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Short communication

New bis-aminomercaptotriazoles and bis-triazolothiadiazoles as possible anticancer agents☆

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Abstract

A series of bis-phenoxyacetic acids **2** were prepared starting from corresponding unsubstituted/substituted 1,4-quinols **1**. The fusion of bis-phenoxyacetic acids **2** with thiocarbohydrazide gave the corresponding bis-[4-amino-5-mercapto-1,2,4-triazol-3-yl-methyleneoxy]phenylenes (**3**) in a one pot reaction. The reaction of bis-triazoles **3** with various reagents afforded *N*-bridged heterocycles **4**–**6** in good yields. The newly synthesised compounds were screened for their anticancer activity against a panel of 60 cell lines derived from seven cancer types namely, lung, colon, melanoma, renal, ovarian, CNS and leukemia. Some of the tested compounds showed promising anticancer properties. © 2002 Published by Éditions scientifiques et médicales Elsevier SAS.

Keywords: Bis-triazoles; Bis-triazolothiadiazoles; *N*-bridged heterocycles; Anticancer activity

1. Introduction

Various 3-substituted-4-amino-5-mercapto-1,2,4-triazoles have been reported to possess analgesic, antibacterial, antifungal, antiinflammatory, antitubercular, antiviral, herbicidal and sedative properties [1–7]. The amino and mercapto groups of these compounds serve as readily accessible nucleophilic centers for the preparation of *N*-bridged heterocycles. The 1,3,4-thiadiazoles exhibit broad spectrum of biological activities, possibly due to the presence of toxophoric >N–C–S moiety [8]. They find applications as antibacterials, -tumour, and -inflammatory agents, pesticides, herbicides, dyes, lubricants and analytical reagents [9]. The 1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazole derivatives obtained by fusing the bio-labile 1,2,4-triazole and 1,3,4-thiadiazole rings together, are reported to possess antibacterial, antifungal, anti-inflammatory, CNS depressant, hypocholesteremic, antiviral, analgesic, anthelmintic, herbicidal and plant growth regulatory effects [10].

It is also observed that incorporation of aryloxymethyl substituent and the halogen atom into the heterocyclic ring systems augments the biological activities considerably [11,12]. Further, it has been reported that many biologically active natural and synthetic products have interesting molecular symmetry [13]. Recently, some bis-triazole derivatives endowed with excellent antibacterial activities have been reported from our laboratory [14–16].

Prompted by these observations and in continuation of our search for bio-active molecules, we designed the synthesis of a series of novel 1,4-bis-[4-amino-5-mercapto-1,2,4-triazol-3-yl-methoxy]phenylenes (**3**) and their triazolothiadiazole derivatives **4**–**6** starting from hydroquinones **1**. The synthesis, characterization and the results of anticancer activity screening studies of the newly synthesised compounds are presented in this paper.

2. Chemistry

1,4-Bis-phenoxyacetic acids (**2**) were synthesised by the reaction of hydroquinones **1** with monochloroacetic

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acid in presence of sodium hydroxide. The fusion of bis-phenoxyacetic acids **2** with thiocarbohydrazide afforded 1,4-bis-[4-amino-5-mercapto-1,2,4-triazol-3-ylmethoxy]phenylenes (**3**) in good yields (Fig. 1). The condensation of bis-triazoles **3** with various aromatic carboxylic acids in presence of phosphorus oxychloride furnished a series of 1,4-bis-(6-aryl-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazol-3-ylmethoxy)phenylenes (**4**). The reaction of bis-triazoles **3** with formic acid in benzene followed by treatment of the product with concentrated sulphuric acid yielded bis-(1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazol-3-ylmethoxy)phenylenes (**5**). The reaction of bis-triazoles with carbon disulphide in the presence of potassium hydroxide in methanol gave 1,4-bis-(6-mercapto-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazol-3-ylmethoxy)phenylenes (**6**).

3. Results and discussion

The characterization data of bis-aminomercaptotriazoles **3a**–**e** and the bis-triazolothiadiazoles **4a**–**w**, **5a**–**e** and **6a**–**e** are given in Tables 1 and 2, respectively. The formation of bis-aminomercaptotriazoles was confirmed by recording the IR, ^{1}H-NMR and mass spectra of a few selected compounds. IR spectrum of compound

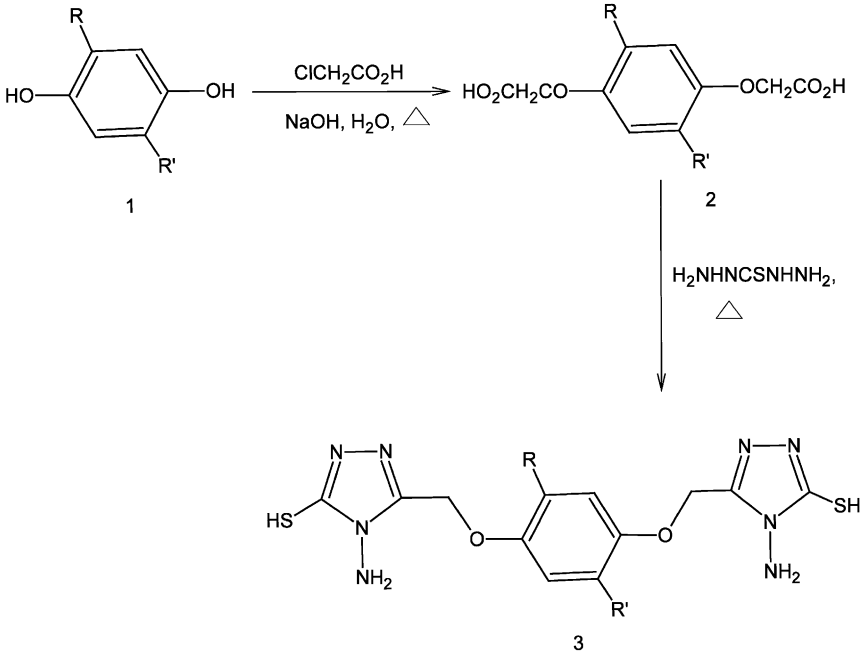

Fig. 1. R = H, Cl, ^{t}Bu; R^1 = H, Cl.

Table 1
Characterization data of compounds **3a**–**e**

Compound	R	R^1	M.p. (°C)	Yield (%)	Molecular formula	Anal. %N found [calculated]
3a	H	H	226–28	70	$C_{12}H_{14}N_8O_2S_2$	30.56 [30.60]
3b	Cl	H	215–17	72	$C_{12}H_{13}ClN_8O_2S_2$	27.94 [27.97]
3c	Cl	Cl	228–30	68	$C_{12}H_{12}Cl_2N_8O_2S_2$	25.71 [25.75]
3d	^{t}Bu	H	164–66	72	$C_{16}H_{22}N_8O_2S_2$	26.57 [26.54]
3e	^{t}Bu	Cl	175–77	76	$C_{16}H_{21}ClN_8O_2S_2$	24.49 [24.53]

IR (KBr, ν_{max} cm^{-1}): **3a**: 3225 (NH_2), 3143 (NH_2), 2947 (C–H), 1614 (C=N), 1583 (C=C); **3b**: 3230 (NH_2), 3146 (NH_2), 3010 (Ar–H), 2936 (C–H), 1609 (C=N), 1591 (C = C); **3c**: 3228 (NH_2), 3152 (NH_2), 3016 (Ar–H), 2940 (C–H), 1610 (C=N), 1585 (C=C); **3d**: 3249 (NH_2), 3148 (NH_2), 2956 (C–H), 1618 (C=N), 1583 (C=C); **3e**: 3218 (NH_2), 3139 (NH_2), 2951 (C–H), 1608 (C=N), 1593 (C=C). ^{1}H-NMR (DMSO-d_6): **3a**: δ 5.14 (bs, 4H, 2 × NH_2), 5.62 (bs, 4H, 2 × OCH_2), 6.92–7.12 (m, 4H, Ar–H), 13.8 (bs, 2H, 2 × SH–NH); **3b**: δ 5.11 (bs, 4H, 2 × NH_2), 5.58 (bs, 4H, 2 × OCH_2), 7.04–7.23 (m, 3H, Ar–H), 13.8 (bs, 2H, 2 × SH–NH); **3c**: δ 4.95 (s, 2H, NH_2), 5.15 (s, 2H, NH_2), 5.64 (bs, 4H, 2 × OCH_2), 6.98 (m, 1H, Ar–H), 7.25 (m, 1H, Ar–H), 13.82 (bs, 2H, 2 × SH–NH); **3d**: δ 1.41 (bs, 9H, *t*-butyl), 5.08 (bs, 4H, 2 × NH_2), 5.32 (bs, 4H, 2 × OCH_2), 6.95–7. 28 (m, 3H, Ar–H), 13.61 (bs, 2H, 2 × SH–NH); **3e**: δ 1.35 (bs, 9H, *t*-butyl), 5.12 (bs, 4H, 2 × NH_2), 5.22 (bs, 4H, 2 × OCH_2), 6.82–7.12 (m, 2H, Ar–H), 13.74 (bs, 2H, 2 × SH–NH). MS: **3a**: *m*/*z* 366 [12%, M$^+$]; 110 [100%, M$^+$ of hydroquinone]; **3b**: *m*/*z* 400 [12%, M$^+$], 144 [100%, M$^+$ of 2-chlorohydroquinone] 41 [50%, M$^+$ of CH_3CN]; **3c**: *m*/*z* 434 [9%, M$^+$]; **3d**: *m*/*z* 422 [22%, M$^+$]; **3e**: *m*/*z* 455 [19%, M$^+$], 200 [100%, M$^+$ of 2-*t*-butyl-5-cholorohydroquinone], 41 [62%, M$^+$ of CH_3CN].

Table 2
Characterization data of compounds **4a–w**

Compound	R	R^1	R^{11}	M.p. (°C)	Yield (%)	Molecular formula	Anal. %N found [calculated]
4a	H	H	C_6H_5	114–16	65	$C_{26}H_{18}N_8O_2S_2$	20.79 [20.82]
4b	H	H	4-$NO_2C_6H_4$	168–70	71	$C_{26}H_{16}N_{10}O_6S_2$	22.25 [22.29]
4c	H	H	2-ClC_6H_4	88–90	75	$C_{26}H_{16}Cl_2N_8O_2S_2$	18.41 [18.45]
4d	Cl	H	4-ClC_6H_4	190–92	66	$C_{26}H_{15}Cl_3N_8O_2S_2$	17.49 [17.46]
4e	Cl	H	C_6H_5	166–68	80	$C_{26}H_{17}ClN_8O_2S_2$	19.49 [19.56]
4f	Cl	H	$C_6H_5CH_2$	106–08	69	$C_{28}H_{21}ClN_8O_2S_2$	18.61 [18.65]
4g	Cl	H	3-pyridyl	58–60	72	$C_{24}H_{15}ClN_{10}O_2S_2$	24.32 [24.37]
4h	Cl	H	4-$NO_2C_6H_4$	236–38	78	$C_{26}H_{15}ClN_{10}O_6S_2$	21.08 [21.13]
4i	Cl	H	2-ClC_6H_4	92–94	65	$C_{26}H_{15}Cl_3N_8O_2S_2$	17.52 [17.46]
4j	Cl	Cl	4-ClC_6H_4	218–20	75	$C_{26}H_{14}Cl_4N_8O_2S_2$	16.62 [16.57]
4k	Cl	Cl	C_6H_5	128–30	73	$C_{26}H_{16}Cl_2N_8O_2S_2$	18.38 [18.45]
4l	Cl	Cl	$C_6H_5CH_2$	102–04	69	$C_{28}H_{20}Cl_2N_8O_2S_2$	17.59 [17.64]
4m	Cl	Cl	4-$NO_2C_6H_4$	196–98	77	$C_{26}H_{14}Cl_2N_{10}O_6S_2$	20.14 [20.09]
4n	Cl	Cl	2-ClC_6H_4	108–10	82	$C_{26}H_{14}Cl_4N_8O_2S_2$	16.50 [16.57]
4o	^{t}Bu	H	C_6H_5	100–02	74	$C_{30}H_{26}N_8O_2S_2$	18.80 [18.86]
4p	^{t}Bu	H	4-$NO_2C_6H_4$	148–50	78	$C_{30}H_{24}N_{10}O_6S_2$	20.52 [20.47]
4q	^{t}Bu	H	2-ClC_6H_4	124–26	84	$C_{30}H_{24}Cl_2N_8O_2S_2$	16.81 [16.89]
4r	^{t}Bu	Cl	4-ClC_6H_4	118–20	81	$C_{30}H_{23}Cl_3N_8O_2S_2$	15.98 [16.06]
4s	^{t}Bu	Cl	C_6H_5	102–04	78	$C_{30}H_{25}ClN_8O_2S_2$	17.75 [17.82]
4t	^{t}Bu	Cl	$C_6H_5CH_2$	98–100	76	$C_{32}H_{29}ClN_8O_2S_2$	17.12 [17.06]
4u	^{t}Bu	Cl	3-pyridyl	118–20	72	$C_{28}H_{23}ClN_{10}O_2S_2$	22.12 [22.20]
4v	^{t}Bu	Cl	4-$NO_2C_6H_4$	138–40	80	$C_{30}H_{23}ClN_{10}O_2S_2$	21.36 [21.39]
4w	^{t}Bu	Cl	2-ClC_6H_4	106–08	79	$C_{30}H_{23}Cl_3N_8O_2S_2$	16.02 [16.06]

IR (KBr, ν_{max} cm^{-1}): **4a**: 3125 (C–H), 1614 (C=N), 1585 (C=C); **4b**: 3132 (C–H), 1610(C=N), 1581 (C=C), 1529 (NO_2), 1348 (NO_2), **4c**: 3113 (C–H), 1609 (C=N), 1586 (C=C), 728 (C–Cl); **4d**: 3120 (C–H), 1612 (C=N), 1584 (C=C), 730 (C–Cl); **4e**: 3126 (C–H), 1608 (C=N), 1585 (C = C); **4g**: 3115 (C–H), 1624(C=N), 1589 (C=C); **4h**: 3123 (C–H), 1604 (C=N), 1589 (C=C), 1531 (NO_2), 1352 (NO_2); **4j**: 3115 (C–H), 1606 (C=N), 1588 (C=C), 726 (C–Cl); **4m**: 3135 (C–H), 1614 (C=N), 1593 (C=C), 1534 (NO_2), 1357 (NO_2); **4q**: 3128 (C–H), 1612 (C=N), 1581 (C=C), 731 (C–Cl); **4v**: 3142 (C–H), 1616 (C=N), 1594 (C=C), 1535 (NO_2), 1356 (NO_2); **4w**: 3121 (C–H), 1613 (C=N), 1589 (C=C), 728 (C–Cl). ^{1}H-NMR (DMSO-d_6–$CDCl_3$): **4a**: δ 5.48 (bs, 4H, 2 × OCH_2), 7.04–7.93 (m, 14H, Ar–H); **4b**: δ 5.45 (bs, 4H, 2 × OCH_2), 7.04–7.63 (m, 12H, Ar–H); **4c**: δ 5.45 (s, 2H, OCH_2), 5.51 (s, 2H, OCH_2), 7.04–7.13 (m, 2H, Ar–H), 7.43–7.63 (m, 7H, Ar–H), 7.79 (d, 1H, Ar–H, $J = 7.6$ Hz), 8.02–8.06 (m, 2H, Ar–H); **4f**: δ 4.26 (bs, 4H, 2 × CH_2), 5.44 (bs, 4H, 2 × OCH_2), 7.12–8.17 (m, 13H, Ar–H); **4h**: δ 5.53 (s, 2H, OCH_2), 5.60 (s, 2H, OCH_2), 7.09 (d, 1H, Ar–H, $J = 6$ Hz), 7.22 (s, 1H, Ar–H), 7.34 (d, 1H, Ar–H, $J = 9.1$ Hz), 7.57 (s, 1H, Ar–H), 8.03 (s,1H, Ar–H), 8.17–8.34 (m, 3H, Ar–H), 8.42 (m, 3H, Ar–H); **4k**: δ 5.42 (bs, 4H, 2 × OCH_2), 7.14–7.84 (m, 12H, Ar–H); **4m**: δ 5.58 (bs, 4H, 2 × OCH_2), 7.09 (d, 1H, Ar–H, $J = 9$ Hz), 7.22 (s, 1H, Ar–H), 7.34 (d, 1H, Ar–H, $J = 9$ Hz), 7.57 (s, 1H, Ar–H), 8.17–8.34 (m, 6H, Ar–H); **4o**: δ 1.32 (bs, 9H, *t*-butyl), 5.50 (s, 2H, OCH_2), 5.54 (s, 2H, OCH_2), 6.94–8.04 (m, 13H, Ar–H,); **4q**: δ 1.34 (bs, 9H, *t*-butyl), 5.53 (s, 2H, OCH_2), 5.55 (s, 2H, OCH_2), 6.94–8.12 (m, 11H, Ar–H,); **4s**: δ 1.29 (bs, 9H, *t*-butyl), 5.53 (bs, 4H, 2 × OCH_2), 6.99–8.14 (m, 12H, Ar–H); **4u**: δ 1.34 (bs, 9H, *t*-butyl), 5.51 (bs, 4H, 2 × OCH_2), 6.92–8.22 (m, 10H, Ar–H,); **4w**: δ 1.37 (bs, 9H, *t*-butyl), 5.51 (bs, 4H, 2 × OCH_2), 7.13–8.32 (m, 10H, Ar–H). MS: **4a**: *m*/*z* 538 [8%, M^+], 430 [15%, M − hydroquinone], 190 (100%), 121 (25%, C_6H_5–C=S), 77 (19%, phenyl cation); **4b**: *m*/*z* 628 [12%, M^+], 148 [100%, 4-$NO_2C_6H_4CN$]; **4d**: *m*/*z* 640 [14%, M^+] 137 [100%, 4-ClC_6H_4CN]; **4f**: *m*/*z* 600 [12%, M^+], 91 (100%, $C_6H_5CH_2$); **4h**: *m*/*z* 662 (46%, M^+); **4o**: *m*/*z* 594 [9%, M^+], 103 [100%, C_6H_5CN]; **4r**: *m*/*z* 696 [9%, M^+], 137 [100%, 4-ClC_6H_4CN], 57 (48%, –$C(CH_3)_3$); **4v**: *m*/*z* 654 [14%, M^+], 148 (100%, 4-$NO_2lC_6H_4CN$), 57 (25%, –$C(CH_3)_3$); **4w**: *m*/*z* 696 [16%, M^+], 137 (100%, 2-ClC_6H_4CN), 57 (41%, –$C(CH_3)_3$).

3a showed absorption bands at 3225, 2947,1614, 1583 and 1026 cm^{-1} corresponding to the NH_2, C–H, C=N, C=C, and C=S groups, respectively. The absence of band due to carbonyl stretching frequency of the parent bis-phenoxyacetic acid clearly indicates the formation of bis-triazole **3a**. The IR spectra of other bis-triazoles of the series showed similar absorption bands.

The ^{1}H-NMR spectrum of **3a** showed a broad singlet at δ 5.62 corresponding to OCH_2 protons. A sharp singlet observed at δ 5.14 is attributed to the N–NH_2 protons. The aromatic protons resonated as a multiplet in the region δ 6.92–7.12 integrating for four protons. The SH protons resonated as a broad singlet at δ 13.8. The ^{1}H-NMR spectra of **3c** and **3e** were also recorded and data are given in Table 1.

Mass spectra of compounds **3a**, **3b** and **3d** showed molecular ion a peaks at *m*/*z* 366, 400 and 422, respectively, in agreement with their molecular formulae.

These bis-aminomercaptotriazoles were converted into bis-triazolothiadiazoles **4**, **5** and **6** in good yields. These *N*-bridged bis-heterocycles were characterised on the basis of IR, ^{1}H-NMR and mass spectral data. IR spectrum of compound **4a** showed an absorption band at 1614 cm^{-1} corresponding to the C=N stretching frequency of thiadiazole ring. The absence of absorption bands due to NH_2, SH and C=O stretching frequencies of starting triazoles and carboxylic acids clearly indicated the formation of cyclised products.

The ^{1}H-NMR spectrum compound **4o** showed broad singlet at δ 1.32 integrating for nine protons of the

t-butyl group. The signal due to the two OCH_2 groups resonated as two singlets at δ 5.50 and 5.54, respectively. The aromatic protons signal appeared as a multiplet in the region δ 6.94–8.04 integrating for 13 protons.

The structures of newly synthesised bis-triazolothiadiazoles were also confirmed by recording their mass spectra. The mass spectra of compounds **4a** and **4d** showed molecular ion peaks at m/z 538 and 640 in conformity with the assigned molecular formulae $C_{26}H_{18}N_8O_2S_2$ and $C_{26}H_{15}Cl_3N_8O_2S_2$, respectively. In both the cases, the molecular ion peaks were fairly intense suggesting the presence of stable triazolothiadiazole ring system in these cyclised products.

The bis-aminomercaptotriazole **3** was converted into compound **6** by refluxing **3** with potassium hydroxide and carbon disulphide. The liberation of hydrogen sulphide gas during the reaction and absence of $-NH_2$ group frequency in the IR spectra of compounds **6** clearly indicated the formation of cyclised product. The 300 MHz ^{1}H-NMR spectrum of **6c** showed a broad singlet at δ 5.42 corresponding to the four protons of two OCH_2 groups. The signals due to two SH protons resonated as a broad singlet at δ 13.7. The aromatic protons appeared as two singlets at δ 7.36 and 7.86. The mass spectra gave further evidence for the formation of **6**. The spectral data are given in Tables 1–3.

4. Pharmacology

The newly synthesised bis-aminomercaptotriazoles and bis-triazolothiadiazoles were screened for their anticancer activities at NIH, Bethesda, MA, USA under the Drug Discovery Programme. Fourteen compounds were submitted for the three cell line one dose primary anticancer assay. The three cell lines used in the present investigation are NCI-H 460 (lung), MCF 7 (breast) and SF 268 (CNS). In this current protocol, each cell line is pre-inoculated, pre-incubated on microtiter plate. The test agents are then added at a single concentration and the culture incubated for 48 h. End point determinations are made with sulphorhodamine B, a protein binding dye. Results for each test agent are reported as the percentage of growth of the treated cells when compared to the untreated control cells. The compounds which reduce the growth of any one of the cells to 32% or less (negative numbers indicate the cell kill) are passed on for the evaluation in the full panel of 60 cell lines over a five-log dose range. Interestingly, six compounds (**3c**, **4d**, **4r**, **4t**, **6c** and **6e**) carrying chloro, ^{t}butyl, ^{t}butyl, chloro and ^{t}butyl substituents, respectively were found to be active in the preliminary screening studies. The results of such studies are given in Table 4. These compounds were further tested against a panel of 60 cell lines derived from seven cancer types namely, lung, colon, melanoma, renal, ovarian, CNS and leukemia. Their GI_{50}, TGI and LC_{50} values were determined.

5. Conclusions

Compound **3c** was found to be active against 16 cancer cell lines: leukemia (5), non-small cell lung cancer (2), colon cancer (1), CNS cancer (1), melanoma (3), renal cancer (1), prostate cancer (1) and breast cancer (2). Compound **4d** was found to be active against 25

Table 3
Characterization data of compounds **5a–e** and **6a–e**

Compound	R	R^1	M.p. (°C)	Yield (%)	Molecular formula	Anal. %N found [calculated]
5a	H	H	78–80	82	$C_{14}H_{10}N_8O_2S_2$	28.96 [29.02]
5b	Cl	H	114–16	79	$C_{14}H_9ClN_8O_2S_2$	26.60 [26.63]
5c	Cl	Cl	108–10	69	$C_{14}H_8Cl_2N_8O_2S_2$	24.58 [24.62]
5d	^{t}Bu	H	96–98	75	$C_{18}H_{18}N_8O_2S_2$	25.28 [25.34]
5e	^{t}Bu	Cl	88–90	80	$C_{18}H_{17}ClN_8O_2S_2$	23.44 [23.50]
6a	H	H	244–46	83	$C_{14}H_{10}N_8O_2S_4$	24.82 [24.89]
6b	Cl	H	200–02	79	$C_{14}H_9ClN_8O_2S_4$	23.03 [23.12]
6c	Cl	Cl	178–80	78	$C_{14}H_8Cl_2N_8O_2S_4$	21.62 [21.58]
6d	^{t}Bu	H	78–80	72	$C_{18}H_{18}N_8O_2S_4$	22.10 [22.13]
6e	^{t}Bu	Cl	104–06	69	$C_{18}H_{17}ClN_8O_2S_4$	20.77 [20.72]

IR (KBr, ν_{max} cm^{-1}): **5a**: 3141 (C–H), 1614 (C=N); **5b**: 3133 (C–H), 1608 (C=N), 732 (C–Cl); **5c**: 3139 (C–H), 1612 (C=N), 728 (C–Cl); **5e**: 3136 (C–H), 1610 (C=N), 730 (C–Cl); **6a**: 3133 (C–H), 1607 (C=N), 1588 (C=C); **6c**: 3121 (C–H), 1602 (C=N), 1594 (C=C), 736 (C–Cl); **6e**: 3141 (C–H), 1617 (C=N), 1594 (C=C), 734 (C–Cl). ^{1}H-NMR (DMSO-d_6–$CDCl_3$): **5a**: δ 5.46 (bs, 4H, $2\times OCH_2$), 6.99–8.48 (m, 6H, Ar–H); **5b**: δ 5.43 (bs, 4H, $2\times OCH_2$), 7.12–8.26 (m, 5H, Ar–H); **5d**: δ 1.34 (s, 9H, *t*-butyl), 5.44 (bs, 4aH, $2\times OCH_2$), 7.05–8.18 (m, 5H, Ar–H); **5e**: δ 1.34 (s, 9H, *t*-butyl), 5.41 (bs, 4H, $2\times OCH_2$), 7.15–8.56 (m, 6H, Ar–H). **6a**: δ 5.46 (bs, 4H, $2\times OCH_2$), 7.21–7.78 (m, 4H, Ar–H), 13.5 (bs, 2H, $2\times$SH); **6c**: δ 5.42 (bs, 4H, $2\times OCH_2$), 7.21(s, 1H, Ar–H), 7.36 (s, 1H, Ar–H), 7.86 (s, 1H, Ar–H), 13.7 (bs, 2H, $2\times$SH); **6e**: δ 1.32 (s, 9H, *t*-butyl), 5.45 (bs, 4H, $2\times OCH_2$), 7.18 (s, 1H, Ar–H), 7.38 (s, 1H, Ar–H), 13.5 (bs, 2H, $2\times$SH). MS: **5a**: m/z 386 (23%); 110 [100%, M$^+$of hydroquinone]; **5b**: m/z 420 [34%, M$^+$]; 144 [100%, M$^+$of 2-chlorohydroquinone]; **5d**: m/z 442 [13%, M$^+$]; 57 (100%, $C(CH_3)_3$ cation); **6a**: m/z 450 [15%, M$^+$], 110 [100%, M$^+$of hydroquinone]; **6c**: m/z 518 [11%, M$^+$]; **6e**: m/z 540 [8%, M$^+$], 505 [5%, M$^+-$Cl], 57 (100%, $C(CH_3)_3$ cation).

Table 4
Anticancer activity screening data of compounds **3**, **4** and **6**

Compound	Growth percentage [a]				
	NCI code no.	NCI-H 460 (lung)	MCF-7 (breast)	SF 268 (CNS)	Activity [b]
3b	NSC 716886	105	80	104	inactive
3c	NSC 716888	18	20	40	active
3e	NSC 716887	95	69	93	inactive
4d	NSC 716889	34	25	−54	active
4f	NSC 716890	74	59	76	inactive
4g	NSC 716892	96	86	82	inactive
4j	NSC 716897	71	44	49	inactive
4l	NSC 716898	64	42	65	inactive
4r	NSC 716893	−20	−57	8	active
4t	NSC 716894	11	−66	18	active
4u	NSC 716896	56	55	74	inactive
6b	NSC 716891	105	74	100	inactive
6c	NSC 716899	79	23	52	active
6e	NSC 716895	6	−27	0	active

Fixed concentration assay (100 μM; standard NCI protocol).
[a] Percent cell growth reduction following 48-h incubation with test compounds (optical density, sulphorhodamine procedure) [19].
[b] 'Active' when growth percentage is $<32\%$ for any one of the three cell lines. Negative numbers indicate the cell kill.

cancer cell lines: leukemia (5), non-small cell lung cancer (2), colon cancer (4), CNS cancer (3), melanoma (2), ovarian cancer (2), renal cancer (5) and breast cancer (2). Compound **4r** was found to be active against 24 cancer cell lines: leukemia (3), non-small cell lung cancer (3), colon cancer (4), CNS cancer (3), melanoma (3), ovarian cancer (1), renal cancer (2) and breast cancer (5). Compound **4t** was found to be active against 19 cancer cell lines: leukemia (4), non-small cell lung cancer (2), colon cancer (4), CNS cancer (2), ovarian cancer (2), renal cancer (2) and breast cancer (3). Compound **6c** was found to be active against 10 cancer cell lines: leukemia (2), non-small cell lung cancer (2), colon cancer (1), melanoma (2), ovarian cancer (1), renal cancer (1) and breast cancer (1). Compound **6e** was found to be active against 24 cancer cell lines: leukemia (4), non-small cell lung cancer (2), colon cancer (1), CNS cancer (2), melanoma (6), ovarian cancer (2), renal cancer (2) and breast cancer (5). Compound **4d** possessed a LC_{50} value less than 50 μM against renal cancer. Compound **4r** possessed LC_{50} values less than 50 μM against leukemia, colon cancer, melanoma and breast cancer. All other tested compounds had LC_{50} values higher than 50 μM. Hence, it is concluded that there is ample scope for further study in developing these compounds as anticancer agents.

6. Experimental

6.1. Chemistry

M.p.s were determined by open capillary method and are uncorrected. The IR spectra (in KBr pellets) were recorded on a Shimadzu FTIR 8700 spectrophotometer. ^{1}H-NMR spectra were recorded ($CDCl_3$/$CDCl_3$–DMSO-d_6 mixture) on a Bruker AC 300 F (300 MHz.) NMR spectometer using TMS as an internal standard. The mass spectra were recorded on a JEOL JMS 300 mass spectrometer operating at 70 eV. The purity of the compounds was checked by thin layer chromatography (TLC) on silica gel plates. The required chlorosubstituted hydroquinones and 1,4-bis-phenoxyacetic acids were synthesised by adopting literature procedures using commercially available hydroquinone [17,18].

*6.1.1. General procedure for the preparation of 1,4-bis-[4-amino-5-mercapto-1,2,4-triazol-3-yl methoxy]phenylenes (**3a–e**)*

A mixture of bis-phenoxyacetic acids **2** (0.01 mol) and thiocarbohydrazide (0.02 mol) contained in a flat-bottomed flask was heated in an oilbath until the contents melted. The mixture was maintained at this temperature for 15–20 min (Fig. 1). The product obtained on cooling was heated with dilute Na_2CO_3 solution to remove the unreacted bis-phenoxyacetic acid, if any. It was then washed with water and collected by filtration. The product was recrystallised from a mixture of dimethylformamide and water to afford pure title compounds **3a**–**e**. Their characterization data are given in Table 1 (Fig. 2).

*6.1.2. General procedure for the preparation of 1,4-bis-(6-aryl-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazol-3-ylmethoxy)phenylenes (**4a–w**)*

To a mixture of aminomercapto triazole **3** (0.01 mol) and aromatic carboxylic acid (0.02 mol) phosphorus oxychloride (20 mL) was added and the contents were

heated under reflux on a waterbath for 4 h. Excess of phosphorus oxychloride was then distilled off and the residue was poured onto crushed ice with vigorous stirring. The resulting solid was washed with cold water, dilute $NaHCO_3$ solution and then recrystallised from dimethylformamide or from a mixture of dioxane and EtOH. The characterization data of title compounds **4a**–**w** are given in Table 2.

*6.1.3. General procedure for the preparation of 1,4-bis-(1,2,4-triazolo[3,4-b]-1,3,4-thiadiazol-3-ylmethoxy)phenylenes (**5a**–**e**)*

A mixture of bis-aminomercaptotriazole **3** (0.01 mol) and HCOOH (1 mL) in C_6H_6 (20 mL) was refluxed for 30 min. The solvent was removed by evaporation and the reaction mixture was cooled. The precipitated product was recrystallised from MeOH. The product (0.01 mol) was then treated with concd. H_2SO_4 (15 mL) in cold whereby a pasty mass was obtained. It was poured into water when the title compound **5** precipitated out. It was recrystallised from DMF or a mixture of dioxane and EtOH. The characterization data of title compounds **5a**–**e** are given in Table 3.

*6.1.4. General procedure for the preparation of 1,4-bis-(6-mercapto-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazol-3-ylmethoxy)phenylenes (**6a**–**e**)*

A mixture of bis-aminomercaptotriazole **3** (0.01 mol), KOH (1 g) and carbon disulphide (2 mL) in MeOH (30 mL) was kept under reflux on a waterbath for 4 h. The solvent was removed. The reaction mixture was cooled and poured on to crushed ice with vigorous stirring. The resulting solid was washed with water, cold dilute EtOH and recrystallised from DMF or a mixture of dioxane and EtOH. The characterization data of these compounds are given in Table 3.

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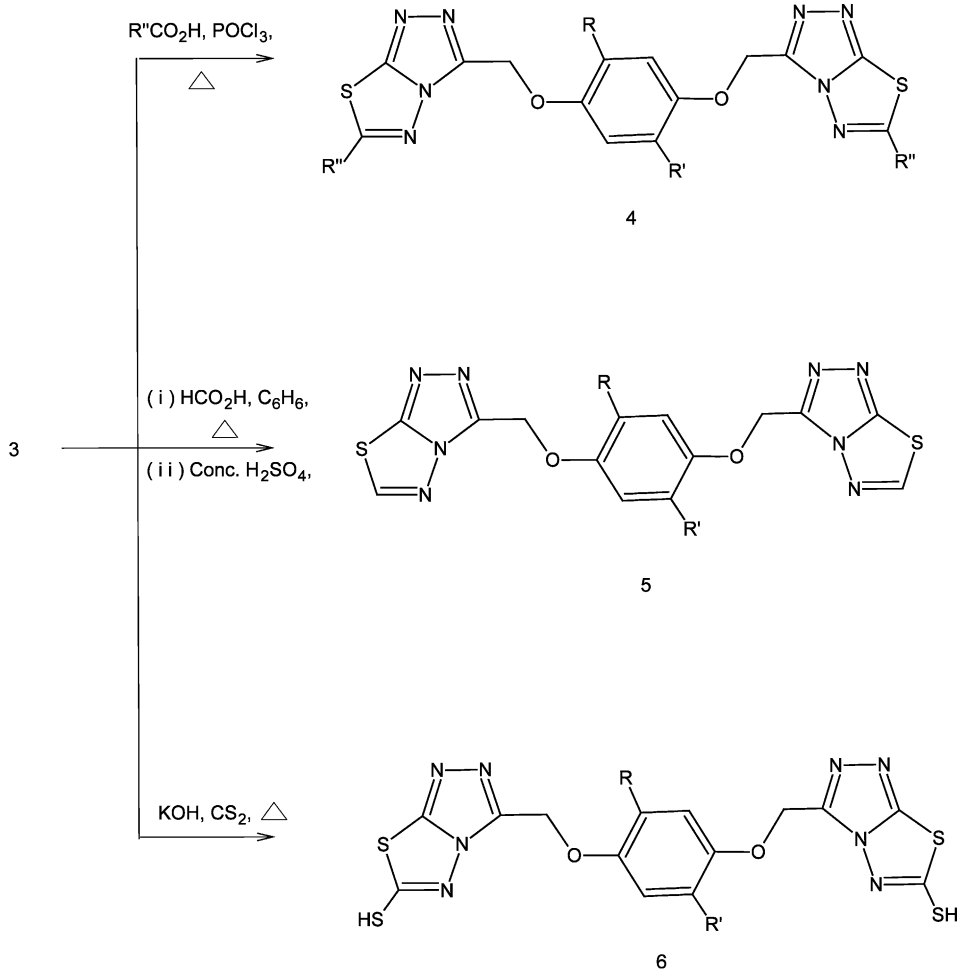

Fig. 2. R = H, Cl, ^{t}Bu; R^1 = H, Cl; R'' = C_6H_5, 4-$NO_2C_6H_4$, 2-ClC_6H_4, 4-ClC_6H_4.

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