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EGFR and PI3K/m-TOR inhibitors: design, microwave assisted synthesis and anticancer activity of thiazole–coumarin hybrids†

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A series of thiazoline and thiazolidinone-based 4-hydroxycoumarin derivatives were synthesized using both conventional synthesis procedures and microwave-assisted techniques. The new compounds were evaluated for their cytotoxic effect against three human cancer cell lines; MCF-7, HCT-116 and HepG2 and one normal human cell line (BJ-1). The promising anti-proliferative compounds **2a**, **2b**, **6a** and **6b** were assessed for inhibiting EGFR and PI3K/mTOR. Compound **6a** showed the highest inhibition activity towards the signaling pathway. The apoptotic effect and cell cycle arrest potential of derivative **6a** were examined. Moreover, the molecular docking, physicochemical properties and pharmacokinetic parameters of the promising compound were investigated, as well.

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1. Introduction

Cancer is a disease of a multifactorial nature that ranks as the second leading cause of death worldwide, resulting in about 10 million deaths in 2020.¹ It has been noted that a number of signaling pathways when dysregulated are subsequently involved in cancer pathogenesis. The PI3K/AKT/mTOR pathway is an intracellular signaling pathway related to a number of biological processes such as apoptosis, cell proliferation and angiogenesis. Dysregulation of this pathway is associated with a vast disturbance in cell growth, survival and metastasis which results in cancer development. Somatic mutations or gaining and losing of key genes are among the factors affecting this pathway in a number of different tumors such as breast, colon and liver cancers.^{2–4}

PI3K/AKT/mTOR pathway is initially activated by the binding of extracellular growth factors to epidermal growth factor receptor (EGFR), then phosphatidylinositol 3-kinase (PI3K) is activated which consequently activates the serine/threonine protein kinase B (PKB/AKT). This leads to some downstream effects as the activation of mechanistic target of rapamycin (mTOR). Agents that target the regulators in this pathway are either single PI3K, AKT or mTOR inhibitors or dual inhibitors of PI3K and mTOR. In theory, inhibiting EGFR and PI3K/mTOR

would probably lead to the strongest inhibition of the whole PI3K/Akt/mTOR pathway.^{5,6}

Coumarin as a privileged heterocyclic ring that displays multifarious biological activities has been utilized to design useful antitumor compounds.^{7–12} A closer look at the coumarin chemical structure and physicochemical properties reveals that the 2H-chromen-2-one ring is planar and lipophilic, which grants the scaffold the ability to interact with a variety of biological counterparts, whereas, the lactone group of the coumarin enable the molecule to form strong polar bonds and to acylate different protein targets.¹³ On the other hand, the thiazole ring is a versatile moiety that demonstrated many desirable biological activities, dabrafenib is an example of a thiazole drug that underwent clinical trials for its selectivity towards tyrosine kinase inhibition.^{14,15} Moreover, thiazoles are reported as potent anticancer agents that are capable of targeting certain kinase pathways, *e.g.* compound **I**, Fig. 1.¹⁶

In an attempt to pursue new antitumor agents, considering the aforementioned information, and as a continuation to our research work in cancer field,^{17–26} the target compounds were rationally developed by using one of the FDA approved drugs that inhibits EGFR (pelitinib) and another that is developed by Novartis and involved in PI3K/AKT/mTOR signaling pathway (NVP-BEZ235) as models, replacing their biologically active quinoline ring with its bioisostere coumarin scaffold, and hybridizing the scaffold with the bioactive thiazole moiety, Fig. 1.

2. Results and discussion

2.1. Chemistry

The target thiazolines and thiazolidinones were prepared from the intermediate thiosemicarbazones **2a–c** through the reaction

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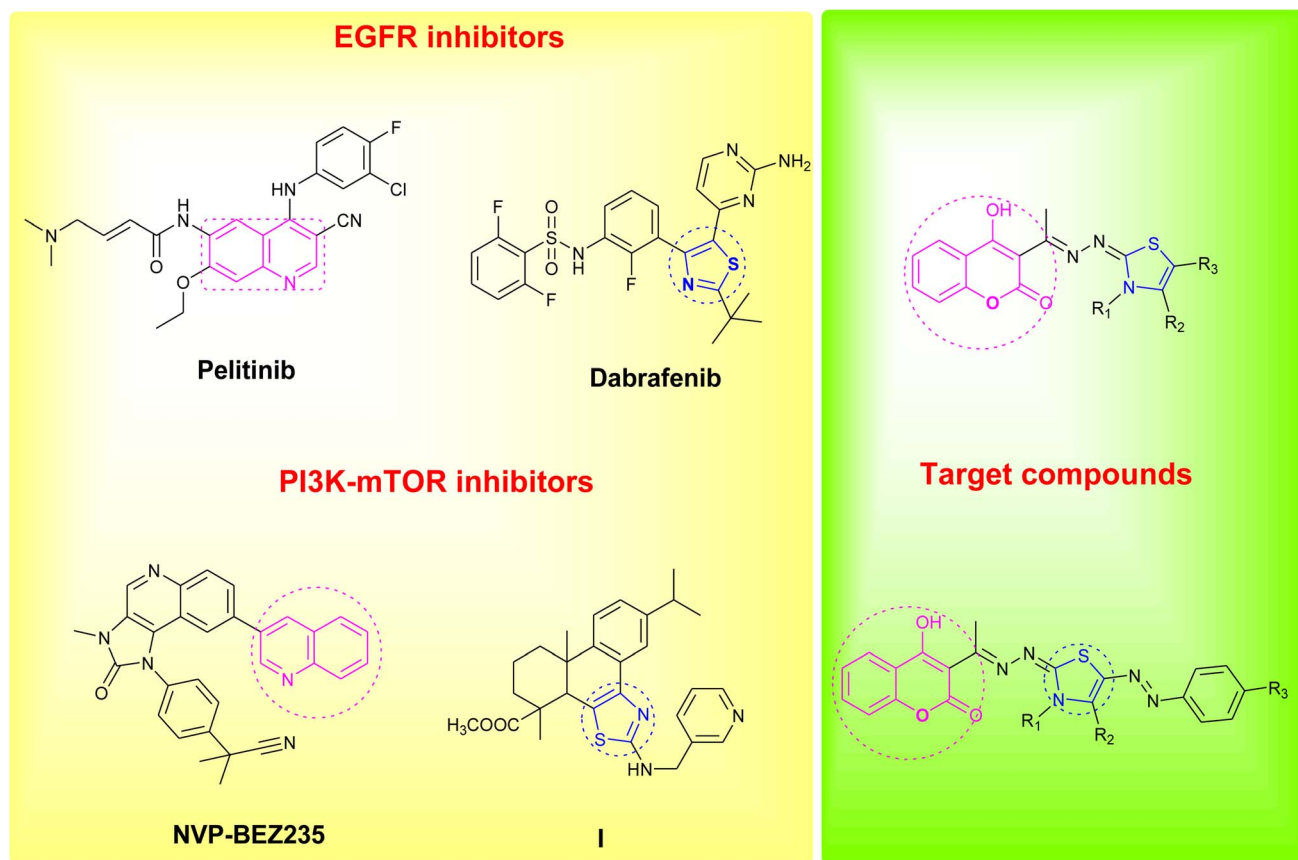
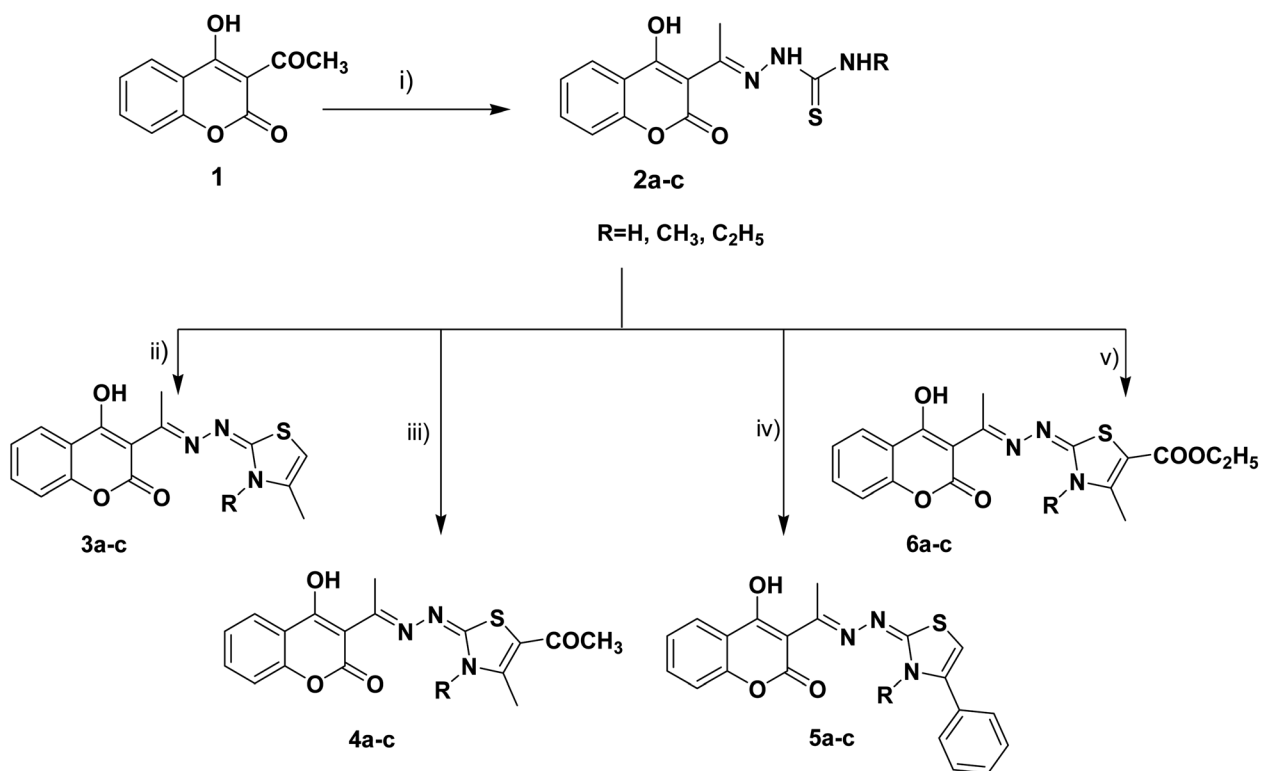
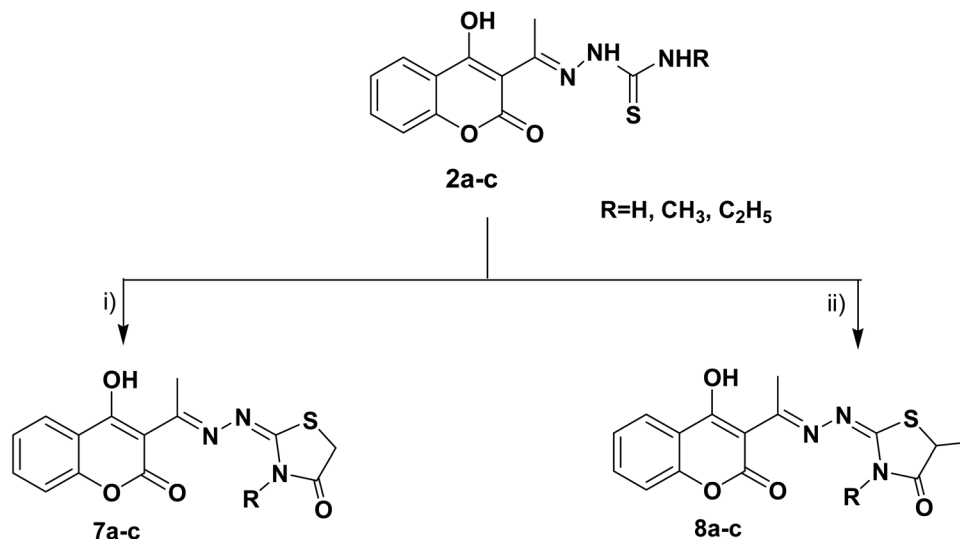


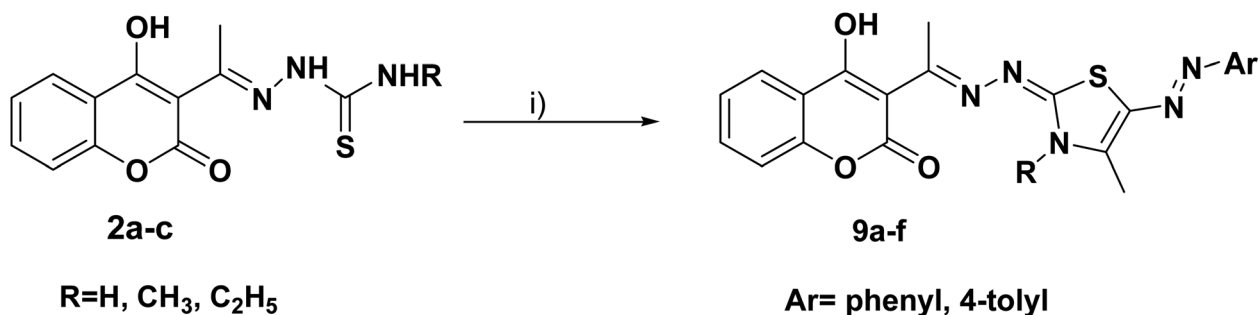
Fig. 1 Reported EGFR and PI3K/mTOR inhibitors and design of the newly synthesized coumarin derivatives.



Scheme 1 Reagents and conditions: (i) $\text{NH}_2\text{NHCSNHR}$, EtOH, AcOH, reflux, (ii) $\text{CH}_3\text{COCH}_2\text{Cl}$, CH_3COONa , EtOH, reflux, (iii) $\text{CH}_3\text{COCH}(\text{Cl})\text{COCH}_3$, CH_3COONa , EtOH, reflux, (iv) PhCOCH_2Br , CH_3COONa , EtOH, reflux, (v) $\text{CH}_3\text{COCH}(\text{Cl})\text{COOC}_2\text{H}_5$, CH_3COONa , EtOH, reflux.



Scheme 2 Reagents and conditions: (i) $CH_3CH_2OCOCH_2Br$, CH_3COONa , EtOH, reflux, (ii) $CH_3CH(Br)COOC_2H_5$, CH_3COONa , EtOH, reflux.



Scheme 3 Reagents and conditions: (i) $CH_3COCH(Cl)N=N-Ar$, dioxane, $N(C_2H_5)_3$.

with different α -haloketones as outlined in Scheme 1. Thus, cyclocondensation of thiosemicarbazones **2a-c** with chloroacetone and/or 3-chloroacetylacetone in ethanol using a catalytic amount of anhydrous sodium acetate yielded the corresponding 4-methylthiazolines **3a-c** and/or 5-acetyl-4-

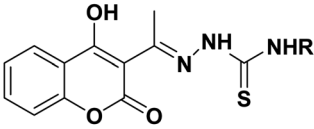
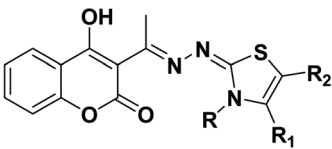
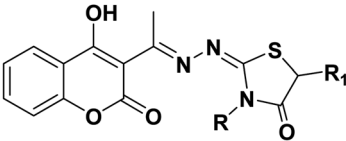
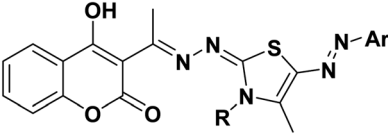
methylthiazolines **4a-c**, respectively. While the reaction of **2a-c** with phenacyl bromide afforded the 4-phenylthiazoline congeners **5a-c**. Similarly, the 4-methylthiazole-5-carboxylates **6a-c** were obtained *via* the reaction with ethyl-2-chloroacetoacetate, Scheme 1.

Table 1 Comparative study of microwave assisted methods and conventional methods

Compound no.	Microwave assisted methods					Conventional method	
	Irradiation power (W)	Reaction temperature ($^{\circ}C$)	Pressure (Pa)	Time (min)	Yield (%)	Time (h)	Yield (%)
2a	500	125	15	20	93.9	5	79.1
2b	500	125	15	20	92.7	6	89.1
2c	500	125	15	20	93.1	7	88.8
3a	500	150	15	15	93.5	6	85.2
4c	500	125	15	20	89.1	8	80.3
5b	500	150	15	15	91.6	7	85.4
5c	500	150	15	15	93.7	10	77.2
6a	500	125	15	20	89.8	9	81.5
7a	500	150	15	15	92.2	7	78.2
7b	500	150	15	15	90.1	8	82.6
8c	500	125	15	20	88.2	8	80.8
9b	500	150	15	15	91.3	10	78.8



Table 2 The cytotoxic IC₅₀ values of the compounds according to the MTT assay on MCF-7, HCT-116 and HepG2 cells

						
2a-c	3-6 a-c				7, 8 a-c	
						
9a-f						

					IC ₅₀ (μM) ± SD		
Compound	R	R1	R2	Ar	MCF-7	HCT-116	HepG-2
2a	H	—	—	—	8.1 ± 1.1	17.8 ± 2.1	61.4 ± 5.1
2b	CH ₃	—	—	—	13.6 ± 1.5	7.2 ± 0.6	48.1 ± 3.9
2c	C ₂ H ₅	—	—	—	22.7 ± 2.6	17.8 ± 1.8	46.4 ± 3.6
3a	H	CH ₃	H	—	34.5 ± 3.6	32.6 ± 3.1	42.5 ± 4.1
3b	CH ₃	CH ₃	H	—	34.9 ± 3.1	27.2 ± 2.9	46.1 ± 3.7
3c	C ₂ H ₅	CH ₃	H	—	36.5 ± 4.2	36.3 ± 3.5	68.2 ± 5.6
4a	H	CH ₃	COCH ₃	—	25.6 ± 3.1	31.4 ± 3.1	48.8 ± 3.1
4b	CH ₃	CH ₃	COCH ₃	—	32.7 ± 3.5	24.3 ± 2.7	57.1 ± 4.3
4c	C ₂ H ₅	CH ₃	COCH ₃	—	33.7 ± 3.2	38.9 ± 3.2	62.5 ± 3.9
5a	H	Ph	H	—	34.8 ± 4.6	31.8 ± 3.2	47.6 ± 4.1
5b	CH ₃	Ph	H	—	34.6 ± 2.9	42.4 ± 4.1	66.4 ± 5.6
5c	C ₂ H ₅	Ph	H	—	25.9 ± 2.3	9.9 ± 0.9	86.9 ± 5.9
6a	H	CH ₃	COOC ₂ H ₅	—	6.4 ± 0.5	20.4 ± 1.9	57.2 ± 4.3
6b	CH ₃	CH ₃	COOC ₂ H ₅	—	11.2 ± 1.1	5.9 ± 0.7	41.4 ± 2.9
6c	C ₂ H ₅	CH ₃	COOC ₂ H ₅	—	20.7 ± 2.1	24.7 ± 1.6	45.1 ± 4.1
7a	H	H	—	—	26.3 ± 4.1	9.5 ± 1.1	97.6 ± 8.2
7b	CH ₃	H	—	—	32.8 ± 3.5	41.2 ± 3.9	65.7 ± 5.1
7c	C ₂ H ₅	H	—	—	24.2 ± 3.7	10.8 ± 1.1	93.8 ± 6.1
8a	H	CH ₃	—	—	27.4 ± 1.9	38.3 ± 4.2	42.4 ± 3.5
8b	CH ₃	CH ₃	—	—	26.1 ± 3.5	44.8 ± 4.5	67.2 ± 4.9
8c	C ₂ H ₅	CH ₃	—	—	7.4 ± 0.9	44.9 ± 4.3	80.2 ± 5.9
9a	H	—	—	Ph	33.4 ± 2.8	33.9 ± 2.8	71.2 ± 5.1
9b	CH ₃	—	—	Ph	32.3 ± 3.5	37.9 ± 2.5	64.1 ± 5.5
9c	C ₂ H ₅	—	—	Ph	32.8 ± 2.8	47.2 ± 4.5	60.9 ± 4.8
9d	H	—	—	4-CH ₃ Ph	26.3 ± 2.5	45.6 ± 4.2	57.2 ± 4.6
9e	CH ₃	—	—	4-CH ₃ Ph	23.3 ± 1.9	55.2 ± 5.1	74.4 ± 5.9
9f	C ₂ H ₅	—	—	4-CH ₃ Ph	17.8 ± 1.1	40.1 ± 4.7	82.1 ± 6.1
Doxorubicin	—	—	—	—	16.7 ± 1.5	21.8 ± 2.9	63.2 ± 5.8

Furthermore, the heterocyclization of the intermediate thiosemicarbazones **2a–c** with ethyl bromoacetate and/or ethyl-2-bromopropionate under the same reaction conditions gave the corresponding thiazolidinones **7a–c** and 5-methylthiazolidinones **8a–c** respectively, Scheme 2.

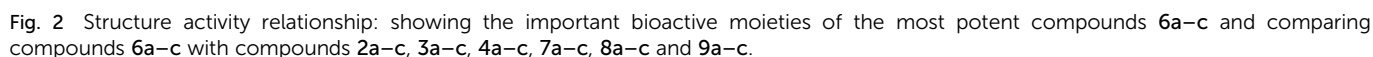
Furthermore, the target compounds **9a–f** were prepared *via* reacting the thiosemicarbazones **2a–c** with hydrazonoyl chlorides in dioxane in alkaline medium, Scheme 3. The chemical structures of the synthesized target compounds were confirmed using elemental and spectral analyses (Experimental section).

Synthesis was carried out using conventional synthesis procedures and reactions were modified to improve the yield

and purity of products *via* microwave-assisted techniques. In order to study the effect of different reaction conditions on the products, microwave-assisted synthesis for the derivatives **2a–c**, **3a**, **4c**, **5b**, **5c**, **6a**, **7a**, **7b**, **8c** and **9b** was also carried out using the same reactants with the same molar ratios by condensation of the key intermediates **2a–c** and the corresponding α -halo-ketones in ethanol under heating and pressure conditions, Table 1.

Based on the results outlined in Table 1, it is clear that the microwave irradiation approach provided products with excellent yields (88.2–93.9%) in a short time (15–20 minutes) compared to the classical synthetic methods. This can be





against HCT-116 human colorectal carcinoma cells, it was shown that five compounds **6b**, **2b**, **7a**, **5c** and **7c** have significantly more cytotoxic activities than doxorubicin ($IC_{50} = 5.9\text{--}10.8\text{ }\mu\text{M}$ vs. $21.8\text{ }\mu\text{M}$ for doxorubicin); three compounds **2a**, **2c** and **6a** have comparable potent cytotoxic activities to doxorubicin ($IC_{50} = 17.8, 17.8$ and $20.4\text{ }\mu\text{M}$, respectively) and compounds **4b**, **6c** and **3b** were slightly less active relative to the standard drug ($IC_{50} = 24.3, 24.7$ and $27.2\text{ }\mu\text{M}$, respectively). As for the activity of the synthesized compounds on HepG2 human liver cancer cells, it was found that twelve compounds **6b**, **8a**, **3a**, **6c**, **3b**, **2c**, **5a**, **2b**, **4a**, **4b**, **6a**, and **9d** showed significant more potent cytotoxic activities than the reference drug ($IC_{50} = 41.4\text{--}57.2\text{ }\mu\text{M}$ vs. $63.2\text{ }\mu\text{M}$ for doxorubicin), three compounds **9c**, **2a**, and **4c** ($IC_{50} = 60.9, 61.4$ and $62.5\text{ }\mu\text{M}$, respectively) have equipotent cytotoxic activities to doxorubicin and five compounds **9b**, **7b**, **5b**, **8b** and **3c** ($IC_{50} = 64.1\text{--}68.2\text{ }\mu\text{M}$) have reasonable cytotoxic activity against HepG2 relative to that of doxorubicin.

In case of MCF-7 human breast cancer cells, five compounds (**6a**, **8c**, **2a**, **6b** and **2b**, respectively) have significantly more potent cytotoxic activities than the standard doxorubicin ($IC_{50} = 6.4\text{--}13.6\text{ }\mu\text{M}$ vs. $16.7\text{ }\mu\text{M}$ for doxorubicin), three compounds **9f**, **6c** and **2c** showed remarkable cytotoxic activity ($IC_{50} = 17.8$, 20.7 and $22.7\text{ }\mu\text{M}$, respectively); the rest of the compounds displayed lower cytotoxic activity against MCF-7 relative to the reference drug, Table 2. When the target compounds were examined

	IC ₅₀ (μM)		
Compounds	EGFR	m-TOR	PI3K
2a	0.311 ± 0.02	1.388 ± 0.08	0.174 ± 0.01
2b	2.275 ± 0.14	2.957 ± 0.18	1.331 ± 0.074
6a	0.184 ± 0.01	0.719 ± 0.04	0.131 ± 0.007
6b	0.563 ± 0.03	1.449 ± 0.08	0.361 ± 0.02
Erlotinib	0.088 ± 0.005	—	—
Rapamycin	—	0.396 ± 0.02	—
LY294002	—	—	0.038 ± 0.002

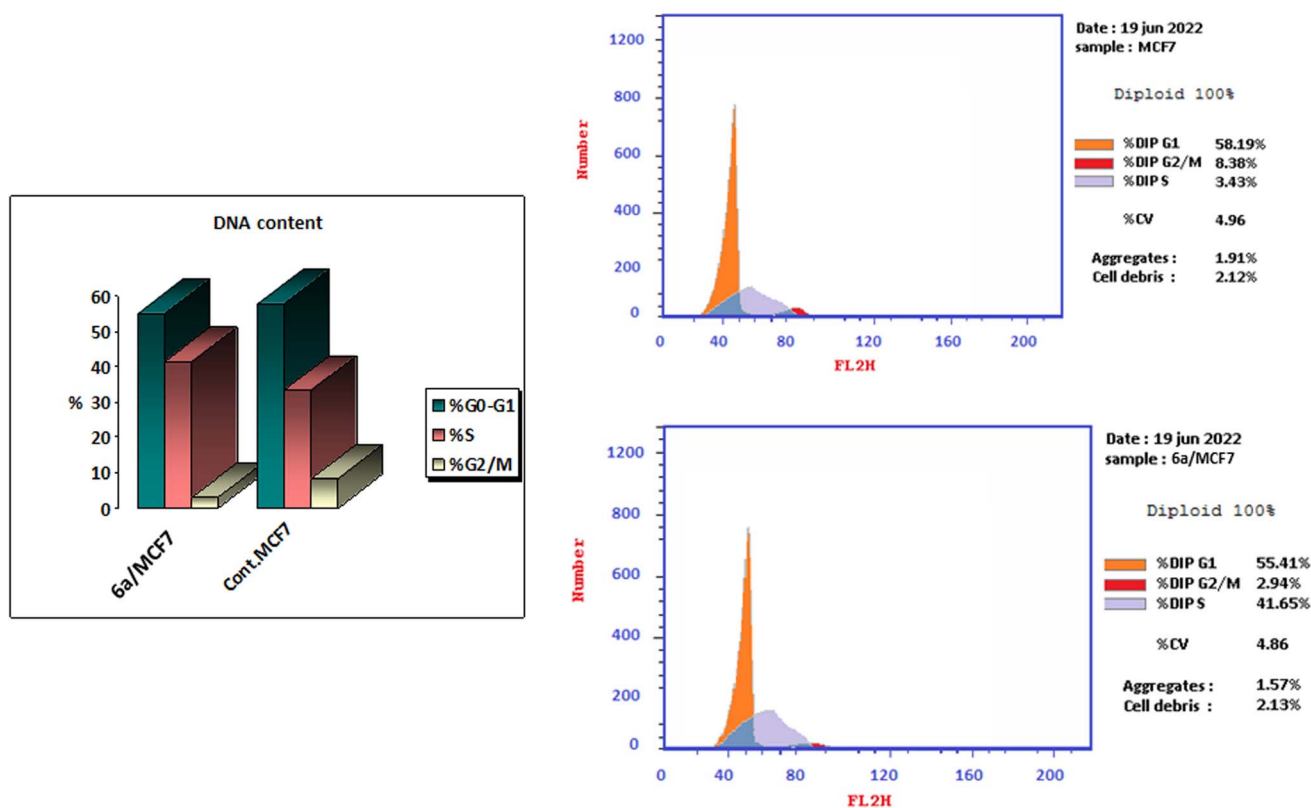


Fig. 3 Cell cycle analysis of MCF-7 after incubation with compound 6a for 48 h.

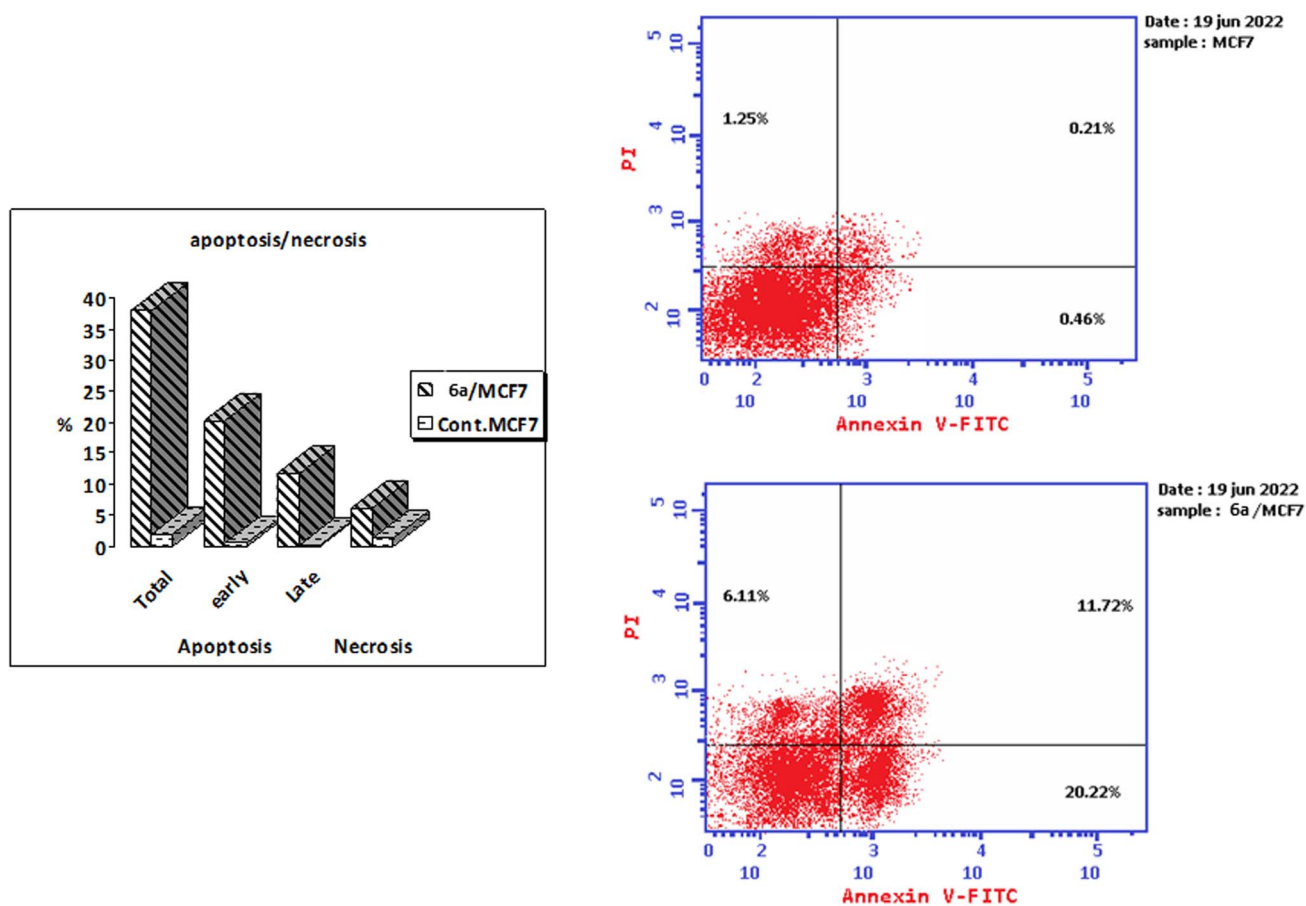


Fig. 4 Induction of apoptosis by compound 6a. Cells were exposed to compound 6a for 48 hours and analyzed by annexin V/PI staining.





Fig. 5 2D interaction diagram showing the co-crystallized ligand docking pose interactions with the key amino acids in EGFR binding site (PDB: 1M17).

From the above results, one can conclude that the six compounds **2a–c** and **6a–c** are non-selectively active on all the three human cancer types. Two compounds (**3b** and **4b**) are active on both human colon cancer HCT-116 and liver cancer cells HepG2 but less active on human breast cancer type. Five compounds (**3a**, **4a**, **5a**, **8a**, and **9d**) are selectively active on only human liver cancer type but less active on both the human breast MCF-7 and human colon HCT-116 cancer types. Two

compounds (**8c**, **9f**) are selectively active on only human breast cancer type MCF-7 but not active on both human colon HCT-116 and liver HepG2 cancer types. Three compounds (**5c**, **7a**, and **7c**) are selectively active on only human colon cancer type HCT-116 but not active on the human liver HepG2 and much less active against breast MCF-7 cancer types.

2.2.2. Structure activity relationship. Compounds **2a**, **2b**, **6a–c**, **8c** and **9f** showed remarkable activity when tested against

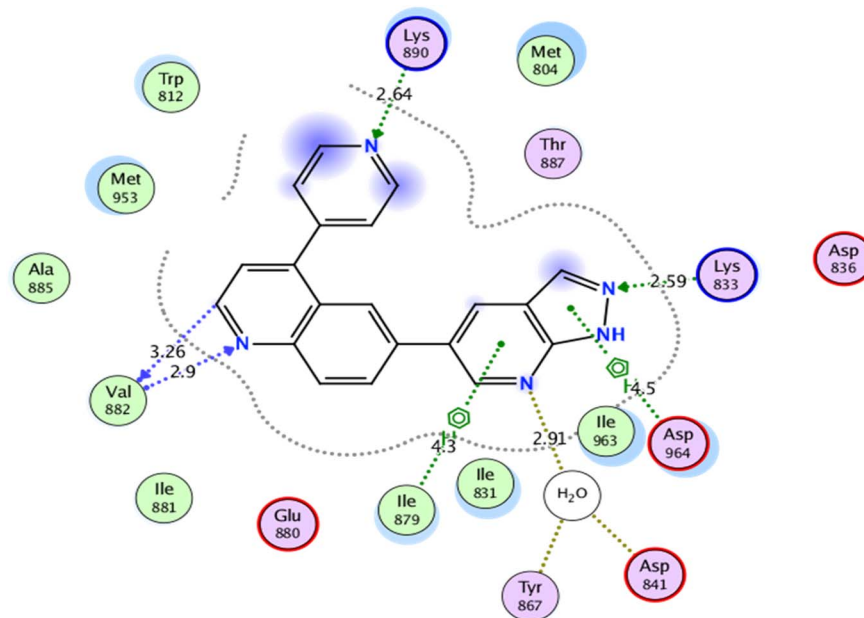


Fig. 6 2D interaction diagram showing the co-crystallized ligand docking pose interactions with the key amino acids in PI3K binding site (PDB: 3L54).



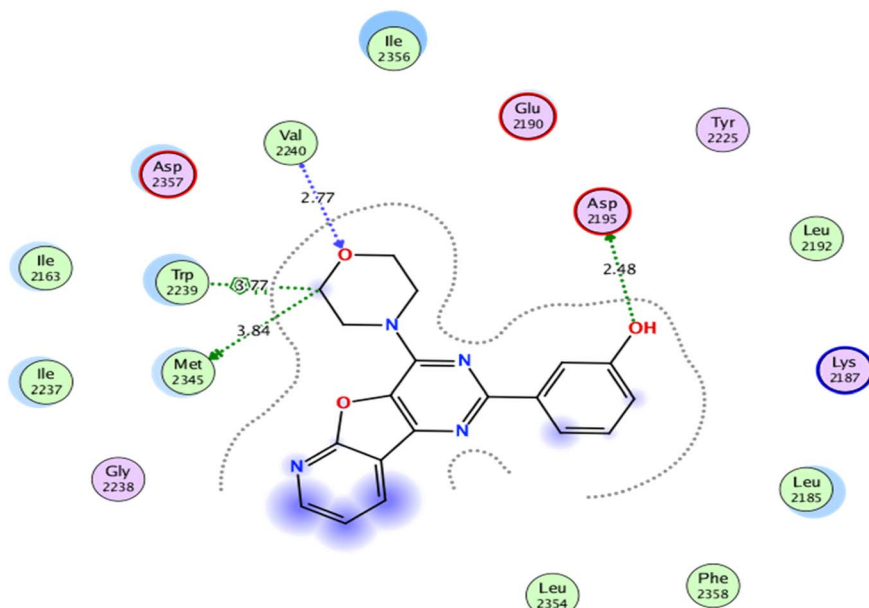


Fig. 7 2D interaction diagram showing the co-crystallized ligand docking pose interactions with the key amino acids in mTOR binding site (PDB: 4JT6).

Table 4 Docking results of compound **6a**

Target protein	Docking score (kcal mol ⁻¹)	Amino acids	Interacting groups	Type of interaction	Bond length (Å)
EGFR	−9.265	Leu694	Coumarin	Arene-H	
		Lys721	O(C=O)CO ₂ C ₂ H ₅	H-bond acceptor	2.40
		Met769	O(C=O)coumarin	H-bond acceptor	2.39
PI3K	−7.320	Lys833	O(C=O)CO ₂ C ₂ H ₅	H-bond acceptor	2.83
		Asp836	(CH ₂)CO ₂ C ₂ H ₅	H-bond donor	2.90
		Val882	O(C=O)coumarin	H-bond acceptor	2.66
		Ile963	Thiazole	Arene-H	
mTOR	−6.855	Trp2239	Coumarin	Arene-arene	
		Trp2239	Coumarin	Arene-arene	
		Val2240	O(C=O)coumarin	H-bond acceptor	2.42
		Asp2357	Thiazole	Arene-H	

MCF-7 breast cancer cell line (IC_{50} = 6.4–20.7 μ M). The 4-methylthiazole-5-carboxylate **6a** was the most potent anticancer agent (IC_{50} = 6.4 μ M), it was 2 fold more active than its *N*-methyl congener **6b** (IC_{50} = 11.2 μ M) and 3 times more potent than the *N*-ethyl derivative **6c** (IC_{50} = 20.7 μ M). The *N*-ethyl derivative of 5-methylthiazolidinones **8c** displayed significant cytotoxic potency (IC_{50} = 7.4 μ M) when compared to the *N*-desethyl and *N*-methyl congeners **8a** and **8b** (IC_{50} = 27.4 and 26.1 μ M, respectively). The non-cyclized thiosemicarbazone intermediates **2a–c** showed a pronounced anticancer activity when compared to doxorubicin (IC_{50} = 8.1, 13.6 and 22.7 μ M, respectively). The tolylazo derivatives **9d–f** were more potent than the phenylazo derivatives **9a–c** with compound **9f** being the most active within the arylazo thiazolylidene series (IC_{50} = 17.8 μ M). The thiazolidinone compound **7a** and its *N*-ethyl derivative **7c** showed similar remarkable cytotoxic effects (IC_{50} = 26.28 and 24.17 μ M, respectively). The *N*-ethyl derivative of 4-

phenylthiazoline **5c** showed higher potency (IC_{50} = 25.9 μ M) than the *N*-methyl and *N*-unsubstituted derivatives (IC_{50} ; **5b** = 34.6 μ M, and **5a** = 34.8 μ M, respectively).

For the activity against HCT cancerous cells, compounds **2a–c**, **4b**, **5c**, **6a–c**, **7a** and **7c** showed significant activity when compared to doxorubicin (IC_{50} = 5.9–24.7 μ M). It was noticed that the 3,4-dimethylthiazole-5-carboxylate **6b** and methyl thiosemicarbazone **2b** were the most potent anticancer agents with IC_{50} = 5.9 and 7.2 μ M, respectively compared to doxorubicin (IC_{50} = 21.8 μ M). The thiazolidinone **7a** and its *N*-ethyl congener **7c** as well as the *N*-ethyl derivative of 4-phenylthiazoline **5c** showed high equipotent activity with IC_{50} values of 9.5, 10.8 and 9.9 μ M, respectively. The thiosemicarbazone **2a** and the ethyl-thiosemicarbazone **2c** showed significant equipotent effect (IC_{50} = 17.8 μ M). The thiazole-5-carboxylate **6a** and its *N*-ethyl congener **6c** displayed remarkable anti-HCT effectiveness (IC_{50} = 20.4 and 24.7 μ M, respectively). Moreover, the *N*-methyl



Table 5 Docking results of metabolite of 6a

Target protein	Docking score (kcal mol ⁻¹)	Amino acids	Interacting groups	Type of interaction	Bond length (Å)
EGFR	−9.638	Lys721	−O(COO) [−]	H-bond acceptor	2.44
		Leu768	O(C=O)coumarin	H-bond acceptor	2.98
		Met769	O(C=O)coumarin	H-bond acceptor	2.52
		Thr830	O(C=O)(COO) [−]	H-bond acceptor	2.73
		Asp831	O(C=O)(COO) [−]	H-bond acceptor	2.58
PI3K	−7.720	Lys833	−O(COO) [−]	Ionic	3.00
		Lys833	O(C=O)(COO) [−]	Ionic	2.59
		Lys833	O(C=O)(COO) [−]	H-bond donor	2.59
		Val882	O(C=O)coumarin	H-bond acceptor	2.82
		Asp964	Thiazole	Arene–H	
mTOR	−6.751	Lys2187	O(C=O)(COO) [−]	H-bond acceptor	3.15
		Glu2190	S(thiazole)	H-bond donor	4.15
		Trp2239	Coumarin	Arene–arene	
		Val2240	O(C=O)coumarin	H-bond acceptor	3.28

derivatives of 4-methylthiazoline **3b** (IC₅₀ = 27.2 μM) and 5-acetyl-4-methylthiazoline **4b** (IC₅₀ = 24.3 μM) showed considerable potency higher than their *N*-unsubstituted and *N*-ethyl congeners.

On the other hand, the 3,4-dimethylthiazole-5-carboxylate **6b**, 5-methylthiazolidinone **8a** and 4-methylthiazoline **3a** are the most active derivatives against human liver cancer cell line HepG2 (IC₅₀ = 41.4, 42.4 and 42.5 μM, respectively, *vs.* 63.2 μM for doxorubicin). Compound **6b** was more potent than the *N*-desmethyl derivative **6a** (IC₅₀ = 57.2 μM) and showed equipotent cytotoxicity to its *N*-ethyl congener **6c** (IC₅₀ = 45.1 μM). Within the thiazolidinone series, compound **8a** was the most active (IC₅₀ = 42.4 μM) being more potent than its *N*-alkylated derivatives **8b, c** (IC₅₀ = 67.2 and 80.2 μM, respectively) and its desmethyl congener **7a** (IC₅₀ = 97.66 μM, respectively). The *N*-methyl derivatives of thiazolidinone **7b** and 5-methylthiazolidinone **8b** showed comparable potency to the reference doxorubicin (IC₅₀ = 65.7 and 67.2 μM, respectively, *vs.* 63.2 μM for doxorubicin). The 5-acetyl-4-methylthiazoline **4a** (IC₅₀ = 48.8 μM) and 4-phenyl thiazoline **5a** (IC₅₀ = 47.6 μM) displayed higher cytotoxic activity against HepG2 cells than their *N*-methyl and *N*-ethyl congeners. Moreover, the methyl and ethyl thiosemicarbazones **2b, c** showed remarkable anticancer effect than the unsubstituted thiosemicarbazone **2a** (IC₅₀ = 48.1 and 46.4 μM, respectively, *vs.* 61.4 μM for **2a**). Within the arylazo thiazolidine series, the 4-tolylazo compound **9d** and the phenylazo derivatives **9c** and **9b** were the most active with comparable effect to the reference drug (IC₅₀ = 57.2, 60.9 and 64.1 μM, respectively) (Fig. 2).

2.2.3. In vitro EGFR, m-TOR and PI3K activity assay. Since compounds **2a, 2b, 6a** and **6b** exhibited an excellent cytotoxic activity against MCF-7, HCT-116 and HepG-2 cancer cell lines and at the same time they showed high safety profile towards the chosen human normal cell line, they were selected for further screening on EGFR and PI3K/mTOR. The results of EGFR inhibition assay revealed the promising inhibitory activity of compound **6a** (IC₅₀ = 0.184 μM) compared to the reference erlotinib (IC₅₀ = 0.088 μM), compounds **2a** and **6b** exhibited potent effect with IC₅₀ values 0.311 and 0.563 μM, respectively,

while compound **2b** showed reduced effectiveness against EGFR inhibition (IC₅₀ = 2.275 μM). Similarly, derivative **6a** showed pronounced m-TOR inhibition activity of (IC₅₀ = 0.719 μM)

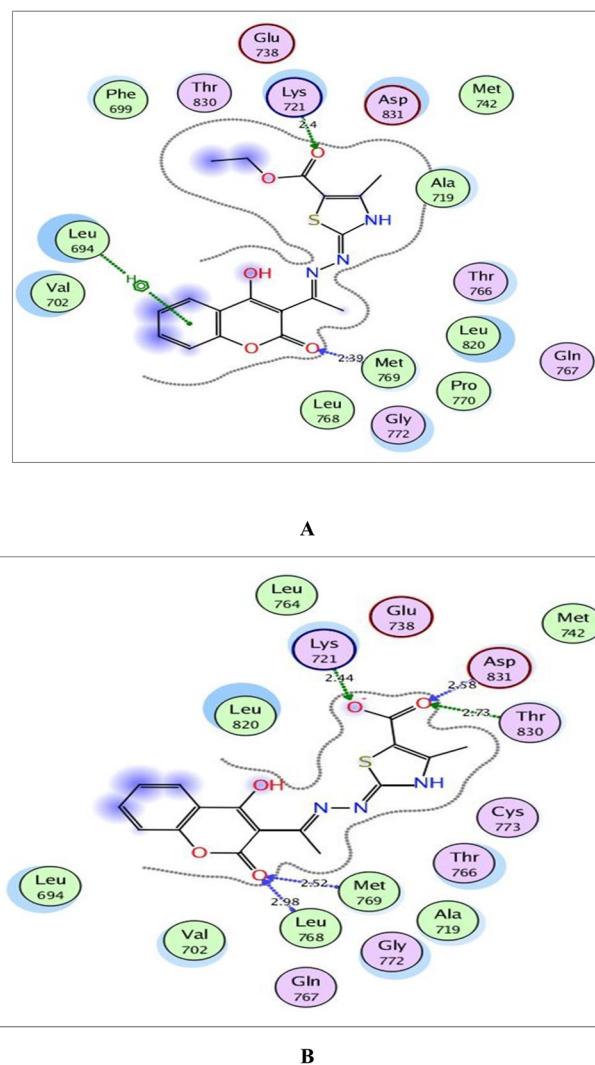


Fig. 8 (A) and (B) Illustrated 2D diagrams of compound **6a** and its metabolite, respectively showing their interactions with the EGFR binding site.



relative to rapamycin as the reference compound which showed an IC_{50} of 0.396 μ M. Moreover, compounds **2a** and **6b** displayed noticeable equipotent effect against m-TOR (IC_{50} = 1.388 and 1.449 μ M), respectively, while compound **2b** showed less inhibition potency (IC_{50} = 2.957 μ M). On the other hand, the selected derivatives **6a** and **2a** exhibited strong comparable PI3K inhibitory activity; at sub-micromolar levels (IC_{50} = 0.131 and 0.174 μ M) versus 0.038 μ M for the reference compound LY294002. Compound **2b** exerted considerable PI3K inhibitory activity showing IC_{50} value of 1.331 μ M. The IC_{50} values of the tested derivatives on EGFR, m-TOR and PI3K were consistent with the promising cytotoxic results (Table 3).

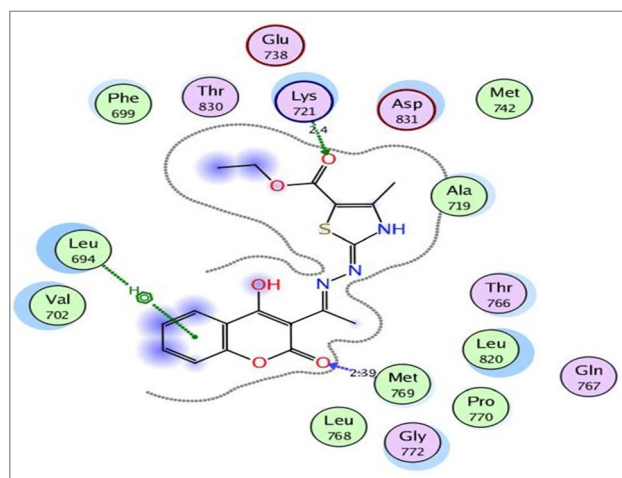
2.2.4. Cell cycle inhibition. Encouraged by the promising cytotoxicity and EGFR/m-TOR/PI3K inhibitory activity of the thiazole derivative **6a**, it was worthy to investigate its effect on cell cycle progression. The results showed that the tested compound inhibited MCF-7 cells proliferation by inducing cell growth arrest at S phase, as shown in Fig. 3.

2.2.5. Cell apoptosis. The effect of the promising derivative **6a** on apoptosis in MCF-7 breast cancer cells was investigated as well. The derivative induced an increase in the early and late cellular apoptosis, Fig. 4.

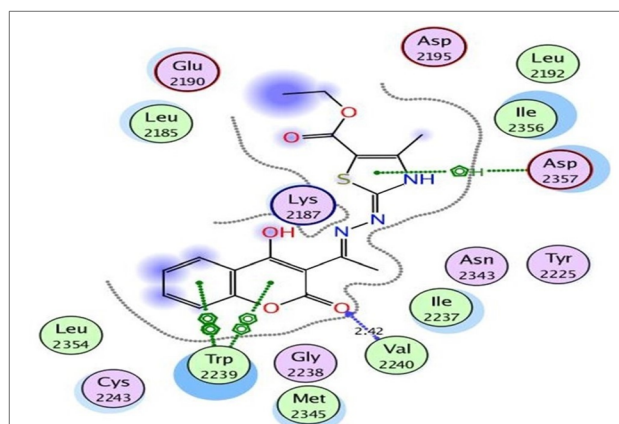
2.3. Molecular docking

In a trial to explore the mode of action of the synthesized derivatives, molecular docking study was performed for the most active compound **6a**. We considered the metabolism of **6a** containing carboxylate group as such; we carried out docking study for its carboxylic acid metabolite as well. Docking setup was first validated by self-docking of the co-crystallized ligand, erlotinib, in the vicinity of the binding site of EGFR showing a binding score of -10.760 kcal mol $^{-1}$ and RMSD value of 1.241 Å, Fig. 5.

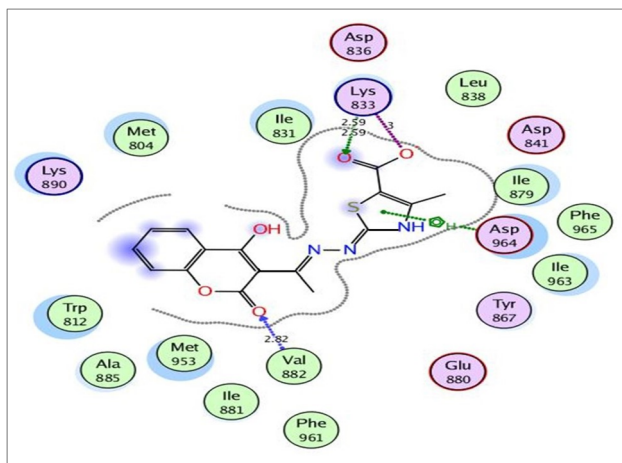
Self-docking of the co-crystallized ligand, 6-(1*H*-pyrazolo[3,4-*b*]pyridin-5-yl)-4-pyridin-4-ylquinoline, in the vicinity of the binding site of PI3K demonstrated a binding score of -7.511 kcal mol $^{-1}$ and RMSD value of 0.564 Å, Fig. 6.



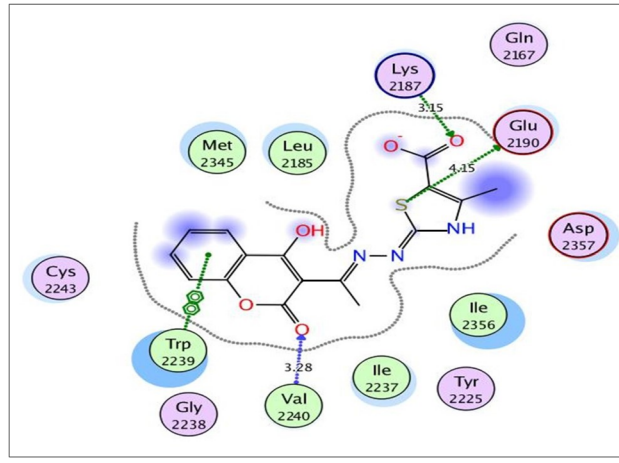
A



A



B



B

Fig. 9 (A) and (B) Illustrated 2D diagrams of compound **6a** and its metabolite, respectively showing their interactions with the PI3K binding site.

Fig. 10 (A) and (B) Illustrated 2D diagrams of compound **6a** and its metabolite, respectively showing their interactions with the mTOR binding site.

and/or ethyl thiosemicarbazide (2.5 mmol) and 0.5 ml glacial acetic acid were added. The reaction mixture was refluxed for 5–7 hours using heating mantle for conventional synthesis and under irradiation power (500 W), reaction temperature (125 °C), pressure (15 Pa) for 20 min using microwave oven for microwave assisted synthesis. The formed precipitate was filtered, dried and recrystallized from acetic acid to yield the target compounds **2a–c**.

4.1.1.1. 4-Methyl-1-(1-(4-hydroxy-2-oxo-2H-chromen-3-yl)ethylidene)thiosemicarbazide (2b). Yellow powder; yield (conventional: 85.2%, microwave: 93.5%); m.p. 210–2 °C; anal. calcd for $C_{13}H_{13}N_3O_3S$ (291.33): C, 53.60; H, 4.50; N, 14.42; S, 11.01. Found: C, 53.69; H, 4.60; N, 14.52; S, 11.08. 1H NMR (DMSO- d_6 , δ , ppm): 2.61 (3H, s, CH_3), 2.87, 2.88 (3H, d, CH_3NH), 7.30–8.00 (4H, m, Ar-H), 8.41 (1H, brs, NH, D_2O exchangeable), 10.46 (1H, s, NH, D_2O exchangeable), 15.23 (1H, s, OH, D_2O exchangeable). ^{13}C NMR (DMSO- d_6 , δ , ppm): 17.75, 31.61, 96.93, 116.80, 124.46, 126.07, 134.83, 153.47, 154.67, 165.84, 183.20.

4.1.2. Preparation of 3-{1-[(3-(un)substituted-4-methyl-3H-thiazol-2-ylidene)-hydrazono]-ethyl}-4-hydroxy-2H-chromen-2-ones (3a–c). To a mixture of compounds **2a–c** (2.5 mmol) and chloroacetone (0.2 ml, 2.5 mmol) in absolute ethanol (20 ml), anhydrous sodium acetate (0.41 g, 5 mmol) was added and the reaction mixture was refluxed for 6–10 hours using heating mantle for conventional synthesis and under irradiation power (500 W), reaction temperature (150 °C), pressure (15 Pa) for 15 min using microwave oven for microwave assisted synthesis of **3a**. After cooling, the precipitate was filtered, washed with water, dried, and crystallized from ethanol to give compounds **3a–c**.

4.1.2.1. 3-{1-[(4-Methyl-3H-thiazol-2-ylidene)-hydrazono]-ethyl}-4-hydroxy-2H-chromen-2-one (3a). Buff powder, yield (conventional: 85.2%, microwave: 93.5%); m.p. > 300 °C. Anal. calcd for $C_{15}H_{13}N_3O_3S$ (315.35): C, 57.13; H, 4.16; N, 13.33; S, 10.17. Found: C, 57.19; H, 4.22; N, 13.40; S, 10.25. 1H NMR (DMSO- d_6 , δ , ppm): 2.27 (3H, s, CH_3), 2.29 (3H, s, CH_3), 6.63 (1H, s, CH-thiazole), 6.64–8.23 (5H, m, Ar-H and NH), 13.04 (1H, s, OH, D_2O exchangeable). ^{13}C NMR (DMSO- d_6 , δ , ppm): 17.44, 17.61, 100.87, 106.97, 116.70, 117.73, 122.50, 132.75, 133.17, 147.10, 154.16, 156.18, 160.91, 163.12.

4.1.2.2. 3-{1-[(3,4-Dimethyl-3H-thiazol-2-ylidene)-hydrazono]-ethyl}-4-hydroxy-2H-chromen-2-one (3b). Buff powder; yield (82.6%); m.p. 241–2 °C. Anal. calcd for $C_{16}H_{15}N_3O_3S$ (329.37): C, 58.34; H, 4.59; N, 12.76; S, 9.74. Found: C, 58.41; H, 4.66; N, 12.86; S, 9.82. 1H NMR (DMSO- d_6 , δ , ppm): 2.20 (3H, s, CH_3), 2.79 (3H, s, CH_3), 3.39 (3H, s, NCH_3), 6.28 (1H, s, CH-thiazole), 7.27–7.99 (5H, m, Ar-H and OH). ^{13}C NMR (DMSO- d_6 , δ , ppm): 14.21, 17.23, 31.84, 95.18, 95.31, 116.61, 119.93, 124.12, 125.63, 133.99, 138.12, 153.34, 161.66, 163.65, 164.33, 177.99.

4.1.2.3. 3-{1-[(3-Ethyl-4-methyl-3H-thiazol-2-ylidene)-hydrazono]-ethyl}-4-hydroxy-2H-chromen-2-one (3c). Yellow powder; yield (87.3%); m.p. 213–4 °C. Anal. calcd for $C_{17}H_{17}N_3O_3S$ (343.4): C, 59.46; H, 4.99; N, 12.24; S, 9.34. Found: C, 59.55; H, 5.09; N, 12.34; S, 9.42. 1H NMR (DMSO- d_6 , δ , ppm): 1.25–1.28 (3H, t, CH_3CH_2 , $J = 7.12$ Hz), 2.23 (3H, s, CH_3), 2.79 (3H, s, CH_3), 3.89–3.94 (2H, q, CH_2CH_3 , $J = 7.08$ Hz), 6.27 (1H, s, CH-

thiazole), 7.28–7.99 (5H, m, Ar-H and OH). ^{13}C NMR (DMSO- d_6 , δ , ppm): 13.24, 13.95, 17.21, 35.04, 95.32, 95.55, 116.63, 119.93, 124.13, 125.65, 134.02, 137.57, 153.35, 161.65, 163.00, 164.43, 178.01.

4.1.3. Preparation of 3-(un)substituted-1-[(5-acetyl-4-methyl-3H-thiazol-2-ylidene)-hydrazono]-ethyl-4-hydroxy-2H-chromen-2-ones (4a–c). To a mixture of compounds **2a–c** (2.5 mmol) and 3-chloroacetylacetone (0.28 ml, 2.5 mmol) in absolute ethanol (20 ml), anhydrous sodium acetate (0.41 g, 5 mmol) was added and the reaction mixture was refluxed for 7–9 hours. After cooling, the precipitate was filtered, washed with water, dried, and crystallized from ethanol to give compounds **4a–c**.

4.1.3.1. 3-{1-[(5-Acetyl-4-methyl-3H-thiazol-2-ylidene)-hydrazono]-ethyl}-4-hydroxy-2H-chromen-2-one (4a). Buff powder; yield (86.1%); m.p. > 300 °C. Anal. calcd for $C_{17}H_{15}N_3O_4S$ (357.38): C, 57.13; H, 4.23; N, 11.76; S, 8.97. Found: C, 57.22; H, 4.29; N, 11.83; S, 9.07. 1H NMR (DMSO- d_6 , δ , ppm): 2.31 (3H, s, CH_3), 2.46 (3H, s, CH_3), 2.59 (3H, s, $COCH_3$), 6.79–8.15 (5H, m, Ar-H and NH), 12.79 (1H, s, OH, D_2O exchangeable). ^{13}C NMR (DMSO- d_6 , δ , ppm): 17.34, 18.75, 30.58, 100.27, 107.01, 116.70, 117.86, 122.43, 124.81, 132.54, 133.37, 155.49, 156.16, 160.70, 163.63, 191.10.

4.1.3.2. 3-{1-[(5-Acetyl-3,4-dimethyl-3H-thiazol-2-ylidene)-hydrazono]-ethyl}-4-hydroxy-2H-chromen-2-one (4b). Buff powder; yield (89.6%); m.p. 250–1 °C. Anal. calcd for $C_{18}H_{17}N_3O_4S$ (371.41): C, 58.21; H, 4.61; N, 11.31; S, 8.63. Found: C, 58.30; H, 4.69; N, 11.38; S, 8.71. 1H NMR (DMSO- d_6 , δ , ppm): 2.36 (3H, s, CH_3), 2.47 (3H, s, CH_3), 2.72 (3H, s, $COCH_3$), 3.37 (3H, s, NCH_3), 7.23–7.96 (5H, m, Ar-H and OH). ^{13}C NMR (DMSO- d_6 , δ , ppm): 14.26, 18.97, 30.08, 32.48, 79.40, 112.74, 113.77, 116.68, 124.60, 125.80, 134.39, 150.72, 158.63, 159.49, 165.50, 167.05, 169.97, 196.78.

4.1.3.3. 3-{1-[(5-Acetyl-3-ethyl-4-methyl-3H-thiazol-2-ylidene)-hydrazono]-ethyl}-4-hydroxy-2H-chromen-2-one (4c). Buff powder; yield (conventional: 85.2%, microwave: 93.5%); m.p. 277–8 °C. Anal. calcd for $C_{19}H_{19}N_3O_4S$ (385.44): C, 59.21; H, 4.97; N, 10.90; S, 8.32. Found: C, 59.32; H, 5.09; N, 10.99; S, 8.43. 1H NMR (DMSO- d_6 , δ , ppm): 1.26–1.30 (3H, t, CH_3CH_2 , $J = 7.04$ Hz), 2.46 (3H, s, CH_3), 2.59 (3H, s, CH_3), 2.78 (3H, s, $COCH_3$), 3.99–4.05 (2H, q, CH_2CH_3 , $J = 7.00$ Hz), 7.27–8.03 (5H, m, Ar-H and OH). ^{13}C NMR (DMSO- d_6 , δ , ppm): 13.04, 13.81, 17.36, 29.88, 40.84, 95.90, 97.96, 114.82, 116.60, 124.25, 125.83, 130.10, 134.39, 153.47, 156.74, 161.38, 164.81, 165.67, 195.92.

4.1.4. Preparation of 3-{1-[(3-(un)substituted-4-phenyl-3H-thiazol-2-ylidene)-hydrazono]-ethyl}-4-hydroxy-2H-chromen-2-ones (5a–c). To a mixture of compounds **2a–c** (2.5 mmol) and phenacyl bromide (0.5 g, 2.5 mmol) in absolute ethanol (20 ml), anhydrous sodium acetate (0.41 g, 5 mmol) was added and the reaction mixture was refluxed for 6–10 hours. After cooling, the precipitate was filtered, washed with water, dried, and crystallized from ethanol to give compounds **5a–c**.

4.1.4.1. 3-{1-[(3-Methyl-4-phenyl-3H-thiazol-2-ylidene)-hydrazono]-ethyl}-4-hydroxy-2H-chromen-2-one (5b). Yellow powder; yield (conventional: 85.2%, microwave: 93.5%); m.p. 206–7 °C. Anal. calcd for $C_{21}H_{17}N_3O_3S$ (391.44): C, 64.43; H, 4.38; N, 10.73; S, 8.19. Found: C, 64.51; H, 4.42; N, 10.79; S, 8.26. 1H NMR (DMSO- d_6 , δ , ppm): 2.82 (3H, s, CH_3), 3.34 (3H, s, NCH_3), 6.62



(1H, s, CH-thiazole), 7.29–8.02 (10H, m, Ar-H and OH). ¹³C NMR (DMSO-*d*₆, δ , ppm): 17.28, 34.03, 95.48, 98.54, 116.62, 119.81, 124.13, 125.66, 129.38, 130.12, 130.27, 134.05, 141.93, 153.35, 161.60, 163.42, 164.95, 178.01.

4.1.5. Preparation of 3-(un)substituted-4-methyl-2-[[1-(4-hydroxy-2-oxo-2H-chromen-3-yl)-ethylidene]-hydrazono]-2,3-dihydro-thiazole-5-carboxylic acid ethyl esters (6a-c). To a mixture of compounds **2a-c** (2.5 mmol) and ethyl-2-chloroacetoacetate (0.35 ml, 2.5 mmol) in absolute ethanol (20 ml), anhydrous sodium acetate (0.41 g, 5 mmol) was added and the reaction mixture was refluxed for 6–10 hours. After cooling, the precipitate was filtered, washed with water, dried, and crystallized from ethanol to give compounds **6a-c**.

4.1.5.1. 4-Methyl-2-[[1-(4-hydroxy-2-oxo-2H-chromen-3-yl)-ethylidene]-hydrazono]-2,3-dihydro-thiazole-5-carboxylic acid ethyl ester (6a). Yellow powder; yield (conventional: 85.2%, microwave: 93.5%); m.p. 289–90 °C. Anal. calcd for C₁₈H₁₇N₃O₅S (387.41): C, 55.80; H, 4.42; N, 10.85; S, 8.28. Found: C, 55.92; H, 4.49; N, 10.93; S, 8.36. ¹H NMR (DMSO-*d*₆, δ , ppm): 1.26–1.29 (3H, t, CH₃ CH₂, *J* = 7.00 Hz), 2.39 (3H, s, CH₃), 2.72 (3H, s, CH₃), 4.18–4.24 (2H, q, CH₂ CH₃, *J* = 7.0 Hz), 6.80–8.00 (5H, m, Ar-H and NH), 12.52 (1H, s, OH, D₂O exchangeable). ¹³C NMR (DMSO-*d*₆, δ , ppm): 14.64, 16.09, 17.42, 60.92, 85.93, 101.74, 116.75, 118.18, 124.60, 132.14, 133.60, 156.00, 156.56, 160.02, 162.79.

4.1.5.2. 3,4-Dimethyl-2-[[1-(4-hydroxy-2-oxo-2H-chromen-3-yl)-ethylidene]-hydrazono]-2,3-dihydro-thiazole-5-carboxylic acid ethyl ester (6b). Yellow powder; yield (87.6%); m.p. 294–5 °C. Anal. calcd for C₁₉H₁₉N₃O₅S (401.44): C, 56.85; H, 4.77; N, 10.47; S, 7.99. Found: C, 57.00; H, 4.85; N, 10.53; S, 8.08. ¹H NMR (DMSO-*d*₆, δ , ppm): 1.28–1.31 (3H, t, CH₃ CH₂, *J* = 7.08 Hz), 2.58 (3H, s, CH₃), 2.80 (3H, s, CH₃), 3.46 (3H, s, NCH₃), 4.23–4.28 (2H, q, CH₂ CH₃, *J* = 7.12 Hz), 7.27–8.04 (5H, m, Ar-H and OH). ¹³C NMR (DMSO-*d*₆, δ , ppm): 13.30, 14.71, 17.37, 32.35, 61.48, 95.78, 100.67, 116.63, 119.43, 124.10, 125.80, 134.35, 149.59, 153.34, 160.18, 161.07, 161.41, 166.83.

4.1.5.3. 3-Ethyl-4-methyl-2-[[1-(4-hydroxy-2-oxo-2H-chromen-3-yl)-ethylidene]-hydrazono]-2,3-dihydro-thiazole-5-carboxylic acid ethyl ester (6c). Orange powder; yield (88.4%); m.p. 205–6 °C. Anal. calcd for C₂₀H₂₁N₃O₅S (415.46): C, 57.82; H, 5.09; N, 10.11; S, 7.72. Found: C, 57.91; H, 5.14; N, 10.18; S, 7.80. ¹H NMR (DMSO-*d*₆, δ , ppm): 1.29–1.31 (6H, m, 2CH₃ CH₂), 2.60 (3H, s, CH₃), 2.78 (3H, s, CH₃), 3.97–4.02 (2H, q, CH₂ CH₃, *J* = 6.72 Hz), 4.24–4.28 (2H, q, CH₂ CH₃, *J* = 7.08 Hz), 7.28–8.03 (5H, m, Ar-H and OH). ¹³C NMR (DMSO-*d*₆, δ , ppm): 12.89, 14.71, 17.29, 39.34, 61.48, 95.76, 100.98, 116.61, 119.41, 124.18, 125.78, 134.30, 148.82, 153.33, 160.18, 161.07, 161.41, 166.83, 178.15.

4.1.6. Preparation of 3-(un)substituted-2-[[1-(4-hydroxy-2-oxo-2H-chromen-3-yl)-ethylidene]-hydrazono]-thiazolidin-4-ones (7a-c). To a mixture of compounds **2a-c** (2.5 mmol) and ethylbromoacetate (0.28 ml, 2.5 mmol) in absolute ethanol (20 ml), anhydrous sodium acetate (0.41 g, 5 mmol) was added and the reaction mixture was refluxed for 7–9 hours. After cooling, the precipitate was filtered, washed with water, dried, and crystallized from ethanol to give compounds **7a-c**.

4.1.6.1. 3-Methyl-2-[[1-(4-hydroxy-2-oxo-2H-chromen-3-yl)-ethylidene]-hydrazono]-thiazolidin-4-one (7b). Yellow powder;

yield (conventional: 85.2%, microwave: 93.5%); m.p. > 300 °C. Anal. calcd for C₁₅H₁₃N₃O₄S (331.35): C, 54.37; H, 3.95; N, 12.68; S, 9.68. Found: C, 54.43; H, 4.02; N, 12.77; S, 9.75. ¹H NMR (DMSO-*d*₆, δ , ppm): 2.82 (3H, s, CH₃), 3.11 (3H, s, NCH₃), 4.23 (2H, s, CH₂-thiazolidinone), 7.31–8.00 (5H, m, Ar-H and OH). ¹³C NMR (DMSO-*d*₆, δ , ppm): 14.47, 29.60, 32.71, 99.15, 117.61, 122.27, 123.77, 126.76, 138.74, 149.72, 157.37, 160.44, 162.36, 163.69, 172.24.

4.1.7. Preparation of 3-(un)substituted-5-methyl-2-[[1-(4-hydroxy-2-oxo-2H-chromen-3-yl)-ethylidene]-hydrazono]-thiazolidin-4-ones (8a-c). To a mixture of compounds **2a-c** (2.5 mmol) and ethyl-2-bromopropionate (0.32 ml, 2.5 mmol) in absolute ethanol (20 ml), anhydrous sodium acetate (0.41 g, 5 mmol) was added and the reaction mixture was refluxed for 7–9 hours. After cooling, the precipitate was filtered, washed with water, dried, and crystallized from ethanol to give compounds **8a-c**.

4.1.7.1. 5-Methyl-2-[[1-(4-hydroxy-2-oxo-2H-chromen-3-yl)-ethylidene]-hydrazono]-thiazolidin-4-one (8a). Yellow powder; yield (87.2%); m.p. 292–3 °C. Anal. calcd for C₁₅H₁₃N₃O₄S (331.35): C, 54.37; H, 3.95; N, 12.68; S, 9.68. Found: C, 54.45; H, 4.05; N, 12.77; S, 9.74. ¹H NMR (DMSO-*d*₆, δ , ppm): 1.60–1.62 (3H, d, CH₃-thiazolidinone, *J* = 7.28 Hz), 2.77 (3H, s, CH₃), 4.51–4.56 (1H, q, CH-thiazolidinone, *J* = 7.20 Hz), 7.31–8.01 (5H, m, Ar-H and NH), 12.34 (1H, s, OH, D₂O exchangeable). ¹³C NMR (DMSO-*d*₆, δ , ppm): 18.91, 19.01, 45.75, 96.15, 116.81, 119.61, 124.43, 125.86, 134.91, 149.55, 153.55, 163.69, 164.86, 176.50.

4.1.7.2. 3,5-Dimethyl-2-[[1-(4-hydroxy-2-oxo-2H-chromen-3-yl)-ethylidene]-hydrazono]-thiazolidin-4-one (8b). Yellow powder; yield (87.9%); m.p. 241–2 °C. Anal. calcd for C₁₆H₁₅N₃O₄S (345.37): C, 55.64; H, 4.38; N, 12.17; S, 9.28. Found: C, 55.72; H, 4.48; N, 12.26; S, 9.37. ¹H NMR (DMSO-*d*₆, δ , ppm): 1.62–1.64 (3H, d, CH₃-thiazolidinone, *J* = 7.20 Hz), 2.81 (3H, s, CH₃), 3.17 (3H, s, NCH₃), 4.51–4.57 (1H, q, CH-thiazolidinone, *J* = 7.12), 7.30–7.99 (5H, m, Ar-H and OH). ¹³C NMR (DMSO-*d*₆, δ , ppm): 17.56, 18.88, 29.99, 44.44, 96.15, 116.82, 119.27, 124.48, 125.87, 134.90, 153.53, 158.91, 161.89, 165.50, 166.70, 174.76.

4.1.7.3. 3-Ethyl-5-methyl-2-[[1-(4-hydroxy-2-oxo-2H-chromen-3-yl)-ethylidene]-hydrazono]-thiazolidin-4-one (8c). Yellow powder; yield (conventional: 85.2%, microwave: 93.5%); m.p. 185–6 °C. Anal. calcd for C₁₇H₁₇N₃O₄S (359.4): C, 56.81; H, 4.77; N, 11.69; S, 8.92. Found: C, 56.89; H, 4.85; N, 11.74; S, 9.01. ¹H NMR (DMSO-*d*₆, δ , ppm): 1.19–1.23 (3H, t, CH₃ CH₂, *J* = 7.12 Hz), 1.62–1.64 (3H, d, CH₃-thiazolidinone, *J* = 7.24 Hz), 2.81 (3H, s, CH₃), 3.74–3.80 (2H, q, CH₂ CH₃, *J* = 7.04 Hz), 4.53–4.59 (1H, q, CH-thiazolidinone, *J* = 7.16 Hz), 7.31–7.99 (5H, m, Ar-H and OH). ¹³C NMR (DMSO-*d*₆, δ , ppm): 12.50, 17.47, 18.92, 38.58, 44.38, 96.14, 116.78, 119.27, 124.38, 125.84, 134.81, 153.52, 157.93, 161.29, 170.57, 174.40, 179.01.

4.1.8. Preparation of 3-(un)substituted-1-[[4-methyl-5-phenylazo-3H-thiazol-2-ylidene]-hydrazono]-ethyl-4-hydroxy-2H-chromen-2-one (9a-f). A mixture of compounds **2a-c** (2.5 mmol) and appropriate hydrazonoyl chlorides (2.5 mmol) in dioxane (20 ml) containing triethylamine (0.25 ml, 1 mmol) was heated under reflux for 8–10 h and then cooled. The solution was poured onto crushed ice and concentrated hydrochloric



acid. The solid produced was collected by filtration and crystallized from the ethanol to give compounds **9a–f**.

4.1.8.1. 3-{1-[(4-Methyl-5-phenylazo-3H-thiazol-2-ylidene)-hydrazono]-ethyl}-4-hydroxy-2H-chromen-2-one (**9a**). Reddish brown powder; yield (78.7%); m.p. 103–5 °C. Anal. calcd for $C_{21}H_{17}N_5O_3S$ (419.46): C, 60.13; H, 4.09; N, 16.70; S, 7.64. Found: C, 60.07; H, 4.01; N, 16.59; S, 7.54. 1H NMR (DMSO- d_6 , δ , ppm): 2.45 (3H, s, CH_3), 2.66 (3H, s, CH_3), 7.26–8.02 (11H, m, Ar-H, OH and NH). ^{13}C NMR (DMSO- d_6 , δ , ppm): 9.00, 12.18, 115.34, 117.26, 119.07, 125.01, 125.26, 125.69, 129.64, 129.85, 130.07, 130.18, 131.07, 137.15, 144.98, 158.20, 159.76, 161.00, 178.38.

4.1.8.2. 3-{1-[(3,4-Dimethyl-5-phenylazo-3H-thiazol-2-ylidene)-hydrazono]-ethyl}-4-hydroxy-2H-chromen-2-one (**9b**). Reddish brown powder; yield (conventional: 85.2%, microwave: 93.5%); m.p. 169–70 °C. Anal. calcd for $C_{22}H_{19}N_5O_3S$ (433.48): C, 60.96; H, 4.42; N, 16.16; S, 7.40. Found: C, 60.85; H, 4.35; N, 16.08; S, 7.31. 1H NMR (DMSO- d_6 , δ , ppm): 2.62 (3H, s, CH_3), 2.69 (3H, s, CH_3), 3.57 (3H, s, CH_3), 7.19–7.96 (10H, m, Ar-H and OH). ^{13}C NMR (DMSO- d_6 , δ , ppm): 17.81, 17.93, 30.16, 116.55, 116.74, 117.27, 123.01, 123.82, 124.05, 125.23, 125.47, 125.75, 126.07, 129.75, 131.85, 137.03, 153.35, 154.31, 154.58, 178.35.

4.1.8.3. 3-{1-[(3-Ethyl-4-methyl-5-phenylazo-3H-thiazol-2-ylidene)-hydrazono]-ethyl}-4-hydroxy-2H-chromen-2-one (**9c**). Reddish brown powder; yield (78.8%); m.p. 126–127 °C. Anal. calcd for $C_{23}H_{21}N_5O_3S$ (447.51): C, 61.73; H, 4.73; N, 15.65; S, 7.17. Found: C, 61.80; H, 4.81; N, 15.74; S, 7.26. 1H NMR (DMSO- d_6 , δ , ppm): 1.36–1.39 (3H, t, CH_3), 2.69 (3H, s, CH_3), 2.79 (3H, s, CH_3), 4.09–4.14 (2H, q, CH_2), 7.26–8.02 (10H, m, Ar-H and OH). ^{13}C NMR (DMSO- d_6 , δ , ppm): 12.54, 17.26, 17.84, 30.05, 116.57, 117.30, 119.29, 122.26, 123.22, 124.13, 124.32, 125.22, 125.68, 129.65, 129.74, 130.04, 137.12, 152.13, 153.29, 161.21, 168.67, 176.89.

4.1.8.4. 3-{1-[(4-Methyl-5-(4-tolyl)azo-3H-thiazol-2-ylidene)-hydrazono]-ethyl}-4-hydroxy-2H-chromen-2-one (**9d**). Reddish brown powder; yield (76.5%); m.p. 145–6 °C. Anal. calcd for $C_{22}H_{19}N_5O_3S$ (433.48): C, 60.96; H, 4.42; N, 16.16; S, 7.40. Found: C, 60.88; H, 4.31; N, 16.06; S, 7.29. 1H NMR (DMSO- d_6 , δ , ppm): 2.39 (3H, s, CH_3), 2.60 (3H, s, CH_3), 2.68 (3H, s, CH_3), 7.17–7.84 (10H, m, Ar-H, OH and NH). ^{13}C NMR (DMSO- d_6 , δ , ppm): 15.71, 17.86, 21.10, 115.36, 116.78, 117.30, 118.43, 122.49, 123.44, 123.56, 125.78, 125.71, 130.06, 130.42, 131.88, 133.77, 141.04, 150.34, 159.55.

4.1.8.5. 3-{1-[(3,4-Dimethyl-5-(4-tolyl)azo-3H-thiazol-2-ylidene)-hydrazono]-ethyl}-4-hydroxy-2H-chromen-2-one (**9e**). Reddish brown powder; yield (74.3%); m.p. 152–3 °C. Anal. calcd for $C_{23}H_{21}N_5O_3S$ (447.51): C, 61.73; H, 4.73; N, 15.65; S, 7.17. Found: C, 61.62; H, 4.64; N, 15.55; S, 7.09. 1H NMR (DMSO- d_6 , δ , ppm): 2.35 (3H, s, CH_3), 2.63 (3H, s, CH_3), 2.74 (3H, s, CH_3), 3.49 (3H, s, CH_3), 7.21–7.97 (9H, m, Ar-H and OH). ^{13}C NMR (DMSO- d_6 , δ , ppm): 9.59, 17.87, 21.11, 25.23, 115.34, 116.92, 117.26, 118.42, 122.30, 123.44, 123.59, 124.66, 126.00, 130.10, 131.55, 135.76, 153.42, 157.46, 161.33, 164.19, 166.71, 168.70.

4.1.8.6. 3-{1-[(3-Ethyl-4-methyl-5-(4-tolyl)azo-3H-thiazol-2-ylidene)-hydrazono]-ethyl}-4-hydroxy-2H-chromen-2-one (**9f**). Reddish brown powder; yield (77.1%); m.p. 159–60 °C. Anal.

calcd for $C_{24}H_{23}N_5O_3S$ (461.54): C, 62.46; H, 5.02; N, 15.17. Found: C, 62.34; H, 5.09; N, 15.06. 1H NMR (DMSO- d_6 , δ , ppm): 1.35–1.39 (3H, t, CH_3), 2.39 (3H, s, CH_3), 2.59 (3H, s, CH_3), 2.66 (3H, s, CH_3), 4.10–4.14 (2H, q, CH_2), 7.26–8.02 (9H, m, Ar-H and OH). ^{13}C NMR (DMSO- d_6 , δ , ppm): 7.44, 11.72, 13.77, 25.45, 34.05, 115.35, 116.78, 117.51, 118.51, 121.59, 123.44, 123.69, 125.73, 127.14, 127.66, 128.00, 130.12, 137.19, 153.35, 154.39, 159.50, 162.91, 164.10, 167.06.

4.2. Biology

4.2.1. MTT anti-proliferative assay. MCF-7 (human breast adenocarcinoma), HCT-116 (human colorectal carcinoma), human liver carcinoma (HepG-2), and the normal human skin fibroblast (BJ-1) cell lines were purchased from the American Type Culture Collection (Rockville, MD, USA) and maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 U ml $^{-1}$ penicillin, and 100 U ml $^{-1}$ streptomycin. The cells were grown at 37 °C in a humidified atmosphere of 5% CO $_2$. The anti-proliferative activities were estimated by the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. This test is based on MTT cleavage by mitochondrial dehydrogenases form viable cells.^{27–29} Cells were placed in a 96 well sterile microplate (5 \times 10 4 cells per well) and incubated at 37 °C in serum-free media containing dimethyl sulfoxide (DMSO) and either a series of various concentrations of each compound or doxorubicin (positive control) for 48 h before the MTT assay. After incubation, the media were removed and 40 μ l MTT (2.5 mg ml $^{-1}$) was added to each well. Incubation was resumed for an additional 4 h. The purple formazan dye crystals were solubilized with 200 μ l DMSO. Absorbance was measured at 590 nm in a Spectra Max Paradigm Multi-Mode microplate reader (Molecular Devices, LLC, San Jose, CA, USA). Relative cell viability was expressed as the mean percentage of viable cells compared to the untreated control cells. All experiments were conducted in technical triplicate and three biological replicates. All values were reported as mean \pm SD. IC $_{50}$ s were determined by SPSS Inc. probit analysis (IBM Corp., Armonk, NY, USA).

4.2.2. In vitro EGFR activity assay. The EGFR kinase activity assay for compounds **2a**, **2b**, **6a** and **6b** was estimated according to the manufacturer procedures, using EGFR kinase assay kit (BPS Bioscience, Cat. #40321). The kit uses Kinase-Glo MAX as the detection reagent (Promega #V6071). Results of the tested compound were assessed by measuring the luminescence using a microplate reader.

4.2.3. In vitro m-TOR activity assay. The assay was conducted for compounds **2a**, **2b**, **6a** and **6b** using the K-LISA TM mTOR Activity Kit which is an ELISA-based assay (Calbiochem kit, Cat. #CBA055) following the manufacturing procedure. The kit utilizes a p70S6K-GST fusion protein as a specific mTOR substrate, where active mTOR phosphorylates p70S6K. The phosphorylated substrate is detected with anti-p70S6K-T389 antibody, followed by detection with HRP-antibody conjugate and TMB substrate and relative activity is determined by reading the absorbance at dual wavelengths of 450/540 nm or 450/595 nm.



4.2.4. *In vitro* PI3K activity assay. The PI3K activity assay was performed for compounds **2a**, **2b**, **6a** and **6b** using the PI3K β (p110 β /p85 α) assay kit per the manufacturer instructions (BPS Bioscience, Cat. #79802), using ADP-Glo ® Kinase Assay as a detection reagent and the luminescence was recorded using a microplate reader.

4.2.5. Cell cycle analysis and apoptosis assay. MCF-7 cells were seeded at 8×10^4 and incubated overnight at 37 °C and supplied with 5% CO $_2$. After 48 h of treatment, cell pellets were collected and centrifuged at 300 g for 5 min. For cell cycle analysis, cell pellets were fixed in 70% ethanol on ice for 15 min. The collected pellets were incubated with propidium iodide (PI) staining solution (50 $\mu\text{g ml}^{-1}$ PI, 0.1 mg ml^{-1} RNaseA and 0.05% Triton X-100) at room temperature for 1 h. Apoptosis detection was performed by FITC Annexin-V/PI kit (Becton Dickinson, Franklin Lakes, NJ, USA) following the manufacturer's protocol. The samples were analyzed by fluorescence-activated cell sorting (FACS) with a Gallios flow cytometer (Beckman Coulter, Brea, CA, USA) within 1 h from the staining. Data were analyzed using Kaluza v1.2 (Beckman Coulter).

4.3. Molecular docking

All the molecular modeling studies were carried out using Molecular Operating Environment (MOE-Dock, 2014.0901) software. All minimizations were performed with MOE until an RMSD gradient of 0.1 kcal mol $^{-1}$ Å $^{-1}$ with MMFF94x force field and the partial charges were automatically calculated.

The X-ray crystallographic structures of EGFR complexed with erlotinib (PDB ID: 1M17), PI3K complexed with 6-(1*H*-pyrazolo [3,4-*b*]pyridin-5-yl)-4-pyridin-4-ylquinoline (PDB ID: 3L54) and mTOR complexed with 3-(4-morpholin-4-ylpyrido[3',2':4,5]furo [3,2-*d*]pyrimidin-2-yl)phenol (PDB ID: 4JT6) were downloaded from the protein data bank. The proteins were prepared for docking by removal of all ligands and water molecules that are not involved in the binding. One conserved water molecule involved in the co-crystallized ligand binding to EGFR binding site was kept. It is worth to mention that four chains make up the mTOR PDB file, and two of them, designated A and B, each include an original ligand whose binding modes to those two chains are noticeably similar. Therefore, only chain A was kept in order to perform molecular docking. All the proteins were then prepared for the docking study using Protonate 3D protocol in MOE with default options. The co-crystallized ligand was used to define the binding site for docking. Triangle Matcher placement method and London dG scoring function were used for docking.

4.4. *In silico* physicochemical properties, pharmacokinetics parameters and medicinal chemistry friendliness study

The physicochemical as well as the leadlike nature and medicinal chemistry friendliness of the promising compound **6a** were predicted using the free SwissADME web tool.

Conflicts of interest

The authors declared that there are no actual or potential conflicts of interest and have approved the article.

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