Journal of Biosciences and Medicines, 2025, 13(8), 138-145
https://www.scirp.org/journal/jbm
ISSN Online: 2327-509X
ISSN Print: 2327-5081

# A Promising TNF-$\alpha$ Inhibition Activity of 2-Aminocyclopentenylphosphonates

**Abed Al Aziz Al Quntar[1*], Ahmad Quntar[2], Mira Shatat[3], Ibrahim Abasy[4], Imad Matouk[5]**

[1]Department of Chemistry and Chemical Technology, Faculty of Science and Technology, Al-Quds University, Jerusalem, Palestine
[2]Faculty of Medicine, Al-Quds University, Jerusalem, Palestine
[3]Faculty of Medicine, Jordan University of Science and Technology, Irbid, Jordan
[4]Department of Microbiology and Immunology, Al-Quds University, Jerusalem, Palestine
[5]Department of Biological Sciences, Faculty of Science and Technology, Al-Quds University, Jerusalem, Palestine
Email: *abedalaziz@staff.alquds.edu

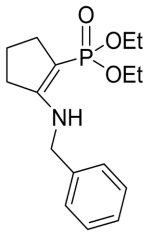
**How to cite this paper:** Al Quntar, A.A.A., Quntar, A., Shatat, M., Abasy, I. and Matouk, I. (2025) A Promising TNF-$\alpha$ Inhibition Activity of 2-Aminocyclopentenylphosphonates. *Journal of Biosciences and Medicines*, **13**, 138-145.
https://doi.org/10.4236/jbm.2025.138012

**Received:** June 21, 2025
**Accepted:** August 10, 2025
**Published:** August 13, 2025

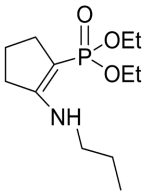


## Abstract

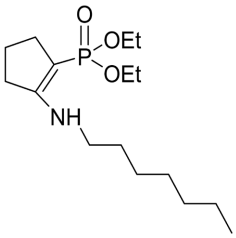
Buoyed by our previous TNF-$\alpha$ results, three 2-amino-1-cyclopentenylphosphonate compounds which were synthesized by the reaction of diethyl 5-chloro-1-pentynylphosphonate with various amines exhibited promising TNF-$\alpha$ inhibitory activity.



1a



1b



1c

## Keywords

Phosphonates, Aminophosphonates, Cyclopentenylphosphonate, TNF-$\alpha$, Inflammation, Budesonide, Rheumatoid Arthritis

## 1. Introduction

Cytokines are important proteins in cell signaling and help to control inflammation in the body. They are released by cells and affect the behavior of other cells, and sometimes the releasing cell itself. There are many types of cytokines including chemokines, interferons, interleukins, lymphokines, and tumor necrosis fac-

tor (TNF-$\alpha$) that are produced by a broad range of cells, including immune cells like macrophages, B lymphocytes, T lymphocytes and mast cells, as well as endothelial cells, fibroblasts, and various stromal cells. The most essential and master kind of cytokines is tumor necrosis (TNF-$\alpha$) [1] [2].

It is well known that TNF-$\alpha$ has an essential role in processes such as immunomodulation, fever, inflammatory response, inhibition of tumor formation, and inhibition of viral replication. In addition, TNF-$\alpha$ and its receptors are ubiquitously expressed in developing organs and they regulate the survival, proliferation, and apoptosis of embryonic stem cells (ESCs) and progenitor cells [1] [2].

However, overexpression of this key pro-inflammatory cytokine (TNF-$\alpha$), together with other mediators play a central role in the pathogenesis of autoimmune inflammatory responses in rheumatoid arthritis (RA), inflammatory bowel disease (IBD), Crohn's disease (CD), and ankylosing spondylitis (AS) [3]-[5].

Recently, TNF-$\alpha$ received much attention in the therapy of these diseases especially the rheumatoid arthritis (RA) which represents one of the most prevalent autoimmune diseases worldwide and characterized by inflammatory arthritis and extra-articular involvement.

In which the immune system can significantly damage the joints and other tissues, ultimately leading to irreversible joint deformity [6] [7].

Accordingly, extensive efforts are employed for TNF-$\alpha$ inhibitors for the treatment of this disease and others [8].

One approach is anti-TNF-$\alpha$ biologics (such as infliximab, adalimumab, etanercept, golimumab, and certolizumab pegol) have markedly improved the outcome of the management of autoimmune inflammatory diseases [9]. However, a considerable proportion of patients do not respond to this anti-TNF-$\alpha$ treatment [10]. Moreover, anti-TNF-$\alpha$ biologics are expensive, and are associated with some adverse side effects.

Recent studies indicate that specifically targeting of one of TNF-$\alpha$ receptors may represent a more effective and safer treatment for autoimmune disorders [11].

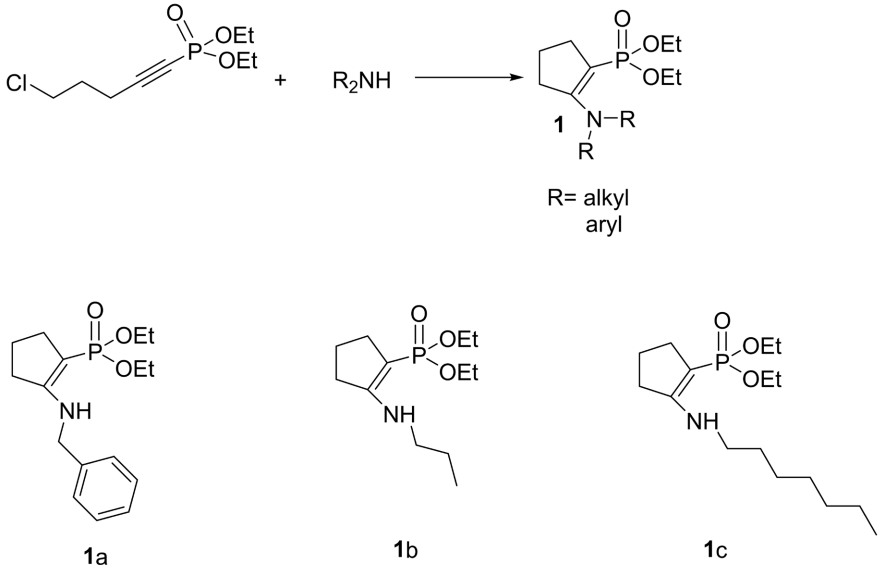
A third option is inhibiting TNF-$\alpha$ converting enzymes (TACE) which is a member of the reprolysin family of the metzincin superfamily that also includes matrix metalloproteinases (MMPs) identified by its ability to cleave the transmembrane form of TNF-$\alpha$ at its physiological processing site [12]. Some inhibitors of MMPs have also shown inhibitory activity of TACE, suggesting some similarities between the active sites of these enzymes. Accordingly, TACE inhibitors have been derived based on the knowledge of the MMP inhibition [13] [14].

Thus, due to the previous diseases that can be caused by the over release of TNF-$\alpha$, there is arising need for new anti-TNF-$\alpha$ compounds with high efficacy and minimum side effects [15]-[18].

We presume that organophosphonates can be a candidate for this purpose due to their extensive biological activity. For instance, they possess anti-inflammatory [19] [20], herbicidal [21], and antitumor activity [22]. Moreover, they showed protective properties against UV radiation [23], and other utilities [24].

## 2. Results and Discussion

This work is an extension of previous reports in which organophosphate compounds prepared by us and were found to be active as anti-TNF- beside others that showed MMPs inhibition [25] [26]. So, three different compounds of 2-amino-1-cyclopentenylphosphonates class **1**a-c which were prepared by the reaction of diethyl 5-chloro-1-pentynylphosphonate with benzylamine, propylamine and heptylamine respectively were explored as TNF-*α* inhibitors as shown in Scheme 1 [27].




Scheme 1. Synthesis of compounds **1**a-c.

The study was carried out on macrophages which were activated using lipopolysaccharide (LPS) in order to induce production and secretion of TNF-*α*. A negative control in which the cells were not treated with LPS in addition to a positive control was taken by treating the macrophages cell with budesonide to check the extent of inhibition. Budesonide is steroid medicine used for asthma and Crohn diseases treatment.

As shown in Figure 1, diethyl (2-(propylamino)cyclopent-1-en-1-yl)phosphonate **1**b showed minimal inhibitory activity at concentrations 50 - 300 μM. In contrast, compounds, diethyl (2-(heptylamino)cyclopent-1-en-1-yl)phosphonate **1**c and diethyl (2-(benzylamino)cyclopent-1-en-1-yl)phosphonate **1**a significantly reduced TNF-*α* levels in the system. In addition, the results indicated that compound **1**a has shown a preferential activity over **1**c. This variation of activities can be attributed to the structure of the compounds. Whereas short chain substituent on the cyclopentenyl ring (**1**b) showed minor activity, longer chain substituent (**1**c) demonstrated higher contribution to the activity.

Besides, the proximity of the phenyl group in the structure significantly enhanced the suppression of TNF-*α* viability.

Accordingly, in order to reduce the relatively high μM concentration activity of the phosphonate **1**a-c, synthesis and trial of compounds with longer chains and more phenyl groups might be more affective.

On the other hand, these results demonstrated that 2-aminocyclopentenylphosphonates **1**a exhibited only a slightly lower inhibition activity compared with budesonide with IC 50 of 150 μM.

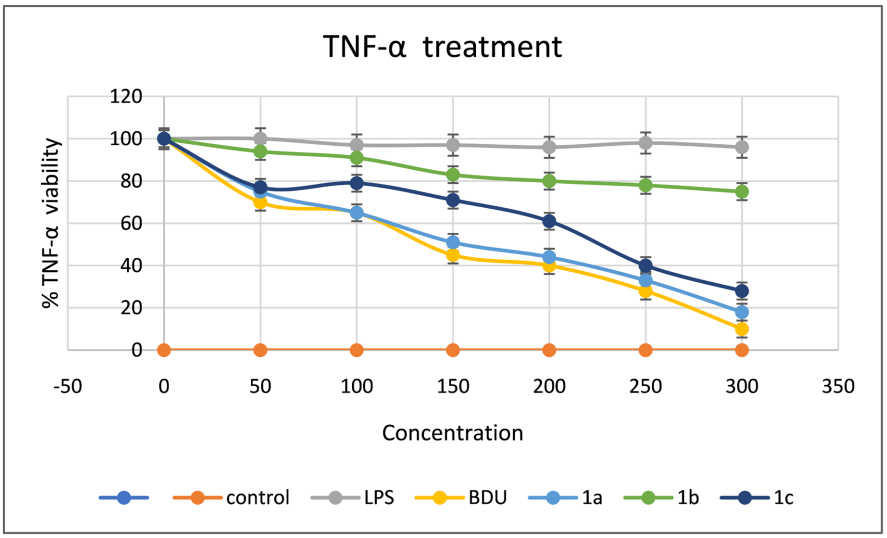



**Figure 1.** Results of compounds **1**a-c TNF-*α* inhibitory activity.

In summary, two 2-aminocyclopentenylphosphonate compounds demonstrated promising TNF-*α* inhibition activity, particularly diethyl (2-(benzylamino)cyclopent-1-en-1-yl)phosphonate which showed proximate activity compared to budesonide medicine.

Motivated by these *in vitro* findings, we plan to extend our research to in vivo studies and to synthesize other phosphonate compounds and explore them for this purpose.

## 3. Experimental

**Chemical experimental section:** [27]

**Procedure for the synthesis of 1**a: To 0.238 g (1 mmol) of diethyl 5-chloro-1-pentylphosphonate was added to 2 mmol (0.214 g) of benzylamine in a 25 ml round-bottom flask. After stirring for 3 hrs r.t., the reaction mixture was washed with 0.1 N $NaHCO_3$ solution, extracted with (2 × 20 ml $CH_2Cl_2$), and separated on silica gel column (10% methanol: 90% dichloromethane), and was analyzed by GCMS, elemental analysis, and NMR spectroscopy in 88% isolated yield.

$^1$H NMR (300 MHz, Acetone d): 1.88 (t, 6H, $J_{HH}$ = 7.2 Hz), 1.93 (qn, 2H, $J_{HH}$ = 7.2 Hz), 2.97 (dt, 2H, $J_{HH}$ = 7.8 Hz, $^3J_{PH}$ = 2.4 Hz), 3.41 (t, 2H, $J_{HH}$ = 6.9 Hz), 3.87 (qn, 4H, $J_{HH}$ = 7.2 Hz), 4.42 (s, 2H), 7.20-7.38 (overlap, 5H). $^{31}$P NMR (121.4 MHZ, Acetone d): $\delta$ 27.85; $^{13}$C NMR (75.5 MHz, Acetone d): $\delta$ 16.1 (d, $^3J_{PC}$ = 6.9 Hz), 21.3, 32.1, 49.5, 52.6, 60.0 (d, $^2J_{PC}$ = 5.2 Hz), 127.3, 127.4, 128.8, 137.2, 164.5 (d,

$^{2}J_{PC}$ = 20.1 Hz); MS(EI):m/z (%) 309 (8.5), 264 (2.1), 207 (1.1), 172 (100), 144 (6.0), 104 (10.3), 91 (80.1), 77 (1.2), 65 (21.3), 41 (10.3); Anal. Calcd for $C_{16}H_{24}NO_3P$: C, 62.12; H, 7.82; N, 4.53; P, 10.01. Found: C, 62.01; H, 7.89; N, 4.49; P, 10.09.

**Procedure for the synthesis of 1**b: Identical procedure for 1a except adding propylamine.

$^{1}$H NMR (300 MHz, Methanol d): $\delta$0.93 (t, 3H, $J_{HH}$ = 7.5 Hz), 1.28 (t, 6H, $J_{HH}$ = 7.2 Hz), 1.68 (m, 2H), 1.92 (qn, 2H, $J_{HH}$ = 6.9 Hz), 2.83 (dt, 2H, $J_{HH}$ = 7.5 Hz, $^{3}J_{PH}$ = 2.1 Hz), 3.16 (t, 2H, $J_{HH}$ = 7.2 Hz), 3.42 (t, 2H, $J_{HH}$ = 6.6 Hz), 3.95 (qn, 4H, $J_{HH}$ = 7.2 Hz). $^{31}$P NMR (121.4 MHZ, Methanol d): $\delta$31.78; $^{13}$C NMR (75.5 MHz, Methanol d): $\delta$10. 8, 15.7 (d, $^{3}J_{PC}$ = 6.8 Hz), 20.8, 21.0, 32.3, 41.2, 52.4, 60.9 (d, $^{2}J_{PC}$ = 5.4 Hz), 165.4 (d, $^{2}J_{PC}$ = 20.9 Hz); MS(EI):m/z (%) 261 (16.7), 232 (26.5), 216 (8.8), 204 (8.8), 146 (17.6), 124 (100), 110 (42.2), 97 (68.6), 83 (68.6), 55 (29.9), 41 (55.9); Anal. Calcd for $C_{12}H_{24}NO_3P$: C, 55.16; H, 9.26; N, 5.36; P, 11.85. Found: C, 55.03; H, 9.20; N, 5.46; P, 11.94.

**Procedure for the synthesis of 1**c: Identical procedure for 1a except adding 1-heptylamine.

$^{1}$H NMR (300 MHz, Methanol d): $\delta$0.90 (t, 3H, $J_{HH}$ = 6.6 Hz), 1.27 (t, 6H, $J_{HH}$ = 7.2 Hz), 1.20-1.56 (overlap, 10H), 1.91 (qn, 2H, $J_{HH}$ = 7.5 Hz), 2.81 (dt, 2H, $J_{HH}$ = 7.8 Hz, $^{3}J_{PH}$ = 2.1 Hz), 3.19 (t, 2H, $J_{HH}$ = 7.8 Hz), 3.39 (t, 2H, $J_{HH}$ = 7.2 Hz), 3.90 (qn, 4H, $J_{HH}$ = 7.2 Hz). $^{31}$P NMR (121.4 MHZ, Methanol d): $\delta$ 29.94; $^{13}$C NMR (75.5 MHz, Methanol d): $\delta$13.1, 16.0 (d, $^{3}J_{PC}$ = 7.2 Hz), 21.3, 22.4, 25.8, 26.1, 27.0, 28.6, 40.5, 45.7, 52.6, 70.0 (d, $^{2}J_{PC}$ = 5.1 Hz), 165.8 (d, $^{2}J_{PC}$ = 21.0 Hz); MS(EI):m/z (%) 317 (3.0), 302 (5.8), 246 (13.8), 233 (40.0), 152 (60.2), 138 (88.8), 79 (100), 83 (15.1), 55 (20.6), 41 (48.8); Anal. Calcd for $C_{16}H_{32}NO_3P$: C, 60.55; H, 10.16; N, 4.41; P, 9.76. Found: C, 58.90; H, 9.79; N, 4.69; P, 9.92.

**Biological experimental section**:

The same protocol of our previous study was used [25].

Peritoneal cells were harvested from the peritoneum of C57BL/6 female mice 4 days after intraperitoneal injection of 1.5 ml of a 3% thioglycollate medium.

The thioglycollate medium macrophages were washed with phosphate-buffered saline, re-suspended in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal calf serum, and plated ($1.2 \times 10^{-5}$) in 96-microwell plates flat-bottom. Following 2 - 3 h of incubation at 37˚C, the non-adherent cells were removed by intensive rinsing. About 95% of the adherent cells were macrophages. The vinylphosphonates were first dissolved in absolute ethanol (1 mg/50 - 100 µl ethanol), and the solutions were further diluted with DMEM. For each compound, various nontoxic concentrations (as determined by MTT assay) were added to the macrophages, followed by addition of 1 µg/ml of lipopolysaccharide (LPS) for activation. The thioglycollate medium macrophages were then cultivated in a humid atmosphere with 5% $CO_2$ for 24 h. The supernatant fluids were harvested and kept at −20˚C until assayed for TNF-$\alpha$.

TNF-$\alpha$ in the supernatants of the vinylphosphonates treated (8 µl/ml) LPS-activated macrophages was determined by ELISA.

The consistency system was checked by negative control in which the cells were not treated with LPS. In addition, a positive control was taken by treating the macrophages cell with budesonide (BDU) to check the extent of inhibition. The TNF-*α* analysis was performed via Enzyme-linked immunosorbent assay ELISA method on triplicates.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

## References

[1] Liu, Y.J., Soumelis, V., Watanabe, N., Ito, T., Wang, Y., de Waal Malefyt, R., *et al.* (2007) TSLP: An Epithelial Cell Cytokine That Regulates T Cell Differentiation by Conditioning Dendritic Cell Maturation. *Annual Review of Immunology*, **25**, 193-219. https://doi.org/10.1146/annurev.immunol.25.022106.141718

[2] Aggarwal, B.B. (2003) Signalling Pathways of the TNF Superfamily: A Double-Edged Sword. *Nature Reviews Immunology*, **3**, 745-756. https://doi.org/10.1038/nri1184

[3] Moudgil, K.D. and Choubey, D. (2011) Cytokines in Autoimmunity: Role in Induction, Regulation, and Treatment. *Journal of Interferon & Cytokine Research*, **31**, 695-703. https://doi.org/10.1089/jir.2011.0065

[4] Sticherling, M. (2016) Psoriasis and Autoimmunity. *Autoimmunity Reviews*, **15**, 1167-1170. https://doi.org/10.1016/j.autrev.2016.09.004

[5] Ellis, J.S. and Braley-Mullen, H. (2017) Mechanisms by Which B Cells and Regulatory T Cells Influence Development of Murine Organ-Specific Autoimmune Diseases. *Journal of Clinical Medicine*, **6**, Article 13. https://doi.org/10.3390/jcm6020013

[6] De Cock, D. and Hyrich, K. (2018) Malignancy and Rheumatoid Arthritis: Epidemiology, Risk Factors and Management. *Best Practice & Research Clinical Rheumatology*, **32**, 869-886. https://doi.org/10.1016/j.berh.2019.03.011

[7] Li, P., Zheng, Y. and Chen, X. (2017) Drugs for Autoimmune Inflammatory Diseases: From Small Molecule Compounds to Anti-TNF Biologics. *Frontiers in Pharmacology*, **8**, Article 460. https://doi.org/10.3389/fphar.2017.00460

[8] Monaco, C., Nanchahal, J., Taylor, P. and Feldmann, M. (2014) Anti-TNF Therapy: Past, Present and Future. *International Immunology*, **27**, 55-62. https://doi.org/10.1093/intimm/dxu102

[9] Meier, F.M., Frerix, M., Hermann, W. and Müller-Ladner, U. (2013) Current Immunotherapy in Rheumatoid Arthritis. *Immunotherapy*, **5**, 955-974. https://doi.org/10.2217/imt.13.94

[10] Roda, G., Jharap, B., Neeraj, N. and Colombel, J.F. (2016) Loss of Response to Anti-TNFs: Definition, Epidemiology, and Management. *Clinical and Translational Gastroenterology*, **7**, e135. https://doi.org/10.1038/ctg.2015.63

[11] Siegmund, D. and Wajant, H. (2023) TNF and TNF Receptors as Therapeutic Targets for Rheumatic Diseases and beyond. *Nature Reviews Rheumatology*, **19**, 576-591. https://doi.org/10.1038/s41584-023-01002-7

[12] Moss, M.L., Jin, S.C., Milla, M.E., Burkhart, W., Carter, H.L., Chen, W., *et al.* (1997) Cloning of a Disintegrin Metalloproteinase That Processes Precursor Tumour-Necrosis Factor-*α*. *Nature*, **385**, 733-736. https://doi.org/10.1038/385733a0

[13] Duan, J.J.W. (2002) Discovery of *γ*-Lactam Hydroxamic Acids as Selective Inhibitors of Tumor Necrosis Factor *α* Converting Enzyme: Design, Synthesis, and Structure-

Activity Relationships. *Journal of Medicinal Chemistry*, **45**, 4954-4957.

[14] Mohan, M.J., Seaton, T., Mitchell, J., Howe, A., Blackburn, K., Burkhart, W., *et al.* (2002) The Tumor Necrosis Factor-*α* Converting Enzyme (TACE): A Unique Metalloproteinase with Highly Defined Substrate Selectivity. *Biochemistry*, **41**, 9462-9469. https://doi.org/10.1021/bi0260132

[15] Heidari, N., Ghannadzadeh Kermani Pour, R., Farshbafnadi, M., Heidari, A. and Ghane, Y. (2025) A Systematic Review of Tumor Necrosis Factor-*α* Blockers, Anti-Interleukins, and Small Molecule Inhibitors for Dissecting Cellulitis of the Scalp Treatment. *Orphanet Journal of Rare Diseases*, **20**, Article No. 236. https://doi.org/10.1186/s13023-025-03720-5

[16] Song, L., Yang, N., Xing, X., Zhao, Y. and Di, J. (2025) Thrombotic Adverse Events Associated with TNF-Alpha Blockers: A Real-World Pharmacovigilance Analysis of the FAERS Database. *Frontiers in Pharmacology*, **16**, Article 1512806. https://doi.org/10.3389/fphar.2025.1512806

[17] Chédotal, H., Narayanan, D., Povlsen, K., Gotfredsen, C.H., Brambilla, R., Gajhede, M., *et al.* (2023) Small-Molecule Modulators of Tumor Necrosis Factor Signaling. *Drug Discovery Today*, **28**, Article 103575. https://doi.org/10.1016/j.drudis.2023.103575

[18] Chen, R., Monaco, G., Stefanini, B., Marseglia, M., De Lorenzo, S. and Tovoli, F. (2024) Combination Makes Strength: A Narrative Review of Transarterial Radioembolization Plus Immune Checkpoint Inhibitors for Hepatocellular Carcinoma. *Chinese Clinical Oncology*, **13**, 71-71. https://doi.org/10.21037/cco-24-27

[19] Zarghi, A. and Arfaei S. (2011) Selective COX-2 Inhibitors: A Review of Their Structure-Activity Relationships. *Iranian Journal of Pharmaceutical Research*, **10**, 655-683. Talley, J.J. (1999) 5 Selective Inhibitors of Cyclooxygenase-2 (COT-2). In: *Progress in Medicinal Chemistry*, Elsevier, 201-234. https://doi.org/10.1016/s0079-6468(08)70048-1

[20] Rao, P. and Knaus, E.E. (2008) Evolution of Nonsteroidal Anti-Inflammatory Drugs (NSAIDs): Cyclooxygenase (COX) Inhibition and Beyond. *Journal of Pharmacy & Pharmaceutical Sciences*, **11**, 81-110. https://doi.org/10.18433/j3t886

[21] Smith, S.C., Clarke, E.D., Ridley, S.M., Bartlett, D., Greenhow, D.T., Glithro, H., *et al.* (2005) Herbicidal Indolizine-5,8-Diones: Photosystem I Redox Mediators. *Pest Management Science*, **61**, 16-24. https://doi.org/10.1002/ps.980

[22] Liu, Z.Y., Wang, Y.M., Han Y.X., Liu, L., Jin, J., Yi, H., Li, Z.R., Jiang, J.D. and Boykin, D. (2013) Synthesis and Antitumor Activity of Novel 3,4-Diaryl Squaric Acid Analogs. *European Journal of Medicinal Chemistry*, **65**, 187-194. https://doi.org/10.1016/j.ejmech.2013.04.046

[23] Ortuno, R., Moglioni, A. and Moltrasio, G. (2005) Cyclobutane Biomolecules: Synthetic Approaches to Amino Acids, Peptides and Nucleosides. *Current Organic Chemistry*, **9**, 237-259. https://doi.org/10.2174/1385272053369088

[24] Sergeiko, A., Poroikov, V.V., Hanus, L.O. and Dembitsky, V.M. (2008) Cyclobutane-containing Alkaloids: Origin, Synthesis, and Biological Activities. *The Open Medicinal Chemistry Journal*, **2**, 26-37. https://doi.org/10.2174/1874104500802010026

[25] Quntar, A.A.A.A., Gallily, R., Katzavian, G. and Srebnik, M. (2007) Potent Anti-Inflammatory Activity of 3-Aminovinylphosphonates as Inhibitors of Reactive Oxygen Intermediates, Nitric Oxides Generation, and Tumor Necrosis Factor-α Release. *European Journal of Pharmacology*, **556**, 9-13. https://doi.org/10.1016/j.ejphar.2006.10.041

[26] Al-Quntar, A.A., Baum, O., Reich, R. and Srebnik, M. (2004) Recently Synthesized

Class of Vinylphosphonates as Potent Matrix Metalloproteinase (MMP-2) Inhibitors. *Archiv der Pharmazie*, **337**, 76-80. https://doi.org/10.1002/ardp.200300828

[27] Al Quntar, A.A.A., Srivastava, H.K., Srebnik, M., Melman, A., Ta-Shma, R. and Shurki, A. (2007) Formation of Diethyl 2-Amino-1-Cyclopentenylphosphonates: A Simple Synthesis with a Unique Mechanism. *The Journal of Organic Chemistry*, **72**, 4932-4935. https://doi.org/10.1021/jo070535b