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Synthesis of Azole Derivatives of *l*-Proline and their Antibacterial Activity

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ABSTRACT

Four azole derivatives of proline, namely 2-[2-(1,3,4-oxadiazol-5-thionyl)]proline 5, 2-[2-(4-N-amino-1,2,4-triazol-5-thiolyl)] proline 6, 2-[2-(1,2,4-triazol-5-thiolyl)] proline 7 and 2-[2-(1,3,4-thiadiazol-5-thionyl)] proline 8 have been synthesized from proline 1via a common synthetic pathway. IR, 1H-NMR, 13C-NMR and DEPT-NMR, characterized all intermediates and final products. The antibacterial activities assessed using the paper disk diffusion method against Gram-negative bacteria: Pseudomonas aeruginosa and Escherichia coli and Gram-positive bacteria, Bacillus cereus, and Staphylococcus aureus. Some of the synthetic compounds showed promising activity against microorganisms under test in comparison to commercially available antibiotics ampicillin and gentamycin.

Keywords: Proline amino acid; 1,3,4-oxadiazole; 1,3,4-thiadiazole; 1,2,4-triazole; antibacterial activity.

INTRODUCTION

Proline is needed for the production of collagen and cartilage. It keeps muscles and joints flexible and helps reduce sagging and wrinkling that accompany UV exposure and normal aging of the skin. Also, [1] helps in prevention from arteriosclerosis by enables the artery walls to release fat buildup into the bloodstream, decreasing the size of the blockages to the heart and surrounding vessels. [2,3] Therefore, proline decreases the pressure built up by these blockages, decreasing the risk of heart disease. [4]

According to Pauling and Rath, cardiovascular health can be strongly supported by providing the body sufficient amounts of Vitamin C (which helps maintain the healthy production of collagen, a vital component for healthy arteries) and the amino acids L-Lysine and L-Proline, which support the body's appropriate use of cholesterol as it pertains to cardiovascular health[5]. L-Proline is an osmoprotectant and therefore they used in many pharmaceutical, biotechnological applications. [6-8] Proline and proline derivatives used as anticonvulsants. [9] Synthetic proline derivatives mimetic and analogs in positions 1, 3, 4 and 5 offer further options to tune the biological, pharmaceutical or physicochemical properties of peptides and proteins. [10,11]

Modifications in carboxylic group have also interested several chemists due to their biological importance such as 1,3,4-oxadiazoles, 1,3,4-thiadiazoles and 1,2,4-triazoles in peptoids containing proline residues were synthesized. [12] These compounds are useful for the treatment or amelioration of symptoms of adult respiratory distress syndrome, septic shock, and multiple organ failure. Processes mediated by human neutrophil elastase (HNE) are also implicated in conditions such as arthritis, periodontal disease, glomerulonephritis, and cystic fibrosis.

In recent years, proline derivatives and analogs such as proline hydrazide are used as enantioselective catalysts based on molecular recognition for aldol reactions between acetone and α-keto acids. [13] In addition, they found increasing popularity as organocatalysts in asymmetric synthesis[14] and in organocatalytic cyclisation. [15]

This work is exclusively dealing with the synthesis of 1,3,4-oxadiazole, 1,3,4-thiadiazole and 1,2,4-triazole and 4-amino-1,2,4-triazole as carboxylic acid derivatives of proline and testing their antibacterial activity.

EXPERIMENTAL SECTION

All reactions were monitored by TLC analysis (silica gel for TLC supplied by MERCK), iodine was used for visualization. The melting points were measured with a BÜCHI 540 melting point apparatus and are uncorrected. The IR spectra exhibited as wave number (ν cm^{-1}) were recorded using KBr discs in a JASCO V-530 spectrophotometer at University of Oran, Es-Senia, Algeria. The ^{1}H NMR and ^{13}C NMR (250 MHz) spectra in DMSO-d_6 exhibited as ppm, were recorded at University of Oran, Es-Senia (Algeria). Microorganisms in this study were supplied by the university hospital of Oran and identified in our laboratory.The Mueller Hinton medium was supplied by Difco.

Methyl (2S)-Pyrrolidine-2-carboxylate: (2)
(2S)-Pyrrolidine-2-carboxylic acid (**1,** 1.30g, 1.1 mmol), methanol (100ml) and H_2SO_4 (2.5ml) were refluxed for 11h at 85C°. Cooled and neutralized by aqueous $NaHCO_3$ (5%) to pH=7. The ester was extracted by dichloromethane, dried over $MgSO_4$, filtered, distilled to dryness to give colorless oil (**2**, 0.98g, 67%).IR υ (cm^{-1}):3404.97 (NH); 1740.13 (C=O).

(2S)-Pyrrolidine-2-carbohydrazide: (3)
Methyl (2S)-Pyrrolidine-2-carboxylate **(2,** 0.30g, 2.3 mmol), ethanol (30ml) and hydrazine hydrate 64% (4ml) were refluxedfor13h. Volatiles were evaporated under vacuum to dryness to give white solid which recrystallized from ethanol to give hygroscopic crystalline (**3,** 0.227g, 92%). IR υ(cm^{-1}): 3394.51(NH, NH_2), 1642(CO-N).

2-[(2S)-pyrrolidine-2-ylcarbonyl]-hydrazinecarbothioamide: (4)
(2S)-Pyrrolidine-2-carbohydrazide **(3,** 0.3g, 2.3 mmol), ammonium thiocyanates (0.6g, 3.9 mmol), ethanol (10 mL) and few drops of HCl were refluxed for 15h. Volatiles removed under vacuum at room temperature. Solid formed was dissolved in water (5 mL), extracted with CH_2Cl_2, dried over $MgSO_4$, filtered and evaporated to dryness. The waxy solid was washed with ethanol to give waxy material (**4**, 0.38g, 87 %.). IR υ (cm^{-1}):3114.26(NH, NH_2); 1618.93(N-C=O); 1380.72 (C=S).

5 [(2S)-pyrrolidine-2-yl]-1, 3, 4-oxadiazole-2(3H)–thione: (5)
L-prolinehydrazide (**3**, 0.2g, 1.6 mmol), ethanol (15 mL) wax added to it an ethanolic solution of KOH (0.3g in ethanol 9 mL). Carbon disulphide (3mL) was dropped gradually and the mixture was refluxed for 17 h. Acidified with HCl (10 %) to pH 6 to give a yellow precipitate , filtered off , washed with ethyl acetate and recrystallized from ethanol to yield a hygroscopic crystalline (**5,** 0.931g, 83%.). IR υ (cm^{-1}): 3390.65(NH), 1630.68(C=N), 1230.63 (C=S), 1051.63(C-O-C).^{1}H NMR, 8.75(s, 1H, HN-C=S), 4.29(s, 1H, ^{2}CH), 3.20(s, 1H, $^{1}NH_{Pyrrolidine}$), 2.23(m, 2H, $^{2}CH_2$), 2.08(m, 1H, $^{3}CH_2$), 1.22(m, 2H, $^{3}CH_2$), 1.07(t, 2H, $^{4}CH_2$), ^{13}C NMR, 206.37(C=S) 170.27(C-SH), 58.61(O-C=N), 45.20(^{5}C, CH_2), 30.61($^{3\&4}C$, CH_2), 27.88 (^{4}C, CH_2).

4-Amino-5-[(2S)-pyrrolidine-2-yl]-2H-1,2,4-triazole-3-thiol: (6)
Oxadiazole (**5,** 0.35 g, 2 mmol) dissolved in ethanol (25mL) ,hydrazine hydrate 64% (4mL) was added and refluxed for 12h. Solid formed after cooling, filtered and recrystallized from ethanol to yellow crystalline (**6**, 0.324g, 87 %.). Mp. 132 C°. IR, υ (cm^{-1}):3420.64 (NH), 1616.69(C=N), 1253(C=S).^{1}H NMR, 8.41(s,1H, HNC=S), 6.46(s, 2H,$^{1}N-NH_2$), 3.57(s, 1H,$^{1}NH_{Pyrrolidine}$), 3.23(s, 1H, SH), 2.64(m, 2H, $^{2}CH_2$), 1.90 (m, 2H, $^{5}CH_2$), 1.53(s, 1H,$^{3}CH_2$), 1.03(t, 2H, $^{4}CH_2$).^{13}C NMR, 179.05(^{6}C, C=S), 167.24(^{1}C, CN), 57.54(^{2}C, CH), 57.56(^{2}C, CH), 45.95(^{5}C, CH_2), 45.53(^{5}C, CH_2), 29.25(^{3}C, CH_2), 23.36 (^{4}C, CH_2); RMN DEPT, 29.62 (^{3}C, CH_2), 23.89 (^{4}C, CH_2).

5-**[(2S)-pyrrolidine-2-yl]-2, 4-dihydro-3H-1, 2, 4-triazole-3-thiol: (7)**
Thiosemicarbazide (**4**, 0.4g**),** ethanol (15 mL), KOH (0.4g) were refluxed for 13h. The reaction mixture was acidified with HCL to pH 6, white ppt formed which recrystallized from ethanol to give hygroscopic crystalline (**7**, 0.30g, 83%). IR, υ(cm^{-1}): 3251.55(N(3)-H), 2622(SH), 1611.92(C=N). ^{1}H NMR**,** 7.62(s, 1H, ^{3}NH), 7.28(s, 1H, ^{1}NH), 3.72(m, 1H, ^{2}CH), 3.18(s, 1H, $NH_{Pyrrolidine}$. 2.11(s, H, SH) 1.88 (s, 1H, $^{5}CH_2$), 1.21(t, 2H, $^{3}CH_2$), 1.09(t, 2H, $^{4}CH_2$), ^{13}C NMR, 170.09(C-SH), 61.40(CN), 58.52(^{2}C, CH), 45.05(^{5}C, CH_2); 27.91(^{3}C, CH_2); 23.03(^{4}C, CH_2).

5-[(2S)-pyrrolidine-2-yl]-1, 3, 4-thiadiazole-2-amine: (8)
Thiosemicarbazide (**4**, 0.209g, 11 mmol), dissolved in H_2SO_4 (3.5 mL) and refluxed for 20h. Neutralized with dilute alcoholic KOH to pH=7, to give white powder. Recrystallized from ethanol to give hygroscopic crystalline, (**8,** 0.24g, 79%).IR υ (cm^{-1}): 3355 (NH_2), 3256. 3157 (N(3)-H), 1610.88(C=N), 760(C-S-C).^{1}H NMR**,** 8.57 (s,1H, NH-aromatic),7.14(d, 2H, NH_2), 4.14(m, 1H, NH-$_{Pyrrolidine}$), 3.76(m, 1H, ^{2}CH), 3.16(t, 2H, $^{5}CH_{2Pyrrolidine}$, 2.15.1.18 (t, 2H,

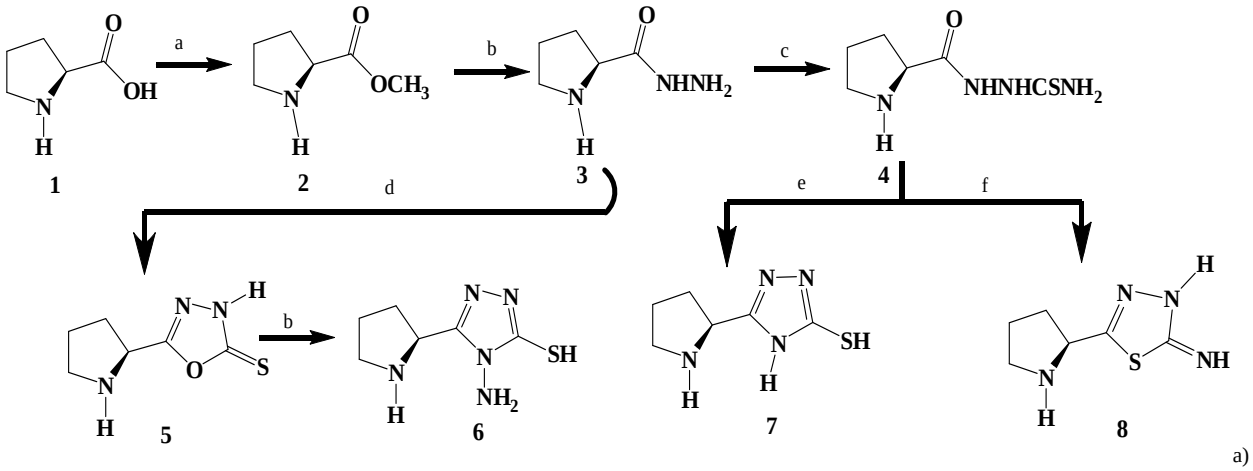
$^{3}CH_2$), 1.06(t, 2H, $^{4}CH_2$),^{13}C NMR, 180.99(^{6}C-NH_2), 165.69(^{1}C=N), 58.48 (^{2}CH), 45.18($^{5}CH_2$), 27.71($^{3}CH_2$), 23.40($^{4}CH_2$).

Antibacterial activity:

Each of the various synthesized compounds dissolved in DMSO at concentrations of 10, 10/2, 10/4, 10/8, 10/16 µg/mL were prepared. Paper discs of Whatman filter paper n°1 were cut and sterilized in an autoclave. The paper discs were saturated with 10 µl of newly synthesized compounds dissolved in DMSO solution. The DMSO as negative control and were placed aseptically in the Petri dishes containing Nutrient agar media inoculated with the above-mentioned two bacteria separately. The petri dishes were incubated at 37°C.Ampicillin and gentamycin were used as a standard drug.

RESULTS AND DISCUSSION

The synthesis of proline derivatives **2-8**, achieved by common synthetic pathway (see Scheme 1). Methyl ester **2** was prepared according to literature procedure, [16] and



a)

CH_3OH, H_2SO_4, b) EtOH, NH_2NH_2, c) EtOH, NH_4SCN, HCl, d) i- EtOH, CS_2, KOH, ii- HCl, e) i- EtOH, KOH, ii- HCl, f) H_2SO_4

Scheme1. **Synthetic pathway for azole derivatives of proline 5-8**

Characterized by IR bands at 3405 cm^{-1} for NH and 1740 cm^{-1} for CO in 67% yield. The hydrazide **3** regarded as the real starting material for the synthesis of the azoles **5-8**. Hydrazide **3** has been synthesized in 92% yield, by treating ester **2** with hydrazine hydrated 64% in ethanol at reflux temperature. IR confirmed that the conversion has taken place by presence of broadband at 3295 cm^{-1} for NH and NH_2 and another band at 1642 cm^{-1} for CO-N. These bands had disclosed the probability of formation diketopiperazine derivative of proline which sometimes-taking place depending on hydrazine concentration and reaction duration. [17] The oxadiazole thione **5** and N-amino triazole thiol **6** were obtained by refluxing hydrazide **3** with CS_2 in ethanol followed by acidification with HCl, resulting oxadiazole thion **5** in 83% yield, as a mixture of (thion ⇄ thiol) tautomers. [18](see Figure 1).This has been confirmed by IR spectroscopic measurements which showed bands at cm^{-1} 3391 (NH), 2642 (SH), 1630 (C=N) and 1230(C=S).^{1}H NMR exhibited signals at 8.8 (HN-C=S), 4.3 (N=C-SH) ppm of oxadiazole and ^{13}C-NMR at 206.37(C=S) and 170.27(N=C-SH) ppm of the tautomer **5**. [19,20] The latter **5a**, after treatment with hydrazine under reflux yielded 4*N*-amino-1,2,4-triazolethiol **6** in 87% yields. IR 3421, 3047(NH, NH_2), 2466(SH),

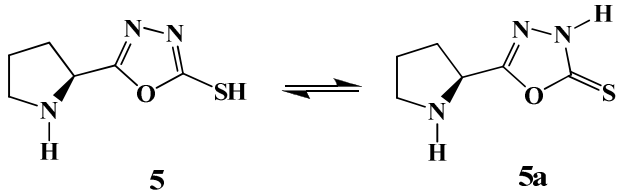


Figure1. Enole (5)- keto (5a) tautomerism of oxadiazole thione (5a)

1617(C=N), 1253(C=S) cm^{-1}. ^{1}H NMR, 8.4(HNC=S), 6.5(N-NH_2), 3.6(N^{+}H, of pyrrolidine), 3.2(SH), 2.64, 1.90, 1.53 and 1.03 H of Pyrrolidine, ^{13}C NMR, 23.36(^{4}C, CH_2), 29.25(^{3}C, CH_2), 45.53(^{5}C, CH_2), 57.54(^{2}C, CH), 167.24(^{1}C, CN), 179.05(^{6}C, C-SH). RMN DEPT, 23.89 (^{4}C, CH_2), 29.62(^{3}C, CH_2), 45.95(^{5}C, CH_2), 57.56(^{2}C, CH).

The other two heterocyclic bases **7** and **8** were synthesized from thiosemicarbazide **4** which was obtained by reacting **3** with ammonium thiocyanate. The synthesis of 1H-1,2,4-triazole-3-thiol **7** achieved by cyclisation of **4** by

heating with KOH followed by acidification with HCl. IR showed the characteristic bands 3420, 2622, 1616 and 1253 cm^{-1} for NH, SH, C=N and C=S bonds respectively, similar to oxadiazole ring's tautomerism (see Figure 1). ^{1}H NMR, also showed the expected signals at 7.62 ppm for $N^{+}H$ of pyrrolidine ring , 7.28 and 3.18 ppm for NH and SH of triazole thiol ring and 7.46 and for NH thione's tautomer .^{13}C NMR, exhibited the signal of N=C(N)-SH at 170 ppm.

When **4** reacted with H_2SO_4 the result was 5-aminothiadiazole **8** in 79% yield. IR showed the characteristic bands 3366, 3257 cm^{-1} for NH in pyrrolidine and thiadiazole rings, 1641 and 1611 for C=N bonds in thiadiazole ring.^{1}H NMR, spectrum exhibited the **signals** at 8.57 and 7.14 ppm for NH in thiadiazole ring and 2.15 ppm for NH of pyrrolidine rings. ^{13}C NMR, showed signals at 180, 165 and 58 ppm for two carbons in thiadiazole and one in pyrrolidine rings.

Antibacterial activity:

The eight compounds **1-8** were screened for their activity against Gram-positive *Staphylococcus aureus* and *Bacillus Cereus* and Gram-negative bacteria, *Escherichia Coli* and *Pseudomonas aeruginosa*. The antibacterial activities of the tested compounds were evaluated using the paper disk diffusion method. DMSO, which is known as bacterial static in the above-mentioned concentration, was used as negative control, standard disks (Mast Diagnostics, UK) saturated with known antibiotics ampicillin, and gentamycin as positive control were applied. After incubation at 37 °C for 24 h, the zone of inhibition of growth around each disk was measured in millimeters and zone diameters were interpreted in accordance with CLSI and NCCLS (for *Campylobacter* spp.) guidelines. [21-23] The experiments were performed in duplicates and the average results are summarized in Table 1.

Table 1, showed that the under-tested organisms Gram positive *Staphylococcus aureus* was almost resistant to all synthesized compounds **1-8** except compounds **6** and **7**, where they showed slight activity only at high concentration. While Gram-positive *Bacillus cereus* was slightly affected by the synthesized compounds, except compound **6** which showed no activity at all even in relatively high concentration in the other hand, the Gram negative bacteria *E.coli* and *P.aeruginosa* were highly affected by all compounds **1-8**.

Microorganisms not affected by compounds tested, no minimum inhibition concentration (MIC) test conducted. Those which showed activity, MIC was done by DMSO till no significant activity, while for highly affected bacteria the dilution was stepped down gradually till reaching 1/16 µg/mL.

Table 1. Antibacterial activity of l-proline 1 and its synthesized derivatives 2-8 in mm

	Gram positive										**Gram negative**									
	Staphylococcus aureus					***Bacillus cereus***					***Escherichia coli***					***Pseudomonas aeruginosa 1139***				
Comp	**Inhibition zone**					**Inhibition zone**					**Inhibition zone**					**Inhibition zone**				
	a*	**b***	**c***	**d***	**e***	**a***	**b***	**c***	**d***	**e***	**a***	**b***	**c***	**d***	**e***	**a***	**b***	**c***	**d***	**e***
1	**0**	**0**	**0**	**0**	**0**	**8**	**0**	**0**	**0**	**0**	**26**	**26**	**21**	**20**	**15**	**28**	**28**	**25**	**21**	**11**
2	**0**	**0**	**0**	**0**	**0**	**8**	**6**	**0**	**0**	**0**	**26**	**24**	**20**	**16**	**12**	**28**	**28**	**26**	**22**	**6**
3	**0**	**0**	**0**	**0**	**0**	**12**	**10**	**0**	**0**	**0**	**32**	**28**	**17**	**15**	**10**	**36**	**36**	**32**	**26**	**8**
4	**0**	**0**	**0**	**0**	**0**	**14**	**14**	**12**	**6**	**0**	**34**	**25**	**18**	**15**	**14**	**38**	**36**	**36**	**32**	**25**
5	**0**	**0**	**0**	**0**	**0**	**15**	**15**	**15**	**12**	**0**	**36**	**22**	**11**	**10**	**8**	**30**	**28**	**26**	**24**	**22**
6	**14**	**8**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**28**	**28**	**28**	**22**	**18**	**38**	**34**	**20**	**19**	**17**
7	**8**	**6**	**0**	**0**	**0**	**13**	**12**	**12**	**8**	**0**	**38**	**31**	**20**	**12**	**7**	**38**	**36**	**30**	**20**	**15**
8	**0**	**0**	**0**	**0**	**0**	**10**	**8**	**8**	**6**	**6**	**36**	**32**	**34**	**28**	**26**	**33**	**31**	**28**	**26**	**22**
†	**0**	-	-	-	-	**10**	-	-	-	-	**12**	-	-	-	-	**0**	-	-	-	-
‡	**12**	-	-	-	-	**8**	-	-	-	-	**27**	-	-	-	-	**28**	-	-	-	-
DMSO	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

a, b*, c*, d*,e* : concentrations :(10, 10/2, 10/4, 10/8, 10/16) µg/mL., respectively† :Ampicillin. ‡: Gentamycin. Key to the inhibition zones activities: Highly active= Inhibition zone > 15mm; Moderately active= Inhibition zone 10-15 mm; Slightly active= Inhibition zone 6-8 mm; Inactive= Inhibition zone < 6 mm.*

Compounds **6** and **8** exhibited significantly high activity even at low concentration upon Gram-negative *Escherichia coli.* Similarly, compounds **4**, **5** and **8** were excreting high activity on Gram-negative *Pseudomonas aeruginosa* at highly diluted solution 1/16 µg/mL.

CONCLUSION

We have described the synthesis of several diazole derivatives of *l*-proline **2-8** starting with *l*-proline *via* six synthetic steps as described in Scheme 1. Intermediate 5 [(2S)-pyrrolidine-2-yl]-1, 3, 4-oxadiazole-2(3H)–thion (**5 a**) was found to exist in equilibrium form with its thiol tautomer (**5**) as indicated by IR, 1H-NMR and 13C-NMR.

The name and structures of all intermediates and final compounds were established on basis of IUPAC, physical and spectral data.

Antibacterial activity of all compounds were tested against Gram-positive *Staphylococcus aureus* and *Bacillus cereus* and Gram-negative bacteria, *Escherichia coli* and *Pseudomonas aeruginosa.* Using commercially known antibiotics ampicillin and gentamycin as positive control. Some of tested compounds have shown promising antibacterial effect against almost all kinds of bacteria under test particularly the Gram-positive *Staphylococcus aureus* and *Bacillus cereus*.

Our future plan is to study some other biological activity and more derivatives of proline.

REFERENCES

[1] A Barbul. *J Nutr*., **2008**, 138(10), 2021S-2024S.
[2] K G McCullough; L A Ehrhart. *Atherosclerosis*., **1974**, 19(1), 13-28.
[3] L A Ehrhart; E H Pezacka. *Arteriosclerosis, Thrombosis and Vascular Biology*., **1997**, 17, 2074-2081.
[4] B Rubin; M J Antonaccio; Z P Horovitz. *Progress in Cardiovascular Diseases.*, **1978**, 21(3), 183-194.
[5] M Rath; L Pauling. *J. Orthomol. Med.*, **1992**, 7(1), 5-12.
[6] J L Milner; D J McClellan; J M Wood. *Microbiology*., **1987**, 133(7) ,1851-1860.
[7] H Gröger; J Wilken. *Angewandte Chemie Inter*., **2001**, 40(3), 529-532.
[8] Y Yulan; S Huakai; L Zhimin; Y Qin. *BMC Biotechnology*., **2014**, 14, 44.
[9] M Dawidowski; J Chońska; W Chońska; J Turło. *Bioorganic & Medicinal Chemistry*., **2014**, 07, 42.
[10] G Brahmachari; S Das. *RSC Adv*., **2014**, 4, 7380-738.
[11] P I Dalko; L Moisan. *Angewandte Chemie International*., **2004**, 43(39), 5138–5175.
[12] M Wieczorek; A Gyorkos; L W Spruce; A Ettinger; S E Ross; H S Kroona; C E Burgos-Lepley; L D Bratton; T S Drennan; D L Garnert; G Von Burg; C G Pilkington; J C Cheronis. *Arch BiochemBiophys.*, **1999**,367(2), 193-201.
[13] L J Wei; Y Yong; C C Feng. *Scientia Sinica Chimica*., **2011**, 41 (8), 1298-1305.
[14] S.S. Makhathini; S.K. Das; T.Singh; P.I. Arvidsson; H.G. Kruger; H. Gunosewoyo; T. Govender; T. Naicker. ARKIVOC., **2016**, (iii), 134-144.
[15] A S Y Lee; C H Chung; Y T Chang; P L Chen. *Journal of Applied Science and Engineering*., **2012**, 15(3), 311-315.
[16] B S Furniss, A J Hannford; P W G Smith; A R Tatchell. Longman Scientific & Technical, 5/e, Vogel's Text Book of Practical Organic Chemistry, **1989**; 1076.
[17] G Mloston; A M Pieczonka; A Wroblewska; A Linden; H Heimgartner. *Tetrahedron Asymmetry*., **2012**, 23, 795-801.
[18] Z Khiati; A A Othman; B Gussase. *S. Afr. J. Chem*., **2007**, *60, 20–24*.
[19] E Pretsch; P Buhlmann; C Affolter. *Springer*, Berlin**, 2000**, 271.
[20] O Tchaicheeyan. *Biophysical characterization of the 117 amino acids long N-terminal segment od D-Raf (Isoform A)*, M. Sc. Thesis, M.Sc Iowa State University, Ames, Iowa, **2007**
[21] M M S. Dallal; M P Doyle; M Rezadehbashi; H Dabiri; M Sanaei; R Modarresi; K Bakhtiari, K Sharifiy; M Taremi; M R Zali; Y M K Sharifi. *Food Control*., **2010**, 21, 388-392.
[22] Clinical and Laboratory Standards Institute. *Performance Standards fir Antimicrobial Susceptibility Testing;* Sixteenth International supplements, **2006**, 26, 11-35.
[23] National committee for Clinical and Laboratory Standards Villanova, PA., *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*, 4/e, **1997**