ANTIBACTERIAL AND CYTOTOXIC ACTIVITY OF 3, 3'-(5,5'-METHYLENE BIS(3-MERCAPTO-4*H*-1,2,4-TRIAZOLE-5,4-DIYL) BIS (AZAN-1-YL-1-YLIDENE) DIINDOLIN-2- ONES

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ABSTRACT

3,3'-(5,5'-methylene bis(3-mercapto-4*H*-1,2,4-triazole-5,4-diyl) bis (azan-1-yl-1-ylidene) diindolin-2-ones (VIIIa-t) have been synthesized by the condensation of an appropriate isatins (IIIa-t) with the intermediate 5, 5[1]-methylene diyl bis (4-amino-4H- 1,2,4-triazole- 3-thiol) (VII). All the title compounds (VIIIa-t) were screened for cytotoxic activity using A-549 Lung cancer and HT-29 Colon cancer cell lines by MTT method and antibacterial activity against *B. subtiliis*, *S.aureus*, *E.coli* and *S.typhi* by cup plate method. The structures of newly synthesized compounds were established on the basis of elemental analysis, IR, ^{1}H NMR and mass spectral data.

KEY WORDS: Isatin, 1,2,4-triazole, cytotoxic activity, antibacterial activity.

1.INTRODUCTION

1,2,4-triazoles belong to an important class of heterocyclic compounds in medicinal chemistry associated with wide range of biological activities such as Antibacterial activity (Pandeya,2000; Askabeer,2001;Hari and Xuyao,1992), antifungal activity (Pandeya,2000; Askabeer,2001), anti-inflammatory (Parlor,1997),antipyretic (Jayasukhalal, 1970) and antitumor activity (Deliwala,1971; Dash,1970; Kahveri,2001; Sengupta and Hijiria,1983). Isatin molecule also possesss various biological activities such as antimicrobial, cytotoxic, anti-inflammatory and antioxidant activities (Pandeya,2005;Beauchard and Ferandin,2006;Knockaert,2002).The biological importance of the compounds inspired us to synthesize some new bisisatin mercapto triazoles to get more potent compounds and screen for cytotoxic activity by the MTT method (Krief,1989;Alley,1988) and antibacterial activity by cup plate method (Seely and Vandemark,1975).

2.MATERIALS AND METHODS

Melting points were determined in open capillary tubes, using Toshniwal melting point apparatus and are uncorrected. IR spectra were recorded on Perkin – Elmer spectrum BX-I series, FT IR spectrophotometer using KBr discs. PMR spectra were recorded on Brucker spectrospin 400 MHz spectrophotometer using TMS as an internal standard. Mass spectra were obtained on a water alliance LC-MS system with combination of a micro mass platform LCZ mass spectrometer (using ESI) a water 2960 separation module and a water 996 photodiode array detector. Purity was checked by TLC using TLC aluminium sheets silica gel 60, supplied by E.Merk, Mumbai, India. The spots were located by keeping the plate in iodine vapor and 2,4,5-trichlorobenzamine was supplied by S. D. Fine Chem Ltd, Mumbai, India. Synthesis of the title compounds were shown in the scheme I. The required istains were prepared by using the method available in literature (Joaquim,2001). The A-549 Lung cancer and HT-29 Colon cancer cell lines were purchased from the National Centre for Cell Science, Pune University Campus, Pune, India.

3.EXPERIMENTAL

Synthesis of malonic acid hydrazide (V):

Diethylmalonate(IV, 0.1 mole) in alcohol(10ml) was refluxed with hydrazine hydrate(99.9%, 0.04 mole, 10ml) for 15 minutes. The resulting compound was cooled and the solvent was removed by distillation . The product thus obtained was recrystallized from ethanol.

Synthesis of 5, 5[1]-methylene diyl bis (1, 3, 4-oxadiazole-2-thiol) (VI):

A mixture of malonic acid hydrazide (V, 0.1mole), 10% alcoholic potassium hydroxide (0.12mole,10ml)

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and carbon disulfide (in excess) was refluxed for 4 hours. The solvent was removed and digested with water and neutralized with dilute hydrochloric acid. The resulting compound was filtered washed several times with cold water, dried, recrystallized from alcohol and purified by column chromatography.

Synthesis of 5, 5^1-methylene diyl bis (4-amino-4H-1,2,4-triazole- 3-thiol) (VII):

A mixture of 5,5^1-methylene diyl bis (1, 3, 4-oxadiazole-2-thiol) (VI, 0.01 mole) and hydrazine hydrate (in excess) in alcohol was refluxed for 12 hours. The solvent was distilled off and resulting white solid was dried and purified by recrystallization from suitable solvent(s) and column chromatography.

Synthesis of 3, 3'-(5, 5'-methylene bis(3-mercapto-4H-1,2,4-triazole-5,4-diyl)) bis(azan-1-yl-1-ylidene) diindolin-2-ones (VIIIa-t):

A mixture of an appropriate indole-2,3-dione (III, 0.02 mol) and 5, 5^1-methylene diyl bis (4-amino-4H- 1, 2, 4-triazole-3-thiol) (VII, 0.01 mol) in methanol (50 ml) was refluxed for 12 hours. The solvent was removed by distillation and resulting white solid was dried and recrystallized from methanol, purified by column chromatography.

As many as twenty compounds have been prepared adopting the above method and the physical data is presented in Table-1. The structure of the title compounds were established by IR, PMR (DMSO-d_6) and Mass spectrum.

SPECRTAL DATA

VI: IR (KBr) (cm^{-1}): 2927.06(C-H),2361.09 (S-H), 2344.62(S-H),1514.77(C=N), 1508.42 (C=N), 1161.77(C-O-C), 1113.04(C-O-C).
^{1}H-NMR (DMSO-d_6, 400 MHz), δ (ppm): 2.5 (s, 2H, CH_2), 4.627(s, 2H, 2SH).
LC-MS (m/z): 217 (M+1).Elemental analysis found: N-22.47%,C-30.94%, H-4.10%, S-4.41%.

VII: IR (KBr) (cm^{-1}): 3330.69 (NH_2), 3194.77(NH_2),2927.66(C-H), 2379.05 (S-H), 2347.69(S-H), 1498.35(C=N).^{1} H-NMR (DMSO-d_6, 400 MHz), δ (ppm): 2.6 (s, 2H, CH_2), 4.62(s, 2H, 2SH), 8.64(s, 4H,2NH_2). LC-MS (m/z): 246.4 (M+1).

VIIIa: IR (KBr) (cm^{-1}): 3274.28(NH), 3215.50(NH), 2921.97(C-H), 2378.65 (S-H), 2346.31 (S-H),1690.02(C=O),1650.80(C=0), 1487.94(C=N). 1427.94(C=N). ^{1}H-NMR (DMSO-d_6, 400 MHz), δ (ppm): 2.89 (s, 2H, CH_2), 4.2(s, 2H, 2SH), 6.5-7.9(m,8H,Ar-H),11.16(s, 1H, indole NH), 11.22 (s, 1H, indole NH). LC-MS (m/z): 503.6 (M+1).

VIIIb: IR (KBr) (cm^{-1}): 3224.28(NH), 3215.50(NH), 2821.97(C-H), 2378.65 (S-H), 2326.31(S-H), 1690.02(C=O), 1650.80(C=0),1467.94(C=N). 1417.94(C=N).
^{1}H-NMR (DMSO-d_6, 400 MHz), δ (ppm): 2.79 (s, 2H, CH_2), 4.3(s, 2H, 2SH), 6.5-7.9(m,8H, Ar-H),11.10(s, 1H, indole NH), 11.02 (s, 1H, indole NH). LC-MS (m/z): 539.6 (M+1).

AntibacterialActivity

The antibacterial activity of all the newly synthesized compounds were determined by cup plate method in nutrient agar (Hi-Media) was used for antibacterial activity. The antibacterial activity of the test compounds was assayed against *Bacillus subtilis, Staphylococcus aureus* (gram – positive) and *Escherichia coli* and *S.typhi* (gram – negative) by cup-plate method. The compounds were tested at a concentration of 10,25, 50 µg/ml were prepared in dimethylformamide (DMF) when compared to the standard Ampicillin as a positive control. The Petri dishes used for antibacterial screening were incubated at 37 ± 1°C for 24h, the diameters of zone of inhibition (mm) surrounding each of the wells were recorded and the screening results were presented in Table 2.

Cytotoxic Activity

New 3,3'-(5,5'-methylene bis(3-mercapto-4*H*-1,2,4-triazole-5,4-diyl)) bis (azan-1-yl-1-ylidene) diindolin-2-ones (VIIIa-t)were subjected to *in vitro* MTT (3-(4,5- Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay to detect cytotoxic antitumor property and *in vivo* test using tumor mouse model to detect noncytotoxic antitumor property were used. MTT assay was used for *in vitro* cytotoxicity test and was performed as per the method of Alley. Cells were harvested from experimental-phase maintenance cultures. Four hundred cells were counted by trypan blue exclusion and dispensed within triplicate 96-well culture plates in 100 µl volumes for each venom concentration. The assay at each concentration was repeated twice. The cell proliferation activity was qualified on A-549 Lung cancer and HT-29 Colon cancer cell lines, by using Cisplatin as a standard. The results are presented in Table 3.

4.RESULTS AND DISCUSSION

The title compounds were obtained in good yields and purity. All the test compounds at the conc. of 10μg/ml, 25 g/ml and 50 μg/ml were taken to evaluate the cytotoxic activity against A-549 Lung cancer and HT-29 Colon cancer cell lines and the results are presented as IC_{50} values. All the compounds showed cytotoxic activity in the range of 30 μM to 190 μM. The structure activity studies reveal that among the test compounds, the compound VIIIi with 5,7-dibromo substitution on indolinone moiety showed relatively high degree of cytotoxic activity with IC_{50} of 30,34μM. The compounds, VIIIg, VIIIc, VIIIh were next in the order of cytotoxic activity with IC_{50} values of 40,38 and 45,30, 48,32 μM against A-549 Lung cancer and HT-29 Colon cancer cell lines respectively. The results are statistically significant and the activity of the compounds were compared with the standard Cisplatin. The test compounds showed antibacterial activity at the concentration of 10,15,25 μg/disc against gram-positive organism (*B. subtilis, S. aureus*) and gram negative (*E. coli, S.typhi*) organisms. The compound VIIIc was more active among all the test compounds followed by compound VIIIe, VIIIi, VIIIg.

5.ACKNOWLEDGEMENTS

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TABLE-1: Physical data of 3, 3'-(5,5'-methylene bis(3-mercapto-4*H*-1,2,4-triazole-5,4-diyl)bis (azan-1-yl-1-ylidene) diindolin-2-ones (VIIIa-t):

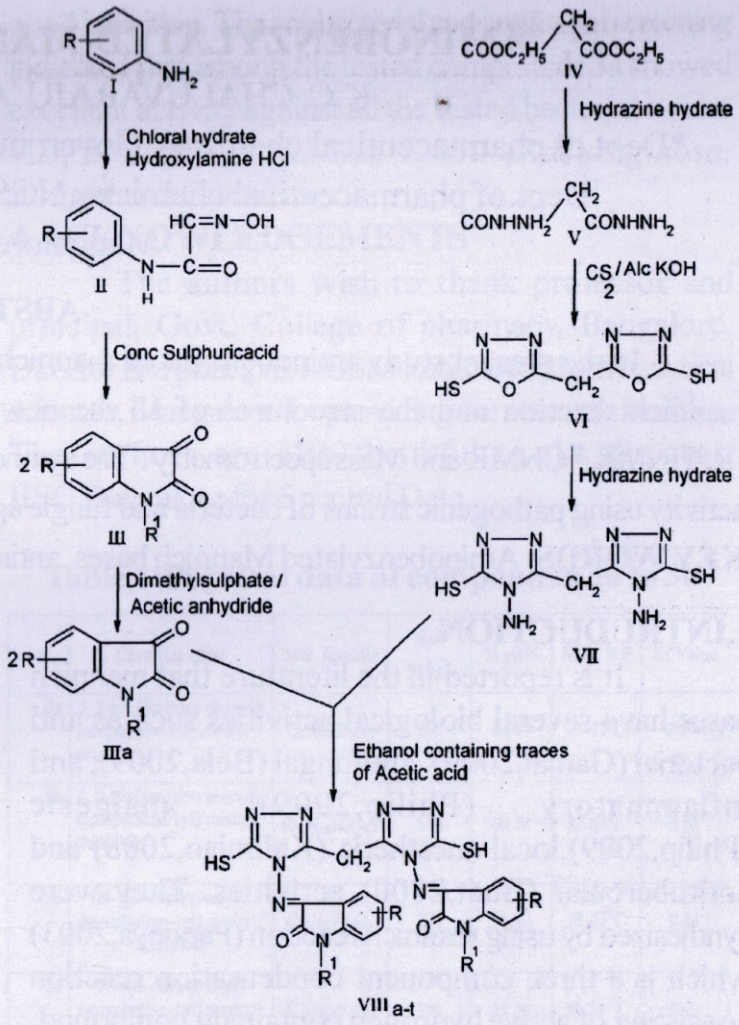
S. No.	Compound	Substituents R	Substituents R^1	Mol. Formula	M.P. °C	Yield %	Mol. Wt
1	VIIIa	H	H	$C_{21}H_{14}N_{10}O_2S_2$	320	90	502
2	VIIIb	5-F	H	$C_{21}H_{12}F_2N_{10}O_2S_2$	200 (decomp)	70	538
3	VIIIc	5-CH_3	H	$C_{23}H_{18}N_{10}O_2S_2$	250 (decomp)	85	530
4	VIIId	6-Br	H	$C_{21}H_{12}Br_2N_{10}O_2S_2$	190	90	664
5	VIIIe	7-$COOCH_3$	H	$C_{25}H_{18}N_{10}O_6S_2$	200 (decomp)	50	586
6	VIIIf	5-NO_2	H	$C_{21}H_{12}N_{11}O_6S_2$	120	50	578
7	VIIIg	5-Cl	H	$C_{21}H_{13}Cl_2N_{10}O_2S_2$	123	70	571
8	VIIIh	7-Cl	H	$C_{21}H_{13}Cl_2N_{10}O_2S_2$	183	70	571
9	VIIIi	5,7-dibromo	H	$C_{21}H_{10}Br_4N_{10}O_2S_2$	185	90	786
10	VIIIj	5-Br	H	$C_{21}H_{12}Br_2N_{10}O_2S_2$	210	40	664
11	VIIIk	7-COOH	H	$C_{23}H_{14}N_{10}O_6S_2$	200	40	590
12	VIIIl	7-CH_3	H	$C_{23}H_{18}N_{10}O_2S_2$	180	80	530
13	VIIIm	5-Br-7-NO_2	H	$C_{21}H_{10}Br_2N_{12}O_6$	150	90	686
14	VIIIn	5-Cl-7-NO_2	H	$C_{21}H_{10}Cl_2N_{12}O_6$	180 (decomp)	70	596
15	VIIIo	5-CH_3-7-NO_2	H	$C_{23}H_{16}N_{12}O_6$	190 (decomp)	70	556
16	VIIIp	4-Cl- 5-F	H	$C_{21}H_{10}Cl_2F_2O_2N_{10}S_2$	340	90	606
17	VIIIq	H	CH_3	$C_{23}H_{18}N_{10}O_2S_2$	200 (decomp)	70	530
18	VIIIr	H	$COCH_3$	$C_{25}H_{18}N_{10}O_4S_2$	280	90	586
19	VIIIs	5-Br	$COCH_3$	$C_{25}H_{16}Br_2N_{10}O_4S_2$	310	80	744
20	VIIIt	7-NO_2	H	$C_{21}H_{12}N_{12}O_6S_2$	130	50	592

Table-2: Antibacterial activity of 3, 3'-(5,5'-methylene bis(3-mercapto-4*H*-1,2,4-triazole-5,4-diyl))bis (azan-1-yl-1-ylidene) diindolin-2-ones (VIIIa-t):

S.No.	Compound	Zone of inhibition (in mm)			
		B.subtilis	*S.aureus*	*E.coli*	*S.typhi*
1	VIIIa	19	18	16	16
2	VIIIb	18	20	22	21
3	VIIIc	26	25	24	26
4	VIIId	19	18	19	21
5	VIIIe	25	20	21	22
6	VIIIf	20	21	22	19
7	VIIIg	24	19	21	21
8	VIIIh	18	16	18	19
9	VIIIi	24	22	22	19
10	VIIIj	16	18	15	19
11	VIIIk	19	18	19	15
12	VIIIl	16	19	22	18
13	VIIIm	16	18	17	21
14	VIIIn	18	16	19	17
15	VIIIo	14	12	11	13
16	VIIIp	22	21	19	18
17	VIIIq	16	17	18	19
18	VIIIr	14	13	12	10
19	VIIIs	18	16	18	20
20	VIIIt	18	15	18	19
21	Ampicilin (standard)	28	26	29	27

Table –3:Cytotoxic activity of 3, 3'-(5,5'-methylene bis(3-mercapto-4*H*-1,2,4-triazole-5,4-diyl)) bis (azan-1-yl-1-ylidene) diindolin-2-ones (VIIIa-t):

S.No.	Compound	A549 Lung cancer cell lines IC_{50} values (μM)	HT-29 Colon cancer cell lines IC_{50} values (μM)
1	VIIIa	185.00	176.00
2	VIIIb	112.00	123.00
3	VIIIc	45.00	30.00
4	VIIId	141.28	154.32
5	VIIIe	187.89	187.12
6	VIIIf	152.72	138.30
7	VIIIg	40.20	38.70
8	VIIIh	48.00	32.00
9	VIIIi	30.23	33.34
10	VIIIj	102.45	90.95
11	VIIIk	123.56	129.90
12	VIIIl	172.32	186.98
13	VIIIm	116.00	110.92
14	VIIIn	58.23	64.70
15	VIIIo	108.00	148.24
16	VIIIp	39.82	46.73
17	VIIIq	154.00	168.00
18	VIIIr	165.78	176.89
19	VIIIs	128.00	156.29
20	VIIIt	102.00	110.23
21	Cisplatin (standard)	25	25



SCHEME - 1

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