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## Synthesis and biological evaluation of new tri-heterocyclic derivatives as anti-colorectal cancer agents

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### ABSTRACT

Colorectal cancer is a major health problem, with a poor prognosis if not detected at early stage. It is the third most common cancer worldwide and the second most deadly. Thus, discovering new treatments became an absolute priority for many research laboratories to improve the prognosis. The involvement of STAT3/5 in the development of colorectal cancer is well established and has led to the development of new inhibitors of these proteins. This work was guided by the previous identification of a potent STAT5 inhibitor in the fight against myeloid leukemias. Pharmacomodulations were strategically performed, and the *in vitro* activities of all tri-heterocyclic derivatives were assessed on HT29 and Lovo colorectal cancer cell lines, as well as on the non-tumoral NCM356 colonic epithelial cell line derived from normal mucosa. Compared with 5-fluorouracil classically used in the treatment strategy for colorectal cancer patients, some of our compounds showed better activity on cancer cells. In particular, compound **1b** blocked the growth of colorectal cancer cells at sub-micromolar concentrations, thus represents a promising lead compound in the fight against colorectal cancer.

### Introduction

For decades, cancer has been a major challenge for the pharmaceutical industry, with considerable efforts made to discover new treatments. In 2022, approximately 20 million new cases alongside 9.7 million deaths were reported. Among these cases, colorectal cancer (CRC) ranks as the third most diagnosed and the second most deadly. It includes cases of cancer of the colon and/or rectum. In 2022, it accounted for 9.3 % of cancer-related deaths [1]. Screening is now widely encouraged, as in most cases surgery to remove polyps in the colon and/or rectum is sufficient to prevent the development of cancer. In fact, a benign tumour can take more than 10 years to develop into cancer, leaving time for regular and effective screening [2]. However, the later CRC is diagnosed, the poorer the prognosis, with only 32.3 % survival at 5 years if diagnosed at stage IV [3].

Numerous solutions exist, including the use of immunotherapy [4] and chemotherapy, either alone or combined with radiotherapy as neoadjuvant treatment [5]. In classical chemotherapy, the aim is to discover therapeutic options capable of targeting proteins responsible

for the disease while minimizing damage to healthy tissues. The most commonly used chemotherapy today typically consist of 5-fluorouracil (5-FU), combined with a chemoprotector, Leucovorin (LV), to reduce side effects [6].

Despite existing solutions, one in two patients still does not survive after a diagnosis of CRC. Therefore, it is important to continuously search for new chemotherapy solutions by targeting potent responsible proteins. For several decades, it has been demonstrated that Signal Transducer and Activator of Transcription 3 (STAT3) and 5 (STAT5) are involved in the formation and progression of numerous cancers. Specifically, they regulate proliferation and inhibit apoptosis in many cancer cell lines, such as hematopoietic diseases [7] as well as solid cancers like breast cancer [8] or CRC [9]. Particularly, Xiong *et al.* have shown by STAT5 depletion with siRNA that STAT5 protein is involved in the proliferation and invasion of CRC cells. This study also suggested a crosstalk between STAT5 and MAPK signalling pathways in the development of CRC [9]. Additionally, Du *et al.* have demonstrated that STAT5 is more expressed in CRC cells than in normal colon tissues. They also showed that isoform STAT5B plays a more important role than

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STAT5A in CRC genesis [10]. These results make STAT5 a potent target to eradicate the disease.

Heterocycles have been extensively used in medicinal chemistry research for many years and play a major role in the structures of biologically active compounds (over 85 % of FDA-approved compounds contain a heterocycle). More specifically, the last decade has seen a significant increase in the number of drugs containing nitrogen heterocycles, particularly in cancer research [11,12]. Over the past decade, our research group developed potent STAT5 inhibitors against myeloid leukemias. Key structural scaffold of these inhibitors includes an indole moiety linked to a tetrahydroquinoline (THQ) core by an ethoxy chain. Based on this structure, Polomski *et al.* [13] identified compounds **1a** and **1b** which exhibited sub-micromolar activities against both chronic (KU812, K562, KCL-22) and acute (MOLM-13, MV-4-11) myeloid leukemia cell lines that constitutively express STAT5 transcription factor. Additional results demonstrated that **1a** inhibits both isoforms of the protein (STAT5A and 5B), specifically targeting their phosphorylation and/or expression. Given the strong correlation between STAT5 expression and CRC progression [9], compounds **1a** and **1b** should have promising *in vitro* activity on CRC. These compounds carry a 2-amino-5-pyrimidinyl ring on the THQ cycle. As 2-substituted-5-pyrimidine moiety seems to be essential to maintain high antileukemic activity, pharmacomodulations were directed toward variations in position 2 of the pyrimidine (Fig. 1). First strategy was to assess the efficiency of electron-withdrawing/donating group like nitrile or chlorine substituents. Second strategy was to evaluate the impact of an additional degree of freedom for hydrogen bonding substituents, like cysteamine, ethanolamine or amidoxime substituent.

## Results

The synthesis of **1a,b**, **5a,b** and **6a,b** has been previously described by Polomski *et al.* [13], starting from brominated compounds **2a,b** (Scheme 1). 2-cyano-5-pyrimidinyl- and 2-chloro-5-pyrimidinylboron derivatives not being commercially available, a palladium-catalyzed Miyaura borylation step was performed on the compounds **2a,b**, brominated at position 5 and 6 of the THQ, with respective yields of 37 % and 71 % [14]. Then, Suzuki-Miyaura coupling was performed in order to obtain compounds **5a,b** and **6a,b**. Compounds **5a,b** and **6a,b**, were synthesized with yields from 35 % to 80 %. In turn, compounds **1a,b** were synthesized directly by Suzuki-Miyaura coupling from starting brominated compounds **2a,b** using commercially available (2-amino-pyrimidin-5-yl)boronic acid. These two compounds were obtained with respective yields of 61 % and 93 %.

Compounds **5a,b** nitrile group offering diverse synthetic possibilities, the amidoxime functional group was chosen for its relevance in medicinal chemistry. Indeed, it has shown relevant impact in numerous studies, including antimicrobial [15] or anticancer [16,17] activities. The work of Sahyoun *et al.* attempted to understand the mechanism of action of this chemical function through the release of nitric oxide [18], a substance that has shown promising anticancer results [19],

particularly in CRC [20]. Based on Festa's work in 2019 [21], carbonitriles **5a-b** reacted with hydroxylamine hydrochloride in the presence of potassium carbonate in methanol at reflux and the amidoxime derivatives **7a** and **7b** were obtained with yields of 52 % and 95 %, respectively (Scheme 2).

Meanwhile, chloropyrimidines **6a,b** offer electrophilic position for nucleophilic attacks of amines. Ethylene diamine was first envisaged to replace compounds **1a,b** amine in order to increase the conformational freedom of the primary amine but all synthesis attempts were unsuccessful. Ethanolamine was then chosen as the alcohol would retain the hydrogen bond donor function. Therefore, alcohols **8a** and **8b** were obtained starting from chloropyrimidines **6a,b** dissolved in ethanolamine at room temperature, with respective yields of 19 % and 47 % [22] (Scheme 3). Additionally, the use of cysteamine was envisaged. Compound **9b** was obtained using chloropyrimidine **6b** in the presence of cysteamine and sodium hydride in DMSO, with a yield of 22 % [23]. Because of the greater nucleophilicity of thiol compared to amine, chlorine substitution was achieved by thiol function, which lead to a primary amine at the end of the ethyl chain. Unfortunately, compound **9a** was never obtained. A trend is observed in these syntheses with lower yields for the compounds at position 5 of the THQ, likely due to steric hindrance caused by the dimethyl group at its position 4.

These molecules were tested *in vitro* on CRC cell lines expressing STAT5, HT29 [9] and Lovo [24], along with 5-FU as control. The Lovo cell line is characterized by microsatellite instability, whereas HT29 cells are microsatellite stable (Table 1).

These results clearly show that a hydrogen bond donor function seems to be crucial to maintain interesting activity in the CRC cell line. Indeed, compounds **5a,b** and **6a,b** demonstrated weak anticancer activity. In addition, compounds **8a,b** showed poor activity, which can be explained either by the lesser relevance of the alcohol function compared with the amine function, or by the conformational freedom offered by the ethyl arm. The same result is observed for compound **9b**, which exhibits poor activity on the two cell lines, suggesting that increasing the degree of freedom has a negative effect on anti-tumour activity. Among all the compounds tested, **1a,b** and **7a** exhibit better activities on these two cell lines. In particular, results clearly demonstrated that **1b** was 14- to 46-more efficient in inhibiting CRC cell growth than 5-FU, with potent sub-micromolar EC<sub>50</sub> for both CRC cell lines.

In previous lab unpublished work, *in vitro* tests showed lower toxicity towards healthy hematopoietic cells with compounds without dimethyl groups at position 4 of the THQ. Furthermore, these dimethyl groups are sterically troublesome and could prevent free rotation of the pyrimidine ring, particularly in the case of substitution at position 5 of the THQ. Therefore, non-methylated analogs of compounds **1a,b**, exhibiting the best activities on HT29 and Lovo cell lines, were synthesized. To do so, commercially available 5- and 6-bromo-1,2,3,4-tetrahydroquinolines were used with previously described [25] protected indole **10** to form **11a** and **11b** via a reductive amination with respective yields of 44 % and 67 %. Deprotection of the indole was carried out in the presence of

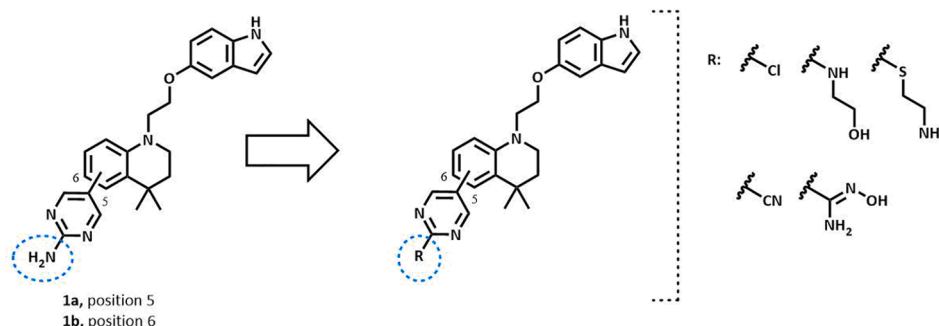
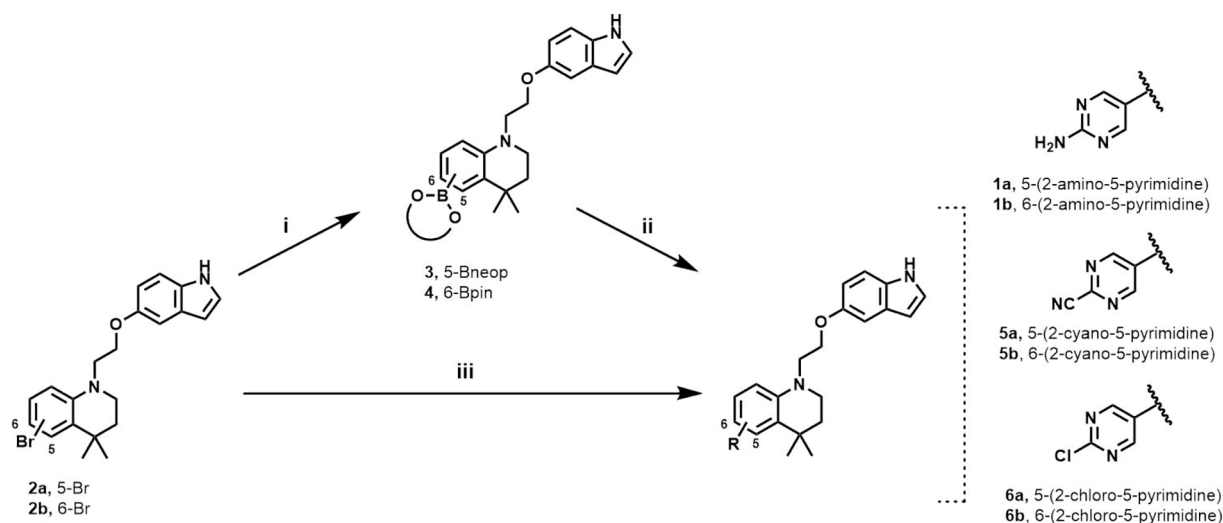
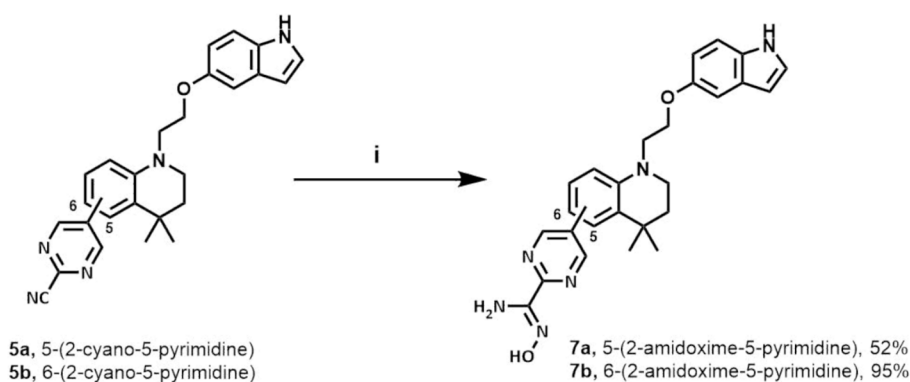


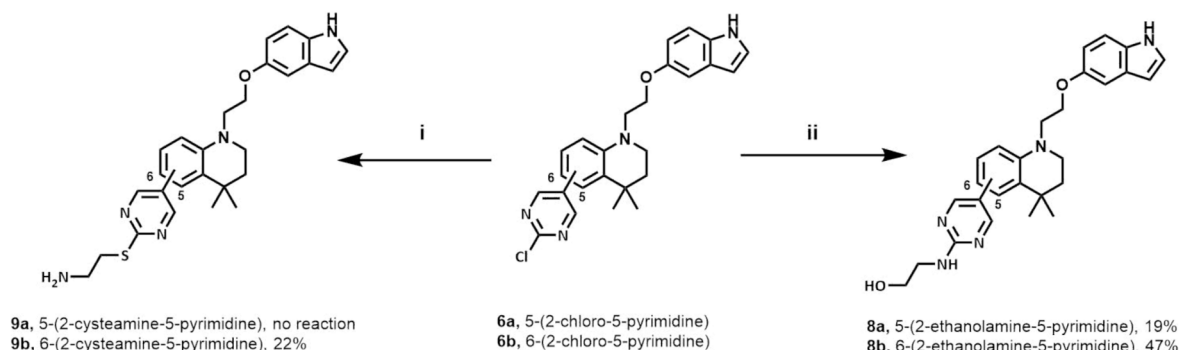
Fig. 1. Design of new anti-CRC inhibitors.



**Scheme 1.** Reagents and conditions: i) (Bneop)<sub>2</sub> or (Bpin)<sub>2</sub>, KOAc, PdCl<sub>2</sub> (dppf), dioxane, reflux, overnight; ii) R-pyrimidine-Br, PdCl<sub>2</sub> (dppf), Na<sub>2</sub> CO<sub>3</sub>, dioxane/water, 110 °C, 2 h; iii) (2-aminopyrimidin-5-yl)boronic acid, PdCl<sub>2</sub> (dppf), K<sub>2</sub> CO<sub>3</sub>, DMF/water, 90 °C, 2 h.



**Scheme 2.** Reagents and conditions: i) hydroxylamine hydrochloride, K<sub>2</sub> CO<sub>3</sub>, methanol, reflux, 3 h.



**Scheme 3.** Reagents and conditions: i) Cysteamine, NaH, DMSO, r.t., 24 h; ii) ethanolamine, r.t., 6 h.

O<sub>2</sub> and potassium *tert*-butoxide in DMSO. Lastly, Suzuki-Miyaura coupling was performed using **12a,b** and (2-aminopyrimidin-5-yl) boronic acid to yield desired compounds **13a** and **13b** with excellent yields (Scheme 4). The activity of these compounds is presented in Table 2.

Compounds **13a,b** showed potent activities against HT29 et Lovo cells. Particularly, **13b** exhibits better activity compared to 5-FU, and was kept for further evaluation.

In summary, based on all biological results, four compounds (**1a**, **1b**, **7a**, and **13b**) were found to exhibit lower cell viability in HT29 and Lovo

cells compared to 5-FU. These results show the importance of hydrogen-bond donor and acceptor functions found in all these structures. This result seems to be confirmed in view of the poor activities of compounds **5a,b** and **6a,b**. We could also highlight the importance of a certain rigidity of the pyrimidine substituent, with better inhibitory activities for compounds with the amine without conformational freedom. Overall, compounds containing a THQ bearing a 2-amino-5-pyrimidine moiety showed the most potent EC<sub>50</sub> values (**1a**, **1b**, and **13b**).

Following the evaluation on CRC cell lines, the selected four compounds were evaluated on non-tumoral NCM356 colonic epithelial cell

**Table 1**

EC<sub>50</sub> values of 2-substituted-5-pyrimidinyl derivatives on CRC cell lines HT29 and Lovo.

Compound	EC <sub>50</sub> (μM)	
	HT29	Lovo
5-FU	7.4 ± 1.8	21.7 ± 6.0
1a	2.8 ± 0.9	1.8 ± 0.5
1b	0.5 ± 0.2	0.5 ± 0.1
5a	38.0 ± 7.3	21.3 ± 5.8
5b	19.6 ± 4.7	14.2 ± 3.1
6a	118.3 ± 27.8	22.5 ± 4.6
6b	13.7 ± 3.2	10.8 ± 1.9
7a	6.3 ± 2.6	5.8 ± 1.2
7b	13.9 ± 4.7	7.6 ± 1.6
8a	38.8 ± 16.0	41.9 ± 12.5
8b	32.6 ± 14.0	23.1 ± 5.2
9b	22.6 ± 7.9	15.0 ± 3.4

line derived from normal mucosa (Table 3).

On non-tumoral colon cell line NCM356, the selected compounds exhibit higher toxicity compared to 5-FU. However, there is still a significant difference in toxicity compared to HT29 and Lovo cell lines for compound 1b, with a better ratio between toxicity on healthy and cancerous cells, compared with 5-FU for Lovo cell line (respectively 8.6 and 4.4). To go further with another healthy cell type, we tested our four selected hit molecules on human skin normal fibroblastic cells (biology platform ImpACcell). Remarkably, all compounds exhibit very low toxicity on this cell line (absolute IC<sub>50</sub> was never reached) and moreover, we were unable to calculate a relative IC<sub>50</sub> for compound 1b (supplementary data, figure S3bis).

Additionally, Daina *et al.* developed a model called “Brain Or Intestinal Estimated permeation method” (BOILED-Egg) [26]. This model uses computational analysis of the lipophilicity and polarity of over 650 small molecules, assessing their gastrointestinal penetration and brain penetration. By gathering all this information, it becomes possible to accurately estimate the gastrointestinal absorption and brain penetration of any small molecule. This model is part of an approach aimed at predicting ADME characteristics in the early stages of drug discovery, helping to reduce failures due to pharmacokinetic parameters in later stages. These computational analyses have significantly improved this aspect in recent years [27]. According to the BOILED-Egg model, the four potent compounds 1a, 1b, 7a and 13b can undergo gastrointestinal absorption but do not cross the blood–brain barrier (Fig. 2A), which is an advantage in the case of CRC. In addition, the Lipinski filter [28] is a useful computational tool to estimate the solubility and permeability of potential future therapeutic molecules. The physicochemical properties to pass the Lipinski filter must be in accordance with the following conditions: MW < 500 g/mol, MLogP < 4.15, number of H-bond donors < 5, and number of H-bond acceptors < 10. Estimation using SwissADME calculation tool ([swissadme.ch](http://www.swissadme.ch)) showed that four hit compounds does not violate any of the Lipinski filter parameters (Fig. 2B) [29,30].

To understand *in vitro* inhibitory activity, the biological activity of

the four hit compounds 1a, 1b, 7a and 13b was evaluated on a panel of 23 kinases by biology platform KissF (residual activities are given in supplementary data, table S1). Only compounds 7a and 13b showed interesting results in the primary screening, on the JAK family’s TYK2 and JAK3 kinases. However, only TYK2 activity (supplementary data, figure S4) could be determined (7a: EC<sub>50</sub> = 14.6 μM; 13b: EC<sub>50</sub> = 4.5 μM). Unfortunately, no kinase biological activity was obtained for the more active compounds 1a and 1b (supplementary data, table S1). TYK2 kinase is involved in the activation of STAT3/5 transcription factors [31] and therefore represents an interesting target in our goal of discovering new STAT5 inhibitors with anti-CRC activity. Indeed, very few studies have investigated this kinase but some have nevertheless shown significant levels of TYK2 expression in the tissues and serum of patients with CRC compared to healthy individuals [32,33], as presented in the Human Protein Atlas, which shows the correlation between high TYK2 expression and low survival rate [34]. With regard to JAK3, Lin *et al.* demonstrated the importance of the JAK3/STAT3 signaling pathway in the proliferation of HT29 and SW480 CRC cells. By inhibiting the JAK3 kinase, they observed a down-regulation of STAT3 phosphorylation, which induces cancer cell apoptosis and cell cycle arrest [35]. Anti-proliferative activity on these two kinases suggests that the compounds 7a and 13b could be indirect STAT3/5 inhibitors through the inhibition of their activation by TYK2 or JAK3. At this stage, we cannot rule out effects on other untested kinases.

## Conclusion

In summary, following the identification of a potent STAT5 inhibitor with sub-micromolar activities on leukemic cells, pharmacomodulations were carried out and all derivatives were tested on CRC cells, also expressing the STAT5 transcription factor. Four tri-heterocyclic analogs

**Table 2**

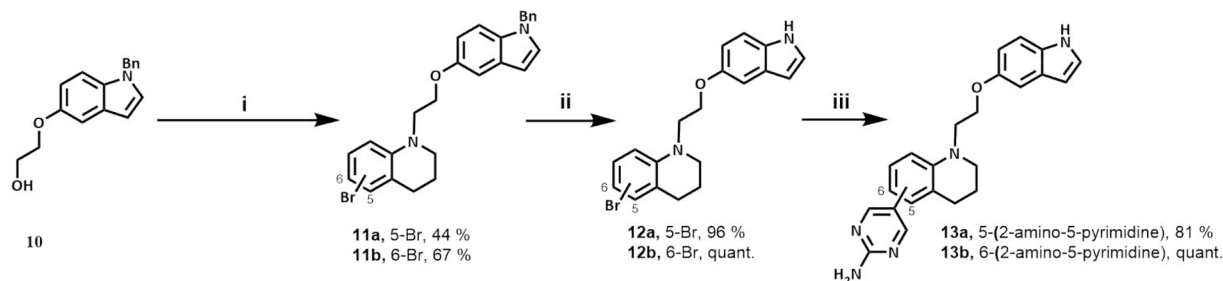
EC<sub>50</sub> values of compounds 13a,b on CRC cell lines HT29 and Lovo.

Compound	EC <sub>50</sub> (μM)	
	HT29	Lovo
5-FU	7.4 ± 1.8	21.7 ± 6.0
13a	9.4 ± 1.4	14.2 ± 1.9
13b	3.9 ± 1.3	3.9 ± 0.9

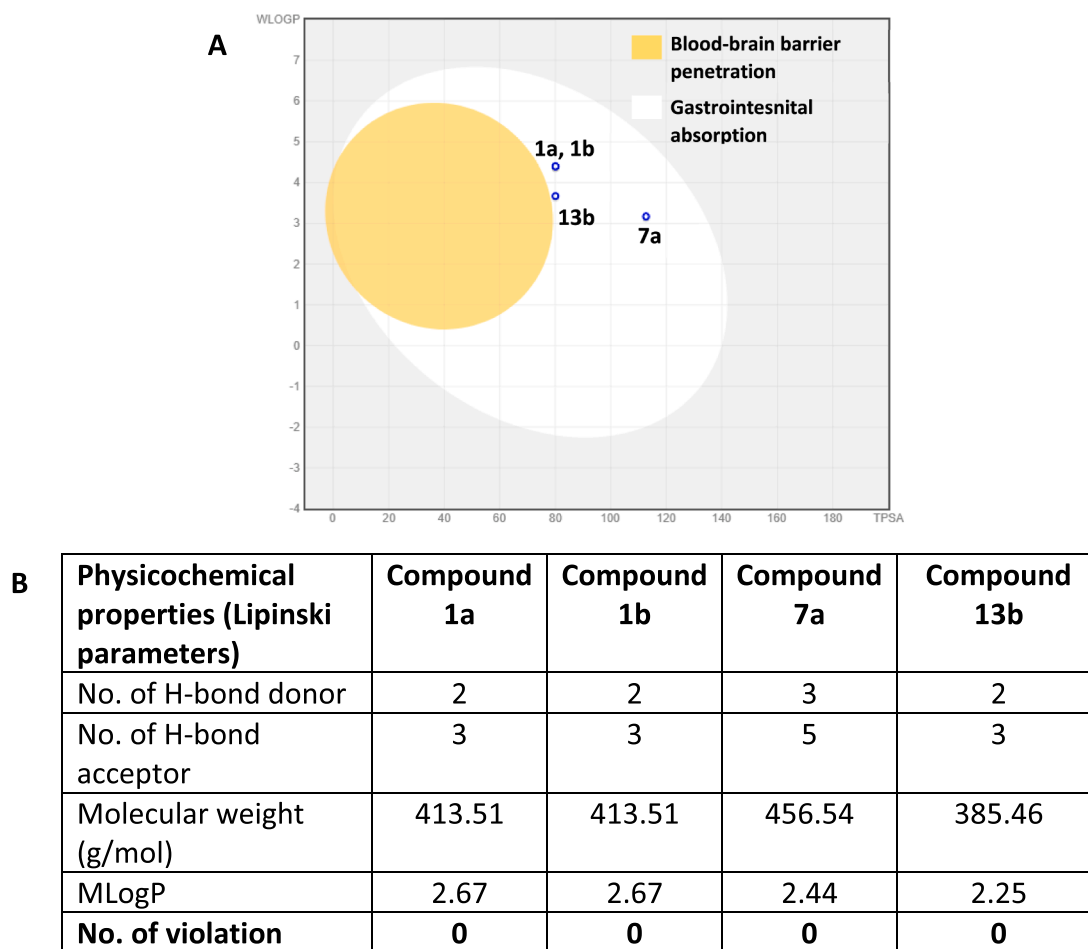
**Table 3**

EC<sub>50</sub> values of selected compounds on non-tumoral colon cells NCM356.

Compound	NCM356 EC <sub>50</sub> (μM)
5-FU	96.1 ± 21.3
1a	7.1 ± 0.7
1b	4.3 ± 1.1
7a	13.1 ± 3.3
13b	9.3 ± 1.0



**Scheme 4.** Reagents and conditions: i) 1) IBX, 1,2-DCE, 80 °C, 2 h; 2) 5- or 6-bromo-1,2,3,4-tetrahydroquinoline, Na(OAc)<sub>3</sub> BH, 1,2-DCE, r.t., 2 h; ii) *t*BuOK, O<sub>2</sub>, DMSO, r.t., 3 h; iii) (2-aminopyrimidin-5-yl)boronic acid, PdCl<sub>2</sub> (dppf), K<sub>2</sub>CO<sub>3</sub>, DMF/water, 90 °C, 2 h.



**Fig. 2.** A) BOILED-Egg model of hit compounds. WLOGP (Wildman and Crippen LogP) for lipophilicity analysis and TPSA (Topological Polar Surface Area) for polarity analysis; B) Computed parameter values for ADME properties estimation ([swissadme.ch](http://swissadme.ch)).

**1a**, **1b**, **7a** and **13b** exhibited potent anti-CRC activities and among them, compound **1b** showed sub-micromolar activities on HT29 ( $EC_{50} = 0.5 \mu\text{M}$ ) and Lovo ( $EC_{50} = 0.5 \mu\text{M}$ ) cell lines, respectively. Moreover, further studies on non-tumoral colon cell line NCM356 showed that **1b** inhibited the growth of healthy cells 9 times less than cancerous cells, and moreover only **1b** gave no relative  $IC_{50}$  on human skin normal fibroblastic cells. Lastly, compounds **7a** and **13b** demonstrated micromolar inhibition activities on JAK family's TYK2 kinase, opening an understanding of the action mechanism. In conclusion, compound **1b** represents a promising lead compound for further optimisation towards a JAK/STAT-targeted therapy for the treatment of CRC. In addition, further pharmacomodulations are ongoing in the laboratory to discover more potent anti-CRC compounds and to better understand their activity on CRC cell lines.

## Material and methods

### Chemistry

Solvents and chemicals were obtained from Aldrich, Fluorochem or VWR and used without further purification. For anhydrous and inert reactions, the glassware was heated with a heat gun, while several vacuum-dry argon cycles were performed. The thin layer chromatography (TLC) studies were performed using commercial precoated aluminum sheets silica gel (60 Å, F254) marketed by Merck and revealed under UV 254 and 365 nm lighting. The purifications by chromatography on silica gel columns were carried out on an ISCO purification unit, Combi Flash RF 75 PSI, with Rediseplash silica gel columns (60 Å,

230 – 400 mesh, grade 9385). Compounds could also be purified by preparative HPLC on a Büchi PURE C-850 Flash Prep using UV 254 nm for detection at 25 °C. The column was a Waters XBridge™ C-18; 5 μm (250 mm x 19,00 mm); elution was performed with 0,1% (by volume) of TFA in water (solvent A), and 0.1 % (by volume) of TFA in acetonitrile (solvent B); gradient 5–100 % of B over 32 min then 100 % of B during 6 min at 17,1 mL/min. NMR spectra (supplementary data) were realized on a Bruker AVANCE AV 300 instrument or on a Bruker Avance Neo 400 spectrometer, HRMS was performed by the mass spectrometry service on a Q-Exactive spectrometer from Thermo Scientific using the electrospray ionization (ESI) technique. The purity was determined by high performance liquid chromatography (HPLC)(supplementary data). HPLC analyses were carried out with a LaChrom Elite system [Hitachi L-2130 (pump) and L-2400 (UV-detector)] using UV 254 nm for detection. The column was a XBridge C-18 (250 × 10 mm, 4 μm, 135 Å); elution was performed with 0.1 % (by volume) of TFA in water (solvent A), and 0.1 % (by volume) of TFA in acetonitrile (solvent B); gradient 20–100 % of B over 35 min with a flow rate of 1 mL.min<sup>-1</sup>; column temperature of 25 °C; injection of 10 μL in DMSO.

### Synthesis of inhibitors

Compounds **1a-b** to **6a-b** were synthesized according to the previous synthesis strategy [13,14,25]. Synthesis and analytic characterizations from compounds **7a-b** to **14a-b** are described below.

*(Z)*-5-(1-(2-((1*H*-indol-5-yl)oxy)ethyl)-4,4-dimethyl-1,2,3,4-tetrahydroquinolin-5-yl)-*N'*-hydroxypyrimidine-2-carboximidamide (**7a**)

To a solution of 5-(1-(2-((1*H*-indol-5-yl)oxy)ethyl)-4,4-dimethyl-1,2,3,4-tetrahydroquinolin-5-yl)pyrimidine-2-carbonitrile **5a** (10 mg, 0.02 mmol) in MeOH (0.5 mL) were added potassium carbonate (5 mg, 0.04 mmol, 1.5 eq.) and hydroxylamine hydrochloride (4 mg, 0.06 mmol, 2.5 eq.). The resulting mixture was stirred at reflux for 3 h. After completion, the crude product was purified by flash chromatography on silica gel (DCM/MeOH; 85/15) to afford the desired product (5.6 mg, 52 %) as a light-yellow oil. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 10.90 (s, 1H), 10.20 (s, 1H), 8.70 (s, 2H), 7.31 – 7.23 (m, *J* = 5.4, 2.6 Hz, 2H), 7.09 – 7.00 (m, *J* = 9.1, 6.6 Hz, 2H), 6.85 (d, *J* = 8.2 Hz, 1H), 6.73 (dd, *J* = 8.7, 2.4 Hz, 1H), 6.36 – 6.25 (m, 1H), 6.20 (d, *J* = 7.1 Hz, 1H), 5.88 (s, 2H), 4.19 (t, *J* = 5.6 Hz, 2H), 3.76 (t, *J* = 5.5 Hz, 2H), 3.48 (t, 2H), 1.61 (t, 2H), 0.93 (s, 6H). <sup>13</sup>C NMR (101 MHz, DMSO-*d*<sub>6</sub>): δ 156.4, 156.0, 152.4, 148.8, 144.6, 138.1, 134.5, 131.1, 128.7, 128.0, 126.3, 125.9, 119.5, 112.0, 112.0, 111.5, 102.8, 100.8, 65.0, 50.9, 45.7, 39.0, 32.9, 31.2. HRMS-ESI (*m/z*): [M + H]<sup>+</sup> caclcd for C<sub>26</sub>H<sub>29</sub>N<sub>6</sub>O<sub>2</sub><sup>+</sup> 457.2347, found 457.2344; purity (HPLC) 99.3 %.

*(Z)*-5-(1-(2-((1*H*-indol-5-yl)oxy)ethyl)-4,4-dimethyl-1,2,3,4-tetrahydroquinolin-6-yl)-*N'*-hydroxypyrimidine-2-carboximidamide (**7b**)

To a solution of 5-(1-(2-((1*H*-indol-5-yl)oxy)ethyl)-4,4-dimethyl-1,2,3,4-tetrahydroquinolin-6-yl)pyrimidine-2-carbonitrile **5b** (18 mg, 0.04 mmol) in MeOH (0.5 mL) were added potassium carbonate (9 mg, 0.06 mmol, 1.5 eq.) and hydroxylamine hydrochloride (7 mg, 0.11 mmol, 2.5 eq.). The resulting mixture was stirred at reflux for 3 h. After completion, MeOH was evaporated and DCM was added. The organic phase was washed three times with water, dried over MgSO<sub>4</sub>, filtered and concentrated in vacuo to afford the desired product without further purification as a yellow oil (18 mg, 95 %). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 10.89 (s, 1H), 10.12 (s, 1H), 9.05 (s, 2H), 7.61 (d, *J* = 2.1 Hz, 1H), 7.48 (dd, *J* = 8.6, 2.1 Hz, 1H), 7.31 – 7.20 (m, *J* = 5.5, 2.8 Hz, 2H), 7.04 (d, *J* = 2.3 Hz, 1H), 6.83 (d, *J* = 8.8 Hz, 1H), 6.72 (dd, *J* = 8.7, 2.4 Hz, 1H), 6.36 – 6.27 (m, 1H), 5.80 (s, 2H), 4.17 (t, *J* = 5.5 Hz, 2H), 3.77 (t, *J* = 5.4 Hz, 2H), 3.51 (t, 2H), 1.71 (t, 2H), 1.30 (s, 6H). <sup>13</sup>C NMR (101 MHz, DMSO-*d*<sub>6</sub>): δ 153.1, 152.4, 144.4, 132.8, 132.8, 131.5, 131.1, 128.0, 125.8, 125.2, 124.0, 119.5, 119.5, 112.00, 111.5, 111.4, 102.8, 100.8, 65.1, 50.1, 46.1, 36.3, 31.8, 30.2. HRMS-ESI (*m/z*): [M + H]<sup>+</sup> caclcd for C<sub>26</sub>H<sub>29</sub>N<sub>6</sub>O<sub>2</sub><sup>+</sup> 457.2347, found 457.2342; purity (HPLC) 98.1 %.

2-((5-(1-(2-((1*H*-indol-5-yl)oxy)ethyl)-4,4-dimethyl-1,2,3,4-tetrahydroquinolin-5-yl)pyrimidin-2-yl)amino)ethan-1-ol (**8a**)

1-(2-((1*H*-indol-5-yl)oxy)ethyl)-5-(2-chloropyrimidin-5-yl)-4,4-dimethyl-1,2,3,4-tetrahydroquinoline (**7a**) was dissolved in ethanolamine (0.5 mL) and the resulting mixture was stirred at room temperature overnight. After completion, the crude product was purified by semi preparative HPLC to afford the desired product as a yellow oil (4.5 mg, 19 %). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 10.90 (s, 1H), 8.08 (s, 2H), 7.31 – 7.23 (m, *J* = 5.4, 2.7 Hz, 2H), 7.05 (d, *J* = 2.3 Hz, 1H), 7.02 – 6.94 (m, 2H), 6.80 – 6.68 (m, 2H), 6.30 (t, *J* = 2.1 Hz, 1H), 6.15 (dd, *J* = 7.3, 1.0 Hz, 1H), 4.68 (t, *J* = 5.6 Hz, 1H), 4.17 (t, *J* = 5.7 Hz, 2H), 3.73 (t, *J* = 5.6 Hz, 2H), 3.53 (q, *J* = 11.9, 6.2 Hz, 2H), 3.45 (t, 2H), 3.37 (q, *J* = 12.1, 6.1 Hz, 2H), 1.60 (t, 2H), 1.00 (s, 6H). <sup>13</sup>C NMR (101 MHz, DMSO-*d*<sub>6</sub>): δ 161.0, 156.7, 152.4, 144.7, 136.17, 131.1, 129.3, 128.0, 127.2, 126.2, 125.8, 120.5, 112.0, 111.5, 111.4, 102.8, 100.8, 65.0, 59.8, 51.1, 45.7, 43.5, 39.8, 32.3, 31.2. HRMS-ESI (*m/z*): [M + H]<sup>+</sup> caclcd for C<sub>27</sub>H<sub>32</sub>N<sub>5</sub>O<sub>2</sub><sup>+</sup> 458.2551, found 458.2547; purity (HPLC) 99.7 %.

2-((5-(1-(2-((1*H*-indol-5-yl)oxy)ethyl)-4,4-dimethyl-1,2,3,4-tetrahydroquinolin-6-yl)pyrimidin-2-yl)amino)ethan-1-ol (**8b**)

1-(2-((1*H*-indol-5-yl)oxy)ethyl)-6-(2-chloropyrimidin-5-yl)-4,4-dimethyl-1,2,3,4-tetrahydroquinoline **7b** (18 mg, 0.04 mmol) was dissolved in ethanolamine (0.5 mL) and the resulting mixture was stirred at room temperature for 6 h. After completion, ethyl acetate was added and

the organic phase was washed twice with a saturated NaCl solution, dried over MgSO<sub>4</sub>, filtered and concentrated in vacuo. Finally, the crude product was purified by flash chromatography on silica gel (DCM/MeOH; 100/0 to 90/10) to afford the desired product as a yellow oil (9 mg, 47 %). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 10.89 (s, 1H), 8.48 (s, 2H), 7.34 (d, *J* = 2.3 Hz, 1H), 7.30 – 7.22 (m, *J* = 5.6, 2.8 Hz, 2H), 7.20 (dd, *J* = 8.6, 2.2 Hz, 1H), 7.04 (d, *J* = 2.4 Hz, 1H), 6.93 (t, *J* = 5.8 Hz, 1H), 6.77 – 6.69 (m, 2H), 6.32 – 6.27 (m, 1H), 4.67 (t, *J* = 5.6 Hz, 1H), 4.15 (t, *J* = 5.7 Hz, 2H), 3.72 (t, *J* = 5.6 Hz, 2H), 3.52 (q, *J* = 6.2 Hz, 2H), 3.46 (t, 2H), 3.37 (q, *J* = 6.0 Hz, 2H), 1.70 (t, 2H), 1.27 (s, 6H). <sup>13</sup>C NMR (101 MHz, DMSO-*d*<sub>6</sub>): δ 161.098, 154.8, 152.4, 142.8, 131.3, 131.1, 128.0, 125.8, 123.8, 123.099, 122.9, 122.0, 112.0, 111.5, 111.3, 102.8, 100.8, 65.1, 59.9, 50.2, 46.0, 43.6, 36.6, 31.7, 30.4. HRMS-ESI (*m/z*): [M + H]<sup>+</sup> caclcd for C<sub>27</sub>H<sub>32</sub>N<sub>5</sub>O<sub>2</sub><sup>+</sup> 458.2551, found 458.2548; purity (HPLC) 97.9 %.

2-((5-(1-(2-((1*H*-indol-5-yl)oxy)ethyl)-4,4-dimethyl-1,2,3,4-tetrahydroquinolin-6-yl)pyrimidin-2-yl)thio)ethan-1-amine (**9b**)

To a solution of 1-(2-((1*H*-indol-5-yl)oxy)ethyl)-6-(2-chloropyrimidin-5-yl)-4,4-dimethyl-1,2,3,4-tetrahydroquinoline **7b** (16 mg, 0.04 mmol) in DMSO (0.5 mL) were added cysteine hydrochloride (5 mg, 0.04 mmol, 1.2 eq.) and sodium hydride (4 mg, 0.10 mmol, 2.6 eq.). The resulting mixture was stirred at room temperature for 1 day. After completion, product was extracted three times with ethyl acetate and the organic phase was washed with a saturated NaCl solution, dried over MgSO<sub>4</sub>, filtered and concentrated in vacuo. Finally, the crude product was purified by flash chromatography on silica gel (DCM/MeOH; 100/0 to 80/20) to afford the desired product as a yellow oil (4 mg, 22 %). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 10.91 (s, 1H), 8.87 (s, 2H), 7.51 (d, *J* = 2.2 Hz, 1H), 7.37 (dd, *J* = 8.6, 2.2 Hz, 1H), 7.29 – 7.22 (m, *J* = 5.7, 3.0 Hz, 2H), 7.03 (d, *J* = 2.3 Hz, 1H), 6.80 (d, *J* = 8.7 Hz, 1H), 6.71 (dd, *J* = 8.7, 2.4 Hz, 1H), 6.32 – 6.26 (m, 1H), 4.16 (t, *J* = 5.5 Hz, 2H), 3.75 (t, *J* = 5.5 Hz, 2H), 3.49 (t, 3H), 3.26 (t, 5H), 3.01 (t, *J* = 6.3 Hz, 2H), 1.70 (t, 2H), 1.28 (s, 6H). <sup>13</sup>C NMR (101 MHz, DMSO-*d*<sub>6</sub>): δ 166.6, 154.2, 152.4, 144.0, 131.5, 131.1, 129.7, 128.0, 125.8, 124.8, 123.7, 119.7, 112.0, 111.4, 111.3, 102.8, 100.8, 65.1, 50.1, 46.0, 40.4 (under DMSO), 36.3, 31.8, 30.9, 30.2. HRMS-ESI (*m/z*): [M + H]<sup>+</sup> caclcd for C<sub>27</sub>H<sub>32</sub>N<sub>5</sub>O<sup>+</sup> 474.2321, found 474.2322; purity (HPLC) 98.9 %.

1-(2-((1-Benzyl-1*H*-indol-5-yl)oxy)ethyl)-5-bromo-1,2,3,4-tetrahydroquinoline (**11a**)

To a solution of 2-((1-benzyl-1*H*-indol-5-yl)oxy)ethanol (0.60 g, 2.24 mmol) in dry 1,2-DCE (20 mL) under argon was added 2-iodoxybenzoic acid (IBX) (1.88 g, 6.72 mmol, 3.0 eq.). The resulting mixture was stirred 2 h at 80 °C and then cooled to rt. The reaction medium was filtered through a sintered glass funnel over a round bottom flask containing 5-bromo-1,2,3,4-tetrahydroquinoline (1.00 g, 4.71 mmol, 2.1 eq.) in dry 1,2-DCE (10 mL). The resulting mixture was stirred for 15 min and then NaBH(OAc)<sub>3</sub> (0.95 g, 4.48 mmol, 2.0 eq.) was added. The reaction medium was stirred 2 h at rt and then hydrolyzed with a saturated aqueous NaHCO<sub>3</sub> solution. After extraction with ethyl acetate, the combined organic extracts were washed with brine and dried with MgSO<sub>4</sub>. After evaporation under reduced pressure, the crude product was purified by flash chromatography on silica gel (cyclohexane/ethyl acetate; 95/5). The obtained mixture of product **11a** and 5-bromo-1,2,3,4-tetrahydroquinoline was dissolved in diethyl ether, washed by HCl 3 M and brine, dried with MgSO<sub>4</sub> and evaporated under reduced pressure to afford the desired compound as a light yellow oil (952 mg, 92 %). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>): δ 7.43 (d, *J* = 3.0 Hz, 1H), 7.32 – 7.21 (m, 4H), 7.17 – 7.12 (m, 2H), 7.05 (d, *J* = 2.4 Hz, 1H), 6.92 – 6.85 (m, 1H), 6.78 – 6.67 (m, 3H), 6.35 (d, *J* = 3.0, 1H), 5.36 (s, 2H), 4.10 (t, *J* = 5.7 Hz, 2H), 3.67 (t, *J* = 5.7 Hz, 2H), 3.42 – 3.38 (m, 2H), 2.67 (t, *J* = 6.5 Hz, 2H), 1.91 – 1.79 (m, 2H). <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>): δ 152.6, 146.8, 138.4, 131.1, 129.7, 128.7, 128.5, 128.0, 127.3, 126.9, 125.1, 120.6, 118.8, 111.6, 110.8, 110.1, 103.3, 100.6, 65.1, 50.4, 49.4, 49.2, 28.3, 21.5. HRMS-ESI (*m/z*): caclcd for C<sub>26</sub>H<sub>26</sub>BrN<sub>2</sub>O<sup>+</sup> [M + H]<sup>+</sup> 461.1223, found 461.1203, and caclcd for C<sub>26</sub>H<sub>26</sub>BrN<sub>2</sub>O<sup>+</sup> [M + H]<sup>+</sup>

463.1203, found 463.1196.

**1-(2-((1-Benzyl-1H-indol-5-yl)oxy)ethyl)-6-bromo-1,2,3,4-tetrahydroquinoline (11b)**

To a solution of 2-((1-benzyl-1H-indol-5-yl)oxy)ethanol (1.20 g, 4.48 mmol) in dry 1,2-DCE (40 mL) under argon was added 2-iodoxybenzoic acid (IBX) (3.80 g, 13.46 mmol, 3.0 eq.). The resulting mixture was stirred 2 h at 80 °C and then cooled to rt. The reaction medium was filtered through a sintered glass funnel over a round bottom flask containing 6-bromo-1,2,3,4-tetrahydroquinoline (2.00 g, 9.40 mmol, 2.1 eq.) in dry 1,2-DCE (20 mL). The resulting mixture was stirred for 15 min and then NaBH(OAc)<sub>3</sub> (1.90 g, 8.96 mmol, 2.0 eq.) was added. The reaction medium was stirred 2 h at rt and then hydrolyzed with a saturated aqueous NaHCO<sub>3</sub> solution. After extraction with ethyl acetate, the combined organic extracts were washed with brine and dried with MgSO<sub>4</sub>. After evaporation under reduced pressure, the crude product was purified by flash chromatography on silica gel (cyclohexane/ethyl acetate; 95/5). The obtained mixture of product **11b** and 6-bromo-1,2,3,4-tetrahydroquinoline was dissolved in diethyl ether, washed by HCl 3 M and brine, dried with MgSO<sub>4</sub> and evaporated under reduced pressure to afford the desired compound as a white solid (1.26 g, 61 %). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>): δ 7.43 (d, *J* = 3.1 Hz, 1H), 7.32 – 7.21 (m, 4H), 7.17 – 7.12 (m, 2H), 7.09 – 7.00 (m, 3H), 6.71 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.61 (d, *J* = 8.8 Hz, 1H), 6.36 (d, *J* = 3.1 Hz, 1H), 5.36 (s, 2H), 4.09 (t, *J* = 5.7 Hz, 2H), 3.63 (t, *J* = 5.7 Hz, 2H), 3.38 (t, *J* = 5.5 Hz, 2H), 2.66 (t, *J* = 6.3 Hz, 2H), 1.87 – 1.75 (m, 2H). <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>): δ 152.5, 144.0, 138.3, 131.1, 130.9, 129.6, 129.2, 128.7, 128.4, 127.2, 126.9, 124.4, 112.4, 111.5, 110.8, 105.8, 103.3, 100.6, 65.1, 50.0, 49.5, 49.2, 27.3, 21.3. HRMS-ESI (*m/z*): caclcd for C<sub>26</sub>H<sub>26</sub><sup>79</sup>BrN<sub>2</sub>O<sup>+</sup> [M + H]<sup>+</sup> 461.1223, found 461.1209, and caclcd for C<sub>26</sub>H<sub>26</sub><sup>81</sup>BrN<sub>2</sub>O<sup>+</sup> [M + H]<sup>+</sup> 463.1203, found 463.1188.

**1-(2-((1H-Indol-5-yl)oxy)ethyl)-5-bromo-1,2,3,4-tetrahydroquinoline (12a)**

To a solution of 1-(2-((1-benzyl-1H-indol-5-yl)oxy)ethyl)-5-bromo-1,2,3,4-tetrahydroquinoline **11a** (0.91 g, 1.98 mmol) in DMSO (60 mL) at rt was added *t*-BuOK (3.12 g, 27.73 mmol, 14.0 eq.). Dry air was then bubbled through solution while stirring 2 h at rt. The reaction mixture was quenched with saturated aqueous NH<sub>4</sub>Cl solution, extracted with ethyl acetate, washed with brine, and dried with MgSO<sub>4</sub>. After evaporation under reduced pressure, the crude product was purified by flash chromatography on silica gel (cyclohexane/ethyl acetate; 80/20) to afford the desired compound **12a** as a dark orange oil (705 mg, 96 %). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>): δ 10.90 (brs, 1H), 7.28 – 7.23 (m, 2H), 7.02 (d, *J* = 2.4 Hz, 1H), 6.89 (dd, *J* = 8.2, 8.2 Hz, 1H), 6.78 – 6.67 (m, 3H), 6.32 – 6.28 (m, 1H), 4.11 (t, *J* = 5.7 Hz, 2H), 3.68 (t, *J* = 5.7 Hz, 2H), 3.42 – 3.37 (m, 2H), 2.68 (t, *J* = 6.5 Hz, 2H), 1.93 – 1.82 (m, 2H). <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>): δ 152.3, 146.8, 131.1, 128.0, 127.9, 125.8, 125.0, 120.6, 118.8, 111.9, 111.4, 110.1, 102.7, 100.8, 65.1, 50.4, 49.3, 28.3, 21.4. HRMS-ESI (*m/z*): caclcd for C<sub>19</sub>H<sub>20</sub><sup>79</sup>BrN<sub>2</sub>O<sup>+</sup> [M + H]<sup>+</sup> 371.0756, found 371.0741 and caclcd for C<sub>19</sub>H<sub>20</sub><sup>81</sup>BrN<sub>2</sub>O<sup>+</sup> [M + H]<sup>+</sup> 373.0733, found 373.0720; purity (HPLC): 98.6 %.

**1-(2-((1H-Indol-5-yl)oxy)ethyl)-6-bromo-1,2,3,4-tetrahydroquinoline (12b)**

To a solution of 1-(2-((1-benzyl-1H-indol-5-yl)oxy)ethyl)-6-bromo-1,2,3,4-tetrahydroquinoline **11b** (1.26 g, 2.73 mmol) in DMSO (81 mL) at rt was added *t*-BuOK (4.30 g, 38.23 mmol, 14.0 eq.). Dry air was then bubbled through solution while stirring 2 h at rt. The reaction mixture was quenched with saturated aqueous NH<sub>4</sub>Cl solution, extracted with ethyl acetate, washed with brine, and dried with MgSO<sub>4</sub>. After evaporation under reduced pressure, the crude product was purified by flash chromatography on silica gel (cyclohexane/ethyl acetate; 80/20) to afford the desired compound **12b** as a dark orange oil (1.01 mg, quantitative). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>): δ 10.90 (s, 1H), 7.28 – 7.23 (m, 2H), 7.07 (dd, *J* = 8.7, 2.6 Hz, 1H), 7.04 – 7.01 (m, 2H), 6.70 (dd, *J* =

8.7, 2.4 Hz, 1H), 6.63 (d, *J* = 8.7 Hz, 1H), 6.32 – 6.28 (m, 1H), 4.10 (t, *J* = 5.5 Hz, 2H), 3.64 (t, *J* = 5.5 Hz, 2H), 3.43 – 3.36 (m, 2H), 2.67 (t, *J* = 6.3 Hz, 2H), 1.88 – 1.78 (m, 2H). <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>): δ 152.3, 144.1, 131.1, 130.9, 129.2, 127.9, 125.78, 124.4, 112.4, 111.9, 111.4, 105.8, 102.8, 100.8, 65.1, 50.1, 49.5, 27.3, 21.3. HRMS-ESI (*m/z*): caclcd for C<sub>19</sub>H<sub>20</sub><sup>79</sup>BrN<sub>2</sub>O<sup>+</sup> [M + H]<sup>+</sup> 371.0753, found 371.0750 and caclcd for C<sub>19</sub>H<sub>20</sub><sup>81</sup>BrN<sub>2</sub>O<sup>+</sup> [M + H]<sup>+</sup> 373.0733, found 373.0729; Purity (HPLC): 97.3 %.

**1-(2-((1H-Indol-5-yl)oxy)ethyl)-5-pyrimidin-1,2,3,4-tetrahydroquinoline (13a)**

A solution of 1-(2-((1H-indol-5-yl)oxy)ethyl)-5-bromo-1,2,3,4-tetrahydroquinoline (55 mg, 0.148 mmol) **12a**, (2-aminopyrimidin-5-yl)boronic acid (62 mg, 0.444 mmol, 3 eq.), and K<sub>2</sub>CO<sub>3</sub> (62 mg, 0.444 mmol, 3 eq.) in DMF (3.0 mL) and water (0.61 mL) was degassed with argon. PdCl<sub>2</sub>(dppf) (10 mg, 0.013 mmol, 0.1 eq.) was added and the resulting mixture was stirred 3 h at 90 °C. The mixture was cooled to room temperature, filtered through a pad of dicalite and extracted with ethyl acetate. The crude product was purified by flash chromatography on silica gel (cyclohexane/ethyl acetate; 40/60), to afford the desired compound **13a** as a yellow oil (58 mg, quantitative). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): 10.89 (s, 1H), 8.17 (s, 2H), 7.30 – 7.23 (m, 2H), 7.07 – 6.98 (m, 2H), 6.74 – 6.71 (m, 2H), 6.63 (s, 2H), 6.39 (dd, *J* = 7.5, 1.0 Hz, 1H), 6.31 – 6.29 (m, 1H), 4.15 (t, *J* = 5.7 Hz, 2H), 3.70 (t, *J* = 5.7 Hz, 2H), 3.42 (t, *J* = 5.6 Hz, 2H), 2.56 (t, *J* = 6.3 Hz, 2H), 1.81 – 1.75 (m, 2H); <sup>13</sup>C NMR (101 MHz, DMSO-*d*<sub>6</sub>): δ 162.4, 157.4, 152.4, 145.2, 135.7, 131.1, 128.0, 126.7, 125.8, 123.6, 119.7, 117.0, 112.0, 111.5, 110.2, 102.7, 100.8, 65.2, 50.6, 49.7, 26.1, 21.8. HRMS-ESI (*m/z*): [M + H]<sup>+</sup> caclcd for C<sub>23</sub>H<sub>23</sub>N<sub>5</sub>O<sup>+</sup> 386.1975, found 386.1968; purity (HPLC) 97 %.

**1-(2-((1H-Indol-5-yl)oxy)ethyl)-6-pyrimidin-1,2,3,4-tetrahydroquinoline (13b)**

A solution of 1-(2-((1H-indol-5-yl)oxy)ethyl)-6-bromo-1,2,3,4-tetrahydroquinoline **12b** (50 mg, 0.134 mmol), (2-aminopyrimidin-5-yl)boronic acid (56 mg, 0.402 mmol, 3 eq.), and K<sub>2</sub>CO<sub>3</sub> (56 mg, 0.402 mmol, 3 eq.) in DMF (2.7 mL) and water (0.55 mL) was degassed with argon. PdCl<sub>2</sub>(dppf) (10 mg, 0.013 mmol, 0.1 eq.) was added and the resulting mixture was stirred 3 h at 90 °C. The mixture was cooled to room temperature, filtered through a pad of dicalite and extracted with ethyl acetate. The crude product was purified by flash chromatography on silica gel (cyclohexane/ethyl acetate; 40/60), to afford the desired compound **13b** as a white solid (42 mg, 81 %). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 10.89 (s, 1H); 8.42 (s, 2H); 7.26 (dd, *J* = 5.6, 2.9 Hz, 2H); 7.21 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.14 (d, *J* = 2.0 Hz, 1H), 7.04 (d, *J* = 2.3 Hz, 1H), 6.75 – 6.69 (m, 2H), 6.51 (s, 2H), 6.30 (d, *J* = 2.0 Hz, 1H), 4.13 (t, *J* = 5.7 Hz, 2H), 3.69 (t, *J* = 5.6 Hz, 2H), 3.48 – 3.40 (m, 3H), 2.73 (t, *J* = 6.1 Hz, 2H), 1.92 – 1.83 (m, 2H). <sup>13</sup>C NMR (101 MHz, DMSO-*d*<sub>6</sub>): δ 162.1, 154.7, 152.4, 144.1, 131.1, 128.0, 125.9, 125.8, 123.9, 122.8, 122.5, 122.0, 112.0, 111.5, 111.1, 102.8, 100.8, 65.2, 50.1, 49.8, 27.6, 21.7. HRMS-ESI (*m/z*): [M + H]<sup>+</sup> caclcd for C<sub>23</sub>H<sub>23</sub>N<sub>5</sub>O<sup>+</sup> 386.1975, found 386.1968; purity (HPLC) 98 %.

**Cell culture and reagents**

CRC cell lines HT29 and Lovo (ATCC, Manassas, VA, USA) were chosen to investigate the role of final derivatives. Cell lines were cultured in RPMI-1640 media + Glutamax (Gibco, Thermo Fisher, Illkirch, France) supplemented with 10 % fetal bovine serum (Eurobio, Les Ulis, France). NCM356 (Incell Corporation, San Antonio, TX, USA) served as a non-tumoral control cell line and was cultured in DMEM media + L-glutamine (Gibco, Thermo Fisher). All cells were cultured in an incubator at 37 °C with 5 % CO<sub>2</sub>.

5-fluorouracil (stock solution 50 mg/ml, Accord) used as the reference standard treatment was evaluated at increasing doses from 1 to 100 μM, and synthesized molecules were evaluated at increasing doses from 0.1 to 100 μM in Falcon 48-well plates (Corning,



ThermoFisher). Cells were seeded at a density of 36,000 cells per well. The treatment began one day after seeding.

#### Cell-based assay

Cell viability/cytotoxicity was evaluated using standard sulforhodamine B (SRB, Sigma-Aldrich, Merck group, Saint-Quentin-Fallavier, France) method after treatment for 48 h. Briefly, cells were fixed with 50 % trichloroacetic acid (Sigma) for 1 h at 4 °C and stained for 15 min with 0.4 % SRB solution. Cells were then washed three times with 1 % acetic acid and dye was dissolved using 10 mM Tris (Sigma) over 10 min. Absorbance at 540 nm was determined with a BioTek spectrophotometer (Winooski, VT, USA). Representative sigmoid curves (3-parameter curve fitting) for estimating EC<sub>50</sub> values of the hit heterocyclic inhibitors are given in the supplementary file, showing log [concentration (μM)] vs normalized response (% in relation to non-treated cells).

#### Skin normal fibroblastic cells – Cytotoxic assay

Skin normal fibroblastic cells are purchased from Lonza (Basel, Switzerland). Culture media are supplemented by 10 % of FBS, 1 % of penicillin–streptomycin and 2 mM glutamine. Chemicals are solubilized in DMSO at a concentration of 10 mM (stock solution) and diluted in culture medium to the desired final concentrations. The dose effect cytotoxic assays (IC<sub>50</sub> determination) is performed by increasing concentrations of each chemical (final well concentrations: 0.1 μM – 0.3 μM – 0.9 μM – 3 μM – 9 μM – 25 μM). Cells are plated in 96 wells plates (4000 cells/well). Twenty-four hours after seeding, cells are exposed to chemicals. After 48 h of treatment, cells are washed in PBS and fixed in cooled 90 % ethanol/5 % acetic acid for 20 min and the nuclei are stained with Hoechst 33,342 (B2261 Sigma). Image acquisition and analysis are performed using a Cellomics ArrayScan VTI/HCS Reader (ThermoScientific). The survival percentages are calculated as the percentage of cell number after compound treatment over cell number after DMSO treatment. The relative IC<sub>50</sub> are calculated using the curve fitting XLfit 5.5.0.5 (idbs) integrates in Microsoft Excel as an add on. The 4 Parameter Logistic Model or Sigmoidal Dose-Response Model is used.

#### Protein kinases assays

Kinase enzymatic activities were assayed in 384-well plates using the ADP-Glo™ assay kit (Promega, Madison, WI) according to the recommendations of the manufacturer. This assay is a luminescent ADP detection assay [36]. It provides a homogeneous and high-throughput screening method to measure kinase activity by quantifying the amount of ADP produced during a kinase reaction. Briefly, the reactions were carried out in a final volume of 6 μl for 30 min at 30 °C in appropriate kinase buffer, with either protein or peptide as substrate in the presence of 10 μM ATP. Assay conditions used for each kinase tested here are described in supplementary file. After that, 6 μl of ADP-Glo™ Kinase Reagent was added to stop the kinase reaction. After an incubation time of 50 min at room temperature (RT), 12 μl of Kinase Detection Reagent was added for one hour at RT. The transmitted signal was measured using the Envision (PerkinElmer, Waltham, MA) microplate luminometer and expressed in Relative Light Unit (RLU). In order to determine the half maximal inhibitory concentration (IC<sub>50</sub>), the assays were performed in duplicate in the absence or presence of increasing doses of the tested compounds. Kinase activities are expressed in % of maximal activity, i.e. measured in the absence of inhibitor.

#### Biological data analysis

Data were collected from at least three independent experiments. The data are presented as the mean ± standard error and were analyzed with GraphPad Prism 6 (GraphPad Software, San Diego, CA, USA). For

each molecule, EC<sub>50</sub> (best-fit values) was obtained using a non-linear curve fitting function (3-parameter).

#### CRediT authorship contribution statement

**Benjamin Victoir:** Writing – original draft, Methodology, Investigation. **Océane Pertegaz:** Investigation. **Elfi Ducrocq:** Investigation. **Marion Polomski:** Investigation. **Maxime Guéguinou:** Supervision, Methodology. **William Raoul:** Writing – review & editing, Validation, Supervision, Methodology, Funding acquisition, Conceptualization. **Cécile Croix:** Writing – review & editing, Validation, Supervision, Methodology, Investigation. **Gildas Prié:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

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#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.rechem.2024.102015>.

#### Data availability

Data will be made available on request.

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