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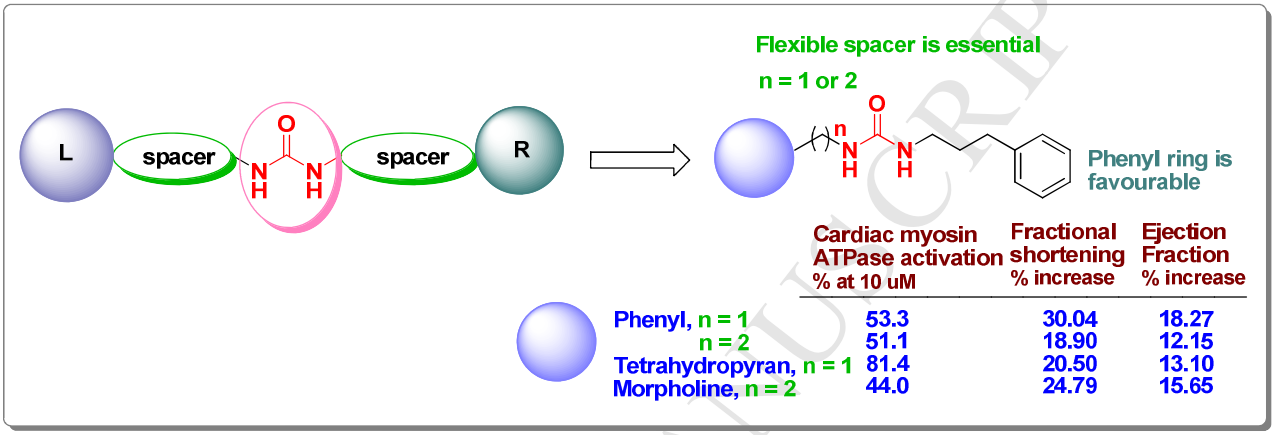
GRAPHICAL ABSTRACT



Exploration of flexible phenylpropylurea scaffold as novel cardiac myosin activators for the treatment of systolic heart failure

Manoj Manickam[a], Hitesh B. Jalani[a], Thanigaimalai Pillaiyar[a], Niti Sharma[a], PullaReddy Boggu[a], Eeda Venkateswararao[a], You-Jung Lee[b], Eun-Seok Jeon[b], Sang-Hun Jung[a]*

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Abstract

A series of flexible urea derivatives have been synthesized and demonstrated as selective cardiac myosin ATPase activator. Among them 1-phenethyl-3-(3-phenylpropyl)urea (**1**, cardiac myosin ATPase activation at 10 μM = 51.1 %; FS = 18.90; EF = 12.15) and 1-benzyl-3-(3-phenylpropyl)urea (**9**, cardiac myosin ATPase activation = 53.3 %; FS = 30.04; EF = 18.27) showed significant activity *in vitro* and *in vivo*. The change of phenyl ring with tetrahydropyran-4-yl moiety *viz*., 1-(3-phenylpropyl)-3-((tetrahydro-2*H*-pyran-4-yl)methyl)urea (**14**, cardiac myosin ATPase activation = 81.4 %; FS = 20.50; EF = 13.10)**,** and morpholine moiety *viz*., 1-(2-morpholinoethyl)-3-(3-phenylpropyl)urea (**21**, cardiac myosin ATPase activation = 44.0 %; FS = 24.79; EF = 15.65), proved to be efficient to activate the cardiac myosin. The potent compounds **1**, **9**, **14** and **21** were found to be selective for cardiac myosin over skeletal and smooth myosins. Thus, these urea derivatives are potent scaffold to develop as a newer cardiac myosin activator for the treatment of systolic heart failure.

Key words: Flexible urea, cardiac myosin activator, inotrope, systolic heart failure.

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1. Introduction

Heart failure is one of the major health and economic burdens worldwide, and its prevalence is continuously increasing. The Global Burden of Disease 2010 study reported that this disease had been the most common cause of death worldwide from 1990 to 2010 [1-3]. Heart failure was responsible for 8.1 million deaths in 2013, which was 15 % of deaths worldwide [4]. Acutely decompensated chronic heart failure concerns about 70 % of patients with acute heart failure syndromes [5], and at least half the patients with heart failure have a low ejection fraction (40 % or less) [6], thus leading to systolic heart failure [7]. Systolic dysfunction is characterized by a decrease in myocardial contractility resulting in a reduction in the left ventricular ejection fraction [8]. There are no safe medical therapies to improve cardiac function of heart failure patients with reduced ejection fraction [9]. Most current therapies are focused on blockade of neurohormonal activation using inhibitors of the renin–angiotensin pathway, β-adrenergic blockers, and aldosterone antagonists and do not improve the contractile function of the heart [10,11]. Currently available inotropes such as adrenergic receptor agonists (*i.e.*, dobutamine), phosphodiesterase inhibitors (*i.e.*, milrinone), increase cardiac contractility at the expense of increased intracellular concentrations of calcium and cAMP, contributing to increased heart rate, hypotension, arrhythmias,and mortality [12,13].

On the other hand, calcium-sensitizing inotropic agents such as levosimendan have been developed to increase the calcium sensitivity of myofibrils without increasing calcium transients for treating systolic heart failure [14]. However, it was found that levosimendan also has

significant phosphodiesterase inhibition activity, this mixed mechanism may be the reason for levosimendan failure in improvement on mortality [15].

To address these limitations, new agents targeting novel mechanisms are being developed: (i) istaroxime, a non-glycoside inhibitor of the sodium-potassium-ATPase with additional stimulatory effects on the sarcoplasmic reticulum calcium pump (SERCA), has shown lusitropic and inotropic properties in experimental and early clinical studies [16]; (ii) gene therapy approaches have also been effectively employed to increase myocardial SERCA2a [17]; (iii) nitroxyl donors have shown positive lusitropic and inotropic as well as potent vasodilatory effects [18]; (iv) the ryanodine receptor stabilizers reduce pathological leak of calcium from the sarcoplasmic reticulum with initial promising pre-clinical results [19] and (v) finally the most attractive class comprises of the cardiac myosin activators, which directly work at the level of the cardiac sarcomere activating the actin-myosin cross-bridges, the smallest force-producing unit involved in the contraction mechanism [20].

The selective cardiac myosin activator, omecamtiv mecarbil, which is currently completing late phase II trials on the verge of phase III trials [21], binds to the myosin catalytic domain and increases the transition rate of myosin into the strongly actin-bound state that generates force, while inhibiting ATP turnover in the absence of actin. This mechanism results in more myosin heads generating force on actin with each beat [22-24]. Studies of healthy volunteers and of patients with chronic stable heart failure have confirmed that omecamtiv mecarbil prolongs the duration of systole, which results in increased stroke volume and fractional shortening with decreased ventricular volumes. Patients with ischemic cardiomyopathy and angina showed no adverse effects of omecamtiv mecarbil [25]. Unlike prior agents that increased intracellular cAMP and calcium and decrease ejection time, omecamtiv mecarbil's unique mechanism of

action prolongs ejection time, allowing for increased stroke volume without significantly increasing myocardial oxygen consumption [26,27].

Although omecamtiv mecarbil is currently undergoing clinical trials, a broader range of drugs that can improve cardiac function is urgently required. Taking advantage of the urea functionality in the omecamtiv mecarbil (Figure 1) and flexible chain in dobutamine (Figure 1), we have rationally designed the target molecule to be a flexible urea derivative as selective cardiac myosin activators for the treatment of systolic heart failure (Figure. 1). Indeed the presence of molecular flexibility or flexible spacers in molecules has a definite role in the field of drug discovery [28-30].

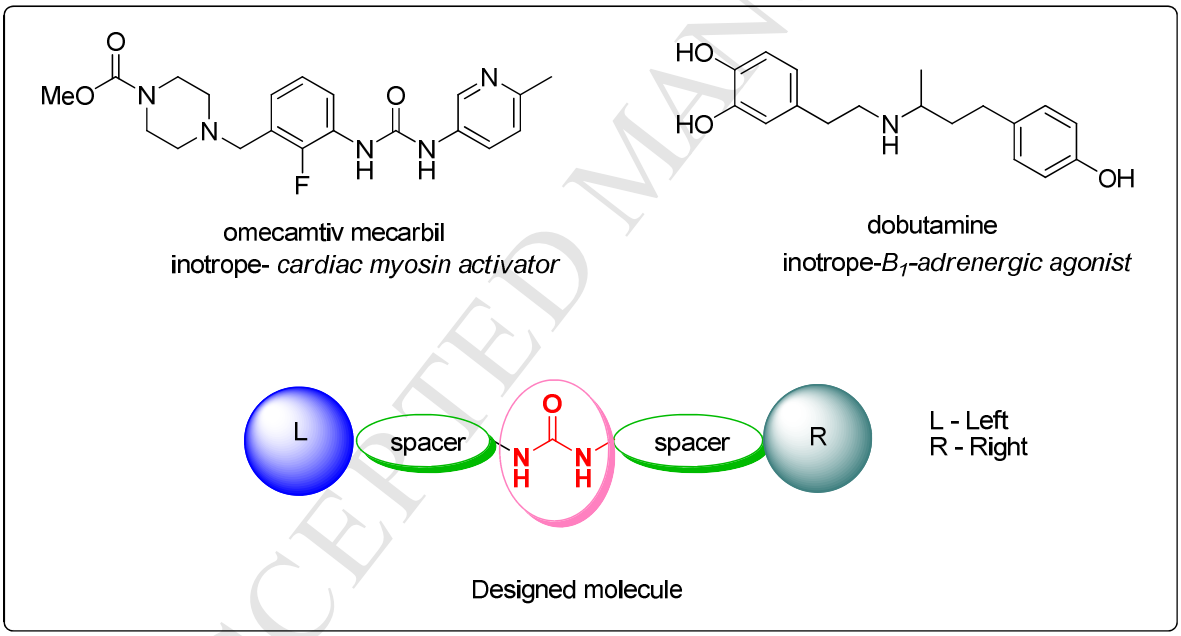
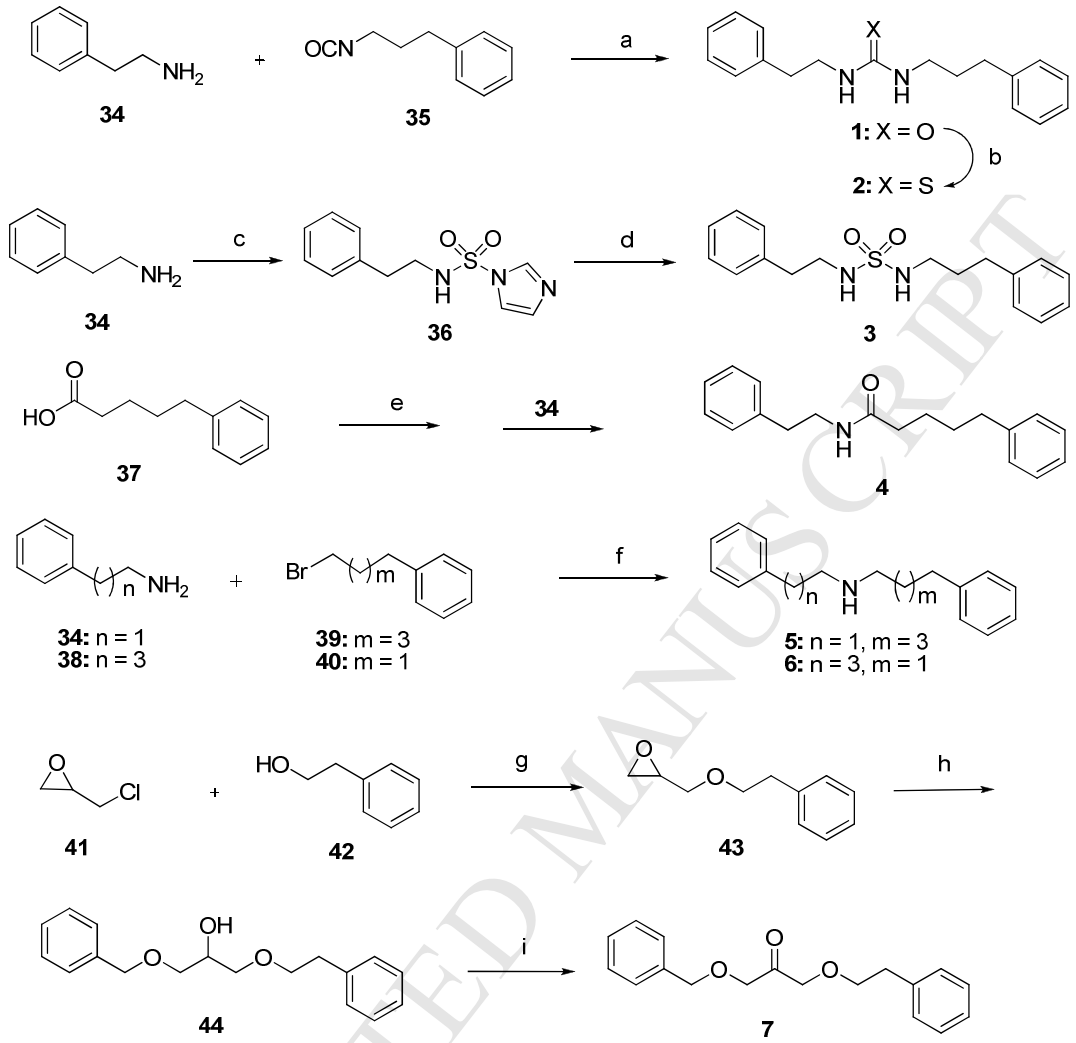


Fig. 1. Background for the design of flexible urea with spacer as novel cardiac myosin activators

2. Chemistry

Scheme 1 represents the synthesis of compounds denoted in Table 1. The typical urea **1** was prepared by the reaction of phenethylamine (**34**) and 3-phenylpropylisocyanate (**35**). Urea **1** was

subsequently converted to its thiourea analog **2** by refluxing with Lawesson's reagent in toluene. The sulfonyldiamide **3** was synthesized from the reaction of the **34** and 1,1-sulfonyldiimidazole to afford the intermediate **36** followed by the substitution with 3-phenylpropylamine. The preparation of amide **4** was achieved by transformation of 5-phenylvaleric acid (**37**) to its acid chloride and then reacting with **34**. The amine derivative **5** and **6** were respectively derived from the substitution reaction of phenylalkylamines **34**, **38** and phenylalkylbromides **39**, **40** using KI as catalyst. In order to prepare the ketone **7**, the epoxide intermediate **43** was prepared from the reaction of epichlorohydrin (**41**) and 2-phenylethanol (**42**) using NaH as base**.** Epoxide ring opening reaction of **43** with benzyl alcohol and KOH yielded the intermediate **44.** The secondary alcohol **44** was then oxidized to the corresponding ketone **7** by Dess-Martin periodinane.

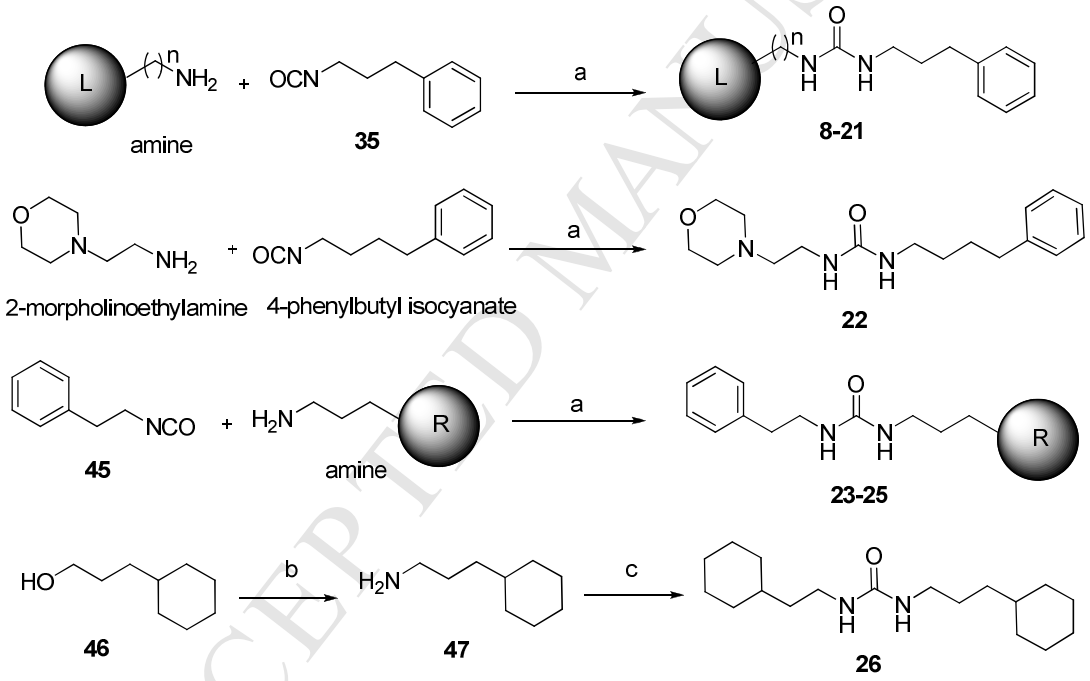


Scheme 1. Synthesis of **1-7** denoted in Table 1**.**

Reagents and conditions: (a) acetonitrile, RT, 8 h; (b) Lawesson's reagent, toluene, reflux, 5 h; (c) 1,1'-sulfonyldiimidazole, TEA, THF, 60 °C, 2 h; (d) 3-phenylpropylamine, TEA, THF, 60 °C, 3 h; (e) (i) $SOCl_2$, CH_2Cl_2, reflux 3 h, (ii) TEA, CH_2Cl_2, 3 h, RT (f) K_2CO_3, KI, DMF, 60 °C, 8 h; (g) NaH, THF, reflux, 15 h; (h) benzylalcohol, KOH, neat condition, 60 °C 3 h; (i) Dess-Martin periodinane, CH_2Cl_2, RT, 1 h.

The reaction of various amine ('L' group and 'n' are in Table 2) with 3-phenylpropylisocyanate (**35**) afforded the corresponding urea derivatives **8-21** (Scheme 2). The preparation of compound

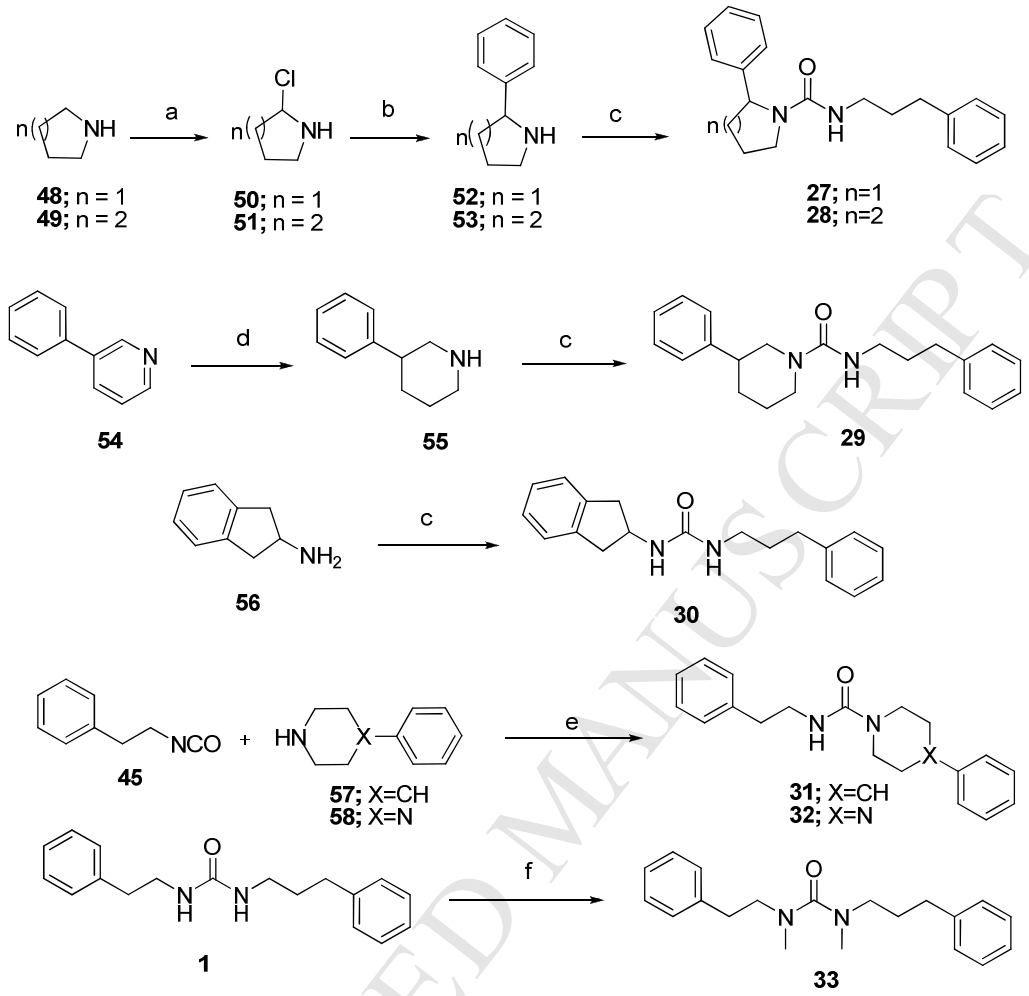
9 was previously reported by our group [31]. To prepare the urea **22**, 2-morpholinoethylamine was reacted with 4-phenylbutyl isocyanate. Similarly the reaction of phenethylisocyanate (**45**) and amine ('R' group is in Table 2) yielded the urea derivatives **23-25**. Urea **26** was prepared by the reaction of 2-cyclohexylethanamine with triphosgene in the presence of *N,N*-diisopropylethylamine for 0.5 h stirring at 0 °C followed by the addition of 3-cyclohexylpropan-1-amine (**47**). The synthesis of 3-cyclohexylpropan-1-amine (**47**) was in turn achieved from 3-cyclohexylpropan-1-ol (**46**) by bromination reaction with HBr and subsequent treatment with phthalimide followed by reaction with hydrazine hydrate.



Scheme 2. Synthesis of **8-26** denoted in Table 2**.**

Reagents and conditions: (a) Acetonitrile, RT, 8 h, (b) (i) HBr, reflux, 5 h (ii) phthalimide, THF, reflux, 2 h (iii) $NH_2NH_2.H_2O$, ethanol, 60 °C, 30 min, (c) 2-cyclohexylethanamine, triphosgene, DIPEA, THF, 0 °C to RT, 8 h. *The 'L, R' group of the amine and 'n,m' are denoted in Table 2.*

To analyze the effect of rigidification of the flexible spacer of **1**, compound **27-32** were prepared as denoted in Scheme 3. Chlorination of pyrrolidine **48**/piperidine **49** with *N*-chlorosuccinimide in diethyl ether afforded **50/51** which was reacted with phenyl lithium at 35 °C in diethyl ether to obtain 2-phenylpyrrolidine (**52**)/2-phenylpiperidine (**53**). The reaction of **52** and **53** with 3-phenylpropylisocyanate (**35**) in acetonitrile afforded the respective ureas **27** and **28**. The urea **29** was synthesized from the reaction of **35** with 3-phenylpiperidine (**55**), which was in turn derived from the Pt/C assisted hydrogenation of 3-phenylpyridine (**54**) in presence of acetic acid at 55 psi. Reaction of 2,3-dihydro-1*H*-inden-2-amine with **35** gave the urea **30**. Similarly the reaction of phenethylisocyanate (**45**) with 4-phenylpiperidine (**57**)/4-phenylpiperazine (**58**) yielded the respective ureas **31** and **32**. To mask the hydrogen bonding donor (NH) group of the urea **1**, it was methylated using methyliodide in presence of sodium hydride as base to give the urea **33**.



Scheme 3. Synthesis of **27-33** denoted in Table 2**.**

Reagents and conditions: (a) *N*-chlorosuccinimide, diethyl ether, RT, 1 h; (b) PhLi diethyl ether, 35°C, 1 h (c) **35**, acetonitrile, RT, 8 h; (d) $Pt/C/H_2$, AcOH, 55psi RT, 20 h; (e) acetonitrile, RT, 8 h, (f) CH_3I, NaH, DMF, RT, 5 h.

3. Pharmacology

In the sarcomere, force generation is directly coupled to ATP hydrolysis by myosin ATPase. Actin stimulated ATPase activity was assayed spectrophotometrically using sarcomere assay [32] with modifications. Compounds that activate the sarcomere were identified by measuring %

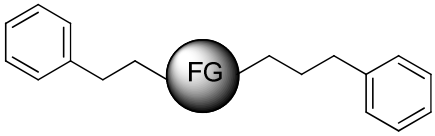
increase in myosin ATPase activity at 10 μM. The results are shown in Table 1 and 2. Compound specificity with respect to muscle type was evaluated by comparing the effect of the compound on actin stimulated ATPase activity of a panel of myosin isoforms including cardiac (bovine (10 μM)) [32], skeletal (rabbit (100 μM)) [33,34,35] and smooth muscle (chicken gizzard (100 μM)) [33,36,37] at a single dose of the compound. Omecamtiv mecarbil was used as positive control for cardiac myosin ATPase activity and (-)-blebbistatin was used as a negative control for measurement of skeletal or smooth muscle myosin ATPase activity [38]. The results are shown in Table 3. Positive inotropic effect was tested by measuring % increase of left ventricle fractional shortening (FS) and ejection fraction (EF) with/without samples using echocardiography [39] in seven-week-old Sprague-Dawley male rats. The results are shown in Table 2.

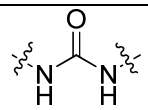
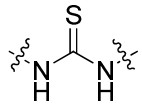
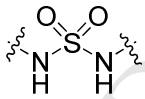
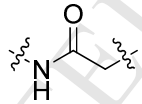
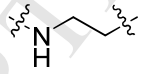
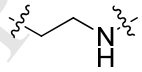
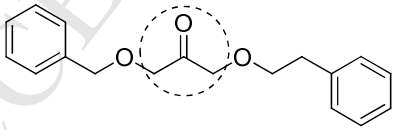
4. Results and Discussion

The importance of the urea functionality is demonstrated from the comparison between similar scaffolds as shown in Table 1. Initially the compound **1** holding a urea functional group showed comparable cardiac myosin ATPase activation (51.1 % at 10 μM) to omecamtiv mecarbil (OM, 58.0 % at 10 μM) and proved potent when compared to its thiourea **2** (cardiac myosin ATPase activation = 10.3 %) and sulfonyldiamide **3** (cardiac myosin ATPase activation = 32.1 %). This demonstrates the importance of enhanced hydrogen bonding acceptor (HBA) property of the carbonyl functional group. Proving once again the same above statement, amide **4** retained the activity (cardiac myosin ATPase activation = 39.1 %), while removing the carbonyl function as shown in the amines **5** and **6** completely abolished the activity (cardiac myosin ATPase activation = 1.6 % and 11.9 % respectively). The importance of C=O functionality in the NH-

C=O linkage was established by evaluating the ketone **7**. Though it retained the activity (cardiac myosin ATPase activation = 42.9 %), its unstability [40] made it unsuitable to develop further as a drug scaffold.

Table 1. Cardiac myosin activation of urea scaffold and its isosteric replacements

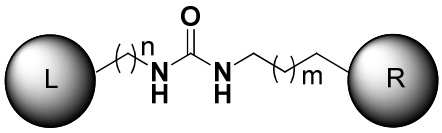


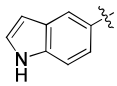
Comp. No	Functional Group (FG)	ATPase activity % at 10 μM	CLogP
1		51.1	4.025
2		10.3	4.305
3		32.1	4.430
4		39.1	4.024
5		1.6	5.113
6		11.9	4.963
7		42.9	3.263
Omecamtiv mecarbil	-	58.0	3.266

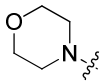
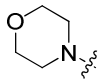
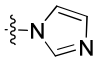
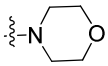
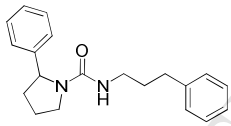
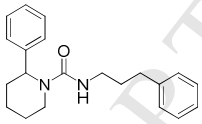
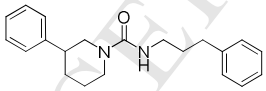
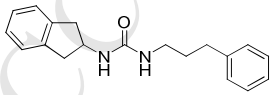
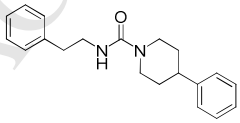
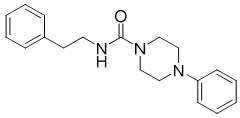
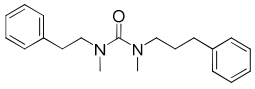
The present work focuses the establishment of urea functionality as selective cardiac myosin activator and therefore we synthesized the compounds listed in Table 2 by varying the spacer and

groups on both sides. Moreover, the importance of the flexible spacer and HBD of urea NH were also established.

Table 2. Optimization of spacer and groups in the urea scaffold



Comp. No	L	R	n	m	ATPase activity % at 10 µM	Fractional Shoretning % increase	Ejection Fraction % increase	CLogP
1	Phenyl	Phenyl	2	1	51.1	18.90	12.15	4.025
8	Phenyl	Phenyl	0	1	46.2	---	---	3.577
9	Phenyl	Phenyl	1	1	53.3	30.04	18.27	3.696
10	Phenyl	Phenyl	3	1	45.5	---	---	4.404
11	Cyclohexyl	Phenyl	0	1	20.3	---	---	3.965
12	Cyclohexyl	Phenyl	1	1	60.2	Precipitated during formulation		4.578
13	Cyclohexyl	Phenyl	2	1	61.4	Precipitated during formulation		5.107
14		Phenyl	1	1	81.4	20.50	13.10	2.179
15		Phenyl	1	1	47.6	---	---	3.342
16		Phenyl	1	1	36.7	---	---	2.872
17		Phenyl	1	1	37.2	---	---	3.686

18		Phenyl	2	1	30.0	---	---	2.528
19		Phenyl	2	1	29.6	---	---	3.671
20		Phenyl	2	1	30.6	---	---	3.201
21		Phenyl	2	1	44.0	24.79	15.65	2.408
22		Phenyl	2	2	42.7	---	---	2.608
23	Phenyl	Cyclohexyl	2	1	37.9	---	---	5.257
24	Phenyl		2	1	32.6	---	---	1.862
25	Phenyl		2	1	32.9	---	---	2.208
26	Cyclohexyl	Cyclohexyl	2	1	44.4	---	---	6.339
27					32.4	---	---	4.216
28					73.0	13.32	9.26	4.775
29					35.2	---	---	4.575
30					61.9	precipitated during formulation		3.910
31					46.6	precipitated during formulation		4.126
32					36.6	---	---	3.676
33					72.4	5.74	3.41	4.307

Omecamtiv mecarbil	---	58.0	20.32	13.35	3.266

---, activity not checked

Based on urea scaffold **1** with good cardiac myosin activation (51.1 % at 10 μM), compounds **8** (n = 0, cardiac myosin ATPase activation = 46.2 %), **9** (n = 1, cardiac myosin ATPase activation = 53.3 %) and **10** (n = 3, cardiac myosin ATPase activation = 45.5 %) were synthesized varying the length of the carbon chain between urea functional group and the phenyl ring (L). The results proved that compound **1** with one carbon spacer or **9** with two carbon spacer retained the cardiac myosin ATPase activity compared to OM (cardiac myosin ATPase activation = 58.0 %). Thus *in vivo* fractional shortening (FS) and ejection fraction (EF) of compounds **1** and **9** were measured. Compound **1** (FS = 18.90; EF = 12.15) showed good activity and compound **9** (FS = 30.04; EF = 18.27) exhibited highly potent activity, which was more potent than the standard omecamtiv mecarbil (FS = 20.32; EF = 13.35).

The replacement of the phenyl ring with cyclohexyl moiety as shown in **11** (n = 0, ATPase activation = 20.3 %), **12** (n = 1, ATPase activation = 60.2 %) and **13** (n = 2, ATPase activation = 61.4 %) confirmed once again that one or two carbon chain is preferred. However, the hydrophobic cyclohexyl compounds **12** (CLog P = 4.578) and **13** (CLog P = 5.107) were not suitable for *in vivo* studies since they were precipitated during formulation.

Introducing an oxygen atom in the cyclohexyl ring of **12** as shown in tetrahydropyran-4-ylurea derivative **14** improved not only the *in vitro* ATPase activity (81.4 %) but also water solubility during formulation and therefore demonstrated good *in vivo* activity (FS = 20.50; EF = 13.10).

Hence we thought to introduce other heterocycles on the left side (L) with the preferred carbon chain length as one or two as shown in **15-22**. The thiophen-2-yl (**15**, ATPase activation = 47.6 %), furan-2-yl (**16**, ATPase activation = 36.7 %), indol-5-yl (**17**, ATPase activation = 37.2 %)

with one carbon chain as well as pyridin-2-yl (**18**, ATPase activation = 30.0 %), thiophen-2-yl (**19**, ATPase activation = 29.6 %), furan-2-yl (**20**, ATPase activation = 30.6 %) with two carbon chain did not improve the activity when compared to the phenyl compounds **1** and **9** and tetrahydropyran-4-yl derivative **14**.

However, the morpholin-1-yl (**21**, ATPase activation = 44.0 %; FS = 20.79; EF = 15.65) showed good activity both *in vitro* and *in vivo*. Further increment in the carbon chain between urea and phenyl ring (R) as demonstrated in the morpholin-1-yl derivative **22** (m = 4) did not improve the activity which clearly say that n = 1 or 2 and m = 3 should be the optimum chain length of the urea pharmacophore for potent cardiac myosin activation.

Replacement of the phenyl ring at the right side (R) of **1** with cyclohexyl ring as shown in compound **23** (ATPase activation = 37.9 %) did not improve the activity.

On the other hand, replacement with other heterocycles such as imidazol-1-yl (**24**, ATPase activation = 32.6 %) and morpholin-1-yl (**25**, ATPase activation = 32.9 %) showed decrement in the activity.

Finally to study the importance of either of the phenyl ring, compound **26** was prepared where both the phenyl rings were replaced with cyclohexyl rings which also did not increase the activity (ATPase activation = 44.4 %) when compared to the compound **1** or **9**.

In order to find the importance of the flexible carbon chain on either side, we synthesized the rigid compounds **27-32**. The rigidification of the flexible spacer of compound **9** (ATPase activation = 53.3 %) with five membered ring as shown in **27** (ATPase activation = 32.4 %) caused the decrement in activity whereas rigidification with six membered ring (**28**, ATPase activation = 73.0 %) showed good *in vitro* activity. However it failed to prove the effectiveness in the animal model contributing low FS and EF (13.32 and 9.26). Similarly rigidification of

compound **1** as demonstrated in compound **29** (ATPase activation = 35.2 %) was not fruitful. However **30** (ATPase activation = 61.9 %) successfully showed good *in vitro* activity but exhibited poor water solubility. On the other hand, rigidification on the other side as shown in compound **31** (ATPase activation = 46.6 %, CLogP = 4.126) retained the cardiac myosin activation. Its poor water solubility obstructed it to formulate for *in vivo* studies. To improve the water solubility additional nitrogen atom was introduced in compound **31** as shown in compound **32**. Unfortunately, the cardiac myosin ATPase activity was decreased to 36.6 %.

Finally to find the importance of the hydrogen bonding donor (HBD) of urea functional group, both the NH were masked with methyl group. Surprisingly the ATPase activity improved to 72.4 %. However, the *in vivo* activity was very weak (FS = 5.74; EF = 3.41).

The potent compounds **1**, **9**, **14** and **21** were analyzed for selectivity in cardiac myosins over skeletal and smooth myosins (Table 3). None of the compound showed significant activity for myosin ATPase using skeletal and smooth myosin S1. Thus, from these results it is evident that these urea derivatives are selective for cardiac myosin S1.

Table 3. Selectivity study in myosins

Compound No.	ATPase % activity		
	Cardiac myosin (at 10 µM)	Skeletal myosin (at 100 µM)	Smooth myosin (at 100 µM)
1	51.1	- 2.1	- 4.7
9	53.3	1.4	- 3.7
14	81.4	4.4	5.1
21	44.0	- 5.3	3.1
Omecamtiv mecarbil	58.0	2.8	- 5.5

(-)Blebbistatin	----	- 50.7	- 35.7

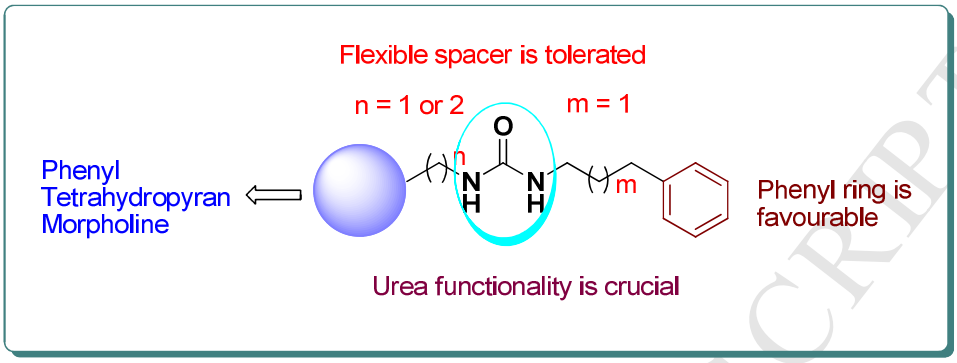


Fig. 2. SAR of urea as a selective cardiac myosin activator

5. Conclusion

Novel urea scaffold with flexible spacers as selective cardiac myosin activator for the treatment of systolic heart failure was explored. The structure activity relationship study (Figure 2) demonstrated that phenyl ring with three carbon spacer is favorable for potent cardiac myosin activation on one side whereas on the other side phenyl ring with one carbon spacer *viz*., 1-benzyl-3-(3-phenylpropyl)urea (**9** cardiac myosin ATPase activation at 10 µM = 53.3 %; FS = 30.04; EF = 18.27) or 1-(3-phenylpropyl)-3-((tetrahydro-2*H*-pyran-4-yl)methyl)urea (**14** cardiac myosin ATPase activation = 81.4 %; FS = 20.50; EF = 13.10) and two carbon spacer *viz*., 1-phenethyl-3-(3-phenylpropyl)urea (**1** cardiac myosin ATPase activation = 51.1 %; FS = 18.90; EF = 12.15) or 1-(2-morpholinoethyl)-3-(3-phenylpropyl)urea (**21** cardiac myosin ATPase activation = 44.0 %; FS = 24.79; EF = 15.65) are favorable. The flexibility of the carbon spacer is well tolerated. Rigidification of the carbon chain on either side has adverse effect on the ATPase activity. Masking the hydrogen bonding donor property of the NH group of the urea is not favorable for cardiac myosin ATPase activity. The potent compounds **1, 9, 14, 21** were highly selective for cardiac myosin over skeletal and smooth myosins and thus proving them

these new urea derivatives is a novel scaffold for discovery of cardiac myosin activators for the treatment of systolic heart failure.

6. Experimental section

6.1 Chemistry

Melting points were determined on Electro thermal 1A 9100 MK2 apparatus and are uncorrected. All commercial chemicals were used as obtained and all solvents were purified by the standard procedures [41] prior to use. Thin layer chromatography was performed on E Merck silica gel GF-254 pre-coated plates and the identification was done with UV light and colorization with spray 10 % phosphomolybdic acid followed by heating. Flash column chromatography was performed with E Merck silica gel (230-400 mesh). Infrared spectrum was recorded by using sample as such on FT-IR spectrum with Nicolet - 380 models. NMR spectra were measured against the peak of tetramethylsilane by JEOL, JNM-AL-400 (Alice) 400 FT-NMR spectrometer. High resolution mass spectra (HRMS) were measured in ESI ionization using AB Sciex TripleTOF 5600 LCMS instrument.

6.1.1. General synthetic procedure for the preparation of Urea derivatives:

To the corresponding amine (1.10 mmol) in acetonitrile (5 mL), isocyanate (1.00 mmol) was added and allowed to stir for 8 h at ambient temperature. The resulting mixture was portioned between water and ethyl acetate. The organic layer was dried over anhydrous sodium sulfate and evaporated under reduced pressure. The crude mixture was subjected to column chromatography to obtain the pure compounds.

6.1.1.1. 1-Phenethyl-3-(3-phenylpropyl)urea ***(1);*** Phenethylamine **34** and 3-phenylpropylisocyanate **35** were reacted according to the general procedure**.** Yield 92 %. White solid. R_f 0.32 (1 : 1 Ethylacetate : Hexane) mp 68-70 $^{\circ}$C. IR (neat): 3340, 2939, 2862, 1620,

1570, 1523, 1454 cm^{-1}. 1H-NMR ($CDCl_3$) δ 1.76-1.83 (m, 2H), 2.63 (t, *J* = 7.20 Hz, 2H), 2.79 (t, *J* = 6.80 Hz, 2H), 3.15 (t, *J* = 7.20 Hz, 2H), 3.41 (t, *J* = 6.80 Hz, 2H), 4.25 (bs, 2H), 7.15-7.32 (m, 10H). 13C-NMR ($CDCl_3$) δ 31.70, 33.09, 36.31, 40.00, 41.55, 125.96, 126.43, 128.39, 128.46, 128.61, 128.85, 139.23, 141.61, 158.21. HRMS Calcd for $C_{18}H_{22}N_2O$ *m/z* [M+H] 283.1811, found 283.1835.

*6.1.1.2. 1-Phenethyl-3-(3-phenylpropyl)thiourea (**2**);* To a solution of the urea (**1,** 0.5 mmol) in toluene (10 mL) Lawesson's reagent (1.00 mmol) was added and the mixture was heated at 100 °C for 5 h. After the completion of the reaction, solvent was evaporated and the crude mixture was portioned between water and ethyl acetate. The organic layer was dried over anhydrous sodium sulfate and evaporated under reduced pressure. The crude mixture was subjected to column chromatography to obtain the pure compounds. Yield 78 %, Pale yellow solid. R_f 0.46 (1 : 1 Ethylacetate : Hexane) mp 104-105 °C. IR (neat): 3352, 2968, 2897, 1565, 1519, 1453, 1160 cm^{-1}. 1H-NMR ($CDCl_3$) δ 1.83-1.90 (m, 2H), 2.65 (t, *J* = 7.60 Hz, 2H), 2.87 (t, *J* = 7.20 Hz, 2H), 3.25-3.33 (m, 2H), 3.58-3.67 (m, 2H), 5.56 (bs, 1H), 5.67 (bs, 1H), 7.14-7.19 (m, 5H), 7.25-7.32 (m, 5H); 13C-NMR ($CDCl_3$) δ 30.17, 33.15, 35.12, 35.14, 45.54, 126.41, 126.96, 128.51, 128.77, 128.91, 128.97, 138.46, 141.00, 181.69. HRMS Calcd for $C_{18}H_{22}N_2S$ *m/z* [M+H] 299.1583, found 299.1606.

*6.1.1.3. 1-Phenethyl-3-(3-phenylpropyl)sulfuricdiamide (**3**);* To a solution of phenethylamine (**34**, 0.15 mmol) in THF (5 mL), triethylamine (0.15 mmol) was added. To this mixture, 1,1'-sulfonyldiimidazole (0.15 mmol) was added and the reaction mixture was stirred at 60 °C for 2 h. After the completion of the reaction monitored by TLC, water was added and the reaction mixture was extracted using ethyl acetate. The organic layer was dried over anhydrous sodium sulfate and concentrated under vacuum. The obtained intermediate **36** was used for next step

without further purification. In the next step, 3-phenylpropylamine (0.15 mmol) was dissolved in THF and triethylamine (0.15 mmol) was added. To this mixture, **36** was added. The reaction was stirred at 60 °C for 3 h. The reaction was monitored by TLC. After the completion of the reaction, the reaction mixture was quenched with water and the whole mixture was extracted using ethyl acetate. The organic layer was dried over anhydrous sodium sulfate and evaporated under reduced pressure. The crude mixture was purified by column chromatography to afford the pure product **3**. Yield 72 %, Pale yellow solid. R_f 0.30 (2 : 1 Ethylacetate : Hexane), mp. 135-137 °C; IR (neat): 3362, 2950, 2822, 1560, 1523, 1345, 1170, 945 cm^{-1}. 1H-NMR ($CDCl_3$) δ 1.79-1.87 (m, 2H), 2.64 (t, *J* = 8.00 Hz, 2H), 2.85 (t, *J* = 7.20 Hz, 2H), 2.92-2.97 (m, 2H), 3.28-3.33 (m, 2H), 4.07-4.13 (m, 2H), 7.15-7.33 (m, 10H); 13C-NMR ($CDCl_3$) δ 31.13, 32.94, 35.81, 42.68, 44.31, 126.35, 127.04, 128.50, 128.71, 128.97, 128.98, 138.10, 141.04. HRMS Calcd for $C_{17}H_{22}N_2O_2S$ *m/z* [M+H] 319.1481, found 319.1507.

*6.1.1.4. N-Phenethyl-5-phenylpentanamide (**4**);* To a solution of 5-phenylvaleric acid **37** (0.30 mol) in methylene chloride (50 mL), thionyl chloride (0.60 mol) was added and the mixture was refluxed for 3 h. After the completion of the reaction, the solvent was removed under reduced pressure to obtain the 5-phenylvaleroyl chloride. To a solution of phenethylamine **34** (0.30 mol) in methylene chloride (50 mL), triethylamine (0.60 mol) was added and cooled to 0 °C. To this reaction mixture the obtained 5-phenylvaleroyl chloride was added and allowed to stir for 3 h at ambient temperature. After the completion of the reaction, the mixture was washed with water. The organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude product was purified by column chromatography. Yield 82 %, White solid, R_f 0.40 (1 : 1 Ethylacetate : Hexane), mp. 73-75 °C; IR (neat): 3323, 2966, 2946, 1650, 1538, 1425, 1211 cm^{-1}1H-NMR ($CDCl_3$) δ 1.60-1.68 (m, 4H), 2.13 (t, *J* = 6.80 Hz, 2H), 2.61 (t, *J* =

6.80 Hz, 2H), 2.80 (d, *J* = 7.20 Hz, 2H), 3.49-3.53 (m, 2H), 5.37 (bs, 1H), 7.15-7.32 (m, 10H); 13C-NMR ($CDCl_3$) δ 25.38, 31.07, 35.72, 35.74, 36.72, 40.52, 125.92, 126.67, 128.47, 128.56, 128.80, 128.92, 139.07, 142.36, 172.99. HRMS Calcd for $C_{19}H_{23}NO$ *m/z* [M+H] 282.1859, found 282.1884.

*6.1.1.5. N-Phenethyl-5-phenylpentan-1-amine (**5**);* To a solution of phenethylamine **34** (0.8 mmol) in DMF (5 mL), K_2CO_3 (1.00 mmol) was added. To this reaction mixture, (5-bromopentyl)benzene **39** (0.8 mmol) and KI (0.2 mmol) was added and heated at 60 °C for 8 h. After the completion of the reaction, water was added to the mixture and extracted using ethyl acetate. The organic layer was dried over anhydrous sodium sulfate and concentrated under vacuum. The crude product was purified by column chromatography. Yield 75 %, Colorless oil, R_f 0.25 (1 : 9 MeOH : CH_2Cl_2), IR (neat): 3373, 2945, 1515, 1211, 967, 868 cm^{-1}; 1H-NMR ($CDCl_3$) δ 1.29-1.37 (m, 2H), 1.49-1.56 (m, 2H), 1.57-1.65 (m, 2H), 2.02 (bs, 1H), 2.57-2.65 (m, 4H), 2.79-2.91 (m, 4H), 7.15-7.31 (m, 10H); 13C-NMR ($CDCl_3$) δ 26.98, 29.70, 31.36, 35.89, 36.16, 49.72, 51.15, 125.79, 126.34, 128.41, 128.55, 128.64, 128.87, 140.07, 142.79. HRMS Calcd for $C_{19}H_{25}N$ *m/z* [M+H] 268.2066, found 268.2095.

*6.1.1.6. 4-Phenyl-N-(3-phenylpropyl)butan-1-amine (**6**);* To a solution of 4-phenylbutylamine **38** (0.8 mmol) in DMF (5 mL), K_2CO_3 (1.00 mmol) was added. Then, (3-bromopropyl)benzene **40** (0.8 mmol) and KI (0.2 mmol) were added and heated at 60 °C for 8 h. After the completion of the reaction, water was added to the mixture and extracted using ethyl acetate. The organic layer was dried over anhydrous sodium sulfate and concentrated under vacuum. The crude product was purified by column chromatography. Yield 72 %, Colorless oil, R_f 0.25 (1 : 9 MeOH : CH_2Cl_2), IR (neat): 3380, 2950, 1510, 1130, 969, 870 cm^{-1}; 1H-NMR ($CDCl_3$) δ 1.60-1.65 (m, 4H), 1.87-1.94 (m, 2H), 2.85-2.70 (m, 8H), 7.15-7.19 (m, 6H), 7.25-7.29 (m, 4H); 13C-NMR

($CDCl_3$) δ 28.79, 29.10, 30.68, 33.53, 35.73, 49.03, 49.36, 125.92, 126.08, 128.47, 128.52, 128.54, 128.55, 141.76, 142.39. HRMS Calcd for $C_{19}H_{25}N$ *m/z* [M+H] 268.2066, found 268.2083.

*6.1.1.7. 1-(Benzyloxy)-3-phenethoxypropan-2-one (**7**)*

*6.1.1.7.1. Preparation of 2-(phenethoxymethyl)oxirane (**43**):* To a cooled suspension of NaH (60 mmol) in THF (30 mL), 2-phenylethanol (**42**, 50 mmol) was added and stirred at ambient temperature for 45 minutes. Then the reaction mixture was cooled and epichlorohydrin (**41,** 60 mmol) was added. The reaction mixture was stirred at reflux for 15 h. Saturated NH_4Cl solution was added to neutralize excess NaH and extracted using ethyl acetate. The organic layer was dried over anhydrous sodium sulfate and concentrated under vacuum. The crude product was purified by column chromatography. Yield 70 %

*6.1.1.7.2. Preparation of 1-(benzyloxy)-3-phenethoxypropan-2-ol (**44**):* Potassium hydroxide (4.76 mmol) was added to benzyl alcohol (4.33 mmol) and heated at 60 °C for 30 minutes to form a white viscous liquid. To that 2-(phenethoxymethyl)oxirane (**43**, 4.33 mmol) was added and heated at 60 °C for 3 h. After the completion of the reaction, water was added to the reaction mixture and the extracted using ethyl acetate. The organic layer was dried over anhydrous sodium sulfate. After the evaporation of the solvent, the crude product was taken for the next step. Yield 65 % Colorless oil; IR (neat): 3330, 2870, 1496, 1268, 1084, 780, 697 cm^{-1}; 1H-NMR ($CDCl_3$) δ 2.40 (d, *J* = 4.40 Hz, 1H), 2.88 (t, *J* = 7.60 Hz, 2H), 3.45-3.55 (m, 4H), 3.66-3.71 (m, 2H), 3.93-3.99 (m, 1H), 4.53 (s, 2H), 7.20-7.22 (m, 3H), 7.27-7.37 (m, 7H).

*6.1.1.7.3. Preparation of (**7**).* To a solution of 1-(benzyloxy)-3-phenethoxypropan-2-ol (**44**, 1.30 mmol) in methylene chloride (10 mL) Dess-Martin Periodinane (1.56 mmol) was added and stirred for 1 h at ambient temperature. The reaction was monitored by TLC. After the completion

of the reaction, diethyl ether (5 mL) was added followed by the addition of 1 % sodium thiosulfate solution. The resulting mixture was stirred for 15 mins. The organic layer was separated and dried over anhydrous sodium sulfate. After the evaporation of the solvent, the crude product was purified by column chromatography. Yield 82 %; Pale yellow oil; R_f 0.48 (1 : 3 Ethylacetate : Hexane), IR (neat): 2829, 1747, 1701, 1686, 1201, 1121, 713, 698 cm^{-1}; 1H-NMR ($CDCl_3$) δ 2.91 (t, *J* = 7.20 Hz, 2H), 3.69 (t, *J* = 7.20 Hz, 2H), 4.18 (s, 2H), 4.21 (s, 2H), 4.56 (s, 2H), 7.19-7.37 (m, 10H). 13C-NMR ($CDCl_3$) δ 36.21, 72.85, 73.57, 73.64, 74.94, 126.55, 128.10, 128.24, 128.59, 128.71, 129.07, 137.23, 138.61, 206.24. HRMS Calcd for $C_{18}H_{20}O_3$ *m/z* [M+H] 285.1491, found 285.1512.

*6.1.1.8. 1-Phenyl-3-(3-phenylpropyl)urea (**8**);* Prepared by general procedure. Yield 80 %; White solid; R_f 0.64 (1 : 1 Ethylacetate : Hexane), mp 88-90 °C; IR (neat): 3328, 3292, 2937, 2357, 2341, 1624 cm^{-1}; 1H-NMR ($CDCl_3$) δ 1.77-1.85 (m, 2H), 2.61 (t, *J* = 8.00 Hz, 2H), 3.22-3.27 (m, 2H), 5.08 (bs, 1H), 6.74 (bs, 1H), 7.03-7.08 (m, 1H), 7.12-7.19 (m, 3H), 7.23-7.30 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 31.62, 33.11, 39.88, 121.17, 123.86, 126.00, 128.38, 128.49, 129.33, 138.63, 141.54, 156.12. HRMS Calcd for $C_{16}H_{18}N_2O$ *m/z* [M+H] 255.1498, found 255.1526.

6.1.1.9. 1-Benzyl-3-(3-phenylpropyl)urea (9); Prepared by general procedure. Yield 89 %; Colorless solid; mp 96-98 °C; R_f 0.30 (1 : 1 Ethylacetate : Hexane), IR (neat): 3308, 2868, 1620, 1559, 1494, 1453 cm^{-1}; 1H-NMR ($CDCl_3$) δ 1.76-1.84 (m, 2H), 2.61 (t, *J* = 7.80 Hz, 2H), 3.17-3.21 (m, 2H), 4.33 (d, *J* = 6.00 Hz, 2H), 4.39 (bs, 1H), 4.66 (bs, 1H), 7.13-7.19 (m, 3H), 7.24-7.34 (m, 7H). 13C-NMR ($CDCl_3$) δ 31.70, 33.02, 39.97, 44.36, 125.94, 127.28, 127.38, 128.38, 128.44, 128.84, 139.32, 141.62, 158.44. HRMS Calcd for $C_{17}H_{20}N_2O$ *m/z* [M+H] 269.1654, found 269.1682.

*6.1.1.10. 1,3-Bis(3-phenylpropyl)urea (**10**);* Prepared by general procedure. Yield 85 %; Colorless solid; mp 94-96 °C; R_f 0.38 (1 : 1 Ethylacetate : Hexane), IR (neat): 3313, 3060, 2862, 1621, 1560, 1493, 1452 cm^{-1}; 1H-NMR ($CDCl_3$) δ 1.76-183 (m, 4H), 2.63 (t, *J* = 8.00 Hz, 4H), 3.13-3.18 (m, 4H), 4.46 (bs, 2H), 7.15-7.28 (m, 10H). 13C-NMR ($CDCl_3$) δ 31.41, 32.80, 39.72, 125.66, 128.08, 128.16, 141.32, 158.05. HRMS Calcd for $C_{19}H_{24}N_2O$ *m/z* [M+H] 297.1968, found 297.1992.

*6.1.1.11. 1-Cyclohexyl-3-(3-phenylpropyl)urea (**11**);* Prepared by general procedure. Yield 78 %; White solid; mp 103-105°C; R_f 0.32 (1 : 2 Ethylacetate : Hexane), IR (neat): 3327, 2927, 2850, 1644, 1618, 1560, 1494 cm^{-1}; 1H-NMR ($CDCl_3$) δ 1.02-1.19 (m, 3H), 1.28-1.39 (m, 2H), 1.57-1.62 (m, 1H), 1.66-1.71 (m, 2H), 1.79-1.86 (m, 2H), 1.89-1.93 (m, 2H), 2.65 (t, *J* = 8.00 Hz, 2H), 3.17-3.22 (m, 2H), 3.42-3.49 (m, 1H), 4.25 (t, *J* = 8.00 Hz, 1H), 4.37 (t, *J* = 8.00 Hz, 1H), 7.17-7.21 (m, 3H), 7.27-7.30 (m, 2H). 13C-NMR ($CDCl_3$) δ 24.84, 25.50, 31.79, 33.15, 33.86, 40.01, 49.00, 125.95, 128.40, 128.46, 141.71, 157.70. HRMS Calcd for $C_{16}H_{24}N_2O$ *m/z* [M+H] 261.1968, found 261.1994.

*6.1.1.12. 1-(Cyclohexylmethyl)-3-(3-phenylpropyl)urea (**12**);* Prepared by general procedure. Yield 49 %; White solid; mp 95-97 °C; R_f 0.35 (1 : 2 Ethylacetate : Hexane), IR (neat): 3322, 2940, 2866, 1667, 1623, 1568, 1511 cm^{-1}; 1H-NMR ($CDCl_3$) δ 0.85-0.94 (m, 2H), 1.12-1.27 (m, 3H), 1.37-1.43 (m, 1H), 1.65-1.72 (m, 5H), 1.81-1.88 (m, 2H), 2.67 (t, *J* = 8.00 Hz, 2H), 2.96 (t, *J* = 6.00 Hz, 2H), 3.18-3.23 (m, 2H), 4.18-4.22 (m, 2H), 7.18-7.31 (m, 5H). 13C-NMR ($CDCl_3$) δ 25.89, 26.48, 30.89, 31.96, 33.28, 38.51, 40.15, 46.90, 126.07, 128.52, 128.58, 141.83, 158.77. HRMS Calcd for $C_{17}H_{26}N_2O$ *m/z* [M+H] 275.2124, found 275.2153.

*6.1.1.13. 1-(2-Cyclohexylethyl)-3-(3-phenylpropyl)urea (**13**);* Prepared by general procedure. Yield 98 %; Colorless oil; R_f 0.40 (1 : 2 Ethylacetate : Hexane), IR (neat): 3325, 2938, 2865,

1662, 1618, 1572, 1510 cm^{-1}; 1H-NMR ($CDCl_3$) δ 0.86-0.94 (m, 2H), 1.12-1.29 (m, 4H), 1.34-1.39 (m, 2H), 1.63-1.70 (m, 5H), 1.80-1.87 (m, 2H), 2.66 (t, *J* = 8.00 Hz, 2H), 3.10-3.15 (m, 2H), 3.18-3.23 (m, 2H), 4.18 (bs, 1H), 4.28 (bs, 1H), 7.17-7.31 (m, 5H). 13C-NMR ($CDCl_3$) δ 26.13, 26.40, 31.78, 33.11, 33.15, 35.18, 37.57, 38.25, 40.04, 125.96, 128.40, 128.46, 141.68, 158.45. HRMS Calcd for $C_{18}H_{28}N_2O$ *m/z* [M+H] 289.2281, found 289.2304.

*6.1.1.14. 1-(3-Phenylpropyl)-3-((tetrahydro-2H-pyran-4-yl)methyl)urea (**14**);* Prepared by general procedure. Yield 80 %; White solid; mp: 123-125 °C; R_f 0.35 (2 : 1 Ethylacetate : Hexane), IR (neat): 3259, 1653, 1558, 1337, 1151, 1108, 1023 cm^{-1}; 1H-NMR ($CDCl_3$) δ 1.22-1.33 (m, 2H), 1.58-1.61 (m, 2H), 1.68-1.72 (m, 1H), 1.80-1.87 (m, 2H), 2.66 (t, *J* = 7.60 Hz, 2H), 3.04 (t, *J* = 6.40 Hz, 2H), 3.17-3.22 (m, 2H), 3.32-3.39 (m, 2H), 3.94-3.98 (m, 2H), 4.32 (bs, 1H), 4.37 (bs, 1H), 7.17-7.21 (m, 3H), 7.27-7.30 (m, 2H). 13C-NMR ($CDCl_3$) δ 30.57, 31.82, 33.16, 35.77, 40.06, 46.09, 67.59, 125.94, 128.30, 128.41, 141.49, 158.48. HRMS Calcd for $C_{16}H_{24}N_2O_2$ *m/z* [M+H] 277.1917, found 277.1942.

*6.1.1.15. 1-(3-Phenylpropyl)-3-(thiophen-2-ylmethyl)urea (**15**);* Prepared by general procedure. Yield 82 %; White solid; mp 89-91 °C; R_f 0.46 (2 : 1 Ethylacetate : Hexane),IR (neat): 3318, 2929, 1633, 1614, 1560, 1454 cm^{-1}; 1H-NMR ($CDCl_3$) δ 1.75-1.83 (m, 2H), 2.61 (t, *J* = 8.00 Hz, 2H), 3.15-3.20 (m, 2H), 4.49 (d, *J* = 8.00 Hz, 2H), 4.60-4.63 (m, 1H), 4.89-4.91 (m, 1H), 6.91-6.93 (m, 2H), 7.13-7.19 (m, 4H), 7.24-7.28 (m, 2H). 13C-NMR ($CDCl_3$) δ 31.70, 33.03, 39.27, 39.96, 124.82, 125.21, 125.93, 126.81, 128.40, 128.44, 141.63, 142.74, 158.15. HRMS Calcd for $C_{15}H_{18}N_2OS$ *m/z* [M+H] 275.1219, found 275.1243.

*6.1.1.16. 1-(Furan-2-ylmethyl)-3-(3-phenylpropyl)urea (**16**);* Prepared by general procedure. Yield 82 %; White solid; mp 70-72°C; R_f 0.42 (2 : 1 Ethylacetate : Hexane), IR (neat): 3319, 1626, 1561, 1496, 1457, 1332, 1183, 1153 cm^{-1}; 1H-NMR ($CDCl_3$) δ 1.78-1.85 (m, 2H), 2.64 (t,

J = 7.60 Hz, 2H), 3.17-3.22 (m, 2H), 4.34 (d, *J* = 6.00 Hz, 2H), 4.49 (bs, 1H), 4.69 (bs, 1H), 6.19-6.20 (m, 1H), 6.30-6.31 (m, 1H), 7.15-7.20 (m, 3H), 7.25-7.29 (m, 2H), 7.32-7.33 (m, 1H); 13C-NMR ($CDCl_3$) δ 31.70, 33.02, 37.32, 39.92, 106.78, 110.37, 125.92, 128.37, 128.43, 141.62, 141.93, 152.63, 158.25. HRMS Calcd for $C_{15}H_{18}N_2O_2$ *m/z* [M+H] 259.1447, found 259.1472.

*6.1.1.17. 1-((1H-Indol-5-yl)methyl)-3-(3-phenylpropyl)urea (**17**);* Prepared by general procedure. Yield 86 %; White solid; mp 128-130 °C; R_f 0.44 (2 : 1 Ethylacetate : Hexane), IR (neat): 3341, 3280, 2982, 1651, 1560, 1492, 1215, 1120 cm^{-1}; 1H-NMR ($CDCl_3$) δ 1.63-1.71 (m, 2H), 2.56 (t, *J* = 7.80 Hz, 2H), 3.00-3.05 (m, 2H), 4.25 (d, *J* = 6.00 Hz, 2H), 5.93 (bs, 1H), 6.18 (bs, 1H), 6.36-6.37 (m, 1H), 6.99 (dd, *J* = 8.40 Hz, *J* = 2.00 Hz, 1H), 7.15-7.33 (m, 7H), 7.40 (s, 1H), 11.01 (bs, 1H). 13C-NMR ($CDCl_3$) δ 31.82, 32.45, 38.87, 43.52, 100.84, 111.15, 118.47, 120.96, 125.49, 125.71, 127.63, 128.31, 131.08, 131.08, 135.02, 141.93, 158.18. HRMS Calcd for $C_{19}H_{21}N_3O$ *m/z* [M+H] 308.1764, found 308.1796.

*6.1.1.18. 1-(3-Phenylpropyl)-3-(2-(pyridine-2-yl)ethyl)urea (**18**);* Prepared by general procedure. Yield 82 %; Pale yellow powder; mp 58-60 °C; R_f 0.35 (1 : 9 MeOH : CH_2Cl_2), IR (neat): 3305, 3010, 2939, 1647, 1616, 1539, 1497 cm^{-1}; 1H-NMR ($CDCl_3$) δ 1.77-1.84 (m, 2H), 2.64 (t, *J* = 8.00 Hz, 2H), 2.98 (t, *J* = 8.00 Hz, 2H), 3.15-3.20 (m, 2H), 3.56-3.61 (m, 2H), 4.47 (bs, 1H), 5.17 (bs, 1H), 7.12-7.30 (m, 7H), 7.59-7.63 (m, 1H), 8.48-8.50 (m, 1H). 13C-NMR ($CDCl_3$) δ 31.73, 33.08, 37.80, 39.68, 40.00, 121.51, 123.63, 125.89, 128.38, 128.41, 136.66, 141.68, 149.08, 158.46, 159.91. HRMS Calcd for $C_{17}H_{21}N_3O$ *m/z* [M+H] 284.1764, found 284.1792.

*6.1.1.19. 1-(3-Phenylpropyl)-3-(2-(thiophen-2-yl)ethyl)urea (**19**);* Prepared by general procedure. Yield 80 %; White solid; mp 68-70 °C; R_f 0.55 (2 : 1 Ethylacetate : Hexane), IR (neat): 3328, 2935, 2856, 1650, 1624, 1560, 1496 cm^{-1}; 1H-NMR ($CDCl_3$) δ 1.77-1.84 (m, 2H), 2.64 (t, *J* =

8.00 Hz, 2H), 3.01 (t, *J* = 6.00 Hz, 2H), 3.14-3.19 (m, 2H), 3.40-3.45 (m, 2H), 4.33-4.35 (m, 1H), 4.43-4.46 (m, 1H), 6.82-6.83 (m, 1H), 6.94 (dd, *J* = 4.00 Hz, 1H), 7.14-7.20 (m, 4H), 7.26-7.29 (m, 2H). 13C-NMR ($CDCl_3$) δ 30.57, 31.69, 33.09, 40.04, 41.71, 123.86, 125.35, 125.98, 127.05, 128.40, 128.48, 141.60, 141.74, 158.14. HRMS Calcd for $C_{16}H_{20}N_2OS$ *m/z* [M+H] 289.1375, found 289.1401.

*6.1.1.20. 1-(2-(Furan-2-yl)ethyl)-3-(3-phenylpropyl)urea (**20**);* Prepared by general procedure. Yield 65 %; White solid; mp 81-83 °C; R_f 0.48 (2 : 1 Ethylacetate : Hexane), IR (neat): 3325, 1630, 1561, 1535, 1280, 1145, 1136, 995 cm^{-1}; 1H-NMR ($CDCl_3$) δ 1.78-1.85 (m, 2H), 2.63 (t, *J* = 7.80 Hz, 2H), 2.92 (t, *J* = 7.80 Hz, 2H), 3.20-3.25 (m, 2H), 3.76 (t, *J* = 7.20 Hz, 2H), 5.90 (bs, 1H), 6.06-6.07 (m, 1H), 6.26-6.27 (m, 1H), 6.47 (s, 1H), 7.17-7.21 (m, 3H), 7.27-7.32 (m, 3H). 13C-NMR ($CDCl_3$) δ 25.50, 31.46, 33.10, 39.65, 49.54, 106.09, 110.33, 125.95, 128.40, 128.48, 141.37, 141.59, 153.25, 160.99. HRMS Calcd for $C_{16}H_{20}N_2O_2$ *m/z* [M+H] 273.1604, found 273.1636.

*6.1.1.21. 1-(2-Morpholinoethyl)-3-(3-phenylpropyl)urea (**21**);* Prepared by general procedure. Yield 83 %; pale yellow powder; mp: 92-94 °C; R_f 0.25 (9 : 1 Ethylacetate : Hexane), IR (neat): 3230, 2912, 2790, 1660, 1611, 1575, 1508 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.81-1.88 (m, 2H), 2.42-2.48 (m, 6H), 2.66 (t, J = 7.80 Hz, 2H), 3.19-3.26 (m, 4H), 3.68-3.70 (m, 4H), 4.82-4.88 (m, 2H), 7.17-7.21 (m, 3H), 7.27-7.31 (m, 2H); ^{13}C-NMR ($CDCl_3$) δ 31.94, 33.29, 36.97, 40.23, 53.47, 58.10, 67.04, 126.11, 128.51, 128.60, 141.78, 158.63. HRMS Calcd for $C_{16}H_{25}N_3O_2$ *m/z* [M+H] 292.2026, found 292.2055.

*6.1.1.22. 1-(2-Morpholinoethyl)-3-(4-phenylbutyl)urea (**22**);* Prepared by general procedure (i). Yield 36 %; Cream solid; mp: 73-76 ^oC; R_f 0.28 (9 : 1 Ethylacetate : Hexane), IR (neat): 3309, 2940, 1624, 1576, 1115, 867, 745, 697 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.50-1.57 (m, 2H), 1.62-1.70

(m, 2H), 2.45-2.49 (m, 6H), 2.61-2.65 (t, 2H), 3.16-3.21 (m, 2H), 3.23-3.27 (m, 2H), 3.67-3.73 (m, 4H), 4.82-4.87 (bs, 2H), 7.16-7.20 (m, 3H), 7.28-7.30 (d, 2H); ^{13}C-NMR ($CDCl_3$) δ 28.90, 30.20, 35.35, 36.75, 39.45, 53.76, 58.77, 66.64, 126.09, 128.68, 128.74, 142.69, 158.48. HRMS Calcd for $C_{17}H_{27}N_3O_2$ *m/z* [M+H] 306.2182, found 306.2207.

*6.1.1.23. 1-(3-Cyclohexylpropyl)-3-phenethylurea (**23**);*

*6.1.1.23.1. Preparation of 3-cyclohexylpropan-1-amine (**47**);* 3-Cyclohexylpropan-1-ol (10.0 mmol) was refluxed with hydrobromic acid (10.0 mmol) for 5 h. After the addition of ice water, the reaction mixture was extracted with ethyl acetate to get 3-(bromopropyl)cyclohexane. The crude mixture was reacted with phthalimide (15.0 mmol) in THF (20 mL) under reflux for 2 h to get a the phthalimide intermediate as white powder. It was filtered and further reacted with hydrazine (98 %, 63 mmol) in absolute ethanol (15 mL) at 60 °C for 30 minutes. The reaction mixture was diluted with water and extracted using ethyl acetate to get the 3-cyclohexylpropan-1-amine **47**. The amine **47** was reacted with phenethylisocyanate according to the general procedure to get **23.** Yield 87 %; White solid; mp 84-86 °C; R_f 0.40 (1 : 2 Ethylacetate : Hexane), IR (neat): 3359, 3307, 2911, 2849, 1625, 1560, 1496 cm^{-1}; 1H-NMR ($CDCl_3$) δ 0.81-0.89 (m, 2H), 1.11-1.26 (m, 6H), 1.41-1.49 (m, 2H), 1.62-1.69 (m, 5H), 2.81 (t, *J* = 8.00 Hz, 2H), 3.05-3.10 (m, 2H), 3.41-3.46 (m, 2H), 4.26-4.33 (m, 2H), 7.19-7.24 (m, 3H), 7.28-7.33 (m, 2H). 13C-NMR ($CDCl_3$) δ 26.24, 26.53, 27.45, 33.24, 34.51, 36.37, 37.33, 40.82, 41.57, 126.40, 128.59, 128.86, 139.30, 158.31. HRMS Calcd for $C_{18}H_{28}N_2O_2$ *m/z* [M+H] 289.2281, found 289.2308.

*6.1.1.24. 1-(3-1H-Imidazol-1-yl)propyl)-3-phenethylurea (**24**);* Prepared by general procedure. Yield 80 %; White solid; mp 72-74 °C; R_f 0.22 (9 : 1 Ethylacetate : Hexane), IR (neat): 3301, 2938, 1637, 1558, 1508, 1475, 1230, 1108 cm^{-1}; 1H-NMR ($CDCl_3$) δ 1.90-1.97 (m, 2H), 2.80 (t,

J = 7.20 Hz, 2H), 3.11-3.16 (m, 2H), 3.41-3.46 (m, 2H), 3.97 (t, *J* = 7.20 Hz, 2H), 4.72 (bs, 1H), 4.91 (bs, 1H), 6.91 (s, 1H), 6.99 (s, 1H), 7.18-7.23 (m, 3H), 7.27-7.31 (m, 2H), 7.37 (s, 1H). 13C-NMR ($CDCl_3$) δ 31.56, 36.36, 36.91, 41.37, 44.34, 119.03, 126.37, 128.55, 128.83, 129.25, 137.14, 139.29, 158.71. HRMS Calcd for $C_{15}H_{20}N_4O$ *m/z* [M+H] 273.1716, found 273.1743.

*6.1.1.25. 1-(3-Morpholinopropyl)-3-phenethylurea (**25**);* Prepared by general procedure. Yield 88 %; White solid; mp 106-108 °C; R_f 0.27 (9 : 1 Ethylacetate : Hexane), IR (neat): 3332, 2974, 1626, 1560, 1356, 1184, 1154, 952 cm^{-1}; 1H-NMR ($CDCl_3$) δ 1.60-1.67 (m, 2H), 2.38-2.41 (m, 6H), 2.82 (t, *J* = 7.00 Hz, 2H), 3.18-3.23 (m, 2H), 3.41-3.46 (m, 2H), 3.60-3.62 (m, 4H), 4.88 (bs, 1H), 5.04 (bs, 1H), 7.19-7.25 (m, 3H), 7.29-7.33 (m, 2H). 13C-NMR ($CDCl_3$) δ 26.07, 36.41, 39.30, 41.71, 53.39, 56.34, 66.86, 126.42, 128.58, 128.90, 139.38, 158.76. HRMS Calcd for $C_{16}H_{25}N_3O_2$ *m/z* [M+H] 292.2026, found 292.2051.

*6.1.1.26. 1-(2-Cyclohexylethyl)-3-(3-cyclohexylpropyl)urea (**26**);* To a cooled solution of triphosgene (1.41 mmol) in THF at 0°C, a mixture of 2-cyclohexylethanamine (3.52 mmol) and *N,N*-diisopropylethylamine (DIPEA, 7.04 mmol) was added. The reaction mixture was stirred at the same temperature for 0.5 h and then the 3-cyclohexylpropan-1-amine **47** (3.52 mmol) (preparation mentioned in the synthesis of compound **23**) was added. The reaction mixture was slowly allowed to attain ambient temperature and further stirred for 8 h. After the completion of the reaction, water was added to the reaction mixture and extracted using ethyl acetate. The organic layer was dried over anhydrous sodium sulfate and evaporated under reduced pressure. The crude mixture was subjected on column chromatography to obtain the pure compounds. Yield 65 %; White solid; mp 80-82 °C; R_f 0.68 (1 : 4 Ethylacetate : Hexane), IR (neat): 3327, 2919, 2848, 1650, 1624, 1560, 1471 cm^{-1}; 1H-NMR ($CDCl_3$) δ 0.82-0.95 (m, 4H), 1.09-1.32 (m, 10H), 1.36-1.41 (m, 2H), 1.45-1.53 (m, 2H), 1.62-1.72 (m, 10H), 3.10-3.20 (m, 4H), 4.28 (bs,

1H), 4.34 (bs, 1H). 13C-NMR ($CDCl_3$) δ 26.46, 26.57, 26.74, 26.86, 27.83, 33.45, 33.57, 34.86, 35.53, 37.67, 37.94, 38.59, 41.19. 158.80. HRMS Calcd for $C_{18}H_{34}N_2O$ *m/z* [M+H] 295.2750, found 295.2781.

3-Cyclohexylpropan-1-ol (10 mmol) was refluxed with hydrobromic acid (10 mmol) overnight. After the addition of ice water, the reaction mixture was extracted with ethyl acetate to get 3-(bromopropyl)cyclohexane. The crude mixture was reacted with phthalimide (15 mmol) in THF (20 mL) to get a the phthalimide derivative as white powder. It was filtered and further reacted with hydrazine (98 %, 63 mmol) in absolute ethanol (15 mL) at 60 C for 10 minutes. The reaction mixture was diluted with water and extracted using ethyl acetate to get the corresponding amine. The amine was reacted with isocyanate to get 1-(3-cyclohexylpropyl)-3-phenethylurea

*Preparation of 2-phenyl-N-(3-phenylpropyl)pyrrolidine-1-carboxamide (**27**) and 2-Phenyl-N-(3-phenylpropyl)piperidine-1-carboxamide (**28**).*

To a slurry of *N*-chlorosuccinimide (103.0 mmol) in dry Et_2O (150mL) under nitrogen atmosphere pyrrolidine **48**/piperidine **49** (58.0 mmol) was added in one portion at 20 °C. After stirring for 1 h at the same temperature, the reaction mixture was filtered and the solid was washed with Et_2O (20mL). The combined Et_2O layers were washed with water and brine and were dried over a mixture of anhydrous sodium sulfate and 4 A° molecular sieves for 1 h and filtered to get the intermediate **50/51**. This solution was filtered and added to a stirred solution of phenyllithium (1.8 M in 7:3 cyclohexane: Et_2O, 144.0 mmol) under argon atmosphere. After 1 h, the obtained dark red mixture was cooled in an ice bath and diluted with water (20 mL) and Et_2O (40mL). The aqueous layer was re-extracted with Et_2O and the combined ether layers were dried over anhydrous sodium sulfate. The ether layer was concentrated and the crude residue was

purified using column chromatography to get **52/53**. Further the amine **52** and **53** were reacted with **35** according to the general procedure to get compound **27** and **28** respectively.

*6.1.1.27. 2-Phenyl-N-(3-phenylpropyl)pyrrolidine-1-carboxamide (**27**)*

Yield 90%; Yellow oil; R_f 0.45 (2 : 1 Ethylacetate : Hexane), IR (neat): 3335, 3025, 2864, 1628, 1527, 1494, 1346 cm^{-1}; 1H-NMR ($CDCl_3$) δ 1.60-1.67 (m, 2H), 1.82-194 (m, 3H), 2.35-2.42 (m, 3H), 3.04-3.11 (m, 1H), 3.15-3.22 (m, 1H), 3.63-3.72 (m, 2H), 4.01 (bs, 1H), 4.64-4.67 (m, 1H), 7.01 (d, *J* = 7.60 Hz, 2H), 7.13-7.37 (m, 8H). 13C-NMR ($CDCl_3$) δ 23.02, 31.58, 32.84, 36.78, 39.87, 47.27, 60.84, 125.74, 125.78, 127.57, 128.33, 128.34, 128.97, 141.76, 143.33, 157.12. HRMS Calcd for $C_{20}H_{24}N_2O$ *m/z* [M+H] 309.1968, found 309.1993.

*6.1.1.28. 2-Phenyl-N-(3-phenylpropyl)piperidine-1-carboxamide (**28**)*

Yield 92 %; Yellow oil; R_f 0.49 (2 : 1 Ethylacetate : Hexane), IR (neat): 3348, 3025, 2936, 2861, 1616, 1533, 1495 cm^{-1}; 1H-NMR ($CDCl_3$) δ 1.41-1.45 (m, 1H), 1.54-1.61 (m, 3H), 1.76-1.83 (m, 2H), 1.88-1.93 (m, 1H), 2.17-2.22 (m, 1H), 2.57 (t, *J* = 7.80 Hz, 2H), 2.92-2.99 (m, 1H), 3.24-3.30 (m, 2H), 3.89-3.92 (m, 1H), 4.32 (bs, 1H), 5.03-5.05 (m, 1H), 7.11-7.18 (m, 3H), 7.22-7.27 (m, 5H), 7.33-7.37 (m, 2H). 13C-NMR ($CDCl_3$) δ 19.03, 24.72, 29.16, 31.61, 33.29, 39.88, 40.57, 54.04, 125.87, 126.46, 126.81, 128.40, 128.43, 128.80, 140.59, 141.88, 158.23. HRMS Calcd for $C_{21}H_{26}N_2O$ *m/z* [M+H] 323.2124, found 323.2159.

*6.1.1.29. 3-Phenyl-N-(3-phenylpropyl)piperidine-1-carboxamide (**29**);* 3-Phenylpyridine (**54**, 3.48 mmol) was dissolved in acetic acid and Pt/C (10%, 20 mg) was added to it under N_2 atmosphere. The reaction mixture was hydrogenated under 55 psi for 20 h. TLC was checked for the product formation. The reaction mixture was filtered through celite and concentrated under vacuum. The crude mixture was purified by column chromatography to get intermediate **55** (Yield 26 %). Further **55** was reacted with **35** according to the general procedure to get **29**. Yield

82 %; Pale yellow oil; R_f 0.52 (2 : 1 Ethylacetate : Hexane), IR (neat): 3325, 3017, 2998, 1660, 1615, 1559, 1497 cm^{-1}; 1H-NMR ($CDCl_3$) δ 1.56-1.65 (m, 2H), 1.74-1.78 (m, 1H), 1.83-1.90 (m, 2H), 2.01-2.05 (m, 1H), 2.66-2.78 (m, 5H), 3.28-3.33 (m, 2H), 3.85-3.92 (m, 2H), 4.36 (bs, 1H), 7.11-7.35 (m, 10H). 13C-NMR ($CDCl_3$) δ 25.25, 31.61, 31.67, 33.50, 40.71, 42.41, 44.21, 50.79, 125.92, 126.71, 127.14, 128.39, 128.46, 128.54, 141.88, 143.46, 157.56. HRMS Calcd for $C_{21}H_{26}N_2O$ *m/z* [M+H] 323.2124, found 323.2154.

*6.1.1.30. 1-(2,3-Dihydro-1H-inden-2-yl)-3-(3-phenylpropyl)urea (**30**);* Compound **30** was prepared by the reaction of 2,3-dihydro-1*H*-inden-2-amine (**56**) with 3-phenylpropylisocyanate (**35**) according to the general procedure. Yield 86 %; White solid; mp 125-127 °C; R_f 0.75 (2 : 1 Ethylacetate : Hexane), IR (neat): 3335, 3020, 2994, 1647, 1616, 1550, 1498 cm^{-1}; 1H-NMR ($CDCl_3$) δ 1.77-1.85 (m, 2H), 2.64 (t, *J* = 8.00 Hz, 2H), 2.76 (dd, *J* = 4, 16 Hz, 2H, A part of AB), 3.18 (t, *J* = 8.00 Hz, 2H), 3.27 (dd, *J* = 4, 16 Hz, 2H, B part of AB), 4.27 (bs, 1H), 4.50-4.52 (m, 2H), 7.15-7.29 (m, 9H). 13C-NMR ($CDCl_3$) δ 31.75, 33.10, 39.95, 40.49, 51.52, 124.80, 125.96, 126.68, 128.37, 128.46, 141.12, 141.64, 158.05. HRMS Calcd for $C_{17}H_{22}N_2O$ *m/z* [M+H] 295.1811, found 295.1843.

*6.1.1.31. N-Phenethyl-4-phenylpiperidine-1-carboxamide (**31**);* Compound **31** was prepared by the reaction of 4-phenylpiperidine (**57**) with phenethylisocyanate (**45**) according to the general procedure. Yield 85 %; White solid; mp 60-62 °C; R_f 0.32 (2 : 1 Ethylacetate : Hexane), IR (neat): 3335, 3010, 2990, 1650, 1621, 1549, 1495 cm^{-1}; 1H-NMR ($CDCl_3$) δ 1.57-1.68 (m, 2H), 1.81-1.84 (m, 2H), 2.62-2.68 (m, 1H), 2.81-2.88 (m, 4H), 3.50-3.55 (m, 2H), 3.98-4.02 (m, 2H), 4.49 (bs, 1H), 7.18-7.34 (m, 10H). 13C-NMR ($CDCl_3$) δ 32.91, 36.31, 42.03, 42.59, 44.57, 126.40, 126.45, 126.78, 128.58, 128.81, 128.92, 139.52, 145.58, 157.55. HRMS Calcd for $C_{20}H_{24}N_2O$ *m/z* [M+H] 309.1968, found 309.1994.

*6.1.1.32. N-Phenethyl-4-phenylpiperazine-1-carboxamide (**32**);* Compound **32** was prepared by the reaction of 1-phenylpiperazine (**58**) with phenethylisocyanate (**45**) according to the general procedure. Yield 87 %; White solid; mp. 135-137 °C; R_f 0.21 (2 : 1 Ethylacetate : Hexane), IR (neat): 3338, 3022, 2986, 1644, 1616, 1551, 1489 cm^{-1}; 1H-NMR ($CDCl_3$) δ 2.85 (t, *J* = 8.00 Hz, 2H), 3.15 (t, *J* = 4.00 Hz, 4H), 3.47 (t, *J* = 4.00 Hz, 4H), 3.50-3.55 (m, 2H), 4.47 (bs, 1H), 6.68-6.93 (m, 3H), 7.20-7.34 (m, 7H). 13C-NMR ($CDCl_3$) δ 36.20, 41.96, 43.58, 49.06, 116.48, 120.33, 126.46, 128.64, 128.88, 129.24, 139.35, 151.05, 157.56. HRMS Calcd for $C_{19}H_{23}N_3O$ *m/z* [M+H] 310.1920, found 310.1951.

*6.1.1.33. 1,3-Dimethyl-1-phenethyl-3-(3-phenylpropyl)urea (**33**);* To a cooled suspension of NaH (1.50 mmol) in DMF (5 mL) 1-phenethyl-3-(3-phenylpropyl)urea (**1**, 0.68 mmol) was added and stirred at ambient temperature for 45 minutes. Then the reaction mixture was cooled and CH_3I (1.63 mmol) was added. The reaction mixture was stirred at ambient temperature for 3 h. Saturated NH_4Cl solution was added to neutralize excess NaH and extracted with ethyl acetate. The organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude product was purified by column chromatography. Yield 75 %; Colorless oil; R_f 0.45 (1 : 1 Ethylacetate : Hexane), IR (neat): 3022, 2980, 2831, 1651, 1610, 1550, 1490 cm^{-1}; 1H-NMR ($CDCl_3$) δ 1.80-1.88 (m, 2H), 2.57 (t, *J* = 8.00 Hz, 2H), 2.84 (t, *J* = 8.00 Hz, 2H), 2.72 (s, 3H), 2.77 (s, 3H), 3.13 (t, *J* = 8.00 Hz, 2H), 3.37 (t, *J* = 8.00 Hz, 2H), 7.18-7.30 (m, 10H). 13C-NMR ($CDCl_3$) δ 29.44, 33.39, 34.28, 36.76, 37.23, 50.30, 52.26, 126.22, 126.57, 128.68, 128.72, 128.78, 129.11, 139.79, 142.10, 165.61. HRMS Calcd for $C_{20}H_{26}N_2O$ *m/z* [M+H] 311.2124, found 311.2158.

6.2. Pharmacology

6.2.1. Sarcomere assay procedure for the measurement of myosin ATPase activity:

In the sarcomere, force generation is directly coupled to ATP hydrolysis. Compounds that activate the sarcomere were identified by measuring the increase in myosin ATPase activity in a sarcomere assay at 10 μM**.**

Actin stimulated ATPase activity was assayed spectrophotometrically as reported previously [32] with modifications. The standard reaction mixture contained 20 mM Tris HCl (pH 7.5), 15 mM KCl, 6 mM $MgCl_2$, 1 mM ATP, S1 myosin (CS-MYS03) and actin thin filament complex (CS-TFC01) with pCa = 6.5 [33]. The reaction was stopped by addition of Cytophos reagent (Cytoskeleton BK054 kit) after 10 min incubation at room temperature, samples were analyzed for inorganic phosphate liberated by taking the absorbance at 650 nm on TECAN Infinite. Omecamtiv was used as a positive control for the selectivity assay. Blank had buffer, ATP and Cytophos mixture while DMSO was used as a negative control.

% Activity = (Mean A - B)-(NC -B)/ (NC-B) x100

Where**,** NC=Negative control; B=Blank, A=Absorbance

6.2.2. Selectivity Studies

Compound specificity with respect to muscle type were evaluated by comparing the effect of the compound on actin stimulated ATPase activity of a panel of myosin isoforms including cardiac (bovine), skeletal (rabbit) and smooth muscle (chicken gizzard) at a single higher dose (100 uM) of the compound.

6.2.2.1. For Skeletal muscle:

Actin stimulated ATPase activity was assayed spectrophotometrically as reported previously [32-35] with modifications. The standard reaction mixture contained 20 mM Tris HCl (pH 7.5), 15

mM KCl, 6 mM $MgCl_2$, 1 mM ATP, S1 myosin (CS-MYS04) and actin thin filament complex (CS-TFC01) with pCa = 6.5. The reaction was stopped by addition of Cytophos reagent (Cytoskeleton BK054 kit) After 10 min incubation at room temperature, samples were analyzed for inorganic phosphate liberated by taking absorbance at 650 nm on TECAN Infinite. Blebbistatin was used as a negative control for selectivity assay [38].

6.2.2.2. For Smooth muscle:

Actin stimulated ATPase activity was assayed spectrophotometrically as reported previously [32,33,36,37] with modifications. The standard reaction mixture contained 20 mM Tris HCl (pH 7.5), 15 mM KCl, 6 mM $MgCl_2$, 1 mM ATP, S1 myosin (CS-MYS05), actin (AD 99) and Tropomyosin (T2400) with pCa = 6.5. The reaction was stopped by addition of Cytophos reagent (Cytoskeleton BK054 kit) After 10 min incubation at room temperature, samples were analyzed for inorganic phosphate liberated by taking absorbance at 650 nm on TECAN Infinite. Blebbistatin was used as a negative control for selectivity assay [38].

6.2.3. Animal study (*in vivo*)

6.2.3.1. The formulation of sample

The stock solution was prepared by dissolving 8 mg of respective compound in 2 mL of DMSO (*i.e.* 4 mg/mL or 4000 µg/mL of DMSO)**.** It was diluted 100 times with saline solution to give 40 µg/mL final solution (% of DMSO is 1 %). The maximum % of DMSO in the final solution was limited to 10 %. The concentration of the final solution (unknown concentration) was measured by HPLC along with three standards (known concentration).

6.2.3.2. Measurement of fractional shortening (FS) and ejection fraction (EF) by echocardiography

6.2.3.2.1. Animals

Seven-week-old Sprague-Dawley male Rats were purchased from the Orient Bio (South Korea). The protocols used in this study [39] conformed to the Guide for the Care and use of Laboratory Animals published by National Institutes of Health (NIH Publication 85-23, revised 1996). All animal experiments were approved by the Institutional Animal Care and Use Committee of Samsung Biomedical Research Institute (SBRI). SBRI is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International and abided by the Institute of Laboratory Animal Resources guide.

6.2.3.2.2. Echocardiography

The rats were anesthetized by 1.5 % isoflurane inhalation method with nosecone. The right internal jugular vein was used as a central venous line for drug administration. Rats were infused with samples at 0, 2, 4, 8, and 16 µg/kg/min for 3 minutes using 40 µg/mL final solution. M-mode echocardiograms were performed at baseline and 16 µg/kg/min. Images were acquired with a MS250 transducer operated at 25 MHz connected to a VisualSonics Vevo2100 (VisualSonics Inc., Toronto, Ontario, Canada). LV end-diastolic interventricular septum, posterior wall thickness, LV dimension, LV fractional shortening (FS) and ejection fraction (EF) were measured. % increase value was calculated according to following formula: % Increase FS(EF) = (FS(EF) data of 16 µg/kg/min - FS(EF) data of 0 µg/kg/min) / baseline data of FS(EF) x 100 %. Three rats per drug were used. A single sonographer who was blinded to treatment information performed all echacardiograms for data acquisition.

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Highlights

- A series of flexible urea derivatives have been explored as novel cardiac myosin ATPase activator for the treatment of systolic heart failure.
- 1-Phenethyl-3-(3-phenylpropyl)urea and 1-benzyl-3-(3-phenylpropyl)urea showed significant activity *in vitro* and *in vivo.*
- Replacement of phenyl ring to tetrahydropyran-4-yl or morpholine moiety enhanced activity.
- These urea analogs are selective activators for cardiac myosin.