Synthesis and antitubercular activity of spiroheterocycles: 2,2',4',5'-tetra-substituted-1,2,2',4'-tetrahydro-4*H*-spiro[isoquinoline-3,3'-pyrazol]-4-ones

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Abstract

Synthesis of a series of fourteen novel 2,2',4',5'-tetra-substituted-1,2,2',4'-tetrahydro-4H-spiro[isoquinoline-3,3'-pyrazol]-4-ones was accomplished in good yield by regio and steroselective 1,3-dipolar cycloaddition of α-chloro-aryliden-phenylhydrazones with (3Z)-3-(arylidene)-2-phenyl-2,3-dihydroisoquinolin-4(1H)-ones **4-8** with dipolarophiles [(3*Z*)-2-phenyl-3-(p-R[1]-phenyl)-2,3-dihydroisoquinolin-4(1*H*)-ones] **1-3**. The structure of the isolated products **9-22** was established through different spectroscopic techniques. X-ray crystal structure analysis of one of the products confirms the structure and the selective region and stereochemistry of this cycloaddition. Their antitubercular activity is evaluated.

Keywords: Spiroheterocycles, 1,3-dipolar cycloaddition,; tuberculosis

Introduction

The spiroisoxazolines derivatives have emerged in recent years as candidates for drugs due to their herbicidal, plant-growth regulatory and antitumor activity.[1,2] We have recently investigated the antitubercular and anti-breast cancer activity of some spiroisoxazolines derivatives.[3,4] With other kind of drugs we had also performed antimicrobial screening of imidazo[1,2-*a*]pyrimidine derivatives. We had shown that compounds bearing a formyl, hydroxyl or nitroso side chains in 3-position are highly active as antitubercular (MIC<6.25 μg/mL; 98% Inhib.) [5] and antibacterial agents (Gram+ and Gram-).[6]

General structure/activity relationship observations allowed us to suggest that functionalized side chain(s) like [O=C-C-O], [O=C-C-O-N] or [X-C-O] and [X-C-O-N] (X=O,S) are usually crucial for the diversity of the bioactivity (Figure 1).

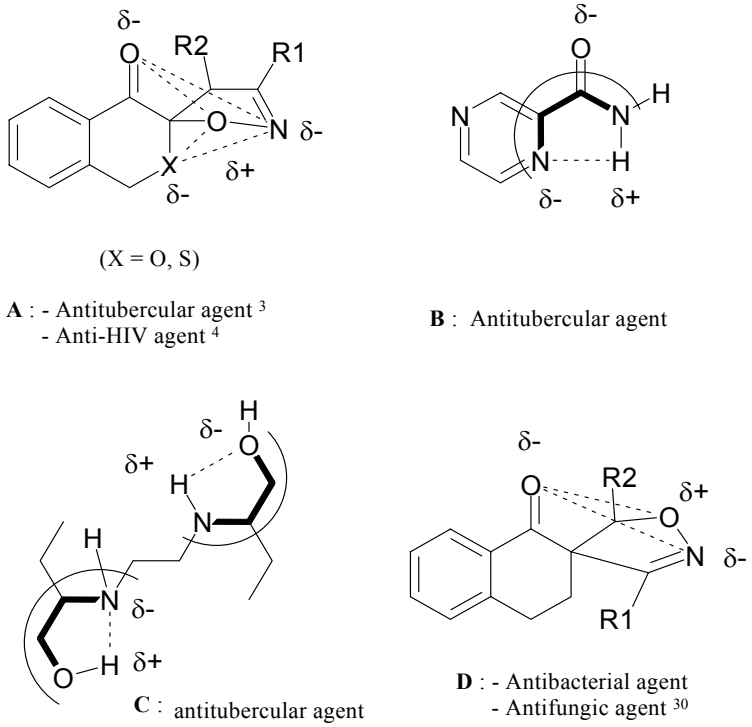


Figure 1. Clinical antitubercular agents (**B**,**C**) and spiroheterocycles (**A,D**).

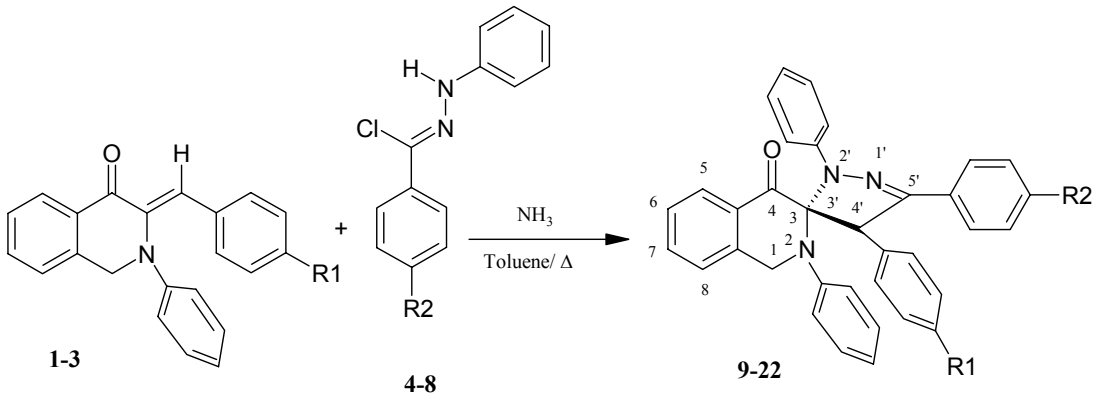
In our earlier studies of the 1,3-dipolar cycloaddition field, we had investigated the reaction of diarylnitrilimines with endocyclic dipolarophiles such as dihydroquinoleine,[7] indene [8] or 3-methoxycarbonyl-4H-1-benzopyran-4-one.[9] We also studied the regio and stereochemistry of the reaction of diarylnitrilimines with the 2-arylidene-indan-1-ones,[10] 3-arylidene-tetraline-4-ones,[11,12] 3-arylidene-isothiochroman-4-ones [13] and recently the 3-toluidene-2,3-dihydro-4(1H)-isoquinolone with exocyclic dipolarophile groups.[14]

In our ongoing programme, we report efficient and short synthesis of new spiroheterocycles in good yields from 1,3-dipolar cycloaddition of dipolarophiles with the appropriate α-chloro-aryliden-phenylhydrazones. The pharmacological investigations as antitubercular activity are also discussed.

Results and Discussion

The dipolarophiles [(3*Z*)-2-phenyl-3-(p-R^1-phenyl)-2,3-dihydroisoquinolin-4(1*H*)-ones] **1-3** were prepared from *para*-substituted benzaldehydes, and 2-phenyl-2,3-dihydroisoquinolin-4(1*H*)-one as reactants.[15]

The cycloadditions of the dipolarophiles **1-3** with the α-chloro-aryliden-phenylhydrazones **4-8** were completely regioselective and stereoselective, to the extent that no other cycloadducts were detected in NMR spectra of the crude product mixtures. This may be attributed to steric effects controlling the reactions, such that the nitrogen of NH group of the compounds **4–8** becomes bonded to the most substituted olefinic carbon of the dipolarophile **1-3**. Good yields of the spiroheterocycles **9–22** were obtained (50-85%) as shown in Scheme 1.



1: R1 = H
2: R1 = CH_3
3: R1 = OCH_3

4: R2 = H
5: R2 = CH_3
6: R2 = OCH_3
7: R2 = Cl
8: R2 = NO_2

9: (R1,R2) = (H,H)
10: (R1,R2) = (H,CH_3)
11: (R1,R2) = (H,OCH_3)
12: (R1,R2) = (H,Cl)
13: (R1,R2) = (CH_3,H)
14: (R1,R2) = (CH_3,CH_3)
15: (R1,R2) = (CH_3,OCH_3)

16: (R1,R2) = (CH_3,Cl)
17: (R1,R2) = (CH_3,NO_2)
18: (R1,R2) = (OCH_3,H)
19: (R1,R2) = (OCH_3,CH_3)
20: (R1,R2) = (OCH_3,OCH_3)
21: (R1,R2) = (OCH_3,Cl)
22: (R1,R2) = (OCH_3,NO_2)

Scheme 1. Synthesis of spiroheterocycles **9-22**.

The cycloaddition could also taken place in toluene and triethylamine. All compounds were characterized using ^{1}H and ^{13}C NMR data's which are in agreement with previously reported similar compounds.[13,16] The selective NMR data are regrouped in Tables 1 and 2.
Following the ORTEP of compound **19**,[17] it appears that the privileged approach of dipolar reagent **4-8** taken place with inverted regiochemistry to that obtained with chromanone exodipolarophile derivatives.[18,19]

Table 1. Selected IR and ^{1}H NMR data of compounds **9-22**

Comp.	(R^1,R^2)	IR (υ CO)	δR^1 δR^2	δH^{1a} δH^{1b}	$\delta H^{4'}$	δH (Aroma)	δH^5
			-	4.45 (d, 1H)		6.75-7.40	8.00 (dd, 1H)
9	(H,H)	1700	-	4.50 (d, 1H)	5.40 (s, 1H)	(m, 23H)	$^4JH^5$-H^7 = 1.15
				$^2JH^{1a}H^{1b}$ = 16.35			$^3JH^5$-H^6 = 7.65
			-	4.45 (d, 1H)		6.90-7.60	8.05 (dd, 1H)
10	(H,CH_3)	1693	2.25 (s, 3H)	4.50 (d, 1H)	5.40 (s, 1H)	(m, 22H)	$^4JH^5$-H^7= 1.20
				$^2JH^{1a}H^{1b}$ = 16.35			$^3JH^5$-H^6 = 7.70
			-	4.45 (d, 1H)		6.65-7.60	8.00 (dd, 1H)
11	(H,OCH_3)	1694	3.70 (s, 3H)	4.50 (d, 1H)	5.35 (s, 1H)	(m, 22H)	$^4JH^5$-H^7= 1.10
				$^2JH^{1a}H^{1b}$ = 16.65			$^3JH^5$-H^6 = 7.75
			-	4.45 (d, 1H)		6.65-7.80	7.95 (dd, 1H)
12	(H,Cl)	1696	-	4.50 (d, 1H)	5.35 (s, 1H)	(m, 22H)	$^4JH^5$-H^7 = 1.25
				$^2JH^{1a}H^{1b}$ = 16.45			$^3JH^5$-H^6 = 7.80
			2.30 (s, 3H)	4.35 (d, 1H)		6.80-7.55	7.95 (dd, 1H)
13	(CH_3,H)	1693	-	4.60 (d, 1H)	5.35 (s, 1H)	(m, 22H)	$^4JH^5$-H^7 = 1.25
				$^2JH^{1a}H^{1b}$ = 16.85			$^3JH^5$-H^6 = 7.75
			2.25 (s, 3H)	4.35 (d, 1H)		6.75-7.65	8.00 (dd, 1H)
14	(CH_3,CH_3)	1694	3.00 (s, 3H)	4.55 (d, 1H)	5.35 (s, 1H)	(m, 21H)	$^4JH^5$-H^7 = 1.30
				$^2JH^{1a}H^{1b}$ = 16.85			$^3JH^5$-H^6 = 7.80
			2.25 (s, 3H)	4.35 (d, 1H)		6.70-7.60	8.00 (dd, 1H)
15	(CH_3,OCH_3)	1695	3.75 (s, 3H)	4.55 (d, 1H)	5.30 (s, 1H)	(m, 21H)	$^4JH^5$-H^7= 1.20
				$^2JH^{1a}H^{1b}$ = 16.75			$^3JH^5$-H^6 = 7.80
			2.30 (s, 3H)	4.35 (d, 1H)		6.75-7.65	8.00 (dd, 1H)
16	(CH_3,Cl)	1696	-	4.60 (d, 1H)	5.30 (s, 1H)	(m, 21H)	$^4JH^5$-H^7 = 1.20
				$^2JH^{1a}H^{1b}$ = 16.75			$^3JH^5$-H^6 = 7.58
			2.25 (s, 3H)	4.50 (d, 1H)		6.75-7.65	7.95 (dd, 1H)
17	(CH_3,NO_2)	1694	-	4.55 (d, 1H)	5.35 (s, 1H)	(m, 21H)	$^4JH^5$-H^7 = 1.35
				$^2JH^{1a}H^{1b}$ = 16.75			$^3JH^5$-H^6 = 7.85
			3.75 (s, 3H)	4.40 (d, 1H)		6.50-7.65	8.00 (dd, 1H)
18	(OCH_3,H)	1693	-	4.60 (d, 1H)	5.35 (s, 1H)	(m, 22H)	$^4JH^5$-H^7= 1.15
				$^2JH^{1a}H^{1b}$ = 16.75			$^3JH^5$-H^6 = 8.85
			3.75 (s, 3H)	4.40 (d, 1H)		6.50-7.65	8.05 (dd, 1H)
19	(OCH_3,CH_3)	1690	2.30 (s, 3H)	4.55 (d, 1H)	5.30 (s, 1H)	(m, 21H)	$^4JH^5$-H^7= 1.25
				$^2JH^{1a}H^{1b}$ = 16.35			$^3JH^5$-H^6 = 7.85
			3.75 (s, 3H)	4.35 (d, 1H)		6.50-7.60	8.00 (dd, 1H)
20	(OCH_3,OCH_3)	1689	3.75 (s, 3H)	4.55 (d, 1H)	5.30 (s, 1H)	(m, 21H)	$^4JH^5$-H^7 = 1.20
				$^2JH^{1a}H^{1b}$ = 16.65			$^3JH^5$-H^6 = 7.75

			3.75 (s, 3H)	4.40 (d, 1H)		6.50-7.65	7.95 (dd, 1H)
21	(OCH_3,Cl)	1691	-	4.60 (d, 1H)	5.30 (s, 1H)	(m, 21H)	$^4JH^5$-H^7= 1.10
				$^2JH^{1a}H^{1b}$ = 16.75			$^3JH^5$-H^6 = 7.75
			3.70 (s, 3H)	4.50 (d, 1H)		6.45-7.65	8.05 (dd, 1H)
22	(OCH_3,NO_2)	1694	-	4.55 (d, 1H)	5.30 (s, 1H)	(m, 21H)	$^4JH^5$-H^7 = 1.25
				$^2JH^{1a}H^{1b}$ = 16.65			$^3JH^5$-H^6 = 7.80

Table 2. Selected ^{13}C NMR data of compounds **9-22**

Comp.	(R^1,R^2)	δC (R^1)	δC (R^2)	δC^1 (N<u>C</u>H_2-)	δC^3 (spiro-C)	δC^4 (-<u>C</u>=O)	δ$C^{4'}$ (-<u>C</u>H-)
		-	-	50.4	91.45	195.0	64.3
9	(H,H)						
		-	21.4	50.4	91.3	195.1	64.4
10	(H,CH_3)						
		-	55.2	50.3	91.3	195.2	64.4
11	(H,OCH_3)						
		-	-	50.4	91.4	194.3	64.3
12	(H,Cl)						
		21.3	-	50.4	91.6	195.0	64.0
13	(CH_3,H)						
		21.2	21.4	50.4	91.4	195.1	64.1
14	(CH_3,CH_3)						
		21.2	55.2	50.4	91.4	195.2	64.1
15	(CH_3,OCH_3)						
		21.2	-	50.4	91.6	194.9	63.7
16	(CH_3,Cl)						
		21.2	-	50.4	91.6	194.9	63.7
17	(CH_3,NO_2)						
		55.2	-	50.4	91.5	195.0	63.6
18	(OCH_3,H)						
		55.2	21.4	50.4	91.4	195.1	63.7
19	(OCH_3,CH_3)						
		55.2	55.3	50.3	91.4	195.2	63.7
20	(OCH_3,OCH_3)						
		55.3	-	50.4	91.0	194.9	63.4
21	(OCH_3,Cl)						
		55.3	-	50.4	92.0	194.5	62.9
22	(OCH_3,NO_2)						

Antimycobacterial activity

The antimycobacterial activity of the compounds was determined with the objective to identify the compounds having inhibitory activity against *M. tuberculosis*.

Interesting results were obtained from these assays and data is reported in Table 3. The *in vitro* antimycobacterial activities of these compounds **9-22** were inferior to that of Isoniazid against *M. tuberculosis* $H_{37}Rv$. Further, the compounds **9-22** had either little or no activity (17-67%inhibition). However, none of the compounds showed activity against *M. tuberculosis* $H_{37}Rv$ suggesting that compounds possess no specific anti tuberculosis activity. This could be probably due to their low absorption (MIC >6.25 µg/mL) and no activity against *M. tuberculosis* may be because of absence of binding antitubercular agent adequate properties.

Structure activity relationship

The antimycobacterial activity data in Table 3 clearly show that the compounds **9-22** having a aryl substituent in *N(2) and C(1)*-positions of the pyrazol ring exhibited low activity against *M. tuberculosi*s. All compounds **9-22** had an almost similar range of MIC values; they differ only by %inhibition, in spite of having two different aryls at C(1) and C(9), (Figure 2).

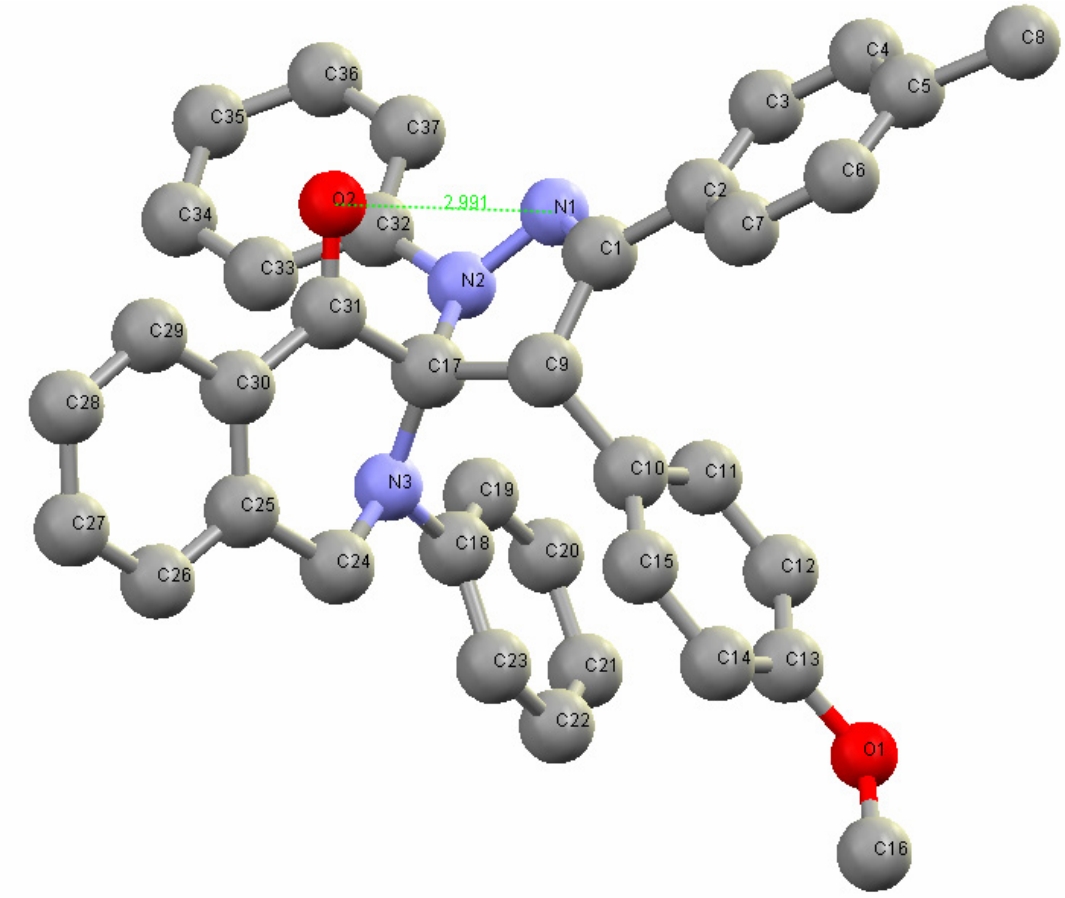


Figure 2. A view of compound (**19**) with the atom-numbering scheme. [17]

The replacement of the nitro substituent (R2) by a methyl or a methoxy group in phenyl ring of C(1) position, as in **19** and **20**, respectively, caused a reduction of activity. Curiously, 1-p-methylphenyl-, and 2-p-nitrophenyl-substitution as in **17** [(R1,R2) = (CH_3,NO_2)], abolished any activity indicating that not only specific electronic but also steric requirements are needed for activity. This suggests that a bulky group or disubstitution on the N(1) and C(1) positions are not favourable for antimycobacterial activity.

Conclusions

In this paper we report efficient and short synthesis of the spiroheterocycles **9-22** in good yields from 1,3-dipolar cycloaddition of dipolarophiles (**1-3**) with the appropriate α-chloro-aryliden-phenylhydrazones (**4-8**). The more interesting result which could be emphasised is the inverted regio and stereochemistry of spirane ring formation in contrast with the result obtained in the litterature with chromanone. This is likely due to the presence of the neighboring N-aryl group. Due to the presence of rigid O=C-C-N-N pharmacophore, antitumorale and anti-HIV screening studies are in progress at National Cancer Institute (NCI) in order to elucidate the structure/activity relationships.

Table 3. Range of %inhibition values (MIC >6.25 μg/mL) of compounds **9-22** against *Mycobacterium tuberculosis* H_{37}Rv strains

Sample ID (TAACF-code)	(R1,R2)	Mol. formula Mol. weight	Assay	MIC (μg/mL)	% Inh
22 (TAACF-299962)	(CH_3O,NO_2)	$C_{36}H_{28}N_4O_4$ 580.63	Alamar	>6.25	69
9 (TAACF-299952)	(H,H)	$C_{35}H_{27}N_3O$ 505.61	Alamar	>6.25	54
16 (TAACF-299957)	(CH_3,Cl)	$C_{36}H_{28}ClN_3O$ 554.08	Alamar	>6.25	41
11 (TAACF-299953)	(H,CH_3O)	$C_{36}H_{29}N_3O_2$ 535.63	Alamar	>6.25	40
13 (TAACF-299954)	(CH_3,H)	$C_{36}H_{29}N_3O$ 519.63	Alamar	>6.25	40
15	(CH_3,CH_3O)	$C_{37}H_{31}N_3O_2$ 549.66	Alamar	>6.25	37

(TAACF-299956)					
17 (TAACF-299958)	(CH_3,NO_2)	$C_{36}H_{28}N_4O_3$ 564.63	Alamar	>6.25	23
14 (TAACF-299955)	(CH_3,CH_3)	$C_{37}H_{31}N_3O$ 533.66	Alamar	>6.25	19
18 (TAACF-299959)	(CH_3O,H)	$C_{36}H_{29}N_3O_2$ 535.63	Alamar	>6.25	19
19 (TAACF-299960)	(CH_3O,CH_3)	$C_{37}H_{31}N_3O_2$ 549.66	Alamar	>6.25	18
20 (TAACF-299961)	(CH_3O, CH_3O)	$C_{37}H_{31}N_3O_3$ 565.66	Alamar	>6.25	17

Experimental Section

General Procedures. Melting points are measured on banc KOFLER without corrections. NMR spectra (^1H, ^{13}C) were recorded on a Bruker Avance (operating at 300 MHz). NMR data are listed in ppm and are reported relative to tetramethylsilane; residual solvent peaks being used as internal standard with external calibration. Infra-red spectra were recorded in KBr pellets using a Perkin-Elmer 1600 FT-IR spectrometer. Mass spectra were recorded on a Hewlett-Packard 5989A Mass Spectrometer (70 eV) Nermag R 1010-C electronic impact and elemental analysis (CNRS, Université Paul Sabatier and Toulouse, France).

Antitubercular activity

Primary screening was conducted at 6.25 μg mL^{-1} against M. tuberculosis $H_{37}Rv$ (ATCC 27294) in BACTEC 12B medium using a broth microdilution assay, the Microplate Alamar Blue Assay (MABA).[20] Compounds exhibiting fluorescence were tested in the BACTEC 460 radiometric system. Compounds demonstrating at least 90% inhibition in the primary screen were retested at lower concentrations against M. tuberculosis $H_{37}Rv$ to determine the MIC using MABA. The MIC is defined as the lowest concentration effecting a reduction in fluorescence of 99% relative to controls.[20]

General procedure of the preparation of spiroheterocycles 9-22

A mixture of a dipolarophile (**1-3**; 11 mmol) and α-chloro-aryliden-phenylhydrazone (**4-8**; 12 mmol) was stirred in dry toluene (20 ml) at refluxed toluene, under nitrogen atmosphere. To this mixture was added 3 ml of triethylamine dropways over 15 min. The mixture was stirred 24-48 h

until TLC indicated complete disappearance of reactants. After cooling, 20 ml of toluene was added and the chloro-triethyl-ammonium salt was eliminated by filtration. The compounds **9-22** were obtained after evaporation and crystallisation in ethanol.

2,2',4',5'-Tetraphenyl-1,2,2',4'-tetrahydro-4*H*-spiro[isoquinoline-3,3'-pyrazol]-4-one (9). White powder. Yield = 67%. M.p. = 178 °C. IR ν (C=O) = 1700 cm^{-1}. ^1H NMR: 4.45 (d, 1H, H^{1a}, $^2JH^{1a}$-H^{1b} = 16.35); 4.50 (d, 1H, H^{1b}, $^2JH^{1b}$-H^{1a} = 16.35); 5.40 (s, 1H, $H^{4'}$); 6.75-7.40 (m, 23 H, Aromatic H); 8.00 (dd, 1 H, H^5, $^4JH^5$-H^7 = 1.15, $^3JH^5$-H^6 = 7.65). ^{13}C NMR: 50.4 (N-$\underline{C}H_2$-); 64.3 (-$\underline{C}$H-); 91.4 (spiro-C); 129.6; 131.8; 134.0; 140.8; 144.1; 147.5; 147.7 (quat. C sp^2); 115.6; 120.6; 121.8; 122.0; 125.9; 126.9; 127.6; 127.9; 128;0; 128.1; 128.2; 128.3; 128.9; 131.2; 134.7 (tert. C sp^2); 195.0 (C=O). MS (EI, 70 eV): $[M]^{+\cdot}$ = m/z : M = 505 [$C_{35}H_{27}N_3O$] (20%); 77 (100%). Anal. Calcd. For $C_{35}H_{27}N_3O$: C, 83.14; H, 5.38; N, 8.31. Found: C, 82.75; H, 5.27; N, 8.15.

2,2',4'-Triphenyl-5'(4-methyl-phenyl)-1,2,2',4'-tetrahydro-4*H*-spiro[isoquinoline-3,3'-pyrazol]-4-one (10). White powder. Yield = 63%. M.p. = 158 °C. IR ν (C=O) = 1693 cm^{-1}. ^1H NMR: 2.25 (s, 3H, CH_3); 4.45 (d, 1H, H^{1a}, $^2JH^{1a}$-H^{1b} = 16.35); 4.50 (d, 1H, H^{1b}, $^2JH^{1b}$-H^{1a} = 16.35); 5.40 (s, 1H, H^4); 6.90-7.60 (m, 22 H, Aromatic H); 8.05 (dd, 1H, H^5, $^4JH^5$-H^7 = 1.20, $^3JH^5$-H^6 = 7.40). ^{13}C NMR: 21.4 ($\underline{C}H_3$); 50.4 (N-$\underline{C}H_2$-); 64.4 (-$\underline{C}$H-); 91.3 (spiro-C); 129.7; 134.2; 138.3; 140.8; 144.2; 147.6; 147.8 (quat. C sp^2); 115.5; 120.5; 121.7; 122.0; 125.9; 126.8; 127.9; 128.0; 128.1; 128.2; 128.9; 128.9; 131.2; 134.7 (tert. C sp^2); 195.1 (C=O). MS (EI, 70 eV): $[M]^{+\cdot}$ m/z : M = 519 [$C_{36}H_{29}N_3O$] (15%); 311 (100%). Anal. Calcd. For $C_{36}H_{29}N_3O$: C, 83.21; H, 5.63; N, 8.09. Found: C, 82.65; H, 5.47; N, 8.15.

2,2',4'-Triphenyl-5'(4-methoxy-phenyl)-1,2,2',4'-tetrahydro-4*H*-spiro[isoquinoline-3,3'-pyrazol]-4-one (11). White powder. Yield = 60%. M.p.= 122°C. IR ν (C=O) = 1694 cm-1. ^1H NMR: 3.70 (s, 3 H, CH_3); 4.35 (d, 1 H, H^{1a}, $^2JH^{1a}$-H^{1b} = 16.65); 4.50 (d, 1H, H^{1b}, $^2JH^{1b}$-H^{1a} = 16.65); 5.35 (s, 1H, H^4); 6.65-7.60 (m, 22 H, Aromatic H); 8.00 (dd, 1H, H^5, $^4JH^5$-H^7 = 1.20, $^3JH^5$-H^6 = 7.40). ^{13}C NMR: 50.3 (N$\underline{C}H_2$-); 55.2 (O$\underline{C}H_3$); 64.4 (-$\underline{C}$H-); 91.3 (spiro-C); 124.5; 129.7; 134.2; 140.8; 144.3; 147.6; 159.7 (quat. C sp^2);113.7; 115.5; 120.4; 121.7; 122.0; 125.9; 127.9; 128.1; 128.2; 128.3; 128.8; 131.2; 134.7 (tert. C sp^2); 195.2 (C=O). MS (EI, 70 eV): $[M]^{+\cdot}$ m/z : M = 535 [$C_{36}H_{29}N_3O_2$] (28%); 77 (100%). Anal. Calcd. For $C_{36}H_{29}N_3O_2$: C, 80.72; H, 5.46; N, 7.84. Found: C, 79.85; H, 5.31; N, 8.05.

2,2',4'-Triphenyl-5'(4-chloro-phenyl)-1,2,2',4'-tetrahydro-4H-spiro[isoquinoline-3,3'-pyrazol]-4-one (12). White powder. Yield = 57%. M.p.=174 °C. IR ν (C=O) = 1696 cm^{-1}. ^1H NMR: 4.45 (d, 1H, H^{1a}, $^2JH^{1a}$-H^{1b} = 16.45); 4.50 (d, 1H, H^{1b}, $^2JH^{1b}$-H^{1a} = 16.45); 5.35 (s, 1H, H^4); 6.65-7.80 (m, 22H, Aromatic H); 7.95 (dd, 1H, H^5, $^4JH^5$-H^7 = 1.25, $^3JH^5$-H^6 = 7.80). ^{13}C NMR: 50.4 (N$\underline{C}H_2$-); 64.3 (-CH-); 91.4 (spiro-C); 124.3; 129.5; 134.2; 140.9; 144.2; 147.5; 147.7 (quat. C sp^2); 113.8; 113.9; 115.8; 121.6; 121.8; 122.4; 123.6; 126.0; 127.0; 128.2; 128.3; 129.0; 129.3; 132.1 (tert. C sp^2); 194.3 (C=O). MS (EI, 70 eV): $[M]^{+\cdot}$ m/z : M = 539 [$C_{35}H_{26}ClN_3O$] (29%); 90 (100%). Anal. Calcd. For $C_{35}H_{26}ClN_3O$: C, 77.84; H, 4.85; N, 7.78. Found: C, 73.95; H, 4.67; N, 7.53.

2,2',5'-Triphenyl-4'(4-methyl-phenyl)-1,2,2',4'-tetrahydro-4*H*-spiro[isoquinoline-3,3'-pyrazol]-4-one (13). White powder. Yield = 87%. M.p.=214 °C. IR ν (C=O) = 1693 cm^{-1}. ^1H NMR: 2.30 (s, 3H, CH_3); 4.35 (d, 1H, H^{1a}, $^2JH^{1a}$-H^{1b} = 16.85); 4.60 (d, 1H, H^{1b}, $^2JH^{1b}$-H^{1a} = 16.85); 5.35 (s, 1H, H^4); 6.80-7.55 (m, 22 H, Aromatic H); 7.95 (dd, 1H, H^5, $^4JH^5$-H^7 = 1.25, $^3JH^5$-H^6 = 7.75). ^{13}C NMR: 21.3 ($\underline{C}H_3$); 50.4 ($N\underline{C}H_2$-); 64.0 ($\underline{C}H$); 91.6 (spiro-C); 129.6; 131.9; 137.7; 140.9; 144.2; 147.6; 147.9 (quat. C sp^2); 115.6; 120.6; 121.7; 122.1; 125.9; 126.9; 128.1; 128.2; 128.3; 128.9; 131.0; 134.7 (tert. C sp^2); 195.1 (C=O). MS (EI, 70 eV): $[M]^{+\cdot}$ m/z : M = 519 [$C_{36}H_{29}N_3O$] (25%); 77 (100%). Anal. Calcd. For $C_{36}H_{29}N_3O$: C, 83.21; H, 5.63; N, 8.09. Found: C, 82.92; H, 5.42; N, 7.85.

2,2'-Diphenyl-4',5'-di(4-methyl-phenyl)-1,2,2',4'-tetrahydro-4*H*-spiro[isoquinoline-3,3'-pyrazol]-4-one (14). White powder. Yield = 85%. M.p.=224 °C. IR ν (C=O) = 1694 cm^{-1}. ^1H NMR: 2.25 (s, 3H, CH_3); 2.35 (s, 3H, CH_3); 4.35 (d, 1H, H^{1a}, $^2JH^{1a}$-H^{1b} = 16.85); 4.55 (d, 1H, H^{1b}, $^2JH^{1b}$-H^{1a} = 16.85); 5.35 (s, 1H, H^4); 6.75-7.65 (m, 21H, Aromatic H); 8.00 (dd, 1H, H^5, $^4JH^5$-H^7 = 1.30, $^3JH^5$-H^6 = 7.80). ^{13}C NMR: 21.2; 21.4 (2 x $\underline{C}H_3$); 50.4 ($N\underline{C}H_2$-); 64.1 (-$\underline{C}H$-); 91.4 (spiro-C); 129.1; 129.7; 131.1; 137.6; 138.3; 140.9; 144.3; 147.6; 148.0 (quat. C sp^2); 115.5; 120.4; 121.6; 122.1; 125.9; 126.8; 127.9; 128.1; 128.8; 128.9; 131.0; 134.6 (tert. C sp^2); 195.1 (C=O). MS (EI, 70 eV): $[M]^{+\cdot}$ = m/z : M = 533 [$C_{37}H_{31}N_3O$] (20%); 77 (100%). Anal. Calcd. For $C_{37}H_{31}N_3O$: C, 83.27; H, 5.86; N, 7.87. Found: C, 82.76; H, 5.67; N, 7.53.

2,2'-Diphenyl-4'(4-methyl-phenyl)-5'(4-methoxy-phenyl)-1,2,2',4'-tetrahydro-4*H*-spiro [isoquinoline-3,3'-pyrazol]-4-one (15). White powder. Yield = 93%. M.p. = 250 °C. IR ν (C=O) = 1695 cm^{-1}. ^1H NMR: 2.25 (s, 3H, CH_3); 3.75 (s, 3H, CH_3); 4.35 (d, 1H, H^{1a}, $^2JH^{1a}$-H^{1b} = 16.75); 4.55 (d, 1H, H^{1b}, $^2JH^{1b}$-H^{1a} = 16.75); 5.30 (s, 1H, H^4); 6.70-7.60 (m, 21 H, Aromatic H); 8.05 (dd, 1H, H^5, $^4JH^5$-H^7 = 1.20, $^3JH^5$-H^6 = 7.80). ^{13}C NMR: 21.2 ($\underline{C}H_3$); 50.4 ($N\underline{C}H_2$-); 55.2 (-$O\underline{C}H_3$); 64.1 (-$\underline{C}H$-); 91.4 (spiro-C); 124.6; 129.7; 131.1; 137.6; 140.9; 144.4; 147.6; 147.8; 159.7 (quat. C sp^2); 113.6; 115.4; 120.2; 121.1; 122.1; 125.8; 127.9; 128.1; 128.3; 128.8; 130.9; 134.6 (tert. C sp^2); 195.20 (C=O). MS (EI, 70 eV): $[M]^{+\cdot}$ = m/z : M = 549 [$C_{37}H_{31}N_3O_2$] (15%); 341 (100%). Anal. Calcd. For $C_{37}H_{31}N_3O_2$: C, 80.85; H, 5.68; N, 7.64. Found: C, 79.87; H, 5.44; N, 7.55.

2,2'-Diphenyl-4'(4-methyl-phenyl)-5'(4-chloro-phenyl)-1,2,2',4'-tetrahydro-4*H*-spiro [isoquinoline-3,3'-pyrazol]-4-one (16). White powder. Yield = 75%. M.p. = 206 °C. IR ν (C=O) = 1696 cm^{-1}. ^1H NMR: 2.30 (s, 3H, CH_3); 4.35 (d, 1H, H^{1a}, $^2JH^{1b}$-H^{1a} = 16.35); 4.60 (d, 1H, H^{1b}, $^2JH^{1b}$-H^{1a} = 16.75); 5.30 (s, 1H, H^4); 6.75-7.65 (m, 21H, Aromatic H); 8.00 (dd, 1H, H^5, $^4JH^5$-H^7 = 1.20, $^3JH^5$-H^6 = 7.58). ^{13}C NMR: 21.2 ($\underline{C}H_3$); 50.4 ($N\underline{C}H_2$-); 63.7 ($\underline{C}H$); 91.6 (spiro-C); 129.5; 130.5; 130.6; 137.0; 137.9; 140.8; 144.1; 146.7; 147.5 (quat. C sp^2); 115.6; 120.8; 121.8; 122.1; 125.9; 128.0; 128.1; 128.4; 128.9; 130.9; 134.7 (tert. C sp^2); 194.9 (C=O). MS (EI, 70 eV): $[M]^{+\cdot}$ m/z : M = 553 [$C_{36}H_{28}ClN_3O$] (35%); 77 (100%). Anal. Calcd. For $C_{36}H_{28}ClN_3O$: C, 78.04; H, 5.09; N, 7.58. Found: C, 77.94; H, 4.97; N, 7.51.

2,2'-Diphenyl-4'(4-methyl-phenyl)-5'(4-nitro-phenyl)-1,2,2',4'-tetrahydro-4*H*-spiro [isoquinoline -3,3'-pyrazol]-4-one (17). White powder. Yield = 78%. M.p. = 208 °C. IR ν (C=O) = 1694 cm^{-1}. ^1H NMR: 2.25 (s, 3H, CH_3); 4.50 (d, 1H, H^{1a}, $^2JH^{1b}$-H^{1a} = 16.75); 4.55 (d,

1H, H^{1b}, $^{2}JH^{1b}$-H^{1a} = 16.75); 5.35 (s, 1H, H^{4}); 6.75-7.65 (m, 21 H, Aromatic H); 7.95 (dd, 1H, H^{5}, $^{4}JH^{5}$-H^{7} = 1.35, $^{3}JH^{5}$-H^{6} = 7.85). ^{13}C NMR: 21.2 ($\underline{C}H_3$); 50.4 (N$\underline{C}H_2$-); 63.7 (-$\underline{C}$H-); 91.6 (spiro-C); 129.5; 130.6; 130.5; 134.1; 137.2; 137.8; 140.7; 144.0; 146.6; 147.5 (quat. C sp^2); 115.5; 120.8; 121.8; 122.1; 125.8; 128.0; 128.1; 128.5; 128.8; 130.8; 134.6 (tert. C sp^2); 194.9 (C=O). MS (EI, 70 eV): $[M]^{+\cdot}$ = m/z : M = 564 [$C_{36}H_{28}N_4O_3$] (25%); 118 (100%). Anal. Calcd. For $C_{36}H_{28}N_4O_3$: C, 76.58; H, 5.00; N, 9.92. Found: C, 75.87; H, 4.87; N, 9.65.

2,2',5'-Triphenyl-4'(4-methoxy-phenyl)-1,2,2',4'-tetrahydro-4*H*-spiro[isoquinoline-3,3'-pyrazol]-4-one (18). White powder. Yield = 64%. M.p.=190 °C. IR ν (C=O) = 1693 cm^{-1}. ^{1}H NMR: 3.75 (s, 3H, CH_3); 4.40 (d, 1H, H^{1a}, $^{2}JH^{1a}$-H^{1b} = 16.75); 4.60 (d, 1H, H^{1b}, $^{2}JH^{1b}$-H^{1a} = 16.75); 5.35 (s, 1H, H^{4}); 6.50-7.65 (m, 22H, Aromatic H); 8.00 (dd, 1H, H^{5}, $^{4}JH^{5}$-H^{7} = 1.15, $^{3}JH^{5}$-H^{6} = 8.85). ^{13}C NMR: 50.4 (N$\underline{C}H_2$-); 55.2 (O-$\underline{C}H_3$); 63.6 ($\underline{C}$H); 91.5 (spiro-C); 129.6; 131.9; 140.8; 144.2; 146.6; 147.9; 150.3 (quat. C sp^2); 113.6; 115.5; 120.6; 121.7; 121.9; 126.0; 126.9; 128.0; 128.2; 128.3; 128.9; 132.2; 134.7 (tert. C sp^2); 195.0 (C=O). MS (EI, 70 eV): $[M]^{+\cdot}$ m/z : M = 535 [$C_{36}H_{29}N_3O_2$] (15%); 91 (100%)]. Anal. Calcd. For $C_{36}H_{29}N_3O_2$: C, 80.72; H, 5.46; N, 7.84. Found: C, 79.83; H, 5.14; N, 7.51.

2,2'-Diphenyl-4'(4-methoxy-phenyl)-5'(4-methyl-phenyl)-1,2,2',4'-tetrahydro-4*H*-spiro [isoquinoline - 3,3'-pyrazol]-4-one (19). White powder. Yield = 74%. M.p. = 222 °C. IR ν (C=O) = 1690 cm^{-1}. ^{1}H NMR: 2.30 (s, 3H, CH_3); 3.75 (s, 3H, OCH_3); 4.40 (d, 1H, H^{1a}, $^{2}JH^{1a}$-H^{1b} = 16.35); 4.55 (d, 1H, H^{1b}, $^{2}JH^{1b}$-H^{1a} = 16.35); 5.30 (s, 1H, H^{4}); 6.50-7.65 (m, 21H, Aromatic H); 8.05 (dd, 1H, H^{5}, $^{4}JH^{5}$-H^{7} = 1.25, $^{3}JH^{5}$-H^{6} = 7.85). ^{13}C NMR: 21.4 (-$\underline{C}H_3$); 50.4 (N$\underline{C}H_2$-); 55.2 (O-$\underline{C}H_3$); 63.7 ($\underline{C}$H); 91.4 (spiro-C); 126.1; 129.1; 129.6; 138.3; 140.8; 144.3; 147.7; 148.0; 159.3 (quat. C sp^2); 113.5; 115.5; 120.4; 121.9; 125.9; 126.8; 128.0; 128.1; 128.8; 128.9; 132.1; 134.6; 138.6 (tert. C sp^2); 195.1 (C=O). MS (EI, 70 eV): $[M]^{+\cdot}$ m/z : M = 549 [$C_{37}H_{31}N_3O_2$] (28%); 77 (100%). Anal. Calcd. For $C_{37}H_{31}N_3O_2$: C, 80.85; H, 5.68; N, 7.64. Found: C, 79.88; H, 5.59; N, 7.51.

2,2'-Diphenyl-4',5'-di(4-methoxy-phenyl)-1,2,2',4'-tetrahydro-4*H*-spiro[isoquinoline-3,3'-pyrazol]-4-one (20). White powder. Yield = 75%. M.p.=242°C. IR ν (C=O) = 1689 cm^{-1}. ^{1}H NMR: 3.75 (s, 6H, 2 x OCH_3); 4.35 (d, 1H, H^{1a}, $^{2}JH^{1a}$-H^{1b} = 16.35); 4.55 (d, 1H, H^{1b}, $^{2}JH^{1b}$-H^{1a} = 16.35); 5.31 (s, 1H, H^{4}); 6.50-7.60 (m, 21H, Aromatic H); 8.00 (dd, 1H, H^{5}, $^{4}JH^{5}$-H^{7} = 1.20, $^{3}JH^{5}$-H^{6} = 7.75). ^{13}C NMR: 50.3 (N$\underline{C}H_2$-); 55.2 (2 x O-$\underline{C}H_3$); 63.7 (-$\underline{C}$H-); 91.4 (spiro-C); 124.6; 126.1; 129.7; 140.8; 144.4; 147.7; 147.8; 159.3; 159.7 (quat. C sp^2); 113.5; 113.6; 115.4; 120.30 121.6; 121.9; 125.9; 128.0; 128.1; 128.3; 128.8; 132.1; 134.6 (tert. C sp^2); 195.2 (C=O). MS (EI, 70 eV): $[M]^{+\cdot}$ m/z : M = 565 [$C_{37}H_{31}N_3O_3$] (34%); 357 (100%). Anal. Calcd. For $C_{37}H_{31}N_3O_3$: C, 78.56; H, 5.52; N, 7.47. Found: C, 77.09; H, 5.28; N, 7.15.

2,2'-Diphenyl-4'(4-methoxy-phenyl)-5'(4-chloro-phenyl)-1,2,2',4'-tetrahydro-4*H*-spiro [isoquinoline -3,3'-pyrazol]-4-one (21). White powder. Yield = 72%. M.p. = 204 °C. IR ν (C=O) = 1691 cm^{-1}. ^{1}H NMR: 3.75 (s, 3H, OCH_3); 4.40 (d, 1H, H^{1a}, $^{2}JH^{1a}$-H^{1b} = 16.75); 4.60 (d, 1H, H^{1b}, $^{2}JH^{1b}$-H^{1a} = 16.75); 5.30 (s, 1H, H^{4}); 6.50-7.65 (m, 21 H, Aromatic H); 7.95 (dd, 1H, H^{5}, $^{4}JH^{5}$-H^{7} = 1.10, $^{3}JH^{5}$-H^{6} = 7.75). ^{13}C NMR: 50.4 (N$\underline{C}H_2$); 55.3 (O-$\underline{C}H_3$); 63.4 (-$\underline{C}$H-); 91.0 (spiro-C); 125.6; 129.5; 130.4; 134.0; 140.8; 144.1; 146.8; 147.6; 159.4 (quat. C sp^2); 113.7;

115.6; 120.8; 121.8; 121.9; 126.0; 128.1; 128.2; 128.4; 128.9; 132.1; 134.8 (tert. C sp^2); 194.9 (C=O). MS (EI, 70 eV): $[M]^{+\cdot}$ = m/z : M = 569 [$C_{36}H_{28}$ Cl N_3O_2] (27%); 77 (100%). Anal. Calcd. For $C_{36}H_{28}ClN_3O_2$: C, 75.85; H, 4.95; N, 7.37. Found: C, 74.25; H, 4.13; N, 7.56.

2,2'-Diphenyl-4'(4-methoxy-phenyl)-5'(4-nitro-phenyl)-1,2,2',4'-tetrahydro-4*H*-spiro [isoquinoline-3,3'-pyrazol]-4-one (22). White powder. Yield = 50%. M.p. = 188 °C. IR ν (C=O) = 1694 cm^{-1}. ^1H NMR: 3.70 (s, 3H, OCH_3); 4.50 (d, 1H, H^{1a}, $^2JH^{1a}$-H^{1b} = 16.65); 4.55 (d, 1H, H^{1b}, $^2JH^{1b}$-H^{1a} = 16.65); 5.31 (s, 1H, H^4); 6.45-7.65 (m, 21H, Aromatic H); 8.05 (dd, 1H, H^5, $^4JH^5$-H^7 = 1.25, $^3JH^5$-H^6 = 7.80). ^{13}C NMR: 50.4 (N<u>C</u>H_2); 55.3 (O-<u>C</u>H_3); 62.9 (<u>C</u>H); 92.0 (spiro-C); 125.0; 138.3; 139.8; 140.7; 143.5; 145.4; 146.8; 147.4; 159.6 (quat. C sp^2); 113.8; 113.9; 115.9; 121.6; 121.8; 122.4; 123.6; 126.0; 127.0; 128.2; 128.3; 129.1; 129.2; 132.0; 134.6 (tert. C sp^2); 194.5 (<u>C</u>=O). MS (EI, 70 eV): $[M]^{+\cdot}$ = m/z : M = 580 [$C_{36}H_{28}N_4O_4$] (15%); 77 (100%). Anal. Calcd. For $C_{36}H_{28}N_4O_4$: C, 74.47; H, 4.86; N, 9.65. Found: C, 73.89; H, 5.17; N, 9.53.

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References

1. Howe, R. K.; Shelton, B. R. *J. Org. Chem*. **1990**, 4603.
2. Smietana, M.; Gouverneur, V.; Mioskowski, C. *Tetrahedron Lett.* **1999**, *40*, 1291.
3. Bennani, B.; Kerbal, A.; Ben Larbi, N.; Ben Hadda, T., OMPIC, *Casablanca-Moroccan Patent* 2004, No. 2769. http://www.taacf.org/publications.htm
4. Bennani, B.; Kerbal, A.; Ben Larbi, N.; Ben Hadda, T., OMPIC, *Casablanca Moroccan Patent* 2004, No. 2771. http://www.taacf.org/publications.htm
5. Benchat, N.; El Bali, B.; Abouricha, S.; Moueqqit, M.; Mimouni, M.; Ben Hadda, T. *Chem. Prep. Server, Med./Pharm. Chem.* **2003**.
6. Anaflous, A.; Benchat, N.; Mimouni, M.; Abouricha, S.; Ben Hadda, T.; El Bali, B.; Hakkou, A.; Hacht, B. *Lett. Drug Des. Disc*. **2004**, *1*, 35.
7. Tshiamala, K.; Kerbal, A.; Vebrel, J.; Laude, B. *J. Chem. Research (S)* **1988**, *3*, 106.
8. Kerbal, A.; Vebrel, J.; Cerutti, E.; Laude, B. *J. Chem. Soc., Chem. Commun*. **1989**, 632.
9. Filali Baba, B.; Kerbal, A.; Bitit, N.; El Bali, B.; Escudie, J.; Ranaivonjatovo, H.; Bolte, M. *Acta. Cryst.* **2000**, *C56*, 205.
10. Kerbal, A.; Tshiamala, K.; Vebrel, J.; Laude, B. *Bull. Soc. Chim. Belg.* **1988**, *97*, 149.
11. Kerbal, A.; Tshiamala, K.; Verbrel, J.; Laude, B.; Mercier, M. F. *Bull. Soc. Chim. Belg.* **1991**, *2*, 159.
12. Theobala, F.; Rodier, N.; Kerbal, A.; Tshiamala, K.; Laude, B. *Acta. Cryst.* **1990**, *C46*, 1312.

13. Kerbal, A.; Vebrel, J. ; Roche, M. ; Laude, B. *Tetrahedron Lett.* **1990**, *31*, 4145.
14. Badri, R. ; Kerbal, A.; El Bali, B.; Escudie, B.; Ranaivonjatovo, H.; Bolte, M. *Acta. Cryst.* **1999**, C55.
15. Pivnenko, N.S.; Orlov, V.D.; Nodel'Man, O.A.; Mikhed'Kina, E.I.; Lavrushin, V.F. *Zh. Org. Khim.* **1980**, *16*, 1945.
16. Bennani, B.; Filali Baba, B.; EL-Fazazi, A.; Al Houari, G.; El Bali, B.; Bolte, M.; Ben Larbi, N.; Kerbal, A. *J. Mar. Chim. Hétéroc*. **2003**, *1*, 1.
17. Akkurt, M.; Yıldırım, S.O.; Badri, R. Kerbal, A.; Ben Hadda, T.; Büyükgüngör, O.; *Acta. Cryst.* **2006**, E62, 5022.
18. Shanmugasundaram, M.; Babu, S.A.; Raghunathan, R.; Malar, E. J. P., *Heteroatom Chemistry* **1999**, *10*, 331.
19. Raghunathan, R.; Shanmugasundaram, M.; Bhanumathi, S.; Malar, E.J.P., *Heteroatom Chemistry* **1998**, *9*, 327.
20. Collins, L. ; Franzblau, S.G. *Antimicrob. Agents Chemother.* **1997**, *41*, 1004.

