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Synthesis, Characterization and Biological Evaluation of New 4-Aryl-6-(2,5-dichlorothiophen-3-yl)-1,2-dihydro-2-oxopyridine-3-carbonitrile

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Condensation of 3-acetyl-2,5-dichlorothiophene with different aldehydes in presence of ethyl cyanoacetate led to the formation of the new oxopyridine derivatives containing a thiophene moiety (**3a-j**) in high yields. All newly synthesized compounds were characterized by standard spectroscopic techniques. Antibacterial evaluation for all new compounds shows no activity against four different types of bacteria.

Keywords: Oxopyridine, Ethyl cyanoacetate, 3-Acetyl-2,5-dichlorothiophene, 3-cyanopyridine-2-one, Condensation.

INTRODUCTION

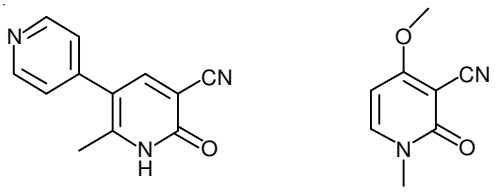
Oxopyridine, a useful *N*-containing heterocyclic compounds, possess interesting pharmacological properties and has received a great attention in previous years due to their biological activities[1,2]. It has been reported that the oxopyridine derivative milrinone (Fig. 1, compound a) is a non-glycosidic cardiotonic agent which has been approved by the United States Food and Drug Administration as a drug used for treatment of patients suffering from heart failure[2-4]. The natural product Ricinine (Fig. 1, compound b) is the first known alkaloid containing oxopyridine derivative[4-6]. The oxopyridine derivatives possess an interesting biological activities, such as antimicrobial[7,8], antitumour[8-11], antiviral activities[12] and anti-inflammatory[13,14].

On the other hand, pyridines-3-carbonitrile derivatives were found to have a significant pharmacological activity such as antimicrobial[15,16] anti-hypertensive[17] and anti-cancer activity[18]. Therefore, we herein report the synthesis of a variety of 2-oxopyridines-3-carbonitrile derivatives containing 2,5-dichlorothiophene moiety (**Scheme-I**).

EXPERIMENTAL

Aldehydes and ethyl cyanoacetate were purchased from Aldrich. 2,5-dichlorothiophene was purchased from acros. 3-Acetyl-2,5-dichlorothiophene was prepared according to literature procedure[19].

The compounds were tested against four bacterial strains: *Staphylococcus* epidermis ATCC 12228, *Bacillus pumilus*, *Escherichia coli* ATCC and *Pseudomonas aeruginosa* by agar diffusion method[20]. Briefly, Mueller-Hinton agar was used as



(a) Milrinone **(b) Ricinine**

Fig. 1. Commercially available and naturally occurring oxopyridine derivatives

cultivation medium. After media preparation, three 6 mm holes were punched in each Mueller-Hinton agar plate using a sterile glass borer. The agar plate was then inoculated with a 24 h-old test bacterium from a broth culture by the vertical and horizontal streaking. The three wells were then filled with 100 µL of a test chemical (20 mg/mL). Inoculated plates were then incubated at 37 ºC overnight. The inhibition zone around the wells were then measured. Dimethyl sulfoxide (DMSO) and amoxicillin were used as negative and positive controls, respectively.

Melting points were determined on an Electrothermal-9002 apparatus. IR spectra were obtained as KBr discs on a Nicolet Impact-400 FT-IR spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AV-300 ultra shield instrument. Chemical shifts are expressed in ppm with reference to TMS as internal standard. Mass spectra (CI), were recorded on a Finnigan MAT SSQ 7000 spectrometer (reaction gas Methane, 100 eV). Electron impact mass spectra (EIMS) were taken with Shimadzo QP instrument at 70 eV and at ion source temperature of 250 °C. For analyses, all new compounds

were further purified on preparative TLC silica-gel plates using chloroform as eluent.

Synthesis

General procedure for the synthesis of oxopyridine (3a-j): Ethanolic solution of potassium hydroxide (1 mmol) was added dropwise to a mixture of aldehydes **2a-j** (1 mmol), 3-acetyl-2,5-dichlorothiophene (1 mmol) and ethyl cyanoacetate (1 mmol) in 50 mL ethanol. The solution mixture was refluxed for 6 h until completion of the reaction. The mixture was left overnight with continuous stirring, whereupon a solid product formed, filtered off, washed with cold ethanol and dried. The product was recrystallized from ethanol to afford analytically and spectroscopically pure 2-oxopyridine-3-carbonitriles (**3a-j**). The progress of all reactions was monitored by thin layer chromatography (TLC).

6-(2,5-Dichlorothiophen-3-yl)-4-(4-fluorophenyl)-1,2-dihydro-2-oxopyridine-3-carbonitrile (3a): Yellow solid, 88 % yield; m.p. 265-267 ºC; ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 12.74 (sb, 1H, NH), 7.79 (dd, 2H, J_F = 5.4 Hz, J = 8.4 Hz, H-2′′,6′′), 7.50 (s,1H, H-5), 7.42 (t, 2H, J = 8.4 Hz, H-3′′,5′′), 6.79 (s, 1H, H-4′); ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) = 163.2 (d, J_{CF} = 263.6 Hz, C-4′′), 161.6 (C=O), 131.3 (J_{CF} = 8.9 Hz, C-2′′,6′′), 128.5 (H-4'), 116.4 (J_{CF} = 21.2 Hz, C-3",5"), 109.5 (C-5), 158.1, 143.9, 136.5, 132.0, 130.8, 126.6, 125.9, 115.8, (C_q) ; ESIMS: *m/z* = 365 [M + H].

6-(2,5-Dichlorothiophen-3-yl)-1,2-dihydro-4-(4-isopropylphenyl)-2-oxopyridine-3-carbonitrile (3b): Yellow solid, 79 % yield; m.p. 251-253 ºC; ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 12.80 (sb, 1H, NH), 7.65 (d, 2H, J = 7.7 Hz, H-2′′,6′′), 7.51 (s,1H, H-5), 7.45 (d, 2H, J = 7.7 Hz, H-3′′,5′′), 6.77 (s, 1H, H-4′), 2.98 (m, 1H, C<u>H</u>$(CH_3)_2$), 1.25 (d, 6H, J = 6.7 Hz, CH(C<u>H</u>$_3)_2$; ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) = 161.6 (C=O), 128.1 (H-4'), 128.3 (C-3",5"), 126.9 (C-2′′,6′′), 108.6 (C-5), 33.3 (<u>C</u>H$(CH_3)_2$), 23.1 (CH$(CH_3)_2$), 151.2, 151.3, 133.1, 133.0, 128.1, 126.5, 125.9, 122.3, 116.2, (C_q); ESIMS: *m/z* = 390 [M + H].

4-(4-*tert*-Butylphenyl)-6-(2,5-dichlorothiophen-3-yl)-1,2-dihydro-2-oxopyridine-3-carbonitrile (3c): Yellow solid, 86 % yield; m.p. 293-295 ºC; ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 12.88 (sb, 1H, NH), 7.65 (d, 2H, J = 8.1 Hz, H-3′′,5′′), 7.58 (d, 2H, J = 8.1 Hz, H-2′′,6′′), 7.49 (s, 1H, H-5), 6.77 (s, 1H, H-4'), 1.33 (s, 9H, -C(C<u>H</u>$_3)_3$; ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) = 161.6 (C=O), 128.0 (C-4′, C-2′,6′), 125.7 (C- H-3′′, 5′′), 108.7 (C-5), 34.5 (<u>C</u>H$(CH_3)_3$), 31.3 (CH(<u>C</u>H$_3)_3$), 159.0, 153.5,152.5,143.8, 132.7, 131.2, 126.6, 125.9, 116.2 (C_q); ESIMS: *m/z* = 404 [M + H].

6-(2,5-Dichlorothiophen-3-yl)-4-[4-(dimethylamino)-phenyl]-1,2-dihydro-2-oxopyridine-3-carbonitrile (3d): Orange solid, 82 % yield; m.p. 308-310 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 12.44 (sb, 1H, NH), 7.64 (d, 2H, J = 7.7 Hz, H-2",6"), 7.49 (s,1H, H-5), 6.83 (d, 2H, J = 7.7 Hz, H-3",5"), 6.71 (s, 1H, H-4'), 3.01 (-N(C<u>H</u>$_3$)2; ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) = 161.9 (C=O), 129.6 (C-2", 6"), 128.0 (C-4'), 111.5 (C- H-3",5"), 107.8 (C-5), 39.6 ((-N(<u>C</u>H$_3)_2$), 158.9, 158.7, 151.9, 131.5, 131.3, 126.3,125.8, 121.5, 117.1, (C_q); ESIMS: *m/z* = 391 [M + H].

6-(2,5-Dichlorothiophen-3-yl)-1,2-dihydro-4-(4-hydroxyphenyl)-2-oxopyridine-3-carbonitrile (3e): Yellow solid, 75 % yield; m.p. 375-377 ºC; ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 12.51 (sb, 1H, NH), 10.11 (sb, 1H, OH), 7.60 (d, 2H, J = 8.5 Hz, H-2",6"), 7.48 (s,1H, H-5), 6.90 (d, 2H, J = 8.5 Hz, H-3",5"), 6.71 (s, 1H, H-4'); ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) = 161.7 (C=O), 159.9 (C-4"), 130.1 (C-2",6"),128.0 (C-4'), 115.7 (C- H-3",5"), 108.2 (C-5), 159.0,148.2, 143.0, 142.9 131.3, 126.4, 125.9, 116.5, (C_q); ESIMS: *m/z* = 364 [M + H].

6-(2,5-Dichlorothiophen-3-yl)-1,2-dihydro-4-(4-hydroxy-3-methoxyphenyl)-2-oxopyridine-3-carbonitrile (3f): Yellow solid, 78 % yield; m.p. 321-323 ºC. ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 12.60 (sb, 1H, NH), 9.74 (sb, 1H, OH), 7.49 (s,1H, H-5), 7.31 (s, H-2"), 7.22 (d, 1H, J = 7.8 Hz, H-6"), 6.94 (d, 1H, J = 7.8 Hz, H-5"), 6.77 (s, 1H, H-4'), 3.85 (s, 3H, -OCH_3) ; ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) = 161.7 (C=O), 128.0 (H-4'), 121.8 (C-6"), 115.6 (C-2"), 112.5 (C-5"), 108.4 (C-5), 55.8 (-OCH_3), 159.1, 149.4, 147.6, 142.9, 131.3, 126.4, 126.1, 125.9, 125.0, 116.6, (C_q); ESIMS: *m/z* = 394 [M + H].

6-(2,5-Dichlorothiophen-3-yl)-4-(3-ethoxy-4-hydroxy-phenyl)-1,2-dihydro-2-oxopyridine-3-carbonitrile (3g): Yellow solid, 92 % yield; m.p. 261-263 ºC. ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 12.55 (sb, 1H, NH), 9.66 (sb, 1H, OH), 7.49 (s, 1H, H-5), 7.30 (s, H-2"), 6.95 (d, 1H, J = 8.2 Hz, H-5"), 6.75 (s, 1H, H-4'), 6.56 (d, 1H, J = 8.2 Hz, H-6"), 4.11 (q, 2H, J = 6.9 Hz, -C<u>H</u>$_2$CH$_3$), 1.37 (t, 3H, J = 6.9 Hz, -CH$_2$C<u>H</u>$_3$); ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) = 161.7 (C=O), 128.0 (H-4'), 121.8 (C-6"), 115.7 (C-5"), 113.7 (C-2"), 108.4 (C-5), 64.0 (-<u>C</u>H$_2$CH$_3$), 14.6 (-CH$_2$<u>C</u>H$_3$), 159.1, 149.6, 146.7, 142.9, 131.2, 126.4, 126.3, 126.2, 125.8, 116.6, (C_q); ESIMS: *m/z* = 408 [M + H].

4-(3-Bromo-4-methoxyphenyl)-6-(2,5-dichloro-thiophen-3-yl)-1,2-dihydro-2-oxopyridine-3-carbonitrile (3h): Pale-yellow solid, 82 % yield; m.p. 360 ºC dec. ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 12.73 (sb, 1H, NH), 7.97 (s, H-2"), 7.77 (d, 1H, J = 8.7 Hz, H-6"), 7.51 (s,1H, H-5), 7.30 (d, 1H, J = 8.7 Hz, H-5"), 6.79 (s, 1H, H-4'), 3.94 (-OCH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) = 161.7 (C=O), 131.7 (C-2"), 128.2 (C-6"), 128.0 (H-4'), 113.8 (C-5"), 108.4 (C-5), 56.67 (-OC<u>H</u>$_3$), 159.1, 156.6, 143.2, 142.9, 131.4, 125.1, 124.5, 116.3, 115.9, 111.0 (C_q); ESIMS: *m/z* = 457 [M + H].

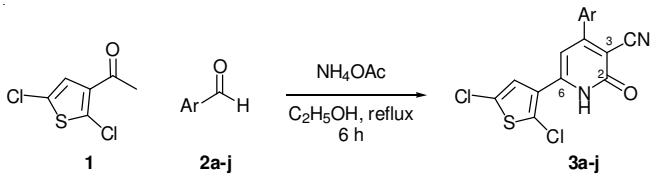
6-(2,5-Dichlorothiophen-3-yl)-1,2-dihydro-4-(2,4-dimethoxyphenyl)-2-oxopyridine-3-carbonitrile (3i): Yellow solid, 84 % yield; m.p. 310-312 ºC. ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 12.63 (sb, 1H, NH), 7.48 (s, 1H, H-5), 7.35 (d, 1H, J = 8.8 Hz, H-6"), 6.74 (d, 1H, J = 8.9 Hz, H-5"), 6.69 (s, 2H, H-3", H-4'), 3.83, (s, 6H, 2-OC<u>H</u>$_3$); ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) = 162.9 (C=O), 130.0 (H-6"), 128.6 (H-4'), 107.5 (H-5"), 107.3 (H-5), 98.3 (H-3"), 56.3, 55.9 (2-O<u>C</u>H$_3$), 160.7, 158.3, 150.1, 147.7, 135.4, 130.0, 127.8, 125.3, 124.5, 121.1, 116.4, (C_q); ESIMS: *m/z* = 408 [M + H].

6-(2,5-Dichlorothiophen-3-yl)-1,2-dihydro-4-(2,5-dimethoxyphenyl)-2-oxopyridine-3-carbonitrile (3j): Yellow solid, 77 % yield; m.p. 263-265 ºC. ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 12.74 (sb, 1H, NH), 7.28 (s, 1H, H-5), 6.93 (d, 1H, J = 8.9 Hz, H-3"), 6.86 (d, 1H, J = 8.9 Hz, H-4"), 6.48 (s, 1H, H-4'), 6.76 (s,1H, H-6"), 3.56, 3.55 (s, 6H, 2-O<u>C</u>H$_3$); ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) = 162.1 (C=O), 128.1 (H-4'), 116.4 (C-6"), 115.1 (C-4"), 113.2 (C-3"),

109.7 (C-5), 56.0, 55.6 (2-O<u>C</u>H$_3$), 156.6, 153.0, 149.8, 144.6, 134.0, 132.0, 127.8, 125.7, 125.4, 116.1, (C$_q$); ESIMS: *m/z* = 408 [M + H].

RESULTS AND DISCUSSION

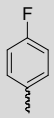
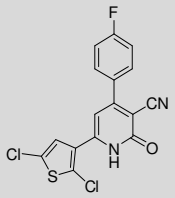
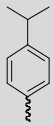
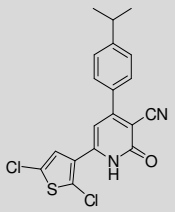
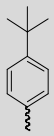
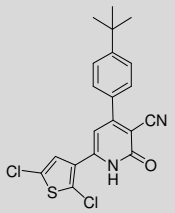
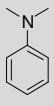
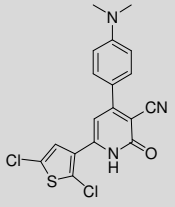
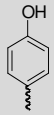
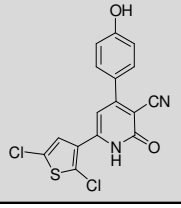
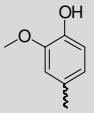
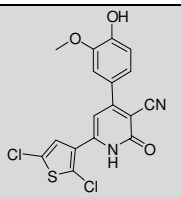
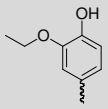
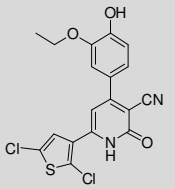
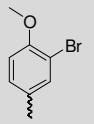
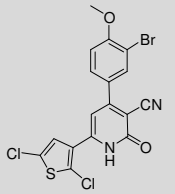
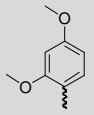
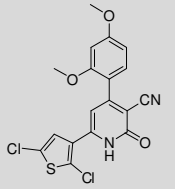
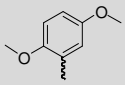
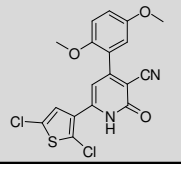
3-Acetyl-2,5-dichlorothiophene was prepared *via* the reaction of 2,5-dichlorothiophene with acetyl chloride using carbon disulphide as a solvent in the presence of anhydrous aluminium chloride[19]. The reactions of 3-acetyl-2,5-dichlorothiophene (**1**) with different aryl aldehydes (**2a-j**) gave 3-cyanopyridin-2-ones derivatives (**3a-j)** in good yields (Table-1; **Scheme-I**).



1 **2a-j** **3a-j**

Scheme-I: Synthesis of substituted 2-oxopyridine-3-carbonitrile derivatives

TABLE-1
SUBSTRATES AND THEIR YIELDS

Entry[a]	Ar	Product	Yield (%)[b]
3a			88
3b			79
3c			86
3d			82
3e			75
3f			78
3g			92
3h			82
3i			84
3j			77

All new 2-oxopyridine-3-carbonitrile (**3a-j**) were characterized by different spectroscopic techniques including IR, ^{1}H NMR, ^{13}C NMR and mass spectrometry. They are in accordance with the assigned structures.

The MS spectra display the correct molecular ion peaks as suggested by their molecular formulae as M + 1. The IR spectra of compounds (**3a-j**) showed a strong peaks in the region 1673-1695 cm^{-1} which attributed to the carbonyl group, as well as a CN bands in the region 2190-1254 cm^{-1}. Moreover, the IR spectra showed peaks at 3320-3168 cm^{-1} which belong to N-H bond.

In the ^{1}H NMR spectra of the compounds **3a-j**, the proton of the NH appeared as a singlet broad peak in the region δ 12.44-12.88 ppm. While, H-4' of thienyl moiety appeared as a singlet at 6.48-6.79 ppm. Complete assignments for all protons are given in the experimental part.

The ^{13}C NMR spectra of oxopyridine compounds **3a-j** showed absorption in the range 161.6-162.9 ppm attributed to the C=O carbon. While, the absorption CN carbon appeared in the range 115.8-117.2 ppm. Assignments for other carbons are given in details in the experimental part.

Antibacterial evaluation: The tested compounds did not show any antibacterial activity against the tested strains *Staphylococcus epidermis* ATCC 12228, *Bacillus pumilus*, *Escherichia coli* ATCC and *Pseudomonas aeruginosa*.

Conclusion

A new series of 2-oxopyridine-3-carbonitrile derivatives have been synthesized. All compounds were characterized by

standard spectroscopic techniques. All compounds show no antibacterial activity against four different types of bactaria.

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REFERENCES

1. A.H. Abadi, T.M. Ibrahim, K.M. Abouzid, J. Lehmann, H.N. Tinsley, B.D. Gary and G.A. Piazza, *Bioorg. Med. Chem.*, **17**, 5974 (2009).
2. P. Fossa, G. Menozzi, P. Dorigo, M. Floreani and L. Mosti, *Bioorg. Med. Chem*., **11**, 4749 (2003).
3. M. Kikura and J.H. Levy, *Int. Anesthesiol. Clin*., **33**, 21 (1995).
4. T. Kishi, K. Nakahashi, H. Ito, S. Taniguchi and M. Takaki, *Clin. Exp. Pharmacol. Physiol*., **28**, 737 (2001).
5. S.R. Johns and L. Marion, *Can. J. Chem.*, **44**, 23 (1966).
6. A. Piroyan and G. Melikyan, *ARKIVOC*, 234 (2006).
7. Y. Fujita, H. Oguri and H. Oikawa, *Tetrahedron Lett*., **46**, 5885 (2005).
8. H.A. El-Sayed, A.H. Moustafa, A.Z. Haikal, R. Abu-El-Halawa and E.H. El Ashry, *Eur. J. Med. Chem*., **46**, 2948 (2011).
9. M.T. Cocco, C. Congiu and V. Onnis, *Eur. J. Med. Chem*., **35**, 545 (2000).
10. L.A. Hasvold, W. Wang, S.L. Gwaltney II, T.W. Rockway, L.T.J. Nelson, R.A. Mantei, S.A. Fakhoury, G.M. Sullivan, Q. Li, N.H. Lin, L. Wang, H. Zhang, J. Cohen, W.Z. Gu, K. Marsh, J. Bauch, S. Rosenberg and H.L. Sham, *Bioorg. Med. Chem. Lett*., **13**, 4001 (2003).
11. N.S. El-Sayed, A.N. Shirazi, M.G. El-Meligy, A.K. El-Ziaty, D. Rowley, J. Sun, Z.A. Nagib and K. Parang, *Tetrahedron Lett*., **55**, 1154 (2014).
12. R.L.T. Parreira, J.O. Abrahão Jr. and S.E. Galembeck, *Tetrahedron*, **57**, 3243 (2001).
13. G. Semple, B.M. Andersson, V. Chhajlani, J. Georgsson, M.J. Johansson, Å. Rosenquist and L. Swanson, *Bioorg. Med. Chem. Lett*., **13**, 1141 (2003).
14. V. Lucchesi, T. Parkkari, J.R. Savinainen, A.M. Malfitano, M. Allarà, S. Bertini, F. Castelli, S. Del Carlo, C. Laezza, A. Ligresti, G. Saccomanni, M. Bifulco, V. Di Marzo, M. Macchia and C. Manera, *Eur. J. Med. Chem*., **74**, 524 (2014).
15. A. M. Abdel-Fattah, F. A. Attaby, M. A. A. Elneairy and M. N. M. Gouda, *Curr. Bioact. Compd*., **12**, 176 (2012).
16. E.R. Kotb, M.M. Anwar, H.A.S. Abbas and S.I. Abd El-Moez, *Acta Pol. Pharm*., **70**, 667 (2013).
17. D.E. McClure, J.J. Baldwin, W.C. Randall, T.F. Lyon, K. Mensler, G.F. Lundell, A.W. Raab, D. Gross and E.A. Risley, *J. Med. Chem*., **26**, 649 (1983).
18. G.T. Wang, X. Wang, W. Wang, L.A. Hasvold, G. Sullivan, C.W. Hutchins, S. O'Conner, R. Gentiles, T. Sowin, J. Cohen, W.Z. Gu, H. Zhang, S.H. Rosenberg and H.L. Sham, *Bioorg. Med. Chem. Lett*., **15**, 153 (2005).
19. G.B. Bachman and L.V. Heise, *J. Am. Chem. Soc*., **70**, 2378 (1948).
20. J.S. Parekh and S. Chanda, *Afr. J. Biomed. Res*., **10**, 175 (2007).