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Identification of diphenylalkylisoxazol-5-amine scaffold as novel activator of cardiac myosin

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ABSTRACT

To identify novel potent cardiac myosin activator, a series of diphenylalkylisoxazol-5-amine compounds **4–7** have been synthesized and evaluated for cardiac myosin ATPase activation. Among the 37 compounds, **4a** (CMA at 10 μM = 81.6%), **4w** (CMA at 10 μM = 71.2%) and **6b** (CMA at 10 μM = 67.4%) showed potent cardiac myosin activation at a single concentration of 10 μM. These results suggested that the introduction of the amino-isoxazole ring as a bioisostere for urea group is acceptable for the cardiac myosin activation. Additional structure–activity relationship (SAR) studies were conducted. Para substitution (-Cl, $–OCH_3$, $-SO_2N(CH_3)_2$) to the phenyl rings or replacement of a phenyl ring with a heterocycle (pyridine, piperidine and tetrahydropyran) appeared to attenuate cardiac myosin activation at 10 μM. Additional hydrogen bonding acceptor next to the amino group of the isoxazoles did not enhance the activity. The potent isoxazole compounds showed selectivity for cardiac myosin activation over skeletal and smooth muscle myosin, and therefore these potent and selective isoxazole compounds could be considered as a new series of cardiac myosin ATPase activators for the treatment of systolic heart failure.

1. Introduction

Heart failure (HF) is a global widespread in health care and a leading cause of mortality and morbidity worldwide[1,2]. The health expenditures for regulating HF are significant and will increase dramatically with an ageing population[1,3]. The weakening of cardiac contractions is expected due to HF. Of the millions of patients with HF worldwide, approximately 50% have HF with reduced ejection fraction (HFrEF). Inotropes have shown advantage in maintaining hemodynamic stability and relieving the patient's pain in systolic HF patients[4,5].

Currently accessible calcitropes[6] such as phosphodiesterase inhibitors and adrenergic receptor agonists are used in the treatment of systolic HF[5]. Inappropriately, these drugs have adverse effects, for instance, increased intracellular concentrations of calcium and cAMP contributing to increased heart rate, hypotension, and mortality[7–10]. To overcome these limitations cardiac myosin activators are being developed, 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[4,11–15].

Omecamtiv mecarbil (OM, Fig. 1), a cardiac-selective myosin activator, is a first in class and effective drug for systolic HF treatment and the achievement of this drug has been tested in small and large animal models, as well as in clinical studies[4,16]. In contrast to calcitropes that increased intracellular concentrations of calcium and cAMP and decrease ejection time, OM is a myotrope[6] that improves the systolic function by increasing myocardial contraction and stroke volume without consuming ATP energy, oxygen, or changing intracellular calcium levels[4,13,15]. Clinical investigations of OM on humans have shown there is a linear association between dose and systolic ejection time[17–20]. Even though the clinical examinations results presented that OM is highly effective in relieving symptoms and improve the quality of life of systolic HF patients, additional drugs to improve cardiac function could be beneficial to patients with HFrEF.

Our group identified flexible dialkyl substituted urea scaffold as a novel cardiac myosin activator with potent cardiac myosin ATPase activation (CMA)[21]. As shown in Figure 1, the urea compound **1**, (CMA at 10 μM = 53.3%) exhibited robust activity at a single concentration of 10 μM[21]. In another study, the lead compound **1** was optimized to enhance the activity and found dimethylsulfonamidediphenylalkylurea

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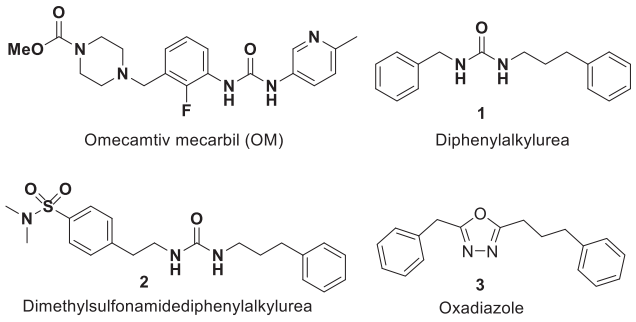
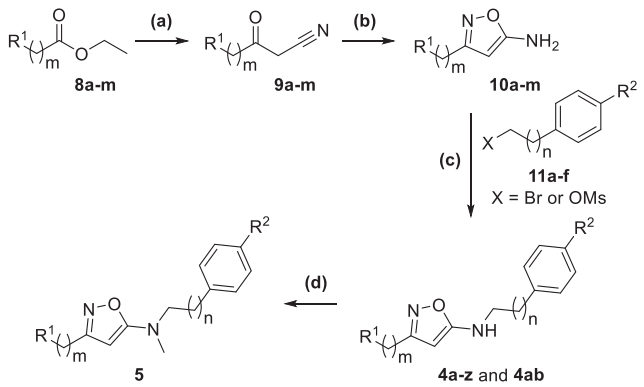


Figure 1. Reported urea analogs and oxadiazole isostere as cardiac myosin activators.



Scheme 1. Synthesis of isoxazol-5-amine compounds (substituents are listed in Table 1). Reagents and Conditions: (a) 60% NaH, CH_3CN, THF, reflux, 3 h; (b) $NH_2OH.HCl$, NaOH, H_2O, reflux, 2 h, 40–82%; (c) Alkyl halide **11a-f**, Cs_2CO_3 or 60% NaH, DMF, 60 °C, 2 h, 30–72%; (d) 60% NaH, MeI, THF, rt, 8 h, 58%.

2 (CMA at 10 μM = 91.6%) as a novel cardiac myosin activator (Figure 1)[22]. Further to enhance the scope of the cardiac myosin activators with new heterocyclic analogs, the urea scaffold was switched over to oxadiazole isostere. However, oxadiazole **3** (CMA at 10 **μ**M = 62.9%) had reduced activity at 10 μM when compared to dimethylsulfonamidediphenylalkylurea **2**.[23] Continuous exploration of new isosteres of urea, parallelly without diminishing the activity of the dimethylsulfonamidediphenylalkylurea **2** is the main objective of this study.

In this respect, the current work aim to investigate possible bioisosteric replacement of the urea scaffold with isoxazole ring. Thus, rigid amino-isoxazoles (**4** and **5**) have been designed and synthesized as selective cardiac myosin activators for the treatment of systolic heart failure. In addition, we also synthesized amide (**6**) and urea (**7**) isoxazole compounds by introduction of additional hydrogen bonding and evaluated cardiac myosin activation (Figure 2).

alkyl halide in presence of Cs_2CO_3 or 60% NaH in *N,N*-dimethylformamide at 60 °C to give desired final amino-isoxazole compounds **4a-z** and **4ab**. The *N*-methylated compound **5** was synthesized from **4a** by alkylation with methyl iodide in the presence of 60% NaH in 58% yield (Scheme 1).

As shown in Scheme 2, the amide compounds **6a, 6b** and **6e** were synthesized from the amine intermediate **10a** and **10 l** by coupling with appropriate carboxylic acid **12** in the presence of EDC.HCl and DMAP in DMF at 50 °C for 16 h. The amide compounds **6c**, **6d** and **6f** were synthesized from intermediate **10a** and **10d** by reaction with the appropriate acid chloride **12** in pyridine at 80 °C for 16 h. Finally, the urea

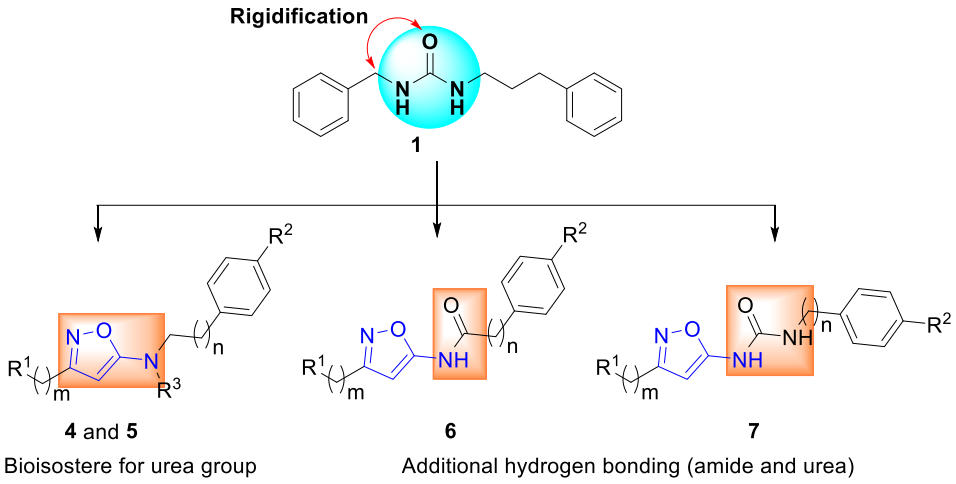
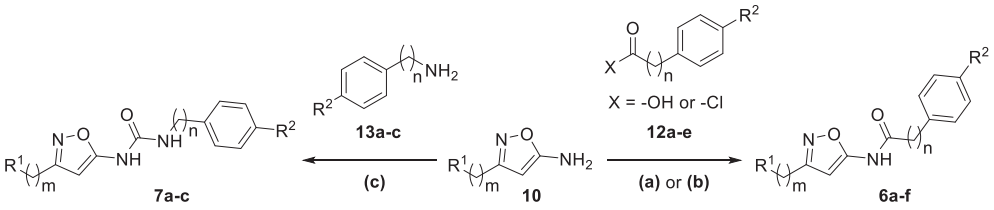


Figure 2. Introduction of isoxazol-5-amine ring as a bioisostere for urea group as cardiac myosin activator.

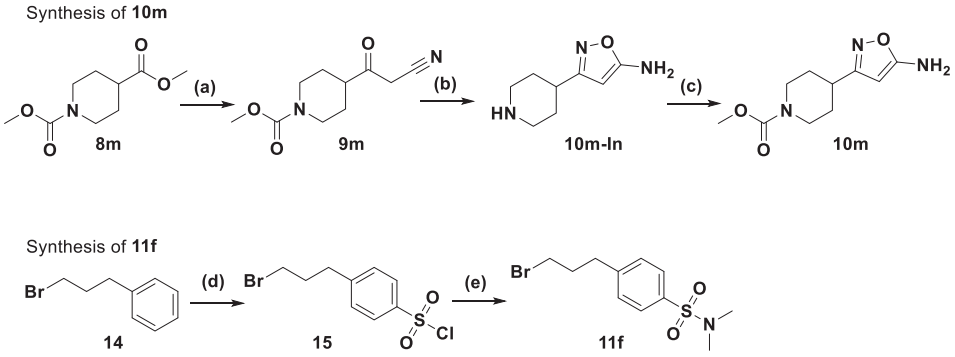
2. Chemistry

The synthetic routes to acquire the target amino-isoxazole compounds **4–7** were outlined in Scheme 1 and Scheme 2. Commercially available various carboxylic esters **8a-m** were mixed with acetonitrile in presence of 60% sodium hydride in tetrahydrofuran under reflux temperature to give nitrile compounds **9a-m** in quantitative yields by using the reported synthetic procedure [24,25]. The reaction of appropriate nitrile compound **9** with hydroxylamine hydrochloride in the presence of sodium hydroxide in water at reflux temperature afforded the key isoxazol-5-amine intermediates **10a-m**[25–31] in excellent yields. The resulted isoxazol-5-amines **10a-m** were stirred with appropriate compounds **7a-c** was synthesized from **10a** in the presence of triphosgene and DIPEA in THF at 0 °C to ambient temperature for 16 h.

Scheme 3 represents the synthesis of intermediate compounds **10 m** and **11f**. The intermediate **10 m** was synthesized similar to that of the preparation of **10a**. The addition of **9 m** to hydroxylamine hydrochloride in the presence of sodium hydroxide in water at reflux temperature yielded the de-protected product **10 m-In** in 62% yield. Later, a conversion of **10 m-In** to **10 m** in 66% yield was smoothly conducted by the reaction with the methyl chloroformate in the presence of DIPEA. The intermediate **11f** was synthesized by mixing starting material **14** with chlorosulfonic acid to give **15**; to **15** was added dimethylamine hydrochloride and TEA in DCM resulting in **11f**.



Scheme 2. Synthesis of amide and urea isoxazole compounds (substituents are listed in Table 1). Reagents and Conditions: (a) Carboxylic acid, EDC.HCl, DMAP, DMF, 50 °C, 16 h, 41–52%; (b) Acid chloride, pyridine, 80 °C, 16 h, 47–67%; (c) Triphosgene, DIPEA, THF, 0 °C to rt, 16 h, 45–58%.

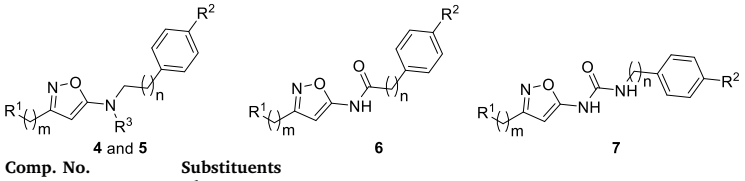


Scheme 3. Synthesis of intermediate **10 m** and **11f**. Reagents and conditions: (a) 60% NaH, CH_3CN, THF, reflux 3 h; (b) $NH_2OH.HCl$, NaOH, H_2O, reflux, 2 h, 62%; (c) $ClCOOCH_3$, DIPEA, DCM, 0 °C to rt, 1 h, 66%; (d) $ClSO_3H$, DCM, rt, 18 h; (e) $(CH_3)_2N.HCl$, TEA, DCM, rt, 2 h, 75%.

3. Pharmacology

In the sarcomere, force generation is directly coupled to ATP hydrolysis by myosin. The Actin stimulated ATPase activity of myosin was evaluated spectrophotometrically using a modified version of a previously reported assay.[32] Compounds **4–7** were tested at a single concentration of 10 μM and were reported for the % increase from baseline of the myosin ATPase at pCa concentration of 6.5. The results from this assay are shown in Table 1. Compound specificity with respect to muscle type was estimated by comparing the effect of the compound on actin stimulated ATPase activity of a panel of myosin isoforms including cardiac (bovine (10 μM))[32], smooth muscle (chicken gizzard (100 μM))[33,34] and skeletal (rabbit (100 μM))[35,36] at a single dose of the compound. Omecamtiv mecarbil (1) was taken as a positive control for cardiac myosin ATPase activity and (-)-blebbistatin was taken as a negative control for measurement of smooth or skeletal muscle myosin ATPase activity[37]. The selectivity data is shown in Table 2.

Table 1
List of the synthesized diphenylalkylisoxazolamine compounds (**4a-z, 4ab, 5, 6a-f** and **7a-c**) as cardiac myosin activator.



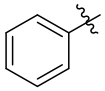
Comp. No.	Substituents R^1	R^{nn}	R^n	m	n	CLogP	Myosin ATPase activity(% at 10 μM)
4a		H	H	0	2	5.139	81.6
4b		H	H	0	3	5.668	44.2
4c		H	H	0	1	4.760	48.5
4d		H	H	0	0	4.111	21.8
4e		H	H	1	2	4.878	46.5
4f		H	H	2	0	4.229	15.0
4 g		H	H	3	0	4.758	53.9
4 h		H	H	0	2	3.839	25.2

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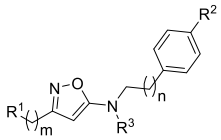
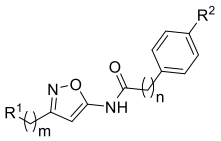
Table 1 (*continued*)


4 and **5**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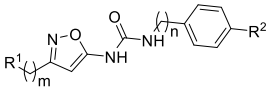
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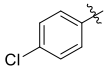
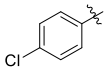
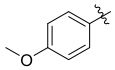
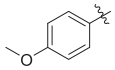
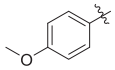
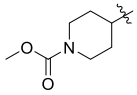
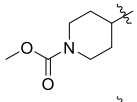
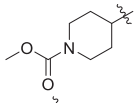
Comp. No.	Substituents R^1	R^{nn}	R^n	m	n	CLogP	Myosin ATPase activity(% at 10 μM)
4i		H	H	0	2	5.869	27.2
4j		H	H	0	1	5.490	31.4
4 k		H	H	0	2	5.155	35.2
4 l		H	H	0	1	4.776	11.5
4 *m*		H	H	0	3	5.685	22.4
4*n*		H	H	0	2	5.431	10.3
4o		H	H	2	2	4.368	15.0
4p		H	H	1	2	3.839	10.2
4q		H	H	0	2	3.310	5.1
4r		H	H	0	1	2.653	45.6
4 s		H	H	0	2	3.032	41.2
4 t		H	H	0	3	3.561	41.8
4u		H	H	0	2	3.708	35.2
4v		–OCH_3	H	0	2	5.058	26.2
4w		-$SO_2N(CH_3)_2$	H	0	2	4.334	71.2
4x		–OCH_3	H	0	2	5.058	20.3
4y		-$SO_2N(CH_3)_2$	H	0	2	3.035	43.2
4z		–OCH_3	H	0	2	3.627	12.0
4ab		-$SO_2N(CH_3)_2$	H	0	2	2.903	33.1
5		H	–CH_3	0	2	5.394	18.0
6a		H	H	0	1	3.581	56.6
6b		H	H	0	2	4.140	67.4
6c		H	H	0	3	4.519	43.2
6d		H	H	0	4	5.048	40.8

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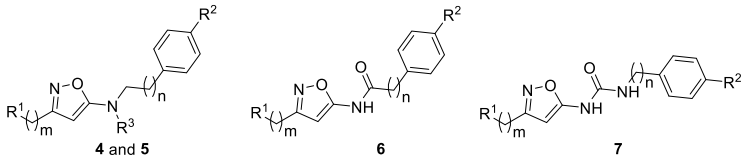
Table 1 (*continued*)



4 and **5** **6** **7**

Comp. No.	Substituents R^1	R^{nn}	R^n	m	n	CLogP	Myosin ATPase activity(% at 10 μM)
6e		H	H	0	2	2.033	42.2
6f		H	H	3	0	4.179	24.6
7a		H	H	0	1	3.891	15.0
7b		H	H	0	2	4.220	30.0
7c		H	H	0	3	4.599	39.4
STD	Omecamtiv mecarbil (**OM**)					3.266	55.3

Table 2
Selectivity study of compounds **4a**, **4e**, **4w** and **6b** in myosins.

Compound No.	ATPase % activity Cardiac myosin(at 10 μM)	Skeletal myosin(at 100 μM)	Smooth myosin(at 100 μM)
4a	81.6	−2.5	1.1
4e	46.5	3.2	2.5
4w	71.2	−1.3	2.1
6b	67.4	1.8	3.2
OM	55.3	3.5	−2.6
(-)Blebbistatin	N.D.	−52.3	−36.4

N.D.: Not determined.

4. Results and discussion

In the first set of experiments, diphenylalkylisoxazol-5-amines **4a-z** and **4ab** were synthesized and determined for their cardiac myosin ATPase activation at a single compound concentration of 10 μM (Table 1). Initially, we synthesized analog **4a** with three carbon spacer between the amine functional group of the isoxazole to the phenyl ring and evaluated for cardiac myosin activation. Surprisingly, analog **4a** (m = 0, n = 2, CMA at 10 μM = 81.6%; cLogP = 5.139) showed greater activity at 10 μM than the previously reported analog **2** (CMA at 10 μM = 53.3%) and **OM** (CMA at 10 μM = 55.3%). This outcome suggested that the bioisosteric replacement of the urea scaffold could be useful for discovery of novel rigid urea molecules such as isoxazol-5-amines as cardiac myosin ATPase activator.

The above result encouraged us to study SAR of the isoxazol-5-amine scaffold. Next, extension and contraction tactics were used to confirm the length of the methylene chain between isoxazol-5-amine and the phenyl ring. Accordingly, analogs **4b**, **4c** and **4d** were prepared. Among them, **4b** (m = 0, n = 3, CMA at 10 μM = 44.2%; cLogP = 5.668), **4c** (m = 0, n = 1, CMA at 10 μM = 48.5%; cLogP = 4.760) showed similar activation at 10 μM when compared to **OM** (CMA = 55.3%) whereas analog **4d** (m = 0, n = 0, CMA at 10 μM = 21.8%; cLogP = 4.111) decreased the activity at 10 μM. These results confirmed that the three carbon spacer "n = 2" is ideal for the activation at 10 μM. On the other side, the methylene spacer to aryl group R^1 to the isoxazol-5-amine ring were sequentially varied as shown in **4e** (m = 1, n = 2, CMA at 10 μM = 46.5%; cLogP = 4.878), **4f** (m = 2, n = 0, CMA at 10 μM = 15.0%; cLogP = 4.229) and **4 g** (m = 3, n = 0, CMA at 10 μM = 53.3%; cLogP = 4.758), which did not improve the ATPase activity. These results show that no spacer between the R^1 (phenyl) and isoxazol-5-amine ring "m = 0" is optimum for the activity at 10 μM.

The change of phenyl ring (R^1) of **4a** with pyridine which could improve the water solubility as represented in analog **4 h** (m = 0, n = 2, CMA at 10 μM = 25.2%; cLogP = 3.839) showed a decrement in the activity at 10 μM. Further, exploration of the effect of substituents on the 3-phenyl ring (left side) was attempted. Accordingly, we prepared 4-chloro analogs such as **4i** (m = 0, n = 2, CMA at 10 μM = 27.2%; cLogP = 5.869) and **4j** (m = 0, n = 1, CMA at 10 μM = 31.4%; cLogP = 5.490), and 4-methoxy analogs such as **4 k** (m = 0, n = 2, CMA at 10 μM = 35.2%; cLogP = 5.155), **4 l** (m = 0, n = 1, CMA at 10 μM = 11.5%; cLogP = 4.776) and **4 m** (m = 0, n = 3, CMA at 10 μM = 22.4%; cLogP = 5.685) which showed moderate to minimal ATPase activation. These results suggested that this substitution on the phenyl ring is not tolerated for robust activation at 10 μM.

Replacement of the phenyl ring (R^1) in **4a** with hydrophobic cyclohexyl ring as denoted in **4n** (m = 0, n = 2, CMA at 10 μM = 15.0%; cLogP = 4.368) revealed reduced activity at 10 μM. Further we prepared **4o** (m = 2, n = 2, CMA at 10 μM = 10.3%; cLogP = 5.431), **4p** (m = 1, n = 2, CMA at 10 μM = 10.2%; cLogP = 3.839) and **4q** (m = 0, n = 2, CMA at 10 μM = 5.1%; cLogP = 3.310) with the R^1 phenyl was replaced with a methyl group and tested for the CMA. The activity of these analogs at 10 μM is dramatically reduced. In addition, replacement of the cyclohexyl ring in **4n** with hydrogen bonding tetrahydropyrane ring as denoted in **4 s** (m = 0, n = 2, CMA at 10 μM = 41.2%; cLogP = 3.032) showed 4-fold more activity at 10 μM than **4n**. However, variation of the chain length as in **4r** (m = 0, n = 1, CMA at 10 μM = 45.6%; cLogP = 2.653) and **4 t** (m = 0, n = 3, CMA at 10 μM = 41.8%; cLogP = 3.561) did not enhance the activity. Moreover, we also synthesized methyl carbamate analog **4u** (m = 0, n = 2, CMA at 10 μM = 35.2%; cLogP = 3.708). Hence, all of these compounds did not show the better activity than the phenyl analog **4a**.

Next, introduction of the methoxy and N,N-dimethylsulfonamide

substituents on the para position (R^2) of the phenyl ring as in **4v** (R^2 = –OCH_3, CMA = 26.2%at 10 μM; cLogP = 5.058) showed decreased CMA activation at 10 μM and **4w** (R^2 = -$SO_2N(CH_3)$, CMA = 71.2%at 10 μM; cLogP = 4.334) showed good CMA activity. Variation of R^1 groups with methoxy and N,N-dimethylsulfonamide substituents as shown in **4x** (CMA = 20.3% at 10 μM; cLogP = 5.058), **4y** (CMA = 43.2% at 10 μM; cLogP = 3.035), **4z** (CMA = 12.0% at 10 μM; cLogP = 3.627) and **4ab** (CMA = 33.1% at 10 μM; cLogP = 2.903) did not enhance the activity. These results suggested that the N,N-dimethylsulfonamide group on phenyl ring play an important role in the CMA activation for this set of analogs.

Introduction of methyl group on nitrogen of **4a** as represented in compound **5** (CMA = 18.1% at 10 μM; cLogP = 5.394) showed dramatic decreased in the in vitro activity at 10 μM. This outcome suggested us substitution on nitrogen is not necessary.

Next objective of our work was to introduce additional hydrogen bonding property next to the amine group. Therefore, we synthesized amide analogs **6a-f** and urea analogs **7a-c**. Among them **6a**, **6b** and **6c** showed greater activation at 10 μM than the OM (Table 1). However, these compounds did not improve the CMA activity when compared with amine compound **4a**. These results suggested that the amine group is important for the CMA activity.

Additional experiments were carried out to determine the cardiac myosin selectivity, the potent isoxazole compounds **4a**, **4e**, **4w** and **6b** were tested in skeletal and smooth myosins. As shown in Table 2, all of them did not activate myosin ATPase of skeletal and smooth myosin S1 which clearly indicate that these isoxazole compounds are selective cardiac myosin activators from these in vitro systems.

5. Conclusion

In summary, novel diphenylalkylisoxazol-5-amines **4–7** have been synthesized and evaluated for cardiac myosin ATPase activation at 10 μM. Among them, compounds **4a**, **4w** and **6b** showed high cardiac myosin activation at 10 μM. These results suggested that the introduction of the amino-isoxazole ring as a bioisostere for urea group is acceptable for the cardiac myosin activation. In addition, SAR (Fig. 3) studies were conducted. Addition of simple functional groups like chlorine, methoxyl and *N,N*-dimethyl sulfonamide on para position of either phenyl rings or change of one phenyl ring to other heterocycles like pyridine, piperidine and tetrahydropyran are not tolerated for the cardiac myosin activation at 10 μM. Additional HBA next to the amino group of the isoxazoles did not enhance the activity. The highly active isoxazole compounds showed selectivity for cardiac myosin activation over skeletal and smooth muscle myosin, and therefore these isoxazole compounds could be considered a new series of cardiac myosin ATPase activators for the treatment of systolic HF.

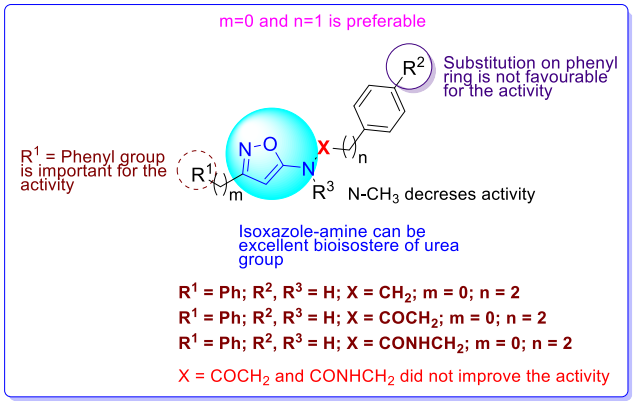


Figure 3. SAR of isoxazoles as a selective cardiac myosin activator.

6. Materials and methods

6.1. Chemistry

All commercial chemicals were used as obtained and all solvents were purified by distillation prior to use applying the standard procedures[38]. Thin layer chromatography (TLC) was performed on E Merck silica gel GF-254 pre-coated plates, identification was performed under UV illumination (λ = 254 nm), and colorization with Iodine and $KMnO_4$. All compounds were purified by flash column chromatography which was performed on E Merck silica gel (230–400 mesh). ^{1}H NMR and ^{13}C NMR spectra were measured against the peak of tetramethylsilane using a Bruker Fourier 300 NMR (300 MHz) and JEOL, JNM-AL400 NMR (400 MHz) spectrometer. Infrared (IR) spectra were recorded on a Nicolet 380 model FTIR. Melting points (mp) were determined on an Electro thermal 1A 9100 MK2 apparatus and are uncorrected. High resolution mass spectrum (HRMS) were measured in ESI ionization using AB Sciex Triple TOF 5600 LCMS instrument.

6.1.1. General procedure for the preparation of compounds *4a-c, 4e-z and 4ab*

To a stirred solution of 3-substituted-isoxazol-5-amine **10** (1.87 mmol) in DMF (5 mL), Cs_2CO_3 (1.87 mmol) and appropriate bromo compound **11** (1.87 mmol) were added at room temperature. The resulting solution was stirred at 60 °C for 16 h. The mixture was cooled, quenched with ice cold water (30 mL), and then extracted with EtOAc (2x times). The combined organic extracts were dried over anhydrous Na_2SO_4 and then concentrated under reduced pressure. The crude mixture was subjected to flash silica gel (230–400 mesh) column chromatography (eluting with 5–10% Ethyl acetate in hexanes) to afford the title compounds **4**.

*6.1.1.1. 3-Phenyl-N-(3-phenylpropyl)isoxazol-5-amine (**4a**).*. Yield 59%; off white solid; mp 54–57 °C; IR (neat) 3265, 2935, 2849, 1611, 1403, 1258, 1176, 1081, 763, 698 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71–7.77 (m, 2H), 7.40–7.46 (m, 3H), 7.29–7.36 (m, 2H), 7.18–7.26 (m, 3H), 5.23 (s, 1H; isoxazole ring proton), 4.55 (t, J = 6.22 Hz, 1H), 3.26 (q, J = 6.99 Hz, 2H), 2.75 (t, J = 7.56 Hz, 2H), 1.94–2.05 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.4, 163.7, 141.0, 130.0, 129.7, 128.7, 128.6, 128.5, 126.6, 126.2, 75.1, 44.0, 32.9, 30.9; HRMS (ESI) calculated for $C_{18}H_{18}N_2O$ $[M + H]^+$ 279.1497, found 279.1521.

*6.1.1.2. 3-Phenyl-N-(4-phenylbutyl)isoxazol-5-amine (**4b**).*. Yield 61%; white solid; mp 75–78 °C; IR (neat) 3267, 2935, 2849, 1611, 1403, 1258, 1176, 1082, 763, 697 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72–7.78 (m, 2H), 7.40–7.47 (m, 3H), 7.27–7.33 (m, 2H), 7.17–7.24 (m, 3H), 5.26 (s, 1H; isoxazole ring proton), 4.52 (t, J = 5.98 Hz, 1H), 3.21–3.30 (m, 2H), 2.68 (t, J = 7.20 Hz, 2H), 1.64–1.80 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5, 163.7, 141.9, 130.0, 129.7, 128.7, 128.5, 128.4, 126.6, 126.0, 75.1, 44.5, 35.4, 29.0, 28.4; HRMS (ESI) calculated for $C_{19}H_{20}N_2O$ $[M + H]^+$ 293.1654, found 293.1675.

*6.1.1.3. N-Phenethyl-3-phenylisoxazol-5-amine (**4c**)*[39].. Yield 30%; colorless oil; IR (neat) 3266, 2935, 2849, 1611, 1403, 1258, 1176, 1081, 763, 698 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71–7.82 (m, 2H), 7.41–7.47 (m, 3H), 7.32–7.41 (m, 2H), 7.27–7.31 (m, 1H), 7.21–7.26 (m, 2H), 5.31 (s, 1H), 4.57 (br. s., 1H), 3.52 (q, J = 6.67 Hz, 2H), 2.96 (t, J = 6.83 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.2, 163.7, 138.2, 129.9, 129.7, 128.9, 128.8, 128.7, 126.9, 126.6, 75.5, 45.7, 35.5; HRMS (ESI) calculated for $C_{17}H_{16}N_2O$ $[M + H]^+$ 265.1341, found 265.1361.

*6.1.1.4. 3-Benzyl-N-(3-phenylpropyl)isoxazol-5-amine (**4e**).*. Yield 51%; Pale yellow solid; mp 63–65 °C; IR (neat) 3253, 3077, 2937, 2918, 1608, 1548, 1494, 1452, 1094, 992, 695 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27–7.35 (m, 5H), 7.13–7.27 (m, 5H), 4.69 (s, 1H), 4.41 (t,

J = 5.12 Hz, 1H), 3.85 (s, 2H), 3.12 (q, J = 6.83 Hz, 2H), 2.68 (t, J = 7.56 Hz, 2H), 1.85–1.97 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.1, 164.6, 141.0, 137.8, 128.9, 128.6, 128.6, 128.4, 126.7, 126.2, 77.1, 43.9, 32.8, 30.9; HRMS (ESI) calculated for $C_{19}H_{20}N_2O$ $[M + H]^+$ 293.1654, found 293.1674.

6.1.1.5. N-Benzyl-3-phenethylisoxazol-5-amine ***(4f)***.. Yield 48%; off white solid; mp 58–61 °C; IR (neat) 3253, 3077, 2937, 2918, 1608, 1548, 1494, 1452, 1094, 823, 693 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27–7.40 (m, 7H), 7.19–7.24 (m, 3H), 4.81 (s, 2H), 4.34 (d, J = 5.85 Hz, 2H), 2.90–3.00 (m, 2H), 2.80–2.87 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.7, 165.0, 141.1, 137.6, 128.8, 128.5, 128.4, 127.9, 127.4, 126.2, 77.5, 48.5, 34.3, 28.2; HRMS (ESI) calculated for $C_{18}H_{18}N_2O$ $[M + H]^+$ 279.1497, found 279.1521.

6.1.1.6. N-Benzyl-3-(3-phenylpropyl)isoxazol-5-amine ***(4 g)***.. Yield 50%; pale yellow oil; IR (neat) 3275, 3025, 2950, 1610, 1420, 1235, 1156, 1089, 885, 695 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26–7.40 (m, 7H), 7.15–7.21 (m, 3H), 4.84 (s, 1H), 4.79 (t, J = 6.22 Hz, 1H), 4.34 (d, J = 5.85 Hz, 2H), 2.63–2.70 (t, J = 7.6 Hz, 2H), 2.51–2.57 (t, J = 7.6 Hz, 2H), 1.89–2.00 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.7, 165.4, 141.8, 137.6, 128.8, 128.5, 128.4, 127.8, 127.4, 125.9, 77.3, 48.5, 35.2, 29.7, 25.93; HRMS (ESI) calculated for $C_{19}H_{20}N_2O$ $[M + H]^+$ 293.1654, found 293.1675.

6.1.1.7. N-(3-Phenylpropyl)-3-(pyridin-4-yl)isoxazol-5-amine ***(4 h)***.. Yield 57%; Pale yellow oil; IR (neat) 3265, 2935, 2849, 1611, 1560, 1508, 1403, 1258, 1324, 1176, 763, 698 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67–8.72 (m, 2H), 7.59–7.64 (m, 2H), 7.29–7.37 (m, 2H), 7.16–7.26 (m, 3H), 5.26 (s, 1H), 4.71 (t, J = 5.24 Hz, 1H), 3.27 (q, J = 6.67 Hz, 2H), 2.75 (t, J = 7.44 Hz, 2H), 2.01 (quin, J = 7.26 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.0, 161.6, 150.4, 140.9, 137.5, 128.6, 128.4, 126.3, 120.9, 74.9, 43.9, 32.8, 30.8; HRMS (ESI) calculated for $C_{17}H_{17}N_3O$ $[M + H]^+$ 280.1450, found 280.1473.

6.1.1.8. 3-(4-Chlorophenyl)-N-(3-phenylpropyl)isoxazol-5-amine ***(4i)***.. Yield 61%; off white solid; mp 87–88 °C; IR (neat) 3276, 3029, 2936, 1613, 1538, 1407, 1255, 1201, 1172, 1081, 821, 760, 690 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63–7.71 (m, 2H), 7.36–7.43 (m, 2H), 7.28–7.35 (m, 2H), 7.15–7.25 (m, 3H), 5.18 (s, 1H), 4.56 (t, J = 6.34 Hz, 1H), 3.25 (q, J = 6.83 Hz, 2H), 2.74 (t, J = 7.44 Hz, 2H), 1.99 (quin, J = 7.26 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.6, 162.7, 141.0, 135.6, 129.0, 128.6, 128.5, 128.5, 127.9, 126.3, 75.0, 44.0, 32.8, 30.9; HRMS (ESI) calculated for $C_{18}H_{17}ClN_2O$ $[M + H]^+$ 313.1107, found 313.1128.

6.1.1.9. 3-(4-Chlorophenyl)-N-phenethylisoxazol-5-amine ***(4j)***.. Yield 35%; off white solid; mp 88–90 °C; IR (neat)3276, 3029, 2936, 1613, 1538, 1407, 1255, 1201, 1172, 1081, 821, 760, 690 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65–7.72 (m, 2H), 7.38–7.44 (m, 2H), 7.32–7.38 (m, 2H), 7.27–7.31 (m, 1H), 7.21–7.26 (m, 2H), 5.27 (s, 1H), 4.52–4.62 (m, 1H), 3.52 (q, J = 6.67 Hz, 2H), 2.96 (t, J = 6.95 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.4, 162.7, 138.2, 135.7, 129.0, 128.9, 128.8, 128.5, 127.9, 126.9, 75.4, 45.7, 35.6; HRMS (ESI) calculated for $C_{17}H_{15}ClN_2O$ $[M + H]^+$ 299.0951, found 299.0973.

6.1.1.10. 3-(4-Methoxyphenyl)-N-(3-phenylpropyl)isoxazol-5-amine ***(4 k)***.. Yield 64%; off white solid; mp 110–112 °C; IR (neat): 3281, 3234, 2938, 1633, 1610, 1477, 1252, 1032, 953, 838, 743, 696 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64–7.71 (m, 2H), 7.28–7.35 (m, 2H), 7.17–7.26 (m, 3H), 6.92–6.98 (m, 2H), 5.18 (s, 1H), 4.52 (s, 1H), 3.85 (s, 3H), 3.21–3.29 (m, 2H), 2.74 (t, J = 7.56 Hz, 2H), 1.94–2.04 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.3, 163.3, 160.8, 141.1, 128.6, 128.5, 128.0, 126.2, 122.6, 114.1, 75.0, 55.3, 44.0, 32.9, 30.9; HRMS (ESI) calculated for $C_{19}H_{20}N_2O_2$ $[M + H]^+$ 309.1603, found 309.1625.

6.1.1.11. 3-(4-Methoxyphenyl)-N-phenethylisoxazol-5-amine ***(4 l)***.. Yield 58%; off white solid; mp 95–98 °C; IR (neat): 3281, 3234, 2938, 1633, 1610, 1477, 1252, 1032, 953, 838, 743, 696 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66–7.74 (m, 2H), 7.31–7.39 (m, 2H), 7.27–7.30 (m, 1H), 7.21–7.27 (m, 2H), 6.91–7.00 (m, 2H), 5.24–5.28 (m, 1H), 4.49–4.57 (m, 1H), 3.84–3.87 (m, 3H), 3.47–3.54 (m, 2H), 2.96 (t, J = 6.95 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.1, 163.3, 160.9, 138.3, 128.9, 128.8, 128.0, 126.9, 122.5, 114.1, 75.3, 55.3, 45.7, 35.6; HRMS (ESI) calculated for $C_{18}H_{18}N_2O_2$ $[M + H]^+$ 295.1446, found 295.1467.

6.1.1.12. 3-(4-Methoxyphenyl)-N-(4-phenylbutyl)isoxazol-5-amine ***(4 m)***.. Yield 68%; off white solid; mp 102–104 °C; IR (neat): 3281, 3234, 2938, 1633, 1610, 1477, 1252, 1032, 953, 838, 743, 696 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65–7.72 (m, 2H), 7.27–7.34 (m, 2H), 7.16–7.23 (m, 3H), 6.92–6.99 (m, 2H), 5.16–5.24 (m, 1H), 4.42–4.51 (m, 1H), 3.82–3.89 (m, 3H), 3.18–3.30 (m, 2H), 2.68 (t, J = 7.20 Hz, 2H), 1.61–1.81 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.4, 163.3, 160.9, 141.9, 128.5, 128.4, 128.0, 126.0, 122.6, 114.1, 74.8, 55.3, 44.6, 35.4, 29.0, 28.4; HRMS (ESI) calculated for $C_{20}H_{22}N_2O_2$ $[M + H]^+$ 323.1696, found 323.1721.

6.1.1.13. 3-Cyclohexyl-N-(3-phenylpropyl)isoxazol-5-amine ***(4n)***.. Yield 71%; pale yellow oil; IR (neat) 3267, 3026, 2926, 2852, 1613, 1471, 1451, 1162, 1090, 740, 693 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28–7.34 (m, 2H), 7.16–7.25 (m, 3H), 4.77 (s, 1H), 4.40 (t, J = 6.46 Hz, 1H), 3.17 (q, J = 6.75 Hz, 2H), 2.71 (t, J = 7.56 Hz, 2H), 2.52–2.63 (m, 1H), 1.94 (quin, J = 7.32 Hz, 4H), 1.68–1.84 (m, 3H), 1.20–1.47 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.2, 169.7, 141.1, 128.6, 128.4, 126.2, 75.2, 43.9, 36.2, 32.8, 32.0, 30.9, 26.0, 25.9; HRMS (ESI) calculated for $C_{18}H_{24}N_2O$ $[M + H]^+$ 285.1967, found 285.1988.

6.1.1.14. N-(3-Phenylpropyl)-3-propylisoxazol-5-amine ***(4o)***.. Yield 42%; colorless oil; IR (neat) 3272, 3028, 2927, 2857, 1613, 1537, 1453, 1369, 1029, 697 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27–7.35 (m, 2H), 7.15–7.25 (m, 3H), 4.77 (s, 1H), 4.33–4.48 (m, 1H), 3.16 (q, J = 6.67 Hz, 2H), 2.70 (t, J = 7.56 Hz, 2H), 2.48 (t, J = 7.56 Hz, 2H), 1.88–2.00 (m, 2H), 1.65–1.70 (m, 1H), 1.59–1.64 (m, 1H), 0.92–1.04 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.9, 165.7, 141.1, 128.6, 128.4, 126.2, 76.6, 43.9, 32.8, 30.9, 28.3, 21.5, 13.7; HRMS (ESI) calculated for $C_{15}H_{20}N_2O$ $[M + H]^+$ 245.1654, found 245.1674.

6.1.1.15. 3-Ethyl-N-(3-phenylpropyl)isoxazol-5-amine ***(4p)***.. Yield 61%; colorless oil; IR (neat) 3275, 3026, 2923, 2856, 1613, 1538, 1453, 1369, 1029, 697 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27–7.34 (m, 2H), 7.15–7.24 (m, 3H), 4.79 (s, 1H), 4.42 (br. s., 1H), 3.17 (q, J = 6.75 Hz, 2H), 2.70 (t, J = 7.56 Hz, 2H), 2.54 (q, J = 7.72 Hz, 2H), 1.94 (td, J = 7.20, 14.88 Hz, 2H), 1.22 (t, J = 7.68 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.9, 167.0, 141.1, 128.6, 128.4, 126.2, 76.3, 43.9, 32.8, 30.9, 19.8, 12.5; HRMS (ESI) calculated for $C_{14}H_{18}N_2O$ $[M + H]^+$ 231.1497, found 231.1519.

6.1.1.16. 3-Methyl-N-(3-phenylpropyl)isoxazol-5-amine ***(4q)***.. Yield 72%; pale yellow oil; IR (neat): 3272, 3025, 2927, 2857, 1613, 1538, 1453, 1369, 1029, 697 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27–7.34 (m, 2H), 7.16–7.25 (m, 3H), 4.77 (s, 1H), 4.38–4.48 (m, 1H), 3.16 (q, J = 6.75 Hz, 2H), 2.70 (t, J = 7.56 Hz, 2H), 2.15 (s, 3H), 1.88–2.00 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.0, 161.5, 141.1, 128.6, 128.4, 126.2, 77.7, 43.9, 32.8, 30.9, 11.7; HRMS (ESI) calculated for $C_{13}H_{16}N_2O$ $[M + H]^+$ 217.1341, found 217.1363.

6.1.1.17. N-Phenethyl-3-(tetrahydro-2H-pyran-4-yl)isoxazol-5-amine ***(4r)***.. Yield 30%; colorless oil; IR (neat) 3276, 2931, 2849, 1611, 1444, 1238, 1126, 1086, 981, 890,695 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31–7.36 (m, 2H), 7.19–7.29 (m, 3H), 4.85 (s, 1H), 4.48 (br. s., 1H), 3.98–4.09 (m, 2H), 3.39–3.56 (m, 4H), 2.79–3.04 (m, 3H), 1.75–1.89

(m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.8, 168.6, 138.3, 128.8, 126.8, 75.3, 67.6, 45.6, 35.6, 33.4, 31.5; HRMS (ESI) calculated for $C_{16}H_{20}N_2O_2$ $[M + H]^+$ 273.1603, found 273.1626.

*6.1.1.18. N-(3-Phenylpropyl)-3-(tetrahydro-2H-pyran-4-yl)isoxazol-5-amine (**4 s**)*.. Yield 58%; off white solid; mp 80–84 °C; IR (neat) 3275, 2931, 2849, 1610, 1444, 1238, 1126, 1086, 981, 889,698 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28–7.34 (m, 2H), 7.17–7.25 (m, 3H), 4.79 (s, 1H), 4.47 (t, J = 6.10 Hz, 1H), 3.98–4.07 (m, 2H), 3.51 (dt, J = 3.17, 11.34 Hz, 2H), 3.13–3.22 (m, 2H), 2.79–2.91 (m, 1H), 2.71 (t, J = 7.56 Hz, 2H), 1.89–2.00 (m, 2H), 1.74–1.87 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.0, 168.6, 141.0, 128.6, 128.4, 126.2, 75.0, 74.9, 67.6, 43.9, 33.4, 33.4, 32.8, 31.5, 31.5, 30.9, 30.8; HRMS (ESI) calculated for $C_{17}H_{22}N_2O_2$ $[M + H]^+$ 287.1759, found 287.1779.

*6.1.1.19. N-(4-Phenylbutyl)-3-(tetrahydro-2H-pyran-4-yl)isoxazol-5-amine (**4 t**)*.. Yield 61%; colorless oil; IR (neat) 3277, 2930, 2849, 1611, 1444, 1238, 1126, 1086, 981, 889, 697 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27–7.32 (m, 2H), 7.16–7.23 (m, 3H), 4.81 (s, 1H), 4.38–4.46 (m, 1H), 3.99–4.07 (m, 2H), 3.46–3.56 (m, 2H), 3.17 (q, J = 6.67 Hz, 2H), 2.79–2.90 (m, 1H), 2.66 (t, J = 7.32 Hz, 2H), 1.62–1.88 (m, 8H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.0, 168.6, 141.9, 128.5, 128.4, 126.0, 74.9, 67.6, 44.4, 35.4, 33.4, 31.5, 31.4, 29.0, 28.4; HRMS (ESI) calculated for $C_{18}H_{24}N_2O_2$ $[M + H]^+$ 301.1916, found 301.1938.

*6.1.1.20. Methyl 4-(5-(3-phenylpropylamino)isoxazol-3-yl)piperidine-1-carboxylate (**4u**)*.. Yield 51%; colorless oil; IR (neat) 3265, 2935, 2849, 1698, 1611, 1560, 1508, 1403, 1278, 1324, 1176, 763, 698 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28–7.34 (m, 2H), 7.16–7.25 (m, 3H), 4.75 (s, 1H), 4.48 (t, J = 6.10 Hz, 1H), 4.19 (br. s., 2H), 3.71 (s, 3H), 3.17 (q, J = 6.75 Hz, 2H), 2.84–2.98 (m, 2H), 2.66–2.81 (m, 3H), 1.86–1.99 (m, 4H), 1.55–1.67 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.0, 168.3, 156.0, 141.0, 128.6, 128.4, 126.2, 75.0, 52.5, 43.9, 43.7, 34.3, 32.8, 30.9, 30.6; HRMS (ESI) calculated for $C_{19}H_{25}N_3O_3$ $[M + H]^+$ 344.1974, found 344.1995.

*6.1.1.21. N-(3-(4-Methoxyphenyl)propyl)-3-phenylisoxazol-5-amine (**4v**)*.. Yield 48%; off white solid; mp 81–85 °C; IR (neat): 3285, 3234, 2938, 1610, 1477, 1262, 1032, 953, 838, 743, 696 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70–7.78 (m, 2H), 7.38–7.46 (m, 3H), 7.08–7.15 (m, 2H), 6.82–6.90 (m, 2H), 5.22 (s, 1H), 4.55 (br. s., 1H), 3.81 (s, 3H), 3.24 (q, J = 6.67 Hz, 2H), 2.68 (t, J = 7.56 Hz, 2H), 1.90–2.00 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.4, 163.7, 158.1, 133.0, 130.0, 129.7, 129.4, 128.7, 126.6, 114.0, 75.1, 55.2, 43.9, 31.9, 31.1; HRMS (ESI) calculated for $C_{19}H_{20}N_2O_2$ $[M + H]^+$ 309.1603, found 309.1625.

*6.1.1.22. N,N-Dimethyl-4-(3-(3-phenylisoxazol-5-ylamino)propyl)benzene sulfonamide (**4w**)*.. Yield 59%; off white solid; mp 149–152 °C; IR (neat): 3255, 3075, 2937, 2918, 1609, 1548, 1494, 1452, 1094, 953, 833, 742, 676 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70–7.77 (m, 4H), 7.41–7.46 (m, 3H), 7.38 (d, J = 8.29 Hz, 2H), 5.26 (s, 1H), 4.60 (s, 1H), 3.30 (q, J = 6.59 Hz, 2H), 2.83 (t, J = 7.68 Hz, 2H), 2.71 (s, 6H), 1.96–2.09 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.2, 163.7, 146.5, 133.5, 129.8, 129.8, 129.0, 128.8, 128.1, 126.6, 75.3, 43.9, 37.9, 32.8, 30.6; HRMS (ESI) calculated for $C_{20}H_{23}N_3O_3S$ $[M + H]^+$ 386.1538, found 386.1561.

*6.1.1.23. N-(3-(4-Methoxyphenyl)propyl)-3-(pyridin-4-yl)isoxazol-5-amine (**4x**)*.. Yield 45%; pale yellow solid; mp 119–121 °C; IR (neat): 3285, 3234, 2938, 1610, 1560, 1508, 1477, 1262, 1032, 953, 838, 743, 696 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.66–8.72 (m, 2H), 7.58–7.64 (m, 2H), 7.08–7.15 (m, 2H), 6.82–6.90 (m, 2H), 5.24 (s, 1H), 4.72 (t, J = 5.73 Hz, 1H), 3.80 (s, 3H), 3.26 (q, J = 6.99 Hz, 2H), 2.69 (t, J = 7.44 Hz, 2H), 1.90–2.02 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.0, 161.6, 158.1, 150.4, 137.5, 132.9, 129.4, 120.9, 114.0, 74.9, 55.2, 43.9, 31.9, 31.0; HRMS (ESI) calculated for $C_{18}H_{19}N_3O_2$ $[M + H]^+$ 310.1555, found 310.1576.

*6.1.1.24. N,N-Dimethyl-4-(3-(3-(pyridin-4-yl)isoxazol-5-ylamino)propyl) benzene sulfonamide (**4y**)*. . Yield 63%; Pale yellow solid; mp122-125 °C; IR (neat): 3346, 2934, 1613, 1560, 1507, 1473, 1458, 1324, 1160, 953, 833, 742, 676 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.66–8.73 (m, 2H), 7.70–7.76 (m, J = 8.29 Hz, 2H), 7.59–7.63 (m, 2H), 7.34–7.40 (m, J = 8.29 Hz, 2H), 5.30 (s, 1H), 4.78 (t, J = 6.22 Hz, 1H), 3.31 (q, J = 6.83 Hz, 2H), 2.79–2.86 (m, 2H), 2.70–2.73 (m, 7H), 1.97–2.09 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8, 161.7, 150.5, 146.3, 137.4, 133.6, 129.0, 128.2, 120.9, 75.1, 43.8, 37.9, 32.7, 30.6; HRMS (ESI) calculated for $C_{19}H_{22}N_4O_3S$ $[M + H]^+$ 387.1491, found 387.1515.

*6.1.1.25. Methyl4-(5-(3-(4-methoxyphenyl) propylamino)isoxazol-3-yl) piperidine-1-carboxylate (**4z**)*. . Yield 55%; colorless oil; IR (neat): 3269, 2938, 2847, 1725, 1611, 1560, 1508, 1403, 1278, 1324, 1176, 953, 833, 763, 676 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.05–7.14 (m, 2H), 6.81–6.87 (m, 2H), 4.75 (s, 1H), 4.46 (t, J = 6.22 Hz, 1H), 4.17 (br. s., 2H), 3.80 (s, 3H), 3.71 (s, 3H), 3.15 (q, J = 6.67 Hz, 2H), 2.83–2.97 (m, 2H), 2.76 (tt, J = 3.78, 11.59 Hz, 1H), 2.64 (t, J = 7.56 Hz, 2H), 1.90 (quin, J = 7.26 Hz, 4H), 1.54–1.69 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.0, 168.3, 158.1, 156.0, 133.0, 129.3, 114.0, 75.0, 55.2, 52.6, 43.8, 43.7, 34.3, 31.9, 31.1, 30.6; HRMS (ESI) calculated for $C_{20}H_{27}N_3O_4$[M + H]$^+$374.2080, found 374.2101.

*6.1.1.26. Methyl 4-(5-(3-(4-(N,N-dimethylsulfamoyl)phenyl)propylamino) isoxazol-3-yl)piperidine-1-carboxylate (**4ab**)*. . Yield 61%; colorless oil; IR (neat): 3265, 2935, 2849, 1725, 1611, 1560, 1508, 1403, 1278, 1324, 1176, 953, 833, 763, 676 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67–7.74 (m, 2H), 7.36 (d, J = 8.05 Hz, 2H), 4.78–4.82 (m, 1H), 4.53 (t, J = 6.22 Hz, 1H), 3.69–3.72 (m, 3H), 3.21 (q, J = 6.67 Hz, 2H), 2.84–2.98 (m, 2H), 2.73–2.83 (m, 3H), 2.68–2.72 (m, 7H), 1.86–2.08 (m, 4H), 1.64–1.69 (m, 1H), 1.55–1.62 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.8, 168.4, 156.0, 146.5, 133.5, 129.0, 128.1, 75.2, 52.6, 43.8, 43.7, 37.9, 34.3, 32.8, 30.6; HRMS (ESI) calculated for $C_{21}H_{30}N_4O_5S$ $[M + H]^+$ 451.2015, found 451.2039.

*6.1.2. Preparation of **4d***

To a stirred solution of 3-phenylisoxazol-5-amine (**10a**, 1.87 mmol) in DMF (5 mL), 60% NaH (2.81 mmol) and benzyl bromide (1.87) were added at 0 °C. The resulting solution was stirred at room temperature for 16 h. The reaction mixture was quenched with ice cold water, and extracted with EtOAc (2x times). The combined ethyl acetate layers were dried over anhydrous Na_2SO_4 and then concentrated under reduced pressure. The crude mixture was subjected to flash silica gel (230–400 mesh) column chromatography (eluting with 5–10% EtOAc in hexanes) to afford the mono- and di-benzylated products.

*6.1.2.1. N-benzyl-3-phenylisoxazol-5-amine (**4d**)*[27]. . Yield 35%; off white solid; mp 91–93 °C; IR (neat) 3265, 2935, 2849, 1611, 1403, 1258, 1176, 1081, 763, 698 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70–7.77 (m, 2H), 7.40–7.46 (m, 3H), 7.30–7.39 (m, 5H), 5.31 (s, 1H), 4.88–4.97 (m, 1H), 4.44 (d, J = 5.85 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.3, 163.7, 137.5, 129.9, 129.7, 128.9, 128.7, 127.9, 127.4, 126.7, 76.0, 48.6; HRMS (ESI) calculated for $C_{16}H_{14}N_2O$ $[M + H]^+$ 251.1184, found 251.1205.

*6.1.2.2. N,N-Dibenzyl-3-phenylisoxazol-5-amine (**4da**)*[40].. Yield 25%; pale yellow solid; mp 77–79 °C; IR (neat): 3027, 2935, 2849, 1611, 1403, 1258, 1176, 1081, 763, 698 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72–7.78 (m, 2H), 7.39–7.45 (m, 3H), 7.25–7.39 (m, 10*H*), 5.30 (s, 1H), 4.53 (s, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.0, 164.0, 136.5, 130.1, 129.7, 128.8, 128.7, 127.8, 126.7, 126.6, 75.6, 52.3; HRMS (ESI) calculated for $C_{16}H_{14}N_2O$ $[M + H]^+$ 251.1184, found 251.1205.

*6.1.3. Preparation of N-methyl-3-phenyl-N-(3-phenylpropyl)isoxazol-5-amine (**5**).*

To a stirred solution of 3-phenyl-N-(3-phenylpropyl)isoxazol-5-amine (**4a)** (0.36 mmol) in THF (5 mL), 60% NaH (0.716 mmol) and MeI (0.716 mmol) were added at 0 °C. The resulting reaction mixture was stirred at rt for 16 h. The reaction mixture was quenched with ice cold water, and then extracted with EtOAc (2x times). The combined organic layers were dried over anhydrous Na_2SO_4 and then concentrated under reduced pressure. The crude residue was subjected to flash silica gel (230–400 mesh) column chromatography (eluting with 5–10% EtOAc in hexanes) to afford the title compound **5**. Yield 58%; colorless oil; IR (neat) 2938, 2848, 1611, 1403, 1258, 1176, 1081, 763, 698 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.67–7.81 (m, 2H), 7.37–7.49 (m, 3H), 7.28–7.35 (m, 2H), 7.16–7.26 (m, 3H), 5.11 (s, 1H), 3.29–3.45 (m, 2H), 2.97–3.05 (m, 3H), 2.69 (t, J = 7.68 Hz, 2H), 1.91–2.07 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.0, 163.8, 141.3, 130.2, 129.6, 128.7, 128.5, 128.4, 126.6, 126.1, 74.6, 51.0, 36.4, 32.9, 28.7; HRMS (ESI) calculated for $C_{19}H_{20}N_2O$ $[M + H]^+$ 293.1654, found 293.1675.

*6.1.4. General procedure for the preparation of **6a,6b**, and **6e***

To a stirred solution of the corresponding isoxazol-5-amine **10** (1.25 mmol) in DMF (10 mL), DMAP (1.5 mmol), appropriate acid **12** (1.25 mmol) and EDC.HCl (1.5 mmol) were added. The resulting solution was stirred at 50 °C for 16 h. The reaction mixture was diluted with EtOAc, washed with water (3x times), dried over anhydrous Na_2SO_4 and then concentrated under reduced pressure. The crude residue was subjected to flash silica gel (230–400 mesh) column chromatography (eluting with 10–15% EtOAc in hexanes) to afford the title compounds.

*6.1.4.1. 2-Phenyl-N-(3-phenylisoxazol-5-yl)acetamide **(6a)**[41]..* This compound was prepared by reaction of **10a** with 2-phenylacetic acid (**12a**). Yield 41%; off white solid; mp 124–126 °C; IR (neat) 3257, 3028, 2937, 1695, 1613, 1538, 1403, 1255, 1222, 1202, 1176, 1082, 763, 690 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.07 (br. s., 1H), 7.75–7.83 (m, 2H), 7.36–7.49 (m, 6H), 7.34 (dd, J = 1.59, 7.93 Hz, 2H), 6.75 (s, 1H), 3.83 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.0, 163.9, 160.2, 132.9, 130.2, 129.6, 129.6, 129.0, 128.9, 128.3, 126.8, 87.1, 43.9; HRMS (ESI) calculated for $C_{17}H_{14}N_2O_2$ $[M + H]^+$ 279.1133, found 279.1156.

*6.1.4.2. 3-Phenyl-N-(3-phenylisoxazol-5-yl)propanamide **(6b)**..* This compound was prepared by reaction of **10a** with 3-phenylpropanoic acid (**12b**). Yield 52%; off white solid; mp 136–138 °C; IR (neat): 3256, 3027, 2938, 1693, 1612, 1538, 1403, 1255, 1222, 1202, 1176, 1082, 763, 691 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.11–8.20 (m, 1H), 7.81 (m, 2H), 7.46 (m, 3H), 7.29–7.35 (m, 2H), 7.20–7.27 (m, 3H), 6.74 (s, 1H), 3.08 (t, J = 7.56 Hz, 2H), 2.77 (t, J = 7.68 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.2, 163.9, 160.2, 139.8, 130.3, 129.0, 128.9, 128.8, 128.3, 126.8, 126.7, 87.1, 38.4, 30.9; HRMS (ESI) calculated for $C_{18}H_{16}N_2O_2$[M + H]$^+$293.1290, found 293.1311.

*6.1.4.3. 3-Phenyl-N-(3-(tetrahydro-2H-pyran-4-yl)isoxazol-5-yl) propanamide **(6e)**..* This compound was prepared by reaction of **10 l** with 3-phenylpropanoic acid (**12b**). Yield 51%; off white solid; mp159-162 °C; IR (neat): 3256, 3029, 2929, 2849, 1613, 1403, 1237, 1201, 1175, 1081, 763, 690 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.26 (br. s., 1H), 7.16–7.38 (m, 6H), 6.28 (s, 1H), 3.97–4.12 (m, 2H), 3.44–3.61 (m, 2H), 3.01–3.13 (m, 2H), 2.88–3.01 (m, 1H), 2.66–2.80 (m, 2H), 1.75–1.94 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.6, 159.9, 139.9, 128.8, 128.3, 126.7, 87.2, 67.5, 38.3, 33.5, 31.1, 30.9; HRMS (ESI) calculated for $C_{17}H_{20}N_2O_3$ $[M + H]^+$ 301.1552, found 301.1573.

*6.1.5. General procedure for the preparation of **6c**, and **6d***

To a stirred solution of the corresponding acid **12** (1.25 mmol) in DCM (5 mL), $SOCl_2$ (3.75 mmol) was added at 0 °C. The resulting reaction mixture was refluxed for 5 h. After cooling to room temperature, the reaction was concentrated under reduced pressure to afford crude compound. The crude compound was dissolved in pyridine (5 mL), and added 3-phenylisoxazol-5-amine **10a** (1.25 mmol). The resulting reaction mixture was stirred at 80 °C for 16 h. After cooling to rt, the reaction was concentrated under reduced pressure and then diluted with EtOAc. The organic phase was washed with 1 *N* HCl solution (2x times), dried over anhydrous Na_2SO_4 and then concentrated under reduced pressure. The crude residue was subjected to flash silica gel (230–400 mesh) column chromatography (eluting with 10–15% EtOAc in hexanes) to afford the title compounds.

*6.1.5.1. 4-Phenyl-N-(3-phenylisoxazol-5-yl)butanamide **(6c)**..* This compound was prepared by reaction of **10a** with 4-phenylbutanoic acid (**12c**). Yield 47%; off white solid; mp 89–92 °C; IR (neat) 3256, 3022, 2926, 1690, 1611, 1538, 1403, 1255, 1205, 1177, 1085, 763, 692 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.11 (br. s., 1H), 7.76–7.86 (m, 2H), 7.43–7.50 (m, 3H), 7.28–7.35 (m, 2H), 7.17–7.26 (m, 3H), 6.73 (s, 1H), 2.73 (t, J = 7.44 Hz, 2H), 2.44 (t, J = 7.44 Hz, 2H), 2.06–2.15 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ168.7, 163.9, 160.3, 140.9, 130.2, 129.0, 128.9, 128.6, 128.5, 126.9, 126.8, 126.8, 126.3, 87.0, 35.7, 34.8, 26.2; HRMS (ESI) calculated for $C_{19}H_{18}N_2O_2$ $[M + H]^+$ 307.1446, found 307.1469.

*6.1.5.2. 5-Phenyl-N-(3-phenylisoxazol-5-yl)pentanamide **(6d)**..* This compound was prepared by reaction of **10a** with 5-phenylpentanoic acid (**12d**). Yield 56%; off white solid; mp 141–143 °C; IR (neat): 3255, 3030, 2937, 1693, 1613, 1538, 1403, 1255, 1201, 1176, 1082, 763, 690 cm^{-1};^1H NMR (400 MHz, $CDCl_3$) δ 8.21 (br. s., 1H), 7.78–7.85 (m, 2H), 7.43–7.50 (m, 3H), 7.27–7.32 (m, 2H), 7.16–7.23 (m, 3H), 6.73 (s, 1H), 2.67 (t, J = 7.32 Hz, 2H), 2.43–2.49 (t, J = 8 Hz, 2H), 1.67–1.84 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.9, 163.9, 160.4, 141.8, 130.2, 129.1, 128.9, 128.5, 128.4, 126.9, 126.8, 126.8, 126.0, 87.0, 36.6, 35.5, 30.7, 24.6; HRMS (ESI) calculated for $C_{20}H_{20}N_2O_2$ $[M + H]^+$ 321.1603, found 321.1624.

*6.1.6. Preparation of N-(3-(3-phenylpropyl)isoxazol-5-yl)benzamide **(6f)***

To a stirred solution of 3-(3-phenylpropyl)isoxazol-5-amine **10d** (1.24 mmol) in pyridine (5 mL), benzoyl chloride **12e** (1.48 mmol) was added at 0 °C. The resulting reaction mixture was stirred at 80 °C for 16 h. After cooling to rt, the reaction was concentrated under reduced pressure, and then diluted with EtOAc. The organic phase was washed with 1 *N* HCl solution (2x times), dried over anhydrous Na_2SO_4 and then concentrated under reduced pressure. The crude residue was subjected to flash silica gel (230–400 mesh) column chromatography (eluting with 10–15% EtOAc in hexanes) to afford the title compound. Yield 67%; White solid; mp: 122–125 °C; IR (neat): 3250, 3025, 2936, 1685, 1610, 1535, 1403, 1255, 1201, 1177, 1085, 763, 690 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.67 (s, 1H), 7.87–7.93 (m, 2H), 7.60–7.66 (m, 1H), 7.50–7.57 (m, 2H), 7.28–7.33 (m, 2H), 7.21 (m, 3H), 6.43 (s, 1H), 2.71 (m, 4H), 1.99–2.10 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.7, 163.2, 160.2, 141.5, 133.0, 132.4, 129.1, 128.5, 128.4, 127.4, 126.0, 88.8, 35.1, 29.5, 25.8; HRMS (ESI) calculated for $C_{19}H_{18}N_2O_2$ $[M + H]^+$ 307.1446, found 307.1468.

*6.1.7. General procedure for the preparation of **7***

To a stirred solution of 3-phenylisoxazol-5-amine **10a** (1.87 mmol) in THF (10 mL), DIPEA (3.74 mmol) and triphosgene (0.75 mmol) were added at 0 °C. The reaction mixture was stirred at 0 °C for 30 min. The appropriate amine **13** (1.87 mmol) was added and then the mixture was allowed to warm to room temperature and stirred for 16 h. The reaction mixture was quenched with 10% $NaHCO_3$ solution and extracted with EtOAc (3x times). The combined organic extracts were dried over anhydrous Na_2SO_4 and then concentrated under reduced pressure. The crude residue was subjected to flash silica gel (230–400 mesh) column chromatography (eluting with 2–5% MeOH in DCM) to afford the title compounds.

*6.1.7.1. 1-Benzyl-3-(3-phenylisoxazol-5-yl)urea (**7a**).*. Yield 49%; off white solid; mp 148–150 °C; IR (neat) 3322, 3273, 3060, 2948, 1662, 1624, 1573, 1473, 1278, 1261, 752, 687 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.65 (br. s., 1H), 7.66–7.73 (m, 2H), 7.34–7.46 (m, 3H), 7.27–7.30 (m, 1H), 7.18–7.27 (m, 4H), 6.41 (s, 1H), 6.08–6.17 (m, 1H), 4.38 (d, J = 5.85 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.1, 162.5, 152.6, 138.0, 130.3, 129.0, 128.8, 127.6, 127.4, 126.8, 84.7, 44.2; HRMS (ESI) calculated for $C_{17}H_{15}N_3O_2$ $[M + H]^+$ 294.1242, found 294.1266.

*6.1.7.2. 1-Phenethyl-3-(3-phenylisoxazol-5-yl)urea (**7b**).*. Yield 58%; off white solid; mp 110–114 °C; IR (neat): 3323, 3276, 3061, 2948, 1660, 1625, 1573, 1473, 1278, 1261, 752, 687 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.39 (br. s., 1H), 7.70–7.78 (m, 2H), 7.40–7.51 (m, 3H), 7.27–7.30 (m, 1H), 7.25 (s, 1H), 7.13–7.23 (m, 3H), 6.38 (s, 1H), 5.64 (br. s., 1H), 3.45–3.56 (m, 2H), 2.80 (t, J = 6.95 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.0, 162.4, 152.3, 138.5, 130.3, 129.1, 129.0, 128.8, 128.8, 126.8, 126.8, 84.7, 41.6, 35.8; HRMS (ESI) calculated for $C_{18}H_{17}N_3O_2$ $[M + H]^+$ 308.1399, found 308.1421.

*6.1.7.3. 1-(3-Phenylisoxazol-5-yl)-3-(3-phenylpropyl)urea (**7c**).*. Yield 45%; off white solid; mp 97–100 °C; IR (neat): 3323, 3276, 3061, 2948, 1660, 1625, 1573, 1473, 1278, 1261, 752, 687 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.55 (br. s., 1H), 7.70–7.80 (m, 2H), 7.37–7.49 (m, 3H), 7.22–7.27 (m, 2H), 7.08–7.21 (m, 3H), 6.42 (s, 1H), 5.67–5.79 (m, 1H), 3.22–3.33 (m, 2H), 2.61 (t, J = 7.68 Hz, 2H), 1.79–1.86 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.1, 162.6, 152.5, 141.2, 130.3, 129.0, 128.5, 128.4, 126.8, 126.8, 126.1, 84.6, 40.0, 33.0, 31.2; HRMS (ESI) calculated for $C_{19}H_{19}N_3O_2$ $[M + H]^+$ 322.1555, found 322.1578.

*6.1.8. General procedure for the preparation of **10***

Step 1: A stirred suspension of NaH (15.6 mmol, 60% dispersion in mineral oil) in THF was heated to 50 °C. To this was added a mixture of ester **8** (10.40 mmol) and acetonitrile (15.60 mmol) dropwise over the course of 15 min. The resulting suspension was heated at reflux temperature for a further 16 h. After cooling to rt, the reaction mixture was poured into H_2O and then the resulting solution was washed with diethyl ether (2x times) (ether extract discarded). The aqueous layer was separated, acidified to pH ~ 2 with aqueous 2 *M* HCl, and extracted with EtOAc (2x times). The combined EtOAc layers were dried over anhydrous Na_2SO_4 and then concentrated under reduced pressure to afford the title compounds **9**, which is used for the next step without further purification.

Step 2: To a stirred solution of nitrile compound **9** (10.14 mmol) and NaOH (20.80 mmol) in water (20 mL), hydroxylamine hydrochloride (11.16 mmol) was added. The resulting mixture was heated at 100 °C for 3 h. After cooling to rt, the precipitated compound was filtered and washed with water and dried under vacuum to afford the title compound **10**. Those which did not obtain precipitation, the mixture was diluted with EtOAc and the organic layer was separated. The aqueous layer was further extracted with EtOAc (2x times), the combined organic layers were dried over anhydrous Na_2SO_4 and then concentrated under reduced pressure to afford the title compound **10**

*6.1.8.1. 3-Phenylisoxazol-5-amine (**10a**)*[25].. Yield 82%; Light brown solid; mp 102 – 104 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 7.70–7.79 (m, 2H), 7.36–7.50 (m, 3H), 5.46 (s, 1H), 4.54 (br. s., 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.9, 164.0, 129.8, 128.8, 126.7, 78.2 (one quaternary peak could be merged with 128.8 peak).

*6.1.8.2. 3-Benzylisoxazol-5-amine (**10b**)*[26].. Yield 65%; Pale yellow solid; mp 60 – 62 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 7.29–7.34 (m, 2H), 7.21–7.28 (m, 3H), 4.89 (s, 1H), 4.30–4.42 (m, 2H), 3.86 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.5, 164.9, 137.7, 128.8, 128.6, 126.7, 80.0, 32.7.

*6.1.8.3. 3-Phenethylisoxazol-5-amine (**10c**)*[27].. Yield 61%; Pale yellow solid; mp 78 – 80 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 7.28–7.35 (m, 2H), 7.19–7.26 (m, 3H), 4.94 (s, 1H), 4.38 (br. s., 2H), 2.90–3.01 (m, 2H), 2.79–2.89 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.4, 165.2, 141.0, 128.5, 128.4, 126.2, 79.9, 34.2, 28.1.

*6.1.8.4. 3-(3-Phenylpropyl)isoxazol-5-amine (**10d**)*[28].. Yield 70%; Light yellow solid; ^1H NMR (400 MHz, $CDCl_3$) δ 7.28–7.32 (m, 2H), 7.16–7.23 (m, 3H), 4.99 (s, 1H), 4.37 (br. s., 2H), 2.67–2.70 (t, J = 8 Hz, 2H), 2.54 – 2.58 (t, J = 7.56 Hz, 2H), 1.96 (quin, J = 7.68 Hz, 2H).

*6.1.8.5. 3-(Pyridin-4-yl)isoxazol-5-amine (**10e**)*[29].. Yield 61%; Pale yellow solid; mp 186 – 188 °C; ^1H NMR (400 MHz, DMSO-d_6) δ 8.66 (d, J = 5.37 Hz, 2H), 7.70 (d, J = 5.37 Hz, 2H), 6.93 (s, 2H), 5.52 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.7, 160.9, 150.4, 137.2, 120.6, 75.2.

*6.1.8.6. 3-(4-Chlorophenyl)isoxazol-5-amine (**10f**)*[27].. Yield 75%; Pale yellow solid; mp 168 – 170 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 7.67 (d, J = 8.54 Hz, 2H), 7.40 (d, J = 8.78 Hz, 2H), 5.42 (s, 1H), 4.55 (br. s., 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.0, 163.0, 135.8, 129.0, 128.3, 127.9, 78.1.

*6.1.8.7. 3-(4-Methoxyphenyl)isoxazol-5-amine (**10 g**)*[27].. Yield 78%; Pale yellow solid; mp 135 – 137 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 7.63–7.73 (m, 2H), 6.90–6.99 (m, 2H), 5.40 (s, 1H), 4.50 (br. s., 2H), 3.85 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.8, 163.6, 160.9, 128.0, 122.3, 114.2, 78.1, 55.3.

*6.1.8.8. 3-Cyclohexylisoxazol-5-amine (**10 h**)*[27].. Yield 81%; Off white solid; mp 136 – 138 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 4.99 (s, 1H), 4.37 (br. s., 2H), 2.50–2.65 (m, 1H), 1.86–1.99 (m, 2H), 1.67–1.85 (m, 3H), 1.19–1.45 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5, 168.1, 78.4, 36.2, 31.9, 25.9, 25.9.

*6.1.8.9. 3-Propylisoxazol-5-amine (**10i**).*. Yield 75%; Light yellow crystals; mp 61 – 63 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 4.99 (s, 1H), 4.38 (br. s., 2H), 2.49 (t, J = 7.56 Hz, 2H), 1.59–1.70 (m, 2H), 0.97 (t, J = 7.44 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.3, 165.9, 79.8, 28.3, 21.4, 13.6.

*6.1.8.10.3-Ethylisoxazol-5-amine (**10j**)*[30].

Yield 68%; Light brown oil; ^1H NMR (400 MHz, $CDCl_3$) δ 5.00 (s, 1H), 4.39 (br. s., 2H), 2.55 (q, J = 7.64 Hz, 2H), 1.22 (t, J = 7.56 Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.3, 167.2, 79.4, 29.6, 19.8.

*6.1.8.10. 3-Methylisoxazol-5-amine (**10 k**)*[25].. Yield 71%; Off white solid; mp 83 – 85 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 4.98 (s, 1H), 4.39 (br. s., 2H), 2.16 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.5, 161.7, 80.7, 11.6.

*6.1.8.11. 3-(Tetrahydro-2H-pyran-4-yl)isoxazol-5-amine (**10 l**)*[31].. Yield 70%; White solid; mp 156 – 158 °C; ^1H NMR (400 MHz, DMSO-d_6) δ 6.52 (s, 2H), 4.85 (s, 1H), 3.82–3.91 (m, 2H), 3.35–3.43 (m, 2H), 2.71 (tt, J = 4.02, 11.46 Hz, 1H), 1.66–1.77 (m, 2H), 1.51–1.65 (m, 2H); ^1H NMR (400 MHz, $CDCl_3$) δ 5.00 (s, 1H), 4.48 (br. s., 2H), 3.99–4.05 (m, 2H), 3.50 (dt, J = 2.93, 11.34 Hz, 2H), 2.81–2.89 (m, 1H), 1.75–1.86 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.8, 168.4, 78.2, 67.6, 33.4, 31.4.

*6.1.8.12. Methyl 4-(5-aminoisoxazol-3-yl)piperidine-1-carboxylate (**10 m**).*. For the preparation of this compound we used dimethyl piperidine-1,4-dicarboxylate(**8 m**) as a starting material. Surprisingly, we obtained decarboxylated product 3-(piperidin-4-yl)isoxazol-5-amine (**10 m-In**). ^1H NMR (400 MHz, $CDCl_3$) δ 5.01 (s, 1H), 4.44 (br. s., 2H), 3.15 (td, J = 3.38, 12.50 Hz, 2H), 2.66–2.78 (m, 3H), 0.78–0.91 (m, 4H).

Furtherly, the compound **10 m-In** was reacted with chloroformate at 0 °C to afford the desired compound **10 m**.

*6.1.8.12.1. Preparation of **10 m** from **10 m to In.***. To a solution of **10 m-In** (2.99 mmol) in DCM (10 mL), DIPEA (4.49 mmol) and methyl chloroformate (2.99 mmol) at 0 °C. The resulting solution was stirred at room temperature for 3 h. The reaction solution was diluted with DCM, washed with water, dried over anhydrous Na_2SO_4 and then concentrated under reduced pressure to afford the title compound as pale yellow solid. Yield 40%; Pale yellow solid; mp 96 – 98 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.97 (s, 1H), 4.48 (br. s., 2H), 4.16 (br. s., 2H), 3.70 (s, 3H), 2.90 (t, J = 12.20 Hz, 2H), 2.77 (tt, J = 3.72, 11.52 Hz, 1H), 1.90 (dd, J = 2.20, 13.17 Hz, 2H), 1.56–1.66 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.5, 168.5, 156.0, 78.1, 52.6, 43.7, 34.2, 30.6.

*6.1.9. Procedure for the preparation of 3-(4-methoxyphenyl)propyl methanesulfonate **11e***[42]

To a solution of 3-(4-methoxyphenyl)propan-1-ol (12.03 mmol) in DCM (20 mL), TEA (24.06 mmol) and methanesulfonyl chloride (14.43 mmol) were added at 0 °C. The solution was stirred for 3 h at rt, diluted with DCM (50 mL), washed with water (2x50 mL), dried over anhydrous Na_2SO_4 and then concentrated under reduced pressure to get the title compound as brownish oil. The crude product was used for the next step without further purification. Yield: 80%.

*6.1.10. Preparation of 4-(3-bromopropyl)-N,N-dimethylbenzenesulfonamide **(11f)***

*6.1.10.1. Preparation of 4-(3-bromopropyl)benzenesulfonyl chloride **(15)***.. Chlorosulfonic acid (6 mL) was taken into a 100 mL round bottom flask, a solution of (3-bromopropyl)benzene**14** (10.05 mmol) in DCM (1 mL) was added at 0 °C. The resulting solution was stirred for 16 h at rt. The reaction solution was slowly poured into crushed ice, and extracted with DCM (2x times). The combined organic layers were dried over anhydrous Na_2SO_4 and then concentrated under reduced pressure to afford the title crude compound 4-(3-bromopropyl)benzene-1-sulfonyl chloride **15** as a brownish oil. The crude product was used for the next step without further purification. Yield 70%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96–8.01 (d, J = 8.54 Hz, 2H), 7.43–7.49 (d, J = 8.54 Hz, 2H), 3.41 (t, J = 6.34 Hz, 2H), 2.94 (t, J = 7.56 Hz, 2H), 2.16–2.27 (m, 2H).

*6.1.10.2. Preparation of 4-(3-bromopropyl)-N,N-dimethylbenzene-sulfonamide **(11f)***.. To a solution of **15** (6.73 mmol) in DCM (20 mL), TEA (20.20 mmol) and dimethyl amine hydrochloride (10.10 mmol) were added at 0 °C. The resulting solution was stirred for 3 h at rt. The reaction solution was diluted with DCM, washed with water, dried over anhydrous Na_2SO_4 and then concentrated under reduced pressure to afford the title compound as light brown solid. The crude product was used for the next step without further purification. Yield 90%; Light Brown solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69–7.74 (d, J = 8.29 Hz, 2H), 7.36–7.41 (d, J = 8.29 Hz, 2H), 3.41 (t, J = 6.46 Hz, 2H), 2.88 (t, J = 7.44 Hz, 2H), 2.68–2.74 (s, 6H), 2.20 (m, 2H).

6.2. Biology

6.2.1. Sarcomere assay method for the measurement of myosin ATPase activity

Actin stimulated ATPase activity was examined spectrophotometrically as reported previously[32] with modifications. The detailed procedure is as follows; The standard reaction solution contained 20 mM Tris HCl (pH 7.5), 6 mM $MgCl_2$,15 mM KCl, S1 myosin (CS-MYS03), 1 mM ATP and actin thin filament complex (CS-TFC01) with pCa = 6.5.[43] The reaction was stopped by adding of Cytophos reagent (Cytoskeleton BK054 kit) after 10 min incubation at rt, samples were examined for inorganic phosphate liberated by taking the absorbance at 650 nm on TECAN Infinite. OM was used as a positive control for the selectivity assay. Blank had buffer, Cytophos and ATP mixture while DMSO was used as a negative control.

$$\text{Activity (\%)} = (\text{Mean A} - \text{B}) - (\text{NC} - \text{B})/(\text{NC} - \text{B}) \times 100$$

where, A = Absorbance; B = Blank; NC = Negative control

6.2.2. Selectivity studies

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

6.2.2.1. Smooth muscle. The standard reaction solution contained 20 mM Tris HCl (pH 7.5), 6 mM $MgCl_2$, 15 mM KCl, 1 mM ATP, S1 myosin (CS-MYS05), actin (AD 99) and Tropomyosin (T2400) with pCa = 6.5. The reaction was stopped by adding of Cytophos reagent (Cytoskeleton BK054 kit) After 10 min incubation at rt, samples were examined for inorganic phosphate liberated by taking absorbance at 650 nm on TECAN Infinite. Blebbistatin was used as a negative control for selectivity assay.

6.2.2.2. Skeletal muscle. The standard reaction solution contained 20 mM Tris HCl (pH 7.5), 6 mM $MgCl_2$, 15 mM KCl, 1 mM ATP, S1 myosin (CS-MYS04) and actin thin filament complex (CS-TFC01) with pCa = 6.5. The reaction was stopped by adding of Cytophos reagent (Cytoskeleton BK054 kit) After 10 min incubation at rt, 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.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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