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Methyl esters of substituted 4-OXO-2-butenic acids for treatment of tuberculosis

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Miles et al.

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(45) **Date of Patent:** Jan. 27, 2004

(54) **METHYL ESTERS OF SUBSTITUTED 4-
OXO-2-BUTENOIC ACID FOR TREATMENT
OF TUBERCULOSIS**

6,066,670 A 5/2000 Brown 514/557
6,080,790 A 6/2000 Boyd et al. 514/650

OTHER PUBLICATIONS

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Konyukova et al, Chemistry of Heterocyclic Compounds, New York, Translation of Khimiya Geterotsiklicheskih Soedinenii , 2001, 37(6), pp. 779-780.*

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

* cited by examiner

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(21) Appl. No.: **10/153,092**

(22) Filed: **May 22, 2002**

(57) **ABSTRACT**

Related U.S. Application Data

(60) Provisional application No. 60/292,985, filed on May 23, 2001.

(51) **Int. Cl.**⁷ **A61K 31/24**; C07C 229/00

(52) **U.S. Cl.** **514/538**; 514/539; 560/35; 560/43

(58) **Field of Search** 560/43, 35; 514/538, 514/539

A novel class of methyl esters of substituted 4-oxo-2-butenic acids and their derivatives are disclosed along with the surprising use property of these compounds as a bacteriostatic agents for humans or non-humans against mycobacteria. These compounds include: 4-(4-Ethoxy-phenyl)-2-(N'-fluoren-9-ylidene-hydrazino)-4-oxo-but-2-enoic acid methyl ester; 4-(4-Ethoxy-phenyl)-2-(N'-fluoren-9-ylidene-hydrazino)-2-hydroxy-4-oxo-butyric acid methyl ester; 2-(4-Bromo-phenylamino)-5,5-dimethyl-4-oxo-hex-2-enoic acid methyl ester; A pharmaceutical composition is disclosed as well as the method of treating human and non-humans infected with *M. tuberculosis* and related mycobacteria.

(56) **References Cited**

U.S. PATENT DOCUMENTS

5,274,002 A 12/1993 Hawkins 514/530
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5,972,363 A 10/1999 Clikeman et al. 424/408

5 Claims, 1 Drawing Sheet

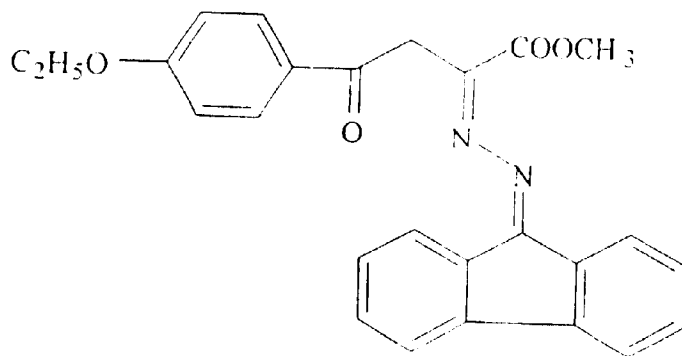


Fig. 1

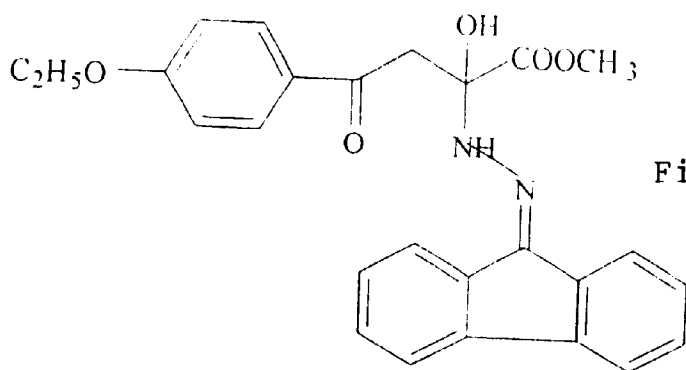


Fig. 2

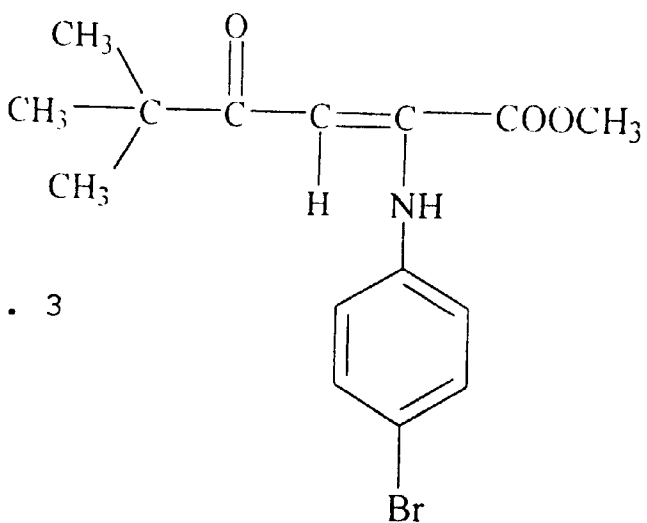


Fig. 3

**METHYL ESTERS OF SUBSTITUTED 4-
OXO-2-BUTENOIC ACID FOR TREATMENT
OF TUBERCULOSIS**

This application claims benefit of Ser. No. 60/292,985
filed May 23, 2001.

FIELD OF THE INVENTION

This invention relates to novel methyl esters of substituted
4-oxo-2-butenoic acid and more particularly to certain moi-
eties of these novel compounds and their close derivatives as
well as to the methods of their production and to the use of
these compounds as bacteriostatic agents (a chemical agent
that stops or inhibits the multiplication of bacteria) for
humans or non-humans against *Mycobacterium*
tuberculosis, and claims the benefit of priority of U.S.
Provisional Application Ser. No. 60/292,985 filed May 23,
2001.

BACKGROUND OF THE INVENTION

The infectious disease, tuberculosis (TB), is the leading
cause of death worldwide from a single human pathogen,
claiming more adult lives than diseases such as acquired
immunodeficiency syndrome (AIDS), malaria, diarrhea, lep-
rosy and all other tropical diseases combined (Zumla A,
Grange J. B M J (1998) 316, 1962–1964). The organism
usually responsible is the tubercle bacillus, *Mycobacterium*
tuberculosis (MT), discovered by Robert Koch in 1882.
However, *M. bovis*, which infects cattle may also infect man
and *M. africanum* is a cause of TB in West Africa.
Furthermore, a number of normally non-pathogenic
mycobacteria, especially *M. avium*, *M. intracellulare* and
M. scrofulaceum, cause opportunistic infectious disease in
patients with AIDS (Horne N. 1996. Tuberculosis and other
mycobacteria diseases. In Mansons Tropical Diseases, 20th
edn, Cook FEG (ed). WB Saunders: London; 971–1015).
Pulmonary TB, the most common type of the disease, is
usually acquired by inhalation of the bacillus from an
infectious patient and causes irreversible lung destruction.

About one third of the world's population is currently
infected with *M. tuberculosis*; 10% of those infected will
develop clinical diseases, particularly those who also have
the human immunodeficiency virus (HIV) infection (Zumla
A, Grange J. B M J (1998) 316, 1962–1964). With the
discovery of effective anti-mycobacteria agents (including
ethambutol, isoniazid, pyrazinamide, rifampicin and
streptomycin) and a reduction in poverty, there was a drastic
decline in the number of TB cases, especially in developed
nations. However, since the late 1980s, the number of cases
of TB throughout the world has been increasing rapidly
partly due to the emergence of multi-drug resistant
M. tuberculosis (C. E. Barry, III, Biochemical Pharmacology
(1997) 54, 1165–1172). According to the World Health
Organization (World Health Organization. 1993 92. per
Besra G S, Brennan P.J. 1997. J Pharm Pharmacol 49 (Suppl.
1):25–30.s), it is expected that the annual death rate caused
by TB will reach an overwhelming 3.5 million people by the
year 2000. Thus the TB problem requires urgent attention.
Short course anti-TB regiments initially using at least three
first-line drugs (including isoniazid, rifampicin and
pyrazinamide) are often not effective due to an increase in
the number of tuberculosis strains that have become resistant
to current drugs. For example the World Health Organization
(WHO) recently reported that the death rate of patients with
multi-drug resistant (MDR) tuberculosis in the US was
approximately 70%. Current treatment is also very expen-

sive: a 3 drugs regimen is needed (more than \$500/month
cost per patient). Thus the major problems faced in tubercu-
losis control are poor infrastructures for diagnosis and
drug supply. The failure of patients to complete therapy as
well as inappropriate mono-therapy has led to the emergence
and distribution of strains of *Mycobacterium tuberculosis*
resistant to every available chemotherapy (Bloom B R and
Murray C J L, Science (1992) 257, 1055–1064). Such
organisms will not remain confined to the Third World or to
the poor and indigent of developed countries. The recent
documentation of the spread of a single clone of multi-drug
resistant *Mycobacterium tuberculosis* (the “W” strain)
throughout the continental United States and Europe high-
lights the danger of an airborne pathogen in our global
society (Bifani P J, et al., JAMA (1996) 275, 452–457).

The patent literature has numerous disclosures of Hetero-
cyclic oxo-butenoic (crotonic) compounds: They include
Pamukci (6,232,312) describes crotonic acid derivatives
(column 22, lines 43–58) for the treatment of colonic
polyps;

Jones et al (6,121,450) discloses crotonic acid derivatives
(column 8, line 34; column 78, line 24 and at example
340) as steroid modifiers in treating breast cancer (column
1, lines 55–58);

Kalden, et al (5,334,612) discloses compounds said to be
useful for treating AIDS including derivatives of carboxy-
lic acid (column 9, line 31) and pyrrolidine (column 7,
line 24);

Nicolai, et al (6,180,651) discloses many anti-inflammatory
and analgesic compounds, including adenocarcinoma
(column 1, line 55), which includes heterocyclic alcohol-
esters (column 11, lines 1–16) and butanoic acid deriva-
tives (many Examples including 47 through 162);

Brown (6,066,670) describes an anti-viral admixture con-
taining crotonic acid for treating tumors (see Abstract);

Girard, et al (5,308,852) discloses many compounds includ-
ing butanoic acid derivatives (see Methods B and C of
schemes II and III) which compounds which are said to
inhibit tumor metastasis (column 7, line 56 and column 8,
line 4);

Horwell, et al (5,580,896) discloses many heterocyclic 4-oxo-
2-butenoic acid derivatives (column 13, lines 21–59; also
in columns 15+, examples 25,26,32,34,40,43–46, 77–79,
97,99,103,106.), which are useful for inhibiting colorectal
cancer (Abstract);

Giordani, et al (6,048,890) discloses 4-oxo-2-butenoic acid
derivatives said to be useful for treatment of AIDS
(column 1, line 8 and column 2, line 61; and,

Yonemeto, et al (6,083,985) recites a number of anti-tumor
or anti-AIDS agents that include heterocyclic butenoic acid
derivatives.

For half a century, the most used anti-microbial agents
referenced above for prophylaxis and treatment of tubercu-
losis since 1952 is isoniazid (isonicotinic acid hydrazide
[INH]). One of the known complications of anti-tuberculosis
chemotherapy caused by this drug is liver dysfunction plus
a great number of other complications. The toxicity of INH
is also a serious problem frequently resulting in poisoning.
It is also known to be an acute/chronic hazards since INH
is an irritant of the skin, eyes, mucous membranes and upper
respiratory tract.

It appears from a review of the above that neither the
heterocyclic oxo-butenoic compounds or the benzoxazine
heterocyclic compounds of interest are disclosed nor is there
any report of activity against *Mycobacterium tuberculosis*.

Consequently, there is a need for an anti-tubercular drug
for humans or non-humans which mitigates the above men-

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tioned disadvantages of current bacteriostatic agents used as drugs against TB bacterium in humans.

SUMMARY OF THE INVENTION

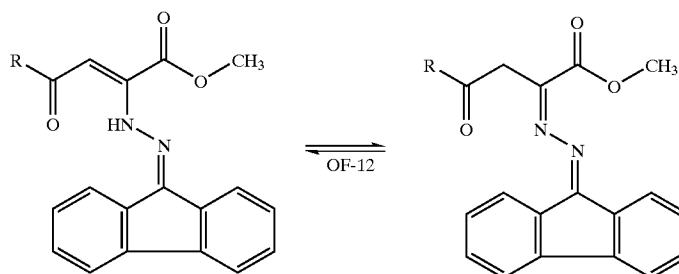
The first objective of the present invention is to provide a bacteriostatic agent effective against *Mycobacterium tuberculosis* and other mycobacteria.

The second object of this invention is to provide a bacteriostatic agent that is effective in humans or non-humans against *Mycobacterium tuberculosis* and other mycobacteria.

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particular arrangement shown since the invention is capable of other embodiments. Also, the terminology used herein is for the purpose of description and not of limitation.

As earlier recited this application has been filed in order to both disclose new methyl esters of substituted 4-Oxo-2-butenic acids and to facilitate a fuller understanding of the chemical scope of the methyl esters of substituted 4-oxo-2-butenic acids.



A third object of this invention is to provide a bacteriostatic agent that is effective in humans or non-humans against *Mycobacterium tuberculosis* and other mycobacteria which is relatively inexpensive as a drug.

Another object of this invention is to provide novel methyl esters of substituted 4-oxo-2-butenic acids.

A preferred embodiment of the invention encompasses a class of heterocyclics having the property of a bacteriostatic agent against *Mycobacterium tuberculosis* and other mycobacterium comprising methyl esters of substituted 4-oxo-2-butenic acids and more specifically those derivatives: 4-(4-Ethoxyphenyl)-2-(N'-fluoren-9-ylidenehydrazino)-4-oxobut-2-enoic acid methyl ester (OF-12); 4-(4-Ethoxyphenyl)-2-(N'-fluoren-9-ylidenehydrazino)-2-hydroxy-4-oxo-butyric acid methyl ester (OF-13); 2-(4-Bromophenylamino)-5,5-dimethyl-4-oxohex-2-enoic acid methyl ester (OF-15); and the use of each in humans or non-humans as therapeutic means for the eradication of *Mycobacterium tuberculosis* and related mycobacteria from the humans and nonhumans.

Further objects and advantages of this invention will be apparent from the following detailed description of presently preferred embodiments which are illustrated structurally in the accompanying drawings.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 illustrates structurally a chemical compound designated as OF-12.

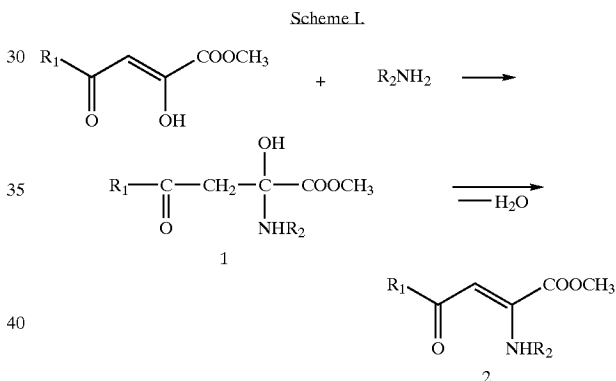
FIG. 2 illustrates structurally a chemical compound designated as OF-13.

FIG. 3 illustrates structurally a chemical compound designated as OF-15.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Before explaining the disclosed embodiments of the present invention in detail, it is to be understood that the invention is not limited in its application to the details of the

The methyl esters can be synthesized according to the amination/dehydration steps designated as scheme I.



More specifically this application is for anti-mycobacteria compounds OF-12, OF-13, OF-15, which have been structurally shown in FIGS. 1-3, respectively. To facilitate a full understanding of the invention:

the compound designated as OF-12 is 4-(4-Ethoxyphenyl)-2-(N'-fluoren-9-ylidenehydrazino)-4-oxobut-2-enoic acid methyl ester;

the compound designated as OF-13 is 4-(4-Ethoxyphenyl)-2-(N'-fluoren-9-ylidenehydrazino)-2-hydroxy-4-oxobutyric acid methyl ester;

the compound designated as OF-15 is 2-(4-Bromophenylamino)-5,5-dimethyl-4-oxohex-2-enoic acid methyl ester;

These compounds are highly active against *Mycobacterium tuberculosis* and have a very low toxicity, a lethal dose 50 (LD₅₀) in animals. The percent activity and animal toxicity for each compound is as follows: OF-12 (81% and LD₅₀>1500 mg/kg), OF-13 (88% and LD₅₀>1500 mg/kg), and OF-15 (98% and LD₅₀>1500 mg/kg).

PREPARATION OF (OF-12)

EXAMPLE 1

The preparation of 4-(4-Ethoxyphenyl)-2-(N'-fluoren-9-ylidene-hydrazino)-4-oxobut-2-enoic acid methyl ester (OF-

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12). A solution of 5.0 g (0.02 moles) of methyl 4-p-ethoxyphenyl-2-hydroxy-4-oxo-2-butenate (1) and 3.88 g (0.02 moles) of hydrazone fluorenone (2) in 80 mL of absolute toluene was refluxed for 3 hr 30 min with a Dean-Stark trap (control for the end of the reaction was carried by TLC). The solution was cooled and the precipitate was filtered and recrystallized from absolute benzene-hexane (1:1) to give 3.4 g (68% yield) of yellow crystals, mp 135–137° C.

Solubility: highly soluble in DMSO, DMFA, dichloroethane, acetonitrile; slightly soluble in ethanol, tetrachloromethane; insoluble in hexane and water.

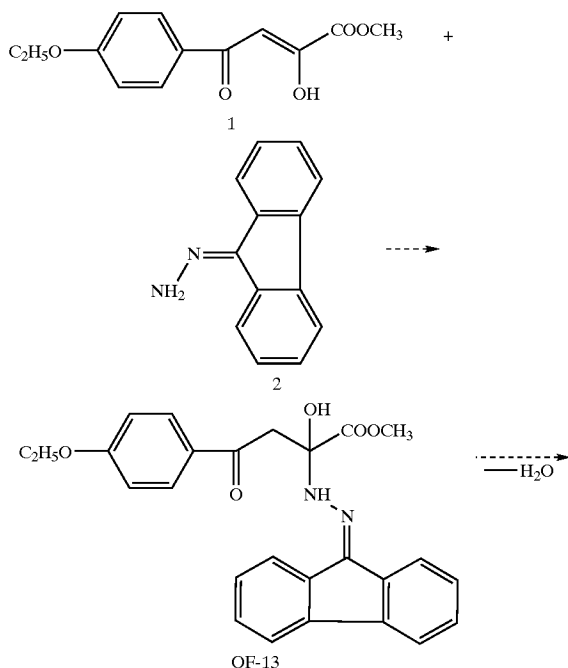
PREPARATION OF (OF-13)

EXAMPLE 2

The preparation of 4-(4-Ethoxyphenyl)-2-(N'-fluorene-9-ylidene-hydrazino)-2-hydroxy-4-oxobutyric acid methyl ester (OF-13). A solution of 5.0 g (0.02 moles) of methyl 4-p-ethoxyphenyl-2-hydroxy-4-oxo-2-butenate (1) and 3.88 g (0.02 moles) of fluorene-9-ylidene-hydrazine (2) in 80 mL of absolute benzene and absolute toluene (1:1) was refluxed for 1 hr 30 min with a Dean-Stark trap (the end of the reaction was determined by TLC), cooled and the precipitate was filtered and recrystallized from benzene-diethyl ether-hexane mixture (1:3:2) to give 2.65 g (53% yield) of colorless crystals with mp 114–116° C.

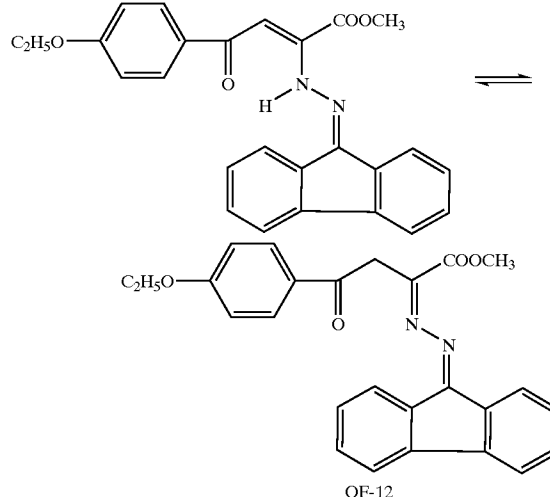
Solubility: highly soluble in DMSO, DMFA, dichloroethane, acetonitrile, insoluble in hexane. The compound is not stable in solutions and decomposes quickly when the solution is heated or stored for a long time with the formation of OF-12.

The synthesis of (OF-12) and (OF-13) has been designated as Scheme I and is further illustrated by the preparation set forth below.



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-continued



It is seen from the Scheme 1 that OF-13 is an intermediate product during the synthesis of OF-12.

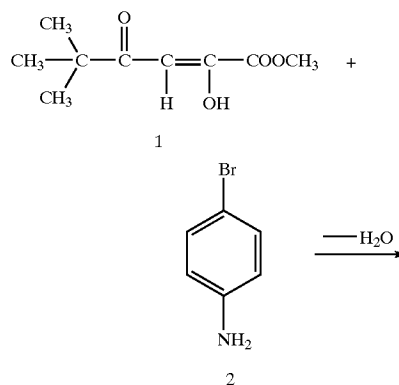
PREPARATION OF OF-15

EXAMPLE 3

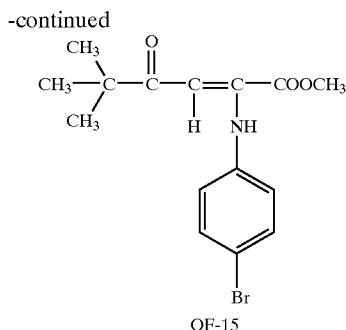
The preparation of 2-(4-Bromophenylamino)-5,5-dimethyl-4-oxohex-2-enoic acid methyl ester (OF-15).

A solution of 5.25 g (0.028 moles) of methyl 2-hydroxy-5,5-dimethyl-4-oxo-2-hexenoate (1) and 4.85 g (0.028 moles) of p-bromoaniline (2) in 30 mL of absolute benzene was refluxed for 2 hr with a Dean-Stark trap (Scheme 2). The solution was cooled and the resulting precipitate of OF-15 was filtered and recrystallized from methanol to give 8.30 g (87%) of yellow crystals with mp of 75–76° C.

The earlier referenced decarbonylation/transesterification reactions designated as Scheme II is readily seen from the following illustrated synthesis of OF-15.



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The bacteriostatic activity against *Mycobacterium tuberculosis* of the novel compounds (as earlier reported) was realized by the following procedure. Compound processing: Synthetic compounds OF-12, OF-13, OF-15, were each first dissolved in 500 ul of dimethylsulfoxide (DMSO) in individual beakers and each placed on a rotary shaker overnight. Distilled water was added to each to provide a final concentration of 10 mg/ml. Each solution was filter-sterilized using Becton Dickinson 5 ml sterile syringes and Whatman 22 um sterile filters. Each of the filtered sterilized synthetic test solutions (designated as synthons hereafter) were stored at -20 degrees centigrade until used.

Culture media and bioassay analytical techniques: BACTEC 12B Mycobacteria also known as Middlebrook 7H12 Medium was purchased from Becton Dickinson (Pittsburgh, Pa.). It contains 7H9 broth base, casein hydrolysate, bovine serum albumin, catalase and palmitic acid labeled with ^{14}C . It is specific for growing mycobacteria and is used in conjunction with the BACTEC brand 460 TB Analyzer. This Middlebrook 7H9 broth base media consist of 4 ml of broth mixture included in a sealed bottle. The culture used in the bioassay was *M. tuberculosis* (ATCC 25177). The synthons were added to Bactec 7H12 B+ bottled liquid media using Becton Dickinson 1 ml sterile syringes to a final concentration of 0.1 mg/ml. To each of these Bactec bottles, 100 ul of Bactec 7H12 B+ cultured *M. tuberculosis* was inoculated using Becton Dickinson 1 ml sterile syringes. Microbial growth activity in this culture medium is indicated by the release of $^{14}\text{CO}_2$ into the atmosphere of the sealed vial following the hydrolysis of ^{14}C -labeled palmitic acid by the microorganism. The BACTEC 460 TB Analyzer: The gas in the atmosphere of the sealed BACTEC 12B vial is aspirated from the vial via a sterile needle attached to a robotic inside the BACTEC 460 TB Analyzer System. The BACTEC Analyzer operates by initially drawing room air through a dust filter, a flush valve, and an ion chamber transferring all $^{14}\text{CO}_2$ into CO_2 trap where it is retained. This process cleans the electrometer and leaves it ready to start the next cycle. During the next cycle, a pair of 18G needles are heated. A pump produces a partial vacuum in the ion chamber used to lower the testing needles through the rubber septum of the vial being tested. A vacuum draws culture gas from the vial to the ion chamber. The electrometer measures the very small current that the radioactive $^{14}\text{CO}_2$ produces in the ion chamber. Following removal of the radioactive culture gas, fresh 5% CO_2 is introduced into the medium headspace every time a vial is tested, enhancing the growth of mycobacterium. The current measured by the electrometer is amplified and displayed as a growth index (GI). The growth index is measured on a scale of 0 to 999 and is an indication of microbial growth activity in the bottle. Usually, a GI of 10 or higher is an indication of definite microbial growth. The percent growth,

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and the percent inhibition in the presence of extract was calculated by the following formulas:

$$\%GR = \frac{GI(\text{with no extract})}{GI(\text{with the extract})} \times 100\%$$

$$\%IN = 100\% - \%GR$$

GR: Percent growth, GI: Growth index, IN: Percent inhibition

Animal Toxicity Bioassay. Acute toxicity was studied on white mice of both sexes with weight ranging between 18–26 grams under intraperitoneal injection of 2% solution of tested compound in starch (the compound was dissolved in starch slime and injected) on the basis of 0.1 ml of solution per 10 g of the animal weight. Each dose was tested on the group of 6 animals that were observed during 14 day period. (This method was approved by the Pharmacology committee of Russian Ministry of Health and has been widely used since 1968.) Averaged lethal dose (LD_{50}) of the compound was computed using results of experiments on 5–7 groups of animals using the method of Litchfield and Wilkinson. (Belenkii M. L. "Elements of quantitative determination of the pharmacological effect," Leningrad, 1963, 71 pages).

The disclosed invention makes it possible to produce and isolate oxo-heterocyclics, which have the property of a bacteriostatic agent toward *Mycobacterium tuberculosis* and other mycobacteria.

ADVANTAGES OF THE DISCOVERY

This discovery has several advantages. In the background there was a short description of one of the most used first-line antimicrobial agents for prophylaxis and treatment of tuberculosis since 1952. The name of this drug is isoniazid (isonicotinic acid hydrazide [INH]). Patients with a recently positive protein positive derivative (PPD) skin test and a normal chest x-ray routinely are given a 6–9 month course of isoniazid. Liver dysfunction is one of known complications of antituberculosis chemotherapy caused by this drug among a great number of other complications when isoniazid is co-administrated with other drugs (W. W. Yew, Clinica Chimica Acta 313 (2001) 31–36).

The toxicity issue is also a serious problem. In the US: A surveillance of cases of isoniazid poisoning by the American Association of Poison Control Centers from 1985–1993 revealed a low number of 138 cases in 1985, with no fatalities, and a high number of 2656 cases in 1991, with 6 fatalities. Mortality/Morbidity: Acute ingestion by adults with as little as 1.5 g of isoniazid can lead to mild toxicity. Ingestion of 6–10 g may be fatal, while ingestion of 15 g is usually fatal if not appropriately treated. The overall mortality rate for acute isoniazid toxicity has been estimated to be 19%. With current methods of supportive care, however, this figure may be high. From 1972–1988, an estimated 152 fatalities were caused by isoniazid-related hepatitis. The overall hepatitis-related death rates among patients using isoniazid prophylaxis is approximately 0.001%. Among individuals who complete a full course of prophylaxis, the hepatitis-related death rate has been calculated to be 23.2–57.9/100,000. The mortality rate among individuals who develop overt isoniazid-induced hepatitis is 10%.

Isoniazid is also known to be an acute/chronic hazard. This compound is an irritant of the skin, eyes, mucous membranes and upper respiratory tract. It can cause harm by ingestion, inhalation and skin absorption [Lenga, Robert E. The Sigma-Aldrich Library of Chemical Safety Data. Edition 1. Sigma-Aldrich Corporation. Milwaukee, Wis. 1985. p. 1114, #A.].

The acute toxicity of isoniazid is as following: LCLo (lowest published lethal concentration) for man is 100 mg/kg. In animal testing with white mouse LD₅₀ (lethal dose 50 percent kill) for isoniazid was shown to be 100 mg/kg (the same intraperitoneal injection method (Krasil'nikov, I. I.; Parfenov, A. I.; Strel'nikov, Yu. E.; Smirnova, S. M. Radiobiologiya (1973), 13(4), 551-4), which was used by us to determine LD₅₀ dose). All toxicity information about isoniazid can also be found on the web site: http://ntp-server.niehs.nih.gov/htdocs/Chem_H&S/NTP_Chem5/Radian54-85-3.html

In addition there are a number of reports in recent literature which suggest the importance of developing a new treatment to combat strains of the deadly disease tuberculosis which have become resistant to current drugs.

By reference to page 6 first paragraph and in the following it is seen that all of the specific compounds of the invention exhibit at least 10 times lower acute toxicity compare to isoniazid and high activity values. These compounds are highly active against *Mycobacterium tuberculosis* and have a very low toxicity, a lethal dose 50 (LD₅₀) in animals. The percent activity and animal toxicity for each compound is as follows: OF-12 (81% and LD₅₀>1500 mg/kg), OF-13 (88% and LD₅₀>1500 mg/kg), and OF-15 (98% and LD₅₀>1500 mg/kg),

The methyl esters of substituted 4-oxo-2-butenic acid compounds of the invention, which are unique in their anti-tubercular activity, can be used in a pharmaceutical composition comprising a non-toxic effective amount of the referenced compound or a tautomeric form thereof or a pharmaceutically acceptable salt thereof or pharmaceutically acceptable solvate thereof, and a pharmaceutically acceptable carrier thereof.

For administration to man in the curative or prophylactic treatment of tuberculosis, in vitro dosages of compounds of the invention will generally be in the range of from 5 to 500 mg daily for an average adult patient (70 kg). Thus for a typical adult patient, individual tablets or capsules contain from 2-500 mg of active compound, in a suitable pharmaceutically acceptable vehicle or carrier, for administration in

single or multiple doses, once or several times per day. Dosages for intravenous, buccal or sublingual administration will typically be within the range of from 5-1000 mg per single dose as required. In practice the physician will determine the actual dosing regimen which will be most suitable for an individual patient and it will vary with the age, weight and response of the particular patient. The above dosages are exemplary of the average case but there can be individual instances in which higher or lower dosage ranges may be merited, and such are within the scope of this invention. The maximum non-toxic one time administration dose for the compound(s) of the invention appears to be 1500 mg.

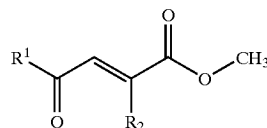
For human use, the compounds of the invention can be administered alone or jointly, but will generally be administered in admixture with a pharmaceutical carrier selected with regard to the intended route of administration and standard pharmaceutical practice. For example, they may be administered orally, buccally or sublingually, in the form of tablets containing excipients such as starch or lactose, or in capsules or ovules either alone or in admixture with excipients, or in the form of elixirs or suspensions containing flavouring or colouring agents. The compounds may also be injected parenterally, for example intravenously, intramuscularly, subcutaneously or intracoronarily. For parenteral administration, they are best used in the form of a sterile aqueous solution which may contain other substances, for example enough salts or glucose to make the solution isotonic with blood.

Thus in a further aspect the invention provides a method for the treatment and/or prophylaxis of *Mycobacterium tuberculosis* and related mycobacteria in a human or non-human which comprises administering an effective, non-toxic amount of a specific compounds according to the invention or a tautomeric form thereof and/or a pharmaceutically acceptable salt thereof and/or a pharmaceutically acceptable solvate thereof, to a tubercular human or non-human mammal in need thereof.

Tables 1, 2 and 3 disclose additional methyl esters of 4-oxo-butenic acids

TABLE 1

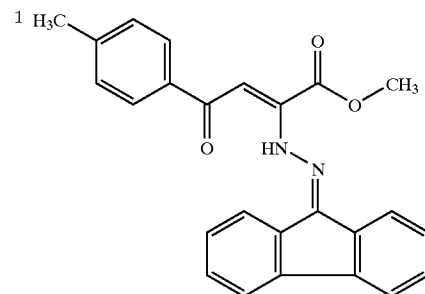
Table of Some Derivatives of the Methyl Esters of 4-oxo-butenic acid.



wherein R₁ and R₂ are organic substituent groups.

Structure

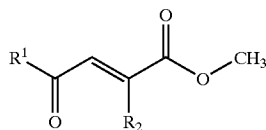
Name



2-(N'-Fluoren-9-ylidene-hydrazino)-4-oxo-4-p-tolyl-but-2-enoic acid methyl ester

TABLE 1-continued

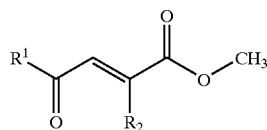
Table of Some Derivatives of the Methyl Esters of 4-oxo-butenoic acid.

wherein R_1 and R_2 are organic substituent groups.

#	Structure	Name
2		4-(4-Ethyl-phenyl)-2-(N'-fluoren-9-ylidene-hydrazino)-4-oxo-but-2-enoic acid methyl ester
3		2-(N'-Fluoren-9-ylidene-hydrazino)-4-oxo-4-o-tolyl-but-2-enoic acid methyl ester
4		2-(N'-Fluoren-9-ylidene-hydrazino)-4-oxo-4-m-tolyl-but-2-enoic acid methyl ester
5		4-(4-Butyl-phenyl)-2-(N'-fluoren-9-ylidene-hydrazino)-4-oxo-but-2-enoic acid methyl ester

TABLE 1-continued

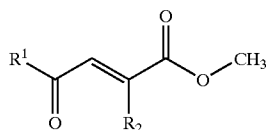
Table of Some Derivatives of the Methyl Esters of 4-oxo-butenoic acid.

wherein R₁ and R₂ are organic substituent groups.

#	Structure	Name
6		4-(4-tert-Butyl-phenyl)-2-(N'-fluoren-9-ylidene-hydrazino)-4-oxo-but-2-enoic acid methyl ester
7		4-(2,4-Dimethyl-phenyl)-2-(N'-fluoren-9-ylidene-hydrazino)-4-oxo-but-2-enoic acid methyl ester
8		4-(3,4-Dimethyl-phenyl)-2-(N'-fluoren-9-ylidene-hydrazino)-4-oxo-but-2-enoic acid methyl ester
9		4-(2,5-Dimethyl-phenyl)-2-(N'-fluoren-9-ylidene-hydrazino)-4-oxo-but-2-enoic acid methyl ester

TABLE 1-continued

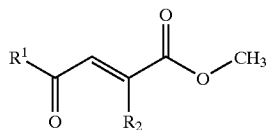
Table of Some Derivatives of the Methyl Esters of 4-oxo-butenoic acid.

wherein R₁ and R₂ are organic substituent groups.

#	Structure	Name
10		2-(N'-Fluoren-9-ylidene-hydrazino)-4-oxo-4-(2,4,6-trimethyl-phenyl)-but-2-enoic acid methyl ester
11		2-(N'-Fluoren-9-ylidene-hydrazino)-4-(4-methoxy-phenyl)-4-oxo-but-2-enoic acid methyl ester
12		4-(2,4-Dimethoxy-phenyl)-2-(N'-fluoren-9-ylidene-hydrazino)-4-oxo-but-2-enoic acid methyl ester
13		4-(3,5-Dimethoxy-phenyl)-2-(N'-fluoren-9-ylidene-hydrazino)-4-oxo-but-2-enoic acid methyl ester

TABLE 1-continued

Table of Some Derivatives of the Methyl Esters of 4-oxo-butenoic acid.

wherein R₁ and R₂ are organic substituent groups.

# Structure	Name
14	4-(2,5-Dimethoxy-phenyl)-2-(N'-fluoren-9-ylidene-hydrazino)-4-oxo-but-2-enoic acid methyl ester

wherein R₁ and R₂ are organic substituent groups.

TABLE II

# Structure	Name
1	2-(2-Bromo-phenylamino)-5,5-dimethyl-4-oxo-hex-2-enoic acid methyl ester
2	2-(3-Bromo-phenylamino)-5,5-dimethyl-4-oxo-hex-2-enoic acid methyl ester
3	2-(2-Chloro-phenylamino)-5,5-dimethyl-4-oxo-hex-2-enoic acid methyl ester
4	2-(3-Chloro-phenylamino)-5,5-dimethyl-4-oxo-hex-2-enoic acid methyl ester

TABLE II-continued

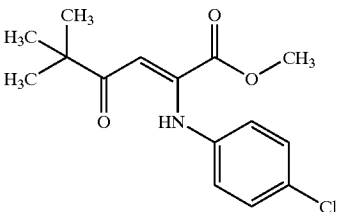
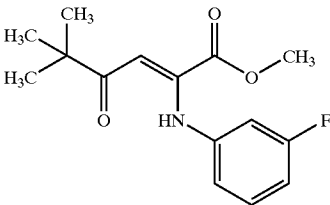
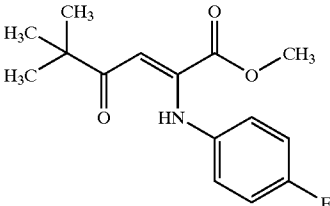
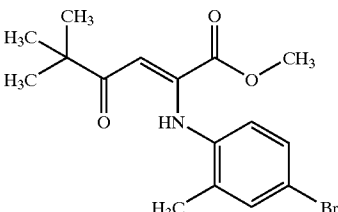
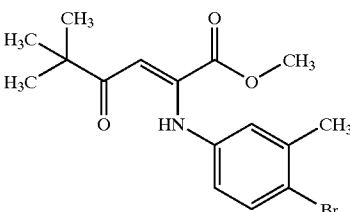
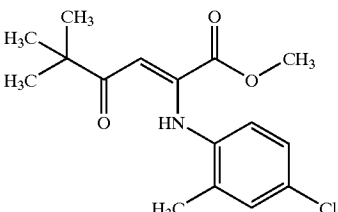
#	Structure	Name
5		2-(4-Chloro-phenylamino)-5,5-dimethyl-4-oxo-hex-2-enoic acid methyl ester
6		2-(3-Fluoro-phenylamino)-5,5-dimethyl-4-oxo-hex-2-enoic acid methyl ester
7		2-(4-Fluoro-phenylamino)-5,5-dimethyl-4-oxo-hex-2-enoic acid methyl ester
8		2-(4-Bromo-2-methyl-phenylamino)-5,5-dimethyl-4-oxo-hex-2-enoic acid methyl ester
9		2-(4-Bromo-3-methyl-phenylamino)-5,5-dimethyl-4-oxo-hex-2-enoic acid methyl ester
10		2-(4-Chloro-2-methyl-phenylamino)-5,5-dimethyl-4-oxo-hex-2-enoic acid methyl ester

TABLE II-continued

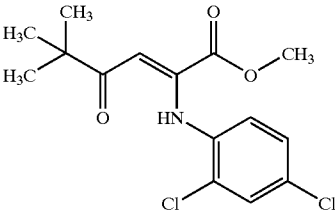
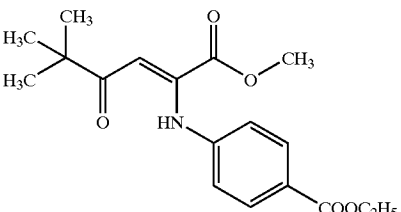
#	Structure	Name
11		2-(2,4-Dichloro-phenylamino)-5,5-dimethyl-4-oxo-hex-2-enoic acid methyl ester
12		4-(1-Methoxycarbonyl-4,4-dimethyl-3-oxo-pent-1-enylamino)-benzoic acid ethyl ester

TABLE 3

Table of Derivatives of Methyl Esters of 4-oxo-butenoic acids.

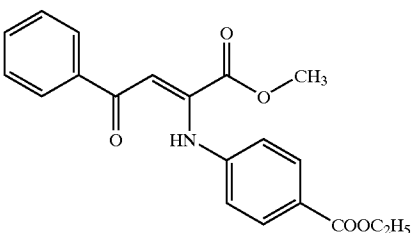
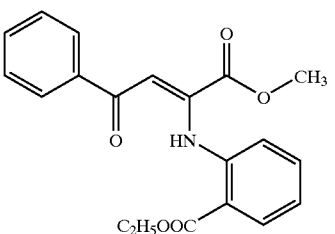
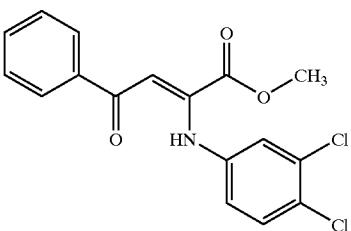
#	Structure	Name
1		4-(1-Methoxycarbonyl-3-oxo-3-phenyl-propenylamino)-benzoic acid ethyl ester
2		2-(1-Methoxycarbonyl-3-oxo-3-phenyl-propenylamino)-benzoic acid ethyl ester
3		2-(3,4-Dichloro-phenylamino)-4-oxo-4-phenyl-but-2-enoic acid methyl ester

TABLE 3-continued

Table of Derivatives of Methyl Esters of 4-oxo-butenoic acids.

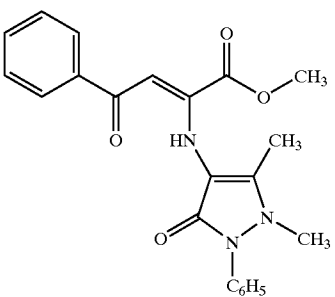
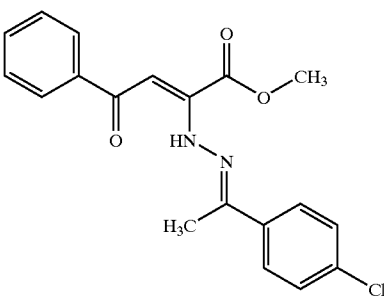
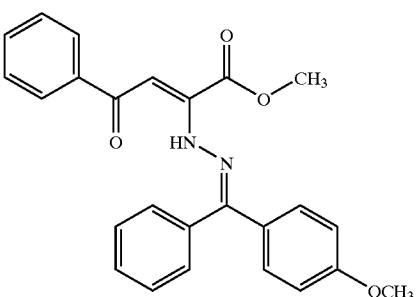
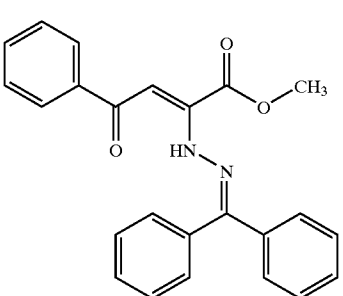
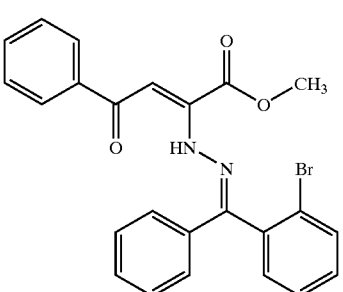
#	Structure	Name
4		2-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-ylamino)-4-oxo-4-phenyl-but-2-enoic acid methyl ester
5		2-{N'-[1-(4-Chloro-phenyl)-ethylidene]-hydrazino}-4-oxo-4-phenyl-but-2-enoic acid methyl ester
6		2-{N'-[1-(4-Methoxy-phenyl)-phenyl-methylene]-hydrazino}-4-oxo-4-phenyl-but-2-enoic acid methyl ester
7		2-(N'-Benzhydrylidene-hydrazino)-4-oxo-4-phenyl-but-2-enoic acid methyl ester
8		2-{N'-[1-(2-Bromo-phenyl)-phenyl-methylene]-hydrazino}-4-oxo-4-phenyl-but-2-enoic acid methyl ester

TABLE 3-continued

Table of Derivatives of Methyl Esters of 4-oxo-butenoic acids.

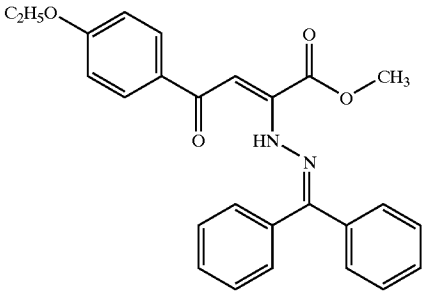
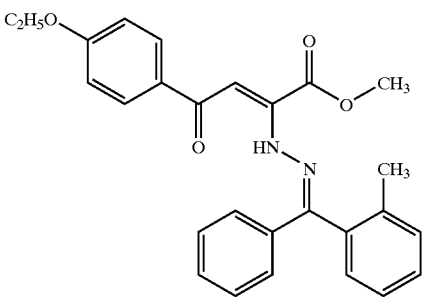
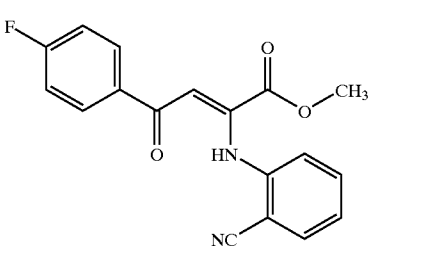
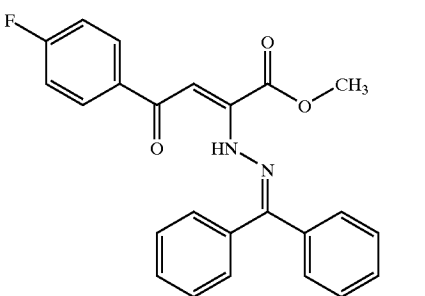
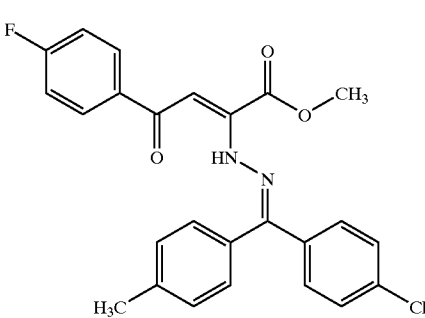
#	Structure	Name
9		2-(N'-Benzhydrylidene-hydrazino)-4-(4-ethoxy-phenyl)-4-oxo-but-2-enoic acid methyl ester
10		4-(4-Ethoxy-phenyl)-4-oxo-2-[N'-(phenyl-o-tolyl-methylene)-hydrazino]-but-2-enoic acid methyl ester
11		2-(2-Cyano-phenylamino)-4-(4-fluoro-phenyl)-4-oxo-but-2-enoic acid methyl ester
12		2-(N'-Benzhydrylidene-hydrazino)-4-(4-fluoro-phenyl)-4-oxo-but-2-enoic acid methyl ester
13		2-(N'-Di-p-tolylmethylene-hydrazino)-4-(4-fluoro-phenyl)-4-oxo-but-2-enoic acid methyl ester

TABLE 3-continued

Table of Derivatives of Methyl Esters of 4-oxo-butenoic acids.

#	Structure	Name
14		2-(N'-Fluoren-9-ylidene-hydrazino)-4-(4-methoxy-phenyl)-4-oxo-but-2-enoic acid methyl ester
15		2-(4-Bromo-phenylamino)-4-(4-ethoxy-phenyl)-4-oxo-but-2-enoic acid methyl ester
16		4-(4-Chloro-phenyl)-4-oxo-2-(2,4,6-trimethyl-phenylamino)-but-2-enoic acid methyl ester
17		2-[3-(4-Chloro-phenyl)-1-methoxycarbonyl-3-oxo-propenylamino]-benzoic acid ethyl ester
18		4-(4-Chloro-phenyl)-2-{N'-[1-(4-ethoxy-phenyl)-ethylidene]-hydrazino}-4-oxo-but-2-enoic acid methyl ester

TABLE 3-continued

Table of Derivatives of Methyl Esters of 4-oxo-butenoic acids.

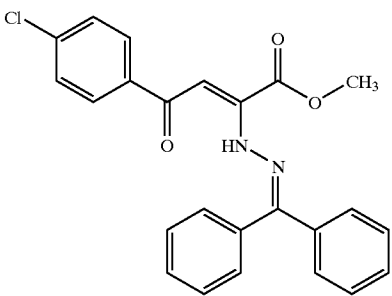
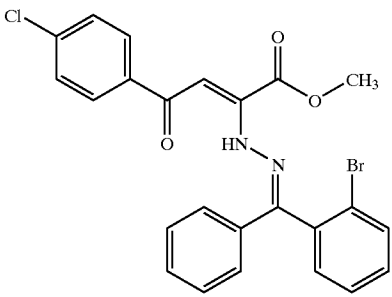
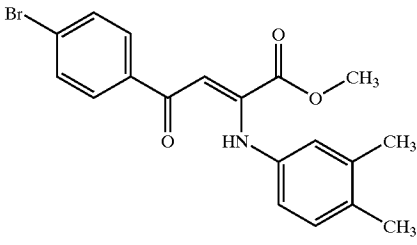
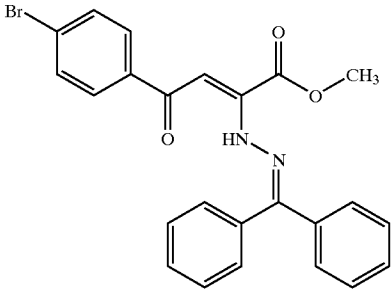
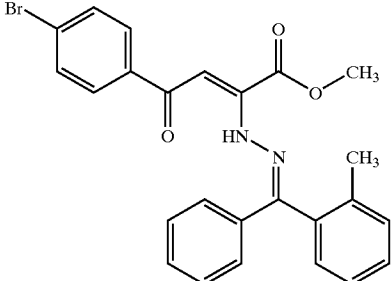
#	Structure	Name
19		2-(N'-Benzhydrylidene-hydrazino)-4-(4-chloro-phenyl)-4-oxo-but-2-enoic acid methyl ester
20		2-{N'-(2-Bromo-phenyl)-phenyl-methylene]-hydrazino}-4-(4-chloro-phenyl)-4-oxo-but-2-enoic acid methyl ester
21		4-(4-Bromo-phenyl)-2-(3,4-dimethyl-phenylamino)-4-oxo-but-2-enoic acid methyl ester
22		2-(N'-Benzhydrylidene-hydrazino)-4-(4-bromo-phenyl)-4-oxo-but-2-enoic acid methyl ester
23		4-(4-Bromo-phenyl)-4-oxo-2-[N'-(phenyl-o-tolyl-methylene)-hydrazino]-but-2-enoic acid methyl ester

TABLE 3-continued

Table of Derivatives of Methyl Esters of 4-oxo-butenoic acids.

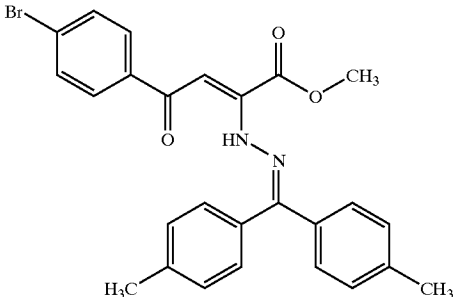
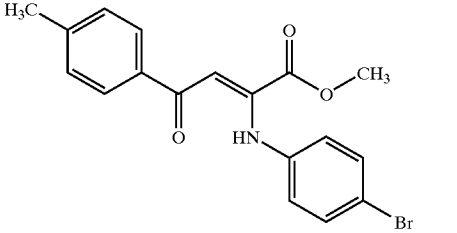
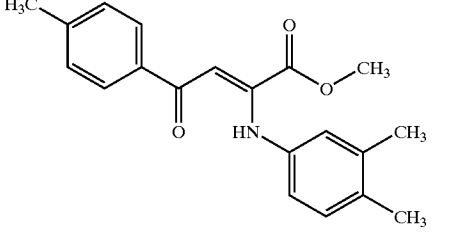
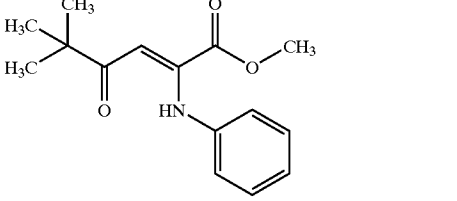
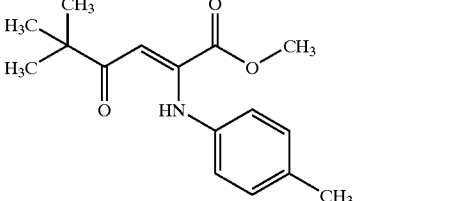
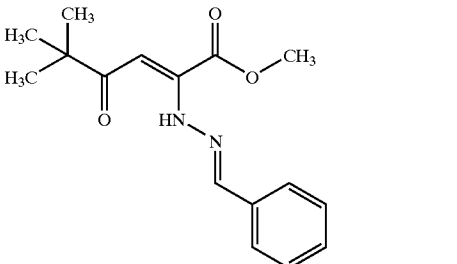
#	Structure	Name
24		4-(4-Bromo-phenyl)-2-(N'-di-p-tolylmethylene-hydrazino)-4-oxo-but-2-enoic acid methyl ester
25		2-(4-Bromo-phenylamino)-4-oxo-4-p-tolyl-but-2-enoic acid methyl ester
26		2-(3,4-Dimethyl-phenylamino)-4-oxo-4-p-tolyl-but-2-enoic acid methyl ester
27		5,5-Dimethyl-4-oxo-2-phenylamino-hex-2-enoic acid methyl ester
28		5,5-Dimethyl-4-oxo-2-p-tolylamino-hex-2-enoic acid methyl ester
29		2-(N'-Benzylidene-hydrazino)-5,5-dimethyl-4-oxo-hex-2-enoic acid methyl ester

TABLE 3-continued

Table of Derivatives of Methyl Esters of 4-oxo-butenoic acids.

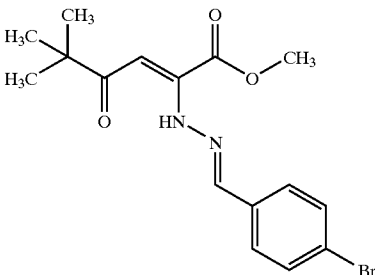
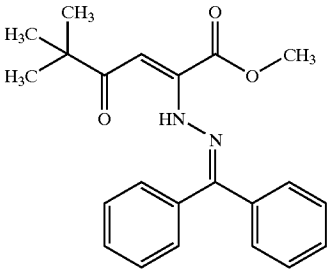
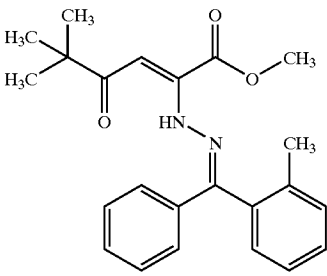
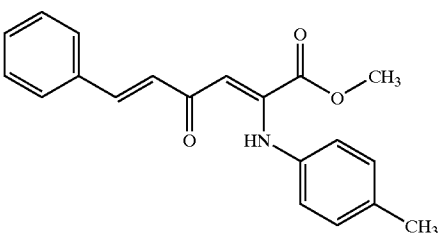
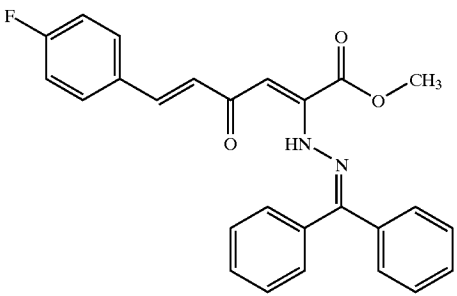
#	Structure	Name
30		2-[N'-(4-Bromo-benzylidene)-hydrazino]-5,5-dimethyl-4-oxo-hex-2-enoic acid methyl ester
31		2-(N'-Benzhydrylidene-hydrazino)-5,5-dimethyl-4-oxo-hex-2-enoic acid methyl ester
32		5,5-Dimethyl-4-oxo-2-[N'-(phenyl-o-tolylmethylene)-hydrazino]-hex-2-enoic acid methyl ester
33		4-Oxo-6-phenyl-2-p-tolylamino-hexa-2,5-dienoic acid methyl ester
34		2-(N'-Benzhydrylidene-hydrazino)-6-(4-fluoro-phenyl)-4-oxo-hexa-2,5-dienoic acid methyl ester

TABLE 3-continued

Table of Derivatives of Methyl Esters of 4-oxo-butenoic acids.

#	Structure	Name
35		2-(4-Bromo-phenylamino)-4-furan-2-yl-4-oxo-but-2-enoic acid methyl ester
36		2-(3,4-Dimethyl-phenylamino)-4-furan-2-yl-4-oxo-but-2-enoic acid methyl ester
37		2-(N'-Benzhydrylidene-hydrazino)-4-furan-2-yl-4-oxo-but-2-enoic acid methyl ester
38		4-Furan-2-yl-4-oxo-2-[N'-(phenyl-o-tolyl-methylene)-hydrazino]-but-2-enoic acid methyl ester
39		2-(N'-Fluoren-9-ylidene-hydrazino)-4-furan-2-yl-4-oxo-but-2-enoic acid methyl ester

TABLE 3-continued

#	Structure	Name
40		2-(N'-Fluoren-9-ylidene-hydrazino)-4-(4-nitro-phenyl)-4-oxo-but-2-enoic acid methyl ester
41		2-(N'-Benzhydrylidene-hydrazino)-4-(4-nitro-phenyl)-4-oxo-but-2-enoic acid methyl ester

While the invention has been described, disclosed, illustrated and shown in various terms of certain embodiments or modifications which it has presumed in practice, the scope of the invention is not intended to be, nor should it be deemed to be, limited thereby and such other modifications or embodiments as may be suggested by the teachings herein are particularly reserved especially as they fall within the breadth and scope of the claims here appended.

We claim:

1. A compound of a 4-oxo-2-butenoic acid methyl ester derivative, having the property of bacteriostatic activity against Mycobacteria, the compound consisting of:

4-(4-Ethoxyphenyl)-2-(N'-fluoren-9-ylidenehydrazino)-2-hydroxy-4-oxobutyric acid methyl ester.

2. A compound of a 4-oxo-2-butenoic acid methyl ester derivative having the property of bacteriostatic activity against Mycobacteria, the compound consisting of:

2-(4-Bromophenylamino)-5,5-dimethyl-4-oxohex-2-enoic acid methyl ester.

3. A method for the treatment and/or prophylaxis of mycobacteria in a human or a non-human which comprises administering to said human or non-human in need thereof, an effective, non-toxic amount of a compound consisting of a 4-oxo-2-butenoic acid methyl ester derivative or a tauto-

meric form thereof and/or a pharmaceutically acceptable salt thereof and/or a pharmaceutically acceptable solvate thereof which compound is 4-(4-Ethoxyphenyl)-2-(N'-fluoren-9-ylidenehydrazino)-4-oxobut-2-enoic acid methyl ester.

4. A method for the treatment and/or prophylaxis of mycobacteria in a human or a non-human which comprises administering to said human or non-human in need thereof, an effective, non-toxic, amount of a compound consisting of a 4-oxo-2-butenoic acid methyl ester derivative or a tautomeric form thereof and/or a pharmaceutically acceptable salt thereof and/or a pharmaceutically acceptable solvate thereof which compound is 4-(4-Ethoxyphenyl)-2-(N'-fluoren-9-ylidenehydrazino)2-hydroxy-4-oxobutyric acid methyl ester.

5. A method for the treatment and/or prophylaxis of mycobacteria in a human or non-human which comprises administering to said human or non-human in need thereof, an effective, non-toxic amount of a compound consisting of a 4-oxo-2-butenoic acid methyl ester derivative or a tautomeric form thereof and/or a pharmaceutically acceptable salt thereof and/or a pharmaceutically acceptable solvate thereof which compound is 2-(4-Bromophenylamino)-5,5-dimethyl-4-oxohex-2-enoic acid methyl ester.

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