

Synthesis and Molluscicidal Properties of Some (1*H*-1,2,4-Triazol-1-yl methyl)anilines, *N*-Alkylanilines and *N,N*-Dialkylanilines

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ABSTRACT

Six of (1*H*-1,2,4-triazol-1-ylmethyl)anilines, *N*-alkylanilines and *N,N*-dialkylanilines were synthesized and their molluscicidal properties against the land snails: white garden, *Theba pisana*, brown garden, *Helix aspersa*, and the aquatic snails, *Biomphalaria alexandrina* was investigated. Some of these compounds in particularly 4-(1*H*-1,2,4-Triazol-1-ylmethyl)-*N,N*-dimethylaniline (2e) and *N*-(1*H*-1,2,4-Triazol-1-ylmethyl)aniline (1) showed strong activity under laboratory conditions against the two types of terrestrial snails. Also, compound 2e gave reasonable activity against the aquatic, *B. alexandrina* snails. The toxicity results are discussed in relation to the chemical structure.

Key words: 1*H*-1,2,4-triazole, anilines, *N*-alkylanilines and *N,N*-dialkylanilines, molluscicidal activity, structure-activity relationship, *Theba pisana*, *Helix aspersa*, *Biomphalaria alexandrina*.

INTRODUCTION

Terrestrial snails are considered of an economic importance to man because of their damage to agricultural crops, horticulture trees, and

forestry. Furthermore, the aquatic snails are of great concern in medical and veterinary vector control due to their main role as intermediate hosts for certain parasitic worms of man and his domestic animals (Godan, 1983). Today, introduction of new molluscicides is regarded as a global goal, since the problems associated with the available molluscicides have not yet been solved (Martin, 1991). Therefore, many commercial pesticides and experimental compounds had been investigated for their molluscicidal effects under laboratory and field conditions in order to identify a potent compound with satisfactory molluscicidal activity (Miller *et al.*, 1988; Radwan *et al.*, 1992 and Schuytema *et al.*, 1994). Following this strategy, a new line of research has been recently initiated in our laboratory in order to find out a new potent and non-conventional molluscicide (El-Zemity and Radwan, 1999).

1,2,4-Triazoles constitute an important class of nitrogen heterocycles. Its representatives possess diverse types of biological activity acting as herbicides, particularly, 3-amino-1,2,4-triazole which has been widely used as the neutral herbicide and defoliant of cotton, and in low concentration it may promote growth (Polya, 1984), fungicides (Gotoda *et al.*, 1998) and insecticides (Ghorab *et al.*, 1996). Also, the antimycotic activity of some (1*H*-1,2,4-triazol-1-ylmethyl)aniline derivatives has been investigated (Scalzo *et al.*, 1989). However, trials on the molluscicidal activity of 1,2,4-triazole derivatives has not been reported and / or developed as commercial molluscicide. Thus, we have synthesized, a series of (1*H*-1,2,4-Triazol-1-ylmethyl)anilines, *N*-Alkyl anilines and *N,N*-Dialkylanilines according to our recently explored and versatile procedure (Katritzky *et al.*, 1994) in order to evaluate their possible molluscicidal activity against terrestrial snails (*Theba pisana* and *Helix aspersa*) and the fresh water snails (*Biomphalaria alexandrina*) as well under laboratory conditions, discussing the aspects of the molluscicidal structure-activity relationships.

MATERIALS AND METHODS

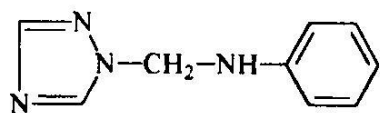
General experimental procedures

^1H NMR spectra were recorded on Varian spectrometer, 300 using TMS as an internal reference in deuterated chloroform (CDCl_3). Melting points were measured with Kofler hot stage apparatus and were uncorrected.

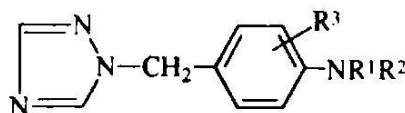
Synthesis

Compounds **2a**, **b** and **c** were obtained in good yields by refluxing the corresponding anilines (*N*-methylaniline, *N*-ethylaniline or *N*-ethyl-3-methylaniline) with 1-hydroxymethyltriazole in concentrated hydrochloric acid for 5 h. Similarly, **2d** and **2e** were prepared in 61% and 65% yields, respectively, by initial treatment of the corresponding *N,N*-dimethyl aniline and *N,N*-dimethyl-3-methylaniline with concentrated HCl to form the chlorides, then to react with 1-hydroxymethyltriazole. Use of the chloride salts, or alternatively, the free aniline or analogs in the presence of a strong acid, is very important for the triazolylalkylation.

This is especially the case where an active hydrogen (N-H) exists in the molecules, because this active hydrogen would react more easily with 1-hydroxymethyltriazole than the aromatic CH-. The N-triazolylmethyl substituted aniline (**1**) has easily been prepared by direct reaction of the aniline with hydroxymethyltriazole in EtOH. Thus, stirring a mixture of aniline with 1-hydroxymethyltriazole in ethanol at room temperature for 2 h afforded **1** in 70% yield. The structures of the triazole derivatives (**1**) and (**2**) were confirmed by their nmr spectral data.



1



2

Table (1): Preparation (1*H*-1,2,4-Triazol-1-ylmethyl)anilines, *N*-Alkyl anilines and *N,N*-Dialkylanilines (1 & 2a-e)

Compound	R ¹	R ²	R ³	Yield (%)	mp (°C)
1	-	-	-	70	oil
2a	H	Me	H	62	99-100
2b	H	Et	H	72	94-95
2c	H	Et	3-Me	63	59-60
2d	Me	Me	H	61	97-98
2e	Me	Me	3-Me	65	79-80

The methylene signals of the derivatives in ¹H nmr spectra were shifted upfield (¹H, 5.20-5.41 ppm;) compared to the signal of methylene in 1-hydroxymethyl-1,2,4-triazole (¹H, 5.47 ppm) due to the loss of the electron-withdrawing -OH group. In the cases of **2a,b** and **d**, the ¹H spectra show two symmetrical aromatic signals. This indicates that para-substituted products were obtained (Katritzky *et al.*, 1994).

This method provides a novel and efficient route for the preparation of (1*H*-1,2,4-triazol-1-ylmethyl) substituted anilines, *N*-alkylanilines and *N,N*-dialkylanilines from readily available starting materials, high yields, short reaction times, and potentially useful for the synthesis of biologically active compounds containing these functionalities.

Synthesis of *N*-(1*H*-1,2,4-Triazol-1-ylmethyl)aniline (1)

A mixture of 1-hydroxymethyl-1,2,4-triazole (94.14 g, 60 mmol) and aniline (5.58 g, 60 mmol) in aqueous EtOH (80 ml, 25%) was stirred at room temperature for 2 h, and kept at 5 °C overnight. The resultant white precipitate was filtered off. The yellow oil was separated from the aqueous media by decantation and dried in vacuo to yield the desired product (7.3 g, 70%)

Table (2): ^1H NMR Spectral Data of (1*H*-1,2,4-Triazol-1-yl-methyl)anilines, *N*-Alkyl anilines and *N,N*-Dialkylanilines (1 & 2a-e).

Comp	OH or NR ¹ R ²	R ³	ArCH ₂ N-	Other groups
1	5.68 (t, 1H, J=7.4)	-	5.47	6.65(dd, 2H, J=7.7), 6.77(t, 1H, J=7.4), 7.14(dt, 2H, J=7.4), 7.93(s, 1H), 8.18(s, 1H)
2a	2.83(s, 3H) 3.88(br s, 1H)	-	5.20	6.60(d, 2H, J=8.5), 7.13(d, 2H, J=8.5), 7.95(s, 1H), 7.97(s, 1H)
2b	1.22(t, 3H, J=7.2) 3.13(q, 2H, J=7.2) 4.05(br s, 1H)		5.20	6.57(d, 2H, J=8.5), 7.10(d, 2H, J=8.5), 7.92(s, 1H), 8.02(s, 1H)
2c	1.23(t, 3H, J=7.1) 3.12(q, 2H, J=7.1) 3.70(br s, 1H)	2.17 (s, 3H)	5.19	6.41(d, 1H, J=6.6), 6.43(s, 1H), 7.02(d, 1H, J=9.0), 7.82(s, 1H), 7.93(s, 1H)
2d	2.95(s, 6H)	-	5.21	6.68(d, 2H, J=8.0), 7.17(d, 2H, J=7.9), 7.93(s, 1H), 7.95(s, 1H)
2e	2.96(s, 6H)	2.23 (s, 3H)	5.24	6.55(m, 2H), 7.10(d, 1H, J=9.1), 7.82(s, 1H), 7.94(s, 1H)

Synthesis of p-(1*H*-1,2,4-Triazol-1-ylmethyl)-*N*-methylaniline (2a-c).

A mixture of *N*-substituted aniline (25 mmol) and 1-hydroxymethyl-1,2,4-triazole (2.47 g, 25) was heated in concentrated hydrochloric acid (25 ml) under reflux for 5 h. The resulting solution

was rendered basic with sodium hydroxide (2 N, 30 ml), extracted with ethyl acetate (3 × 150 ml) and dried with MgSO₄. The extracts were then removed in vacuo, and the residue was chromatographed with hexane and ethyl acetate (5:1). The nmr spectral data of the products are given in Table 2.

Synthesis of p-(1H-1,2,4-Triazol-1-ylmethyl)-N,N-dialkylanilines (2d-e).

A mixture of N,N-dimethylaniline (or N,N-dimethyl-3-methylaniline) (25 mmol) and concentrated HCl (25 ml) was heated under reflux for 1 h. then the 1-hydroxymethyl-1,2,4-triazole (2.47 g, 25 mmol) was added and the whole was refluxed for another 11 h. The resulting solution was rendered basic with NaOH (5 N, 30 ml), extracted with ethyl acetate (3 × 150 ml) and dried with MgSO₄. Evaporation of the solvent gave a crude product which was chromatographed with hexane and ethyl acetate (5:1). The nmr spectral data of the products are shown in Tables 1 and 2.

Molluscicidal Tests

Terrestrial snails:-

Specimens of the herbivorous snails, *Theba pisana* (Muller) and *Helix aspersa* (Muller) were collected during autumn 1998, from untreated nursery plants and farms in Alexandria Governorate, Egypt. The adult of both species were identified according to the key given by Godan (1983), and allowed to acclimatize to the laboratory conditions for three weeks and were fed on bran baits *ad libitum*.

Fresh water snails:-

The intermediate hosts for schistosomiasis (Bilharziasis) of human disease, *Biomphalaria alexandrina* snails were collected from Kafr El-Dwar, Behera Governorate during July and August of 1998, and were maintained in glass aquaria containing 5 liters of dechlorinated water supplied with vegetation and dry lettuce leaves as a food, oxygenated for one hour daily, for two weeks before testing.

Chemicals:-

Stock solutions of each compound including pure methiocarb as a reference for terrestrial snails as well as niclosamide and / or copper sulphate for the fresh water snails were prepared in dimethyl sulfoxide (DMSO), and serially diluted with the same solvent or dechlorinated water to achieve the desired concentrations.

Testing procedure:-

Topical application (Contact toxicity): The method of Hussein *et al* (1994) was used. Preliminary experiments were carried out to establish the range of dosage of the tested chemicals. Five different concentrations, ranged from 0.5 % to 2.5 % for each compound were prepared and three replicates (10 snails for each) were kept in 0.5 liter glass jar covered with cloth netting and secured with a rubber band to prevent snails from escaping. Control snails were treated with DMSO. The tested dose was gently applied on the surface of the snail body inside the shell using a micropipet contained in 30 μ l in case of *H. aspersa* and 5 μ l in case of *T. pisana*. Snails were provided with lettuce leaves to feed on after 24 hr. of treatment. Dead snails were recorded 24, 48, and 72 hr. after treatment by loss of response to a thin stainless steel needle according to the WHO procedure (1965).

Toxic baits (Stomach toxicity): The toxic bran baits 1% and 2% (w/w) of the tested 1,2,4-triazole derivatives were used for poison baits toxicity against *H. aspersa* and *T. pisana* snails. Preparation of the bran baits was carried out according to the method of El-Sebae *et al.* (1982). For each treatment, three glass jars (0.5 liter), ten adult snails per jar, were introduced and tightly covered with cloth netting secured with a rubber band. Three jars were also prepared for control group containing bran baits free of chemicals. Two ml of water were added daily into each jar to offer the suitable humidity required for snails activity. Mortality counts were recorded daily up to 7 days by checked for the dead snails which were removed.

Fresh water snails

Tests with aquatic snails, *B. alexandrina* were performed in one liter glass aquaria. Five different concentrations, ranged from 10-400 ug/ml for each compound were prepared in DMSO (10 ml), and added to the aquaria which were filled up to 500 ml of dechlorinated water to give the desired concentration (w/v). Three aquaria (10 snails for each) were set up for each concentration of each compound. Control assays with DMSO were carried out with the appropriate dilutions in water without chemicals. Death was recorded 24 hr. after exposure by the WHO procedure as mentioned above.

Statistical procedure: Percentage mortality was corrected using Abbotts formula (Abbott, 1925). Toxicity parameters for each treatment were analyzed according to probit-analysis method of Finney (1971). Analysis of variance between LD₅₀ values of various chemical structures was carried out according to Steel and Torrie (1980).

RESULTS AND DISCUSSION

The activity of the test chemicals has been evaluated against terrestrial and fresh water snails and the significant results have been compared with a reference standard molluscicide, methiocarb as well as niclosamide and/ or copper sulphate, respectively (Tables 3, 4, and 5). The molluscicidal properties of (1*H*-1,2,4-Triazol-1-ylmethyl)- anilines, *N*-Alkylanilines and *N,N*-Dialkylanilines which are presented in Table (3) against the terrestrial snails, *Helix aspersa* and *Theba pisana* by topical application showed that there are some promising compounds which could be developed in this area.

Table (3): Molluscicidal properties of six triazole derivatives against *Helix aspersa* and *Theba pisana* snails by topical application method.

Comp	<i>H. aspersa</i>		<i>T. pisana</i>	
	LD ₅₀ (ug/snail) with 95% Fiducial limits ^a	Slope ± variance	LD ₅₀ (ug/snail) with 95% Fiducial limits ^a	Slope ± variance
1	338.98 (315.74-362.93)	2.65 ± 0.068	256.00 (244.11-258.09)	2.49 ± 0.066
2a	375.74 (323.61-436.26)	1.76 ± 0.059	501.33 (490.04-673.36)	4.32 ± 0.015
2b	488.45 (249.69-693.60)	2.56 ± 0.076	189.10 (154.76-230.91)	2.09 ± 0.064
2c	392.46 (264.40-582.41)	1.30 ± 0.056	184.59 (156.26-217.94)	2.59 ± 0.074
2d	377.18 (363.38-434.76)	2.65 ± 0.070	390.69 (286.49-528.41)	4.43 ± 0.012
2e	170.22 (122.10-237.14)	1.36 ± 0.057	185.83 (162.00-213.09)	3.17 ± 0.089
Stand. ^b	210.72 (189.20-244.90)	1.19 ± 0.055	107.34 (87.83-124.35)	2.43 ± 0.062
LSD _{0.05}	81.17		137.28	

^a n = 30, in three replicates of 10 each

^b Standard compound (methiocarb)

The structure-activity relationship studies of the tested compounds revealed that the methyl substituted compounds (R³) showed maximum activity in comparison to the unsubstituted one of this series (2c versus 2b and 2e versus 2d). The methyl group in position 3 as shown in compound (2e) was the most active in the whole series and exhibited much superior activity to methiocarb against *H. aspersa* snails (LD₅₀ = 170.22 ug/snail). On the other hand, compounds 2c and 2e

showed the highest activity against *T. pisana* snails with LD₅₀ = 184.59 and 185.83 ug/snail, respectively, but less active than that of methiocarb, indicating that the methyl substituent at position 3 appears to be required for the molluscicidal activity. The compound with two methyl substituents in the amine group was more active against *T. pisana* snails compared to the compound which has a single methyl group (2d versus 2a). However, the molluscicidal properties of both compounds were approximately equal against *H. aspersa* snails. Replacement of the methyl group (R¹) as represented in compound 2a by ethyl group in compound 2b caused an increase of the molluscicidal activity against *T. pisana* snails and vice versa against *H. aspersa* snails.

Table (4) shows the comparative toxicities after 7 days of the tested chemicals as 1% and 2% bran baits against the two tested terrestrial snails.

Table (4): Toxic effect of six triazole derivatives as bran baits against *H. aspersa* and *T. pisana* snails.

compound	Mortality % $\pm$ SE ^a of snails exposed to bran baits (7 days)			
	<i>H. aspersa</i>		<i>T. pisana</i>	
	1% bait	2% bait	1% bait	2% bait
1	19.63 $\pm$ 3.15	35.89 $\pm$ 3.71	42.33 $\pm$ 4.15	53.33 $\pm$ 5.44
2a	37.78 $\pm$ 1.57	43.33 $\pm$ 7.19	54.45 $\pm$ 5.67	70.56 $\pm$ 5.15
2b	15.67 $\pm$ 1.14	33.33 $\pm$ 2.36	36.67 $\pm$ 1.95	40.00 $\pm$ 1.67
2c	0.00 $\pm$ 0.00	43.33 $\pm$ 2.70	53.33 $\pm$ 0.70	73.33 $\pm$ 1.15
2d	0.00 $\pm$ 0.00	20.00 $\pm$ 5.12	46.67 $\pm$ 0.52	63.67 $\pm$ 1.62
2e	43.67 $\pm$ 3.20	66.67 $\pm$ 0.60	78.00 $\pm$ 0.92	93.67 $\pm$ 1.42
Methiocarb ^b	68.75 $\pm$ 6.25	37.50 $\pm$ 5.40	66.67 $\pm$ 0.71	53.33 $\pm$ 1.58
LSD _{0.05}	4.43	7.58	10.15	7.56

^a n = 30, in three replicates of 10 each

^b Standard compound.

Most of the tested compounds prepared as 2% bran baits showed superior molluscicidal activity compared to the standard, methiocarb, against *H. aspersa* and *T. pisana* snails with the exception of compound 1 which showed equivalent activity.

Higher mortality percentage was observed in 2% poison baits against *T. pisana* than *H. aspersa* snails, indicating that *T. pisana* was more susceptible to the tested chemicals than *H. aspersa*. These results are in agreement with that reported by different authors: Judge, 1969; Godan, 1983 and Radwan, 1993.

Similarly, high molluscicidal activity due to the presence of methyl group in position 3 at the phenyl ring was noticed as previously mentioned in the topical application (compounds 2e and 2c). Replacing the ethyl group (R^1) with methyl, enhanced the activity against both tested snails. However, converting the monomethyl amine into dimethyl (2a versus 2d) reduced the molluscicidal activity.

The obtained results in Table (5) showed that this class of triazole derivatives have reasonable molluscicidal activity against *Biomphalaria alexandrina*. The order of activity of the compounds 1, 2a, 2d, 2c and 2e as judged by their 24 h. LC_{50} values (ug/ml) was :- 2e (88) > 1 (170) > 2d (180) > 2c (270) > 2a (520). Compound 2e was significantly the more potent derivative, followed by 1 and 2d which were not significantly different. These compounds were less potent than the standard niclosamide and/or copper sulphate. Generally these triazole derivatives seems to need more structure modification to improve their molluscicidal activity against the fresh water snails.

In conclusion, these series of 1,2,4-triazole derivatives represent a potential for a new potent molluscicides, in which methyl substituent at position 3 on the phenyl ring plays an important role and seems to be vital for the molluscicidal activity. So, compound 2e, which could be used as a good lead in this area, gave promising potential for both terrestrial and aquatic tested snails comparable to the standard.

Table (5): Molluscicidal action of six triazole derivatives against *Biomphalaria alexandrina* snails.

Comp.	LC ₅₀ (ug/ml) with 95% Fiducial limits ^a	Slope ± variance
1	170 (144.68 – 199.75)	1.91 ± 0.041
2a	520 (382.35 – 709.37)	3.46 ± 0.082
2b	NT	NT
2c	270 (254.24 – 286.78)	1.50 ± 0.056
2d	180 (162.75 – 199.20)	1.27 ± 0.073
2e	88 (79.28 - 97.84)	1.53 ± 0.032
Niclosamide ^b	0.39 (0.25- 0.59)	1.14 ± 0.090
Copper sulphate ^b	2.9 (2.32- 3.63)	1.88 ± 0.018
LSD _{0.05}	103.81	

^a n = 30, in three replicates of 10 each

^b Standard compound

N.T = Not Tested

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الملخص العربي

التخليق لبعض مركبات ١و٢و٤-ترايازول-١-يل ميثايل انيلين، ن، ن ثنائي الكيل انيلين و دراسة الخواص الالابدية لهما على القواقع

مسعد رشاد الزميتي و محمد علي رضوان
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تم تحضير ستة مركبات من مشتقات ٤.٢.١-ترايازول ودرست خواصها الالابدية ضد نوعين من القواقع الارضية هما (*Theba pisana*, *Helix aspersa*) وضد قوقع البلهارسيا *Biomphalaria alexandrina*. اظهرت بعض المركبات وبخاصة المركب (٤.٢.١-ترايازول-١-يل ميثايل) ن، ن-ثنائي ميثل انيلين و ن-(٤.٢.١-ترايازول-١-يل ميثايل) انيلين فاعلية عالية تحت الظروف المعملية ضد نوعي القواقع الارضية تحت الدراسة. ايضا اظهر المركب (٤.٢.١-ترايازول-١-يل ميثايل) ن، ن-ثنائي ميثايل انيلين نشاط ابادي مقبول ضد قوقع البلهارسيا. بالاضافة الى ذلك، ناقش البحث العلاقة بين التركيب الكيماوي والنشاط الالابدي ضد القواقع تحت الاختبار.