

RATIONAL-BASED DRUG DESIGN OF NOVEL, HIGHLY POTENT MER INHIBITORS AS POTENTIAL TREATMENT OF CANCERS

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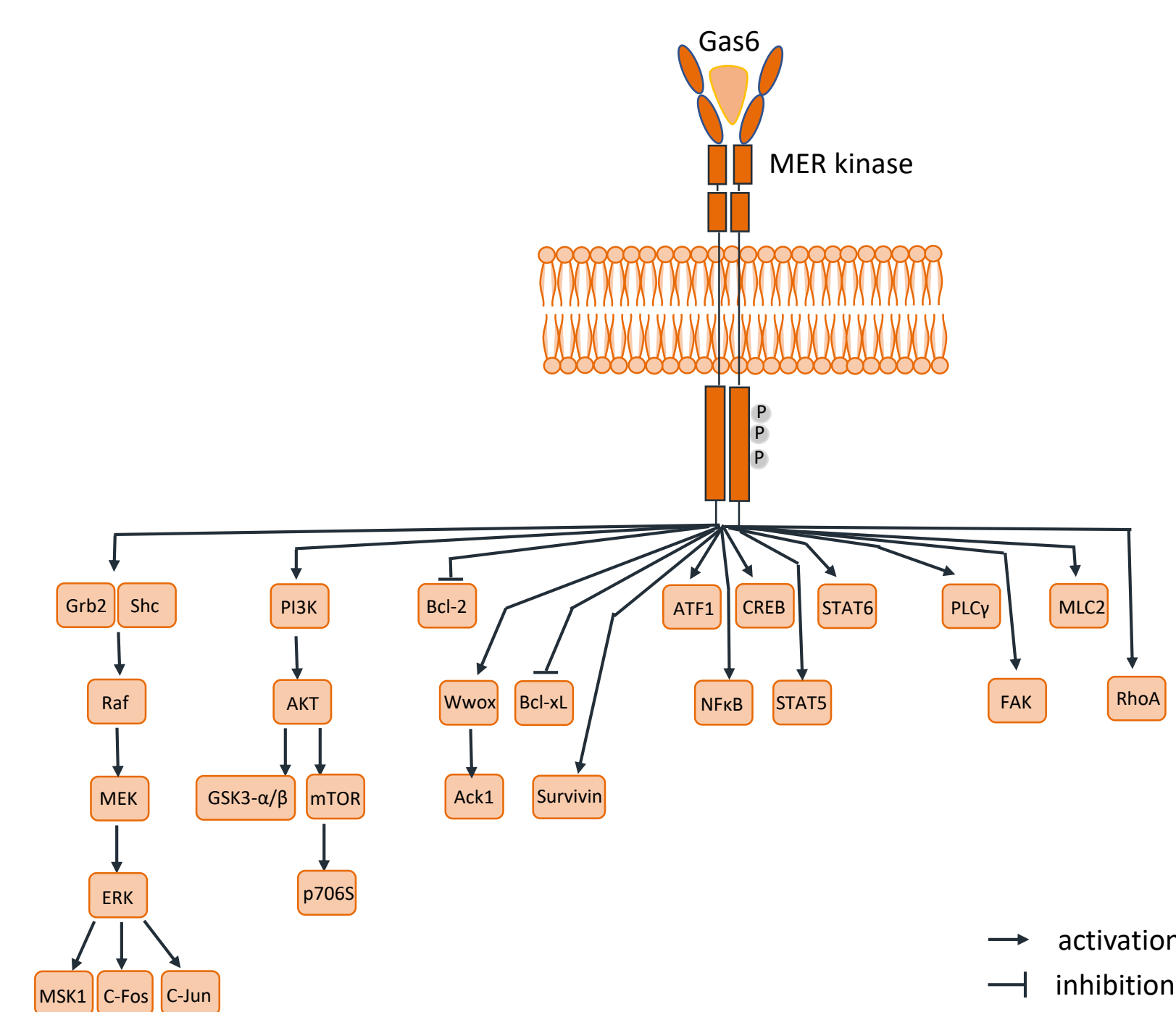
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INTRODUCTION

MER is a member of TAM family of receptor tyrosine kinases (TYRO3, AXL, MER). MER is aberrantly expressed in several types of solid and haematological tumours, playing a substantial role in tumour cell survival, growth, invasion and metastasis. Inhibition of MER solely can be a particularly prominent therapy in solid tumours, whereas dual targeting of MER and other kinases may potentially bring higher therapeutic benefit, both in solid and haematological cancers e.g. dual targeting of MER/AXL in NSCLC or MER/FLT3 in leukaemia. Despite the intensive development of inhibitors, such as UNC2025 and MRX2843 (MER/FLT3) or INCB081776 (AXL/MER), no mono nor dual MER/FLT3 and MER/AXL inhibitors has been approved for therapy. Celon Pharma is carrying out intensive work to develop an innovative inhibitor targeting MER kinase.

MER SIGNALING



Activation of MER kinase occurs through binding of one of the natural ligands like Gas6 or Pros1. This induces the activation of downstream signalling pathways such as MAPK/ERK, PI3K/AKT, JAK/STAT, or related to apoptotic processes.

MATERIALS AND METHODS

Compounds and cell lines

H1299 cell line was purchased from ATCC. Compounds were designed and synthesized at Celon Pharma Medicinal Chemistry Department.

In Vitro Kinase Inhibition Assays

All recombinant kinases were purchased from Carma Biosciences. The inhibitory activity of compounds against tested protein was evaluated based on their ability to converse ATP to ADP in the presence of 30 μM ATP using ADP-Glo assays (Promega Corp.) according to the manufacturer's protocol. In ATP-competitive assay, ATP concentrations in the range of 10–300 μM were used.

Immunoblot Analysis

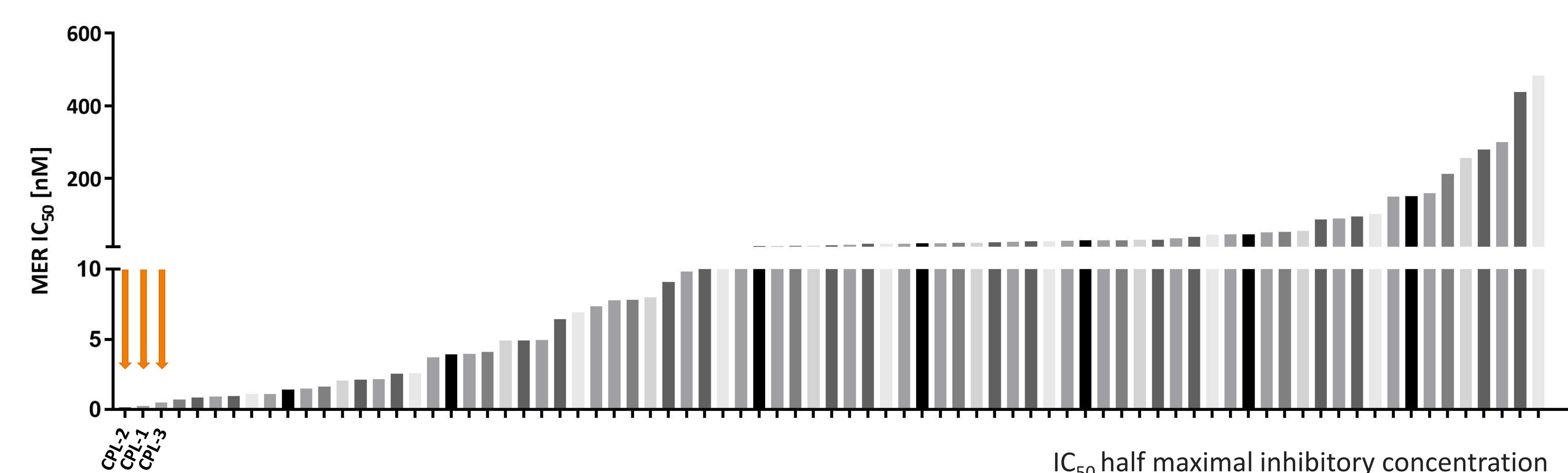
H1299 cells (NSCLC cell line) were seeded in 6-well plates in serum-enriched medium. After 24 hours, cells were starved for 3h in serum-free medium, treated with inhibitors at the indicated concentrations for 1h and stimulated for 10 minutes with 400 ng/ml Gas6 (R&D systems). After stimulation cells were lysed and examined by Western blotting according to the protocols provided by the antibody suppliers. The antibodies against phosphoAKT and total AKT were purchased from Cell Signaling Technology.

Cell cycle analysis

H1299 cells (NSCLC cell line) were seeded in 6-well plates in an inhibitor-free medium. After 24 hours cells were treated with inhibitors at the indicated concentrations for 24h. Then, cells were fixed in cold 70% alcohol, stained with propidium iodide, and analysed by the flow cytometry with the use of FACS Calibur and Cell Image Quant software (Becton Dickinson).

RESULTS

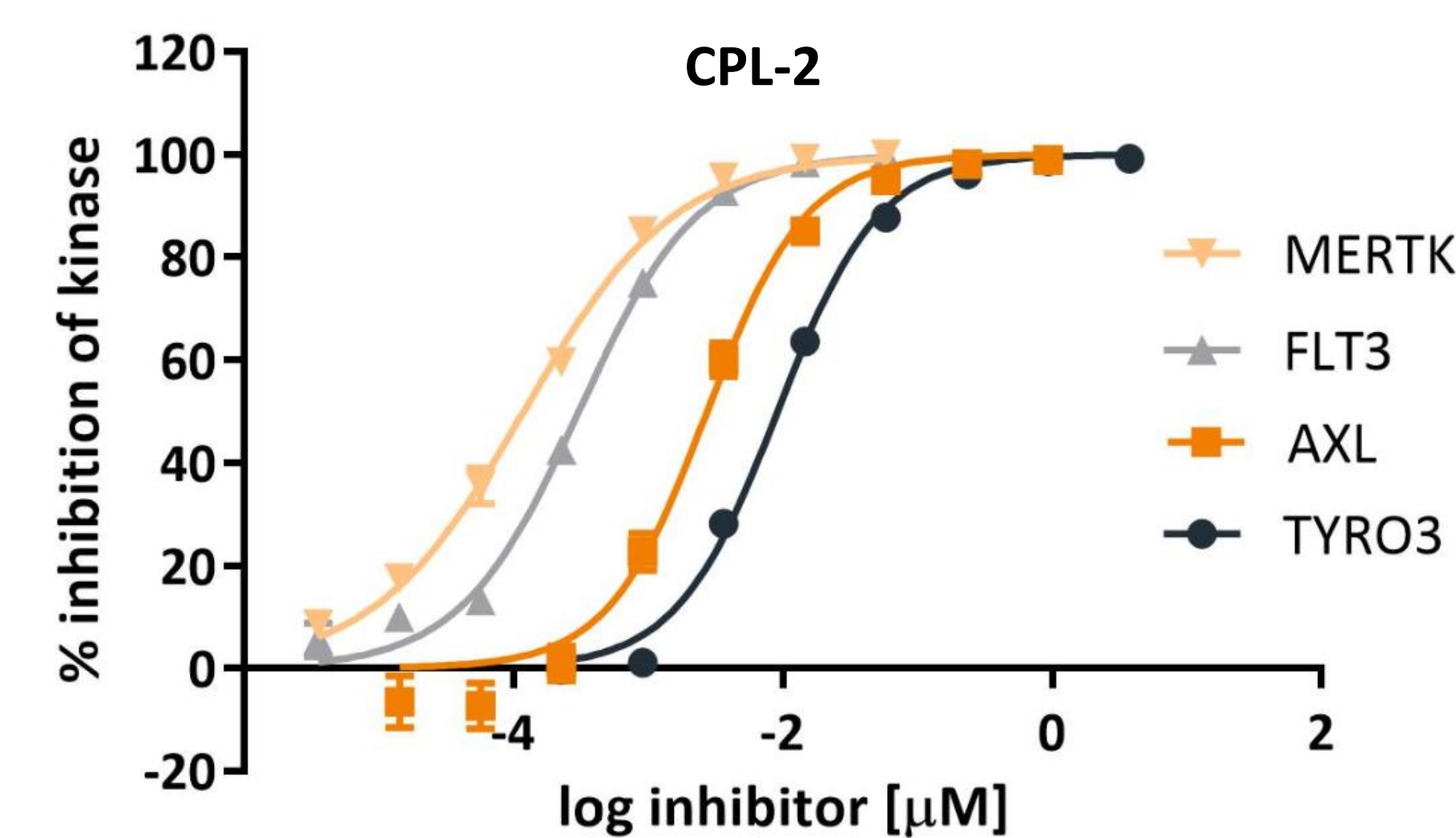
In vitro kinase activity



Celon Pharma is expanding a library of compounds, potential inhibitors of MER kinase. Up to date, a large group of chemical compounds with very high, nanomolar activity against MER kinase has been obtained.

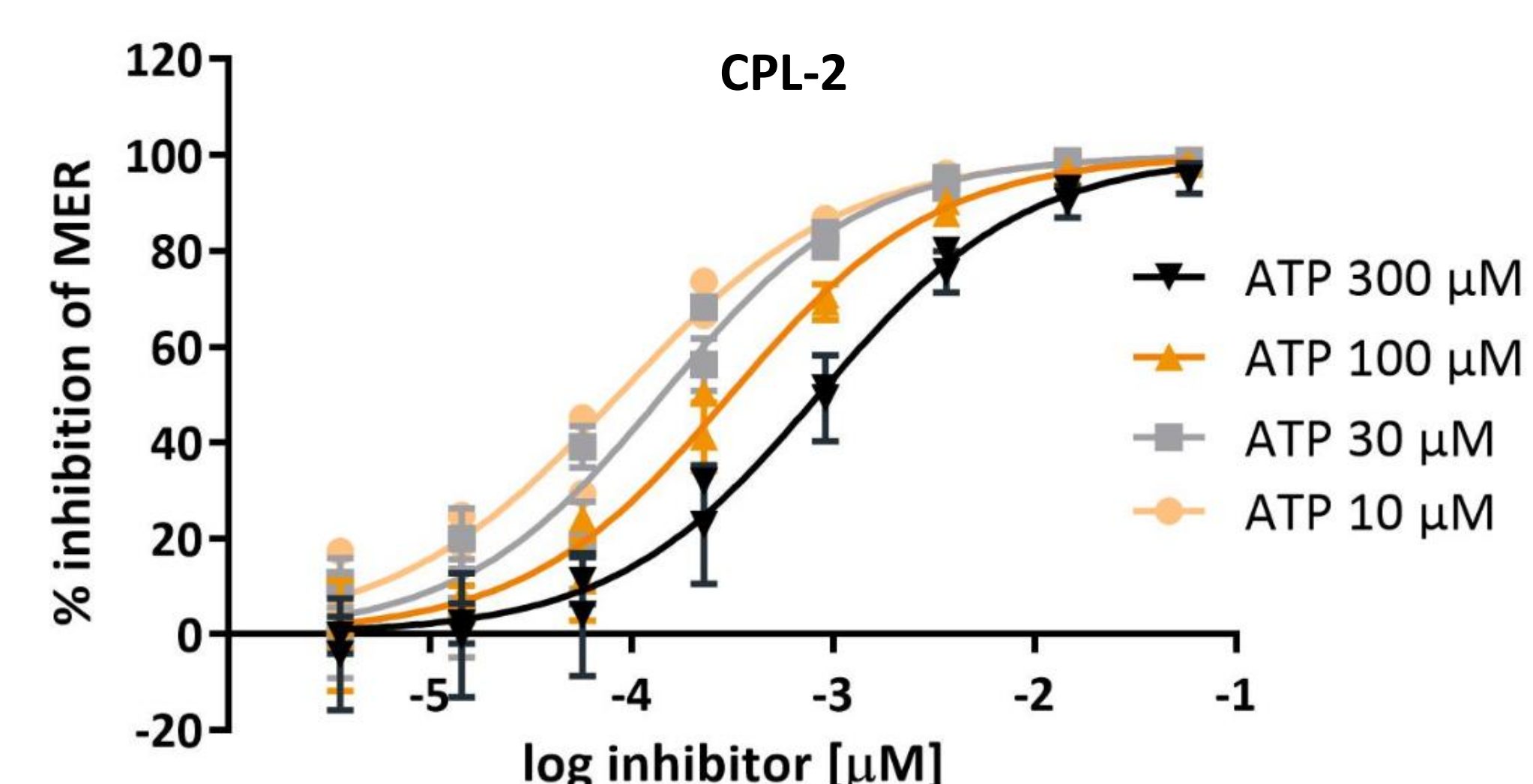
In vitro kinase selectivity

| IC ₅₀ [nM] | MER | AXL | TYRO3 | FLT3 |
|-----------------------|------|------|-------|------|
| UNC2025 | 0.21 | 1.85 | 11.09 | 0.26 |
| MRX2843 | 0.25 | 1.05 | 11.9 | 0.44 |
| CPL-1 | 0.49 | 8.8 | 36.32 | 0.07 |
| CPL-2 | 0.17 | 2.13 | 8.6 | 0.36 |
| CPL-3 | 0.27 | 8.73 | 14.5 | 0.13 |



Activity of the newly synthesized compounds has been tested, in addition to MER kinase, on other TAM family proteins as well as on the FLT3 kinase. The most potent compound, CPL-2, inhibits MER and FLT3 kinase activity at low subnanomolar IC₅₀ (0.17 nM and 0.36 nM, respectively) and AXL kinase at nanomolar level (8.6 nM).

ATP-competitive mode of inhibition



Using different ATP concentrations in ADPGlo kinase assay (Promega Corp.), ATP-competitive mode of action of novel MER inhibitors was confirmed. As an example, a response curve for one of the compounds is shown.

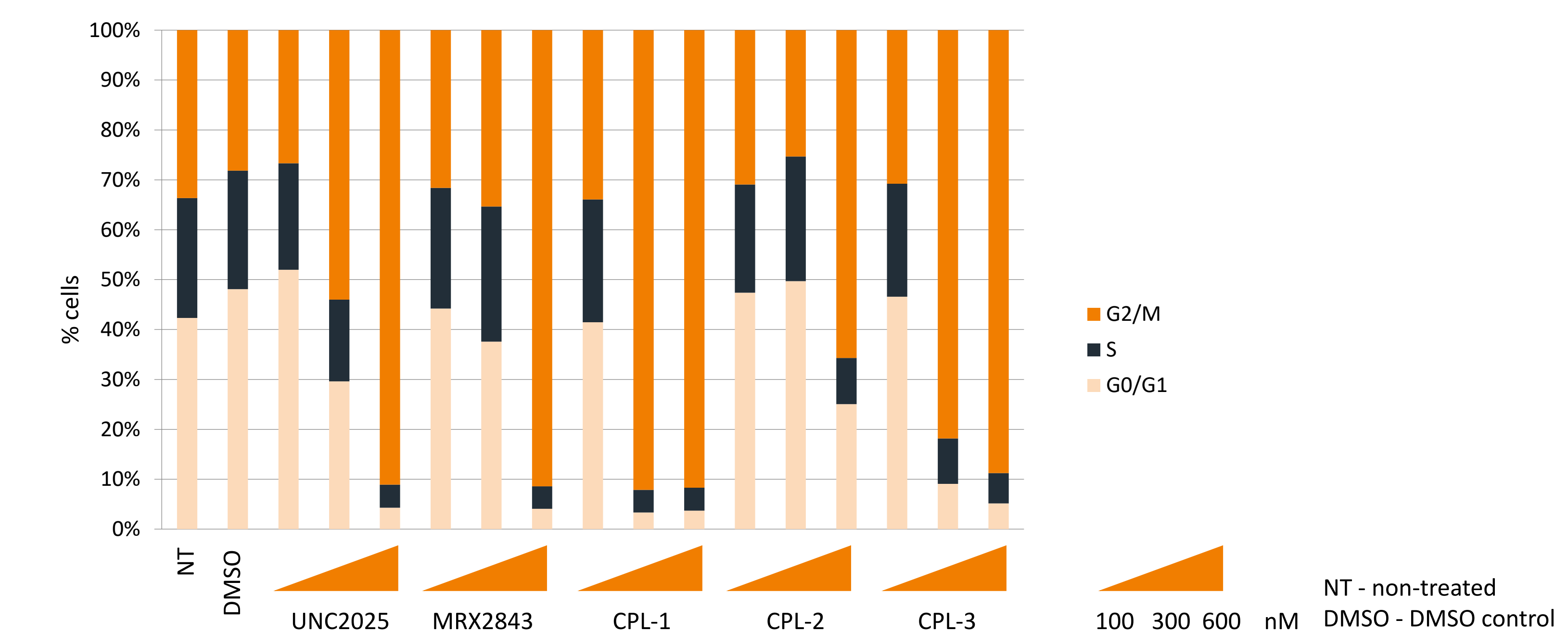
RESULTS

Downstream signalling inhibition



Western blot analysis has shown that tested compounds significantly inhibited Gas6-induced phosphorylation of AKT in H1299 cell line.

Cell cycle analysis



Analysis of the cell cycle distribution after 24h treatment of H1299 cells showed a strong cell cycle arrest in G2/M phase.

CONCLUSIONS

Celon Pharma has synthesized a rich library of compounds highly potent against MER kinase. Presented compounds also exhibit high activity against FLT3 kinase, and lower against AXL kinase. Preliminary confirmation of the activity of compounds in cell-based assays was made using the NSCLC cell line. High activity of a number of chemical compounds, also in relation to FLT3 kinase, allows to presume the benefits that may occur in leukaemia models. Extensive work will be carried out to further develop a library of compounds, investigate the mechanism of action of compounds, as well as to confirm activity in *in vivo* models, both solid tumour and leukemic.

REFERENCES

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