Med Chem Res (2012) 21:174–184
DOI 10.1007/s00044-010-9517-9

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, in vitro cytotoxicity, and anti-microbial studies of 1,4-bis(4-substituted-5-mercapto-1,2,4-triazol-3-yl)butanes

Madhusudan Purohit · Yergeri C. Mayur

Received: 26 March 2010 / Accepted: 15 November 2010 / Published online: 8 December 2010
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Abstract Synthesis and evaluation of cytotoxicity and anti-microbial activity of a series of 1,4-bis(4-substituted-5-mercapto-1,2,4-triazol-3-yl)butane derivatives comprising thioether functionality and other pharmacophore modifications are described. All the newly synthesized compounds were characterized by IR, NMR, elemental analyses, and mass spectral studies. The compounds **4a–f**, **5a–f**, and **6a–f** were evaluated for in vitro cytotoxicity potential using the standard MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay against a panel of three human cancer cell lines: Lung carcinoma A-549, Colon carcinoma HT-29, and Breast Cancer MDA MB-231. All the compounds were subjected to in vitro anti-bacterial activity against *Bacillus subtilus* (ATCC 6633), *Staphylococcus aureus* (ATCC-25923), *Escherichia coli* (ATCC-25922), and *Pseudomonas aeruginosa* (ATCC-27853) and their minimal inhibitory concentrations were determined.

Keywords 1,2,4-Triazoles · Cytotoxicity · Anti-microbial activity · MTT assay

M. Purohit
Department of Pharmaceutical Chemistry,
JSS College of Pharmacy, JSS University,
Mysore 570015, Karnataka, India

Y. C. Mayur
Medicinal Chemistry Research Division,
V.L. College of Pharmacy, Raichur, India

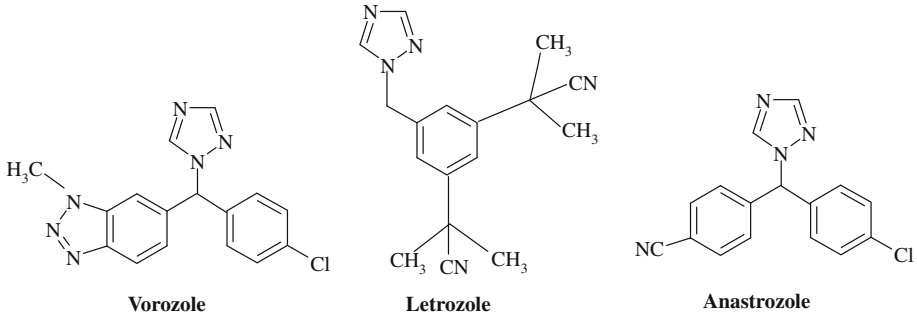
Y. C. Mayur (✉)
Center for Drug Design, SVERI's College of Pharmacy,
Pandharpur, India
e-mail: mayuryc@rediff.com; mayuryc@hotmail.com

Introduction

Over the last few years the increasing number of neoplastic diseases together with the accompanied high mortality rates has stimulated an unparallel level of research directed towards the development of new lead molecules that might be of use in designing novel anti-neoplastic agents. Dimeric analogues of various heterocyclic compounds are drawing much attention in the recent past. Many dimeric compounds designed as bis-DNA intercalators were evaluated as anti-cancer agents. Dimers of more lipophilic compounds have shown potent and broad spectrum activity against human solid tumor cell lines both in culture and as xenografts in nude mice. Some of the bis-intercalators were found to possess high selective toxicity against human colon carcinoma (Yong *et al*., 2005; Denny, 2003). In addition to DNA intercalation, the bis-heterocyclic molecules were also shown to exhibit such diverse pharmacological activity as anti-microbial (Holla *et al*., 1998), anti-protozoal (Coro *et al*., 2005), anti-inflammatory (Sondhi *et al*., 2007), anti-HIV (Pomarnacka and Kornicka, 2001), and cytotoxicity (Antonini *et al*., 2008; Brana *et al*., 1997; Rewcastle *et al*., 1987; Gamage *et al*., 1999; Spicer *et al*., 2000; Dabholkar and Ansari, 2008). Many of the bis-1,2,4-triazoles have also been reported to possess wide spectrum of biological activity (Holla *et al*., 2000; Holla *et al*., 2002; Ghorab *et al*., 2000; Al-Soud and Al-Masoudi, 2004). The triazole derivatives vorozole, letrozole, and anastrozole are non-steroidal drugs used for the treatment of breast cancer (Clemons *et al*., 2004) (Fig. 1).

Based on the above literature, the authors have tried to design the bis-triazole derivatives by incorporating various modifications using groups such as diethyl carbamoyl, thioester, and hydrazide functional groups. The incorporation of thioester moiety being attached to different

Fig. 1 Clinically useful triazole derivatives



heterocyclic systems such as 1,2,4-triazolo[4,3-a]quinoxaline (Gulerman *et al*., 2001; Badran *et al*., 2003) and 1,3,4-oxadiazole has shown to enhance the anti-microbial activity (Berghot, 2001). The well-known anti-filarial agent diethylcarbamazine contains the diethylcarbamoyl moiety; moreover, the fact that hydrazino derivatives are themselves capable of exerting anti-cancer activity by alkylation of DNA through free radical intermediate (Remers, 2004). These studies prompted the authors to synthesize some more novel bis-triazole derivatives hooked with thioester, diethylcarbamoyl, and hydrazine moieties and carry out their evaluation as cytotoxic agents.

On the other hand, the effectiveness of commercially available anti-microbials has become unreliable, due to the emergence of resistant microorganisms like methicillin-resistant *Staphylococcus aureus* (MRSA), chloroquine-resistant *Plasmodium falciparam*, multi-drug-resistant *Mycobacterium tuberculosis*, and vancomycin-resistant *Enterococcus faecium* (VRE) (Rostom *et al*., 2009). Hence such type of infections continue to be the driving force for the search and discovery of novel, more potent and selective non-traditional anti-microbial agents with the less likeliness of development of cross-resistance.

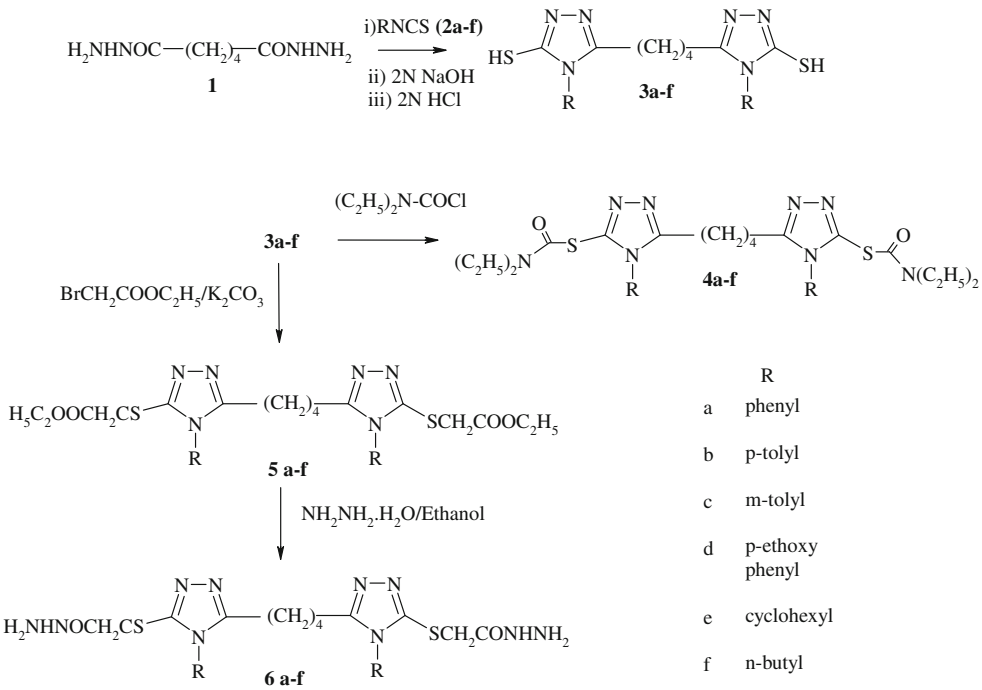
Keeping these observations in mind and in continuation of our study on the synthesis of bis-heterocyclic compounds (Purohit *et al*., 2006); we report herein the synthesis and in vitro cytotoxic activity and anti-bacterial studies of certain novel bis-1,2,4-triazole derivatives.

Results and discussion

Chemistry

Synthesis of the intermediate and target compounds was accomplished according to the steps depicted in Scheme 1. The bis-1,2,4 triazoles **3a–f**, which served as the key intermediates were prepared starting from adipoyldihydrazide **1**

Scheme 1 Scheme of synthesis



according to the previously reported reaction conditions (Mahmoud Omar, 1997) using six different substituted isothiocyanates namely phenyl, *p*-tolyl, *m*-tolyl, *p*-ethoxyphenyl, cyclohexyl, and *n*-butyl. Stirring **3a–f** with *N*,*N*-diethyl carbamoyl chloride in the presence of dry acetone and anhydrous potassium carbonate at reflux temperature yielded respective diethyl carbamoyl thio derivatives **4a–f** in moderate yields. Similarly, when **3a–f** were reacted with ethyl bromoacetate under similar reaction conditions as above-yielded corresponding thioesters **5a–f** in moderate to good yield. Refluxing **5a–f** with excess of hydrazine hydrate in absolute ethanol afforded respective acid hydrazides **6a–f** in good yield. The structures of all the compounds were elucidated on the basis of elemental analysis, IR, ^{1}H-NMR, ^{13}C-NMR, and mass spectral data.

The IR spectra of compound **4a** clearly showed the C=O stretching band at 1675 cm^{-1}, which is absent in the IR spectrum of its starting compound **3a.** Further, the ^{1}H-NMR spectrum shows the signal due to –NCH_2 protons at 3.3 ppm as quartet and the terminal methyl protons resonated at 1.0 ppm as triplet. The absence of tautomeric form of mercapto proton signal at 12.3 ppm also supports the formation of **4a**. The protons of the methylene bridge appeared at 2.45 and 1.54 ppm. The aromatic protons of the phenyl ring were observed as multiplet between 7.2 and 7.5 ppm. In the ^{13}C-NMR spectrum of the same compound, the chemical shift was observed at 163.30 ppm, this was assigned to the carbonyl carbon of the side chain. The signals at 155.22 and 148.56 ppm were due to the C_3 and C_5 of the triazole moiety. The signals at 43.72 and 13.09 ppm were assigned to carbon atom of the NCH_2 and CH_3 groups of the diethyl carbamoyl side chain, respectively. The chemical shifts at 132.56, 129.89, 128.95, and 127.15 were assigned to the aromatic carbon atoms of the phenyl ring. The chemical shifts at 32.33 and 25.10 ppm were assigned to the methylene carbons (C_2 and C_3) and (C_1 and C_4) of the butyl bridge, respectively.

The IR spectrum of compounds **5a–f** showed C=O stretching bands between 1685 and 1715 cm^{-1}. The ^{1}H-NMR spectrum of compound **5a** shows a quartet at 4.1 ppm and a triplet at 0.9 ppm assigned to the –OCH_2 and –CH_3 group of ethyl ester, respectively. The protons of the methylene bridge appeared at 2.4 and 1.5 ppm. The –SCH_2 protons resonated as singlet at 3.8 ppm. The ten aromatic protons of the phenyl ring appeared between 7.1 and 7.4 ppm as multiplet. The ^{13}C-NMR spectrum of the compound **5a** showed 12 signals corresponding to the 12 magnetically different carbon atoms. The signal at δ 168.09 ppm was assigned to carbonyl carbon of the ester functional group. The signals at 155.27 and 148.88 ppm were due to the C_3 and C_5 of the triazole moiety. The chemical shifts at 132.86, 129.99, 129.04, and 127.11 were assigned to the aromatic carbon atoms of the phenyl ring. The carbon atoms of –OCH_2 and –SCH_2 of the side chain resonated at 61.20 and 33.97 ppm. The chemical shifts at 32.37 and 25.30 ppm were assigned to the methylene carbons (C_2 and C_3) and (C_1 and C_4) of the butyl bridge. The aliphatic methyl carbon of the ester resonated at 13.72 ppm.

The ^{1}H-NMR spectrum of compound **6a** showed a singlet at 9.31 and 4.34 ppm which were assigned to –CONH and NH_2 of the acid hydrazide group. The absence of the characteristic group signals of the ethoxy group of the ester (a quartet at 4.1 ppm and a triplet at 0.9 ppm) also confirmed the formation of the acid hydrazide. The protons of the methylene bridge appeared at 2.44 and 1.45 ppm. The protons of –SCH_2 group resonated as singlet at 3.8 ppm. The multiplet signal between 7.3 and 7.6 ppm was attributed due to ten aromatic protons of the phenyl ring. The ^{13}C-NMR spectrum of the compound **6a** showed a signal at δ 166.11 ppm due to the –CONH functional group. The signal at 155.18 and 149.41 ppm was attributed to the C_3 and C_5 of the triazole moiety. The chemical shifts at 132.94, 130.88, 129.94, and 127.18 were assigned to the aromatic carbon atoms of the phenyl ring. The carbon atom of –SCH_2 of the side chain resonated at 35.06 ppm. The chemical shifts at 34.24 and 25.33 ppm were assigned to the methylene carbons (C_2 and C_3) and (C_1 and C_4) of the butyl bridge. Similar explanation for assigning the carbon holds good for the rest of the compounds. In conclusion, ^{1}H-NMR and ^{13}C-NMR spectral data were consistent with the proposed structures. The mass spectra of all the triazole derivatives were analyzed under ESI conditions. Molecular ions were observed in the form of M + H. Most of the compounds yield abundant molecular ions in the form of M + H peaks. Similarly, the elemental analyses of all the compounds have been performed and the data given under the physical data in Table 1.

Biological activity

Lipophilicity

The efficiency of the cytotoxicity of the drug depends on the accumulation of the compound into the cell and thus lipophilic character plays a major role in the cytotoxic effect of the compounds. The partition coefficient ($\log_{10}P$) of the compounds which is a measure of lipophilicity was calculated using the software Bioloom (version 1) from Biobyte Corp. (201, West 4th St. Suite 204, Claremont, CA 91711). The lipophilicity data of all the compounds vary between 0.35 and 5.82, which is expressed in $\log_{10}P$, are given along with other physical data in Table 1. Presence of a methyl group or ethoxy group on the phenyl ring markedly increased the $\log_{10}P$ values, while the replacement of aryl ring with cyclohexyl moiety slightly decreased

Table 1 Physical data of synthesized compounds

Compound	R	Mol. wt	Mol. formula	Clog P[a]	Yield	MP(°C)[b]	Elemental analysis calculated (found)		
							C	H	N
4a	Phenyl	607	$C_{30}H_{38}N_8O_2S_2$	4.50	57	165	59.38 (59.25)	6.31 (6.12)	18.47 (18.28)
4b	*p*-Tolyl	635	$C_{32}H_{42}N_8O_2S_2$	5.50	55	183	60.54 (60.40)	6.67 (6.35)	17.65 (17.74)
4c	*m*-Tolyl	635	$C_{32}H_{42}N_8O_2S_2$	5.50	50	180	60.54 (60.56)	6.67 (6.55)	17.65 (17.55)
4d	*p*-Ethoxy phenyl	695	$C_{34}H_{46}N_8O_4S_2$	5.57	52	197	58.76 (58.80)	6.67 (6.65)	16.12 (16.10)
4e	Cyclohexyl	619	$C_{30}H_{50}N_8O_2S_2$	4.39	48	110	58.22 (58.34)	8.14 (8.18)	18.11 (18.20)
4f	*n*-butyl	567	$C_{26}H_{46}N_8O_2S_2$	3.50	45	104	55.09 (55.24)	8.18 (8.23)	19.77 (19.82)
5a	Phenyl	580	$C_{28}H_{32}N_6O_4S_2$	4.75	65	197	57.91 (57.85)	5.55 (5.65)	14.47 (14.55)
5b	*p*-Tolyl	608	$C_{30}H_{36}N_6O_4S_2$	5.75	72	210	59.19 (59.30)	5.96 (6.05)	13.80 (13.75)
5c	*m*-Tolyl	608	$C_{30}H_{36}N_6O_4S_2$	5.75	70	214	59.19 (59.43)	5.96 (5.87)	13.80 (13.73)
5d	*p*-Ethoxy phenyl	668	$C_{32}H_{40}N_6O_6S_2$	5.82	65	235	57.47 (57.65)	6.03 (6.10)	12.57 (12.45)
5e	Cyclohexyl	592	$C_{28}H_{44}N_6O_4S_2$	4.64	60	182	56.73 (56.87)	7.48 (7.65)	14.18 (14.24)
5f	*n*-Butyl	540	$C_{24}H_{40}N_6O_4S_2$	3.75	63	176	56.73 (56.85)	7.48 (7.54)	14.18 (14.32)
6a	Phenyl	552	$C_{24}H_{28}N_{10}O_2S_2$	0.46	78	215	51.04 (51.22)	7.14 (7.23)	24.80 (24.65)
6b	*p*-Tolyl	580	$C_{26}H_{32}N_{10}O_2S_2$	1.46	75	224	53.77 (53.86)	5.55 (5.47)	24.12 (23.95)
6c	*m*-Tolyl	580	$C_{26}H_{32}N_{10}O_2S_2$	1.46	75	240	53.77 (53.65)	5.55 (5.45)	24.12 (24.32)
6d	*p*-Ethoxy phenyl	640	$C_{28}H_{36}N_{10}O_4S_2$	1.53	65	257	52.48 (52.34)	5.66 (5.76)	21.86 (21.65)
6e	Cyclohexyl	564	$C_{24}H_{40}N_{10}O_2S_2$	0.35	70	205	51.04 (51.23)	7.14 (6.95)	24.80 (24.26)
6f	*n*-Butyl	512	$C_{20}H_{36}N_{10}O_2S_2$	0.54	68	190	56.73 (56.34)	7.48 (7.70)	14.18 (14.35)

[a] Determined using software Bioloom (BioByte Corp, USA)

[b] Average of three trials

the $\log_{10}P$ values. The diethylcarbamoyl and thioesters derivatives (**4a–f** and **5a–f**, respectively) were found to be more lipophilic as indicated by higher $\log_{10}P$ values. The conversion of thioester group into the acid hydrazide drastically decreased the $\log_{10}P$ values due to more polar hydrazide functionality. The triazoles **4b** and **4c**, **5b** and **5c**, and **6b** and **6c** are the positional isomers which exhibit identical $\log_{10}P$ values. It is clear from $\log_{10}P$ data given in Table 1 that the triazoles have shown lipophilicity in the following order, **5d** > **5b** > **4d** > **4b** > **5a** > **5e** > **4a** > **4e** > **5f** > **4f** > **6d** > **6b** > **6f** > **6a** > **6e**. The analysis of the relationship between $\log_{10}P$ values and the efficiency of the compounds cytotoxicity in cancer cells showed a poor correlation. The hydrazide derivative **6d** with ethoxy phenyl substitution having a $\log_{10}P$ value (1.53) showed significant cytotoxicity, while the diethyl carbamoyl derivative **4d** having same substitution and a higher $\log_{10}P$ value was not effective against cancer cell lines. It is also speculated that the bis-triazole nucleus with substitution may also exhibit higher affinity for membranes or be more readily taken up into cells than that with hydrogen atom present. Therefore, we can conclude that the degree of lipophilicity of each drug would seem to be important, but it is not the sole determinant of potency for the anticancer activity of the triazoles.

Cytotoxicity studies

The cytotoxicity of all the compounds was evaluated in vitro against the following human cancer cell lines: A-549 lung carcinoma, HT-29 colon adenocarcinoma, and MDA-MB-231 breast carcinoma. The standard MTT assay was used to determine IC_{50} values, i.e., the drug concentration that causes 50% cell growth inhibition after 72 h of continuing exposure to the test compounds and the mean of the results obtained from triplicate assays are shown in Table 2 (Molinari *et al*., 2009; Manjula *et al*., 2009). The IC_{50} values were compared with that of anti-cancer antibiotic doxorubicin. From the evaluation of the data reported in Table 2, these observations can be made. All the synthesized compounds were far less potent cytotoxic than the standard drug doxorubicin (IC_{50} value 0.07–0.1 μM) as evident from higher IC_{50} values. However, among the synthesized compounds, the triazoles with diethylcarbamoyl and thioester functionality were found to be more effective than the acid hydrazides possibly due to their higher lipophilicity and higher membrane penetrating potency. The compounds which possess aromatic phenyl or substituted phenyl group at the 4th position of the triazole moiety are found to be more effective against the three cell lines assayed. The methyl and ethoxy substitution at the

Table 2 In vitro anti-cancer activity of bis-1,2,4-triazoles

Compound	IC_{50} (μM)[a]		
	A-549[b]	HT-29[c]	MDA-MB-231[d]
4a	19.31 ± 0.98	14.32 ± 1.89	14.91 ± 1.05
4b	3.23 ± 2.23	3.45 ± 1.67	8.17 ± 2.53
4c	15.25 ± 2.02	10.23 ± 3.53	17.21 ± 1.26
4d	51.87 ± 2.07	75.03 ± 1.09	71.63 ± 3.90
4e	51.81 ± 3.07	69.85 ± 1.84	66.17 ± 1.74
4f	63.06 ± 2.80	64.52 ± 2.34	70.34 ± 2.44
5a	34.47 ± 1.46	51.25 ± 2.45	32.17 ± 1.13
5b	9.13 ± 3.33	13.52 ± 1.62	24.31 ± 2.03
5c	23.27 ± 1.35	25.81 ± 2.53	25.19 ± 3.51
5d	9.30 ± 3.16	13.18 ± 5.75	15.13 ± 3.68
5e	51.87 ± 1.10	65.31 ± 4.72	70.36 ± 4.09
5f	61.50 ± 2.78	69.82 ± 2.15	71.39 ± 1.51
6a	63.16 ± 1.87	65.31 ± 1.48	69.90 ± 2.08
6b	36.94 ± 1.72	31.18 ± 2.49	32.20 ± 4.50
6c	39.15 ± 1.39	38.25 ± 2.66	40.17 ± 1.48
6d	9.13 ± 2.53	5.16 ± 1.75	13.21 ± 1.33
6e	65.26 ± 2.49	66.19 ± 3.11	70.20 ± 4.16
6f	69.33 ± 1.60	63.39 ± 1.65	59.82 ± 3.83
Doxorubicin	0.09 ± 0.01	0.07 ± 0.01	0.10 ± 0.02

[a] The drug concentration that causes 50% cell growth inhibition

[b] Lung carcinoma

[c] Colon adenocarcinoma

[d] Breast carcinoma

SEM: average of three experiments

Table 3 In vitro anti-bacterial potential of bis-1,2,4-triazoles

Compounds	Minimal inhibitory concentrations (MIC, in μg/ml)			
	B.substilus	*S. aureus*	*E. coli*	*P. aeruginosa*
4a	25	50	12.5	50
4b	12.5	25	12.5	25
4c	25	50	12.5	25
4d	50	ND	100	ND
4e	100	ND	100	ND
4f	ND	ND	ND	ND
5a	100	100	50	100
5b	100	100	100	100
5c	50	100	50	100
5d	25	50	25	100
5e	100	ND	100	ND
5f	100	ND	100	ND
6a	25	25	25	100
6b	12.5	12.5	12.5	100
6c	25	25	25	ND
6d	6.25	12.5	12.5	12.5
6e	100	ND	50	ND
6f	100	ND	100	ND
Ampicillin	12.5	6.25	6.25	12.5

ND not determined due to solubility problem

para position of the phenyl ring appears to increase the effectiveness of the molecule, while methyl group at *meta* position slightly decreased the activity. The compounds with cyclohexyl and *n*-butyl substitution exhibited poor cytotoxicity. Among the six acid hydrazides synthesized *para* ethoxy phenyl-substituted triazole **6d** only exhibited significant cytotoxic activity.

Anti-microbial studies

All the compounds were tested for in vitro anti-microbial activity against the following microorganisms: *Bacillus subtilus* (ATCC 6633), *Staphylococcus aureus* (ATCC-25923) (Gram positive bacteria), *Pseudomonas aeruginosa* (ATCC-27853), *Escherichia coli* (ATCC-25922) (Gram negative bacteria). Ampicillin was used as a reference standard. The minimal inhibitory concentration (MIC) values for compounds tested, defined as the lowest concentration of the compound preventing the visible growth, were determined using serial dilution method and are given in Table 3 (Rostom *et al*., 2009). From the data given in the Table 3, it is clear that the compounds **4d**, **4e**, and **5a**–**f** did not show good anti-bacterial activity when compared to Ampicillin, while compounds **4a**, **4b**, **4c**, and **6a** exhibited fairly good anti-bacterial activity. The compounds **6b** and **6d** were found to be more potent anti-bacterial agents with MIC value closer to that of the reference standard. The lipophilic nature of the compounds appears to be an important factor as higher lipophilicity of **4d**, **4e**, and **5a**–**f** failed to exhibit anti-bacterial activity. However, the lipophilicity is not the sole determinant for the activity since the compounds **4a**, **4b**, and **4c** having higher lipophilicity showed moderate to fairly good anti-bacterial activity. This clearly indicates that the thioester moiety is not essential for anti-bacterial activity, while diethyl carbamoyl moiety and hydrazide functional group are essential for better anti-bacterial activity of the triazole derivatives. The compound **6d** showed better anti-bacterial activity against gram positive *B. subtilus* with lowest MIC of (6.25 μg/ml) compared to the other compounds. The acid hydrazide **6b** also showed good anti-bacterial activity with MIC of (12.5 μg/ml) against *B. subtilus*, *E. coli*, and *S. aureus*. The investigation of structure activity relationship clearly revealed that the compounds **4b**, **6b**, and **6d** showed better anti-bacterial activity and this may be attributed due to the presence of electron donating groups like methyl and ethoxy substituents on the *para* position of

the phenyl group and also due to polar hydrazide functional groups.

Conclusion

Eighteen bis-1,2,4-traizole derivatives containing diethylcarbamoyl, thioester, and hydrazide functional groups were prepared and characterized. The compounds were evaluated for their in vitro cytotoxicity against three human cell lines (A-549 lung carcinoma, HT-29 colon adenocarcinoma, and MDA-MB-231) and anti-microbial potential against *B. subtilus*, *S. aureus*, *E. coli*, and *P. aeruginosa*. The triazoles substituted with *p*-tolyl and *p*-ethoxy phenyl groups showed greater effectiveness than other compounds. The triazoles **4b**, **5b**, **5d**, and **6d** were found to be potent cytotoxic molecules. Probably the cytotoxicity is conferred via DNA intercalation and further studies are necessary to confirm the possible mechanism of cytotoxicity and to validate the lead molecule. The anti-microbial activity evaluations suggest as indicated by higher MIC values that they lack any significant anti-bacterial activity probably due to higher lipophilicity. However, anti-bacterial activity of triazoles **6b** and **6d** is probably attributed to polar carbohydrazide functional group and electron donating groups like methyl and ethoxy substituents on the *para* position of the phenyl ring.

Experimental

Materials and methods

The melting points were determined in open glass capillaries and are uncorrected. IR spectra were recorded on Shimadzu FT-IR 8400-S spectrophotometer by KBr pellet technique. Elemental analyses were performed and found values are within 0.4% of theoretical values unless otherwise noted. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on AMX-400 NMR spectrophotometer at 400 MHz using DMSO-d_6 as the solvent and tetramethylsilane (TMS) as internal standard. The chemical shifts are expressed in δ ppm. The splitting patterns were designated as follows; s: singlet; d: doublet; q: quartet; m: multiplet. LCMS were recorded by using Shimadzu LCMS-2010A instrument by ESI.

Chemistry

The starting compounds 1,4-bis-[5-mercapto-4-substituted-1,2,4-triazol-3-yl]-butane **3a–f** were synthesized from adipoyl dihydrazide **1** by reacting with six different substituted isothiocyantes **2a–f** as per the reported method (Mahmoud Omar 1997).

*Synthesis of 1,4-bis[5-(N,N-diethylcarbamoyl)-thio-4-phenyl-1,2,4-triazol-3-yl]-butane **4a***

A mixture of **3a** (0.5 g, 0.0012 mol), diethylcarbamoyl chloride (0.34 g, 0.32 ml, 0.0025 mol), and anhydrous K_2CO_3 (0.5 g, 0.0036 mol) in dry acetone (15 ml) was heated under reflux for 8 h with continuous stirring. The resulting mixture was filtered. The residue was washed twice with 5 ml each of hot acetone and the washings were added to the filtrate. The excess solvent was concentrated under reduced pressure and the residue was poured into the crushed ice. The precipitate obtained was filtered and washed with cold methanol, dried, and crystallized using DMF. The physical data is presented in Table 1.

IR: 1675 cm^{-1} (C=O stretching); ^{1}H-NMR δ: 7.2–7.5 (m, Ar–H, 10H), 3.3 (q, 8H, NCH_2), 2.45 (t, 4H, C_1, and C_4 methylene protons of butyl chain), 1.54 (t, 4H, C_2 and C_3 methylene protons of butyl chain), 1.0 (t, 12H, CH_3); ^{13}C-NMR δ: 163.3 (C=O), 155.22 (C_3 of triazole), 148. 56 (C_5 of triazole), 132.56 (C_1 of phenyl ring), 129.89 (C_2 and C_6 of phenyl ring), 128.95 (C_3 and C_5 of phenyl ring), 127.15 (C_4 of phenyl ring), 43.72 (NCH_2), 32.33 (C_2 and C_3 of butyl chain), 25.10 (C_1 and C_4 of butyl chain), 13.09 (terminal methyl); MS *m*/*z*: 608 $[M + H]^+$ (75).

*1,4-bis[5-(N,N-diethylcarbamoyl)-thio-4-(p-tolyl)-1,2,4-triazol-3-yl]-butane **4b***

The procedure used for preparation of **4a** was followed using **3b** (0.52 g, 0.0012 mol), diethylcarbamoyl chloride (0.34 g, 0.32 ml, 0.0025 mol) and anhydrous K_2CO_3 (0.5 g, 0.0036 mol). The physical data is presented in Table 1.

IR: 1672 cm^{-1} (C=O stretching); ^{1}H-NMR δ: 7.0–7.6 (m, Ar–H, 8H), 3.4 (q, 8H, NCH_2), 2.44 (t, 4H, C1, and C_4 methylene protons of butyl chain), 2.25 (s, 6H, tolyl methyl), 1.51 (t, 4H, C_2 and C_3 methylene protons of butyl chain), 0.92 (t, 12H, CH_3); ^{13}C-NMR δ: 165.3 (C=O), 155.34 (C_3 of triazole), 147. 86 (C_5 of triazole), 132.67 (C_1 of phenyl ring), 129.99 (C_2 and C_6 of phenyl ring), 127.95 (C_3 and C_5 of phenyl ring), 127.10 (C_4 of phenyl ring), 43.62 (NCH_2), 32.33 (C_2 and C_3 of butyl chain), 25.10 (C_1 and C_4 of butyl chain), 20.27 (tolyl methyl), 13.12 (terminal methyl); MS *m*/*z*: 636 $[M + H]^+$ (79).

*1,4-bis[5-(N,N-diethylcarbamoyl)-thio-4-(m-tolyl)-1,2,4-triazol-3-yl]-butane **4c***

The experimental steps for the **4a** was repeated using **3c** (0.52 g, 0.0012 mol), diethylcarbamoyl chloride (0.34 g, 0.32 ml, 0.0025 mol) and anhydrous K_2CO_3 (0.5 g, 0.0036 mol).

IR: 1671 cm^{-1} (C=O stretching); ^{1}H-NMR δ: 7.1–7.6 (m, Ar–H, 8H), 3.33 (q, 8H, NCH_2), 2.42 (t, 4H, C_1, and C_4 methylene protons of butyl chain), 2.23 (s, 6H, tolyl methyl),1.53 (t, 4H, C_2 and C_3 methylene protons of butyl chain), 0.95 (t, 12H, CH_3); ^{13}C-NMR δ: 165.43 (C=O), 155.39 (C_3 of triazole), 147. 86 (C_5 of triazole), 126.30 (C_1 of phenyl ring), 124.83 (C_2 of phenyl), 138.78 (C_3 of phenyl ring), 129.89 (C_4 of phenyl ring), 128.12 (C_5 of phenyl), 126.89 (C_6 of phenyl ring), 127.90 (C_3 and C_5 of phenyl ring), 127.17 (C_4 of phenyl ring), 43.76 (NCH_2), 32.33 (C_2 and C_3 of butyl chain), 25.13 (C_1 and C_4 of butyl chain), 20.25 (tolyl methyl), 13.15 (terminal methyl); MS *m/z*: 636 $[M + H]^+$ (79).

1,4-bis[5-(N,N-diethylcarbamoyl)-thio-4-(p-ethoxyphenyl)-1,2,4-triazol-3-yl]-butane ***4d***

A mixture of **3d** (0.5 g, 0.001 mol), diethylcarbamoyl chloride (0.27 g, 0.25 ml, 0.002 mol), and anhydrous K_2CO_3 (0.5 g, 0.0036 mol) was treated similar to the method described in **4a**. The residue obtained was washed three times with 10 ml each of diethyl ether to obtain the product. Crystallization was done using dichloromethane.

IR: 1681 cm^{-1} (C=O stretching); ^{1}H-NMR δ: 7.1–7.6 (m, 8H, Ar–H), 4.3(q, 4H, OCH_2), 3.30 (q, 8H, NCH_2), 2.46 (t, 4H, C_1, and C_4 methylene protons of butyl chain), 1.50 (t, 4H, C_2 and C_3 methylene protons of butyl chain), 1.13 (t, 6H, CH_3 of ethoxy group), 0.93 (t, 12H, CH_3); ^{13}C-NMR δ: 165.55 (C=O), 155.35 (C_3 of triazole), 147. 87 (C_5 of triazole), 132.65 (C_1 of phenyl ring), 129.88 (C_2 and C_6 of phenyl ring), 127.93 (C_3 and C_5 of phenyl ring), 127.18 (C_4 of phenyl ring), 62.37 (OCH_2), 43.76 (NCH_2), 32.33 (C_2 and C_3 of butyl chain), 25.15 (C_1 and C_4 of butyl chain), 15.11 (methyl of ethoxy group), 13.11 (terminal methyl); MS *m/z*: 696 $[M + H]^+$ (65).

1,4-bis[5-(N,N-diethylcarbamoyl)-thio-4-cyclohexyl-1,2,4-triazol-3-yl]-butane ***4e***

A mixture of **3e** (0.5 g, 0.0012 mol), diethylcarbamoyl chloride (0.27 g, 0.25 ml, 0.002 mol), and anhydrous K_2CO_3 (0.5 g, 0.0036 mol) was treated similar to the method described in the preparation of **4a.** The residue obtained was washed three times with 10 ml each of diethyl ether to obtain the product. Crystallization was done using mixture of DMF and ethanol.

IR: 1660 cm^{-1} (C=O stretching); ^{1}H-NMR δ: 3.69 (m, 2H, C_1 protons of cyclohexyl), 3.25 (q, 8H, NCH_2), 2.44 (t, 4H, C_1 and C_4 methylene protons of butyl chain), 2.05 (m, 8H, C_2 and C_6 protons of cyclohexyl), 1.75 (m, 8H, C_3 and C_5 protons of cyclohexyl), 1.64 (m, 4H, C_4 protons of cyclohexyl), 1.45 (t, 4H, C_2 and C_3 methylene protons of butyl chain), 0.93 (t, 12H, CH_3); MS *m/z*: 620 $[M + H]^+$ (45).

1,4-bis[5-(N,N-diethylcarbamoyl)-thio-4-(n-butyl)-1,2,4-triazol-3-yl]-butane ***4f***

The experimental steps for the **4a** was repeated using **3f** (0.5 g, 0.0013 mol), diethylcarbamoyl chloride (0.34 g, 0.32 ml, 0.0025 mol), and anhydrous K_2CO_3 (0.5 g, 0.0036 mol) in dry acetone (15 ml).

IR: 1663 cm^{-1} (C=O stretching); ^{1}H-NMR δ: 3.79 (t, 4H, C_1 protons of *n*-butyl), 3.35 (q, 8H, NCH_2), 2.45 (t, 4H, C_1 and C_4 methylene protons of butyl chain), 1.65 (m, 4H, C_2 protons of *n*-butyl), 1.47 (t, 4H, C_2 and C_3 methylene protons of butyl chain), 1.30 (m, 4H, C_3 protons of *n*-butyl), 1.03 (t, 12H, CH_3), 0.91(t, 6H, C_4 protons of *n*-butyl); MS *m/z*: 568 $[M + H]^+$ (45).

1,4-bis-[5-(carbethoxy-methyl)-thio-4-phenyl-1,2,4-triazol-3-yl]-butane ***5a***

A suspension of **3a** (0.5 g, 0.0012 mol) in 10 ml dry acetone was mixed with a solution of ethyl bromoacetate (0.67 g, 0.5 ml, 0.004 mol) in 10 ml of acetone in an Erlenmeyer flask and freshly fused anhydrous K_2CO_3 (0.5 g, 0.0036 mol) was added. The resulting reaction mixture was refluxed for 8 h on boiling water bath with constant stirring. After the completion of the reaction (monitored by TLC), the reaction mixture was cooled and filtered. The solid residue was washed four times with 10 ml of each of hot acetone. The filtrate and the washings were mixed and the excess solvent was evaporated under reduced pressure. The mother liquor was kept in a refrigerator over night whereby the ester was solidified. The product was filtered and washed with cold methanol, dried, and recrystallized with dichloromethane.

IR: 1680 cm^{-1} (C=O stretching); ^{1}H-NMR δ: 7.0–7.6 (m, Ar–H, 10H), 4.1(q, 4H, OCH_2), 3.8 (s, 4H, SCH_2), 2.45 (t, 4H, C_1 and C_4 methylene protons of butyl chain), 1.54 (t, 4H, C_2 and C_3 methylene protons of butyl chain), 0.90 (t, 6H, CH_3); ^{13}C-NMR δ: 168.09 (C=O), 155.27 (C_3 of triazole), 148.88 (C_5 of triazole), 132.86 (C_1 of phenyl ring), 129.99 (C_2 and C_6 of phenyl ring), 129.04 (C_3 and C_5 of phenyl ring), 127.11 (C_4 of phenyl ring), 61.20 (OCH_2), 33.97 (SCH_2), 32.37 (C_2 and C_3 of butyl chain), 25.30 (C_1 and C_4 of butyl chain); MS *m/z*: 581 $[M + H]^+$ (100).

1,4-bis[5-(carbethoxy-methyl)-thio-4-(p-tolyl)-1,2,4-triazol-3-yl]-butane ***5b***

The experimental procedure was followed as per the synthesis of **5a** using **3b** (0.52 g, 0.0012 mol), ethyl

bromoacetate (0.67 g, 0.5 ml, 0.004 mol) and anhydrous K_2CO_3 (0.5 g, 0.0036 mol).

IR: 1673 cm^{-1} (C=O stretching); ^{1}H-NMR δ: 7.2–7.4 (m, 8H, ArH), 4.1 (q, 4H, OCH_2), 4.0 (s, 4H, SCH_2), 2.5 (t, 4H, C_1 and C_4 methylene protons of butyl chain), 2.4 (s, 6H, tolyl CH_3), 1.4 (t, 4H, C_2 and C_3 methylene protons of butyl chain), 1.1 (t, 6H, ester CH_3); ^{13}C-NMR δ: 168.07 (C=O), 155.31 (C_3 of triazole), 148.95 (C_5 of triazole), 129.46 (C_1 of phenyl ring), 130.38 (C_2 and C_6 of phenyl ring), 126.81 (C_3 and C_5 of phenyl ring), 139.77 (C_4 of phenyl ring), 61.16 (OCH_2), 33.89 (S-CH_2), 32.34 (C_2 and C_3 of butyl chain), 25.29 (C_1 and C_4 of butyl chain), 20.70 (tolyl CH_3), 13.9 (CH_3); MS *m/z*: 609 $[M + H]^+$ (100).

1,4-bis[5-(carbethoxy-methyl)-thio-4-(m-tolyl)-1,2,4-triazol-3-yl]-butane ***5c***

The procedure described in the synthesis of **5a** was repeated using **3c** (0.52 g, 0.0012 mol), ethyl bromoacetate (0.67 g, 0.5 ml, 0.004 mol) and anhydrous K_2CO_3 (0.5 g, 0.0036 mol).

IR: 1678 cm^{-1} (C=O stretching); ^{1}H-NMR δ: 7.2–7.5 (m, 8H, ArH), 4.15 (q, 4H, OCH_2), 3.85 (s, 4H, SCH_2), 2.54 (t, 4H, C_1 and C_4 methylene protons of butyl chain), 2.42 (s, 6H, tolyl CH_3), 1.54 (t, 4H, C_2 and C_3 methylene protons of butyl chain), 1.1 (t, 6H, ester CH_3); ^{13}C-NMR δ: 167.98 (C=O), 155.18 (C_3 of triazole), 148.75 (C_5 of triazole), 127.29 (C_1 of phenyl ring), 123.99 (C_2 of phenyl), 139.72 (C_3 of phenyl ring), 132.71 (C_4 of phenyl ring), 130.54 (C_5 of phenyl), 129.61 (C_6 of phenyl), 61.09 (OCH_2), 33.88 (S-CH_2), 32.15 (C_2 and C_3 of butyl chain), 25.26 (C_1 and C_4 of butyl chain), 20.59 (tolyl CH_3), 13.85 (CH_3); MS *m/z*: 609 $[M + H]^+$ (100).

1,4-bis[5-(carbethoxy-methyl)-thio-4-(p-ethoxyphenyl)-1,2,4-triazol-3-yl]-butane ***5d***

The method described in the synthesis of **5a** was repeated using **3d** (0.5 g, 0.001 mol), ethyl bromoacetate (0.67 g, 0.5 ml, 0.004 mol), and anhydrous K_2CO_3 (0.5 g, 0.0036 mol).

IR: 1680 cm^{-1} (C=O stretching); ^{1}H-NMR δ: 7.0–7.3 (m, 8H, Ar–H), 4.2(q, 4H, OCH_2), 4.1(q, 4H, OCH_2), 3.9 (s, 4H, SCH_2), 2.4 (t, 4H, C_1 and C_4 methylene protons of butyl chain), 1.45 (t, 4H, C_2 and C_3 methylene protons of butyl chain), 1.3 (t, 6H, CH_3 of ester group), 1.1 (t, 6H, CH_3 of ethoxy group); MS *m/z*: 669 $[M + H]^+$ (75).

Synthesis of 1,4-bis[5-(carbethoxy-methyl)-thio-4-cyclohexyl-1,2,4-triazol-3-yl]-butane ***5e***

The method described in the synthesis of **5a** was repeated using **3e** (0.5 g, 0.0012 mol), ethyl bromoacetate (0.67 g, 0.5 ml, 0.004 mol), and anhydrous K_2CO_3 (0.5 g, 0.0036 mol).

IR: 1670 cm^{-1} (C=O stretching); ^{1}H-NMR δ: 4.12 (q, 4H, OCH_2), 3.9 (s, 4H, SCH_2), 3.65 (m, 2H, protons of C_1 of cyclohexyl), 2.5 (t, 4H, C_1 and C_4 methylene protons of butyl chain), 2.10 (m, 8H, C_2 and C_6 protons of cyclohexyl), 1.75 (m, 8H, C_3 and C_5 protons of cyclohexyl), 1.64 (m, 4H, C_4 protons of cyclohexyl), 1.4 (t, 4H, C_2 and C_3 methylene protons of butyl chain), 1.1 (t, 6H, CH_3 of ester group); MS *m/z*: 593 $[M + H]^+$ (68).

1,4-bis[5-(carbethoxy-methyl)-thio-4-butyl-1,2,4-triazol-3-yl]-butane ***5f***

The method described in the synthesis of **5a** was followed using **3d** (0.5 g, 0.001 mol), ethyl bromoacetate (0.67 g, 0.5 mL, 0.004 mol), and anhydrous K_2CO_3 (0.5 g, 0.0036 mol).

IR: 1673 cm^{-1} (C=O stretching); ^{1}H-NMR δ: 4.1 (q, 4H, OCH_2), 3.9 (s, 4H, SCH_2), 3.73 (t, 4H, C_1 protons of *n*-butyl), 2.35 (t, 4H, C_1 and C_4 methylene protons of butyl chain), 1.75 (m, 4H, C_2 protons of *n*-butyl), 1.47 (t, 4H, C_2 and C_3 methylene protons of butyl chain), 1.30 (m, 4H, C_3 protons of *n*-butyl), 1.03 (t, 6H, CH_3), 0.91 (t, 6H, C_4 protons of *n*-butyl); ^{13}C-NMR δ: 169.07 (C=O), 154.33 (C_3 of triazole), 149.19 (C_5 of triazole), 62.16 (OCH_2), 36.21 (C_1 of butyl), 33.88 (S-CH_2), 32.90 (C_2 of butyl), 32.10 (C_2 and C_3 of butyl chain), 25.2 (C_1 and C_4 of butyl chain), 20.12 (C_3 of butyl), 14.5 (CH_3 of ester), 12.32 (C_4 of butyl); MS *m/z*: 541 $[M + H]^+$ (55).

1,4-bis(5[hydrazinocarbonylmethylthio]-4-phenyl-1,2,4 triazol-3-yl)butane ***6a***

A mixture of **5a** (0.58 g, 0.001 mol) and hydrazine hydrate (0.15 g, 0.14 ml, 0.003 mol) in absolute ethanol (25 ml) was heated under reflux for 8 h on boiling water bath. The resulting solution was concentrated and cooled to room temperature during which a crystalline white precipitate separated out. The solid obtained was filtered, washed with cold aqueous ethanol, and recrystallized from a mixture of DMF and methanol.

IR: 1663 cm^{-1} (C=O stretching); ^{1}H-NMR δ: 9.3 (bs, 2H, CONH), 7.2–7.4 (m, 10H, Ar–H), 4.0 (s, 4H, NH_2), 3.8 (s, 4H, SCH_2), 2.5 (t, 4H, C_1 and C_4 methylene protons of butyl chain), 1.47 (t, 4H, C_2 and C_3 methylene protons of butyl chain); ^{13}C-NMR δ: 166.01 (C=O), 155.18 (C_3 of triazole), 149.40 (C_5 of triazole), 132.12 (C_1 of phenyl), 129.16 (C_2 and C_6 of phenyl), 128.31 (C_3 and C_5 of phenyl), 126,87 (C_4 of phenyl), 34.24 (S-CH_2), 32.90 (C_2 and C_3 of butyl chain), 25.33 (C_1 and C_4 of butyl chain); MS *m/z*: 553 $[M + H]^+$ (100).

1,4-bis(5[hydrazinocarbonylmethylthio]-4-p-tolyl-1,2,4 triazol-3-yl)butane ***6b***

The experimental procedure was repeated as with **6a** using **5b** (0.61 g, 0.001 mol) and hydrazine hydrate (0.15 g, 0.14 ml, 0.003 mol) in absolute ethanol (25 ml).

IR: 1667 cm^{-1} (C=O stretching); ^{1}H-NMR δ: 9.33 (bs, 2H, CONH), 7.1–7.5 (m, 8H, Ar–H), 4.3 (s, 4H, NH_2), 3.8 (s, 4H, SCH_2), 2.52 (t, 4H, C_1 and C_4 methylene protons of butyl chain), 2.32 (s, 6H, tolyl CH_3), 1.47 (t, 4H, C_2 and C_3 methylene protons of butyl chain); ^{13}C-NMR δ: 166.20 (C=O), 155.28 (C_3 of triazole), 149.50 (C_5 of triazole), 126.9 (C_1 of phenyl), 128.24 (C_2 and C_6 of phenyl), 130.31 (C_3 and C_5 of phenyl), 139.73 (C_4 of phenyl), 34.20 (S-CH_2), 32.85 (C_2 and C_3 of butyl chain), 25.35 (C_1 and C_4 of butyl chain), 20.7 (tolyl CH_3); MS *m/z*: 581 $[M + H]^+$ (100).

1,4-bis(5[hydrazinocarbonylmethylthio]-4-m-tolyl-1,2,4 triazol-3-yl)butane ***6c***

The method described as in the preparation of **6a** was followed using **5c** (0.61 g, 0.001 mol) and hydrazine hydrate (0.15 g, 0.14 ml, 0.003 mol) in absolute ethanol (25 ml).

IR: 1677 cm^{-1} (C=O stretching); ^{1}H-NMR δ: 9.34 (s, 2H, CONH), 7.1–7.5 (m, 8H, Ar–H), 4.32 (s, 4H, NH_2), 3.78 (s, 4H, SCH_2), 2.51 (t, 4H, C_1 and C_4 methylene protons of butyl chain), 2.30 (s, 6H, tolyl CH_3), 1.45 (t, 4H, C_2 and C_3 methylene protons of butyl chain); ^{13}C-NMR δ: 166.26 (C=O), 153.15 (C_3 of triazole), 148.55 (C_5 of triazole), 126.9 (C_1 of phenyl), 124.24 (C_2 of phenyl), 138.31 (C_3 of phenyl), 129.23 (C_4 of phenyl), 128.12 (C_5 of phenyl), 126.72 (C_6 of phenyl), 34.25 (S-CH_2), 32.81 (C_2 and C_3 of butyl chain), 25.33 (C_1 and C_4 of butyl chain), 20.67 (tolyl CH_3); MS *m/z*: 581 $[M + H]^+$ (100).

1,4-bis(5[hydrazinocarbonylmethylthio]-4-p-ethoxyphenyl-1,2,4-triazol-3-yl)butane ***6d***

A mixture of **5d** (0.7 g, 0.001 mol) and hydrazine hydrate (0.15 g, 0.14 ml, 0.003 mol) in absolute ethanol (25 ml) was treated similar to the method explained as in the preparation of **6a**.

IR: 1680 cm^{-1} (C=O stretching); ^{1}H-NMR δ: 9.2 (s, 2H, CONH), 7.0–7.3 (m, 8H, Ar–H), 4.3 (s, 4H, NH_2), 4.0 (q, 4H, OCH_2), 3.8 (s, 4H, SCH_2), 2.3 (t, 4H, C_1 and C_4 methylene protons of butyl chain), 1.4 (t, 4H, C_2 and C_3 methylene protons of butyl chain), 1.3 (t, 6H, CH_3 of ethoxy group); MS *m/z*: 641$[M + H]^+$ (100).

1,4-bis(5[hydrazinocarbonylmethylthio]-4-cyclohexyl-1,2,4 triazol-3-yl)butane ***6e***

The experimental procedure described as in the preparation of **6a** was followed using **5e** (0.59 g, 0.001 mol) and hydrazine hydrate (0.15 g, 0.14 ml, 0.003 mol) in absolute ethanol (25 ml).

IR: 1650 cm^{-1} (C=O stretching); ^{1}H-NMR δ: 9.3 (s, 2H, CONH), 4.3 (s, 4H, NH_2), 3.9 (s, 4H, SCH_2), 3.60 (m, 2H, protons of C_1 of cyclohexyl), 2.44 (t, 4H, C_1 and C_4 methylene protons of butyl chain), 2.16 (m, 8H, C_2 and C_6 protons of cyclohexyl), 1.85 (m, 8H, C_3 and C_5 protons of cyclohexyl), 1.69 (m, 4H, C_4 protons of cyclohexyl), 1.4 (t, 4H, C_2 and C_3 methylene protons of butyl chain); ^{13}C-NMR δ: 166.12 (C=O), 155.05 (C_3 of triazole), 147.49 (C_5 of triazole), 55.16 (C_1 of cyclohexyl), 35.09 (S-CH_2), 33.23 (C_2 and C_3 of butyl chain), 30.71 (C_2 and C_6 of cyclohexyl), 26.11 (C_4 of cyclohexyl), 25.18 (C_1 and C_4 of butyl chain), 24.49 (C_3 and C_5 of cyclohexyl); MS *m/z*: 565 $[M + H]^+$ (76).

1,4-bis(5[hydrazinocarbonylmethylthio]-4-butyl-1,2, 4-triazol-3-yl)butane ***6f***

The method described as in the preparation of **6a** was followed using **5f** (0.54 g, 0.001 mol) and hydrazine hydrate (0.15 g, 0.14 ml, 0.003 mol) in absolute ethanol (25 ml).

IR: 1676 cm^{-1} (C=O stretching); ^{1}H-NMR δ: 9.3 (bs, 2H, CONH), 4.3 (s, 4H, NH_2), 3.9 (t, 4H, C_1 protons of *n*-butyl), 3.8 (s, 4H, SCH_2), 2.65 (t, 4H, C_1 and C_4 methylene protons of butyl chain), 1.7 (t, 4H, C_2 and C_3 methylene protons of butyl chain), 1.5 (m, 4H, C_2 protons of *n*-butyl), 1.25 (m, 4H, C_3 protons of *n*-butyl), 0.91 (t, 6H, C_4 protons of *n*-butyl); ^{13}C-NMR δ: 166.14 (C=O), 155.17 (C_3 of triazole), 148.15 (C_5 of triazole), 42.85 (C_1 of butyl), 35.03 (S-CH_2), 31.43 (C_2 and C_3 of butyl chain), 25.81 (C_2 of butyl), 23.76 (C_1 and C_4 of butyl chain), 19.13 (C_3 of butyl), 13.29 (C_4 of butyl); MS *m/z*: 513 $[M + H]^+$ (75).

The physical data of the compounds prepared is presented in Table 1.

Biological activity

In vitro cytotoxicity activity

The cytotoxicity of the compounds was evaluated in vitro against the following human cancer cell lines: A-549 lung carcinoma, HT-29 colon adenocarcinoma and MDA-MB-231 breast carcinoma. The cell lines were procured from National Centre for Cell Sciences, Pune, India, and were cultured in DMEM medium supplemented with 10% FBS, 1% L-glutamine, and 50 μg/ml gentamicin sulfate in a CO_2

incubator in a humidified atmosphere of 5%CO_2 and 95% air. The in vitro cytotoxicity was determined using a standard MTT assay (Molinari *et al*., 2009, Manjula *et al*., 2009). Briefly, the exponentially growing cells were plated in 96-well plates (10^4 cells/well in 100 µl of medium) and incubated for 24 h for attachment. The test compounds were prepared prior to the study by dissolving in 0.1% DMSO and diluted with medium. The cells were then exposed to different concentration of test compounds (10, 20, and 50 µM) in a volume of 100 µl/well. The cells in the growth control wells received only the same volume of medium containing 0.1%DMSO. After 72 h of exposure, the medium was removed and the cell cultures were incubated with 100 µl of MTT reagent (0.1%) for 4 h at 37°C. The pink colored formazan was dissolved in 100 µl of DMSO and absorbance of each well was read in an ELISA micro plate reader at 570 nm. The experiment was performed in triplicate and the percentage cytotoxicity was calculated using the following formula.

$$\% \text{ Cytotoxicity} = (\text{control abs} - \text{test abs}) \times 100/\text{control abs}.$$

The drug concentration that causes 50% cell growth inhibition after 72 h of continuous exposure to the test compounds (IC_{50}) was determined by plotting the graph of concentration of the drug against the percent cytotoxicity and performing the regression analysis. The IC_{50} values of the test compounds are shown in Table 2.

Anti-microbial activity

All the compounds were tested for in vitro anti-microbial activity against the following microorganisms: *Bacillus subtilus*, *Staphylococcus aureus* (Gram positive bacteria), *Pseudomonas aeruginosa*, *Escherichia coli* (Gram negative bacteria). The minimal inhibitory concentration (MIC) values for compounds tested, defined as the lowest concentration of the compound preventing the visible growth, were determined by using by serial dilution method (Rostom *et al*., 2009). The inocula were adjusted to 0.5 McFarland Standard (1.5×10^8 CFU/ml) were prepared from 24 h broth cultures and used for the study. The test compounds dissolved in DMSO was first diluted to the highest concentration (400 µg/ml) to be tested. Drugs (10.0 mg) were dissolved in DMSO (1 ml) and the solution was diluted with water (9 ml). Then serial 2-fold dilutions were made in concentration ranging from 6.25 to 200 µg/ml in 10-ml sterile tubes. A prepared suspension of the standard microorganisms was added to each dilution in a 1:1 ratio. Growth (or its absence) of microorganisms was determined visually after incubation for 24 h at 37°C. At the end of the incubation period, the MIC was determined, which is the lowest concentration of the test compound that resulted in no visible growth on the plate. A control test was also performed with test medium supplemented with DMSO at the same dilutions as used in the experiment to ensure that the solvent had no influence on bacterial growth. Ampicillin was used as standard drug for the comparison. The mean of the values of MIC obtained from three independent measurements is presented in Table 3.

Acknowledgments Authors are thankful to The Principal, JSS College of Pharmacy, Mysore, India for providing necessary facilities. Authors also thankful to The Director, NMR research centre, Indian Institute of Science, Bangalore for spectral data. Thanks are due to Mr. V.M. Chandrashekhar, Asst. Professor, Department of Pharmacology, HSK college of Pharmacy, Bagalkot, India for carrying out anti-cancer activity.

Conflict of interest The authors have declared no conflict of interest.

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