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SYNTHESIS AND CHARACTERISATION OF NOVEL AZO N-OXIDE COMPOUNDS

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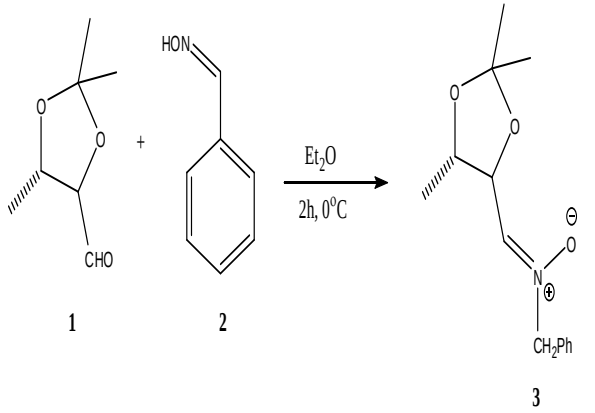
ABSTRACT
In this study novel aldehyde were synthesised through azo coupling between amino pyrimidine and aldehyde. The resulting aldehyde coupled with hydroxyl amine forming N-oxides which were characterised by the use of infra red, proton NMR and carbon 13.

KEYWORD: azo, N-oxide, pyrimidine, nitrones.

INTRODUCTION

One of the most common routes to the synthesis of nitrone (**3**) involves the condensation of aldehydes or ketones (**1**) with *N*-monosubstituted hydroxylamines (**2**), followed by treatment with a mild base to afford the[1] desired nitrone.[2,3,4] The aldehydes react immediately with *N*-monosubstituted hydroxylamines at room temperature, while the less reactive ketones consistently require longer reaction times or heating.[5,6] This can sometimes hinder their value within synthetic strategies. A useful example of aldonitrone formation is illustrated in scheme 1.[1,7,8]



1 2 3

Scheme 1

The condensation of aldehyde 1 with *N*-benzylhydroxylamine affords *N*-benzyl nitrone 3, only as the *Z*-isomer, in 93% yield. Due to the high reactivity of the aldehyde, this reaction usually proceeds at room temperature.[9,10,11]

This method is used extensively in the synthesis of nitrones and has been used to synthesis a wide variety of nitrone derivatives, generally in good yields.[2]

Some exceptions are reported however, particularly with aliphatic ketones having bulky alkyl groups, in which case no nitrones are formed.[2,12,13,14]

Furthermore, in other cases, dimeric products were produced, as is the case of the reaction between phenylhydroxylamine 4 and acetone the product has the structure shown in figure (1).[15,16,17]

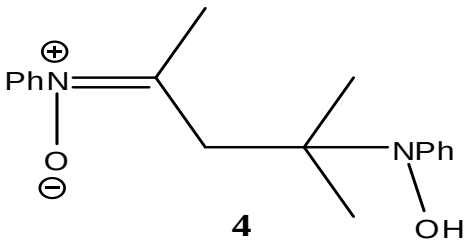


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Figure 1

In this study, novel aldehydes were synthesis through azo coupling reactions followed reaction with the corresponding hydroxyl amine. The following step was condensation reaction between aldehyde 5 and hydroxyl amine which were formed in situ

Experimental

All the chemicals were reagent grade unless stated otherwise and resourced from Sigma and Aldrich. Silica gel (Merck 7736), and silica gel plates for column and thin layer chromatography were Aldrich products, the separated components were detected using iodine vapour. Anhydrous sodium sulfate was used to dry organic solutions. Infrared (IR) spectra were carried out

on a Infrared Reflection Absorption Spectroscopy (IRRAS) (4000-400 cm^{-1}) and recorded using Perkin-Elmer tensor 27 as thin film. Melting points were measured using a SMP31melting point apparatus. ^{1}H NMR spectra were carried out on a VARIAN spectrophotometer (300 MHz). The ^{13}C-NMR spectra were recorded, using VARIAN spectrophotometer (75 MHz).

General procedure of azo dye preparation.

Preparation of 4-hydroxy-3-(pyrimidin-2-yldiazenyl) benzaldehyde 6a

Following the same procedure above 2-amino pyrimidine (0.95 g, 0.01 mole) and 4-hydroxy benzaldehyde (1.22 g, 0.01 mole) to give the title compound 2.1 g, 92% as white solid with m.p 84-85^{o}C.

^{1}H-NMR (d_6-DMSO) δ: 9.78 (1H, s), 7.76-7.73 (3H, m), 6.94-6.90 (3H, m).
^{13}C NMR: 190.37, 164.25, 163.64, 162.88, 133.06, 131.56, 127.98, 127.87, 115.83, 115.33, 114.74.

IR: 3159.68, 3043.51, 2967.18, 1905.46, 1663.42, 1587.27, 1516.94, 1449.22, 1382.42, 1314.16, 1281.95, 1237.89, 1214.32, 1154.97, 1112.27, 830.99, 787.20, 697.53, 640.03, 600.86.

preparation of 2-nitro-5-(pyrimidin-2-yldiazenyl) benzaldehyde 6b

A solution of $NaNO_2$ (0.69 g, 0.01 mole) in water (3 ml) was added to 2-nitro benzyldehyde (1.51 g, 0.01 mole) in 10% NaOH aq (5 ml) and cooled to 0^{o}C. The resulting solution was added slowly with stirring to a solution of 2-amino pyrimidine (0.95 g, 0.01 mole) in HCl aq (3 ml cone HCl: 3 ml water) keeping the temp. at 0^{o}C. Following the addition (10 min) the pH of the mixture was adjusted to 7 and the precipitate which formed was filtered off, purified by recrystallized from EtOH to give 2.1 g (84%) of yellow solid crystals with m.p. 36-38^{o}C.

^{1}H-NMR (d_6-DMSO) δ: 10.24 (1H, s), 8.17-7.88 (6H, m)
^{13}C-NMR: 189.35, 149.39, 149.03, 138.15, 134.21, 134.13, 133.70, 133.62, 130.00, 129.29, 123.76.

IR: 3103.54, 3057.75, 2904.62, 2865.05, 1692.56, 1607.51, 1571.38, 1447.67, 1396.99, 1347.66, 1270.34, 1187.75, 1142.94, 1077, 997.57, 964.06, 891.95, 675.12.

Preparation of 4-(dimethylamino)-3-(pyrimidin-2-yldiazenyl) benzaldehyde 6c

Following the same procedure above 2-amino pyrimidine (0.95 g, 0.01 mole) and (1.49 g, 0.01 mole) dimethyl amino benzaldehyde to give 2.2 g (88%) of the title compound as a cream crystalline solid with m.p 56-57^{o}C.

^{1}H-NMR (d_6-DMSO) δ: 9.67 (1H, s), 7.70 (1H, s), 7.69-7.66 (2H, m), 6.79-6.76 (3H, m), 3.03 (6H, s).

^{13}C-NMR ($CDCl_3$) δ: 189.20, 160.20, 154.43, 153.58, 153.08, 130.90, 123.92, 122.77, 116.76, 115.36, 110.43, 39.45, 39.33.

IR: 2902.49, 2795.52, 2713.57, 1681.67, 1655.26, 1588.18, 1546.67, 1529.67, 1483.89, 1415.23, 1366.80, 1334.50, 1162.11, 1122.95, 1064.59, 999.18, 593.65.

Preparation of 4-chloro-3-(pyrimidin-2-yldiazenyl) benzaldehyde 6d

Following the same procedure above 2-amino pyrimidine (0.95 g, 0.01 mole) and (1.40 g, 0.01 mole) 4-chloro benzaldehyde to give the title compound 2.1 g, (87%) as cream solid with m.p 44-46 ^{o}C

^{1}1H-NMR (D6-DMSO) δ: 10.01 (1H, s,), 7.95-7.91 (3H, m), 7.70-7.67 (3H, m).

^{13}C-NMR ($CDCl_3$) δ: 191.55, 152.21, 140.02, 139.00, 138.82, 138.04, 134.30, 133.37, 130.61, 129.10, 128.81.

IR: 3089.50, 2859.15, 1919.16, 1690.80, 1676.30, 1587.33, 1573.80, 1483.73, 1451.02, 1420.13, 1385.08, 1285.16, 1206.17, 1153.41, 1091.15, 1010.77, 971.95, 812.56, 701.74, 682.81, 582.81, 517.08.

PREPARATION OF NITRONES

preparation of N-(4-hydroxy-3-(pyrimidin-2-yldiazenyl) benzylidene)aniline oxide 7a

In 50 ml round bottom flask (0.5 g, 0.004 mole) of n-phenyl hydroxylamine was dissolve in 15 ml absolute ethanol and worm to 40^{o}C. 4- hydroxy-3-(pyrimidin-2-yldiazenyl)benzaldehyde (0.91 g, 0.004 mole) was added the reaction mixture was stirred overnight. The reaction was monitored by TLC. The volume of reaction mixture were reduced under reduce pressure and recrystallize from ethanol to give the title compound 1.01 g, 84% (m.p. 88-90 ^{o}C).

^{1}1H-NMR (D6-DMSO) δ: 8.40 (1H, d, J=9Hz), 8.34 (1H, s), 8.27 (1H, d, J=9 Hz), 8.09 (1H, d, J=9 Hz), 7.89 (1H, d, J=9Hz), 7.77 (2H, d, J=9Hz), 7.55-7.45 (3H, m), 6.94-6.86 (3H, m).

^{13}C-NMR: 162.84, 159.12, 147.91, 132.64, 131.56, 130.64, 128.79, 128.77, 128.43, 128.41, 127.87, 124.44, 121.53, 120.74, 115.32, 114.73.

IR: 3164.55, 1665.61, 1588.14, 1515.17, 1473.53, 1437.92, 1383.23, 1313.68, 1283.21, 1214.53, 1155.81, 1112.48, 1069.60, 1024.27, 927.51, 857.67, 831.18, 762.42, 682.58, 640.88, 601.60.

Preparation of N-(4-(dimethylamino)-3-(pyrimidin-2-yldiazenyl) benzylidene) aniline oxide 7b

Following the same procedure above (0.5 g, 0.004 mole) n-phenyl hydroxylamine and (1.02 g, 0.004 mole) 4-(dimethylamino)- 3- (pyrimidin-2- yldiazenyl) benzaldehyde mixed together to give the title compound 1.1 g, 84% as cream solid with m.p. 54-56^{o}C.

^{1}1H-NMR (D6-DMSO) δ: 8.39 (1H, d, J=9 Hz), 8.28 (1H, s, J=9 Hz), 8.10 (1H, d, J= 9Hz), 7.90 (1H, t, J=9Hz), 7.70 (3H, d, J= 9Hz), 7.56-7.44 (3H, m), 6.79 (2H, d, J=9 Hz), 3.03 (6H, s).

^{13}C-NMR: 153.65, 150.87, 147.83, 132.52, 131.66, 130.98, 130.24, 129.23, 128.77, 128.39, 128.32, 123.99, 121.50, 120.44, 118.39, 110.51, 110.48, 43.39, 43.07.

IR: 2903.60, 2814.77, 2795.59, 2713.55, 1659.77, 1590.32 , 1549.40, 1527.41, 1482.27, 1433.49, 1367.88, 1311.58, 1230.49, 1161.23, 1123.41, 1057.60, 999.42, 940.28, 885.57, 811.00, 766.82, 726.45, 687.30, 658.60, 631.15, 593.86.

Preparation of N-(4-chloro-3-(pyrimidin-2-yldiazenyl) benzylidene)aniline oxide 7c

Following the same procedure above mixed n-phenyl hydroxyl amine (0.5 g, 0.004 mole) and chloro-3-(pyrimidin-2-yldiazenyl)benzaldehyde 4- (0.99 g, 0.004 mole) together to gave the title compound 1.02 g, (78%) as cream solid with m.p. 136-138 ^{o}C.

^{1}1H-NMR (D6-DMSO) δ: 8.56 (2H, d, J=9Hz), 8.50 (1H, s), 7.93 (2H, d, J=9 Hz), 7.90 (1H, s), 7.58 (1H, m), 7.56-7.53 (5H, m).

^{13}C-NMR: 147.84, 133.96, 132.23, 131.86, 130.03, 129.66, 129.41, 129.22, 128.77, 128.38, 128.22, 127.83, 127.57, 126.77, 121.30, 120.97, 120.91.

IR: 3058.34, 3042.07, 1588.76, 1545.86, 1484.16, 1456.43, 1401.80, 1301.69, 1285.67, 1191.81, 1171.72, 1070.42, 1012.48, 916.73, 893.52, 840.71, 812.36, 764.11, 685.62, 634.67.

Preparations of N-(2-nitro-5-(pyrimidin-2-yldiazenyl) benzylidene)aniline oxide 7d

Following the same procedure above n-phenyl hydroxylamine (0.5 g, 0,004 mole) and (1.02 g, 0.004 mole) 2-nitro-5-(pyrimidin-2-yldiazenyl) benzaldehyde were mixed together to give the title compound 1.1 g, (84%) as yellowish solid with m.p. 74-76 ^{o}C.

^{1}1H-NMR (D6-DMSO) δ: 8.76 (1H, s), 8.50 (1H, d, J=9Hz), 8.10 (1H, d, J=9Hz), 7.90-7.84 (5H, m), 7.71 (1H, t, J=9 Hz), 7.58 (3H, t, J=9Hz).
^{13}C-NMR: 147.87, 147.43, 147.30, 132.74, 130.23, 130.02, 129.76, 128.84, 128.75, 124.49, 124.44, 123.97, 123.70, 121.52, 120.94, 119.80, 116.32.

IR: 3103.59, 3072.91, 2918.28, 2851.05, 1700.20, 1599.76, 1568.07, 1482.99, 1466.61, 1439.40, 1411.21, 1338.78, 1224.08, 1191.58, 1166.96, 1089.92, 1068.39, 1022.93, 1001.26, 968.37, 916.74, 886.07, 860.53, 805.05, 763.39, 735.82, 685.04, 586.53.

Preparation of N-(4-hydroxy-3-(pyrimidin-2-yldiazenyl) benzylidene)aniline oxide 7e

Following the same procedure above (0.5 g, 0.004 mole) N-p-tolylhydroxylamine and (0.91 g, 0.004 mole) 4-hydroxy-3-(pyrimidin-2-yldiazenyl)benzyldehyde were mixed together to give the title compound 1 g, (76%) as white solid with m.p 58-60 ^{o}C.

^{1}1H-NMR (D6-DMSO) Δ : 8.38 (1H , d, J=9Hz), 8.15 (1H, d, J=9Hz), 8.30 (1H, s), 8.06(1H, d, J=6Hz), 7.40 (1H, t, J=6Hz), 7.33(1H, t, J=6Hz), 7.74(1H, d, J=9Hz), 6.94(2H, d, J=9Hz), 6.88(2H , t, J=9Hz), 2.38(3H, s).

^{13}C-NMR: 162.82, 158.99, 145.58, 145.08, 141.71, 140.76, 139.38, 138.46, 131.56, 129.11, 128.86, 127.89, 124.65, 121.30, 120.46, 115.32, 114.71, 21.24.

IR: 3161.12, 1905.57, 1665.62, 1596.58, 1508.89, 1450.61, 1384.13, 1314.83, 1284.07, 1239.46, 1215.85, 1159.22, 1110.86, 1053.04, 1014.01, 857.71, 820.73, 787.88, 705.21, 641.05, 602.31.

Preparation N-(4-dimethylamino)-3-(pyrimidin-2-yldiazenyl)benzylidene) aniline oxide 7f

Following the same procedure as above (0.5 g, 0.004 mole) N-p-tolylhydroxylamine and (1.02 g, 0.004 mole) 4-dimethylamino)-3-(pyrimidin-2-yldiazenyl)benzyldehyde were mixed together to give 1.2 g (85%) of the title compound as white solid with m.p 42-43^{o}C.

^{1}1H-NMR (D6-DMSO) Δ: 8.38(1H, d, J=6Hz), 8.14(1H, d, J=6Hz), 8.06(1H, d, J=6Hz), 8.24(1H, s), 7.79-7.74(1H, m), 7.41(1H, d, J=9Hz), 7.35-7.27(1H, d, J=9Hz), 7.69(2H, d, J=9Hz), 6.79(2H, d , J=9Hz), 3.03(6H, s), 2.37(3H, s).

^{13}C-NMR: 158.40, 153.58, 144.93, 141.60, 140.68, 139.28, 137.86, 130.90, 129.01, 128.76, 128.61, 124.57, 123.94, 121.19, 120.11, 110.84, 110.44, 20.46, 20.18, 19.94.

IR: 2912.60, 2795.44, 2713.68, 1659.86, 1591.79, 1548.03, 1529.97, 1462.52, 1432.56, 1368.09, 1312.50, 1230.73, 1161.37, 1123.57, 1063.49, 1016.12, 999.71, 939.55, 811.26, 726.46, 707.25, 612.33, 516.42.

Preparation of N-(2-nitro-5-(pyrimidin-2-yldiazenyl) benzylidene)aniline oxide 7g

Following the procedure as above (0.5 g, 0.004 mole) N-p-tolylhydroxylamine and (1.02 g, 0.004 mole) 2-nitro-5-(pyrimidin-2-yldiazenyl)benzyldehyde were mixed together to give the title compound 1.1 g, (78%) as cream solid with m.p 86-88 ^{o}C.

^{1}1H-NMR (D6-DMSO) Δ: 8.72(1H, s), 8.51(1H, d, J=9Hz), 8.14(1H, d, J=9Hz), 8.06 (1H, t, J=9Hz), 7.85(1H, t, J=9Hz), 7.79(1H, d, J=9Hz), 7.64(1H, s, J=9Hz), 7.41(2H, d, J=9Hz), 7.35(2H, d, J=9Hz), 2.38(3H, s).

^{13}C-NMR: 147.39, 145.07, 140.91, 139.92, 139.37, 133.34, 132.68, 132.45, 129.88, 128.97, 124.65, 123.95, 123.79, 122.19, 121.27, 120.78, 120.63, 20.24.
IR: 3097.35, 3032.85, 2918.14, 2855.72, 1700.18, 1602.00, 1567.74, 1516.90, 1499.32, 1463.71, 1342.12, 1310.89, 1292.08, 1188.56, 1175.93, 1163.35, 1087.55, 1068.52, 1041.45, 1015.63, 963.03, 898.99, 863.32, 806.232, 732.51, 632.06.

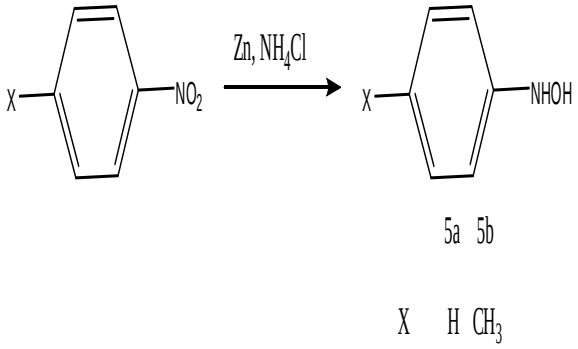
Biological study

The antibacterial activities of the prepared samples were determined by the agar well diffusion method, using laboratory strains of four bacterial species which included gram-positive bacteria, *Bacillus cereus* and gram-negative bacteria, *Enterobacter*. Erythromycin was used as the standard antibacterial agent.

The prepared compounds in this study, 2a-d and 3a-h were tested for their activity against the above bacteria using the disc diffusion method.[18,19,20] Mueller Hinton Agar was sterilized in a round bottom flask, cooled to 40–50 °C and homogenously spread onto pre-sterilized Petri dishes. The bacteria were separately introduced on the agar plates. The test compounds were introduced to the Petri dishes by soaking discs in a 0.25 mg concentration of the test compounds (2a-d) and (3a-h) and then applying then to the surface of the agar plates. A sterile disc was use as blank and this disc was soaked in the solvent (DMSO) and applied to the agar as a negative control on each Petri dish along with the standard drug (Erythromycin). The Petri dishes were incubated at 37°C (overnight) for bacterial strains.

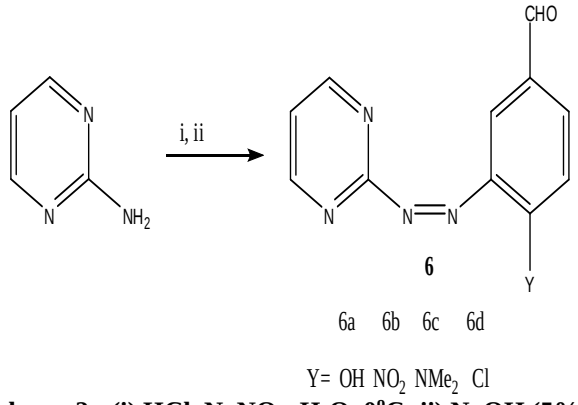
RESULTS AND DISCUSSION

As in the other synthesis of N-oxides, the molecules were constructed from two fragments with one fragment containing a carbonyl group, and the second group containing the hydroxyl amine part. These two moieties were linked together using a condensation reaction. The first part was the preparation of hydroxyl amine (**5a** and **5b**) through a reduction reaction of two nitro compounds.[21,17,22] The reduction was done using zinc as this is a mild reducing agent in order to prevent the reaction from going a further step and forming an amine group.[23] The formation of hydroxyl amine was confirmed by IR which showed broad absorption at 3300 cm^{-1} belonging to the hydroxyl group, scheme 1.



Scheme 1: reduction of nitro group

The second part was the preparation of the aldehydes (**6a-d**) via a diazonium coupling reaction between amino pyrimidine and one of four benzaldehyde derivatives (**6a-d**). The diazonium reaction gave a yield of only 30-40 % since this reaction is sensitive to two conditions: the reaction temperature which should be maintained between 0-5 degrees and the concentration of the acid, scheme 2.



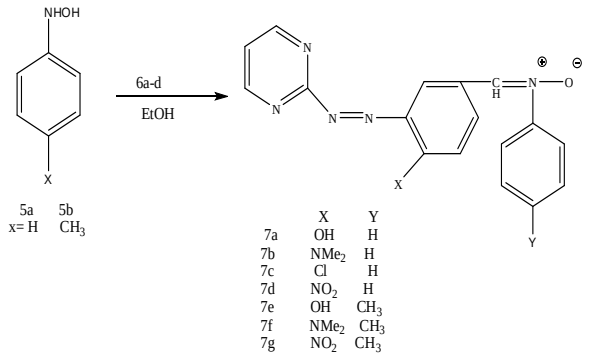
Scheme 2: (i) HCl, $NaNO_2$, H_2O, 0^{o}C; ii) NaOH (5%), H_2O, benzaldehyde dervitives

The formation the aldehydes (**6a-d**) were confirmed using infrared spectra which gave an absorbance peak between 1690-1663 cm^{-1} for the carbonyl group, while the azo group showed absorbance between 1449-1483 cm^{-1}. The proton and carbon NMR provided further conformation of the success of the reaction (see table 1).

Table 1: NMR data for the novel aldehyde 6a-d

	Proton NMR		Carbon NMR	
	CHO	Para proton	CHO	para pyrimidine
6a	10.59	6.91	190.87	136.38 , 136.06
6b	10.24	8.17-7.88	198.86	149.39, 149.04
6c	9.57	7.70	189.20	157.91, 156.73
6d	10.01	7.70-7.67	190.55	152.21 , 149.92

The last step in this study was the coupling of aldehydes (**6 a-d**) and the hydroxyl amines (**5 a-b**) using a condensation reaction, scheme 3.



Scheme 4: Nitrones prepared in this study

The formation of the synthesized compounds was confirmed by infra red spectrometry which showed a lack of absorbance in the carbonyl region around 1700 cm^{-1} and new absorbance at 1045-1065 cm^{-1} belonging to the N-O group. The proton NMR of the compounds **7a-g** resonates at 7-8 ppm while the nitrone protons resonate at 7.8-8.5ppm.

The ^{13}C NMR provided further conformation of forming the nitrons (7a-g).

Compound	Enterobacter Inhibition zone(mm)	Bacillus Inhibition zone(mm)
6a	29	37
6b	33	36
6c	11	24
6d	0	43
7a	18	16
7b	24	14
7c	11	11
7d	16	18
7e	15	9
7f	18	11
7g	14	16
Erythromycin	28	0

The compound 6b showed the highest effective on both gram positive and negative bacteria while the compound 6d showed effective on gram negative unlike Erythromycin which showed effective on gram positive.

REFERENCES

1. M. Gulla, L. Bierer, S. Schmidt, L. Redcliffe, and V. Jäger, *Zeitschrift fur Naturforsch. - Sect. B J. Chem. Sci.*, 2006; 61: 471–485.
2. G. R. Delpierre and M. Lamchen, *Q. Rev. Chem. Soc.*, 1965; 19: 329.
3. F. Nikbakht, A. Heydari, D. Saberi, and K. Azizi, *Tetrahedron Lett.*, 2013; 54: 6520–6523.
4. V. K. Aggarwal, R. S. Grainger, H. Adams, P. L. Spargo and K. Ct, 1998; 3263: 3481–3485.
5. C. Zhou and R. C. Larock, *J. Am. Chem. Soc.*, 2004; 126: 2302–3.
6. X. Zhou and X. Xiang, *Procedia Environ. Sci.*, 2013; 18: 540–546.
7. A. Dondoni, D. Perrone, and M. Rinaldi, *J. Org. Chem.*, 1998; 63: 9252–9264.
8. Y. Inouye, K. Takaya, and H. Kakisawa, *Magn. Reson. Chem.*, 1985; 23: 101–103.
9. *Organic Functional Group Preparations*, Elsevier, 1989.
10. H. M. I. Osborn, N. Gemmell, and L. M. Harwood, *J. Chem. Soc. Perkin Trans.* 2002; *1:* 2419–2438.
11. D. D. Dhavale, S. M. Jachak, N. P. Karche, and C. Trombini, *Tetrahedron*, 2004; 60: 3009–3016.
12. T. Kano, T. Hashimoto, and K. Maruoka, *J. Am. Chem. Soc.*, 2005; 127: 11926–7.
13. A. Brandi, S. Cicchi, F. M. Cordero, and A. Goti, *Chem. Rev.*, 2003; 103: 1213–1269.
14. A. Corma, A. Leyva-Pérez, and M. J. Sabater, *Chem. Rev.*, 2011; 111: 1657–712.
15. K. Maruyama, *Bull. Chem. Soc. Jpn.*, 1964; 37: 1013–1017.
16. W. C. AGOSTA, *J. Org. Chem.*, 1961; 26: 1724–1728.
17. Y. Ogata, Y. Sawaki, J. Mibae, and T. Morimoto, *J. Am. Chem. Soc.*, 1964; 86: 3854–3858.
18. M. Balouiri, M. Sadiki, and S. K. Ibnsouda, *J. Pharm. Anal.*, 2015; 6: 71–79.
19. M.. Okeke, C. . Iroegbu, E. . Eze, A.. Okoli, and C. . Esimone, *J. Ethnopharmacol.*, 2001; 78: 119–127.
20. M. Gulluce, A. Aslan, M. Sokmen, F. Sahin, A. Adiguzel, G. Agar and A. Sokmen, *Phytomedicine*, 2006; 13: 515–21.
21. and F. A. S. George H. Coleman, Chester M. McCloskey, *Org. Synth.*, 1945; 25: 80.
22. C. Matassini, C. Parmeggiani, F. Cardona and A. Goti, *Org. Lett.*, 2015; 17: 4082–5.
23. R. J. Rahaim and R. E. Maleczka, *Org. Lett.*, 2005; 7: 5087–90.