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Review

Natural antimycobacterial metabolites: current status

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Abstract

Over the years the introduction of very effective drugs has revolutionized the treatment of tuberculosis. In recent years, however, emerging multiple drug resistance has become a major threat and thus calls for an urgent search for new and effective treatments for this deadly disease. This review is complementary to earlier reviews and covers more recent reports of naturally occurring compounds, and in some cases synthetic analogs, largely from plants, fungi and marine organisms that demonstrate significant activity in the in vitro bioassays against *Mycobacterium tuberculosis*, and other mycobacterial species. Included also are traditional medicinal uses of specific plants when utilized to treat tuberculosis and other pulmonary diseases.

Keywords: Review; Antimycobacterial metabolites; Plants; Fungi; Marine organisms

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1. Introduction

The genus *Mycobacterium* (Mycobacteriaceae) is highly diverse with 85 species known. Some cause human and animal diseases, and others are omnipresent in nature. The three most common human mycobacterial diseases are the primary pulmonary disease, tuberculosis, and two skin diseases, leprosy (*M. leprae*) and buruli ulcer (*M. ulcerans*). This review concerns efforts to identify phytochemicals that are inhibitory in vitro against *Mycobacterium* pertaining to C6 (glycolytic) metabolism.

Historically tuberculosis (TB) is one of the oldest and most pervasive diseases in history. Worldwide, TB is caused by *Mycobacterium tuberculosis* (MTB), and to a lesser degree *M. bovis* and *M. africanum*, and continues to be a major disease of global importance infecting at least one third, or two billion, of the world's population. It is a highly infective airborne and chronic bacterial disease usually infecting the lungs, although other organs are sometimes involved. Most healthy individuals are able to control the infection with a vigorous immune response, halting the progression of the disease, but not necessarily eradicating the organism (McKinney, 2000). Most cases are asymptomatic but can be reactivated under certain debilitating circumstances that impair the immune system such as malnutrition, diabetes, malignancy, and especially, AIDS (acquired immunodeficiency syndrome). For example, in sub-Saharan countries over 50% of AIDS related deaths have been attributed to tuberculosis (WHO, 2002). Also in these compromised cases, individuals are also susceptible to infection from *Mycobacterium* species from ubiquitous sources. Late stage AIDS patients are particularly susceptible to the ubiquitous *M. avium* ssp. *avium,* also referred to as the *Mycobacterium avium* complex (MAC). Once considered a rare zoonotic nodular wound infection of bird handlers, the disease in immunocompromised persons can result in pulmonary infections, lymphadenopathy and disseminated disease. Pulmonary disease can be caused by other ubiquitous mycobacteria such as *M. kansasii, M. intracellularae, M. xenopi* and *M. scrofulaceum* (Czajkowska et al., 2002). Of these, *M. kansasii* disease is associated more closely with levels of immunosuppression and progression of Human Immunodeficiency Virus (HIV) infection than are infections caused by MTB (Canueto-Qintero et al., 2003). By the mid 1950s the use of Streptomyces-based antibiotics (streptomycin, rifampin) and other chemotherapeutic antimycobacterial agents (pyrazinamide, ethambutol, isonizid) became epicentral to the treatment of TB. Often used in combination, to prevent the development of drug resistance during prolonged drug therapy, these agents can dramatically reduce the incidence of the disease, particularly when treatment is carefully supervised (Mitnick et al., 2003).

However, in the early 1990s it was becoming evident that this promising trend was being reversed by a number of factors that continue to promote the emergence of high rates of pulmonary disease and the development of antibiotic resistant strains. This tendency is particularly evident in southeastern Asia, subSaharan Africa, and eastern Europe where overcrowding (particularly in prisons and in long-term facilities), poor nutrition, ineffective diagnosis, and poor treatment regimens, as well as AIDS, all contribute to the amplification of cases that are observed. Immigrants from these regions continue to be the primary source of multiple-drug resistant strains seen elsewhere. In developed countries, particularly in inner-city populations, the debilitating effects of increased poverty, homelessness and drug use are also contributory features. When ubiquitous mycobacteria are involved, treatment is particularly challenging and often requires the use of a macrolide antibiotic (either clarithromycin or azithromycin) along with one or more other antimycobacterial agents, such as ciprofloxacin, ethambutol, and rifabutin. Unfortunately, rifabutin and clarithromycin can interact with protease inhibitors, and rifabutin with some non-nucleoside reverse transcriptase inhibitors, making treatment of AIDS patients even more difficult (Pablos-Mendez, 1998; WHO, 2003).

It has only been recently determined that MTB populations present during infection are physiologically heterogenic, and therapeutic agents selected to inhibit the glycolytic phase may not affect the slow and non-growing phase that predominates in lung granulomas; hence the need for prolonged therapy (Bishai, 2000). In this sequestered environment, where oxygen is depleted, a glyoxylate shunt occurs which allows for continuing survival in spite of long-term therapy. Unlike strains employed in most functional screening assays, these "persisters", that can vary in their susceptibility to current antimycobacterial antibiotics, can only be isolated from clinical specimens, and prefer fatty acids to carbohydrates. Therefore, to achieve appropriate resolution of an infection in an expedited manner, there is a need to identify compounds that affect this aspect as well as other suitable pharmacological targets that affect cell wall synthesis (Scherman et al., 2003), bacterial survival (virulence factors such as extracellular repeat protein—Erp) and inhibit the mechanism by which these organisms overcome the early immune response of the host (e.g., gene clusters related to the export of a complex cell wall-associated lipid, phthiocerol dimycocerosate, PDIM) (McKinney et al., 2000).

With the development of relatively time-saving, shorter incubation period assay techniques with lower risk of contamination (Heifets, 1986, 1988; Ashtekar et al., 1988; Chung et al., 1995; Collins and Franzblau, 1997), the past decade has witnessed a surge in the number of compounds that have been added into the scientific literature as possible antimycobacterial agents.

Naturally occurring pure compounds as well as extracts from higher and lower forms of plants, microorganisms and marine organisms have indicated that inhibitory activity against MTB is widespread in nature. Many compounds isolated using preliminary functional assays have been provided from investigators interested in phytochemical biodiversity. Usually their potential pharmaceutical worth remains unknown since data to show that these compounds are adversely affecting mycobacterial survival mechanisms in humans, or have been derived from medicinal plants, is lacking.

Preliminary results utilizing functional and mechanistic assays are indicating that our Peruvian Amazon plant collection of primarily medicinal plants is a potentially valuable source for identifying new antimycobacterial agents. In functional screening assays using the virulent human strain H37Rv, both published (Lewis et al., 1999; Lewis, 2003) and unpublished data show that numerous (750/1507) plant species were bioreactive against MTB. This 49.8% hit rate showing 80–100% inhibition of MTB at 100 μg/mL is significantly higher than those derived from broad screening programs (<5%). There are several possible reasons why this success rate is higher than for other collections examined elsewhere, such as the uniqueness of the flora, the tropical ecosystem in which they grow, and the type of phytoalexins that are elicited to control certain plant pathogens within the related *Streptomyces*. Also our specimens were unlike other plant extract libraries submitted for initial screening in that they were derived from primarily medicinal plants expected to contain bioreactive compounds, detannified to remove large molecules, and Cs-sterilized to prevent spurious bacterial contamination during the culture process.

More recently, 182 randomly selected extracts testing positive (80–100% inhibition of MTB) were further screened in a mechanistic assay to examine their ability to inhibit the conversion of dTDP-Glc (thymidine diphospate-α-D-glucose) to dTDP-Rha (deoxythymidine diphosphate rhamnose). This mechanistic assay is designed to identify inhibition of cell wall synthesis, a target of interest to the pharmaceutical industry. This is a pathway common to both MTB and *M. ulcerans*. Eight extracts elicited strong inhibitory activity with IC_{50} (inhibitory concentration 50%) ranging from 0.4–ca. 5.0 μg/mL and 12 additional ones proved moderately inhibitory with $IC_{50} > 5.0$ μg/mL (Ma et al., 2002 and unpublished).

There have been recent reviews showing antimycobacterial activity of plant species and natural products (Asres et al., 2001; Newton et al., 2002; Cantrell et al., 2001). Also 39 marine compounds were recently assessed for their antimycobacterial activity (König et al., 2000). In their review Newton et al. (2000) reported extracts from 138 plant species and no fewer than 112 pure metabolites having moderate to high antimycobacterial activities. Cantrell et al. (2001) also reviewed the antimycobacterial activities of plant terpenoids. Justifiably, there continues to be a renewed effort at isolating and assaying compounds from natural sources. This review emphasizes the structural diversities of the naturally occurring compounds with antimycobacterial properties at minimal inhibitory concentrations (MICs) of <200 μg/mL, which were reported since the last reviews. It includes active compounds from different classes, such as alkaloids, terpenoids, coumarins/chromones, peptides and phenolics obtained from plants, marine organisms, fungi and one bacterium. It is our intent that this will continue to generate sustained interest by natural product chemists, synthetic chemists and biochemists to attempt to generate different classes of compounds and analogs that can be further investigated and can serve as leads for drug development. Also included are some synthetic analogs having antimycobacterial activity against those associated with AIDS infections. The MICs provided are obtained from in vitro assays.

2. Alkaloids

In the review by Newton et al. (2000), 12 pure alkaloids were among the metabolites reported to have antimycobacterial activities. Since then 23 new and known naturally occurring alkaloids and analogs (Table 1, Fig. 1) have been assayed and found to possess antimycobacterial activities. The carbazoles **1**–**4** obtained from the rhizomes and roots of *Clausena excavata* (Rutaceae) and the other indole alkaloid **6**, all have modest activity against MTB. The indoloquinoline alkaloid **5** has significant activity against *M. fortuitum*, a species that has recently been shown to be an alternative screening model to MTB for potential antitubercular drugs (Gillespie et al., 2001). It also has significant activity against a related *M. bovis* and four other models with MICs close to and in some cases less than those obtained with the standard generic drugs ethambutol and isoniazid. The benzoxazole alkaloids **7**–**9**, marine metabolites, are also strong inhibitors of MTB. They all contain the oxazole moiety found in the synthetic oxazolidinones, which showed modest in vivo activity against MTB (Cynamon et al., 1999). The oxazole alkaloid texalin reported earlier (Rastogi et al., 1998) is significantly active against MTB and two other related species, *M. kansasii* and *M. avium*. The iminium salts **10** and **11** demonstrate appreciable activity against *M. avium*, *M. bovis* BCG and *M. smegmatis*. It is believed that the iminium ion improves the lipophilicity and hence increases the bioavailability of the alkaloids to the organisms. Alkaloids **13**–**14** and **16**–**21**, synthetic analogs of the naturally occurring cleistopholine **12** (from *Cleistopholis patens*, Annonaceae) and sampangine **15**

Table 1
Antimycobacterial compounds by class, source, model used, and activity

Compound class Compound name	Source/type of organism[a]	Model[b]	Activity MIC (μg/mL)	Reference
Alkaloids				
3-formylcarbazole (**1**)	*Clausena excavata* (P)	MTB	100	Sunthitikawinsakul et al. (2003)
3-methoxycarbonylcarbazole (**2**)	*C. excavata* (P)	MTB	50	
2-hydroxy-3-formyl-7-methoxycarbazole (**3**)	*C. excavata* (P)	MTB	100	
clauszoline J (**4**)	*C. excavata* (P)	MTB	100	
cryptolepine HCl (**5**)	*Cryptolepis sanguinolenta* (P)			
	(ATCC6841)	MF	16	Gibbons et al. 2003
	BCG	MB	12.5	
echinuline (**6**)	*Chaetomium globosum* (F)	MTB	169.9[b]	Kanokmedhalkul et al. (2002)
pseudopteroxazole (**7**)	*Pseudopterogorgia elisabethae* (MO)	MTB	12.5	Rodríguez and Rodríguez (2003) Rodríguez and Ramírez (2001)
seco-pseudopteroxazole (**8**)	*P. elisabethae* (MO)	MTB	12.5	Rodríguez and Rodríguez (2003) Rodríguez and Ramírez (2001)
homopseudopteroxazole (**9**)	*P. elisabethae* (MO)	MTB	12.5	Rodríguez and Rodríguez (2003)
sanguinarine (**10**)	*Sanguinaria canadensis* (P)	MB	24.5[c]	Newton et al. (2002)
chelerythrine (**11**)	*S. canadensis* (P)	MB	14.3[c]	
cleistopholine (**12**)[d]	*Cleistopholis patens* (P)	MI	12.5	Waterman and Muhammad (1985) Peterson et al. (1992)
cleistopholine analog (**13**)	synthetic	MI	12.5	Peterson et al. (1992)
cleistopholine analog (**14**)	synthetic	MI	1.56	
sampangine (**15**)[d]	*Cananga odorata* (P)	MI	0.78	Peterson et al. (1992); Rao et al. (1986)
sampangine analog (**16**)	synthetic	MI	3.12	Peterson et al. (1992)
sampangine analog (**17**)	synthetic	MI	25	
sampangine analog (**18**)	synthetic	MI	3.12	
sampangine analog (**19**)	synthetic	MI	3.12	
3-methoxy sampangine (**20**)[d]	*Cleistopholis patens* (P)	MI	1.56	Peterson et al. (1992); Liu et al. (1990)
sampangine analog (**21**)	synthetic	MI	0.39	Peterson et al. (1992)
ascididemin analog (**22**)	synthetic	MI	0.39	
ascididemin (**23**)	*Didemnum* sp. (MO)	MAu	0.25	Chung et al. (1995) Kobayashi et al. (1988)
Flavonoids, coumarins, chromone, chalcone				
flavonols **24a–c** (**24**)	*Haplopappus sonoriensis* (P)	MTB	–	Murillo et al. (2003)
flavone (**25**)	*Lysionotus pauciflorus* (P)	MTB	–	Xu et al. (1979)
ostruthin (**26**)	*Peucedanum ostruthium* (P)	MF	6.7[b]	Schinkovitz et al. (2003)
		MAu	3.4[b]	
		MS	6.7[b]	
		MPh	6.7[b]	
dentatin (**27**)	*Clausena excavata* (P)	MTB	50	Sunthitikawinsakul et al. (2003)
nor-dentatin (**28**)	*C. excavata* (P)	MTB	100	
methyl clausenidin (**29**)	synthetic	MTB	50	
chaetomanone (**30**)	*Chaetomium globosum* (F)	MTB	216.6[b]	Kanokmedhalkul et al. (2002)
licochalcone A (**31**)	*Glycyrrhiza inflata* (P)	MTB	7.1[f]	Fris-Moller et al. (2002)
Terpenoids				
erogorgiaene (**32**)	*Pseudopterogorgia elisabethae* (MO)	MTB	12.5	Rodríguez and Rodríguez (2003)
7-hydroxy erogorgiaene (**33**)	*P. elisabethae* (MO)	MTB	6.25	
aureol *N*,*N*-dimethyl-thiocarbamate (**34**)	*Smenospongia aurea* (MO)	MTB	6.25	Hu et al. (2002)
potamogetonin (**35**)	*Potamogeton malaianus* (MP)	MTB	100	Kittakoop et al. (2001)
potamogetonin analog (**36**)	*P. malaianus* (MP)	MTB	100	
potamogetonyde (**37**)	*P. malaianus* (MP)	MTB	100	
potamogetonol (**38**)	*P. malaianus* (MP)	MTB	50	
(+)-totarol (**39**)	*Xanthocyparis nootkatensis* (P)	MTB	16	Constatine et al. (2001)
secokauranes (**40**)	*Croton kongensis* (P)	MTB	25	Thongtan et al. (2003)
secokauranes (**41**)	*C. kongensis* (P)	MTB	6.25	
secokauranes (**42**)	*C. kongensis* (P)	MTB	6.25	

Table 1 (*continued*)

Compound class Compound name	Source/type of organism[a]	Model[b]	Activity MIC (μg/mL)	Reference
phorbol ester (**43**)	*Sapium indicum* (P)	MTB	50	Chumkaew et al. (2003)
phorbol ester (**44**)	*S. indicum* (P)	MTB	3.12	
phorbol ester (**45**)	*S. indicum* (P)	MTB	25	
phorbol ester (**46**)	*S. indicum* (P)	MTB	12.5	
phorbol ester (**47**)	*S. indicum* (P)	MTB	25	
phorbol ester (**48**)	*S. indicum* (P)	MTB	25	
dustanin (**49**)	*Aschersonia tubulata* (F)	MTB	12.5	Boonphong et al. (2001)
15-acetoxydustain (**50**)	*A. tubulata* (F)	MTB	12.5	
cycloartenol (**51**)	*Morinda citrifolia* (P)	MTB	32	Saludes et al. (2002)
Steroids, saponins				
stigmasta-4-en-3-one (**52**)	*Morinda citrifolia* (P)			
stigmasta-4,22-dien-3-one (**53**)	(mixture of **52** and **53**)	MTB	<2.0	
β-sitosterol (**54**)	*M. citrifolia* (P)	MTB	128	
stigmasterol (**55**)	*M. citrifolia* (P)	MTB	32	
epidioxysterol (**56**)	*M. citrifolia* (P)	MTB	2.5	
pregnene saponin (**57**)	*Eunicea pinta* (MO)	MTB	6.25	Shi et al. (2002)
jujubogenin analog (**58**)	*Colubrina retusa* (P)	MTB	10	ElSohly et al. (1999)
jujubogenin analog (**59**)	*C. retusa* (P)	MTB	50	
physalin B (**60**)	*Physalis angulata* (P)	MTB	>128	Januário et al. (2002)
physalin D (**61**)	*P. angulata* (P)	MTB	32	
Phenols, polyphenols				
hydroxybenzene analog (**62**)	*Ardisia japonica* (P)	MTB	[e]	Huang et al. (1980)
hydroxybenzene analog (**63**)	*Ardisia japonica* (P)	MTB	[e]	
bakuchiol (**64**)	*Psoralea corylifolia* (P)	MB	21.4[c]	Newton et al. (2002)
preussomerin (**65**)	*Microsphaeropsis* sp. (F)	MTB	3.12	Seephonkai et al. (2002)
preussomerin (**66**)	*Microsphaeropsis* sp. (F)	MTB	25	
preussomerin (**67**)	*Microsphaeropsis* sp. (F)	MTB	6.25	
preussomerin (**68**)	*Microsphaeropsis* sp. (F)	MTB	3.12, 6.25	
preussomerin (**69**)	*Microsphaeropsis* sp. (F)	MTB	12.5	
preussomerin (**70**)	*Microsphaeropsis* sp. (F)	MTB	25	
deoxypreussomerin (**71**)	*Microsphaeropsis* sp. (F)	MTB	1.56, 3.12	
deoxypreussomerin (**72**)	*Microsphaeropsis* sp. (F)	MTB	50	
punicalagin (**73**)	*Combretum molle* (P)	MTB	0.6, 1.2[f]	Asres et al. (2001)
Peptides				
hirsutellide (**74**)	*Hirsutella kobayasii* (F)	MTB	6-12	Vongvanich et al. (2002)
beauvericin (**75**)	*Paecilomyces tenuipes* (F)	MTB	12.5	Nilanonta et al. (2000)
beauvericin A (**76**)	*P. tenuipes* (F)	MTB	25	
enniatin B (**77**)	*Verticillium hemipterigenum* (F)	MTB	3.12	Nilanonta et al. (2003)
enniatin B_4 (**78**)	*V. hemipterigenum* (F)	MTB	3.12	
enniatin G (**79**)	*V. hemipterigenum* (F)	MTB	6.25	
enniatin C (**80**)	*V. hemipterigenum* (F)	MTB	6.25	
enniatin H (**81**)	*V. hemipterigenum* (F)	MTB	6.25	
enniatin I (**82**)	*V. hemipterigenum* (F)	MTB	6.25	
enniatin analog (**83**)	*V. hemipterigenum* (F)	MTB	1.56	
syringomycin (**84**)	*Pseudomonas syringae* (B)	MS	1.5	Buber et al. (2002)
oceanapia (**85**)	*Oceanapia* sp. (MO)	MTB	3.0[g]	Nicholas et al. (2003)
psammaplysin A (**86**)	*Pseudoceratina* sp. (MO)	MTB	30.0[g]	
oceanapiside (**87**)	*Oceanapia* sp. (MO)	MTB	10.0[g]	
1,3-pyridinium polymers (**88**)	*Amphimedon* sp. (MO)	MTB	0.1[g]	

[a] Source and types of organisms: P = plant; F = fungus; MO = marine organism; MP = marine plant (most species aquatic); B = bacterium; *Mycobacterium tuberculosis* (MTB), *M. aurum* A+ (MAu), *M. bovis* BCG (MB), *M. fortuitum* (MF), *M. intracellularae* (MI), *M. phlei* (MPh), *M. smegmatis* (MS).

[b] MIC in μM.

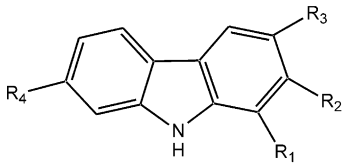
[c] IC_{50} in μg/mL.

[d] Synthetic compound.

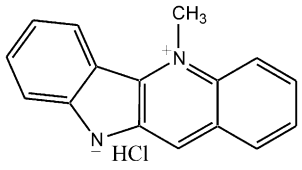
[e] In vivo test.

[f] MIC in mg/mL.

[g] IC_{50} μM.



1 R_1, R_2, R_4 = H, R_3 = CHO
2 R_1, R_2, R_4 = H, R_3 = CO_2CH_3
3 R_1, = H, R_2, = OH, R_3 = CHO, R_4 = OCH_3
4 R_1, = H, R_2, R_4 = OCH_3, R_3 = CO_2H,



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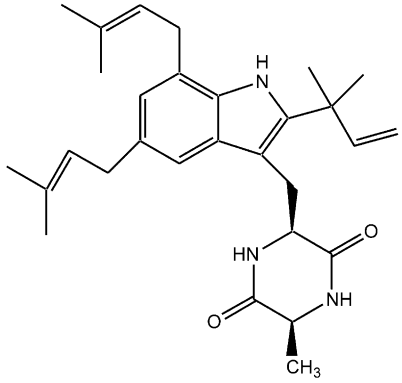

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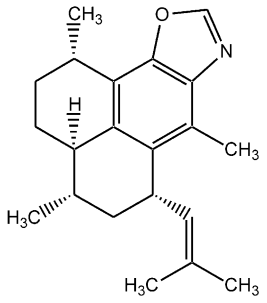

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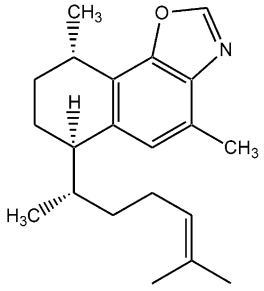

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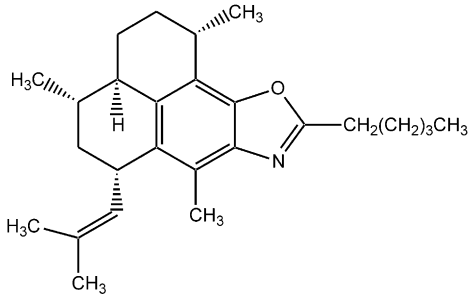

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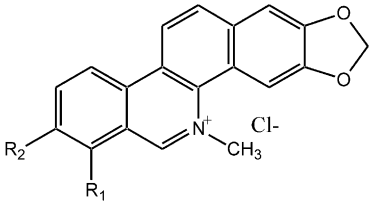
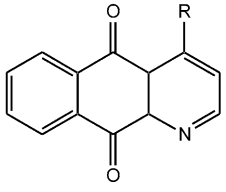
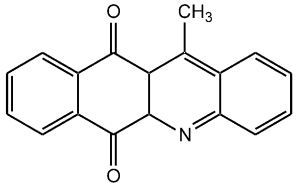


10 R_1 + R_2 = OCH_2O
11 R_1 = R_2 = OCH_3



12 R = CH_3
13 R = CH_2CH_3



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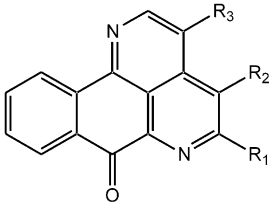
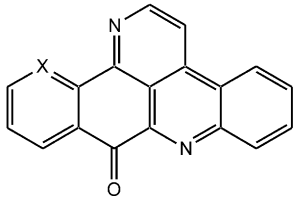


15 R_1 = R_2 = R_3 = H
16 R_1 = R_3 = H, R_2 = Br
17 R_1 = OEt, R_2 = Br, R_3 = H
18 R_1 = R_3 = H, R_2 = Cl
19 R_1 = R_3 = H, R_2 = OCH_3
20 R_1 = R_2 = H, R_3 = OCH_3
21 R_1 = R_2 = H, R_3 = CH_3



22 X = CH
23 X = N

Fig. 1. Alkaloids.

(from *Cananga odorata*, Annonaceae) respectively, were tested against the AIDS pathogen *M. intracellularae*. **15**, **21** and **22**, an analog of **23**, are very strong inhibitors of *M. intracellularae* with MICs equal to or less than those recorded for the control drug rifampin. Ascididemin **23** metabolite from the marine animal *Didemnum* sp. is also very active against the rapidly growing *M. aurum* A+ strain. In African traditional medicine *Cleistopholis patens* has a history of use to treat respiratory disease: in Zaire sap from pounded bark, or a bark decoction, is drunk for treating TB and simple bronchial afflictions, while in Nigeria the Yoruba use parts of the tree for curing coughs (Burkill, 1985). *Cananga odorata* is used in Malaysia as a source of a floral paste for treating asthma while the bark is used to treat scabies (Perry, 1980).

3. Flavones, coumarins, chromone and chalcone

Flavonols **24a–c** isolated from *Haplopappus sonoriensis* (Asteraceae), (Murillo et al., 2003) and flavone **25** from *Lysionotus pauciflorus* (Gesneriaceae) (Xu et al., 1979) are believed to be the active principles of extracts from these plants. A leaf extract of *H. rigidus*, a Chilean species of *Haplopappus*, is used locally to treat coughs. Even though these extracts have significant antimycobacterial activity, no MICs are recorded for the pure compounds. The coumarins **27–30** (Table 1, Fig. 2) are modestly active against MTB and it is believed that the prenyl at the C-8 position is essential for the antimycobacterial activity. Ostruthin **26** from *Peucedanum ostrothium* (Apiaceae) with the prenyl group at C-6 equally demonstrates significant activity against

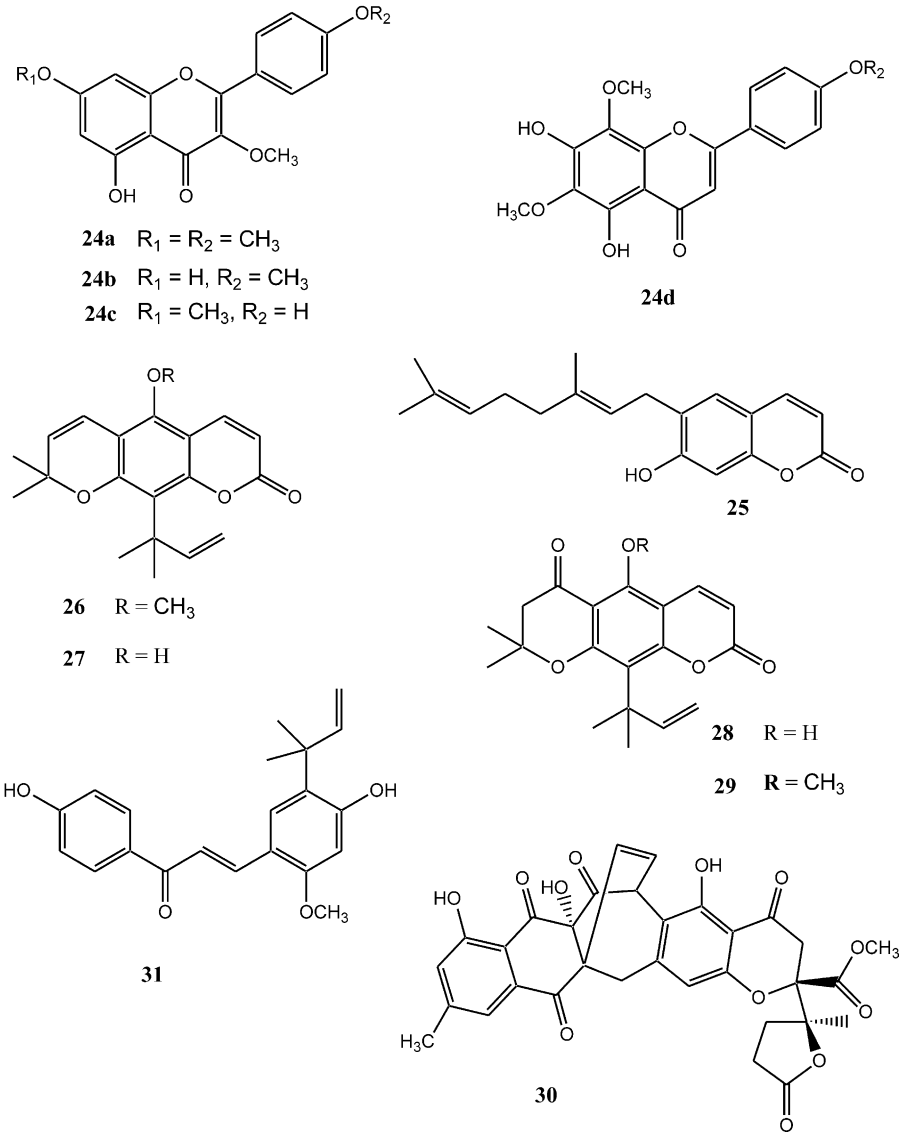


Fig. 2. Flavonoids, coumarins, chromone and chalcone.

different strains of the rapidly growing mycobacteria, *M. aurum, M. fortuitum*, *M. phlei* and *M. smegmatis,* with MICs close to those obtained for the control isoniazid. 7-Hydroxycoumarin on the other hand is only weakly active against *M. fortuitum* (Schinkovitz, 2003). It thus appears that the presence of the prenyl group is important to the activity of the coumarins, since it increases the lipophilicity and hence the bioavailability to the organism as earlier observed in some sesquiterpenes (Cantrell et al., 2001). The anthraquinone-chromanone **30** is a fungal metabolite and exhibits a modest activity against MTB compared to the controls isoniazide and kanamycin sulfate (MICs 0.37 and 4.29 μM, respectively) (Kanokmedhalkul et al., 2002). MTB,

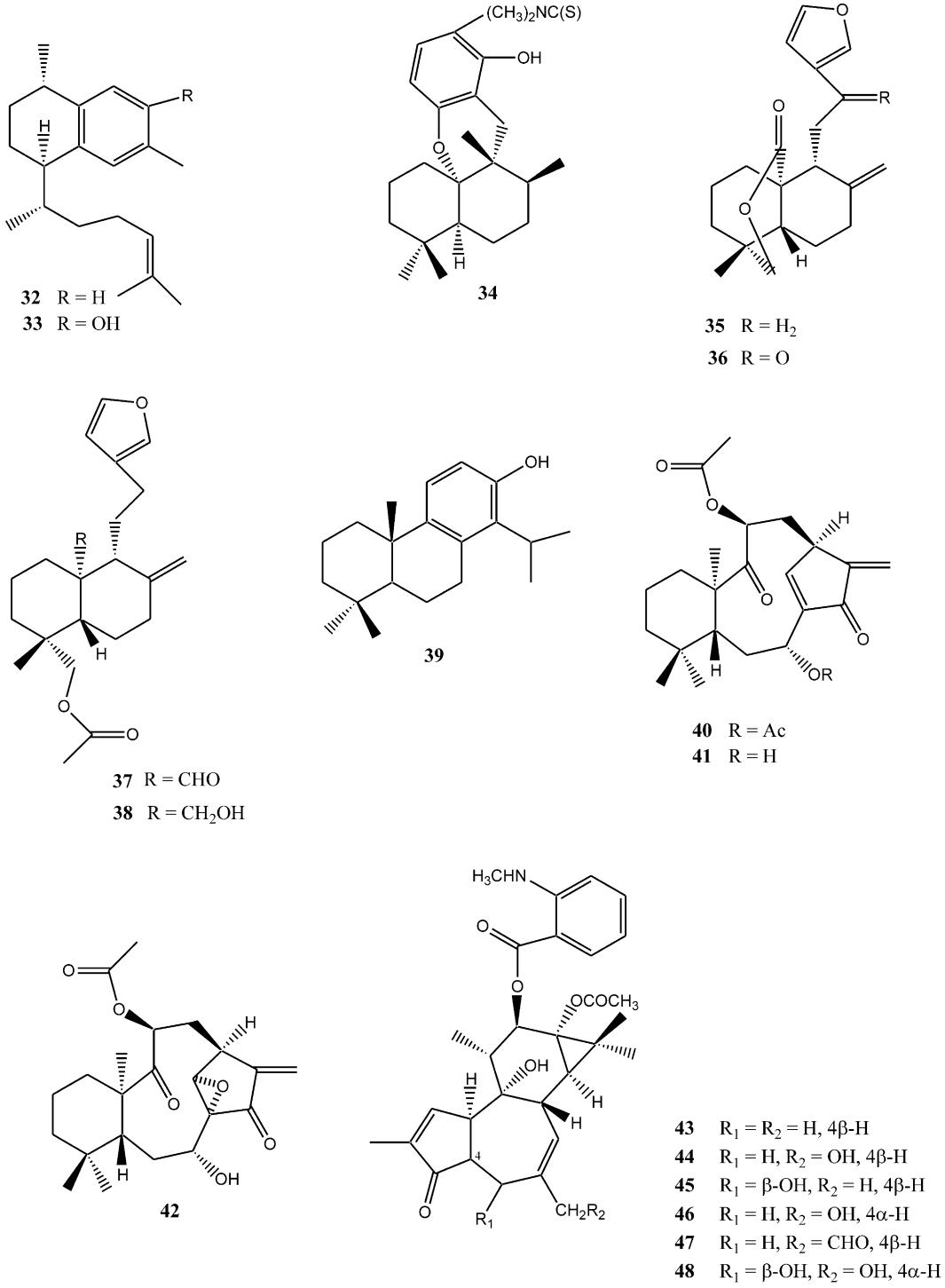


Fig. 3. Terpenoids.

M. avium and *M. bovis* are strongly inhibited by licochalcone **31** obtained from *Glycyrrhiza inflata* (Fabaceae) with low MICs in the range of 5–20 mg/L (MIC of the control rifampicin <2 mg/L). A number of *Glycyrrhiza* species are used as throat demulcents.

4. Terpenoids

In a comprehensive review by Cantrell et al. (2001), 118 synthetic and natural plant terpenoids were reported to have moderate to high antimycobacterial activity against MTB. The nor-diterpenoid 12-demethylmulticauline has an MIC comparable with rifampin and even less than that for ethambutol. In this review, of the 25 new terpenoids (Table 1, Figs. 3, 4), the most active compound is the phorbol ester **44** from the tree *Sapium indicum* (Euphorbiaceae), which shows activity comparable to the positive control, kanamycin sulfate, but not as active as isoniazide. In contrast, the 4α-H isomer of **44** is inactive, suggesting that the orientation of the proton at position 4 is very important to the activity of the compound. The serulatane diterpenoids **32**, **33** obtained from the marine coral *Pseudopterogorgia elisabethae*, as well as thiocarbamate **34,** from the marine sponge *Smenospongia aurea*, are significantly active against MTB. In the case of **34**, it is believed that the thiocarbamate moiety plays an important role in the activity of this compound. The secokauranes **41** and **42** from *Croton kongensis* (Euphorbiaceae) (Thongtan et al., 2003) also show significant activity. (+)-Totarol **39** from the outerbark of *Xanthocyparis nootkatensis* (synonym *Chamaecyparis nootkatensis*) (Cupressaceae) is reported to have activity against MTB (Constantine et al., 2001) and other species of *Mycobacterium* (Muhammad et al., 1995). Other compounds in this class that are very active and require further study are the hopane terpenoids **49** and **50**, obtained from the insect pathogenic fungus *Aschersonia tubulata*. Woldemichael et al. (2003) reported the antimycobacterial activity against MTB of eight diterpenes and three triterpenes from *Calceolaria pinnifolia* (Scrophulariaceae) with MIC values between 4 and 128 μg/mL.

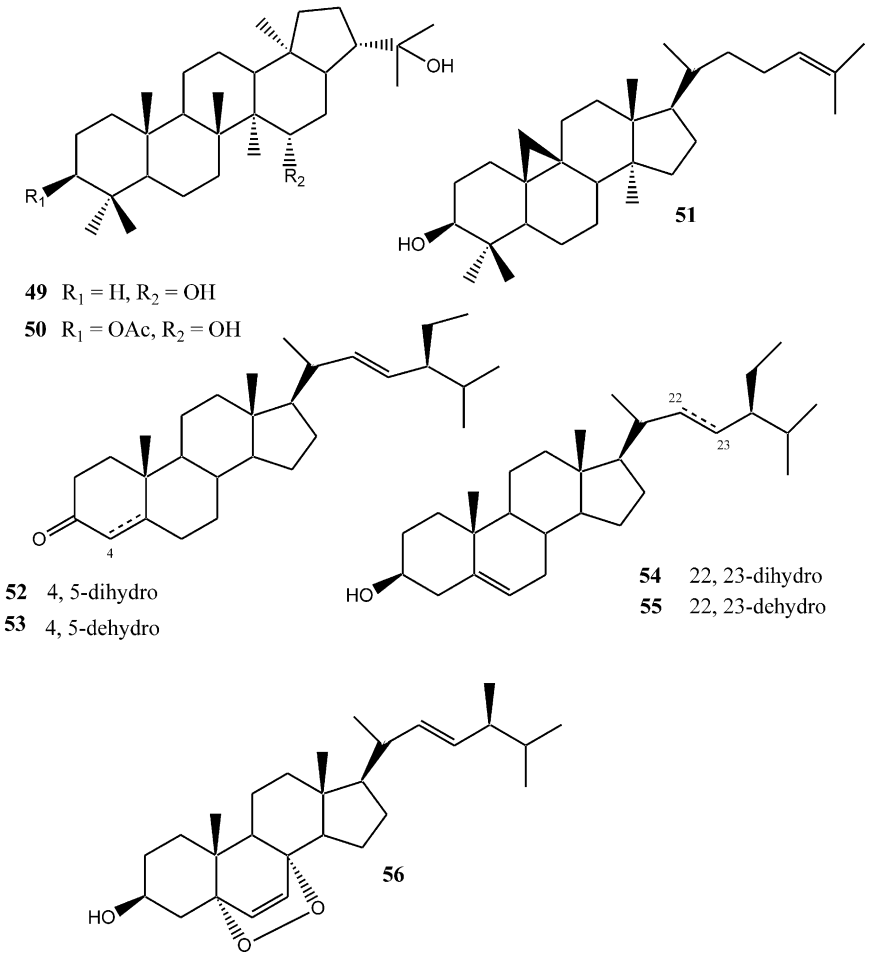


Fig. 4. Terpenoids/sterols.

5. Steroids and saponins

The mixture of **52** and **53** (Table 1, Fig. 4) obtained from *Morinda citrifolia* (Rubiaceae), as well as epidioxysterol **56**, are significantly active against MTB. The sterol **56** is said to be an auto-oxidation product of campesta-5,7,22-trien-3β-ol. The human use of *M. citrifolia* root decoctions as a medication to treat TB was reported by Okabe (1940) in the western Pacific (Palau Islands). The saponin **57** (Fig. 5) obtained from the gorgonian octocoral *Eunicea pinta*, as well as the jujubogenin analog **58** from *Colubrina retusa* (Rhamnaceae), are also significantly active, while the seco-steroids phasalin B, **60** and D, **61** from *Physalis angulata* (Solanaceae) are moderately active against MTB. Plant infusions of *P. angulata* are used in Colombia, Ecuador, Ivory Coast and Upper Volta (Burkina Faso) to treat asthma and also in Colombia and Ecuador as an anti-inflammatory (Burkill, 2000; Lescure et al., 1987). Woldemichael et al. (2003) also reported some sterols from *Ruprechtia triflora* (Polygonaceae) with moderate to very good antimycobacterial activity against MTB. MIC values range from 2 to 128 μg/mL.

6. Phenols and polyphenols

The benzenoid compounds **62**, **63** (Table 1, Fig. 6) were isolated over two decades ago by Huang et al. (1980) from *Ardisia japonica* who reported that the compounds were tested in vivo using 201 patients infected with MTB and they found that both compounds were over 80% effective. This medicinal plant is used locally for the treatment of bronchitis and as an antitussive and expectorant (Chang et al., 1985). The third benzenoid compound, bakuchiol **64** with prenyl substituent, isolated from the seeds of *Psoralea corylifolia* (Fabaceae), is also reported to have significant activity

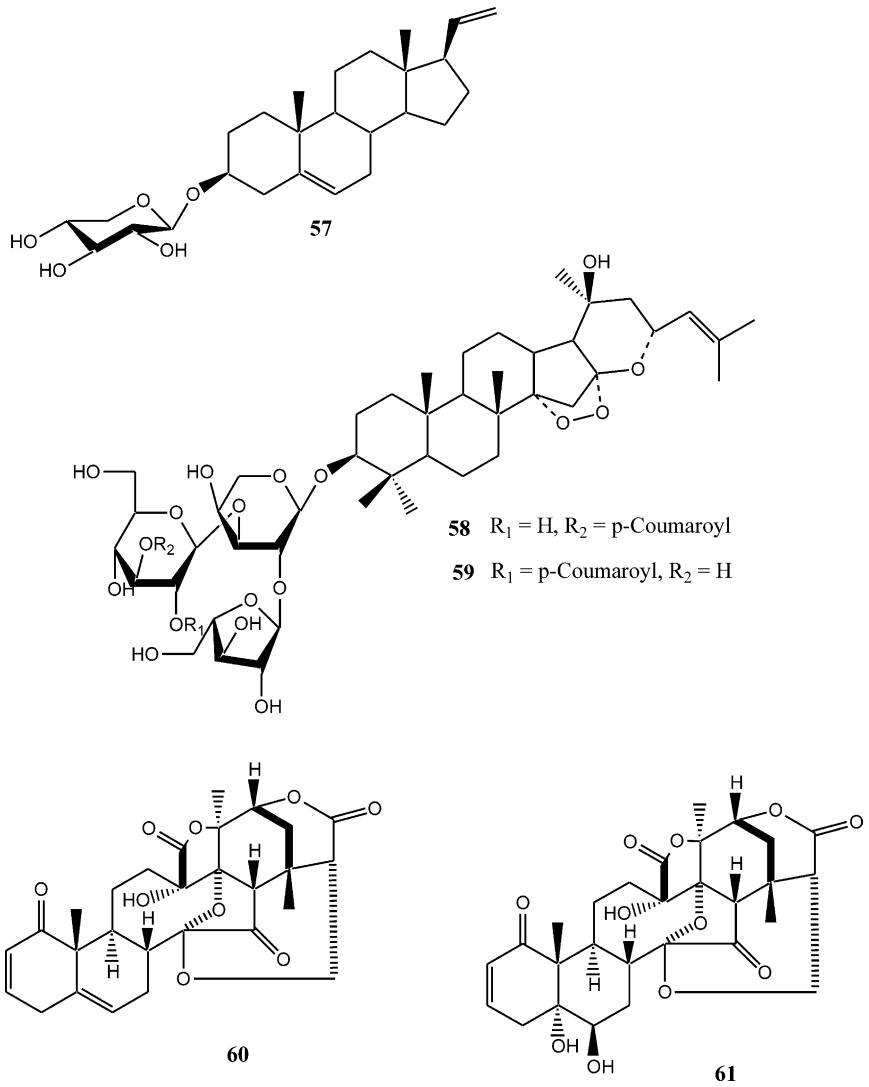


Fig. 5. Saponins.

against *M. aurum* and *M. bovis* BCG. In traditional Hindu medicine, seed preparations of *P. corylifolia* are specially recommended for the treatment of leprosy, psoriosis and inflammatory diseases of the skin, and are used both orally and topically (Wealth of India, 1969). The preussomerins **65**–**72** are lichenous fungal metabolites from *Microsphaeropsis* sp. All have high to modest antimycobacterial activity against MTB; MICs range from 1.56 to 50 μg/mL with compounds **65**, **68** and **71** having MICs close to that of the control compound kanamycin (2.5 μg/mL). The synthesis of this class of compounds has recently been reported (Chi and Heathcock, 1999; Ragot et al., 1999). The antimycobacterial activity of punicalagin **73** from *Combretum molle* (Combretaceae) was evaluated using two strains of MTB, typus humanus and an unidentified patient strain. Even though the reported MICs for this compound (Table 1) are greater than 200 μg/mL, it is however reported here because of the specific use of the plant from which it is obtained and it is the only tannin so far associated with antimycobacterial activity. The East Africa leaves of this species are chewed or pounded, soaked in water, and the juice drunk to treat chest complaints, or as an inhalant in hot steam baths (Kokwaro, 1976).

7. Peptides

The cyclodepsipeptides **74-83** (Table 1, Fig. 7) from fungi all have modest to high antimycobacterial activity against MTB ranging from 1.56 to 25 μg/ml, while **84** shows a high antimycobacterial activity against a

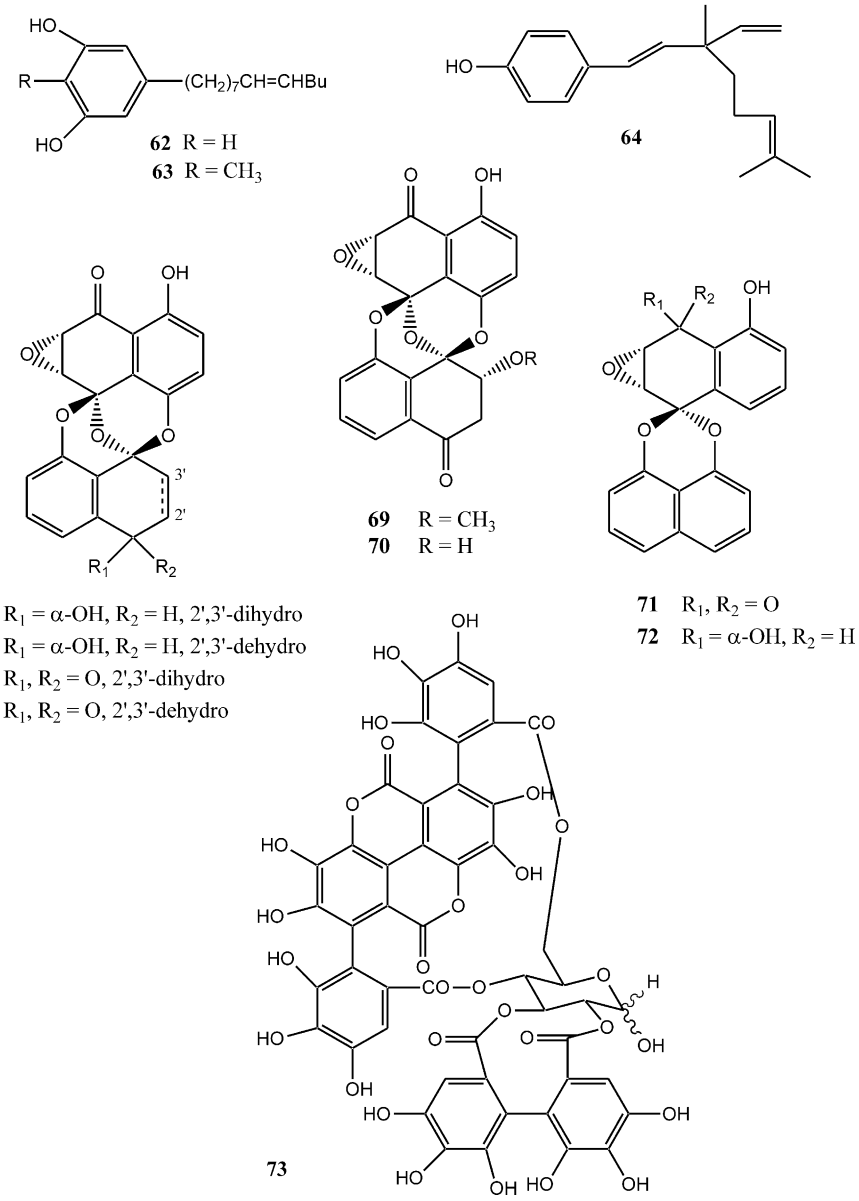


Fig. 6. Phenols/polyphenols.

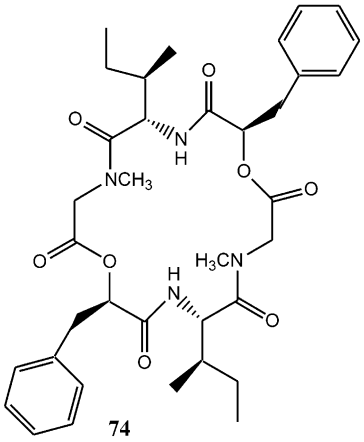
related model *M. smegmatis*. The MIC of 1.50 μg/mL obtained for **84** was lower than those obtained for the primary drugs pyrazinamide and streptomycin, but comparable to values obtained for the secondary drugs kanamycin and cycloserine. The mechanism of antimycobacterial activity of this class of compounds is under study (Jackson et al., 2000).

8. Other classes of compounds

Recently Nicholas et al. (2003) identified 13 natural product inhibitors of a novel detoxification enzyme, mycothiol-*S*-conjugate amidase (MCA) unique to the Actinomycetes. They represent six different structural classes and have IC_{50} (μM) ranging from 0.1 to 100. By conducting enzyme inhibition assays using varied inhibitor and substrate concentrations, they determined the mode of inhibition of *M. tuberculosis*' MCA for four of these compounds. The compounds **85**, **87** (Fig. 8) are obtained from the marine sponge *Oceanapia* sp., while **86** is obtained from *Pseudoceratina* sp and **88** is an isolate from *Amphimedon* sp. (Table 1). A novel piperine dimer, chabamide from *Piper chaba* (Piperaceae) also has a moderate activity against against MTB with MIC value of 12.5 μg/mL (Rukachaisirikul et al., 2002)

9. Conclusions

Of the 88 compounds reviewed, the preussomerins **65**, **68** and **71** have high activities against MTB (MICs 1.56–



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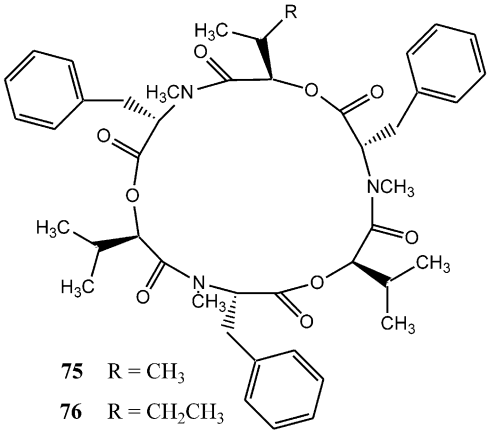
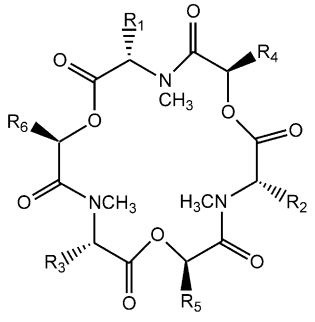


75 R = CH_3

76 R = CH_2CH_3



	R_1	R_2	R_3	R_4	R_5	R_6
77	i-Pr	i-Pr	i-Pr	i-Pr	i-Pr	i-Pr
78	i-Bu	i-Pr	i-Pr	i-Pr	i-Pr	i-Pr
79	i-Bu	i-Bu	i-Pr	i-Pr	i-Pr	i-Pr
80	i-Bu	i-Bu	i-Bu	i-Pr	i-Pr	i-Pr
81	i-Pr	i-Pr	i-Pr	s-Bu	i-Pr	i-Pr
82	i-Pr	i-Pr	i-Pr	s-Bu	s-Bu	i-Pr
83	i-Pr	i-Pr	i-Pr	s-Bu	s-Bu	s-Bu

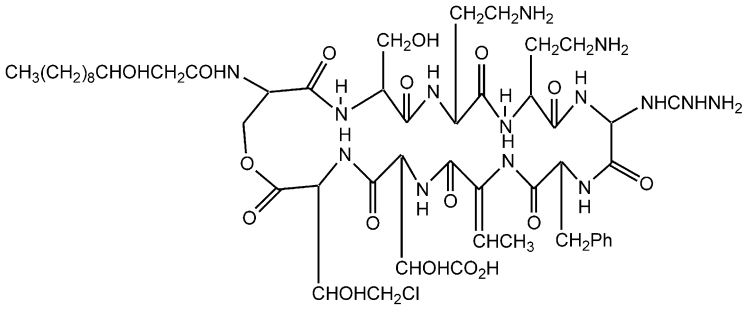


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Fig. 7. Peptides.

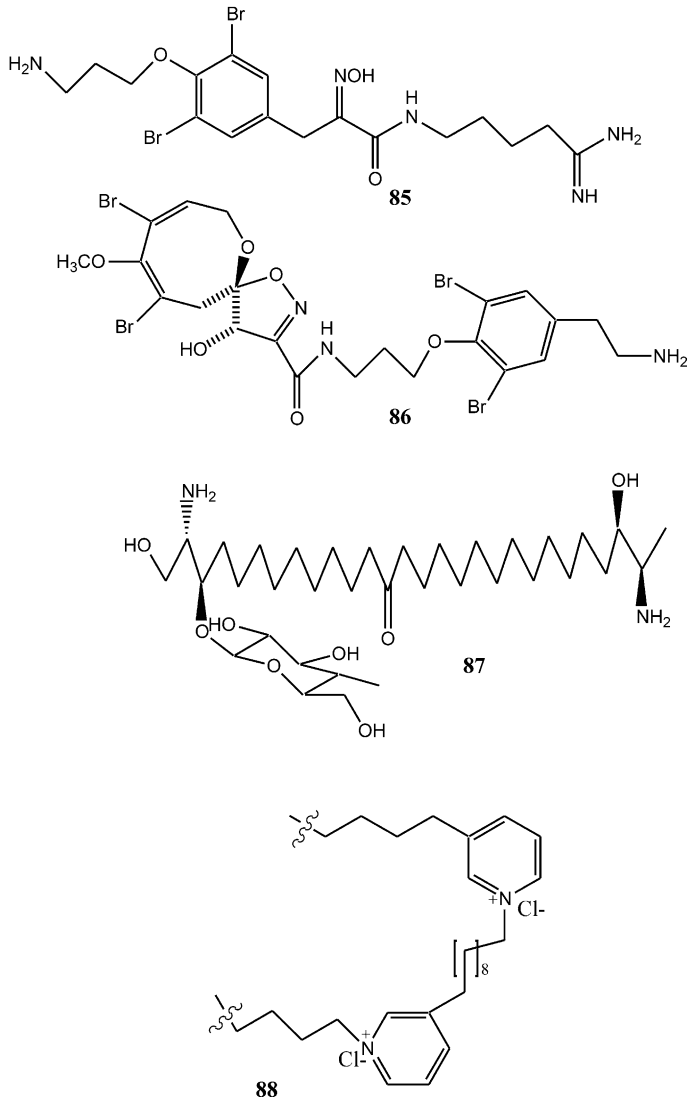

Fig. 8. Other classes of compounds.

3.12 μg/mL). The MICs are comparable to that of the control compound kanamycin (2.5 μg/mL). Interestingly, these compounds with an unusual bis-acetal ring system have attracted the attention of the synthetic chemists. The central bis-acetal is readily obtained in multigram quantities (Ragot et al., 2000). It is therefore possible to generate many analogs and semisynthetics for further assays. Even though most of these compounds are cytotoxic, it is noteworthy that **71**, the most active of them, showed weak cytotoxicity to Vero cells, while retaining its antimycobacterial activity. The enniatins **77**–**83** are also a group of compounds worth further investigation as potential antimycobacterial compounds. They have MICs of 1.56–6.25 μg/mL and except for the analog **83** which is relatively toxic to Vero cells, the others are not cytotoxic. The most active compound reported in this review is the marine pyridoacridine **23** with a MIC of 0.25 μg/mL against the fast growing *M. aurum* A+. It has been shown that activity against this strain of *M. aurum* is highly predictive of activity against MTB (Chung et al., 1995). The IC_{90} for *M. aurum* A+ obtained using **23** (0.21 μg/mL) is less than those obtained with the standard antibiotics rifampin (0.57 μg/mL) and ethambutol (0.5 μg/mL). The IC_{90} correlates well with MIC data (Table 1) in this experiment. The cleistopholine and sampangine alkaloids **12**–**21** are in general very active against *M. intracellulare*. Other compounds with high activity against MTB include the phorbol ester **44** (MIC 3.12 μg/mL), the steroid mixture of **52** and **53** (<2 μg/mL) and the sterol **56** (2.5 μg/mL), and against *M. smegmatis* the lipodepsipeptide **84** (1.5 μg/mL).

The low molecular weight phenols **62** and **63** from *Ardisia japonica* were reported to have in vivo efficacy. This plant is native to some parts of Asia and is currently marketed as a berry-bearing ornamental plant in South Korea (Lee et al., 2003). Bergenin was also isolated from this plant and shown to have antitussive (Xie

et al., 1981) and anti-HIV (Piacente et al., 1996) activities. A detailed study of the antimycobacterial activity of constituents from this plant could be worthwhile. Furthermore, the antimycobacterial activities of some other compounds covered in this review are comparable to the standard anti-TB drugs, such that they warrant further investigation in search for new drugs.

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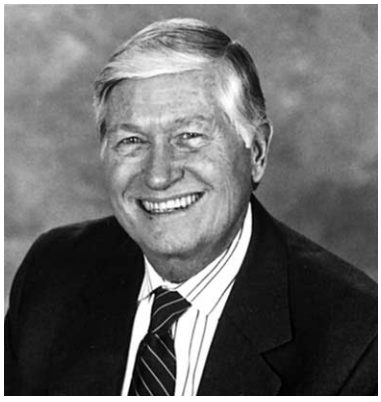

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