Abstract

Aim: Pharmacological screening and evaluation of novel chemical entities in cardiometabolic disorders

Material and Methods: Pharmacological screening of multitargeted quinazoline derivatives for antagonism on α_1 and AT_1 receptor by rat aortic strip preparation was carried out. Selected potent compounds **(18)** and **(24)** were challenged against acute challenge of agonists and blood pressure was determined by invasive blood pressure measurement. Toxicological evaluation was performed by MTT assay on HEK 293 cells and acute toxicity according to OECD guidelines. Compound **(18)** and **(24)** were further analysed for their drug likeliness and ADMET prediction by SWISS ADME and pkCSM tool. Further, compounds were evaluated in two models of hypertension i.e.,1) Uninephrectomized+ hypertension and 2) blockade of nitric oxide by L-NAME induced hypertension. Evaluation of compound **(18)** and **(24)** for cardiometabolic disorders was performed by docking study with PPARγ and DPP4 enzyme. Further, both the compounds were evaluated in 20% fructose induced cardiometabolic disorders. Evaluation of *in-vivo* study was done by battery of parameters such as blood pressure, vascular reactivity, endothelial dysfunction, biochemical and inflammatory markers and histological analysis.

For the screening of novel factor Xa inhibitors, compounds were screened by enzyme inhibition assay and IC_{50} values were determined. Novel compounds were also studied for *ex-vivo* profile by prothrombin (PT) and activated partial thromboplastin time (aPTT). Most potent compounds **(14)** and **(50)** were studied for their interaction with FXa enzyme via docking study. Potent compounds were also scrutinised for their target specificity over thrombin by enzyme inhibition assay. Compound **(14)** and **(50)** were further analysed for their drug likeliness and ADMET prediction by SWISS ADME and pkCSM tool. These compounds were also studied for toxicological concern by MTT assay and acute toxicity studies. Both the novel selective inhibitors were studied for their *in-vivo* anti-thrombotic potential in $FeCl_3$ and AV shunt induced thrombosis. Possible side effect of Compound **(14)** and **(50)** was assessed by tail bleeding model.

Result: Library of 90 novel multitargeted compounds bearing different substitutions on dimethoxyquinazoline scaffold were screened for pA_2 value determination. It was found that compound **(18)** [pA_2 for $\boldsymbol{\alpha_1}$**=9.47±0.06,** $\boldsymbol{AT_1}$**= 8.54±0.07**]and **(24)**[pA_2 for $\boldsymbol{\alpha_1}$**=8.34±0.14,** $\boldsymbol{AT_1}$**= 8.73 ±0.10**]possess potent and balance inhibition at α_1 and AT_1 receptors.Moreover,

docking interactions supplemented the results obtained in tissue studies exhibiting potential binding with receptors. Further, both the compounds showed excellent druggability and ADMET profile. *In-vivo* evaluation of compound **(18)** and **(24)** exhibited significant amelioration of hypertension induced by DOCA salt and L- NAME. Among two compounds, compound (24) was found to be better in amelioration of cardiorenal hypertension induced by unilateral nephrectomy and DOCA salt treatment. While similar degree of benefits was offered by both compound (18) and (24) in L-NAME induced hypertension. Both the doses of compounds 5mg/kg and 10mg/kg offered protection against detrimental changes however novel compounds offered dose dependent action. Cardiometabolic abnormalities inflicted by fructose consumption are characterised by hyperglycemia with insulin resistance, elevated TG level and reduced HDL level with endothelial dysfunction were efficiently managed by multitargeted therapy. Both investigational compound were found to possess positive effect target of CMets i.e . PPARγ receptor and DPP4 enzyme. This additional effect with its inherent properties of dual antagonism on α_1 and AT_1 receptor provides superior effects compared to combination of standard drugs by losartan and terazosin.

For investigation of potential FXa inhibitors, screening of 78 novel compounds yielded compound **(14)** (IC_{50} = **0.7 ± 0.2**) and **(50)** (IC_{50} = **0.22 ± 0.08**) with potent factor Xa inhibition. Compounds showed excellent selectivity for FXa over thrombin by exhibiting IC_{50} of >80 µM for thrombin. Compound **(14)** and **(50)** were found to be non-toxic according to OECD and ADMET prediction observations. Finally, both compounds revealed significant prevention of thrombus formation at 15 mg/kg and 30 mg/kg with equal potency for thrombus reduction and lesser bleeding risk in *in-vivo* models which was comparable with standard drug.

Conclusion: Current investigations provide insights regarding efficacy of novel therapeutics in cardiometabolic disorders. This discovery can provide platform for identification of potential leads and therapeutic alternatives which can efficiently target the multifactorial disease like cardiometabolic disorders.

Keywords: Cardiometabolic disorders, multifactorial disease, Deoxycorticosterone acetate (DOCA), L-nitroarginine methyl ester (L-NAME), FXa inhibitors.