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Original article

Design and synthesis of potent 1,2,4-trisubstituted imidazolinone derivatives with dual p38αMAPK and ERK1/2 inhibitory activity

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ABSTRACT

The synthesis of new 1,2,4-trisubstituted imidazolinone derivatives was described. The new compounds were designed as dual p38αMAPK and ERK1/2 inhibitors through hybridization of pharmacophoric elements associated with inhibition of these kinases. The kinase inhibition assay revealed excellent activity in the nanomolar range; especially compounds **6d** and **7h** which seemed promising candidates for such dual activity with IC_{50} values of 4.5 and 4.7 nM against p38αMAP, 25.0 and 24.0 nM against ERK1, and 3.2 and 3.5 nM against ERK2, respectively. These compounds were further tested for their antiproliferative activity against nine cancer cell lines, where they elicited high activity in the sub-micromolar range against breast, prostate and melanoma cells.

1. Introduction

The oncogenic process is a result of multiple dysfunctional pathways and pathway genes. Therefore, targeting multiple pathways simultaneously is necessary for sustained tumor remission. Signal transduction in cancer cells is a sophisticated process that involves receptor tyrosine kinases (RTKs) that eventually trigger multiple cytoplasmic kinases, which are often serine/threonine kinases. In clinical trials, highly selective or specific inhibitors of only one of the kinases involved in these signaling pathways has been associated with limited or sporadic responses. Therefore, simultaneous inhibition of several key kinases at the level of receptors and/or downstream serine/threonine kinases may help to optimize the overall therapeutic benefit associated with molecularly targeted anticancer agents [1–3].

Mamalian mitogen-activated protein kinases (MAPKs) are serine/threonine protein kinases that participate in intracellular signaling during proliferation, differentiation, cellular stress responses and apoptosis. Inhibition of MAPKs, including extracelluar signal-regulated kinases 1 and 2 (ERK1/2), p38MAPK (having α, β, γ, δ isoforms) and the stress activated protein kinase (SAPK)/c-Jun N-terminal kinase (JNK), has been implicated in the activity of numerous chemotherapy and genotoxic drugs [4,5]. p38MAPK regulates production of cytokines by the tumor microenvironment and its activation enables cancer cells to survive in the presence of oncogenic stress, radiation, chemotherapy and targeted therapies [6]. In addition, p38MAPK is implicated in angiogenesis which is required for tumor growth and metastasis, and many new cancer therapies are therefore directed against the tumor vasculature. LY2228820 dimesylate **I** is a highly selective small molecule inhibitor of p38α and p38β MAPKs that is currently under clinical investigation for human malignancies [7]. Another example is the potent p38αMAP kinase inhibitor isoxazole isostere **II** [8] (Fig. 1).

On the other hand, upon activation of ERK1/2 by growth factors, cytokines and osmotic stresses, they can phosphorylate and regulate multiple substrates such as cytoskeletal proteins, kinases and transcription factors within various cellular compartments. These events in turn result in gene expression changes and alteration in cell proliferation, differentiation and survival [9]. For this reason, inhibitors of the ERK1/2 are entering clinical trials as potential anticancer agents [10]. The 3,4-diarylsubstituted pyrazole derivative **III** demonstrated potent cytotoxic activity through ERK1/2 inhibition [11] (Fig. 1).

Based on these observations, it seemed beneficial to co-target p38αMAPK and ERK1/2, an approach that might lead to a

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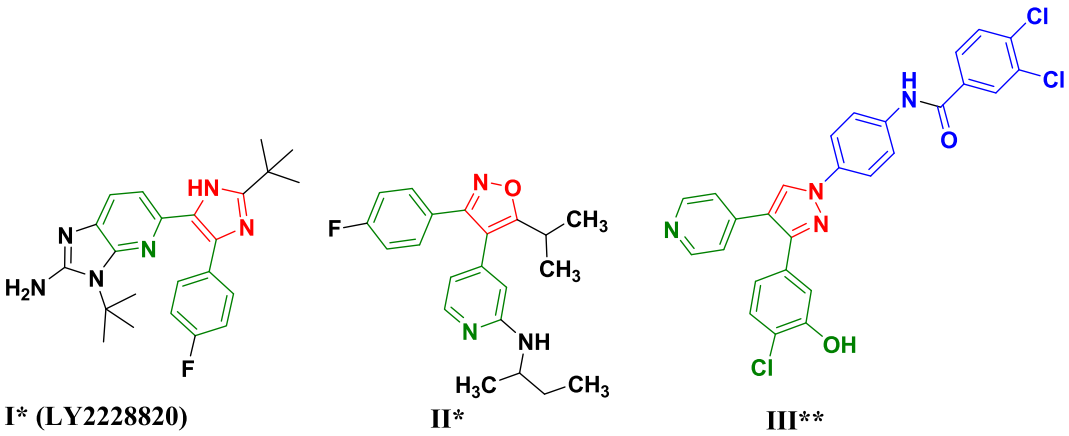


Fig. 1. Structure of some reported inhibitors of p38αMAPK* and ERK1/2**.

synergistic effect on inhibiting cell proliferation and on controlling metastasis. Moreover, it was reported that the inhibition of p38MAPK resulted in stimulation of ERK with subsequent induction of proliferation [12,13]. Therefore, dual targeting of p38MAPK and ERK would prevent activation of ERK following p38MAPK inhibition, and would potentiate the antiproliferative activity of the new compounds.

With the goal of producing new antitumor agents and based on the aforementioned facts, the design of the new target compounds relied on a hybrid pharmacophoric approach in which two molecular fragments were combined. The first was the vicinal diarylsubstituted 5-membered heterocyclic nucleus, which is typical for p38MAPK inhibition, as could be featured in compounds **I** and **II**. Accordingly, the vicinal diarylsubstituted imidazolinone moiety was selected as a core for our target compounds **6** and **7**. On the other hand, it could be observed that the ERK1/2 inhibitor **III** was characterized by a structure extension at position 1 of the pyrazole ring which was believed to contribute to ERK activity. Hence, a second molecular fragment deemed essential to extend the imidazolinone core as to empower ERK inhibition [11]. Searching for a bioactive moiety of interest in the field of chemotherapy, the literature survey revealed the broad spectrum anticancer activity of compound **IV** against lung, prostate, breast cancers and leukemia [14]. Appropriately, the benzylidene tail of the thiazolidinedione derivative **IV** was selected to extend the imidazolinone scaffold with the hope to obtain new compounds with potential activity against many types of cancer cells and acting via inhibition of p38αMAPK and ERK1/2 (Fig. 2).

Herein, we report the synthesis of novel series of diversely substituted imidazolinone derivatives **6a**–**j** and **7a**–**j** together with the study of their inhibitory activity against p38αMAPK and ERK1/2, and their antiproliferative activity toward selected cell lines.

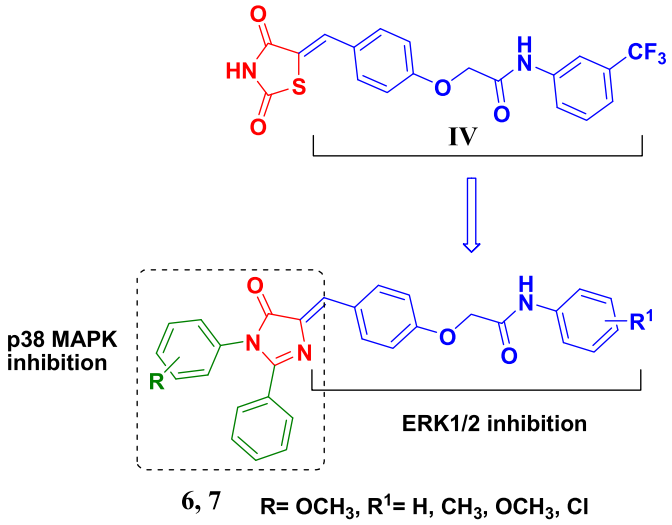
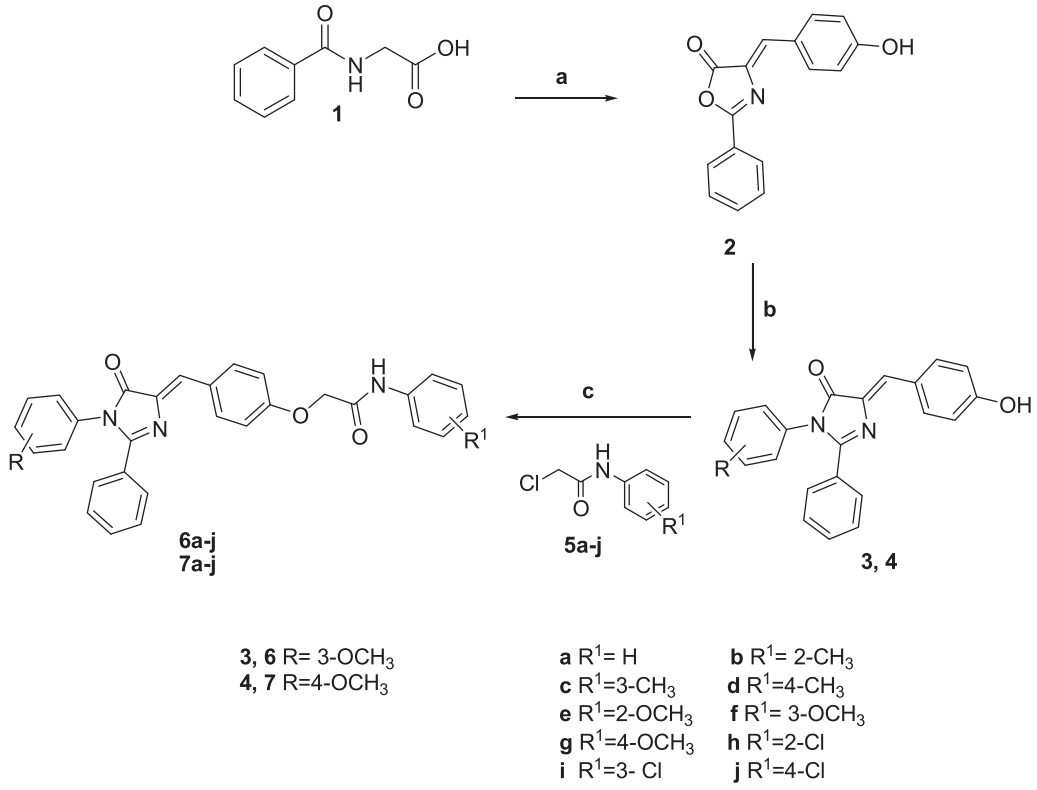


Fig. 2. Design of the target compounds **6** and **7**.

2. Discussion

2.1. Chemistry

The synthetic strategy for the preparation of the target compounds **6a**–**j** and **7a**–**j** was illustrated in Scheme 1. 4-(4-Hydroxybenzylidene)-2-phenyloxazol-5(4*H*)-one **2** was synthesized according to the literature method [15] via the Erlenmyer reaction of hippuric acid with 4-hydroxybenzaldehyde in acetic anhydride. Subsequently, 4-(4-hydroxybenzylidene)-1-(3/4-methoxyphenyl)-2-phenyl-1*H*-imidazol-5(4*H*)-ones **3** and **4** were obtained through the reaction of **2** with 3/4-methoxyaniline in glacial acetic acid in the presence of anhydrous sodium acetate. The mechanism of this reaction proceeded through open intermediates which were recyclized in the presence of glacial acetic acid to afford the imidazolinone derivatives [16]. IR spectra of compounds **3** and **4** revealed the appearance of bands at 3327 and 3290 cm^{-1}, respectively assigned to the OH group. Also, ^1H NMR showed the exchangeable singlet signal corresponding to OH proton. Reaction of **3**, **4** with 2-chloro-*N*-un/substituted phenylacetamide **5a**–**j**; previously synthesized according to the literature method [17,18]; afforded the acetamide derivatives **6a**–**j** and **7a**–**j** in good yields. IR spectra revealed the presence of an additional carbonyl band assigned to the phenylacetamido function. ^1H NMR revealed the presence of a singlet signal integrated for 2 protons corresponding to the CH_2 protons at 4.67–4.76 ppm and an exchangeable singlet signal corresponding to NH proton mostly at δ 8.16–9.01 ppm. Also, additional singlet signals corresponding to the 3 methyl or 3 methoxy protons resonating at 2.27–2.39 or 3.83–3.90 ppm in case of **6b**–**d**, **7b**–**d** or **6e**–**g**, **7e**–**g**, respectively, were observed. Moreover, ^{13}C NMR showed the characteristic signals of the CH_2 signal at 67.4–67.6 ppm in addition to signals at 17.4–21.4 ppm or 55.4–55.9 ppm for compounds bearing methyl or methoxy groups, respectively.



Scheme 1. Synthesis of the target compounds **6** and **7**. Reagents and reaction conditions: **a**: 4-hydroxybenzaldehyde, acetic anhydride, anh. CH_3COONa; **b**: 3/4-methoxyaniline, acetic acid, anh.CH_3COONa; **c**: 2-chloro-*N*-un/substituted phenylacetamide **5a**–**j**, DMF, K_2CO_3.

2.2. Anticancer activity

2.2.1. In vitro kinase inhibitory activity

The target compounds were evaluated for their potential inhibitory effect on p38αMAPK and ERK1/2 protein kinases using LanthaScreen Kinase activity assay kits (Invitrogen). The activity was expressed by median growth inhibitory concentration (IC_{50}) and provided in Table 1. Sorafenib was used as reference drug. The results indicated that, the new imidazolinones exhibited potent inhibitory activity against the three kinases at nanomolar level (IC_{50} ranges: p38α = 3.7–354, ERK1 = 21–95, ERK2 = 2.3–89 nM). Moreover, the majority of the tested compounds were more potent against p38αMAPK and/or ERK2 than ERK1. Several derivatives showed potency higher than sorafenib against p38αMAPK and ERK2 but all of them were less potent than the reference drug against ERK1. In particular, compounds **6d**, **6f**–**6j**, **7h** and **7i** displayed excellent kinase suppressive effect at single digit nanomolar concentrations against p38αMAPK and ERK2 (IC_{50} range = 3.7–6.5 and 3.2–7.0 nM, respectively). Compound **6e**, **7g** and **7j** preferentially inhibited ERK2 with IC_{50} = 4.7, 2.3 and 4.5 nM, respectively.

Analysis of p38αMAPK enzymatic assay results revealed that the inhibitory potency of N^1-3-methxoyphenyl imidazolinones **6a**–**j** and their N^1-4-methxoyphenyl analogs **7a**–**j** was primarily affected by the electronic nature and position of the substituent on the pendant phenyl ring of the aryl acetamide moiety. The inhibitory potency of N^1-3-methxoyphenyl imidazolinones **6a**–**j** was enhanced by grafting electron-withdrawing substituent like 3-methoxy group **6f** (IC_{50} = 6.5 nM) or chlorine atom **6h**–**i** (IC_{50} = 3.7–5.6 nM) to the phenyl ring regardless of its position. The potency was also favored by *para*-substitution on the phenyl with 4-methyl **6d** (IC_{50} = 4.5 nM) or 4-methoxy group **6g** (IC_{50} = 4.3 nM), (c.f. **6a** R = H, IC_{50} = 35 nM). However, *ortho*- and *meta*-methylphenyl **6b**, **c** or *ortho*-methoxyphenyl **6e** derivatives were the least active in this series. Considering N^1-4-methxoyphenyl imidazolinones **7a**–**j**, compound **7a**, having unsubstituted phenyl ring, was the least active kinase inhibitor (IC_{50} = 354 nM). Similar to the N^1-3-methxoyphenyl imidazolinones **6a**–**j**, compounds with electron-withdrawing 3-methoxy group **7f** (IC_{50} = 23 nM) or chloro **7h**–**i** (IC_{50} = 4.5–44 nM) substituent on the pendant phenyl were significantly more potent than those with electron-donating methyl group **7b**–**d** or 2/4-methoxy group **7e** and **7g** (IC_{50} = 34–345 nM). However, substitution at the *para* position (**7d**, **7g** and **7j**) was not well tolerated compared to *ortho* (**7b**, **7e** and **7h**) and *meta* (**7c**, **7f** and **7i**) substituted counterparts.

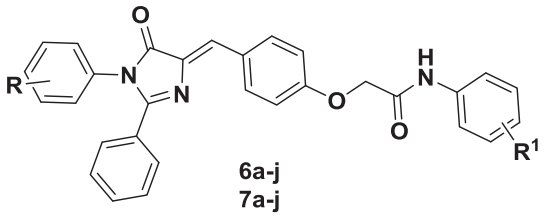
As per the activity against ERK2, it could be noticed that there was a high degree of homogeneity between the activity of the compounds on p38αMAPK and ERK2; so that compounds highly effective against p38αMAPK were similarly effective against ERK2 except for derivatives **6e**, **7g** and **7j** which were more active on ERK2 than p38αMAPK.

Finally, looking up for our target dual p38αMAP-ERK1/2 inhibitors, compounds **6d** and **7h** seemed promising candidates for such dual activity; exerting inhibitory activity of 4.5 and 4.7 nM against p38αMAP, 25 and 24 nM against ERK1, and 3.2 and 3.5 nM against ERK2, respectively.

2.2.2. Antiproliferative activity of compounds *6d* and *7h*

Constitutive activation of MAPK pathway was found in patient tumor samples in breast, colon, melanoma, lung, blood and thyroid [19,20]. Therefore, the antiproliferative activity of compounds **6d** and **7h** which demonstrated the best overall kinase inhibitory activity was evaluated against nine human tumor cell lines derived from different cancer types. The growth inhibitory activity

Table 1
IC_{50} values (nM) of tested compounds **6a–j** and **7a–j** against p38αMAPK and ERK1/2.



Compound	R	R^1	IC_{50} (nM) ± SEM[a]		
			P38αMAPK	ERK1	ERK2
6a	3-OCH_3	H	35.0 ± 2.1	65.0 ± 5.9	54.0 ± 4.3
6b	3-OCH_3	2-CH_3	66.0 ± 3.8	43.0 ± 3.2	44.0 ± 3.6
6c	3-OCH_3	3-CH_3	87.0 ± 5.2	20.0 ± 1.7	43.0 ± 2.7
6d	3-OCH_3	4-CH_3	4.5 ± 0.3	25.0 ± 1.9	3.2 ± 0.32
6e	3-OCH_3	2-OCH_3	54.0 ± 3.7	46.0 ± 2.8	4.7 ± 0.45
6f	3-OCH_3	3-OCH_3	6.5 ± 0.5	57.0 ± 4.7	5.4 ± 0.27
6g	3-OCH_3	4-OCH_3	4.3 ± 0.3	76.0 ± 7.3	4.3 ± 0.34
6h	3-OCH_3	2-Cl	5.6 ± 0.3	87.0 ± 6.2	5.3 ± 0.41
6i	3-OCH_3	3-Cl	3.7 ± 0.3	89.0 ± 7.4	3.4 ± 0.30
6j	3-OCH_3	4-Cl	4.6 ± 0.3	95.0 ± 8.9	4.6 ± 0.3
7a	4-OCH_3	H	354.0 ± 20.6	23.0 ± 2.4	23.0 ± 2.1
7b	4-OCH_3	2-CH_3	56.0 ± 4.1	32.0 ± 2.5	23.0 ± 1.7
7c	4-OCH_3	3-CH_3	77.0 ± 5.7	89.0 ± 5.2	78.0 ± 6.3
7d	4-OCH_3	4-CH_3	345.0 ± 29.5	23.0 ± 1.6	43.0 ± 3.3
7e	4-OCH_3	2-OCH_3	34.0 ± 2.4	43.0 ± 3.8	89.0 ± 6.7
7f	4-OCH_3	3-OCH_3	23.0 ± 1.6	21.0 ±.1.2	21.0 ± 1.2
7g	4-OCH_3	4-OCH_3	345.0 ± 30.3	23.0 ± 2.0	2.3 ± 0.2
7h	4-OCH_3	2-Cl	4.7 ± 0.4	24.0 ± 2.1	3.5 ± 0.3
7i	4-OCH_3	3-Cl	4.5 ± 0.4	35.0 ± 2.3	7.0 ± 0.6
7j	4-OCH_3	4-Cl	44.0 ± 4.2	47.0 ± 3.1	4.5 ± 0.4
Sorafenib	–	–	37.4 ± 3.5	18.1 ± 1.6	109.5 ± 9.8

[a] IC_{50} values were calculated from the mean values of data from three separate experiments.

of the compounds was evaluated using MTT method [21] and sorafenib was employed as a reference compound. The results are expressed as IC_{50} values and are presented in Table 2. The results revealed that both compounds exhibited potent antiproliferative activity against most of the tested cell lines (IC_{50} range = 0.05–9.40 μM), but were inactive against leukemia K562. Compounds **6d** and **7h** possessed superior activity at sub-micromolar level, even higher than that of sorafenib, against breast cancer (MCF7 and T47D), melanoma (LOX IMVI and MDA-MB-435) and prostate (PC3) cell lines with IC_{50} ranging from 0.05 to 0.99 μM. However, unlike sorafenib, the tested compounds were less effective against colon cancer (Colo-205 and HT29) and lung cancer (A549) cell lines (IC_{50} range = 4.50–9.40 μM).

Table 2
Antiproliferative activity of compounds **6d** and **7h** against different cancer cell lines and normal Vero cells.

Cancer type	Cell line	IC_{50} (μM) ± SEM[a]				
		6d	**7h**	**Sorafenib**	SI[b](**6d**)	SI[b](**7h**)
Breast	MCF7	0.37 ± 0.02	0.53 ± 0.05	0.78 ± 0.05	46.86	24.90
	T47D	0.67 ± 0.05	0.50 ± 0.04	0.61 ± 0.04	25.88	26.40
Colon	Colo-205	9.40 ± 0.71	4.50 ± 0.03	0.87 ± 0.07	1.85	2.93
	HT29	5.80 ± 0.05	4.50 ± 0.04	0.39 ± 0.03	2.98	2.93
Leukemia	K562	>100	>100	1.22 ± 0.01	–	–
Lung	A549	6.50 ± 0.05	4.90 ± 0.04	3.19 ± 0.03	2.66	2.69
Melanoma	LOX IMVI	0.76 ± 0.06	0.13 ± 0.01	4.25 ± 0.03	22.81	101.54
	MDA-MB-435	0.23 ± 0.02	0.05 ± 0.003	1.67 ± 0.01	75.39	264.00
Prostate	PC3	0.86 ± 0.08	0.99 ± 0.07	4.13 ± 0.03	20.16	13.33
Vero cells	–	17.34 ± 1.42	13.20 ± 1.20	–	–	–

[a] IC_{50} values were calculated from the mean values of data from three separate experiments.
[b] SI (selectivity index) = IC_{50} of Vero cells/IC_{50} of cancer cell. SI > 3 means that the compound is selective to cancer cell.

Comparing the activity of **6d** and **7h** against the sensitive cell lines, revealed that compound **7h** displayed higher potency than **6d** toward breast (MCF7), colon (Colo-205 and HT29), lung (A549) and melanoma (LOX IMVI and MDA-MB-435) cancer cell lines. On the other hand, **6d** was more effective against breast (T47D) and prostate (PC3) cell lines.

To examine the selectivity and hence the safety of compounds **6d** and **7h**, their antiproliferative activity was assessed against normal mammalian cells (Vero cells). The data indicated that the tested compounds had relatively low cytotoxic effect on normal cells (IC_{50} = 17.34 and 13.20 μM, respectively). The selectivity index calculated in relation to IC_{50} on Vero cells revealed high selectivity toward most tested cancer cells (SI > 20) except colon (Colo-205, HT-29) and lung (A-549) cells (Table 2).

3. Conclusion

The design and synthesis of new 1,2,4-trisubstituted imidazolinone derivatives of dual p38αMAPK and ERK1/2 inhibitory activity was described. The structure of the new compounds featured a vicinal diarylsubstituted imidazolinone core contributing to p38αMAPK activity and an extended substitution at position 4 of the imidazolinone nucleus that favored the ERK1/2 activity. In vitro kinase activity revealed preferential activity against p38αMAPK and ERK2 with IC_{50} in the nanomolar level and the activity of some derivatives exceeded that of sorafenib. Generally, the N^1-3-methoxyphenyl-imidazolinones were more potent than their N^1-4-methoxyphenyl isomers. Compounds **6d** and **7h** exhibited the highest activity against the three tested kinases. Therefore, their antiproliferative activity was assessed against several cell lines belonging to breast, colon, lung, leukemia, melanoma and prostate cancers. These compounds displayed activity in the sub-micromolar range and were found superior to sorafenib against breast cancer (MCF7 and T47D), melanoma (LOX IMVI and MDA-MB-435) and prostate (PC3) cell lines with IC_{50} ranging from 0.05 to 0.99 μM. Also, the compounds were highly selective to the same cancer cell lines as compared to normal Vero cells. Finally, it could be claimed that the rationale behind the design of the target compounds succeeded in achieving dual p38αMAPK and ERK1/2 inhibition, which in turn secured potent antiproliferative activity against several cancer cell lines.

4. Experimental

4.1. Chemistry

Melting Points were carried out by open capillary tube method using Stuart SMP3 Melting Point apparatus and they were uncorrected. Elemental Microanalysis was carried out at the Regional Center for Mycology and Biotechnology, Al-Azhar University. Infrared spectra were recorded on Shimadzu Infrared spectrometer IR Affinity-1 (FTIR- 8400S-Kyoto-Japan), and expressed in wave number (cm^{-1}), using potassium bromide discs. NMR spectra were recorded on Bruker Avance III 400 MHz high performance digital FT-NMR spectrophotometer (^1H: 400, ^{13}C: 100 MHz) in chloroform-*d*, using TMS as an internal standard. Chemical shifts were expressed in δ units and were related to that of the solvents. All the reactions were followed up by TLC using silica gel F254 plates (Merck), using chloroform: methanol 9:1 or chloroform as eluting system and were visualized by UV-lamp. Compounds **2** [15] and **5a-j** [17,18] were prepared according to reported methods.

4.2. Chemistry

*4.2.1. General procedure for the synthesis of compounds (**3**, **4**)*

A mixture of **2** (10 mmol, 2.65 g), 3/4-methoxyaniline (12 mmol, 1.47 g) and freshly prepared anhydrous sodium acetate (0.3 g) in glacial acetic acid (10 mL) was heated in a boiling water bath for 3 h. The crystalline product separated on cooling was filtered off, washed with water, dried, and recrystallized from ethanol.

*4.2.1.1. 4-(4-Hydroxybenzylidene)-1-(3-methoxyphenyl)-2-phenyl-1H-imidazol-5(4H)-one (**3**).* Yield 87%; m.p. 252–253 °C; IR (KBr, cm^{-1}: 3327 (OH), 3061 (aromatic CH), 2954 (CH aliphatic), 1685 (C=O);^{1}H NMR: δ 3.72 (s, 3H, OCH_3), 6.79 (d, 2H, aromatic H, J = 7.72 Hz), 6.81 (s, 1H, aromatic H), 6.88 (d, 1H, aromatic H, J = 8.60 Hz), 6.91 (s, ex, 1H, OH), 6.99, 7.01 (dd, 1H, J = 2.24, 8.30 Hz), 7.20 (s, 1H, CH), 7.33–7.39 (m, 3H, aromatic H), 7.46 (t, 1H, aromatic H, J = 7.36 Hz), 7.53 (d, 2H, aromatic H, J = 7.16 Hz), 8.24 (d, 2H, aromatic H, J = 8.68 Hz); ^{13}C NMR: δ 55.8, 114.2, 114.3, 116.6, 120.4, 125.6, 128.8, 129.1, 129.4, 130.4, 131.5, 135.2, 135.8, 136.3, 158.9, 160.1, 161.2, 169.9. Anal. Calcd. For $C_{23}H_{18}N_2O_3$ (370.12): C, 74.58; H, 4.90; N, 12.96%. Found: C, 74.69; H, 4.93; N, 7.65%.

*4.2.1.2. 4-(4-Hydroxybenzylidene)-1-(4-methoxyphenyl)-2-phenyl-1H-imidazol-5(4H)-one (**4**).* Yield 82%; m.p. 273–274 °C; IR (KBr, cm^{-1}): 3290 (OH), 3062 (aromatic CH), 2960 (CH aliphatic), 1689 (C=O); ^{1}H NMR: δ 3.76 (s, 3H, OCH_3), 6.89 (d, 2H, aromatic H, J = 8.80 Hz), 6.96 (d, 2H, aromatic H, J = 6.76 Hz), 7.16 (d, 2H, aromatic H, J = 6.96 Hz), 7.18 (s, 1H, CH), 7.34–7.38 (m, 3H, aromatic H), 7.51 (d, 2H, aromatic H, J = 8.52 Hz), 8.24 (d, 2H, aromatic H, J = 8.76 Hz), 10.49 (s, ex, 1H, OH); ^{13}C NMR: δ 55.8, 114.9, 116.4, 127.8, 128.1, 128.8, 129.2, 129.4, 129.5, 131.5, 135.2, 136.1, 159.4, 160.7, 170.4; Anal. Calcd. For $C_{23}H_{18}N_2O_3$ (370.12): C, 74.58; H, 4.90; N, 12.96%. Found: C, 74.72; H, 4.87; N, 7.68%.

*4.2.2. General procedure for the synthesis of compounds (**6a–j**, **7a–j**)*

Potassium carbonate (10 mmol, 1.38 g) was added to a solution of compound **3** or **4** (10 mmol, 3.70 g) in dry DMF (15 mL) and stirred at room temperature for 0.5 h. Then, the appropriate 2-chloro-*N*-un/substituted phenylacetamide **5a–j** (10 mmol) was added and heated at 100 °C for 8 h. The reaction mixture was poured onto ice/water and the precipitated solid was filtered off, washed with water, dried and crystallized from ethanol.

*4.2.2.1. 2-(4-((1-(3-Methoxyphenyl)-5-oxo-2-phenyl-1H-imidazol-4(5H)-ylidene)methyl) phenoxy) -N-phenylacetamide (**6a**).* Yield: 82%; m.p. 220–221 °C; IR (KBr, cm^{-1}): 3332 (NH), 3061 (aromatic CH), 2924 (CH aliphatic), 1706, 1685 (C=O); ^{1}H NMR: δ 3.78 (s, 3H, OCH_3), 4.71 (s, 2H, CH_2), 6.74 (s, 1H, aromatic H), 6.76–6.78 (m, 1H, aromatic H), 6.94, 6.96 (dd, 1H, aromatic H, J = 2.16, 8.40 Hz), 7.09 (d, 2H, aromatic H, J = 8.80 Hz), 7.12 (t, 1H, aromatic H, J = 7.40 Hz), 7.28 (s, 1H, CH), 7.31–7.41 (m, 5H, aromatic H), 7.44 (t, 1H, J = 7.44 Hz), 7.61 (d, 4H, aromatic H, J = 8.40 Hz), 8.25 (s, ex, 1H, NH), 8.34 (d, 2H, aromatic H, J = 8.40 Hz); ^{13}C NMR: δ 55.4, 67.5, 113.0, 114.3, 115.1, 119.6, 120.1, 120.2, 125.0, 128.3, 128.4, 128.6, 129.0, 129.1, 130.0, 131.3, 134.7, 135.7, 136.6, 137.6, 158.5, 160.0, 160.2, 165.6, 170.4. Anal. Calcd. For $C_{31}H_{25}N_3O_4$ (503.55): C, 73.94; H, 5.00; N, 8.34%. Found: C, 74.02; H, 5.04; N, 8.42%.

*4.2.2.2. 2-(4-((1-(3-Methoxyphenyl)-5-oxo-2-phenyl-1,5-dihydro-4H-imidazol-4-ylidene) methyl)phenoxy)-N-(o-tolyl)acetamide (**6b**).* Yield: 65%; m.p. 159–161 °C; IR (KBr, cm^{-1}): 3410 (NH), 3050 (aromatic CH), 2924 (CH aliphatic), 1728, 1693 (C=O); ^{1}H NMR: δ 2.38 (s, 3H, CH_3), 3.78 (s, 3H, OCH_3), 4.68 (s, 2H, CH_2), 6.74 (s, 1H, aromatic H), 6.76–6.78 (m, 1H, aromatic H), 6.93, 6.95 (dd, 1H, aromatic H, J = 2.24, 8.32 Hz), 6.99 (t, 1H, aromatic H, J = 7.50 Hz), 7.08 (d, 2H, aromatic H, J = 8.80 Hz), 7.26 (s, 1H, CH), 7.24–7.48 (m, 7H, aromatic H), 7.61 (d, 2H, aromatic H, J = 7.32 Hz), 8.23 (s, ex, 1H, NH), 8.33 (d, 2H, aromatic H, J = 8.76 Hz); ^{13}C NMR: δ 21.4, 55.4, 67.5, 113.0,114.3, 115.1, 117.3, 120.7,120.8, 125.8, 128.3, 128.4, 128.8, 128.9, 129.0, 129.1, 130.0, 131.3, 134.7, 135.7, 136.6, 137.5, 139.1, 158.5,159.9,160.2,165.6, 170.4. Anal. Calcd. For $C_{32}H_{27}N_3O_4$ (517.58): C, 74.26; H, 5.26; N, 8.12%. Found: C, 74.34; H, 5.25; N, 8.22%.

*4.2.2.3. 2-(4-((1-(3-Methoxyphenyl)-5-oxo-2-phenyl-1,5-dihydro-4H-imidazol-4-ylidene) methyl)phenoxy)-N-(m-tolyl)acetamide (**6c**).* Yield: 86%; m.p. 167–169 °C; IR (KBr, cm^{-1}): 3414 (NH), 3066 (aromatic CH), 2924 (CH aliphatic), 1724, 1701(C=O); ^{1}H NMR: δ 2.27 (s, 3H, CH_3), 3.79 (s, 3H, OCH_3), 4.76 (s, 2H, CH_2), 6.75 (s, 1H, aromatic H), 6.77–6.78 (m, 1H, aromatic H), 6.94, 6.96 (dd, 1H, aromatic H, J = 1.96, 8.44 Hz), 7.10 (d, 2H, aromatic H, J = 8.80 Hz), 7.13 (d, 1H, aromatic H, J = 7.72 Hz), 7.22 (t, 1H, aromatic H, J = 7.60 Hz), 7.28 (s, 1H, CH), 7.32 (s,1H, aromatic H), 7.33–7.37 (m, 3H, aromatic H), 7.44 (t, 1H, aromatic H, J = 7.44 Hz), 7.61 (d, 2H, aromatic H, J = 7.32 Hz), 7.98 (d, 1H, aromatic H, J = 7.92 Hz), 8.22 (s, ex, 1H, NH), 8.35 (d, 2H, aromatic H, J = 8.80 Hz); ^{13}C NMR: δ 17.4, 55.4, 67.5, 113.0, 114.3, 115.0, 119.6, 122.4, 125.5, 126.9, 128.3, 128.6, 128.8, 129.0, 129.1, 130.0, 130.5, 131.3, 134.7, 135.7, 137.5, 158.5, 160.0, 160.2, 165.6, 170.4. Anal. Calcd. For $C_{32}H_{27}N_3O_4$ (517.58): C, 74.26; H, 5.26; N, 8.12%. Found: C, 74.35; H, 5.29; N, 8.25%.

*4.2.2.4. 2-(4-((1-(3-Methoxyphenyl)-5-oxo-2-phenyl-1,5-dihydro-4H-imidazol-4-ylidene) methyl)phenoxy)-N-(p-tolyl)acetamide (**6d**).* Yield: 84%; m.p. 151–153 °C; IR (KBr, cm^{-1}): 3323 (NH), 3050 (aromatic CH), 2922 (CH aliphatic), 1710, 1683 (C=O); ^{1}H NMR: δ 2.36 (s, 3H, CH_3), 3.78 (s, 3H, OCH_3), 4.70 (s, 2H, CH_2), 6.74 (s, 1H, aromatic H), 6.76–6.78 (m, 1H, aromatic H), 6.94, 6.96 (dd, 1H, aromatic H, J = 2.08, 8.32 Hz), 7.09 (d, 2H, aromatic H, J = 8.80 Hz), 7.18 (d, 2H, aromatic H, J = 8.24 Hz), 7.28 (s, 1H, CH), 7.33–7.38 (m, 3H, aromatic H), 7.44–7.50 (m, 3H, aromatic H), 7.61 (d, 2H, aromatic H, J = 7.32 Hz), 8.19 (s, ex, 1H, NH), 8.34 (d, 2H, aromatic H, J = 8.76 Hz); ^{13}C NMR: δ 20.9, 55.4, 67.5, 113.0, 114.3, 115.1, 119.6, 120.1, 128.3, 128.4, 128.8, 129.0, 129.1, 129.6, 130.0, 131.3, 134.1, 134.7, 135.7, 137.5, 158.5, 160.0, 160.2, 165.5, 170.4. Anal. Calcd. For $C_{32}H_{27}N_3O_4$ (517.58): C, 74.26; H, 5.26; N, 8.12%. Found: C, 74.39; H, 5.30; N, 8.25%.

*4.2.2.5. N-(2-Methoxyphenyl)-2-(4-((1-(3-methoxyphenyl)-5-oxo-2-phenyl-1,5-dihydro-4H-imidazol-4-ylidene)methyl)phenoxy)acetamide (**6e**).* Yield: 76%; m.p. 172–174 °C -; IR (KBr, cm^{-1}): 3402 (NH), 3060 (aromatic CH), 2924 (CH aliphatic), 1710, 1683 (C=O); ^{1}H NMR: δ 3.78 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 4.72 (s, 2H, CH_2), 6.75 (s, 1H, aromatic H), 6.77–6.78 (m, 1H, aromatic H), 6.91–6.96 (m, 3H, aromatic H), 6.99 (t, 1H, aromatic H, J = 7.52 Hz), 7.10 (d, 2H, aromatic H, J = 8.84 Hz), 7.28 (s, 1H, CH), 7.31–7.37 (m, 3H, aromatic H), 7.44 (t, 1H, J = 7.44 Hz), 7.61 (d, 2H, aromatic H, J = 7.48 Hz), 8.34 (d, 2H, aromatic H, J = 8.76 Hz), 8.41 (d, 1H, aromatic H, J = 7.92 Hz), 8.96 (s, ex, 1H, NH); ^{13}C NMR: δ 55.4, 55.9, 67.6, 110.1, 113.0, 114.3, 115.2, 119.6, 120.0, 121.1, 124.4, 126.6, 128.3, 128.5, 128.8, 129.1, 130.0, 131.3, 134.7, 135.7, 137.4, 148.3, 158.7, 159.9, 160.2, 165.4, 170.4. Anal. Calcd. For $C_{32}H_{27}N_3O_5$ (533.58): C, 72.03; H, 5.10; N, 7.88%. Found: C, 72.19; H, 5.07; N, 8.02%.

*4.2.2.6. N-(3-Methoxyphenyl)-2-(4-((1-(3-methoxyphenyl)-5-oxo-2-phenyl-1,5-dihydro-4H-imidazol-4-ylidene)methyl)phenoxy)acetamide (**6f**).* Yield: 87%; m.p. 137–140 °C; IR (KBr, cm^{-1}): 3383 (NH), 3062 (aromatic CH), 2924 (CH aliphatic), 1706, 1676 (C=O); ^{1}H NMR: δ 3.78 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 4.70 (s, 2H, CH_2), 6.73–6.77 (m, 3H, aromatic H), 6.94, 6.96 (dd, 1H, aromatic H, J = 2.20, 8.40 Hz), 7.08–7.11 (m, 3H, aromatic H), 7.25 (s, 1H,

aromatic H), 7.28 (s, 1H, CH), 7.33–7.37 (m, 4H, aromatic H), 7.44 (t, 1H, $J = 7.40$ Hz), 7.61 (d, 2H, aromatic H, $J = 7.32$ Hz), 8.24 (s, ex, 1H, NH), 8.34 (d, 2H, aromatic H, $J = 8.76$ Hz); ^{13}C NMR: δ 55.3, 55.4, 67.5, 105.9, 110.8, 112.3, 113.0, 114.3, 115.1, 119.6, 128.3, 128.4, 128.8, 129.0, 129.1, 129.8, 130.0, 131.3, 134.7, 135.7, 137.5, 137.8, 158.5, 160.0, 160.2, 160.3, 165.6, 170.4. Anal. Calcd. For $C_{32}H_{27}N_3O_5$ (533.58): C, 72.03; H, 5.10; N, 7.88%. Found: C, 72.15; H, 5.09; N, 8.02%.

*4.2.2.7. N-(4-Methoxyphenyl)-2-(4-((1-(3-methoxyphenyl)-5-oxo-2-phenyl-1,5-dihydro-4H-imidazol-4-ylidene)methyl)phenoxy)acetamide (**6g**).* Yield: 72%; m.p. 204–206 °C; IR (KBr, cm^{-1}): 3361 (NH), 3055 (aromatic CH), 2927 (CH aliphatic), 1714, 1683 (C=O); ^{1}H NMR: δ 3.83 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 4.70 (s, 2H, CH_2), 6.74 (s, 1H, aromatic H), 6.76–6.79 (m, 1H, aromatic H), 6.91–6.96 (m, 3H, aromatic H), 7.09 (d, 2H, aromatic H, $J = 8.48$ Hz), 7.28 (s, 1H, CH), 7.31–7.37 (m, 3H, aromatic H), 7.46 (d, 1H, aromatic H, $J = 7.76$ Hz), 7.50 (d, 2H, aromatic H, $J = 8.60$ Hz), 7.61 (d, 2H, aromatic H, $J = 7.60$ Hz), 8.16 (s, ex, 1H, NH), 8.34 (d, 2H, aromatic H, $J = 8.52$ Hz); ^{13}C NMR: δ 55.4, 55.5, 67.4, 113.0, 114.2, 115.1, 119.6, 122.0, 122.1, 128.3, 128.4, 128.8, 129.0, 129.1, 129.7, 130.0, 131.3, 134.7, 137.5, 156.9, 158.6, 160.0, 160.2, 160.3, 165.6, 170.4. Anal. Calcd. For $C_{32}H_{27}N_3O_5$ (533.58): C, 72.03; H, 5.10; N, 7.88%. Found: C, 72.13; H, 5.13; N, 7.96%.

*4.2.2.8. N-(2-Chlorophenyl)-2-(4-((1-(3-methoxyphenyl)-5-oxo-2-phenyl-1,5-dihydro-4H-imidazol-4-ylidene)methyl)phenoxy)acetamide (**6h**).* Yield: 78%; m.p. 162–164 °C; IR (KBr, cm^{-1}): 3381 (NH), 3066 (aromatic CH), 2935 (CH aliphatic), 1712, 1685 (C=O), 837 (C–Cl); ^{1}H NMR: δ 3.77 (s, 3H, OCH_3), 4.71 (s, 2H, CH_2), 6.74 (s, 1H, aromatic H), 6.76–6.78 (m, 1H, aromatic H), 6.93, 6.95 (dd, 1H, aromatic H, $J = 2.08$, 8.36 Hz), 7.07–7.10 (m, 3H, aromatic H), 7.28 (s, 1H, CH), 7.30–7.41 (m, 5H, aromatic H), 7.43 (t, 1H, aromatic H, $J = 7.40$ Hz), 7.61 (d, 2H, aromatic H, $J = 7.52$ Hz), 8.33 (d, 2H, aromatic H, $J = 8.72$ Hz), 8.46 (d, 1H, aromatic H, $J = 8.24$ Hz), 9.00 (s, ex, 1H, NH); ^{13}C NMR: δ 55.4, 67.4, 113.0, 114.2, 114.7, 119.6, 121.3, 123.1, 125.2, 127.8, 128.3, 128.4, 128.7, 129.0, 129.1, 130.0, 130.3, 131.0, 131.3, 134.7, 135.7, 137.5, 158.4, 159.9, 160.2, 165.7, 170.4. Anal. Calcd. For $C_{31}H_{24}ClN_3O_4$ (538.00): C, 69.21; H, 4.50; N, 7.81%. Found: C, 69.30; H, 4.53; N, 7.92%.

*4.2.2.9. N-(3-Chlorophenyl)-2-(4-((1-(3-methoxyphenyl)-5-oxo-2-phenyl-1,5-dihydro-4H-imidazol-4-ylidene)methyl)phenoxy)acetamide (**6i**).* Yield: 74%; m.p. 157–159 °C; IR (KBr, cm^{-1}): 3309 (NH), 3066 (aromatic CH), 2935 (CH aliphatic), 1710, 1685 (C=O), 839 (C–Cl); ^{1}H NMR: δ 3.77 (s, 3H, OCH_3), 4.68 (s, 2H, CH_2), 6.73 (s, 1H, aromatic H), 6.75–6.77 (m, 1H, aromatic H), 6.93, 6.95 (dd, 1H, aromatic H, $J = 2.12$, 8.12 Hz), 7.07 (d, 2H, aromatic H, $J = 8.76$ Hz), 7.14 (d, 1H, aromatic H, $J = 8.04$ Hz), 7.27 (s, 1H, CH), 7.28–7.29 (m, 3H, aromatic H), 7.31–7.37 (m, 2H, aromatic H), 7.44 (t, 1H, aromatic H, $J = 7.66$ Hz), 7.61 (d, 2H, aromatic H, $J = 7.48$ Hz), 7.74 (s, 1H, aromatic H), 8.32 (s, ex, 1H, NH), 8.34 (d, 2H, aromatic H, $J = 8.56$ Hz); ^{13}C NMR: δ 55.4, 67.4, 113.0, 114.2, 114.7, 115.1, 118.1, 119.6, 120.2, 125.0, 128.3, 128.4, 128.8, 129.1, 130.0, 130.1, 131.3, 134.7, 135.7, 137.6, 137.8, 158.4, 160.0, 160.2, 165.8, 170.4. Anal. Calcd. For $C_{31}H_{24}ClN_3O_4$ (538.00): C, 69.21; H, 4.50; N, 7.81%. Found: C, 69.33; H, 4.52; N, 7.95%.

*4.2.2.10. N-(4-Chlorophenyl)-2-(4-((1-(3-methoxyphenyl)-5-oxo-2-phenyl-1,5-dihydro-4H-imidazol-4-ylidene)methyl)phenoxy)acetamide (**6j**).* Yield: 75%; m.p. 215–217 °C; IR (KBr, cm^{-1}): 3369 (NH), 3060 (aromatic CH), 2935 (CH aliphatic), 1707, 1674 (C=O), 831 (C–Cl); ^{1}H NMR: δ 3.78 (s, 3H, OCH_3), 4.69 (s, 2H, CH_2), 6.74 (s, 1H, aromatic H), 6.76–6.77 (m, 1H, aromatic H), 6.93, 6.95 (dd, 1H, aromatic H, $J = 2.16$, 8.32 Hz), 7.07 (d, 2H, aromatic H, $J = 8.80$ Hz), 7.28 (s, 1H, CH), 7.30–7.37 (m, 5H, aromatic H), 7.44 (t, 1H, aromatic H, $J = 7.40$ Hz), 7.57 (d, 2H, aromatic H, $J = 8.76$ Hz), 7.61 (d, 2H, aromatic H, $J = 7.36$ Hz), 8.29 (s, ex, 1H, NH), 8.33 (d, 2H, aromatic H, $J = 8.56$ Hz); ^{13}C NMR: δ 55.4, 67.4, 113.0, 114.2, 114.7, 115.1, 119.6, 121.3, 121.4, 128.3, 128.4, 128.8, 129.1, 129.2, 130.0, 130.1, 131.3, 134.7, 135.3, 135.7, 137.6, 158.4, 160.0, 160.2, 165.7, 170.4. Anal. Calcd. For $C_{31}H_{24}ClN_3O_4$ (538.00): C, 69.21; H, 4.50; N, 7.81%. Found: C, 69.32; H, 4.53; N, 7.91%.

*4.2.2.11. 2-(4-((1-(4-Methoxyphenyl)-5-oxo-2-phenyl-1,5-dihydro-4H-imidazol-4-ylidene) methyl)phenoxy)-N-phenylacetamide (**7a**).* Yield: 70%; m.p. 229–230 °C-; IR (KBr, cm^{-1}): 3338 (NH), 3052 (aromatic CH), 2923 (CH aliphatic), 1710, 1689 (C=O); ^{1}H NMR: δ 3.86 (s, 3H, OCH_3), 4.71 (s, 2H, CH_2), 6.95 (d, 2H, aromatic H, $J = 8.88$ Hz), 7.09 (d, 2H, aromatic H, $J = 6.84$ Hz), 7.13 (d, 2H, aromatic H, $J = 6.84$ Hz), 7.19 (t, 1H, aromatic H, $J = 7.44$ Hz), 7.28 (s, 1H, CH), 7.31–7.48 (m, 5H, aromatic H), 7.61 (d, 4H, aromatic H, $J = 8.36$ Hz), 8.25 (s, ex, 1H, NH), 8.34 (d, 2H, aromatic H, $J = 8.76$ Hz); ^{13}C NMR: δ 55.5, 67.5, 114.7, 115.1, 120.2, 125.0, 127.3, 128.2, 128.3, 128.5, 128.9, 129.1, 129.2, 131.3, 134.7, 136.6, 137.6, 158.4, 159.4, 160.2, 165.6, 170.8. Anal. Calcd. For $C_{31}H_{25}N_3O_4$ (503.55): C, 73.94; H, 5.00; N, 8.34%. Found: C, 73.98; H, 4.97; N, 8.51%.

*4.2.2.12. 2-(4-((1-(4-Methoxyphenyl)-5-oxo-2-phenyl-1,5-dihydro-4H-imidazol-4-ylidene) methyl)phenoxy)-N-(o-tolyl)acetamide (**7b**).* Yield: 93%; m.p. 231–233 °C; IR (KBr, cm^{-1}): 3309 (NH), 3062 (aromatic CH), 2960 (CH aliphatic), 1716, 1697 (C=O); ^{1}H NMR: δ 2.27 (s, 3H, CH_3), 3.86 (s, 3H, OCH_3), 4.75 (s, 2H, CH_2), 6.95 (d, 2H, aromatic H, $J = 8.88$ Hz), 7.09–7.14 (m, 4H, aromatic H), 7.21 (t, 1H, aromatic H, $J = 7.28$ Hz), 7.27–7.37 (m, 5H, aromatic H and CH), 7.44 (t, 1H, aromatic H, $J = 7.40$ Hz), 7.61 (d, 2H, aromatic H, $J = 7.31$ Hz), 7.98 (d, 1H, aromatic H, $J = 7.91$ Hz), 8.23 (s, ex, 1H, NH), 8.35 (d, 2H, aromatic H, $J = 8.80$ Hz); ^{13}C NMR: δ 17.4, 55.5, 67.5, 114.7, 115.0, 122.4, 125.5, 126.9, 127.3, 128.1, 128.3, 128.5, 128.6, 128.9, 129.1, 129.2, 130.5, 131.3, 134.7, 134.8, 137.6, 158.4, 159.4, 160.2, 165.6, 170.8. Anal. Calcd. For $C_{32}H_{27}N_3O_4$ (517.58): C, 74.26; H, 5.26; N, 8.12%. Found: C, 74.35; H, 5.24; N, 8.27%.

*4.2.2.13. 2-(4-((1-(4-Methoxyphenyl)-5-oxo-2-phenyl-1,5-dihydro-4H-imidazol-4-ylidene) methyl)phenoxy)-N-(m-tolyl)acetamide (**7c**).* Yield: 87%; m.p. 239–241 °C -; IR (KBr, cm^{-1}): 3350 (NH), 3050 (aromatic CH), 2927 (CH aliphatic), 1710, 1678 (C=O); ^{1}H NMR: δ 2.39 (s, 3H, CH_3), 3.86 (s, 3H, OCH_3), 4.70 (s, 2H, CH_2), 6.95 (d, 2H, aromatic H, $J = 8.84$ Hz), 7.00 (d, 1H, aromatic H, $J = 7.96$ Hz), 7.09–7.13 (m, 4H, aromatic H), 7.28 (m, 1H, CH), 7.25–7.46 (m, 6H, aromatic H), 7.61 (d, 2H, aromatic H, $J = 7.40$ Hz), 8.19 (s, ex, 1H, NH), 8.34 (d, 2H, aromatic H, $J = 8.72$ Hz); ^{13}C NMR: δ 21.4, 55.5, 67.5, 114.7, 115.1, 117.3, 120.8, 125.8, 127.3, 128.2, 128.3, 128.5, 128.9, 129.0, 129.1, 129.2, 131.3, 134.7, 136.5, 137.6, 139.1, 158.5, 159.4, 160.2, 165.6, 170.8. Anal. Calcd. For $C_{32}H_{27}N_3O_4$ (517.58): C, 74.26; H, 5.26; N, 8.12%. Found: C, 74.33; H, 5.28; N, 8.25%.

*4.2.2.14. 2-(4-((1-(4-Methoxyphenyl)-5-oxo-2-phenyl-1,5-dihydro-4H-imidazol-4-ylidene) methyl)phenoxy)-N-(p-tolyl)acetamide (**7d**).* Yield: 80%; m.p. 244–246 °C; IR (KBr, cm^{-1}): 3361 (NH), 3051 (aromatic CH), 2918 (CH aliphatic), 1716, 1683 (C=O); ^{1}H NMR: δ 2.36 (s, 3H, CH_3), 3.85 (s, 3H, OCH_3), 4.69 (s, 2H, CH_2), 6.94 (d, 2H, aromatic H, $J = 8.84$ Hz), 7.01 (d, 2H, aromatic H, $J = 8.68$ Hz), 7.11 (d, 2H, aromatic H, $J = 7.68$ Hz), 7.18 (d, 2H, aromatic H, $J = 8.24$ Hz), 7.28 (s, 1H, CH), 7.30–7.35 (m, 3H, aromatic H), 7.48 (d, 2H, aromatic H, $J = 8.36$ Hz), 7.59 (d, 2H, aromatic H, $J = 7.64$ Hz), 8.21 (s, ex, 1H, NH), 8.33 (d, 2H, aromatic H, $J = 8.76$ Hz); ^{13}C NMR: δ 20.9, 55.4, 67.5, 114.6, 115.1, 120.3, 127.3, 128.2, 128.3, 128.5, 128.9, 129.2, 129.3, 129.6, 130.3, 130.8, 131.3, 134.7, 138.0, 158.2, 159.2, 159.4, 165.5, 170.9. Anal. Calcd. For $C_{32}H_{27}N_3O_4$ (517.58): C, 74.26; H, 5.26; N,

8.12%. Found: C, 74.41; H, 5.29; N, 8.19%.

*4.2.2.15. N-(2-Methoxyphenyl)-2-(4-((1-(4-methoxyphenyl)-5-oxo-2-phenyl-1,5-dihydro-4H-imidazol-4-ylidene)methyl)phenoxy)acetamide (**7e**).* Yield: 77%; m.p. 188–190 °C; IR (KBr, cm^{-1}): 3367 (NH), 3055 (aromatic CH), 2927 (CH aliphatic), 1710, 1689 (C=O); ^{1}H NMR: δ 3.85 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 4.72 (s, 2H, CH_2), 6.91–6.99 (m, 4H, aromatic H), 7.01–7.13 (m, 5H, aromatic H), 7.28 (s, 1H, CH), 7.30–7.47 (m, 3H, aromatic H), 7.61 (d, 2H, aromatic H, J = 7.48 Hz), 8.34 (d, 2H, aromatic H, J = 8.60 Hz), 8.41 (d, 1H, aromatic H, J = 7.52 Hz), 8.97 (s, ex, 1H, NH); ^{13}C NMR: δ 55.5, 55.7, 67.6, 110.1, 114.7, 115.1, 119.9, 121.1, 127.3, 128.3, 128.4, 128.5, 128.9, 129.1, 129.2, 131.0, 131.2, 134.7, 134.8, 137.5, 148.3, 158.7, 159.4, 160.0, 165.4, 170.9. Anal. Calcd. For $C_{32}H_{27}N_3O_5$ (533.58): C, 72.03; H, 5.10; N, 7.88%. Found: C, 72.09; H, 5.14; N, 7.96%.

*4.2.2.16. N-(3-Methoxyphenyl)-2-(4-((1-(4-methoxyphenyl)-5-oxo-2-phenyl-1,5-dihydro-4H-imidazol-4-ylidene)methyl)phenoxy)acetamide (**7f**).* Yield: 74%; m.p. 196–197 °C; IR (KBr, cm^{-1}): 3438 (NH), 3062 (aromatic CH), 2938 (CH aliphatic), 1720, 1654 (C=O); ^{1}H NMR: δ 3.82 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 4.67 (s, 2H, CH_2), 6.09 (s, ex, 1H, NH), 6.59 (d, 1H, aromatic H, J = 7.76 Hz), 6.75–6.78 (m, 3H, aromatic H), 6.94 (d, 2H, aromatic H, J = 8.48 Hz), 7.08 (d, 2H, aromatic H, J = 7.68 Hz), 7.25 (s, 1H, aromatic H), 7.28 (s, 1H, CH), 7.31–7.42 (m, 4H, aromatic H), 7.58 (d, 2H, aromatic H, J = 7.16 Hz), 8.31 (d, 2H, aromatic H, J = 8.76 Hz); ^{13}C NMR: δ 55.3, 55.5, 67.4, 105.9, 110.8, 112.3, 114.7, 115.9, 127.3, 128.2, 128.5, 128.6, 128.8, 129.1, 129.2, 130.2, 131.0, 134.7, 135.9, 137.8, 145.8, 158.5, 159.4, 160.2, 165.6, 170.9. Anal. Calcd. For $C_{32}H_{27}N_3O_5$ (533.58): C, 72.03; H, 5.10; N, 7.88%. Found: C, 72.11; H, 5.12; N, 7.99%.

*4.2.2.17. N-(4-Methoxyphenyl)-2-(4-((1-(4-methoxyphenyl)-5-oxo-2-phenyl-1,5-dihydro-4H-imidazol-4-ylidene)methyl)phenoxy)acetamide (**7g**).* Yield: 80%; m.p. 239–240 °C; IR (KBr, cm^{-1}): 3332 (NH), 3064 (aromatic CH), 2933 (CH aliphatic), 1712, 1699 (C=O); ^{1}H NMR: δ 3.84 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 4.70 (s, 2H, CH_2), 6.91 (d, 2H, aromatic H, J = 9.08 Hz), 6.95 (d, 2H, aromatic H, J = 8.48 Hz), 7.09 (d, 2H, aromatic H, J = 8.89 Hz), 7.17 (d, 2H, aromatic H, J = 8.84 Hz), 7.28 (s, 1H, aromatic H), 7.31–7.37 (m, 3H, aromatic H), 7.50 (d, 2H, aromatic H, J = 8.96 Hz), 7.61 (d, 2H, aromatic H, J = 7.60 Hz), 8.15 (s, ex, 1H, NH), 8.34 (d, 2H, aromatic H, J = 8.72 Hz); ^{13}C NMR: δ 55.5, 55.6, 67.4, 114.2, 114.7, 115.1, 122.1, 125.4, 127.3, 127.7, 128.2, 128.3128.6, 129.0, 129.2, 130.0, 131.3, 134.7, 134.8, 137.6, 149.2, 158.5, 159.4, 160.2, 165.6, 170.8. Anal. Calcd. For $C_{32}H_{27}N_3O_5$ (533.58): C, 72.03; H, 5.10; N, 7.88%. Found: C, 72.14; H, 5.15; N, 7.94%.

*4.2.2.18. N-(2-Chlorophenyl)-2-(4-((1-(4-methoxyphenyl)-5-oxo-2-phenyl-1,5-dihydro-4H-imidazol-4-ylidene)methyl)phenoxy)acetamide (**7h**).* Yield: 94%; m.p. 225–227 °C; IR (KBr, cm^{-1}): 3383 (NH), 3053 (aromatic CH), 2928 (CH aliphatic), 1714, 1710 (C=O), 833 (C–Cl); ^{1}H NMR: δ 3.85 (s, 3H, OCH_3), 4.74 (s, 2H, CH_2), 6.95 (d, 2H, aromatic H, J = 8.84 Hz), 7.09–7.13 (m, 5H, aromatic H), 7.28 (s, 1H, CH), 7.30 (t, 1H, aromatic H, J = 7.80 Hz), 7.37–7.45 (m, 4H, aromatic H), 7.61 (d, 2H, aromatic H, J = 7.40 Hz), 8.34 (d, 2H, aromatic H, J = 8.68 Hz), 8.47 (d, 1H, aromatic H, J = 8.20 Hz), 9.01 (s, ex, 1H, NH); ^{13}C NMR: δ 55.5, 67.4, 114.7, 115.1, 117.3, 121.3, 125.2, 127.8, 128.2, 128.3, 128.5, 128.9, 129.1, 129.2, 131.0, 131.2, 133.7, 134.5, 134.7, 137.6, 158.3, 159.3, 160.1, 165.7, 170.8. Anal. Calcd. For $C_{31}H_{24}ClN_3O_4$ (538.00): C, 69.21; H, 4.50; N, 7.81%. Found: C, 69.29; H, 4.51; N, 7.89%.

*4.2.2.19. N-(3-Chlorophenyl)-2-(4-((1-(4-methoxyphenyl)-5-oxo-2-phenyl-1,5-dihydro-4H-imidazol-4-ylidene)methyl)phenoxy)acetamide (**7i**).* Yield: 93%; m.p. 267–269 °C; IR (KBr, cm^{-1}): 3375 (NH), 3055 (aromatic CH), 2925 (CH aliphatic), 1708, 1681 (C=O), 832 (C–Cl); ^{1}H NMR: δ 3.86 (s, 3H, OCH_3), 4.71 (s, 2H, CH_2), 6.95 (d, 2H, aromatic H, J = 8.72 Hz), 7.08–7.09 (m, 4H, aromatic H), 7.16 (d, 1H, aromatic H, J = 7.96 Hz), 7.28 (s, 1H, CH), 7.31–7.38 (m, 3H, aromatic H), 7.44–7.48 (m, 2H, aromatic H), 7.61 (d, 2H, aromatic H, J = 7.48 Hz), 7.75 (s, 1H, aromatic H), 8.26 (s, ex, 1H, NH) 8.34 (d, 2H, aromatic H, J = 8.72 Hz); ^{13}C NMR: δ 55.5, 67.4, 114.7, 115.1, 118.1, 120.2, 125.1, 128.1, 128.3, 128.5, 129.2, 130.1, 131.3, 134.7, 134.8, 137.7, 137.8, 158.3, 159.4, 160.1, 165.7, 170.8. Anal. Calcd. For $C_{31}H_{24}ClN_3O_4$ (538.00): C, 69.21; H, 4.50; N, 7.81%. Found: C, 69.31; H, 4.50; N, 7.92%.

*4.2.2.20. N-(4-Chlorophenyl)-2-(4-((1-(4-methoxyphenyl)-5-oxo-2-phenyl-1,5-dihydro-4H-imidazol-4-ylidene)methyl)phenoxy)acetamide (**7j**).* Yield: 91%; m.p. 276–278 °C; IR (KBr, cm^{-1}): 3354 (NH), 3054 (aromatic CH), 2926 (CH aliphatic), 1714, 1699 (C=O), 831 (C–Cl); ^{1}H NMR: δ 3.86 (s, 3H, OCH_3), 4.70 (s, 2H, CH_2), 6.95 (d, 2H, aromatic H, J = 8.84 Hz), 7.08–7.13 (m, 4H, aromatic H), 7.28 (s, 1H, CH), 7.30–7.37 (m, 4H, aromatic H), 7.44 (t, 1H, aromatic H, J = 7.40 Hz), 7.57 (d, 2H, aromatic H, J = 8.72 Hz), 7.61 (d, 2H, aromatic H, J = 7.48 Hz), 8.24 (s, ex, 1H, NH) 8.32 (d, 2H, aromatic H, J = 8.72 Hz); ^{13}C NMR: δ 55.5, 67.4, 114.7, 115.1, 121.4, 127.3, 128.1, 128.3, 128.5, 128.9, 129.1, 129.2, 130.0, 131.3, 134.7, 135.2, 137.7, 158.3, 159.4, 160.3, 165.7, 170.8. Anal. Calcd. For $C_{31}H_{24}ClN_3O_4$ (538.00): C, 69.21; H, 4.50; N, 7.81%. Found: C, 69.34; H, 4.52; N, 7.94%.

4.3. Anticancer activity screening

4.3.1. In vitro kinase inhibition

All kinase reactions were performed using LanthaScreen Kinase activity assay kits (Invitrogen), following the manufacturer's instructions. In summary, kinase reactions were performed in a 10 μL volume in low-volume 384-well plates. Typically, Corning model 3676 (black) or 3673 (white) plates were used. The concentration of substrate [GFP-ATF2] in the assay was 400 nM, and the kinase reaction buffer consisted of 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM $MgCl_2$, and 1 mM EGTA, plus any additional additives that might be required for a specific kinase. Kinase reactions were allowed to proceed for 1 h at room temperature with substrate and ATP in the presence or absence of inhibitor before a 10 μL preparation of EDTA (20 mM) and Tb-labeled antibody [Tb-pAT F2(pThr71), 4 nM] in TR-FRET dilution buffer were added. The final concentration of the antibody in the assay well was 2 nM, and the final concentration of EDTA was 10 mM. The plate was allowed to incubate at room temperature for at least 30 min. The data were generated using a BMG Pherastar plate reader using the LanthaScreen™ filter block available from BMG. Inhibition studies were performed against a dilution series of tested compounds and IC_{50} values determined from the resulting inhibition curves.

4.3.2. MTT assay for antiproliferative activity

The cancer cell lines and Vero cells were trypsinized and washed with Ca^{2+}/Mg^{2+}-free PBS (pH 7.2). Cells were adjusted to 4×10^4 cells/ml with DMEM supplemented with 10% fetal calf serum (Hyclone, Logan, UT,USA) and plated (50 μL/well) in 96-well cell culture plate (Corning, Corning, NY, USA) overnight at 37 °C with 5% CO_2 and 95% humidity. Fifty microliters of serial 10-fold diluted sterile tested compounds were added to final concentrations of 0–100 μM. Culture medium was used as negative control. Cultures were incubated for 72 h. Supernatants were discarded, 20 μL/well of methylthiazolyldiphenyl-tetrazolium bromide (MTT) reagent (Promega, Madison, WI, USA) was added and incubated for 4 h at 37 °C with 5% CO_2. Sterile sodium dodecyl sulfate (10% v/v in PBS) was added (25 μL/well) and the plate was kept at room temperature

for 18 h before measuring optical density (OD) at 490 nm. DMEM was used as a blank control. Results from three separate experiments were recorded and the percentage of viable cells was calculated as [(O.D. of cell control−O.D. of treated cells)/(O.D. of cell control−O.D. of initiated cells)] × 100.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2015.03.008.

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