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Research Article

ANTIOXIDANT AND DNA BINDING STUDY 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

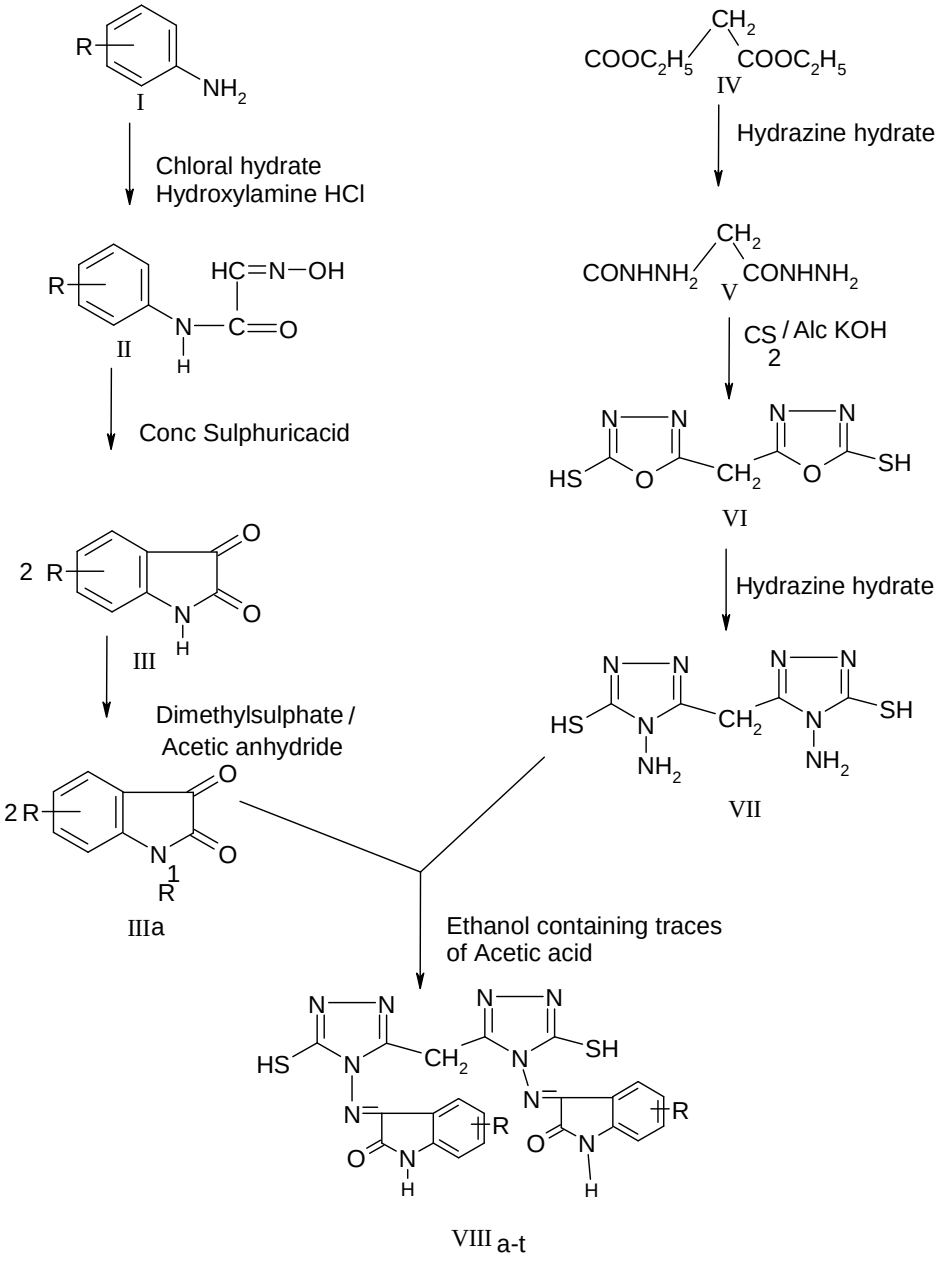
3,3'-(5,5'-methylene bis(3-mercapto-4*H*-1,2,4-triazole-5,4-diyl) bis (azan-1-yl-1-ylidene) diindolin-2-ones (VIIIa-t) have been synthesized by the condensation of an appropriate isatins (IIIa-t) with the intermediate 5, 5[1]-methylene diyl bis (4-amino-4H- 1,2,4-triazole- 3-thiol) (VII). All the title compounds (VIIIa-t) were screened for Antioxidant activity and DNA Binding study, for this evaluation Ascorbic acid is employed as standard, for DNA binding Study employed DNA is Herring sperm DNA. Almost all the compounds showed free radical scavenging activity and DNA binding to some extent. 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, Antioxidant activity, DNA Binding study

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[1-3], antifungal activity[1,2], anti-inflammatory[4] , antipyretic[5] and antitumor activity[6-9]. Isatin molecule also

possesss various biological activities such as antimicrobial, cytotoxic, anti-inflammatory and antioxidant activities[10-12].The biological importance of the compounds inspired us to synthesize some newbisisatin mercapto triazoles to get more potent compounds and screen for antioxidant activity by DPPH method [13,] and DNA Binding Study[14]. The synthetic approach to the title compounds are outlined in SchemeI.



SCHEME - 1

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.

Purity was checked by TLC using TLC aluminum 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 [15]. The DPPH was purchased from Sigma-Aldrich,USA. Herring sperm DNA was purchased from Himedia,Mumbai,India.

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) 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.

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	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

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).

Anti oxidant activity

The compounds synthesized were evaluated for antioxidant activity and compared with standard drug(ascorbic acid).The activity is done by DPPH method. One ml of 0.3mM DPPH methanol solution was added to 2.5ml of sample solutions of different Concentrations (2,4,6,8,10µg/ml) and allowed to react at room temperature. After 30min the absorbance values were measured at 518 nm and converted in to the percentage antioxidant activity(AA%) using the following formula:AA%=100-{(Abs $_{sample}$-Abs $_{blank}$)} X100/Abs $_{control}$}.Ethanol(1ml) plus drug solution(2.5ml) was used as a blank .DPPH solution (1.0ml,0.3Mm) plus (2.5ml) was used as a negayive control. The positive control is ascorbic acid. The IC_{50} values were calculated by linear regression plots, where abscissa represented the concentration of test drug solution (2,4,6,8,10µg/ml) and ordinate the average percentage of antioxidant activity from three separate tests and results are tabulated in table 2.

DNA Binding Activity

New 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) were subjected to DNA Binding study, when drug binds to the DNA the amount of DNA detected in HPLC is reduced and can be estimate by the reduction in peak area. In this investigation employed DNA is Herring sperm DNA. Cisplatin is employed as a standard,100µl of DNA solution was taken in an eppendorff tube and to it100µl of test/standard solution was added and the mixture was incubated at 37^{o}C for 30 min,40 µl of the mixture was then injected in to HPLC with 90:10(methanol:water) and detected at 254nm wave length. The peak area of the test/standard is compared with that of blank and get the % binding. The results are presented in Table 2.

Table-2: Antioxidant and DNA Binding 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	Substituent		Antioxidant activity IC_{50} values (µM)	% DNA Binding
		R	R^1		
1	VIIIa	H	H	15.00	55
2	VIIIb	5-F	H	11.00	82
3	VIIIc	5-CH_3	H	14.00	61
4	VIIId	6-Br	H	11.28	64
5	VIIIe	7-$COOCH_3$	H	12.76	57
6	VIIIf	5-NO_2	H	14.72	66
7	VIIIg	5-Cl	H	13.20	57
8	VIIIh	7-Cl	H	10.00	75
9	VIIIi	5,7-dibromo	H	10.23	80
10	VIIIj	5-Br	H	11.45	69
11	VIIIk	5-COOH	H	14.98	58
12	VIIIl	7-CH_3	H	12.32	62
13	VIIIm	5-Br-7-NO_2	H	11.00	76
14	VIIIn	5-Cl-7-NO_2	H	10.23	70
15	VIIIo	5-CH_3- 7-NO_2	H	10.00	77
16	VIIIp	4-Cl-5-F	H	9.08	73
17	VIIIq	H	CH_3	14.00	68
18	VIIIr	H	$COCH_3$	13.82	67
19	VIIIs	5-Br	$COCH_3$	12.00	81
20	VIIIt	7-NO_2	H	10.09	79
21	Cisplatin			5.87	89

Results and Discussion

The title compounds were obtained in good yields and purity. All the test compounds Showed antioxidant activity in the range of 10µm to15 µm. Among the test compounds compound VIIIp showed highest percentage of free radical scavenging activity with IC_{50}

Value of 9.08 µm. The result of DNA binding activity showed that all the compounds bind the DNA in the range of 55% to 82%.Among all the test compounds compound VIIIb showed good DNA Binding capacity with 82%.

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