

A screening of TNF- α inhibition activity by cyclopropylmethylphosphonate

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ABSTRACT

TNF- α is a member of a family of proteins called cytokines that are important components of the immune system. Their physiological role in inflammation is well recognized. An imbalance in their production contributes to various pathological disorders.

Encouraged by our previous TNF- α result, four cyclopropylmethylphosphonate compounds which were synthesized using 1-alkynylphosphonates with the reagent system $Cp_2ZrCl_2/2EtMgBr/2AlCl_3$ and were found to be active as TNF- α inhibitors.

Keywords: Cyclopropanes, phosphonates, cyclopropylmethylphosphonate, TNF- α , Inflammation, Budesonide, rheumatoid arthritis.

1. INTRODUCTION

Cytokines are proteins generally of low molecular weight that mediate communication between immune cells and also non-immune cells and play pivotal roles in diverse cellular processes, including immune cellular activation, differentiation, survival and proliferation (Dinarello, 1984). They also play basic roles in both innate and adaptive immunity. Apart from their role in host defense, cytokines can participate in tissue development, angiogenesis, and wound healing (Barrientos et al., 2008). Deregulated production and signaling of cytokines, contributes to a variety of pathological conditions that is extending beyond immune mediated diseases such as autoimmune disease and chronic inflammation disorder, but also to cancer (Grivennikov et al., 2010).

Cytokines are diverse and, based on their structures and functions, are grouped into families including interleukins, interferons, colony-stimulating factors, and tumor necrosis factors (Oppenheim et al., 2001). They exert their biological function through binding specifically to their cognate receptor to initiate a signaling cascade that can modulate gene expression (Darnell et al., 1994). They also mediate their effect through various signaling modes. Paracrine, autocrine or endocrine modes of signaling were reported, and this seems to depend on their concentrations and biological context.

One of the most studied and thus most popular cytokine is tumor necrosis factor (TNF- α). Initially discovered Carswell et al in 1970s as a serum factor that is capable of inducing hemorrhagic necrosis in tumors in mice treated with endotoxin (Carswell et al., 1975). Subsequent studies indicate that this cytokine is rapidly induced following immune stimulation. This discovery positions it as an early-response cytokine that are later proved to play pivotal role in modulating and shaping the subsequent immune response (Beutler & Cerami, 1986).

In response to bacterial products such as lipopolysaccharide (LPS), TNF- α is primarily produced by macrophages and monocytes to mediate immune activation (Beutler et al., 1985). Additional immune and non-immune components are also reported to produce TNF- α including T-lymphocytes and natural killers, among other types. Endothelial cells and fibroblasts are also reported to produce TNF- α (Sung et al., 1988; Vassalli, 1992). TNF- α biological activity is mediated through binding to two structurally distinct receptors. Signaling through TNFR1 (p55) is complicated and can trigger seemingly opposite effect that is related to cell survival and apoptosis. Whereas signaling through TNFR2 (p75) is more restricted and is associated mainly with immune regulation and tissue repair (Hsu et al., 1996; Grell et al., 1995).

The transient and physiological role of TNF- α once deregulated contributes directly to chronic inflammation and tissue damage (Tracey & Cerami, 1994). Its increased expression is well recognized as a hallmark of both autoimmune and inflammatory diseases. Rheumatoid arthritis, inflammatory bowel disease, psoriasis, ankylosing spondylitis, and multiple sclerosis are well-documented examples (Feldmann & Maini, 2001; Neurath, 2014; Locksley et al., 2001). Moreover, systemic inflammatory diseases like sepsis and cytokine release syndromes are characterized by high circulating TNF- α that contributes to various pathological phenotypes associated with these conditions (Hotchkiss & Karl, 2003).

In cancer TNF- α can play a dual contradictory role depending on whether the exposure is acute or chronic. Whereas acute exposure can trigger apoptosis, chronic exposure can promote inflammatory signals that promote tumor growth and progression, angiogenesis and immune evasion (Balkwill, 2006; Pikarsky et al., 2004).

Much effort have been devoted for the development of TNF- α targeted biological therapies owing to the pivotal pathological role as a central mediator of inflammation played by TNF- α .

These efforts were particularly successful in Rheumatoid arthritis, one of the most prevalent autoimmune diseases worldwide and characterized by inflammatory arthritis and extra-articular involvement. This represents a major milestone in immunology and clinical medicine (Feldmann et al., 1998).

Monoclonal antibodies and soluble TNF receptors have demonstrated significant efficacy in treating chronic inflammatory diseases and substantially improve the clinical outcomes and the quality of life (Maini et al., 1998). Anti-TNF- α such as infliximab, adalimumab, etanercept, golimumab, and certolizumab pegol has markedly improved the outcome of the management of autoimmune inflammatory diseases (Lipsky et al., 2000; Keystone et al., 2004; Mease et al., 2000).

More recent efforts have been invested in targeting TNF- α receptors and this may represent a more effective and safer treatment for disorders (Fischer et al., 2017). In addition, inhibiting TNF- α converting enzymes (TACE) can represent another promising approach in limiting the availability of TNF- α (Black et al., 1997; Moss et al., 1997).

Despite all of these remarkable efforts and the effectiveness of TNF- α inhibitors major limitations associated with increased susceptibility to infection, high treatment costs, and loss of therapeutic response in some patients remain significant challenges

(Bongartz et al., 2006). These limitations underscore the urgent need for novel anti-TNF- α compounds with high efficacy and reduced adverse effects.

Recently, we found that organophosphonate compounds are potent as anti-pancreatic and prostate cancer. (Al Quntar et al., 2024; Al Quntar et al., 2025). In addition, other analogs possessed significant TNF- α and MMPs inhibition (Al Quntar et al., 2007; Al Quntar et al., 2004).

2. RESULTS AND DISCUSSION

This work is an extension of continuous efforts to explore the biological utility of various classes of phosphonates. Thus, four different cyclopropylmethylphosphonate compounds 2a-d were prepared using 1-alkynylphosphonates 1 with the reagent system $\text{Cp}_2\text{ZrCl}_2/2\text{EtMgBr}/2\text{AlCl}_3$ and were investigated as TNF- α inhibitors as shown in Figure 1. (Al Quntar, 2009)

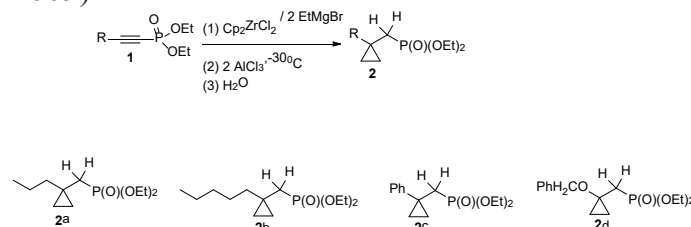


Figure 1. Synthesis of compounds 2a-d.

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 2, unlike diethyl ((1-propylcyclopropyl)methyl)phosphonate 2a and diethyl ((1-pentylcyclopropyl)methyl)phosphonate 2b which did not show significant TNF- α inhibitory activity at concentrations 50-300 μM , compounds, diethyl ((1-phenylcyclopropyl)methyl)phosphonate 2c and diethyl ((1-(benzyloxy)cyclopropyl)methyl)phosphonate 2d remarkably reduced TNF- α concentration in the system. Moreover, it is obvious that compound 2d has shown a slightly higher activity compared 2c. This indicates that there is a structure activity relationship in which the proximity of a phenyl ring to the cyclopropyl moiety (2c, 2d) enhanced the activity compared to the chain (2a, 2b).

In addition, it is notable that the activity of the compounds is a dose dependent i.e. the higher the concentration of the compounds the higher the activity is.

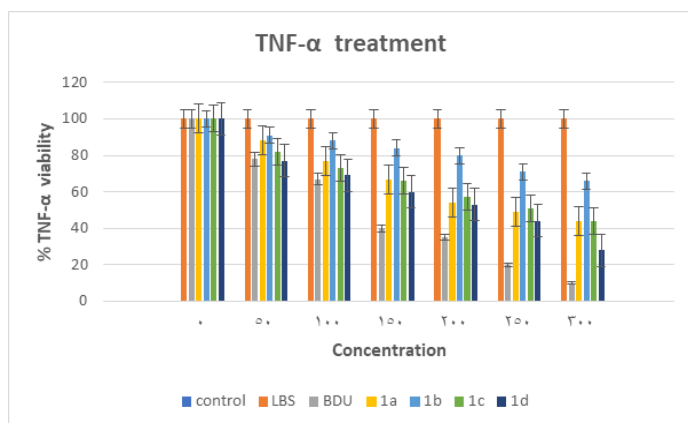


Figure 2: Results of compounds 2a-d TNF- α inhibitory activity.

In conclusion, four diethyl cyclopropylmethylphosphonate compounds were screened in order to test their TNF- α inhibition activity. It has been found that phenyl substituted cyclopropanes demonstrated satisfactory activity compared to chain substituents. This finding might stimulate preparation of other phosphonate compounds and explore their TNF- α activity.

3. EXPERIMENTAL

3.1. Chemical experimental section: (Al Quntar, 2009)

Procedure for the synthesis of 2: Ethyl magnesium bromide EtMgBr (2.1 mmol) was added dropwise 0.306g (1.05 mmol) of zirconocene dichloride dissolved in 7 ml of dry THF at -78°C . The mixture was stirred for 5 min at -78°C and 1 mmol of diethyl 1-alkynylphosphonate was added. Then, the reaction was gradually warmed to 25°C and stirred for 2 hr. Again it was cooled to -30°C and 2 equiv. of AlCl_3 were added. After stirring for 1 hr at -30°C and was worked up with dilute HCl. The product was extracted with diethyl ether (2 X 15ml), separated on silica gel column (80% petroleum ether: 20% ethyl acetate), and analyzed by GCMS, elemental analysis, and NMR spectroscopy.

2a). Diethyl (1-propylcyclopropyl) methylphosphonate. $^1\text{H NMR}$ (300 MHz): δ 0.34 (m, 2H), 0.41 (m, 2H), 0.87 (t, 3H, $J_{\text{HH}} = 6.6$ Hz), 1.30 (dt, 6H, $J_{\text{HH}} = 6.9$, $^4J_{\text{PH}} = 0.2$ Hz), 1.32-1.47 (overlap, 4H), 1.77 (d, 2H, $^2J_{\text{PH}} = 18.0$ Hz), 3.98-4.18 (m, 4H). $^{31}\text{P NMR}$ (121.4 MHz): δ 31.88. $^{13}\text{C NMR}$ (75.5 MHz): δ 12.7, 12.8, 14.1, 15.0 (d, $^2J_{\text{PC}} = 4.2$ Hz), 16.42 (d, $^3J_{\text{PC}} = 6.0$ Hz), 22.8, 32.3 (d, $^1J_{\text{PC}} = 140.0$ Hz), 36.7, 61.3 (d, $^2J_{\text{PC}} = 6.6$ Hz).

2b). Diethyl (1-pentylcyclopropyl)

methylphosphonate. $^1\text{H NMR}$ (300 MHz): δ 0.34 (m, 2H), 0.42 (m, 2H), 0.84 (t, 3H, $J_{\text{HH}} = 6.6$ Hz), 1.28 (dt, 6H, $J_{\text{HH}} = 6.9$, $^4J_{\text{PH}} = 0.2$ Hz), 1.35-1.47 (overlap, 8H), 1.71 (d, 2H, $^2J_{\text{PH}} = 17.7$ Hz), 3.97-4.17 (m, 4H). $^{31}\text{P NMR}$ (121.4 MHz): δ 31.64. $^{13}\text{C NMR}$ (75.5 MHz): δ 12.7, 12.8, 14.0, 16.1 (d, $^2J_{\text{PC}} = 6.9$ Hz), 16.4 (d, $^3J_{\text{PC}} = 6.0$ Hz), 22.6, 26.2, 31.9, 32.3 (d, $^1J_{\text{PC}} = 140.1$ Hz), 36.9, 61.2 (d, $^2J_{\text{PC}} = 6.6$ Hz).

2c). diethyl ((1-phenylcyclopropyl)methyl) phosphonate. $^1\text{H NMR}$ (300 MHz): δ 0.40 (m, 2H), 0.45 (m, 2H), 1.26 (dt, 6H, $J_{\text{HH}} = 6.9$, $^4J_{\text{PH}} = 0.2$ Hz), 1.74(d, 2H, $^2J_{\text{PH}} = 17.5$ Hz, 3.91-4.20 (m, 4H), 7.15-7.35 (m, 5H). $^{31}\text{P NMR}$ (121.4 MHz): δ 31.26. $^{13}\text{C NMR}$ (75.5 MHz): δ 12.6, 12.8, 16.1 (d, $^2J_{\text{PC}} = 6.6$ Hz), 16.4 (d, $^3J_{\text{PC}} = 6.0$ Hz), 32.7 (d, $^1J_{\text{PC}} = 139.8$ Hz), 61.2 (d, $^2J_{\text{PC}} = 6.6$ Hz), 127.4, 127.5, 127.5, 127.6, 128.2, 138.5.

2d). Diethyl (1-(2-(benzyloxy)ethylcyclopropyl) methylphosphonate. $^1\text{H NMR}$ (300 MHz): δ 0.44 (m, 2H), 0.47 (m, 2H), 1.27 (dt, 6H, $J_{\text{HH}} = 6.9$, $^4J_{\text{PH}} = 0.2$ Hz), 1.76 (d, 2H, $^2J_{\text{PH}} = 17.7$ Hz), 3.92-4.15 (m, 4H), 4.47 (s, 2H), 7.10-7.30 (m, 5H). $^{31}\text{P NMR}$ (121.4 MHz): δ 31.38. $^{13}\text{C NMR}$ (75.5 MHz): δ 12.6, 12.8, 16.4 (d, $^3J_{\text{PC}} = 6.0$ Hz), , 32.7 (d, $^1J_{\text{PC}} = 139.8$ Hz), 61.2 (d, $^2J_{\text{PC}} = 6.6$ Hz), 67.9, 127.3, 127.7, 127.5, 127.6, 128.2, 139.3.

3.2. Biological experimental section:

The same protocol of our previous study was used. (Al Quntar et al., 2007).

Peritoneal cells were harvested from the peritoneum of C57BL/6 female mice 4 days after intraperitoneal injection of 1.5 ml of a 3% thioglycolate 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 (1.2×10^5) 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 $\mu\text{g}/\text{ml}$ of lipopolysaccharide (LPS) for activation.

The thioglycolate medium macrophages were then cultivated in a humid atmosphere with 5% CO₂ for 24 h. The supernatant fluids were harvested and kept at -20 °C until assayed for TNF- α .

TNF- α in the supernatants of the vinylphosphonates treated (8 μ l/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.

REFERENCES

- Al Quntar, A. A. (2009). Direct formation of cyclobutenylphosphonates from 1-alkynylphosphonates and Cp₂ZrCl₂/2EtMgCl/2CuCl. *Tetrahedron Letters*, 50, 867–869. <https://doi.org/10.1016/j.tetlet.2008.11.108>.
- Al Quntar, A. A., Raich, R., & Srebniak, 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>.
- Al Quntar, A. A., et al. (2007). Potent anti-inflammatory activity of 3-aminophosphonates as inhibitors of ROI, NO generation and TNF- α release. *European Journal of Pharmacology*, 556, 9–1. <https://doi.org/10.2174/1874104500802010026>.
- Al Quntar, A. A., et al. (2024). Aminated Cyclopropylmethylphosphonates as potent prostate cancer inhibitors. *Journal of Biosciences and Medicine*, 12, 239–244. <https://doi.org/10.4236/jbm.2024.127022>.
- Al Quntar, A. A., et al. (2025). A promising TNF- α inhibition activity of 2-aminocyclopentenylphosphonates. *Journal of Biosciences and Medicine*, 13(8), 138–145. <https://doi.org/10.4236/jbm.2025.138012>.
- Balkwill, F. (2006). TNF- α in promotion and progression of cancer. *Cancer Metastasis Reviews*, 25(3), 409–416. <https://doi.org/10.1007/s10555-006-9008-3>.
- Barrientos, S., Stojadinovic, O., Golinko, M. S., Brem, H., & Tomic-Canic, M. (2008). Growth factors and cytokines in wound healing. *Wound Repair and Regeneration*, 16(5), 585–601. <https://doi.org/10.1111/j.1524-475X.2008.00499.x>.
- Beutler, B., & Cerami, A. (1986). Cachectin and tumor necrosis factor as two sides of the same biological coin. *Nature*, 320(6058), 584–588. <https://doi.org/10.1038/320584a0>.
- Beutler, B., et al. (1985). Tumor necrosis factor: Cachexia, shock, and inflammation. *The Journal of Experimental Medicine*, 161(6), 984–995. <https://doi.org/10.1084/jem.161.6.984>.
- Black, R. A., et al. (1997). A metalloproteinase disintegrin that releases tumor-necrosis factor- α from cells. *Nature*, 385(6618), 729–733.
- Bongartz, T., et al. (2006). Anti-tumor necrosis factor antibody therapy in rheumatoid arthritis and the risk of serious infections. *JAMA*, 295(19), 2275–2285.
- Carswell, E. A., et al. (1975). An endotoxin-induced serum factor that causes necrosis of tumors. *PNAS*, 72(9), 3666–3670. <https://doi.org/10.1073/pnas.72.9.3666>.
- Darnell, J. E., Jr., Kerr, I. M., & Stark, G. R. (1994). Jak-STAT pathways and transcriptional activation in response to IFNs. *Science*, 264(5164), 1415–1421.
- Dinarello, C. A. (1984). Interleukin-1 and the pathogenesis of the acute-phase response. *New England Journal of Medicine*, 311(23), 1413–1418. <https://doi.org/10.1056/NEJM198412063112301>.
- Feldmann, M., et al. (1998). Anti-TNF therapy of rheumatoid arthritis. *Annual Review of Immunology*, 16, 479–503.
- Feldmann, M., & Maini, R. N. (2001). TNF defined as a therapeutic target for rheumatoid arthritis. *Cell*, 104(4), 481–490.
- Fischer, R., et al. (2017). Selective TNFR1 inhibition for the treatment of chronic inflammatory diseases. *Nature Communications*, 8, 1–13.
- Grell, M., et al. (1995). The transmembrane form of TNF is the prime activating ligand of TNFR2. *Cell*, 83(5), 793–802.
- Grivennikov, S. I., Greten, F. R., & Karin, M. (2010). Immunity, inflammation, and cancer. *Cell*, 140(6), 883–899.
- Hotchkiss, R. S., & Karl, I. E. (2003). The pathophysiology and treatment of sepsis. *New England Journal of Medicine*, 348(2), 138–150.
- Hsu, H., et al. (1996). TRADD-TRAF2 and TRADD-FADD interactions in TNFR1 signaling. *Cell*, 84(3), 299–308.
- Keystone, E. C., et al. (2004). Adalimumab in active rheumatoid arthritis. *Arthritis & Rheumatism*, 50(5), 1400–1411.
- Lipsky, P. E., et al. (2000). Infliximab and methotrexate in rheumatoid arthritis. *New England Journal of Medicine*, 343(21), 1594–1602.
- Locksley, R. M., Killeen, N., & Lenardo, M. J. (2001). The TNF and TNF receptor superfamilies. *Cell*, 104(4), 487–501.
- Maini, R. N., et al. (1998). Therapeutic efficacy of multiple intravenous infusions of anti-TNF monoclonal antibody. *Arthritis & Rheumatism*, 41(9), 1552–1563.

- Mease, P. J., et al. (2000). Etanercept in the treatment of psoriatic arthritis. *New England Journal of Medicine*, 342(2), 118–124.
- Moss, M. L., et al. (1997). Cloning of a disintegrin metalloproteinase that processes precursor tumor-necrosis factor- α . *Nature*, 385(6618), 733–736.
- Neurath, M. F. (2014). Cytokines in inflammatory bowel disease. *Nature Reviews Immunology*, 14(6), 329–342.
- Oppenheim, J. J., et al. (2001). Cytokines: Past, present, and future. *International Journal of Hematology*, 74(1), 3–8.
- Pikarsky, E., et al. (2004). NF- κ B functions as a tumour promoter in inflammation-associated cancer. *Nature*, 431(7007), 461–466.
- Sung, S. S., et al. (1988). Production of TNF- α by human T lymphocytes. *The Journal of Immunology*, 141(10), 3507–3512.
- Tracey, K. J., & Cerami, A. (1994). Tumor necrosis factor: A pleiotropic cytokine and therapeutic target. *Annual Review of Medicine*, 45, 491–503.
- Vassalli, P. (1992). The pathophysiology of tumor necrosis factors. *Annual Review of Immunology*, 10, 411–452.