RESEARCH ARTICLE

Journal of Erzincan Binali Yıldırım University Health Sciences Institute - (JEBYUHSI)

Nitrofurantoin with Anticholinergic Effect: A Different in Vitro Approach to Alzheimer's Disease

Şeyma KANDEMİR[1], Cüneyt TÜRKEŞ[1]

[1]Erzincan Binali Yıldırım Üniversitesi Department of Biochemistry, Faculty of Pharmacy, Erzincan, Türkiye

Received: *06.08.2024,* ***Accepted:*** *27.08.2024,* ***Publication Date:*** *08.10.2024*

ABSTRACT

Alzheimer's disease (AD), a leading cause of dementia, severely affects cognitive function, with the depletion of acetylcholine being a pivotal factor in its pathogenesis. This study delves into the inhibitory potential of the nitrofuran analogue, nitrofurantoin on cholinesterases (ChEs) using comprehensive in vitro approaches. Our findings indicate that nitrofurantoin exhibits differential inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), with inhibition constants (KI) of 6.77 ± 0.56 μM for AChE and 9.48 ± 0.69 μM for BChE. These values, although less potent than those of the reference drug tacrine (KIs of 0.17 ± 0.01 μM for AChE and 0.13 ± 0.01 μM for BChE), suggest a noteworthy anticholinergic capability. By providing detailed insights into the enzyme inhibition dynamics, this study lays the groundwork for optimizing nitrofuran derivatives in the therapeutic landscape of AD. The implications of these findings extend to the broader context of pharmacological advancements, highlighting the significance of targeted enzyme inhibition in managing neurodegenerative diseases. Future research building on these results could lead to the development of more effective treatments, enhancing the quality of life for individuals affected by AD and offering new avenues for clinical intervention.

Keywords: Acetylcholinesterase, Alzheimer's Disease, Butyrylcholinesterase, Enzyme-ligand Interactions Nitrofuran Derivative.

Antikolinerjik Etkili Nitrofurantoin: Alzheimer Hastalığına Farklı Bir in Vitro Yaklaşım

ÖZET

Demansın önde gelen nedenlerinden biri olan Alzheimer hastalığı (AD), bilişsel işlevi ciddi şekilde etkiler ve asetilkolinin tükenmesi patogenezinde önemli bir faktördür. Bu çalışma, kapsamlı in vitro yaklaşımlar kullanarak nitrofuran analoğu olan nitrofurantoin'in kolinesterazlar (ChE'ler) üzerindeki inhibitör potansiyelini araştırmaktadır. Bulgularımız, nitrofurantoin'in asetilkolinesteraz (AChE) ve bütirilkolinesteraz (BChE) enzimleri üzerinde farklı inhibisyon etkileri sergilediğini, AChE için 6.77 ± 0.56 μM ve BChE için 9.48 ± 0.69 μM inhibisyon sabitleri (KI)'ne sahip olduğunu göstermektedir. Bu değerler, referans ilaç takrin'in KI değerlerinden (AChE için 0.17 ± 0.01 μM ve BChE için 0.13 ± 0.01 μM) daha az etkili olsa da, dikkate değer bir antikolinerjik kapasiteye işaret etmektedir. Bu çalışma, enzim inhibisyon dinamiklerine dair ayrıntılı içgörüler sağlayarak, nitrofuran türevlerinin AD'nin terapötik yaklaşımının optimize edilmesi için temel oluşturmaktadır. Bu bulguların çıkarımları, farmakolojik gelişmelerin daha geniş bağlamına uzanmakta ve nörodejeneratif hastalıkların yönetiminde hedeflenen enzim inhibisyonunun önemini vurgulamaktadır. Bu sonuçlara dayanarak yapılacak gelecekteki araştırmalar, daha etkili tedavilerin geliştirilmesine, AD'den etkilenen bireylerin yaşam kalitesinin artırılmasına ve klinik müdahale için yeni yollar sunulmasına yol açabilir.

Anahtar kelimeler: Asetilkolinesteraz, Bütirilkolinesteraz, Alzheimer Hastalığı, Nitrofuran Türevi, Enzim-Ligand Etkileşimleri.

Corresponding Author: Cüneyt TÜRKEŞ, Erzincan Binali Yıldırım Üniversitesi Department of Biochemistry, Faculty of Pharmacy, Erzincan, Türkiye.
E-mail: cuneyt.turkes@erzincan.edu.tr

Cite this article: Kandemir, Ş. & Türkeş, C. (2024). Nitrofurantoin with Anticholinergic Effects: A Different in Vitro Approach to Alzheimer's Disease. *Journal of Erzincan Binali Yıldırım University Health Sciences Institute, 1*(1), 1-12.

1. INTRODUCTION

The global incidence of Alzheimer's disease (AD) is escalating, currently impacting over 50 million individuals (Scheltens et al., 2021). A hallmark of AD pathology is the cholinergic deficit, leading to significant impairments in memory, cognition, and behavior (Hampel et al., 2018). This deficit arises from the hydrolytic breakdown of acetylcholine by cholinesterase enzymes, culminating in cognitive dysfunction characteristic of AD (Bandyopadhyay, 2021). Present therapeutic strategies, including donepezil, galanthamine, rivastigmine, and tacrine (THA), aim to mitigate these symptoms by inhibiting cholinesterases (ChEs) and enhancing cholinergic transmission in the synaptic cleft (Bortolami et al., 2021) (Figure 1). The human brain expresses two principal cholinesterase enzymes: acetylcholinesterase (AChE, EC 3.1.1.7) (Wu et al., 2020) and butyrylcholinesterase (BChE, EC 3.1.1.8) (Türkan, 2021), which share a 65% sequence homology and feature a 20 Å deep hydrophobic active site gorge (Xing et al., 2021). Structural differences between these enzymes account for their distinct substrate specificities, influenced by variations in the amino acid sequences within their active sites (De Boer et al., 2021). The AChE active site is further subdivided into the peripheral anionic site (PAS) and the catalytic anionic site (CAS), with PAS playing a role in allosteric regulation and amyloid-beta (Aβ) aggregation (Roca et al., 2018). BChE, predominantly found in blood plasma and, to a lesser extent, in the brain, primarily resides in glial cells and participates in cholinergic modulation (Rossi et al., 2021). Notably, BChE activity is elevated in AD brains' hippocampus and temporal cortex, where AChE levels are conversely reduced (Reid & Darvesh, 2024). This increased BChE activity with age, particularly in AD, contrasts with the stable activity levels of AChE (Li et al., 2021). Research has demonstrated that BChE inhibition correlates with memory improvements in AD patients (Li et al., 2020). Initially, AChE inhibition was the primary focus for AD treatment, but recent studies underscore the advantages of dual inhibition of both AChE and BChE (AlFadly et al., 2019). Given BChE's significant role in cholinergic regulation and AD progression, dual inhibition is poised to offer long-term therapeutic benefits (Gao et al., 2021). Consequently, numerous dual inhibitors have been developed over the past decade, reflecting a promising avenue for enhanced AD treatment outcomes (Turgutalp et al., 2022).

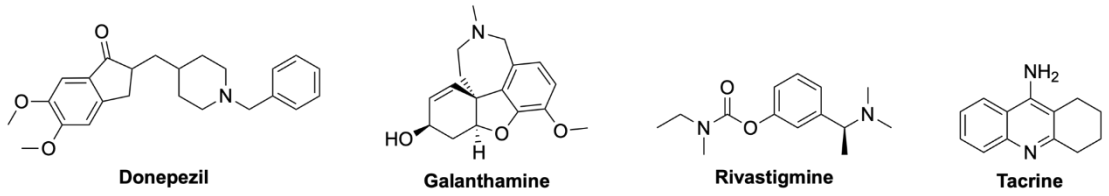

Figure 1. Depictions of some cholinesterase inhibitors are employed in the pharmacotherapy of AD.

Although 5-nitrofurans (NFs) have been utilized for over six decades due to their broad-spectrum efficacy, recent years have seen limited advancements in developing new clinical variants (Bailly, 2019). Historically, NFs have found applications in both animal feed and pharmaceuticals (Suarez-Torres et al., 2021). However, prior to 1995, these compounds were widely employed as feed additives in the livestock industry to enhance growth in poultry, pigs, and cattle, and were also utilized in aquaculture and bee colonies. (Mologni et al., 2021). Clinically, NFs function as broad-spectrum redox-active antibiotics (Zuma et al., 2020), exhibiting bacteriostatic or bactericidal effects against both Gram-positive and Gram-negative bacteria (Lewkowski et al., 2019). Additionally, derivatives of 5-nitrofuraldehyde, known as Schiff bases, demonstrate efficacy against a variety of pathogens, including tuberculosis (Kumar Sahoo et al., 2022), malaria (Melekhin et al., 2021), leishmaniasis (Kannigadu et al., 2022), trypanosomiasis (Foscolos et al., 2016), urinary tract infections (UTIs) (Gallardo-Garrido et al., 2020), and even cancer (Ding et al., 2020). These yellow, crystalline agents are aromatic xenobiotic compounds characterized by a nitro group attached to a furan ring (Penning et al., 2022). Notably, NFs possess a secondary pharmacophore, a hydrazone moiety, which features zwitterionic properties that contribute to the chemical stability of the nitrofuran ring (Ndlovu et al., 2023) (Figure 2).

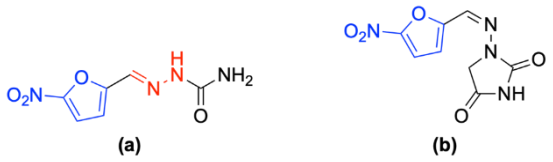

Figure 2. (a) Diagram showcasing the pharmacophoric groups found in 5-nitrofuran medications. (b) Structure of nitrofurantoin.

The hydrazone group exhibits significant anti-pathogenic properties (El-Wakil et al., 2021), including antituberculosis (Yan et al., 2020), antibacterial (Popiołek et al., 2020), anti-trypanosomal (Fernando da Silva Santos-Júnior et al., 2022), and anticancer activities (Mohamed et al., 2023). Specifically, the anticancer efficacy of hydrazones against breast

carcinoma is primarily mediated through inhibiting the phosphoinositide 3-kinase pathway. This pathway plays a vital role in cancer cell survival by facilitating the phosphorylation of lipids within cell membranes (Mushtaq et al., 2024) (Figure 3).

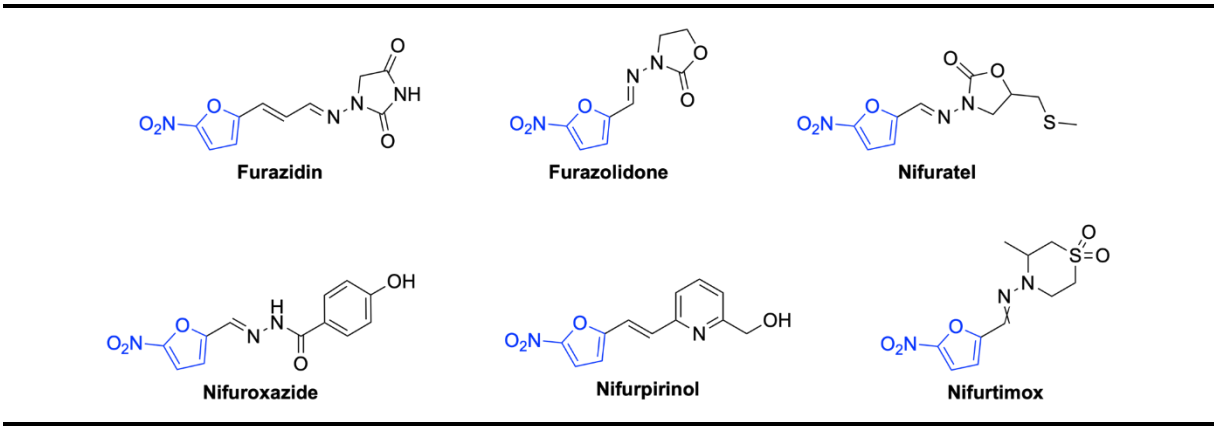

Figure 3. Structures of some clinical nitrofuran derivatives.

Nitrofurantoin (see Figure 2b) is a cyclic amide derived from NF, primarily employed for treating UTIs (Konwar et al., 2022). Its efficacy against UTIs is notably heightened under acidic conditions (Vasudevan et al., 2020). This drug is formulated as a suspension within a hydrophilic methylcellulose medium, making it suitable for pediatric and geriatric populations (Huang et al., 2022). Additionally, nitrofurantoin acts as a hypoxic agent (Munsimbwe et al., 2021), suggesting its potential applicability in combating anaerobic pathogens, including latent tuberculosis (Kalinin et al., 2021). There have been reports of its activity against Mycobacterium tuberculosis, with an MIC_{50} in the micromolar range (Murugasu-Oei & Dick, 2000). Notably, despite being in use for over fifty years, significant resistance to this medication has not emerged, likely due to its action on multiple biological targets (Uddin et al., 2021).

The simultaneous administration of bioactive macromolecules that exhibit complementary pharmacophoric attributes or distinct mechanisms of action frequently results in synergistic outcomes. Expanding on earlier findings, the current investigation offers a comprehensive *in vitro* analysis of nitrofurantoin, particularly emphasizing its interactions with AChE and BChE. This specific drug has not been extensively studied regarding its relationship with ChEs. The findings aim to furnish a thorough understanding of the interactions between nitrofurans and ChEs, which is essential for advancing novel therapeutics, clarifying the biochemical mechanisms underlying ChEs, and insight into how this compound influences biomolecular dynamics and particular metabolic pathways. Furthermore, this research will contribute to

refining clinical dosing strategies and underscore the potential for drug interactions when these antibiotics are co-administered with other therapeutic agents.

2. MATERIALS and METHODS

1.1. General Information

The substrates, acetylthiocholine iodide (AChI, Sigma A5751, PubChem CID: 74629) and butyrylthiocholine iodide (BChI, Sigma B3253, PubChem CID: 74630), 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB, Sigma D8130, PubChem CID: 6254), AChEs (Sigma C2888), BChE (Sigma C1057), and nitrofurantoin (Sigma 46502, PubChem CID: 6604200) were procured from Sigma-Aldrich Chemie GmbH (Germany). The preparation of all assay solutions involved the use of ultra-pure water.

1.2. Biological Screening

The inhibitory efficacy of nitrofurantoin was evaluated quantitatively through an *in vitro* spectrophotometric technique utilizing a modified version of Ellman's method (Ellman et al., 1961). THA (Sigma A3773, PubChem CID: 1549120) was employed as a control for the assay. The experimental procedure involved preparing a reaction mixture consisting of a 50 mM buffer solution, varying concentrations of the test compound, and 10 µL of the ChEs enzyme solution. This mixture was preincubated at 37 °C for 5 minutes. Following this, 10 mM of the AChI or BChI was added to initiate the enzymatic activity, and then 0.5 mM DTNB was included as a chromogenic agent. Absorbance was measured at 412 nm, and each sample was assessed in triplicate. In alignment with our earlier research (Muğlu et al., 2024), Lineweaver-Burk plots (Lineweaver & Burk, 1934; Lolak et al., 2023) (refer to Figure 4) were generated for each antibiotic. The inhibition constants (K_Is) and the types of inhibition were deduced from the collected data. Data evaluation for nitrofurantoin was conducted utilizing GraphPad Prism 10 (GraphPad Software) for MacOS, while K_Is against AChE and BChE were calculated using SigmaPlot 12 (Systat Software) for Windows. Comparative statistical analyses among the datasets were performed employing the extra sum-of-squares F test along with the Akaike Information Criterion method, with a significance level set at $p < 0.05$. Results are presented as mean ± SEM, encompassing 95% confidence intervals.

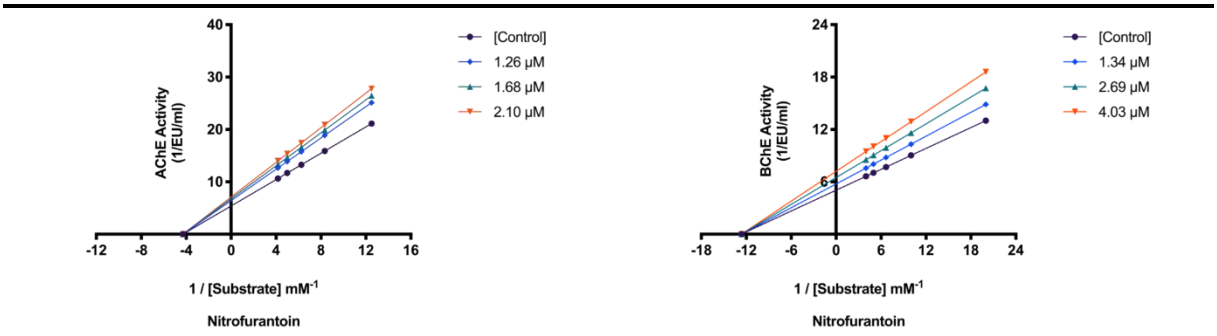

Figure 4. The Noncompetitive Inhibitory Effects of Nitrofurantoin Against AChE and BChE Were Evaluated *In Vitro.*

The Lineweaver-Burk curves were used to determine the K_Is and the types of inhibition caused by this drug. Various concentrations of the nitrofurantoin were examined across five levels using acetylthiocholine iodide (PubChem CID: 74629; 0.080, 0.120, 0.160, 0.200, and 0.240 mM for AChE) and butyrylthiocholine iodide (PubChem CID: 74630; 0.050, 0.100, 0.150, 0.200, and 0.250 mM for BChE) as substrate to assess AChE and BChE activity.

3. RESULTS and DISCUSSION

1.3. Biological Evaluation

The application of nitroheterocyclic drugs (NHCDs) in treating bacterial, protozoal, and cancerous conditions is well-documented. Nitrofurans were among the first NHCDs introduced into chemotherapy and have been widely used for many years. Similar to other NHCDs, their cytotoxicity is believed to result from the reduction of the nitro group, leading to subsequent DNA damage, though the precise mechanism remains partially elucidated (Squella et al., 1996). Consequently, despite extensive experimental research on nitrofurans, they continue to be a focal point of scientific investigation.

In this study, we examined the inhibitory capacity of the 5-nitrofuran analogue, nitrofurantoin, against AChE and BChE enzymes implicated in AD pathology using Ellman's assay. The inhibitory effects of nitrofurantoin were compared to THA, a well-known competitive ChE inhibitor, which was used as a positive control. The calculated K_Is and their respective coefficients of determination (R^2) are presented in Table 1. Nitrofurantoin exhibited mild inhibition of ChEs, with K_I values of 6.77 ± 0.56 µM for AChE and 9.48 ± 0.69 µM for BChE, suggesting it is a weaker inhibitor compared to THA, which has K_I constants of 0.17 ± 0.01 µM for AChE and 0.13 ± 0.01 µM for BChE. Furthermore, nitrofurantoin demonstrated a noncompetitive inhibition mechanism against both AChE and BChE.

Table 1. Inhibition Data of Ache and Bche with Nitrofurantoin Compared to Reference İnhibitor Tacrine

Compounds	**AChE**		**BChE**	
	K_I [a] **(µM)**	R^2	K_I [a] **(µM)**	R^2
Nitrofurantoin	6.77 ± 0.56	0.9824	9.48 ± 0.69	0.9827
Tacrine	0.17 ± 0.01	0.9881	0.13 ± 0.01	0.9877

[a] The analysis outcomes were presented as means of triplicate assays ± SEM.

Despite the extensive body of research examining the effects of various therapeutic drugs on many metabolic enzymes documented in the literature, there remains a notable absence of comprehensive studies specifically targeting nitrofurantoin. This gap is particularly evident in the context of human carbonic anhydrase (*h*CA) VII, a promising molecular target for treating epileptic seizures and other central nervous system disorders due to its nearly exclusive expression in neurons. Gantner et al. (2022) highlight this through their development of an *in silico* protocol using AutoDock to identify new inhibitors for *h*CA VII via virtual screening. Their findings indicate that nitrofurantoin exhibits significant activity versus *h*CA VII at low nanomolar levels and demonstrates a favorable selectivity index for *h*CA VII over *h*CA II. These results underscore the importance of incorporating docking ligand efficiency as a critical selection criterion and highlight nitrofurantoin's potential as a therapeutic agent. This study not only bridges a crucial research gap but also sets a foundation for future investigations into the therapeutic applications of nitrofurantoin, emphasizing the need for more detailed and targeted studies on this drug.

4. CONCLUSION

In conclusion, our comprehensive study underscores the differential inhibitory effects of nitrofurantoin on AChE and BChE, highlighting its potential as a therapeutic agent in the management of AD. Nitrofurantoin demonstrated notable inhibitory activity, with inhibition constants of 6.77 ± 0.56 µM for AChE and 9.48 ± 0.69 µM for BChE. Although its inhibitory potency is less than that of the reference drug THA (KI constants of 0.17 ± 0.01 µM for AChE and 0.13 ± 0.01 µM for BChE), nitrofurantoin's capacity to modulate these key enzymes suggests it could be a valuable candidate for further development. This research significantly contributes to ongoing efforts aimed at developing effective inhibitors for AChE and BChE,

facilitating the optimization of nitrofuran-based therapeutics. Moreover, our findings enhance the understanding of the mechanistic roles these enzymes play in AD pathology, potentially leading to new insights and strategies in treating this debilitating disease. Exploring nitrofurantoin's inhibitory mechanisms could pave the way for novel therapeutic approaches and improve the efficacy of AD management protocols.

Author Contributions

Having an idea/opinion or contributing to the creation and maintenance of the article/work: Ş.K., C.T.; Planning and designing: Ş.K., C.T.; Collection of data or processing of collected data in preparation for analysis: Ş.K., C.T.; Data analysis or interpretation of the analysis: Ş.K., C.T.; Review of the literature: Ş.K., C.T.; Writing the article/study: Ş.K., C.T.; Final checking and review: Ş.K., C.T.

Declaration of Competing Interest

The authors declare no conflict of interest.

Financial Support

No financial support was received for this study.

REFERANCES

AlFadly, E. D., Elzahhar, P. A., Tramarin, A., Elkazaz, S., Shaltout, H., Abu-Serie, M. M., . . . & Belal, A. S. F. (2019). Tackling neuroinflammation and cholinergic deficit in Alzheimer's disease: Multi-target inhibitors of cholinesterases, cyclooxygenase-2 and 15-lipoxygenase. *European Journal of Medicinal Chemistry, 167*, 161-186.

Bailly, C. (2019). Toward a repositioning of the antibacterial drug nifuroxazide for cancer treatment. *Drug Discovery Today, 24*(9), 1930-1936.

Bandyopadhyay, S. (2021). Role of Neuron and Glia in Alzheimer’s Disease and Associated Vascular Dysfunction. *Frontiers in Aging Neuroscience, 13*.

Bortolami, M., Rocco, D., Messore, A., Di Santo, R., Costi, R., Madia, V. N., . . . & Pandolfi, F. (2021). Acetylcholinesterase inhibitors for the treatment of Alzheimer’s disease – a patent review (2016–present). *Expert Opinion on Therapeutic Patents, 31*(5), 399-420.

De Boer, D., Nguyen, N., Mao, J., Moore, J., & Sorin, E. J. (2021). A Comprehensive Review of Cholinesterase Modeling and Simulation. *Biomolecules, 11*(4), 580.

Ding, Z., Li, F., Zhong, C., Li, F., Liu, Y., Wang, S., . . . & Li, W. (2020). Structure-based design and synthesis of novel furan-diketopiperazine-type derivatives as potent microtubule inhibitors for treating cancer. *Bioorganic & Medicinal Chemistry, 28*(10), 115435.

El-Wakil, M. H., Meheissen, M. A., & Abu-Serie, M. M. (2021). Nitrofurazone repurposing towards design and synthesis of novel apoptotic-dependent anticancer and antimicrobial agents: Biological evaluation, kinetic studies and molecular modeling. *Bioorganic Chemistry, 113*, 104971.

Ellman, G. L., Courtney, K. D., Andres Jr, V., & Featherstone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical pharmacology, 7*(2), 88-95.

Fernando da Silva Santos-Júnior, P., Rocha Silva, L., José Quintans-Júnior, L., & Ferreira da Silva-Júnior, E. (2022). Nitro compounds against trypanosomatidae parasites: Heroes or villains? *Bioorganic & Medicinal Chemistry Letters, 75*, 128930.

Foscolos, A.-S., Papanastasiou, I., Foscolos, G. B., Tsotinis, A., Kellici, T. F., Mavromoustakos, T., . . . & Kelly, J. M. (2016). New hydrazones of 5-nitro-2-furaldehyde with adamantanealkanohydrazides: synthesis and *in vitro* trypanocidal activity. *MedChemComm, 7*(6), 1229-1236.

Gallardo-Garrido, C., Cho, Y., Cortés-Rios, J., Vasquez, D., Pessoa-Mahana, C. D., Araya-Maturana, R., . . . & Faundez, M. (2020). Nitrofuran drugs beyond redox cycling: Evidence of Nitroreduction-independent cytotoxicity mechanism. *Toxicology and Applied Pharmacology, 401*, 115104.

Gantner, M. E., Prada Gori, D. N., Llanos, M. A., Talevi, A., Angeli, A., Vullo, D., . . . & Gavernet, L. (2022). Identification of New Carbonic Anhydrase VII Inhibitors by Structure-Based Virtual Screening. *Journal of Chemical Information and Modeling, 62*(19), 4760-4770.

Gao, H., Jiang, Y., Zhan, J., & Sun, Y. (2021). Pharmacophore-based drug design of AChE and BChE dual inhibitors as potential anti-Alzheimer's disease agents. *Bioorganic Chemistry, 114*, 105149.

Hampel, H., Mesulam, M.-M., Cuello, A. C., Farlow, M. R., Giacobini, E., Grossberg, G. T., . . . & Khachaturian, Z. S. (2018). The cholinergic system in the pathophysiology and treatment of Alzheimer's disease. *Brain, 141*(7), 1917-1933.

Huang, L., Huang, C., Yan, Y., Sun, L., & Li, H. (2022). Urinary Tract Infection Etiological Profiles and Antibiotic Resistance Patterns Varied Among Different Age Categories: A Retrospective Study From a Tertiary General Hospital During a 12-Year Period. *Frontiers in Microbiology, 12*.

Kalinin, S., Vedekhina, T., Paramonova, P., & Krasavin, M. (2021). Antimicrobial activity of 5-membered nitroheteroaromatic compounds beyond nitrofurans and nitroimidazoles: Recent progress. *Current Medicinal Chemistry, 28*(29), 5926-5982.

Kannigadu, C., Aucamp, J., & N'Da, D. D. (2022). Exploring novel nitrofuranyl sulfonohydrazides as anti-Leishmania and anti-cancer agents: Synthesis, *in vitro* efficacy and hit identification. *Chemical Biology & Drug Design, 100*(2), 267-279.

Konwar, M., Gogtay, N. J., Ravi, R., Thatte, U. M., & Bose, D. (2022). Evaluation of efficacy and safety of fosfomycin versus nitrofurantoin for the treatment of uncomplicated lower

urinary tract infection (UTI) in women – A systematic review and meta-analysis. *Journal of Chemotherapy, 34*(3), 139-148.

Kumar Sahoo, S., Maddipatla, S., Nageswara Rao Gajula, S., Naiyaz Ahmad, M., Kaul, G., Nanduri, S., … & Madhavi Yaddanapudi, V. (2022). Identification of nitrofuranylchalcone tethered benzoxazole-2-amines as potent inhibitors of drug resistant Mycobacterium tuberculosis demonstrating bactericidal efficacy. *Bioorganic & Medicinal Chemistry*, *64*, 116777.

Lewkowski, J., Rogacz, D., & Rychter, P. (2019). Hazardous ecotoxicological impact of two commonly used nitrofuran-derived antibacterial drugs: Furazolidone and nitrofurantoin. *Chemosphere*, *222*, 381-390.

Li, Q., Chen, Y., Xing, S., Liao, Q., Xiong, B., Wang, Y., . . . & Sun, H. (2021). Highly Potent and Selective Butyrylcholinesterase Inhibitors for Cognitive Improvement and Neuroprotection. *Journal of Medicinal Chemistry, 64*(10), 6856-6876.

Li, Q., Xing, S., Chen, Y., Liao, Q., Xiong, B., He, S., . . . & Sun, H. (2020). Discovery and Biological Evaluation of a Novel Highly Potent Selective Butyrylcholinsterase Inhibitor. *Journal of Medicinal Chemistry, 63*(17), 10030-10044.

Lineweaver, H., & Burk, D. (1934). The determination of enzyme dissociation constants. *Journal of the American chemical society, 56*(3), 658-666.

Lolak, N., Akocak, S., Durgun, M., Duran, H. E., Necip, A., Türkeş, C., . . . & Beydemir, Ş. (2023). Novel bis-ureido-substituted sulfaguanidines and sulfisoxazoles as carbonic anhydrase and acetylcholinesterase inhibitors. *Molecular Diversity, 27*(4), 1735-1749.

Melekhin, A. O., Tolmacheva, V. V., Shubina, E. G., Dmitrienko, S. G., Apyari, V. V., & Grudev, A. I. (2021). Determination of nitrofuran metabolites in honey using a new derivatization reagent, magnetic solid-phase extraction and LC–MS/MS. *Talanta*, *230*, 122310.

Mohamed, M. S., Elamin, K. M., Alenazy, R., Mohamed Eltayib, E., Timan Idriss, M., Alhudaib, N. A. A., . . . & Awadalla Mohamed, M. (2023). Synthesis, Antimicrobial, and Anticancer Activities of Novel Nitrofuran Derivatives. *Journal of Chemistry, 2023*(1), 1481595.

Molognoni, L., Daguer, H., & Hoff, R. B. (2021). Chapter 12 - Analysis of nitrofurans residues in foods of animal origin. In C. M. Galanakis (Ed.), *Food Toxicology and Forensics* (pp. 379-419): Academic Press.

Muğlu, H., Yakan, H., Erdoğan, M., Topal, F., Topal, M., Türkeş, C., & Beydemir, Ş. (2024). Novel asymmetric biscarbothioamides as Alzheimer's disease associated cholinesterase inhibitors: synthesis, biological activity, and molecular docking studies. *New Journal of Chemistry, 759*, 110099.

Munsimbwe, L., Seetsi, A., Namangala, B., N'Da, D. D., Inoue, N., & Suganuma, K. (2021). *In Vitro* and *In Vivo* Trypanocidal Efficacy of Synthesized Nitrofurantoin Analogs. *Molecules, 26*(11), 3372.

Murugasu-Oei, B., & Dick, T. (2000). Bactericidal activity of nitrofurans against growing and dormant Mycobacterium bovis BCG. *Journal of Antimicrobial Chemotherapy, 46*(6), 917-919.

Mushtaq, A., Wu, P., & Naseer, M. M. (2024). Recent drug design strategies and identification of key heterocyclic scaffolds for promising anticancer targets. *Pharmacology & Therapeutics, 254*, 108579.

Ndlovu, K., Kannigadu, C., Aucamp, J., van Rensburg, H. D. J., & N'Da, D. D. (2023). Exploration of ethylene glycol linked nitrofurantoin derivatives against Leishmania: Synthesis and *in vitro* activity. *Archiv der Pharmazie, 356*(5), 2200529.

Penning, T. M., Su, A. L., & El-Bayoumy, K. (2022). Nitroreduction: A Critical Metabolic Pathway for Drugs, Environmental Pollutants, and Explosives. *Chemical Research in Toxicology, 35*(10), 1747-1765.

Popiołek, Ł., Rysz, B., Biernasiuk, A., & Wujec, M. (2020). Synthesis of promising antimicrobial agents: hydrazide-hydrazones of 5-nitrofuran-2-carboxylic acid. *Chemical Biology & Drug Design, 95*(2), 260-269.

Reid, G. A., & Darvesh, S. (2024). Interaction of exogenous acetylcholinesterase and butyrylcholinesterase with amyloid-β plaques in human brain tissue. *Chemico-Biological Interactions, 395*, 111012.

Roca, C., Requena, C., Sebastián-Pérez, V., Malhotra, S., Radoux, C., Pérez, C., . . . & Campillo, N. E. (2018). Identification of new allosteric sites and modulators of AChE through computational and experimental tools. *Journal of Enzyme Inhibition and Medicinal Chemistry, 33*(1), 1034-1047.

Rossi, M., Freschi, M., de Camargo Nascente, L., Salerno, A., de Melo Viana Teixeira, S., Nachon, F., . . . & Bolognesi, M. L. (2021). Sustainable Drug Discovery of Multi-Target-Directed Ligands for Alzheimer's Disease. *Journal of Medicinal Chemistry, 64*(8), 4972-4990.

Scheltens, P., De Strooper, B., Kivipelto, M., Holstege, H., Chételat, G., Teunissen, C. E., . . . & van der Flier, W. M. (2021). Alzheimer's disease. *The Lancet, 397*(10284), 1577-1590.

Squella, J. A., Letelier, M. E., Lindermeyer, L., & Nuñez-Vergara, L. J. (1996). Redox behaviour of nifuroxazide: generation of the one-electron reduction product. *Chemico-Biological Interactions, 99*(1), 227-238.

Suarez-Torres, J. D., Orozco, C. A., & Ciangherotti, C. E. (2021). The numerical probability of carcinogenicity to humans of some antimicrobials: Nitro-monoaromatics (including 5-nitrofurans and 5-nitroimidazoles), quinoxaline-1,4-dioxides (including carbadox), and chloramphenicol. *Toxicology in Vitro, 75*, 105172.

Turgutalp, B., Bhattarai, P., Ercetin, T., Luise, C., Reis, R., Gurdal, E. E., . . . & Yarim, M. (2022). Discovery of Potent Cholinesterase Inhibition-Based Multi-Target-Directed Lead Compounds for Synaptoprotection in Alzheimer's Disease. *Journal of Medicinal Chemistry, 65*(18), 12292-12318.

Türkan, F. (2021). Investigation of the toxicological and inhibitory effects of some benzimidazole agents on acetylcholinesterase and butyrylcholinesterase enzymes. *Archives of Physiology and Biochemistry*, *127*(2), 97-101.

Uddin, T. M., Chakraborty, A. J., Khusro, A., Zidan, B. M. R. M., Mitra, S., Emran, T. B., . . . & Koirala, N. (2021). Antibiotic resistance in microbes: History, mechanisms, therapeutic strategies and future prospects. *Journal of Infection and Public Health*, *14*(12), 1750-1766.

Vasudevan, S., Thamil Selvan, G., Bhaskaran, S., Hari, N., & Solomon, A. P. (2020). Reciprocal Cooperation of Type A Procyanidin and Nitrofurantoin Against Multi-Drug Resistant (MDR) UPEC: A pH-Dependent Study. *Frontiers in Cellular and Infection Microbiology*, *10*.

Wu, J., Pistolozzi, M., Liu, S., & Tan, W. (2020). Design, synthesis and biological evaluation of novel carbamates as potential inhibitors of acetylcholinesterase and butyrylcholinesterase. *Bioorganic & Medicinal Chemistry*, *28*(5), 115324.

Xing, S., Li, Q., Xiong, B., Chen, Y., Feng, F., Liu, W., & Sun, H. (2021). Structure and therapeutic uses of butyrylcholinesterase: Application in detoxification, Alzheimer's disease, and fat metabolism. *Medicinal Research Reviews*, *41*(2), 858-901.

Yan, M., Xu, L., Wang, Y., Wan, J., Liu, T., Liu, W., . . . & Li, Q. (2020). Opportunities and challenges of using five-membered ring compounds as promising antitubercular agents. *Drug Development Research*, *81*(4), 402-418.

Zuma, N. H., Smit, F. J., Seldon, R., Aucamp, J., Jordaan, A., Warner, D. F., & N'Da, D. D. (2020). Single-step synthesis and *in vitro* anti-mycobacterial activity of novel nitrofurantoin analogues. *Bioorganic Chemistry*, *96*, 103587.