1. INTRODUCTION

Cardiometabolic disorders (CMets) have turned into an epidemic represented by constellation of metabolic and vascular dysfunctions characterized by hypertension, insulin resistance (IR) with hyperglycemia, atherogenic dyslipidemia, intra-abdominal adiposity, hypercoagulability and pro-inflammatory stage(1). Repercussions of CMets include Atherosclerotic Cardiovascular Diseases (ASCVDs), diabetes mellitus and chronic renal failure. Statistics from the World Health Organization (WHO) reflects that only cardiovascular mortality accounts for 18.5 million death (around 32.8%) globally(2). Among them ischemic heart disease (49.2%), ischemic stroke disease (17.7%), hypertensive heart disease and circulatory disease (9.5%) contribute to be the leading causes (3). Statistics from India are also in line with these staggering numbers reflecting 1.63 million deaths in India (4). Individuals at risk of CVD may exhibited with raised blood pressure, glucose and lipids as well as overweight and obesity. Recent clinical studies have demonstrated that hypertension and metabolic syndrome play significant role in initiation and progression of cardiovascular events either alone or in combination leading to significant increase in risk of sudden cardiac death(5),(6).

Hypertension is one of the major modifiable risk factors of the cardiovascular associated events (7). Definitions of hypertension have been revised over time owing to clinical studies in diverse populations and research elaborating the etiopathology of hypertension(8). It can be generalized on the basis of recent guidelines of ACC/AHA 2020 that an individual is hypertensive if his/her blood pressure (BP) is above 130/80 mm Hg(9). The etiology of hypertension involves the complex interplay of environmental and pathophysiological factors including genetic predisposition that affect multiple systems. The regulation of BP is a very complex physiological function, which depends on a continuum of actions of cardiovascular, neural, renal and endocrine systems. Briefly, sympathetic and parasympathetic regulation constitute neural control, renin angiotensin aldosterone system (RAAS) represents renal control while local endothelium derived factors such as nitric oxide, bradykinin, substance P make up the endocrine system. These systems work in tandem to maintain the hemostasis of blood pressure (10).

Sympathetic nervous system (SNS) and RAAS are recognized as the major governing regulatory mechanism for maintenance of BP. SNS modulates wide spectrum of responses ranging from mild to massive and from acute to chronic changes in BP(11). Release of catecholamines such as epinephrine and norepinephrine upon SNS stimulation leads to

vasoconstriction in the peripheral vasculature, along with an increase in the chronotropic and inotropic action of the heart(12). The SNS is the sole system of the body capable of managing both momentary and sustained regulation of BP. On the other hand, RAAS maintains the blood pressure via directly and indirect pathways. Directly, through the vasoconstrictive action of the octapeptide angiotensin II (Ang II) released upon ACE-mediated hydrolysis of angiotensin I and indirectly, through stimulating aldosterone release leading to sodium-water retention ultimately contributing to increase in total blood volume. Moreover, there are ample evidences suggesting that these two systems do not only work independently but are connected with tight and reciprocal regulation(13). It is reported that heightened activation of sympathetic flow promotes activation of RAAS system by potentiating renin secretion and aldosterone production in renal tubule ultimately causing blood volume expansion. Simultaneously, release of renin from juxtaglomerular cells stimulates release of catecholamines from presynaptic nerve terminals. Considering molecular interactions, norepinephrine modulates Ang II receptors via interactions with α_1 adrenergic receptors (14, 15) while intracerebral injection of Ang II increase SNS controlled rise in blood pressure(16-20). Stimulation of presynaptic Ang II receptors can stimulate norepinephrine release from nerve terminals (20-22) and Ang II may amplify vasoconstrictor responses affected by the α_1 receptors.

Hypertension is also an integral part, usually associated with other metabolic abnormalities such as insulin resistance, glucose intolerance, obesity and dyslipidemia. Impact of mentioned risk factors either independently or collectively increase global burden of cardiovascular morbidity and mortality. Around 91.3% of the hypertensive patients possess least one associated metabolic or cardiovascular risk factor(23). Based on past statistics, presence of CMets increased the risk of mortality by two-fold because of coronary events while occurrence of heart attack and stroke increased mortality by three-fold in humans (24). The relationship between HTN and CMets is complex and encompasses many interactive dysfunctional regulatory systems that all contribute to increased CVDs(25). It involves not only vascular and hemodynamic changes presented with HTN, but also a myriad of complex metabolic abnormalities that collectively constitute full-fledged CMets. Activation of overwhelming RAAS activity is reported in metabolic patients depicting the role of Ang II in insulin resistance, body weight regulation and oxidative stress(26),(27). Multiple studies have supported the role of sympathetic nervous system (SNS) activation in obesity-related HTN(28),(29). Persistent hypertension also induces sympatho-toxication

which leads to diminished insulin signaling, insulin resistance and lipid abnormalities. Over activation of SNS in turn activates RAAS which advances the vicious loop of metabolic disturbances. The relationship between insulin and hypertension can be understood by cause-effect response i.e. presence of insulin resistance promotes hypertension or vice versa (30). Insulin is believed to elevate blood pressure via several mechanisms: enhanced renal sodium reabsorption, stimulation of the SNS, change in ion transport across the cell and hypertrophy of resistance vessels (31),(32). On the other hand, hypertension can cause insulin resistance by altering the delivery of insulin and glucose to skeletal muscle cells, resulting in impaired glucose uptake.

Controlling hypertension and metabolic disorder mainly involves lifestyle modifications (exercises and diet modifications) and pharmacological therapy. However, lifestyle medications prove inefficient for the control and progression of disease and combination therapy is mainly prescribed to regulate these conditions. Usually, two or more pills are prescribed for the control of hypertension (33),(34) and if the metabolic anomalies are present with HTN, it further increases the pill burden, renders poor adherence, adverse effects and unpredictable PK-PD relationships.

Recently, it is extensively appreciated that deliberately designed multitargeted ligands (DML) can provide superior therapeutic alternative for the treatment of multifactorial diseases such as cancer, neurological disorders and multiple cardiovascular complications. Despite the number of therapeutic alternatives available for the management of cardiovascular diseases, a considerable number of patients suffer from uncontrolled blood pressure. This bridge can be filled by developing efficacious drug molecules or procedures which significantly increases the patient compliance and improves the therapeutic outcome.The deliberately designed multifunctional ligands certainly can provide superior therapeutic efficacy, predictable pharmacokinetic properties, lesser off target effects and ultimately enhanced patient compliance. Different complimentary systems have been identified and explored for development of multitargeted ligands such as angiotensin-converting enzyme / neutral endopeptidase inhibitors(35), neutral endopeptidase / endothelin-converting enzyme inhibitors, angiotensin-converting enzyme/neutral endopeptidase/endothelin-converting enzyme inhibitors(36), dual angiotensin/endothelin receptor(37),(38).

In the quest of developing DMLs for multifactorial complex diseases such as hypertension and cardiometabolic disorders, there are certain primary requirements that have to be taken

into consideration such as selection of targets, identification of lead molecules, rational design of small molecules, with balance modulation of desirable targets and eliminating off target effects(39). Regarding the first issue at hand, selection of appropriate target is the primary concern which demands thorough understanding of the pathomechanism of disease (40). Moreover, selection of target relies on the fact that simultaneous modulation of two targets will provide either synergistic or additive effect. Selection of targets from same signaling pathway offer additive effect while synergistic effect is obtained when both targets are placed on different pathway complementing the function of each other (41). Secondly, design of DMLs involves integration of pharmacophores with suitable linkers. These involve size, druggability and solubility of molecules under investigation(42).

This is one of the few studies utilizing interlink between two major governing systems i.e. RAAS and SNS and their involvement on hypertension and metabolic abnormalities. In line with this notion, the novel dual receptor antagonists were synthesized by Medicinal Chemistry Laboratory, Faculty of Pharmacy, The Maharaja Sayajirao University of Baroda. Subsequently, screening of series of new chemical entities for potential dual-antagonist activity on the AT_1 and α_1 receptors was evaluated. These compounds belong to a series of dimethoxyquinazolines with different substitutions using framework combination involving prazosin and losartan as parent molecules and were assumed to show a balanced modulation of both the receptors in question.

Newly synthesized compounds were pharmacologically evaluated by *in-vitro* screening on aorta and were further evaluated for their toxicological and pharmacokinetic profile. Compounds exhibited potent and balance modulations at the targets and were further explored for their potential in *in-vivo* animal models of hypertension. Potent compounds were evaluated for in vivo potential by determining their efficacy in Unilateral nephrectomy and DOCA salt induced hypertension as it involves the cardinal features of cardiorenal hypertension with profound involvement of SNS and RAAS response. Selected compounds were studied for potential in chronic blockade offered by L-NAME treatment. This model was selected with a view to checking effect of multitargeting on two systems in resistant form of hypertension which marked by significant depletion of nitric oxide.

Current series of DMLs were initially designed and studied for their potential role in treatment of hypertension. However, as discussed earlier, involvement of plethora of mechanisms involved in CMets sparked an idea to explore the role of novel molecules in the management of CMets. Involvement of SNS and RAAS in the initiation and progression

of insulin resistance has been reported in literature. In order to evaluate the therapeutic potential novel compounds in CMets, they were challenged against 20% fructose induced metabolic alterations. During the course of study, compounds were further evaluated for their interactions of recognized targets of CMets such as PPAR-gamma, DPP4, SGLT 2, α-glucosidase inhibition. This hierarchal approach and detailed investigation can provide superior therapeutic alternative for the management of CMets.

The current research is also focused on another important risk factor i.e., Coagulation and Thrombosis, involved in CVDs mortality. As mentioned earlier, metabolic syndrome and hypertension are major culprit for cardiovascular disease (43),(44). While the presence of prothrombotic stage, impaired endothelial function and hyperfibronolysis as the propagator of Mets, it is highly likely to assume that MetS can increase the risk of Venous Thromboembolism occurrence(VTE) (45),(46).

Attributes of CMets are closely related for the development of arterial and venous thrombosis to varying degrees. Presence of metabolic triad, Hypertension, insulin resistance and the metabolic syndrome predisposes the patients to developing the risk of CVD by multifold (47), (48). Recently, one of largest cohort study on deep vein thrombosis have shown percent recurrence of thromboembolic events increased in a stepwise fashion with each addition of MetS component, ranging from 7% recurrence in patients without any MetS complications to 37% recurrence in those with all 4 components (49).

Increasing clinical and laboratory evidence suggests that hypertension may cause prothrombotic or hypercoagulable state. The damaged endothelium could provide the connecting link that despite exposure of the blood vessels to high pressures, the main complications of hypertension is thrombotic in nature rather than hemorrhagic(50). Some individual components of the metabolic syndrome have been associated with VTE. It primarily involves dyslipoproteinemia involving high triglyceride (TG) levels, small LDL particles and low HDL particles (51),(52). A recent large clinical study has assessed the association between cardiovascular risk factors and VTE. A total of 63,552 subjects met the inclusion criteria showed profound prevalence of MetS and VTE. Compared to healthy individual, obese patients are 2.33 time prone to VTE while diabetes increases the likelihood of VTE by 1.42-fold. HDL cholesterol and VTE are in inverse relationship (49). These clinical evidences emphasize the significant impact of hypercoagulation and its association with mortality and morbidity in patients with CMets.

Blood clotting is a complex process that has been well explained by the cascade model (2). coagulation cascade is proceeds via two arms that runs parallelly, one is the extrinsic and other is intrinsic pathway (53) – both converge at the common point where FXa is activated.The activated FXa couple FVa to make a complex that induces breakdown of prothrombin and converted to its active form, thrombin. Subsequently, thrombin cause conversion soluble fibrinogen to insoluble fibrin, the polymerized fibrin sheets and activated platelets form cluster to form stable clot at the injury site (54).

To regulate blood coagulation, various enzymes involved in blood coagulation process have gained great attention as potential target for the development of new antithrombotic agents. Numerous data on the effectiveness of approved conventional anticoagulants such as warfarin, heparin analogues and dabigataran (direct thrombin inhibitor) are utilised for the prevention and treatment of a diverse set of thromboembolic arterial and venous diseases (55). The use of warfarin and other VKAs particularly poses serious complications, despite their convenience of oral administration offered by these anticoagulants. Unfortunately, warfarin possesses number of drug and food interactions, , unpredictable pharmacokinetic (PK) and pharmacodynamic (PD) relationships and considerable intra/inter-patient variability due to different genomic response to metabolism cause variable drug response, narrow therapeutic window (56). As a direct consequence, constant monitoring is imperative and regular dose adjustment should be made. This unpredictable profile of warfarin often cause subtherapeutic effect and higher risk of thromboembolism or excessive anticoagulant action leads to fatal bleeding risk (57).

Another line of therapy includes, fondaparinux (as indirect FXa inhibitor), UFH, LMWHs, hirudin derivatives (as direct thrombin inhibitors) for short term therapy(58). These medications require intravenous administration that rises the concern regarding their uses without a medical professional and frequently produce injection site induced hematomas. Heparin analogues like UFH and LMWHs bear the potential risk of thrombocytopenia which could be life threatening and as they are isolated from animal tissues, serious immunogenic reactions are often observed. In addition, with that uncontrolled bleeding, unpredictable PK profile and requirement of therapeutic monitoring are major problems(59). These pitfalls associated with parenteral anticoagulants pose concerns regarding their use and safety in clinical setting. Dabigatran, orally acting inhibitor of thrombin, has been linked with instances of uncontrolled bleeding and cause life threating situations(60). These obstacles linked with clinical use existing drugs prompt researchers

to develop new orally bioavailable antithrombotic drugs with enhanced efficacy and better safety profile.

Different strategies were employed for the selective inhibition of specific enzymes within thecoagulation cascade. Factor Xa (FXa) and factor IIa (thrombin) belong to the family of serine proteases, have emerged as promising targets. Amongst them, FXa is placed at a very critical juncture of blood hemostasis cascade and acts as a rate limiting step in clot formation. Direct inhibition of FXa has appeared as the most effective strategy to achieve anticoagulation with minimal bleeding risks by maintaining the normal hemostasis (61). The upstream position of FXa and limited function outside the coagulation cascade, direct inhibition of FXa is more prudent strategy compared to direct inhibition of thrombin. Generation of one molecule of FXa can lead to the activation of hundreds of thrombin molecules and thus inhibition Fxa could provide amplifies response to prevent hypercoagulation They inhibit both free factor Xa in solution and within a clot, and have no direct effect on platelet aggregation (62). Selective blockade of FXa is associated with less bleeding risks because absence of significant interactions with existing thrombin level and process of platelets aggregation. Research regarding their anti-thrombotic potential, preclinical studies have revealed that inhibition of FXa offers larger therapeutic window in comparison to direct thrombin inhibitors (63),(64). Thus, FXa inhibition has been widely appreciated strategy to develop novel antithrombotic drugs by researchers.

In line with this notion, novel compounds were screened for their FXa inhibitory potential, specificity and anticoagulant activity. Finally, compounds were studied for their toxicity and *in-vivo* antithrombotic potential by ferric chloride and arteriovenous shunt induced thrombosis in rats.