Journal of Pharmacology and Toxicology

ISSN 1816-496X

Academic Journals Inc.

www.academicjournals.com

Journal of Pharmacology and Toxicology 1 (5): 471-477, 2006
ISSN 1816-496X

Neuropsychobehavioral Effects and Anticancer Activity of Some Substituted Triazolo[4,3-a][1,4]Benzodiazepines

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Abstract: Neuropsychobehavioral effects and anticancer screening of some 8-chloro-6-(2-fluorophenyl)-1-(aryl)-4H-[1,2,4]triazolo[4,3-a][1,4]benzodiazepines (3a-f) are discussed. The compounds were evaluated for antianxiety properties, effects on coordination and effects on learning and memory. The compounds were also screened for their anticancer activity. All the compounds possessed variable antianxiety properties and three of them possessed skeletal muscle relaxant property. One of the compound exhibited promising anticancer activity.

Key words: Triazolobenzodiazepine, neuropsychobehavioral effect, anticancer activity

Introduction

Many compounds of the 1,4-benzodiazepine series display tranquilizing, muscle-relaxant, anticonvulsant and sedative effects (Randall and Kappel, 1973; Randall, 1974; Vida, 1995). There are many benzodiazepines that are marketed either exclusively as hypnotics or used extensively as sleep inducers in addition to their use as antianxiety agents (Greenblatt and Shader, 1974). Since their introduction as tranquilizer drugs, the synthesis of new molecules bearing 1,4-benzodiazepine moiety has become increasingly important (Archer and Sternbatch, 1968; Sharp, 1984; Tucker and Lee Count, 1996). In our recent publication (Narayana *et al*., 2006) we reported the synthesis and anticonvulsant activity of some new triazolo[4,3-a][1,4]-benzodiazepine derivatives. Most of the compounds exhibited comparable anticonvulsant activity with standard drug diazepam. The compounds we reported resemble well-known CNS depressant drugs such as alprazolam and midazolam in their structure with an aryl substitution at C-1. Therefore the main objective of the present study focuses on the neuropsychobehavioral effects of these compounds. The study is also extended to evaluate their anticancer activity.

This study presents the evaluation of 8-chloro-6- (2-fluorophenyl)-1-(aryl)-4H-[1,2,4]triazolo[4,3-a][1,4]benzodiazepines (3a-f) for antianxiety properties, effects on coordination and effects on learning and memory along with their anticancer activity. As we reported earlier (Narayana *et al*., 2006), the compounds bearing pyridine moiety (3a and 3f), 4-flourophenyl (3d) and 2-bromo-5-methoxyphenyl (3e) substituents at C-1 had shown excellent anticonvulsant activity in comparison with standard drug diazepam, used in the PTZ animal model. The same compounds 3a, 3d, 3e and 3f were more effective for shortening the duration of MES-induced tonic seizures too. It is

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in this background we decided to carry out the continuation of our studies. Antianxiety properties were carried out by elevated plus maze model (Kulkarni and Reddy, 1996; Vogel and Vogel, 1997) and effect on coordination by Rotarod test (McIlwain *et al*., 2001) and effect of learning and memory by transfer latency method (Kulkarni, 1999; Itoh *et al*., 1990). All the studies were carried out with the approval of Institutional Ethics Committee.

Out of the six compounds submitted to National Cancer Institute, NCI, USA, four (3a, 3c, 3d and 3f) were accepted by them and screened for three cell lines (prescreen). The compound 3f was selected for 60 cell line screening.

Materials and Methods

The neuropsychobehavioural studies were carried out at Department of Chemistry, Mangalore University, Karnataka, India. All the studies were carried out with the approval of institutional ethics committee. The anticancer studies were carried out at National Institute of Health, Bethesda, Maryland, USA under the Drug Discovery Programme of NCI.

Neuropsychobehavioral Effects

Swiss albino mice of either sex weighing around 25-30 g were used for the study. Diazepam was used as standard drug. Test compounds 3a-f (dose = 50 mg kg^{-1}) and standard drug (dose = 2 mg kg^{-1}) were suspended in 2% gum acacia and were administered in a volume of 10 mL kg^{-1}.

Elevated plus Maze Model of Anxiety

The plus maze apparatus, consisting of two open arms (16×5 cm) and two closed arms (16×5×12 cm) having an open roof, with plus maze elevated (25 cm) from the floor. Each mouse was placed at the center of elevated plus maze with its head facing the open arm. During the 5 min of experiment, the behavior of the mouse was recorded as number of entries in to open and closed arms, time sent in open and closed arms and number of rears minimum open and close arms. The rationale is that the open arms are more fear provoking and the ratio of either time spent in open and closed arms or entries in to open arms to close arms reflect the relative safety of closed arms compared with the relative fearfulness of open arms. Anxiolytic drugs are expected to increase the proportion of entries into and time spent on open arms. The results are presented in Table 1.

Effect on Coordination-Rotarod Test

Motor coordination and balance were tested using accelerating rotarod (TSE Rotarod system). Mice were placed on a horizontal metal-coated rod with rubber (3 cm diameter) rotating at an initial speed of 10 rpm min^{-1}. Rotational velocity of the rod was linearly increased from 10 to 20 rpm within 10 sec. The time each animal was able to maintain its balance walking on top of the rod was measured. Mice were given two trials with a maximum time of 300 sec and a 30 to 60 min intertrial rest interval. Before the beginning of all experiments, the riding ability of the animals in the rotarod was checked and mice that immediately dropped off (within 30 sec) were removed from the experiment. The results are presented in Table 2.

Effects on Learning and Memory

The elevated plus maze was used to measure the anxiety state in animals. However, transfer latency that is the time elapsed between the movements of the animal from an open to an enclosed arm was markedly shortened if the animal had previously experienced entering open and closed arms and this shortened transfer latency has shown to be related with memory process. In the EPM, acquisition (learning) can be considered as transfer latency on the first day trials and the retention/consolidation (memory) is examined 24 h later.

On the first day, each mouse was placed at the end of an open arm, facing away from the central platform. Transfer Latency (TL) is the time taken by the mouse with all its four legs to move into one

Table 1: Antianxiety studies (Elevated plus maze model)

	No. of entries				Time spent (s)		No. of rears	
	Open	Close	Total	% of open/total	Open	Close	Open	Close
Control	1	7	8	12.90	6	282	0	8
Diazepam	6	27	33	18.18	54	223	2	31
3a	2	16	14	14.28	42	249	0	6
3b	1	1	2	50.00	20	294	0	3
3c	2	14	16	12.90	45	259	0	7
3d	2	9	11	18.18	27	287	0	16
3e	7	13	20	39.00	89	208	0	19
3f	14	29	43	32.55	127	188	0	25

Table 2: Effects on coordination by accelerated test

Treatment	First trial	Second trial
Control	300.0	300.0
Diazepam	10.2	28.3
3a	8.2	12.0
3b	300.0	300.0
3c	8.4	12.4
3d	14.2	22.4
3e	300.0	300.0
3f	14.2	28.6

Table 3: Effects on learning and memory

Treatment	Latent period (1st day) (s)	Latent period (2nd day)(s)
Control	52	24
3a	60	17
3b	54	22
3c	63	19
3d	65	30
3e	53	25
3f	90	45

of the enclosed arms. Transfer latency was recorded on the first day. If the animal did not enter into one of the enclosed arms within 90 sec, it was gently pushed into one of the enclosed arms and the transfer latency was assigned as 90 sec. The mouse was allowed to explore the maze for 10 sec and then returned to its home cage. Retention was examined 24 h after the first day trial. The results are presented in Table 3.

Anticancer Activity

Four of the triazolobenzodiazepines such as 3a, 3c, 3d and 3f were screened for their antitumor activities at NIH, Bethesda, Maryland, USA under the Drug Discovery Programme of NCI as per the procedure suggested by Boyd and Paul (1995) in a primary three cell line-one dose antitumor assay against NCI-H (Lung), MCF-7 (Breast) and SF 268 (CNS). In the current protocol each cell line is inoculated on a preincubated microtiter plate. Test agents are added at a single concentration and culture is incubated for 48 h. End point of determinations is made with sulpharhodamine B, a protein binding dye. Compounds which reduce the growth of any one of the cell line to 32% or less (negative numbers indicate cell kill) are passed for evaluation in a panel of 60 cell lines over a 5-long dose range. Prescreen results are presented in Table 4.

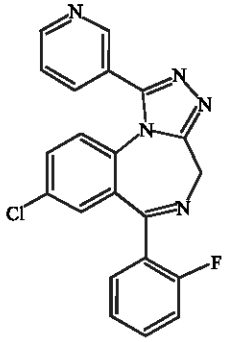
Among the four tested compounds the compound 3f, 8-Chloro-6-(2-fluorophenyl)-1-pyridin-3-yl-4H-[1,2,4]triazolo[4,3-a][1,4]benzodiazepine was selected for 60-cell screening and considered as active. The 60 cell screening result showed that, the compound 3f showed good antiproliferative activity on the whole panel of 60 cells derived from nine cancer cells namely Leukemia, lung, colon, melanoma, renal, ovarian, CNS, prostrate and breast cells. Their GI_{50} (Growth Inhibition), TGI (Total Growth Inhibition) and LC_{50} (Lethal Concentration) values were determined. The 60 cell results are presented in Table 5.

Table 4: Anticancer activity screening data of the compounds 3a, 3c, 3d and 3f

		Growth percentage			
Compound	NCI code	NCI-H	MCF7	SF-268	Activity[a]
3a	NSC 736962	89	117	115	Inactive
3c	NSC 736963	80	85	71	Inactive
3d	NSC 736964	89	95	96	Inactive
3f	NSC 736965	0	1	1	Active

Fixed concentration (100 μM; standard NCI protocol), [a]Active when growth percentage is <32% for any one of the three line cell

Table 5: Sixty cell line *in vitro* antitumour screening data of 3f (GI_{50}, TGI and LC_{50} in μM)



3f

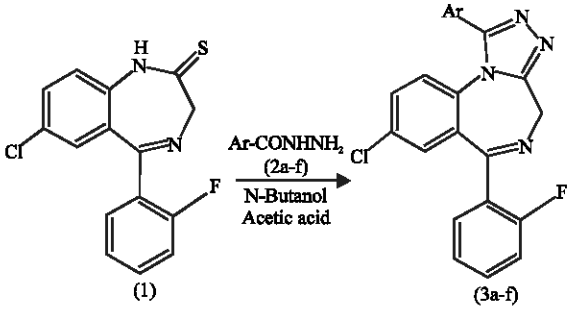
Panel/cell line	GI_{50}	TGI	LC_{50}
Leukemia			
CCRF-CEM	13.5	>100	>100
HL-60 (TB)	17.4	44.8	>100
K-562	23.2	92.3	>100
MOLT-4	21.4	60.1	>100
RPMI-8226	12.1	41.0	>100
SR	17.2	48.1	>100
Non-small cell lung cancer			
A549/ATCC	23.0	>100	>100
EKVX	34.4	>100	>100
HOP-62	22.3	67.5	>100
HOP-92	14.1	51.6	>100
NCI-H226	24.2	86.5	>100
NCI-H23	21.4	56.9	>100
NCIH322M	31.5	>100	>100
NCI-H460	15.3	38.3	95.6
NCI-H522	30.5	>100	>100
Colon cancer			
COLO 205	25.9	97.0	>100
HCC-2998	17.7	35.4	70.8
HCT-15	28.7	>100	>100
HT29	30.0	99.1	>100
KM12	20.7	88.4	>100
SW-620	24.7	>100	>100
CNS cancer			
SF-268	25.9	>100	>100
SF-295	18.4	64.7	>100
SF-539	20.0	37.2	68.3
SNB-19	23.6	>100	>100
SNB-75	24.2	63.3	>100
U251	21.2	96.0	>100
Melanoma			
LOX IMVI	12.7	29.6	69.0
MALME-3M	26.0	73.7	>100
SK MEL-2	26.2	>100	>100
SK MEL-28	21.6	83.4	60.6
SK MEL-5	14.5	29.7	>100

Table 5: Continued

Panel/cell line	GI_{50}	TGI	LC_{50}
UACC-257	27.9	91.3	>100
UACC-62	17.9	43.5	>100
Ovarian cancer			
IGRVO1	37.2	>100	>100
OVCAR-3	22.5	56.9	>100
OVCAR-4	31.0	>100	>100
OVCAR-5	46.1	>100	>100
OVCAR-6	27.0	>100	>100
SK-OV-3	26.1	>100	>100
Renal cancer			
786-O	32.3	>100	>100
A498	21.4	65.0	>100
ACHN	25.5	>100	>100
CAKI-1	30.0	>100	>100
RXF-393	41.5	>100	>100
SN12C	27.1	>100	>100
TK-10	30.7	>100	>100
UO-31	29.3	>100	>100
Prostate cancer			
PC-3	31.3	>100	>100
DU-145	24.5	>100	>100
Breast cancer			
MCF7	21.4	60.6	>100
NCI/ADR-RES	18.1	45.0	>100
MDA-MB-231/ATCC	22.3	71.0	>100
HS-578T	32.9	>100	>100
MDA-MB-435	19.2	73.5	>100
BT-549	20.9	48.9	>100
T-47D	36.4	>100	>100

Results and Discussion

We reported (Narayana *et al*., 2006) the synthesis and characterization of the 8-chloro-6- (2`-fluorophenyl)-1-(aryl)-4H-[1,2,4]triazolo[4,3-a][1,4]benzodiazepines (3a-f). According to the process described in present study 7-chloro-5- (2-fluorophenyl)-1,3-dihydro-2H-1,4-benzodiazepine-2-thione (1) was made to react with aromatic acid hydrazides (2a-f) by refluxing in n-butanol with catalytic amount of acetic acid which resulted in the formation of 8-chloro-6- (2-fluorophenyl)-1-(aryl)-4H-[1,2,4]triazolo[4,3-*a*][1,4] benzodiazepine (3a-f); Scheme 1.



	Ar-
3a-	Pyridin-4-yl
3b-	4-nitro-1H-indol-2-yl
3c-	6-methoxy-2-naphthyl
3d-	4-fluorophenyl
3e-	2-Bromo-5-methoxyphenyl
3f-	Pyridin-3-yl

Scheme 1

The compounds, 8-chloro-6- (2-fluorophenyl)-1-(aryl)-4*H*-[1,2,4]triazolo[4,3-*a*][1,4] benzodiazepines (3a-f) were evaluated for antianxiety properties, effects on coordination and effects on learning and memory. New compounds were also screened for their anticancer activity.

In the present study, the standard drug diazepam (2 mg kg^{-1}) increased number of entries to open arms, time spent and rearing in open and close arms. The percentile ratio of open arm to total arm entries was also increased by diazepam. The compounds 3e and 3f increased number of entries to open arm. The compounds 3a, 3b, 3d, 3e and 3f increased the percentile ratio of open arm to total arm entries. All of the compounds increased time spent in open arms. Rearing in close arm was increased by 3d, 3e and 3f. In accelerod performance, 3a, 3c, 3d and 3f showed a decline in motor control. Mice given the compounds 3a, 3c, 3d and 3f also looked sedated. None of the compounds had significant effect on the latent periods on both the days. The compound 3f looked sedated, which may have caused increase in the latency on first day. The studies are supportive to our previous findings and highlight of the present findings is that triazolo[4,3-a][1,4] benzodiazepines with aryl substitution at C-1 exhibited promising CNS depressant activity like the compounds with alkyl substitution at C-1 as in alprazolam and midazolam. These conclusions are based on a pilot study in a limited number of animals in the respective models used. Further studies using larger samples have to be done for obtaining conclusive data.

Anticancer studies reveal that the compound 3f, 8-Chloro-6-(2-fluorophenyl)-1-pyridin-3-yl-4H-[1,2,4]triazolo[4,3-a][1,4]benzodiazepine is most active. The 60 cell screening result showed that, the compound showed promising antiproliferative activity on the whole panel of 60 cells derived from nine cancer cells namely leukemia, lung, colon, melanoma, renal, ovarian, CNS, prostrate and breast cells. The highest activity was observed against Leukemia, CNS cancer and Melanoma cancer cells in comparison with other cells. The examination of the 60 cell result reveals that 3f showed highest activity against Leukemia RPMI-8226 cell line [GI_{50} = 12.1, TGI = 41.0 and LC_{50} >100], Non-small cell lung cancer NCI-H460 [GI_{50} = 15.3, TGI = 38.3 and LC_{50} = 95.6], Colon cancer HCC-2998 [GI_{50} = 17.7, TGI = 35.4 and LC_{50} = 70.8], CNS cancer SF-539 [GI_{50} = 20.0, TGI = 37.2 and LC 50 = 68.3] and Melanoma cancer SK MEL-28 [GI_{50}= 21.6, TGI = 83.4 and LC_{50} = 60.6]. The compound was moderately active against prostrate cancer cells and renal cancer cells. The compound 3f was already proved to have highest antconvulsant activity along with 3a. Both of these contains pyridine ring as the substituent at C-1. The presence of pyridine ring may the reason for higher activity.

Conclusions

The present study reveals that all the tested compounds have variable antianxiety properties. Compounds 3a, 3c, 3d and 3f have muscle relaxant property. None of the compounds affected learning and memory. Compound 3f exhibited promising anticancer activity in comparison with other compounds tested. Hence the compound 3f, 8-Chloro-6-(2-fluorophenyl)-1-pyridin-3-yl-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepine emerges to be most active compound and recommended for further studies.

Acknowledgments

The authors are thankful to Dr. V.L. Narayana, Chief, Drug Synthesis, National Institute of Health, Bethesda, USA for his help in screening the compounds for anticancer activity. Authors are also thankful to Dr. Mohandas Rai, K.S. Hegde Medical College for their help in carrying out the neuropsychobehavioral studies.

References

Archer, G.A. and L.H. Sternbatch, 1968. Chemistry of benzodiazepines. Chem. Rev., 68:747 .

Boyd, M.R. and K.D. Paull, 1995. Some practical considerations and applications of the national cancer institute *in vivo* anticancer drug discovery scheme. Drug Dev. Res., 34: 91-109.

Greenblatt, D.J. and R.I. Shader, 1974. Benzodiazepines in Clinical Practice. New York, Raven Press, pp: 183-196.

Itoh, J., T. Nabeshima and T. Kameyama, 1990. Utility of an elevated plus maze for the evaluation of memory in mice: Effect of nootropics, scopolamine and electroconvulsive shock. Psychopharmacology, 101: 27-33.

Kulkarni, S.K. and D.S. Reddy, 1996. Animal behavioural models for testing antianxiety activity. Method Find Exp. Clin. Pharmacol., 18: 219-240.

Kulkarni, S.K., 1999. Handbook of Experimental Pharmacology. 3rd Edn., Delhi, Vallabh Prakashan.

McIlwain, K.L., M.Y. Merriweather, L.A. Yuva Paylor and R. Paylor, 2001. The use of behavioral test batteries: Effect of training history. Physiol. Behav., 73: 705-717.

Narayana, B., K.K. Vijaya Raj, B.V. Ashalatha and N. Suchetha Kumari, 2006. Synthesis of some new substituted triazolo[4,3-a][1,4]benzodiazepine derivatives as potent anticonvulsants. Eur. J. Med. Chem., 41: 417-422.

Randall, L.O. and B. Kappell, 1973. The Benzodiazepines, Garattini, S., E. Mussini and L.O. Randall, (Eds.), New York, Raven Press, pp: 27-51.

Randall, L.O., 1974. Psychopharmacological Agents. Gordon, M. (Ed.) New York, Academic Press, 3: 175-281.

Sharp, J.T., 1984. Comprehensive Heterocyclic Chemistry II. Katritzky, A.R. and C.W. Rees (Eds.). Pergamon: Amsterdam, 7: 593.

Tucker, H and D.J. Lee Count, 1996. Comprehensive Heterocyclic Chemistry II, Katritzky, A.R, C.W. Rees, E.F.E. Scriven (Eds.). Elsevier, Amsterdam, 9: 151.

Vida, J.A., 1995. Anticonvulsants, Foye, W.O., T.L. Lemke and D.A. Williams (Eds.). Principles of Medicinal Chemistry, London, Williams and Wilkins.

Vogel, H.G. and W.H. Vogel, 1997. Drug Discovery and Evaluation. Germany, Heidelberg. Springer Verlag, pp: 378-379.