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SYNTHESIS AND CYTOTOXIC ACTIVITY OF N-(2-PYRIDYLSULFENYL)UREA DERIVATIVES. A NEW CLASS OF POTENTIAL ANTINEOPLASTIC AGENTS.

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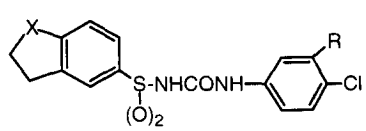
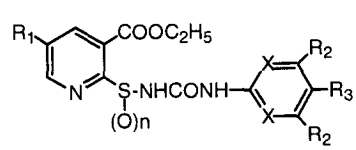
Abstract.- Starting from a 3D-model for the antineoplastic activity of diarylsulfonylureas several new features were proposed and tested. Both types of assayed compounds, the N-(2-pyridylsulfonyl)urea and N-(2-pyridylsulfenyl)urea derivatives, inhibited by 50% the growth of the CCRF-CEM cell line at a dosage near to 1 μM. The N'-(2-pyrimidinyl) derivative of the sulfenylurea **6c** showed a better profile against HT-29, K-562 and HTB-54 tumor cell lines than the corresponding sulfonylurea **6b**. Structural modifications on aryl systems affected differently to the cytotoxic activity shown by the compounds against each cell line.

Diarylsulphonylureas (DSU's) represent a new class of antitumor agents with significant therapeutic activity against rodent and human models of cancer.[1] Inhibition of a drug-responsive NADH oxidase activity located at the external surface of the plasma membrane of cancer cells has been proposed as a mechanism of action for DSU's.[2] Despite the exciting preclinical activity of sulofenur, the prototypic agent, its clinical activity was poor.[3] In an attempt to identify additional clinical candidates, sulfonimidamide analogs were recently checked but, unfortunately, they only exhibited moderate activity against human tumor xenografts.[1] A QSAR[4] study on DSU's ($Ar_1SO_2NHCONHAr_2$), limited to the exploration of the diaryl domains, established the physico-chemical requeriments of substituents in *meta* and/or *para* from the sulfonylurea bridge to bring the *in vivo* inhibition of growth of the 6C3HED lymphosarcoma. These requirements are:

- Ar_1 and Ar_2 must be near to planar systems without bulky substituents that go away from the aryl plane. The bulk of the two pockets for Ar_1 and Ar_2 is limited at about 8 Å and 7.5 Å from the sulfonylurea bridge towards the *para* and *meta* positions, respectively. The Ar_2 substituents at these positions should not to be bonded.
- The Ar_1 substituents which create both a negative electrostatic field near to the *para* position and a positive one near to the *meta* position increase the inhibition.
- The lipophilicity (logP) of the molecule seems to have a calculated[4] optimum value of 4.6.



X = CH_2, R=H Sulofenur
X = O, R=Cl LY295501



6a-d

Compound LY295501, a second generation DSU, represents an example of the above model. Interestingly it brings some physiological properties better from those than sulofenur.[5] The DSU's are extensively bound to albumin and so their *in vitro* cytotoxic activity in serum-containing medium poorly correlates with their in vivo

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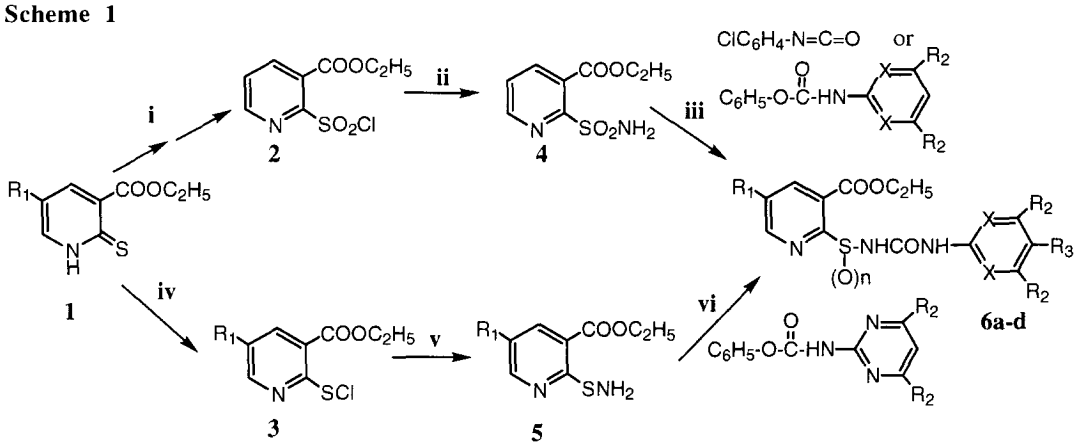
antitumor activity.[6] In spite of that, the *in vitro* CCRF-CEM citotoxicity of this type of compounds gives a rough estimation of their *in vivo* ability to inhibit the growth of the C63HED lymphosarcoma.[7]

Here we describe the synthesis and cytotoxic activity of the new N-(2-pyridylsulfenyl)urea system (**6**, n=0) and we compare it with that from the N-(2-pyridylsulfonyl)urea oxidized system (**6**, n=2). Aryl rings in **6** were selected to obtain DSU's less lipophilic than sulofenur, and so to diminish their binding to plasmatic proteins. The LogP's of **6** were spread from 0.6 to 3.2 units[8]. In the proposed molecules, the ester group limits the freedom of rotation of the pyrido-sulfur bond covering an area not studied before. The nitro group in R_1 roughly reproduces the electrostatic field recommended by the QSAR model for Ar_1, and the chlorine and methoxy groups in R_2-R_3 obey the steric conditions proposed for Ar_2 by the model.

The N-(2-pyridylsulfonyl)urea derivatives **6a** and **6b** were prepared from ethyl 2-chlorosulfonylnicotinate[9] **2** following Scheme 1. The sulfenylurea derivatives **6c-6d** were obtained by refluxing the 2-pyridinesulfenamides **5** with carbamates in xylene. The ethyl 5-nitro-2-sulfenamoylnicotinate **5** (R_1=NO_2) was synthesized with a 75% yield by treatment of the 2-chlorothiopyridine derivative **3** (R_1=NO_2) with ammonia. On the contrary, the 5-hydrogen derivative of **5** was the minority product (20% yield) when **3** (R_1=H) reacted with ammonia under similar conditions (Scheme 1). The corresponding sulfenimides are the side products of the last kind of reactions. Finally, the ethyl 2-chlorothionicotinate derivatives **3** (R_1=H, NO_2) were easily obtained by treating the mercapto compounds **1**, as thioxo form when R_1=NO_2[10] or as disulfide when R_1=H[11], with dry chlorine. Spectroscopic properties of all compounds are in agreement with their structures.[12]

Scheme 1



i: Ref.9; **ii**: NH_3, C_2H_5OH, 0°C, 15min.;
iii: NaH, DMF, 0°C, 10h.; **iv** : Cl_2 (g), $CHCl_3$, 0°C, 30 min.; **v**: NH_3 (g), dioxane, rt, 30 min.;
vi: xilene, reflux, 2h.

6a: R_1=H, R_2=H, R_3=Cl, X=CH, n=2
6b: R_1=H, R_2=H, R_3=H, X=N, n=2
6c: R_1=H, R_2=H, R_3=H, X=N, n=0
6d: R_1=H, R_2=OCH_3, R_3=H, X=N, n=0
6e: R_1=NO_2, R_2=H, R_3=H, X=N, n=0

The cytotoxic activity results obtained against five human tumor cell lines are included in Table 1. All tested compounds **6a-6e** showed a higher cytotoxic activity against the CCRF-CEM lymphocytic leukemia cell line than sulofenur (IC_{50}=32 μM[13]). When the N'-(4-chlorophenyl) group in **6a** was replaced by N'-(2-

pyrimidinyl) to give the sulfonylurea **6b** it also appeared significant inhibition of the growth of the lung carcinoma (HTB-54) and melanoma (MEL-AC) cell lines. The reduction of **6b** to the sulfenylurea **6c** notably increased the cytotoxic activity against both the colon carcinoma (HT29) and myelocitic leukemia (K-562) cell lines.

Table 1. Antineoplastic activities (IC_{50}, μM inhibition of cell growth[14]).

Compound	CCRF-CEM	HT-29	HTB-54	MEL-AC	K-562
6a	4.3	>100	>100	>100	14.0
6b	3.5	>100	1.6	1.2	53.0
6c	15.0	4.8	1.8	34.0	1.0
6d	0.7	96.0	74.0	43.0	43.0
6e	1.5	>100	14.0	0.2	0.2
Doxorubicin	0.1	0.7	1.1	1.2	1.0

Antineoplastic sulfenylureas seem to follow an aryl substitution pattern similar to the one exposed above for DSU's. As shown in Table 1, sulfenylureas with a methoxy group in R_2 (**6d**) or a nitro group in R_1 (**6e**) had a higher cytotoxic activity against the CCRF-CEM cell line than the R_1-R_2 unsubstituted compound **6c**. However, these model was not valuable for the remaining cell lines.

The sulfenylurea **6c** also showed cytotoxic activity against normal PMBC cells. The clonogenic assay[15] of **6c** gave an IC_{50}=13.6 μM. For comparative purposes, the IC_{50} of the positive control doxorubicin was 39.4 μM. Depicted results point to **6c** as a possible lead compound for this new family of potential antineoplastic agents.

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12. Spectroscopic properties of compound **6c**, ethyl 2-[*N*-(2-pyrimidinylcarbamoyl)sulfenamoyl]nicotinate: Mp: 215-216°C. EA: Calc: (C) 48.90, (N) 21.94, (S) 10.03, (H) 4.07. Found: (C) 48.71, (N) 21.36, (S) 9.82, (H) 4.03. ^{1}H-NMR ($CDCl_3$): 1.41 (t, 3H, CH_3), 4.41 (q, 2H, CH_2), 6.94 (t, $J_{5'6'}$= 5 Hz, 1H, H-5'), 7.07 (c, J_{54}=7.6 Hz, J_{56}=4.8 Hz, 1H, H-5), 8.20 (dd, 1H, H-4), 8.54 (dd, J_{64}=2 Hz, 1H, H-6), 8.59 (d, 2H, H-4' y H-6'), 8.92 (s, 1H, NH), 10.26 (s, 1H, NH). Spectroscopic properties of compound **5** (R_1=NO_2), ethyl 5-nitro-2-sulfenamoylnicotinate: Mp: 120-121°C. EA: Calc: (C) 39.50, (N) 17.28, (S) 13.16, (H) 3.70. Found: (C) 39.99, (N) 17.74, (S) 12.91, (H) 3.46. ^{1}H-RMN ($CDCl_3$): 1.43 (t, 3H, CH_3), 3.01 (s, 2H, NH_2), 4.44 (q, 2H, CH_2), 8.91 (d, J_{46}=2.4 Hz, H-4), 9.41 (d, 1H, H-6).
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15. PBMC cells were obtained as described by Boyun (Boyun, A. Scand. J. Inmunol. **1983**, *17*, 429-436.) and dissolved at a cellular density of 2.0-2.5 10^6 cells/mL in Isocove's media. For the clonogenic assay 0.8 mL of a mixture including 0.3 mL of the cell suspension, 0.9 mL of fetal calf serum (final concentration 30%), 0.3 mL of PHA-LCM (final concentration 10%), 1.4 mL of methyl cellulose (final concentration 0.9%), 6 U of human recombinant erithropoietine and 150 μL of the tested compound at the appropriate concentration were incubated for 14 days at 37°C in 6-well plates. After incubation formed clones were counted. At least 3 independent experiments, each done in triplicates, were performed for every compound and concentration tested.