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Antimicrobial Activity of Some Substituted Triazoloquinazolines

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Dedicated to Professor Ján Garaj, DrSc., on the occasion of his 70th birthday.

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ABSTRACT. Fifteen substituted 1,2,4-triazolo[4,3-*c*]quinazolines were tested for antibacterial and antifungal effects. The most effective derivatives had the triazoloquinazoline skeleton substituted with the pharmacologically active chromophores – morpholine, chlorine and nitro group. The broadest antimicrobial activity was found with 5-morpholin-4-yl-3-(5-nitrothien-2-yl)[1,2,4]triazolo[4,3-*c*]quinazoline in concentration of 10 mg/L for *B. subtilis*, 50 mg/L for *S. aureus* and 100 mg/L for *C. tropicalis*. The highest tested concentration of derivative caused 83 % growth inhibition of *R. nigricans*.

Abbreviation

DHFR dihydrofolate reductase (EC 1.5.1.3)

Many quinazoline derivatives are used in pharmaceutical industry, in medicine because of their anti-inflammatory (Farghaly *et al.* 1990), diuretic (Pomarnacka *et al.* 1984), anticonvulsant (Gursoy and Karali 1995), antiallergic (Peet *et al.* 1986), antihypertensive (Kurogi *et al.* 1996), anticancer (Takemura and Jackman 1997) and other properties.

Some medicinal applications were reported for (1,2,4)-triazolo- and (1,2,4)-triazino[4,3-*c*]quinazolines, such as being antihistaminics, blood-platelet-aggregation inhibitors, analgesics and antidepressants (Špirková *et al.* 1997; Nasr *et al*. 2003).

Some derivatives of quinazoline have antimicrobial activity (Farghaly *et al.* 1990, 2002; Elzohry *et al.* 1992; Abdelmegeed *et al.* 1994; Jantová *et al.* 1994, 1995; Gottasová *et al.* 1998; Nawrocka and Stasko 2002). The molecular target for the development of medical antimicrobial agents is DHFR. Quinazolines can be species-selective or -nonselective inhibitors of DHFR. Such species-selective inhibition of DHFR has been proven clinically useful in other therapeutic applications. For example, the therapeutic value of the antibacterial agent trimethoprim lies in its ability to selectively inhibit the bacterial DHFR. Certain pyrrolo-2,4-diaminoquinazolines which are potent but nonselective inhibitors of DHFR, have been reported to have *in vitro* antifungal activity (Chan *et al.* 1995).

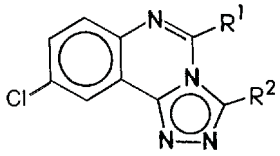
We studied antibacterial activity of some substituted tricyclic quinazolines and their synthetic precursors, the synthesis of which had been reported by Špirková *et al.* (1997). 3-(Chloroacetyl)-2-methylquinazoline-4(3*H*)-thione and 5-phenyl-[1,2,4]triazolo[4,3-*c*]quinazolin-3(2*H*)-one had the highest antibacterial effect against *B. subtilis*, the MIC values being 50 mg/L. Two derivatives were more active against *P. aeruginosa* than ampicillin, the IC_{50} being 80 and 100 mg/L. The most effective derivatives contained in the structure generally pharmacologically active chromophores, a methyl group in position 2 and chloromethyl arrangement on the carbonyl group in position 3.

The aim of this paper was to investigate the antimicrobial effects of 15 new substituted tricyclic quinazolines – 1,2,4-triazoloquinazolines (**1**–**15**; Špirková *et al.* 1991, 1993) and the relationship between this antimicrobial effect and the structure of synthetically prepared quinazoline derivatives.

MATERIALS AND METHODS

Materials. Bacterial strains *Escherichia coli* CCM 3988, *Pseudomonas aeruginosa* CCM 3955, *Bacillus subtilis* CCM 1718, *Staphylococcus aureus* CCM 3953, the yeasts *Candida utilis* CCY 29-38-86, *C. tropicalis* CCY 29-7-48, *C. parapsilosis* CCY 29-20-19, *Saccharomyces cerevisiae* CCY 21-4-19 (6C Val-

tice) and the filamentous fungi *Rhizopus nigricans* 8075, *Aspergillus niger* CCM F-237 (obtained from the *Collection of Microorganisms* of the *Department of Biochemistry and Microbiology, Slovak University of Technology*) were used. The preparation of compounds **1**–**15** has been described by Špirková *et al.* (1991, 1993). The reported quinazolines were obtained by an oxidative cyclization of the corresponding arylhydrazones.



1–15

	R^1	R^2		R^1	R^2
1	Mo	3-indolyl	**10**	Ph	4-chlorophenyl
2	Mo	5-nitro-2-thienyl	**11**	Ph	5-nitro-2-furyl
3	Mo	5-nitro-2-furyl	**12**	Ph	5-chloro-2-furyl
4	Mo	5-phenylsulfonyl-2-furyl	**13**	Ph	2-furyl
5	Mo	5-acetyl-2-furyl	**14**	Ph	5-bromo-2-furyl
6	Mo	5-bromo-2-furyl	**15**	Ph	4-*N*,*N*-dimethylaminophenyl
7	Mo	4-acetamidophenyl			
8	Mo	4-nitrophenyl	Mo – morpholin-4-yl		
9	Mo	4-chlorophenyl	Ph – phenylpiperazinyl		

The compounds were used at concentrations of 100, 50, 10, 1 and 0.1 mg/L. Chromatographically pure derivatives were dissolved in Me_2SO whose final concentration never exceeded 1 % (*V*/*V*) in either control or treated samples.

The *antimicrobial effect* was determined by IC_{50} values, *i.e.* the minimal concentration of a substance that inhibits growth of microorganisms by 50 %, and MIC values, *i.e.* the minimal concentration of a substance that completely inhibits growth, both values being determined from toxicity curves.

Antibacterial assay. The antibacterial effect was assayed by a microdilution method in 96-well microtitration plates (Jantová *et al.* 1995). The bacteria were cultured on a Mueller–Hinton medium at 30 °C. An overnight inoculum was prepared 12–16 h before the test. The growing inoculum was filtered and a 1.5 % suspension of bacteria was prepared. This suspension (180 μL) was added to 20 μL of the tested complex solution and cultured for 6 h on a reciprocal shaker in a thermostat at 30 °C. The time course of absorbance A_{630} was then determined in triplicates. To compare the antibacterial activity, ampicillin (100, 10, 1, 0.1 and 0.01 mg/L) was used as standard. The antibacterial effect was characterized by IC_{50} and MIC values.

Determination of the effect on yeasts. The yeasts were cultured on Sabouraud-glucose medium at 28 °C (Jantová *et al.* 1997). Seven mL of culture medium was inoculated with 0.5 mL of growing overnight culture and 75 μL solution of quinazolines. The cultures were then grown for 6 h on a reciprocal shaker in a thermostat at 28 °C. The A_{650} of triplicate sets of tubes were measured at 2-h intervals.

Antifungal assay. The effects on filamentous fungi were tested during static culturing (Hudecová *et al.* 1996). One tenth mL of the tested quinazoline in Me_2SO was added to Petri dishes (diameter 100 mm) immediately before pouring 10 mL Sabourad–glucose agar to obtain the desired concentration of inhibitors. The solidified plates were then inoculated in the center with 5 μL of the spore suspension (100/μL). Triplicate sets of agar plates were incubated at 25 °C and the diameter of growing colonies was measured at intervals.

RESULTS AND DISCUSSION

In preliminary experiments antibacterial activity of 11 substituted tricyclic quinazolines and their synthetic precursors against 4 selected strains was surveyed. Three of them were 1,2,4-triazolo[4,3-*c*]quinazolines. 5-Phenyl-[1,2,4]triazolo[4,3-*c*]quinazolin-3(2*H*)-one (substituted on the pyrimidine ring of quinazoline) was more active than unsubstituted [1,2,4]triazolo[4,3-*c*]quinazolin-3-one. That is why we studied the effect of 15 substituted 1,2,4-triazolo[4,3-*c*]quinazolines on bacteria, yeasts and filamentous fungi further. Their activity against 4 bacteria strains is given in Table I. Derivatives were active only on G^+ bacteria. The broadest antibacterial effect was found with 5-morpholin-4-yl-3-(5-nitrothien-2-yl)[1,2,4]triazolo[4,3-*c*]quinazoline (**2**), whose concentration of 10 mg/L for *B. subtilis* and of 50 mg/L for *S. aureus* induced 100 %

inhibition of cell growth. A certain antibacterial effect on G^+ bacteria was manifested by derivatives **1**, **3**, **6** and **11**. Their MIC values were 100 or 50 mg/L (derivative 3 for *S. aureus*). No antibacterial activity was found with derivatives **4**, **5**, **7**–**10**, and **12**–**15** (MIC values were >100 mg/L). In general, the sensitivity of the G^+ bacteria was higher than that of the G^- bacteria. Any of the tested derivatives was more effective than ampicillin.

Table I. Antimicrobial activity (mg/L) of triazoloquinazolines

Compound	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC
				Bacteria[a,b]				
	B. subtilis		*S. aureus*					
1	3.98	50[c]	2.56	100[c]				
2	1.92	100[c]	2.66	100[c]				
3	1.95	100[c]	0.46	100[c]				
6	76.3	>100	14.8	100[c]				
8	9.12	>100	36.5	>100				
11	4.53	100[c]	12.3	100[c]				
Ampicillin	0.71	10[c]	0.02	0.04[c]				
				Yeasts[b]				
	C. utilis		*C. tropicalis*		*C. parapsilosis*		*S. cerevisiae*	
1	>100	≅100	>100	≅100	5.48	100[d]	4.05	100[d]
2	7.14	>100	0.20	100[d]	>100	≅100	25.3	>100
3	0.51	50[c]	22.4	100[d]	>100	≅100	>100	≅100
6	>100	≅100	>100	≅100	60.5	>100	>100	≅100
				Fungi[b]				
	A. niger		*R. nigricans*					
2	>100	≅100	54.8	>100				
3	>100	≅100	75.3	≅100				
5	>100	≅100	89.9	≅100				
6	>100	≅100	18.9	50				
9	>100	≅100	72.7	≅100				

[a]All compounds were inactive against *E. coli* and *P. aeruginosa* (IC_{50} and MIC were >100 mg/L).
[b]Other compounds were inactive (IC_{50} and MIC were >100 mg/L).
[c]Bacteriostatic effect. [d]Microbistatic effect.

Figs 1–3 represent the growth curves of the selected microorganism treated with effective derivatives. The highest concentration of derivatives induced a total inhibition of cell proliferation, the other concentrations inhibited the cell division proportionally. The sensitivity of *B. subtilis* to derivative **2** was higher than that of *S. aureus*.

The higher sensitivity of G^+ bacteria than that of G^- ones was found by Elslager *et al.* (1978, 1984), Khalil and Habib (1987), Baiocchi *et al.* (1993) and Shiba *et al.* (1997). On the other hand, Elslager *et al.* (1978), Aboushady *et al*. (1979), Farghaly *et al.* (1990) and Nasr *et al.* (2003) found that the tested quinazolines were active on both G^+ and G^- bacteria. They synthesized from corresponding thiosemicarbazide derivatives two novel series of imidazo[2′,1′,5,1]-1,2,4-triazolo[4, 3-*c*]quinazolines bearing 5-thioxo-1,2,4-triazoles and 4-oxothiazolidines. All their compounds were screened for *in vitro* antibacterial activity against various G^+ and G^- bacteria. Some compounds were found to possess potent antibacterial activity. One derivative exhibited much higher potency than the reference standard ciprofloxacin, against both types of bacteria, particularly G^+ organisms. It probably has something to do with the fact that some quinazolines are species-selective of DHFR and other of quinazoline derivatives are nonselective DHFR inhibitors (Chan *et al.* 1995).

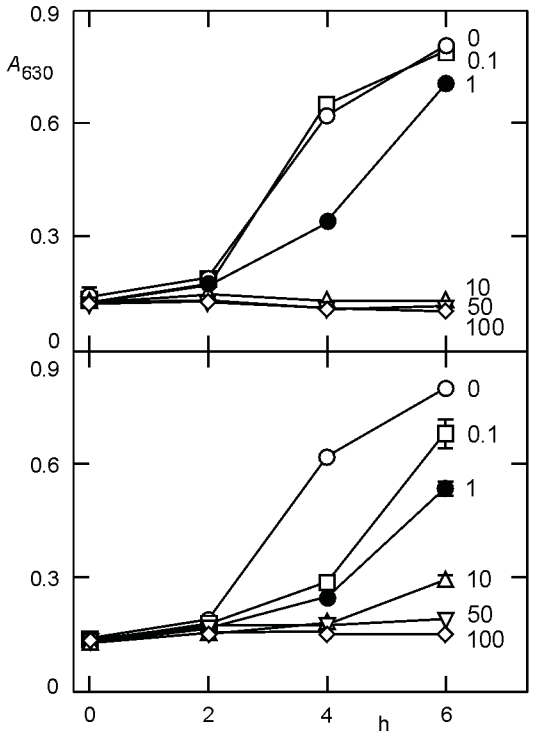


Fig. 1. Effect of 5-morpholin-4-yl-3-(5-nitrothien-2-yl)[1,2,4]triazolo[4,3-*c*]quinazoline (**2**) on growth (absorbance A_{630}) of *B. subtilis* (*top*) and *S. aureus* (*bottom*); *numbers at curves* – concentration of quinazoline (mg/L).

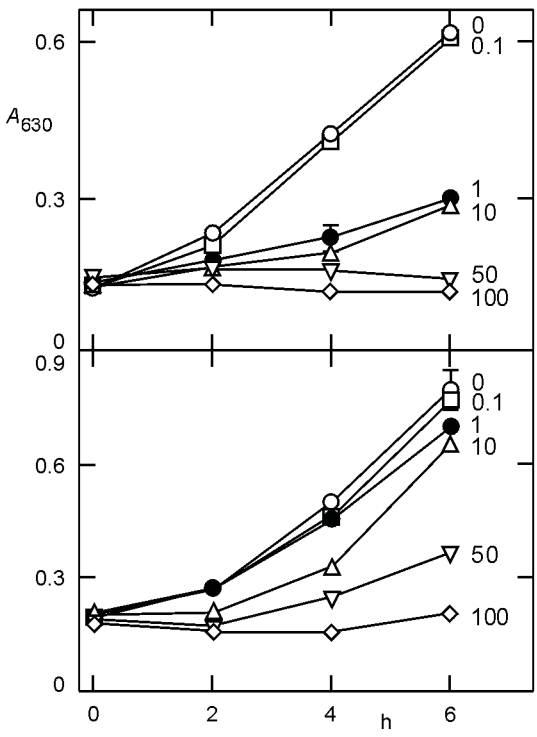


Fig. 2. Effect of 5-morpholin-4-yl-3-(5-nitro-2-furyl)[1,2,4]triazolo[4,3-*c*]quinazoline (**3**) on growth (absorbance A_{630}) of *C. utilis* (*top*) and *C. tropicalis* (*bottom*); *numbers of curves* – concentration of quinazoline (mg/L).

From the results it follows that the substitution by morpholine in position 5 on the pyrimidine ring and by 5-nitro-2-thienyl- (derivative **2**) or 5-nitro-2-furyl- (derivative **3**) in position 3 on the triazole ring exhibited an increase of antibacterial effect.

The activity of substituted 1,2,4-triazolo[4,3-*c*]quinazolines against 4 selected yeast strains (IC_{50} and MIC) is given in Table I. Derivatives **1**–**3** markedly influenced the growth of yeasts. Derivative **1** influenced the growth of *C. parapsilosis* and *S. cerevisiae*, the MIC values being 100 mg/L. Derivative **2** influenced the growth of *C. tropicalis*, the MIC being 100 mg/L. Derivative **3** inhibited the cell proliferation of *C. utilis* and *C. tropicalis* (MIC 50 and 100 mg/L). The other 1,2,4-triazolo[4,3-*c*] quinazolines were inactive (MIC >100 mg/L). Most sensitive was *C. tropicalis*.

The activity of substituted 1,2,4-triazolo[4,3-*c*]quinazolines against two filamentous fungal strains (IC_{50} and MIC) is given in Table I. Any quinazoline was effective on *A. niger* (IC_{50} and MIC were >100 mg/L). 5-Morpholin-4-yl-3-(5-bromo-2-furyl)[1,2,4]triazolo[4,3-*c*]quinazoline (**6**) was most effective on *R. nigricans*, the MIC being 100 mg/L. Derivatives **2**, **3**, **5** and **9** had certain antifungal activity, IC_{50} ranging from 18.9 to 89.9 mg/L; on the other hand, their highest concentration did not totally inhibit the growth of *R. nigricans*.

Quinazolines as well as triazoles are compounds showing a therapeutic antimicrobial effect. The increase of antimicrobial activity was expected from the connection of two biologically active structures (benzopyrimidine and 1,2,4-triazole skeleton) by their synthesis. Comparison of the structure of the most active derivatives showed that the triazoloquinazoline skeleton substituted with a morpholine, chlorine and nitro group.

These preliminary results allow us to consider 5-morpholin-4-yl-3-(5-nitrothien-2-yl)[1,2,4]triazolo[4,3-*c*]quinazoline (**2**) as a potential antimicrobial compound.

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REFERENCES

CHAN J.H., HONG J.S., KUYPER L.F., BACCANARI D.P., JOYNER S.S., TANSIK R.L., BOYTOS C.M., RUDOLH S.K.: Selective inhibitors of *Candida albicans* dihydrofolate reductase: activity and selectivity of 5-(arylthio)-2,4-diaminoquinazolines. *J.Med.Chem.* **38**, 3608–3616 (1995).

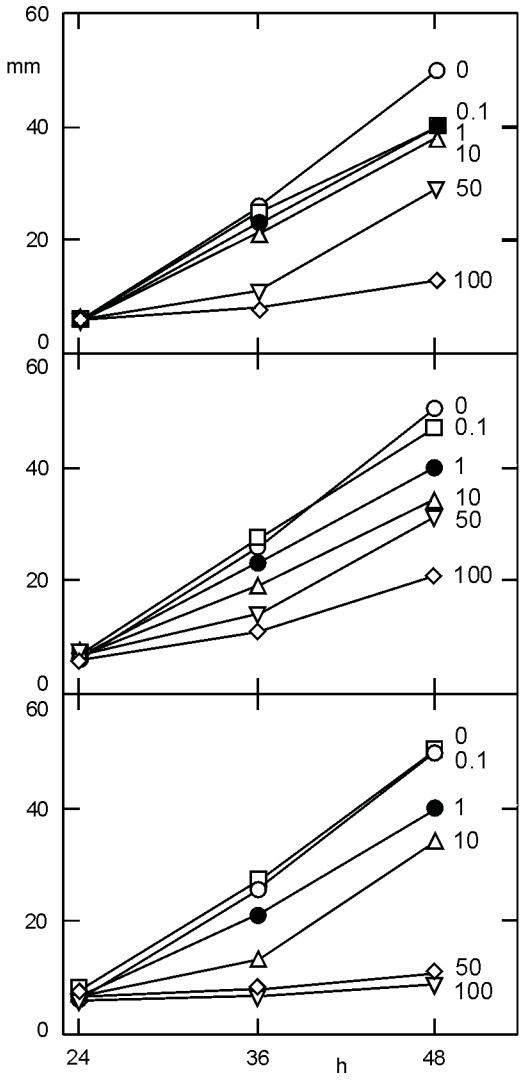

Fig. 3. Effect of 5-morpholin-4-yl-3-(5-bromo-2-furyl)-[1,2,4]triazolo[4,3-*c*]quinazoline (**6**, *top*), 5-morpholin-4-yl-3-(5-nitrothien-2-yl)[1,2,4]triazolo[4,3-*c*]quinazoline (**2**, *middle*) and 5-morpholin-4-yl-3-(5-nitro-2-furyl)[1,2,4]triazolo-[4,3-*c*]quinazoline (**3**, *bottom*) (all three at concentrations of 0–100 mg/L) on growth (diameter of colonies in mm) of *R. nigricans.*

FARGHALY A.M., MONSEN A., OMAR M.E., KHALIL M.A., GABER M.A.: Synthesis of 3-aryl-2-substituted-4(3*H*)-quinazolines as potential antimicrobial agents. *Farmaco* **45**, 431–438 (1990).

FARGHALY A.M., SOLIMAN R., KHALIL M.A., BEKHIT A.A., EL-DIN A., BEKHIT A.: Thioglycolic acid and pyrazole derivatives of 4(3*H*)-quinazolinone: synthesis and antimicrobial evaluation. *Boll.Chim.Farm.* **5**, 372–378 (2002).

GURSOY A., KARALI N.: Synthesis and anticovulsant activity of new acylthiosemicarbazides and thiazolidones. *Farmaco* **50**, 857–866 (1995).

HUDECOVÁ D., JANTOVÁ S., MELNÍK M., UHER M.: New azido metalkojates and their biological activity. *Folia Microbiol.* **6**, 473–476 (1996).

JANTOVÁ S., HUDECOVÁ D., STANKOVSKÝ Š., ŠPIRKOVÁ K., RUŽEKOVÁ Ľ.: Antibacterial effect of substituted 4-quinazolylhydrazines and their arylhydrazones determined by modified microdilution method. *Folia Microbiol.* **40**, 611–614 (1995).

JANTOVÁ S., LABUDA J., VOLLEK V., ZASTKOVÁ M.: Antimicrobial effects of the macrocyclic $Cu^{(II)}$-tetraanhydroaminobenzaldehyde complex. *Folia Microbiol.* **4**, 324–326 (1997).

KUROGI Y., INOUE Y., TSUTSUMI K., NAKAMURA S., NAGAO K., YOSHITSUGU H., TSUDA Y.: Synthesis and hypolipidemis activities of novel 2-(4-((diethoxyphosphoryl)methyl)phenyl)quinazolines and 4(3*H*)-quinazolinones. *J.Med.Chem.* **39**, 1433–1437 (1996).

NASR M.N., GINEINAH M.M., EL-BENDARY E.R.: Synthesis and *in vitro* antibacterial evaluation of novel imidazo-[2′,1′:5,1]-1,2,4-triazolo[4,3-*c*]-quinazoline derivatives of 5-thioxo-1,2,4-triazole, 4-oxothiazolidine, and their open-chain counterparts. *Arch.Pharm.(Weinheim)* **12**, 560–566 (2003).

NAWROCKA W., STASKO J.J.: 4(3*H*)-Quinazolinones. Antimicrobial 4(3*H*)-quinazolinones. III. *Boll.Chim.Farm.* **1**, 84–91 (2002).

PEET N.P., BAUGH L.E., LEWIS J.E., MATTHEWS E.H., OLBERDING E.L., SHAN D.N., SUNDER S.: Chemistry and antiallergic activity of 3-(1*H*-tetrazol-5-yl)-4-(3*H*)-quinazoline sodium-salt (MDL-427). *Abstr.Papers Amer.Chem.Soc.* **192**, 13 (1986).

POMARNACKA E., AGIELSKI S., HOPPE A.: Derivatives of 4-chloro-5-sulfanoylbenzoic acid. 8. Synthesis and diuretic activity of pyrazole (3,2)-quinazoline and 1-benzoylpyrazole derivatives. *Acta Polon.Pharm.* **41**, 141–151 (1984).

ŠPIRKOVÁ K., STANKOVSKÝ Š.: Amidinoyl isothiocyanates in the synthesis of condensed quinazolines. Preparation of 3-aryl-5,9-disubstituted-*s*-triazolo[4,3-c]quinazolines. *Collect.Czech. Chem.Commun.* **56**, 1719–1724 (1991).

ŠPIRKOVÁ K., HORŇÁČEK J., STANKOVSKÝ Š.: Amidinoyl isothiocyanates in the synthesis of condensed quinazolines. Preparation of 3-aryl-9-chloro-5-morpholino[1,2,4]triazolo[4,3-*c*]quinazolines and their [1,5-*c*] isomers. *Chem.Papers* **47**, 382–385 (1993).

ŠPIRKOVÁ K., STANKOVSKÝ Š., ZAHRADNÍK M.: Annelation to the quinazoline ring. Preparation and utilization of 3-quinazolylcarboxylates in the synthesis of fused systems. *Chem.Papers* **51**, 403–407 (1997).

TAKEMURA Y., JACKMAN A.L.: Folate-based thymidylate synthase inhibitors in cancer chemotherapy. *Anti-Cancer Drugs* **8**, 3–16 (1997).