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Research Article

Synthesis and Biological Evaluation of S-Substituted Perhalo-2-nitrobuta-1,3-dienes as Novel Xanthine Oxidase, Tyrosinase, Elastase, and Neuraminidase Inhibitors

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S-substituted perhalo-2-nitrobuta-1,3-dienes **3a**, **b** were synthesized by the reaction of polyhalo-2-nitrobuta-1,3-dienes **1a**, **b** with allyl mercaptan. 1-(2,3-Dibromopropanethio)-4-bromo-1,3,4-trichloro-2-nitrobuta-1,3-diene **4** was obtained from the addition of bromine to S-substituted polyhalo-2-nitrobuta-1,3-diene **3b** in carbon tetrachloride. Sulfoxides **5a**, **b**, and **6** were obtained from the reaction of thiosubstituted polyhalonitrobutadienes **3a**, **b**, and **4** with *m*-CPBA in $CHCl_3$. The structures of the new compounds were determined by spectroscopic data (FTIR, ^{1}H NMR, ^{13}C NMR, MS). These compounds exhibited antixanthine oxidase, antityrosinase, antielastase, and antineuraminidase activities.

1. Introduction

Nitro-substituted polyhalobuta-1,3-dienes, regarding their intriguing chemical and biological properties, have been in the organic compound families that were preferred by researchers. Nitro-containing polyhalobuta-1,3-dienes especially play a role in building blocks in organic syntheses. Due to the presence of the nitro group it contains, the compound 2-nitropolyhalobuta-1,3-diene easily gives the vinylic substitution (S_NVin) reactions. These nitro derivatives are used as building pioneering blocks for polyfunctional acyclic and heterocyclic compounds' syntheses with biological activity. These synthesized compounds are important in pharmacology. S-, N-, N,S-substituted polyhalonitrobutadiene compounds obtained by the reactions of polyhalonitrobutadiene compounds with various nucleophilic reagents such as thiols, dithiols, and amines are given in the literature [1–9]. There are studies on antifungal, antibacterial, anticancer, and anti-HIV properties of these compounds [10–16]. Moreover, there are patents about the properties of these substances [17–19].

Allylthiol derivatives, selected as the nucleophile, have diverse biological activities. These derivatives show antibacterial, anti-inflammatory, antithrombotic, anticancer, and antiatherosclerotic properties. Most of these effects are due to the SH group [20–24]. At the same time, allylthio compounds are seen to decrease cholesterol and triglyceride levels in humans and animals [25, 26].

Sulfoxide compounds, also synthesized in our work, have unique synthetic application to be used in synthetic studies. In the literature, these compounds have been termed as "chemical chameleons." They are used as synthetic mediators in the syntheses of several organic compounds having antiulcer, antibacterial, antifungal, and antihypertensive properties [27–29].

Xanthine oxidase (XO, EC: 1.1.3.22) is a complex metalloflavoprotein. This enzyme catalyzes the oxidation of hypoxanthine to xanthine and produces uric acid which leads to many diseases like gout and hyperuricemia [30]. High XO activity leads to accumulation of the uric acid in blood. Hyperuricemia and gout diseases are risks of cardiovascular disease [31] and chronic kidney disease. Excessive uric acid levels in blood cause insulin resistance. Also, hyperuricemia is related to type 2 diabetes, metabolic syndrome, hypertension, kidney disease, hepatitis, inflammation, ischemia-reperfusion, carcinogenesis, and ageing. XO is responsible for

oxidative damage that causes many pathological diseases, such as gout, hyperuricemia, atherosclerosis [32], hepatitis, carcinogenesis [33], metabolic syndrome [34], vascular endothelium damage, and ageing. For this reason, inhibition of XO has been an obvious target to control uric acid level in blood. It has been shown that XO inhibitors may be useful for the treatment of hepatic disease, cancer, inflammation, and gout which is caused by the generation of uric acid and superoxide anion radical [35].

Tyrosinase (EC: 1.14.18.1) is a copper containing bifunctional enzyme. This enzyme is widely distributed in plants, microorganisms, fungi, and animals [36]. Tyrosinase is also responsible for the production of neuromelanin. Excessive production of dopaquinone by oxidation of dopamine results in neuronal damage and cell death [37]. For this reason, this enzyme contributed to the neurodegeneration associated with Parkinson's disease and other neurodegenerative disease processes [38]. Also, cancers are characterized by abnormalities in tyrosinase activity [39]. Tyrosinase is also associated with the host defense, wound healing, melting, and sclerotization of insects [40]. Tyrosinase inhibitors were widely used in food, agriculture, and cosmetic industries and in the control of molting process of insects. Many natural and synthetic tyrosinase inhibitors have been reported such as ascorbic acid, arbutin, kojic acid, aromatic alcohols, azelaic acids, and aldehydes and polyphenol compounds such as resveratrol in recent years [41]. However, most of them suffered from limitations such as low activity, high toxicity, and insufficient penetrative ability. Also, these substances have lower therapeutic effect, mutagenicity, and cytotoxicity potential. For this reason, the development of novel, potent nontoxic and stable tyrosinase inhibitors has a great importance in the medical, cosmetic, and agricultural fields.

Elastase (EC: 3.4.21.37) is a serine protease of the chymotrypsin family that hydrolytically degrades extracellular matrix components such as elastin, fibronectin, collagen, proteoglycans, laminin, and some matrix metalloproteinases [42]. This enzyme is particularly abundant in the skin, arteries, lungs, and ligaments. Neutrophil elastase plays an important role in some processes like blood coagulation, apoptosis, and inflammation. Inhibition of the elastase activity could be used for protection against various diseases such as chronic obstructive pulmonary disease, cystic fibrosis, acute respiratory distress syndrome, acute lung injury, ischemia, and reperfusion injury [42]. Neutrophil elastase also plays a main role in both acute pathogenesis and chronic functional recovery after traumatic brain injury [43].

Neuraminidase (NA, EC: 3.2.1.18) is one of the two glycoproteins on the surface of influenza virus. NA activity is increased in the serum patients and animal models of sepsis. This event indicates that NA is associated with the pathogenesis of inflammation during infection [44]. NA is a key validated drug target in commercially available antiviral drugs. NA inhibitors, such as zanamivir, peramivir, and oseltamivir are commonly used for prevention and treatment of influenza [45]. NA inhibitors are in wide use for the treatment of influenza [46]. In the literature, thiourea and thiadiazole compounds have been reported to be effective against HIV and to have bacterial action [47].

Enzymes are biological macromolecules that accelerate or catalyze chemical reactions in biological systems. Some chemical, natural compounds and drugs alter normal enzyme activities by specific enzyme inhibition [48].

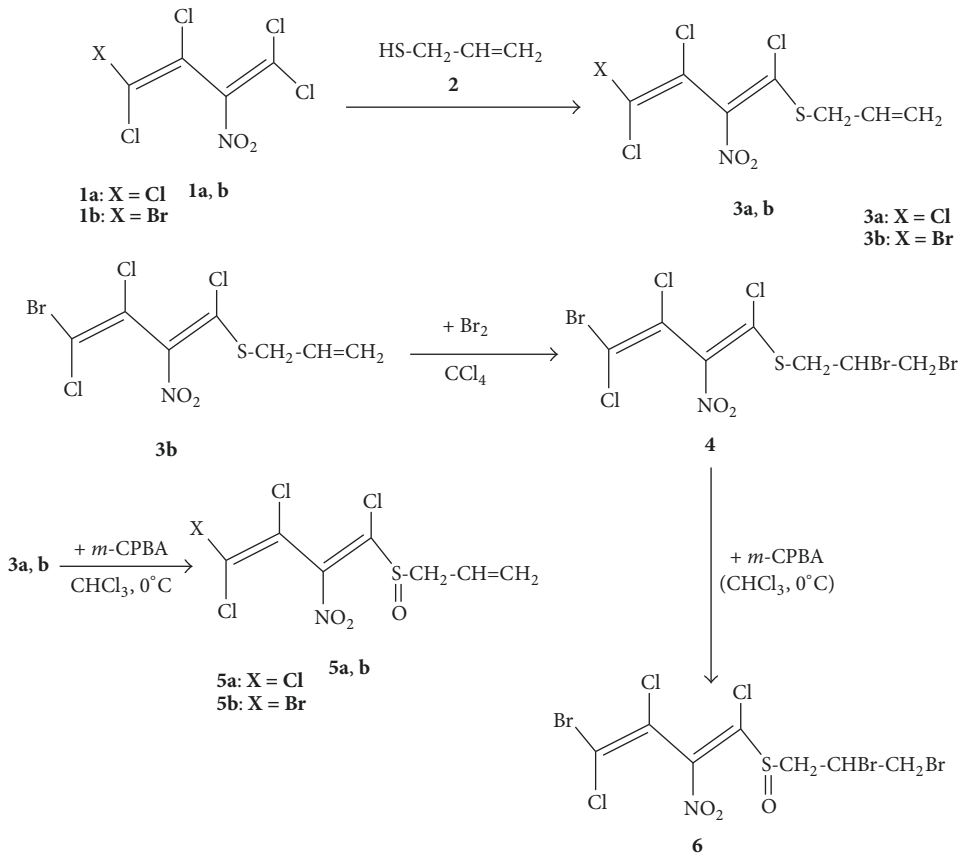
The increasing activities of enzyme cause different types of damage in the body. In this study, firstly we aimed to synthesize S-substituted perhalonitrobutadienes. The synthesized compounds were characterized by FTIR, ^{1}H NMR, ^{13}C NMR, MS. The second aim of this study is to investigate the xanthine oxidase, tyrosinase, elastase, and neuraminidase inhibition effect of these S-substituted perhalonitrobuta-1,3-diene compounds. The inhibition of these enzymes is important for the treatment of many diseases such as gout, influenza, and cancer.

2. Results and Discussion

2.1. Synthesis of S-Substituted Perhalonitrobuta-1,3-dienes. In our previous works, polyhalonitrobutadienes reacted with several thiols, and mono-, di-, and tri-(thio)substituted polyhalonitrobutadiene derivatives were synthesized [49–53]. In this work, 1,1,3,4,4-pentachloro-2-nitrobuta-1,3-diene **(1a)** [54] and 4-bromo-1,1,3,4-tetrachloro-2-nitro-1,3-butadiene **(1b)** [55] reacted with allyl mercaptan **(2)** in equimolar ratio at room temperature. Compounds **1a** and **1b** gave vinylic substitution reaction at room temperature and with the absence of solvent because of the reactivity of terminal carbon having nitrodichlorovinyl, and they yielded monosubstituted polyhalobutadiene compounds **3a** [49] and **3b** [50]. It has been reported that mono(thio)substitution nitrodienes are *(E)* isomers in previous studies [56–58]. As a consequence, it might be thought that derived new S-Substituted nitrodiene products were probably *(E)* isomers. The thiosubstituted **3b** compound gave the reaction of addition of bromine to the double bond in the allyl group in the presence of carbon tetrachloride. In the second phase of the study, the thiosubstituted compounds obtained were subjected to oxidation reactions.

Compounds **3a**, **3b**, and **4** were subjected to oxidation with m-chloroperbenzoic acid in chloroform at 0°C to oxidize the thioether group. After the reactions, new sulfoxide derivatives **5a**, **5b**, and **6** were obtained (Scheme 1).

The compound **3b** yielded ^{13}C-NMR peaks as the following: methylene carbon bound to sulfur at δ = 37.7 ppm, CH= group for allyl substituent at δ = 129 ppm, and the $=CH_2$ carbon at δ = 120.4 ppm. The compound **4**, which is the bromine added product of **3b**, yielded the following ^{13}C-NMR data: CH-Br carbon in the propyl group at δ = 46.1 ppm and the CH_2-Br carbon at δ = 33.6 ppm. At the same time, ^{1}H-NMR spectrum shows the propyl group protons instead of the characteristic CH= group for allyl system and CH_2= group. Methylene protons bound to sulfur, in compound **4**, yielded ^{1}H-NMR peaks in the range δ = 3.3–3.5 ppm, CH_2 protons bound to bromine atoms at δ = 3.6–3.9, and CH proton at δ = 4.2–4.3 ppm. These data show that the double bond in the allyl group formed an added product with bromine. Meanwhile, in MS, the compound **4**, which is an addition product, had a molecular ion peak at 531.3 $[M + NH_4]^+$. Oxidation reactions are successful when the sulfoxide compounds **5a**, **5b**, and **6** are viewed in terms of spectroscopic



Scheme 1: Synthesis of S-substituted perhalonitrobuta-1,3-diene compounds.

data. FTIR data of **5a** and **5b** showed characteristic S=O band at 1071 cm^{-1}, and FTIR data of **6** showed characteristic S=O band observed at 1073 cm^{-1}. Compound **5a** yielded molecular ion peak at 326 $[M + H]^+$ in the mass spectrum, whereas compound **5b** yielded molecular ion peak at 369.9 $[M + H]^+$. When the mass spectrum of compound **6** is investigated, the ion peak is at 548.15 $[M + NH_4]^+$ and a disintegration product which is the result of a loss of chlorine atom is at 492.5 $[M + NH_4\text{-Cl}]^+$.

2.2. Enzyme Inhibition. The xanthine oxidase inhibitory activities of S-substituted perhalonitrobuta-1,3-diene compounds were given in Table 1. All the test compounds exhibited antixanthine oxidase activities (Table 1). The xanthine oxidase inhibitory activities of S-substituted perhalonitrobuta-1,3-diene compounds were found to increase in dose depending manner. Low IC_{50} values indicate the higher enzyme inhibitory activity. Antixanthine oxidase activity of S-substituted perhalonitrobuta-1,3-diene compounds and standard decreased in the order of allopurinol > **4** > **5b** > **6** > **5a**. All these compounds showed variable inhibition values ranging from 86.72 to 4094.61 μM for XO while compound **4** showed the lowest IC_{50} values (86.72 ± 3.20 μM). We also compared our inhibition values to allopurinol that is frequently used as XO inhibitor in gout disease. IC_{50} value of allopurinol was 3.61 ± 0.31 μM lesser than our inhibition results of S-substituted perhalonitrobuta-1,3-dienes. However, allopurinol shows some adverse effects such as rash hepatitis, nephropathy, allergic reactions, vasculitis eosinophilia, and renal failure [59]. For this reason, new inhibitor, which has nonadverse effects, is needed. **4** was the most potent inhibitor against XO among the tested compounds. Compound **5a** has low XO inhibitory activity. Compounds **5b** and **6** show similar XO inhibitory activities. Their IC_{50} values are 3145.08 ± 79.08 and 3480.15 ± 32.84 μM. In the compound **4**, there is a free sulfur group and this sulfur group has two unpaired electrons. Molybdenum is in the active center of XO. Molybdenum and sulfur atoms can be found in a complex and this complex formation shows inhibitory effects on XO. It has been pointed out in the literature that compounds with sulfur atoms in the structure exhibit xanthine oxidase inhibitor activity [60].

It has been reported by Song et al. (2015) that febuxostat with sulfur in its structure inhibits the enzyme XO at a high rate. It was determined that the enzyme inhibited is similar to allopurinol in compound 4 which had a free sulfur group in its structure [61].

All compounds clearly showed a concentration dependent inhibition against tyrosinase activity (Table 2). Low IC_{50} values indicate higher enzyme inhibitor activity. The results show that compounds **5b** and **6** could inhibit tyrosinase. As shown in Table 2 the IC_{50} values of **5b** and **6** were similar.

Table 1: The xanthine oxidase inhibitory activity of different concentrations of S-substituted perhalonitrobuta-1,3-diene compounds.

Chemical compounds and standard	Concentration (μM)	Inhibition (%)*	IC_{50} (μM)*
4	100	55.07 ± 1.93	86.72 ± 3.20
	50	33.82 ± 0.97	
	25	21.26 ± 0.97	
	10	15.77 ± 1.46	
	1	3.38 ± 0.84	
5a	500	8.14 ± 0.48	4094.61 ± 213.20
	250	6.46 ± 0.24	
	100	4.90 ± 0.17	
	50	3.55 ± 0.18	
	25	1.92 ± 0.83	
5b	1000	16.59 ± 1.81	3145.08 ± 79.08
	750	12.69 ± 0.25	
	500	7.66 ± 0.48	
	250	4.90 ± 1.26	
	100	2.55 ± 1.21	
6	500	10.77 ± 0.72	3480.15 ± 32.84
	250	7.50 ± 0.55	
	100	6.19 ± 0.55	
	50	3.47 ± 0.50	
	25	2.80 ± 0.14	
Allopurinol	10	96.29 ± 1.61	3.61 ± 0.31
	5	77.78 ± 2.45	
	1	28.40 ± 1.93	
	0.5	24.36 ± 0.54	
	0.1	5.25 ± 1.42	

*Mean ± SD.

Table 2: The tyrosinase inhibitory activity of different concentrations of S-substituted perhalonitrobuta-1,3-diene compounds.

Chemical compounds and standard	Concentration (μM)	Inhibition (%)*	IC_{50} (μM)*
4	2000	40.98 ± 3.04	2497.42 ± 196.40
	1000	22.62 ± 1.04	
	500	13.82 ± 0.57	
	250	10.24 ± 2.12	
	125	5.37 ± 1.29	
5a	2000	30.53 ± 0.93	3465.46 ± 174.24
	1000	22.90 ± 1.09	
	500	17.34 ± 1.00	
	250	11.94 ± 1.44	
	125	5.09 ± 1.40	
5b	2000	90.89 ± 0.94	538.19 ± 82.47
	1000	68.54 ± 1.54	
	500	57.05 ± 2.63	
	250	43.20 ± 2.50	
	125	26.28 ± 4.30	
6	2000	99.37 ± 0.44	513.26 ± 6.97
	1000	86.61 ± 0.22	
	500	58.59 ± 1.44	
	250	38.01 ± 2.79	
	125	20.46 ± 4.22	
Kojic acid	1000	98.24 ± 0.22	291.01 ± 8.13
	500	85.35 ± 1.33	
	250	58.71 ± 2.96	
	125	32.95 ± 2.63	
	60	14.52 ± 1.53	

*Mean ± SD.

However, IC_{50} values of **4** and **5a** compounds were higher. Antityrosinase activity of S-substituted perhalonitrobuta-1,3-diene compounds and standard decreased in the order of kojic acid > **6** > **5b** > **4** > **5a**. Sulfur containing compounds, such as thiourea, thiosemicarbazone, and thiocarbonyl compounds can form chelate with transition metal ions. In the literature, thiosemicarbazone derivatives had been reported to be efficient tyrosinase inhibitors [62]. The inhibitory effect of thiosemicarbazone derivatives on tyrosinase exhibited potent inhibitory activity. The reason of this may be that the sulfur atom in the structure of the compound can chelate the two copper ions in the active side of enzyme. Many thiourea derivatives were known to be competitive inhibitors of tyrosinase due to their chelating ability to copper ions at the active sites [63]. It was reported in the literature that sulfur atoms inhibit tyrosinase via bonding the copper ions in the active site of the enzyme [64]. Liu et al. found that thiosemicarbazone derivatives showed high tyrosinase inhibition [65]. According to a previous study thio groups containing compounds act as o-dopaquinone scavengers [66]. In our study, it can be claimed that sulfur atom which is found in compound **6** inhibited the enzyme by binding to the copper ion at the active site.

Inhibition effects of elastase activities are shown in Table 3. The elastase inhibitor activities of S-substituted perhalonitrobuta-1,3-diene compounds were found to increase in a dose depending manner (Table 3). The inhibition was increased with increasing S-substituted perhalonitrobuta-1,3-dienes concentration. Amongst the compounds, the best inhibition was found for the compound **4**, followed by the **6**, **5b**, and **5a**. A high elastase inhibition (58.55 ± 2.37%) was seen at 2000 μM for **4** and its IC_{50} value was 1476.79 ± 56.03 μM. However, **5b** and **6** showed nearly the same inhibition. The inhibitory potency of compounds was in the order of ursolic acid > **4** > **6** > **5b** > **5a**. In our study, it was seen that S-substituted perhalonitrobuta-1,3-diene compounds inhibit elastase in low ratio. It was reported by Stolk et al. [67] that thiol agent has an effect on elastase activity. In addition, Rodis and Digenis [68] reported that low molecular weight thiocarbamate compounds also have inhibitory effect on leukocyte elastase activity. Sokmen et al. showed that triazole compounds which include halogen, carboxylate, heterocyclic sulfide, sulfone, and/or methoxy groups in their structure inhibit elastase enzyme [42]. In our study, it can be assumed that compounds which have halogen and sulfoxide groups in their structure inhibit elastase enzyme, yet, not in high ratio.

The neuraminidase inhibition activities of S-substituted perhalonitrobuta-1,3-diene compounds were given in Table 4. NA inhibitory activities of S-substituted perhalonitrobuta-1,3-diene compounds were found to increase in a dose dependent manner. Amongst them, the best inhibition was found for the compound **6**. All compounds clearly showed a concentration dependent inhibition against neuraminidase activity. The inhibitory effect of compound **6** on neuraminidase activity was better than quercetin. Antineuraminidase activity of **4**, **5a**, **5b**, and **6** decreased in the order of **6** > quercetin > **5b** > **5a** > **4**. It was found that the compounds which have sulfoxide groups have higher NA inhibitory activity. A higher NA inhibitor activity is associated with a lower IC_{50} value. Compound **6** that contained halogen atoms and sulfoxide group showed excellent inhibitory activities. The inhibitory potential of compounds **5b** and **5a** was found greater than that of **4**. Compound **6** demonstrated higher neuraminidase inhibition activity than that of quercetin (Table 4). A Ki value is the binding affinity constant of each inhibitor. Compound **6** was the best neuraminidase inhibitor (Ki = 108.37 ± 14.65 μM). Quercetin showed a Ki value of 124.83 ± 14.95 μM against neuraminidase activity. The inhibition mechanisms of compound **6** and quercetin were competitive. The quercetin which is a natural flavonoid has antiviral property [69–71]. This substance was used as standard substance for antineuraminidase activity assay in our study. However, the synthesized compound 6 inhibited neuraminidase at higher rate than quercetin. It was declared that the flavonoid and phenolic compounds which are obtained from plants have antineuraminidase activity [72, 73]. The hydroxyl groups exist mostly in the structure of these compounds. There exists sulfur in the synthesized compounds in our study. Sulfoxides have antibacterial and antifungal activities [74]. It can be assumed that the compound 6 which has sulfoxide groups has higher inhibition activity than the compounds that have hydroxyl groups.

This indicates that sulfoxide and halogens bind to active sites of NA enzyme. In our current study, the compound **6** which has sulfoxide and halogen groups in its structure has the highest antineuraminidase activity. Compound **6** could be a new lead for further modifications to discover more potent inhibitors.

3. Materials and Methods

3.1. General. All chemicals were obtained from commercial suppliers and without further purification. Thin layer chromatography was performed on Merck (60 F 254) TLC-plates (aluminum based). Purifications were carried out by means of column chromatography on silica gel 60 (Merck, 63–200 μm particle size, 60–230 mesh). Melting points were measured on a Buchi B-540 apparatus and were uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded with Varian^{UNITY} INOVA spectrometers with 500 MHz frequency for ^{1}H and 125 MHz frequency for ^{13}C NMR in ppm (δ). ^{1}H NMR spectra and ^{13}C NMR spectra in $CDCl_3$ refer to the solvent signal center at δ 7.2 and δ 77 ppm, respectively. IR spectra were recorded by ATR on Thermo Scientific Nicolet 6700 spectrometer. Mass spectra were recorded on Shimadzu LCMS-8030 triple quadrupole spectrometer in ESI (+) polarity.

*3.2. Synthesis of S-Substituted Polyhalonitrobuta-1,3-dienes **3a**, **b**.* In this study compounds **1a** and **1b** were used as starting compounds. Pentachloro-2-nitrobuta-1,3-diene **(1a)** was prepared from 2H-pentachlorobuta-1,3-diene in 43% yield (bp. 90–96°C) following the literature procedure [54]. 4-Bromo-tetrachloro-2-nitrobuta-1,3-diene **(1b)** was prepared from 2H-4-bromo-tetrachlorobuta-1,3-diene in 40% yield (bp. 68–70°C) following the literature procedure [55]. 1-(Allylthio)-1,3,4,4-tetrachloro-2-nitrobuta-1,3-diene **(3a)** and 1-(Allylthio)-4-bromo-1,3,4-trichloro-2-nitrobuta-1,3-diene **(3b)** were prepared using the procedure according to the reported literature [49, 50].

Table 3: The elastase inhibitory activity of different concentrations of S-substituted perhalonitrobuta-1,3-diene compounds.

Chemical compounds and standard	Concentration (μM)	Inhibition (%)*	IC_{50} (μM)*
4	2000	58.55 ± 2.37	1476.79 ± 56.03
	1000	45.19 ± 0.23	
	500	34.12 ± 2.25	
	250	13.32 ± 1.99	
	125	8.24 ± 0.55	
5a	2000	20.23 ± 0.78	9116.60 ± 1068.43
	1000	14.36 ± 0.30	
	500	13.66 ± 0.08	
	125	12.74 ± 0.04	
	60	10.09 ± 0.42	
5b	2000	36.81 ± 1.65	2605.75 ± 150.87
	1000	30.03 ± 1.67	
	500	18.68 ± 1.10	
	250	4.66 ± 1.37	
	125	3.94 ± 0.05	
6	1000	27.09 ± 0.66	2024.74 ± 139.82
	500	23.31 ± 1.07	
	250	16.19 ± 1.44	
	125	10.22 ± 0.81	
	60	8.23 ± 1.66	
Ursolic acid	1000	76.74 ± 0.38	424.10 ± 5.15
	500	72.27 ± 0.51	
	250	49.33 ± 0.06	
	125	27.60 ± 1.56	
	60	12.20 ± 0.50	

*Mean ± SD.

Table 4: The neuraminidase inhibitory activity of different concentrations of S-substituted perhalonitrobuta-1,3-diene compounds.

Chemical compounds and standard	Concentration (μM)	Inhibition (%)*	IC_{50} (μM)*
4	100	18.16 ± 1.26	458.90 ± 62.62
	10	13.70 ± 0.23	
	1	10.73 ± 0.23	
	0.1	7.97 ± 0.02	
	0.01	7.20 ± 0.12	
5a	100	21.77 ± 2.51	329.45 ± 48.44
	10	16.95 ± 0.52	
	1	14.78 ± 0.27	
	0.1	11.48 ± 1.29	
	0.01	4.89 ± 0.56	
5b	100	28.85 ± 0.49	200.23 ± 5.92
	10	11.33 ± 0.38	
	1	8.89 ± 0.57	
	0.1	7.99 ± 0.79	
	0.01	6.19 ± 1.34	
6	25	56.57 ± 18.45	23.12 ± 9.62
	10	23.00 ± 2.79	
	1	13.77 ± 2.62	
	0.1	8.02 ± 0.35	
	0.01	4.30 ± 0.4	
Quercetin	100	32.31 ± 3.50	161.54 ± 18.21
	50	21.37 ± 0.88	
	10	13.06 ± 0.29	
	1	4.58 ± 0.76	
	0.1	3.26 ± 0.05	

*Mean ± SD.

1-(Allylthio)-1,3,4,4-tetrachloro-2-nitrobuta-1,3-diene ***(3a)***. Yellow crystals. M.p. = 72-73°C. Yield 40%. FTIR (ATR) ν (cm^{-1}) 2900, 1600, 1510, 1310, 1295. ^{1}H-NMR ($CDCl_3$): 5.7–6 (m, 1H, $CH=_{allyl}$), 5.2–5.6 (m, 2H, $=CH_{2\ allyl}$), 3.6–4 (m, 2H, $CH_{2\ allyl}$). ^{13}C-NMR ($CDCl_3$): 37.7 ($S\text{-}CH_{2\ allyl}$), 120.3 ($=CH_{2\ allyl}$), 129 ($CH=_{allyl}$), 120.4, 127.7, 128.9, 155.9 ($C_{butadiene}$). MS (ESI), (m/z%): 327.20 $[M + NH_4]^+$. $C_7H_5Cl_4NO_2S$ (M = 308.945 g/mol).

1-(Allylthio)-4-bromo-1,3,4-trichloro-2-nitrobuta-1,3-diene ***(3b)***. Yellow oil. Yield 20%. FTIR (ATR) ν (cm^{-1}) 2950, 2900, 1600. ^{1}H-NMR ($CDCl_3$): 5.8–6 (m, 1H, $CH=_{allyl}$), 5.2–5.5 (m, 2H, $=CH_{2\ allyl}$), 3.6–3.8 (m, 2H, $CH_{2\ allyl}$). ^{13}C-NMR ($CDCl_3$): 37.7 ($S\text{-}CH_{2\ allyl}$), 120.4 ($=CH_{2\ allyl}$), 129 ($CH=_{allyl}$), 113, 129, 137.9, 155.9 ($C_{butadiene}$). MS (ESI), (m/z%): 372 $[M + NH_4]^+$. $C_7H_5BrCl_3NO_2S$ (M = 353.45 g/mol).

3.3. Synthesis of 1-(2,3-Dibromopropanethio)-4-bromo-1,3,4-trichloro-2-nitrobuta-1,3-diene ***(4)***. This compound was synthesized from **3b** (0.1 g, 0.28 mmol) and bromine (0.045 g, 0.28 mmol) in CCl_4. Bromine was added slowly dropwise and stirring continued for 3 hours at room temperature. The progress of the reaction was monitored by TLC. A solution of sodium sulfide and ether was added to the reaction mixture. The organic layer was separated and dried with Na_2SO_4. The solvent was evaporated under low pressure. The product was purified by column chromatography. Yellow oil. Yield 21%. FTIR (ATR) ν (cm^{-1}) 2957, 2921, 2851, 1592, 1529, 1290. ^{1}H-NMR ($CDCl_3$): 4.2–4.3 (m, 1H, CH-Br), 3.6–3.9 (m, 2H, CH_2-Br), 3.3–3.5 (m, 2H, $S\text{-}CH_2$). ^{13}C-NMR ($CDCl_3$): 31 ($S\text{-}CH_2$), 33.6 (CH_2-Br), 46.1 (CH-Br), 114, 121.3, 123.08, 153 ($C_{butadiene}$). MS (ESI), (m/z%): 531.30 $[M + NH_4]^+$, 497.50 $[M + NH_4\text{-}Cl]^+$, 461.90 $[M + NH_4\text{-}2Cl]^+$. $C_7H_5Br_3Cl_3NO_2S$ (M = 513.16 g/mol).

3.4. General Procedure for the Synthesis of Sulfoxides ***5a***, ***5b***, ***6***. Compound **3a**, **3b**, or **4** (1 mmol) in 30 mL of chloroform was mixed with *m*-chloroperbenzoic acid (4 mmol) in 30 mL of chloroform at 0°C for 24 h. The progress of the reaction was monitored by TLC. A solution of 2 M NaOH and chloroform was added to the reaction mixture. The organic layer was separated and dried with Na_2SO_4. The solvent was evaporated under low pressure. The product was purified by column chromatography.

1-(Allylsulfinyl)-1,3,4,4-tetrachloro-2-nitrobuta-1,3-diene ***(5a)***. This compound was synthesized from **3a** (0.058 g, 0.18 mmol) and *m*-chloroperbenzoic acid (0.12 g, 0.72 mmol) according to the general procedure. White crystals. M.p = 195-196°C. Yield 28%. FTIR (ATR) ν (cm^{-1}) 2957, 2914, 2856, 1570, 1462, 1267, 1071. ^{13}C-NMR ($CDCl_3$): 40.4 ($SO\text{-}CH_{2\ allyl}$), 109.9 ($=CH_{2\ allyl}$), 131 ($CH=_{allyl}$), 128, 133, 134, 166 ($C_{butadiene}$). MS (ESI), (m/z%): 343.0 $[M + NH_4]^+$, 326.0 $[M + H]^+$. $C_7H_5Cl_4NO_3S$ (M = 325 g/mol).

1-(Allylsulfinyl)-4-bromo-1,3,4-trichloro-2-nitrobuta-1,3-diene ***(5b)***. This compound was synthesized from **3b** (0.026 g, 0.84 mmol) and *m*-chloroperbenzoic acid (0.18 g, 0.72 mmol) according to the general procedure. White oil. Yield 17%. FTIR (ATR) ν (cm^{-1}) 2957, 2924, 2855, 1578, 1461, 1271, 1071. MS (ESI), (m/z%): 387.20 $[M + NH_4]^+$, 369.90 $[M + H]^+$. $C_7H_5BrCl_3NO_3S$ (M = 369.45 g/mol).

1-(2,3-Dibromopropylsulfinyl)-4-bromo-1,3,4-trichloro-2-nitrobuta-1,3-diene ***(6)***. This compound was synthesized from **4** (0.02 g, 0.038 mmol) and *m*-chloroperbenzoic acid (0.006 g, 0.15 mmol) according to the general procedure. White crystals. M.p = 103–105°C. Yield 15%. FTIR (ATR) ν (cm^{-1}) 2918, 2849, 1690, 1573, 12891, 1073. MS (ESI), (m/z%): 548.15 $[M + NH_4]^+$, 492.50 $[M + NH_4\text{-}Cl]^+$. $C_7H_5Br_3Cl_3NO_3S$ (M = 529.26 g/mol).

3.5. Enzyme Inhibition

3.5.1. Xanthine Oxidase Inhibitory Activity. XO inhibitory activity was determined spectrophotometrically by using xanthine as the substrate [75]. The assay mixture consisted of samples, phosphate buffer (pH: 7.5), and xanthine oxidase enzyme solution (0,1 U/mL in phosphate buffer, pH: 7.5) which was prepared immediately before use. After preincubation at 25°C for 15 mins, the reaction was initiated by substrate solution. The assay mixture was incubated for 30 minutes. Then, the reaction was stopped by adding HCl. Absorbance value was measured at 290 nm by using UV spectrophotometer. XO inhibitory activity was expressed as the percentage of the inhibition of XO in the assay system above, calculated as

$$\text{inhibition } (\%) = \left(1 - \left[\frac{B}{A}\right]\right) \times 100, \quad (1)$$

where A represents the activity of the enzyme without sample and B is the activity of XO in the presence of the sample. Allopurinol was used as a standard compound as control.

3.5.2. Tyrosinase Inhibitory Activity. Tyrosinase inhibitory activity was determined spectrophotometrically [76]. Briefly, test reaction mixtures were prepared by adding mushroom tyrosinase, sample, L-tyrosine, and sodium phosphate buffer (pH 6.5). The resulting mixture was incubated for 10 mins at 37°C and absorption value was measured at 475 nm. The percentage of the inhibition of tyrosinase activity was calculated according to the following equation:

$$\text{inhibition } (\%) = \left[\frac{(A - B)}{A}\right] \times 100, \quad (2)$$

where A represents the activity of the enzyme without sample and B is the activity of tyrosinase in the presence of the sample. Kojic acid was used as a standard compound as control.

3.5.3. Elastase Inhibitory Activity. Elastase inhibitory activity was examined in this study by using N-Succinyl-Ala-Ala-Pro-Phe p-nitroanilide (STANA) as a substrate and by measuring the release of p-nitroanilide at 410 nm [77]. The percentage

of the inhibition of elastase was calculated according to the following equation:

$$\text{inhibition } (\%) = \left[\frac{(A-B)}{A}\right] \times 100, \quad (3)$$

where A represents the activity of the enzyme without sample and B is the activity of elastase in the presence of the sample. Ursolic acid was used as a standard compound as control.

3.5.4. Neuraminidase Inhibitory Activity. NA inhibitory activity was determined by using the method described by Myers et al. [78] with some modification. The reaction was carried out with the enzyme (2.5×10^{-3} U/mL), acetate buffer (pH: 5.0), and sample substrate (4-methylumbelliferyl-a-D-N-acetylneuraminic acid sodium salt hydrate, 0.125 mM) and was incubated for 10 minutes at 37°C. After reaction was stopped by adding glycine-NaOH buffer (pH: 10.4), the fluorescence of reactions was measured spectrofluorometrically. The emission wavelength was 440 nm, while excitation wavelength was 360 nm. The percentage of the inhibition was obtained by the equation below:

$$\text{inhibition } (\%) = \left[(\text{rate of control reaction}) - \frac{(\text{rate of sample reaction})}{(\text{rate of control reaction})}\right] \times 100. \quad (4)$$

Quercetin was applied as a standard compound as control.

The Ki values and inhibition types for compound **6** and quercetin were calculated by Lineweaver Burk curves for neuraminidase enzyme [79].

For all the enzyme inhibition, the results are given as half maximal inhibitory concentrations (IC_{50}) values, calculated from the regression equations prepared from the concentrations of samples.

4. Conclusions

Nitro and sulfoxide compounds have many biological activities such as antiulcer, antibacterial, antifungal, anticancer, and antihypertensive activities [10–16, 27–29]. However, antixanthine oxidase, antityrosinase, antielastase, and antineuraminidase activities of these compounds have not been investigated until now. Therefore, we have synthesized some new derivatives of S-substituted perhalonitrobutadiene and sulfoxide. Many of them have shown good xanthine oxidase, tyrosinase, and neuraminidase inhibitions in this study. For this reason, S-substituted perhalonitrobuta-1,3-dienes may be considered as main neuraminidase, tyrosinase, and xanthine oxidase inhibitors. These compounds can be used in pharmacy, cosmetic, and food industries due to their excellent antixanthine oxidase, antityrosinase, and antineuraminidase activities.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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