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Synthesis, Docking Study and Biological Evaluation of Naproxen-Based Heterocyclic Derivatives

A series of Naproxen-based heterocyclic derivatives (NA1-NA4) were designed, synthesized, and evaluated for their antibacterial and anticancer activities. These heterocyclic derivatives were developed by integrating Naproxen with various heterocycles, including indole, benzothiophene, benzothiazole, and pyrazole, in order to enhance efficacy while reducing gastrointestinal side effects. The synthesized compounds were characterized using FT-IR, 1H NMR, and 13C NMR spectroscopy. The antibacterial activity was evaluated against *S. aureus* (Gram-positive) and two Gram-negative bacteria (*P. aeruginosa* and *K. pneumonia*) by measuring the diameter of the zone of inhibition. Compounds NA2 and NA3 showed promising inhibitory activity against the tested bacteria compared to amoxicillin. The anticancer activity of NA1-NA4 compounds against the MDA-MB-231 human breast cancer cell line was assessed by determining the IC50 values (the concentration required to inhibit 50 % of cell viability). NA1 and NA3 exhibited notable antiproliferative effects with IC50 values of 11.81 and 11.08 μg/mL, respectively. Molecular docking studies of compounds NA1-NA4 were performed against COX-2 enzyme (PDB Code: 3NT1) using MOE software. The compounds showed strong binding affinities, indicating potential anti-inflammatory properties. Collectively, the antibacterial, anticancer, and molecular docking data suggest that these Naproxen derivatives possess promising multifunctional therapeutic potential.

Keywords: Naproxen derivatives, heterocyclic compounds, COX-2 inhibition, MTT assay, antibacterial, anticancer, breast cancer, anti-inflammatory properties

Introduction

Cancer is characterized by the rapid growth of abnormal cells that can spread to other parts of the body, eventually leading to death [1]. Globally, cancer has become the leading cause of mortality, with the number of deaths projected to reach 16.4 million by 2040 [2]. It is estimated that 30–50 % of cancer-related deaths could be prevented through early detection, effective treatment, and long-term care. Breast, lung, and colorectal cancers currently represent the most prevalent cancer types based on incidence rates. In terms of treatment, cancer has remained at the forefront of advancements in surgery, radiotherapy, chemotherapy, and hormonal therapies. However, most currently available anticancer agents are associated with dose-limiting toxicity, drug resistance, and limited selectivity [3]. Therefore, the development of new chemotherapeutic agents capable of overcoming these challenges is of critical importance.

Naproxen is both a COX inhibitor and a strong anti-inflammatory agent. However, one of the most common problems associated with oral Naproxen administration is gastrointestinal disorders [4, 5]. This adverse effect is primarily attributed to the presence of a free carboxylic acid group. Therefore, masking this acidic group has been proposed as an effective strategy to reduce or eliminate gastrointestinal side effects [6]. It has been reported that the therapeutic index for NSAIDs can be improved by synthesizing ester prodrugs of Naproxen, which serve as promoieties [7]. Compounds containing naproxen amide have also shown consistent anti-inflammatory properties [8]. Additionally, it has been observed that amide derivatives of Naproxen, such as Naproxen glycolamide, exhibit anti-inflammatory activity with significantly less gastric damage compared to the parent drug [9]. Compared to conventional Naproxen, amide prodrugs of Naproxen derivatives have demonstrated strong anti-inflammatory activity [10].

The anticancer properties of Naproxen and its derivatives have also been extensively studied, with several derivatives reported to inhibit the proliferation of various cancer cell lines [11, 12]. For instance, Naproxen derivatives such as propanamide and urea analogues have shown promising activity in suppressing colon cancer growth [13]. Moreover, 1,3,4-oxaadiazole derivatives exhibit significant activity against epidermal growth factor receptor (EGFR) [14], while hydrazide and hydrazone derivatives display potent inhibition of vascular endothelial growth factor receptor-2 (VEGFR-2) [15]. Triazole derivatives have also been found to effectively target histone deacetylases (HDACs) [16]. Naproxen's propanamide derivatives have demonstrated notable antimicrobial activity against both Gram-positive and Gram-negative bacteria, including *Escherichia coli* and *Pseudomonas aeruginosa* [10, 17]. These antibacterial effects are comparable to those of standard antibiotics such as ciprofloxacin (for Gram-negative bacteria) and ampicillin (for Gram-positive bacteria) [18]. Furthermore, Naproxen administration has been shown to reduce tumor progression in tumor-bearing rats [11, 12]. Strong suppression of histone deacetylase has been demonstrated by a naproxen hydroxamic acid derivative [5]. Overall, Naproxen represents a promising scaffold for the development of novel therapeutic agents with antiviral, anticancer activities [17, 19, 20], including potential applications in bladder cancer prevention [21].

Heterocycles are considered a vital component in medicinal chemistry. Benzofused and five-membered heterocycles motifs, in particular, represent a significant class of compounds with well-documented anticancer and antibacterial activities [22–25]. Derivatives of benzothiazole, benzothiophene, and indole, for example, have demonstrated notable antimicrobial and antitumor properties [26].

Based on the importance of these heterocycles, we designed a new series of Naproxen-based heterocyclic derivatives. The heterocycles were conjugated to the Naproxen core via its carboxylic acid moiety, aiming to mask this functional group and thereby reduce its associated gastrointestinal side effects. The synthesized analogs were evaluated for their anticancer and antibacterial activities (Fig. 1). Furthermore, molecular docking study was conducted to assess the potential of these compounds as analgesic and anti-inflammatory agents.

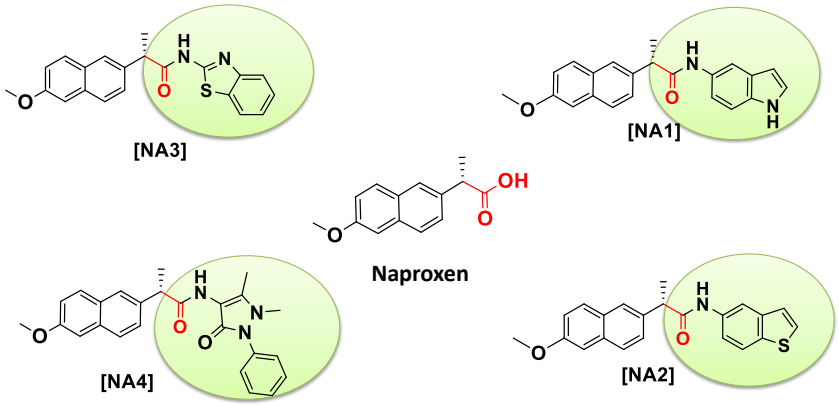


Figure 1. Chemical structures of the newly designed Naproxen-based heterocyclic analogues (NA1–NA4)

Experimental

Material and Methods

All chemical reagents used in this study were of analytical grade and were supplied by Sigma-Aldrich, Fluka, CDH, and Thomas Baker. Naproxen was purchased from Leyan Company (China). Melting points were determined using a Gallenkamp MFB-600 melting point apparatus (Stuart). FT-IR spectra were recorded on a Shimadzu IRAffinity-1S spectrometer. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker Multinuclear Spectrometer.

Synthesis of Naproxen acid chloride [27]

To a stirred solution of Naproxen (1.0 g, 4.34 mmol, 1 equiv.) in dichloromethane (DCM, 20 mL), phosphorous trichloride (128 µL, 1.450 mmol, 0.33 equiv.) was added. The resulting mixture was stirred at 0 °C for one hour, then allowed to warm to room temperature (RT) and stirred overnight. The solution was then transferred to a 50 mL round-bottom flask to remove the precipitated phosphorous acid. Thin-layer

chromatography (TLC) was performed to confirm the complete consumption of Naproxen. The resulting mixture was used directly in the subsequent steps without further workup.

Synthesis of (N-(1H-indol-5-yl)-2-(6-methoxynaphthalen-2-yl) propenamide [NA1]

To a solution of acid chloride, [27], in DCM, (0.57 g, 4.34 mmo of 1l)H-indol-5-amine and triethylamine (0.73 ml, 5 mmol) were added with stirring at RT for 18 hours. Upon completion of the reaction, the organic layer was removed. The crude product was dissolved in 50 mL of ethyl acetate, then washed sequentially with 50 mL of 5 % NaOH solution and 50 mL of 2 N HCl. The organic phase was dried over anhydrous magnesium sulfate, and the solvent was removed under reduced pressure using a rotary evaporator. The desired product was obtained as a pink solid (0.96 g, 64 % yield).

FT-IR ν_{max}, cm^{-1}: 3211 (N–H, and NH, Amide), 3066 and 3024 (C–H, aromatic), 2929 (C–H, aliphatic), 1653 (CO, Amide), 1595–1435 (aromatic rings); ^{1}H NMR (500 MHz, DMSO), ppm: 10.65 (s, 1H, CO–NH–), 9.68 (s, 1H, Ar–NH–), 7.88–7.26 (11 H, Ar–H), 3.97 (s, 3H, O–CH_3), 3.91 (m, 1H, CO–CH–), 1.57 (d, J = 1 Hz, 3H, –CH_3). ^{13}C NMR (125 MHz), ppm: 172.48 (1C, CO), 152.79 (1C, MeO–C), 132.63–101.06 (17C, Ar–C), 51.21 (1C, –OCH_3), 33.30 (1C, Ar (Me)–CH–CO), 14.14 (1C, –CH_3) (Figures S1-S3).

Synthesis of N-(benzo[b]thiophen-5-yl)-2-(6-methoxynaphthalen-2-yl) propenamide [NA2]

To a pre-cooled solution of NAC in DCM, benzo[b]thiophen-5-amine (0.647 g, 4.34 mmol) and triethylamine (0.73 mL, 5.00 mmol) were added and left to stir at RT for 18 hours.

After completion of the reaction, the solvent was evaporated, and the crude product was dissolved in 50 mL of ethyl acetate. The solution was washed sequentially with 50 mL of 5 % NaOH solution, 50 mL of 2 N HCl, and a saturated NaCl solution. The organic layer was dried over anhydrous magnesium sulfate, and the solvent was removed under reduced pressure using a rotary evaporator, affording the product as brown crystals (1.2 g, 76 % yield). FT-IR ν_{max}, cm^{-1}: 3255 (–NH, Amide), 3064 (C–H, aromatic), 1662 (CO, Amide), 1556–1435 (aromatic carbons). ^{1}H NMR (500 MHz, DMSO), ppm: 10.25 (s, 1H, CO–NH–), 7.81–7.14 (m, 11 H, Ar–H), 3.86 (s, 3H, methoxy), 3.81 (*d*, 1H, J = 1 Hz, CO–CH–), 1.45 (d, J = 2 Hz, 3H, –methyl). ^{13}C NMR (125 MHz), ppm: 173.22 (1C, CO), 157.09 (1C, Methoxy-C), 145–105.69 (17C, Aromatic-C), 55.12 (1C, Methoxy), 44.64 (1C, Aromatic (Me)-CH-CO), 18.44 (1C — Methyl) (Figures S4-S6).

Synthesis of N-(benzo[d]thiazol-2-yl)-2-(6-methoxynaphthalen-2-yl)propanamide [NA3]

To a pre-cooled [27] solution in DCM, (0.65 g, 4.34 mmol) of benzo[d]thiazol-2-amine and (0.73 ml, 5 mmol) of triethylamine was added and left to stir at RT for 18 hours. After completion of the reaction, the solvent was evaporated, and the crude product was recrystallized from 70 % ethyl acetate/hexane mixture (50 mL of ethyl acetate) to afford off-white crystals (0.80 g, 50 % yield). FT-IR ν_{max}, cm^{-1}: 3343 (–NH, Amide), 2953, 2908, and 2868 (C–H, aliphatic), 1666 (CO, Amide), 1606-1427 (aromatic rings); ^{1}H NMR (500 MHz, DMSO), ppm: 12.52 (s, 1H, amide NH), 7.98–7.12 (m, 11 H, aromatic-H), 3.95 (s, 1H, –CH–), 3.86 (s, 3H, methoxy), 1.53 (d, J = 2 Hz, 3H, methyl). ^{13}C NMR (125 MHz), ppm: 175.48 (thiazole-C–NH–), 172.69 (1C, CO) 157.51 (1C, methoxy–C), 150.38-104.56 (16 C, aromatic–C), 56.16 (1C, –methoxy), 45.02 (1C, Ar(methyl)–CH–CO), 19.11 (1C, methyl) (Figures S7-S9).

Synthesis of N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-2-(6-methoxynaphthalen-2-yl) propanamide [NA4]

To a pre-cooled [27] solution in DCM, (0.88 g, 4.34 mmol) of 4-Aminoantipyrine and (0.73 ml, 5 mmol) of triethylamine were added and the reaction mixture was stirred at RT for 18 hours. After completion of the reaction, the solvent was evaporated, and the crude product was dissolved in 50 mL of ethyl acetate, washed successively with 5 % NaOH solution (50 mL) and 2 N HCl (50 mL). The organic layer was then washed with saturated NaCl solution, dried over anhydrous magnesium sulfate, and concentrated using a rotary evaporator to afford the desired product as brown crystals (1.50 g, 83 % yield). FT-IR ν_{max}, cm^{-1}: 3257 (–NH, Amide), 3043 (C–H, Aromatic) 2972 and 2920 (C–H, aliphatic), 1660 (CO, Amide), 1620–1427 (aromatic rings); ^{1}H NMR (500 MHz, DMSO): 9.01 (s, 1H, amide-NH–), 8.00–7.37 (m, 11 H, aromatic-H), 3.25 (s, 3H, methoxy-CH_3), 3.09 (q, J = 2 Hz, 1H,), 2.25 (s, 3H, methyl), 2.23 (s, 3H, methyl), 1.18 (d, J = 2 Hz, 3H, methyl). ^{13}C NMR (125 MHz), ppm: 172.97 (1C, CO–NH), 168.64 (CO), 158.16 (1C, MeO–C), 134–116.20 (18 C, Ar–C), 58.28 (1C, –OCH_3), 46.10 (1C, Ar(Me)–CH–CO), 24.17, 16.20, and 12.96 (3C, 3 CH_3) (Figures S10–S12).

Antibacterial Activity

The antibacterial activity of the synthesized compounds (50 mg/mL) was evaluated against three pathogenic bacterial strains, namely *S. aureus* (Gram-positive) and *P. aeruginosa* and *E. coli* (Gram-negative). Dimethyl sulfoxide (DMSO) was used as a negative control, and amoxicillin at a concentration of 50 μg/mL was used as the standard reference drug. All tests were performed in duplicate at 37 °C. After 24 hours of incubation, the diameter of the inhibition zone was measured and recorded.

Culture and Cell Lines

The human breast cancer cell line (MDA-MB-231) was obtained from the Pasteur Institute, Iran. The cells were cultured in RPMI-1640 medium (Gibco) supplemented with 10 % fetal bovine serum (FBS), penicillin (100 U/mL**)**, and streptomycin (0.1 mg/mL). The cancer cells were incubated under standard conditions: humidified atmosphere with 5 % CO_2 at 37 °C.

MTT Test for Cell Viability in MCF7 Cells

To evaluate cell viability, the MTT assay was performed following standard protocols described by Wang, Rui, et al. and Mahdi, Zainab H., et al. [28, 29]. The absorbance was measured at 570 nm using an ELISA microplate reader. The results were expressed as IC_{50} values, determined as the concentration of compound required to cause 50 % inhibition of cell viability.

Molecular Docking Studies

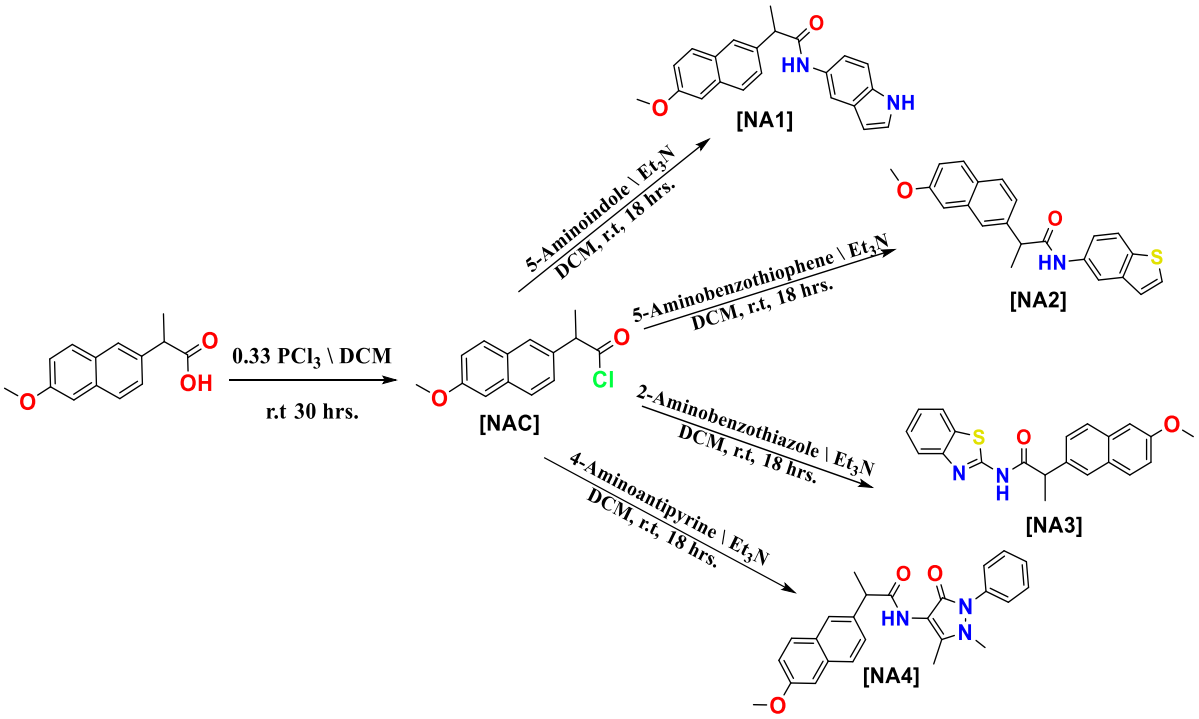
Molecular docking facilitates understanding of ligand-enzyme interactions, predicts the binding conformations of ligands within a protein's active site, and assists in the design of novel, potent inhibitors. In this study, molecular docking analysis was performed using MOE docking software (version 2019) [30] to evaluate the binding behavior of the naproxen-COX-2 complex (oxidoreductase, PDB Code: 3NT1) [31]. The protein structure files were obtained from the Protein Data Bank (PDB). Water molecules (H_2O) were removed prior to docking to avoid potential interference with ligand binding. The docking analysis using MOE revealed key insights into the position, size, and characteristics of the COX-2 binding site within the 3NT1 structure, which are critical parameters for the development of selective COX-2 inhibitors [32]. The COX-2 active site is located within a hydrophobic pocket that plays a crucial role in ligand accommodation. This binding cavity is sufficiently spacious to allow the binding of structurally diverse ligands [30]. Ser530, Val523, and Leu531 were identified as the key amino acid residues involved in ligand-enzyme interactions, contributing significantly to ligand specificity and binding affinity [33]. All compounds were sketched and cleaned using ChemDraw 22.2.0, and geometry optimization was carried out using MOE software prior to docking. The docking scores and binding interactions of the designed and synthesized compounds are summarized in Table 3.

Results and Discussion

Chemistry

The newly synthesized compounds (NA1-NA4) were obtained via direct amidation of various aromatic amines with the acyl derivative of Naproxen, as illustrated in Scheme. In the first step of the synthesis, Naproxen acyl chloride was prepared by treating naproxen with phosphorous trichloride (PCl_3) in dichloromethane (DCM) at RT for 30 hours. Completion of the reaction was confirmed by the formation of a white solid precipitate of phosphorous acid, and by monitoring the complete consumption of Naproxen using thin-layer chromatography (TLC). After formation, the Naproxen acyl chloride solution was cooled, and the solution of the respective amine was added dropwise under continuous stirring. The mixture was stirred for 18 hours, which was also the maximum reaction time required for the synthesis of compound NA4.

The structures of compounds NA1-NA4 were confirmed by spectroscopic analysis. The FT-IR spectra of all synthesized compounds showed the disappearance of the broad bands corresponding to the COOH group and the carbonyl of Naproxen, and the appearance of characteristic amide (NH) bands at 3211, 3343, 3255, and 3257 cm^{-1}, along with amide carbonyl (C=O) stretches at 1653, 1662, 1666, and 1660 cm^{-1}, respectively. The ^1H NMR spectra exhibited characteristic amide proton signals at 10.65, 10.25, 12.52, and 9.01 ppm, respectively, with the disappearance of the broad signal previously attributed to the carboxylic acid proton of Naproxen. Additionally, the ^{13}C NMR spectra showed distinct signals for the amide carbonyl carbons at 172.48, 173.22, 172.29, and 172.97 ppm.



Scheme. Synthesis of compounds NA1-NA4

Antibacterial Activity

A solution of each synthesized compound (NA1-NA4) at a concentration of 5 μg/mL in 1 mL of DMSO was prepared to evaluate the preliminary antibacterial activity. The susceptibility testing technique [34] was employed against *E. Coli*, *P. aeruginosa*, *Proteus*, *K. pneumonia* and *S. aureus*.

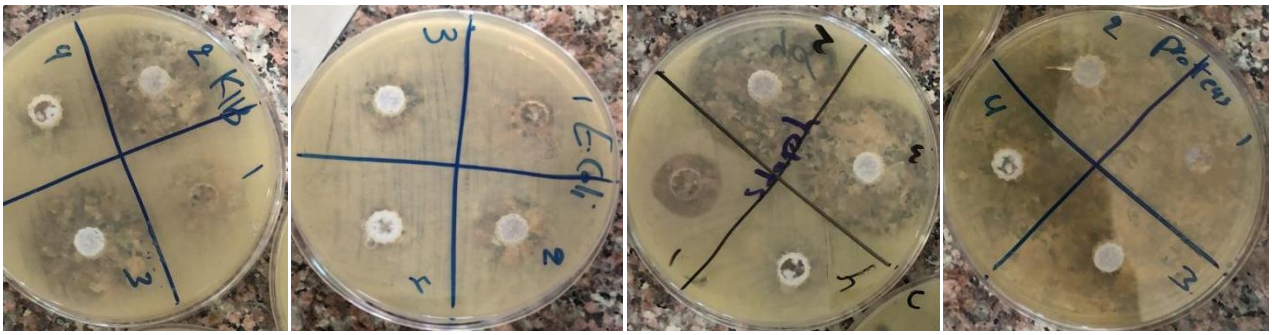
Furthermore, dimethyl sulfoxide was used as a negative control, as it has no effect on bacterial growth. The antibacterial activity of each compound is expressed by the diameter of the inhibition zone (in millimeters, mm), as shown in Table 1 and Figure 2.

Table 1

The diameter of inhibition zone of compounds (NA1–NA4) against *P. aeruginosa, E. Coli, K. pneumonia, Proteus* and *S. aureus*

Sample code	*P. aeruginosa*	*E. Coli*	*K. pneumoniae*	*Proteus*	*S. aureus*
NA1	12	20	13	0	12
NA2	26	20	22	10	30
NA3	32	16	30	18	35
NA4	13	10	0	0	5
Amoxicillin	28	26	20	16	30

Compounds NA2 and NA3 exhibited the highest antibacterial activity among the tested compounds, particularly against *P. aeruginosa*, *K. pneumonia*, and *S. aureus*. The inhibition zone diameters for NA2 were 26 mm, 22 mm, and 30 mm, respectively, while those for NA3 were 32 mm, 30 mm, and 35 mm, demonstrating comparable or even superior activity to the standard drug amoxicillin. This enhanced activity may be attributed to the presence of sulfur-containing heterocyclic moieties, which are known to contribute significantly to antimicrobial efficacy [35].

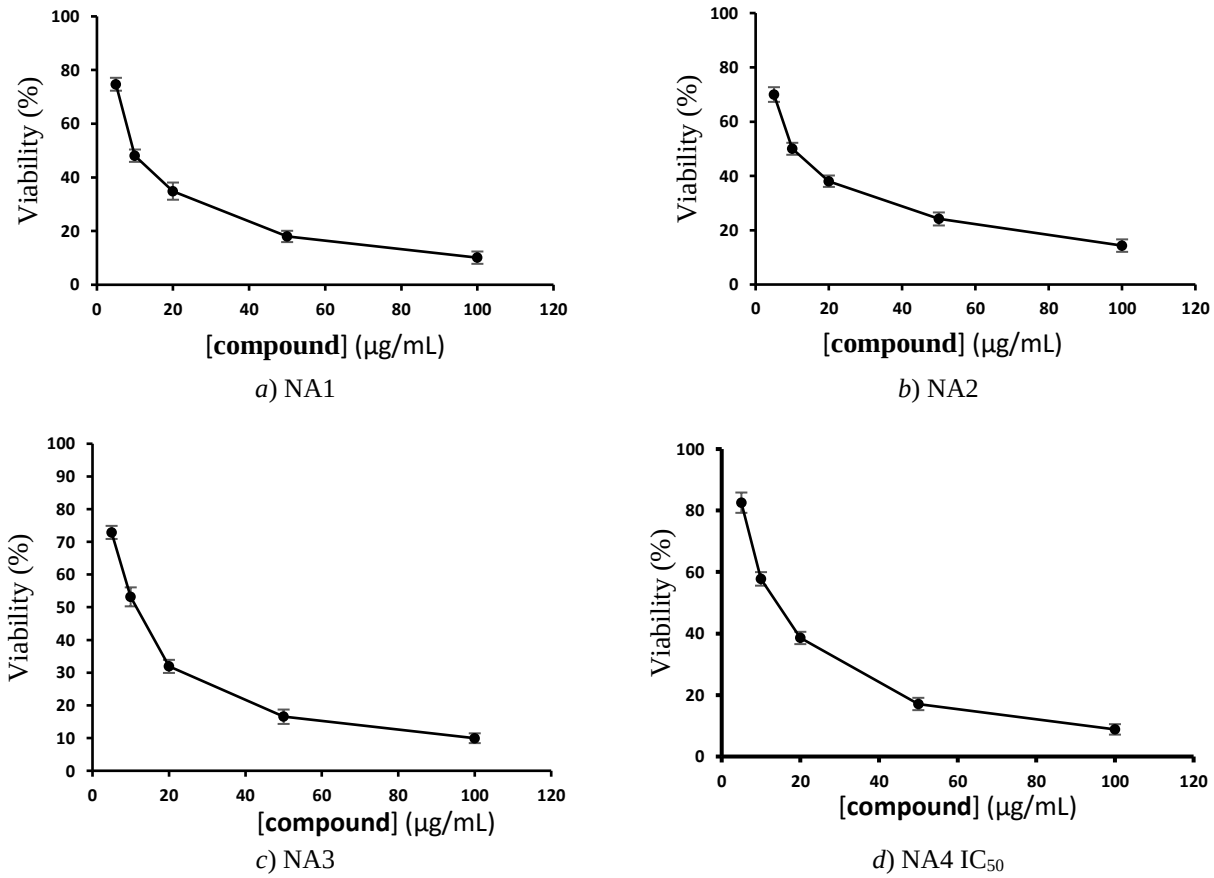


1 — NA1; *2* — NA2; *3* — NA3; *4* — NA4

Figure 2. Antibacterial activity of NA1–NA4

Cell Viability Assay

Based on the results, the synthesized compounds NA1-NA4 exhibited notable cytotoxic activity against the selected cancer cell line when compared to the standard drug, doxorubicin. Compounds NA1 and NA3 demonstrated potent inhibitory effects, with IC_{50} values of 11.81 μg/mL and 11.08 μg/mL, respectively. Compounds NA2 and NA4 also showed considerable inhibitory activity, both with IC_{50} values of 12.05 μg/mL, as presented in Figure 3A–D.



a) NA1 *b*) NA2

c) NA3 *d*) NA4 IC_{50}

Figure 3. Cell viability diagram for compound NA1 (*a*), NA2 (*b*), NA3 (*c*), and NA4 (*d*)

Table 2

IC_{50} values for the tested compounds compared to the standard

Compound	MDA-MB-231
	IC50 (μM)
Standard (Doxorubicin)	9.81
NA1	11.81
NA2	12.05
NA3	11.08
NA4	15.16

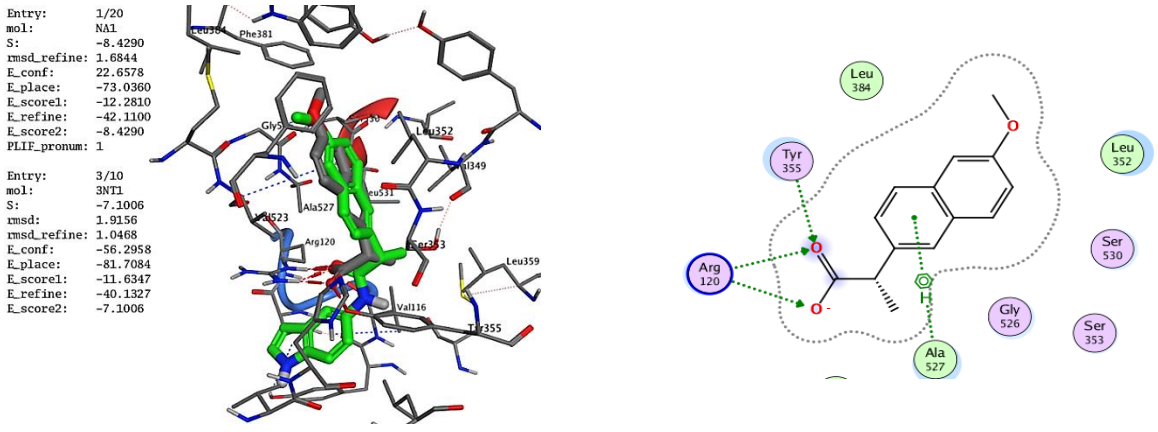
Molecular Docking Studies

Molecular docking simulations were performed to evaluate the potential anti-inflammatory activity of the synthesized analogues (NA1–NA4) against COX-2. The study employed Molecular Operating Environment (MOE) software, which enables detailed visualization, characterization, and assessment of protein-ligand interactions. MOE provided detailed information on the ligand positioning and key interactions with receptor-binding residues, along with high-quality graphical representations. Docking analysis revealed that the proposed compounds (NA1-NA4) bind at the same active site as Naproxen within the COX-2 enzyme (oxidoreductase), with additional interactions observed at conserved residues of the Naproxen-binding pocket. The newly synthesized compounds exhibited improved binding affinities, with S-scores ranging from — 8.7078 to–8.3094 kcal/mol, and low root-mean-square deviation (RMSD) values ranging from 1.0012 to 1.6844, in comparison to Naproxen, which showed a binding energy of –7.1006 kcal/mol and RMSD value of 1.9156. These results suggest that the designed derivatives possess a stronger binding affinity toward the COX-2 active site, potentially enhancing their anti-inflammatory efficacy. Furthermore, the higher structural congruency of these ligands with the target site as visualized in Figures 4–8 supports their improved binding performance, as summarized in Table 3.

Table 3

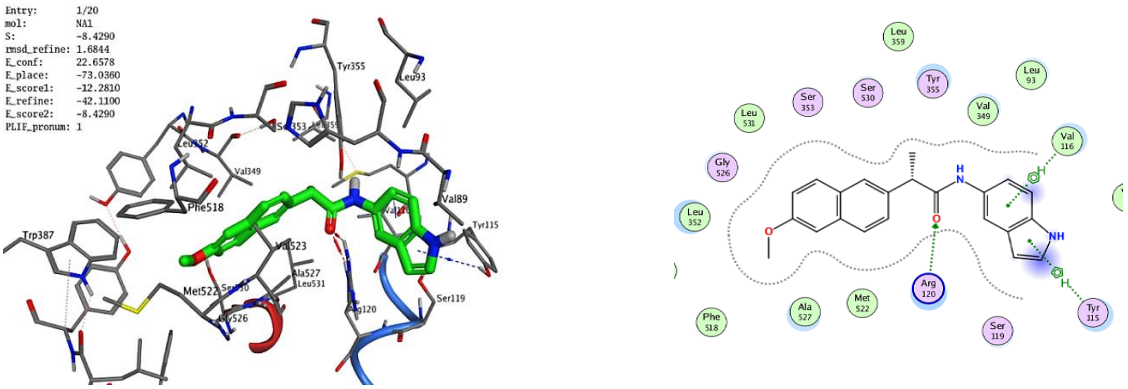
Binding properties of the synthesized compounds with Oxidoreductase (PDB code: 3NT1)

Compound	*S*-score (Kcal/mol)	RMSD	No. of binding sites	Binding amino acids
Naproxen 3NT1	–7.1006	1.9156	3	Arg120, Tyr 355, Ala527
NA 1	–8.4290	1.6844	3	Arg120, Tyr 115, Val 116
NA2	–8.3094	1.4927	2	Arg120, Val 116
NA3	–8.7078	1.0012	2	Two Arg120
NA4	–8.4155	1.4196	4	Arg120, Tyr 355, Val 349, Glu 524



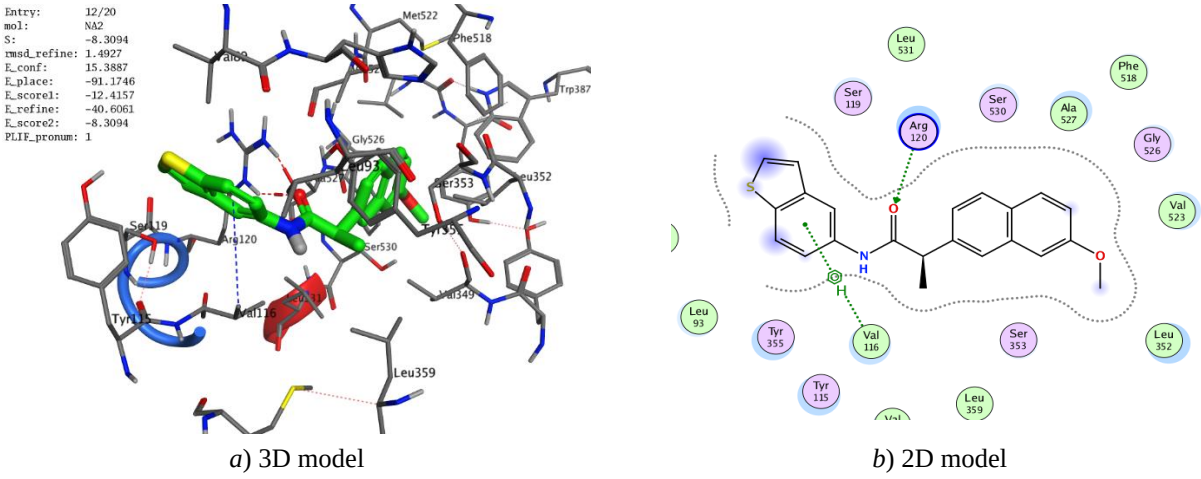
a) 3D model *b*) 2D model

Figure 4. Interactions of native ligand Naproxen and NA1 with COX-2 oxidoreductase (PDB Code: 3NT1)



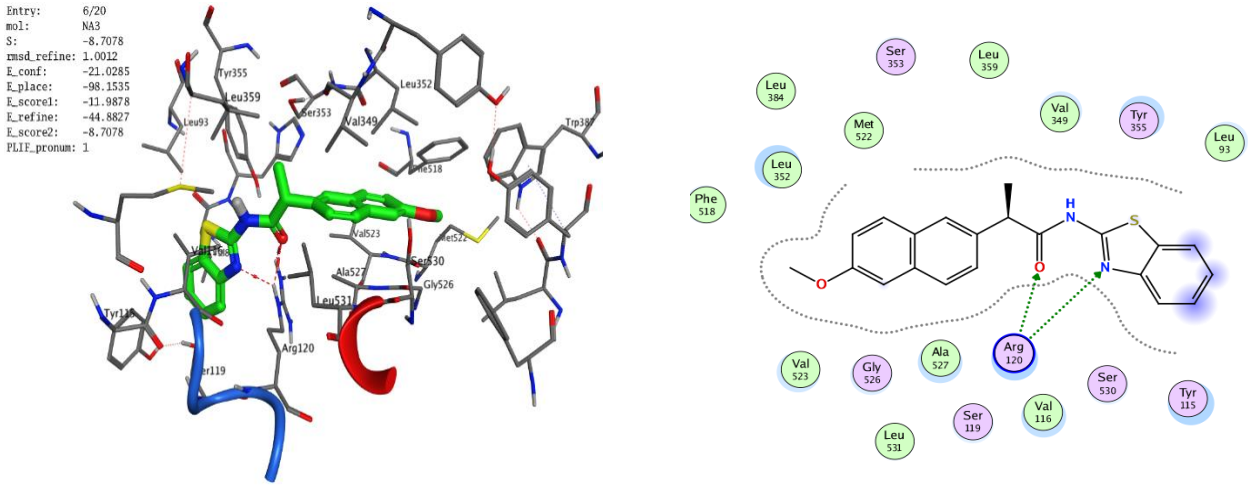
a) 3D model *b*) 2D model

Figure 5. Interactions of NA1 ligand with COX-2 oxidoreductase (PDB Code: 3NT1)



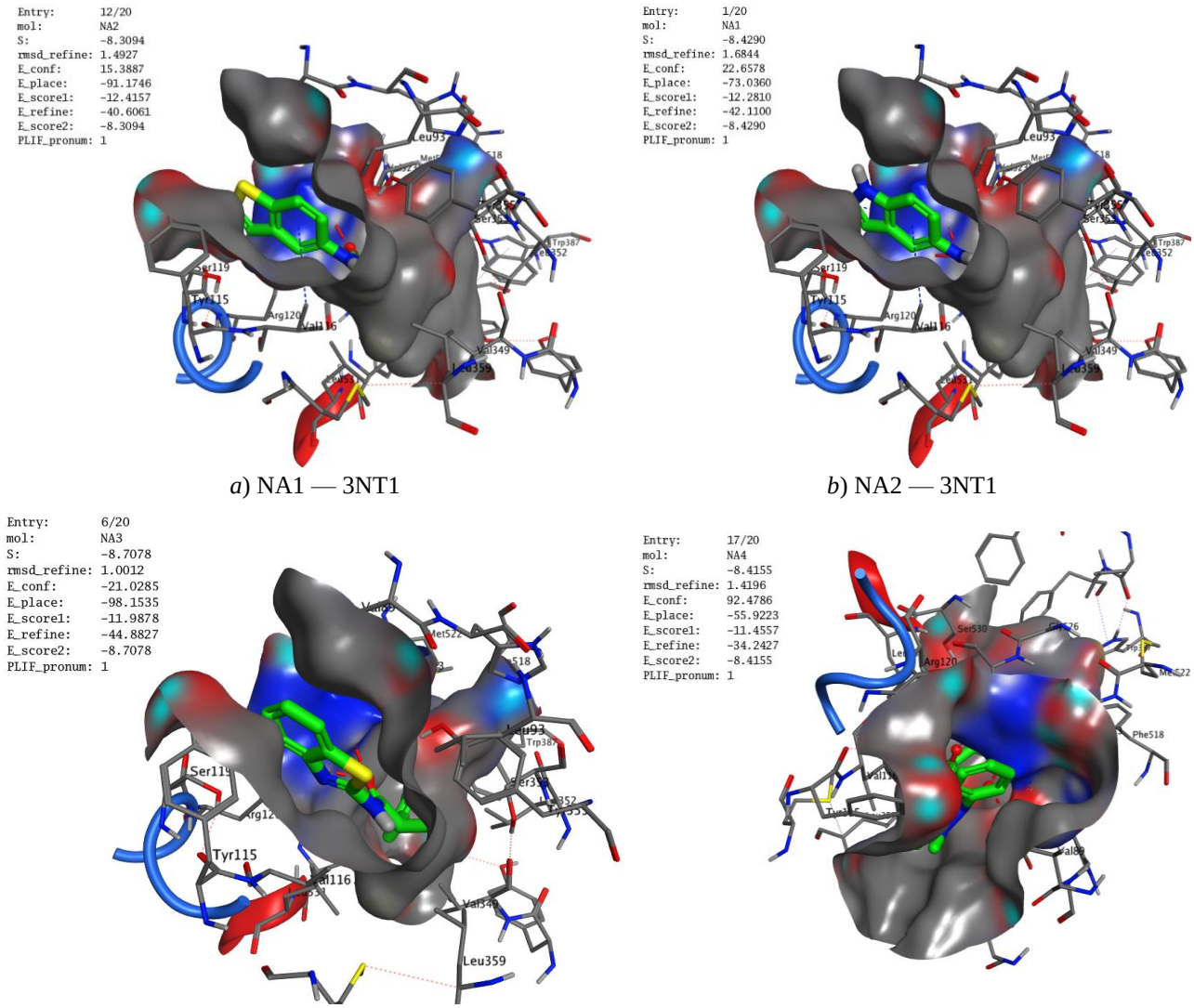
a) 3D model *b*) 2D model

Figure 6. Interactions of NA3 ligand with COX-2 oxidoreductase (PDB Code: 3NT1)



a) 3D model *b*) 2D model

Figure 7. Interactions of NA4 ligand with COX-2 oxidoreductase (PDB Code: 3NT1)



a) NA1 — 3NT1 *b*) NA2 — 3NT1

c) NA3 — 3NT1 *d*) NA4 — 3NT1

Figure 8. The 3D receptor surface interactions of NA1-NA4 ligand with COX-2 oxidoreductase (PDB Code: 3NT1)

Conclusion

In conclusion, the newly designed Naproxen-based heterocyclic derivatives (NA1-NA4) have been synthesized and demonstrated strong biological activity as antibacterial and anticancer agents. Antibacterial screening revealed that NA2 and NA3 exhibited significant inhibitory effects, with inhibition zones reaching 32 mm against *P. aeruginosa* and 35 mm against *S. aureus*, in some cases surpassing the standard antibiotic amoxicillin. Similarly, anticancer evaluations showed that NA1 and NA3 effectively inhibited the growth of MDA-MB-231 breast cancer cells, with IC_{50} values of 11.81 μg/mL and 11.08 μg/mL, respectively, comparable to standard chemotherapeutic agents. Furthermore, molecular docking studies revealed strong binding interactions between NA1–NA4 and COX-2 enzyme, indicating potential anti-inflammatory properties. These findings emphasize the value of incorporating heterocyclic moieties into the Naproxen scaffold to develop multifunctional therapeutic agents. The promising biological activities of these derivatives suggest that they hold potential for further development as antimicrobial, anticancer, and anti-inflammatory drug candidates.

Supporting Information

The Supporting Information is available free at https://ejc.buketov.edu.kz/index.php/ejc/article/view/230/191

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. CRediT: **Ammar Abdul Hussein Awad** conceptualization, data curation, formal analysis, funding acquisition, resources, supervision, validation, writing-original draft, writing-review & editing; **Mohammed Nawfal Abdul Maged** conceptualization, data curation, investigation, methodology, validation, visualization, writing-review & editing; **Dhulfiqar Ali Abed** data conceptualization, data curation, investigation, methodology, validation, visualization, writing-review & editing; **Osamah Ne'meh Wennas** data curation, formal analysis, visualization; **Noor Zuhair Kbah** data curation, formal analysis; **Ayad Ali Disher** conceptualization, data curation, investigation.

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Conflicts of Interest

The authors declare no conflict of interest.

References

1 Alam, M. M., Malebari, A. M., Syed, N., Neamatallah, T., Almalki, A. S., Elhenawy, A. A., Obaid, R. J., & Alsharif, M. A. (2021). Design, synthesis and molecular docking studies of thymol based 1, 2, 3-triazole hybrids as thymidylate synthase inhibitors and apoptosis inducers against breast cancer cells. *Bioorganic & medicinal chemistry*, *38*, 116136. https://doi.org/10.1016/j.bmc.2021.116136

2 Lorenzoni, V., Chaturvedi, A. K., Vignat, J., Laversanne, M., Bray, F., & Vaccarella, S. (2022). The current burden of oropharyngeal cancer: a global assessment based on GLOBOCAN 2020. *Cancer Epidemiology, Biomarkers & Prevention*, *31*(11), 2054–2062. https://doi.org/10.1158/1055-9965.EPI-22-0642

3 El-Sayed, A. A., El-Hashash, M. A., & El-Sayed, W. M. (2022). Synthesis, Antiproliferative Activity, and Apoptotic Profile of New Derivatives from the Meta Stable Benzoxazinone Scaffold. *Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents)*, *22*(6), 1226–1237. https://doi.org/10.2174/1871520621666210706152632

4 Ranatunge, R. R., Augustyniak, M. E., Dhawan, V., Ellis, J. L., Garvey, D. S., Janero, D. R., Letts, L. G., Richardson, S. K., Shumway, M. J., & Trocha, A. M. (2006). Synthesis and anti-inflammatory activity of a series of N-substituted naproxen glycola-

mides: Nitric oxide-donor naproxen prodrugs. *Bioorganic & medicinal chemistry*, *14*(8), 2589–2599. https://doi.org/10.1016/j.bmc.2005.11.040

5 Elhenawy, A. A., Al-Harbi, L., Moustafa, G. O., El-Gazzar, M., Abdel-Rahman, R. F., & Salim, A. E. (2019). Synthesis, comparative docking, and pharmacological activity of naproxen amino acid derivatives as possible anti-inflammatory and analgesic agents. *Drug design, development and therapy*, 1773–1790. https://doi.org/10.2147/dddt.s196276

6 Ullah, N., Huang, Z., Sanaee, F., Rodriguez-Dimitrescu, A., Aldawsari, F., Jamali, F., Bhardwaj, A., Islam, N. U., & Velázquez-Martínez, C. A. (2016). NSAIDs do not require the presence of a carboxylic acid to exert their anti-inflammatory effect–why do we keep using it? *Journal of enzyme inhibition and medicinal chemistry*, *31*(6), 1018–1028. https://doi.org/10.3109/14756366.2015.1088840

7 Shah, K., Gupta, J. K., Chauhan, N. S., Upmanyu, N., Shrivastava, S. K., & Mishra, P. (2017). Prodrugs of NSAIDs: A review. *The open medicinal chemistry journal*, *11*, 146. https://doi.org/10.2174/1874104501711010146

8 Nedeljković, N., Dobričić, V., Bošković, J., Vesović, M., Bradić, J., Anđić, M., Kočović, A., Jeremić, N., Novaković, J., & Jakovljević, V. (2023). Synthesis and investigation of anti-inflammatory activity of new thiourea derivatives of naproxen. *Pharmaceuticals*, *16*(5), 666. https://doi.org/10.3390/ph16050666

9 Sohail, R., Mathew, M., Patel, K. K., Reddy, S. A., Haider, Z., Naria, M., Habib, A., Abdin, Z. U., Chaudhry, W. R., & Akbar, A. (2023). Effects of non-steroidal anti-inflammatory drugs (NSAIDs) and gastroprotective NSAIDs on the gastrointestinal tract: a narrative review. *Cureus*, *15*(4). https://doi.org/10.7759/cureus.37080

10 Gouda, A. M., Beshr, E. A., Almalki, F. A., Halawah, H. H., Taj, B. F., Alnafaei, A. F., Alharazi, R. S., Kazi, W. M., & Al-Matrafi, M. M. (2019). Arylpropionic acid-derived NSAIDs: New insights on derivatization, anticancer activity and potential mechanism of action. *Bioorganic Chemistry*, *92*, 103224. https://doi.org/10.1016/j.bioorg.2019.103224

11 Han, M. İ., & Küçükgüzel, Ş. G. (2020). Anticancer and antimicrobial activities of naproxen and naproxen derivatives. *Mini reviews in medicinal chemistry*, *20*(13), 1300–1310. https://doi.org/10.2174/1389557520666200505124922

12 Kumar, G., Madka, V., Singh, A., Farooqui, M., Stratton, N., Lightfoot, S., Mohammed, A., & Rao, C. V. (2021). Naproxen inhibits spontaneous lung adenocarcinoma formation in KrasG12V mice. *Neoplasia*, *23*(6), 574–583. https://doi.org/10.1016/j.neo.2021.05.010

13 Nirogi, R. V., Bandyala, T. R., Konda, J. B., Reballi, V., Gudla, P., Kambhampati, R., & Khagga, M. (2012). Design, synthesis and biological activity of novel substituted 2-Aryl sulfonyl methyl tryptamines as potential 5-HT6 Receptor ligands. *Der Pharma Chem*, *4*(4), 1552–1566. https://www.derpharmachemica.com/pharma-chemica/design-synthesis-and-biological-activity-of-novel-substituted-2aryl-sulfonyl-methyl-tryptamines-as-potential-5ht6-recept.pdf.

14 Alam, M. M., Nazreen, S., Almalki, A. S., Elhenawy, A. A., Alsenani, N. I., Elbehairi, S. E. I., Malebari, A. M., Alfaifi, M. Y., Alsharif, M. A., & Alfaifi, S. Y. (2021). Naproxen based 1,3,4-oxadiazole derivatives as EGFR inhibitors: Design, synthesis, anticancer, and computational studies. *Pharmaceuticals*, *14*(9), 870. https://doi.org/10.3390/ph14090870

15 Han, M. I., Atalay, P., Tunç, C. Ü., Ünal, G., Dayan, S., Aydın, Ö., & Küçükgüzel, Ş. G. (2021). Design and synthesis of novel (S)-Naproxen hydrazide-hydrazones as potent VEGFR-2 inhibitors and their evaluation in vitro/in vivo breast cancer models. *Bioorganic & medicinal chemistry*, *37*, 116097. https://doi.org/10.1016/j.bmc.2021.116097

16 Chen, P. C., Patil, V., Guerrant, W., Green, P., & Oyelere, A. K. (2008). Synthesis and structure–activity relationship of histone deacetylase (HDAC) inhibitors with triazole-linked cap group. *Bioorganic & medicinal chemistry*, *16*(9), 4839–4853. https://doi.org/10.1016/j.bmc.2008.03.050

17 Mohamed, S. K., El Bakri, Y., Abdul, D. A., Ahmad, S., Albayati, M. R., Lai, C. -H., Mague, J. T., & Tolba, M. S. (2022). Synthesis, crystal structure, and a molecular modeling approach to identify effective antiviral hydrazide derivative against the main protease of SARS-CoV-2. *Journal of Molecular Structure*, *1265*, 133391. https://doi.org/10.1016/j.molstruc.2022.133391 Get rights and content

18 Paes Leme, R. C., & da Silva, R. B. (2021). Antimicrobial activity of non-steroidal anti-inflammatory drugs on biofilm: Current evidence and potential for drug repurposing. *Frontiers in microbiology*, *12*, 707629. https://doi.org/10.3389/fmicb.2021.707629

19 Terrier, O., Dilly, S., Pizzorno, A., Chalupska, D., Humpolickova, J., Bouřa, E., Berenbaum, F., Quideau, S., Lina, B., & Fève, B. (2021). Antiviral properties of the NSAID drug naproxen targeting the nucleoprotein of SARS-CoV-2 coronavirus. *Molecules*, *26*(9), 2593. https://doi.org/10.3390/molecules26092593

20 Deb, J., Majumder, J., Bhattacharyya, S., & Jana, S. S. (2014). A novel naproxen derivative capable of displaying anti-cancer and anti-migratory properties against human breast cancer cells. *BMC cancer*, *14*, 1–8. https://doi.org/10.1186/1471-2407-14-567

21 Lubet, R. A., Steele, V. E., Juliana, M. M., & Grubbs, C. J. (2010). Screening agents for preventive efficacy in a bladder cancer model: study design, end points, and gefitinib and naproxen efficacy. *The Journal of urology*, *183*(4), 1598–1603. https://doi.org/10.1016/j.juro.2009.12.001

22 Keri, R. S., Chand, K., Budagumpi, S., Somappa, S. B., Patil, S. A., & Nagaraja, B. M. (2017). An overview of benzo[b]thiophene-based medicinal chemistry. *European journal of medicinal chemistry*, *138*, 1002–1033. https://doi.org/10.1016/j.ejmech.2017.07.038

23 Mo, X., Rao, D. P., Kaur, K., Hassan, R., Abdel-Samea, A. S., Farhan, S. M., Bräse, S., & Hashem, H. (2024). Indole Derivatives: A Versatile Scaffold in Modern Drug Discovery—An Updated Review on Their Multifaceted Therapeutic Applications (2020–2024). *Molecules*, *29*(19), 4770. https://doi.org/10.3390/molecules29194770

24 Paoletti, N., & Supuran, C. T. (2024). Benzothiazole derivatives in the design of antitumor agents. *Archiv der Pharmazie*, e2400259. https://doi.org/10.1002/ardp.202400259

25 Saha, M., Mandal, S., Sarkar, S., Biswas, A., Ghati, A., Cordes, D. B., Slawin, A. M., & Saha, N. C. (2024). Anticancer, antimicrobial and photocatalytic activities of a new pyrazole containing thiosemicarbazone ligand and its Co (III) and Ni (II) complexes: Synthesis, spectroscopic characterization and X-ray crystallography. *Journal of Inorganic Biochemistry*, *257*, 112577. https://doi.org/10.1016/j.jinorgbio.2024.112577

26 Kashyap, P., Verma, S., Gupta, P., Narang, R., Lal, S., & Devgun, M. (2023). Recent insights into antibacterial potential of benzothiazole derivatives. *Medicinal Chemistry Research*, *32*(8), 1543–1573. https://doi.org/10.1007/s00044-023-03077-z

27 Bresciani, A., Missineo, A., Gallo, M., Cerretani, M., Fezzardi, P., Tomei, L., Cicero, D. O., Altamura, S., Santoprete, A., Ingenito, R., Bianchi, E., Pacifici, R., Dominguez, C., Munoz-Sanjuan, I., Harper, S., Toledo-Sherman, L., & Park, L. C. (2017). Nuclear factor (erythroid-derived 2)-like 2 (NRF2) drug discovery: Biochemical toolbox to develop NRF2 activators by reversible binding of Kelch-like ECH-associated protein 1 (KEAP1). *Arch. Biochem. Biophys.*, *631*, 31–41. https://doi.org/https://doi.org/10.1016/j.abb.2017.08.003

28 Mahdi, Z. H., Alsalim, T. A., Abdulhussein, H. A., Majed, A. A., & Abbas, S. (2024). Synthesis, molecular docking, and anti-breast cancer study of 1-H-indol-3-Carbohydrazide and their derivatives. *Results in Chemistry*, *11*, 101762. https://doi.org/10.1016/j.rechem.2024.101762

29 Wang, R., Wang, J., Dong, T., Shen, J., Gao, X., & Zhou, J. (2019). Naringenin has a chemoprotective effect in MDA‑MB‑231 breast cancer cells via inhibition of caspase‑3 and‑9 activities. *Oncology letters*, *17*(1), 1217–1222. https://doi.org/10.3892/ol.2018.9704

30 Ayaz, M., Alam, A., Zainab, Assad, M., Javed, A., Islam, M. S., & Ahmad, M. (2023). Biooriented synthesis of ibuprofen-clubbed novel bis-schiff base derivatives as potential hits for malignant glioma: In vitro anticancer activity and in silico approach. *ACS omega*, *8*(51), 49228–49243. https://doi.org/10.1021/acsomega.3c07216

31 Duggan, K. C., Walters, M. J., Musee, J., Harp, J. M., Kiefer, J. R., Oates, J. A., & Marnett, L. J. (2010). Molecular basis for cyclooxygenase inhibition by the non-steroidal anti-inflammatory drug naproxen. *Journal of biological chemistry*, *285*(45), 34950–34959. https://doi.org/10.1074/jbc.m110.162982

32 Mohammad-Aghaie, D., & Jafari, Z. (2013). Docking and Molecular Dynamics Simulation Studies of Interactions between Cyclooxygenases Enzymes and Celecoxib drug. *1st Tabriz International Life Science Conference and 12th Iran Biophysical Chemistry Conference*. https://doi.org/10.1080/07391102.2020.1823884

33 Nekkaz, K., Daoud, I., Younes, K., Merghache, S., Khebichat, N., & Ghalem, S. (2014). Docking Studies on Cyclooxygenases-2 Inhibitors based on Potential Ligand Binding Sites. *International Journal of Computer Applications*, *87*(1). https://doi.org/10.5120/15173-3086

34 Awad, A., Kareem, M., Rasheed, O., & Eesa, M. (2024). Synthesis, Biological Screening, and Molecular Dockingof Drug Carrier Maleimide Derivatives. *Russian Journal of Bioorganic Chemistry*, *50*(3), 991–1000. https://doi.org/10.1134/s1068162024030245

35 Pathania, S., Narang, R. K., & Rawal, R. K. (2019). Role of sulphur-heterocycles in medicinal chemistry: An update. *European journal of medicinal chemistry*, *180*, 486–508. https://doi.org/10.1016/j.ejmech.2019.07.043