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Synthesis of potential bioactive benzo-, pyrido- or pyrazinothieno[3,2-*d*]pyrimidin-4-amine analogs of MPC-6827

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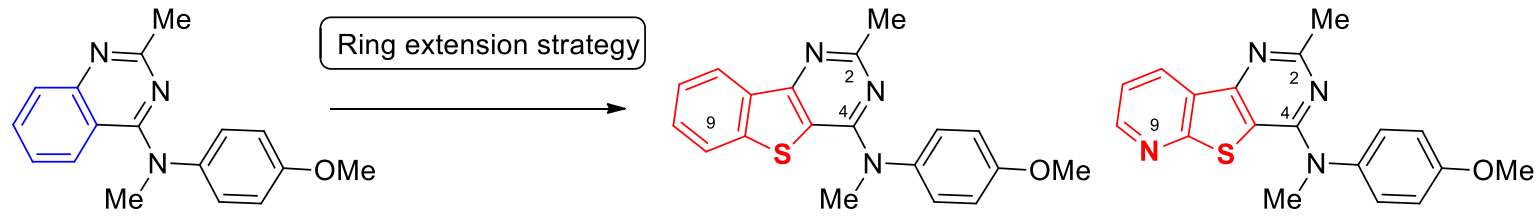
Graphical Abstract



MPC-6827
(Azixa or Verubulin)
Microtubule destabilizing agent

4a

4c

Antiproliferative Activity on HT-29 and Caco-2 Colorectal Cancer Cells

Abstract: Efficient microwave-assisted chemical processes were applied to the synthesis of an array of novel *N*-(4-methoxyphenylamino)-2-methyl benzo-, pyrido- or pyrazino-thieno[3,2-*d*]pyrimidin-4-amine derivatives. These heteroaromatic systems were envisioned as potent bioisosteric analogues of MPC-6827, an anticancer agent previously developed until phase II clinical studies. A brief evaluation and comparison of their antiproliferative activity on HT-29 and Caco-2, two human colorectal cancer cell lines, were also reported. At the tested concentrations (5 and 10 µM), thieno[3,2-*d*]pyrimidin-4-amines **4a** and **4c** exhibited an inhibitory effect similar to **MPC-6827** on human colorectal cancer cell proliferation.

Keywords: Microwave-assisted chemistry; thieno[3,2-*d*]pyrimidines; colorectal cancer; HT-29 cells; caco-2 cells; antiproliferative activity

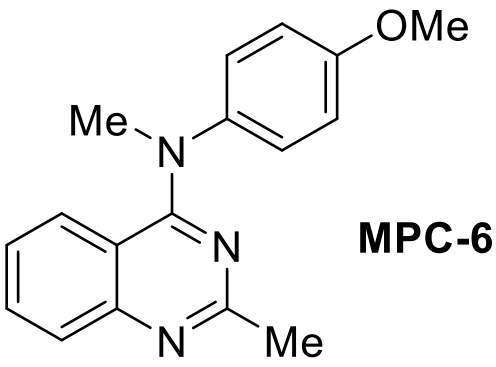
Introduction

MPC-6827 (*N*-(4-methoxyphenylamino)-*N*,2-dimethylquinazoline) has been extensively studied for its therapeutic use against cancer.

Also named Azixa or Verubulin, **MPC-6827** is a microtubule destabilizing agent with a dual mode of action :

- It leads to apoptosis by blocking cell cycle and to growth inhibition on several types of cancer such as breast, colon or ovarian.

- It reduces blood supply to the tumors.



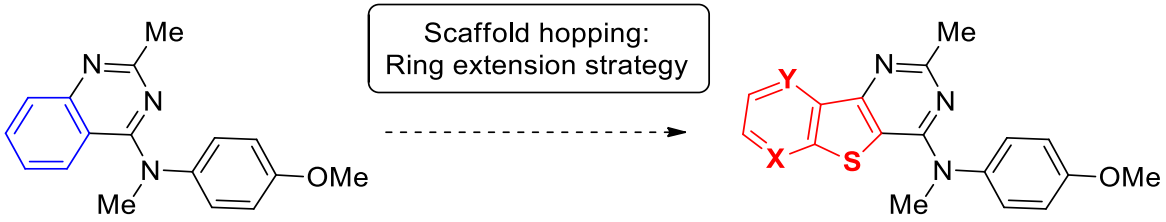
MPC-6827

- *Cancer Res.* **2007**, *67*, 5865–5871, doi:10.1158/0008-5472.CAN-07-0127.
- *J. Med. Chem.* **2008**, *51*, 4771–4779, doi:10.1021/jm8003653.
- *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3536–3540, doi:10.1016/j.bmcl.2009.04.145.
- *J. Med. Chem.* **2009**, *52*, 2341–2351, doi:10.1021/jm801315b.
- *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2330–2334, doi:10.1016/j.bmcl.2010.01.155.
- *ChemMedChem* **2014**, *9*, 847–854, doi:10.1002/cmdc.201300531.

MPC 6827 was as a good candidate for phase I and phase II clinical trials in patients with metastatic melanoma and multiform glioblastoma. These investigations revealed some cardiotoxicity, leading to suspend its clinical development in Phase II.

MPC 6727 remains an excellent model for the design of potential cytotoxic agents.

Bioisosteric analogs of **MPC-6827** were envisioned by extending and replacing the benzene part of this small molecule into an arylthiophene ring.



MPC-6827
(Azixa or Verubulin)

(**X** and **Y** = CH or N)

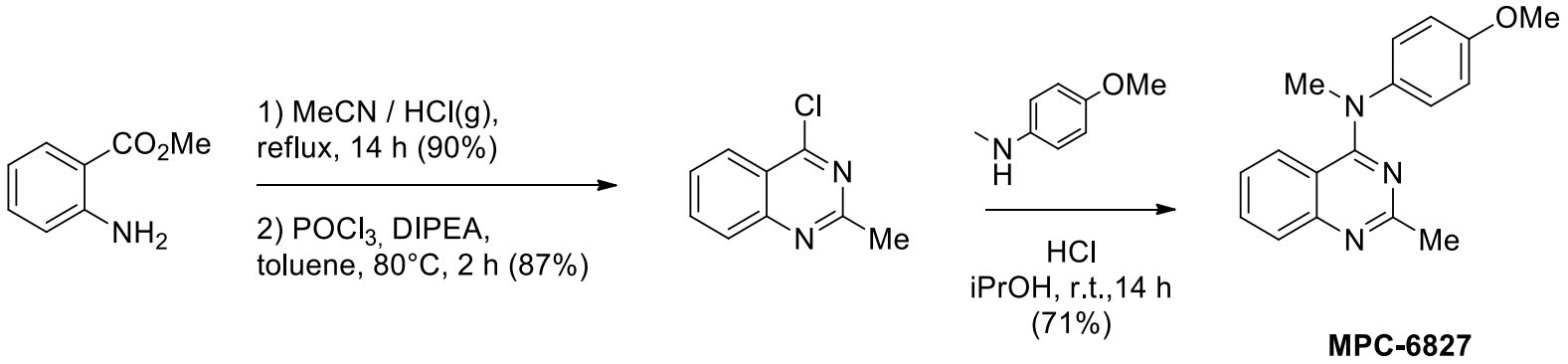
This work

N-(4-methoxyphenylamino)-2-methyl benzo-pyrido- or pyrazino- thieno[3,2-*d*]pyrimidine derivatives envisioned in this work.

- *Mol. Cancer Ther.* **2010**, *9*, 3410–3419, doi:10.1158/1535-7163.MCT-10-0516.
- *J. Neurooncol*. **2012**, *110*, 257–264, doi:10.1007/s11060-012-0964-7.
- *J. Neurooncol*. **2014**, *118*, 335–343, doi:10.1007/s11060-014-1437-y.
- *Oncologist* **2011**, *16*, 1120–1130, doi:10.1634/theoncologist.2010-0432.
- *Bioorg. Chem.* **2019**, *83*, 380–390, doi:10.1016/j.bioorg.2018.10.027.

Results and discussion: chemistry

Initial work of Sirisoma *et al.* described synthetic route of **MPC-6827**, from anthranilic acid methyl ester.
In the last step, 4-chloro-2-methylquinazoline was reacted with *N*-methyl-4-methoxyaniline in anhydrous propanol to give the target product in an overall yield of 55%.

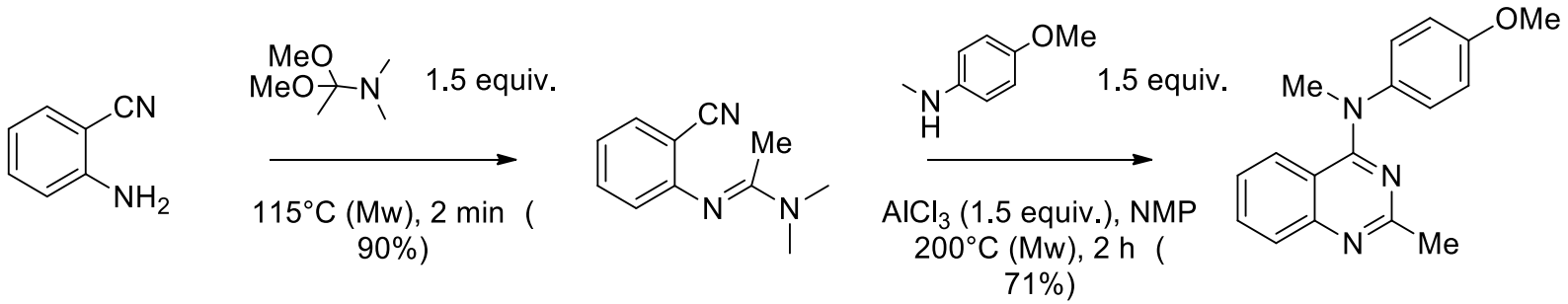


Sirisoma et al.: Three-step traditional route, overall yield: 55%

Sirisoma, N. *et al. J. Med. Chem.* **2009**, *52*, 2341–2351, doi:10.1021/jm801315b.

Developing sustainable and convenient multicomponent processes our group investigated a novel and efficient two-step synthesis of **MPC-6827**: reaction of anthranilonitrile with *N,N*-dimethylacetamide dimethylacetal (DMA-DMA) at 115°C for 2 min and intense heating (200°C for 2 h) with *N*-methyl-*p*-anisidine, in *N*-methylpyrrolidone (NMP), in the presence of aluminum chloride ($AlCl_3$).

MPC-6827 was obtained in 71% yield, *i.e.* 64% over the two steps.

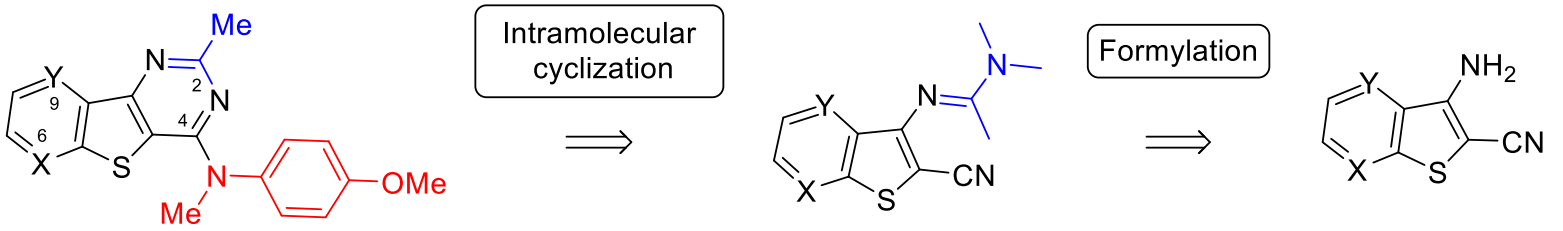


Our work: Two-step microwave-assisted synthesis, overall yield: 64%

MPC-6827

Foucourt, A. *et al. Tetrahedron* **2010**, *66*, 4495–4502, doi:10.1016/j.tet.2010.04.066.

The retrosynthetic route to the novel heteroarenes was envisioned *via* intramolecular cyclization of the key *N,N*-dimethylacetamidamidines (**2**), itselves obtained by formylation of the corresponding cyanoenamines (**1**).



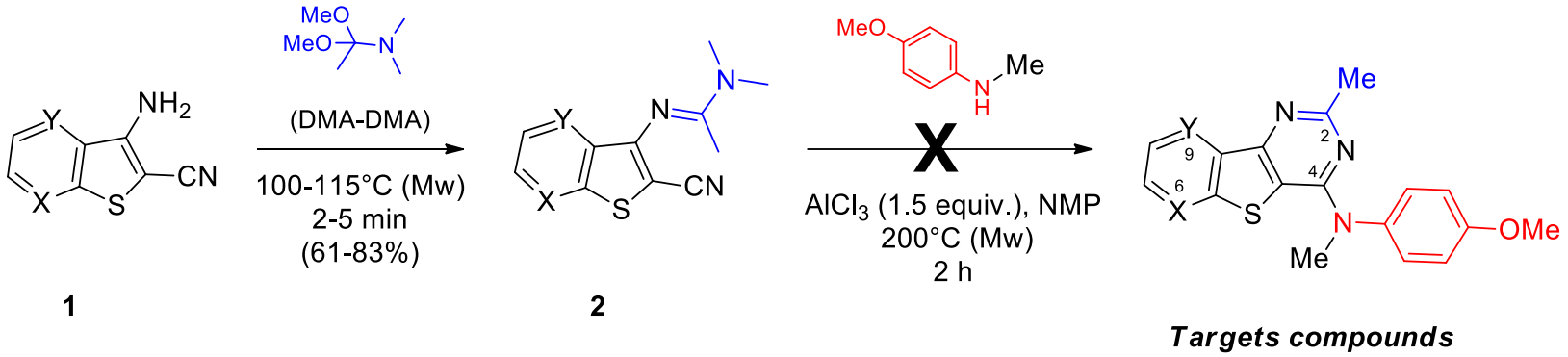
Target compounds

N,N-dimethylacetimidamides **2**

X = CH or N; Y = CH or N

Cyanoenamines **1**

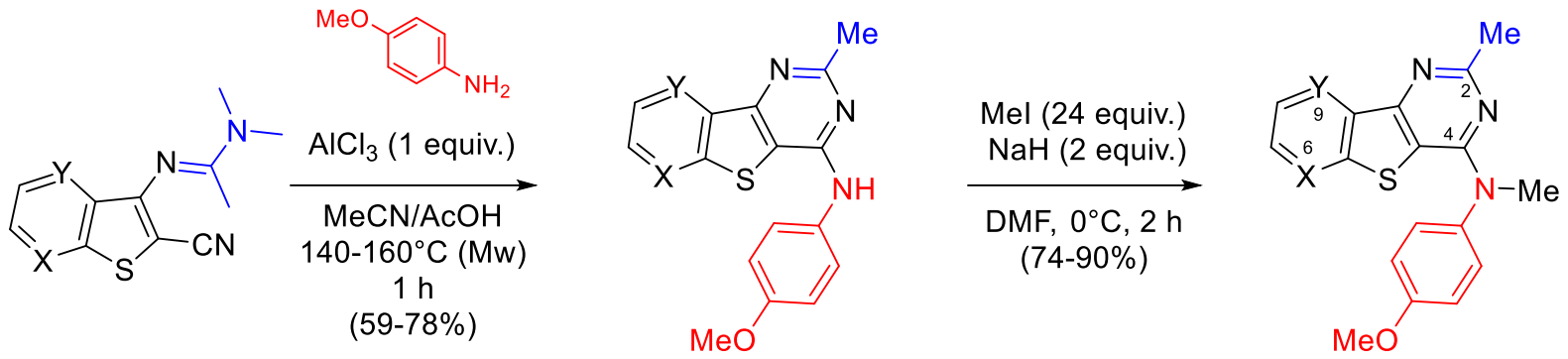
First approach: via previous « **MPC-6827** route »



According preceding works on **MPC-6827**, compound **2** was heated at 200°C for 2 h under microwave irradiation in the presence of *N*-methyl-*p*-anisidine and $AlCl_3$ in *N*-methylpyrrolidone (NMP). These operating conditions were unable to generate the target compounds.

Foucourt, A. *et al. Tetrahedron* **2010**, *66*, 4495–4502, doi:10.1016/j.tet.2010.04.066.
Loidreau, Y. *et al. Tetrahedron* **2011**, *67*, 4852–4857, doi:10.1016/j.tet.2011.05.010.

Second approach: alternative two-step procedure from acetimidamides **2**



2a-d **3a-d** **4a-d**

For all series:

a : X = CH, Y = CH
b : X = CH, Y = N
c : X = N, Y = CH
d : X = N, Y = N

Compound 2	Temp.[1] (°C)	Time[2] (min)	Yield[3] (%)	Compound 3	Temp.[1] (°C)	Yield[3] (%)	Compound 4	Yield[3] (%)
a	115	5	72	a	160	78	a	86
b	115	5	74	b	160	37	b	90
c	115	5	83	c	160	27	c	83
d	100	2	61	d	140	59	d	74

[1] Temperature; [2] Reaction time; [3] Isolated yields

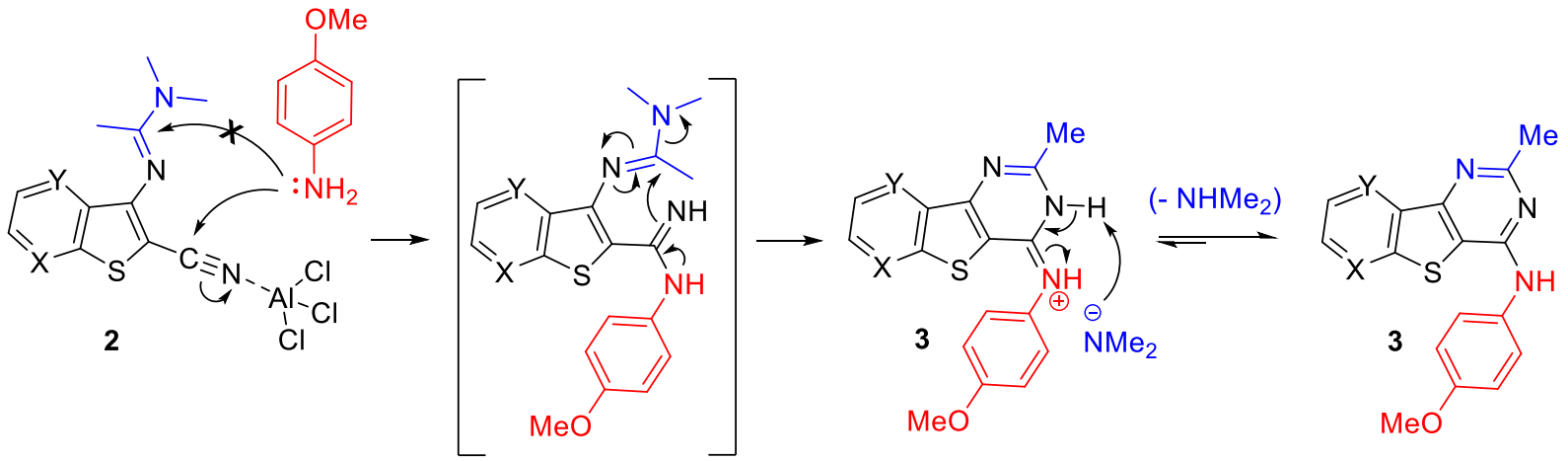
For experimental details see: Loidreau, Y. *et al. Pharmaceuticals* **2020**, *13*, 89, doi : https://doi.org/10.3390/ph13050089.

Comments on chemistry part:

Microwave-assisted heating is still an efficient technology allowing reproducible and safe operating conditions when traditional multistep processes would need long reaction times and unstable and toxic reagents.

The innovative conditions previously described for the synthesis of **MPC-6827** failed and a more traditional approach was investigate to provide compounds **4a-d**.

The crucial part of our synthetic pathway is the cyclization step in which cyanoenamines **2** were converted into tricyclic compounds **3**. Suggested mechanism is described below.

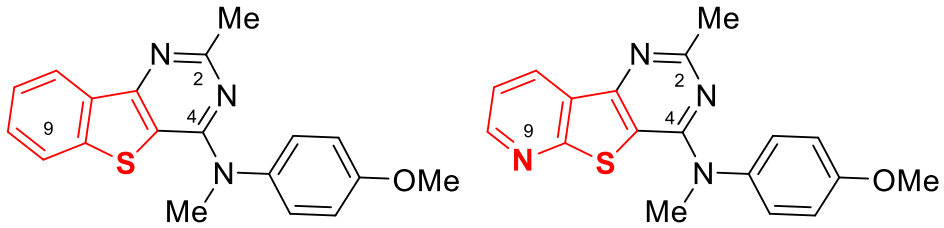


Results and discussion:

Antiproliferative activity on colorectal cancer cell lines (Caco-2 and HT29)

Preliminary results:

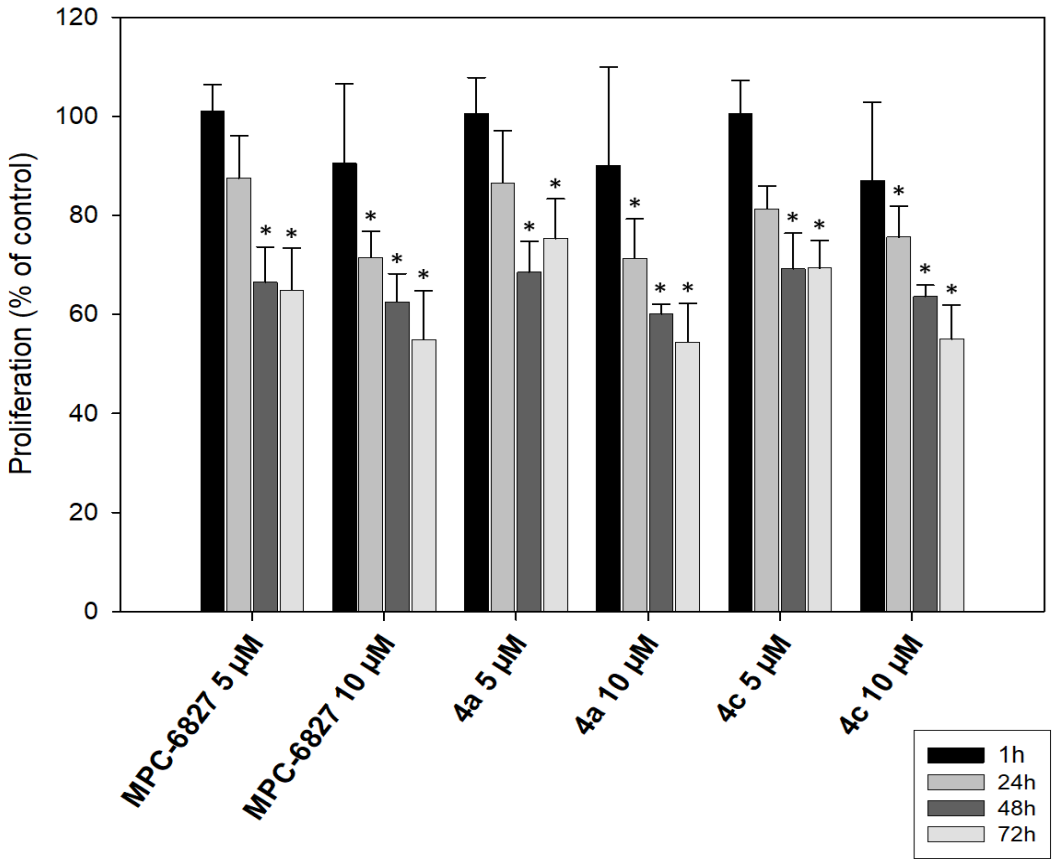
Anti-proliferative effects of compounds (**3b-d**) and (**4a-d**) was evaluated on Caco-2 cells and compared to **MPC-6827**. Each molecule was tested at two concentrations (5 and 10 µM), for 1, 24, 48 and 72 h. The benzo[4,5]thieno[3,2-*d*] pyrimidine **3a** was not tested because of its insolubility in the conditions used. Proliferation of Caco-2 cells was not altered with molecules **3b**, **3c**, **3d**, **4b** and **4d** (data not shown).



4a **4c**

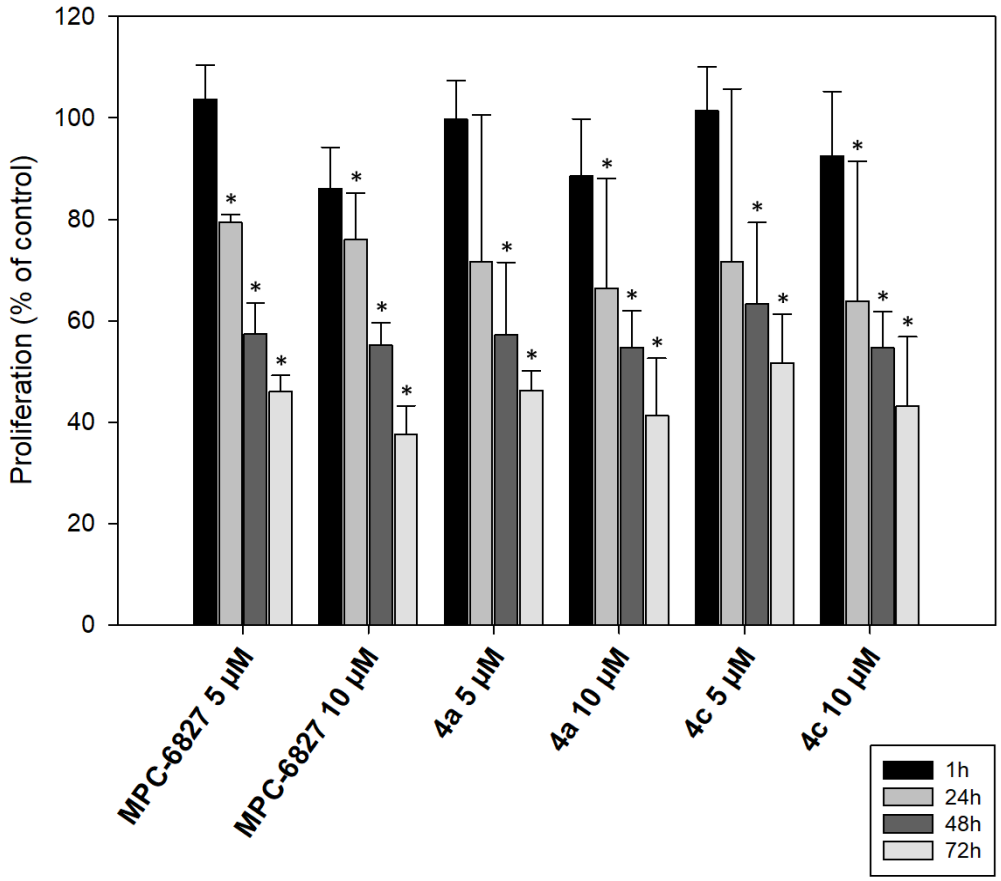
The two most interesting compounds (**4a** and **4c**) were also tested on HT29 cells.

*1) Results obtained on Caco-2 cells for **4a**, **4c** and **MPC-6827**.*



Proliferation of Caco-2 cells treated with **MPC-6827** (5 and 10 µM), **4a** (5 and 10 µM) and **4c** (5 and 10 µM) after 1, 24, 48 and 72 h of treatment. Proliferation was evaluated with MTT assay. Data are reported as the mean ± SD in percentage from three independent experiments. *P < 0.05 vs control that represented 100% of proliferation.

*2) Results obtained on HT-29 cells for **4a**, **4c** and **MPC-6827**.*



Proliferation of HT-29 cells treated with **MPC-6827** (5 and 10 µM), **4a** (5 and 10 µM) and **4c** (5 and 10 µM) after 1, 24, 48 and 72 h of treatment. Proliferation was evaluated with MTT assay. Data are reported as the mean ± SD in percentage from three independent experiments. *P < 0.05 vs control that represented 100% of proliferation.

Comments on biological part

The results of the biological evaluation highlighted the interest of two compounds (**4a** and **4c**) that possess antiproliferative properties on Caco-2 and HT-29 as interesting as **MPC-6827** (Azixa or Verubulin).

Overall, the number of surviving Caco-2 cancer cells decreased in a time-dependent manner. More precisely, inhibition of the proliferation of Caco-2 colon cancer cells was 25% after 24 h of treatment with 10 µM concentration of **4c**. Higher inhibitory effects measured after 48 h incubation are close to the final results observed after 72 h of experiment. In this case, proliferation of Caco-2 cells was significantly inhibited in the presence of **4a** and **4c**, in a manner equivalent to **MPC-6827**, with inhibitory effects of 30% and 45% at 5 µM and 10 µM, respectively.

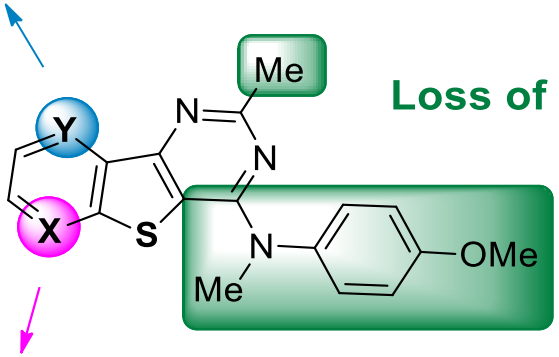
As Caco-2 cells, HT-29 cancer cell proliferation was significantly inhibited in the presence of **MPC-6827** and compounds **4a** and **4c**, after 72 hours of treatment. The growth inhibition of this HT-29 colorectal cancer cell line appeared also time-dependent. In addition, more important effects were observed compared to those described for Caco-2 cells (around 50% and 60% inhibition at 5 µM and 10 µM concentration, respectively).

For experimental details see : Loidreau, Y.; et al. *Pharmaceuticals* **2020**, *13*, 89, doi : https://doi.org/10.3390/ph13050089.

Conclusion

This work described the convenient microwave-assisted synthesis of novel thieno[3,2-*d*]pyrimidin-4-amines (**4a**-**d**). It demonstrates that **4a** and **4c** exhibit a similar inhibitory effect on colon cancer cells proliferation as **MPC-6827**. Preliminary SAR results are summarized below:

Y = N loss of antiproliferative activity



Loss of activity if changed

X = CH or N maintaining antiproliferative activity

The development of such compounds is in course in the hope of identifying new leads.

Acknowledgments

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