Discovery of Small Molecules Targeting the Synergy of Cardiac Transcription Factors GATA4 and NKX2-5

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Background: Transcription factors are fundamental regulators of gene transcription, and many diseases, such as heart diseases, are associated with deregulation of transcriptional networks. In the heart, the transcription factors GATA4 and NKX2-5 are required for cardiogenesis. GATA4 and NKX2-5 interact physically, and the activation of GATA4, in cooperation with NKX2-5, is essential for stretch-induced cardiomyocyte hypertrophy. In the adult heart, GATA4 is a critical regulator of cardiac repair and remodelling. Therefore, the functional modulation of the GATA4 protein or the GATA4-NKX2-5 interaction could represent an innovative therapeutic approach for cardiac regeneration or other pathophysiological cardiac conditions. Here we report the identification and biological characterization of small molecules that either inhibit or enhance the GATA4-NKX2-5 transcriptional synergy.

Methods: A fragment-based screening, reporter gene assay and pharmacophore search were utilized for the small molecule screening, identification and optimization. The effect of compounds on natriuretic peptide gene expression was examined by employing an in vitro mechanical stretch model of cultured neonatal rat cardiomyocytes and inducing cardiomyocyte hypertrophy with endothelin-1 and phenylephrine. The cardiac actions of the most potent hit were also investigated in experimental models of myocardial ischemic injury and pressure overload.

Results: We identified four small molecule families that produce either an agonistic or antagonist effect on the GATA4-NKX2-5 transcriptional synergy. The most potent inhibitor of GATA4-NKX2-5 interaction, N-[4-(diethylamino)phenyl]-5-methyl-3-phenylisoxazole-4-carboxamide (3i-1000, IC50 3 microM), exhibited no activity on the protein kinases involved in the regulation of GATA4 phosphorylation. The compound inhibited stretch-, endothelin-, and phenylephrine-induced hypertrophic response in neonatal rat cardiomyocytes. In mice after myocardial infarction, 3i-1000 significantly improved left ventricular ejection fraction and fractional shortening, and attenuated myocardial structural changes. The compound also improved cardiac function in an experimental model of angiotensin II-mediated hypertension in rats. Furthermore, the up-regulation of cardiac gene expression induced by myocardial infarction and ischemia reduced with treatment of 3i-1000 or when micro- and nanoparticles loaded with 3i-1000 were injected intramyocardially or intravenously, respectively.

Conclusions: These results indicate significant potential for small molecules targeting GATA4-NKX2-5 interaction to promote myocardial repair after myocardial infarction and other cardiac injuries.