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Synthesis, Structure and Antimicrobial Activity of new 3- and 2- arylmethyl and arylacyl-3H[1,2,4]triazino[3,2-b]-quinazoline-2,6(1H)diones as expected as DNA fluorphores

Break L.M.[1] and Mosselhi M.A.N.[*2]
[1]Department of Chemistry, Faculty of Science (Girls), Taif University, 58- Taif, SAUDI ARABIA
[2]Dept. of Chem., Faculty of Science, Taif University,888-Taif, SAUDI ARABIA and Department of Chemistry, Cairo University, Giza, EGYPT

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Abstract

Starting from 2,3-diaminoquinazoline 3, a series of two isomers of new expected fluorescent 3- and 2-arylmethyl and arylacyl-3H[1,2,4]triazino[3,2-b]quinazoline-2,6(1H)diones 6 and 7 have been synthesized via its condensation with each of aryl pyruvic acid 4 and ethyl aroylpyruvates 5 depending upon the pH of the reaction medium. The structure of products was elucidated on the basis of their spectral, elemental analyses and alternate synthesis. The products 6 and 7 obtained are tested for antimicrobial activity and reported.

Keywords: 2,3-Diaminoquinazoline, acidity, triazinoquinazoline, aryl pyruvic acid, ethyl aroylpyruvates.

Introduction

In the broad class of heterocyclic compounds, the nitrogen heterocycles play an important role. Among them, quinazolines are most important class of compounds and have received much attention from synthetic as well as medicinal chemists, because of the diverse range of their pharmacological properties[1-5]. Moreover, quinazoline derivatives are reported to be used in several areas as materials in electronics, in electrochemistry as anticorrosive agents, as polymers or optical materials and fluorescent tags in DNA sequencing[6]. Fusedquinazolines are reported to be bioactive members, such as benzimidazo and benzothiazoloquinazoline derivatives as cytotoxic compounds with potential antitumoral[7]. Also, it was reported that benzimidazo[1,2-c]quinazoline derivatives show various therapeutic activities, such as anticancer[8] and anticonvulsants[9]. Furthermore, fluorescence is a powerful and sensitive tool for studying the biochemistry of DNA and protein–DNA interactions. Because DNA is inherently only slightly fluorescent, for most studies, highly fluorescent probes with more favorable spectral characteristics, such as fluorescein, are attached to the DNA molecule through a carbon linker[10]. So quinazoline derivatives are reported to be good DNA fluorphores[11,12].

In the present work we wish to report our attempts to synthesize new two isomers of triazinoquinazolines as expected as DNA fluorophores..

Material and Methods

All chemicals were supplied by Sigma-Aldrich and Merck (Germany). IR spectra were recorded for KBr discs on Fourier Transform infrared and Pye Unicam SP 300 Infrared Spectrophotometers, ^{1}H and ^{13}C NMR spectra were recorded in DMSO-d6 using a Varian Gemini 200 NMR Spectrometer and Bruker AM-400 spectrometers. Mass spectra were recorded on a HP-5988A mass spectrometer at 70ev. Thin layer chromatography (TLC) was performed on silica gel sheets F 1550 LS 254 of Schleicher & Schull and column chromatography on Merck silica gel 60 (particle size 0.063–0.20 mm). Melting points were measured on Gallenkamp melting point apparatus (UK) and are uncorrected. Elemental analyses were carried out at Microanalytical Centre (Cairo University, Egypt). The starting materials, 2,3-diaminoquinazoline **3**[13], arylpyruvic acid **4a,b**[14], ethyl aroylpyruvate **5c-e**[15] were prepared as in the literature reported methods.

3-Arylmethyl and arylacyl-(*3H*)[1,2,4]triazino-[3,2-b]quinazoline-2,6(*1H*)diones (6a-e). General procedure: A mixture of 2,3-diamino-4(3H)quinazolinone **3** (10 mmole) and the appropriate arylpyruvic acid **4a,b** or ethyl aroylpyruvate **5c-e** (10 mmole) was heated under reflux in 50 ml of dry pyridine or acetic acid for 3 hours and cooled . The solid precipitated was collected, washed with ethanol and crystallized from acetic acid to give pale yellow crystals of 2-arylacyl-(*3H*)[1,2,4]triazino-[3,2-b]quinazoline-2,6(*1H*)diones (**6a-e**).

3-Benzyl-(*3H*)[1,2,4]triazino-[3,2-b]quinazoline-2,6 (*1H*) dione (6a): Yield 65%, mp 290-292^{o}C. IR (KBr) ν = 3363 (N–H), 3060 (C–H aromatic), 1700, 1666 (2C= O). ^{1}H NMR (DMSO-d6) δ = 4.00 (s, 2H, CH_2), 7.20–7.80 (m, 9H, Ar-H), 12.00 (s, 1H, N–H) (D_2O exchangeable). EIMS, m/z: 304 (M^+),

Elemental analysis ($C_{17}H_{12}N_4O_2$); calcd. C, 67.11; H, 3.95; N, 18.42; Found C, 67.08; H, 3.83; N, 18.40%.

3-(4-Chlorophenyl)methyl-(*3H*)[1,2,4]triazino-[3,2-b]quinazoline-2,6 (*1H*)dione (6b) : Yield 65%, mp 200-202°C. IR (KBr) ν = 3400 (N–H), 3060 (C–H aromatic), 1715, 1655 (2C= O). ^{1}H NMR (DMSO-d6) δ = 4.00 (s, 2H, CH_2), 7.20–8.00 (m, 8H, Ar-H), 12.01 (s, 1H, N–H) (D_2O exchangeable). EIMS, m/z: 339.5 (M^++1), Elemental analysis ($C_{17}H_{11}N_4O_2Cl$); calcd. C, 60.26; H, 3.25; N, 16.54; Found C, 60.15; H, 3.10; N, 16.35%.

3-Phenacyl-(*3H*)[1,2,4]triazino-[3,2-b]quinazoline-2,6(*1H*)dione (6c): Yield 70%, mp 305-307°C. IR (KBr) ν = 3350 (N–H), 3050 (C–H aromatic), 1670, 1600 (2C= O). ^{1}H NMR (DMSO-d6): δ = 6.85 (s, 1H, =CH), 7.50–7.80 (m, 9H, Ar-H), 14.00 (s, 2H, 2N–H) (D_2O exchangeable). ^{13}C NMR (DMSO-d6): δ = 115, 122-134, 137, 145, 155, 160, 162, 164, 181. EIMS, m/z: 332 (M^+), Elemental analysis ($C_{18}H_{12}N_4O_3$); calcd. C, 61.45; H, 3.61; N, 16.87; Found C, 61.38; H, 3.58; N, 16.72%.

3-(4-Methoxy)phenacyl-(*3H*)[1,2,4]triazino-[3,2-b]quinazoline-2,6(*1H*)dione (6d): Yield 65%, mp 290-291°C. IR (KBr) ν = 3400 (N–H), 3100 (C–H aromatic), 1640, 1600 (2C= O). ^{1}H NMR (DMSO-d6) δ = 3.90 (s, 3H, OCH_3), 6.80 (s, 1H, =CH), 7.20–8.00 (m, 8H, Ar-H), 13.90 (s,2H, 2N–H) (D_2O exchangeable). EIMS, m/z: 362 (M^+), Elemental analysis ($C_{19}H_{14}N_4O_4$); calcd. C, 62.98; H, 3.87; N, 15.47; Found C, 62.81; H, 3.76; N, 15.32%.

3-(4-Bromo)phenacyl-(*3H*)[1,2,4]triazino-[3,2-b]quinazoline-2,6(*1H*)dione (6e): Yield 65%, mp 310-312°C. IR (KBr) ν= 3420 (N–H), 3090 (C–H aromatic), 1680, 1595 (2C= O). ^{1}H NMR (DMSO-d6) δ = 6.75 (s, 1H, =CH), 7.50–8.00 (m, 8H, Ar-H), 14.00 (s, 2H, 2N–H) (D_2O exchangeable). EIMS, m/z: 412 (M^++1), Elemental analysis ($C_{18}H_{11}N_4O_3Br$); calcd. C, 52.55; H, 2.68; N, 13.62; Found C, 52.20; H, 2.53; N, 13.54%.

2-Arylmethyl and arylacyl-(*3H*)[1,2,4]triazino-[3,2-b]quinazoline-2,6(*1H*)diones (7a-e). General procedure: A suspension of 2,3-diamino-4(3H)quinazolinone **3** (10 mmole) and the appropriate arylpyruvic acid **4a,b** or ethyl aroylpyruvate **5c-e** (10 mmole) was heated under reflux in 100 mL 1N hydrochloric acid for one hour and cooled . The solid precipitated was collected and purified by dissolving in 5% sodium carbonate and precipitated by addition of dilute hydrochloric acid. The purification process was repeated until a pure product of **7** (TLC) was obtained.

2-Benzyl-(*3H*)[1,2,4]triazino-[3,2-b]quinazoline-2,6(*1H*)dione (7a): Yield 65%, mp 200-201°C. IR (KBr) ν = 3360 (N–H), 3060 (C–H aromatic), 1715, 1666 (2C= O). ^{1}H NMR (DMSO-d6) δ = 4.05 (s, 2H, CH_2), 7.50–7.80 (m, 9H, Ar-H), 12.20 (s, 1H, N–H) (D_2O exchangeable). EIMS, m/z: 304 (M^+), Elemental analysis ($C_{17}H_{12}N_4O_2$); calcd. C, 67.11; H, 3.95; N, 18.42; Found C, 67.03; H, 3.13; N, 18.38%.

2-(4-Chlorophenyl)methyl-(*3H*)[1,2,4]triazino-[3,2-b]quinazoline-2,6(*1H*)dione (7b): Yield 60%, mp >300°C. IR (KBr) ν = 3350 (N–H), 3060 (C–H aromatic), 1715, 1670 (2C= O). ^{1}H NMR (DMSO-d6) δ = 4.00 (s, 2H, CH_2), 7.40–7.89 (m, 8H, Ar-H), 12.10 (s, 1H, N–H) (D_2O exchangeable). EIMS, m/z: 338 (M^+), Elemental analysis ($C_{17}H_{11}N_4O_2Cl$); calcd. C, 60.26; H, 3.25; N, 16.54; Found C, 60.12; H, 3.15; N, 16.38%.

2-Phenacyl-(*3H*)[1,2,4]triazino-[3,2-b]quinazoline-2,6(*1H*)dione (7c): Yield 65%, mp 242-244°C. IR (KBr) ν = 3250 (N–H), 3050 (C–H aromatic), 1688, 1600 (2C= O). ^{1}H NMR (DMSO-d6) δ = 6.90 (s, 1H, =CH), 7.40–8.10 (m, 9H, Ar-H), 14.00 (s, 2H, 2N–H) (D_2O exchangeable). ^{13}C NMR (DMSO-d6): δ = 115, 122-134, 137, 145, 155, 159, 161, 164, 180. EIMS, m/z: 332 (M^+), Elemental analysis ($C_{18}H_{12}N_4O_3$); calcd. C, 61.45; H, 3.61; N, 16.87; Found C, 61.29; H, 3.50; N, 16.70%.

2-(4-Methoxy)phenacyl-(*3H*)[1,2,4]triazino-[3,2-b]quinazoline-2,6(*1H*)dione (7d): Yield 65%, mp 290-291°C. IR (KBr) ν = 3300 (N–H), 3100 (C–H aromatic), 1680, 1663 (2C= O). ^{1}H NMR (DMSO-d6) δ = 3.95 (s, 3H, OCH_3), 6.85 (s, 1H, =CH), 7.30–8.00 (m, 8H, Ar-H), 12.00 (s, 1H, 1N–H), 13.50 (s,1H, 1N–H) (D_2O exchangeable). EIMS, m/z: 362 (M^+), Elemental analysis ($C_{19}H_{14}N_4O_4$); calcd. C, 62.98; H, 3.87; N, 15.47; Found C, 62.76; H, 3.69; N, 15.36%.

2-(4-Bromo)phenacyl-(*3H*)[1,2,4]triazino-[3,2-b]quinazoline-2,6(*1H*)dione (7e): Yield 60%, mp 235-236°C. IR (KBr) ν = 3350 (N–H), 3055 (C–H aromatic), 1690, 1650 (2C= O). ^{1}H NMR (DMSO-d6) δ = 6.90 (s, 1H, =CH), 7.30–8.10 (m, 8H, Ar-H), 14.00 (s, 2H, 2N–H) (D_2O exchangeable). EIMS, m/z: 412 (M^++1), Elemental analysis ($C_{18}H_{11}N_4O_3Br$); calcd. C, 52.55; H, 2.68; N, 13.62; Found C, 52.31; H, 2.50; N, 13.50%.

Results and Discussion

The nature of the products obtained from the condensation of 1,2-diaminoheterocycles with β–ketoacids or β–ketoesters was reported to be dependent on the acidity of the reaction[13,16-20] . Since, it has been reported[21-24] that the reaction of 2,3-diaminopyridine with ethyl benzoylpyruvate **5a** afforded two isomers **8** and **9** (chart 1) when carried out in aqueous sulfuric acid and acetic acid solution respectively.

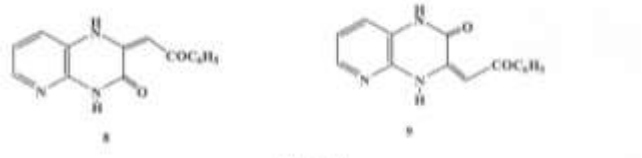
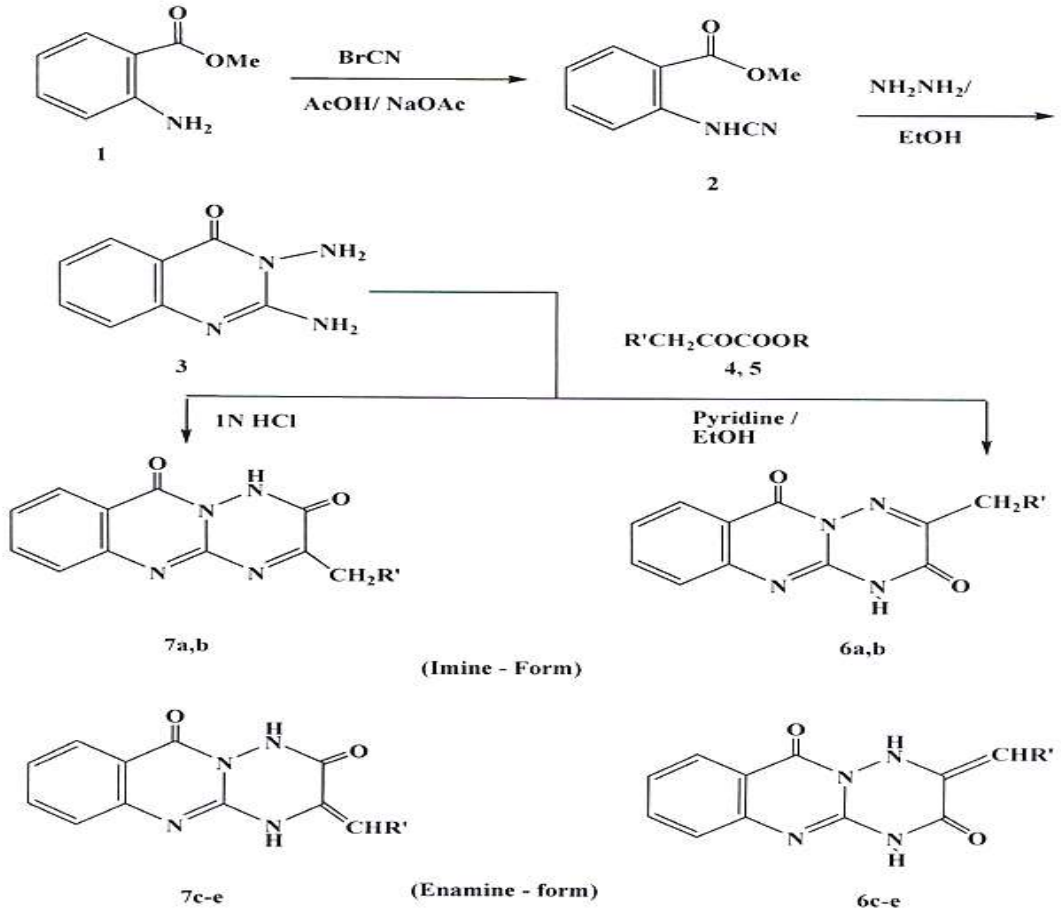


Chart-1
Two isomers of reaction of 2,3-diaminopyridine with ethyl benzoylpyruvate, 8 and 9

It was considered of interest to examine the reaction products of 2, 3-diaminoquinazoline **3** with arylpyruvic acid **4a,b** and ethyl aroylpyruvate **5c-e**. The starting , 2,3-diaminoquinazoline **3**[13] required in this study was prepared as reported in scheme 1 by reaction of methyl anthranilate **1** with cyanogen bromide to give **2** and followed by heating in hydrazine hydrate. In our hands the condensation of 2,3-diaminoquinazoline **3** with each of **4a,b** and **5c-e** gave the product isomers **6** when it was carried out in pyridine. On the other hand, isomers **7** were obtained when the reaction was carried out in 1N hydrochloric acid solution (scheme 1).

It might that this finding is dependent upon the difference in acidity of amino groups at positions 2 and 3[25] hence the 3-amino group is more basic than 2-amino group. So in basic medium the condensation occurred with the 3-amino group to give **6**. While in acidic medium the 3-amino group is protonated and the condensation occurred with the 2-mino group to afford the other isomers 7.



1 2 3 7a,b 6a,b 7c-e 6c-e

	6, 7	R	R'
4	a	H	C_6H_5
	b	H	C_6H_4-Cl-4
5	c	Et	COC_6H_5
	d	Et	COC_6H_5-OCH_3-4
	e	Et	COC_6H_5-Br-4

Scheme-1

Synthesis of 3- & 2- arylmethyl and arylacyl *3H*[1,2,4]triazino[3,2-b]quinazoline-2,6(*1H*)diones

The structures of the products **6** and **7**, were confirmed by comparison of **6a** with an authentic sample prepared by reaction of 3-methylthio-6-benzyl-1,2,4-triazin-5(*2H*)-one with anthranilic acid[26]. Both samples of **6a** were found to be identical in all respects (mp., mixed mp., ir, ^{1}H nmr). On the other hand, the authentic substance **6a** was not identical in all respects (mp., mixed mp., ir, ^{1}H nmr) with the other isomer 2-benzyl-3*H*-[1,2,4]triazino[3,2-b]quinazolin-3,6-dione **7a** (See Experimental). According to ^{1}H nmr study of the products **6a-e** and **7a-e** we have found that the products **6a,b** and **7a,b** are present in their imine forms as their spectra revealed the presence of CH_2 proton signal at $\delta = 4.00$ and the absence of =CH protons in their ^{1}H nmr. The products **6c-e** and **7c-e** are present in their enamine forms (Chart 2), however. This is because the spectra of the products **6c-e** and **7c-e** have no signals of CH_2 protons while exhibit signals of =CH protons at δ = 6.75-6.90 and signals of the 2-NH protons. The latter signals disappeared upon exchange with deuterium oxide. As shown in experimental the signals of =CH and NH protons of enamine structures of **6c-e** and **7c-e** appeared at down field. Furthermore, the ^{13}C NMR in DMSO-d6 of **6c** revealed no peak corresponding to CH_2, while the observed peaks at δ = 115, 122-134, 137, 145, 155, 160, 162, 164, 181 correspond the structure **6c**. This indicates the products **6c-e** and **7c-e** have the chelated Z-configurations. Such findings are similar to those reported for the products of the reaction of 2,3-diaminopyridine with dicarbonyl compounds[24].

Antimicrobial Activity: Ten products, **6a-e** and **7a-e** were evaluated for their antibacterial and antifungal activities against three bacteria species namely *Escherichia coli* EC, *Pseudomonas aeruginosa* PA and *Staphylococcus aureus* SA as well as three fungal species, namely *Aspergillus fumigatus* AF, *Aspergillus niger* AN and *Candida albicans* CA.

The antibacterial and antifungal activity were carried out in the Microbiology Division of Microanalytical Center of Cairo university, using the diffusion plate method[27,28] a bottomless cylinder containing a measured quantity (1mI, mg/mL) of the sample is placed on (9 cm diameter) containing a solid bacterial medium (nutrient agar broth) or fungal medium (Dox`s medium) which has been heavily seeded with the spore suspension of the test organism. After incubation (24 h for bacteria and 5 days for fungi), the diameter of the clear zone of inhibition surrounding the sample is taken as measure of the inhibitory power of the sample against the particular test organism.

Most of the compounds were tested *in vitro* against gram negative bacteria [Escherichia coli (EC) and Pseudomonas aeruginosa (PA)], gram positive bacteria [Staphylococcus aureus (SA)] and antifungal activity against Aspergillus fumigatus (AF), Aspergillus niger (AN) and Candida albicans (CA). The reference antibiotics Ampicillin and Tetracycline were used as references to evaluate the potency of the tested compounds under the same condition. The test results are depicted in table 1 on the following basis: The solvent used was dimethylsulfoxide and concentration of the sample in 100 µg/ml.

The results indicate that compounds tested have moderate degree of inhibition against the bacteria species and exhibited no activity against fungal species.

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Conclusion

In this report, a simple method for the synthesis of a series 3- & 2- arylmethyl and arylacyl-*3H*[1,2,4]triazino[3,2-*b*]quinazoline-2,6(*1H*)diones depending upon pH value of the reaction medium. The structure of products was elucidated on the basis of their spectral, elemental analyses and alternate synthesis. Their spectral data indicate 3- and 2- arylmethyl-*3H*[1,2,4]triazino[3,2-b]quinazoline-2,6(*1H*)diones exist predominantly in the imine forms **6a,b** and **7a,b**, while 3- and 2-arylacyl-*3H*[1,2,4]triazino[3,2-b]quinazoline-2,6(*1H*) diones exist in enamine forms **6c-e** and **7c-e**. In addition, the antimicrobial activity of some of the products showed moderate activities against the bacteria species.

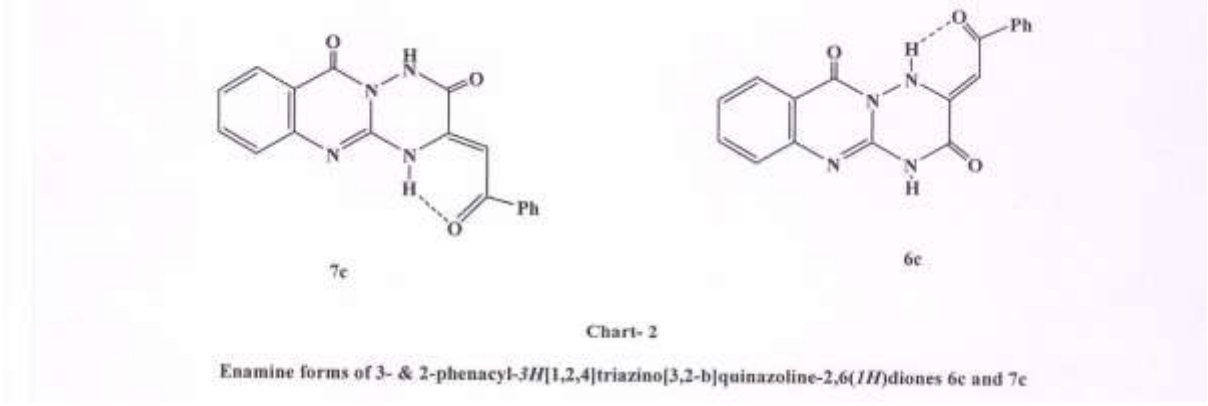

Chart- 2

Enamine forms of 3- & 2-phenacyl-*3H*[1,2,4]triazino[3,2-b]quinazoline-2,6(*1H*)diones 6c and 7c

Table-1

Antibacterial and Antifungal Activities of the Synthesized Compounds **6a-e** and **7a-e**
Inhibition Zone Diameter (IZD*) (mm/mg Compound Tested)

Compound No.	(EC) G^-	(PA) G^-	(SA) G^+	(AF) Fungus	(AN) Fungus	(CA) Fungus
Control: DMSO	0.0		0.0	0.0		0.0
Tetracycline Antibacterial agent	28 +++	28 +++	26 +++	-	-	-
Amphotericin B Antifungal agent	-	-	-	16 ++	15 ++	15 ++
6a	25 +++	16 ++	22 ++	00	00	00
6b	16 ++	15 ++	16 ++	00	00	14 ++
6c	22 ++	14 ++	15 ++	00	00	00
6d	19 ++	11 ++	14 ++	00	00	00
6e	14 ++	12 ++	15 ++	00	00	00
7a	12 ++	18 ++	20 ++	00	00	00
7b	14 ++	17 ++	15 ++	00	00	00
7c	17 ++	12 ++	16 ++	00	00	00
7d	11 ++	15 ++	11 ++	00	00	00
7e	14 ++	20 ++	18 ++	00	00	00

ZD = 2-10 mm beyond control = + (low activity).
:D = 11-24 mm beyond control = ++ (moderate activity).
:D = 25-35 mm beyond control = +++ (high activity)

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