Ministry of higher Education
and scientific research
Babylon University
College of Science for Girls
Department of chemistry

Synthesis of new thiourea derivatives and study of its activity as anticancer agents molecular docking study

A dissertation submitted to the College of Science for women to Babylon university in partial fulfillment to the requirement of bachelor degree in chemistry

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بِسْمِ اللَّـهِ الرَّحْمَـٰنِ الرَّحِيمِ

يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ ۚ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ ﴿١١﴾

صدق الله العلي العظيم

المجادلة : الأية (11)

الاهداء والتقدير

وصلت رحلتي الجامعية إلى نهايتها بعد تعب ومشقَّة..

وها أنا ذا أختم بحث تخرُّجي بكل همَّة ونشاط،

وأمتنُّ لكل من كان له فضل في مسيرتي،

وساعدني ولو باليسير،

الأبوين، والأهل، والأصدقاء، والأساتذة المُبجَّلين..

أُهديكم بحث تخرُّجي.....

كما أقدم كامل تقديري وشكري للدكتور الذي اشرف على هذا البحث (الدكتور الفاضل احمد حسن شنتاف) , والذي اسأل الله به ان يضيف قيمة الى هذا العلم

Introduction:

Thiourea or thiocarbamide is an organosulfur compound with the formula SC(NH2)2. The term “thioureas” is used for the class of compounds with the general formula (R1R2N)(R3R4N)C═S [1]. None of any other class of organic compounds has such a wide diversity and multiple applications as thioureas have. Indeed, in almost every branch of chemistry, thioureas have played their exceptional role [2]. Commercially, industrially, and academically, they are of huge importance. On commercial scale, thioureas are frequently used in photographic film, plastics, dyes, elastomers, and textiles [3,4]. Several derivatives of thioureas are used as pharmaceuticals, preservatives, rodenticides, and insecticides [5,6,7,8]. Thioureas have valuable uses in organic synthesis and are used as intermediates in several organic synthetic reactions [9]

These have structural resemblance to ureas, except that the oxygen atom of ureas is replaced by a sulfur atom; the chemical properties of urea and thiourea are quite different from each other.

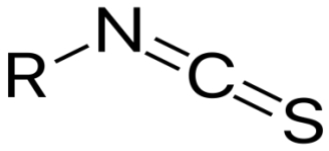
Thioureas Ureas

1 2

Several derivatives of thioureas are used as pharmaceuticals, preservatives, rodenticides, and insecticides [5,6,7,8]. Thioureas have valuable uses in organic synthesis and are used as intermediates in several organic synthetic reactions [9]. Another very important, diverse, and effective area of thiourea applications is their biological activities. Again, none other class of organic compounds has as much biological activities as thioureas. These have been reported to have antifungal, antiviral, antibacterial, catalytic, antitubercular, analgesic, insecticidal, anti-inflammatory, herbicidal, anticonvulsant, anti-cancer, anti-thyroid, anthelmintic, and anti-phenoloxidase activities [9,10,11,12,13,14,15,16]. They also act as rodenticide and have anti-HIV, antiviral, high density lipoprotein (HDL)-elevating, antibacterial, analgesic, antidiabetic, anti-hypertensive, anti-epileptic, anticancer, DNA-binding, hypnotic, and anesthetic properties [9,10,11,12,13,14,15,16].

Isothiocyanate:

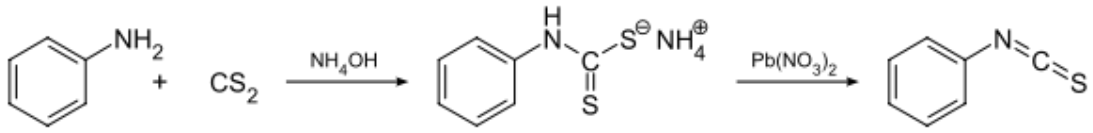
is the chemical group –N=C=S, formed by substituting the oxygen in the isocyanate group with a sulfur. Many natural isothiocyanates from plants are produced by enzymatic conversion of metabolites called glucosinolates. These natural isothiocyanates, such as allyl isothiocyanate, are also known as mustard oils. An artificial isothiocyanate, phenyl isothiocyanate, is used for amino acid sequencing in the Edman degradation.



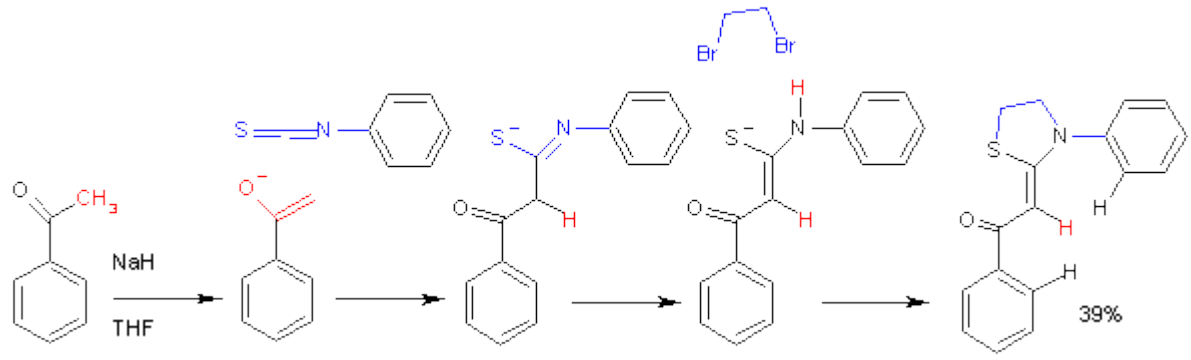
Cruciferous vegetables, such as bok choy, broccoli, cabbage, cauliflower, kale, and others, are rich sources of glucosinolate precursors of isothiocyanates.[1] Although there has been some basic research on how isothiocyanates might exert biological effects in vivo, there is no high-quality evidence to date for its efficacy against human diseases.

Synthesis and reactions:

Isothiocyanates are generally prepared by the reaction of a primary amine (e.g. aniline) and carbon disulfide in aqueous ammonia.[1] This combination results in precipitation of the solid ammonium dithiocarbamate salt, which is then treated with lead nitrate to yield the corresponding isothiocyanate.[3] Another method relies on a tosyl chloride mediated decomposition of dithiocarbamate salts that are generated in the first step above.[4]

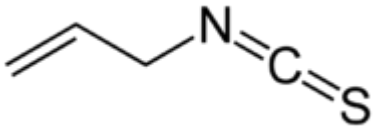


Isothiocyanates may also be accessed via the thermally-induced fragmentation reactions of 1,4,2-oxathiazoles.[5] This synthetic methodology has been applied to a polymer-supported synthesis of isothiocyanates



Allyl isothiocyanate:

Allyl isothiocyanate (AITC) is the organosulfur compound with the formula CH2CHCH2NCS. This colorless oil is responsible for the pungent taste of mustard, radish, horseradish, and wasabi. This pungency and the lachrymatory effect of AITC are mediated through the TRPA1 and TRPV1 ion channels.[1][2][3] It is slightly soluble in water, but more soluble in most organic solvents.[4]



Allyl isothiocyanate (AITC) is produced by the hydrolysis of its glucosinolate precursor, sinigrin, which can be found in many commonly consumed cruciferous vegetables and is particularly abundant in mustard, horseradish and wasabi where it is responsible for the pungent taste1. Because of the pungent flavor, AITC is also used as a food additive known as mustard oil. AITC has been shown to possess a broad spectrum of anticancer activities in both cultured cancer cell lines and animal tumor models1,2,3. The mode of action for the chemopreventive activity of AITC is attributed primarily to the detoxification of carcinogens through activation of nuclear factor erythroid-related factor (Nrf2) AITC also inhibited the growth of various human cancer cell lines such as colorectal

carcinoma, lung cancer, leukemia, breast adenocarcinoma, bladder cancer, neuroblastoma

Allyl Isothiocyanate Interactions:

1- Interaction of oxidized glutathione with allyl isothiocyanate:

Isothiocyanates formed from glucosinolates in Brassica species have a strong affinity for amino acids and proteins, especially for their thiol, sulphide and terminal amino groups. To investigate the action of isothiocyanate on cystine residues in proteins and peptides, the present study on the interaction between allyl isothiocyanate and oxidized glutathione under physiological conditions was undertaken. Oxidized glutathione was oxidatively cleaved to some modified glutathiones by the attack of allyl isothiocyanate on its disulphide bond. Two new modified products were isolated from the reaction mixture by gel chromatography and HPLC, and their structures were determined by NMR and mass spectral analyses as glutathionyl N-allyldithiocarbaramate and its allyl thiohydantoin derivative. The formation of these products indicated oxidative cleavage of the disulphide bond in the cystine residue; the electrophilic attack of the isothiocyanate on the sulphur atom must cleave the disulphide bond oxidatively to dithiocarbamate and sulphenate, as in the case of cystine.

2- The interaction between β-Lactoglobulin and allyl-isothiocyanate:

Beta-Lactoglobulin (β-Lg) is a globular milk protein and a major component of whey (~50% dry weight). β-Lg binds strongly to various hydrophobic ligands and may act as a transporter for these molecules. Monomeric β-Lg has two disulfide bonds and one free thiol group that have important roles in the formation of the tertiary structures of β-Lg. As a result, β-Lg reacts to pH changes in a very specific manner. In this study, isothermal titration calorimetry (ITC) and circular dichroism (CD) were used to investigate the interaction between β-Lg and allyl isothiocyanate (AITC) at different pH values 3.0, 6.5 and 8.5. The interaction was characterized using total reflectance Fourier-transform infrared spectroscopy and molecular docking. The final thermograms from ITC were fitted to three sites of a sequential binding model (Koshland model), showing the high affinity between

β-Lg and AITC with binding constants of 104–105 M−1. ITC and CD results showed that AITC binding at different pH changes the secondary structure of β-Lg. FTIR results showed the binding of AITC with strong isothiocyanate peaks between 1900 and 2150 cm−1. Three different AITC-binding sites to β-Lg were confirmed using molecular docking. The results showed that the characteristic properties of the interaction between β-Lg and AITC can be used to predict the nanotransporter capacity of β-Lg for bioactive materials at different pH values.

3- Allyl isothiocyanate (AITC) inhibits pregnane X receptor (PXR) and constitutive androstane receptor (CAR) activation and protects against acetaminophen- and amiodarone-induced cytotoxicity:

Antagonizing the action of the pregnane X receptor (PXR) may have important clinical implications for preventing inducer-drug interactions and improving therapeutic efficacy. We identified a widely distributed isothiocyanate, allyl isothiocyanate (AITC), which acts as an effective antagonist of the nuclear receptor pregnane X receptor (PXR, NR1I2) and constitutive androstane receptor (CAR, NR1I3). HepG2 cells were used to assay reporter function, mRNA levels, and protein expression. Catalytic activities of the PXR and CAR target genes, CYP3A4 and CYP2B6, respectively, were also assessed in differentiated HepaRG cells. Protective effects of AITC on rifampin-induced cytotoxicity were observed, and transient transfection assays showed that AITC was able to effectively attenuate the agonist effects of rifampin and CITCO on human PXR and CAR activity, respectively. AITC-mediated reduction in the transcriptional activity of PXR and CAR correlated well with the suppression of CYP3A4 and CYP2B6 expression in HepG2 cells, which reflected the reduced catalytic activities of both of these genes following AITC treatment in differentiated HepaRG cells. Furthermore, AITC disrupts the co-regulations of PXR with several important co-regulators. Furthermore, the antagonist effect of AITC against PXR was found in HepaRG cells upon addition of acetaminophen (APAP) and amiodarone, indicating that AITC protects cells from drug-induced cytotoxicity. Taken together, our results show that AITC inhibits the transactivation effects of PXR and CAR and reduces the expression and function of CYP3A4 and CYP2B6. Additionally, AITC

reversed the cytotoxic effects of APAP and amiodarone induced by PXR ligand. Results from this study suggest that AITC could be a powerful agent for reducing potentially dangerous interactions between transcriptional inducers of CYP enzymes and therapeutic drugs.

Allyl isothiocyanate as a cancer chemopreventive phytochemical:

Allyl isothiocyanate (AITC), also known as mustard oil, is one of the most common naturally occurring isothiocyanates (ITCs) [1, 2]. ITCs occur primarily in cruciferous vegetables, many of which show significant cancer chemopreventive activities, and therefore are widely suspected to account in part for the cancer preventive activities of these vegetables in humans [3]. Sulforaphane is perhaps the most widely known crucifer-derived cancer chemopreventive ITC [4]. ITCs are synthesized and stored in cruciferous vegetables as glucosinolates (β-thioglucoside N-hydroxysulfate), which are believed to be chemically and biologically inert, and formed from the latter when plant tissues are damaged. The conversion is catalyzed by myrosinase (a thioglucoside glucohydrolase), first forming thiohydroximate-O-sulfonates, which rapidly and spontaneously rearrange to give rise to ITCs. Myrosinase coexists with but is physically separated from glucosinolates under normal conditions. Conversion (up to 40%) to ITCs of ingested glucosinolates that escape plant myrosinase may take place in vivo, as the intestinal microflora of both humans and animals also possess myrosinase activity

Allyl isothiocyanate (AITC), which occurs in many common cruciferous vegetables, is widely and often frequently consumed by humans. Besides antimicrobial activity against a wide spectrum of pathogens, it showed anticancer activity in both cultured cancer cells and animal models, although the underlining mechanisms remain largely undefined. Bioavailability of AITC is extremely high, as nearly 90% of orally administered AITC is absorbed. AITC absorbed in vivo is metabolized mainly through the mercapturic acid pathway and excreted in urine. Available data suggest that urinary concentrations of AITC equivalent are at least ten times higher than in the plasma, and tissue levels of AITC equivalent in the urinary bladder were 14-79 times higher than in other organs after oral

AITC administration to rats. These findings suggest that AITC may be most effective in the bladder as a cancer chemopreventive compound. AITC at high-dose levels also exhibit a low degree of cytotoxicity and genotoxicity in animal studies, but such adverse effects are unlikely in humans exposed to dietary levels of AITC. Overall, AITC exhibits many desirable attributes of a cancer chemopreventive agent, and further studies are warranted in order to elucidate its mechanism of action and to assess its protective activity in humans.

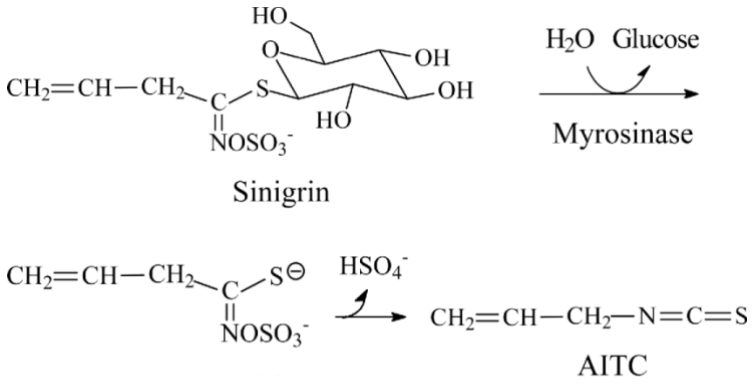
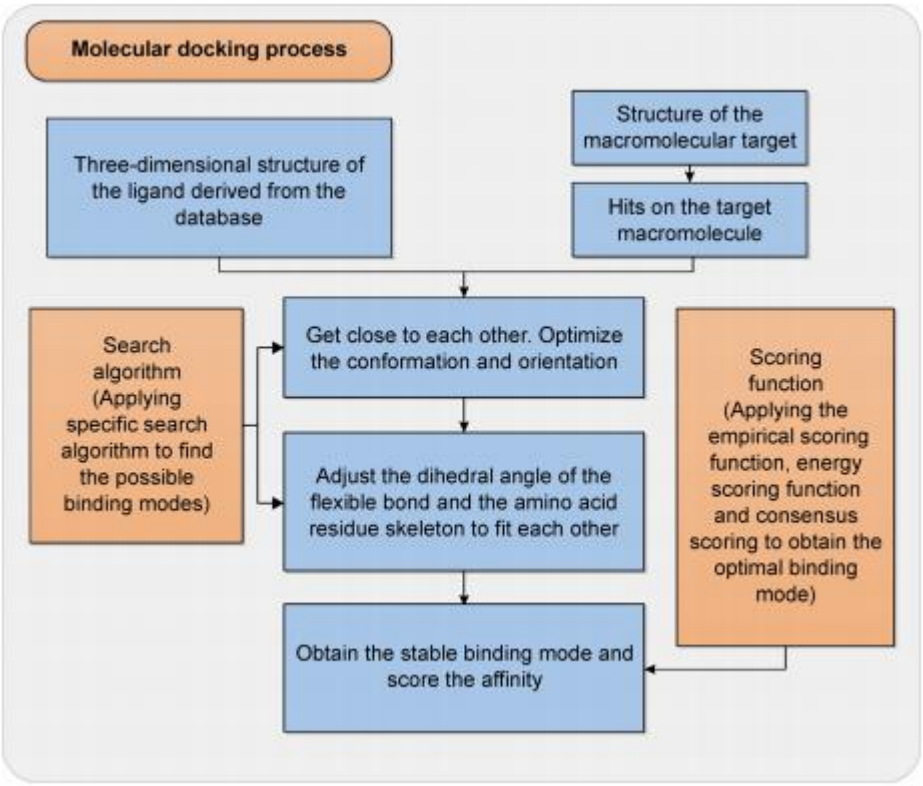


Figure 1.
Myrosinase-catalyzed conversion from sinigrin to AITC

Molecular Docking

Molecular docking is a kind of bioinformatics modeling which involves simulates the ligands and proteins interaction and predicts the binding mode and affinity between ligands and proteins, fit together to give the stable adduct. The molecular docking has played a crucial role in drug design research field. The molecular docking software can help the researchers to predict the optimal conformation and orientation according to complementarity and pre-organization with specific algorithm, followed by applying a scoring function to predict the binding affinity and analyze the interactive mode.



Molecular docking process

Applications of Molecular Docking:

Molecular docking could be used to prove the feasibility of any biochemical reaction as it is accomplished before experimental part of any study. There are some field, where molecular docking has widespread applications in understanding the interaction between small molecules (ligands) and protein targets (may be an enzyme) may predict the activation or drug's binding properties to nucleic acid. This information explain the correlation between drug's molecular structure and its cytotoxicity.

Method :

A mixture of aniline derivatives (0.01 mol), Allyl isothiocyanate (0.01 mol, 1 g) and ethanol (30 ml) was refluxed for (10 h). It was then cooled and kept overnight in refrigerator. The precipitated was filtered, washed with ethanol, dried and recrystallized from ethanol.

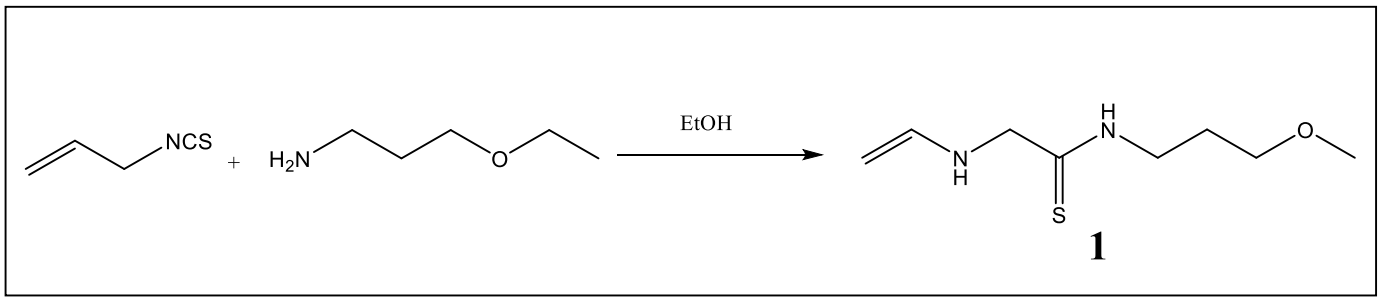
Results and Discussion:

Introduction in the synthesis of compounds:

The physicals and chemicals properties of the synthesized compounds were studies and identification by use spectrophotometric methods (Infrared spectrophotometer (FTIR).

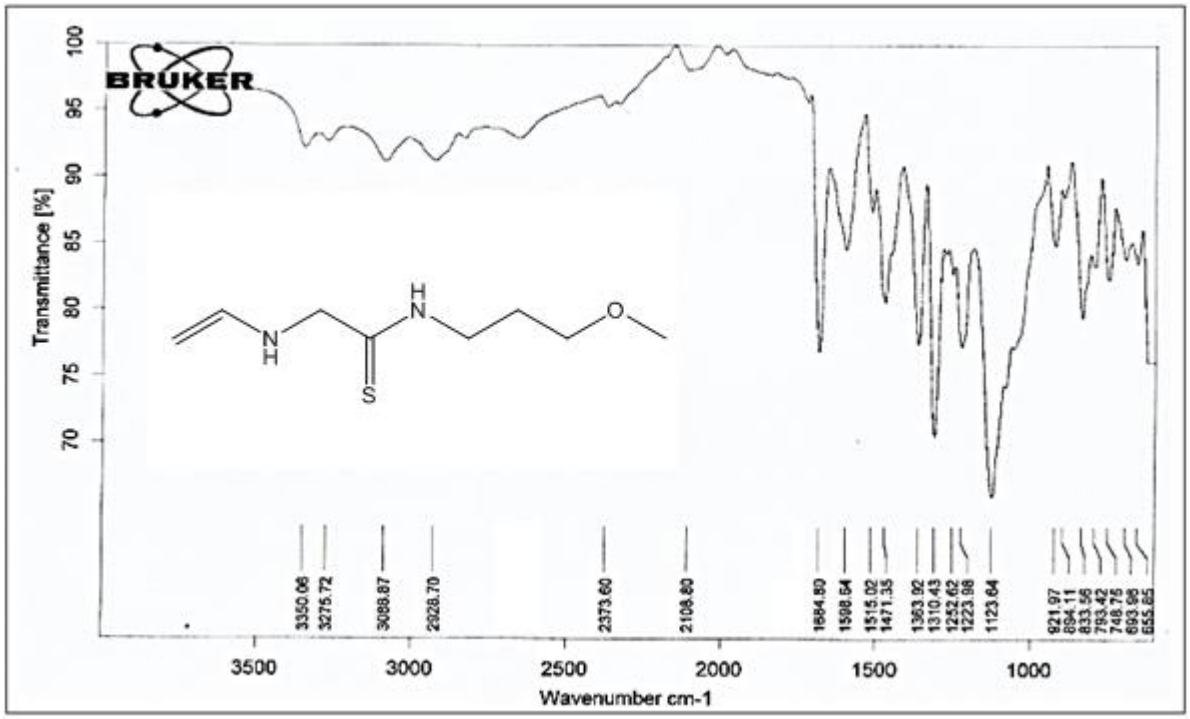
Synthesis and Identification of N-(3-methoxypropyl)-2-(vinylamino) ethanethioamide [1]

Compound [1] was synthesized according to the following equation:



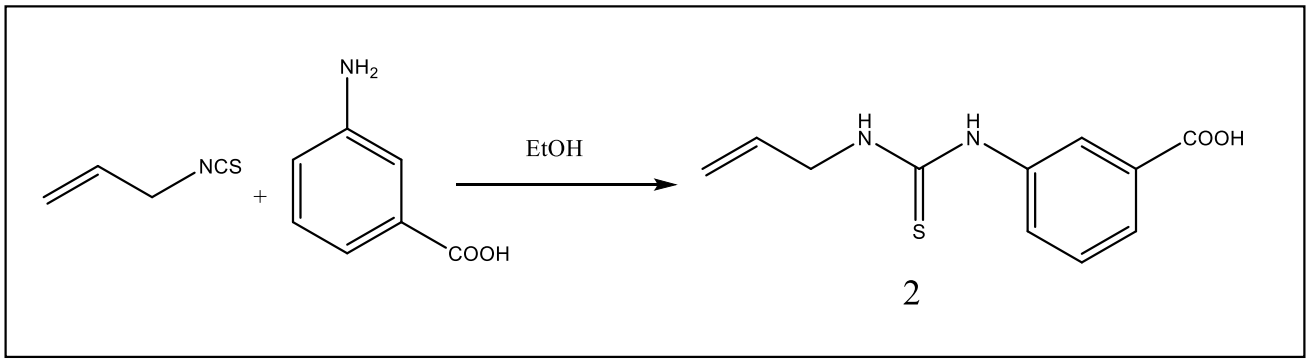
molecular formula: $C_8H_{16}N_2OS$, Molecular Weight: 188.29, Color: Dark brown, yield: 90%.

The FTIR spectrum of compound [1] was exhibited appearance absorption bands of str N-H of amines group at (3350, 3275) cm^{-1}, str =C-H at (3088), str C=C at (1684) cm^{-1} and appearance absorption bands of str C=S at 1310 cm^{-1}.



Synthesis and Identification of 3-(3-allylureido)benzoic acid [2]

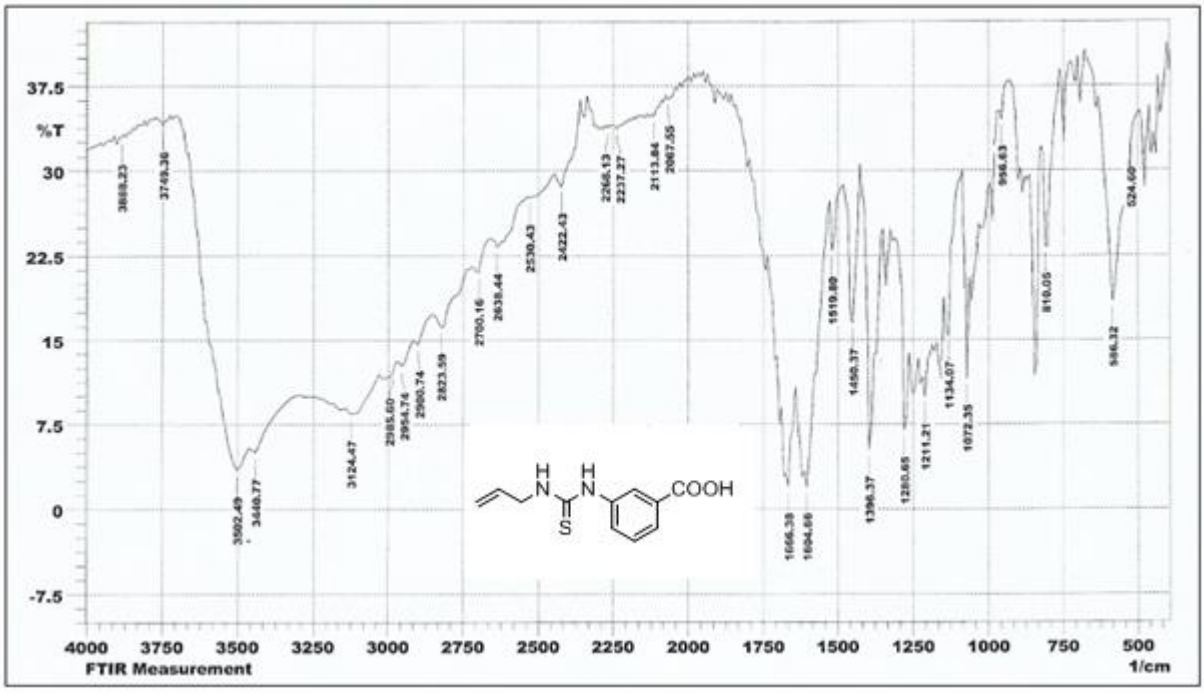
Compound [1] was synthesized according to the following equation:



2

molecular formula: C11H12N2O2S, Molecular Weight: 236.29, Color: yellow, yield: 75%.

The FTIR spectrum of compound [2] was exhibited appearance absorption bands of str OH group at 3502 cm^{-1}, str N-H of amines group at (3440) cm^{-1}, str =C-H at (3124), str C=C at (1664) cm^{-1} and appearance absorption bands of str C=S at 1366 cm^{-1}.

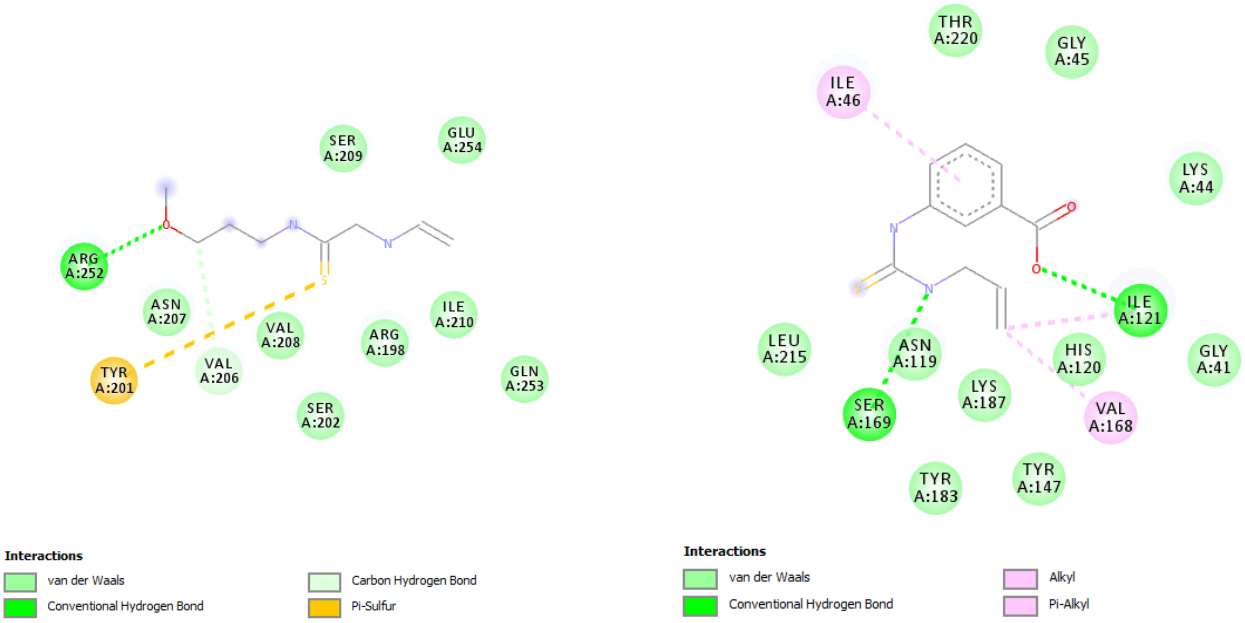


The study of COX-2 inhibitory effect of synthesized compounds *in silico* study:

Molecular docking was used in order to predict the molecular orientation of the synthesized compounds into the 11β-hydroxysteroid dehydrogenase type 1active site and to explain the biological activities results. In addition to the docking score

Molecular docking results of the synthesized compounds

No	compound	Binding energy (Kcal/mol)
1.	1	-3.38
2.	2	-2.88



Interaction of synthesis compounds with active site of enzyme

References:

1- Schroeder DC. Thioureas. Chem Rev. 1955;55:181–228.10.1021/cr50001a005Search in Google Scholar
2- Mertschenk B, Knott A, Bauer W. Thiourea and thiourea derivatives. Ullmann's Encyclo Ind Chem. 2000; Jun 15:1–15.10.1002/14356007.a26_803.pub3Search in Google Scholar
3- Schreiner PR. Metal-free organocatalysis through explicit hydrogen bonding interactions. Chem Soc Rev. 2003;32:289–96.10.1039/b107298fSearch in Google Scholar
4- KILCIGİL GA, Altanlar N. Synthesis and antifungal properties of some benzimidazole derivatives. Turk J Chem. 2006;30:223–8.Search in Google Scholar
5- Fengling C, Yanrui C, Hongxia L, Xiaojun Y, Jing F, Yan L. Interaction of APT with BSA or HSA. Chin Sci Bull. 2006;51:2201–7.10.1007/s11434-006-2108-ySearch in Google Scholar
6- Kaneko C, Sugimoto A, Tanaka S. A facile one-step synthesis of cis-2-cyclopentene- and cis-2-cyclohexene-1,4-diols from the corresponding cyclodienes. Synthesis. 1974;1974:876–7.10.1002/chin.197513207Search in Google Scholar
7- Gupta D, Soman R, Dev S. Thiourea, a convenient reagent for the reductive cleavage of olefin ozonolysis products. Tetrahedron. 1982;38:3013–8.10.1016/0040-4020(82)80187-7Search in Google Scholar
8- Alcolea V, Plano D, Karelia DN, Palop JA, Amin S, Sanmartín C, et al. Novel seleno- and thio-urea derivatives with potent in vitro activities against several cancer cell lines. Eur J Med Chem. 2016;113:134–44.10.1016/j.ejmech.2016.02.042Search in Google ScholarPubMed
9- "Isothiocyanates". Micronutrient Information Center, Linus Pauling Institute, Oregon State University. 1 April 2017. Retrieved 14 April 2019.
10- Dains FB; Brewster RQ; Olander CP (1926). "Phenyl Isothiocyanate". Organic Syntheses. 6: 72. doi:10.15227/orgsyn.006.0072.
11- Wong, R; Dolman, SJ (2007). "Isothiocyanates from tosyl chloride mediated decomposition of in situ generated dithiocarbamic acid salts". The Journal of Organic Chemistry. 72 (10): 3969–3971. doi:10.1021/jo070246n. PMID 17444687.
12- O'Reilly, RJ; Radom, L (2009). "Ab initio investigation of the fragmentation of 5,5-diamino-substituted 1,4,2-oxathiazoles". Organic Letters. 11 (6): 1325–1328. doi:10.1021/ol900109b. PMID 19245242.

13- Burkett, BA; Kane-Barber, JM; O'Reilly, RJ; Shi, L (2007). "Polymer-supported thiobenzophenone : a self-indicating traceless 'catch and release' linker for the synthesis of isothiocyanates". Tetrahedron Letters. 48 (31): 5355–5358. doi:10.1016/j.tetlet.2007.06.025.

14- *Everaerts, W.; Gees, M.; Alpizar, Y. A.; Farre, R.; Leten, C.; Apetrei, A.; Dewachter, I.; van Leuven, F.; et al. (2011). "The Capsaicin Receptor TRPV1 is a Crucial Mediator of the Noxious Effects of Mustard Oil". Current Biology. **21** (4): 316–321. doi:10.1016/j.cub.2011.01.031. PMID 21315593. S2CID 13151479.*

15-rône, B.; Peeters, P. J.; Marrannes, R.; Mercken, M.; Nuydens, R.; Meert, T.; Gijsen, H. J. (2008). "Tear gasses CN, CR, and CS are potent activators of the human TRPA1 receptor". *Toxicology and Applied Pharmacology*. **231** (2): 150–156. doi:10.1016/j.taap.2008.04.005. PMID 18501939.

16- *Ryckmans, T.; Aubdool, A. A.; Bodkin, J. V.; Cox, P.; Brain, S. D.; Dupont, T.; Fairman, E.; Hashizume, Y.; Ishii, N.; et al. (2011). "Design and Pharmacological Evaluation of PF-4840154, a Non-Electrophilic Reference Agonist of the TrpA1 Channel". Bioorganic & Medicinal Chemistry Letters. **21** (16): 4857–4859. doi:10.1016/j.bmcl.2011.06.035. PMID 21741838.*

17- *Romanowski, F.; Klenk, H. "Thiocyanates and Isothiocyanates, Organic". Ullmann's Encyclopedia of Industrial Chemistry. Weinheim: Wiley-VCH. doi:10.1002/14356007.a26_749.*