SYNTHESIS OF 3-AMINO-2-ETHOXYCARBONYL PYRIDO[1,2-a]THIENO-[2,3-d]-4-PYRIMIDONE DERIVATIVES AND THEIR ANTIMALARIAL AND CYTOTOXIC ACTIVITIES *IN VITRO*.

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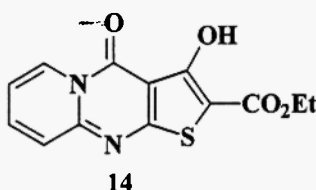
Abstract: A series of 3-amino-2-ethoxycarbonyl pyrido[1,2-a]thieno[2,3-d]-4-pyrimidone **10-13** were prepared in order to investigate their cytotoxicity and antimalarial activities. Some of the them showed a promising activity. The detailed synthesis, spectroscopic, computational and biological data are described.
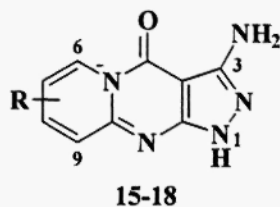
Introduction

Structures such as [1,2-a]4-pyrimidone have been studied intensively due to their biological importance (1-3). The pharmacological activities and the therapeutic effects of some pyridopyrimidones have a wide application in clinic (2). As a part of our study directed toward the synthesis of tricyclic ring systems possessing a pyrido [1,2-a] pyrimidine nucleus, we reported the synthesis of some new pyrimidone derivatives as cytotoxic and antimalarial agents.

Results and discussion

2-ethoxycarbonyl-3-hydroxypyrido[1,2-a]thieno[2,3-d]-4-pyrimidone **14** has been obtained in a multistep procedure from ethyl 2-methylthio-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylate and ethyl mercapto acetate in acetonitrile, under the presence of potassium carbonate (4).

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Synthesis of 3-amino-2-ethoxycarbonyl pyridol[1,2-a]thieno-[2,3-d]-4-pyrimidone derivatives and their antimalarial and cytotoxic activities in vitro

Recently, we have reported a general procedure for the synthesis and antimalarial activity of 3-amino-pyrido[1,2-a] pyrazolo[2,3-d] pyrimidin-4-ones **15-18**, by the reaction of the corresponding 2-methylthio-pyrido[1,2-a]pyrimidine-4-oxo-3-carbonitrile derivatives **6-9** with hydrazine (3).

15-18

No	15	16	17	18
R	H	7-Me	8-Me	9-Me

In view of the interesting biological activity exhibited by the above mentioned compounds, now we wanted to explore the effect caused by a modification on the fused hetrocyclic ring c by introducing a thiophene moiety. Thus, the synthesis of compounds **10-13** were accomplished by refluxing the corresponding nitriles **6-9** with an equimolecular amount of ethyl mercaptoacetate in the presence of sodium metoxide under an inert atmosphere of nitrogen. The reaction took place by a nucleophilic attack of thiolate anion on the carbon bearing the methylthio substituent, followed by a subsequent cyclization to afford compounds **10-13** as stable solids in good yields, as shown in the scheme 1.

Scheme 1.
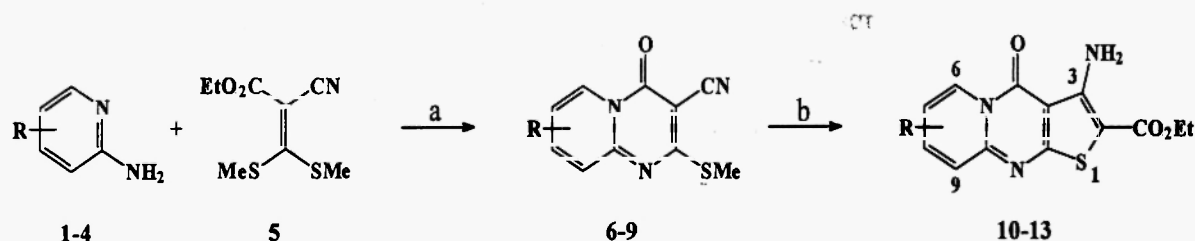
a. n-butanol, Δ; b. HSCH₂CO₂Et, Na, EtOH, Δ.

No	10	11	12	13
R	H	7-Me	8-Me	9-Me

The starting nitriles **6-9** were prepared as previously described (3). The obtained compounds **10-13** were fully characterized by their analytical and spectroscopical data. Thus, they showed the presence of an NH_2 group in the IR spectra (3456-3438 cm^{-1}), and a carbonyl from the ester (1696-1705 cm^{-1}). In the ^{1}H NMR spectra, the NH_2 group appears at 6.87-6.89 ppm as a broad singlet. It is important to show that for compounds **11, 12** the methyl groups located at positions C_7 and C_8 respectively appeared as doblets at 2.45-2.40 ppm with a J: 0.99 Hz, while in compound **13** this

coupling was not observed. Using NMR techniques, compounds **11**, **12** were irradiated at the corresponding signals to H$_6$ at 8.74 ppm and H$_9$ at 7.32 ppm affording enhancements on the of CH$_3$ signal, (20%) and (15%) respectively, and a disappearance of the doublet. This clearly indicated that the methyl groups at compounds **11**, **12** were coupling with protons at 8.74 and 7.32 ppm respectively. These unexpected effects were not observed by us in previously synthesized compounds **16-18**. Thus, for a better understanding of the above experimental results, we conducted a conformational and electronic study on the derivatives **10-13** and their parent compounds **15-18**. The purpose was to obtain more precise information about the stereoelectronic aspects that may aid in the understanding of the different accoupling obtained for these compounds in ^{1}H NMR spectra. We determined the low-energy conformations of these compounds by molecular orbital calculations. Both semiempirical AM1 and ab-initio (RHF 3-21G*) calculations, predict that the most stable forms are those in which the side-chain is located almost co-planar with respect to the rings system. Conformational potential energy surfaces (PES) of the compounds varing the torsional angles T1 vs T2 are shown in Figure 1 and 2.
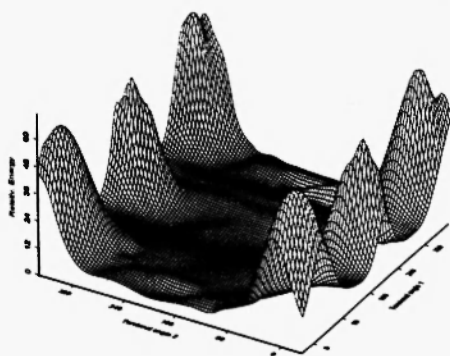

Figure 1: *Conformational Potential Energy Surfaces for compounds* **10-13**.
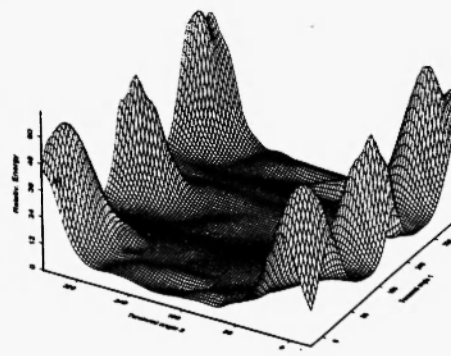
Figure 2: *Conformational Potential Energy Surfaces for compounds* **15-18**.

Observing these PESs it is clear that the conformational behavior of these compounds is very similar. The best way to take into account all of the possible electrostatic contributions is by means of the molecular electrostatic potential maps (MEPs), evaluated at ab-initio RHF/3-21G* level. Comparing these MEPs, at least two significative differences should be noted. The first is a different electronic distribution in the vicinity of the N-H group (ring c) of compounds **15-18** in comparison with that observed near to the sulphur group of compounds **10-13**. The other is the different electronic distribution on the hydrogen atoms in (ring a). This is particularly evident after

examining the calculated partial charges in compounds **10-13** and **15-18** respectively. These results emphasize the importance of electronic effects rather than conformational factors in explaining the results observed in the ^{1}H NMR. The calculated partial charges are shown in Table1. These molecular modeling results are in perfect agreement with the data obtained by irradiation spectrometric measurements.

Table 1: Calculated Partial Charge for derivatives 10-13 and 15-18.

X: S or N Y: C or N
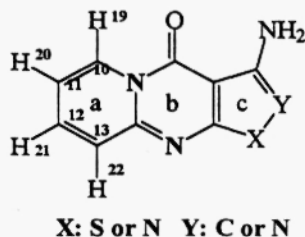
Compound	C_{10}	C_{11}	C_{12}	C_{13}	H_{19}	H_{20}	H_{21}	H_{22}
10	0.005	-0.213	-0.057	-0.163	0.191	0.160	0.150	0.173
11	-0.004	-0.147	-0.056	-0.159	0.191	-	0.149	0.173
12	0.006	-0.212	0.008	-0.174	0.190	0.157	-	0.172
13	-0.005	0.202	-0.070	-0.09i	0.193	0.160	0.149	-
15	0.005	-0.218	-0.056	-0.166	0.192	0.158	0.148	0.171
16	0.000	-0.155	-0.054	-0.165	0.191	-	0.148	0.171
17	0.007	-0.216	0.007	-0.175	0.191	0.157	-	0.171
18	-0.002	-0.211	-0.067	-0.097	0.191	0.158	0.148	-

The ^{13}C NMR assignments were confirmed using a combination of DEPT 135$^\alpha$ and 90$^\alpha$, and HETCOR experiments. The mass spectra showed the presence of the respective m/z M$^+$ (100% intensity) and a peak appearing in all cases at m/z: 257, corresponding to the lost of the Et$_2$OH group to form a stable ion.

The cytotoxicity of compounds **10-13** were studied against the growth of B16, MCF7, RD and FOG cancer cells, Vero (renal monkey cells) and primary culture from mouse and human fibroblast. The results are shown in Table 2. These data showed that compound **10** exhibited a significant cytotoxicity toward the above four cells lines. Compounds **11-13** exhibited a significant activity against the growth of RD cancer cells, and a moderate cytotoxicity toward B16. Compounds **11-13** were found to be inactive against Vero, MCF7 and FOG cell lines, mouse embryo fibroblast and human fibroblast. The above results indicate that the methyl group may be responsible for the selective cytotoxicity observed toward some cancer lines and that no methyl group presence increase the cytotoxic activity, which has been proposed by others (5), and further could be helpful in designing of new therapeutic agents. Preliminary antimalarial activity was evaluated against

Plamodium falciparum in vitro, and the results are shown in Table 3. These data show that compounds **10, 12, 13** exhibited significant activity against the inhibition of ^{3}[H]-hypoxanthine incorporation at concentrations of 1×10^{-3} and 1×10^{-4}M.

Table 2. Evaluation of the Cytotoxic Potential of Compounds (**10-13**).
Cell lines LC$_{50}$ (μg/ml)

Compound	HF	MF	Vero	MCF7	B16	RD	FOG
10	60	55	60	98	>>100	>>100	>>100
11	>>100	>>100	>>100	>>100	>100	50	>100
12	>>100	>>100	>100	>>100	>100	61	>>100
13	75	>>100	>>100	>>100	76	57	>>100

HF: human fibroblast; MF: mouse fibroblast; Vero: renal monkey cells; MCF7: breast cancer; B16: melanoma; RD: rabdomiosarcome; FOG: astrocitoma.

Table 3. Evaluation of the Antimalarial Activity (1×10^{-3}, 1×10^{-4} M)of compounds (**10-13**) against a chloroquine-resistant strain of *Plasmodium falciparum*.

Compound	% inhibition [a]	% inhibition [b]
10	82	71
11	c	c
12	70	63
13	83	81

a. 1×10^{-3} M; b. 1×10^{-4} M; c. untested

Experimental

Melting points were determined in a Fisher melting point apparatus and are uncorrected. The IR spectra were recorded on a Shimadzu 470 spectrophotometer in KBr discs. The NMR spectra were recorded on a JEOL 270 MHz spectrometer using TMS as an internal standard. The chemical shifts are expressed as δ ppm. Mass spectra were obtained using an HP 5890 spectrometer with 70 eV ionization potential. Elemental analysis were obtained from Atlantic Microlab Inc (Norcross, GA) and are within $\pm$ 0.4% of the theoretical values. All chemicals and solvents were purchased from Aldrich Chemical. In the case where dry solvent were required, EtOH were destilled from Mg metal. Theoretical calculations were performed using the AM1 semi-empirical method contained in the MOPAC package (6) and ab-initio RHF/3-21G* computations from the gaussian 94 suite of program (7). We made a complete molecular geometry optimization in each case, without any symmetry constraint, via the energy gradient method (8-10) contained in the gaussian 94 program. The electronic study of the molecules was carried out using Molecular Electrostatic Potentials (MEP) from the SPARTAN program (11).

Pyrido[1,2-a]pyrimido-4-one **6-9**.

These compounds were obtained by following the method previously reported (3).

General procedure for synthesis of 3-amino-2-ethoxycarbonylpyrido[1,2-a]thieno[2,3-d]pyrimido-4-one derivatives **10-13**.

A mixture of the appropiate pyridopyrimidine 6-9 0.3g (1.3 mmol), ethyl 2-mercatoacetate 0.15g (1.3 mmol), sodium (0.04at/g) in dry ethanol (5 ml) was refluxed for 4 hours. The solvent was evaporated to dryness under reduced pressure, water was added (10 ml) and the solid thus obtained was colected by filtration. Further purification was accomplished by recrystalization from DMF/EtOH (1:3).

3-amino-2-ethoxycarboylpyrido[1,2-a]thieno[2,3-d]pyrimido-4-one **10**.

The compound was obtained as yellow solid yield (83%), mp 186-187˚C; IR (KBr): 3456 (NH_2), 1699(C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$): 1,36(3H, t, CH_2CH_3, J: 7.2Hz), 4.30(2H, q, CH_2CH_3, J: 7.2Hz), 6.88(2H, brs, NH_2), 7.05(1H, dd, H_8, J: 6.9Hz), 7.54(2H, d, H_9, J: 8.9Hz), 7.70(1H, dd, H_7, J: 6.9Hz), 8.94(1H, d, H_6, J:7.2Hz); ^{13}C NMR: 14.24, 60.10, 91.25, 107.49, 114.43, 125.96, 127.19, 136.64, 149.94, 151.39, 155.27, 165.16, 167.07; MS: m/z (intensity %) 289 (M^+, 100%).

Anal. Calcd. for $C_{13}H_{11}N_3O_3S$ (289.32): C, 53.97; H, 3.83; N, 14.52. Found: C,53.80; H; 3.85; N, 14.54.

3-amino-2-ethoxycarbonyl-7-methylpyrido[1,2-a]thieno[2,3-d]pyrimido-4-one **11**.

The compound was obtained as yellow solid yield (79%), mp 174-176˚C; IR (KBr): 3456 (NH_2), 1693 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$): 1.36(3H, t, CH_2CH_3, J: 7.2 Hz), 2.40(3H, d, CH_3, J: 0.99Hz), 4.29(2H, q, CH_2CH_3, J: 7.2 Hz), 6.89(2H, brs, NH_2), 7.48(1H, dd, H_9, J_1: 9.2Hz, J_2: 0.74Hz), 7.55(1H, dd, H_8, J_1: 9.2Hz, J_2: 2.0Hz), 8.74(1H, m, H_6,), ^{13}C NMR: 14.22, 17.94, 60.07, 91.77, 107.44, 124.39, 124.75, 125.36, 139.77, 149.02, 151.24, 155.24, 165.23, 167.23; MS: m/z (intensity, %) 303 (M^+, 100%).

Anal. Calcd. for $C_{14}H_{13}N_3O_3S$ (303.34): C,55.43; H, 4.32; N, 13.55. Found: C, 55.12; H, 4,39; N, 13.67.

3-amino-2-ethoxycarbonyl-8-methylpyrido[1,2-a]thieno[2,3-d]pyrimido-4-one **12**.

The compound was obtained as yellow solid yield (91 %), mp 239-240˚C; IR (KBr): 3472 (NH_2), 1705 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$): 1.36(3H, t, CH_2CH_3, J: 7.2 Hz), 2.46(3H, d, CH_3, J: 0.99Hz), 4.29(2H, q, CH_2CH_3, J: 7.2 Hz), 6.87(2H, brs, NH_2), 6.88(1H, dd, H7, J_1: 7.5Hz, J_2: 1.7Hz), 7.32(1H, m, H_9), 8.84(1H, d, H_6, J: 7.5Hz), ^{13}C NMR: 14.22, 21.24, 60.04, 91.70, 106.72, 117.27, 123.86, 126.45, 149.16, 150.06, 151.58, 155.51, 166.52, 167.94; MS: m/z (intensity, %) 303 (M^+, 100%).

*Anal.*Calcd. for $C_{14}H_{13}N_3O_3S$ (303.34): C, 55.43; H, 4.32; N, 13.55. Found: C, 55.10; H, 4.35; N, 13.54.

3-amino-2-ethoxycarbonyl-9-methylpyrido[1,2-a]thieno[2,3-d]pyrimido-4-one **13**.

The compound was obtained as yellow solid yield (87 %), mp 204-206 C; IR (KBr): 3483 (NH$_2$), 1696 (C=O) cm^{-1}; ^{1}H NMR (CDCl$_3$): 1.36(3H, t, CH$_2$CH$_3$, J: 7.2 Hz), 2.54(3H, s, CH$_3$), 4.30(2H, q, CH$_2$CH$_3$, J: 7.2 Hz), 6.87(2H, brs, NH$_2$), 6.96(1H, dd, H$_7$, J$_1$: 6.9 Hz, J$_2$: 7.2 Hz), 7.55(1H, dd, H$_8$, J$_1$: 6.7Hz, J$_2$: 1.5Hz), 8.86(1H, dd, H$_6$, J$_1$: 7.2Hz, J$_2$: 1.5Hz), ^{13}C NMR: 14.25, 19.17, 60.23, 91.69, 107.23, 119.35, 124.25, 126.18, 139.18, 150.19, 151.37, 155.36, 166.01, 167.78; MS: m/z (intensity, %) 303 (M$^+$, 100%).

Anal. Calcd. for $C_{14}H_{13}N_3O_3S$ (303.34): C, 55.43; H, 4.32; N, 13.55. Found: C, 54.91; H, 4.31; N, 13.55.

Cytotoxicity, antimalarial testing.

B16, MCF7, RD, FOG cells were kindly provided by Laboratorie of Marie France Poupon, Institute Curie, Paris, France; and Vero cell line, mose embrion and human fibroblast cells were purchased from the Instituto de Biología Experimental, Facultad de Ciencias, Universidad Central de Venezuela, Caracas, Venezuela. The cytotoxic activities of tested compounds against the above seven cell lines were assayed with a modification of the MTT [3-(4,5-dimethylthiazol-2-yl)-diphenyltetrazolium bromide]colorimetric method (12). The cultured cells were treated at four concentration of pure test compound ranging from 10, 50, 100 and 400 µg/ml. All assays were performed in triplicate. The duration of the treatment was 24 h. The LC$_{50}$ value was defined as the concentration of test compound resulting in a 50% reduction of absorbance compared to untreated cells in the MTT assay.

The FCB1, a chloroquine-resistant strain, was originally obtained from a Colombian Patient, and maintained in continuos culture according to the techniques of Trager and Jensen (13). The culture was synchronized until ring trophozoytes using the method described by Lambros and Vanderberg (14). Drugs effects on *P. falciparum* proliferation were measured as inhibition of ^{3}H-hypoxanthine incorporation into nucleic acid, using the method described by Desjardin (15).

Acknowledgments

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