doi:10.2533/chimia.2025.241 Chimia 79 (2025) 241–244

Natural Products as Timeless Remedies – Unlocking Nature's Treasure Trove

Tamara Balsiger§*, Robin Teufel, and Eliane Garo

§SCS-dsm-firmenich Award for the best poster presentation in Medicinal Chemistry

Abstract: Natural products are an essential source of medicines, accounting for a large proportion of approved drugs nowadays. However, the isolation of active natural products from complex extracts is challenging. To address this bottleneck, a drug discovery strategy was developed in our lab, that combines the screening of an in-house crude plant extract library of more than 2,500 samples with an HPLC-based activity profiling approach. This workflow is used routinely in our group and was successfully applied to numerous natural product drug discovery projects.

Keywords: Activity profile · Drug discovery · Natural products

Tamara Balsiger is a passionate natural product scientist and football fan. She received her master's degree in pharmacy from the University of Basel and the Swiss federal diploma as a pharmacist in 2021. Currently, is conducting her PhD in the Pharmaceutical Biology group at the University of Basel in the lab of Prof. Robin Teufel under the supervision of PD Dr. Eliane Garo investigating plant extracts in drug discovery projects (*i.e.* melanoma & forgetting).

1. Natural Products in Drug Discovery

Natural products (NPs) have been and continue to be an invaluable source of drugs. Morphine, used as part of opium from *Papaver somniferum* since the 3rd millennium BC, was the first ever NP to be isolated, and remains one of the most prescribed drugs to treat severe pain in clinics today.[1] The breakthrough discovery of penicillin from the fungus *Penicillium notatum* at the beginning of the 20th century allowed the first effective treatment of bacterial infections.[2] NPs are also an essential source of inspiration for medicinal chemistry and chemical biology, as illustrated by atorvastatin, a compound inspired by fungal NP that has become a blockbuster treatment for hypercholesterolemia,[3] and more recently, the highly prized semaglutide used to treat type 2 diabetes (Ozempic®, Rybelsus®) and obesity (Wegowy®), which is derived from the NP exendin-4, a peptide found in the Gila monster venom.[4] These examples demonstrate the importance of NPs in modern medicine. Thus, it is not surprising that NPs, semi-synthetic NPs, and small molecules inspired by NPs still account for 2/3 of all approved small molecule drugs today.[5]

NPs are so-called secondary (or specialized) metabolites produced by plants, animals, and microorganisms. They are not essential for living but provide evolutionary advantages to the producer.[6] For example, the bitter-tasting nicotine in the tobacco plant's leaves wards off insects and herbivores,[7] while conotoxins from the cone snail venom serve as offensive weapons to hunt prey.[8] NPs can also mediate (interspecies) communication like essential oils in flowers to attract pollinators.[9] The structures of NPs were refined through co-evolution over millions of years, expressing nature's exploration of the biologically relevant chemical space. As a result, NPs are nature's treasure trove filled with unique and diverse structures having interesting features that provide lead compounds for drug discovery.

However, the challenge of how to access this chemical treasure remains, as the path from a crude extract to a pure bioactive compound is costly, time-consuming and challenging. Therefore, a strategy to tackle some of these issues was developed in our lab and is illustrated in the following by two ongoing projects focusing on identifying NPs that inhibit melanoma cells (section 2.2) and improve memory function by impeding forgetting (section 2.3).

2. Natural Product Drug Discovery Platform

The drug discovery platform was initially established by Prof. em. Matthias Hamburger, former PI of our group, and is built around two core elements: a pre-formatted extract library and the HPLC-based activity profiling approach.[10] This platform is routinely used and was successfully applied for over a decade to various drug discovery projects[11,12] while being constantly optimized.

Our extract library is highly diverse with more than 2,500 plant extracts (140 plant families and >700 species) from different parts of the world, mainly Panama, China and Europe. All extracts are compliant with the Nagoya protocol to ensure the preservation of biodiversity and fair sharing of genetic material with the countries of origin. The extracts are systematically prepared using standardized extraction methods and stored at a standard concentration (10 mg/mL in DMSO) in a 96-well plate format in matrix tubes, making them suitable for high-throughput screening. Transfer plates (used for screening) are generated by using a pipetting robot to precisely adjust the amounts of extract needed for individual screening assays. After the initial screening of crude extracts, hits are further analyzed *via* HPLC-based activity profiling to link the observed activity with specific peaks within the chromatogram. Based upon these results, the most promising extracts are then prioritized for scale-up isolation and purification of the bioactive NPs corresponding to these peaks. The pure compounds are then fully characterized by HRMS, as well as 1D and 2D NMR; if necessary, absolute stereochemistry is determined by electronic circular dichroism spectroscopy (ECD) or X-ray crystallography. Finally, the IC_{50} values of the pure compounds are measured. Fur-

*Correspondence: T. Balsiger, E-mail: tamara.balsiger@unibas.ch
Department of Pharmaceutical Sciences, University of Basel, CH-4056 Basel

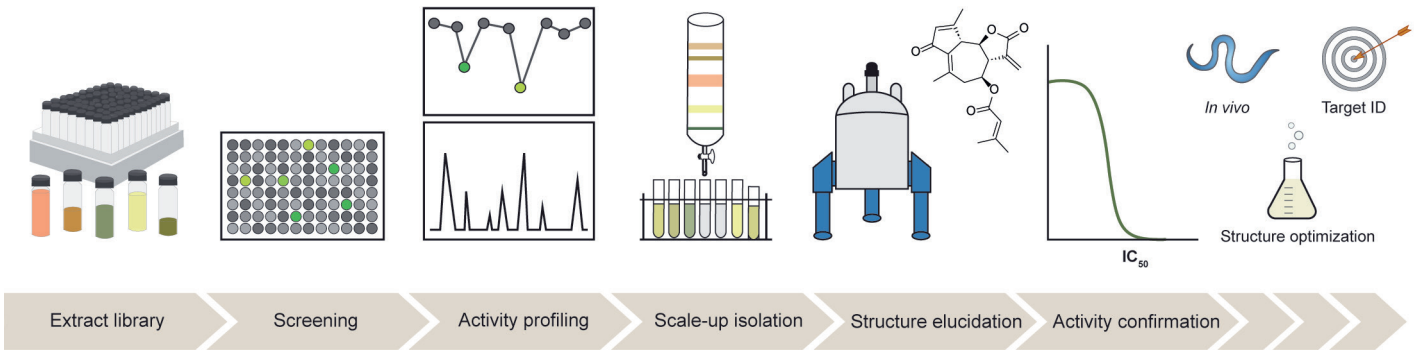

Fig. 1. Natural product drug discovery workflow – Our in-house plant extract library is screened across various biological assays to identify crude extracts as hits. The active extracts are then subjected to HPLC-based activity profiling to prioritize and select the most promising candidates for scale-up isolation. Structures of the isolated compounds are elucidated using NMR and HRMS analysis. The biological activity of these pure compounds is further assessed, including IC_{50} determination. Depending on the project, subsequent steps may include *in vivo* validation, target identification, and structure optimization.

ther proceedings depend on the project and may include target identification in the case of phenotypic screening assays (*e.g.* for the melanoma project), validation of *in vivo* activity (*e.g.* for the forgetting project), or structure optimization through both synthetic and enzymatic approaches. (Fig. 1.)

2.1 *HPLC-based Activity Profiling*

For HPLC-based activity profiling, the active extracts are analyzed by analytical HPLC-CAD-UV-MS. The samples are loaded twice on the instrument, once to obtain analytical data (UV and MS) and once to collect microfractions. Simultaneous analysis and collection of the extracts would require a complete rewiring of the eluent splitting including the use of a different splitter. The microfractions are then dried, redissolved in DMSO and tested for activity. Finally, the HPLC-based activity profiles are generated by overlaying the analytical HPLC traces with the activity values of each fraction. This allows the identification of peaks eluting in the active windows that likely correspond to active NPs. The associated MS and UV spectra provide valuable information used for annotating these peaks through comparison with in-house and online databases.

Several points need to be addressed while working with HPLC-based activity profiling. First, the HPLC method as well as the amounts of extracts loaded onto the HPLC system should be individually adapted to each bioassay, which ensures the efficient transfer of this approach to almost all types of miniaturized assays (functional, cellular, and biochemical). For example, to generate microfractions that will be tested in a cellular assay (*e.g.* in the melanoma project), a sub-milligram amount of extract is loaded (in portions) on the column, while typically microgram amounts suffice for a biochemical assay (*e.g.* in the forgetting project). Moreover, some compound classes such as tannins are known to unspecifically bind to proteins, which can result in false positive hits. Fluorescent-based assay-readouts (*e.g.* fluorescence polarization) might be susceptible to autofluorescence of NPs, necessitating further refinements such as the time-resolved fluorescence energy transfer (TR-FRET) to circumvent these effects (*e.g.* in the forgetting project). Having these considerations in mind, the entire approach is using only microgram amounts of extract and is suitable to almost all current screening formats (96- and 384-wells) with medium or even large throughput. Lastly, the quality of peak annotation, currently based on low-resolution MS, should also be addressed. In a recent project, we successfully combined our HPLC-based activity profiling with HRMS/MS analysis resulting in a multilayer molecular network, which significantly improved the quality of annotation.[6] This later methodology will therefore be implemented as routine in our future drug discovery projects.

2.2 *Screening for MAPK/ERK and PI3K/AKT Pathway Inhibitors in Melanoma*

Advanced melanoma is the most aggressive form of skin cancer, and its incidence is increasing worldwide. High mutation rates are key drivers of melanoma and occur mostly in the MAPK/ERK (mitogen-activated protein kinase/extracellular signal-regulated kinase) and PI3K/AKT (phosphoinositide-3-kinase/protein kinase B) signaling pathways, leading to uncontrolled cell proliferation.[13] Half of the patients harbor a mutation in the BRAF kinase from the MAPK/ERK pathway. The approval of BRAF inhibitors led to impressive initial patient responses, however fast occurring resistances of the tumor causes rapid relapse and often prevents long-term effectiveness.[14] Combination of several inhibitors targeting different kinases in the pathway show promising results by delaying the onset of drug resistances.[15] Therefore, new lead compounds targeting the MAPK/ERK and PI3K/AKT pathways are needed to broaden the spectrum of drugs available for advanced combinational therapies.

An innovative high-content screening assay was thus developed to identify new natural ERK and/or AKT pathway inhibitors in melanoma cells, which were engineered with genetically encoded biosensors using KTR (kinase translocation reporters) technology[16] to quantify ERK and AKT activity in single cells. In brief, the ERK-KTR and AKT-KTR sensors are phosphorylated by ERK and AKT, respectively. Aberrant activation of the ERK and AKT pathways accordingly leads to the phosphorylation of the sensors that become visible in the cytosol. Upon treatment with inhibitors, the KTR sensors then relocalize to the nucleus (Fig. 2).

To this end, our extract library was screened on two different melanoma cell lines bearing different mutations (A2058 and the patient-derived cells MM121224, both engineered with KTR-sensors). The screening campaign identified 140 active extracts, from which 44 were selected for HPLC-based activity profiling, primarily based on taxonomy (*i.e.* only one species per genus was analyzed). The results so far enabled the prioritization of seven plants for scale-up isolation and led to the identification of a total of 80 compounds, comprising eight NP scaffolds. Among these, some of the brevipolides, methoxylated flavonoids, sesquiterpene lactones (guaianolides), and thymol derivatives displayed the most potent activities with IC_{50} values below 20 μM and were thus considered for further investigations.[11,17–19] (Fig. 3)

As this work was based on a phenotypic assay that measured the activity of downstream ERK and AKT, direct conclusions on specific target proteins in the pathways are not possible. Hence, the challenge of target identification remains; current efforts focus on identifying the actual target(s) of all active compounds with different methodologies, ranging from *in silico* to wet lab experi-

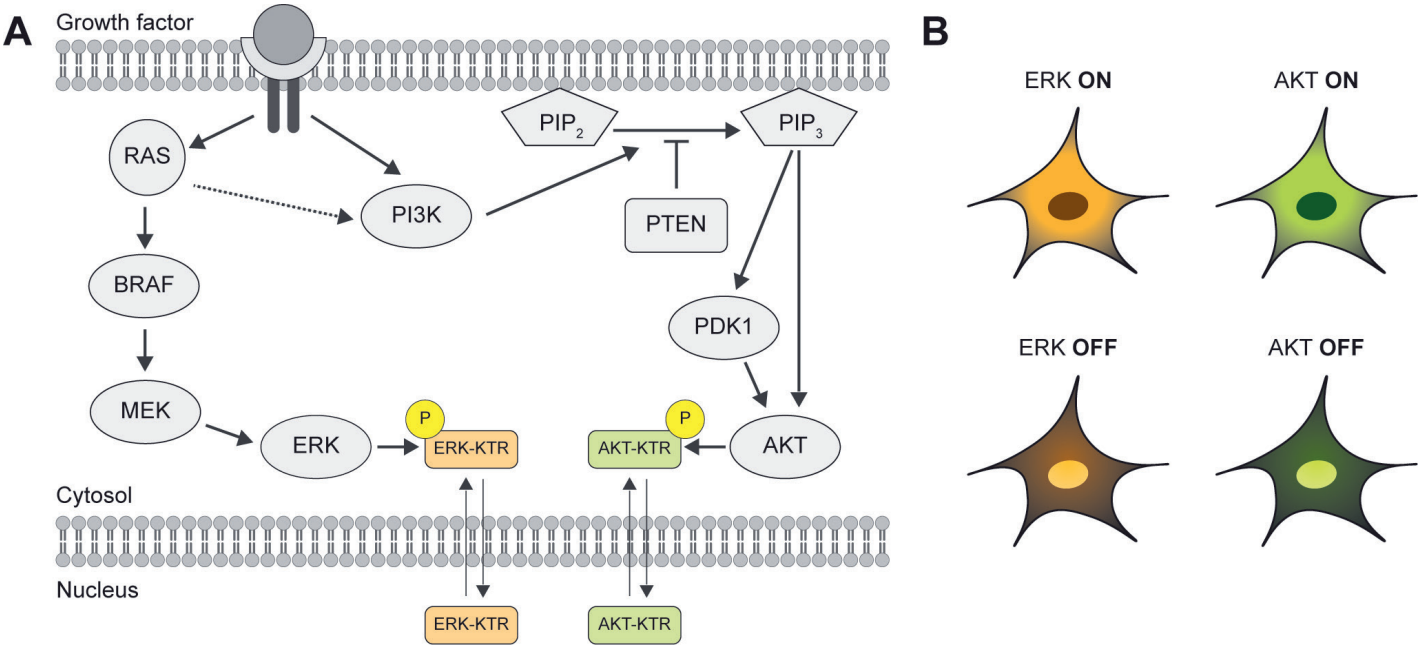

Fig. 2. **A**) Simplified schematic representation of the MAPK/ERK and PI3K/AKT pathways including, in addition to the transcriptional regulators AKT and ERK, selected receptors and upstream kinases such as BRAF for signal transduction. The activity of ERK and AKT is quantified in single cells by using genetically encoded biosensors (ERK-KTR and AKT-KTR) based on kinase translocation technology (KTR). When the ERK or AKT pathway is active (ON), the respective ERK- and AKT-KTR is phosphorylated and thus present in the cytosol. When the pathways are inhibited (OFF), their respective biosensors shuffle into the nuclei. ERK and AKT activities are therefore calculated as the ratio of cytosolic over nuclear fluorescence measured for each biosensor. **B**) Simplified representation of cells with the fluorescent read-out.

ments (*e.g.* binding assays). In the future, target-specific structure optimization of the identified lead compounds will be pursued.

2.3 *Screening for Musashi Inhibitors Tackling Forgetting*

Memory maintenance and forgetting are fundamental processes in our lives. However, the mechanisms underlaying forgetting are still not completely understood. Recently, a protein called Musashi (MSI) was discovered to be crucial for the process of forgetting in *Caenorhabditis elegans* (*C. elegans*).[20,21] MSI proteins are translational regulators (repressors) that bind to different mRNAs.[22] Genetic deletion of MSI in *C. elegans* tremendously improved memory.[20,21] This prompted us to screen for new NP-based MSI inhibitors. Therefore, our crude extract library was screened with a biochemical fluorescence polarization assay leading to the identification of 97 hits. After taxonomical considerations, HPLC-based activity profiling was applied on 61 extracts. Currently, scale-up isolation of the most promising extracts is ongoing, and the first pure compound confirmed its activity not only *in vitro*, but also *in vivo* in a short- and long-term associative memory test in *C. elegans*. These exciting results will be published soon – stay tuned.

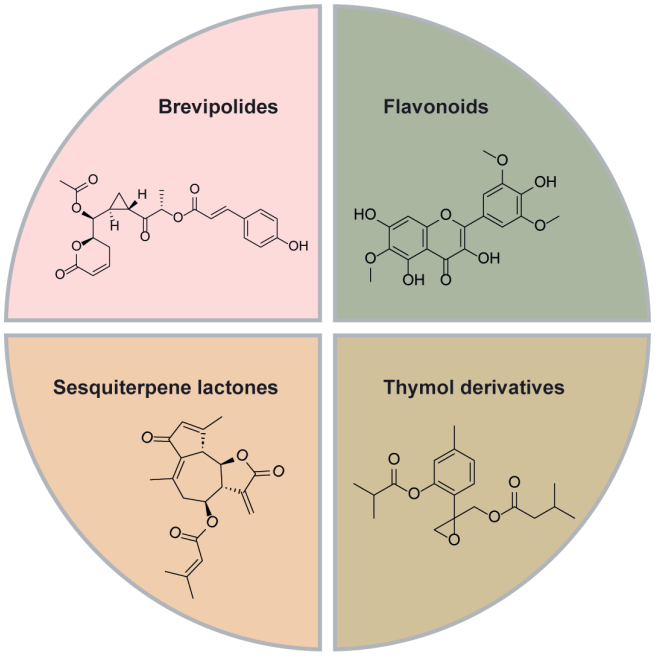

Fig. 3. Representative examples of the NP scaffolds identified as active in the high-content screening assay used in the melanoma project. Compounds were considered as active if they were inhibiting the MAPK/ERK and/or PI3K/AKT pathway in melanoma cells with an IC_{50} value < 20 μM.

3. Conclusion

Nature offers a treasure trove of unique structures that have evolved over millions of years, forming the basis of many drugs nowadays. However, this rich chemical space remains underexplored, mainly due to the inherent challenges when working with complex extracts. Our integrated approach applied to NP drug discovery is aiming to find the 'key' – or at least part of it – to study this bioactive chemical space. As illustrated by two current projects, this journey is demanding but certainly worth the effort. Who knows, the next NP-based drug might just be around the corner.

Acknowledgements

We kindly acknowledge SCS and dsm-firmenich for the organization and sponsoring of the best poster awards. Special thanks to all current and former workers and collaborators of the lab of Prof. Robin Teufel and Prof. em. Matthias Hamburger who contributed to the development of the presented drug discovery platform and projects.

Received: January 29, 2025

[1] C. Krishnamurti, S. S. C. C. Rao, *Indian J. Anaesth*. **2016**, *60*, 861, https://doi.org/10.4103/0019-5049.193696.
[2] A. Fleming, *Br. J. Exp. Pathol*. **1929**, 10.
[3] A. Sadowska, P. Osiński, A. Roztocka, K. Kaczmarz-Chojnacka, E. Zapora, D. Sawicka, H. Car, *Int. J. Mol. Sci*. **2024**, *25*, https://doi.org/10.3390/ijms25010466.
[4] M. Lodato, V. Plaisance, V. Pawlowski, M. Kwapich, A. Barras, E. Buissart, S. Dalle, S. Szunerits, J. Vicogne, R. Boukherroub, A. Abderrahmani, *Cells* **2023**, *12*, 940, https://doi.org/10.3390/cells12060940.
[5] D. J. Newman, G. M. Cragg, *J. Nat. Prod*. **2020**, *83*, 770, https://doi.org/10.1021/acs.jnatprod.9b01285.
[6] P. Nuhn, 'Chemie der Naturstoffe', De Gruyter, **1981**, https://doi.org/10.1515/9783112650882.
[7] C. F. Pinto, D. Torrico-Bazoberry, M. Penna, R. Cossio-Rodríguez, R. Cocroft, H. Appel, H. M. Niemeyer, *J. Chem. Ecol.* **2019**, *45*, 708, https://doi.org/10.1007/s10886-019-01089-x.
[8] S. Dutertre, A. H. Jin, Q. Kaas, A. Jones, P. F. Alewood, R. J. Lewis, *Mol. Cell. Proteomics* **2013**, *12*, 312, https://doi.org/10.1074/mcp.M112.021469.
[9] L. J. Cseke, P. B. Kaufman, A. Kirakosyan, *Nat. Prod. Commun*. **2007**, *2*, 1317, https://doi.org/10.1177/1934578X0700201225.
[10] O. Potterat, M. Hamburger, *Planta Med*. **2014**, *80*, 1171, https://doi.org/10.1055/s-0034-1382900.
[11] L. Dürr, T. Hell, M. Dobrzyński, A. Mattei, A. John, N. Augsburger, G. Bradanini, J. K. Reinhardt, F. Rossberg, M. Drobnjakovic, M. P. Gupta, M. Hamburger, O. Pertz, E. Garo, *J. Nat. Prod*. **2022**, *85*, 1006, https://doi.org/10.1021/acs.jnatprod.1c01154.
[12] M. Y. Heng, B. Thuerig, O. Danton, J. Ramseyer, M. P. Gupta, L. Tamm, M. Hamburger, O. Potterat, *Phytochem*istry **2022**, *199*, 113183, https://doi.org/10.1016/j.phytochem.2022.113183.
[13] I. Yajima, M. Y. Kumasaka, N. D. Thang, Y. Goto, K. Takeda, O. Yamanoshita, M. Iida, N. Ohgami, H. Tamura, Y. Kawamoto, M. Kato, *Dermatol. Res. Pract*. **2012**, *2012*, https://doi.org/10.1155/2012/354191.
[14] A. Boutros, E. Croce, M. Ferrari, R. Gili, G. Massaro, R. Marconcini, L. Arecco, E. T. Tanda, F. Spagnolo, *Crit. Rev. Oncol. Hematol.* **2024**, *196*, 104276, https://doi.org/10.1016/j.critrevonc.2024.104276.
[15] G. V. Long, D. Stroyakovskiy, H. Gogas, E. Levchenko, F. de Braud, J. Larkin, C. Garbe, T. Jouary, A. Hauschild, J. J. Grob, V. Chiarion Sileni, C. Lebbe, M. Mandalà, M. Millward, A. Arance, I. Bondarenko, J. B. A. G. Haanen, J. Hansson, J. Utikal, V. Ferraresi, N. Kovalenko, P. Mohr, V. Probachai, D. Schadendorf, P. Nathan, C. Robert, A. Ribas, D. J. DeMarini, J. G. Irani, M. Casey, D. Ouellet, A.-M. Martin, N. Le, K. Patel, K. Flaherty, *N. Engl. J. Med*. **2014**, *371*, 1877, https://doi.org/10.1056/nejmoa1406037.
[16] S. Regot, J. J. Hughey, B. T. Bajar, S. Carrasco, M. W. Covert, *Cell* **2014**, *157*, 1724, https://doi.org/10.1016/j.cell.2014.04.039.
[17] T. Hell, M. Dobrzyński, F. Gröflin, J. K. Reinhardt, L. Dürr, O. Pertz, M. Hamburger, E. Garo, *Biomed. Pharmacother*. **2022**, *156*, 113754, https://doi.org/10.1016/j.biopha.2022.113754.
[18] L. Dürr, J. K. Reinhardt, M. Dobrzyński, T. Hell, M. Smieško, O. Pertz, M. Hamburger, E. Garo, *J. Nat. Prod*. **2022**, *85*, 2557, https://doi.org/10.1021/acs.jnatprod.2c00471.
[19] T. Hell, A. Rutz, L. Dürr, M. Dobrzyński, J. K. Reinhardt, T. Lehner, M. Keller, A. John, M. Gupta, O. Pertz, M. Hamburger, J. L. Wolfender, E. Garo, *J. Nat. Prod*. **2022**, *85*, 1540, https://doi.org/10.1021/acs.jnatprod.2c00146.
[20] P. Mastrandreas, C. Boglari, A. Arnold, F. Peter, D. J. F. de Quervain, A. Papassotiropoulos, A. Stetak, *PLoS Genet.* **2022**, *18*, e1010420, https://doi.org/10.1371/journal.pgen.1010420.
[21] N. Hadziselimovic, V. Vukojevic, F. Peter, A. Milnik, M. Fastenrath, B. G. Fenyves, P. Hieber, P. Demougin, C. Vogler, D. J. F. De Quervain, A. Papassotiropoulos, A. Stetak, *Cell* **2014**, *156*, 1153, https://doi.org/10.1016/j.cell.2014.01.054.
[22] A. E. Kudinov, J. Karanicolas, E. A. Golemis, Y. Boumber, *Clin. Cancer Res*. **2017**, *23*, 2143, https://doi.org/10.1158/1078-0432.CCR-16-2728.

License and Terms

The definitive version of this article is the electronic one that can be found at https://doi.org/10.2533/chimia.2025.241