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## S6.1

### Pharmaceutical care for a patient with mediastinitis treated by vancomycin based on population pharmacokinetics

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To explore the pharmacy practice that clinical pharmacists carry out vancomycin individual treatment based on vancomycin population pharmacokinetics and therapeutic drug monitoring in order to expand new work pattern of clinical pharmacists. Clinical pharmacists carry out vancomycin individual treatment based on population pharmacokinetic formula of elderly patients in our hospital. What's more, we remind the doctors to do therapeutic drug monitoring timely. Medication adjustments are made according to the therapeutic drug monitoring results and patient's condition. According to the patient's initial creatinine clearance rate (87.784 mL/min), initial therapeutic regimen of 1.0 g, once every 12 h. After giving 5 doses, therapeutic drug monitoring is done having the result of 12.05 g/mL. Because the creatinine clearance rate decreased, we adjust therapeutic regimen to 0.5 g, once every 8 h and 0.5 g, once 12 h successively. The results of therapeutic drug monitoring are maintained among 10–20 g/mL. The patient finally recovered and discharged. Clinical pharmacists have carried out reasonable and effective individual treatment. It is helpful to improve the safety and effectiveness of vancomycin on the basis of population pharmacokinetics.

**Keywords**: clinical pharmacist; population pharmacokinetics; mediastinitis; vancomycin; therapeutic drug monitoring

## S6.2

### Gold nanostructures for gambogic acid delivery

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Nanotechnology provides a novel strategy for the delivery of anticancer drugs. In this study, titanium dioxide coated gold nanorods (GNR/$TiO_2$) was used as the drug carrier for gambogic acid in order to improve its anticancer effect. Biocompatibility and cellular uptake of GNR/$TiO_2$ was studied in human glioblastoma U-87 MG cells. Cell viability was evaluated by ATP assay and calcein AM staining. LysoSensor Green DND-189 and Hoechst 33342 were used to analyze the intracellular location of GNR/$TiO_2$. The *in vitro* anticancer effect of gambogic acid loaded nanoparticles was compared with free drug. The results showed that GNR/$TiO_2$ is biocompatible, and they are localized at the intracellular acidic compartments of endosomes and lysosomes. The intracellular drug content delivered via GNR/$TiO_2$ was 6 fold higher than the free form, thus dramatically enhancing the anticancer effect of gambogic acid. Furthermore, mild photothermal therapy also showed synergistic effect with the drug. Our study suggested that GNR/$TiO_2$ is a promising anticancer drug carrier.

**Keywords**: gold nanorods; titanium dioxide; photothermal therapy; gambogic acid; drug delivery

**Acknowledgements**: The project was supported by the Macao Science and Technology Development Fund (FDCT) (No 014/2014/A1).

## S6.3

### Mass spectrometric multi-reaction simultaneous monitoring of acrolein-deoxynucleoside adducts and its application to screen human leukocytes

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The exposure to genotoxic chemicals and the occurrence of reactive electrophiles formed in endogenous cellular processes can induce chemical modifications of DNA (DNA adducts or adductomics) and thus trigger harmful responses in the body. The present study aimed to develop a method for simultaneous and untargeted detection of both known and unknown acrolein-deoxynucleoside adduts. In this work, a liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) using multiple reaction monitoring (MRM) was adopted to qualitative profiling of various acrolein-deoxynucleoside adduts. The chemical derivatization conditions were optimized by adjusting incubation buffer, pH, ion strength, temperature and reaction time. The *in vitro* incubation in 50 mmol/L phosphate buffer notably improved the MS-based detection sensitivity (10-fold) of acrolein-deoxynucleoside adducts with the limit of detection of 5 fmol. By applying the newly developed method, six acrolein adducts of all four nucleotides (dG, dC, dA, dT), including 3 Acr-dG isomers (γ-, α- and AcrdG) and 3 non-dG adducts (Acr-dC, Acr-dA and Acr-dT) can be detected in human leukocytes. These findings suggest that the modification adducts can be spontaneously generated via normal cellular metabolism. Considering the difference in mutation potency of these adducts, this new wide-scope approach can improve untargeted profiling and accurate monitoring of the circulating acrolein-deoxynucleoside addults, as potential biomarkers or epigenetic regulators of DNA oxidative damages, toward understanding the functions of this modification in related human diseases.

**Keywords:** DNA adductomics; acrolein; multiple reaction monitoring; untargeted profiling; leukocytes

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## S6.4

### Strategies for enhancing the delivery of poorly soluble compounds in drug discovery and development

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Discovery candidates with poor aqueous solubility pose technical challenges in formulating oral dosage forms during drug development. Available enabling formulations can promote oral bioavailability by either increasing the drug's equilibrium solubility (*eg*, prodrugs), enhancing the apparent solubility by forming drug-carrier complexes (*eg*, surfactant micelles), or creating a supersaturated drug solution (*eg*, amorphous solid dispersions (ASDs) in water-soluble carriers) during dissolution in the gastrointestinal (GI) microenvironment. In the latter case, precipitation within the intestinal lumen owing to the unstable nature of the supersaturated drug solution could reduce the absorption rate resulting in suboptimal systemic exposure. Supersaturating formulations are often subjected to in vitro dissolution testing under nonsink conditions to gain better understanding of their supersaturation kinetics and potential in vivo precipitation behavior. Such dissolution profiles are typically designed to exhibit a rapidly dissolving and supersaturating "spring" with a precipitation retarding "parachute". However, current evidence shows that an exceedingly high rate of supersaturation generation (*ie*, rapid dissolution) can actually result in a suboptimal dissolution profile due to crystallization events with reduced oral bioavailability. In this presentation, we outline recent research findings on the effects of the rate and extent of supersaturation generation on the overall kinetic solubility profiles of supersaturating formulations. Additional insights into the design of suitable supersaturating formulations to best improve the dissolution behavior and oral bioavailability of poorly water-soluble drugs will also be highlighted.

**Keywords**: poorly water-soluble drugs; amorphous formulations; solid dispersions; supersaturation; precipitation; crystallization

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## S6.5

### Systems biology and drug discovery

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With the rapid development of computational and network biology, achievements have been gained in the drug discovery. Since many diseases are caused by complex mechanisms, in the past decade, we used network pharmacology and molecular docking and other biological methods to construct the network of diseases (such as

colonic malignant transformation network, oral mucosal leukoplakia network, *etc*), identify the targets or study the off target effect of drugs (GRP78, AKAP, AQP1, NE, S100A9, *etc*), and by using molecular docking and virtual screening methods, we try to find new candidates for cancer treatments (for example AQP1 inhibitor, BH3 mimetics, NE inhibitors). As one example, we investigated the angiogenic effects of curcumin on an ischemia and lung cancer model. To induce ischemia combined with lung cancer models, unilateral femoral arteries of C57BL/6 mice were disconnected and Lewis lung carcinoma (LLC) cells were xenografted on the opposite side. Angiogenic effects and underlying mechanisms associated with curcumin were investigated. Molecular target (s), signaling cascades and binding affinities were detected by Western blot, two-dimensional gel electrophoresis (2-DE), network biology, computer simulations and surface plasmon resonance (SPR) techniques. We found that curcumin promoted post-ischemic blood recirculation and suppressed lung cancer progression in inbred C57BL/6 mice via regulation of the HIF1α/mTOR/VEGF/VEGFR cascade oppositely. Inflammatory stimulation induced by neutrophil elastase (NE) promoted angiogenesis in lung cancer tissues, but these changes were reversed by curcumin through directly reducing NE secretion and stimulating α1-antitrypsin (α1-AT) and insulin receptor substrate-1 (IRS-1) production. Meanwhile, curcumin dose-dependently influenced endothelial cells (EC) tube formation and chicken embryo chorioallantoic membrane (CAM) neovascularization. We concluded that curcumin had opposite effects on blood vessel regeneration under physiological and pathological angiogenesis, which was affected through negative or positive regulation of the HIF1α/mTOR/VEGF/VEGFR cascade. Curcumin had the promise as a new treatment modality for both ischemic conditions and lung cancer simultaneously in the clinic.

**Keywords**: systems biology; network pharmacology; drugable target; cancer; ischemia; angiogenic

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## S6.6

### Determination of the structure and activation mechanism of the human angiotensin II receptor AT1 by biochemical probes

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Structures of G-protein coupled receptors are mostly determined by protein X-ray crystallography. Most such receptors need important stabilization efforts either by using stabilizing protein inserts and/or very tightly binding inverse agonists; consequently in most cases such receptors were of inactive conformations. Different approaches are needed to evidence receptor mobility and structural changes during receptor activation. We have devised a doubly iterative chemoselective labelling approach that allowed ligand-receptor contact determination along the whole length of the peptide ligand under physiological conditions in living cells expressing receptors at 37 ºC.

We found 52 individual ligand-receptor contact points between the human AT1 receptor and Angiotensin II. This information allowed to construct the agonist occupied AT1 receptor structure by homology modelling and to elucidate the molecular network responsible for receptor activation. This structure was later confirmed by a crystallographic approach by another laboratory. These results evidence an important interaction network and receptor movements that are necessary for receptor activation, receptor bias and constitutive activity.

## S6.7

### Targeting transverse tubular structure in skeletal muscle physiology and diseases

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Transverse tubule (TT) networks are critical structures for excitation contraction coupling in striated muscles. Mitsugumin 29 (MG29) is a muscle-specific member of the synaptophysin family protein that participates in controlling the biogenesis of TT structure and maintenance of skeletal muscle functions. Genetic ablation of MG29 leads to TT network defects in skeletal muscle, which resembles the abnormal TT structure observed in dystrophic muscles. MG29 protein level is drastically reduced in animal models and human patients with muscular dystrophy. Interestingly, SYPL2 gene that encodes MG29 protein contains a unique 3' untranslated region (UTR) with potential binding sites for inhibitor factors. Molecular biology studies revealed that miR-181a could target a region in the 3'UTR of SYPL2. Overexpression of antagomir miR-181a by electroporation into mouse flexor digitorum brevis (FDB) muscle significantly increased the expression of MG29. To further determine the function of MG29 in muscle physiology and regeneration, co-immunoprecipitation was performed and we found that MG29 can bind to Bin1, another TT protein. Two-color STORM super-resolution imaging analysis confirmed co-localization of MG29 and Bin1 on TT. Distribution of Bin1 is severely disrupted in $mg29^{-/-}$ muscle. For testing the role of MG29 in muscle regeneration, we injured gastrocnemius muscle with cardiotoxin (CTX) and tracked muscle regeneration. Immunoblotting showed that following CTX injury, MG29 protein levels were transiently reduced from day 1 to day 3, followed by recovery associated with muscle regeneration, while Bin1 remained relatively stable. Compared with wild type muscle, the $mg29^{-/-}$ muscle displayed delayed regeneration with significantly reduced levels of Bin1 following CTX-induced injury. Our data suggest that the 3'UTR of MG29 and functional interaction between MG29 and Bin1 might be potential targets for treatment of muscle diseases.

**Keywords**: transverse tubule; microRNAs; muscular dystrophy; Mitsugumin29; SYPL2

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## S6.8

### Association of the genetic polymorphism of HLA with the the onset and prognosis of Anaphylactoid Purpura/Henoch-Schonlein Purpura and Henoch-Schonlein Purpura nephritis

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Anaphylactoid Purpura/Henoch-Schonlein Purpura (HSP), the most usual systematic disease in childhood, is a systemic vasculitis with the small vessel vasculitis as the main pathological change. In clinical pediatrics, Henoch-Schonlein Purpura Nephritis (HSPN) is the most serious long-term complication of HSP.

The etiology and pathogenesis of purpura remains unclear. Immune abnormalities play an important role in the pathogenesis of HSP. Significant polyclonal activation of B cells was observed.

The key to the prognosis of HSP is the degree of kidney involvement. The occurrence of the disease had a close relationship with polymorphism of HLA gene. In this review, the association between genetic polymorphism of HLA and the onset and prognosis of HSP and HSPN is summarized.

**Keywords**: genetic polymorphism; anaphylactoid purpura; Henoch-Schonlein Purpura nephritis

## S6.9

### Genomewide association study and weighted gene coexpresion network analysis identify novel genetic loci that modify antiplatelet effects and pharmacokinetics of clopidogrel

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Genetic variants in the pharmacokinetic (PK) mechanism are the main underlying factors affecting the anti-platelet response to clopidogrel. Using a genome-wide association study (GWAS) to identify new genetic loci that modify antiplatelet effects in Chinese patients with coronary heart disease, we identified novel variants in two transporter genes (SLC14A2 rs12456693, ATP-binding cassette [ABC]A1 rs2487032) and in N6AMT1 (rs2254638) associated with P2Y12 reaction unit (PRU)

and plasma active metabolite (H4) concentration. These new variants dramatically improved the predictability of PRU variability to 37.7%. The associations between these loci and PK parameters of clopidogrel and H4 were observed in additional patients, and its function on the activation of clopidogrel was validated in liver S9 fractions ($P$<0.05). Rs2254638 was further identified to exert a marginal risk effect for major adverse cardiac events in an independent cohort. To further identify the hub genes in the genes with SNPs suggestively associated with the H4 formation, we performed a weighted gene coexpresion network analysis in 32 human liver tissues and found MEF2A as a regulating factor of CYP2C19 gene expression, which was confirmed by a cell model. In conclusion, new genetic variants were systematically identified as risk factors for the reduced efficacy of clopidogrel treatment. Our study findings enhanced the understanding of the absorption and metabolic mechanisms that influence PD responses to clopidogrel treatment.

**Keywords**: clopidogrel; pharmacokinetics; antiplatelet effects; genome-wide association study; weighted gene co-expression network analysis

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## S6.10

### Effects of tetramethylpyrazine on regeneration of cochlear hair cells after gentamicin poisoning

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This study aimed to observe the effects of tetramethylpyrazine on the regeneration of cochlear hair cells exposed to gentamicin by auditory brainstem response test under scanning electron microscope. Totally 160 healthy guinea pigs were randomized into gentamicin, tetramethylpyrazine+gentamicin, tetramethylpyrazine, normal control (normal saline) groups. After 10 days of gentamicin medication, stereocilia on outer hair cells were fused, twisted, lodging, missing or incomplete. The cells exhibit chromatin condensation and margination, nuclear depression and shrinkage, and mitochondrial vacuolar degeneration. Along with the prolonged drug withdrawal time, the thresholds of auditory brainstem response (ABR) in the gentamicin group were restored partially; at 28 days after drug withdrawal, the third turn of outer hair cells exhibited slender cilia bundles, and meanwhile, the ABR thresholds were restored, but still lower than that in the normal control group ($P$<0.01). After 10 days of tetramethylpyrazine+gentamicin medication, the damage to cochlear hair cells was milder than that in the gentamicin group; at 28 days after drug withdrawal, the ABR thresholds in the tetramethylpyrazine+gentamicin group were similar to those in the normal control group ($P$>0.05). In the cochlea with functional recovery, newborn cells migrated from the basal plane of the basement membrane and the middle layer of the injured sensory epithelium to the cell layer of cavity surface. Overall, tetramethylpyrazine can antagonize damage to the cochlea hair cells in guinea pig after gentamicin-induced cytotoxicity and promote hair cell regeneration, with protection and improvement of the auditory function.

**Keywords:** tetramethylpyrazine; gentamicin; scanning electron microscopy; cochlea

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## S6.11

### Pdxdc1 (pyridoxal-dependent decarboxylase domain containing 1) as a novel antipsychotic drug target

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All existing antipsychotic medications target the dopamine D2 receptor, but many patients with schizophrenia do not respond to these treatments. In an attempt to identify new potential antipsychotic treatment targets, we searched for novel modulators of prepulse inhibition (PPI), the most commonly studied endophenotype for schizophrenia. We profiled mRNA transcripts across six different mouse strains with different levels of PPI. We then identified a number of transcripts that varied in expression level proportional or inversely proportional to PPI for each strain. We verified the correlation between transcript level and PPI for a selected group of mRNAs and chose Pdxdc1 for additional experiments. Using a lentiviral vector containing an anti-Pdxdc1 shRNA, we suppressed Pdxdc1 protein levels and saw a corresponding increase in PPI. The function of Pdxdc1 remains unknown, so further characterization of this protein could be useful for developing new antipsychotic medications.

## S6.12

### Gasotransmitter in physiology and pharmacology

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Gasotransmitters are endogenously generated molecules of gas. They are freely permeable to cell membranes and their effects do not depend on specific membrane receptors or second messengers. These gaseous signaling molecules functions at physiologically relevant concentrations. The concept of "gasotransmitter" opens new windows for understanding the complex and interweaved cellular signaling networks. Wide application and critical biological importance of gasotransmitters have been realized over the last decade. Nitric oxide (NO) is produced from *L*-arginine. Carbon monoxide (CO) is a product of heme metabolism and regulates numerous physiological processes as NO does. Hydrogen sulfide ($H_2S$) is the third gasotransmitter in terms of its discovery chronology relative to that of NO and CO. While NO takes the role of an endothelium-derived relaxing factor (EDRF), $H_2S$ fills the gap as an endothelium-derived hyperpolarizing factor (EDHF). These are examples of the physiological and pathophysiological roles of gasotransmitters. Gasotransmitter are biologically irreplaceable. They SAVE life. The challenges for future gasotransmitter research are plentiful. One of them is the complexity of the interactions among gasotransmitters and the significance of their cross-talks for cellular signaling network. Gasotransmitters share many common molecular targets but modulate their activities through different mechanisms. They also act on different targets but affect the common outcome. Finally, guess who's coming to dinner as new member(s) of the modern family of gasotransmitters. The next wave of gasotransmitter research will be as big as one can envisage–you can take my prediction to the bank.

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## S6.13

### Hydrogen sulfide and glucose homeostasis

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Glucose homeostasis is critical for maintaining physiological function of our body, whereby its sustained disturbance may lead to the metabolic syndrome. Among many endogenous mediators, the gasotransmitter hydrogen sulfide ($H_2S$) plays an important role in the regulation of glucose homeostasis. In the present study, we mainly focused on the role of cystathionine γ-lyase (CSE)-generated $H_2S$ in the regulation of hepatic glucose homeostasis including glucose update, glycolysis, glycogen and gluconeogenesis under different conditions. The current study may offer clues for the homeostatic regulation of glucose metabolism under physiological conditions and its dysregulation in the metabolic syndrome.

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## S6.14

### Non-human primate models of motor and non-motor symptoms and molecular pathology of Parkinson's disease as platforms for drug discovery

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Non-human primate models of Parkinson's disease (PD) have had a tremendous impact on the drug development process over the past 30 years. In particular, monkeys administered MPTP have provided models of advanced PD and proved especially successful in predicting Phase II clinical efficacy of many classes of symptomatic therapy for motor symptoms and complications of dopamine-replacement therapy. More recent, these models have been focused upon non-motor problems, particularly in the cognitive domain.

However, toxin based models have not had the same impact on identification of

clinically-efficacious disease-modifying agents. In the last few years, non-human primate models of PD based upon over-expression of alpha-synuclein, focally in the substantia nigra, have emerged. For the first time in non-human primate, viral vector-delivered alpha-synuclein has been shown to accumulate, in Lewy body-like aggregates, produce degeneration of dopaminergic neurons.

This presentation will review the pathology, behavioural and imaging finding in MPTP compared to alpha-synuclein models in non-human primates and discuss how best to employ both in the search for novel therapeutics for PD, whether they be symptomatic or disease-modifying.

**Keywords**: Parkinson's disease; non-human primate; alpha-synuclein; dyskinesia; cognition

## S6.15

### A non-human primate model of Parkinson's disease based on viral vector mediated overexpression of alpha-synuclein

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The present study aimed to develop a new non-human primate model of Parkinson's disease (PD) based on viral vector mediated overexpression of A53T alpha-synuclein (αSyn) to serve as a preclinical testing platform for evaluating potential therapeutics. To this end, 12 cynomolgus macaques were enrolled in the study (female, 9 years old). Each received baseline behavioural assessments on motor activity, non-human primate parkinsonian disability rating scale (MPPrs) and were trained to conduct a fine motor task (mMAP test). Behaviour was assessed once monthly. Baseline PET scans were obtained using $^{18}$F-labelled AV133 (VMAT-2) and FDG (fluorodeoxyglucose) and were both conducted every 2 months. Animals were bilaterally injected with either AAV1/2 A53T αSyn or empty vector (EV) (both,$1.7\times10^{12}$ gp/mL) into each hemisphere of the substantia nigra (SN). Animals were sacrificed 8 months post surgery. Postmortem results showed a 42% and a 39% reduction in putaminal DA and DAT, respectively, compared to controls (all, $P<0.05$), along with a reduction in TH positive neurons in the SN. Immunolabeling of the SN showed Lewy pathology revealed by accumulations of αSyn using LB509 and pS129 antibodies and furthermore accumulations were positive for thioflavin-S. Neurites also showed transgene expression throughout the striatum and had a dystrophic Lewy morphology. Behaviourally, by 5 months post surgery, A53T αSyn macaques showed 45% less motor activity in the 2–4 h period of a 4 h observation, compared to EV controls ($P<0.05$). This deficit persisted through to the final month (53%, 56% and 60%, respectively, to month 8, [all, $P<0.05$]). No significant effect was observed on mMAP performance at any time point. PET imaging showed no significant change in striatal VMAT-2 to assess dopaminergic activity or change in FDG uptake evaluated to derive the Parkinson related pattern (PrP). The macaque model of PD alpha-synucleinopathy produced here is in a position to assess therapeutics aimed at reducing or preventing αSyn accumulation in the nigrostriatal system, endpoints include: striatal neurochemistry and DAT, αSyn load per DA neuron, striatal αSyn levels, number of TH neurons remaining and locomotor activity. Furthermore, this primate model, with its robust αSyn expression, can be used to screen potential aSyn PET ligands with the goal of identifying an agent to use for assessment of disease modification.

**Keywords:** Parkinson's disease; viral vector; VMAT-2; non-human primate

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## S6.16

### Epigenetic chemical probes for target validation

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The Structural Genomics Consortium (SGC) is in the midst of a project to discover chemical probes for epigenetic targets. Epigenetic signaling is responsible for the control of gene expression through the regulation of chromatin packing. At the molecular level this is accomplished by proteins which catalyze methylation/demethylation and acetylation/deacetylation of histone tails. In addition, a large family of proteins recognizes or "reads" the epigenetic marks. In order to understand the role of these proteins in diseases, potent, selective inhibitors or antagonists of these processes are highly desired. A variety of strategies are being employed to discover epigenetic chemical probes, including HTS and structure-based design. Multiple examples of how compounds are chemically optimized and characterized in biochemical and epigenetic assays are presented.