SYNTHESIS, ^{1}H-NMR AND ^{13}C-NMR SPECTRAL CHARACTERIZATION OF SOME ETHYL 3-AMINO-9-METHYLTHIENO[2,3-b]-4-QUINOLONE-2-CARBOXYLATES AS POTENTIAL ANTIMALARIAL AGENTS

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Abstract: We report the synthesis, ^{1}H-NMR and ^{13}C-NMR chemical shifts and J(H,H), J(H,F) and J(C,F) coupling constants (Hz) of several ethyl 3-amino-9-methylthieno[2,3-b]-4-quinolone-2-carboxylate derivatives, some of them with a moderate activity against *in vitro* non-enzymatic heme polymerization. They were characterized and assigned on the basis of ^{1}H, ^{13}C and ^{13}C-^{1}H (short and long range) correlated spectra.

Keywords: ^{1}H-NMR, ^{13}C-NMR, quinolones, thiopheno, antimalarial.

Introduction

Malaria remains endemic in more than 90 countries, principally in the developing world, with between 300 and 500 million new infections each year and several million people vulnerable to infection. As well as causing more than one million deaths every year, mainly in children and pregnant women[1]. The emergence and spread of resistance to antimalarial drugs has highlighted the need for the discovery and development of novel antimalarial molecules. To achieve this goal, antimalarial drug research, on the one hand, needs to focus on validated targets in order to generate new drug candidates and, on the other hand, needs to identify the targets for the future by studying the basic metabolic and biochemical processes of the malaria parasite[2-4]. We have recently reported the synthesis and spectral characterization of some 3-amino-9-methyl or phenylpyrazolo[3,4-b]-4-quinolones and 2,4-diamino-10-methyl or phenylpyrimido-[4,5-b]-5-quinolones[5-9]. Some of these compounds proved to be an interesting family of antimalarial agents *in vitro* and *in vivo* against *P. berghei*[10]. In view of the interesting biological activity exhibited by the above mentioned compounds, now we wanted to explore the effect caused by a modification on the fused heterocyclic ring by introducing a thiophene moiety. In this paper we present the synthesis, ^{1}H-NMR and ^{13}C-NMR data for **1-15** derivatives (Scheme-1). The ethyl ester derivatives have demonstrated a moderate activity against the *in vitro* non-enzymatic heme polymerization. However, the hydrolyzed derivatives **14-15** were as active as chloroquine against in vitro non-enzymatic heme polimerization (see Table-1).

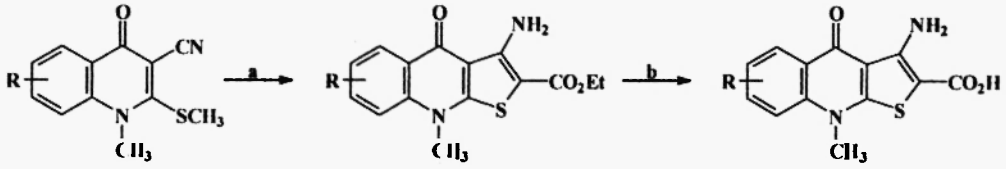
Result and Discussions

Compounds **1-13** were prepared by the synthetic route shown in Scheme-1. The final products **III** were obtained when **I**, was reacted with ethyl 2-mercaptoacetate, triethylamine in dry ethanol under an inert atmosphere of nitrogen. The hydrolysis of the ethyl ester on **5-6** with aqueous sodium hydroxide gave the corresponding carboxylic acids **14-15**. ^{1}H and ^{13}C-NMR chemical shift assignments are given in the Tables-2 and 3, respectively. The resonance were taken from the data provided by ^{13}C-^{1}H (short and long range) HETCOR, HMQC, FLOCK[11] and HMBC experiments. Short range correlations provided an unambiguous assignment of all the methine carbons. The long-range correlations determined by FLOCK and HMBC were useful in the assignment of quaternary carbons; particularly the C-4a, 8a , 9a and 4, which showed a quite similar chemical shift for almost all of the compounds. In all the structures, a three-bond connectivity was observed between the proton located at position C-5 and that of the carbon at position C-4, 7 and 8a. Also, all these structures showed a three-bond connectivity between the proton of the methyl group at position 9 and that of the carbon at position 8a and 9a. The chemical shift for the methyl group at position 9 was significantly different for compounds **4, 6** (Tables-2, 3), a reason should be an steric effect caused by a methoxy substitution in position 8.

The ^{1}H-NMR for **1-13** included two broad singlet around 6.9 and 8.2 ppm which were assigned to the amine group linked to C-3 (see Table-2). This is a clear evidence of the presence of hydrogen bonding between one of the NH_2 protons and the carboxyl group attached to the carbon at C-4. The ^{1}H-^{19}F and ^{13}C-^{19}F coupling constants of compounds, are in agreement with the published data[12].

In this work we found that structures **1-13**, have a moderate activity against *in vitro* non-enzymatic heme polymerization compared with the activity presented for related compounds reported previously[13]. This moderate activity could be attributed to the hydrogen bonding between the NH_2 group linked to C-3 and the

carboxyl group at C-4. However, hydrolyzed compound **14-15**, were as active against *in vitro* non-enzymatic heme polymerization as chloroquine (see Table-2). The acid derivatives represents an important step in the development of a novel potential anti-malarial agent.



I **II** **III**

a. $SHCH_2CO_2Et$, Na, EtOH; **b**. NaOH, $EtOH/H_2O$, HCl

Scheme-1: Preparation of compounds **1-15**

Experimental

Melting points were determined on a Thomas micro hot stage apparatus and are uncorrected. Infrared spectra were determined as KBr pellets on a Shimadzu model 470 spectrophotometer. NMR spectra were recorded on a JEOL EX 270 Fourier transform (FT) NMR spectrometer and Bruker AMX 500 FT (500MHz) instrument using DMSO-d_6 or $CDCl_3$; and are reported in ppm dowenfield from $CHCl_3$ or DMSO residual. Elemental analysis were performed by (Atlantic Microlab, Norcross, GA, USA), and analytical results were within ±0.4% of the calculate values. All solvent were destilled and dried with the usual dessicants. N-metylquinolone 2,3-substitutes **I** were obtained by following the method previously reported [7].

General procedure for the synthesis of ethyl 3-amino-9-methylthieno[2,3-b]-4-quinolone-2-carboxilates II.
A mixture of the appropiate quinolone **I** (1.3mmol), ethyl mercaptoacetate (1.3mmol), ethylamine (3 mmol) in dry ethanol (5 ml) was refluxed for 5 hours. The solvent was evaporated to dryness under reduced pressure, water was added (8 ml) and the solid thus obtained was collected by filtration. Further purification was accomplished by recrystallization from ethanol/water (4:1).

General procedure for the synthesis of 3-amino-9-methylthieno[2,3-b]-4-quinolone-2-carboxylic acids III.
To a suspension of **5**, 6 (0.2 mmol) in dioxane (5 ml) was added 5N sodium hydroxide (2 ml) and the mixture was heated at 100°C until tlc analysis (hexane:ethyl acetate, 3:2) showed that no more starting material remained (3-4 hours). The mixture was then concentrated in vacuo, the solids were dissolved in water (4 ml), and the clear solution was acidified with concentrated hydrochloric acid to give a solid, rinsed well with water, and air dried. Recrystallization from ethanol.

NMR Spectroscopy

Processing was performed using the program DELTA V1.8, and XWIN NMR V2.5, respectively, running on a Silicon Graphic Workstation.

In ^{1}H-NMR experiments, the parameters were as follows: spectral window 15 ppm; width of 30° pulse, 2 μs; relaxation delay, 4 s; and number of scans, 8. In the ^{13}C-NMR experiments, the parameters were as follows: spectral window, 250 ppm; width of 30° pulse, 2.8 μs; relaxation delay, 2 s; and number of scans, 9000-10000. ^{1}H, ^{13}C, COSY, HETCOR and FLOCK spectra were obtained using standard JEOL software.

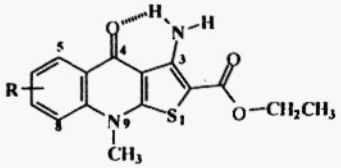
Heteronuclear ^{13}C-^{1}H HETCOR experiments were carried out with a spectral width of 17000 Hz for ^{13}C (F_2) and 4000 Hz for ^{1}H (F_1). The spectra were acquired with 1024 x 128 data points. The data were processed by exponential multiplication (LB: 3Hz) in F_2 and sinusoidal multiplication in F_1 and zero filling was applied in F_1. The mixing delay for single-bond correlation was 3.4 ms and for long-range bond correlation it was 70 ms and the relaxation delay was 1.5 s.

Two-dimensional inverse hydrogen detected heteronuclear shift correlation HMQC spectra and long-rang correlation HMBC were obtained with the standard Bruker pulse program [^{1}J(C,H): 140 Hz, F_2 27930 Hz and F_1 5040 Hz, relaxation delay 1.5 s, 2K x 128 data points. ^{2}J (C,H): 7Hz, F_2 27930 Hz and F_1 6666 Hz, relaxation delay 2.0 s, 2K x 128 data points].

Inhibition of Haem Polymerization

The haem polymerization assay was performed according to[14].

Table-1: Melting-points, yields, IR, biological data and elemental analyses for compounds **1-15**.



No	R	M.P (°C)	Yield (%)	IR(cm^{-1}) NH_2, CO	%[b c]	IC_{50} μM[d]	Elemental analysis: calcd (found %)		
							C	H	N
1	*H*	*206-208*	*72*	*3440, 1703*	*<40*		*58.97(58.71)*	*4.68(4.68)*	*8.92(9.12)*
2	*6-OMe*	*256-258*	*88*	*3444, 1697*	*<40*		*57.72(57.81)*	*4.82(4.85)*	*8.33(8.43)*
3	*7-OMe*	*212-214*	*79*	*3442, 1690*	*47*	*NA*	*57.72(57.43)*	*4.82(4.67)*	*8.33(8.48)*
4	*8-OMe*	*182-184*	*78*	*3445, 1693*	*55*	*NA*	*57.72(57.70)*	*4.82(4.86)*	*8.33(8.35)*
5	*6,7-OMe*	*238-240*	*54*	*3480, 1695*	*<40*		*56.01(56.34)*	*5.08(5.00)*	*7.63(7.72)*
6	*6,8-OMe*	*210-212*	*58*	*3490, 1699*	*<40*		*56.01(55.83)*	*5.08(5.16)*	*7.63(7.62)*
7	*5-Me*	*206-208*	*35*	*3480, 1690*	*<40*		*60.74(60.86)*	*5.10(5.16)*	*8.85(8.87)*
8	*6-Me*	*226-228*	*54*	*3470, 1695*	*49*	*NA*	*60.74(60.58)*	*5.10(5.01)*	*8.85(9.05)*
9	*7-Me*	*214-216*	*35*	*3450, 1690*	*<40*		*60.74(60.93)*	*5.10(5.12)*	*8.85(8.54)*
10	*6-Cl*	*220-222*	*30*	*3450, 1705*	*<40*		*53.49(53.86)*	*3.89(3.70)*	*8.32(8.39)*
11	6,7-Cl	231-233	35	3460, 1699	<40		48.53(48.82)	3.25(3.38)	7.54(7.95)
12	*6-F*	*244-246*	*45*	*3452, 1695*	*<40*		*56.29(55.88)*	*4.09(4.08)*	*8.74(8.39)*
13	*6,7-F*	*278-280*	*39*	*3475, 1700*	*64*		*55.73(55.28)*	*4.19(4.14)*	*7.56(7.45)*
14	*6,7-OMe*[a]	*280-282*	*75*	*3500, 1725*	*93*	*2.14*	*53.89(54.05)*	*4.22(4.16)*	*8.38(8.35)*
15	*6,8-OMe*[a]	*271-276*	*64*	*3500, 1725*	*82*	*3.89*	*53.89(53.93)*	*4.22(4.16)*	*8.38(8.63)*

[a] carboxylic acid, [b] % of inhibition $1x10^{-6}$M, [c] Chloroquine 93%, [d] IC_{50}: 2.00 μM. NA: not available

Acknowledments

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Table-2: ^{1}H NMR chemical shifts (ppm) and J(H,H) and J(F,H) coupling constants (Hz) for compounds **1-15**

No	NH_2[a]	NH_2[a]	CH_3[b]	CH_2[c]	OCH_3[d]	CH_3[d]	N-CH_3[d]	5-H	6-H	7-H	8-H
1	7.0	7.8	1.3, J: 7.1	4.2, J: 7.1			3.9	8.3dd J 8.3, 2.6	7.8[e]	7.5[e]	7.8[e]
2	7.0	7.9	1.3, J: 6.9	4.2, J: 6.9	3.8		3.9	7.7d J 8.8		7.5dd J:2.5, 9.4	8.8d, J 9.4
3	6.9	7.9	1.3, J: 6.9	4.2, J 6.9	3.9		3.8	8.2d J:8.8	7.0dd, J 2.2, 8.8		7.1d J 2.2
4	7.0	7.8	1.3, J: 7.1	4.2, J 7.1	3.9		4.2	7.9dd J:1.5,7.9	7.4dd J 7.7, 7.9	7.5dd J:6.6, 1.4	
5	6.9	7.9	1.3, J: 6.0	4.3, J: 6.0	3.9, 4.0		3.7	7.7[d]			6.7[d]
6	6.9	7.8	1.3, J: 7.2	4.3, J 7.2	3.8, 3.9		4.1	6.7d J:2.9		7.4d, J:2.8	
7	7.1	7.9	1.4, J: 7.3	4.3, J 7.3		2.9	3.8		7.3dd J:2.2, 7.5	7.5d J, 7.5, 7.5	7.1dd J:2.2, 7.5
8	6.9	7.8	1.4 J: 7.5	4.3, J: 7.5		2.4	3.7	8.1d, J:1.7		7.5dd J7.9, 1.7	7.3d J 7.9
9	7.0	7.5	1.3, J: 7.3	4.3 J: 7.3		2.9	3.7	7.1[e]	7.1[e]		7.5d J:2.2
10	6.9	8.2	1.3 J: 7.3	4.2 J: 7.3			3.9	7.9d, J:2.1		7.7dd, J:8.6, 2.1	7.3d, J:8.6
11	7.1	8.1	1.4, J: 7.3	4.3 J: 7.3			3.8	7.9[f]			7.5[f]
12	6.9	7.8	1.3, J: 7.4	4.2, J: 7.4			3.8	7.7[f]		7.6[f]	7.7[f]
13	7.2	7.8	1.4, J: 7.3	4.3, J: 7.3			3.8	8.1dd, J:8.7,1.8			7.5dd, J:7.4, 1.4
14					3.8, 3.9		3.6	7.4[d]			7.3[d]
15					3.8, 3.9		3.7	7.1d, J: 2.6			6.9d, J:2.6

[a] Broad singlet, [b] triplet, [c] quartet, [d] singlet, [e] multiplet.

Table-3: ^{13}C NMR chemical shifts (ppm) for compounds **1-15**

Carbon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
C-2	112.77	112.55	112.49	112.75	112.43	112.45	112.56	113.09	113.06	112.83	112.27	112.16	112.38	115.16	115.86
C-3	153.35	153.49	152.00	152.01	153.56	153.34	154.38	153.98	154.68	153.56	152.95	152.17	153.47	150.24	150.26
C-3a	80.76	85.70	82.02	82.02	85.63	80.17	82.60	80.76	83.52	82.17	82.87	83.77	82.09	86.16	86.55
C-4	174.35	173.91	173.84	173.85	173.35	174.69	177.82	175.09	177.52	174.39	173.68	174.01	173.88	169.43	168.31
C-4a	124.72	126.15	118.78	127.22	121.22	127.54	123.45	124.65	124.05	124.16	122.74	123.67	119.49	118.15	118.63
C-5	126.35	122.67	128.25	116.81	106.60	97.31	141.22	126.31	125.78	125.80	128.32	110.93[a]	113.93[e]	98.06	97.45
C-6	133.71	156.25	112.66	127.22	157.29	156.11	131.83	140.18	130.05	125.12	126.33	157.03[b]	151.93[f]	156.24	155.01
C-7	123.92	107.46	163.74	118.33	147.02	105.47	126.88	134.15	140.28	132.50	139.90	122.13[c]	120.29[g]	154.25	106.68
C-8	116.64	118.15	99.49	150.27	99.16	150.73	114.30	114.20	116.15	116.54	113.96	117.13[d]	103.73	105.25	149.97
C-8a	131.71	136.25	143.26	132.75	137.47	127.99	142.73	133.24	142.75	141.64	143.94	139.97	140.39	127.42	127.02
C-9a	158.02	157.34	157.82	159.95	157.29	157.75	157.16	157.45	158.26	157.72	158.03	157.75	157.01	159.85	161.45
NCH_3	39.02	39.00	39.82	42.00	38.2	43.16	37.96	37.07	38.06	39.80	39.76	38.09	40.06	39.22	35.18
OCH_3		56.28	56.49	57.48	56.41;56.90	55.69;56.38								57.28,	55.73; 57.05
CH_3							24.33	20.80	25.13					58.31	158.56
CO	163.97	164.05	163.98	163.94	164.03	164.56	164.65	164.48	164.75	164.05	164.28	164.12	163.17	159.48	
CH_2	60.03	59.93	59.97	60.03	59.86	59.90	59.88	59.95	59.70	60.00	59.65	58.96	59.65		
CH_3	15.13	15.02	15.12	15.11	15.05	14.74	14.75	14.74	15.05	15.10	14.93	14.10	15.07		

J_a: 22.87; J_b: 243.65; J_c: 23.99; J_d: 5.19; J_e: 25.30; J_f: 242.35; J_g: 224.78 Hz.