Democratic and Popular Republic of Algeria
Ministry of Higher Education and Scientific Research
Echahid Cheikh Larbi-Tébessi University
Faculty of Exact Sciences and Nature and Life Sciences
Department of Matter Sciences

MASTER'S THESIS

Field: Matter Sciences

Discipline: Chemistry

Option: Chemistry of natural products

Synthesis and biological activities of α-sulfamido/α-amino phosphonate derivatives of N-heterocycle

Theme:

Presented by:

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Academic Year: 2023/2024

Acknowledgment

First and foremost, I would like to convey my heartfelt appreciation to my supervisor, ***Dr. Zinelaabidine CHERAIET****. I am honored that I worked under his guidance and help, which improved my academic experience in ways that I cannot express in just one page. I am grateful for his invaluable advice, trusted monitoring, and endless patience. Working and studying under his guidance was a great pleasure and honor.*

Second, I highly appreciate the efforts of the broad examiners, ***Pr. Louisa BOUDIBA*** *and* ***Dr. Meriem BOUMEDJOUT****, and I thank them for devoting their time to read and evaluate my dissertation I extend my gratitude to them for being great and competent teachers throughout my academic experience.*

I must further thank ***Pr. Sameh Boudiba*** *for her great help, kindness, and moral support.*

I would also like to acknowledge the ***PhD****. student* ***Khaled AGGOUN*** *for his help.*

I would like to thank the student ***Maroua SAIGHI*** *for her endless support and help.*

Finally, I would give my deepest appreciation to ***Nariman ALIOUA****, the friend who continuously supported me, giving encouragement and delight, and gave me the strength to pursue my academic ambitions with a newfound drive.*

Dedication

All praise for his Mighty, Allah alone for his guidance and blessings. This research is dedicated to my dear parents, who encouraged and supported me through thick and thin.

To my dear sisters and brothers who showed me love, motivation, and empathy in moments of difficulty and stress.

To Meriem,my sister, my best friend and greatest supporter. Your patience, understanding, and encouragement have been invaluable throughout this Journey .

This work is dedicated to all my friends and colleagues who helped me prayed for me and believed in me. Thank you from the bottom of my heart.

Abstract

In this study, we describe a simple *one-pot* synthesis of novel α-aminophosphonates *via* the *Kabachnik-Fields* reaction bearing *N*-Sulfamoyl aminopyridine **3a-7a** and **3b-4b** and 5-(ethylthio)-1, 3, 4-thiadiazol-2-yl) α-aminophosphonate derivatives. The synthesized molecules were rationally designed and synthesized according to the principle of synergic bioactive substructures. The synthesized compounds were obtained with a moderate yield without chromatographic work-up and were characterized by the usual spectroscopic methods: ^{1}H NMR, ^{13}C NMR, HSQC, and HMBC.

Some compounds were evaluated for *in vitro* antioxidant (DPPH and SPF) and anti-inflammatory activity in order to demonstrate the potential spectrum of the obtained α-aminophosphonates.

Keywords: sulfamide, 1, 3, 4-thiadiazol, α-aminophosphonates, *Kabachnik-Fields* reaction, antioxidant, and anti-inflammatory.

RESUME

Dans cette étude, nous décrivons une synthèse *one-pot* simple de nouveaux α-aminophosphonates *via* la réaction de *Kabachnik-Fields* portant de la *N*-sulfamoyl aminopyridine **3a-7a** et **3b-4b** et du 5-(éthylthio)-1, 3, 4-thiadiazol- Dérivés 2-yl) α-aminophosphonates. Les molécules synthétisées ont été conçues et synthétisées de manière rationnelle en se basant sur le principe de synergie des molecules bioactives. Les composés synthétisés ont été obtenus avec des rendements modérés sans traitement chromatographique et ont été caractérisés par les méthodes spectroscopiques usuelles : RMN ^{1}H, RMN ^{13}C, HSQC et HMBC.

Certains composés ont été évalués pour leur activité antioxydante (DPPH et SPF) et anti-inflammatoire *in vitro* afin de démontrer le potentiel spectre d'activites des α-aminophosphonates obtenus.

Mots clés: sulfamide, 1, 3, 4-thiadiazole, α-aminophosphonates, réaction de *Kabachnik-Fields*, antioxydant et anti-inflammatoire.

الملخص

في هذه الدراسة، قمنا بوصف توليف بسيط من وعاء واحد لـ α-aminophosphonates الجديدة عبر تفاعل *Kabachnik-Fields*الذي يحمل *N*-سلفامويل أمينوبيريدين a3-a7 وb3-b4 و5-(إيثيلثيو)-1، 3، 4-ثياديازول-2-ييل) ألفا-أمينوفوسفونات. تم تصميم وتوليف الجزيئات المركبة وفقًا لمبدأ البنى النشطة بيولوجيًا. تم الحصول على المركبات المحضرة بإنتاجية معتدلة بدون معالجة كروماتوغرافية وتم التاكد من بنيتها بالطرق الطيفية المعتادة: ^{1}H NMR, ^{13}C NMR, HSQC، وHMBC.

تم تقييم بعض المركبات لمضادات الأكسدة في المختبر (DPPHوSPF) والنشاط المضاد للالتهابات من أجل إظهار الطيف المحتمل لألفا-أمينوفوسفونات التي تم الحصول عليها.

الكلمات المفتاحية: سلفوناميد، 1، 3، 4-ثياديازول، ألفا-أمينوفوسفونات، تفاعل *Kabachnik-Fields* ، مضادات للأكسدة، ومضادات للالتهابات.

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List of Symbols

%	Percentage
°C	Degree Celsius
eq	Number of gram equivalents
δ	Chemical shifts
J	Coupling constant
pKa	Negative base-10 logarithm of the acid dissociation constant (K_a) of a solution

List of Abbreviations

Abs	Absorbance
BHA	Butylhydroxyanisole
Bht	Butylhydroxytolune
BSA	Bovine serum albumin
Boc	tert-butyloxycarbonyl
CDCl3	Deuterated chloroform
CI50	Concentration at inhibition of 50%
CNS	Central nervous system
CSI	Chlorosulfonyl isocyanate
DCM	Dichloromethane
DEAD	Diethyl azodicarboxylate
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
DPPH	2,2-Diphenyl-1-picrylhydrazyl
EDG	Electron-donating groups
EtOH	Ethanol
EWG	Electron-withdrawing groups
g	Gram
h	hour
HCl	Hydrochloric acid
HIV	Human Immunodeficiency Virus

HMBC	Heteronuclear multiple-bond correlation spectroscopy
HSQC	Heteronuclear single-quantum correlation spectroscopy
Hz	Hertz
m	Mass
M	molarity
MCRs	Multi-component reactions
MED	Minimum erythematic dose
MeOH	Methanol
min	Minute(s)
mL	mili liter
mol	Mole
mmol	milli mole
Mp	Melting point
NaOH	Sodium hydroxide
nm	Nanometer
NMR	Nuclear magnetic resonance
P2A	Pridin-2amine
P3A	Pridin-3-amine
PPh_3	Triphenylphosphine
Ppm	Parts per million
R	Radical
R_f	Retardation factor
RT	Room temperature

SPF	Sun Protection Factor
TLC	Thin-layer chromatography
T	Temperature
TEA	Triethylamine
UA	Ursolic acid
UV	Ultraviolet
UV-Vis	Ultraviolet-Visible
µl	Microlitre

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Introduction

Introduction

Currently, applications of organic chemistry are present everywhere in our daily lives: medicines, cosmetics, household products, materials, fuels…etc. Consequently, in recent years, scientific research has focused on discovering new flexible and adaptable synthesis methodologies to acquire new biologically active molecules. In this context, developing a synthesis pathway for bioactive substances based on heterocycles is one of the most exciting challenges for research chemists. This challenge requires a lot of imagination, but also extensive knowledge and long experience in the field.

Heterocycles are a class of compounds in which one or more carbon atoms are replaced by a heteroatom such as oxygen, nitrogen, phosphorus, sulfur, etc. However, the most common ones contain nitrogen, sulfur, and oxygen. They are very interesting both synthetically and biologically due to their potential applications in various fields, which is why their synthesis has become a very important and constantly relevant topic. There are several types of heterocycles: sugars, purine bases, pyridine bases, etc.

Various pharmacological studies have been conducted on heterocyclic compounds with interesting biological activities, leading to the development of new agents to treat various infections[1-2].Compounds bearing the pyrimidine motif are well-known in synthetic biomolecules, and their synthesis is a field of interest in organic synthesis due to their broad range of applications in therapeutic chemistry.

On the other hand, the development of novel bioactive molecules is crucial to address the growing issue of antibiotic resistance and to create effective treatments for bacterial, viral, and fungal infections. α-Aminophosphonic acid derivatives are known for their biological activity due to their structural similarity to natural α-amino acids. Notably, various α-aminophosphonate derivatives have been utilized in diverse fields of medicinal chemistry.[3] Incorporating a

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[1] Kočí, J., Klimešová, V., Waisser, K., Kaustová, J., Dahse, H. M., & Möllmann, U. **(2002)**. Heterocyclic benzazole derivatives with antimycobacterial in vitro activity. *Bioorganic & medicinal chemistry letters*, *12*(22), 3275-3278.

[2] Rana, A., Siddiqui, N., & Khan, S. A. **(2007)**. Benzothiazoles: A New Profile of Biological Activities. *Indian Journal of Pharmaceutical Sciences*, *69*(1).

[3] (a)Sieńczyk, M., Winiarski, Ł., Kasperkiewicz, P., Psurski, M., Wietrzyk, J., & Oleksyszyn, J. **(2011)**. Simple phosphonic inhibitors of human neutrophil elastase. *Bioorganic & medicinal chemistry letters*, *21*(5), 1310-1314. (b) Vassiliou, S., Weglarz-Tomczak, E., Berlicki, Ł., Pawełczak, M., Nocek, B., Mulligan, R., ... & Mucha, A. **(2014)**. Structure-guided, single-point modifications in the phosphinic dipeptide structure yield highly potent and selective inhibitors of neutral aminopeptidases. *Journal of medicinal chemistry*, *57*(19), 8140-8151. (c) Arya, T., Reddi, R., Kishor, C., Ganji, R. J., Bhukya, S., Gumpena, R., ... & Addlagatta, A. **(2015)**. Identification of the molecular basis

phosphorus-containing group with a heterocyclic fragment has produced heterocyclic phosphonates with significant chemical and biological properties[4] **(Figure 1)**. These new hybrid biomolecules are subsequently tested *in vitro* and *in vivo* to evaluate their efficacy, selectivity, and toxicity.

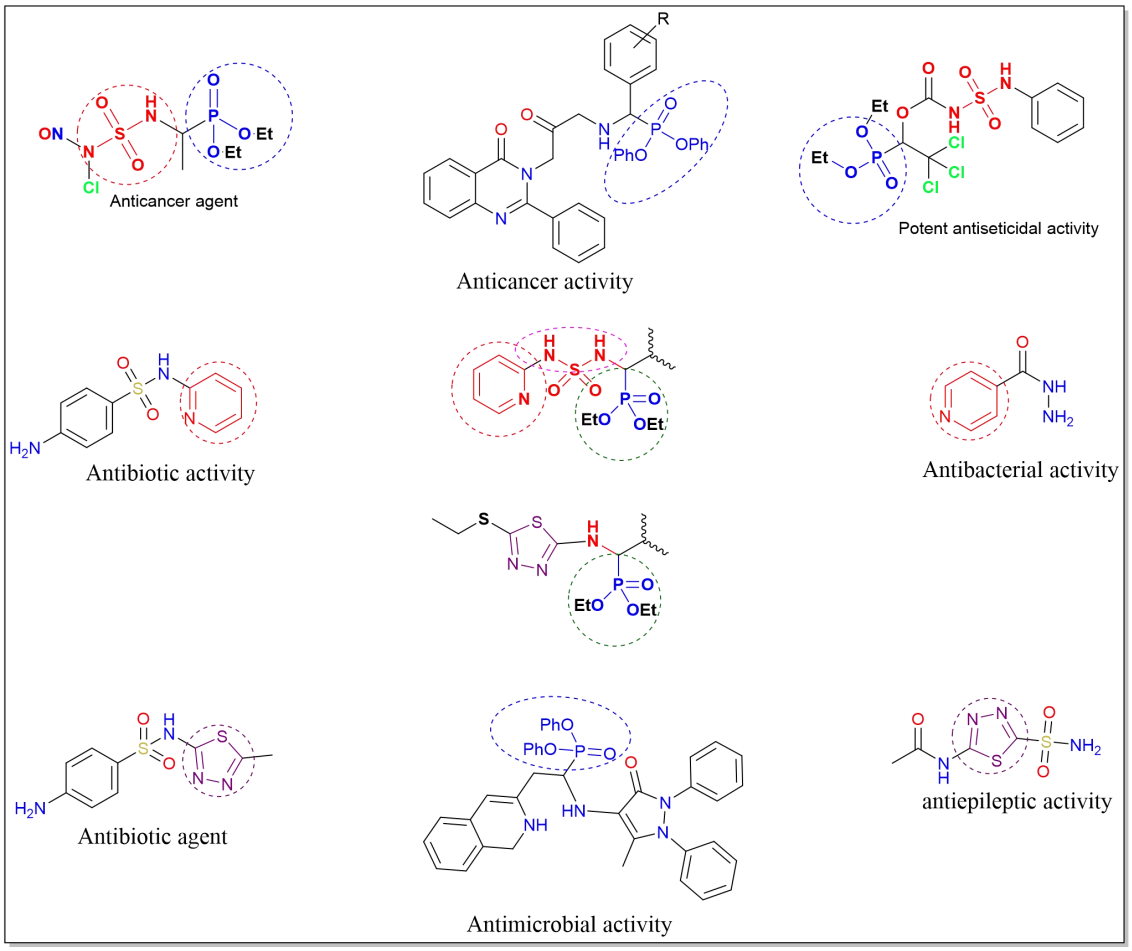

Figure 1: Design of α-aminophosphonates bearing a pyridine and thiadiazole heterocycle.

of inhibitor selectivity between the human and streptococcal type I methionine aminopeptidases. *Journal of medicinal chemistry*, *58*(5), 2350-2357.

[4] (a)Bouchareb, F., & Berredjem, M. **(2022)**. Recent progress in the synthesis of phosphoramidate and phosphonamide derivatives: A review. *Phosphorus, Sulfur, and Silicon and the Related Elements*, *197*(7), 711-731.(b) Reddy, G. S., Rao, K. U. M., Sundar, C. S., Sudha, S. S., Haritha, B., Swapna, S., & Reddy, C. S. **(2014)**. Neat synthesis and antioxidant activity of α-aminophosphonates. *Arabian Journal of Chemistry*, *7*(5), 833-838. (c) Awad, M. K., Abdel-Aal, M. F., Atlam, F. M., & Hekal, H. A. **(2019)**. Molecular docking, molecular modeling, vibrational and biological studies of some new heterocyclic α-aminophosphonates. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *206*, 78-88. (d) Elsherbiny, D. A., Abdelgawad, A. M., El-Naggar, M. E., El-Sherbiny, R. A., El-Rafie, M. H., & El-Sayed, I. E. T. **(2020)**. Synthesis, antimicrobial activity, and sustainable release of novel α-aminophosphonate derivatives loaded carrageenan cryogel. *International Journal of Biological Macromolecules*, *163*, 96-107.

Introduction

The importance of α-aminophosphonates in the biological and chemical fields has spurred the development of new derivatives. Developing new drugs by combining two pharmacophores involves synthesizing chemical compounds that merge the active characteristics of two distinct molecular groups, or pharmacophores. This strategy aims to harness the beneficial pharmacological properties of each pharmacophore, resulting in a more effective drug with potentially reduced side effects.

Therefore, our research is based on the synthesis of biomolecules, we anticipate that incorporating phosphonate and pyridine, and/or thiadiazol into the same structure using α-aminophosphonate motif could increase the spectrum of this motif, such as its antimicrobial and anti-inflammatory activities. We have successfully synthesized a new series of hybrid analogs of α-aminophosphonates bearing an *N*-Sulfamoylpyridine and 1,3,4-thiadiazol heterocycle. The newly synthesized derivatives were then evaluated for their antioxidant and anti-inflammatory activity.

Our work will be presented as follows: In the first chapter, we will provide a literature review on the chemical and biological applications of phosphorus compounds. The synthesis and structural study of α-sulfamidophosphonate-pyridine and α-aminophosphonate derivatives of thiadiazole and their antioxidant activity will be described in the second chapter. In the third chapter, we will present the experimental protocols.

Chapter I: Bibliographic Review

I. General information on organophosphorus compounds

An organophosphorus compound is a type of organic compound with at least one phosphorus atom bonded directly to a carbon. Organophosphorus compounds are classified into different categories based on the degree of oxidation of phosphorus and the nature of substituents, which may include the presence of an oxygen atom or other chalcogen[5] **(Figure I-1).**

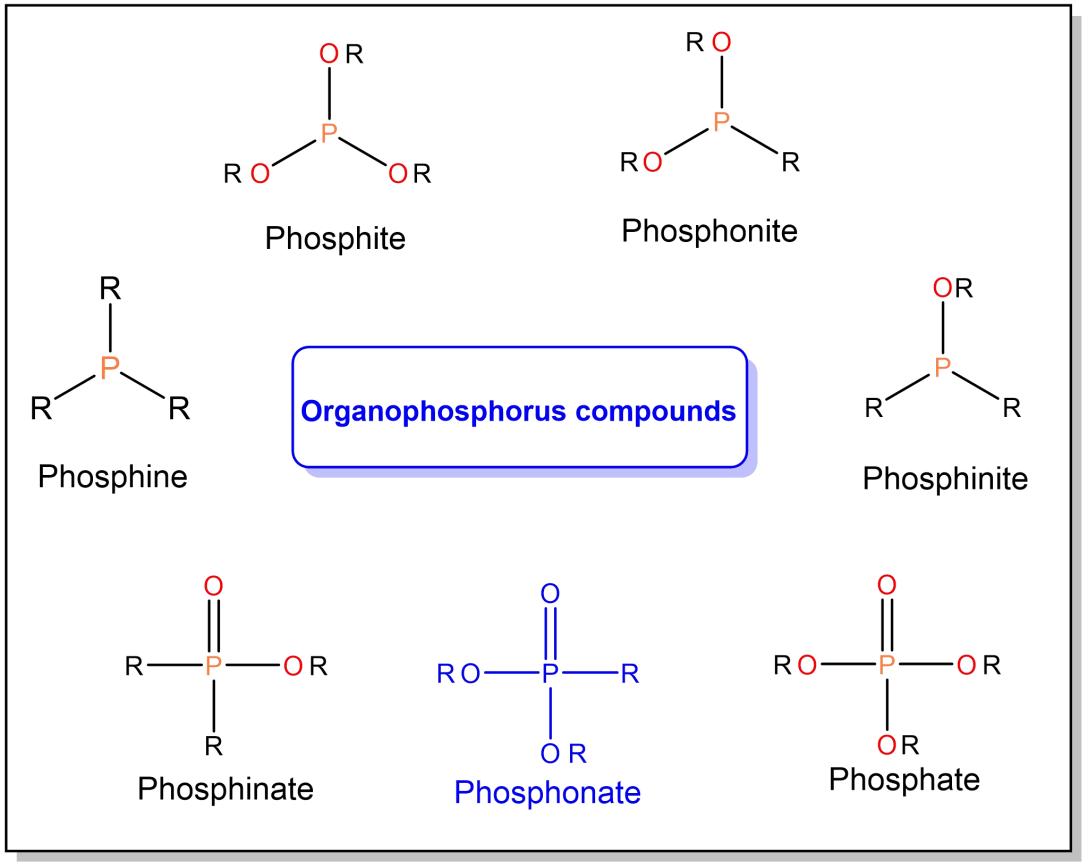

Figure I-1: The principal classes of organophosphorus.

I.1. Phosphorus Generalities (P)

Phosphorus is one of the widespread elements on earth: it constitutes about 0.04% of the total number of atoms, it has the symbol **P** with an atomic number of 15, it belongs to the chemical family of non-Phosphorus can form bonds with a variable number of atoms (coordination

[5] Troev, K. D. **(2006)**. *Chemistry and application of H-phosphonates*. Elsevier.

number) ranging from 1 to 6. In addition, it can have different valences 3 or 5, and empty orbitals that easily accept electrons[6].

I.1.1. Phosphonates

Phosphonates are compounds that have a phosphonate group **-C-PO(OR)$_2$** attached to the molecule through a **P-C** bond. These act as non-hydrolyzable phosphate mimetics in a variety of biomedical applications.

❖ ***General properties***

In solution, these compounds exist in an equilibrium of two tautomeric forms, phosphonate and phosphite form.

Phosphonates are characterized by the presence of **-C-PO(OR)$_2$** groups and have the following properties:

- Highly soluble in water, nonvolatile, and poorly soluble in organic solvents.
- A threshold effect on salt crystal development.
- Ability to bind metal ions.
- Less toxic to the environment.
- Biodegradable in soil: release of phosphates.
- Very stable product even under harsh chemical conditions It is a very biologically active compound.
- Phosphonates are effective chelating agents that bind strongly to divalent and trivalent metal ions.
- The stability of metal complexes increases as the number of phosphonate groups increases[7].

❖ ***Phosphonate derivatives***

Phosphonates are generally subdivided into six main classes:

➢ ***α-aminophosphonates***

α-Aminophosphonic acids are bioisosteres of α-amino acids, replacing carboxylic groups with tetrahedral phosphonic acid. Because of their diverse biological activity, they are valuable

[6] Kukhar, V. P., & Hudson, H. R. **(2000)**. *Aminophosphonic and aminophosphinic acids: Chemistry and biological activity*. John Wiley & Sons.
[7] Mucha, A., Kafarski, P., & Berlicki, Ł. **(2011)**. Remarkable potential of the α-aminophosphonate/phosphinate structural motif in medicinal chemistry. *Journal of Medicinal Chemistry*, *54*(17), 5955-5980.

targets in biochemistry[6-8], pharmaceutical chemistry[9-10-11-12], and pesticide chemistry[13-14-15] . Since 1952, about 1,500 papers have been published on the production and applications of α-aminophosphonic acid derivatives **(Figure I-2)**. The *Kabachnik-Fields* condensation and *aza-Pudovik* reaction are the most frequently used synthetic methods to produce α-aminophosphonates.

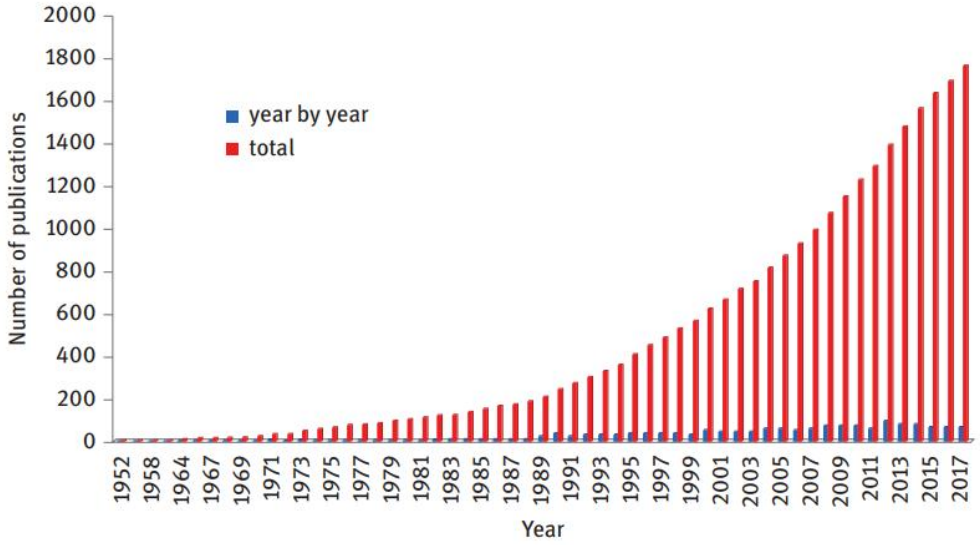

Figure I-2: The number of publications on α-aminophosphonic acid derivatives (1952–2017).

The α-aminophosphonate compounds constitute a specific family of widely distributed phosphonates, they participate in many important biological processes, the presence of the

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[8] Mucha, A., Kafarski, P., & Berlicki, Ł. **(2011)**. Remarkable potential of the α-aminophosphonate/phosphinate structural motif in medicinal chemistry. *Journal of Medicinal Chemistry*, *54*(17), 5955-5980.

[9] Allen, J. G., Atherton, F. R., Hall, M. J., Hassall, C. H., Holmes, S. W., Lambert, R. W., ... & Ringrose, P. S. **(1978)**. Phosphonopeptides, a new class of synthetic antibacterial agents. *Nature*, *272*(5648), 56-58.

[10] Lavielle, G., Hautefaye, P., Schaeffer, C., Boutin, J. A., Cudennec, C. A., & Pierre, A. **(1991)**. New. alpha.-amino phosphonic acid derivatives of vinblastine: chemistry and antitumor activity. *Journal of medicinal chemistry*, *34*(7), 1998-2003.

[11] Grembecka, J., Mucha, A., Cierpicki, T., & Kafarski, P. **(2003)**. The most potent organophosphorus inhibitors of leucine aminopeptidase. Structure-based design, chemistry, and activity. *Journal of medicinal chemistry*, *46*(13), 2641-2655.

[12] Sienczyk, M., & Oleksyszyn, J. **(2009)**. Irreversible inhibition of serine proteases-design and in vivo activity of diaryl α-Aminophosphonate derivatives. *Current medicinal chemistry*, *16*(13), 1673-1687.

[13] Forlani, G., Berlicki, Ł., Duò, M., Dziędzioła, G., Giberti, S., Bertazzini, M., & Kafarski, P. **(2013)**. Synthesis and evaluation of effective inhibitors of plant δ1-pyrroline-5-carboxylate reductase. *Journal of agricultural and food chemistry*, *61*(28), 6792-6798.

[14] Forlani, G., Occhipinti, A., Berlicki, Ł., Dziedzioła, G., Wieczorek, A., & Kafarski, P. **(2008)**. Tailoring the structure of aminobisphosphonates to target plant P5C reductase. *Journal of agricultural and food chemistry*, *56*(9), 3193-3199.

[15] Long, N., Cai, X. J., Song, B. A., Yang, S., Chen, Z., Bhadury, P. S., ... & Xue, W. **(2008)**. Synthesis and antiviral activities of cyanoacrylate derivatives containing an α-aminophosphonate moiety. *Journal of agricultural and food chemistry*, *56*(13), 5242-5246.

nitrogen atom in the α-aminophosphonate increases their chelating power against metals, and the stability of the formed complexes[16].

[16] Kafarski, P., & Lejczak, B. **(2001)**. Aminophosphonic acids of potential medical importance. *Current Medicinal Chemistry-Anti-Cancer Agents*, *1*(3), 301-312.

➢ *Bisphosphonates*

Bisphosphonates are organophosphate derivatives characterized by a **P-C-P** structural backbone; their general structure is presented as follows **(Figure I-3).**

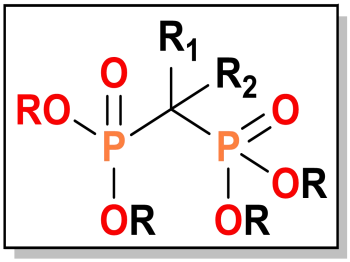


Figure I-3: The General structure of bisphosphonates.

Bisphosphonates are highly soluble in water, while phosphonic acids are only slightly soluble. They are non-volatile and have low solubility in organic solvents.

➢ *Nucleoside phosphonates*

Nucleoside phosphonates have molecular structures similar to natural nucleosides and nucleic acids **(Figure I-4)**.

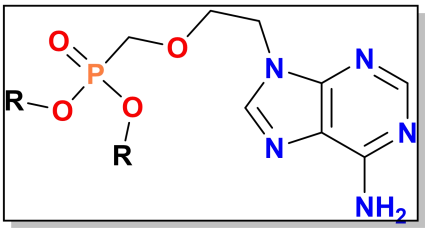


Figure I-4: The structure of nucleoside phosphonates.

➢ *α -hydroxyphosphonates*

In this family of organophosphonates, the α-carbon atom bonded to phosphorus carries a hydroxyl **(-OH)** moiety **(Figure I-5)**.

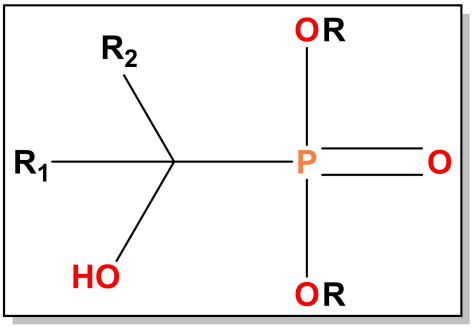


Figure I-5: The structure of α –hydroxyphosphonates.

➢ *Alkyl-phosphonates and aryl-phosphonates*

These are organophosphate derivatives where the phosphonate group is bound to a radical alkyl or aryl **(Figure I-6)**.

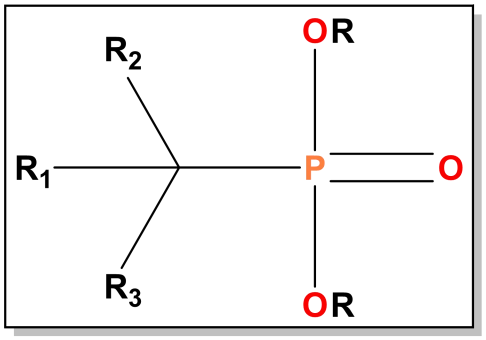


Figure I-6: The structure of alkyl-phosphonates and aryl-phosphonates.

➢ *Polyphosphonates*

This category of organophosphate macromolecules is characterized by the repetition of one or several types of monomer patterns that carry one or more groupings **(Figure I-7)**.

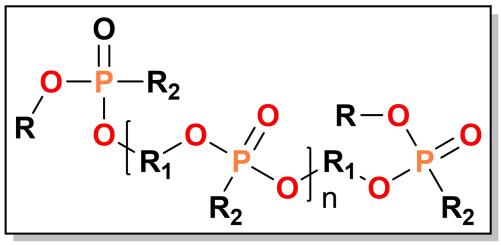


Figure I-7: The general structure of polyphosphonates.

I.2. Synthesis of α-Aminophosphonates

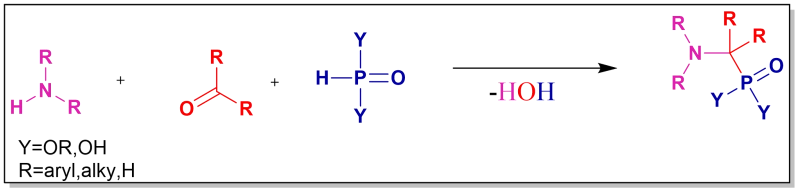
Due to their many actions, α-Aminophosphonates are a promising target for organic and medicinal chemistry.

Over the past decade, new and more efficient methods for synthesizing α-Aminophosphonates have emerged**.** The standard procedure is synthesizing imines in situ and producing an **N-C-P** bond by phosphite nucleophiles and imine electrophiles in a phospha-Mannich reaction.

There are many techniques for synthesizing phosphonates, including the *Aza-Pudovic* reaction, *Kabachnik-Fields*[17-18-19], *Pudovik* reactions[20], *Mitsunobu* reaction, and other recent methods. However, these catalysts are costly and require hard conditions. The catalyst-free synthesis of α-aminophosphonates is limited[21].

I.2.1. Kabachnik–Field reaction

In 1952, *Martin Izrailevich Kabachnik*[18]and *Ellis K. Fields*[47] discovered a one-pot multi-component process to synthesize α-aminophosphonate from an amine, carbonyl molecule, and dialkyl phosphite. The multicomponent reaction was named after them, the *Kabachnik-Field* reaction. This reaction has many combinatorial chemistry applications[22]**(Scheme I-1)**.



Scheme I-1: The general scheme for the Kabachnik–Fields reaction.

In general, the *Kabachnik-Fields* reaction has two different directions **(Scheme I-2)**. One occurs when the carbonyl compound and the main amine react, forming an imine (Schiff base)

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[17] Kabachnik, M. I., & MEDVED, T. I. **(1953)**. Certain derivatives of aminomethylphosphinic acid. *Izvestiia Akademii nauk SSSR, Otdelenie khimicheskikh nauk*, *6*, 1126-1128.

[18] Kabachnik, M. I., & Medved, T. Y. **(1952)**. New synthesis of aminophosphonic acids. In *Dokl. Akad. Nauk SSSR* (Vol. 83, pp. 689-692).

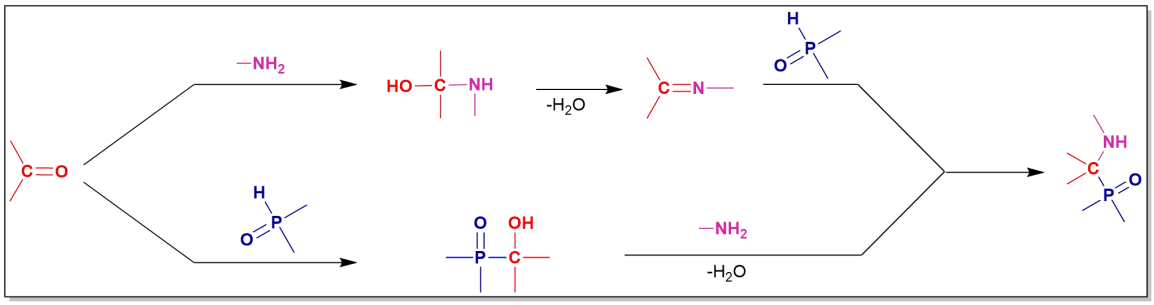
[19] Fields, E. K. **(1952)**. The synthesis of esters of substituted amino phosphonic acids1a. *Journal of the American Chemical Society*, *74*(6), 1528-1531.

[20] Pudovik, A. N. **(1952)**. New method of synthesis of esters of phosphonocarboxylic acids and their derivatives. In *Doklady Akademii Nauk* (Vol. 85, pp. 349-351).

[21] Ranu, B. C., & Hajra, A. **(2002)**. A simple and green procedure for the synthesis of α-aminophosphonate by a one-pot three-component condensation of carbonyl compound, amine and diethyl phosphite without solvent and catalyst. *Green Chemistry*, *4*(6), 551-554.

[22] LaPointe, A. M. **(1999)**. Parallel synthesis of aminomethylphosphine ligands. *Journal of Combinatorial Chemistry*, *1*(1), 101-104.

intermediate, and then adding the **P**-reagent to the **C=N** unit. The alternative process involves adding dialkyl phosphites to the carbonyl group of the oxo component, resulting in an α-hydroxyphosphonate that is then substituted by the amine to yield the α-aminophosphonate. *Kinetic* studies led to the conclusion that the mechanism is determined by the reactants' nature[23-24-25].



Scheme I-2: Two possible mechanistic pathways for the *Kabachnik–Fields* reaction.

Recently, dehydrating or water-tolerant catalysts, such as rare earth metal triflates, have been used in *Kabachnik-Field*s reactions. The biological significance of α-aminophosphonates has led to research into numerous catalytic systems for this reaction, including tetramethylguanidine, dodecatungestophosphoric acid, silica-gel, magnesium perchlorate, $TiCl_4$, $FeCl_3$, $YbCl_3$, [bmim]Cl-$AlCl_3$ ionic liquid, montmorillonite K10, bismuth(III) chloride, gallium triiodide, $LiClO_4$, CAN, samarium diiodide, CF_3COOH, $TaCl_5$-SiO_2, indium(III) chloride and zirconium(IV) compounds, $Bi(NO_3)_3.5H_2O$, Amberlite IR-120 (acidic) etc[26] **(Figure I-8)**.

[23] Rafael'A, C., & Galkin, V. I. **(1998)**. The Kabachnik–Fields reaction: synthetic potential and the problem of the mechanism. *Russian Chemical Reviews*, *67*(10), 857-882.
[24] Keglevich, G., & Bálint, E. **(2012)**. The Kabachnik–Fields reaction: Mechanism and synthetic use. *Molecules*, *17*(11), 12821-12835.
[25] Matveeva, E. D., & Zefirov, N. S. **(2008, June)**. On the mechanism of the Kabachnik-Fields reaction: Does a mechanism of nucleophilic amination of α-hydroxyphosphonates exist?. In *Doklady Chemistry* (Vol. 420, pp. 137-140). SP MAIK Nauka/Interperiodica.
[26] Varga, P. R., & Keglevich, G. **(2021)**. Synthesis of α-aminophosphonates and related derivatives; The last decade of the Kabachnik–Fields reaction. *Molecules*, *26*(9), 2511.

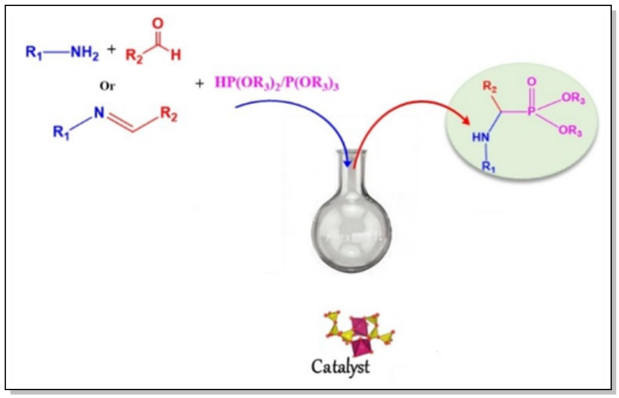
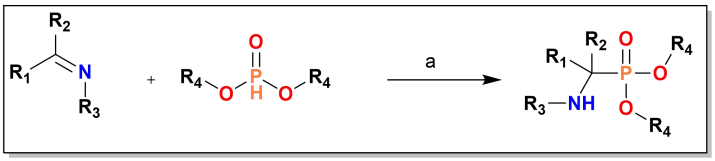


Figure I-8: The synthesis of aminophosphonates by the catalyst method.

I.2.2. Aza-Pudovik reaction

α-Aminophosphonates are produced by adding phosphites to imines **(Scheme I-3)**, also known as the hydrophosphynylation reaction. In this reaction, phosphonates were employed as P-nucleophiles. The phosphite's reactivity is caused by the phosphonate-phosphite tautomerism, which occurs with the phosphite form as the active nucleophilic species and the phosphonate tautomer as the nearly exclusively preferred but non-nucleophilic. The *Pudovik* reaction refers to the hydrophosphynylation of imines. The production of αaminophosphonates consists of two steps: generation of imine and hydrophosphynylation. This reaction is capable of being catalyzed by either basic or acid[27].

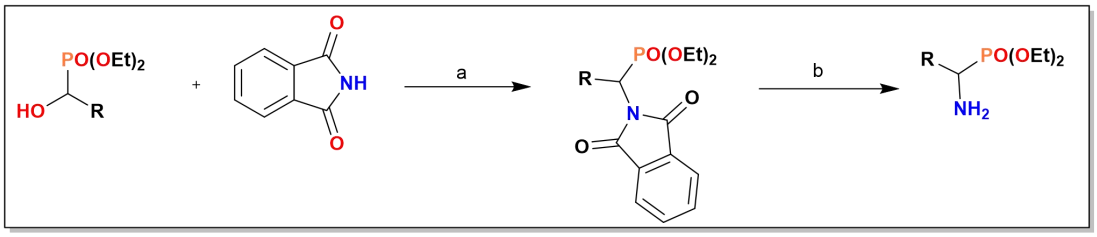


Scheme I-3: Reagents and conditions: (a) acid or base.

I.2.3. Mitsunobu reaction

The *Mitsunobu* reaction can also be used to produce α-aminophosphonates[28].In this technique, the hydroxyphosphonate is treated to the *Mitsunobu* reaction with an amine, such as phthalimide. Finally, α-aminophosphonates are produced by deprotecting the phthalimide group **(Scheme I-4)**.

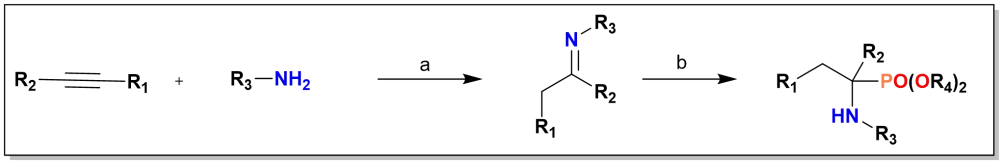
[27] Varga, P. R., & Keglevich, G. **(2021)**. Synthesis of α-aminophosphonates and related derivatives; The last decade of the Kabachnik–Fields reaction. *Molecules*, *26*(9), 2511.
[28]PG, B. **(1982)**. Guarneri M. Moroder F. Polloni GP. Simoni D. *Synthesis*, *653*.



Scheme I-4: Reagents and conditions: (a) Ph_3P, DEAD, THF; (b) Hydrazine hydrate.

I.2.4. Other methods

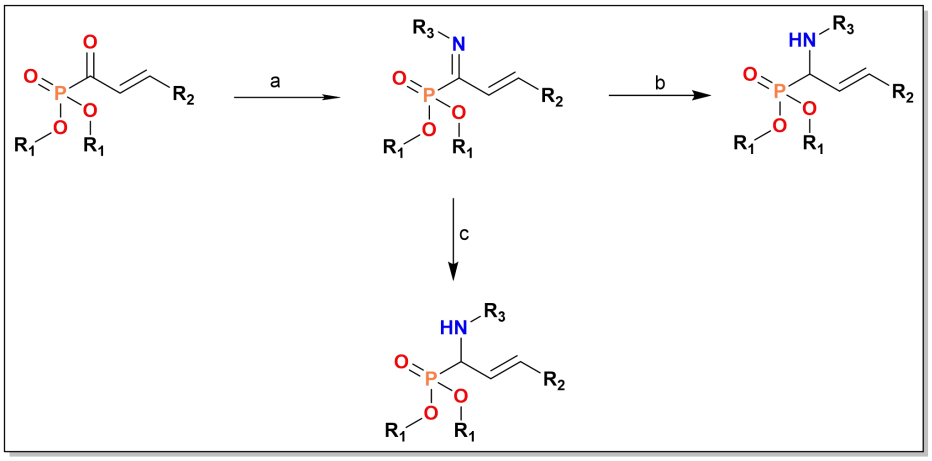
Doye et al[29]. developed a new method for synthesizing α-aminophosphonates using alkynes, primary amines, and diethyl or dimethyl phosphites. The reaction is a one-pot procedure. Cp_2TiMe_2 catalyzes alkyne hydroamination, followed by nucleophilic addition of diethyl or dimethyl phosphite in the presence of Me_2AlCl to produce desirable α-aminophosphonates **(Scheme I-5)**.



Scheme I-5: Reagents and conditions : (a) 3.0-5.0 mol%, Cp_2TiMe_2, 110°C; (b) $HPO(OR_4)_2$, 5.0mol%, Me_2AlCl, 25°C.

Palacios et al[30]. had reported synthesis of α-aminophosphonates fromβ,γ- unsaturated α-ketophosphonates. This approach utilized the *aza-Wittig* reaction of trimethyl phosphazenes followed by hydrogenation to furnish α-aminophosphonates**. (Scheme I-6)**.

[29] Haak, E., Bytschkov, I., & Doye, S. **(2002)**. A One-Pot Procedure for the Synthesis of α-Amino Phosphonates from Alkynes. *European Journal of Organic Chemistry*, *2002*(3), 457-463.
[30] Palacios, F., Vicario, J., Maliszewska, A., & Aparicio, D. **(2007)**. Synthesis of α-phosphorylated α, β-unsaturated imines and their selective reduction to vinylogous and saturated α-aminophosphonates. *The Journal of Organic Chemistry*, *72*(7), 2682-2685.



Scheme I-6: Reagents and conditions: (a) PMe_3, toluene, 0°C; (b) H_2, Pd/C, 80 psi. RT; (c) $BH_3.SMe_2$, -78°C.

I.3. The pharmacological activity of α-Aminophosphonates

α-Aminophosphonates are a significant class of organophosphorus compounds. Expanding the structural similarity between α-amino acids and **P**-analogues, where a phosphonic acid or comparable group replaces a carboxylic moiety, makes them interesting[31-32].They are regarded as an important class of substances having fascinating biological properties[33-34-35-36-37] There are many pharmacological properties displayed by certain α-Aminophosphonates and their related α-Aminophosphonic acids, including antibacterial[38-39], anticancer agents[40-41], herbicides[42-

[31] Naydenova, E. D., Todorov, P. T., Mateeva, P. I., Zamfirova, R. N., Pavlov, N. D., & Todorov, S. B. **(2010)**. Synthesis and biological activity of novel small peptides with aminophosphonates moiety as NOP receptor ligands. *Amino Acids*, *39*, 1537-1543.

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Hiratake, J., & Oda, J. I. **(1997)**. Aminophosphonic and aminoboronic acids as key elements of a transition state analogue inhibitor of enzymes. *Bioscience, biotechnology, and biochemistry*, *61*(2), 211-218.

[36] Kafarski, P., & LeJczak, B. **(1991)**. Biological activity of aminophosphonic acids. *phosphorus, Sulfur, and Silicon and the Related Elements*, *63*(1-2), 193-215..

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[40] Kafarski, P., & Lejczak, B. **(2001)**. Aminophosphonic acids of potential medical importance. *Current Medicinal Chemistry-Anti-Cancer Agents*, *1*(3), 301-312.

[43], Pesticides[44], enzyme inhibitors [45-46-47], peptide mimics[48], antioxidants[49], antivirals[50], antithrombotic agents[51], pharmacological agents[52], anti-HIV agents[53], anti-inflammatory properties[54], antidepressants[55], and plant growth regulators[56]. They are also employed as ligands in organic and transition-metal catalysis[57-58-59] **(Figure I-9)**.

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[43] Che, J. Y., Xu, X. Y., Tang, Z. L., Gu, Y. C., & Shi, D. Q. **(2016)**. Synthesis and herbicidal activity evaluation of novel α-amino phosphonate derivatives containing a uracil moiety. *Bioorganic & Medicinal Chemistry Letters*, *26*(4), 1310-1313.

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[49] Rao, A. J., Rao, P. V., Rao, V. K., Mohan, C., Raju, C. N., & Reddy, C. S. **(2010)**. Microwave assisted one-pot synthesis of novel α-aminophosphonates and their biological activity. *Bull. Korean Chem. Soc*, *31*(7), 1863.

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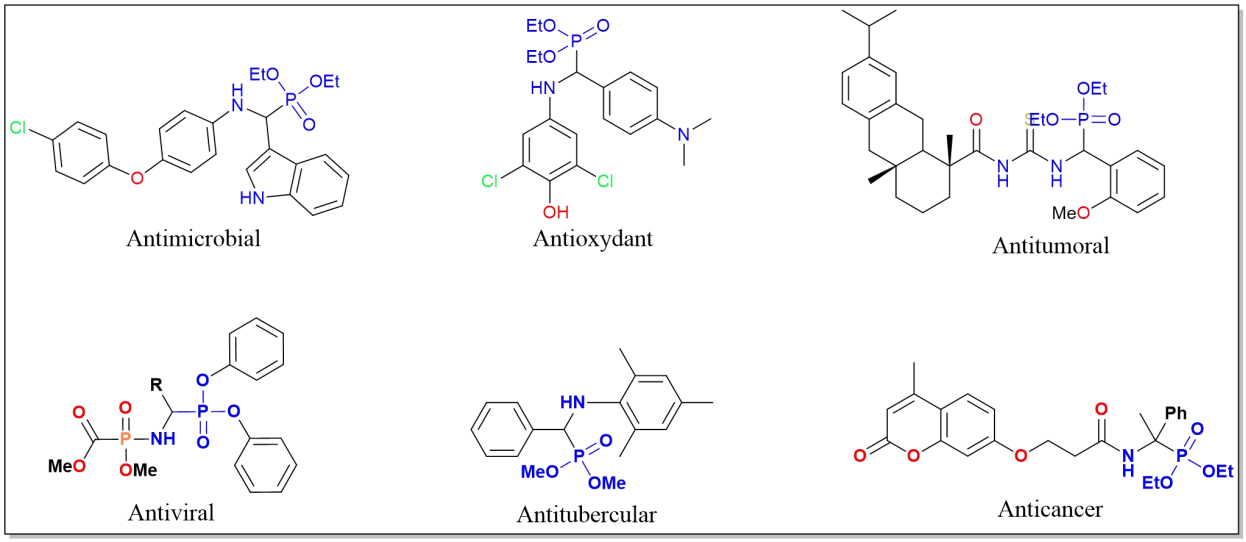

Figure I-9: Some examples of biologically active α-aminophosphophosphonate derivatives.

I.4. Biological Activities of α-Aminophosphonates

In recent years, significant attention has been given to the synthesis of α-aminophosphonate, which are known as analogs of amino acids and have a wide variety of antibiotic, antibacterial, antiviral, and anti-cancer activities[38-65].

Troev et al. Synthesized new α-aminophosphonates bearing furane heterocycle compound 1 and other derivatives, these compounds were evaluated for their antiproliferative activities against four human leukemic cell lines[60]. Among each compound evaluated, compound 1 was the most effective cytotoxic agent. The other compounds showed less effectiveness than cisplatin, the reference anticancer drug **(Figure I-10)**.

[59] Birkholz, M. N., Freixa, Z., & van Leeuwen, P. W. **(2009)**. Bite angle effects of diphosphines in C–C and C–X bond forming cross coupling reactions. *Chemical Society Reviews*, *38*(4), 1099-1118.
[60] Kraicheva, I., Bogomilova, A., Tsacheva, I., Momekov, G., & Troev, K. **(2009)**. Synthesis, NMR characterization and in vitro antitumor evaluation of new aminophosphonic acid diesters. *European journal of medicinal chemistry*, *44*(8), 3363-3367.

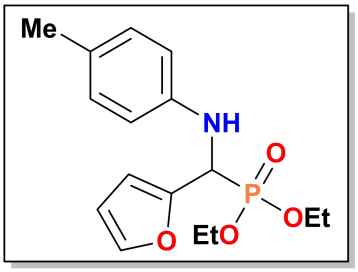

Figure I-10: α-aminophosphonates bearing furane heterocycle with anti-proliferative activity Compound 1.

Song et al. Prepared α-aminophosphonates compounds, which included benzothiazole moiety, and substituted with fluorine. The synthesized compounds were evaluated for *in vitro* anticancer activity against PC3, A431, A375, and Bcap37 cells, applying the MTT technique. Compound 2 shows great efficiency against PC3 cells and weak effectiveness against A431 cells[38]

(Figure I-11).

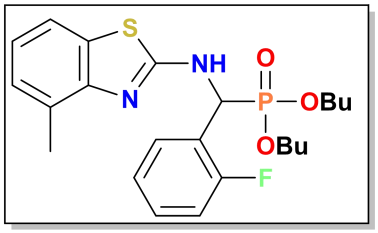

Figure I-11: α-amino (benzothiazole)phosphonates with anti-cancer activity compound 2.

α-Aminophosphonates having isoxazole and fluorine moiety were synthesized by *Song et al*[61].These compounds were evaluated for their antitumor activities against PC3 and A431 cells and were found to possess lower antitumor activities**(Figure I-12)**.

[61] Song, B., Yang, S., Hong, Y., Zhang, G., Jin, L., & Hu, D. **(2005)**. Synthesis and bioactivity of fluorine compounds containing isoxazolylamino and phosphonate groups. *Journal of fluorine chemistry*, *126*(9-10), 1419-1424.

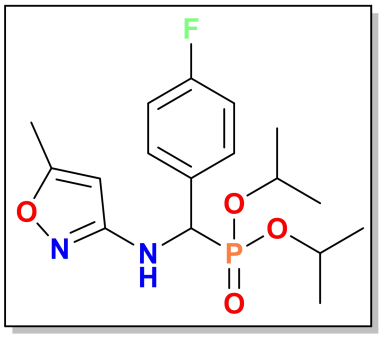

Figure I-12: α-amino (isoxazolyl)phosphonates with antitumor activity.

Deng et al[62].Synthesized α-aminophosphonate derivatives of ursolic acid (UA). It showed that the UA derivatives have particular anti-HIV activity. They inhibit the interaction between gp120 and CD4, making them HIV-1 entry inhibitors **(Figure I-13)**.

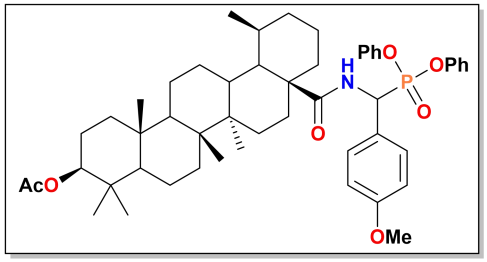

Figure I-13: α-aminophosphonate derivatives of ursolic acid with anti-HIV activity.

Conclusion

This chapter reviewed compounds bearing α-aminophosphonate motif, which were synthesized to discover novel biomolecules that can selectively interact or interfere with biological processes. It becomes clear that the presence of the phosphonate group pattern in diverse molecules results in a variety of interesting biological activities. Obtained with a friendly approach.

[62] Deng, S. L., Baglin, I., Nour, M., Flekhter, O., Vita, C., & Cavé, C. **(2007)**. Synthesis of ursolic phosphonate derivatives as potential anti-HIV agents. *Phosphorus, Sulfur, and Silicon and the Related Elements*, *182*(5), 951-967.

Chapter II: Synthesis of α - aminophosphonates

We report herein a synthesis of novel α-sulfamidophosphonate bearing pyridine moiety and α-aminophosphonate derivatives of 1, 3, 4-thiadiazole *via Kabachnik-Fields* reaction with the belief that the incorporation of more than one biological potent scaffold into a single structure may furnish novel compounds with interesting biological activities.

II. Synthesis of α-sulfamidophosphonates and 1, 3, 4-thiadiazol-2-yl α-aminophosphonates

II.1. α-sulfamidophosphonates

The sulfa drugs have received a great deal of attention, one of the sulfamides is Considered an appealing target for medicinal chemists, it has been explored as an HIV protease inhibitor[63-64]agonist of the 5-HTID receptor, active components of epinephrine analogs[65], non-hydrolysable components of peptide mimics[66], and carbonic anhydrase inhibitors[67-68-69] **(Figure II-1).** This functionality has been identified as the bioisosteric replacement of the polar portion of the pharmacophore[1] (**Figure II-2)**.

Figure II-1: Sulfonamide derivatives with antibacterial activity.

[63] Gavernet, L., Cabrera, M. J. D., Bruno-Blanch, L. E., & Estiú, G. L. **(2007)**. 3D-QSAR design of novel antiepileptic sulfamides. *Bioorganic & medicinal chemistry*, *15*(3), 1556-1567.
[64] Bäckbro, K., Löwgren, S., Österlund, K., Atepo, J., Unge, T., Hultén, J., ... & Hallberg, A. **(1997)**. Unexpected binding mode of a cyclic sulfamide HIV-1 protease inhibitor. *Journal of medicinal chemistry*, *40*(6), 898-902.
[65] Acheson, R. M., Bite, M. G., & Kemp, J. E. **(1981)**. Acidic epinephrine analogs derived from 1H, 3H-2, 1, 3-benzothiadiazole 2, 2-dioxide and from trifluoromethanesulfonanilide. New synthesis of 1H, 3H-2, 1, 3-benzothiadiazole 2, 2-dioxide. *Journal of Medicinal Chemistry*, *24*(11), 1300-1304.
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[69] Casini, A., Winum, J. Y., Montero, J. L., Scozzafava, A., & Supuran, C. T. **(2003)**. Carbonic anhydrase inhibitors: inhibition of cytosolic isozymes I and II with sulfamide derivatives. *Bioorganic & medicinal chemistry letters*, *13*(5), 837-840.

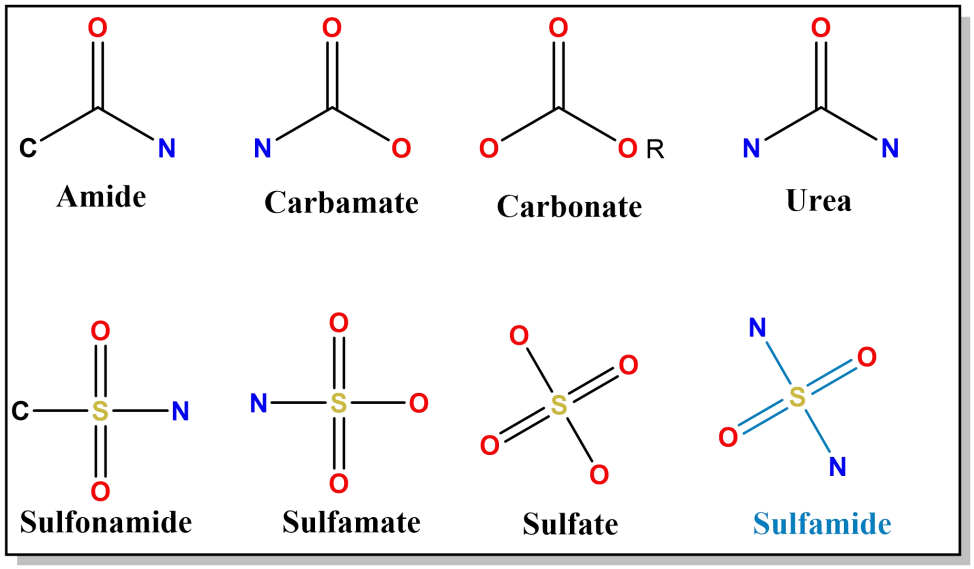

Figure II-2: Chemical functions involved analogs of sulfonyl pharmacophore.

The relatively simple compound sulfamide[70] **($H_2NSO_2NH_2$)**, structurally linked to the commonly used building block in medicinal chemistry, sulfonamide **(SO_2NH_2)**, might be considered the parent molecule of a vast class of biologically active compounds.

Sulfamide **($R_2NSO_2NR_2$)** is an electron-rich functional group that has found its application in a variety of medicinal chemistry applications[71-72]. Doripenem, the clinically marketed broad-spectrum antibiotic, includes a mono-substituted sulfamide.

Quinagolide, a hyperprolactinemia medication, includes a trisubstituted sulfamide. The sulfur atom in sulfamide is tetrahedral, resulting in a conformationally rich topological arrangement of atoms when presented to a protein goal. The sulfamide functional group is not as commonly represented in medicinal chemistry as the urea or sulfonamide functionalities, but it could provide a druggable and valuable alternative.

In the last decade, there has been a lot of interest in the production and reactivity of sulfamides (sulfonyl urea analogs)[73]. A wide variety of sulfamide derivatives have been listed to have biological activities.

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[70] Supuran, C. T., Scozzafava, A., & Casini, A. **(2003)**. Carbonic anhydrase inhibitors. *Medicinal research reviews*, *23*(2), 146-189.

[71] Winum, J. Y., Scozzafava, A., Montero, J. L., & Supuran, C. T. **(2006)**. Therapeutic potential of sulfamides as enzyme inhibitors. *Medicinal research reviews*, *26*(6), 767-792.

[72] Winum, J. Y., Scozzafava, A., Montero, J. L., & Supuran, C. T. **(2006)**. The sulfamide motif in the design of enzyme inhibitors. *Expert Opinion on Therapeutic Patents*, *16*(1), 27-47.

[73] Spillane, W., & Malaubier, J. B. **(2014)**. Sulfamic acid and its N-and O-substituted derivatives. *Chemical reviews*, *114*(4), 2507-2586.

Numerous compounds with sulfamide and cyclo sulfamide were listed in the literature as diuretics [74], potential HIV protease inhibitors-1[75], antioxidants[76], anti-thyroids[77], carbon anhydrase inhibitors[78], and anti-tumor agents[79] **(FigureII-3)**.

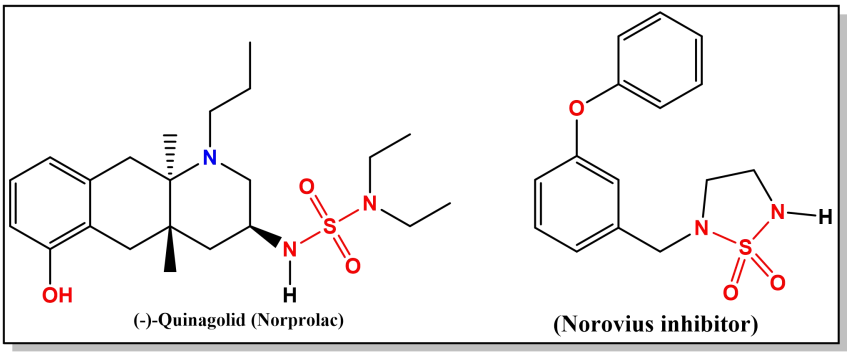

Figure II-3: Drugs bearing sulfamide moiety.

The use of such moieties as precursors in the synthesis of novel α-aminophosphonates compounds could affect a biomolecule's properties, including chemical reactivity, lipophilicity, solubility, metabolic stability, conformation, and hydrogen-bond ability[80]. **(Figure II-4)**. depicts some chemical structures of phosphonates having sulfonamide/sulfamide moieties, as well as their biological activities.

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[74] Maren, T. H. **(1967)**. Carbonic anhydrase: chemistry, physiology, and inhibition. *Physiological reviews*, *47*(4), 595-781.

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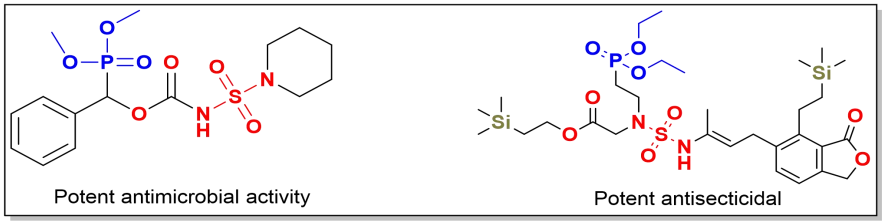


Figure II-4: Structures of sulfamidophosphonates having biological activities.

Several investigations in the scientific literature indicate that synthetic compounds with heterocyclic structures can reduce acute and chronic inflammatory diseases, potentially facilitating healing and comfort. Their structural diversity, biological activity, target specificity, and clinical relevance make them suitable building blocks for developing effective and safer inflammatory treatments[81-82]. Among others, medicinal chemists frequently use the pyridine ring as a scaffold to build and modify molecules with specified pharmacological characteristics.

The incorporation of the pyridine motif into drug molecules has the advantage of acting as a bioisostere for the benzene ring, and such isosteric replacement with pyridine may result in changes in physicochemical properties such as acidity/basicity and lipophilicity, which affect drug-receptor interactions.

In addition, pyridine is a weak base with a pKa value of approximately 5.2. This makes it more basic than benzene and allows pyridine-containing compounds to interact with acidic functional groups in biological molecules, such as the protonated amine functions of proteins. The nitrogen atom in the pyridine ring may act as a hydrogen bond acceptor. This property is essential for mediating interactions with biological macromolecules such as enzymes and receptors. due to its excellent physicochemical and biological profiles, pyridine template is a necessary moiety in many drugs, such as metyrapone, piroxicam (anti-inflammatory), phenazopyridine (urinary tract analgesic), omeprazole, pantoprazole (antiulcer), Tacrine (anti-Alzheimer's), isoniazid (antitubercular), nicorandil (vasodilator), and nikethamide (respiratory stimulant) (**Figure II-5)**.

[81] Sharma, S., Kumar, D., Singh, G., Monga, V., & Kumar, B. **(2020)**. Recent advancements in the development of heterocyclic anti-inflammatory agents. *European Journal of Medicinal Chemistry*, *200*, 112438.
[82] Sondhi, S. M., Dinodia, M., Singh, J., & Rani, R. **(2007)**. Heterocyclic compounds as anti-inflammatory agents. *Current Bioactive Compounds*, *3*(2), 91-108.

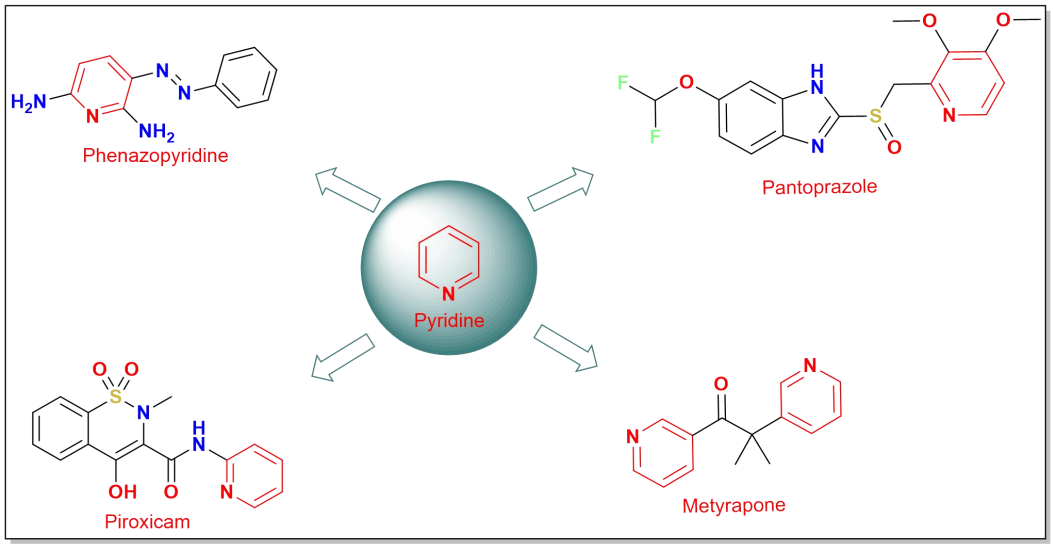


Figure II-5: Structures of pyridine contain drug molecules.

Our interest is focusing on the preparation of sulfamide derivatives, so we decided to include the three motifs, pyridine, sulfamide, and phosphonate, on each targeted compound and then obtain new hybrid molecules**(Figure II-6)**.

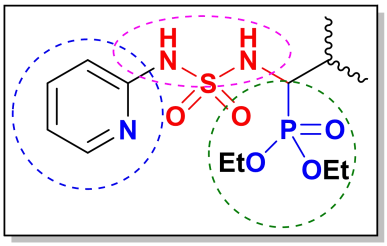


Figure II-6: Design of α-sulfamido-pyrinyl-phosphonates.

II.2. Synthesis of α-sulfamidophosphonates

II.2.1. Synthesis of N-Boc sulfamide

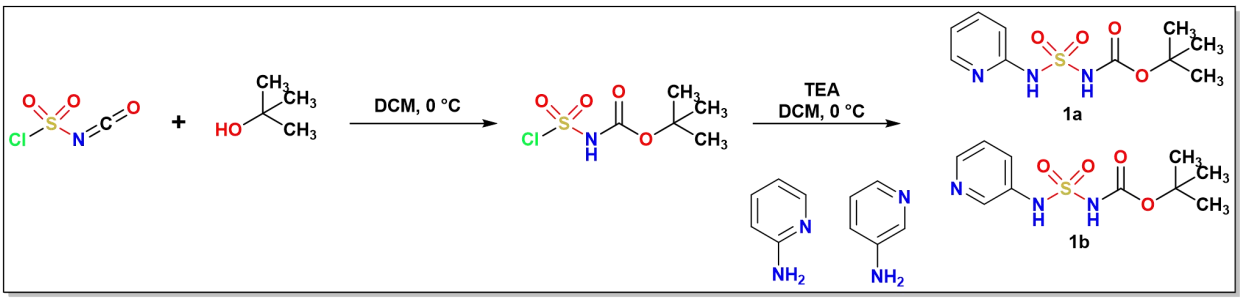
The synthesis of these precursors consists of three successive stages. First, carboxyl sulfamides are formed in two sequential reactions (carbamoylation-sulfamoylation), based on the CSI's dual functional structure[83], followed by a deprotection reaction of the *tert*-butyloxycarbonyl (Boc) protecting group for obtaining the required sulfamides.

The first step is to prepare chlorosulfonylcarbamate by reacting tert-butyl alcohol with the chlorosulfonyl isocyanate at 0°C in dichloromethane for half an hour.

[83] a)Dewynter, G., & Montero, J. L. **(1993)**. Use of Chlorosulfonyl Isocyanate as a Trifunctional Reagent: Insertion of an Activated Sulfamoyl Group, Application to Biomolecules. *ChemInform*, *24*(16), no-no. ; b) Abdaoui, M., Dewynter, G., & Montero, J. L. **(1996)**. Expedient synthesis of 2-chloroethylnitrososulfamides (CENS) via the decarboxylative reopening of sulfamoyloxazolidinones. *Tetrahedron letters*, *37*(32), 5695-5698.

We used tert-butyl alcohol to create an intermediate with a BOC grouping, allowing us to deprotect the amine function quickly.

The second stage of sulfamoylation consists of adding the resulting carbamate to a primary amino solution (pyridin-2-amine and pyridin-3-amine) in an alkaline medium. After 2 hours of processing, carboxyl sulfamides are formed with high yields **(Scheme II-1)**.



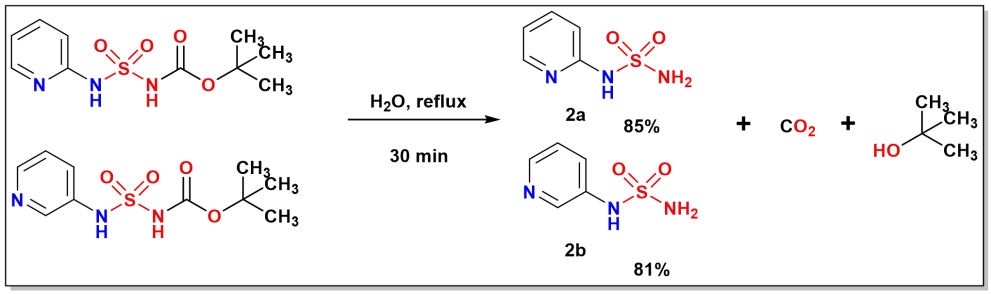
Scheme II-1: Synthesis of carboxyl sulfamides.

The two tributyl carboxyl sulfamide derivatives of P2A and P3A were obtained after purification using a mixture of diethyl ether and dichloromethane (7-3), with reasonable yields of 65 and 70%, respectively. The results can be explained by the limited reactivity of P2A and P3A's amine functions. The non-bonding electronic doublet of the amine function is very engaged in resonance with the pyridine heterocycle.

II.2.2. N-tertiobutoxycarbonyl sulfamide deprotection

Different N-Boc deprotection procedures using several mediums (acids or bases) have been the subject of several works carried out. the method of *N*-Boc deprotection has been developed by Cheraiet et *al*[84], which seems easy compared to conventional methods. The resulting carboxyl sulfamides were previously deprotected in distilled water, and reflux for 30 minutes **(Scheme II-2)**. A TLC plate was used to monitor the progress of the reaction, which showed the formation of a more polar product revealed by ninhydrin. The obtained sulfamides were used in the next step without any chromatographic column purification.

[84] Zinelaabidine, C., Souad, O., Zoubir, J., Malika, B., & Nour-Eddine, A. **(2012)**. A simple and efficient green method for the deprotection of N-Boc in various structurally diverse amines under water-mediated catalyst-free conditions. *International journal of Chemistry*, *4*(3), 73.



Scheme II-2: Deprotection of N-Boc sulfamides in boiling water.

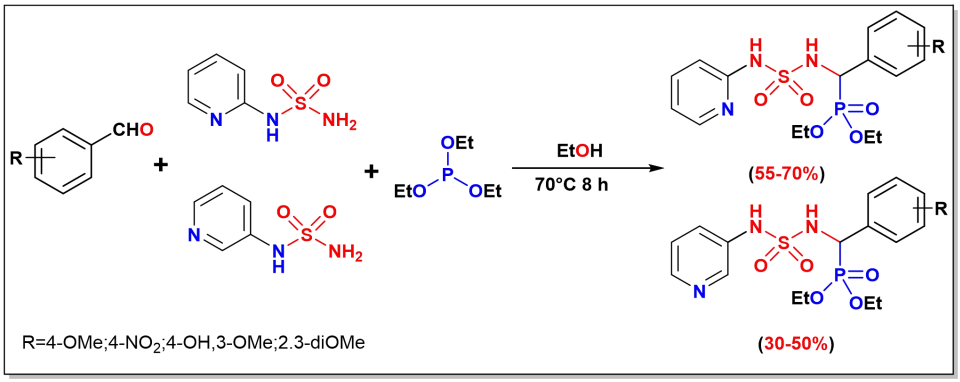
II.2.3. Synthesis of α-sulfamidophosphonates derivatives

The synthesis of the α-sulfamidophosphonates can be done using the "one-pot" reaction according to the *Kabachnik-Fields* reaction as highlighted in **(Scheme II-3)**. In our case, the reaction is achieved by reacting the sulfamide derivative of P2A, 4-methoxy benzaldehydes, and triethyl phosphite with a molar ratio (1: 1: 1 mmol), under refluxed conditions in ethanol. without using a catalyst. After 8hs the diethyl ((4-methoxyphenyl)((N-(pyridin-2-yl)sulfamoyl)amino)methyl)phosphonate **(3a)** was obtained with a moderate yield of 58 %.

To assess the effect of temperature, the same reaction, showing the synthesis of α-sulfamidophosphonates **(3a)**, the different molar ratio of components was replicated at room temperature and then at 60°C. However, **(3a)** was obtained with a reduced yield in comparison to the initial reaction conditions.

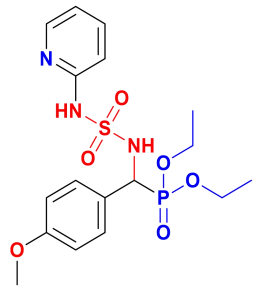
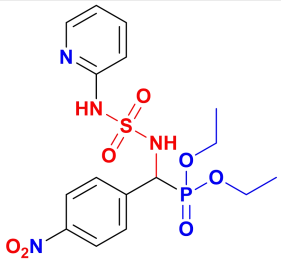
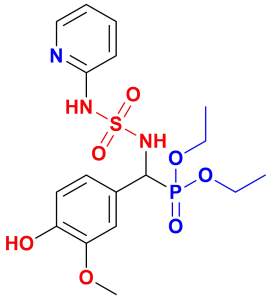
Encouraged by the initial findings and aiming to broaden the scope of this reaction, we expanded our investigation to include substituted benzaldehydes. The results are summarized in **(Table II-1)**.

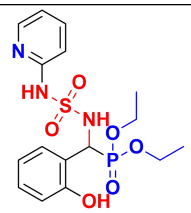
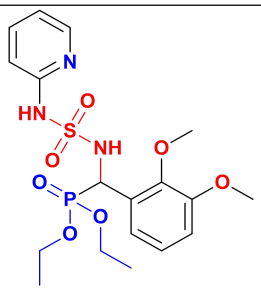
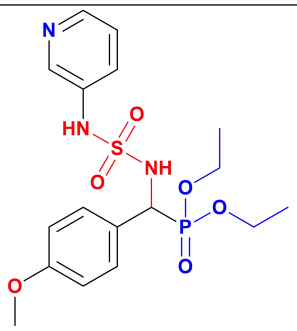
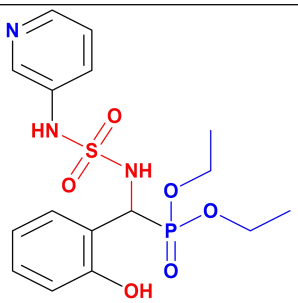
As illustrated in **(Table II-1)**, the desired products were obtained in all instances, with yields ranging from moderate to good between 55-70 %. The best result was obtained with **(7a)**, yielding sulfamidophosphonate in 70 %.



Scheme II-3: Synthesis of α-sulfamidophosphonates.

Table II- 1: Derivatives of synthesized α-sulafamidophosphonates.

Entry	Compound	Yield (%)
3a		58
4a		Not purified
5a		62

6a		59
7a		70
3b		64
4b		62

In all cases summarized in **(Table II-1)**, aromatic aldehydes containing electron-withdrawing groups (EWG) and electron-donating groups (EDG) react in the same manner with the sulfamide derivatives of pyridine.

❖ *Spectroscopic characterization:*

Two newly synthesized compounds (**3a** and **5a**) were characterized by ^{1} H and C^{13} NMR, ^{31}P-NMR, 2D NMR [HSQC] and [HMBC]. The detailed data is found in the experimental section.

^{1}H NMR analysis revealed the protons of the substituted benzene ring, pyridines, and phosphonate moiety with accurate integration to confirm the molecular structures of all products. The **N-H** amine proton was found at around 5.56 ppm for compounds (**3a**), and the signal at 5.43 ppm represented the proton of the chiral carbon **(PC*H)**. two triplets at 1.03-1.17 correspond to the methyl of **CH_3- CH_2-O-P** and 4.00 ppm signals correspond to the methylene of the same group. The presence of dt, ddd, td, and dd with coupling constants 7.98 (dd, J = 5.4, 1.8 Hz, 1H), 7.25 (td, J = 2.0, 0.8 Hz, 1H), 6.47 (ddd, J = 7.2, 5.0, 0.9 Hz, 1H), 6.35 (dt, J = 8.3, 1.0 Hz, 1H) confirmed the presence of the substituted pyridine at **C-2**.

In **^{13}C NMR** chemical shift for **P-C*** was observed as a doublet (d) in the region 51,19–52.72 ppm (J_{C-P} = 154 Hz coupling constant) confirming the formation of phosphonate groups. **^{31}P NMR** spectra which confirmed the presence of the phosphonate function (22.92ppm).

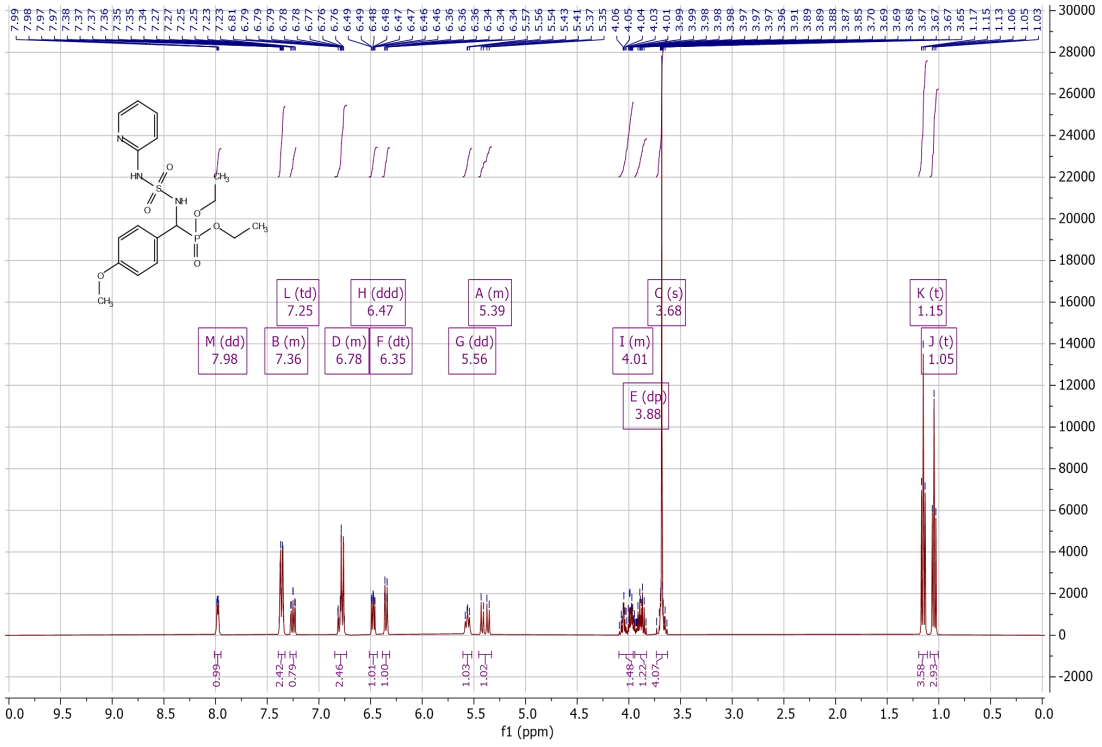


Figure II-7: ^{1}H NMR Spectrum of diethyl ((4-methoxyphenyl)((N-(pyridin-2-yl)sulfamoyl)amino)methyl)phosphonate **(3a)**.

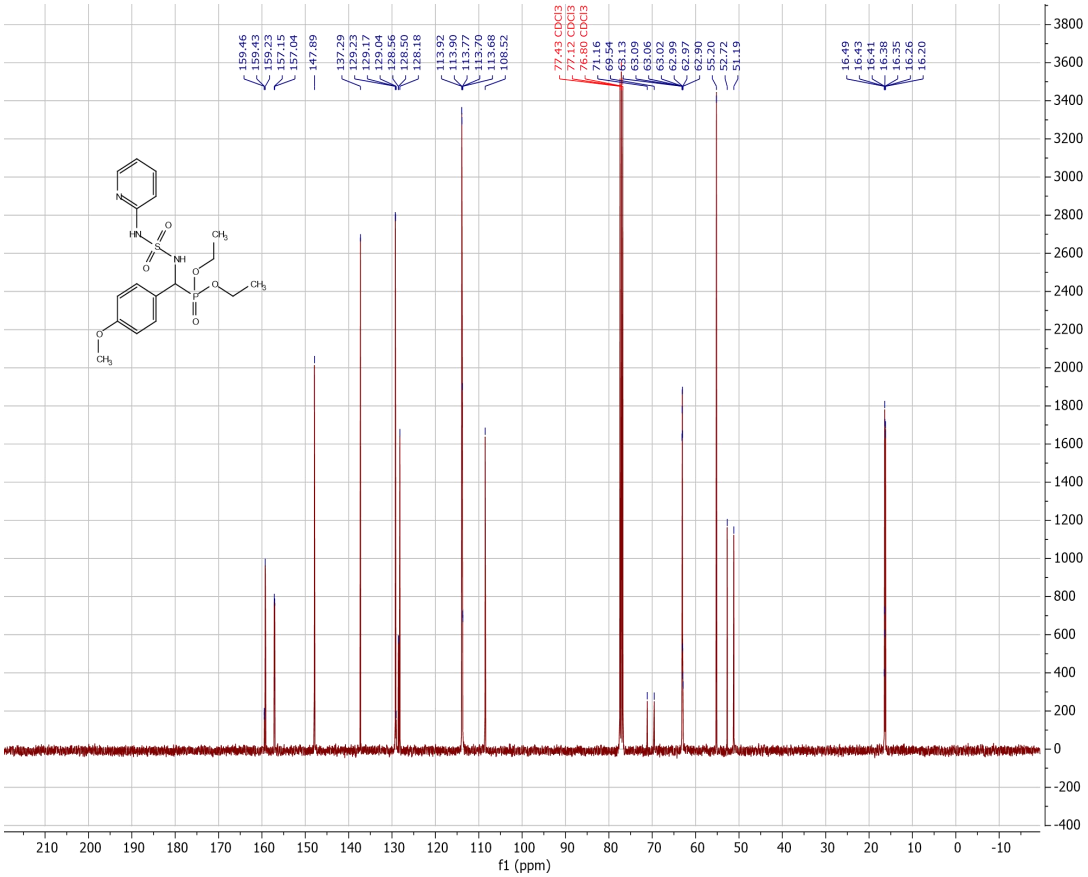


Figure II-8: ^{13}C NMR Spectrum of diethyl ((4-methoxyphenyl)((N-(pyridin-2-yl)sulfamoyl)amino)methyl)phosphonate **(3a)**.

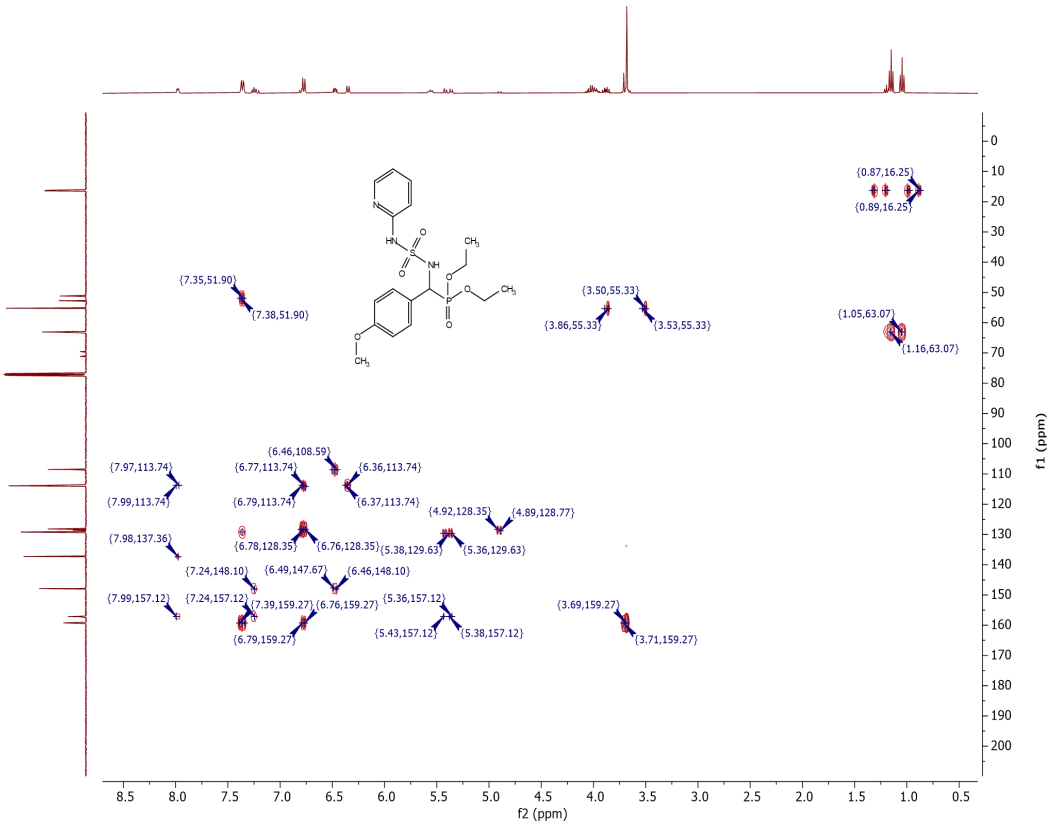


Figure II-9: 2D NMR [HMBC] Spectrum of diethyl ((4-methoxyphenyl)((N-(pyridin-2-yl)sulfamoyl)amino)methyl)phosphonate **(3a)**.

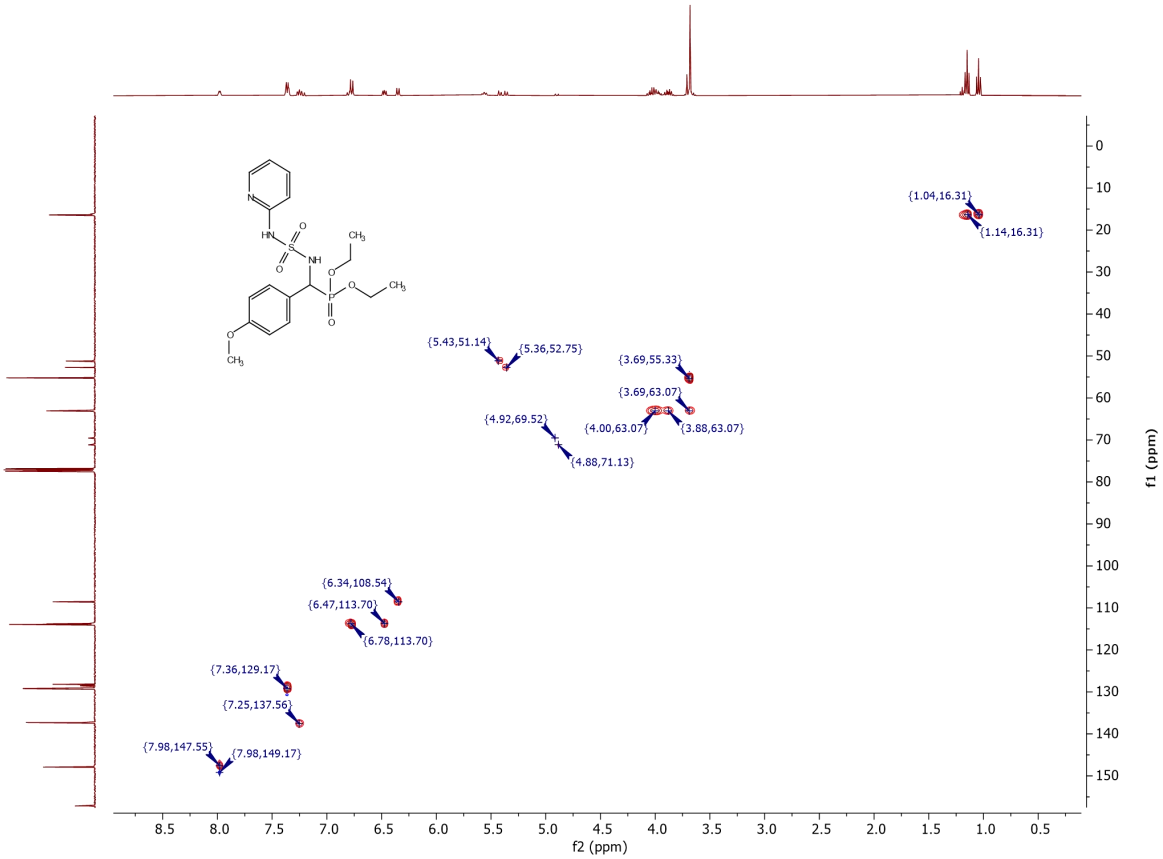


Figure II-10:2D NMR [HSQC] Spectrum of diethyl ((4-methoxyphenyl)((N-(pyridin-2-yl)sulfamoyl)amino)methyl)phosphonate **(3a)**.

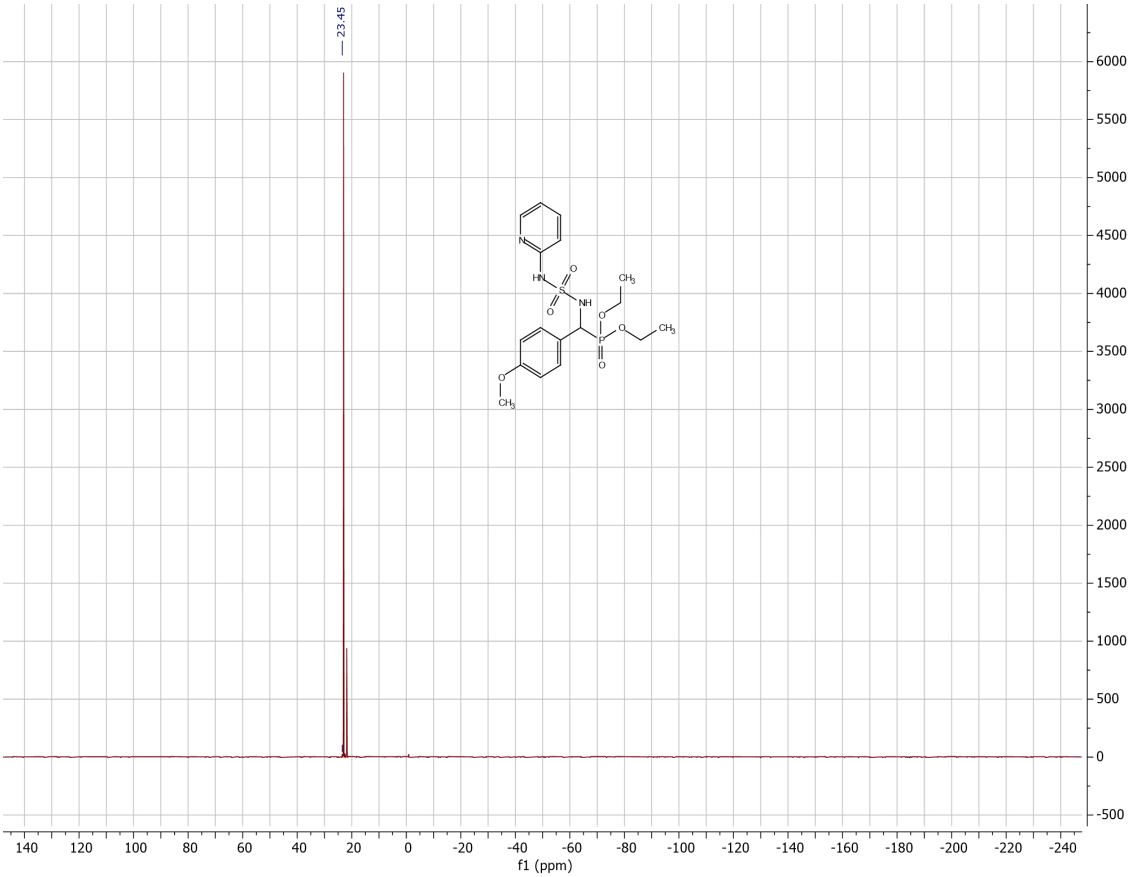


Figure II-11: ^{31}P NMR Spectrum of diethyl ((4-methoxyphenyl)((N-(pyridin-2-yl)sulfamoyl)amino)methyl)phosphonate **(3a)**.

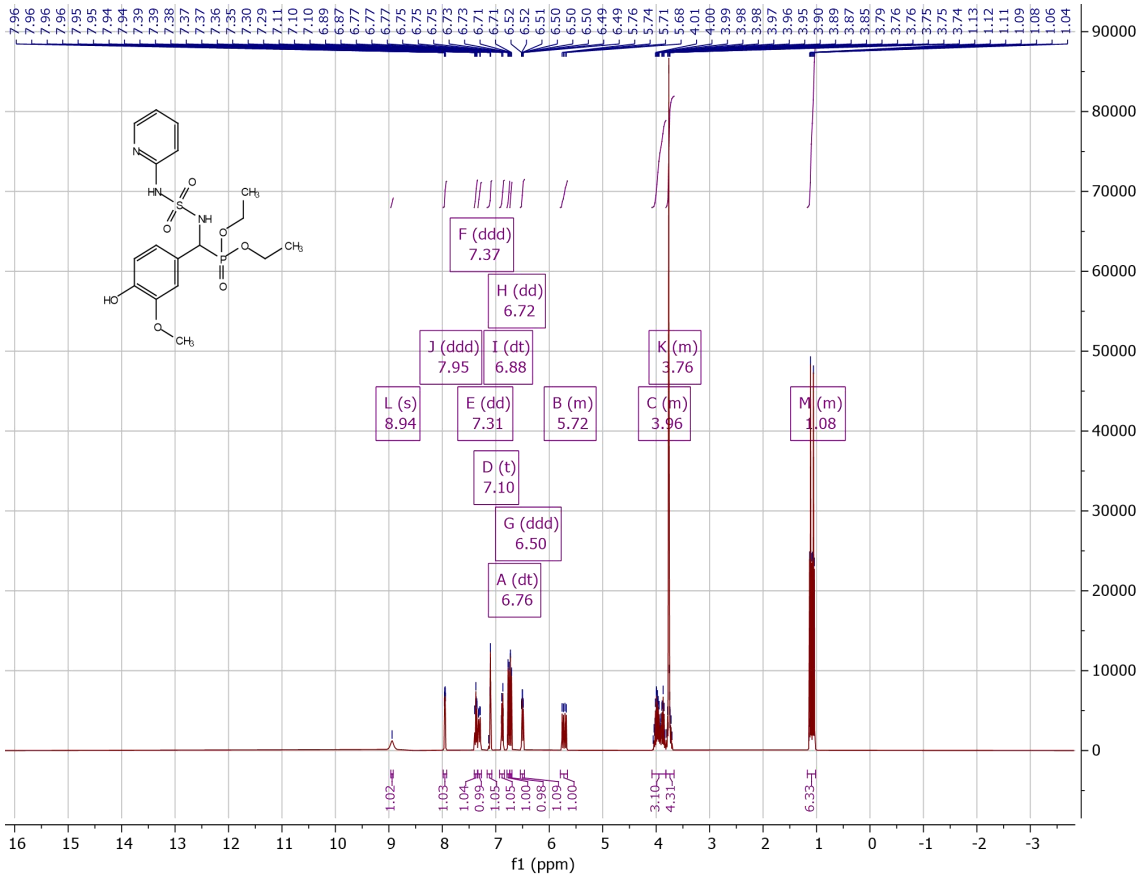


Figure II-12:[1] H NMR Spectrum of diethyl ((4-hydroxy-3-methoxyphenyl)((N-(pyridin-2-yl)sulfamoyl)amino)methyl)phosphonate (**5a**).

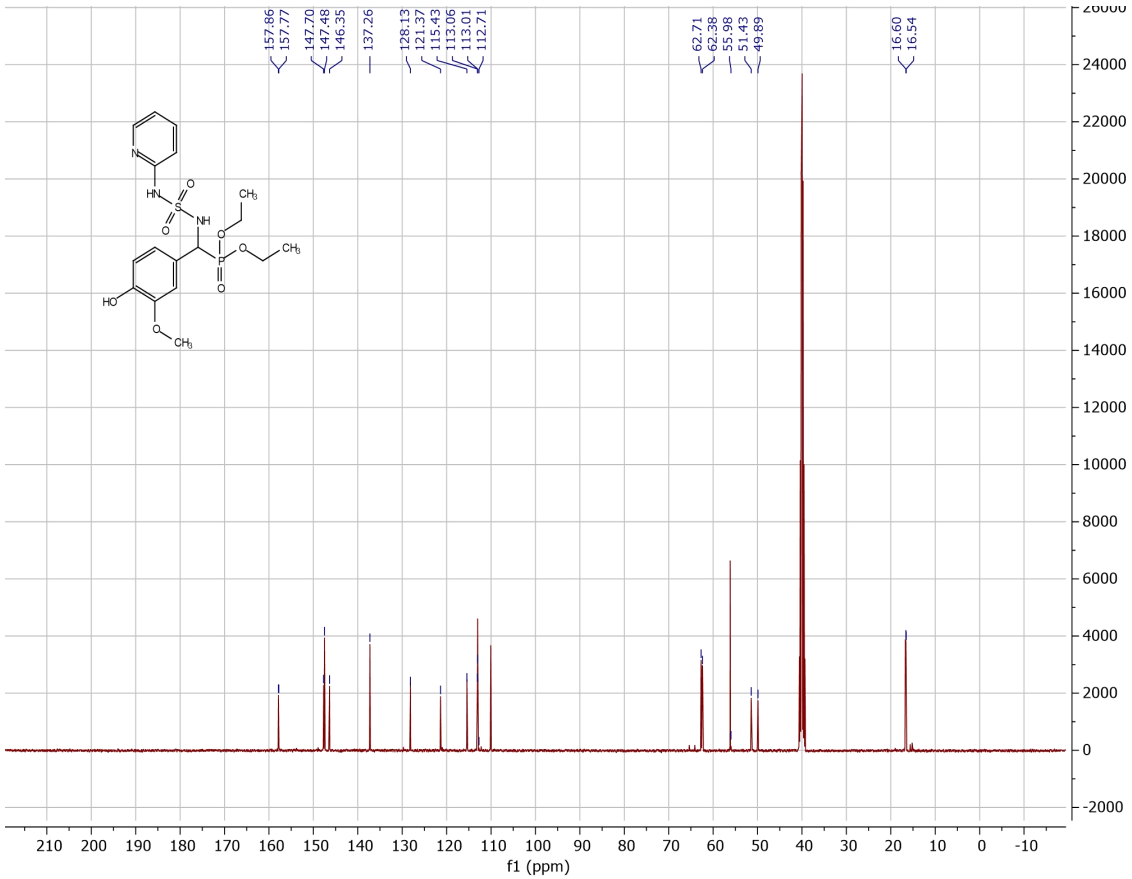


Figure II-13:[13] C NMR Spectrum of diethyl ((4-hydroxy-3-methoxyphenyl)((N-(pyridin-2-yl)sulfamoyl)amino)methyl)phosphonate (**5a**).

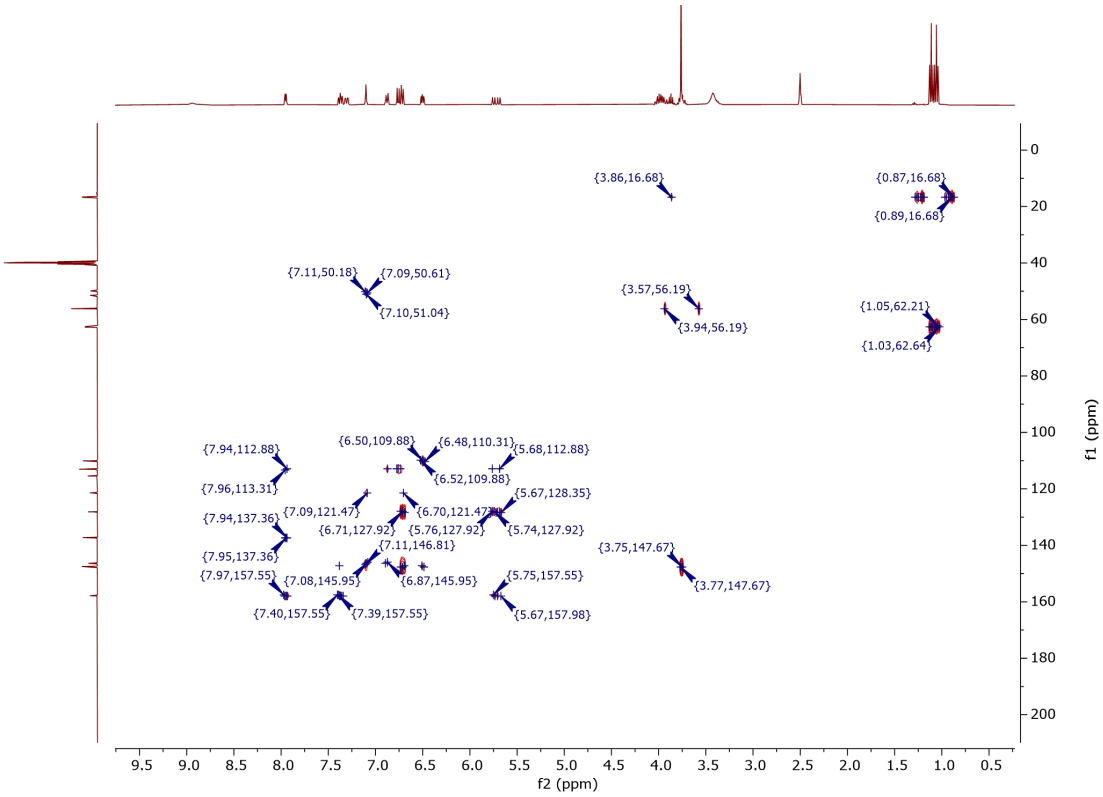


Figure II-13:HMBC Experience of NMR Spectrums of diethyl ((4-hydroxy-3-methoxyphenyl)((N-(pyridin-2-yl)sulfamoyl)amino)methyl)phosphonate (**5a**).

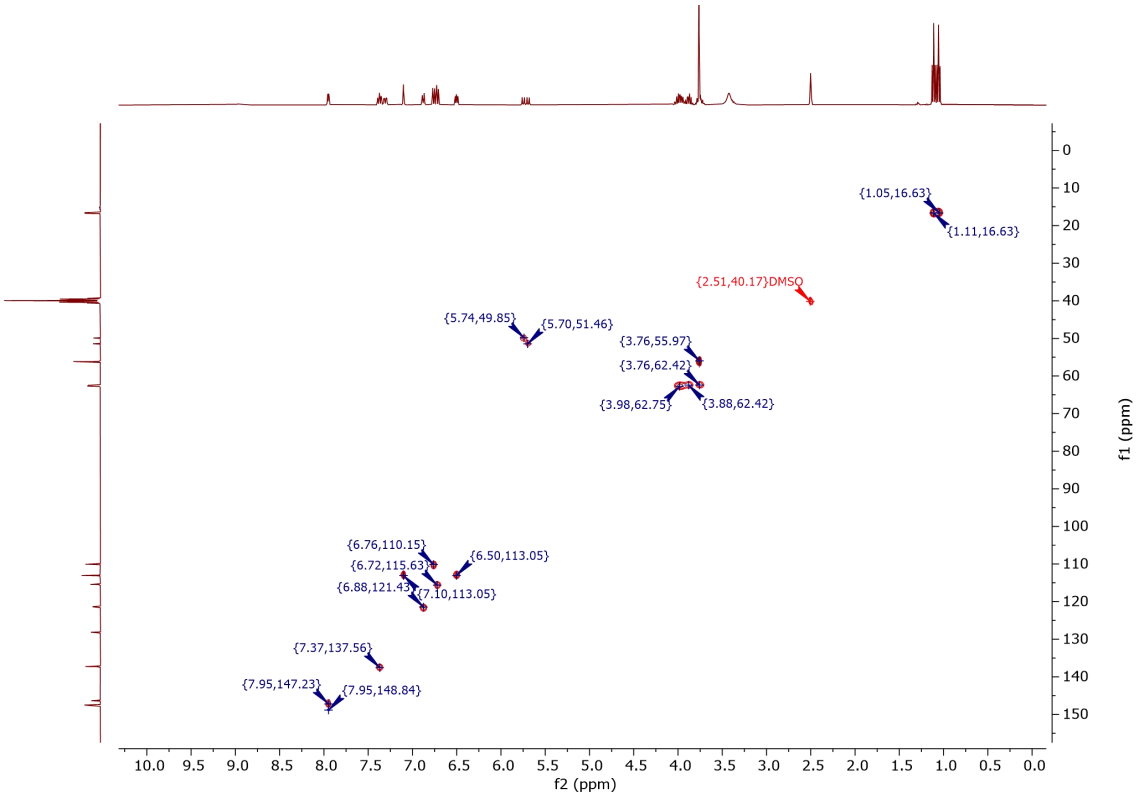


Figure II-14: HSQC Experience of NMR Spectrums of diethyl ((4-hydroxy-3-methoxyphenyl)((N-(pyridin-2-yl)sulfamoyl)amino)methyl)phosphonate (**5a**).

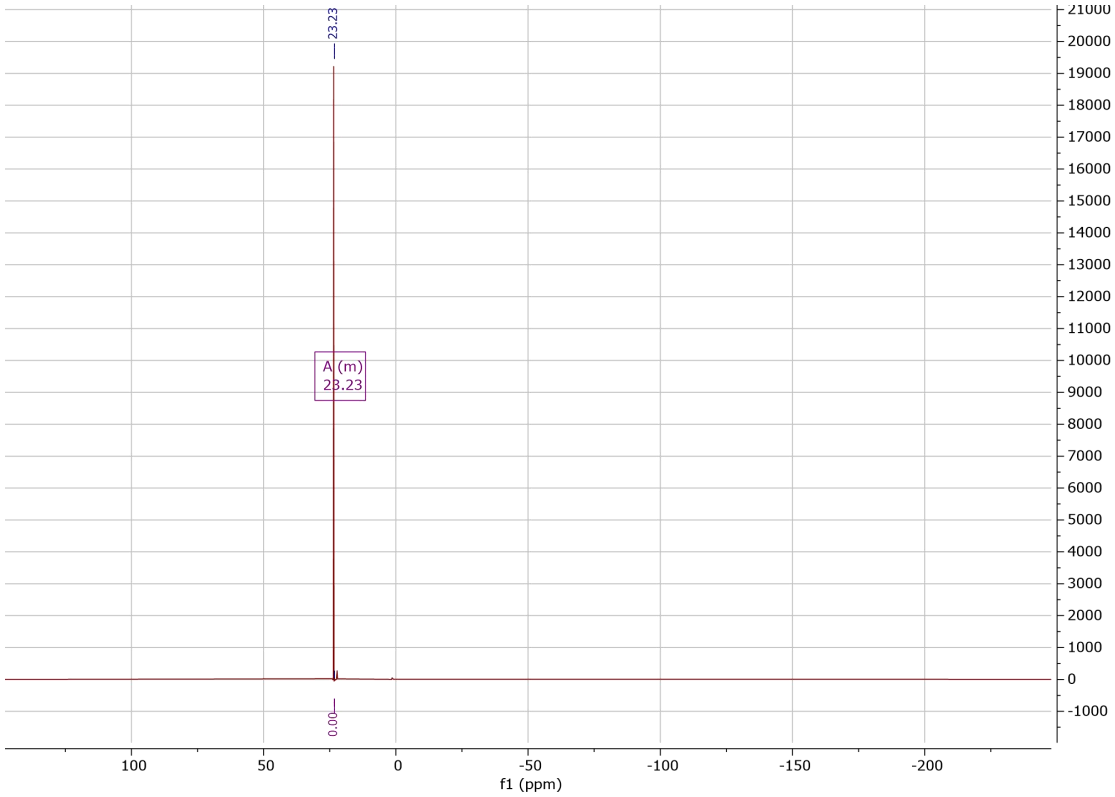


Figure II-16: ^{31}P NMR Spectrum of diethyl ((4-hydroxy-3-methoxyphenyl)((N-(pyridin-2-yl)sulfamoyl)amino)methyl)phosphonate (**5a**).

II.3. α-aminophosphonates derivatives of 1, 3, 4-thiadiazole

Heterocyclic compounds are important chemical compounds bringing biological activity that are applied as medications in human and veterinary medicine, as well as insecticides and pesticides in agriculture. Chemical rings, which are included in many commercial drugs, may have pharmacological impacts or act as a platform for pharmacophoric groups that interact with receptors[85].

Thiadiazoles belong to the class of nitrogen-sulfur heterocycles that have a wide range of applications as structural units of biologically active compounds and valuable intermediates in medicinal chemistry. The thiadiazole ring has various isomers, including 1,2,3-thiadiazole, 1,2,4-thiadiazole, 1,2,5-thiadiazole, and 1,3,4-thiadiazole**(Figure II-17)**.

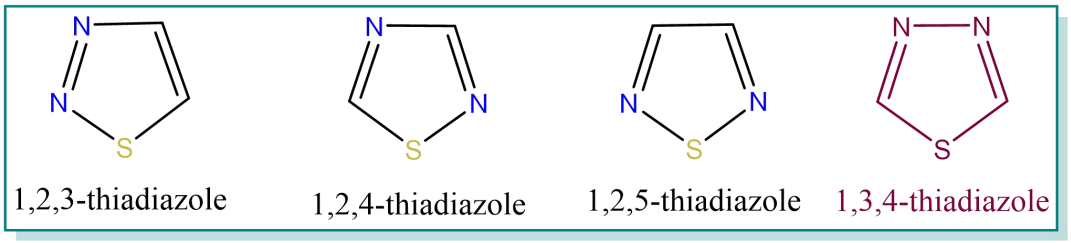


Figure II-17: Isomers of thiadiazole.

For the reason of their numerous pharmacological properties, substituted 1,3,4-thiadiazole derivatives have attracted a lot of attention and research lately. The presence of the **=N-C-S-** moiety is believed to lead 1,3,4-thiadiazole derivatives to exhibit a variety of biological activities[86]. The biological activities of 1,3,4-thiadiazole derivatives are due to the ring's strong aromaticity, which additionally provides this five-membered ring system with excellent in vivo stability and low toxicity to higher vertebrates, including humans[87].

Some studies indicate the importance of isosterism for the pharmacological profile of a compound. Based on these researches, 1,2,4-thiadiazole is the bioisostere of pyrimidine, while 1,3,4-thiadiazole is the bioisostere of pyridazine via the substitution of **-CH=CH- by -S- (Figure II-18)**[88-89].

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[85] Wermuth, C. G., Ciapetti, P., Giethlen, B., & Bazzini, P. **(2007)**. Comprehensive medicinal chemistry II. *Bioisosterism, 2nd edn. Elsevier, New York*, 649-711.
[86]Holla, B. S., Poojary, K. N., Rao, B. S., & Shivananda, M. K. **(2002)**. New bis-aminomercaptotriazoles and bis-triazolothiadiazoles as possible anticancer agents. *European Journal of Medicinal Chemistry*, *37*(6), 511-517.
[87] Yousif, E., Majeed, A., Al-Sammarrae, K., Salih, N., Salimon, J., & Abdullah, B. **(2017)**. Metal complexes of Schiff base: preparation, characterization and antibacterial activity. *Arabian Journal of Chemistry*, *10*, S1639-S1644.
[88] Li, Y., Geng, J., Liu, Y., Yu, S., & Zhao, G. **(2013)**. Thiadiazole—A promising structure in medicinal chemistry. *ChemMedChem*, *8*(1), 27-41.

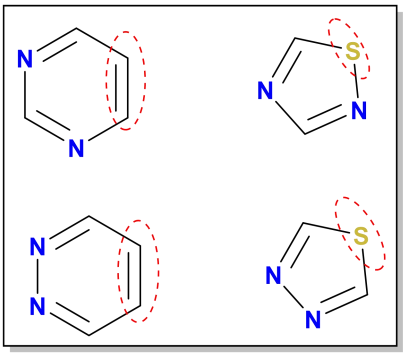

Figure II-18: Isosterism between the six-membered diaza-heterocycles and thiadiazole derivatives.

Bio-isosteric ring substitution can improve compounds' lipophilicity and biological properties. due to the presence of sulfur atom thiadiazole derivatives have excellent liposolubility, oral absorption, and cell permeability, resulting in high bioavailability. Substituting a heterocycle for a homocyclic ring allows for the production of novel analogs that interact more effectively with receptors[4-90-91].

Heterocyclic compounds are among the most frequent and essential scaffolds found in a wide range of bioactive natural products, synthetic drugs, pharmaceuticals, and agrochemicals, and they are one of the most important classes of organic compounds used in a variety of biological fields due to their anti-disease properties. Among the various heterocycles, 1,3,4-thiadiazole was initially defined in 1882 by *Fischer* and later advanced by *Busch* and his colleagues[92]. Thiadiazoles are a heterocycle class with a five-membered ring including sulfur and two nitrogen atoms, and they demonstrate a wide range of biological activities[93-94]. Several compounds containing the thiadiazole moiety have numerous biological properties, which include antimicrobial, antiproliferative, antitumor, antitubercular, anti-inflammatory,

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[89] Wermuth, C. G. (Ed.). **(2011)**. *The practice of medicinal chemistry*. Academic Press.
[90] Brown, N. **(2012)**. Bioisosterism in medicinal chemistry. *Bioisosteres in medicinal chemistry*, 1-14.
[91] Tripathy, R., Ghose, A., Singh, J., Bacon, E. R., Angeles, T. S., Yang, S. X., ... & Mallamo, J. P. **(2007)**. 1, 2, 3-Thiadiazole substituted pyrazolones as potent KDR/VEGFR-2 kinase inhibitors. *Bioorganic & medicinal chemistry letters*, *17*(6), 1793-1798.
[92] Al-Zubiady, S. F., Al-Khafaji, Z. H. K., & Mohamed, I. M. **(2018)**. Synthesis, characterization of new1, 3, 4-thiadiazole derivatives with studying their biological activity. *Research Journal of Pharmacy and Technology*, *11*(1), 284-293.
[93] Mojallal-Tabatabaei, Z., Foroumadi, P., Toolabi, M., Goli, F., Moghimi, S., Kaboudanian-Ardestani, S., & Foroumadi, A. **(2019)**. 2-(Bipiperidin-1-yl)-5-(nitroaryl)-1, 3, 4-thiadiazoles: Synthesis, evaluation of in vitro leishmanicidal activity, and mechanism of action. *Bioorganic & Medicinal Chemistry*, *27*(16), 3682-3691.
[94] Yan, L., Deng, M., Chen, A., Li, Y., Zhang, W., Du, Z. Y., ... & Chen, H. **(2019)**. Synthesis of N-pyrimidin [1, 3, 4] oxadiazoles and N-pyrimidin [1, 3, 4]-thiadiazoles from 1, 3, 4-oxadiazol-2-amines and 1, 3, 4-thiadiazol-2-amines via Pd-catalyzed heteroarylamination. *Tetrahedron letters*, *60*(20), 1359-1362.

anticonvulsant, antioxidant, antileishmanial, antibacterial, antiviral, analgesic, antipsychotic, antihistamine, anti-depressive, and antihypertensive[95] **(Figure II-19)**.

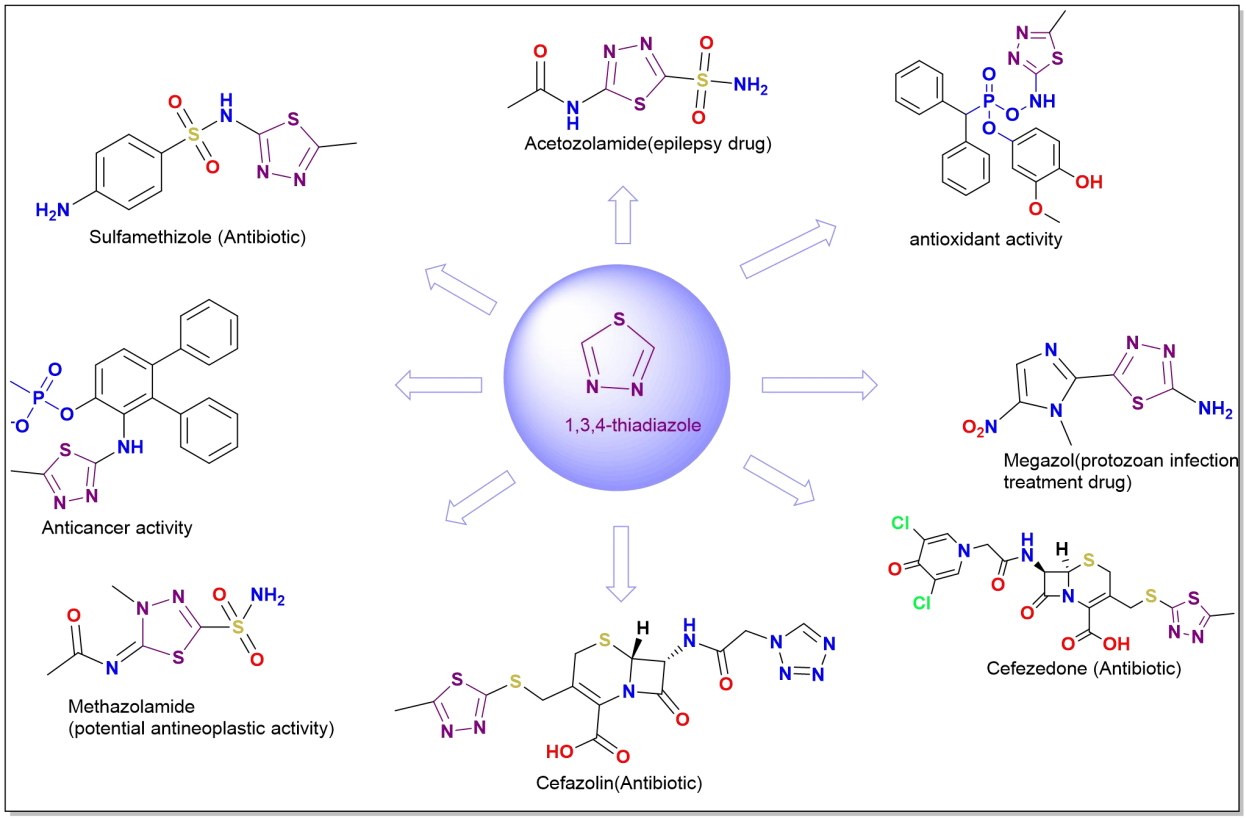


Figure II-19: Some examples of biologically active 1, 3, 4-thiadiazole derivatives.

[95] Serban, G., Stanasel, O., Serban, E., & Bota, S. **(2018)**. 2-Amino-1, 3, 4-thiadiazole as a potential scaffold for promising antimicrobial agents. *Drug design, development and therapy*, 1545-1566.

II.3. 1. Synthesis of 5-(ethylthio)-1, 3, 4-thiadiazol-2-yl) α-aminophosphonates derivatives

The coupling of two bioactive nuclei has been assumed a promising strategy in drug design and discovery. Since **α**-aminophosphonates and 1,3,4-thiadiazol ring are important exhibits many pharmacological properties such as anticancer, antioxidant, antifungal, antiviral, CNS depressant, antitubercular, and anti-inflammatory.[96] Our objective was to integrate both moieties by attaching a bioactive thiadiazole ring with the structure of α-aminophosphonates. Herein, we report the hybridization of 1, 3, 4-thiadiazoles with α-aminophosphonates to obtain single biologically modified entities and preliminary investigations for their anti-inflammatory potentials.

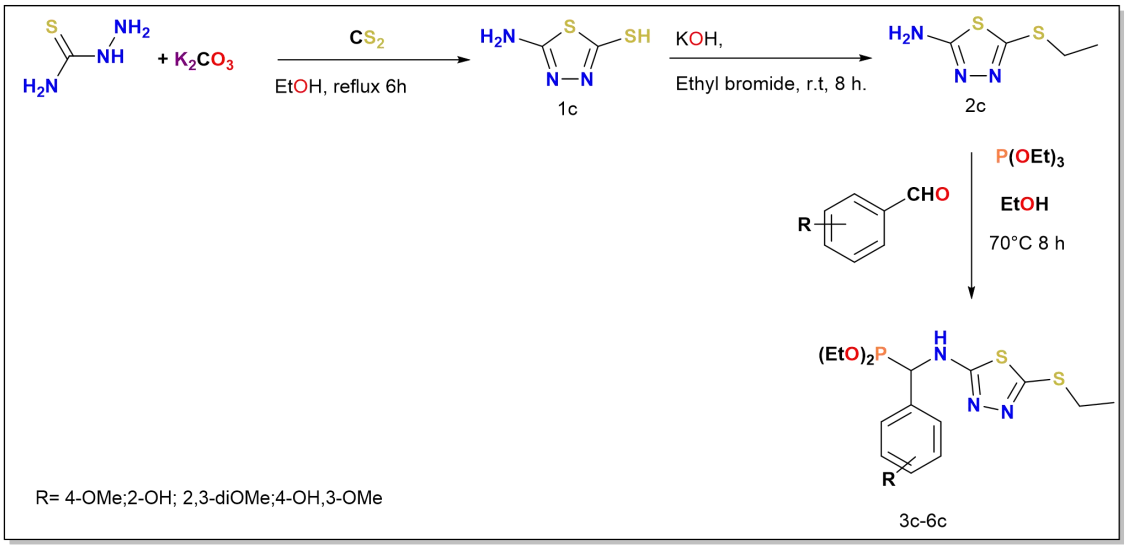
The synthetic strategy to prepare the target compounds was depicted in **(Scheme II-4)**.

Firstly, 5-amino-1,3,4-thiadiazole-2-thiol was synthesized according to the literature procedure[97], after the reaction of thiosemicarbazide with potassium carbonate in ethanol at reflux for 30 min, carbon disulfide was added and refluxed for 8h to obtain 5-amino-1,3,4-thiadiazole-2-thiol **(1c)**. After 5-amino-1,3,4-thiadiazole-2-thiol and KOH are dissolved in water, Ethyl bromide is added for 6h and refluxed for 3h to obtain compound **(2c)** 5-(ethylthio)-1,3,4-thiadiazol-2-amine.

We investigated the reaction of 5-(ethylthio)-1,3,4-thiadiazol-2-amine with *para*-methoxybenzaldehyde and triethylphosphite in DMF at room temperature for 6h, no product were formed. Consequently, the same reactions are achieved at refluxed conditions in ethanol, diethyl (((5-(ethylthio)-1,3,4-thiadiazol-2-yl)amino)(4-methoxyphenyl)methyl)phosphonate **3c** is obtained and purified by crystallization from ethanol to yield 54% **(Scheme II-4) (Table II-2)**.

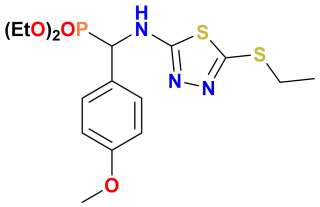
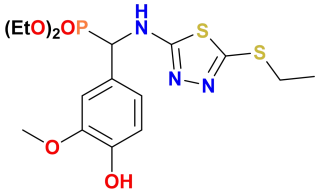
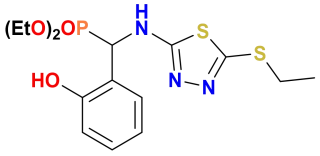
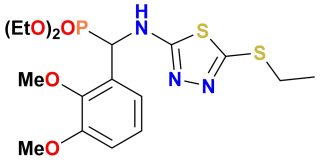
[96] Mohamed, A. E., Elgammal, W. E., Dawaba, A. M., Ibrahim, A. G., Fouda, A., & Hassan, S. M. **(2022)**. A novel 1, 3, 4-thiadiazole modified chitosan: synthesis, characterization, antimicrobial activity, and release study from film dressings. *Applied Biological Chemistry*, *65*(1), 54.

[97] Petrow, V., Stephenson, O., Thomas, A. J., & Wild, A. M. **(1958)**. 302. Preparation and hydrolysis of some derivatives of 1: 3: 4-thiadiazole. *Journal of the Chemical Society (Resumed)*, 1508-1513.



Scheme II-4: Synthesis of α-aminophosphonates derivatives of 1, 3, 4-thiadiazole.

Table II- 2: Derivatives of synthesized 5-(ethylthio)-1, 3, 4-thiadiazol-2-yl) α-aminophosphonates.

Entry	Compound	Yield (%)
3c		54
4c		45
5c		52
6c		59

- ***Spectroscopic characterization***

Two of the title compounds **5c** and **3c** were fully characterized spectral NMR data. In ^{1}H NMR spectra, the chemical shifts in 8 ppm correspond NH proton, the region of 7.49 –6.79 ppm are due to aromatic protons, the doublet at d 5.53 with J = 19.7Hz confirmed the P-C**H** protons of assymetric carbone. The multiplet with 2H integration, doublet of pentuplet with 1H and doublet doublet of quadruplet *ddq* in the region of 4. 4.22 –3.75 and two triplet at 1.00 and 1.23 ppm are due to the two O–CH2–CH3. The S-CH2–CH3 multiplets are responsible for the triplet at 1.31ppm and quadruplet at 3.06 ppm.

In 13C NMR spectra, the chemical shifts in the region of 169.3–119.1 ppm are assigned to carbons of the aromatic ring and thiadiazol moeity; the signals in the region of 51.45 ($J_{C\text{-}P}$ = 159.9 Hz) confirmed H**C**–P in accordance with littérature coupling constant.

The ^{31}P NMR, chemical shifts of the title compounds appeared in 21.82 ppm.

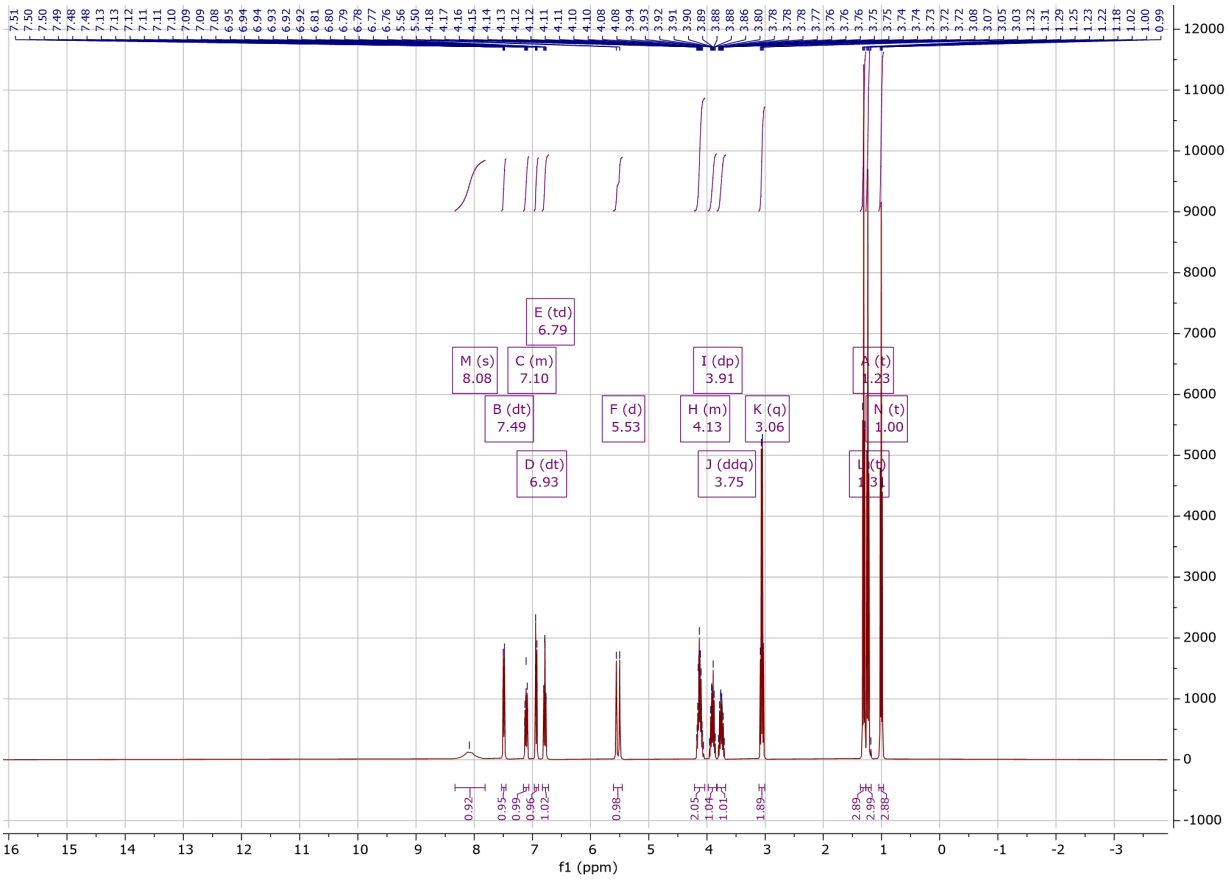


Figure II-20: [1] H NMR Spectrum of diethyl (((5-(ethylthio) -1,3,4-thiadiazol-2-yl) amino) (2-hydroxyphenyl) methyl)phosphonate **(5c)**.

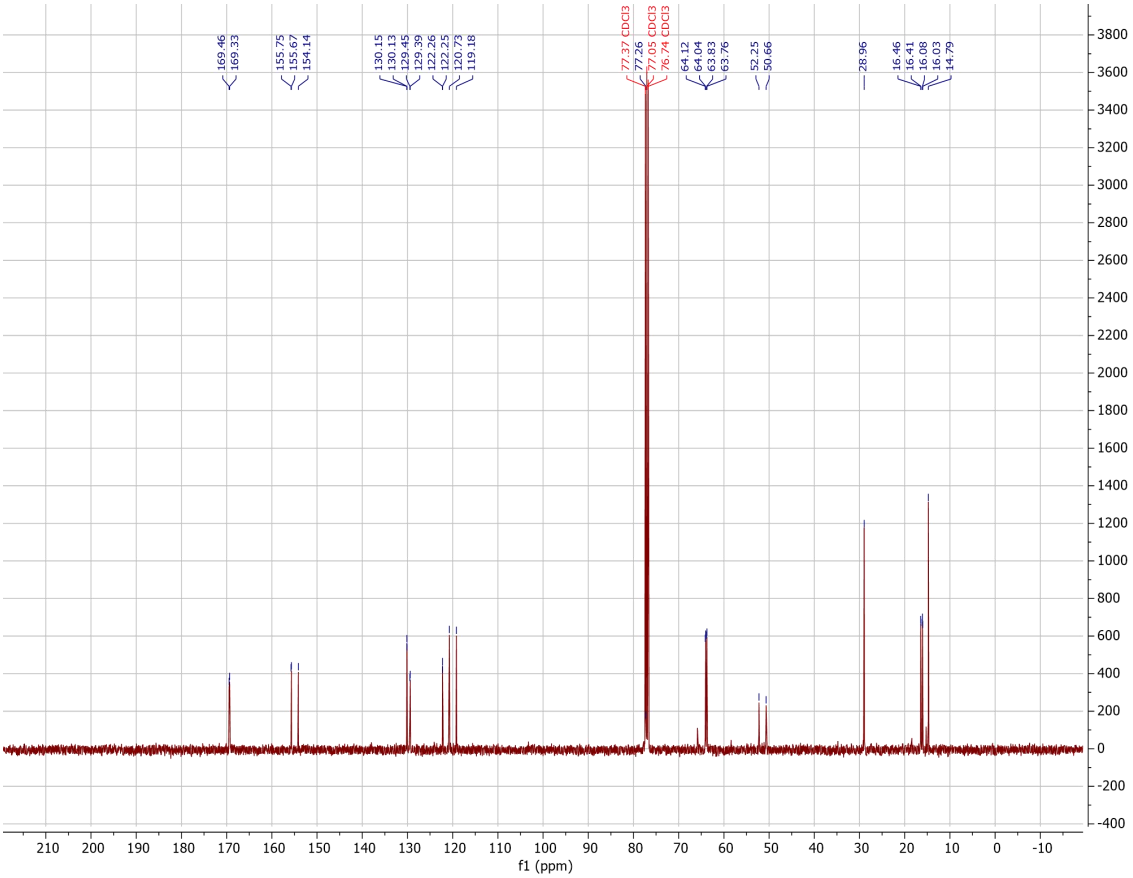


Figure II-21: ^{13}C NMR Spectrum of diethyl (((5-(ethylthio) -1,3,4-thiadiazol-2-yl) amino) (2-hydroxyphenyl) methyl)phosphonate **(5c)**.

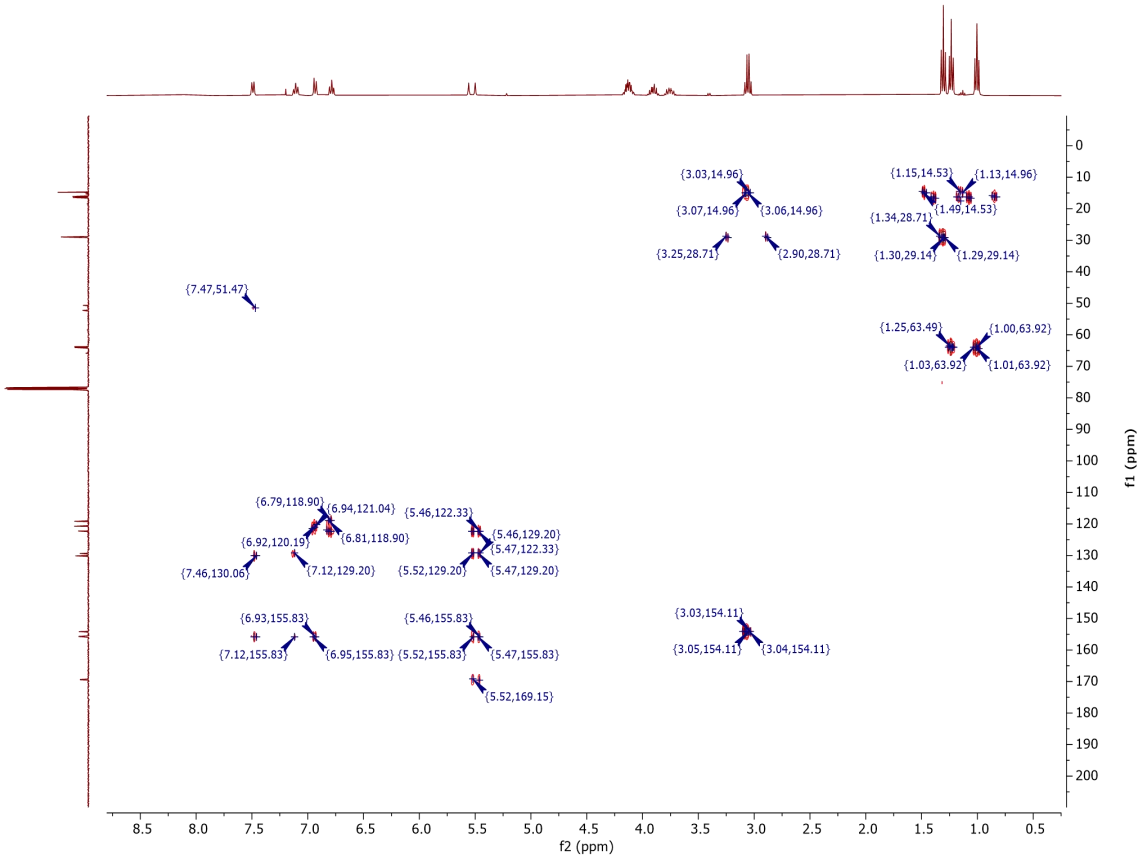


Figure II-22: 2D NMR [HMBC] Spectrum of diethyl (((5-(ethylthio) -1,3,4-thiadiazol-2-yl) amino) (2-hydroxyphenyl) methyl)phosphonate**(5c)**.

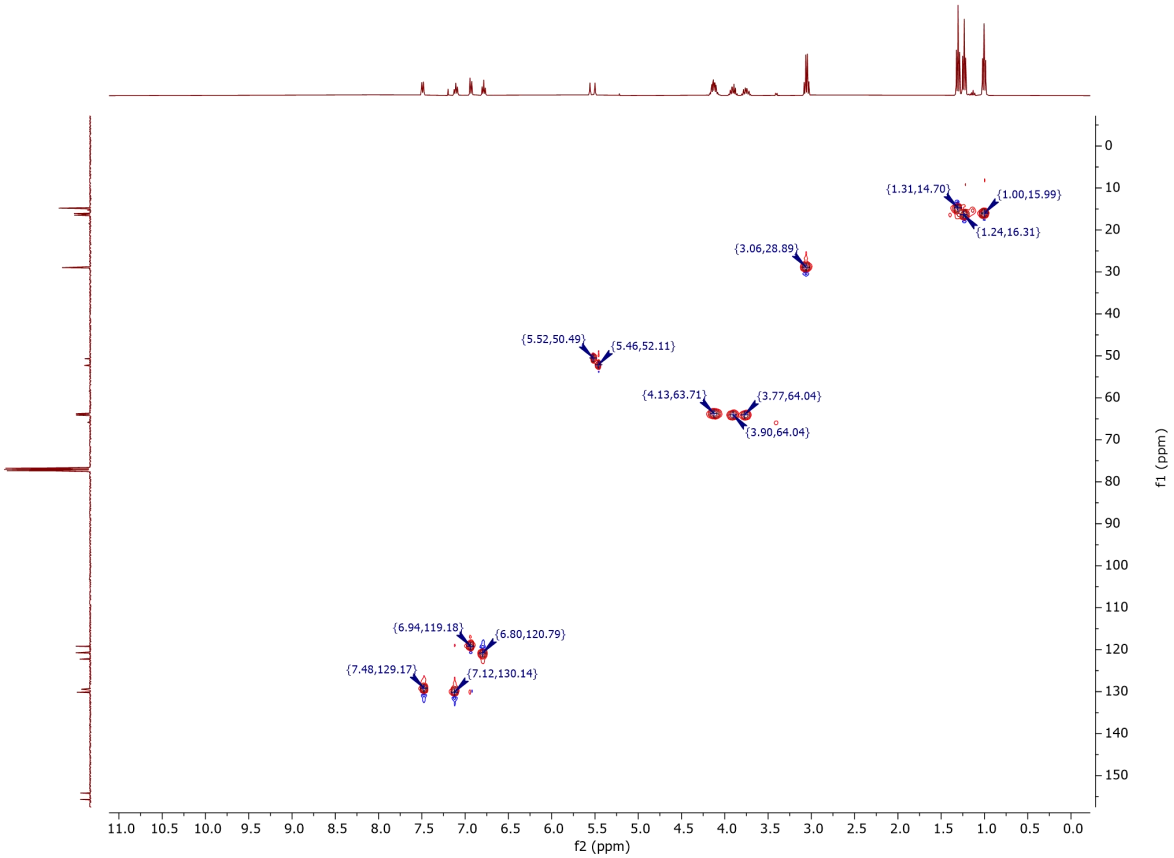


Figure II-23: 2D NMR [HSQC] Spectrum of diethyl (((5-(ethylthio) -1,3,4-thiadiazol-2-yl) amino) (2-hydroxyphenyl) methyl)phosphonate **(5c)**.

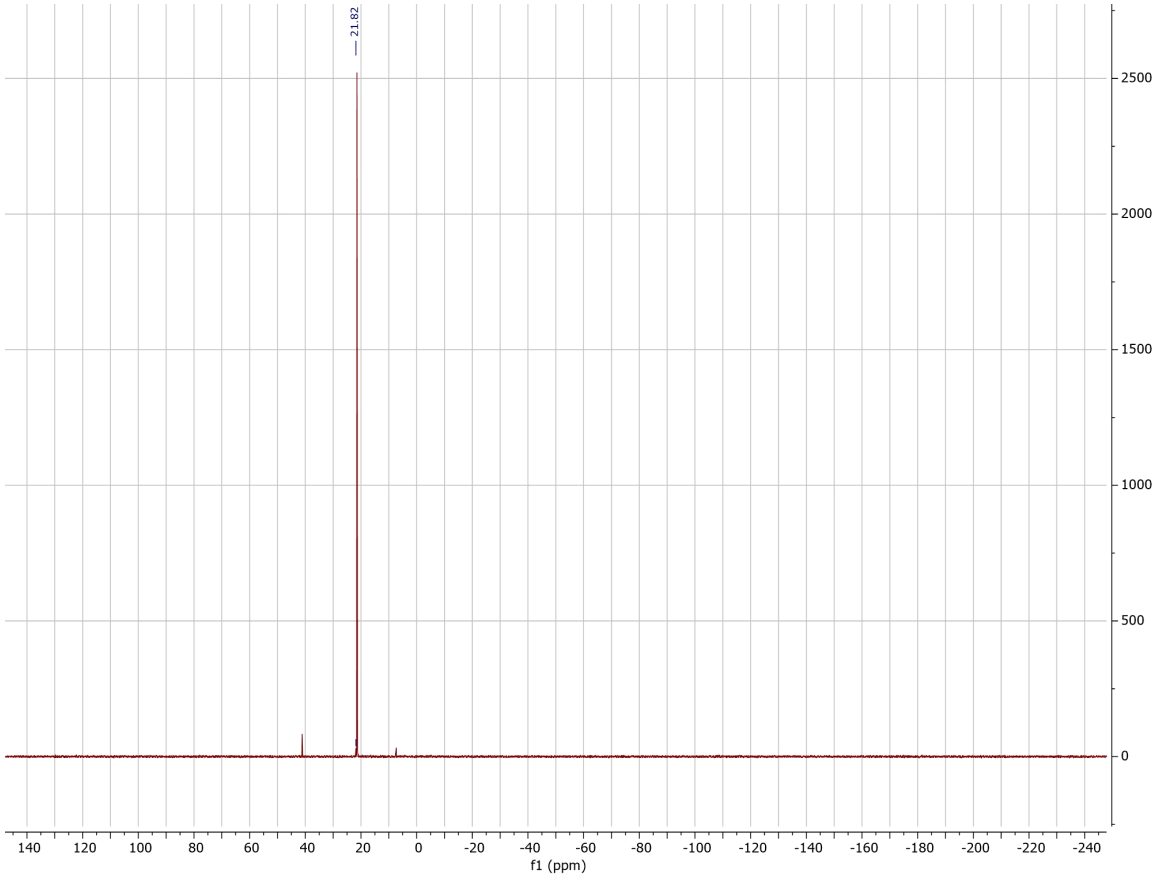


Figure II-24: ^{31}P NMR Spectrum of diethyl (((5-(ethylthio) -1,3,4-thiadiazol-2-yl) amino) (2-hydroxyphenyl) methyl)phosphonate **(5c)**.

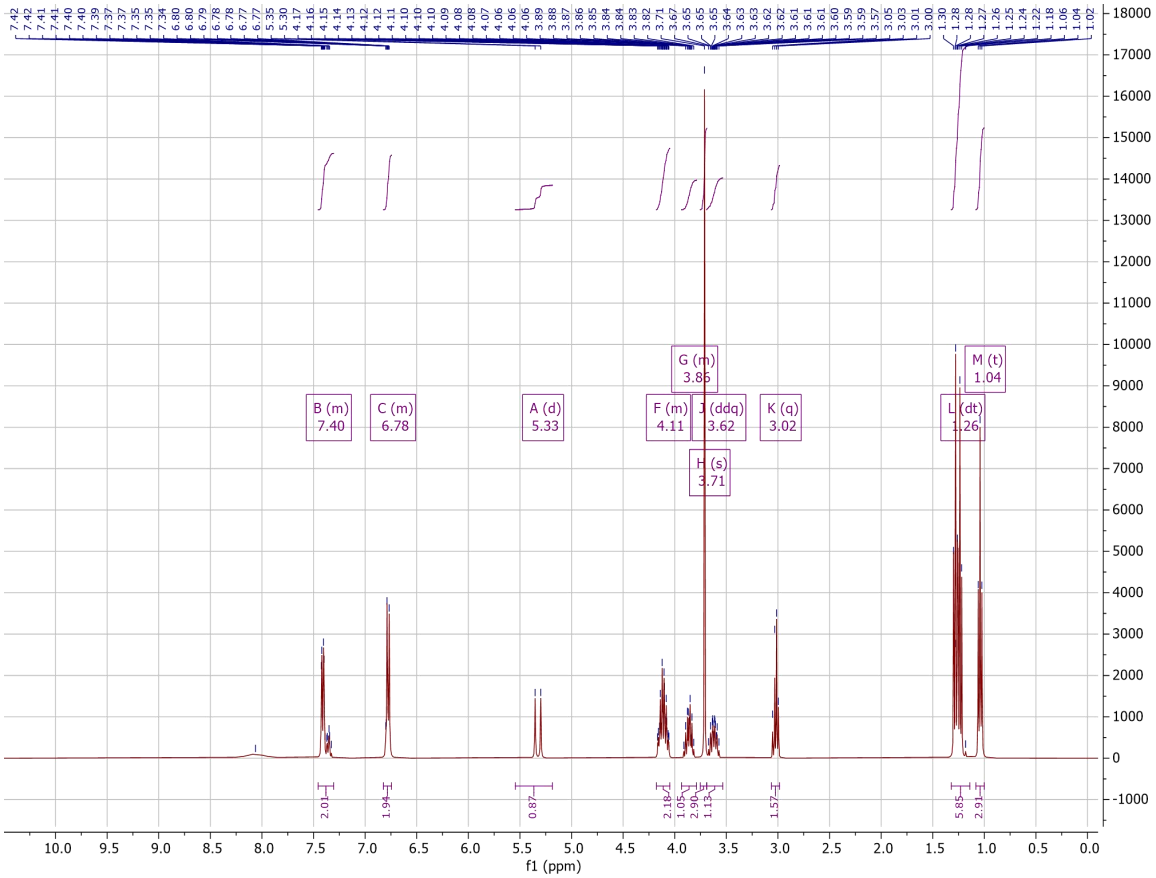


Figure II-25 : ^{1}H NMR Spectrum of diethyl (((5-(ethylthio) -1,3,4-thiadiazol-2-yl)amino)(4-methoxyphenyl)methyl)phosphonate **(3c)**.

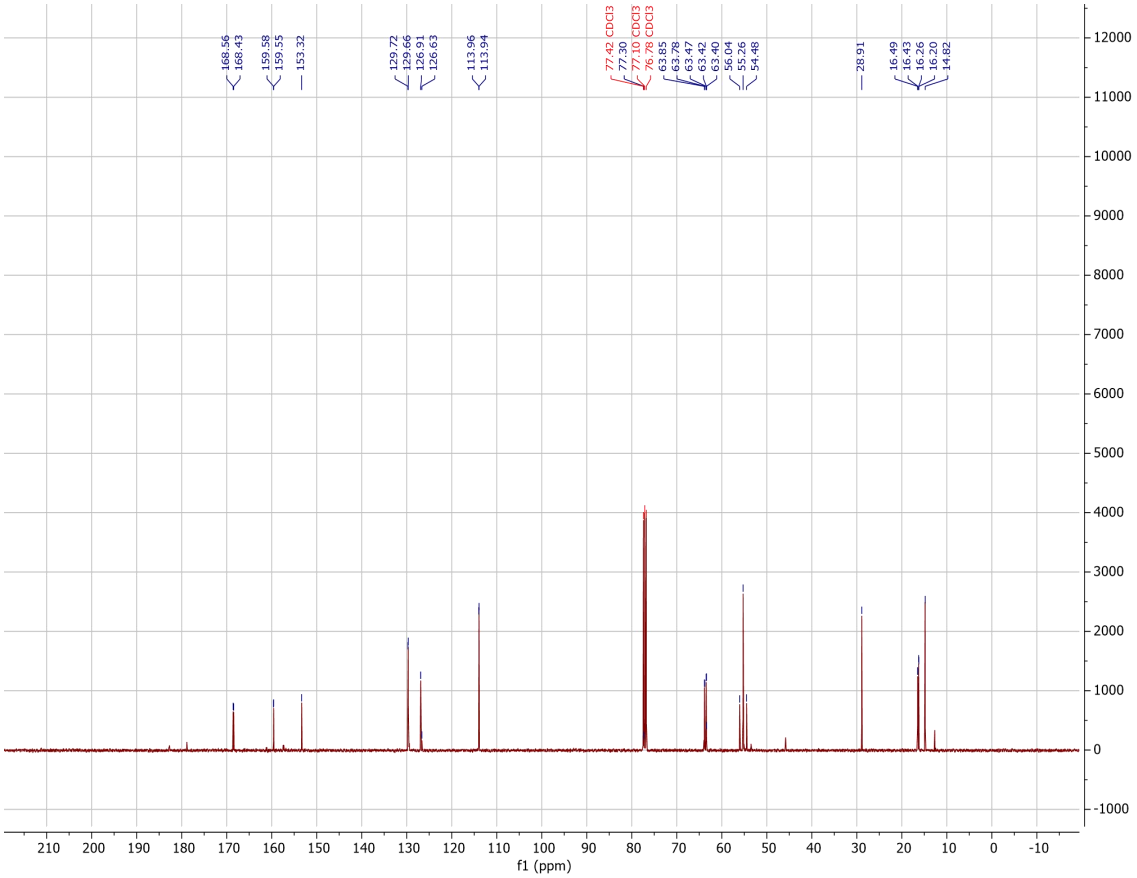


Figure II-26: ^{13}C NMR Spectrum of diethyl (((5-(ethylthio) -1,3,4-thiadiazol-2-yl) amino) (4-methoxyphenyl) methyl) phosphonate **(3c)**.

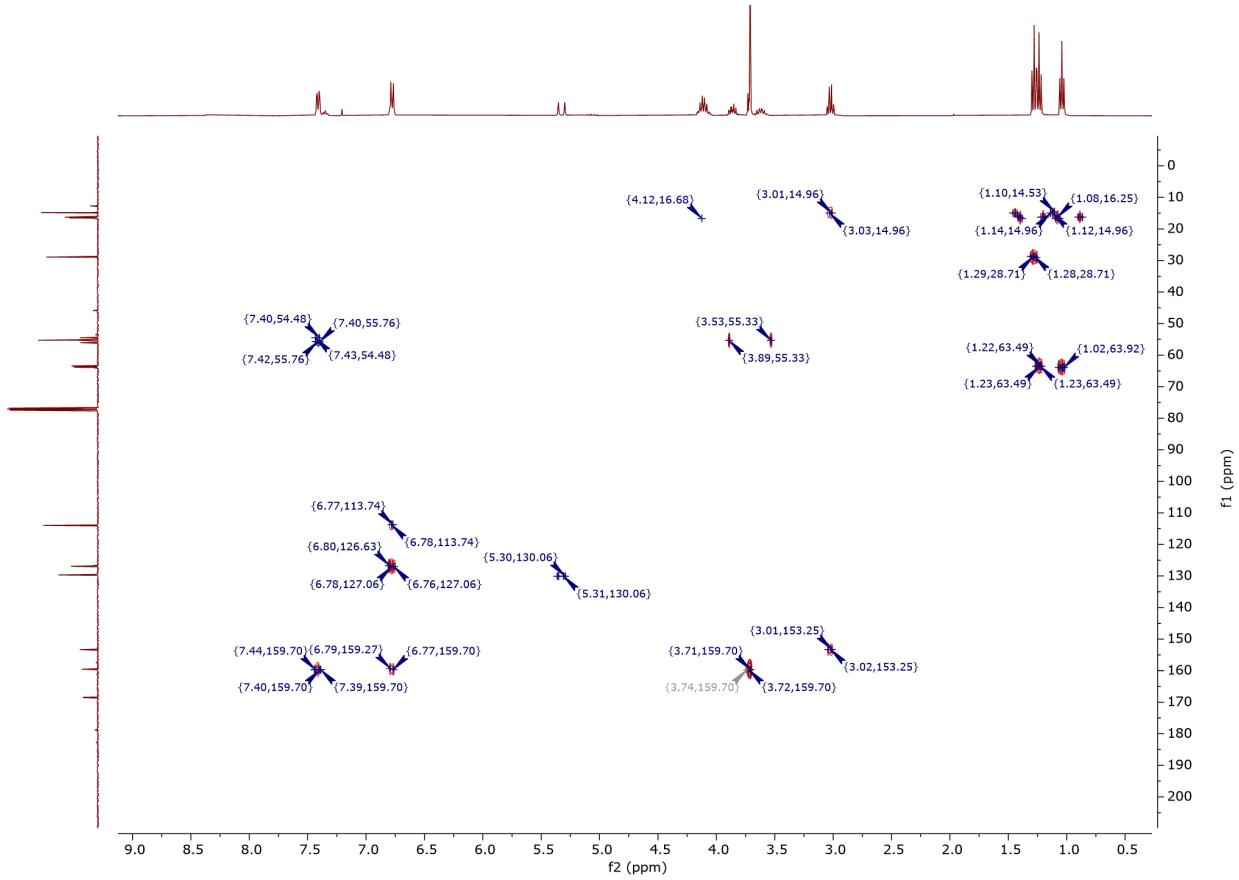


Figure II-27:2D NMR [HMBC] Spectrum of diethyl (((5-(ethylthio) -1,3,4-thiadiazol-2-yl) amino) (4-methoxyphenyl)methyl)phosphonate **(3c)**.

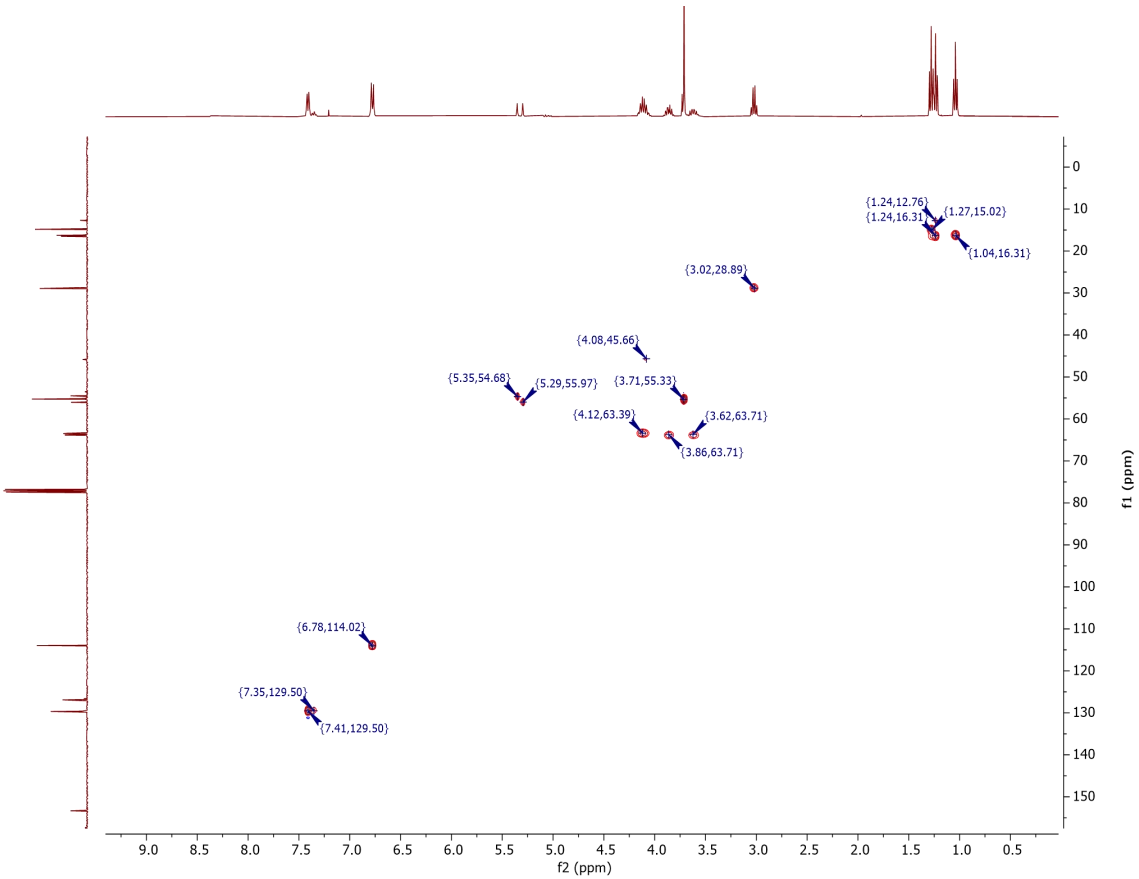


Figure II-28:2D NMR [HSQC] Spectrum of diethyl (((5-(ethylthio) -1,3,4-thiadiazol-2-yl) amino) (4-methoxyphenyl)methyl)phosphonate **(3c)**.

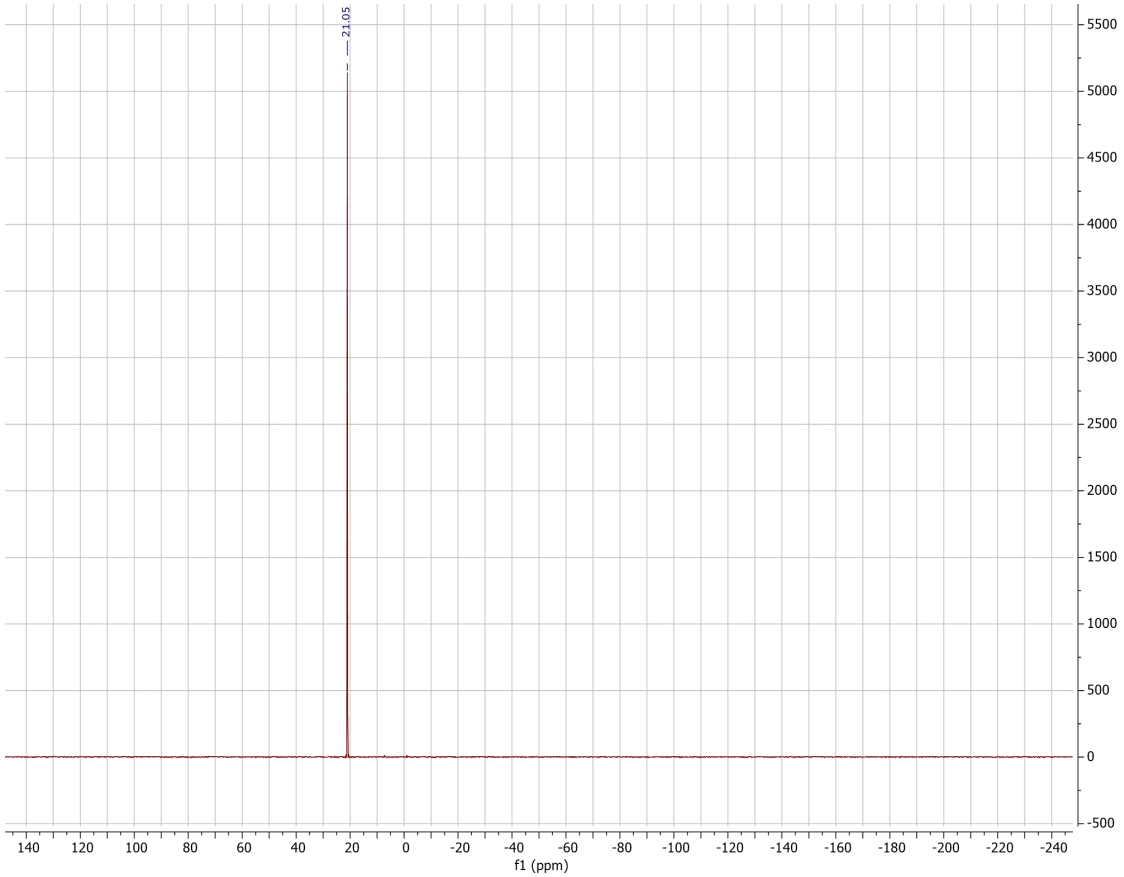


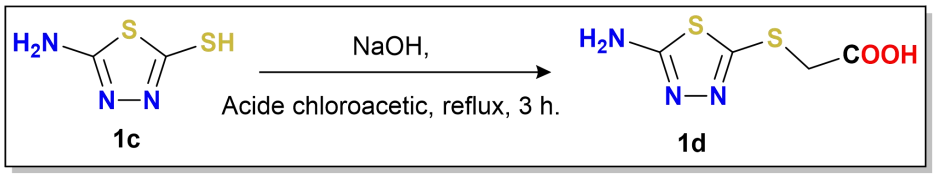
Figure II-29: ^{31}P NMR Spectrum of diethyl (((5-(ethylthio) -1,3,4-thiadiazol-2-yl) amino) (4-methoxyphenyl)methyl)phosphonate **(3c)**.

II.1.3. Synthesis of 2-((5-amino-1,3,4-thiadiazol-2-yl)thio)acetic acid

Surprisingly, we didn't come across the synthesis of functionalized 1,3,4-thiadiazol with carboxylic acid in the literature. In this context, our research is based on the development of novel potential biomolecules.

Herein, we report the synthesis of 2-((5-amino-1,3,4-thiadiazol-2-yl)thio)acetic acid, in order to obtain two single biologically modified entities 1,3,4-thiadiazol & modified acetic acid and investigations for their anti-inflammatory potentials *via* BSA denaturation.

The synthesis of target compounds was achieved using synthetic procedures depicted in **(Scheme II-5)**. The starting material, chloroacetic acid, was subjected to nucleophilic substitution reaction with **(1c)** to generate 2-((5-amino-1,3,4-thiadiazol-2-yl)thio)acetic acid **(1d)** with 83% of yield.



Scheme II-5:synthesis of 2-((5-amino-1,3,4-thiadiazol-2-yl)thio)acetic acid **(1d)**.

^{1}H, ^{13}C, HMBC, and HSQC nuclear magnetic resonance (NMR) were used to characterize the target compound. The appearance peak of a singlet peak at 3.91 ppm (attributable to the **CH_2** moiety of acid) and the singlet peak around 7.29ppm is attributed to the amine proton (**NH_2**) on the proton NMR of compound **((3c).1d)** implies that the nucleophilic substitution reaction was successful and was achieved on the thiol group but not on the amine group.

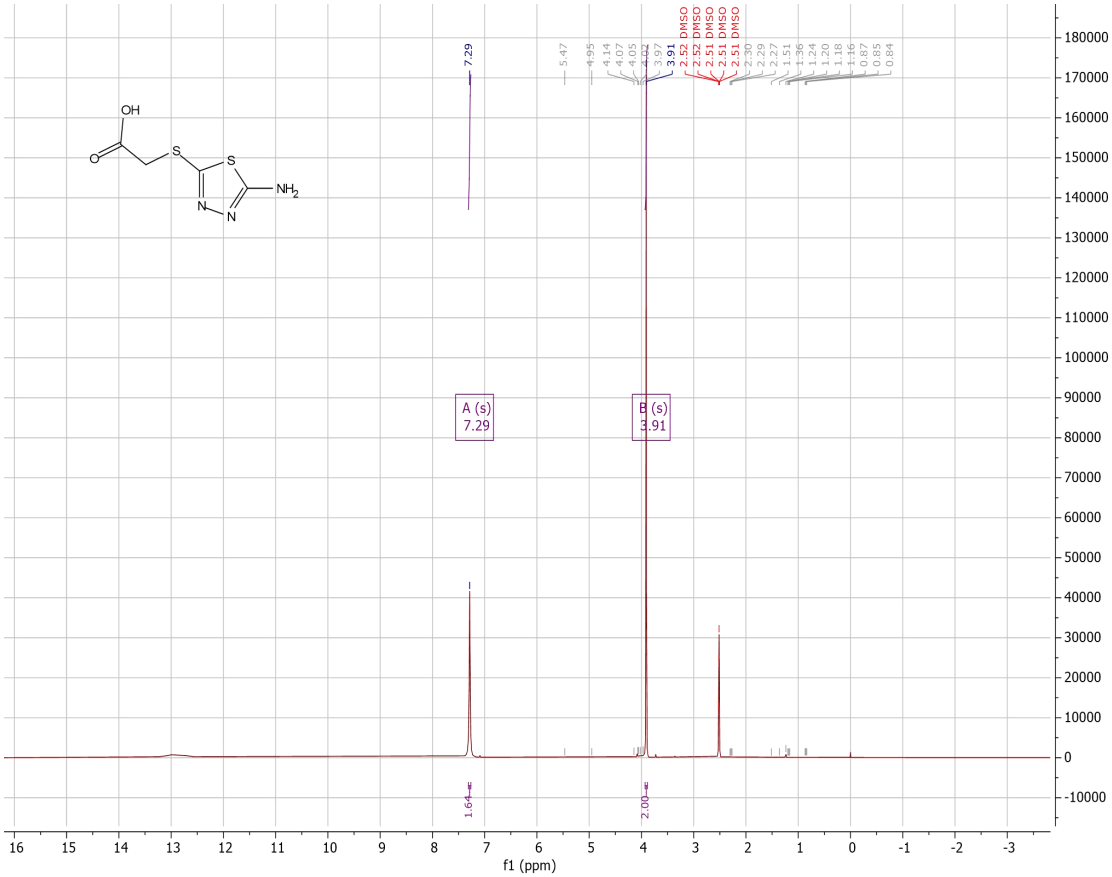


Figure II-30 : ^{1}H NMR spectrum of 2-((5-amino-1,3,4-thiadiazol-2-yl)thio)acetic acid **(1d)**.

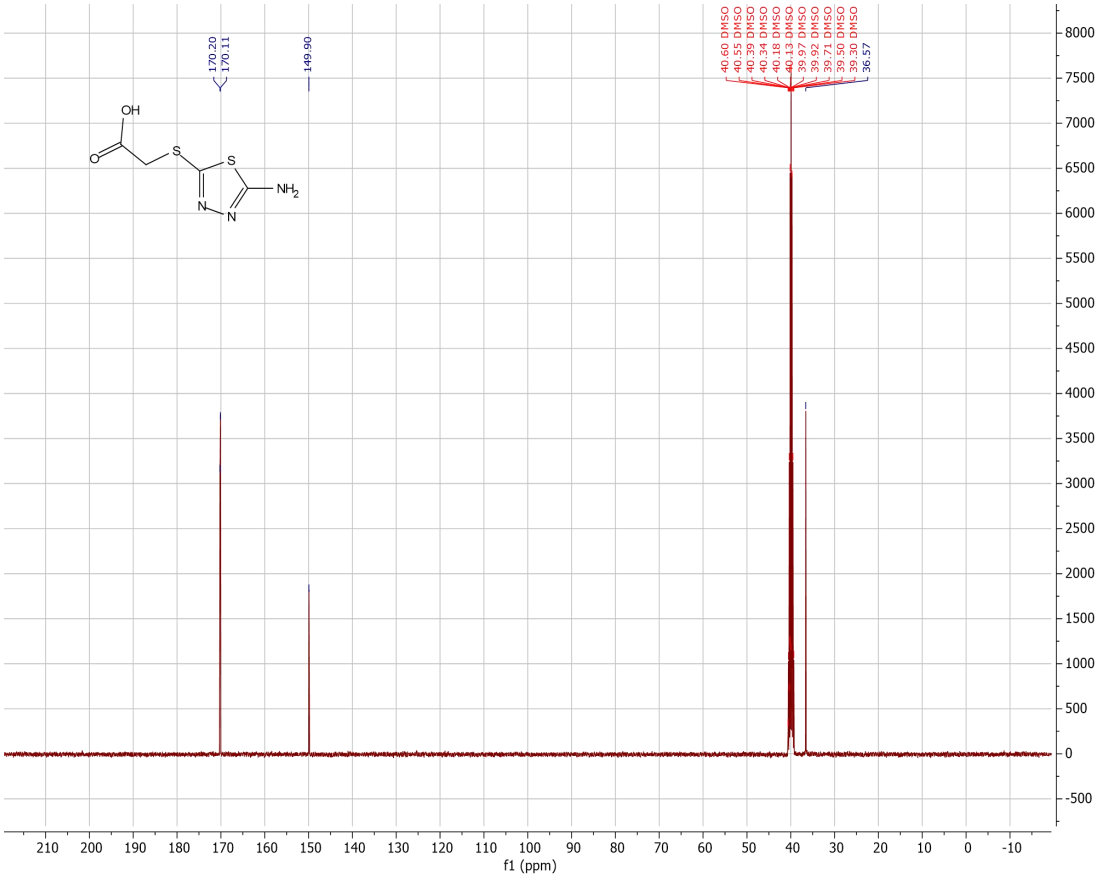


Figure II-31 : ^{13}C NMR spectrum of 2-((5-amino-1,3,4-thiadiazol-2-yl)thio)acetic acid **(1d)**.

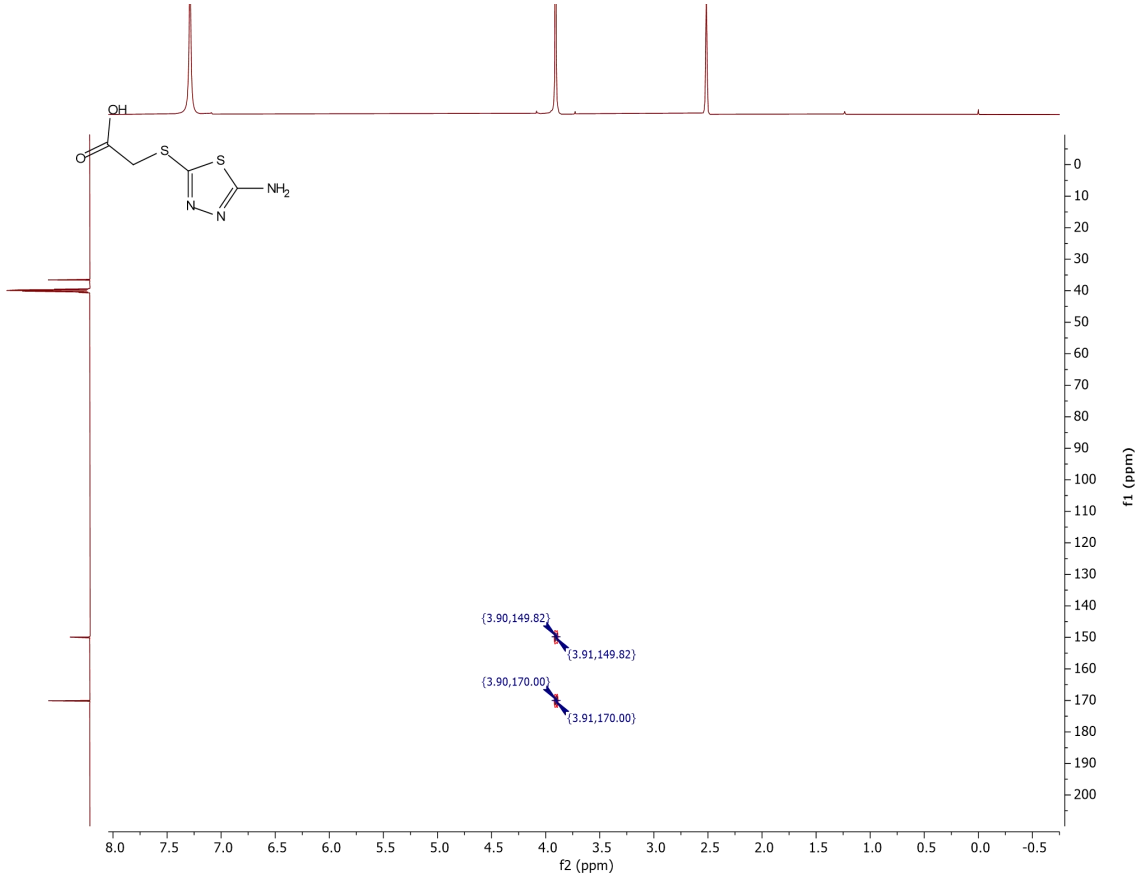


Figure II-32 : 2D NMR [HMBC] spectrum of 2-((5-amino-1,3,4-thiadiazol-2-yl)thio)acetic acid **(1d)**.

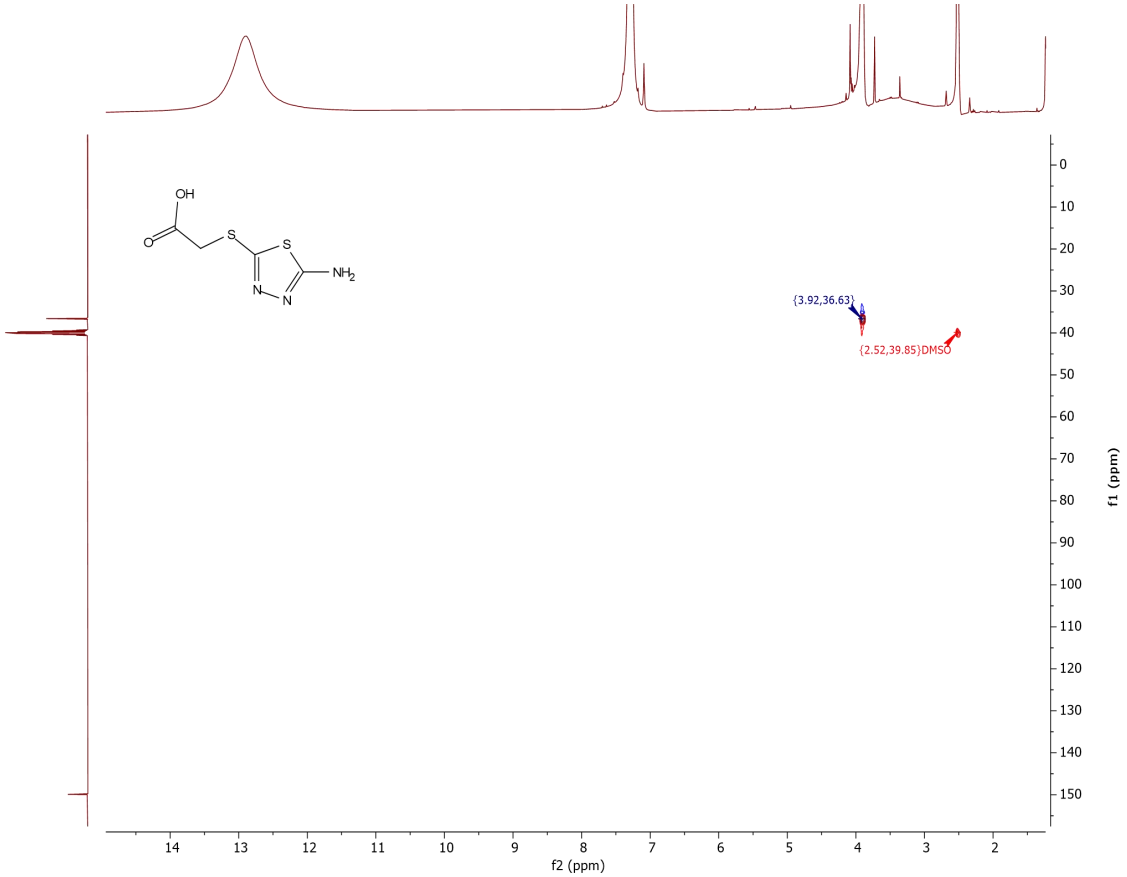


Figure II-33: 2D NMR [HSQC] spectrums of 2-((5-amino-1,3,4-thiadiazol-2-yl)thio)acetic acid **(1d)**.

II.2. Biological Activities

II.2.1. In-vitro Anti-inflammatory activity of synthesized compounds

Inflammation is the immune system's required defensive response for sustaining tissue homeostasis under diverse unfavorable situations[98]. Inflammation resolution is necessary for health because unresolved inflammation can contribute to the pathogenesis of chronic inflammatory and autoimmune diseases such as asthma, psoriasis, and rheumatoid arthritis[99].

Every year, many people suffer from these diseases, which are a serious global public health concern. Analgesics and anti-inflammatories are required for treating inflammatory diseases[100]. There is an ongoing need to develop novel anti-inflammatory drugs with higher effectiveness and safety qualities while avoiding side effects, particularly for long-term treatment.

Organophosphorus compounds, particularly α-aminophosphonates, have been recognized for their biological and pharmacological potential[101]. These compounds are known for their

[98] Medzhitov, R. **(2010)**. Inflammation 2010: new adventures of an old flame. *Cell*, *140*(6), 771-776.
[99] Lang, Y., Chu, F., Shen, D., Zhang, W., Zheng, C., Zhu, J., & Cui, L. **(2018)**. Role of inflammasomes in neuroimmune and neurodegenerative diseases: a systematic review. *Mediators of inflammation*, *2018*(1), 1549549.
[100] Romero-Estudillo, I., Viveros-Ceballos, J. L., Cazares-Carreño, O., González-Morales, A., de Jesús, B. F., López-Castillo, M., ... & Ordóñez, M. **(2019)**. 000Synthesis of new α-aminophosphonates: Evaluation as anti-inflammatory agents and QSAR studies. *Bioorganic & Medicinal Chemistry*, *27*(12), 2376-2386.
[101] Chen, Y. Y., Bai, Y. P., Li, B., Zhao, X. B., Yang, C. J., Liu, Y. Q., ... & Xu, C. R. **(2021)**. Design and synthesis of novel 20 (S)-α-aminophosphonate derivatives of camptothecin as potent antitumor agents. *Bioorganic Chemistry*, *114*, 105065.

reduced toxicity[102], antifungal activity[103-104], antibacterial activity[105-106], antitumor effects[107-108-109], and antiviral activity[110], have gained more attention and are an important class of compounds[111].

❖ *Results:*

In vitro, the anti-inflammatory effect of the synthesized compound samples was evaluated by BSA denaturation, and the results are shown in **(Table II-3)**. The results showed that **(1d)** exhibited the highest activity against protein denaturation when compared to Voltarene; it gives a very high level of inhibition of 83,13%, followed by the compound α-aminophosphonate derivative of 2,3-dimethoxybenzaldehyde **(6c)** with a percentage inhibition of 52,61%.

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[102] Oniţa, N., Şişu, I., Penescu, M., Purcarea, V. L., & Kurunczi, L. **(2010)**. Synthesis, characterization and biological activity of some α-aminophosphonates. *SYNTHESIS*, *58*, 5.

[103] Qin, Y., Xing, R., Liu, S., Yu, H., Li, K., Hu, L., & Li, P. **(2014)**. Synthesis and antifungal properties of (4-tolyloxy)-pyrimidyl-α-aminophosphonates chitosan derivatives. *International Journal of Biological Macromolecules*, *63*, 83-91.

[104] Aissa, R., Guezane-Lakoud, S., Gali, L., Toffano, M., Ignaczak, A., Adamiak, M., ... & Aribi-Zouioueche, L. **(2022)**. New promising generation of phosphates α-aminophosphonates: Design, synthesis, in-vitro biological evaluation and computational study. *Journal of Molecular Structure*, *1247*, 131336.

[105] Dake, S. A., Raut, D. S., Kharat, K. R., Mhaske, R. S., Deshmukh, S. U., & Pawar, R. P. **(2011)**. Ionic liquid promoted synthesis, antibacterial and in vitro antiproliferative activity of novel α-aminophosphonate derivatives. *Bioorganic & medicinal chemistry letters*, *21*(8), 2527-2532.

[106] Alotaibi, S. H., & Amer, H. H. **(2020)**. Synthesis, spectroscopic and molecular docking studies on new schiff bases, nucleosides and α-aminophosphonate derivatives as antibacterial agents. *Saudi Journal of Biological Sciences*, *27*(12), 3481-3488.

[107] Ma, J., Li, J., Guo, P., Liao, X., & Cheng, H. **(2021)**. Synthesis and antitumor activity of novel indole derivatives containing α-aminophosphonate moieties. *Arabian Journal of Chemistry*, *14*(8), 103256.

[108] Guo, Y. C., Li, J., Ma, J. L., Yu, Z. R., Wang, H. W., Zhu, W. J., ... & Zhao, Y. F. **(2015)**. Synthesis and antitumor activity of α-aminophosphonate derivatives containing thieno [2, 3-d] pyrimidines. *Chinese Chemical Letters*, *26*(6), 755-758.

[109] Huang, X. C., Wang, M., Pan, Y. M., Tian, X. Y., Wang, H. S., & Zhang, Y. **(2013)**. Synthesis and antitumor activities of novel α-aminophosphonates dehydroabietic acid derivatives. *Bioorganic & medicinal chemistry letters*, *23*(19), 5283-5289.

[110] Xie, D., Zhang, A., Liu, D., Yin, L., Wan, J., Zeng, S., & Hu, D. **(2017)**. Synthesis and antiviral activity of novel a-aminophosphonates containing 6-fluorobenzothiazole moiety. *Phosphorus, Sulfur, and Silicon and the Related Elements*, *192*(9), 1061-1067.

[111] Ghosh, R., Maiti, S., Chakraborty, A., & Maiti, D. K. **(2004)**. In (OTf) 3 catalysed simple one-pot synthesis of α-amino phosphonates. *Journal of Molecular Catalysis A: Chemical*, *210*(1-2), 53-57.

Table II- 3: The in vitro anti-inflammatory effect of the synthesized compounds.

Sample	Inhibition %
Voltarene (1000µg/mL)	85,27
6c (2000µg/mL)	52,61
5c (2000µg/mL)	27,60
3c (2000µg/mL)	22,73
1d (2000µg/mL)	83,13

The majority of our data suggests that the compounds revealed to have anti-inflammatory activities in the present study have structural similarities and/or are derivatives of phosphonates and thiadiazol motifs. This group of molecules may be a starting point for the development of novel compounds to reduce the deleterious effects of inflammatory diseases.

II.2.2. In-vitro Anti-oxidant activity of synthesized compounds

In this work, we evaluate the antioxidant potential of some synthesized compounds, which were tested using *in vitro* DPPH and SPF (Sun Protection Factor assays).

❖ *DPPH radical scavenging activity*

The free radical scavenging activity of the synthesized molecules was realized by the DPPH (1,1-diphenyl-2-picrylhydrazyl) to quantify free radical scavenging ability using Blois methodology[112]. The samples were dissolved in ethanol to achieve an initial concentration of 4 mM and were plated out in triplicate. A well was filled with 400 µL of samples and 1600 µL of DPPH solution. A negative control was obtained by replacing the inhibitors with ethanol. The reaction mixture was incubated for 30 minutes at room temperature in darkness. Then, the absorbance was determined using a spectrophotometer at 517nm. BHA and BHT were used as reference compounds and the inhibition percentage of DPPH was calculated via **Equation (1)**.

$$PI\% = [(Abs0 - Abs1) / Abs0] \, x \, 100 \ldots(1)$$

[112] Blois, M. S. **(1958)**. Antioxidant determinations by the use of a stable free radical. *Nature*, *181*(4617), 1199-1200.

Where :

PI= the percentage of anti-radical activity.

Abs_0= the absorbance of the control reaction.

Abs_1= the absorbance in the presence of a standard sample.

Table II- 4: Antiradical activity of DPPH• and percentage inhibition.

Compounds	DPPH radical scavenging IC_{50} (µg/mL)
2	NA
3a	NA
5a	198.45±0.30
6a	>400
5c	NA
6c	>400
3c	225.75±4.67
1d	240±0.43
BHT *	12.99±0.41
BHA *	6.14±0.41

NA: no activity, * reference compounds, IC_{50}: 50% inhibition concentration of DPPH•

The DPPH spectrophotometric antiradical colorimetric test is applied to measure and determine the antioxidant activity of the different compounds. The results are shown in **(Table II-4)** expressed as the IC_{50} of DPPH activity calculated from the graph of antioxidant activity percentage against product concentration. It demonstrates that except for the three molecules **(5a)**, **(3c)**, and **(1d)**, which exhibit moderate antioxidant properties in all the remaining compounds owing to the presence of the hydroxyl and methoxy groups in the peripheral benzene ring, they influence reducing DPPH activity via their ability to generate hydrogen.

❖ *Study of Sun protection factor (SPF)*

Sun Protection Factor (SPF) is an important indicator of how efficiently a sunscreen protects the skin from the damaging effects of ultraviolet (UV) radiation, the SPF solely measures protection versus UVB radiation, which is the main cause of sunburn. nevertheless,

both UVA and UVB radiations may lead to skin damage and enhance the chance of skin cancer. Understanding SPF is necessary for making better choices regarding sunscreen and skincare.

Every year, over one million people are diagnosed with skin cancer, with around 10,000 dying from malignant *melanoma*. Most skin cancers develop around parts of the body that are frequently exposed to the sun, such as the face, neck, head, and the back of the hands[113]. Solar radiation is primarily damaging in the ultraviolet (UV) part of the electromagnetic spectrum. Long-term exposure to UV light causes oxidative stress.

It should be mentioned that the spectral range runs from 200 to 400 nm. Three types of UV radiation have been identified based on their biological activities and penetration power into the skin:

UV-A: wavelengths 320–400 nm,

UV-C: wavelengths 200–280 nm,

UV-B radiation, with a wavelength of 290 to 320nm, is extremely dangerous to human skin and is primarily responsible for sunburn and bronzed skin. When human skin is exposed to UV-B radiation, keratinocyte DNA absorbs it [114].

- ***Sun Protection Factor (SPF)***

The sun protection factor (SPF) measures how effective sunscreens are. The SPF is determined by the UV energy needed to produce a minimum erythematic dose (MED) in protected skin divided by the UV energy required to produce an unprotected MED (Equation 2)[115] The higher the SPF, the more effective the sunscreen is at preventing sunburn.

$$\text{SPF}=\frac{\text{dose capable of causing an erythema on the protected skin}}{\text{dose capable of causing erythema on unprotected skin}}\ldots(2)$$

The minimum erythema dose (MED) is the amount of UV radiation that causes the smallest erythema (sunburn or redness caused by capillary blockage) on a human's skin within a few hours of sun exposure[116].

[113]Allen, M. W., & Bain, G. **(1994)**. Measuring the UV protection factor of fabrics. Retrieved March 25, 2008.
[114] Mishra, A. K., Mishra, A., & Chattopadhyay, P. **(2011)**. Herbal cosmeceuticals for photoprotection from ultraviolet B radiation: A review. *Tropical Journal of Pharmaceutical Research*, *10*(3).
[115] Shaath, N. **(2005)**. *Sunscreens: Regulations and commercial development*. CRC Press.
[116] Heckman, C. J., Chandler, R., Kloss, J. D., Benson, A., Rooney, D., Munshi, T., ... & Oslin, D. W. **(2013)**. Minimal erythema dose (MED) testing. *JoVE (Journal of Visualized Experiments)*, (75), e50175.

➢ *Determination of sun protection factor (SPF)*

The sun protection factor (SPF) of a sunscreen is a measure of the photoprotector's effectiveness against the effects of short-term UV exposure. This factor represents the level of UV-B protection provided by the tested product. The SPF of the compounds was determined by spectrophotometric analysis of the diluted solution (2 mg/mL). The compounds were dissolved in ethanol or DMSO, and absorbance values were measured at 290-320 nm at 5 nm intervals in 1cm quartz cuvettes using a UV-Vis spectrophotometer. The SPF value was obtained by applying the equation of Mansur et al. (1986). SPF values were calculated with the Mansur equation[117] (Equation 3).

$$SPF = CF \sum_{290}^{320} EE(\lambda) \cdot I(\lambda) \cdot Abs(\lambda) \ldots (3)$$

CF: correction factor (= 10).

EE = erythemogenic effect.

I = intensity of the sun.

Abs = absorbance of the sample.

Table II- 5: Normal function of the constants used to calculate SPF[118].

Wavelength λ (nm)	EE (λ) x I(λ) (Normes)
290	0,0150
295	0,0817
300	0,2874
305	0,3278
310	0,1864
315	0,0837
320	0,0180
Total	1

[117] Rezine, F., & Fedaouche, M. S. **(2017)**. Coumarines a intérêt thérapeutique: synthèse et contrôle analytique. *Université Abou Bekr Belkaîd. Faculte De Médecine Dr Benzerdjeb B. Tlemcen*.

[118] Remesy, C., Manach, C., Demigne, C., Texier, O., & Regerat, F. **(1996)**. Intérêt nutritionnel des flavonoïdes. *Médecine et nutrition*, *32*(1), 17-27.

Table II- 6: Protection categories displayed on solar products based on measured protection factors, according to the European Commission Recommendation 2006.

Indicated category	Indicated protection factor	Measured sun protection factor
« Low protection »	6	6 - 9,9
	10	10 - 14,9
« Medium protection »	15	15 - 19,9
	20	20 - 24,9
	25	25 - 29,9
« High protection »	30	30 - 49,9
	50	50 - 59,9
« Very high protection »	50+	60 ≤

The obtained results of the tested compounds were compared with two sunscreens (Nivea and Vichy) and are listed in **(Table II-7)**.

Table II- 7: Photoprotective efficacy of tested compounds.

Compounds	SPF
2a	31.33±0.04
3a	32.34±0.19
5a	35.58±0.16
6a	36.80±0.19
3c	37.28±1.03
5c	36.91±1.10
6c	37.43±1.04
1d	37.82±0.48
Vichy *	50.11±0.53
Nivea *	44.22 ±0.35

* Reference compounds

Based on the SPF values obtained, it is found that the photoprotective activity of the compounds ranges from 31.33 to 37.82, which corresponds to high protection according to the European Commission Recommendation, located in the range of [30 - 49.9] (Table II-6). The

compounds 1d, 3c, and 6c show the highest photoprotective activity against UV-B among all of the tested compounds, with SPF values of 37.82±0.48, 37.28±1.03, and 37.43±1.04, respectively.

Conclusion

In conclusion, a simple and efficient MCRs reaction was used for the synthesis of novels of α-(pyridyl)sulfamidofosphonate and (1, 3, 4-thiadiazolyl)α-aminophosphonate derivatives using various structurally Benzaldehydes, triethyl phosphite without the use of any chromatographic purification. The corresponding compounds were obtained with moderate yields and were determined based on NMR spectrums.

The obtained products were evaluated for their *in-vitro* antioxidant (DPPH and SPF) and anti-inflammatory activities.

General Conclusion

General Conclusion

The aim of this work was to synthesize α-aminophosphonates derived from *N*-sulfamoyl pyridine and thiadiazole *via* the *Kabachnik-Fields* reaction. We began our study with a bibliographic overview of organophosphorus compounds, highlighting their importance, and more specifically, the α-aminophosphonates, which hold a significant position in organic and medicinal chemistry.

For the first time, we successfully incorporated sulfamides derived from pyridine with the α-aminophosphonate motif. We synthesized α-aminophosphonates derived from 5-(ethylthio)-1,3,4-thiadiazol-2-amine using a simple synthesis method with acceptable yields, a relatively short reaction time, and without chromatographic purification, which is recommended in organic synthesis. We also conducted a preliminary study of the antioxidant (DPPH and SPF) and anti-inflammatory activities *in vitro* of some synthesized products to anticipate structure-activity relationships. The structure of the synthesized products was well confirmed by spectroscopic methods: ^{1}1H NMR, ^{13}C NMR, HSQC, and HMBC.

Chapter III: Experimental

III. GENERAL CONDITIONS

All chemicals and solvents used were sourced from Sigma-Aldrich and were used without purification unless otherwise stated.

III.1.Chromatography

Reaction monitoring was performed by thin-layer chromatography (TLC) on Merck 60 F254 silica gel plates (Art. 5554), visualized using UV light and a detector (ninhydrin).

III.2. Nuclear Magnetic Resonance (NMR)

NMR spectra (^{1}H, ^{13}C, HSQC, and HMBC) were recorded at room temperature on a Brüker AC 400 MHz instrument. The deuterated solvents used are indicated in each case. Chemical shifts (δ) are expressed in parts per million (ppm) and coupling constants (J) in Hz. Multiplicities are designated by the following abbreviations: s (singlet), sl (broad singlet), d (doublet), dd (double doublet),ddd (double double doublet), t (triplet), q (quadruplet), and m (multiplet, for multiplicities > 4).

III.3. Synthesis Procedures and Spectroscopic Data

III.3.1.General procedure of synthesis of 1a and 1b

The sulfamide *N*-Boc was prepared according to the literature[119], to a flask filled with dichloromethane (10 mL), was added chlorosulfonyl isocyanate (12 mmol) at room temperature. The reaction mixture was cooled to about 0°C and a solution of tertio-butanol (12,12 mmol) in dichloromethane (3 mL) was added dropwise while maintaining the temperature between 0 and 10°C. The reaction mixture was stirred at the same temperature for 30 min. A mixture of 2-aminopyridine or 3-aminopyridine (12 mmol) and triethylamine (15 mmol) in dichloromethane (14 mL) was then added dropwise while maintaining the temperature between 0 and 10°C. The reaction mixture was decanted and the separated organic layer was washed with 0.1 M aqueous HCl (100 mL) and then with water (100 mL). The organic layer was diluted with 100 mL water and as much as possible of dichloromethane was removed under vacuum at a temperature below 25° C. The resulting suspension was stirred for 2.5 h at room temperature, filtered, rinsed twice with sufficient water, and dried under vacuum overnight to afford **1a** and **1b.**

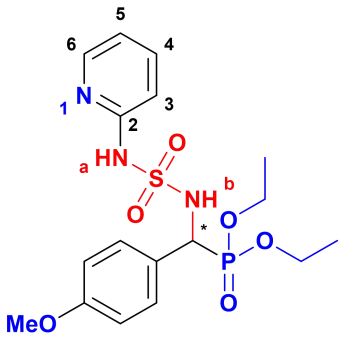
[119] Montero, J. L., Dewynter, G., Agoh, B., Delaunay, B., & Imbach, J. L. **(1983)**. Selective synthesis of sulfonylureas and carboxysulfamides a novel route to oxazolidinones. *Tetrahedron Letters*, *24*(30), 3091-3094.

III.3.2.General procedure of synthesis of 2-(sulfamoylamino) pyridine (2a) *and 3-(sulfamoylamino) pyridine* (2b)

(1 mmol) *N*-Boc sulfamide, kept in a round-bottomed flask, is dissolved in (5 mL) of water and stirred at a temperature between 90 and 100 °C for 10 min. The progress of the reactions is monitored by TLC. Each reaction is then cooled to room temperature. Dichloromethane (5 mL) is added to the mixture. The organic extract is dried over anhydrous magnesium sulfate and concentrated under reduced pressure to give the desired product without purification by silica gel column chromatography.

III.3.3.General procedure of synthesis of α-sulfamidophosphonates

In a 10 mL round-bottom flask, a mixture of benzaldehyde (1 mmol) and **2a** or **2b** (1 mmol) and then triethylphosphite (1 mmol) in 4 mL of ethanol. The reaction mixture was subjected to 80°C for 6 hours. After completion of the reaction, as indicated by TLC, the pure products were crystallized in a mixture of diethyl ether/DCM (4: 1) at room temperature overnight. Finally, the desired product was obtained with modest yields after simple filtration and drying. This procedure was followed for the preparation of all the α-sulfamidophosphonat listed in **(Table II-1)**.

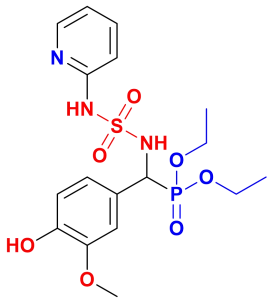


Diethyl((4-methoxyphenyl)((N-(pyridin-2-yl)sulfamoyl)amino)methyl)phosphonate (**3a**): white powder, R_f (DCM/MeOH)(90/10)= 0.35

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (dd, *J* = 5.4, 1.8 Hz, 1H), 7.39 – 7.33 (m, 2H), 7.25 (td, *J* = 2.0, 0.8 Hz, 1H), 6.85 – 6.73 (m, 2H), 6.47 (ddd, *J* = 7.2, 5.0, 0.9 Hz, 1H), 6.35 (dt, *J* = 8.3, 1.0 Hz, 1H), 5.56 (dd, *J* = 9.2, 6.9 Hz, 1H), 5.45 – 5.33 (m, 1H), 4.10 – 3.96 (m, 1H), 3.88 (dp, *J* = 10.1, 7.1 Hz, 1H), 3.68 (s, 4H), 1.15 (t, *J* = 7.1 Hz, 4H), 1.05 (t, *J* = 7.1 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 159.23, 157.10 (J_{C-P} = 11.3 Hz), 147.89, 137.29, 129.20 (J_{C-P}= 5.9 Hz), 128.53 (J_{C-P} = 6.1 Hz), 128.18, 114, 108.52, 70.35 (J_{C-P} = 162.0 Hz), 63.07 (J_{C-P}= 7.0, 4.1 Hz), 55.20, 51.95 (J_{C-P} = 154.0 Hz), 16.31 (J_{C-P} = 15.1, 5.8 Hz).

^{31}P NMR (162 MHz, $CDCl_3$) δ 22.92.



Diethyl ((4-hydroxy-3-methoxyphenyl)((N-(pyridin-2-yl)sulfamoyl) amino) methyl) phosphonate (**5a**): yellow paste, R_f (DCM/MeOH)(90/10)= 0.40

^{1}H NMR (400 MHz, DMSO) δ 8.94 (s, 1H), 7.95 (ddd, J = 5.0, 1.9, 0.8 Hz, $1H_6$), 7.37 (ddd, J = 8.8, 7.0, 1.9 Hz, $1H_4$), 7.31 (dd, J = 10.2, 3.3 Hz, 1H), 7.10 (t, J = 2.0 Hz, 1H), 6.88 (dt, J = 8.2, 2.2 Hz, $1H_3$), 6.76 (dt, J = 8.5, 1.0 Hz, $1H_5$), 6.72 (dd, J = 8.1, 0.8 Hz, $1H_3$), 6.50 (ddd, J = 7.0, 5.0, 1.0 Hz, 1H), 5.79 – 5.66 (m, 1H), 4.08 – 3.82 (m, 3H), 3.82 – 3.67 (m, 4H), 1.17 – 1.02 (m, 6H).

^{13}C NMR (101 MHz, DMSO) δ 157.82 (J_{C-P} = 8.8 Hz), 147.70, 147.48, 146.35, 137.26, 128.13, 121.37, 115.43, 113.06, 113.01, 62.55 (J_{C-P} = 33.4 Hz), 55.98, 50.66 (J_{C-P} = 155.2 Hz), 16.57 (J_{C-P} = 5.5 Hz).

^{31}P NMR (162 MHz, DMSO) δ 23.23.

III.3.1.Synthesis of 5-amino-1, 3, 4-thiadiazole-2-thiol[97]

Thiosemicarbazide (0.25 mmol) was suspended in absolute ethanol and anhydrous sodium carbonate (0.25 mmol) and carbon disulphide 0.75 mmol were added slowly. The mixture was stirred under reflux for 1 h and later heated at 75–80 °C for 4 h. Solvent was removed and the residue was dissolved in water (200 mL), acidified with conc. HCl to give the product as hydrochloride salt, TLC (silica gel), mp 232 °C, yield 84%

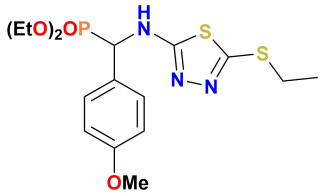
III.3.2. Synthesis of 5-(ethylthio)-1,3,4-thiadiazol-2-amine (2c)

5-amino-1, 3, 4-thiadiazole-2-thiol (0.01 mol) was added to a mixture of bromoethane (0.01 mol) and KOH (0.01 mol) in 100 mL H_2O. The reaction mixture was stirred at room temperature for

12 h. The mixture was neutralized with concentrated HCl, the formed precipitate was then filtered under reduced pressure to afford crude product **(2c)** as a light-yellow powder.

III.3.3. Synthesis α-aminophosphonate derivatives of thiadiazol (3c-6c)

The three-component reaction takes place between 5-(ethylthio)-1,3,4-thiadiazol-2-amine (0.01 mol), benzaldehyde derivatives (0.01 mol), and triethlylphosphite (0.01 mol). The three components were dissolved in 5 mL of EtOH, followed by adding a few drops of acetic acid with continuous stirring under reflux for 12h, the completion of the reaction was followed by TLC. The solvent was evaporated under low pressure, the remaining amount was neutralized with diethyl ether to give the corresponding α-aminophosphonates in moderate yields as indicated in **(Table II-2)**.

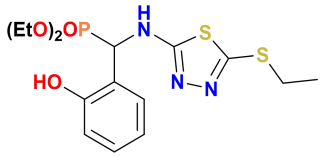


diethyl (((5-(ethylthio)-1,3,4-thiadiazol-2-yl)amino)(4-methoxyphenyl)methyl) phosphonate (3c): R_f (DCM/MeOH)(90/10)= 0.65

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 – 7.30 (m, 2H), 6.82 – 6.74 (m, 2H), 5.33 (d, J = 23.0 Hz, 1H), 4.18 – 4.05 (m, 2H), 3.93 – 3.79 (m, 1H), 3.71 (s, 3H), 3.62 (ddq, J = 10.2, 8.7, 7.0 Hz, 1H), 3.02 (q, J = 7.8 Hz, 2H), 1.26 (dt, J = 16.6, 7.2 Hz, 6H), 1.04 (t, J = 7.0 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 168.50 (J = 13.1 Hz), 159.57 (J = 2.6 Hz), 153.32, 129.69 (J = 6.0 Hz), 126.91, 113.95 (J = 2.0 Hz), 63.81 (J = 7.2 Hz), 63.43 (J = 7.3 Hz), 56.04, 55.26, 54.48, 28.91, 16.46 (J = 5.9 Hz), 16.23 (J = 5.6 Hz), 14.82.

^{31}P NMR (162 MHz, $CDCl_3$) δ 21.05.



diethyl (((5-(ethylthio)-1,3,4-thiadiazol-2-yl)amino)(2-hydroxyphenyl)methyl)phosphonate (5c): R_f (DCM/MeOH)(90/10)= 0.70

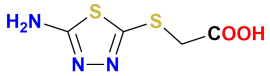
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (s, 1H), 7.49 (dt, J = 7.7, 1.7 Hz, 1H), 7.11 (dt, J = 8.3, 1.2 Hz, 1H), 6.93 (dt, J = 8.2, 1.3 Hz, 1H), 6.79 (td, J = 7.5, 1.3 Hz, 1H), 5.53 (d, J = 19.7 Hz, 1H), 4.22 – 4.04 (m, 2H), 3.91 (dp, J = 10.1, 7.1 Hz, 1H), 3.75 (ddq, J = 10.1, 8.4, 7.1 Hz, 1H), 3.06 (q, J = 7.4 Hz, 2H), 1.31 (t, J = 7.4 Hz, 3H), 1.23 (t, J = 7.1 Hz, 3H), 1.00 (t, J = 7.1 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 169.39 ($J_{C\text{-}P}$ = 13.1 Hz), 155.71 ($J_{C\text{-}P}$ = 8.1 Hz), 154.14, 130.14 ($J_{C\text{-}P}$ = 1.8 Hz), 129.42 ($J_{C\text{-}P}$ = 5.9 Hz), 122.25 ($J_{C\text{-}P}$ = 1.4 Hz), 120.73, 119.18, 64.08 ($J_{C\text{-}P}$ = 7.3 Hz), 63.80 ($J_{C\text{-}P}$ = 7.3 Hz), 51.45 ($J_{C\text{-}P}$ = 159.9 Hz), 28.96, 16.43 ($J_{C\text{-}P}$ = 5.7 Hz), 16.05 ($J_{C\text{-}P}$ = 5.7 Hz), 14.79.

^{31}P NMR (162 MHz, $CDCl_3$) δ 21.82.

III.3.3. synthesis of 2-((5-amino-1,3,4-thiadiazol-2-yl)thio)acetic acid (1d)

5-amino-1, 3, 4-thiadiazole-2-thiol (0.01 mol) was added to a mixture of Chloroacetic acid (0.01 mol) and KOH (0.025 mol) in 100 mL H_2O. The reaction mixture was stirred at 50 °C for 3 h. The mixture was neutralized with concentrated HCl, the formed precipitate was then filtered under reduced pressure to afford crude product **(1d)** as a light-yellow powder.



2-((5-amino-1,3,4-thiadiazol-2-yl)thio)acetic acid (1d): yellow powder, *Rf* (DCM:MeOH)(85:15)= 0.3

^{1}H NMR (400 MHz, DMSO) δ 7.29 (s, 2H, N**H$_2$**), 3.91 (s, 2H, C**H$_2$**). ^{13}C NMR (101 MHz, DMSO) δ 170.20, 170.11, 149.90, 36.57.