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Full Length Article

6-(2-Morpholinoethyl)-thiazolo[3,2-*a*]pyrimidin-5-one: A novel scaffold for the synthesis of potential PI3kα inhibitors

Ahmed R. Ali, Eman R. El-Bendary, Mariam A. Ghaly *, Ihsan A. Shehata

Department of Medicinal Chemistry, Faculty of Pharmacy, Mansoura University, Mansoura 35516, Egypt

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ABSTRACT

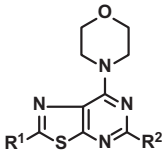
The present study involves the development of certain thiazolo[3,2-*a*]pyrimidin-5-ones linked through an ethylene bridge to various amines. The newly synthesized compounds **4–6(a–c)** were subjected to *in vitro* anticancer evaluation using NCI antitumor screening. The target compounds showed observed activity against *Renal UO-31* cancer cell line with cell growth promotion 52.72–64.52%. COMPARE analyses revealed compounds **4a** and **4b** exhibiting high correlation levels with rapamycin (mTOR inhibitor). Kinase assays were performed for compounds **4a** and **4b** on mTOR and structurally-related PI3Kα. They displayed moderate activity against PI3Kα with IC_{50} values of 120 and 151 μM, respectively. Compounds **4a** and **4b** could thus be considered as a promising leading scaffold for further development of potential PI3Kα inhibitors.

1. Introduction

Fused thiazoles are an important class of compounds which have attracted much attention to make use of their remarkable biological and pharmacological properties. Several publications have pointed to the antitumor activity of fused thiazole compounds e.g. thiazolo[5,4-*c*]pyridin-4(5*H*)-one [1], and thiazolo[3,2-*a*]pyrimidine derivatives [2–5].

Based on these findings, and in continuation to our efforts to synthesize biologically active compounds against cancer [6,7], we became interested in the evaluation of a series of thiazolo[3,2-*a*]pyrimidin-5-one derivatives with various substituents at the 3-, 6- and 7-positions, aiming at identifying potent anticancer agents.

The phosphoinositide 3-kinase (PI3K) pathway is an intracellular signaling pathway that has regulatory roles in cell survival, proliferation, and differentiation, and a critical role in tumorigenesis [8,9]. In cancer, multiple studies have investigated the therapeutic targeting of the PI3K pathway, and multiple inhibitors targeting PI3K and its isoforms, protein kinase B/AKT, and mammalian target of rapamycin (mTOR), have been developed [8]. A US patent reported thiazolopyrimidine compounds, substituted with a morpholine ring, of formulae I and II, with anticancer activity, and more specifically with PI3 kinase inhibitory activity. The compounds may inhibit tumor growth in mammals and may be useful for treating human cancer patients [10].



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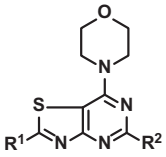
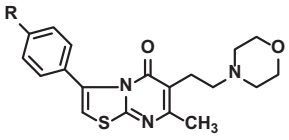


(II)

Taking the above mentioned compounds as lead, a part of the research undertaken here involved the combination of a morpholinoalkyl moiety with the thiazolo[3,2-*a*]pyrimidin-5-one series in a single molecular frame of the general structure (**A**) with the hope of finding interesting antitumor activity through inhibiting PI3K. A morpholine ring is often introduced to enhance water solubility. The group is attached through alkyl chain in order to protrude from the binding site and be exposed to the surrounding aqueous environment [11].



(A)

* Corresponding author.
E-mail addresses: mariamaghaly2@yahoo.com, mariamaghaly@mans.edu.eg (M.A. Ghaly).

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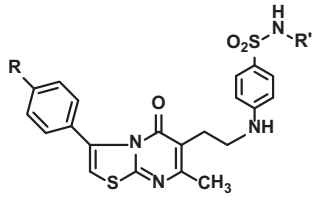
Moreover, it is well documented that aryl/heteroaryl sulfonamides, where the nitrogen of -SO_2NH_2 group is either free or substituted, exhibited substantial antitumor activity *in vitro* and/or *in vivo* [12–13]. The discovery of E-7010 [14] and vemurafenib (PLX4032) [15], fused heterocyclic compounds incorporating sulfonamide moiety, emphasized the role of sulfonamides as an important class of anticancer agents which interact with a wide range of different cellular targets. In addition, series of novel compounds containing benzenesulfonamide moiety and incorporating benzoquinones [16], quinazolin-2-ones [17] or coumarins [18] have revealed promising anticancer activities.

In view of the preceding information, it was envisaged to construct a system which combines both thiazolo[3,2-*a*]pyrimidin-5-ones and sulfonamides in a single molecular frame (**B**), in order to explore the additive effects towards their anticancer activities.



(B)

2. Results and discussion

2.1. Chemistry

A general approach to synthesize the designed compounds **4–6 (a–c)** is shown in Scheme 1. 2-Amino-4-arylthiazoles **2a–c** were prepared utilizing either phenacyl chloride or bromide according to a reported procedure [19] which is considered to be an easy, rapid and purification-free procedure. Thiourea was allowed to react with phenacyl halide at room temperature for 2–3 min to yield the corresponding arylthiazole. The reaction of **2a–c** with α -acetyl-γ-butyrolactone in phosphorus oxychloride afforded 6-(2-chloroethyl)-7-methyl-3-(un)substituted phenyl-5*H*-thiazolo[3,2-*a*]pyrimidin-5-ones **3a–c** without isolating the intermediates [20,21] in quantitative yields.

Heating compounds **3a–c** with morpholine, sulfacetamide, and sulfaguanidine in dry DMF in the presence of triethylamine gave the corresponding target compounds **4–6(a–c)** in moderate yields.

The structures of the synthesized compounds **4–6(a–c)** were confirmed by microanalyses and spectral data (IR, ^{1}H NMR, ^{13}C NMR and EI-MS) which showed full agreement with their structures. In the ^{1}H NMR spectra of compounds **4a–c**, the triplet signals of the morpholine ring protons resonated at the expected regions integrating for eight protons. In the ^{13}C NMR spectra of compound **4a**, new bands appeared at 53.16 and 66.15 ppm, attributed to ((CH_2)$_2$N-morpholine) and ((CH_2)$_2$O-morpholine), respectively. For compounds **5a–c**, the aromatic protons (—NH-**C$_6$H$_4$**-SO_2NH—) in the ^{1}H NMR spectra and the ^{13}C signals of CO**CH_3** and **CO**CH_3 in compound **5a** were observed at the expected regions. The ^{13}C signal for guanidine moiety in compound **6a** was observed at 159.23 ppm. The mass spectral data of the synthesized compounds **4–6(a–c)** displayed molecular ion peaks which confirmed their molecular weights.

2.2. Biological evaluation

2.2.1. In vitro anticancer screening

The target compounds **4–6(a–c)** were submitted to the National Cancer Institute (NCI) [22], Bethesda, Maryland, USA, under the Developmental Therapeutic Program (DTP). The operation of this screen utilizes 60 different human tumor cell lines, representing leukemia, melanoma and cancers of the lung, colon, brain, ovary, breast, prostate, and kidney. Structures are generally selected for screening based on their ability to add diversity to the NCI small molecule compound collection. Compounds with drug-like properties based on computer-aided design are to be prioritized in the NCI screening service. All compounds submitted to the NCI 60 cell screen were tested initially at a single high dose (10^{-5} M) in the full NCI 60 cell panel. The compounds were added at a single concentration (10^{-5} M) and the culture was incubated for 48 h. End point determinations were made with a protein binding dye, Sulforhodamine B [23–25].

The mean percentage growth percentages and the growth percentage with the most sensitive cell lines of all of the tested compounds over the full panel of cell lines are illustrated in Table 1.

In light of the NCI results, the following could be considered:

- Regarding the sensitivity against individual cell lines in Table 1, all target compounds **4–6(a–c)** showed observed low cell growth promotion against *Renal UO-31*cancer cell line with cell growth promotion varying from 52.72% to 64.52%.
- By comparing the results from different series, it was found that the introduction of sulfacetamide in compounds **5a–c** or sulfaguanidine in compounds **6a–c** instead of morpholine moiety in compounds **4a–c** proved to enhance the potency towards *Renal UO-31*cancer cell line and reduce potency towards *Leukemia SR* cancer cell line.
- It is worth mentioning that compounds **4–6(a)** exhibited increased potency towards *Leukemia SR* cancer cell line and reduced the potency towards *Non-Small Cell Lung HOP-92* cancer.

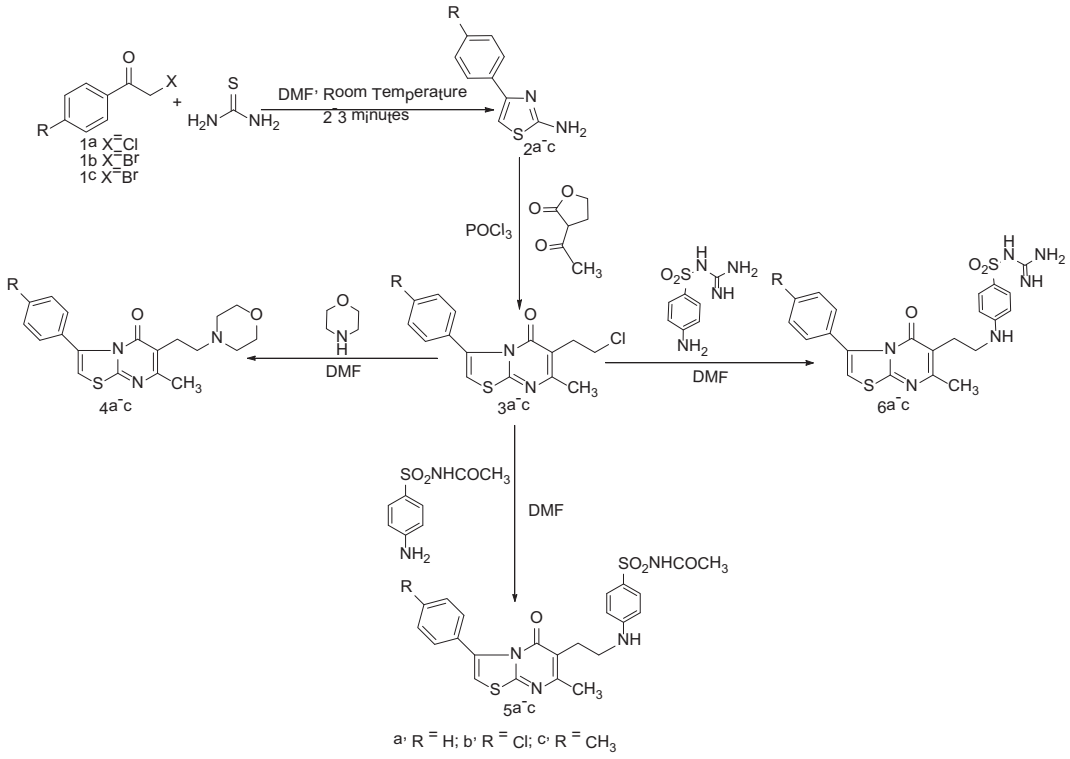
2.2.2. COMPARE analyses

We performed COMPARE [26] analyses for compounds **4–6(a–c)** in order to investigate the similarity of their cytotoxicity pattern (mean graph fingerprints) with those of known anticancer standard agents, NCI active synthetic compounds and natural extracts, which are present in public available databases. If the data pattern correlates well with that of compounds belonging to a standard agent database (Pearson's correlation coefficient (PCC > 0.6), the compound of interest may have the same mechanism of action [27,28]. On the other hand, if the activity pattern does not correlate with any standard agent, it is possible that the compound has a novel mechanism of action. Standard COMPARE analyses were performed at the GI_{50} level.

It was established that compounds **4a** and **4b** demonstrated high correlation levels with rapamycin (NSC S226080) with PCC values of 0.607 and 0.629, respectively. Considerable correlations between compounds **4c**, **5a**, **5b**, **5c**, **6a**, **6b,** and **6c**, and rapamycin were noted with PCC values of 0.523, 0.507, 0.496, 0.53, 0.448, 0.453, and 0.538, respectively. Such similarity in COMPARE results could indicate the resemblance in mechanisms of action with rapamycin. Rapamycin is reported to be mTOR inhibitor which is considered to be a key enzyme in regulation of cellular metabolism, growth, and proliferation [29,30].

2.2.3. Enzymatic screening

Compounds **4a** and **4b**, exhibiting the highest correlation levels with rapamycin (mTOR inhibitor), were selected to explore their biological targets at the molecular level. They were subjected to *in vitro* enzymatic screening against mTOR and structurally-related phosphatidylinositol 3-kinase-alpha (PI3Kα) to determine their potential affinity as inhibitors for those enzymes. A literature survey was conducted to search for similar compounds with previously reported mTOR or PI3Kα activities [31]. It was found that our



Scheme 1. Synthesis of compounds **4**–**6**(**a**–**c**).

Table 1
Mean percentage growth and screening data of the final compounds with the most sensitive cell lines represented as percent cell growth.

Comp. NO.	NSC code	Mean Percentage Growth	Range of Growth	Leukemia SR	Non-Small Cell Lung Cancer HOP-92	CNS Cancer SNB-75	Renal Cancer UO-31	Prostate Cancer PC-3	Breast Cancer MDA-MB-231/ATCC	Breast Cancer T-47D
4a	768162	96.24	77.60	70.28	84.40	71.77	58.46	81.37	79.26	84.65
4b	768163	96.91	84.42	81.39	81.60	84.59	57.53	87.50	78.32	76.61
4c	768178	96.88	77.94	81.78	70.44	73.56	64.52	79.48	79.66	88.13
5a	768164	97.82	73.08	75.05	82.43	82.31	55.24	84.74	81.15	78.49
5b	768165	99.22	78.84	91.86	80.43	87.92	57.26	86.69	83.53	82.39
5c	768186	95.98	78.77	89.43	62.58	75.52	62.94	73.44	73.92	84.24
6a	768166	96.18	79.86	81.71	79.02	87.97	52.72	83.75	79.61	89.62
6b	768167	95.54	71.13	92.00	66.69	80.01	53.92	80.27	79.32	84.05
6c	768187	99.94	90.94	95.05	70.69	73.13	61.63	85.53	83.98	85.76

synthesized compounds have several differences from those preceding studies. They contain a new central core thiazolo[3,2-*a*] pyrimidin-5-one with aryl group at the 3-position and having a morpholine ring that is essential for mTOR/PI3Kα inhibitory activity. Our compounds attained relative structural similarity with thiazolopyrimidinone series of selective PI3Kβ inhibitory activity [32]. In addition, the central scaffold is not directly attached to a morpholine moiety, but through ethylene bridge. It was reported that mTOR and PI3Kα inhibitors share common feature which is critical hydrogen bond to morpholine oxygen. This interaction is established with the backbone NH of Val2240 in mTOR, in an equivalent position to Val882 in PI3Kα [33]. Furthermore, enzyme docking studies indicated that a single amino acid difference exists between mTOR and PI3K in the vicinity of the hinge binding region. Modeling indicates that Phe961 of PI3K is too large to comfortably accommodate the 2,6-ethylene-bridged morpholine, causing displacement of the morpholine oxygen away from its hydrogen bonding partner, the backbone NH of Val882. However, in mTOR, the smaller amino acid substitution leucine (Phe961Leu) creates a deeper pocket, which accommodates the bridged morpholine without causing significant displacement relative to the corresponding morpholine-containing compounds. This causes increased selectivity for analogues containing 2,6-ethylene-bridged morpholine [34].

In addition, it was reported that PI3Kα inhibition will interfere with glucose homeostasis [35]. It was envisioned that compounds **4a** and **4b** having morpholine ring extending from the fused heterocyclic system will accomplish a selective inhibitor of mTOR that could display less side effects compared to a dual mTOR/PI3Kα inhibitor. Moreover, on literature survey, similar framework to our compounds was also observed in 3,4-dihydropyrazino[2,3-*b*] pyrazin-2(1*H*)-ones having ethyl bridge to tetrahydro-2*H*-pyran. They exhibited excellent mTOR potency and maintain selectivity over the related PI3Kα lipid kinase [36]. We wanted to extend our work to discover other scaffolds and report herein our findings with mTOR/PI3Kα inhibitory activity. In addition, those compounds showed compliance to Lipinski's rule of five and might have good oral bioavailability. Encouraged by those promising findings, kinase assays were performed at Reaction Biology Corporation at a single dose concentration of 100 μM over mTOR/PI3Kα

to evaluate the kinase inhibitory activity of the synthesized mor pholinoethyl-thiazolopyrimidinone. Regarding activity against mTOR, compounds **4a** and **4b** displayed low inhibitory activity at testing concentration of 100 μm. Meanwhile, they exhibited moderate activity against PI3Kα with IC_{50} values of 120 and 151 μm, respectively. Table 2 shows % inhibition and IC_{50} values of kinase activity as to the known dual mTOR/PI3Kα kinase inhibitor, PI-103, as a positive control.

We tried to investigate the possible causes for the decrease in the activity of screened compounds. A possible cause of the decrease in inhibitory activity of the screened compounds may be due to the non-coplanarity. Compounds **4a** and **4b** were energy minimized using MMFF94x and after that aligned using MOE.2009.10 [37]. It was found that a morpholine moiety, attached to thiazolopyrimidinone ring system through two carbon spacer, is not co-planar with the central core, Fig. 1. Previous case was reported with the same enzymes and was responsible for the decrease in activity. It was found that the inclusion of the tetrahydropyranyl scaffold instead of dihydropyranyl into pyrazolopyrimidine derivatives was associated with a great decrease in both mTOR and PI3K potency. This decline in activity was explained by the differing minimum energy conformation of these two cycles. The dihydropyran (DHP) ring was found to be co-planar with the pyrazolopyrimidine core, whereas the tetrahydropyran (THP) ring is rotated about 90° out-of-plane with the core [38].

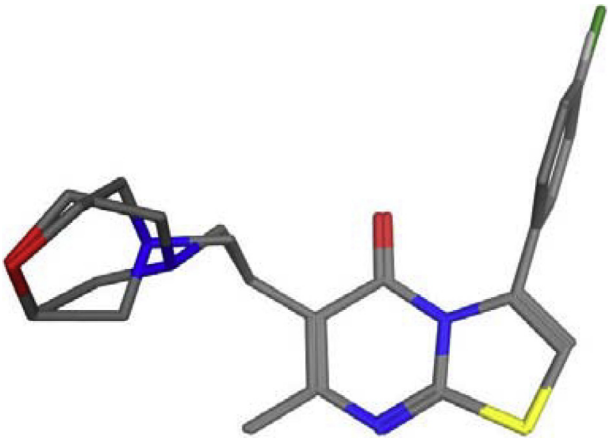


Fig. 1. Aligned energy minimized, using MMFF94x force field, structures for compounds 4a and 4b showing the non-coplanarity of the morpholine moiety with the central core.

3. Conclusion

On the basis of results obtained, it was found that the synthesized compounds showed observed activity against *Renal UO-31* cancer cell line with cell growth inhibition 36 to 48% at a dose of 10 μM. Compounds **4a** and **4b** displayed moderate activity against PI3Kα with IC_{50} values of 120 and 151 μM, respectively. Taking into account the *in vitro* anticancer evaluation and kinase assay, compounds **4a** and **4b** could be considered as promising leading skeleton for further development of more potent PI3kα inhibitors.

4. Experimental

4.1. General

All the reagents and solvents were obtained from commercial suppliers, and used without purification. TLC was monitored on Fluka silica gel TLC aluminum cards (0.2 mm thickness) with fluorescent indicator 254 nm using a mixture of petroleum ether/ethyl acetate in various proportions.

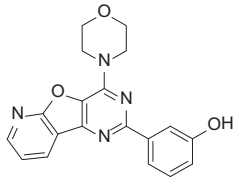
Melting points (°C) were recorded using a Fischer-Johns melting point apparatus and are uncorrected. The IR spectra (KBr) were recorded on Mattson 5000 FT-IR spectrophotometer (νin cm^{-1}) in the Microanalytical Unit, Faculty of Science, Mansoura University. ^1H and ^{13}C NMR for compounds **4a**, **5a** and **6a** were recorded on Bruker 500 MHz FT NMR spectrometer and NMR spectra for remaining compounds were carried out at the National Research Centre using a Varian Gemini 500 MHz FT NMR. Deuteriodimethylsulfoxide (DMSO d_6) was used as a solvent with the chemical shift being expressed in δ (ppm) and downfield from tetramethylsilane (TMS) as internal standard.

Electron impact mass spectra (EI-MS), recorded on a Shimadzu GC/MS QP-2010 Plus mass spectrometer, and elemental analyses (in accord with the calculated values) were carried out in the Microanalytical Unit, Faculty of Science, Cairo University. Anticancer evaluation was performed at National Cancer Institute (NCI), Bethesda, Maryland, USA.

4.2. General procedure for synthesis of 2-amino-4-(un)substituted phenylthiazoles (**2a–c**) [19]

A mixture of phenacyl halide (either phenacyl chloride or phenacyl bromide) **1a–c** (10 mmol) and thiourea (0.76 g, 10 mmol) in

Table 2
Inhibitory activity and IC_{50} values of compounds **4a** and **4b** against mTOR and PI3Kα.[a]

Compd No.	Structure	mTOR		PI3Kα	
		% Inhibition[b]	IC_{50} (μM)[c]	% Inhibition[b]	IC_{50} (μM)[c]
4a	Scheme 1	10	ND[d]	44	120
4b	Scheme 1	14	ND[d]	40	151
Control[e]		–	0.091	–	0.001

[a] Enzymatic assay was conducted by Reaction Biology Corporation (http://www.reactionbiology.com).
[b] Compounds were tested at 100 μM in the presence of 10 μM ATP. Inhibition is calculated by subtracting% enzyme activity from 100.
[c] Compounds were tested in 10-dose IC_{50} mode with 3-fold serial dilution starting at 100 μM. Control compound tested in 10-dose IC_{50} with 3-fold serial dilution starting at 10 μM.
[d] Not determined.
[e] PI-103 (http://www.nature.com/leu/journal/v22/n9/full/leu2008144a.html).

DMF (10 mL) was stirred at room temperature until completion of the reaction 2 and 3 min). The progress of the reaction was monitored by thin-layer chromatography. On completion of the reaction, the reaction mixture was poured onto crushed ice, treated with an excess of aqueous Na_2CO_3 solution. The precipitate was separated by filtration and washed with water. The product was pure enough (single spot on TLC) for all practical purposes.

*4.2.1. 4-Phenylthiazol-2-amine (**2a**)*

Yield: 95%; mp 146–148 °C (lit. mp 146 °C) [19]; IR (KBr, ν, cm^{-1}): 3436 (N—H), 1598, 1539, 1516 (C=N, C=C).

*4.2.2. 4-(4-Chlorophenyl)thiazol-2-amine (**2b**)*

Yield: 93%; mp 161–163 °C (lit. mp 161 °C) [39].

*4.2.3. 4-p-Tolylthiazol-2-amine (**2c**)*

Yield: 94%; mp 126–128 °C (lit. mp 126 °C) [39].

*4.3. Synthesis of 6-(2-chloroethyl)-7-methyl-3-(un)substituted phenyl 5H-thiazolo[3,2-a]pyrimidin-5-ones (**3a–c**) [20,21]*

α-Acetyl-γ-butyrolactone (1.08 mL, 10 mmol) was added slowly to a solution of 2-amino-4-arylthiazole **2a–c** (10 mmol) in phosphorous oxychloride (15 mL). The mixture was refluxed for 18 h, allowed to cool and poured onto crushed ice. The crude product was filtered, dried and crystallized from DMF/EtOH.

*4.3.1. 6-(2-Chloroethyl)-7-methyl-3-phenyl-5H-thiazolo[3,2-a] pyrimidin5-one (**3a**)*

Yield: 52%; mp 138–141 °C (lit. mp 136 °C) [20]; IR (KBr, ν, cm^{-1}): 3079 (CH aromatic), 2960, 2915 (CH aliphatic), 1651 (C=O), 1598, 1539, 1499 (C=N, C=C).

*4.3.2. 6-(2-Chloroethyl)-3-(4-chlorophenyl)-7-methyl-5H-thiazolo [3,2-a]pyrimidin-5-one (**3b**)*

Yield: 55%; mp 156–158 °C (lit. mp 161–163 °C) [20].

*4.3.3. 6-(2-Chloroethyl)-7-methyl-3-p-tolyl-5H-thiazolo[3,2-a] pyrimidin-5-one (**3c**)*

Yield: 48%; mp 188–190 °C (lit. mp 188 °C) [20].

*4.4. General procedure for the synthesis of compounds **4–6(a–c)***

An equimolar amount of 6-(2-chloroethyl)-7-methyl-3-(un)sub stitutedphenyl-5*H*-thiazolo[3,2-*a*]pyrimidin-5-one **3a–c** (10 mmol) and the appropriate amine (10 mmol) was heated at 90 °C in dry DMF (15 mL) containing triethylamine (2 mL) for 18 h. The reaction mixture was cooled to 20 °C and poured onto ice-water. The crude product was filtered, dried, and crystallized from DMF/EtOH to yield the desired compounds.

*4.4.1. 7-Methyl-6-(2-morpholinoethyl)-3-phenyl-5H-thiazolo[3,2-a] pyrimidin-5-one (**4a**)*

Yield: 35%; mp 140–142 °C; ^{1}H NMR (δ, ppm): 2.33 (t, 2H, —**CH$_2$**CH$_2$N—), 2.41 (s, 3H, —CH$_3$), 2.44 (t, 2H, —CH$_2$**CH$_2$**N—), 2.59 (t, 4H, (CH$_2$)$_2$N-morpholine), 3.55 (br s, 4H, (CH$_2$)$_2$O-morpholine), 7.37 (t, 2H, Ar-H), 7.42 (t, 1H, Ar-H), 7.75 (s, 1H, H-thiazole), 7.94 (d, 2H, Ar-H); ^{13}C NMR (δ, ppm, DMSO d_6): 21.90 (CH$_3$), 29.53 (CH$_2$-CH$_2$N), 53.16 ((CH$_2$)$_2$N-morpholine), 56.51 (CH$_2$CH$_2$N), 66.15 ((CH$_2$)$_2$O-morpholine), 102.44, 109.36, 125.78, 127.02, 128.68, 133.93, 137.13 (aromatic C), 154.82 (C7 of thiazolopyrimidine), 159.77 (C=O), 168.13 (—S—C(N)=N— of thiazolopyrimidine); EI-MS (70 eV) *m*/*z* (Rel. Int.): 355 (M$^+$, 24.35), 278 (2.28), 255 (8.80), 192 (4.40), 176 (15.23), 149 (5.31), 134 (20.51), 114 (7.76); Anal. for $C_{19}H_{21}N_3O_2S$ (355.45) C, H, N.

*4.4.2. 3-(4-Chlorophenyl)-7-methyl-6-(2-morpholinoethyl)-5H-thiazolo[3,2-a]pyrimi-din-5-one (**4b**)*

Yield: 40%; mp 134–136 °C; ^{1}H NMR (δ, ppm): 2.29 (t, 2H, —**CH$_2$**CH$_2$N—), 2.42 (s, 3H, —CH$_3$), 2.47 (t, 2H, –CH$_2$**CH$_2$**N—), 2.71 (t, 4H, (CH$_2$)$_2$N-morpholine), 3.53 (t, 4H, (CH$_2$)$_2$O-morpholine), 7.46 (d, 2H, Ar-H), 7.63 (s, 1H, H-thiazole), 7.86 (d, 2H, Ar-H); ^{13}C NMR (δ, ppm, DMSO d_6): 21.51 (CH$_3$), 25.65 (CH$_2$CH$_2$N), 52.78 ((CH$_2$)$_2$N-morpholine), 62.88 (CH$_2$CH$_2$N), 66.75 ((CH$_2$)$_2$O-morpholine), 111.72, 127.04, 130.93, 131.12, 131.16, 133.20 (aromatic C), 154.62 (C7 of thiazolopyrimidine), 159.25 (C3 of thiazolopyrimidine), 160.51 (C=O), 168.13 (—S—C(N)=N— of thiazolopyrimidine); EI-MS (70 eV) *m*/*z* (Rel. Int.): 391 (M$^+$+2, 0.83), 389 (M$^+$, 2.42), 278 (1.28), 275 (6.32), 223 (3.96), 210 (5.78), 168 (13.07), 114 (9.56); Anal. for $C_{19}H_{20}ClN_3O_2S$ (389.90) C, H, N.

*4.4.3. 7-Methyl-6-(2-morpholinoethyl)-3-p-tolyl-5H-thiazolo[3,2-a] pyrimidin-5-one (**4c**)*

Yield: 31%; mp 130–132 °C; ^{1}H NMR (δ, ppm): 2.30 (m, 5H, —**CH$_2$**CH$_2$N— and Ar-CH$_3$), 2.42 (s, 3H, —CH$_3$), 2.46 (t, 2H, —CH$_2$-**CH$_2$**N-), 2.69 (t, 4H, (CH$_2$)$_2$N-morpholine), 3.39 (t, 4H, (CH$_2$) $_2$O-morpholine), 7.18 (d, 2H, Ar-H), 7.23 (s, 1H, H-thiazole), 7.63 (d, 2H, Ar-H); ^{13}C NMR (δ, ppm, DMSO d_6): 20.87 (CH$_3$), 21.11 (CH$_3$), 29.53 (CH$_2$CH$_2$N), 53.14 ((CH$_2$)$_2$N-morpholine), 56.51 (CH$_2$CH$_2$N), 66.10 ((CH$_2$)$_2$O-morpholine), 100.57, 125.49, 127.64, 128.51, 129.06, 129.26, 137.13 (aromatic C), 154.82 (C7 of thiazolopyrimidine), 159.77 (C=O), 168.10 (—S—C(N)=N— of thiazolopyrimidine); EI-MS (70 eV) *m*/*z* (Rel. Int.): 369 (M$^+$, 14.37), 278 (12.23), 255 (14.17), 203 (17.48), 192 (19.81), 190 (95.73), 176 (12.62), 148 (32.43), 114 (16.31); Anal. for $C_{20}H_{23}N_3O_2S$ (369.48) C, H, N.

*4.4.4. N-(4-(2-(7-Methyl-5-oxo-3-phenyl-5H-thiazolo[3,2-a] pyrimidin-6-yl)ethylamino)-phenylsulfonyl)acetamide (**5a**)*

Yield: 38%; mp 102–104 °C; ^{1}H NMR (δ, ppm): 1.93 (s, 3H, —COCH$_3$), 2.34 (t, 2H, —**CH$_2$**CH$_2$NH—), 2.51 (s, 3H, —CH$_3$), 3.43 (t, 2H, —CH$_2$**CH$_2$**NH-), 5.88 (s, 1H, —CH$_2$CH$_2$**NH**-Ar), 5.99 (s, 1H, —ArSO$_2$**NH**—), 6.59 (d, 2H, NH-**Ar-H**-SO$_2$NH-), 7.38 (t, 2H, H-Ar-thiazole), 7.42 (t, 1H, H-Ar-thiazole), 7.52 (s, 1H, H-thiazole), 7.78 (d, 2H, NH-**Ar-H**-SO$_2$NH-), 7.94 (d, 2H, H-Ar-thiazole); ^{13}C NMR (δ, ppm, DMSO d_6): 21.45 (CH$_3$), 25.16 (COCH$_3$), 29.59 (CH$_2$CH$_2$N), 55.77 (CH$_2$CH$_2$N), 101.31, 109.04, 112.59, 125.67, 127.87, 128.39, 128.89, 131.91, 134.31, 137.15 (aromatic C), 148.80 (NH-aromatic C), 156.58 (C7 of thiazolopyrimidine), 162.27 (CO), 168.05 (—S—C(N)=N— of thiazolopyrimidine), 171.04 (COCH$_3$); EI-MS (70 eV) *m*/*z* (Rel. Int.): 482 (M$^+$, 0.20), 439 (0.09), 424 (0.19), 405 (0.04), 348 (0.05), 284 (1.50), 160 (0.96), 134 (8.45); Anal. for $C_{23}H_{22}N_4O_4S_2$ (482.58) C, H, N.

*4.4.5. N-(4-(2-(3-(4-Chlorophenyl)-7-methyl-5-oxo-5H-thiazolo[3,2-a]pyrimidin-6-yl)ethylamino)phenylsulfonyl)acetamide (**5b**)*

Yield: 43%; mp 126–128 °C; ^{1}H NMR (δ, ppm): 1.92 (s, 3H, —COCH$_3$), 2.31 (t, 2H, —**CH$_2$**CH$_2$NH—), 2.45 (s, 3H, —CH$_3$), 3.36 (t, 2H, —CH$_2$**CH$_2$**NH—), 5.72 (s, 1H, —CH$_2$CH$_2$**NH**-Ar), 5.90 (s, 1H, -ArSO$_2$**NH**—), 6.44 (d, 2H, NH-**Ar-H**-SO$_2$NH-), 7.38 (d, 2H, H-Ar-thiazole), 7.63 (s, 1H, H-thiazole), 7.71 (d, 2H, NH-**Ar-H**-SO$_2$NH—), 7.86 (d, 2H, H-Ar-thiazole); ^{13}C NMR (δ, ppm, DMSO d_6): 21.75 (CH$_3$), 26.50 (COCH$_3$), 29.54 (CH$_2$CH$_2$N), 59.19 (CH$_2$CH$_2$N), 102.33, 111.66, 112.56, 112.93, 127.05, 128.38, 131.04, 131.09, 133.14 (aromatic C), 147.47 (C3 of thiazolopyrimidine), 152.42 (NH-aromatic C), 159.27 (C7 of thiazolopyrimidine), 160.27 (C=O), 168.78 (—S—C(N)=N— of thiazolopyrimidine), 170.41 (COCH$_3$); EI-MS (70 eV) *m*/*z* (Rel. Int.): 518 (M$^+$+2, 1.07), 516 (M$^+$,

1.52), 473 (1.07), 458 (0.96), 405 (1.67), 348 (1.37), 318 (1.30), 210 (79.80), 194 (4.46), 168 (37.89); Anal. for $C_{23}H_{21}ClN_4O_4S_2$ (517.02) C, H, N.

*4.4.6. N-(4-(2-(7-Methyl-5-oxo-3-p-tolyl-5H-thiazolo[3,2-a] pyrimidin-6-yl)ethylamino)-phenylsulfonyl)acetamide (**5c**)*

Yield: 35%; mp 152–154 °C; ^{1}H NMR (δ, ppm): 1.92 (s, 3H, —COCH$_3$), 2.30 (m, 5H, —**CH$_2$**CH$_2$N- and Ar-CH$_3$), 2.47 (s, 3H, —CH$_3$), 3.32 (t, 2H, —CH$_2$**CH$_2$**NH—), 5.78 (s, 1H, —CH$_2$CH$_2$**NH**-Ar, D$_2$O exchangeable), 5.86 (s, 1H, -ArSO$_2$**NH**-, D$_2$O exchangeable), 6.55 (d, 2H, NH-**Ar-H**-SO$_2$NH-), 7.13 (d, 2H, H-Ar-thiazole), 7.20 (s, 1H, H-thiazole), 7.49 (d, 2H, NH-**Ar-H**-SO$_2$NH—), 7.64 (d, 2H, H-Ar-thiazole); ^{13}C NMR (δ, ppm, DMSO d_6): 20.86 (CH$_3$), 26.50 (CO**C**H$_3$), 30.78 (**C**H$_2$CH$_2$N), 35.81 (CH$_3$), 50.57 (CH$_2$**C**H$_2$N), 100.56, 110.59, 112.41, 112.54, 125.48, 127.62, 128.35, 129.04, 129.23 (aromatic C), 149.00 (C3 of thiazolopyrimidine), 149.90 (NH-aromatic C), 158.79 (C7 of thiazolopyrimidine), 162.33 (C=O), 168.09 (—S—C(N)=N— of thiazolopyrimidine), 170.41 (**C**OCH$_3$); EI-MS (70 eV) *m*/*z* (Rel. Int.): 496 (M$^+$, 15.32), 453 (48.65), 405 (63.06), 348 (63.06), 190 (42.34), 174 (72.97), 148 (25.23); Anal. for $C_{24}H_{24}N_4O_4S_2$ (496.60) C, H, N.

*4.4.7. N-Carbamimidoyl-4-(2-(7-methyl-5-oxo-3-phenyl-5H-thiazolo [3,2-a]pyrimidin-6-yl)ethylamino)benzenesulfonamide (**6a**)*

Yield: 41%; mp 134–136 °C; ^{1}H NMR (δ, ppm): 2.28 (t, 2H, —**CH$_2$**CH$_2$NH—), 2.51 (s, 3H, —CH$_3$), 3.46 (t, 2H, —CH$_2$**CH$_2$**NH—), 4.12 (s, 1H, -ArSO$_2$NH-C(**NH**)-), 4.33 (s, 2H, **H$_2$N**-C(NH)—), 6.05 (s, 1H, —CH$_2$CH$_2$**NH**-Ar), 6.58 (d, 2H, NH-**Ar-H**-SO$_2$NH—), 7.04 (s, 1H, -ArSO$_2$**NH**—), 7.37 (t, 2H, H-Ar-thiazole), 7.40 (t, 1H, H-Ar-thiazole), 7.63 (s, 1H, H-thiazole), 7.91 (d, 2H, NH-**Ar-H**-SO$_2$NH—), 7.94 (d, 2H, H-Ar-thiazole); ^{13}C NMR (δ, ppm, DMSO d_6): 21.49 (CH$_3$), 29.47 (**C**H$_2$CH$_2$N), 59.79 (CH$_2$**C**H$_2$N), 101.46, 109.98, 118.31, 125.63, 127.15, 128.61, 129.12, 130.63, 132.01, 137.15 (aromatic C), 148.80 (NH-aromatic C), 157.75 (C7 of thiazolopyrimidine), 159.23 (C(NH)NH$_2$), 161.30 (C=O), 168.17 (—S—C(N)=N— of thiazolopyrimidine); EI-MS (70 eV) *m*/*z* (Rel. Int.): 482 (M$^+$, 0.01), 284 (0.42), 241 (4.24), 238 (0.83), 189 (11.31), 176 (100); Anal. for $C_{22}H_{22}N_6O_3S_2$ (482.58) C, H, N.

*4.4.8. N-Carbamimidoyl-4-(2-(3-(4-chlorophenyl)-7-methyl-5-oxo-5H-thiazolo[3,2-a]pyrimidin-6-yl)ethylamino)benzenesulfonamide (**6b**)*

Yield: 49%; mp 148–150 °C; ^{1}H NMR (δ, ppm): 2.31 (t, 2H, —**CH$_2$**CH$_2$NH—), 2.46 (s, 3H, —CH$_3$), 3.05 (t, 2H, —CH$_2$**CH$_2$**NH—), 4.12 (s, 1H, -ArSO$_2$NH-C(**NH**)—), 4.53 (s, 2H, **H$_2$N**-C(NH)—), 5.99 (s, 1H, —CH$_2$CH$_2$**NH**-Ar), 6.54 (d, 2H, NH-**Ar-H**-SO$_2$NH—), 7.06 (s, 1H, -ArSO$_2$**NH**—), 7.38 (d, 2H, H-Ar-thiazole), 7.63 (s, 1H, H-thiazole), 7.78 (d, 2H, NH-**Ar-H**-SO$_2$NH—), 7.89 (d, 2H, H-Ar-thiazole); ^{13}C NMR (δ, ppm, DMSO d_6): 20.98 (CH$_3$), 29.53 (**C**H$_2$CH$_2$-N), 59.20 (CH$_2$**C**H$_2$N), 102.34, 108.65, 109.46, 111.68, 127.03, 127.24, 128.50, 128.80, 131.06 (aromatic C), 147.48 (C3 of thiazolopyrimidine), 148.59 (NH-aromatic C), 159.03 (C7 of thiazolopyrimidine), 159.41 (C(NH)NH$_2$), 160.21 (C=O), 168.36 (—S—C(N)=N— of thiazolopyrimidine); EI-MS (70 eV) *m*/*z* (Rel. Int.): 518 (M$^+$+2, 0.31), 516 (M$^+$, 0.27), 481 (0.26), 318 (0.34), 275 (0.59), 272 (0.37), 223 (1.57), 210 (100); Anal. for $C_{22}H_{21}ClN_6O_3S_2$ (517.02) C, H, N.

*4.4.9. N-Carbamimidoyl-4-(2-(7-methyl-5-oxo-3-p-tolyl-5H-thiazolo [3,2-a]pyrimidin-6-yl)ethylamino)benzenesulfonamide (**6c**)*

Yield: 35%; mp 168–170 °C; ^{1}H NMR (δ, ppm): 2.29 (m, 5H, —**CH$_2$**CH$_2$N- and Ar-CH$_3$), 2.40 (s, 3H, —CH$_3$), 3.32 (t, 2H, —CH$_2$**CH$_2$**-NH-), 4.10 (s, 1H, -ArSO$_2$NH-C(**NH**)-, D$_2$O exchangeable), 4.53 (s, 2H, **H$_2$N**-C(NH)-, D$_2$O exchangeable), 5.99 (s, 1H, —CH$_2$CH$_2$**NH**-Ar, D$_2$O exchangeable), 6.55 (d, 2H, NH-**Ar-H**-SO$_2$NH—), 6.98 (s, 1H, -ArSO$_2$**NH**—), 7.13 (d, 2H, H-Ar-thiazole), 7.20 (s, 1H, H-thiazole), 7.64 (d, 2H, H-Ar-thiazole), 7.79 (d, 2H, NH-**Ar-H**-SO$_2$NH—); ^{13}C NMR (δ, ppm, DMSO d_6): 20.81 (CH$_3$), 21.10 (CH$_3$), 30.78 (**C**H$_2$CH$_2$-N), 59.02 (CH$_2$**C**H$_2$N), 100.55, 112.30, 125.48, 125.57, 127.27, 128.35, 129.03, 129.22, 129.32, 137.15 (aromatic C), 150.00 (NH-aromatic C), 158.00 (C7 of thiazolopyrimidine), 158.60 (C(NH)NH$_2$), 162.33 (C=O), 168.08 (—S—C(N)=N— of thiazolopyrimidine); EI-MS (70 eV) *m*/*z* (Rel. Int.): 496 (M$^+$, 40.00), 481 (34.84), 255 (7.10), 252 (37.42), 203 (2.58), 190 (50.97); Anal. for $C_{23}H_{24}N_6O_3S_2$ (496.60) C, H, N.

4.5. Full NCI 60 cell panel in vitro anticancer assay

The synthesized compounds **4–6(a–c)** were subjected to the National Cancer Institute (NCI) *in vitro* disease-oriented human cells screening panel assay for *in vitro* antitumor activity according standard procedure which is previously reported [22–25].

4.6. Enzymatic screening

Enzymatic assays were performed at Reaction Biology Corporation using the HTRF (Homogenous Time-Resolved Fluorescence) assay platform [40]. Testing compounds were dissolved in 100% DMSO to 10 mM stock. The serial dilution was conducted by epMotion 5070 in DMSO. Compounds were tested in 10-dose IC_{50} mode with 3-fold serial dilution starting at 100 μM using PI-103 as positive control (20 μM as starting concentration). The reaction was carried out at 10 μM ATP concentration. The nonlinear regression to obtain the standard curve and IC_{50} values are performed using Graphpad Prism software.

4.6.1. mTOR kinase assay protocol

Base reaction buffer: 20 mM HEPES (pH 7.5), 10 mM $MgCl_2$, 1 mM EGTA, 0.02% Brij35, 0.02 mg/ml BSA, 0.1 mM Na_3VO_4, 2 mM DTT, 1% DMSO. Reaction Procedure: Substrate (4EBP1/E1F4EBP1) was prepared in freshly prepared base reaction buffer. Required cofactors were delivered to the substrate solution above as 2 mM final concentration in reaction. mTOR/FRAP1 kinase was delivered into the substrate solution and gently mixed till 300 nM final concentration in reaction. Compounds **4a** and **4b** were delivered in 100% DMSO into the kinase reaction mixture by Acoustic technology (Echo550; nanoliter range) and incubated for 20 min at room temperature. ^{33}P-ATP (specific activity 10 μCi/μl) was delivered into the reaction mixture to initiate the reaction. After that, compounds were incubated for 2 h at room temperature. Kinase activity was detected by filter-binding method.

4.6.2. PI3Kα (p110α/p85α) kinase assay protocol

Reaction buffer: HEPES 50 mM (pH 7.0), NaN_3 0.02%, BSA 0.01%, Orthovanadate 0.1 mM, 1% DMSO. Detection buffer: HEPES 10 mM (pH 7.0), BSA 0.02%, KF 0.16 M, EDTA 4 mM. Reaction Procedure: PIP2 substrate (10 μM) was prepared in freshly prepared reaction buffer. PI3Kα kinase was delivered into the substrate solution and gently mixed. Compounds **4a** and **4b** were delivered in 100% DMSO into the kinase reaction mixture by Acoustic technology (Echo550; nanolitter range), and then incubated for 10 min at room temperature. ATP was delivered into the reaction mixture to initiate the reaction and then incubated for 30 min at 30 °C. The reaction was quenched with stop solution followed by detection mixture, and incubation for overnight. HTRF was measured as follows: Ex = 320 nm, ratio of Em = 615 nm and Em = 665 nm.

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