Chemistry of Heterocyclic Compounds, Vol. 47, No. 5, August 2011 (Russian original Vol. 47, No. 5, May, 2011)

SYNTHESIS, SPECTRAL ANALYSIS, AND *IN VITRO* MICROBIOLOGICAL EVALUATION OF ETHYL 7,9-DIARYL-1,4-DIAZASPIRO[4.5]DEC-9-ENE-6-CARBOXYLATES AS A NEW CLASS OF ANTIBACTERIAL AND ANTIFUNGAL AGENTS

J. Thanusu[1], V. Kanagarajan[1], and M. Gopalakrishnan[1]*

A series of novel ethyl 7,9-diaryl-1,4-diazaspiro[4.5]dec-9-ene-6-carboxylates was synthesized by the reaction of ethyl 4,6-diaryl-2-oxocyclohex-3-ene-1-carboxylates with ethylenediamine in the presence of p-toluenesulfonic acid without a solvent under focused microwave irradiation. The title compounds were screened for their antimicrobial activities against a spectrum of clinically isolated microorganisms.

Keywords: ethyl 7,9-diaryl-1,4-diazaspiro[4.5]dec-9-ene-6-carboxylates, ethylenediamine, *p*-toluenesulfonic acid, antibacterial activity, antifungal activity.

Heterocycles form by far the largest of the classical divisions of organic chemistry and are of immense importance biologically and industrially. The majority of pharmaceuticals and biologically active agrochemicals are heterocyclic, while countless additives and modifiers used in industrial applications, including cosmetics, reprography, information storage, and plastics, are also heterocyclic in nature. The development of simple, efficient, and environmentally benign chemical processes for widely used organic compounds from readily available reagents is one of the major challenges for chemists in organic synthesis.

Imidazoline is a nitrogen-containing heterocycle derived from imidazole. The ring contains an imine bond, and the carbon atoms at the positions 4 and 5 are singly rather than doubly bonded as in the case of imidazole. The importance of the imidazoline unit rests on its presence in many biologically active compounds [1, 2]. Imidazoline units are also used in organic synthesis as synthetic intermediates [3, 4], chiral auxiliaries [5], chiral catalysts [6], and ligands for asymmetric catalysis [7]. Like imidazole, imidazoline-based compounds have been used as N-heterocyclic carbene ligands [8] with various transition metals, for example, in the commercially available second generation Grubbs' catalyst.

A good number of bioactive imidazoline derivatives bear a substituent (aryl or alkyl group) on the carbon atom between the nitrogen centers. Oxymetazoline, generally available as a nasal spray [9], is a selective

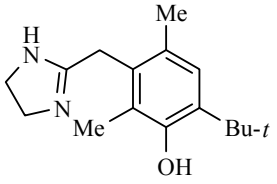
* To whom correspondence should be addressed, e-mail: profmgk@yahoo.co.in.

[1] Synthetic Organic Chemistry Laboratory, Department of Chemistry, Annamalai University, Annamalai Nagar-608 002, Tamil Nadu, India

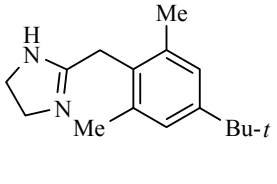
Translated from Khimiya Geterotsiklicheskikh Soedinenii, No. 5, pp. 696–705, May, 2011. Original article received November 2, 2009, in revised form April 6, 2011.

0009-3122/11/4705-0575©2011 Springer Science+Business Media, Inc.

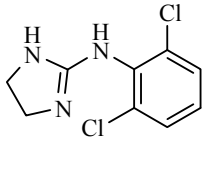
α_1-adrenoceptor agonist and partial α_2-adrenoceptor agonist topical decongestant, used in the form of hydrochloride in products such as Afrin. Oxymetazoline causes down-regulation of α_2-adrenoceptors, and therefore becomes less effective after a few doses. Xylometazoline (also known as Xylomethazoline) is a drug which is used as a nasal decongestant. It is a topical decongestant that is applied directly into the nose either as a spray or as drops. Tetrahydrozoline, a derivative of imidazoline, is an α-adrenoceptor agonist [10], and its main mechanism of action is the constriction of conjunctival blood vessels. This serves to relieve the redness of the eye caused by minor ocular irritants. Naphazoline is a sympathomimetic agent [11] with marked α-adrenergic activity. It is a vasoconstrictor with a rapid action in reducing swelling when applied to mucous membrane. It acts on α-receptors in the arterioles of the conjunctiva to produce constriction, resulting in decreased congestion. It is an active ingredient in Naphcon-A and Clearine eye drops. Clonidine is a medication used to treat several medical conditions. It is a direct-acting α_2-adrenergic agonist. An imidazole ring forms a part of some antitumor drugs such as dacarbazine (5-(3,3-dimethyl-1-triazenyl)imidazole-4-carboxamide) and Azathioprine (Imuran, 6-[(1-methyl-4-nitro-1H-imidazol-5-yl)sulfanyl]-7H-purine) [12], as well as the tumor growth-inhibiting iminoimidazolidine (Cyclocreatine) [13].



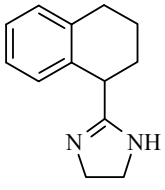
Oxymetazoline



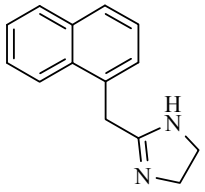
Xylometazoline



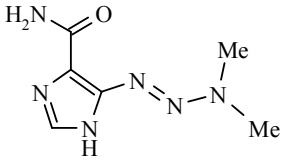
Clonidine



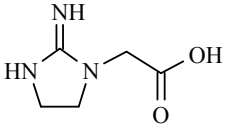
Tetrahydrozoline



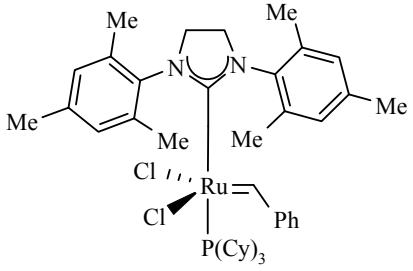
Naphazoline



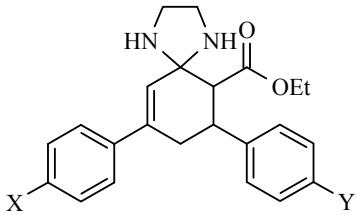
Dacarbazine



Cyclocreatine



Second generation Grubbs' catalyst



Imidazolidine derivative. Target molecule

A variety of methods has been reported for the synthesis of imidazolines, which include conversion of esters using aluminum reagents [14], the reaction between N-ethoxycarbonylthioamides with 1,2-diamines [15], and the reaction of aldehydes with 1,2-diamines followed by N-halosuccinimides [16]. Recently, several methods have been developed where azalactones [17], 2-aryl-1,1-dibromoethanes [18], nitriles [19], and amino amides [20] are used as starting materials for this synthesis. However, many of the synthesis protocols reported so far suffer from disadvantages such as the need for anhydrous conditions [14], use of organic solvents [14–20], severe reaction conditions [14], prolonged reaction time [16], use of metals, and expensive reagents [14]. Therefore the development of a cost-effective, safe, and environmentally friendly reagent system is desirable.

In recent years, there has been a great deal of interest in exploiting more than one proximal functional group for designing novel structures capable of performing a variety of functions. The present study describes

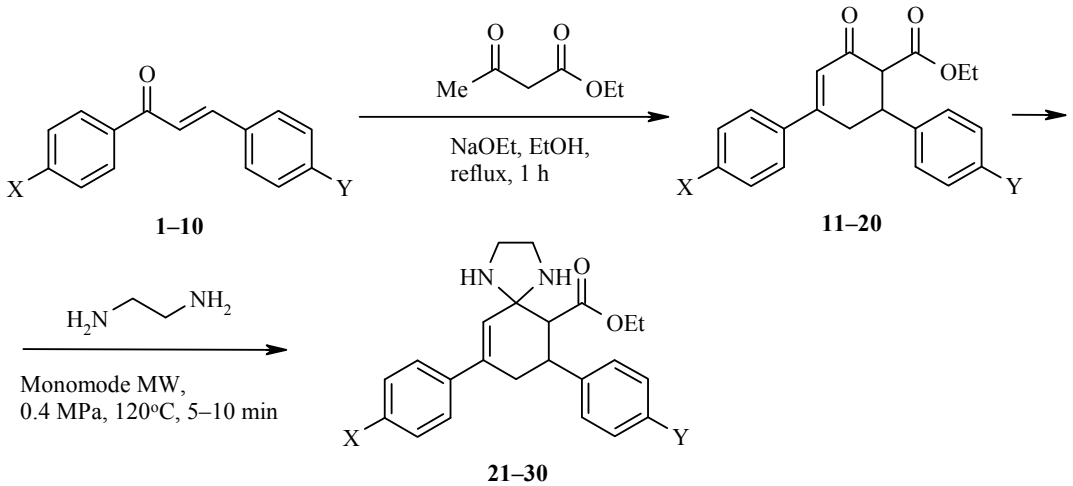
the use of ethyl 4,6-diaryl-2-oxocyclohex-3-ene-1-carboxylates [21], intermediates with three versatile functional groups, i.e., ketone, olefin, and ester, for the synthesis of imidazoline derivatives. In continuation of our earlier work on the synthesis of structurally diverse biologically active hybrid heterocyclic ring systems and as part of our ongoing research program [22–26], we plan to design imidazoline derivatives bearing a cyclohexene substituent bound by a spiro-linkage to the carbon atom between the nitrogen centers to obtain a new series of heterocycles, namely, ethyl 7,9-diaryl-1,4-diazaspiro[4.5]dec-9-ene-6-carboxylates since most of the bioactive imidazoline derivatives bear a substituent (aryl or alkyl group) on this carbon center [11–13]. In order to evaluate the effect of the structure on the biological activity of the target compounds, we chose different substituents at the phenyl rings of the starting reactants.

Condensation of appropriate acetophenone derivatives with appropriate benzaldehydes in the presence of sodium hydroxide yielded the respective 1,3-diarylprop-2-en-1-ones **1–10**. On treatment with ethyl acetoacetate in the presence of sodium ethoxide, α,β-unsaturated ketones **1–10** gave cyclohex-2-ene derivatives **11–20** by the Knoevenagel reaction. Synthesis of ethyl 7,9-diaryl-1,4-diazaspiro[4.5]-deca-9-ene-6-carboxylates **21–30** was carried out by the reaction of compounds **11–20** with ethylenediamine in the presence of *p*-toluenesulfonic acid under solvent-free conditions and under focused microwave irradiation. The applications of microwave technology to rapid synthesis of biologically significant heterocyclic molecules under solvent-free conditions are very promising and numerous and have recently been recognized as a useful tool for a drug-discovery program, especially in combinatorial chemistry [27–29]. The reactions were performed at 120°C under 4 bar pressure within 5–10 min. After completion of the reaction, as indicated by the TLC, the reaction mixture was poured into ice water and worked up using standard methods.

The synthetic route for the formation of compounds **21–30** and their physical and analytical data are given in Scheme 1 and Table 1, respectively. The structures of all the synthesized compounds **21–30** are determined with the help of elemental analysis, FT-IR, MS, one-dimensional ^{1}H NMR, D_2O-exchanged ^{1}H NMR, ^{13}C NMR, and two-dimensional HSQC spectra. In order to investigate the spectral assignments, ethyl 7,9-diphenyl-1,4-diazaspiro[4.5]dec-9-ene-6-carboxylate **21** was chosen as a representative compound.

The FT-IR spectrum of ethyl 7,9-diphenyl-1,4-diazaspiro[4.5]dec-9-ene-6-carboxylate **21** shows characteristic absorption frequencies in the region of 3267–3528 cm^{-1} (NH stretching frequency). The absorption bands at 1738 cm^{-1} and 1607 cm^{-1} are due to carbonyl stretching of the ester group and C=C stretching, respectively. The presence of the band at 1445 cm^{-1} (C–N) adds more evidence for the formation of compound **21**. The mass spectrum of compound **21** shows a protonated molecular ion peak at *m/z* 363. This is consistent with its proposed empirical formula which is confirmed also by elemental analysis.

Scheme 1



1–5, **11–15, 21–25** X = H, **1**, **11**, **21** Y = H, **2**, **12**, **22** Y = Cl, **3**, **13**, **23** Y = F,
4, **14**, **24** Y = Me, **5**, **15**, **25** Y = OMe, **6**, **16**, **26** X = Cl, Y = H, **7**, **17**, **27** X = OMe, Y = H,
8, **18**, **28** X = Cl, Y = Me, **9**, **19**, **29** X = OMe, Y = Cl, **10**, **20**, **30** X = Cl, Y = OMe

TABLE 1. Physical and Analytical Data for the Title Compounds **21–30**

Compound	*m/z* $[M+1]^+$ Empirical formula	Found, % / Calculated, %			mp, °C	Yield, %
		C	H	N		
21	363 $C_{23}H_{26}N_2O_2$	76.11 76.21	7.15 7.23	7.63 7.73	77	92
22	397 $C_{23}H_{25}ClN_2O_2$	69.48 69.60	6.24 6.35	6.95 7.06	100	93
23	381 $C_{23}H_{25}FN_2O_2$	72.52 72.61	6.57 6.62	7.23 7.36	65	92
24	377 $C_{24}H_{28}N_2O_2$	76.49 76.56	7.43 7.50	7.32 7.44	117	90
25	393 $C_{24}H_{28}N_2O_3$	73.33 73.44	7.10 7.19	7.01 7.14	112	93
26	397 $C_{23}H_{25}ClN_2O_2$	69.52 69.60	6.30 6.35	6.97 7.06	68	91
27	393 $C_{24}H_{28}N_2O_3$	73.32 73.44	7.11 7.19	7.03 7.14	65	92
28	411 $C_{24}H_{27}Cl_2O_2$	70.04 70.15	6.53 6.62	6.78 6.82	73	93
29	427 $C_{24}H_{27}ClN_2O_3$	67.42 67.52	6.25 6.37	6.51 6.56	69	94
30	427 $C_{24}H_{27}N_2O_3Cl$	67.44 67.52	6.29 6.37	6.48 6.56	99	93

The assignments of signals in the ^1H and ^{13}C NMR spectrum have been done with the help of HSQC one-bond ^1H–^{13}C correlations, which allowed us to confirm the formation of the imidazolidine ring. Among the signals important for the structure determination are two NH protons of the imidazoline moiety (H-1, H-4) in the form of a broad singlet at 5.98 ppm which disappears upon addition of D_2O.

The methylene protons (H-2, H-3) of the imidazolidine moiety were observed as one multiplet at 4.02–4.13 ppm. The ^{13}C resonance of a quaternary carbon at 80.1 ppm is due to the spiro carbon (C-5).

The antibacterial activity of compounds **21–30** was tested *in vitro* against *Bacillus subtilis, Vibrio cholerae, Shigella flexneri, Salmonella typhi*, and *Escherichia coli*. The potency of the synthesized compounds expressed as the minimum inhibitory concentration (MIC) was compared with a broad-spectrum antibiotic, Ciprofloxacin (Table 2). A close survey of the MIC values indicate that all the compounds exhibited a varied range (6.25–200 μg/ml) of antibacterial activity against all the tested bacterial strains except **21** and **29** which did not show activity against *E. coli* and *S. flexneri*, respectively, even at the maximum concentration of 200 μg/ml. Compound **22,** having an electron-withdrawing *p*-chloro substituent at the phenyl ring attached to C-7 of the cyclohexenone moiety, shows moderate activity against *B. subtilis, V. cholerae*, and *S. typhi* and good activity against *S. flexneri* and *E. coli*. Compound **23** with an electron-withdrawing *p*-fluoro substituent at the same position was more active than Ciprofloxacin against all the tested bacterial strains, thus showing a broad spectrum of antibacterial activity.

The *in vitro* antifungal activity of compounds **21–30** was studied against the fungal strains *Aspergillus flavus, Aspergillus niger, Mucor*, and *Rhizopus*. Fluconazole was used as the standard drug. The corresponding MIC values are given in Table 3. All the compounds **21–30** exhibited a varied range (6.25–200 μg/ml) of antifungal activity against all the tested fungal strains except compounds **21** and **25** which were not active against *A. niger* and *A. flavus*, respectively. Compound **22** with a *p*-chloro substituent at the 7-phenyl ring shows good activity against *A. niger* and *Rhizopus* and moderate activity against *Mucor*, being more active than Fluconazole against those three species. Likewise, compound **29**, which has an electron-withdrawing chloro substituent at the same position and an electron-donating *p*-methoxy substituent at the phenyl ring attached to C-9, performed better than Fluconazole against *A. flavus*, *A. niger*, and *Rhizopus*.

TABLE 2. *In vitro* Antibacterial Activity of Compounds **21–30** against Clinically Isolated Bacterial Strains

Compound	MIC, μg/ml				
	B. subtilis	*V. cholerae*	*S. flexneri*	*S. typhi*	*E. coli*
21	200	25	25	100	–*
22	25	25	12.5	25	6.25
23	6.25	25	12.5	25	12.5
24	100	50	50	100	6.25
25	100	50	50	6.25	100
26	100	6.25	50	50	100
27	50	100	100	12.5	100
28	100	12.5	100	100	50
29	12.5	12.5	–*	6.25	50
30	100	6.25	25	100	100
Ciprofloxacin	25	50	25	50	25

* No inhibition even at concentration 200 μg/ml.

TABLE 3. *In vitro* Antifungal Activity of Compounds **21–30** against Clinically Isolated Fungal Strains

Compound	MIC, μg/ml			
	A. flavus	*A. niger*	*Mucor*	*Rhizopus*
21	200	–*	50	25
22	100	6.25	12.5	6.25
23	6.25	100	6.25	25
24	50	100	50	100
25	–*	50	50	100
26	25	100	12.5	25
27	100	12.5	50	100
28	50	100	6.25	50
29	25	12.5	25	6.25
30	50	6.25	50	25
Fluconazole	50	50	25	25

* No inhibition even at concentration 200 μg/ml.

Thus we have synthesized a series of novel biologically active ethyl 7,9-diaryl-1,4-diazaspiro[4.5]dec-9-ene-6-carboxylates and established their structures by their spectral data. A survey of their *in vitro* antibacterial and antifungal activity was performed, which may be considered as an important step for construction of novel chemical entities with comparable pharmacological profiles to that of the standard drugs.

EXPERIMENTAL

All the reported melting points were measured in open capillaries and were uncorrected. IR spectra were recorded in KBr pellets on a Thermo Nicolet Avatar 330 FT-IR spectrophotometer, and noteworthy absorption

values alone are listed. One-dimensional ^{1}H and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively, on a Bruker AMX 400 NMR spectrometer using DMSO-d_6 as solvent and TMS as internal standard. Two-dimensional HSQC spectra were recorded at 500 MHz on a Bruker DRX 500 NMR spectrometer using DMSO-d_6 as solvent. The electron spray ionization (ESI) positive mode mass spectra were recorded on a Bruker Daltonics Esquire 300 LC-MS spectrometer. Satisfactory microanalysis was obtained on a Carlo Erba 1106 CHN analyzer. TLC was used to control the reactions and the purity of the products. A Gyan Easi Flash column chromatography system was used for flash chromatography. A Biotage Initiator microwave synthesizer was used for the irradiation.

1,3-Diarylprop-2-en-1-ones **1–10** [30] and 4,6-diaryl-2-oxocyclohex-3-enecarboxylates **11–20** [21] were prepared by adopting published procedures.

Synthesis of Ethyl 7,9-Diaryl-1,4-diazaspiro[4.5]dec-9-ene-6-carboxylates 21–30 (General Method). An appropriate cyclohexenone **11–20** (0.01 mol), ethylenediamine (0.01 mol), and *p*-toluenesulfonic acid (10 mg) were placed in a 10 ml Pyrex glass vial which was sealed with a Teflon cap. The vial was inserted in a Teflon outer jacket and then placed into the holder in the microwave cavity. The sample was irradiated under focused monomode irradiation at 120ºC for 5–10 min at 0.4 MPa. After allowing the mixture to cool to room temperature, the reaction vessel was opened, and the contents were poured into ice water. The organic material was extracted with ethyl acetate. The organic layer was washed with 10% sodium hydrogen carbonate and brine, and dried over anhydrous Na_2SO_4. After evaporation of the ethyl acetate under reduced pressure, a solid mass was obtained which was subjected to flash column chromatography using toluene–ethyl acetate as eluent.

Ethyl 7,9-Diphenyl-1,4-diazaspiro[4.5]dec-9-ene-6-carboxylate (21). IR spectrum, ν, cm^{-1}: 3448, 3388, 3175, 3054, 3022, 2972, 2931, 2837, 1738, 1607, 1445, 1027, 827, 758, 698. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 0.90 (3H, t, *J* = 7.1, CH_3 ester); 2.97–3.02 (2H, m, H-8); 3.04–3.22 (1H, m, H-7); 3.51–3.67 (1H, m, H-6); 3.89 (2H, q, *J* = 6.8, CH_2 ester); 4.02–4.13 (4H, m, 2CH_2 imidazolidine); 5.98 (2H, s, 2NH imidazolidine); 6.54 (1H, s, H-10); 7.10–7.52 (8H, m, H Ar); 7.65–7.79 (2H, d, *J* = 2.7, H Ar). ^{13}C NMR spectrum, δ, ppm: 13.7 (CH_3 ester); 35.2 (C-7); 43.8 (C-8); 49.3 (C-2, 3); 58.7 (CH_2 ester); 59.9 (C-6); 80.1 (C-5); 122.8 (C-10); 126.4 (*p*-C Ar); 127.0 (*o*-C Ar); 127.5 (*p*-C Ar); 128.3 (*o*-C Ar); 128.8 (*m*-C Ar); 130.0 (*m*-C Ar); 130.4 (C-9); 137.3 (*ipso*-C Ar); 141.4 (*ipso*-C Ar); 169.2 (C=O).

Ethyl 7-(4-Chlorophenyl)-9-phenyl-1,4-diazaspiro[4.5]dec-9-ene-6-carboxylate (22). IR spectrum, ν, cm^{-1}: 3535, 3437, 3382, 3295, 3065, 2978, 2924, 2847, 1735, 1601, 1445, 1018, 824, 758, 693. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 0.92 (3H, t, *J* = 7.2, CH_3 ester); 2.97–3.07 (2H, m, H-8); 3.10–3.14 (1H, m, H-7); 3.57–3.76 (1H, m, H-6); 3.92 (2H, q, *J* = 6.6, CH_2 ester); 4.02–4.15 (4H, m, 2CH_2 imidazolidine); 6.12 (2H, s, 2NH imidazolidine); 6.55 (1H, s, H-10); 7.13–7.48 (7H, m, H Ar); 7.69–7.79 (2H, d, *J* = 2.5, H Ar). ^{13}C NMR, δ, ppm: 13.7 (CH_3 ester); 34.9 (C-7); 43.1 (C-8); 48.4 (C-2, 3); 58.5 (CH_2 ester); 60.0 (C-6); 80.2 (C-5); 121.9 (C-10); 122.8 (*o*-C Ar); 128.1 (*p*-C Ar); 128.6 (*m*-C Ar); 128.8 (*m*-C Ar); 129.1 (*o*-C Ar); 130.6 (C-9); 135.6 (*ipso*-C Ar); 136.4 (*p*-C Ar); 140.4 (*ipso*-C Ar); 169.0 (C=O).

Ethyl 7-(4-Fluorophenyl)-9-phenyl-1,4-diazaspiro[4.5]dec-9-ene-6-carboxylate (23). IR spectrum, ν, cm^{-1}: 3519, 3388, 3262, 3186, 3058, 2977, 2930, 2863, 1739, 1604, 1443, 1023, 832, 758, 694. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 0.89 (3H, t, *J* = 7.0, CH_3 ester); 2.74–2.94 (2H, m, H-8); 2.98–3.13 (1H, m, H-7); 3.56–3.88 (1H, m, H-6); 3.90 (2H, q, *J* = 6.8, CH_2 ester); 3.95–4.13 (4H, m, 2CH_2 imidazolidine); 6.02 (2H, s, 2NH imidazolidine); 6.57 (1H, s, H-10); 6.83–7.28 (7H, m, H Ar); 7.80–7.94 (2H, d, *J* = 2.6, H Ar). ^{13}C NMR spectrum, δ, ppm: 13.7 (CH_3 ester); 35.2 (C-7); 42.9 (C-8); 48.4 (C-2,3); 59.8 (CH_2 ester); 61.0 (C-6); 113.1 (*m*-C Ar); 122.3 (C-10); 125.3 (*o*-C Ar); 127.9 (*p*-C Ar); 128.6 (*m*-C Ar); 128.7 (*o*-C Ar); 130.5 (C-9); 136.1 (*ipso*-C Ar); 139.8 (*ipso*-C Ar); 158.2 (*p*-C Ar); 169.1 (C=O).

Ethyl 7-(4-Methylphenyl)-9-phenyl-1,4-diazaspiro[4.5]dec-9-ene-6-carboxylate (24). IR spectrum, ν, cm^{-1}: 3454, 3399, 3267, 3054, 3038, 2981, 2921, 2863, 1739, 1604, 1023, 832, 758, 694. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 0.92–0.96 (3H, t, *J* = 6.9, CH_3 ester); 2.26 (3H, s, CH_3 tolyl); 2.72–2.93 (2H, m, H-8); 2.97–3.12 (1H, m, H-7); 3.56–3.76 (1H, m, H-6); 3.91 (2H, q, *J* = 6.6, CH_2 ester); 4.00–4.09 (4H, m, 2CH_2 imidazolidine); 5.94 (2H, s, 2NH imidazolidine); 6.53 (1H, s, H-10); 6.88–7.48 (7H, m, H Ar); 7.82–7.91 (2H, d, *J* = 2.3, H Ar).

^{13}C NMR spectrum, δ, ppm: 13.7 (CH_3 ester); 20.5 (CH_3 tolyl); 35.3 (C-7); 43.3 (C-8); 48.9 (C-2,3); 59.8 (CH_2 ester); 60.9 (C-6); 80.2 (C-5); 121.9 (C-10); 122.5 (*o*-C Ar); 128.2 (*p*-C Ar); 128.4 (*o*-C Ar); 128.7 (*m*-C Ar); 129.3 (*m*-C Ar); 131.1 (C-9); 138.3 (*ipso*-C Ar); 138.4 (*p*-C Ar); 139.6 (*ipso*-C Ar); 169.1 (C=O).

Ethyl 7-(4-Methoxyphenyl)-9-phenyl-1,4-diazaspiro[4.5]dec-9-ene-6-carboxylate (25). IR spectrum, ν, cm^{-1}: 3524, 3424, 3267, 3054, 2989, 2929, 2830, 1739, 1445, 1607, 1033, 832, 760, 694. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 0.93 (3H, t, *J* = 7.1, CH_3 ester); 2.69–2.93 (2H, m, H-8); 2.97–3.12 (1H, m, H-7); 3.58–3.64 (1H, m, H-6); 3.72 (3H, s, OCH_3); 3.90 (2H, q, *J* = 6.9, CH_2 ester); 3.96–4.36 (4H, m, $2CH_2$ imidazolidine); 5.94 (2H, s, 2NH imidazolidine); 6.61 (1H, s, H-10); 6.85–7.51 (7H, m, H Ar); 7.63–7.78 (2H, d, *J* = 2.6, H Ar). ^{13}C NMR spectrum, δ, ppm: 13.7 (CH_3 ester); 35.4 (C-7); 43.0 (C-8); 48.5 (C-2,3); 54.9 (OCH_3); 59.8 (CH_2 ester); 60.9 (C-6); 80.3 (C-5); 113.1 (*m*-C Ar); 121.9 (C-10); 122.9 (*o*-C Ar); 128.2 (*p*-C Ar); 128.7 (*m*-C Ar); 129.2 (*o*-C Ar); 130.2 (C-9); 135.7 (*ipso*-C Ar); 140.3 (*ipso*-C Ar); 160.6 (*p*-C Ar); 169.2 (C=O).

Ethyl 9-(4-Chlorophenyl)-7-phenyl-1,4-diazaspiro[4.5]dec-9-ene-6-carboxylate (26). IR spectrum, ν, cm^{-1}: 3524, 3386, 3175, 3059, 3029, 2978, 2927, 2858, 1738, 1584, 1448, 1013, 824, 762, 699. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 0.91 (3H, t, *J* = 7.2, CH_3 ester); 2.71–2.97 (2H, m, H-8); 3.00–3.15 (1H, m, H-7); 3.60–3.78 (1H, m, H-6); 3.91 (2H, q, *J* = 6.9, CH_2 ester); 3.97–4.13 (4H, m, $2CH_2$ imidazolidine); 6.08 (2H, s, 2NH imidazolidine); 6.59 (1H, s, H-10); 6.88–7.39 (7H, m, H Ar); 7.64–7.79 (2H, d, *J* = 2.4, H Ar). ^{13}C NMR spectrum, δ, ppm: 14.2 (CH_3 ester); 35.1 (C-7); 43.0 (C-8); 48.3 (C-2,3); 59.9 (CH_2 ester); 61.0 (C-6); 80.2 (C-5); 122.8 (C-10); 122.9 (*o*-C Ar); 128.0 (*p*-C Ar); 128.7 (*m*-C Ar); 129.3 (*m*-C Ar); 130.2 (*o*-C Ar); 130.9 (C-9); 137.6 (*ipso*-C Ar); 140.8 (*ipso*-C Ar); 141.4 (*p*-C Ar); 168.9 (C=O).

Ethyl 9-(4-Methoxyphenyl)-7-phenyl-1,4-diazaspiro[4.5]dec-9-ene-6-carboxylate (27). IR spectrum, ν, cm^{-1}: 3450, 3444, 3065, 3033, 2924, 2852, 1736, 1605, 1447, 1037, 757, 695. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 0.91 (3H, t, *J* = 7.0, CH_3 ester); 2.72–2.98 (2H, m, H-8); 3.01–3.16 (1H, m, H-7); 3.59–3.71 (1H, m, H-6); 3.78 (3H, s, OCH_3); 3.91 (2H, q, *J* = 6.8, ester CH_2); 3.96–4.08 (4H, m, $2CH_2$ imidazolidine); 5.93 (2H, s, 2NH imidazolidine); 6.51 (1H, s, H-10); 6.83 (7H, m, H Ar); 7.70–7.81 (2H, d, *J* = 2.1, H Ar). ^{13}C NMR spectrum, δ, ppm: 13.7 (CH_3 ester); 36.0 (C-7); 43.7 (C-8); 49.2 (C-2,3); 54.3 (OCH_3); 59.9 (CH_2 ester); 61.0 (C-6); 80.2 (C-5); 116.2 (*m*-C Ar); 122.3 (C-10); 123.1 (*o*-C Ar); 128.3 (*p*-C Ar); 128.6 (*m*-C Ar); 129.3 (*o*-C Ar); 131.1 (C-9); 135.6 (*ipso*-C Ar); 141.4 (*ipso*-C Ar); 159.2 (*p*-C Ar); 169.0 (C=O).

Ethyl 9-(4-Chlorophenyl)-7-(4-methylphenyl)-1,4-diazaspiro[4.5]dec-9-ene-6-carboxylate (28). IR spectrum, ν, cm^{-1}: 3459, 3386, 3273, 3169, 3049, 2983, 2923, 2856, 1739, 1612, 1447, 1013, 817, 744, 711. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 0.93 (3H, t, *J* = 7.1, CH_3 ester); 2.19 (3H, s, CH_3 tolyl); 2.75–2.98 (2H, m, H-8); 2.98–3.15 (1H, m, H-7); 3.57–3.77 (1H, m, H-6); 3.90 (2H, q, *J* = 6.9, CH_2 ester); 4.00–4.10 (4H, m, $2CH_2$ imidazolidine); 6.00 (2H, s, 2NH imidazolidine); 6.55 (1H, s, H-10); 6.89–7.52 (7H, m, H Ar); 7.83–7.98 (2H, d, *J* = 2.5, H Ar). ^{13}C NMR spectrum, δ, ppm: 14.2 (CH_3 ester); 20.5 (CH_3 tolyl); 35.5 (C-7); 43.2 (C-8); 48.8 (C-2,3); 59.8 (CH_2 ester); 61.0 (C-6); 80.2 (C-5); 122.2 (C-10); 122.8 (*o*-C Ar); 128.2 (*p*-C Ar); 128.4 (*o*-C Ar); 128.7 (*m*-C Ar); 129.4 (*m*-C Ar); 130.8 (C-9); 137.2 (*p*-C Ar); 138.4 (*ipso*-C Ar); 140.7 (*ipso*-C Ar); 169.0 (C=O).

Ethyl 7-(4-Chlorophenyl)-9-(4-methoxyphenyl)-1,4-diazaspiro[4.5]dec-9-ene-6-carboxylate (29). IR spectrum, ν, cm^{-1}: 3442, 3393, 3284, 3065, 2962, 2923, 2850, 1738, 1598, 1457, 1140, 826, 718. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 0.93 (3H, t, *J* = 7.3, CH_3 ester); 2.77–2.98 (2H, m, H-8); 2.77–2.98 (1H, m, H-7); 3.48–3.75 (1H, m, H-6); 3.79 (3H, s, OCH_3); 3.90 (2H, q, *J* = 6.9, CH_2 ester); 4.01–4.06 (4H, m, $2CH_2$ imidazolidine); 5.96 (2H, s, 2NH imidazolidine); 6.55 (1H, s, H-10); 6.96–7.42 (6H, m, H Ar); 7.70–7.82 (2H, d, *J* = 2.3, H Ar). ^{13}C NMR spectrum, δ, ppm: 13.7 (CH_3 ester); 36.0 (C-7); 43.7 (C-8); 49.2 (C-2,3); 55.2 (OCH_3); 59.8 (CH_2 ester); 60.9 (C-6); 80.2 (C-5); 119.9 (C-10); 120.9 (*m*-C Ar); 122.8 (*o*-C Ar); 128.7 (*m*-C Ar); 128.9 (*ipso*-C Ar); 129.4 (*o*-C Ar); 131.3 (C-9); 135.6 (*ipso*-C Ar); 140.6 (*p*-C Ar); 161.2 (*p*-C Ar); 169.2 (C=O).

Ethyl 9-(4-Chlorophenyl)-7-(4-methoxyphenyl)-1,4-diazaspiro[4.5]dec-9-ene-6-carboxylate (30). IR spectrum, ν, cm^{-1}: 3453, 3393, 3262, 3065, 2951, 2927, 2830, 1738, 1608, 1456, 1032, 824, 749, 711. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 0.93 (3H, t, *J* = 7.2, CH_3 ester); 2.87–3.09 (2H, m, H-8); 3.12–3.19 (1H, m, H-7); 3.59–3.70 (1H, m, H-6); 3.79 (3H, s, OCH_3); 3.89 (2H, q, *J* = 6.7, CH_2 ester); 4.05–4.20 (4H, m, $2CH_2$ imidazolidine); 5.96 (2H, s, 2NH imidazolidine); 6.55 (1H, s, H-10); 7.24–7.62 (6H, m, H Ar); 7.78–7.91 (2H,

d, J = 2.5, H Ar). ^{13}C NMR spectrum, δ, ppm: 14.3 (CH_3 ester); 35.6 (C-7); 43.1 (C-8); 48.4 (C-2,3); 55.2 (OCH_3); 59.9 (CH_2 ester); 61.0 (C-6); 79.9 (C-5); 119.9 (C-10); 120.9 (*m*-C Ar); 122.7 (*o*-C Ar); 128.2 (*ipso*-C Ar); 128.7 (*m*-C Ar); 129.9 (*o*-C Ar); 131.0 (C-9); 131.7 (*ipso*-C Ar); 139.8 (*p*-C Ar); 161.2 (*p*-C Ar); 169.1 (C=O).

***In vitro* Antibacterial and Antifungal Activity**. All the clinically isolated bacterial and fungal strains were obtained from the Faculty of Medicine, Annamalai University, Annamalainagar-608 002, Tamil Nadu, India. MIC values were measured by the twofold serial dilution method [31]. The respective test compounds **21–30** were dissolved in DMSO to obtain 1 mg/ml stock solution. Seeded broth (broth containing microbial spores) was prepared in nutrient broth (NB) from 24 h old bacterial cultures on nutrient agar (Hi-media, Mumbai) at 37±1°C, while fungal spores from 1 to 7 days old Sabouraud agar (Hi-media, Mumbai) slant cultures were suspended in Sabouraud dextrose agar broth (SDB). The colony-forming units (cfu) of the seeded broth were determined by the plating technique and adjusted in the range of 10^4–10^5 cfu/ml. The final inoculum size was 10^5 cfu/mL for the antibacterial assay and $1.1–1.5\times10^2$ cfu/ml for the antifungal assay. Testing was performed at pH 7.4±0.2 for bacteria (NB) and at pH 5.6 for fungi (SDB). Exactly 0.4 ml of the solution of the test compound was added to 1.6 ml of seeded broth to form the first dilution. One milliliter of this was diluted with a further 1 ml of seeded broth to give the second dilution and so on until six such dilutions were obtained. A set of assay tubes containing only seeded broth was kept as control. The tubes were incubated in BOD incubators at 37±1°C for bacteria and 28±1°C for fungi. MIC values were determined by visual observation after 24 h (for bacteria) and 72–96 h (for fungi) of incubation. Ciprofloxacin was used as standard for bacterial studies, and Fluconazole was used as standard for fungal studies.

The authors are thankful to the NMR Research Centre, Indian Institute of Science, Bangalore for recording spectra. One of the authors (V. K.) is grateful to the Council of Scientific and Industrial Research (CSIR), New Delhi, Republic of India for providing financial support in the form of a CSIR-Senior Research Fellowship (SRF) in Organic Chemistry. Another author (J. T.) is highly thankful to Annamalai University authorities for providing a University Studentship.

REFERENCES

1. M. R. Grimmett, in: A. R. Katritzky, C. W. Rees, and E. F. V. Scriven (editors), *Comprehensive Heterocyclic Chemistry*, (Pergamon, Oxford, Vol. 3, (1996), p. 77.
2. J. V. Greenhill and L. Lue, in: G. P. Ellis and D. K. Luscombe (editors), *Progress in Medicinal Chemistry*, Elsevier, New York, Vol. 3, (1993), p. 170.
3. R. C. F. Jones and J. R. Nichols, *Tetrahedron Lett.*, **31**, 1771 (1990).
4. T. Hayashi, E. Kishi, V. A. Soloshonok, and Y. Uozumi, *Tetrahedron Lett.*, **37**, 4969 (1996).
5. R. C. F. Jones, I. Turner, and K. J. Howard, *Tetrahedron Lett.* **34**, 6329 (1993).
6. T. Isobe, K. Fukuda, Y. Araki, and T. Ishikawa, *Chem. Commun.*, 243 (2001).
7. N. A. Boland, M. Casey, S. J. Hynes, J. W. Matthews, H. Müller-Bunz, and P. Wilkes, *Org. Biomol. Chem.*, **2**, 1995 (2004).
8. J. Huang, E. D. Stevens, S. P. Nolan, and J. Petersen, *J. Am. Chem. Soc.*, **121**, 2674 (1999).
9. N. MacInnes and S. Duty, *Br. J. Pharmacol.*, **143**, 952 (2004).
10. M. Dahlström, F. Lindgren, K. Berntsson, M. Sjögren, L. G. E. Mårtensson, P. R. Jonsson, and H. Elwing, *J. Exp. Zool.*, **303A**, 551 (2005).
11. J. A. A. Zavala, E. R. Pereira, V. H. F. Zétola, H. A. G. Teive, É. M. Nóvak, and L. C. Werneck, *Arq. Neuropsiquiatr.*, **62**, 889 (2004).
12. R. M. Silverman, *The Organic Chemistry of Drug Design and Drug Action,* Academic Press, New York, 1992.
13. K. J. Martin, S.-F. Chen, G. M. Clark, D. Degen, M. Wayma, D. D. Von Hoff, and R. Kaddurah-Daouk, *J. Natl. Cancer Inst.*, **86**, 608 (1994).

14. G. Neef, U. Eder, and G. Sauer, *J. Org. Chem*., **46**, 2824 (1981).
15. B. George and E. P. Papadopoulos, *J. Org. Chem.*, **42**, 441 (1977).
16. H. Fujioka, K. Murai, Y. Ohba, A. Hiramatsu, and Y. Kita, *Tetrahedron Lett*., **46**, 2197 (2005).
17. S. Peddibhotla and J. J. Tepe, *Synthesis*, 1433 (2003).
18. D. H. Huh, H. Ryu, and Y. G. Kim, *Tetrahedron*, **60**, 9857 (2004).
19. J. M. Mitchell and N. S. Finney, *Tetrahedron Lett.*, **41**, 8431 (2000).
20. S.-L. You and J. W. Kelly, *Org. Lett*., **6**, 1681 (2004).
21. M. Balasubramanian and A. D'Souza, *Tetrahedron*, **24**, 5399 (1968).
22. M. Gopalakrishnan, P. Sureshkumar, V. Kanagarajan, and J. Thanusu, *Lett. Org. Chem*., **2**, 444 (2005).
23. M. Gopalakrishnan, P. Sureshkumar, V. Kanagarajan, J. Thanusu, R. Govindaraju, and M. R. Ezhilarasi, *Lett. Org. Chem*., **3**, 484 (2006).
24. M. Gopalakrishnan, P. Sureshkumar, J. Thanusu, and V. Kanagarajan, *Chem. Heterocycl. Comp*., **44**, 950 (2008). [*XTC*, 1183 (2008)].
25. M. Gopalakrishnan, P. Sureshkumar, J. Thanusu, and V. Kanagarajan, *J. Enz. Inhib. Med. Chem.*, **23**, 974 (2008).
26. M. Gopalakrishnan, P. Sureshkumar, J. Thanusu, and V. Kanagarajan, *J. Enz. Inhib. Med. Chem.*, **23**, 347 (2008).
27. C. Oliver Kappe and D. Dallinger, *Nature Rev. Drug Disc*., **5**, 51 (2006).
28. K. Bougrin, A. Loupy, and M. Soufiaoui, *J. Photochem. Photobiol. C. Photochem. Rev.*, **6**, 139, (2005).
29. C. Oliver Kappe, *Curr. Opinions Chem. Biol.*, **6**, 314, (2002).
30. A. T. Nielsen and W. J. Houlihan, *Organic Reactions*, J. Wiley & Sons, New York, 1968, Vol.16.
31. M. H. Dhar, M. M. Dhar, B. N. Dhawan, B. N. Mehrotra, and C. Ray. *Indian J. Exp. Biol.*, **6**, 232 (1968).