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Synthesis and evaluation of antitumoral activity of ester and amide derivatives of 2-arylamino-6-trifluoromethyl-3-pyridinecarboxylic acids

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Abstract—The synthesis and antitumoral activity of ester and amide derivatives of 2-arylamino-6-trifluoromethyl-3-pyridinecarboxylic acids **8**–**58** is described. Trifluoromethylpyridine derivatives **8**–**58** were evaluated for their anticancer activity toward human tumoral cell lines by the National Cancer Institute (NCI). Most of them possess encouraging anticancer activity, having GI_{50} values in the low micromolar to nanomolar concentration range. The 3,4,5-trimethoxyphenylamide **44** was the most active, and it is now under review by NCI Biological Evaluation Committee for possible further studies.

1. Introduction

Fluorinated organic molecules are known to perform a wide range of biological functions and fluorinated anticancer agents have become a focus in the development of new therapies for cancer. An increasing number of fluorinated antitumor agents have now become available for cancer treatment.[1,2] Trifluoromethylated drugs such as nilutamide, flutamide, hydroxyflutamide, and bicalutamide are non-steroidal anti-androgens which are widely used for the treatment of metastatic prostate cancer.[3,4] Furthermore, several studies have been devoted to the antiproliferative activity of trifluoromethyl bearing molecules. Thus, Panomifene (EGIS-5656, GYKI-13504), a trifluoromethyl tetra-substituted alkene, is a follow-up molecule to tamoxifen and is reported to exhibit anti-estrogenic activity superior to that of tamoxifen in the treatment of breast cancer.[5] A number of trifluoromethylated compounds demonstrated their antiproliferative activity through inhibition of kinases. For example, the biaryl urea BAY 43-9006 (sorafenib), that is now in phase II clinical trials, is known to inhibit a broad range of kinases including the vascular endothelial growth factor receptor tyrosine kinases (VEGFR-1 and VEGFR-2).[6] While trifluoromethyl-anthranilamides[7] are potent and selective VEGFR-1 and VEGFR-3 inhibitors, possessing potent antiangiogenic and antitumor properties. Furthermore, Nilonitib is a novel and selective inhibitor of BCR-ABL more potent than imanitib and also significantly active against imatinib-resistant BCR-ABL-expressing cells.[8] 4-Amino-5-(4-((2-fluoro-5-(trifluoromethyl)phenyl)aminocarbonylamino) phenyl)furo[2,3-*d*]pyrimidine is a potent dual VEGFR-2 and Tie-2 inhibitor, and showed marked tumor growth inhibition and anti-angiogenic activity in mouse HT-29 xenograft model via once-daily oral administration.[9] Furthermore, quite a few of trifluoromethylated molecules demonstrated their antiproliferative activity through modulation of cell cycle proteins. Thus, 1,1-bis(3′-indolyl)-1-(*p*-trifluoromethylphenyl)methane induced peroxisome proliferator-activated receptor gamma (PPARγ)-mediated transactivation in SW480 colon cancer cells and also it inhibited cell proliferation and modulated some cell cycle proteins.[10] While *N*-(3,5-bis(trifluoromethyl)phenyl)-5-chloro-2-hydroxybenzamide is an IKK^{β} inhibitor that inhibited NF-KB activity in HMC-1 cells, resulting in complete repression of growth factor-independent proliferation of mast cells. Inhibition of NF-KB activity decreased the expression of cyclin D_3 and the phosphorylation of pRb, leading to cell cycle arrest and apoptosis.[11] Apoptosis induction in tumoral cell lines has been also reported for molecules such as 4-(3-methoxyanilino)-2-(2-pyridinyl)-6-(trifluoromethyl)pyrimidine[12] and *N*-(2-aminoethyl)-4-[5-(4-tolyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl]benzene-

Keywords: Trifluoromethylpyridine; Ester derivatives; Amide derivatives; Synthesis; Antitumoral activity; SAR.
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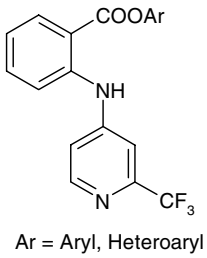


Figure 1. *N*-(2-(Trifluoromethyl)pyridin-4-yl)anthranilic acid derivatives.

sulfonamide.[13] We have recently reported on some derivatives of *N*-(2-(trifluoromethyl)pyridin-4-yl)anthranilic acid (Fig. 1) demonstrating antiproliferative activity in nanomolar to low micromolar concentrations against a wide array of human tumor cell lines.[14]

Prompted by the above findings, in continuation of our ongoing research on pyridine derivatives endowed with anticancer activity,[15–18] we became interested in novel compounds containing the trifluoromethylpyridine scaffold. In this paper, we report the synthesis and evaluation for in vitro antitumoral efficacy against human cancer cell lines of a series of ester and amide derivatives of 2-arylamino-6-trifluoromethyl-3-pyridinecarboxylic acids. The interest for these analogs is also derived from antitumor activities associated with the structure of 2-(substituted amino)-3-pyridinecarboxamide derivatives.[19–23]

2. Results and discussion

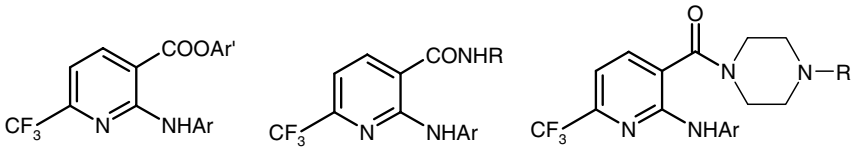
2.1. Chemistry

The target derivatives of 2-arylamino-6-trifluoromethyl-3-pyridinecarboxylic acids **8**–**58** (Table 1) were synthesized as shown in Schemes 1–3.

We have previously reported an easy and convenient method for the synthesis of 2-arylamino-6-trifluoromethyl-3-pyridinecarboxylic acids.[24]

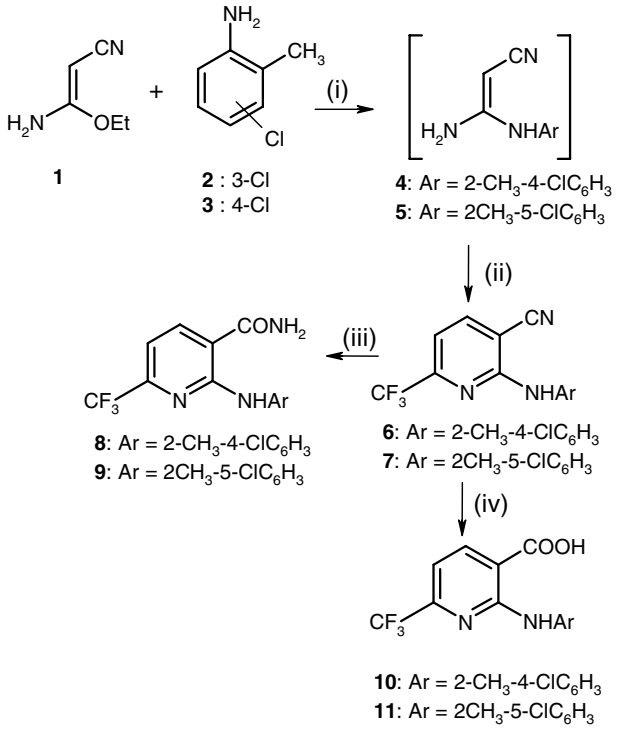
According to Scheme 1, 3-amino-3-ethoxypropenenitrile **1** was first treated with the appropriate substituted arylamines **2** and **3** in MeCN solution to give the non-isolable 3-amino-3-arylaminopropenenitriles **4**, **5** and then with 1,1,1-trifluoro-4-iso-butoxy-3-buten-2-one. The temperature was gradually allowed to reach 60 °C. After 3 h, pyridinecarbonitriles **6**, **7** are obtained in good yields. Sodium hydroxide catalyzed hydrolysis of compounds **6**, **7** afforded pyridine-3-carboxamides **8**, **9**. High

Table 1. Ester (**15**–**32**) and amide (**33**–**58**) derivatives of 2-arylamino-6-trifluoromethylpyridin-3-carboxylic acids



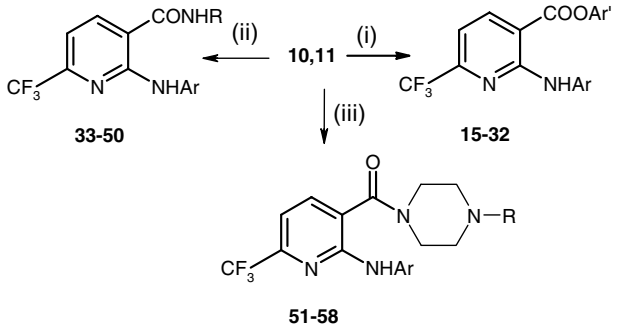
15-32 **33-50** **51-58**

Compound	Ar	Ar′	Compound	Ar	R
15	2-Me-4-Cl-phenyl	2-Cl-phenyl	**33**	2-Me-4-Cl-phenyl	3-Me-phenyl
16	2-Me-5-Cl-phenyl	2-Cl-phenyl	**34**	2-Me-5-Cl-phenyl	3-Me-phenyl
17	2-Me-4-Cl-phenyl	3-Cl-phenyl	**35**	2-Me-4-Cl-phenyl	3-CF_3-phenyl
18	2-Me-5-Cl-phenyl	3-Cl-phenyl	**36**	2-Me-5-Cl-phenyl	3-CF_3-phenyl
19	2-Me-4-Cl-phenyl	4-Cl-phenyl	**37**	2-Me-4-Cl-phenyl	3-MeO-phenyl
20	2-Me-5-Cl-phenyl	4-Cl-phenyl	**38**	2-Me-5-Cl-phenyl	3-MeO-phenyl
21	2-Me-4-Cl-phenyl	2,4-Cl_2-phenyl	**39**	2-Me-4-Cl-phenyl	3,4-$(MeO)_2$-phenyl
22	2-Me-5-Cl-phenyl	2,4-Cl_2-phenyl	**40**	2-Me-5-Cl-phenyl	3,4-$(MeO)_2$-phenyl
23	2-Me-4-Cl-phenyl	2,4,6-Cl_3-phenyl	**41**	2-Me-4-Cl-phenyl	3,5-$(MeO)_2$-phenyl
24	2-Me-5-Cl-phenyl	2,4,6-Cl_3-phenyl	**42**	2-Me-5-Cl-phenyl	3,5-$(MeO)_2$-phenyl
25	2-Me-4-Cl-phenyl	3-MeO-phenyl	**43**	2-Me-4-Cl-phenyl	3,4,5-$(MeO)_3$-phenyl
26	2-Me-5-Cl-phenyl	3-MeO-phenyl	**44**	2-Me-5-Cl-phenyl	3,4,5-$(MeO)_3$-phenyl
27	2-Me-4-Cl-phenyl	4-MeO-phenyl	**45**	2-Me-4-Cl-phenyl	3-Cl-phenyl
28	2-Me-5-Cl-phenyl	4-MeO-phenyl	**46**	2-Me-5-Cl-phenyl	3-Cl-phenyl
29	2-Me-4-Cl-phenyl	3,5-$(MeO)_2$-phenyl	**47**	2-Me-4-Cl-phenyl	3-Cl-4-OMe-phenyl
30	2-Me-5-Cl-phenyl	3,5-$(MeO)_2$-phenyl	**48**	2-Me-5-Cl-phenyl	3-Cl-4-OMe-phenyl
31	2-Me-4-Cl-phenyl	3-Pyridyl	**49**	2-Me-4-Cl-phenyl	3-Pyridyl
32	2-Me-5-Cl-phenyl	3-Pyridyl	**50**	2-Me-5-Cl-phenyl	3-Pyridyl
			51	2-Me-4-Cl-phenyl	COOEt
			52	2-Me-5-Cl-phenyl	COOEt
			53	2-Me-4-Cl-phenyl	3-Cl-phenyl
			54	2-Me-5-Cl-phenyl	3-Cl-phenyl
			55	2-Me-4-Cl-phenyl	4-F-phenyl
			56	2-Me-5-Cl-phenyl	4-F-phenyl
			57	2-Me-4-Cl-phenyl	4-MeO-phenyl
			58	2-Me-5-Cl-phenyl	4-MeO-phenyl



Scheme 1. Reagents and conditions: (i) MeCN, rt; (ii) 1,1,1-trifluoro-4-iso-butoxy-3-buten-2-one, reflux; (iii) 20% NaOH, 100 °C; (iv) H_2SO_4, H_2O, 100 °C.

Scheme 2. Reagents and conditions: (i) $ArNH_2$(**2**, **3**),MeCN, rt; (ii) 1,1,1-trifluoro-4-iso-butoxy-3-buten-2-one, reflux.



Scheme 3. Reagents and conditions: (i) Ar′OH, EDCI, HOBt, MeCN, rt; (ii) RNH_2, EDCI, HOBt, MeCN, rt; (iii) substituted piperazine, EDCI, HOBt, MeCN, rt.

yields of 2-arylamino-6-trifluoromethyl-3-pyridinecarboxylic acids **10**, **11** were achieved by hydrolysis of derivatives **6**, **7** in 50% aqueous sulfuric acid. A chemistry similar to that described in the synthesis of compounds **4**, **5** was used for the preparation of ethyl esters **13**, **14**. As reported in Scheme 2, ethyl 3-amino-3-ethoxypropenoate **12** was sequentially treated with the appropriate substituted arylamines **2**, **3**, then with 1,1,1-trifluoro-4-iso-butoxy-3-buten-2-one in MeCN solution to give pyridine derivatives **13**, **14**.

Aryl esters and amides were prepared as reported in Scheme 3. Treatment of acids **10**, **11** with the appropriate phenol in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) and hydroxybenzotriazole (HOBt) in MeCN solution gave esters **15**–**32** (Table 1) in 73–97% yields. Amide derivatives **33**–**58** (Table 1) were obtained in 73–96% yields by reaction of acids **10**, **11** with the appropriate amine by EDCI method.

All the newly synthesized compounds gave corrected analytical data. The IR and NMR spectral data were consistent with the assigned structure.

2.2. Pharmacology

The compounds **8**–**58** were submitted to the US National Cancer Institute (NCI; Bethesda, MD), for in vitro testing against a panel of approximately 60 tumor cell lines, derived from nine different cancer types: leukemia, lung, colon, CNS, melanoma, ovarian, renal, prostate, and breast. The compounds were tested at five concentrations at 10-fold dilution. A 48-h continuous drug exposure protocol was used and sulforhodamine B (SRB) protein assay was used to estimate cell growth. Details of this system and the information, which is encoded by the activity pattern over all cell lines, have been published.[25–27] The antitumoral activity of tested compounds is given by three parameters for each cell line: $\log GI_{50}$ value (GI_{50} = molar concentration of the compound that inhibits 50% net cell growth), $\log TGI$ value (TGI = molar concentration of the compound leading to total inhibition), and $\log LC_{50}$ value (LC_{50} = molar concentration of the compound leading to 50% net cell death). Furthermore, a mean graph midpoint (MG_MID) is calculated for each of the mentioned parameters, giving an averaged activity parameter over all cell lines. For the calculation of the MG_MID, insensitive cell lines are included with the highest concentration tested. Selectivity of the compound with respect to one or more cell lines of the screen is characterized by a high deviation (Δ) of the particular cell line parameter compared to the MG_MID value. From the analysis of the data reported in Tables 2 and 3 we can evince that acids **10**, **11** were inactive, while the primary amide **9** showed cytostatic activity against 36 different tumoral cell lines at micromolar GI_{50} values ($\log GI_{50}$ values between −5.84 and −5.07). Amide **9** displayed −5.18 MG_MID value with the best results against HS 578T cell line at GI_{50} ($\log GI_{50}$ −6.09) and TGI levels ($\log TGI$ −5.40). In the ester series, an aryl substituent appears to favorably modulate antiproliferative activity. Thus, replacement of phenyl moiety with an alkyl led to compounds **13** and **14** that had no antiproliferative activity. Furthermore, the presence of functionality as 4-chlorophenyl (compound **20**) or 3-methoxyphenyl (compound **26**) led to compounds endowed with high antitumoral activity (GI_{50} MG_MID

Table 2. Overview of the results[a] of the anticancer screening for compounds **9**, **20**, **26**, **35**–**37**, **43**, **44**, **47**, **51**, **52**, **54**, **56**, and **58**[b]

Compound	No. of the cell lines investigated	Number of the cell lines giving positive $\log GI_{50}$, $\log TGI$, and $\log LC_{50}$[c]								
		$\log GI_{50}$ (M)			$\log TGI$ (M)			$\log LC_{50}$ (M)		
		No.	Range	MG_MID[d]	No.	Range	MG_MID[d]	No.	Range	MG_MID[d]
9	54	54	−4.36 to −6.09	−5.18	37	−4.04 to −5.40	−4.37	14	−4.06 to −4.58	−4.06
20	56	56	−4.54 to −5.72	−5.03	44	−4.20 to −5.05	−4.45	32	−4.03 to −4.46	−4.11
26	56	54	−4.51 to −6.61	−5.10	36	−4.02 to −6.01	−4.36	11	−4.01 to −4.36	−4.03
35	57	57	−5.32 to −5.73	−5.50	57	−4.56 to −5.33	−4.89	52	−4.03 to −4.76	−4.28
36	59	59	−5.22 to −5.77	−5.51	58	−4.52 to −5.47	−4.91	55	−4.03 to −5.18	−4.34
37	52	52	−4.76 to −5.93	−5.08	52	−4.29 to −5.14	−4.61	46	−4.01 to −4.55	−4.25
43	57	57	−4.93 to −6.02	−5.40	52	−4.18 to −5.25	−4.63	30	−4.01 to −4.68	−4.12
44	59	59	−4.97 to −8.00	−5.54	59	−4.48 to −7.23	−4.78	52	−4.04 to −5.14	−4.27
47	60	60	−4.69 to −5.81	−5.34	60	−4.25 to −5.31	−4.77	52	−4.06 to −4.74	−4.26
51	59	21	−4.05 to −5.95	−4.23	1	−4.06	−4.00			
52	59	56	−4.13 to −5.86	−4.79	34	−4.02 to −4.66	−4.18	7	−4.06 to −4.18	−4.02
54	57	21	−4.06 to −5.51	−4.17						
56	59	59	−4.71 to −8.00	−5.01	59	−4.09 to −4.85	−4.51	45	−4.04 to −4.32	−4.15
58	58	58	−4.83 to −7.29	−5.22	58	−4.31 to −5.10	−4.63	48	−4.01 to −4.48	−4.18

[a] Data obtained from the NCI's in vitro disease-oriented human tumor cells screen (see Refs. 25–27 for details).

[b] Compounds **8**, **10**, **11**, **15**–**19**, **21**–**25**, **33**, **34**, **38**–**42**, **45**, **46**, **48**–**50**, **53**, **55**, and **57** were inactive.

[c] The response parameters: $\log GI_{50}$, $\log TGI$, and $\log LC_{50}$ are interpolated values representing the molar concentrations at which percentage growth is +50, 0 and −50, respectively.

[d] MG_MID = mean graph midpoint = arithmetical mean value for all tested cancer cell lines. If the indicated effect was not attainable within the used concentration interval, the highest concentration was used for the calculation.

values −5.03 and −5.10, respectively). Compounds **20** and **26** showed an in vitro chemosensitive profile toward **28** and **32** different cancer cell lines, respectively, with GI_{50} values lying in the concentration range between submicromolar to micromolar. Compound **26** displayed selectivity on renal cancer RXF 393 cell line (Table 3) at GI_{50} ($\log GI_{50}$ value −6.61) and TGI levels ($\log TGI$ value −6.01). Simply moving 4-chlorine or 3-methoxy groups into other position of phenyl ring (esters **15**–**18**) as well as the introduction of the same group on more position of phenyl ring (esters **21**–**24**) resulted in loss of activity. Among amide derivatives, piperazinocarbonyl compounds **52**–**54**, **56**, and **58** as well as aromatic amides **35**–**37**, **43**, **44**, and **47** exhibited moderate to excellent activity. In the piperazinocarbonyl series (**51**–**58**) the results indicated that inhibition of cell growth was strongly dependent on the kind of substituent at N-4 of piperazine ring. A comparison of substituent effects revealed that the introduction in this position of the ethoxycarbonyl group led to compound **52** that is endowed with weak antiproliferative activity (GI_{50} MG_MID values −4.79). Replacement of the ethoxycarbonyl by a 3-chlorophenyl ring (amides **53** and **54**) resulted in marginal reduction of activity (GI_{50} MG_MID values −4.23 and −4.17, respectively). However, substitution of the ethoxycarbonyl by a 4-fluorophenyl (amide **56**, GI_{50} MG_MID −5.01) or by a 4-methoxyphenyl ring (amide **58**, GI_{50} MG_MID −5.22) caused an increase in activity. Amide **58** inhibited the growth of 38 cell lines with micro and nanomolar GI_{50} values, showing high selectivity (Table 3) against ovarian cancer IGROV1 cell line ($\log GI_{50}$ −7.29, $\Delta \log GI_{50}$ 2.07). Amide **56** selectively exhibited high potency against CNS cancer cell line SNB-75 ($\log GI_{50} < -8$, $\Delta \log GI_{50}$ 2.99) (Table 3). In the aromatic amide series (**33**–**50**), the results indicated that the 3,4,5-trimethoxyphenyl substitution (compounds **43** and **44**) was crucial for potent cell growth inhibition. Compound **44** showed the best activity, as a matter of fact it inhibited the growth of all tested cell lines at submicromolar to micromolar concentrations with MG_MID value −5.54, and it is now under review by Biological Evaluation Committee of the NCI for possible further studies. Amide **44** displayed cytostatic activity at nanomolar concentrations against four cell lines: HOP-92, HCC-2998, CCRF-CEM, and MOLT-4 as well as high selectivity on the same cell lines ($\log GI_{50} < -8$, $\Delta \log GI_{50}$ 2.46). The growth of leukemia MOLT-4 and SR cell lines was totally inhibited by submicromolar concentrations of compound **44** ($\log TGI$ −7.38 and −6.52, $\Delta \log TGI$ 2.60 and 1.74, respectively). In addition, **44** was very active against leukemia HL-60 (TB) cell line at GI_{50} ($\log GI_{50}$ value −7.64, $\Delta \log GI_{50}$ 2.1), TGI ($\log TGI$ value −5.79, $\Delta \log TGI$ 1.01), and LC_{50} levels ($\log LC_{50}$ value −5.14). Although less potent when compared to **44**, compound **43** inhibited the growth of 50 cell lines at micromolar concentrations (GI_{50}MG_MID −5.40). Substitution of the 3,4,5-trimethoxyphenyl with a 3,4-dimethoxyphenyl or 3,5-dimethoxyphenyl moiety (compounds **39**, **40** and **41**, **42**, respectively) led to the loss of growth inhibition activity. However, a moderate loss of activity was observed with methoxy substituents at C-3 of phenyl ring (amide **37**, GI_{50}MG_MID −5.08).

Table 3. The in vitro activity[a] and selectivity toward most sensitive tumor cell lines for compounds **9**, **20**, **26**, **35**–**37**, **43**, **44**, **47**, **52**–**54**, **56**, and **58**

Compound	Most sensitive tumor cell lines	$\log GI_{50}$ (M)	log TGI (M)	$\log LC_{50}$ (M)	Selectivity toward tumor cell lines (Δ) for $\log GI_{50}$/TGI (M). The value is shown if $\Delta > 1$[b,c]	Mean value for all tested cell lines (MG_MID) for $\log GI_{50}$ (M)
9	Non-small cell lung: NCI-H226	−5.84	−5.01			−5.18
	Non-small cell lung: NCI-322M	−5.69				
	Melanoma: LOX IMVI	−5.65				
	Melanoma: SK-MEL-5	−5.72	−5.18			
	Breast: HS 578T	−6.09	−5.40			
20	Non-small cell lung: HOP-92	−5.72				−5.03
	Ovarian:OVCAR-3	−5.59	−5.05			
26	Leukemia: CCRF-CEM	−5.67	−5.29			−5.10
	Leukemia: K-562	−5.51	−5.03			
	Leukemia: MOLT-4	−5.51	−5.10			
	Non-small cell lung: HOP-92	−5.79	−5.16			
	Renal: RXF 393	−6.61	−6.01		1.51/1.65	
	Breast: HS 578T	−5.91	−5.32			
35	Leukemia: HL-60(TB)	−5.69	−5.33			−5.50
	Leukemia: SR	−5.59	−5.08			
	Non-small cell lung: EKVX	−5.73				
	Non-small cell lung: HOP-92	−5.66	−5.09			
	Colon: COLO205	−5.60	−5.21			
	Colon: HCT-15	−5.59	−5.13			
	Colon: KM12	−5.68				
	CNS: U251	−5.52	−5.01			
	Melanoma: LOX IMVI	−5.55	−5.07			
	Melanoma: MALME-3M	−5.70	−5.04			
	Melanoma: SK-MEL-5	−5.55	−5.03			
	Ovarian: SK-OV-3	−5.61				
	Renal: A498	−5.55	−5.13			
	Renal: ACHN	−5.56	−5.05			
	Breast: MCF7	−5.64	−5.11			
	Breast: MDA-MB-231/ATCC	−5.69	−5.09			
36	Leukemia: CCRF-CEM	−5.55				−5.51
	Leukemia: MOLT-4	−5.56	−5.00			
	Leukemia: RPMI-8226	−5.63				
	Non-small cell lung: HOP-92	−5.66	−5.06			
	Non-small cell lung: NCI-H460	−5.55	−5.00			
	Non-small cell lung: NCI-H522	−5.75	−5.20			
	Colon: COLO205	−5.76	−5.47			
	Colon: HCC2998	−5.75	−5.35			
	Colon: HCT-116	−5.62				
	Melanoma: SK-MEL-2	−5.61	−5.18			
	Melanoma: SK-MEL-5	−5.66	−5.35			
	Melanoma: UACC-257	−5.53	−5.08			
	Ovarian: IGROV1	−5.77				
	Ovarian: SK-OV-3	−5.76	−5.26			
	Renal: A498	−5.53	−5.07			
	Renal: RXF 393	−5.57	−5.10			
	Prostate: PC-3	−5.68				
	Breast: MCF7	−5.60	−5.02			
	Breast: NCI/ADR-RES	−5.67	−5.12			
	Breast: MDA-MB-231/ATCC	−5.64	−5.02			
	Breast: T-47D	−5.65	−5.05			
37	Non-small cell lung: NCI-H522	−5.89	−5.14			−5.08
	Ovarian:OVCAR-5	−5.60				
	Renal: A498	−5.78				
	Renal: CAKI-1	−5.56				
	Renal: RXF 393	−5.93				
	Renal: UO-31	−5.84	−5.08			
43	Leukemia: CCRF-CEM	−5.80				−5.40
	Leukemia: HL-60(TB)	−5.76				
	Non-small cell lung: H23	−5.66				
	Non-small cell lung: NCI-H522	−5.79				

(*continued on next page*)

Table 3 (*continued*)

Compound	Most sensitive tumor cell lines	logGI_{50} (M)	logTGI (M)	logLC_{50} (M)	Selectivity toward tumor cell lines (Δ) for logGI_{50}/TGI (M). The value is shown if $\Delta > 1$[b,c]	Mean value for all tested cell lines (MG_MID) for logGI_{50} (M)
43	Colon: HCC2998	−5.72	−5.25			
	Colon: KM12	−5.81				
	Ovarian: IGROV1	−6.02				
	Ovarian: OVCAR-3	−5.62				
	Prostate: PC-3	−5.73				
	Breast: MCF7	−5.62				
	Breast: T-47D	−5.61				
44	Leukemia: CCRF-CEM	<−8.00			2.46	−5.54
	Leukemia: HL-60(TB)	−7.64	−5.79	−5.14	2.10/1.01	
	Leukemia: K-562	−6.17				
	Leukemia: MOLT-4	<−8.00	−7.38		2.46/2.60	
	Leukemia: RPMI-8226	−6.34				
	Leukemia: SR	−7.73	−6.52		2.19/1.74	
	Non-small cell lung: EKVX	−5.56				
	Non-small cell lung: HOP-92	<−8.00	−5.78		2.46	
	Non-small cell lung: H23	−5.66				
	Non-small cell lung: NCI-H522	−5.64	−5.13			
	Colon: HCC2998	<−8.00	−5.27		2.46	
	Colon: HCT-116	−5.60				
	CNS: SNB-75	−5.97				
	Melanoma: LOX IMVI	−6.14	−5.62			
	Melanoma: SK-MEL-5	−5.93	−5.00			
	Melanoma: UACC-62	−6.02				
	Ovarian: IGROV1	−6.39				
	Ovarian: OVCAR-4	−5.76				
	Ovarian: SK-OV-3	−5.69				
	Renal: A498	−5.63				
	Renal: ACHN	−5.75				
	Renal: CAKI-1	−5.62				
	Prostate: PC-3	−5.92	−5.27			
	Breast: MDA-MB-231/ATCC	−5.78	−5.05			
	Breast: T-47D	−5.67	−5.02			
47	Leukemia: HL-60(TB)	−5.62	−5.27			−5.34
	Leukemia: K-562	−5.62				
	Leukemia: MOLT-4	−5.81	−5.31			
	Leukemia: RPMI-8226	−5.68	−5.31			
	Leukemia: SR	−5.77	−5.19			
	CNS: SF-295	−5.63				
	Breast: MCF7	−5.62				
	Breast: MDA-MB-231/ATCC	−5.78	−5.17			
52	CNS: SNB-75	−5.85			1.06	−4.79
	Ovarian: IGROV1	−5.86			1.07	
53	CNS: SNB-75	−5.95			1.72	−4.23
	Ovarian: IGROV1	−5.75			1.52	
54	Leukemia: SR	−5.56			1.34	−4.17
56	CNS: SNB-75	<−8.00			2.99	−5.01
	Ovarian: IGROV1	−6.88			1.87	
	Renal: CAKI-1	−5.82				
58	Non-small cell lung: HOP-92	−5.83	−5.10			−5.22
	Melanoma: SK-MEL-5	−6.02				
	Ovarian: IGROV1	−7.29			2.07	
	Renal: CAKI-1	−5.86				
	Renal: UO-31	−5.77				

[a] Data obtained from the NCI's in vitro disease-oriented human tumor cells screen (see Refs. 25–27 for details).
[b] The reported data represent the logarithmic difference between the parametric value referred to the most sensitive cell line and the same mean parameter, Δ is considered low if <1, moderate >1 and <3, high if >3.
[c] The value is shown if $\Delta > 1$.

Amide **37** totally inhibited the growth of lung cancer NCI-H522 cell line (logGI_{50} −5.89, logTGI −5.14) and renal cancer UO-31 cell line (logGI_{50} −5.84, logTGI −5.08). The replacement of C-3 methoxy with a C-3 methyl (amides **33** and **34**) and C-3 chlorine (amides **45** and **46**) gave inactive compounds. We observed the

opposite effect by substitution of C-3 methoxy with a C-3 trifluoromethyl (amides **35** and **36**, GI_{50}MG_MID −5.5) that caused an increase in activity with respect to amide **37** but a decrease in activity with respect to amides **43** and **44**. Activity intermediate between the activities of **44** and **37** was observed with **47**, bearing a 3-chloro-4-methoxyphenyl, that inhibited the growth of 55 cell lines at micromolar concentrations (GI_{50}MG_MID −5.34). As a general trend, esters and amides bearing the 2-(5-chloro-2-methylphenylamino) substituent on pyridine ring are significantly more potent than the isomeric 2-(4-chloro-2-methylphenylamino) analogs.

A COMPARE[28] analysis was performed with the more active compounds to investigate whether they resemble anticancer drugs of the NCI standard agent database and to probably predict its mechanism of action. The COMPARE algorithm was developed to determine the degree of similarity of mean graph fingerprints obtained from the in vitro anticancer screen with patterns of activity of standard agents. The hypothesis is that, if the data pattern of a compound correlates well with the data pattern of compounds belonging to the standard agent database, the compound of interest may have the same mechanism of action as those agents with known mechanism. A Pearson's correlation coefficient (PCC) of 0.55–0.6 is considered the lowest correlation that suggests a relationship with another compound.[27] When tested as seeds against the NCI 'Standard Agents' Database (Table 4), compounds **37**, **43**, **52**, and **56** showed a response pattern that correlated their activity to those of DNA antimetabolite agents, including 6-methoxyguanosine and 2α-deoxy-6-thioguanosine (PCC > 0.60 at GI_{50} level).

COMPARE analysis also indicates that compounds **43**, **44**, **52**, and **47** shared a response pattern with topoisomerase II inhibitors, including menogaril, chloroquinoxaline sulfonamide, and dactinomycin. Using GI_{50} values of compounds **26** and **43** as seed, COMPARE analysis showed correlation with the inhibitor of protein synthesis anguidin, while using those of compounds **56** and **44** a correlation was found with proapoptotic agents such as didemnin (caspase activator), triciribine (inhibitor of the Akt signaling pathway), and L-buthionine sulfoximine (irreversible inhibitor of γ-glutamylcysteine synthetase).

All in all COMPARE analysis indicates that the tested compounds might exert their antiproliferative activity through alteration and/or inhibition of processes crucial for the cell cycle progression.

In summary, a series of ester and amide derivatives of 2-arylamino-6-trifluoromethyl-3-pyridinecarboxylic acids have been successfully synthesized in good yield. They were screened for their activity against a panel of about 60 human tumor cell lines. Most of them possess encouraging anticancer activity having GI_{50} values in the low micromolar to nanomolar concentration range. The cell lines derived from leukemia, renal, lung, and ovarian cancers appeared to be the most sensitive to the new agents herein described.

3. Experimental

3.1. Chemistry

Melting points were determined on a Stuart Scientific Melting point SMP1 and are uncorrected. Proton NMR spectra were recorded on a Varian Unity 300 spectrometer. The chemical shift are reported in parts per million (δ, ppm) downfield from tetramethylsilane (TMS), which was used as internal standard. Infrared spectra were obtained with a Bruker Vector 22 spectrophotometer. Elemental analyses were carried out with a Carlo Erba model 1106 Elemental Analyzer and the values found were within 0.4% of theoretical values. The compounds **1**,[29] **6**, **7**, **9–11**, **14**,[24] **12**,[30] and 1,1,1-tri-

Table 4. COMPARE correlation coefficients (PCC) using GI_{50} values of compounds **9**, **26**, **31**, **37**, **43**, **44**, **47**, **52**, **56**, and **58** as seeds, tested in the US NCI 60 Cell lines in vitro screen

Compound	Standard agent	Endpoint level	PCC	No. of common cell lines
9	Rifamicin	GI_{50}	0.656	52
26	Anguidin	GI_{50}	0.855	45
31	Flavone acetic acid	GI_{50}	0.589	27
37	6-Methoxyguanine	GI_{50}	0.645	42
43	Anguidin	GI_{50}	0.491	57
	Chloroquinoxaline sulfonamide	GI_{50}	0.810	11
	6-Methoxyguanine	GI_{50}	0.780	11
44	Dihydrolenperone	GI_{50}	0.730	23
	Dactinomycin	TGI	0.811	19
	Triciribine	TGI	0.761	23
	L-Buthionine sulfoximine	GI_{50}	0.631	23
	L-Buthionine sulfoximine	TGI	0.731	23
47	Menogaril	GI_{50}	0.578	21
52	α-2′-Deoxythioguanosine	GI_{50}	0.610	25
	Chloroquinoxaline sulfonamide	GI_{50}	0.930	11
56	α-2′-Deoxythioguanosine	GI_{50}	0.810	50
	Didemnin B	GI_{50}	0.665	59
58	Chloroquinoxaline sulfonamide	GI_{50}	0.616	37

fluoro-4-iso-butoxy-3-buten-2-one[31] were obtained with previously described procedures.

3.1.1. 2-((4-Chloro-2-methylphenyl)amino)-6-trifluoromethylpyridine-3-carboxamide (8). Pyridine-3-carbonitrile **6** (3.11 g, 10 mmol) was added 20% aqueous sodium hydroxide solution (10 mL). The resulting mixture was heated in boiling water bath for 4 h. After cooling the reaction mixture was diluted with water (50 mL) and the formed precipitate was separated by filtration, dried, and crystallized from toluene. Yield 85%. Mp 234–235 °C. ^{1}H NMR (DMSO-d_6): δ 2.28 (s, 3H, CH_3), 7.13–8.45 (m, 7H, aryl and NH_2), 10.43 (s, 1H, NH). IR (Nujol) 3488, 3165, 3124, 1663, 1619, 1592 cm^{-1}. Anal. Calcd for $C_{14}H_{11}ClF_3N_3O$: C, 51.00; H, 3.36; N, 12.74. Found: C, 50.97; H, 3.35; N, 12.77.

3.1.2. Ethyl 2-((4-chloro-2-methylphenyl)amino)-6-trifluoromethylpyridine-3-carboxylate (13). 4-Chloro-2-methylaniline (1.41 g, 10 mmol) was added to a solution of ethyl 3-amino-3-ethoxypropenoate **12** (1.60 g, 10 mmol) in anhydrous acetonitrile (20 mL). The resulting solution was kept at room temperature for 6 days and then 1,1,1-trifluoro-4-iso-butoxy-3-buten-2-one (1.96 g, 10 mmol) was added. The resulting mixture was stirred at room temperature for 0.5 h and then refluxed for 3 h. Then solvent was removed to dryness and the resulting residue was treated with isopropyl ether, separated by filtration, and crystallized from *n*-hexane. Yield 80%. Mp 99–110 °C. ^{1}H NMR (DMSO-d_6): δ 1.30 (t, J = 7.0 Hz, 3H, CH_3), 2.23 (s, 3H, CH_3), 4.33 (q, J = 7.0 Hz, 2H, CH_2), 7.26 (m, 3H, aryl), 8.06 (d, 1H, J = 8.8 Hz, pyridyl), 8.46 (d, 1H, J = 8.8 Hz, pyridyl), 10.04 (s, 1H, NH). IR (Nujol) 3280, 1696, 1620 cm^{-1}. Anal. Calcd for $C_{16}H_{14}ClF_3N_2O_2$: C, 53.57; H, 3.93; N, 7.81. Found: C, 53.61; H, 3.94; N, 7.78.

3.1.3. General procedure for the synthesis of esters (15–32). A mixture of acids **10** or **11** (0.33 g, 1 mmol), EDCI (1.92 g, 1.1 mmol), and HOBt (0.13 g, 1 mmol) in dry MeCN (10 mL) was stirred at room temperature for 30 min and then treated with the appropriate phenol (1 mmol). The mixture was stirred at room temperature for an additional 24 h. Then the solution was evaporated to dryness in vacuo. The residue was dissolved in ethyl acetate (20 mL) and washed with brine (2× 5 mL), 5% aqueous sodium hydroxide (2× 5 mL), and water (2× 5 mL). The organic layer was dried over anhydrous magnesium sulfate. Concentration of the dried extract yielded a residue which was triturated with isopropyl ether. The formed precipitate was filtered off and purified by crystallization from the adequate solvent to give the ester derivatives **15**–**32**.

3.1.3.1. 2-Chlorophenyl 2-(4-chloro-2-methylphenylamino)-6-(trifluoromethyl)nicotinate (15). Yield 86%. Mp 194–195 °C (2-propanol). ^{1}H NMR (DMSO-d_6): δ 2.20 (s, 3H, CH_3), 7.24–7.65 (m, 7H, aryl), 8.03 (d, 1H, J = 8.8 Hz, pyridyl), 8.77 (d, 1H, J = 8.8 Hz, pyridyl), 9.74 (s, 1H, NH). IR (Nujol) 3344, 3311, 1712, 1605, 1590 cm^{-1}. Anal. Calcd for $C_{20}H_{13}Cl_2F_3N_2O_2$: C, 54.44; H, 2.97; N, 6.35. Found: C, 54.37; H, 2.98; N, 6.37.

3.1.3.2. 2-Chlorophenyl 2-(5-chloro-2-methylphenylamino)-6-(trifluoromethyl)nicotinate (16). Yield 94%. Mp 159–160 °C (cyclohexane). ^{1}H NMR ($CDCl_3$): δ 2.31 (s, 3H, CH_3), 7.04–8.72 (m, 9H, aryl and pyridyl), 9.92 (s, 1H, NH). IR (Nujol) 3313, 1712, 1667, 1618, 1591 cm^{-1}. Anal. Calcd for $C_{20}H_{13}Cl_2F_3N_2O_2$: C, 54.44; H, 2.97; N, 6.35. Found: C, 54.48; H, 2.99; N, 6.33.

3.1.3.3. 3-Chlorophenyl 2-(4-chloro-2-methylphenylamino)-6-(trifluoromethyl)nicotinate (17). Yield 86%. Mp 177–180 °C (cyclohexane). ^{1}H NMR ($CDCl_3$): δ 2.29 (s, 3H, CH_3), 7.11–7.40 (m, 7H, aryl), 8.22 (d, 1H, J = 6.7 Hz, pyridyl), 8.59 (d, 1H, J = 6.7 Hz, pyridyl), 9.93 (s, 1H, NH). IR (Nujol) 3340, 3310, 1712, 1678, 1618, 1590 cm^{-1}. Anal. Calcd for $C_{20}H_{13}Cl_2F_3N_2O_2$: C, 54.44; H, 2.97; N, 6.35. Found: C, 54.41; H, 2.98; N, 6.37.

3.1.3.4. 3-Chlorophenyl 2-(5-chloro-2-methylphenylamino)-6-(trifluoromethyl)nicotinate (18). Yield 94%. Mp 179–180 °C (cyclohexane). ^{1}H NMR ($CDCl_3$): δ 2.33 (s, 3H, CH_3), 7.15–7.44 (m, 7H, aryl), 8.26 (d, 1H, J = 6.7 Hz, pyridyl), 8.63 (d, 1H, J = 6.7 Hz, pyridyl), 9.97 (s, 1H, NH). IR (Nujol) 3341, 3306, 1713, 1619, 1590 cm^{-1}. Anal. Calcd for $C_{20}H_{13}Cl_2F_3N_2O_2$: C, 54.44; H, 2.97; N, 6.35. Found: C, 54.49; H, 2.99; N, 6.32.

3.1.3.5. 4-Chlorophenyl 2-(4-chloro-2-methylphenylamino)-6-(trifluoromethyl)nicotinate (19). Yield 97%. Mp 164–165 °C (2-propanol). ^{1}H NMR (DMSO-d_6): δ 2.19 (s, 3H, CH_3), 7.22–7.52 (m, 7H, aryl), 8.20 (d, 1H, J = 8.1 Hz, pyridyl), 8.70 (d, 1H, J = 8.1 Hz, pyridyl), 9.77 (s, 1H, NH). IR (Nujol) 3343, 3301, 1717, 1620, 1590 cm^{-1}. Anal. Calcd for $C_{20}H_{13}Cl_2F_3N_2O_2$: C, 54.44; H, 2.97; N, 6.35. Found: C, 54.49; H, 2.96; N, 6.31.

3.1.3.6. 4-Chlorophenyl 2-(5-chloro-2-methylphenylamino)-6-(trifluoromethyl)nicotinate (20). Yield 91%. Mp 168–170 °C (cyclohexane). ^{1}H NMR ($CDCl_3$): δ 2.31 (s, 3H, CH_3), 7.04–7.19 (m, 7H, aryl), 7.87 (d, 1H, J = 6.1 Hz, pyridyl), 8.08 (d, 1H, J = 6.1 Hz, pyridyl), 8.68 (s, 1H, NH). IR (Nujol) 3550, 3306, 1667, 1614, 1597 cm^{-1}. Anal. Calcd for $C_{20}H_{13}Cl_2F_3N_2O_2$: C, 54.44; H, 2.97; N, 6.35. Found: C, 54.39; H, 2.98; N, 6.38.

3.1.3.7. 2,4-Dichlorophenyl 2-(4-chloro-2-methylphenylamino)-6-(trifluoromethyl)nicotinate (21). Yield 73%. Mp 184–185 °C (2-propanol). ^{1}H NMR (DMSO-d_6): δ 2.20 (s, 3H, CH_3), 7.25–7.57 (m, 6H, aryl), 8.01 (d, 1H, J = 6.4 Hz, pyridyl), 8.76 (d, 1H, J = 6.4 Hz, pyridyl), 9.70 (s, 1H, NH). IR (Nujol) 3348, 3310, 1720, 1620, 1591 cm^{-1}. Anal. Calcd for $C_{20}H_{12}Cl_3F_3N_2O_2$: C, 50.50; H, 2.54; N, 5.89. Found: C, 50.43; H, 2.55; N, 5.94.

3.1.3.8. 2,4-Dichlorophenyl 2-(5-chloro-2-methylphenylamino)-6-(trifluoromethyl)nicotinate (22). Yield 82%. Mp 164–165 °C (cyclohexane). ^{1}H NMR ($CDCl_3$): δ 2.31 (s, 3H, CH_3), 7.04–7.54 (m, 6H, aryl), 8.25 (d, 1H,

J = 6.3 Hz, pyridyl), 8.68 (d, 1H, J = 6.3 Hz, pyridyl), 9.87 (s, 1H, NH). IR (Nujol) 3301, 1720, 1666, 1620, 1592 cm^{-1}. Anal. Calcd for $C_{20}H_{12}Cl_3F_3N_2O_2$: C, 50.50; H, 2.54; N, 5.89. Found: C, 50.54; H, 2.55; N, 5.86.

3.1.3.9. 2,4,6-Trichlorophenyl 2-(4-chloro-2-methylphenylamino)-6-(trifluoromethyl)nicotinate (23). Yield 78%. Mp 179–180 °C (cyclohexane). ^1H NMR ($CDCl_3$): δ 2.30 (s, 3H, CH_3), 7.04–7.46 (m, 5H, aryl), 8.26 (d, 1H, J = 6.7 Hz, pyridyl), 8.70 (d, 1H, J = 6.7 Hz, pyridyl), 9.78 (s, 1H, NH). IR (Nujol) 3324, 1725, 1621, 1591 cm^{-1}. Anal. Calcd for $C_{20}H_{11}Cl_4F_3N_2O_2$: C, 47.09; H, 2.17; N, 5.49. Found: C, 47.03; H, 2.19; N, 5.52.

3.1.3.10. 2,4,6-Trichlorophenyl 2-(5-chloro-2-methylphenylamino)-6-(trifluoromethyl)nicotinate (24). Yield 84%. Mp 180–182 °C (cyclohexane). ^1H NMR ($CDCl_3$) δ 2.32 (s, 3H, CH_3), 7.16–8.73 (m, 7H, aryl and pyridyl), 9.80 (s, 1H, NH). IR (Nujol) 3322, 3301, 1725, 1621, 1592 cm^{-1}. Anal. Calcd for $C_{20}H_{11}Cl_4F_3N_2O_2$: C, 47.09; H, 2.17; N, 5.49. Found: C, 47.15; H, 2.18; N, 5.46.

3.1.3.11. 3-Methoxyphenyl 2-(4-chloro-2-methylphenylamino)-6-(trifluoromethyl)nicotinate (25). Yield 87%. Mp 184–185 °C (2-propanol). ^1H NMR (DMSO-d_6): δ 2.23 (s, 3H, CH_3), 3.55 (s, 3H, OCH_3), 7.20–7.66 (m, 7H, aryl), 8.16 (d, 1H, J = 6.9 Hz, pyridyl), 8.41 (d, 1H, J = 6.9 Hz, pyridyl), 10.57 (s, 1H, NH). IR (Nujol) 3344, 1748, 1705, 1621, 1591 cm^{-1}. Anal. Calcd for $C_{21}H_{16}ClF_3N_2O_3$: C, 57.74; H, 3.69; N, 6.41. Found: C, 57.79; H, 3.70; N, 6.38.

3.1.3.12. 3-Methoxyphenyl 2-(5-chloro-2-methylphenylamino)-6-(trifluoromethyl)nicotinate (26). Yield 88%. Mp 164–165 °C (cyclohexane). ^1H NMR ($CDCl_3$): δ 2.32 (s, 3H, CH_3), 3.85 (s, 3H, OCH_3), 6.76–7.40 (m, 7H, aryl), 8.25 (d, 1H, J = 6.3 Hz, pyridyl), 8.65 (d, 1H, J = 6.3 Hz, pyridyl), 10.03 (s, 1H, NH). IR (Nujol) 3337, 3309, 1705, 1619, 1590 cm^{-1}. Anal. Calcd for $C_{21}H_{16}ClF_3N_2O_3$: C, 57.74; H, 3.69; N, 6.41. Found: C, 57.80; H, 3.70; N, 6.38.

3.1.3.13. 4-Methoxyphenyl 2-(4-chloro-2-methylphenylamino)-6-(trifluoromethyl)nicotinate (27). Yield 80%. Mp 169–170 °C (2-propanol). ^1H NMR (DMSO-d_6): δ 2.22 (s, 3H, CH_3), 3.74 (s, 3H, OCH_3), 6.97–7.36 (m, 7H, aryl), 8.10 (d, 1H, J = 8.1 Hz, pyridyl), 8.71 (d, 1H, J = 8.1 Hz, pyridyl), 9.91 (s, 1H, NH). IR (Nujol) 3336, 3299, 1713, 1619, 1590 cm^{-1}. Anal. Calcd for $C_{21}H_{16}ClF_3N_2O_3$: C, 57.74; H, 3.69; N, 6.41. Found: C, 57.69; H, 3.68; N, 6.42.

3.1.3.14. 4-Methoxyphenyl 2-(5-chloro-2-methylphenylamino)-6-(trifluoromethyl)nicotinate (28). Yield 92%. Mp 171–173 °C (2-propanol). ^1H NMR (DMSO-d_6): δ 2.21 (s, 3H, CH_3), 3.74 (s, 3H, OCH_3), 6.97–7.35 (m, 7H, aryl), 8.09 (d, 1H, J = 8.1 Hz, pyridyl), 8.70 (d, 1H, J = 8.1 Hz, pyridyl), 9.90 (s, 1H, NH). IR (Nujol) 3336, 3298, 1713, 1619, 1590 cm^{-1}. Anal. Calcd for $C_{21}H_{16}ClF_3N_2O_3$: C, 57.74; H, 3.69; N, 6.41. Found: C, 57.79; H, 3.70; N, 6.37.

3.1.3.15. 3,5-Dimethoxyphenyl 2-(4-chloro-2-methylphenylamino)-6-(trifluoromethyl)nicotinate (29). Yield 87%. Mp 184–185 °C (2-propanol). ^1H NMR (DMSO-d_6): δ 2.19 (s, 3H, CH_3), 3.72 (s, 6H, OCH_3), 6.44 (s, 1H, aryl), 6.53 (s, 2H, aryl), 7.24–7.36 (m, 3H, aryl), 8.07 (d, 1H, J = 8.1 Hz, pyridyl), 8.68 (d, 1H, J = 8.1 Hz, pyridyl), 9.86 (s, 1H, NH). IR (Nujol) 3336, 3295, 3223, 3148, 3114, 1703, 1617 cm^{-1}. Anal. Calcd for $C_{22}H_{18}ClF_3N_2O_4$: C, 56.60; H, 3.89; N, 6.00. Found: C, 56.67; H, 3.90; N, 5.96.

3.1.3.16. 3,5-Dimethoxyphenyl 2-(5-chloro-2-methylphenylamino)-6-(trifluoromethyl)nicotinate (30). Yield 77%. Mp 185–186 °C (2-propanol). ^1H NMR (DMSO-d_6): δ 2.22 (s, 3H, CH_3), 3.71 (s, 6H, OCH_3), 6.44–7.36 (m, 6H, aryl), 8.06 (d, 1H, J = 8.1 Hz, pyridyl), 8.68 (d, 1H, J = 8.1 Hz, pyridyl), 9.85 (s, 1H, NH). IR (Nujol) 3334, 3295, 1704, 1619 cm^{-1}. Anal. Calcd for $C_{22}H_{18}ClF_3N_2O_4$: C, 56.60; H, 3.89; N, 6.00. Found: C, 56.54; H, 3.88; N, 6.04.

3.1.3.17. 3-Pyridyl 2-(4-chloro-2-methylphenylamino)-6-(trifluoromethyl)nicotinate (31). Yield 88%. Mp 194–195 °C (2-propanol). ^1H NMR (DMSO-d_6): δ 2.22 (s, 3H, CH_3), 7.24–8.75 (m, 9H, aryl and pyridyl), 9.77 (s, 1H, NH). IR (Nujol) 3348, 3305, 1720, 1624, 1592 cm^{-1}. Anal. Calcd for $C_{19}H_{13}ClF_3N_3O_2$: C, 55.96; H, 3.21; N, 10.30. Found: C, 55.91; H, 3.22; N, 10.34.

3.1.3.18. 3-Pyridyl 2-(5-chloro-2-methylphenylamino)-6-(trifluoromethyl)nicotinate (32). Yield 86%. Mp 184–185 °C (2-propanol). ^1H NMR (DMSO-d_6): δ 2.21 (s, 3H, CH_3), 7.25–8.76 (m, 9H, aryl and pyridyl), 9.80 (s, 1H, NH). IR (Nujol) 3345, 3304, 1720, 1668, 1624, 1592 cm^{-1}. Anal. Calcd for $C_{19}H_{13}ClF_3N_3O_2$: C, 55.96; H, 3.21; N, 10.30. Found: C, 56.01; H, 3.20; N, 10.27.

3.1.4. General procedure for the synthesis of amides (33–58). A mixture of acids **10** or **11** (0.33 g, 1 mmol), EDCI (1.92 g, 1.1 mmol), and HOBt (0.13 g, 1 mmol) in dry MeCN (10 mL) was stirred at room temperature for 30 min and then treated with the appropriate amine (1 mmol). The mixture was stirred at room temperature for an additional 24 h. Then the solution was evaporated to dryness in vacuo. The residue was dissolved in ethyl acetate (20 mL) and washed sequentially with brine (2× 5 mL), 10% aqueous sodium carbonate (2× 5 mL), 10% aqueous citric acid (2× 5 mL), and water (2× 5 mL). The organic layer was dried over anhydrous magnesium sulfate. Concentration of the dried extracts yielded a solid residue which was washed with ethyl ether, filtered off, and dried to give the amide derivatives in analytically pure form without additional purification by crystallization if not elsewhere indicated.

3.1.4.1. 2-(4-Chloro-2-methylphenylamino)-*N*-(3-methylphenyl)-6-(trifluoromethyl)nicotinamide (33). Yield 95%. Mp 179–180 °C. ^1H NMR (DMSO-d_6): δ 2.25 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 6.94–7.48 (m, 7H, aryl), 8.11 (d, 1H, J = 8.1 Hz, pyridyl), 8.43 (d, 1H,

J = 8.1 Hz, pyridyl), 10.29 (s, 1H, NH), 10.55 (s, 1H, NH). IR (Nujol) 3293, 1639, 1609 cm^{-1}. Anal. Calcd for $C_{21}H_{17}ClF_3N_3O$: C, 60.08; H, 4.08; N, 10.01. Found: C, 60.14; H, 4.06; N, 10.04.

3.1.4.2. 2-(5-Chloro-2-methylphenylamino)-*N*-(3-methylphenyl)-6-(trifluoromethyl)nicotinamide (34). Yield 85%. Mp 174–175 °C (cyclohexane). ^1H NMR (DMSO-d_6): δ 2.24 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 6.93–7.48 (m, 7H, aryl), 8.11 (d, 1H, J = 7.7 Hz, pyridyl), 8.42 (d, 1H, J = 7.7 Hz, pyridyl), 10.29 (s, 1H, NH), 10.54 (s, 1H, NH). IR (Nujol) 3294, 1639, 1610 cm^{-1}. Anal. Calcd for $C_{21}H_{16}ClF_3N_2O_2$: C, 59.94; H, 3.83; N, 6.66. Found: C, 60.00; H, 3.82; N, 6.70.

3.1.4.3. 2-(4-Chloro-2-methylphenylamino)-6-(trifluoromethyl)-*N*-(3-(trifluoromethyl)phenyl)nicotinamide (35). Yield 77%. Mp 130–132 °C. ^1H NMR (DMSO-d_6): δ 2.24 (s, 3H, CH_3), 7.06–8.46 (m, 9H, aryl and pyridyl), 10.12 (s, 1H, NH), 10.89 (s, 1H, NH). IR (Nujol) 3452, 3184, 1671, 1613, 1536 cm^{-1}. Anal. Calcd for $C_{21}H_{14}ClF_6N_3O$: C, 53.24; H, 2.98; N, 8.87. Found: C, 53.29; H, 2.97; N, 8.83.

3.1.4.4. 2-(5-Chloro-2-methylphenylamino)-6-(trifluoromethyl)-*N*-(3-(trifluoromethyl)phenyl)nicotinamide (36). Yield 73%. Mp 149–150 °C (cyclohexane). ^1H NMR (DMSO-d_6): δ 2.23 (s, 3H, CH_3), 7.18–7.74 (m, 5H, aryl), 7.95–8.07 (m, 3H, aryl and pyridyl), 8.44 (m, 1H, pyridyl), 10.12 (s, 1H, NH), 10.86 (s, 1H, NH). IR (Nujol) 3453, 3233, 3182, 3133, 1670, 1613, 1537 cm^{-1}. Anal. Calcd for $C_{21}H_{14}ClF_6N_3O$: C, 53.24; H, 2.98; N, 8.87. Found: C, 53.20; H, 2.99; N, 8.90.

3.1.4.5. 2-(4-Chloro-2-methylphenylamino)-6-(trifluoromethyl)-*N*-(3-(methoxy)phenyl)nicotinamide (37). Yield 73%. Mp 155–156 °C (cyclohexane). ^1H NMR ($CDCl_3$): δ 2.34 (s, 3H, CH_3), 3.84 (s, 3H, OCH_3), 6.74–8.29 (m, 9H, aryl and pyridyl), 10.21 (s, 1H, NH), 10.53 (s, 1H, NH). IR (Nujol) 3362, 3273, 1642, 1613, 1595 cm^{-1}. Anal. Calcd for $C_{21}H_{17}ClF_3N_3O_2$: C, 57.87; H, 3.93; N, 9.64. Found: C, 57.82; H, 3.94; N, 9.67.

3.1.4.6. 2-(5-Chloro-2-methylphenylamino)-6-(trifluoromethyl)-*N*-(3-(methoxy)phenyl)nicotinamide (38). Yield 83%. Mp 154–155 °C. ^1H NMR (DMSO-d_6): δ 2.25 (s, 3H, CH_3), 3.72 (s, 3H, OCH_3), 6.80–7.36 (m, 7H, aryl), 8.10 (d, 1H, J = 7.7 Hz, pyridyl), 8.42 (d, 1H, J = 7.7 Hz, pyridyl), 10.25 (s, 1H, NH), 10.59 (s, 1H, NH). IR (Nujol) 3361, 3273, 1642, 1613, 1595 cm^{-1}. Anal. Calcd for $C_{21}H_{17}ClF_3N_3O_2$: C, 57.87; H, 3.93; N, 9.64. Found: C, 57.93; H, 3.92; N, 9.60.

3.1.4.7. 2-(4-Chloro-2-methylphenylamino)-6-(trifluoromethyl)-*N*-(3,4-(dimethoxy)phenyl)nicotinamide (39). Yield 86%. Mp 174–175 °C. ^1H NMR (DMSO-d_6): δ 2.24 (s, 3H, CH_3), 3.71 (s, 6H, OCH_3), 6.91–7.35 (m, 6H, aryl), 8.11 (d, 1H, J = 8.8 Hz, pyridyl), 8.41 (d, 1H, J = 8.8 Hz, pyridyl), 10.38 (s, 1H, NH), 10.50 (s, 1H, NH). IR (Nujol) 3370, 3286, 1637, 1611, 1598 cm^{-1}. Anal. Calcd for $C_{22}H_{19}ClF_3N_3O_3$: C, 56.72; H, 4.11; N, 9.02. Found: C, 56.67; H, 4.10; N, 9.06.

3.1.4.8. 2-(5-Chloro-2-methylphenylamino)-6-(trifluoromethyl)-*N*-(3,4-(dimethoxy)phenyl)nicotinamide (40). Yield 79%. Mp 156–157 °C. ^1H NMR (DMSO-d_6): δ 2.24 (s, 3H, CH_3), 3.71 (s, 6H, OCH_3), 6.94–7.35 (m, 6H, aryl), 8.12 (d, 1H, J = 8.4 Hz, pyridyl), 8.41 (d, 1H, J = 8.4 Hz, pyridyl), 10.37 (s, 1H, NH), 10.49 (s, 1H, NH). IR (Nujol) 3371, 3284, 1637, 1611, 1598 cm^{-1}. Anal. Calcd for $C_{22}H_{19}ClF_3N_3O_3$: C, 56.72; H, 4.11; N, 9.02. Found: C, 56.78; H, 4.12; N, 8.97.

3.1.4.9. 2-(4-Chloro-2-methylphenylamino)-6-(trifluoromethyl)-*N*-(3,5-(dimethoxy)phenyl)nicotinamide (41). Yield 90%. Mp 148–150 °C. ^1H NMR (DMSO-d_6): δ 2.25 (s, 3H, CH_3), 3.70 (s, 6H, OCH_3), 6.29 (s, 1H, aryl), 6.93 (s, 2H, aryl), 7.20–7.35 (m, 3H, aryl), 8.08 (d, 1H, J = 8.1 Hz, pyridyl), 8.40 (d, 1H, J = 8.1 Hz, pyridyl), 10.20 (s, 1H, NH), 10.53 (s, 1H, NH). IR (Nujol) 3429, 3402, 3248, 1661, 1613 cm^{-1}. Anal. Calcd for $C_{22}H_{19}ClF_3N_3O_3$: C, 56.72; H, 4.11; N, 9.02. Found: C, 56.66; H, 4.10; N, 9.05.

3.1.4.10. 2-(5-Chloro-2-methylphenylamino)-6-(trifluoromethyl)-*N*-(3,5-(dimethoxy)phenyl)nicotinamide (42). Yield 96%. Mp 123–125 °C. ^1H NMR (DMSO-d_6): δ 2.25 (s, 3H, CH_3), 3.70 (s, 6H, OCH_3), 6.29 (s, 1H, aryl), 6.93 (s, 2H, aryl), 7.20–7.35 (m, 3H, aryl), 8.08 (d, 1H, J = 8.1 Hz, pyridyl), 8.40 (d, 1H, J = 8.1 Hz, pyridyl), 10.20 (s, 1H, NH), 10.53 (s, 1H, NH). IR (Nujol) 3401, 3246, 1661, 1610 cm^{-1}. Anal. Calcd for $C_{22}H_{19}ClF_3N_3O_3$: C, 56.72; H, 4.11; N, 9.02. Found: C, 56.68; H, 4.10; N, 8.99.

3.1.4.11. 2-(4-Chloro-2-methylphenylamino)-6-(trifluoromethyl)-*N*-(3,4,5-(trimethoxy)phenyl)nicotinamide (43). Yield 84%. Mp 208–210 °C. ^1H NMR (DMSO-d_6): δ 2.24 (s, 3H, CH_3), 3.61 (s, 3H, OCH_3), 3.73 (s, 6H, OCH_3), 7.06 (m, 2H, aryl), 7.20–7.35 (m, 3H, aryl), 8.05 (d, 1H, J = 7.7 Hz, pyridyl), 8.40 (d, 1H, J = 7.7 Hz, pyridyl), 10.22 (s, 1H, NH), 10.53 (s, 1H, NH). IR (Nujol) 3411, 3118, 1666, 1610, 1540 cm^{-1}. Anal. Calcd for $C_{23}H_{21}ClF_3N_3O_4$: C, 55.71; H, 4.27; N, 8.47. Found: C, 55.80; H, 4.28; N, 8.43.

3.1.4.12. 2-(5-Chloro-2-methylphenylamino)-6-(trifluoromethyl)-*N*-(3,4,5-(trimethoxy)phenyl)nicotinamide (44). Yield 91%. Mp 210–211 °C. ^1H NMR (DMSO-d_6): δ 2.24 (s, 3H, CH_3), 3.61 (s, 3H, OCH_3), 3.73 (s, 6H, OCH_3), 7.06 (m, 2H, aryl), 7.19–7.35 (m, 3H, aryl), 8.05 (d, 1H, J = 7.7 Hz, pyridyl), 8.39 (d, 1H, J = 7.68 Hz, pyridyl), 10.24 (s, 1H, NH), 10.52 (s, 1H, NH). IR (Nujol) 3407, 3117, 1666, 1613 cm^{-1}. Anal. Calcd for $C_{23}H_{21}ClF_3N_3O_4$: C, 55.71; H, 4.27; N, 8.47. Found: C, 55.66; H, 4.26; N, 8.51.

3.1.4.13. 2-(4-Chloro-2-methylphenylamino)-6-(trifluoromethyl)-*N*-(3-(chloro)phenyl)nicotinamide (45). Yield 93%. Mp 165–166 °C. ^1H NMR (DMSO-d_6): δ 2.24 (s, 3H, CH_3), 7.17–7.82 (m, 7H, aryl), 8.07 (d, 1H, J = 7.7 Hz, pyridyl), 8.41 (d, 1H, J = 7.7 Hz, pyridyl), 10.16 (s, 1H, NH), 10.72 (s, 1H, NH). IR (Nujol) 3451, 3345, 3289, 3190, 1639, 1613, 1588 cm^{-1}. Anal. Calcd for $C_{20}H_{14}Cl_2F_3N_3O$: C, 54.56; H, 3.21; N, 9.54. Found: C, 54.63; H, 3.22; N, 9.50.

3.1.4.14. 2-(5-Chloro-2-methylphenylamino)-6-(trifluoromethyl)-*N*-(3-(chloro)phenyl)nicotinamide (46). Yield 86%. Mp 179–180 °C. ^{1}H NMR (DMSO-d_6): δ 2.24 (s, 3H, CH_3), 7.16–8.46 (m, 9H, aryl and pyridyl), 10.17 (s, 1H, NH), 10.71 (s, 1H, NH). IR (Nujol) 3344, 3288, 1639, 1612, 1588 cm^{-1}. Anal. Calcd for $C_{20}H_{14}Cl_2F_3N_3O$: C, 54.56; H, 3.21; N, 9.54. Found: C, 54.51; H, 3.20; N, 9.58.

3.1.4.15. 2-(4-Chloro-2-methylphenylamino)-*N*-(3-chloro-4-methoxyphenyl)-6-(trifluoromethyl)nicotinamide (47). Yield 80%. Mp 173–175 °C. ^{1}H NMR (DMSO-d_6): δ 2.24 (s, 3H, CH_3), 3.80 (s, 3H, OCH_3), 7.12–8.78 (m, 8H, aryl and pyridyl), 10.28 (s, 1H, NH), 10.58 (s, 1H, NH). IR (Nujol) 3454, 3294, 3254, 1641, 1613, 1594 cm^{-1}. Anal. Calcd for $C_{21}H_{16}Cl_2F_3N_3O_2$: C, 53.63; H, 3.43; N, 8.94. Found: C, 53.67; H, 3.44; N, 8.90.

3.1.4.16. 2-(5-Chloro-2-methylphenylamino)-*N*-(3-chloro-4-methoxyphenyl)-6-(trifluoromethyl)nicotinamide (48). Yield 90%. Mp 168–170 °C. ^{1}H NMR (DMSO-d_6): δ 2.24 (s, 3H, CH_3), 3.81 (s, 3H, OCH_3), 7.12–8.42 (m, 8H, aryl and pyridyl), 10.28 (s, 1H, NH), 10.59 (s, 1H, NH). IR (Nujol) 3288, 3251, 1640, 1612, 1594 cm^{-1}. Anal. Calcd for $C_{21}H_{16}Cl_2F_3N_3O_2$: C, 53.63; H, 3.43; N, 8.94. Found: C, 53.58; H, 3.44; N, 8.97.

3.1.4.17. 2-(4-Chloro-2-methyl-phenylamino)-*N*-pyridin-3-yl-6-trifluoromethylnicotinamide (49). Yield 89%. Mp 175–176 °C. ^{1}H NMR (DMSO-d_6): δ 2.25 (s, 3H, CH_3) 7.08–8.83 (m, 9H, aryl and pyridyl) 10.24 (s, 1H, NH), 10.85 (s, 1H, NH). IR (Nujol) 3370, 3299, 3251, 3191, 3134, 3082, 1672, 1623, 1592 cm^{-1}. Anal. Calcd for $C_{19}H_{14}ClF_3N_4O$: C, 56.10; H, 3.47; N, 13.77. Found: C, 56.15; H, 3.45; N, 13.81.

3.1.4.18. 2-(5-Chloro-2-methyl-phenylamino)-*N*-pyridin-3-yl-6-trifluoromethylnicotinamide (50). Yield 91%. Mp 194–195 °C. ^{1}H NMR (DMSO-d_6): δ 2.25 (s, 3H, CH_3) 7.06–8.84 (m, 9H, aryl and pyridyl) 10.23 (s, 1H, NH), 10.81 (s, 1H, NH). IR (Nujol) 3419, 3311, 3248, 3188, 1672, 1623, 1593 cm^{-1}. Anal. Calcd for $C_{19}H_{14}ClF_3N_4O$: C, 56.10; H, 3.47; N, 13.77. Found: C, 56.16; H, 3.46; N, 13.80.

3.1.4.19. Ethyl 4-(2-(4-chloro-2-methylphenylamino)-6-(trifluoromethyl)nicotinoyl)piperazine-1-carboxylate (51). Yield 84%. Mp 188–190 °C. ^{1}H NMR (DMSO-d_6): δ 1.13 (t, 3H, J = 6.9 Hz, CH_3), 2.10 (s, 3H, CH_3), 3.42 (m, 8H, piperazinyl), 4.02 (q, 2H, J = 6.9 Hz, CH_2), 7.17–8.48 (m, 5H, aryl and pyridyl) 11.17 (s, 1H, NH). IR (Nujol) 3463, 3300, 3190, 1703, 1680, 1613, 1597 cm^{-1}. Anal. Calcd for $C_{21}H_{22}ClF_3N_4O_3$: C, 53.57; H, 4.71; N, 11.90. Found: C, 53.53; H, 4.72; N, 11.87.

3.1.4.20. Ethyl 4-(2-(5-chloro-2-methylphenylamino)-6-(trifluoromethyl)nicotinoyl)piperazine-1-carboxylate (52). Yield 88%. Mp 123–125 °C. ^{1}H NMR (DMSO-d_6): δ 1.13 (t, 3H, J = 6.8 Hz, CH_3), 2.10 (s, 3H, CH_3), 3.40 (m, 8H, piperazinyl), 4.01 (q, 2H, J = 6.8 Hz, CH_2), 7.19–7.76 (m, 5H, aryl and pyridyl) 8.45 (s, 1H, NH). IR (Nujol) 3319, 1703, 1634, 1613, 1597 cm^{-1}. Anal. Calcd for $C_{21}H_{22}ClF_3N_4O_3$: C, 53.57; H, 4.71; N, 11.90. Found: C, 53.60; H, 4.72; N, 11.93.

3.1.4.21. (2-(4-Chloro-2-methylphenylamino)-6-(trifluoromethyl)pyridin-3-yl)(4-(3-chlorophenyl) piperazin-1-yl)methanone (53). Yield 91%. Mp 129–130 °C (cyclohexane). ^{1}H NMR (DMSO-d_6): δ 2.10 (s, 3H, CH_3), 3.24 (m, 4H, piperazinyl), 3.59 (m, 4H, piperazinyl), 6.75–7.82 (m, 9H, aryl and pyridyl) 8.50 (s, 1H, NH). IR (Nujol) 3356, 1614, 1593 cm^{-1}. Anal. Calcd for $C_{24}H_{21}Cl_2F_3N_4O$: C, 56.59; H, 4.16; N, 11.19. Found: C, 56.63; H, 4.27; N, 11.36.

3.1.4.22. 2-(5-Chloro-2-methylphenylamino)-6-(trifluoromethyl)pyridin-3-yl)(4-(3-chlorophenyl) piperazin-1-yl) methanone (54). Yield 83%. Mp 178–180 °C. ^{1}H NMR (DMSO-d_6): δ 2.11 (s, 3H, CH_3), 3.23 (m, 4H, piperazinyl), 3.59 (m, 4H, piperazinyl), 6.75–7.79 (m, 9H, Ar and pyridyl) 8.49 (s, 1H, NH). IR (Nujol) 2770, 2721, 2486, 1595, 1571 cm^{-1}. Anal. Calcd for $C_{24}H_{21}Cl_2F_3N_4O$: C, 56.59; H, 4.16; N, 11.19. Found: C, 56.63; H, 4.17; N, 11.16.

3.1.4.23. (2-(4-Chloro-2-methylphenylamino)-6-(trifluoromethyl)pyridin-3-yl)(4-(4-fluorophenyl) piperazin-1-yl)methanone (55). Yield 87%. Mp 169–170 °C. ^{1}H NMR (DMSO-d_6): δ 2.10 (s, 3H, CH_3), 3.10 (m, 4H, piperazinyl), 3.56 (m, 4H, piperazinyl), 6.93–7.77 (m, 9H, aryl and pyridyl) 8.49 (s, 1H, NH). IR (Nujol) 3374, 1630, 1615, 1599 cm^{-1}. Anal. Calcd for $C_{24}H_{21}ClF_4N_4O$: C, 58.48; H, 4.29; N, 11.37. Found: C, 58.42; H, 4.27; N, 11.40.

3.1.4.24. (2-(5-Chloro-2-methyl-phenylamino)-6-(trifluoromethyl)pyridin-3-yl)(4-(4-fluorophenyl) piperazin-1-yl)methanone (56). Yield 87%. Mp 139–140 °C. ^{1}H NMR (DMSO-d_6): δ 2.11 (s, 3H, CH_3), 3.10 (m, 4H, piperazinyl), 3.57 (m, 4H, piperazinyl), 6.93–7.77 (m, 9H, aryl and pyridyl) 8.48 (s, 1H, NH). IR (Nujol) 3374, 1679, 1614, 1599 cm^{-1}. Anal. Calcd for $C_{24}H_{21}ClF_4N_4O$: C, 58.48; H, 4.29; N, 11.37. Found: C, 58.53; H, 4.30; N, 11.33.

3.1.4.25. (2-(4-Chloro-2-methylphenylamino)-6-(trifluoromethyl)pyridin-3-yl)(4-(4-methoxyphenyl) piperazin-1-yl)methanone (57). Yield 83%. Mp 179–180 °C. ^{1}H NMR (DMSO-d_6): δ 2.25 (s, 3H, CH_3), 3.24 (m, 4H, piperazinyl), 3.59 (s, 4H, piperazinyl), 3.63 (s, 3H, OCH_3), 6.76–8.48 (m, 9H, aryl and pyridyl) 11.17 (s, 1H, NH). IR (Nujol) 3464, 3357, 3189, 1681, 1614, 1598 cm^{-1}. Anal. Calcd for $C_{25}H_{24}ClF_3N_4O_2$: C, 59.47; H, 4.79; N, 11.10. Found: C, 59.50; H, 4.80; N, 11.14.

3.1.4.26. 2-(5-Chloro-2-methyl-phenylamino)-6-(trifluoromethyl)pyridin-3-yl)(4-(4-methoxyphenyl) piperazin-1-yl)methanone (58). Yield 95%. Mp 198–200 °C. ^{1}H NMR (DMSO-d_6): δ 2.24 (s, 3H, CH_3), 3.24 (m, 4H, piperazinyl), 3.59 (m, 4H, piperazinyl), 3.65 (s, 3H, OCH_3), 6.75–7.82 (m, 9H, aryl and pyridyl) 11.18 (s, 1H, NH). IR (Nujol) 3310, 3183, 1680, 1609, 1532 cm^{-1}. Anal. Calcd for $C_{25}H_{24}ClF_3N_4O_2$: C, 59.47; H, 4.79; N, 11.10. Found: C, 59.45; H, 4.78; N, 11.16.

3.2. Determination of GI_{50}, TGI, and LC_{50} values

A total of 60 human tumor cell lines, derived from nine cancer types (leukemia, lung, colon, brain, melanoma, ovarian, renal, prostate, and breast) formed the basis of this test. The tumor cells were cultured in RPMI1640 medium supplemented with 5% fetal calf serum and 2 mM L-glutamine. The tumor cells are inoculated over a series of standard 96-well microtiter plates in 100 mL of medium.[32,33] Density of inoculum depends on the type of tumor cell and its growth characteristics.[27] These cells are then preincubated on the microtiter plate for 24 h before adding the compounds. These were tested in DMSO solution at five different concentrations (10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8} M). After an incubation of the chemical agent for 48 h with the tumor cell lines a sulforhodamine B (SRB) protein assay was used to estimate cell viability or growth. The cytotoxic effects are evaluated and the assay results and dose–response parameters were calculated as previously described.[34]

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References and notes

1. Cozzi, P.; Mongelli, N.; Suarato, A. *Curr. Med. Chem. Anticancer Agents* **2004**, *4*, 93–121.
2. Isanbor, C.; O'Hagan, D. *J. Fluorine Chem.* **2006**, *12*, 303–319.
3. Nair, V. A.; Mustafa, S. M.; Mohler, M. L.; Fisher, S. J.; Dalton, J. T.; Miller, D. D. *Tetrahedron Lett.* **2004**, *45*, 9475–9477, and references cited therein.
4. Ohtsu, H.; Xiao, Z.; Ishida, J.; Nagai, M.; Wang, H. K.; Itokawa, H.; Su, C. Y.; Shih, C.; Chiand, T.; Chang, E.; Lee, Y.; Tsai, M. Y.; Chang, C.; Lee, K. H. *J. Med. Chem.* **2002**, *45*, 5037–5042, and references cited therein.
5. Konno, T.; Daitoh, T.; Noiri, A.; Chae, J.; Ishihara, T.; Yamanaka, H. *Org. Lett.* **2004**, *6*, 933–936.
6. Harris, P. A.; Cheung, M.; Hunter, R. N., III; Brown, M. L.; Veal, J. M.; Nolte, R. T.; Wang, L.; Liu, W.; Crisby, R. M.; Johnson, J. H.; Epperly, A. H.; Kumar, R.; Luttrell, D. K.; Stafford, J. A. *J. Med. Chem.* **2005**, *48*, 1610–1619.
7. Manley, P. W.; Furet, P.; Bold, G.; Bruggen, J.; Mestan, J.; Meyer, T.; Schnell, C. R.; Wood, J. *J. Med. Chem.* **2002**, *45*, 5687–5693.
8. Weisberg, E.; Manley, P.; Mestan, J.; Cowan-Jacob, S.; Ray, A.; Griffin, J. D. *Br. J. Cancer.* **2006**, *94*, 1765–1769.
9. Miyazaki, Y.; Tang, J.; Maeda, Y.; Nakano, M.; Wang, L.; Nolte, R. T.; Sato, H.; Sugai, M.; Okamoto, Y.; Anne, T.; Truesdale, A. T.; Hassler, D. F.; Nartey, E. N.; Patrick, D. R.; Ho, M. L.; Ozawa, K. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1773–1778.
10. Chintharlapalli, S.; Papineni, S.; Safe, S. *Mol. Cancer Ther.* **2006**, *5*, 1362–1370.
11. Tanaka, A.; Konno, M.; Muto, S.; Kambe, N.; Morii, E.; Nakahata, T.; Itai, A.; Matsuda, H. *Blood* **2005**, *105*, 2324–2331.
12. Sirisoma, N.; Kasibhatla, S.; Nguyen, B.; Pervin, A.; Wang, Y.; Claassen, G.; Tseng, B.; John Drewe, J.; Cai, S. X. *Bioorg. Med. Chem.* **2006**, *14*, 7761–7773.
13. Kusunoki, N.; Ito, T.; Sakurai, N.; Handa, H.; Kawai, S. *Anticancer Res.* **2006**, *26*(5A), 3229–3236.
14. Congiu, C.; Cocco, M. T.; Lilliu, V.; Onnis, V. *J. Med. Chem.* **2005**, *48*, 8245–8252.
15. Cocco, M. T.; Congiu, C.; Onnis, V. *Eur. J. Med. Chem.* **2000**, *35*, 545–552.
16. Cocco, M. T.; Congiu, C.; Onnis, V. *Eur. J. Med. Chem.* **2003**, *38*, 37–47.
17. Cocco, M. T.; Congiu, C.; Lilliu, V.; Onnis, V. *Eur. J. Med. Chem.* **2005**, *40*, 1365–1372.
18. Cocco, M. T.; Congiu, C.; Lilliu, V.; Onnis, V. *Bioorg. Med. Chem.* **2007**, *15*, 1859–1867.
19. Elbaum, D.; Askew, B.; Booker, S.; Germain, J.; Habgood, G.; Handley, M.; Kim, T. S.; Li, A.; Nishimura, N.; Patel, V. F.; Yuan, C. C.; Kim, J. L. U.S. Pat. Appl. Publ., 2003, 134–836.
20. Patel, V. F.; Askew, B.; Booker, S.; Chen, G.; Dipietro, L. V.; Germain, J.; Habgood, G. J.; Huang, Q.; Kim, T. S.; Li, A.; Nishimura, N.; Nomak, R.; Riahi, B.; Yuan, C. C.; Elbaum, D. U.S. Pat. Appl. Publ. US 2003, 203922, 2003.
21. Chen, G.; Booker, S.; Cai, G.; Croghan, M.; Dipietro, L.; Dominguez, C.; Elbaum, D.; Germain, J.; Uuang, Q.; Kim, J. L.; Kim, T. S.; Patel, V. F.; Smith, L. M.; Tasker, A.; Xi, N.; Xu, S.; Yuan, C. C. PCT Int. Appl. WO 025550, 2002.
22. Girgis, A. S.; Hosni, H. M.; Barsoum, F. F. *Bioorg. Med. Chem.* **2006**, *14*, 4466–4476.
23. Yaun, C. C.; Yang, K.; Van der Plas, S.; Riahi, B.; Potashman, M.; Patel, V. F.; Nomak, R.; Li, A.; Huang, Q.; Harmange, J.-C.; Askew Jr., B.C. U.S. Pat. Appl. Publ. US20060040966A1, 2006.
24. Cocco, M. T.; Congiu, C.; Onnis, V.; Morelli, M.; Felipo, V.; Cauli, O. *Bioorg. Med. Chem.* **2004**, *12*, 4169–4177.
25. Boyd, M. R. *Am. Assoc. Cancer Res.* **1989**, *30*, 652–663.
26. Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Vaigro-Wolff, A.; Gray-Goodrich, M.; Campbell, H.; Mayo, J.; Boyd, M. *J. Natl. Cancer Inst.* **1991**, *83*, 757–766.
27. Weinstein, J. N.; Myers, T. G.; O'Connor, P. M.; Friend, S. H.; Fornance, A. J., Jr.; Kohn, K. W.; Fojo, T.; Bates, S. E.; Rubinstein, L. V.; Anderson, N. L.; Buolamwini, J. K.; van Osdol, W. W.; Monks, A. P.; Scudiero, D. A.; Sausville, E. A.; Zaharevitz, D. W.; Bunow, B.; Viswanadhan, V. N.; Johnson, G. S.; Wittes, R. E.; Paull, K. D. *Science* **1997**, *275*, 343–349.
28. Paull, K. D.; Shoemaker, R. H.; Hodes, L.; Monks, A.; Scudiero, D. A.; Rubinstein, L.; Plowman, J.; Boyd, M. R. *J. Natl. Cancer Inst.* **1989**, *81*, 1088–1092.
29. McElvain, S. M.; Schoeder, J. P. *J. Am. Chem. Soc.* **1949**, *71*, 40.
30. Glickman, S. A.; Cope, A. C. *J. Am. Chem. Soc.* **1945**, *67*, 1017.
31. Moriguchi, T.; Endo, T.; Takata, T. *J. Org. Chem.* **1995**, *60*, 3523–3528.
32. Grever, M. R.; Schepartz, S. A.; Chabner, B. A. *Semin. Oncol.* **1992**, *19*, 622.
33. Boyd, M. R.; Paull, K. D. *Drug Dev. Res.* **1995**, *34*, 91.
34. Boyd, M. R. *Princ. Pract. Oncol.* **1989**, *3*, 1.