us the opportunity to be able to make predictions regarding inactivation mechanisms for hypothetical new structural classes of inactivators. Since the different mechanistic pathways lead to different types of enzyme adducts, inactivator design may be driven by the class of adduct that is desired. This mechanistic approach should be useful to the rational design of GABA aminotransferase inactivators and, therefore, of potential anticonvulsant drugs.

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Articles

Synthesis and Antitubercular Activity of *N*-(2-Naphthyl)glycine Hydrazide Analogues

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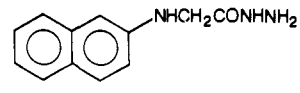
N-(2-Naphthyl)glycine hydrazide analogues were synthesized and tested for possible in vitro antitubercular activity. *N*-(2-Naphthyl)alanine hydrazide (**3**), *N*-methyl-*N*-(2-naphthyl)glycine hydrazide (**5**), *N*-(6-methoxy-2-naphthyl)glycine hydrazide (**7**), and 3-(2-naphthylamino)butyric acid hydrazide (**23**) showed potent inhibitory action against *Mycobacterium tuberculosis* $H_{37}R_v$ in Youman's medium at concentrations ranging from 0.5 to 10.0 μg/mL. These compounds showed significant inhibitory action against isonicotinic acid hydrazide and streptomycin-resistant strains of *M. tuberculosis*. *N*-(6-Quinolyl)glycine hydrazide (**18**) and 3-(2-quinolylamino)butyric acid hydrazide (**24**), which are bioisosteres of compounds **1** and **23**, showed loss of antitubercular activity at low concentrations.

Tuberculosis is generally regarded as the most important chronic communicable disease in the world. This disease continues to be one of the major health problems in India and other South Asian countries. The main reason for this has been the emerging resistance of *Mycobacterium tuberculosis*, the causative organism of this communicable disease, to currently available antitubercular drugs. The major problems associated with chemotherapy of tuberculosis are emergence of resistant strains, the variation of causative species, the failure of current antitubercular drugs to eradicate the mycobacterial infections quickly, and severe toxicity of certain available antitubercular drugs. Hence, many studies have been attempted in the past to develop new antitubercular compounds in order to resolve some of these problems.[1-10] Earlier, we found *N*-(2-naphthyl)glycine hydrazide (**1**) and its dihydrochloride to be potent in vitro inhibitors of *M. tuberculosis* $H_{37}R_v$ and the toxicity of compound **1** dihydrochloride was lower in animals compared to that of the primary antitubercular drug isonicotinic acid hydrazide.[7] Compound **1** dihydrochloride had certain favorable in vivo antitubercular activity.[11,12] Some of the favorable biological activities observed with compound **1** in our earlier studies[7,13] gave the impetus to synthesize new analogues of compound **1** and study their possible in vitro antitubercular activity. This paper describes the synthesis of *N*-(2-naphthyl)-glycine hydrazide analogues and their in vitro antitubercular activity.

Chemistry

Hydrazides shown in Chart I were obtained by condensation of arylamines with ethyl haloacetate in aqueous medium followed by hydrazinolysis in ethanol as described in the Experimental Section.

Chart I



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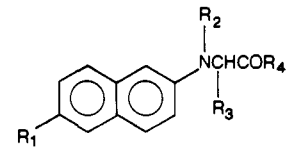
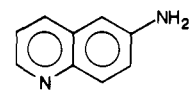
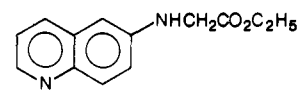


2: R_1 = H; R_2 = H; R_3 = CH_3; R_4 = OC_2H_5
3: R_1 = H; R_2 = H; R_3 = CH_3; R_4 = $NHNH_2$
4: R_1 = H; R_2 = CH_3; R_3 = H; R_4 = OC_2H_5
5: R_1 = H; R_2 = CH_3; R_3 = H; R_4 = $NHNH_2$
6: R_1 = OCH_3; R_2 = H; R_3 = H; R_4 = OC_2H_5
7: R_1 = OCH_3; R_2 = H; R_3 = H; R_4 = $NHNH_2$
8: R_1 = OCH_3; R_2 = H; R_3 = CH_3; R_4 = OC_2H_5
9: R_1 = OCH_3; R_2 = H; R_3 = CH_3; R_4 = $NHNH_2$
10: R_1 = H; R_2 = H; R_3 = H; R_4 = OH

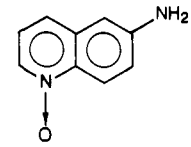
Chart II



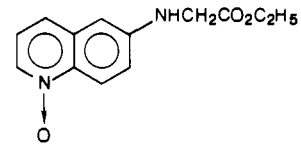
11



12



13



14

Reactions of various aromatic amines with ethyl haloacetate produced the corresponding condensed product

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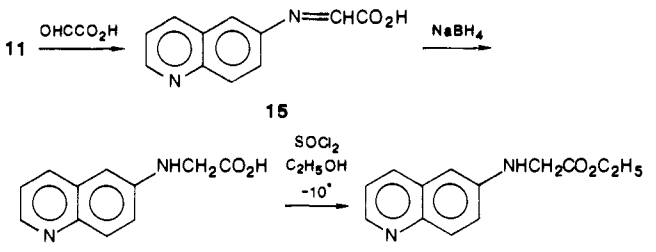
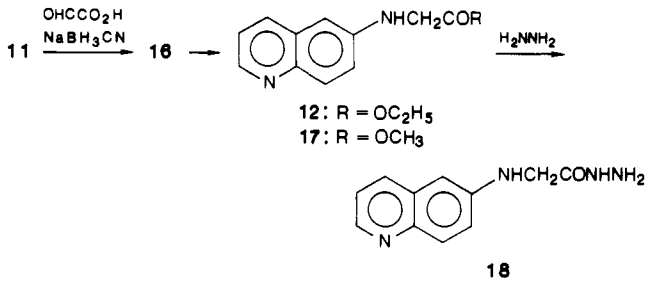
0022-2623/89/1832-2421$01.50/0

Scheme I

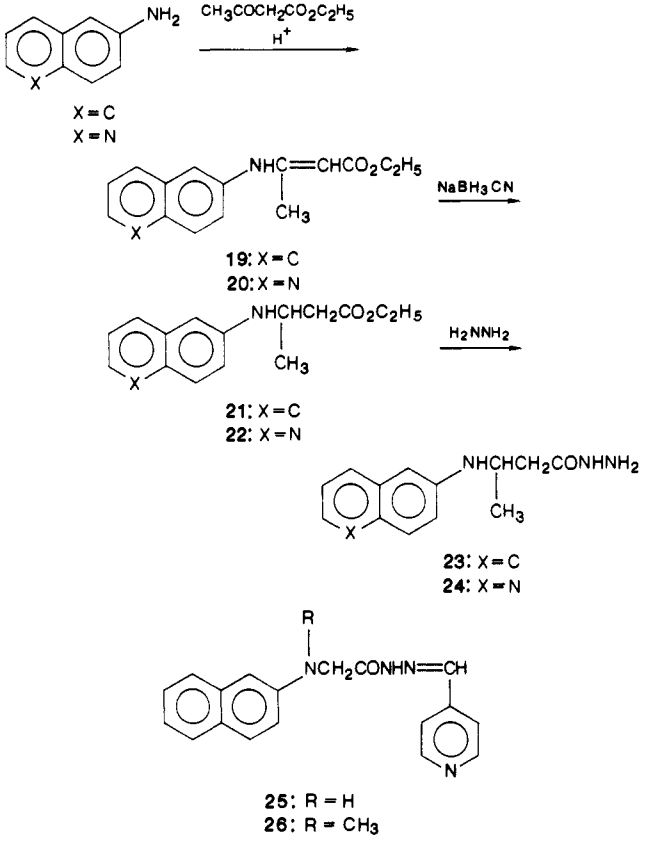


Scheme II



(Chart I) in good yield. But the reaction of 6-aminoquinoline (11) with ethyl haloacetates under a variety of conditions did not yield the expected product (12). Failure to get the expected product (12) could possibly be due to quaternization of ethyl haloacetates in the ring nitrogen of 6-aminoquinoline (11). Hence, as given in Chart II, the ring nitrogen was protected as an *N*-oxide (13)[14] with the aim that the expected product (12) could ultimately be obtained by cleavage of *N*-oxide 14 using a suitable reagent. A series of experimentation for condensation of 6-aminoquinoline *N*-oxide (13) with ethyl haloacetates have failed to yield the desired product (14). Thus, we turned our attention toward utilization of glyoxylic acid in order to get a Schiff base with compound 11. When we treated 6-aminoquinoline (11) with glyoxylic acid, it resulted in a highly hygroscopic bright yellow compound, possibly the Schiff base 15 (Scheme I). The Schiff base 15 upon reduction with $NaBH_4$ using ethanol as a solvent gave a product that was identified as *N*-(6-quinolyl)glycine (16)

Scheme III



by IR and NMR spectra. Compound 16 upon esterification by the $SOCl_2$–ethanol method[15] gave the desired product (12). However, the overall yield obtained was poor. Hence we planned to obtain product 16 by reductive amination of glyoxylic acid with 6-aminoquinoline (11) using $NaBH_3CN$ (Scheme II). When we carried out reductive amination of glyoxylic acid with 6-aminoquinoline (11) using $NaBH_3CN$[16] in methanol, it gave a poor yield of *N*-(6-quinolyl)glycine (16). The change of solvent system to THF–methanol (15:1) in the above reductive amination reaction did not improve the yield. However, reductive amination of glyoxylic acid with compound 11 in aqueous acetonitrile resulted in good yield of compound 16. The compound was converted to esters 12 and 17, which was followed by hydrazinolysis to yield *N*-(6-quinolyl)glycine hydrazide (18).

Treatment of 2-naphthylamine and 6-aminoquinoline with ethyl acetoacetate in the presence of a catalytic amount of HCl gave corresponding crotonates 19 and 20 in an 85% yield (Scheme III). *N*-(2-Naphthyl)crotonate (19) and *N*-(6-quinolyl)crotonate (20) were reduced with $NaBH_3CN$ in THF–methanol (15:1) to yield ethyl 3-(2-naphthylamino)butyrate (21) and ethyl 3-(6-quinolylamino)butyrate (22). Upon hydrazinolysis these esters (21 and 22) gave hydrazides 23 and 24. Schiff bases 25 and 26 were obtained by the treatment of isonicotinaldehyde with the corresponding hydrazides 1 and 5.

Biological Evaluation and Discussion

All hydrazides synthesized were tested for in vitro antitubercular activity against *M. tuberculosis* $H_{37}R_v$ in Youman's medium (nonprotein medium) and in the presence of serum. Further, some of these compounds

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Table I. In Vitro Antitubercular Activities of Naphthylglycine Hydrazide Analogues on *M. tuberculosis* $H_{37}R_v$

compd	concn (μg/mL) at complete (100%) growth inhibn[a]		concn (μg/mL) at partial (50 ± 10%) growth inhibn[a]	
	A[b]	B[c]	A	B
1	1	50	0.5	10
3	1	50	0.5	10
5	10	50	2.0	10
7	10	50	5.0	10
9	50	100	10	50
10	1000	–	100	–
16	100	100	10	50
18	100	100	25	50
23	10	100	5	50
24	100	150	50	100
25	1	100	0.5	10
26	10	100	1	10
INH	1	1	0.1	0.5

[a] The growth inhibition observed at the end of 21 days. [b] A = Youman's medium. [c] B = Youman's medium with 10% (v/v) bovine serum.

were evaluated for antitubercular activity against INH and streptoymcin-resistant strains (clinical isolates).

Hydrazides such as glycine hydrazide and alanine hydrazide have been reported[17] to possess in vitro antitubercular activity at concentrations greater than 300 μg/mL. Our earlier studies, especially *N*-aryl substitution of glycine hydrazide with the *N*-(2-naphthyl) group, resulted in a potent inhibitor, compound **1**, which inhibited *M. tuberculosis* $H_{37}R_v$ at a concentration of 1 μg/mL.[7] The dihydrochloride of compound **1** was less toxic to animals in comparison to the primary antituberular drug INH. Compound **1** dihydrochloride showed certain favorable in vivo antitubercular activity, but its activity was lower compared to that of INH.[11,12] The lowering of in vivo activity possibly is due to the binding of compound **1** to serum albumin. One approach to resolve this problem is to know whether analogues of compound **1** retain potent in vitro antitubercular activity in Youman's medium (nonprotein medium). Hence, analogues of compound **1** were synthesized and evaluated for possible antitubercular action. *N*-(2-Naphthyl)alanine hydrazide (**3**), wherein the glycine moiety of compound **1** had been changed to alanine, showed potent antitubercular activity at a concentration of 1 μg/mL (Table I). Thus, the changing of the glycine moiety in compound **1** to alanine showed retention of antitubercular activity in Youman's medium atlower concentrations. However, compound **3** exhibited lowered activity (about 50-fold) in the presence of bovine serum (Table I). *N*-Methyl-*N*-(2-naphthyl)glycine hydrazide (**5**), the aromatic N-methylated analogue of compound **1**, showed antitubercular activity at a concentration of 10 μg/mL. Its activity was lowered (about 50-fold) in the presence of bovine serum (Table I). Similarly, *N*-(6-methoxy-2-naphthyl)glycine hydrazide (**7**) showed antitubercular activity at a concentration of 10 μg/mL (Table I) and its activity was lowered (about 50-fold) in the presence of bovine serum (Table I). *N*-(6-Methoxy-2-naphthyl)alanine hydrazide (**9**), wherein the glycine moiety of compound **7** is replaced with an alanine moiety, showed antitubercular activity at a concentration of 50 μg/mL in Youman's medium (Table I). The activity of compound **9** was lowered (about 5-fold) compared to that of compound **7** in Youman's medium (Table I) and its activity was lowered (about 2-fold) in the presence of serum (Table I). *N*-(6-Quinolyl)glycine hydrazide (**18**), which is a bioisostere of compound **1** and expected to be more hydrophilic compared to compound **1**, showed antitubercular activity at a concentration of 100 μg/mL in Youman's medium. It had partial activity at a concentration of 10 μg/mL. Thus, bioisosteric substitution of a naphthalene ring in compound **1** with the quinoline ring results in a loss of antitubercular activity at lower concentrations in the in vitro conditions employed at normal physiological pH. However, it is known that pyrazinamide, a clinically useful antitubercular drug, when tested in vitro, is affected by the pH of the medium.[18,19] At normal physiological pH pyrazinamide showed little activity against *M. tuberculosis* $H_{37}R_v$ but at pH 5.0–5.5 it is highly active. To a lesser extent, the activity of nicotinamide is also affected by the pH of the medium.[20] In this context, it would be of interest to study the antitubercular activity of compound (**18**) at a lower pH. The ammonium salt of *N*-(6-quinolyl)glycine (**16**), an intermediate compound obtained during the synthesis of compound **18**, showed antitubercular activity at a concentration of 100 μg/mL and retained its activity at the same concentration in the presence of the serum. 3-(2-Naphthylamino)butyric acid hydrazide (**23**), the compound with a chiral center in the molecule and closely related to compound **3**, showed antitubercular activity at a concentration of 10 μg/mL in Youman's medium. Its activity was lower (about 10-fold) when compared with *N*-(2-naphthyl)alanine hydrazide (**3**), while its activity was lowered about 10-fold in the presence of the serum (Table I). 3-(2-Quinolylamino)butyric acid hydrazide (**24**), which is a bioisostere of compound **23**, showed antitubercular activity at a concentration of 100 μg/mL in Youman's medium (Table I). Thus, bioisosteric substitution of a naphthalene ring in compound **23** with the quinoline ring resulted in about a 10-fold decrease in activity.

Schiff bases such as 4-pyridal *N*-(2-naphthyl)glycinoyl hydrazone (**25**) and 4-pyridal *N*-methyl-*N*-(2-naphthyl)-glycinoyl) hydrazone (**26**) retained the antitubercular activities of the parent compounds **1** and **5** at lower concentrations (Table I). *N*-(2-Naphthyl)glycine (**10**), without the hydrazide group of parent compound **1**, was found to be inactive at 1 or 10 μg/mL. This suggests the requirement of hydrazide group in the parent molecule in order to exhibit antitubercular activity at a lower concentrations. *N*-(1-Naphthyl)glycine hydrazide, a positional isomer of naphthylglycine hydrazide, was found to be inactive at the 1 or 10 μg/mL level. The compound showed complete inhibition at a concentration of 100 μg/mL or more. This indicates the importance of the positional specificity of glycine hydrazide moiety in the naphthalene ring for antitubercular action.

One of the major problems in the use of most of the available chemotherapeutic agents is the emergence of resistant strains.[21] In order to control the emergence of resistant strains and to successfully manage tuberculosis by short-term chemotherapy,[22] it will be of interest to evaluate compound **1** and some of its analogues for possible

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Table II. In Vitro Antitubercular Activities of Naphthylglycine Hydrazides on Drug-Resistant Strain of *M. tuberculosis*

compd	concn (μg/mL) at complete (100%) growth inhibn[a]	concn (μg/mL) at partial (50 ± 10%) growth inhibn[a]
1	1	0.5
3	1	0.5
5	10	2
7	10	5
23	10	5
INH[b]	150	100
INH[c]	100	50
Streptomycin[d]	2500	1000

[a] The growth inhibition observed at the end of 28 days. [b] INH-resistant strain no. 22443. [c] INH-resistant strain no. 312. [d] *Streptomycin*-resistant strain no. 14029.

inhibitory action against drug-resistant strains of *M. tuberculosis*, particularly against INH-resistant strains. Since compound 1 does not have the structural features of 4-pyridinecarboxylic acid hydrazide (INH), except for the acid hydrazide group, it is unlikely that the mechanism by which this compound inhibits *M. tuberculosis* $H_{37}R_v$ will be the same as that of INH,[23] and hence is expected to have no cross resistance with INH-resistant strains. When we evaluated compounds 1 and 3 for antitubercular activity against INH-resistant strains, both compounds showed potent inhibitory action at a concentration of 1 μg/mL (Table II). Compounds 5, 7, and 23 also were active against INH-resistant strains, but at a concentration of 10 μg/mL (Table II). *M. tuberculosis* strains were found to be resistant to INH up to concentrations of 50 and 100 μg/mL. Compounds 1, 3, 5, 7, and 23 were also active against streptomycin-resistant strains of *M. tuberculosis* (Table II).

Thus in the present study some analogues of compound 1 were found to be potent inhibitors of *M. tuberculosis* $H_{37}R_v$ at concentrations ranging from 0.5 μg/mL to 10 μg/mL. *N*-(2-Naphthyl)glycine hydrazide (1), *N*-(2-naphthyl)alanine hydrazide (3), *N*-methyl-*N*-(2-naphthyl)glycine hydrazide (5), *N*-(6-methoxy-2-naphthyl)glycine hydrazide (7), and 3-(2-naphthylamino)butyric acid hydrazide (23) were found to be potent inhibitors of INH-resistant strains as well as streptomycin-resistant strains. There is an interesting report indicating that compound 1 inhibits DNA-dependent RNA polymerase of *M. tuberculosis* $H_{37}R_v$ at low concentrations, whereas it showed insignificant inhibition of the eukaryotic enzyme.[24] It will be of interest to study naphthylglycine hydrazide analogues, including quinoline analogues 18 and 24, for possible inhibitory action against RNA polymerase, as this will lead to the design of inhibitors based on blocking a critical enzyme in *M. tuberculosis* $H_{37}R_v$. Our earlier studies with compound 1 dihydrochloride showed certain favorable results in in vivo antitubercular evaluation. It will be of interest to evaluate compounds 3, 5, 7, and 23 for in vivo antitubercular activity either alone or in combination with the primary antitubercular drug INH for short-term chemotherapy. Further, synthesis of compounds resulting in the subsitution of a suitable hydrophilic group in the naphthalene ring or in its side chain of compounds 1, 3, 5, 7, and 23 could lead to newer inhibitors that retain activity in Youman's medium (nonprotein medium) without an increased reduction of antitubercular activity in the presence of the serum.

Experimental Section

Melting points and boiling points reported are uncorrected. The IR spectra were recorded on Perkin-Elmer Model 700, 137-B, 397, and 599 spectrometers. The NMR spectra were recorded on Varian T60 and Brucker WH-270 spectrometers. Chemical shifts are quoted related to tetramethylsilane (TMS) ($\delta = 0$ ppm) as internal standard. Before removal of solvents, all organic extracts were washed with water and brine and then dried over anhydrous sodium sulfate. Ion-exchange resin (Dowex-50, H^+ form) used was from Sigma Chemical Co., St. Louis, MO. Analytical results indicated by element symbols were within ±0.4% of the theoretical values. Elemental analysis were performed in the Analytical Chemistry section of Organic Chemistry Department, Indian Institute of Science, Bangalore, India.

Ethyl *N*-(2-Naphthyl)alaninate (2). A stirring mixture of 2-naphthylamine (4.0 g, 28 mmol), redistilled (±)-ethyl 2-bromopropionate (5.02 g, 27.7 mmol), and sodium acetate (4.6 g, 56 mmol) (dissolved in 5.0 mL of water) was heated at 90–93 °C for $2^1/_2$ h. The solid material obtained upon cooling was extracted into benzene. Evaporation of benzene gave a crude product which was crystallized from hexane to yield 4.65 g (68.5%) of ethyl *N*-(2-naphthyl)alaninate (2), mp 84–85 °C.[25]

***N*-(2-Naphthyl)alanine Hydrazide (3).** A solution of ethyl *N*-(2-naphthyl)alaninate (2.7 g, 11.1 mmol) and hydrazine hydrate (1.24 g, 24.8 mmol) in ethanol was refluxed for 10 h. The solvent and excess of reagent were distilled off in vacuo. The crude compound obtained was crystallized from ethanol to yield 1.8 g (71%) of *N*-(2-naphthyl)alanine hydrazide (3), mp 152–153 °C. Anal. ($C_{13}H_{15}N_3O$) C, H, N.

Ethyl *N*-Methyl-*N*-(2-naphthyl)glycinate (4). This was prepared from *N*-methyl-2-naphthylamine and ethyl chloroacetate, yield 82.3%, bp 187 °C/1 mm. Anal. ($C_{15}H_{17}NO_2$) C, H, N.

***N*-Methyl-*N*-(2-naphthyl)glycine Hydrazide (5).** This was prepared by hydrazinolysis of the ester 4, yield 80.6%, mp 135–136 °C (EtOH–H_2O). Anal. ($C_{13}H_{15}N_3O$) C, H, N.

Ethyl *N*-(6-Methoxy-2-naphthyl)glycinate (6). This was prepared from 2-amino-6-methoxynaphthalene and ethyl chloroacetate, yield 60%, mp 125–126 °C (hexane). Anal. ($C_{15}H_{17}NO_3$) C, H, N.

***N*-(6-Methoxy-2-naphthyl)glycine Hydrazide (7).** This was prepared by hydrazinolysis of ester 6, yield 80.3%, mp 167–168 °C (EtOH). Anal. ($C_{13}H_{15}N_3O_2$) C, H, N.

Ethyl *N*-(6-Methoxy-2-naphthyl)alaninate (8). This was prepared from 2-amino-6-methoxynaphthalene and (±)-ethyl 2-bromopropionate, yield 52.4%, mp 60–61 °C (petroleum ether). Anal. ($C_{16}H_{19}NO_3$) C, H, N.

***N*-(6-Methoxy-2-naphthyl)alanine Hydrazide (9).** This was prepared by hydrazinolysis of ester 8, yield 78.9%, mp 146–147 °C (EtOH). Anal. ($C_{14}H_{17}N_3O_2$) C, H, N.

Ethyl *N*-(6-Quinolyl)glycinate (12). Procedure A. To a stirring solution of 6-aminoquinoline[26] (11) (2.88 g, 20 mmol) in dry ether (100 mL) was added a solution of glyoxylic acid (1.84 g, 20 mmol) in dry ether (100 mL). Immediately a bright yellow precipitate was formed. The mixture was stirred further for 2 h and then filtered and washed with a small volume (10 mL) of dry ether. The yellow compound obtained, possibly the Schiff base 15 (2.7 g, 67.5%), was transferred immediately into a dessicator since it was a highly hygroscopic material. To the stirring solution of yellow compound 15 (2.0 g, 10 mmol) in dry ethanol (25 mL) was added solid $NaBH_4$ (0.65 g, 17.2 mmol), and the reaction mixture was set aside overnight at room temperature. The reaction mixture was then refluxed for 30 min and cooled, water was added (25 mL), and the precipitate formed was filtered. The filtrate was neutralized and evaporated in vacuo. The compound obtained was dissolved in a minimum amount of water, and the pH was adjusted to 6. Upon cooling, the precipitate formed was filtered and dried. The reduced product (0.2 g) showed the following: IR (Nujol) ν_{max} 1710 cm^{-1} (acid C=O); NMR

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(DMSO-d_6) δ 4.0 (s, 2 H, NCH_2), 6.6–8.5 (m, 6 H, Ar), which indicated formation of *N*-(6-quinolyl)glycine (**16**).

To a mixture of the above compound (**16**) (0.15 g) and dry ethanol (1.0 mL) cooled to −10 °C was added dropwise redistilled $SOCl_2$ (0.22 g) with good stirring. The reaction mixture was allowed to stir at room temperature overnight. Ethanol and excess thionyl chloride were evaporated in vacuo. The ester hydrochloride obtained was suspended in dry ether and neutralized by bubbling dry NH_3 gas. After the ammonium chloride was filtered, the ether extract was evaporated in vacuo. The viscous crude product obtained upon purification by thin-layer chromatography (ethyl acetate–hexane) (3:2) yielded 0.030 g of ethyl *N*-(6-quinolyl)glycinate (**12**), mp 121–122 °C (EtOH–H_2O).

Procedure B. To a stirring solution of 6-aminoquinoline (**11**) (0.72 g, 5 mmol) in acetonitrile (20 mL) was added 33% of aqueous glyoxylic acid (1.85 g, 20 mmol) solution (5.6 mL). To the above cold reaction mixture (bath temperature 3 °C) was added a solution of $NaBH_3CN$ (0.64 g, 10.2 mmol) in acetonitrile (20 mL) over a 20-min period. The reaction mixture was gradually raised to room temperature and allowed to stir for 48 h. Acetonitrile was evaporated in vacuo, water (20 mL) was added to the residue, the pH of the solution was adjusted to alkaline (~9.5), and unreacted amine was extracted with ether (5 × 30 mL). To the aqueous solution was added concentrated HCl (25 mL) and the mixture was stirred at 25 °C for 1 h. The solvent was evaporated in vacuo. The residue was dissolved in water (20 mL) and added to a Dowex 50 (H^+ form) column containing 600 molar equiv capacity. The column was washed with deionized water (2 L), and the compound was eluted with 1 N NH_4OH (1.2 L). The fraction, upon evaporation in vacuo, gave 0.5 g of ammonium *N*-(6-quinolyl)glycinate (**16**).

To dry ethanol (50 mL) saturated with dry HCl was added ammonium *N*-(6-quinolyl)glycinate (**16**) (0.55 g, 2.5 mmol). The reaction mixture was stirred overnight and then refluxed for 3 h. The alcohol was evaporated in vacuo, the ester hydrochloride was suspended in dry ether, and ammonia gas was bubbled. The ammonium chloride was filtered. Evaporation of ether gave ethyl *N*-(6-quinolyl)glycinate (**12**) (0.5 g, 86.9%), mp 121–122 °C (EtOH–H_2O). Anal. ($C_{13}H_{14}N_2O_2$) C, H, N.

Methyl *N*-(6-Quinolyl)glycinate (17). This was prepared by esterification of compound **16** with methanol, yield 82.4%, mp 67–68 °C (EtOH–H_2O). Anal. ($C_{12}H_{12}N_2O_2$) C, H, N.

***N*-(6-Quinolyl)glycine Hydrazide (18).** This was prepared by hydrazinolysis of either ester **12** or **17**, yield 76.5%, mp 168–169 °C (EtOH–H_2O). Anal. ($C_{11}H_{12}N_4O$) C, H, N.

Ethyl 3-(2-Naphthylamino)crotonate (19). To a mixture of 2-naphthylamine (4.3 g, 30.0 mmol) and ethyl acetoacetate (3.88 g, 29.8 mmol) was added a drop of methanolic HCl as a catalyst. The clean solution became turbid in 20–25-min period and then the reaction mixture was set aside at room temperature overnight. The crystals formed were washed with methyl alcohol (1.0 mL) and then recrystallized from aqueous methanol to give ethyl 2-naphthylamino crotonate (**19**) (6.53 g, 85.2%), mp 66–67 °C.[27]

Ethyl 3-(6-Quinolylamino)crotonate (20). This was prepared from 6-aminoquinoline (**11**) and ethyl acetoacetate by a similar procedure as described above for compound **19** except for evacuation of the crude reaction mixture in vacuo for 4 days, yield 78.1%; **20** was a gum.

Ethyl 3-(2-Naphthylamino)butyrate (21). A solution of ethyl 3-(2-naphthylamino)crotonate (**19**) (1.8 g, 7.06 mmol) in dry THF–methanol mixture (3:1) (30 mL) was adjusted to pH 3.0 by addition of 2 N HCl–methanol solution. $NaBH_3CN$ (0.6 g, 9.54 mmol) was added with efficient stirring and during the course of addition the solution was maintained at pH 3 by the addition of HCl–methanol solution. After the completion of the addition, the stirring was continued further for 3 h at room temperature with the solution being maintained at pH 3. The solvent was distilled off in vacuo, and the residue obtained was dissolved in water and made alkaline with 0.1 N NaOH solution. The aqueous solution after saturation with NaCl was extracted with ether, and evaporation of ether in vacuo gave crude ethyl 3-(2-naphthylamino)butyrate (**21**). The crude material was added to a silica gel column and eluted with ethyl acetate–hexane (1:1) solvent system. Evaporation of pooled solvent fractions gave ethyl 3-(2-naphthylamino)butyrate (**21**) (1.35 g, 74.5%) as a gum. Along with the above compound (**21**) a small amount of methyl ester of 3-(2-naphthylamino)butyric acid was obtained due to transesterification of the ethyl ester of 3-(2-naphthylamino)butyric acid in methanolic medium.

Ethyl 3-(6-Quinolylamino)butyrate (22). This was prepared by reduction of compound **20** as described above for compound **21**, yield 70%; **22** was a gum.

3-(2-Naphthylamino)butyric Acid Hydrazide (23). This was prepared by hydrazinolysis of ester **21**, yield 83.6%, mp 151–152 °C (EtOH–H_2O). Anal. ($C_{14}H_{17}N_3O$) C, H, N.

3-(6-Quinolylamino)butyric Acid Hydrazide (24). This was prepared by hydrazinolysis of ester **22**, yield 78.7%, mp 200–201 °C (EtOH–H_2O). Anal. ($C_{13}H_{16}N_4O$) C, H, N.

4-Pyridal (*N*-(2-Naphthyl)glycinoyl)hydrazone (25). A mixture of *N*-(2-naphthyl)glycine hydrazide (**1**) (1.075 g, 5 mmol) and pyridine-4-aldehyde (0.54 g, 5 mmol) in ethanol was refluxed for 3 h. The hydrazone that crystallized upon cooling was filtered, washed with a small quantity of alcohol, and recrystallized from ethanol to give 4-pyridal (*N*-(2-naphthyl)glycinoyl)hydrazone (**25**) (1.2 g, 78.9%), mp 194–195 °C. Anal. ($C_{18}H_{16}N_4O$) C, H, N.

4-Pyridal (*N*-Methyl-*N*-(2-naphthyl)glycinoyl)hydrazone (26). This was prepared from compound **5** and pyridine-4-aldehyde, yield 81.9%, mp 192–193 °C. Anal. ($C_{19}H_{18}N_4O$) C, H, N.

Antitubercular Evaluation. Preparation of Solutions of Test Compounds. Weighed amounts of all compounds tested in the present study were dissolved either in ethylene glycol or 0.01 N HCl and sterilized by passing through a Millipore filter (0.45 μm). Stock solutions of test compounds were diluted under aseptic conditions in sterilized Youman's medium to obtain various concentrations of test compounds.

Microorganisms. A. *M. tuberculosis* $H_{37}R_v$ culture obtained from NCTC and maintained by regular subculture on Petrik's solid medium[28] was used to study in vitro inhibitory activity of test compounds. The infectivity of *M. tuberculosis* $H_{37}R_v$ was studied by inoculating the organism in guinea pigs, as reported.[11,12]

B. INH and streptomycin-resistant strains (clinical isolates) of *M. tuberculosis* were obtained from the National Tuberculosis Institute, Bangalore, India. These resistant strains maintained in Lowenstern Jensen medium[29] or Petrik's medium[28] were transferred into Youman's media, and 14-day-old culture was used to study antitubercular activity.

Susceptibility Testing Method. A. Tubes (each tube 19 × 150 mm) containing varying concentrations of test compounds in Youman's media[30] were inoculated with 14-day-old cultures of *M. tuberculosis* $H_{37}R_v$ grown on the same medium. Approximately equal inocula (3-mm-diameter loopful) were floated on the surface of media (5.0 mL) containing the test compounds. Controls containing only the Youman's medium and solvent (ethylene glycol) in Youman's medium were also inoculated in the same way. All the tubes were then sealed with paraffin and incubated at 37 °C for 3 weeks. The inhibitory action of the compound was assessed by comparing the growth of bacilli in the experimental tubes with that in the controls. Readings were taken at the end of each week for up to 3 weeks. The results of experiments carried out in triplicate agreed, and the results observed at the end of 3 weeks are presented in Table I.

B. The in vitro conditions in Youman's media described above to evaluate antitubercular activity cannot be said to resemble those encountered in vivo. Hence, substance of biological origin, such as serum was added to the chemically defined medium (Youman's medium) to determine inhibitory action of naphthylglycine hydrazide analogues against *M. tuberculosis* $H_{37}R_v$.[31] The effect of bovine serum (freshly prepared) on the activity of test com-

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pounds were studied at a level of 10% (v/v). The results of experiments carried out in triplicate agreed and growth inhibitory action at the end of 3 weeks are presented in Table I.

C. Tubes containing varying concentrations of test compounds in Youman's media were inoculated with 14-day-old cultures of INH and streptomycin-resistant strains of *M. tuberculosis*. Controls containing only the Youman's medium, solvent (ethylene glycol), INH (various concentrations), and streptomycin (various concentrations) were also inoculated in the same way. All the tubes were then sealed with paraffin and incubated at 37 °C for 4 weeks. The inhibitory action of the compound was assessed by comparing the growth of bacilli in the experimental tubes with that in the controls. Readings were taken at the end of each week up to 4 weeks. The results of experiments carried out in triplicate agreed, and results observed at the end of 4 weeks are presented in Table II.

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Registry No. 1, 70955-02-1; 2, 104411-19-0; 3, 121810-69-3; 4, 121810-70-6; 5, 100850-37-1; 6, 121810-71-7; 7, 121810-72-8; 8, 121810-73-9; 9, 121810-74-0; 10, 89504-69-8; 11, 580-15-4; 12, 121810-75-1; 15, 121810-76-2; 16, 75793-57-6; 16·NH_3, 121810-86-4; 17, 121810-77-3; 18, 121810-78-4; 19, 98797-17-2; 20, 121810-79-5; 21, 121810-80-8; 21 (methyl ester), 121810-87-5; 22, 121810-81-9; 23, 121810-82-0; 24, 121810-83-1; 25, 121810-84-2; 26p, 121810-85-3; (±)-$CH_3CHBrCOOEt$, 41978-69-2; $ClCH_2COOEt$, 105-39-5; OHCCOOH, 298-12-4; CH_3COCH_2COOEt, 141-97-9; 2-naphthylamine, 91-59-8; *N*-methyl-2-naphthylamine, 2216-67-3; 2-amino-6-methoxynaphthalene, 13101-88-7; pyridine-4-aldehyde, 872-85-5.

Synthesis of Methotrexate–Antibody Conjugates by Regiospecific Coupling and Assessment of Drug and Antitumor Activities

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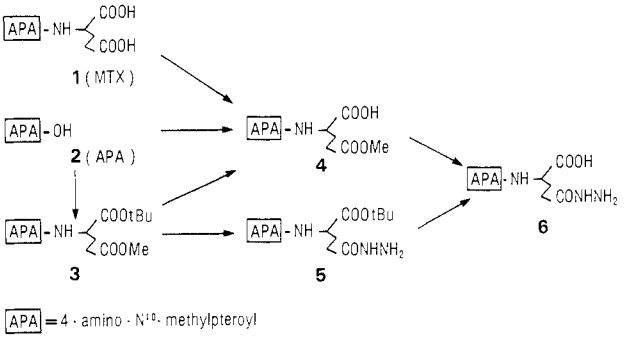
In order to increase the retention of drug activity, regiospecific coupling has been used to synthesize conjugates of methotrexate (MTX, 1) with normal rabbit IgG (NRG) and a mouse anti-human renal cancer monoclonal IgG (Dal K-20). MTX γ-methyl ester (4) was produced either by selective esterification of MTX or by coupling of 4-amino-4-deoxy-N^{10}-methylpteroic acid (2) with suitable glutamic acid derivatives. The MTX γ-methyl ester (4) was then converted to the corresponding hydrazide 6. An amide-linked conjugate was formed when the MTX γ-hydrazide (6) was converted to reactive acylating species 7 by using *tert*-butyl nitrite or trifluoroacetaldehyde, which were reacted with nucleophilic centers, presumably ε-amino groups, in native IgG. A hydrazone-linked conjugate was formed when MTX γ-hydrazide (6) was reacted directly with IgG that had first been oxidized with periodate to form polyaldehyde IgG. The regiospecifically synthesized conjugates were somewhat more effective inhibitors in vitro of dihydrofolate reductase and of colony formation by human renal cancer (Caki-1) cells than were control nonregiospecific conjugates.

Antibody-mediated drug targeting offers a potentially important approach for increasing the therapeutic efficiency of antineoplastic agents and therefore opens a new dimension in cancer treatment.[1-3] The choice of methotrexate (MTX) as a cytotoxic component of drug–antibody conjugates is based on the fact that it has proved to be a very effective anticancer agent in clinical use.[4] It is stable in an acidic milieu such as the lysosomal compartment where the conjugate may be degraded to release MTX or its low molecular weight derivatives.[5] MTX acts as a potent inhibitor of dihydrofolate reductase (DHFR), an enzyme responsible for recycling 7,8-dihydrofolate to its reduced, physiologically active, 6(*R*)-tetrahydro form, and therefore the cytotoxic effect of MTX has been ascribed to the depletion of the intracellular pool of reduced folates.[6-8]

A key objective in conjugate synthesis is that the linkage incorporating the drug into the conjugate must preserve optimal drug activity either in the intact conjugate per se or in a moiety cleaved by target tumor cells. MTX has been coupled to IgG after activating carboxyl groups by formation of the *N*-hydroxysuccinimide active ester which reacts with nucleophilic centers, presumably amino groups in the protein.[9] Alternatively, the active ester can be converted to the corresponding hydrazide, which reacts with aldehyde groups produced in the IgG by periodate oxidation.[10,11] When conjugated by these methods, bound MTX retains only 10–25% of the DHFR inhibitory effect of equimolar amounts of free MTX. Stereochemical factors play an important role in the interaction between

Scheme I



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