine the most crucial factor between cell-free and cell-based systems of drug testing. Such two types of data from cell-free and cell-based assays were collected. The four ligand cellular systems related to nuclear receptors include estrogen analogs, androgen analogs, progestagen analogs and clucocorticoid analogs. The working equation in the context is "cell-based data=a (cell-free data)+b (descriptor to be chosen)+c". The top captured descriptors stand for the difference between cell-free and cell-based system. **Results:** The most frequent top captured descriptors for such four kinds of analogs are polar surface area, polar solvent accessible surface or the related, highlighting the similar critical drug action in the ligand cellular system of nuclear receptors. Moreover, it is interesting to note that those descriptors have been proved to be extremely correlated with the drug action of membrane transport. **Conclusion:** We can therefore step by step squeeze out the descriptor-drug-action relationship inside the ligand cellular system of nuclear receptor, leading to the identification of critical drug action. In the end, the whole picture of mapping molecular descriptor onto subcellular drug action is thus able to be established.

Keywords: molecular-descriptor-drug-action relationship; ligand cellular system; nuclear receptor; drug action of membrane transport

S4.9

Zebrafish-based traditional Chinese medicine (TCM) drug discovery and assessment platform

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Zebrafish as a vertebrate animal model is increasingly used for *in vivo* drug discovery and for assessing compound toxicity and safety, for its several compelling experimental advantages including transparency, high throughput, short test period, low cost, small amount of compound required, easy manipulation and so on. Traditional Chinese medicine usually are complex mixtures comprising of multicomponent and have multi-targets, the traditional *in vitro* and *in vivo* drug research approaches are not very suitable for TCM. In present study, we used zebrafish to assess TCM (eg liquid, powder, pill and ointment) toxicity and safety; and build several zebrafish models of human diseases for TCM pharmacology study and new treatment indications research. zebrafish models could be used for assessing TCM toxicity and safety including acute toxicity, development toxicity & teratogenicity, cardiovascular toxicity, liver toxicity, renal toxicity, nerve toxicity, bone toxicity, reproductive toxicity and anaphylactoid reaction, and have a overall predictivity ≥80%; and zebrafish models of human diseases are also very useful for TCM drug discovery including anti- and pro-angiogenesis models, cancer models, cardiovascular disease models, CNS disease models, liver and metabolic disease models, inflammatory and bone disease models, aging models, visual and otoprotectant models, skin care model and target therapy models. Zebrafish TCM drug discovery platform can reduce the time and cost required for the drug development process, increase the compound success rate and decrease the failure ratio due to adverse side effects throughout the drug research and development process.

S4.10

The auxiliary GABA(B) receptor subunits KCTD12 and KCTD16 differentially modulate receptor function

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Aim: KCTD12 and KCTD16 were recently identified as $GABA_B$ receptor auxiliary subunits, yet their functional significance has yet to be clearly elucidated. Here we characterised the impact of human KCTD12 and KCTD16 on $GABA_B$ receptor kinetics and pharmacology *in vitro*. **Methods:** Using a *Xenopus laevis* oocyte based automated two-electrode voltage clamp assay, $GABA_B$ receptor activated GIRK current was used to characterize the effects of the $GABA_B$ receptor agonist baclofen or the positive allosteric modulator CGP7930 on receptor kinetics in the absence and presence of KCTD subunits. **Results:** Co-expression of KCTD12 with $GABA_B$ receptor accelerated the kinetics of response to agonist application (20%-80% rise time from 2.8 s to 1.3 s) and the kinetics of subsequent receptor desensitization (increased from 3.9% to 54.7%, compared with $GABA_B$ receptor only expression, n>7 oocytes). In contrast, KCTD16 co-expression did not significantly alter $GABA_B$ receptor kinetics. Analysis of baclofen dose-response curves showed that neither KCTD12 nor KCTD16 co-expression altered the EC_{50} or Hill slope (n>10 oocytes at

each agonist concentration). However, the potentiating effect of CGP7930 on an EC_{20} concentration of GABA was enhanced in the presence of either KCTD12 or KCTD16 (from 11.5% to 20.3% and 24.4% respectively, n>33 oocytes). **Conclusion:** KCTD12 and KCTD16 differentially modulate $GABA_B$ receptor kinetics and pharmacology, raising the possibility of selective targeting of interactions between KCTDs and $GABA_B$ receptors.

Keywords: $GABA_B$ receptor; subunit; KCTD

S4.11

A potential drug target CLK1 from host cell for anti-influenza drug discovery

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Aim: Influenza (including seasonal epidemic and pandemic influenza) caused by inlfuenza virus, seriously threaten human life and health. High mutation rates facilitate the generation of viral escape mutants, rendering vaccines and drugs directed against virus-encoded targets potentially ineffective. In contrast, drugs targeting host cell determinants temporarily dispensable for the host but crucial for virus replication could prevent viral escape. CDC-like kinase 1 (CLK1) in human host cells as the crucial protein in influenza A virus replication process, is expected to become a new target for anti-influenza drugs. In this study, we aim to express CLK1 protein, establish CLK1 activity assay which can be used to do drug screening on CLK1 inhibitors. **Methods:** The full length CLK1 gene from HUVEC was amplified by PCR, cloned into the pFastBac1 plasmid and transformed into the DH10Bac E.coli strain. The recombinant His6-CLK1 protein was expressed in Sf9 cells by the Baculovirus Expression Systerm. After purification by Ni/NTA affinity chromatography, His6-CLK1 inhibitor screening model was established by Kinase-Glo assay. Compounds selected by structure-based virtual screening were evaluated for their CLK1 inhibitory activities. Their *in vitro* anti-influenza virus activities were evaluated. **Results:** A large amount of recombinant His6-CLK1 protein was acquired with high purity. The activity assay parameters were optimized, containing His6-CLK1 (149 ng/mL), substrate MBP peptide (50 μg/mL), ATP(1 μmol/L), Mg^{2+} (10 mmol/L), Mn^{2+} (2 mmol/L), time (90 min) and temperature (25 °C). The Z′ factor of the assay was 0.7, which demonstrated its robustness and reliability. 100 compounds from 21,758 compounds were selected by virtural screening for drug screening. As a result, 14 among them exhibited significant CLK1 inhibitory activities. The further study on their *in vitro* anti-influenza virus activty showed that they all down regulated the protein expression level of M2 in the A/PR/8/34 (H1N1) influenza virus infected A549 cells in a dose dependent manner at the concentration 0.4–10 μg/mL. **Conclusion:** As one of the host factors essential for influenza virus replication, CLK1 is a potential drug target for anti-influenza virus drugs, which involved in the alternative splicing of viral M2 pre-mRNA. CLK1 inhibitors might inhibit the viral replication through down regulating the expression of M2 mRNA. Our study on CLK1 protein expression, CLK1 activity assay and rational CLK1 inhibitor discovery will lay important theory and experiment foundation for the anti-influenza drug development, and provide guidance to the study of other host factors related with influenza virus.

Keywords: Host cell factor; CDC-like kinase 1 (CLK1); influenza virus; virtual screening; drug discovery

Acknowledgements: This work was supported by the National Great Science and Technology Projects (2012ZX09301002) and the International Collaboration Project (2011DFR31240).

S4.12

Rational drug screening on neuraminidase (NA) inhibitors of influenza virus using support vector machine (SVM)

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Aim: Neuraminidase (NA) is one of the most important drug targets for anti-influenza drugs, NA inhibitors are currently the main clinical medicines for the treatment of influenza virus infection, and many new NA ihnibitors are under development. However, reliable models for predicting NA inhibitors are rare. In this study, several computational models were built, and used to guide new drug discovery from our compound library. **Methods:** Based on the data set with 776 distinct structures, several computational models were built to predict whether or not a compound is a NA inhibitor. Each molecule was represented by ADRIANA. Code. The models were built using a support vector machine (SVM) and a Kohonen's self-organizing map (SOM). The virtual screening of our compoud library was carried out using the better model, and the NA inhibition of the selected compounds was evaluated. **Results:** One SVM model and one SOM model were built and verified by the test data set, the correct prediction rate of the two models were 95.72% and 91.44%, respectively, so the better model-SVM model was chose to perform virtual screening of our partial compoud library with 65571 compounds, and 560 compounds were predicted as active compounds, which were evaluated by NA activity assay, the assay results showed that 51 compounds displayed NA inhibitory activity, two among them exhibited the highest activity with IC_{50} values less than 20 nmol/L. **Conclusion:** For the first time, one model was built using SVM and successfully used in the rational drug screening on NA inhibitors of influenza virus, several active compounds with novel structures were discovered. This study will provide important information for drug design and drug development.

Keywords: neuraminidase (NA) inhibitors; rational drug screening; support vector machine (SVM)

Acknowledgements: This research work was supported by the National Great Science and Technology Projects (2012ZX09301002) and the International Collaboration Project (2011DFR31240).

S4.13

Application and construction of pharmacology evaluation system of treating cerebrovascular disease

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Aim: To discover the new drugs of treating cerebrovascular disease by conatructing a new pharmacology evaluation system. **Methods:** Under the thoughts of "mimic clinics", we first developed animal model to close the clinical features by integrating literature information and clinical experiment and animal experiment,then select a lot of pharmacology indexes to construct pharmacology evaluation system. **Results:** We have developed a Chinese miniature swine model with coronary heart disease by mechanical vascular trauma combining with diet-induced hypercholesterolemia, and evaluated MG53 and quyu huatan tongmai granula. **Conclusion:** we have constructed a new pharmacology evaluation system for drugs of treating cerebrovascular disease.

Keywords: cerebrovascular disease; mimic clinic; pharmacology

S4.14

Antisense antibiotics: mission possible or impossible?

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The nightmare of multi-drug resistant bacteria will still haunt if no panacea is ever found. Efforts on seeking desirable natural products with bactericidal property and screening chemically modified derivatives of traditional antibiotics have lagged behind the emergence of new multi-drug resistant bacteria. The concept of using antisense antibiotics, now as revolutionary as is on threshold has experienced ups and downs in the past decade. In the past five years, however, significant technology advances in the fields of microbial genomics, structural modification of oligonucleotides and efficient delivery system have led to fundamental progress in the research and *in vivo* application of this paradigm. The wealthy information provided in the microbial genomics era has allowed the identification and/or validation of a number of essential genes that may serve as possible targets for antisense inhibition; antisense oligodeoxynucleotides (ODNs) based on the 3rd generation of modified structures, eg, peptide nucleic acids (PNAs) and phosphorodiamidate morpholino oligomers (PMOs) have shown great potency in gene expression inhibition in a sequence-specific and dose-dependent manner at low micromolar concentrations; and cell penetrating peptide mediated delivery system has enabled the effective display of intracellular antisense inhibition of targeted genes both *in vitro* and *in vivo*. The new methods show promise in the discovery of novel gene-specific antisense antibiotics that will be useful in the future battle against drug-resistant bacterial infections.

Keywords: antisense antibiotics; multi-drug resistant bacteria; microbial genomics; structural modification; delivery system

S4.15

A novel accurate method of human protein tyrosine phosphatase 1B inhibitors high-throughput screening

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Aim: A high-throughput screening model was established on 384-well microplate with 50 μL volume in order to screen potential human soluble protein tyrosine phosphatase 1B (PTP1B) inhibitors. **Methods:** Recombinant PTP1B was cloned and expressed in *E. coli*. the specific phosphotyrosine peptides substrate [TRDIY(P)ETDYYRK, PPS], base on the insulin receptor (GenBank: ADQ39592.1) 371–381 amino acid sequence, was syntheticed; A chromogenic assay based on the Malachite Green method was employed for the detection of inorganic phosphate (Pi) released from phospho-peptides by PTP1B. This assay, modified so as to improve its sensitivity, adapted to a 384-well microplate format, was established for PTP1B inhibitors high-throughput screening model (Z′=0.75). **Results:** Using this model, A total of 105920 samples were screened, of which 183 samples with inhibition rates more than 70% were selected for further re-screening. Finally, four compounds (J11022, J22153, J26925, and W10353) with high inhibitory activity were identified, whose IC_{50} value were 126.57, 56.26, 57.75, and 60.59 μg/mL respectively. **Conclusion:** The results indicated that the assay was stable, sensitive, reproducible and suitable for high-throughput screening of PTP1B inhibitions.

Keywords: protein tyrosine phosphatase 1B; inhibitor; high-throughput screening; insulin receptor

S4.16

Tumor glutamine metabolism: regulation & therapeutic targeting

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Elevated glutamine metabolism is an essential feature of malignant transformation. Regardless of their origin, tumor cells constantly require a large amount of glutamine supply to support their characteristic unabated growth. We and other colleagues report that the transcriptional regulatory properties of the oncogene Myc coordinate the expression of genes necessary for cells to engage in glutamine catabolism to sustain cellular viability and TCA cycle anapleurosis. The stimulation of mitochondrial glutamine metabolism resulted in reduced glucose carbon entering the TCA cycle and a decreased contribution of glucose to the mitochondrial-dependent synthesis of phospholipids. MYC-transformed cells predominantly depend on mitochondrial glutamate-oxaloacetate transaminase (GOT2) to maintain Gln homeostasis and suppress apoptosis. Consequently, GOT2 inhibitors potently induce apoptosis in vitro and inhibit tumor growth in vivo. These results reveal mechanisms whereby oncogene MYC regulates tumor metabolic reprogramming and validate inhibitors of Gln metabolism may be effective therapeutic strategies for treating the ≥40% of human cancers that overexpress Myc.

S4.17

Establishment of standardization models to screen for drugs against prostate diseases

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Aim: To establish the standardization models to screen for drugs against prostate diseases, including prostatitis, benign prostate hyperplasia (BPH) and prostate cancer. **Methods:** The standardization platform to screen for drugs against prostate diseases was established by Good Laboratory Practice (GLP) rules, in addition to biology principles. This platform, including nearly 50 people, will provide relatively necessary condition for prostate pharmacology research and the development of drugs. **Results:** A series of prostate disease drugs screening model were established in our department, including the model of rat prostatitis induced by chemical substrates, by immune adjuvant and by estrogen, rat and beagle dog BPH induced by testosterone propionate, old dog spontaneous BPH, steroidal 5α-reductase inhibitors *in vitro*, hyperplasia of rat prostate tissue generation in primary culture cells *in vitro*, prostate cancer cells (PC-3, DU145, LNCap, and CWR22Rv1) *in vitro* and *in vivo* drugs screening and prostate cancer cells (PC-3M) infiltration model *in vitro* and *in vivo* drugs screening. **Conclusion:** The standardization platform established would promote the research and development of drugs anti-prostate disease.

Keywords: prostate; benign prostate hyperplasia; prostatitis; prostate cancer; biology model screening drugs

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S4.18

Application and practice of high content analysis (HCA) technology in the R&D of drugs

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HCA, a multiparametric analysis of cell populations or subcellular events that measures spatial and temporal changes of phenotypic parameters, is developed with the aim of improving the quality and efficiency of lead compound generation. Due to steady improvement in the technology and development in application, HCA is not only used in primary HTS for improving target validation and lead compound selection, but are already used in the entire drug discovery and development process. Here we introduce our experiences on application and practice of HCA technology in drug R&D, including lead compound identification by high content screening (HCS), mechanism rapidly probing of anti-tumor drug, pharmacological profiles study on GPCRs-target drug, pathway and phenotype profiles assay of candidate and market drug, discovery toxicological study, drug repurposing and even cytokinetic analysis of antibody. Taken together, HCS offers a unique, efficiency and powerful tool for new drug researcher, especially pharmacologist.

Keywords: HCA; new drug R&D; pharmacological profiles; discovery toxicology
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S4.19

The feasibility of use of radionuclide-labeled drugs in human mass balance study

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Mass balance study, in which the radionuclide labeling technique can be used to obtain more detailed pharmacokinetic data, is very important for drug research and development. The radioactive drugs are used in human subjects to obtain basic information relating to metabolism, human physiology, pathophysiology, and biochemistry, rather than to accomplish immediate therapeutic or diagnostic purposes or to determine the safety and effectiveness of the drug. The FDA guidance recommends that the safety assessment should be considered in nonclinical studies, if the human metabolites exposure exceeds 10% of the parent drug systemic exposure at steady-state. ADME studies with radiolabeled drug are considered to be able to provide more detailed metabolism data. Mass balance study had been carried out for 40 years in foreign countries and their experiences showed that in the prerequisite of reasonable design and standard operation, the radiation will not harm investigators and subjects. In China, due to the limitations of the laws and regulations, as well as the ethical factors, so far, there is no human test instance yet. However, the feasibility of the implementation of this technology in China is discussed in this article.

Keywords: mass balance; radionuclide; pharmacokinetics; radiolabeled human study

S4.20

Optimization study on the influential factors for simulation calcium oxalate calculus models *in vitro* by orthogonal design with multi-targets

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Aim: To optimize the influential factors on simulation calcium oxalate calculus model *in vitro* with the targets of the CaOxa crystal growth rate and the ratio of CaOxa crystals. **Methods:** To establish the model of calcium oxalate stone agar gel system by evaluating the influential factors which include the concentration of agar gel, calcium chloride, sodium oxalate, the incubation time and temperature in orthogonal test and each factor with three levels, affecting formation of calcium oxalate stone under the microscope. And to optimize the formation conditions of calcium oxalate stone *in vitro*. **Results:** As incubation extension of time, different concentrations of agar gel was rendered in a certain percentage of COM, COD, COT as well as aggregates, which gathered rate was the quickest and easy to form COM under 1% agar concentration. the cultivation condition of 37°C was the most suitable for COM nucleation, growth, as well as gather. With concentration of calcium chloride/sodium oxalate as 0.1/0.2 (mol/L), incubating in 37°C for 3 d,

COM and aggregate accounted for more 90% of all Crystal. **Conclusion:** The optimal conditions for the model of calcium oxalate stone formation *in vitro* were incubated in 37°C for 3 d with 1% agar gel, $[Ca^{2+}]/[C_2O_4^{2-}]$ (0.1/0.2 mol/L) at pH 7.4.

Keywords: calcium oxalate stone; simulation model; *in vitro*; influential factors

S4.21

A virtual screening approach for Cdk5/p35 inhibitors based on common feature pharmacophore modeling and molecular docking

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Aim: Cdk5 is an a typical cyclin-dependent kinase localized in the brain, and its activity is dependent upon binding to p35. Cdk5/p35 plays an important role in the hyperphosphorylation of tau protein, which has been observed in Alzheimer's Disease (AD) patients. Cdk5/p35 represents an attractive drug target in AD. In this study, we aim to find the inhibitors of cdk5/p35 using virtual screening technology. **Methods:** A total of 23 266 compounds were filtered by using Lipinski's rule of five to make them more drug-like. Then 6 known representative cdk5/p35 inhibitors were used to develop common feature pharmacophore models. The enrichment factor calculation was performed to validate the predictive ability of the top 1 model. After the model validation, the top 1 model was employed as a 3D search query to screen compounds that passed Lipinski's rule. Furthermore, the hit compounds were subsequently subjected to docking studies to refine the retrieved hits. **Results:** A total of 16 383 compounds passed Lipinski's rule. The best pharmacophore model contains five features: two hydrogen-bond donors (D), two ring aromatic features (R), and one hydrophobic aliphatic moiety (L). The enrichment factor (EF) of test set (including 286 inhibitors and 40000 non-inhibitors) is 12.4, which shows the pharmcophore model has a high predictive ability. A total of 1604 compounds can match at least four features of the best pharmacophore, and the hit compounds were subsequently subjected to docking studies to retrieved hits. **Conclusion:** Through docking studies, the top 200 compounds were selected for further biological test.

Keywords: Cdk5/p35 inhibitors; virtual screening; pharmacophore; molecular docking; Alzheimer's Disease

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Screening super-spectrum β-lactamase producing gram-negative bacilli by modified three-dimensional test and PCR method

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Aim: The purpose of this study was to established a method to Screen bacteria producing Super-spectrum -lactamase. **Methods:** 326 Gram-negative bacteria were collected from our hospital. MIC values of ceftazidime and cefoxitin acting on them were got by agar double dilution method. Based on the MIC values, ceftazidime- and cefoxitin-resistant Gram-negative bacilli were obtained. Modified three-dimensional test, susceptibility test and PCR method were used to harvest SSBLs-producing strains and identify resistant phenotypes and genotypes of ESBLs and AmpC, respectively. **Results:** Among the screened 136 Gram-negative bacteria with cefoxitin- and ceftazidime-resistance 3 SSBLs-producing strains were obtained by modified three-dimensional tests and plasmid conjugation tests. It was found that the gene types of plasmid-coded ESBLs were SHV, TEM and CTX-M, whereas AmpC was ACT. **Conclusion:** Modified three dimensional tests and PCR method can be used to confirm SSBLs strain.

Keywords: Gram-negative Bacilli; Super-spectrum -lactamase; ESBLs; AmpC; resistance

S4.23

Network pharmacology: a novel strategy for antidepressant discovery

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Aim: Advances in systems biology suggest that network pharmacology may be a novel and more effective strategy for drug discovery than the single-target strategy for complex diseases, such as depression. The present study was designed to screen novel antidepressants from 3655 US Food and Drug Administration (FDA)-approved drugs, based on a novel network pharmacology platform. **Methods:** This novel network pharmacology platform was created according to the information of drugs-diseases networks, drugs-targets networks and diseases-genes networks. These information were assembled and integrated together to develop the computational program to predict probable antidepressants from 3655 drugs. The tail suspension test (TST) and the forced swimming test (FST) in mice were used to evaluate the antidepressant effect of drugs predicted by the network pharmacology platform. Learned helplessness (LH) test was performed to further vefify the antidepressant potential of Alverine. Drug induced depression models, including yohimbine induced toxicity potentiation test and 5-HTP or DOI induced head twitch test, were used to analyze the possible mechanisms of Alverine. The affinities to hSERT, hNET as well as h5-HT_{1A}R of Alverine were detected by radioligand binding assays. The MAO-A and MAO-B activity were also measured using the Amplex Red Monoamine Oxidase Assay Kit. **Results:** According to the predictive results from the established network pharmacology platform and the pharmacology properties of these drugs, 10 drugs were selected and screened for their antidepressant potential. Among these drugs, Alverine, an antispasmodic drug showed better antidepressant activity. Alverine (100 mg/kg, *po*) significantly decreased the duration of immobility time in both the TST and FST. Furthermore, Alverine markedly improved the escapable situations in mice in LH test. In drug induced depression model, Alverine raised the mortality rate in the yohimbine induced toxicity potentiation test, and reduced the number of head twitches induced by 5-HTP and (±) DOI (a selective 5-HT_2 agonist). In addition, Alverine showed mild affinities to hSERT (IC_{50}=7.6 μmol/L), hNET(IC_{50}=3 μmol/L) and h5-HT_{1A}R (IC_{50}=0.86 μmol/L) while had no effect on the activities of MAO-A or MAO-B. **Conclusion:** Network pharmacology may be a novel and effective strategy for antidepressant discovery. According to this platform,Alverine is screened as a potential antidepressant, and the underlying mechanism may be related to multi-targets action including monoamine reuptake inhibition and influence on various neurotransmitter receptors.

Keywords: network pharmacology; antidepressant discovery; Alverine; depression models; multi-targets

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S4.24

High-throughput screening for the promoter agonist of LAIR-1

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Aim: Leukocyte-associated Ig-like receptor-1 (LAIR-1) is a surface molecule that functions as an inhibitory receptor. Recent studies have shown LAIR-1 was closely related with the development of inflammation, and the expression of it declined rapidly in LPS-activated murine macrophages. In this study, we established a high-throughput screening assay for the promoter agonist of LAIR-1, in order to find new candidate compounds. **Methods:** Firstly, we established a stable HEK293 cell line contained the promoter of LAIR-1. Secondly, we optimized the conditions like microbiomation density, serum level and brood time of cell with drug through detecting the reporter gene while PMA is used as agonist for the promoter of LAIR-1. Then we performed the receptor agonist screening for the 42880 compounds on the LAIR-1/HEK293 cells though the activity of luciferase. After finding the new Candidate compounds, LPS-activated murine macrophages RAW264.7 was used to assess the potent anti-inflammatory effects of LAIR-1 agonist. **Results:** 13 compounds exerted LAIR-1 agonism in the second round of screening. 7 compounds exerted a dose-dependent inhibition of the LPS-stimulated release of the NO. Moreover, the 7 compounds can significantly increase the expression of LAIR-1. **Conclusion:** We established a high-throughput screening model for the promoter agonists of LAIR-1, it is stable, sensitive, reproducible and

high efficiency, and it discovered novel anti-inflammatory candidates.
Keywords: inflammation; LAIR-1 receptor; drug screening

S4.25

Zebrafish: a predictive model for assessing compound toxicity, safety and efficacy

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Zebrafish as a vertebrate animal model is increasingly used for in vivo drug discovery and for assessing compound toxicity and safety and numerous studies confirm that mammalian and zebrafish physiology, development, metabolism and pathways are strikingly similar and that zebrafish is extremely predictive of mammalian responses. This convenient and predictive animal model offers several compelling experimental advantages including transparency, high throughput, short test period, low cost, small amount of compound required, easy manipulation, direct compound dilivery, and so on. FDA and EMEA have accepted zebrafish toxicity and safety assessment data for IND approval and more than one dozen of new drugs discovered primarily based on zebrafish model are now in clinical trials. An important recent development impacting wider use of zebrafish is that the OECD is developing standards for using zebrafish to assess chemical toxicity and the European Union anacted Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) that requires toxicity assessment using animal testing including zebrafish for any chemical imported or manufactured in the region. In this presentation, I will briefly introduce: (1) the background of zebrafish drug screening and compound assessment; (2) zebrafish models for assessing compound toxicity and safety including acute toxicity, LC_{50}, nanotoxicity, development toxiciy & teratogenicity, cardiovascular toxicity, liver toxicity, renal toxicity, nerve toxicity, behavior toxicity, bone toxicity, blood toxicity, skin and muscle toxicity, visual toxicity, ototoxicity, gene toxicity, reproductive toxicity and anaphylactoid reaction; and (3) zebrafish models of human diseases for drug discovery including anti- and pro-angiogenesis models, cancer models, cardiovascular disease models, CNS disease models, liver and metabolic disease models, inflammatory and bone disease models, aging models, visual and otoprotectant models, skin care model and target therapy models. Zebrafish drug discovery platform can reduce the time and cost required for the drug development process, increase the compound success rate and decrease the failure ratio due to adverse side effects throughout the drug research and development process.
Keywords: Zebrafish; toxicity assay; safety assessment; disease model; drug discovery

S4.26

The role and molecular mechanism of antibacterial enhancer

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Aim: To review the development of the role and molecular mechanism of antibacterial enhancer. **Methods and results:** Bacterial resistance continually increased because of wide use even abuse, therefore, it is focus to search for new and effective antibacterial agents. There are two strategies: the first one is direct antibacterial such as structure modification of present antibacterial agents, another is indirect strategy such as antibacterial enhancer. Antibacterial enhancer increased the sensitivity of present antibacterial agents although it has no antibacterial activity; its advantages are less dosage and lower toxicity. The drug-targets of antibacterial enhancer are resistance protein such as Penicillin binding protein 2a (PBP2a) of Methicillin-resistant *Staphylococcus aureus* (MRSA) and key protein during bacterial growth and multiplication. Natural products are valuable sources of antibacterial enhancer. Recent decade, components with antibacterial enhancement activity are isolated from Fructus crataegi (hawthorn) under guide of bioactivity. The results from *in vitro* and *in vivo* experiments demonstrate components possesses good bioactivity with novel antibacterial mechanism. **Conclusion:** Antibacterial enhancer is hopeful strategy and worthy of further investigation in the future.
Keywords: bacterial resistance; antibacterial strategy; antibacterial enhancer; components

S4.27

Study on the best compatibility and spectrum-activity relationship of Xiaoyaosan antidepressant effective fractions based on CUMS model

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Aim: Two fractions (A, B_3) of XYS were demonstrated to be the active fractions which possessed the antidepressant-like effects in our previous study. In this paper, The compatibility of A and B_3 were used to screen the best prescription group, and the active compounds were screened by spectrum-activity relationship based on rat model of chronic unpredictable mild stress (CUMS). **Methods:** Behavior research and metabonomics method were used for efficacy study of different compatibility groups. Each group was chemically analyzed using Gas Chromatography and Mass Spectrometry (GC-MS) and bioassayed using behavioral indicators and the contents of plasma metabolic markers. The spectrum-effect relationships were analyzed with multivariate correlation analysis. **Results:** In the behavior research and pattern recognition analysis of plasma metabolites, When A and B_3 both were 92.6 g herb/kg, compatibility group shows the best antidepressant effect. Close correlation existed in the spectrum-effect relationships, 6 compounds which might contribute to the antidepressant-like effects were selected. **Conclusion:** This work provides a general model of the combination of chromatographic analysis and pharmacological effect to study the spectrum-effect relationships based on compatibility of active fractions.
Keywords: Xiaoyaosan; compatibility; spectrum-effect relationship
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