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NEW TRENDS IN PHARMACEUTICAL SCIENCES
Oporto, 13th to 15th October 2011

Pre-Congress Symposium

NEW REGULATORY DEVELOPMENTS IN PHARMACOKINETIC ASSESSMENT
Lisbon, 12th October 2011

ABSTRACTS

CRS Spain-Portuguese Local Chapter

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SYNTHESIS OF AMINODIARYLAMINES IN THE THIENO[3,2-*b*]PYRIDINE SERIES AND EFFECTS ON TUMOR CELL GROWTH INHIBITION, CELL CYCLE AND APOPTOSIS

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INTRODUCTION

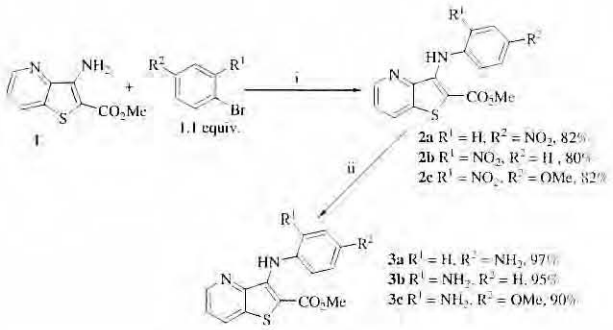
Several series of compounds that include the thienopyridine scaffold have been reported as inhibitors of known cancer therapeutic targets or as inhibitors of cell proliferation in tumor cell lines [1,2]. Our research group has already synthesized several thieno[3,2-*b*]pyridine derivatives by Pd-catalyzed C-C (Suzuki and Sonogashira) and C-N (Buchwald-Hartwig) couplings and some of them have presented tumor cell growth inhibitory activity in cell lines [3-5].

In the present work, three new aminodiarylamines of the mentioned series were synthesized, fully characterized and further submitted to evaluation of their growth inhibitory effect on three human tumor cell lines, representing different tumor models, MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer) and A375-C5 (melanoma), and on non-tumor primary cells (porcine liver primary cell culture). For the most active compound, a study of its effects on normal cell cycle distribution and apoptosis induction was performed in the NCI-H460 cell line.

MATERIAL AND METHODS

Chemistry

Three di(hetero)arylamines were prepared by Buchwald-Hartwig palladium-catalyzed C–N coupling of the methyl 3-aminothieno[3,2-*b*]pyridine-2-carboxylate with bromonitrobenzenes and further reduced in almost quantitative yields to the amino compounds **1a-c** (Scheme 1).



i) $Pd(OAc)_2$ (15 mol%), xantphos (18 mol%), Cs_2CO_3 (2 equiv.), dry dioxane, 2h, 120 °C
ii) NH_4Cl (1 equiv.), Fe (8 equiv.), EtOH/THF/H_2O (3:1:0.5), 100 °C, 2h.

Scheme 1. Synthesis of di(hetero)arylnitro compounds **2** by Buchwald-Hartwig C-N coupling and their reduction to the di(hetero)arylamines **3**.

Antitumoral activity and toxicity to non-tumor cells

The effect of the aminodiarylamines on the growth of three human tumor cell lines (MCF-7, A375-C5 and NCI-H460) was studied using the sulforhodamine B (SRB) assay. Doxorubicin and ellipticine were used as positive controls. Furthermore, to investigate the possible toxicity of the compounds to non-tumor cells, the *in vitro* cell growth inhibition assay was also performed in non-tumor porcine liver primary cells.

Cell cycle and apoptosis

The effect of compound **3c** on cell cycle profile and apoptosis were analysed by flow cytometry following propidium iodide (PI) or Annexin/PI staining, respectively.

RESULTS AND DISCUSSION

The effects of the aminodiarylamines on the growth of the tumour cell lines (MCF-7, A375-C5, and NCI-H460) are summarized in Table 1.

Table 1 – GI_{50} values[a] (μM) obtained for the aminodiarylamines **3** and the positive controls.

	3a	3b	3c	Standard
MCF-7	>125	33.80 ± 1.70	1.40 ± 0.20	0.04 ± 0.00[b]
A375-C5	111.80 ± 5.00	26.00 ± 2.30	1.30 ± 0.10	0.13 ± 0.01[b]
NCI-H460	>125	31.30 ± 2.90	1.40 ± 0.40	0.09 ± 0.00[b]
PLP1	>125	61.27 ± 1.83	12.49 ± 0.09	4.19 ± 0.08[c]

[a]Results are given in concentrations that were able to cause 50% of cell growth inhibition (GI_{50}) after a continuous exposure of 48 h. [b]Positive control doxorubicin. [c]Positive control ellipticine.

The aminodiarylamine **3c** provided the lowest GI_{50} values (≤ 1.40 μM) in all the tested human tumor cell lines and did not present toxicity to the non-tumor cells at those concentrations.

The effect of compound **3c** on cell cycle profile and induction of apoptosis was analyzed in the NCI-H460 cell line (Figure 2).

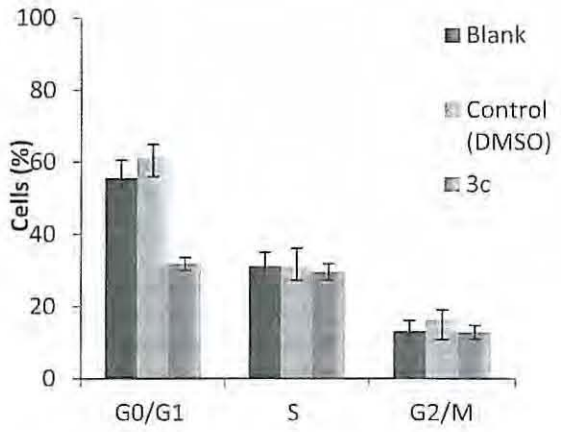


Figure 2 – Cell cycle analysis of NCI-H460 cells treated with compound **3c** at its GI_{50} concentration (1.4 μM). Untreated cells (Blank) and compound vehicle (DMSO) were used as controls. Results are the mean±SEM of three independent experiments.

This compound changed the cell cycle profile, causing a decrease in the percentage of cells in the G0/G1 phase. Furthermore, it caused and increase in the percentage of cells with a sub-G1 DNA content, which was suggestive of apoptosis.
Results from the Annexin V/PI assay confirmed that treatment of NCI-H460 cells with compound **3c** caused an increase in the percentage of apoptotic cells.

CONCLUSIONS

The aminodiarylamine **3c** gave the lowest GI_{50} values in the tested breast, melanoma and non-small cell lung cancer cell lines, and did not show toxicity to porcine liver non-tumor cells at those concentrations. Furthermore, all the compounds presented lower toxicity to porcine liver non-tumor cells than the positive control ellipticine. Compound **3c** changed the cell cycle profile and increased apoptosis of the non-small cell lung cancer (NCI-H460) cells.

REFERENCES

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OPORTO
PORTUGAL
13-15 OCTOBER, 2011

III CONGRESS OF THE PORTUGUESE SOCIETY OF PHARMACEUTICAL SCIENCES
and **IX SPANISH-PORTUGUESE CONFERENCE ON CONTROLLED DRUG DELIVERY**

New Trends in Pharmaceutical Sciences

AUDITORIUM OF ASSOCIATION OF PORTUGUESE PHARMACIES, OPORTO

PROGRAMME

THURSDAY, 13th OCTOBER

09h00m-13h00m **Registration**

14h30m **Opening Ceremony**
José Guimarães Morais (President of SPCF) and **Carlos Maurício Barbosa** (President of SPLC-CRS)

SESSION I
Chairpersons: Rogério Gaspar and Maria Jesus Vicent

15h00m **Opening Lecture**
Leslie Benet (Univ. California San Francisco, USA)
"Forty Years of Biopharmaceutical Sciences and Its Impact on Drug Development"

15h45m **Oral Communications**

15h45m In silico prediction of the tissue: blood partition coefficient in the rat
N. Aniceto, L.F. Gouveia, J.G. Morais and P. Paixão

16h00m Pharmacokinetic profile of tocotrienols after topical application of an sub-micron emulsion hydrogel in various droplet sizes
Tommy Julianto, Rosa Pereira, Yuen Kah Hay and Abu Bakar Abdul Majeed

16h15m Norfloxacin impregnation and release from hydrogels suitable as intraocular lenses
C. González-Chomón, M.E.M. Braga, H.C. de Sousa, A. Concheiro and C. Alvarez-Lorenzo

16h30m Valproate does not deplete hepatic carnitine: a study in rat tissues
P.B.M. Luís, L. IJlst, H. van Lenthe, S. Violante, M.F. Moedas, W. Kulik, M. Duran, I. Tavares de Almeida, R.J.A. Wanders and M.F.B. Silva

16h45m **Coffee-break**

17h15m **Oral Communications**
Chairpersons: J.M. Sousa Lobo and Antonio M. Rabasco

17h15m Targeting of Epidermal Growth Factor Receptor in colon cancer cell lines with PEGylated liposomes coupling to different types of ligands
S. Zalba, I. Navarro, L. de Pablo, I.F. Trocóniz, C. Tros de Ilarduya and M.J. Garrido

17h30m Antiangiogenic and anticancer polymer-drug conjugates in combination therapy
A. Eldar-Boock, K. Miller, J. Sanchis, R. Lupu, M.J. Vicent and R. Satchi-Fainaro

17h45m Intracellular targeting, distribution and activity of hydrophobic gentamicin loaded polymeric nanoparticles
E. Imbuluzqueta, S. Lemaire, F. Van Bambeke, C. Gamazo and M.J. Blanco-Prieto

18h00m **Invited Lecture**
João Nuno Moreira (Univ. Coimbra, Portugal)
"Non-viral vectors and the new opportunities for cellular and molecular targeting"

18h45m **General Assembly of SPLC-CRS**

19h00m **Welcome Reception at the Pharmacy Museum**

FRIDAY, 14th OCTOBER

SESSION II
Chairpersons: João Nuno Moreira and Consuelo Montejo Rubio

09h30m **Invited Lecture**
Rui Medeiros (Instituto Português de Oncologia, Porto, Portugal)
"Genomics, Proteomics & Other "omics" in Oncology"

10h15m **Oral Communications**

10h15m Fast/slow release ibuprofen formulations containing lipidic microparticles and solid dispersions
C.A. Pinho, M.H. Amaral and J.M. Sousa Lobo

10h30m Gold nanoparticles for drug delivery based on imidazolium-derived ligand
A. Calpena, M. Rodrigues and L. Pérez-García

10h45m Establishment of a new in vitro triple intestinal co-culture cell model to evaluate and correlate in vivo intestinal absorption of nanoparticles and therapeutic proteins
B. Sarmento, F. Antunes, F. Andrade, F. Araújo and D. Ferreira

11h00m **Coffee-break and Poster Session**

11h30m **Oral Communications**
Chairpersons: Rui Moreira and Juan M. Irache

11h30m Activity of anti-PLK-1 siRNA on cancer cell lines of different histological origin
Carla Gomes, Lígia G. Silva, Nuno A. Fonseca, José S. Ramalho and João N. Moreira

11h45m Improvement of *in vitro* and *in vivo* antileishmanial activities of bisnaphthalimidopropyl--diaaminooctane by encapsulation in poly(D,L-lactide-co-glycolide nanoparticles
S.C. Lima, J.Tavares, M. Resende, R. Silvestre, P.K.T. Lin and A.Cordeiro-da-Silva

12h00m Effect of protamine on the transfection capacity of solid lipid nanoparticles: application to the treatment of Fabry disease with gene therapy
A.P. Ruiz de Garibay, D. Delgado, A. del Pozo-Rodríguez, M.A. Solinís and A.R. Gascón

12h15m Optimization of polymeric nanoparticle formulations for siRNA delivery to tumour cells
A.D. Oliveira, R. Pereira, M. Teixeira, G.M. Almeida and C.M. Barbosa

12h30m Cannabinoid-Loaded Solid Lipid Nanoparticles for Oral Drug Delivery
M. Durán, R. Lopes, L. Martín-Banderas, M. Fernández-Arévalo, L.M.D. Gonçalves, A. J. Almeida

12h45m Design of anti-PLK1 siRNA-containing liposomes and targeted to cancer cells and the tumor microenvironment
Lígia C. G. da Silva, José S. Ramalho, Sérgio Simões and João N. Moreira

13h00m **Lunch**

SESSION III
Chairpersons: C. Maurício Barbosa and Maria Adfolfina Ruiz

14h30m **Invited Lecture**
Claus-Michael Lehr (Univ. Saarland, Saarbrücken, Germany)
"Nanoparticles for drug delivery across biological barriers"

15h15m **Oral Communications**

15h15m Mannosamine nanoparticles for ocular vaccination against brucellosis
R. da Costa Martins, C. Gamazo, M. Sánchez, I. Peñuelas and J.M. Irache

15h30m Design of a melanoma therapeutic vaccine candidate using polymeric nanoparticles
J.A. Silva, M.A. Videira, H.F. Florindo and V. Préat

15h45m *In vivo* evaluation of tamoxifen-loaded biodegradable polymeric microspheres
Ana Fernandez, Cesar Tejión, Rosa Olmo, Elena Perez, Rafael Lozano and Jose Mª Tejión

16h00m Tamoxifen-loaded nanoparticles based on modified albumin and thiolated alginate: optimization and evaluation in carcinoma cell lines
A. Martínez, M. Benito-Miguel, A. Fernández, S. Guerrero, I. Iglesias and M.D. Blanco

16h15m **Coffee-break and Poster Session**

16h45m-18h00m **Round Table**
"Future Role of Controlled Release in Therapy"
Chairpersons: José G. Morais and Ana Isabel Torres Suarez

Leslie Benet (Univ. California San Francisco, USA)
"The Changing Environment of the Pharmaceutical Industry and the Impact of Controlled Release Formulations"

Malcolm Rowland (Univ. Manchester, UK)
"Controlled Release: The PK/PD Partnership"

Ruth Duncan (Univ. Cardiff, UK)
"Definition of the nanomedicine-specific biomarkers that will improve safety and efficacy"

Vinod P. Shah (Univ. of Kentucky, USA)
"Future role of CR in therapy"

18h30m **General Assembly of SPCF**

21h30m **Congress Dinner**

SATURDAY, 15th OCTOBER

SESSION IV
Chairpersons: Maribel Teixeira and Manuel Guzman

09h30m **Invited Lecture**
Madalena Pinto (Univ. Porto, Portugal)
"At the crossroads of Chemistry, Biology, and Nanothecnology"

10h30m **Oral Communications**

10h30m Oxysterols as selective cytotoxic and chemosensitizer agents. Synthesis and cell proliferation studies
J.F.S. Carvalho, M.M.C. Silva, J.N. Moreira, S. Simões and M.L. Sá e Melo

10h45m Synthesis of aminodiarylamines in the thieno[3,2-b]pyridine series and effects on tumor cell growth inhibition, cell cycle and apoptosis
Ricardo C. Calhelha, Isabel C.F.R. Ferreira, Rui M.V. Abreu, Luís A. Vale-Silva, Eugénia Pinto, Raquel T. Lima, M. Inês Alvelos, M. Helena Vasconcelos and Maria-João R.P. Queiroz

11h00m **Coffee-break**

11h30m **Oral Communications**

11h30m The marine fungi Eurotium cristatum: chemical study, evaluation of growth inhibition effect on human tumor cell lines and development of HPLC analysis
A.P. Almeida, B. Macedo, S. Cravo, T. Dethoup, R.T. Lima, M.H. Vasconcelos, M. Pinto and A. Kijjoa

11h45m Multidimensional optimization of xanthone derivatives with potential antitumor activity
C.M.G. Azevedo, C.M.M. Afonso, J.X. Soares, S. Reis, R.T. Lima, M. Pedro and M. Pinto

CLOSING SESSION
Chairpersons: José G. Morais and C. Maurício Barbosa

12h00m **Closing Lecture**
Malcolm Rowland (Univ. Manchester, UK)
"Physiologically based pharmacokinetics: coming of age"

12h45m **SPLC-CRS PhD Thesis Award**
Oral Communication Award
Poster Award

13h10m **Closing Ceremony**

13h30m **Lunch**