Supplemental Data

Validation of pharmacophore model

In this validation process the complete strategy was followed to ensure that the acceptability of the pharmacophore model for further studies. For this purpose Hypo-3 was used to predict the external test set of 20 compounds from literature. The chemical structures and fit value generated by CFPMA using Hypo-3 are given below (**Supplemental Data** Table 1 and Figure I)(1-21). The ligand pharmacophore mapping protocol of DS 2.5 was used to map all the hit compounds with best flexible search option.

Pharmacokinetic data analysis

The pharmacokinetic parameters were determined using the extra-vascular non compartmental analysis module of WinNonlin (version 1.5, Pharsight, MountainView, CA). The area under the curve ($AUC_{0-\infty}$) was estimated using the linear trapezoidal method from 0-t last and extrapolation from t_{last} to infinity based on the observed concentration at the last time point divided by the terminal elimination rate constant (λ). For intravenous administration, Clearance (CL) and the apparent volume of distribution of the elimination phase (V_d) were calculated as Dose/$AUC_{0-\infty}$ and Dose/K_{el}.$AUC_{0-\infty}$, respectively. CL, V_d for oral data, was calculated as F.Dose/$AUC_{0-\infty}$ and F.Dose/K_{el} .$AUC_{0-\infty}$, respectively. Oral and *I.V.* (Intra venous) mean residence time (MRT) was calculated as $AUMC_{0-\infty}/AUC_{0-\infty}$. The absolute bioavailability (F) was calculated as the ratio between the $AUC_{0-\infty}$ from oral and intravenous routes, after dose normalization using the following equation:

$$(\%)\ \boldsymbol{F_{absolute}} = \frac{\mathbf{AUC_{Oral}} \times \mathbf{Dose_{I.V.}}}{\boldsymbol{AUC_{I.V.}} \times \boldsymbol{Dose_{Oral}}} \times \mathbf{100}$$

HPLC-UV chromatogram of rohitukine:

The Waters HPLC system, Milford USA consisted of a quatry pump (model 600), auto sampler (model 717) and UV detector (model 2487). The rohitukine resolution and better

peak shape were achieved on a Phenomex C_{18} column (4.6 ×250 mm, particle size 5 µm) protected with a Phenomenex C_{18} guard column. Phenacetin was used as internal standard (IS). The mobile phase consisting of methanol: 10 mM sodium acetate buffer pH 5.5 (38:62, v/v) with flow rate of 1.0 mL/min was found to be suitable during LC optimization. Under these optimum conditions, Rohitukine and I.S. were free of interference from endogenous substances and the observed retention times were 7.79 min for RH and 17.8 min for IS. The retention times of the rohitukine and IS showed less variability with a relative standard deviation (R.S.D.) well within the acceptable limit of 5%. There were no interfering peaks within the elution times for either reference standard and tested samples (**Supplemental Data Figure** III). The method was validated as per FDA guideline.

Effect of rohitukine administration on high fat diet induced dyslipidemic Syrian golden hamster model

This was initial experiment performed before the experiment explained in the manuscript. All animal handling and experimental procedure were same as provided in manuscript. The animals with identification marks were acclimatized for seven days before experiment. The control group was fed on normal chow diet. The dyslipidemic groups were HFD Fed with vehicle treatment, HFD fed with 30mg/Kg body weight rohitukine treatment, HFD fed with 100 mg/Kg body weight rohitukine treatment were fed with 45% kcal HDF diet from day 1-day10. Rohitukine was suspended in the 0.1% gum acacia solution and gavaged orally once daily at fixed time for seven consecutive days (day 4-10) to treated group and vehicle to the parallel control. All other experimental conditions were similar as mentioned in methods section of manuscript. Blood collection and serum separation procedures were followed and lipid parameters were measured as mentioned earlier.

Results of *in vivo* experiments

Rohitukine at 30 mg/kg body weight dose did not had any significant effect in case of total triglycerides, total cholesterol, body weight changes and food consumption. Rohitukine at this dose increased HDLc significantly while decreased LDLc significantly. Rohitukine

treatment reduced diet consumption at higher dose (100 mg/Kg body weight) over the treatment duration. Food intake was significantly reduced from day 7 onwards. This also was reflected in reduced body weight in this group. The lipid parameters in serum were significantly reduced in this group including total triglycerides, total cholesterol, LDL-cholesterol and HDL cholesterol. The ratio of HDLc/LDLc and HDLc/TC was found to be increased significantly (**Supplemental Data** Figure IV). As food intake and body weight reduced significantly with increasing dose of rohitukine, it was necessary perform pair fed experiment which is presented in main manuscript .

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Table figure captions/legends:

Supplemental Data Table 1. The fit values of the external test set used to validate the pharmacophore model.

Supplemental Data Figure I. Chemical structures of external test set molecules a used to validate the common feature (Hiphop) pharmacophore model.

Supplemental Data Figure II. Pharmacophore mapping of the flavoperidol and P-00-267 on Hypo-3 Fit value 2.791 and 2.969 respectively.

Supplemental Data Figure III: Representative HPLC-UV overlay chromatograms of blank analytical standard and analytical standard of rohitukine and internal standard.

Supplemental Data Figure IV: Effect of Rohitukine administration on ND,HFD fed dyslipidemic Syrian golden hamsters (A) Absolute amount of diet consumption per group (B) Body Weight Gain (C) Total cholesterol (D) Triglyceride (E) LDL-Cholesterol (F) HDL-Cholesterol (G) HDL-c/TC (H) HDL-c/LDL-c .