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Synthesis and cytotoxic activity of novel 2,6-disubstituted-4-morpholinothieno[3,2-*d*]pyrimidines as potent anti-tumor agents

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Abstract

A series of 2,6-disubstituted-4-morpholinothieno[3,2-*d*]pyrimidine derivatives were synthesized and their cytotoxic activity against H460, HT-29, MDA-MB-231, U87MG and H1975 cancer cell lines were evaluated *in vitro*. Most of the target compounds exhibited moderate to excellent activity to the tested cell lines. The most promising compound **23** (0.84 μmol/L, 0.23 μmol/L, 2.52 μmol/L, 1.80 μmol/L) was 1.0, 2.9, 29.3 and 4.3 times more active than GDC-0941 (0.87 μmol/L, 0.66 μmol/L, 73.8 μmol/L, 7.77 μmol/L) against H460, HT-29, MDA-MB-231 and U87MG cell lines, respectively.

Keywords: 4-Morpholinothieno[3,2-*d*]pyrimidine; Synthesis; Cytotoxic activity

The derivatives of thienopyrimidine have attracted great attentions over many years due to their broad bioactivities, including anti-tumor [1–3], antimicrobial [4], anti-inflammatory [5], *etc*. Among them, 4-morpho-linothieno[3,2-*d*]pyrimidines (*e.g.* **1**, GDC-0941 and GNE-477, Fig. 1) have been reported to show marked anti-tumor activities [2,3,6]. For example, GDC-0941, which is a potent, selective, orally bioavailable small inhibitor of PI3K, exerted antiproliferative effects against an array of human tumor cell lines and is currently under going Phase I clinical trials [2,7]. Further studies of GDC-0941 led to a backup clinical candidate GNE-477, which possessed potent kinase and cytotoxic activity [3]. The SARs studies revealed that the 4-morpholinothieno[3,2-*d*]pyrimidine scaffold played a key role in their anti-tumor activities. Furthermore, the introduction of 4-(methylsulfonyl)-piperazin-1-ylmethyl substituent at C6-position remarkably enhanced the solubility and metabolic stability, which may attribute to the hydrogen bonds formed between sulfonylpiperazine moiety and the residues located near the mouth of the ATP-binding pocket [2,3].

The benzylidenehydrazinyl group or its mimics are key pharmacophores in the design of different potential anticancer agents [8–10]. In the past few years, many of the benzylidenehydrazine derivatives are identified as potent anticancer agents, such as, PAC-1 [8] and compound **5** [9] (Fig. 1). In addition, we have reported a series of 2-(2-benzylidenehydrazinyl)pyrido[2,3-*b*]pyrazin-3(4*H*)-ones derivatives and most of them showed potent *in vitro* antitumor activities against several cell lines [10,11]. Encouraged by these observations, we planned to synthesize new

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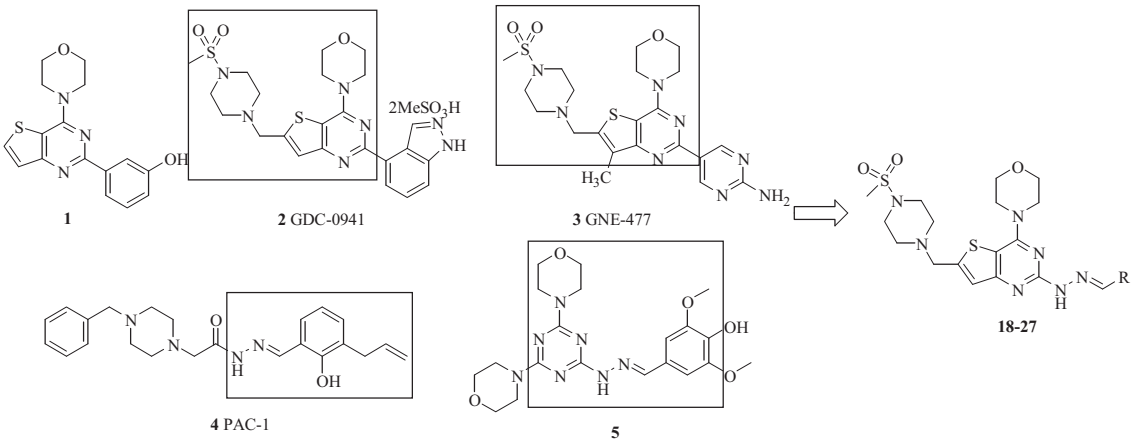
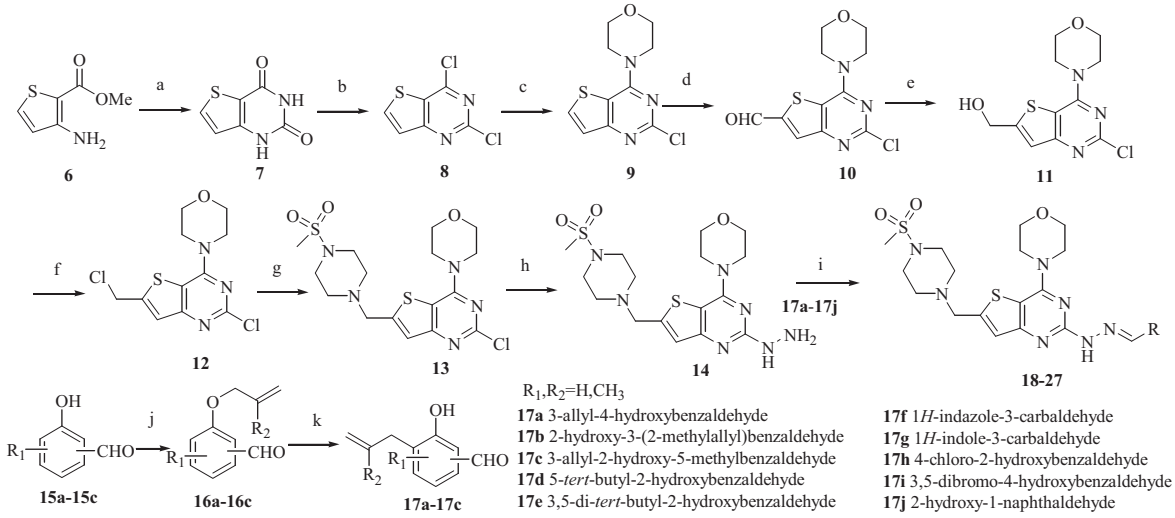


Fig. 1. Structures of some reported thieno[3,2-*d*]pyrimidines (**1**, GDC-0941, GNE-477), hydrazones (PAC-1, **5**) and target compounds.

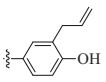
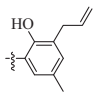
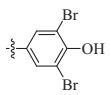
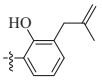
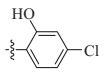
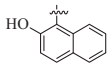
chemical entities incorporating the two active pharmacophores, 4-morpholinothieno[3,2-*d*]pyrimidine and (hetero)arylmethylenehydrazinyl group in a single molecular framework. Therefore, a series of 2,6-disubstituted-4-morpholinothieno[3,2-*d*]pyrimidine derivatives, containing arylmethylene- or heteroarylmethylene-hydrazinyl group at C2-position and 4-(methylsulfonyl)piperazin-1-ylmethyl substituent at C6-position (Fig. 1), were designed and synthesized. Their cytotoxic activity against H460 (human lung cancer), HT-29 (human colorectal cancer), MDA-MB-231 (human breast cancer), U87MG (human glioblastoma) and H1975 (human lung cancer) cell lines were evaluated *in vitro* in attempt to find novel and potent anti-tumor agents.

The synthesis of title compounds is illustrated in Scheme 1 [2]. The commercially available methyl 3-amino-2-thiophenecarboxylate **6** was condensed with urea at 190 °C for 2 h to give thienopyrimidinedione **7**, which was chlorinated with phosphorus oxychloride to afford **8** as a pale yellow solid. Regioselective nucleophilic displacement of the 4-chloride with morpholine gave rise to **9** in 85–87% yields. Subsequently, lithiation of **9**, followed by the addition of DMF, gave the aldehyde **10** in 75% yield. Treatment of **10** with $NaBH_4$ in MeOH at room temperature provided **11** as a primary alcohol, which reacted with thionyl chloride and then substituted with 1-(methylsulfonyl)piperazine to furnish



Scheme 1. Reagents and conditions: (a) 5 equiv. urea, 190 °C, 2.5 h; (b) $POCl_3$, DMF (*cat.*), reflux, 8 h; (c) 2.1 equiv. morpholine, MeOH, 0 °C, 30 min, r.t., 1–2 h; (d) DMF, *n*-BuLi, THF, −78 °C to r.t., 5 h; (e) $NaBH_4$, MeOH, r.t., 3 h; (f) $SOCl_2$, CH_2Cl_2, r.t., 2 h; (g) 1-(methylsulfonyl)piperazine, DIPEA, isopropanol, r.f., 11 h; (h) 80% $NH_2NH_2{\cdot}H_2O$, r.f., 6–8 h; (i) R-CHO, EtOH, reflux, 4–8 h; (j) allyl bromide, CH_3CN, K_2CO_3, 60 °C, 5–8 h; (k) 180 °C, 6–8 h.

Table 1
The structures of compounds **18**–**27**.

Compd.	R	Compd.	R	Compd.	R	Compd.	R	Compd.	R
18		**20**		**22**		**24**		**26**	
19		**21**		**23**		**25**		**27**	

compound **13**. Next, substitution reaction of **13** with an excess of 80% hydrazine hydrate in ethanol gave the key intermediate **14**, which was then condensed with the corresponding (hetero)aromatic aldehyde **17a**–**17j** in refluxing ethanol to yield the target compounds **18**–**27** as white to yellow solids. The substituted aromatic aldehyde **17a**–**17c** were prepared *via* a two-step reaction. Reaction of substituted hydroxybenzaldehyde **15a**–**15c** with alkyl bromide in the presence of K_2CO_3 in CH_3CN gave **16a**–**16c**, which were then heated at 190 °C to afford **17a**–**17c** *via* Claisen rearrangement. The structures of target compounds were listed in Table 1. All of them were confirmed by IR, ^{1}H NMR and MS spectra [12].

All the target compounds and their precursor **14**, along with the reference compounds GDC-0941 and PAC-1, were screened for their *in vitro* cytotoxic activity against H460, HT-29, MDA-MB-231, U87MG and H1975, using standard MTT assay. The results, expressed as IC_{50} values, were summarized in Table 2.

As illustrated in Table 2, most of the synthesized compounds showed potent cytotoxic activities against one or more cancer cell lines. Several compounds (**19**, **21**–**23**) exhibited enhanced cytotoxic activities than PAC-1 against H460 cancer cell line. The most promising compound **23** (0.84 μmol/L, 0.23 μmol/L, 2.52 μmol/L, 1.80 μmol/L) was 1.0, 2.9, 29,3 and 4.3 times more active than positive control GDC-0941 (0.87 μmol/L, 0.66 μmol/L, 73.8 μmol/L, 7.77 μmol/L) against H460, HT-29, MDA-MB-231 and U87MG cell lines, respectively.

The pharmacological data indicated that the introduction of substituted arylmethylene group was favorable for increasing the cytotoxic activity, as all the target compounds showed greater cytotoxic effects than the precursor **14** against all tested cell lines. Furthermore, the substituents in benzene ring had an important influence on antitumor activity. In most cases, the presence of electron-donating groups (such as *tert*-butyl, allyl, hydroxyl in this study) in the benzene ring (**19**–**22**) with the exception of hydroxyl group at 4-position (**18**, **26**), enhanced the cytotoxic activity. By contrast, the introduction of electron-withdrawing groups (such as chloro, bromo) caused a dramatic decrease in biological activity (**25**, **26**). In addition, hydrogen atom at 5-position of the benzene ring was more favorable, as compound **19** was more potent than compounds **20**–**22** against all the tested cancer cell lines.

On the other hand, replacement of arylmethylene with indolyl methylene (**24**) or 2-hydroxynaphthalen-1-ylmethylene (**27**) group resulted in a considerable loss in potency. Interestingly, the alternative indazol-3-ylmethylene group (**23**) showed significantly improved potency. Therefore, the nature of the (hetero)arylmethylene group appears

Table 2
Cytotoxic activities of compounds against H460, HT-29, MDA-MB-231, U87MG and H1975 cancer cell lines *in vitro*.

Compd.	IC_{50} (μmol/L)					Compd.	IC_{50} (μmol/L)				
	H460	HT29	MDA-MB-231	U87MG	H1975		H460	HT29	MDA-MB-231	U87MG	H1975
14	39.81	46.22	51.41	ND	ND						
18	31.98	6.82	20.99	ND	ND	**23**	0.84	0.23	2.52	1.80	28.82
19	1.88	2.90	>100	25.63	>100	**24**	18.38	15.65	>100	ND	ND
20	4.61	6.83	7.00	30.73	>100	**25**	>100	>100	>100	ND	ND
21	3.51	3.67	4.23	ND	ND	**26**	15.96	17.41	6.38	ND	ND
22	3.57	2.64	11.57	ND	ND	**27**	31.57	ND	>100	ND	ND
GDC-0941[a]	0.87	0.66	73.82	7.77	0.27	PAC-1[a]	3.57	0.97	6.11	ND	ND

ND: not determined.
[a] Used as a positive control.

to have a significant impact on anti-tumor activity, presumably due to the putative hydrogen bonds between the (hetero)aryl (*e.g.* indazole, **23**) and the enzyme necessary for activity. Next our attention will turn to replacement of indazolylmethylene with other heterocyclic methylene groups. Further studies in this sense are currently underway and will be reported in the future.

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[12] Data for new compounds. **18**: Yield: 77%; mp: 185–187 °C; ESI-MS *m/z*: 572.1 $(M+H)^+$; IR (KBr, cm^{-1}): 3434.6, 1630.2, 1548.3, 1197.1, 1054.8, 937.7, 782.7; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.40 (s, 4H), 3.00 (s, 3H), 3.19 (s, 4H), 3.83 (s, 6H), 4.07 (s, 4H), 4.65 (s, 2H), 5.07 (m, 2H), 6.02 (d, 1H, J = 6.9 Hz), 6.92 (d, 1H, J = 8.2 Hz), 7.62 (d, 2H, J = 7.3 Hz), 7.72 (s, 1H), 8.18 (s, 1H), 12.43 (s, 1H), 13.01 (s, 1H). **19**: Yield: 82%; mp: 265–265 °C; ESI-MS *m/z*: 586.0 $(M+H)^+$, 609.6 $(M+Na)^+$; IR (KBr, cm^{-1}): 3440.4, 3308.2, 2920.7, 2855.3, 1554.5, 1516.3, 1158.3, 960.9; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.70 (s, 3H), 2.57 (s, 4H), 2.90 (s, 3H), 3.15 (s, 4H), 3.98–3.67 (m, 12H), 4.68 (s, 1H), 4.77 (s, 1H), 6.84 (s, 1H), 7.11 (m, 3H), 8.20 (s, 1H), 11.18 (s, 1H), 12.58 (s, 1H). **20**: Yield: 82%; mp: 195–196 °C; ESI-MS *m/z*: 586.6 $(M+H)^+$; IR (KBr, cm^{-1}): 3432.0, 2853.5, 1558.5, 1514.7, 1326.1, 1159.7, 960.1; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.35 (s, 7H), 2.57 (s, 3H), 3.20 (s, 4H), 3.83(s, 2H), 3.87 (s, 6H), 4.08 (s, 4H), 4.67 (s, 2H), 5.96–5.99 (m, 1H), 7.23 (s, 2H), 7.72 (s, 1H), 8.17 (s, 1H), 12.55 (s, 1H), 13.15 (s, 1H). **21**: Yield: 78%; mp: 273–273 °C; ESI-MS *m/z*: 585.6 $(M-H)^-$, 665.6 $(M+Br)^-$; IR (KBr, cm^{-1}): 3425.0, 3295.0, 2958.2, 2859.6, 1556.0, 1516.3, 1160.2, 959.3; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.27 (s, 9H), 2.57 (s, 4H), 2.89 (s, 3H), 3.14 (s, 4H), 3.76 (s, 4H), 3.87 (d, 6H, J = 10.1 Hz), 6.83 (d, 1H, J = 8.4 Hz), 7.11 (s, 1H), 7.39–7.18 (m, 2H), 8.20 (s, 1H), 11.16 (s, 1H), 11.92 (s, 1H). **22**: Yield: 72%; mp: 248–250 °C; ESI-MS *m/z*: 641.9 $(M-H)^-$; IR (KBr, cm^{-1}): 3438.0, 2958.3, 1626.2, 1548.5, 1204.9, 1159.4, 1039.5, 782.2; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.29 (s, 9H), 1.42 (s, 9H), 2.38 (s, 4H), 2.99 (s, 3H), 3.22 (s, 4H), 3.83 (s, 4H), 4.08 (s, 4H), 4.66 (s, 2H), 7.34 (s, 2H), 7.66 (s, 1H), 8.47 (s, 1H), 12.79–11.93 (m, 1H). **23**: Yield: 76%; mp: 249–251 °C; ESI-MS *m/z*: 555.6 $(M+H)^+$; IR (KBr, cm^{-1}): 3426.9, 1625.7, 1548.2, 1327.2, 1196.1, 1055.7, 782.4; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.58 (s, 4H), 2.90 (s, 3H), 3.14 (s, 4H), 3.89 (m, 10H), 7.15 (s, 1H), 7.25–7.16 (m, 1H), 7.40 (t, 1H, J = 7.5 Hz), 7.54 (d, 1H, J = 8.3 Hz), 8.39 (s, 1H), 8.50 (d, 1H, J = 8.1 Hz), 10.95 (s, 1H), 12.15 (s, 1H). **24**: Yield: 68%; mp: 256–257 °C; ESI-MS *m/z*: 554.3 $(M+H)^+$; IR (KBr, cm^{-1}): 3426.0, 3009.0, 1629.2, 1551.8, 1195.3, 1055.5, 782.2; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.39 (s, 4H), 2.99 (s, 3H), 3.17 (s, 4H), 3.75 (s, 4H), 3.83 (s, 4H), 4.62 (s, 2H), 7.03 (s, 1H), 7.06 (s, 1H), 7.70 (s, 1H), 7.79 (d, 3H, J = 8.3 Hz), 8.18 (s, 1H), 12.42 (s, 1H), 12.97 (s, 1H). **25**: Yield: 73%; mp: 251–251 °C; ESI-MS *m/z*: 565.5 $(M+H)^+$; IR (KBr, cm^{-1}): 3423.2, 3006.0, 1638.0, 1593.1, 1549.0, 1198.3, 937.3, 780.1; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.57 (s, 4H), 2.89 (s, 3H), 3.14 (s, 4H), 4.01–3.55 (m, 10H), 5.91–6.02 (m, 1H), 6.89 (s, 1H), 6.96 (s, 1H), 7.14 (s, 1H), 8.15 (s, 1H), 11.13 (s, 1H), 12.29 (s, 1H). **26**: Yield: 79%; mp: 210–211 °C; ESI-MS *m/z*: 690.5 (Br = 79), 692.5 (Br = 81) $(M+H)^+$; IR (KBr, cm^{-1}): 3427.8, 1626.1, 1592.1, 1548.6, 1328.4, 1199.7, 780.5; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.43 (s, 4H), 3.01 (s, 3H), 3.43 (s, 4H), 3.84 (s, 4H), 4.08 (s, 4H), 4.72 (s, 2H), 7.76 (s, 1H), 8.16 (s, 3H), 12.66 (s, 1H), 13.15 (s, 1H). **27**: Yield: 69%; mp: >300 °C; ESI-MS *m/z*: 580.6 $(M-H)^-$; IR (KBr, cm^{-1}): 3433.0, 3281.4, 1547.2,1515.6, 1345.2, 1163.0, 962.2,776.9; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.59 (s, 4H), 2.90 (s, 3H), 3.16 (s, 4H), 4.06–3.59 (m, 10H), 7.15 (s, 1H), 7.22 (d, 1H, J = 9.0 Hz), 7.38 (t, 1H, J = 7.4 Hz), 7.57 (t, 1H, J = 7.7 Hz), 7.95–7.76 (m, 2H), 8.08 (d, 1H, J = 8.5 Hz), 9.14 (s, 1H), 11.17 (s, 1H), 13.35 (s, 1H).