

Synthesis of some bis-triazole derivatives as probes for cytotoxicity study

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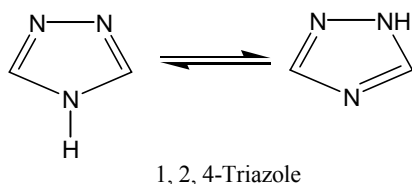
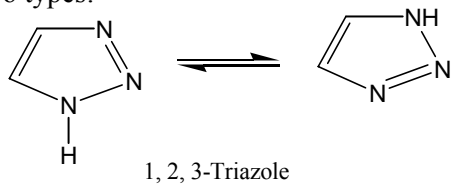
Abstract

A series of bis-[4-N-amino-5-mercapto-1,2,4-triazol-3-yl] alkanes (**1a-e**) and their Schiff bases with 2-adamanta-nine (**2a-d**) and bis - [1, 2, 4-triazolo [3, 4-b] - 1, 3, 4-thiadiazol-4-yl] alkanes (**3a-e**) have been synthesized with high yields. The cytotoxicity study of these newly synthesized compounds against brine shrimp lethality test as well as Structure-Activity-Relationship (SAR) has been discussed.

Key words: bis-triazole, cytotoxicity, shrimp

Introduction

Triazoles are five membered heterocyclic compounds having three nitrogen atoms. They are of two types:



If two triazole units are linked by carbon atoms, then they form bis-triazole. Various 1,2,4-triazols are found to be linked with diverse pharmacological activities (Hirota et al., 1991; Yale and Piala, 1966; Andotra and Sharma, 1988). The 1,2,4-triazol nucleus has been incorporated into a wide variety of therapeutically interesting drug candidates including H_1/H_2 histamine receptor blockers, cholinesterase active agents, CNS stimulants, antianxiety agents and sedative (Heindel and Reid, 1980). It was also found that the thiadiazoline nucleus which incorporates a toxophoric N-C-S linkage exhibits a large number of biological activities. A number of 1,3,4-thiadiazoline possessed antibacterial properties comparable with sulphonamide drugs (Omar and Aboul-Wafa, 1986). Subsequently, thiadiazole derivatives have found applications as antitumour

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Materials and Methods

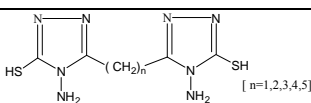
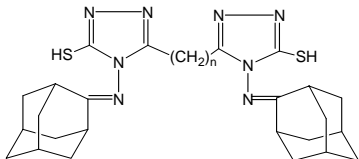
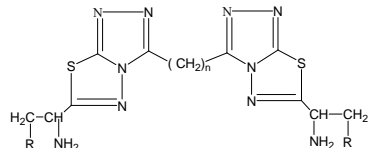
All melting points were recorded by thin disk method on a "Fischer Johns" electrothermal melting point apparatus and are not corrected. Infrared spectra were recorded on DR-8001, SHIMADZU FT-IR spectrophotometer as a solid which was finely grounded in a small agate mortar with a drop of nujol (liquid hydrocarbon) as a mull and also in KBr disk. ¹H-NMR spectra were measured by WP 400-NMR spectrometer, deuterated solvents such as dimethyl-sulphoxide (DMSO-d₆), methanol (CD₃OD) and also chloroform (CDCl₃) were used as solvents and the chemical shifts were quoted as δ-value relative to tetramethyl silane (TMS, δ = 0) as an internal standard. The ¹³C-NMR spectra were measured by WP 50 NMR spectrometer. The purity of compounds was checked by TLC on silica gel plates and iodine was used as a visualizing agent.

Bis-(4-N-amino-5-mercapto-1,2,4-triazol-3-yl) alkanes, 1a-e: A mixture of dicarboxylic acids (malonic acid, succinic acid, gluteric acid, adipic acid, palmilic acid) and thiocarbohydrazide in the ratio of 1:2 contained in a 100 ml. round-bottom flask was heated in an oil bath until the contents melted. The mixture was maintained at melting temperature for 15-20 minutes. The product obtained on cooling was treated with sodium bicarbonate solution to dissolve the unreacted dicarboxylic 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 the title compounds **1a-e** and characterized spectroscopically. The melting points, yields and IR data of the compounds, **1a-e** are given in Table 1.

Table 1 – Physical and spectral data of compounds, **1a-e**, **2a-d** and **3a-e**

Compounds	n	Yield (%)	m.p. (°C)	Nature of compounds	IR(cm ⁻¹)
1-a	1	80	240-245	White crystals	3315&3298(νN-H),2926 & 2855 (νC-H,aliphatic),2359(νS-H),1600 (νC=N).
1-b	2	85	280-282	White crystalline solid	3315&3155(νN-H),2924 & 2855(νC-H,aliphatic),2361(νS-H),1595 (νC=N).
1-c	3	75	240-242	White crystalline solid	3310&3295(νN-H),2925 & 2855(νC-H,aliphatic),2359(νS-H),1598 (νC=N).
1-d	4	80	250-252	White crystalline solid	3325&3285(νN-H), 2924& 2855 (νC-H,aliphatic),2361(νS-H),1590 (νC=N).
1-e	5	78	215-217	White crystalline solid	3244&3200(νN-H), 2928& 2856 (νC-H,aliphatic),2360(νS-H),1600 (νC=N).
2-a	1	60	265-270	White crystalline solid	2926&2855(νC-H,aliphatic),2359 (νS-H),1608(νC=N).
2-b	2	65	255-257	White crystalline solid	2924&2855(νC-H,aliphatic),2359 (νS-H),1635(νC=N).
2-c	3	75	208-210	White crystalline solid	2926&2855(νC-H,aliphatic),2359 (νS-H),1608(νC=N).
2-d	5	79	235-237	White crystalline solid	2928&2853(νC-H,aliphatic),2361 (νS-H),1615(νC=N).
3-a	1	60	210-215	Gray crystalline solid	3315(νN-H),3100(νC-H,aromatic), 2924&2855(νC-H,aliphatic), 1593(νC=N),1508 (νC=C, aromatic).
3-b	5	65	165-168	Gray crystalline solid	3240(νN-H), 3092 (νC-H,aromatic), 2952&2865(νC-H,aliphatic),1599 (νC=N),1600&1508(νC=C,aromatic).
3-c	5	74	250-252	Brown crystalline solid	3240 (νN-H), 3100 (νC-H, aromatic), 2924&2855(νC-H,aliphatic),1687 (νC=N),1600&1500(νC=C, aromatic).
3-d	1	60	210-212	Gray crystalline solid	3240(νN-H/OH),3092(νC-H, aromatic),2952&2860(νC-H, aliphatic),1599(νC=N),1600&1500 (νC=C, aromatic).
3-e	4	73	220-222	Gray crystalline solid	3395(νN-H/OH),3065(νC-H, aromatic),2945&2865(νC-H, aliphatic),1651(νC=N),1600,1558 & 1506 (νC=C, aromatic).

Table 2. Cytotoxicity study of newly synthesized compounds

Tested compounds	Concentration (µg/ml)	Percentage of mortality	LC ₅₀ (µg/ml)
	50	16.66	2.40
	100	42.86	
	150	50.00	
2-a (n=1)	50	86.15	1.20
	100	81.82	
	150	92.86	
	50	56.25	1.15
	100	85.71	
	150	92.86	
2-c (n=3)	50	69.23	1.10
	100	87.50	
	150	100.00	
2-d (n=5)	50	91.66	1.05
	100	93.33	
	150	100.00	
3-a (n=1, R=C ₆ H ₅)	50	42.85	1.40
	100	66.67	
	150	100.00	
3-b (n=5, R=C ₆ H ₅)	50	73.33	1.05
	100	100.00	
	150	100.00	
	50	66.67	1.05
	100	100.00	
	150	100.00	
3-d (n=1, R=C ₆ H ₄ OH)	50	63.64	1.10
	100	91.67	
	150	100.00	
3-e (n=4, R=C ₆ H ₄ OH)	50	53.33	1.10
	100	86.67	
	150	100.00	

Bis-[4-N-(adamantyl) imino-5-mercapto-1,2,4-triazol-3-yl] alkanes, 2a-d: A mixture of bis-[4-N-amino-1,2,4-triazol-3-yl] alkanes, **1-a**, **1-b**, **1-c** and **1-e** and 2- adamantanone in the ratio of 1:2 in dimethylformamide + ethanol (5 + 15 ml) media was heated under reflux on an oil bath for 4-5 hours after the addition of a few drops of concentrated sulfuric acid. The solid mass obtained on cooling the reaction mixture was collected by filtration and recrystallised from dimethylformamide to obtain Schiff bases, **2a-d** and characterized spectroscopically.

Bis- (6-phenylalanyl/tryptopheno/tyrosino-1, 2, 4- triazolo-[3,4-b]-1,3,4-thiadiazol-4-yl) alkanes, 3a-e: A three-necked quick fit flask was fitted with a dropping funnel and a condenser. To a mixture of 1-a/phenylalanine, 1-e/phenylalanine, 1-e/tryptophan, 1-a/tyrosine, 1-d/tyrosine and

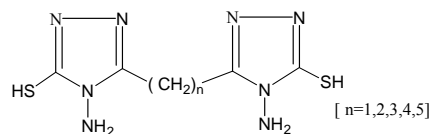
phosphorus oxychloride was added and the contents were heated under reflux for 2 hours on an oil bath. Excess of phosphorus oxychloride was then distilled off and the residue was poured onto crushed ice and stirred well. These were then washed with sodium bicarbonate solution (5%) and the resulting solids were then washed with water and recrystallised from dimethylformamide to obtain the compounds **3a-e** respectively and characterized spectroscopically.

Screening test: Because of the continuing interest of bis-triazoles derivatives, we conducted cytotoxicity investigation of the newly synthesized compounds by brine shrimp lethality bioassay and the test results show significant activity, as recorded in Table 2.

LC₅₀: The LC₅₀ of an agent is the concentration, which will kill, or inactive 50% of the test animal. LC₅₀ is inversely proportional to the toxicity of a compound, i.e. the lower is the LC₅₀, the higher is the cytotoxicity.

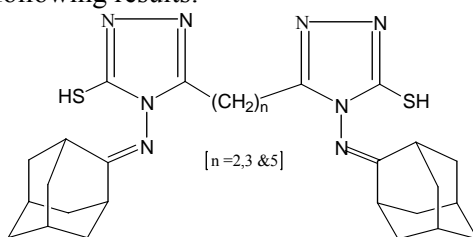
Structural-Activity-Relationship (SAR) according to the brine shrimp lethality test: The chemical structure of a drug is important as the relatively minor modification in the drug molecule may result a major change in pharmacological properties. This does not mean that changes in molecular configuration always alter all actions and effects of drug. So we have been able to recognize the functional groups/ring and determine which one is important. By synthesizing different compounds, one particular group of the molecule is removed or altered, to find out which groups are essential for biological activity and which are not. In this study we have the following results:

(1) The compounds, **1a-e** having free amino (NH₂) groups showing very little cytotoxic activity.



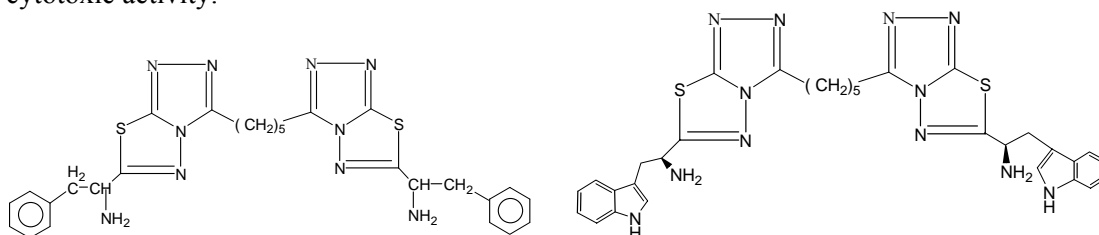
Sample: 1a-e LC₅₀ : **2.40**

(2) The Schiff bases like **2-b**, **2-c** and **2-d** have very good cytotoxic activity. That is, these compounds may act as potent cytotoxic agents. It should be mentioned here that, as the chain length increases, the cytotoxic activity also increases, i.e., the big molecule can easily interact with the DNA molecule.



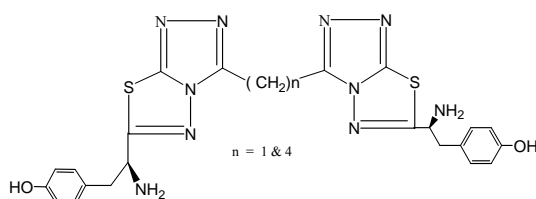
2-b: LC₅₀ =1.15 **2-c: LC₅₀ =1.10**
2-d: LC₅₀ =1.05

(3) The compounds containing amino acid moiety such as, compounds **3b-e** have very high cytotoxic activity.



3-b: LC₅₀ =1.05

3-c: LC₅₀ =1.05



3-d (n=1): LC₅₀ =1.10
3-e (n=4): LC₅₀ =1.10

Results and Discussions

All the newly synthesized compounds analyzed satisfactory for their nitrogen content. Characterization of the compounds was done on the basis of spectral analysis. The IR spectrum of compound **1-b** shown absorption bands at 3315 and 3155 cm^{-1} indicating the presence of primary amino (R-NH_2) group in the molecule. The band at 1685 cm^{-1} indicating the presence of C=N in the ring and the band at 2361 cm^{-1} corresponded the SH functional group in the molecule. In $^1\text{H-NMR}$ spectrum of the compound, **1-b** the methylene protons (CH_2) appear as a singlet at δ_{H} 5.6, relatively a higher value which may be due to the anisotropic effect of the C=N group. The singlet at δ_{H} 3.4 arises due to labile protons in NH_2 . The singlet at δ_{H} 2.5 appear due to the proton in SH. In $^{13}\text{C-NMR}$ spectrum of the synthesized compound **1-b**, clearly indicated the three signals at δ ^{13}C 21.223, 151.220 and 165.348 corresponded to the nonequivalent carbons respectively.

The IR spectrum of the Schiff base, **2-b** did not show any absorption bands corresponding to the NH stretching frequencies of the parent triazolyl-alkane, **1-b**. However, a sharp absorption band was seen around 1576 cm^{-1} , corresponding to the C=N linkage. The band at 2359 cm^{-1} corresponded to the $\nu\text{S-H}$ stretching. In the $^1\text{H-NMR}$ spectrum, the two labile protons in SH appeared as a singlet at δ_{H} 3.4, relatively a higher value which may be due to the electron withdrawing effect of nitrogen atoms (electronegativity 3.5). The CH_2 protons appeared as a triplet at δ_{H} 2.5 which may be due to the coupling with neighboring CH_2 protons. The adamantyl protons appear as multiplets at δ_{H} 2.0-1.6. In $^{13}\text{C-NMR}$ spectrum of the synthesized compound **2-b**, clearly indicated the signals at δ ^{13}C 20.397, 150.187, 165.520 and 161.626 and also δ ^{13}C 26-34 corresponded to the nonequivalent carbons respectively.

In the IR Spectrum of the synthesized compounds **3a-e**, the absorption band around at 1593

cm^{-1} come into sight due to the presence of $\nu\text{C=N}$ stretching in the ring. At 2360 cm^{-1} did not show any absorption bands corresponding to the SH stretching frequencies of the parent triazolyl-alkanes, **1a-e**, confirmed the involvement of the SH groups of the parent bis-triazoles in the cyclisation. In the IR spectra of the cyclised products, **3a-e**, the absorption band corresponding to the carbonyl stretching frequency (due to COOH groups) was absent, which again gave a conclusive evidence for the cyclisation.

In our present research work, the synthesized compounds were investigated for their property as cytotoxic agents by brine shrimp lethality bioassay. Among these compounds **2-b**, **2-c**, **2-d**, **3-b**, **3-c**, **3-d** and **3-e** were found to be very active and compounds **2-a**, **3-a** were moderately active and compounds **1a-e** shown very poor activity against brine shrimp.

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