SUMMARY
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In an effort to identify a novel therapeutic agent for hypertension, that acts as multi-targeted ligand towards the α_1 and AT_1 receptors, different series of 6,7-dimethoxyquinazolines were screened on rat aortic strip preparation using specific agonists like phenylephrine and angII for α_1 and AT_1 receptors respectively. This method allowed calculation of pA_2 values for different test compounds. One interesting speculation was raised during the screening studies that if different substituents on the quinazoline nucleus may antagonise the effects of phenylephrine and angII on rat aortic strips, it is likely that the known drugs of this category might also show these effects. To substantiate this speculation prazosin and other compounds were cross-screened for angII antagonism and the *sartans* were cross-screened for phenylephrine antagonism likewise. In a remarkable and motivating observation, it was found that only prazosin, no other agent screened, was found to antagonize the effects of angII mediated contractions on rat aortic strips. It was concluded that the potency of prazosin rests not only in its α_1 blocking activity but in the angII–antagonizing property as well.

Further, the test compounds from different series of 6,7-dimethoxyquinazolines were screened and the compound showing highest as well as balanced inhibition of agonist-mediated contractions of rat aortic strips, i.e. **MCR-1329**, was chosen for further analysis. Toxicological evaluations were performed by administration of the test compound, **MCR-1329**, through oral route as it was the intended route of administration. Acute toxicity study revealed that the compound showed no signs of toxicity at a single dose administration of 2000 mg/kg. Further to this, repeat dose oral toxicity was performed at the intended dose for *in vivo* studies. The intended dose presented deviation from the guideline, and the dose range study mentioned in the guideline suggested a pilot study at 1000 mg/kg which is 100 times the intended dose. Hence the pilot study was not performed and safety over a repeat-dosing period of 28-days was evaluated at 10 mg/kg. It was observed that animals exhibited normal behavioural patterns, eating and drinking habits were regular and no untoward observations were made in any regard. Hence, it was concluded that repeat dosing at the intended dose-level is safe in rats. Oral dose pharmacokinetics was performed assuming non-compartmental models of analysis. The dose disposition curve for **MCR-1329** was asymmetric and represented a linear time-dependent absorption pattern, reaching a peak

followed by a time-dependent elimination pattern. A minor anomaly was noticed as a shoulder in the elimination curve which was treated as an incidental observation since exclusion of that data did not affect the results of the study. *In vitro* plasma protein binding study was performed with human plasma and it was found that over a period of 24 hrs, only a small fraction of **MCR-1329** is secreted into the physiological buffer. On the basis of oral dose-disposition and the plasma-protein binding study, it was concluded that **MCR-1329** remains highly bound to plasma proteins, has a relatively faster absorption rate suggesting its absorption in the upper part of the GIT, compared with a moderate elimination rate. Peak plasma levels were obtained around 4 hrs post-dosing with an elimination half-life of 3.77 hrs.

Acute *in vivo* studies involving measurement of pressor responses to phenylephrine and angII revealed important particulars about **MCR-1329**. Initial studies showed that inhibition of pressor response by prazosin and losartan was more significant as compared to that of MCR-1329. This was intriguing since the *in vitro* potency of **MCR-1329** was significantly better than the respective standards. It was thus discerned that **MCR-1329** might distribute on both the receptors *in vivo* leading to a submissive response. This inconsistency was improved when the pressor response was recorded under masked conditions under which the pressor response to phenylephrine was recorded under losartan pre-administration to block the effects on angII receptors and responses to angII were recorded under prazosin administered in tandem. Results of this data assisted in concluding our previous findings showing that prazosin shows affinity towards angII receptors and blocks the angII mediated contractile responses. Since prazosin also antagonises both the agonists studied herewith, results produced by prazosin masking of angII pressor responses could not be justified for MCR-1329. It was thus decided to use terazosin as the masking agent for recording angII mediated contractions since terazosin was found not to have antagonistic actions on angII mediated responses. At the end of the study, it was concluded that **MCR-1329** showed a similar inhibition of pressor response as compared to prazosin and losartan both.

Efficacy of **MCR-1329** in chronic hypertension was evaluated using the DOCA-salt model of renal hypertension. DOCA-salt administration coupled with uninephrectomy of animals was utilised for the induction of hypertension in animals. Results of the study demonstrated that administration of **MCR-1329** could prevent

development of hypertension mediated through salt intake and aldosterone turnover. This conclusion was supplemented from tail-cuff data and mean arterial pressures of animals recorded at the terminal stage of the study. At the same time, it was also revealed that **MCR-1329** prevented hypertension mediated endothelial dysfunction and that this kind of dysfunction in rats is unrelated to serum uric acid levels in rats. Electrolyte imbalance and urinary markers of hypertension were also controlled by **MCR-1329** as indicated by urinary indices. Other parameters including urine output, albuminuria, glucosuria, creatininuria, natriuria and kaluria were also significantly improved in animals treated with **MCR-1329** as compared to the DOCA-salt group. Urine osmolality and creatinine clearance, surrogate indices based on preliminary electrolyte and solute levels, were also improved in **MCR-1329** treated in animals. All the findings were in close agreement with the animals from the standard group in which losartan & prazosin were co-administered so as to assess the worth of multi-targeting. It could be concluded that **MCR-1329** is safe and effective in the management of mineralocorticoid induced hypertension in rats.

Overall, the study sheds new light on the paradigm of multiply-targeted ligands for the effective management of multifactorial conditions. The era of serendipitous discoveries takes a backseat with designed multiple ligands entering the mainframe of the therapeutic armamentarium. The results of this study indicate that safe and efficacious multiply-targeted ligands can be designed to show an evenhanded modulation of the desired targets to achieve therapeutic goals. Further, the compounds presented in the present study may serve as potential examples or lead candidates which could be further explored for multiply targeting complex disorders.

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Based on the review of literature, the urea-based test compounds were required to be screened for their ACAT-inhibition potential. Since, the methods available in the literature involved mostly the use of radioactive compounds it was decided to develop a planar-chromatography based method for the quantitative determination of cholesteryl esters, which could be used for the screening of compounds having their effect on ACAT catalytic activity. An HPTLC-based ACAT assay was developed using alumina-backed silica gel 60F254 as the stationary phase, *n*-Hexane: diethyl ether: acetic acid (90: 10: 1; v/v/v) as the mobile phase and anisaldehyde-sulphuric acid as the detection reagent. This method was validated for quantification of cholesteryl esters as products of the ACAT assay and it was also found to be suitable for quantification of cholesteryl esters in plasma samples. It was validated for linearity over the selected range, accuracy, precision, sensitivity and specificity. Robustness was also studied by making minor alterations in the parameters of analysis. This method was then utilized for the screening of five series of urea-based test compounds for their potential to block ACAT-mediated catalysis. Based on the screening results, one compound **MCR-788** (or **IIIn**) was chosen for further evaluation. Toxicity studies with this compound revealed that the compound did not show any signs of distress/morbidity. Single dose and repeat-dose studies confirmed that the expected adverse effect of this category of compounds, *i.e.* cutaneous xanthomatosis, was not observed with **MCR-788** at the doses intended for the studies. The animals, dosed with **MCR-788**, neither showed deviation from their normal behaviour, food and water intake nor in locomotor and grooming functions. The test compound was assumed to be safe for evaluation in further animal models.

Phamacodynamic studies performed with the chosen test compound **MCR-788**, presented interesting observations. An acute model of triglyceride turnover, poloxamer-407 mediated LPL inhibition, was utilized to evaluate the effects of **MCR-788** on 24-hr triglyceride turnover in rats. This model has been summarily utilized to study the compound showing positive effects on lipid turnover. It was found that prevention of TG elevation showed by **MCR-788** was comparable to the standard ACAT inhibitor, avasimibe. It could be concluded that this compound may reduce the availability of cholesteryl esters to be incorporated in the chylomicrons. This effect can certainly be of

therapeutic advantage to increase the excretion of chylomicrons and reduce their recycling. This acute model presented the efficacy of **MCR-788** *in vivo* and hence it was decided to evaluate this compound in a chronic model of atherosclerosis as a proof of efficacy. A model of diet-induced atherogenesis in rats over a period of 8-weeks was utilized for this purpose. A long protocol was selected to allow development of atherogenic lesions in the test animals. An atherogenic diet was custom-prepared to meet the needs of the study and to allow reproduction of the pathogenic condition of atherosclerosis in rats. Different parameters relevant to the study like body weight, serum lipid profile, plaque formation and development of atherogenic lesions along the aortae were studied as endpoints for the study. **MCR-788** was administered at two doses, 10 and 30 mg/kg. It was observed that both the dose groups resulted in beneficial effect considering the levels of lipoproteins in serum. This beneficial effect was also dose-dependent. It was found that administration of **MCR-788** prevented elevation of total cholesterol and simultaneously also prevented a decrease in HDL-C levels. Modest prevention in the elevation of triglyceride and VLDL-C levels were also evident in both the treatment groups. Parameters derived on the basis of these lipid levels, i.e. LDL-C and atherogenic index, were consequently found to be improved in the treated groups. These surrogate parameters pointed towards the protective effects of **MCR-788** on the lipid profile however, arterial deposition of lipids was evaluated to estimate a *bonafide* protective action of **MCR-788**. This study indicated reduced deposition of intra-arterial lipids. Further to this, plaque formation in the aortic root was also evaluated since this site is known to be a critical region for the formation of atherosclerotic plaques in animal models. It was concluded that **MCR-788** not only prevents the disparaging effects of an atherogenic diet on the lipid profile but also prevents lipid deposition in the aortae and prevents plaque development.

Finally, it may be summarized that the planar chromatography-based technique is suitable for the quantification of cholesteryl esters in different biological samples. The method is sensitive, accurate, precise, cost-effective and robust for use with different biological matrices. It can be applied to screening of multiple compounds due to the relative ease with which samples may be handled on an HPTLC. Further to this, urea based inhibitors were screened and one potent compound, **MCR-788**, was identified. This compound was subjected to toxicity studies and was found to be safe for repeat-dose oral administration. This compound showed protective action against

triglyceride turnover and was found to be efficacious in an animal model of atherogenesis. The synthetic series utilized in preparing such compounds may serve as lead for the synthesis and evaluation of better ACAT-inhibitors.