Supplementary Material

Microwave assisted practical synthesis of 4-imino-3-phenyl-3,4-dihydro-1*H*-chromeno[2,3-*d*]pyrimidine-2(5*H*)-thione derivatives

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Biochemistry section

Cell culture and survival assays

Skin diploid fibroblastic cells were provided by BIOPREDIC International Company (Rennes, France). Caco2 (differentiated colorectal adenocarcinoma, Ref ECACC: 86010202), Huh-7D12 (differential hepatocellular carcinoma, Ref ECACC: 01042712), MDA-MB-231 (breast carcinoma, Ref ECACC: 92020424), HCT-116 (actively proliferating colorectal adenocarcinoma, Ref ECACC: 91091005), PC3 (prostate carcinoma, Ref ECACC: 90112714), NCI-H727 (lung carcinoma, Ref ECACC: 94060303) cell lines were obtained from the ECACC collection and HaCaT (keratinocyte from Cell Lines Service, Eppelheim, Germany). Cells were grown according to ECACC recommendations (Nakabayashi et al., 1982). The toxicity test of the compounds on these cells was as follows: 2 x 10^3 cells for HCT-116 cells or 4 x 10^3 for the other cells were seeded in 96 multiwell plates in triplicate and left for 24 h for attachment, spreading and growing. Then, cells were exposed for 48 h to increasing concentrations of the compounds, ranging from 0.1 to 25 mM in a final volume of 120 mL of culture medium. Cells were fixed in cooled 90% ethanol/5% acetic acid solution, nuclei were stained with Hoechst 3342 (Sigma) and counted using automated imaging analysis (Cellomics Arrayscan VTI/HCS Reader, Thermo/Scientific). The IC_{50} were graphically determined.

Kinase preparations and assays

Kinase activities for each enzyme were assayed in:

- buffer A (25 mM Tris-HCl pH 7.5, 10 mM $MgCl_2$, 1 mM EGTA, 1 mM DTT, 50 µg/mL Heparin, BSA 0.15 mg/mL)
- or buffer B (60 mM β-glycerophosphate, 30 mM *p*-nitrophenylphosphate, 25 mM MOPS, 5 mM EGTA, 15 mM $MgCl_2$, 1 mM DTT, 0.1 mM Na vanadate)
- or buffer C ($MgCl_2$ 10 mM, 1 mM ethylene glycol-bis(2-aminoethylether)-*N,N,N',N'*-tetraacetic acid (EGTA), 1 mM dithiothreitol (DTT), 25 mM Tris-HCl pH 7.5, 50 µg heparin/mL) or buffer D (25 mM MOPS, pH 7.2, 12.5 mM b-glycerophosphate, 25 mM $MgCl_2$, 5 mM EGTA, 2 mM EDTA, 0.25 mM DTT)
- or buffer H (MOPS 25 mM, pH 7.5; 10 mM $MgCl_2$)

with their corresponding substrates, in the presence of 15 µM [γ-^{33}P] ATP (3,000 Ci/mmol; 10 mCi/ml) in a final volume of 30 µL. After 30 min incubation at 30°C, the reaction was stopped by harvesting, using a FilterMate harvester (Packard), onto P81 phosphocellulose papers (GE Healthcare) which were washed in 1% phosphoric acid. Scintillation fluid was added and the radioactivity measured in a Packard counter. Blank values were subtracted and activities calculated as pmoles of phosphate incorporated during the 30 min incubation. The activities were expressed in % of the maximal activity, i.e. in the absence of inhibitors. Controls were performed with appropriate dilutions of DMSO. Peptide substrates were obtained from Proteogenix (OberhausBergen, France).

- *HsCDK5/p25* (human, recombinant) was prepared as previously described (Leclerc et al., 2001). Its kinase activity was assayed in buffer B, with 1 mg histone H1/ml.
- *GSK-3*α/β (porcine brain, native) was assayed, as described for CDK5/p25 but in Buffer A and using a GSK-3 specific substrate (GS-1: YRRAAVPPSPSLSRHSSPHQSpEDEEE) (pS stands for phosphorylated serine) (Primot et al., 2000).
- *CLK1* (Human, recombinant, expressed in *E. coli* as GST fusion protein) was assayed in buffer C (+0.15 mg BSA/mL) with RS peptide (GRSRSRSRSRSR) (1 µg/assay).
- *Haspin* kinase domain (*HsHaspin*-kd aa 470 to 798) encoding cDNA, obtain by RT-PCR, was cloned into pGex-6P-3. The fusion protein was expressed in *E. coli* strain BL21-KRX

(Promega) and purified by affinity chromatography on glutathione-agarose beads (Sigma). Haspin-kd activity was assayed in buffer H with 3 µM Histone H3 (1-21) peptide, a specific Haspin substrate, (ARTKQTARKSTGGKAPRKQLA).

- *Hs*PIM1 (human proto-oncogene, recombinant, expressed in bacteria) was assayed in buffer B with 0.8 µg/µl of histone H1 (Sigma #H5505) as substrate.
- *Hs*Aurora B (human, recombinant, expressed by baculovirus in Sf9 insect cells, SignalChem, product #A31-10G) was assayed in buffer D with 0.2 µg/µl of MBP as substrate.

*^{1}H NMR spectrum of 8-diethylamino-4-imino-3-phenyl-3,4-dihydro-1H-chromeno[2,3-d]pyrimidine-2(5H)-thione (**6a**)*

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*^{13}C NMR spectrum of 8-diethylamino-4-imino-3-phenyl-3,4-dihydro-1H-chromeno[2,3-d]pyrimidine-2(5H)-thione (**6a**)*

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*Details on the ^{13}C NMR spectrum (from 100 to 145 ppm) of 8-diethylamino-4-imino-3-phenyl-3,4-dihydro-1H-chromeno[2,3-d]pyrimidine-2(5H)-thione (**6a**)*

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*^{1}H NMR spectrum of 4-imino-3-phenyl-3,4-dihydro-1H-chromeno[2,3-d]pyrimidine-2(5H)-thione (**6b**)*

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*^{13}C NMR spectrum of 4-imino-3-phenyl-3,4-dihydro-1H-chromeno[2,3-d]pyrimidine-2(5H)-thione (**6b**)*

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*^{1}H NMR spectrum of 4-imino-6-methoxy-3-phenyl-3,4-dihydro-1H-chromeno[2,3-d]pyrimidine-2(5H)-thione (**6c**)*

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*^{1}H NMR spectrum of 4-imino-7-methoxy-3-phenyl-3,4-dihydro-1H-chromeno[2,3-d]pyrimidine-2(5H)-thione (**6d**)*

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*^{1}H NMR spectrum of 4-imino-8-methoxy-3-phenyl-3,4-dihydro-1H-chromeno[2,3-d]pyrimidine-2(5H)-thione (**6e**)*

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*^{13}C NMR spectrum of 4-imino-8-methoxy-3-phenyl-3,4-dihydro-1H-chromeno[2,3-d]pyrimidine-2(5H)-thione (**6e**)*

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*^{1}H NMR spectrum of 8,12-dihydro-11-imino-10-phenyl-9H-naphtho[1',2':5,6]pyrano[2,3-d]pyrimidine-9-thione (**6f**)*

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*^{13}C NMR spectrum of 8,12-dihydro-11-imino-10-phenyl-9H-naphtho[1',2':5,6]pyrano[2,3-d]pyrimidine-9-thione (**6f**)*

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*Details on the ^{13}C NMR (from 126 to 132 ppm) of 8,12-dihydro-11-imino-10-phenyl-9H-naphtho[1',2':5,6]pyrano[2,3-d]pyrimidine-9-thione (**6f**)*

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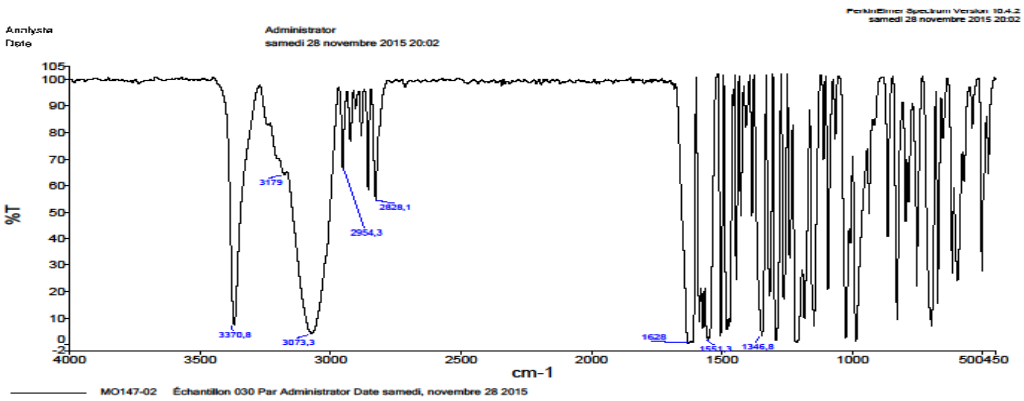
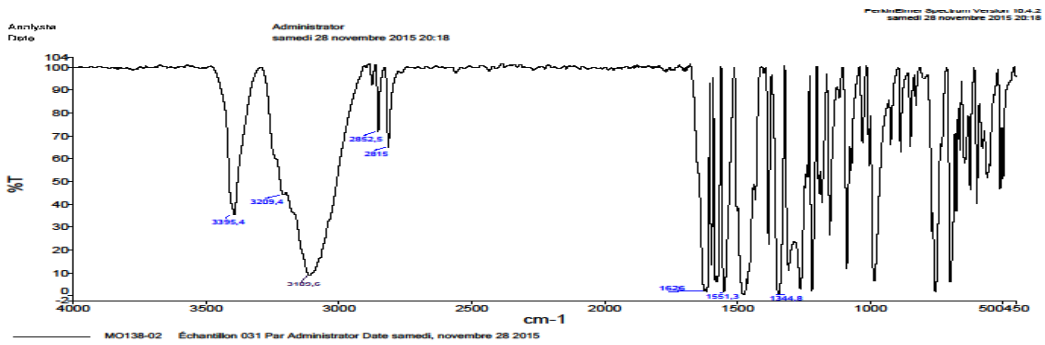
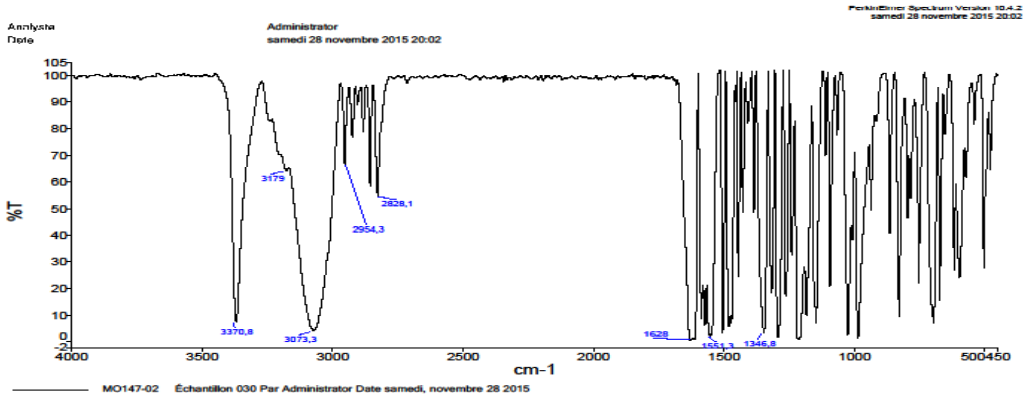
*IR spectrum of 8-diethylamino-4-imino-3-phenyl-3,4-dihydro-1H-chromeno[2,3-d]pyrimidine-2(5H)-thione (**6a**)*



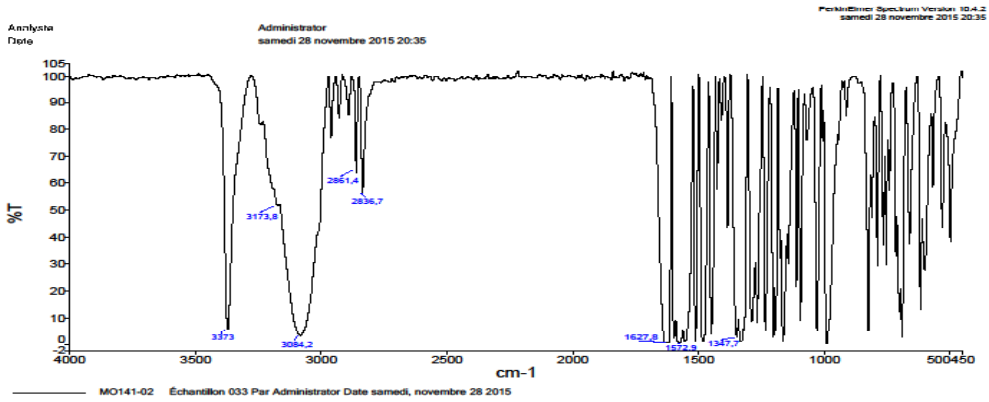
*IR spectrum of 4-imino-3-phenyl-3,4-dihydro-1H-chromeno[2,3-d]pyrimidine-2(5H)-thione (**6b**)*



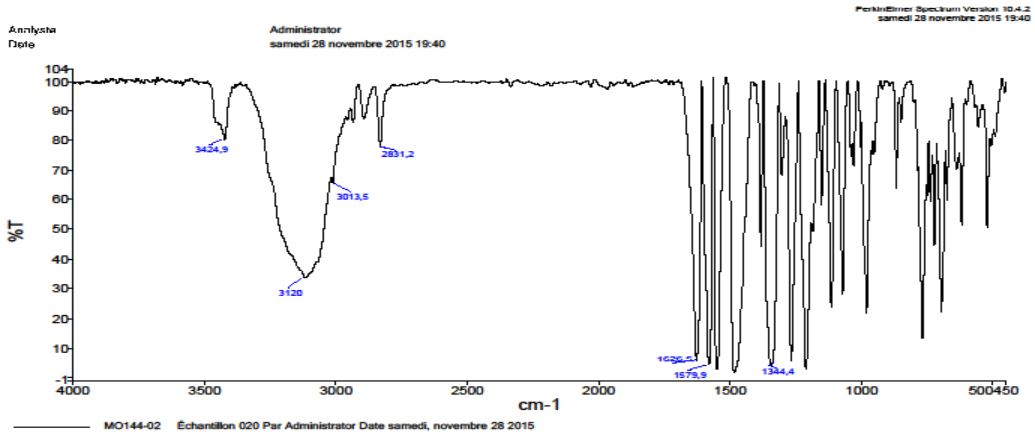
*IR spectrum of 4-imino-6-methoxy-3-phenyl-3,4-dihydro-1H-chromeno[2,3-d]pyrimidine-2(5H)-thione (**6c**)*



*IR spectrum of 4-imino-7-methoxy-3-phenyl-3,4-dihydro-1H-chromeno[2,3-d]pyrimidine-2(5H)-thione (**6d**)*



*IR spectrum of 4-imino-8-methoxy-3-phenyl-3,4-dihydro-1H-chromeno[2,3-d]pyrimidine-2(5H)-thione (**6e**)*



*IR spectrum of 8,12-dihydro-11-imino-10-phenyl-9H-naphtho[1',2':5,6]pyrano[2,3-d]pyrimidine-9-thione (**6f**)*