Review
JOURNAL OF UNIVERSITY OF BABYLON
For Pure and Applied Sciences (JUBPAS)
Vol. 33 ; No.4 | 2025

Mass Spectrometry for the Structural Elucidation of Peptide-Based Therapeutics

Eateman Salah Mahdi[1], Ali Taleb Bader[1*], Nada Ahmed Rasheed Al-Qasii[2]

1-Department of Chemistry, College of Sciences for Woman, University of Babylon, Hilla, 51001, Iraq

2-Department of Chemistry, College of Science, University of Baghdad, Baghdad, 11001, Iraq

*Corresponding Author wsc.ali.taleb@uobabylon.edu.iq

Accepted: 2/12/2025 **Published:** 31/12/2025

ABSTRACT

In recent decades, the life sciences have undergone a paradigm shift in the strategies used to characterize genes, proteins, transcriptomes, and metabolites. This transformation has been propelled by the advent of high-throughput analytical platforms, which enable the simultaneous interrogation of vast numbers of biomolecules with unprecedented efficiency and resolution. Among these technologies, mass spectrometry has emerged as a cornerstone methodology, owing to its unrivaled sensitivity, selectivity, and quantitative power in ion detection. By facilitating the accurate reconstruction of the molecular and chemical architecture of complex biological systems, mass spectrometry has become indispensable to contemporary biological and biomedical research, driving advances in systems biology, drug discovery, and personalized medicine.

MS enables high-throughput measurement of complex protein or peptide mixtures, sequencing and quantifying proteins or peptides, detecting post-translational modifications (PTMs) of proteins, and predicting their 3D folding and topology. Peptides and proteins are typically studied using mass spectrometry because traditional ionization techniques easily generate protonated species. Peptide drug conjugates (PDC) are a prodrug type in which drug molecules are conjugated to peptides by covalent bonding via specific linkers. The fragmentation of this PDC type depends on several parameters, such as instrument settings, solvent composition, and fragment ion intensities and types, which also depend on the structure. A novel conjugate of Anthracycline and peptide provides safe and effective cancer therapy. For its structure elucidation via MS, several detection techniques would be complementary.

Keywords: peptides, conjugates, Tandem mass spectrometry

ISSN: 2312-8135 | Print ISSN: 1992-0652
info@journalofbabylon.com | jub@itnet.uobabylon.edu.iq | www.journalofbabylon.com

ISSN: 2312-8135 | Print ISSN: 1992-0652
info@journalofbabylon.com | jub@itnet.uobabylon.edu.iq | www.journalofbabylon.com

2. MASS SPECTROMETRY OF BIOLOGICAL MOLECULES

The 3D structure of a macromolecular complex is usually necessary to understand the biological and molecular mechanisms that permit the functional activity of the complex[1]. The molecular structure of biomolecules has been studied using classic techniques, such as cryo-electron microscopy (cryo-EM), crystallography (X-ray diffraction), [2] and nuclear magnetic resonance (NMR). Structure-based proteomics makes considerable use of MS to study 3D structures [3-5]. Several MS-based techniques have been developed to detect morphological and structural changes in biological molecules and analyse their non-covalent interactions and complex biological environments such as whole cell-lysates, the responsive target stability test, protein oxidation rate stability , cellular thermal shift assay with MS, chemical cross-linking [6, 7], and hydroxyl radical foot printing [8-11].

Electrospray Ionization (ESI) is the most frequently ionization method used in proteomics.. This soft ionization approach produces a single or many protonated species of intact peptides. Tandem MS is used to sequence and verify peptide modification sites, it also employed to measure and validate the molecular mass of peptide. The tandem MS analysis of daunomycin-containing peptides is complicated as the molecules breaking apart during the ESI test.

biological cell (e.g., human or microbial) or to a component of the mass spectrometry instrument (e.g., ion trap or collision cell). A more precise term is recommended [12]. During the high vacuum, the glycosidic bonds which conjugate the peptide with daunomycin spontaneously break, resulting in MS fragments missing the sugars part in the analysed sample. In deep-scan ESI-MS spectra, daunomycin-containing peptide conjugates frequently appears as base peaks in spectroscopic patterns [13].

2.1. Protonated peptides in mass spectrometry

Peptide-ion disintegration based on Matrix-Assisted Laser Desorption/Ionization MALDI has been explored in several instances. Zhiqiang and Chao [14] have studied the behaviour of over 200 peptides during fragmentation using MALDI ion trap equipment. The amino acid concentration does not affect the fragmentation performance of peptides ions with m/z values less than 1500

Pepsins that do not contain arginine (Arg) residues behave in a manner comparable to pepsins whose sequences include lysine (Lys) as their only basic residue, whether in terms of cleavage specificity or analytical response.[15-17]. The mobile protons concept of polypeptide

مجلة جامعة بابل للعلوم الصرفة والتطبيقية مجلة جامعة بابل للعلوم الصرفة والتطبيقية

ISSN: 2312-8135 | Print ISSN: 1992-0652
info@journalofbabylon.com | jub@itnet.uobabylon.edu.iq | www.journalofbabylon.com

fragmentation was used to examine this issue. According to this view, the most stable forms of protonated peptides rarely undergo fast fragmentation processes [18, 19]. Indeed, the fragmentation can occur without ion stimulation-induced structural changes.

Fragmentation often proceeds through a series of intermediate minima rather than an instantaneous onset under certain conditions. The MS/MS spectra of backbone amide bonds typically contain rich sequences of b- and y-type ions, which are most apparent in systems with high proton mobility. However, side chains of certain amino acids can sequester multiple protons, thereby reducing the availability of mobile protons and leading to spectra with limited structural information [18].

2.1.1. Amino acids and peptides

Because amino acids are utilized in both chemical and biological studies, having a solid understanding of them is absolutely necessary. They serve as precursors in medicinal chemistry, being part of the chiral pool, and as chiral auxiliaries and catalysts, all of which are essential functions in the overall process of bioactive molecule synthesis [20]. A peptide is made up of two or more than two amino acids joined together by a covalent bond called peptide bond as shown in Figure 2.1. Peptides have the potential to be used as therapeutic agents because of the diverse biological effects they can produce, they have the ability to be mechanically stable, structurally simple (easy modification), have low toxicity, and are easily compatible with bio-systems, as well as selective to their targets.

On the other hand, peptide drug design faces a variety of challenges, the most significant of which is a lack of in vivo stability, being protease sensitive and therefore plasma degradable. Additionally, peptide drugs have unfavourable physicochemical features, such as low oral bioavailability, short half-life, low membrane permeability (long peptide chains), high conformational freedom, and low binding affinity [21]. Synthetic mutagenesis, also known as the synthetic substitution of amino acids, is a proven way to overcome these deficiencies [22]. Moreover, N- heteroaromatics are also highly attractive for medicinal applications due to the fact that they concurrently affect the solubility, metabolism, and binding affinity of the medicines. Therefore, synthetic amino acids containing heteroarene moiety have a lot of potential in the field of peptide therapy[23] . In addition, in the presence of heterocycles such as pyridine, both amino acids and peptides experience profound shifts in their structures, such

ISSN: 2312-8135 | Print ISSN: 1992-0652
info@journalofbabylon.com | jub@itnet.uobabylon.edu.iq | www.journalofbabylon.com

as, the coplanar conformation stabilizes due to conjugation between the carboxylate groups and adjacent pyridine rings which in turn stabilizes the whole structure [24].

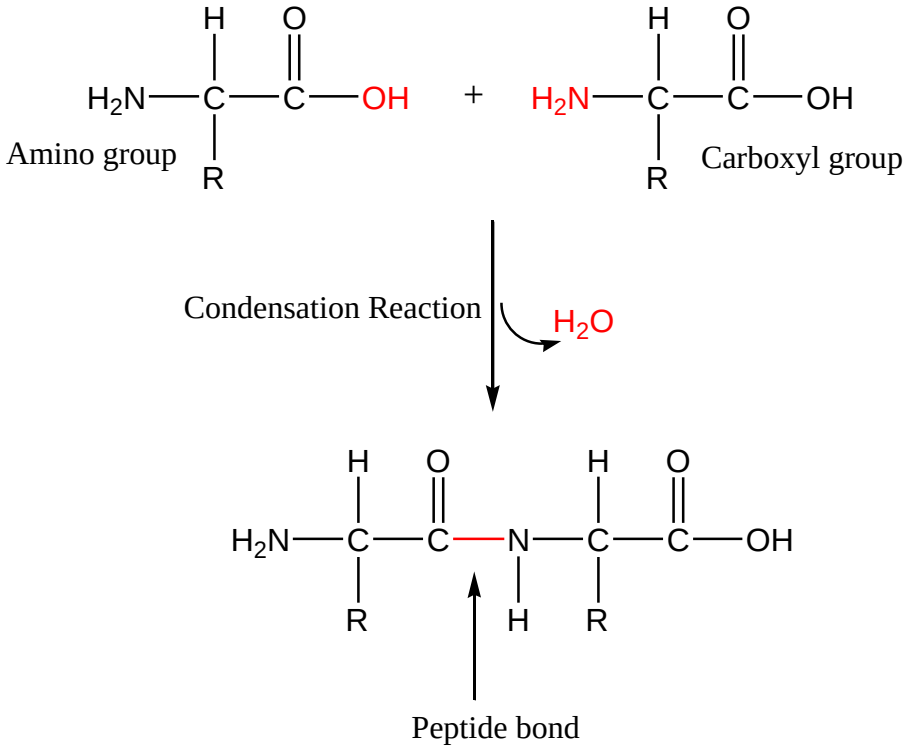


Figure 2.1. Amide bond formation via amino acid condensation.

In order to conduct mass spectrometric analysis for the amino acid composition of peptides, proteins are digested using specialized enzymes such as trypsin, chymotrypsin, pepsin, and elastase. These enzymes cleave proteins at specific peptide bonds [25]. Trypsin, for instance, is capable of breaking the peptide bond on the C-terminal side of lysine and arginine residues. This procedure results in the production of short peptide fragments (bx-ion and Yz-ion), as shown in **scheme 2.2**, which are easily analyzed by mass spectrometry [26, 27].

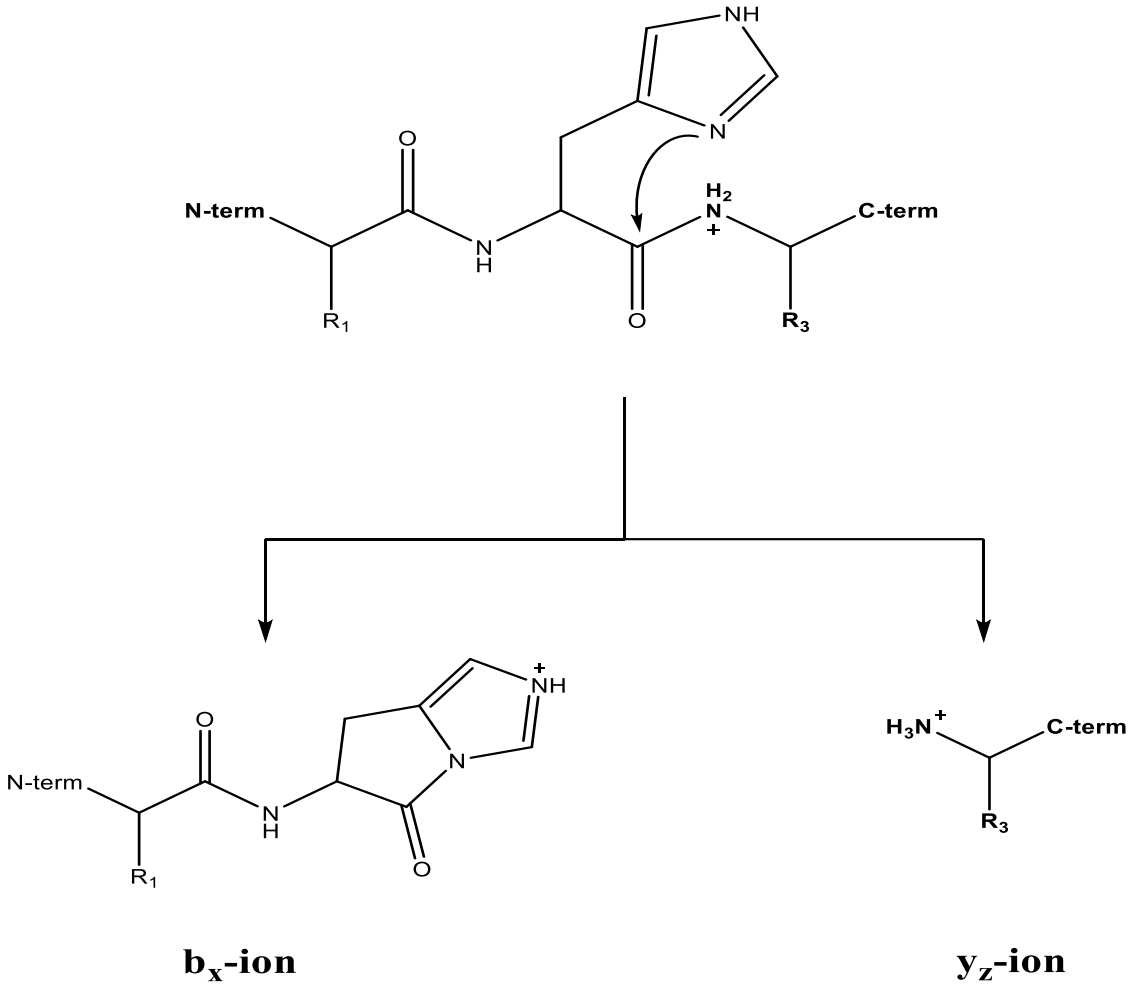
2.1.2 Sidechain effects

Drug transfer strategies for cancer treatment have been created when anti-cancer medicines (such as anthracycline derivatives) were connected to the Lys side chain. The *in vitro* cytostatic efficacy of anthracycline derivative bioconjugates of Gonadotropin-Releasing Hormone II (GnRH-III) revealed low concentration IC50 values. Furthermore, it was found that

bioconjugates of GnRH-III and daunorubicin (Dau) linked by oxime bonds administered intraperitoneally suppress tumour formation in mice with colon cancer [28]. As a model peptide, $[RGD+H]^+$ offers various advantages [29]. Various fragments are generated when $[RGD+H]^+$ dissociates, notably backbone fragmentation and specific molecular losses (such as water or ammonia) [30]. There have long been discussions about creating these fragments via MS. Recent infrared multiple proton dissociation spectroscopy studies [31] have found these fragments for a large peptide that contained this sequence and similar sequences [32]. Recently, the $[RGD+H]^+$ mobile phase composition and two possible sources of the COOH proton has been identified [33]. Because of the short length of the peptide, fragmentation mechanisms has also been approximated [34]. Based on their basic characteristics, the sequestration of Arg is more effective than that of Lys. Arg tends to sequester protons; therefore, exciton fragmentation pathways may be more successful than charge-directed fragmentation routes [35]. Transporting entrapped ionising protons is one example of this type of transport. C-terminal fragments dominate MS/MS spectra that originate from peptide C-terminal division. There are few sequence-informative peaks in these spectra, so it may be difficult to distinguish them. These restrictions apply to both MALDI- and ESI-generated tryptic peptide ions; however, the higher concentration of ionising protons generated by ESI makes it more difficult to employ [36].

ISSN: 2312-8135 | Print ISSN: 1992-0652
info@journalofbabylon.com | jub@itnet.uobabylon.edu.iq | www.journalofbabylon.com

ISSN: 2312-8135 | Print ISSN: 1992-0652
info@journalofbabylon.com | jub@itnet.uobabylon.edu.iq | www.journalofbabylon.com



Scheme 2.2. Pathway of the preferential cleavage of the C-terminal in histidine containing peptide.

2.2. Methodology of bioconjugation

the bioconjugation can be : a chemical process that creates a stable, covalent link between a biomolecule (like a protein or nucleic acid) and another molecule, such as a drug, nanoparticle, or fluorescent tag.. It involves combining two or more biomolecules to create functional properties of interest. The field of bioconjugate technology gained momentum when researchers successfully developed bioconjugate materials. These compounds are components in numerous biochemical assays used in biopharmaceutical settings like antibody-drug conjugates (ADCs). Moreover, biochemical tests also utilize biological conjugates, in nanotherapeutics. [37, 38].

ISSN: 2312-8135 | Print ISSN: 1992-0652
info@journalofbabylon.com | jub@itnet.uobabylon.edu.iq | www.journalofbabylon.com

When the elemental composition of multi-component bioconjugates is determined, the usual approach involves time-consuming chemistry methods. Because it is rapid and accurate, mass spectrometry should be employed to determine chemical substances and their products. Doxorubicin (Dox) as well as anti-cancer drug derivatives undergo different fragmentation proteins during MS evaluation, which makes the quality of these drugs very hard to determine well Protonated peptides and proteins are produced whole by such methods of electrospray ionization (ESI), and matrix-assisted laser desorption ionization (MALDI). Bioconjugated daunomycin mass spectra differ in fragmentation patterns depending on whether the antibiotic is present in some ready manner when examined under traditional mass spectrometric conditions. [13].

Drug delivery through receptors may prove to be a better cancer treatment than chemotherapy, which involves injecting uncharged anti-cancer drugs. It may be safer for the body and more selective. Moreover, anthracycline bioconjugates and GnRH-III derivatives have already been synthesized and thoroughly characterized chemically[39]. The anticancer drug Dau is one of the most effective drug carriers and is usually attached to GnRH-III derivatives via an oxime bond, with the fourth amino acid Ser substituted by Lys. [40].

To reduce systemic toxicity as much as possible while increasing selectivity, we can attach Dau to the targeting moieties based on GnRH. It was observed that the bioconjugates were highly selective and substantially inhibited the tumor growth of a large variety of tumors [32]. Toxic substances can be liberated at an early stage due to the action of carboxylesterases; in this case, the toxicity is not related to the receptor, because the ester bond is cleaved too quickly in rapidly growing cells. With 2-pyrrolino-Dox myelotoxicity was noted as the main side effect of the compound [41-46].

2.2.1. Bioconjugation chemical linkages

The amide bonds in the sugar moiety and the linkages of the aglycone part (oxime or hydrazone)[47] are exposed to in-source fragmentation that could produce t a complex MS spectra which generated due to the split of the daunosamine (Dau) bioconjugated molecule . Cross-conventional MS has its limitations. Conjugate protonation causes its glycosidic bond to cleave and removes the daunosamine moiety. Much of the biological activity [48] is therefore lost. The search for new conjugate structure modifications will therefore be helped. This study, by using anti-cancer drug conjugates of ester bond type as an example, demonstrated that drugs

ISSN: 2312-8135 | Print ISSN: 1992-0652
info@journalofbabylon.com | jub@itnet.uobabylon.edu.iq | www.journalofbabylon.com

of identical weight may have entirely different biological effects[34] .Because the structure is what decides whether or not something is of value as a drug, proper structural characterization is essential.

Pharmacological applications need peptide-based therapeutics to be stable against enzyme-catalyzed hydrolysis. This is particularly pertinent if the drugs are delivered orally since the drugs could be hydrolyzed by stomach and intestinal enzymes. For example, chymotrypsin hydrolyses GnRH and its analogues at the Trp-Ser-peptide bond preferentially. Though trypsin-catalyzed hydrolysis occurs, the levels of hydrolysis of these GnRH derivatives were lower than elastase[49].

2.2.2. Linkage generation and thiol reactions

Peptide bioconjugates that include **thiol (–SH)** groups — for example, via cysteine residues or engineered thiol-linkers — are widely used in drug development (e.g., antibody-drug conjugates), diagnostics, and chemical biology. Characterizing these conjugates by **mass spectrometry (MS)** (especially tandem MS, MS/MS) is critical to confirm the conjugation site, to detect side-products (e.g., disulfide exchange), and to ensure product integrity. However, the presence of sulfur (S) bonds — especially **S–C**, **S–S**, or thioester linkages — introduces specific fragmentation behavior, which must be understood and leveraged in MS analysis[50].As a consequence of the cysteine residue's connections to the polypeptide present in newly approved ADCs, selective thiol methods are of utmost importance to generate effective bioconjugates [51]. Cysteine is a versatile residue selected during bioconjugation due to the variety of thiol-selective methods adopted to make modifications [52]. Although there are numerous thiol-selective conjugation methods available, maleimides are the most widely utilized method of cysteine modification. Maleimide reagents have a rapid biomolecular labelling rate and exhibit adequate cysteine specificity [53]. Additionally, maleimide derivative products, which are commercially available, such as affinity probes, dyes, and cross-linkers, can be easily obtained without the necessity of having specialized knowledge. However, the stability of maleimides does pose an issue and can result in unsatisfactory and dangerous conjugates. Innovative solutions to stability issues associated with maleimide-based bioconjugates have emerged, and have been recently discussed in a comprehensive article [54].There has been an increase in the development of ADCs for the treatment of cancer in recent years, primarily due to mixed results from clinical trials, including the approval of brentuximab and ado-trastuzumab [55, 56]. A unique observation includes a variety of deadly small compounds that are chemically conjugated to monoclonal antibodies with relatively broad specificity. Different linkers have

ISSN: 2312-8135 | Print ISSN: 1992-0652
info@journalofbabylon.com | jub@itnet.uobabylon.edu.iq | www.journalofbabylon.com

been used in the clinic to make ADCs [57] One of the more widely used approaches in ADCs has been thiol-maleimide linkages due to their adequate specificity, aqueous compatibility, and rapid reaction times. In the case of T-DM1, A polyketide derivative of maytansine DM1 is added to the maleimide moiety of the linker to produce T-DM1 by conjugating Trastuzumab's lysine residues to the N-hydroxy succinimidyl ester of the SMCC linker [58, 59].

2.2.3. Conjugation of C–N double bonds

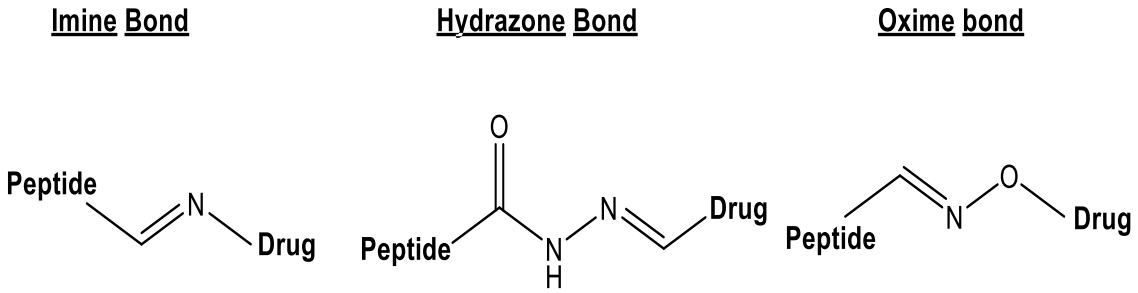
Condensation of nitrogenous bases with aldehydes and ketones at pH= 7 gives C = N bonds, therefore it can perfectly used for bioconjugation. When hydrazine is used as the nitrogen source, hydrazones (C=N–N) are formed whereas when an alkoxyamine nitrogen base is used the product obtained is oximes (C=N–O) (**Scheme 2.3**) [51]. As a summary, the process of covalent bioconjugation can be summarized as follows:

1) In general nanoparticles contain active functional groups which are grafted.

2) The thiol groups on the side chain of the protein is chemically activated by a particular reductant.

3) The reduction agent is also separated from the system[60].

Extraction of unbound proteins, as well as stripping off additional residues are sub-processes under post-conjugation operations. Covalent bioconjugation is slow and has the disadvantage of changing protein properties and conformation; the protein experiences partial denaturation. [61].



Scheme 2.3. Structures of peptide drug conjugates' linkers.

مجلة جامعة بابل للعلوم الصرفة والتطبيقية مجلة جامعة بابل للعلوم الصرفة والتطبيقية

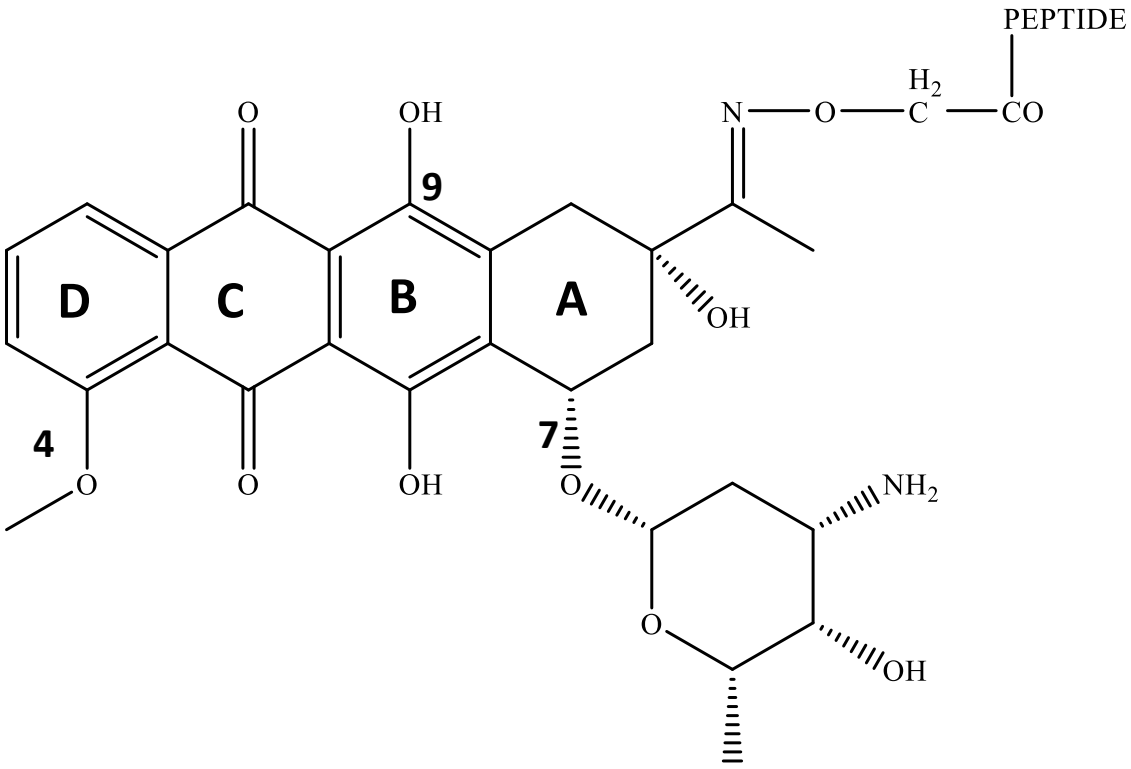
ISSN: 2312-8135 | Print ISSN: 1992-0652
info@journalofbabylon.com | jub@itnet.uobabylon.edu.iq | www.journalofbabylon.com

2.3. Anthracycline's chemical structure and modes of action

Anthracyclines are chemotherapeutic medicines with a broad therapeutic range. They were intended to be antibiotics, but their toxicity makes it impossible. These aminoglycosides contain tetracyclic quinine and are polar and mildly basic. Their discovery in a pigment-producing strain of *Streptomyces* has led to their widespread use as an anti-cancer medication. daunorubicin (DAU), doxorubicin (DOX) , and epirubicin (Epi) are the three most important anthracyclines. [62]. Over the past 30 years, have been used in cancer treatment as a key class of chemotherapeutic medications and are recognised as some of the most potent antineoplastics available today. Many cancer cell lines responded well to the anthracycline Dau, which was the first to be identified in 1963. Epi and Dox, which are the next anthracyclines to be identified, were identified in the 1960s and 1970s. Despite being identified in 1976, idarubicin, a synthetic Dnr homolog, is infrequently used in chemotherapy. Most patients with advanced breast or gastrointestinal cancer receiving systemic chemotherapy receive anthracyclines during their treatment [63]. Owing to their similar structures, anthracyclines and sugars can be combined to form antibiotics with anti-cancer properties [64, 65].

Compared to Dox, the hydroxyl group at the 4’ position of Epi has been reoriented, which possesses a daunosamine ring, and has various distinct pharmacological characteristics. The pKa of Epi is lower than that of Dox (7.7 vs. 8.4), and thus, Epi is more lipophilic and capable of penetrating cells. Epi has a higher therapeutic index than Dox, allowing for higher doses [62].

ISSN: 2312-8135 | Print ISSN: 1992-0652
info@journalofbabylon.com | jub@itnet.uobabylon.edu.iq | www.journalofbabylon.com

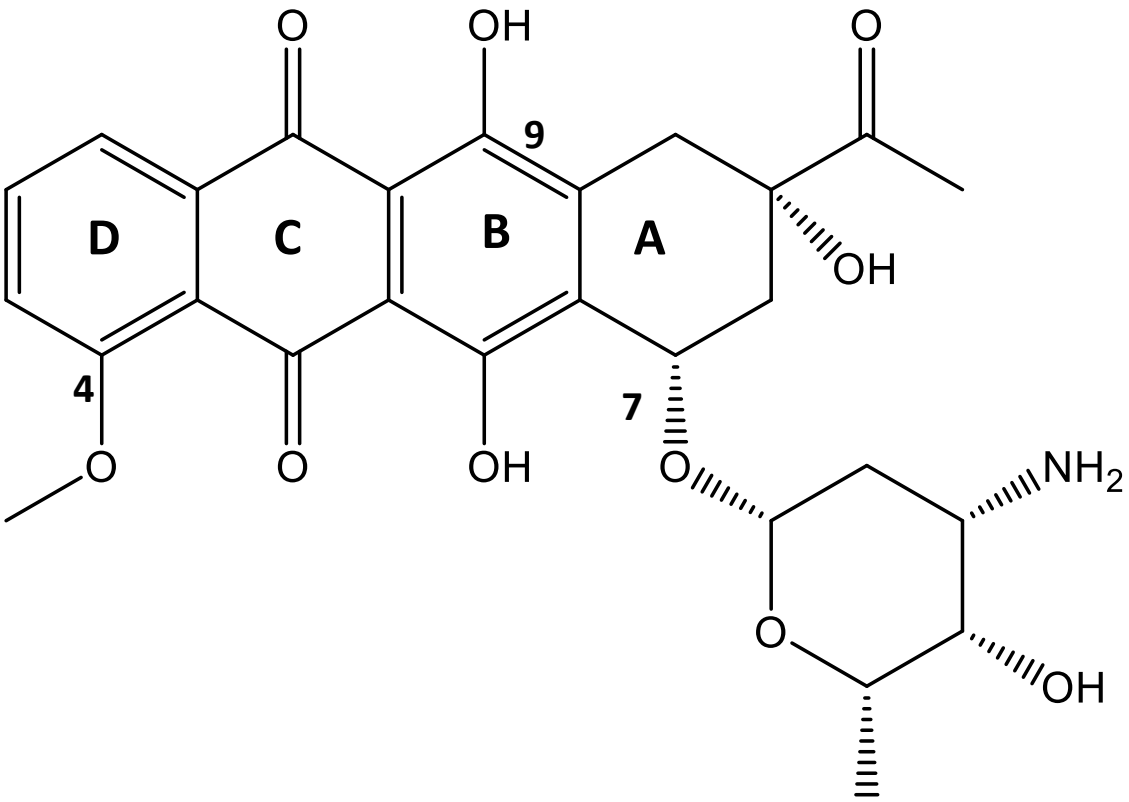


Scheme 2.4. Chemical structure of a daunomycin-containing peptide conjugate with an oxime bond.

Because of their complex architectures, anthracyclines have nearly limitless possibilities for structural modification (**Scheme 2.5**). This small structural difference results in Epi exhibiting markedly lower toxicity to myocardium and bone marrow than Dox, and comparable efficacy in a wide range of hematologic malignancies, including ovarian cancer, lung cancer, Hodgkin's carcinoma of the breast, gastric cancer, and non-resectable hepatocellular [65]. In addition to lung, breast, head, ovary, and bladder cancer, anthracyclines work well against a wide range of solid cell tumours. Adults with soft tissue sarcomas are more likely to benefit from these drugs [66].

مجلة جامعة بابل للعلوم الصرفة والتطبيقية مجلة جامعة بابل للعلوم الصرفة والتطبيقية

ISSN: 2312-8135 | Print ISSN: 1992-0652
info@journalofbabylon.com | jub@itnet.uobabylon.edu.iq | www.journalofbabylon.com



Scheme 2.5. Chemical structure of anthracycline.

Dox and Dau are two anthracycline anti-cancer drugs that are commonly used in cancer therapy. However, their therapeutic value is restricted due to potentially lethal dose-related side effects, such as nephrotoxicity, cardiotoxicity, myelosuppression, and extravasation, from cytotoxicity that affects all developing cells. Drug delivery strategies that deliver antineoplastic drugs specifically to tumours are needed to improve their therapeutic index (drug targeting). Liposomes, monoclonal antibodies, serum proteins, hydroxyl propyl methacrylamide polymers, and polyethylene glycol have all been considered as macromolecular carriers [67]. Peptides have overexpressed receptors on tumour cells when they are used as carriers for the peptides. Chemotherapy drugs can be covalently attached to peptides to specifically target tumour cells. Neuropeptide Y (NPY) was chosen as a model peptide because it has receptors in various neuroblastomas and cell lines originating from neuroblastomas. NPY, which is 36 amino acids long and produced by the pancreas, is a neuropeptide that may be present in the brain as well as the peripheral nervous system. Scientists have found five distinct NPY receptor subtypes, including Y1, which is the most common [68]. After binding to G-protein receptors, the ligand-receptor complex is internalised, making receptor-mediated endocytosis the fastest route inside the cell [69]. Due to the well-known structure-activity correlations (SARs) of menaimide anthracycline derivatives, they have been added to NPY at position 15, which should not result

مجلة جامعة بابل للعلوم الصرفة والتطبيقية مجلة جامعة بابل للعلوم الصرفة والتطبيقية

in any significant loss of Y1 receptor binding activity. Compared to Dauno-MBS, Doxo-MBS has stronger anthracycline-amide bonds, but Dauno-HYD has a weaker 13-ketohydrazone linker, making it less suited for clinical studies. D- and Dox were given maleimide moiety additions [70].

CONCLUSION

This study delved into the importance of mass spectrometry (MS) when studying biological compounds like peptides and proteins. Show casing its vital role in their analysis and characterization process. MS plays a role in pinpointing and measuring ions accurately while also aiding in the recreation of complex biomolecular structures and spotting any modifications post-protein synthesis. A closer examination of drug conjugates (PDCs) particularly those based on Anthracycline compounds emphasizes their promising potential to offer safe and efficient treatments, for cancer patients. The research highlighted how PDC fragmentation relies on factors like instrument configurations and solvent makeup. We also explored bioconjugation techniques that show how important it is to use chemicals to improve drug effectiveness and lower overall toxicity. The development of Anthracycline and GnRH III derivatives showcases progress in treating cancer effectively by reducing tumor size and enhancing treatment success rates. Additionally, the study discussed obstacles in designing peptide-based drugs such as stability and how accessible they are in the body suggesting an approach, through mutagenesis to address these issues. Using spectrometry allows us to analyze complex drug combinations in detail and helps in creating better treatment options for patients. Essentially this study confirms the role of MS in studying biomolecules and developing drugs. It also provides information for potential use, in proteomics and cancer treatment going forward.

Acknowledgments

Authors thankful for all researchers who support us us in completing this study.

ISSN: 2312-8135 | Print ISSN: 1992-0652
info@journalofbabylon.com | jub@itnet.uobabylon.edu.iq | www.journalofbabylon.com

مجلة جامعة بابل للعلوم الصرفة والتطبيقية مجلة جامعة بابل للعلوم الصرفة والتطبيقية

Conflict of interests:

There are non-conflicts of interest.

References

[1] X. Xu, C. Yan, R. Wohlhueter, and I. Ivanov, "Integrative Modeling of Macromolecular Assemblies from Low to Near-Atomic Resolution," *Computational and Structural Biotechnology Journal,* vol. 13, pp. 492-503, 2015/01/01/ 2015, doi: https://doi.org/10.1016/j.csbj.2015.08.005.

[2] Y. Jiang, Z. Wang, and S. Scheuring, "A structural biology compatible file format for atomic force microscopy," *Nature Communications,* vol. 16, no. 1, p. 1671, 2025/02/15 2025, doi: 10.1038/s41467-025-56760-7.

[3] A. N. Calabrese and S. E. J. M. Radford, "Mass spectrometry-enabled structural biology of membrane proteins," vol. 147, pp. 187-205, 2018.

[4] U. Kaur, D. T. Johnson, E. E. Chea, D. J. Deredge, J. A. Espino, and L. M. J. A. c. Jones, "Evolution of structural biology through the lens of mass spectrometry," vol. 91, no. 1, pp. 142-155, 2018.

[5] J. R. J. F. Yates III, "Recent technical advances in proteomics," vol. 8, 2019.

[6] M. Matzinger and K. J. J. o. P. R. Mechtler, "Cleavable cross-Linkers and mass spectrometry for the ultimate task of profiling protein–Protein interaction networks in vivo," vol. 20, no. 1, pp. 78-93, 2020.

[7] B. A. Kochert, R. E. Iacob, T. E. Wales, A. Makriyannis, and J. R. J. M. i. m. b. Engen, "Hydrogen-Deuterium Exchange Mass Spectrometry to Study Protein Complexes," vol. 1764, pp. 153-171, 2018.

[8] S. D. Maleknia, K. M. J. P. Downard, and p. letters, "Protein footprinting with radical probe mass spectrometry-Two decades of achievement," vol. 26, no. 1, pp. 4-15, 2019.

[9] J. R. Engen, T. Botzanowski, D. Peterle, F. Georgescauld, and T. E. J. A. C. Wales, "Developments in Hydrogen/Deuterium Exchange Mass Spectrometry," vol. 93, no. 1, pp. 567-582, 2020.

[10] E. B. Erba, L. Signor, and C. J. J. o. p. Petosa, "Exploring the structure and dynamics of macromolecular complexes by native mass spectrometry," vol. 222, p. 103799, 2020.

[11] M. M. Attwood, D. Fabbro, A. V. Sokolov, S. Knapp, and H. B. J. N. R. D. D. Schiöth, "Trends in kinase drug discovery: targets, indications and inhibitor design," pp. 1-23, 2021.

[12] L. Pethő, G. Mező, and G. J. M. Schlosser, "Overcharging effect in electrospray ionization mass spectra of daunomycin-tuftsin bioconjugates," vol. 24, no. 16, p. 2981, 2019.

[13] M. Al-Majidi, D. Szabó, L. Dókus, A. Steckel, G. Mező, and G. J. J. o. M. S. Schlosser, "Energy-resolved HCD fragmentation of daunorubicin-peptide conjugates," vol. 55, no. 10, p. e4641, 2020.

[14] J. Qin and B. T. J. I. j. o. m. s. Chait, "Collision-induced dissociation of singly charged peptide ions in a matrix-assisted laser desorption ionization ion trap mass spectrometer," vol. 190, pp. 313-320, 1999.

[15] B. Niu and M. L. J. M. S. B. C. P. Gross, "MS-based hydroxyl radical footprinting: Methodology and application of fast photochemical oxidation of proteins (FPOP)," pp. 363-416, 2019.

[16] R. Singla, S. M. Abidi, A. I. Dar, and A. J. A. o. Acharya, "Inhibition of glycation-induced aggregation of human serum albumin by organic–inorganic hybrid nanocomposites of Iron oxide-functionalized Nanocellulose," vol. 4, no. 12, pp. 14805-14819, 2019.

[17] Y. A. Lyon, D. Riggs, L. Fornelli, P. D. Compton, and R. R. J. J. o. t. A. S. f. M. S. Julian, "The ups and downs of repeated cleavage and internal fragment production in top-down proteomics," vol. 29, no. 1, pp. 150-157, 2017.

[18] P. Maitre, D. Scuderi, D. Corinti, B. Chiavarino, M. E. Crestoni, and S. J. C. r. Fornarini, "Applications of infrared multiple photon dissociation (IRMPD) to the detection of posttranslational modifications," vol. 120, no. 7, pp. 3261-3295, 2019.

ISSN: 2312-8135 | Print ISSN: 1992-0652
info@journalofbabylon.com | jub@itnet.uobabylon.edu.iq | www.journalofbabylon.com

[19] L. Konermann, H. Metwally, Q. Duez, and I. J. A. Peters, "Charging and supercharging of proteins for mass spectrometry: recent insights into the mechanisms of electrospray ionization," vol. 144, no. 21, pp. 6157-6171, 2019.
[20] S. Boz, "A computational study on the structures and proton affinities of B3+ ions; peptide mass fragment product," Izmir Institute of Technology (Turkey), 2015.
[21] L. Konermann, H. Metwally, Q. Duez, and I. Peters, "Charging and supercharging of proteins for mass spectrometry: recent insights into the mechanisms of electrospray ionization," *Analyst,* 10.1039/C9AN01201J vol. 144, no. 21, pp. 6157-6171, 2019, doi: 10.1039/C9AN01201J.
[22] J. M. Rogers, "Peptide Folding and Binding Probed by Systematic Non-canonical Mutagenesis," *Frontiers in Molecular Biosciences,* vol. 7, p. 100, 2020.
[23] H. Ouldali *et al.*, "Electrical recognition of the twenty proteinogenic amino acids using an aerolysin nanopore," *Nature Biotechnology,* vol. 38, no. 2, pp. 176-181, 2020/02/01 2020, doi: 10.1038/s41587-019-0345-2.
[24] V. Apostolopoulos *et al.*, "A Global Review on Short Peptides: Frontiers and Perspectives," *Molecules,* vol. 26, no. 2, p. 430, 2021. [Online]. Available: https://www.mdpi.com/1420-3049/26/2/430.
[25] R. Aycock, D. Vogt, and N. T. Jui, "A practical and scalable system for heteroaryl amino acid synthesis," *Chemical Science,* vol. 8, no. 12, pp. 7998-8003, 2017.
[26] T. W. Bell, A. B. Khasanov, and M. G. B. Drew, "Role of Pyridine Hydrogen-Bonding Sites in Recognition of Basic Amino Acid Side Chains," *Journal of the American Chemical Society,* vol. 124, no. 47, pp. 14092-14103, 2002/11/01 2002, doi: 10.1021/ja0273694.
[27] K. O. Zhurov, L. Fornelli, M. D. Wodrich, Ü. A. Laskay, and Y. O. Tsybin, "Principles of electron capture and transfer dissociation mass spectrometry applied to peptide and protein structure analysis," *Chemical Society Reviews,* 10.1039/C3CS35477F vol. 42, no. 12, pp. 5014-5030, 2013, doi: 10.1039/C3CS35477F.
[28] I. Ranđelović *et al.*, "Improved in vivo anti-tumor and anti-metastatic effect of GnRH-III-daunorubicin analogs on colorectal and breast carcinoma bearing mice," vol. 20, no. 19, p. 4763, 2019.
[29] J. R. J. J. o. t. A. S. f. M. S. Yates III, "The Journey Is the Reward, a Taoist Proverb: John B. Fenn Award for Distinguished Contribution in Mass Spectrometry Lecture," vol. 31, no. 7, pp. 1327-1336, 2020.
[30] H. A. Aguilar, A. B. Iliuk, I.-H. Chen, and W. A. J. N. p. Tao, "Sequential phosphoproteomics and N-glycoproteomics of plasma-derived extracellular vesicles," vol. 15, no. 1, pp. 161-180, 2020.
[31] E. Largy *et al.*, "Mass Spectrometry of Nucleic Acid Noncovalent Complexes," 2021.
[32] S. Bakels, M.-P. Gaigeot, and A. M. J. C. r. Rijs, "Gas-Phase Infrared Spectroscopy of Neutral Peptides: Insights from the Far-IR and THz Domain," vol. 120, no. 7, pp. 3233-3260, 2020.
[33] S. Guan and B. J. J. J. o. t. A. S. f. M. S. Bythell, "Size Dependent Fragmentation Chemistry of Short Doubly Protonated Tryptic Peptides," vol. 32, no. 4, pp. 1020-1032, 2021.
[34] R. Goyal *et al.*, "Molecular hybridization combining tumor homing and penetrating peptide domains for cellular targeting," pp. 1-8, 2021.
[35] R. J. J. o. T. A. S. f. M. S. R. Julian, "The mechanism behind top-down UVPD experiments: making sense of apparent contradictions," vol. 28, no. 9, pp. 1823-1826, 2017.
[36] D. J. Ryan *et al.*, "MicroLESA: integrating autofluorescence microscopy, in situ micro-digestions, and liquid extraction surface analysis for high spatial resolution targeted proteomic studies," vol. 91, no. 12, pp. 7578-7585, 2019.
[37] M. Sharifi *et al.*, "Plasmonic gold nanoparticles: Optical manipulation, imaging, drug delivery and therapy," vol. 311, pp. 170-189, 2019.
[38] W. K. Nevala, J. T. Butterfield, S. L. Sutor, D. J. Knauer, and S. N. J. S. r. Markovic, "Antibody-targeted paclitaxel loaded nanoparticles for the treatment of CD20+ B-cell lymphoma," vol. 7, no. 1, pp. 1-9, 2017.

مجلة جامعة بابل للعلوم الصرفة والتطبيقية مجلة جامعة بابل للعلوم الصرفة والتطبيقية
ISSN: 2312-8135 | Print ISSN: 1992-0652
info@journalofbabylon.com | jub@itnet.uobabylon.edu.iq | www.journalofbabylon.com

[39] P. Liu *et al.*, "Receptor-mediated targeted drug delivery systems for treatment of inflammatory bowel disease: Opportunities and emerging strategies," *Acta Pharmaceutica Sinica B,* vol. 11, no. 9, pp. 2798-2818, 2021/09/01/ 2021, doi: https://doi.org/10.1016/j.apsb.2020.11.003.

[40] S. Y. van der Zanden, X. Qiao, and J. J. T. F. J. Neefjes, "New insights into the activities and toxicities of the old anticancer drug doxorubicin," 2020.

[41] E. I. Vrettos, G. Mező, and A. G. Tzakos, "On the design principles of peptide–drug conjugates for targeted drug delivery to the malignant tumor site," *Beilstein Journal of Organic Chemistry,* vol. 14, pp. 930-954, // 2018, doi: 10.3762/bjoc.14.80.

[42] C. Chen, D. Y. W. Ng, and T. Weil, "Polymer bioconjugates: Modern design concepts toward precision hybrid materials," *Progress in Polymer Science,* vol. 105, 2020.

[43] Y. Yamashita, T. Yasukawa, W.-J. Yoo, T. Kitanosono, and S. Kobayashi, "Catalytic enantioselective aldol reactions," *Chemical Society Reviews,* 10.1039/C7CS00824D vol. 47, no. 12, pp. 4388-4480, 2018, doi: 10.1039/C7CS00824D.

[44] M. P. van der Helm, B. Klemm, and R. Eelkema, "Organocatalysis in aqueous media," *Nature Reviews Chemistry,* vol. 3, no. 8, pp. 491-508, 2019/08/01 2019, doi: 10.1038/s41570-019-0116-0.

[45] A. R. Nanna *et al.*, "Generation and validation of structurally defined antibody–siRNA conjugates," *Nucleic Acids Research,* vol. 48, no. 10, pp. 5281-5293, 2020, doi: 10.1093/nar/gkaa286.

[46] A. K. Agrahari *et al.*, "Cu(I)-Catalyzed Click Chemistry in Glycoscience and Their Diverse Applications," (in eng), *Chem Rev,* vol. 121, no. 13, pp. 7638-7956, Jul 14 2021, doi: 10.1021/acs.chemrev.0c00920.

[47] K. El-Boubbou, "Magnetic Iron Oxide Nanoparticles As Drug Carriers: Clinical Relevance," *Nanomedicine,* vol. 13, no. 8, pp. 953-971, 2018/04/01 2018, doi: 10.2217/nnm-2017-0336.

[48] D. Kaushik and G. Bansal, "Four new degradation products of doxorubicin: An application of forced degradation study and hyphenated chromatographic techniques," *Journal of Pharmaceutical Analysis,* vol. 5, no. 5, pp. 285-295, 2015/10/01/ 2015, doi: https://doi.org/10.1016/j.jpha.2015.05.003.

[49] L. Feni *et al.*, "Kiss and Run: Promoting Effective and Targeted Cellular Uptake of a Drug Delivery Vehicle Composed of an Integrin-Targeting Diketopiperazine Peptidomimetic and a Cell-Penetrating Peptide," *Bioconjugate Chemistry,* vol. 30, no. 7, pp. 2011-2022, 2019/07/17 2019, doi: 10.1021/acs.bioconjchem.9b00292.

[50] S. Na, E. Paek, J. S. Choi, D. Kim, S. J. Lee, and J. Kwon, "Characterization of disulfide bonds by planned digestion and tandem mass spectrometry," (in eng), *Mol Biosyst,* vol. 11, no. 4, pp. 1156-64, Apr 2015, doi: 10.1039/c4mb00688g.

[51] J. M. Chalker, G. J. L. Bernardes, Y. A. Lin, and B. G. Davis, "Chemical Modification of Proteins at Cysteine: Opportunities in Chemistry and Biology," *Chemistry – An Asian Journal,* vol. 4, no. 5, pp. 630-640, 2009, doi: https://doi.org/10.1002/asia.200800427.

[52] S. B. Gunnoo and A. Madder, "Chemical Protein Modification through Cysteine," *ChemBioChem,* vol. 17, no. 7, pp. 529-553, 2016, doi: https://doi.org/10.1002/cbic.201500667.

[53] P. Ochtrop and C. P. R. Hackenberger, "Recent advances of thiol-selective bioconjugation reactions," *Current Opinion in Chemical Biology,* vol. 58, pp. 28-36, 2020/10/01/ 2020, doi: https://doi.org/10.1016/j.cbpa.2020.04.017.

[54] J. M. J. M. Ravasco, H. Faustino, A. Trindade, and P. M. P. Gois, "Bioconjugation with Maleimides: A Useful Tool for Chemical Biology," *Chemistry – A European Journal,* vol. 25, no. 1, pp. 43-59, 2019, doi: https://doi.org/10.1002/chem.201803174.

[55] A. Beck, L. Goetsch, C. Dumontet, and N. Corvaïa, "Strategies and challenges for the next generation of antibody–drug conjugates," *Nature Reviews Drug Discovery,* vol. 16, no. 5, pp. 315-337, 2017/05/01 2017, doi: 10.1038/nrd.2016.268.

[56] A. M. Newland, J. X. Li, L. E. Wasco, M. T. Aziz, and D. K. Lowe, "Brentuximab Vedotin: A CD30-Directed Antibody-Cytotoxic Drug Conjugate," *Pharmacotherapy: The Journal of Human*

ISSN: 2312-8135 | Print ISSN: 1992-0652
info@journalofbabylon.com | jub@itnet.uobabylon.edu.iq | www.journalofbabylon.com

Pharmacology and Drug Therapy, vol. 33, no. 1, pp. 93-104, 2013, doi: https://doi.org/10.1002/phar.1170.

[57] S. J. Walsh *et al.*, "Site-selective modification strategies in antibody–drug conjugates," *Chemical Society Reviews,* vol. 50, no. 2, pp. 1305-1353, 2021.

[58] P. Khongorzul, C. J. Ling, F. U. Khan, A. U. Ihsan, and J. Zhang, "Antibody–Drug Conjugates: A Comprehensive Review," *Molecular Cancer Research,* vol. 18, no. 1, pp. 3-19, 2020, doi: 10.1158/1541-7786.Mcr-19-0582.

[59] H. Amani *et al.*, "Controlling Cell Behavior through the Design of Biomaterial Surfaces: A Focus on Surface Modification Techniques," *Advanced Materials Interfaces,* vol. 6, no. 13, p. 1900572, 2019, doi: https://doi.org/10.1002/admi.201900572.

[60] W. Zhang *et al.*, "Nanoscale bioconjugates: A review of the structural attributes of drug-loaded nanocarrier conjugates for selective cancer therapy," *Heliyon,* vol. 8, no. 6, p. e09577, 2022/06/01/ 2022, doi: https://doi.org/10.1016/j.heliyon.2022.e09577.

[61] M. Bilal, M. Asgher, H. Cheng, Y. Yan, and H. M. J. C. r. i. b. Iqbal, "Multi-point enzyme immobilization, surface chemistry, and novel platforms: a paradigm shift in biocatalyst design," vol. 39, no. 2, pp. 202-219, 2019.

[62] S. I. Kaya, S. Kurbanoglu, E. Yavuz, S. D. Mustafov, F. Sen, and S. A. J. S. R. Ozkan, "Carbon-based ruthenium nanomaterial-based electroanalytical sensors for the detection of anticancer drug Idarubicin," vol. 10, no. 1, pp. 1-12, 2020.

[63] N. Ahmad *et al.*, "Daunorubicin oral bioavailability enhancement by surface coated natural biodegradable macromolecule chitosan based polymeric nanoparticles," vol. 128, pp. 825-838, 2019.

[64] Y. Pashaei, M. Mehrabi, and M. J. T. T. i. A. C. Shekarchi, "A review on various analytical methods for determination of anthracyclines and their metabolites as anti–cancer chemotherapy drugs in different matrices over the last four decades," p. 115991, 2020.

[65] F. Guo *et al.*, "Menstrual blood derived mesenchymal stem cells combined with Bushen Tiaochong recipe improved chemotherapy-induced premature ovarian failure in mice by inhibiting GADD45b expression in the cell cycle pathway," vol. 17, no. 1, pp. 1-12, 2019.

[66] R. X. Zhang *et al.*, "Sample extraction and simultaneous chromatographic quantitation of doxorubicin and mitomycin C following drug combination delivery in nanoparticles to tumor-bearing mice," no. 128, 2017.

[67] Y.-Q. Hu *et al.*, "Quinoline hybrids and their antiplasmodial and antimalarial activities," vol. 139, pp. 22-47, 2017.

[68] S. Wittrisch, N. Klöting, K. Mörl, R. Chakaroun, M. Blüher, and A. G. J. M. m. Beck-Sickinger, "NPY1R-targeted peptide-mediated delivery of a dual PPARα/γ agonist to adipocytes enhances adipogenesis and prevents diabetes progression," vol. 31, pp. 163-180, 2020.

[69] L. Ma, C. Wang, Z. He, B. Cheng, L. Zheng, and K. J. C. m. c. Huang, "Peptide-drug conjugate: a novel drug design approach," vol. 24, no. 31, pp. 3373-3396, 2017.

[70] D. J. Worm, S. Els-Heindl, and A. G. J. P. S. Beck-Sickinger, "Targeting of peptide-binding receptors on cancer cells with peptide-drug conjugates," vol. 112, no. 3, p. e24171, 2020.

مجلة جامعة بابل للعلوم الصرفة والتطبيقية مجلة جامعة بابل للعلوم الصرفة والتطبيقية
ISSN: 2312-8135 | Print ISSN: 1992-0652
info@journalofbabylon.com | jub@itnet.uobabylon.edu.iq | www.journalofbabylon.com

ISSN: 2312-8135 | Print ISSN: 1992-0652
info@journalofbabylon.com | jub@itnet.uobabylon.edu.iq | www.journalofbabylon.com

مجلة جامعة بابل للعلوم الصرفة والتطبيقية مجلة جامعة بابل للعلوم الصرفة والتطبيقية

الخلاصة :

شهدت خلال العقود الأخيرة تحولًا نوعيًا في الأساليب والمنهجيات المعتمدة لتوصيف الجينات والبروتينات والمُعَبِّرات الجينية (الترانسكريبتومات) والمواد الأيضية. وقد جاء هذا التحول نتيجةً للتطور الكبير في منصّات التحليل عالية الإنتاجية، التي أتاحت إمكانية فحص أعداد واسعة من الجزيئات الحيوية بصورة متزامنة، وبمستويات متقدمة من الكفاءة والدقة والقدرة التمييزية. وفي هذا الإطار، برزت مطيافية الكتلة كإحدى الركائز الأساسية في هذا المجال، نظرًا لما تمتاز به من حساسية فائقة وانتقائية عالية وقدرات كمية دقيقة في الكشف عن الأيونات. وقد مكّن هذا التطور من إعادة بناء دقيقة للمعمار الجزيئي والكيميائي للأنظمة البيولوجية المعقدة، الأمر الذي جعل مطيافية الكتلة أداة لا غنى عنها في البحوث البيولوجية والطبية الحديثة، وأسهم في دعم التقدم الحاصل في بيولوجيا الأنظمة، واكتشاف الأدوية، والطب الدقيق.