The Egyptian Journal of Hospital Medicine (October 2022) Vol. 89 (2), Page 7314- 7320

Synthesis and Characterization of Some New 1,2,4-Triazole linked to Schiff bases Derived From Ibuprofen as a Possible to Inhibit TNF-α

***Ahmed .M. Abdulwahid [1], Mazin. N. Mousa [2], Husam Hamza Salman[3]**

[1]Basrah Health Directorate, Ministry of Health, Basrah, Iraq

[2] Department of Pharmaceutical Chemistry, College of Pharmacy, University of Basrah, Basrah, Iraq

Corresponding author: Ahmed. M. Abdulwahid, **Email**: Ahmedmohsin199222@gmail.com, **Mobile:** 009647725578088

ABSTRACT

Background: Pro-inflammatory cytokines trigger immune cells to produce and release interleukin, interferon, and Tumor necrosis factors. In pathological conditions, the excessive release of these cytokines can cause various autoimmune diseases such as (rheumatoid arthritis, psoriasis, and irritable bowel syndrome).

Methods: This study was conducted to synthesize four derivatives of 1,2,4-triazole linked to Schiff bases.The synthesis was started by converting of ibuprofen to ibuprofen ethyl ester. Then converted to ibuprofen hydrazide by reaction with hydrazine hydrate. After that ibuprofen hydrazide is converted to an intermediate salt of potassium carbazinate by the reaction with an alcoholic solution of KOH and CS_2 in a cold condition which undergoes the cyclization reaction with hydrazine hydrate in an aqueous condition to form 1,2,4 triazole. The derivatives of triazole were converted to Schiff base by reaction with different aromatic aldehydes.

Results: The structure of prepared compounds was confirmed by using FTIR, ^{1}HNMR, ^{13}CNMR, and MS. The inhibitory effect of newly synthesized compounds against TNF-α was evaluated in comparison with standard ibuprofen. It showed good to moderate activity upon inhibition of TNF-α

Conclusion: The synthesized compounds are prepared successfully according to 1H-NMR, 13C-NMR, FT-IR, and mass spectroscopy data, and they showed a good to moderate inhibition rate of TNF-α compared to reference drug (Ibuprofen) they may use in the treatment of various autoimmune disease.

Keywords: Triazole, Schiff base, Anti-inflammatory, TNF-α, Ibuprofen.

INTRODUCTION

Cytokines are considered small proteins produced by cells that have a particular influence on cell interactions and communication.

Proinflammatory cytokines are produced by activated macrophages and have a role in the control of inflammatory responses. There is considerable evidence that pro-inflammatory cytokines such as (interleukin IL-1, IL-6) and tumor necrosis factor TNF- are involved in the pathological pain process and different autoimmune diseases, **Xie *et al*. [(1)]**.

TNF-α belongs to cytokine with a multifunctional molecule that regulates a wide range of biological processes such as cell proliferation, differentiation, death, lipid metabolism, and coagulation. TNF-α protein is primarily made by macrophages, and considerable quantities of it are released in response to lipopolysaccharide, other bacterial products, and Interleukin-1 (IL-1). TNF-α is associated with carcinogenesis and has a role in cancer therapy, **Horiuchi *et al*. [(2)].**

High levels of TNF-α result in inflammation and different autoimmune diseases such as rheumatoid arthritis, psoriatic arthritis, ulcerative colitis, and Crohn's disease. Several TNF-α inhibitors have been authorized for clinical use, such as setanercept, infliximab, adalimumab, golimumab, and certolizumab, as well as the assessment of existing TNF-α inhibitors employed as therapeutic medications against autoimmune disorders. The expression of COX2 is induced by TNF-α in human gingival fibroblast (HGF) by tyrosine kinase signaling resulting in the releasing of PGE2 formation, **Bauer *et al*. [(3)]**.

Triazole has a broad spectrum of biological activity such as antifungal by **Groll [(4)]**, anti-inflammatory by **Colanceska *et al*. [(5)]**, analgesic by **Xu *et al*. [(6)]**, anticancer activity by **Millson [(7)]**, antimigraine by **Sancak *et al*. [(8)]**, antioxidant by **Bektas *et al*. [(9)]**, anti-urease by **Asif [(10)]**, anti-parasitic by **Plech *et al*. [(11)]**, anticonvulsant by **Gupta *et al*. [(12)]**, and antimicrobial by **Al-Omar *et al*. [(13)]**.

Received: 15/7/2022
Accepted: 18/9/2022

It has activity against TNF-α as shown in the study by **Joshua *et al*. [14]**, in this study they synthesized three new derivatives of triazole and measuring their activity in the LPS-induced TNF-α assay. The pyridyl triazole derivative showed good inhibition against TNF-α while the pyrimidinyl triazole derivative showed excellent inhibition against TNF-α and the 3 acetamido-phenoxy-pyrimidinyl triazole derivative showed excellent inhibition against TNF-α and also showed good pharmacokinetic profile in the rat.

Haider *et al*. [15] synthesized a new triazole derivative of benzoxazolinone that exhibited significant TNF-α activity with 50.95% inhibition compared to the standard drug indomethacin with 64.01% inhibition.

Schiff base reaction resulted from the condensation of a primary amine with aldehydes or ketones. The newly synthesized compounds are produced from the reaction of 1,2,4 triazole linked to primary amine reacting with the different aromatic aldehyde to form Schiff base. It has a wide biological activity such as anti-microbial, anti-oxidant, anti-cancer activity, anti-Alzheimer, anti-inflammatory, antioxidants, urease inhibitors, and anti-tuberculosis [16-17].

This study aimed to synthesize new compounds of 1,2,4 triazole linked to different chemical groups with possible affinity to inhibit TNF-α could use in the treatment of various autoimmune diseases such as (rheumatoid arthritis, psoriasis, and irritable bowel syndrome).

MATERIAL AND METHODS

Synthesis of ethyl 2-(4-isobutylphenyl) propanoate (I)

Compound (**I**) was synthesized according to the method published in the literature by **Mustafa *et al*. [18].**

Synthesis of 2-(4-isobutylphenyl) propane hydrazide (II)

Compound (**II**) was synthesized according to a process published in the literature by **Mustafa *et al*. [18].**

Synthesis of 4-amino-5-(1-(4-isobutylphenyl) ethyl)-4H-1,2,4-triazole-3-thiol (III).

In a 100 m round-bottomed flask, 7.8 g (0.01 mmol) of compound (**II**) was dissolved in 10 ml of absolute ethanol and 3.98 g (0.02 mmol) of KOH was dissolved in 10 ml of absolute ethanol.

The mixture was cooled and 6.81 ml (0.02 mmol) of CS_2 was added drop by drop to the mixture. Then stirred for 12 hours in cold conditions resulted in the formation of an intermediate salt of potassium carbazinate. After that, the precipitate was filtrated, dried, and used for the next step without further purification. Yellow powder, Yield 91%, Melting point 290-292 0^oC.

7.5 g (0.01 mmol) of salt of potassium carbazinate was dissolved in 10 ml of water and 3 ml (0.03 mmol) of hydrazine hydrate (99%) was added to form the suspension. The mixture was refluxed for 20 hours until the evolution of H_2S gas ceased until getting a homogenous green solution. Then cooled solution, treated with 10 ml of ice water, and acidified with dilute HCl until a white precipitate formed.

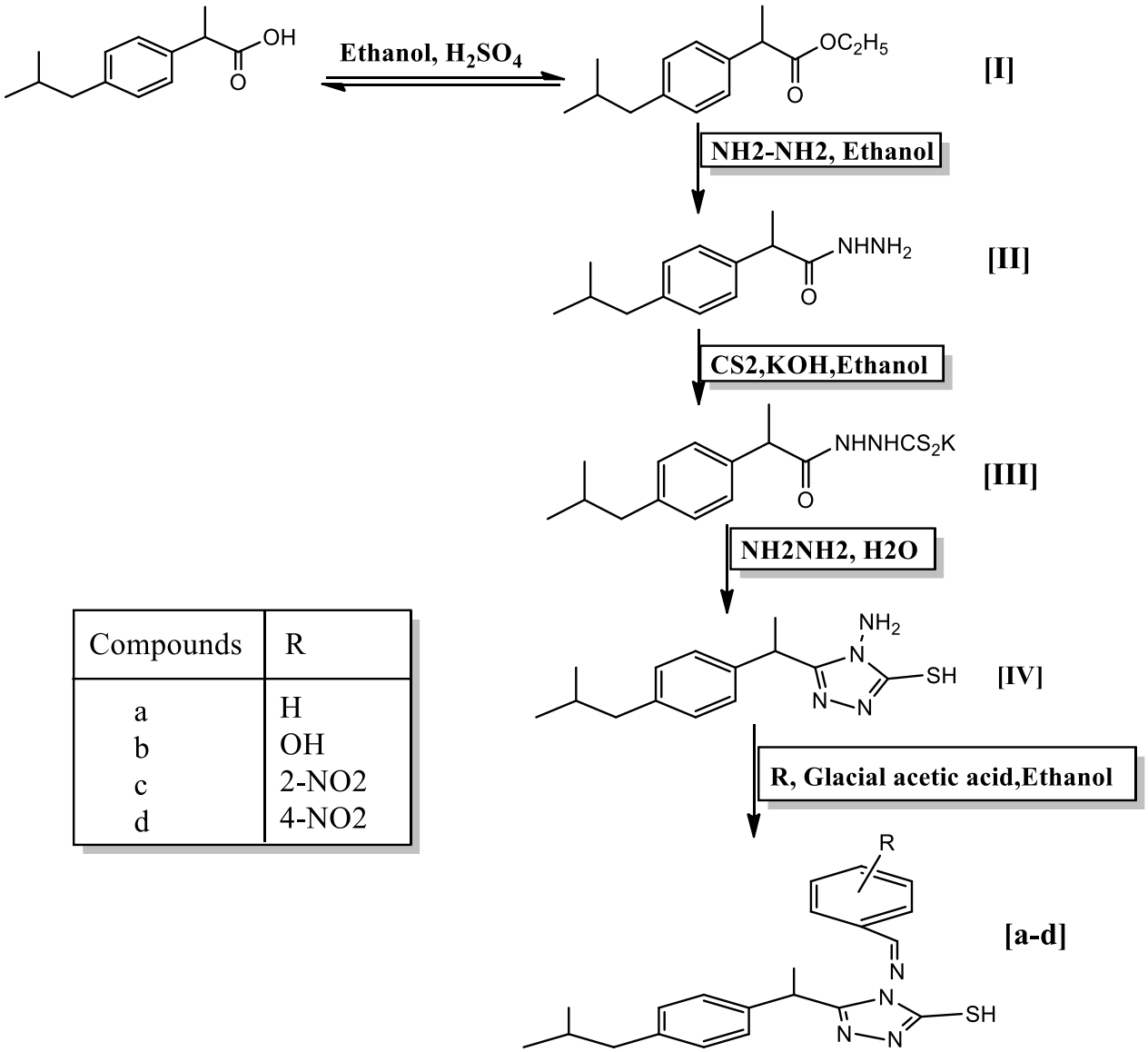
The physicochemical properties of compound (**III**) are shown in **Table (1).** In addition, the spectrophotometer data are shown in **Table** (**2**), **Al-Masoudi *et al*. [19].**

Synthesis of Schiff base derivatives (a-d)

In a 100 ml round-bottomed flask, (0.01 mmol) of compound (**III**) was dissolved in 10 ml of absolute ethanol and (0.01 mmol) of different aromatic aldehydes dissolved in 5 ml of absolute ethanol and 5 ml of glacial acetic acid then mixed.

The solution was refluxed for 6 hours. After that, the mixture was cooled and leave it overnight until getting the precipitate. The precipitate was filtrated and recrystallized from absolute ethanol. The physicochemical data of the newly synthesized Schiff bases were summarized in **Table (1).** The spectrophotometer data of the desired Schiff base were summarized in **Table (2)** [20, 21].

The chemical synthesis of new derivatives of 1,2,4 triazole is summarized in **Scheme (1)** below.



Compounds	R
a	H
b	OH
c	2-NO2
d	4-NO2

Scheme (1): The synthesis pathway of new derivatives of 1,2,4 triazole.

Ethical Approval

Ethical approval for this study was obtained from the research and publication committee in Baghdad region.

RESULTS

The newly synthesized compounds are prepared by converting Ibuprofen into 1,2,4 triazole-3-thiol linked to Schiff bases derivatives, and the chemical structures are confirmed by FT-IR, 1H-NMR, 13C-NMR, and mass spectroscopy. The data of synthesized compounds are shown in **Tables (1 and 2).**

Table 1: Physiochemical properties of the synthesized compounds

Compounds	M.wt	Appearance	Yield %	Melting point, ^{0}C
I	234.16	Pale yellow oil	90	Boiling point (264-266 ^{0}C)
II	220.16	White crystals	86	70-72
III	276	White crystals	56	165-167
a	364	White crystals	86	161-163
b	398	Yellow crystals	82	163-165
c	409	Yellow crystals	88	159-161
d	394	White crystals	80	174-176

Table 2: Spectrophotometer date of the synthesized compounds

Compounds	Molecular formula	Molecular ion peak M/Z	Characteristic absorption band of FT-IR (cm^{-1})	Chemical shifts of ^{1}H-NMR (ppm)	Chemical shifts of ^{13}C-NMR (ppm)
III	$C_{14}H_{20}N_4S$	276.2	3320.37-3440.40 w (NH2), 3143.67 s (NH), 2620.50 w (S-H)	4.45 (s, 2H, NH2), 11.52,(s, 1H, SH), 5.99, (d, 6H, CH3)	No signal of Schiff base
a	$C_{21}H_{24}N_4S$	364.2	3435,19 w (NH) 1611,91 w (HC=N)	10.03 (s, 1H, CH=N) 10.91 (s, 1H, SH) 0.814 (q, 6H,CH3)	161.81 (s, 1C, C=N)
b	$C_{21}H_{24}N_4OS$	380.2	3302.91 w (OH) 1505.25 w (HC=N)	10.36, (s, 1H, CH=N), 13.86 (s, H, OH) 0.76 (q, 6H,CH_3)	161.91 (s, 1C, C=N), 162.14 (s, 1C, C-OH)
c	$C_{21}H_{23}N_5O_2S$	409.1	3448.87 w (NH) 1603.92 w (HC=N)	10.87 (s, 1H, CH=N), 0.86 (q, 6H,CH_3)	155.06 (s, 1C, C=N), 148.68 (s, 1C, C-NO2)
d	$C_{21}H_{23}N_5O_2S$	409.2	3439.71 w (NH), 1596.29 w (HC=N)	10.75 (s, 1H, CH=N) 11.04 (s, 1H, SH) 0.84 (t, 6H,CH_3)	155.25 (s, 1C, C=N), 149.73 (s, 1C, C-NO2)

BIOLOGICAL ACTIVITY

Inhibition of Tumor necrosis factor-alpha assay (TNF-α)

TNF-α expression was measured using the eLabscience (TNF-α) ELISA Kit. In a 12-well plate, 1106 cells were treated with 500 g/ml LPS for 12 hours, then with 5mM ATP for 30 minutes. The supernatant was then collected. Control cells with the lowest concentration of TNF-α while LPS+ATP cells with the highest concentration of TNF-α to compare with the standard (ibuprofen) and new derivatives of triazole. The concentration used in this method was 100 g/ml for the standard and samples. While the concentration of (TNF-a) for the control cells and samples after three replications was summarized in **Table (3)** and **Figure (1).**

Table (3): TNF-a concentration activity of newly synthesized compounds

Compounds	Concentration of TNF-a pg/ml
Controlled cells	8.8
LPS+ATP cells	603.62
Ibuprofen	404.76
III	92.85
a	136.77
b	161.59
c	195.43
d	206.15

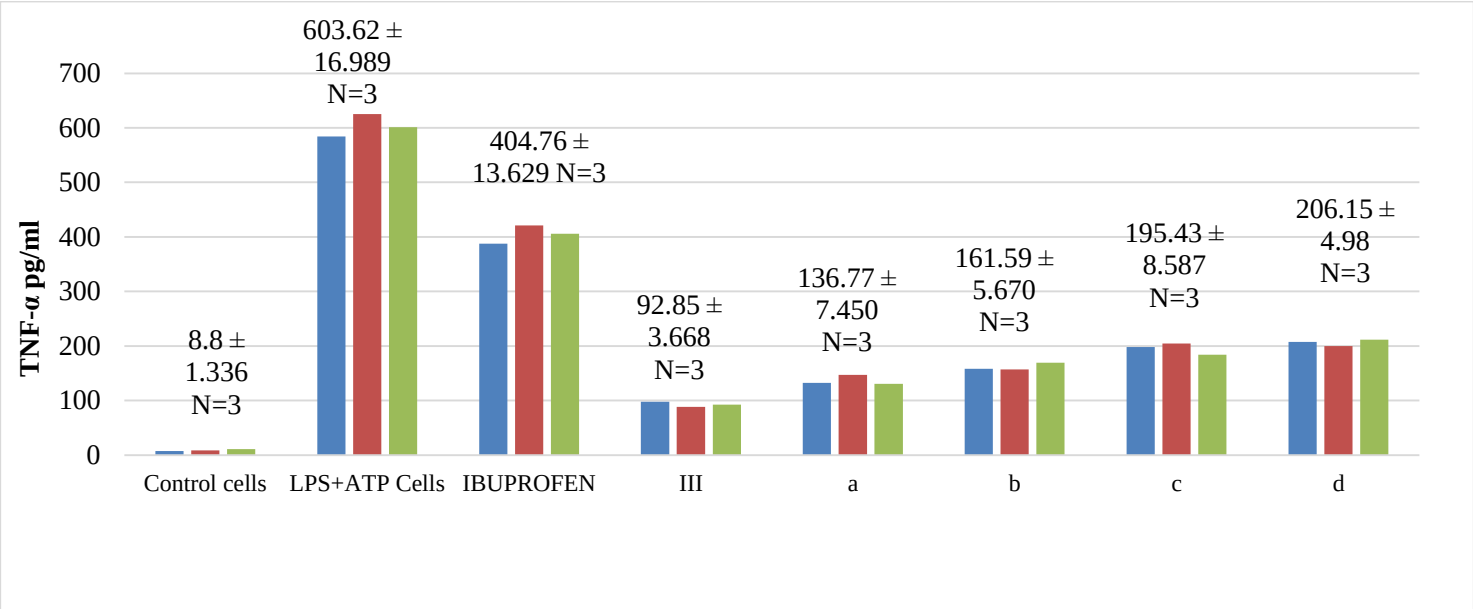


Figure (1): TNF-α concentration of new derivatives compounds

Ibuprofen was used as the standard compound. All the samples and standards were done in three replicates, and the results of the inhibition percent of TNF-α were calculated by the equation below. The results are shown in **Figure (2).**

$$\text{Percent of inhibition} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

Where A control is the concentration of TNF-a of LPS cells, A sample is the concentration of TNF-α of the samples.

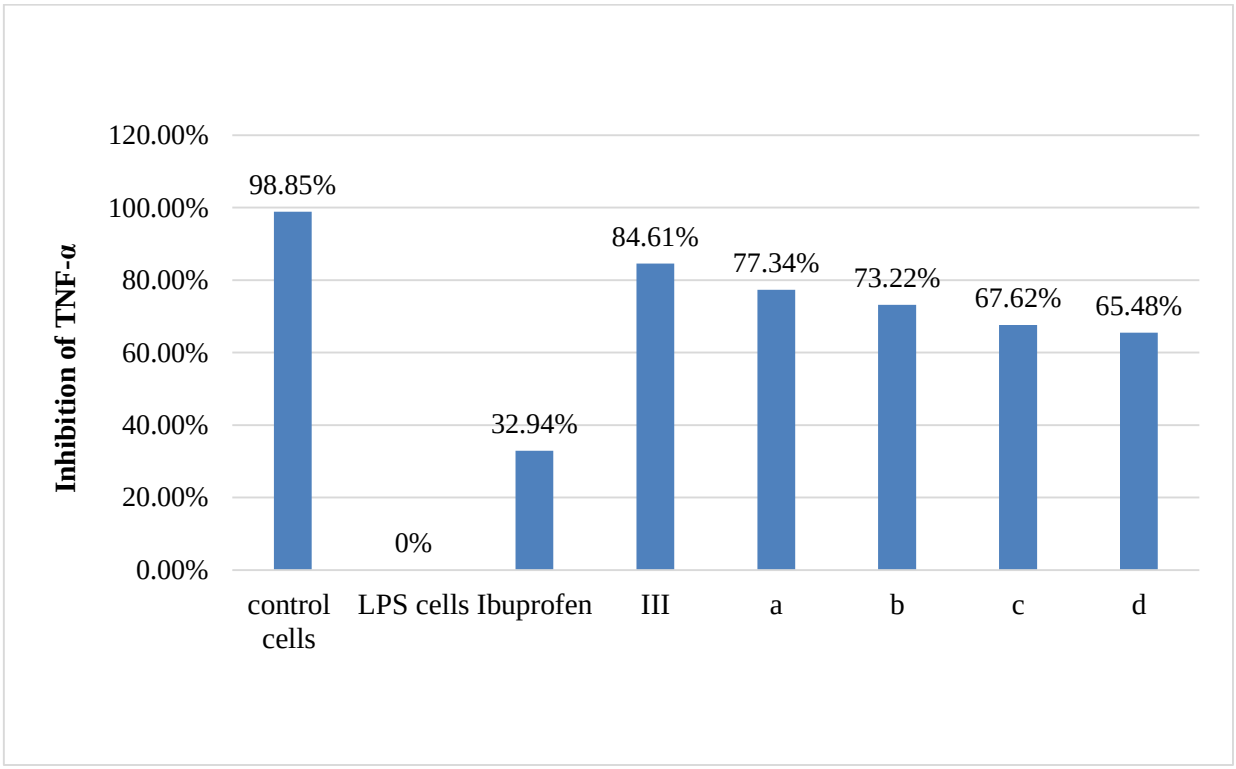


Figure (2): TNF-α inhibition percent of the synthesized compounds

DISCUSSION

The new derivatives are confirmed by FT-IR such as compound (I) showed a peak at 1716 cm^{-1} related to the (C=O) group of ester and that proves the formation of ester instead of carboxylic acid group, while compound (**II**) showed shifting a peak at 1685 cm^{-1} belongs to C=O group and two new signals at 3275 and 3313 cm^{-1} belongs to NH_2 group and one vibration at 3176 cm^{-1} corresponding to NH that prove the formation of compound (**II**) by **Nyquist** [(22)]. While compound (**III**) showed a new weak signal at 2620.50 cm^{-1} belonging to (S-H) and the main new signal that appears in the new derivatives of triazole at (1505-1610 cm^{-1}) corresponding to (HC=N) and disappearing signal range at (3275-3313 cm^{-1}) that related to NH2. Compound b showed a signal at 3302.91 cm^{-1} related to (OH). The ^{1}H-NMR spectroscopy of compound (**III**) showed a signal at 4.45 ppm (s,2H, NH_2) while the newly synthesized compounds showed disappearing that signal and appeared new signal range at (9.74-10.88 ppm) belonging to (HC=N). The ^{13}C-NMR of new derivatives of triazole showed the main singlet signal range at (155.06-161.91 ppm) corresponding to (HC=N). The Mass spectrophotometer of newly synthesized compounds showed that molecular ion peaks are equal to the calculated molecular weight of the synthesized compounds. The new compounds are successfully prepared according to the date of the spectrophotometer by **Gunther** [(23)].

The activity of compound (**III**) showed excellent inhibition against TNF-α about 84.61% compared to the standard and other derivatives while the new derivatives of triazole (**a-d**) showed good to moderate inhibition against TNF-α (77.34% to 65.84%). The compound (**III**) showed excellent inhibition against TNF-α can be due to the steric effect of (NH2) in comparison to other derivatives with a bulk group and it may increase the binding fit of the drug within the receptors and also can be attributed to hydrogen bonding of (NH2) within the receptors. Also compound (**III**) showed good inhibition against TNF-α due to the NH2 electron-donating group in the structure that increases the electron density and may increase the activity upon inhibition of TNF-α. While the other derivatives showed good to moderate activity and less than compound (**III**) can be due to the steric effect of additional aromatic aldehydes that binding to new derivatives resulted in decreasing the binding to the receptors and reducing their activity. The compound **a** showed good inhibition percent of TNF-α may be due to the steric effect of the size of benzaldehyde smaller than other derivatives of aromatic aldehydes that may increase the binding to the receptor that supports the previous explanation. The compound **b** showed good inhibition may be due to the electron donating group (OH) increasing the electron density and increasing the effect of inhibition of TNF-α. The compound (**c, d**) showed moderate inhibition against TNF-α due to the NO2 electron withdrawing group in the structure that decreases the electron density and may decrease the activity upon the inhibition of TNF-α by **Marie *et al.*** [(24)].

CONCLUSION

The new compounds were prepared successfully which showed a good to moderate inhibition activity against TNF-α and can be used in the treatment of different autoimmune diseases such as (rheumatoid arthritis, psoriasis, and irritable bowel syndrome).

DECLARATIONS

Consent for Publication: I confirm that all authors accept the manuscript for submission.

Availability of data and material: Available
Competing interests: None
Funding: No fund
Conflict of interest: The authors declare no conflicts of interest regarding the publication of this paper.

REFERENCES

1. **Xie R, Deng H, Li H *et al.* (2006):** Robust increase of cutaneous sensitivity, cytokine production and sympathetic sprouting in rats with localized inflammatory irritation of the spinal ganglia. Neuroscience, 142: 809–822.
2. **Horiuchi T, Mitoma H, Harashima S, Tsukamoto H, Shimoda T *et al.* (2010):** Transmembrane TNF-alpha: Structure, function, and interaction with anti-TNF agents**.** Rheumatology, 49:1215-1228.
3. **Bauer K, Lieb K, Schulze K, Berger M, GebickeHaerter J, Bauer J, Fiebich L *et al.* (1997):** Expression and regulation of cyclooxygenase-2 in rat microglia. European Journal of Biochemistry, 243: 726-731.
4. **Groll H, Walsh J (2002):** Antifungal chemotherapy: Advances and perspectives. Swiss Medical Weekly, 132:303–311.
5. **Colanceska K, Dimova V, Kakurinov V, Molnar D, Buzarovska A *et al.* (2001):** Synthesis, Antibacterial and Antifungal Activity of 4-Substituted-5Aryl-1,2,4-Triazoles. Molecules, 6: 815–824.
6. **Xu Z, Zhao J, Liu Y *et al.* (2019):** 1, 2, 3-Triazole-containing hybrids as potential anticancer agents: Current developments, action mechanisms, and structure-activity relationships. European Journal of Biochemistry, 183:111700–111713.
7. **Millson D, Tepper S (2000**): Migraine pharmacotherapy with oral triptans: a rational approach

to clinical management. Expert Opinion on Pharmacotherapy, 1(3): 391-404.

8. **Sancak K, Unver Y, Unluer D, Dugdu E, Kor G, Celic F, Birinci E *et al.* (2012):** Synthesis, characterization, and antioxidant activities of new tri-substituted triazoles. Turkish Journal of Chemistry, 36: 457–466.
9. **Bektas H, Ceylan S, Demirba N, Alpay S, Sokmen B *et al.* (2013):** Antimicrobial and antiurease activities of newly synthesized morpholine derivatives containing an azole nucleus. Medicinal Chemistry Research, 22(8): 3629–3639.
10. **Asif M (2015):** Antiviral and antiparasitic activities of various substituted triazole derivatives: a mini-review. Chemistry International**,** 1(2):71–80.
11. **Plech T, Kapron B, Luszczki J, Wujec M, Paneth A, Siwek A, Nowak G *et al.* (2014):** Studies on the anticonvulsant activity and influence on GABA-ergic neurotransmission of 1,2,4-triazole-3-thione-based compounds. Molecules, 19(8): 11279-11299.
12. **Gupta K, Prachand S, Patel A, Jain S *et al.* (2012):** Synthesis of some 4-amino-5-(substituted-phenyl)-4H-[1,2,4] triazole-3-thiol derivatives and antifungal activity. International Journal of Pharmaceutical and Life Sciences, 3(7): 1848-1857.
13. **Al-Omar A, Al-Abdullah S, Shehata A, Habib E, Ibrahim M, El-Emam A, *et al.* (2010):** Synthesis, antimicrobial and anti-inflammatory activities of novel 5-(1-adamantyl)-4-arylideneamino-3-mercapto-1,2,4-triazoles and related derivatives. Molecules, 15:2526–2550.
14. **Joshua S, John C, Michael G, Michael P, Biswanath D, Lily C, Michael J *et al.* (2003):** The development of new triazole-based inhibitors of tumor necrosis factor-α (TNF-α) production. Bioorganic & Medicinal Chemistry, 13(10), 1665–1668.
15. **Haider S, Alam S, Hamid H, Shafi S, Nargotra A, Mahajan P *et al.* (2013):** Synthesis of novel 1,2,3-triazole based benzoxazolinones: Their TNF-a based molecular docking with in-vivo anti-inflammatory, antinociceptive activities, and ulcerogenic risk evaluation. European Journal of Biochemistry, 70: 579-588.
16. **Kajal A, Bala S, Kamboj S *et al.* (2013):** Schiff bases: a versatile pharmacophore. Journal of Catalysts, 13:1-14.
17. **Abudief M, Mohamed M (2015):** A review on versatile applications of transition metal complexes incorporating Schiff bases. Beni-Suef University Journal, 4(2):119-33.
18. **Mustafa Q, Leaqaa A. Raheem A, Falah H *et al.* (2020):** Synthesis, Characterization of Ibuprofen N-Acyl-1,3,4- Oxadiazole Derivatives and Anticancer Activity against MCF-7 Cell Line. The Systematic Reviews in Pharmacy, 11(4): 681 689.
19. **Al-Masoudi A, Al-Soud Y, Al-Salihi N, Al-Masoudi N *et al.* (2006):** 1,2,4-triazoles: synthetic approaches and pharmacological importance. Chemistry of Heterocyclic Compounds, 42(11): 1377-1403.
20. **Zainab J, Mariam A (2020):** Synthesis and Antibacterial Activity of Schiff Base Compounds Derived from Glyox, Vanillin with Their Complexes with Iron (ш).The Egyptian Journal of Chemistry, 63(10): 105-114.
21. **Mohammed Q (2011):** Synthesis and characterization of new Schiff bases and evaluation as Corrosion inhibitors. Journal of Basrah Researches Sciences, 37(4): 116-130.
22. **R. Nyquist (2001):** Interpreting Infrared, Raman and Nuclear Magnetic Resonance Spectra. Academic Press, 2: 105-110.
23. **H. Gunther (1995):** NMR Spectroscopy: Basic Principles, Concepts, and Applications in Chemistry, John Wiley & Sons, 2nd edition, 78-88.
24. **Marie L, Lohmann M, Domzig M, Meerpohl H *et al.* (1997):** Mouse bone marrow-cultured macrophage as indicator cells for mouse and human migration inhibitory factor (MIF). Eur. J. Immunol., 8: 585-588