（一） 代表性新药研发进展

2005年上海药物研究所针对国家重大需求，围绕严重危害人民健康的恶性肿瘤、神经系统疾病和感染性疾病等重大疾病，以基础研究和平台建设促进新药创制，使药物研究开发各阶段都有一批候选新药在进展中，形成了良好的梯次发展态势。一批结构新颖、生物活性显著的先导化合物被先后发现；多种候选化合物正处于临床前新药研究阶段；抗老年性痴呆药希普林、抗肿瘤药沙尔威辛、抗心律失常药硫酸舒欣啶、抗菌药安妥沙星4个具有自主知识产权的新药进入临床研究；特别是治疗心血管疾病二类中药丹参多酚酸盐和治疗心律失常一类新药盐酸关附甲素等重大新药项目本年度取得新药证书，抗老年痴呆一类新药希普林在欧洲结束II期临床研究，标志着上海药物研究所新药研发取得重大突破。

1．治疗心血管疾病现代中药注射用丹参多酚酸盐取得新药证书

丹参是传统的活血化瘀中药，临床上广泛用于治疗冠心病、心绞痛、缺血性中风等疾病。我国目前生产的丹参及其复方制剂品种很多，仅注射剂的年使用量就达30亿支以上。但这些产品有效成分不明确，因此质量难以控制，导致临床疗效不稳定，不能适应中药现代化和国际化的要求。

在中国科学院、国家科技部和上海市的支持下，上海药物研究所开展了中药丹参的系统研究，发现以丹参乙酸镁为主要成分的多酚酸盐是丹参治疗心血管疾病最重要的有效成分。在此基础上，创新性地提出了以丹参乙酸镁作为质量控制标准，建立了拥有专利的提取精制工艺，成功研制出丹参多酚酸盐（Depsides Salt）及其注射用丹参多酚酸盐，新的质量标准能充分反映该药的临床疗效，并与原有的丹参制剂存在本质上的不同。该药有效成分明确，总多酚酸含量近100%，其中丹参乙酸镁含量达到80%以上，并且运用了指纹图谱技术对药材、原料药和制剂的质量进行全面控制 。

经过大量的临床前药理学研究及临床试验，证明丹参多酚酸盐对于冠心病、心绞痛疗效显著、确切，病人使用安全，显著优于现有丹参注射剂。作为治疗冠心病心绞痛的现代中药，丹参多酚酸盐首次在大规模的临床试验病例中，采用了国际公认的运动试验作为药物疗效的评价标准，显示该药能显著地增加治疗前后患者的运动耐量和运动级别。同时，开展了人体药物代谢动力学的研究，这是国内第一个进行人体药代研究的中药，为该药在临床上的广泛应用提供了充分的实验依据 。

2005年5月25日，丹参多酚酸盐和注射用丹参多酚酸盐的新药注册申请经国家食品药品监督管理局（SFDA）批准，获得了新药证书和生产批文。6月被国家发改委批准为现代中药产业化示范项目。丹参多酚酸盐的相关技术已获得中国专利和美国专利的授权，成为拥有自主知识产权的现代化中药新药。研究所还与绿谷（集团）有限公司达成合作开发协议，在张江高科技园区共同组建了上海绿谷制药有限公司，合作开发丹参多酚酸盐注射剂，现已投入市场。该药是现代中药的一个典范，将会带来巨大的社会和经济效益。

2．治疗心律失常新药盐酸关附甲素取得新药证书

盐酸关附甲素（Acehytisine Hydrochloride）是从中药关白附子的根块中提取分离得到的二萜类生物碱，由上海药物研究所科研人员筛选发现的具有抗心律失常活性的新天然化合物。

经药理实验证明，盐酸关附甲素对高钙、结扎冠状动脉及乌头碱诱发的室性心动过速和心室颤动有拮抗作用，并可对抗乙酰胆碱诱发的心房扑动和心房颤动。该药对快反应心肌细胞的Na^+内流具有抑制作用。人体电生理研究显示，盐酸关附甲素能延缓心房、房室结、希蒲系统和心室内传导，是该药抗心律失常的主要机制。

上海药物研究所与中国药科大学共同合作，经过15年的临床前研究和临床试验，完成了创新药物盐酸关附甲素注射液的研发。该药临床试验结果表明，盐酸关附甲素对室性早搏的总有效率为85.7%，对阵发性室上性心动过速的总有效率为78.1%，治疗室性早搏和终止阵发性室上性心动过速安全有效，不良反应少。

本项目曾获国家医药管理局“七五”国家重点科学技术项目、“十五”重大科技专项“创新药物和中药现代化”的资助，于2005年8月22日经国家食品药品监督管理局（SFDA）批准，获得了新药证书。

该药由深圳大佛药业受让，吉林马应龙制药有限公司生产。

3．治疗老年性痴呆候选新药希普林（ZT-1）在欧洲完成II期临床

希普林（Schiperine）是在上海药物研究所发明的抗老年性痴呆新药——石杉碱甲的基础上，创新研制的新一代抗老年痴呆药物，具有我国自主知识产权，在国际上倍受关注。经药理和动物模型研究表明，该药在体内、外对胆碱酯酶抑制的选择性、对多种动物的多种认知障碍模型的恢复效果、生物利用度、毒性等方面，均优于上世纪90年代以来国际研制的抗老年痴呆药物多奈派齐、他克林和利瓦司替明(Rivastigmine)，也优于我国在90年代研制的药物石杉碱甲，有望成为国际上治疗早老性痴呆症患者认知缺损更有效的、具有自主知识产权的新药。

上海药物研究所从2000年起与瑞士德彪集团合作共同研发希普林。I期临床研究显示其具有良好的安全性、耐受性和提高记忆活性。II期临床研究于2004年1月份开始，通过评估希普林和石杉碱甲不同时段的浓度，测定希普林对中度AD病人的疗效。该随机双盲对照临床研究，在法国、瑞士、南非等6个国家38家医院同时展开，已于2005年底完成揭盲，临床疗效优于阳性对照组药多奈派齐，即将进入III期临床试验。

国内临床试验已于2005年7月11日获取II期临床试验批件（2005L02368），11月份II期临床已在首都药科大学北京天坛医院、北京医院、北京大学第六医院、第四军医大学西京医院等正式开始，计划在一年内完成。

希普林具有全球知识产权保护，已获美国、欧盟、日本等国专利授权。该候选新药在国内外同步开展临床研究和新药注册，有望成为真正意义上进入国际医药主流市场，并且具有良好市场前景的由我国科学家自行研制的首个新药。希普林后续技术开发同步跟进，现已进行皮下包埋缓释给药系统的研究，以开发更安全和方便给药的长效新剂型。

为了确保合成希普林用的原料提取植物资源的可持续开发，上海药物研究所正在进行药材的种植和共生菌的培养等研究，并取得了良好的效果。人工种植药材“千层塔”已经取得成功，正在推广中。该技术不仅为希普林的国内外医药市场提供稳定的原料保证，也对环境资源保护和促进农村高附加值养殖业具有重要意义。

4．抗菌候选新药盐酸安妥沙星临床研究进展顺利

盐酸安妥沙星（Antuofloxacin）是具有自主知识产权的氟喹诺酮类创新药物。与左旋氧氟沙星对照相比，盐酸安妥沙星是一种安全、有效、质量可控（稳定）并有优良药物代谢特性的新型抗菌药物，2005 年 11 月已进入 III 期临床，预计 2007 年初取得新药证书和生产批件。盐酸安妥沙星有可能成为我国自主研发的第一个氟喹诺酮类抗菌新药，具有很大的经济效益和社会效益。

盐酸安妥沙星的临床研究显示出高效、长效抗菌作用。II 期临床研究以左旋氧氟沙星为对照，采用双盲双模拟法治疗慢性支气管炎急性发作和肾盂肾炎，在不揭盲的情况下中期分析结果表明：首次 400 毫克 / 天，以后 200 毫克 / 天（与同类药物每次 400-600 毫克相比，有十分明显的优势），盐酸安妥沙星有效率高达98.0-100.0%。

慢性支气管炎急性发作：治疗 1 天后痊愈显效率高达 96.55%，治疗 7 天后痊愈显效率高达 100.0%。治疗 1 天后细菌完全清除率为 96.5％，治疗 7 天后细菌完全清除率为 100.0%。轻度不良事件发生率为 10.34%，病人依从性好。

肾盂肾炎：治疗 1 天后痊愈显效率高达 97.78%，治疗 7 天后痊愈显效率高达 95.45%。治疗 1 天后细菌完全清除率为97.72％，治疗7天后细菌完全清除率为97.62%。轻度不良事件发生率为4.35%，病人依从性好。

根据上述结果，2005年10月20日开始同时进行III期临床研究。计划于在2006年完成III期临床并申报新药证书和生产批件。

5．抗心律失常候选新药硫酸舒欣啶进入临床研究

硫酸舒欣啶（Sulcardine Sulfate）是上海药物研究所通过对具有抗心律失常作用的先导化合物进行结构修饰，从中筛选出具有很强生物活性的化合物。其化学结构新颖，作用机理独特。实验研究显示，硫酸舒欣啶对心肌细胞的钠通道、钙通道和钾通道均有明显的抑制作用，属于新型作用机制的抗心律失常药，具有更安全和高效的特点。动物实验也证明，硫酸舒欣啶是一个安全、高效的抗心律失常药物，为该药进行临床试验提供了充分的实验依据。硫酸舒欣啶性质稳定，制备时原料易得，合成比较简便，适合工业化生产，具有良好的发展前景。

硫酸舒欣啶临床前研究工作目前已全部完成，于2004年8月通过了药品审评中心技术审评，获得国家食品药品监督管理局（SFDA）的新药临床研究申请批件，并于 2005 年底正式进入临床研究 。

硫酸舒欣啶已获得了中国和美国的发明专利授权；同时，PCT 专利也获得批准，将进入德国、法国、瑞士、西班牙和意大利等国家。2002 年 10 月，本项目获得国家“十五”重大科技专项

“创新药物和中药现代化”的资助。

硫酸舒欣啶的成功研制，将成为我国具有自主知识产权的创新药物，可产生显著的经济和社会效益。

（二） 重大项目研究成果

积极争取和努力实施国家重大科研任务是上海药物研究所的中心工作。2005 年是“十五”的最后一年，各部委和中科院集中对“十五”期间承担的项目进行结题验收，并着重进行“十一五”和中长期战略规划。研究所在积极组织好各类项目总结验收工作的同时，还积极争取重大项目，承担了一大批国家、地方重大科研项目，取得了一批具有原始创新性的科研成果。2005 年研究所共获国家部委（含中科院）重大科研项目共 12 项，在研重大科研项目共 56 项。

在本年度进行的各重大项目中期评估和验收中，上海药物研究所承担的 4 个“973”计划项目子课题均通过了科技部组织的中期评估。其中，蒋华良研究员主持的国家“973”项目在本年进行的项目中期评估中被评为优秀。此外，上海药物研究所在“十五”期间承担的一系列“863”计划课题与《创新药物和中药现代化》国家重大科技专项课题全部顺利通过了科技部组织的专家组验收。特别是由陈凯先院士负责的“新药筛选关键技术平台”和由丁健研究员负责的“抗肿瘤、抗心血管病新药药效学关键技术及平台研究”两大项目获得专家组的好评。通过上述两项目的实施，逐步建立和发展完善了我国自主的新药筛选技术平台体系和抗肿瘤、抗心血管病新药药效学评价体系，这标志着我国新药筛选研究和药效学评价研究取得了重要的技术进步，对我国药物研究的源头创新和持续创新将发挥积极的推动作用。

1. “新药筛选关键技术平台”项目获专家组好评

新药筛选是药物研究源头创新、持续创新的关键环节。《药物筛选技术平台研究》课题围绕新靶点、新模型、新技术、新化合物四个关键点，全面提升我国药物自主创新能力，推动我国新药研究逐步实现由仿制为主到创新为主的历史性转变，推动我国医药产业逐步完成由生产主导型产业向研发驱动型产业的战略转轨。

项目依托中国科学院上海药物研究所，组织了国家新药筛选中心、中国医学科学院药物所、中国医学科学院医药生物技术研究所、中国药科大学、北京大学药学院和福建微生物研究所承担本课题，在全体研究人员的共同努力下，全面超额完成了预定指标。本课题各承担单位针对肿瘤、心脑血管疾病、神经退行性疾病、代谢性疾病及严重感染性疾病等重大疾病的关键靶点，进一步完善了各具特长的新药筛选中心和实验室，在整体上形成了比较系统的国家新药筛选技术平台体系。

上海药物研究所承担的主要工作有：使样品库有了快速增长，初步形成了一定规模的化合物样品库，特别是建立了具有我国资源特色的植物天然产物和微生物样品库。同时，在国内首先应用了多种高通量筛选新技术和新方法，包括处于世界前沿水平的高内涵药物筛选技术，于2005年完成

了从96孔向384孔平板的升级和关键仪器设备的更新换代，引进了与国际接轨的样品信息管理系统，从而极大地提高了药物筛选的技术能力、研究水平和工作效率，日筛选量由2万样次提高至10万样次。总计发表文章93篇（包括研究论文和综述），申请专利40个，授权9个。新建了68个筛选模型，其中10%未见国内外在同类靶点上报道活性小分子化合物，约50%模型国内外尚无同类机理的新药上市。这些筛选新模型的建立，为我国开展自主药物创新研究，参与国际竞争，奠定了十分重要的基础，为全国29个省市、自治区241个研究机构、大学及企业提供了筛选服务。

上述具有国际先进水平的技术平台加上正在不断建设中的国家化合物样品库和国内一流的创新药物研究队伍，为我国医药工业实现从仿制为主到创新为主的历史性转变和跨跃式发展做出了重要的贡献。目前已经研究发现的胰高血糖素样肽受体（GLP-1R）小分子激动剂、具有体内神经保护作用的新型Caspase-3抑制剂（CH95）和新型非核苷类抗乙肝病毒先导化合物（W28）等内在的11种新药候选化合物不仅代表了这一公共技术平台的创新能力，而且具有巨大的市场前景。

课题注重应用生命科学前沿的新成果，丰富和完善筛选技术，建立了适应基因组时代生命科学发展的药物研究新模式。一方面，把一批现代分子生物学、细胞生物学的先进研究方法应用于高通量药物筛选，建立了先进的高通量筛选检测方法。另一方面，把握功能基因组时代大量靶标不断发现的发展趋势，建立了若干集群式家族靶标体系，利用高通量平行筛选，获取每个化合物对于同一家族不同亚型的作用信息；积极引进和发展了高内涵筛选技术，在保持细胞结构和功能完整性的前提下，同时检测被筛样品对细胞形态、生长、分化、迁移、凋亡、代谢途径及信号转导各个环节的影响，在同一细胞、单一实验中获取大量相关信息，确定其生物活性和潜在毒性，发展了富有创新性的集群式高通量筛选和高内涵筛选相结合的新模式，使我国的药物筛选技术基本达到或接近国际水平。

筛选技术平台的研究显著增强了我国开展药物源头创新、自主研发创新药物的能力。“国家新药筛选体系的建设和高通量筛选技术的研究和应用”获得2002年度上海市科技进步一等奖，“现代新药筛选体系和高通量筛选技术的研究和应用”获得2003年度国家科技进步二等奖。

2．“抗肿瘤、抗心血管病新药药效学关键技术及平台研究”获专家组好评

恶性肿瘤及心脑血管疾病是危害我国人民生命健康的重大疾病，且发病率仍呈逐年上升趋势，已经成为城市主要疾病死亡率及死亡原因的前两位。建立和完善符合国际规范标准的、科学系统的抗肿瘤及治疗心脑血管疾病药物的药效学评价技术平台，是研发有效药物的前提和基础，对于推动创新药物的产业化和国际化，促进中药走向国际市场具有重要现实意义。

中国科学院上海药物研究所为项目依托单位，联合中国中医研究院西苑医院、第二军医大学、中国医学科学院医药生物技术研究所和中国医学科学院药物研究所共同承担本项目，包括了抗肿瘤药物和抗心、脑血管病药物药效学评价关键技术与平台。

上海药物研究所主要承担了抗肿瘤药物药效学关键技术与平台研究。建立和完善了抗肿瘤药物药效学评价技术平台和数类新型抗肿瘤药物评价技术及标准，包括具有中国特色的达到国际标准的

人癌体内外抗肿瘤药物药效学评价技术平台；细胞、组织、体内多水平的肿瘤新生血管生成抑制剂系列评价模型以及相应的药效评价标准，并有多个候选新生血管生成抑制剂正在进行药效评价；建立了 8 个分子水平的受体或非受体型酪氨酸激酶模型和系列酪氨酸激酶高表达肿瘤细胞（肺癌、乳腺癌）模型，并利用这些模型对大量化合物进行了抑制酪氨酸激酶活性的测定，发现了数十个具有不同的酪氨酸激酶抑制活性的化合物；检测酪氨酸激酶抑制剂的先进的荧光偏振技术及其标准操作规程和评价标准；DNA损伤模型的标准操作规程以及评价标准；系统的微管抑制剂评价模型的标准操作规程以及评价标准；通过原位接种和克隆选择，建立了人乳腺癌 MDA-MB-435、MDA-MB-231两个裸小鼠移植瘤高转移模型以及四种抗转移评价方法。本课题搭建了抗肿瘤药物的临床前药效学评价系统，建立了抗肿瘤药物药效学评价的标准操作规范 29 项。利用上述平台完成了 33 个候选新药的临床前药效学评价，其中 2 个进入了临床研究，6 个正在申报临床，另外 25 个候选新药正在进行其他的临床前研究，特别是为国外医药公司完成了 2 个候选新药的临床前药效评价，评价报告得到该公司的认可和好评。申请国家发明专利 28 项,国际专利 3 项，获国际专利授权 1 项；在国际 SCI 学术期刊上发表论文 37 篇，总影响因子 119.98；获 2003 年国家科技进步二等奖一项、第六届 DEBIO-CCRF（德彪 -CCRF）中国奖 三等奖、首届上海药学科技奖一等奖、中国科学院院长特别奖、全国百篇优秀博士论文、美国 AACR Scholar traning Award 等奖项 13 项；培养了博士后 3 名、博士 16 名、硕士 1 名，建立起了一支抗肿瘤药物临床前药效学评价的中青年骨干队伍，为我国的抗肿瘤新药研发提供了良好的药效药理学技术平台。

3．973 项目“基于生物信息学的药物新靶标的发现和功能研究”中期评估优秀

该项目共包括七个子课题，其中，上海药物研究所蒋华良研究员承担了“药物靶标模拟筛选结构生物信息体系的建立”，拟建立药物靶标模拟筛选生物信息公共平台系统，与第一子课题合作，服务于其他子课题。主要任务是发展高通量蛋白质三维结构模建方法、高通量蛋白质－小分子相互作用虚拟筛选方法、已知活性分子(包括中药有效成分)的作用靶标或作用途径搜寻的方法，用于新候选靶标的进一步验证及其活性分子的发现；建立其它课题获得的候选药物靶蛋白的三维结构数据库和蛋白质动态结构(构象变化)数据库，用高通量虚拟筛选方法从现有的小分子数据库中筛选或用全新药物设计方法设计新的化合物，用于候选靶标的验证。

项目实施以来，组织了上海药物研究所分子模拟和药物研究方面的优秀科研人员，与其他子课题紧密合作，共同协作努力，圆满完成了本子课题前两年的研究任务，实现了预期目标。特别是在2003年SARS爆发期间，本子课题建立的平台系统用于SARS感染机理和抗SARS药物研究，取得了较好的结果。进行了药物靶标模拟筛选生物信息公共平台系统建设和药物靶标模拟筛选生物信息平台系统应用研究。本年度共在 SCI 期刊上发表论文 49 篇，其中国际刊物 42 篇，影响因子(IF)大于 10 的 1 篇，大于 5 的 9 篇，大于 4 的 18 篇，总影响因子大于 150。发表论文的重要杂志有 PNAS(IF>10)、JBC(IF>7)、JACS(IF>6)、Chemistry & Biology(IF>6)。被邀请为 Curr. Med. Chem.

(IF>5)和 Curr. Pharmac. Design(IF>5)撰写综述文章 4 篇。翻译了 Julio Licinio & Ma Li 主编的专著《Pharmacogenomics - The Search for Individualized Therapies，药物基因组学 - 寻求个性化治疗》(大部分课题成员参加翻译工作，科学出版社，2005，北京)。申请中国专利 18 项，国际专利 2 项。应邀 12 次在国际学术会议上作邀请报告，2 次在国内学术会议上作邀请报告。

上海药物研究所岳建民研究员承担的子课题“以中草药有效成分为探针发现和验证药物靶标”，主要研究内容包括：①发现 3-5 个新的可用于探针的中草药有效成分，初步确认能与探针小分子特异性结合的蛋白质；②发现能与探针小分子作用的特异性结合的蛋白质 3-5 个；③发表论文 5-7 篇，申请专利 1-2 项；④培养和建立一支高素质和年轻化的研究队伍。两年来，本子课题组织了上海药物研究所天然药物化学和细胞分子生物学方面的优秀科研人员，与其他子课题紧密合作，特别是本项目的课题二，共同协作努力，圆满完成了本课题第一阶段的研究任务。

本子课题利用天然活性成分为探针分子探索药物靶标，并建立利用该靶标筛选和发现药物活性化合物的平台系统，与其他课题合作，并服务于其他子课题。主要任务是在已取得的成果基础上，利用相关子课题发展的高通量蛋白质三维结构模建方法、高通量蛋白质－小分子相互作用虚拟筛选方法、已知活性分子(包括中药有效成分)的作用靶标或作用途径搜寻等方法，结合研究所现有的生化和生物物理技术平台（如，配体垂钓技术、靶标垂钓技术等）发现和验证药物靶标。进行了抗幽门螺旋杆菌的活性化合物的发现与研究以及抗肿瘤活性化合物的发现与研究。在前期对相关幽门螺旋杆菌种属进行了系统的基因组分析，利用其他课题组相关技术平台，并结合研究所前期的实验结果，对可能作为药物靶点蛋白质进行表达，并建立相应的筛选模型。与相关课题合作，共在 SCI 期刊上发表论文 50 篇，其中国外刊物 40 篇。应邀在国内和国际学术会议上作邀请报告 4 次。

（三） 在抗禽流感药物研究中快速反应，取得重要进展

上海药物研究所在知识创新工程中一直将严重影响公共安全的感染性疾病的治疗药物列入新药研究重点，已经启动针对禽流感、肝炎、血吸虫病等重大传染性疾病防治药物研究项目，其中，“禽流感病毒蛋白结构与功能研究及相应小分子抑制剂设计”获得上海市科学技术委员会2005年启明星计划支持，“抗禽流感药物的筛选”项目获得上海市科委攻关项目支持。

2005 年入冬以来，由高致病性 H5N1 病毒株引起的禽流感，在国际上许多国家和地区蔓延，中试放大工作。上海药物研究所合成的磷酸奥司他韦原料做成制剂后，经上海市药品检验所检测，与罗氏达菲产品一致。根据与上海医药集团签订的合作开发合同，研究所已将磷酸奥司他韦的原料及制剂工艺移交给上海医药集团。此外，上海药物研究所又相继完成相关申报资料的准备工作，为中国科学院仿制磷酸奥司他韦解决知识产权问题奠定了基础；与此同时，研究所还进一步研究解决了“达菲”生产原料的问题。在上海药物研究所的技术支持下，上海医药集团已经取得罗氏公司

在中国生产磷酸奥司他韦的许可，解决了我国的抗禽流感药物技术和战略储备的问题。目前上海药物研究所也已与上海医药集团合作，共同向SFDA提请磷酸奥司他韦的药品注册申请。此项工作得到院领导的高度赞扬，路甬祥院长亲自发贺信表示祝贺。

此外，上海药物所还启动了扎那米韦、帕拉米韦等抗病毒药物的仿制和me too类药物的研发，以期尽快获得自主知识产权的抗禽流感新药。科研人员根据H5N1禽流感病毒蛋白质的结构，进行了高通量虚拟筛选和全新药物设计，争取从现有的药物中发现抗禽流感药物，迅速进行临床研究，获得全新结构的化合物进行临床前研究。目前，通过高通量虚拟筛选已完成了200万个化合物的筛选，获得了候选化合物，合成了部分化合物，大部分化合物的合成和筛选工作正在进行之中。

（四） 研究技术平台建设

围绕中国科学院围绕中国科学院“人口健康与医药创新基地”和“张江国家生物医药产业化基地”的建设，上海药物研究所在2005年进一步完整、健全创新药物研发技术平台，为全国、全院和上海市药物创新研究提供药学专业的技术支撑和配套服务。针对各平台的不同情况，采取“壮大、完善、强化、新建”相结合的措施，各技术平台建设取得显著成绩。在国家科技部、中科院及上海市的领导和大力支持下，研究所的创新药物研发技术平台建设取得显著成绩，新药筛选技术、药效学关键技术、新药临床前安全评价技术等平台得到进一步加强，其服务国内医药产业、参与国际竞争的能力显著增强。新药筛选技术、药效学关键技术、新药临床前安全评价技术等平台在2005年均通过科技部组织的平台项目验收；药物代谢研究技术平台和中药现代化平台建设取得明显成效，在软件、硬件建设上均已经形成规范和规模，在2005年获得批准，将建设成为上海市的关键技术平台，并为中科院知识创新工程三期中“人口健康与医药创新基地”的建设提供服务和技术保证；药物制剂技术、新药质量控制技术等平台得到了一定的发展，被确立为知识创新工程三期中重点建设的技术平台。2005年的平台建设和部署工作，为在知识工程创新三期中建成具有国内先进水平、能够参与国际竞争的创新药物研发平台体系奠定了坚实基础。

1．药物筛选技术平台

国家新药筛选中心已经建立了分子或细胞水平的高通量筛选模型，在国内首先应用了处于世界前沿水平的高内涵药物筛选技术等多种高通量筛选新技术和新方法。迄今为止，国家新药筛选中心已建立232个分子或细胞水平的高通量筛选模型(含亚型和功能型)，其中除个别模型尚在完善外，其他均投入使用，累计完成大规模随机筛选133余万样次，发现活性化合物327个，对25个活性化合物正在进行体内外的深入研究。2005年，中心顺利完成了从96孔向384孔平板的升级和关键仪器设备的更新换代，在信息管理上与国际接轨，极大地提高了药物筛选的技术能力、研究水平和工作效率。

截至2005年底，国家新药筛选中心为全国29个省、市和自治区的241家科研院所、大学医院和医药企业提供了多种形式的筛选服务，筛选了2.2余万个化合物或天然提取物。2005年总计发表文章38篇（包括研究论文和综述），申请专利18项，其中PCT申请2项。

国家新药筛选中心的国际合作范围持续扩大，与美国Invitrogen、美国Cellomics、日本田边、法国Servier、瑞士Actelion和丹麦Lundbeck等国际知名医药公司形成了战略伙伴关系，合作研究已经取得一系列重要成果，并于2005年4月召开了全国首届高内涵药物筛选研讨会，聘请多名海外专家前来授课，向全国各科研院校推广药物研究新技术。

2．药物设计技术平台

基于多学科交叉研究策略，上海药物研究所建立的超级计算机辅助药物设计平台和药物筛选与评价平台，已在创新药物先导结构设计和评价研究中发挥重要作用。今年在创新药物设计新方法探索、药物作用新靶标发现与确证、中草药药效物质基础研究等领域取得一系列重大进展。在国产万亿次集群式超级计算机上建立起了高效快速的药物发现与设计系统，发展了新的打分函数、组合库设计系统和类药性评价系统。针对糖尿病、老年性痴呆及艾滋病等疾病，建立起了可靠的筛选平台，发现了有价值的活性化合物数百个，其中2个新药先导化合物已进入临床前研究阶段。2005年共发表论文30余篇，包括PNAS、JBC、JACS等期刊杂志。共递交申请专利12项，获得发明专利证书1项。同时，还发现新靶标线索13个，成功地测定了其中一个蛋白质与抑制剂复合物的晶体结构；协助验证了3个药物新靶标。2005年8月还举办了国际“生化结构与功能研讨会”，邀请了包括诺贝尔奖获得者在内的14名欧美一流科学家进行学术交流。

3．中药现代化研究中心

2005年是中药现代化研究中心正式运转的第一年，相关工作取得重要进展，中心围绕科技能力建设（实验室建设和管理、科研管理、科技开发管理、知识产权管理）、信息网络建设（信息化建设和管理）等方面，启动各项工作并逐步走向正常化、规范化。中心从多学科、多途径、多角度研究中药的化学成分、药理作用、体内代谢、作用靶点和机制，研制现代中药，探索进行中药现代化研究的方法体系。中心建立了中药成分快速分离、纯化、鉴定平台，中药体内代谢过程研究平台，中药药效的细胞和分子机制研究平台以及中药新药研发平台，并为中药研究单位和产业提供服务。

2005年中心开展中药样品库和中药数据库建设，加强基础能力建设。选择临床应用广泛且有效的常用中药，从其道地产地采集道地药材样品，准确鉴定其学名，按常规方法进行粗提物、部位、类别成分或单体化合物等的提取分离和制备工作，形成专人负责保管的中药实物样品库。样品库可用于新靶点、新活性筛选，对于中药新药的开发具有重要价值；建成了以“药物化学基因组学数据管理系统”和“中药数据库信息管理系统”两大数据库体系为支撑的中药数据信息管理系统。

中心在创新中药方面与绿谷药业进行全方位合作，产品研发主要定位在肿瘤、心脑血管疾病、

妇科及糖尿病等疾病领域，通过“三品并行、多源并重、内外并举、远近结合”等战略手段，更好更快地引进新品，建立最佳的新产品储备体系。已针对市售产品“绿谷双灵固本散”成功进行了二次开发研究。

中药现代化研究中心参与完成了上海生物公共服务平台多中心合作以及上海市产、学、研联盟的大量工作，在科学研究、产品开发、成果转化、学术交流、资源共享、人才培养与使用、队伍管理建设等方面开展了广泛深入的合作。

4．药物安全性评价平台

本年度制定和规范了毒性病理学检测标准，编写了两本毒性病理学相关的专业技术书籍，基本形成了系统的毒性病理学检测体系。在我国率先建立了用于新药安全药理研究的遥测试验系统，积累了经验和数据。成功建立了短期大鼠致肝癌模型和快速肝脏毒性评价体系，并深入进行了相应的机理研究，研究成果得到了国内外专家的认可。

2005 年，中心承担了“用于新药快速致癌性检测和致癌机制研究的动物模型的建立”、“新药临床前安全评价 GLP 标准化研究”、“新药临床前安全评价 GLP 标准化研究（子课题）”和“重要神经系统疾病治疗药物作用靶点的寻找、先导化合物的发现及药理毒理学研究”等系列药物安全性相关的基础研究课题，并积极为所内外提供安全性评价服务，对 8 个重要新药进行了系统的安全性研究，对一批候选新药进行毒性快速评价。中心以先进的技术平台和良好的服务质量，积极开展与国外制药企业的合作，在 2005 年里已与多家国外公司合作开展新药的毒性评价试验项目。如与美国的礼来公司合作，已完成了一个试验，正在按照国际标准进行第二个化合物的毒性试验。此外，还与美国的 Ascenta Therapeutics Inc.、日本 Phytopharma 和香港大学开展了多项合作项目。

5．药物代谢技术平台

本年度围绕创新药物研发，形成了从药物早期发现的体外药代筛选，中期动物药代评价和体内未知代谢产物鉴定，到后期临床人体药代评价的新药药代研究平台，部分研究工作已达到国际先进水平。另外，在围绕中药现代化所涉及的核心科学问题而开展的复方中药药代研究方面，已有良好的基础和较强的实力。2005 年以本中心为基础，组建上海药物代谢研究中心，可行性方案获得通过，完成市科委组织的项目答辩。

中心目前配备了 5 台 LC/MS/MS 大型仪器，为进一步扩大研究规模、做好与国外先进的研发标准和规范接轨及参与国际新药研发工作创造了条件。

2005 年，中心共完成 6 项一类新药临床前药代动力学试验，10 项临床药物动力学试验和 3 类新候选化合物的动力学筛选试验；获得 3 项“药物临床试验批件”；另有 5 项一类新药临床前药代动力学试验，9 项一类新药临床前毒代动力学研究和 1 项一类新药临床药代动力学研究工作正在进行中。此外，中心还完成 2 项中药制剂药代动力学研究；17 项化合物动物体内预初药代快速评价，共 34 个先导化合物；在体外药代研究方面，中心完成了 23 个化合物的 Caco-2 膜通透性研究，18

个化合物对5种CYP450同工酶抑制活性的测定以及7个化合物对5种CYP450同工酶代谢稳定性的测试 。

在本年度中心申请了一系列国家及地方重大研究项目，其中包括：国家科技部“863”项目“治疗及预防日本血吸虫病青蒿素衍生物新药的研究”和“临床前药物代谢动力学关键技术及平台研究”、国家科技部“973”项目“中药组分的体内变化过程及配伍对其影响的研究”、上海市科委项目“药物代谢及上海药代技术平台建设”等。

2005年中心在本学科领域发表了一批具有重要学术价值的研究论文。中心的钟大放研究员还主持并参加了全国有机质谱第13届学术大会。

6．药物制剂平台

2005年药物制剂平台在平台建设和科研工作方面均取得了一系列可喜的成绩。作为项目或课题负责人有2项国家纵向项目获得批准：国家基金委面上项目（30572259）和中科院重点方向性项目(kjcx2-sw-h12-01) ；本年度承担的国家基金委面上项目（30371691）和973项目课题（2004CB518802）均按计划完成了年度工作任务（已递送年度总结）；参与国家科技部“863”项目课题“治疗及预防日本血吸虫病青蒿素衍生物新药的研究”（课题编号：2004AA2Z3210，课题负责人：李川），解决了其活性成分SM618口服生物利用度低的难题，并已基本完成了新药（一类）临床前的制剂学研究工作。

在药剂学科的国际著名期刊 *Int.J.Pharm.*和 *Biomaterials* 上各公开发表论文1篇。

参加了抗禽流感药物磷酸奥司他韦的联合攻关，承担了该药制剂研究部分的工作。在有限的时间内，认真细致地完成了相关研究，并获得协作单位上海医药集团有限公司的好评。此外，还参与了上药集团和瑞士罗氏公司关于许可生产该药的技术谈判工作，为上药集团最终获得授权做出了贡献。

深入开展微乳制剂技术平台研究，完成注射用纳米乳剂等一批新药项目的临床前研究工作，并即将展开注册申报工作。与此同时，药物制剂平台还添置了和靶向制剂与生物技术药物释药系统研究相关的仪器设备。

（五）基础和应用基础研究

2005年，上海药物研究所在基础和应用基础研究领域取得一大批成果，发表科学论文的数量与水平显著提高，呈现快速攀升的势头，在PNAS、JBC、JACS、Chemistry & Biology等一系列国际一流的学术刊物上发表了一大批高水平优秀论文。本年度共发表论文283篇，在SCI期刊上发表论文达200篇，影响因子(IF)总数478.27，平均影响因子2.391，影响因子3以上的论文达51篇。

1. 2005 年在影响因子 3.0 以上 SCI 刊物上发表的论文统计

期刊名称	影响因子	论文篇数	单本期刊影响因子小计
Proceedings of the National Academy of Sciences USA	10.452	1	10.452
Diabetes	8.848	1	8.848
Journal of the American Chemical Society	6.903	4	27.612
Journal of Biological Chemistry	6.355	1	6.355
Molecular Membrane Biology	5.667	1	5.667
Clinical Cancer Research	5.623	1	5.623
Diabetologia	5.583	1	5.583
Journal of Molecular Biology	5.542	1	5.542
Journal of Virology	5.398	1	5.398
Molecular Pharmacology	5.08	2	10.16
Journal of medicinal chemistry	5.076	3	15.228
Neuropsychopharmacology	4.941	1	4.941
Journal of Neurochemistry	4.824	1	4.824
Biophysical Journal	4.585	1	4.585
Proteins: Structure, Function, and Bioinformatics	4.429	1	4.429
International Journal of Cancer	4.416	1	4.416
Journal of Pharmacology and Experimental Therapeutics	4.335	2	8.670
Antimicrobial Agents and Chemotherapy	4.216	1	4.216
Journal of Combinatorial Chemistry	4.197	1	4.197
Organic Letters	4.195	2	8.390
Protein Science	4.116	2	8.232
Biochemistry	4.008	2	8.016
FEBS Letters	3.843	2	7.686
Drug Metabolism and Disposition	3.836	1	3.836
Journal of Physical Chemistry B	3.834	6	23.004
Biochimie	3.814	2	7.628
Neurosignals	3.585	1	3.585
BBA-General Subjects	3.369	2	6.738
Br J Pharmacol	3.325	1	3.325
Cancer Biology & Therapy	3.279	3	9.837
Microbiology	3.114	1	3.114
合 计		51	

2．2005 年研究所发表的代表性学术论文摘要

[1] Conformational Transition of Amyloid β-Peptide

Yechun Xu, Jianhua Shen, Xiaomin Luo, Weiliang Zhu, Kaixian Chen, Jianpeng Ma, and Hualiang Jiang

Proceedings of the National Academy of Sciences USA

2005,102:5403-5407.

The amyloid β-peptides (Aβs), containing 39–43 residues, are the key protein components of amyloid deposits in Alzheimer's disease. To structurally characterize the dynamic behavior of Aβ_{40}, 12 independent long-time molecular dynamics (MD) simulations for a total of 850 ns were performed on both the wide-type peptide and its mutant in both aqueous solution and a biomembrane environment. In aqueous solution, an α-helix to β-sheet conformational transition for Aβ_{40} was observed, and an entire unfolding process from helix to coil was traced by MD simulation. Structures with β-sheet components were observed as intermediates in the unfolding pathway of Aβ_{40}. Four glycines (G_{25}, G_{29}, G_{33}, and G_{37}) are important for Aβ_{40} to form β-sheet in aqueous solution; mutations of these glycines to alanines almost abolished the β-sheet formation and increased the content of the helix component. In the dipalmitoyl phosphatidylcholine (DPPC) bilayer, the major secondary structure of Aβ_{40} is a helix; however, the peptide tends to exit the membrane environment and lie down on the surface of the bilayer. The dynamic feature revealed by our MD simulations rationalized several experimental observations for Aβ_{40} aggregation and amyloid fibril formation. The results of MD simulations are beneficial to understanding the mechanism of amyloid formation and designing the compounds for inhibiting the aggregation of Aβ and amyloid fibril formation.

[2] Glycosidic Bond Cleavage of Pyrimidine Nucleosides by Low-Energy Electrons: A Theoretical Rationale

Jiande Gu, Yaoming Xie, Henry F. Schaefer

Journal of the American Chemical Society

2005,127:1053-1057.

DNA damage by attachment of low-energy secondary electrons is a very interesting and important mechanism. Electron capture and subsequent base release are thought to be the elementary steps of this mechanism. The process of the N1-glycosidic bond breaking of anion radicals of pyrimidine nucleosides, specifically the 2'-deoxyribothymidine (dT) and 2'-deoxyribocytidine (dC) anions, has been investigated theoretically at the B3LYP/DZP++ level of theory. The release of nucleobases by the attachment of low-energy electrons depends on the formation of a stable anion radical of the nucleoside. The lower bond-breaking activation energy and the higher vertical electron detachment energy for dT enables the heterolytic cleavage of the N1-glycosidic bond. However, with the higher bond-breaking activation energy and the lower vertical electron detachment energy for dC, the release of cytosine might be impractical when the incident electrons have high kinetic energy. Furthermore, the release of cytosine would have a quantum

yield much lower than that of dT when the incident electrons have lower kinetic energy. This study also demonstrates the importance of the proton at O5' of 2'-deoxyribose in the base release process. Extending this investigation from dT to dC advances the insight into the mechanism of the N1-glycosidic bond-breaking process. The information from this extensive investigation should be valuable for further experimental studies of cytosine release in irradiated DNA.

[3] Conformational Dynamics of the Nicotinic Acetylcholine Receptor Channel: A35-ns Molecular Dynamics Simulation Study

Yechun Xu, Francisco J, Barrantes, Xiaomin Luo, Kaixian Chen, Jianhua Shen, and Hualiang Jiang

Journal of the American Chemical Society

2005,127:1291-1299.

The nicotinic acetylcholine receptor (AChR) is the paradigm of ligand-gated ion channels, integral membrane proteins that mediate fast intercellular communication in response to neurotransmitters. A 35-ns molecular dynamics simulation has been performed to explore the conformational dynamics of the entire membrane-spanning region, including the ion channel pore of the AChR. In the simulation, the 20 transmembrane (TM) segments that comprise the whole TM domain of the receptor were inserted into a large dipalmitoylphosphatidylcholine (DPPC) bilayer. The dynamic behavior of individual TM segments and their corresponding AChR subunit helix bundles was examined in order to assess the contribution of each to the conformational transitions of the whole channel. Asymmetrical and asynchronous motions of the M1-M3 TM segments of each subunit were revealed. In addition, the outermost ring of five M4 TM helices was found to convey the effects exerted by the lipid molecules to the central channel domain. Remarkably, a closed-to-open conformational shift was found to occur in one of the channel ring positions in the time scale of the present simulations, the possible physiological significance of which is discussed.

[4] Dynamic Mechanism for the Autophosphorylation of CheA Histidine Kinase: Molecular Dynamics Simulations

Jian Zhang, Yechun Xu, Jianhua Shen, Xiaomin Luo, Jiagao Chen, Kaixian Chen, Weiliang Zhu, and Hualiang Jiang

Journal of the American Chemical Society

2005,127:11709-11719.

The two-component system (TCS) is an important signal transduction component for most bacteria. This signaling pathway is mediated by histidine kinases via autophosphorylation between P1 and P4 domains. Taking chemotaxis protein CheA as a model of TCS, the autophosphorylation mechanism of the TCS histidine kinases has been investigated in this study by using a computational approach integrated homology modeling, ligand-protein docking, protein-protein docking, and molecular dynamics (MD) simulations. Four nanosecond-scale MD simulations were performed on the free P4 domain, P4-ATP, P4-

TNPATP, and P1-P4-ATP complexes, respectively. Upon its binding to the binding pocket of P4 with a folded conformation, ATP gradually extends to an open state with help from a water molecule. Meanwhile, ATP forms two hydrogen bonds with His413 and Lys494 at this state. Because of the lower energy of the folded conformations, ATP shrinks back to its folded conformations, leading to the rupture of the hydrogen bond between ATP and Lys494. Consequently, Lys494 moves away from the pocket entrance, resulting in an open of the ATP lid of P4. It is the open state of P4 that can bind tightly to P1, where the His45 of P1 occupies a favorable position for its autophosphorylation from ATP. This indicates that ATP is not only a phosphoryl group donor but also an activator for CheA phosphorylation. Accordingly, a mechanism of the autophosphorylation of CheA is proposed as that the ATP conformational switch triggers the opening of the ATP lid of P4, leading to P1 binding tightly, and subsequently autophosphorylation from ATP to P1.

[5] A Highly Efficient and Direct Approach for Synthesis of Enantiopure β-Amino Alcohols by Reductive Cross-Coupling of Chiral N-tert-Butanesulfinyl Imines with Aldehydes

Yu-Wu Zhong, Yi-Zhou Dong, Kai Fang, Kenji Izumi, Ming-Hua Xu, and Guo-Qiang Lin

Journal of the American Chemical Society

2005, 127: 11956-11957.

Optically active β-amino alcohols are versatile building blocks for medicinal chemistry and natural product synthesis.[1] They have also been used as powerful chiral ligands or auxiliaries in asymmetric synthesis.[2] Due to their great importance, considerable efforts have been made to develop efficient methods for their preparation.[3] Among them, the pinacol-type cross-coupling between carbonyls and imines is one of the most direct ways to construct β-amino alcohols. However, a serious issue is that achievement of both good chemoselectivity and stereoselectivity is often difficult. Because of this, only a few examples of intermolecular crosscoupling to form racemic β-amino alcohols have been reported;[4] a highly diastereo- and enantioselective crossed pinacol coupling remains a significant synthetic challenge. To our knowledge, only recently has asymmetric synthesis of β-amino alcohols by cross pinacol coupling using planar chiral substrates been realized,[5] but the products are limited to ferrocenyl or $Cr(CO)_3$ aromatic derivatives; new approaches with broader substrate generality are still in high demand. In this communication, we wish to report a highly promising asymmetric pinacol-type coupling of chiral N-tert-butanesulfinyl imines with aldehydes, leading to enantiopure β-amino alcohols directly.

[6] Severe Acute Respiratory Syndrome Coronavirus 3C-like Proteinase N Terminus Is Indispensable for Proteolytic Activity but Not for Enzyme Dimerization: Biochemical and Thermodynamic Investigation In Conjunction with Molecular Dynamics Simulations

Shuai Chen, Lili Chen, Jinzhi Tan, Jing Chen, Li Du, Tao Sun, Jianhua Shen, Kaixian Chen, Hualiang Jiang, and Xu Shen

Journal of Biological Chemistry

2005, 280: 164-173.

Severe acute respiratory syndrome (SARS) coronavirus is a novel human coronavirus and is responsible for SARS infection. SARS coronavirus 3C-like proteinase (SARS $3CL^{pro}$) plays key roles in viral replication and transcription and is an attractive target for anti-SARS drug discovery. In this report, we quantitatively characterized the dimerization features of the full-length and N-terminal residues 1–7 deleted SARS $3CL^{pro}$s by using glutaraldehyde cross-linking SDS-PAGE, size-exclusion chromatography, and isothermal titration calorimeter techniques. Glutaraldehyde cross-linking SDS-PAGE and size-exclusion chromatography results show that, similar to the full-length SARS $3CL^{pro}$, the N-terminal deleted SARS $3CL^{pro}$ still remains a dimer/monomer mixture within a wide range of protein concentrations. Isothermal titration calorimeter determinations indicate that the equilibrium dissociation constant (K_d) of the N-terminal deleted proteinase dimer (262 μM) is very similar to that of the full-length proteinase dimer (227 μM). Enzymatic activity assay using the fluorescence resonance energy transfer method reveals that N-terminal deletion results in almost complete loss of enzymatic activity for SARS $3CL^{pro}$. Molecular dynamics and docking simulations demonstrate the N-terminal deleted proteinase dimer adopts a state different from that of the full-length proteinase dimer, which increases the angle between the two protomers and reduces the binding pocket that is not beneficial to the substrate binding. This conclusion is verified by the surface plasmon resonance biosensor determination, indicating that the model substrate cannot bind to the N-terminal deleted proteinase. These results suggest the N terminus is not indispensable for the proteinase dimerization but may fix the dimer at the active state and is therefore vital to enzymatic activity.

[7] Antimetastatic Effect of Salvicine on Human Breast Cancer MDA-MB-435 Orthotopic Xenograft Is Closely Related to Rho-Dependent Pathway

Jing-Yu Lang, Hua Chen, Jin Zhou, Yi-Xiang Zhang, Xiong-Wen Zhang, Mei-Hong Li, Li-Ping Lin, Jin-Sheng Zhang, Michael P. Waalkes, and Jian Ding

Clinical Cancer Research

2005, 11: 3455-3464.

PURPOSE: Salvicine is a novel DNA topoisomerase II inhibitor with potent anticancer activity. In present study, the effect of salvicine against metastasis is evaluated using human breast carcinoma orthotopic metastasis model and its mechanism is further investigated both in animal and cellular levels.

EXPERIMENTAL DESIGN: The MDA-MB-435 orthotopic xenograft model was applied to detect the antimetastatic effect of salvicine. Potential target candidates were detected and analyzed by microarray technology. Candidates were verified and explored by reverse transcription-PCR and Western blot. Salvicine activities on stress fiber formation, invasion, and membrane translocation were further investigated by immunofluorescence, invasion, and ultracentrifugal assays.

RESULTS: Salvicine significantly reduced the lung metastatic foci of MDA-MB-435 orthotopic xenograft, without affecting primary tumor growth obviously. A comparison of gene expression profiles of primary tumors and lung metastatic focus between salvicine-treated and untreated groups using the CLOTECH Atlas human Cancer 1.2 cDNA microarray revealed that genes involved in tumor metastasis, particularly those closely related to cell adhesion and motility, were obviously down-regulated, including fibronectin,

integrin α3, integrin β3, integrin β5, FAK, paxillin, and RhoC. Furthermore, salvicine significantly down-regulated RhoC at both mRNA and protein levels, greatly inhibited stress fiber formation and invasiveness of MDA-MB-435 cells, and markedly blocked translocation of both RhoA and RhoC from cytosol to membrane.

CONCLUSION: The unique antimetastatic action of salvicine, particularly its specific modulation of cell motility *in vivo* and *in vitro*, is closely related to Rho-dependent signaling pathway.

[8] pH-dependent Conformational Flexibility of the SARS-CoV Main Proteinase (M^{pro}) Dimer: Molecular Dynamics Simulations and Multiple X-ray Structure Analyses

Jinzhi Tan, Koen H.G., Verschueren, Kanchan Anand, Jianhua Shen, Maojun Yang, Yechun Xu, Zihe Rao, Janna Bigalke, Burkhard Heisen, Jeroen R. Mesters, Kaixian Chen, Xu Shen, Hualiang Jiang, and Rolf Hilgenfeld

Journal of Molecular Biology

2005, 354: 25-40.

The SARS coronavirus main proteinase (M^{pro}) is a key enzyme in the processing of the viral polyproteins and thus an attractive target for the discovery of drugs directed against SARS. The enzyme has been shown by X-ray crystallography to undergo significant pH-dependent conformational changes. Here, we assess the conformational flexibility of the M^{pro} by analysis of multiple crystal structures (including two new crystal forms) and by molecular dynamics (MD) calculations. The MD simulations take into account the different protonation states of two histidine residues in the substrate-binding site and explain the pH-activity profile of the enzyme. The low enzymatic activity of the M^{pro} monomer and the need for dimerization are also discussed.

[9] Cinanserin Is an Inhibitor of the 3C-Like Proteinase of Severe Acute Respiratory Syndrome Coronavirus and Strongly Reduces Virus Replication In Vitro

Lili Chen, Chunshan Gui, Xiaomin Luo, Qingang Yang, Stephan Günther, Elke Scandella, Christian Drosten, Donglu Bai, Xichang He, Burkhard Ludewig, Jing Chen, Haibin Luo, Yiming Yang, Yifu Yang, Jianping Zou, Volker Thiel, Kaixian Chen, Jianhua Shen, Xu Shen, and Hualiang Jiang

Journal of Virology

2005, 79: 7095-7103.

The 3C-like proteinase ($3CL^{pro}$) of severe acute respiratory syndrome-associated coronavirus (SARS-CoV) is one of the most promising targets for anti-SARS-CoV drugs due to its crucial role in the viral life cycle. In this study, a database containing structural information of more than 8,000 existing drugs was virtually screened by a docking approach to identify potential binding molecules of SARS-CoV $3CL^{pro}$. As a target for screening, both a homology model and the crystallographic structure of the binding pocket of the enzyme were used. Cinanserin (SQ 10,643), a well-characterized serotonin antagonist that has undergone preliminary clinical testing in humans in the 1960s, showed a high score in the screening and was chosen for

further experimental evaluation. Binding of both cinanserin and its hydrochloride to bacterially expressed $3CL^{pro}$ of SARS-CoV and the related human coronavirus 229E (HCoV-229E) was demonstrated by surface plasmon resonance technology. The catalytic activity of both enzymes was inhibited with 50% inhibitory concentration (IC_{50}) values of 5 μM, as tested with a fluorogenic substrate. The antiviral activity of cinanserin was further evaluated in tissue culture assays, namely, a replicon system based on HCoV-229E and quantitative test assays with infectious SARS-CoV and HCoV-229E. All assays revealed a strong inhibition of coronavirus replication at nontoxic drug concentrations. The level of virus RNA and infectious particles was reduced by up to 4 log units, with IC_{50} values ranging from 19 to 34 μM. These findings demonstrate that the old drug cinanserin is an inhibitor of SARS-CoV replication, acting most likely via inhibition of the 3CL proteinase.

[10] Stereospecific Induction of Nuclear Factor-κB Activation by Isochamaejasmin

Qinghai Tian , Jing Li , Xin Xie, Meiling Sun, Hairong Sang, Caihong Zhou, Tianying An, Lihong Hu, Richard D. Ye, and Ming-wei Wang

Molecular Pharmacology

2005,68:1534-1542.

The root of Stellera chamaejasme L. is a traditional Chinese herb termed Rui Xiang Lang Du and has been used to treat solid tumors, tuberculosis and psoriasis. Exactly how S. chamaejasme L. regulates cellular responses remains unclear. We examined four biflavonoids isolated from S. chamaejasme L., including isochamaejasmin, two of its stereo-isomers and a methyl derivative, in functional assays originally designed to screen ligands for the G protein-coupled formyl peptide receptor-like 1 (FPRL1). Isochamaejasmin was found to induce the expression of a nuclear factor (NF)- κB-directed reporter gene in transfected Hela cells with an EC_{50} of 3.23 μM, independently of FPRL1. The isochamaejasmin-stimulated NF-κB reporter activity was accompanied by nuclear translocation of NF-κB proteins and was blocked by a dominant-negative construct of IκBα. Isochamaejasmin also induced time-dependent phosphorylation of the mitogen-activated protein kinases extracellular signal-regulated kinase 1/2 and p38, and a novel protein kinase C (PKCδ). Likewise, inhibition of these kinases with the respective pharmacological inhibitors significantly reduced the isochamaejasmin-stimulated NF-κB and were more cytotoxic than isochamaejasmin, which could partially rescue cycloheximide-induced apoptosis. Inhibition of NF-κB activation reversed the anti-apoptotic effect of isochamaejasmin. These results provide the first evidence for a potential mechanism of action by S. chamaejasme L., and indicate that structurally similar compounds derived from S. chamaejasme L. may have different pharmacological properties.

[11] Reactive Oxygen Species Elicit Apoptosis by Concurrently Disrupting Topoisomerase II and DNA-Dependent Protein Kinase

Lu HR, Zhu H, Huang M, Ding J

Molecular Pharmacology

2005,68:983-994.

Reactive oxygen species (ROS) are produced by all aerobic cells and have been implicated in the regulation of diverse cellular functions, including intracellular signaling, transcription activation, proliferation, and apoptosis. Salvicine, a novel diterpenoid quinone compound, demonstrates a broad spectrum of antitumor activities. Although salvicine is known to trap the DNA-topoisomerase II (Topo II) complex and induce DNA double-strand breaks (DSBs), its precise antitumor mechanisms remain to be clarified. In this study, we investigated whether salvicine altered the levels of ROS in breast cancer MCF-7 cells and whether these ROS contributed to the observed antitumoral activity. Our data revealed that salvicine stimulated intracellular ROS production and subsequently elicited notable DSBs. The addition of N-acetyl cysteine (NAC), an antioxidant, effectively attenuated the salvicine-induced ROS enhancement and subsequent DNA DSBs. Heat treatment reversed the accumulation of DNA DSBs, and the addition of NAC attenuated the Topo II-DNA cleavable complexes formation and the growth inhibition of salvicine-treated JN394top2-4 yeast cells, collectively indicating that Topo II is a target of the salvicine-induced ROS. On the other hand, when examining the impact of salvicine on DNA repair pathways, we unexpectedly observed that salvicine selectively down-regulated the catalytic subunit of DNA-dependent protein kinase (DNA-PK_{CS}) protein levels and repressed DNA-PK kinase activity; both of these effects were attenuated by NAC pretreatment of MCF-7 cells. Finally and most importantly, NAC attenuated salvicine-induced apoptosis and cytotoxicity in MCF-7 cells. These results indicate that apart from its direct actions, salvicine generates ROS that modulate DNA damage and repair, contributing to the comprehensive biological consequences of salvicine treatment, such as DNA DSBs, apoptosis, and cytotoxicity in tumor cells. The finding of salvicine-induced ROS provides new evidence for the molecular mechanisms of this compound. Moreover, the effects of salvicine-induced ROS on Topo II and DNA-PK give new insights into the diverse biological activities of ROS.

[12] Bis-huperzine B: Highly Potent and Selective Acetylcholinesterase Inhibitors

Song Feng, Zhifei Wang, Xuchang He, Suxin Zheng, Yu Xia, Hualiang Jiang, Xican Tang, and Donglu Bai

Journal of Medicinal Chemistry

2005, 48: 655-657.

By targeting dual active sites of AChE, a series of bis-huperzine B analogues with various lengths of the tether were designed, synthesized, and tested for their inhibition and selectivity. The most potent bis-huperzine B (**5g**) exhibited 3900-fold increase in AChE inhibition and 930-fold greater in selectivity for AChE vs BuChE than its parent huperzine B.

[13] Synthesis and Immunosuppressive Activity of New Artemisinin Derivatives.

1. [12 (β or α)-Dihydroartemisininoxy] Phen(ox)yl Aliphatic Acids and Esters

Zhong-shun Yang, Wen-liang Zhou, Yi Sui, Jun-Xia Wang, Jin-Ming Wu, Yu Zhou, Yu Zhang, Pei-Lan He, Ji-Ye Han, Wei Tang, Ying Li, and Jian-Ping Zuo

Journal of Medicinal Chemistry
2005, 48: 4608-4617.

A series of novel dihydroartemisinin derivatives were synthesized and evaluated on their immunosuppressive activity in the search for potential immunosuppressive agents with high efficacy and low toxicity. These compounds were assayed in their cytotoxicity of lymphocyte, inhibition activity on concanavalin A (ConA) induced T cell proliferation and lipopolysaccharide (LPS) induced B cell proliferation. Among them, **11b**, **13b**, **14d**, **15b**, **16**, and **17** remarkably exhibited lower cytotoxicity and higher inhibition activity on the mitogen-induced T cell and B cell proliferation in comparison with artemisinin, artesunate, and artemether in vitro. More significantly, compound **11b** displayed reduced cytotoxicity by over 100-fold compared with cyclosporin A (CsA) and comparable inhibition activity (SI = 848) on ConA-induced T cell proliferation to CsA (SI = 963) and more than 4000 times the inhibitory effect (SI = 28473) on LPS-induced B cell proliferation compared with CsA (SI = 7) in vitro. The in vivo experimental results showed that compound **16** could inhibit 2,4-dinitrofluorobenzene (DNFB)-induced delayed-type hypersensitivity (DTH) reaction and sheep red blood cell (SRBC) induced antibody production, respectively. The structure and activity relationships (SAR) of these compounds were also discussed.

[14] Effects of l-Stepholidine on Forebrain Fos Expression: Comparison with Clozapine and Haloperidol
Yi-Qing Mo, Xi-Lu Jin, You-Ting Chen, Guo-Zhang Jin, and Wei-Xing Shi
Neuropsychopharmacology
2005, 30: 261-267.

l-Stepholidine (SPD) is a tetrahydroprotoberberine alkaloid and a mixed dopamine D1 agonist/D2 antagonist. Preliminary clinical trials suggest that SPD improves both positive and negative symptoms of schizophrenia without producing significant extrapyramidal side effects. Here, we report that SPD mimics the effect of the atypical antipsychotic drug clozapine, preferentially increasing Fos expression in corticolimbic areas. Thus, at 10 mg/kg (i.p.), SPD induced Fos expression in the medial prefrontal cortex (mPFC), nucleus accumbens (NAc), and lateral septal nucleus (LSN) without significantly affecting the dorsolateral striatum (DLSt). At higher doses (20–40 mg/kg), SPD also increased Fos expression in the DLSt. The increase, however, was less pronounced than the increase seen in the NAc. Within the NAc, SPD also induced more Fos expression in the shell than in the core. In all subcortical areas examined, the Fos expression induced by SPD was mimicked by the D2 antagonist sulpiride and reversed by the D2 agonist quinpirole, suggesting that the effect is due to blockade of D2-like receptors by SPD. In the mPFC, however, the effect was not mimicked by sulpride or reversed by quinpirole. It was also not mimicked by the D1 agonist SKF38393 or SKF38393 plus sulpride, and not reversed by the D1 antagonist SCH23390. These results suggest that, in the mPFC, SPD may induce Fos expression through a non-DA mechanism. Whether the mechanism involves an interaction of SPD with other neurotransmitters such as 5-HT and norepinephrine remains to be determined.

[15] NT3 Inhibits FGF2-Induced Neural Progenitor Cell Proliferation Via the PI3K/GSK3 Pathway

Lu Jin, Xinhua Hu, and Linyin Feng

Journal of Neurochemistry (IF 4.824)

2005, 93: 1251–1261.

Neurotrophin 3 (NT3), a member of the neurotrophin family, antagonizes the proliferative effect of fibroblast growth factor 2 (FGF2) on cortical precursors. However, the mechanism by which NT3 inhibits FGF2-induced neural progenitor (NP) cell proliferation is unclear. Here, using an FGF2-dependent rat neurosphere culture system, we found that NT3 inhibits both FGF2-induced neurosphere growth and bromodeoxyuridine (BrdU) incorporation in a dose-dependent manner. U0126, a mitogen-activated protein kinase kinase 1/2 (MEK1/2) inhibitor, and LY294002, a phosphatidylinositol 3-kinase (PI3K) inhibitor, both inhibited FGF2-induced BrdU incorporation,

suggesting that the extracellular signal-regulated kinase1/2 (ERK1/2) and PI3K pathways are required for FGF2-induced NP cell proliferation. NT3 significantly inhibited FGF2-induced phosphorylation of Akt and glycogen synthase kinase 3β (GSK3β), a downstream kinase of Akt, whereas phosphorylation of ERK1/2 was unaffected. The inhibitory effect of NT3 on FGF2-induced NP cell proliferation was abolished by LY294002, and treatment with SB216763, a specific GSK3 inhibitor, antagonized the NT3 effect, rescuing both neurosphere growth and BrdU incorporation. Moreover, experiments with anti-NT3 antibody revealed that endogenous NT3 also plays a role in inhibiting FGF2-induced NP cell proliferation, and that anti-NT3 antibody enhanced phospho-Akt and phospho-GSK3β levels in the presence of FGF2. These findings indicate that FGF2-induced NP cell proliferation is inhibited by NT3 via the PI3K/GSK3 pathway.

[16] Computational Analysis of Molecular Basis of 1:1 Interactions of NRG-1β Wild-Type and Variants With ErbB3 and ErbB4

Cheng Luo, Lingfei Xu, Suxin Zheng, Xiaomin Luo, Jianhua Shen, Hualiang Jiang, Xifu Liu, and Mingdong Zhou

Proteins: Structure, Function, and Bioinformatics

2005, 59: 742-756.

The neuregulin/ErbB system is a growth factor/receptor cascade that has been proven to be essential in the development of the heart and the sympathetic nervous system. However, the basis of the specificity of ligand–receptor recognition remains to be elucidated. In this study, the structures of NRG-1β/ErbB3 and NRG-1β/ErbB4 complexes were modeled based on the available structures of the homologous proteins. The binding free energies of NRG-1β to ErbB3 and ErbB4 were calculated using the molecular mechanics Poisson–Boltzmann surface area (MM-PBSA) computational method. In addition, computational alanine-scanning mutagenesis was performed in the binding site of NRG-1β and the difference in the binding free energies between NRG-1β mutants and the receptors was calculated. The results specify the contribution of each residue at the interaction interfaces to the binding affinity of NRG-1β with ErbB3 and ErbB4, identify-

ing several important interaction residue pairs that are in agreement with previously acquired experimental data. This indicates that the presented structural models of NRG-1β/ErbB3 and NRG-1β/ErbB4 complexes are reliable and could be used to guide future studies, such as performing desirable mutations on NRG-1β to increase the binding affinity and selectivity to the receptor and discovering new therapeutic agents for the treatment of heart failure.

[17] Philinopside A, a Novel Marine-Derived Compound Possessing Dual Anti-Angiogenic and Anti-Tumor Effects

Yuaguang Tong, Xiongwen Zhang, Fang Tian, Yanghua Yi, Qiangzhi Xu, Ling Li, Linjiang Tong, Liping Lin and Jian Ding

International Journal of Cancer

2005, 114: 843-853.

Philinopside A is a novel sulfated saponin isolated from the sea cucumber, *Pentacta quadrangulari*. The effects of philinopside A on angiogenesis and tumor growth were assessed in a series of models *in vitro* and *in vivo*. Our results demonstrated that philinopside A significantly inhibited the proliferation, migration and tube formation of human microvascular endothelial cells (HMECs) in a dose-dependent manner, with average IC_{50} values of 1.4 ± 0.17, 0.89 ± 0.23 and 0.98 ± 0.19 μM, respectively. Rat aortas culture assay provides a close imitation of *in vivo* angiogenesis process and 2–10 μMphilinopside A suppressed the formation of new microvessels in cultured rat aortas. Philinopside A 2–10 nmol/egg obviously inhibited angiogenesis in chick embryo chorioallantoic membrane assay. In addition, philinopside A manifested strong anti-tumor activities both *in vitro* and *in vivo*. Through immunofluorescent analysis, we found the compound reduced mouse sarcoma 180 tumor volume by inducing apoptosis of tumor and tumor-associated endothelial cells. An examination of the effects of philinopside A on the angiogenesis-related receptor tyrosine kinases (RTKs) showed that philinopside A broadly inhibited all tested RTKs, including vascular endothelial growth factor (VEGF) receptor, fibroblast growth factor (FGF) receptor- 1, platelet-derived growth factor (PDGF) receptor-β and epithelial growth factor (EGF) receptor, with IC_{50} values ranging from 2.6–4.9 μM. These results suggest that philinopside A is a promising anti-cancer agent that possesses dual cytotoxic and anti-angiogenic effects that were at least partly due to its inhibitory effects on RTKs.

[18] Scalaradial Inhibition of Epidermal Growth Factor Receptor-Mediated Akt Phosphorylation Is Independent of Secretory Phospholipase A_2

Yili Xie, Lunhua Liu, Xiaochun Huang, Yuewei Guo, and Liguang Lou

The Journal of Pharmacology and Experimental Therapeutics

2005, 314: 1210-1217.

The marine natural product 12-epi-scalaradial (SLD) is a specific secretory phospholipase A_2 ($sPLA_2$) inhibitor. However, little is known about whether this compound has other pharmacological effects. Here, we revealed a novel effect of SLD on epidermal growth factor receptor (EGFR)-mediated Akt phosphorylation.

SLD dose- and time-dependently inhibited epidermal growth factor (EGF)-stimulated Akt phosphorylation, which is required for Akt activation. SLD also blocked the EGF-stimulated membrane translocation of 3-phosphoinositide-dependent protein kinase 1 and inhibited phosphatidylinositol 3-kinase activity. This inhibition is specific for SLD because other phospholipase inhibitors, including $sPLA_2$ inhibitor thioetheramide-phosphatidylcholine, cytosolic PLA_2 inhibitor arachidonyl trifluoromethyl ketone, cytosolic PLA_2 and Ca^{2+}-independent PLA_2 inhibitor methyl arachidonyl fluorophosphonate, phospholipase C inhibitor U73122, and cyclooxygenases inhibitor indomethacin, failed to inhibit EGF-stimulated Akt phosphorylation. Furthermore, arachidonic acid, the main $sPLA_2$-catalyzed metabolite, was not able to rescue SLD inhibition of EGF-stimulated Akt phosphorylation. Overexpression of group IIA or group X $sPLA_2$ did not reverse the inhibitory effect of SLD on Akt phosphorylation, either. Our results demonstrate that SLD inhibits EGFR-mediated Akt phosphorylation, and this novel effect of SLD is independent of $sPLA_2$.

[19] Inhibition of S-Adenosyl-L-homocysteine Hydrolase Induces Immunosuppression

Qing-Li Wu, Yun-Feng Fu, Wen-Liang Zhou, Jun-Xia Wang, Yong-Hong Feng, Jing Liu, Jian-Yi Xu, Pei-lan He, Ru Zhou, Wei Tang, Gui-Feng Wang, Yu Zhou, Yi-Fu Yang, Jian Ding, Xiao-Yu Li, Xiao-Ru Chen, Chong Yuan, Brian R. Lawson, and Jian-Ping Zuo

The Journal of Pharmacology and Experimental Therapeutics

2005,313:705-711.

Lymphocytes depend on transmethylation reactions for efficient activation and function. These reactions are primarily catalyzed by S-adenosylmethionine-dependent methyltransferases, which convert S-adenosylmethionine to S-adenosyl-Lhomocysteine. S-adenosyl-L-homocysteine is then hydrolyzed by S-adenosyl-L-homocysteine hydrolase to prevent feedback inhibition of transmethylation reactions. By impeding S-adenosyl-L-homocysteine hydrolase, a build-up of S-adenosyl-L-homocysteine occurs, and most intracellular transmethylation reactions cease. Thus, a nontoxic inhibitor of this enzyme might be a useful immunosuppressive therapeutic agent. We identified a potent reversible type III inhibitor of S-adenosyl-L-homocysteine hydrolase, DZ2002 [methyl 4-(adenin-9-yl)-2-hydroxybutanoate], and determined its cytotoxic and immunologic effects. We demonstrated that DZ2002 blocked S-adenosyl-L-homocysteine hydrolase more effectively than a type I inhibitor, but cytotoxicity from DZ2002 was greatly reduced. Although DZ2002 did not prevent concanavalin A-induced T cell proliferation or interleukin (IL)-2 production, it significantly reduced both a mixed lymphocyte reaction and IL-12 production from in vitro-stimulated splenocytes. In addition, levels of CD80 and CD86 on human monocytic THP-1 cells were decreased in a dose-dependent manner in the presence of 0.1 to 10 μM DZ2002, and decreases were also seen in IL-12 and tumor necrosis factor-α production from both mouse thioglycollatestimulated peritoneal macrophages and THP-1 cells. In vivo, DZ2002 significantly suppressed a delayed-type hypersensitivity reaction as well as antibody secretion. We conclude that DZ2002's immunosuppressive effects are likely not solely attributed to T cell inhibition but also to the obstruction of macrophage activation and function through reductions in cytokine output and/or T cell costimulation. These data suggest an important dual role for the S-adenosyl-L-homocysteine hydrolase in both macrophage and T cell function.

[20] Bactericidal and Morphological Effects of NE-2001, a Novel Synthetic Agent Directed against *Helicobacter pylori*

Guofei Dai, Ni Cheng, Lei Dong, Mutsumi Muramatsu, Shudong Xiao, Ming-Wei Wang, and De-Xu Zhu

Antimicrobial Agents and Chemotherapy

2005, 49: 3468-3473.

The antibacterial activities of NE-2001 were tested against 24 clinical isolateds of *Helicobacter pylori* and compared with those of amoxicillin, clarithromycin, metronidazole, and furazolidone. The MIC_{50} and MIC_{90} of this synthetic compound on the isolates were 8 and 16 μg/ml, respectively. This action was highly selective against *Helicobacter pylori*; there was a >4-fold difference between the concentration of NE-2001 required to inhibit the growth of *Helicobacter pylori* and that required to inhibit the growth of common aerobic and anaerobic bacteria. Exposure of *Helicobacter pylori* (ATCC43504) to NE-2001 at the MIC (4 μg/ml), or at a greater concentration, resulted in an extensive loss of viability. The phenomenon was also observed at pH levels between 3.0 and 7.0. When two clinical *Helicobacter pylori* strains were successively cultured at subinhibitory concentration of NE-2001, no significant changes in the bactericidal effects were found. The morphological alterations of *Helicobacter pylori* cells (ATCC43504), exposed to NE-2001 at various concentrations for 6h, were observed using transmission electron microcopy. The bacterium displayed features such as swelling, vacuole-like structures in the cytoplasm, and cell destruction following exposure to NE-2001. The efficacy of NE-2001 was maintained when evaluated in eight clinical isolates resistant to metronidazole and five isolates resistant to both metronidazole and clarithromycin (MIC ranging between 4 and 16 μg/ml). The above-described results suggest that NE-2001 may have the potential to be developed as a candidate agent for the treatment of *Helicobacter pylori* infection.

[21] Focused Combinatorial Library Design Based on Structural Diversity, Druglikeness and Binding Affinity Score

Gang Chen, Suxin Zheng, Xiaomin Luo, Jianhua Shen, Weiliang Zhu, Hong Liu, Chunshan Gui, Jian Zhang, Mingyue Zheng, Chum Mok Puah, Kaixian Chen, and Hualiang Jiang

Journal of Combinatorial Chemistry

2005, 25: 398-406.

The advent of focused library and virtual screening has reduced the disadvantage of combinatorial chemistry and changed it to a realizable and cost-effective tool in drug discovery. Usually, genetic algorithms (GAs) are used to quickly finding high-scoring molecules by sampling a small subset of the total combinatorial space. Therefore, scoring functions play essential roles in focused library design. Reported here is our initial attempt to establish a new approach for generating a target-focused library using the combination of the scores of structural diversity and binding affinity with our newly improved druglikeness scoring functions. Meanwhile, a software package, named LD1.0, was developed on the basis of the new approach. One test on a cyclooxygenase (COX)2-focused library successfully reproduced the structures

that have been experimentally studied as COX2-selective inhibitors. Another test is on a peroxisome proliferator-activated receptors γ-focused library design, which not only reproduces the key fragments in the approved (thiazolidinedione) TZD drugs, but also generates some new structures that are more active than the approved drugs or published ligands. Both of the two tests took~15% of the running time of the ordinary molecular docking method. Thus, our new approach is an effective, reliable, and practical way for building up a properly sized focused library with a high hit rate, novel structure, and good ADME/T profile.

[22] Lathyranoic Acid A: First Secolathyrane Diterpenoid in Nature from *Euphorbia lathyris*

Shang-Gao Liao, Zha-Jun Zhan, Sheng-Ping Yang, and Jian-Min Yue

Organic Letters

2005, 7: 1379-1382.

Lathyranoic acid A (1), the first secolathyrane diterpenoid with an unprecedented skeleton, and a new diterpenoid *Euphorbia* factor L_{11} (2) were isolated from the seeds of *Euphorbia lathyris*. Their structures were elucidated by spectroscopic analysis and chemical methods. A biogenetic route involving an enzymatic Baeyer-Villiger oxidation as the key step was postulated for the transformation of 2 to 1 and mimicked by an unusual chemical Baeyer-Villiger oxidation.

[23] Organocatalytic Asymmetric Michael Addition of 2,4-Pentandione to Nitroolefins

Jian Wang, Hao Li, Wenhu Duan, Liansuo Zu, and Wei Wang

Organic Letters

2005, 7: 4713-4716.

A novel binaphthyl-derived amine thiourea organocatalyst has been developed and demonstrated to efficiently catalyze Michael addition reactions (using as low as 1 mol % loading) of diketones to nitroalkenes with remarkably high enantioselectivities.

[24] Ligand-Binding Regulation of LXR/RXR and LXR/PPAR Heterodimerizations: SPR Technology Based Kinetic Analysis Correlated with Molecular Dynamics Simulation

Liduo Yue, Fei Ye, Chunshan Gui, Haibin Luo, Jianhua Cai, Jianhua Shen, Kaixian Chen, Xu Shen, and Hualiang Jiang

Protein Science

2005, 14: 812-822.

Liver X receptor (LXR) and peroxisome proliferator-activated receptor (PPAR) are two members of nuclear receptors involved in the nutrient metabolisms of dietary fatty acid and cholesterol. They are found to be of cross-talk function in that LXR regulates fatty acid synthesis and PPAR controls fatty acid degradation. LXRs (LXRα and LXRβ) function by forming obligate heterodimers with the retinoid X receptor

(RXR), and subsequently binding to specific DNA response elements within the regulatory regions of their target genes. In this work, the kinetic features concerning LXR/RXR and LXR/PPAR interactions have been fully investigated using surface plasmon resonance (SPR) technology. It is found that LXRs could bind to all the three PPAR subtypes, PPARα, PPARγ and PPARδ with different binding affinities, and such receptor/receptor interactions could be regulated by ligand binding. Moreover, molecular dynamics (MD) simulations were performed on six typical complex models. The results revealed that ligands may increase the interaction energies between the receptor interfaces of the simulated receptor/receptor complexes. The MD results are in agreement with the SPR data. Further analyses on the MD results indicated that the ligand binding might increase the hydrogen bonds between the interfaces of the receptor/receptor complex.

[25] Solution Structure of the Ubiquitin-Like Domain of Human DC-UbP from Dendritic Cells

Yong-Guang Gao, Ai-Xin Song, Yan-Hong Shi, Yong-Gang Chang, Shu-Xun Liu, Yi-Zi Yu, Xue-Tao Cao, Dong-Hai Lin, and Hong-Yu Hu

Protein Science

2005, 14: 2044-2050.

The previously identified dendritic cell-derived ubiquitin-like protein (DC-UbP) was implicated in cellular differentiation and apoptosis. Sequence alignment suggested that it contains a ubiquitin-like (UbL) domain in the C terminus. Here, we present the solution NMR structure and backbone dynamics of the UbL domain of DC-UbP. The overall structure of the domain is very similar to that of Ub despite low similarity (<30%) in amino-acid sequence. One distinct feature of the domain structure is its highly positively charged surface that is different from the corresponding surfaces of the well-known UbL modifiers, Ub, NEDD8, and SUMO-1. The key amino-acid residues responsible for guiding polyubiquitinated proteins to proteasome degradation in Ub are not conserved in the UbL domain. This implies that the UbL domain of DC-UbP may have its own specific interaction partners with other yet unknown cellular functions related to the Ub pathway.

[26] SR-Rich Motif Plays a Pivotal Role in Recombinant SARS Coronavirus Nucleocapsid Protein Multimerization

Haibin Luo, Fei Ye, Kaixian Chen, Xu Shen, and Hualiang Jiang

Biochemistry

2005, 44: 15351-15358.

The nucleocapsid (N) protein of SARS coronavirus (SARS-CoV) is reported to function in encapsidating the viral genomic RNA into helical nucleocapsid, and its self-association is believed to be vital in coating the viral genomic RNA. Characterization of SARS-CoV N multimerization may thereby help us better understand the coronavirus assembly. In the current work, using the yeast two-hybrid technique, an unexpected interaction between residues 1-210 and 211-290 (central region) of the SARS-CoV N protein was detected,

and SPR results further revealed that the SR-rich motif (amino acids 183-197) of SARS-CoV N protein is responsible for such an interaction. Chemical cross-linking and gelfiltration analyses indicated that the residues 283-422 of the SARS-CoV N protein have multimeric ability, although the full-length N protein is prone to exist predominantly as dimers. In addition, the multimeric ability of the C-terminal domain of SARS-CoV N protein could be weakened by the SR-rich motif interaction with the central region (amino acids 211-290). All of these data suggested that the SR-rich motif of the SARS-CoV N protein might play an import role in the transformation of the SARS-CoV N protein between the dimer and multimer during its binding to its central region for self-association or dissociation. This current paper will hopefully provide some new ideas in studying SARS-CoV N multimerization.

[27] Folding of the SARS Coronavirus Spike Glycoprotein Immunological Fragment (SARS_S1B): Thermodynamic and Kinetic Investigation Correlating with Three-Dimensional Structural Modeling

Changying Yu, Chunshan Gui, Haibin Luo, Lili Chen, Liang Zhang, Hao Yu, Sheng Yang, Weihong Jiang, Jianhua Shen, Xu Shen, and Hualiang Jiang

Biochemistry

2005,44:1453-1463.

Spike glycoprotein of SARS coronavirus (S protein) plays a pivotal role in SARS coronavirus (SARS_CoV) infection. The immunological fragment of the S protein (Ala251-His641, SARS_S1b) is believed to be essential for SARS_CoV entering the host cell through S protein-ACE-2 interaction. We have quantitatively characterized the thermally induced and GuHCl-induced unfolding features of SARS_S1b using circular dichroism (CD), tryptophan fluorescence, and stopped-flow spectral techniques. For the thermally induced unfolding at pH 7.4, the apparent activation energy (E_{app}) and transition midpoint temperature (T_m) were determined to be 16.3 ± 0.2 kcal/mol and 52.5±0.4 °C, respectively. The CD spectra are not dependent on temperature, suggesting that the secondary structure of SARS_S1b has a relatively high thermal stability. GuHCl strongly affected SARS_S1b structure. Both the CD and fluorescent spectra resulted in consistent values of the transition middle concentration of the denaturant (C_m, ranging from 2.30 to 2.45 M) and the standard free energy change ($\Delta G°$, ranging from 2.1 to 2.5 kcal/mol) for the SARS_S1b unfolding reaction. Moreover, the kinetic features of the chemical unfolding and refolding of SARS_S1b were also characterized using a stopped-flow CD spectral technique. The obvious unfolding reaction rates and relaxation times were determined at various GuHCl concentrations, and the C_m value was obtained, which is very close to the data that resulted from CD and fluorescent spectral determinations. Secondary and three-dimensional structural predictions by homology modeling indicated that SARS_S1b folded as a globular-like structure by β-sheets and loops; two of the four tryptophans are located on the protein surface, which is in agreement with the tryptophan fluorescence result. The three-dimensional model was also used to explain the recently published experimental results of S1-ACE-2 binding and immunizations.

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1. 成果转化

2005 年，上海药物研究所共签订四技合同（其合同类别包括：技术转让、委托或合作技术开发、技术咨询以及技术服务）共 120 项，其中转让项目与合作开发项目 90 项，合同成交额 2618 万元。

2. 新 药

研究所全年共取得新药证书 10 项(其中一类新药 2 项、二类新药 6 项、三类新药 2 项)，完成临床研究正在申请审批新药证书的项目共 10 项；2005 年取得新药临床批件 3 项，目前正处于临床研究阶段的新药共有 20 余项，完成临床前研究并提出申请临床研究的有 9 项。

2005 年度上海药物研究所获取的新药证书

序号	新药名称	证书号	类　别	批 准 日
1	丹参多酚酸盐	国药证字 Z20050258	中药第 2 类	2005 年 5 月 25 日
2	注射用丹参多酚酸盐	国药证字 Z20050259	中药第 2 类	2005 年 5 月 25 日
3	那格列奈	国药证字 H20050536	化学药品第 2 类	2005 年 4 月 11 日
4	那格列奈片	国药证字 H20050537	化学药品第 2 类	2005 年 4 月 11 日
5	那格列奈胶囊	国药证字 H20051336	化学药品第 2 类	2005 年 9 月 26 日
6	盐酸关附甲素	国药证字 H20051128	化学药品第 1 类	2005 年 8 月 22 日
7	盐酸关附甲素注射液	国药证字 H20051129	化学药品第 1 类	2005 年 8 月 22 日
8	利拉萘酯	国药证字 H20051737	化学药品第 3.1 类	2005 年 12 月 15 日
9	利拉萘酯乳膏	国药证字 H20051738	化学药品第 3.1 类	2005 年 12 月 15 日
10	依普黄酮	国药证字 20040487	化学药品第 2 类	2004 年 4 月 30 日

2005 年度上海药物研究所获取的新药临床批文

序号	新药名称	临床研究批文号	类　别	批 准 日
1	盐酸安妥沙星片	2005L00472	化学药品第 1 类	2005 年 2 月 2 日
2	醋酸烯诺孕酮埋植剂	2005L02570	化学药品第 1 类	2005 年 7 月 28 日
3	希普林片	2005L02368	化学药品第 1 类	2005 年 7 月 11 日

3. 专 利

2005 年，上海药物研究所共申请专利 80 项（其中实用新型专利 1 项；发明专利 79 项，包含国际申请 4 项），专利实施数 2 项，已获授权的专利数为 15 项（其中发明专利 15 项，包括美国专利 1 项）。截至目前，研究所共累计拥有发明专利 54 项。

2005 年度上海药物研究所获授权的专利

序号	专利名称	类型	国家	发明人	专利号	申请单位
1	新土槿皮酸类衍生物及其制备方法和用途	发明	美国	岳建民、杨升平、丁健、肖东、袁声涛、吴艳、童云广、董蕾	US 6,887,895 B2	药物所
2	呋甾皂甙的类似物、分离方法和用途	发明	中国	朱大元、顾仁樾、蒋山好、曾佳锋、蒋福祥、刘传海	ZL 00135190.7	药物所
3	一种双膦酸衍生物及其制备方法和应用	发明	中国	朱友成，吴颖，邹永，原伟芳	ZL 00117064.3	药物所
4	可溶性 SARS 病毒 3CL 蛋白酶的表达和纯化	发明	中国	沈旭、蒋华良、汪恒、陈静、沈建华、叶飞、罗小民、陈凯先、庄贤韩	ZL 03129147.3	药物所 生科院 先导药业
5	银杏内酯针剂的制备方法	发明	中国	陈仲良、殷梦龙	ZL 00115134.7	药物所
6	六种异喹啉生物碱	发明	中国	潘俊芳、余琛、朱大元、张慧、任建英	ZL 00114970.9	药物所 徐汇区中心医院
7	2－丁基－1，3－二氮杂螺环【4，4】壬烷－1－烯－4－酮的改进的生产工艺	发明	中国	沈敬山、李剑峰、毛睿、刘为四、穆汝运	ZL 01145583.7	药物所
8	坎地沙坦酯的合成新路线	发明	中国	沈敬山、李剑锋、严铁马、杨洁、穆汝运	ZL 00135191.5	药物所
9	替米沙坦的一种制备路线	发明	中国	沈敬山、严铁马、刘为四、毛睿、李剑锋、穆汝运	ZL 01131915.1	药物所 特化医药
10	抗肿瘤药物 4-[1-(3,5,5,8,8-五甲基-5,6,7,8-四氢－2－萘基）乙烯基]苯甲酸合成工艺	发明	中国	吕伟、阳海、朱勤	ZL 01145585.3	药物所 华拓医药
11	一类萘茜衍生物及其制备方法和用途	发明	中国	陆群、段文虎、蔡俊超、刘卫军、丁健	ZL 01132243.8	药物所
12	L－N－保护基－氮杂环丁烷－2－羧酸合成方法	发明	中国	秦欣荣、谢雨礼、罗会兵、谢毓元	ZL 01113124.1	药物所
13	11,11'-二去氧沃替西林的医学用途	发明	中国	赵维民、丁健、袁盛涛、施玉花、童云广、林莉萍、唐坚	ZL 01145719.8	药物所
14	青蒿素芳香醚类衍生物及其制备方法	发明	中国	李英、韩继烨、吴光韶、王方道、吴锦明、隋毅、张瑜、范崇光	ZL 01113407.0	药物所
15	一类取代哌嗪化合物的制备方法	发明	中国	杨玉社、郭柏淑、穆汝运、陈凯先	ZL 01145560.8	药物所