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Research Article

Design, Synthesis and Biological Evaluation of Nitrogen Heterocycles Containing 4(3h) Quinazolinone and 1,2,4-Triazole Nuclei

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ABSTRACT

3,5-Dibromo anthranilic acid **(1)** reacted with acetic anhydride to give 6,8-dibromo-2-methyl-(1,3)benzoxazin-4-one **(2).** On the other hand, the aryl/aryloxy acid hydrazides **(3a1-a8)** were converted into their corresponding potassium dithiocarbazinates **(4a1-a8)** by a reaction with carbon disulphide in presence of alcoholic potassium hydroxide. The mixture of potassium dithiocarbazinates **(4a1-a8)** and isonicotinic acid hydrazide were heated for 6-8 hr to produce 3- aryl/aryloxy-4-(N-pyridyl carboxamido)-5-mercapto-1,2,4-triazoles **(5a1-a8).** The required title compounds N-(3-substituted aryl/aryloxy methyl-5-(6,8-dibromo-2-methyl-4-oxoquinazolin-3(4H)-ylamino)-4H-1,2,4-triazol-4-yl) isonicotinamide derivatives **(6a1-a8)** were obtained in excellent yields in one pot reaction by heating a mixture of the above triazole **(5**) (0.01mol), hydrazine hydrate (99%, 0.01mol) and 6,8-dibromo-2-methyl-(1,3)benzoxazin-4-one **(2)** (0.01mol) in ethanol (50ml) till the evolution of hydrogen sulphide was ceased. The structures of the compounds were confirmed by IR, ^{1}HNMR and Mass spectral analysis. All the compounds have been evaluated for their antimicrobial, antitubercular and anticancer properties. Most of the compounds have shown promising biological activity.

INTRODUCTION

In recent years, there has been increased interest in the chemistry of 4(3H)-Quinazolinones since these have been known as promising class of biologically active compounds[1,2]**.** Many of them have been reported to possess antifungal, antibacterial, anticancer, anti-inflammatory, anticonvulsant, immunotropic, hypolipidemic, antitumor, antiulcer, analgesic and antiproliferative activities as well as inhibitory effects for thymidylate synthase and poly-(ADP-ribose) polymerase (PARP) enzymes[3]**.** Several plants containing alkaloids with 4-quinazolinone nuclei were reported to have anti-malerial, anti-inflammatory, antibacterial, diuretic and CNS sedative properties[4]**.** Since, quinazolinones are excellent reservoirs of bioactive substances and the stability of the quinazoline nucleus has inspired medicinal chemists to introduce many bioactive

moieties into this nucleus and synthesize new potential medicinal agents.

Literature survey reveals that, a number of heterocyclic systems incorporating 1,2,4-triazole nucleus are associated with diverse pharmacological activities such as analgesic, antiasthmatic, diuretic, antihypersensitive, anticholinergic, antibacterial, antifungal and anti-inflammatory activities [5-12].

In view of the above facts and in continuation of our research programmes in developing new biologically active compounds[13-16], we report herein, the synthesis of title compounds comprising of 4(3H)-quinazolinone and 1,2,4-triazole nuclei and their evaluation for antimicrobial, antitubercular and anticancer properties.

EXPERIMENTAL

All the compounds in the study were synthesized by following **scheme-1.** Melting points were determined on a Toshniwal apparatus in open capillaries and are uncorrected. The purity of the compounds was checked by TLC on silica gel-G plates using chloroform-ethyl acetate (1:1) solvent system as irrigant and iodine vapor as visualizing agent. IR spectra in KBr (cm^{-1}) were recorded on a Schimadzu FTIR-8000 series spectrophotometer and ^1H NMR spectra (DMSO-d_6) on EM-390MHz spectrometer using TMS as internal standard (Chemical shift, δ ppm). Mass spectra were recorded on a Jeol JMSD-300 Mass Spectrometer operating at 70 eV. All the compounds showed satisfactory micro analytical results for C, H and N.

The starting material 6,8-dibromo-2-methyl-(1,3)benzoxazin-4-one **(2)** was prepared by the reaction of 3,5-dibromo anthranilic acid **(1)** with acetic anhydride by known method[17]. The aryl/aryloxy acid hydrazides **(3a1-a8)** were prepared from their corresponding esters by a reaction with hydrazine hydrate following known method[18]. These hydrazides were then converted into their corresponding potassium dithiocarbazinates **(4a1-a8)** by a reaction with carbon disulphide in presence of alcoholic potassium hydroxide[19]. The mixture of potassium dithiocarbazinates **(4a1-a8)** and isonicotinic acid hydrazide were heated for 6-8 hr to produce 3-aryl/aryloxy-4-(N-pyridyl carboxamido)-5-mercapto-1,2,4-triazoles **(5a1-a8)**[15]. The required title compounds N-(3-substituted aryl/aryloxy methyl-5-(6,8-dibromo-2-methyl-4-oxoquinazolin-3(4H)-ylamino)-4H-1,2,4-triazol-4-yl) isonicotinamide **(6a1-a8)** were obtained in excellent yields in one pot reaction by heating a mixture of the above triazole **(5)** (0.01mol), hydrazine hydrate (99%, 0.01mol) and 6,8-dibromo-2-methyl-(1,3)benzoxazin-4-one **(2)** (0.01mol) in ethanol (50ml) till the evolution of hydrogen sulphide was ceased[20].

Preparation of 6,8-dibromo-2-methyl-(1,3)benzoxazin-4-one (2): A mixture of 3,5-dibromoanthranilic acid (1) (0.12mol) and acetic anhydride (0.2mol) with few drops of dry pyridine was refluxed for 3 hr. The excess of solvent was distilled off under reduced pressure. The reaction mixture was filtered, washed, dried and recrystallized from absolute ethanol. [**IR (KBr, cm^{-1}):** 1774(C=O str), 1579(C=N str), 530,554 (C-Br str)]

Synthesis of 3-aryl/aryloxy-4-(pyridyl carboxamido)-5-mercapto-1,2,4-triazoles (5a1-a8): Potassium dithiocarbazinates **(4a1-a8)** were prepared by reacting aryl/aryloxy acid hydrazides **(3a1-a8)** with carbon disulphide in the presence of alcoholic KOH following the reported procedure (yields were between 75-82%). A suspension of potassium dithiocarbazinate (**4**, 0.1 mol), isonicotinic acid hydrazide (0.1 mol) and water (5ml) was heated under reflux for 5-6 hr when hydrogen sulphide was evolved and a clear solution resulted. Dilution of the reaction mixture with cold water (50 ml) and subsequent acidification with hydrochloric acid gave the required product which was filtered, washed with water and crystallized from aqueous ethanol. The compounds of the series **(5a1-**

a8) were prepared following the same procedure.

3-phenoxy methyl-4-(pyridyl carbox amido)-5-mercapto-1,2,4-triazole (5a4): IR: 3250 (NH of CONH), 3050 (aromatic C-H stretching), 2910 (OCH_2), 2610 (SH), 1665 (CONH), 1635 (NH in plane bending), 1620 (C=N), 1605 (C=C), 1490 (C-N), 1098 (C-O-C), 740 (mono substituted benzene), 695 (C-S); **^{1}HNMR (DMSO-d_6,δ ppm):** 5.91-8.82 (11 H, m, 5H of aromatic group, 4H of pyridyl group and 2H of –OCH_2), 11.11 (1H, s, 1H of CONH), -SH group which generally appears around 14 and above is not observed as the spectrum is taken in the δ scale of 0-14. **MS:** m/z 327 (M^+).

3-(2-Tolyloxy methyl)-4-(pyridyl carbox amido)-5-mercapto-1,2,4-triazole (5a7): IR: 3248 (NH of CONH), 3066 (aromatic C-H stretching), 2950 (C-H stretching), 2905 (OCH_2), 2608 (SH), 1662 (CONH), 1640 (NH in plane bending), 1615 (C=N), 1608 (C=C), 1486 (C-N), 1130 (C-O-C), 840 (disubstituted benzene), 700 (C-S); **^{1}HNMR (DMSO-d_6,δ ppm):** 2.51 (3H, s, 3H of CH_3), 3.42 (2H, bs, 2H of –OCH2), 7.79-8.72 (8 H, m, 4H of aromatic group, 4H of pyridyl group), a weak peak at 10.1 (1H, s, 1H of CONH), 14 (1H, of –SH); **MS:** m/z 341 (M^+).

Preparation of N-(3-substituted aryl/aryloxy methyl-5-(6,8-dibromo-2-methyl-4-oxoquinazolin-3(4H)-ylamino)-4H-1,2,4-triazol-4-yl)isonicotinamide (6a1-a8): The title compounds N-(3-substituted aryl/aryloxy methyl-5-(6,8-dibromo-2-methyl-4-oxoquinazolin-3(4H)-ylamino)-4H-1,2,4-triazol-4-yl)isonicotinamides **(6a1-a8)** were obtained in excellent yields in one pot reaction by heating a mixture of the above triazole **(5)** (0.01mol), hydrazine hydrate (99%, 0.01mol) and 6,8-dibromo-2-methyl-(1,3)benzoxazin-4-one **(2)** (0.01mol) with few drops of glacial acetic acid using the solvent ethanol (50ml) till the evolution of hydrogen sulphide was ceased (8 hr). The reaction mixture was cooled to room temperature, filtered, dried and recrystallized from absolute ethanol. The compounds of the series **(6a1-a8)** were prepared following the same procedure. The characterization data is given in **Table-1**.

N-(3-phenoxy methyl-5-(6,8-dibromo-2-methyl-4-oxoquinazolin-3(4H)-ylamino)-4H-1,2,4-triazol-4-yl)isonicotinamide (6a4): IR (KBr, cm^{-1}): 3284 & 3368 broad band, hydrogen bonded NH & NH of CONH; 3060 aromatic C-H stretching; 2972 C-H stretching of CH_2 of OCH_2; 1685 C=O of –CONH; 1620 C=N; 1610, 1590, 1480 C=C ring stretching; 1465 C-H bending of CH_2 of OCH_2; 1430 C-N; 1080 C-O-C; 760 mono substituted aromatic ring. **^{1}HNMR (DMSO-d_6, δ ppm):** 1.97 (3H, s, 3H of CH_3), 3.6 (2H, weak peak, 2H of –OCH_2), 6.83-8.83 (11H, m, 4H of Ar-H & 6H of heteroaryl), 10.99 (1H, s, NH of N-NH), 11.82 (1H, s, -CONH).**MS:** m/z **M^+**626.

N-3(2-tolyloxy methyl)-5-(6,8-dibromo-2-methyl-4-oxoquinazolin-3(4H)-ylamino)-4H-1,2,4-triazol-4-yl) isonicotinamide (6a7): IR (KBr, cm^{-1}): 3286 & 3367 broad band, hydrogen bonded NH & NH of CONH; 3063 aromatic C-H stretching; 2972 & 2890 C-H stretching of CH_2 of OCH_2 and CH_3; 1690 C=O of –CONH; 1615 C=N; 1606, 1586, 1482 C=C ring stretching; 1465 C-H bending of CH_2 of OCH_2; 1430 C-N; 1080 C-O-C; 840 1,2-di substituted aromatic ring. **^{1}HNMR (DMSO-d_6, δ ppm):** 1.16 (3H, s, CH_3 of phenyl ring), 1.77 (3H, s, CH_3 of heterocyclic ring), 3.50 (2H, bs, 2H of –OCH_2), 6.73-8.27 (10H, m, 4H of phenyl ring & 6H of heterocyclic ring), 8.78 (1H, s, NH of N-NH), 9.71 (1H, s, -CONH). **MS:** m/z **M^+**640.

BIOLOGICAL ACTIVITY

a) **ANTIFUNGAL AND ANTIBACTERIAL ACTIVITIES:** The compounds of the series **(6a1-a8)** have been subjected to invitro evaluation of their antibacterial and antifungal activities against the fungi ***Candida albicans, Aspergillus niger*** and against the bacteria

Staphylococcus aureus, ***Enterococcus fecalis***, ***Escherichia coli, Klebsiella pneumoniae*** by Minimum Inhibitory Concentration method [21]**.** The results obtained are tabulated in **Table-2**.

b) **ANTITUBERCULAR ACTIVITY STUDIES:** All the compounds in the series **(6a1-a8)** have been evaluated for their invitro antitubercular activity against the standard strain of ***Mycobacterium tuberculosis*** **H_{37} RV** using **Middlebrook 7H-9** broth referring to the standard procedure [22]**.** The results of the antitubercular studies are given in **Table-3.**

c) **ANTICANCER ACTIVITY STUDIES:** All the synthesized compounds in the above scheme were evaluated for anticancer activity against the cell lines A-549 (Human: lungs:carcinoma), MDA:MB (Human:Adenocarcinoma; mammary gland), HT-29 (Human:colorectal adenocarcinoma) using microculture tetrazolium assay (MTT assay) [23]. The results of the anticancer activity are tabulated in **Table-4** and graphically depicted in figure **1, 2 and 3** separately for all the three cell lines**.**

RESULTS AND DISCUSSION

ANTIFUNGAL ACTIVITY: Against ***C. albicans***, **6a6** was the compound to show better activity at 12.5µg itself which was followed by the compound **6a8** at 25µg. The compounds **6a3**, **6a4**, **6a7** have MIC of 50µg where as the remaining the compounds **6a1**, **6a2** and **6a5** were effective only at 100µg. Against ***A. niger***, the compounds **6a4**,**6a5**, **6a8** were effective at 25µg however, all the remaining compounds showed to possess activity only at 100µg. The antifungal activity of the compounds revealed that, the compounds possess weak to moderate activity.

ANTIBACTERIAL ACTIVITY: Among the derivatives evaluated for antibacterial activity, the compound **6a3** found to be most effective with MIC of 25µg against ***S.aureus*** & ***E.fecalis*** and 50µg against ***E.coli*** & ***K.pneumoniae***. The compound **6a6** was the other effective compound having MIC of 25µg against ***S.aureus*** & ***E.fecalis***. The growth of the organisms was inhibited at 50µg by the compounds **6a1** (***S.aureus***), **6a3** (***K.pneumoniae***) and **6a4** (***S.aureus*** & ***E.fecalis***). The organism ***E.coli*** was susceptible to all the compounds either at 50 or 100µg; however, all the other organisms in the present study were resistant to the compound **6a7** at all the concentration levels tested. Thus the antibacterial results reveal that, the compounds exhibit the weak activity.

ANTITUBERCULAR ACTIVITY:
Remarkable antitubercular activity has been showed by the compound **6a3** which was effective against ***Mycobacterium tuberculosis*** **H_{37} RV** at all the tested dose levels. This is followed by the compound **6a6** which was effective at 6.25µg. The compounds **6a4** and **6a8** were found to be the next effective compounds in this study with MIC value of 12.5µg. The compound **6a1** possessed MIC of 25µg where as the remaining compounds **6a2**, **6a5** and **6a7** were effective at 50µg concentration level. The antitubercular activity of the compounds revealed that the compound **6a3** possess excellent activity and the compounds **6a1**, **6a4**, **6a6**, **6a8** possesses very significant activity. The compounds appear to be promising antitubercular agents, if necessary toxicity studies are carried out.

ANTICANCER ACTIVITY: The anticancer activity of the compounds revealed that, the compounds except **6a4** and **6a5** which are inactive, rest of the compounds exhibited anticancer activity against cell lines HT-29. The results also indicated that the compounds are active against cell line MDB and A-549 except the compounds **6a3** & **6a4** which failed to show the activity. The results indicated that the compounds

appear to be good anticancer agents and further work with necessary modification is quite desirable.

SUMMARY AND CONCLUSION

The title compounds in present work, were synthesized as per given **Scheme-1**. 3-aryloxy methyl-4-(N-pyridin-2-yl)-5-mercapto-1,2,4-triazoles prepared were reacted with hydrazine hydrate and substituted benzoxazin-4-one described in the procedure that resulted required title compounds. The synthesized compounds were characterized by IR, ^{1}HNMR and Mass spectral studies. The compounds were evaluated for their biological activities like antibacterial, antifungal, antitubercular and anticancer activities. It was observed from results of each activity that majority of the synthesized compounds possess poor antibacterial and antifungal activity. However, the compounds are proved to be effective antitubercular and anticancer agents as some of them were very sensitive even at very low concentration. Hence, the detailed study on their toxicity trials and slight modification of the molecule may yield much therapeutically useful agents of the present need.

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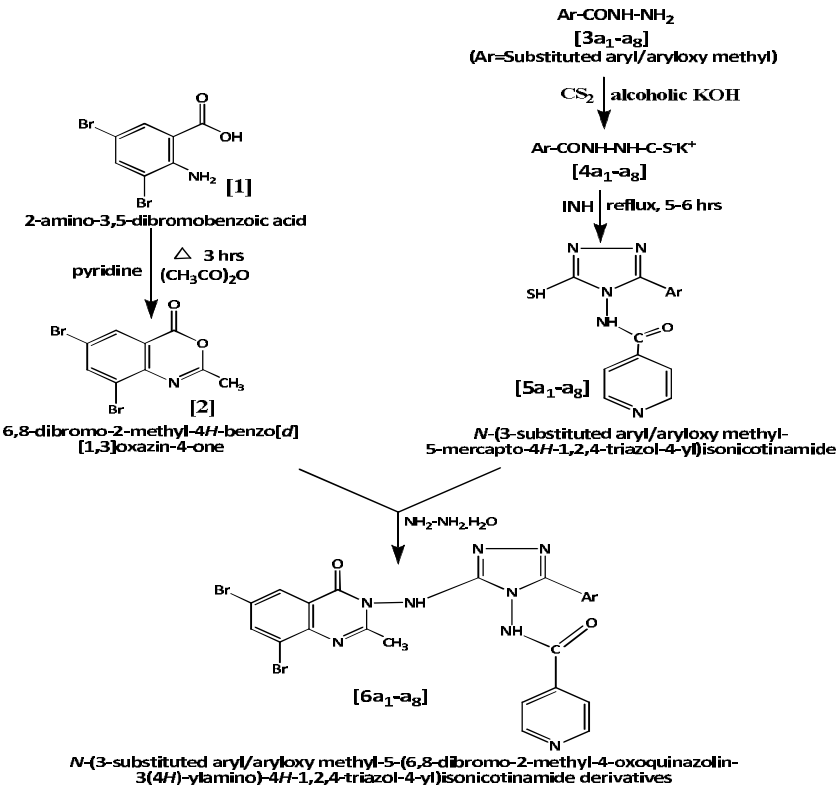
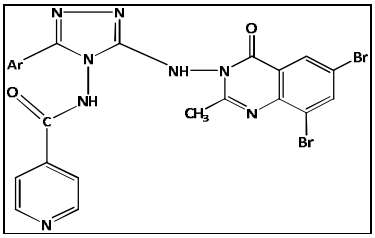


SCHEME-1

Table 1: Characterization data of 3-aryl/aryloxy-4-(pyridyl carboxamido) -5-([1,2,4]triazol-4-yl amino) -1,2,4-triazoles (6a1-a8)



S. No.	Code	Ar (substituent)	Molecular Formula	Molecular weight	Melting Point (^{0}C)
1.	6a1	Phenyl	$C_{23}H_{16}O_2N_8Br_2$	596	242
2.	6a2	4-Chloro phenyl	$C_{23}H_{15}O_2N_8Br_2Cl$	630	188
3.	6a3	2-Amino phenyl	$C_{23}H_{17}O_2N_9Br_2$	611	248
4.	6a4	Phenoxy methyl	$C_{24}H_{18}O_3N_8Br_2$	626	235
5.	6a5	2-Chloro phenoxy methyl	$C_{24}H_{17}O_3N_8Br_2Cl$	660	168
6.	6a6	4-Tolyloxy methyl	$C_{26}H_{20}O_3N_8Br_2$	640	127
7.	6a7	2-Tolyloxy methyl	$C_{26}H_{20}O_3N_8Br_2$	640	142
8.	6a8	4-Amino phenoxy methyl	$C_{24}H_{19}O_3N_9Br_2$	601	255

Table 2: Data showing the results of antifungal and antibacterial activity studies of the compounds of the series (6a1-a8) by MIC method

S. No.	Samples	Minimum Inhibitory Concentration (MIC in µg)					
		Antifungal activity		Antibacterial activity			
		C. albicans	*A. niger*	*S. aureus*	*E. fecalis*	*E. coli*	*K. pneumoniae*
1	6a1	100	100	50	100	100	100
2	6a2	100	100	100	100	100	100
3	6a3	50	100	25	25	50	50
4	6a4	50	25	50	100	50	100
5	6a5	100	25	100	100	100	100
6	6a6	12.5	100	25	25	100	100
7	6a7	50	100	R	R	100	R
8	6a8	25	25	100	100	100	100

Table 3: Data showing the results of antitubercular activity of the compounds of the series (6a1-a8) against *Mycobacterium tuberculosis H37 RV*

S. No.	Samples	Concentration in µg									
		100	50	25	12.5	6.25	3.125	1.6	0.8	0.4	0.2
1.	6a1	S	S	S	R	R	R	R	R	R	R
2.	6a2	S	S	R	R	R	R	R	R	R	R
3.	6a3	S	S	S	S	S	S	S	S	S	S
4.	6a4	S	S	S	S	R	R	R	R	R	R
5.	6a5	S	S	R	R	R	R	R	R	R	R
6.	6a6	S	S	S	S	S	R	R	R	R	R
7.	6a7	S	S	R	R	R	R	R	R	R	R
8.	6a8	S	S	S	S	R	R	R	R	R	R

Table 4: Anticancer activity data of the compounds 6a-1 to 6a-8

Sl. No	Cell lines→ / Samples↓ / Concentr-ation→	Cell line HT-29			Cell line –MDB			Cell line –A549		
		10µl	20µl	30µl	10µl	20µl	30µl	10µl	20µl	30µl
1	6a1	62.27	61.36	61.81	60.00	59.58	57.5	80.00	79.09	78.18
2	6a2	65.90	61.81	60.90	77.91	77.5	70.41	84.09	83.18	82.27
3	6a3	62.27	60.45	55.90	94.1	93.33	92.91	97.27	95.45	95.00
4	6a4	82.27	95.90	99.54	83.75	91.25	90.00	98.63	97.27	95.90
5	6a5	82.72	99.09	97.27	71.66	70.83	69.58	82.27	95.45	81.81
6	6a6	64.54	63.18	60.45	75.41	73.33	71.66	79.54	78.63	77.72
7	6a7	70.00	67.27	66.81	72.5	71.66	70.41	83.63	82.72	94.54
8	6a8	66.36	62.72	61.36	76.66	76.25	75.41	79.09	78.18	77.27
Green shade= Lysis					Yellow shade= No Lysis					

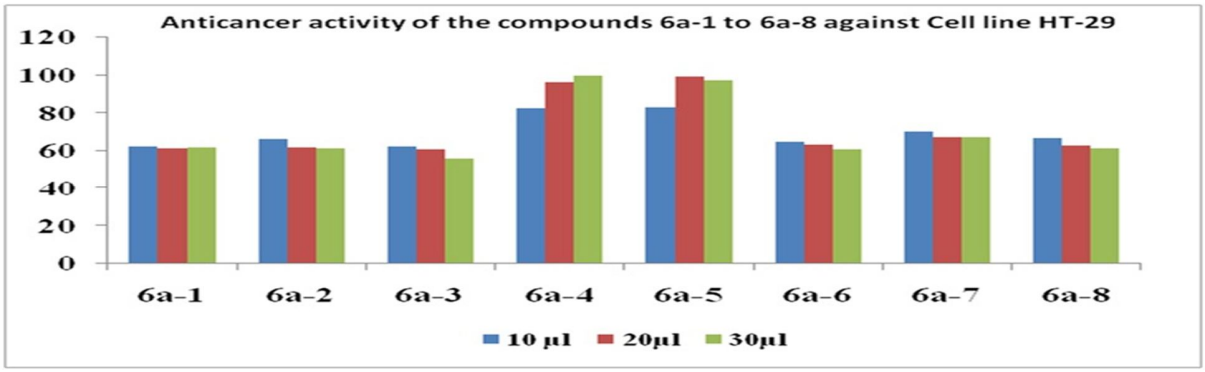


Fig. 1: Anticancer activity of the compounds 6a1-to 6a8 against Cell line HT-29

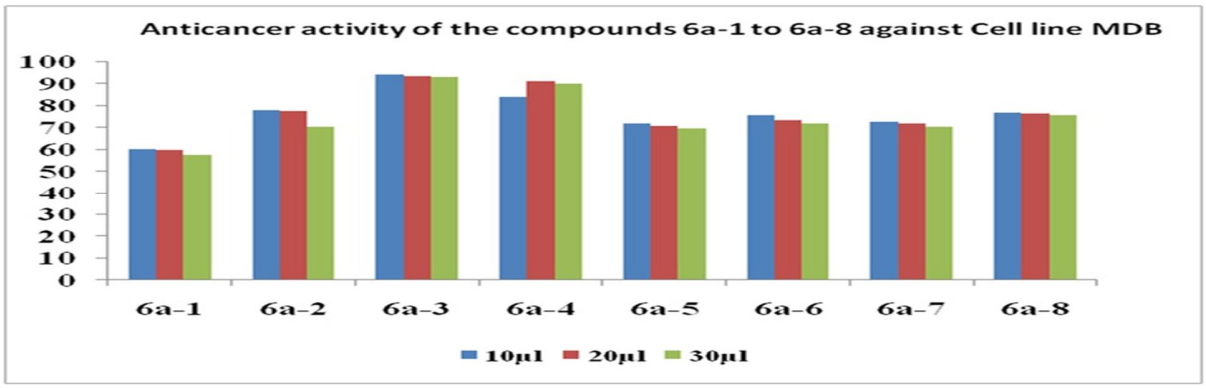


Fig. 2: Anticancer activity of the compounds 6a1-to 6a8 against Cell line MDB

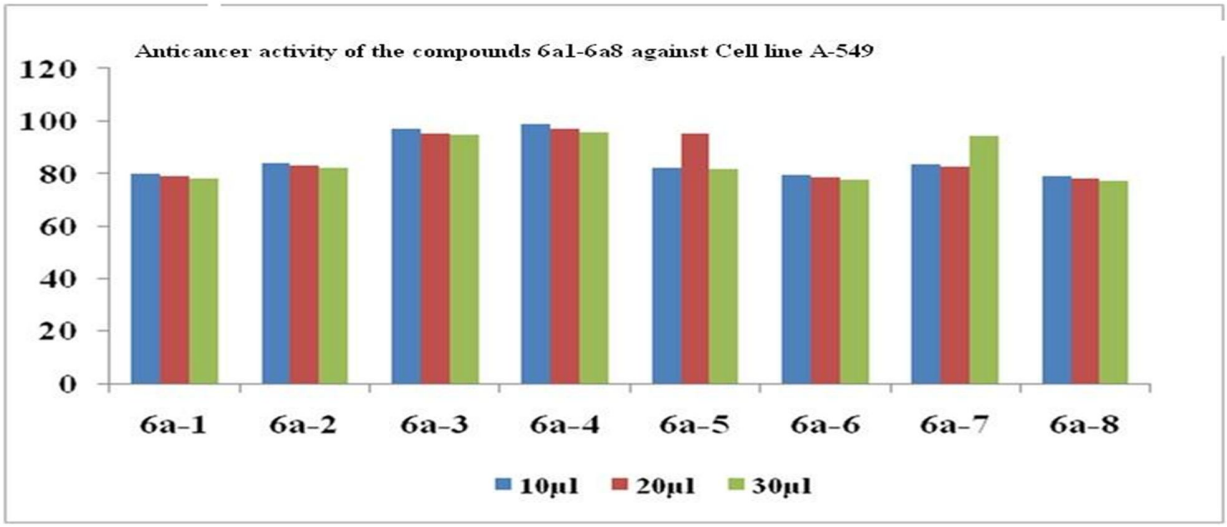


Fig. 3: Anticancer activity of the compounds 6a1-to 6a8 against Cell line A-549

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