Synthesis of N^{α}-protected aminoacid/peptide Weinreb amides employing *N,N′*-carbonyldiimidazole as activating agent; studies on docking and antibacterial activities

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Abstract

An efficient method for the synthesis of N^{α}-protected amino/ peptide Weinreb amides (*N*-methoxy-*N*-methylamides) employing *N,N′*-carbonyldiimidazole (CDI) has been achieved. N^{α}-protected amino/peptide acids were treated with *N,N′*-carbonyldiimidazole, followed by the addition of *N,O*-dimethylhydroxylamine hydrochloride salt to yield the desired compounds. The synthesized compounds were mainly gums, a few were solids, after the simple workup, and were characterized by IR, ^{1}H NMR, ^{13}C NMR and HRMS. The Weinreb amides were subjected to *in silico* studies, to predict the preferred orientation and binding affinity between the molecules using scoring functions. The ligand *N*-Fmoc-L-Phe-N(OCH_3)CH_3 showed minimum binding energy -29.85 kcal/mol with *Escherichia coli* and the ligand *N*-Fmoc-L-Ala-N(OCH_3)CH_3 showed minimum binding energy -24.79 kcal/mol with *Pseudomonas aeruginosa*, -25.01 kcal/mol with *Staphylococcus aureus*. Based on the minimum binding energies, antibacterial activities have been conducted for a few of the synthesized compounds.

Key words: Weinreb amides, *N,O*-dimethylhydroxylamine hydrochloride, *N,N′*-carbonyl-diimidazole, *in silico* molecular docking studies, *in vitro* antibacterial activities

Introduction

Weinreb amides have wide importance in organic synthesis due to their versatile reactivity with nucleophiles and selective reduction to aldehydes. Weinreb amides derived from amino acids have been extensively used as precursors in the preparation of α-amino aldehydes and α-amino ketones. They are selectively reduced to aldehydes using $LiAlH_4$ and form ketones upon reaction with

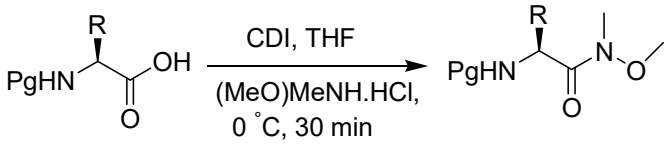
Grignard reagents. These *α*-amino ketones are the intermediates in the synthesis of many drugs and biologically active compounds.[1,2,8] They are also useful in the synthesis of acetylenes which are starting materials for the popular click reactions.

The reported protocols to convert carboxylic acids into the corresponding Weinreb amides follow the method of activation of the carboxylic group mainly *via* mixed anhydride using chloroformates followed by coupling with *N,O*-dimethylhydroxylamine. Quite a few common peptide coupling reagents such as BOP, DCC, CDMT, DMTMM, COMU *etc*., are alternatives for this transformation.[3,12] One-pot synthesis of *N*-Boc α-amino Weinreb amides using acid fluorides as key intermediates employing [bis-(2-methoxyethyl)amino]sulfur trifluoride (Deoxo-fluor) has also been discussed.[4,5] Sureshbabu *et al.* synthesized Weinreb amides employing acid chlorides as key intermediates. Further the use of carboxylic acid-activating reagents like *N*,*N*′-dicyclohexylcarbodiimide, propylphosphonic anhydride/*N*-ethylmorpholine, *N*-benzotriazole derivatives, *S*,*S*′-di-(2-pyridyl)dithiocarbonate, and 2-bromo-1-methylpyridinium iodide are reported.[6] Palladium-catalyzed preparation of Weinreb amides from boronic acids is described.[7] Deagostino and co-workers developed a protocol for the synthesis of Weinreb amides *via* Pd-catalyzed aminocarbonylation of heterocyclic-derived triflates.[9] The combination of PPh_3/I_2 and T3P/DBU has proved to be effective for conversion of N^{α}-protected amino acids into the corresponding Weinreb amides.[10,11] However, many of the procedures mentioned are unattractive due to disadvantages such as longer reaction times, low yields, multi-step reactions, *etc*.

In this paper we report the activation of carboxylic acids with *N,N*′-carbonyldiimidazole (CDI). Carbonyl diimidazole is a useful coupling reagent that promotes peptide bond formation and is also used for the preparation of ureas and carbamates from amines and alcohols respectively.[13] Aspirin prodrugs in one pot were synthesized employing CDI.[14] This reagent is commonly used on a large scale in peptide chemistry and its use can be extended to the formation of esters and thioesters.[15] The reaction of secondary amines using *N,N*′-carbonyldiimidazole, was described in the literature for the synthesis of tertiary amides.[16] Synthesis of N^{α}-protected amino acid azides employing CDI and its application for the preparation of ureidopeptides was investigated.[17] Azolide[18] and oxadiazolone synthesis in good yield by treatment of the hydrazide with CDI in refluxing dioxane was reported.[19] Aromatic amides and esters have been synthesized by *in situ* activation of hydroxy acids using CDI mediated coupling.[20] *N,N*′- carbonyldiimidazole is one of several universally used reagents for the activation carboxyl groups. It is relatively cheap and the byproducts are carbon dioxide and imidazole. Therefore, an efficient method for the synthesis of N^{α}-protected-amino-peptidyl Weinreb amides is desirable. Herein, we report an efficient, one pot synthesis of N^{α}-protected amino acid/peptide acid-derived Weinreb amides employing CDI as activating agent. The prepared compounds were screened for *in silico* molecular docking studies and *in vitro* antibacterial activities. Docking aims to predict accurately the structure of a ligand within the constraints of a receptor binding site and correctly to estimate the strength of binding between the molecules.[21,22]

Results and Discussion

Chemistry: For the synthesis of the N^{α}-protected Weinreb amides (**2a**-**2m**), N^{α}-protected amino acid was dissolved in THF; CDI was added at 0° C and the solution was stirred for about 10 min. Then, *N,O*-dimethylhydroxylamine hydrochloride salt in dry DCM neutralized by the addition of *N*-methylmorpholine (NMM) was added. The reaction mixture was stirred till the completion of the reaction as indicated by TLC. After simple work up, the desired products were obtained in good yield (Scheme 1). In this way several Weinreb amides were synthesized from N^{α}-protected Fmoc/Cbz/Boc amino acids (Table 1).



1 **2a-2m**

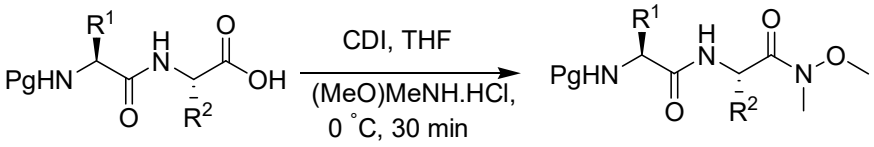
Pg = Fmoc/Boc or Cbz protecting group; R = Amino acid side chains

Scheme 1. Synthesis of N^{α}-protected Weinreb amides.

Table 1. List of amino acid Weinreb amides prepared following Scheme 1

Compound	Weinreb amides	Yield (%)	M.p./ °C
2a	*N*-Fmoc-L-Phe-N(OCH_3)CH_3	89	130
2b	*N*-Fmoc-L-Ala-N(OCH_3)CH_3	85	114
2c	*N*-Fmoc-L-Val-N(OCH_3)CH_3	83	109
2d	*N*-Fmoc-L-Leu-N(OCH_3)CH_3	82	125
2e	*N*-Fmoc-L-Trp-N(OCH_3)CH_3	87	Gum
2f	*N*-Fmoc-L-Pro-N(OCH_3)CH_3	90	Gum
2g	*N*-Cbz -L-Ala-N(OCH_3)CH_3	86	110
2h	*N*-Cbz -L-Ser-N(OCH_3)CH_3	80	Gum
2i	*N*-Cbz -L-Gly-N(OCH_3)CH_3	88	Gum
2j	*N*-Boc-L-Phe-N(OCH_3)CH_3	90	106
2k	*N*-Boc-L-Met-N(OCH_3)CH_3	81	Gum
2l	*N*-Boc-L-Ile-N(OCH_3)CH_3	80	Gum
2m	*N*-Boc-L-Leu-N(OCH_3)CH_3	86	Gum

N$^{\alpha}$-protected peptidyl Weinreb amides (Table 2, **4n**-**4p**) were also prepared starting from Fmoc-peptide acids (Scheme 2). Chiral HPLC analysis was carried out for the enantiomeric pair of Fmoc-L-Ala- and -D-Ala-Weinreb amide and the D, L mixture. The samples showed distinct peak with retention times at 10.8 min and 14.6 min respectively. The equimolar mixture of D- and L-enantiomers under similar conditions showed a significant difference in the retention times between D- and L-amino acid derivatives with retention times 11.0 and 14.3 min.



3 **4n-4p**

Pg = Fmoc/Boc or Cbz group; R^1, R^2 = Amino acid side chains

Scheme 2. Synthesis of *N*$^{\alpha}$-protected peptidyl Weinreb amides.

Table 2. Peptidyl Weinreb amides prepared following Scheme 2

Compound	Peptidyl Weinreb amides	Yield (%)	M.p./ °C
4n	*N*-Fmoc-L-Gly-Pro-N(OCH_3)CH_3	90	Gum
4o	*N*-Fmoc-L-Arg-Ala-N(OCH_3)CH_3	84	Gum
4p	*N*-Fmoc-L-Arg-Gly-N(OCH_3)CH_3	88	Gum

The docking results for top five compounds against all the three different receptor proteins are tabulated in table 3.

Table 3(a). Docking results of Weinreb amides with *Escherichia coli*

Entry	Compound name	*Escherichia coli* Docking score
1.	Fmoc-L-Phe-N(OCH_3)CH_3	-29.85
2.	Fmoc-L-Ala-N(OCH_3)CH_3	-27.46
3.	Fmoc-L-Arg-N(OCH_3)CH_3	-26.06
4.	Fmoc-L-Asn-N(OCH_3)CH_3	-25.61
5.	Fmoc-L-Cys-N(OCH_3)CH_3	-24.21
6.	Fmoc-L-Ser-N(OCH_3)CH_3	-24.01

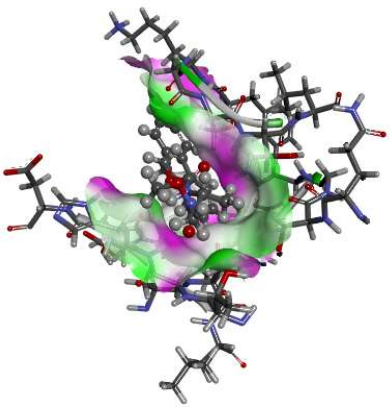
Table 3(b). Docking results of Weinreb amides with *Pseudomonas aeroginosa*

Entry	Compound name	*Pseudomonas aeroginosa* Docking score
1	Fmoc-L-Ala-N(OCH_3)CH_3	-24.79
2	Fmoc-L-Phe-N(OCH_3)CH_3	-23.63
3	L-Glu-N(OCH_3)CH_3	-21.34
4	Fmoc-L-Gln-N(OCH_3)CH_3	-21.08
5	L-Arg-N(OCH_3)CH_3	-20.20
6	L-Gln-N(OCH_3)CH_3	-19.65

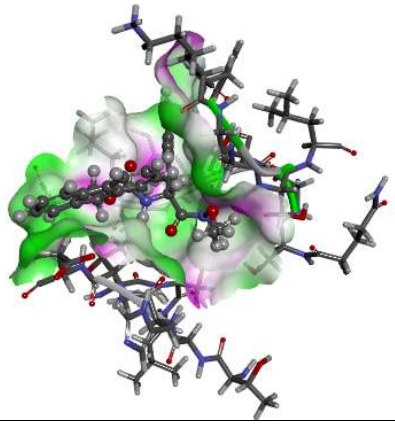
Table 3(c). Docking results of Weinreb Amides with *Staphylococcus aureus*

Entry	Compound name	*Staphylococcus aureus* Docking score
1	Fmoc-L-Ala-N(OCH_3)CH_3	-25.01
2	Fmoc-L-Phe-N(OCH_3)CH_3	-21.60
3	Fmoc-L-Arg-N(OCH_3)CH_3	-13.28
4	L-His-N(OCH_3)CH_3	-10.08
5	L-Pro-N(OCH_3)CH_3	-9.13
6	Fmoc-L-Trp-N(OCH_3)CH_3	-8.98

Figure 1 shows the pictorial representation of the binding modes of synthesized molecules with target proteins. The ligand *N*-Fmoc-L-Phe-N(OCH_3)CH_3 showed minimum binding energy -29.85 kcal/mol with *Escherichia coli* and the ligand *N*-Fmoc-L-Ala-N(OCH_3)CH_3 showed minimum binding energy -24.79 kcal/mol with *Pseudomonas aeruginosa*, -25.01 kcal/mol with *Staphylococcus aureus. In silico* results indicate, *N*-Fmoc-L-Phe-N(OCH_3)CH_3 and *N*-Fmoc-L-Ala-N(OCH_3)CH_3 were showing least binding energy. These lead molecules were evaluated in *in vitro* studies.



N^{α}-Fmoc-L-Ala-N(OCH_3)CH_3 with *Escherichia coli*



N^{α}-Fmoc-L-Phe-N(OCH_3)CH_3 with *Escherichia coli*

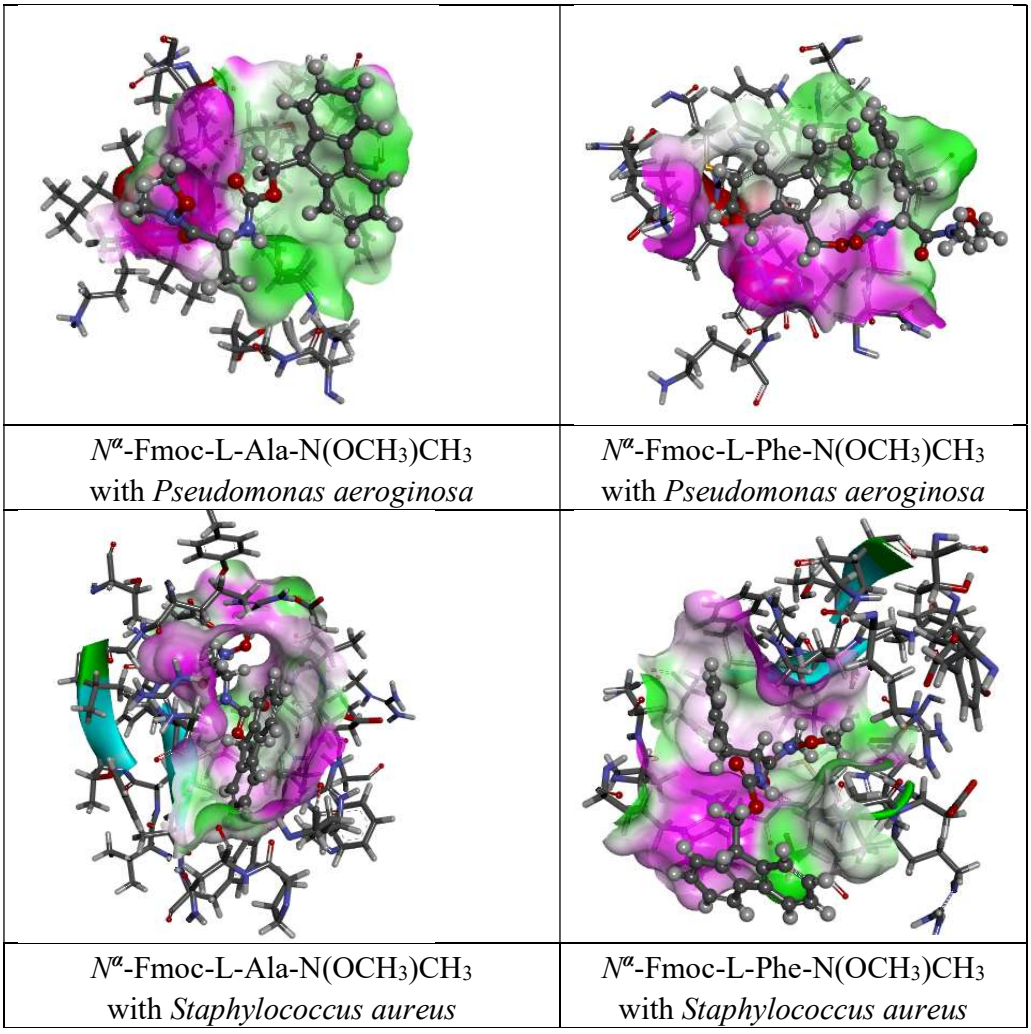


Figure 1. Pictorial representations of the binding modes of synthesized molecules with target proteins.

The antibacterial results of *Escherichia coli, Pseudomonas aeruginosa*, and *Staphylococcus aureus* are tabulated in Table 4. Based on the docking results the *N*-Fmoc-L-Ala-N(OCH_3)CH_3 and *N*-Fmoc-L-Phe-N(OCH_3)CH_3 were showing good activity in *in vitro* studies. The compound *N*-Fmoc-L-Phe-N(OCH_3)CH_3 showed inhibition zone of 14 mm against *Escherichia coli, N*-Fmoc-L-Ala-N(OCH_3)CH_3 showed inhibition zone of 14 mm against *Pseudomonas aeruginosa* and *N*-Fmoc-L-Ala-N(OCH_3)CH_3 showed inhibition zone of 14 mm against *Staphylococcus aureus* compared to standard compound.

Table 4. Antibacterial test for protected Weinreb amides

Entry	Sample name	Standard	*Sample concentration*		
	Escherichia coli		**50**	**100**	**200**
1.	*N*-Fmoc-L-Ala-N(OCH_3)CH_3	14 mm	10 mm	12 mm	12 mm
2.	*N*-Fmoc-L-Phe-N(OCH_3)CH_3	14 mm	14 mm	10 mm	12mm
	Pseudomonas aeruginosa		**50**	**100**	**200**
1.	*N*-Fmoc-L-Ala-N(OCH_3)CH_3	14 mm	14 mm	13 mm	13 mm
2.	*N*-Fmoc-L-Phe-N(OCH_3)CH_3	14 mm	8 mm	10 mm	10 mm
	Staphylococcus aureus		**50**	**100**	**200**
1.	*N*-Fmoc-L-Ala-N(OCH_3)CH_3	13 mm	14 mm	8 mm	10 mm
2.	*N*-Fmoc-L-Phe-N(OCH_3)CH_3	13 mm	9 mm	11 mm	10 mm

Conclusions

In the present work, we have used Fmoc/Cbz/Boc α-amino/peptide acids as precursors for the preparation of Fmoc/Cbz/Boc α-amino/peptidyl Weinreb amides. The carboxylic group of amino acids was activated using CDI followed by the coupling reaction with *N, O*- dimethyl hydroxylamine hydrochloride salt to obtain the title products. This protocol is an efficient method for the synthesis of Weinreb amides. All the products were isolated after simple work up and were fully characterized by IR, ^{1}H NMR, ^{13}C NMR and mass spectroscopy. Finally, the synthesized products were subjected to molecular docking studies and antibacterial activities employing *Escherichia coli, Pseudomonas aeroginosa* and *Staphylococcus aureus*. The lead compound, *N*-Fmoc-L-Phe-N(OCH_3)CH_3 showed least binding energy of -29.85 kcal/mol with enoyl-ACP reductase (1C14) *Escherichia coli,* the lead compound *N*-Fmoc-L-Ala-N(OCH_3)CH_3 showed least binding energy of -24.79 kcal/mol with LasR ligand binding domain bound to its natural ligand *n*-3-oxododecanoyl-L-homoserine lactone (2UV0) *Pseudomonas aeroginosa* and the lead compound *N*-Fmoc-L-Ala-N(OCH_3)CH_3 showed least binding energy -25.01 kcal/mol with dehydrosqualene synthase (2ZCP) *Staphylococcus aureus.*

Experimental Section

General. All chemicals were purchased from Sigma-Aldrich and Merck and used without purification. The solvents were freshly distilled before use. Melting points were taken in open capillaries. TLC analysis was carried out using precoated silica gel F_{254}. IR spectra were recorded on Agilent Cary 620 FT-IR spectrometer. ^{1}H NMR spectra were done on a Bruker AMX 400 MHz spectrometer using Me_4Si as an internal standard and $CDCl_3$ as a solvent. Mass spectra were recorded on a Micromass Q-ToF Micro Mass Spectrometer. Acronyms in this section are defined

as follows: CDI carbonyldiimidazole; THF tetrahydrofuran; DCM dichloromethane; NMM *N*-methylmorpholine; Boc, Fmoc, Cbz – as defined in the compound names.

General procedure for the synthesis of N^{α}- protected amino/peptidyl Weinreb amides. To a stirred solution of protected amino/peptide acid (1 mmol) in THF, NMM (1.5 mmol) and CDI (1.5 mmol) was added at 0 °C, followed by the addition of *N,O*-dimethylhydroxylamine hydrochloride (1.1 mmol) in dry DCM (5-6 mL), neutralized with NMM. The reaction mixture was stirred till the completion of reaction. THF was removed and the product was extracted into ethyl acetate and the organic layer was washed with hydrochloric acid solution (10 mL) or citric acid solution (in case of Boc-protected compounds), sodium carbonate solution (15mL × 2), water (15 mL) and brine (15 mL). It was dried over anhydrous sodium sulfate and concentrated.

Physical and spectral data of the synthesized compounds

N^{α}-Fmoc-Phe-N(OCH$_3$)CH$_3$ (2a). (*S*)-(9*H*-Fluoren-9-yl)methyl-1-(methoxy(methyl)amino)-1-oxo-3-phenylpropan-2-ylcarbamate. Yield 89%, mp 130 °C. R_f (ethyl acetate/ *n*-hexane 2:8) 0.6, IR (KBr, cm^{-1}): 1660; ^{1}H NMR (400 MHz, CDCl$_3$): δ (ppm) 1.59 (s, 3H), 2.91-3.12 (m, 2H), 3.65 (s, 3H), 4.16-4.19 (t, *J* 8.0 Hz, 1H), 4.24-4.39 (m, 2H), 5.00-5.05 (m, 1H), 5.48-5.50 (d, *J* 8.0 Hz, 1H), 7.15-7.76 (m, 13H). ^{13}C NMR (100 MHz, CDCl$_3$): δ (ppm) 32.05, 38.65, 47.10, 52.04, 61.52, 66.95, 125.09, 125.15, 126.88, 127.00, 127.62, 128.38, 129.41, 136.26, 141.23, 143.85, 155.73, 171.87. MS: Calc. for $C_{26}H_{26}N_2O_4$: *m/z* 453.1790 (M^++Na), found: 453.1793.

N^{α}-Fmoc-Ala-N(OCH$_3$)CH$_3$(2b). (*S*)-(9*H*-fluoren-9-yl)methyl-1-(methoxy(methyl)amino)-1-oxopropan-2-ylcarbamate. Yield 85%, mp 114 °C. R_f (ethyl acetate/*n*- hexane 2:8) 0.4, IR (KBr, cm^{-1}): 1635. ^{1}H NMR (400 MHz, CDCl$_3$): δ (ppm) 1.36-1.38 (d, *J* 8.0 Hz, 3H), 3.22 (s, 3H), 3.77 (s, 3H), 4.20-4.24 (t, *J* 8.0 Hz, 1H), 4.35-4.37 (d, *J* 8.0 Hz, 2H), 4.74-4.77 (t, *J* 4.0 Hz, 1H), 5.57-5.61 (d, *J* 8.0 Hz, 1H), 7.29-7.77 (m, 8H). ^{13}C NMR (100 MHz, CDCl$_3$): δ (ppm) 18.65, 32.14, 47.11, 47.15, 61. 59 (59, 66.93, 125.12, 125.15, 127.02, 127.64, 141.25, 143.83, 143.95, 155.72. MS: Calc. for $C_{20}H_{22}N_2O_4$: *m/z* 377.1477 (M^++Na), found: 377.1479.

N^{α}-Fmoc-Val-N(OCH$_3$)CH$_3$ (2c). (S)-(9*H*-fluoren-9-yl)methyl-1-(methoxy(methyl)amino)-3-methyl-1-oxobutan-2-ylcarbamate. Yield 83%, mp 109 °C. R_f (ethyl acetate/ *n*-hexane 2:8) 0.55, IR (KBr, cm^{-1}): 1654. ^{1}H NMR (400 MHz, CDCl$_3$): δ (ppm) 0.86-0.98 (m, 6H), 1.98-2.07 (m, 1H), 3.22 (s, 3H), 3.77 (s, 3H), 4.20-4.23 (t, *J* 8.0 Hz, 1H), 4.30-4.42 (m, 1H), 4.62- 4.66 (d, *J* 8.0 Hz, 1H), 5.46-5.48 (d, *J* 8.0 Hz, 1H), 7.25-7.64 (m, 8H). ^{13}C NMR (100 MHz, CDCl$_3$): δ (ppm) 17.61, 29.63, 31.86, 47.16, 55.55, 61.51, 68.73, 119.88, 124.37, 126.99, 129.22, 141.24, 143.81, 156.41, 172.56. MS: Calc. for $C_{22}H_{26}N_2O_4$ *m/z*: 405.1900 (M^++Na), found: 405.1901.

N^{α}-Fmoc-L-Leu-N(OCH$_3$)CH$_3$ (2d). (*S*)-(9*H*-fluoren-9-yl)methyl-1-(methoxy-(methyl)amino)-4-methyl-1-oxopentan-2-ylcarbamate. Yield 82%, mp 125 °C, R_f (ethyl acetate/ *n*- hexane 2:8) = 0.58, IR (KBr, cm^{-1}): 1645. ^{1}H NMR (400 MHz, CDCl$_3$): δ (ppm) 0.84-0.99 (m, 6H), 1.25-1.32 (m, 2H), 1.63-1.72 (m, 1H), 2.29 (s, 3H), 3.14 (s, 3H), 4.04-4.23 (m, 1H), 4.35-4.41 (m, 1H), 5.16 (d, *J* 8.0 Hz, 1H), 5.36- 5.39 (d, br, *J* 12.0 Hz, 2H), 7.17-7.76 (m, 8H). ^{13}C NMR (100 MHz, CDCl$_3$): δ (ppm) 22.05, 22.92, 33.10, 41.56, 46.64, 47.58, 61.83, 67.40, 126.60,

128.20, 128.40, 128.80, 141.00, 143.60, 154.00, 156.06. MS: Calc. for $C_{23}H_{28}N_2O_4$: *m/z* 419.2000 (M^++Na), found: 419.2040.

N$^{\alpha}$-Fmoc-L-Trp-N(OCH$_3$)CH$_3$ (2e). (9*H*-Fluoren-9-yl)methyl-3-(1*H*-indol-3-yl)-1-(methoxy (methyl)amino)-1-oxopropan-2-ylcarbamate. Yield 87%, gum. R_f (ethyl acetate/n- hexane 2:8) = 0.62, IR (KBr, cm^{-1}): 1608; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 2.75 (s, 3H), 3.00 (m, 2H) , 3.35 (s, 3H), 4.48 (m, 1H) , 4.72 -4.74 (d, *J* 8.0 Hz, 2H), 4.90-4.91 (t, *J* 4.0 Hz, 1H), 6.74 (s, 1H), 7.18 (s, 4H), 7.28-7.84 (m, 8H), 5.00 (d, br, *J* 8.0 Hz, 1H,), 5.45 (d, br, *J* 8.0 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 31.05, 33.26, 47.10, 50.18, 61.84, 67.00, 110.84, 111.10, 119.00, 120.26, 122.00, 122.70, 126.65, 127.00, 128.20, 128.40, 128.80, 136.56, 141.00, 143.50, 154.58, 156.00. MS: Calc. for $C_{28}H_{27}N_3O_4$: *m/z* 492.2000 (M^++Na), found: 492.2002.

***N*$^{\alpha}$-Fmoc-L-Pro-N(OCH$_3$)CH$_3$ (2f). (*S*)-1-(9*H*-Fluoren-9-yl)methyl-2-(methoxymethyl) carbamoylpyrrolidine-1-carboxylate.** Yield 90%, gum. R_f (ethyl acetate/*n*- hexane 2:8): 0.65, IR (KBr, cm^{-1}): 1667; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.46 (m, 2H) 1.74 (m, 2H), 2.59 (s, 3H), 3.20 (m, 2H), 3.45 (s, 3H), 4.28-4.30 (t, *J* 4.0 Hz, 1H), 4.40-4.42 (t, *J* 4.0 Hz, 1H), 4.66-4.70 (d, *J* 8.0 Hz, 2H), 7.28-7.84 (m, 8H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 22.00, 29.22, 32.94, 47.00, 47.35, 56.00, 61.55, 67.26, 126.80, 128.20, 128.40, 128.80, 141.00, 143.60, 154.50, 156.80. MS: Calc. for $C_{22}H_{24}N_2O_4$: *m/z* 403.1700 (M^++Na), found: 403.1736.

N$^{\alpha}$-Cbz-Ala-N(OCH$_3$)CH$_3$ (2g). (*S*)-Benzyl 1-(Methoxy(methyl)amino)-1-oxopropan-2-ylcarbamate. Yield 86%, mp 110 ºC. R_f (ethyl acetate/n- hexane 2:8) = 0.32, IR (KBr, cm^{-1}): 1650; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.33-1.35 (d, *J* 8.0 Hz, 3H), 3.21 (s, 3H) , 3.77 (s, 3H), 5.05-5.14 (m, 1H), 5.30 (s, 2H), 5.52-5.54 (d, br, *J* 8.0 Hz, 1H), 7.29-7.35 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 18.59, 32.14, 47.09, 61.57, 66.69, 127.96, 128.04, 128.45, 136.39, 154.55, 156.67. MS: Calc. for $C_{13}H_{18}N_2O_4$: *m/z* 289.1164 (M^++Na), found: 289.1166.

***N*$^{\alpha}$-Cbz-Ser-N(OCH$_3$)CH$_3$ (2h). (*S*)-benzyl 3-Hydroxy-1-(methoxy(methyl)amino)-1-oxopropan-2-ylcarbamate.** Yield 80%, gum. R_f (ethyl acetate/n- hexane 2:8) = 0.44, IR (KBr, cm^{-1}): 1673; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.98 (s, 1H), 2.58 (s, 3H), 3.16 (s, 3H), 3.49-3.79 (m, 2H), 4.10 - 4.20 (m, 1H), 5.10 (s, 2H), 5.98- 6.0 (d, *J* 8.0 Hz, 1H), 7.24 - 7.32 (s, 5H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 30.15, 52.96, 61.37, 62.66, 64.24, 127.84, 127.91, 128.25, 136.13, 154.69, 155.82. MS: Calc. for $C_{13}H_{18}N_2O_5$: *m/z* 305.1216 (M^++Na), found: 305.1220.

N$^{\alpha}$-Cbz-Gly-N(OCH$_3$)CH$_3$ (2i). Benzyl 2-(methoxy(methyl)amino)-2-oxoethylcarbamate. Yield 88%, gum. R_f (ethyl acetate/*n*- hexane 2:8) = 0.22, IR (KBr, cm^{-1}): 1630; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 2.5 (s, 3H), 3.28 (s, 3H), 3.65 (s, 2H), 5.10 (d, br, *J* 4.0 Hz, 1H), 5.37 (s, 2H), 7.18 (s, 5H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 31.2, 38.0, 62.5, 66.4, 127.2, 127.8, 128.2, 141.0, 156.4, 164.3. MS: Calc. for $C_{12}H_{16}N_2O_4$: *m/z* 275.1100 (M^++Na), found: 275.1096.

N$^{\alpha}$-Boc-Phe-N(OCH$_3$)CH$_3$ (2j). (*S*)-*tert*-Butyl 1-(methoxy(methyl)amino)-1-oxo-3-phenyl-propan-2-ylcarbamate. Yield 90%, mp 106 ºC, R_f (ethyl acetate/*n*- hexane 2:8) = 0.29, IR (KBr, cm^{-1}): 1648; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.37 (s, 9H), 2.70 (s, 3H), 3.15-3.17 (d, *J* 8.0 Hz, 2H), 3.36 (s, 3H), 4.79-4.80 (t, *J* 4.0 Hz, 1H), 5.24 (d, br, *J* 8.0 Hz, 1H), 7.12-7.24 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 28.18, 31.94, 38.41, 51.44, 61.95, 77.31, 126.62, 128.27,

129.23, 136.50, 155.07, 157.10. MS: Calc. for $C_{16}H_{24}N_2O_4$: *m/z* 331.1700 (M^+ +Na), found: 331.1710.

N$^{\alpha}$-Boc-Met-N(OCH$_3$)CH$_3$ (2k). (*S*)-*tert*-Butyl 1-(methoxy(methyl)amino)-4-(methylthio)-1-oxobutan-2-ylcarbamate. Yield 81%, gum. R_f (ethyl acetate/n- hexane 2:8) = 0.36, IR (KBr, cm^{-1}): 1653; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.44 (s, 9H), 2.09 (s, 3H), 2.51-2.61 (m, 2H), 2.90-2.93 (t, *J* 4.0 Hz, 2H), 3.21 (s, 3H), 3.78 (s, 3H), 4.21- 4.23 (t, *J* 4.0 Hz, 1H), 5.25- 5.27 (d, br, *J* 8.0 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 15.25, 27.59, 30.04, 31.42, 32.07, 49.72, 61.54, 79.54, 155.43, 172.48. MS: Calc. for $C_{12}H_{24}N_2O_4S$ *m/z*: 315.1500 (M^+ +Na), found: 315.1550.

N$^{\alpha}$-Boc-Ile-N(OCH$_3$)CH$_3$ (2l). *tert*-Butyl (2*S*,3*S*)-1-(methoxy(methyl)amino)-3-methyl-1-oxopentan-2-ylcarbamate. Yield 80%, gum. R_f (ethyl acetate/*n*- hexane 2:8) = 0.35, IR (KBr, cm^{-1}):1622; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 0.94-.95 (t, *J* 4.0 Hz, 3H), 1.15-1.17 (d, *J* 8.0 Hz, 3H), 1.29 (m, 2H), 1.40 (s, 9H), 2.55 (m, 1H), 2.74 (s, 3H), 3.45 (s, 3H), 4.48-4.50 (d, *J* 8.0 Hz, 1H), 5.31 (d, br, *J* 8.0 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 10.85, 14.70, 24.75, 28.50, 33.26, 37.48, 51.55, 61.85, 79.00, 154.00, 156.00. MS: Calc. for $C_{13}H_{26}N_2O_4$ *m/z* 297.1900 (M^+ +Na), found: 297.1942.

N$^{\alpha}$-Boc-Leu-N(OCH$_3$)CH$_3$ (2m). (*S*)-*tert*-Butyl 1-(methoxy(methyl)amino)-4-methyl-1-oxopentan-2-ylcarbamate. Yield 86%, gum, R_f (ethyl acetate/ *n*-hexane 2:8) = 0.46, IR (KBr, cm^{-1}): 1640; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 0.98-1.00 (d, *J* 8.0 Hz, 6H), 1.35 (s, 9H), 1.72 (m, 2H), 1.80 (m, 1H), 2.68 (s, 3H), 3.45 (s, 3H), 4.64-4.67 (t, *J* 4.0 Hz, 1H), 5.18 (d, br, *J* 8.0 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 22.00, 22.35, 28.52, 31.90, 40.74, 46.57, 61.46, 79.00, 154.40, 156.50. MS: Calc. for $C_{13}H_{26}N_2O_4$: *m/z* 297.1900 (M^+ +Na), found: 297.1890.

N$^{\alpha}$-Fmoc-L-Gly-Pro-N(OCH$_3$)CH$_3$ (4n). (*R*)-(9*H*-Fluoren-9-yl)methyl-2-(2-(methoxy(methyl)carbamoyl)pyrrolidin-1-yl)-2-oxoethylcarbamate. Yield 90%, gum. R_f (ethyl acetate/ *n*-hexane 2:8) = 0.26, IR (KBr, cm^{-1}): 1655; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.85-1.86 (m, 2H), 2.05 (m, 2H), 2.70 (s, 3H), 3.29 (s, 3H), 3.40-3.42 (t, *J* 4.0 Hz, 2H), 3.78 (s, 2H), 4.37-4.39 (t, *J* 4.0 Hz, 1H), 4.48- 4.50 (t, *J* 4.0 Hz, 1H), 4.58- 4.60 (d, *J* 8.0 Hz, 2H), 5.05 (d, br, *J* 4.0 Hz, 1H) 7.25-7.84 (m, 8H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 22.25, 29.74, 33.44, 42.00, 44.98, 46.80, 54.55, 61.90, 67.25, 126.80, 128.20, 128.40, 128.80, 141.00, 143.55, 154.00, 156.25, 167.80. MS: Calc. for $C_{24}H_{27}N_3O_5$: *m/z* 447.1872 (M^+ +Na), found: 447.1868.

N$^{\alpha}$-Fmoc-L-Arg-Gly-N(OCH$_3$)CH$_3$ (4o). (*S*)-(9*H*-fluoren-9-yl)methyl 5-guanidino-1-(2(methoxy(methyl)amino)-2-oxoethylamino)-1-oxopentan-2-ylcarbamate. Yield 88%, gum. R_f (ethyl acetate/*n*-hexane 2:8) = 0.38, IR (KBr, cm^{-1}): 1665; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.50 (m, 2H), 1.76 (m, 2H), 2.00 (s, 3H), 2.58-2.59 (t, *J* 4.0 Hz, 2H), 2.70 (s, 3H), 3.00 (s, 1H), 3.24 (s, 2H), 4.05 (s, 1H), 4.36 (s, 2H), 4.45 (m, 1H), 4.58 (m, 1H), 4.70- 4.74 (d, *J* 8.0 Hz, 2H), 5.10 (d, br, *J* 8.0 Hz, 2H), 7.28-7.84 (m, 8H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 24.05, 28.65, 32.70, 37.24, 37.56, 47.00, 54.55, 61.76, 66.95, 126.80, 128.20, 128.40, 128.80, 141.00, 143.60, 156.00, 158.50, 164.55, 171.00. MS: Calc. for $C_{25}H_{32}N_6O_5$: *m/z* 519.2434 (M^+ +Na), found: 519.2430.

N$^{\alpha}$-Fmoc-L-Arg-Ala-N(OCH$_3$)CH$_3$ (4p). (9*H*-fluoren-9-yl)methyl (*S*)-5-guanidino-1-((*R*)-1-(methoxy(methyl)amino)-1-oxopropan-2-ylamino)-1-oxopentan-2-ylcarbamate. Yield 84%,

gum. R_f (ethyl acetate/ *n*-hexane 2:8)= 0.3, IR (KBr, cm^{-1}): 1657; ^1H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.50 (m, 2H), 1.76 (m, 2H), 2.00 (s, 3H), 2.58-2.59 (t, *J* 4.0 Hz, 2H), 2.70 (s, 3H), 3.00 (s, 1H), 3.24 (s, 2H), 4.05 (s, 1H), 4.36 (s, 2H), 4.45 (m, 1H), 4.58 (m, 1H), 4.70- 4.74 (d, *J* 8.0 Hz, 2H), 5.10 (d, br, *J* 8.0 Hz, 2H), 7.28-7.84 (m, 8H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 24.05, 28.65, 32.70, 37.24, 37.56, 47.00, 54.55, 61.76, 66.95, 126.80, 128.20, 128.40, 128.80, 141.00, 143.60, 156.00, 158.50, 164.55, 171.00. MS: Calc. for $C_{26}H_{34}N_6O_5$: *m/z* 533.2591 (M^+ +Na), found: 533.2589.

Biological Studies

To evaluate the binding efficacy and inhibitory effects of synthesized compounds, three protein targets from three different organisms, namely *Escherichia coli*, *Pseudomonas aeroginosa* and *Staphylococcus aureus*, were selected for this study. The X-ray crystallographic structure of enoyl-ACP reductase (1C14) *Escherichia coli*, X-ray crystallographic structure of LasR ligand binding domain bound to its natural ligand *n*-3-oxododecanoyl-L-homoserine lactone (2UV0) *Pseudomonas aeroginosa* and X-ray crystallographic structure of dehydrosqualene synthase (2ZCP) *Staphylococcus aureus* [24-26] respectively were obtained from Protein Data Bank (PDB)[23]. The prepared targets were then used for molecular docking studies[27] which will predict possible ligand interactions with the active site residues that can inhibit protein activity. To perform docking, FlexX module of LeadIT software was used.[28] The LeadIT score obtained after the docking were considered to know the free binding energy (ΔG). Based on the very low binding energy and maximum possible intermolecular interactions, best lead compounds with strong binding efficacy were finalized. 2D structures of all the ligands were drawn in ChemDraw Ultra 8.0 and were exported as mol file for further processing in DS 3.5. The generated conformers were optimized using CHARMm force field[29] and then minimized. Each selected conformers were then grouped into one library file and were subjected to docking. The FlexX docking score correlates with the binding affinity of the molecules with the target.

Antibacterial activity was screened by Agar well diffusion method[30] against three pathogenic bacterial strains, *Escherichia coli* MTCC1692, *Pseudomonas aeroginosa* MTCC1688 and *Staphylococcus aureus* MTCC3160 (one gram +ve and two gram –ve). Preliminary screening was done to check antibacterial activities of synthesized compounds over Muller-Hinton agar plates. The inhibition zones obtained were measured in millimeters against the Streptomycin sulfate standard. Finally, the average values were considered for the ultimate antibacterial activity.

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