Thiophene Analogs of Sulfanilamide: Bacterial Growth Inhibitors

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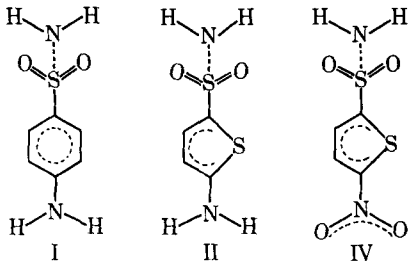
Structure *versus* activity study of thiophene analogs of sulfanilamides indicated: (*a*) the parent aminothiophene-2-sulfonamide is a better bacterial growth inhibitor than is sulfanilamide (I); (*b*) 5-aminothiophene-2-sulfonamide (II) is a better inhibitor than the 4-amino isomer (III); (*c*) substitution of the sulfonamide N with groups which enhance I's antibacterial activity does not necessarily similarly enhance the activity of the thiophene analog (VI, VII); (*d*) 5-(IV) and 4-nitrothiophene-2-sulfonamides(V) are more inhibitory than the corresponding 5- and 4-amino compounds (II, III) with the 5-nitro compound (IV) being bactericidal at very low concentrations. Folic acid or PABA reversed the growth inhibition produced by the thiophene-2-sulfonamides, indicating both the nitro and amino thiophene-2-sulfonamides prevent intracellular folic acid biosynthesis.

THE MODERN era of drug treatment of many bacterial infections originated with the finding of a selective effect of sulfanilamide on organisms which synthesize their own folic acid from *p*-aminobenzoate (1–3). The essential nucleus, a *p*-aminophenyl-sulfonamide, has been modified and sometimes improved extensively, mostly by addition of groups to the sulfonamide nitrogen (4). In the increasing frequency of instances of bacterial drug resistance transmitted by the multiple-drug resistance RTF episome, most often resistance to streptomycin, chloramphenicol, and tetracycline are gained simultaneously by the invading pathogen; there is a smaller incidence of concomitant sulfanilamide (I) resistance (5). Improved forms of I might be effective in such cases of multiple-drug resistance, or in cases where current I derivatives are only marginally effective. The authors report here the superior antibacterial effectiveness of modified forms of I, in which the phenyl ring of the parent molecule of the sulfanilamide family of drugs has been replaced by a thiophene ring (II, IV).



I II IV

MATERIALS AND METHODS

Salmonella typhimurium and *Proteus vulgaris* were obtained from the University of California, San Francisco, collection.[1] These bacterial strains were maintained on sensitivity test (ST) agar (Colab, Chicago) or brain heart infusion (BHI) agar (Difco, Detroit) slants or plates and subcultured on the same media at 37° in a candle jar as needed for anaerobic-high CO_2 conditions (8). The bacterial cells used as inoculum were grown overnight in tubes containing 8 ml. of BHI broth for approximately 15 hr., centrifuged at about 1500 × g in an International Clinical centrifuge (I.E.C. Co., Boston) at 25° and suspended in a pH 7.0 sodium phosphate buffer. The growth flasks consist of 300-ml. conical flasks, fitted with a closed-tube side-arm about 2.5 cm. from the flask bottom. The drug, if any, was added to these flasks and diluted with the growth medium consisting of 15 ml. of BHI broth supplemented with 0.5% lactalbumin hydrolysate (Difco, Detroit) and 0.6 m*M* $NaHCO_3$. To this medium the bacterial cells were added to about 25 mcg../ml. of dry weight equivalent and flasks placed at 37° on a gyrotary shaker (New Brunswick Scientific, New Brunswick, N. J.) at two shakes per sec. The bacterial growth was followed spectrophotometrically at 30-min. intervals.

Details of the synthesis and properties of the thiophene analogs will be published (6, 7). The compounds used and their melting points were:

	Compounds	m.p.
II	5-aminothiophene-2-sulfonamide	137–138°
III	4-aminothiophene-2-sulfonamide	132–133°
IV	5-nitrothiophene-2-sulfonamide	135–136°
V	4-nitrothiophene-2-sulfonamide	165–166°
VI	2-(5-aminothiophene-2-sulfonamide) pyridine	162°
VII	2-(5-aminothiophene-2-sulfonamide) pyrimidine	204–205°

To estimate *in vivo* toxicity of a compound, 25-Gm. male albino mice, random bred Webster-Simonsen Swiss (Simonsen Laboratories, Gilroy, Calif.), were given a single intraperitoneal injection each morning for five consecutive mornings. The animals were weighed daily and survival followed for 30 days. A tolerated dose was considered that dose which produced a readily reversible weight loss not exceeding 12% at any time and which caused no mouse deaths during the 30-day period.

RESULTS AND DISCUSSION

Superior Antibacterial Activity Associated with Thiophene Ring Modification—As seen in Fig. 1, the thiophene ring modification (Structure II) produced an increase in the antibacterial activity against streptococcus AHT, known to be a human pathogen, above that seen under these conditions with the phenyl ring compound (Structure I). The 5-aminothiophene analog, II, is considerably more effective as a growth inhibitor than is the 4-amino isomer (Fig. 1). The medium of cultures containing II turned a violet-brown after 24 hr.

The growth inhibition by II is much greater than I when tested on *Salmonella*, *Streptococcus*, and *Proteus* (Table I). Growth rate was significantly

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[1] Streptococcus, human type, "AHT," was obtained from D. D. Zinner.

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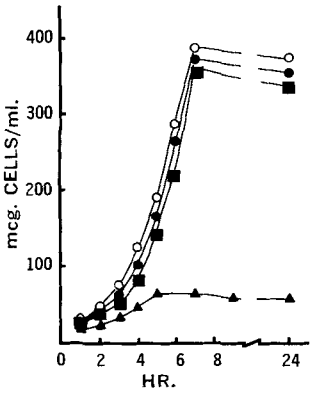

Fig. 1—Growth of Streptococcus *in the presence and absence of I and thiophene analogs of I. All drugs at 500 mcg./ml. Key: ○, control (no drugs); ●, I; ■, III; ▲, II.*

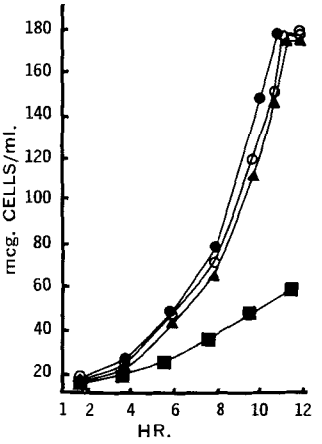

Fig. 2—Inhibition of Streptococcus *by IV and its reversal by PABA or folic acid. IV was added to 5 mcg./ml. Key: ○, control (no drugs); ■, IV only; ●, IV + 300 mcg./ml. PABA; ▲, IV + 100 mcg./ml. folic acid.*

slowed by II, which about doubled the time between cell divisions at concentrations at which I had no significant effect (Table I). Substitution of a pyrimidine on the thiophene sulfonamide nitrogen (Table I) produced a drug which was much more active than I.

Nitrothiophene Analogs As More Effective Growth Inhibitors Than Aminothiophene Analogs—The nitrothiophene compounds were both more inhibitory than the corresponding aminothiophene compounds (Table I). For example, I had no effect on *Salmonella* growth at 1 mg./ml., II inhibited *Salmonella* growth by 60% at 1 mg./ml., but both III and IV inhibited *Salmonella* growth by over 95% at concentrations of only 100 mcg./ml. (Table I). At 100 mcg./ml., IV was a more effective growth inhibitor than III against both *Salmonella* and *Streptococcus* (Table I).

Based on relative inhibition per mcg./ml. concentration, IV is about 1,000-fold more effective than I (*cf.* Fig. 2 and Table I).

Mechanism of Bacterial Growth Inhibition: Inhibition Reversal by Folate and p-Aminobenzoate—BHI growth medium contains folic acid and its metabolites. After limited growth at the slower rate by *Streptococcus* in the presence of II, the culture was found to be sterile: no viable cells could be found by the plating-out procedure using drug-free BHI or ST agar (9). The latter observation was

Table I—Effect of Sulfanilamide and its Thiophene Analogs on Growth of Three Diverse Bacteria

Drug Name Microorganism	Drug Concn., mcg./ml.	7-Hr. Growth, mg./L.	Inhibition, %	Division Time, min.
Streptococcus AHT				
None	—	400	—	75
Sulfanilamide (I)	1,000	300	25	85
	500	400	0	75
4-Aminothiophene-2-sulfonamide (III)	1,000	250	40	—
5-Aminothiophene-2-sulfonamide (II)	1,000	100	75	150
	500	150	60	—
4-Nitrothiophene-2-sulfonamide (V)	1,000	25	95	>600
	500	200	50	—
	100	335	15	—
5-Nitrothiophene-2-sulfonamide (IV)	1,000	20	95	—
	500	25	95	>600
	100	25	95	—
2-(5-Aminothiophene-2-sulfonamido) pyridine (VI)	500	300	25	100
2-(5-Aminothiophene-2-sulfonamido) pyrimidine (VII)	1,000	115	70	—
	500	250	40	—
Salmonella typhimurium				
None	—	4,700	—	26
Sulfanilamide (I)	1,000	4,700	0	27
5-Aminothiophene-2-sulfonamide (II)	1,000	1,850	60	50
4-Nitrothiophene-2-sulfonamide (V)	100	200	95	100
5-Nitrothiophene-2-sulfonamide (IV)	100	35	99	190
Proteus vulgaris				
None	—	3,000	—	28
Sulfanilamide (I)	1,000	2,250	25	30
5-Aminothiophene-2-sulfonamide (II)	1,000	900	70	65

also made for *Streptococcus* exposed to either IV or V at the second lowest concentrations used (Table I), suggesting the organisms incorporate the drugs as they grow and the drugs then diminish some essential activity to a lethal extent, in a manner similar to the less effective I.

Consistent with the possible mode of action of these thiophene analogs in antagonizing *p*-aminobenzoate metabolism, II is probably closer in its three-dimensional structure to *p*-aminobenzoate than is III, since the large S atom essentially replaces two carbon atoms.

Experiments with *Streptococcus* using 100 mcg./ml. of the nitro compounds or 500 mcg./ml. of the amino compounds showed no significant inhibition reversal by 25 mcg./ml. of PABA except in the case of III. The inhibition by V (150 mcg./ml.) was reversed by 300 mcg./ml. PABA. The latter concentration of PABA also served to release the inhibition induced by 2–5 mcg./ml. of IV (Fig. 2).

The reversal of the IV-induced inhibition by PABA or folate (Fig. 2) suggests the biosynthesis of folate is the site of action of the nitrothiophene-2-sulfonamides as well as the amino compounds. This could be accomplished by intracellular reduction of the nitro compounds to the corresponding amino compounds, making the amino compounds the active forms. The same concentration of PABA (300 mcg./ml.), completely reversing effective inhibition by IV, produced only a slight reversal of the same extent of inhibition by II.

However, nothing is yet known about the relative transport rates of these thiophenes into cells, so no conclusions can be drawn about the relative effective concentration; it appears the nitro compounds, if they are reduced to the amino form, may be transported to the site of intracellular activity at a greater rate than the corresponding amino compound.

Antibacterial Activity of II and IV Exceeds That of Similar Compounds—The carboxamide corresponding to II, 5-aminothiophene-2-carboxamide, was inactive against *Streptococcus*, *Escherichia*, or *Diplococcus*, even at concentrations many times that at which sulfanilamide was active (10). The 5-amino-3-thiophene sulfonamide isomer of II was not inhibitory to a wide variety of bacterial genera concentrations at least tenfold greater than those used for II (11). Thus, II is a new structure with much increased biological activity over similar structures.

Although the 5-nitrothiophene-3-sulfonamide isomer has antibacterial activity, it is 500–1,000-fold less active against *Streptococcus* per μM concentration than IV (6). More significantly, compared with the data of Woods on sulfanilamide reversal (1), IV is at least 10,000-fold less susceptible to reversibility by PABA than is sulfanilamide.

In Vivo Toxicity—The substitution of the thiophene ring for the phenyl ring of I increases the water solubility and thus decreases the partition coefficient. Such changes are less significant to this study *in vitro* than they would be to study of the drug in pathogen-infected animals. Although a few mcg./ml. of IV appeared to be bactericidal, a daily injection (5 days) of 100 mg./Kg. of IV to adult Swiss mice was tolerated with a reversible weight loss. This suggests a body fluid level of up to several hundred mcg./ml. could be attained.

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Keyphrases

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Structure–activity relationship—sulfanilamide analogs
Antibacterial activity—*in vitro*
Toxicity—*in vivo*, *in vitro*