Looking for a new antitubercular pharmacophore site: synthesis and bioactivity of spiroheterocycles 2,3',4'-tri-subsituted-1,2-dihydro-4*H*,4'*H*-spiro[isoquinoline-3,5'-isoxazol]-4-ones

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Abstract
Synthesis of a series of fourteen novel 2,3',4'-tri-subsituted-1,2-dihydro-4*H*,4'*H*-spiro[isoquinoline-3,5'-isoxazol]-4-ones was accomplished in good yield by regio and stereoselective 1,3-dipolar cycloaddition of *p*-R^2-benzadoxime **4-8** with dipolarophiles (3*Z*)-3-[(4-substituted-phenyl)methylidene]-2-phenyl-2,3-dihydroisoquinolin-4(1*H*)-ones **1-3**. The structure of the isolated products **9-23** 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 also evaluated.

Keywords: Spiroisoxazolines, 1,3-dipolar cycloaddition, antitubercular activity

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 activity of some spiroisoxazolines derivatives.[3,4] With other kind of drugs we had also performed antimicrobial screening of 3-nitrozo-imidazo[1,2-a]pyrimidines. 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).

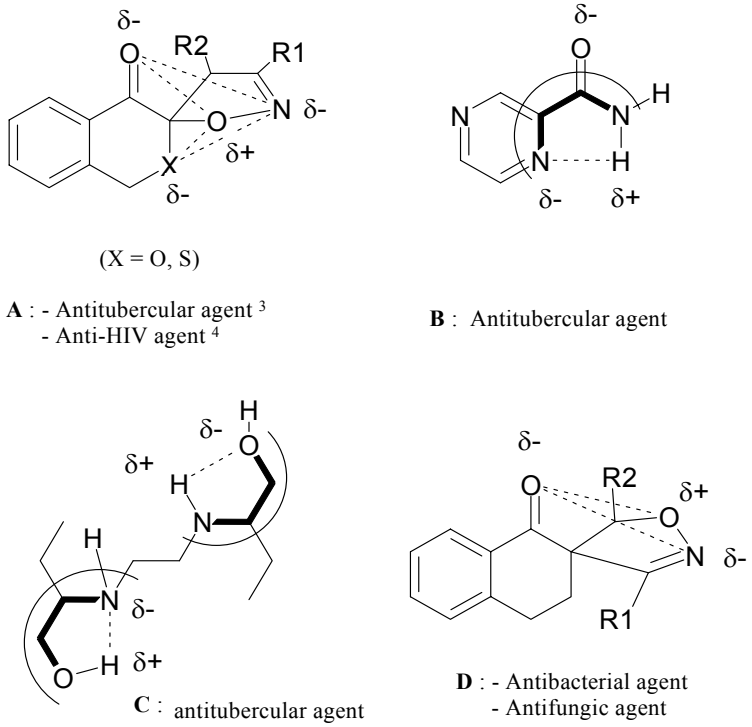


Figure 1. Structures of clinical antitubercular agents (**B**,**C**) and spiroheterocycles (**A,D**).

In our earlier studies of the 1,3-dipolar cycloaddition field, we had investigated the reaction of diarylnitrilimines with endocyclic dipolarophiles such as dihydroquinoline,[7] indene [8] or 3-methoxycarbonyl-4*H*-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(1*H*)-isoquinolone with exocyclic dipolarophile groups.[14]

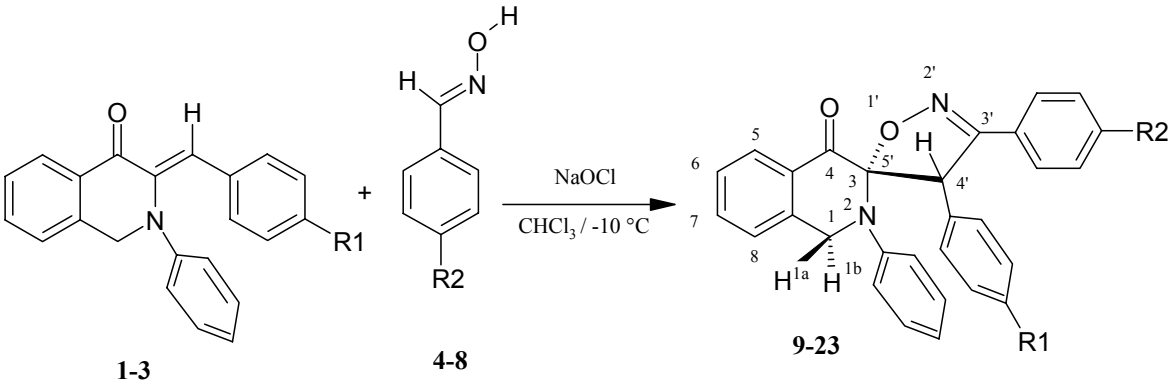
In our ongoing program, we report an efficient and short synthesis of new spiroheterocycles in good yields from 1,3-dipolar cycloaddition of dipolarophiles with the appropriate *p*-R^2-benzadoxime. The pharmacological investigations as antitubercular activity are also discussed.

Results and Discussion

Chemistry

The precursors or dipolarophiles (**1-3**); (3*Z*)-3-[(4-substituted-phenyl)methylidene]-2-phenyl-2,3-dihydroisoquinolin-4(1*H*)-ones 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 *p*-R^2-benzadoxime **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 atom of the NOH group of the compounds **4–8** becomes bonded to the most substituted olefinic carbon of the dipolarophile **1-3**. Modest to good yields of the spiroheterocycles **9–23** were obtained (40-80%) 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) = (H,NO_2)
14: (R1,R2) = (CH_3,H)
15: (R1,R2) = (CH_3,CH_3)
16: (R1,R2) = (CH_3,OCH_3)
17: (R1,R2) = (CH_3,Cl)
18: (R1,R2) = (CH_3,NO_2)
19: (R1,R2) = (OCH_3,H)
20: (R1,R2) = (OCH_3,CH_3)
21: (R1,R2) = (OCH_3,OCH_3)
22: (R1,R2) = (OCH_3,Cl)
23: (R1,R2) = (OCH_3,NO_2)

Scheme 1. Synthesis of spiroheterocycles **9-23**.

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.[13,16,17] The selective NMR data are represented in Tables 1 and 2.
The crystallographic structure of compound **18** has been previously deteremined.[18] The ^{1}H NMR and crystallographic data of compound **18**, for example, show that the 1,3-dipolar cycloaddition occurs with inverted regiochemistry to that obtained with chromanone.[18] The same observation have been noted elsewhere for similar other spiroheterocycles.[19]

Table 1. Selected IR and ^{1}H NMR data of compounds **9-23**

Comp.	(R1,R2)	IR (υ C=O)	δR1 δR2	δH^{1a} δH^{1b}	$\delta H^{4'}$	δH (Aroma)	δH^{5}
			-	4.05 (d, 1H)		6.70-7.75	8.25 (dd, 1H)
9	(H,H)	1703	-	5.05 (d, 1H)	6.15 (s, 1H)	(m, 18H)	^{4}J = 1.25
				^{2}J = 17.35			^{3}J = 7.13
			-	4.00 (d, 1H)		6.55-7.55	8.15 (dd, 1H)
10	(H,CH_3)	1686	2.25 (s, 3H)	5.00 (d, 1H)	6.05 (s, 1H)	(m, 17H)	^{4}J = 1.25
				^{2}J = 17.20			^{3}J = 7.70
			-	4.00 (d, 1H)		6.70-7.50	8.20 (dd, 1H)
11	(H,OCH_3)	1693	3.70 (s, 3H)	4.95 (d, 1H)	6.00 (s, 1H)	(m, 17H)	^{4}J = 1.30
				^{2}J = 17.60			^{3}J = 7.60
			-	4.00 (d, 1H)		6.50-7.55	8.20 (dd, 1H)
12	(H,Cl)	1685	-	4.95 (d, 1H)	6.05 (s, 1H)	(m, 17H)	^{4}J = 1.35
				^{2}J = 17.20			^{3}J = 7.80
			-	3.85 (d, 1H)		6.50-7.60	8.15 (dd, 1H)
13	(H,NO_2)	1706	-	4.60 (d, 1H)	6.00 (s, 1H)	(m, 17H)	^{4}J = 1.65
				^{2}J = 17.25			^{3}J = 7.80
			2.20 (s, 3H)	4.75 (d, 1H)		6.90-7.50	7.60 (dd, 1H)
14	(CH_3,H)	1694	-	4.80 (d, 1H)	6.00 (s, 1H)	(m, 17H)	^{4}J = 1.20
				^{2}J = 17.00			^{3}J = 7.35
			2.20 (s, 3H)	4.10 (d, 1H)		6.45-7.55	8.20 (dd, 1H)
15	(CH_3,CH_3)	1703	2.30 (s, 3H)	5.05 (d, 1H)	6.05 (s, 1H)	(m, 16H)	^{4}J = 1.25
				^{2}J = 17.25			^{3}J = 7.80
			2.15 (s, 3H)	4.10 (d, 1H)		6.50-7.55	8.20 (dd, 1H)
16	(CH_3,OCH_3)	1704	3.75 (s, 3H)	5.05 (d, 1H)	6.00 (s, 1H)	(m, 16H)	^{4}J = 1.15
				^{2}J = 17.30			^{3}J = 7.70
			2.20 (s, 3H)	4.10 (d, 1H)		6.40-7.55	8.20 (dd, 1H)
17	(CH_3,Cl)	1710	-	5.05 (d, 1H)	6.05 (s, 1H)	(m, 16H)	^{4}J = 1.45
				^{2}J = 17.25			^{3}J = 7.75
			2.15 (s, 3H)	4.00 (d, 1H)		6.35-7.75	8.15 (dd, 1H)
18	(CH_3,NO_2)	1708	-	4.95 (d, 1H)	6.10 (s, 1H)	(m, 16H)	^{4}J = 1.45
				^{2}J = 17.35			^{3}J = 7.80
			3.85 (s, 3H)	4.10 (d, 1H)		6.50-7.65	8.05 (dd, 1H)
19	(OCH_3,H)	1697	-	5.05 (d, 1H)	6.00 (s, 1H)	(m, 17H)	^{4}J = 1.40
				^{2}J = 17.30			^{3}J = 7.25
			3.65 (s, 3H)	4.10 (d, 1H)		6.35-7.55	8.20 (dd, 1H)
20	(OCH_3,CH_3)	1691	2.30 (s, 3H)	5.05 (d, 1H)	6.05 (s, 1H)	(m, 16H)	^{4}J = 1.40
				^{2}J = 17.30			^{3}J = 7.70
			3.65 (s, 3H)	4.10 (d, 1H)		6.35-7.55	8.15 (dd, 1H)

21	(OCH_3,OCH_3)	1698	3.75 (s, 3H)	5.05 (d, 1H)	6.00 (s, 1H)	(m, 16H)	^4J = 1.35
				^2J = 17.30			^3J = 7.60
			3.65 (s, 3H)	4.10 (d, 1H)		6.35-7.60	8.20 (dd, 1H)
22	(OCH_3,Cl)	1695	-	5.05 (d, 1H)	6.05 (s, 1H)	(m, 16H)	^4J = 1.45
				^2J = 17.30			^3J = 7.80
			3.65 (s, 3H)	4.05 (d, 1H)		6.40-7.75	8.15 (dd, 1H)
23	(OCH_3,NO_2)	1705	-	4.95 (d, 1H)	6.10 (s, 1H)	(m, 16H)	^4J = 1.30
				^2J = 17.35			^3J = 7.65

Table 2. Selected ^{13}C NMR data of compounds **9-23**

Comp.	(R1,R2)	δC (R1)	δC (R2)	δC^1 ($N\underline{C}H_2$-)	$\delta C^{3,5'}$ (spiro-C)	δC^4 (-$\underline{C}$=O)	$\delta C^{4'}$ (-$\underline{C}$H-)
9	(H,H)	-	-	55.40	102.40	187.25	53.70
10	(H,CH_3)	-	21.50	55.34	102.40	187.65	54.50
11	(H,OCH_3)	-	54.70	55.35	102.20	187.85	55.30
12	(H,Cl)	-	-	55.35	103.00	187.35	54.30
13	(H,NO_2)	-	-	55.30	102.80	187.80	54.00
14	(CH_3,H)	21.15	-	52.15	103.95	187.35	57.40
15	(CH_3,CH_3)	21.10	21.50	55.35	102.40	187.80	54.35
16	(CH_3,OCH_3)	21.10	54.50	55.35	102.15	187.95	55.30
17	(CH_3,Cl)	21.10	-	55.35	102.95	187.50	54.10
18	(CH_3,NO_2)	21.10	-	55.40	103.95	187.00	53.70
19	(OCH_3,H)	53.80	-	55.35	102.65	187.55	55.20
20	(OCH_3,CH_3)	54.00	21.45	55.35	102.15	187.85	55.17
21	(OCH_3,OCH_3)	54.15	55.20	55.35	101.90	187.00	55.30
22	(OCH_3,Cl)	53.90	-	55.35	102.35	187.75	55.20
23	(OCH_3,NO_2)	53.40	-	55.40	103.65	187.10	55.20

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 **9-23** were inferior to that of Isoniazid against *M. tuberculosis* H_{37}Rv. Further, the compounds **9-23** had either little or no activity (17-64% inhibition).

Table 3. Range of % inhibition values (MIC>6.25 μg/mL) of compounds **9-23** against *Mycobacterium tuberculosis* $H_{37}Rv$ strains

Compd ID (TAACF ID)	(R1, R2)	M.W. (g/mole)	MIC (μg/mL)	% Inhibition (Alamar test)	Activity
9 (299937)	(H, H)	430	>6.25	42	negative
10 (299938)	(H, CH_3)	444.5	>6.25	19	negative
11 (299939)	(H, OCH_3)	460.5	>6.25	44	negative
12 (299940)	(H, Cl)	468.9	>6.25	48	negative
13 (299941)	(H, NO_2)	477.5	>6.25	24	negative
14 (299942)	(CH_3, H)	444.5	>6.25	20	negative
15 (299943)	(CH_3, CH_3)	458.5	>6.25	19	negative
16 (299944)	(CH_3, OCH_3)	474.5	>6.25	42	negative
17 (299945)	(CH_3, Cl)	478.9	>6.25	33	negative
18 (299946)	(CH_3, NO_2)	489.5	>6.25	64	negative
19 (299951)	(OCH_3, H)	460.5	>6.25	60	negative
20 (299948)	(OCH_3, CH_3)	474.5	>6.25	49	negative
21 (299949)	(OCH_3, OCH_3)	490.5	>6.25	34	negative
22 (299950)	(OCH_3, Cl)	494.9	>6.25	53	negative
23 (299947)	(OCH_3, NO_2)	505.5	>6.25	44	negative

The replacement of the nitro substituent (R2) by a methyl or a methoxy group in phenyl ring of C(3') position, as in **10** and **11**, respectively, did not cause any apparent change of activity (% inhibition <90%). Curiously, 4'-*p*-methylphenyl-, and 3'-*p*-nitrophenyl-substitution as in **18** [(R1,R2) = (CH_3,NO_2)], abolished any activity indicating that not only specific electronic but

also steric requirements are needed for activity (% Inhibition = 64%). This suggests that a bulky group or disubstitution on the N(2) and C(4') positions are not favourable for antimycobacterial activity.

Structure activity relationship

The antimycobacterial activity data in Table 3 clearly show that the compounds **9-23** having an aryl substituent in N(2) position of the isoquinoline ring exhibited low activity against *M. tuberculosi*s (Scheme 1).

All compounds **9-23** had an almost similar range of MIC values; they differ only by % inhibition, in spite of having two different aryls at C(3) and C(4), as shown in Figure 2.

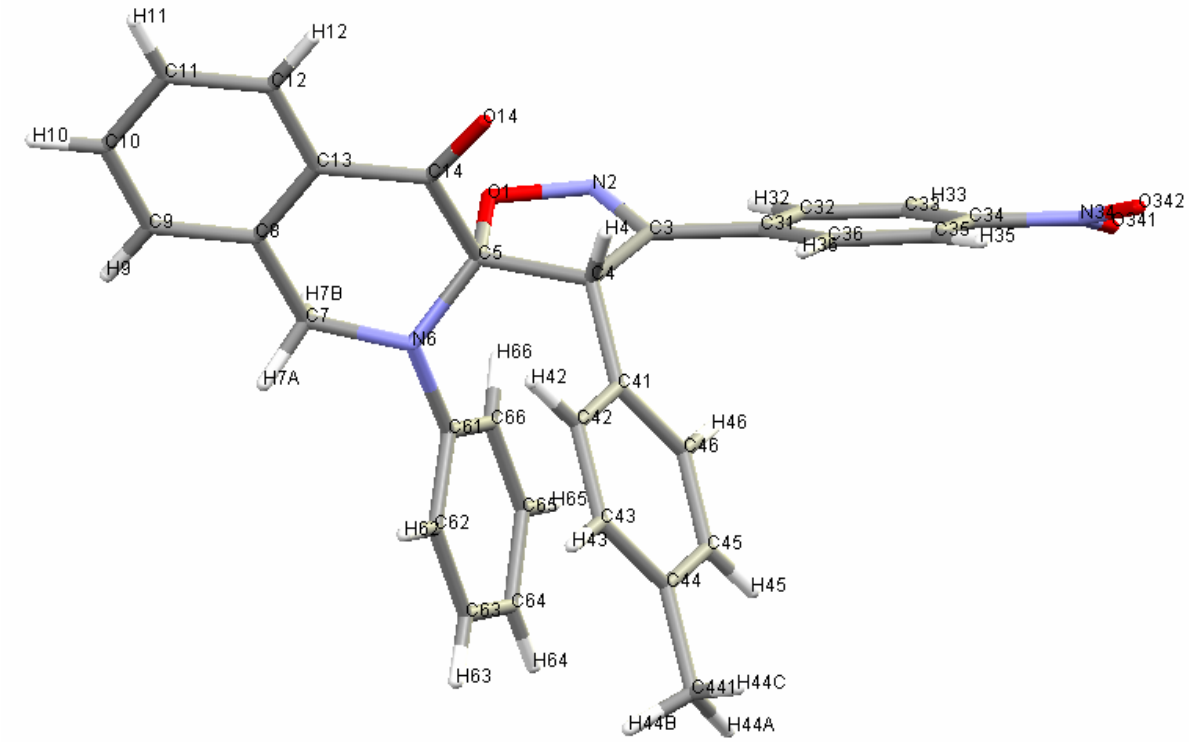


Figure 2. A view of compound (**18**) with the atom-numbering scheme.[21] Distance: O(1) --- O(14) = 3.03 Å; O(1) --- N(6) = 2.43 Å; O(14) --- N(2) = 3.62 Å. Angle: (O^{1},C^{5},N^{6}) = 112°; torsion angle: (O^{14}-C^{14}-C^{5}-O^{1}) = 89°; (O^{14}-C^{14}-C^{3}-N^{2}) = 115°; (C^{4},C^{3},C^{31},C^{36}) = 20°; (N^{2},C^{3},C^{31},C^{32}) = 23°; (O^{341},N^{34},C^{34},C^{33}) = 14°.

It contains an almost planar isoxazoline ring. The C atoms of the isoquinoline moiety lie in a common plane, with the N atom deviating by 0.474 (2) Å from that plane.

Conclusions

In this paper we report efficient and short synthesis of the spiroheterocycles **9-23** in good yields from 1,3-dipolar cycloaddition of dipolarophiles *N*-phenyl-3-(*p*-R^1-phenyl)-4-isoquinolone (**1-3**) with the appropriate *p*-R^2-benzadoxime (**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 literature with chromanone. This is likely due to the presence of the neighboring N-aryl group. Due to the presence of rigid O=$C^{5'}$-$O^{1'}$-$N^{2'}$ pharmacophore, anti-HIV screening studies are in progress at National Cancer Institute (NCI/ USA) in order to elucidate the structure/activity relationships.

Experimental Section

General Procedures. Melting points are measured on banc KOFLER without corrections. NMR spectra (^{1}H, ^{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 9-23

A mixture of a dipolarophile (**1-3**; **6.8** mmol) and *p*-R^2-benzadoxime (**4-8**; 7 mmol) was stirred in chloroform (20 ml) at cold temperature (-10°C). To this mixture was added 15 ml of sodium hypochlorite (degree = 12 g $NaClO_4$/100 g solution) drop ways over 20 min. The temperature was controlled and has not to excess (-5°C). After complete addition of NaOCl, the mixture was stirred 4-8 h at room temperature, until TLC indicated complete disappearance of reactants. The compounds were extracted by chloroform, washed by water and dried on sodium sulphate. The compounds **9-23** were obtained after evaporation and crystallisation in ethanol.

2,3',4'-Tri-phenyl-1,2-dihydro-4*H*,4'*H*-spiro[isoquinoline-3,5'-isoxazol]-4-one (9). This compound was obtained as white-yellow powder. Yield 70%. M.p. 174-176 °C. IR, ν (C=O):1703 cm^{-1}. ^1H NMR: 4.05 (d, 1H, H^{1a}, $^2JH^{1a}$-H^{1b} = 17.35); 5.05 (d, 1H, H^{1b}, $^2JH^{1b}$-H^{1a} = 17.35); 6.15 (s, 1H, $H^{4'}$); 6.70-7.75 (m, 18H, Aromatic H); 8.25 (dd, 1H, H^5, ^4J = 1.25, ^3J = 7.13). ^{13}C NMR: 55.40 (N-$\underline{C}H_2$-); 53.70 (-$\underline{C}^{4'}$H-); 120.40 (spiro-C); 126.10; 128.65; 132.30; 140.05; 140.45; 148.65; 159.60 (quat. C sp^2); 125.40; 125.65; 126.00; 126.35; 126.85; 127.00; 128.15; 128.85; 128;95; 130.15; 134.00; (tert. C sp^2). MS (EI, 70 eV): $[M]^{+\cdot}$ m/z: M (430.5) [$C_{29}H_{22}N_2O_2$] (15%); 118 (100%). Anal. Calcd. For $C_{29}H_{22}N_2O_2$: C, 80.91; H, 5.15; N, 6.51. Found: C, 81.02; H, 4.98; N, 6.43.

2-Phenyl-4'-phenyl-3'-(4-methylphenyl)-1,2-dihydro-4*H*,4'*H*-spiro[isoquinoline-3,5'-isoxazol]-4-one (10). This compound was obtained as white-yellow powder. Yield 72%. M.p. 247-251 °C. IR, ν (C=O): 1686 cm^{-1}. ^1H NMR: 2.25 (s, 3H, CH_3); 4.00 (d, 1H, H^{1a}, $^2JH^{1a}$-H^{1b} = 17.20); 5.00 (d, 1H, H^{1b}, $^2JH^{1b}$-H^{1a} = 17.20); 6.05 (s, 1H, $H^{4'}$); 6.55-7.55 (m, 17 H, Aromatic H); 8.15 (dd, 1H, $H^{5'}$, ^4J = 1.25, ^3J = 7.70). ^{13}C NMR: 21.50 ($\underline{C}H_3$); 55.35 (N-$\underline{C}H_2$-); 54.50 (-$\underline{C}$H-); 102.40 (spiro-C); 126.25; 128.85; 132.75; 140.20; 140.90; 148.70; 161.00 (quat. C sp^2); 125.20; 125.75; 126.25; 126.45; 127.40; 127.50; 128.50; 128.75; 129.25; 130.20; 134.25; (tert. C sp^2); 187.65 (C=O). MS (EI, 70 eV): $[M]^{+\cdot}$ m/z: M (444.5) [$C_{30}H_{24}N_2O_2$] (15%); 444 (10%); 207 (100%). Anal. Calcd. For $C_{30}H_{24}N_2O_2$: C, 81.06; H, 5.44; N, 6.30. Found: C, 81.15; H, 5.48; N, 6.17.

2-Phenyl-4'-phenyl-3'-(4-methoxyphenyl)-1,2-dihydro-4*H*,4'*H*-spiro[isoquinoline-3,5'-isoxazol]-4-one (11). This compound was obtained as white-yellow powder. Yield 57-59%. M.p. 228-231°C. IR, ν (C=O): 1693 cm^{-1}. ^1H NMR: 3.70 (s, 3 H, CH_3); 4.00 (d, 1 H, H^{1a}, $^2JH^{1a}$-H^{1b} = 17.60); 4.95 (d, 1H, H^{1b}, $^2JH^{1b}$-H^{1a} = 17.60); 6.00 (s, 1H, $H^{4'}$); 6.70-7.50 (m, 17 H, Aromatic H); 8.20 (dd, 1H, H^5, ^4J = 1.30, ^3J = 7.75). ^{13}C NMR: 55.30 (-$\underline{C}$H-); 55.35 (N$\underline{C}H_2$-); 102.20 (spiro-C); 1241.50; 128.90; 132.85; 140.90; 148.70; 160.65; 160.95 (quat. C sp^2); 113.95; 125.10; 125.75; 126.35; 126.50; 127.40; 127.50; 128.45; 128.75; 129.10; 130.25; 134.20 (tert. C sp^2); 187.85 (C=O). MS (EI, 70 eV): $[M]^{+\cdot}$ m/z: M (460.5) [$C_{30}H_{24}N_2O_3$] (8%); 223 (100%). Anal. Calcd. For $C_{30}H_{24}N_2O_3$: C, 78.24; H, 5.25; N, 6.08. Found: C, 79.05; H, 5.34; N, 6.12.

2-Phenyl-4'-phenyl-3'-(4-chlorophenyl)-1,2-dihydro-4*H*,4'*H*-spiro[isoquinoline-3,5'-isoxazol]-4-one (12). This compound was obtained as white-yellow powder. Yield 71%. M.p. 242-245°C. IR, ν (C=O): 1685 cm^{-1}. ^1H NMR: 4.00 (d, 1H, H^{1a}, $^2JH^{1a}$-H^{1b} = 17.20); 4.95 (d, 1H, H^{1b}, $^2JH^{1b}$-H^{1a} = 17.20); 6.05 (s, 1H, $H^{4'}$); 6.50-7.55 (m, 17H, Aromatic H); 8.20 (dd, 1H, H^5, ^4J = 1.35, ^3J = 7.60). ^{13}C NMR: 54.30 (-CH-); 55.35 (N$\underline{C}H_2$-); 103.00 (spiro-C); 132.35; 135.95; 140.90; 148.55; 160.05; (quat. C sp^2); 125.40; 125.75; 126.55; 126.70; 127.55; 128.55; 128.80; 130.15; 134.35 (tert. C sp^2); 187.35 (C=O). MS (EI, 70 eV): $[M]^{+\cdot}$ m/z: M (464) [$C_{29}H_{21}ClN_2O_2$] (15%); 466 (8%); 277 (100%). Anal. Calcd. For $C_{29}H_{21}ClN_2O_2$: C, 74.91; H, 4.55; N, 6.03. Found: C, 74.65; H, 4.48; N, 6.13.

2-Phenyl-4'-phenyl-3'-(4-nitrophenyl)-1,2-dihydro-4*H*,4'*H*-spiro[isoquinoline-3,5'-isoxazol]-4-one (13). This compound was obtained as white-yellow powder. Yield 50%. M.p. 194-197 °C. IR, ν (C=O): 1696 cm^{-1}. ^1H NMR: 3.85 (d, 1H, H^{1a}, $^2JH^{1a}$-H^{1b} = 17.25); 4.60 (d, 1H, H^{1b},

$^{2}JH^{1b}$-H^{1a} = 17.25); 6.00 (s, 1H, $H^{4'}$); 6.50-7.60 (m, 17H, Aromatic H); 8.15 (dd, 1H, H^{5}, ^{4}J = 1.25, ^{3}J = 7.80). ^{13}C NMR: 54.00 (-CH-); 55.30 (N<u>C</u>H_2-); 102.80 (spiro-C); 132.55; 136.00; 140.95; 148.50; 161.00 (quat. C sp^2); 125.40; 125.80; 126.55; 126.85; 127.55; 128.65; 128.85; 130.20; 134.40 (tert. C sp^2); 187.80 (C=O). MS (EI, 70 eV): $[M]^{+\cdot}$ m/z: M (475) [$C_{29}H_{21}N_3O_4$] (25%); 90 (100%). Anal. Calcd. For $C_{29}H_{21}N_3O_4$: C, 73.25; H, 4.45; N, 8.84. Found: C, 73.02; H, 4.39; N, 8.57.

2,3'-Di-phenyl-4'-(4-methylphenyl)-1,2-dihydro-4*H*,4'*H*-spiro[isoquinoline-3,5'-isoxazol]-4-one (14). This compound was obtained as white-yellow powder. Yield 40%. M.p. 260-264 °C. IR, ν (C=O): 1695 cm^{-1}. ^{1}H NMR: 2.20 (s, 3H, CH_3); 4.75 (d, 1H, H^{1a}, $^{2}JH^{1a}$-H^{1b} = 17.00); 4.80 (d, 1H, H^{1b}, $^{2}JH^{1b}$-H^{1a} = 17.00); 6.00 (s, 1H, $H^{4'}$); 6.90-7.50 (m, 16 H, Aromatic H); 7.60 (dd, 1H, H^{5}, ^{4}J = 1.20, ^{3}J = 7.35). ^{13}C NMR: 21.15 (<u>C</u>H_3); 52.15 (N<u>C</u>H_2-); 57.40 (-CH-);103.95 (spiro-C);108.15; 130.35; 137.30; 139.95; 146.45; 158.45 (quat. C sp^2); 125.30; 126.40; 127.20; 127.35; 127.50; 127.75; 128.40; 129.05; 129.30; 129.50; 129.80; 133.55 (tert. C sp^2); 192.35 (C=O). MS (EI, 70 eV): $[M]^{+\cdot}$ m/z: M (444) [$C_{30}H_{24}N_2O_2$] (15%); 207 (100%). Anal. Calcd. For $C_{30}H_{24}N_2O_2$: C, 81.06; H, 5.44; N, 6.30. Found: C, 80.93; H, 5.52; N, 6.14.

2-Phenyl-3',4'-di-phenyl-1,2-dihydro-4*H*,4'*H*-spiro[isoquinoline-3,5'-isoxazol]-4-one (15). This compound was obtained as white-yellow powder. Yield 70%. M.p. 202-205 °C. IR, ν (C=O): 1703 cm^{-1}. ^{1}H NMR: 2.20 (s, 3H, CH_3); 2.30 (s, 3H, CH_3); 4.10 (d, 1H, H^{1a}, $^{2}JH^{1a}$-H^{1b} = 17.25); 5.05 (d, 1H, H^{1b}, $^{2}JH^{1b}$-H^{1a} = 17.25); 6.05 (s, 1H, $H^{4'}$); 6.45-7.55 (m, 16H, Aromat H); 8.20 (dd, 1H, $H^{5'}$, ^{4}J = 1.25, ^{3}J = 7.80). ^{13}C NMR: 21.10 and 21.50 (2 <u>C</u>H_3); 54.35 (-<u>C</u>H-); 55.35 (N<u>C</u>H_2-); 102.40 (spiro-C); 126.35; 128.90; 129.65; 136.05; 140.10; 140.95; 148.80; 161.10 (quat. C sp^2); 125.15; 125.70; 126.50; 127.50; 128.10; 128.45; 128.60; 129.20; 130.05; 134.20 (tert. C sp^2); 187.80 (C=O). MS (EI, 70 eV): $[M]^{+\cdot}$ m/z : M (458) [$C_{31}H_{26}N_2O_3$] (25%); 90 (100%). Anal. Calcd. For $C_{31}H_{26}N_2O_3$: C, 81.22; H, 5.67; N, 6.11. Found: C, 81.06; H, 5.66; N, 6.00.

2-Phenyl-4'-(4-methylphenyl)-3'-(4-methoxyphenyl)-1,2-dihydro-4*H*,4'*H*-spiro[isoquinoline-3,5'-isoxazol]-4-one (16). This compound was obtained as white-yellow powder. Yield 66%. M.p. 192-195 °C. IR, ν (C=O): 1704 cm^{-1}. ^{1}H NMR: 2.15 (s, 3H, CH_3); 3.75 (s, 3H, CH_3); 4.10 (d, 1H, H^{1a}, $^{2}JH^{1a}$-H^{1b} = 17.30); 5.05 (d, 1H, H^{1b}, $^{2}JH^{1b}$-H^{1a} = 17.30); 6.00 (s, 1H, $H^{4'}$); 6.50-7.55 (m, 16H, Aromatic H); 8.20 (dd, 1H, H^{5}, ^{4}J = 1.15, ^{3}J = 7.70). ^{13}C NMR: 21.10 (<u>C</u>H_3); 54.50 (-O<u>C</u>H_3); 55.30 (-<u>C</u>H-); 55.35 (N<u>C</u>H_2-);102.15 (spiro-C); 121.60; 128.95; 129.75; 136.10; 140.90; 148.80; 160.75; 160.90 (quat. C sp^2); 113.95; 125.05; 125.70; 126.35; 127.50; 128.15; 128.40; 128.60; 129.10; 130.05; 134.15 (tert. C sp^2); 187.95 (C=O). MS (EI, 70 eV): $[M]^{+\cdot}$ m/z: M (474) [$C_{31}H_{26}N_2O_3$] (18%); 90 (100%). Anal. Calcd. For $C_{31}H_{26}N_2O_3$: C, 78.46; H, 5.52; N, 5.90. Found: C, 77.96; H, 5.43; N, 6.01.

2-Phenyl-4'-(4-methylphenyl)-3'-(4-chlorophenyl)-1,2-dihydro-4*H*,4'*H*-spiro[isoquino-line-3,5'-isoxazol]-4-one (17). This compound was obtained as white-yellow powder. Yield 82%. M.p. 223-225 °C. IR, ν (C=O): 1710 cm^{-1}. ^{1}H NMR: 2.20 (s, 3H, CH_3); 4.10 (d, 1H, H^{1a}, $^{2}JH^{1b}$-H^{1a} = 17.25); 5.05 (d, 1H, H^{1b}, $^{2}JH^{1b}$-H^{1a} = 17.25); 6.05 (s, 1H, $H^{4'}$); 6.40-7.55 (m, 16H, Aromat H); 8.20 (dd, 1H, H^{5}, ^{4}J = 1.45, ^{3}J = 7.75). ^{13}C NMR: 21.10 (<u>C</u>H_3); 54.10 (-<u>C</u>H-); 55.35 (N<u>C</u>H_2-);

102.95 (spiro-C); 127.75;129.25; 135.90; 136.35; 140.90; 148.65; 160.15 (quat. C sp^2); 125.35; 125.75; 126.60; 127.60; 128.30; 128.50; 128.65; 128.80; 130.00; 134.30 (tert. C sp^2); 187.50 (C=O). MS (EI, 70 eV): $[M]^{+\cdot}$ m/z: M (478) [$C_{30}H_{23}ClN_2O_2$] (15%); 480 (8%); 90 (100%). Anal. Calcd. For $C_{30}H_{23}ClN_2O_2$: C, 75.23; H, 4.84; N, 5.85. Found: C, 74.96; H, 4.76; N, 5.61.

2-Phenyl-4'-(4-methylphenyl)-3'-(4-nitrophenyl)-1,2-dihydro-4*H*,4'*H*-spiro[isoquinoline-3,5'-isoxazol]-4-one (18). This compound was obtained as white-yellow powder. Yield 72%. M.p. 242-244 °C. IR, ν (C=O): 1697 cm^{-1}. ^{1}H NMR: 2.15 (s, 3H, CH_3); 4.00 (d, 1H, H^{1a}, $^2JH^{1b}$-H^{1a} = 17.35); 4.95 (d, 1H, H^{1b}, $^2JH^{1b}$-H^{1a} = 17.35); 6.10 (s, 1H, $H^{4'}$); 6.35-7.75 (m, 16H, Aromat H); 8.15 (dd, 1H, H^5, ^4J = 1.45, ^3J = 7.80). ^{13}C NMR: 21.10 ($\underline{C}H_3$); 53.70 (-$\underline{C}$H-); 55.40 (N$\underline{C}H_2$-); 103.95 (spiro-C); 135.45; 136.65; 140.85; 148.25; 148.85; 159.35 (quat. C sp^2); 123.75; 125.60; 125.75; 126.75; 127.70; 128.25; 128.45; 128.60; 128.75; 129.90; 134.45 (tert. C sp^2); 187.00 (C=O). MS (EI, 70 eV): $[M]^{+\cdot}$ m/z: M (489) [$C_{30}H_{23}N_3O_4$] (25%); 208 (100%). Anal. Calcd. For $C_{30}H_{23}N_3O_4$: C, 73.61; H, 4.74; N, 8.58. Found: C, 72.89; H, 4.56; N, 8.63.

2-Phenyl-4'-(4-methoxylphenyl)-3'-phenyl-1,2-dihydro-4*H*,4'*H*-spiro[isoquinoline-3,5'-isoxazol]-4-one (19). This compound was obtained as white-yellow powder. Yield 70%. M.p. 170-173 °C. IR, ν (C=O): 1697 cm^{-1}. ^{1}H NMR: 3.85 (s, 3H, CH_3); 4.10 (d, 1H, H^{1a}, $^2JH^{1a}$-H^{1b} = 17.30); 5.05 (d, 1H, H^{1b}, $^2JH^{1b}$-H^{1a} = 17.30); 6.00 (s, 1H, $H^{4'}$); 6.50-7.65 (m, 17H, Aromatic H); 8.05 (dd, 1H, H^5, ^4J = 1.40, ^3J = 7.25). ^{13}C NMR: 53.80 (O-$\underline{C}H_3$); 55.20 (-$\underline{C}$H-); 55.35 (N$\underline{C}H_2$-); 102.65 (spiro-C); 124.25; 127.70; 135.90; 140.85; 148.70; 158.35; 160.15 (quat. C sp^2); 113.15; 125.35; 126.50; 127.55; 128.45; 128.80; 131.15; 134.25 (tert. C sp^2); 187.55 (C=O). MS (EI, 70 eV): $[M]^{+\cdot}$ m/z: M (460) [$C_{30}H_{24}N_2O_3$] (15%); 223 (100%)]. Anal. Calcd. For $C_{30}H_{24}N_2O_3$: C, 78.24; H, 5.25; N, 6.08. Found: C, 79.01; H, 5.34; N, 5.92.

2-Phenyl-4'-(4-methoxylphenyl)-3'-(4-methylphenyl)-1,2-dihydro-4*H*,4'*H*-spiro[isoquinoline-3,5'-isoxazol]-4-one (20). This compound was obtained as white-yellow powder. Yield 68%. M.p. 184-186 °C. IR, ν (C=O): 1690 cm^{-1}. ^{1}H NMR: 2.30 (s, 3H, CH_3); 3.65 (s, 3H, OCH_3); 4.10 (d, 1H, H^{1a}, $^2JH^{1a}$-H^{1b} = 17.30); 5.05 (d, 1H, H^{1b}, $^2JH^{1b}$-H^{1a} = 17.30); 6.05 (s, 1H, $H^{4'}$); 6.35-7.55 (m, 16H, Aromatic H); 8.20 (dd, 1H, H^5, ^4J = 1.40, ^3J = 7.70). ^{13}C NMR: 54.00 (O-$\underline{C}H_3$); 21.45 (-$\underline{C}H_3$); 55.17 (-$\underline{C}$H-); 55.35 (N$\underline{C}H_2$-);102.14 (spiro-C); 124.75; 126.27; 128.93; 140.13; 140.90; 148.83; 158.18; 161.13 (quat. C sp^2); 112.99; 125.17; 125.71; 126.39; 127.50; 128.43; 128.70; 129.21; 131.21; 134.17 (tert. C sp^2); 187.85 (C=O). MS (EI, 70 eV): $[M]^{+\cdot}$ m/z: M (474) [$C_{31}H_{26}N_2O_3$] (10%); 237 (100%). Anal. Calcd. For $C_{31}H_{26}N_2O_3$: C, 78.46; H, 5.52; N, 5.90. Found: C, 79.03; H, 5.61; N, 5.41.

2-Phenyl-3',4'-(4-methoxylphenyl)-1,2-dihydro-4*H*,4'*H*-spiro[isoquinoline-3,5'-isoxazol]-4-one (21). This compound was obtained as white-yellow powder. Yield 56%. M.p. 198-201°C. IR, ν (C=O): 1698 cm^{-1}. ^{1}H NMR: 3.65 (s, 3H,OCH_3); 3.75 (s, 3H,OCH_3); 4.10 (d, 1H, H^{1a}, $^2JH^{1a}$-H^{1b} = 17.30); 5.05 (d, 1H, H^{1b}, $^2JH^{1b}$-H^{1a} = 17.30); 6.00 (s, 1H, $H^{4'}$); 6.35-7.55 (m, 16H, Aromatic H); 8.15 (dd, 1H, $H^{5'}$, ^4J = 1.35, ^3J = 7.60). ^{13}C NMR: 54.15 and 55.20 (2 x O-$\underline{C}H_3$); 55.30 (-$\underline{C}$H-);55.35 (N$\underline{C}H_2$-);101.90 (spiro-C); 121.55; 124.80; 128.95; 140.90; 148.85; 158.20; 160.75; 160.90 (quat. C sp^2); 113.00; 113.95; 125.10; 125.70; 126.30; 127.45; 128.40; 128.70; 129.10; 131.20; 134.15 (tert. C sp^2); 188.00 (C=O). MS (EI, 70 eV): $[M]^{+\cdot}$ m/z: M (490)

[$C_{31}H_{26}N_2O_4$] (14%); 253 (100%). Anal. Calcd. For $C_{31}H_{26}N_2O_4$: C, 75.90; H, 5.34; N, 5.71. Found: C, 75.13; H, 5.27; N, 5.65.

2-Phenyl-4'-(4-methoxylphenyl)-3'-(4-chlorophenyl)-1,2-dihydro-4*H*,4'*H*-spiro[isoquinoline-3,5'-isoxazol]-4-one (22). This compound was obtained as white-yellow powder. Yield 78%. M.p. 200-2003 °C. IR, ν (C=O): 1695 cm^{-1}. ^1H NMR: 3.65 (s, 3H, OCH_3); 4.10 (d, 1H, H^{1a}, $^2JH^{1a}$-H^{1b} = 17.30); 5.05 (d, 1H, H^{1b}, $^2JH^{1b}$-H^{1a} = 17.30); 6.05 (s, 1H, $H^{4'}$); 6.35-7.60 (m, 16H, Aromatic H); 8.20 (dd, 1H, H^5, ^4J = 1.45, ^3J = 7.80). ^{13}C NMR: 53.90 (O-$\underline{C}H_3$); 55.20 (-$\underline{C}$H-); 55.35 (N$\underline{C}H_2$-); 102.35 (spiro-C); 124.60; 128.90; 129.15; 140.90; 148.80; 158.20; 161.15 (quat. C sp^2); 113.00; 125.25; 125.70; 126.45; 127.55; 128.50; 128.70; 129.95; 131.20; 134.20 (tert. C sp^2); 187.75 ($\underline{C}$=O). MS (EI, 70 eV): $[M]^{+\cdot}$ m/z: M (494) [$C_{30}H_{23}ClN_2O_3$] (10%); 496 (4%); 257 (100%). Anal. Calcd. For $C_{30}H_{23}ClN_2O_3$: C, 72.80; H, 4.68; N, 5.66. Found: C, 71.95; H, 4.53; N, 5.58.

2-Phenyl-4'-(4-methoxylphenyl)-3'-(4-nitrophenyl)-1,2-dihydro-4*H*,4'*H*-spiro[isoquinoline-3,5'-isoxazol]-4-one (23). This compound was obtained as white-yellow powder. Yield 57%. M.p. 228-231 °C. IR, ν (C=O): 1698 cm^{-1}. ^1H NMR: 3.65 (s, 3H, OCH_3); 4.05 (d, 1H, H^{1a}, $^2JH^{1a}$-H^{1b} = 17.35); 4.95 (d, 1H, H^{1b}, $^2JH^{1b}$-H^{1a} = 17.35); 6.10 (s, 1H, $H^{4'}$); 6.40-7.75 (m, 16H, Aromatic H); 8.15 (dd, 1H, $H^{5'}$, ^4J = 1.30, ^3J = 7.65). ^{13}C NMR: 53.40 (O-$\underline{C}H_3$); 55.20 (-$\underline{C}$H-); 55.40 (N$\underline{C}H_2$-); 103.65 (spiro-C); 123.65; 135.40; 140.85; 148.25; 148.50; 158.45; 159.45 (quat. C sp^2); 113.30; 113.90; 116.85; 123.75; 125.65; 125.80; 126.65; 127.70; 128.30; 128.55; 128.85; 129.25; 131.10; 134.50 (tert. C sp^2); 187.10 ($\underline{C}$=O). MS (EI, 70 eV): $[M]^{+\cdot}$ m/z: M (505) [$C_{30}H_{23}N_3O_5$] (15%); 208 (100%). Anal. Calcd. For $C_{30}H_{23}N_3O_5$: C, 71.28; H, 4.59; N, 8.31. Found: C, 70.93; H, 4.48; N, 8.25.

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