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Synthesis of new *N*-(2-(trifluoromethyl)pyridin-4-yl)anthranilic acid derivatives and their evaluation as anticancer agents

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Abstract—The *N*-(2-(trifluoromethyl)pyridin-4-yl)anthranilic acid **6** and a series of its ester and amide derivatives were synthesized and evaluated for their in vitro cytotoxic activity against human cancer cells. Ester derivatives **13** and **18** exhibited potent growth inhibitory activity with GI_{50} values at nanomolar concentrations. Among amide derivatives, *N*-anthraniloylglycinate **19** shown moderate inhibitory activity in the full panel cancer cell line screening.

1. Introduction

Among the wide variety of chemical structures in development as new anticancer drugs, molecules designed on anthranilic acid scaffold have attracted great interest in recent years. In experimental models, a number of these compounds exhibited preventive or inhibitory activity because they can act through different biological mechanisms that are involved in the development and maintenance of tumoral cells. For example (Fig. 1), Tranilast,[1] also known as anti-allergic drug, inhibited the proliferation, chemotaxis, and tube formation of human microvascular endothelial cells in vitro and angiogenesis in vivo by VEGF-mediated mechanism. Farnesyl anthranilate[2] suppressed the growth of murine melanomas in in vivo and in vitro models, in part by arresting cells in the G1/S interface of the cell cycle and in part by initiating apoptosis. The anthranilamide PD 184352 (CI-1040), developed by Parke-Davis,[3] is an inhibitor of both Mitogen Activated Extracellular Kinases MEK1 and MEK2. In vivo, PD 184352 was shown to inhibit the growth of colon and pancreatic tumors. Tariquidar (XR9576),[4] is a potent and specific inhibitor of P-gp, commonly associated with the development of multidrug resistance (MDR). In in vitro and in vivo experimental studies, Tariquidar was shown to restore the antitumor activity of several drugs including doxorubicin, paclitaxel, etoposide, and vincristine

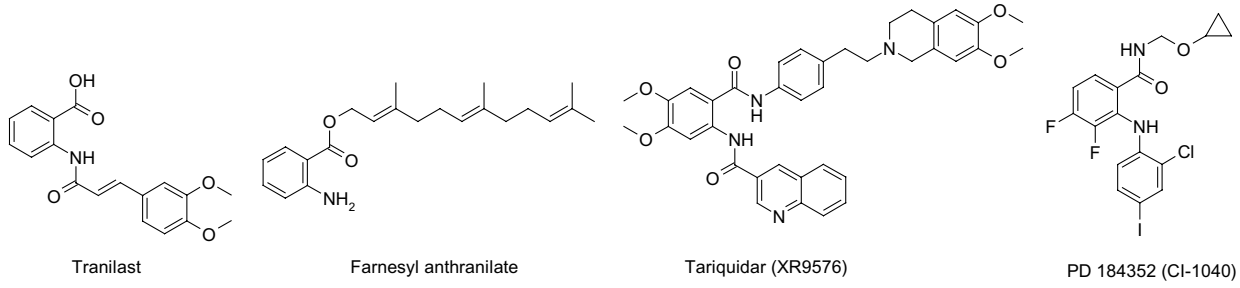

Figure 1. Anthranilic acid derivatives as anticancer agents.

Keywords: Anthranilic acid; Anticancer activity; Antitumoral drugs; Pyridine derivatives.
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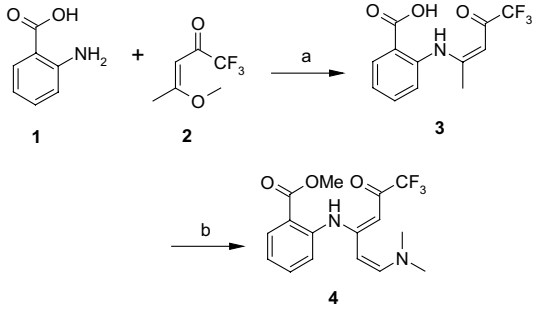
against two highly resistant MDR human tumor cells.[5] Other anthranilamides showed activity as VEGF receptor tyrosine kinase[6] and MMP inhibitors.[7]

Prompted by the above findings, in continuation of our ongoing research on pyridine derivatives endowed with anticancer activity,[8] we became interested in novel compounds containing the anthranilic acid scaffold bearing the pyridine moiety. In this communication we report the synthesis and evaluation for in vitro antitumoral efficacy against human cancer cell lines of the *N*-(2-(trifluoromethyl)pyridin-4-yl)anthranilic acid **6** and a series of its ester and amide derivatives.

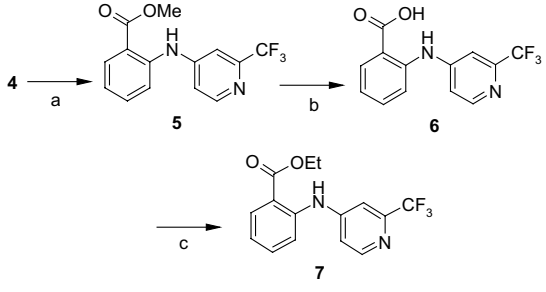
2. Chemistry

The synthesis of target compounds was conveniently performed as outlined in Schemes 1–3. By slight modifications of the procedure previously described by us,[9] the anthranilic acid **1** was reacted with trifluoroacetylvinyl ether **2** in 1:1.5 M ratio, in refluxing acetonitrile to give the *N*-alkenylanthranilic acid **3** in almost quantitative yield (Scheme 1).

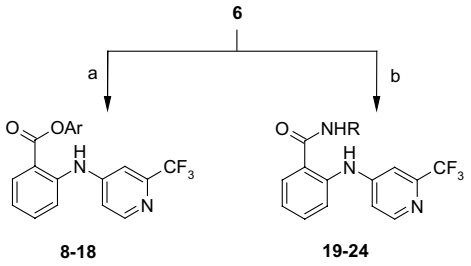
Upon reaction with excess of *N*,*N*-dimethylformamide dimethyl acetal (DMF-DMA) in boiling toluene, compound **3** was converted into *N*-hexadienyl-anthranilic acid ester **4** in a one-pot procedure, resulting in esterification–condensation reaction sequence, in 92% overall yield.



Scheme 1. Reagents and conditions: (a) MeCN, reflux, 2 h; then rt, 24 h; (b) DMF-DMA (3 equiv), toluene, reflux, 2 h.



Scheme 2. Reagents and conditions: (a) ammonium acetate (2 equiv), DMF, reflux, 1 h; (b) 10% aq NaOH, reflux, 30 min, then H^+; (c) EtOH anhyd, $SOCl_2$ (4 equiv), reflux, 3 h.



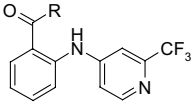
Scheme 3. Reagents and conditions: (a) ArOH, DCC, $CHCl_3$, rt, 24 h; (b) R–NH_2, CDI, THF, rt, 24 h.

The key intermediate **4** underwent pyridine ring closure upon treatment with ammonium acetate in hot DMF to give **5** in 76% yield (Scheme 2). Hydrolysis of **5** in 10% aq NaOH solution afforded acid **6**. The ethyl ester **7** was obtained in 93% yield by refluxing an ethanolic solution of **6** in the presence of thionyl chloride.

Aryl esters and amides were prepared as reported in Scheme 3. Treatment of **6** with the appropriate phenol in the presence of DCC gave esters **8**–**18** in 56–92% yields. Amide derivatives **19**–**24** were obtained in 46–82% yields by reaction of **6** with the appropriate amine by CDI method.[10]

The stability of ester and amide derivatives was investigated both toward acid (0.1 M aq trifluoroacetic acid)

Table 1. Comparison of effects of anthranilic acid derivatives, at 10^{-4} M concentrations, on the proliferation in a three-cell line prescreen, expressed as percentage of proliferation of vehicle treated cells



Compd	R	Cell lines		
		MCF7 (breast)	NCI-H460 (lung)	SF-268 (CNS)
5	OMe	96	100	103
6	OH	105	100	102
7	OEt	98	102	103
8	OPh-3-Me	nt[a]	nt	nt
9	OPh-3-OMe	42	13	43
10	OPh-4-OMe	74	49	105
11	OPh-4-OPr	nt	nt	nt
12	OPh-4-SMe	80	68	81
13	OPh-2-Cl	6	3	17
14	OPh-3-Cl	12	3	5
15	OPh-4-Cl	24	9	28
16	OPh-2,4-Cl_2	3	1	9
17	OPh-2,4,6-Cl_3	12	9	55
18	O-3-Py	62	22	75
19	$NHCH_2COOEt$	60	19	59
20	$NH(CH_2)_3OMe$	70	105	97
21	NH-2-Py	66	86	94
22	NH-3-Py	56	62	98
23	NH-4-Py	79	99	96
24	$NHCH_2$-3-Py	nt	nt	nt

[a] Not tested.

Table 2. GI_{50} values, in μM concentrations, of anthranilic acid derivatives **9**, **13**–**19**

Panel/cell line	Compd							
	9	**13**	**14**	**15**	**16**	**17**	**18**	**19**
Leukemia								
CCRF-CEM	nt[a]	0.032	0.89	4.9	3.5	nt	0.033	2.2
HL-60(TB)	2.1	0.47	13	6.4	6.6	3.9	44	33
K-562	0.74	0.051	3.2	3.6	3.0	2.4	0.043	18
MOLT-4	0.69	0.028	0.93	100	100	100	0.039	16
RPMI-8226	1.0	0.24	1.8	2.9	2.4	nt	0.054	11
SR	nt	0.030	0.44	4.0	0.77	nt	0.010	17
Nonsmall cell lung cancer								
A549/ATCC	3.2	0.73	18	15	11	3.9	0.72	29
EKVX	6.6	8.6	22	25	19	3.3	0.39	31
HOP-62	2.8	2.7	25	nt	nt	2.7	0.019	21
HOP-92	5.7	0.027	8.7	21	12	100	0.48	0.057
NCI-H226	8.1	0.29	2.1	2.9	3.4	5.9	0.51	100
NCI-H23	2.0	0.25	2.4	2.8	3.5	2.6	0.066	22
NCI-H322M	7.4	4.8	23	17	14	20	2.9	32
H460	4.1	0.66	11	10	4.3	3.4	0.34	20
H522	7.6	0.51	4.2	4.3	4.6	5.7	0.41	22
Colon cancer								
COLO 205	2.2	nt	nt	nt	nt	6.3	0.14	19
HCC-2998	nt	2.2	3.5	13	3.2	nt	0.36	71
HCT-116	1.1	1.8	1.6	nt	nt	1.7	0.036	21
HCT-15	2.8	0.22	3.7	2.1	2.2	2.7	0.29	29
HT29	3.7	0.39	nt	nt	nt	4.6	0.055	29
KM12	2.6	0.33	9.5	8.9	3.7	4.1	0.31	27
SW-620	4.4	0.36	4.4	6.6	4.6	4.6	0.062	20
CNS cancer								
SF-268	4.7	0.22	3.0	4.4	3.1	3.9	0.063	20
SF-295	2.5	0.24	18	63	15	4.2	0.31	71
SF-539	nt	0.58	2.8	15	3.8	nt	0.010	28
SNB-19	3.5	7.4	nt	nt	nt	7.1	0.33	30
SNB-75	nt	0.68	nt	nt	nt	nt	nt	nt
U251	2.9	0.27	nt	nt	nt	3.0	0.52	18
Melanoma								
LOX IMVI	1.1	0.026	nt	nt	nt	2.0	0.052	17
M14	1.3	0.036	1.8	2.1	1.9	2.1	0.031	25
SK-MEL-28	12	nt	nt	nt	nt	24	0.66	23
SK-MEL-5	2.2	0.26	2.6	17	2.8	3.1	0.17	10
UACC-257	5.4	nt	nt	4.5	11	12	55	69
UACC-62	3.1	0.31	2.0	2.6	2.3	1.7	0.35	17
Ovarian cancer								
OVCAR-3	1.6	0.22	3.8	11	11	4.3	0.14	24
OVCAR-4	nt	0.26	nt	nt	nt	nt	0.46	35
OVCAR-5	3.5	0.46	14	26	7.9	4.1	0.49	26
OVCAR-8	3.6	0.24	3.5	4.1	4.8	3.0	0.69	26
SK-OV-3	nt	8.0	nt	nt	nt	nt	0.25	29
Renal cancer								
786-0	3.4	0.25	3.5	2.7	1.9	2.9	0.27	29
A498	nt	3.9	nt	nt	21	nt	0.52	64
ACHN	3.4	0.28	nt	11	13	2.4	0.26	34
CAKI-1	2.7	0.29	2.1	2.3	2.1	7.1	0.052	16
RXF 393	21	nt	nt	nt	nt	9.5	0.34	19
SN12C	5.4	0.33	3.0	3.0	3.0	3.1	0.41	24
TK-10	11	0.65	85	18	4.1	3.4	0.27	37
Prostate cancer								
PC-3	3.2	0.30	7.4	17	8.3	15	0.42	22
DU-145	nt	4.3	14	19	16	nt	0.22	27
Breast cancer								
MCF7	7.6	0.28	4.8	3.7	3.6	4.7	0.16	25
NCI/ADR-RES	nt	0.32	2.2	16	3.6	nt	0.081	51
MDA-MB-231/ATCC	2.1	0.32	1.7	1.8	2.2	3.2	0.39	24

(*continued on next page*)

Table 2 (*continued*)

Panel/cell line	Compd							
	9	**13**	**14**	**15**	**16**	**17**	**18**	**19**
HS 578T	4.9	0.32	21	30	25	2.6	0.57	46
MDA-MB-435	1.0	0.13	3.0	2.1	2.9	1.6	0.064	39
BT-549	8.1	0.34	6.8	15	6.8	2.4	0.016	36

[a] Not tested.

and base (0.1 M aq NaOH) hydrolysis. After 48 h at room temperature, the formation of acid **6** was monitored by TLC (chloroform/*n*-hexane 4:1). In all cases, examination of TLC plates revealed a single spot with rf identical to starting compound. Thus, anthranilic esters and amides do not appear to be hydrolytically labile.

3. Results and discussion

The anthranilate derivatives examined in this study were evaluated at National Cancer Institute (NCI) for cytotoxicity in the NCI's disease-oriented antitumor screening.[11–13]

Cytotoxic activities of synthesized compounds were first evaluated in vitro against NCI-H460 (nonsmall cell lung), MCF7 (breast), or SF-268 (CNS) human cancer cell lines in a three-cell line, one dose primary anticancer assay. Results for each test compound are reported as the percentage of growth of the treated cells when compared to the untreated control cells and are shown in Table 1. Compounds which reduced the growth of any one of the cell lines to less than 32% were passed on for evaluation in the full panel of 60-cell lines.

As shown in Table 1, aryl esters **9** and **13**–**18**, and ethyl *N*-anthraniloylglycinate **19** fulfilled this condition. These compounds were then assayed for cytotoxic activity against leukemia, lung, colon, CNS, melanoma, ovarian, renal, prostate, and breast human cancer cell lines. The test compounds were evaluated using five concentrations at 10-fold dilutions, in the 10^{-4}–10^{-8} M range. The anticancer activity of each compound was deduced from dose–response curves and is presented in Table 2 according to the data provided by NCI. The response parameter GI_{50} refers to the drug concentration that produced 50% of growth inhibition.

Although acid **6** and alkyl esters **5** and **7** are devoid of cytotoxic activity, their conversion into (hetero)aryl esters leds to compounds **9** and **13**–**18** that exhibit antiproliferative activity. In this series, growth inhibition is strongly sensitive to the nature of aryloxycarbonyl moiety and position of substituents on aromatic ring. The 3-pyridyl ester **18** exhibits potent cytotoxic activity, with GI_{50} values in the range of 0.010–0.72 μM against almost all cancer cell lines. In contrast, HL-60 (leukemia), NCI-H322M (lung), SK-MEL-2, and UACC-257 (melanoma) cell lines are inhibited by higher concentrations (44, 2.9, 32, and 55 μM, respectively) of **18**.

Esters bearing chlorine atom(s) on the phenyloxy moiety show antiproliferative activity decreasing approximately in the order: **13** (2-chloro) ≫ **14** (3-chloro) > **17** (2,4,6-trichloro) > **16** (2,4-dichloro) > **15** (4-chloro). This fact suggests that steric and electronic effects of substituent(s) on phenyloxy moiety might be implicated in the activity of these types of compound. Among these, the ester **13**, bearing a 2-chlorophenyl ring, exhibits potent antiproliferative activity against all tested cell lines, with GI_{50} values in the range of 0.026–8.6 μM. Compound **13** shows particular selectivity against melanoma LOX-IMVI (GI_{50} 0.026 μM), leukemic MOLT-4 (GI_{50} 0.028 μM), CCRF-CEM (GI_{50} 0.032 μM), and K-562 (GI_{50} 0.051 μM) cell lines.

On other hand, compound **9**, bearing a 3-methoxy-phenyl ring, is effective against all the tested cell lines. Compound **9** shows GI_{50} values between 0.69 and 21 μM, the best result being against leukemic MOLT-4 and K-562 cell lines, with GI_{50} values of 0.69 and 0.74 μM, respectively. Comparison of antiproliferative activity of **9** with the 4-methoxy isomer **10** shows that shifting of 3-methoxy group to 4-position results in dramatic loss of inhibitory activity. Introduction of methylthio group in 4-position of phenyloxy moiety, also results in inactive compound **12**.

Among amide derivatives, only ethyl *N*-anthraniloylglycinate **19** exhibits moderate inhibitory activity in the full panel cell lines test; however, this compound shows selective inhibitory activity against nonsmall cell lung cancer HOP-92 (GI_{50} 0.057 μM) and leukemic CCRF-CEM (GI_{50} 2.2 μM) cell lines.

4. Conclusions

In summary, we report the synthesis and anti-proliferative activity against human cancer cell lines of a series of *N*-(2-(trifluoromethyl)pyridin-4-yl)-anthranilic acid derivatives. The first results confirm the validity of our approach providing practical access to anhranilate-based derivatives possessing potent in vitro antiproliferative activity against human tumor cells. Compounds **13** and **18** were found to have GI_{50} values at nanomolar concentrations in most of the cell lines assayed.

Evaluation in in vivo models of these compounds is underway at NCI and the results will be disclosed in due course.

Acknowledgements

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10. All compounds gave correct analytical and spectral data. For example compound **7**; ^{1}HNMR (DMSO-d_6): δ 1.34 (t, J = 6.9 Hz, 3H, CH_3), 4.36 (q, J = 6.9 Hz, 2H, CH_2), 7.24 (d, J = 5.4 Hz, H-5 Py), 7.37 (t, J = 7.7 Hz, 1H, Ph), 7.45 (s, H-3, Py), 7.64 (m, 1H, Ph), 7.75 (m, 1H, Ph), 8.05 (d, J = 7.7 Hz, 1H, Ph), 8.47 (d, J = 5.4 Hz, H-6, Py), 9.52 (s, 1H, NH). Melting point = 82–84 °C (from *n*-hexane). Infra red (Nujol mull), v = 3252 (NH), 1677 (C=O) cm^{-1}.
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