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Full Length Article

[](http://crossmark.crossref.org/dialog/?doi=10.1016/j.ejbas.2018.02.001&domain=pdf)6-(2-Morpholinoethyl)-thiazolo[3,2-*a*]pyrimidin-5-one: A novel scaffold for the synthesis of potential PI3ka inhibitors

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# a r t i c l e i n f o

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PI3ka

# a b s t r a c t

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 anal- yses 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 PI3Ka. They

displayed moderate activity against PI3Ka with IC50 values of 120 and 151 lM, respectively. Compounds 4a and 4b could thus be considered as a promising leading scaffold for further development of potential PI3Ka inhibitors.

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1. Introduction

Fused thiazoles are an important class of compounds which have attracted much attention to make use of their remarkable bio- logical 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]](#_bookmark10), and thiazolo[3,2-*a*] pyrimidine derivatives [[2–5]](#_bookmark10).

Based on these findings, and in continuation to our efforts to

**R1**

**(I)**

**O N**

**R2**

**N**

**N**

**S N**

**R1**

**(II)**

**O N**

**R2**

**S**

**N**

**N N**

synthesize biologically active compounds against cancer [[6,7]](#_bookmark10), 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 intracellu- lar signaling pathway that has regulatory roles in cell survival, pro- liferation, and differentiation, and a critical role in tumorigenesis [[8,9]](#_bookmark11). 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]](#_bookmark11). A US patent reported thiazolopyrimidine compounds, substituted with a mor- pholine ring, of formulae I and II, with anticancer activity, and more specifically with PI3 kinase inhibitory activity. The com- pounds may inhibit tumor growth in mammals and may be useful

for treating human cancer patients [[10]](#_bookmark16).

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Taking the above mentioned compounds as lead, a part of the research undertaken here involved the combination of a morpholi- noalkyl 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]](#_bookmark17).

**R**



**O**

**O**

**N**

**S N**

**N**

**CH3**

**(A)**

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Moreover, it is well documented that aryl/heteroaryl sulfon- amides, where the nitrogen of -SO2NH2 group is either free or sub- stituted, exhibited substantial antitumor activity *in vitro* and/or *in vivo* [[12–13]](#_bookmark20). The discovery of E-7010 [[14]](#_bookmark22) and vemurafenib (PLX4032) [[15]](#_bookmark25), fused heterocyclic compounds incorporating sul- fonamide 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 com- pounds containing benzenesulfonamide moiety and incorporating benzoquinones [[16]](#_bookmark27), quinazolin-2-ones [[17]](#_bookmark28) or coumarins [[18]](#_bookmark31) have revealed promising anticancer activities.

In view of the preceding information, it was envisaged to con- struct 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.

**H O2SN R'**

**O**

**N**

**NH**

**S N CH3**

**R**

**R'=COCH , C(NH)NH**

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 proper- ties 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 concen- tration (10—5 M) and the culture was incubated for 48 h. End point determinations were made with a protein binding dye, Sulforho- damine B [[23–25]](#_bookmark12).

The mean percentage growth percentages and the growth per- centage with the most sensitive cell lines of all of the tested com- pounds over the full panel of cell lines are illustrated in [Table 1](#_bookmark2).

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

* Regarding the sensitivity against individual cell lines in [Table 1](#_bookmark2), 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

1. Results and discussion
   1. *Chemistry*

**3 2**

**(B)**

sulfaguanidine in compounds 6a–c instead of morpholine moi- ety in compounds 4a–c proved to enhance the potency towards *Renal UO-31*cancer cell line and reduce potency towards *Leuke- mia 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.

A general approach to synthesize the designed compounds 4–6 (a–c) is shown in [Scheme 1](#_bookmark1). 2-Amino-4-arylthiazoles 2a–c were prepared utilizing either phenacyl chloride or bromide according to a reported procedure [[19]](#_bookmark12) 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 a

-acetyl-c-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]](#_bookmark12) 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, 1H NMR, 13C NMR and EI-MS) which showed full agreement with their structures. In the 1H NMR spectra of compounds 4a–c, the triplet signals of the morpholine ring protons resonated at the expected regions integrat- ing for eight protons. In the 13C NMR spectra of compound 4a, new bands appeared at 53.16 and 66.15 ppm, attributed to ((CH2)2N- morpholine) and ((CH2)2O-morpholine), respectively. For com-

pounds 5a–c, the aromatic protons (ANH-C6H4-SO2NHA) in the 1H

NMR spectra and the 13C signals of COCH3 and COCH3 in compound 5a were observed at the expected regions. The 13C signal for guani- dine 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.

* 1. *Biological evaluation*
     1. *In vitro anticancer screening*

The target compounds 4–6(a–c) were submitted to the National Cancer Institute (NCI) [[22]](#_bookmark12), Bethesda, Maryland, USA, under the

* + 1. *COMPARE analyses*

We performed COMPARE [[26]](#_bookmark12) 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 stan- dard 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]](#_bookmark12). 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 per- formed at the GI50 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 rapa- mycin. Rapamycin is reported to be mTOR inhibitor which is con- sidered to be a key enzyme in regulation of cellular metabolism, growth, and proliferation [[29,30]](#_bookmark13).

* + 1. *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 (PI3Ka) to determine

their potential affinity as inhibitors for those enzymes. A literature survey was conducted to search for similar compounds with previ- ously reported mTOR or PI3Ka activities [[31]](#_bookmark14). It was found that our

R

O

* + - 1. 1a X=Cl

X S

+

H2N

DMF, Room Temperature NH2 2-3 minutes

N

* + - 1. - NH2

1b X=Br 1c X=Br

R R

O O O

N N

N

H

2a c

O O



O

N

O

CH3

Cl

H O2S N

NH2

NH2 R NH

H O2S N

O

N NH

NH2 NH

S N

4a-c

CH3

DMF

S N

3a c

-

CH3

S N

6a c

-

CH3

SO2NHCOCH3

DMF

NH2 R

SO2NHCOCH3

O

N NH

S N

-

5a c

CH3

a, R = H; b, R = Cl; c, R = CH3

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 pre- ceding 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/PI3Ka inhibitory activ- ity. Our compounds attained relative structural similarity with thi-

azolopyrimidinone series of selective PI3Kb inhibitory activity [[32]](#_bookmark15). In addition, the central scaffold is not directly attached to a mor- pholine moiety, but through ethylene bridge. It was reported that

mTOR and PI3Ka inhibitors share common feature which is critical

hydrogen bond to morpholine oxygen. This interaction is estab- lished with the backbone NH of Val2240 in mTOR, in an equivalent position to Val882 in PI3Ka [[33]](#_bookmark18). 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 dis- placement 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 corre- sponding morpholine-containing compounds. This causes increased selectivity for analogues containing 2,6-ethylene- bridged morpholine [[34]](#_bookmark19).

In addition, it was reported that PI3Ka inhibition will interfere

with glucose homeostasis [[35]](#_bookmark21). It was envisioned that compounds 4a and 4b having morpholine ring extending from the fused hete- rocyclic system will accomplish a selective inhibitor of mTOR that

could display less side effects compared to a dual mTOR/PI3Ka

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 PI3Ka lipid kinase [[36]](#_bookmark23). We wanted to extend

our work to discover other scaffolds and report herein our findings with mTOR/PI3Ka inhibitory activity. In addition, those com- pounds 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 Corpo- ration at a single dose concentration of 100 lM over mTOR/PI3Ka

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 lm. Meanwhile, they exhibited mod-

erate activity against PI3Ka with IC50 values of 120 and 151 lm, respectively. [Table 2](#_bookmark4) shows % inhibition and IC50 values of kinase activity as to the known dual mTOR/PI3Ka 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]](#_bookmark24). 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](#_bookmark3). Previous case was reported with the same enzymes and was responsible for the decrease in activity. It was found that the inclusion of the tetrahy- dropyranyl scaffold instead of dihydropyranyl into pyrazolopyrim- idine 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]](#_bookmark26).

1. Conclusion

On the basis of results obtained, it was found that the synthe- sized compounds showed observed activity against *Renal UO-31* cancer cell line with cell growth inhibition 36 to 48% at a dose of 10 lM. Compounds 4a and 4b displayed moderate activity against

PI3Ka with IC50 values of 120 and 151 mM, respectively. Taking into

account the *in vitro* anticancer evaluation and kinase assay, com- pounds 4a and 4b could be considered as promising leading skele- ton for further development of more potent PI3ka inhibitors.

1. Experimental
   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 fluo-

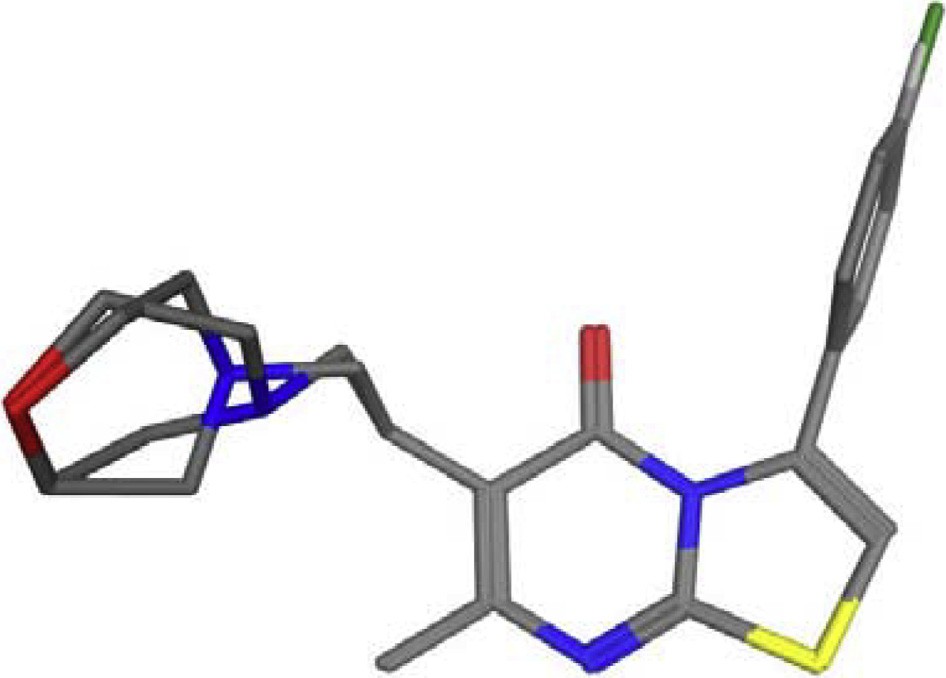


Fig. 1. Aligned energy minimized, using MMFF94x force field, structures for compounds 4a and 4b showing the non-coplanarity of the morpholine moiety with the central core.

rescent 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 (*m*in cm—1) in the Microanalytical Unit, Faculty of Science, Mansoura University.

1H and 13C 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. Deuteriodimethyl- sulfoxide (DMSO *d*6) was used as a solvent with the chemical shift being expressed in *d* (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. Anti- cancer evaluation was performed at National Cancer Institute (NCI), Bethesda, Maryland, USA.

* 1. *General procedure for synthesis of 2-amino-4-(un)substituted phenylthiazoles (2a–c)* [*[19]*](#_bookmark12)

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

Table 2

Inhibitory activity and IC50 values of compounds 4a and 4b against mTOR and PI3Ka.[a](#_bookmark5)

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Compd No. | Structure |  | mTOR |  |  | PI3Ka |  |  |
|  |  |  | % Inhibition[b](#_bookmark6) | IC50 (lM)[c](#_bookmark7) |  | % Inhibition[b](#_bookmark6) | IC50 (lM)[c](#_bookmark7) |
| 4a | [Scheme 1](#_bookmark1) |  | 10 | ND[d](#_bookmark8) |  | 44 | 120 |  |
| 4b | [Scheme 1](#_bookmark1) |  | 14 | ND[d](#_bookmark8) |  | 40 | 151 |  |
| Control[e](#_bookmark9) |  | O | – | 0.091 |  | – | 0.001 |  |
|  |  | N |  |  |  |  |  |  |

OH

O

N

N

N

a Enzymatic assay was conducted by Reaction Biology Corporation ([http://www.reactionbiology.com](http://www.reactionbiology.com/)).

b Compounds were tested at 100 lM in the presence of 10 lM ATP. Inhibition is calculated by subtracting% enzyme activity from 100.

c Compounds were tested in 10-dose IC50 mode with 3-fold serial dilution starting at 100 lM. Control compound tested in 10-dose IC50 with 3-fold serial dilution starting at 10 lM.

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 moni- tored by thin-layer chromatography. On completion of the reac- tion, the reaction mixture was poured onto crushed ice, treated with an excess of aqueous Na2CO3 solution. The precipitate was separated by filtration and washed with water. The product was pure enough (single spot on TLC) for all practical purposes.

* + 1. *4-Phenylthiazol-2-amine (2a)*

Yield: 95%; mp 146–148 °C (lit. mp 146 °C) [[19]](#_bookmark12); IR (KBr,

*m*, cm—1): 3436 (NAH), 1598, 1539, 1516 (C@N, C@C).

* + 1. *4-(4-Chlorophenyl)thiazol-2-amine (2b)*

Yield: 93%; mp 161–163 °C (lit. mp 161 °C) [[39]](#_bookmark29).

* + 1. *4-p-Tolylthiazol-2-amine (2c)*

Yield: 94%; mp 126–128 °C (lit. mp 126 °C) [[39]](#_bookmark29).

* 1. *Synthesis of 6-(2-chloroethyl)-7-methyl-3-(un)substituted phenyl 5H-thiazolo[3,2-a]pyrimidin-5-ones (3a–c)* [*[20,21]*](#_bookmark12)

a-Acetyl-c-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 pro- duct was filtered, dried and crystallized from DMF/EtOH.

* + 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]](#_bookmark12); IR (KBr, *m*,

cm—1): 3079 (CH aromatic), 2960, 2915 (CH aliphatic), 1651

(C@O), 1598, 1539, 1499 (C@N, C@C).

* + 1. *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]](#_bookmark12).

* + 1. *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]](#_bookmark12).

* 1. *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.

* + 1. *7-Methyl-6-(2-morpholinoethyl)-3-phenyl-5H-thiazolo[3,2-a] pyrimidin-5-one (4a)*

1

* + 1. *3-(4-Chlorophenyl)-7-methyl-6-(2-morpholinoethyl)-5H- thiazolo[3,2-a]pyrimi-din-5-one (4b)*

Yield: 40%; mp 134–136 °C; 1H NMR (*d*, ppm): 2.29 (t, 2H,

ACH2CH2NA), 2.42 (s, 3H, ACH3), 2.47 (t, 2H, –CH2CH2NA), 2.71

(t, 4H, (CH2)2N-morpholine), 3.53 (t, 4H, (CH2)2O-morpholine),

7.46 (d, 2H, Ar-H), 7.63 (s, 1H, H-thiazole), 7.86 (d, 2H, Ar-H); 13C NMR (*d*, ppm, DMSO *d*6): 21.51 (CH3), 25.65 (CH2CH2N), 52.78 ((CH2)2N-morpholine), 62.88 (CH2CH2N), 66.75 ((CH2)2O-

morpholine), 111.72, 127.04, 130.93, 131.12, 131.16, 133.20 (aro-

matic C), 154.62 (C7 of thiazolopyrimidine), 159.25 (C3 of thia- zolopyrimidine), 160.51 (C@O), 168.13 (ASAC(N)@NA 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 C19H20ClN3O2S (389.90) C, H, N.

* + 1. *7-Methyl-6-(2-morpholinoethyl)-3-p-tolyl-5H-thiazolo[3,2-a] pyrimidin-5-one (4c)*

Yield: 31%; mp 130–132 °C; 1H NMR (*d*, ppm): 2.30 (m, 5H,

ACH2CH2NA and Ar-CH3), 2.42 (s, 3H, ACH3), 2.46 (t, 2H, ACH2-

CH2N-), 2.69 (t, 4H, (CH2)2N-morpholine), 3.39 (t, 4H, (CH2) 2O-

morpholine), 7.18 (d, 2H, Ar-H), 7.23 (s, 1H, H-thiazole), 7.63 (d,

2H, Ar-H); 13C NMR (*d*, ppm, DMSO *d*6): 20.87 (CH3), 21.11 (CH3),

29.53 (CH2CH2N), 53.14 ((CH2)2N-morpholine), 56.51 (CH2CH2N),

66.10 ((CH2)2O-morpholine), 100.57, 125.49, 127.64, 128.51,

129.06, 129.26, 137.13 (aromatic C), 154.82 (C7 of thiazolopyrim- idine), 159.77 (C@O), 168.10 (ASAC(N)@NA of thiazolopyrim- idine); 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 C20H23N3O2S (369.48) C, H, N.

* + 1. *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; 1H NMR (*d*, ppm): 1.93 (s, 3H,

ACOCH3), 2.34 (t, 2H, ACH2CH2NHA), 2.51 (s, 3H, ACH3), 3.43 (t,

2H, ACH2CH2NH-), 5.88 (s, 1H, ACH2CH2NH-Ar), 5.99 (s, 1H,

AArSO2NHA), 6.59 (d, 2H, NH-Ar-H-SO2NH-), 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-SO2NH-), 7.94 (d, 2H, H-Ar-thiazole); 13C NMR

(*d*, ppm, DMSO *d*6): 21.45 (CH3), 25.16 (COCH3), 29.59 (CH2CH2N),

55.77 (CH2CH2N), 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 (ASAC(N)@NA of thiazolopyrimidine), 171.04 (COCH3);

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 C23H22N4O4S2 (482.58) C, H, N.

* + 1. *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; 1H NMR (*d*, ppm): 1.92 (s, 3H,

Yield: 35%; mp 140–142 °C; H NMR (*d*, ppm): 2.33 (t, 2H,

ACH2CH2NA), 2.41 (s, 3H, ACH3), 2.44 (t, 2H, ACH2CH2NA), 2.59

(t, 4H, (CH2)2N-morpholine), 3.55 (br s, 4H, (CH2)2O-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); 13C NMR (*d*, ppm, DMSO *d*6): 21.90 (CH3), 29.53 (CH2- CH N), 53.16 ((CH ) N-morpholine), 56.51 (CH CH N), 66.15

ACOCH3), 2.31 (t, 2H, ACH2CH2NHA), 2.45 (s, 3H, ACH3), 3.36 (t, 2H, ACH2CH2NHA), 5.72 (s, 1H, ACH2CH2NH-Ar), 5.90 (s, 1H,

-ArSO2NHA), 6.44 (d, 2H, NH-Ar-H-SO2NH-), 7.38 (d, 2H, H-Ar-

thiazole), 7.63 (s, 1H, H-thiazole), 7.71 (d, 2H, NH-Ar-H- SO2NHA), 7.86 (d, 2H, H-Ar-thiazole); 13C NMR (*d*, ppm, DMSO *d*6):

2 2 2 2 2

((CH2)2O-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 (ASAC(N)@NA 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 C19H21N3O2S (355.45) C, H, N.

21.75 (CH3), 26.50 (COCH3), 29.54 (CH2CH2N), 59.19 (CH2CH2N),

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 (ASAC(N)@NA of thiazolopyrimidine), 170.41

(COCH3); 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 C23H21ClN4O4S2 (517.02) C, H, N.

* + 1. *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; 1H NMR (*d*, ppm): 1.92 (s, 3H,

ACOCH3), 2.30 (m, 5H, ACH2CH2N- and Ar-CH3), 2.47 (s, 3H,

ACH3), 3.32 (t, 2H, ACH2CH2NHA), 5.78 (s, 1H, ACH2CH2NH-Ar,

D2O exchangeable), 5.86 (s, 1H, -ArSO2NH-, D2O exchangeable),

6.55 (d, 2H, NH-Ar-H-SO2NH-), 7.13 (d, 2H, H-Ar-thiazole), 7.20

(s, 1H, H-thiazole), 7.49 (d, 2H, NH-Ar-H-SO2NHA), 7.64 (d, 2H, H-Ar-thiazole); 13C NMR (*d*, ppm, DMSO *d*6): 20.86 (CH3), 26.50

(COCH3), 30.78 (CH2CH2N), 35.81 (CH3), 50.57 (CH2CH2N), 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 (ASAC(N)@NA of thiazolopyrimidine), 170.41 (COCH3);

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 C24H24N4O4S2 (496.60) C, H, N.

* + 1. *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; 1H NMR (*d*, ppm): 2.28 (t, 2H,

ACH2CH2NHA), 2.51 (s, 3H, ACH3), 3.46 (t, 2H, ACH2CH2NHA),

4.12 (s, 1H, -ArSO2NH-C(NH)-), 4.33 (s, 2H, H2N-C(NH)A), 6.05 (s,

1H, ACH2CH2NH-Ar), 6.58 (d, 2H, NH-Ar-H-SO2NHA), 7.04 (s, 1H,

-ArSO2NHA), 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 NHA), 7.94 (d, 2H, H-Ar-thiazole); 13C NMR (*d*, ppm, DMSO *d* ):

NH-), 4.10 (s, 1H, -ArSO2NH-C(NH)-, D2O exchangeable), 4.53 (s, 2H, H2N-C(NH)-, D2O exchangeable), 5.99 (s, 1H, ACH2CH2NH-Ar, D2O exchangeable), 6.55 (d, 2H, NH-Ar-H-SO2NHA), 6.98 (s, 1H,

-ArSO2NHA), 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-SO2NHA); 13C

NMR (*d*, ppm, DMSO *d*6): 20.81 (CH3), 21.10 (CH3), 30.78 (CH2CH2-

N), 59.02 (CH2CH2N), 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) NH2), 162.33 (C@O), 168.08 (ASAC(N)@NA 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 C23H24N6O3S2 (496.60) C, H, N.

* 1. *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]](#_bookmark12).

* 1. *Enzymatic screening*

Enzymatic assays were performed at Reaction Biology Corpora- tion using the HTRF (Homogenous Time-Resolved Fluorescence) assay platform [[40]](#_bookmark30). Testing compounds were dissolved in 100% DMSO to 10 mM stock. The serial dilution was conducted by epMo-

tion 5070 in DMSO. Compounds were tested in 10-dose IC50 mode with 3-fold serial dilution starting at 100 lM using PI-103 as pos- itive control (20 lM as starting concentration). The reaction was carried out at 10 lM ATP concentration. The nonlinear regression

2 6 to obtain the standard curve and IC50 values are performed using

21.49 (CH3), 29.47 (CH2CH2N), 59.79 (CH2CH2N), 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 thiazolopyrim- idine), 159.23 (C(NH)NH2), 161.30 (C@O), 168.17 (ASAC(N)@NA of

thiazolopyrimidine); EI-MS (70 eV) *m*/*z* (Rel. Int.): 482 (M+, 0.01),

Graphpad Prism software.

* + 1. *mTOR kinase assay protocol*

Base reaction buffer: 20 mM HEPES (pH 7.5), 10 mM MgCl2, 1 mM EGTA, 0.02% Brij35, 0.02 mg/ml BSA, 0.1 mM Na VO , 2 mM

284 (0.42), 241 (4.24), 238 (0.83), 189 (11.31), 176 (100); Anal. 3 4

for C22H22N6O3S2 (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; 1H NMR (*d*, ppm): 2.31 (t, 2H,

ACH2CH2NHA), 2.46 (s, 3H, ACH3), 3.05 (t, 2H, ACH2CH2NHA),

4.12 (s, 1H, -ArSO2NH-C(NH)A), 4.53 (s, 2H, H2N-C(NH)A), 5.99

(s, 1H, ACH2CH2NH-Ar), 6.54 (d, 2H, NH-Ar-H-SO2NHA), 7.06 (s,

1H, -ArSO2NHA), 7.38 (d, 2H, H-Ar-thiazole), 7.63 (s, 1H, H-

thiazole), 7.78 (d, 2H, NH-Ar-H-SO2NHA), 7.89 (d, 2H, H-Ar- thiazole); 13C NMR (*d*, ppm, DMSO *d*6): 20.98 (CH3), 29.53 (CH2CH2-

N), 59.20 (CH2CH2N), 102.34, 108.65, 109.46, 111.68, 127.03,

127.24, 128.50, 128.80, 131.06 (aromatic C), 147.48 (C3 of thia-

zolopyrimidine), 148.59 (NH-aromatic C), 159.03 (C7 of thia- zolopyrimidine), 159.41 (C(NH)NH2), 160.21 (C@O), 168.36

(ASAC(N)@NA 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 C22H21ClN6O3S2 (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; 1H NMR (*d*, ppm): 2.29 (m, 5H,

ACH2CH2N- and Ar-CH3), 2.40 (s, 3H, ACH3), 3.32 (t, 2H, ACH2CH2-

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 con- centration in reaction. Compounds 4a and 4b were delivered in 100% DMSO into the kinase reaction mixture by Acoustic technol-

ogy (Echo550; nanoliter range) and incubated for 20 min at room temperature. 33P-ATP (specific activity 10 lCi/ll) was delivered into the reaction mixture to initiate the reaction. After that, com- pounds were incubated for 2 h at room temperature. Kinase activ-

ity was detected by filter-binding method.

* + 1. *PI3Ka (p110a/p85a) kinase assay protocol*

Reaction buffer: HEPES 50 mM (pH 7.0), NaN3 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 lM) was prepared in freshly prepared reaction

buffer. PI3Ka 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 detec- tion 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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