UNIVERSITÀ DEGLI STUDI
DELL'INSUBRIA

"Organic Synthesis Laboratory and Enzymatic Studies on In Vitro Release of Drugs and Agrochemicals from Conjugates"

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Keywords: drug conjugates, agro-drug conjugates, cleavable linkers, organic synthesis, peptide synthesis

Purpose: The research carried out in the Organic Synthesis Laboratory and Enzymatic Studies on the In Vitro Release of Drugs and Agrochemicals from Conjugates is aimed at the design, chemical synthesis, and characterization of conjugates containing active pharmaceutical and agrochemical compounds, as well as the study of controlled release mechanisms of the corresponding drugs and agrochemicals through enzymatic cleavage processes in vitro. The final objective is the development of pro-active systems and innovative delivery strategies, evaluating their stability, selectivity, and enzymatic activation profiles under controlled conditions.

Location: Department of Science and High Technology – "Cubo dei Chimici", Via Valleggio 9, 22100 Como.

Organization: The facility is fully coordinated by the SynthMedChem research group (Prof. Gazzola – Prof. Piarulli), which manages the instruments and equipment required for organic synthesis and enzymatic studies of in vitro drug and agrochemical release from conjugates.

Connection with CRIETT Technological Platforms and University Scientific Platforms:

1) *Sustainability* Platform, within Theme 7: *Sustainability and Protection of Natural Resources – "Sustainable Agriculture".*

2) *Technologies for Energy, Health and Environment* Platform, within Theme 3: *Technologies, Methods and Approaches for Biomedical Investigation – "Drug Design".*

Brief Description: The facility consists of:

1) Organic Synthesis: Two fully equipped organic synthesis laboratories (fume hoods, rotary evaporators, nitrogen/vacuum lines, classical and Schlenk glassware, analytical and technical balances, vacuum pumps, etc.). In addition, a flash chromatography system and an automatic peptide synthesizer are available (see "Organic Synthesis Facility").

2) Molecular Characterization and Enzymatic Assays: Two centrifuges, a thermo-shaker, a -80°C freezer for enzyme storage, and a single quadrupole LC-MS system for routine analysis (see "Molecular Characterization and Enzymatic Testing Facility").

Publications:

1. Synthesis and biological evaluation of a novel Dual-Targeting Small Molecule Drug Conjugate Modulating the crosstalk between α5β1 Integrin and MDM2 in Glioblastoma
   Arrigoni F., Ferrari, Ana, Prpić, H., Markeviciute, E., Muzi, A., Roscilli, G., Gazzola, S., Piarulli, U. - ACS MedChem Lett. 2026, DOI: 10.1021/acsmedchemlett.5c00669

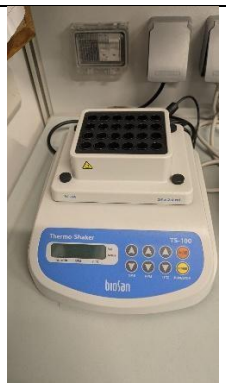
2. AgroDrug Conjugates for Sustainable Crop Protection: Molecular Architectures, Mechanisms, and Critical Perspectives. Cazzaniga, G., Orru, R., Barber, D.M., Gazzola S. - Top Curr Chem, 2026, 384, 1. https://doi.org/10.1007/s41061-025-00531-x
3. Comparative Enzymatic and Stability Assays Reveal GPLG as an Effective Cathepsin B Cleavable Linker for Tumor-Targeting Drug Conjugates. Cazzaniga, G., Zambra, M., Bongiolo, S., Prpić, H., Fasola, E., Arrigoni, F., Piarulli, U.*, Gazzola, S.. ACS Omega, 2025, 10, 36, 41783-41798.
4. Impairing protein–protein interactions in an essential tRNA modification complex: An innovative antimicrobial strategy against Pseudomonas aeruginosa. Bollati, M., Fasola, E., Pieraccini, Freddi, F., Cocomazzi, P., Oliva, F., Klußmann, M., Maspero, A., Piarulli, U., Ferrara, S., Pellegrino, S. Bertoni, G.,* Gazzola, S.* - J. Pep. Sci., 2025, 31:e3658.
5. Design, Synthesis and Preliminary In-Vitro Activity of 6-Hydroxyalkyl β-Carboline Derivatives for the Development of Drug Conjugates Targeting MDM2. *Arrigoni, F., Prpic, H., Ferrari, A., Zambra, M., Roscilli, G., Gazzola, S.,* Piarulli. U.*- Eur. J. Org. Chem., 2024, e202400915*
6. Optimizing the Enzymatic Release of MMAE from isoDGR-based Small Molecule Drug Conjugate by Incorporation of a GPLG-PABC Enzymatically Cleavable Linker. Zambra, M., Ranđelović, I., Talarico, F., Borbély, A., Svajda, L., Tóvári, J., Mező, G., Bodero, L. A., Colombo, S., Arrigoni, F., Fasola, E., Gazzola, S.,* Piarulli. U.*- Front. Pharmacol., 2023, 14, 1215694.
7. Conformational switch and multiple supramolecular structures of a newly identified self-assembling protein-mimetic peptide from Pseudomonas aeruginosa YeaZ protein. Fasola, E., Alboreggia, G., Pieraccini, S., Oliva, F., Agharbaoui, F. E., Bollati, M., Bertoni, G., Recchia, S., Marelli, M., Piarulli, U., Pellegrino, S. Gazzola, S.* Front. Chem., 2022, 10, 1038796.
8. A combined fragment-based virtual screening and STD-NMR approach for the identification of E-cadherin ligands. Vasile, F., Lavore, F., Gazzola, S., Vettraino, C., Parisini, E., Piarulli, U., Belvisi, L., Civera, M. - Front. Chem., 2022, 10, 946087.
9. Synthesis and Biological Evaluation of an isoDGR-Paclitaxel Conjugate Containing a Cell-Penetrating Peptide to Promote Cellular Uptake. Bodero L., Parente S., Arrigoni F., Klimpel A., Neundorf I., Gazzola S.,* Piarulli U.* – Eur. J. Org. Chem., 2021, 17, 2383.

- **A) Organic Synthesis Facility**

	**Interchim puriFlash System:** Automated flash chromatography system equipped with fraction collector and UV detector for the purification of organic molecules. The system can operate in both normal-phase and reverse-phase modes, depending on experimental requirements.
	**Biotage Initiator+ Alstra™ Microwave Peptide Synthesizer:** Fully automated single-channel microwave-assisted peptide synthesizer designed for small- and large-scale peptide synthesis. Microwave heating accelerates reactions, improving yield and purity while significantly reducing synthesis time. The system supports the synthesis of peptoids, PNA, and peptidomimetics, with flexible scales ranging from 5 µmol to 2 mmol.

- **A) Molecular Characterization and Enzymatic Testing Facility**

	**LC-MS: LC–MS System** Compact single-quadrupole mass spectrometer (m/z range 10–1200) equipped with an ESI (electrospray ionization) source for direct liquid sample introduction, interfaced with a Jasco HPLC system (Series 900/1500) including: 1. Binary pump with flow rates from 0.001 to 10 mL/min, maximum pressure 500 bar. 2. 50-position autosampler with variable injection volume. 3. UV/Vis detector.
	Chest-type −80 °C freezer for the storage of small biological samples (e.g., enzymes), ensuring long-term stability under ultra-low temperature conditions.

	**Thermo-shaker BioSan TS-100C:** The TS-100C thermal shaker provides intensive mixing and precise temperature control of samples in PCR plates or microtest tubes. The instrument is also capable of cooling samples down to +4 °C. Heating and mixing functions can be performed either simultaneously or independently. Thirteen interchangeable heating and cooling blocks are available, including a block with a plastic lid for PCR plates. All blocks are easily replaceable and can be installed on the shaker.
 	Centrifuges for Eppendorf tubes and for 15 mL Falcon tubes.
	Laboratory ultrasonic processor operating at 30 kHz, equipped with a 2 mm titanium probe, suitable for sample volumes ranging from 2 to 50 mL.