Synthesis of Sulfanilamido-Naphthoquinones as Potential Antituberculous Agents

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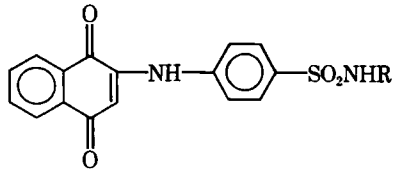
Abstract □ *p*-Aminobenzoic acid, the acid hydrazides of benzoic acid, salicylic acid and isonicotinic acid, and 4,4′-diaminodiphenyl sulfone were condensed with *p*-aminobenzenesulfonyl chloride to give the corresponding N^1-substituted sulfanilamides. These molecules were then treated with 1,4-naphthoquinone to yield 2-substituted-1,4-naphthoquinones. The partition coefficient for the substituted quinones showed, in some cases, high diffusion rates to the organic phase, benzene, from physiological Tyrode's solution. These compounds are effective in low concentration in dioxane against the human sensitive strain of *Mycobacterium tuberculosis* H_{37} Rv. Sulfanilamides obtained from *p*-aminosalicylic acid and thiosemicarbazide failed to react with 1,4-naphthoquinone. These sulfanilamides also showed high activity against the same *Mycobacterium*.

Keyphrases □ Antituberculous agents—potential, synthesis of sulfanilamido-naphthoquinones □ Sulfanilamides—synthesis of sulfanilamido-naphthoquinones as potential antituberculous agents

It has been postulated that the penetration through the Tubercle bacilli cell wall is a limiting factor in the effectiveness of the tuberculostatic agents. Consequently, trials were made to improve the cell penetration of these drugs. Surface active agents (1, 2), used to promote cell penetration, were found to be effective through modification of the surface lipids of the bacilli (3, 4).

The isolation of phthiocol (2-hydroxy-3-methyl-1,4-naphthoquinone) from the human bacilli (5, 6), indicated that the compound could be a natural metabolite of the organism; thus showing the tolerance of the latter to the *p*-quinonoid structure. Earlier reports have also indicated the use of *p*-quinonoid moieties as carriers for certain tuberculostatic agents (7, 8). These facts, beside the high lipid solubility of naphthoquinone suggested using 1,4-naphthoquinone as a carrier of certain sulfonamides, with the objective that the *p*-quinonoid moiety will facilitate the diffusion of the compounds through the cell wall giving them a better opportunity to exert their effects on the organism. The present work is concerned with the synthesis of 2-(substituted sulfanilamido)-1,4-naphthoquinones:



The routes by which these compounds were synthesized are shown in Scheme I.

RESULTS AND DISCUSSION

Chemistry—The key intermediates, sulfanilamide compounds, were prepared by adding *p*-acetamidobenzenesulfonyl chloride to *p*-aminobenzoic acid, *p*-aminosalicylic acid, isonicotinic acid hydrazide, benzoic acid hydrazide, salicylic acid hydrazide, and 4,4′-diaminodiphenyl sulfone according to a previously used technique (9). The products were then deacetylated. The sulfanilamide compounds obtained were allowed to react with 1,4-naphthoquinone. The 2-substituted-1,4-naphthoquinones were obtained, where the sequence of the reaction could follow the same route proposed for the reaction of the arylamine and 1,4-naphthoquinone (10, 11). The sulfanilamides obtained from *p*-aminosalicylic acid and thiosemicarbazide (II, VII) did not react with the quinone, while (VI) the ethyl ester of (II) underwent the reaction successfully. The sulfanilamide obtained from *p*-aminobenzoic acid was esterified after combining it with the 1,4-naphthoquinone (XVI, XVII). The unsubstituted 4,4′-diaminodiphenyl sulfone reacted with one molecule of 1,4-naphthoquinone (XV) and the same compound, condensed as sulfanilamide derivative, reacted with two molecules of the quinone (XIV).

Table I—Partition Coefficient $\left(\frac{A_2}{A_1 - A_2}\right)$ between Benzene and Tyrode's Solution [a]

Compound	A_1	A_2	$\frac{A_2}{A_1 - A_2}$
IX	0.085	0.050	1.43
X	0.050	0.010	0.25
XI	0.390	0.345	7.67
XII	0.650	0.640	64.00
XIII	0.055	0.030	1.20
XIV	0.620	0.600	30.00
XV	0.380	0.380	α
XVI	0.065	0.055	5.50
XVII	0.030	0.010	0.50

[a] Absorbance measured at wavelength 450 nm.

Table II—Sensitivity Pattern of *Mycobacterium tuberculosis* H_{37} Rv Against Tested Compounds

Compound	Concentrations in μg/ml in Dioxane I	II	III
II	1.0 R[a]	1.5 S	2.0 S
VII	1.0 S[b]	1.5 S	2.0 S
IX	0.5 S	1.0 S	2.0 S
X	0.06 R	0.12 S	0.24 S
XI	0.06 R	0.12 S	0.24 S
XII	0.03 S	0.06 S	0.12 S
XIII	1.0 R	1.5 S	2.0 S
XIV	1.0 R	1.5 R	2.0 S
XV	1.0 R	1.5 S	2.0 S
XVI	1.0 R	1.5 R	2.0 S
XVII	1.0 R	1.5 R	2.0 S

[a] Resistant. [b] Sensitive.

The purity and the identity of the compounds were determined by TLC on silica gel using different solvent systems, melting points (uncorrected), IR spectra using a spectrophotometer[1] as nujol mull on sodium chloride plates and elemental microanalysis.

A number of the compounds prepared (IX–XVII) showed high diffusion rates to the organic solvent, benzene, from physiological Tyrode's solution. The values of the partition coefficient between the solvents were calculated using the ratio $A_2/A_1 - A_2$, where A_1 and A_2 are the absorbances in benzene before and after extraction with the Tyrode's solution at wavelength 450 nm (Table I).

Biology—Most of the prepared compounds proved to be effective in low concentrations against the human strain of *Mycobacterium tuberculosis* H_{37} Rv[2].

A concentration of 0.1 μg/ml wet weight of 3-week-old cultures of the *Mycobacterium* was found suitable. Lowenstein–Jensen medium without

[1] Unicam 2000.
[2] Received from National Collection of Type Cultures, UK.

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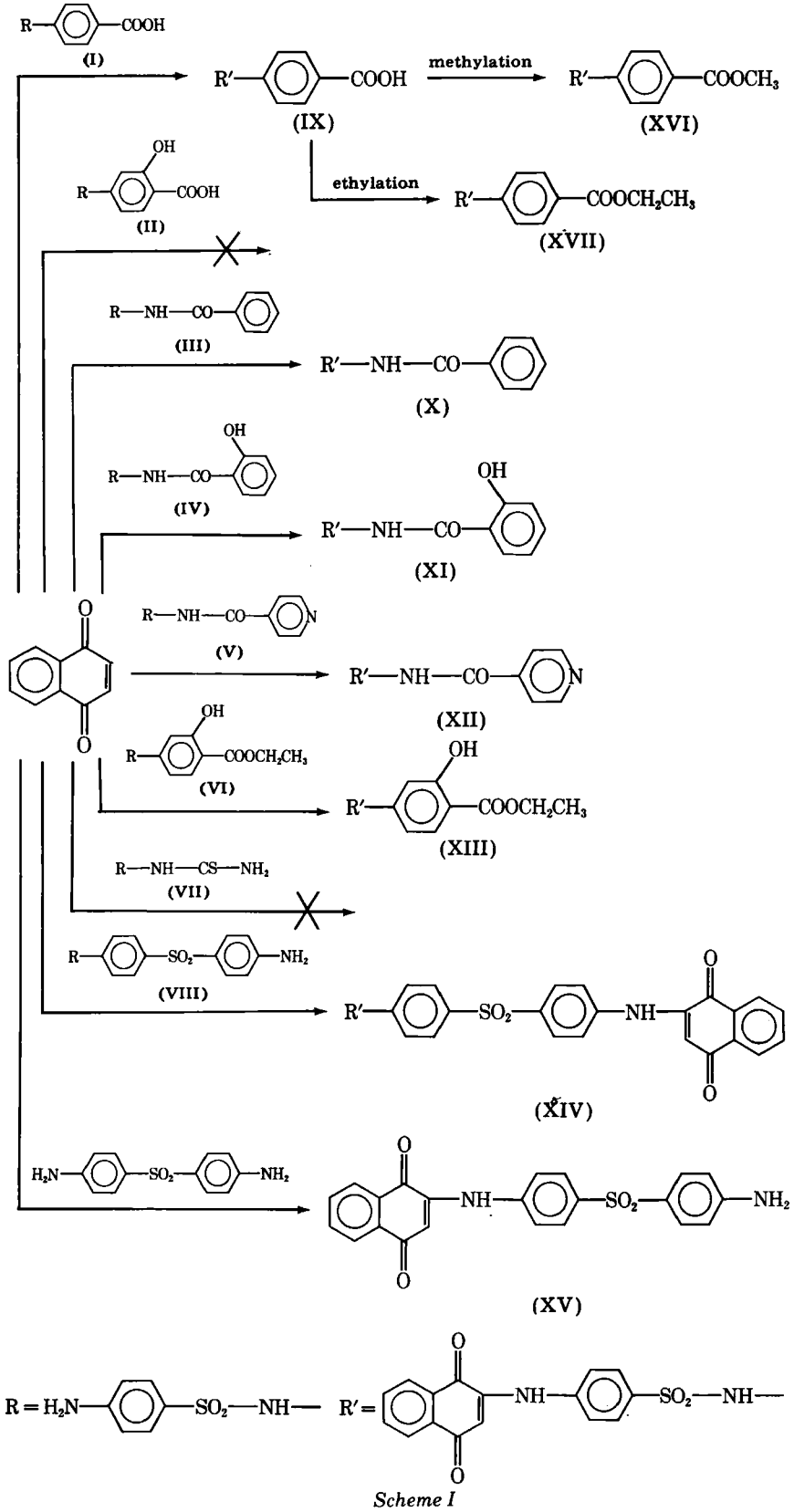


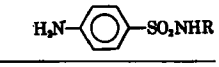
Scheme I

potato was used. No growth or growth of <20 colonies indicated that the strain was sensitive to the compounds in the given concentrations, provided that the compound-free control yielded >100 colonies.

All compounds tested were found effective against the human sensitive strain of the *Mycobacterium* in concentrations of 1–2 μg/ml. However, compounds IX–XII gave the same effect in lesser concentrations (Table II).

On comparing data from Tables I and II, structural variations of the compounds appear to show uncorrelated effects on the activities and the rates of diffusion of the compounds into the organic phase. Some of the highly active compounds were also highly diffusible, *e.g.*, compounds XI and XII. Such a relationship does not hold with the other compounds. A detailed comparative study of the effect of the structural variations involving quinonoid and unquinonoid compounds on both activities and

Table III—The *p*-Aminobenzenesulfonamide Derivatives



Compound	R	mp°	Yield, %	Molecular Formula	Analysis Calc.	Analysis Found
I	*p*-Carboxyphenyl	158	52	$C_{13}H_{12}N_2O_4S$	C: 53.42	53.09
					H: 4.14	4.05
					N: 9.58	9.36
					S: 10.97	11.08
II	*p*-Carboxy-*m*-hydroxyphenyl	165—166	38	$C_{13}H_{12}N_2O_5S$	C: 50.64	50.40
					H: 3.89	3.97
					N: 9.09	9.01
					S: 10.38	10.73
III	Benzamido	185–186	52	$C_{13}H_{13}N_3O_3S$	C: 53.60	53.23
					H: 4.50	4.63
					N: 14.43	14.24
					S: 11.01	10.68
IV	*o*-Hydroxybenzamido	164–165	46	$C_{13}H_{13}N_3O_4S$	C: 50.81	50.80
					H: 4.26	4.34
					N: 13.67	13.62
					S: 10.43	10.52
V	*p*-Pyridoylamino	210–211	59	$C_{12}H_{12}N_4O_3S$	C: 49.31	49.39
					H: 4.14	4.30
					N: 19.17	19.08
					S: 10.97	10.83
VII	Thiouriedo	223	42	$C_7H_{10}N_4O_2S_2$	C: 34.14	34.14
					H: 4.06	4.14
					N: 22.76	22.81
					S: 26.01	26.03
VIII	[*p*(*p*-Aminobenzene) sulfonyl]phenyl	194–195	90	$C_{18}H_{17}N_3O_4S_2$	C: 53.58	53.08
					H: 4.25	4.17
					N: 10.42	10.53
					S: 15.86	15.45

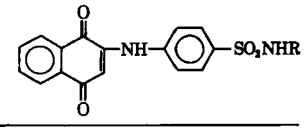


Table IV—The Sulfanilamido-1,4-naphthoquinone Derivatives

Compound	R	mp°	Yield, %	Molecular Formula	Analysis Calc.	Analysis Found
IX	*p*-Carboxyphenyl	245–245	93	$C_{23}H_{16}N_2O_6S$	C: 61.60	62.03
					H: 3.57	3.70
					N: 6.25	6.06
					S: 7.41	6.98
X	Benzamido	258–259	38	$C_{23}H_{17}N_3O_5S$	C: 61.75	62.10
					H: 3.80	3.71
					N: 8.39	7.97
					S: 7.15	7.26
XI	*o*-Hydroxybenzamido	263–264	50	$C_{23}H_{17}N_3O_6S$	C: 59.61	60.03
					H: 3.67	3.89
					N: 9.09	8.86
					S: 6.91	6.76
XII	*p*-Pyridolyamino	248–249	40	$C_{22}H_{16}N_4O_5S$	C: 58.93	58.59
					H: 3.56	3.81
					N: 12.50	12.08
					S: 7.14	6.74
XIII	*p*-Carboethoxy-*m*-hydroxyphenyl	268–269	30	$C_{25}H_{20}N_2O_7S$	C: 60.77	60.27
					H: 4.06	3.98
					N: 5.69	5.66
					S: 6.50	6.86
XIV	(*p*-{*p*-[2-(1,4-naphthoquinonyl)]aminobenzene}sulfonyl)phenyl	310–311	92	$C_{38}H_{25}N_3O_8S_2$	C: 63.98	64.25
					H: 3.49	3.92
					N: 5.87	6.03
					S: 8.25	7.84

rates of diffusion and their relationship to each other is presently being conducted.

EXPERIMENTAL

All compounds mentioned in Tables III and IV were prepared by the following general procedures. All the figures of analysis and the IR spectra of the compounds were in agreement with the structures.

***p*-Acetamidobenzenesulfanilamido Derivatives**—*p*-Acetamidobenzenesulfonyl chloride (10 mmoles) was added gradually to a solution of the appropriate derivative (20 mmoles) in 2 *N* NaOH solution (30 ml) with constant stirring. The stirring continued for 10–15 min. The alkaline solution was extracted with ether, then acidified with hydrochloric acid, when the *p*-acetamidobenzenesulfonamido compound separated. The compound was then filtered and crystalized from 95% ethyl alcohol. The IR spectra of the pure compounds showed absorption bands at 1740 cm^{-1} (C=O in $COCH_3$), 1600—1590 cm^{-1} (C=C aromatic), 1680 cm^{-1}, 1570 cm^{-1} (CONH amides I & II), 1370–1335 cm^{-1}, 1180 cm^{-1} (SO_2NH) beside others for different compounds.

Benzenesulfanilamido Derivatives—The hydrolysis (deacetylation) of the *p*-acetamidobenzene derivatives was carried out either by heating with alkali on a steam bath or by gently refluxing for 30 min with twice its weight of diluted hydrochloric acid. The IR spectra of the hydrolyzed compounds showed the disappearance of the C=O band of the $COCH_3$ group at 1740 cm^{-1} and the appearance of a typical primary N—H pair of bands at 3300 and 3130 $cm.^{-1}$ (Table III).

***N*¹-(*p*-Carboethoxy-*m*-hydroxyphenyl) Sulfanilamide (VI)**—A solution of II (10 mmoles) was esterified by warming with excess 95% ethyl alcohol in the presence of concentrated sulfuric acid. The ethyl ester, mp 230–231° was obtained in a 60% yield. The IR spectrum marked the disappearance of the bands at 1690 cm^{-1} (C=O in COOH) and 3000 cm^{-1}

(OH carboxylic) and the appearance of the band at 1665 cm^{-1} (C=O in $COOC_2H_5$).

Anal.—Calc. for $C_{15}H_{16}N_2O_5S$: C, 53.56; H, 4.79; N, 8.33; S, 9.53. Found: C, 53.06; H, 4.71; N, 8.30; S, 9.66.

Sulfanilamido-1,4-naphthoquinone Derivatives—A solution of 1,4-naphthoquinone (20 mmoles in 40 ml of 95% ethyl alcohol) was gradually added over a period of 30 min to a solution of the appropriate sulfanilamido derivative (10 mmoles in 10–30 ml of glacial acetic acid). Sodium acetate (20 mg) was added with constant stirring at 60°. Stirring was continued for 30 min, then refluxed for 1 hr, and left overnight at room temperature. A black precipitate was separated and filtered. Water was added to the filtrate when a brownish material separated. It was then filtered, washed with hot water, dried at 80°, and crystallized from 95% ethyl alcohol as light crystals. The IR spectra of the compounds showed the appearance of two bands at 1650 and 1630 cm^{-1} for the C=O of the naphthoquinone. The pair of bands of the primary NH_2 at 3300 and 3130 cm^{-1} disappeared, while the N—H stretching absorption was shown at 3245 cm^{-1} (Table IV).

4-Amino-4′-[2-(1,4-naphthoquinonyl)]aminodiphenylsulfone (XV)—This was obtained by the same procedure for the preparation of the sulfanilamido-1,4-naphthoquinone derivatives, from 1,4-naphthoquinone (20 mmoles in 40 ml of 95% ethyl alcohol) and 4,4′-diaminodiphenyl sulfone (10 mmoles in 35 ml of glacial acetic acid). The product was crystallized from 95% ethyl alcohol. Yield: 91%, mp 306–307°.

Anal.—Calc. for $C_{22}H_{16}N_2O_4S$: C, 65.34; H, 3.96; N, 6.93; S, 7.92. Found: C, 65.25; H, 4.14; N, 6.72; S, 7.71.

2-{N^4-[N^1-(*p*-Carbomethoxy)phenyl]sulfanilamido}-1,4-naphthoquinone (XVI)—A solution of IX (10 mmoles) was esterified by warming with excess methyl alcohol in the presence of concentrated sulfuric acid. The methyl ester recrystallized from glacial acetic acid was obtained in 37% yield, mp 253°. In the IR spectrum the bands at 1700 cm^{-1} (C=O in COOH) and 3245 cm^{-1} (OH carboxyl) disappeared and a band at 1750 cm^{-1} (C=O in $COOCH_3$) appeared.

Anal.—Calc. for $C_{24}H_{18}N_2O_6S$: C, 62.34; H, 3.89; N, 6.06; S, 6.90. Found: C, 61.93; H, 3.67; N, 5.64; S, 6.68.

2-[N^4-[N^1-(*p*-Carboethoxy)phenyl]sulfanilamido}-1,4-naphthoquinone (XVII)—Obtained as XVI from IX by esterification with 95% ethyl alcohol. Yield: 35%, mp 261–262°. In the IR spectrum the bands at 1700 cm^{-1} (C=O in the carboxyl group) and 3245 cm^{-1} (OH carboxyl) disappeared, and a band at 1665 cm^{-1} (C=O in $COOC_2H_5$) appeared. appeared.

Anal.—Calc. for $C_{25}H_{20}N_2O_6S$: C, 63.02; H, 4.20; N, 5.88; S, 6.72. Found: C, 62.68; H, 3.90; N, 5.48; S, 6.82.

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High-Performance Liquid Chromatographic Determination of Alizapride, a New Antiemetic Compound, and Its Application to a Dose-Dependent Pharmacokinetic Study

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Received November 20, 1980, from the **Department of Pharmacology, Faculty of Medicine of Paris XII, 8 Rue du Général Sarrail—94000 Creteil*, and the ‡*Department of Medical Statistics, Hopital du Bocage—21000 Dijon, France.* Accepted for publication March 4, 1982.

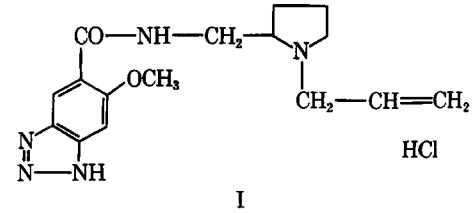
Abstract □ An assay was developed to measure alizapride (a new antiemetic compound) in biological specimens. The method involved reversed-phase high-performance liquid chromatography and fluorescence detection. The detection limit was 5 ng/ml in plasma or urine samples. The value of the assay was demonstrated with a dose-dependent pharmacokinetic study. It showed a two-phase decrease in plasma concentrations, after intravenous injection, with half-lives of 7.5-min and 2.5-hr, respectively. From plasma and urine results, pharmacokinetic parameters remained constant in the dose range of 50–200 mg.

Keyphrases □ High-performance liquid chromatography—determination and dose-dependent pharmacokinetic application of alizapride, new antiemetic compound □ Alizapride—new antiemetic compound, high-performance liquid chromatographic determination and dose-dependent pharmacokinetic application □ Antiemetic compound—high-performance liquid chromatographic determination and dose-dependent pharmacokinetic application of alizapride □ Pharmacokinetic application—dose-dependent, alizapride, new antiemetic compound, high-performance liquid chromatographic determination

Alizapride[1], *N*-[(1-allyl-2-pyrrolidinyl)methyl]-6-methoxy-1*H*-benzotriazole-5-carboxamide (I), is a new compound with antiemetic properties (1, 2). For bioavailability studies a sensitive and specific assay for plasma and urine concentrations was needed. According to the physicochemical properties of the compound, a high-performance liquid chromatographic (HPLC) assay with a fluorescence detector was selected. This study describes the procedure used for the analysis of biological samples and the preliminary results on pharmacokinetic parameters calculated in a dose-dependency study.

EXPERIMENTAL

Materials—Alizapride was obtained from commercial suppliers and showed no impurities in two different TLC systems. Methanol, chloroform, and tris(hydroxymethyl)aminomethane buffer (pH 8.1) were commercially available analytical grades and used without further purification.



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[1] Plitican, Delagrange Laboratories, Paris, France.

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