ELSEVIER

Il Farmaco 57 (2002) 171–174

IL FARMACO

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Short Communication

Synthesis and antimicrobial activity of some new hydrazones of 4-fluorobenzoic acid hydrazide and 3-acetyl-2,5-disubstituted-1,3,4-oxadiazolines

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Received 25 October 2001; accepted 27 October 2001

Abstract

A series of hydrazide hydrazones and 1,3,4-oxadiazolines of 4-fluorobenzoic acid hydrazide were prepared and evaluated as potential antimicrobial agents and were tested for their antibacterial and antifungal activities against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*. From these compounds, 4-fluorobenzoic acid[(5-nitro-2-furanyl)methylene]hydrazide (**1a**) showed equal activity with ceftriaxone against *S. aureus*. In addition, the MIC values of compounds **1c**, **1d** and **2a** for the same strain were in the range of those reported for ceftriaxone according to NCCLS 1997.

Keywords: Antimicrobial activity; Hydrazones; 4-Fluorobenzoic acid hydrazide

1. Introduction

Hydrazone derivatives are reported to possess antimicrobial [1], antitubercular [2], anticonvulsant [3] and anti-inflammatory [4] activities. 3-Acetyl-2,5-disubstituted-2,3-dihydro-1,3,4-oxadiazolines are also known to exhibit antimicrobial [5,6] activity. Following these observations, we have designed and synthesized a series of 4-fluorobenzoic acid(substituted methylene) hydrazides and 3-acetyl-2,5-disubstituted-2,3-dihydro-1,3,4-oxadiazoles. These derivatives were evaluated in vitro for their antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*.

2. Experimental

2.1. Chemistry

Melting points were determined by using a Buchi-530 melting point apparatus and are uncorrected. Spectroscopic data were recorded on the following instruments: UV: Shimadzu UV 2100S spectrophotometer, IR: Perkin Elmer 1240 and 1600 FTIR spectrophotometers, ^{1}H NMR and ^{13}C NMR: Bruker AC 200L spectrometer, EI MS: VG Zabspec GC MS spectrometer. Elemental analyses were performed by Carlo Erba 1106 instrument. The purity of the compounds was confirmed by thin layer chromatography on silica gel HF_{254} (Merck).

2.1.1. 4-Fluorobenzoic acid(substituted methylene)hydrazide **1a–d**

Equimolar amounts of 4-fluorobenzoic acid hydrazide (0.005 mol) and appropriate aldehyde (0.005 mol) were refluxed in ethanol (25 ml) for 1 h. The mixture was cooled, filtered and recrystallized from ethanol.

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0014-827X/02/$ - see front matter
PII: S0014-827X(01)01192-2

*2.1.1.1. 4-Fluorobenzoic acid[(5-nitro-2-furanyl)methylene]hydrazide (**1a**).* ^{1}H NMR (DMSO-d_6) δ (ppm): 7.24 (d, 1H, $H_{5\text{-nitrofuranyl}}$ C_3–*H*, $J = 3.82$), 7.37 (t, 2H, $H_{4\text{-fluorophenyl}}$, C_3–*H*, C_5–*H*, $J = 8.69$), 7.75 (d, 1H, $H_{5\text{-nitrofuranyl}}$ C_4–*H*, $J = 3.80$), 7.99 (dd, 2H, $H_{4\text{-fluorophenyl}}$, C_2–*H*, C_6–*H*), 8.40 (s, 1H, N=C*H*), 12.13 (s, 1H, CON*H*).

*2.1.1.2. 4-Fluorobenzoic acid[(4-nitrophenyl)methylene]hydrazide (**1b**).* ^{1}H NMR (DMSO-d_6) δ (ppm): 7.38 (t, 2H, $H_{4\text{-fluorophenyl}}$, C_3–*H*, C_5–*H*, $J = 8.82$), 8.01 (t, 4H, $H_{4\text{-fluorophenyl}}$, C_2–*H*, C_6–*H* and $H_{4\text{-nitrophenyl}}$ C_2–*H*, C_6–*H*), 8.31 (d, 2H, $H_{4\text{-nitrophenyl}}$ C_3–*H*, C_5–*H*, $J = 8.69$), 8.55 (s, 1H, N=C*H*), 12.11 (s, 1H, CON*H*). EI MS [*m*/*z* (rel.%)]: 288(11), 287(48), 165(21), 139(62), 124(100), 123(62), 122(18), 121(13), 118(35), 102(7), 96(35), 95(62), 94(22), 91(17), 83(51), 77(11), 75(66), 65(6), 63(37).

*2.1.2. 3-Acetyl-5-(4-fluorophenyl)-2-substituted-2,3-dihydro-1,3,4-oxadiazole **2a**–**d***

A mixture of **1a**–**d** (0.003 mol) and acetic anhydride (6 ml) were refluxed at 140–200 °C for 45 min. The solution was poured into ice cold water and the solid was recrystallized from ethanol or ethanol/water (1/1).

*2.1.2.1. 3-Acetyl-5-(4-fluorophenyl)-2-(5-nitro-2-furanyl)-2,3-dihydro-1,3,4-oxadiazole (**2a**).* ^{1}H NMR ($CDCl_3$) δ (ppm): 2.38 (s, 3H, $COCH_3$), 6.81 (d, 1H, $H_{5\text{-nitrofuranyl}}$ C_3–*H*, $J = 3.78$), 7.08 (s, 1H, OC*H*R), 7.15 (t, 2H, $H_{4\text{-fluorophenyl}}$, C_3–*H*, C_5–*H*, $J = 8.59$), 7.30 (d, 1H, $H_{5\text{-nitrofuranyl}}$ C_4–*H*, $J = 3.69$), 7.88 (dd, 2H, $H_{4\text{-fluorophenyl}}$, C_2–*H*, C_6–*H*). EI MS [*m*/*z* (rel.%)]: 320(5), 319(28), 277(57), 165(19), 154(6), 139(12), 138(35), 137(26), 124(30), 123(100), 122(5), 121(7), 95(68), 94(7), 83(16), 79(25), 75(20), 57(7).

*2.1.2.2. 3-Acetyl-5-(4-fluorophenyl)-2-(4-nitrophenyl)-2,3-dihydro-1,3,4-oxadiazole (**2b**).* ^{1}H NMR ($CDCl_3$) δ (ppm): 2.35 (s, 3H, $COCH_3$), 7.14 (s, 1H, OC*H*R), 7.16 (t, 2H, $H_{4\text{-fluorophenyl}}$, C_3–*H*, C_5–*H*, $J = 8.71$), 7.68 (d, 2H, $H_{4\text{-nitrophenyl}}$ C_2–*H*, C_6–*H*, $J = 8.75$), 7.90 (dd, 2H, $H_{4\text{-fluorophenyl}}$, C_2–*H*, C_6–*H*), 8.27 (d, 2H, $H_{4\text{-nitrophenyl}}$ C_3–*H*, C_5–*H*, $J = 8.75$). ^{13}C NMR ($CDCl_3$) δ (ppm): 21.33 ($CO\mathit{C}H_3$), 90.91 (oxadiazoline C_2), 115.98, 116.92 ($C_{4\text{-fluorophenyl}}$, C_3, C_5), 120.12 ($C_{4\text{-fluorophenyl}}$, C_1), 124.02 ($C_{4\text{-nitrophenyl}}$ C_3, C_5), 127.23 ($C_{4\text{-nitrophenyl}}$ C_2, C_6), 129.24, 129.41 ($C_{4\text{-fluorophenyl}}$, C_2, C_6), 142.21 ($C_{4\text{-nitrophenyl}}$ C_1), 148.25 ($C_{4\text{-nitrophenyl}}$ C_4), 162.30 (oxadiazoline C_5), 167.21 ($C_{4\text{-fluorophenyl}}$, C_4), 168.79 ($\mathit{C}OCH_3$).

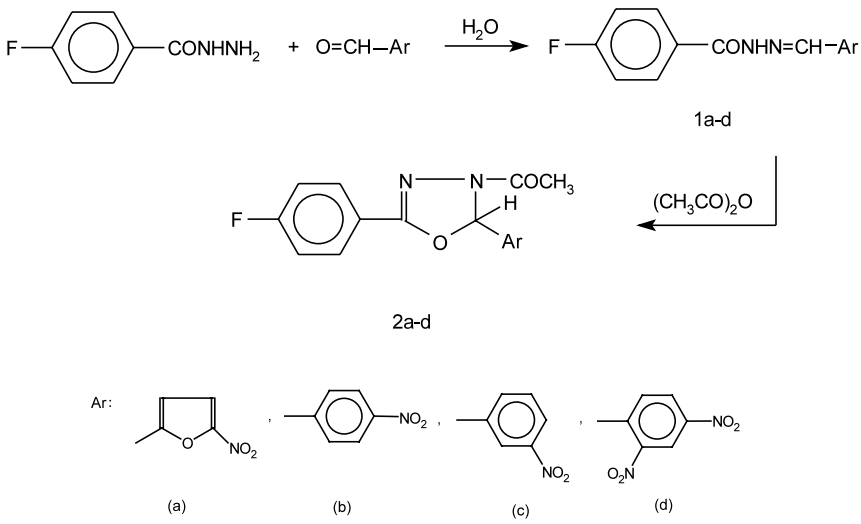
2.2. Microbiology

All of the compounds synthesized were screened in vitro for antimicrobial activity by microdilution susceptibility method. In the determination of antibacterial activity *S. aureus* ATCC 29213, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were used as the reference strains and Mueller–Hinton Broth (pH 7.3) was used as the medium. In the investigation of antifungal activity, *C. albicans* ATCC 2091 and Yeast Nitrogen Base (pH 7) were used.

All the compounds were dissolved in DMSO and concentration were compared with those of ceftriaxone (Roche) and miconazole (Sigma) as reference antibacterial and antifungal agents, respectively. Ceftriaxone were used at concentration of 0.015–32 μg/ml, 0.0019–3.84 μg/ml and 0.12–256 μg/ml against *S. aureus*, *E. coli* and *P. aeruginosa*, respectively. For the limit of MIC ranges of miconazole for *C. albicans*, concentrations 0.05–100 μg/ml were examined. Each test was performed twice and the average of the results was calculated [7–9].

3. Results and discussion

The synthetic pathway followed for the preparation of the compounds is represented in Scheme 1. 4-Fluorobenzoic acid(substitutedmethylene)hydrazides (**1a**–**d**) were prepared by condensation of 4-fluorobenzoic acid hydrazide with appropriate aldehydes [10]. 3-Acetyl-2,5-disubstituted-2,3-dihydro-1,3,4-oxadiazoles (**2a**–**d**) were then synthesized by heating these compounds (**1a**–**d**) with acetic anhydride [11]. The structure of the synthesized compounds was elucidated using UV, IR, ^{1}H NMR (**1a**, **1b**, **2a**, **2b**), ^{13}C NMR (**2b**) and mass (**1b**, **2a**) spectroscopic methods besides elemental analyses (Table 1). The IR spectra of **1a**–**d** showed hydrazone N–H bands in 3210–3170 cm^{-1} and C=O bands in 1680–1670 cm^{-1} region. ^{1}H NMR spectra of **1a** and **1b** showed two signals in the δ 8.40, 8.55 ppm and δ 12.13, 12.11 ppm which were attributed to the N=CH and NH protons, respectively [12,13]. The EI–MS spectrum of selected prototype **1b** showed the molecular ion at *m*/*z* 287 and this fragmentation pattern was also cited in similar structures [14,15]. IR spectra of **2a**–**d** had different characteristics as they showed no N–H stretching bands and only C=O bands in the 1685–1674 cm^{-1} region which were attributed to the C=O stretching of acetyl group. ^{1}H NMR spectra of **2a**,**b** displayed the O–CHR–N resonance of the oxadiazoline ring in the δ 7.08, 7.14 ppm in accordance with the literature [16]. The formation of the oxadiazolines was further supported by recording the ^{13}C NMR spectrum of selected compound **2b**. While the N=CH carbon of the compounds in the hydrazone structures were observed at δ 143.5–149.8 ppm in their ^{13}C NMR spectra; the same carbon atoms (OCHR–N) were observed at δ 89.5–91.7 ppm after cyclization of oxadiazoline ring [16,17]. In the ^{13}C NMR of **2b**, this signal was also observed at δ 90.91 ppm due to the sp^3 hybridization. The EI MS

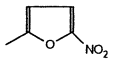
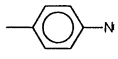
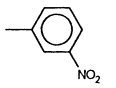
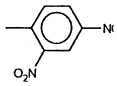
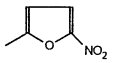
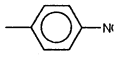
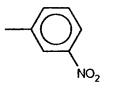
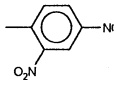


Scheme 1.

spectrum of **2a** showed the molecular ion peak at *m/z* 319 corresponding to $C_{14}H_{10}FN_3O_5$ and the base peak at *m/z* 123. The possible fragmentation pattern was in accordance with the literature values [18].

Antimicrobial activities of the hydrazide hydrazones (**1a**–**d**) and 1,3,4-oxadiazolines (**2a**–**d**) listed in Table 2 were evaluated against *S. aureus*, *E. coli*, *P. aeruginosa* and *C. albicans* by the microdilution susceptibility method [7–9]. In our work, the MIC value of the ceftriaxone was 2 μg/ml for the *S. aureus* strain. According to NCCLS 1997, the MIC value of ceftriaxone is 1–8 μg/ml for the same strain [9]. From the

Table 1
Some characteristics of **1a**–**d** and **2a**–**d**

Comp.	R	Yield [%]	m.p. [°C]	UV (EtOH) λ max, nm	IR (KBr) [cm^{-1}] NH	C=O	Formula (mol. Mass)	Elemental Analysis calc./ found. %C	%H	%N
1a		91	243[a]	202, 276 (sh), 359	3170	1670	$C_{12}H_8FN_3O_4$ (277.214)	51.99 51.99	2.91 2.81	15.16 15.23
1b		87	239-240[a]	204, 329	3180	1680	$C_{14}H_{10}FN_3O_3$ (287.25)	58.54 58.77	3.51 3.42	14.63 14.45
1c		87	196[a]	203, 226 (sh), 342	3210	1675	$C_{14}H_{10}FN_3O_3$ (287.25)	58.54 58.20	3.51 3.59	14.63 14.42
1d		83	244-245[a]	206, 293	3175	1675	$C_{14}H_9FN_4O_5$ (332.252)	50.61 51.05	2.73 2.68	16.86 16.51
2a		63	175-178[a]	202, 220 (sh), 271	-	1678	$C_{14}H_{10}FN_3O_5$ (319.25)	52.67 52.73	3.16 3.05	13.16 13.32
2b		66	121[b]	203, 220 (sh), 271	-	1675	$C_{16}H_{12}FN_3O_4$ (329.30)	58.36 58.13	3.67 3.61	12.76 12.65
2c		65	144-145[a]	206, 289	-	1685	$C_{16}H_{12}FN_3O_4$ (329.30)	58.36 57.89	3.67 3.56	12.76 12.67
2d		69	166[a]	205, 226, 263	-	1674	$C_{16}H_{11}FN_4O_6$ (374.30)	51.34 51.15	2.96 2.83	14.97 14.73

[a] Recrystallized from ethanol.
[b] Recrystallized from ethanol–water (1/1).

Table 2
Antimicrobial activity of **1a–d** and **2a–d**

Comp.	MIC values (μg/ml)			
	S. aureus ATCC 29213	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27853	*C. albicans* ATCC 2091
1a	2	250	250	62.5
1b	16	250	125	125
1c	8	125	125	125
1d	4	125	125	125
2a	8	250	250	8
2b	62.5	500	250	250
2c	16	250	250	250
2d	125	125	125	125
Ceftriaxone	2	0.06	8	
Miconazole				0.05

synthesized compounds, **1a**, **1c**, **1d** having hydrazone structure and **2a** in the structure of 3-acetyl-1,3,4-oxadiazoline were found to have reasonable activity against *S. aureus*. In regard to structure, the most important variable effecting the antimicrobial activity against *S. aureus* was the substituted phenyl/furanylnitro group on the hydrazone moiety. A remarkable activity was found in compound **1d** carrying 2,4-dinitrophenyl moiety. Compound **1c** having *meta* nitrophenyl was more active than the corresponding *para* nitrophenyl derivative. The most active compound was **1a** (2 μg/ml) having 5-nitro-2-furanyl moiety. Cyclization of hydrazones into corresponding 3-acetyl-1,3,4-oxadiazolines resulted in lowering their ability of antimicrobial activity against *S. aureus* strain. However, 1,3,4-oxadiazoline derivative (**2a**), which was the cyclization product of **1a**, exhibited a MIC value 8 μg/ml against *S. aureus* and the tested strain of *C. albicans*. The results obtained show that some of the prepared and tested compounds, especially the nitrophenyl/furanyl derivatives, may be considered promising for the development of new antibacterial and antifungal agent.

References

[1] H.M. Eisa, A.S. Tantawy, M.M. El-Kerdawy, Synthesis of certain 2-aminoadamantane derivatives as potential antimicrobial agents, Pharmazie 46 (1991) 182–184.

[2] P.P.T. Sah, S.A. Peoples, Isonicotinyl hydrazones as antitubercular agents and derivatives for identification of aldehydes and ketones, J. Am. Pharm. Ass. Sci. Ed. 43 (1954) 513–524.

[3] S.S. Parmar, A.K. Gupta, T.K. Gupta, V.I. Stenberg, Synthesis of substituted benzyldinohydrazines and their monoamine oxidase inhibitory and anticonvulsant properties, J. Pharm. Sci. 64 (1975) 154–157.

[4] R. Kalsi, K. Pande, T.N. Bhalla, J.P. Bartwall, G.P. Gupta, S.S. Parmar, Anti-inflammatory activity of quinazolinoformazans, J. Pharm. Sci. 79 (1990) 317–320.

[5] E. Hassan, M.I. Al-Ashmawi, B. Abdel-Fattah, Synthesis and antimicrobial testing of certain oxadiazoline and triazole derivatives, Pharmazie 38 (1983) 833–835.

[6] M.A. Khalil, O.A. El-Sayed, H.A. El-Shamny, Synthesis and antimicrobial evaluation of novel oxa(thia)diazolylquinolines and oxa(thia)diazepino[7,6-*b*]quinolines, Arch. Pharm. (Weinheim) 326 (1993) 489–492.

[7] R. Guinet, D. Nerson, F. De Closets, J.D. Camet, D. Kures, M. Mariollet, J.L. Poriot, A. Ros, J.T. Maugein, P.J. Volle, Collaborative evaluation in seven laboratories of standardized micromethod for yeast susceptibility testing, J. Clin. Microbiol. 26 (1988) 2307–2311.

[8] A.E. Ingroff, M.A. Pfaller, Antifungal agents and susceptibility testing, in: P.R. Murray, E. Jo Baron (Eds.), Manual of Clinical Microbiology, American Society for Microbiology, Washington, 1995, pp. 1405–1414.

[9] National Committee for Clinical Laboratory Standards, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, 4th ed., Approved Standard, NCCLS document M 100-S7, Villanova, 1997.

[10] S. Rollas, S. Büyüktimkin, N. Büyüktimkin, M. Ülgen, E. Yemni, Evaluation of some aroylhydrazones of N^2-arylidenebenzylic acid hydrazide as antimicrobial agents, Pharmazie 43 (1988) 511.

[11] N. Ergenç, S. Rollas, Y. Topaloglu, G. Ötük, Synthesis and characterization of new 1,3,4-oxadiazolines, Arch. Pharm. (Weinheim) 322 (1989) 837–838.

[12] S. Rollas, Synthesis and spectrometric analysis of some hydrazide hydrazones I, J. Fac. Pharm. Istanbul 17 (1981) 41–50; Chem. Abstr. 97 (1982) 55729c.

[13] N. Ergenç, S. Rollas, G. Çapan, N. Dogan, Y. Özger, Studies on azopyrazole derivatives. Part 8: Synthesis and characterization of new 1-(1-substituted-3,4-dimethylpyrazolylazo)-N^2-(substituted benzylidene)benzohydrazides, Pharmazie 44 (1989) 573–574.

[14] S.G. Kömürcü, S. Rollas, M. Ülgen, J.W. Gorrod, A. Çevikbas, Evaluation of some arylhydrazones of *p*-aminobenzoic acid hydrazide as antimicrobial agents and their in vitro hepatic microsomal metabolism, Boll. Chim. Farm. 134 (1995) 375–379.

[15] D.G.I. Kingston, H.P. Tannenbaum, G.B. Baker, J.R. Dimmock, W.G. Taylor, Mass spectrometry of organic compounds. Part V. Mass spectra of substituted aroyl hydrazones, J. Chem. Soc. (C) (1970) 2574–2577.

[16] B. Durgun, G. Çapan, N. Ergenç, S. Rollas, Synthesis, characterization and biological evaluation of new benzylidenebenzohydrazides and 2,5-disubstituted-2,3-dihydro-1,3,4-oxadiazoles, Pharmazie 48 (1993) 942–943.

[17] L. Somogyi, Notes on the reactions of ketone acylhydrazones under acylation conditions, Tetrahedron 41 (1985) 5187–5190.

[18] I. Kramer, I. Szelenyi, W. Schunack, H_2-Antihistaminika, 34 Mitt. 1,3,4-Oxadiazol-2,5-diamine mit H_2-antagonistischer aktivitat, Arch. Pharm. (Weinheim) 320 (1987) 120–130.