CYTOTOXIC ACTIVITY OF NOVEL ISATIN DERIVATIVES

Nataraj K.S[*1], Venkateshwara Rao J[2], Jayaveera, K.N[3]
Srinivas Ampaty[1].

[1]SRR College of Pharmaceutical Sciences, Valbhapur, Elkathurthy, Karimangar – 505 476 (India.)

[2]Talla Padmavathi college of Pharmacy, Warangal.

[3] OTRI.JNTU, Ananthapur

***Corresponding author**: E-mail: kalakondan@yahoo.com Mobile: +91 9866250050

Summary

Forty two New 2-{(benzalamino-4-hydroxybenzyl) (1,3,4)-oxadiazino[6,5-b]} Indole derivatives (**V**) have been synthesized by condensing 2-Amino-4-[(1,3,4)oxadiazino[6,5-b]indole-3-yl]-phenol (**IV**) with various aromatic aldehydes. The intermediates, on the other hand, have been synthesized by the cyclization of 3-Amino-4-hydroxy-benzoic acid (2-oxo-1,2-dihydro-indol-3-ylidene)-hydrazide (**III**) in presence of Concentrated H_2SO_4. The title compounds have been purified and characterized by their analytical and spectral data.All compounds have been evaluated for their cytotoxic activity against CHO cell lines and MCF-7 Cell lines by MTT method. The results of the evaluation have been viewed by taking cisplatin as the standard drug.Compound $V_{25}$was the most potent compound with IC50 value of 25.9 and 95um against CHO and MCF-7 cell lines.Compounds V_{32} and V_{31} were next potent compounds with IC50 values of 31.26, 98.2 and 32.10, 120.2um among all the forty two compounds. The rest of the compounds showed moderate cytotoxic activity.

Key words: (1,3,4)-oxadiazino-[6,5-b]indole, Cytotoxic activity,Isatin derivatives.

Introduction

It is known from the literature that indole derivatives exhibit varied biological and pharmacological properties[1-8] viz. antimicrobial, antiviral, anti neoplastic, analgesic, CNS activities. In view of theses observations the synthesis of New 2-{(benzalamino-4-hydroxybenzyl) (1,3,4)-oxadiazino[6,5-b]} Indole derivatives (**V**) has been carried out.

For this purpose the required indole-2,3-diones (I) were prepared and condensed with 3-amino-4-hydroxybenzoicacidhydrazide(II) in ethanol to get the respective 3-Amino-4-hydroxy-benzoic acid(2-oxo-1,2-dihydro-indol-3-ylidene)-hydrazide(III).These compounds were cyclized using concentrated sulfuric acid to get respective 2-Amino-4-[(1,3,4)oxadiazino[6,5-b]indole-3-yl]-phenol (**IV**). These compounds were refluxed with aromatic aldehyde,ethanol and few drops of acetic acid to get the title compounds. The compounds were characterized by their physical, analytical and spectral data (IR and PMR,MASS). The data on Cytotoxic activity is presented in Table I

Materials Methods

Cytotoxic activity [9-12]:

The Cytotoxic activity of the test compounds was determined by Microculture tetrazolium (MTT) assay method. The principle of Microculture tetrazolium assay (MTT) is based on the metabolic reduction of 3-(4,5-dimethylthiazol-2,5-diphenyl)tetrazolium bromide (MTT) to water insoluble formazan crystals with mitochondrial dehydrogenase enzyme, which gives direct correlation of viable cells.In this method Cell suspension (0.1 ml containing 5 x 10^5 cells / 100 µl), 0.1 ml of the compound solution (10, 20, 50, 100, 150 and 200 µg in DMSO such that the final concentration of DMSO in media is less than 1%) was added to the 96 well plates and kept in carbon dioxide incubator with 5% CO_2 at 37^oC for 72 hours. Blank contains only cell suspension and control wells contain 1% DMSO and cell suspension. After 72 hours, 20 µl of MTT was added and kept in carbon dioxide incubator for 2 hours followed by 80 µl of lysis buffer (15% SLS in 1:1 DMF and water). The plate was covered with aluminium foil to protect from light, then the 96 well plate was kept on rotary shaker for 8 hours. After 8 hours the 96 well plates were processed on ELISA reader for absorption at 562 nm. The readings were averaged and viability of the test samples was compared with DMSO control.

Results and Discussion:

All compounds have been evaluated for their cytotoxic activity against CHO cell lines and MCF-7 Cell lines by MTT method. The results of the evaluation have been viewed by taking cisplatin as the standard drug.The IC50 values of the test compounds are presented in table1and the values are compared with the IC50 values of the standard cisplatin.

CompoundsV_{1-10} indicates that all the compounds have noticeable degree of cytotoxic activity against CHO and MCF-7 Cell lines. Among them, compound V_7 was found to be relatively more effective against CHO cell lines and MCF-7 cell lines with IC50 values of 131 and 160um respectively,the rest of the compounds showed moderate cytotoxic activity in the range of 198 to 352µM and 194 to 368µM against CHO cell lines and MCF-7 cell lines.The IC50 values of standard cisplatin is 25µM.

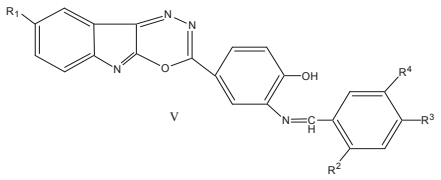
Componds11-20 indicates that the all the compounds have a noticeable degree of Cytotoxic activity against CHO cell lines and MCF-7 cell lines. IC50 values of these compounds are in the range of 50.02 to 141.20µM against CHO cell lines and 63.92 to 209 µM against MCF-7 cell lines. Among these compounds, compound V_{16} was found to be more effective in Cytotoxic activity against CHO cell lines and MCF-7cell lines with IC50 values of 50.20 and 63.9µM respectively.

Compounds21-31 in this Some of the compounds showed more cytotoxic activity against CHO and MCF-7 cell lines similar to cisplatin IC50 value 25µM. Among the test compounds, compoud V_{25} showed more Cytotoxic activity with IC_{50} value of 25.9 and 95 µM against CHO and MCF-7 cell lines respectively. This series of compounds were more sensitive to CHO cell lines compared to MCF-7 cell lines. Rests of the compounds were in the range from 69.54 to 120.4 µM and 120.8 to 180.84µM.

Compounds V_{32-42} showed more Cytotoxic activity against CHO cell lines similar to standard cisplatin (IC50 value 25µM). Among these compounds, compound V_{32} showed more Cytotoxic activity with IC50 values of 31.26. and 98.2µM against CHO and MCF-7 cell lines.

Compounds V_{31},V_{30},andV_{34} were next in the order of Cytotoxic activity with IC50 values of 32.10, 42.40 and 55.04 respectively against CHO cell lines, where as the IC50 values of rest of the compounds were in the range of 84.03 to 130.45µM against CHO cell lines.Compounds of this series showed more Cytotoxic activity against CHO cell lines compared to MCF-7 cell lines.

Table I: Data on Cytotoxic activity of New 2-{(benzalamino-4-hydroxybenzyl) (1,3,4)-oxadiazino[6,5-b] } Indole derivatives (**V**)



V

Standard: Cisplatin at all concentration ranges tested showed an IC_{50} of 25µM

Compound	Substituents				CHO cell lines IC_{50} Value (µM)	MCF-7 cell Lines IC_{50} Value (µM)
	R^1	R^2	R^3	R^4		
Standard	--	--	--	--	25	25
V(1)	H	H	H	H	352	368
V(2)	H	H	Cl	H	320	301
V(3)	H	OH	H	H	322	258
V(4)	H	H	OCH_3	H	248	201
V(5)	H	H	OCH_3	OCH_3	256	240
V(6)	H	H	$N(CH_3)_2$	H	198	296
V(7)	H	H	OH	OCH_3	131	160
V(8)	Br	H	H	H	296	284
V(9)	Br	H	Cl	H	210	194
V(10)	Br	OH	H	H	141.20	209.0
V(11)	Br	H	OCH_3	H	82.92	180.6
V(12)	Br	H	OCH_3	OCH_3	69.07	152.2
V(13)	Br	H	$N(CH_3)_2$	H	64.20	120.4
V(14)	Br	H	OH	OCH_3	55.92	110.1
V(15)	NO_2	H	H	H	82.50	168.8
V(16)	NO_2	H	Cl	H	50.20	63.9

V(17)	NO_2	OH	H	H	94.63	183.5
V(18)	NO_2	H	OCH_3	H	80.01	125.4
V(19)	NO_2	H	OCH_3	OCH_3	120.8	180.8
V(20)	NO_2	H	$N(CH_3)_2$	H	56.4	160.7
V(21)	NO_2	H	OH	OCH_3	46.58	124.4
V(22)	F	H	H	H	42.75	102.2
V(23)	F	H	Cl	H	35.08	74.3
V(24)	F	OH	H	H	77.9	121.6
V(25)	F	H	OCH_3	H	25.9	95.0
V(26)	F	H	OCH_3	OCH_3	89.06	168.8
V(27)	F	H	$N(CH_3)_2$	H	69.54	120.4
V(28)	F	H	OH	OCH_3	130.45	186.6
V(29)	Cl	H	H	H	125.7	172.6
V(30)	Cl	H	Cl	H	42.4	160.0
V(31)	Cl	OH	H	H	32.10	120.2
V(32)	Cl	H	OCH_3	H	31.26	98.2
V(33)	Cl	H	OCH_3	OCH_3	84.03	94.6
V(34)	Cl	H	$N(CH_3)_2$	H	55.04	210.0
V(35)	Cl	H	OH	OCH_3	135.6	154.5
V(36)	CH_3	H	H	H	68.7	110.9
V(37)	CH_3	H	Cl	H	325.4	306.8
V(38)	CH_3	OH	H	H	309.4	254
V(39)	CH_3	H	OCH_3	H	302.9	233.8
V(40)	CH_3	H	OCH_3	OCH_3	118	131.7
V(41)	CH_3	H	$N(CH_3)_2$	H	204	220
V(42)	CH_3	H	OH	OCH_3	282	294.2

Conclusion

Compound $V_{25}$was the most potent compound with IC50 value of 25.9 and 95uM against CHO and MCF-7 cell lines.Compounds $V_{32}$and V_{31} were next potent compounds with IC50 values of 31.26, 98.2 and 32.10, 120.2um among all the forty two compounds. The rest of the compounds showed moderate cytotoxic activity

References:

[1] Sarangapani M, Jessy Jacob, Srinivas B and Raghunandan N, ``Synthesis and CNS Activity of New Indole Derivatives``, Indian Drugs 38(5) May 2001(264-268).

[2] S. N. Pandeya, V.S.Laximi and A.Pandeya, ``Biological Activity of Mannich Bases``, Indian J. Pharma. Science. 2003, 65(3):213-222.

[3] M. Sarangapani, A. Narayana Reddy, Y. Jayamma, V.M. Reddy, ``Synthesis and Pharmacological Activity of New Hydrazones``, Indian Drugs 35(6) June 1998 (336-343).

[4] M S Y Khan and M Akhtar, ``Synthesis of Some New 2,5-Disubstituted 1,3,4 – Oxadiazole Derivatives and Their Biological Activity``, Indian journal of Chemistry Vol. 42B, April 2003 (903-904).

[5] S.P. Singh, S.K. Shukla and L.P. Awasthi, ``Synthesis of Some 3-(4`-Nitrobenzoyl hydrazono)-2-Indolinone as Potential Anti-Viral Agents``, Current Science, Aug 20, 1983, Vol. 52, No.16

[6] Anku Patel, Sanjay bari, Gokul Talele, Jitendra Patel and Manda Sarangapani, Synthesis and Antimicrobial Activity of Some New Isatin Derivatives``, Iranian Journal of Pharmaceutical research, (2006)4:249-254.

[7] B. Bari, A.O. Agrawal, and U.K.Patel, ``Synthesis and Pharmacological Evalution of Some Novel Isatin derivatives for Antimicrobial Activity, Journal of Science, Islamic Republic of Iran 19(3):217-221(2008).

[8] M. Sarangapani amd V.M.Reddy, " Synthesis and screening of Isatin-3-[N^2-(alkoxyethyloxy) acetyl] hydrazones", Indian drugs 36(6) June 1999(357-362).

[9] G.J. Kapadia, Y.N. Shukla, S.P. Basak, E.A. Sokoloski and H.M. Fales, *Tetrahedron,* **36** (1980) 2441.

[10] G.J. Kapadia, Y.N. Shukla, *Planta Med.,* **59** (1993) 568.

[11] (a) U. Grafe and L. Radics, *J. Antibiotics,* **39** (1986) 162;
(b) U. Grafe, W. Schade and W. Fleck, *Ger (East) DD,* **241** (1986) 749 (CA 107: P216174k).

[12] J. Breinholt, H. Demuth, M. Heide, G.W. Jensen, I.L. Moller, R.I. Nielsen, C.E. Olsen and C.N. Rosendahl, *Acta Chem. Scand.,* **50** (1996) 443.