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Article

Synthesis and Anti-Platelet Evaluation of New Tricyclic PAF Antagonists, Designed as Structurally Related to Hetrazepine Class - Web 2086

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No âmbito de um programa de pesquisas que visa a síntese e a avaliação farmacológica de novos agentes antitrombóticos explorando princípios racionais de modificação molecular, descrevemos neste trabalho a síntese de duas séries de amidas (3a-f) e (4) derivadas do novo sistema heteroaromático tricíclico pirazolo[3,4-b]tieno[2,3-d]piridina funcionalizado, com bons rendimentos globais a partir do intermediário-chave (5), como antagonistas do PAF análogos ao WEB2086 (2). A avaliação preliminar das propriedades antiagregantes plaquetárias das amidas (3a-f) e (4) no modelo induzido pelo PAF (1) permitiu evidenciar o perfil antitrombótico desses novos derivados. A fraca atividade antiagregante observada para esses compostos possivelmente se deve à importância da unidade espaçadora flexível na cadeia lateral, como a presente na substância protótipo (2).

In the scope of a research program aiming at the synthesis and pharmacological evaluation of new antithrombotic agents exploring rational principles of molecular designing, we describe in this paper the synthesis of two amide series (3a-f) and (4) derived from new functionalized tricyclic heteroaromatic pyrazolo[3,4-b]thieno[2,3-d]pyridine system, in good overall yields from key intermediate (5), as PAF antagonists related to WEB2086 (2). The preliminary platelet antiaggregating evaluation of amides (3a-f) and (4) in model induced by PAF permitted to evidenciate an antithrombotic profile to these new derivatives. The poor anti-platelet action observed to these compounds are due probably to the importance of a flexible spacer unit in the lateral chain, as present in the lead compound (2).

Keywords: *PAF antagonists, WEB2086 analogues, functionalized pyrazolo[3,4-b] thieno[2,3-d]pyridine system*

Introduction

Platelet activating factor (PAF, 1) is an important autacoid, bioformed from membrane phospholipids by phospholipase A_2 action[1], which was characterized by Benveniste *et al.*[2] as the more potent natural thrombogenic agent, displaying still hipotensive properties.[3] Thus, the designing of new PAF receptor antagonists consist of an

import therapeutical strategy to development of new potent and selective antithrombotic agents. Among these, the amides derived from fused triazolothieno-1,4-diazepine system, referred to hetrazepines, as the lead compound WEB2086 (2) were described by Weber *et al.*[4] as potent, non structurally related, competitive PAF antagonist agent, which displayed important platelet antiaggregatory properties (IC_{50} = 0.17 μM, human PRP[5]) (Chart 1).

Unfortunately, the clinical application of (2) is drastically limited due the low duration of action and poor pharmacokinetic profile, as function of the metabolic vulnerability of the 1,4-diazepine methylene group to enzymatic liver hydroxylation in the man[6].

The structural diversity of the PAF antagonists agents carried Bures *et al.*[7] to disclose a theoretical three point model (Fig. 1) to pharmacophoric groups of some PAF receptor antagonists, including the WEB2086 (2), applying new molecular modeling tools. The set of interaction points of this model comprised of hydrogen bonding donating and accepting site, an aromatic nitrogen ligand site and hydrophobic region site, distant each other adequately, as follows illustrated to (2).

As part of a research program aiming at the synthesis and pharmacological evaluation of heterocyclic bioactive compounds[8-14] we describe in the present paper the synthesis and the preliminary platelet antiaggregatory profile of two series of new heteroaromatic amides (3a-f) and (4)[15], derived from new pyrazolo[3,4-b]thieno[2,3-d]pyridine system[16], planned as PAF receptor antagonists, analogues to WEB2086 (2) (Chart 1).

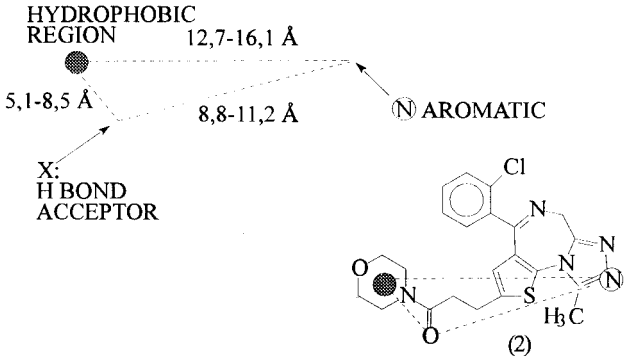
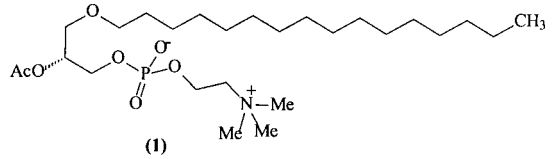
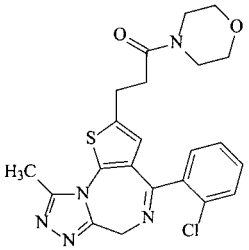


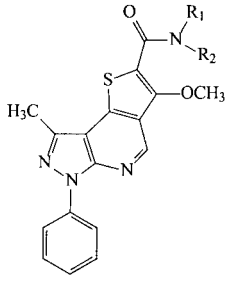
Figure 1. Three point model proposed to interaction of antagonists, as WEB2086 (2), with the PAF bioreceptor (Adapted of Bures *et al.* [7]).



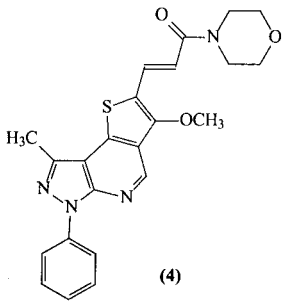
(1)



(2)



(3a) R_1 = Et, R_2 = Et
(3b) R_1 = R_2 = -$CH_2(CH_2)_3CH_2$-
(3c) R_1 = R_2 = -$CH_2CH_2OCH_2CH_2$-
(3d) R_1 = R_2 = -$CH_2CH_2N(CH_3)CH_2CH_2$-
(3e) R_1 = R_2 = -$CH_2CH_2N(Ph)CH_2CH_2$-
(3f) R_1 = H, R_2 = Ph



(4)

Chart 1.

The analogues (3a-f) and (4) were designed exploring the classical principles of bioisosterism[17] between aromatic rings, as the triazolothieno-1,4-diazepine nucleus present in (2) and condensed pyrazolothienopyridine system, maintaining the minimum structural requirements to interaction anticipated by Bures model. The suppression of the methylene group present in position 6 of the 1,4-diazepine ring, was sketched to prevent the metabolic lability increasing the plasmatic half life of the new derivatives. In the other hand, the substitution of the aryl group from C-4 of the hetrazepine nucleus to N-6 of pyrazolo[3,4-b]thieno[2,3-d]pyridine system respected the number of the electrons and the lipophylic properties among the two families of derivatives.

The nature of the substituints R_1 and R_2, in the derivatives (3a-f), was rationally defined based on the structure activity relationships (SAR) described previously to compounds related to (2)[18], aiming to infer the effect of the change of the hydrophobic character of this region in the PAF receptor to antagonist activity. Already, the α,β-unsaturated morpholine amide derivative (4) was designed as a conformational restrict analogue at lateral side chain level, to assure a real analogy in the molecular arrangement among the lead compound (2) and these new synthetic derivatives.

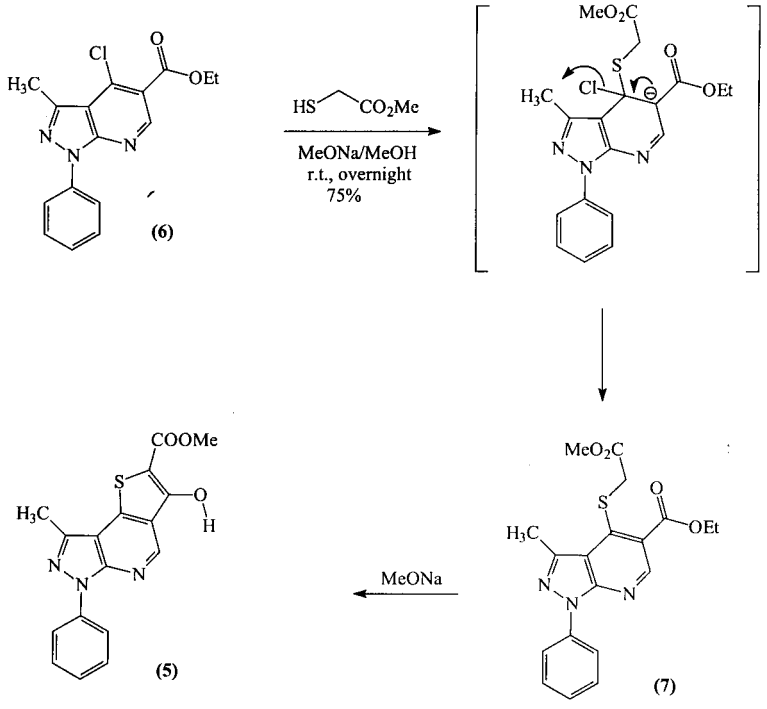
Results and Discussion

Preparation of 2-carbomethoxy-3-hydroxy-6-phenyl-8-methyl-pyrazolo[3,4-b]thieno[2,3-d]pyridine (5), which was identified as a key common intermediate to synthesis of the 2-alkylcarboxamides (3a-f) and 2-alkenylcarboxamides (4), was achieved as previously described[16], in 75% yield, exploring a new one-pot sequence: heteroaromatic nucleophilic substitution of the chloroester derivative[13] (6) with sodium methylthioglycolate, followed by Dieckman condensation of the transient thioarylether derivative (7) (Scheme 1).

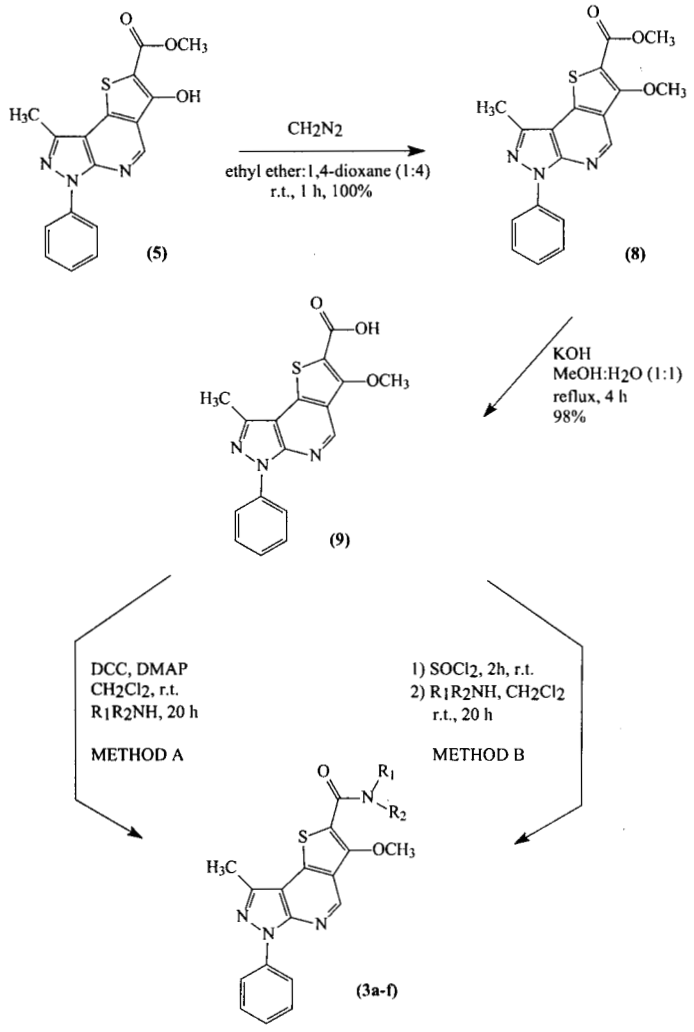
The adequate manipulation of functional groups of the hydroxy ester derivative (5) permit us to transform it in the methoxy acid derivative (9), in 98% overall yield for the two steps, by treatment of (5) with diazomethane in ethyl ether[19] and subsequent hydrolysis of the respective methoxy ester derivative (8) by reflux with a methanolic aqueous solution of KOH[10] (Scheme 2).

The derivatives (8) and (9), unlike the precursor (5)[16] do not displayed the formation of the deep purple color when submitted to ferric chloride test[20] indicating the lack of the -hydroxy ester character. In fact, the ^{1}H-NMR analysis of (8) showed the occurrence of two singlets at δ 3.97 and δ 4.12 ppm referred to methyl of the methoxy groups attached to carbonyl and thiophene ring, respectively, while that the ^{1}H-NMR analysis of (9) showed only one singlet at δ 4.24 ppm inputed to methyl of the methoxy ether moiety.

Finally, the desired amide derivatives (3a-f) was prepared in good chemical yield, as showed in the Table 1, by condensation of (9) with the respective amines exploring classical methodologies, using DCC as coupling agent[21] (Method A) or thionyl chloride[22] (Method B) to formation

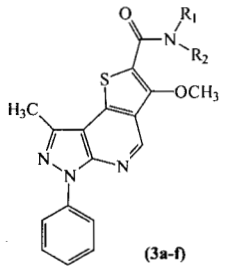


Scheme 1.



Scheme 2.

Table 1. Preparation of amides (3a-f) employing methods A and B.



Compound	R_1	R_2	Method A yield (%)	Method B yield (%)	MP (°C)
3a	Et	Et	88	90	198
3b	$-CH_2(CH_2)_3CH_2-$		30	40	108
3c	$-CH_2CH_2OCH_2CH_2-$		46	49	167
3d	$-CH_2CH_2-N(CH_3)-CH_2CH_2-$		48	70	119
3e	$-CH_2CH_2-N(Ph)-CH_2CH_2-$		33	45	145
3f	H	Ph	53	86	178

of the more eletrophilic acid derivatives iminoanhydride or acyl chloride, respectively (Scheme 2).

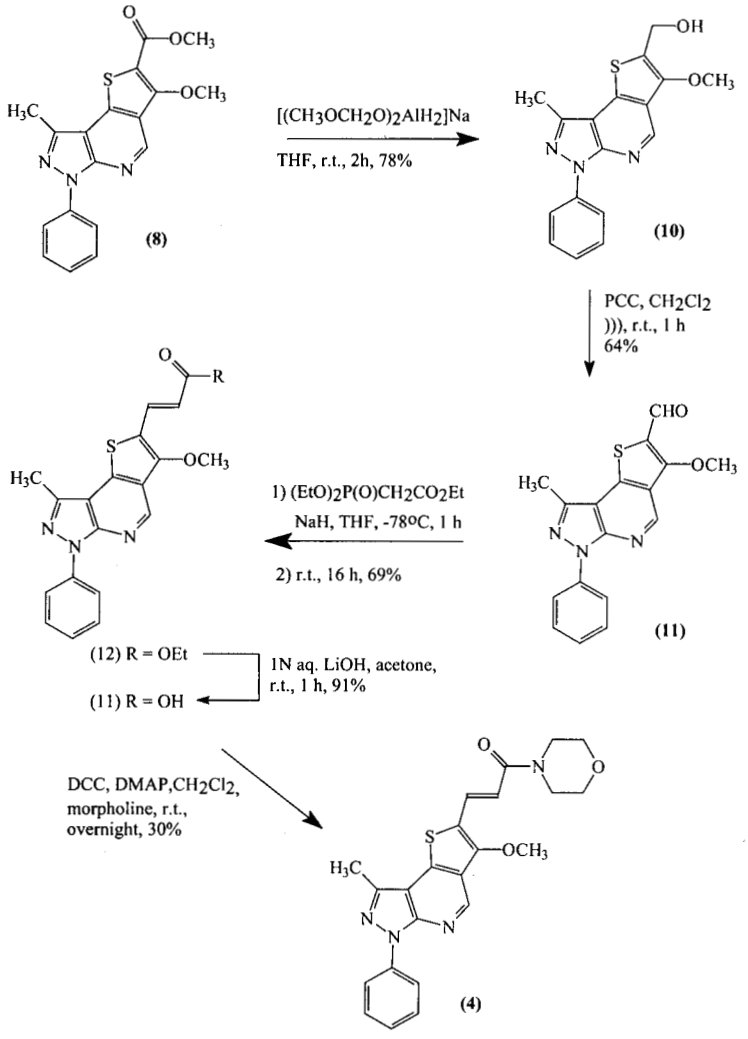
The initial step in the planned synthetic route to 2-alkenyl carboxamide (4) would be to submit the functionalized heterocyclic derivative (8) to a quimiosselective reduction of the ester group. After successive misfortunes using several experimental conditions[23-25] employing classical reduction agents as $LiAlH_4$ and $NaBH_4$, we were finally able to obtain the hydroxymethylene derivative (10), in 78% yield, using Red-Al[26] as a hydride donor, wherein the aluminum atom is covalently bonded to Lewis base sites. Subsequently, treatment of the crystalline derivative (10) with PCC in dichloromethane under sonication[23] conditions, furnished the corresponding unstable formyl derivative (11), in 64% yield, which was submitted to next step without additional purification (Scheme 3).

The homologation of two carbon atoms in the derivative (11) was achieved diastereossectively employing the Wadsworth-Emmons-Horner methodology for olefination of aldehydes[27]. Thus, the unsaturated α,β-unsaturated ester derivative (12) was prepared in 69% yield from (11) by treatment with sodium salt of triethylfosfonoacetate in THF at -78 °C (Scheme 3).

The ^{1}H-NMR spectrum of this α,β-unsaturated ester showed a typical AB pattern centered at δ 6.1 and δ 8.07 ppm (J = 15.6 Hz) for the vinyl protons with the expected E-configuration.

The mild hydrolysis of (12) by treatment with an aqueous 1N LiOH solution in acetone[28] furnished the α,β-unsaturated acid (13), in 91% yield, which showed in the ^{1}H-NMR spectra a similar AB pattern described for (12) due the olefinic hydrogens.

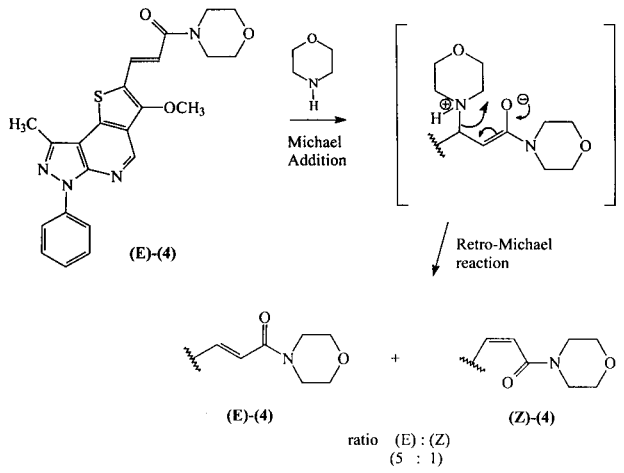
With the compound (13) in hands, the synthesis of the homologue amide derivative (E)-(4) was concluded, in 30% yield, employing the method A (Scheme 3). However, this procedure carried out to partial isomerization of the (E)-configuration of the derivative (4) due the Michael 1,4-addition/Retro-Michael sequence, giving a mixture of



Scheme 3.

the (E)/(Z)-amides, in a relative proportion of 5:1, which was carefully purified by silica gel column chromatography (Scheme 4).

The platelet antiaggregatory profile of these compounds will be described in due course. Meanwhile, the preliminary results of the antiplatelet activity of these new



Scheme 4.

WEB2086 analogues (3a-f) and (4) are given in Table 2. The inhibitory activities of these new compounds in blood platelet aggregation[29,30], performed using rabbit platelet-rich plasma stimulated by PAF (1), was unfortunately poor in the tested model.

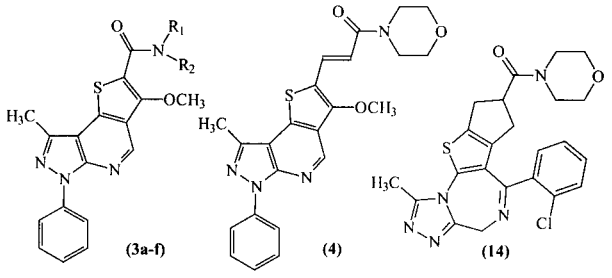
Conclusion

In conclusion, the synthetic route described herein represents a useful approach to different PAF receptor antagonists exploring the new pyrazolo[3,4-b]thieno[2,3-d]pyridine derivative (5) as starting material. The poor antithrombotic activity observed for these new derivatives (3a-f) prompt us to plan new others appropriate structural modifications, as the increase of the flexibility in lateral side chain, in order to improve the observed antiplatelet activity.

Experimental

Melting points were determined with a Thomas-Hoover apparatus and are uncorrected. Proton magnetic ressonance (^{1}H-NMR), unless otherwise stated, was determined in deuterated dimethylsulfoxide containing *ca.* 1% tetramethylsilane as an internal standard with Brucker AC 200,

Table 2. Effect of the new tricyclic amides (3a-f) and (4) in the platelet aggregation[29,30] induced by PAF (5 x 10 $^{-8}$ M) in rabbit PRP, using DMSO as vehicle and WEB2170 (14) as standard



Compound	R_1	R_2	C (μM)	n	% aggregation	% inhibition
Control 1	---	---	---	7	39.3 ± 1.3	---
WEB2170 (14)	---	---	0.15	3	25.1 ± 4.0	36.1*
3b	$-CH_2(CH_2)_3CH_2-$		100	4	34.4 ± 2.5	12.5 ns
3c	$-CH_2CH_2OCH_2CH_2-$		100	4	35.7 ± 2.1	9.2 ns
3e	$-CH_2CH_2-N(Ph)-CH_2CH_2-$		100	4	35.4 ± 1.6	9.9 ns
Control 2	---	---	---	7	44.5 ± 1.8	---
WEB 2170 (14)	---	---	0.15	3	28.5 ± 4.9	35.9*
3a	Et	Et	100	4	38.9 ± 1.3	12.6 ns
3d	$-CH_2CH_2-N(CH_3)-CH_2CH_2-$		100	4	38.4 ± 1.6	13.7 ns
3f	H	Ph	100	4	37.2 ± 1.8	16.4
4	---	---	100	3	39.0 ± 2.1	12.4 ns

[a] The values of the aggregation represents the means ± SD; C = final concentration; n = number of the experiments in triplicate; ns = no significative.
[b] p 0.05 ("t"student test).

Brucker AG 50 and Brucker DRX 300 spectrometers at 200 MHz and 300 MHz respectively. Splitting patterns are as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Carbon magnetic ressonance (^{13}C-NMR) was determined in the same spectrometers described above at 50 MHz and 75 MHz respectively, using deuterated dimethylsulfoxide as internal standard. Infrared (IR) spectra were obtained with Nicolet-205, Nicolet-550 Magna and Perkin-Elmer-257 spectrophotometers by using potassium bromide plates. The mass spectra (MS) were obtained by electron impact (70 eV) with a GC/VG Micromass 12 spectrometer.

The progress of all reactions was monitored by tlc, which was performed on 2.0 cm x 6.0 cm aluminum sheets precoated with silica gel 60 (HF-254, Merck) to a thickness of 0.25 mm. The developed chromatograms were viewed under ultraviolet light. For column chromatography Merck silica gel (70-230 mesh) was used. Solvents used in the reactions were generally redistilled prior to use and stored over 3-4 Å molecular sieves. Reactions were generally stirred under a dry nitrogen atmosphere. The usual work-up means that the organic extracts prior to concentration, under reduced pressure, were treated with a saturated aqueous sodium chloride solution, referred to as brine, dried over anhydrous sodium sulfate and filtered.

2-Methoxycarbonyl-3-methoxy-6-phenyl-8-methyl-pyrazolo[3,4-b]thieno[2,3-d]pyridine (8)[19]

To a solution of 0.1 g (0.29 mmol) of the ester hydroxy derivative (5) in 20 mL of 1,4-dioxane was added dropwise an ethereal solution of diazomethane until that the tlc indicated that all starting material had been consumed. Then, was added acetic acid (*ca.* 1 mL) to reaction mixture until ceased the gaseous evolution. The organic layer was dried with anhydrous potassium carbonate, filtered and submitted at usual work-up affording 0.1 g (100%) of the respective methyl ether derivative (8) as an orange precipitate, mp 75 °C; ^{1}H-NMR (200 MHz): δ 2.73 (s, 3H, Ar-CH$_3$), 3.97 (s, 3H, O=C-O-CH$_3$), 4.12 (s, 3H, Ar-O-CH$_3$), 7.32 (t, 1H, Ar-H$_{3'}$, J = 7.3 Hz), 7.52 (t, 2H, Ar-H$_{2'}$, J = 7.4 Hz), 8.16 (d, 2H, Ar-H$_{1'}$, J = 7.7 Hz), 8.81 (s, 1H, pyridine-H) ppm; ^{13}C-NMR (50 MHz): δ 14.5 (Ar-CH$_3$), 52.3 (COOCH$_3$), 63.0 (Ar-OCH$_3$), 110.9 (C$_9$), 111.4 (C$_{8a}$), 121.4 (C$_{10}$), 121.6 (C$_{12}$), 126.2 (C$_{3a}$), 129.0 (C$_{11}$), 139.1 (C$_2$), 143.5 (C$_8$), 145.7 (C$_{5a}$), 153.8 (C$_4$), 165.6 (C=O) ppm; IR (KBr) cm^{-1}: 1716 (ν C=O), 1594 (ν C=N), 1558 (ν C=C), 1271 and 1250 (ν C-O); ms (m/z): 297 (M$^{+\cdot}$ - 56, 100%), 266 (M$^+$ - 87, 52%), 223 (M$^{+\cdot}$ -130, 9%), 77 (M$^{+\cdot}$ - 276, 10%).

2-Carboxy-3-methoxy-6-phenyl-8-methyl-pyrazolo [3,4-b]thieno[2,3-d]pyridine (9)[10]

The methyl ester (8) (0.48 g, 1.36 mmol) was refluxed in 40 mL of aqueous methanolic solution (1:1) containing 0.78 g (14 mmol) of potassium hydroxide for 4 hours. Then, the reaction mixture was neutralized with hydrochloric acid and the resulting precipitate was filtered out, washed with water (10 mL) and air dried affording 0.45 g (98%) of the respective acid derivative (9) as a light yellow solid, mp 167 °C; ^{1}H-NMR (200 MHz): δ 2.70 (s, 3H, Ar-CH$_3$), 4.24 (s, 3H, O-CH$_3$), 7.30 (t, 1H, Ar-H$_{3'}$, J = 7.4 Hz), 7.53 (t, 2H, Ar-H$_{2'}$, J = 7.5 Hz), 8.25 (d, 2H, Ar-H$_{1'}$, J = 7.5 Hz), 8.79 (s, 1H, pyridine-H) ppm; ^{13}C-NMR (50 MHz): δ 13.5 (Ar-CH$_3$), 61.8 (Ar-OCH$_3$), 110.6 (C$_9$), 120.6 (C$_{10}$), 125.5 (C$_{12}$), 126.9 (C$_{3a}$), 128.9 (C$_{11}$), 137.1 (C$_2$), 139.33 (C$_8$), 141.1 (C$_{8b}$), 143.5 (C$_4$), 148.23 (C=O) ppm; IR (KBr) cm^{-1}: 3440 (ν OH), 1718 (ν C=O), 1690 (ν C=N) 1564 (ν C=C), 1502 (ν C-O-H), 1283 and 1216 (ν C-O); C-H); ms (m/z): 339 (M$^+$, 2%), 295 (M$^+$ -44, 10%), 283 (M$^{+\cdot}$ - 56, 100%), 251 (M$^+$ - 88, 8%), 239 (M$^+$ -100, 69%), 223 (M$^{+\cdot}$ -116, 13%), 77 (M$^+$ - 262, 21%).

General Procedure for the Preparation of the Amides (3a-f) using DCC (Method A)[21]

To a mixture of 0.33 g (1.62 mmol) of dicyclohexylcarbodiimide (DCC) and 0.03 g (0.35 mmol) of 4-N,N-dimethylaminopyridine (DMAP) were added 0.10 g (0.29 mmol) of the acid derivative (9) diluted in 50 mL of dry methylene chloride. Then, 1.16 mmol of the respective amine were added and the reaction mixture was stirred at room temperature for 20 h. After this time, 90 mL of methylene chloride were added and the organic layer was washed with a saturated sodium bicarbonate solution and submitted at usual work-up to give the respective amide derivatives (3a-f) as described bellow;

General Procedure for the Preparation of the Amides (3a-f) using Thionyl Chloride (Method B)[22]

A solution of 0.15 g (0.44 mmol) of acid (9) in 3.2 mL (44 mmol) of freshly distilled thionyl chloride was vigorously stirred under reflux for 2 h. After this time, the solvent was careful evaporated at reduced pressure and a solution of 1.76 mmol of the respective amine in 5 mL of dry methylene chloride was added. The reaction mixture was stirred for 20 h at room temperature, then poured into 30 mL of water and extracted with methylene chloride (3 x 30 mL). The organic layers were jointed and submitted at usual work-up to give the respective amide derivatives (3a-f) as described bellow;

3-Methoxy-6-phenyl-8-methyl-pyrazolo[3,4-b]thieno [2,3-d]pyridine 2-N,N-diethyl-carboxamide (3a)

This compound was obtained using diethylamine, in 88% (method A) and 90% (method B) yield, respectively, as a yellow powder, mp 198 °C; ^{1}H-NMR (200 MHz): δ 1.60 (m, 6H, CH$_2$-CH$_3$), 2.54 (s, 3H, Ar-CH$_3$), 3.24 (s, 4H, CH$_2$-CH$_3$), 4.09 (s, 3H, O-CH$_3$), 7.30 (t, 1H, Ar-H$_{3'}$, J = 7.4

Hz), 7.53 (t, 2H, Ar-H$_{2'}$, J = 7.4 Hz), 8.12 (d, 2H, Ar-H$_{1'}$, J = 7.8 Hz), 8.28 (s. 1H. pyridine-H) ppm; ^{13}C-NMR (50 MHz): δ 13.2 (-N-CH$_2$-CH$_3$), 14.2 (Ar-CH$_3$), 39.9 (-N-CH$_2$-CH$_3$), 59.4 (Ar-OCH$_3$), 108.5 (C$_9$), 114.7 (C$_{8a}$), 120.1 (C$_{10}$), 125.3 (C$_{12}$), 128.7 (C$_{11}$), 138.7 (C$_2$), 142.2 (C$_8$), 149.8 (C$_4$), 166.2 (C=O) ppm; IR (KBr) cm^{-1}: 1623 (ν C=O), 1594 (ν C=N), 1564 (ν C=C), 1216 (ν C-O); ms (m/z): 394 (M$^+$, 68%), 363 (M$^+$ -31, 4%), 322 (M$^{+\cdot}$ - 41, 88%), 307 (M$^+$ - 15, 23%), 295 (M$^+$ -12, 100%), 252 (M$^{+\cdot}$ -43, 27%), 77 (M$^{+\cdot}$ - 317, 35%).

Anal. Calcd. for $C_{21}H_{22}N_4O_2S$: C, 63.94; H, 5.62; N, 14.20. Found: C, 63.93; H, 5.63; N, 14.25.

3-Methoxy-6-phenyl-8-methyl-pyrazolo[3,4-b]thieno [2,3-d]pyridine 2-piperidine carboxamide (3b)

This compound was obtained using piperidine, in 30% (method A) and 40% (method B) yield, respectively, as a yellow powder, mp 108 °C; ^{1}H-NMR (200 MHz): δ 1.61 (m, 6H, -CH$_2$CH$_2$CH$_2$-), 2.65 (s, 3H, Ar-CH$_3$), 3.31 (m, 4H, -CH$_2$-N), 4.07 (s, 3H, -O-CH$_3$), 7.30 (t, 1H, Ar-H$_{3'}$, J = 9.0 Hz), 7.52 (t, 2H, Ar-H$_{2'}$, J = 7.0 Hz), 8.18 (d, 2H, Ar-H$_{1'}$, J =7.0 Hz), 8.29 (s, 1H, pyridine-H) ppm; ^{13}C-NMR: δ 13.7 (Ar-CH$_3$), 23.6 (N-CH$_2$-CH$_2$-CH$_2$), 47.2 (N-CH$_2$-CH$_2$-CH$_2$), 59.0 (Ar-OCH$_3$), 113.7 (C$_{8a}$), 119.6 (C$_{10}$), 124.8 (C$_{12}$), 128.2 (C$_{11}$), 138.3 (C$_2$), 141.7 (C$_{8b}$), 149.6 (C$_4$), 164.6 (C=O) ppm; IR (KBr) cm^{-1}: 1723 (ν C=O); 1594 (ν C=N), 1561 (ν C=C), 1275 (ν C-O); ms (m/z): 406 (M$^+$, 9%), 364 (M$^+$ -42, 9%), 350 (M$^{+\cdot}$ - 56, 43%), 336 (M$^+$ -14, 56%), 295 (M$^+$ -41, 16%), 266 (M$^+$ -29, 100%), 52 (M$^{+\cdot}$ -14, 55%), 223 (M$^+$ - 29, 54%).

Anal. Calcd. for $C_{22}H_{22}N_4O_2S$: C, 65.00; H, 5.45; N, 13.78. Found: C, 65.03; H, 5.42; N, 13.75.

3-Methoxy-6-phenyl-8-methyl-pyrazolo[3,4-b]thieno [2,3-d]pyridine 2-morpholine carboxamide (3c)

This compound was obtained using morpholine, in 46% (method A) and 49% yield, respectively, as a yellow powder, mp 167 °C; ^{1}H-NMR (200 MHz): δ 2.71 (s, 3H, Ar-CH$_3$), 3.57 (m, 8H, -NCH$_2$CH$_2$O-), 4.00 (s, 3H, O-CH$_3$), 7.31 (t, 1H, Ar-H$_{3'}$, J = 7.2 Hz), 7.5 (t, 2H, Ar-H$_{2'}$, J = 6.2 Hz), 8.23 (d, 2H, Ar-H$_{1'}$, J = 7.6 Hz), 8.94 (s, 1H, pyridine-H) ppm; ^{13}C-NMR (50 MHz): δ 14.2 (Ar-CH$_3$), 47.3 (N-CH$_2$-CH$_2$-O), 59.8 (Ar-OCH$_3$), 65.6 (N-CH$_2$-CH$_2$-O), 113.5 (C$_{8a}$), 120.1 (C$_{10}$), 125.4 (C$_{12}$), 128.7 (C$_{11}$), 142.2 (C$_{8b}$), 150.3 (C$_4$), 165.5 (C=O) ppm; IR (KBr) cm^{-1}: 1623 (ν C=O), 1593 (ν C=N), 1569 (ν C=C), 1247 (ν C-O); ms (m/z): 408 (M$^+$, 58%), 322 (M$^{+\cdot}$ - 86, 100%), 307 (M$^+$ -15, 15%).

Anal. Calcd. for $C_{21}H_{20}N_4O_3S$: C, 61.75; H, 4.93; N, 13.72. Found: C, 61.78; H, 4.92; N, 13.74.

3-Methoxy-6-phenyl-8-methyl-pyrazolo[3,4-b]thieno [2,3-d]pyridine 2-(N-methyl)piperazine carboxamide (3d)

This compound was obtained using 1-N-methyl-piperazine, in 48% (method A) and 70% (method B) yield, respectively, as a yellow powder, mp 119 °C; ^{1}H-NMR (200 MHz): δ 2.35 (s, 3H, -N-CH$_3$), 2.54 (m, 4H, O=C-N-CH$_2$-, J = 4.5 Hz), 2.76 (s, 3H, Ar-CH$_3$), 3.72 (m, 4H, CH$_3$-N-CH$_2$-, J = 4.6 Hz), 4.17 (s, 3H, -O-CH$_3$), 7.39 (t, 1H, Ar-H$_{3'}$, J = 7.3 Hz), 7.60 (t, 2H, Ar-H$_{2'}$, J = 7.4 Hz), 8.31 (d, 2H, Ar-H$_{1'}$, J = 7.7 Hz), 8.99 (s, 1H, pyridine-H) ppm; ^{13}C-NMR (50 MHz): δ 12.9 (Ar-CH$_3$), 44.2 (N-CH$_2$-CH$_2$-N-CH$_3$), 44.9 (-N-CH$_2$-CH$_2$-N-CH$_3$), 54.0 (-N-CH$_2$-CH$_2$-N-CH$_3$), 60.5 (Ar-OCH$_3$), 109.9 (C$_{8a}$), 120.4 (C$_{10}$), 123.4 (C$_{3a}$), 125.4 (C$_{12}$), 128.5 (C$_{11}$), 138.7 (C$_2$), 140.6 (C$_8$), 141.7 (C$_{8b}$), 143.1 (C$_4$), 160.9 (C=O) ppm; IR (film) cm^{-1}: 1629 (ν C=O), 1594 (ν C=N), 1563 (ν C=C), 1124 (ν C-O). 757 (ν C-H); MS (m/z): 421 (M$^+$, 21%), 364 (M$^+$ -57, 27%), 338 (M$^{+\cdot}$ - 83, 18%), 322 (M$^+$ -99, 51%), 307 (M$^+$ -15, 13%).

Anal. Calcd. for $C_{22}H_{23}N_5O_2S$: C, 62.69; H, 5.50; N, 16.61. Found: C, 62.66; H, 5.52; N, 16.60.

3-Methoxy-6-phenyl-8-methyl-pyrazolo[3,4-b]thieno [2,3-d]pyridine 2-(N-phenyl)piperazine carboxamide (3e)

This compound was obtained using 1-N-phenyl-piperazine, in 33% (method A) and 45% (method B) yield, respectively, as a yellow powder, mp 145 °C; ^{1}H-NMR (200 MHz): δ 2.68 (s. 3H. Ar-CH$_3$), 3.11 (t, 4H, -CH$_2$-N-Ph J = 5.2 Hz), 3.80 (t, 4H, -CO-N-CH$_2$-, J = 4.8 Hz), 4.13 (s, 3H, -O-CH$_3$), 6.80 (m, 3H, H$_{3''}$ & H$_{1''}$), 7.19 (m, 3H, H$_{3'}$ & H$_{2''}$), 7.44 (m, 2H, H$_{2'}$); 8.18 (d, 2H, H$_{1'}$ J = 8.7 Hz), 8.85 (s, 1H, pyridine-H) ppm; ^{13}C-NMR (50 MHz): δ 13.8 (Ar-CH$_3$), 38.8 (N-CH$_2$-CH$_2$-N-Ph), 61.4 (Ar-OCH$_3$), 68.1 (-N-CH$_2$-CH$_2$-N-Ph), 110.9 (C$_{8a}$), 116.7 (C$_{14}$) 120.7 (C$_{16}$), 121.6 (C$_{16}$); 126.1 (C$_{10}$); 130.7 (C$_{11\text{-}15}$); 138.8 (C$_2$); 139.4 (C$_8$); 141.3 (C$_{8b}$); 143.7 (C$_4$); 162.5 (C=O) ppm; IR (KBr): 1726 (ν C=O), 1624 (ν C=N), 1595 (ν C=C), 1129 (ν C-O); ms (m/z): 483 (M$^+$, 21%), 364 (M$^+$ -119, 16%), 322 (M$^{+\cdot}$ - 42, 32%).

Anal. Calcd. for $C_{27}H_{25}N_5O_2S$: C, 67.06; H, 5.21; N, 14.48. Found: C, 67.03; H, 5.22; N, 14.49.

3-Methoxy-6-phenyl-8-methyl-pyrazolo[3,4-b]thieno [2,3-d]pyridine 2-N-phenyl carboxamide (3f)

This compound was obtained using aniline, in 53% (method A) and 86% (method B) yield, respectively, as a yellow powder, mp 178 °C; ^{1}H-NMR (200 MHz): δ 2.71 (s, 3H, Ar-CH$_3$), 4.34 (s, 3H, -O-CH$_3$), 7.10 (t, 1H, H$_{3''}$, J = 7.4 Hz), 7.37 (m, 3H, H$_{3'}$ & H$_{2''}$), 7.53 (t, 2H, H$_{2'}$, J = 8.0 Hz), 7.71 (d, 2H, H$_{1''}$, J = 7.6 Hz), 8.21 (d, 2H, H$_{1'}$, J = 7.8 Hz), 9.15 (s, 1H, pyridine-H), 9.58 (s, 1H, NH) ppm; ^{13}C-NMR (50 MHz): δ 13.1 (Ar-CH$_3$), 62.7 (Ar-OCH$_3$), 110.0 (C$_9$), 118.7 (C$_{8a}$), 120.1 (C$_{13}$), 120.6 (C$_{10}$), 123.8 (C$_{12}$), 125.7 (C$_{16}$), 128.4 (C$_{15}$), 128.6 (C$_{11}$), 138.6 (C$_2$), 141.1 (C$_8$); 144.3 (C$_{8b}$); 147.5 (C$_{5a}$); 151.3 (C$_4$); 164.6 (C=O), 173.61 (C$_3$) ppm; IR (KBr): 3352 (ν N-H), 1652 (ν C=O), 1594 (ν C=N), 1550 (ν C=C), 1248 (ν C-O): ms

(m/z): 358 (M^+ -56, 27%), 266 (M^+ -92, 100%), 223 ($M^{+\cdot}$ -191, 11%), 77 ($M^{+\cdot}$ - 337, 12%).

Anal. Calcd. for $C_{23}H_{18}N_4O_2S$: C, 66.65; H, 4.38; N, 13.52. Found: C, 66.64; H, 4.38; N, 13.50.

2-Hydroxymethyl-3-methoxy-6-phenyl-8-methyl-pyrazolo [3,4-b]thieno[2,3-d] pyridine (10)[26]

To a solution of 0.1 g (0.28 mmol) of ester (8) in 20 mL of dry THF were slowly added 0.1 mL (0.67 mmol) of Red-Al and the reaction mixture was stirred at room temperature for 2 h. Then, 0.4 mL of 40% aqueous NaOH solution were added and the white suspension was filtered through of an anhydrous sodium sulfate column. The organic layer was submitted at usual work-up furnishing 0.07 g (78%) of the hydroxymethyl derivative (10) as a yellow precipitate, mp 169 °C; ^{1}H-NMR (200 MHz): δ 2.51 (s, 3H, Ar-CH$_3$), 4.04 (s, 3H, -och$_3$), 4.80 (d, 2H, -ch$_2$OH, J = 5.2 Hz), 5.75 (t, 1H, D_2O exchangeable, -OH, J = 5.4 Hz), 7.33 (t, 1H, Ar-H$_{3'}$, J = 7.3 Hz), 7.52 (t, 2H, Ar-H$_{2'}$, J = 7.5 Hz), 8.25 (d, 2H, Ar-H$_{1'}$, J = 7.6 Hz), 8.87 (s, 1H, pyridine-H) ppm; ^{13}C-NMR (50 MHz): δ 13.4 (Ar-CH$_3$), 54.9 (CH$_2$-OH), 61.8 (Ar-OCH$_3$), 110.6 (C_{8a}); 120.4 (C_{10}); 125.6 (C_{12}); 126.2 (C_{3a}); 128.9 (C_{11}); 139.0 (C_2); 140.6 (C_8); 142.5 (C_4); 146.5 (C_9) ppm; IR (KBr) cm^{-1}: 3369 (ν OH), 1593 (ν C=N), 1572 (ν C=C), 1313 (ν C-O); ms (m/z): 325 (M^+, 95%), 308 (M^+ -17, 47%), 293 ($M^{+\cdot}$ - 32, 100%), 278 ($M^{+\cdot}$ - 47, 20%), 252 ($M^{+\cdot}$ -73, 15%), 239 (M^+ -86, 39%), 207 (M^+ -118, 16%), 149 (M^+ -176. 13%), 77 (M^+ -248, 32%).

Formyl-3-methoxy-6-phenyl-8-methyl-pyrazolo[3,4-b] thieno[2,3-d] pyridine (11)[23]

To a suspension of 0.03 g (0.16 mmol) of pyridine chlorochromate (PCC) in 1.5 mL of dry methylene chloride was quickly added, at room temperature, a solution of 0.05 g (0.16 mmol) of the alcohol (10) in 4 mL of dry methylene chloride and the resulting brown mixture was sonicated for 1 h. Then, solvent was removed at reduced pressure and the residue suspended in dry diethyl ether. The organic layer was filtered through of a Florisil column and submitted at usual work-up to give 0.03 g (64%) of the formyl derivative (11) as a yellow solid, mp 193 °C; IR (KBr) cm^{-1}: 1637 (ν C=O), 1589 (ν C=N), 1401 (ν C-H).

Ethyl 2-(E)-3-methoxy-6-phenyl-8-methyl-pyrazolo [3,4-b]thieno[2,3-d] pyridine 2-(3-propenoate) (12)[27]

To a mixture of sodium triethylphosphonoacetate in THF [from 0.026 g (80% dispersion in mineral oil, 0.86 mmol) of sodium hydride and 0.183 mL (0.92 mmol) of triethylphosphonoacetate in 9.6 mL of dry THF stirred under nitrogen at room temperature for 1 h] at -78 °C was added 0.23 g (0.71 mmol) of aldehyde (11) in 4 mL of dry THF. After stirring for 1 h at -78 °C and 16 h at 25-30 °C the temperature of reaction mixture was cooled to 0 °C and added dropwise *ca.* 0.6 mL of acetic acid. The solvent was evaporated and the resulting syrup was diluted with water (10 mL) and extracted with methylene chloride (3 x 30 mL). The organic extracts were dried, evaporated and the resulting light yellow solid was chromatographed on silica gel G with a mixture of n-hexane:ethyl acetate (95:5 to 80:20) to furnish 0.193 g (69%) of α,β-unsaturated ester (12), as a yellow solid, mp 171 °C; ^{1}H-NMR (200 MHz, $CDCl_3$): δ 1.31 (q, 3H, CH$_2$-CH$_3$, J = 7.1 Hz), 2.74 (s, 3H, Ar-CH$_3$), 4.17 (s, 3H, -OCH$_3$), 4.26 (t, 2H, -CH$_2$-CH$_3$, J = 7.1 Hz), 6.16 (d, 1H, -HC=CH-CO-, J = 15.6 Hz), 7.32 (t, 1H, Ar-H$_{3'}$, J = 8.5 Hz), 7.50 (t, 2H, Ar-H$_{2'}$, J = 7.1 Hz), 8.00 (d, 1H, -HC=CH-CO-, J = 15.6 Hz), 8.21 (d, 2H, Ar-H$_{1'}$, J = 6.4 Hz), 8.88 (s, 1H, pyridine-H) ppm; ^{13}C-NMR (50 MHz, $CDCl_3$): δ 13.8 (Ar-CH$_3$), 14.3 (COOCH$_2$CH$_3$), 60.6 (COOCH$_2$CH$_3$), 63.1 (Ar-OCH$_3$), 111.2 (C_{8a}), 117.2 (-HC=CH-CO-), 120.2 (C_9), 121.5 (C_{10}); 124.6 (C_{3a}); 126.18 (C_{12}); 129.0 (C_{11}); 133.2 (-HC=CH-CO); 139.0 (C_2); 139.4 (C_8); 141.5 (C_{8b}); 143.3 (C_4); 148.7 (C_{5a}); 153.9 (C_{16}); 166.5 (C=O) ppm; IR (KBr) cm^{-1} : 1716 (ν C=O), 1621 (ν C=C), 1593 (ν C=N), 1036 (ν C-O); ms (m/z): 393 (M^+, 3%), 337 (M^+ -56, 26%), 307 (M^+ -30, 27%), 267 (M^+ -40, 23%), 167 (M^+ -100, 18%), 149 (M^+ -18, 63%).

2-(E)-3-methoxy-6-phenyl-8-methyl-pyrazolo[3,4-b] thieno[2,3-d] pyridine 2-(3-propenoic acid) (13)[28]

To a solution of the 0.042 g (0.11 mmol) of the α,β-unsaturated ester (12) in 20 mL of acetone were added 9 mL of 1N aqueous LiOH solution. The resulting mixture was stirred at room temperature for 1.5 h, then neutralized with 1 N aqueous HCl (*ca.* 3 mL) until pH 4 and extracted with diethyl ether (2 x 5 mL). The organic extracts were jointed and submitted at usual work-up to give 0.035 g (91%) the respective acid derivative (13) as a light yellow solid, mp 165C; ^{1}H-NMR (200 MHz): δ 2.71 (s, 3H, Ar-CH$_3$), 4.16 (s, 3H, -OCH$_3$), 5.65 (s, 1H, D_2O exchangeable, -OH), 6.18 (d, 1H, -HC=CH-CO, J = 15.6 Hz), 7.32 (m, 1H, Ar-H$_{3'}$, J = 7.0 Hz), 7.50 (t, 2H, Ar-H$_{2'}$, J = 7.0 Hz), 7.90 (d, 1H, -HC=CH-CO-, J = 15.6 Hz), 8.26 (d, 2H, Ar-H$_{1'}$, J = 6.6 Hz), 9.0 (s, 1H, pyridine-H) ppm; IR (film) cm^{-1}: 2950 (ν O-H), 1688 (ν C=O), 1611 (ν C=C), 1589 (ν C=N), 1213 (ν C-O); ms (m/z): 365 (M^+, 100%), 348 (M^+ -17, 1%), 305 (M^+ -43, 17%), 77 (M^+ -288, 18%).

2-(E)-3-methoxy-6-phenyl-8-methyl-pyrazolo[3,4-b] thieno[2,3-d]pyridine 2-(3-propenoic acid) morpholine carboxamide (4)[21]

To a mixture of 0.25 g (1.21 mmol) of dicyclohexylcarbodiimide (DCC) and 0.024 g (0.28 mmol) of 4-N,N-dimethylaminopyridine (DMAP) was added a solution of 0.08 g (0.21 mmol) of the α,β-unsaturated acid derivative (13) in 50 mL of dry methylene chloride. Then, 0.08 mL

(0.84 mmol) of distilled morpholine were added and the reaction mixture was stirred at room temperature for 20 h. After this time, 90 mL of methylene chloride were added and the organic layer was washed with a saturated sodium bicarbonate solution and submitted at usual work-up to give a solid residue which was chromatographed on silica gel G with a mixture of n-hexane:ethyl acetate (95:5 to 80:20) to furnish 0.023 g (30%) of diastereomerically pure (E)-amide (4), as a yellow solid, mp 204 °C; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.76 (s, 3H, Ar-CH_3), 3.81 (m, 11H, -OCH_3 and -NCH_2CH_2O-), 6.51 (d, 1H, -HC=CH-CO-, J = 15 Hz), 7.32 (t, 1H, Ar-$H_{3'}$, J = 8.5 Hz), 7.50 (t, 2H, Ar-$H_{2'}$, J = 7.8 Hz), 8.00 (d, 1H, -HC=CH-CO-, J = 15 Hz), 8.13 (d, 2H, Ar-$H_{1'}$, J = 8.7 Hz), 8.7 (s, 1H, pyridine-H) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$): δ 13.7 (Ar-CH_3), 47.0 (-NCH_2CH_2O-), 63.2 (-OCH_3), 65.0 (-NCH_2CH_2O-), 110.9 (C_{8a}), 118.57 (-HC=CH-CO-), 120.9 (C_9), 121.7 (C_{10}), 124.4 (C_{3a}), 126.1 (C_{12}), 129.0 (C_{11}), 132.1 (HC=CH-CO-), 138.5 (C_2) 139.1 (C_8), 141.3 (C_{8b}), 143.2 (C_4), 148.3 (C_{5a}), 153.8 (C_{16}), 165.4 (C=O) ppm; IR (KBr) cm^{-1} : 1698 (ν C=O), 1642 (ν C=C), 1591 (ν C=N), 1221 (ν C-O).

Anal. Calcd. for $C_{23}H_{22}N_4O_3S$: C, 63.58; H, 5.10; N, 12.89. Found: C, 63.55; H, 5.12; N, 12.86.

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