CSIR – Central Drug Research Institute, Lucknow

Candidate Drugs / Leads / Hits open for licensing

Neglected Parasitic and Infectious Diseases
Candidate Drug 97/78 (Antimalarial)
Novel oral combination formulation (SMEDDS) for treatment of Malaria
Compound S011-1793 (Antimalarial)
Dry powder inhalation of anti-tuberculosis drugs
Compound 98/288 (anti-leishmanial)
Life Style Diseases
Standardized fraction of 4655 (anti-dyslipidemic)
Compound S013-0431 (Anti-dyslipidemic)
Compound S013-1593 (Anti-obesity)
Compound S007-867 (Anti-platelet)
Compound S002-333 (Anti-platelet)
Compound S013-1304 (For major depression)
Reproductive Health and Osteoporosis
Candidate Drug 99/373 (Anti-osteoporotic)
Compound S007-1500 (Novel Oral Rapid Fracture Healing agent)
CDR2492C002 formulation for post-menopausal osteoporosis and associated fractures
Cancer
Compound S007-1235 (Anti-colon cancer)
Novel cancer biomarker for rapid screening of cervical cancer

For Further Enquiries, Please Contact

Dr Madhu Dikshit, FNA, FASc, FNASc, JC Bose Fellow

Director

CSIR-Central Drug Research Institute

Sector 10, Jankipuram Extn., Sitapur Road

Lucknow - 226 031, UP, India

Tel: 091-522-2771940; Fax: 091-522-2771941

Email: director@cdri.res.in http://www.cdriindia.org

Candidate Drug 99/373 (Anti-osteoporotic)

Highlights:

- ❖ Compound 99/373 is antiresorptive agent useful for Osteoporosis accompanying menopause and other estrogen deficiency states.
- ❖ Compound has no/negligible estrogenic profile (Uterotrophic side effect) on genital tract tissue.
- ❖ Compound has low thrombogenic activity in comparison to known drug (Raloxifene)
- ❖ Completed preclinical studies and Permission for conducting phase I Clinical trial has been obtained from DCGI

Background:

Osteoporosis, which has been defined as a “state of low bone mass” is one of the major aging problems of the society. Osteoporosis is a metabolic disorder characterized by microarchitectural deterioration of bone tissue leading to enhanced bone fragility and consequent increase in fracture risk in older members of the population. Osteoporosis fractures occur most commonly in the spine, hip, distal radius and ribs. The risk is high in women as compared to men and increases sharply after 50 years of age. Factors predisposing towards osteoporosis include family history, genetic factors, hormonal factors, inadequate nutrition, and intake of certain medications, immobility and disease. The quality of life is greatly impaired in persons with sever osteoporosis. It is known to affect >50% of women and 30% men over the age of 50 years. In women, there is also an accelerated rate of bone loss immediately and for variable number of years following menopause.

Most of the pharmacological agents available for clinical use such as calcium, vitamin D and its analogue, calcitonin, bisphosphonates, raloxifene, hormone replacement therapy (HRT) etc. act by decreasing the rate of bone resorption, thereby slowing the rate of bone loss. Timely administration of such antiresorptive agents prevents bone loss.

The only side effect of calcium therapy is development of renal stones. The major disadvantage in calcitonin use is its high cost. Tachyphylaxis can develop in some individuals under calcitonin treatment. Bisphosphonates are poorly absorbed and may cause gastrointestinal irritation, diarrhea and constipation. Raloxifene has been reported to increase incidence of hot flashes, deep vein thrombosis, pulmonary embolism and leg cramps.

Hormone replacement therapy, though effective in preventing bone loss following ovariectomy or menopause in women, is associated with increased risk of endometrial hyperplasia and carcinoma, breast cancer, and thromboembolic diseases. Parathyroid hormone [PTH(1–34)], the sole clinically used anabolic agent, has been recommended recently by the US Food and Drug Administration to carry a black box warning because it is associated with an increased risk of osteosarcoma in rats. Intermittently administered PTH (iPTH) not only increases bone mass but also improves bone quality and strength by positive effects on the microarchitecture and geometry of bone.

In India the estimated number of fracture related cases range in the order of 50 million per year. A course of daily oral treatment for fracture healing is expected to last for 90 to 120 days per patient per incident. In the fracture healing incidence alone, 50 billion or 5000 crores of formulary dosages will be utilized annually. Bone anabolic therapy is also expected to impact secondary osteoporosis as well as primary osteoporosis. The prevalence of osteoporosis in India alone is estimated at 300 million cases. The market potential of an affordable bone anabolic therapy in India is enormous. If this treatment were to be taken onto the global stage, the market potential is limitless. There is no currently available oral therapy in the market or in clinical trials.

Unique Features CDRI Comp 99/373

CDRI Compound 99/373 is a promising alternative option in the prevention and treatment of osteoporosis. CDRI COMP 99/373 is an orally active achiral compound. It has excellent antiresorptive activity, backed by elegant bone biology studies. It has favourable animal PK profile. Found safe in preclinical regulatory pharmacology and toxicity studies. CDRI has obtained DCGI permission to carry out Phase-I clinical trial. National and international patents granted which are active. Product has advantage over existing clinically used drugs like raloxifene and bisphoaphonates in terms of its efficacy as well as toxicity. Cost effective due to “one pot synthesis”

Patent Status:

APPLICATION NUMBER	FILING DATE	PATENT NUMBER	DATE OF GRANT	TITLE	INVENTOR(S)
0246DEL2004	20-Feb-04	237915	12-Jan-10	Novel mercapto phenyl naphthyl methane derivatives and preparation thereof	Sangita, Atul Kumar, Man Mohan Singh, Girish Kumar Jain, Puvvada Sri Ramchandra Murthy & Suprabhat Ray
CA-2524568	02-Nov-05	2524568	17-Jul-12	Substituted mercapto phenyl naphthyl methane derivatives as SERM for the prevention and treatment of esteoporosis and other estrogen dependent disorders and as contraceptives	
CN-ZL200380110327.4	29-Nov-05	200380110327	16-Dec-09		
EP-20030780487	17-Nov-05	1692101	06-Jul-11		
FR-20030780487	17-Nov-05	1692101	06-Jul-11		
Gb-20030780487	17-Nov-05	1692101	06-Jul-11		
US-11/812251	15-Jun-07	7582653	01-Sep-09	Novel mercaptophenyl naphthyl methane compounds and synthesis thereof	
US-10/809845	26-Mar-04	7250446	31-Jul-07	Substituted mercapto phenyl naphthyl methane derivatives as SERM for the prevention and treatment of esteoporosis and other estrogen dependent disorders and as contraceptives	
PCT/IB03/006247	23-Dec-03			Mercapto-phenyl-naphthyl-methane derivatives and preparation thereof	

Candidate Drug 97/78 (Anti-malarial)

Highlights:

- Synthetic molecule (Artemesinine Derivative)
- Pre-Clinical Studies completed
- Pharmacologically safe, effective & Toxicologically safe
- Clinical Trial permission from DCGI to carry out Phase-I studies
- Phase-I Single dose clinical trial in normal healthy volunteers already completed along with human single dose pharmacokinetic studies.
- Safe in Phase-I single dose studies in normal healthy volunteers

Background

Malaria is one of the most widespread tropical parasitic diseases, caused by the protozoa of the genius plasmodium and transmitted by the female anopheles mosquito. According to the world malaria report 2011 of the World Health Organization (WHO), there were 216 million cases of malaria in the world in 2010. As much as 81% of these were in Africa and 13% is South-East Asia. There were an estimated 655,000 deaths in that year. Most of the deaths occurred in sub-Saharan Africa and the victims were mostly children under the age of five. Malaria remains the major public health problem in India. Northeastern region of India is one of the hot spots for malaria transmission. Focal outbreaks of malaria are of common occurrence especially in forest-fringed villages of Assam, bordering Arunachal Pradesh. Orissa alone contributes to more than 40% of P. falciparum deaths in India; south Orissa is a known as hyper-endemic area of the state.

Commonly used drugs to treat malaria are quinine (1), chloroquine (2) mefloquine (3), amodiaquine (4), primaquine (5), artemisinine (6), piperquine (7) dapsone (8) and sulfadiazine (9). But the rapidly increasing resistance of P. falciparum malaria parasites to drugs such as quinine, chloroquine, proguanil and pyrimethamine has made malaria chemotherapy ineffective. Most widely used antimalarial drugs, chloroquine (CQ) is a 4-aminoquinoline is the gold standard for the treatment of malaria that was previously characterized by its efficacy and low toxicity. But now malaria parasite developed resistance to Chloroquine with complex point mutations in the gene encoding PfCRT ultimately enhances efflux of Chloroquine from the food vacuole resulting low concentration of drug which makes the drug ineffective. Literature strongly suggests the involvement of compound specific resistance. To overcome the challenges in malaria chemotherapy in view of the fast growing resistance against existing drugs, there is an urgent need to develop new drugs. Structural modification of existing drugs offers promise for the development of new drugs as their mechanism of action, pharmacokinetic and toxicity profiles are already known.

Current Status

CDRI Comp 97/78 is a trioxane peroxide, a synthetic derivative of artemesinin. Pre-clinical regulatory studies have been completed. It is found safe in preclinical regulatory pharmacology and toxicity studies. DCGI permission to carry out phase-I clinical trial and pharmacokinetic studies has been obtained.

Phase-I single dose studies have been completed in PGIMER, Chandigarh and found safe and well-tolerated in normal healthy subjects. PK studies in normal healthy subjects in single dose shows rapid absorption and rapid conversion to its precursor 97/63; this confirmed preclinical data on the molecule. The higher t1/2 may help maintain the therapeutic efficacy and reduce the occurrence of recrudescence of malarial parasite during therapy and follow-up.

Patent Status:

TITLE	INVENTOR(S)	APPLICATION NUMBER	FILING DATE	PATENT NUMBER	DATE OF GRANT
An improved process for the preparation of ether derivatives of dihydroartemisinin	Chandan Singh & Rani Kanchan	1258DEL1997	13-May-97	186127	25-Jan-02
Novel substituted 1,2,4-trioxanes useful as antimalarial agents	Chandan Singh & Sunil Kumar Puri	1579DEL1999	28-Dec-99	232539	18-Mar-09
		BD-0058/2000	31-Mar-00	1003409/2000	
		ID-P-20000261	31-Mar-00	ID0011878	13-Oct-03
		ML-PI-20001330	31-Mar-00		
		PK-819/2001 DIV	22-Aug-01		
		PK-0288/2000	31-Mar-00	137301	31-Jul-02
		SG-200001862-2	31-Mar-00		
		SL-200001862.2	31-Mar-00	0084585	30-Sep-04
		LK-12044	31-Mar-00	12044	27-Oct-00
		TH-056618	31-Mar-00		
		US-09/539574	31-Mar-00	6316493	13-Nov-01
		Vl-S20000281	31-Mar-00		
		VN-S20000281	31-Mar-00	4230	13-Apr-04
		ZA-2000/1642	31-Mar-00	2000/1642	27-Dec-00

Candidate Drug 80/574 (Anti-dyslipidemic)

Highlights:

- ❖ Synthetic hypolipidemic drug
- ❖ Safe in all preclinical studies
- ❖ Phase-I , Phase-II , Phase-III clinical trials completed and found to be safe and effective lipid lowering agent
- ❖ International patent granted
- ❖ Ready for licensing

Current Status:

CDRI comp 80/574 is a new synthetic hypolipidemic with unique mode of action through possible FXR Receptor Antagonism – proven by various international groups. Compound found safe in preclinical regulatory pharmacology and toxicity studies and in clinical trial Phase-I, II & III data all duly submitted to DCGI and accepted. It is safe as shown by clinical trials and does not have proximal myopathy, myotoxicity, muscle enzyme enhancing adverse effects all hallmarks of statins.

Being triglyceride centric it maybe most suited anti-dyslipedemic in indians who are most prone to diabetes, metabolic syndrome and obesity where major abnormality is high triglyceride excellent preclinical data. National and international patents granted which are active. Cost benefit ratio very significant. Huge possible market for this lifestyle disorder and a Potential blockbuster for India and developing world.

Patent Status:

APPLICATION NUMBER	FILING DATE	PATENT NUMBER	DATE OF GRANT	TITLE	INVENTOR(S)
1052MUM2009	22-Apr-10			Combination of BAR antagonist and HMG-CoA reductase inhibitor for treatment of dyslipidemia	Indravadan Ambalal Modi, Bakulesh Mafatlal Khamar, Chhitar Mal Gupta, Anju Puri, Rabi Sankar Bhatta, Ram Pratap, Girish Kumar Jain, Smrati Bhadauria, Ashok Kumar Khanna, Omkar Prasad Aana & Ashim Ghatak
PCT/IB2010/000898	21-Apr-10				
2302DEL1995	13-Dec-95	193304	16-Dec-05	A process for the preparation of an anti-hyperlipidimic composition	Ram Pratap, Ram Chandra Gupta, Narendra Kumar Kapoor, Ramesh Chander, Ashok Kumar Khanna, Asheem Ghatak, Omkar Prasad Asthana, Swarn Nityanand, Sukh Dev & Nitya Anand
EP-99302556.8	31-Mar-99				
1361DEL2003	06-Nov-03			An anti-hyperlipidemic composition	Ram Pratap, Ram Chandra Gupta, Ramesh Chander, Ashok Kumar Khanna, Omkar Prasad Asthana, Swarn Nityanand, Nitya Anand, Ashim Ghatak, Narinder Kumar Kapoor & Sukh Dev
0067DEL1999	12-Jan-99			Method of treating hyperlipidemic and hyperglycemic conditions in mammals using pregnadienols and pregnadienones	Ram Pratap, Ram Chandra Gupta, Ramesh Chander, Ashok Kumar Khanna, Arvind Kumar Srivastava, Deepak Raina, Savita Srivastava, Satyawan Singh, Anil Kumar Rastogi, Asheem Ghatak, Omkar Prasad Asthana, Swarn Nitya Anand & Nitya Anand
DE-99302556.8	31-Mar-99	1020191	24-Dec-08		
FR-99302556.8	31-Mar-99	1020191	24-Dec-08		
GB-99302556.8	31-Mar-99	1020191	24-Dec-08		
IT-99302556.8	31-Mar-99	1020191	24-Dec-08		
EP-99302556.8	31-Mar-99	1020191	24-Dec-08		
US-10/385936 DIV	14-Mar-03	6875758	05-Apr-05		
US-09/280448	30-Mar-99	6579862	17-Jun-03		

Lead Compound S007-867 (Anti-platelet)

Unique Features of the Molecule:

- ❖ Unique collagen mediated platelet activation Inhibitor
- ❖ Inhibits collagen induced platelet activation
- ❖ Novel mechanism of action as a specific inhibitor of collagen induced platelet aggregation and adhesion

Executive Summary

CDRI has a novel small molecule anti-platelet compound which may be useful in treating intravascular arterial thrombosis. This novel compound (chiral) is patented and has unique scaffold. The compound was picked after extensive SAR studies, which are active and selectively inhibit collagen mediated platelet activation. The compound is relatively simple to synthesize (MW < 500) and can easily be chemically modified to obtain the desired ratio of anti-platelet activity. The anti-platelet molecule has shown definite activity in animal models of arterial thrombosis. Preclinical studies show the compound to have lower incidences of bleeding as compared to currently used agents such as aspirin and clopidogrel. Acute toxicity studies in rats and mice studies demonstrate a favourable safety profile of this compound.

Highlights

Pharmacological inhibition of collagen activation is considered to be protective and lesion specific target. S007-867 is a potent and specific inhibitor of collagen induced platelet activation.

- It is a specific inhibitor of collagen induced platelet activation.
- It has no effect on coagulation cascade proteins (TT, PT aPTT) and has no adverse effect on vasoreactivity.
- It is orally active, as evident by sustained efficacy in mice model of thrombosis.
- It has mild effect on bleeding time with better efficacy, in contrast to existing anti-platelet drugs Aspirin and Clopidogrel at the similar efficacy doses administered by oral route.
- It significantly prolonged time of occlusion in $FeCl_3$ induced arterial thrombosis in rat, mice and hamsters, and reduced thrombus weight in arterio-venous shunt model in rat and hamsters
- Preclinical safety pharmacological, mutagenic and limited toxicity studies conducted so far have demonstrated no adverse effect. It has been tested *in vitro* for binding to 451 kinases and important GPCRs, which predict its safety.
- Good pharmacokinetic properties and bioavailability.

Advantages of CDRI Compound

Clinicians have used combination anti-thrombotic drug therapies to improve the antithrombotic efficacy. The combined use of antiplatelet agents, however, is considered high-risk by many clinical centers due to increased bleeding tendencies. In addition identification of resistance of these drugs is also a matter of great concern. Potent antiplatelet action selectively against collagen mediated platelet activation offers a novel approach to resolve the bleeding problem. The key

advantage of the new compound is related to a novel mechanism of action: Collagen antagonism. Unlike the known anti-platelet drugs, it does not enhance the bleeding time to a very high level as observed with clinically used anti-platelet drugs, thus it is expected to reduce bleeding risks.

Intellectual Property Right Protection (IPR)/ Patent Status

Patent filed: Chiral 3-aminomethylpiperidine derivatives as inhibitors of collagen induced platelet activation and adhesion

Indian Application No. 208DEL2011, Dt. 25-Jan-12

Europe Application No. 12705463.3, Dt. 23-Jul-13

United states Application No., 13/995336, Dt. 18-Jun-13

PCT Application No. PCT/IN2011/000032, Dt. 12-Jan-12

Lead Compound S007-1500 (Novel Oral Rapid Fracture Healing Agent)

Unique Features of the Molecule:

- Potential osteogenic property and shows accelerated fracture repairing. New bone formation at the fracture site is increased by ~40% in rats.
- Increases callus formation at only 1 mg/kg dose and restores trabecular microarchitecture at fractured site in normal female and osteopenic rats
- It leads to accelerated fracture repair by BMP-2/Smad signaling pathway.
- Compound found safe in single dose toxicity studies in rodents and in 10 days DRF studies
- Essential Safety Pharmacology study as per schedule Y is completed and no mortality/adverse effect was observed.

Background & Scientific Basis of the Project

Osteoporosis and related bone metabolic diseases have emerged as leading health care issues worldwide. The overall weakening of bone that results in osteoporosis is caused by a shift in the equilibrium of bone remodeling in which bone formation by osteoblasts is overtaken by the resorption of old bone by osteoclasts. India has the largest population in world suffering from osteoporosis. One out of three female and one out of eight male are suffering from osteoporosis which predisposes to greater fracture risks. In addition, the accidental fracture rates in India are one of the highest in world. Despite the biologically optimized nature of the repair process, patients still require several months before a fracture achieves complete return to mechanical stability. Consequently, there is significant interest in treatments that could enhance the rate of repair providing for a more rapid return to an active lifestyle and work. The identification of a compound that could effectively enhance repair under these less than optimal conditions would be of significant social and economic benefit.

Highlights of results:

Medicarpin, a natural pterocarpan, was identified as the most potent osteogenic agent. Subsequently new synthetic derivatives based on Medicarpin scaffold were synthesized. Out of these, a synthetic pterocarpan, CDRI-S007-1500 was taken up for further studies based on the preliminary studies which revealed enhanced osteoblastogenesis and chondrogenesis by CDRI-S007-1500 (CSIR-CDRI PCT Pub. No. WO/2010/052734. We identified S007-1500 (M.W. 294) to be the most potent among the other members of the series. S007-1500 required estrogen receptor (ER) function for its osteogenic action. S007-1500 stimulated several osteogenic bone morphogenetic proteins (BMPs), including BMP-2, 6 &-7. Pharmacological blockade of ER signalling abrogated the stimulatory effect of S007-1500 on BMP secretion from osteoblasts. Presence of endogenous BMP inhibitor, noggin also abrogated differentiation induction of osteoblasts by S007-1500. S007-1500 also stimulated BMP-2&-7 synthesis in human mesenchymal stem cells and this action exhibited ER dependence. Despite requiring ER function for its osteogenic effect, S007-1500 has no uterine estrogenicity/anti-estrogenicity. *In vivo*, S007-1500 stimulated new bone formation and bone biomechanical strength in growing female rats at a minimum oral dose of 1.0 mg/kg dose. S007-1500 also increased cortical microarchitectural parameters in the growing rats that further confirmed its favourable effect on bone strength. In OVx rats (with established osteopenia), S007-1500 at 10.0 mg/kg oral dose increased mineral apposition rate (MAR) and bone formation rate (BFR) over sham + vehicle, OVx + vehicle and

OVx + PTH groups. qCT measurements demonstrates that S007-1500 treatment to OVx rats also improved cortical thickness over OVx + vehicle group. Although, S007-1500 stimulated BMP synthesis by osteoblasts, it has no effect on the BMP-2 mRNA levels of aorta and cardiac valves of rats treated with this compound, suggesting absence of off-target effect. S007-1500 at 1.0 mg/kg oral dose also accelerated fracture healing in normal adult as well as in OVx (osteopenic) rats. S007-1500 accelerated fracture repairing by stimulating BMP-2/Smad signaling pathway. Single-dose toxicity studies and 10 days DRF studies suggest that S007-1500 is well tolerated. Together, pharmacological data suggest that S007-1500 possesses potential osteogenic property and shows accelerated fracture repairing by significantly increasing the callus formation at the fracture site in the test models i.e. normal adult females and osteopenic OVx rats at 1.0 mg/kg dose and also restoration of trabecular microarchitecture as determined by microCT. **These data suggest that S007-1500 has therapeutic potential as rapid fracture repairing agent and possesses several beneficial effects on improving bone health in the management of osteoporosis and bone related disorders.**

- S007-1500 increases the alkaline phospatase activity and nascent calcium deposition in primary calvarial osteoblast cells
- S007-1500 increases the mineralization from bone marrow derived osteoblast cells
- S007-1500 increases mRNA levels of osteogenic genes in osteoblasts
- S007-1500 enhances osteoblastogenesis via ER/BMP2 pathway
- Increased BMP-2- and -7 expression in human marrow stromal cells
- S007-1500 promotes osteoid formation and bone strength in growing rats
- S007-1500 improves cortical microarchitecture in growing rats
- S007-1500 promotes osteoid formation in ovariectomized rats
- S007-1500 improves cortical microarchitecture in OVx rats
- S007-1500 has no uterine estrogenicity
- S007-1500 does not increases BMP-2 expression in heart valve (bicuspid and tricuspid) and common aortic arch suggestive of absence of any off-target effects
- S007-1500 enhances chondrocyte mineralization
- Accelerated fracture repairing by S007-1500 in a BMP-2/Smad dependent manner

Comparative superiority over the marketed drugs

Current therapeutic approaches in India for the management of Osteoporosis and bone related disorders include calcium, vitamin D, hormone replacement therapy, raloxifene, alendronate and risedronate out of which bisphosphonates (alendronate) constitute the mainstay of treatment in India. All of these treatments attempt to reduce fracture risk primarily by reducing the rate of bone resorption and remodeling and in some cases, leading to minimal increases in bone mass. The reduction of bone loss alone, however, does not routinely lead the restoration of normal bone mass or strength and only leads to modest reductions in fracture risk. PTH has been shown to significantly increase bone mineral density and reduce fracture risk but is associated with other side effects (Matthew et al 2011). Recombinant human BMP2 (INFUSE® Bone Graft) has also been approved for open tibial fractures by FDA. However, the use of BMP2 is hampered by numerous clinical complications which include postoperative inflammation, cyst-like bone formation and life-threatening cervical swelling. Food and Drug Administration (FDA) has in fact issued a warning that in anterior cervical spine surgery, use of BMP/INFUSE posed the risks of dysphagia, hematoma and swelling. Consequently, there is an urgent need to discover safe and economical orally active agents that promote bone repairing and

regeneration. Currently, no orally active rapid fracture repairing agent is available nationally and internationally**.** Our Studies have led to identification of osteogenic activity in molecules like medicarpin (Tyagi et al 2010; Bhargavan et al 2012). Subsequently, several synthetic analogues of Medicarpin have been designed. Of these, CDRI-S007-1500 has been found to have bone regeneration potential as assessed by its effect on chondrogenesis and fracture callus formation.

Intellectual Property Right Protection (IPR) / Patent Status:

- Indian Patent App No. 2511DEL2008, Filed on 06 Nov 2008
- Chinese Patent App No. 200980152325.9, Filed on 23 Jun 2011
- European Patent App No. 09787590.0, Filed on 05 May 2011
- Japanese Patent App No. 2011-535212, Filed on 06 May 2011; **Granted: JP 535212 dt 03.03.2015)**.
- Korean Patent App No. 10-2011-7012523, Filed on 31 May 2011
- US Patent App No. 13/127913, Filed on 05 May 2011 **Granted: US-8686028 dated 01-04-2014**
- PCT Patent App No. PCT/IN2009/000285, Filed on 14 May 2009

Lead Compound S002-333 (Anti-platelet)

Unique Features of the Molecule

- ❖ Unique collagen mediated platelet activation Inhibitor
- ❖ Inhibits collagen induced platelet activation
- ❖ Novel mechanism of action as a specific inhibitor of collagen induced platelet aggregation and adhesion
- ❖ Good pharmacokinetic properties and bioavailability

Executive Summary

Platelet collagen interaction through its receptors (glycoprotein VI [GPVI] and α2β1 or GIa/IIa) is crucial for the initiation of intravascular thrombosis. Recent data suggest collagen receptors as promising target for a novel anti-platelet therapy. In the present study, a novel synthetic compound S002-333, conferred significant and better protection in mice model of collagen-epinephrine induced thrombosis with comparable prolongation in bleeding tendency in mice to that of standard drug Aspirin. Compound S002-333 specifically inhibited platelet aggregation induced by collagen, but not by other agents including ADP, arachidonic acid and TRAP. Furthermore, the compound also inhibited platelet aggregation induced by collagen-related peptide and convulxin, the specific agonists of the major collagen signaling receptor GP VI. Moreover, the compound exhibited a specific inhibitory activity against GP VI mediated platelet adhesion over collagen in a concentration dependent manner in both *in vitro (*human) and *ex vivo* (mice) assays. It also inhibited collagen induced dense granule secretion, thromboxane A_2 generation, intra-platelet calcium mobilization as well as tyrosine phosphorylation of various proteins. S002-333 significantly prolonged the time of occlusion in mouse carotid artery following endothelial injury by ferric chloride. Therefore these results suggest that the compound S002-333 seems to target GPVI receptor and inhibit platelet activation by collagen. S002-333 is a safe and well-tolerated molecule that prevents platelet adhesion and consecutive thrombus formation at the site of vascular injury. The strategy is to block the very early source of acute vascular complications, thereby excluding the disadvantages of existing anti-platelet drugs that primarily rely upon targeting the consequences of platelet activation.

Highlights:

The compound S002-333 inhibits collagen induced platelet activation:

- It has a novel mechanism of action as a specific inhibitor of collagen induced platelet aggregation and adhesion possibly mediated by the inhibition of platelet collagen receptors
- It has no effect on the coagulation cascade (TT, PT, aPTT) and no adverse effect on vasoreactivity.
- It is orally active, and offered significant protection against collagen and epinephrine induced thrombosis, and prevented thrombus formation in AV-shunt and ferric chloride induced thrombosis.
- it has moderate effect on mice tail bleeding time with better efficacy, in contrast to existing anti-platelet drugs Aspirin and Clopidogrel

- Preclinical safety pharmacological studies have been completed, exhibiting no adverse effect on cardiovascular, respiratory and neurological parameters. It has been tested *in vitro* for binding with 451 kinases, important GPCRs and hERG channel, which predict its safety.
- Good pharmacokinetic properties and bioavailability.

Intellectual Property Right Protection (IPR)/ Patent Status:

Patent filed: 2-alkyl/aryl sulphonyl-1,2,3,4-tetrahydro-9H-pyrido (3,4-b) indole-3-carboxylic acid esters /amides as antithrombotic agents

- Indian Application No. 01258DELNP2005, Dt. 31-Mar-05
- United States Application No. 11/842674, Dt. 21-Aug-07
- PCT Application No. PCT/IN04/00417, Dt. 27-Dec-04

Novel oral combination formulation (SMEDDS) for treatment of malaria

Unique Features of the Molecule:

- SMEDDS are for oral treatment of MDR *P. falciparum.*
- Oral formulation containing three anti-malarial drugs α/β-arteether, sulfadoxine and pyrimethamine
- No formulation of α,β-arteether is so far available for oral administration.
- Prepared SMEDDS have the blend of drug, oil and surfactants. These surfactants were known to increase the permeability by disturbing the cell membrane and thus enhance the absorption of poorly soluble drugs.

Intellectual Property Right Protection (IPR)/ Patent Status:

Patent Filed: Novel combination kit for treatment of malaria. Tripathi Renu, Mishra Prabhat Ranjan, Dwivedi Pankaj, Dwivedi Hemlata, Singh Sunil Kumar, Puri Sunil Kumar, Dwivedi Anil Kumar. Indian Patent Application No: 1983/DEL/2014.

Dry powder inhalation of particles containing anti-tuberculosis drugs

[Game Changing Technology, with strong indications of being able to reduce the duration of treatment of drug-sensitive TB to one month from the current requirement of six months]

Unique Features of the Product

- Completely eradicates tuberculosis (TB)-causing bacteria from the lungs of experimentally-infected mice and guinea pigs in four weeks (20 doses of 80-100 micrograms).
- If combined with half the recommended oral dose of the same drugs, extra-pulmonary site of infection (spleen) is also completely cleared of bacteria, with no relapse over the next four weeks if animals receive no further treatment.
- Activates macrophages infected with TB-causing bacteria to mobilize innate bactericidal responses when they pick up inhaled particles (host-directed therapy in addition to chemotherapy).
- Completely safe for chronic (90-days, repeated daily doses, 5 days/week) inhalation by Rhesus monkeys—also in acute/subacute inhalation safety/toxicity studies in mice.
- Potential for “therapeutic isolation” of TB patients who receive inhalations (since some inhaled particles will come out every time they cough), thereby blocking transmission of TB.
- Potential to reduce total drug intake for successful cure by one order of magnitude, significantly ameliorating toxic/adverse effects.
- Potential to reduce total duration of treatment, significantly reducing probability of infecting naïve individuals.

Scientific Basis of the Project

The scientific basis of the project resides in the realization that TB bacteria live and proliferate within lung macrophages, which are the very cells that have evolved to kill microbes landing on the lung surface. If inhaled particles containing anti-TB drugs are picked up by macrophages harboring TB bacteria, a very high drug concentration will develop in close vicinity of the pathogen. Simultaneously, the event of phagocytic uptake will rescue the macrophage from its state of ‘alternative activation’ imposed by the pathogen in order to survive within the phagocyte. Abundant evidence for each of these assertions has been collected over the past years [1-25].

Lead Compound S011-1793 (Antimalarial)

Unique Features of the Molecule:

- Exhibits activity against drug-resistant *P. falciparum* lines *in vitro*
- Curative against chloroquine-resistant parasite strain in mouse model
- Molecule has good solid state properties
- *In vitro* ADME parameters have indicated favourable drug-like profile with no off-target ion channel activities

Executive Summary

A novel 4-aminoquinoline derivative exhibiting curative activity against drug resistant malaria has been identified for preclinical development as a blood schizontocidal agent. The lead molecule, selected after detailed SAR studies, has good solid state properties and has promising activity against *in vitro* and *in vivo* experimental malaria models. The *in vitro* ADME parameters have indicated favourable drug-like profile with no off–target ion channel activities.

Scientific Basis of the Project

Aminoquinolines such as chloroquine had been the most important animalarials for more than four decades in view of their efficacy against all species of human malaria and their safety profile. Emergence of resistance to this class of compounds during 1980's created a genuine health crisis in the developing world. Studies on elucidation of mechanism of resistance and general trend emerging from the SAR-studies revealed that chloroquine resistance does not involve any change to the target of this class of drugs but involves compound specific efflux mechanism. Based on this premise a number of research groups have developed short chain analogues of 4-aminoquinoline, which are active against CQ-resistant strains of *P. falciparum*. However, these derivatives undergo biotransformation (de-alklylation) significantly affecting lipid solubility of the drug. In view of this background information, we introduced modification in the side chain that would prevent de-alkylation leading to improved activity. This prompted us to explore the amino acid conjugates with a view to achieve improved antimalarial activity and to the best of our knowledge such amino acid conjugates have not been hitherto reported in the literature in the case of 4-aminoquinolines. The seminal finding of our study is that a new series of compounds having significant activity against CQ resistant parasites has been identified.

Intellectual Property Right Protection (IPR)/ Patent Status:

Patent Filed: 4-Amino quinolines and process of preparation thereof Indian Application No. 2291DEL2013 Dt. 31 Jul 13

Standardized fraction of 4655 as antidyslipidemic herbal formulation

Introduction/Background: Clerodane diterpene, a natural compound isolated from leaves (a renewable resource) of *Polyalthia longifolia* (False Ashoka), is a new class of HMG-CoA reductase inhibitor.

Highlight Description: Our studies provide the first evidence that clerodane diterpene inhibits triglyceride accumulation *in-vitro* in adipocytes. Furthermore, clerodane diterpene ameliorates dyslipidemia and obesity like FDA approved drug (Orlistat) when given in mice. Taken together, clerodane diterpene is good candidate as a translational lead for metabolic disorders.

Importance/Unique Salient Feature: Clerodane diterpene, although belonging to a distinct chemical class, possess triglyceride lowering activity specifically targeting Indian population for treating dyslipidemia.

Patent Status

Title: A process for isolation of 16a-hydroxycleroda-3,13(14)Z-dien-15,16-olide from Polyalthia longifolia

Inventors: Koneni Venkata Sashidhara, Anju Puri & Jammikuntla Naga Rosaiah

Application Number: 0773DEL2008; Date: 26-Mar-08

Title: Method of treating dyslipidemia using naturally occurring diterpene

Inventors: Koneni Venkata Sashidhara, Anju Puri & Jammikuntla Naga Rosaiah

Appl No. US-12/323156 Date of Filing: 25-Nov-08 Grant No. 89215417 Grant Date: 30-Dec-14

Lead compound S007-1235 (anti-colon cancer)

Unique Features of the Molecule:

- S007-1235 inhibits proliferation and induces apoptosis in cell-lines from breast, colon or haematological malignancies.
- Inhibits proliferation and induces apoptosis in chronic myelogenous leukemia patient (CML) samples with higher efficacy than marketed drugs Imatinib and Dasatinib.
- Mechanism of action is membrane receptor mediated and thus is not likely to be influenced by drug efflux by ABC transporters.

Background & Scientific Basis of the Project

S007-1235 is a novel synthetic compound that was identified as a robust anti-cancer agent out of a series of compounds (designed based on commonality of the cytotoxic scaffold of a number of receptor or non-receptor tyrosine kinase inhibitors that are either in market as drugs or are currently undergoing clinical trials) tested for their anti-cancer activities. S007-1235 displayed robust cytotoxicity in cancer cells (cell lines corresponding to both solid tumor and haematological malignancies) while in normal cells its cytotoxic effects were obtained at a much higher dose. S007-1235 induced apoptotic cell death as evident from Annexin V and TUNEL staining. It caused cell growth arrest at the G0-G1 stage of cell cycle. S007-1235 also inhibited cell migration, invasion and angiogenesis in respective breast cancer cellular models. Further, S007-1235 induced cell death in chronic myelogenous leukemia (CML) patient samples, including in CML patient samples harbouring multi-drug resistant T315I mutation in BCR-ABL oncogene. At non-cytotoxic doses S007-1235 caused differentiation of blast cells in cell-lines of CML and acute myelogenous leukemia (AML). While it induced megakaeryocytic differentiation in a CML cell-line (K562), it induced neutrophil differentiation in an AML cell-line (HL-60). This differentiation inducing capacity of S007-1235 was also evident in samples from CML patients in an accelerated phase. Due to the lack of an appropriate leukemia model system, the *in vivo* efficacy of S007-1235 was tested in an MCF-7 xenograft mouse model, where at a daily oral dose of 16mg/Kg body weight, S007-1235 robustly reduced tumor progression and induced favorable histological changes in the tumors. These histological changes were associated with reduced number or proliferating cells and enhanced TUNEL-positive apoptotic cells in the tumors. Further, S007-1235 also robustly reduced CD133+ colon cancer and ALDH+ breast cancer stem cell population, where its efficacy was comparable to salinomycin, the positive control reagent, used widely as an anti-cancer stem cell experimental agent. Mechanistic analysis of its cytotoxic properties revealed that S007-1235 reduced EGFR and Her2 phosphorylation in breast cancer cells. It suppressed both BCR-ABL and Raf-Rac-ERK pathway in CML cells and activated p38 MAPK. S007-1235-mediated signalings were not due to direct modulation of kinases as it failed to affect the activity of none of the kinases tested in cell-free biochemical assays. S007-1235 also did not directly modulate the activities of any phosphatase. However, S007-1235 treatment was associated with an enhancement of cellular cAMP, and it's cytotoxic activity could be blocked by pertussis toxin (PTX), indicating that S007-1235 may function through one or more PTX-sensitive G-protein coupled receptors (GPCRs).

Together, these data suggest that S007-1235 is a robust anti-cancer agent that specifically causes cell-death in different cancer cells including cancer stem cells. Given that, S007-1235 also induces differentiation of blast cells from haematological malignancies, it appears that this compound may have a robust therapeutic potential in different cancers. Further development of S007-1235 as an anti-cancer agent however is warranted.

Highlights:

- S007-1235 inhibits proliferation and induces apoptosis in cell-lines from breast, colon or haematological malignancies.
- S007-1235 inhibits proliferation and induces apoptosis in chronic myelogenous leukemia patient (CML) samples with higher efficacy than marketed drugs Imatinib and Dasatinib.
- S007-1235 inhibits proliferation and induces apoptosis in mutant BCR-ABL harbouring patient samples (T315I) refractory to Imatinib or Dasatinib.
- S007-1235 reduced breast, colon and leukemia cancer stem cell population and its activity was comparable to Salinomycin, the only experimental reagent capable of inhibiting cancer stem cells.
- S007-1235 induced robust myeloid differentiation in leukemia cell-lines as well as blast cells from CML patients in accelerated phase.
- Apart from patient samples, S007-1235 also exhibited robust anti-tumor activity in an MCF-7 breast cancer xenograft mouse model.
- Detailed mechanistic studies revealed that S007-1235-mediated cell-death in routed through one or more pertussis toxin-sensitive G-protein coupled receptors.

Intellectual Property Right Protection (IPR)/ Patent Status

Patent Title: Novel Aryl Naphthyl methanone oxime derivatives for the treatment of Hematological Malignancies and solid tumors

Indian Patent App No. 2567DEL2013 Filed on 30 Aug 2013

PCT Application No. PCT/IN2014/000556 Filed on 29-Aug-14

Advantage over existing therapeutics

S007-1235 displays clear advantage over existing drugs in terms of its efficacy against drug-resistant cancers by virtue of following observations.

- **Mutations**: S007-1235 works on T315I & other BCR-ABL mutant-harboring cells that are refractive to existing therapeutics.
- **Differentiation:** Apart from inducing apoptosis, S007-1235 also induces differentiation of CML blasts.
- **Cancer stem cells**: CSCs are root cause of drug resistance and relapse of cancers. S007-1235 shows robust cytotoxicity towards CD34+, CD133+ or ALDH+ CSCs of CML, colon or breast cancer origin. Currently no drug in market is capable of efficiently killing CSCs.
- **Efflux**: S007-1235 mechanism of action is membrane receptor mediated and thus is not likely to be influenced by drug efflux by ABC transporters
- **Inability of immune system in eliminating surviving cancer cells**: S007-1235 induces p38 activation. Chance of immunomodulatory action (TH1>TH2 response)