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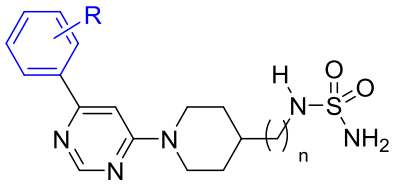

28 compounds
R = alkyl, halogen, ester, etc.
n = 1 or 2

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Synthesis of novel substituted pyrimidine derivatives bearing a sulfamide group and their *in vitro* cancer growth inhibition activity

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ABSTRACT

The synthesis of two series of novel substituted pyrimidine derivatives bearing a sulfamide group have been described and their *in vitro* cancer growth inhibition activities have been evaluated against three human tumour cell lines (HT-29, M21, and MCF7). In general, growth inhibition activity has been enhanced by the introduction of a bulky substituent on the aromatic ring with the best compound having $GI_{50} < 6$ μM for all the human tumour cell lines. The MCF7 selective compounds were evaluated on four additional human invasive breast ductal carcinoma cell lines (MDA-MB-231, MDA-MB-468, SKBR3, and T47D) and were selective against T47D cell line in all cases except one, suggesting a potential antiestrogen activity.

Cancer is one of the most devastating diseases in the world with millions of deaths every year.[1] Despite the development of new treatments,[2] deleterious effects and drug-resistance sometimes result in therapy failure.[3] Thus, the continuous development of new chemotherapeutic scaffolds that could potentially circumvent these shortcomings is still of primary importance.

On the one hand, pyrimidine and its analogues are naturally present in nucleobases which composed deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Importantly, pyrimidine derivatives are found in many bioactive compounds with a broad spectrum of biological effects including antibiotic and anticancer activities.[4,5] On the other hand, the sulfamide group is a valuable and versatile substituent in medicinal chemistry as it can be considered as a bioisostere of sulfamate, sulfonamide, urea, carbamate and amide functionalities. Hence, it is not surprising to find that bioactive molecules bearing a sulfamide group display a wide range of activity including antiepileptic, antibacterial and antiviral activities.[6,7]

In the context of a screening campaign for inhibition of ectonucleotide pyrophosphatase/phosphodiesterase 1 (NPP1),[8] we had access to some compounds bearing both functionalities. Considering the above-mentioned elements, these compounds were also tested for their potential antitumour activities and demonstrated promising results against MCF-7 cancer cell line (Figure 1). Hence, we decided to further investigate the potential antitumour activities of this class of compounds. We report herein the synthesis of novel substituted pyrimidines and their *in vitro* growth inhibition activity against various cancer cell lines. The targeted compounds were built around 4,6-dichloropyrimidine (**3**), and prepared by a Suzuki-Miyaura cross-coupling using various arylboronic acids to functionalize one chloro-position while a S_NAr reaction using sulfamide-containing piperidine **4** or **5** allowed us to obtain the final compounds **1** or **2** (Figure 1).

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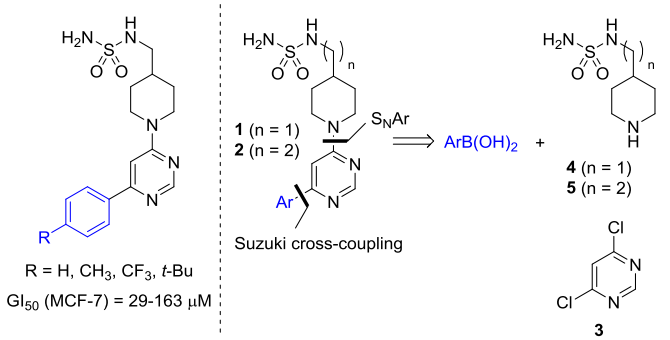
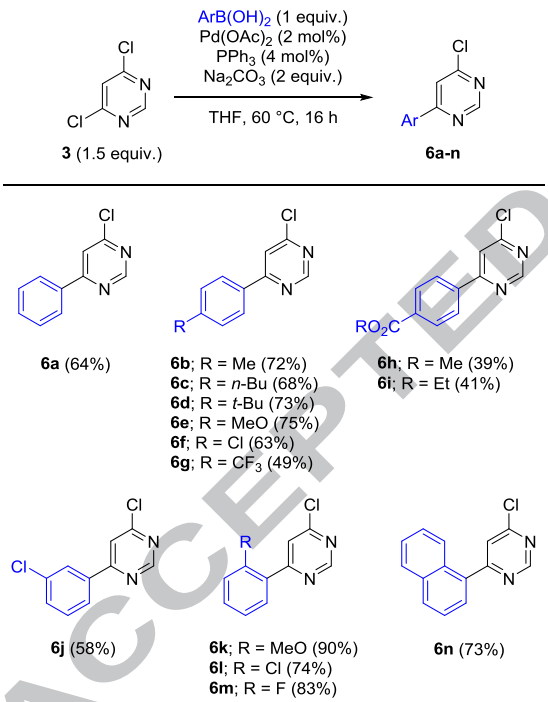


Figure 1. Initial results, targeted substituted pyrimidine derivatives bearing a sulfamide group, and retrosynthetic analysis.

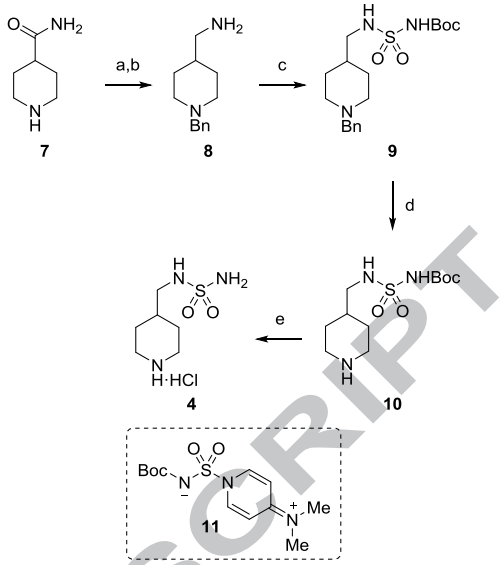
We first investigated the monoarylation of 4,6-dichloropyrimidine (**3**) as shown in Scheme 1. Suzuki-Miyaura conditions inspired by ones developed for the arylation of 6-aryl-2,4-dichloropyrimidine were used.[9] To avoid diarylation and thus facilitate purification, the stoichiometry was adjusted by increasing the amount of 4,6-dichloropyrimidine. Under those conditions, a wide range of monoarylated pyridimine (**6a-n**) were obtained in moderate to excellent yields (39-90%) using 4-, 3- or 2-substituted arylboronic acids (Scheme 1).



Scheme 1. Synthesis of 4-chloro-6-arylpyridimine **6a-n**.

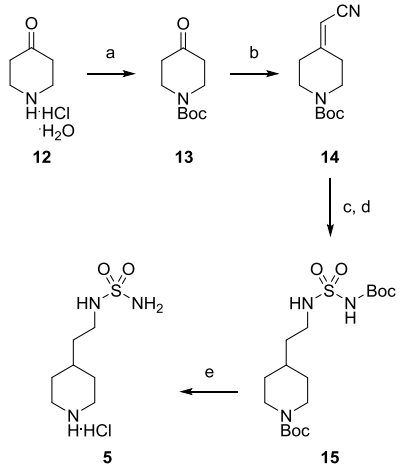
Inspired by a partial synthetic sequence reported by Patel,[10] the synthesis of the sulfamide-containing piperidines **4** and **5** are shown in Schemes 2 and 3, respectively.

For the synthesis of **4**, the primary amine **8** was first obtained from 4-piperidinecarboxamide (**7**) through a two-step sequence.[11] Introduction of a benzyl group on the amine followed by reduction of the amide using $LiAlH_4$ provided **8** in 81% over two steps. Sulfamoylation using reagent **11**[12] gave **9** in 77% yield. Removal of the benzyl group was achieved under palladium catalysis to provide amine **10** in 71% yield. Finally, Boc-deprotection was performed using 4 M HCl/dioxane to give amine **4** as the hydrochloride salt in a quantitative yield.



Scheme 2. Synthesis of piperidine **4** (n = 1). Reagents and conditions: (a) BnBr, K_2CO_3, EtOH, reflux, 16 h; (b) $LiAlH_4$, THF, reflux, 5 h, 81% from **7** (2 steps); (c) **11**, *i*-Pr_2EtN, CH_2Cl_2, rt, 16 h, 77%; (d) H_2, Pd/C, $PdCl_2$, CH_3OH, 16 h, 71%; (e) 4 M HCl/dioxane, 2.5 h, 100%.

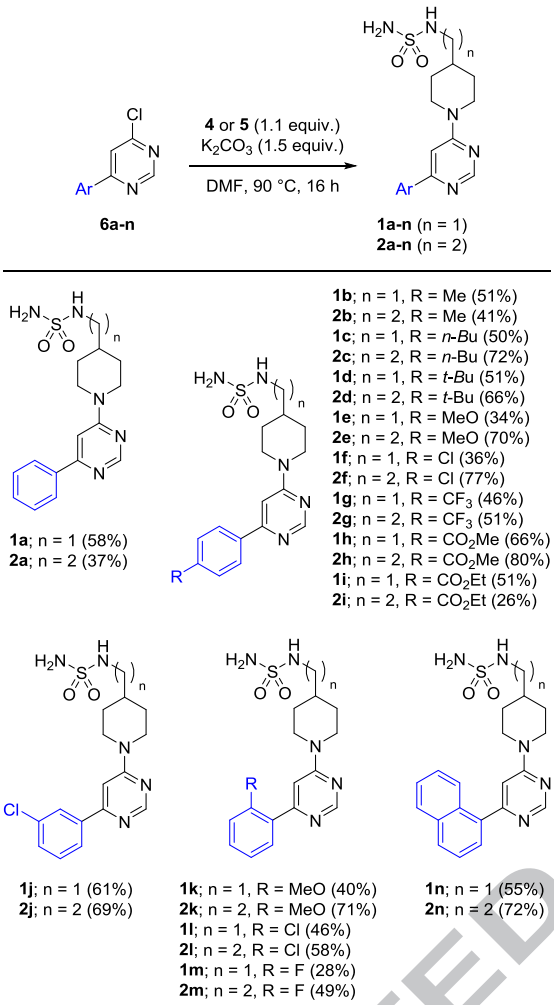
For the synthesis of **5**, 4-piperidone monohydrate hydrochloride (**12**) was first Boc-protected under standard conditions to afford **13**[13] in 93% yield. A Horner–Wadsworth–Emmons reaction between **13** and diethyl(cyanomethyl)phosphonate provided the acrylonitrile derivative **14** in 93% yield.[14] Reduction of the alkene and the nitrile[12] followed by sulfamoylation using reagent **11**[12] gave product **15** in 61% for the 2 steps. Finally, removal of the Boc was accomplished using 4M HCl/dioxane to give amine **5** as the hydrochloride salt in a quantitative yield.



Scheme 3. Synthesis of piperidine **5** (n = 2). Reagents and conditions: (a) Boc_2O, NaOH, THF/H_2O, rt, 2 h, 93%; (b) diethyl cyanomethylphosphonate, Et_3N, LiBr, THF, rt, 16 h. 96%; (c) H_2, Ni Raney, Pd/C, $LiOH{\cdot}H_2O$, dioxane/H_2O, rt, 16 h; (d) **11**, *i*-Pr_2EtN, CH_2Cl_2, rt, 16 h, 61% from **14** (2 steps); (e) 4 M HCl/dioxane, 2.5 h, 100%.

With both partners in hands, the S_NAr reaction could be realized using K_2CO_3 as a base and DMF as the solvent at 90 °C for 16 h (Scheme 4). Moderate yields (28-66%) in the case of **4**, and moderate to good yields (26-80%) in the case of **5** were obtained. The use of a different reaction conditions (K_2CO_3,

CH_3CN, reflux, 16 h) was also evaluated, but generally provided lower yields.



Scheme 4. Synthesis of **1a-n** and **2a-n**.

The antiproliferative activity of the compounds **1a-n** and **2a-n** was evaluated on three human tumour cell lines, namely, HT-29 colon carcinoma cells, M21 skin melanoma, and MCF7 estrogen-dependent breast adenocarcinoma. Cell growth inhibition was assessed according to the NCI/NIH Developmental Therapeutics Program.[15] The results are summarized in Table 1 and expressed as the concentration, in μM, of drug inhibiting the cell growth by 50% (GI_{50}).

Table 1. Growth inhibition activity of compounds **1a-n** and **2a-n** on three different human cancer cell lines.

Compd	GI_{50} (μM)[a]		
	HT-29	M21	MCF7
1a	>100	>100	34.3
2a	66.5	>100	53.2
1b	33.9	71.0	30.6
2b	52.3	57.6	47.5
1c	15.2	47.2	21.8
2c	13.3	15.1	11.3
1d	8.9	19.3	19.7
2d	10.3	18.4	13.1
1e	>100	>100	35.3
2e	72.3	69.8	56.6
1f	37.7	50.3	25.5
2f	30.5	45.2	19.7
1g	29.1	43.1	22.5
2g	22.6	34.8	15.2
1h	22.9	14.1	17.7
2h	19.6	19.6	17.6
1i	5.7	5.0	4.1
2i	6.8	9.0	6.2
1j	47.5	62.5	47.1
2j	31.6	50.7	31.2
1k	>100	>100	10.1
2k	74.3	96.9	69.8
1l	>100	>100	62.4
2l	52.9	93.9	44.8
1m	80.4	>100	48.5
2m	51.8	81.8	>100
1n	6.8	6.6	12.1
2n	3.5	1.8	5.9

[a] Values are means of three experiments and the deviation from the mean is <10% of the mean value.

The *in vitro* growth inhibitory data revealed that substituted pyrimidine derivatives **2c**, **1-2d**, **2h**, **1-2i**, and **1-2n** had significant GI_{50} values below 20 μM for all the cell lines. For those compounds, the length of the linker chain between the piperidine and the sulfamide group (n = 1 for **1**, n = 2 for **2**) had no significant effect on the activity except for **1-2c**. Comparison of the nature of the aryl substituent showed that bulky substituents were well tolerated, in particular naphthalene-substituted compound **2n** was the most active against HT-29, M21, and MCF7 cell lines with GI_{50} = 3.5, 1.8, and 5.9 μM. As a point of comparison, tamoxifen showed a GI_{50} value of 11.2 μM against MCF7 cell line in a similar assay.[16] High growth inhibitory activity was also obtained for naphthalene-substituted derivatives **1-2n**. A *tert*-butyl substituent introduced at the *para* position, as in compounds **1-2d**, displayed better activity than compounds bearing a methyl group at the same position (**1-2b**). Meanwhile, compounds containing an electron-withdrawing group at the *para* position such as an ester (**1-2i**) were potent for the three cell lines with GI_{50} < 10 μM whereas compounds containing an electron-donating group on the aromatic ring at either the *ortho* or the *para* position were selective for MCF7 cell line with, for instance, GI_{50} = 35.3 μM (**1e**) and GI_{50} = 10.1 μM (**1k**). In these cases, the linker length appeared to have an important effect on the selectivity with better results being observed for the shorter linker (n = 1). A similar phenomenon was observed for compound **1l** and **1m** bearing respectively at the *ortho* position a chlorine and a fluorine atom. The ethyl esters (**1i** and **2i**) showed a 3-fold improvement over the methyl esters (**1h** and **2i**). These results are in line with previous observations suggesting that bulkier substituents are beneficial for the antiproliferative activity.

Antiproliferative activity of the MCF7 selective compounds **1a**, **1e**, **1k**, and **1l** was evaluated on four additional human invasive breast ductal carcinoma cell lines, namely, MDA-MB-231, MDA-MB-468, SKBR3, and T47D.[17] The GI_{50} results are presented in Table 2. Among the four compounds tested, **1a**, **1e**, and **1l** showed activity against only one cell line (T47D) with GI_{50} = 24.1, 24.5, and 65.5 μM respectively, while **1k** had no antiproliferative activity. Both MCF7 and T47D are accepted models for ER+ tumors,[17] hence, compounds **1a**, **1e** and **1l** may function as antiestrogens while the mechanism of action of **1k** might be different. Further experiments should be conducted to confirm this hypothesis.

Table 2. Growth inhibition activity of compounds **1a**, **1e**, **1k**, and **1l** on four different human cancer cell lines.

Compd	GI_{50} (μM)[a]			
	MDA-MB-231	MDA-MB-468	SKBR3	T47D
1a	>100	>100	>100	24.1
1e	>100	>100	>100	24.5
1k	>100	>100	>100	>100
1l	>100	>100	>100	65.5

[a] Values are means of three experiments and the deviation from the mean is <10% of the mean value.

Finally, we evaluated the toxicity of the MCF7 selective compounds against HaCat (human spontaneously transformed aneuploid immortal keratinocytes) and HDFn (normal human dermal fibroblasts-neonatal) cells by measuring their growth inhibition activity. All the compounds showed no effect, i.e., GI_{50} >100 μM, for HaCat. For HDFn, compounds **1a** and **1k** had no influence (GI_{50} >100 μM) whereas compound **1e** and **1l** exhibited limited inhibition with GI_{50} values of 77 μM and 93 μM respectively.

In summary, the synthesis of two series of novel substituted pyrimidine derivatives bearing a sulfamide group (**1** and **2**) have been described and their *in vitro* cancer growth inhibition activities have been evaluated against three human tumour cell lines (HT-29, M21, and MCF7). In general, growth inhibition activity has been enhanced by the introduction of a bulky substituent on the aromatic ring with the best compound (**2n**) having GI_{50} < 6 μM for all the human tumour cell lines. In addition, the results revealed that a shorter linker from the piperidine to the sulfamide group was beneficial for selective activity. The MCF7 selective compounds were evaluated on four additional human invasive breast ductal carcinoma cell lines (MDA-MB-231, MDA-MB-468, SKBR3, and T47D) and were selective against T47D cell line in all cases except one, suggesting a potential antiestrogen activity, which may help to address the problem of cancer therapy resistance.

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Supplementary Material

Supplementary data (experimental procedures and spectroscopic characterizations of the compounds) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/...

References and notes

1. Stewart, B. and Wild, C.P. (Eds.), World Cancer Report 2014, 2014. International Agency for Research on Cancer, WHO. http://publications.iarc.fr/Non-Series-Publications/World-Cancer-Reports/World-Cancer-Report-2014 (accessed August 5, 2016).
2. Varmus, H. *Science* **2006**, *312*, 1162.
3. Prakash, O.; Kumar, A. P.; Kumar, Ajeet *Am. J. Pharmacol. Sci.* **2013**, *1*, 104.
4. (a) Undeim, K.; Benneche, T. In *Comprehensive Heterocyclic Chemistry II*; Katritzky, A. R., Scriben, E. F. V., Eds.; Elsevier: Oxford, U.K., 1996; Vol. 6, pp 93−231 and references cited therein. (b) Radi, M.; Schenone, S.; Botta, M. *Org. Biomol. Chem.* **2009**, *7*, 2841.
5. (a) Fabbro, D.; Ruetz, S.; Buchdunger, E.; Cowan-Jacob, S. W.; Fendrich, G.; Liebetanz, J.; Mestan, J.; O'Reilly, T.; Traxler, P.; Chaudhuri, B.; Fretz, H.; Zimmermann, J.; Meyer, T.; Caravatti, G.; Furet, P.; Manley, P. W. *Pharmacol. Ther.* **2002**, *93*, 79. (b) Tavares, F. X.; Boucheron, J. A.; Dickerson, S. H.; Griffin, R. J.; Preugschat, F.; Thomson, S. A.; Wang, T. Y.; Zhou, H.-Q. *J. Med. Chem.* **2004**, *47*, 4716. (c) Selvam, T. P.; James, C. R.; Dniandev, P. V.; Valzita, S. K. *Res. Pharm.* **2012**, *2*, 1.
6. Reviews: (a) Winum, J.-Y.; Scozzafava, A.; Montero, J.-L.; Supuran, C. T. *Med Res Rev.* **2006**, *26*, 767. (b) Reitz, A. B.; Smith, G. R.; Parker, M. H. *Expert Opin Ther Pat.* **2009**, *19*, 1449.
7. For selected recent examples of bioactive sulfamides, see (a) Dou, D.; Tiew, K.-C.; He, G.; Mandadapu, S. R.; Aravapalli, S.; Alliston, K. R.; Kim, Y.; Chang, K.-O.; Groutas, W. C. *Bioorg. Med. Chem.* **2011**, *19*, 5975. (b) Suthagar, K.; Watson, A. J. A.; Wilkinson, B. L.; Fairbanks, A. J. *Eur. J. Med. Chem.* **2015**, *102*, 153. (c) Villalba, M. L.; Enrique, A. V.; Higgs, J.; Castaño, R. A.; Goicoechea, S.; Taborda, F. D.; Gavernet, L.; Lick, I. D.; Marder, M.; Bruno Blanch, L. E. *Eur. J. Pharmacol.* **2016**, *774*, 55. (d) Villalba, M. L.; Palestro, P.; Ceruso, M.; Gonzales Funes, J. L.; Talevi, A.; Bruno Blanch, L.; Supuran, C. T.; Gavernet, L. *Bioorg. Med. Chem.* **2016**, *24*, 894.
8. Shayhidin, E. E.; Forcellini, E.; Boulanger, M.-C.; Mahmut, A.; Dautrey, S.; Barbeau, X.; Lagüe, P.; Sévigny, J.; Paquin, J.-F.; Mathieu, P. *Br. J. Pharmacol.* **2015**, *172*, 4189.
9. Peng, Z.-H.; Journet, M.; Humphrey, G.; *Org. Lett.* **2006**, *8*, 395.
10. Patel, S. D.; Habeski, W. M.; Cheng, A. C.; de la Cruz, E.; Loh, C.; Kablaoui, N. M. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3339.
11. Keita, M.; Vandamme, M.; Mahé, O.; Paquin, J.-F. *Tetrahedron Lett.* **2015**, *56*, 461.
12. Winum, J.-Y.; Toupet, L.; Barragan, V.; Dewynter, G.; Montero, J.-L. *Org. Lett.* **2001**, *3*, 2241.
13. Wang, Z.; Miller, E. J.; Scalia, S. J. *Org. Lett.* **2011,** *13*, 6540.
14. Renslo, A. R.; Atuegbu, A.; Herradura, P.; Jaishankar, P.; Ji, M.; Leach, K. L.; Husband, M. D.; Dermyer, M. R.; Wu, L.; Prasad, J. V. N. V.; Gordeev, M. F. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5036.
15. Shoemaker, R. H. *Nat. Rev. Cancer* **2006**, *6*, 813.
16. Malo-Forest, B.; Landelle, G.; Roy, J.-A.; Lacroix, J.; C.-Gaudreault, R.; Paquin, J.-F. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 1712.
17. Lacroix, M.; Leclercq, G. *Breast Cancer Res Treat.* **2004**, *83*, 249.