Received: 7 September 2017 | Revised: 4 February 2018 | Accepted: 6 February 2018
DOI: 10.1002/ardp.201700195

FULL PAPER

DPhG ARCH PHARM
Archiv der Pharmazie

Synthesis, anticancer activity, and molecular modeling of etodolac-thioether derivatives as potent methionine aminopeptidase (type II) inhibitors

Işıl Çoruh[1] | Özge Çevik[2] | Kemal Yelekçi[3] | Teodora Djikic[3] | Ş. Güniz Küçükgüzel[1]

[1] Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Marmara University, İstanbul, Turkey

[2] Department of Biochemistry, School of Medicine, Adnan Menderes University, Aydın, Turkey

[3] Faculty of Engineering and Natural Sciences, Department of Bioinformatics and Genetic, Kadir Has University, İstanbul, Turkey

Correspondence
Prof. Ş. Güniz Küçükgüzel, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Marmara University, Haydarpaşa, 34668 İstanbul, Turkey.
Email: gkucukguzel@marmara.edu.tr

Funding information
Scientific Research Project Commission of Marmara University, Grant number: SAG-C-DRP-041213-0451

Abstract

A series of (*R*,*S*)-1-{[5-(substituted)sulfanyl-4-substituted-4*H*-1,2,4-triazole-3-yl]-methyl}-1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indoles (**5a–v**) were designed and synthesized using a five-step synthetic protocol that involves substituted benzyl chlorides and (*R*,*S*)-5-[(1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indole-1-yl)methyl]-4-substituted-2,4-dihydro-3*H*-1,2,4-triazole-3-thiones in the final step. The synthesized derivatives were evaluated for cytotoxicity and anticancer activity *in vitro* using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) colorimetric method against VERO, HEPG2 (human hepatocellular liver carcinoma), SKOV3 (ovarian carcinoma), MCF7 (human breast adenocarcinoma), PC3 and DU145 (prostate carcinoma) cells at 10^{-5} M (10 μM) for 24 h. Compounds **5d** and **5h** showed the best biological potency against the SKOV3 cancer cell line (IC_{50} = 7.22 and 5.10 μM, respectively) and did not display cytotoxicity toward VERO cells compared to etodolac. Compounds **5k**, **5s**, and **5v** showed the most potent biological activity against the PC3 cancer cell line (IC_{50} = 8.18, 3.10, and 4.00 μM, respectively) and did not display cytotoxicity. Moreover, these compounds were evaluated for caspase-3, -9, and -8 protein expression and activation in the apoptosis pathway for 6, 12, and 24 h, which play a key role in the treatment of cancer. In this study, we also investigated the apoptotic mechanism and molecular modeling of compounds **5k** and **5v** on the methionine aminopeptidase (type II) enzyme active site in order to get insights into the binding mode and energy.

KEYWORDS
anticancer activity, apoptosis, etodolac, molecular docking, thioethers

Abbreviations: AO/EB, acridine orange/ethidium bromide; DMF, dimethylformamide; FT-IR, Fourier transform infrared; HR-MS, high resolution mass spectrometry; MCF7, human breast adenocarcinoma cell line; MetAP, methionine aminopeptidase; MTT, (3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyltetrazolium bromide) colorimetric method; NMR, nuclear magnetic resonance; SAR, structure–activity relationship; TLC, thin layer chromatography.

This work was partly presented at the 3rd International BAU Drug Design Congress, 1–3 October, 2015.

1 | INTRODUCTION

Cancer is a diverse group of diseases characterized by the proliferation and spread of abnormal cells and a major worldwide problem. Therefore, the discovery and development of new potent

and selective anticancer drugs are of high importance in modern research.

Methionine aminopeptidase (MetAP) is a cobalt-containing metalloprotease and protein-processing enzyme responsible for the removal of initiator methionine residues from polypeptide chains during protein synthesis. In eukaryotes, there are two isoforms of MetAP, MetAP1 and MetAP2. MetAP2 plays a critical role in cell proliferation and tumor growth. Due to the pivotal role of these enzymes for angiogenesis, MetAP2 has been one of the major targets in the anticancer drug development area. 1,2,4-Triazole-3-thiones have wide spectrum biological activity.[1] In recent years, 1,2,4-triazole derivatives and thioethers are reported as potential MetAP2 inhibitors.[2,3] JNJ-4929821, which contains triazole structure and 3-anilino-5-benzylthio-1,2,4-triazole derivatives, was identified as the inhibitors of the human metalloprotease (MetAP2).[4,5] Hou and co-workers reported that 1,4-benzodioxan fragment is an important pharmacophore on biological activity, interaction 1,4-benzodioxane and the hydrogen of some amino in the active site of MetAP2 and this condition increased biological activity.

Etodolac, (*R*,*S*)-2-[1,8-diethyl-1,3,4-tetrahydropyrano[3,4-*b*]indole-1-yl]acetic acid, is a non-steroidal anti-inflammatory drug which has anticancer activities reported in the literature.[6,7] In addition, etodolac hydrazones,[8] thiosemicarbazides,[9] 1,2,4-triazoles,[10] 1,2,3-triazoles,[11] and 4-thiazolidinones[8] have been reported to have anticancer effect and to be hepatitis C NS5B polymerase inhibitors. Etodolac have tetrahydropyrano group. There was no study showing the MetAP2 interaction of the tetrahydropyrano group. In the light of these literature, etodolac was chosen as a starting substance to synthesize thioether containing 1,2,4-triazole compounds.

The aim of this study was to synthesize novel and original 19 thioether compounds starting from etodolac, racemic form, and to evaluate and confirm their structures by FTIR, ^{1}H NMR, ^{13}C NMR, high resolution mass spectrometry (HR-MS) spectral data and elemental analysis data. The synthesized compounds were evaluated on the several cancer cell lines in the following studies. Our strategy opens up possibilities for the design and synthesis of new thioether moieties with anticancer activities.

In continuation to extend these research areas on anticancer compounds with MetAP2 structure inhibitory activity, in the present work a series of etodolac-thioether derivatives derived from 1,2,4-triazole-3-thione have been studied. Our study may also allow the identification of key substrates by database mining.

2 | RESULTS AND DISCUSSION

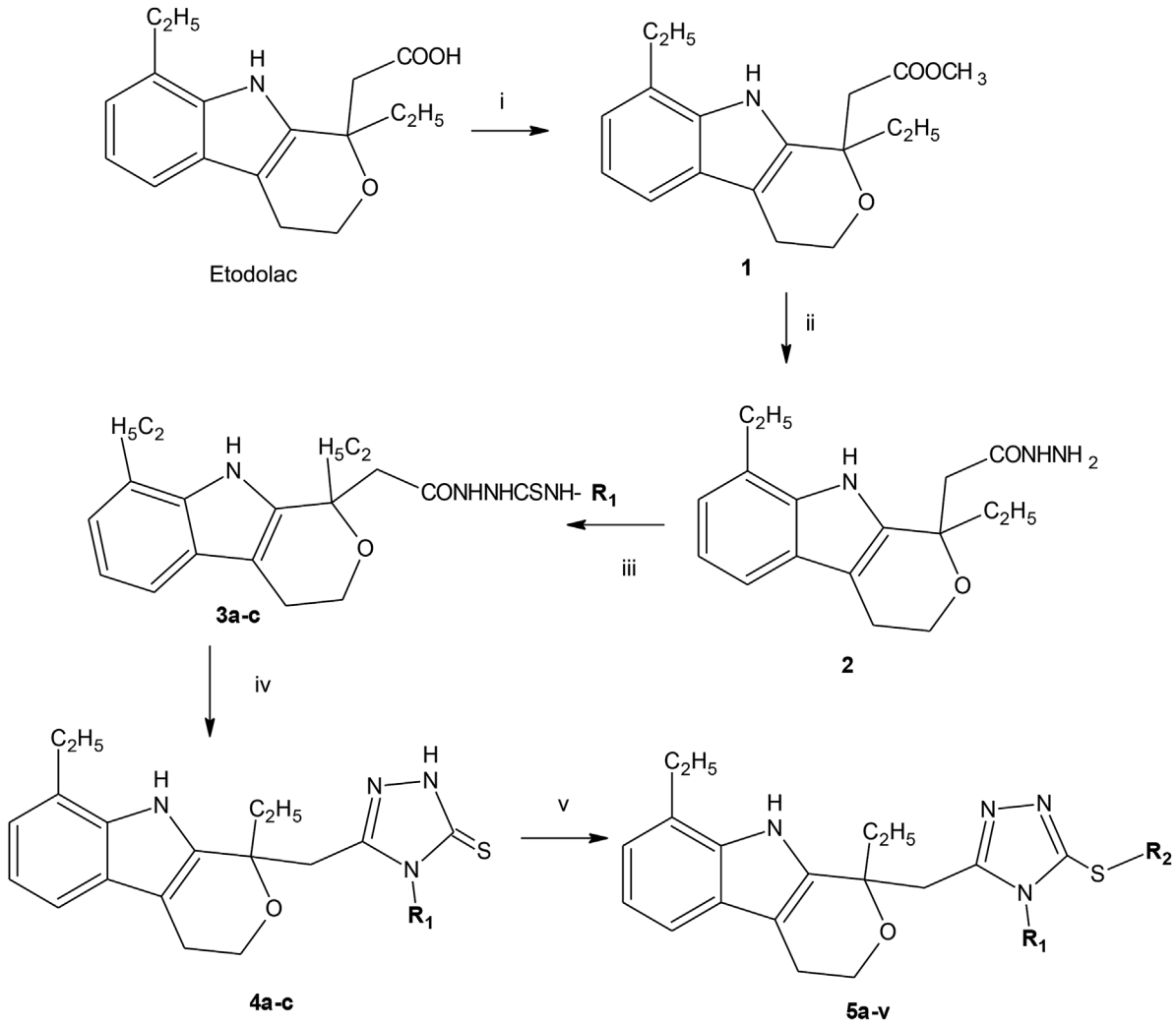
2.1 | Chemistry

Etodolac, (*R*,*S*)-2-[1,8-diethyl-1,3,4-tetrahydropyrano[3,4-*b*]indole-1-yl]acetic acid, was refluxed in the presence of methanol by catalyst saturated sulfuric acid to form methyl 2-(1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indole-1-yl)acetate **1**. Methanolic solution of this compound and hydrazine hydrate were refluxed to obtain methyl 2-(1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indole-1-yl)-acetate **1** and 2-(1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indole-1-yl)acetohydrazide **2**. Compound **2** and several isothiocyanates yield 1-[2-(1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indole-1-yl)-acetyl]-4-alkyl/aryl thiosemicarbazides **3a–c**. The thiosemicarbazides react with sodium hydroxide solutions (2 N) and after cyclization reaction 5-[(1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]-indole-1-yl)methyl]-4-substituted-2,4-dihydro-3*H*-1,2,4-triazole-3-thiones **4a–c** are gained. To a suspension of 4-substituted-1,2,4-triazole-3-thione in DMF containing anhydrous K_2CO_3, substituted benzyl chlorides were added to form the target compounds 1-{[5-(substituted)sulfanyl)-4-substituted-4*H*-1,2,4-triazole-3-yl]methyl}-1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indoles **5a–v**. The synthesis of novel series of etodolac-thioethers **5a–v** (Table 1) was performed as outlined in Scheme 1 according to the literature.[12,13] The etodolac-thioethers **5a–v** are original compounds. All synthesized compounds were checked for purity using TLC and were characterized by their melting points, elemental analysis, ^{1}H NMR, ^{13}C NMR, and HR-MS spectral data.

The FT-IR spectral data of etodolac thioethers **5a–v** have exhibited bands in the region 3169–3293 cm^{-1} which may be attributed to indole N—H stretching. The N—H bending and C—N stretching bands of the indole structure and the aromatic C═C stretching bands of the compounds, whose practical results are in agreement with literature, are located at the range of 1421–1622 cm^{-1}. These values are in agreement with the values reported.[14]

TABLE 1 Structural profiles of compounds **5a–v**

Compounds	R_1	R_2
5a	CH_3–	C_6H_5–CH_2–
5b	CH_3–	(4)F–C_6H_4–CH_2–
5c	CH_3–	(2)Cl–C_6H_4–CH_2–
5d	CH_3–	(4)Cl–C_6H_4–CH_2–
5e	CH_3–	(2,6)Cl_2–C_6H_3–CH_2–
5f	CH_3–	(4)CH_3–C6H_4–CH_2–
5g	CH_3–	(4)NO_2–C_6H_4–CH_2–
5h	CH_3–CH_2–	C_6H_5–CH_2–
5i	CH_3–CH_2–	(4)F–C_6H_4–CH_2–
5j	CH_3–CH_2–	(2)Cl–C_6H_4–CH_2–
5k	CH_3–CH_2–	(4)Cl–C_6H_4–CH_2–
5l	CH_3–CH_2–	(2,6)Cl_2–C_6H_3–CH_2–
5m	CH_3–CH_2–	(4)CH_3–C_6H_4–CH_2–
5n	C_6H_5–	C_6H_5–CH_2–
5p	C_6H_5–	(4)F–C_6H_4–CH_2–
5r	C_6H_5–	(4)Cl–C_6H_4–CH_2–
5s	C_6H_5–	(2,6)Cl_2–C_6H_3–CH_2–
5t	C_6H_5–	(4)CH_3–C_6H_4–CH_2–
5v	C_6H_5–	(4)NO_2–C_6H_4–CH_2–



SCHEME 1 Synthesis of compounds **5a–v**. Reagents and conditions: (i) CH_3OH/H_2SO_4; (ii) $NH_2NH_2.H_2O$; (iii) R_1—NCS (R_1: —CH_3, —CH_2CH_3, C_6H_5)/EtOH; (iv) NaOH (2 N), HCl; (v) DMF, K_2CO_3, R_2—CH_2—Cl

When ^{1}H NMR spectra of etodolac thioethers **5a–v** are considered, the first spectroscopic evidence of thioether structure formation is the observation of integral values at approximately 4.00 ppm, which is assigned to S-CH_2 protons.[15–17] Mostly, the S-CH_2 protons are determined as singlets. Depending on the substituent in the compounds **5e**, **5g**, **5j–l**, **5n**, **5s**, **5t**, and **5v**, geminal protons in the form of double doublets were detected and coupling constants were calculated as 12 and 15 Hz.

The indole N—H proton of the etodolac thioethers **5a–v** in the main skeleton of etodolac was determined as a singlet between 9.78 and 10.72 ppm. These values are in agreement with the values of etodolac starting material whose indole N—H value is observed at 10.57 ppm in the ^{1}H NMR spectrum.

Looking at ^{13}C NMR for the compounds **5a**, **5c–e**, **5h**, **5j**, and **5n**, chemical shifts of the S-CH_2 appeared upfield at 39.16, 37.90, 34.97, 38.79, 38.83, and 37.63 ppm, respectively, C═N appeared downfield at 149.64, 149.84, 149.69, 149.00, 149.98, 149.33, and 151.31 ppm, respectively, and C—S carbon appeared also downfield at 153.58, 153.43, 153.52, 153.85, 152.65, 152.77, and 153.46 ppm, respectively. The ^{1}H and ^{13}C NMR spectra of the compound **5d** were correlated for the HMBC spectrum evaluation. The ^{1}H and ^{13}C NMR spectra of the compound are in agreement with the HMBC spectrum at 600 MHz.

The HR-MS results of the compounds **5a**, **5c**, **5e**, **5k**, **5n** in the etodolac-thioether structure were detected by electron ionization (EI^+) technique. Molecular weight and elemental composition of the compounds, *m/z* values of the resulting mass fractions were determined by monoisotopic mass values from closed formulas. It has been found that calculated and found *m/z* values of the molecular ion peaks of the compounds, the elemental composition and the monoisotopic mass data belonging to them, are compatible with each other.

2.2 | Biological evaluation

2.2.1 | Anticancer activity and apoptosis of etodolac thioethers

The synthesized 19 compounds were tested for anticancer activity in six different cell lines; VERO cells (African green monkey kidney cell),

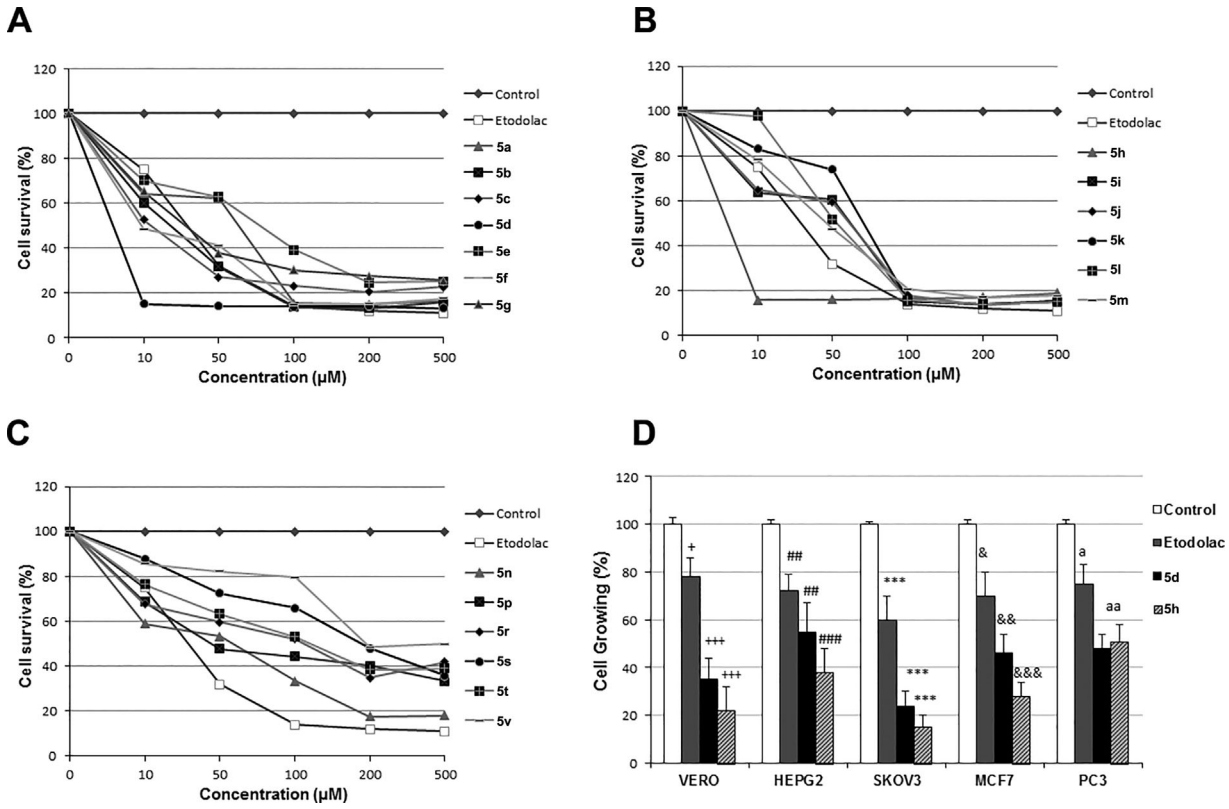


FIGURE 1 Cell survival of VERO cells incubated with different concentrations of etodolac and (A–C) derivative compounds **5a–v** for 24 h. (D) Cell survival of different cell lines with incubated 10 μM concentration of etodolac, **5d** and **5h** compounds for 24 h. ($^{+}p < 0.05$, $^{+++}p < 0.001$ compare to control groups in VERO cells; $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ compare to control groups in HEPG2 cells; $^{***}p < 0.001$ compare to control groups in SKOV3 cells; $^{\&}p < 0.05$, $^{\&\&}p < 0.01$, $^{\&\&\&}p < 0.001$ compare to control groups in MCF7 cells; $^{a}p < 0.05$, $^{aa}p < 0.01$, compare to control groups in PC3 cells)

HEPG2 (human hepatocellular liver carcinoma), SKOV3 (ovarian carcinoma), MCF7 (human breast adenocarcinoma), PC3 and DU145 (human prostate carcinoma) cells using MTT assay method at 10^{-5} M (10 μM).[18–20] Dose-dependent cytotoxicity tests were performed in VERO cells for etodolac thioethers **5a–v**. 1-{[5-(4-Chlorobenzyl)-sulfanyl-4-methyl-4*H*-1,2,4-triazol-3-yl]methyl}-1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indole **5d** and 1-{[5-benzylsulfanyl-4-ethyl-4*H*-1,2,4-triazol-3-yl]methyl}-1,8-diethyl-1,3,4,9-tetrahydropyrano [3,4-*b*]indole **5h** compounds were found to be cytotoxic at 10^{-5} M concentration (Figure 1).

1-{[5-(4-Chlorobenzyl)sulfanyl)-4-methyl-4*H*-1,2,4-triazole-3-yl]-methyl}-1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indole **5d** and 1-{[5-benzylsulfanyl]-4-ethyl-4*H*-1,2,4-triazole-1,3,4,9-tetrahydropy-rano[3,4-*b*]indole **5h** compounds have anticancer activity in SKOV3 cancer cells. While etodolac showed no inhibition against SKOV3 cells. Compounds **5a–v** inhibited cell proliferation with IC_{50} values between 5.10 and 266.9 μM as shown in Table 2. As a result, 1-{[5-(4-chlorobenzyl)sulfanyl-4-methyl-4*H*-1,2,4-triazol-3-yl]methyl}-1,8-di-ethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indole **5d** and 1-{[5-benzylsul-fanyl-4-ethyl-4*H*-1,2,4-triazole-1,3,4,9-tetrahydropyrano[3,4-*b*]in-dole **5h** were found to have anticancer activity in SKOV3 cell lines at an IC_{50} of 7.22 and 5.10 μM, respectively (Figure 2). Compared with etodolac, these compounds do not show cytotoxicity against VERO cells.

Apoptosis, known as programmed cell death, involves the controlled dismantling of the cell by cleaving key cellular components while damaged or redundant. Caspases are a family of genes important for maintaining cell death via demolishing key structural proteins and endoproteases that cysteinyl aspartate proteinases (cysteine proteases that cleave their substrates following an Asp residue). Nonetheless, caspase-3 is known as effector caspases, caspase-9 is responsible for the intrinsic mitochondrial apoptosis and caspase-8 is initiate of extrinsic death receptor-mediated apoptosis.

These compounds have also been assessed for caspase 3, 9, and 8 protein expression and activity at the 6th, 12th, and 24th hours of apoptosis, which can be said to play a key role in cancer treatment. Following this study apoptotic caspases protein expression Western blotting bands in SKOV3 ovarian cancer cells with incubated 10 μM concentration of etodolac, **5d** and **5h** compounds. AO/EB staining was performed in SKOV3 cancer cells to demonstrate cell death by fluorescence microscopy and time-dependent cell growth in SKOV3 ovarian cancer was investigated (Figure 3).

TABLE 2 IC_{50} values of compounds **5a–v** on SKOV3 cancer cell lines

Compound	IC_{50} (μM)
Etodolac	9.44
5a	110.2
5b	16.00
5c	31.00
5d	7.22
5e	57.2
5f	89.2
5g	246.20
5h	5.10
5i	83.8
5j	113.6
5k	6.48
5l	40.3
5m	266.9
5n	19.77
5p	27.5
5r	216.49
5s	9.03
5t	47.575
5v	11.08

Etodolac thioethers **5a–v** also were studied on the dose-dependent cytotoxicity effect of PC3 (androgen-independent prostate cancer) cells. This study was examined by time-dependent cell growth on PC-3 prostate cancer cell lines. These compounds have also been assessed for caspase 3 activity at the 12th, 24th, and 48th hours of apoptosis (Figures 4 and 5).

While etodolac showed no inhibition against PC3 cells, compounds **5a–v** inhibited cell proliferation with IC_{50} values between 3.10 and 575.93 μM as shown in Table 3. As a result, 1-{[5-(4-chlorobenzyl)-sulfanyl-4-ethyl-4*H*-1,2,4-triazole-3-yl]methyl}-1,8-diethyl-tetrahydro-pyrano[3,4-*b*]indole **5k**, 1-{[5-(2,6-dichlorobenzyl)sulfanyl-4-phenyl-4*H*-1,2,4-triazol-3-yl]methyl}-1,8-diethyl-1,3,4,9-tetrahydropyrano-[3,4-*b*]indole **5s**, and 1-{[5-(4-nitrobenzyl)sulfanyl-4-phenyl-1,2,4-triazol-3-yl]methyl}-1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indole **5v** showed the most anticancer activity at 8.18 μM, 3.10, and 4.00 μM IC_{50} levels in PC3 cancer cell lines.

The structure–activity relationship (SAR) analysis stated that compounds containing electron-withdrawing group in benzyl group showed stronger anticancer activity against SKOV3 and PC3 cells. The electron-withdrawing group containing NO_2 (**5v**) and Cl (**5s**) groups in benzyl group for phenyl containing at 4-position of 1,2,4-triazole and Cl (**5k**) groups in benzyl group for ethyl containing at 4-position of 1,2,4-triazole in prostate cancer cell line. The electron-withdrawing group containing phenyl (**5h**) in benzyl group for ethyl containing at 4-position of 1,2,4-triazole and Cl (**5d**) groups in benzyl group for methyl containing at 4-position of 1,2,4-triazole in ovarian cancer cell line.

2.3 | Molecular modeling

Compounds were docked into human MetAP2 (hMetAP2) according to the method described in Ref. [21]. The results showed that all of these compounds have good binding affinity for MetAP2 (Table 4).

Most important amino acids of the binding pocket that were making non-bonded hydrophobic interactions with compounds were HIS231, LEU328, ASN329, HIS331, HIS339, THR343, ASP376, TYR444, PRO443, and LEU447. Additionally, compounds with —NO_2 group in *para* position of the phenyl ring made hydrogen bonds with ARG337 and PHE387. According to docking results, the compound **5k** with $\Delta G = -11.21$ kcal/mol and $K_i = 6.10$ nM, compound **5s** with $\Delta G = -11.02$ kcal/mol and $K_i = 8.36$ nM, and **5v** 11.02 kcal/mol and $K_i = 7.27$ nM were shown to be the best inhibitors.

Furthermore from these results we may conclude that the nitro group in *para* position (**5v**) for compounds containing phenyl in position 4 of 1,2,4-triazole and dichloro groups at 2,6-positions (**5k**) for compounds containing ethyl group at position 4 of 1,2,4-triazole ring turned out to be important for binding. These compounds showed good binding for the MetAP2 protein (Figures 6 and 7). In the same time, they were also most active in PC3 cancer cell line.

3 | CONCLUSION

The selective COX-2 inhibitor etodolac, the carboxylic acid derivative containing the pyrano[3,4-*b*]indole structure, has been a non-steroidal agent with analgesic, antipyretic, and anti-inflammatory effects since 1985. The indole structure is important in the development of new drug candidates, especially in terms of anticancer activity. Indole-based pharmacophores are the most preferred structures among researchers in structural modifications, as indole derivatives respond to anticancer agents in a significant way. The main reason for selecting etodolac as the starting material in the synthesis is its anticancer activity.

Etodolac thioethers were synthesized with substituted benzyl chlorides and their corresponding etodolac 1,2,4-triazoles. The cytotoxicity and anticancer activity of synthesized compounds were evaluated *in vitro* using the MTT colorimetric method against VERO cells, HEPG2 (human hepatocellular liver carcinoma), SKOV3 (ovarian carcinoma), MCF7 (human breast adenocarcinoma), PC3 and DU145 (prostate carcinoma) cells at 10^{-5} M for 24 h. Spectroscopic and elemental analysis methods of the synthesized etodolac thioether derivatives have been elucidated.

In this study, anticancer activities of etodolac derivatives were screened in cell lines VERO (monkey kidney cancer), HEPG2 (human liver cancer), SKOV3 (human ovarian cancer), MCF7 (human breast cancer), PC3 and DU145 (human prostate cancer). Among these compounds, compounds **5d** and **5h** were found to have anticancer activity in SKOV3 cell line at IC_{50} values of 7.22

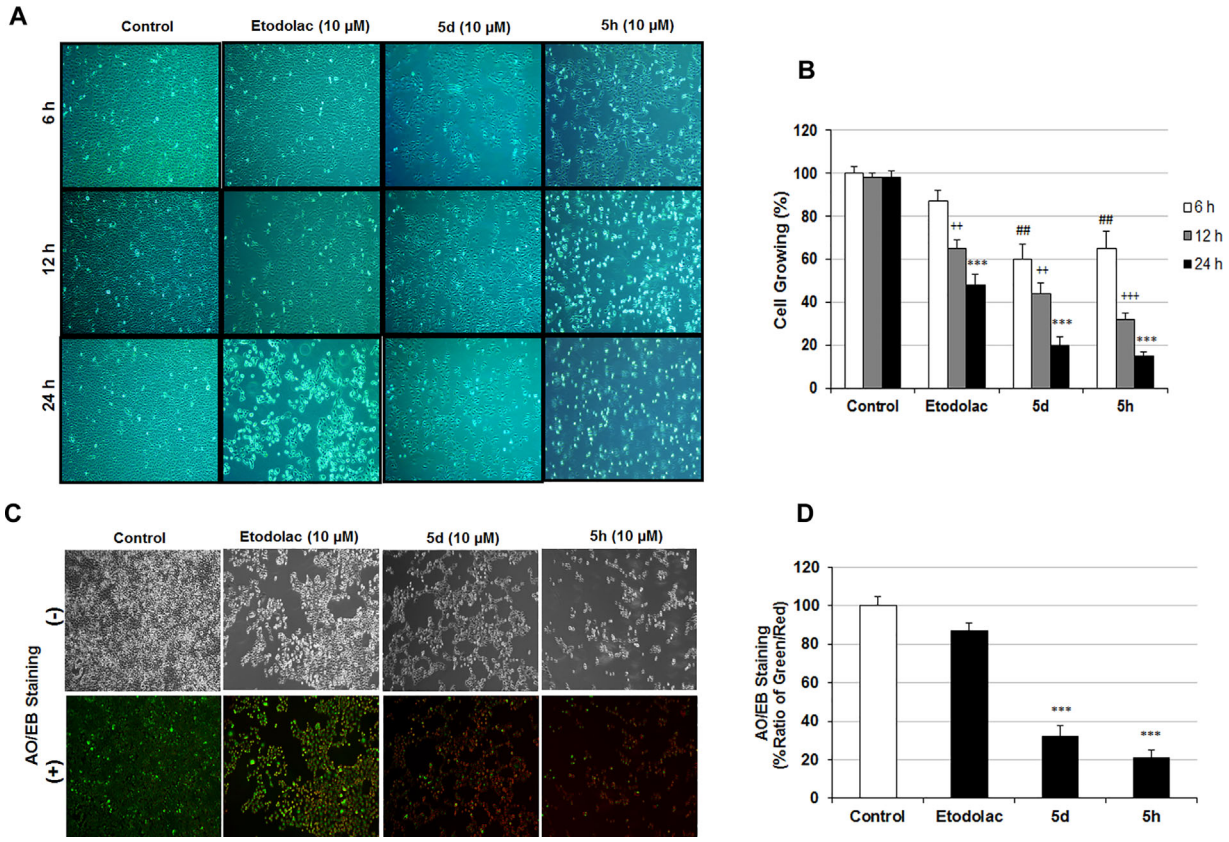


FIGURE 2 (A) Cell growing imaging and (B) percentage of cell growing SKOV3 ovarian cancer cells with incubated 10 µM concentration of etodolac, **5d** and **5h** compounds for 6, 12, and 24 h. ($^{\#\#}p < 0.01$ compare to control groups in 6 h; $^{++}p < 0.01$, $^{+++}p < 0.001$ compare to control groups in 12 h, $^{***}p < 0.001$ compare to control groups in 24 h. (C) AO/EB staining image and (D) ratio of AO/EB staining in SKOV3 ovarian cancer cells with incubated 10 µM concentration of etodolac, **5d** and **5h** compounds for 24 h ($^{***}p < 0.001$ compare to control groups)

and 5.10 µM, respectively, and IC_{50} values of **5k**, **5s**, and **5v** compounds at PC3 cell line at 8.18 µM, 3.10 and 4.00 µM, respectively.

A molecular modeling study using MetAP2 enzyme to support these findings were performed and compound **5k** with the values of $\Delta G = -11.21$ kcal/mol, $K_i = 6.10$ nM, **5s** with the values of $\Delta G = -11.02$ kcal/mol, $K_i = 8.36$ nM, and **5v** with the values of $\Delta G = -11.02$ kcal/mol, $K_i = 7.27$ nM were found to be the best MetAP2 inhibitors. With these results, there was a correlation between molecular modeling and anticancer activity results.

4 | EXPERIMENTAL

4.1 | Chemistry

4.1.1 | General

All solvents and chemicals used in this study were supplied from Sigma–Aldrich, Merck and used without purification. Melting points (mp) were determined using Schmelzpunktbestimmer SMP II melting point apparatus and expressed in degrees centigrade (°C). Reactions were monitored by analytical thin layer chromatography (TLC) plates precoated with silica gel F254 using solvent systems M_1, petroleum ether/acetone (60:40, v/v); M_2, petroleum ether/acetone (50:50, v/v); M_3, petroleum ether/acetone (70:30, v/v). The spots were located under UV light (254 nm, $t = 25$°C). Elemental analyses of compounds were performed on a VarioMICRO V1.5.7. instrument. Infrared spectra of compounds were recorded on Shimadzu FTIR-8400S spectrophotometer and expressed in wavenumber v (cm^{-1}). ^{1}H NMR spectra were recorded as solutions in $CDCl_3$ and DMSO-d_6 on Bruker Ultrashield TM and Varian Mercury spectrometer operating at 300 and 600 MHz. ^{13}C NMR spectra were recorded on Bruker Avance DPX 400 as solutions in $CDCl_3$ operating at 75 and 150 MHz. The ^{1}H and ^{13}C chemical shifts were expressed in ppm relative to tetramethylsilan (TMS) as an internal standard, where TMS (δ) = 0.0 ppm. The multiplicity of the signal is indicated as: d, doublet; m, multiplet; s, singlet; t, triplet; q, quarted. Coupling constants are expressed in hertz (Hz). All NMR spectra and elemental analyses were carried out by the Inonu University Scientific and Technological Research Center (IBTAM).

The IR and NMR spectra as well as the InChI codes of the investigated compounds together with some biological activity data are provided as Supporting Information.

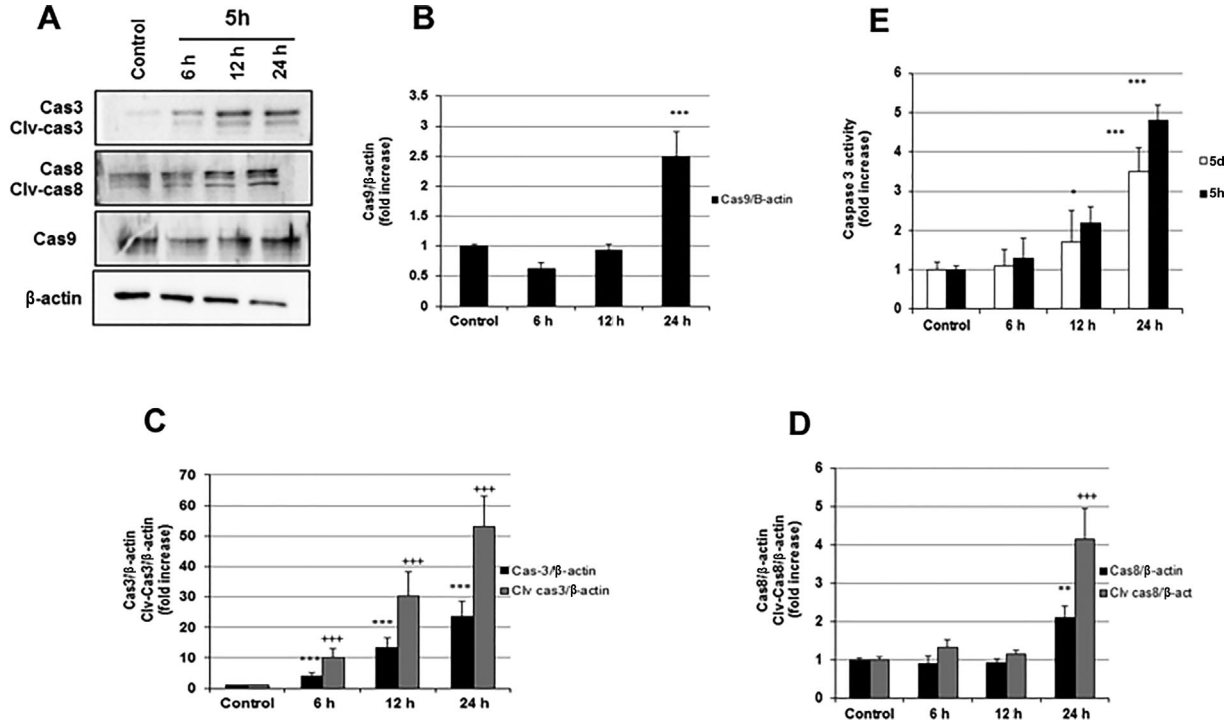


FIGURE 3 (A) Apoptotic caspases protein expression Western blotting bands in SKOV3 ovarian cancer cells with incubated 10 μM concentration of etodolac, **5d** and **5h** compounds for 6, 12, and 24 h. (B) Densitometry analysis of Cas3/β-actin and Clv-Cas3/β-actin protein expression levels (***$p < 0.001$ compare to Cas3 bands of control groups; $^{+++}p < 0.001$ compare to Clv-Cas3 bands of control groups). (C) Densitometry analysis of Cas8/β-actin and Clv-Cas8/β-actin protein expression levels (**$p < 0.01$ compare to Cas8 bands of control groups; $^{++}p < 0.001$ compare to Clv-Cas8 bands of control groups). (D) Densitometry analysis of Cas9/β-actin protein expression levels (***$p < 0.001$ compare to Cas9 bands of control groups). (E) Caspase3 activity in SKOV3 ovarian cancer cells with incubated 10 μM concentration of **5d** and **5h** compounds for 6, 12, and 24 h (*$p < 0.05$, ***$p < 0.001$ compare to control groups of each compound)

4.1.2 | Preparation of (*R*,*S*)-methyl-2-(1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indole-1-yl)acetate (1, CAS no. 122188-02-07) and (*R*,*S*)-2-(1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indole-1-yl)-acetohydrazide (2, CAS no. 946848-58-4)

Etodolac (0.01 mol) and methanol (16 mL) were refluxed for 3 h in a few drops of concentrated sulfuric acid. The contents of the flask were subsequently cooled and neutralized by using sodium bicarbonate ($NaHCO_3$) (5%). The resulting precipitate was filtered, dried, and recrystallized twice from ethanol. Methanolic solution of etodolac ester (0.01 mol) and hydrazine hydrate (80%, 7 mL) were refluxed for 3 h. The reaction mixture was then cooled, diluted with water, and allowed to stand overnight. The precipitated solid was washed with water, dried, and recrystallized twice from petroleum ether. (For compound **2** mp 186–188°C[8].)

4.1.3 | General procedure for the synthesis of (*R*,*S*)-1-[2-(1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]-indole-1-yl)acetyl]-4-alkyl/arylthiosemicarbazides (3a–c; 3a CAS no. 1526911-69-2, 3b CAS no. 1528514-00-2, 3c CAS no. 1528514-12-6)

A solution of compound **2** (0.01 mol) and equimolar amount of appropriate isothiocyanate in 20 mL of ethanol was heated under reflux for 2 h. The precipitate obtained was filtered off, washed with water, and followed by two washings with boiling ethanol.[9]

4.1.4 | General procedure for synthesis of (*R*,*S*)-5-[(1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indole-1-yl)-methyl]-4-substituted-2,4-dihydro-3*H*-1,2,4-triazole-3-thiones (4a–c; 4a CAS no. 1526911-68-1, 4b CAS no. 1528513-78-1, 4c CAS no. 1528513-91-8)

A sample of etodolac thiosemicarbazides **3a–c** (0.001 mol) and sodium hydroxide (NaOH) solution (2 N) was refluxed for 4 h on water bath. On cooling, it was solidified. This was dissolved in water and acidified with hydrochloric acid (HCl) solution (10%). The precipitate was washed with water and recrystallized from ethanol.[10]

4.1.5 | General procedure for synthesis of (*R*,*S*)-1-{[5-(substituted)sulfanyl-4-substituted-4*H*-1,2,4-triazole-3-yl]methyl}-1,8-diethyl-1,3,4,9-tetrahydropyrano-[3,4-*b*]indole (5a–v)

To a suspension of substituted-1,2,4-triazole-3-thione **4a–c** (0.7 g, 1.9 mmol) in *N*,*N*-dimethylformamide (DMF) (5 mL) containing anhydrous potassium carbonate (K_2CO_3) (0.26 g, 1.9 mmol), substituted

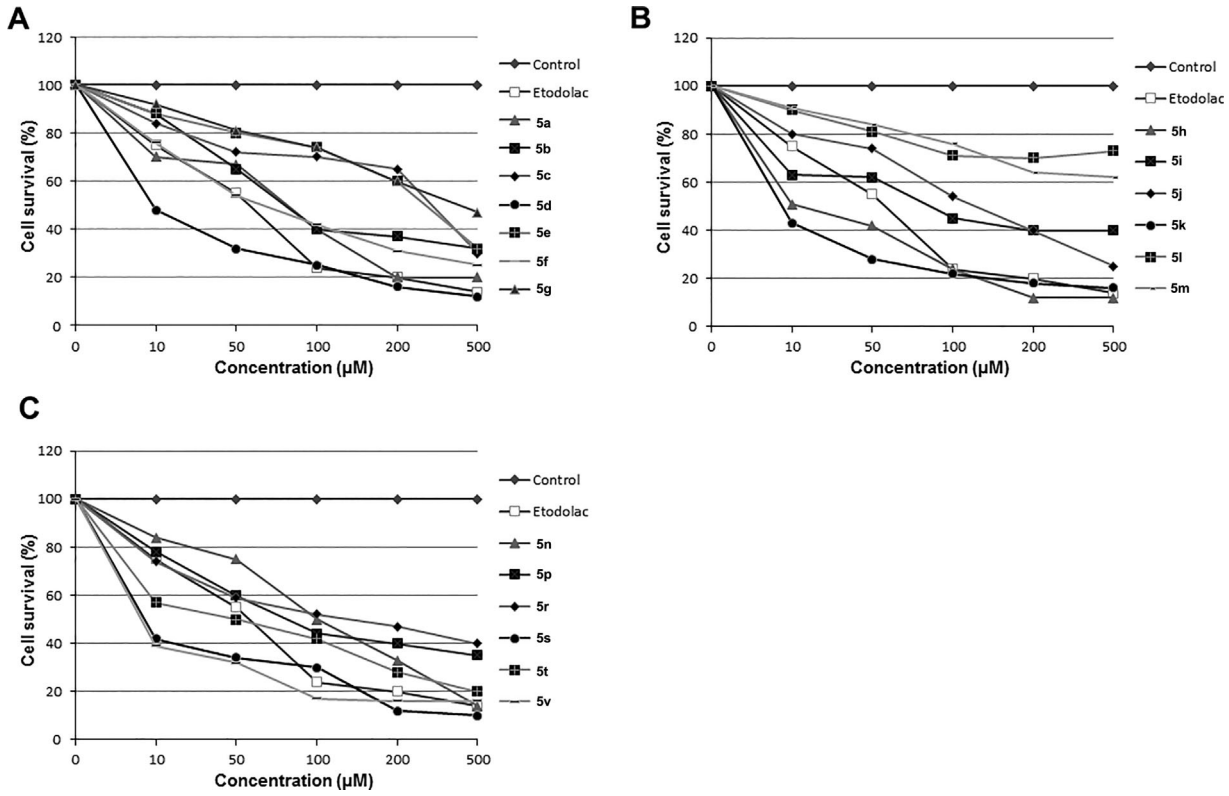


FIGURE 4 Cell survival of PC3 cells incubated with different concentrations of etodolac and (A–C) derivative compounds **5a–v** for 24 h

benzyl chloride (1.9 mmol) was added. The reaction mixture was stirred at room temperature for overnight, then poured onto cold water and the formed precipitate was filtered, washed with water, dried, and crystallized.

(*R*,*S*)-1-{[5-Benzylsulfanyl-4-methyl-4*H*-1,2,4-triazol-3-yl]-methyl}-1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indole (5a)

White solid, yield 62.55%, mp 162.0–163.0°C. R_f: 0.37 (M_1). FT-IR (ν_{max}, cm^{-1}): 3182 (indole N—H); 3084, 3028, 2964, 2928, 2860 (C—H); 1504, 1496, 1471, 1458, 1440 ((N—H, C═C, triazole C═N); 1369, 1329 (C—H); 1249 (C—S); 1076 (pyran C—O); 801, 754 (monosubstituted benzene); 696 (C—S). ^{1}H NMR (600 MHz, DMSO-d_6): δ 0.58 (t, 3H, —CH_2—CH_3 at C_1, J = 7.2 Hz, J = 7.8 Hz); 1.27 (t, 3H, —CH_2—CH_3 at C_8, J = 7.2 Hz, J = 7.8 Hz); 1.56–2.07 (m, 2H, —CH_2—CH_3 at C_1); 2.50–2.89 (m, 4H, —CH_2—CH_3 at C_8, —CH_2 at C_1); 3.31–3.44 (m, 5H, —CH_2 at C_4 and N—CH_3); 3.78–3.90 (m, 2H, —CH_2 at C_3); 4.26 (s, 2H, S—CH_2); 6.89–7.29 (m, 8H, Ar—H); 10.70 (s, 1H, indole N—H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 7.61 (C-12); 13.79 (C-10); 22.49 (N-CH_3); 24.29 (C-9); 30.05 (C-4); 30.45 (C-11); 34.81 (C-13); 36.83 (S-CH_2); 60.98 (C-3); 76.24 (C-1); 108.08 (C-4a); 115.77 (C-6); 119.45 (C-5); 120.27 (C-7); 126.01 (C-5a); 126.98, 127.08 (C-2′ and C-6′); 129.38 (C-4′); 129.80, 131.00 (C-3′ and C-5′); 134.17 (C-8); 134.64 (C-1′); 134.68 (C-1a); 135.78 (C-8a); 149.64 (triazole C═N); 153.58 (C—S). HR MS [(EI$^+$), *m/z* (%)]: 446.2143 ([M^+], 9.16), 417.1760 (2.33), 229.1422 (17.54), 228.1390 (100.0), 227.1308 (15.58), 219.0832 (5.81), 198.0920 (2.34), 156.0813 (2.71), 91.0548 (11.86), 57.0339 (4.00). Analysis for $C_{26}H_{30}N_4OS.1/2H_2O$; calcd. (%): C, 68.54; H, 6.86; N, 12.30; S, 7.04. Found: C, 68.78; H, 6.61; N, 12.34; S, 6.81.

(*R*,*S*)-1-{[5-(4-Fluorobenzyl)sulfanyl-4-methyl-4*H*-1,2,4-triazole-3-yl]methyl}-1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indole (5b)

``Pale yellow solid, yield 47.30%, mp 161.1°C. R_f: 0.10 (M_1). FT-IR (ν_{max}, cm^{-1}): 3254 (indole NH), 3017, 2963, 2920, 2872 (C—H); 1601, 1508, 1472, 1460, 1413 (N—H, C═C, triazole C═N); 1375, 1292 (C—H); 1225 (C—S); 1080 (pyran C—O); 742, 696 (benzene); 657 (C—S). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.77 (t, 3H, —CH_2—CH_3 at C_1, J = 7.2 Hz); 1.37 (t, 3H, —CH_2—CH_3 at C_8, J = 7.5 Hz, J = 7.8 Hz); 1.98–2.3 (m, 2H, —CH_2—CH_3 at C_1); 2.80–2.93 (m, 4H, —CH_2—CH_3 at C_8 and —CH_2— at C_1); 3.09–3.25 (m, 5H, —CH_2— at C_4 and —N—CH_3); 3.98–4.13 (m, 2H, —CH_2 at C_3); 4.31 (s, 2H, S—CH_2); 6.95–7.36 (m, 7H, Ar—H); 10.04 (s, 1H, indole N—H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 7.58 (C-12); 13.78 (C-10); 22.47 (N—CH_3); 24.26 (C-9); 30.14 (C-4); 30.56 (C-11); 34.73 (C-13); 37.92 (S—CH_2); 60.96 (C-3); 76.30 (C-1); 108.19 (C-4a); 115.48 (C-6); 119.48 (C-5); 120.29 (C-7); 126.03 (C-5a); 127.04, 130.58 (C-2′ and C-6′); 130.69 (C-4′); 132.61, 132.65 (C-3′ and C-5′); 134.66 (C-8); 135.70 (C-1′); 149.83 (C-1a); 153.46 (C-8a); 160.66 (triazole C═N); 163.94 (C—S). Analysis for $C_{26}H_{29}FN_4OS$ calcd. (%): C, 67.21; H, 6.29; N, 12.06; S, 6.90. Found (%): C, 66.55; H, 6.68; N, 11.36; S, 6.35.

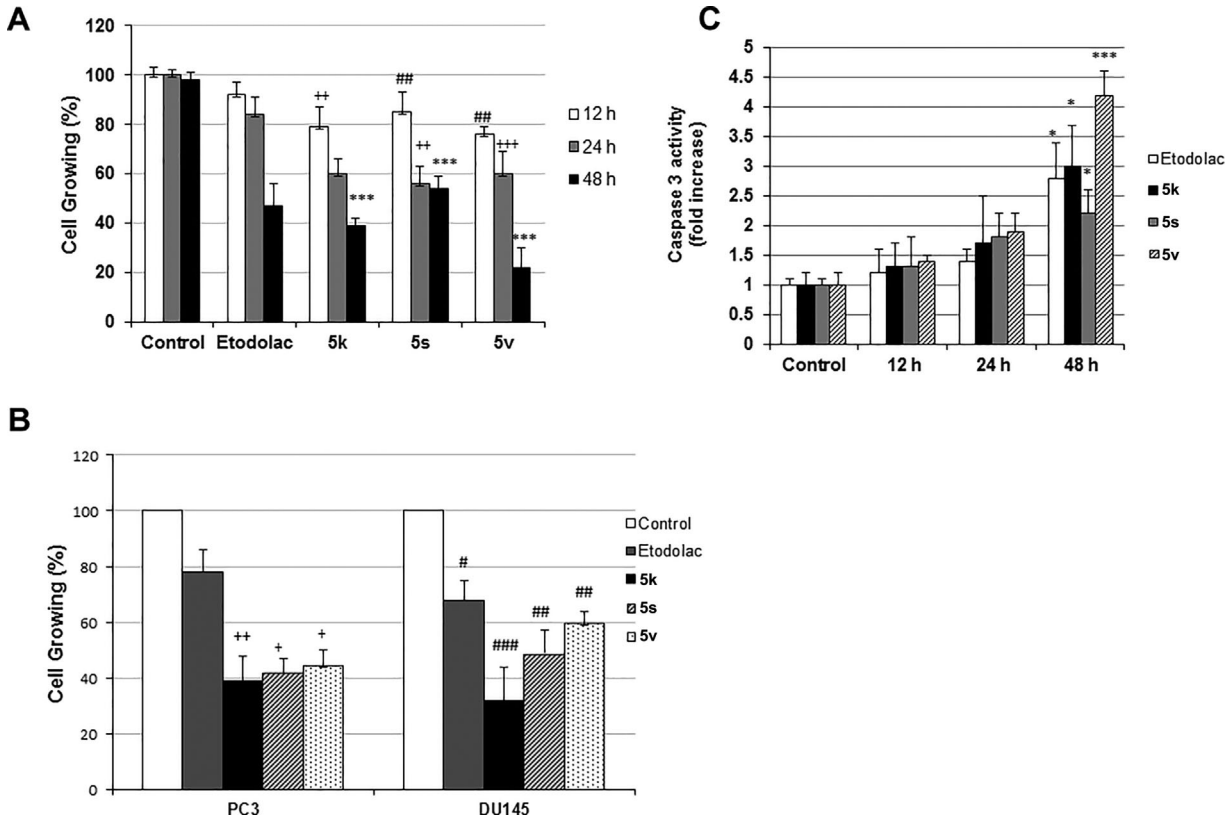


FIGURE 5 (A) Percentage of cell growing PC3 prostate cancer cells incubated with 10 μM concentration of etodolac, **5k**, **5s**, and **5v** compounds for 12, 24, and 48 h ($^{\#\#}p < 0.01$ compare to control groups in 12 h; $^{++}p < 0.01$, $^{+++}p < 0.001$ compare to control groups in 24 h, $^{***}p < 0.001$ compare to control groups in 48 h). (B) Cell survival of different prostate cancer PC3 and DU145 cell lines incubated with 10 μM concentration of etodolac, **5k**, **5s**, and **5v** compounds for 36 h ($^{+}p < 0.05$, $^{++}p < 0.01$ compare to control groups in PC3 cells; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ compare to control groups in DU145 cells). (C) Caspase3 activity in PC3 prostate cancer cells incubated with 10 μM concentration of **5k**, **5s**, and **5v** compounds for 12, 24, and 48 h ($^{*}p < 0.05$, $^{***}p < 0.001$ compare to control groups of each compound)

(*R*,*S*)-1-{[5-(2-Chlorobenzyl)sulfanyl-4-methyl-4*H*-1,2,4-triazole-3-yl]methyl}-1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indole (5c)

White solid, yield 52.0%, mp 153.4°C. R_f: 0.19 (M_1). FT-IR (ν_{max}, cm^{-1}): 3526 (O—H); 3246 (indole N—H); 3061, 2970, 2918, 2862 (C—H); 1601, 1582, 1520, 1476, 1447, 1422 (N—H, C═C, triazole C═N); 1329, 1319 (C—H); 1252 (C—S); 1072 (pyran C—O); 743, 692 (*o*-substituted benzene); 677 (C—S). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.76 (t, 3H, —CH_2—CH_3 at C_1, *J* = 7.2 Hz, *J* = 7.5 Hz); 1.37 (t, 3H, —CH_2—CH_3 at C_8, *J* = 7.5 Hz, *J* = 7.8 Hz); 1.97–2.29 (m, 2H, —CH_2—CH_3 at C_1); 2.79–2.94 (m, 4H, —CH_2 —CH_3 at C_8 and —CH_2— at C_1); 3.07–3.24 (m, 5H, —CH_2— at C_4 and —N—CH_3); 3.98–4.13 (m, 2H, —CH_2 at C_3); 4.41 (s, 2H, S—CH_2); 7.01–7.38 (m, 7H, Ar—H); 10.13 (s, 1H, indole N—H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 7.57 (C-12); 13.75 (C-10); 22.47 (N—CH_3); 24.24 (C-9); 30.03 (C-4); 30.45 (C-11); 34.76 (C-13); 39.16 (S—CH_2); 60.95 (C-3); 76.26 (C-1); 108.05 (C-4a); 115.73 (C-6); 119.42 (C-5); 120.22 (C-7); 126.01 (C-5a); 127.08 (C-2′ and C-6′); 127.81 (C-4′); 128.70 (C-3′ and C-5′); 128.89 (C-8); 134.67 (C-1′); 135.80 (C-1a); 136.80 (C-8a); 149.84 (triazole CN); 153.43 (C—S). HR-MS [(EI$^+$), *m/z* (%)]: 482.1728 ([M+2]$^+$, 2.78), 480.1747 ([M$^+$], 6.80), 253.0437 (4.47), 229.1422 (16.77), 228.1383 (100.0), 227.1305 (15.49), 226.1231 (3.19), 198.0914 (2.03), 156.0810 (2.20), 125.0157 (5.60), 57.0338 (4.29). Analysis for $C_{26}H_{29}ClN_4OS.H_2O$ calcd. (%): C, 62.57; H, 6.26; N, 11.23; S, 6.42. Found (%): C, 61.78; H, 6.15; N, 11.30; S, 6.32.

(*R*,*S*)-1-{[5-(4-Chlorobenzyl)sulfanyl-4-methyl-4*H*-1,2,4-triazole-3-yl]methyl}-1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indole (5d)

White solid, yield 38.4%, mp 116.0–119.0°C. R_f: 0.53 (M_1). FT-IR (ν_{max}, cm^{-1}): 3600 (O—H); 3169 (indole N—H); 3082, 2963, 2920, 2872, 2861 (C—H); 1592, 1503, 1489, 1470, 1449 (N—H, C═C, triazole C═N); 1375, 1314 (C—H); 1246 (C—S); 1080 (pyran C—O); 750, 669 (*p*-substituted benzene); 628 (C—S). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.77 (t, 3H, —CH_2—CH_3 at C_1, *J* = 7.5 Hz, *J* = 7.2 Hz); 1.36 (t, 3H, —CH_2—CH_3 at C_8, *J* = 7.5 Hz, *J* = 7.8 Hz); 1.98–2.30 (m, 2H, —CH_2—CH_3 at C_1); 2.76–2.92 (m, 4H, —CH_2—CH_3 at C_8 and —CH_2— at C_1); 3.14–3.25 (m, 5H, —CH_2— at C_4 and —N—CH_3); 3.98–4.13 (m, 2H, —CH_2 at C_3); 4.30 (s, 2H, S—CH_2); 6.99–7.37 (m, 7H, Ar—H); 10.0317 (s, 1H, indole N—H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 7.62 (C-12); 13.81 (C-10); 22.48 (N—CH_3); 24.27 (C-9); 30.18 (C-4); 30.62 (C-11); 34.72 (C-13); 37.90 (S—CH_2); 60.97 (C-3); 76.32 (C-1); 108.24 (C-4a); 115.81 (C-6); 119.50 (C-5); 120.30 (C-7); 126.05 (C-5a); 127.05 (C-2′ and C-

TABLE 3 IC_{50} values of **5a–v** on PC3 prostate cancer cell line

Compound	IC_{50} (μM)
Etodolac	20.29
5a	40.75
5b	77.97
5c	208.12
5d	11.20
5e	234.43
5f	48.83
5g	575.93
5h	15.01
5i	75.06
5j	88.79
5k	8.18
5l	ND
5m	ND
5n	62.12
5p	80.53
5r	15.05
5s	3.10
5t	28.24
5v	4.00

ND, not determined.

TABLE 4 Docking scores of compounds **5a–v**

AutoDock 4.2.6		
Compounds	**ΔG (kcal/mol)**	**K_i (nM)**
5a	−9.20	179.32
5b	−8.82	345.21
5c	−9.06	227.02
5d	−9.52	104.89
5e	−9.90	55.30
5f	−9.39	129.97
5g	−8.45	639.86
5h	−9.06	227.39
5i	−9.09	216.94
5j	−9.31	150.68
5k	−11.21	6.10
5l	−9.83	62.05
5m	−9.73	74.16
5n	−10.42	23.18
5p	−10.35	26.03
5r	−10.79	12.41
5s	−11.02	8.36
5t	−10.82	11.64
5v	−11.02	7.27

6′); 128.86 (C-4′); 130.31 (C-3′ and C-5′); 133.72 (C-8); 134.70 (C-1′); 135.41 (C-1a); 135.69 (C-8a); 149.69 (triazole C═N); 153.52 (C—S). Analysis for $C_{26}H_{29}ClN_4OS.3/2\ H_2O$ calcd. (%): C, 61.46; H, 6.35; N, 11.03; S, 6.31. Found (%): C, 61.43; H, 5.97; N, 10.32; S, 5.81.

(*R*,*S*)-1-{[5-(2,6-Dichlorobenzyl)sulfanyl-4-methyl-4*H*-1,2,4-triazole-3-yl]methyl}-1,8-diethyl-tetrahydropyrano[3,4-*b*]indole (5e)

White solid, yield 68.0%, mp 145.0–147.0°C. R_f: 0.57 (M_2). FT-IR (ν_{max}, cm^{-1}): 3293 (indole N—H); 3053, 2964, 2926, 2868 (C—H); 1558, 1501, 1468, 1434 (N—H, C═N, C═C); 1374, 1335 (C—H); 1250 (C—S); 1075 (pyran C—O); 783, 741 (substituted benzene); 693 (C—S). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.78 (t, 3H, —CH_2—CH_3 at C_1, *J* = 7.2 Hz, *J* = 7.5 Hz); 1.35 (t, 3H, —CH_2—CH_3 at C_8, *J* = 7.8 Hz, *J* = 7.5 Hz); 1.98–2.26 (m, 2H, —CH_2—CH_3 at C_1); 2.81–2.92 (m, 4H, —CH_2 —CH_3 at C_8 and —CH_2— at C_1); 3.11–3.28 (m, 5H, —CH_2— at C_4 and —N—CH_3); 4.01–4.12 (m, 2H, —CH_2 at C_3); 4.45–4.59 (dd, 2H, *J* = 12.6 Hz, *J* = 12.9 Hz, S—CH_2); 7.0–7.36 (m, 6H, Ar—H); 10.33 (s, 1H, indole N—H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 7.64 (C-12); 13.76 (C-10); 22.51 (N—CH_3); 24.26 (C-9); 30.28 (C-4); 34.91 (C-11, C-13); 34.97 (S—CH_2); 60.99 (C-3); 76.29 (C-1); 107.93 (C-4a); 115.73 (C-6); 119.41 (C-5); 120.19 (C-7); 126.01 (C-5a); 127.17 (C-2′ and C-6′); 128.50 (C-4′); 129.52 (C-3′ and C-5′); 133.00 (C-8); 134.69 (C-1′); 135.75 (C-1a); 135.93 (C-8a); 149.00 (triazole C═N); 153.85 (C—S). HR-MS [(EI$^+$), *m/z* (%)]: 518.1311 ([M+4]$^+$, 0.85), 516.1322 ([M+2]$^+$, 3.84), 514.1348 ([M$^+$], 5.29), 287.0041 (4.13), 229.1415 (16.80), 228.1377 (100.0), 227.1299 (15.15), 160.9733 (4.59), 158.9763 (7.15), 156.0806 (2.17), 57.0338 (4.08). Analysis for $C_{26}H_{28}Cl_2N_4OS.1/2H_2O$ calcd. (%): C, 59.54; H, 5.57; N, 10.68; S, 6.11. Found (%): C, 59.01; H, 5.19; N, 10.52; S, 6.47.

(*R*,*S*)-1-{[5-(4-Methylbenzyl)sulfanyl-4-methyl-4*H*-1,2,4-triazole-3-yl]methyl}-1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indole (5f)

Pale yellow solid, yield 68.5%, mp 104.2–105.5°C. R_f: 0.55 (M_3). FT-IR (ν_{max}, cm^{-1}): 3500 (O—H); 3271 (indole NH), 3055, 2972, 2922, 2858 (C—H); 1674, 1622, 1514, 1498, 1464 (N—H, C═N, CC); 1373, 1329 (C—H); 1209 (C—S); 1072 (pyran C—O); 792, 742 (substituted benzene); 688 (C—S). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.57 (t, 3H, —CH_2—CH_3 at C_1, *J* = 7.2 Hz, *J* = 7.5 Hz); 1.27 (t, 3H, —CH_2—CH_3 at C_8, *J* = 7.5 Hz); 1.53–2.07 (m, 2H, —CH_2—CH_3 at C_1); 2.26 (s, 3H, Ar—CH_3); 2.53–2.90 (m, 4H, —CH_2 —CH_3 at C_8 and —CH_2— at C_1); 3.31–3.47 (m, 5H, —CH_2— at C_4 and —N—CH_3); 3.77–3.95 (m, 2H, —CH_2 at C_3); 4.22 (s, 2H, S—CH_2); 6.87–7.25 (m, 6H, Ar—H); 10.72 (s, 1H, indole N—H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 7.55 (C-12); 13.75 (C-10); 22.48 (N—CH_3); 24.25 (C-9); 30.08 (C-4); 30.41 (C-11); 34.77 (C-13); 38.87 (S—CH_2); 60.97 (C-3); 76.27 (C-1); 108.01 (C-4a); 115.73 (C-6); 119.42 (C-5); 120.22 (C-7); 126.00 (C-5a); 127.10 (C-2′ and C-6′); 128.82 (C-4′); 129.37 (C-3′ and C-5′); 133.63 (C-8); 134.66 (C-1′); 135.83 (C-1a); 137.64 (C-8a); 150.03 (triazole C═N); 153.35 (C—S). Analysis for $C_{27}H_{32}N_4OS.2H_2O$ calcd. (%): C, 65.29; H, 7.31; N, 11.28; S, 6.49. Found (%): C, 65.09; H, 6.90; N, 11.41; S, 6.52.

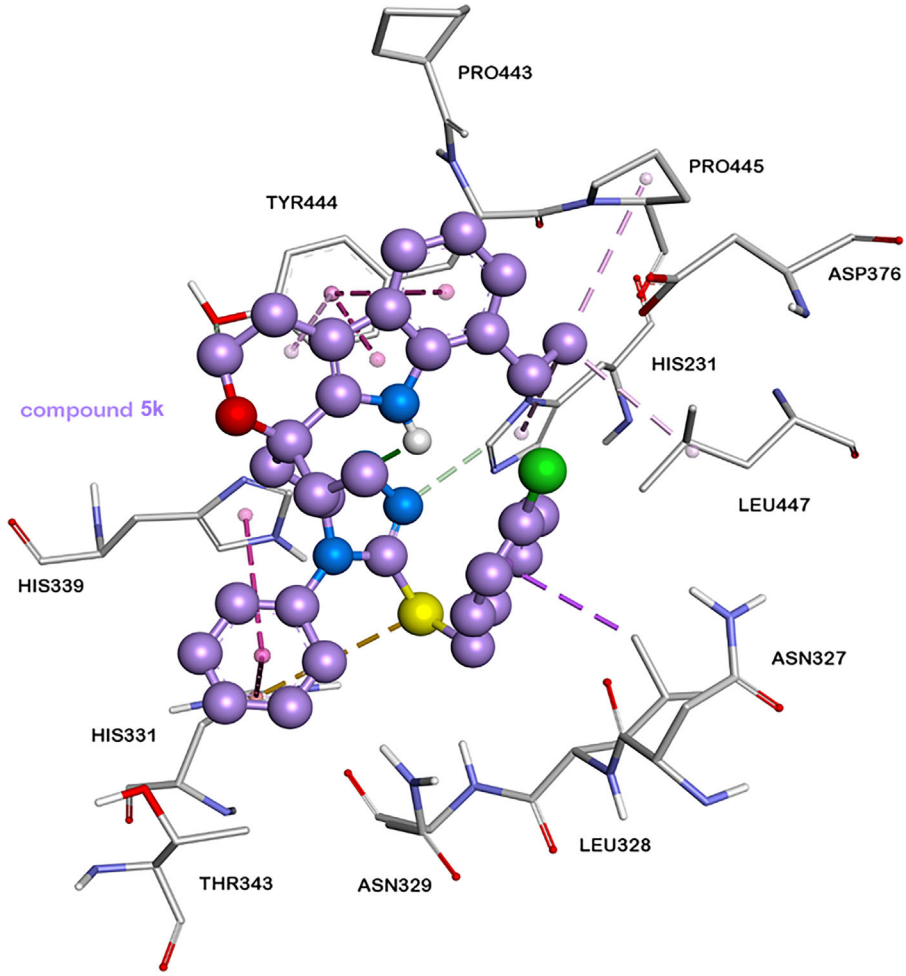


FIGURE 6 Molecular interactions of compound **5k**

(*R*,*S*)-1-{[5-(4-Nitrobenzyl)sulfanyl-4-methyl-4*H*-1,2,4-triazole-3-yl]methyl}-1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indole (5g)

Yellow solid, yield 46.4%, mp 133.4–136.8°C. R_f: 0.35 (M_1). FT-IR (ν_{max}, cm^{-1}): 3600 (O—H); 3190 (indole NH), 3050, 2965, 2932, 2874 (C—H); 1600, 1518, 1479 (N—H, C═N, C═C); 1343, (C—H); 1250 (C—S); 1076 (pyran C—O); 745, 719 (*p*-substituted benzene); 698 (C—S). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.77 (t, 3H, —CH_2—$C\underline{H_3}$ at C_1, J = 7.2 Hz, J = 7.5 Hz); 1.31–1.38 (t, 3H, —CH_2—$C\underline{H_3}$ at C_8, J = 7.5 Hz, J = 7.8 Hz); 1.99–2.29 (m, 2H, —$C\underline{H_2}$—CH_3 at C_1); 2.78–2.90 (m, 4H, —$C\underline{H_2}$ —CH_3 at C_8 and —$C\underline{H_2}$— at C_1); 3.12–3.31 (m, 5H, —$C\underline{H_2}$— at C_4 and —N—CH_3); 3.97–4.12 (m, 2H, —$C\underline{H_2}$ at C_3); 4.40–4.50 (dd, 2H, J = 15 Hz, J = 12 Hz, S—$C\underline{H_2}$); 6.99–7.36 (m, 3H, Ar—H); 8.11 (d, 2H, Ar—NO_2, *ortho*, J = 8.7 Hz); 7.49 (d, 2H, Ar—NO_2, *para*, J = 8.7 Hz); 9.75 (s, 1H, indole N—H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 7.60 (C-12); 13.81 (C-10); 22.39 (N—CH_3); 24.26 (C-9); 30.24 (C-4); 30.75 (C-11); 34.68 (C-13); 36.89 (S—CH_2); 60.94 (C-3); 76.33 (C-1); 108.41 (C-4a); 115.87 (C-6); 119.59 (C-5); 120.45 (C-7); 126.03 (C-5a); 127.37, 127.48 (C-2′ and C-6′); 128.99 (C-4′); 129.90, 130.05 (C-3′ and C-5′); 134.63 (C-8); 135.44 (C-1′); 144.47 (C-1a); 147.39 (C-8a); 149.33 (triazole $\underline{C}$═N); 153.69 ($\underline{C}$—S). Analysis for $C_{26}H_{29}N_5O_3S.H_2O$ calcd. (%): C, 61.28; H, 6.13; N, 13.74; S, 6.29. Found (%): C, 61.24; H, 5.39; N, 13.54; S, 5.40.

(*R*,*S*)-1-{[5-Benzylsulfanyl-4-ethyl-4*H*-1,2,4-triazol-3-yl]methyl}-1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indole (5h)

White solid, yield 54.8%, mp 107.7°C. R_f: 0.26 (M_1). FT-IR (ν_{max}, cm^{-1}): 3485 (O—H); 3221 (indole N—H); 2970, 2870 (C—H); 1600, 1504, 1475, 1456, 1438 (N—H, C═C, triazole C═N); 1383 (C—H); 1238 (C—S); 1078 (pyran C—O); 748, 696 (mono-substituted benzene); 680 (C—S). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.77 (t, 3H, —CH_2—$C\underline{H_3}$ at C_1, J = 7.2 Hz, J = 7.5 Hz); 1.06 (t, 3H, triazole —CH_2—$C\underline{H_3}$, J = 7.5 Hz, J = 7.2 Hz); 1.38 (t, 3H, —CH_2—$C\underline{H_3}$ at C_8, J = 7.5 Hz, J = 7.8 Hz); 2.03–2.31 (m, 2H, —$C\underline{H_2}$—CH_3 at C_1); 2.81–2.96 (m, 4H, —$C\underline{H_2}$—CH_3 at C_8 and —$C\underline{H_2}$— at C_1); 3.10–3.25 (q, 2H, triazole —$C\underline{H_2}$—CH_3); 3.62–3.7 (m, 2H, —$C\underline{H_2}$— at C_4); 3.99–4.13 (m, 2H, —$C\underline{H_2}$ at C_3); 4.39 (s, 2H, S—$C\underline{H_2}$); 6.99–7.37 (m, 8H, Ar—$\underline{H}$ and $C\underline{H}Cl_3$); 10.41 (s, 1H, indole N—$\underline{H}$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 7.64 (C-12); 13.81 (C-10); 14.89 (N—CH_2—CH_3); 18.48 (C-9); 22.52 (C-4); 24.31 (C-11); 30.30 (N—$\underline{C}H_2$—CH_3); 34.62 (C-13); 38.79 (S—CH_2); 60.99 (C-3); 76.34 (C-1); 107.86 (C-4a); 115.76 (C-6); 119.40 (C-5); 120.20 (C-7); 126.03 (C-5a); 127.14 (C-2′ and C-6′); 127.85 (C-4′); 128.73 (C-3′ and C-5′);

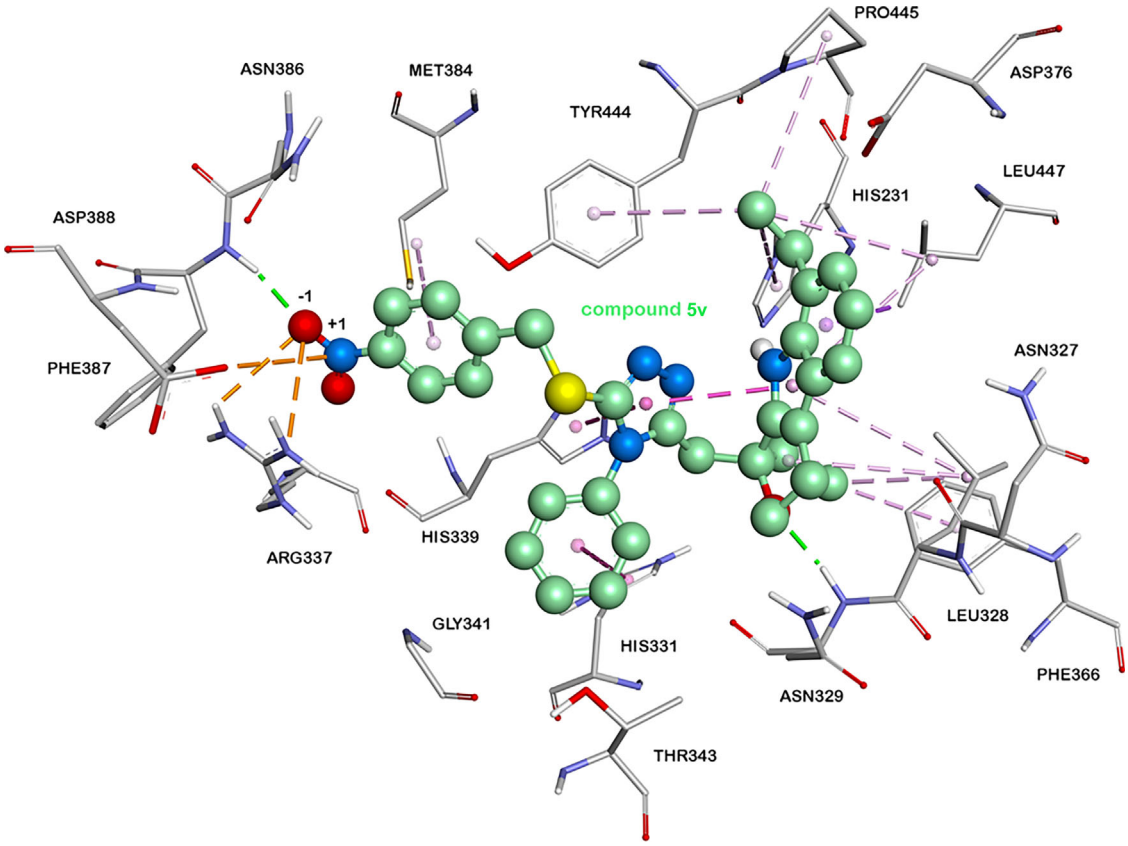


FIGURE 7 Molecular interactions of compound **5v**

128.95 (C-8); 134.65 (C-1′); 136.01 (C-1a); 136.73 (C-8a); 149.58 (triazole C═N); 152.65 (═C—S). $C_{27}H_{32}N_4OS.H_2O$ calcd. (%): C, 67.75; H, 7.16; N, 11.71; S, 6.70. Found (%): C, 67.73; H, 6.89; N, 11.91; S, 6.44.

(*R*,*S*)-1-{[5-(4-Fluorobenzyl)sulfanyl-4-ethyl-4*H*-1,2,4-triazole-3-yl]methyl}-1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indole (5i)

White solid, yield 63.8%, mp 90.0–92.0°C. R_f: 0.47 (M). FT-IR (ν_{max}, cm^{-1}): 3630 (O—H); 3246 (indole NH); 3022, 2970, 2931, 2850 (C—H); 1600, 1506, 1473, 1456 (N—H, C═C, triazole C═N); 1381, 1311 (C—H); 1211 (C—S); 1080 (pyran C—O); 790, 746 (mono-substituted benzene); 680 (C—S). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.77 (t, 3H, —CH_2—CH_3 at C_1, J = 7.5 Hz, J = 7.2 Hz); 1.10 (t, 3H, triazole —CH_2—CH_3, J = 7.2 Hz, J = 7.5 Hz); 1.37 (t, 3H, —CH_2—CH_3 at C_8, J = 7.5 Hz); 2.05–2.29 (m, 2H, —CH_2—CH_3 at C_1); 2.81–2.95 (m, 4H, —CH_2—CH_3 at C_8 and —CH_2— at C_1); 3.16–3.27 (q, 2H, triazole —CH_2—CH_3); 3.67–3.74 (m, 2H, —CH_2— at C_4); 3.99–4.13 (m, 2H, —CH_2 at C_3); 4.37 (s, 2H, S—CH_2); 6.94–7.37 (m, 8H, Ar—H and $CHCl_3$); 10.31 (s, 1H, indole N—H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 7.61 (C-12); 13.79 (C-10); 14.92 (N—CH_2—CH_3); 22.51 (C-9); 24.31 (C-4); 30.33 (C-11); 34.60 (N—CH_2—CH_3); 37.67 (C-13); 38.85 (S—CH_2); 60.99 (C-3); 76.29 (C-1); 107.91 (C-4a); 115.88 (C-6); 119.42 (C-5); 120.23 (C-7); 126.03 (C-5a); 127.08, 130.65 (C-2′ and C-6′); 130.76 (C-4′); 132.59, 132.61 (C-3′ and C-5′); 134.61 (C-8); 135.97 (C-1′); 149.41 (C-1a); 152.49 (C-8a); 161.49 (triazole C═N); 163.13 (═C—S). Analysis for $C_{27}H_{31}FN_4OS.H_2O$ calcd. (%): C, 65.33; H, 6.70; N, 11.28; S, 6.46. Found (%): C, 65.10; H, 6.58; N, 10.94; S, 6.30.

(*R*,*S*)-1-{[5-(2-Chlorobenzyl)sulfanyl-4-ethyl-4*H*-1,2,4-triazole-3-yl]methyl}-1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indole (5j)

White solid, yield 56.8%, mp 107.7–109.4°C. R_f: 0.47 (M_3). FT-IR (ν_{max}, cm^{-1}): 3532 (O—H); 3198 (indole N—H); 3087, 2961, 2920 (C—H); 1598, 1510, 1474, 1438 (N—H, C═C, triazole C═N); 1377, 1350 (C—H); 1238 (C—S); 1078 (pyran C—O); 743, 694 (*o*-substituted benzene); 665 (C—S). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.77 (t, 3H, —CH_2—CH_3 at C_1, J = 7.2 Hz, J = 7.5 Hz); 1.06 (t, 3H, triazole —CH_2—CH_3, J = 7.2 Hz); 1.38 (t, 3H, —CH_2—CH_3 at C_8, J = 7.5 Hz, J = 7.8 Hz); 2.08–2.30 (m, 2H, —CH_2—CH_3 at C_1); 2.81–2.96 (m, 4H, —CH_2 —CH_3 at C_8 and —CH_2— at C_1); 3.08–3.29 (q, 2H, triazole —CH_2—CH_3); 3.63–3.71 (m, 2H, —CH_2— at C_4); 3.99–4.13 (m, 2H, —CH_2 at C_3); 4.50–4.51 (dd, 2H, J = 15 Hz, J = 15 Hz, S—CH_2); 7.07–7.38 (m, 7H, Ar—H); 10.44 (s, 1H, indole N—H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 7.67 (C-12); 13.84 (C-10); 14.92 (N—CH_2—CH_3); 22.55 (C-9); 24.36 (C-4); 30.29 (C-11); 34.66 (N—CH_2—CH_3); 36.55 (C-13); 38.83 (S—CH_2); 61.01 (C-3); 76.29 (C-1); 107.86 (C-4a); 115.79 (C-6); 119.41 (C-5); 120.23 (C-7); 126.06 (C-5a); 127.00 (C-2′ and C-6′); 127.11 (C-4′); 129.38 (C-3′ and C-5′); 129.79 (C-8); 131.10 (C-1′); 134.16 (C-1a); 134.67 (C-8a); 149.33 (triazole C═N); 152.77 (═C—S). Analysis for

$C_{27}H_{31}ClN_4OS.1/2H_2O$ calcd. (%): C, 64.33; H, 6.40; N, 11.11; S, 6.36. Found (%): C, 64.71; H, 6.25; N, 11.00; S, 6.11.

(*R*,*S*)-1-{[5-(4-Dichlorobenzyl)sulfanyl-4-ethyl-4*H*-1,2,4-triazole-3-yl]methyl}-1,8-diethyl-tetrahydropyrano[3,4-*b*]indole (5k)

White solid, yield 51.5%, mp 184.9–185.8°C. R_f: 0.41 (M_3). FT-IR (ν_{max}, cm^{-1}): 3509 (O—H); 3185 (indole N—H); 3080, 2962, 2920, 2872, 2860 (C—H); 1609, 1491, 1471, 1435 (N—H, C═C, triazole C═N); 1411, 1377 (C—H); 1236 (C—S); 1078 (pyran C—O); 742, 723 (*p*-substituted benzene); 641 (C—S). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.77 (t, 3H, —CH_2—CH_3 at C_1, *J* = 7.2 Hz, *J* = 7.5 Hz); 1.10 (t, 3H, triazole —CH_2—CH_3, *J* = 7.2 Hz, *J* = 7.5 Hz); 1.37 (t, 3H,—CH_2—CH_3 at C_8, *J* = 7.5 Hz); 2.07–2.30 (m, 2H, —CH_2—CH_3 at C_1); 2.83–2.95 (m, 4H, —CH_2—CH_3 at C_8 and —CH_2— at C_1); 3.11–3.26 (q, 2H, triazole —CH_2—CH_3); 3.66–3.74 (m, 2H, —CH_2— at C_4); 3.99–4.12 (m, 2H, —CH_2 at C_3); 4.32–4.42 (dd, 2H, *J* = 12 Hz, *J* = 12 Hz, S—CH_2); 6.99–7.37 (m, 8H, Ar—H and $CHCl_3$); 10.32 (s, 1H, indole N—H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 7.60 (C-12); 13.79 (C-10); 14.93 (N—CH_2—CH_3); 22.51 (C-9); 24.31 (C-4); 30.33 (C-11); 34.60 (N—CH_2—CH_3); 37.61 (C-13); 38.86 (S—CH_2); 60.99 (C-3); 76.28 (C-1); 107.92 (C-4a); 115.78 (C-6); 119.42 (C-5); 120.24 (C-7); 126.03 (C-5a); 127.07 (C-2′ and C-6′); 128.85 (C-4′); 130.33 (C-3′ and C-5′); 133.73 (C-8); 134.60 (C-1′); 135.34 (C-1a); 135.94 (C-8a); 149.27 (triazole CN); 152.72 (═C—S). HR MS [(EI$^+$), *m/z* (%)]: 496.1864 ([M+2]$^+$, 2.98), 494.1882 ([M$^+$], 7.24), 228.1369 (100.0), 227.1293 (16.29), 142.0461 (2.92), 125.0150 (7.05), 57.0333 (4.04). Analysis for $C_{27}H_{31}ClN_4OS.1/2H_2O$ calcd. (%): C, 64.33; H, 6.40; N, 11.11; S, 6.36. Found (%): C, 64.10; H, 6.14; N, 10.89; S, 5.60.

(*R*,*S*)-1-{[5-(2,6-Dichlorobenzyl)sulfanyl-4-ethyl-4*H*-1,2,4-triazole-3-yl]methyl}-1,8-diethyl-tetrahydropyrano[3,4-*b*]indole (5l)

White solid, yield 42.2%, mp 106.0–107.0°C. R_f: 0.25 (M_3). FT-IR (ν_{max}, cm^{-1}): 3510 (O—H); 3213 (indole N—H); 3011, 2965, 2922, 2872 (C—H); 1579, 1562, 1510, 1473, 1437 (N—H, C═C, triazole C═N); 1375, 1348 (C—H); 1236 (C—S); 1076 (pyran C—O); 779, 762, 742 (substituted benzene); 677 (C—S). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.79 (t, 3H, —CH_2—CH_3 at C_1, *J* = 7.2 Hz, *J* = 7.5 Hz); 1.16 (t, 3H, triazole —CH_2—CH_3, *J* = 7.5 Hz, *J* = 7.2 Hz); 1.37 (t, 3H, —CH_2—CH_3 at C_8, *J* = 7.5 Hz, *J* = 7.8 Hz); 2.10–2.30 (m, 2H, —CH_2—CH_3 at C_1); 2.87–2.95 (m, 4H, —CH_2—CH_3 at C_8 and —CH_2— at C_1); 3.14–3.29 (q, 2H, triazole —CH_2—CH_3); 3.74–3.82 (m, 2H, —CH_2— at C_4); 4.03–4.13 (m, 2H, —CH_2 at C_3); 4.60–4.70 (dd, 2H, *J* = 12 Hz, *J* = 12 Hz, S—CH_2); 7.01–7.37 (m, 7H, Ar—H); 10.55 (s, 1H, indole N—H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 7.63 (C-12); 13.73 (C-10); 15.14 (N—CH_2—CH_3); 22.52 (C-9); 24.26 (C-4); 30.06 (C-11); 34.75 (N—CH_2—CH_3); 34.97 (C-13); 38.99 (S—CH_2); 60.99 (C-3); 76.29 (C-1); 107.66 (C-4a); 115.69 (C-6); 119.34 (C-5); 120.11 (C-7); 126.00 (C-5a); 127.18, 128.47 (C-2′ and C-6′); 128.54 (C-4′); 129.52, 130.25 (C-3′ and C-5′); 132.88 (C-8); 134.62 (C-1′); 135.86 (C-1a); 1366.14 (C-8a); 48.85 (triazole C═N); 152.91 (═C—S). Analysis for $C_{27}H_{30}Cl_2N_4OS.1/2H_2O$ calcd. (%): C, 60.22; H, 5.80; N, 10.40; S, 5.95. Found (%): C, 59.44; H, 5.86; N, 9.91; S, 5.43.

(*R*,*S*)-1-{[5-(4-Methylbenzyl)sulfanyl-4-ethyl-4*H*-1,2,4-triazole-3-yl]methyl}-1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indole (5m)

Pale yellow solid, yield 76.5%, mp 80.0–81.0°C. R_f: 0.96 (M_2). FT-IR (ν_{max}, cm^{-1}): 3500 (O—H); 3252 (indole N—H); 3051, 2962, 2931, 2872 (C—H); 1564, 1512, 1458, 1421 (N—H, CC, triazole C═N); 1379 (C—H); 1238 (C—S); 1076 (pyran C—O); 785, 742 (mono-substituted benzene); 694 (C—S). ^{1}H NMR (300 MHz, $CDCl_3$-*d*): δ 0.77 (t, 3H, —CH_2—CH_3 at C_1, *J* = 7.5 Hz, *J* = 7.2 Hz); 1.09 (t, 3H, triazole —CH_2—CH_3, *J* = 7.5 Hz, *J* = 7.2 Hz); 1.38 (t, 3H, —CH_2—CH_3 at C_8, *J* = 7.5 Hz, *J* = 7.8 Hz); 2.09–2.24 (m, 2H, —CH_2—CH_3 at C_1); 2.30 (s, 3H, Ar—CH_3); 2.82–2.96 (m, 4H, —CH_2 —CH_3 at C_8 and —CH_2— at C_1); 3.11–3.27 (q, 2H, triazole —CH_2—CH_3); 3.64–3.72 (m, 2H, —CH_2— at C_4); 3.99–4.14 (m, 2H, —CH_2 at C_3); 4.36 (s, 2H, S—CH_2); 6.99–7.37 (m, 8H, Ar—H ve $CHCl_3$); 10.42 (s, 1H, indole N—H). Analysis for $C_{28}H_{34}N_4OS.2H_2O$ calcd. (%): C, 65.85; H, 7.50; N, 10.97; S, 6.28. Found (%): C, 66.34; H, 7.07; N, 11.53; S, 6.39.

(*R*,*S*)-1-{[5-Benzylsulfanyl-4-phenyl-4*H*-1,2,4-triazole-3-yl]-methyl}-1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indole (5n)

White solid, yield 87.5%, mp 120.0–122.0°C. R_f: 0.14 (M_3). FT-IR (ν_{max}, cm^{-1}): 3219 (indole N—H); 3059, 2962, 2929, 2872 (C—H); 1597, 1496, 1452, 1444 (N—H, C═N, C═C); 1369, 1336 (C—H); 1236 (C—S); 1076 (pyran C—O); 765, 747 (mono-substituted benzene); 694 (C—S). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.71 (t, 3H, —CH_2—CH_3 at C_1, J = 7.5 Hz, *J* = 7.2 Hz); 1.41 (t, 3H, —CH_2—CH_3 at C_8, *J* = 7.5 Hz, *J* = 7.2 Hz); 2.10–2.30 (m, 2H, —CH_2—CH_3 at C_1); 2.65–2.98 (m, 4H, —CH_2 —CH_3 at C_8 and CH_2— at C_1); 3.09, 3.12 (d, 2H, —CH_2— at C_4); 3.68–3.93 (m, 4H, —CH_2 at C_3); 4.38–4.43 (dd, 2H, *J* = 12.6 Hz, *J* = 12.6 Hz, S—CH_2); 7.01–7.46 (m, 13H, Ar—H); 10.11 (s, 1H, indole N—H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 7.56 (C-12); 13.80 (C-10); 22.38 (C-9); 24.29 (C-4); 30.46 (C-11); 34.50 (C-13); 37.63 (S—CH_2); 60.74 (C-3); 76.36 (C-1); 108.08 (C-4a); 115.77 (C-6); 119.47 (C-5); 120.25 (C-7); 126.06 (C-5a); 127.06, 127.2, 127.80, 128.68 (C-2′ and C-6′); 129.13, 129.88 (C-4′); 130.14 (C-3′ and C-5′); 132.75 (C-8); 134.62 (C-1′); 135.87 (C-1a); 136.32 (C-8a); 151.31 (triazol C═N); 153.46 (═C—S). HR MS [(EI$^+$), *m/z* (%)]: 508.2284 ([M$^+$], 7.81), 281.0976 (5.50), 229.1413 (16.90), 228.1377 (100.0), 227.1300 (9.88), 91.0543 (8.00), 57.0335 (3.96). Analysis for $C_{31}H_{32}N_4OS.1/2H_2O$ calcd. (%): C, 71.92; H, 6.43; N, 10.82; S, 6.19. Found (%): C, 71.78; H, 6.43; N, 10.60; S, 5.94.

(*R*,*S*)-1-{[5-(4-Fluorobenzyl)sulfanyl-4-phenyl-4*H*-1,2,4-triazole-3-yl]methyl}-1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indole (5p)

White solid, yield 56.8%, mp 98.1°C. R_f: 0.34 (M_1). FT-IR (ν_{max}, cm^{-1}): 3446 (O—H); 3223 (indole N—H); 3016, 2962, 2950, 2853 (C—H); 1597, 1510, 1497, 1447, 1424 (N—H, C═C, triazole C═N); 1338 (C—H); 1223 (C—S); 1076 (pyran C—O); 748, 692 (*p*-substituted benzene); 664 (C—S). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.71 (t, 3H, —CH_2—CH_3 at C_1, *J* = 7.2 Hz); 1.40 (t, 3H, —CH_2—CH_3 at C_8, *J* = 7.5 Hz, *J* = 7.8 Hz); 2.01–2.31 (m, 2H, —CH_2—CH_3 at C_1); 2.59–3.15 (m, 6H, —CH_2 —CH_3 at C_8 and —CH_2 at C_1 and —CH_2— at C_4);

3.61–3.99 (m, 2H, —C$\underline{H_2}$ at C_3); 4.38 (s, 2H, S—C$\underline{H_2}$); 6.92–7.50 (m, 12H, Ar—H); 9.95 (s, 1H, indole N—H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 7.52 (C-12); 13.77 (C-10); 22.35 (C-9); 24.26 (C-4); 30.54 (C-11); 34.47 (C-13); 36.54 (S-CH_2); 60.71 (C-3); 76.33 (C-1); 108.17 (C-4a); 115.45 (C-6); 119.48 (C-5); 120.28 (C-7); 126.05 (C-5a); 126.98, 127.20, 129.91, 130.19 (C-2′ and C-6′); 130.78, 130.83 (C-4′); 132.18, 132.20 (C-3′ and C-5′); 134.59 (C-8); 135.75 (C-1′); 151.12 (C-1a); 153.50 (C-8a); 161.46 (triazol C═N); 163.10 (═C—S). Analysis for $C_{31}H_{31}FN_4OS.2H_2O$ calcd. (%): C, 66.17; H, 6.27; N, 9.96; S, 5.70. Found (%): C, 66.11; H, 6.23; N, 10.03; S, 5.47.

(*R*,*S*)-1-{[5-(4-Chlorobenzyl)sulfanyl-4-phenyl-4*H*-1,2,4-triazole-3-yl]methyl}-1,8-diethyl-1,3,4,9-tetrahidropirano[3,4-*b*]indole (5r)

White solid, yield 68.2%, mp 100.0–102.0°C. R_f: 0.37 (M_1). FT-IR (ν_{max}, cm^{-1}): 3510 (O—H); 3184 (indole N—H); 3015, 2963, 2930, 2872 (C—H); 1597, 1491, 1444, 1423 (N—H, C═C, triazole C═N); 1371, 1339 (C—H); 1238 (C—S); 1076 (pyran C—O); 745, 694 (*p*-substituted benzene). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.71 (t, 3H, —CH_2—C$\underline{H_3}$ at C_1, *J* = 7.2 Hz, *J* = 7.5 Hz); 1.40 (t, 3H, —CH_2—C$\underline{H_3}$ at C_8, *J* = 7.2 Hz, *J* = 7.8 Hz); 2.02–2.31 (m, 2H, —C$\underline{H_2}$—CH_3 at C_1); 2.76–3.14 (m, 6H, —C$\underline{H_2}$ —CH_3 at C_8 and —C$\underline{H_2}$ at C_1 and —C$\underline{H_2}$— at C_4); 3.59–3.95 (m, 2H, —C$\underline{H_2}$ at C_3); 4.36 (s, 2H, S—C$\underline{H_2}$); 7.00–7.48 (m, 13H, Ar—H and C$\underline{H}Cl_3$); 9.96 (s, 1H, indole N—H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 7.54 (C-12); 13.80 (C-10); 22.36 (C-9); 24.29 (C-4); 30.58 (C-11); 34.49 (C-13); 36.57 (S—CH_2); 60.73 (C-3); 76.34 (C-1); 108.20 (C-4a); 115.80 (C-6); 119.50 (C-5); 120.31 (C-7); 126.07 (C-5a); 126.99, 127.20, 128.79, 129.93 (C-2′ and C-6′); 130.22, 130.48 (C-4′); 132.66, 133.66 (C-3′ and C-5′); 134.60 (C-8); 135.04 (C-1′); 135.74 (C-1a); 150.98 (C-8a); 153.57 (triazol C═N); 163.10 (═C—S). Analysis for $C_{31}H_{31}ClN_4OS.CH_3CH_2OH$ calcd. (%): C, 67.27; H, 6.33; N, 9.51; S, 5.44. Found (%): C, 67.26; H, 5.74; N, 9.06; S, 4.88.

(*R*,*S*)-1-{[5-(2,6-Dichlorobenzyl)sulfanyl-4-phenyl-4*H*-1,2,4-triazole-3-yl]methyl}-1,8-diethyl-tetrahydropyrano[3,4-*b*]indole (5s)

White solid, yield 88.0%, mp 85.0–86.0°C. R_f: 0.71 (M_3). FT-IR (ν_{max}, cm^{-1}): 3400 (O—H), 3210 (indole N—H); 3072, 2963, 2927, 2872 (C—H); 1560, 1498, 1435 (N—H, C═N, C═C); 1368, 1336 (C—H); 1239 (C—S); 1076 (pyran C—O); 765, 747 (mono-substituted benzene); 693 (C—S). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.72 (t, 3H, —CH_2—C$\underline{H_3}$ at C_1, *J* = 7.2 Hz, *J* = 7.5 Hz); 1.22–1.27 (t, 3H, OH—CH_2—C$\underline{H_3}$, *J* = 6.9 Hz); 1.38–1.43 (t, 3H, —CH_2—C$\underline{H_3}$ at C_8, *J* = 7.5 Hz); 2.07–2.28 (m, 2H, —C$\underline{H_2}$—CH_3 at C_1); 2.65–3.19 (m, 6H, —C$\underline{H_2}$ —CH_3 at C_8, —C$\underline{H_2}$— at C_1 and OH—C$\underline{H_2}$—CH_3); 3.67–3.94 (m, 4H, —C$\underline{H_2}$— at C_4 and —C$\underline{H_2}$ at C_3); 4.62–4.70 (dd, 2H, *J* = 12 Hz, *J* = 12 Hz, S—C$\underline{H_2}$); 7.08–7.49 (m, 12H, Ar—H); 10.18 (s, 1H, indole N—H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 7.54 (C-12); 13.75 (C-10); 22.38 (C-9); 24.25 (C-4); 30.35 (C-11); 33.78 (C-13); 34.65 (S—CH_2); 60.75 (C-3); 76.37 (C-1); 107.98 (C-4a); 115.72 (C-6); 119.43 (C-5); 120.18 (C-7); 126.03 (C-5a); 127.10, 127.24, 128.40, 129.47 (C-2′ and C-6′); 129.89 (C-4′); 130.11, 132.25 (C-3′ and C-5′); 132.82 (C-8); 134.63 (C-1′); 135.93 (C-1a); 136.04 (C-8a); 150.56 (triazole C$\underline{N}$); 153.77 (═C—S). Analysis for $C_{31}H_{30}Cl_2N_4OS.CH_3CH_2OH.H_2O$ calcd. (%): C, 61.77; H, 5.97; N, 8.73; S, 5.00. Found (%): C, 61.52; H, 5.73; N, 8.60; S, 5.40.

(*R*,*S*)-1-{[5-(4-Methylbenzyl)sulfanyl-4-phenyl-4*H*-1,2,4-triazole-3-yl]methyl}-1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indole (5t)

White solid, yield 56.3%, mp 126.8°C. R_f: 0.49 (M_1). FT-IR (ν_{max}, cm^{-1}): 3426 (O—H); 3225 (indole N—H); 3035, 2965, 2934, 2874 (C—H); 1595, 1516, 1497, 1445, 1425 (N—H, C═C, triazole C═N); 1337 (C—H); 1240 (C—S); 1076 (pyran C—O); 746, 692 (*p*-substituted benzene); 665 (C—S). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.71 (t, 3H, —CH_2—C$\underline{H_3}$ at C_1, *J* = 7.2 Hz, *J* = 7.5 Hz); 1.24 (t, 3H, OH—CH_2—C$\underline{H_3}$, *J* = 6.9 Hz, *J* = 7.2 Hz); 1.41 (t, 3H, —CH_2—C$\underline{H_3}$ at C_8 *J* = 7.5 Hz); 2.04–2.28 (m, 2H, —C$\underline{H_2}$—CH_3 at C_1); 2.30 (s, 3H, Ar—CH_3); 2.62–3.14 (m, 6H, —C$\underline{H_2}$ —CH_3, at C_8 and —C$\underline{H_2}$ at C_1 and —C$\underline{H_2}$— at C_4); 3.63–3.93 (m, 2H, —C$\underline{H_2}$ at C_3); 4.33–4.42 (dd, 2H, *J* = 12 Hz, *J* = 12 Hz, S—C$\underline{H_2}$); 7.00–7.48 (m, 12H, Ar—H); 9.96 (s, 1H, indole N—H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 7.52 (C-12); 13.76 (C-10); 22.37 (C-9); 24.27 (C-4); 30.43 (C-11); 34.51 (C-13); 37.29 (S-CH_2); 60.73 (C-3); 76.35 (C-1); 108.03 (C-4a); 115.74 (C-6); 119.44 (C-5); 120.21 (C-7); 126.04 (C-5a); 127.05, 127.26, 129.02, 129.31 (C-2′ and C-6′); 129.84 (C-4′); 130.09, 132.78 (C-3′ and C-5′); 133.14 (C-8); 134.61 (C-1′); 135.89 (C-1a); 137.57 (C-8a); 151.44 (triazole C═N); 153.37 (═C—S). Analysis for $C_{32}H_{34}N_4OS.CH_3CH_2OH.H_2O$ calcd. (%): C, 69.59; H, 7.210; N, 9.55; S, 5.46. Found (%): C, 69.19; H, 6.75; N, 10.27; S, 5.57.

(*R*,*S*)-1-{[5-(4-Nitrobenzyl)sulfanyl-4-phenyl-4*H*-1,2,4-triazole-3-yl]methyl}-1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indole (5v)

Yellow solid, yield 48.5%, mp 88.0–91.0°C. R_f: 0.23 (M_1). FT-IR (ν_{max}, cm^{-1}): 3225 (indole N—H); 3025, 2963, 2930, 2872 (C—H); 1599, 1518, 1497, 1445 (N—H, C═C, triazole C═N); 1343, 1304 (C—H); 1238 (C—S); 1076 (pyran C—O); 745, 694 (*p*-substituted benzene). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.72 (t, 3H, —CH_2—C$\underline{H_3}$ at C_1 *J* = 7.2 Hz); 1.39 (t, 3H, —CH_2—C$\underline{H_3}$ at C_8 *J* = 7.5 Hz); 2.05–2.33 (m, 2H, —C$\underline{H_2}$—CH_3 at C_1); 2.75–3.27 (m, 6H, —C$\underline{H_2}$ —CH_3, at C_8 and —C$\underline{H_2}$ at C_1 and —C$\underline{H_2}$— at C_4); 3.61–3.93 (m, 2H, —C$\underline{H_2}$ at C_3); 4.43–4.53 (dd, 2H, *J* = 15 Hz, *J* = 15 Hz, S—C$\underline{H_2}$); 6.83–7.48 (m, 8H, Ar—H); 8.13 (d, 2H, Ar—NO_2, *ortho*, *J* = 8.7 Hz); 7.53 (d, 2H, Ar—NO_2, *para*, *J* = 8.7 Hz); 9.775 (s, 1H, indole N—H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 7.53 (C-12); 13.80 (C-10); 22.31 (C-9); 24.28 (C-4); 30.72 (C-11); 34.42 (C-13); 35.85 (S—CH_2); 60.69 (C-3); 76.30 (C-1); 108.36 (C-4a); 115.84 (C-6); 119.55 (C-5); 120.40 (C-7); 123.79 (C-5a); 126.05, 126.86, 127.10, 130.03 (C-2′ and C-6′); 130.37 (C-4′); 132.46 (C-3′ and C-5′); 134.54 (C-8); 135.51 (C-1′); 144.29 (C-1a); 147.36 (C-8a); 150.45 (triazole C$\underline{N}$); 153.80 (═C—S). Analysis for $C_{31}H_{31}N_5O_3S.H_2O$ calcd. (%): C, 65.13; H, 5.82; N, 12.25; S, 5.61. Found (%): C, 65.91; H, 5.76; N, 12.23; S, 5.38.

4.2 | Biological assays

4.2.1 | Cell culture and cell viability assay

Cell lines were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). Monkey kidney epithelial cell line VERO, human ovarian cancer cell line SKOV3, human breast cancer cell line MCF7, human hepatocellular cancer cell line HEPG2,

human prostate cancer cell line PC3 cells were maintained in Dulbecco's modified Eagle's medium (DMEM, Invitrogen) supplemented with 10% FBS, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin and kept in a humidified atmosphere at 37°C incubator with 5% CO_2 in air. To determine the cell viability cells were plated onto 96-well plates (1×10^4 cells/well). The cells were treated with different concentrations (0, 10, 50, 100, 200, and 500 µM) of synthesized compound (**5a–v**) derivatives from etodolac and incubated for 24 h. After the incubation cells were washed with PBS and added to 100 µL DMEM. A total of 10 µL of the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Vybrant, Invitrogen) labeling reagent was added to each well and incubated for 4 h in humidified atmosphere at 37°C incubator with 5% CO_2 in air. After the incubation, 100 µL of the SDS buffer was added into each well for solubilization of formazan precipitate. Then absorbance was measured by microplate reader at 570 nm and was carried out in triplicate of each assay.[18]

4.2.2 | Cell survival assays

In order to investigate the effects of compounds on cell survival of SKOV3, HEPG2, MCF7, VERO cells, we treated cells with selected etodolac derivatives of **5d** and **5h** at a final concentration of 10 µM in growth medium (without antibiotics) for 24 h. After the incubation, methylene blue staining was used for cell survivals. The cells were extracted with 1% SDS in PBS solution and stained with methylene blue solutions. The absorbance was measured at 600 nm with microplate reader.[19]

4.2.3 | Determination of apoptosis

SKOV3 ovarian cancer cells were treated with 10 µM concentration of etodolac and derivatives **5d** and **5h** at different times (0, 6, 12, 24 h) in 12-well plates at density of 2×10^4 cells/well in triplicate. Cell growing was imaged using a Zeiss Axio inverted microscope (10×) imaging system. Methylene blue staining was used for quantification for cell growing.

Apoptosis in the SKOV3 ovarian cancer cell line was measured using Acridine orange and ethidium bromide (AO/EB). After 24 h incubation with 10 µM concentrations of etodolac and derivatives in 12-well plates at density of 2×10^5 cells/well in triplicate. Cells were then stained for 1 µg/mL AO/EB solutions and the fluorescent intensities specific of followed by microscope (Zeiss). Living cells grouped with green intensity and apoptotic cells grouped with red intensity.

4.2.4 | Measurement of caspase-3 activity

SKOV3 cells were incubated with 10 µM concentrations of **5d** and **5h** at different times (0, 6, 12, 24 h) in 12-well plates. After the incubations, cells were harvested with cell lysis buffer. Caspase-3 activity assay was performed with caspase-3 activity assay kit according to the manufacturer's instructions (Invitrogen).[20]

Similarly, PC3 cells were incubated with 10 µM concentrations of **5k**, **5s**, and **5v** at different times (0, 12, 24, 48 h) in 12-well plates. After the incubation, cells were harvested with cell lysis buffer. Caspase-3 activity assay was performed with caspase-3 activity assay kit according to the manufacturer's instructions (Invitrogen).

4.2.5 | Western blot analysis

SKOV3 cells were homogenized in cell lysis buffer and protein concentrations were determined by Bradford reagent (Biorad). Proteins were seperated by SDS-PAGE and transferred onto PVDF membrane (Santa Cruz). Membrane was blocked with 3% BSA at room temperature and was incubated with primary antibodies. The following primary antibodies at 4°C overnight: anti-Caspase-3 (1:500), anti-Caspase-8 (1:500), anti-Caspase-9 (1:500) from Santa Cruz. Then incubated with a primary antibody, membrane was incubated with a horseradish peroxidase-conjugated secondary antibodies for room temperature. Bands were visualized by the chemiluminescence Western blotting detection reagent in imaging system. β-Actin protein levels were used as a control to verify equal protein loading.

4.3 | Molecular docking

The preparation of the protein human MetAP2 as well as compounds was performed in BIOVIA Discovery Studio 4.5. The X-ray crystal structure of the receptor was downloaded from PDB database-accession code: 1QZY, resolution: 1.60 A (http://www.rcsb.org/pdb/). The protein structure was cleaned from inhibitor, water molecules, and non-interacting ions. All hydrogens were added and protein was minimized using "Clean Geometry" toolkit, then for more complete optimization it was submitted to "Prepare Macromolecule" protocol. Missing hydrogen atoms were added based on the protonation state of the titratable residues at a pH of 7.4. The CHARMm force field was assigned.

Compounds were drawn using "small molecules" toolkit and minimized using the same force field. After preparation docking was performed in AutoDock 4.2 programme (http://autodock.scripps.edu). All the compounds were set to be flexible, and protein is set to be rigid. AutoDock adds a free-energy scoring function created from a linear regression analysis, the AMBER force field, and a large set of diverse protein-ligand complexes with known inhibition constants. For the center of binding pocked (grid box) HIS231 was used. The size grid box was set to be 60, 60, and 60. The searching algorithms include Lamarckian genetic algorithm.

For visualization of non-bonded interactions BIOVIA, and creating the pictures Discovery Studio 4.5 was used.

ACKNOWLEDGMENTS

This study has been financially supported by the Scientific Research Project Commission of Marmara University (Project number: SAG-C-DRP-041213-0451). The authors are grateful to Jürgen Gross from the

Institute of Organic Chemistry, University of Heidelberg, for his generous help in obtaining HR-EI mass spectra of the synthesized compounds.

CONFLICT OF INTEREST

The authors have declared no conflicts of interest.

ORCID

Ş. Güniz Küçükgüzel http://orcid.org/0000-0001-9405-8905

REFERENCES

[1] Ş. G. Küçükgüzel, P. Süzgün, *Eur. J. Med. Chem*. **2015**, *97*, 830.
[2] Y.-P. Hou, J. Sun, Z.-H. Pang, P.-C. Lv, D.-D. Li, L. Yan, H.-J. Zhang, E. X. Zheng, J. Zhao, H.-L. Zhu, *Bioorg. Med. Chem*. **2011**, *19*, 5948.
[3] S.-Q. Yin, R. A. Jr. Galemmo, Z.-P. Liu, *Curr. Med. Chem*. **2012**, *19*, 1021.
[4] T. Garrabrant, R. W. Tuman, D. Ludovici, R. Tominovich, R. L. Simoneaux, R. A. Galemmo, Jr., D. L. Johnson, *Angiogenesis* **2004**, *7*, 91.
[5] J. P. Jr. Marino, P. W. Fisher, G. A. Hofmann, R. B. Kirkpatrick, C. A. Janson, R. K. Johnson, C. Ma, M. Mattern, T. D. Meek, M. D. Ryan, C. Schulz, W. W. Smith, D.G Tew, T. A. Jr. Tomazek, D. F Veber, W. C. Xiong, Y. Yamamoto, K. Yamashita, G. Yang, S. K. Thompson, *J. Med. Chem*. **2007**, *50*, 3777.
[6] M. Kobayashi, S. Nakamura, K. Shibata, N. Sahara, K. Shigeno, K. Shinjo, K. Naito, K. Ohnishi, *Eur. J. Haematol*. **2005**, *75*(3), 212.
[7] S. Murata, M. Adachi, M. Kioi, S. Torigoe, K. Ijichi, Y. Hasegawa, T. Ogawa, M. K. Bhayani, S. Y. Lai, K. Mitsudo, I. Tohnai, *Anticancer Res*. **2011**, *31*(9), 2893.
[8] P. Çıkla, D. Özsavcı, Ö. Bingöl-Özakpınar, A. Şener, Ö. Çevik, S. Özbaş-Turan, J. Akbuğa, F. Şahin, Ş. G. Küçükgüzel, *Arch. Pharm*. **2013**, *346*, 367.
[9] P. Çıkla, P. Arora, A. Basu, T. T. Talele, N. Kaushik-Basu, Ş. G. Küçükgüzel, *Marmara Pharm. J*. **2013**, *17*, 138.
[10] P. Çıkla-Süzgün, N. Kaushik-Basu, A. Basu, P. Arora, T.T. Talele, İ. Durmaz, R. Cetin-Atalay, Ş. G. Kucukguzel, *J. Enzyme Inhib. Med. Chem*. **2015**, *30*(5), 778.
[11] B. Kummari, N. Polkam, P. Ramesh, H. Anantaraju, P. Yogeeswari, J. S. Anireddy, S. D. Guggilapud, B. N. Babud, *RSC Adv*. **2017**, *7*, 23680.
[12] İ. Küçükgüzel, Ş. G. Küçükgüzel, S. Rollas, G. Ötük-Sanış, O. Özdemir, İ. Bayrak, T. Altuğ, J. P. Stables, *Il Farmaco*. **2004**, *59*(11), 893.
[13] İ. Küçükgüzel, Ş. G. Küçükgüzel, S. Rollas, M. Kiraz, *Bioorg. Med. Chem. Lett*. **2001**, *11*(13), 1703.
[14] K. Zamani, K. Faghihi, T. Tofighi, M. R. Shariatzadeh, *Turk. J. Chem*. **2004**, *28*, 95.
[15] L. Popiolek, U. Kosikowska, L. Mazur, M. Dobosz, A. Malm, *Med. Chem. Res*. **2013**, *22*, 3134.
[16] G. Tu, Y. Yan, X. Chen, Q. Lv, J Wang, S. Li, *Drug Discov. Ther*. **2013**, *7*(2), 58.
[17] N. Ulusoy, G. Çapan, N. Ergenç, A. C. Ekinci, A. Vidin, *Acta Pharm. Turcica* **1998**, *1*, 5.
[18] S. H. Baek, Y. O. Kim, J. S. Kwag, K. E. Choi, W. Y. Jung, D. S. Han, *Arch. Pharm. Res*. **1998**, *21*, 353.
[19] A. H. Sheila, S. K. Frank, *TCA Manual/Tissue Culture Association* **1975**, *1*(2), 103.
[20] H. Jaeschke, M. A. Fisher, J. A. Lawson, C. A. Simmons, A. Farhood, D. A. Jones, *J. Immunol*. **1998**, *160*(7), 3480.
[21] H. Towbin, K. W. Bair, J. A. DeCaprio, M. J. Eck, S. Kim, F. R. Kinder, A. Morollo, D. R. Mueller, P. Schindler, H. K. Song, J. van Oostrum, R. W. Versace, H. Voshol, J. Wood, S. Zabludoff, P. E. Phillips, *J. Biol. Chem*. **2003**, *278*(52), 52964.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Çoruh I, Çevik Ö, Yelekçi K, Djikic T, Küçükgüzel ŞG. Synthesis, anticancer activity, and molecular modeling of etodolac-thioether derivatives as potent methionine aminopeptidase (type II) inhibitors. *Arch Pharm Chem Life Sci*. 2018;351:e1700195. https://doi.org/10.1002/ardp.201700195