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Research Article

Thiourea Derivatives as Estrogen Receptor Alpha Inhibitors for Breast Cancer Therapy: An *In Silico* Evaluation with ADMET Prediction and Molecular Docking

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Abstract

Breast cancer remains a significant public health concern, necessitating the discovery of novel therapeutic agents. This study investigates the potential of thiourea derivatives, specifically HU, HTMX, and BMPTU compounds, as estrogen receptor alpha (ERα) inhibitors using computational approaches. Drug-likeness assessments using Lipinski's Ro5 confirmed the oral bioavailability of all compounds. Additionally, ADMET analysis indicated favorable pharmacokinetic properties, with minimal metabolic interactions and acceptable safety profiles, except for BMPTU2, which showed potential hepatotoxicity. Molecular docking simulations revealed strong binding affinities between BMPTU derivatives, particularly BMPTU2, BMPTU3, and BMPTU4, and key ERα residues. These interactions suggest their potential as ERα modulators, warranting further *in silico* and experimental validation. In conclusion, the findings highlight the potential of BMPTU derivatives, especially BMPTU2, BMPTU3, and BMPTU4, as promising lead compounds for developing novel ERα-targeted breast cancer therapies. Further optimization and validation are crucial to fully elucidate their therapeutic potential.

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INTRODUCTION

Breast cancer remains a significant global health burden, with millions of cases diagnosed annually and substantial mortality rates[1]. Despite advancements in conventional treatments like surgery, chemotherapy, and radiation therapy, drug resistance and adverse effects continue to hinder optimal patient outcomes[2]. The urgent need for novel therapeutic agents with enhanced efficacy and safety profiles has spurred research into compounds targeting specific molecular pathways involved in breast cancer progression[3]. Such targeted therapies hold the promise of improving treatment outcomes and potentially extending survival rates[4].

Thiourea derivatives have emerged as a promising class of compounds with diverse pharmacological activities, including anticancer potential[5-8]. These compounds have been shown to inhibit key enzymes, induce apoptosis, and modulate cellular signaling pathways, suggesting their potential as anticancer agents[9]. Their ability to interact with biological macromolecules further underscores their versatility in drug development, prompting further investigation into their therapeutic applications, particularly in the context of breast cancer[10].

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Estrogen receptor alpha (ERα) plays a pivotal role in the pathogenesis of hormone-sensitive breast cancers, making it a prime therapeutic target[11]. The ERα activation by estrogen triggers downstream signaling cascades that promote tumor growth, survival, and metastasis[12]. Consequently, inhibiting ERα signaling has emerged as a promising approach for treating ER-positive breast cancers, a significant subset of breast cancer cases[13].

Thiourea derivatives have demonstrated potential as ERα inhibitors, disrupting estrogen-mediated signaling pathways and attenuating tumor growth in preclinical studies[14]. This evidence underscores their potential as targeted agents for combating hormone-driven breast cancers. Thiourea derivatives have emerged as promising scaffolds for drug discovery, with N-benzoyl-N'-methoxyphenilthiourea (BMPTU) serving as a lead compound. This study explores the structural modification of BMPTU's aromatic ring, guided by the Topliss approach, to optimize its physicochemical properties as defined by the Hansch model[15].

Computational methods have revolutionized drug discovery by enabling efficient identification and optimization of potential drug candidates[16]. *In silico* techniques, such as molecular docking and absorption, distribution, metabolism, excretion, and toxicity (ADMET) prediction, offer valuable insights into drug-target interactions, pharmacokinetics, and toxicology[7]. By leveraging these computational tools, researchers can streamline drug development, reduce costs, and accelerate innovation[17,18]. Furthermore, *in silico* methods facilitate the exploration of chemical space, guiding the rational design of novel compounds with improved potency, selectivity, and bioavailability[19,20].

This study aims to harness the potential of *in silico* methods to identify novel thiourea derivatives with optimized pharmacological profiles for the treatment of breast cancer. Through integrating computational and experimental techniques, this research seeks to elucidate the therapeutic potential of thiourea derivatives as anti-breast cancer agents. By employing *in silico* docking studies, we aim to elucidate the molecular interactions between thiourea derivatives and ERα, providing mechanistic insights into their inhibitory effects. Additionally, ADMET predictions will help evaluate the pharmacokinetic properties and safety profiles of the identified compounds, guiding subsequent experimental validation. Ultimately, this interdisciplinary approach aims to accelerate the discovery and development of novel therapeutics for breast cancer, offering new hope for patients and advancing the paradigm of personalized medicine.

MATERIALS AND METHODS

Materials

All calculations and visualizations were performed on a laptop equipped with an AMD A6-7310 quad-core processor (2.00 GHz), 6 GB RAM, and a Windows 10 Pro (64-bit) operating system. The following software packages were utilized: Chem Bio Draw Ultra version 12 (PerkinElmer, Inc.), Chem Bio 3D Ultra version 12 (PerkinElmer, Inc.), Molegro Virtual Docker 5.5 (CLC bio), and pkCSM (https://biosig.unimelb.edu.au/pkCSM/prediction). Protein structures were retrieved from the Protein Data Bank (https://www.rcsb.org). As shown in **Table I**, hydroxyurea (HU), 4-hydroxytamoxifen (HTMX), and a BMPTU derivative were employed as chemical structures in this study. Hydroxyurea served as a positive control, while HTMX acted as both a positive control and an internal ligand within the PDB file[21].

Methods

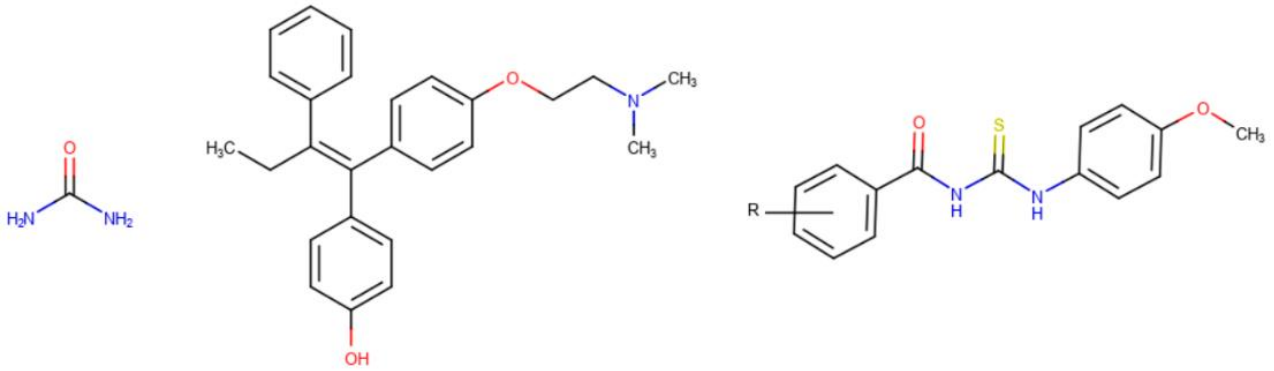
The ADMET properties of BMPTU derivatives were predicted using the pkCSM. The 3D structure of the ERα protein was obtained from the Protein Data Bank (PDB ID: 3ERT)[21,22]. This protein was selected due to its high resolution (1.9 Å), and the ligand HTMX was used to identify the receptor's binding site. The BMPTU derivative was constructed using Chem Bio Draw Ultra version 12 and energy-minimized using Chem Bio 3D Ultra Version 12 with the Merck Molecular Force Field 94 (MMFF94) method. Molecular docking was performed using Molegro Virtual Docker 5.5, with BMPTU docked in the same position as HTMX (x = 34.21; y = -2.42; z = 20.71; Radius 13) using the MolDock Score (GRID) scoring function and MolDock SE search algorithm.

Data analysis

Molecular docking simulations were performed to evaluate the binding affinity between the identified ligands and the target proteins. The docking results were ranked based on the calculated rerank score (RS), a measure of the binding energy

between the ligand and receptor. A lower RS value indicates a stronger predicted binding affinity, suggesting a greater potential for the compound to exhibit anticancer activity[23].

Table I. Chemical structures of HU, HTMX, and BMPTU derivative compounds.



HU **HTMX** **BMPTU**

No	R	Compound name	Compound code
1	-	Hydroxyurea	HU
2	-	4-hydroxytamoxifen	HTMX
3	H	N-benzoyl-N′-4-methoxyphenylthiourea	BMPTU1
4	4-NO_2	N-4-nitronbenzoyl-N′-4-methoxyphenylthiourea	BMPTU2
5	4-CN	N-4-cyanobenzoyl-N′-4-methoxyphenylthiourea	BMPTU3
6	3-NO_2	N-3-nitrobenzoyl-N′-4-methoxyphenylthiourea	BMPTU4
7	4-OCH_3	N-4-methoxybenzoyl-N′-4-methoxyphenylthiourea	BMPTU5
8	3,4-diCl	N-3,4-dichlorobenzoyl-N′-4-methoxyphenylthiourea	BMPTU6
9	2,4-diCl	N-2,4-dichlorobenzoyl-N′-4-methoxyphenylthiourea	BMPTU7
10	3-CF_3	N-3-trifluoromethylbenzoyl-N′-4-methoxyphenylthiourea	BMPTU8
11	4-CF_3	N-4-trifluoromethylbenzoyl-N′-4-methoxyphenylthiourea	BMPTU9
12	4-Br	N-4-bromobenzoyl-N′-4-methoxyphenylthiourea	BMPTU10
13	4-Cl	N-4-chlorobenzoyl-N′-4-methoxyphenylthiourea	BMPTU11
14	2-CH_3	N-2-methoxybenzoyl-N′-4-methoxyphenylthiourea	BMPTU12
15	2-Cl	N-2-chlorobenzoyl-N′-4-methoxyphenylthiourea	BMPTU13
16	2-OCH_3	N-2-methoxybenzoyl-N′-4-methoxyphenylthiourea	BMPTU14
17	4-$C(CH_3)_3$	N-4-tertiarybutylbenzoyl-N′-4-methoxyphenylthiourea	BMPTU15
18	4-NH_2	N-4-aminobenzoyl-N′-4-methoxyphenylthiourea	BMPTU16
19	3-Cl	N-3-chlorobenzoyl-N′-4-methoxyphenylthiourea	BMPTU17
20	4-CH_3	N-4-methylbenzoyl-N′-4-methoxyphenylthiourea	BMPTU18
21	4-OH	N-4-hidroxybenzoyl-N′-4-methoxyphenylthiourea	BMPTU19
22	4-F	N-4-fluorobenzoyl-N′-4-methoxyphenylthiourea	BMPTU20

RESULTS AND DISCUSSION

Drug-likeness

All compounds adhered to Lipinski's Rule of Five (Ro5), suggesting favorable oral bioavailability (**Table II**)[24]. Hydroxyurea, with its low molecular weight and favorable hydrogen bond donor/acceptor balance, is a promising candidate for oral administration. Its negative log P value indicates hydrophilicity. 4-hydroxytamoxifen, a known estrogen receptor modulator, also meets Ro5 but exhibits moderate lipophilicity (log P slightly exceeding 5), potentially influencing its distribution[25]. All BMPTU2, BMPTU3, and BMPTU4 demonstrated balanced lipophilicity with log P values around 2.7-2.9, despite slightly higher hydrogen bond acceptor counts. BMPTU5, BMPTU6, and BMPTU7 exhibited moderate lipophilicity (log P around 3.0-4.1), suggesting a good balance of hydrophilic and lipophilic properties. All BMPTU10, BMPTU11, and BMPTU13 showed similar moderate lipophilicity (log P around 3.5-3.8). In contrast, BMPTU15, BMPTU16, and BMPTU17 displayed higher hydrophilicity (log P around 0.1-0.4), which might affect membrane permeability but could favor solubility.

Drug development is a lengthy and costly process, with a high failure rate often attributed to the inability to therapeutically modulate drug targets[26]. Lipinski's Rule of Five, introduced in 1997, provides a set of guidelines for assessing the drug-likeness of compounds based on their physicochemical properties[27]. The Ro5 criteria stipulate that orally active drugs

typically possess a molecular weight below 500 Da, no more than five hydrogen bond donors and ten hydrogen bond acceptors, and a log P value less than or equal to 5[28]. By applying the Ro5, researchers can identify and eliminate compounds with low likelihood of success in clinical trials, thereby improving drug development efficiency and reducing costs[29].

Table II. The drug-likeness analysis using Lipinski's Ro5.

Compound code	Molecular weight	Hydrogen bond acceptors	Hydrogen bond donors	Log P	Lipinski's Ro5
	≤500 DA	≤10	≤5	≤5	
HU	76.055	2	3	-0.956	Yes
HTMX	371.524	2	0	5.996	Yes
BMPTU1	286.386	3	2	2.822	Yes
BMPTU2	331.351	5	2	2.730	Yes
BMPTU3	311.266	4	2	2.694	Yes
BMPTU4	331.353	5	2	2.730	Yes
BMPTU5	316.238	4	2	2.831	Yes
BMPTU6	355.246	3	2	4.129	Yes
BMPTU7	355.246	3	2	4.129	Yes
BMPTU8	354.353	3	2	3.841	Yes
BMPTU9	354.353	3	2	3.841	Yes
BMPTU10	365.252	3	2	3.585	Yes
BMPTU11	320.801	3	2	3.475	Yes
BMPTU12	303.383	3	2	3.130	Yes
BMPTU13	320.801	5	2	3.475	Yes
BMPTU14	316.382	4	2	2.381	Yes
BMPTU15	342.464	3	2	4.119	Yes
BMPTU16	301.371	4	2	2.404	Yes
BMPTU17	320.801	3	2	3.475	Yes
BMPTU18	300.383	3	2	3.130	Yes
BMPTU19	302.335	3	2	2.528	Yes
BMPTU20	304.346	3	2	2.961	Yes

All BMPTU derivatives adhere to Lipinski's Ro5, indicating their potential for oral bioavailability. Variations in the R group do not significantly affect their fundamental drug-like characteristics. The observed range of log P values suggests a balance between hydrophilicity and lipophilicity, which is crucial for optimal drug absorption and distribution. Compounds with higher log P values (e.g., BMPTU6 and BMPTU7) might exhibit enhanced membrane permeability but require careful consideration of potential solubility issues.

Hydroxyurea exhibited a pronounced hydrophilic character compared to the more lipophilic HTMX, highlighting the diverse physicochemical profiles within this drug class. The BMPTU derivatives demonstrated intermediate properties, suggesting a potential balance between solubility and permeability, which could translate into improved therapeutic profiles. Compounds like BMPTU6 and BMPTU7, with their moderate log P values and adherence to Lipinski's Ro5, emerged as promising candidates for further development due to their favorable physicochemical properties. Compounds with extreme log P values may require structural modifications to optimize their bioavailability while maintaining efficacy. Despite the favorable predictions based on Lipinski's Ro5, further analysis and toxicity studies are warranted to validate these initial assessments and ensure safety and efficacy.

All BMPTU derivatives evaluated in this study demonstrated favorable drug-like properties, indicating their potential suitability for oral administration. The diverse log P values observed among these compounds suggest a range of hydrophilicity and lipophilicity, providing a promising foundation for further pharmacokinetic and pharmacodynamic investigations. Similar to previous research on N-benzoyl-N'-phenylthiourea derivatives[30], all BMPTU compounds adhered to Lipinski's Ro5, exhibiting acceptable log P values, molecular weight, hydrogen bond donors (HBDs), and hydrogen bond acceptors (HBAs). To further explore the therapeutic potential of BMPTU derivatives as ERα inhibitors, subsequent analyses focusing on ADMET properties and molecular docking simulations are warranted. These investigations will provide valuable insights into the compounds' drug-like characteristics and their potential interactions with the ERα.

ADMET prediction

Hydroxyurea demonstrated favorable intestinal solubility and a safe profile, with limited brain-blood barrier penetration and minimal interactions with CYP2D6 or OCT2 (**Table III**). These characteristics suggest a low likelihood of central nervous system effects and a relatively high therapeutic dose. In contrast, HTMX exhibited high intestinal solubility but

significant brain-blood barrier penetration, indicating potential central nervous system effects. Its maximum recommended dose is lower than HU, suggesting a narrower therapeutic window. Both HU and HTMX exhibited minimal risk of drug-drug interactions due to their lack of interaction with CYP2D6 and OCT2.

All BMPTU derivatives demonstrated generally good intestinal solubility, ranging from 83.235 to 93.148, suggesting adequate absorption. However, their brain-blood barrier penetration varied, with most compounds showing low to moderate permeability. None of the derivatives were substrates for CYP2D6 or renal OCT2, minimizing the risk of drug-drug interactions in these pathways. Maximum recommended doses varied across derivatives (log mg/kg/day: 0.015 to 0.411), reflecting varying safety profiles. Notably, BMPTU2 displayed potential hepatotoxicity.

Table III. ADMET prediction.

No	Compound code	Absorption	Distribution	Metabolism	Excretion	Toxicity	
		Intestinal solubility	Blood-brain barrier penetration	CYP2D6 substrate	Renal OTC2 substrate	Max dose (log mg/kg/day)	Hepatotoxic
1	HU	73.601	-0.664	No	No	1.934	No
2	HTMX	96.885	1,329	No	No	0.313	No
3	BMPTU1	90.737	0.243	No	No	0.348	No
4	BMPTU2	83.836	-0.438	No	No	0.09	Yes
5	BMPTU3	93.148	-0.135	No	No	0.304	No
6	BMPTU4	83.235	-0.333	No	No	0.015	No
7	BMPTU5	92.321	-0.058	No	No	0.411	No
8	BMPTU6	89.531	0.2	No	No	0.36	No
9	BMPTU7	88.792	0.127	No	No	0.34	No
10	BMPTU8	89.665	0.059	No	No	0.269	No
11	BMPTU9	91.163	0.097	No	No	0.303	No
12	BMPTU10	89.927	0.22	No	No	0.355	No
13	BMPTU11	89.994	0.221	No	No	0.365	No
14	BMPTU12	91.765	0.149	No	No	0.31	No
15	BMPTU13	90.307	0.136	No	No	0.322	No
16	BMPTU14	91.861	0.167	No	No	0.332	No
17	BMPTU15	89.685	0.221	No	No	0.101	No
18	BMPTU16	91.174	-0.768	No	No	0.349	No
19	BMPTU17	91,178	0.079	No	No	0.369	No
20	BMPTU18	92.48	0.107	No	No	0.354	No
21	BMPTU19	90.896	-0.893	No	No	0.216	No
22	BMPTU20	91.44	0.246	No	No	0.323	No

BMPTU2 demonstrated moderate intestinal solubility but exhibited low BBB penetration and potential hepatotoxicity at a maximum dose of 0.09 log mg/kg/day. This suggests caution in its use due to the risk of liver toxicity and a narrow therapeutic window. BMPTU4, similar to BMPTU2, exhibited low hepatotoxicity but possessed a very narrow therapeutic window, with a maximum dose of only 0.015 log mg/kg/day. BMPTU7 and BMPTU8 displayed higher intestinal solubility and moderate BBB penetration, suggesting their potential suitability for conditions requiring moderate central nervous system (CNS) exposure. BMPTU11 and BMPTU13 demonstrated favorable pharmacokinetic properties, including good intestinal solubility and moderate BBB penetration, indicating potential CNS effects with an acceptable safety profile. BMPTU16 and BMPTU17 exhibited high intestinal solubility and promising safety profiles, making them potential candidates for further development due to their balanced ADMET properties.

All compounds demonstrated favorable oral absorption characteristics due to their good intestinal solubility, a crucial factor for developing orally administered drugs. While most compounds exhibited low to moderate blood-brain barrier (BBB) penetration, which is advantageous for avoiding CNS side effects, compounds with higher BBB penetration, such as TMX, could be explored for CNS-targeted therapies. The absence of CYP2D6 substrate interactions across all BMPTU derivatives suggests a reduced risk of metabolic drug-drug interactions, making them potentially safer for patients taking multiple medications. Additionally, the lack of interactions with renal OCT2 transporters indicates minimal renal excretion issues, which is beneficial for compounds requiring prolonged systemic exposure. Most compounds in this study showed no hepatotoxicity, indicating a favorable safety profile. However, BMPTU2 and BMPTU4 warrant caution due to their narrow therapeutic windows and potential for liver toxicity.

N-benzoyl-N'-methoxyphenilthiourea derivatives, compared to the established benchmarks of HU and HTMX, demonstrate improved intestinal solubility and more consistent BBB penetration. Moreover, their broader safety profiles,

characterized by a lack of CYP2D6 and OCT2 interactions, suggest potential advantages over existing therapies. Compounds such as BMPTU7 and BMPTU16, with their balanced ADMET profiles, emerge as promising candidates for further development. However, compounds exhibiting potential hepatotoxicity or narrow therapeutic windows warrant either structural modifications or rigorous monitoring in subsequent studies.

An ADMET analysis was conducted to assess the pharmacokinetic and safety profiles of the compounds[31]. The pkCSM database was utilized to predict intestinal solubility, BBB penetration, CYP2D6 substrate status, renal OCT2 substrate status, maximum recommended therapeutic dose, and hepatotoxicity. Intestinal solubility is a crucial factor in oral absorption[32]. BBB penetration is essential for compounds targeting CNS[33]. CYP2D6 substrate status determines the compound's susceptibility to metabolism by the CYP2D6 enzyme, which can impact drug-drug interactions[34]. Renal OCT2 substrate status indicates the compound's potential for renal excretion[35]. The maximum recommended therapeutic dose and hepatotoxicity parameters provide insights into safety and potential liver toxicity[36]. While the compounds exhibited varying ADMET profiles, most did not show significant hepatic toxicity.

The ADMET analysis revealed favorable pharmacokinetic properties for the BMPTU derivatives, including good absorption, minimal metabolic interactions, and acceptable safety profiles. These characteristics suggest promising potential for further development, especially for compounds like BMPTU7 and BMPTU16. Previous research on thiourea derivatives has identified hepatotoxic potential in some compounds, such as N-(benzoyl)-N'-phenyltiourea, N-(4-methylbenzoyl)-N'-phenyltiourea, N-(4-tertiarybutylbenzoyl)-N'-phenyltiourea, N-(4-propoxybenzoyl)-N'-phenyltiourea, N-(3,4-ditrifluoromethylbenzoyl)-N'-phenyltiourea, N-(3,5-ditrifluoromethylbenzoyl)-N'-phenyltiourea, N-(4-dimethylaminobenzoyl)-N'-phenyltiourea, and N-(3-nitrobenzoyl)-N'-phenyltiourea[30]. However, it's important to note that these derivatives also demonstrate good human intestinal absorption (>80%)[15]. Future research should focus on optimizing BMPTU derivative compounds to enhance their efficacy and safety. *In vivo* studies are crucial to validate their potential for therapeutic applications.

Molecular docking

Molecular docking simulations were employed to predict the binding affinity of BMPTU derivatives to ERα. Hydroxyurea, a known ERα modulator, served as a positive control. The docking scores obtained revealed that all BMPTU derivatives exhibited significantly stronger binding affinities to ERα compared to HU with RS range from -75.1959 to -96.820, suggesting their potential as ERα modulators[37,38]. Among the BMPTU derivatives, BMPTU2 demonstrated the highest binding affinity, indicating a promising lead compound for further development. BMPTU3 and BMPTU4 also exhibited strong binding affinities, warranting further investigation. BMPTU8 and BMPTU6 displayed moderate binding affinities, while BMPTU22, despite being the weakest among BMPTU derivatives, still exhibited a significantly higher affinity than HU (**Table IV**).

BMPTU derivatives demonstrated strong binding affinities for ERα, as evidenced by their negative RS scores. Compounds containing nitro (e.g., BMPTU2 and BMPTU4), cyano (e.g., BMPTU3), and methoxy groups (e.g., BMPTU5) exhibited particularly high binding affinities, suggesting their contribution to enhanced receptor interactions. Additionally, halogenated derivatives (e.g., BMPTU6 and BMPTU11) displayed strong binding affinities, highlighting the importance of these functional groups for receptor interaction. Hydroxyurea, a reference compound, exhibited low binding affinity, underscoring the superior binding potential of BMPTU derivatives.

Among the BMPTU derivatives, BMPTU2, BMPTU3, and BMPTU4 emerged as promising candidates with the highest binding affinities. BMPTU8 and BMPTU6 also demonstrated significant potential and warrant further investigation. Future research should focus on optimizing these lead compounds to enhance their binding affinities while maintaining favorable ADMET properties. Structural modifications could be explored to fine-tune interactions with the ERα receptor, potentially improving efficacy and reducing off-target effects.

To gain insights into the ligand-receptor interaction, the specific amino acid residues on the receptor that interact with the ligand were identified. These interactions can be categorized as hydrogen bonds, electrostatic interactions, or steric interactions. These interactions play a pivotal role in determining the binding affinity and stability of the ligand-receptor complex[39].

Table IV. Molecular docking results.

No	Compound code	Rerank score	Amino residue interaction		
			Hydrogen bond	Electrostatic interaction	Steric interaction
1	HTMX	-124.289	Arg394	Asp351	Ala350, Asp351, Glu353
2	BMPTU2	-96.82	-	-	Met343, Leu346, Leu387, Met388, Leu391
3	BMPTU3	-92.5796	His524	-	Leu346, Leu391, Leu525
4	BMPTU4	-89.2612	Arg394	-	Leu346, Thr347, Ala350, Leu387, Met388
5	BMPTU5	-89.058	Thr347, His524	-	Leu346, Thr347, His524, Leu525
6	BMPTU6	-88.9712	-	-	Leu346, Leu384, His524
7	BMPTU7	-86.554	Thr347	-	Met343, Leu346, Thr347, Met388, Phe404, Leu525
8	BMPTU8	-85.9682	Leu346, Ala350, His524	-	Met343, Leu346, Ala350, Leu387, Met388, Leu391, Gly420
9	BMPTU9	-85.4756	Thr347	-	Met343, Leu346, Thr347, Ala350, Gly420, Gly521, His524, Leu525
10	BMPTU10	-85.3747	Leu346, Thr347, Arg394	-	Ala350, Asp351, Leu387, Arg394
11	BMPTU12	-84.7357	Thr347	-	Leu349, Ala350, Met388, Leu387, Arg394, Leu391
12	BMPTU11	-84.5448	Thr347	-	Met343, Leu346, Ala350, Leu428, His524, Leu525
13	BMPTU13	-84.5182	His524	-	Leu346, Leu349, Leu387, Met388, Phe404, Ile424, His524, Leu525
14	BMPTU14	-83.2699	Arg394	-	Leu346, Met388, Leu391, Arg394
15	BMPTU15	-82.443	-	-	Met321, Leu387, Met388, Leu391, Ile424
16	BMPTU1	-82.046	Leu346, Arg394	-	Leu346, Ala350, Glu353, Leu384, Met388
17	BMPTU16	-80.7878	Glu353	-	Ala350, Asp351, Leu387
18	BMPTU17	-80.3469	Thr347, Arg394	-	Arg350, Glu353, Trp383, Leu384, Leu387, Leu391, Arg394
19	BMPTU18	-79.0584	His524	-	Leu346, Gly521, Leu525
20	BMPTU19	-76.6632	Leu346, His524	-	Leu346, Leu349, Leu387, Met388, Phe404, Ile424, His524, Leu525
21	BMPTU20	-75.1959	-	-	Leu346, Ala350, Glu353, Leu387, Arg394
22	HU	-34.895	Glu353, Leu387, Arg394	-	-

Molecular docking simulations revealed that HU forms hydrogen bonds with key residues such as Glu353, Leu387, and Arg394, contributing to its binding to ERα. However, its lower binding affinity may be attributed to a lack of significant electrostatic and steric interactions. 4-hydroxytamoxifen, in contrast, forms hydrogen bonds with Arg394 and engages in electrostatic interactions with Asp351. Additionally, steric interactions with Ala350, Asp351, and Glu353 contribute to its enhanced binding stability, explaining its potent modulatory effects on ERα.

The majority of derivatives studied formed multiple hydrogen bonds, primarily with Thr347, Arg394, and His524. Electrostatic interactions were less frequent but played a significant role when present. Steric interactions, involving residues like Leu346, Ala350, and Met388, also contributed to overall binding stability. BMPTU2 exhibited strong steric interactions with multiple leucine residues, while methionine contributed to its highest binding affinity among the derivatives. BMPTU3 demonstrated a hydrogen bond with His524 and significant steric interactions with leucine residues, resulting in strong ERα binding. BMPTU4, with its dual hydrogen bonds and extensive steric interactions, exhibited the most potent binding affinity to ERα.

Hydrogen bonding plays a crucial role in stabilizing ligand-receptor interactions. Residues like Arg394, Thr347, and His524 are commonly involved in these interactions. Compounds forming multiple hydrogen bonds often exhibit stronger binding affinities. While less frequent, electrostatic interactions, as observed with Asp351 in HTMX, can significantly enhance binding stability. Additionally, steric interactions, mediated by residues such as Leu346, Leu387, and Met388, contribute to the stability of the ligand-receptor complex. Compounds with extensive steric interactions, like BMPTU2 and BMPTU4, tend to have higher binding affinities.

Hydroxyurea exhibited fewer molecular interactions with the target protein compared to the BMPTU derivatives, aligning with its lower binding affinity. The BMPTU derivatives demonstrated significantly stronger binding, facilitated by additional steric and hydrogen bond interactions. 4-hydroxytamoxifen, with its extensive hydrogen bonding, electrostatic interactions, and steric interactions, established a high benchmark for binding affinity. Several BMPTU derivatives approached or surpassed this level of interaction complexity, suggesting their potential efficacy. Specific functional groups, including nitro, cyano, and methoxy, contributed to enhanced hydrogen bonding and steric interactions, leading to higher

binding affinities. Halogenated derivatives, such as those containing chloro groups, also exhibited significant steric interactions, crucial for binding stability.

ERα, a nuclear receptor, regulates gene expression in response to estrogen. Ligand binding induces conformational changes in ERα, enabling it to distinguish between agonists and antagonists[40]. Agonist binding to ERα leads to H12 adopting a conformation that recruits coactivators, facilitating gene transcription. Residues Glu353, Arg394, and His524 stabilize H12 in this conformation through hydrogen bonding. Conversely, antagonists disrupt H12 positioning, preventing coactivator recruitment and favoring corepressor binding, which inhibits transcription. Asp351 forms a salt bridge with antagonists, further stabilizing the antagonistic conformation[21].

The ERα ligand binding domain (LBD) undergoes distinct conformational changes depending on the ligand bound. Hydrogen bonding with His524 is crucial for differentiating agonists and antagonists. Mutations in His524, even if hydrogen bonding with Glu353 or Arg394 is maintained, can result in ERα antagonism[22,41]. Understanding these molecular interactions is essential for designing selective ERα modulators with therapeutic potential.

To elucidate the mode of action of BMPTU derivatives, we investigated their interactions with ERα. Our analysis revealed that HU forms hydrogen bonds with key residues similar to known ERα agonists, suggesting potential agonistic activity. However, its low binding affinity indicates weak potency. In contrast, HTMX, a known ERα antagonist, forms a hydrogen bond with Asp351 but lacks the critical hydrogen bond with His524, leading to displacement of the H12 helix[42]. Several BMPTU derivatives (BMPTU3, BMPTU5, BMPTU8, BMPTU13, BMPTU18, and BMPTU19) demonstrated hydrogen bonding with His524, suggesting potential agonistic activity. However, further *in silico* molecular dynamics simulations are necessary to confirm whether their steric interactions can effectively displace the H12 position. *In vitro* and *in vivo* studies are warranted to definitively validate these predictions and characterize the biological activity of BMPTU derivatives as ERα modulators.

CONCLUSION

This *in silico* study evaluated BMPTU derivatives as potential ERα inhibitors for breast cancer therapy. All compounds adhered to Lipinski's Ro5, suggesting favorable oral bioavailability. ADMET analysis revealed promising pharmacokinetic profiles, with minimal metabolic interactions and acceptable safety margins, except for BMPTU2, which warrants further assessment due to potential hepatotoxicity. Molecular docking simulations identified strong binding affinities between BMPTU derivatives, particularly BMPTU2, BMPTU3, and BMPTU4, and key ERα residues. These findings suggest that certain BMPTU derivatives could act as ERα agonists or antagonists, warranting further investigation through molecular dynamics simulations and experimental studies. Collectively, these results highlight the potential of BMPTU derivatives, especially BMPTU2, BMPTU3, and BMPTU4, as promising lead compounds for developing novel breast cancer therapies targeting ERα. Further optimization and validation are necessary to fully realize their therapeutic potential.

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DATA AVAILABILITY

None.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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