Asian Journal of Chemistry; Vol. 28, No. 5 (2016), 1027-1030

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2016.19576

Synthesis and Microbiological Activities of Novel Acyclic Nitrones

CHERIFA MAHIEDDINE*, MOHAMED SALAH BOUKHECHEM, SAID ZERKOUT and ABDELGHANI ZITOUNI

Laboratoire de Recherche sur les Produits Bioactifs et la Valorisation de la Biomasse, Ecole Normale Supérieure-Vieux Kouba, B.P.: 92, 16038 Kouba, Alger, Algeria

*Corresponding author: E-mail: mahieddinec@gmail.com

Received: 24 August 2015; *Accepted*: 12 October 2015; *Published online*: 30 January 2016; AJC-17740

In this article, the synthesis, antibacterial and antifungal activities of α-(5-(R)-2-thiophyl)-N-(4-R') phenyl nitrones are reported. The synthesis was achieved by the condensation of N-substituted hydroxylamine [4-R'-phenylhydroxylamine] with 5-R-2-thiophene-carboxaldehyde in boiling absolute ethanol. The chemical structure of this nitrones was identified by spectroscopic techniques (IR and NMR). The antibacterial and antifungal activities were evaluated against bacteria: *B. substilis*, *S. aureus*, *E. coli*, *S. enterica* and fungus: *M. ramannianus* and *F. oxysporium f.sp. albedinis*. The reactivity of this reaction was making clear according the director condition.

Keywords: Antibacterial, Antifungal, Nitrones, Thiophene.

INTRODUCTION

The syntheses of acyclic and cyclic nitrones are considered as intermediary molecules for the preparation of therapeutic products [1-4] because of their easiness preparation which are more stable and reactive than other compounds containing C=N group [5]. They have an effect of carbonyl group and exhibit geometric isomerism [6]. They also contribute well in 1,3-dipolar cycloaddition reactions, nucleophilic additions and synthetic utility [7]. Previous work also showed that the nitrones was used as spin traps, antioxidants in biological systems and antibacterial [8].

The method for synthesis of nitrone was selected according to chemical structure of nitrone which is synthesized, chemical and physical properties of initial substances, the catalyst and the solvent or reactions conditions that will be affected in the course of interaction in addition by Torssell *et al.* [4] and Coutts *et al.* [9]. The general synthetic method of the acyclic nitrones was from the condensation reaction between the N-substituted hydroxylamine and aldehyde that is used as a direct synthetic method of many acyclic nitrones [4,6,10].

EXPERIMENTAL

Ethanol (Merck), butanol (Merck), diethyl ether (Reidel de Haën), ethyl acetate (Reidel de Haën), dichloromethane (Reidel de Haën), petroleum ether (Fluka), nitrobenzene (Merck), 2-thiophene carboxaldehyde 98 % (Fluka), 5-methyl-2-thiophene carboxaldehyde 98 % (Fluka), 5-nitro-2-thiophene carboxaldehyde 98 % (Fluka), 4-nitophenol (Fluka), 4-chloro nitro benzene m.p. 78 °C (lit. 80-83 °C) and 4-bromo nitrobenzene m.p. 122 °C (lit. 124-126 °C) were prepared according the method of nitration of aromatic cycle [11] using chlorobenzene (Merck) and bromobenzene (Merck) respectively. Ammonium chloride (BDH), zinc dust (Prolabo) were purchased from market.

IR spectra were measured with a FTIR-8300 Shimadzu. ^{1}H and ^{13}C NMR spectra were obtained in $CDCl_3$ solution at 300 MHz with a Bruker Nanobay. Melting points were measured on traditional methods and are uncorrected.

Preparation of nitrone

Preparation of β-phenylhydroxylamine: To a magnetically stirred solution of nitrobenzene (12.5 g, 0.101 mol) in aqueous solution of ammonium chloride (6.25 g, 0.126 mol) was added zinc dust partly (14.25 g, 21.79 mmol). The reaction mixture was stirred at 60-65 °C for 15 min after the stir was completed for another 15 min without heating. At this time, the reaction mixture was filtered and then was extracted with diethyl ether. Also, diethyl ether was evaporated on a rotary evaporator that gave a yellow crystalline solid. Recrystallization of this solid from petroleum ether (40-60°) gave (7.56 g) colourless needles crystalline of β-phenyl-hydroxylamine [12].

Preparation of 4-R′-phenylhydroxylamine: To a magnetically stirred solution of 4-R′-nitrobenzene (12.5 g, 10.1

mmol) in alcoholic solution (ethanol) of ammonium chloride (1 g, 18.6 mmol) was added zinc dust partly (4.5 g, 68.8 mmol). The reaction mixture was stirred at a cold (12-16 °C) for 2h. At this time the reaction mixture filtered. The filtrate was used directly for the second stage of the synthesis of α-(2-thiophyl)-N-(4-R′-phenyl)nitrone.

α-(2-Thiophenyl)-N-phenyl nitrone (4a): A mixture of β-phenylhydroxylamine (3 g, 27.4 mmol) in (40 mL) of absolute ethanol and 2-thiophene carboxaldehyde (3.08 g, 27.4 mmol) in (50 mL) of ethanol was refluxed for 3 h leaving the mixture under room temperature for the whole night. Evaporation of solvent on the rotary evaporator gave brown oil, which was triturated with petroleum ether (60-80°) to give crystals. Recrystallization from buthanol gave pistachio green crystals. Yield: 62.47 %; m.p.: 75 °C; UV (EtOH) λ_{max} (log ε): 343.0 (4.39), 260.5 (3.78), 222.5 (4.05), 203.0 (4.28) nm; IR (KBr, ν_{max}, cm^{-1}): 3057.3 (N=C-H), 1557.3 (C=N), 1067.7 (N→O), 770.83, 757.3. ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 8.49 (s, 1H, N=C-H); 7.84 (2H, 3, 4, CH, Ar); 7.62 (m, 1H, CH, Ar); 7.57(m, 1H, CH, Ar); 7.48 (3H, o, p, CH, Ar); 7.22 (1H, 5, CH, Ar); ^{13}C NMR ($CDCl_3$, 300 MHz): δ (ppm) 147.19, 133.75, 131.60, 130.74-130.55, 129.89-129.47, 127.66, 121.73.

α-[5-(Methyl)-2-thiophenyl]-N-phenylnitrone (4b): A mixture of β-phenylhydroxylamine (3 g, 27.4 mmol) in (40 mL) of absolute ethanol and 5-methyl-2-thiophene carboxaldehyde (3.46 g, 27.4 mmol) in (50 mL) of ethanol was refluxed for 3 h, leaving the mixture under room temperature for the whole night. Evaporation of solvent on the rotary evaporator gave yellow oil which was triturated and recrystallized with petroleum ether (60-80°) to give (5.73 g) of yellow needles. Yield: 95.98 %; m.p.: 139 °C; UV (EtOH) λ_{max} (log ε): 348.5 (4.14), 205.0 (4.31) nm; IR (KBr, ν_{max}, cm^{-1}): 3062.7 (N=C-H), 1554.5 (C=N), 1068.5 (N→O), 779.2, 752.2; ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 8.39 (s, 1H, N=C-H); 7.84 (d, 2H, 3, 4, CH, Ar); 7.49 (4H, o, m, CH, Ar); 7.04 (d, 1H, p, CH, Ar); 2.48 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 300 MHz): δ (ppm) 165.13, 146.57, 145.24, 138.64, 133.82, 130.91, 126.52.

α-(5-(Nitro)-2-thiophenyl)-N-phenylnitrone (4c): A mixture of β-phenylhydroxylamine (1.74 g, 15.9 mmol) in (40 mL) of absolute ethanol and 5-nitro-2-thiophene carboxaldehyde (2.5 g, 15.9 mmol) in (60 mL) of ethanol was refluxed for about 1 h, shiny orange plates separated, recrystallization from dichloromethane afforded (3.09 g) of **4c**. Yield: 78.17 %; m.p.: 228 °C; UV (EtOH) λ_{max} (log ε): 403.5 (4.25), 275.0 (4.21), 204.0 (4.34) nm; IR (KBr, ν_{max}, cm^{-1}): 3052.08 (N=C-H), 1551.6 (C=N), 1072.9 (N→O), 772.9, 762.5; ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 8.59 (s, 1H, N=C-H); 7.99 (d, 1H, 4, CH, Ar) ; 7.84 (dd, 2H, m, CH, Ar); 7.55 (t, 3H, o, p, CH, Ar); 7.46 (d, 1H, 3, CH, Ar); ^{13}C NMR ($CDCl_3$, 300 MHz): δ (ppm) 145.90, 138.56, 131.10, 129.5-127.90-127.84-127.55, 121.09.

α-(2-Thiophenyl)-N-(4-hydroxyphenyl)nitrone (4d): A mixture of the filtrate of 4-hydroxyphenyl hydroxylamine (3.34 g, 26.7 mmol) in (70 mL) of ethanol and 2-thiophene carboxaldehyde (3 g, 26.7 mmol) in (50 mL) of ethanol was refluxed for 3 h, leaving the mixture under room temperature for the whole night. Removal of the solvent gave a dark violet crystalline solid. This solid was recrystallized from ethyl acetate to give (2.97 g) of yellow needles. Yield: 50.69 %; m.p.: 207 °C; UV (EtOH) λ_{max} (log ε): 364.5 (3.75), 292.5 (3.56), 270.5 (3.52), 238.0 (3.40), 201.0 (3.88) nm; IR (KBr, ν_{max}, cm^{-1}): 3052.08 (N=C-H), 1593.7 (C=N), 1050.0 (N→O), 841.6, 805.2; ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 9.10 (s, 1H, OH); 8.52 (s, 1H, N=C-H); 7.40 (2H, 3, 4, CH, Ar); 7.05 (dd, 3H, o, 5, CH, Ar); 6.76 (dd, 2H, m, CH, Ar); ^{13}C NMR ($CDCl_3$, 300 MHz): δ (ppm) 151.28, 142.64, 131.62, 130.04, 127,69, 122.44, 111.58, 105.79, 103.92.

α-(2-Thiophenyl)-N-(4-chlorophenyl)nitrone (4e). A mixture of the filtrate of 4-chlorophenyl hydroxylamine (3.86 g, 26.7 mmol) in (70 mL) of ethanol and 2-thiophene carboxaldehyde (3 g, 26.7 mmol) in (50 mL) of ethanol was refluxed for 3 h, leaving the mixture under room temperature for the whole night. Removal of the solvent gave yellow solid. Recrystallization from ethyl acetate gave (3 g) of yellow needles. Yield: 58.00 %; m.p.: 170 °C; UV (EtOH) λ_{max} (log ε): 358.5 (4.06), 261.5 (3.50), 203.0 (4.08) nm; IR (KBr, ν_{max}, cm^{-1}): 3058.7 (N=C-H), 1570.4 (C=N), 1091.3 (N→O), 723.4, 710.4; ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 8.63 (s, 1H, N=C-H); 8.59 (1H, 5, CH, Ar); 8.03-7.93 (d, 2H, 3, 4, CH, Ar); 7.73 (2H, o, CH, Ar); 7.31 (2H, m, CH, Ar); ^{13}C NMR ($CDCl_3$, 300 MHz): δ (ppm) 149.72, 145.69, 136.01, 132.62-132.31, 131.96-129.37, 127.61, 124.94, 118.23, 115.64.

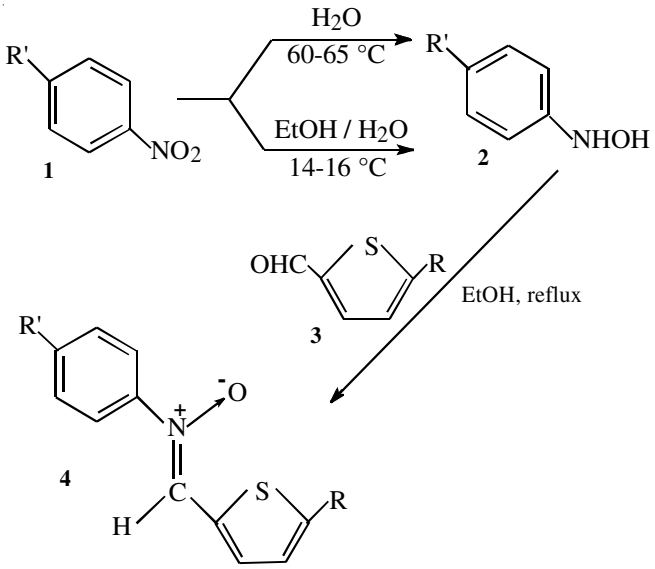
α-(2-Thiophenyl)-N-(4-bromophenyl)nitrone (4f): A mixture of the filtrate of 4-bromo-phenyl hydroxylamine (5.04 g, 26.7 mmol) in (70 mL) of ethanol and 2-thiophenecarboxaldehyde (3 g, 26.7 mmol) in (50 mL) of ethanol was refluxed for 3 h, leaving the mixture under room temperature for the whole night. A green yellow plates separated, which was recrystallized from ethyl acetate to give (6.14 g) of yellow plates. Yield: 81.18 %; m.p.: 193 °C; UV (EtOH) λ_{max} (log ε): 347.0 (4.32), 265.0 (3.78), 245.0 (3.85), 222.5 (3.98), 203.0 (4.25) nm; IR (KBr, ν_{max}, cm^{-1}): 3071.7 (N=C-H), 1535.2 (C=N), 1078.2 (N→O), 758.7, 745.6; ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 8.51 (s, 1H, N=C-H); 7.83 (dd, 2H, 3, 4, CH, Ar); 7.64 (dd, 2H, o, CH, Ar); 7.49 (2H, m, CH, Ar); 7.27 (d, 1H, 5, CH, Ar); ^{13}C NMR ($CDCl_3$, 300 MHz): δ (ppm) 149.75, 145.71, 136.02, 132.60, 132.33, 131.97, 129.35, 127.60, 124,96, 118.23, 115.66.

RESULTS AND DISCUSSION

In this study, we have studied the efficacy of the presence of thiophene ring in the acyclic nitrone molecular, that we have prepared six types of acyclic nitrones **4** which contained a heterocyclic ring [11,13] of thiophene by the condensation of the β-phenyl-hydroxyl-amine derivatives **2** and the commercially available 5-R-2-thiophene carboxaldehyde **3.** Before that one, we have prepared β-phenylhydroxylamine derivatives by the reduction of 4-R′-nitrobenzene **1** with zinc dust in aqueous or alcoholic solution of ammonium chloride NH_4Cl (the weak acid) to count of substituent R′ [4,12] without making it under the nitrogen gas and to take in the consideration of the instability of β-phenyl hydroxylamine derivatives [3] that we have achieved the synthesis of β-phenylhydroxylamine and the condensation reaction successively. All that were clarified in **Scheme-I** [13], substituents and yield were illustrated in Table-1.

TABLE-1
NITRONES, SUBSTITUENT AND INFRARED DATA

Nitrone	Substituent		Yield (%)	m.p. (°C)	Reaction time (h)	Frequency (cm^{-1})	
	R	R'				N→O	C=N
4a	H	H	62.47	75	3	1067.7	1557.3
4b	Me	H	95.98	139	3	1068.5	1554.5
4c	NO_2	H	78.17	228	2-3	1072.9	1554.6
4d	H	OH	50.69	207	3	1050.0	1593.7
4e	H	Cl	58.00	170	3	1091.3	1570.4
4f	H	Br	81.18	193	3	1078.2	1535.2



Scheme-I: Synthesis of nitrones [α-(5-(R)-2-thiophyl)-N-(4-R′)phenyl nitrone]

The reactivity of the condensation of the N-substituted hydroxylamine (4-R′-phenylhydroxylamine) and aldehyde (5-R-2-thiophenecarboxaldehyde) in boiling ethanol as a polar protic solvent to produce nitrones [α-(5-(R)-2-thiophyl)-N-(4-R′)phenyl nitrone] depended on the nucleophilicity of 4-R′-phenylhydroxylamine or the electrophilicity of 5-R-2-thiophene carboxaldehyde and also the effect of the substituents (electron-donating or withdrawing) on the aromatic rings. The electron donating substituent located in the 4-position of benzene nucleus imposes a positive mesomeric depending on the electro-negativity of –OH, –Cl and –Br which is diminishing as well as the reaction solvent is a polar protic solvent, the basicity of the molecular entity (4-R′-phenylhydroxylamine) is decreased respectively for these substituents this means that the nucleophilicity strength of this molecular entity was best in the state of bromo the less electro negativity resulting in the best and acceptable yield (Table-1). The other hand, the presence of the methyl group (electron-donating) located at the 5-position of the thiophene ring of the aldehyde reactive (5-R-2-thiophene carboxaldehyde) imposes a positive mesomeric effect on contrary to the nitro group which imposes a negative mesomeric effect, it was expected that the reactivity of the reaction was better in the case of nitro group than methyl group but the high electro negativity of the nitrogen atom in nitro group (electron-withdrawing) in compared the carbon atom of methyl made us conclude that the basicity of 5-nitro-2-thiophene carboxaldehyde in a polar protic solvent was better than 5-methyl-2-thiophene carboxaldehyde which indicates that the electophilicity strength of the molecular entity (5-R-2-thiophenecarboxaldehyde) is greater in the state of methyl group that justifies the high value of yield.

Infrared and ^{1}H NMR spectra: The infrared spectra of the synthesized nitrones characteristic an absorption bands of the (N→O) group and (C=N) group and their stretching are listed in Table-1. which according to results of precedent study [14,15] about α,N-diphenylnitrone that the (N→O) stretching frequencies were assigned to, respectively, 1088 and 1172 cm^{-1} and the (C=N) stretching frequencies were assigned to, respectively, 1587 and 1548 cm^{-1} [6]. Through these results the change of the benzene nucleus by thiophene nucleus in the α-position (C-position) of the molecule α-(5-(R)-2-thiophyl)-N-(4-R′) phenyl nitrone **4** (**Scheme-I**) caused a blue shift of frequencies values of (N→O) and (C=N) groups because of the increases of the μ values and also the change of the benzene nucleus by thiophene led to a weakening of resonance energy of the α,N-diphenylnitrone compared with the molecule **4** which decreased the value of the bond constant of Hook law.

On the other hand, the ^{1}H NMR spectra of nitrones [16] prepared in this study attribute single signals of the CH proton of azomethine N-oxide group (N=CH) that is observed in the region 8.39-8.63 ppm, while the multiple signals of the CH proton of aromatic rings (thiophenyl and phenyl) are shifted at 6.76-8.46 ppm. According to results of previous study about acyclic nitrones in particular α,N-diphenylnitrone [13,15-17]. The chemical shift of the carbon atom of the nitrone group in the ^{13}C NMR spectra [18] of α-(5-(R)-2-thiophenyl)-N-(4-R′) phenyl nitrone **4** is located in the range of 117 to 152 ppm and depends on the electrophilic character of substituent in position 5 and 4' of the aromatic ring of thiophene and benzene (Table-2).

TABLE-2
PROTON AND CARBON CHEMICAL SHIFT OF NITRONES

Nitrone	δ (ppm)		
	Hα		α-C
4a	8.49	–	147.19
4b	8.39	2.48 (CH_3)	165.13
4c	8.59	–	145.90
4d	8.52	9.10 (OH)	151.28
4e	8.63	–	149.72
4f	8.51	–	149.75

Antibacterial and antifungal activities: To uncover the biological properties of the synthesized nitrones **4**, we have

TABLE-3
MICROBIAL ACTIVITY OF NITRONES (THE DIAMETER OF THE ZONE OF INHIBITED MICROBIAL GROWTH AROUND A DISC)

Microorganism tested	Zone of inhibition (mm)					
	4a	**4b**	**4c**	**4d**	**4e**	**4f**
Escherichia coli	4	–	–	–	7	–
Salmonella enterica	11	–	8	12	–	7
Staphylococcus aureus	–	11	–	–	10	–
Bacillus substilis	–	–	8	16 (11N)[b]	–	–
Fusarium oxysporium f.sp. albedinis	12	12	–	–	–	14
Mucor ramannianus	–	8	16	11	–	9

[b]Diameter of clear zone, –No zone of inhibition

accredited the Kirby-Bauer [19] that is assimilated to grow a sterilized paper disc (5 mm), which was impregnated with nitrone solution (0.25 mg per a disk) in ISP_2 [20] medium of cultivation in petri dish. All this was accomplished at an isolated milieu. The culture medium contained bacteria: Gram-positive (*Bacillus substilis, Staphylococcus aureus*), Gram-negative (*Escherichia coli, Salmonella enterica*) and fungus: *Fusarium oxysporium f.sp. albedinis* and *Mucor ramannianus*. Once reading the diameter of the zone of inhibition, the results obtained are summarized in Table-3.

These nitrones exhibited a good activity despite their small quantity per disc. The molecule α-2-thiophyl-N-phenyl nitrone **(4a)** (Table-3) is active against both *F.o. albedinis* fungus and *S. enterica* bacteria Gram-negative, which remainder stable in the presence of methyl group located in the 5-position of the thiophene nucleus (4**b**) against *F.o. albedinis* and *S. aureus* bacteria Gram-positive, while exist bromo group located in the 4-position of the benzene nucleus (**4f**) the 4-position of the benzene nucleus of this molecule was increased against *F.o. albedinis*. When hydroxyl group was located in the 4-position of the benzene nucleus (**4d**), the activity was best against *B. substilis* bacteria Gram-positive (16 mm, 11 mm clear); medium against both *M. ramannianus* fungus and *S. enterica*; that is quite active against *E. coli* bacteria Gram-negative. The presence of nitro group in the 5-position of the thiophene nucleus (**4c**), the activity of this compound was best against *M. ramannianus* and feeble against both *B. substilis* and S. *enteric*. To find out that the activity of the compound (**4a**) was improved by the withdrawing group than the donor group located in the 5-position of the thiophene nucleus whilst the hydroxyl and bromo groups (donor group) located in the 4-position of the benzene nucleus increased this activity. Thus, we find that these compounds have shown equally effective towards selected bacteria and fungi.

Conclusion

In conclusion, the successful preparation of α-(5-(R)-2-thiophyl)-N-(4-R′) phenyl nitrone as an acyclic nitrones possess heterocyclic ring of thiophene, that was confirmed by the IR spectrum, ^{1}H NMR and ^{13}C NMR spectrum of the synthesized compounds. On the other hand this nitrones obvious an activity in opposition to certain microbe chosen (bacteria and fungus).

Further the preparation of other acyclic nitrones and their activity are currently underway in our laboratory.

ACKNOWLEDGEMENTS

The authors are indebted to Pr. N. Sabaou and Dr. A. Badji for their assistance in microbial activity test and to O. Fabre and A. Addou for the measurements of NMR spectra. Thanks are also due to Pr. A. Nedjmi and Mr. A. Chouchane for their useful discussions.

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