Synthesis and evaluation of the antitumor potential of novel methyl 3-(hetero)arylthieno[3,2-*b*]pyridine-2-carboxylates

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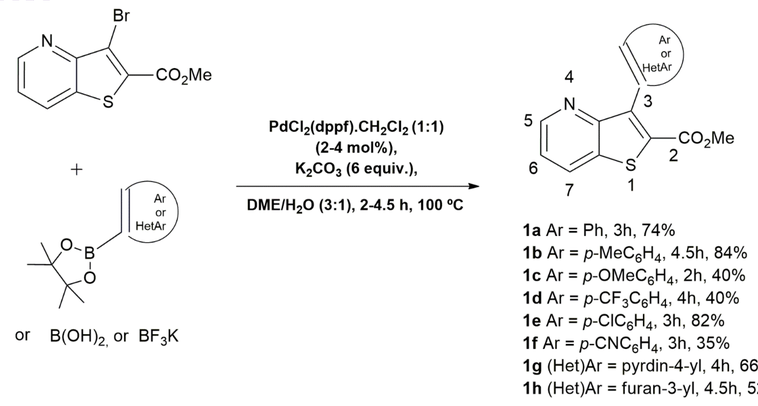
INTRODUCTION

Recently, we have been interested in the synthesis of thieno[3,2-*b*]pyridine derivatives functionalized on the thiophene ring and in their potential antitumor activity [1].

CHEMISTRY

Herein, by C-C Pd-catalyzed Suzuki-Miyaura cross-coupling of methyl 3-bromothieno[3,2-*b]*pyridine-2-carboxylate with (het)aryl pinacol boranes, trifluoro potassium boronate salts or boronic acids, novel methyl 3-(hetero)arylthieno[3,2-*b*]pyridine-2-carboxylates **1a-1h** were synthesized in moderate to high yields after column chromatography (**Scheme 1**), and were fully characterized by:

- **^{1}H**
- **^{13}C-NMR**
- **HRMS**



1a Ar = Ph, 3h, 74%
1b Ar = *p*-MeC_6H_4, 4.5h, 84%
1c Ar = *p*-OMeC_6H_4, 2h, 40%
1d Ar = *p*-$CF_3C_6H_4$, 4h, 40%
1e Ar = *p*-ClC_6H_4, 3h, 82%
1f Ar = *p*-CNC_6H_4, 3h, 35%
1g (Het)Ar = pyrdin-4-yl, 4h, 66%
1h (Het)Ar = furan-3-yl, 4.5h, 52%

Scheme1. Synthesis of novel methyl 3-(het)arylthieno[3,2-*b*]pyridine-2-carboxylates by C-C Suzuki Miyaura cross-coupling.

MATERIALS AND METHODS

- **Sulforhodamine B (SRB) assay** was used to screen 8 synthetized compounds (48h treatments) against different cancer cell line models - pancreatic adenocarcinoma (PANC-1 and BxPC3), non-small cell lung cancer (NCI-H460) and triple negative breast cancer (MDA-MB-231 and MDA-MB-468). The cytotoxicity of the best compounds against the non-tumorigenic cell line MCF-12A was also evaluated by SRB
- **Trypan Blue Exclusion Assay** allowed to determine the number of viable cells
- The effect of the selected compounds on cell cycle profile was evaluated using **Flow Cytometry** with Propidium Iodide
- The expression of specific proteins was analysed by **Western blot**
- **Chick Chorioallantoic Membrane (CAM) assay** was performed to evaluate the angiogenesis and/or tumorigenesis

RESULTS AND DISCUSSION

Table 1 – GI_{50} concentrations (μM) for each compound in different human tumor cell lines, using SRB the assay.

Cell line and respective GI_{50}* concentration (μM) for each compound					
	PANC-1	BxPC3	NCI-H460	MDA-MB-231	MDA-MB-468
1a	>10	>20	>20	>20	>20
1b	>10	>10	>10	>10	>10
1c	>10	>10	>10	>10	>10
1d	>50	>30	>30	>10	>30
1e	>10	>14	>10	**12.56 ± 1.88**	>14
1f	>50	>30	>50	28.67 ± 1.34	**8.73 ± 1.73**
1g	>50	>75	>75	>75	>75
1h	>50	>75	>75	>75	**4.67 ± 0.68**

*GI_{50} values correspond to the mean ± S.E.M. of at least three independent experiments, all performed in duplicated. Doxorubicin was used as a positive control. Doxorubicin GI_{50} values were 68.34 ± 5.69 nM and 81.30 ± 8.99 nM in MDA-MB-231 and MDA-MB-468 cells, respectively. The concentrations tested were the ones possible without appearance of crystals or aggregates in culture.

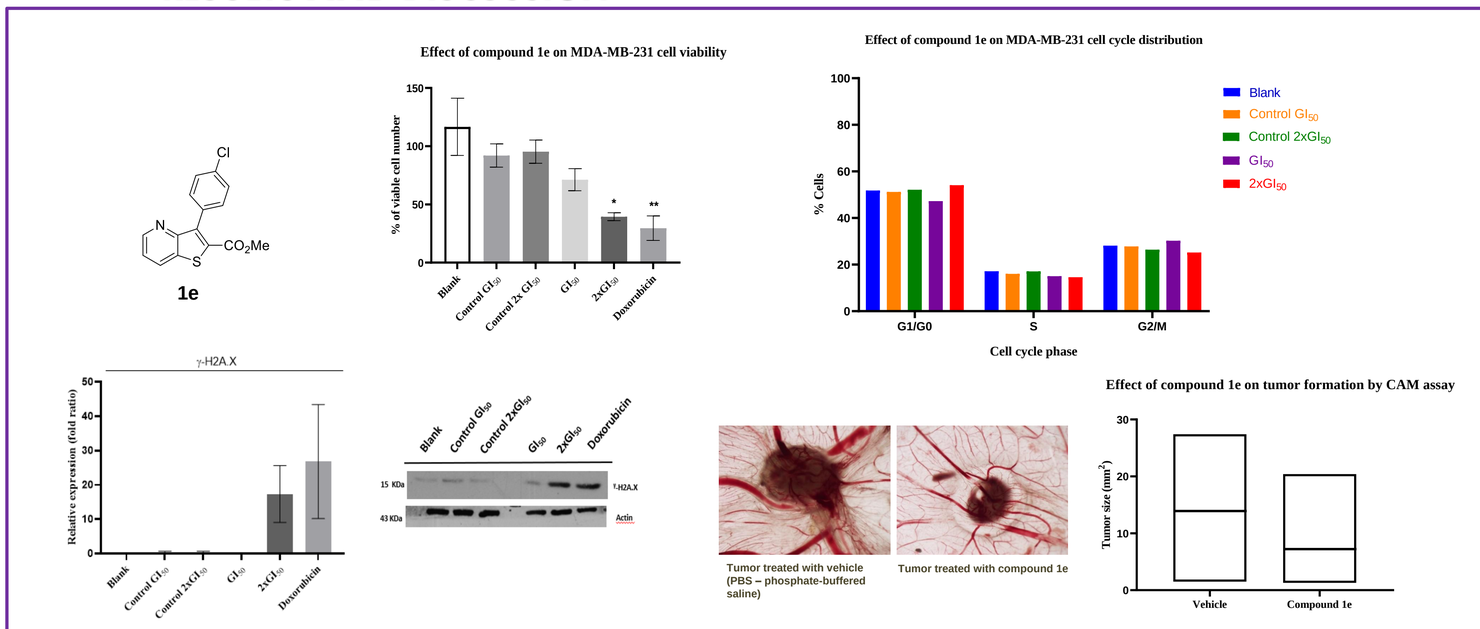


Figure 1. Results of viability, cell cycle profile analysis, Western Blot and CAM assay of compound **1e** after 48h of treatments GI_{50} and 2x GI_{50} concentrations**.**

Table 2 – Evaluation of the toxicity of the most promising compounds in the non-tumorigenic cell line MCF-12A by SRB assay.

Compound	GI_{50} concentration (μM) in the tumor cell lines	Tumor cell line tested	% of MCF-12A Cell Growth at GI_{50} concentration
Compound 1e (*p*-Cl)	12.56 ± 1.88	MDA-MB-231	88.62 ± 4.04
Compound 1f (*p*-CN)	8.73 ± 1.73	MDA-MB-468	117.73 ± 3.22
Compound 1h (furan)	4.67 ± 0.68	MDA-MB-468	82.13 ± 4.78

GI_{50} concentrations values correspond to the mean ± S.E.M. of at least three independent experiments, all performed in duplicate. The % of growth of the cell line MCF-12A was achieved using the GI_{50} concentrations of each compound in the indicated tumour cell lines.

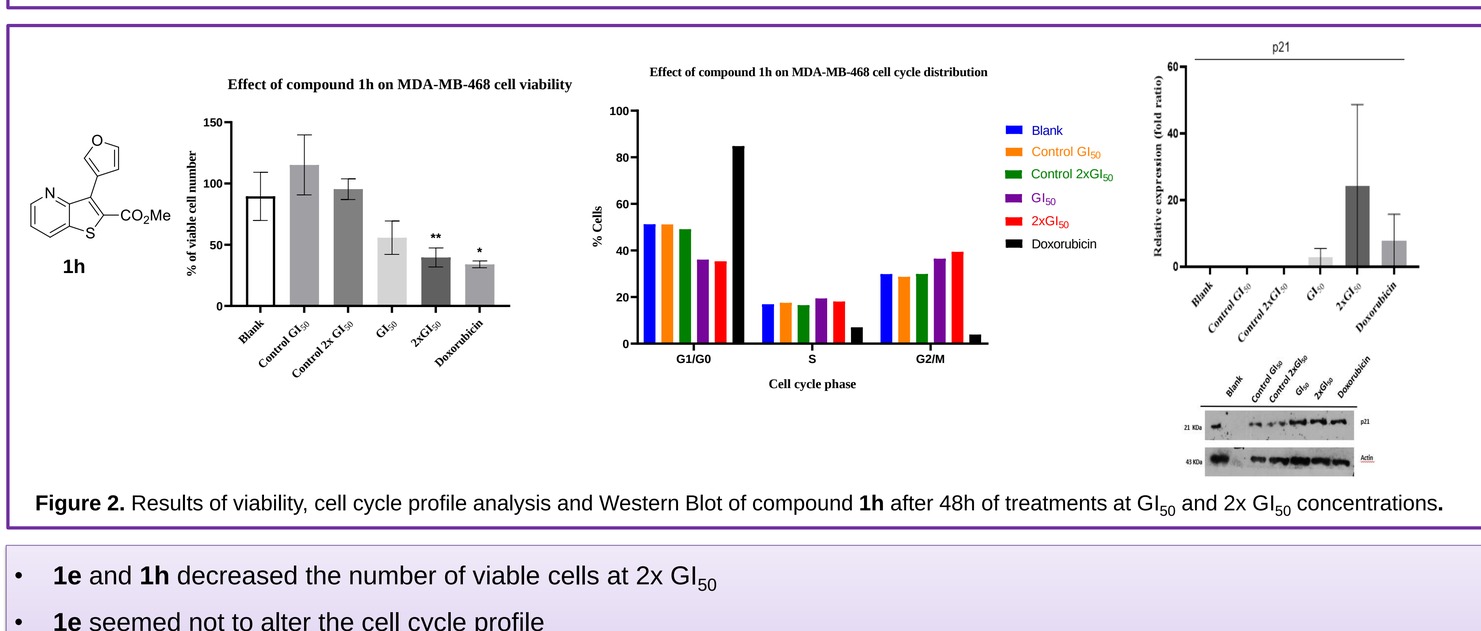


Figure 2. Results of viability, cell cycle profile analysis and Western Blot of compound **1h** after 48h of treatments at GI_{50} and 2x GI_{50} concentrations**.**

- **1e** → lowest GI_{50} value for the MDA-MB-231 cells
- **1f** and **1h** → very low GI_{50} values for MDA-MB-468 cells
- **1e**, **1f** and **1h** → none or little toxicity against MCF12-A cells

- **1e** and **1h** decreased the number of viable cells at 2x GI_{50}
- **1e** seemed not to alter the cell cycle profile
- **1h** increased G2/M phase with concomitant decrease of G1/G0
- **1e** induced high expression of the DNA damage marker γ-H2A.X at 2x GI_{50} concentration
- **1h** presented high expression of the cell cycle marker p21
- **1e** decreased the xenografted tumors size of the cells at GI_{50} concentration by the *in ovo* CAM assay

CONCLUSIONS

We found 3 compounds in this series (**1e**, **1f** and **1h**) that were able to cause growth inhibition of TNBC cell lines at low GI_{50} (5-13μM) without showing much toxicity against a non-tumorigenic cell line. Compound **1e** caused an induction of DNA damage and decreased tumor size in CAM assay. Compound **1h** induced cell cycle arrest at G2/M phase with high expression of p21 in the cells.

ACKNOWLEDGMENTS
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REFERENCES
1. Rodrigues, J. M.; Buisson, P.; Pereira, J. M.; Pinheiro, I. M.; Fernández-Marcelo, T.; Vasconcelos, M. H.; Berteina-Raboin, S.; Queiroz, M.-J. R. P. Synthesis of novel 8-(het)aryl-6H-pyrano[4′,3′:4,5]thieno[3,2-*b*]pyridines by 6-endo-dig cyclization of Sonogashira products and halolactonizations with Cu salts/NXS. Preliminary antitumor evaluation. Tetrahedron. **2019**, 75, 1387-1397.