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Synthesis, anticancer activity and molecular docking studies of new 4-nitroimidazole derivatives

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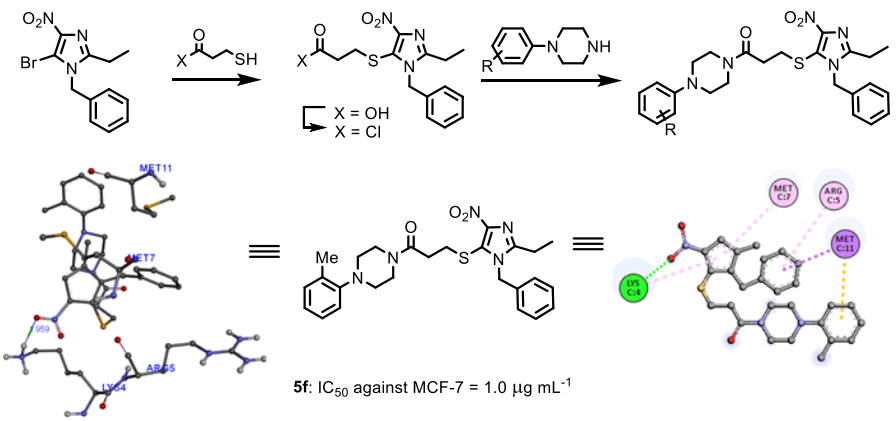
Abstract

Imidazoles have occupied a unique position in heterocyclic chemistry, and its derivatives have attracted considerable interests in recent years for their versatile properties in chemistry and pharmacology. Herein, we report the synthesis of 3-(1-benzyl-2-ethyl-4-nitro-1H-imidazol-5-ylsulfanyl)-1-(4-substituted phenyl-piperazin-1-yl)-propan-1-one **5a**-**p** by reaction of 3-(1-benzyl-2-methyl-4-nitro-1H-imidazol-5-ylsulfanyl)-propanoyl chloride (**3**) with piperazine nucleophiles. Eighteen compounds were assessed for their antiproliferative inhibition potency against four human cancer cell lines (MCF-7, PC3, MDA MB231 and Du145). Compounds **5f** and **5k** were the most potent anticancer agents on MCF-7 cell lines cell line with IC_{50} value of 1.0 µg/mL, while **5d** and **5m** exhibited cytotoxic effect on PC3 and DU145 cell lines with IC_{50} values of 4.0 and 5.0 µg/mL, respectively. The molecular docking of compounds **5f**, **5d** and **5m** has been studied.



Keywords: Anticancer activity, imidazoles, piperazines, molecular docking study

Introduction

Imidazoles have attracted attention since imidazole ring presents in the essential amino acid, histidine which is existed in many proteins and enzymes and plays an important role in the structure and binding function of hemoglobin. Biological studies showed great numbers of substituted imidazoles with wide spectrum of biological activities, such as antitumor, antimicrobial, anti-HIV, antibacterial, antihypertensive, antifungal and anticonvulsant activity.[1-6] Imidazole nucleus and its derivatives are considered as privileged scaffold in medicinal chemistry, they constitute an important class of therapeutic agents and well known as drugs. For example; Dacarbazine (DTIC) (5-(3,3-dimethyl-1-triazeno)imidazol-4-carboxamide was synthesized as an alkylating agent[7] and used in the treatment of metastatic melanoma[8,9] as well as a part of the ABVD chemotherapy regimen to treat Hodgkin's lymphoma[10,11] and in the MAID regimen for sarcoma. [12] Temozolomide (Temodar) is also classified as one of alkylating agents commonly used to treat certain types of brain tumors such as glioblastoma multiforme or anaplastic astrocytoma.[13,14] Furthermore, clotrimazole [1-(2-chlorotrityl)-1*H*-imidazole] is an azole antimycotic agent (antifungal) and used to treat skin infections such as athlete's foot, jock itch, ringworm, and other fungal skin infections (candidiasis).[15,16] Moreover, imidazole ring substituted with nitro group (nitroimidazoles) are also biologically active compounds commonly used as therapeutic agents for treatment of different diseases such as; metronidazole [2-(2-methyl-5-nitroimidazol-1-yl)ethanol] (Flagyl) (antibiotic) is used to treat trichomoniasis, amoebiasis, and giardiasis. [17] Misonidazole (1-methoxy-3-(2-nitroimidazol-1-yl)propan-2-ol) (radiosensitizer and antineoplastic) is one of the imidazole drugs which used for treatment of hypoxic tumors,[18] meanwhile cimetidine is considered as a potential histamine H2 receptor antagonist that inhibits stomach acid production.[19] In addition, secnidazole (hydroxy-2-propyl)-l-methyl-2-nitro-5-imidazole) and tinidazole (1-[2-(ethylsulfonyl)ethyl]-2-methyl-5-nitroimidazole) has been described for treatment of bacterial vaginosis.[20,21] Some selected structures of biologically active imidazole compounds are shown in Fig 1.

Based on the imidazole pharmacological importance of imidazole derivatives and in contituation of our previous work on imidazole analogues with their antiviral and anticancer activity,[22-29] we report here new derivatives of nitroimidazole-containing piperazine derivatives and evaluation of their anticancer activity as well as the molecular docking study.

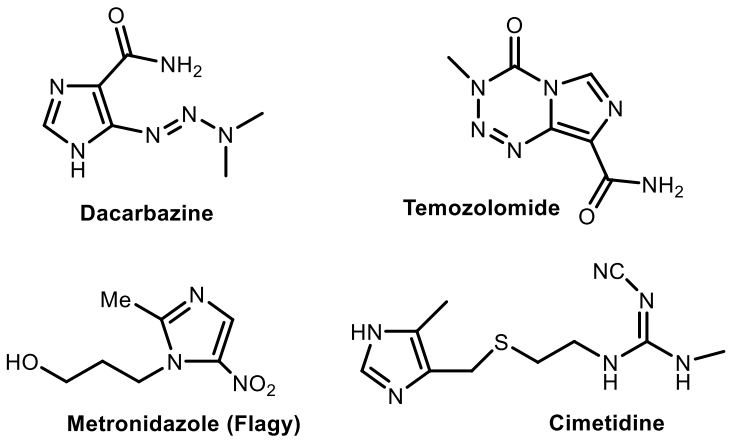


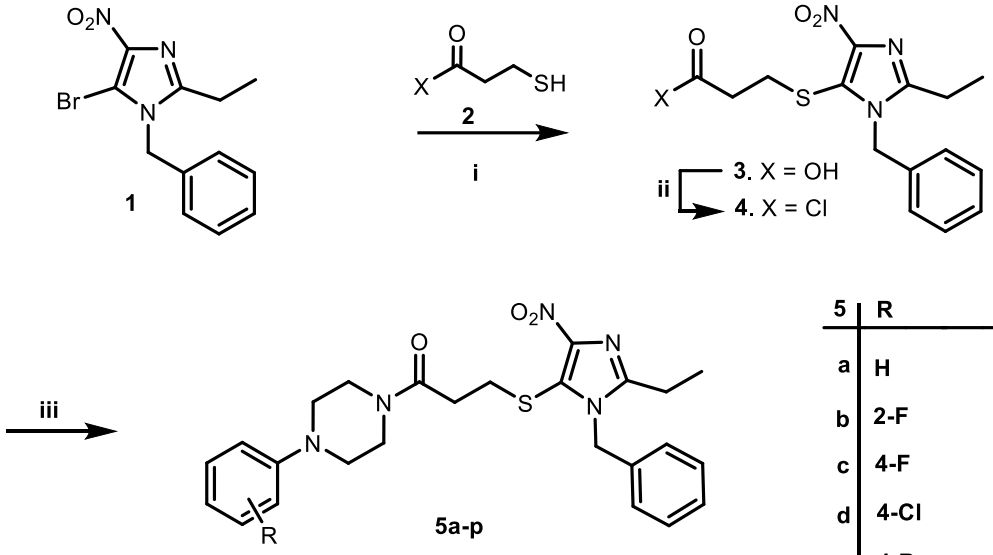
Fig.1. Some biologically active imidazole compounds

Results and Discussion

Chemistry

Over the last fifteen years, our laboratory synthesized several new derivatives of 4-nitroimidazoles, including some new 5-alkylsulfanyl derivatives of imidazoles[27] *via* the nucleophilic displacements of the bromine atom activated by an adjacent nitro group. Our efforts are continued in preparation of such compounds carrying various potential groups aiming to evaluate their anticancer activity might leading to active candidates.

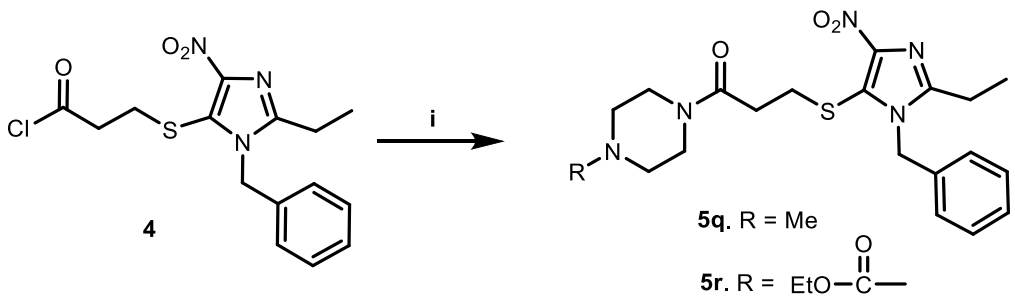
Compound **1** has been selected as a key intermediate for the synthesis of our targets by treatment with 3-mercaptopropanoic in the presence of K_2CO_3 in hot *i*-PrOH to give, after purification, **4** in 60% yield. The structure assignment of **4** follows from the ^{1}H- and ^{13}C-NMR spectra, and was confirmed by X-ray diffraction.[30] The crude product of 3-(1-benzyl-2-ethyl-4-nitro-1*H*-imidazol-5-ylsulfanyl)propionyl chloride (**5**), prepared by treatment of **4** with excess thionyl chloride, was used directly for the next step without further purification. Substituted-piperazine nucleophiles were treated with **4** to furnish the target products **5a**-**r** in 68-77% yield. (Scheme1).



5	R	5	R
a	H	i	3-MeO
b	2-F	j	4-MeO
c	4-F	k	2-OH
d	4-Cl	l	4-OH
e	4-Br	m	2-CN
f	2-Me	n	4-CN
g	3-Me	o	4-NO_2
h	2-MeO	p	4-$COCH_3$

Scheme 1. *Reagents and conditions*: (i). KOH, isopropanol, 60-80 ^{o}C, 4 h; ii.$SOCl_2$, reflux, 3 h; (iii) substituted piperazine, $CHCl_3$, pyridine, rt, 18 h.

Analogously, aliphatic residues derived piperazine moiety of 5-nitroimidazole analog have prepared, aiming to study the effect of such group on the cancer cell lines. Thus, compounds **5q** and **5r** have been prepared in 75 and 71% yield, respectively, from **4** using the same procedure described for **5a**-**5p**. (Scheme 2)



Scheme 2. *Reagents and conditions*: (i) substituted piperazine, $CHCl_3$, pyridine, rt, 18 h.

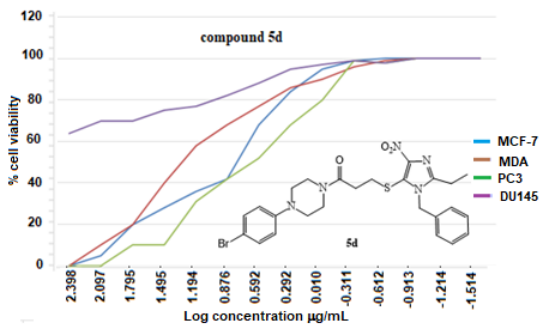
The structures of the newly prepared compounds **5a-5p** were confirmed on the basis of their ^{1}H, ^{13}C NMR, DEPT experiments (for distinguishing between CH_3, CH_2, CH carbons and the quaternary carbons) and

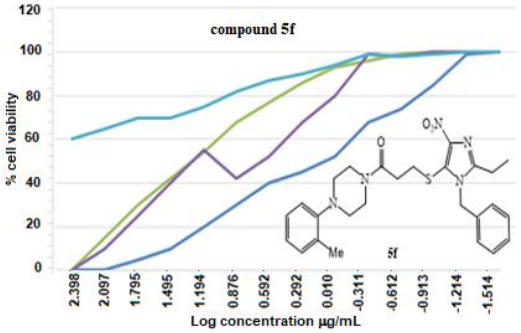
mass spectroscopic data in addition to elemental analysis. In the ^{1}H NMR spectra, the phenyl, and ethyl protons of the imidazole ring showed rather similar patterns, whereas the singlets in the region δ 5.40-5.43 ppm were attributed to the methylene of benzyl group. The $COCH_2CH_2S$ protons appeared in the region δ 2.63-3.20 ppm. The eight methylene protons of the piperazine ring are equivalent, while the ring protons appear as a broad hump. In the ^{13}C NMR spectra of **5a-5p,** resonances in the region δ 31.3-31.9 ppm were assigned to SCH_2 carbon atoms, whereas the signals at δ 32.3-32.7 ppm were attributed to the methylene carbons adjacent to the carbonyl group of the amide residue. The Ph*C*H$_2$ carbon atom appeared in the region δ 47.0-51.4 ppm, while the downfield resonances at the region δ 168.5-168.9 ppm were assigned to the carbonyl group. Carbon atoms of the imidazole and piperazine moieties have been fully analyzed (*c.f*. Experimental section).

Additional support for identification of the synthesized compounds came from LC-MS and LC-MS/MS, which revealed the correct molecular ion $[M+H]^+$, as suggested by their molecular formulas.

In vitro cytotoxic activity

The cytotoxic potential of the newly synthesized hybrid compounds **5a-5r** was evaluated *in vitro* against a panel of human tumor cell lines using MTT assay.[31] The panel consisted of breast canrcinoma (MCF-7 and MDA MB231), and human prostate cancer (PC-3, and DU-145) cell lines. Paclitaxil and docitaxil were used as the reference drugs. The results are summarized in Table 1, which showed that nitroimidazole scaffold bearing substituted-piperazine moieties have a significant effect on the cytotoxixic activity. Compounds **5f**, and **5k** showed a good activity cytotoxic activity against MCF-7 cell lines with IC_{50} values of 1.0 µg/mL. IC_{50} values of the other analogues against MCF-7 cell lines were ranged between 2.0-8.0 µg/mL, except compounds **5g**, **5j**, **5o** , **5q** and **5r** with IC_{50} value of >100 µg/mL. In terms of the substituents with different positions of phenyl-piperazine residue, the IC_{50} values of Table 1 clearly showed that the replacement of the H atom of the phenyl-piperazine with 2-Me or 2-OMe groups produced a significant increase in the inhibitory growth effect on the MCF-7 cell lines (compounds **5f** and **5h**). On the other hand, the synthesized compounds were inactive against breast cancer (MDA MB231 cell lines), except **5k** and **5m** which exhibited IC_{50} values of 12.02 and 10.83 µg/mL. Additionally, compounds **5d** and **5m** having the chlorine and cyano groups 4- and 2-position of the phenyl-piperazine ring, respectively, exhibited moderate cytotoxic effect on PCa (PC3 and Du145) cell lines with IC_{50} values of 4.0 and 5.0 µg/mL, respectively. Figure 2 demonstrates the cell viability (%) of cell lines, MCF-7, MDA, PC3 and DU145, against compounds **5d**, **5f** and **5m**.





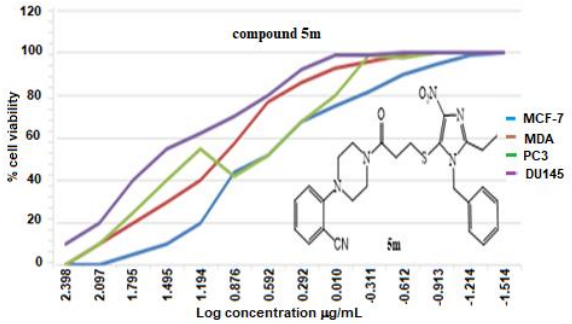


Fig. 3. Cell viability % of cell lines (MCF-7, MDA, PC3 and DU145) against compounds **5d**, **5f** and **5m**

Table 1.

In vitro cytotoxicity[a] of some 4-nitroimidazole analogues given as IC_{50}[b] in µg/mL

Compd.	Cell line			
	MCF-7	MDA MB231	PC3	Du145
5a	7.0±0.2	>100	48.0±0.6	>100
5b	8.0±0.26	24.88±1.92	>100	>100
5c	6.0±0.1	40.23±4.79	>100	>100
5d	5.0±0.7	18.68±1.03	4.0±0.2	>100
5e	5.0±0.17	25.11±3.55	44.0±8.3	>100
5f	1.0± 0	20.12±1.55	18.0±0.6	>100
5g	>100	20.72±1.91	17.0±0.2	>100
5h	2.0±0.1	21.68±0.76	>100	>100
5i	5.0±0.17	25.09±2.20	6.0±0.1	>100
5j	>100	>100	>100	>100
5k	1.0 ± 0	12.02±1.25	58.0±1.6	>100
5l	2.0± 0.1	40.18±1.42	>100	>100
5m	4.0±0.1	10.83±2.58	41.0±0.9	5.0±0.3
5n	2.0±0.1	18.46±1.58	73±1.0	>100
5o	>100	23.38± 2.38	>100	>100
5p	2.0±0.1	NT	>100	>100
5q	>100	>100	>100	>100
5r	>100	13.72±2.59	79.0±0.6	>100
Paclitaxil	0.021	0.0310	0.010	0.07
Docitaxil	0.230	0.105	0.010	0.1

NT: Not tested. [a] Cytotoxicity as IC_{50} (± SD values) for each cell line is the concentration of tested compound with reduced by 50% the optical density of treated cells with respect to untreated cells using the MTT assay. [b] Data represent the mean values of three independent determinations.

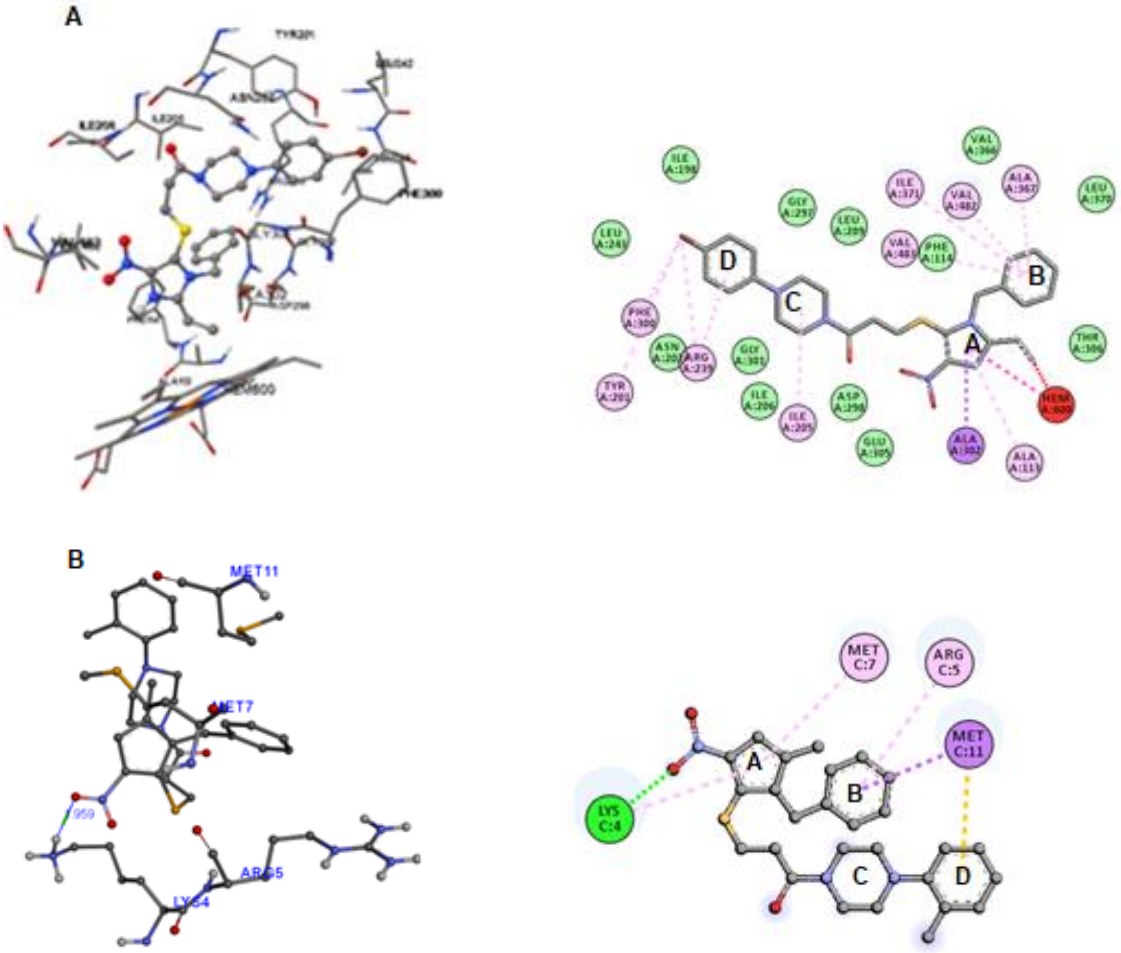
Molecular docking study

In silico study using molecular docking was undertaken against targets of imidazol analogues to verify the potential affinity of the most active compounds of the series **5d**, **5f** and **5m** to the target proteins. In docking calculations, compound **5d** was docked to the binding pockets of proteins with PDB code 3RUK (chain A), while

5f and **5m** were docked with 3U2B (B chain) and 5T8E (C chain), respectively, using Autodock4[32] and the docking results were viewed and analysed by MGLTools.

MCF-7, PC-3 and DU145 cell lines, respectively. The binding energy scores of **5d**, **5f** and **5f** were found -9.97, 7.68 and -7.17 kcal mol^{-1}, respectively, indicating selectivity and potency profiles of these analogs to bind the active site of proteins pockets. Detailed analysis of the binding mode showed that compound **5d** is settled down in the protein active site properly. Fig. 2 (A) demonstrated π - π stacking interactions between the aromatic ring (ring B) of **5d** and Phe114, together with the same interaction between the pyrrole ring of HEM600 and imidazole scaffold (ring A). Additionally, it showed seven π⍰H interactions: three interactions between the aromatic ring at N-1 (ring B) and Ala367, Val482 and 371 were observed, while other interactions were indicated between imidazole ring and Ala113 and Ala302. Furthermore, π⍰H interactions between rings C and D with Ile205 and Arg239, respectively, in addition to the same interaction between OMe group at ring D and Phe300.

Fig 2 B showed π⍰H interactions between imidazole ring and Met7 and Lys4, while ring B revealed the same interaction with Me11 and Arg5. The phenyl group (ring D) presented a π⍰H interaction with Met11, in addition to aliphatic hydrophobic action between nitro group and Lys4. Fig. 2 (C) demonstrated that compound **5m** was able to show π–π interactions between imidazole ring and Trp718 as well as ring D with Phe764. Further π⍰H and aliphatic hydrophobic interactions with protein receptors-binding residues including His714, Val715, Pro682, Ala748, Met749, Arg752, Met745, Leu707, Leu873 and Met742 were witnessed.



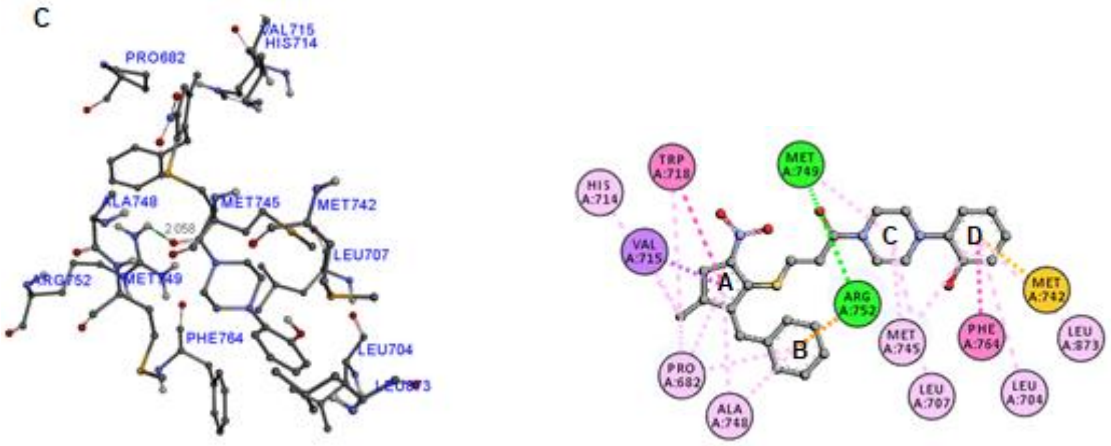


Fig. 2. The interactions mode of compounds **5d** (A) and **5f** (B) and **5m** (C) with the active site amino acids of the proteins (PDB ID's: 3RUK, 3U2B and 5T8E), respectively.

Conclusions

In conclusion, we have reported the synthesis of new 4-nitroimidazole derived substituted arylpiperazine at C-5. The structures of the new synthesized 4-nitroimidazole derivatives were confirmed by the spectral and mass data. The synthesized compounds were evaluated for their activity against breast cancer (MCF-7 and MDA MB231) and prostate cancer (PC3 and DU145) cell lines. Two derivatives, **5f** and **5h** exhibited significant cytotoxic activity on MCF cell lines (IC_{50} 1.0 µg/mL), while compounds **5d** and **5m** showed cytotoxic effect on PC3 and DU145 cell lines with IC_{50} values of 4.0 and 5.0 µg/mL, respectively. These studies revealed that such molecules have high scope and potential for further investigations. Molecular docking studies were in agreement with the anticancer activity data. Studies on extensive diversification, mechanistic analysis and application of pharmacognosy principles, especially compounds **5f** and **5h**, are currently under process to come up with better leads.

Experimental Section

General. Melting points were measured on a Mettler FP1 melting point apparatus and are uncorrected. Reaction progress was monitored by thin layer chromatography (TLC) on Alugram SIL G UV254 (Macherey-Nagel). All new compounds were analyzed for C, H, and N using a 2400 CHN Elemental Analyzer by Perkin Elmer. The observed results agreed with the calculated percentages to within ±0.4%. ^{1}H and ^{13}C-NMR spectra were recorded on a Bruker DRX-300 instrument. Chemical shifts are given in parts per million (ppm), and tetramethylsilane (TMS) was used as internal standard for spectra obtained in $CDCl_3$. Mass spectra were measured on LC-MS 8050 triple quadrupole mass spectrometer (Shimadzu, Kyoto, Japan) equipped with a binary solvent delivery system (LC-30AD), a controller (CBM 20A), an autosampler (SIL-30A), column thermostat (CTO-20AC).

Synthesis of 3-(1-benzyl-2-ethyl-4-nitro-1*H*-imidazol-5-ylsulfanyl)propionyl chloride (4). A solution of **3** (0.5 mmol) and thionyl chloride (5 mL) was heated under reflux at 75-80 °C for 3-4 h. Excess of thionyl chloride was removed under vacuum to afford compound **4.** The crude product **4** was used directly for the next step without further purification.

General procedure for the preparation of 4-nitroimidazole analogues (5a-p). Compound **4** (0.5 mmol) was dissolved in $CHCl_3$ (15 mL), piperazine derivatives (0.7 mmol) and three drops of pyridine were added, the reaction mixture was stirred at room temperature for 18 h. After cooling, the mixture was evaporated to dryness. The residue was partitioned between $CHCl_3$ (50 mL) and water (50 mL) and the combined organic extracts were dried over anhydrous sodium sulfate (Na_2SO_4), filtered and evaporated to dryness. The residue was purified by thin-layer chromatography (TLC) and eluted with ($CHCl_3$-MeOH; 9.5:0.5) to give **5a-p**.

3-(1-Benzyl-2-ethyl-4-nitro-1*H*-imidazol-5-ylsulfanyl)-1-(4-phenyl-piperazin-1-yl)-propan-1-one (5a). Brown amorphous; Yield: (166 mg, 70%). ^{1}H NMR (300 MHz, DMSO-d_6): δ_H 1.13 (t, *J* 7.3 Hz, 3H, CH_3CH_2), 2.64 (m, 2H, CH_2CH_3), 3.04-3.06 (m, 4H, SCH_2CH_2), 3.71-3.76 (br. s, 8H, $H_{piperazine}$), 5.41 (s, 2H, CH_2Ph), 6.8-6.95 (m, 1H, $H_{arom.}$), 6.9-7.1 (m, 4H, $H_{arom.}$), 7.2-7.5 (m, 5H, $H_{arom.}$). ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ_C 10.7 (CH_3), 20.3 (CH_2CH_3), 31.8 (CH_2CO), 32.6 (CH_2S), 40.8, 44.3, 47.0, 48.2 ($C_{piperazine}$), 48.5 (CH_2Ph), 115.8, 119.3, 126.1, 127.7, 128.9, 129.0 ($C_{arom.}$), 135.8 (C-5), 148.0 (C-4), 150.7 (C-2), 168.5 (C=O). LCM-MS (*m/z*): 480 $[M+H]^+$. Anal. Calcd. for $C_{25}H_{29}N_5O_3S$: C, 62.61; H, 6.10; N, 14.60. Found: C, 62.85; H, 6.30; N, 14.83.

3-(1-Benzyl-2-ethyl-4-nitro-1*H*-imidazol-5-ylsulfanyl)-1-[4-(2-fluoro-phenyl)-piperazin-1-yl]-propan-1-one (5b). Brown amorphous; Yield: (340 mg, 71%). ^{1}H NMR (300 MHz, DMSO-d_6): δ_H 1.14 (t, *J* 7.3 Hz, 3H, CH_3), 2.57-2.65 (m, 4H, CH_2CH_3+CH_2CO), 288-2.92 (m, 4H, CH_2S+2$H_{piperazine}$), 3.07 (t, *J* 6.3 Hz, 2H, $H_{piperazine}$), 3.43-3.54 (m, 4H, $H_{piperazine}$), 5.40 (s, 2H, CH_2Ph), 6.99-7.18 (m, 6H, $H_{arom.}$), 7.30-7.39 (m, 3H, H_{arom}). ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ_C 10.7 (CH_3), 20.3 (CH_2CH_3), 31.8 (CH_2CO), 32.6 (CH_2S), 41.1, 44.62, 47.0, 50.0 ($C_{piperazine}$), 50.2 (CH_2Ph), 115.5 (d, $^2J_{CF}$ 20.4, C-3), 116.1, 119.5, 124.9, 125.7, 126.1, 127.7 ($C_{arom.}$), 122.8 (d, $^4J_{CF}$ 8.06, C-5), 128.9 (C-5), 135.9 (C-4), 139.5 (d, $^3J_{CF}$ 8.3, C-6), 150.0 (C-2), 154.9 (d, J_{CF} 244, C-2), 168.5 (C=O). LCM-MS (*m/z*): 498 $[M+H]^+$. Anal. Calcd. for $C_{25}H_{28}FN_5O_3S$: C, 60.35; H, 5.67; N, 14.07. Found: C, 60.56; H, 5.79; N, 14.22.

3-(1-Benzyl-2-ethyl-4-nitro-1*H*-imidazol-5-ylsulfanyl)-1-[4-(4-fluoro-phenyl)-piperazin-1-yl]-propan-1-one (5c). Brown amorphous; Yield: (180 mg, 72%). ^{1}H NMR (300 MHz, DMSO-d_6): δ_H 1.13 (t, *J* 7.3 Hz, 3H, CH_3), 2.64-2.66 (m, 4H, CH_2CH_3+CH_2CO), 2.90-2.95 (m, 6H, CH_2S+$H_{piperazine}$), 3.64-3.7 (m, 4H, $H_{piperazine}$), 5.40 (s, 2H, CH_2Ph), 6.9-7.09 (m, 6H, $H_{arom.}$), 7.31-7.34 (m, 3H, $H_{arom.}$). ^{13}C-NMR (DMSO-d_6, 75.5 MHz): δ_C 10.7 (CH_3), 20.3 (CH_2CH_3), 31.8 (CH_2CO), 32.6 (CH_2S), 40.9, 44.4, 47.0, 49.0 ($C_{piperazine}$), 49.3 (CH_2Ph), 115.5, 117.7, 126.1, 127.7, 128.9, 125.7, 135.9, 147.6, 148.0 ($C_{arom.}$+C-5), 150.0 (C-4); 154.7 (C-2), 168.5 (C=O). LCM-MS (*m/z*): 498 $[M+H]^+$. Anal. Calcd. for $C_{25}H_{28}FN_5O_3S$: C, 60.35; H, 5.67; N, 14.07. Found: C, 60.49; H, 5.80; N, 14.30.

3-(1-Benzyl-2-ethyl-5-nitro-1*H*-imidazol-5-ylsulfanyl)-1-[4-(4-chloro-phenyl)-piperazin-1-yl]-propan-1-one (5d). Reddish brown powder; Yield: (193 mg, 75%); mp 84-87 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ_H 1.13 (t, *J* 7.3 Hz, 3H, CH_3), 2.57-2.91 (m, 6H, CH_2CH_3+SCH_2CH_2), 2.99-3.25 (m, 6H, $H_{piperazine}$), 3.42-3.69 (m, 2H, $H_{piperazine}$), 5.40 (s, 2H, CH_2Ph), 6.94-7.13 (m, 4H, $H_{arom.}$), 7.23-7.45 (m, 5H, $H_{arom.}$). ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ_C 10.7 (CH_3), 20.3 (CH_2CH_3), 31.8 (CH_2CO), 32.6 (CH_2S), 40.7, 44.2, 47.0, 47.9 ($C_{piperazine}$), 48.2 (CH_2Ph), 117.2, 122.8, 125.7, 126.1, 127.7, 128.6, 128.9, 129.0 ($C_{arom.}$), 135.9 (C-5), 149.5 (C-4), 150.0 (C-2), 168.5 (C=O). LCM-MS (*m/z*): 514/516 $[M+H]^+$. Anal. Calcd. for $C_{25}H_{28}ClN_5O_3S$: C, 58.41; H, 5.49; N, 13.62. Found: C, 58.59; H, 5.60; N, 13.85.

3-(1-Benzyl-2-ethyl-4-nitro-1*H*-imidazol-5-ylsulfanyl)-1-[4-(4-bromo-phenyl)-piperazin-1-yl]-propan-1-one (5e). Brown powder; Yield: (215 mg, 77%); mp 85-88 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ_H 1.13 (t, *J* 7.3 Hz, 3H, CH_3), 2.57-2.88 (m, 6H, CH_2CH_3+SCH_2CH_2); 2.9-3.27 (m, 6H, $H_{piperazine}$), 3.3-3.9 (m, 2H, $H_{piperazine}$), 5.40 (s, 2H, CH_2Ph), 6.86-6.91 (m, 2H, $H_{arom.}$), 7.00-7.06 (m, 2H, $H_{arom.}$), 7.30-7.34 (m, 5H, $H_{arom.}$). ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ_C 10.4 (CH_3), 20.0 (CH_2CH_3), 31.6 (CH_2CO), 32.4 (CH_2S), 40.41, 43.88, 46.7, 47.5 ($C_{piperazine}$), 47.8 (CH_2Ph), 117.4, 122.7, 125.7, 126.1, 127.7, 128.6, 131.2, 135.9, 148.0 ($C_{arom.}$+C-5), 149.8 (C-4), 150.0 (C-2), 168.5 (C=O). LCM-MS (*m/z*): 558/560 $[M+H]^+$. Anal. Calcd. for $C_{25}H_{28}BrN_5O_3S$: C, 53.77; H, 5.05; N, 14.31. Found: C, 53.99; H, 5.18; N, 14.58.

3-(1-Benzyl-2-ethyl-4-nitro-1*H*-imidazol-5-ylsulfanyl)-1-(4-o-tolyl-piperazin-1-yl)-propan-1-one (5f). Brown powder; Yield: (183 mg, 74%); mp 95-98 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ_H 1.13 (t, *J* 7.3 Hz; 3H, CH_3), 2.25 (s, 3H, CH_3Ph), 2.59-2.92 (m, 10H, CH_2CH_3+SCH_2CH_2+$H_{piperazine}$), 3.05-3.95 (m, 4H, $H_{piperazine}$), 5.43 (s, 2H, CH_2Ph), 6.96-7.25 (m, 6H, $H_{arom.}$), 7.29-7.36 (m, 3H, $H_{arom.}$). ^{13}C NMR (DMSO- d_6, 75.5 MHz): δ_C 10.7 (CH_3), 17.5 (CH_3Ph), 20.3 (CH_2CH_3), 31.9 (CH_2CO), 32.7 (CH_2S), 41.6, 45.0, 47.0, 51.2 ($C_{piperazine}$), 51.4 (CH_2Ph), 118.9, 123.2, 125.8, 126.1, 126.6, 127.7, 128.9, 130.8, 131.9, 135.9, 148 ($C_{arom.}$+C-5), 150.0 (C-4), 150.8 (C-2), 168.6 (C=O). LCM-MS (*m/z*): 494 $[M+H]^+$. Anal. Calcd. for $C_{26}H_{31}N_5O_3S$: C, 63.26; H, 6.33; N, 14.19. Found: C, 63.45; H, 6.25; N, 14.34.

3-(1-Benzyl-2-ethyl-4-nitro-*1H*-imidazol-5-ylsulfanyl)-1-(4-m-tolyl-piperazin-1-yl)-propan-1-one (5g). Brown amorphous; Yield: (169 mg, 68%). ^{1}H NMR (300 MHz, DMSO-d_6): δ_H 1.14 (t, *J* 7.3 Hz, 3H, CH_3CH_2), 2.25 (s, 3H, CH_3Ph), 2.57-2.65 (m, 4H, CH_2CH_3+CH_2CO), 3.01-3.06 (m, 6H, CH_2S+$H_{piperazine}$), 3.5 (br s, 4H, $H_{piperazine}$), 5.43 (s, 2H, CH_2Ph), 6.74-6.7 (m, 3H, $H_{arom.}$), 7.04-7.13 (m, 3H, $H_{arom.}$), 7.3 (m, 3H, $H_{arom.}$). ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ_C 10.7 (CH_3), 20.3 (CH_2CH_3), 21.4 (CH_3Ph), 31.8 (CH_2CO), 32.6 (CH_2S), 40. 9, 44.3, 46.9, 48.2 ($C_{piperazine}$), 48.6 (CH_2Ph), 113.1, 116.5, 120.2, 125.6, 126.0, 126.1, 127.7, 128.8, 128.9, 135.9 ($C_{arom.}$), 138.0 (C-5), 150.0 (C-4), 150.7 (C-2), 168.5 (C=O). LCM-MS (*m/z*): 494 $[M+H]^+$. Anal. Calcd. for $C_{26}H_{31}N_5O_3S$: C, 63.26; H, 6.33; N, 14.19. Found: C, 63.49; H, 6.35; N, 14.44.

3-(1-Benzyl-2-ethyl-4-nitro-1*H*-imidazol-5-ylsulfanyl)-1-[4-(2-methoxy-phenyl)-piperazin-1-yl]-propan-1-one (5h). Brown powder; Yield: (178 mg, 70%); mp 85-88 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ_H 1.13 (t, *J* 7.3 Hz, 3H, CH_3), 2.50-2.62 (m, 5H, CH_3O+CH_2CH_3), 2.84-2.87 (m, 4H, SCH_2CH_2), 3.06 (t, *J* 6.3 Hz, 2H, $H_{piperazine}$), 3.40-3.89 (m, 6H, $H_{piperazine}$), 5.42 (s, 2H, CH_2Ph), 3.87-7.06 (m, 6H, $H_{arom.}$), 7.36 (m, 3H, $H_{arom.}$). ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ_C 10.7 (CH_3), 20.3 (CH_2CH_3), 31.9 (CH_2CO), 32.6 (CH_2S), 41.3, 44.8, 57.0, 49.9 ($C_{piperazine}$), 50.3 (CH_2Ph), 55.3 (CH_3O), 111.7, 118.2, 120.8, 123.0, 125.8, 126.1, 127.7, 128.9, 130.0, 135.9, 140.6 ($C_{arom.}$+C-5), 150.0 (C-4), 151.9 (C-2), 168.5 (C=O). LCM-MS (*m/z*): 510 $[M+H]^+$. Anal. Calcd. for $C_{26}H_{31}N_5O_4S$: C, 61.28; H, 6.13; N, 13.74. Found: C, 61.47; H, 6.33; N, 13.98.

3-(1-Benzyl-2-ethyl-4-nitro-1*H*-imidazol-5-ylsulfanyl)-1-[4-(3-methoxy-phenyl)-piperazin-1-yl]-propan-1-one (5i). Brown powder; Yield: (190 mg, 75%); mp 65-68 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ_H 1.12 (t, *J* 7.3 Hz, 3H, CH_3), 2.50-2.66 (m, 5H, CH_3O+CH_2CH_3), 2.99-3.05 (m, 4H, SCH_2CH_2), 3.51-3.73 (m, 8H, $H_{piperazine}$), 5.41 (s, 2H, CH_2Ph), 6.34-6.62 (m, 3H, $H_{arom.}$), 7.0-7.2 (m, 3H, $H_{arom.}$), 7.25-7.54 (m, 3H, $H_{arom.}$). ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ_C 10.7 (CH_3), 20.3 (CH_2CH_3), 31.8 (CH_2CO), 32.6 (CH_2S), 40.8, 44.3, 47.0, 48.1 ($C_{piperazine}$), 48.4 (CH_2Ph), 54.8 (CH_3O), 101.9, 104.6, 107.7, 108.4, 125.7, 126.1, 127.7, 128.9, 129.7, 135.8, 150 ($C_{arom.}$+C-5), 152.0 (C-4), 160.1 (C-2), 168.5 (C=O). LCM-MS (*m/z*): 510 $[M+H]^+$. Anal. Calcd. for $C_{26}H_{31}N_5O_4S$: C, 61.28; H, 6.13; N, 13.74. Found: C, 61.37; H, 6.40; N, 13.86.

3-(1-Benzyl-2-ethyl-4-nitro-1*H*-imidazol-5-ylsulfanyl)-1-[4-(4-methoxy-phenyl)-piperazin-1-yl]-propan-1-one (5j). Brown amorphous; Yield: (176 mg, 69%). ^{1}H NMR (300 MHz, DMSO-d_6): δ_H 1.12 (t, *J* 7.3 Hz, 3H, CH_3), 2.56-2.66 (m, 4H, CH_2CH_3+CH_2CO), 2.83-3.2 (m, 6H, CH_2S+$H_{piperazine}$), 3.2-3.9 (m, 7H, CH_3O+$H_{piperazine}$), 5.41 (s, 2H, CH_2Ph), 6.83-7.04 (m, 6H, $H_{arom.}$), 7.28-7.33 (m, 3H, $H_{arom.}$). ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ_C 10.7 (CH_3), 20.3 (CH_2CH_3), 31.8 (CH_2CO), 32.6 (CH_2S), 41.1, 44.6, 47.0, 49.7 ($C_{piperazine}$), 50.0 (CH_2Ph), 55.1 (CH_3O), 114.5, 118.0, 126.7, 127.7, 128.9, 129.0, 135.8, 145.0, ($C_{arom.}$+C-5), 148.0 (C-4), 153.3 (C-2), 168.5 (C=O). LCM-MS (*m/z*): 510 $[M+H]^+$. Anal. Calcd. for $C_{26}H_{31}N_5O_4S$: C, 61.28; H, 6.13; N, 13.74. Found: C, 61.33; H, 6.29; N, 14.01.

3-(1-Benzyl-2-ethyl-4-nitro-1*H*-imidazol-5-ylsulfanyl)-1-[4-(2-hydroxy-phenyl)-piperazin-1-yl]-propan-1-one (5k). Brown powder; Yield: (186 mg, 75%); mp 80-82 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ_H 1.14 (t, *J* 7.3 Hz, 3H, CH_3), 2.69-2.74 (m, 6H, CH_2CH_3+SCH_2CH_2), 2.81-2.85 (m, 4H, $H_{piperazine}$), 2.90-3.72 (m, 4H, $H_{piperazine}$), 5.40 (s, 2H, CH_2Ph), 6.71-6.93 (m, 4H, $H_{arom.}$), 7.00-7.10 (m, 2H, $H_{arom.}$), 7.28-7.30 (m, 3H, $H_{arom.}$). ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ_C 10.7 (CH_3), 20.3 (CH_2CH_3), 31.9 (CH_2CO), 32.6 (CH_2S), 41.3, 44.9, 47.0, 50.0 ($C_{piperazine}$), 50.4 (CH_2Ph), 115.6, 118.8, 119.4, 123.3, 126.1, 127.6, 128.8, 125.71, 129.0, ($C_{arom.}$), 135.9 (C-5), 139.9 (C-4), 150.2 (C-2),

168.4 (C=O). LCM-MS (*m/z*): 496 $[M+H]^+$. Anal. Calcd. for $C_{25}H_{29}N_5O_4S$: C, 60.59; H, 5.90; N, 14.13. Found: C, 60.78; H, 6.02; N, 14.28.

3-(1-Benzyl-2-ethyl-4-nitro-1*H*-imidazol-5-ylsulfanyl)-1-[4-(4-hydroxy-phenyl)-piperazin-1-yl]-propan-1-one (5l). Brown amorphous; Yield: (183 mg, 74%). ^{1}H NMR (300 MHz, DMSO-d_6): δ_H 1.14 (t, *J* 7.3 Hz, 3H, CH_3), 2.68-2.74 (m, 6H, CH_2CH_3+SCH_2CH_2), 2.80-2.85 (m, 4H, $H_{piperazine}$), 2.90-3.72 (m, 4H, $H_{piperazine}$), 5.40 (s, 2H, CH_2Ph), 6.71-6.93 (m, 4H, $H_{arom.}$), 7.09-7.12 (m, 2H, $H_{arom.}$), 7.30-7.34 (m, 3H, $H_{arom.}$). ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ_C 10.7 (CH_3), 20.3 (CH_2CH_3), 31.9 (CH_2CO), 32.6 (CH_2S), 41.3, 44.9, 47.0, 50.0 ($C_{piperazine}$), 50.4 (CH_2Ph), 112.5, 125.6, 125.7, 126.1, 127.7, 128.9, 135.9, 136.9, 148.0 ($C_{arom.}$+C-5), 150.1 (C-4), 154.2 (C-2), 168.4 (C=O). LCM-MS (*m/z*): 496 $[M+H]^+$. Anal. Calcd. for $C_{25}H_{29}N_5O_4S$: C, 60.59; H, 5.90; N, 14.13. Found: C, 60.81; H, 5.99; N, 14.37.

2-{4-[3-(1-Benzyl-2-ethyl-4-nitro-1*H*-imidazol-5-ylsulfanyl)-propionyl]-piperazin-1-yl}-benzonitrile (5m). Brown amorphous; Yield: (190 mg, 75%). ^{1}H NMR (300 MHz, DMSO-d_6): δ_H 1.15 (t, *J* 7.3 Hz, 3H, CH_3), 2.57-2.64 (m, 4H, CH_2CH_3+CH_2CO), 3.04-3.08 (m, 6H, CH_2S+$H_{piperazine}$), 3.61-3.65 (m, 4H, $H_{piperazine}$), 5.40 (s, 2H, CH_2Ph), 7.03-7.24 (m, 4H, $H_{arom.}$), 7.29-7.38 (m, 3H, $H_{arom.}$), 7.59-7.79 (m, 2H, $H_{arom.}$). ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ_C 10.7 (CH_3), 20.3 (CH_2CH_3), 31.3 (CH_2CO), 32.7 (CH_2S), 41.1, 44.6, 46.9, 50.7 ($C_{piperazine}$), 51.3 (CH_2Ph), 104.9 (CN), 118.0, 119.2, 122.4, 125.6, 126.0, 126.1, 127.6, 128.8, 134.1, 134.3 ($C_{arom.}$), 135.8 (C-5), 150.0 (C-4), 154.8 (C-2), 168.7 (C=O). LCM-MS (*m/z*): 505 $[M+H]^+$. Anal. Calcd. for $C_{26}H_{28}N_6O_3S$: C, 61.89; H, 5.59; N, 16.65. Found: C, 62.03; H, 5.71; N, 16.89.

4-{4-[3-(1-Benzyl-2-ethyl-4-nitro-1*H*-imidazol-5-ylsulfanyl)-propionyl]-piperazin-1-yl}-benzonitrile (5n). Brown amorphous; Yield: (184 mg, 73%). ^{1}H NMR (300 MHz, DMSO-d_6): δ_H 1.13 (t, *J* 7.3 Hz, 3H, CH_3), 2.38-2.94 (m, 8H, CH_2CH_3+SCH_2CH_2+$H_{piperazine}$), 2.99-3.88 (m, 6H, $H_{piperazine}$), 5.43 (s, 2H, CH_2Ph), 7.03-7.06 (m, 4H, $H_{arom.}$), 7.33-7.36 (m, 3H, $H_{arom.}$), 7.57-7.61 (m, 2H, $H_{arom.}$). ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ_C 10.7 (CH_3), 20.0 (CH_2CH_3), 31.4 (CH_2CO), 32.3 (CH_2S), 40.1, 43.5, 45.6, 45.8 ($C_{piperazine}$), 46.7 (CH_2Ph), 98.4 (CN), 113.7, 119.6, 125.8, 126.1, 127.4, 128.6, 133.0, 135.9, 148.0 ($C_{arom.}$ + C-5), 149.7 (C-4), 152.7 (C-2), 168.6 (C=O). LCM-MS (*m/z*): 505 $[M+H]^+$. Anal. Calcd. for $C_{26}H_{28}N_6O_3S$: C, 61.89; H, 5.59; N, 16.65. Found: C, 62.11; H, 5.48; N, 16.91.

3-(1-Benzyl-2-ethyl-4-nitro-1*H*-imidazol-5-ylsulfanyl)-1-[4-(4-nitro-phenyl)-piperazin-1-yl]-propan-1-one (5o). Brown powder; Yield: (199 mg, 76%); mp 96-98 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ_H 1.14 (t, *J* 7.3 Hz, 3H, CH_3), 2.60-2.63 (m, 6H, CH_2CH_3+SCH_2CH_2), 3.07 (t, 2H, *J* 6.2 Hz, $H_{piperazine}$), 3.26-3.9 (m, 6H, $H_{piperazine}$), 5.40 (s, 2H, CH_2Ph), 6.93-7.14 (m, 4H, $H_{arom.}$), 7.26-7.46 (m, 3H, $H_{arom.}$), 8.0-8.014 (m, 2H, $H_{arom.}$). ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ_C 10.7 (CH_3), 20.3 (CH_2CH_3), 31.7 (CH_2CO), 32.6 (CH_2S), 40.4, 43.6, 45.7, 45.8 ($C_{piperazine}$), 47.0 (CH_2Ph), 112.4, 125.6, 125.7, 126.1, 127.7, 128.9, 135.9, 136.9, 148.06 ($C_{arom.}$+C-5), 150.0 (C-4), 154.3 (C-2), 168.8 (C=O). LCM-MS (*m/z*): 525 $[M+H]^+$. Anal. Calcd. for $C_{25}H_{28}N_6O_5S$: C, 57.24; H, 5.38; N, 16.02. Found: C, 57.41; H, 5.44; N, 16.27.

3-(1-Benzyl-2-ethyl-4-nitro-1*H*-imidazol-5-ylsulfanyl)-1-[4-(2-oxo-2-phenyl-ethyl)-piperazin-1-yl]-propan-1-one (5p). Brown amorphous; Yield: (185 mg, 71%). ^{1}H NMR (300 MHz, DMSO-d_6): δ_H 1.14 (t, *J* 7.3 Hz, 3H, CH_3), 2.17-2.86 (m, 11H, CH_2CH_3 +SCH_2CH_2+CH_3CO+$H_{piperazine}$), 2.89-4.01 (m, 6H, $H_{piperazine}$), 5.43 (s, 2H, CH_2Ph), 6.84-7.15 (m, 4H, $H_{arom.}$), 7.16-7.68 (m, 3H, $H_{arom.}$), 7.7-8.14 (m, 2H, $H_{arom.}$). ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ_C 10.7 (CH_3), 20.3 (CH_2CH_3), 26.1 (CH_3CO), 31.8 (CH_2CO), 32.7 (CH_2S), 40.6, 43.9, 46.2, 46.5 ($C_{piperazine}$), 47.0 (CH_2Ph), 113.2, 125.6, 126.1, 126.8, 127.7, 128.9, 129.0, 130.1 ($C_{arom.}$), 135.9 (C-5), 150.0 (C-4), 153.4 (C-2), 168.7 (NC=O), 195.6 (Ph*C*=*O*). LCM-MS (*m/z*): 522 $[M+H]^+$. Anal. Calcd. for $C_{27}H_{31}N_5O_4S$: C, 62.17; H, 5.99; N, 13.43. Found: C, 62.35; H, 6.11; N, 13.60.

3-(1-Benzyl-2-ethyl-4-nitro-1*H*-imidazol-5-ylsulfanyl)-1-(4-methyl-piperazin-1-yl)-propan-1-one (5q). Yellow amorphous; Yield: (164 mg, 75%). ^{1}H NMR (300 MHz, DMSO-d_6): δ_H 1.09-1.15 (m, 6H, 2xCH_3), 2.5-2.67 (m, 10H, CH_2CH_3+$H_{piperazine}$), 3.04 (t, *J* 6.3 Hz, 2H, CH_2CO), 4.04 (t, *J* 7.0 Hz, 2H, CH_2S), 5.41 (s, 2H, CH_2Ph), 7.04 (d, *J* =7.0

Hz, 2H, $H_{arom.}$), 7.29-7.38 (m, 3H, $H_{arom.}$). ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ_C 10.7 (CH_3), 20.3 (CH_2CH_3), 31.7 (CH_2CO), 32.4 (CH_2S), 41.6, 52.2 ($C_{piperazine}$), 42.2 (CH_3N), 47.0 ($CH_2Ph.$), 125.5, 126.1, 127.7, 128.9 ($C_{arom.}$), 135.8 (C-5), 150.1 (C-2), 168.9 (C=O). LCM-MS (*m/z*): 418 $[M+H]^+$. Anal. Calcd. for $C_{20}H_{27}N_5O_3S$: C, 57.53; H, 6.52; N, 16.77. Found: C, 57.75; H, 6.61; N, 16.93.

4-[3-(1-Benzyl-2-ethyl-4-nitro-1*H*-imidazol-5-ylsulfanyl)-propionyl]-piperazine-1-carboxylic acid ethyl ester (5r). Brown amorphous; Yield: (168 mg, 71%). ^{1}H NMR (300 MHz, DMSO-d_6): δ_H 1.10-1.13 (m, 6H, 2xCH_3), 2.50-2.72 (m, 10H, CH_2CH_3+$H_{piperazine}$), 3.02-3.18 (m, 4H, SCH_2CH_2), 5.42 (s, 2H, CH_2Ph), 7.04 (d, *J* 7.0 Hz, 2H, $H_{arom.}$), 7.03-7.39 (m, 3H, $H_{arom.}$). ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ_C 10.7 (CH_3), 14.5 (CH_3CH_2O), 20.3 (CH_3CH_2C), 31.8 (CH_2CO), 32.7 (CH_2S), 40.8, 42.9, 43.2, 44.3 ($C_{piperazine}$), 47.0 ($CH_2Ph.$), 60.9 (CH_2O), 125.6, 126.1, 127.7, 128.9 ($C_{arom.}$), 135.8 (C-4), 148.1 (C-5); 150.1 (C-2), 154.6, 168.7 (C=O). LCM-MS (*m/z*): 476 $[M+H]^+$. Anal. Calcd. for $C_{22}H_{29}N_5O_5S$: C, 55.56; H, 6.15; N, 14.73. Found: C, 55.69; H, 6.01; N, 14.58.

Biological assays

Cell Lines and Culture Conditions. Human breast adenocarcinoma MCF-7 (HTB-22TM), epithelial breast cancer MDA MB231, human androgen-resistant (PC-3) and androgen-sensitive (DU145) prostate cancer cell lines, cells were from the American Type Culture Collection (ATCC, Rockville, MD, USA). MCF-7 cells were cultured in DMEM. PC3, and DU145 cells were cultured in RPMI-1640 medium; media contained 10% heat-inactivated fetal bovine serum (FBS), 1% (v/v) of penicillin (10,000 units/mL)-streptomycin (10 mg/mL), and 1% (v/v) L-glutamine (200 mM) (all from Sigma-Aldrich). All cell lines were cultured at 37 OC in a 5% CO_2, fully humidified atmosphere.

Cytotoxicity Assay. Cell lines were seeded in 96-well flat-bottomed microplates in 100 µL culture medium at the following densities: MCF-7, MDA MB231, PC-3, and DU145 cells (3 x 10^3 cells/well). Cells were allowed to adhere for 24 h. Then, the medium was replaced with fresh medium alone or with the tested compounds at increasing concentrations from 0 to 250 uM for cancerous cell lines and to 500 uM for the normal dermal fibroblast cells. The reference drugs cisplatin (0-100 uM) and doxorubicin (0-10 uM) were included as positive controls for growth inhibition. After 72 h, cell viability was assayed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [31]. All experimental conditions were tested in triplicate and the experiment was performed three times. Half maximal inhibitory concentrations (IC_{50}, the concentration required for 50% in vitro inhibition of growth) were calculated for each experiment using Graphpad prism software (Version 8, San Diego, CA, USA). IC_{50} values were reported as mean $\pm$ SD

Dock and virtual screening

Preparations of ligands and proteins. The structures of ligands **5d**, **5f** and **5m** were prepared by Avogadro (v. 1.0.1)[33] software and saved as PDB file formate. Then, the two ligands were prepared selecting torsions and the structures were converted from PDB formate to PDBQT. The PDBQT files for the proteins and the ligands, united atom Kollman charges, fragmental volumes, and solvation parameters were performed by the MGLTools software. Ligand structures were energy minimized with the MMFF94 force field. The native ligands and crystallographic water molecules were removed from the PDB structures and the polar hydrogens were added before docking.

Grid map calculations. AutoDock grid maps were calculated for each compound using AutoGrid4, based on the active site coordinates of each protein crystal structure. The size of all grid boxes 60 x 60 x 60 xyz points

with a grid spacing of 0.375 Å. The grid center dimensions were 85.44, 52.99, and 46.41for x, y and z respectively. Maps were calculated for each atom type in each ligand along with an electrostatic and desolavation map using dielectric value of -0.1465.

Molecular docking simulations. Molecular docking simulations were undertaken using the Autodock program.[32] Protein structures were prepared using UCSF Chimera 1.15.[34] In Autodock program, the Lamarkian Genetic Algorithm (LGA) was used for pose sampling and the number of energy simulations was set to 2500000. The default scoring function was used for calculating the docking scores. Autogrid was used to prepare the maps. The results of molecular docking were visualized in Biovia Discovery Studio 2020 software[35] and then analyzing the docking results. All docking simulations performed to validate the method, using the ligands present in crystal structures, were able to reproduce the ligand-protein interaction geometries. The image of the native ligands for 3RUK, 3U2B and 5T8E against the redocked native ligand with AutoDock is shown in Fig. 2, meanwhile the root-mean-square deviation of atomic positions (RMSD) was 0.405 Å.

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Supplementary Material

Supplementary material is attached

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