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Synthesis, Docking Study, and Cytotoxicity Evaluation of New Hydroxy benzoic Acid Derivatives

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Abstract

Hydroxy benzoic acid derivatives are an important scaffold in medicinal chemistry. A review of the literature revealed that they have a wide range of biological activities, including anti-inflammatory, antibacterial, and anti-tumor properties. The current study aimed to synthesize three new hydroxy benzoic acid derivatives (compounds 8, 9, and 10) and test them as epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors *in silico* and *in vitro*. Traditional organic synthesis methods were applied to produce these compounds. Docking studies revealed that compound 8 had nearly equal binding energy (ΔG) to erlotinib, the standard EGFR tyrosine kinase inhibitor. -8.56 and -8.75 kcal/mol, respectively. Using the MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) viability assay, the cytotoxicity of the new hydroxy benzoic acid derivatives was tested against DLD-1 colorectal adenocarcinoma cells, HeLa cervical cancer cells, and MCF-7 breast cancer cells. Compound 8 showed cytotoxicity against the three cancer cell lines tested (25.05, 23.88, and 48.36 µM, respectively), while compound 9 showed cytotoxicity against HeLa cells only (37.67 µM), and compound 10 was cytotoxic to DLD-1 and HeLa cells (27.26 and 19.19 µM), respectively. The half maximal inhibitory concentrations (IC_{50}) of compound 8 were comparable to those of the standard tyrosine kinase inhibitor drug erlotinib (13.86, 36.41, and 87.34 µM, respectively). In conclusion, the findings of the present study indicate that compound 8, through its EGFR inhibitory activity, is a promising cytotoxic agent.

تخليق ودراسة الرسو ,وتقييم النشاط السمي للخلايا لمشتقات جديدة من حامض الهيدروكسي بينزويك

الخلاصة

تعتبر مشتقات حامض الهيدروكسي بنزويك هي مجموعة مهمة في الكيمياء الطبية. كشفت مراجعة الأدبيات أن لديهم مجموعة واسعة من الأنشطة البيولوجية ، بما في ذلك الخصائص المضادة للالتهابات والمضادة للبكتيريا والمضادة للأورام. ان الدراسة تهدف الى تخليق وتقييم مشتقات حامض الهيدروكسي البنزويك الجديدة (المركبات ٨ و ٩ و ١٠) ، والتي تم اختبارها في المختبر وفي اسلوب المحاكاة بالحاسوب كمثبطات انزيم EGFR التيروزين كينيز. لقد تم تطبيق طرائق التخليق العضوية التقليدية لإنتاج المركبات المنشودة. ان دراسة الرسو الجزيئي , اظهر ان المركب ٨ له الفة ارتباط حرة مقاربه للمادة القياسيه المستعمله (ارلوتنيب), كمثبط لانزيم EGFR, تايروزين كينيز.

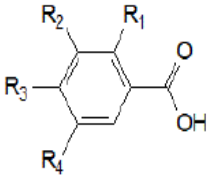
تم استعمال تحليل التترازوليوم (MTT assay), لاختبار السمية لمشتقات حامض البنزويك الجديدة باستعمال الخلايا السرطانية وهي(DLD-1 خلايا سرطان القولون والمستقيم ، وخلايا سرطان عنق الرحم HeLa cells ، وخلايا سرطان الثدي MCF-7 ، وقد بينت النتائج ان المركب ٨ له سميه واضحه ضد الخلايا الثلاثة المستعملة. بينما اظهر المركب ٩ مختبريا اعلى سمية ضد الخلايا سرطان عنق الرحم Hela cells . اضف الى ذلك, فقد وجد ان المركب **١٠ له** سمية واضحه ضد خلايا سرطان القولون والمستقيمDLD-1 , وايضا ضد خلايا سرطان عنق الرحم Hela cells . اذن فان المحصلة النهائية لهذه الدراسة, تبين ان المركب ٨ , يمكن اعتباره دواء واعدا , مثبطا للانزيم EGFR, التيروزين كينيز.

Introduction

Cancer remains a major threat with high mortality and morbidity rates due to its complicated and heterogeneous nature. There is an urgent need to create new anticancer agents to counteract the increased toxicity and resistance of existing anticancer medications. Apoptosis is a major target for the discovery of new anticancer candidates because it plays a significant role in cancer progression. As a result, anticancer drugs that target different apoptotic signaling pathways have emerged as an important mechanism in cancer treatment [1].

The epidermal growth factor receptor (EGFR) tyrosine kinase regulates a variety of cellular activities including the cell cycle, adhesion, and motility [2]. Thus, EGFR overexpression or mutations promote cell proliferation and metastasis and inhibit apoptosis, leading to a variety of epidermal carcinomas including colon, breast, and bladder cancer [3-6].

Hydroxy benzoic acids can be formed directly from intermediates early in the shikimate pathway. However, they are more commonly formed in plants by the degradation of C6-C3 cinnamic acid derivatives. Examples of these acids include p-hydroxybenzoic, protocatechuic, vanillic, and syringic acids (**Figure 1**) [7].



Radical	Hydroxybenzoic acid
R_1 = OH; R_2 = R_3 = R_4 = H	Salicylic acid
R_1 = R_4 = OH; R_2 = R_3 = H	Gentisic acid
R_1 = R_2 = R_4 = H; R_3 = OH	*p*-hydroxybenzoic acid
R_1 = R_4 = H; R_2 = R_3 = OH	Protocatechuic acid
R_1 = H; R_2 = OCH_3; R_3 = OH; R_4 = H	Vanillic acid
R_1 = H; R_2 = R_3 = R_4 = OH	Gallic acid
R_1 = H; R_2 = R_4 = OCH_3; R_3 = OH	Syringic acid

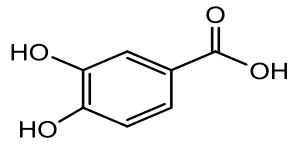
Figure 1. Natural hydroxyl benzoic acids. drawn using ChemDraw Professional version 19.0.022.

It was reported that 3-O-Methylgallic acid (Megal) and gallic acid (Gal), two hydroxy benzoic acids, decreased cell viability in SW-480 and Caco-2 colorectal cancer cells. This reduction was primarily due to Megal and Gal's ability to inhibit cell cycle progression during the G0/G1 phase. These two hydroxy benzoic acids also inhibited the activities of survival transcription factors known to be activated in colorectal carcinoma such as NF-kB, AP-1, STAT-1, and OCT-1. The ability of these compounds to inactivate these transcription factors might have contributed to Megal and Gal's anti-proliferative effects and induction of apoptosis [8].

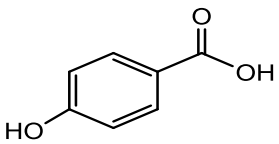
Different studies have reported that dihydroxy benzoic acid (**Figure 2A**) and 4-hydroxybenzoic acid (**Figure 2B**) inhibited activity of histone deacetylases (HDACs), enzymes which modulate the expression of genes, leading to cancer cell growth inhibition through the induction of reactive oxygen species (ROS) and apoptosis mediated by caspase-3. 4-hydroxybenzoic acid was able to successfully reverse adriamycin (ADM), a drug used to treat clinical breast cancer, resistance in human breast cancer cells by acting as histone deacetylase 6 (HDAC6) inhibitor [9-11].

Thiosemicarbazides (hydrazine-1-carbothioamide) are a class of compounds with high bioactivity. These compounds have numerous biological activities, including antifungal, anticancer, antimicrobial, sodium channel blocker, and antiviral properties [12]. In addition, thiosemicarbazide derivatives such as triapine (**Figure 2C**), and methisazone (**Figure 2D**) have anticancer activity due to their ability to suppress ribonucleotide reductase enzyme [13].

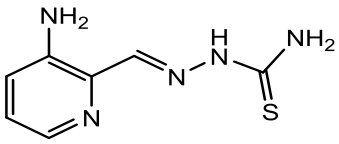
The present study aimed to design and synthesize new hydroxy benzoic acid derivatives exhibiting anticancer activity based on their predicted EGFR tyrosine kinase inhibition from molecular docking studies. The purified compounds containing (3-hydroxy,4-methoxy) 3,4-dimemethoxy or (3-butoxy,4-methoxy) benzoic acid, were characterized spectroscopically and were assessed for their anti-proliferation activities *in silico*, and *in vitro* against DLD-1, HeLa, and MCF-7 cancer cell lines.



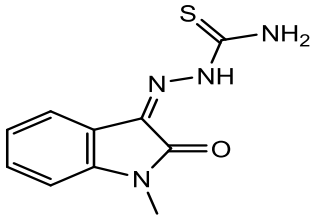
A: 3,4-dihydroxybenzoic acid



B: 4-hydroxybenzoic acid



C: triapine



D: methisazone

Figure 2. Chemical structure of hydroxy benozoic acid derivatives exhibiting anticancer activities. drawn using ChemDraw Professional version 19.0.022.

Experimental part

Material and methods

All of the chemicals and reagents used were purchased commercially from hyper chem. Limited (China) and used as received with no further purification. ^{1}HNMR spectra were recorded at 400 MHz (Bruker BioSpin), and chemical shift values (ppm) were reported to TMS, as an internal reference. $DMSO_{d6}$ was the solvent used . Tensor II Bruker-Optics FT-IR spectrophotometer was used to measure KBr disc FT-IR spectra.

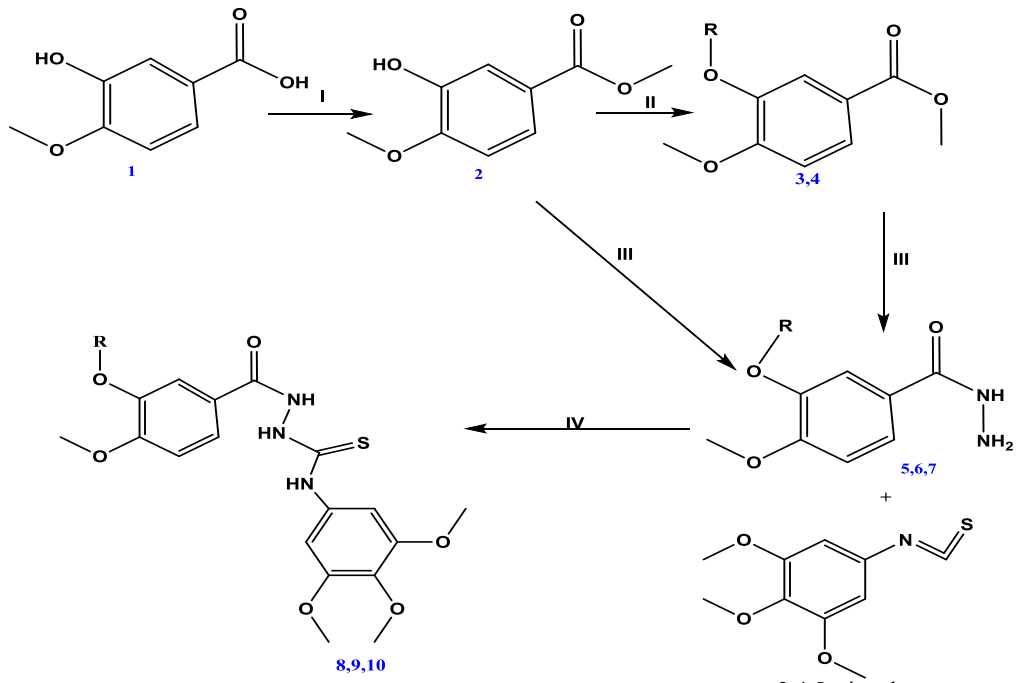
Chemical synthesis

General procedure for synthesis of compound (1-7)

Starting from 3-hydroxy,4-methoxybenzoic acid **(1**), The following compounds were synthesized and characterized in accordance with previously published articles: compound **(2**) [14], compounds **3** and **4** [15], and compounds **5**, **6**, and **7** [16,17].

General procedure for synthesis of compounds(8-10)[18]

In 20 mL abs. EtOH, containing (1g, 0.0044mol) of **5-isothiocyanato-1,2,3-trimethoxybenzene** and the appropriate benzohydrazide (added separately) as follow: 0.8g, 0.0044 mol of 3-hydroxy, 4-mthoxy benzohydrazide **(5)**, 0.86g, 0.0044 of 3,4-dimethoxy benzohydrazide **(6),** and 1.06g, 0.0044mol of 3-butoxy,4-methoxy benzohydrazid (**7)** were separately dissolved in abs. EtOH, and heated under reflux for 4 h, filtered, washed by ethanol and diethyl ether then recrystallized from EtOH 75%, to yield compounds **8, 9,** and **10**, respectively (**Scheme 1**).



I. methanol,5dr, H_2SO_4 ,reflux 8h
II. RX=CH_3-I to give 3,But-I to give **4**
,K_2CO_3, acetone, relux 4 h
III. $NH_2NH_2.H_2O$, ethanol, reflux 10 h
IV. ethanol, reflux 4h
R=H,comp. **5,8**
R=CH_3,comp. **3,6,9**
R=-CH_2-CH_2-CH_2-CH_3, comp.**4,7,10**

Scheme 1. The synthetic pathway for the preparation of the new compounds (2-10).

2-(3-hydroxy-4-methoxybenzoyl)-N-(3,4,5-trimethoxyphenyl) hydrazine-1-carbothioamide (8)

White crystal, yield 87%, R_f=0.31 (*n*-hexane/ethylacetate7 :3), m.p = 205-208 ^oC.
FT-IR (ύ, cm^{-1}): 3332.8(OH)str, 3301.2(Ar-N-H)str, 3265.8 (H-N-C=S)str, 3153.2(H-N-C=O), 3084.6 Ar(CH)str, 2838.8(CH_3)str, 1670.0(C=O), 1658(NH)bend, 1596.4, 1555.7, 1504, and 1457 Ar(C=C)str, 1219.5(C-O-C)str, 1177.5(C=S)str, 1107.8(C-N) (**Figure 3**).

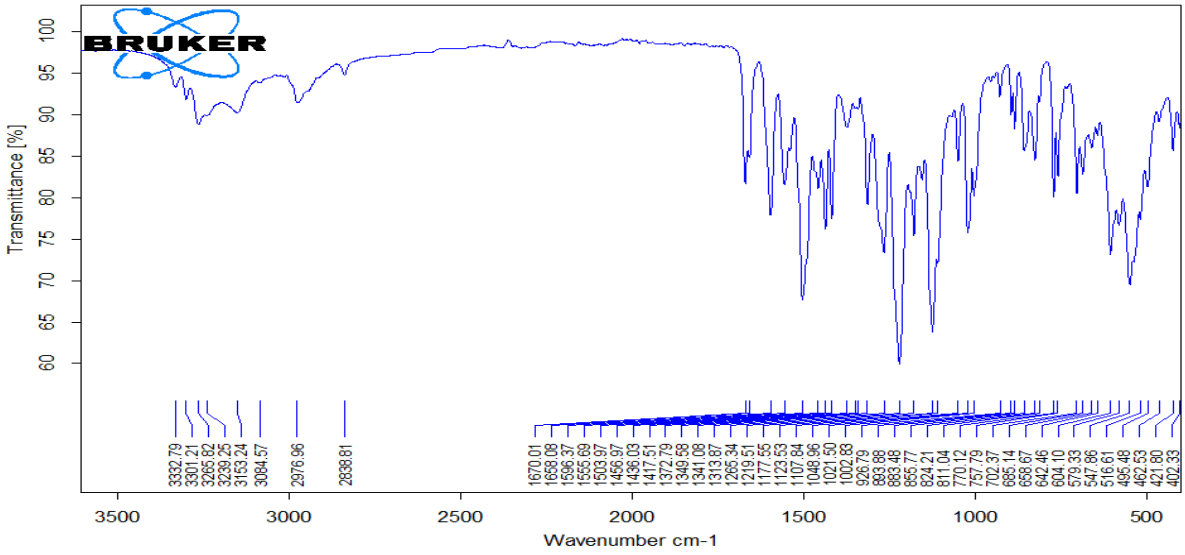


Figure 3. FT-IR spectrum of compound **8**.

^{1}HNMR (400 MHz, DMSO$_{d6}$; δ, ppm): 10.3(s,1H, NH-Ar-thiomide), 9.6(s,1H,NH-thiomide), 9.3(s,1H,OH), 7.5(d, *J*=7.85 Hz,1H, Ar-H), 7.4(s,1H, Ar-H), 7.0(d, *J*= 7.85 Hz,1H, Ar-H), 3.8(s, 3H, O-CH$_3$), 3.7(s,6H, 2xO-CH$_3$), 3.4(s,3H,O-CH$_3$) (**Figure 4**).

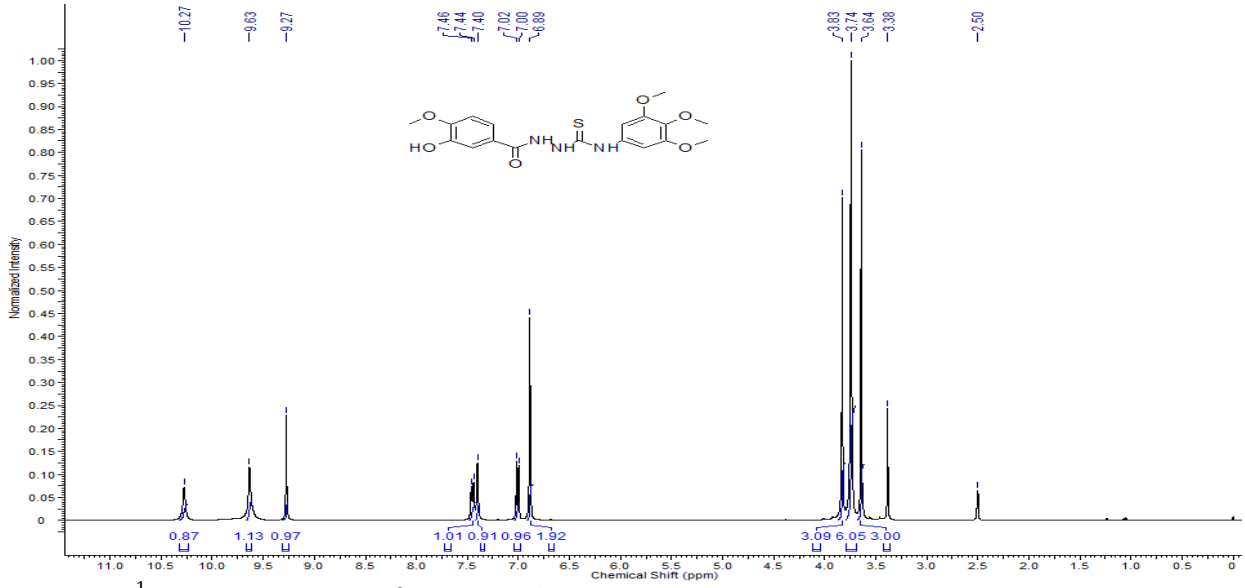


Figure 4. ^{1}HNMR spectrum of compound **8**.

2-(3,4-dimethoxybenzoyl)-N-e(3,4,5-trimethoxyphenyl)ehydrazine-1-carbothioamide (9)

Off White powder, yield 81.5%, R$_f$=0.36 (*n*-hexane/ethylacetate7:3), m.p = 228-230^{o}C. FT-IR(ύ, cm^{-1}): 3290.4(Ar-N-H)str, 3263.2(H-N-C=S)str, 3162.4(H-N-C=O), 3109.4Ar(CH)str, 2980.4(CH$_3$)str, 1678.3(C=O), 1598.7, 1550.0, 1503.7, and 1460 Ar(C=C)str, 1228.3(C-O-C)str, 1150.4(C=S)str, 1136.2(C-N) (**Figure 5**).

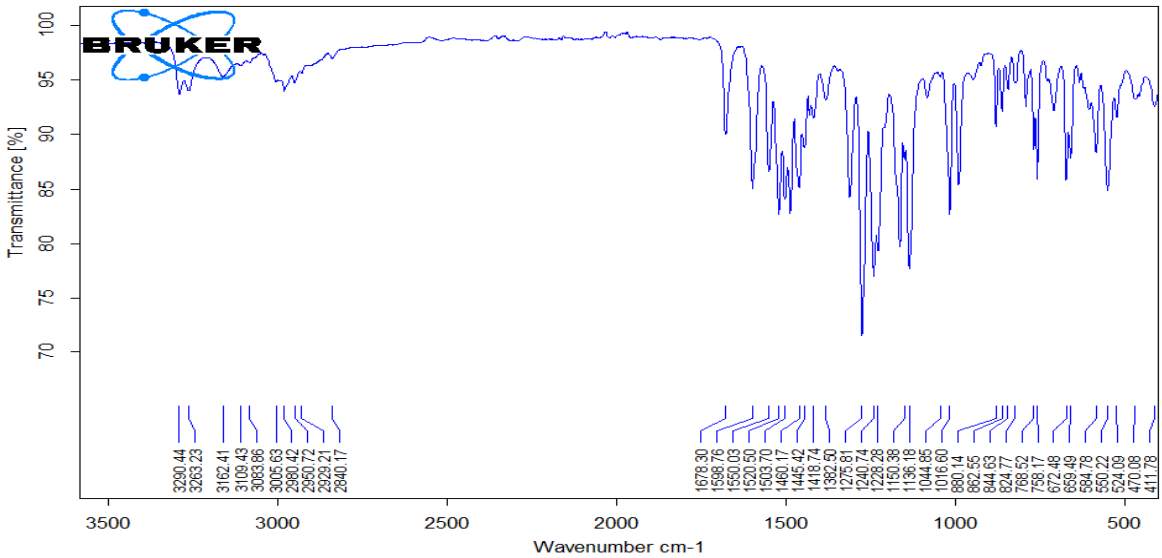


Figure 5. FT-IR spectrum of compound **9**.

^{1}HNMR (400 MHz, DMSO$_{d6}$; δ, ppm): 10.4(s,1H,NH Ar-thiomide), 9.7(s,2H,NH amide and thiomide), 7.6(d,*J*=8, 1H, Ar-H), 7.5(s, 1H, Ar-H), 7.1(d, *J*=8.6 Hz,1H, Ar-H), 6.9(s,2H, Ar-H), 3.8(s,6H,2xO-CH$_3$), 3.7(s,3H,O-CH$_3$), 3.4(s,6H,2x O-CH$_3$) (**Figure 6**).

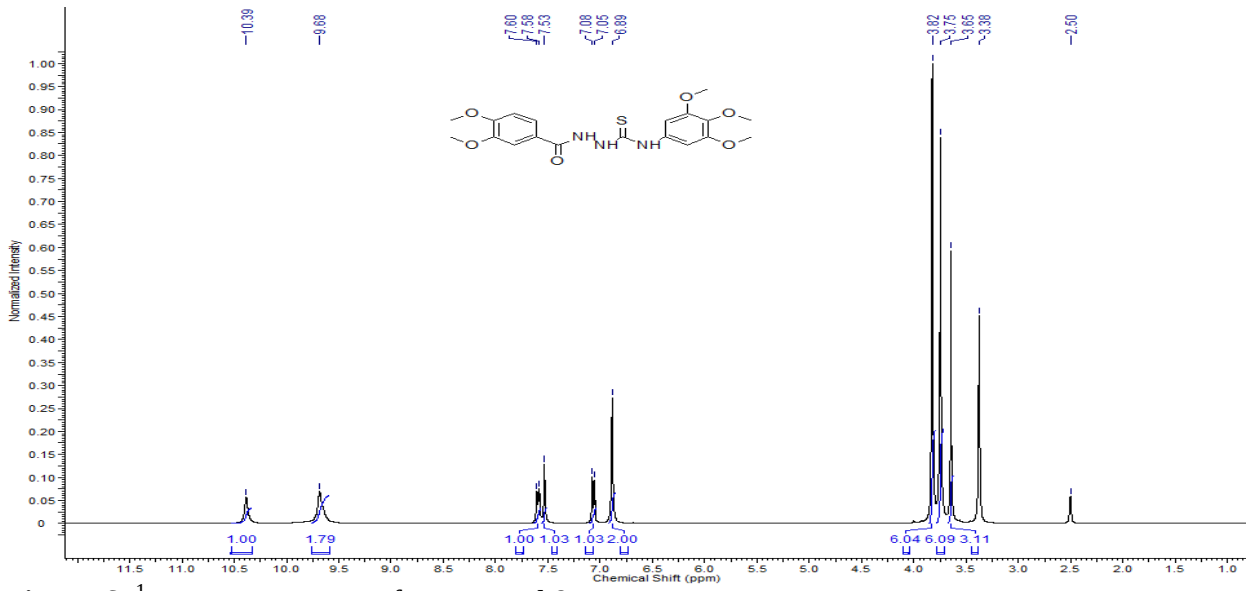


Figure 6. [1]HNMR spectrum of compound **9**.

2-(3-butoxy-4-methoxybenzoyl)-N-(3,4,5-trimethoxyphenyl)ihydrazine-1-carbothioamide (10)

White fluffy powder, yield 83.7%, R_f=0.46 (*n*-hexane/ethylacetate10:4.5), m.p = 158-161^{o}C.

FT-IR(ύ, cm^{-1}): 3291.6(Ar-N-H)str, 3265.1(H-N-C=S)str, 3161.1(H-N-C=O), 3004.9 Ar(CH)str, 2951.6 and 2839.2 *aliph*(CH)str, 1681.0(C=O), 1598.9, 1550.2, 1519.0, 1504.9, and 1484.0 Ar(C=C)str, 1241.0(C-O-C)str, 1177.3(C=S)str, 1132.6(C-N) (**Figure7**).

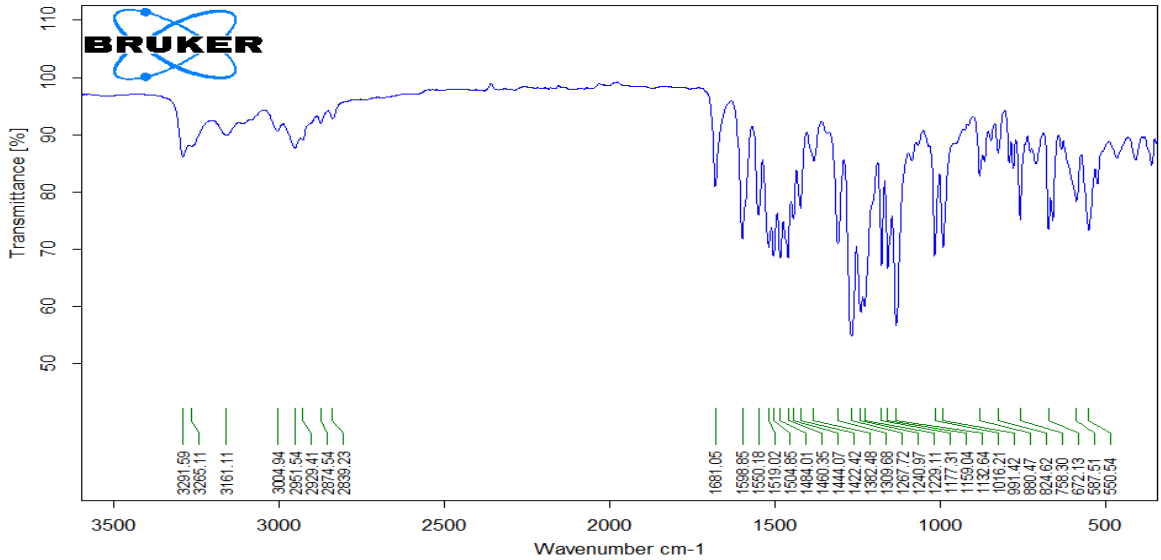


Figure 7. FT-IR spectrum of compound **10**.

[1]HNMR (400 MHz, $DMSO_{d6}$; δ, ppm): 10.4(s,1H, NH Ar-thiomide), 9.7(s, 2H,NH amide and thioamide), 7.6(dd, *J*=8.48Hz,and 2Hz,1H, Ar-H), 7.5(s,1H, Ar-H), 7.1 (d,*J*=8 Hz,1H), 6.9(s,2H,Ar-H), 4.0(t, *J*=6.5 Hz, 2H, O-CH_2), 3.8(s, 6H, 2x O-CH_3), 3.7(s,3H,O-CH_3), 3.4(s, 3H, O-CH_3), 1.7-1.69(m,2H, CH_2), 1.5-1.4(m,2H, CH_2), 0.9(t, *J*=7.4 Hz, 3H, CH_3) (**Figure 8**).

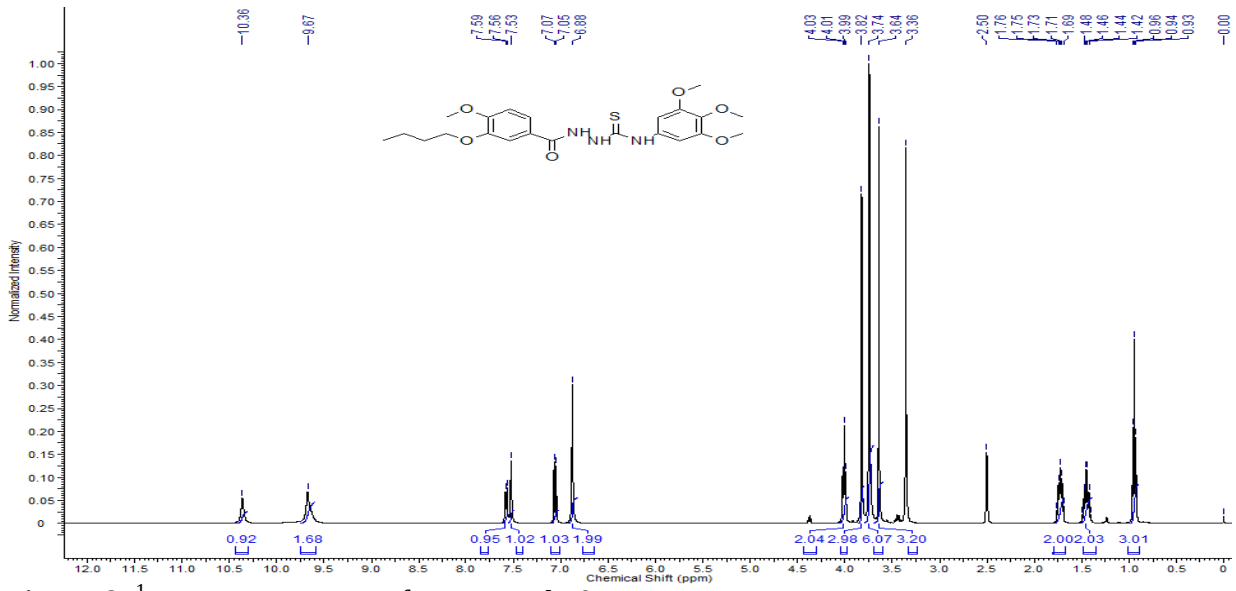


Figure 8. [1]HNMR spectrum of compound **10**.

Molecular docking studies

Method of docking process

For the molecular docking process, the CDOCKER protocol was used. EGFR was kept rigid during the method, while the ligands were made flexible. Each molecule was allowed to create ten different interaction poses with EGFR tyrosine kinase. When the best-fitting poses were identified, docking scores (-CDOCKER interaction energy) were recorded. The protein data bank (https://www.rcsb.org) was used to identify molecular targets for the newly synthesized compounds **(8-10),** compare them to other ligands, and determine the pharmacophoric functionality that may enable binding to the critical amino acids at tyrosine kinase domain of EGFR.

Following the selection of a specific protein, EGFR in the current study, some procedures were performed that provide an understanding of the molecular binding modes of the test compounds inside of the pockets of the proteins (EGFR tyrosine kinase), by using MOE 19.0901 Software. The co-crystallized ligand was used to produce the binding sites within the crystal protein (PDB codes: 4HJO) (https://www.rcsb.org). Water molecules were initially removed from the complex. The crystallographic disorders and unfilled valence atoms were then corrected using protein report, utility, and clean protein options. Protein energy was minimized by applying MMFF94 force fields to it. The essential amino acids of the protein are outlined and ready for docking. The 2D structures of the compounds tested were sketched in Chem-Bio Draw Ultra17.0 and saved in MDL-SD file format using MOE 19.0901 software. The saved file was opened, 3D structures protonated, and energy was kept to a minimum by using a .05 RMSD kcal/mol MMFF94 force field. The minimized structures were then ready for docking with the prepared ligand protocol [14].

ADMET studies

Erlotinib was used as a reference drug to the newly synthesized compounds in absorption, distribution, metabolism, excretion, and toxicity (ADMET) studies, in which the Discovery Studio 2019 Software was used.

Biological study

Cell culture

Human DLD-1 colorectal cancer cells were maintained in high glucose DMEM (Lonza, Switzerland), human HeLa cervical cancer cell line was maintained in low glucose DMEM (Euroclone, Italy), and human MCF-7 breast cancer cells were maintained in RPMI-1640 media (Lonza, Switzerland), media were supplemented with 10% fetal bovine serum (Euroclone, Italy). Trypsin-EDTA

(Millipore-Merck, USA) was used for cell passaging. Cells were incubated in 5% CO_2, 100% humidity at 37°C.

MTT *in vitro* anti-proliferation assay

The MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) cytotoxicity assay was performed according to Mosmann, 1983 [19]. The different compounds were diluted in 0.2% DMSO and 8 different concentrations for each compound were prepared (100, 30, 10, 3, 1, 0.3, 0.1, and 0.03 µM) in the specific growth medium.

Results and Discussion

Chemistry

The general route for the synthesis of the desired compounds is summarized in Scheme (1). Using FT-IR and ^{1}HNMR, all chemically synthesized compounds were identified.

The IR spectra for compounds **8, 9** and **10** showed bands at ύ =3301.2, 3290.4 and 3291.6 cm^{-1}, respectively, for (Ar-N-H) groups, at ύ= 3265.8, 3263.2 and 3265.1 cm^{-1}, for (S=C-N-H) groups, and ύ= 3153.2, 3162.4, and 3161.1 cm^{-1} attributed to (O=C-N-H) groups.

^{1}HNMR spectrum of compound **8** showed a signal at δ= 10.3 and 9.6 ppm assigned to distinct (NH) *sec.* amine, and a signal at δ =9.3 ppm due to (O-H). The ^{1}HNMR spectrum of compound **9** showed signals at δ= 10.4 and 9.7 ppm assigned to (NH) *sec*. amine. The ^{1}HNMR spectrum of compound **10** showed signals at δ =10.4 and 9.8 ppm due to (NH) *sec.* amine. The aromatic protons appeared clearly at their respective region.

Molecular docking results

Ten poses for each compound were generated, then the best orientations were captured, and affinity scores with RMSD (root-mean-square deviation) values were collected. The binding modes and interaction energy (ΔG, docking score) of **erlotinib**, and the newly synthesized hydroxy benzoic acid derivatives against human EGFR target site are presented in **Table 1**.

The binding mode of the crystal ligand **erlotinib** exhibited binding energy of -8.75 kcal/mol against EGFR tyrosine kinase. The quinazolin-4-amine moiety formed five *pi*-alkyl interactions with Leu694, Leu820, Ala719, and one H-bond with Met769, additionally, the bis(2-methoxyethoxy) moiety interacted with Cys773 by two H- bonds. Moreover the 3-ethynylphenyl formed one *pi*-alkyl interaction with Lys721 **(Figure 9)**.

Table 1. Binding energy (Docking score, ΔG, kcal/mol) of compounds (8-10) against EGFR tyrosine kinase target site PDB ID: 4HJO.

Compound	RMSD (Å)	Docking score, (ΔG) Kcal/mol	Interactions	
			H.B	*pi* interactions
8	0.98	-8.56	4	6
9	1.05	-8.04	2	5
10	1.32	-8.36	4	4
Erlotinib	1.09	-8.75	3	6

The binding mode of compound **8** exhibited binding energy of -8.56 kcal/mol against EGFR tyrosine kinase. It created six *pi*-alkyl interactions with Leu768, Leu820, Lys721, Ala719, Leu694, and Val702, additionally, it interacted with Lys721, Lys704, and Met769 by four H- bonds with a distance of 3.02, 2.71, 2.49 and 2.38 Å (**Figure 10**). Compound **9** exhibited binding energy of -8.04 kcal/mol against EGFR tyrosine kinase. Compound **9** produced five *pi*-alkyl interactions with Leu694, Leu820, Lys721, Ala719, and Val702. Additionally, it formed two H-bonds with Met769 and Lys704 (2.57, 2.52 Å) **(Figure 11).** Compound **10** binding mode showed -8.36 kcal/mol binding energy to EGFR tyrosine kinase. It created four *pi*-akyl and *pi*-cation interactions with Cys773, Lys704, Lys721 and Val702. Moreover, compound **10** formed four H-bonds with Met769, Lys704, Lys692, and Cys773 (1.99, 2.47, 2.14 and 2.84 Å) **(Figure 12**).

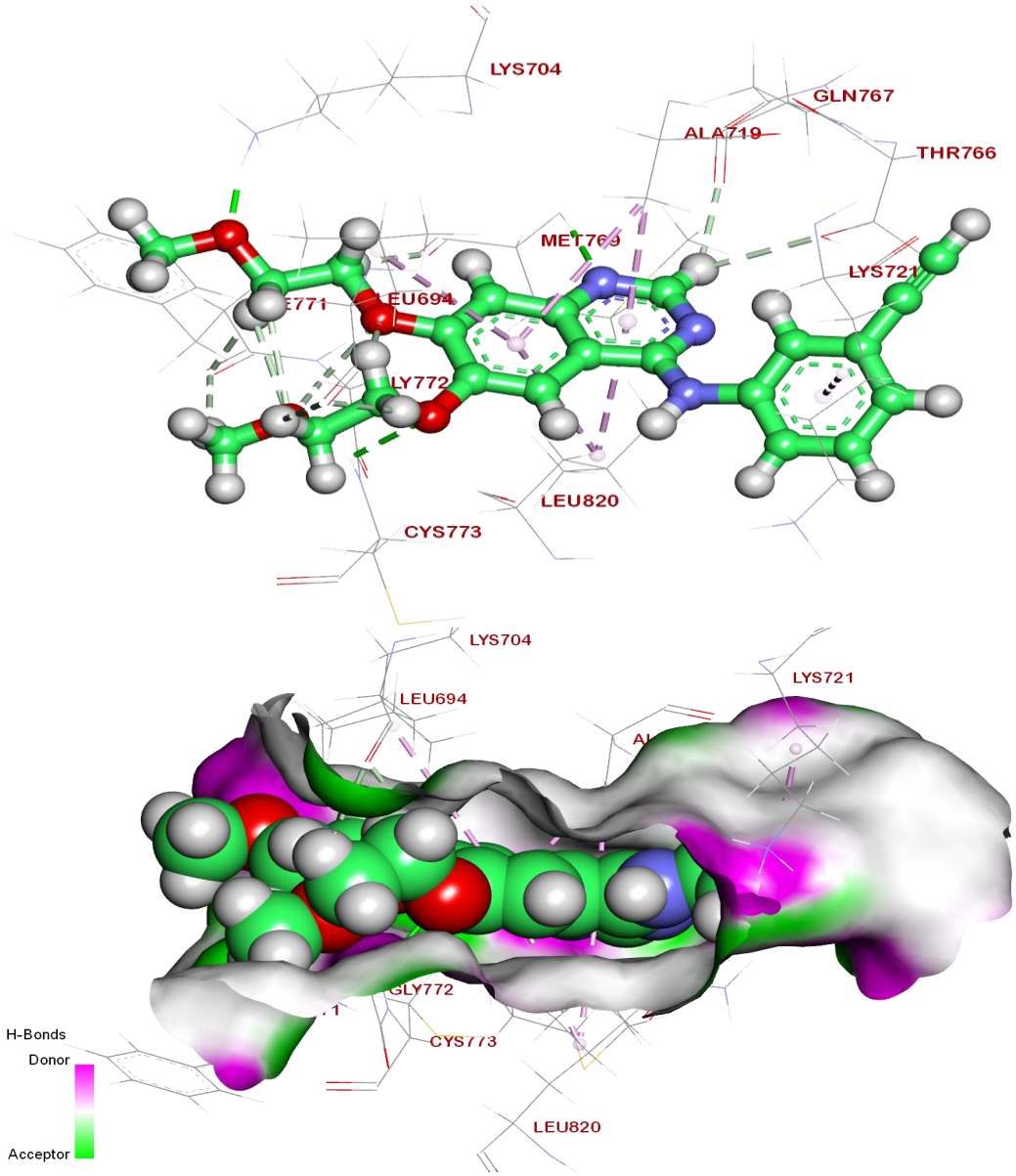


Figure 9. A) The crystal ligand (**erlotinib**) docked in EGFR tyrosine kinase, hydrogen bonds are represented in green lines and the *pi* interactions are represented in purple lines. **B**) Mapping surface showing the crystal ligand **erlotinib** occupying the active pocket of EGFR tyrosine kinase.

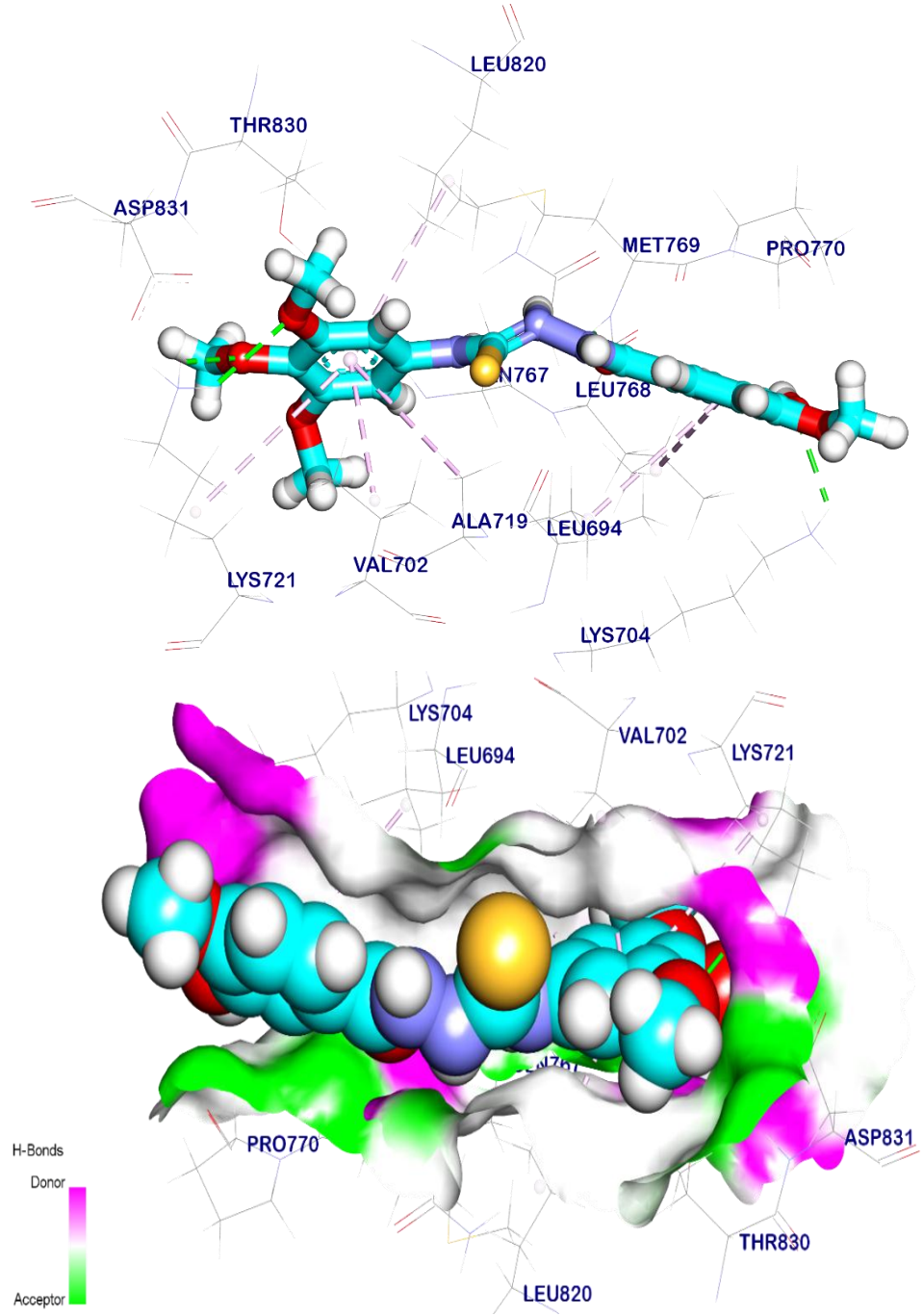


Figure 10. **A)** Compound **8** docked in EGFR tyrosine kinase, hydrogen bonds are represented in green lines and the *pi* interactions are represented in purple lines. **B**) Mapping surface showing compound **8** occupying the active pocket of EGFR tyrosine kinase.

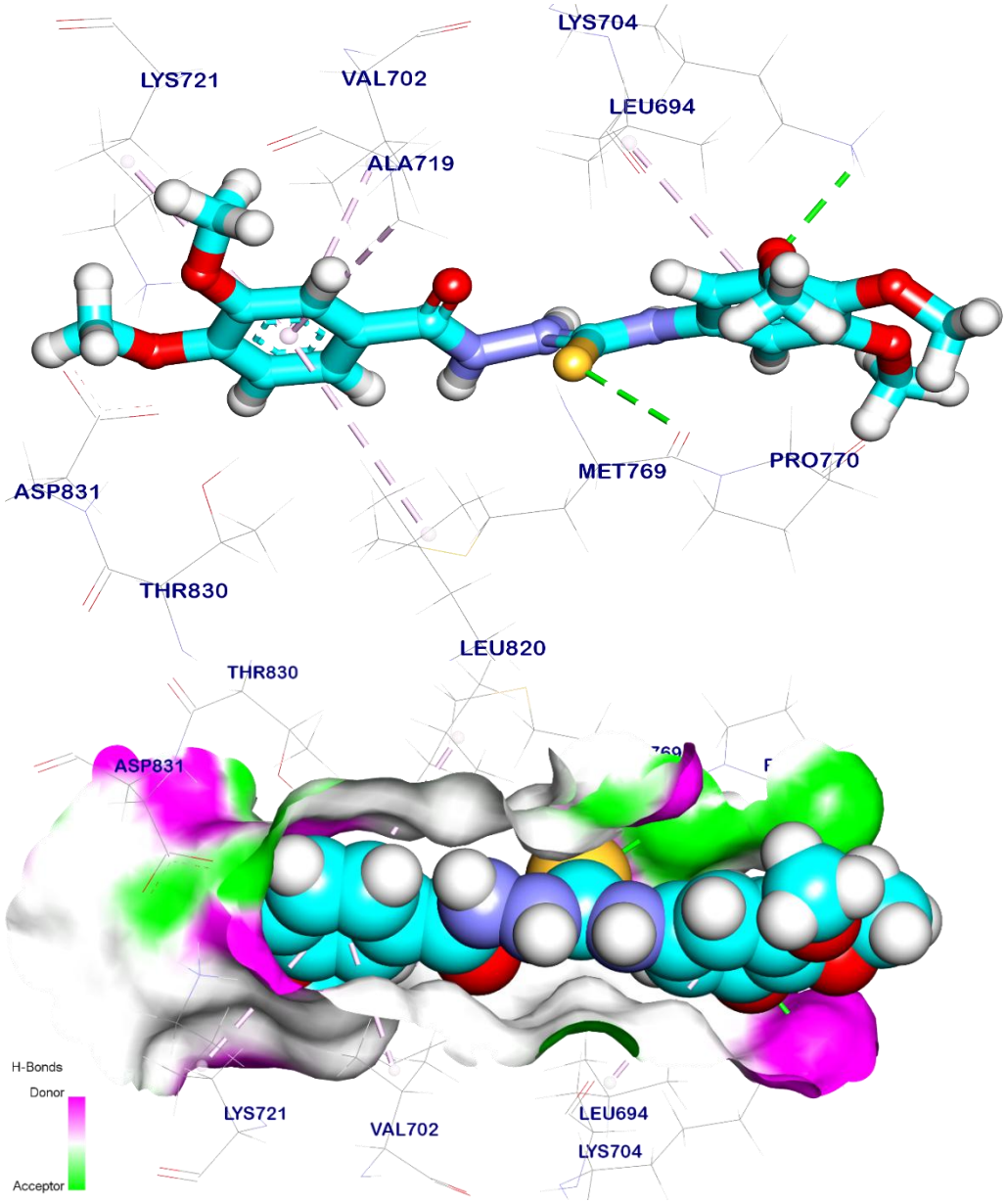


Figure 11. **A)** Compound **9** docked in EGFR tyrosine kinase, hydrogen bonds are represented in green lines and the *pi* interactions are represented in purple lines. **B**) Mapping surface showing compound **9** occupying the active pocket of EGFR tyrosine kinase .

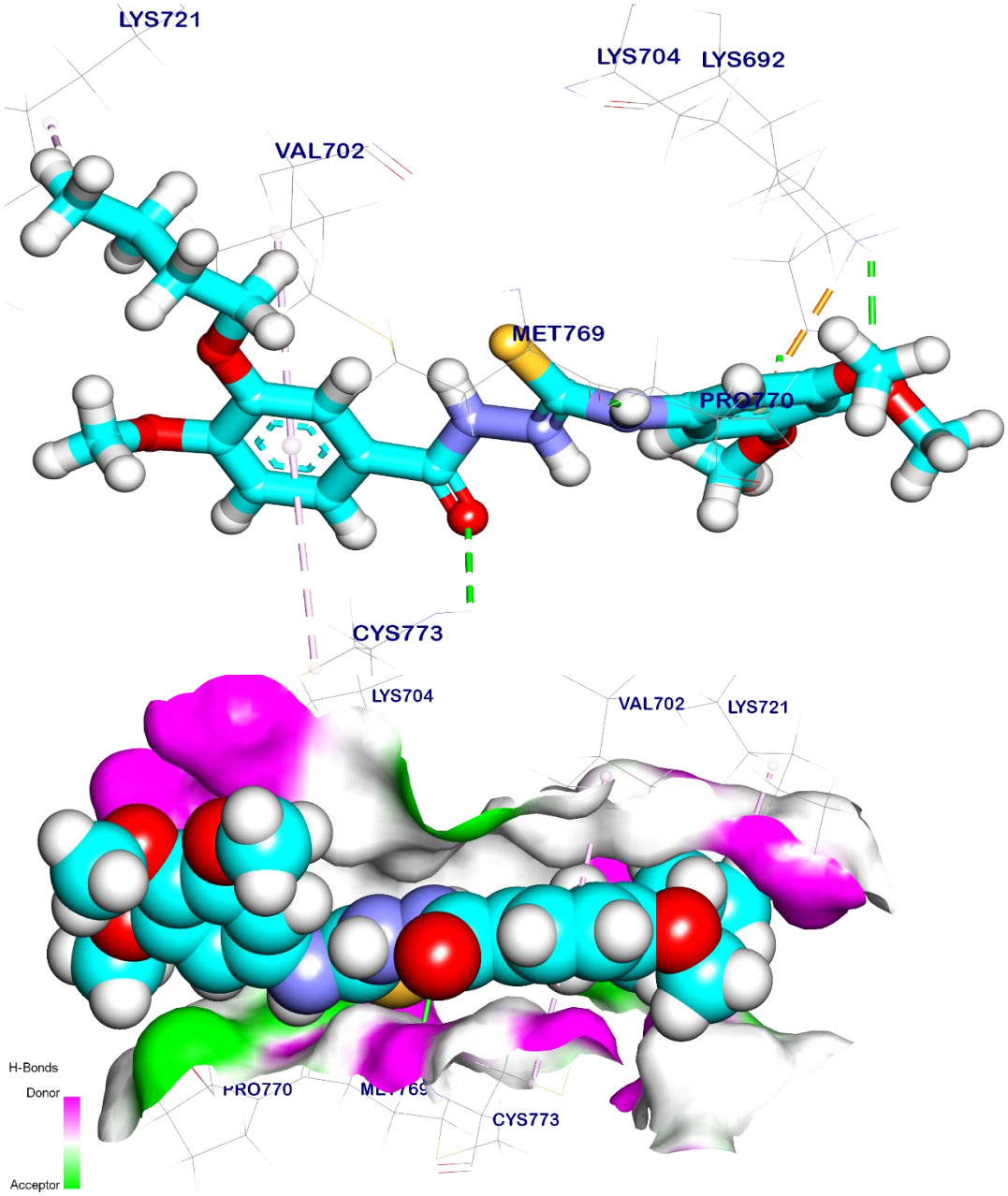


Figure 12. A) Compound **10** docked in EGFR tyrosine kinase, hydrogen bonds are represented in green lines and the *pi* interactions are represented in purple lines. **B**) Mapping surface showing compound **10** occupying the active pocket of EGFR tyrosine kinase.

ADMET studies

Six ADMET parameters were evaluated using the Discovery Studio 2019 Software and **erlotinib** as a reference drug (**Figure 13**). The findings summarized in **Table 2** show that the synthesized compounds displayed high penetration of blood-brain barrier (BBB). The solubility level of all compounds was low except for compound **8** which showed good solubility level. Additionally, all compounds showed some *in silico* hepatotoxicity except for compound **10**. Finally, the new hydroxy benzoic acid derivatives showed an optimal absorption value with good distribution in different organs in the human body.

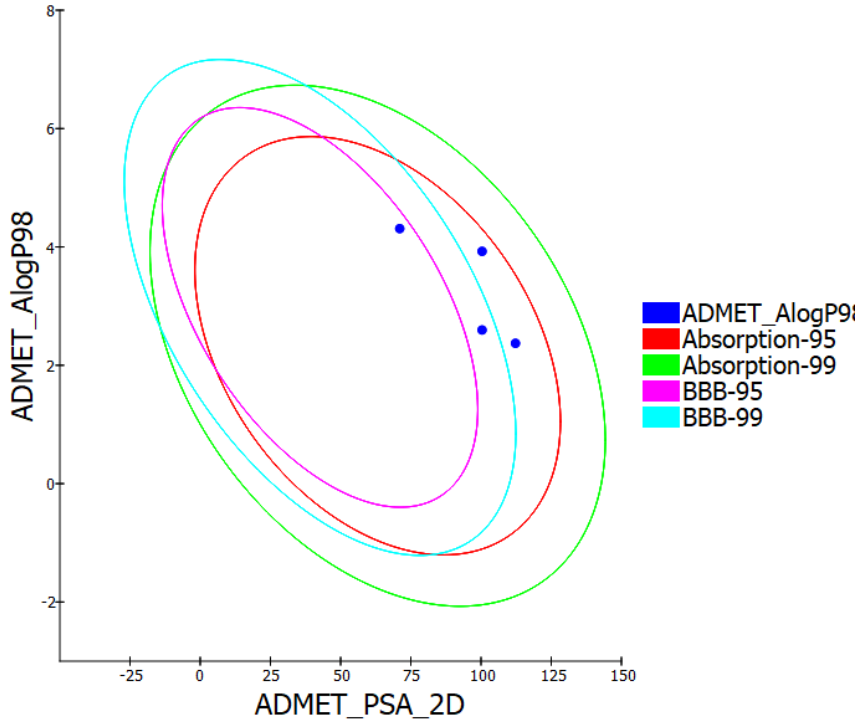


Figure 13. Predicted ADMET chart for the synthesized compounds.

Table 2. Predicted ADMET parameters for the synthesized compounds **8-10**.

Compound	BBB level [a]	Solubility level [b]	Absorption level [c]	Hepatotoxicity	CYP2D6 prediction [d]	PPB prediction [e]
8	5	3	0	True	False	False
9	4	2	0	True	False	True
10	5	2	0	False	False	True
Erlotinib	2	2	0	True	False	True

[a] BBB (blood brain barrier) level: 5 = very high, 4 = high, 3= medium, 2 = low, 1= very low.
[b] Solubility level: 1 = very low, 2 = low, 3 = good, 4 = optimal.
[c] Absorption level: 0 = good, 1 = moderate, 2 = poor, 3 = very poor.
[d] CYP2D6 (cytochrome P2D6): True = inhibitor, False = non inhibitor. The classification of whether a compound is a CYP2D6 inhibitor using the cutoff Bayesian score of 0.161.
[e] PPB (plasma protein binding): True = more than 90%, False = less than 90%. The classification of whether a compound is highly bounded (≥ 90% bound) to plasma proteins using the cutoff Bayesian score of -2.209.

Biological study

The *in vitro* anti-proliferation activities of the novel hydroxy benzoic acid derivatives

To assess the effects of the newly synthesized compounds on cell proliferation, the cytotoxicity of the novel hydroxy benzoic acid derivatives was investigated by means of the MTT assay. The results presented in **Table 3** indicate that compound **8** showed cytotoxicity against the three cancer cell lines tested, while compound **9** showed cytotoxicity against HeLa cells only, and compound **10** was cytotoxic to DLD-1 and HeLa cells. Compound **8** IC_{50} concentrations were comparable to those of the standard tyrosine kinase inhibitor drug **erlotinib** (**Table 3**).

Table 3. *In vitro* anti-proliferation cytotoxicity results of the newly synthesized compounds. Cells were treated for 72 hours, and the experiment was repeated three times. Data are shown as mean ± SEM.

	Average IC_{50} (µM)		
Compound	**DLD-1**	**HeLa**	**MCF-7**
8	25.05±5.09	23.88±5.47	48.36±8.35
9	X	37.67±7.21	X
10	27.26±2.16	19.19±5.03	X
Erlotinib	13.86± 1.06	36.41±0.21	87.34±1.57

Conclusion

A series of hydroxy benzoic acid derivatives (compounds **8-10**) were successfully prepared and structurally characterized by FT-IR and ^{1}HNMR spectroscopy. According to the molecular docking studies, compound **8** had the highest docking score (ΔG) and showed favorable pharmacokinetic profile. Moreover, compound **8** exhibited cytotoxicity against the three cancer cell lines tested, and compound **8** IC_{50} concentrations were comparable to those of the standard tyrosine kinase inhibitor drug **erlotinib**. The findings of the present study indicate that compound **8**, through its EGFR inhibitory activity, is a promising cytotoxic agent.

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