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Research Article

Bazı Antidepresanların Antikanser Hedefi Olan Tiyoredoksin Redüktaz Enziminin İnhibitörleri Olarak Değerlendirilmesi

İkranur Felek[1], Ebru Akkemik[2*]

[1]Bingöl University, Faculty o Health Sciences, Department of Nutrition and Dietetics, Bingöl, Turkey (ORCID ID:0000-0001-9503-5772) ikranurfelek@bingol.edu.tr
[2*]Siirt University, Faculty of Engineering, Department of Food Engineering, Siirt, Turkey (ORCID ID: 0000-0002-4177-4884), eakkemik@siirt.edu.tr

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Öz

Tiyoredoksin redüktaz (TrxR), tiyoredoksinin indirgenmesini katalize ederek detoksifikasyondan radikallerin indirgenmesine kadar pek çok metabolik yolda yer almaktadır. Bu nedenle kanser de dahil olmak üzere birçok fizyolojik süreçle ilişkili bir enzimdir. Bu enzimin inhibitörleri antikanser hedefleri olarak kabul edilir. Geçmişte yapılan çalışmalarda bazı antidepresanların çeşitli mekanizmalar yoluyla antikanser etki gösterdiği bulunmuş ve bu nedenle antidepresanların antikanser ilaç olarak tekrar kullanılması araştırmacıların ilgisini çekmiştir. Bu çalışmada bazı antidepresanların (neferin (1), amoksapin (2), mirtazapin (3), agomelatin (4), trazodon hidroklorür (5), amitrptilin hidroklorür (6)) sitozolik hormon üzerindeki inhibisyon etkisinin araştırılması amaçlanmıştır. Sıçan karaciğeri TrxR aktivitesi üzerine bu moleküllerin inhibisyon etkileri IC_{50} ve K_i değerleri ile belirlenmiştir. 1 (IC_{50}:220 µM, K_i: 1,3±0,79 µM), 2 (IC_{50}:337 µM, K_i: 5,2±2,1 µM), 3 (IC_{50}:487 µM, K_i: 5,6±1,99 µM) ve 4 (IC_{50}: 545) uM, K_i: 7,0±1,83 uM), sitozolik sıçan karaciğeri TrxR üzerinde güçlü inhibisyon etkisi sergilediği tespit edilmiştir. Sonuç olarak, bu sonuçların hem bu antidepresanların antikanser mekanizmasını açıklamaya hem de antikanser etkileri olan yeni TrxR inhibitörlerinin sentezlenmesine katkı sağlayacağı düşünülmektedir.

Anahtar Kelimeler: Antidepresan, Kanser, Tioredoksin redüktaz.

Evaluation of Some Antidepressants as Inhibitors of Thioredoxin Reductase Enzyme, which is an Anticancer Target

Abstract

Thioredoxin reductase (TrxR) is an enzyme that is involved in many metabolic pathways from detoxification to reduction of radicals by catalyzing the reduction of thioredoxin, and is therefore associated with many physiological processes, including cancer. Inhibitors of this enzyme are considered anticancer targets. In past studies, some antidepressants have been found to have anticancer effects through various mechanisms, and therefore the reuse of antidepressants as anticancer drugs has attracted the attention of researchers. In this study, it was aimed to investigate the inhibition effect of some antidepressants (neferine (**1**), amoxapine (**2**), mirtazapine (**3**), agomelatine (**4**), trazodone hydrochloride (**5**), amitrptyline hydrochloride (**6**)) on cytosolic rat liver TrxR activity. The inhibition effects of these molecules were determined by IC_{50} and K_i values. **1** (IC_{50}:220 µM, K_i: 1.3±0.79 µM), **2** (IC_{50}:337 µM, K_i: 5.2±2.1 µM), **3** (IC_{50}:487 µM, K_i: 5.6±1.99 µM) and **4** (IC_{50}: 545 µM, K_i: 7.0±1.83 µM) exhibited potent inhibition effect on cytosolic rat liver TrxR. As a result, it was hoped that these results might contribute to both explaining the anticancer mechanism of these antidepressants and synthesizing new TrxR inhibitors with anticancer effects.

Keywords: Antidepressant, Cancer, Thioredoxin reductase

[*] Sorumlu Yazar: eakkemik@siirt.edu.tr

1. Introduction

Thioredoxin Reductase (TrxR, EC 1.6.4.5) is a homodimeric flavoenzyme that catalyzes the reduction of thioredoxin using NADPH, containing a redox active disulfide bond and a tightly bound FAD molecule in each subunit (Bjørklund vd., 2021). TrxR is required for all biochemical pathways in which Trx is used as a reducing substrate such as reduction of nucleotides to deoxyribonucleotides, detoxification of xenobiotics, oxidants, and radicals (Collet & Messens, 2010; Saccoccia vd., 2014). Moreover, TrxR enzyme is associated with various pathological processes such as apoptosis (Tonissen & Di Trapani, 2009), cancer (Selenius vd., 2010), parasitosis (Prast-Nielsen vd., 2011; Boumis vd., 2012; Saccoccia vd., 2012), chronic inflammatory, immune system (Becker vd., 2000; Holmgren & Lu, 2010) and neurodegenerative diseases (Cimini vd., 2013). Inhibition of TrxR has become an important target in clinical trials because of its relevance to various pathological processes (Kuntz vd., 2007; Saccoccia vd., 2014). Aurothiomalate and auranofin are TrxR inhibitors approved for use as drugs (Saccoccia vd., 2014). TrxR is overexpressed in various tumor types and is associated with resistance to tumor chemotherapy, which leads to the growth of tumor angiogenesis (Arnér & Holmgren, 2000; Kim vd., 2005; Patwardhan vd., 2022). For this reason, TrxR is seen as an anticancer target and inhibitors of this enzyme are thought to be effective therapeutic agents against cancer (Zhang vd., 2017; Patwardhan vd., 2022).

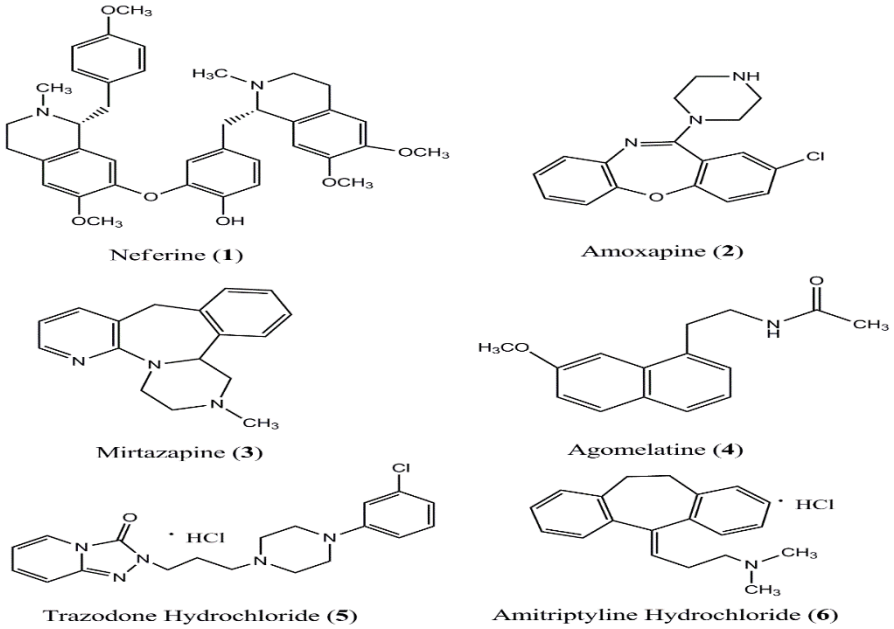

Figure 1. Chemical structures of antidepressants were used in this study.

Although some studies suggest that antidepressants are associated with increased cancer risk, many epidemiological and preclinical studies point to a predominantly inhibitory effect of antidepressants on cancer prognosis. In addition, some studies indicate that antidepressants can control tumor growth by reducing malignant cell turnover and inducing apoptosis in tumor cells with their ability to activate the immune system (Frick & Rapanelli, 2013; Low vd., 2020). In the light of these informations in the literature, we aimed to investigate the inhibition effects of some antidepressants (Figure 1) (neferine (**1**), amoxapine (**2**), mirtazapine (**3**), agomelatine (**4**), trazodone hydrochloride (**5**), amitrptyline hydrochloride (**6**)), on TrxR, which is an anticancer target. It has been determined in recent studies that amitriptyline (Cordero vd., 2010; Parker vd., 2012; Higgins vd., 2012; Zhang vd., 2013), mirtazapine (Fang vd., 2012; Uzawa vd., 2014; Bilici vd., 2012), agomelatine (Galeti vd., 2021) have anticancer effects with different mechanisms in various cancer types. And neferin has also been determined to exhibit anticancer effects (Marthandam Asokan vd., 2018). However, no study investigating the inhibitory effects of these antidepressants (**1-6**) on TrxR activity was found in the literature.

In this study, the inhibition effects of **1-6** antidepressants on TrxR activity were determined by IC_{50} and K_i values for each antidepressant in order to contribute to both the elucidation of the anticancer effect mechanisms of **1-6** antidepressants and the studies of new anticancer drug design.

2. Material and Method

2.1. Chemicals

Thioredoxin reductase (from rat liver), 5,5′-Dithiobis-(2-nitrobenzoic acid); DTNB, β-Nicotinamide adenine dinucleotide 2′-phosphate reduced tetrasodium salt hydrate; NADPH Ethylenediaminetetraacetic acid; EDTA, DL-Dithiothreitol; DTT, bovine serum albumin; BSA, dimethyl sulfoxide; DMSO, acetic acid, antidepressants (Amitrptyline Hydrochloride, Neferine, Amoxapine, Mirtazapine, Agomelatine, Trazodone Hydrochloride) were obtained from Sigma-Aldrich Co. (Sigma-Aldrich Chemie GmbH Export Department Eschenstrasse 5, 82024 Taufkirchen, Germany).

2.2. TrxR Activity Assay

The DTNB (5,5'-dithiobis-2-nitrobenzoic acid) method was used for the measurement of TrxR activity. In this method, TrxR catalyzes the reduction of disulfide bonds in DTNB using NADPH (Hill vd., 1997). For activity measurement, reaction mixture was prepared to contain the folowing: 200 µL 100 mM pH:7 K-phosphate buffer, 100 µL 10 mM EDTA, 100 µL 0.02 mM NADPH, 100 µL 0.2 mg/mL bovine serum albumin, 100 µL 1% ethanol, 100 µL 5 mM DTNB and 50 µL enzyme. The final volume was made up to 1000 µL with distilled water. The increase in absorbance at 412 nm versus blank was measured spectrophotometrically for 3 minutes at 60 second intervals. The amount of DTNB spent for 1 minute was calculated using the Lambert-Beer equation. The basis of TrxR activity measurement is based on the determination of TNB oxidation per minute.

2.3 Determination of *in vitro* inhibitory effect of antidepressants on TrxR Activity

The rat liver TrxR enzyme we used in our study was commercially available. Stock solutions of antidepressants to be used in inhibition studies were prepared with 1 mg of substance per mL. DMSO was used as the solvent. During the inhibition studies, necessary dilutions were made with distilled water. *In vitro* inhibition effects of antidepressants (**1-6**) on rat liver TrxR activity were also determined by IC_{50} and K_i values. To determine the IC_{50} values, TrxR activity was measured by the DTNB method (Hill vd., 1997). at least five different concentrations of each antidepressant. The control activity of the enzyme was accepted as 100%. Activity%/[inhibitor concentration] graphs were plotted and the IC_{50} value of each molecule was calculated from these graphs. TrxR activity was measured at least five different substrate concentrations to determine K_M and V_{max} values. Lineweaver-Burk graph was drawn and K_M and V_{max} values were calculated from this graph (Lineweaver & Burk, 1934). K_i values of antidepressants with inhibitory effect were calculated from the formula (1) below using K_M and V_{max} values.

$$V = \frac{V_{max} \times [S]}{K_M \times \left(1 + \frac{[I]}{K_i}\right) + [S]} \quad (1)$$

According to this equation (Zhang vd., 2020), [I] is the inhibitor concentration, K_i is the inhibition constant, [S] is the concentration of the substrate.

3. Results and Discussion

The use of existing drugs for new purposes to treat different diseases is referred to as drug repurposing, which offers much more advantages than new drug discovery (Low vd., 2020). The drug repurposing method has also achieved success in the field of anticancer drug development in recent years (Frick & Rapanelli, 2013; Low vd., 2020). Various studies have stated that some antidepressant drugs exhibit anticancer effects by different mechanisms (Frick & Rapanelli, 2013; Low vd., 2020). Tranylcypromine, phenelzine and desipramine are approved antidepressants and have entered clinical trials for use in cancer treatment (Fang vd., 2019; Low vd., 2020; Riess vd., 2020), This has made the reuse of antidepressants an extremely important strategy for anticancer drug discovery. In this study, the inhibition effects of some antidepressants (neferine (**1**), amoxapine (**2**), mirtazapine (**3**), agomelatine (**4**), trazodone hydrochloride (**5**), amitrptyline hydrochloride (**6**)) on cytosolic rat liver TrxR enzyme activity, which is known as an anticancer target, were investigated.

The inhibition effects of **1-6** antidepressants on TrxR enzyme were determined by IC_{50} and K_i parameters. IC_{50} values were found by Activity%/[Inhibitor] graphs (Figure 2) to be 220, 337, 487 and 545 µM for **1**, **2**, **3** and **4**, respectively. K_i values were determined as 1.3±0.79 µM, 5.2±2.1 µM, 5.6±1.99 µM and 7.0±1.83 µM for **1**, **2**, **3** and **4**, respectively. The IC_{50} value represents the inhibitor concentration that halves the enzyme activity, while the K_i value is the dissociation equilibrium constant of the enzyme and the inhibitor. At the same time, the K_i value indicates the inhibitor's affinity for the enzyme. The low IC_{50} and K_i value emphasize the strong inhibitory effect. According to these, the inhibition powers of **1-4** antidepressants on TrxR were listed as follows: neferine (**1**)> amoxapine (**2**)> mirtazapine (**3**)> agomelatine (**4**). Trazodone hydrochloride (**5**) and amitrptyline hydrochloride (**6**) did not show any significant effect on inhibition or activation of TrxR activity.

When we looked at the K_i values, it was seen that **1**, **2**, **3** and **4** molecules had a strong inhibition effect on cytosolic rat liver TrxR activity at micromolar level. In this study, neferine was the molecule with the strongest inhibitory effect on cytosolic rat liver TrxR. Neferine is a bisbenzylisoquinoline alkaloid, a member of the isoquinolines (Yeh vd., 2020). Neferine is a unique molecule with a wide range of biological activity, from antidepressant to anticancer and even anti-HIV effects (Marthandam Asokan vd., 2018; Yeh vd., 2020).

*Table 1. IC_{50} and K_i values of **1-4** molecules for cytosolic rat liver TrxR*

Molecules	IC_{50} (µM)	K_i (µM)
1	220	1.3±0.79 µM
2	337	5.2±2.1 µM
3	487	5.6±1.99 µM
4	545	7.0±1.83 µM

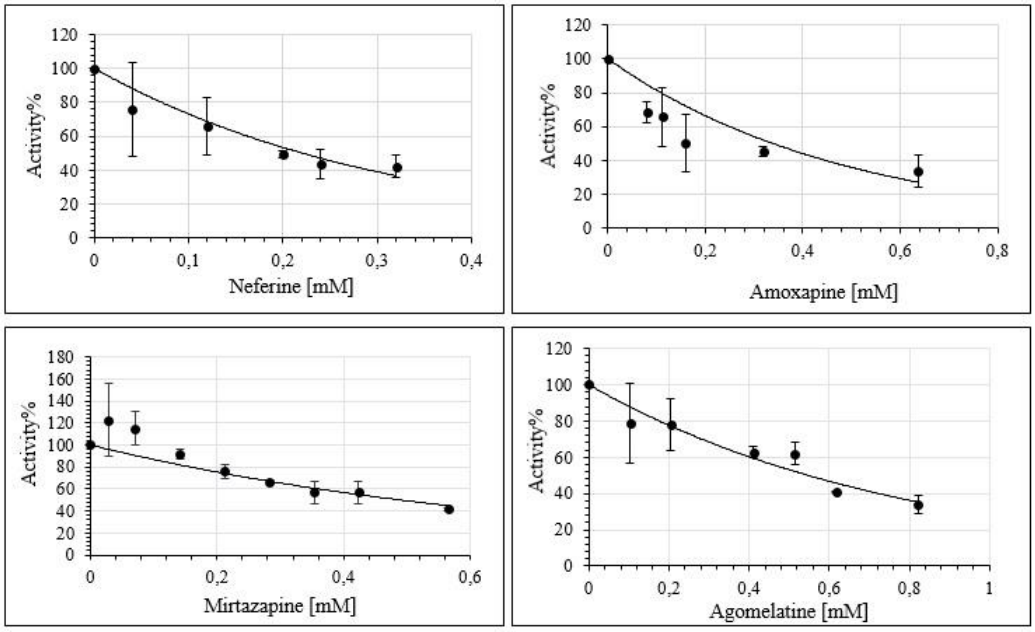

Figure 2. IC_{50} graphs of neferine, amoxapine, mirtazapine, agomelatine for cytosolic rat liver TrxR

4. Conclusions

TrxR is an anticancer target enzyme and inhibitors of this enzyme are thought to be effective therapeutic agents against cancer (Zhang vd., 2017; Patwardhan vd., 2022). Many studies have stated that antidepressants also have anticancer effects (Frick & Rapanelli, 2013; Low vd., 2020). In this study, the inhibition effect of some antidepressants (neferine, amoxapine, mirtazapine, agomelatine, trazodone hydrochloride, amitrptyline hydrochloride) on cytosolic rat liver TrxR activity was investigated. of these, neferine, amoxapine, mirtazapine, agomelatine molecules exhibited a micromolar inhibition effect on cytosolic rat liver TrxR. These results may contribute to both explaining the anticancer mechanism of these antidepressants and synthesizing TrxR inhibitors with new anticancer effects.

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