Accepted Manuscript

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PII: S0223-5234(13)00422-4

DOI: 10.1016/j.ejmech.2013.04.069

Reference: EJMECH 6282

To appear in: *European Journal of Medicinal Chemistry*

Received Date: 4 January 2013

Revised Date: 12 March 2013

Accepted Date: 24 April 2013

Please cite this article as: R.-A. Tucaliuc, V.V. Cotea, M. Niculaua, C. Tuchilus, D. Mantu, I.I. Mangalagiu, New Pyridazine–Fluorine Derivatives: Synthesis, Chemistry and Biological Activity. Part II, *European Journal of Medicinal Chemistry* (2013), doi: 10.1016/j.ejmech.2013.04.069.

- synthesis and antimicrobial activity of new pyridazine–fluorine (**PYF**)
- the first synthesis of **PYF** in phase-transfer catalysis under microwave
- regiochemistry and chorochemistry involved in these reactions are discussed
- trifluoromethyl group on pyridazine skeleton increase the antimicrobial activity

New Pyridazine–Fluorine Derivatives: Synthesis, Chemistry and Biological Activity. Part II

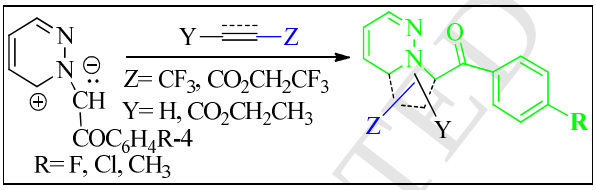
Roxana-Angela Tucaliuc,[1] Valeriu V. Cotea,[1] Marius Niculaua,[1] Cristina Tuchilus,[2] Dorina Mantu,[3] Ionel I. Mangalagiu[3]*

1. University of Agriculture and Veterinary Medicine "Ion Ionescu de la Brad" Iasi, Aleea Mihail Sadoveanu, Iasi, Romania,
2. "Gr.T.Popa" University of Medicine and Pharmacy, Iasi, School of Pharmacy, Str. Universitatii 16, 700115 Iasi, Romania.
3. "Al. I. Cuza" University of Iasi, Organic Chemistry Department, Bd. Carol 11, 700506 Iasi, Romania; e-mail: ionelm@uaic.ro

Abstract

A comprehensive study concerning synthesis, structure and biological activity of new pyridazine–fluorine (**PYF**) derivatives is presented. The first synthesis of **PYF** derivatives in phase-transfer catalysis (PTC) under microwave (MW) and conventional thermal heating (TH) is reported. Under MW irradiation the consumed energy decreases considerably, the amount of used solvent in liquid phase is at least five-fold less comparative with conventional TH, while PTC did not use solvents. Consequently these reactions could be considered environmentally friendly. Also, the reaction time decrease substantially and, in some cases, the yields are higher. A feasible explanation for MW efficiency is presented. Regiochemistry and chorochemistry involved in these reactions are also discussed; the reactions are regioselective and chorospecific or choroselective, respectively. Ten new pyridazine–fluorine cycloadducts are obtained. The *in vitro* antibacterial and antifungal activities of the **PYF** compounds were tested. Introduction of a trifluoromethyl moiety on the pyridazine skeleton is leading to an increasing of the antimicrobial activity. Structure - activity correlationships have been done.

Graphical abstract



The design, synthesis and *in vitro* antimicrobial activity of new pyridazine–fluorine (**PYF**) derivatives are presented. The first synthesis of **PYF** derivatives in phase-transfer catalysis (PTC) under microwave (MW) and conventional thermal heating (TH) is also reported. Introduction of a trifluoromethyl moiety on the pyridazine skeleton is leading to an increasing of the antimicrobial activity.

Keywords: Pyridazine; Fluorine; Chorochemistry; Microwaves; Biological activities.

Article Outline

1. Introduction

Over the last decades, pyridazine and its derivatives have been extensively investigated because of their important role especially in medicinal chemistry, a large variety of biological activities being described: antibacterial, antifungus, antituberculosis, antiviral, anti-inflammatory, anticancer, cardiovascular disorders, etc.[1,2]. On the other hand, the importance of fluorine in medicinal chemistry is well recognized, more than 150 drugs from the pharmaceuticals market being fluorinated compounds [3-7].

A simple and flexible method to generate fused pyridazine derivatives, involves 1,3-dipolar cycloaddition of cycloimmonium ylides to various symmetric and non-symmetric dipolarophiles [8-16]. The reaction of ylides with non-symmetrical dipolarophiles raises supplementary issues regarding regiochemistry and chorochemistry, being a problem widely discussed and far away from being solved [8,9].

Within the past decade, microwave (MW) assisted reactions have became a widely accepted method in organic synthesis [11,14-19]. Furthermore, phase-transfer catalytic (PTC) reactions under MW conditions have the great advantage of using small amounts of solvents, or even no organic solvents ('solvent free'), thus being environmentally friendly [17,18].

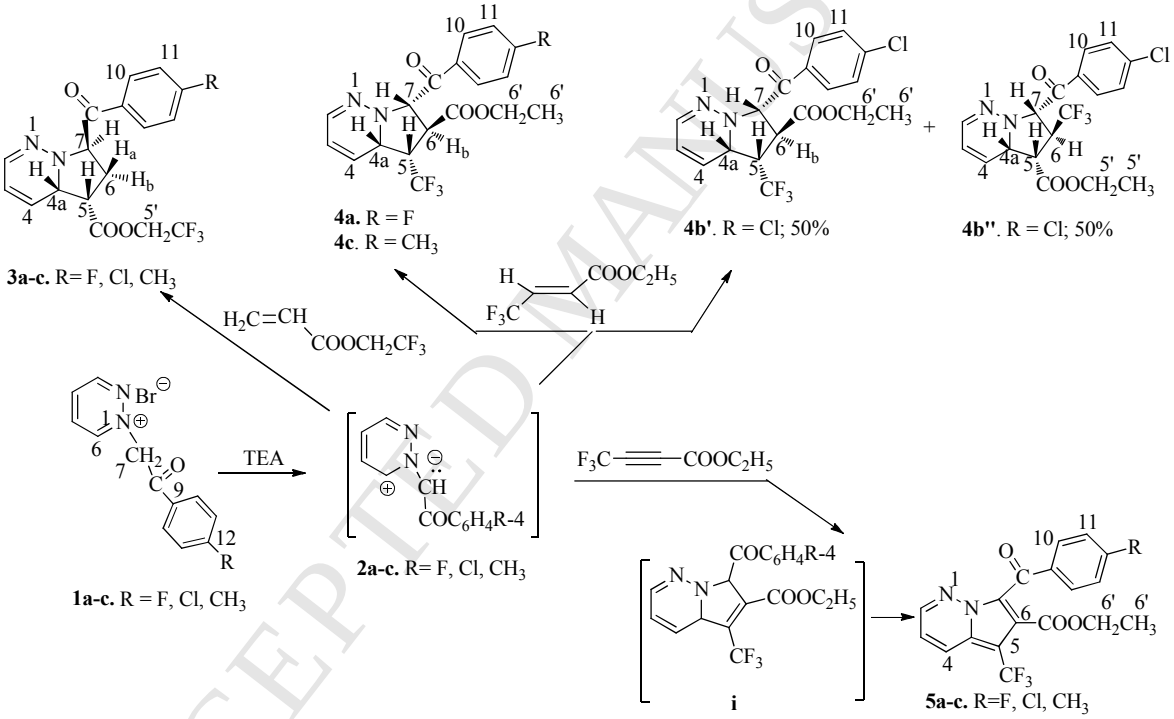
In continuation of our work in the field of biologically active pyridazine [11,13,20,21], we decided to synthesize new pyridazine-fluorine (**PYF**) derivatives in

order to test their respective biological potentials. In equal measure we were interested in developing a new environmentally friendly method for preparation of these derivatives by using MW technologies in PTC, and in study in the regiochemistry and chorochemistry involved in the cycloaddition reactions.

2. Results and Discussion

2.1. Chemistry

In a previous paper [11], we designed a general and straightforward synthetic route for obtaining fused pyrrolopyridazine using MW irradiation. In order to reach our goal we decided to adapt this strategy, thus pyridazinium ylides **2** (generated '*in situ*' from the corresponding cycloimmonium salts, **1**) were treated with various dipolarophiles containing fluorine moiety (non-symmetrically substituted *Z*-alkenes and Z-alkynes) *via* a [3+2] dipolar cycloaddition leading to fused **PYF** derivatives (Scheme 1).



Scheme 1. Reaction pathway for preparation of pyridazine-fluorine derivatives

The cycloaddition reaction of the ylides **2** with 2,2,2- trifluoroethyl acrylate took place in accordance with our expectation; we obtained a single isomer, the tetrahydropyrrolopyridazine **PYF 3**. Because a single regioisomer is obtained, these types of reactions are considered highly regioselective under charge control in accordance with the usual electronic effects in dipolarophiles: one bond is formed between the ylide carbanion and the non-substituted carbon atom of the dipolarophile and the second bond between the positively charged carbon of the ylide and the substituted carbon of the dipolarophile). For the saturated structure proposed for tetrahydropyrrolopyridazine **PYF 3** the main spectral data confirm the proposed structure.

The structures of the compounds were proved by elemental and spectral analysis [IR, ^{1}H-NMR, ^{13}C NMR, two-dimensional experiments 2D-COSY, 2D-HETCOR (HMQC), long range 2D-HETCOR (HMBC) and MS (ES) - see Supporting Information]. In the ^{1}H NMR spectrum of the **PYF** derivatives **3**, the most informative signals are those of the H_5, H_{6a}, H_{6b}, H_7 and H_{4a} atoms. H_7 appears as a doublet of doublet at 5.60 ppm (**3a**), 5.57 ppm (**3b**) and 5.64 ppm (**3c**), having two different coupling constants ($J_{7,6a}$ = 8.4 Hz, $J_{7,6b}$ = 3.6 Hz), which proves the *E*-configuration to H_{6a} proton and *Z*- configuration to H_{6b} proton. The H_{6a} (dtd) proton which appears at 2.33 ppm (**3a**), 2.32 ppm (**3b**) and 2.32 (**3c**), has three different coupling constants ($J_{6a,6b}$ =13.2 Hz, $J_{6a,7}$ = 8.8 Hz, $J_{6a,5}$ = 4.8 Hz) which proves the *Z*-configuration with respect to H_5. An additional evidence for the assigned structures is the great chemical shifts difference between the protons: H_7 gives the signal around 5.60 ppm, H_{4a} around 4.30 ppm, H_5 around 3.30 ppm, H_{6b} around 2.40 ppm and H_{6a} around 2.30 ppm. In the ^{13}C NMR spectra the carbons C_{4a}, C_5, C_6 and C_7 appear at chemical shifts in accordance with the saturated structures proposed for the adducts **3** (e.g. (**3a**): C_{4a} at 57.18 ppm, C_5 at 50.12 ppm, C_6 at 25.47 ppm and C_7 at 71.19 ppm).

When the dipolarophile was ethyl 4,4,4-trifluorocrotonate (*E*-isomer, non-symmetrically olefine) the reactions involved additional stereo- and regiochemical problems, that is chorochemistry.[7] While for ylides **2a** and **2c** the reaction occur chorospecifically, for ylide **2b** (R = Cl) it occur choroselectively, after flash chromatography and crystallization from an appropriate solvent, we recovered an inseparable mixture of two regisomers (**4b'**and **4b''**, ratio 1:1).

In the ^{1}H NMR spectrum of the tetrahydropyrrolopyridazine **PYF** derivatives **4a** and **4c**, the position of the signals for H_7 protons (d, 5.76 ppm and 5.77 ppm) and the coupling constants ($J_{7,6b}$ = 7.2 Hz) prove the *E*-configuration with $H_{6b.}$ Also, the different coupling constants for the H_{4a} (dd) proton ($J_{4a,5}$ = 8.8 Hz, $J_{4a,4}$ = 5.2 Hz) proves the *Z*-configuration with H_5. In the ^{13}C NMR spectra carbons C_{4a}, C_5, C_6 and C_7 appear at chemical shifts in accordance with the saturated structures proposed for the adducts **4a** and **4c** (**4a**: C_{4a} at 56.40 ppm, C_5 at 48.47 ppm, C_6 at 51.10 ppm and C_7 at 69.54 ppm).

For the **PYF** regisomers **4b'** and **4b''** the structure was elucidated by ^{1}H-NMR, ^{13}C NMR, and two-dimensional experiments 2D-COSY, 2D-HETCOR (HMQC), long range 2D-HETCOR (HMBC) - all signals are recurrent and in accordance with the proposed structures.

The reaction with ethyl 4,4,4-trifluorobutinoate leads to the aromatized pyrrolopyridazine **PYF** derivatives **5**. Aromatisation of the initially hydrogenated diazine **i** occurs spontaneously and could be explained by oxidative dehydrogenation.

For the pyrrolopyridazines **PYF 5**, the main spectral data confirms the proposed structures. The lack of signals until 7.04 ppm and the position of H_4 proton which appears at 8.23-8.21 ppm as a doublet in the proton ^{1}H NMR spectra, combined with the fact that in the ^{13}C NMR spectra the carbons C_{4a}, C_5, C_6 and C_7 appear at high chemical shifts are a good evidence of the proposed structures for the aromatized pyrrolopyridazines **PYF 5**.

The major disadvantages of the synthesis carried out under conventional thermal heating (TH) include long reaction time (180 min), high energy consumption, low to moderate yields, etc. As an alternative route we have performed the synthesis under MW irradiation (it was used a monomode reactor STAR-2, CEM corporation, USA). Table 1 lists the optimized reaction conditions, under TH and MW irradiation.

Using MW irradiation, in liquid phase, the best results were obtained by applying a constant irradiation power (25% of the full power of the magnetron, 800 W) while for phase-transfer catalysis (PTC) the best results were obtained using a constant temperature (50 ^{o}C) and varying the irradiation power. As far as the PTC reactions, a mix of potassium fluoride and N-(*p*-R-phenacyl)-pyridazinium bromides was used as solid phase, while the liquid phase consisted of dipolarophiles dissolved in trioctyl-methyl-ammonium chloride–Aliquat 336.

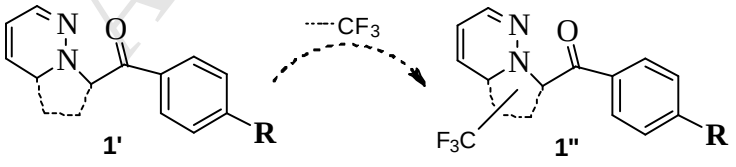
Table 1: The synthesis of **PYF** derivatives under conventional TH and MW irradiation

Compd.	Conventional TH		Microwaves			
			Liquid phase		PTC (KF-Aliquat)	
	Reaction time/min	Yield %	Reaction time/min	Yield %	Reaction time/min	Yield %
3a	180	22	5	16	15	14
3b	180	30	5	24	15	21
3c	180	23	5	19	15	17
4a	180	14	5	10	-	-
4b' + 4b''	180	16	5	11	-	-
4c	180	9	5	7	-	-
5a	180	38	5	59	15	56
5b	180	41	5	59	15	52
5c	180	46	5	68	15	58

As indicated in Table 1, under MW irradiation these reactions could be considered environmentally friendly because the consumed energy decreased considerably, the amount of used solvent in liquid phase is at least five-fold less comparative with conventional TH, while PTC does not use solvents. The reaction time decreases substantially and, in some cases, the yields are higher. We presume that the MW heating approach is more effective in [3+2] dipolar cycloaddition reactions due to two factors: i) the mode of action under MW irradiation, and ii) the structure of the ylide intermediate. It is well known that the magnetic field component of MW radiation is responsible for the dielectric heating effect [19]. The greater the dipole moment of the molecule, the larger the effect of the MW energy will be. The ylides having a 1, 2- dipolar structure are excellent dipoles and, therefore, the efficiency of MW heating increases considerably when compared with TH.

2.2. Design and biological activity

In a previously published paper [11] we proved the antibacterial and antifungal activity of pyridazine derivatives which contain a pyridazine–acetophenone skeleton (**1'**) as pharmacophoric moiety. Considering the antimicrobial potential of fluorine moiety [3-5], we decided to introduce a trifluoromethyl group, in order to combine and increase the respective biological potentials. The synthesized compounds were tested, at a 4 mg/disk concentration, for their *in vitro* antimicrobial activity against different strains of *Gram-positive*, *Gram-*



1' **1"**

negative bacteria and fungus. The primary screen was carried out by the disc diffusion method [22, 23] using a nutrient agar medium (Mueller Hinton agar for antibacterial tests and Sabouraud agar for antifungal tests). Chloramphenicol (antibiotic) and Nysatin (antimycotic) were used as control drugs. Table 2 summarizes the antimicrobial activity of the compounds and control drugs. The results are expressed as zone diameter (mm).

Table 2. The inhibition zone (diameter mean, in mm) as a criteria of antibacterial and antifungal activity for some diazine derivatives described in the text.

Strain→ Product and reference drug ↓	*S. aureus ATCC 25923*	*S. luteea ATCC 9341*	*B. subtillis*	*P. Aeruginosa ATCC 27853*	*E. coli ATCC 25922*	*Candida albicans ATCC 10231*
Chloramphenicol 30 μg/disc	*25*	*30*	*25*	*19*	*29*	-
Nysatin, 100 μg/disc	-	-	-	-	-	*29*
3a (R=F)	28	**55**	31	19	26	27
3b (R=Cl)	30	**58**	33	20	35	**37**
3c (R=CH_3)	30	**59**	**39**	20	28	30
4a (R=F)	28	**58**	**38**	21	31	29
4(b'+b") (R=Cl)	**35**	**61**	**42**	19	38	**39**
4c (R=CH_3)	29	**61**	**38**	21	27	31
5a (R=F)	30	**41**	31	16	34	29
5b (R=Cl)	29	**44**	**38**	18	**42**	26
5c (R=CH_3)	**49**	**60**	**36**	19	**40**	32

The comparative analysis of the data from Table 2, leads to the following main conclusions:

- the **PYF** derivatives have a spectacular nonselective antimicrobial activity against *Gram positive* germs *Sarciria Luteea* and *B. subtillis*;
- against the remaining *Gram-positive* bacteria, compounds bearing a methyl group in *para* position of acetophenone moiety (**3c**, **4c** and **5c**), show a higher activity tendency;
- the **PYF** derivatives **3** and **4** have a better antibacterial activity than **5**;
- compounds bearing a methyl group in *para* position of acetophenone moiety (**3c**, **4c** and **5c**), seem to be more active;
- only the **PYF** derivatives (**3b)** and (**4b'+b''**) have a significant activity against fungus *Candida albicans*.

Taking into consideration the above results, we decided to determine the MICs for compounds bearing a methyl group in *para* position of the acetophenone moiety. The compounds MICs against the sensitive microbial strains were determined using Mueller–Hinton Broth (MHB) culture media in comparison to chloramphenicol [24], Table 3.

Table 3. The antibacterial activities measured by MICs of some diazine derivatives described in the text

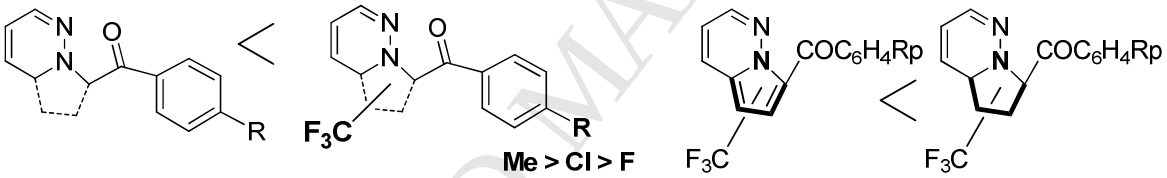
Strain→ Product and reference drug ↓	*S. luteea ATCC 9341*	*B. subtillis*	*S. aureus ATCC 25923*
Chloramphenicol, μg/mL	0.25	0.25	0.25
3c (R=CH_3) , *mg/mL*	0.11	0.18	3.80
4c (R=CH_3) , *mg/mL*	0.09	0.20	4.20
5c (R=CH_3) , *mg/mL*	0.10	0.22	0.16

These results show again that our pyridazine–fluorine derivatives have a good antibacterial activity, especially against *Gram positive* germs *Sarciria Luteea* and *B. subtillis*.

Having in view the above results, the following structure-activity relationships correlations could be done:

- if we compare the antimicrobial activity of **PYF** derivatives, with our previously reported results [11], we can see an increasing effect of the trifluoromethyl group concerning antibacterial activity;

Antibacterial activity



- the **PYF** derivatives with the hydrogenated pyrrolo ring (**3** and **4**) have a better antibacterial activity that their aromatized analogues (**5**);

- there is a certain influence of the isoster R substituent from the *para*- position of the benzoyle ring, the antibacterial activity decreasing in the order Me > Cl > F.

3. Conclusion

Here we report a straightforward method for preparation of new pyridazine–fluorine (**PYF**) derivatives, by using PTC synthesis under MW and TH. Under MW irradiation the reactions could be considered as environmentally friendly, because the consumed energy decreased considerably and the amount of used solvent in liquid phase is at least five-fold less comparative with conventional TH, while PTC does not use solvents. Also, the reaction time decreased substantially under MW and, in some cases, the yields are higher. We presume that the MW efficiency in [3+2] dipolar cycloaddition reactions can be explained by two factors: the mode of action under MW irradiation and the structure of the ylide intermediate. The regiochemistry studies indicate that the reactions occur highly regioselective (a single regioisomer being formed), under charge control. The chorochemistry studies show that the reactions occurs either chorospecifically, either choroselectively, according with the influence of the *para*-R-phenyl-substituent in the 7- position of the **PYF** moiety. The *in vitro* antimicrobial activity of the **PYF** compounds indicated that the introduction of a trifluoromethyl moiety on the pyridazine skeleton is beneficial for antimicrobial activity. Practically, all the **PYF** derivatives have

a spectacular antimicrobial activity against *Gram positive* germs, *Sarciria Luteea* and *B. subtillis*, and very good activity against *Gram negative* germ *S. aureus*. Regarding fungus *Candida albicans* no significant activity of **PYF** derivatives was observed.

4. Experimental protocols

4.1. Chemistry

^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 DRX spectrometer operating at 400/100 MHz, downfield from an internal standard, $SiMe_4$ in $CDCl_3$. Chemical shifts are given in ppm (δ-scale), coupling constants (*J*) in Hz. IR spectra were recorded with a Shimadzu Prestige 8400s FTIR spectrophotometer in KBr. Mass spectral (MS) data were obtained using a Brucker APEX-Q IV 7T system. A monomode reactor (STAR-2, CEM corporation, USA, (800 W) was used for carrying out the reaction under MW. Melting points were determined using a MELTEMP II apparatus and are uncorrected.

4.1.1. General procedure for [3+2] dipolar cycloaddition in the liquid phase

A mixture of cycloimmonium salt **2** (2 mmol) and dipolarophile (2.2 mmol) was suspended in chloroform (50 mL under conventional TH and 10 mL under MW heating). Then, triethylamine (2.2 mmol) was added.
Under conventional TH, the solution was refluxed for 3 h on an oil bath. Using MW irradiation, the best results were obtained using a constant irradiation power (25% from the full power of the magnetron, 800 W) and varying the temperature ('power control'). Under MW heating, the solution mixture was placed in the reaction vessel (Pyrex glass) and exposed to microwaves for 5 min. Once the heating cycle was completed, the tube was cooled to ambient temperature and the resulting mixture was washed thoroughly three times with water, dried with sodium sulfate, filtered and the solvent evaporated. The crude product was purified by chromatography on silica gel with dichloromethane /methanol (99/1) as eluent.

4.1.2. General procedure for [3+2] dipolar cycloaddition in phase-transfer catalysis

The corresponding cycloimmonium salt **2** (2 mmol), dipolarophile (2.2 mmol), KF (2 mmol) and 0.1 ml trioctyl-methyl-ammonium chloride – Aliquat 336, were grounded in an agate mortar until a fine homogeneous mixture was obtained. The mixture was exposed to MW for 15 min; the best results were obtained by applying a constant temperature (50 ^{o}C) and varying the irradiation power ('temperature control'). The resulting mixture was washed thoroughly three times with chloroform. The crude product was purified by chromatography on silica gel with dichloromethane/methanol (99/1) as eluent.

2,2,2-Trifluoroethyl 7-(4-fluorobenzoyl)-4a,5,6,7-tetrahydropyrrolo[1,2-b]pyridazine-5-carboxylate (3a): White crystals, mp: 94-95 ^{o}C. IR (KBr): ν/cm^{-1}: 3092, 2903, 1740, 1686, 1596, 1504, 1481, 1266. ^{1}H NMR (400 MHz, $CDCl_3$): δ_{ppm}: 2.35-2.31 (1H, dd, J_{HH} = 4.4 Hz, 8.4 Hz, 13.2 Hz, H_{6a}), 2.45-2.42 (1H, dd, J_{HH} = 3.6 Hz, 8.8 Hz, 13.2 Hz, H_{6b}), 3.32-3.26 (1H, ddd, J_{HH} = 3.6 Hz, 4.4 Hz, 8.4 Hz, H_5), 4.26-4.23

(1H, dd, J_{HH} = 4.8 Hz, 7.6 Hz, H_{4a}), 4.39-4.34 (1H, dd, J_{HH} = 8.4 Hz, 12.8 Hz, $H_{5'}$), 4.56-4.51 (1H, dd, J_{HH} = 8.4 Hz, 12.8 Hz, $H_{5'}$), 5.61-5.58 (1H, dd, J_{HH} = 3.2 Hz, 8.4 Hz, H_7), 5.87-5.84 (1H, dd, J_{HH} = 3.6 Hz, 10.0 Hz, H_3), 6.00-5.96 (1H, dd, J_{HH} = 4.8 Hz, 10.0 Hz, H_4), 6.69-6.68 (1H, dd, J_{HH} = 3.2 Hz, H_2), 7.17-7.13 (2H, dd, J_{HH} = 8.8 Hz, H_{11}), 8.21-8.18 (2H, dd, J_{HH} = 8.8 Hz, H_{10}). ^{13}C NMR (100 MHz, $CDCl_3$): δ_{ppm}: 25.47 (C_6), 50.12 (C_5), 57.18 (C_{4a}), 61 (CH_2-5'), 71.19 (C_7), 115.91-115.69 (C_{11}), 118.67 (C_3), 121.52 (CF_3), 124.27 (C_4), 131.56 (C_{12}), 132.17 (C_{10}), 134.07 (C_2), 164.76 (C_9), 172.13 ($C_{5'}$, keto ester), 195.01 (C_8, keto). Anal. Calcd. $C_{17}H_{14}F_4N_2O_3$: C, 55.14; H, 3.81; N, 7.57; Found: C, 55.19; H, 3.77; N, 7.41. MS (ESI) m/z: 371 (M+1), 372 (M+2), 393 ($M+Na^+$), 394 [$(M+1)+Na^+$], 763 ($2M+Na^+$).

2,2,2-Trifluoroethyl 7-(4-chlorobenzoyl)-4a,5,6,7-tetrahydropyrrolo[1,2-b]pyridazine-5-carboxylate (3b): Yellow crystals, mp: 118-120 ^{o}C. IR (KBr): ν/cm^{-1}: 3094, 2914, 1746, 1687, 1592, 1498, 1266. ^{1}H NMR (400 MHz, $CDCl_3$): δ_{ppm}: 2.34-2.31 (1H, dd, J_{HH} = 4.8 Hz, 8.8 Hz, 13.2 Hz, H_{6a}), 2.44-2.41 (1H, dd, J_{HH} = 3.6 Hz, 8.8 Hz, 13.2 Hz, H_{6b}), 3.31-3.26 (1H, ddd, J_{HH} = 3.6 Hz, 4.4 Hz, 8.4 Hz, H_5), 4.24-4.21 (1H, dd, J_{HH} = 4.8 Hz, 7.2 Hz, H_{4a}), 4.39-4.34 (1H, dd, J_{HH} = 8.4 Hz, 12.8 Hz, $H_{5'}$), 4.56-4.50 (1H, dd, J_{HH} = 8.4 Hz, 12.8 Hz, $H_{5'}$), 5.58-5.55 (1H, dd, J_{HH} = 3.2 Hz, 8.4 Hz, H_7), 5.86-5.83 (1H, dd, J_{HH} = 2.4 Hz, 10.0 Hz, H_3), 5.99-5.95 (1H, dd, J_{HH} = 6.4 Hz, 10.0 Hz, H_4), 6.68-6.67 (1H, d, J_{HH} = 3.2 Hz, H_2), 7.47-7.44 (2H, d, J_{HH} = 8.8 Hz, H_{11}), 8.11-8.09 (2H, dd, J_{HH} = 8.4 Hz, H_{10}). ^{13}C NMR (100 MHz, $CDCl_3$): δ_{ppm}: 25.31 (C_6), 49.84 (C_5), 57.19 (C_{4a}), 61 (CH_2-5'), 71.24 (C-7), 118.68 (C_3), 121.72 (CF_3), 125.13 (C_4), 128.39 (C_{11}), 134.29 (C_{10}), 131.42 (C_{12}), 134.29 (C_2), 146.07 (C_9), 172.45 ($C_{5'}$, keto ester), 195.12 (C_8, keto). Anal. Calcd. $C_{17}H_{14}ClF_3N_2O_3$: C, 52.79; H, 3.65; N, 7.24; Found: C, 52.77; H, 3.67; N, 7.18.

2,2,2-Trifluoroethyl 7-(4-methylbenzoyl)-4a,5,6,7-tetrahydropyrrolo[1,2-b]pyridazine-5-carboxylate (3c): Yellow crystals, mp: 147-149 ^{o}C. IR (KBr): ν/cm^{-1}: 3092, 2914, 1741, 1679, 1607, 1503, 1436, 1271. ^{1}H NMR (400 MHz, $CDCl_3$): δ_{ppm}: 2.41 (3H, s, CH_3-12), 2.36-2.34 (1H, dd, J_{HH} = 4.8 Hz, 8.0 Hz, 13.2 Hz, H_{6a}), 2.39-2.37 (1H, dd, J_{HH} = 4.0 Hz, 8.8 Hz, 13.2 Hz, H_{6b}), 3.31-3.26 (1H, ddd, J_{HH} = 3.2 Hz, 4.8 Hz, 8.4 Hz, H_5), 4.33-4.30 (1H, dd, J_{HH} = 4.8 Hz, 8.0 Hz, H_{4a}), 4.42-4.34 (1H, dd, J_{HH} = 8.4 Hz, 12.8 Hz, $H_{5'}$), 4.58-4.48 (1H, dd, J_{HH} = 8.4 Hz, 12.8 Hz, $H_{5'}$), 5.65-5.62 (1H, dd, J_{HH} = 4.0 Hz, 8.4 Hz, H_7), 5.87-5.83 (1H, dd, J_{HH} = 3.2 Hz, 10.0 Hz, H_3), 5.99-5.95 (1H, dd, J_{HH} = 5.2 Hz, 10.0 Hz, H_4), 6.92-6.68 (1H, dd, J_{HH} = 3.2 Hz, H_2), 7.29-7.26 (2H, d, J_{HH} = 8.0 Hz, H_{11}), 8.05-8.03 (2H, dd, J_{HH} = 8.0 Hz, H_{10}). ^{13}C NMR (100 MHz, $CDCl_3$): δ_{ppm}: 21.66 (CH_3-12), 27.56 (C_6), 43.71 (C_5), 57.31 (C_{4a}), 61 (CH_2-5'), 70.92 (C_7), 117.08 (C_3), 121.65 (CF_3), 124.84 (C_4), 129.47 (C_{11}), 134.95 (C_{10}), 133.52 (C_{12}), 134.70 (C_2), 145.38 (C_9), 172.03 ($C_{5'}$, keto ester), 195.26 (C_8, keto). Anal. Calcd. $C_{18}H_{17}F_3N_2O_3$: C, 59.02; H, 4.68; N, 7.65; Found: C, 59.11; H, 4.61; N, 7.55. MS (ESI) m/z: 389 ($M+Na^+$), 390 [$(M+1)+Na^+$], 755 ($2M+Na^+$), 756 [$(2M+1)+Na^+$].

Ethyl 7-(4-fluorobenzoyl)-5-(trifluoromethyl)-4a,5,6,7-tetrahydropyrrolo[1,2-b] pyridazine -6-carboxylate (4a): White crystals, mp: 141-142 ^{o}C. IR (KBr): ν/cm^{-1}: 3090, 2981, 1730, 1684, 1590, 1502, 1470, 1262. ^{1}H NMR (400 MHz, $CDCl_3$): δ_{ppm}: 1.29-1.26 (3H, t, J_{HH} = 7.2 Hz, CH_3-6'), 3.63-3.57 (1H, dd, J_{HH} = 8.8 Hz, H_6), 3.67-3.63 (1H, dd, J_{HH} = 8.8 Hz, H_5), 4.22-4.17 (2H, q, J_{HH} = 7.2 Hz, $CH_{26'}$), 5.02-4.99 (1H, dd, J_{HH} = 5.2 Hz, 8.4 Hz, H_{4a}), 5.77-5.75 (1H, d, J_{HH} = 7.2 Hz, H_7), 5.88-5.85 (1H, dd, J_{HH}

= 3.2 Hz, 9.6 Hz, H_3), 5.96-5.92 (1H, dd, J_{HH} = 5.2 Hz, 10.0 Hz, H_4), 6.78-6.77 (1H, d, J_{HH} = 3.2 Hz, H_2), 7.29-7.27 (2H, dd, J_{HH} = 8.0 Hz, H_{11}), 7.95-7.92 (2H, dd, J_{HH} = 8.0 Hz, H_{10}). ^{13}C NMR (100 MHz, $CDCl_3$): δ_{ppm}: 14.30 (CH_3-5'), 48 (C_5), 51.10 (C_6), 56.40 (C_{4a}), 61.49 (CH_2-6'), 69.54 (C_7), 118.67 (C_3), 124.38 (C_4), 127.58 (CF_3), 128.11 (C_{11}), 128.99 (C_{10}), 136.61 (C_2), 144.95 (C_{12}), 158.27 (C_9), 171.39 ($C_{5'}$, keto ester), 194.93 (C_9, keto). Anal. Calcd. $C_{18}H_{16}F_4N_2O_3$: C, 56.25; H, 4.20; N, 7.29; Found: C, 56.31; H, 4.13; N, 7.21. MS (ESI) m/z: 407 ($M+Na^+$), 791 ($2M+Na^+$).

Ethyl 7-(4-chlorobenzoyl)-5-(trifluoromethyl)-4a,5,6,7-tetrahydropyrrolo[1,2-b] pyridazine-6-carboxylate (4b'): White crystals, mp: 117-118 ^{o}C. IR (KBr): ν/cm^{-1}: 1725, 1696, 1306, 1273, 1249, 1216, 1207, 1159 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ_{ppm}: 1.27-1.23 (3H, t, J_{HH} = 6.4 Hz, CH_3-6'), 3.63-3.56 (1H, dd, J_{HH} = 9.2 Hz, 8.8 Hz, H_6), 3.69-3.63 (1H, dd, J_{HH} = 9.2 Hz, 8.8 Hz, H_5), 4.22-4.19 (2H, q, J_{HH} = 7.2 Hz, CH_2-6'), 4.38-4.34 (1H, dd, J_{HH} = 8.4 Hz, 5.6 Hz, H_{4a}), 4.75-4.72 (1H, dd, J_{HH} = 9.2 Hz, H_3), 5.89-5.86 (1H, dd, J_{HH} = 8.0 Hz, 4.0 Hz, H_7), 5.97-5.94 (1H, dd, J_{HH} = 10.0 Hz, 4.8 Hz, H_4), 6.79-6.67 (1H, d, J_{HH} = 7.2 Hz, H_2), 7.45-7.43 (2H, d, J_{HH} = 8.0 Hz, H_{11}), 7.99-7.97 (2H, d, J_{HH} = 8.4 Hz, H_{10}). ^{13}C NMR (100 MHz, $CDCl_3$): δ_{ppm}: 14.18 (CH_3-6'), 44.34 (CH_2-6'), 50.18 (C_6), 56.36 (C_5), 61.60 (C_{4a}), 69.65 (C_7), 118.69 (C_3), 124.30 (CF_3), 124.89 (C_4), 128.94 (C_{11}), 134.24 (C_{10}), 134.26 (C_{12}), 136.85 (C_2), 139.90 (C_9), 170.44 ($C_{6'}$, keto ester), 190.33 (C_8, keto). MS (ESI) m/z: 423 ($M+Na^+$), 425 [$(M+2)+Na^+$], 425 [$(M+3)+Na^+$], 823 ($2M+Na^+$), 824 [$(2M+1)+Na^+$].

Ethyl 7-(4-chlorobenzoyl)-5-(trifluoromethyl)-4a,5,6,7-tetrahydropyrrolo[1,2-b] pyridazine-6-carboxylate (4b''): White crystals, mp: 117-118 ^{o}C. IR (KBr): ν/cm^{-1}: 1725, 1696, 1306, 1273, 1249, 1216, 1207, 1159 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ_{ppm}: 1.30 -1.27 (3H, t, J_{HH} = 6.4 Hz, CH_3-5'), 3.25-3.21 (1H, dd, J_{HH} = 8.4 Hz, 5.2 Hz, H_6), 4.17-4.13 (1H, dd, J_{HH} = 7.6 Hz, 3.6 Hz, H_5), 4.26-4.22 (2H, q, J_{HH} = 7.2 Hz, CH_2-5'), 4.96-4.93 (1H, dd, J_{HH} = 8.8 Hz, 4.8 Hz, H_{4a}), 5.72-5.71 (1H, dd, J_{HH} = 7.2 Hz, H_3), 5.91-5.89 (1H, dd, J_{HH} = 8.0 Hz, 4.0 Hz, H_7), 6.21-6.18 (1H, dd, J_{HH} = 9.6 Hz, 5.6 Hz, H_4), 6.79-6.67 (1H, d, J_{HH} = 7.2 Hz, H_2), 7.47-7.45 (2H, d, J_{HH} = 8.0 Hz, H_{11}), 8.16-8.14 (2H, d, J_{HH} = 7.6 Hz, H_{10}). ^{13}C NMR (100 MHz, $CDCl_3$): δ_{ppm}: 14.29 (CH_3-5'), 48.38 (CH_2-5'), 50.97 (C_6), 57.76 (C_5), 61.60 (C_{4a}), 70.59 (C_7), 118.78 (C_3), 128.15 (CF_3), 126.46 (C_4), 129.15 (C_{11}), 130.66 (C_{10}), 134.38 (C_{12}), 137.21 (C_2), 140.52 (C_9), 171.22 (C_5, keto ester), 193.87 (C_8, keto). MS (ESI) m/z: 423 ($M+Na^+$), 425 [$(M+2)+Na^+$], 425 [$(M+3)+Na^+$], 823 ($2M+Na^+$), 824 [$(2M+1)+Na^+$].

Ethyl 7-(4-methylbenzoyl)-5-(trifluoromethyl)-4a,5,6,7-tetrahydropyrrolo[1,2-b] pyridazine-6-carboxylate (4c): Beige crystals, mp: 140-142 ^{o}C. IR (KBr): ν/cm^{-1}: 3094, 2989, 1725, 1696, 1598, 1480, 1257. ^{1}H NMR (400 MHz, $CDCl_3$): δ_{ppm}: 1.29-1.22 (3H, t, J_{HH} = 7.2 Hz, CH_3-6'), 2,41 (3H, s, CH_3-12), 3.61-3.57 (1H, dd, J_{HH} = 8.8 Hz, H_6), 3.68-3.63 (1H, dd, J_{HH} = 8.4 Hz, H_5), 4.22-4.17 (2H, q, J_{HH} = 7.2 Hz, CH_2-6'), 5.03-4.99 (1H, dd, J_{HH} = 5.2 Hz, 8.8 Hz, H_{4a}), 5.78-5.76 (1H, d, J_{HH} = 7.2 Hz, H_7), 5.88-5.85 (1H, dd, J_{HH} = 3.2 Hz, 10.0 Hz, H_3), 5.96-5.92 (1H, dd, J_{HH} = 4.8 Hz, 9.6 Hz, H_4), 6.78-6.77 (1H, d, J_{HH} = 3.2 Hz, H_2), 7.29-7.27 (2H, d, J_{HH} = 8.4 Hz, H_{11}), 7.95-7.93 (2H, d, J_{HH} = 8.4 Hz, H_{10}). ^{13}C NMR (100 MHz, $CDCl_3$): δ_{ppm}: 14.29 (CH_3-5'), 21.73 (CH_3-12), 48.46-47.59 (C_5), 51.08 (C_6), 56.40 (C_{4a}), 61.49 (CH_2-6'), 69.53 (C_7), 118.66 (C_3), 124.86 (C_4), 128.10 (CF_3), 128.98 (C_{11}), 129.30 (C_{10}), 133.50 (C_{12}), 136.62 (C_2), 150.75 (C_9), 171.39 ($C_{5'}$, keto ester), 194.68 (C_9, keto). Anal. Calcd. $C_{19}H_{19}F_3N_2O_3$: C,

60.00; H, 5.03; N, 7.36; Found: C, 60.08; H, 5.00; N, 7.31. MS (ESI) m/z: 403 ($M+Na^+$), 404 [$(M+2)+Na^+$], 783 ($2M+Na^+$), 784 [$(2M+1)+Na^+$].

Ethyl 7-(4-fluorobenzoyl)-5-(trifluoromethyl)pyrrolo[1,2-b]pyridazine-6-carboxylate (5a): Yellow crystals, mp: 104-106 ^oC. IR (KBr): ν/cm^{-1}: 3106, 2958, 1690, 1669, 1599, 1506, 1457, 1457, 1217. ^1H NMR (400 MHz, $CDCl_3$): δ_{ppm}: 1.44-1.41 (3H, t, J_{HH} = 7.2 Hz, CH_3-6'), 4.46-4.41 (2H, q, J_{HH} = 7.2 Hz, CH_2-6'), 7.04-7.00 (1H, dd, J_{HH} = 4.4 Hz, 9.2 Hz, H_3), 7.16-7.12 (2H, d, J_{HH} = 8.8 Hz, H_{11}), 7.86-7.82 (2H, d, J_{HH} = 8.8 Hz, H_{10}), 8.23-8.21 (1H, dd, J_{HH} = 4.4 Hz, H_4), 8.67-8.64 (1H, dd, J_{HH} = 9.2 Hz, H_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ_{ppm}: 14.13 (CH_3-6'), 60.95 (CH_2-6'), 102.45 (C_6), 116.36 (C_{11}), 116.81 (C_3), 117.18 (C_5), 123 (CF_3), 126.35 (C_7), 129.28 (C-4), 130.48 (C_{4a}), 132 (C_{10}), 133.22 (C_{12}), 145.12 (C_2), 162,20 (C_9), 167.90 ($C_{6'}$, keto ester), 185.80 (C_8, keto). Anal. Calcd. $C_{18}H_{12}F_4N_2O_3$: C, 56.85; H, 3.18; N, 7.37; Found: C, 56.96; H, 3.11; N, 7.24. MS (ESI) m/z: 381 (M+1), 423 ($M+Na^+$), 783 ($2M+Na^+$).

Ethyl 7-(4-chlorobenzoyl)-5-(trifluoromethyl)pyrrolo[1,2-b]pyridazine-6-carboxylate (5b): White crystals, mp: 124-126 ^oC. IR (KBr): ν/cm^{-1}: 3100, 2960, 1705, 1676, 1585, 1502, 1467, 1218. ^1H NMR (400 MHz, $CDCl_3$): δ_{ppm}: 1.45-1.41 (3H, t, J_{HH} = 8.0 Hz, CH_3-6'), 4.47-4.41 (2H, q, J_{HH} = 8.0 Hz, CH_2-6'), 7.05-7.01 (1H, dd, J_{HH} = 4.0 Hz, 8.0 Hz, H_3), 7.46-7.44 (2H, d, J_{HH} = 8.0 Hz, H_{11}), 7.77-7.75 (2H, d, J_{HH} = 8.0 Hz, H_{10}), 8.23-8.22 (1H, dd, J_{HH} = 4.0 Hz, H_4), 8.69-8.66 (1H, dd, J_{HH} = 8.0 Hz, H_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ_{ppm}: 14.12 (CH_3-6'), 60.97 (CH_2-6'), 102.47 (C_6), 116.66 (C_3), 117.18 (C_5), 126.09 (CF_3), 126.09 (C_7), 129.30 (C_4), 129.36 (C_{11}), 130.52 (C_{4a}), 130.91 (C_{10}), 135.08 (C_{12}), 145.14 (C_9), 145.10 (C_2), 162.20 ($C_{6'}$, keto ester), 186.16 (C_8, keto). Anal. Calcd. $C_{18}H_{12}ClF_3N_2O_3$: C, 54.49; H, 3.05; N, 7.06; Found: C, 54.96; H, 3.00; N, 6.92. MS (ESI) m/z: 419 ($M+Na^+$), 815 ($2M+Na^+$).

Ethyl 7-(4-methylbenzoyl)-5-(trifluoromethyl)pyrrolo[1,2-b]pyridazine-6-carboxylate (5c): White crystals, mp: 138-139 ^oC. IR (KBr): ν/cm^{-1}: 3194, 2958, 1706, 1674, 1600, 1502, 1444, 1220. ^1H NMR (400 MHz, $CDCl_3$): δ_{ppm}: 1.26 -1.21 (3H, t, J_{HH} = 7.2 Hz, CH_3-6'), 2.41 (3H, s, CH_3-12), 4.46-4.41 (2H, q, J_{HH} = 7.2 Hz, CH_2-6'), 7.01-6.98 (1H, dd, J_{HH} = 4.4 Hz, 9.2 Hz, H_3), 7.27-7.25 (2H, d, J_{HH} = 8.4 Hz, H_{11}), 7.72-7.70 (2H, d, J_{HH} = 8.4 Hz, H_{10}), 8.21-8.19 (1H, dd, J_{HH} = 4.0 Hz, H_4), 8.66-8.63 (1H, dd, J_{HH} = 9.2 Hz, H_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ_{ppm}: 14.13 (CH_3-6'), 21.86 (CH_3-12), 60.87 (CH_2-6'), 102.20 (C_6), 116.50 (C_3), 117.18 (C_5), 126.95 (CF_3), 126.93 (C_7), 129.18 (C_4), 129.70 (C_{11}), 129.76 (C_{10}), 130.35 (C_{4a}), 134.26 (C_{12}), 145.01 (C_2), 145.81 (C_9), 162.31 ($C_{6'}$, 'keto ester), 187.02 (C_8, keto). Anal. Calcd. $C_{19}H_{15}F_3N_2O_3$: C, 60.64; H, 4.02; N, 7.44; Found: C, 60.66; H, 3.98; N, 7.39. MS (ESI) m/z: 399 ($M+Na^+$), 401 [$(M+2)+Na^+$], 775 ($2M+Na^+$), 776 [$(2M+1)+Na^+$].

4.2. Microbiology

Agar diffusion assay

The antibacterial and antifungal activity of the compounds were determined by the disc diffusion method [22, 23]. The microbial suspension (0.5 McFarland turbidity standard) was mixed (1/10 ratio) with agar nutrient (Mueller Hinton agar for antibacterial tests, respectively Sabouraud agar for antifungal tests). Sterile filter paper discs (5 mm diameter), moistened with the test compound solution in chloroform of

specific concentration (4 mg/disc), were carefully placed on the agar culture plates [filter paper discs were previously introduced in sterile stainless steel cylinders (5 mm internal diameter, 10 mm height)]. After 24 h of incubation at 30 °C for bacteria and 48 h at 28 °C for fungi, the diameter of the inhibition zone (mm) was measured (Table 2). Chloramphenicol and nysatin were purchased from the market (Himedia, Mumbai, India) and used in a concentration of 30 μg/disc, respectively 100 μg/disc as control drugs for antibacterial and antifungal activities.

Broth microdilution assay

The minimum inhibitory concentrations (MICs) were determined using a broth microdilution assay [24]. The microbial suspensions were adjusted to the turbidity of a 0.5 McFarland standard. The test compound were dissolved in 50% dimethylsulfoxide at a concentration of 250 mg/mL and then subjected (in the well plates) to a serial of two-fold dilution in Mueller Hinton broth (antibacterial tests, concentration range from 250 to 0.12 mg/mL; final volume in each well 100 μL). Other 95 μL of broth and 5 μL of microbial inoculums were further dispensed into each well. The plates were incubated at 37 °C for 24 h. The test compounds were screened twice against each microorganism. The lowest concentration of the tested compound that visibly inhibited the growth of the microorganisms was recorded as the MIC. The MIC values of chloramphenicol (μg/mL) towards bacteria strains were also evaluated.

Acknowledgements

This work was co-financed from the European Social Fund Sectoral Operational Human Resources Development 2007-2013, project number POSDRU/I.89/1.5/S62371 „Postdoctoral School in Agriculture and Veterinary Medicine area".

Supporting information

Supporting information associated with this article could be found, in the online version, at……..

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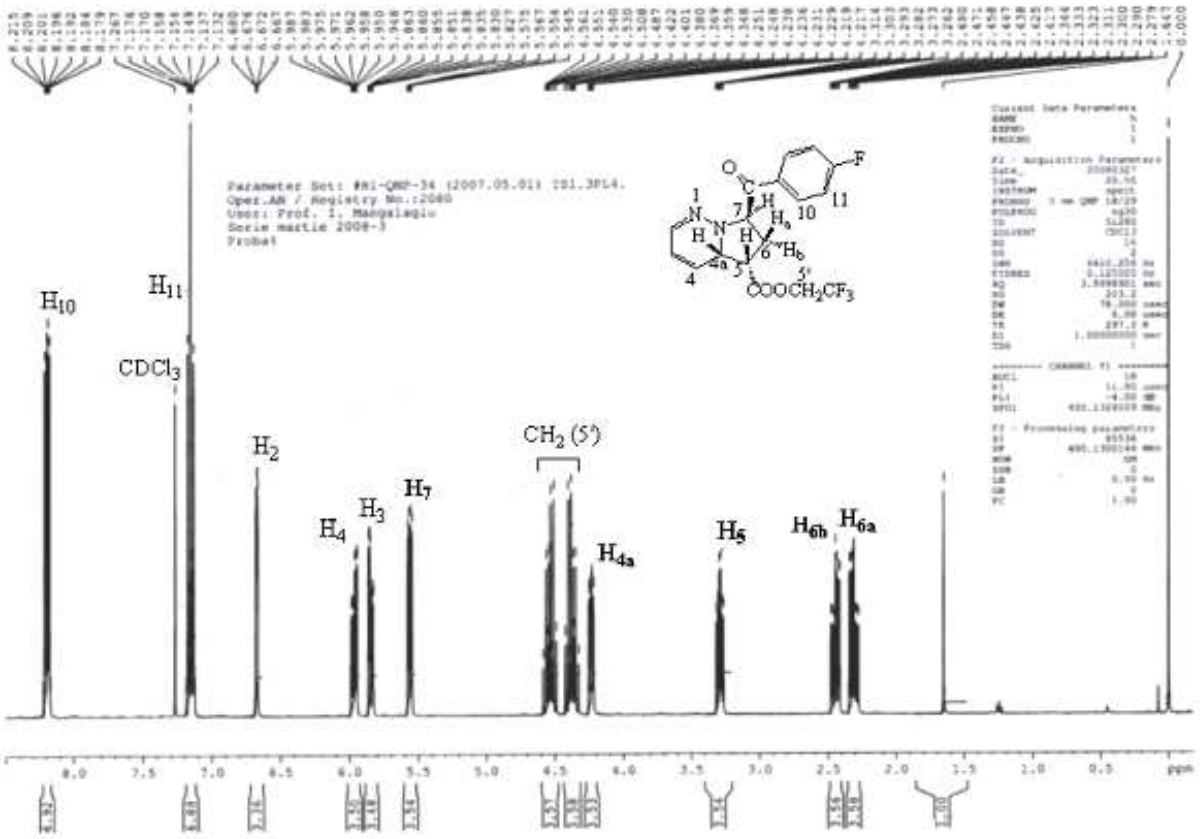
New Pyridazine–Fluorine Derivatives: Synthesis, Chemistry and Biological Activity. Part II

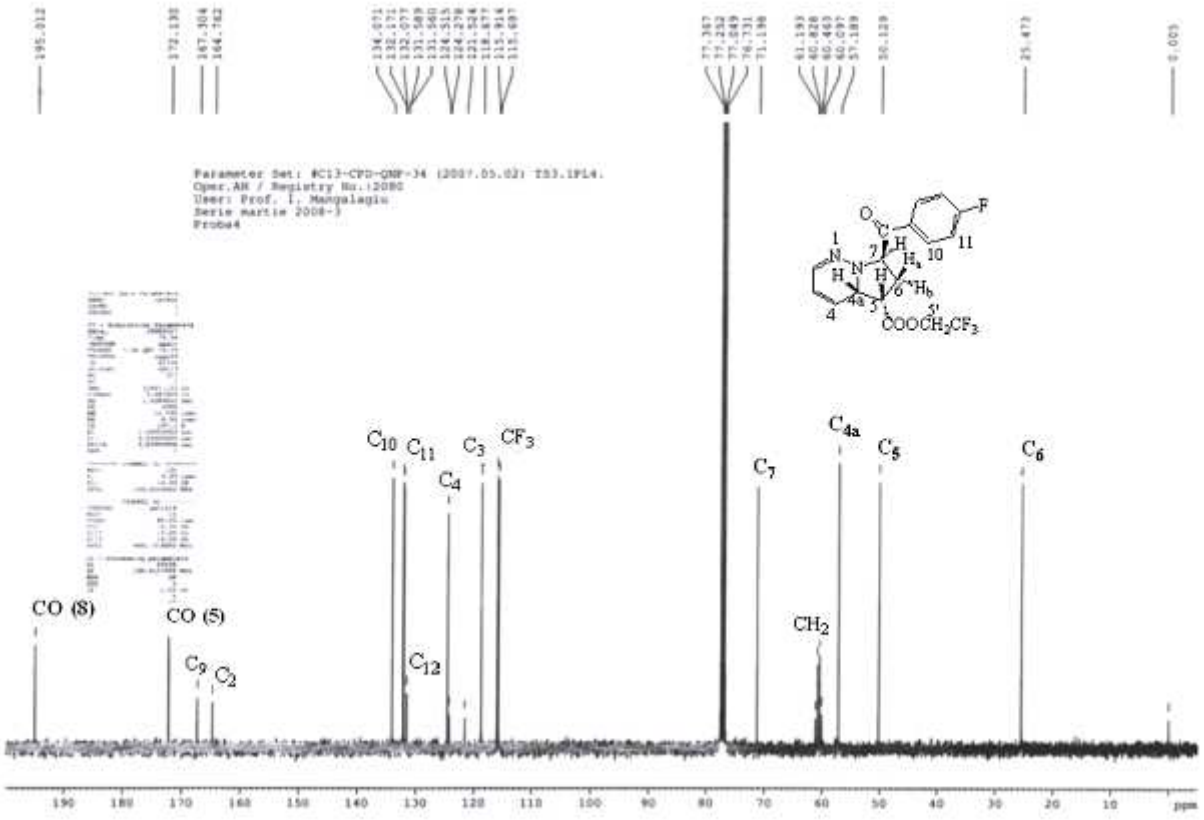
Roxana-Angela Tucaliuc,[1] Valeriu V. Cotea,[1] Marius Niculaua,[1] Cristina Tuchilus,[2] Dorina Mantu,[3] Ionel I. Mangalagiu[4]*

1. University of Agriculture and Veterinary Medicine "Ion Ionescu de la Brad" Iasi, Aleea Mihail Sadoveanu, Iasi, Romania,
2. "Gr.T.Popa" University of Medicine and Pharmacy, Iasi, School of Pharmacy, Str. Universitatii 16, 700115 Iasi, Romania.
3. "Al. I. Cuza" University of Iasi, Organic Chemistry Department, Bd. Carol 11, 700506 Iasi, Romania; e-mail: ionelm@uaic.ro

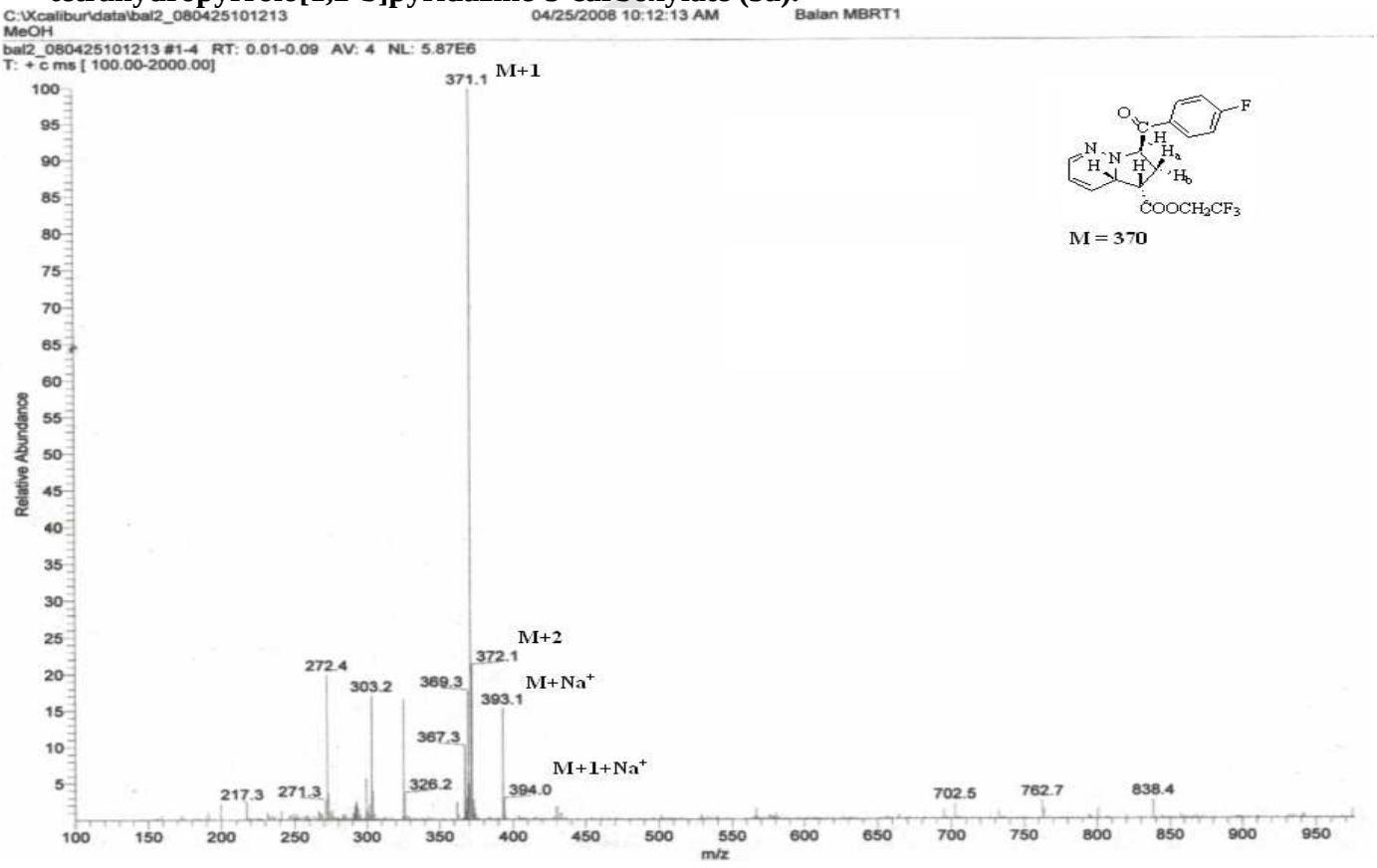
1. ^{1}H NMR spectrum of compound 2,2,2-Trifluoroethyl 7-(4-fluorobenzoyl)-4a,5,6,7-tetrahydropyrrolo[1,2-b]pyridazine-5-carboxylate (3a).



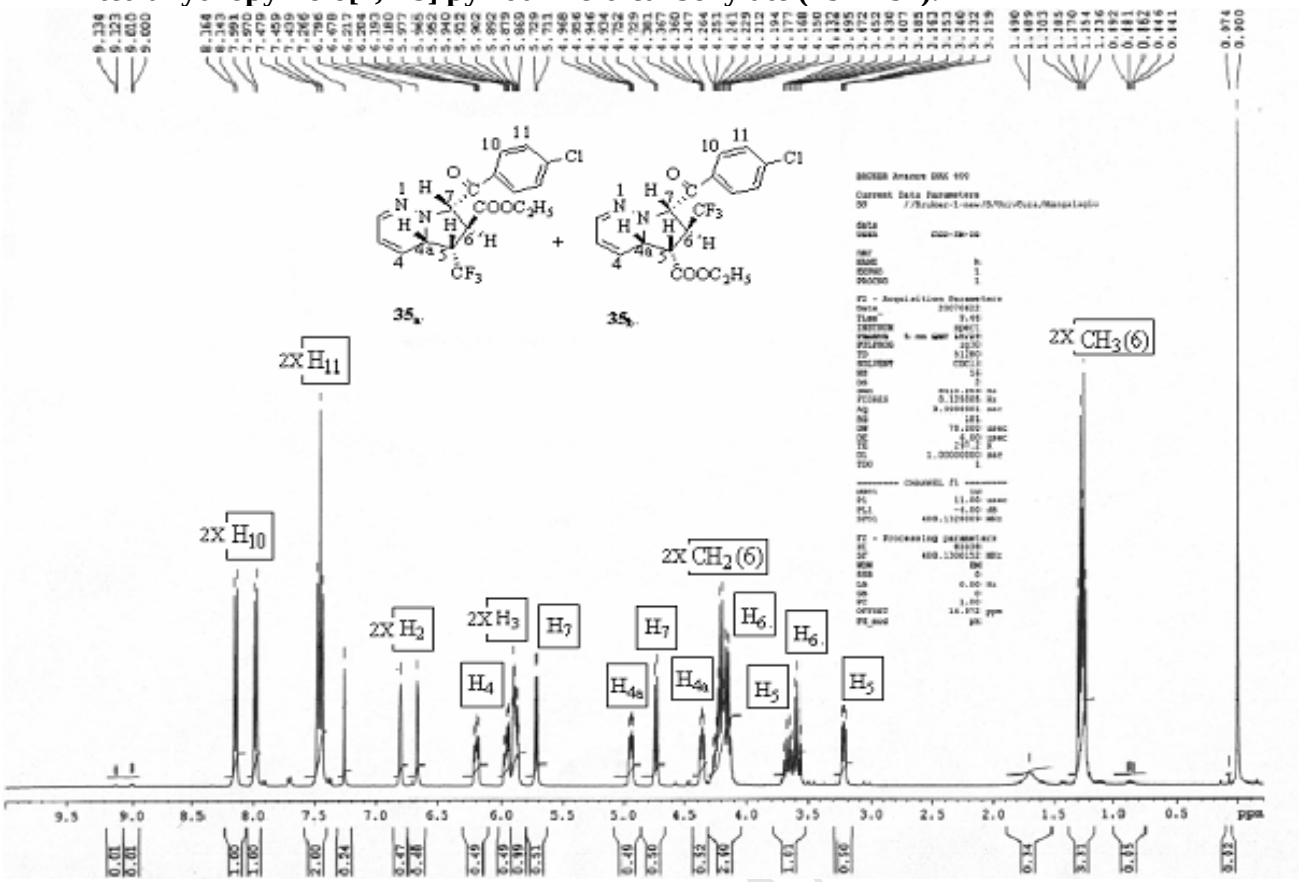
2. ^{13}C NMR spectrum of compound 2,2,2-Trifluoroethyl 7-(4-fluorobenzoyl)-4a,5,6,7-tetrahydropyrrolo[1,2-b]pyridazine-5-carboxylate (3a).



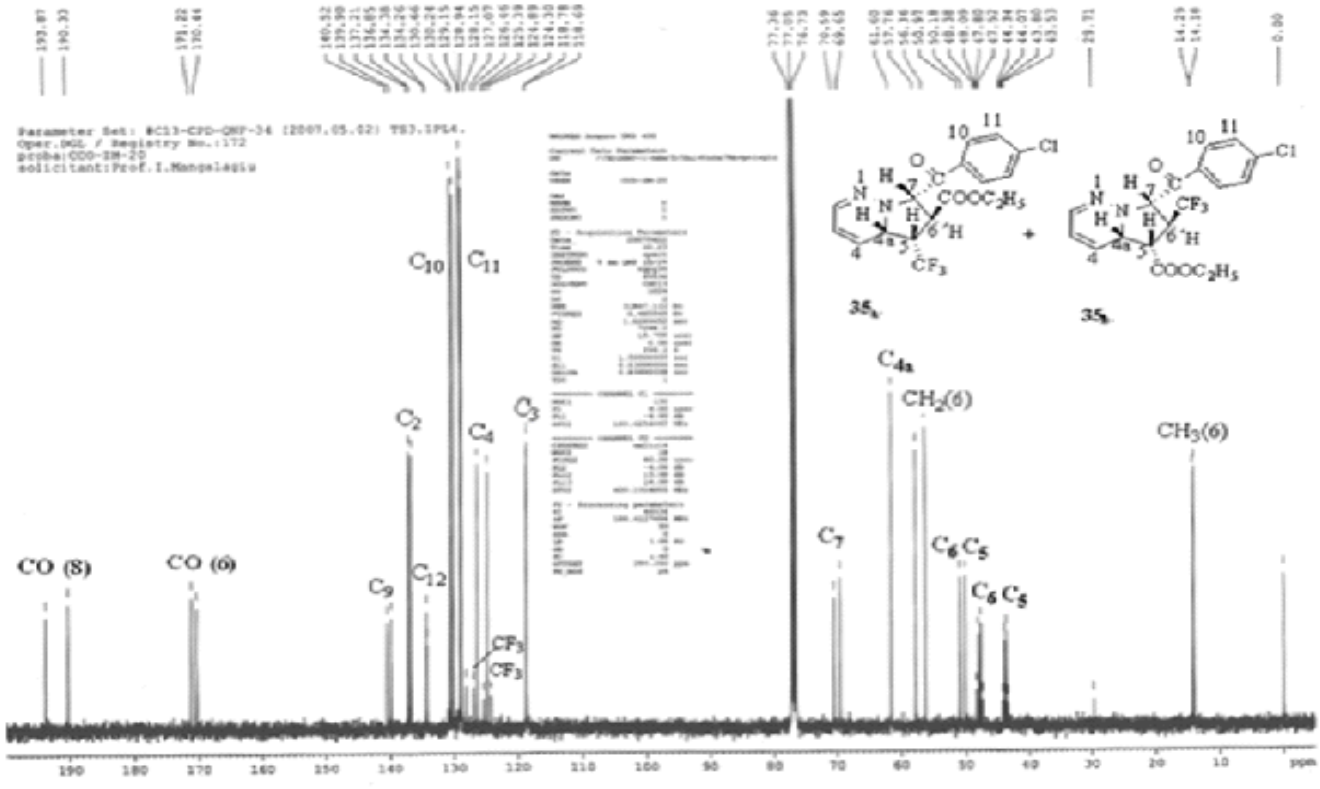
3. **ES-MS spectrum of compound 2,2,2-Trifluoroethyl 7-(4-fluorobenzoyl)-4a,5,6,7-tetrahydropyrrolo[1,2-b]pyridazine-5-carboxylate (3a).**



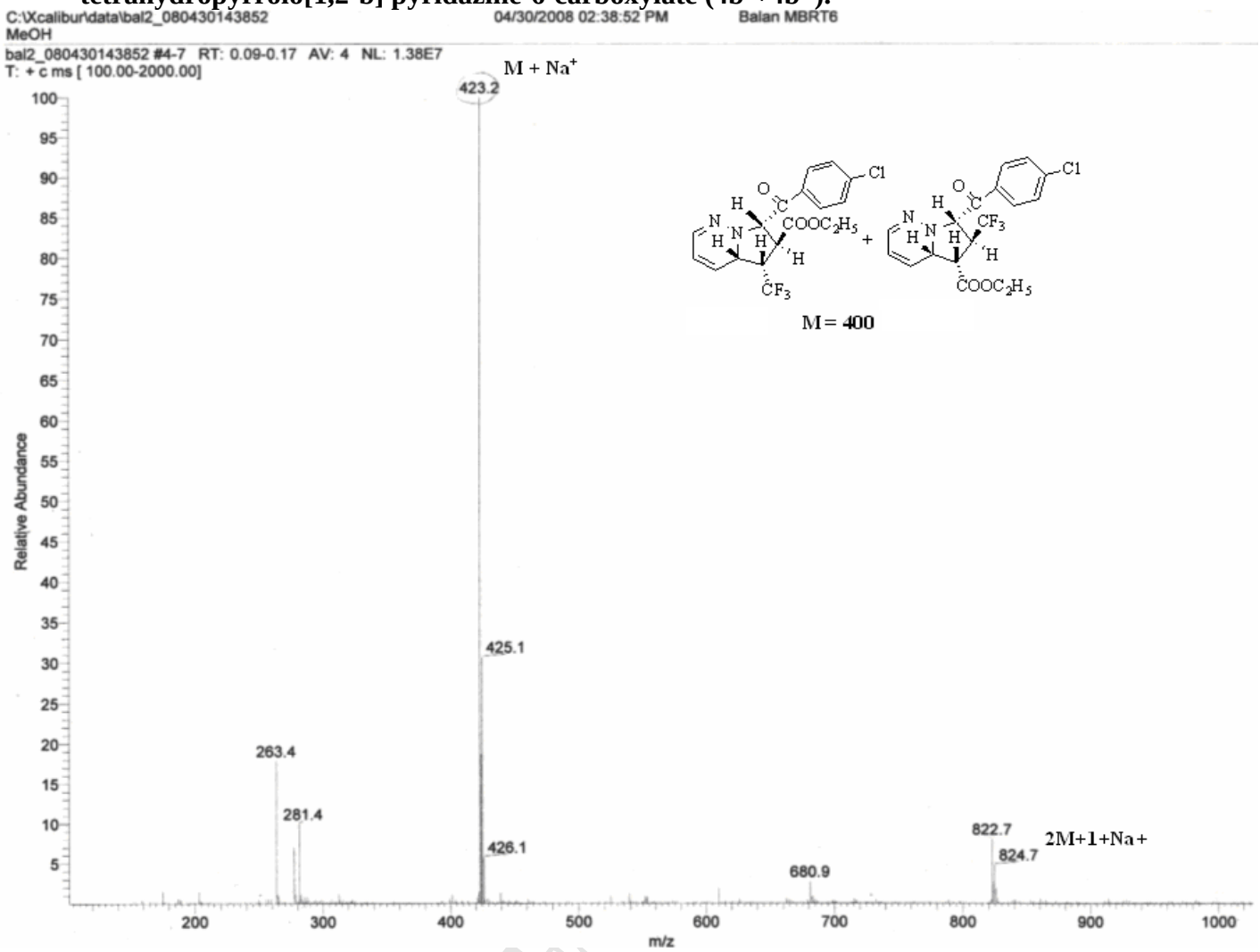
4. **^{1}H NMR spectrum of compounds Ethyl 7-(4-chlorobenzoyl)-5-(trifluoromethyl)-4a,5,6,7-tetrahydropyrrolo[1,2-b] pyridazine-6-carboxylate (4b'+4b").**



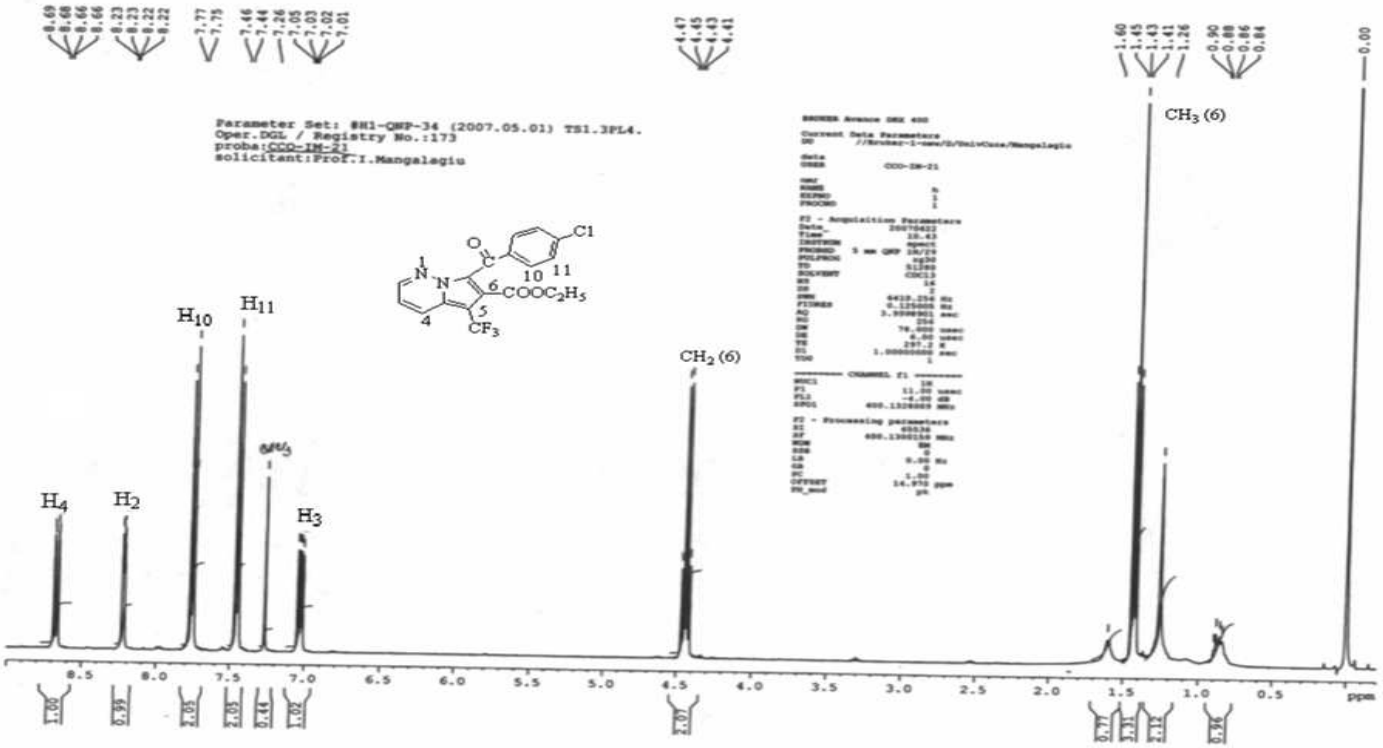
5. **^{13}C NMR spectrum of compounds Ethyl 7-(4-chlorobenzoyl)-5-(trifluoromethyl)-4a,5,6,7-tetrahydropyrrolo[1,2-b] pyridazine-6-carboxylate (4b'+4b").**



6. **ES/MS- spectrum of compounds Ethyl 7-(4-chlorobenzoyl)-5-(trifluoromethyl)-4a,5,6,7-tetrahydropyrrolo[1,2-b] pyridazine-6-carboxylate (4b'+4b").**



7. **^{1}H NMR spectrum of compounds Ethyl 7-(4-chlorobenzoyl)-5-(trifluoromethyl)pyrrolo[1,2-b]pyridazine-6-carboxylate (5b)**



8. **^{13}C NMR spectrum of compounds Ethyl 7-(4-chlorobenzoyl)-5-(trifluoromethyl)pyrrolo[1,2-b]pyridazine-6-carboxylate (5b)**