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Novel Synthetic Unnatural β^3 aminoacids via acid hydrolysis of diazepinones as Synthons for Antibiotics

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ABSTRACT

A convenient two-step method for the preparation of β^3-amino acids is described. This paper describes an efficient and straightforward two-step synthetic sequence leading in excellent yields to a relatively inaccessible lipophilic β^3-amino acids with antimicrobial potential from easily available starting materials. Schmidt reaction of substituted piperidin-4-ones with HN_3 generated *in situ* afforded the corresponding diazapinones. Acid hydrolysis of chosen diazapinones yielded β^3-amino acids. Selective cleavage of only the lactam bond is achieved using 4N HCl–MeOH (1:1) mixture leading to the formation of N-alkyl β^3-amino acids in quantitative yields. synthesized aminoacids are subjected to antimicrobial activity for gram positive, gram negative bacteria, fungi. compounds 3e & 3h possess excellent antimicrobial and antifungal activity comparable to standard streptomycin and ketoconazole. Therefore obtained β^3-amino acids serves as a scaffold for synthetic antibiotics as they have desired structural moieties necessary for antibiotic action. Further refinement of the β^3-amino acids using either conjugation or structural modification or incorporation of these aminoacids into synthetic peptides would bring about excellent antimicrobial activity.

Keywords: Mannich reaction, Piperidin-4-ones, Schmidt reaction, Diazapinone, Acid hydrolysis, β-amino acids

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INTRODUCTION

β-aminoacids are key structural elements of peptides, peptidomimetics, small molecule pharmaceuticals and other Natural products.[1] In addition, they are essential chiral building blocks for the Synthesis of valuable antibiotics.[2] β-amino acids show interesting pharmacological properties by themselves, But most are valuable intermediates in routes to novel molecules with biological and pharmacological activity.[3] β-peptides have secondary structures comparable to their α-amino acid analogs, β-amino acids are subdivided into β^2,β^3,$\beta^{2,3}$aminoacids depending on the position of the side chain at the N-alkyl 3- amino carboxylic acid core. Synthetic derivative of biologically relevant peptides incorporating β-amino acids often display interesting pharmacological activity with increased potency and enzymatic stability relative to native counterparts.[4]

Until recently, methods for the synthesis of β-amino acids relied predominantly on classical resolution, stoichiometric use of chiral auxiliaries or homologation of α-amino acids, catalytic asymmetric synthesis using transition metals, organocatalyst and biocatalysts.[5] However, in many cases *high catalyst loadings* have to be used, and reaction times are in some cases rather long. some transition metals are highly toxic and harmful for the environment. Therefore, biocatalysis would provide an important alternative, but up to now no enzymes are known that have a broad substrate scope in combinations with high turnover numbers. Therefore, the catalytic asymmetric synthesis of β-amino acids starting from simple and cheap starting materials and using recyclable sustainable catalysts remains an important challenge for synthetic organic chemists. Accordingly, the development of efficient methods in their preparation has been a mainstay in organic synthesis.

MATERIALS AND METHOD

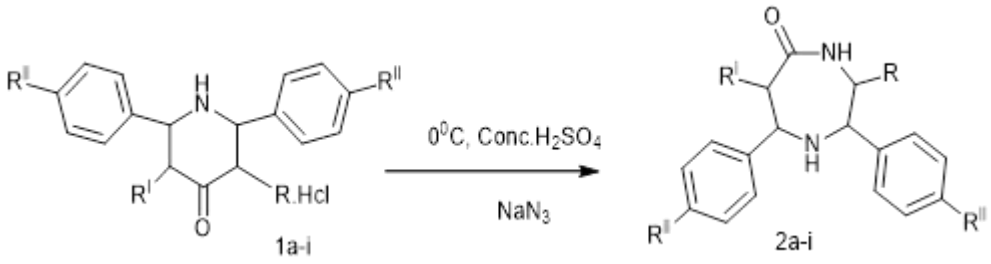
All the chemicals used in this study were purchased from Sigma Aldrich without further purification. Melting points were determined in open capillary tubes and are uncorrected. FT-IR spectra were taken as KBr pellets for solids on a Perkin Elmer Spectrum RXI FTIR. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded in $CDCl_3$ solutions with TMS as an internal standard on a JEOL instrument and Bruker Avance instrument. Mass spectra were recorded on a Thermo Finnigan LCQ Advantage MAX 6000 ESI spectrometer. The obtained lactams were purified by recrystallization with ethanol. piperidinones (1a-i) were synthesized as previously reported by Thennarasu et al. 2002

Bacterial Strains and Growth Condition

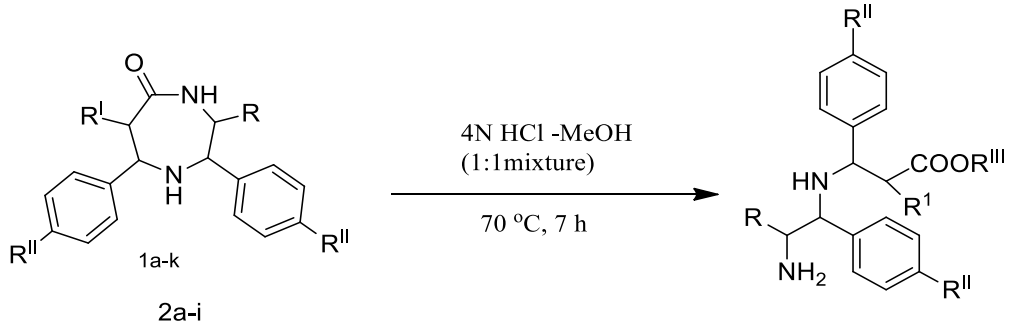
The following Gram positive, Gram negative bacteria, clinical isolate and fungi were used for the experiment. Gram positive bacteria: *Staphylococcus aureus* MTCC 96, *Enterobacter aerogenes* MTCC 111 and *Bacillus subtilis* MTCC 441; Gram negative bacteria: *Klebsiella pneumoniae* MTCC 109, *Proteus vulgaris* MTCC 1771, *Salmonella typhimurium* MTCC 1251 and *Escherichia coli*; clinical isolate: *Staphylococcus aureus* (MRSA- methicillin resistant, clinical pathogen). Fungi: *Candida albicans* MTCC 227 and *Malassesia pachydermatis*, were used for antifungal assays. The reference cultures were obtained from Institute of Microbial Technology (IMTECH), Chandigarh, India-160 036. All the cultures were obtained from the Department of Microbiology, Christian Medical College, Vellore, Tamil Nadu, India. Bacterial inoculums were prepared by growing cells in Mueller Hinton broth (MHB) (Himedia) for 24 h at 37°C. The filamentous fungi were grown on sabouraud dextrose agar (SDA) slants at 28°C for 10 days and the spores were collected using sterile doubled distilled water and homogenized. Yeast was grown on sabouraud dextrose broth (SDB) at 28°C for 48 h.

RESULTS AND DISCUSSION

Three component Mannich-type condensation involving an aryl aldehyde, a ketone and ammonium acetate resulted in the quantitative formation of 2,6-diaryl piperidin-4-ones. Schmidt rearrangement of 2,6-diaryl piperidin-4-ones into 2,7-diaryl 1,4-diazepan-5-ones(2a-i) occurred at extremely acidic conditions with sodium azide at ice cold condition (scheme 1). selective cleavage of the amide bond in 1,4-diazepan-5-ones was achieved using 4N HCl-MeOH (1:1)mixture at 70 °C and the β-amino acids (3a-i)obtained was in excellent yields.(scheme 2).



Scheme 1: Schidmt rearrangement of piperidinones to diazepinones

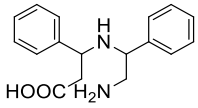
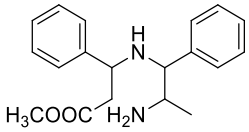
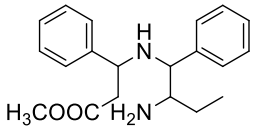
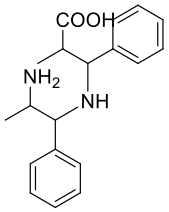


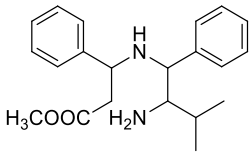
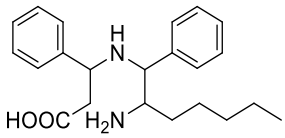
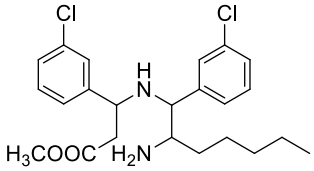
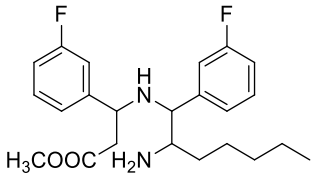
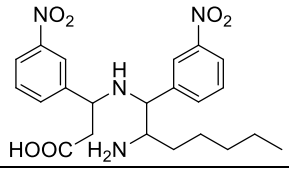
R-H,CH_3,CH_2CH_3,$CH(CH_3)_2$,$(CH_2)_4CH_3$
R^I- H,CH_3
R^{II}- H,Cl,F,NO_2
R^{III}- H,CH_3

Scheme 2: Acid hydrolysis of diazepinones to β-amino acids

In FT-IR spectrum of the compound **3c ,** the two stretching bands at ~ 3417 and ~ 1719 cm^{-1} corresponds to NH stretching and acid carbonyl stretching modes respectively. In ^{13}C NMR spectrum, the peak resonating at 173.3 ppm reveals the acid carbonyl carbon in compound **3c**. Unambiguous assignment of the ^{1}H and ^{13}C-NMR spectra of the β-amino acid 3**c** was accomplished *via* the corresponding HETCOR spectrum .The expected mass of β-amino acids **3c** Ms m/z = 327 $[MH]^+$ was also observed by mass spectrometry .out of 11 aminoacids synthesized, almost 6 aminoacids Possess significant antimicrobial activity when compared to standard streptomycin and ketoconazole. synthesized aminoacids were presented in table1

Table 1: Series of β^3-amino acids

NO	List of derivatives	YIELD (%)
3a		85%
3b		78%
3c		85%
3d		82%

3e		73%
3f		70%
3g		69%
3h		72%
3i		70%

Experimental section

General procedure for the preparation of diazepin-5-ones (**2a-i**)

Portion wise addition of hydrochloride salt of 1a-i (dry powder, 10.0 g, 27.9 mmol) into ice-cold concentrated sulphuric acid (50 mL) taken in a conical flask generated fumes, when stirred using a magnetic stirrer. After the addition was complete, the solution was allowed to equilibrate to room temperature. Portion wise addition of sodium azide (3.0 g, 46.15 mmol) over a period of one hour into the acid solution generated effervescence. After the addition was over and the effervescence ceased, the reaction mixture was poured into crushed ice, and the pH was adjusted to ~8.0 using 4N NaOH solution to obtain the product diazepin-5-one(**2a-i)**. The white solid formed was isolated by filtration and purified further by crystallization from ethanol.

General procedure for the preparation of β^3-amino acids (**3a-i**)

Dry solid **1a** (5.0 g, 14.9 mmol) was dissolved in 4N HCl-methanol mixture (150 mL, 1:1 v/v) in a 250 mL round-bottom flask and refluxed for 7 hours at 70 ^{o}C. The resulting solution was evaporated under reduced pressure to dryness to afford the methyl ester of β^3-amino acid **3a-i**.

Spectral data for β-amino acids:

3-((2-amino-1-phenylethyl)amino)-3-phenylpropanoic acid (**3a)** : White solid; m.p 220-222 °C; Yield 85 %,^{1}H NMR (500 MHz, DMSO-d_6): δ ppm 2.50–2.51 (m, 1H), 3.11 (dd, 1H, J = 16, 11.5

Hz), 3.40 (dd, 1H, *J* = 16, 4 Hz), 3.78 (d, 1H, *J* = 7.5 Hz), 4.21 (dd, 1H, *J* = 10.5, 3.5 Hz), 4.69 (m, 1H), 7.38–7.39 (m, 3H, -Ar-H), 7.45–7.49 (m, 5H, -Ar-H), 7.59–7.60 (m, 2H, -Ar-H), 8.49 (br s, 3H, -NH_2 with 2HCl), 9.82 (br s, 1H, -NH), 11.65 (br s, 1H, -COOH); ^{13}C NMR (125 MHZ, DMSO-d_6): δ ppm 37.1, 41.0, 57.1, 57.6, 129.1, 129.2, 129.3, 129.8, 130.4, 132.5, 135.4, 170.3; FT-IR (KBr cm^{-1}): 3397, 2957, 2788, 1702, 1618, 1528, 1445, 1302, 1163, 1089, 989, 766; MS *m*/*z* = 285 $[MH]^+$.

Methyl 3-(2-amino-1-phenylpropylamino)-3-phenylpropanoate,(3b) obtained as white crystalline solid. Hygroscopic.yield-78%, Mp: 260-270^{0}C. ^{1}H NMR (500MHz, $CDCl_3$): δ ppm 10.39(s, 1H, NH), 9.04-8.92 (d, 3H, 2 amino proton with HCL proton),7.55-6.88 (m, 10H, Ar), 5.59-5.48 (d,1H), 4.89-4.79 (d,Hz, 1H), 3.81-2.88 (d, 2H overlapped with (s,3H),1.30-1.22(d,3H).^{13}C NMR (125MHz, $CDCL_3$): δ ppm 173.0, 169.2, 134.3, 133.6, 131.0, 130.7, 130.0, 129.5, 129.3, 129.2, 128.8, 128.1, 58.7, 52.1, 50.4, 50.2, 18.1, 17.6. IR (KBr): (cm^{-1}) 3453, 2965, 1727 ,1492, 1458, 1212, 990, 762, 699, 548. MS m/z = 313 $[MH]^+$

Methyl 3-(2-amino-1-phenylbutylamino)-3-phenylpropanoate(3c) obtained as white crystalline solid. Hygroscopic. yield-85%, Mp: 250-260^{0}C^{1}H NMR (500MHz, $CDCl_3$): δ ppm0.88 (t, 3H, *J* = 7 Hz), 1.38–1.45 (m, 1H), 1.76–1.81 (m, 1H), 3.06 (dd, 1H, *J* = 17, 8 Hz), 3.25 (dd, 1H, *J* = 17, 6 Hz) 3.87–3.91 (m, 1H), 4.57–4.60 (m, 2H), 7.16–7.28 (m, 7H, -Ar-H), 7.30–7.39 (m, 3H, -Ar-H); ^{13}C NMR (125MHz, $CDCL_3$): δ ppm 170.5, 130.2, 130.1, 129.7 , 129.6, 129.4, 129.2, 129.1, 129, 62.4, 62.3, 58.3,58.2, 53.6,52.2, 23, 22.9, 9.6, 9.5. IR (KBr): (cm^{-1}) 3407,2951,1736,1569,1459,1217,763,702,565. MS m/z = 327 $[MH]^+$

3-((2-amino-1-phenylpropyl)amino)-2-methyl-3-phenylpropanoic acid (3d) : White solid; m.p 150-152 °C; Yield 82%; ^{1}H NMR (500 MHz, D_2O): δ ppm 0.81 (d, 3H, *J* = 7 Hz), 1.16 (d, 3H, *J* = 6.5 Hz), 3.11 – 3.18 (m, 1H), 3.97 – 4.03 (m, 1H), 4.38 – 4.43 (m, 2H), 7.05 – 7.08 (m, 4H, -Ar-H), 7.16 – 7.32 (m, 6H, -Ar-H); ^{13}C NMR (125 MHz, D_2O): δ ppm 13.5, 14.3, 42.4, 48.3, 64.1, 65.6, 128.6, 128.8, 129.3, 129.5, 129.7, 130.4, 132.7, 178.6; FT-IR (KBr cm^{-1}): 3421, 2893, 1710, 1608, 1553, 1457, 1403, 1202, 764, 701; MS m/z = 313 $[MH]^+$.

Methyl 3-(2-amino-3-methyl-1-phenyl butyl amino) -3-phenylpropanoate,(3e) obtained as white crystalline solid. Hygroscopic.Yield-73%.Mp: 248-258^{0}C, ^{1}H NMR (500MHz, $CDCl_3$): δ ppm 10.28 (s, 1H, NH), 8.81 (s, 3H, NH_2 with Hcl proton), 7.45-7.17(m, 10H, Ar), 5.41 (s, 1H), 4.83 (s,1H), 4.28 - 4.26 (d, J = 9.94Hz, 1H), 3.96 -3.93 (d, 2H, J = 15.29Hz), 3.55 - 3.49 (s, 3H), 1.55 (s, 1H), 1.26 - 1.21(d, J = 6.88Hz, 3H), 1.03 (s, 3H). ^{13}C NMR (125MHz, $CDCL_3$): δ ppm 169.9, 134.3, 131.8, 130.2, 129.4, 128.9, 128.7, 63.9, 58.7, 58.1, 52.0, 37.0, 28.8, 21.0, 14.9. IR

(KBr) : (cm^{-1})3424, 2955, 2369, 2342, 1954, 1896, 1737, 1629, 1524, 1566, 1436, 1461, 1300, 1245, 1159, 1060, 981, 919, 765, 699, 627, 586, 551, 425. MS m/z = 341 $[MH]^+$

Methyl 3-(2-amino-1-phenylheptylamino)-3-phenylpropanoate,(3f) obtained as white crystalline solid.Hygroscopic.Yield-70%. Mp:140-144^{0}C. ^{1}H NMR (400MHz, $CDCl_3$): δ ppm 10.29(s,1H),8.99-8.90(s,3H,aminoprotonswithHclproton),7.52-7.13(m,10H,Ar),5.47(s,1H),4.66(s,2H),3.60-3.57(s,1H),3.01(s,1H),1.54-1.04(m,8H,Aliphatic ,0.77-0.68(m,3H).^{13}CNMR (125MHz, DMSO-D_6):δppm170.6,130.1,129.6,129.3,129.1,128.9, 62.3,58.2,52.5,52.2,39.2,36.9,31.1,29.6,24.0,22.0,14.1.IR(KBr):(cm^1)3424,2944,2369,2342,1954,1896,1737,1629,1524,1566,1524,1461,1436,1366,1245,1159,1060,981,919,627,586,551,425. MS m/z = 369 $[MH]^+$

Methyl 3-((2-amino-1-(4-chlorophenyl)heptyl)amino)-3-(4-chlorophenyl)propanoate (3g) : oily mass; Yield 69%; ^{1}H NMR (500 MHz, D_2O): δ ppm 0.59 (t, 3H, J = 6 Hz), 0.85 – 1.03 (m, 7H), 1.08 – 1.18 (m, 1H), 2.60 (dd, 1H, J = 16, 8 Hz), 2.92 (dd, 1H, J = 15.5, 6 Hz), 3.18 – 3.26 (m, 1H), 3.38 (s, 3H), 3.67 (d, 1H, J = 9 Hz), 3.93 – 3.96 (m, 1H), 6.97 – 7.06 (m, 8H, -Ar-H); ^{13}C NMR (125 MHz DMSO-d_6): δ ppm 13.9, 21.8, 24.3, 29.2, 30.9, 40.5, 52.2, 55.0, 58.3, 63.1, 128.4, 128.7, 129.5, 129.8, 132.7, 133.1, 136.7, 139.4, 172.1; FT-IR (KBr cm^{-1}): 3416, 2933, 1719, 1601, 1493, 1414, 1094, 1012, 829; Ms m/z = 437 [M+], 439 [M+2].

Methyl 3-((2-amino-1-(4-fluorophenyl)heptyl)amino)-3-(4-fluorophenyl)propanoate (3h) :oily mass; Yield 72%; ^{1}H NMR (400 MHz, D_2O): δ ppm 0.70 (t, 3H, J = 6.6 Hz), 1.09 – 1.41 (m, 7H), 1.61 – 1.68 (m, 1H), 3.04 (dd, 1H, J = 16.5, 8.6 Hz), 3.21 – 3.27 (m, 1H), 3.50 (s, 3H), 3.81 – 3.87 (m, 1H), 4.58 – 4.62 (m, 1H), 6.87 – 6.92 (m, 2H, -Ar-H), 6.97 – 7.02 (m, 2H, -Ar-H), 7.12 – 7.16 (m, 4H, -Ar-H); ^{13}C NMR (100 MHz, D_2O): δ ppm 13.0, 21.4, 23.9, 27.7, 30.4, 37.9, 52.7, 53.1, 59.4, 62.9, 116.4, 115.9, 129.9, 130.30, 130.39, 130.8, 130.9, 163.2, 163.0, 172.0; FT-IR (KBr cm^{-1}): 3421, 2934, 1730, 1607, 1514, 1440, 1235, 1167, 839; Ms m/z = 405 $[MH]^+$.

3-((2-amino-1-(3-nitrophenyl)heptyl)amino)-3-(3-nitrophenyl)propanoic acid (3i) : oily mass; Yield 70%; ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 0.74 (t, 3H, J = 7.0 Hz), 0.98 – 1.15 (m, 6H), 1.27 – 1.32 (m, 2H), 2.62, (dd, 1H, J = 16, 8 Hz), 3.04 (dd, 1H, J = 16, 4 Hz), 3.27 (m, 1H), 3.80 (d, 1H, J = 8 Hz), 4.09 – 4.13 (m, 1H), 7.34 – 7.44 (m, 2H, -Ar-H), 7.56 – 7.59 (m, 2H, -Ar-H), 7.84 – 7.87 (m, 1H, -Ar-H), 7.92 – 7.96 (m, 3H, -Ar-H), 8.21 (br s, 3H, -NH_2 with 2HCl), 9.42 (br s, 1H, -NH), 12.35 (br s, 1H, -COOH); ^{13}C NMR (100 MHz DMSO-d_6): δ ppm 14.1, 22.0, 24.6, 29.5, 31.2, 41.7, 55.5, 58.4, 63.0, 122.0, 122.4, 122.6, 123.0, 129.7, 130.1, 134.8, 142.9, 145.8,

147.7, 147.8, 172.9; FT-IR (KBr cm^{-1}): 3118, 2928, 1724, 1607, 1533, 1479, 1349, 1107, 737; Ms m/z = 445 $[MH]^+$.

Biological evaluation:

Antimicrobial activity

Antimicrobial activities were carried out using well plate method (Balachandran et al., 2012). Petri plates were prepared with 20 ml of sterile Mueller Hinton agar (MHA) (Hi-media, Mumbai). The test cultures were swabbed on the top of the solidified media and allowed to dry for 10 min and a specific amount of synthesized compound at 1mg/well was added to each well separately. The loaded wells were left for 30 min at room temperature for compound diffusion. Negative control was prepared using respective solvents. Streptomycin was used as positive control against bacteria. Ketoconazole was used as positive control for fungi. The plates were incubated for 24 h at 37°C for bacteria and for 48 h at 28°C for fungi. Zones of inhibition were recorded in millimetres and the experiment was repeated twice. The results were presented in table2

Table 2: Antimicrobial activity of synthesized β^3-amino acids

Organism	3b	3c	3e	3f	3g	3h	Streptomycin
Gram positive bacteria							
Enterobacter aerogenes	6	-	14	11	13	18	22
Bacillus subtilis	8	6	22	17	13	17	22
Staphylococcus aureus	6	6	12	13	12	18	14
Gram negative bacteria							
Klebsiella pneumoniae	7	8	9	9	10	17	20
Salmonella typhimurium	7	7	18	14	12	20	24
Proteus vulgaris	7	10	15	13	10	18	30
Escherichia coli	8	7	19	14	11	20	20
Clinical isolates							
*Staphylococcus aureus((*MRSA)	7	6	20	16	10	19	24
Fungi							Ketoconazole
Candida albicans	8	7	16	11	13	20	22
Malassesia pachydermatis	7	7	17	12	13	20	24

Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration studies of the synthesized compounds were performed according to the standard reference methods for bacteria (Duraipandiyan and Ignacimuthu, 2009), for filamentous fungi (CLSI, 2008). The required concentrations (1000 µg/mL, 500 µg/mL, 250 µg/mL, 125µg/mL, 62.5 µg/mL, 31.25 µg/mL, 15.62 µg/mL and 7.81µg/mL) of the compound were dissolved in DMSO (2%), and diluted to give serial two-fold dilutions that were added to each medium in 96 well plates. An inoculum of 100µl from each well was inoculated. The antifungal agents Ketoconazole for fungi and Streptomycin for bacteria were included in the assays

as positive controls. For fungi, the plates were incubated for 48 to 72 hours at 28°C and for bacteria the plates were incubated for 24 h at 37°C. The MIC for fungi was defined as the lowest concentration, showing no visible fungal growth after incubation time. 5µl of tested broth was placed on the sterile MHA plates for bacteria and incubated at respective temperature. The MIC for bacteria was determined as the lowest concentration of the compound inhibiting the visual growth of the test cultures on the agar plate.MIC values are given in table3

Table 3: **MIC (minimum inhibitory concentration) of selected compounds**

Organism	**3e**	**3f**	**Streptomycin**
Gram positive bacteria			
Enterobacter aerogenes	**250**	**500**	**6.25**
Bacillus subtilis	**31.25**	**62.5**	6.25
Staphylococcus aureus	**125**	**250**	6.25
Gram negative bacteria			
Klebsiella pneumoniae	**500**	**500**	6.25
Salmonella typhimurium	**62.5**	**125**	30
Proteus vulgaris	**125**	**125**	6.25
Escherichia coli	**31.25**	**125**	6.25
Clinical isolates			
Staphylococcus aureus MRSA	**31.25**	**62.5**	6.25
Fungi			Ketoconazole
Candida albicans	**62.5**	**250**	12.5
Malassesia pachydermatis	**125**	**250**	12.5

CONCLUSION

A convenient route to synthesis of β^3-amino acids is explained. Yield of corresponding aminoacids obtained is good. The compounds numbered 3e and 3h showed excellent antimicrobial activity to gram positive, gram negative, fungi.

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