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*RESEARCH ARTICLE*

# Synthesis and Biological Evaluation of Novel Paracetamol-Triazole Conjugates

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***Synthesis and Biological Evaluation of Novel Paracetamol-Triazole Conjugates***

***SUMMARY***

*Some new triazole containing acetamide derivatives **9-20** using paracetamol as starting material were synthesized, and their structures were verified by FTIR, NMR ($^{1}H$ and $^{13}C$) and mass spectral data. Compounds **9-20** were tested against five human cancer cell lines (lung cancer A549, chronic myelogenous leukemia K562, breast cancer MCF-7, prostate cancer PC-3, neuroblastoma SH-SY5Y cell lines) for in vitro cytotoxic activities. They were also evaluated their cytotoxic effect on mouse embryonic fibroblast cells (NIH/3T3) to define selectivity by MTT assay. Additionally, we evaluated the potential mPGES-1 and COX-1/2 inhibitory effect of twelve target compounds **9-20**. While none of the synthesized compounds exhibited significant inhibition against both cancer cells and mPGES-1 as well as COX-1/2, it was determined that they were not cytotoxic against healthy cells, too. Finally, ADMET properties of newly synthesized compounds were estimated using in silico methods.*

***Key Words:*** *Paracetamol, 1,2,4-triazole, cancer, mPGES-1, COX-1/2.*

***Yeni Parasetamol-Triazol Konjugatlarının Sentezi ve Biyolojik Değerlendirmesi***

***ÖZ***

*Başlangıç maddesi olarak parasetamol kullanılarak bazı yeni triazol içeren asetamid türevleri **9-20** sentezlendi ve yapıları FTIR, NMR ($^{1}H$ and $^{13}C$) ve kütle spektral verileri ile karakterize edildi. Beş insan kanser hücre hattına (akciğer kanseri A549, kronik miyelojenöz lösemi K562, meme kanseri MCF-7, prostat kanseri PC-3, nöroblastoma SH-SY5Y hücre hatları) karşı sentezlenen tüm moleküllerin in vitro sitotoksik aktiviteleri incelendi ve ayrıca seçiciliği tanımlamak için fare embriyonik fibroblast hücreleri (NIH/3T3) üzerinde sitotoksik etkileri MTT yöntemiyle test edildi. Ek olarak, on iki hedef bileşik **9-20**, mPGES-1 ve COX-1/2 inhibe edici etkileri açısından tarandı. Sentezlenen bileşiklerin hiçbiri hem kanser hücrelerine hem de mPGES-1 ve COX-1/2 enzimlerine karşı anlamlı bir inhibisyon göstermezken, sağlıklı hücrelere karşı da sitotoksik olmadıkları belirlendi. Son olarak yeni sentezlenen bileşiklerin ADMET özellikleri in siliko yöntemler kullanılarak tahmin edildi.*

***Anahtar Kelimeler:*** *Parasetamol, 1,2,4-triazol, kanser, mPGES-1, COX-1/2.*

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## INTRODUCTION

Characterized by uncontrolled cell division, cancer is one of the significant causes of death worldwide. According to the World Health Organization (WHO) data, cancer caused about 10 million deaths (nearly one in six deaths) in 2020 (WHO, 2022). Today, the primary method of treating cancer is surgery-based therapy, but patients undergoing surgical treatment are often at risk of death and perioperative complications. Apart from surgical interventions, radiotherapy and chemotherapy, which are known to inhibit tumor cell growth, proliferation and metastasis, play a critical role in cancer treatment. However, the used drugs can cause more problems due to their undesired side effects (Zhai et al., 2022). Hence, ongoing research endeavors are focused on identifying novel anticancer agents characterized by improved side effect profiles.

Cancer formation, development, and metastasis are closely linked to the tumor microenvironment, and clinical studies have reported increasing levels of microsomal prostaglandin E2 synthase-1 (mPGES-1) and cyclooxygenase-2 (COX-2) in several human cancers (Murakami, 2011) bind to their cognate G protein-coupled receptors (GPCRs). Even the relationship of breast (Howe et al., 2013), colon (Sasaki, Nakatani, & Hara, 2015), prostate (Hanaka et al., 2009), lung (Chang et al., 2012), neuroblastoma (Kock et al., 2018) and many more cancer types with COX-2 and mPGES-1 enzymes has been reported in the literature. Cyclooxygenases (COXs) are rate-limiting enzymes, which are involved in the conversion of arachidonic acid to prostaglandin E2 (PGE2). It has two structurally analogous isoforms (COX-1 and COX-2). Long-term use of COX-1 and COX-2 inhibitors has been reported to beget gastrointestinal and cardiovascular side effects, respectively (Crofford, 1997). It is known that COX-1 has also been known to be regulated, for example, Interleukin 4 (IL-4) and results in increased production of PGs (Shay et al., 2017), and the excessive of PGE2 is synthesized by COX-2 enzyme and mPGES-1 enzyme (Akasaka, So, & Ruan, 2015). Therefore, mPGES-1 and COX-1/2 enzymes have been the focus of attention as new targets in cancer therapy. The triazole ring is an electron-rich aromatic structure. It exhibits a wide range of biological activities such as anti-tuberculosis (Küçükgüzel, Küçükgüzel, Rollas, & Kiraz, 2001), anti-inflammatory (Küçükgüzel et al., 2007) and anticancer activities (Kulabaş et al., 2016) due to their ability to easily bind to different macromolecular targets with weak interactions. Moreover, many 1,2,4-triazole-thioether derivatives were evaluated as anticancer (Demirbolat et al., 2022; Kulabaş et al., 2016; Zengin, Unsal Tan, Arafa, & Balkan, 2022) agents whereas several reports indicate them as potential inhibitors of mPGES-1 (Bülbül et al., 2022; Erensoy et al., 2023; He, Li, Liu, & Lai, 2013). Paracetamol, a non-opioid antipyretic and analgesic agent, is used in the treatment of fever and pain as it inhibits COX-1/2 enzymes (Graham, Davies, Day, Mohamudally, & Scott, 2013). In light of previous studies on the anticancer and anti-inflammatory effect of triazolyl-sulfanyl acetamides, as shown in Figure 1, we decided to design novel paracetamol-triazole conjugates bearing acetanilide moiety.

## MATERIAL AND METHOD

### Chemicals and instruments

All solvents and reagents utilized in this study were of analytical grade and were procured from commercially reputable sources. The compounds' purity was ascertained through thin-layer chromatography (TLC) conducted on Merck silica gel 60 F254 aluminum sheets (Merck, Darmstadt, Germany). UV light at a wavelength of $\lambda$= 254 nm was employed to visualize the spots. The determination of all melting points (°C, uncorrected) was carried out utilizing the Electro thermal IA9300 melting point apparatus. Infrared spectra (FTIR) were captured using a Shimadzu FTIR 8400s, with data presented in wavenumbers ($cm^{-1}$). Nuclear Magnetic Resonance (NMR) spectra were acquired employing a Bruker Avance NMR spectrometer operating at frequencies of 300 MHz for $^{1}H$ NMR and 75 MHz for $^{13}C$ NMR.

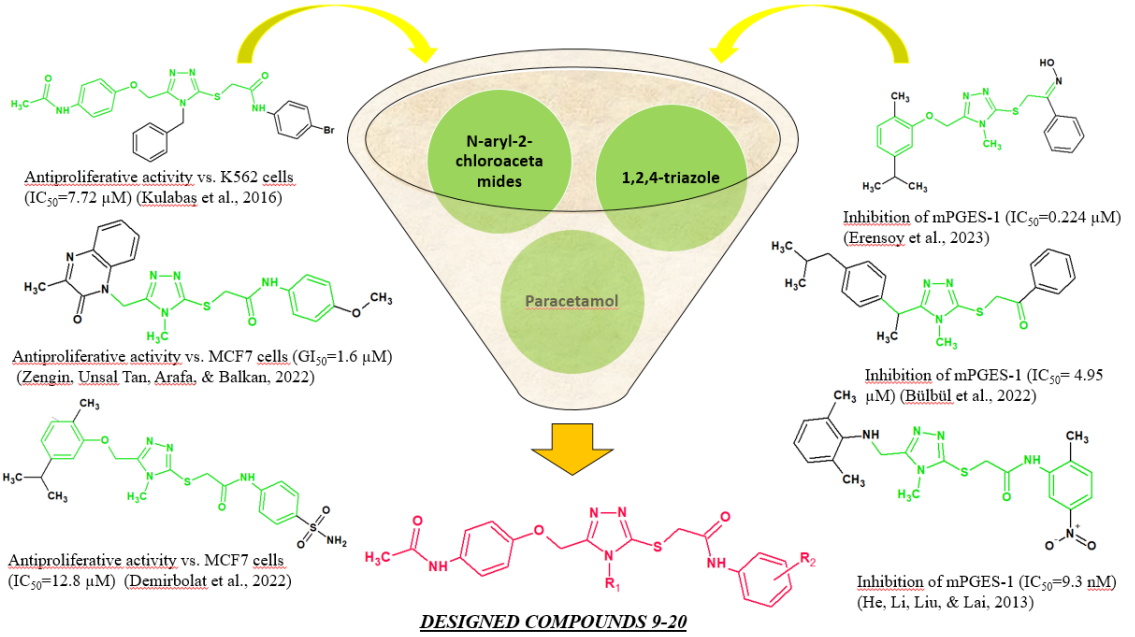



**Figure 1.** The reported 1,2,4-triazoles with antiproliferative and mPGES-1 inhibitory effects and our designed paracetamol-triazole conjugates **9**-**20**.

Chemical shifts were denoted in parts per million (ppm), referenced downfield from tetramethylsilane (TMS), with DMSO-$d_6$ serving as the solvent. The mass spectra were obtained using the Shimadzu LC-MS/MS-8030 system (Shimadzu Corporation, Kyoto, Japan). Operating within the realm of positive electrospray ionization (ESI) mode, the instrument entailed a nebulizing gas flow rate of 2.8 L/min, a drying gas flow of 15 L/min, a DL temperature of 250 °C, a heat block temperature of 400 °C, CID gas at 230 kPa, collision energy set at -5.0 V, a scan speed of 15000 u/sec, and an event time of 0.030 sec. Elemental analyses were conducted using the Leco CHNS 932 apparatus.

**Chemistry**

**General synthesis method of *N*-aryl-2-chloroacetamides (I-IV)**

The reaction mixtures consisted of dissolved substituted aniline derivatives (0.003 mol) in dichloromethane (DCM), followed by the addition of trimethylamine (TEA) (0.5 ml). 2-Chloroacetyl chloride (0.005 mol ) was dropped onto the mixture at 0-5°C. Then the flask content was stirred under reflux at 50°C for 4 h. After completion of the reaction according to TLC; the mixtures were cooled, filtered, dried, and recrystallized from methanol to obtain products **I**-**IV** (Demirci et al., 2018; Kulabaş et al., 2022).

2-Chloro-*N*-(4-acetylphenyl)acetamide (**I**): M.p. 146.2-147.4°C (lit. 169.8-172 °C, (Haider & Hamada, 2012)), yield 85.9%. TLC Rf: 0.36. IR $cm^{-1}$: 3281 (N-H stretching band (str)), 1703 (C=O str, ketone), 1651 (C=O str, anilide).

2-Chloro-*N*-(4-nitrophenyl)acetamide (**II**): M.p. 176.2-177 °C (lit. 177-180 °C, (Zhaowen et al., 2007)), yield 70%. TLC Rf: 0.66. IR $cm^{-1}$: 3266 (N-H str), 1684 (C=O str, anilide).

2-Chloro-*N*-(2-methyl-5-nitrophenyl)acetamide (**III**): M.p. 155.5-155.7 °C (lit. 144-146 °C, (Svetkin, Minlibaeva, & Khafizova, 1961)), yield 63%. TLC Rf: 0.59. IR $cm^{-1}$: 3252 (N-H str), 1672 (C=O str, anilide).

Ethyl 4-[(chloroacetyl)amino]benzoate (**IV**): M.p. 112.4-112.7 °C (MeOH) (lit. 106-107 °C,(Haider & Hamada, 2012)), yield 78%. TLC Rf: 0.67. IR $cm^{-1}$:

3273 (N-H str), 1717 (C=O str, ester), 1674 (C=O str, anilide).

**General synthesis method of ethyl (4-acetamidophenoxy)acetate (1)**

Paracetamol (0.05 mol) and 0.075 mol anhydrous potassium carbonate were dissolved in dry acetone and stirred at 65 °C for 4 h under reflux. Then ethyl 2-bromoacetate (0.055 mol) was added dropwise to this mixture during 1 h, and refluxed for another 8 h. Following the termination of the reaction, the mixture underwent filtration, and the excess acetone was subjected to distillation. Afterward, the resulting product underwent a washing process with water, followed by drying and recrystallization from ethanol (Küçükgüzel, Tatar, Küçükgüzel, Rollas, & De Clercq, 2008).

Ethyl [4-(acetylamino)phenoxy]acetate (**1**): M.p. 103.2-103.6 °C (MeOH) (lit. 106-107 °C,(Morar, Cost, Lameiras, Antheaume, & Darabantu, 2015)), yield 68.5%. TLC Rf: 0.50. IR $cm^{-1}$: 3379 (N-H), 1738 (C=O, ester), 1674 (C=O, anilide).

**General synthesis method of 2-(4-acetamidophenoxy) acetohydrazide (2)**

The ethanol solution containing compound **1** (0.015 mol) was subjected to reflux with hydrazine monohydrate (0.045 mol) for 3 h. After cooling the reaction mixture, the resulting residue was washed with ice-cold water and subsequently filtered. Compound **2** was acquired by recrystallization from ethanol (Küçükgüzel et al., 2008).

*N*-[4-(2-Hydrazinyl-2-oxoethoxy)phenyl] acetamide (**2**): M.p. 193.5-194.1°C (MeOH) (lit. 194-195 °C,(Nargund, Reddy, & Hariprasad, 1994)), yield 85.6%. TLC Rf: 0.30. IR $cm^{-1}$: 3322, 3298, 3190 (N-H), 1676 (C=O, anilide), 1642 (C=O, hydrazide).

**General synthesis method of *N*-methyl/ethyl/phenyl-2-(substituted aryloxyacetyl) hydrazinecarbothioamide (3-5)**

Compound **2** (0.03 mol) was mixed with methyl/ethyl/phenylisothiocyanate (0.03 mol) in methanol (40 ml), and this mixture was refluxed for 6 h at 95°C. The cooled solution was filtered and then washed with water. The resulting solid product was subjected to recrystallization from methanol, resulting in the acquisition of compounds **3**-**5** (Kulabaş et al., 2016).

*N*-(4-{2-[2-(Methylcarbamothioyl)hydrazinyl]-2-oxoethoxy}phenyl)acetamide (**3**): M.p. 195.4-196.8 °C (MeOH) (lit. 222-226 °C,(İlkay Küçükgüzel et al., 2008)), yield 62%. TLC Rf: 0.48. IR *ν* ($cm^{-1}$): 3223, 3131, 3072 (N-H str), 2953-2870 (C-H str), 1720, 1640 (C=O str), 1229 (C=S str).

*N*-(4-{2-[2-(Ethylcarbamothioyl)hydrazinyl]-2-oxoethoxy}phenyl)acetamide (**4**): M.p. 191.5-192.1°C (MeOH) (lit. 182-184 °C,(İlkay Küçükgüzel et al., 2008)), yield 60%. TLC Rf: 0.57. IR *ν* ($cm^{-1}$): 3264, 3131 (N-H str), 3070-2918 (C-H str), 1690, 1643 (C=O str), 1215 (C=S str).

*N*-(4-{2-[2-(Phenylcarbamothioyl)hydrazinyl]-2-oxoethoxy}phenyl)acetamide (**5**): M.p. 196.7-196.9 °C (MeOH) (lit. 190-192 ° C,(İlkay Küçükgüzel et al., 2008)), yield 51%. TLC Rf: 0.07. IR v ($cm^{-1}$): 3295, 3243, 3129 (N-H str), 3101-2906 (C-H str), 1699, 1661 (C=O str), 1238 (C=S str).

**General synthesis method of 4-methyl/ethyl/phenyl-5-(substituted aryloxymethyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (6-8)**

A mixture of compounds **3**, **4,** or **5** (0.02 mol) and TEA (8 ml) in ethanol (40 ml) was refluxed for 6 h at 95 °C. After the reaction was terminated, the flask content was neutralized by 5% HCl to give desired compounds. The obtained solid product was subjected to filtration, followed by drying and recrystallization from ethanol (Kulabaş et al., 2016).

*N*-{4-[(4-Methyl-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)methoxy]phenyl}acetamide (**6**): M.p. 221.7-211.9 °C, yield 71%. TLC Rf: 0.26. IR *ν* ($cm^{-1}$): 3289, 3133 (N-H str), 3055-2949 (C-H str), 1669 (C=O str), 1539 (C=N str), 1275 (C=S str). $^1$H NMR (300 MHz, DMSO-$d_6$) δ ppm: 1.06 (3H, t, *J= 6 Hz*, C**H**$_3$CH$_2$OH); 2.01 (3H, s, -COC**H**$_3$); 2.33 (3H, s, Ar-C**H**$_3$); 3.49 (3H, s, N-C**H**$_3$); 5.17 (2H, s, -OC**H**$_2$); 7.01 (2H, d, J= 9 Hz, Ar-**H**); 7.51 (2H, d, *J= 9 Hz*, Ar-

**H**); 9.86 (1H, s, amide -N**H**-); 13.83 (1H, s, triazole -N**H**-). Anal. calc. for $C_{12}H_{14}N_4O_2S$.1/3 mol $C_2H_5OH$ C: 51.80, H: 5.49, N: 19.08, S: 10.92; found C: 51.78, H: 5.07, N: 20.13, S: 11,52.

*N*-{4-[(4-Ethyl-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)methoxy]phenyl}acetamide (**7**): M.p. 198.4-198.8 °C, yield 69%. TLC Rf: 0.33. IR *ν* ($cm^{-1}$): 3349 (N-H str), 3084-2851 (C-H str), 1672 (C=O str), 1539 (C=N str), 1275 (C=S str). $^1$H NMR (300 MHz, DMSO-$d_6$) δ ppm: 1.06 (3H, t, *J= 7 Hz*, C**H**$_3$CH$_2$OH); 1.31 (3H, t, *J= 7.2 Hz*, N-CH$_2$C**H**$_3$); 2.01 (3H, s, -COC**H**$_3$); 3.39-3.49 (m, CH$_3$C**H**$_2$OH and DMSO); 4.04 (2H, q, *J= 6.6 Hz* and *7 Hz*, N-C**H**$_2$CH$_3$); 4.39 (1H, t, *J= 5.1* Hz, CH$_3$CH$_2$O**H**); 5.18 (2H, s, -OC**H**$_2$); 7.00 (2H, d, *J= 9 Hz*, Ar-**H**); 7.51 (2H, d, *J= 9 Hz*, Ar-**H**); 9.86 (1H, s, amide -N**H**-); 13.84 (1H, s, triazole -N**H**-). Anal. calc. for $C_{13}H_{16}N_4O_2S$.1/2 mol $C_2H_5OH$ C: 53.31, H: 6.07, N: 17.76, S: 10.17; found C: 53.48, H: 5.52, N: 19.16, S: 10.97.

*N*-{4-[(4-Phenyl-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)methoxy]phenyl}acetamide (**8**): M.p. 213.9-214.1 °C, yield 63%. TLC Rf: 0.38. IR v ($cm^{-1}$): 3293 (N-H str), 3094-2920 (C-H str), 1659 (C=O str), 1543 (C=N str), 1240 (C=S str). $^1$H NMR (300 MHz, DMSO-$d_6$) δ ppm: 1.99 (3H, s, -COCH$_3$); 4.91 (2H, s, -OCH$_2$); 6.75 (2H, d, J= 9 Hz, Ar-H); 7.38-7.54 (7H, m, Ar-H); 9.81 (1H, s, amide -NH-); 14.06 (1H, s, triazole -NH-). Anal. calcd. for $C_{17}H_{16}N_4O_2S$ C: 59.62, H: 4.48, N: 16.30, S: 9.29; found C: 59.98, H: 4.74, N: 16.46, S: 9.42.

**General synthesis method of 2-{[3-(substituted aryloxymethyl)-4-methyl/ethyl/phenyl-4,5-dihydro-1*H*-1,2,4-triazol-5-yl]sulfanyl}-*N*-(4-substituted phenyl)acetamide (9-20)**

The solution of compounds **6**, **7**, or **8** (0.001 mol) was prepared in dimethylformamide (DMF) (10 ml), and subsequently, anhydrous potassium carbonate (0.002 mol) was introduced into the solution. Following an hour of stirring at room temperature, the solution containing compounds **I**-**IV** (0.001 mol) in DMF (3 ml) was introduced into the mixture. The reaction medium was stirred by refluxing for 6 h at 50-60 °C. After the reaction was terminated, the solution was cooled, and the product was filtered, and subjected to washing with water. Finally, compounds **9**-**20** were recrystallized from ethanol (Kulabaş et al., 2016).

2-[(5-{[4-(Acetylamino)phenoxy]methyl}-4-methyl-4*H*-1,2,4-triazol-3-yl)sulfanyl]-*N*-(4-acetylphenyl)acetamide (**9**): M.p. 280.9-281.8 °C, yield 50%. TLC Rf: 0.25. IR *ν* ($cm^{-1}$): 3237, 3187 (N-H str), 3102-2863 (C-H str), 1694, 1659 (C=O str), 1541 (C=N str), 671 (C-S str). $^1$H NMR (300 MHz, DMSO_d6) δ ppm: 2.01 (3H, s, -COC**H**$_3$); 2.53 (3H, s, -COC**H**$_3$); 3.61 (3H, s, N-C**H**$_3$); 2.73 and 2.89 (s, DMF peak); 4.16 (2H, s, -S-C**H**$_2$-); 5.23 (2H, s, -OC**H**$_2$); 7.00 (2H, d, *J= 9 Hz*, Ar-**H**); 7.49 (2H, d, *J= 9 Hz*, Ar-**H**); 7.70 (2H, d, *J= 9 Hz*, Ar-**H**); 7.95 (2H, d, *J= 9 Hz*, Ar-**H**); 9.83 (1H, s, -N**H**-); 10.67 (1H, s, -N**H**). $^{13}$C NMR (75 MHz, DMSO-$d_6$/TMS): δ ppm: 24.28, 26.90, 30.93 (aliphatic **C**), 37.94 (S-**C**H$_2$), 60.79 (O-**C**H$_2$), 115.59, 118.87, 120.88, 130.02, 132.43, 133.95, 143.50, 151.01 (Ar-**C**), 152.67 and 153.71 (triazole **C**3 and **C**5), 166.77 and 168.70 (**C**O, amide), 196.97 (Ar-**C**OCH$_3$). Anal. calc. for $C_{22}H_{23}N_5O_4S$ C:58.26, H:5.11, N:15.44, S:7.07; found C:58.06, H:5.25, N:15.37, S:7.07. LR-MS (ESI) (m/z): calc. for $(M+Na)^+$: 476.15, found: 476.15. Calc. for $(M-H)^-$: 452.15, found: 452.15.

2-[(5-{[4-(Acetylamino)phenoxy]methyl}-4-methyl-4*H*-1,2,4-triazol-3-yl)sulfanyl]-*N*-(4-nitrophenyl)acetamide (**10**): M.p. 279.5-280.0 °C, yield 58%. TLC Rf: 0.29. IR *ν* ($cm^{-1}$): 3219, 3159 (N-H str), 3096-2834 (C-H str), 1694, 1659 (C=O str), 1547 (C=N str), 688 (C-S str). $^1$H NMR (300 MHz, DMSO-$d_6$) δ ppm: 2.01 (3H, s, -COC**H**$_3$); 2.73 and 2.89 (s, DMF peak); 3.62 (3H, s, N-C**H**$_3$); 4.19 (2H, s, -S-C**H**$_2$-); 5.23 (2H, s, -OC**H**$_2$); 7.00 (2H, d, *J= 9 Hz*, Ar-**H**); 7.49 (2H, d, *J= 9 Hz*, Ar-**H**); 7.81 (2H, d, *J= 9 Hz*, Ar-**H**); 8.23 (2H, d, *J= 9 Hz*, Ar-**H**); 9.83 (1H, s, -N**H**-); 10.94 (1H, s, -N**H**). $^{13}$C NMR (75 MHz, DMSO-$d_6$/TMS): δ ppm: 19.03, 24.25 (aliphatic **C**), 37.32 (S-**C**H$_2$), 60.58 (O-**C**H$_2$), 115.47, 119.32, 120.79, 125.54, 127.43, 130.28, 130.62, 133.03, 133.88, 142.86, 145.34,

151.83 (Ar-**C**), 152.33 and 153.47 (triazole **C**3 and **C**5), 167.00 and 168.27 (**C**O, amide). Anal. calc. for $C_{20}H_{20}N_6O_5S$.1/5 mol DMF C:52.52, H:4.58, N:18.43, S:6.81; found C:51.24, H:4.55, N:18.12, S:6.94. LR-MS (ESI) (m/z): Calc. for $(M+Na)^+$: 479.12, found: 479.10. Calc. for $(M-H)^-$: 455.12, found: 455.10.

2-[(5-{[4-(Acetylamino)phenoxy]methyl}-4-methyl-4*H*-1,2,4-triazol-3-yl)sulfanyl]-*N*-(2-methyl-5-nitrophenyl)acetamide (**11**): M.p. 216.4-217.7 °C, yield 47%. TLC Rf: 0.27. IR *ν* ($cm^{-1}$): 3243, 3190 (N-H str), 3026-2834 (C-H str), 1669 (C=O str), 1537 (C=N str), 681 (C-S str). $^1$H NMR (300 MHz, DMSO-$d_6$) δ ppm: 1.06 (0.3 H, t, *J= 6 Hz*, 0.1 mol C**H**$_3$CH$_2$OH); 2.01 (3H, s, -COC**H**$_3$); 2.33 (3H, s, Ar-C**H**$_3$); 3.62 (3H, s, N-C**H**$_3$); 4.21 (2H, s, -S-C**H**$_2$-); 5.24 (2H, s, -OC**H**$_2$); 7.01 (2H, d, *J= 9 Hz*, Ar-**H**); 7.46-7.52 (3H, m, Ar-**H**); 7.95 (1H, dd, *J= 2.4 Hz* and *9 Hz*, Ar-**H**); 8.50 (1H, d, *J= 2.4 Hz*, Ar-**H**); 9.83 (1H, s, -N**H**-); 9.99 (1H, s, -N**H**-). $^{13}$C NMR (75 MHz, DMSO-$d_6$/TMS): δ ppm: 18.52, 24.28, 30.96 (aliphatic **C**), 37.23 (S-**C**H$_2$), 60.76 (O-**C**H$_2$), 115.57, 118.25, 119.90, 120.88, 131.92, 133.95, 137.41, 139.07, 146.18, 151.20 (Ar-**C**), 152.72 and 153.70 (triazole **C**3 and **C**5), 167.21 and 168.30 (**C**O, amide). Anal. calc. for $C_{21}H_{22}N_6O_5S$.0.1 mol $C_2H_5OH$ C: 53.59, H:4.79, N:17.69, S:6.75; found C:53.66 H:4.75, N:17.38, S:6.87. LR-MS (ESI) (m/z): Calc. for $(M+Na)^+$: 493.14, found: 493.15. Calc. for $(M-H)^-$: 469.14, found: 469.15.

Ethyl 4-[({[5-{[4-(acetylamino)phenoxy]methyl}-4-methyl-4*H*-1,2,4-triazol-3-yl]sulfanyl}acetyl)amino]benzoate (**12**): M.p. 241.0-241.3 °C, yield 56%. TLC Rf: 0.33. IR *ν* ($cm^{-1}$): 3256, 3244 (N-H str), 3107-2932 (C-H str), 1686 (C=O str), 1525 (C=N str), 635 (C-S str). $^1$H NMR (300 MHz, DMSO-$d_6$) δ ppm: 1.31 (3H, t, *J= 6 Hz*, C**H**$_3$CH$_2$O-); 2.01 (3H, s, -COC**H**$_3$); 3.61(3H, s, N-C**H**$_3$); 4.16 (2H, s, -S-C**H**$_2$-); 4.29 (2H, q, *J= 7.2 Hz*, CH$_3$C**H**$_2$O-); 5.22 (2H, s, -OC**H**$_2$); 7.00 (2H, d, J= 9 Hz, Ar-**H**); 7.49 (2H, d, *J= 9 Hz*, Ar-**H**); 7.70 (2H, d, *J= 9 Hz*, Ar-**H**); 7.93 (2H, d, *J= 9 Hz*, Ar-**H**); 9.83 (1H, s, -N**H**-); 10.67 (1H, s, -N**H**). $^{13}$C NMR (75 MHz, DMSO-$d_6$/TMS): δ ppm: 14.67, 24.28, 30.92 (aliphatic **C**), 37.91 (S-**C**H$_2$), 60.94 (O-**C**H$_2$), 115.58, 118.98, 120.88, 125.00, 130.79, 133.95, 143.52, 151.02 (Ar-**C**), 152.66 and 153.71 (triazole **C**3 and **C**5), 165.73 (**C**O, ester), 166.78 and 168.29 (**C**O, amide). Anal. calc. for $C_{23}H_{25}N_5O_5S$ C: 57.13, H:5.21, N:14.48, S:6.63; found %C:56.54, H:4.82, N:14.40, S:6.58. LR-MS (ESI) (m/z): Calc. for $(M+Na)^+$: 506.16, found: 506.15. Calc. for $(M-H)^-$: 482.16, found: 482.20.

2-[(5-{[4-(Acetylamino)phenoxy]methyl}-4-ethyl-4*H*-1,2,4-triazol-3-yl)sulfanyl]-*N*-(4-acetylphenyl)acetamide (**13**): M.p. 262.1-262.8 °C, yield 61%. TLC Rf: 0.39. IR *ν* ($cm^{-1}$): 3241, 3188 (N-H str), 3119-2843 (C-H str), 1661 (C=O str), 1541 (C=N str), 662 (C-S str). $^1$H NMR (300 MHz, DMSO-$d_6$) δ ppm: 1.31 (3H, t, *J= 6 Hz*, N-CH$_2$C**H**$_3$); 2.01 (3H, s, -COC**H**$_3$); 2.73 and 2.89 (s, DMF peak); 4.06 (2H, q, *J= 6 Hz* and *9 Hz*, N-C**H**$_2$CH$_3$); 4.24 (2H, s, -S-C**H**$_2$-); 5.24 (2H, s, -OC**H**$_2$); 7.00 (2H, d, *J= 9 Hz*, Ar-**H**); 7.50 (2H, d, *J= 9 Hz*, Ar-**H**); 7.70 (2H, d, *J= 9 Hz*, Ar-**H**); 7.95 (2H, d, *J= 9 Hz*, Ar-**H**); 9.84 (1H, s, -N**H**-); 10.70 (1H, s, -N**H**). $^{13}$C NMR (75 MHz, DMSO-$d_6$/TMS): δ ppm: 15.53, 24.27, 26.90 (aliphatic **C**), 37.86 (S-**C**H$_2$), 60.51 (O-**C**H$_2$), 115.37, 118.88, 120.96, 130.01, 132.40, 133.89, 143.57, 150.54 (Ar-**C**), 152.09 and 153.62 (triazole **C**3 and **C**5), 166.70 and 168.30 (**C**O, amide), 196.97 (Ar-**C**OCH$_3$). Anal. calc. for $C_{23}H_{25}N_5O_4S$ C:59.08, H:5.39, N:14.98, S:6.86; found C:58.46, H:5.22, N:14.79, S:6.71. LR-MS (ESI) (m/z): Calc. for $(M+Na)^+$: 490.16, found: 490.20. Calc. for $(M-H)^-$: 466.16, found: 466.10.

2-[(5-{[4-(Acetylamino)phenoxy]methyl}-4-ethyl-4*H*-1,2,4-triazol-3-yl)sulfanyl]-*N*-(4-nitrophenyl)acetamide (**14**): M.p. 272.9-273.9 °C, yield 62%. TLC Rf: 0.30. IR *ν* ($cm^{-1}$): 3217 (N-H str), 3055-2857 (C-H str), 1697, 1653 (C=O str), 1574 (C=N str), 665 (C-S str). $^1$H NMR (300 MHz, DMSO-$d_6$) δ ppm: 1.31 (3H, t, *J= 6 Hz*, N-CH$_2$C**H**$_3$); 2.01 (3H, s, -COC**H**$_3$); 2.73 and 2.89 (s, DMF peak); 4.06 (2H, q, *J= 6 Hz* and *9 Hz*, N-C**H**$_2$CH$_3$); 4.26 (2H, s, -S-C**H**$_2$-); 5.24 (2H, s, -OC**H**$_2$); 7.00 (2H, d, *J= 9 Hz*, Ar-**H**); 7.49 (2H, d, *J= 9 Hz*, Ar-**H**); 7.82 (2H, d, *J= 9 Hz*, Ar-**H**); 8.24 (2H, d, *J= 9 Hz*, Ar-**H**); 9.83 (1H, s, -N**H**-); 10.97 (1H, s, -N**H**). $^{13}$C NMR (75 MHz, DMSO-$d_6$/TMS): δ

ppm: 24.27, 30.93 (aliphatic **C**), 37.90 (S-**C**$H_2$), 60.78 (O-**C**$H_2$), 115.58, 119.32, 120.87, 125.53, 133.95, 142.88, 145.31, 150.94 (Ar-**C**), 152.69 and 153.69 (triazole **C**3 and **C**5), 167.22 and 168.29 (**C**O, amide). Anal. calc. for $C_{21}H_{22}N_6O_5S$ C: 53.61, H:4.71, N:17.86, S:6.82; found C:53.53, H:4.50, N:17.44, S:6.74. LR-MS (ESI) (m/z): Calc. for $(M+Na)^+$: 493.14, found: 493.15. Calc. for $(M-H)^-$: 469.14, found: 469.15.

2-[(5-{[4-(Acetylamino)phenoxy]methyl}-4-ethyl-4*H*-1,2,4-triazol-3-yl)sulfanyl]-*N*-(2-methyl-5-nitrophenyl)acetamide (**15**): M.p. 207.6-208.5 °C, yield 45%. TLC Rf: 0.36. IR *ν* ($cm^{-1}$): 3237, 3187 (N-H str), 3134-2843 (C-H str), 1667 (C=O str), 1534 (C=N str), 664 (C-S str). $^1$H NMR (300 MHz, DMSO-$d_6$) δ ppm: 1.06 (0.3H, t, *J= 6 Hz*, 0.1 mol C**H**$_3$CH$_2$OH); 1.31 (t, 3H, *J= 6 Hz*, N-CH$_2$C**H**$_3$); 2.01 (3H, s, -COC**H**$_3$); 2.34 (3H, s, Ar-C**H**$_3$); 4.06 (2H, q, *J= 6 Hz* and *9 Hz*, N-C**H**$_2$CH$_3$); 4.27 (2H, s, -S-C**H**$_2$-); 5.25 (2H, s, -OC**H**$_2$); 7.01 (2H, d, *J= 9 Hz*, Ar-**H**); 7.48-7.52 (3H, m, Ar-**H**); 7.95 (1H, dd, *J= 2.7 Hz* and *8.1 Hz*, Ar-**H**); 8.50 (1H, d, *J= 2.7 Hz*, Ar-**H**); 9.84 (1H, s, -N**H**-); 10.02 (1H, s, -N**H**-). $^{13}$C NMR (75 MHz, DMSO-$d_6$/TMS): δ ppm: 15.52, 18.51, 19.03, 24.27 (aliphatic **C**), 37.17 (S-**C**$H_2$), 60.47(O-**C**$H_2$), 115.37, 118.29, 119.91, 120.96, 131.92, 133.89, 137.42, 139.09, 146.18, 150.68 (Ar-**C**), 152.18 and 153.61 (triazole **C**3 and **C**5), 167.16 and 168.30 (**C**O, amide). Anal. calc. for $C_{22}H_{24}N_6O_5S$ C: 54.53, H:4.99, N:17.34, S:6.62; found C:54.43 H:5.17, N:17.13, S:6.66. LR-MS (ESI) (m/z): Calc. for $(M+Na)^+$: 507.15, found: 507.20. Calc. for $(M-H)^-$: 483.15, found: 483.20.

Ethyl 4-[({[5-{[4-(acetylamino)phenoxy]methyl}-4-ethyl-4*H*-1,2,4-triazol-3-yl]sulfanyl}acetyl) amino] benzoate (**16**): M.p. 242.1-243.3 °C, yield 55%. TLC Rf: 0.41. IR *ν* ($cm^{-1}$): 3258, 3181 (N-H str), 3151-2955 (C-H str), 1696, 1659 (C=O str), 15413 (C=N str), 662 (C-S str). $^1$H NMR (300 MHz, DMSO-$d_6$) δ ppm: 1.31 (6H, t, *J= 6 Hz*, C**H**$_3$CH$_2$O- and N-CH$_2$C**H**$_3$); 2.01 (3H, s, -COC**H**$_3$); 2.73 and 2.89 (s, DMF peak); 4.05 (2H, q, *J= 6 Hz* and *9 Hz*, N-C**H**$_2$CH$_3$); 4.23-4.32 (4H, m, -S-C**H**$_2$- and CH$_3$C**H**$_2$O-); 5.24 (2H, s, -OC**H**$_2$); 7.00 (2H, d, *J= 9 Hz*, Ar-**H**); 7.50 (2H, d, *J= 9 Hz*, Ar-**H**); 7.71 (2H, d, *J= 9 Hz*, Ar-**H**); 7.93 (2H, d, *J= 9 Hz*, Ar-**H**); 9.84 (1H, s, -N**H**-); 10.70 (1H, s, -N**H**). $^{13}$C NMR (75 MHz, DMSO-$d_6$/TMS): δ ppm: 14.67, 15.53, 24.27 (aliphatic **C**), 37.81 (S-**C**$H_2$), 60.93 (O-**C**$H_2$), 115.37, 118.98, 120.96, 124.99, 130.78, 133.89, 143.53, 150.54 (Ar-**C**), 152.09 and 153.63 (triazole **C**3 and **C**5), 165.73 (**C**O, ester), 166.68 and 168.29 (**C**O, amide). Anal. calc. for $C_{24}H_{27}N_5O_5S$ C: 59.08, H:5.39, N:14.98, S:6.86; found C:58.46, H:5.22, N:14.79, S:6.71. LR-MS (ESI) (m/z): Calc. for $(M+Na)^+$: 520.17, found: 520.20. Calc. for $(M-H)^-$: 496.17, found: 496.20.

2-[(5-{[4-(Acetylamino)phenoxy]methyl}-4-phenyl-4*H*-1,2,4-triazol-3-yl)sulfanyl]-*N*-(4-acetyl phenyl)acetamide (**17**): M.p. 238.1-239.0 °C, yield 52%. TLC Rf: 0.25. IR *ν* ($cm^{-1}$): 3256, 3183 (N-H str), 3100-2930 (C-H str), 1672, 1667 (C=O str), 1541 (C=N str), 667 (C-S str). $^1$H NMR (300 MHz, DMSO-$d_6$) δ ppm: 1.99 (3H, s, -COC**H**$_3$); 2.53 (3H, s, -COC**H**$_3$); 4.24 (2H, s, -S-C**H**$_2$-); 5.03 (2H, s, -OC**H**$_2$); 6.79 (2H, d, *J= 9 Hz*, Ar-**H**); 7.41 (2H, d, *J= 9 Hz*, Ar-**H**); 7.50-7.58 (5H, m, Ar-**H**); 7.71 (2H, d, *J= 9 Hz*, Ar-**H**); 7.95 (2H, d, *J= 9 Hz*, Ar-**H**); 9.79 (1H, s, -N**H**-); 10.70 (1H, s, -N**H**). $^{13}$C NMR (75 MHz, DMSO-$d_6$/TMS): δ ppm: 24.25, 26.91 (aliphatic **C**), 37.34 (S-**C**$H_2$), 60.58 (O-**C**$H_2$), 115.48, 118.86, 120.80, 127.44, 130.02, 130.27, 130.61, 132.42, 133.05, 133.88, 143.52, 151.93 (Ar-**C**), 152.30 and 153.49 (triazole **C**3 and **C**5), 166.53 and 168.28 (**C**O, amide), 196.99 (Ar-**C**OCH$_3$). Anal. calc. for $C_{27}H_{25}N_5O_4S$ C: 62.90, H:4.89, N:13.58, S:6.22; found C:63.00 H:5.07, N:13.10, S:5.81. LR-MS (ESI) (m/z): Calc. for $(M+Na)^+$: 538.16, found: 538.15. Calc. for $(M-H)^-$: 514.16, found: 514.20.

2-[(5-{[4-(Acetylamino)phenoxy]methyl}-4-phenyl-4*H*-1,2,4-triazol-3-yl)sulfanyl]-*N*-(4-nitro phenyl)acetamide (**18**): M.p. 273.0-274.0 °C (lit. 250 °C, (Yurttaş, Evren, Kubilay, & Temel, 2021)), yield 57%. TLC Rf: 0.35. IR *ν* ($cm^{-1}$): 3225, 3161 (N-H str), 3107-2841 (C-H str), 1697, 1667 (C=O str), 1559 (C=N str), 681 (C-S str). $^1$H NMR (300 MHz, DMSO-$d_6$) δ ppm: 1.06 (3H, t, *J= 6.6 Hz*, 1/3 mol C**H**$_3$CH$_2$OH); 1.99 (3H, s, -COC**H**$_3$); 3.40-3.49 (2H, m, 1/3 mol CH$_3$C**H**$_2$OH); 4.26 (2H, s, -S-C**H**$_2$-); 4.37

(1H, t, *J= 5.1 Hz*, 1/3 mol $CH_3CH_2O$**H**); 5.03 (2H, s, -OC**H**$_2$); 6.78 (2H, d, *J= 9 Hz*, Ar-**H**); 7.41 (2H, d, *J= 9 Hz*, Ar-**H**); 7.49-7.59 (5H, m, Ar-**H**); 7.82 (2H, d, *J= 9 Hz*, Ar-**H**); 8.25 (2H, d, *J= 9 Hz*, Ar-**H**); 9.79 (1H, s, -N**H**-); 10.97 (1H, s, -N**H**). $^{13}$C NMR (75 MHz, DMSO-$d_6$/TMS): δ ppm: 15.52, 24.27 (aliphatic **C**), 37.78 (S-**C**H$_2$), 60.48 (O-**C**H$_2$), 115.36, 119.32, 120.94, 125.54, 133.88, 142.86, 145.32, 150.46 (Ar-**C**), 152.12 and 153.60 (triazole **C**3 and **C**5), 167.15 and 168.30 (**C**O, amide). Anal. calc. for $C_{25}H_{22}N_6O_5S$.1/3 mol $C_2H_5OH$ C: 57.78 H:4.47, N:15.85, S:6.05; found C:57.35 H:4.65, N:15.56, S:6.20. LR-MS (ESI) (m/z): Calc. for $(M+Na)^+$: 541.14, found: 541.00. Calc. for $(M-H)^-$: 517.14, found: 517.00.

2-[(5-{[4-(Acetylamino)phenoxy]methyl}-4-phenyl-4*H*-1,2,4-triazol-3-yl)sulfanyl]-*N*-(2-methyl-5-nitrophenyl)acetamide (**19**): M.p. 158.9-160.7 °C, yield 49%. TLC Rf: 0.28. IR *ν* ($cm^{-1}$): 3258, 3185 (N-H str), 3127-2863 (C-H str), 1688, 1657 (C=O str), 1535 (C=N str), 693 (C-S str). $^1$H NMR (300 MHz, DMSO-$d_6$) δ ppm: 1.06 (3H, t, *J= 6.6 Hz*, 0.5 mol C**H**$_3$CH$_2$OH); 1.98 (3H, s, -COC**H**$_3$); 2.36 (3H, s, Ar-C**H**$_3$); 3.40-3.49 (2H, m, 0.5 mol $CH_3$C**H**$_2$OH); 4.26 (2H, s, -S-C**H**$_2$-); 4.38 (1H, t, *J= 5 Hz*, 0.5 mol $CH_3CH_2O$**H**); 5.05 (2H, s, -OC**H**$_2$); 6.79 (2H, d, *J= 8 Hz*, Ar-**H**); 7.41 (2H, d, *J= 8 Hz*, Ar-**H**);7.50-7.59 (6H, m, Ar-**H**); 7.95 (1H, dd, *J= 2.4 Hz* and *9 Hz*, Ar-**H**); 8.49 (1H, d, *J= 2.4 Hz*, Ar-**H**); 9.80 (1H, s, -N**H**-); 10.01 (1H, s, -N**H**-). $^{13}$C NMR (75 MHz, DMSO-$d_6$/TMS): δ ppm: 18.55, 19.03, 24.25 (aliphatic **C**), 36.77 (S-**C**H$_2$), 60.59 (O-**C**H$_2$), 115.48, 118.31, 119.90, 120.80, 127.44, 130.27, 130.62, 131.92, 133.04, 133.89, 137.45, 139.13, 146.18, 152.08 (Ar-**C**), 152.37 and 153.48 (triazole **C**3 and **C**5), 167.00 and 168.27 (**C**O, amide). Anal. calc. for $C_{26}H_{24}N_6O_5S$.1/2 mol $C_2H_5OH$ C: 57.93, H:4.86, N:15.01, S:5.54; found C:57.24 H:4.81, N:14.70, S:5.42. LR-MS (ESI) (m/z): Calc. for $(M+Na)^+$: 555.15, found: 555.15. Calc. for $(M-H)^-$: 531.15, found: 531.15.

Ethyl 4-[({[5-{[4-(acetylamino)phenoxy]methyl}-4-phenyl-4*H*-1,2,4-triazol-3-yl]sulfanyl}acetyl)amino]benzoate (**20**): M.p. 251.4-252.0 °C, yield 60%. TLC Rf: 0.31. IR *ν* ($cm^{-1}$): 3256, 3185 (N-H str), 3120-2870 (C-H str), 1711, 1663 (C=O str), 1545 (C=N str), 694 (C-S str). $^1$H NMR (300 MHz, DMSO-$d_6$) δ ppm: 1.31 (3H, t, *J= 6 Hz*, C**H**$_3$CH$_2$O-); 1.99 (3H, s, -COC**H**$_3$); 2.73 and 2.89 (s, DMF peak); 4.23 (2H, s, -S-C**H**$_2$-); 4.29 (2H, q, *J= 7 Hz*, $CH_3$C**H**$_2$O-); 5.03 (2H, s, -OC**H**$_2$); 6.78 (2H, d, *J= 9 Hz*, Ar-**H**); 7.41 (2H, d, *J= 9 Hz*, Ar-**H**); 7.49-7.59 (5H, m, Ar-**H**); 7.71 (2H, d, *J= 9 Hz*, Ar-**H**); 7.94 (2H, d, *J= 9 Hz*, Ar-**H**); 9.79 (1H, s, -N**H**-); 10.70 (1H, s, -N**H**). $^{13}$C NMR (75 MHz, DMSO-$d_6$/TMS): δ ppm: 14.68, 24.25 (aliphatic **C**), 37.31 (S-**C**H$_2$), 60.94 (O-**C**H$_2$), 115.47, 118.97, 120.79, 124.97, 127.44, 130.27, 130.60, 130.79, 133.05, 133.88, 143.55, 151.92 (Ar-**C**), 152.29 and 153.49 (triazole **C**3 and **C**5), 165.74 (**C**O, ester), 166.52 and 168.26 (**C**O, amide). Anal. calc. for $C_{28}H_{27}N_5O_5S$ C: 61.64, H:4.99, N:12.84, S:5.88; found C:62.32, H:5.09, N:13.09, S:5.90. LR-MS (ESI) (m/z): Calc. for $(M+Na)^+$: 568.17, found: 568.15. Calc. for $(M-H)^-$: 544.17, found: 544.20.

**Biological methods**

**Cell culture**

Cell culture studies were conducted using lung cancer (A549), chronic myelogenous leukemia (K562), breast cancer (MCF-7), prostate cancer (PC-3), neuroblastoma (SH-SY5Y), and mouse embryonic fibroblast (NIH/3T3) cells as human cancer cell lines. The cells were grown, and cell passage was carried out according to previously reported methods (Demirbolat et al., 2022).

**Cell viability assay**

Cell viability was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cells were seeded in 96-well plates at a density of $1\times10^4$ cells per well and incubated overnight. Subsequently, the cells were exposed to varying concentrations (1-50 μM) of the synthesized compounds (**9**-**20**) for 48 hours. Following the initial incubation period, MTT was introduced to each well at a final concentration of 0.5 mg/mL, and the cells were further incubated for an additional 4 hours. After aspirating the growth medium, 100 μl of SDS buffer was introduced to dissolve the purple formazan product. Subsequently, a microplate reader (BioTek, Winooski,

VT, USA) was employed to measure the absorbances at 570 nm and 630 nm wavelengths (Demirbolat et al., 2022).

**Preparation of human mPGES-1 membrane fraction and PGES activity assay**

Human mPGES-1 membrane fractions were prepared as described before (Demirbolat et al., 2022). The inhibitory potential of the selected compounds was assessed through the 2-thiobarbituric acid (TBA) – malondialdehyde (MDA) assay, following a previously established protocol (Larsson et al., 2019). For reference, 1-{6-chloro-5-methyl-1-[6-(trifluoromethyl)pyridin-2-yl]-1*H*-benzimidazol-2-yl}-*N*-(oxolan-3-yl)piperidine-4-carboxamide (compound **118**), a recognized potent mPGES-1 inhibitor, was employed (Larsson et al., 2019).

***In vitro* COX-1/2 enzyme inhibition assay**

The inhibitory activities of all synthesized compounds on COX-1/2 were assessed using an enzyme immunoassay-based approach, specifically the 'COX (ovine/human) Inhibitor Screening Assay' kit (#560131, Cayman Chemical, Ann Arbor, MI, USA). To ascertain their selectivity for mPGES-1 compared to COX-1/2, a combination of equal amounts of COX-1 and COX-2 enzymes was prepared for this study. Each assay was conducted in triplicate, and the inhibitory efficacy of the tested compounds was quantified as the percentage inhibition against COX-1/2 enzymes at a concentration of 100 μM. The COX-1/2 determination experiments were carried out according to the method specified in the literature (Demirbolat et al., 2022).

***In silico* methods**

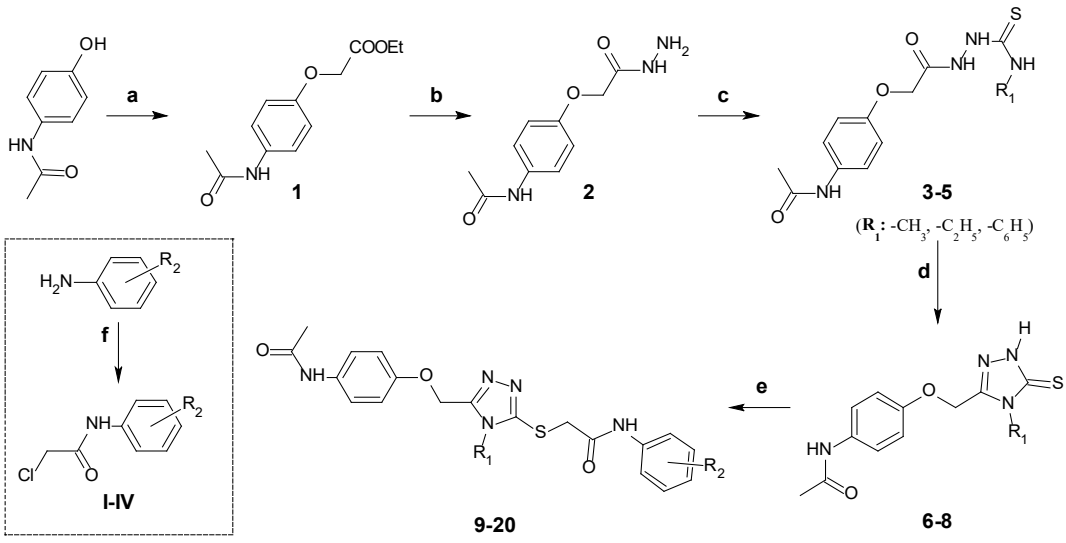
**Prediction of ADMET profiles**

The solubility properties and structural descriptors of the novel paracetamol-triazole conjugates, compounds **9**-**20**, were evaluated. Target compounds were assessed for their LogP, molecular weight, solubility, topological polar surface area, count of hydrogen bond acceptors/donors and rotatable bonds, %ABS, etc. Moreover, compounds **9**-**20** were examined for Lipinski's Rule of 5 for their compliance (Lipinski, Lombardo, Dominy, & Feeney, 1997). All the data mentioned above were procured from the online web server SwissADME (Daina, Michielin, & Zoete, 2017). *In silico* toxicological parameters were predicted by OSIRIS Data Warrior software (Sander, Freyss, Von Korff, & Rufener, 2015)which molecular families are present, which structural motifs correlate with measured properties, and which tiny structural changes cause large property changes. Data visualization and analysis software with sufficient chemical intelligence to support chemists in this task is rare. In an attempt to contribute to filling the gap, we released our in-house developed chemistry aware data analysis program DataWarrior for free public use. This paper gives an overview of DataWarrior's functionality and architecture. Exemplarily, a new unsupervised, 2-dimensional scaling algorithm is presented, which employs vector-based or nonvector-based descriptors to visualize the chemical or pharmacophore space of even large data sets. DataWarrior uses this method to interactively explore chemical space, activity landscapes, and activity (Sander, Freyss, Von Korff, & Rufener, 2015) (http://www.openmolecules.org/datawarrior).

## RESULTS AND DISCUSSION

**Synthesis and characterization**

Compounds **1**-**5** were synthesized starting from paracetamol as described in previously reported methods (Demirbolat et al., 2022). The synthesis of compounds **9**-**20** followed the reaction protocol as outlined in Scheme 1. 3H-1,2,4-Triazole-3-thione derivatives (**6**-**8**) were synthesized through the cyclocondensation process of compounds **3**-**5**. This reaction occurred in the presence of TEA in ethanol to prevent the hydrolysis of the acetamido moiety. Conversely, **N**-aryl-2-chloroacetamides (**I**-**IV**) were prepared by reacting substituted anilines with 2-chloroacetylchloride under the influence of TEA. In final step, triazoles **6**-**8** were treated with compounds **I**-**IV** in DMF to yield target compounds **9**-**20** (Kulabaş et al., 2016).




**Scheme 1.** Synthetic route to compounds **9-20**. Key to reagents: **a.** $BrCH_2COOC_2H_5$, $K_2CO_3$, acetone; **b.** $NH_2NH_2.H_2O$, EtOH; **c.** $R_1NCS$, EtOH; **d.** TEA/EtOH, reflux; **e. I-IV**, $K_2CO_3$, DMF **f.** $ClCOCH_2Cl$, TEA, DCM.

| Compound | $R_1$ | $R_2$ | Compound | $R_1$ | $R_2$ |
|---|---|---|---|---|---|
| 9 | $-CH_3$ | 4-$COCH_3$ | 15 | $-C_2H_5$ | 2-$CH_3$-5-$NO_2$ |
| 10 | $-CH_3$ | 4-$NO_2$ | 16 | $-C_2H_5$ | 4-$COOC_2H_5$ |
| 11 | $-CH_3$ | 2-$CH_3$-5-$NO_2$ | 17 | $-C_6H_5$ | 4-$COCH_3$ |
| 12 | $-CH_3$ | 4-$COOC_2H_5$ | 18 | $-C_6H_5$ | 4-$NO_2$ |
| 13 | $-C_2H_5$ | 4-$COCH_3$ | 19 | $-C_6H_5$ | 2-$CH_3$-5-$NO_2$ |
| 14 | $-C_2H_5$ | 4-$NO_2$ | 20 | $-C_6H_5$ | 4-$COOC_2H_5$ |

Among the prepared triazoles (**6-8**), only compound 8 was synthesized by Yurttaş et al. (Yurttaş et al., 2021). Still, the ring-closing reaction was carried out in 2N KOH whereas we used triethylamine in the ethanolic medium. Our study is unique in this respect, and the structures of compounds **6-8** were confirmed with spectral data for compound **8** and other triazole rings. In the IR spectra of triazoles **6-8**, the detection of a C=O band at 1679-1652 $cm^{-1}$ belonging to paracetamol moiety as well as the C=S band at 1275-1240 $cm^{-1}$ supported the formation of 1,2,4-triazole-3-thione. Moreover, 1,2,4-triazole N-H proton was detected at 13.83-14.06 ppm in a higher energy field than the thiosemicarbazide NH protons (Tatar et al., 2015).

The synthesized compounds **9-20** were assessed for purity using TLC as well as elemental analysis, and their structures were elucidated by FTIR, $^1$H NMR, $^{13}$C-NMR and LC-MS spectral data. The IR spectra of compounds **9-20** exhibited distinctive N-H and C=O stretching bands, appearing at 3258-3159 $cm^{-1}$ and 1669-1653 $cm^{-1}$, respectively. Also, the disappearance of the C=S stretching band of the 1,2,4-triazole-3-thione and the detection of the C-S-C stretching band at 693-635 $cm^{-1}$ confirms these structures. In the $^1$H NMR spectra of **9-20**, peaks at around 9.79-9.84 ppm and 9.99-10.97 ppm were attributed to the N-H protons of paracetamol and thioacetanilide moieties, respectively, and N-H signals of the 1,2,4-triazol-3-thione were not detectable (Kulabaş et al., 2016). Moreover, S-$CH_2$ protons were observed at 4.16-4.27 ppm as a singlet peak with 2H integration. In the $^{13}$C NMR spectra of compound **9-20**, signals at about 166.52-168.70 ppm as two peaks that were attributed to the paracetamol and thioacetanilide carbonyl peak (C=O) while C3 and C5 carbon of the 1,2,4-triazole

ring were observed between 152.09-153.71 ppm (Demirbolat et al., 2022). On the other hand, the resonances at 196.97-196.99 ppm were attributed to the acetyl C=O for compounds **9, 13, 17** and signals at about 165.73-165.74 ppm were attributed to the ester C=O for compounds **12, 16, 20.** Low-resolution mass spectra (LR-MS) confirmed the molecular weights of compounds **9-20** with determined both $[M+Na]^+$ and $[M-H]^-$ ion peaks. Finally, elemental analysis data obtained for all synthesized compounds demonstrated consistency with their assigned structures. Among the target compounds, compound **18** was previously synthesized by Yurttaş et al., (Yurttaş et al., 2021) in the presence of $K_2CO_3$ in acetone, and its matrix metalloproteinase-9 (MMP-9) inhibition potential was reported. Our study differs from the other reported study in terms of the difference in the synthesis method and the bioactivity studies performed.

**Biological activity studies**

**Cytotoxic effects of compounds 9-20**

Compounds **9-20** were screened for their antiproliferative properties against A549, K562, MCF-7, PC-3, SH-SY5Y, and cytotoxic effects in NIH/3T3 cells using MTT assay. Table 1 provides a comprehensive summary of the outcomes from the initial screening conducted at a concentration of 10 μM. Notably, negligible cell inhibitory effects were observed in the samples treated with DMSO. Among the various cell lines tested, MCF-7 exhibited the most pronounced inhibitory response to the synthesized compounds, yielding inhibition values ranging from 17.29% to 25.06%. It was found that these compounds did not show significant inhibition against all selected cell lines in general. However, the highest inhibition value against healthy cell NIH3T3 was 23.04%, and therefore, they were considered not to be cytotoxic.

**Table 1.** Cytotoxicity profiles of compounds **9-20** against cancer cell lines and NIH3T3 cell line.

| Compound | Lab ID codes | Inhibition % | | | | | |
|---|---|---|---|---|---|---|---|
| | | **A549** | **K562** | **MCF7** | **PC-3** | **SH-SY5Y** | **NIH3T3** |
| **9** | KUC16D590 | 9.12 | -3.86 | 13.28 | -9.44 | 5.67 | 0.18 |
| **10** | KUC16D577 | 2.34 | -3.72 | 9.52 | -6.92 | -5.16 | -2.14 |
| **11** | KUC16D584 | 0.58 | -6.41 | 8.02 | -12.03 | -4.59 | -7.32 |
| **12** | KUC16D588 | 6.43 | 2.33 | 19.17 | -13.11 | -4.85 | 7.32 |
| **13** | KUC16D621 | 3.51 | -3.86 | 12.91 | -1.87 | 1.48 | 5.89 |
| **14** | KUC16D609 | 10.53 | -1.60 | 17.42 | 7.93 | -9.65 | 13.57 |
| **15** | KUC16D616 | 4.68 | 13.63 | -2.26 | 0.50 | 0.82 | -8.39 |
| **16** | KUC16D620 | 9.71 | -2.04 | 18.55 | 0.72 | 0.01 | 10.18 |
| **17** | KUC16D683 | 17.31 | 4.01 | 17.29 | 5.48 | 3.47 | 13.04 |
| **18** | KUC16D671 | 15.91 | -4.37 | 21.30 | 8.93 | 8.98 | 21.07 |
| **19** | KUC16D678 | 6.32 | 9.62 | 9.52 | -0.65 | 7.50 | 7.86 |
| **20** | KUC16D682 | 10.76 | 0.95 | 25.06 | 18.37 | 8.01 | 23.04 |

**Inhibition of mPGES-1 and COX-1/2 enzymes**

Inhibitory activities of compounds 9-20 were screened towards mPGES-1 and COX-1/2 enzymes. Compound 118 was used as a reference compound for the mPGES-1 enzyme (Larsson et al., 2019). Preparation of a protein mixture containing equal amounts of COX-1 and COX-2 enzymes was performed to evaluate whether the tested compound had the potential to significantly inhibit COX-1 or COX-2. This approach enables the determination of substantial inhibitory activity against the combined COX-1 and COX-2 enzymes. Dexketoprofen, ibuprofen, and celecoxib were used as reference compounds for COXs enzymes. As seen in Table 2, it was observed that the target compounds had a significant effect against neither the mPGES-1 enzyme at 10 μM dose nor the COX-1/2 enzyme mix at 100 μM dose.

**Table 2.** Inhibitory properties of compounds **9-20** against mPGES-1 and COX-1/2.

| Compound | mPGES-1 | | | % Inhibition of COX-1/2 at 100 μM |
|---|---|---|---|---|
| | % Inhibition | P value | P value (-log10) | |
| **9** | 3.895 | 0.601 | 0.221 | 19.1 |
| **10** | 0.034 | 0.938 | 0.028 | 17.5 |
| **11** | -1.087 | 0.495 | 0.306 | 16.6 |
| **12** | 2.506 | 0.435 | 0.362 | 14.7 |
| **13** | 1.686 | 0.341 | 0.467 | 20.0 |
| **14** | -2.152 | 0.067 | 1.174 | 10.8 |
| **15** | 0.294 | 0.611 | 0.214 | 12.2 |
| **16** | 1.912 | 0.326 | 0.487 | 10.6 |
| **17** | 0.203 | 0.891 | 0.050 | 15.8 |
| **18** | 0.072 | 0.951 | 0.022 | 9.1 |
| **19** | 1.363 | 0.284 | 0.547 | 5.8 |
| **20** | -1.663 | 0.371 | 0.431 | 16.6 |
| Compd 118[a] | 73.780 | 0.000 | 4.030 | |
| Dexketoprofen | | | | 65.3 |
| Ibuprofen | | | | 68.1 |
| Celecoxib | | | | N.D.[b] |

[a] Compound **118**, reported by Larsson et. al.(Larsson et al., 2019) , was used as reference compound.

[b] Not determined; inhibition at this concentration is above the linear value of the calibration plot (too high inhibition)

***In silico* ADMET predictions**

SMILES codes of the compounds **9**-**20** were prepared using the ACD/ChemSketch v. 12.0 to estimate their ADMET parameters through SwissADME software (http://www.swissadme.ch). The range of hydrogen bond acceptors in the tested compounds was either 6 or 7, and the number of rotatable bonds exhibited variability within the scope of 11 to 14. Notably, none of the tested compounds had more than two hydrogen bond donors (Table 3). The association between low aqueous solubility and limited absorption is well-established. Remarkably, the synthesized compounds demonstrated favorable logS values exceeding -5.2.

Furthermore, the estimated intestinal absorption (%ABS) of the synthesized compounds was calculated according to the methodology reported by Zhao et al. (Zhao et al., 2002)diffusion, or perfusion processes can become the rate-limited step. The absorption data of 238 drugs have been classified into either dissolution or diffusion rate-limited based on an equilibrium method developed from solubility, dose, and percentage of absorption. A nonlinear absorption model derived from first-order kinetics has been developed to identify the relationship between percentage of drug absorption and molecular descriptors. Regression analysis was performed between percentage of absorption and molecular descriptors. The descriptors used were ClogP, molecular polar surface area, the number of hydrogen-bonding acceptors and donors, and Abraham descriptors. Good relationships were found between absorption and Abraham descriptors or ClogP. The absorption models can predict the following three BCS (Biopharmaceutics Classification Scheme, and determined between 50.6-60.5% for compounds **9**-**20**. Adhering to Lipinski's rule of five, it is conventionally recommended that the molecular weight remains below 500 Da. However, it is noteworthy that compounds **17**-**20**, characterized by a phenyl ring at the 4th position of the triazole moiety, appear to deviate from this criterion.

**Table 3.** Solubility and molecular descriptors of compounds **9**-**20** from SwissADME.

| Compound | $LogP_{o/w}$ | LogS (ESOL) | nON | nOHN | nRot | TPSA | %ABS | Lipinski Rule nviol |
|---|---|---|---|---|---|---|---|---|
| 9 | 2.04 | -3.29 | 6 | 2 | 11 | 140.51 | 60.5 | 0 |
| 10 | 1.35 | -3.40 | 7 | 2 | 11 | 169.26 | 50.6 | 0 |
| 11 | 1.55 | -3.70 | 7 | 2 | 11 | 169.26 | 50.6 | 0 |
| 12 | 2.45 | -3.65 | 7 | 2 | 13 | 149.74 | 57.3 | 0 |
| 13 | 2.37 | -3.49 | 6 | 2 | 12 | 140.51 | 60.5 | 0 |
| 14 | 1.68 | -3.59 | 7 | 2 | 12 | 169.26 | 50.6 | 0 |
| 15 | 1.93 | -3.90 | 7 | 2 | 12 | 169.26 | 50.6 | 0 |
| 16 | 2.72 | -3.85 | 7 | 2 | 14 | 149.74 | 57.3 | 0 |
| 17 | 3.32 | -4.71 | 6 | 2 | 12 | 140.51 | 60.5 | 1 |
| 18 | 2.81 | -4.83 | 7 | 2 | 12 | 169.26 | 50.6 | 1 |
| 19 | 3.10 | -5.13 | 7 | 2 | 12 | 169.26 | 50.6 | 1 |
| 20 | 3.66 | -5.09 | 7 | 2 | 14 | 149.74 | 57.3 | 1 |

LogPo/w: Consensus; LogS (ESOL): Estimating aqueous solubility from molecular structure; nON: Number of hydrogen acceptors; nOHN: Number of hydrogen donors; nRot: Number of rotatable bonds; TPSA: Topological polar surface area; %ABS: Percentage of absorption.

SwissADME server was also used to boiled egg plot denotes. In this graphic, the white area is defined as intestinal absorption, while the gray area is poor intestinal absorption. The yellow region, which designates effective brain penetration and favorable intestinal absorption, is demarcated. None of the synthesized compounds exhibited the potential to cross the blood-brain barrier as predicted. All target compounds that exhibit poor intestinal absorption have been found to be potential substrates of P-glycoprotein except compounds **18** and **19**. According to the estimated toxicity profiles of the compounds, only compounds **9, 13**, and **17** containing the acetyl group showed mutagenic effects, while none of the compounds showed tumorigenic and irritant profiles (Figure 2).

## CONCLUSION

In conclusion, a new series of paracetamol-triazole conjugates **9**-**20** were synthesized, and their structures were characterized by elemental analyses besides spectral methods. Cytotoxic effects of the target compounds were tested on A549, K562, MCF-7, PC-3, SH-SY5Y, and NIH-3T3 cells. Furthermore, a comprehensive evaluation was conducted on compounds **9**-**20** to assess their inhibitory potential against mPGES-1 and COX-1/2 enzymes. None of these compounds exhibited significant antiproliferative effect and mPGES-1 inhibition, as well as COX-1/2 inhibition, too. However, compounds **9**-**20** showed no cytotoxic effect against NIH-3T3 cells, concluding that different biological effects of these safe compounds can be evaluated in our future studies.

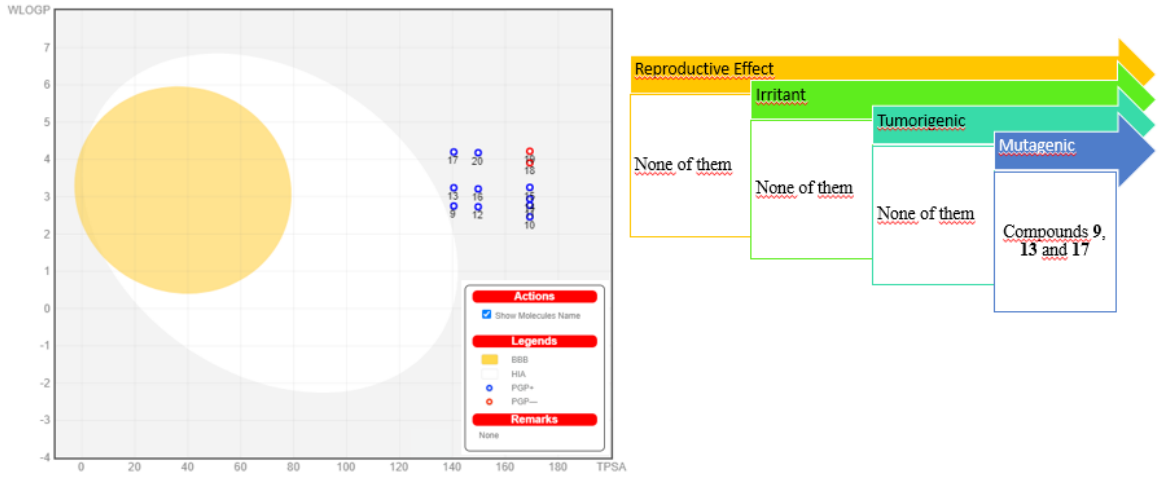



**Figure 2.** Graphical distribution of compounds according to the boiled egg predictive model and their toxicity profiles.

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**CONFLICT OF INTEREST**

The authors declare that there is no conflict of interest.

**AUTHOR CONTRIBUTION STATEMENT**

The hypothesis of this study were developed and literature research was carried out by N.K. and İ.K. Compounds were designed, synthesized and characterized by N.K. and İ.K. The antiproliferative activity of synthesized compounds were determined by M.G. and Ö.B.Ö. Their inhibitory activity against mPGES-1 enzyme were tested by J.L. and P.-J.J. Then inhibition potential of these compounds were evaluated against COX-1/2 enzymes by Ö.D. and A.O. Finally, manuscript preparation and critical reviews were realized by N.K., M.G., Ö.B.Ö., J.L., J.J., Ö.D., A.O. and İ.K.

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