Highlights from the Society for Medicines Research Case Histories in Drug Discovery and Design meeting held December 4, 2003, in London, United Kingdom.

Successes in Drug Discovery and Design

by the SMR Committee

Summary

The Society for Medicines Research (SMR) held a one-day meeting on case histories in drug discovery on December 4, 2003, at the National Heart and Lung Institute in London. These meetings have been organized by the SMR biannually for many years, and this latest meeting proved extremely popular, attracting a capacity audience of more than 130 registrants. The purpose of these meetings is educational; they allow those interested in drug discovery to hear key learnings from recent successful drug discovery programs. There was no overall linking theme between the talks, other than each success story has led to the introduction of a new and improved product of therapeutic use. The drug discovery stories covered in the meeting were extremely varied and, put together, they emphasized that each successful story is unique and special. This meeting is also special for the SMR because it presents the "SMR Award for Drug Discovery" in recognition of outstanding achievement and contribution in the area. It should be remembered that drug discovery is an extremely risky business and an extremely costly and complicated process in which the success rate is, at best, low.

Dr. Helmut Haning (Bayer Healthcare, Germany) opened the Society for Medicines Research (SMR) meeting on case histories in drug discovery on December 4, 2003, at the National Heart and Lung Institute in London. He described the story behind the phosphodiesterase 5 (PDE5) inhibitor **vardenafil**, which is used to treat erectile dysfunction. PDE5 is a 99 kDa homodimeric enzyme of the PDE family comprising 11 members. It is characterized by its specificity for cGMP and allosteric binding sites for the substrate. It is known that suitably substituted purinones can behave as bioisosteres for cGMP and are potent PDE inhibitors. However, the team at Bayer demonstrated that although the purinones were potent *in vitro*, they lacked *in vivo* efficacy. These researchers recognized early on that xanthine oxidase plays a major role in metabolizing the purinone nucleus through hydroxylation (Fig. 1). The Bayer team hypothesized that substitution of the carbon atom for a heteroatom may increase the metabolic stability of the heterocyclic core. A great variety of purine-isosteric heterocyclic systems were synthesized. Among those systems, the imidazo[5,1-*f*][1,2,4]triazin-4(3*H*)-ones (Fig. 2) turned out to be the optimal heterocyclic core for inhibition of PDE5.

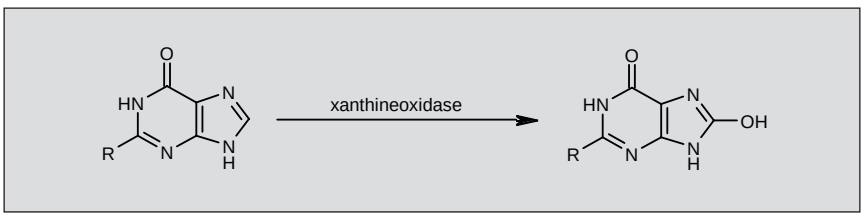

Fig. 1. Xanthine oxidase plays a major role in metabolizing the purinone nucleus through hydroxylation.

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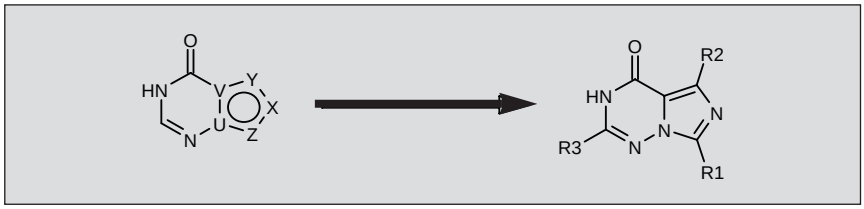

Fig. 2. Optimal heterocyclic core for inhibition of phosphodiesterase 5.imidazo[5,1-*f*][1,2,4]triazin-4(3*H*)-ones

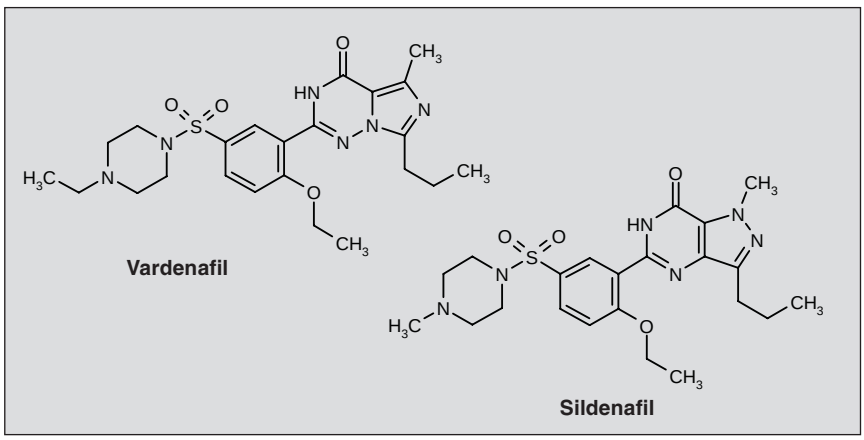

Fig. 3. Phosphodiesterase 5 inhibitors to treat erectile dysfunction.

With the use of this heterocyclic skeleton, **vardenafil** (Fig. 3) was discovered and was determined to be at least an order of magnitude more potent than **sildenafil** (Fig. 3), while also displaying greater selectivity with respect to PDE1. In addition to the heterocyclic core, the two molecules differ in the substituent on the piperazine nitrogen. It was clearly demonstrated that the superior potency is due to the change in the heterocycle; however, the potency enhancement observed cannot currently be explained through X-ray co-crystallization analysis. Vardenafil is also more potent than sildenafil in the conscious rabbit model of erectile dysfunction. Clinically, vardenafil reaches its T_{max} early and is efficacious in more than 90% of patients. Side effects have been reported to be mild and transient.

Dr. George Muller (Celgene, U.S.A.) gave a presentation focusing on the history, and current and future therapeutic indications for **thalidomide** (Fig. 4). By the late 1950s, thalidomide had become a popular sedative but was removed from the market when its use as a treatment for morning sickness was linked to serious birth defects. The serendipitous discovery of its antiinflammatory activity in the mid 1960s for the treatment of erythema nodosum leprosum (ENL) in leprosy resulted in its renewed use. The discovery of thalidomide's tumor necrosis factor-α (TNF-α) inhibitory activity, antiangiogenic activity and clinical efficacy in cancer trials has resulted in renewed interest in thalidomide and its analogues. Celgene began development of thalidomide in 1992 and received U.S. FDA approval in 1998 for the treatment of cutaneous manifestations of ENL in leprosy. The major use of thalidomide in the United States is now in oncology, with particular focus on blood cancers. A large number of clinical trials have demonstrated clinical efficacy of thalidomide in the treatment of multiple myeloma. Thalidomide is currently being investigated in more than 140 clinical trials. Because the mechanism of action relating to the side effect profile was unclear, Celgene began a drug discovery program at the end of 1992 to discover thalidomide analogues with improved activity that lacked the side effects of teratogenicity, neuropathy, constipation and sedation. This research resulted in the discovery of a new class of thalidomide analogues termed IMiDs™. The IMiDs are thalidomide analogues with greatly improved *in vitro* activity that show potent antiinflammatory activity and anticancer activity. *Revimid*™ (**ENMD-0997**; Fig. 4) and *Actimid*™ (**CC-4047**; Fig. 4) are potent inhibitors of not only TNF-α (IC_{50} > x2000 that of thalidomide) but also a range of cytokines and cyclooxygenase-2 induction. Like thalidomide, the IMiDs also possess a chiral center that rapidly epimerizes. Importantly, *Revimid* displays none of the teratogenetic, sedating or constipating side effects of thalidomide use. *Revimid* also displays excellent bioavailability in the rat, dog and monkey (>50%) and is currently in a number of early to late clinical trials.

Dr. Stan Vanboeckel (Organon, The Netherlands) took the audience on a journey focusing on the discovery and development of **fondaparinux**

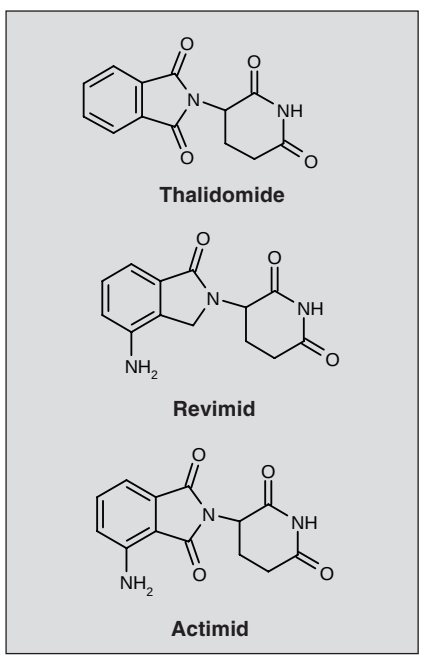

Fig. 4. Thalidomide and analogues that show antiinflammatory and anticancer activity.

sodium, a story that involved 50–70 steps in the synthesis of a clinical candidate! Since 1936, **heparin** has been used in clinics for the prevention and treatment of thrombosis. Its main antithrombotic activity is in its ability to potentiate the activity of the serine protease inhibitor antithrombin III (AT-III), which inactivates a number of serine proteases such as thrombin and factor Xa in the coagulation cascade. By the end of the 1970s, heparin fragments, obtained by chemical or enzymatic degradation, had been isolated by affinity chromatography on immobilized AT-III. From these studies, it was deduced in 1981 that a unique pentasaccharide (PS) fragment that occurs in about one third of the heparin polysaccharide chains constitutes the minimal binding domain for AT-III. The PS fragment (also known as the DEFGH part of heparin) was synthesized a couple of years later to confirm the earlier proposal. A key moment in the discovery of fondaparinux sodium was when the researchers recognized that the metabolically liable cyclic acetal could be stabilized via methylation. This modified synthetic PS fragment was found to elicit a very selective antithrombotic mode of action, in that it only accelerates the AT-III–mediated inhibition of coagulation factor Xa but not that of thrombin. Interestingly, both Organon and Sanofi Synthelabo arrived at the same molecule (ORG-31540/SR-90107; fondaparinux sodium), and as the project was perceived high risk, the two companies decided to collaborate. The results of four phase III clinical trials demonstrated that PS provides a superior benefit over low-molecular-weight heparin in preventing deep-vein thrombosis in major orthopedic surgery patients, with an overall relative risk reduction of 50% and a similar safety profile. In 2002, the FDA approved this PS as a new antithrombotic drug called *Arixtra*®. The specificity of the interaction of the PS with AT-III was confirmed when PS analogues were synthesized and tested for inhibition of blood coagulation factor Xa. Structure–activity relationship (SAR) analysis established which of the charged groups played an important role in the activation of AT-III, and by the contemplation of molecular modeling data, a simplified AT-III/PS interaction model was postulated. On the basis of this model, an extra sulfate group at position 3 of unit H of the naturally occurring fragment was introduced. This extra-sulfated analogue displayed higher affinity toward AT-III and an enhanced AT-III–mediated anti-Xa activity. Subsequent attention turned to a simplified series in which all hydroxyl groups were methylated and in which all *N*-sulfate groups were replaced by *O*-sulfate groups. In this series, several analogues methylated at the 2-*O* and 3-*O* positions of both uronic acid moieties were prepared. One of these methylated analogues (**SanOrg-34006**; **idraparinux sodium**) turned out to be highly potent. This analogue binds much stronger to AT-III (K_d = 20 nM), relative to the PS (K_d = 700 nM), and as a result its elimination half-life is much longer.

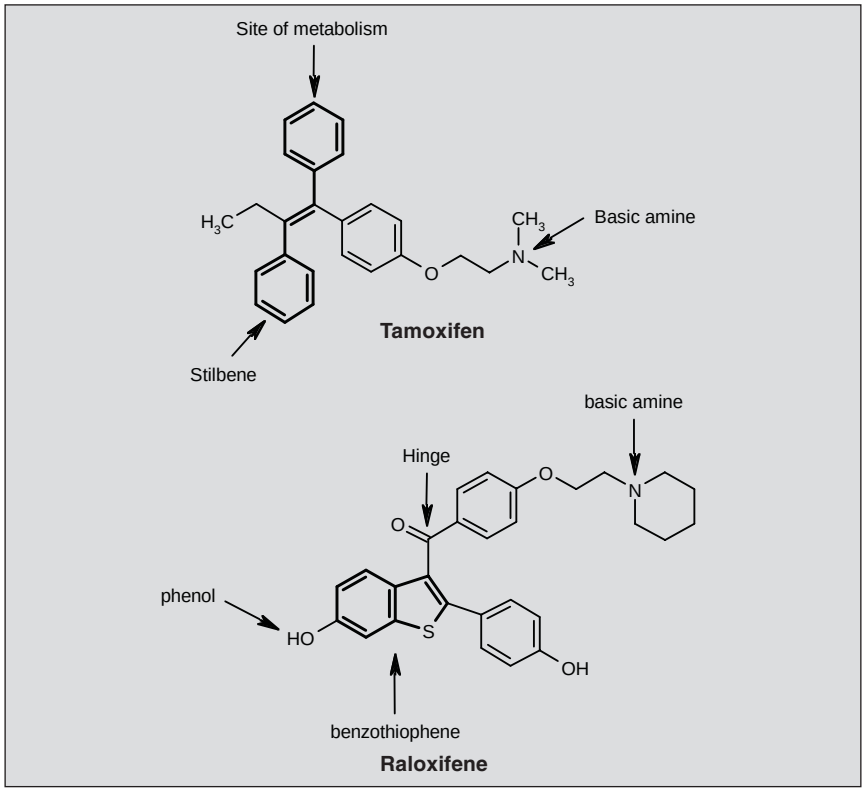

Fig. 5. Selective modulators for the treatment of postmenopausal osteoporosis.

Dr. Jeffrey Dodge (Lilly, U.S.A.) talked of the quest for selective estrogen receptor modulators for the treatment of postmenopausal osteoporosis. The first estrogen antagonist was discovered in the 1950s, and **tamoxifen** (Fig. 5) was approved in 1973 for the treatment of breast cancer, although the therapy was later discontinued after it was found that tamoxifen was a partial agonist in uterine tissue. This uterine agonist activity has been associated with an increased risk of endometrial cancer. The desire was to identify an agent that would antagonize the effects of estrogen on the mammary tissue while mimicking its effects on the bone. Interestingly, the geometrical isomers of tamoxifen have opposing biological activities. It was hypothesized that structural changes to the ligand may influence the conformation of the receptor/ligand complex and thereby affect which estrogen-responsive genes are modulated in various tissues. SAR analysis focused on varying the central template, with particular attention on identifying a replacement for the stilbene scaffold. From this analysis, **raloxifene** (Fig. 5) was identified in the early 1980s.

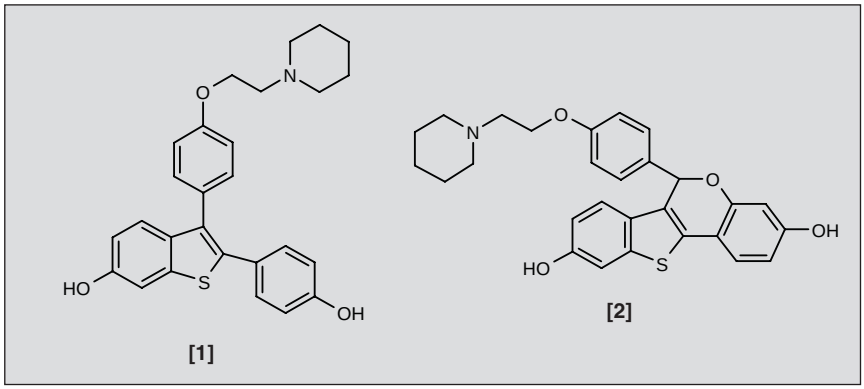

Fig. 6. Compounds prepared to establish whether the benzothiophene ring system or carbonyl hinge was responsible for the enhanced tissue selectivity in raloxifene.

The biological activity of **raloxifene** differs from that of **tamoxifen** in that it is an antagonist in uterine tissue. Compounds **[1]** and **[2]** (Fig. 6) were prepared to establish whether the benzothiophene ring system and/or carbonyl "hinge" was responsible for the enhanced tissue selectivity.

In compound **[1]**, the hinge carbonyl has been removed, while in compound **[2]**, the orientation of the basic side chain has been rigidified by incorporation of the carbonyl into a benzopyran ring. Interestingly, while the *in vitro* estrogen action of compounds [1] and [2] were similar, compound [1] produced a significant increase in uterine eosinophilia and uterine weight. From the SAR analysis, it was apparent that the hydroxylation pattern was important for receptor binding and *in vitro* activity, and the presence and nature of the basic side chain was critical for determining estrogen antagonist activity. However, the shift from an acylic olefin in **tamoxifen** to a benzothiophene system in **raloxifene** is the most striking structural difference between the two molecules. Dodge and co-workers proposed that it was this modification together with the inclusion of a carbonyl "hinge" that was responsible for the differences in tissue selectivity observed between tamoxifen, raloxifene and compounds [1] and [2]. Modeling studies suggested that these simple modifications produce a dramatic change in the position of the basic side chain from a nearly orthogonal orientation in raloxifene to a coplanar orientation for tamoxifen. Thus, it was hypothesized that the coplanar orientation of the side chain of tamoxifen and compound [1] were responsible for the uterine stimulation observed.

The 2003 SMR Award for Drug Discovery was presented to the key members of the team that led to the discovery of **imatinib** (*Gleevec*™; STI-571), developed for the treatment of chronic myeloid leukemia (CML). Juerg Zimmermann, Elisabeth Buchdunger, Ulrike Pfaar, Peter Graf, John Ford and Renaud Capdeville, key scientists from the Novartis program team, each received a framed certificate in recognition of their achievement, presented by the chairman of the SMR, Dr. Malcolm Duckworth. Dr. Juerg Zimmerman delivered the award lecture. Until recently, a patient suffering from CML, a cancer of the blood cells, had few options. Radiation, the first treatment for CML, was introduced in the 1920s. Chemotherapeutic agents followed in the 1950s and 1960s. Superior to radiation therapy, chemotherapy increased survival among patients with CML to about 5 years. Bone marrow transplantation arrived in the 1970s, and interferon alfa debuted in the 1980s. Of all these treatments, only bone marrow transplantation currently provides a potential "cure," that is, long-term remission from cancerous cell growth. Imatinib represents a remarkable story that began more than 40 years ago with the discovery of the Philadelphia chromosome, the first cancer-related genetic abnormality to be recognized. The discovery hinged on the clarification of the role of Bcr-Abl in CML, which later provided Novartis with the unique opportunity to discover and develop this targeted anticancer therapy.

The Novartis effort toward the discovery of **imatinib** (Fig. 7) started with a desire to discover potent, selective and orally active ATP-competitive protein kinase inhibitors based on a central phenylamino-pyrimidine template. This scaffold was thoroughly optimized, eventually leading to compounds with potent activity against protein kinase C (PKC). Excellent cellular activity was obtained with analogues bearing a 3′-pyridyl group at the 3 position of the pyrimidine (**[3]**; Fig. 7). During the optimization of this structural class on the inhibition of PKC, a serine/threonine kinase, it was observed that the presence of an amide group on the phenyl ring also gave rise to inhibition of tyrosine kinases, such as the Bcr-Abl kinase (**[4]**; Fig. 7). In this case, the amide bond was required to be stable toward hydrolysis because the release of an unprotected diaminophenyl moiety has to be avoided to exclude potential toxicological issues. In fact, high hydrolytic stability could be achieved with derivatives with R_1 = phenyl. However, selectivity was the next hurdle, since these compounds inhibit PKC as well as tyrosine kinases.

At this point, a key observation was made from analysis of SAR: substitution at position 6 of the diamino phenyl ring was not tolerated for PKC inhibition. Indeed, introduction of a simple "flag-methyl" led to loss of activity against PKC, while the activity against protein–tyrosine kinases could be retained or even enhanced (**[5]**; Fig. 7). Disappointingly, the first series of selective inhibitors that were prepared showed low aqueous solubility and poor oral bioavailability. This drawback was eventually circumvented by the introduction of a solubilizing side chain in a region of the molecule that did not interfere with the binding

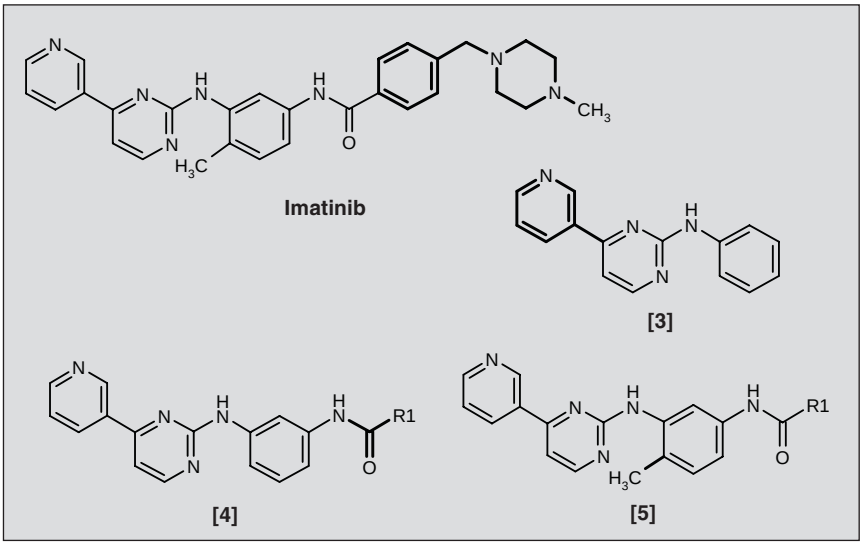

Fig. 7. Potent, selective and orally active ATP-competitive protein kinase inhibitors that led to the discovery of imatinib.

affinity. Importantly, this modification dramatically increased not only aqueous solubility but also the oral bioavailability. The attachment of basic groups at 4 position of the phenyl ring did, however, raise an "aniline alert" (mutagenic potential), which had to be avoided. This was achieved by the introduction of a spacer between the phenyl ring and the nitrogen atom. The best compound from this series was the methyl-piperazino derivative **STI-571**, which was selected as the most promising candidate for clinical development. STI-571, which eventually came to be known as *Gleevec*, was the first protein kinase inhibitor to reach the market and was also the first example of a targeted drug therapy for cancer. Not since the interferon craze in the early 1980s has a cancer drug captured the public imagination like Novartis's STI-571. It won approval from the FDA on May 10, 2001, for the treatment of CML after a lightning-fast 2½-month review. It represents a monumental leap forward in cancer chemotherapy. It proves a principle. It justifies an approach. It demonstrates that highly specific, nontoxic therapy is possible. It does not guarantee success of similar efforts, because CML may not be typical of most other malignancies. Congratulations to the Novartis team for accomplishing the equivalent of the 4-minute mile.

Dr. Howard Fox (Novartis, U.K.) delivered a lecture that focused on the developmental issues overcome along the journey to the discovery of **omalizumab** (*Xolair*®), an antibody and a first-in-class treatment for asthma. Developments in the antiinflammatory treatment of asthma currently provide patients with more choices for reducing and controlling the symptoms associated with the disease. Inhaled corticosteroids, prescribed since the 1980s, help control airways inflammation, and inhaled short-acting B_2 receptor agonists provide symptom relief for asthma patients. Since then, incremental advances in asthma treatment have led to the development of inhaled long-acting B_2 agonists and oral leukotriene antagonists. Despite these advances, asthma remains a heavy financial and social burden for many of the +3 million U.K. patients. Omalizumab is the first recombinant DNA-derived humanized monoclonal antibody developed to intervene in the critical common pathway of pathophysiological expression of asthma and allergy, namely immunoglobulin E (IgE). Omalizumab consists of a humanized IgG1 κ framework (95%), with a variable murine antibody sequence (5%) grafted onto the framework. This avoids any potential sensitization to murine antibodies, as they are not detected by the human immune system when omalizumab binds to the IgE. Omalizumab was also designed to be nonanaphylactogenic because of the fact that it is unable to bind to IgE already bound to the high-affinity IgE receptors of mast cells. Omalizumab binds to free IgE, forming a biologically inert complex that is unable to bind to effector cells, therefore blocking the allergic response of asthma. By adjusting omalizumab's dose according to body weight and IgE levels, IgE can be reduced by up to 95%; this also leads to a downregulation of the high-affinity IgE receptors on basophils and, potentially, mast cells. The results from clinical trials of more than 6,000 patients indicate that treatment with omalizumab reduces asthma exacerbations, time to exacerbation, inhaled corticosteroid use, rescue medication use, asthma symptom scores and healthcare utilization. Omalizumab was approved in Australia in 2002, and in June 2003 the FDA licensed *Xolair* for the treatment of adolescents and adults with allergic asthma.

The final lecture of the meeting focused on emerging treatments for opioid dependence and was delivered by a former SMR chairman, Dr. Chris Chapleo (Reckitt Benckiser Healthcare, U.K.). Efforts to tackle the problems associated with opiate (**heroin**) addiction are driven by the recognition of a need for effective harm reduction policies. This encompasses a range of activities and issues including health and risk behaviors from transmission of HIV and hepatitis, criminal behavior and social functioning. Maintenance therapy (also referred to as substitution therapy) has a clear role to play in any program aimed at reducing harm associated with addiction. **Methadone**, as a once-a-day therapy, has been the maintenance therapy of choice for more than 30 years. Unfortunately, methadone is also highly addictive, resulting in overdose situations, and it is also very difficult to achieve a state of abstinence because of severe withdrawal problems. Even if patients successfully reduce their dose until they reach the drug-free state, it is estimated that 90–95% of patients

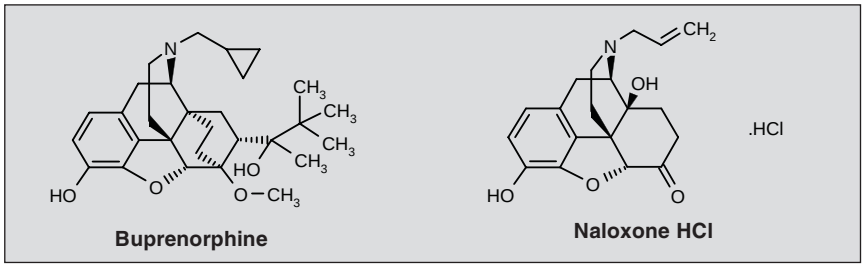

Fig. 8. Opioid antagonists for the treatment of narcotics dependence.

relapse. **Buprenorphine**'s (Fig. 8) potential as a treatment for heroin addiction was first recognized during the 1970s. Discovered in 1966, buprenorphine was developed as a potent analgesic of the morphine class in 1978, from which time effort focused on buprenorphine's potential as a new indication for addiction treatment. Its unique qualities result from it being a partial agonist at the mu receptors in the brain. Other agents acting at this receptor (heroin, **morphine** and methadone) are full agonists, producing very high levels of physical dependence. The physical dependence of buprenorphine has been evaluated in rodents, dogs and monkeys, and is markedly lower than that of full agonists. Current evidence supports the theory that the low level of physical withdrawal following chronic buprenorphine treatment is due to slow receptor kinetics. Therefore, when drug administration is stopped after chronic dosing, buprenorphine leaves the receptors very slowly, such that the biochemical systems involved in the dependence process return to their pretreatment levels while maintaining homeostasis. With the full agonists, opiate withdrawal results in an abrupt return of the system to predependence levels, and this is responsible for "spontaneous withdrawal."

Buprenorphine also possesses an improved safety profile. In a dose-ranging study with sublingual doses of 1–32 mg, a nonstatistical decrease in respiration rate at the 4-mg dose level was observed; thereafter, no further decrease in respiratory rate was observed as the dose increased. Blood levels increased over this dose range; thus, decreased absorption was not a possible reason for decreased effect on respiration. Tolerance is another aspect of full agonists that is a major concern in addiction treatment, where addicts over a period of time have to consume higher levels of their opiate to achieve the same effects. Addicts who have stopped "consumption" are in danger if they return to their habit, using the same dose level used prior to the period. Buprenorphine does not suffer from this tolerance problem, which in effect is a measure of its safety. As with all opiates, buprenorphine has been the subject of abuse by the injectable route, and diversion of products containing buprenorphine has occurred in a number of countries.

Naloxone (Fig. 8) is a competitive mu antagonist and produces an opioid withdrawal syndrome when administered intravenously to an opioid-dependent individual. However, naloxone is not well absorbed when administered sublingually, and it has been shown that the drug does not interfere with **buprenorphine**'s absorption or pharmacological effects when administered in combination by the sublingual route. The optimum ratio of buprenorphine to naloxone in a combination product is 4 to 1, and this combination is sufficiently unpleasant to the opioid-dependent individual who might abuse the product, but it does not attenuate the "good" opioid agonist effects. Taken sublingually, the combination product *Suboxone*® is equivalent to the buprenorphine-alone product *Subutex*®. However, if abused intravenously, the combination product precipitates withdrawal in opioid dependents and is perceived to be naloxone by those who inject the product. New dosage formulations were essential to clinically probe the safety and efficacy of buprenorphine and to determine the dose range for treatment. As buprenorphine is not orally active, a number of routes of administration have been examined, and for convenience a sublingual liquid has been developed.

The SMR Committee organizes conferences on behalf of the Society for Medicines Research four times a year. These one-day conferences are of a multidisciplinary nature, therapeutically focused and normally staged in or around London. Details about forthcoming meetings can be obtained from: SMR Secretariat, Triangle House, Broomhill Road, London, SW18 4HX, U.K. Tel: +44 (0)20 8875-2431; Fax: +44 (0)20 8875-2424; E-mail: secretariat@socmr.org; URL: http://www.socmr.org.