Arch Pharm Res Vol 29, No 1, 46-49, 2006

Archives of Pharmacal Research
http://apr.psk.or.kr

A One-Step Synthesis and Antimicrobial Activities of New Substituted Dihydro-1,3,4-Thiadiazoles

Asma M. Mahran and Nasser A. Hassan
Department of Photochemistry, National Research Centre, Dokki, Cairo, Egypt

(Received November 17, 2004)

In this study, 2-*N*-arylimino-2,3-dihydro-1,3,4-thiadiazoles derivatives (**6a-h**) were synthetized. The mechanism of the studied reactions was discussed. The chemical structures of the compounds were elucidated by their IR, ^{1}H-NMR, ^{13}C-NMR, and Mass spectral data and elemental analyses. The compounds were tested for antimicrobial activity using diffusion agar technique.

Key words: Hydrazonoyl halides, Cyanothioformamides, Thiadiazoles, Antimicrobial activity

INTRODUCTION

Hydrazonoyl halides are highly versatile intermediates for synthesis of a variety heterocyclic incorporating different functionalities (Shawali, 1993). 1,3,4-Thiadiazoles represent an important heterocyclic system due to their pharmacological activity (Zaidi *et al.*, 1977; Antonardi *et al.*, 1992). They were found to have antihypertensive, anticonvulsive activities (Chapleo *et al.*, 1988), antibacterial (Helmut *et al.*, 1975), antifungal (Gulerman *et al.*, 2001), and biological activities (Andotra *et al.*, 1993), also some 1,3,4-thiadiazole have industrial importance (Miyake *et al.*, 1970), act as semiconductors (Schneider *et al.*, 1980). Recently, we reported (Al-Masoudi *et al.*, 1998; El-Gazzar *et al.*, 2002) the synthesis of 1,3,4-thiadiazols and (glucosylimino)-1,3,4-thiadiazoles formed by the reaction of 1-aza-2-azoniallene salts with alkylisothiocyanates and 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate respectively. These cycloaddition could have occurred either across the C=S double bond to give the 2,3-dihydo-1,3,4-thiadiazole or across the C=N bond with the formation of an isomeric 4,5-dihydro-1*H*-1,3,4-triazole-5-thione in a competitative manner under [3+2] cycloaddition reactions.

As a part of a program directed for developing new biologically active compounds (Shawali *et al.*, 2004), it is reported here on the utility of hydrazonoyl halides and cyanothioformamides as candidates for a facile synthetic route to heterocyclic substituted 1,3,4 thiadiazoles.

Correspondence to: Asma M. Mahran, Department of Photochemistry, National Research Centre, Dokki, Cairo, Egypt.
Tel: (+2010) 4095333, Fax: (+202) 3370931
E-mail: Dr asma chem@Hotmail.com

EXPERIMENTAL

Melting points were determined on a Gallenkamp apparatus and are uncorrected. IR spectra were recorded in potassium bromide using PU 9712 spectrophotometer. ^{1}H-NMR and ^{13}C-NMR spectra were recorded using a Varian Gemini 300 spectrometer (300 MHz). Mass spectra were recorded on a 75 Kratos spectrometer. Elemental analyses were carried out at the Microanalytical Laboratory of National Research Center, Giza, Egypt. Antimicrobial activities carried out at the medical mycology lab.

Synthesis of compounds 6a-h

General procedure

To a stirred ethanolic sodium ethoxide solution, prepared from sodium metal (0.23 g, 10 mg atom) and absolute ethanol (30 mL) was added cyanothioformamide (**2**) (10 mmol). To the resulting solution was added the appropriate hydrazonoyl chloride (**1**) (10 mmol) portionwise while stirring the mixture. After the addition was complete, the reaction mixture was refluxed for 2-4 h then cooled. The solid that precipitated was filtered off, washed with water, air dried and finally crystallized from the appropriate solvent to give the respective thiadiazoles (**6a-h**). The compounds prepared together with their physical constants are listed below.

3-Phenyl-5-acetyl-2-*N*-phenylimino-2,3-dihydro-1,3,4-thiadiazole (6a)

(2.32 g, 80%) as orange crystals; m.p. 112°C (MeOH) [Lit. m.p. 111-113°C (Zohdi *et al.*, 1998)]; IR (KBr) υ_{max} (cm^{-1}): 1682(C=O, acetyl); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 3.14(3H, s, $\underline{CH_3}$), 7-7.6 (10H, m, $\underline{Ar\text{-}H}$). ^{13}C-NMR (75 MHz,

DMSO-d_6) δ (ppm): 24.9, 120.2, 123.5, 124.4, 127.6, 129.0, 129.9, 138.3, 145.5, 150.9, 155.4, 189.5; Ms *m/z* (%), 295 (M^+, 79), 252 (2), 225 (6), 135 (5), 118 (100), 91 (83), 77 (46); Anal. found C, 65.12; H, 4.40; N, 14.24; S, 10.88. Calcd. for $C_{16}H_{13}N_3OS$ (295.1) C, 65.11; H, 4.40; N, 14.23;S, 10.86%.

3-Phenyl-5-acetyl-2-*N*-(1-methylphenylimino)-2,3-dihydro-1,3,4-thiadiazole (6b)

(2.44 g, 79%) as orange crystals; m.p. 110°C (EtOH); IR (KBr) υ_{max} (cm^{-1}), 1688 (C=O, acetyl); ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.12 (3H, s, $\underline{CH_3}$), 2.60 (3H, s, $\underline{CH_3}$), 2.60 (3H, s, $\underline{CH_3}$), 6.9-8.05 (9H, m, Ar-H); MS *m/z* (%), 309 (M^+, 9), 266 (2), 239 (3), 149 (3), 118 (74), 91 (106), 77 (40); Anal. found C, 66.07; H, 4.84; N, 13.59; S, 10.37. Calcd. for $C_{17}H_{15}N_3OS$ (309.1) C, 66.05; H, 4.85; N, 13.58; S, 10.37%.

3-Phenyl-5-acetyl-2-*N*-(*p*-methoxyphenylimino)-2,3-dihydro-1,3,4-thiadiazole (6c)

(2.47 g, 76%) as brown crystals; m.p. 122°C (EtOH); IR (KBr) υ_{max} (cm^{-1}): 1676 (C=O, acetyl); ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.59 (3H, s, $\underline{CH_3}$), 3.80 (3H, s, $\underline{CH_3}$), 6.88-7.99 (9H, m, Ar-H); MS *m/z* (%), 325 (M^+, 40), 282 (2), 257 (2), 224 (10), 118 (100), 91 (90), 77 (57); Anal. found C, 62.76; H, 4.62; N, 12.91; S, 9.90. Calcd. for $C_{17}H_{15}N_3O_2S$ (325.2) C, 62.77; H, 4.61; N, 12.91; S, 9.85%.

3-Phenyl-5-acetyl-2-*N*-(4-chlorophenylimino)-2,3-dihydro-1,3,4-thiadiazole (6d)

(2.63 g, 80%) as golden crystals; m.p. 113°C (EtOH); IR (KBr) υ_{max} (cm^{-1}): 1678 (C=O, acetyl); ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.60 (3H, s, $\underline{CH_3}$), 6.95-7.95 (9H, m, Ar-H), ^{13}C-NMR (DMSO-d_6) δ (ppm): 24.9, 122.2, 123.6, 127.7, 128.2, 129.0, 129.8, 138.2, 146.7, 149.6, 158.0, 189.4; MS *m/z* (%), 331 (M+2, 12), 329 (M^+, 30), 286 (2), 259 (1), 169 (5), 118 (92), 91 (100), 77 (9); Anal. found C, 58.40; H, 3.62; N, 12.76; S, 9.75. Calcd. for $C_{16}H_{12}N_3OSCl$ (329) C, 58.40; H, 3.64; N, 12.76; S, 9.74 %.

Ethyl-3-phenyl-2-*N*-(phenylimino)-2,3-dihydro-1,3,4-thiadiazole-5-carboxylate (6e)

(2.53 g, 78%) as yellow crystals; m.p. 98°C(MeOH) [Lit. m.p. 96-98°C (Zohdi *et al.*, 1998)]; IR (KBr) υ_{max} (cm^{-1}) : 1715(C=O, ester); ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.41 (3H, t, CH_2-$\underline{CH_3}$), 4.43 (2H, q, $\underline{CH_2}$-CH_3), 7.01-7.99 (10H, m, Ar-H); MS *m/z* (%) 325 (M^+, 81), 252 (2), 225 (9), 135 (32), 118 (16), 91 (100), 77 (61); Anal. found C, 62.62; H, 4.63; N, 12.82; S, 9.80. Calcd. for $C_{17}H_{15}N_3O_2S$ (325.39) C, 62.75; H, 4.65; N, 12.91; S, 9.85%.

Ethyl-3-phenyl-2-*N*-(*O*-methylphenylimino)-2,3-dihydro-1,3,4-thiadiazole-5-carboxylat (6f)

(2.54 g, 75%) as yellow crystals; m.p. 92°C (MeOH); IR (KBr) υ_{max} (cm^{-1}): 1741 (C=O, ester); ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.37 (3H, t, CH_2-$\underline{CH_3}$), 2.12 (3H, s, $\underline{CH_3}$), 4.4 (2H, q, $\underline{CH_2}$-CH_3), 6.92-8.02 (9H, m, Ar-H); ^{13}C-NMR (DMSO-d_6) δ (ppm): 15.9, 55.2, 62.7, 114.9, 121.2, 123.5, 127.5, 129.0, 137.9, 138.4, 144.1, 154.6, 156.1, 158.3; MS *m/z* (%) 339 (M^+, 60), 266 (2), 239 (10), 149 (2), 91 (100), 77 (20); Anal. Found C, 63.76; H, 5.02; N, 12.38; S, 9.44. Calcd for $C_{18}H_{17}N_3O_2S$ (339.1). C, 63.75; H, 5.01; N, 12.38, S, 9.45%.

Ethyl-3-phenyl-2-*N*-(*p*-methoxyphenylimino)-2,3-dihydro-1,3,4-thiadiazole-5-carboxylate (6g)

(2.84 g, 80%) as yellow crystals; m.p. 90°C (MeOH); IR (KBr) υ_{max} (cm^{-1}): 1715(C=O, ester); ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.04 (3H, t, CH_2-$\underline{CH_3}$), 3.80 (3H, s, $\underline{CH_3}$), 4.40 (2H, q, $\underline{CH_2}$-CH_3), 6.91-7.9 (9H, m, Ar-H); MS *m/z* (%) 355 (M^+, 60), 282 (1), 255 (2), 165 (45), 118 (2), 91 (100), 77 (46); Anal. Found. C, 60.88; H, 4.78; N, 11.83; S, 9.11. Calcd for $C_{18}H_{17}N_3O_3S$ (355.2). C, 66.86; H, 4.78; N, 11.82; S, 9.02%.

Ethyl-3-phenyl-2-*N*-(*p*-chlorophenylimino)-2,3-dihydro-1,3,4-thiadiazole-5-carboxylate (6h)

(2.87 g, 80%) as yellow crystals; m.p. 110°C (MeOH); IR (KBr) υ_{max} (cm^{-1}): 1752 (C=O, ester); ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.38 (3H, t, CH_2-$\underline{CH3}$), 4.43 (2H, q, $\underline{CH_2}$-CH_3), 7.0-7.9 (9H, m, Ar-H); MS *m/z* (%), 361 (M+2, 2), 359 (M^+, 5), 345 (60), 286 (5), 224 (10), 176 (50), 91 (100), 77 (20); Anal. Found C, 56.82; H, 3.89; N, 11.89; S, 8.91. Calcd for $C_{17}H_{14}N_3O_2SCl$ (359.2): C, 56.84; H, 3.89; N, 11.99; S, 8.92%.

Antimicrobial assay

Cultures of four fungal species *namely Aspergillus fumigatus* (AF), *Penicillium italicum* (PI), *Syncephalastrum racemosum* (SR), and *Candida albicans*. (CA) as well as four bacterial species namely *Staphylococcus aureus* (SA), *Pseudomonas aeruginosa* (PA), *Bacillus subtilis* (BS), and *Escherichia coli* (EC) were used to investigate the antimicrobial activity of the compounds **6a-h**. The antimicrobial activity was assayed biologically using the diffusion plate technique. The latter technique was carried out by pouring a spore suspension of the fungal species (one ml of sterile water contains approximately 10^8 conidia) or spreading bacterial suspension over a solidified malt agar medium. The layer is allowed to set for 30 min. A solution of the test compound **6** (5, 2.5, and 1 mg/mL) in chloroform was placed onto sterile 5 mm filter paper discs and allowed to dry, then the discs were placed on the centre of the malt agar plate and incubated at optimum incubation temperature 28 ± 2°C. Test organism growth may be affected by the inhibitory action of the test compound, so a clear zone around the disc appears as an indication of

Table I. Antimicrobial activity of the products **6a-h**

Sample	6a			6b			6c			6d			6e			6f			6g			6h			St.		
Conc.	5	2.5	1	5	2.5	1	5	2.5	1	5	2.5	1	5	2.5	1	5	2.5	1	5	2.5	1	5	2.5	1	5	2.5	1
	Mg/mL			mg/mL			mg/mL			mg/mL			mg/mL			mg/mL			mg/mL			mg/mL			mg/mL		
AF	+	+	0	+	+	+	++	+	+	++	++	++	+	0	0	0	0	0	+	0	0	0	0	0	+++	+++	++
PI	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+	0	0	0	0	+	0	0	+++	+++	++
SR	+	0	0	+	+	0	+	+	+	0	0	0	0	0	0	+	+	0	+	+	0	0	0	0	+++	+++	+++
CA	+	+	+	+	0	0	0	0	0	0	0	0	++	++	+	++	++	+	+	+	0	+	+	0	++	++	++
SA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+	0	+	0	0	0	0	0	++	++	++
PA	+	+	0	0	0	0	+	0	0	+	+	0	0	0	0	0	0	0	+	+	0	0	0	0	+++	+++	++
BS	0	0	0	+	+	+	+	0	0	+	+	+	+	0	0	+	+	+	+	0	0	0	0	0	+++	+++	++
EC	+	0	0	+	+	+	0	0	0	+	0	0	+	0	0	+	+	+	0	0	0	+	0	0	++	++	++

The organisms were tested against the activity of different concentrations of the sample.

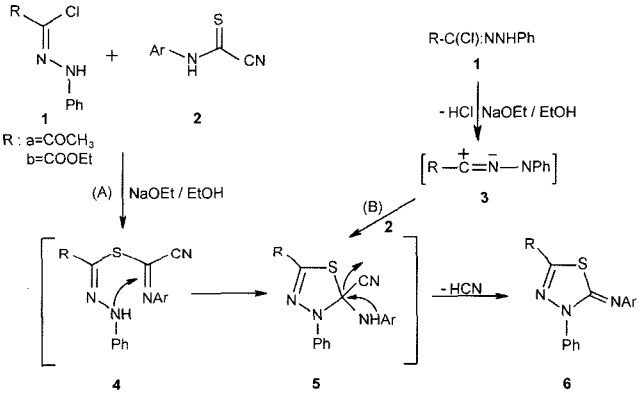
AF =Aspergillus fumigatus
PI =Pencillium italicum
SR =Syncephalastrum racemosum
CA =Candida albicans
SA =Staphylococcus aureus
PA =Pseudomonas aeruginosa
BS =Bacillus subtillis
EC =Escherichia coli

St. =Reference standard; Chloramphenicol was used as a standard antibacterial agent and Terbinafin was used as standard antifungal agent.
The test was done using the diffusion agar technique.
Well diameter: 0.6 cm .....(100 uL of each conc. Was tested).
Inhibition values = 0.1- 0.5 cm beyond control = + ; Inhibition values = 0.6-1.0 cm beyond control= ++
Inhibition values = 1.1- 1.5 cm beyond control = +++ ; 0 = Not detected

the inhibition of test organism growth. The size of the clearing zone is proportional to the inhibitory action of the compound. The fungicide Terbinafin and the bactericide Chloramphenicol were used as standards under the same conditions. Measurements were considered after 72 h for fungi and 24 h for bacteria. The results are summarized in Table I.

RESULTS AND DISCUSSION

In addition of our interest in the synthesis of novel polyfunctionalized heterocycles of biological importance (Abdel-Megeid *et al.*, 1998) we report here a facile one-pot synthesis of the title compounds **6a-h**. The required cyanothioformamides (**2a-d**) and hydrazonoyl chloride (**1a-b**) were prepared by literature methods (Walter *et al.*, 1966; Lozinskii *et al.*, 1967). Treatment of cyanothioformamides (**2a-d**) with hydrazonoyl chlorides (**1a-b**) in the presence of sodium ethoxide in refluxing ethanol for 2-4 h afforded a single product as evidenced by TLC. The structure of the isolated products was established by analytical and spectroscopic data [IR, ^{1}H-NMR, ^{13}C-NMR, MS] and identified as 3-phenyl 5-subistituted 2-*N*-(arylimino)-1,3,4-thiadiazoles (**6a-h**) (Scheme 1), for example the IR spectra of each of the compounds **6a-h** are characterized by the absence of the nitrile absorption band around 2225 cm^{-1} and NH absorption band in the region 3271 cm^{-1} respectively, also the IR of **6a-d** revealed the acetyl carbonyl band near 1680, ester carbonyl band near 1715-1735 cm^{-1} for **6e-h** and C=N band at 1630 cm^{-1}, also the mass spectra of each of the products **6a-h** exhibited a molecular ion beak of high intensity, for example Ms *m/z* (%) for **6a** M$^+$ = 295 (79), **6e** M$^+$ = 395 (81)% (c.f. experimental). Furthermore the ^{1}H-NMR spectra of **6** are characterized by the absence of exchangeable protons and the appearance of the expected resonance signals of the aliphatic protons



6	R / Ar
a	$COCH_3$ / Ph
b	$COCH_3$ / 1-MeC_6H_4
c	$COCH_3$ / 4-$MeOC_6H_4$
d	$COCH_3$ / 4-ClC_6H_4
e	$COOCH_2CH_3$ / Ph
f	$COOCH_2CH_3$ / 1-MeC_6H_4
g	$COOCH_2CH_3$ / 4-$MeOC_6H_4$
h	$COOCH_2CH_3$ / 4-ClC_6H_4

Scheme 1. Preparation of compounds **6a-h**

(CH_3-CO- and $COOCH_2CH_3$-) and aromatic protons. The structures **6a-h** are corroborated by ^{13}C-NMR data for some of the compounds **6a**, **6d**, and **6f** which showed the absence of the CN signals at 118 ppm and the appearance of the expected **sp** and **sp²** carbon signals. The products seemed to have the dihydrothiadiazole structure **6a-h**. To account for the formation of **6a-h** from the reaction of **1** with **2**, the reaction sequence outlined in (Scheme 1) is suggested. The product **6a-h** may result *via* elimination of hydrogen cyanide from the corresponding cycloadduct (**5**) which is formed from the acyclic hydrazon (**4**) (*Bath A*) or may be also formed *via* 1,3-dipolar cycloaddition of the nitrile imide (**3**) intermediate to C=S of cyanothioformamide (**2**) (*Bath B*) (Scheme 1).

REFERENCES

Abdel-Megeid, F. M. E., Hassan, N. A., Zahran, M. A., and Rashed, A. E., Synthesis of 5,6-dihydronaphtho [1',2':4,5] thieno[2,3-d]pyrimidines, 5,6-dihydronaphtho[1',2':4,5][3,2-e] [1,2,4]triazolo[1,5-c]pyrimidines and some of their nucleosides. *Sulfur letter.*, 21, 269-284(1998).

Al-Masoudi, N., Hassan, N. A., Al-Soud, Y. A., Schmidt, P., Gaafar A.M., Weng, M., Marino, S., Schoch, A., Amer, A., and Jochims J. C., Synthesis of *C*- and *N*-nucleosides from 1-aza-2-azoniallene and 1,3-diaza-2-azoniallene salts. *J. Chem. Soc., Perkin Trans1.*, 947-953 (1998).

Andotra, C. S., Langer, T. C., Dhan, S., and Kour, P., Synthesis of some 2,6-disubstitutedimidazo[2,1-b]-1,3,4-thiadiazoles and their biological activities. *Proc. Nalt. Acad. Sci., India,* Sect. A 63(4), 589-593 (Eng) (1993).

Antonardi, S., Bianco, A., Brufani, M., Cellai, L., Baido, G. L., Potier, E., Bonomi, L., Parfetti, S., Fiaschi, A. I., and Segre, G., Acetazolamide- like carbonic Anhydrase Inhibitors with topical ocular Hypotensive Activity. *J. Med. Chem.*, 35, 2697-2703 (1992).

Chapleo, C. B., Myers, P. L., Smith, A. C. B., Stillings, M. R., Tulloch, I. F., and Walter, D. S., Substituted 1,3,4-thiadiazoles with Anticonvulstant Activity. 4. Amidines. *J. Med. Chem.*, 31, 7-11 (1988).

El-Gazzar, A. B. A., Hegab, M. I., and Hassan, N. A., One-pot synthesis of *N*-nucleosides *via* 1,3-dipolar cycloadditions of 1-aza-2-azonialline salts to 2,3,4,6-tetra-*O*-acetyl *β*-D-glucopyranosyl isothiocyanate. *Sulfur letters.*, 25, 161-171 (2002).

Gulerman, N., Nehir, Rollas, Sevim, Erdeniz, Habibe, and Kiraz, Muammer., Antibacterial, antifungal and antimycobacterial activities of some substituted thiosemicarbazides and 2,5-disubstituted 1,3,4-thiadiazoles. *Journal of pharm. Sci.*, 26, 1-5(Eng), (2001).

Helmut, H. and Helmut, F., Bactericidal, and Fungicidal [(nitrofury)vinyl] thiadiazoles. *Basefa FA-G., Ger.,* offen 2, 329, 653 (Cl. Co 7D 417/06), 09 Jan, 1975, Appl. 09 Jan, 13pp, 1973. C. A. 189963F, 86.

Lozinskii, M. O., Kukota, S. K., and Pel'kis, P. S., Ethylarylazochloroacetates and their reactions with morpholine and hydrazine hydrate. *UKr. Khim, Zh.*, 33, 1295-1296(Russ), (1967).

Miyake, Hideo., Ogino, Yoshinori., and Yokawa, Akio., Stabilized polyamide composition. *Toyobo Co., Ltd., Japan.* 74, 10, 986(Cl. C 089, D 01*f*), 14 Mar 1974, Appl. 70.13, 562, 16 Mar (1970).

Schneider, O. and Hanack, M., Phthalocyaninatoeisen mit Pyrazin als zweizähnigen Brückenliganden. *Angew. Chem.*, 1980, 92, 391; *Int. Ed. Engl.*, 19, 392-399 (1980).

Shawali, A. S., Reaction of heterocyclic compounds with nitrilimines and their precursors. *Chem. Rev.*, 93, 2731-2777 (1993).

Shawali, A. S., Abbas, I. M., and Mahran, A. M., Facile Entries for regioselective synthesis of [1,2,4]triazolo[4,3-a]pyrimidin-5(1*H*)-ones from 2-thiouracil. *J. Iranian Chem. Soc.*, 1, 33-39 (2004).

Walter, W. and Klaus, D. Bode., Oxydationsreaktionen an Thiocarbonsäureami-den mit elektronenanziehenden substituenten am C-Atom. *Liebig Ann. Chem.*, 698, 131-144 (1966).

Zaidi, S. A. A., Farouqi, A. S., Varshney, D. K., Islam, V., and Siddiqi, K. S., Spectral and magnetic studies on the complexes of 2,5-dimercapto-1,3,4-thiazole(Bismuthiol 1) with Co(II), Ni(II), Zn(II), Cd(II) and Hg(II), *J. inorg. mol. Chem.*, vol. 39, pp. 581-583 (1977).

Zohdi, H. F., Rateb, N. M., Sallam, M. M. M., and Abdelhamid, A. O., Reactions with Hydrazonoyl Halides. Part 20. Synthesis of New unsymmetrical Azines, Dihydro-1,3,4-thiadiazoles and 5-Arylazo- thiazoles. *J. Chem. Research(s).*, 742-743 (1998).