Digest Journal of Nanomaterials and Biostructures Vol. 5, No 1, March 2010, p. 177-184

PRELIMINARY ANTICANCER ACTIVITY OF SOME PROP-2-ENEAMIDO, THIAZOLE AND 1-ACETYL-PYRAZOLIN DERIVATIVES OF AMINOBENZOTHIAZOLES

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The scaffold 2-aminobenzothiazole fused with prop-2-eneamido, 1-acetyl-pyrazolin and thiazole moieties have been evaluated for their anticancer activity at the National Cancer Institute for testing against a panel of approximately 60 different human tumor cell lines derived from nine neoplastic cancer types. Relations between structure and activity are discussed, the most efficient anticancer compound (**1**) was found to be active with selective influence on renal cancer cell lines, especially on RXF 393 with a growth % of -71.40.

(Received March 2, 2010; accepted March 16, 2010)

Keywords: Aminobenzothiazole; Prop-2-eneamide; Pyrazoline; Thiazole; Anticancer activity.

1. Introduction

Cancer is a disease of striking significance in the world today. It is the second leading cause of death in the world after cardiovascular diseases and it is projected to beginning the primary cause of death there within the coming years [1,2]. The identification of novel structures that can be potentially useful in designing new, potent selective and less toxic anticancer agents is still a major challenge to medicinal chemistry researchers [3]. Despite of the important advances achieved over recent decades in the research and development of various cancerostatic drugs, current antitumor chemotherapy still suffers from two major limitations–the first is the lack of selectivity of conventional chemotherapeutic agents for cancer tissues, bringing about unwanted side effects. The second is the acquisition by cancer cells of multiple-drug resistance. Unwanted side effects of antitumor drugs could be overcome with agents capable of discriminating tumor cells from normal proliferative cells and the resistance is minimized using combined modality approach with different complementary mechanism of action [4].

The current scenario highlights the need for the discovery and development of new lead compounds of simple structure, exhibiting optimal *in vivo* antitumor potency and new mechanisms of action. Recent advances in clinical techniques, including large co-operative studies are allowing more rapid and reliable evaluation of new drugs. The combination of these advantages with improved preliminary screening systems is enhancing the emergence of newer and more potent compounds. In this regard, it should be emphasized that National Cancer Institute (NCI) *in vitro* primary anticancer drug screen represents a valuable research tool to facilitate the drug discovery of new structural/ mechanistic types of antitumor agents [5].

The main sources of lead compounds for drug development are natural products, new synthetic compounds and analogues of new agents [6]. Benzothiazole template is a privileged structure fragments in modern medicinal chemistry considering its broad pharmacological spectrum and affinity for various biotargets of these class heterocyclic compounds. It is among the usually occurred heterocyclic nuclei in many marine as well as natural plant products possessing

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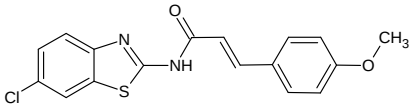
the wide range of biological applications [7-9]. Some of benzothiazole derivatives, especially 2-aminobenzothiazoles, possessed diuretic [10], antimicrobial [11], antihistamine [12], anticonvulsant [13,14], anti-inflammatory [15] effects. Aminobenzothiazoles and related heterocycles represents a novel class of potent and selective antitumor agents which exhibit nanomolar inhibitory activity against a range of human breast, lung, colon, leukemia, CNS, melanoma, ovarian, renal and prostate cell lines *in vitro* [16–20]. It must be emphasized that combination of 2-aminobenzothiazoles with other heterocycles is a well-known approach for drug-like molecules build-up, which allows achieving new pharmacological profile, action strengthening or toxicity lowering [21]. Thus, a single molecule containing more than one pharmacophore, each with different mechanism of action could be beneficial for the treatment of cancer. Taking inspiration from the above, and as a part of our enduring research on the "chemistry-driven" approach of aminobenzothiazoles, we have struck a rich lode of novel bioactive agents and report herein the influence of prop-2-eneamido, 1-acetyl-pyrazoline and thiazole moieties with 2-aminobenzothiaole scaffold combination on the anticancer effect.

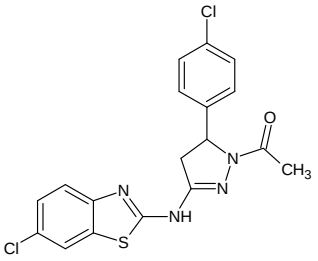
2. Experimental

2.1. Chemistry

The scaffold benzothiazole analogues viz. 6-chloro-[3-(4-methoxyphenyl)-prop-2-eneamido]benzothiazole (**1**; NSC: D-751324), 6-chloro-2-[(1-acetyl-5-(4-chlorophenyl))-2-pyrazolin-3-yl]aminobenzothiazole (**2**; NSC: D-751325), 6-chloro-2-[(1-acetyl-5-(4-methoxyphenyl))-2-pyrazolin-3-yl]aminobenzothiazole (**3**; NSC: D-751326), 4-(6-fluoro-1,3-benzothiazol-2-yl)amino-2-(4-hydroxyphenyl)amino-1,3-thiazole (**4**; NSC: D-751327) and 4-(6-fluoro-1,3-benzothiazol-2-yl)amino-2-(4-methoxyphenyl)amino-1,3-thiazole (**5**; NSC: D-751328) were synthesized and characterized as reported earlier [13, 22].



(**1**); (NSC: D-751324)



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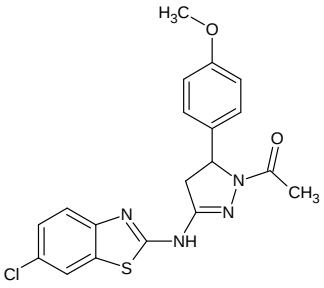

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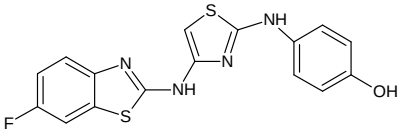
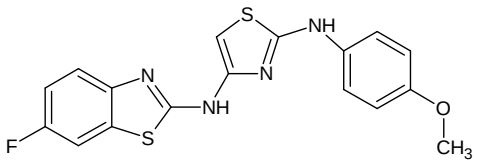


(**4**); (NSC: D-751327)



(**5**); (NSC: D-751328)

2.2. Anticancer activity

The compounds (**1–5**) were screened for preliminary anticancer assay by National Cancer Institute (NCI), Bethesda, Maryland, USA in an *in vitro* 60 human tumor cell lines panel, derived from nine neoplastic cancer types.

2.2.1. Criterion for submission and selection of compounds for testing in the NCI screens

The compounds (**1–5**) were submitted to NCI under the Developmental Therapeutic Program (DTP) which operates a tiered anticancer compound screening course for the benefit of the general research community with the goal of identifying novel chemical leads and biological mechanisms. Structures of the compounds were selected for screening based on their ability to add diversity to the NCI small molecule compound collection. In addition, the submission of compounds with drug-like properties utilizing the concept of privileged scaffold or structures based on computer-aided design were preferred [23].

2.2.2. Process of NCI–60 DTP Human Tumor Cell Line Screen

The screening of the compounds (**1–5**) operated with the *In Vitro* Cell Line Screening Project (IVCLSP) which is a dedicated service, providing direct support to the DTP anticancer drug discovery program. The process utilized 60 different human tumor cancers of the lung, colon, brain, ovary, breast, prostate and kidney which was aimed in showing selective growth inhibition or cell killing of particular tumor cell lines by specific compound. The screening begins with the evaluation of all selected compounds against these 60 cell lines at a single dose of 10^{-5} M.

2.2.3. Methodology of in vitro cancer screen

The human tumor cell lines of the cancer screening panel were grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells were inoculated into 96 well microtiter plates in 100 µL at plating densities ranging from 5,000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37° C, 5% CO_2, 95% air and 100% relative humidity for 24 h prior to addition of experimental drugs.

After 24 h, two plates of each cell line were fixed *in situ* with TCA, to represent a measurement of the cell population for each cell line at the time of drug addition (T_z). Experimental drugs were solubilized in dimethyl sulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate was thawed and diluted to twice the desired final test concentration (10^{-5} M) with complete medium containing 50 µg/ml gentamicin. Aliquots of 100 µl of these drug dilutions were added to the appropriate microtiter wells already containing 100 µl of medium, resulting in the required final drug concentrations.

Following drug addition, the plates were incubated for an additional 48 h at 37°C, 5% CO_2, 95% air, and 100% relative humidity. For adherent cells, the assay was terminated by the addition of cold TCA. Cells were fixed *in situ* by the gentle addition of 50 µl of cold 50% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 minutes at 4°C. The supernatant was discarded, and the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (100 µl) at 0.4 % (w/v) in 1% acetic acid was added to each well, and plates were incubated for 10 minutes at room temperature. After staining, unbound dye was removed by washing five times with 1% acetic acid and the plates were air dried. Bound stain was subsequently solubilized with 10 mM trizma base, and the absorbance was read on an automated plate reader at a wavelength of 515 nm. Using the seven absorbance measurements [time zero, (T_z), control growth, (C), and test growth in the presence of drug at the 10^{-5} M concentration level (T_i)], the percentage growth was calculated at each of the drug concentrations levels. Percentage growth inhibition was calculated as:

$[(T_i - T_z)/(C - T_z)]$ x 100 for concentrations for which $T_i \geq T_z$,

$[(T_i - T_z)/T_z]$ x 100 for concentrations for which $T_i < T_z$.

3. Results and discussion

In the present study, some new prop-2-eneamido, 1-acetyl-pyrazoline and thiazolyl substituted 2-aminobenzothiazoles (compounds **1–5**) were submitted to NCI for *in vitro* human tumor cell lines screening. The compounds were evaluated at single concentration of 10^{-5} M towards the panel of approximately 60 cancer cell lines derived from nine different cancer types: leukemia, lung, colon, CNS, melanoma, ovarian, renal, prostrate and breast cancers. Preliminary anticancer assay was performed according to the US NCI protocol [23]. All the compounds (**1–5**) were added to a previously prepared cell culture at a single concentration. The cell culture was incubated for 48 h. End point determinations were made with a protein binding dye, sulforhodamine B (SRB). The results for each compound were reported as the percent growth of treated cell lines/panel when compared to untreated control cells (Table 1).

*Table 1. Percent tumor growth inhibition recorded on some panel/cell lines at 10^{-5}M of compounds (**1–5**).*

Panel/ Cell Line	**Growth % of tested compounds**				
	(**1**)	(**2**)	(**3**)	(**4**)	(**5**)
Leukemia					
HL-60(TB)	88.99	100.69	112.87	115.70	97.90
K-562	85.77	91.57	91.54	90.40	85.23
MOLT-4	86.79	102.35	106.87	101.85	92.28
RPMI-8226	84.59	94.53	95.93	91.07	92.27
SR	105.16	117.13	95.16	124.48	90.40
Non-Small Cell Lung Cancer					
A549/ATCC	92.71	99.49	82.64	98.19	101.01
EKVX	97.25	107.03	99.47	111.77	108.64
HOP-62	78.43	75.32	85.84	93.10	92.99
HOP-92	NT	NT	65.06	53.87	NT
NCI-H226	94.57	96.88	98.04	101.25	104.27
NCI-H23	93.32	104.25	92.00	91.78	105.03
NCI-H322M	112.77	121.44	122.77	115.04	113.97
NCI-H460	95.16	97.83	NT	111.25	105.05
NCI-H522	78.67	55.44	58.61855	60.80	74.64
Colon Cancer					
COLO 205	99.68	96.99	100.96	102.99	106.86
HCT-116	83.35	89.64	91.17	86.40	99.34
HCT-15	96.72	100.08	87.41	97.38	92.86
HT29	93.38	98.77	90.33	86.32	93.85
KM12	94.03	100.50	100.64	106.33	104.48

Panel/ Cell Line	**Growth % of tested compounds**				
	(1)	**(2)**	**(3)**	**(4)**	**(5)**
SW-620	94.09	99.73	NT	98.98	104.02
CNS Cancer					
SF-268	88.77	99.62	97.88	98.58	105.10
SF-295	112.24	107.39	106.40	112.34	110.08
SF-539	92.99	91.45	87.52	90.72	94.04
SNB-19	98.25	107.80	115.09	108.23	101.16
SNB-75	88.03	71.98	80.46	86.29	90.51
U251	95.10	94.68	95.39	103.38	100.82
Melanoma					
LOX IMVI	89.96	89.27	89.04	89.31	89.54
MALME-3M	115.83	109.53	115.32	102.72	126.40
M14	95.67	100.53	101.48	96.68	108.52
MDA-MB-435	123.85	108.87	113.13	112.82	117.83
SK-MEL-2	92.17	123.14	101.75	91.47	96.08
SK-MEL-28	109.26	99.60	102.48	96.25	99.93
SK-MEL-5	103.24	102.62	104.47	110.70	104.41
UACC-62	99.16	89.01	102.59	106.86	95.93
Ovarian Cancer					
IGROV1	101.82	110.91	114.54	112.11	115.33
OVCAR-3	96.32	94.43	97.42	100.18	103.18
OVCAR-4	94.05	81.40	81.61	95.55	101.47
OVCAR-5	102.18	110.90	114.06	101.20	105.78
OVCAR-8	91.65	81.54	88.56	79.06	82.66
NCI/ADR-RES	101.17	97.48	94.90	96.14	105.36
SK-OV-3	90.37	88.84	87.83	91.71	95.04
Renal Cancer					
786-0	87.24	94.27	99.72	91.02	98.51
A498	81.25	89.08	NT	98.63	83.87
ACHN	104.89	98.35	90.46	93.61	96.83
CAKI-1	100.71	89.17	94.04	92.72	104.24
RXF 393	28.59	78.78	86.98	91.97	91.79
SN12C	95.34	113.28	105.08	103.57	114.04
TK-10	97.40	107.56	89.17	95.89	104.35
UO-31	82.38	86.66	94.13	92.93	90.84

Panel/ Cell Line	**Growth % of tested compounds**				
	(**1**)	(**2**)	(**3**)	(**4**)	(**5**)
Prostate Cancer					
PC-3	75.15	88.25	87.86	76.88	83.85
DU-145	98.43	111.38	111.60	110.41	108.18
Breast Cancer					
MCF7	93.79	94.06	92.16	91.55	96.25
MDA-MB-231/ATCC	110.95	131.01	113.76	110.72	100.96
HS 578T	91.65	85.93	NT	95.08	103.26
T-47D	82.36	73.79	80.80	103.08	101.40
MDA-MB-468	99.90	94.91	101.21	98.73	103.89

NT; not tested at this molar concentration.

The mean growth %, range of growth % and growth % relative to most sensitive cell line is depicted in Table 2. The tested compounds showed a broad spectrum of growth inhibitory activity against human tumor cells, as well as some distinctive patterns of selectivity. Compound (**1**) was found to be a highly active growth inhibitor of the renal cancer cell line (RXF 393) with a growth % of most sensitive cell line to be -71.40, whilst least active over other cell lines. The mean growth % for compound (**1**) was observed 93.96 % and fall in a range of -71.4 to 95.25. Compounds (**2**), (**3**) and (**5**) showed selectivity on non-small cell lung cancer NCI-H522 with a growth % of most sensitive cell line to be -44.55, -46.12 and -25.35, respectively. These compounds showed varying range of growth % -44.55 to 75.57 for compound (**2**), -41.38 to 64.16 for compound (**3**) and -25.35 to 51.75 for compound (**5**). The hydroxyphenyl substituted thiazole derivative, compound (**4**) possessed significant activity on non-small cell lung cancer cell line HOP-92 with growth % of most sensitive cell line as -46.12. The range of growth % was found to be -46.12 to 70.61.

Table 2. Anticancer screening data of tested compounds

Compd No. (NSC code)	60 Cell lines in assay in 1-dose 10^{-5} M concentration			
	Mean growth (%)	Range of growth (%)	Most sensitive cell line	Growth of most sensitive cell line (%)
(1) (D-751324)	93.96	-71.40 to 95.25	Renal Cancer (RXF 393)	-71.40
(2) (D-751325)	97.23	-44.55 to 75.57	Non-Small Cell Lung Cancer (NCI–H522)	-44.55
(3) (D-751326)	96.47	-41.38 to 64.16	Non-Small Cell Lung Cancer (NCI–H522)	-41.38
(4) (D-751327)	97.65	-46.12 to 70.61	Non-Small Cell Lung Cancer (HOP–92)	-46.12
(5) (D-751328)	99.94	-25.35 to 51.75	Non-Small Cell Lung Cancer (NCI–H522)	-25.35

The SAR study revealed that anticancer activity of compounds (**1–5**) is sensitive to the nature of substitutents in position-2 of aminobenzothiazole. Among the compounds tested, compound (**1**) with prop-2-eneamido derivative and p-hydroxyphenyl substitution demonstrates the most marked effect and possessed significant activity (Fig. 1).

The compounds with pyrazoline and thiazole substitution were found to have moderate sensitivity. Amongst all, the compound (**5**) with p-methoxyphenyl substituted thiazole derivative was found to be least active. The results also states that heterocyclic rings, 1-acetyl-pyrazoline and thiazole do not support eminently for the anticancer activity. In fact, the chloro substituted aminobenzothiazoles were found to have encouraging sensitivity to cell lines compared to fluoro group.

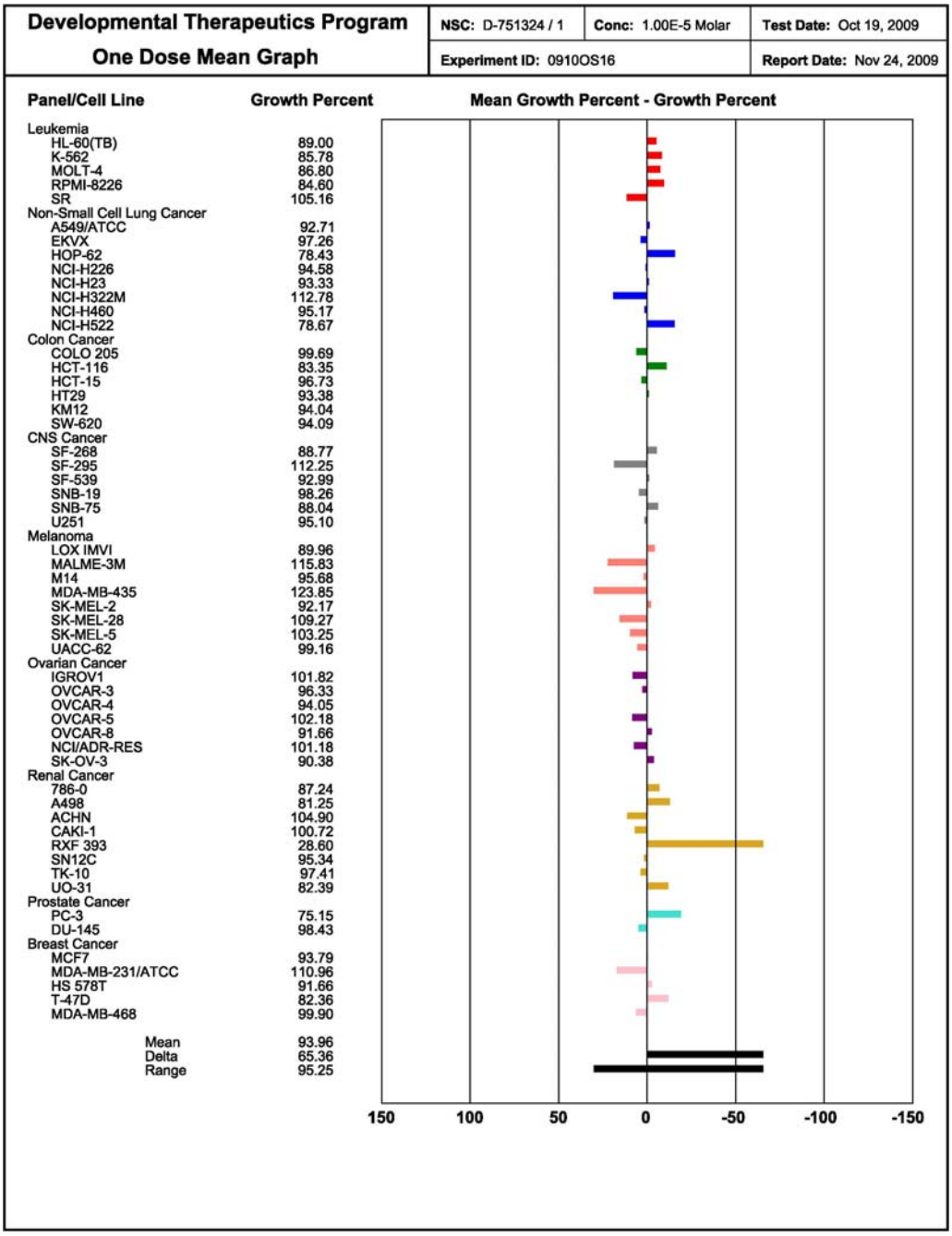


Fig. 1. Selected NCI sixty cell screening data highlighting the potency of compound (1; NSC: D-751324) against renal cancer cell line (RXF 393). Bars to the right of the mean line represent cell lines more sensitive to test compound compared to mean, whereas bars to the left represent less sensitive cell lines.

4. Conclusion

In the present paper five compounds were tested and most of them displayed antitumor activity on renal cancer cell and non-small cancer cell lung cancer cell lines. The most active compound (**1**) showed selective influence on renal cancer cell lines RXF 393. The obtained results prove the necessity for further investigations to clarify the features underlying the antitumor potential of tested compounds.

Acknowledgements

We thank the National Cancer Institute (NCI), Bethesda, MD, USA for *in vitro* screening of our compounds in human cancer cell lines.

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