New Potential Anticancer Agents Based on the Anthranilic Acid Scaffold. Synthesis and Evaluation of Biological Activity

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The synthesis and anticancer activity of new compounds designed on the anthranilic acid scaffold are reported. The antiproliferative activity was assayed by the National Cancer Institute in established in vitro and in vivo anticancer experimental models. Structural variations based on the flufenamic acid motif afforded a series of (hetero)aryl esters of *N*-(2-(trifluoromethyl)-pyridin-4-yl)anthranilic acid, which showed in vitro growth inhibitory properties against human tumor cell lines in nanomolar to low micromolar concentrations. The pyridinyl ester **25** exhibited very potent in vitro antiproliferative efficacy, with a chemosensitive profile showing a number of GI_{50} values at concentrations lower than 10^{-7} M in the full panel of human tumor cell lines. Compound **25** was also tested in vivo as a potential anticancer agent in the hollow fiber assay and in human tumor xenografts, showing moderate inhibitory properties. Analysis of biological activities and the COMPARE procedure was utilized to support putative biochemical mechanisms implicated with the antiproliferative activity.

Introduction

Among the wide variety of synthetic compounds recognized as potential anticancer drugs, molecules based on the anthranilic acid scaffold have attracted great interest in recent years. Experimental and preclinical models demonstrated that a number of these compounds elicited outstanding anticancer activity through a range of biological activities implicated with the development and maintenance of tumor cells. In this context, several reports describing the antitumor evaluation of anthranilate derivatives appeared in the recent literature. For example, Tranilast (Figure 1) has been reported to exhibit antiproliferative activity against cultured leiomyoma cells, through the suppression of cyclin-dependent kinase (CDK) 2 activity.[1] Also, Yashiro et al. have described that Tranilast decreases the production of matrix metallo-proteinase-2 (MMP-2) and transforms the growth factor-β1 (TGF-β1) from fibroblasts, resulting in significant suppression of the invasion ability of gastric cancer cells.[2] Farnesyl anthranilate has been shown to reveal tumor growth-suppressive action in experimental murine melanomas models, as a probable consequence of down regulation of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase activity.[3]

Antitumor activity of the anthranilamide CI-1040 has been demonstrated in preclinical models, particularly for pancreas, colon, and breast cancers.[4] The CI-1040 activity has been correlated with its inhibition of mitogen-activated protein kinase (MAPk) cascade pathway. Moreover, the anthranilamide AAL993 has been described as a lead compound of a new structural class of vascular endothelial growth factor (VEGF) kinase inhibitors, which possess potent antiangiogenic and antitumor properties.[5] Flufenamic acid (FA), a nonsteroidal antiinflammatory drug (NSAID) belonging to the

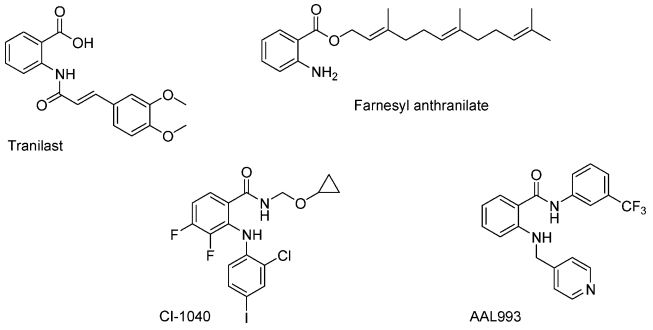

Figure 1.

structural class of fenamates, has been recently found to exhibit inhibitory properties against cell proliferation. Pharmacological activity of NSAIDs is mainly attributed to inhibition, to different extents, of the two isoforms of prostaglandin H synthase, cyclooxygenase (COX) 1 and 2, the key enzyme that converts arachidonic acid to prostaglandin H_2, the precursor of a wide group of biologically active mediators such as prostaglandins, prostacyclin, and thromboxane A_2.[6] In recent years, an important development in oncology is the assessment that various NSAIDs played an important role as antiproliferative agents against a broad spectrum of in vivo and in vitro models of human malignancies, resulting in cell cycle arrest, increased apoptosis, and inhibition of angiogenesis.[7] Although it has become widely accepted that the antiinflammatory and antineoplastic activities of NSAIDs are interrelated and mediated by COX-2 inhibition,[8] it has been pointed out that both COX isoforms and/or their products may act in promoting and maintaining the neoplastic state.[9] Whether the NSAIDs block tumor progression only by blocking COX activity is a theme still being debated. On the other hand, several research groups have shown that NSAIDs can produce some of their cancer chemopreventive and antiproliferative effects via mechanisms that are independent of COX inhibition.[10] Thus, recent experimental

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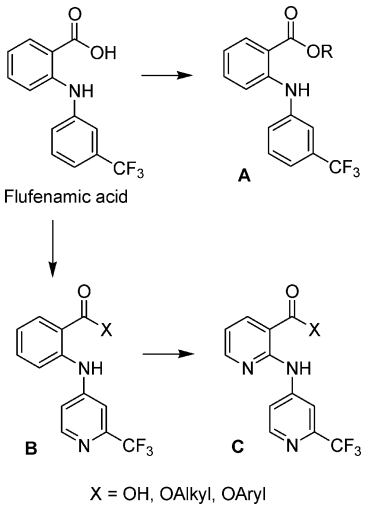


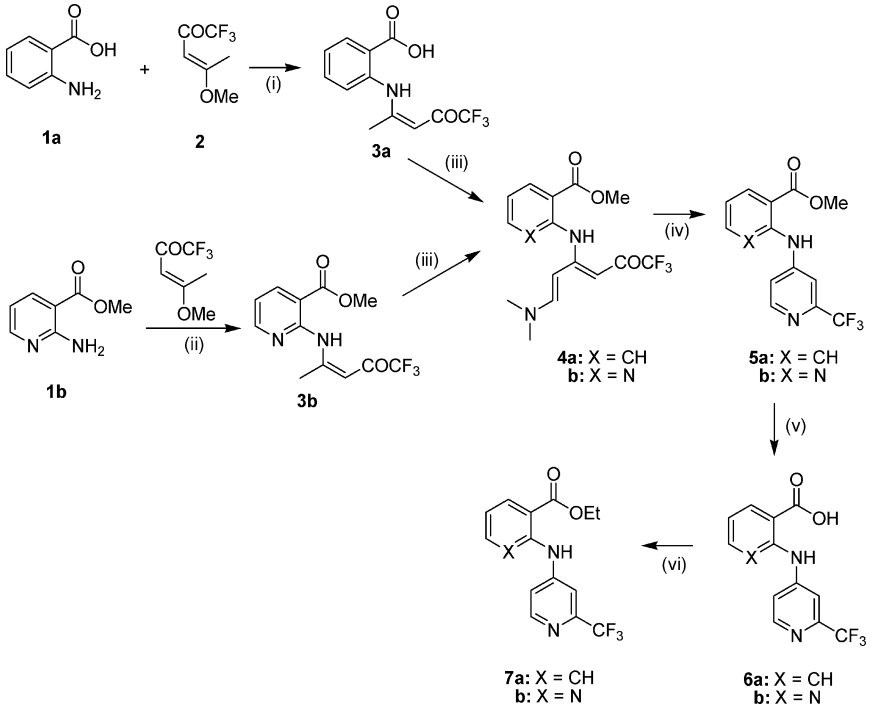
Figure 2. Structural variations of the flufenamic acid motif.

findings have shown FA to elicit antiproliferative activity by COX inhibition independent pathways. Weiser et al. have reported that FA exhibits growth inhibition properties on a mouse fibroblast cell line LM(TK−) through blockage of a 28-pS nonselective cation channel.[11] Zhu et al. have shown FA to inhibit the androgen receptor (AR) expression in human prostate cancer (LNCaP) cells, providing a new possible therapeutic approach in prostate cancer prevention and/or treatment.[12] Because of our ongoing interest in the search for novel antitumor compounds arising from anthranilic acid, we started a study aimed to evaluate new derivatives bearing the FA motif. Recently, we have reported the preliminary results of the noteworthy anticancer activity demonstrated by some aromatic esters of *N*-(2-trifluoromethylpyridin-4-yl)anthranilic acid, which possess the aza skeleton of FA.[13] Encouraged by the above findings, we planned to explore putative key portions of the FA molecule, i.e., the carboxylic group, the anthranilic portion, and the lipophilic trifluoromethylphenyl moiety, by introducing structural modifications in these regions and evaluating the effects of the changes on the activity. In this paper, we describe the synthesis and biological properties as potential antitumor agents of FA aryl esters **A**, pyridinyl analogs **B**, and nicotinic congeners **C** (Figure 2). Selected compounds were assayed at the National Cancer Institute (NCI) for their cytotoxicity and antitumor properties in the in vitro anticancer screening. NCI also evaluated the most potent compound **25** for its in vivo anticancer efficacy in the hollow fiber assay and human tumor xenografts.

Chemistry

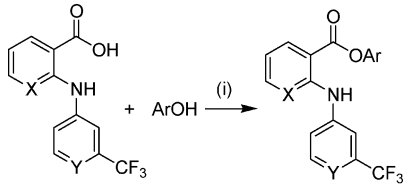
The synthesis of the target compounds was conveniently undertaken as outlined in Schemes 1 and 2. Aryl esters **A** were obtained by coupling FA with an appropriate phenolic compound by the dicyclohexylcarbodiimide (DCC) method (vide infra). The first step of the synthetic approach to compounds **B** and **C** was based on a previously described procedure that allows the construction of the trifluoromethylpyridine moiety linked by an amino nitrogen to an aromatic ring.[14] With some modifications, this synthetic pathway is now successfully extended to the preparation of intermediates **5a,b** by using anthranilic acid and 2-aminonicotinic acid as easily available starting materials. Anthranilic acid **1a** was reacted with trifluoroacetylvinyl ether **2** in 1:1.5 molar ratio in refluxing acetonitrile solution to yield the acid **3a** in almost quantitative yield (Scheme 1). Under the same conditions, 2-aminonicotinic acid failed to react with **2**.

Scheme 1. Synthesis of Compounds 4−7[a]



[a] Reagents and conditions: (i) MeCN, reflux; (ii) 120 °C; (iii) DMF−DMA, 3 equiv, toluene, reflux; (iv) ammonium acetate, 2 equiv, DMF, reflux; (v) 10% aq NaOH, reflux, then H_3O^+; (vi) anhyd EtOH, $SOCl_2$, 4 equiv, reflux.

Scheme 2. Synthesis of the Aryl Esters 8−31[a]



Flufenamic acid: X = Y = CH
6a: X = CH; Y = N
6b: X = Y = N

8 - 31

8: Ar = 2-methoxyphenyl; X = Y = CH
9: Ar = 3-methoxyphenyl; X = Y = CH
10: Ar = 2-chlorophenyl; X = Y = CH
11: Ar = 3-chlorophenyl; X = Y = CH
12: Ar = 4-chlorophenyl; X = Y = CH
13: Ar = 2,4-dichlorophenyl; X = Y = CH
14: Ar = 2,4,6-trichlorophenyl; X = Y = CH
15: Ar = pyridin-3-yl; X = Y = CH
16: Ar = 2-methoxyphenyl; X = CH; Y = N
17: Ar = 3-methoxyphenyl; X = CH; Y = N
18: Ar = 4-methoxyphenyl; X = CH; Y = N
19: Ar = 4-methylthiophenyl; X = CH; Y = N
20: Ar = 2-chlorophenyl; X = CH; Y = N
21: Ar = 3-chlorophenyl; X = CH; Y = N
22: Ar = 4-chlorophenyl; X = CH; Y = N
23: Ar = 2,4-dichlorophenyl; X = CH; Y = N
24: Ar = 2,4,6-trchlorophenyl; X = CH; Y = N
25: Ar = pyridin-3-yl; X = CH; Y = N
26: Ar = 3-methoxyphenyl; X = Y = N
27: Ar = 2-chlorophenyl; X = Y = N
28: Ar = 3-chlorophenyl; X = Y = N
29: Ar = 4-chlorophenyl; X = Y = N
30: Ar = 2,4-dichlorophenyl; X = Y = N
31: Ar = pyridin-3-yl; X = Y = N

[a] Reagents: (i) DCC, $CHCl_3$.

Attempts to favor the reaction by either prolonging reaction time or heating the reactant mixture without solvent resulted in poor yields or extensive decomposition of the starting materials. However, upon heating the mixture of methyl ester **1b**[15] and **2** in 1:5 molar ratio without solvent, nicotinate **3b** was successfully obtained in 82% yield. Upon reacting with an excess of *N*,*N*-dimethylformamide dimethylacetal (DMF−DMA) in refluxing toluene, compounds **3a**,**b** were converted into 1,1,1-trifluorohexadienones **4a,b** in 92 and 84% yields, respectively. Compound **3a** was converted in a one-pot procedure into ester **4a**, resulting in an esterification−condensation reaction sequence. Upon treatment with ammonium acetate in hot DMF, the key intermediates **4a,b** readily underwent pyridine ring closure to give compounds **5a,b** in 86 and 82% yields, respectively. Hydrolysis of **5a,b** in 10% aqueous sodium hydroxide afforded acids **6a,b**. Upon refluxing an ethanolic solution of **6a,b** in the presence of thionyl chloride, the ethyl esters **7a,b** were obtained in 93 and 91% yields, respectively. Finally (Scheme 2), treatment of FA and **6a,b** with appropriate phenol in the presence of DCC in chloroform solution afforded the ester derivatives **8**−**31** in moderate to good yields.

Biological Results and Discussion

In Vitro Antitumor Activity. The in vitro growth inhibition and cytotoxicity assay was performed by the NCI according to well-established procedures.[16−19] First, selected compounds were evaluated in a one dose primary anticancer screen at 10^{-4} M concentration for their in vitro cytotoxic activity against NCI-H460 (non-small cell lung), MCF7 (breast), and SF-268 (central nervous system, CNS) human cancer cell lines. Compounds that reduced the growth of any one of the cell lines to less than 32% were then evaluated in the full cell line panel. Compounds **10**, **17**, **20**−**25**, **27**, and **31**, which fulfilled this condition, were assayed for their antiproliferative activity against a panel of almost 60 cell lines derived from leukemia, lung, colon, CNS, melanoma, ovarian, renal, prostate, and breast human cancers. In the full cell line panel screening, the test compounds were evaluated using five concentrations at 10-fold dilutions, ranging from 10^{-4} to 10^{-8} M. For each compound, anticancer activity was deduced from dose−response curves and expressed by three parameters (GI_{50}, TGI, LC_{50}) calculated for each cell line. The GI_{50} value indicates the concentration of the compound required to cause 50% inhibition of net cell growth. The TGI value represents the concentration of the compound resulting in total inhibition of net cell growth. The LC_{50} value refers to the concentration of the compound leading to 50% net cell death. Moreover, for each antitumor activity parameter, the mean graph midpoint (MG_MID) was calculated giving an averaged activity parameter over all cell lines. For the calculation of MG_MID, insensitive cell lines were included with the highest concentration tested. Selective activity of a compound against a certain cancer cell line from a specific organ is characterized by a high deviation of the particular cell line parameter compared to the MG_MID value. Table 1 shows the GI_{50} values of a subset of the 60 cell line panel, as well as the MG_MID values over all cell lines. As reported in Table 1, tested compounds exhibit moderate to excellent activity in the full cell line panel screening, expressed by pGI_{50} MG_MID values ranging from 4.67 (compound **10**) to 6.38 (compound **25**). Although compounds **10**, **27**, and **31** possess moderate activity, all the other tested compounds show antiproliferative activity with pGI_{50} MG_MID values higher than 5.0. Compounds **20** and **25** demonstrate the best inhibitory properties with pGI_{50} MG_MID value of 6.21 and 6.38, respectively, which compares favorably to standard compounds such as etoposide (6.00), fluorouracil (6.05), and 6-mercaptopurine (5.13). Compounds **20** and **25** also exhibit very potent growth inhibitory effects at nanomolar concentrations against various cell lines. The chemosensitive profile across the full cell line panel reveals that all the tested compounds showed particular efficacy against colonic SW-620 cell line, with

Table 1. Selected Growth Inhibitory Properties of Tested Compounds in the NCI's Full Cancer Cell Line Panel Screening and the Average pGI_{50} Value over All Cell Lines[a]

	pGI_{50} (M)[b]							
compd	leu-kemia K-562	lung NCI-H23	colon SW-620	CNS SF-268	mela-noma UACC-62	renal SN12C	breast MCF-7	MG_ MID[c]
10	5.57	4.70	5.09	4.69	4.77	4.63	4.47	4.67
17	6.13	5.69	5.39	5.33	5.51	5.27	5.14	5.39
20	7.29	6.59	6.44	5.65	6.51	6.48	6.55	6.21
21	5.49	5.62	5.36	5.52	5.69	5.53	5.32	5.28
22	5.44	5.55	5.18	5.36	5.58	5.53	5.43	5.08
23	5.52	5.45	5.34	5.51	5.64	5.52	5.44	5.24
24	5.62	5.59	5.34	5.41	5.76	5.51	5.33	5.28
25	7.37	7.44	7.39	6.48	6.54	7.15	7.34	6.38
27	5.34	5.06	5.43	5.00	5.21	5.39	5.44	4.90
31	5.29	5.20	5.26	5.25	5.24	5.19	5.57	4.84

[a] Data obtained from the NCI's in vitro disease-oriented human tumor cells screen. [b] pGI_{50} is the −log of the molar concentration that cause 50% inhibition of net cell growth. [c] MG_MID = mean graph midpoint = arithmetical mean value for all tested cell lines.

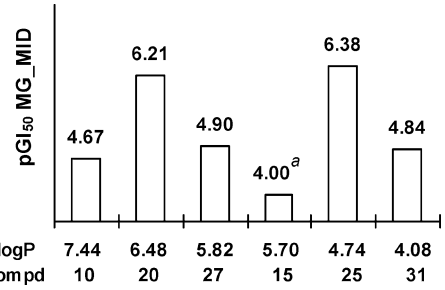


Figure 3. Correlation between the averaged activity (pGI_{50} MG_MID) parameter and calculated log *P* values for representative anthranilates. [a]Extrapolated value from the three-cell-line prescreen.

GI_{50} values ranging from nanomolar to low micromolar concentrations. A similar pattern of activity was also found against leukemic K-562 cell line. This result acquires notable significance, since previous studies indicated that K-562 cells are resistant to the cytotoxic effect of a variety of different agents, including cytarabine and etoposide.[20]

Among (hetero)aryl ester derivatives of FA, only 2-chlorophenyl ester **10** was endowed with moderate activity. Although carboxylic acids **6**, as well as alkyl esters **5** and **7**, appeared devoid of cytotoxicity, their conversion into (hetero)aryl esters resulted in generation of antiproliferative agents. Within the series of the *N*-(trifluoromethylpyridine)anthranilates, compounds **17** and **20**−**25** exhibited good to excellent inhibitory properties, with 2-chlorophenyl (compound **20**) and pyridin-3-yl (compound **25**) esters being the most potent agents. In contrast, among the nicotinic esters, 2-chlorophenyl and pyridin-3-yl esters, compounds **27** and **31** respectively, revealed moderate to good inhibitory activity. The 2-chlorine substituent on the phenoxy group appears to be the most favorable to produce different extents of antiproliferative efficacy within the three series of derivatives. Both shifting of the 2-chlorine atom to other positions of phenyl ring and introducing further chlorine atoms result in decreasing activity of esters **21**−**24**. In contrast, the same changes on both flufenamic and nicotinic esters result in loss of antiproliferative effect. Replacement of the chlorine atom with an electron-donating substituent, such as the methoxy group, results in inactive compounds, with the exception of 3-methoxyphenyl ester **17**, which maintains a good level of activity. Esterification with 3-hydroxypyridine produces different effects. Flufenamate **15** is devoid of activity and nicotinate **31** shows moderate cytotoxicity, while the *N*-(trifluoromethylpyridine)anthranilate **25** exhibits the highest levels of inhibitory activity among all the tested compounds.

Moreover, a correlation can be found between the averaged activity parameter and increasing hydrophilicity, expressed by calculated log *P* values,[21] due to introduction of pyridine ring(s) (Figure 3).

With respect to flufenamic ester **10** (log $P = 7.44$), a single isosteric modification of the phenyl bearing a trifluoromethyl group with a pyridine ring to give compound **20** (log $P = 6.48$) produced 30-fold enhancement of inhibitory potency. In contrast, the introduction of a further nitrogen atom in the anthranilic moiety resulted in reduction of activity. In fact, nicotinic ester **27** (log $P = 5.82$) was 20-fold less potent than **20**.

Table 2. Hollow Fiber Assay for 25

ip score	sc score	total score	cell kill
14	14	28	no

Similarly, with respect to **15** (log $P = 5.70$), **25** (log $P = 4.74$) showed 240-fold enhancement of potency, while **31** (log $P = 4.08$) was 35-fold less potent than **25**. Probably, electronic and/or steric effects, due to ester moiety, although important, remain no longer the main structural determinant for the observed antiproliferative effect. However, differences in hydrophilicity due to the introduction of pyridine ring(s) appear to be more important in affecting both potency and selectivity in these classes of compound.

In Vivo Antitumor Activity. Compound **25**, the most potent of the series, showed interesting in vitro chemosensitive profile with a number GI_{50} values at concentrations lower than 10^{-7} M in the full panel of human tumor cell lines. Leukemia (3/4) and colon (5/7) cancers were the more sensitive, renal (3/8), breast (2/7), melanoma (2/7), CNS (1/6), ovarian (1/6), and lung (1/9) cancers were the more resistant tumor types. Moreover, **25** exhibited noteworthy selectivity, with inhibitory values in submicromolar range, against certain colon cell lines at TGI (COLO 205, HCT-116, and KM12) and LC_{50} (COLO 205, and KM12) levels. To evaluate whether the in vitro activity of aryl *N*-(trifluoromethylpyridine)anthranilates can be translated into anticancer efficacy in human tumor in vivo, compound **25** was assayed by NCI in further in vivo experiments as described below.

Hollow Fiber Assay. Compound **25**, found to have reproducible activity in the in vitro anticancer drug screening, was evaluated by NCI in the hollow fiber assay as the preliminary in vivo experiment, which provides quantitative indications of drug efficacy.[22,23] In the hollow fiber model, polyvinylidene fluoride fibers containing various human cancer cell cultures were implanted intraperitoneally (ip) and subcutaneously (sc) into athymic nude mice and compounds were administrated by ip route. The effects of the compounds on reduction of viable cancer cell mass compared to those of controls were determined. To simplify evaluation, the NCI protocol adopts a point system that allows rapid viewing of the activity of a given compound. For this, a value of 2 is assigned for each compound dose that results in a 50% or greater reduction in viable cell mass. Compounds with a combined ip + sc score ≥ 20, a sc score ≥ 8, or a net cell kill of one or more cell lines were considered significantly active. Compound **25** exhibited a combined ip + sc score of 28, with low cytotoxicity (no cell kill) (Table 2).

Activity against Human Tumor Xenografts. According to the results of the in vitro antiproliferative activity and in vivo hollow fiber assay, the human melanoma xenograft LOX IMVI, human nonsmall cell lung tumor xenograft H522, and human CNS tumor xenograft U251 were selected by NCI as potentially sensitive tumor types to evaluate the in vivo efficacy of compound **25**. In the NCI's protocol, human tumor cells were implanted subcutaneously into the axillary region of pathogen-free athymic nude mice and allowed to establish into a sizable tumor as determined by calliper measurements. Compound **25** was formulated as a

Table 3. Antitumor Activity and Toxicity Data in Human CNS Tumor Xenograft U251 for 25[a]

dose, mg/kg/day	no. of mice	route	schedule	opt %T/C (d)[b]	% growth delay[c]	max % wt loss	drug deaths[d]
100	10	ip	Q4D × 3, day 5	27 (24)	67	no wt loss	0
67	10	ip	Q4D × 3, day 5	75 (24)	15	no wt loss	0

[a] Data obtained from the NCI Division of Cancer Treatment and Diagnosis. [b] Opt % T/C (d): optimum tumor weight of treated/control animals in percent (day). [c] Tumor growth delay according to the formula $[(T - C)/C] \times 100$. [d] Number of mice in the treatment group which were lost as result of test agent related toxicity.

solution in 10% DMSO in saline/Tween 80 and administrated by ip route. In the CNS tumor xenograft U251, **25** was administrated in groups of 10 mice. A group of 20 mice served as a vehicle control group. Doses of 100, 67, and 45 mg/kg were administered every 4 days, for a total of three treatments, with the first treatment given on day 5 postimplant (Q4D × 3, day 5) (Table 3). All the ip dosages were very well tolerated and resulted in no signs of toxicity or body weight loss (maximally tolerated dose was not achieved). Treatment with **25** resulted in moderate growth inhibition (67% of control at 100 mg/kg/day). In contrast, the melanoma xenograft LOX IMVI and human nonsmall cell lung tumor xenograft H522 were resistant toward therapy with compound **25**.

On the basis of structural similarity, the possibility exists that anthranilate derivatives herein described share some pharmacological properties with NSAIDs fenamates, i.e., COX-inhibitory activity, which accounted, at least in part, for the observed anticancer activity. To investigate whether these compounds are endowed with NSAIDs-type activity, preliminary experiments (data not shown) were carried out on 2-chlorophenyl ester **10** and pyridyl ester **25**. In the acetic acid-induced writhing test in rats,[24] after intraperitoneal administration, the tested compounds exhibited inhibition of stretching on the same order of magnitude as compared to flufenamic acid. In addition, all compounds that were active in the full cell line panel screening exhibited noteworthy activity against both SW-620 colon cancer cells, which express a high level of COX-2 protein,[25] and MCF-7 breast cancer cells, which constitutively express COX-1.[26] These results provide evidence supporting the hypothesis that COX-inhibitory activity may be involved, at least in part, in biochemical mechanisms, which resulted in the antiproliferative effect of compounds **10** and **25** and, by inference, of the other active compounds. Nevertheless, all the tested compounds also displayed remarkable inhibitory activity against HCT-15 and HCT-116 colon cancer cell lines, which, in contrast, have been found intrinsically deficient in COX-1 and COX-2 expression.[27,28] Taken together, these data indicate that compounds herein described could exert their antiproliferative activity through COX-dependent mechanisms, and suggest that their cytotoxicity might result in targeting other proteins or pathways.

COMPARE Analysis. The patterns of activity of compounds **10**, **17**, **20**, **25**, **27**, and **31** were also analyzed by the COMPARE algorithm.[29] COMPARE searches the NCI database of screened agents for those most similar to the tested compound in their patterns of activity against the panel of 60 cell lines. Similarity in pattern often indicates similarity in mechanism of action and molecular structure. In this analysis, a Pearson correlation coefficient (PCC) > 0.60 is considered significant. When tested as seeds against the NCI "Standard Agents" Database (Table 4), compounds **10**, **17**, and **27** show, with PCC > 0.60 at GI_{50} level, a response pattern that correlated their activity to those of DNA antimetabolite agents, including inosine dialdehyde, 6-mercaptopurine, and 2′-deoxy-6-thioguanosine. COMPARE analysis also indicates that pyridyl esters **25** and **31** shared a response pattern with topoisomerase II inhibitors, including morpholino-ADR and etoposide. Interestingly, results of the COMPARE procedure indicate that the tested compounds might exert their antiproliferative activity through alteration and/or inhibition of processes crucial for the cell cycle progression.

Conclusions

An efficient synthesis of new anthranilic acid derivatives led us to identify a series of potential anticancer agents. The in vitro anticancer screening performed by the NCI reveals that some esters of *N*-(2-(trifluoromethyl)pyridin-4-yl)anthranilic acid demonstrated interesting inhibitory properties against a wide array of human tumor cell lines. In particular, compounds **17**, **20**, and **25** exhibited antiproliferative activity in nanomolar to low micromolar concentrations against most of the tested cell lines. On the basis of observed biological activities and COMPARE analysis, putative COX-dependent/independent mechanisms responsible for antitumor activity were proposed.

Table 4. Results of COMPARE Analysis Procedure against the NCI Standard Agents Database, Using Compounds 10, 17, 20, 25, 27, and 31 as Seeds

compd	standard agent	mechanism of action	endpoint level	correlation	no. of common cell lines
10	inosine dialdehyde	DNA antimetabolite	GI_{50}	0.61	41
17	vincristine	antimitotic agent	GI_{50}	0.68	48
	6-mercaptopurine	DNA antimetabolite	GI_{50}	0.67	35
20	flavone acetic acid[a]		GI_{50}	0.68	32
	2-deoxy-6-thioguanosine	DNA antimetabolite	GI_{50}	0.58	32
25	morpholino-ADR	topoisomerase II inhibitor	GI_{50}	0.62	21
	vincristine	antimitotic agent	TGI	0.61	31
27	macbecin II	DNA antimetabolite	GI_{50}	0.66	38
31	etoposide	topoisomerase II inhibitor	GI_{50}	0.61	34

[a] NCI's Standard Agents Database does not report the mechanism of action. Recently, it has been reported that flavone acetic acid induces a G2/M cell cycle arrest in mammary carcinoma cells.[30]

Experimental Section

Chemistry. Melting points were determined on a Stuart Scientific Melting point SMP1 and are uncorrected. Infrared spectra were run on Bruker Vector 22 spectrophotometer. Absorption band position is given in cm^{-1}. ^1H NMR spectra were recorded on a Varian Unity 300 spectrometer (300 MHz). Chemical shifts are expressed in ppm relative to tetramethylsilane. Silica gel thin-layer chromatography (TLC) sheets from Fluka (silica gel precoated aluminum sheets with fluorescent indicator at 254 nm) were used for TLC. Developed plates were visualized by a Spectroline ENF 260C/F UV apparatus. Concentration and evaporation of the solvent after reaction or extraction were carried out on a rotary evaporator (Büchi Rotavapor) operating at reduced pressure. Petroleum ether refers to the 40−60 °C boiling range fractions. Elemental analyses were carried out with a Carlo Erba model 1106 elemental analyzer and the values found to be within 0.4% of the theoretical values.

General Procedure for the Synthesis of 5a,b. To a solution of **4a** or **4b** (2 mmol) in dry DMF (5 mL) was added ammonium acetate (0.308 g, 4 mmol) and the mixture was gently refluxed for 3 h. The mixture was carefully concentrated in vacuo and to the residue was added ice−water (15 mL). The formed solid was filtered off, washed with water, air-dried, and then crystallized from *n*-hexane to give **5a** or **5b**.

***N*-(2-(Trifluoromethyl)pyridin-4-yl)anthranilic acid methyl ester (5a)**: 0.510 g, 86%, mp 87−89 °C. Anal. ($C_{14}H_{11}F_3N_2O_2$) C, H, N.

2-(2-(Trifluoromethyl)pyridin-4-ylamino)nicotinic acid methyl ester (5b): 0.487 g, 82%, mp 110−112 °C. Anal. ($C_{13}H_{10}F_3N_3O_2$) C, H, N.

General Procedure for the Hydrolysis of 5a,b. A mixture of **5a** or **5b** (1 mmol) in 10% aqueous sodium hydroxide (15 mL) was refluxed for 30 min, during which a homogeneous solution was formed. After cooling, the solution was acidified with 20% aqueous hydrochloric acid to pH 3−4. The formed solid was filtered off, washed with water, air-dried, and crystallized from ethanol to give **6a** or **6b**.

***N*-(2-(Trifluoromethyl)pyridin-4-yl)anthranilic acid (6a)**: 0.260 g, 92%, mp 222−224 °C. Anal. ($C_{13}H_9F_3N_2O_2$) C, H, N.

2-(2-(Trifluoromethyl)pyridin-4-ylamino)nicotinic acid (6b): 0.252 g, 89%, mp 242−244 °C. Anal. ($C_{12}H_8F_3N_3O_2$) C, H, N.

General Procedure for the Synthesis of 7a,b. To an ice-cooled solution of **6a** or **6b** (1 mmol) in dry ethanol (10 mL) was added thionyl chloride (0.476 g, 4 mmol) dropwise with stirring. The ice bath was removed and the mixture was refluxed for 6 h. The volatile components were carefully eliminated in vacuo, and to the residue was added aqueous sodium bicarbonate (15 mL, saturated). The formed solid was filtered off, washed with water, air-dried, and then crystallized from *n*-hexane to give **7a** or **7b**.

***N*-(2-(Trifluoromethyl)pyridin-4-yl)anthranilic acid ethyl ester (7a)**: 0.288 g, 93%, mp 82−84 °C. Anal. ($C_{15}H_{13}F_3N_2O_2$) C, H, N.

2-(2-(Trifluoromethyl)pyridin-4-ylamino)nicotinic acid ethyl ester (7b): 0.283 g, 91%, mp 65−67 °C. Anal. ($C_{14}H_{12}F_3N_3O_2$) C, H, N.

General Procedure for the Synthesis of 8−31. A mixture of FA, **6a** or **6b** (1 mmol), and DCC (0.227 g, 1.1 mmol) in dry chloroform (10 mL) was stirred at room temperature for 1 h and then treated with the appropriate phenol (1.1 mmol). The mixture was stirred at room temperature for an additional 24 h. The formed precipitate was eliminated by filtration and the solution evaporated to dryness in vacuo. To the residue was added aqueous hydrochloric acid (20 mL, 1 M) and the mixture was extracted with ethyl ether (3 × 10 mL). The combined organic layers were washed with brine and then dried over anhydrous magnesium sulfate. Concentration of the dried extracts yielded a residue which was purified by crystallization to give the ester derivatives **8−31**.

Flufenamic Acid 2-Methoxyphenyl Ester (8). Following the general procedure, the title compound was obtained from FA and 2-methoxyphenol: 0.225 g, yield 58%; mp 43−45 °C (petroleum ether). ^1H NMR (DMSO-d_6) δ 3.89 (s, 3H, CH_3), 6.87 (m, 1H), 7.13 (m, 2H), 7.30−7.68 (m, 8H), 8.28 (m, 1H), 9.38 (s, 1H, NH); IR (Nujol) 3330 (NH), 1700 (CO), 1630, 1582 cm^{-1}. Anal. ($C_{21}H_{16}F_3NO_3$) C, H, N.

Flufenamic Acid 3-Methoxyphenyl Ester (9). Following the general procedure, the title compound was obtained from FA and 3-methoxyphenol: 0.256 g, yield 66%; mp 47−48 °C (petroleum ether); ^1H NMR (DMSO-d_6) δ 3.88 (s, 3H), 6.46 (m, 1H), 6.97 (m, 3H), 7.12 (m, 1H), 7.46 (m, 3H), 7.64 (m, 3H), 8.26 (m, 1H), 9.38 (s, 1H, NH); IR (Nujol) 3329 (NH), 1699 (CO), 1582 cm^{-1}. Anal. ($C_{21}H_{16}F_3NO_3$) C, H, N.

Flufenamic Acid 2-Chlorophenyl Ester (10). Following the general procedure, the title compound was obtained from FA and 2-chlorophenol: 0.207 g, yield 53%; mp 64−66 °C (*n*-hexane); ^1H NMR (DMSO-d_6) δ 7.15 (m, 1H), 7.49 (m, 3H), 7.57 (m, 2H), 7.68 (m, 3H), 7.74 (m, 2H), 8.32 (m 1H), 9.30 (s, 1H, NH); IR (Nujol) 3321 (NH), 1702 cm^{-1}. Anal. ($C_{20}H_{13}ClF_3NO_2$) C, H, N.

Flufenamic Acid 3-Chlorophenyl Ester (11). Following the general procedure, the title compound was obtained from FA and 3-chlorophenol: 0.180 g, yield 46%; mp 56−58 °C (petroleum ether); ^1H NMR (DMSO-d_6) δ 7.14 (m, 1H), 7.38−7.70 (m, 10H), 8.26 (m, 1H), 9.31 (s, 1H, NH); IR (Nujol) 3322 (NH), 1700 (CO) cm^{-1}. Anal. ($C_{20}H_{13}ClF_3NO_2$) C, H, N.

Flufenamic acid 4-Chlorophenyl Ester (12). Following the general procedure, the title compound was obtained from FA and 4-chlorophenol: 0.282 g, yield 72%; mp 76−78 °C (petroleum ether); ^1H NMR (DMSO-d_6): δ 7.13 (m, 1H), 7.43−7.70 (m, 10H), 8.26 (m, 1H), 9.32 (s, 1H, NH); IR (Nujol) 3318 (NH), 1693 (CO) cm^{-1}. Anal. ($C_{20}H_{13}ClF_3NO_2$) C, H, N.

Flufenamic Acid 2,4-Dichlorophenyl Ester (13). Following the general procedure, the title compound was obtained from FA and 2,4-dichlorophenol: 0.336 g, yield 79%; mp 104−106 °C (isopropyl ether); ^1H NMR (DMSO-d_6) δ 7.14 (m, 1H), 7.49 (m, 2H), 7.67 (m, 6H), 7.96 (s, 1H), 8.32 (m, 1H), 9.27 (s, 1H, NH); IR (Nujol) 3326 (NH), 1703 (CO) cm^{-1}. Anal. ($C_{20}H_{12}Cl_2F_3NO_2$) C, H, N.

Flufenamic Acid 2,4,6-Trichlorophenyl Ester (14). Following the general procedure, the title compound was obtained from FA and 2,4,6-trichlorophenol: 0.290 g, yield 63%; mp 128−130 °C (isopropyl ether); ^1H NMR (DMSO-d_6) δ 7.15 (m, 1H), 7.48 (m, 2H), 7.74 (m, 4H), 8.03 (s, 1H), 8.36 (m, 1H), 9.23 (s, 1H, NH); IR (Nujol) 3290 (NH), 1716 (CO) cm^{-1}. Anal. ($C_{20}H_{11}Cl_3F_3NO_2$) C, H, N.

Flufenamic Acid Pyridin-3-yl Ester (15). Following the general procedure, the title compound was obtained from FA and 3-hydroxypyridine: 0.262 g, yield 73%; mp 46−48 °C (petroleum ether); ^1H NMR (DMSO-d_6) δ 7.14 (m, 1H), 7.47 (m, 2H), 7.65 (m, 5H), 7.91 (m, 1H), 8.30 (m, 1H), 8.67 (m, 2H), 9.30 (s, 1H, NH); IR (Nujol) 3334 (NH), 1702 (CO), 1584 cm^{-1}. Anal. ($C_{19}H_{13}F_3N_2O_2$) C, H, N.

***N*-(2-(Trifluoromethyl)pyridin-4-yl)anthranilic Acid 2-Methoxyphenyl Ester (16).** Following the general procedure, the title compound was obtained from **6a** and 2-methoxyphenol: 0.248 g, yield 64%; mp 64−66 °C (isopropyl ether); ^1H NMR (DMSO-d_6) δ 7.38 (m, 1H), 7.68 (m, 3H), 8.16 (m, 1H), 8.53 (s, 1H), 8.68 (m, 1H), 8.83 (m, 2H), 10.41 (s, 1H, NH); IR (Nujol) 3277 (NH), 1702 (CO), 1623, 1587 cm^{-1}. Anal. ($C_{20}H_{15}F_3N_2O_3$) C, H, N.

***N*-(2-(Trifluoromethyl)pyridin-4-yl)anthranilic Acid 3-Methoxyphenyl Ester (17).** Following the general procedure, the title compound was obtained from **6a** and 3-methoxyphenol: 0.280 g, yield 72%; mp 86−88 °C (isopropyl ether); ^1H NMR (DMSO-d_6) δ 3.87 (s, 3H, CH_3), 6.88−7.87 (m, 9H), 8.28 (m, 1H), 8.49 (m, 1H), 9.54 (s, 1H, NH); IR (Nujol) 3288 (NH), 1703 (CO), 1610, 1588 cm^{-1}. Anal. ($C_{20}H_{15}F_3N_2O_3$) C, H, N.

***N*-(2-(Trifluoromethyl)pyridin-4-yl)anthranilic Acid 4-Methoxyphenyl Ester (18).** Following the general procedure, the title compound was obtained from **6a** and 4-methoxyphenol: 0.299 g, yield 77%; mp 103−105 °C (isopropyl ether); ^1H NMR (DMSO-d_6) δ 3.46 (s, 3H, CH_3), 7.08−7.86 (m,

9H), 8.27 (m, 1H), 8.49 (m, 1H), 9.55 (s, 1H, NH); IR (Nujol) 3323 (NH), 1695 (CO), 1613, 1588 cm^{-1}. Anal. ($C_{20}H_{15}F_3N_2O_3$) C, H, N.

***N*-(2-(Trifluoromethyl)pyridin-4-yl)anthranilic Acid 4-Methylthiophenyl Ester (19).** Following the general procedure, the title compound was obtained from **6a** and 4-methylthiophenol: 0.328 g, yield 81%; mp 112−114 °C (isopropyl ether/MeOH 3:1); ^1H NMR (DMSO-d_6) δ 3.45 (s, 3H, CH_3), 7.28−7.88 (m, 9H), 8.29 (m, 1H), 8.50 (m, 1H), 9.54 (s, 1H, NH); IR (Nujol) 3317 (NH), 1697 (CO), 1612, 1591 cm^{-1}. Anal. ($C_{20}H_{15}F_3N_2O_2S$) C, H, N.

***N*-(2-(Trifluoromethyl)pyridin-4-yl)anthranilic Acid 2-Chlorophenyl Ester (20).** Following the general procedure, the title compound was obtained from **6a** and 2-chlorophenol: 0.306 g, yield 78%; mp 132−134 °C (isopropyl ether); ^1H NMR (DMSO-d_6) δ 7.30 (m, 1H), 7.51 (m, 4H), 7.56 (m, 1H), 7.72 (m, 2H), 7.87 (m, 1H), 8.35 (m, 1H), 8.48 (m, 1H), 9.49 (s, 1H, NH); IR (Nujol) 3323 (NH), 1704, 1614, 1596 cm^{-1}. Anal. ($C_{19}H_{12}ClF_3N_2O_2$) C, H, N.

***N*-(2-(Trifluoromethyl)pyridin-4-yl)anthranilic Acid 3-Chlorophenyl Ester (21).** Following the general procedure, the title compound was obtained from **6a** and 3-chlorophenol: 0.314 g, yield 80%; mp 80−82 °C (isopropyl ether); ^1H NMR (DMSO-d_6) δ 7.32 (m, 3H), 7.48 (m, 3H), 7.60 (m, 1H), 7.73 (m, 1H), 7.85 (m, 1H), 8.29 (m, 1H), 8.49 (m, 1H), 9.51 (s, 1H, NH); IR (Nujol) 3322 (NH), 1700 (CO) cm^{-1}. Anal. ($C_{19}H_{12}ClF_3N_2O_2$) C, H, N.

***N*-(2-(Trifluoromethyl)pyridin-4-yl)anthranilic Acid 4-Chlorophenyl Ester (22).** Following the general procedure, the title compound was obtained from **6a** and 4-chlorophenol: 0.362 g, yield 92%; mp 84−85 °C (isopropyl ether); ^1H NMR (DMSO-d_6) δ 7.30 (m, 1H), 7.40 (m, 2H), 7.46 (d, J = 8.8 Hz, 2H), 7.64 (d, J = 8.8 Hz, 2H), 7.73 (m, 1H), 7.85 (m, 1H), 8.29 (m, 1H), 8.49 (m, 1H), 9.51 (s, 1H, NH); IR (Nujol) 3321 (NH), 1697 (CO) 1613, 1592 cm^{-1}. Anal. ($C_{19}H_{12}ClF_3N_2O_2$) C, H, N.

***N*-(2-(Trifluoromethyl)pyridin-4-yl)anthranilic Acid 2,4-Dichlorophenyl Ester (23).** Following the general procedure, the title compound was obtained from **6a** and 2,4-dichlorophenol: 0.291 g, yield 68%; mp 100−102 °C (isopropyl ether/MeOH 3:1); ^1H NMR (DMSO-d_6) δ 7.31 (m, 1H), 7.48−7.85 (m, 7H), 8.35 (m, 1H), 8.49 (m, 1H), 9.47 (s, 1H, NH); IR (Nujol) 3325 (NH), 1698 (CO) 1628, 1588 cm^{-1}. Anal. ($C_{19}H_{11}Cl_2F_3N_2O_2$) C, H, N.

***N*-(2-(Trifluoromethyl)pyridin-4-yl)anthranilic Acid 2,4,6-Trichlorophenyl Ester (24).** Following the general procedure, the title compound was obtained from **6a** and 2,4,6-trichlorophenol: 0.259 g, yield 56%; mp 120−122 °C (isopropyl ether/MeOH 3:1); ^1H NMR (DMSO-d_6) δ 7.32 (m, 3H), 7.54 (m, 2H), 7.77 (m, 2H), 7.91 (m, 1H), 7.99 (s, 1H), 8.43 (m, 1H), 8.49 (m, 1H), 9.45 (s, 1H, NH); IR (Nujol) 3328 (NH), 1724 (CO) 1613, 1593 cm^{-1}. Anal. ($C_{19}H_{11}Cl_3F_3N_2O_2$) C, H, N.

***N*-(2-(Trifluoromethyl)pyridin-4-yl)anthranilic Acid Pyridin-3-yl Ester (25).** Following the general procedure, the title compound was obtained from **6a** and 3-hydroxypyridine: 0.298 g, yield 83%; mp 66−68 °C (isopropyl ether); ^1H NMR (DMSO-d_6) δ 7.31 (m, 1H), 7.47 (m, 2H), 7.65 (m, 1H), 7.74 (m, 1H), 7.86 (m, 2H), 8.33 (m, 1H), 8.49 (m, 1H), 8.63 (s, 1H), 9.52 (s, 1H, NH); IR (Nujol) 3260 (NH), 1713 (CO), 1613, 1597 cm^{-1}. Anal. ($C_{18}H_{12}F_3N_3O_2$) C, H, N.

2-(2-(Trifluoromethyl)pyridin-4-ylamino)nicotinic Acid 3-Methoxyphenyl Ester (26). Following the general procedure, the title compound was obtained from **6b** and 3-methoxyphenol: 0.285 g, yield 73%; mp 76−78 °C (isopropyl ether); ^1H NMR (DMSO-d_6) δ 4.05 (s, 3H, CH_3), 7.08 (m, 2H), 8.09 (m, 1H), 8.51 (m, 3H), 8.68 (m, 4H), 10.62 (s, 1H, NH); IR (Nujol) 3286 (NH), 1700 (CO), 1614, 1586 cm^{-1}. Anal. ($C_{19}H_{14}F_3N_3O_3$) C, H, N.

2-(2-(Trifluoromethyl)pyridin-4-ylamino)nicotinic Acid 2-Chlorophenyl Ester (27). Following the general procedure, the title compound was obtained from **6b** and 2-chlorophenol: 0.248 g, yield 63%; mp 64−66 °C (isopropyl ether); ^1H NMR (DMSO-d_6) δ 7.38 (m, 1H), 7.68 (m, 3H), 8.16 (m, 1H), 8.53 (s, 1H), 8.68 (m, 1H), 8.83 (m, 2H), 10.41 (s, 1H, NH); IR (Nujol) 3277 (NH), 1702 (CO), 1623, 1587 cm^{-1}. Anal. ($C_{18}H_{11}ClF_3N_3O_2$) C, H, N.

2-(2-(Trifluoromethyl)pyridin-4-ylamino)nicotinic acid 3-Chlorophenyl Ester (28). Following the general procedure, the title compound was obtained from **6b** and 3-chlorophenol: 0.268 g, yield 68%; mp 68−70 °C (isopropyl ether); ^1H NMR (DMSO-d_6) δ 7.34 (m, 1H), 7.69−7.47 (m, 4H), 8.13 (m, 1H), 8.51 (s, 1H), 8.74 (m, 3H), 10.43 (s, 1H, NH); IR (Nujol) 3288 (NH), 1703 (CO), 1610, 1588 cm^{-1}. Anal. ($C_{18}H_{11}ClF_3N_3O_2$) C, H, N.

2-(2-(Trifluoromethyl)pyridin-4-ylamino)nicotinic Acid 4-Chlorophenyl Ester (29). Following the general procedure, the title compound was obtained from **6b** and 4-chlorophenol: 0.315 g, yield 80%; mp 86−88 °C (isopropyl ether); ^1H NMR (DMSO-d_6) δ 7.35 (m, 1H), 7.53 (d, J = 8.2 Hz, 2H), 7.69 (d, J = 8.2 Hz, 2H), 8.10 (m, 1H), 8.52 (s, 1H), 8.79 (m, 3H), 10.45 (s, 1H, NH); IR (Nujol) 3283 (NH), 1695 (CO), 1610, 1584 cm^{-1}. Anal. ($C_{18}H_{11}ClF_3N_3O_2$) C, H, N.

2-(2-(Trifluoromethyl)pyridin-4-ylamino)nicotinic Acid 2,4-Dichlorophenyl Ester (30). Following the general procedure, the title compound was obtained from **6b** and 2,4-dichlorophenol: 0.368 g, yield 86%; mp 116−118 °C (isopropyl ether); ^1H NMR (DMSO-d_6) δ 7.36 (s, 1H), 7.73 (s, 2H), 8.00 (s, 1H), 8.13 (m, 1H), 8.52 (s, 1H), 8.79 (m, 3H), 10.34 (bs, 1H, NH); IR (Nujol) 3298 (NH), 1716 (CO), 1610, 1587 cm^{-1}. Anal. ($C_{18}H_{10}Cl_2F_3N_3O_2$) C, H, N.

2-(2-(Trifluoromethyl)pyridin-4-ylamino)nicotinic Acid Pyridin-3-yl Ester (31). Following the general procedure, the title compound was obtained from **6b** and 3-hydroxypyridine: 0.278 g, yield 77%; mp 96−98 °C (isopropyl ether); ^1H NMR (DMSO-d_6) δ 4.05 (s, 3H, CH_3), 7.36 (m, 1H), 7.71 (m, 1H), 8.01 (m, 1H), 8.13 (m, 1H), 8.52 (s, 1H), 8.69 (m, 2H), 8.78 (m, 3H), 10.42 (s, 1H, NH); IR (Nujol) 3263 (NH), 1722 (CO), 1597 cm^{-1}. Anal. ($C_{17}H_{11}F_3N_4O_2$) C, H, N.

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Supporting Information Available: Experimental details and structural assignment for compounds **3** and **4**. Elemental analyses, spectral data, and tables of NCI's in vitro testing results. This material is available free of charge via the Internet at http://pubs.acs.org.

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