Synthesis of Novel Pyrido[3′,2′:4,5]furo[3,2-*d*]pyrimidine Derivatives and Their Cytotoxic Activity

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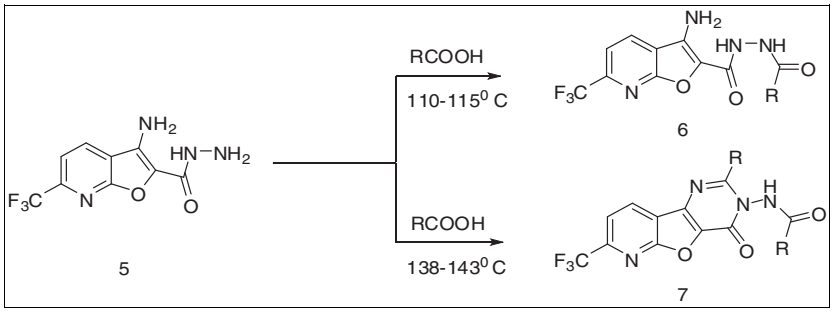

The 3-amino-6-(trifluoromethyl)furo[2,3-*b*]pyridine-2-carbohydrazide (**5**) was prepared from 3-cyano-6-trifluoromethyl-2(1*H*)pyridone (**2**) in series of steps via selective *O*-alkylation, Thorpe–Ziegler cyclization followed by reaction with hydrazine hydrate. The 2-carbohydrazide (**5**) was further reacted with aliphatic acids under different reaction temperatures to form a series of novel *N*-acylfuro[2,3-*b*]pyridine-2-carbohydrazide (**6**) and pyrido[3′,2′:4,5]furo[3,2-*d*]pyrimidine derivatives (**7**). All the compounds **6** and **7** were screened for cytotoxic activity against breast carcinoma MD Anderson-Metastatic Breast (MDA-MB) 231 (aggressive) cell lines at 10 μM concentration. Compounds **6a**, **6b**, and **6c** showed promising activity.

INTRODUCTION

Pyridine [1,2] or pyrimidine [3] nucleus present in many biologically active compounds and combination of both also proved as potential molecules because of their cumulative effect. Recent efforts on synthesis of pyrido [3′,2′:4,5]furo[3,2-*d*]pyrimidine derivatives resulted selective mGluR1 antagonists [4], potential PI3 kinase P110α inhibitors [5], and their isosters, that is, phenyl analogs [6] identified as potent inhibitors of the epidermal growth factor receptor tyrosine kinase. In recent past, it was found that the presence of fluorine [7] or trifluoromethyl [8] group in a strategic position of organic molecule dramatically alters the properties of a molecule in terms of lipophilicity, oxidative thermal stability, and oral bioavailability thereby improves the transport mechanism and efficacy of drug molecule. However, the fluorinated pyrido furo pyrimidine nucleus is not been extensively exploited except our reports [9,10]. On the basis of the importance of these compounds and in continuation of our efforts [11–13] on synthesis of fluorinated molecules, we have prepared a series of novel trifluoromethyl substituted pyrido[3′,2′:4,5]furo[3,2-*d*]pyrimidine derivatives in series steps. All the compounds were screened for cytotoxic activity against breast carcinoma MD Anderson-Metastatic Breast (MDA-MB) 231 (aggressive) cell lines at 10 μM concentration, and promising compounds have been identified.

CHEMISTRY

The 3-cyano-6-trifluoromethyl-2(1*H*)pyridone (**2**) [14] was prepared by acylation of vinyl butyl ether in pyridine at RT, followed by reaction with cyanoacetamide in presence of sodium ethoxide in ethanol. The 2(1*H*)pyridone (**2**) was reacted with α-bromoethylacetate in acetone using potassium carbonate as base and potassium iodide as catalyst and obtained selectively *O*-alkylated product, that is, ethyl 2-(3-cyano-6-(trifluoromethyl)pyridine-2-yloxy) acetate (**3**) [15]. Compound **3** was cyclized under Thorpe–Ziegler conditions, that is, DMF/K_2CO_3 at 110°C to give 2-carbethoxy-3-amino-6-trifluoromethylfuro[2,3-*b*]pyridine (**4**). The furo[2,3-*b*]pyridine (**4**) was further reacted with hydrazine hydrate in ethanol and obtained 3-amino-6-trifluoromethylfuro[2,3-*b*]pyridine-2-carbohydrazide (**5**). The hydrazide (**5**) was reacted with various aliphatic acids at 110–115°C for 12–18 h, and resulted exclusively 3-amino-*N*′-acyl-6-trifluoromethylfuro[2,3-*b*]pyridine-2-carbohydrazide (**6a–g**). The structure of the products was established on the basis of the ^{1}H-NMR data that shows the presence of C—NH_2 in the region δ 6.15 to 6.29 and absence of N—NH_2 protons at δ 4.21. In addition, the presence of two amide NH protons having different chemical shifts confirms the structure. The reaction of compound **5** with different aliphatic acids at 138–143°C for 24–30 h, resulted pyrido[3′,2′:4,5]furo[3,2-*d*]pyrimidine derivatives **7a–f**.

The sequence of reaction is mainly selective acylation of N—NH_2, followed by cyclization to form products **7a–f**. The details of reactions outlined in Scheme 1, and products are tabulated in Table 1.

RESULTS AND DISCUSSION

Cytotoxic activity. Compounds **6a–g** and **7a–f** were screened for cytotoxic activity against breast carcinoma MDA-MB 231 (aggressive) cell lines at 10 μM concentration by MTT assay method. Compounds **6a**, **6b**, and **6c** found to show promising cell death, whereas compounds **6d**, **6g**, and **7a** showed moderate activity. The structure verses activity study reveals that the furo [2,3-*b*]pyridine derivatives found to show high activity than pyrido[3′,2′:4,5]furo[3,2-*d*]pyrimidine derivatives, and the activity is independent of increase in chain length of alkyl. The details on percentage of cell viability verses cell death with standard deviation for all the compounds outlined in Table 2, and the data is outlined pictorially in Figure 1.

EXPERIMENTAL

General procedure. Melting points of all the compounds was recorded on Casia-Siamia (VMP-AM) melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer FTIR 240-C spectrophotometer using KBr optics. ^{1}H-NMR spectra were recorded on Bruker AV 300 MHz INOVA 400 MHz and INOVA 500 MHz in DMSO-d_6 or $CDCl_3$ using TMS as an internal standard. EI and chemical ionization mass spectra were recorded on a VG 7070 H instrument at 70 eV. All high-resolution spectra were recorded on QSTARXL hybrid MS/MS system (Applied Biosystems, USA) under ESI. All the reactions were monitored by TLC on pre-coated silica gel 60 F_{254} (mesh); spots were visualized with UV light. Merck silica gel (60–120 mesh) was used for column chromatography.

Preparation of 4-butoxy-1,1,1-trifluorobut-3-en-2-one (1) [14].

Preparation of 2-oxo-6-trifluoromethyl-1,2-dihydropyridine-3-carbonitrile (2) [14].

Preparation of ethyl 2-(3-cyano-6-(trifluoromethyl)pyridine-2-yloxy)acetate (3) [15].

Ethyl 3-amino-6-(trifluoromethyl)furo[2,3-b]pyridine-2-carboxylate (4). Ethyl 2-(3-cyano-6-(trifluoromethyl)pyridine-2-yloxy)acetate (**3**) (5.0 g, 18.2 mmol) and potassium carbonate (3.0 g, 21.7 mmol) were taken in dry dimethylformamide

Scheme 1

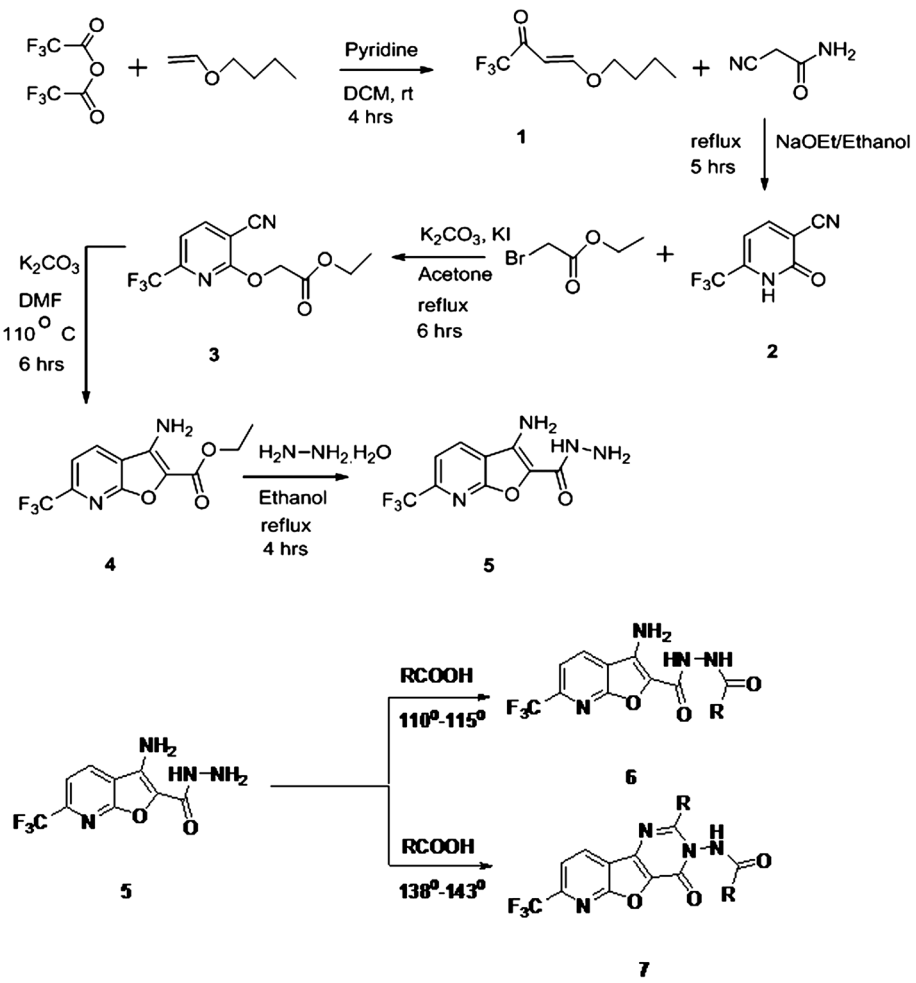

Table 1

Preparation of compounds **6a-g** and **7a-f**.

S. no.	Compound	R	mp (°C)	Yield (%)
1	**6a**	CH_2CH_3	251–254	84
2	**6b**	$(CH_2)_2CH_3$	239–242	82
3	**6c**	$CH(CH_3)_2$	253–256	80
4	**6d**	$CH_2CH(CH_3)_2$	243–246	78
5	**6e**	$(CH_2)_4CH_3$	241–244	81
6	**6f**	$(CH_2)_5CH_3$	226–229	78
7	**6g**	CF_3	>260	79
8	**7a**	CH_3	233–235	80
9	**7b**	C_2H_5	140–143	78
10	**7c**	$CH_2CH_2CH_3$	133–136	76
11	**7d**	$CH(CH_3)_2$	176–179	78
12	**7e**	$CH_2CH(CH_3)_2$	150–153	69
13	**7f**	$CH_2(CH_2)_3CH_3$	95–98	67

Table 2

Cytotoxic activity of **6a-g** and **7a-f**.

S. no.	Compound no:	% Cell viability ± *SD*	% Cell death
1	Control	100 ± 0	0
2	**6a**	69.67 ± 9.14	30.33
3	**6b**	63.37 ± 5.46	36.63
4	**6c**	69.53 ± 3.69	30.47
5	**6d**	77.54 ± 3.81	22.46
6	**6e**	79.11 ± 0.22	20.89
7	**6f**	84.30 ± 8.52	15.7
8	**6g**	74.57 ± 0.98	25.43
9	**7a**	77.51 ± 14.14	22.49
10	**7b**	96.97 ± 4.45	3.03
11	**7c**	105.69 ± 2.16	−5.69
12	**7d**	106.70 ± 7.31	−6.70
13	**7e**	116.45 ± 12.77	−16.45
14	**7f**	102.76 ± 13.47	−2.7

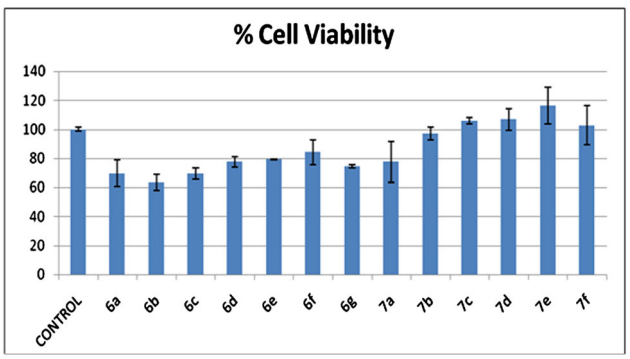

Figure 1. Pictorial representation of cytotoxic activity.

(50 mL), raised the reaction temperature to 80°C and allowed to stir for 6 h, at the same temperature. The progress of the reaction was monitored by TLC. After completion of reaction, the reaction mixture was allowed to cool to RT and poured into ice cold water. The separated solid was collected by filtration, washed with water, and dried. The crude product was recrystallized from ethanol.

Pale yellow solid. Yield: 78%. mp 199–202°C. IR (KBr) cm^{-1}: 3492, 3339 (NH_2), 1689 (ester, CO), 1633 (C═N), 1599 (C═C). ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.54 (t, 3H, J = 7.55 Hz, CH_3), 4.43 (q, 2H, J = 7.55 Hz, OCH_2), 5.05 (s, 2H, NH_2), 7.63 (d, 1H, J = 8.30 Hz, Ar—H), 8.07 (d, 1H, J = 8.30 Hz, Ar—H). ESI–MS: 275 (M + 1), 297 (M + 23).

3-Amino-6-(trifluoromethyl)furo[2,3-b]pyridine-2-carbohydrazide (5). Ethyl 3-amino-6-(trifluoromethyl)furo[2,3-*b*]pyridine-2-carboxylate (**4**) (4.0 g, 14.6 mmol) was taken in ethanol (32 mL) and was added hydrazine hydrate (4.3 g, 87.5 mmol), and the reaction mixture was allowed to reflux for 4 h. After completion of reaction, ethanol was removed under vacuum. The crude residue was treated with ice cold water, and separated solid was collected by filtration, washed with water, dried, and used in reactions without further purification.

Orange solid. Yield: 80%. mp 225–228°C. IR (KBr) cm^{-1}: 3381, 3289 (NH_2), 1650 (amide, CO), 1583 (C═N), 1527 (C═C). ^{1}H-NMR (DMSO-d_6, 500 MHz): δ 4.21 (s, 2H, $NHNH_2$), 6.05 (s, 2H, NH_2), 7.65 (d, J = 8.54 Hz, 1H, Ar—H), 8.51 (d, J = 8.54 Hz, 1H, Ar—H), 9.13 (br, s, 1H, NH). ESI–MS: 261 (M + 1), 283 (M + 23).

Preparation of 3-amino-N′-substituted-6-(trifluoromethyl) furo[2,3-b]pyridine-2-carbohydrazide derivatives (6a–g): General procedure. The 3-amino-6-(trifluoromethyl)furo [2,3-*b*]pyridine-2-carbohydrazide (100 mg, 0.38 mmol) was taken in different aliphatic acids (5 mL) and heated at 110–115°C during 12–18 h. The progress of the reaction was monitored by TLC. After completion of reaction, the reaction mixture was cooled to RT and neutralized with saturated sodium bicarbonate solution. Aqueous solution was extracted with ethyl acetate (2 × 50 mL); combined extracts were washed with distilled water till washings are neutral to pH and dried over anhydrous sodium sulfate. Concentrated under vacuum, the crude product was purified by column chromatography with 60–120 mesh silica gel.

3-Amino-N′-propionoyl-6-(trifluoromethyl)furo[2,3-b]pyridine-2-carbohydrazide (6a). Pale yellow solid. Yield: 84%. mp 251–254°C. IR (KBr) cm^{-1}: 3298, 3404 (—NH_2), 1668, 1693 (amide, C═O), 1627 (C═N), 1599 (C═C); ^{1}H-NMR (DMSO-d_6, 500 MHz): δ 1.13 (t, J = 7.08 Hz, 3H, CH_3), 2.27 (q, 2H, J = 7.08 Hz, —CH_2—), 6.18 (s, br, 2H, —NH_2), 7.62 (d, 1H, J = 7.80 Hz, Ar—H), 8.53 (d, 1H, J = 7.80 Hz, Ar—H), 9.56 (br, s, 1H, —NH—), 9.60 (br, s, 1H, —NH—), ESI–MS: 317 (M + 1), 336 (M + 23); HRMS *m/z* Calcd for $C_{12}H_{12}F_3N_4O_3$ ($[M+H]^+$): 317.0856, Found 317.0858.

3-Amino-N′-butyroyl-6-(trifluoromethyl)furo[2,3-b]pyridine-2-carbohydrazide (6b). Yellow solid. Yield: 82%; mp 239–242°C; IR (KBr) cm^{-1}: 3296, 3354, (NH_2), 1692, 1665 (amide, C═O), 1627 (C═N), 1532 (C═C). ^{1}H-NMR (DMSO-d_6, 500 MHz): δ 0.98 (t, J = 7.68 Hz, 3H, CH_3), 1.62 (m, 2H, CH_2), 2.22 (t, J = 7.70 Hz, 2H, CH_2), 6.16 (br, s, 2H, NH_2), 7.69 (d, J = 7.74 Hz, 1H, Ar—H), 8.59 (d, J = 7.74 Hz, 1H, Ar—H), 9.72 (br, s, 1H, NH), 9.85 (br, s, 1H, NH), ESI–MS: 331 (M + 1), 353 (M + 23). HRMS *m/z* Calcd for $C_{13}H_{14}F_3N_4O_3$ ($[M+H]^+$): 331.1012, Found: 331.1019.

3-Amino-N′-isobutyroyl-6-(trifluoromethyl)furo[2,3-b] pyridine-2-carbohydrazide (6c). Yellow solid. Yield: 80%; mp 253–256°C; IR (KBr) cm^{-1}: 3247, 3371, (—NH_2), 1696, 1643 (amide, C═O), 1578 (C═N), 1529 (C═C); ^{1}H-NMR (DMSO-d_6, 500 MHz): δ 1.15 (d, J = 6.92 Hz, 6H, 2 × CH_3), 2.51–2.60 (m, 1H, CH), 6.17 (br, s, 2H, NH_2), 7.62 (d, J = 7.91 Hz, 1H, Ar—H), 8.53 (d, J = 7.91 Hz, 1H, Ar—H), 9.59 (br, s, 2H, 2 × NH). ESI–MS: 331 (M + 1), 332 (M + 2), 353 (M + 23). HRMS *m/z* Calcd for $C_{13}H_{14}F_3N_4O_3$ ($[M+H]^+$): 331.1012. Found: 331.1015.

3-Amino-N′-(3-isopentanoyl)-6-(trifluoromethyl)furo[2,3-b] pyridine-2-carbohydrazide (6d). Orange solid. Yield: 78%. mp 243–246°C. IR (KBr) cm^{-1}: 3251, 3378, (NH_2), 1690, 1641 (amide, C═O), 1532 (C═N), 1529 (C═C). ^{1}H-NMR (DMSO-d_6, 500 MHz): δ 1.01 (d, J = 5.18 Hz, 6H, 2 × CH_3), 1.25 (m, 1H, CH), 2.16 (d, J = 5.12 Hz, 2H, CH_2), 6.15 (s, 2H, NH_2), 7.65 (d, J = 7.25 Hz, 1H, Ar—H), 8.53 (d, J = 7.25 Hz, 1H, Ar—H), 9.53 (br, s, 1H, NH), 9.67 (br, s, 1H, NH). ESI–MS: 345 (M + 1), 367 (M + 23). HRMS *m/z* Calcd for $C_{14}H_{16}F_3N_4O_3$ ($[M+H]^+$): 345.1169. Found 345.1170.

3-Amino-N′-hexanoyl-6-(trifluoromethyl)furo[2,3-b]pyridine-2-carbohydrazide (6e). Orange solid. Yield: 81%. mp 241–244°C. IR (KBr) cm^{-1}: 3248, 3385, (NH_2), 1693, 1641 (amide, C═O), 1577 (C═N), 1528 (C═C). ^{1}H-NMR (DMSO-d_6, 500 MHz): δ 0.91 (t, J = 6.98 Hz, 3H, CH_3), 1.28–1.40 (m, 4H, 2 × CH_2), 1.56–1.69 (m, 2H, CH_2), 2.22 (t, J = 7.55 Hz, 2H, CH_2), 6.30 (s, 2H, NH_2), 7.71 (d, J = 7.93 Hz, 1H, Ar—H), 8.60 (d, J = 7.93 Hz, 1H, Ar—H), 9.71 (br, s, 1H, NH), 9.89 (br, s, 1H, NH). ESI–MS: 359 (M + 1), 360 (M + 2), 381 (M + 23). HRMS *m/z* Calcd for $C_{15}H_{18}F_3N_4O_3$ ($[M+H]^+$): 359.1325. Found: 359.1331.

3-Amino-N′-heptanoyl-6-(trifluoromethyl)furo[2,3-b]pyridine-2-carbohydrazide (6f). Orange solid. Yield: 78%. mp 226–229°C; IR (KBr) cm^{-1}: 3248, 3385, (—NH_2), 1693, 1641 (amide, C═O), 1577 (C═N), 1528 (C═C). ^{1}H-NMR (DMSO-d_6, 500 MHz): δ 0.91 (t, J = 6.47 Hz, 3H, CH_3), 1.24–1.41 (m, 6H, 3 × CH_2), 1.58–1.67 (m, 2H, CH_2), 2.23 (t, J = 7.40 Hz, 2H, CH_2), 6.18 (s, 2H, NH_2), 7.63 (d, J = 8.30 Hz, 1H, Ar—H), 8.54 (d, J = 8.30 Hz, 1H, Ar—H), 9.65 (br, s, 1H, NH). ESI–MS: 373 (M + 1), 374 (M + 2), 395 (M + 23). HRMS *m/z* Calcd for $C_{16}H_{20}F_3N_4O_3$ ($[M+H]^+$): 373.1482. Found: 373.1498.

3-Amino-N′-(2,2,2-trifluoroacetyl)-6-(trifluoromethyl)furo [2,3-b]pyridine-2-carbohydrazide (6g). Pale yellow solid. Yield: 79%. mp >260°C. IR (KBr) cm^{-1}: 3307, 3355 (NH_2), 1747 ($COCF_3$), 1653 (amide, C═O), 1578 (C═N), 1507 (C═C). ^{1}H-NMR ($CDCl_3$ + DMSO-d_6, 500 MHz): δ 6.29 (s, 2H, NH_2), 7.77 (d, J = 7.91 Hz, 1H, Ar—H), 8.65 (d, J = 7.91 Hz, 1H, Ar—H), 10.33 (br, s, 1H, NH), 11.09 (br, s, 1H, NH). ESI–MS: 357 (M + 1), 379 (M + 23). HRMS *m/z* Calcd for $C_{11}H_7F_6N_4O_3$ ($[M+H]^+$): 357.0325. Found: 357.0329.

Preparation of pyrido[3′,2′:4,5]furo[3,2-d]pyrimidine derivatives (7a–f): General procedure. The 3-amino-6-(trifluoromethyl)furo[2,3-*b*]pyridine-2-carbohydrazide (**5**) (100 mg, 0.38 mmol) was taken in different aliphatic acids (5.0 mL) and heated to 138–143°C during 24–30 h, and after completion of reaction, it was cooled to RT and neutralized with saturated sodium bicarbonate solution. Aqueous solution was extracted with ethyl acetate (2 × 50 mL); combined extracts were washed with distilled water and dried over sodium sulfate. Concentrated under vacuum, the crude product was purified by column chromatography with 60–120 mesh silica gel.

N-3-Methyl-1-oxo-7-(trifluoromethyl)pyrido[3′,2′:4,5]furo [3,2-d]pyrimidin-2(1H)-acetamide (7a). White solid. Yield: 80%. mp 233–235°C. IR (KBr) cm^{-1}: 3306, 3280 (NH_2), 1678 (amide, C═O), 1562 (C═N), 1502 (C═C). ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 2.38 (s, 3H, CH_3), 2.72 (s, 3H, $COCH_3$), 7.73 (d, J = 9.11 Hz, 1H, Ar—H), 9.08 (d, J = 9.11 Hz, 1H, Ar—H), 9.36 (br, s, 1H, NH). ESI–MS: 327 (M + 1), 349 (M + 23); HRMS *m/z* Calcd for $C_{13}H_{10}F_3N_4O_3$ ($[M+H]^+$): 327.0699. Found: 327.0705.

N-3-Ethyl-1-oxo-7-(trifluoromethyl)pyrido[3′,2′:4,5]furo[3,2-d]pyrimidin-2(1H)-propionamide (7b). White solid. Yield: 78%. mp 140–143°C. IR (KBr) cm^{-1}: 3250 (NH_2) , 1704 (amide, C═O), 1564 (C═N), 1517 (C═C). ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 1.25 (t, J = 7.08 Hz, 3H, CH_3), 1.34 (t, J = 7.13 Hz, 3H, CH_3), 2.54 (q, J = 7.08 Hz, 2H, CH_2), 2.97 (q, J = 7.13 Hz, 2H, CH_2) 7.78 (d, J = 7.79 Hz, 1H, Ar—H), 8.58 (d, J = 7.79 Hz, 1H, Ar—H), 9.57 (br, s, 1H, NH). ESI–MS: 355 (M + 1), 377 (M + 23). HRMS *m/z* Calcd for $C_{15}H_{14}F_3N_4O_3$ ($[M+H]^+$): 355.1012. Found: 355.1010.

***N-1-Oxo-3-propyl-7-(trifluoromethyl)pyrido[3′,2′:4,5]furo [3,2-d]pyrimidin-2(1H)*-butyramide (7c).** White solid. Yield: 76%. mp 133–136°C. IR (KBr) cm^{-1}: 3243 (NH_2), 1704 (amide, C═O), 1560 (C═N), 1520 (C═C). ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 1.03 (t, J = 7.42 Hz, 3H, CH_3), 1.05 (t, J = 7.44 Hz, 3H, CH_3), 1.76–1.84 (m, 2H, CH_2), 1.84–1.92 (m, 2H, CH_2) 2.51 (t, 2H, J = 7.45 Hz), 2.84 (t, 2H, J = 7.47 Hz), 7.84 (d, J = 7.46 Hz, 1H, Ar—H), 8.57 (br, s, 1H, NH), 8.62 (d, J = 7.46 Hz, 1H, Ar—H). ESI–MS: 383 (M + 1), 405

(M + 23). HRMS *m/z* Calcd for $C_{17}H_{18}F_3N_4O_3$ ($[M+H]^+$): 383.1325. Found: 383.1331.

N-(3-Isopropyl-1-oxo-7-(trifluoromethyl)pyrido[3′,2′:4,5]furo [3,2-d]pyrimidin-2(1H)-yl)isobutyramide (7d). Yellow solid. Yield: 78%. mp 176–179°C. IR (KBr) cm^{-1}: 3187 (NH_2), 1700 (amide, C═O), 1549 (C═N). ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 1.18 (d, *J* = 7.00 Hz, 6H, 2 × CH_3), 1.34 (d, *J* = 7.04 Hz, 6H, 2 × CH_3), 2.76–2.82 (m, 1H, CH), 3.25–3.30 (m, 1H, CH), 7.79 (d, *J* = 8.00 Hz, 1H, Ar—H), 8.60 (d, *J* = 8.00 Hz, 1H, Ar—H) 9.14 (br, s, 1H, NH). ESI–MS: 383 (M + 1), 405 (M + 23). HRMS *m/z* Calcd for $C_{17}H_{18}F_3N_4O_3$ ($[M+H]^+$): 383.1325. Found: 383.1325.

N-(3-Isobutyl-1-oxo-7-(trifluoromethyl)pyrido[3′,2′:4,5]furo [3,2-d]pyrimidin-2(1H)-yl)-3-methylbutanamide (7e). Pale yellow solid. Yield: 69%. mp 150–153°C. IR (KBr) cm^{-1}: 3184 (NH_2), 1699 (amide, C═O), 1549 (C═N). ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 0.99 (d, *J* = 6.76 Hz, 6H, 2 × CH_3), 1.03 (d, *J* = 6.79 Hz, 6H, 2 × CH_3), 2.18–2.32 (m, 2H, 2 × CH), 2.38 (d, *J* = 6.95 Hz, 2H, CH_2), 2.75 (d, *J* = 6.98 Hz, 2H, CH_2), 7.84 (d, *J* = 7.93 Hz, 1H, Ar—H), 8.64 (d, *J* = 7.93 Hz, 1H, Ar—H) 8.79 (br, s, 1H, NH); ESI–MS: 411 (M + 1), 433 (M + 23); HRMS *m/z* Calcd for $C_{19}H_{22}F_3N_4O_3$ ($[M+H]^+$): 411.1638. Found: 411.1638.

N-(1-Oxo-3-pentyl-7-(trifluoromethyl)pyrido[3′,2′:4,5]furo [3,2-d]pyrimidin-2(1H)-yl)-hexanamide (7f). Orange solid. Yield: 67%. mp 95–98°C. IR (KBr) cm^{-1}: 3209 (NH_2), 1705 (amide, C═O), 1550 (C═N), 8.57 (br, s, 1H, NH). ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 0.92 (t, *J* = 6.98 Hz, 6H, 2 × CH_3), 1.32–1.43 (m, 6H, 3 × CH_2), 1.73–1.85 (m, 6H, 3 × CH_2), 2.51 (t, *J* = 7.55 Hz, 2H, CH_2), 2.87 (t, *J* = 7.36 Hz, 2H, CH_2), 7.84 (d, *J* = 7.93 Hz, 1H, Ar—H), 8.63 (d, *J* = 7.93 Hz, 1H, Ar—H), 9.01 (br, s, 1H, NH). ESI–MS: 439 (M + 1), 461 (M + 23). HRMS *m/z* Calcd for $C_{21}H_{26}F_3N_4O_3$ ($[M+H]^+$): 439.1951. Found: 439.1951.

CYTOTOXIC ACTIVITY

Procedure. Compounds **6a–g** and **7a–f** were screened for cytotoxic activity against breast carcinoma MDA-MB 231(aggressive) cell lines at 10 μM concentration for period of 48 h and was measured by standard MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), a yellow tetrazole assay method. At the end of the treatment, medium was removed, and cells were washed with Dulbecco's phosphate buffered saline (DPBS) and 50 μL of 5 mg/mL MTT solution in 500 μL of culture medium was added and incubated for 1 h at 37°C. Cells were solubilized with 500 μL of dimethyl sulfoxide, and absorbance was measured at 562 nm in a spectrophotometer.

CONCLUSION

A series of novel pyrido[3′,2′:4,5]furo[3,2-*d*]pyrimidine derivatives were prepared via selective *O*-alkylation of 2 (1H)pyridone, Thorpe–Ziegler cyclization, and reaction with hydrazine hydrate followed by reaction with different aliphatic acids. All the derivatives **6a–g** and **7a–f** were screened for cytotoxic activity against breast carcinoma MDA-MB 231 (aggressive) cell lines. Compounds **6a**, **6b**, and **6c** showed promising activity.

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