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Hantzsch-Type Dihydropyridines and Biginelli-Type Tetra-hydropyrimidines: A Review of their Chemotherapeutic Activities

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ABSTRACT - Years after the first report on 1,4-dihydropyridines (1,4-DHPs) and 1,2,3,4-tetrahydropyrimidines (1,2,3,4-THPMs) appeared, they are revisited as plausible therapeutic agents. This is mainly due to the convenient methods that exist for their synthesis and the diverse pharmacologic properties that these scaffolds present. 1,4-Dihydropyridines and 1,2,3,4-tetrahydropyrimidines are usually regarded as analogous in several aspects. They are both prepared in multi-component reactions using very similar starting materials and synthesis protocols. This leads to common structural features between 1,4-DHPs and 1,2,3,4-THPMs, as well several related biological effects. For example, they share many pharmacological features such as analgesic, anti-tumor, antioxidant, anti-inflammatory, antitubercular, antibacterial, cardiovascular and adrenoceptor blocking activities. Numerous reviews have been devoted to the chemistry and cardiovascular effects of these compounds. However, the lack of a comprehensive literature overview on the chemotherapeutic ability of these scaffolds is behind the present attempt to provide a detailed survey of 1,4-DHPs and 1,2,3,4-THPMs and their structural features as chemotherapeutic agents.

INTRODUCTION

In the last two decades of the nineteenth century two analogous chemical scaffolds were introduced to organic chemistry, which were later to have a great impact on pharmacology and medicinal chemistry. Arthur Hantzsch in 1882, and Pietro Biginelli in 1893, reported methods for the synthesis of 1,4-dihydropyridine (1,4-DHP) and 1,2,3,4-tetrahydropyrimidine (1,2,3,4-THPM) structures, respectively, which now bear their names (1,2). They were similar scaffolds in several aspects, each being prepared in a multi-component reaction (MCR), a very useful method in organic synthesis, which was still in its early years. Acetoacetate esters and aromatic aldehydes were the two components common in both reactions. This led to common structural features between 1,4-DHPs and 1,2,3,4-THPMs, the latter of which could be regarded as the aza analogue of the former from a structural point of view. After years of being neglected, both these chemical scaffolds attracted considerable attention by medicinal chemists in the last three decades of the twentieth century. This was due to their diverse pharmacologic properties, which included analgesic (3,4) anti-tumor (5-7), antioxidant (8,9), anti-inflammatory (10,11), antitubercular (12,13), cardiovascular (14,15), adrenoceptor blocking (16), and antibacterial (17,18) activities. Nonetheless, 1,4-dihydropyridines are mostly known for their cardiovascular effects because, since their introduction into clinical practice in 1975, they have primarily been used as calcium channel modulators (19). 1,2,3,4-Tetrahydropyrimidines have also been evaluated as calcium channel modulating agents due to their structural similarity to 1,4-dihydropyridines (20), and several reviews have been devoted to the chemistry and synthesis of these two chemical scaffolds (21-35). The calcium channel modulating activity of both scaffolds has also led to numerous reviews dedicated to biological responses initiated by this property (36-

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51). However, little is about other biological capacities of 1,4-DHPs and 1,2,3,4-THPMs (52-57).

In this review, we provide a literature overview on the chemotherapeutic ability of these two scaffolds. A detailed survey of the antitubercular, antibacterial, antifungal and cytotoxic activities described for 1,4-dihydropyridine and 1,2,3,4-tetrahydropyrimidine analogues, from 2001 to the present is made. Since the general methods for the synthesis of these scaffolds, known as the Biginelli and Hantzsch reactions, and different strategies for the preparation of the more complicated derivatives have been discussed in several reviews, nothing is provided here about this subject and only their pharmacological properties are discussed.

1,4-DIHYDROPYRIDINES

ANTITUBERCULAR ACTIVITY

Somebody somewhere in the world is newly infected with tuberculosis (TB) bacilli every second and one-third of the world's population currently suffers from this disease. An estimated 1.5 million people die from TB each year (58).

Tuberculosis is principally caused by *Mycobacterium tuberculosis,* a remarkably successful airborne pathogen (59). The infection spreads through the air, but is both preventable and curable. The major solution to the problem is chemotherapy, which requires the development of effective and non-toxic antitubercular agents. The emergence of multi-drug resistance (MDR) has forced the development of new structural classes of antitubercular agents, several of which have shown promising activity. In this respect, 1,4-dihydropyridines and 1,2,3,4-tetrahydropyrimidines are excellent starting scaffolds for the development of antiTB agents against the best-characterized strain of *Mycobacterium tuberculosis*, $H_{37}Rv$ (60-62).

The first report of 1,4-dihydropyridine as an antitubercular scaffold dates back to 2001, when Desai *et al.* described the high antitubercular activity for of 1,4-dihydropyridine 3,5-dicarboxamide and 3,5-dicarboxylate ester derivatives (63). Since that time, it has been demonstrated that some features of dihydropyridine substituents have important effects on the antiTB activity of these compounds. The most studied features are the type of C-3, C-4 and C-5 substituents and also the lipophilicity of the compound. Most of the reported antitubercular 1,4-DHPs fall into one of the three structural categories shown in Figure 1.

The first antitubercular 1,4-DHPs introduced were 3,5-dicarboxamide derivatives rather than 3,5-dicarboxylate esters, which had been known as antihypertensive agents for decades (63). Other researchers identified more antitubercular derivatives of this scaffold (12,64,65). Asymmetric 1,4-DHPs bearing one carboxylate ester group and symmetric analogues with carboxylate ester groups in both C-3 and C-5 positions were also reported as potent antitubercular agents (62,66-68), C-3 and C-5 derivatives in most cases being a (substituted) phenylcarboxamide moiety. There are also some reports of using heteroaromatic carboxamides in these positions (12,69). In C-4 position, substituted phenyl (61,63,66,69) and several heteroaromatic rings (12,62,64,67,68,70) have been studied. N-1 (substituted) phenyl substitutions exist in the structure of some of the antitubercular 1,4-DHPs (61,62,68).

Lipophilicity seems to be an important property since the microorganism cell wall is lipophilic in character and C-3, C-4 and C-5 substituents provide the necessary lipophilicity to the compounds. These positions are always substituted by lipophilic aromatic moieties. It is believed that phenyl substituted 3,5-dicarboxamides are in fact lipophilic prodrugs of the active dicarboxylic derivatives which cannot easily pass through the cell wall (63). The 3,5-dicarboxamide groups undergo hydrolysis to the parent dicarboxylic acid derivatives at the site of action (12,61-70).

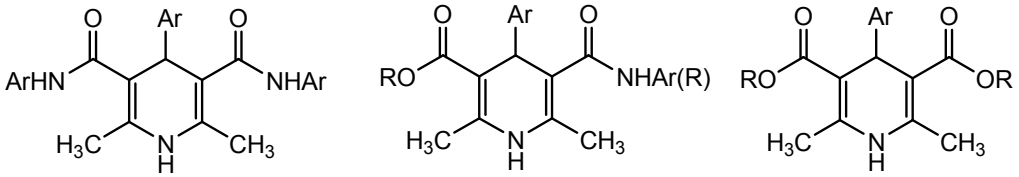
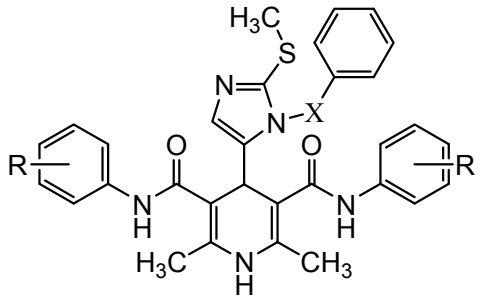


Figure 1. Structural categories of antitubercular 1,4-DHPs

Kharkar *et al.* (70) concluded that *ortho* substitution on the C-3 and C-5 phenyl moieties sterically reduce the rate of amide bond hydrolysis, decreasing the antitubercular activity of the compound. Examples of this are **1a** and **1b,** the former compound being substantially more active than the latter (12) Figure 2.

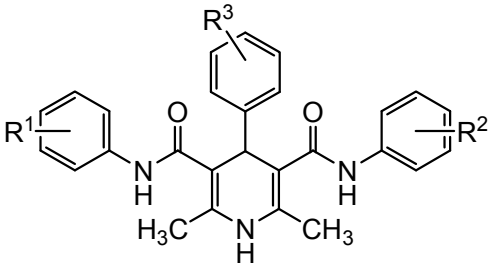


	X	R	MIC[a] (μg/mL)
1a	CH_2	*p*-ClC_6H_5	1
1b	CH_2	*o*-ClC_6H_5	>16
Rifampicin			1
Isoniazide			0.2

[a] MIC= Minimum Inhibitory Concentration

Figure 2. Chemical structures and antitubercular activities of **1a** and **1b**

Other examples can be found in the report provided by Manvar *et al.* (61). Compound **2a** with *ortho*-Cl is less active than **2b** with *meta*-Cl (Figure 3).

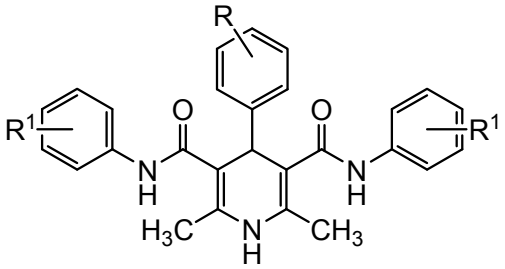


	R^1 , R^2	R^3	
2a	*o*-Cl	*o*-Cl	(PI[a] at >12.5μg/mL = 11)
2b	*m*-Cl	*o*-Cl	(PI at > 6.25μg/mL = 72)
Rifampicin			(PI at > 6.25μg/mL = 97)

[a] PI= Percentage of Inhibition

Figure 3. Chemical structures and PIs (%) of **2a** and **2b**

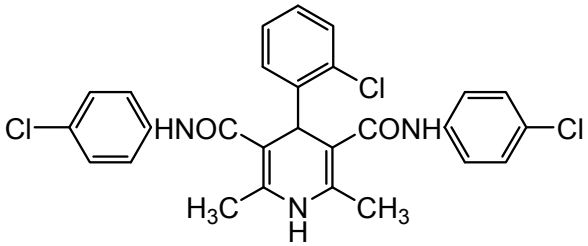
Kharkar *et al.* (70) also concluded that electron-withdrawing groups in *meta* and *para* positions of the C-3 and C-5 phenyl carboxamide moieties increase the antiTB potency. This is not exemplified well in their report but a good example can be seen in other reports. **3a** (PI at 2.5 μg/mL = 98) and **3b** (PI at 2.5μg/mL = 97) are *meta*/*para* nitro phenyl compounds with high antitubercular activity as indicated in (70) Figure 4.



	R^1	R
3a	*m*-NO_2	*p*-SCH_3
3b	*p*-NO_2	*p*-SCH_3

Figure 4. Chemical structures of **3a** and **3b**

It is interesting that **2c** with *para*-Cl is also very weak compared with **2b** with its *meta*-Cl substitution (61) Figure 5. The reason is that the chloro group in *para* position is an electron-releasing rather electron-withdrawing group, while the chloro group in *meta* position is electron-withdrawing.



2c	(PI at >12.5mg/mL = 6)	
Rifampicin	(PI at > 6.25mg/mL = 97)	

Figure 5. Structure and antitubercular activity of compound **2c**

Asymmetric 3,5-dicarboxamide 1,4-dihydro-pyridines with two different dicarboxamide derivatives have also been evaluated as antiTB agents but, compared to the symmetric equivalents, they show weaker activity (61). Asymmetric 1,4-

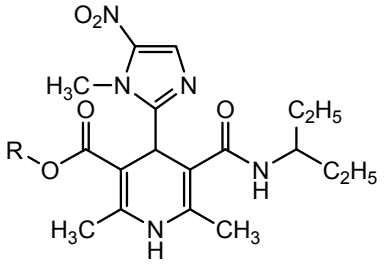
DHPs bearing a carboxylate ester and a dicarboxamide group have also been studied for antitubercular activity as mentioned above. Khoshneviszadeh *et al.* (67) introduced into alkyl (**4a-e**) or aralkyl (**4f-j**) esters to 1,4-dihydropyridines substituted with one carboxamide group at the C-5 position (Figure 6).

Aralkyl esters were found to be more potent, with a potency that depended on the number of methylen groups they contained in the aralkyl ester group. Benzyl, phenethyl and phenpropyl esters were most potent and further increases in the length of the chain led to a fifty percent reduction of the activity (67). This can be explained by the effect of the optimum lipophilicity on the potency (discussed below). Although alkyl esters were weaker antitubercular agents, they showed the same relationship between potency and lipophilicity.

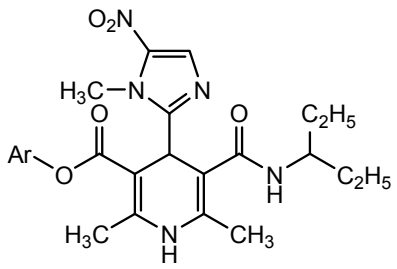
Optimum lipophilicity is probably necessary for the compound to become an active antiTB agent, as was suggested by Kharkar *et al.* (70), who developed 3D-quantitative structure–activity relationship (3D-QSAR) models, and observed that the parameter CLogP had no effect on the significance of the CoMFA and CoMSIA models obtained. Compound **3c** (PI at 2.5 μg/mL = 98) was less lipophilic than **3d** (PI at 2.5 μg/mL = 11) Figure 7, confirming that the third chloro group in the latter compound does not have a positive effect on the antiTB activity (6,14). Compound **3e**, which was even less lipophilic than **3c**, showed high antitubercular activity (PI at 2.5 μg/mL = 94).

In the same vein, compound **4j** (MIC = 2 μg/mL), with its higher LogP (2.23 *vs* 0.79) than compound **4f** (MIC = 1 μg/mL) had half of its antitubercular activity (68) Figure 6. Thus, besides this physicochemical property, other structural features also determine the potency; for example, the effect of the polar electron-withdrawing nitro groups substituted on the C-3 and C-5 phenyl carboxamide moieties discussed above (61,70).

Analysis of the structure of the antiTB 1,4-DHPs reported by Fassihi *et al.* (12) revealed that replacing the NH moiety with CH_2 in the less lipophilic compound **1c** increased its potency. *Meta*-Cl isomer **1d** was much weaker, than the active *para*-isomer **1a**. This is in contrast with the explanation of the differences between **2b** and **2c** activities (60) Figures 2,8, mentioned previously, which suggests that other factors are determinants of the potencies.

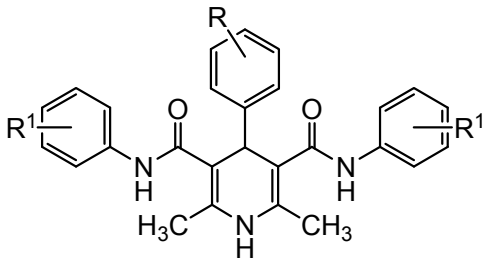


	R	MIC (μg/mL)
4a	CH_3	>500
4b	CH_2CH_3	250
4c	$CH(CH_3)_2$	>500
4d	$(CH_2)_2CH_3$	2
4e	$(CH_2)_3CH_3$	1



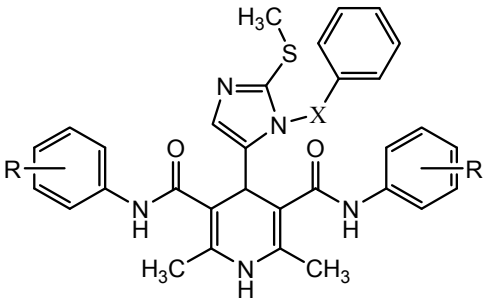
	Ar	MIC (μg/mL)
4f	CH_2-C_6H_5	1
4g	$(CH_2)_2C_6H_5$	1
4h	$(CH_2)_3C_6H_5$	1
4i	$(CH_2)_4C_6H_5$	2
4j	$(CH_2)_5C_6H_5$	2
Isoniazide		1
Rifampicin		1

Figure 6. Chemical structures and MICs (μg/mL) of asymmetric 1,4-dihydropyridines



	R^1	R
3c	*m*-NO_2	*o,p*-$(Cl)_2$
3d	*o*-Cl	*o,p*-$(Cl)_2$
3e	*m*-NO_2	H

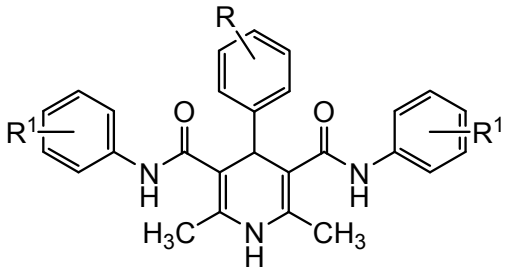
Figure 7. Structures of **3c-e**



	X	R	MIC (μg/mL)
1c	NH	*p*-ClC_6H_5	>16
1d	CH_2	*m*-ClC_6H_5	>16
Rifampicin			1
Isoniazide			0.2

Figure 8. Chemical structures and MICs of **1c** and **1d**

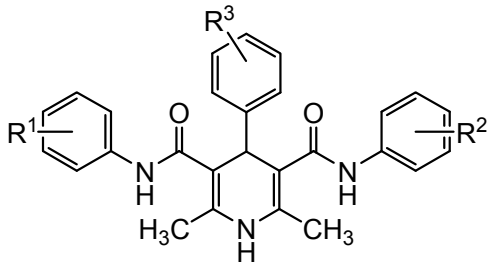
The presence of different aromatic moieties at the C-4 position of the dihydropyridine ring has been seen to affect the antiTB activity, partly by providing the desired lipophilicity to the molecule (62,64,65,67,68,70). Phenyl substituted groups were the first aromatic moieties evaluated in this position (61,63,66). Compound **3f** (LogP = -0.3888) was reported to be less reactive than **3g** with a LogP of 1.1949, confirming the effect of the lipophilicity on the potency (63,70) Figure 9.



	R^1	R	PI at 2.5μg/mL
3f	*p*-Cl	*m*-NO_2	29
3g	*p*-Cl	*m,p,m′*-$(OCH_3)_3$	94
Rifampicin			95

Figure 9. Structure and antitubercular activity (PI) of **3f** and **3g**

Here again some controversy exists between the different reports, confirming the involvement of other factors as stated previously. For example, **2d** with one more nitro group in its structure was more potent than **2e** and even more potent than **2f** and **2g** (PIs at > 6.25 μg/mL = 64, 42, 29 and 28, respectively), which contain halogen atoms in their structures (61) Figure 10.

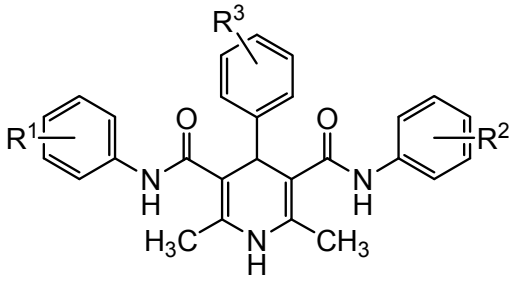


	R^1, R^2	R_3
2d	*m*-NO_2	*o*-NO_2
2e	*m*-NO_2	H
2f	*p*-F	*o*-NO_2
2g	*p*-F	*o*-Cl

Figure 10. Chemical structures of **2d-g**

Presumably some kinds of electrostatic interactions or hydrogen bonding is involved in the attachment of the molecule to its site of action. According to a 3D-QSAR study published by Kharkar *et al.* (70), the presence of *para* substituents with rotatable bonds at the C-4

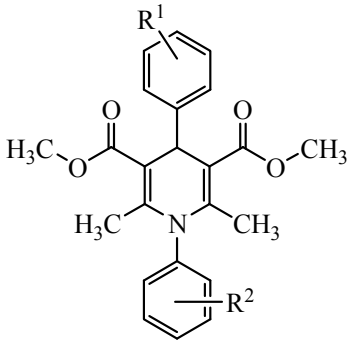
position, such as -$N(CH_3)_2$, -OCH_3 and -SCH_3, increases antiTB activity. The authors assumed the involvement of proper hydrogen bonding between these substituents and the receptor due to the free rotation of these substituents (70). This finding was confirmed by Manvar *et al.* (61) in a more recent 3D-QSAR study. Some disagreement concerning this relationship can be observed in the experimental data, again suggesting that other factors are determining the potency of the compounds. For example, **2h** and **2i** (PIs at > 12.5 μg/mL = 93 and 84, respectively) were strong compounds but, **2j** (PI at > 12.5 μg/mL = 23) was weak, although all had a *para*-dimethylamino phenyl group at the C-4 position with slight differences in other substituents (61). In the case of the methoxy group in this position, most of the compounds were weak: **2k** (PI at > 12.5 μg/mL = 30) was weak and **2l** (PI at > 6.25 μg/mL = 71) has been shown to be a moderate antitubercular agent (Figure 11). Several weak examples can be extracted from the data provided by Manvar *et al.* (61).The lack of the data for defining the resonance or inductive effects of the substituents on the C-3 and C-5 phenyl moieties makes it difficult to establish a relationship between the effect of the above-mentioned C-4 substitutions and the antitubercular activity. The assumption made by Kharkar *et al.* (70) that the *ortho* substitution on the C-3 and C-5 phenyl moieties sterically reduces the rate of amide bond hydrolysis could be an explanation for the weak antitubercular activities of some of these compounds.



	R^1, R^2	R^3
2h	*o*-OCH_3	*p*-$N(CH_3)_2$
2i	*o*-CH_3	*p*-$N(CH_3)_2$
2j	*o*-Cl	*p*-$N(CH_3)_2$
2k	*o*-Cl	*p*-OCH_3
2l	*m*-Cl	*p*-OCH_3

Figure 11. Structures of **2h-l**

N-1 substitution of the 1,4-dihydropyridine 3,5-dicarboxylate esters with (substituted) phenyl moiety **5a** and **5b** resulted in compounds with lower antiTB activity than unsubstituted compounds (61) Figure 12.

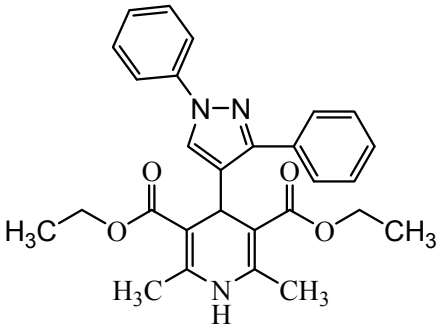


	R^1	R^2	PI at > 6.25 μg/mL
5a	*m*-OC_6H_5	H	33
5b	*p*-Cl	*p*-OCH_3	5
Rifampicin			97

Figure 12. PIs of antitubercular N-1 substituted 1,4-dihydropyridine 3,5-dicarboxylate esters

However, this cannot be regarded as a rule: for example, N-1 substitution (5) did not change the potency. This can be concluded by comparing the potencies of **6** and **7** (68) Figure 13.

In an attempt to suggest a putative mechanism of action for this class of antitubercular agents Mahnam *et al.* (71) focused on the structural similarities of the 1,4-dihydropyridine scaffold to NADH, the coenzyme for a key regulatory enzyme in fatty acid elongation, *trans*-2-enoyl-ACP reductase. The authors determined the high binding affinity of a series of 1,4-dihydropyridine-3,5-dicarboxamide derivatives to enoyl reductase from *Mycobacterium tuberculosis* using docking and molecular dynamics simulation methods. Using the same methods, they also obtained high binding affinity for the 3,5-dicarboxylic acid derivatives and concluded that aryl or heteroaryl substituents at C-3 and C-5 are not necessary for receptor binding and only provide the necessary lipophilicity to the molecule. This agrees with the observations of Desai *et al.* and Kharkar *et al.* that carboxamide groups are hydrolyzed to 1,4-dihydropyridine-3,5-carboxylic acid derivatives with antibuercular activity (12, 61-71).



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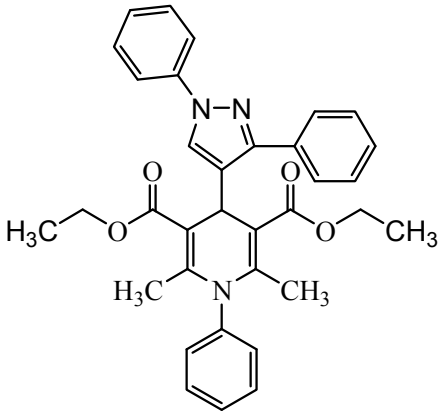


7

	MIC (μg/mL)	PI at 1.56 μg/mL
6	1.56	95
7	1.56	96
Isoniazide	0.03	-

Figure 13. Comparison of the antitubercular activity of N-1 substituted and N-1 unsubstituted 1,4-dihydropyridines

ANTIMICROBIAL ACTIVITY

Despite the recent advances in medicine, antimicrobial chemotherapy still remains a problem in most developing and even developed countries. The narrow spectrum of activity of some antimicrobial drugs on the market and the many serious adverse effects reported explain the reasons for the failure of antimicrobial chemotherapy and why there is a search for more acceptable compounds. The inevitable emergence of resistant strains of pathological microorganisms and of the growing list of multi-drug resistant strains is another serious problem in clinical practice (72). Among the many different chemical scaffolds screened, 1,4-dihydropyridine compounds have attracted attention both as antimicrobial agents and MDR-reversing entities because of their ability to exert synergistic antibacterial effects in combination with known antibiotics (73-99). It has been demonstrated that the type of C-3, C-4 and C-5 substituent and the lipophilicity of the molecule have important effects on the antimicrobial activity of 1,4-dihydropyridine compounds. Three structural categories are found among the antimicrobial 1,4-DHPs as shown in Figure 14.

1,4-DHP derivatives containing diethyl carbamoyl and ester substituents at C-3 and C-5, and substituted aromatic or heteroaromatic ring at C-4 position have also been reported as potential antimicrobial agents (73-76). An N-1 (substituted) phenyl substitution exists in the structure of some of the antimicrobial 1,4-DHPs (96-99).

Maya *et al.* (75) concluded that the presence of a fused ring attached to the dihydropyridine ring through the C2=C3 bond of the dihydropyridine moiety decreases the antimicrobial activity of the compound. Examples of this type of dihydropyridine derivatives are **8a**, **8b** and **8c** (Figure 15).

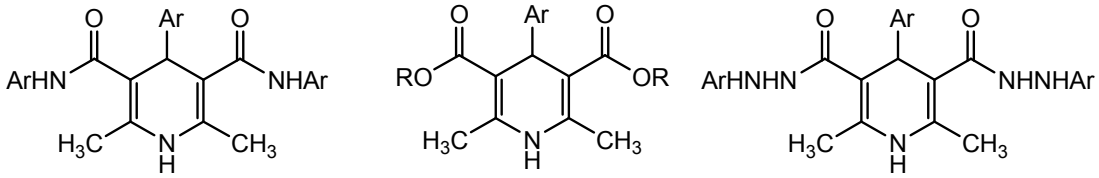
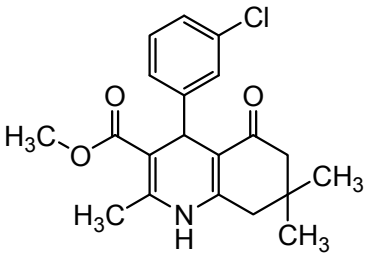
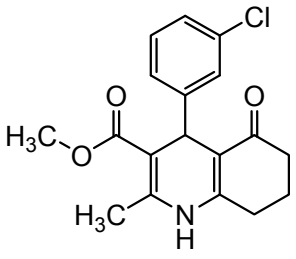


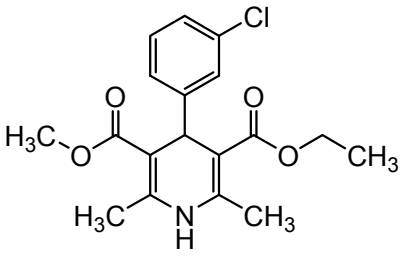
Figure 14. Three structural classes of antimicrobial 1,4-DHPs



8a



8b

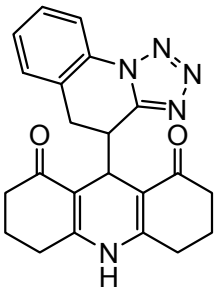


8c

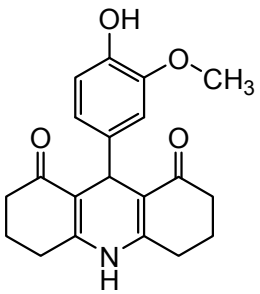
	PI at 10 μM *T. cruzi*
8a	10
8b	No Inhibition
8c	40
Nicardipine	40
Felodipine	32

Figure 15. Chemical structures and antimicrobial activities of **8a-c**

In addition, Ladani *et al.* (76) reported antimicrobial dihydropyridin derivatives as possessing two cyclic ketones fused to the main scaffold, instead of the C-3 and C-5 carboxylate derivatives. Compound **9a** is an example with good fungicidal and poor bactericidal activity, while compound **10** (17) displayed good bactericidal properties (Figure 16).



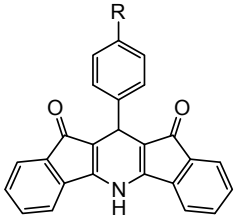
9a



10

Figure 16. Antimicrobial dihydropyridines with cyclic ketones fused to the main scaffold

Other examples were reported by Dabholkar *et al.* (77). Compounds **11a** and **11b** showed almost equal potency against *Escherichia coli* and very similar activities against *Corynebacterium diphtheria* (gram positive bacteria), *Pseudomonas aeruginosa* and *Staphylococcus aureus* (gram negative bacteria) to the reference drug, Ampicillin trihydrate (Figure 17).



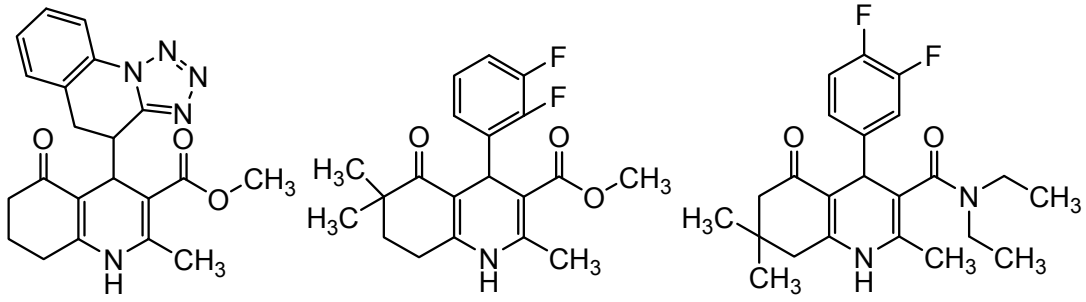
		Zone of inhibition at 100 μg/mL (mm)			
	R	***S. aureus***	***C. diphtheria***	***P. aeruginosa***	***E. coli***
11a	Cl	22	20	21	19
11b	OCH_3	21	18	20	18
Ampicillin		26	28	24	21

Figure 17. Chemical structures and zone of inhibition values (ZIs) of **11a** and **11b**

Ladani *et al* (76) and Gunduz *et al.* (78) prepared dihydropyridines with a fused cyclic ketone and a carboxylate or carboxamide moiety at the C-3 and C-5 positions of the DHP ring, finding the compounds to be antimicrobial agents (**9b**, **12a** and **12b**) (Figure 18).

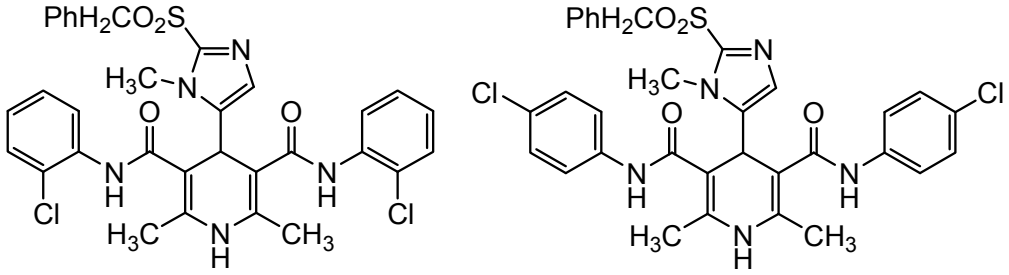
Akbarzadeh *et al.* (79) in 2008 prepared antimicrobial 1,4-DHP 3,5-dicarboxamides, which were found to be inactive when used as antimicrobials alone, but active when used in conjunction with Amoxicillin. Comparison of the activities of the synthesized symmetrical amides indicated that the presence of withdrawing groups, especially at the *para* position of the phenyl carboxamide moieties, was essential enhancing activity; however, substitution at the *meta* or *ortho*

positions reduced or eliminated this enhancing effect. **13a** had no inhibitory activity against *S. aureus*, unlike **13b** (79) Figure 19.



9b **12a** **12b**

Figure 18. 1,4-DHPs with a fused cyclic ketone and a carboxylate or carboxamide moiety at the C-3 and C-5 positions



13a **13b**

	Zone of inhibitionat 25 μg/mL (mm)
	S. aureus
13a+Amoxicillin	No Inhibition
13b+Amoxicillin	14

Figure 19. Chemical structures and antimicrobial activity of **13a** and **13b** in combination with Amoxicillin

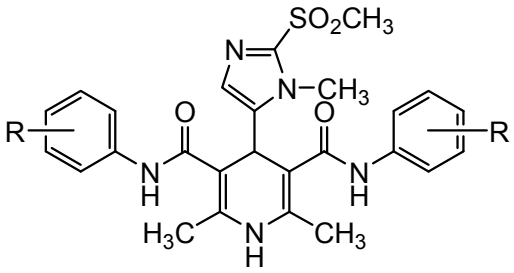
The same research group in 2010 reported that disubstitution at *meta* and *para* positions of the phenylcarboxamide moieties with chlorine atoms also increased the activity, while the presence of an *ortho*-Cl substitution decreased the potency. This conclusion can be exemplified by compounds **14a** and **14b** in combination with Cloxacillin, whose joint antibacterial activities were studied against Methicillin-resistant *S. aureus* (MRSA) (80) Figure 20.

Sirisha *et al.* also demonstrated that 1,4-DHP-3,5-dicarboxamides were more active than 1,4-DHP-3,5-dicarboxylate esters. Most of the compounds they prepared were relatively more active against gram negative bacteria. Compound **15a** was almost equipotent to Streptomycin/ Tetracyclin against gram negative bacteria [zone of inhibition (ZI) = 23, 26 and 25, 21 millimeters in 50 μg/mL for *Escherichia coli* and *Proteus vulgaris*, respectively] (Figure 20). But, among the compounds they prepared, **15b** was more potent against a gram positive bacterium, *Bacillus subtilis*, unlike other compounds. The ZI for this compound was 18 mm against *B. subtilis* (Tetracycline: 20 mm) and 12 against *S. aureus* (Tetracycline: 22 mm) both in 50 μg/mL (69) Figure 21.

Sirisha *et al.* reported on some more antibacterial 1,4-DHPs with higher activities against gram negative bacteria in 2011. For example, **16a** and **16b** had significant inhibitory activity against *E. coli* and *P. vulgaris*. **16a** was also found to exhibit considerable activity against MRSA (81) Figure 22. Compounds with aromatic or heteroaromatic rings at C-3 and C-5 arylcarboxamide moieties were active against the pathogenic microorganisms studied (69,81).

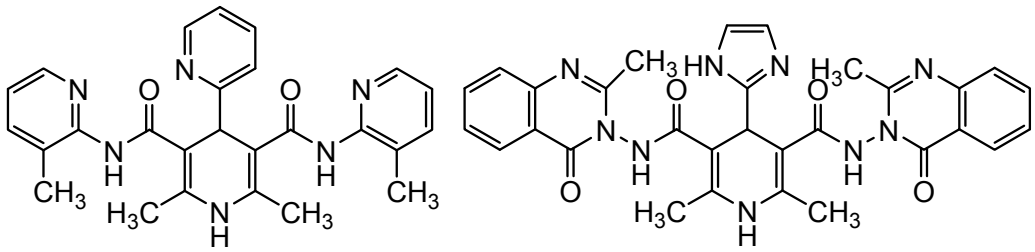
The presence of bulk groups at C-3 and C-5 positions in some novel 1,4-dihydropyridines prepared by Rao *et al.* confirmed that lipophilicity is important for antimicrobial effects. Here again,

the results obtained confirmed that compounds with a dicarboxylic acid ester moiety at C-3 and C-5 were less potent than dicarboxamide analogues (82). They also showed that 3,5- dicarboxamide compounds possessing a *para* substituent (-OH , -OCH_3 or -Cl) on the C-4 phenyl ring showed high inhibitory activities against gram positive and gram negative bacteria and fungi. For instance, **17a** (ZI at 100 µg/mL = 24 mm, ZI for Ciprofloxacin = 23 mm) was found to be highly active against *S. aureus*. **17b** (ZI at 100 µg/mL = 26 mm) was another potent compound against the same bacterium. **17b** was also a good antifungal agent (ZI at 100 µg/mL = 24 mm against *Aspergillus niger*, for Clotrimazole = 23). Another compound of this series, **17c**, showed high antifungal activity against *Chrysosporium Sp.* (82) Figure 23.



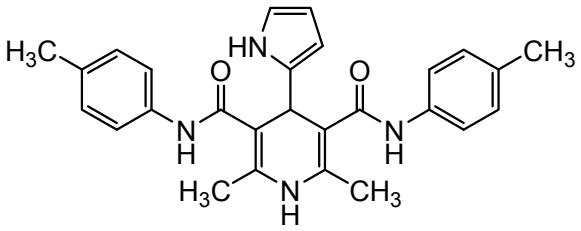
	R	Zone of inhibition at 125 µg/disc (mm)
14a+Cloxacillin	*p,o*-Cl	9
14b+Cloxacillin	*o*-Cl	No Inhibition

Figure 20. Chemical structures and antibacterial activities of **14a** and **14b** in combination with Cloxacillin

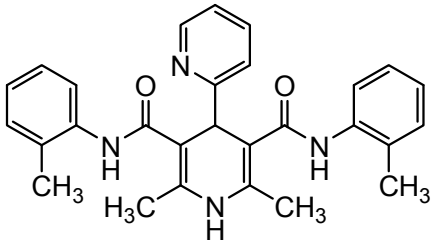


15a **15b**

Figure 21. Chemical structures of **15a** and **15b**

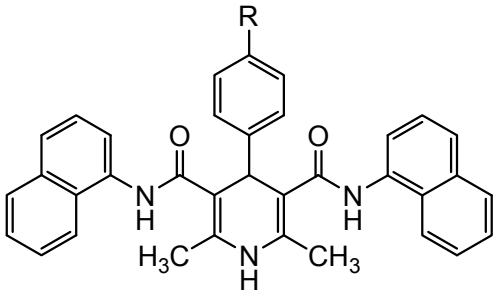


	Zone of inhibition (mm)		
	E. coli	***P. vulgaris***	***MRSA***
16a	22 (50 μg/mL)	18 (50 μg/mL)	10 (1000 μg/mL)
Streptomycin	23 (50 μg/mL)	26 (50 μg/mL)	18 (1000 μg/mL)
Tetracyclin	25 (50 μg/mL)	21 (50 μg/mL)	15 (1000 μg/mL)



	Zone of inhibition (mm)		
	E. coli	***P. vulgaris***	***MRSA***
16b	19 (50 μg/mL)	16 (50 μg/mL)	8 (1000 μg/mL)
Streptomycin	23 (50 μg/mL)	26 (50 μg/mL)	18 (1000 μg/mL)
Tetracyclin	25 (50 μg/mL)	21 (50 μg/mL)	15 (1000 μg/mL)

Figure 22. Antimicrobial activity of **16a** and **16b**

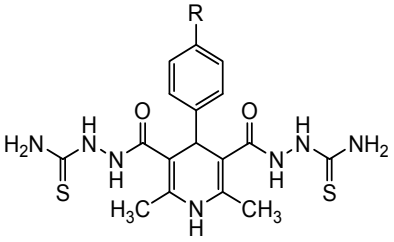


17a **R:** Cl
17b **R:** OH
17c **R:** OCH_3

Figure 23. Antimicrobial 1,4-DHPs with bulk groups at C-3 and C-5 positions

In 2011, 1,4-DHPs possessing thiosemicarbazide moieties at C-3 and C-5 were subjected to antimicrobial studies (83). It was demonstrated that these compounds were better antibacterial and antifungal agents than 3,5-diester derivatives. **18a** was found to be highly active against *Klebsiella pneumoniae* and **18b** was the most potent compound against *E. coli* according to the antimicrobial evaluations. **18a** and **18b** were also both excellent antifungals against *Cryptococcus*

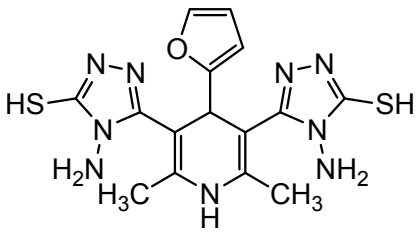
neoformans and *Candida albicans*, respectively (Figure 24). Kumar *et al.* showed that compounds with thiosemicarbazide moieties at C-3 and C-5 and, at the same time, electron donor substituents (-OH and -OCH_3) on the C-4 position of the phenyl, had good inhibitory activities against gram positive and gram negative bacteria and fungi (83).



		Zone of inhibition at 100 µg/mL (mm)			
	R	*K. pneumoniae*	*E. coil*	*C. neoformans*	*C. albicans*
18a	OCH_3	18	-	24	-
18b	OH	-	25	-	26
Ciprofloxacin		19	27	-	-
Clotrimazole		-	-	25	24

Figure 24. Structures of 1,4-DHPs possessing thiosemicarbazide moieties at C-3 and C-5

Kumar *et al.* also explained the antimicrobial activities of structures mimicking the thiosemicarbazide moieties at C-3 and C-5 positions of 1,4-DHP ring in another report (30). Molecule **19** was potent against *A. niger* and *C. albicans* at 100 µg/mL (ZI = 17 and 20 mm, respectively; ZIs for Clotrimazole were 22 and 18 mm, respectively) (84) Figure 25.

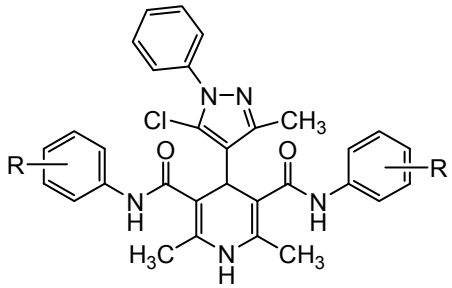


19

Figure 25. Chemical structure of compound **19**

Solanki *et al.* demonstrated that the presence of bulk substituents on C-3 and C-5 made the compound more potent against bacteria and fungi (85). They also concluded that the presence of -Cl on the phenyl rings of C-3 and C-5 phenyl carboxamide moieties, along with a variation in solubility, play an important role in determining the antimicrobial activity of the compounds. For example, compound **20a** possessed effective antibacterial activity against *Streptococcus pyogenes* (Figure 26). Akbarzadeh *et al.* (79,80) also e concluded that compounds with a chloro group at the *meta* position of this ring have high antimicrobial potency (Figures 19,20).

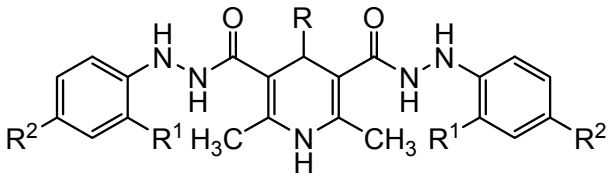
Solanki *et al.* demonstrated that the presence of fluoro and nitro groups on any position of the C-3 and C-5 phenyl carboxamide moieties increases the activity, whereas any other group at *para* reduces the activity (**20b** and **20c**) (85) Figure 26.



		Zone of inhibition at 25 µg/mL(mm)
	R	*S. pyogenes*
20a	*m*-Cl	11
20b	*m*-NO_2	12
20c	*p*-F	12
Chloramphenicole		13
Ciprofloxacin		19

Figure 26. Chemical structures and antibacterial activities of **20a**-c against *Streptococcus pyogenes*

In 2013 a research group reported the antibacterial and antifungal activities of a series of C-3 and C-5 diphenyl carbohydrazide derivatives of 1,4-DHPs (86). The presence of -NO_2 and Cl groups at the *para* and *ortho* positions of phenylhydrazine moieties were found to favor antibacterial (both gram positive and gram negative) and also antifungal activities. **21a**, **21b** and **21c** showed half of the activity of Ciprofloxacin and more than half of the activity of Ketoconazole against *S. aureus*, *E. coli*, *C. albicans* and *A. niger* (Figure 27).



	R	R^1	R^2
21a	C_6H_4OH	H	Cl
21b	H	H	Cl
21c	H	NO_2	NO_2

Figure 27. Structures of C-3 and C-5 diphenyl carbohydrazide derivatives of 1,4-DHPs

Another research group synthesized several C-3 and C-5 dicarbohydrazide derivatives. Among the compounds evaluated for antibacterial activity, **22a** showed good activity against *Bacillus cereus* and *E. coli*. The same was true for compound **22b** against *S. aureus* and *B. subtilis*. This compound also displayed good antifungal activity against *C. albicans* (87) Figure 28.

Samaunnisa *et al.* reported compounds having bis pyrazolidine-3,5-dione moieties at C-3 and C-5 to be antibacterial and antifungal agents but less potent than the corresponding phenylhydrazine derivatives (88) Figure 29.

Abu-Melha explained that compounds with heterocyclic moieties at C-3 and C-5 positions showed better antimicrobial activities than their corresponding open chain ones. The tested compounds were more active against gram positive than against gram negative bacteria. **24a** (MIC = 3.125 µg/mL) was equipotent to Chloramphenicol (MIC = 3.125 µg/mL) against *B. subtilis* and eight times stronger than **24b** (MIC = 25 µg/mL). The structures of these compounds are illustrated in Figure 30 (89).

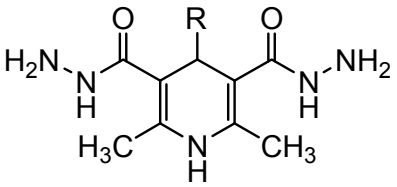
As previously mentioned, the C-4 substituent has a determining effect on the antimicrobial activity. In almost all of the reported antimicrobial 1,4-dihydropyridines the C-4 substituent was a (substituted) phenyl ring. It is demonstrated that substitution on this ring is important for the antifungal activities (90). Compounds including OCH_3 at *para* position displayed good inhibitory activity against the growth of *Aspergillus fumigatus*, e.g. **25a**, but **25b,** with -OH instead of -OCH_3, had no inhibitory effect (Figure 31).

Compounds possessing heterocyclic systems substituted with phenyl derivatives have been associated with moderate antimicrobial effects. Prakash *et al.* provided a new series of 1,4-DHPs and evaluated their antibacterial and antifungal activities. The results showed that **26a** (Figure 32) with CH_3 substituent on the phenyl ring of C-4 pyrazol was the best antibacterial among the studied compounds (MIC = 64 µg/mL against *S. aureus*; Ciprofloxacin: 5 µg/mL). On the other hand, **26b** without this group was the best antifungal derivative (PI of 51.1 and 58.8 against *A.niger* and *A. flavus*, respectively, while the corresponding values for Fluconazole were 81.1 and 77.7, respectively) (73).

In the report provided by Vijesh *et al.,* other examples can be found of compounds including a pyrazole ring with different substituents on the C-4 moiety. Among these compounds, **27** showed excellent antibacterial activity, and was equipotent to Streptomycin, against *E. coli*, *S. aureus* and *P. aeruginosa*. The same compounds demonstrated poor antifungal activity (74) Figure 33.

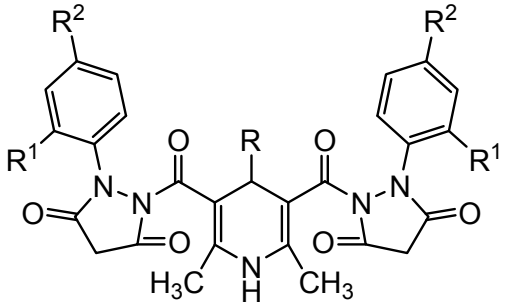
Ahmed *et al.* reported the antimicrobial screening of several 1,4-DHPs against different bacteria and fungi. Compounds **28a** and **28b** shown in Figure 34 exhibited good inhibitory activity against *E. coli*, *S. aureus* and *A. Niger*, concluding that the presence of electron-releasing -OH and -OCH_3 enhances the antimicrobial activity, probably by interaction at a receptor site in the microorganism through hydrogen bonding. They offered confirmation of their conclusion by reference to the effects of the drugs such as Methicilline and Amoxycilline, which also possess -OH and -OCH_3 in their structure (91).

N-1 substitution of the 1,4-dihydropyridines which possess heteroaromatic moieties at C-3 and C-5 positions was reported by Rajput *et al*.



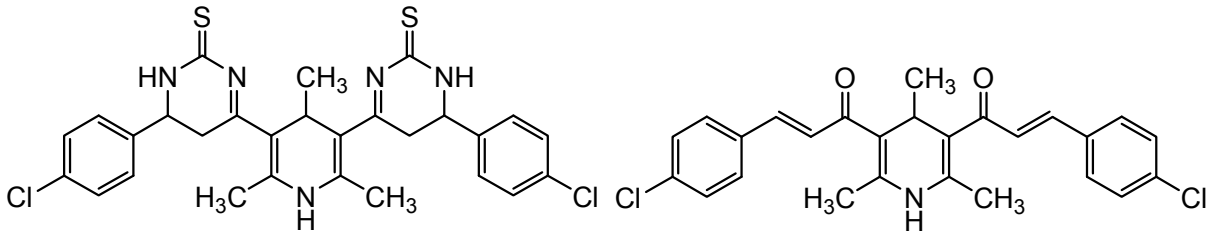
		Zone of inhibition at 100 μg/mL (mm)				
	R	***B. cereus***	***E. coli***	***S. aureus***	***B. subtilis***	***C. albicans***
22a	*p*-Styryl	12	12	-	-	-
22b	*p*-chlorophenyl	-	-	12	11	10
Streptomycin		24	No Inhibition	20	No Inhibition	-
Miconazole		-	-	-	-	17

Figure 28. The C-3 and C-5 dicarbohydrazide derivatives of 1,4-DHPs and their antimicrobial activity



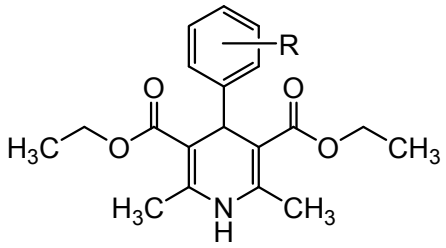
				Zone of inhibition at 1000 μg (mm)			
	R	**R^1**	**R^2**	***B. subtilis***	***E. coli***	***C.albicans***	***A. niger***
23a	C_6H_4OH	H	Cl	14	-	-	-
23b	H	H	Cl	-	12	-	-
23c	H	NO_2	NO_2	-	-	14	12
Ciprofloxacin (10 μg/mL)				36	31	-	-
Clotrimazole (10 μg/mL)				-	-	29	25

Figure 29. Antibacterial and antifungal activities of **23a-c**



24a **24b**

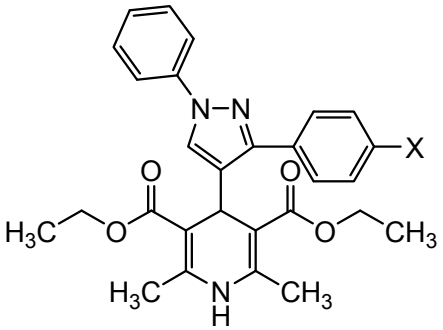
Figure 30. Chemical structures of **24a** and **24b**



	R	DDA[a] MIC (μg/disc) *A. fumigatus*	MDA[b] MIC (μg/mL) *A. fumigatus*
25a	*p*-OCH_3	2.92	15.62
25b	*m*-OH	No Inhibition	No Inhibition
Amphotericin B		0.73	1.95

[a] DDA= Disc Diffusion , [b] MDA= Microbroth Dilution

Figure 31. Chemical structures and antifungal activity of **25a** and **25b**



	X
26a	CH_3
26b	H

Figure 32. Structures of compounds **26a** and **26b**

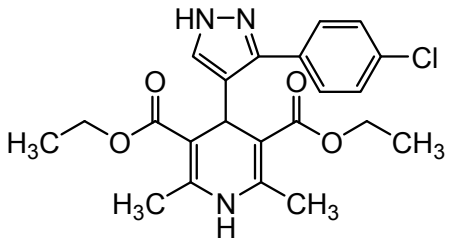
Antimicrobial evaluation of these compounds identified moderate to good antimicrobial activity (92), and all were found to be less active against *A. niger*. Compound **29** was more potent than the standard against *C. albicans*. The structure of this compound is given in (92) Figure 35.

The same research group screened more compounds for their antimicrobial activity. They revealed that compounds **30a**, **30b** and **30c** were weak against *B. subtilis*, *E. coli* and *P. aeruginosa* but, **30d** (Figure 36) showed good antibacterial activity against *P. aeruginosa*, *B. subtilis*, *E. coli* and *S. aureus*. All of these compounds were found to be more potent than standard antifungal (Nystatin) against *A. niger*. The mean ZI for **30e** was 19.39 mm and for Nyastatin 9.53 mm, both at 100 μg/mL (93).

It seems that the presence of a thiazolidinone moiety favors antifungal activity, while compounds with isoxazole rings at the same positions show poor potency against *A. niger* (92,93).

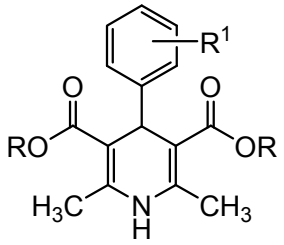
Mithlesh *et al.* reported the preparation and high antibacterial and antifungal activities of a series of 1,4-dihydropyridine derivatives. Compounds **31a** and **31b**, shown in Figure 37 exhibited very high antibacterial and antifungal potencies. It was concluded that the nitro group, with its lone pair of electrons, plays an important role in the antimicrobial activity by forming complexes with microbial metaloenzymes (94).

N-1 phenyl substituted 1,4-dihydropyridines which have another phenyl ring substituted on the C-4 moiety, bearing substituents on both phenyl rings have been subjected to antimicrobial evaluations. All the compounds with a chloro group substituted on the N-1 phenyl ring were better antibacterials than antifungals. **32a** inhibited *P. vulgaris* growth with a 21 mm zone of inhibition (Amoxicillin = 25 mm). Compound **32b** was almost as strong as amoxicillin against *E. coli* (ZI = 23 mm, for Amoxicillin = 25 mm). Replacing the *para*-chloro group in **32c** with *para*-OCH_3 caused a change in the antimicrobial potency in favor of antifungal activity. **32d** inhibited *A. Niger* growth with 23 mm zone of inhibition, while Griseofulvin, as the standard drug, had ZI = 24 mm (95) Figure 38.



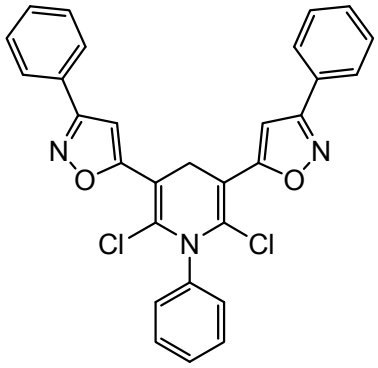
	Zone of inhibition at 1 μg/mL (mm)					
	E. coli	***S. aureus***	***P. aeruginosa***	***A. flavus***	***C. keratinophilum***	***C. albicans***
27	17	15	16	2	3	4
Streptomycin	16	15	16	13	17	22

Figure 33. Chemical structures and antimicrobial activity of compound **27**



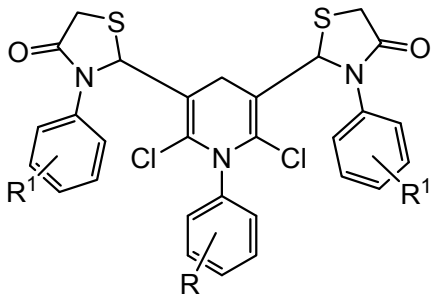
			Zone of inhibition at 50 μg/mL (mm)			
	R	**R^1**	***S. aureus***	***E. coli***	***A. niger***	***C. albicans***
28a	OC_2H_5	*p*-OH	14	11	12	10
28b	OC_2H_5	*p,m*-OCH_3	11	10	12	9
Gentamicin			20	19	-	-
Fluconazole			-	-	18	18

Figure 34. Chemical structures and antimicrobial activity of **28a** and **28b**



	Zone of inhibition at 100 μg/mL (mm)
	C. albicans
29	9.57
Nyastatin	9.53

Figure 35. Structure of **29** and its antifungal activity against *C. albicans*



	R	R^1
30a	*p*-OCH_3	*p*-CH_3
30b	*m*-Cl	*p*-CH_3
30c	*p*-OCH_3	*p*-Cl
30d	H	*p*-Cl

Figure 36. Chemical structures of some antimicrobial N-1 substituted 1,4-dihydropyridines

ANTICANCER ACTIVITY

One of the major clinical problems during antitumor chemotherapy is the development of multidrug-resistance (MDR) in tumor cells (96). MDR is defined as the increased efflux of a broad class of hydrophobic cytotoxic drugs mediated by one of a family of energy-dependent transporters, known as ATP-binding cassette (ABC) transporters. Several members of the ABC transporter family, such as P-glycoprotein (P-gp, also known as MDR1), can induce multidrug-resistance. Researchers have worked to develop drugs that either evade efflux or inhibit the function of efflux transporters. A large number of structurally diverse compounds have been identified as MDR inhibitors (98). These compounds in general have aromatic moieties in their structure, are highly lipophilic and possess monocationic or dicationic side chains. Most of them also have a protonated nitrogen group at physiological pH.

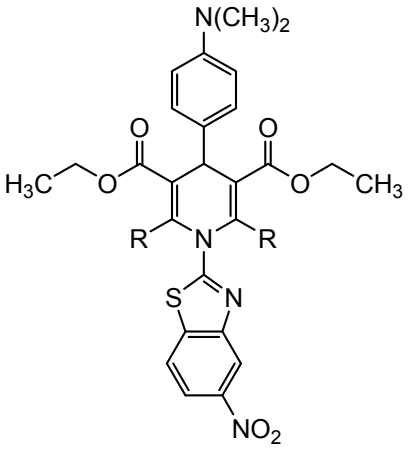
Because of their affinity for P-gp, 1,4-dihydropyridines have become the most extensively investigated compounds as P-gp inhibiting agents (98). It is interesting that 1,4-DHPs which lack calcium antagonistic activity or are weak calcium channel blockers possess this property (98-100). In addition, the presence of a hetaryl group at 4-position of DHP was found to be effective at increasing MDR-inhibiting activity (101). The literature shows that the type of C-3, C-4 and C-5 substituents and also the lipophilicity of the molecule have important effects on the anticancer activity of 1,4-dihydropyridine compounds.

Based on QSAR/QSPKR (quantitative structure-activity/pharmacokinetics relationship) prediction models, 1,4-DHPs with potent P-gp inhibitory effects and minimal Ca^{2+} channel blocking activity have been reported. The results suggested LogP as a highly correlated parameter with P-gp inhibitory effect in Daunomycin cytotoxicity (102).

Tasaka *et al.* (2001) reported MDR-reversing symmetric 1,4-dihydropyridines possessing an *n*-pentyl group at the C-4 position. Based on Ford *et al.,* compounds with one or more tertiary amino groups had a strong MDR-reversing effect and a cyclic amine was more effective than a noncyclic one (103). Compounds with these characteristics were designed for the investigation by Tasaka *et al.* (104). Some of the results are summarized in Figure 39. The reported resistance index was determined from the IC_{50} of Doxorubicine (DXR) with the test compounds (1 µg/mL) in KB/VJ300 cells divided by the IC_{50} of DXR without test compounds in the KB cells. Antitumor activity was determined by the survival days of mice treated with Vincristine (VCR) (0.1 µg/kg, ip) with the test compounds (100 µg/kg, ip) divided by the survival days of mice treated by VCR (0.1 µg/kg, ip) alone.

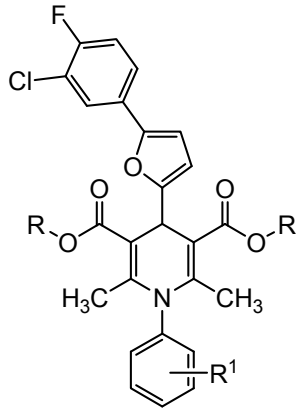
The results showed that **33a** had no antitumor activity, presumably because it lacked a tertiary amino group. **33b** and **33c** were effective antitumor

agents; **33b** was a better MDR-reversing compound according to the resistance index values obtained. Replacement of one of the 3-pyridylpropylesters with a substituted piperazinylpropyl group provided compounds with lower resistance index values *in vitro* and *in vivo*, amongst which **33d** showed the best activity. **33e** indicated that the nitrogen atom at the 2-position in 2-pyridylpropylester is probably sterically hindered and therefore less effective than 3- or 4-pyridylpropylesters. Compound **33f** revealed that substitution by 4-pyridylpropylester was associated with high MDR activity. Compounds such as **33b** and **33e**, with weak calcium antagonistic activities showed effective MDR-inhibiting activities both *in vitro* and *in vivo*. In particular, **33e** was expected to be the most suitable compound to overcome MDR (104).



		Zone of inhibition at 200 μg/mL (mm)					
	R	***P. aeruginosa***	***S. aureus***	***M. leutius***	***K. rosea***	***A. candidus***	***A. niger***
31a	CH_3	21	20	25	20	20	22
31b	OEt	24	19	27	22	22	24
Gentamicin		24	18	35	24	-	-
Ampicilline		-	-	-	-	30	32

Figure 37. Antibacterial and antifungal activities of **31a** and **31b**



	R	**R^1**
32a	C_2H_5	*o*-Cl
32b	CH_3	*m*-Cl
32c	C_2H_5	*p*-Cl
32d	C_2H_5	*p*-OCH_3

Figure 38. Structures of N-1 substituted 1,4-DHPs with antibacterial and antifungal activities

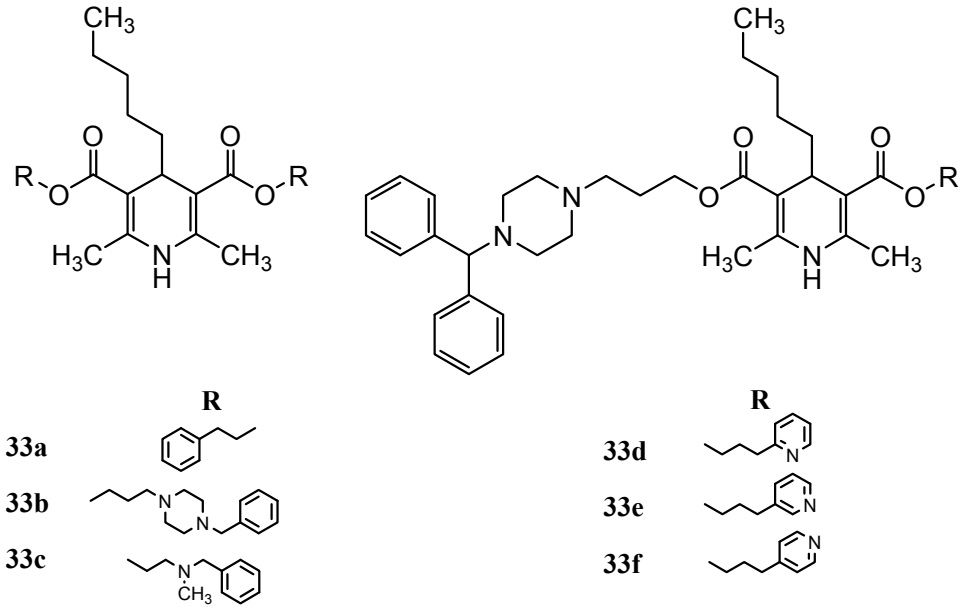


Figure 39. Chemical structures of 1,4-dihydropyridines possessing an *n*-pentyl group at the C-4 position

In another study, asymmetric compounds with a bulk group at C-3 position were synthesized and their P-glycoprotein-inhibitory activities were evaluated in human breast cancer MCF-7 cells. These compounds restored the intracellular drug accumulation by inhibiting P-glycoprotein efflux activity. For instance, compounds **34a** and **34b** demonstrated a 15-fold increase in Vinblastine accumulation in P-glycoprotein-overexpressed MCF-7/adr cells. Pyridine derivatives such as **35a** and **35b** demonstrated stronger inhibitory effects than the corresponding 1,4-dihydro analogues, **34a** and **34b**. Thus, the oxidation of the 1,4-dihydropyridine ring to the corresponding pyridine may favor P-glycoprotein interaction (105). Results from the Daunomycin cytotoxicity assays were in agreement with those from the Vinblastine accumulation assays. The IC_{50} values in Figure 40 are the inhibitory concentrations of Daunomycin in the presence of 3 μM of the studied 1,4-dihydropyridines or Niguldipine.

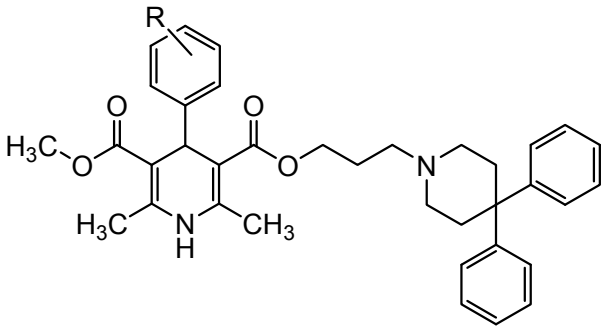
In 2002, Kawase *et al.* investigated the MDR-reversal activities of 3,5-dibenzoyl-1,4-dihydropyridines against mouse lymphoma cells transfected with human MDR1 gene. They also studied the cytotoxic activity of the compounds against human oral tumor cell lines. The compounds demonstrated very variable cytotoxic activity against HSC-2 and HSG tumor cell lines. This variation was dependent on the difference in the substituents at the C-4 position. Compounds **36a** and **36b** displayed the highest cytotoxic activity against HSC-2 (IC_{50} = 7.0 μM) and HSG (IC_{50} = 8.7 μM) (Figure 41). Cytotoxicity was nearly the same as that of Doxorubicine (IC_{50} = 4.1 μM and 5.3 μM, respectively). **36a** was found to be more active than the others. It seems that Cl substitution at *metha* position on the phenyl ring leads to a higher cytotoxic and MDR-inhibiting activity than that at the *ortho* or *para* positions. However, *metha*-Br derivative **36c** was inactive (5). Lipophilicity was one of the most important parameters affecting MDR-modulating efficiency in the structure-activity relationship studies of MDR-modulating drugs (98). The LogP values of molecules were calculated and found to be higher (LogP = 4.26–7.47) than the corresponding values of Verapamil (LogP = 3.71) or Nifedipine (LogP = 2.35). It was also mentioned that lipophilicity alone was not an essential parameter of direct MDR-modulating activity of this series of compounds (5).

Morshed *et al.* reported that compounds **36a** and **36b** from the above research had higher cytotoxicity against the tumor cell lines (HSC-2, HSC-3, HSG, HL-60) than normal cells (HGF, HPC, HPLF), yielding a tumor specific (TS) cytotoxicity value of >33 and >53, respectively. Western blot analysis showed that **36a** and **36b** transiently increased the expression of both the anti-apoptotic protein (Bcl-2) and pro-apoptotic proteins

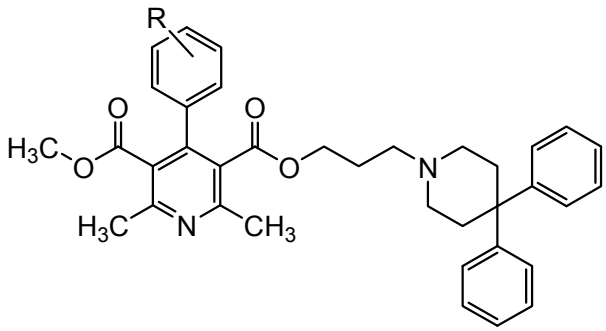
(Bax, Bad) at lower concentrations (2.5-40 μM), and reduced their expression at higher concentrations in HL-60 cells (6) Figure 41. They concluded that 3,5-dibenzoyl-1,4-dihydropyridines, such as **36a** and **36b**, with both MDR reversal activity (5) and tumor-specific cytotoxicity, may be possible antitumor candidates (6).

Another research group explained the antiproliferative effects of 1,4-dihydropyridine derivatives in comparison with those of Verapamil (VP) and Doxorubicin. The human breast cancer T47D cell line and its MDR1 over-expressed and moderately resistant cells (RS) were used in this research. They also examined the effects of these compounds on cytotoxicity of Doxorubicin in these two cell types. The cytotoxicity of the molecules was similar to that of Verapamil, and significantly less than that of Doxorubicin. Among these compounds, **37a** and **37b** showed promise as potential new MDR1 reversal agents (106) Figure 42.

Thiosemicarbazide derivatives of 1,4-dihydropyridines had significant antitumor activity (107). Kumar *et al.* explained the anticancer activity of a new series of 1,4-dihydropyridine derivatives against HepG2 and Hela cancer cell lines. Compound **38a** was more active than the other compounds against HepG2 and MCF-7. Likewise, **38b** was more active than the other molecules against Hela cancer cells (108) Figure 43.

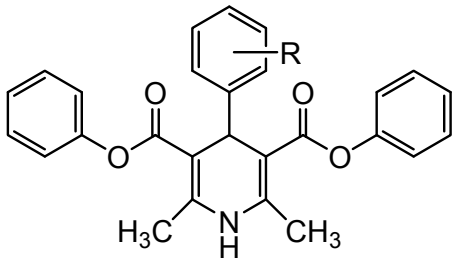


	R	IC_{50} (μM) (MCF-7)
34a+Daunomycin	*p*-O-nPr	0.79
34b+Daunomycin	*p*-O-nBu	0.92
Niguldipine+Daunomycin		1.09



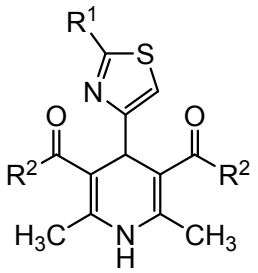
	R	IC_{50} (μM) (MCF-7)
35a+Daunomycin	*p*-O-nPr	0.52
35b+Daunomycin	*p*-O-nBu	0.61
Niguldipine+Daunomycin		1.09

Figure 40. Structures and anticancer activity of **34a**, **34b**, **35a** and **35b** in combination with Daunomycin



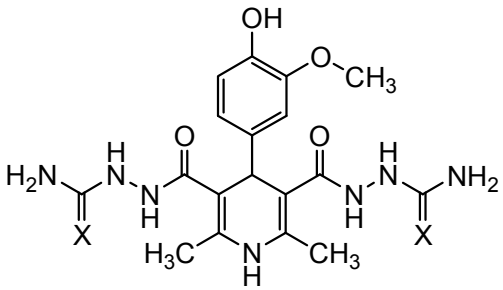
	R
36a	*m*-Cl
36b	*o*-CF_3
36c	*m*-Br

Figure 41. Anticancer 3,5-dibenzoyl-1,4-dihydropyridines



			IC_{50} (μM)	
	R^1	R^2	T47D	RS
37a	*o*-methyl	phenyl	113	141
37b	*o*-chlorophenyl	methyl	121	126
DXR			0.399	0.545
VP			123	108
36a+DXR			0.296	0.327
36b+DXR			0.267	0.288
VP+DXR			0.267	0.316

Figure 42. The antiproliferative effects of **37a** and **37b** in combination with Verapamil and Doxorubicin



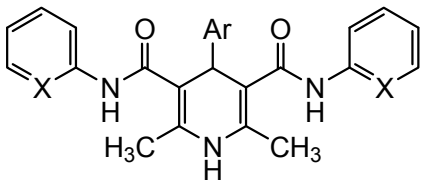
		GI_{50}[a] (μM)		
	X	HepG2	MCF-7	Hela
38a	O	31.2	32.3	-
38b	S	-	-	31.1
Doxorubicin		0.01	0.02	0.05

[a] Values reported in GI_{50}: concentration (μM) of compound causing 50% inhibition of cell growth

Figure 43. (Thio)semicarbazide derivatives of 1,4-DHPs and their antitumor activity

The structural requirements of 1,4-dihydropyridines for MDR reversal were also investigated using the molecular docking technique on multidrug resistance protein 1 (MRP1). Sirisha *et al.* subjected a series of 1,4-DHP derivatives to MDR inhibiting activity assessment and molecular docking studies. It was observed that only two test compounds, **39a** and **39b**, induced inhibition of MRP1 basal ATPase activity at concentrations above 10 μM. Compound **39b** was shown to be relatively more potent than **39a** according to the docking results. The structures of these compounds and the experimental and computational results are provided in Figure 44. The IC_{50} values corresponding to the inhibitory concentration of the compounds against the MRP1 and Ki values are the *in silico* calculated inhibitory concentrations. A correlation between docking scores and experimental binding data suggested that

this technique might be useful for developing a pharmacophore model to help interpret the affinities observed and to design new MRP1 antagonists (109).

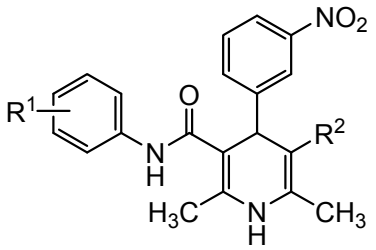


	X	Ar	IC_{50} (μM) MRP1	K_i^a (μM)
39a	CH	*o*-furyl	20	0.95
39b	N	*o*-pyrrolyl	14	0.66
Benzbromarone			4	0.19

[a] Ki= Inhibition Constant

Figure 44. Experimental and computational values for the inhibition of MRP1 by **39a** and **39b**

Inhibition of the MDR reversal activity of 1,4-dihydropyridine derivatives using a multidrug-resistant human colon cancer cell line expressing MDR1/LRP and a human *mdr1* gene-transfected mouse lymphoma cells was reported by Engi *et al.* The tumor-specific cytotoxicity of these compounds against human tumor and normal cell lines was also investigated. Compound **40a** displayed a marked inhibition of the MDR of mouse lymphoma cells. **40b** had a very strong tumor-specific cytotoxic action, with TS (CC_{50} normal/ CC_{50} tumor) of 5.9 μM (TS of Doxorubicin was >14.6 μM). The dihydropyridine derivatives were more cytotoxic against tumor cell lines as compared with normal cell lines (110) Figure 45.



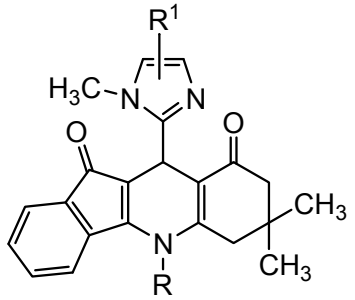
	R^1	R^2
40a	*o*-OCH_3	CN
40b	*o*-OCH_3	$COOC_2H_5$

Figure 45. Chemical structures of **40a** and **40b**

Evaluation of the antiproliferative effects of a series of new imidazole-substituted indeno[1,2-*b*]quinoline-9,11-dione derivatives on HeLa, LS180, MCF-7 and Jurkat human cancer cell lines was reported in 2013. These molecules showed more activity against the Jurkat cell line than the LS180, MCF-7 and Hela cell lines. It was concluded that compounds bearing an imidazole-2-yl moiety at the C-11 position of DHP have stronger antiproliferative activity than Cisplatin against the Jurkat cell line. Also the presence of electron-withdrawing groups on the imidazole ring increased the antiproliferative potential of the molecules (111) Figure 46.

1,2,3,4-TETRAHYDROPYRIMIDINES ANTITUBERCULAR ACTIVITY

As mentioned above, 1,2,3,4-tetrahydropyrimidines share many biological properties with 1,4-dihydrpyridines. 1,2,3,4-THPMs and some full aromatic pyrimidine derivatives have also shown antitubercular activities. Structural features affecting the antitubercular potency of these compounds are the nature of C-2, C-4 and C-5 substituents and also the lipophilicity of the compound. Most of the reported antitubercular 1,2,3,4-THPM derivatives have one of the general structures shown in Figure 47.



			IC_{16} (μM)			
	R	**R^1**	**Hela**	**LS180**	**MCF-7**	**Jurkat**
41a	H	3-NO_2	9.2	12.5	>25	0.7
41b	Ph	3,4-*di*-Cl	10.7	19.4	13.6	1.7
Cisplatin			5.6	5.0	4.0	1.9

Figure 46. Fused-ring anticancer 1,4-DHPs

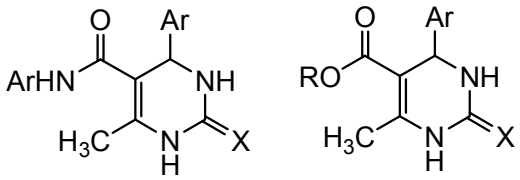


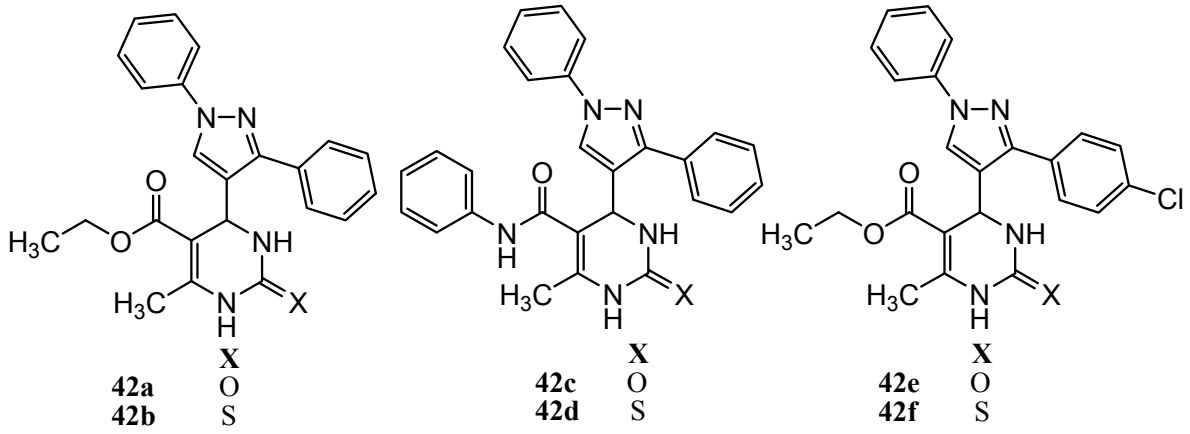
Figure 47. Structural categories of antitubercular 1,2,3,4-THPMs

Carbonyl and thiocarbonyl groups are the most common types of functional groups in the C-2 position of 1,2,3,4-THPM derivatives. As with 1,4-DHPs, substituted phenyl and heteroaromatic rings have been studied as the C-4 substituents of both 1,2,3,4-THPMs and pyrimidine derivatives. Both the 5-phenylcarboxamide and 5-alkylcarboxylate esters of the 1,2,3,4-THPM scaffold have been seen to possess antitubercular properties (13,112-116).

Based on the structures of the active derivatives, lipophilicity seems to be an important physicochemical property affecting the compound's potency. However, the lipophilicity of the substituents at any position of the molecule cannot be considered the only determining factor. Regarding the C-2 substituent, no specific relationship between the potency and the type of this substituent (carbonyl or thiocarbonyl) can be found. For example, compound **42a** (MIC = 16 μg/mL) was more potent than **42b**, with an MIC of 32 μg/mL, but **42c** (MIC = 32 μg/mL) was as potent as **42d,** while **42e** (MIC = 0.25 μg/mL) was weaker than **42f** (MIC = 0.125 μg/mL) as reported by Yadlapalli *et al*. The minimum inhibitory concentration of Isoniazid was determined to be 0.03μg/mL by the same author (13) Figure 48. The same is true for the report of Trivedi *et al.* (114), who also changed the thiocarbonyl to a thiomethyl moiety, observing a decrease in the potency of most of the compounds, which did not agree with the increase in their lipophilicity values.

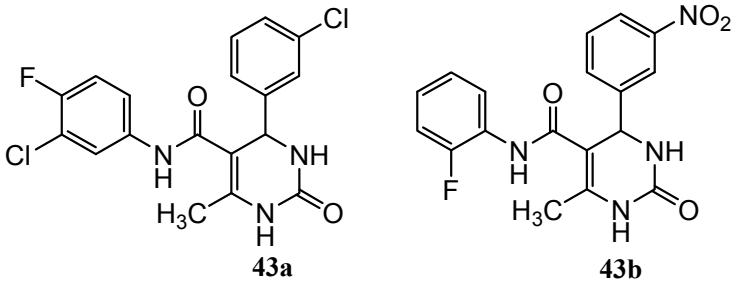
The C-4 derivatives are lipophilic aromatic or heteroaromatic moieties and it seems that the amount of this physicochemical property affects potency. This can be exemplified by the two THPM derivatives illustrated in Figure 49. Compound **43a** with LogP = 4.77 was more potent than **43b** (LogP = 4.27) (115).

The position of the substitution on the C-4 phenyl moiety was discussed by Virsodia *et al*. (115). Substituted N-aryl-6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydro-pyrimidine-5-carboxamides which had antitubercular activity were subjected to a 3D-QSAR study by this group, who concluded, from the CoMFA electrostatic contours, that electropositive groups are favored at the *ortho'*, *meta* and *para* positions of the 4-phenyl ring. The substitution pattern of these compounds is illustrated in Figure 50.



	X
42a	O
42b	S
42c	O
42d	S
42e	O
42f	S

Figure 48. Molecular structures of **42a-f**



	PI at > 6.25 μg/mL
43a	48
43b	24
Rifampicin	99

Figure 49. Chemical structures and PIs of antitubercular 1,2,3,4-THPMs

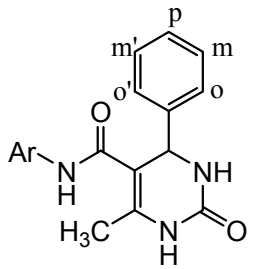
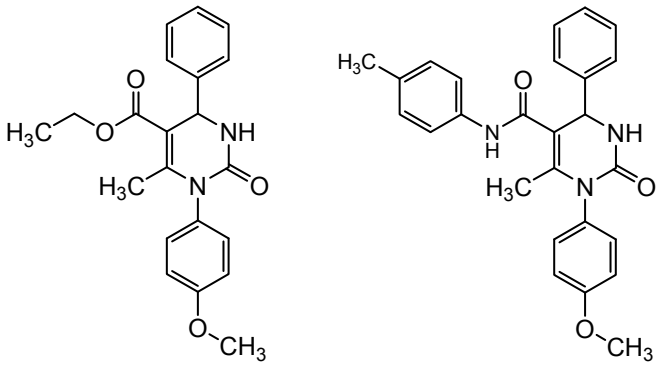


Figure 50. The substitution pattern of substituted N-aryl-6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydro pyrimidine-5-carboxamides

They also indicated that electronegative groups at the *meta* position of this ring increase the activity. Using the CoMSIA electrostatic contours, they confirmed that electropositive groups are favored at the *para* and *ortho* positions of the 4-phenyl ring. According to the CoMFA steric fields and steric CoMSIA contours reported by this group, bulky substituents are not favored at the *para* position of the 4-phenyl ring. Mapping the desired and undesired hydrophobic regions on this ring, they demonstrated that hydrophobic groups at the *meta'* and *para* positions decrease the activity. Hydrogen bonding with an H-bond donor at the *meta'* and *para* positions of the 4-phenyl ring was also confirmed to be favorable (115).

Between the 5-phenylcarboxamides and 5-alkylcarboxylate esters of the 1,2,3,4-THPMs scaffold, the latter derivatives proved to have superior antitubercular properties. For example, **42f** was 256 and **42e** was 128 times more potent than **42c** and **42d**, respectively (13) Figure 48. A smaller difference in the magnitude of the activity was mentioned in the report of Zalavadiya *et al.* (116).

5-Alkylcarboxylate ester **44a** exhibited a growth inhibition percentage of 65, which was stronger than that for the corresponding phenylcarboxamide derivative, **44b**, with a growth inhibition percentage of 3.0, while Rifampicin, as the standard drug, showed a growth inhibition percentage of 98, all at 6.25 μg/mL (Figure 51). The results of other research articles confirm the higher potency of the ester derivatives (114).



44a **44b**

Figure 51. Chemical structures of **44a** and **44b**

The 3D-QSAR study by Virsodia *et al.* also clarified some structural features of the 5-phenylcarboxamide moiety and its influence on potency. According to the CoMFA electrostatic contours obtained in this study, electropositive groups are favored at the *meta* position of the 5*N*-phenyl ring. The CoMSIA electrostatic contours favored the electronegative groups at the *para* position of this ring. The CoMFA steric fields confirmed *meta* position on the 5*N*-phenyl moiety as the favored position for bulky substituents. Using hydrophobic contours of CoMSIA, Virsodia *et al.* indicated that hydrophobic groups positioned at *meta′* decrease the activity, but small hydrophobic groups are accepted at the *meta* position of the 5*N*-phenyl ring (115). This latter observation can be better understood by comparing **45a** and **45b**. As can be seen, the small hydrophobic methyl group is not tolerated at *meta′* position in **45a**, but the same group in the *meta* position of the 5*N*-phenyl ring lends higher activity to the compound (Figure 52). H-bond acceptor groups were disfavored in the *ortho* and *meta* positions of the ring.

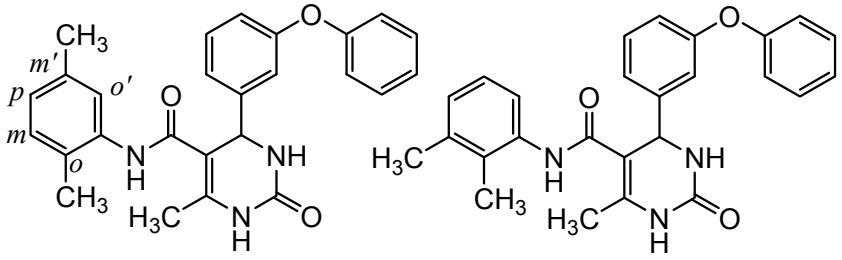
Full aromatic analogues of Biginelli pyrimidines have also shown antitubercular activities, but weaker compared with the 1,2,3,4-THPMs (113). **46a** and **46b** shown in Figure 53 are two examples of such weak antitubercular compounds.

N-1 substitution on both 5-phenylcarboxamide and 5-alkylcarboxylate esters of 1,2,3,4-THPMs led to weak to moderate compounds (116) Figure 54.

Ring fusion to the C5=C6 bond of the tetrahydropyrimidine moiety (reported by Trivedi *et al*.) led to some pyrazolo[3,4-*d*]pyrimidines being regarded as promising antiTB compounds (114) Figure 55.

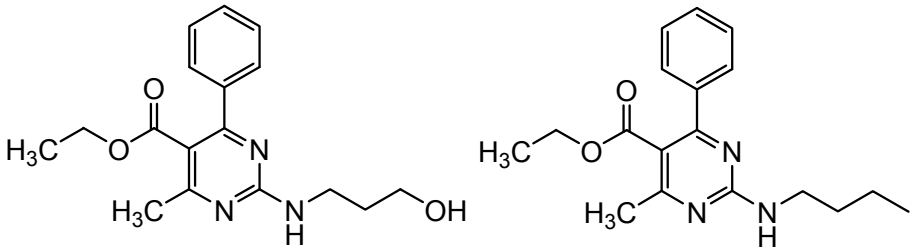
ANTIMICROBIAL ACTIVITY

Microbial infections comprise a group of diseases that have been common since the beginning of humankind. Even with the enormous achievements that have been made in the field of antimicrobial medications, there is still no perfect solution to many of the deadly diseases caused by bacteria (117).



45a **45b**

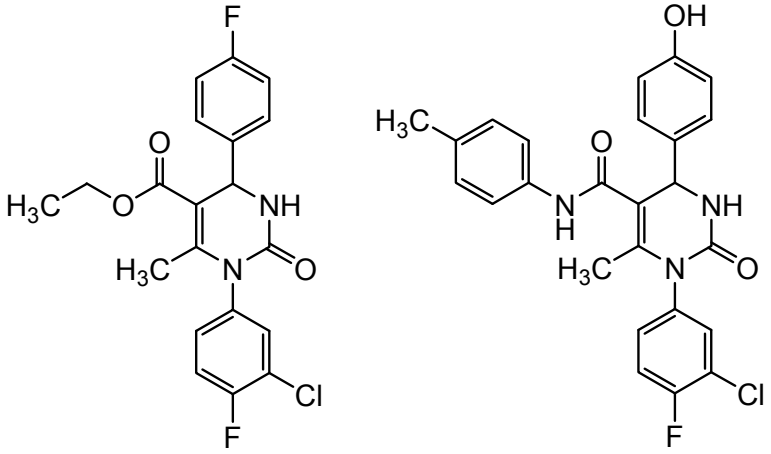
Figure 52. Molecular structures of **45a** and **45b**



46a **46b**

	MIC (μM)
46a	> 128
46b	117.7
Moxifloxacin	0.47
Isoniazid	0.12

Figure 53. Full aromatic analogues of Biginelli pyrimidines and their antitubercular activity

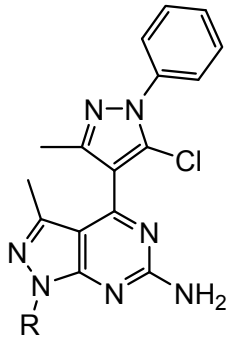


47a **47b**

	PI at 6.25 μg/mL
47a	74
47b	46
Rifampicin	98

Figure 54. Antitubercular activity of N-1 substitututed 5-phenylcarboxamide and 5-alkylcarboxylate esters of 1,2,3,4-THPMs

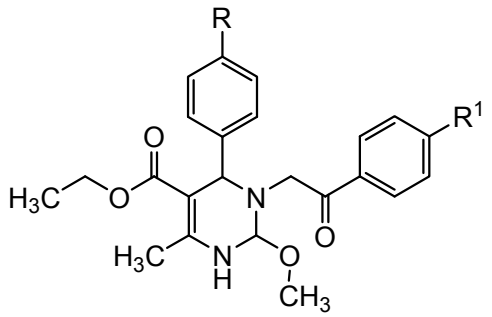
Several reports of antimicrobial activities of substituted 2 – oxo / thioxo – 1 , 2 , 3 , 4 - tetrahydropyrimidines have been published in the recent years (118-142). For example, Sawant *et al.* described the antimicrobial activity of some THPM derivatives in 2008. They screened them against the gram-positive bacteria, *S. aureus*, and observed that two of the compounds, **49a** and **49b**, were more effective than others. The structures of these compounds are illustrated in Figure 56. The same research group also investigated the results using the QSAR approach and observed a good correlation between the structural features and biological activity. They concluded that molecules with a less positive partial charge and a negative polar Van der Waals surface area make a higher contribution rather than the ratio of Van der Waals surface area to molar refractivity (119).



	R	MIC (μg/mL)
48a	*o*-chlorophenyl	1.20
48b	*p*-methylphenyl	1.20
Isoniazid		0.20

Figure 55. Fused ring 1,2,3,4-THPMs and their antitubercular activity

Another series of THPM derivatives with a group substituted at N-3 providing higher Van der Waals surface area, as compounds **49a** and **49b**, and different groups at C-2, C-4 and C-5 positions, were screened against gram negative (*E. coli* and *Salmonella typhi*) and gram positive (*S. aureus* and *B. subtilis*) microorganisms. Compound **50a** showed good activities against *S.aureus*, *B. subtilis*, *E. coli* and *S. typhi* (MICs = 3, 1, 1 and 2 μg/mL, respectively). Chloramphenicol was used as one of the standards in this study. The corresponding MIC values for this drug were 5, 7, 6 and 7 μg/mL. Compound **50b** was also a good inhibitor of *S. typhi* growth, with an MIC = 3 μg/mL. It was concluded that compounds which had OCH_3 and/or Cl substitution at any position of the C-4 phenyl group showed antimicrobial activities at lower concentrations (117) Figure 57.



	R	R^1
50a	OCH_3	OCH_3
50b	OCH_3	Cl

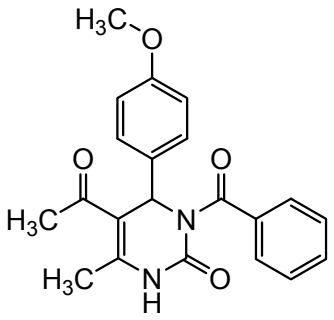
Figure 57. Chemical structures of **50a** and **50b**

A substituted benzoyl methyl thio group located on the C-2 position of the THPM ring seemed to be effective in the antimicrobial activity, as effective as a moiety located on N-3 position. Hussein *et al.* revealed that the most active compounds of this series contained an electron-withdrawing group (R^1 = Cl, Br; R^2 = Br, Cl or NO_2) in their structures, while the least active ones contained an electron-donating group (R^1 = H, CH_3; R^2 = OCH_3, CH_3). **51a** was effective against *S. aureus, E. coli* and *P. aeruginosa*, while **51b** (Figure 58) had antimicrobial activity against *B. cereus, E. coli* and *P. aeruginosa* (120).

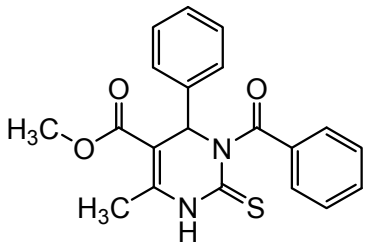
An investigation into compounds with bulk moieties at C-4, or C-5 or a heterocyclic ring fused to the 1,2,3,4-THPM ring (Figure 59) revealed variable antimicrobial activities ranging from zero to moderate (121).

Tetrahydropyrimidines possessing bulkier groups at C-4 position were also subjected to antimicrobial assessments. Different examples of these structures will be discussed below.

Nagawade *et al.* described the antibacterial activity of a series of THPMs with substituted biphenyl ring against both gram positive and gram negative strains. Most of the compounds (e.g. compound **55**) displayed moderate activity against the studied strains, while all the tested compounds were inactive (MIC > 32 μg/mL) against *P. aeruginosa* and *Enterococcus faecium*, unlike the standards (Ciprofloxacin, Sparfloxacin, and Trovafloxacin) (122) Figure 60.



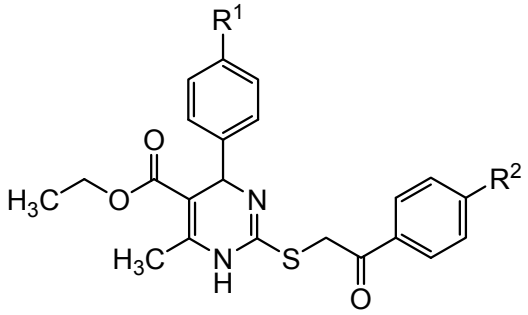
49a



49b

	MIC (μg/mL) *S. aureus*
49a	32
49b	32

Figure 56. Chemical structures of **49a** and **49b** and their antibacterial activity against *S. aureus*

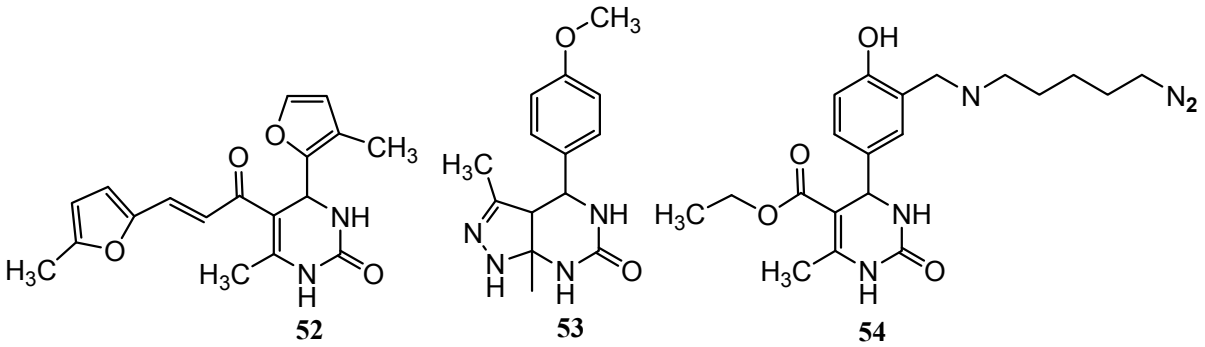


			Zone of inhibition at 100 μmol/mL (mm)			
	R^1	**R^2**	***S. aureus***	***E. coli***	***P. aeruginosa***	***B. cereus***
51a	Cl	Br	23	22	21	-
51b	Br	NO_2	-	21	20	25
Chloramphenicol			27	30	24	32

Figure 58. Chemical structures and zones of inhibition at 100 μmol/mL (mm) for **51a** and **51b**

The replacement of one of the C-4 position phenyl rings by tetrahydropyran or tetrahydropyrrolidine led to compounds with high antimicrobial activity. For example, **56a** and **56b** had significant activity against the selected bacteria and fungi, with zones of inhibition almost comparable to those of the standard drugs (Figure 61). It is interesting that the substituted 4-diethylaminophenyl compounds (**56c** and **56d**) were also as potent as their closed-ring analogues **56a** and **56b** (123).

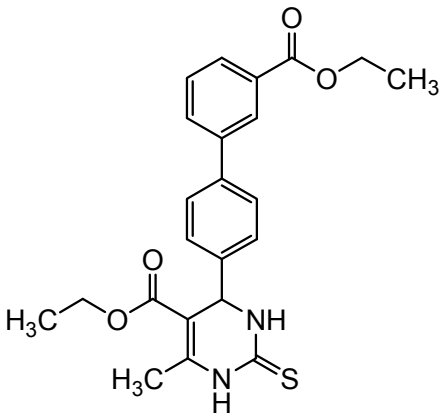
1,2,3,4-tetrahydropyrimidin-2(1*H*)-ones having a substituted urea on the C-4 phenyl group have been evaluated for antibacterial and antifungal activities against various gram positive and gram negative bacteria as well as fungal strains. These compounds were similar to the molecules studied by Nagawade *et al.* (122) but differed in their C-4 substituent. The antibacterial activity data suggested that the presence of lipophilic moieties or H-bond acceptors such as F, Cl, CF_3, OCF_3 and OCH_3 at *ortho* position of the C-4 substituent favors high antibacterial activity (Figure 62).



52 **53** **54**

	Zone of inhibition at 1mg/mL (mm)					
	C. albicans	***B. subtilis***	***B. cereus***	***E. coli***	***P. aeruginose***	***S. aureus***
52	14	12	-	-	-	-
53	15	8	8	3	-	3
54	15	2	9	-	-	-
Ref.	44	40	30	20	35	50

Figure 59. Bulk moieties at C-4, or C-5 or a heterocyclic ring fused to the 1,2,3,4-THPM and the corresponding antimicrobial activities



55

	MIC (μg/mL)				
	S. aureus	***E. coli***	***S. epidermidis***	***Klebsiella sp.***	***E. faecalis***
55	8	8	8	8	8
Ciprofloxacin	1	0.007	1	0.01	2
Sparfloxacin	1	0.007	1	0.01	1
Trovafloxacin	0.5	0.002	0.5	0.025	1

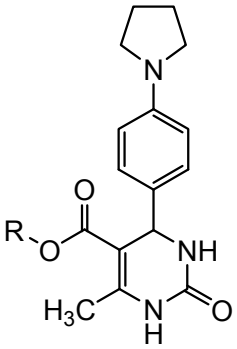
Figure 60. Chemical structure and antibacterial activity of compound **55**

Compounds **57a** and **57b**, both with MIC=10 μg/mL against *S. aureus*, and *E. coli* are good examples. Ciprofloxacin, which was used as the standard, had an MIC of 25 and 15 μg/mL against the same bacteria, respectively). But substitution at *meta* position or disubstitution at *meta* and *para* positions of the C-4 substituent led to moderate (or even no) activity with respect to the standard drug against the test strains. Compound **57c** had no activity against *E. coli* up to 200 μg/mL and was a moderate antibacterial against *S. aureus* (MIC=90 μg/mL). The presence of nonpolar lipophilic groups such as isopropyl or *n*-butyl at the C-4 terminal phenyl ring had no major effect on the activity (e.g. **57d** with MIC=85, 55 and 95 μg/mL against *S. aureus*, *E. coli* and *Salmonella typhimurium*,

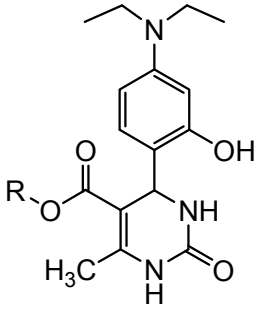
respectively). The structure-activity relationships (SAR) of the molecules for antibacterial activity strongly correlated with their SAR for antifungal activity. For instance, MIC values of µg/mL against *C. albicans*, *A. niger* and *A. flavus* were observed for **57a.** In the case of Miconazole, which was used as reference drug, the MICs were 25, 20 and 20 µg/mL against *C. albicans*, *A. niger* and *A. flavus*, respectively (124).

Other compounds with bulk groups at C-4 position of the THPM ring containing a rotatable bond were reported by Balaji *et al.* Among the tested compounds, **58** was the most active against *S. aureus*, *B. cereus*, *E.coli*, and *P.aeruginosa* (125) Figure 63.

THPMs containing a naphthalene ring as the C-4 substituent have also been investigated for their antimicrobial properties. According to a report by Borse *et al.*, such compounds are not strong antimicrobials. The best reported compound, **59**, shown in Figure 64, had an MIC=250 µg/mL against four representative pathogens: *E. coli*, *P. neumoniae*, *A. niger* and *C. albicans*. Streptomycin was the standard drug used in the antibacterial assays, with MIC=125 µg/mL, while Fluconazole was the standard antifungal agent (MIC=125µg/mL) (126).

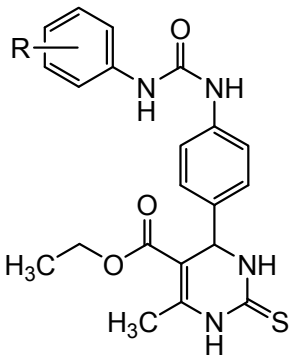


		Zone of inhibition at 100 ppm (%)			
	R	***S. aureus***	***E. coli***	***A. niger***	***H. oryzae***
56a	C_2H_5	23	23	21	20
56b	CH_3	23	23	18	19
Penicillin		20	20	-	-
Griseofulvin		-	-	20	20



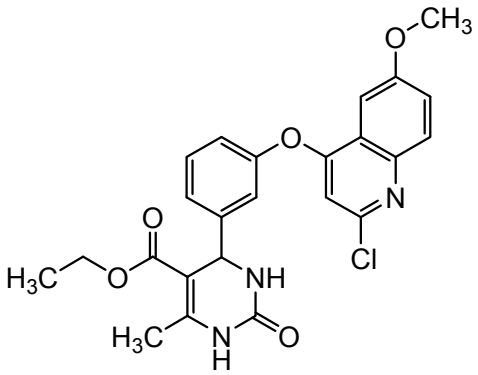
			Zone of inhibition at 100 ppm (%)			
	R	**X**	***S. aureus***	***E. coli***	***A. niger***	***H. oryzae***
56c	C_2H_5	S	23	22	19	15
56d	CH_3	NH	21	21	20	19
Penicillin			20	20	-	-
Griseofulvin			-	-	20	20

Figure 61. Chemical structures and antimicrobial activity of **56a-d**



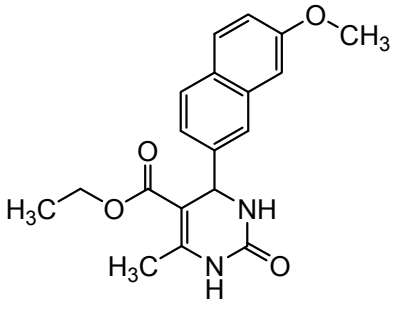
	R
57a	*o*-F
57b	*o*-Cl
57c	*m,m′*-F
57d	*p*-isopropyl

Figure 62. 1,2,3,4-THPMs having a substituted urea on the C-4 phenyl group



	Zone of inhibition at 250 μg/mL (mm)			
	S. aureus	***B. cereus***	***E. coli***	***P. aeruginosa***
58	15	15	13	14
Streptomycin	17	17	16	15

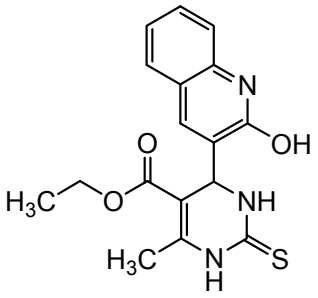
Figure 63. Chemical structure and antimicrobial activity of **58**



59

Figure 64. 1,2,3,4-THPM containing a naphthalene ring at the C-4 position

Adhikari *et al.* explained the antimicrobial activity of THPMs substituted by quinoline at C-4 position. These compounds were all stronger than the naphthalene analogues and showed moderate to good antimicrobial activities. Compound **60** showed good activity against *E. coli, P. aeruginosa, S. aureus*, *C. albicans* and *A. flavus* with zones of inhibition of 20, 19, 16, 18 and 20 (all at 100 µg/mL), respectively (Figure 65). The corresponding values for the standard drug (Chloramphenicol) were 19, 18, 16, 20 and 21 (all at the same concentration), respectively (127).



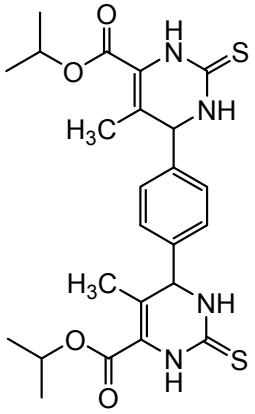
60

Figure 65. 1,2,3,4-THPM containing a quinoline moiety at the C-4 position

Two 1,2,3,4-tetrahydropyrimidin-2(1*H*)-one (thione) scaffolds attached through a phenylene linker were also investigated by Chellakili *et al.* as possible antimicrobial compounds. They connected two tetrahydropyrimidine rings from their C-4 positions through a phenyl ring. These compounds showed good inhibitory activity against the growth of a wide variety of the microorganisms. Compound **61** shown in Figure 66 is an example of this series (128).

Arylamide derivatives of the 1,2,3,4-tetrahydropyrimidine-2(1*H*)-one-5-carboxylic acid scaffold were also subjected to antimicrobial evaluations. Baldev *et al.* reported a series of compounds with this scaffold and claimed that very few of the compounds were less active than standard drugs (129) Figure 67. Substitution on the arylamide moiety was methyl, methoxy or halide in this research.

Sedaghati *et al.* prepared carboxylate ester and arylcarboxamide derivatives of the THPM ring with bulky substitutions containing heteroaromatic moieties at C-4 position of the ring. This heteroaromatic moiety was a substituted imidazole or a simple thienyl or furyl ring. Of note was the observation that the antimicrobial THPMs investigated in this research were stronger antifungals rather antibacterial agents. For example, **62a** and **62b**, which are shown in Figure 68, inhibited the fungal growth at lower concentrations (130).



61

	MIC (µg/mL)			
	B. subtilis	***K. pneumonia***	***A. niger***	***C. albicans***
61	12.5	6.25	25	25
Ciprofloxacin	12.5	12.5	-	-
Fluconazole	-	-	12.5	25

Figure 66. Two 1,2,3,4-tetrahydropyrimidin-2(1*H*)-thione scaffolds attached through a phenylene linker and the corresponding antimicrobial activities

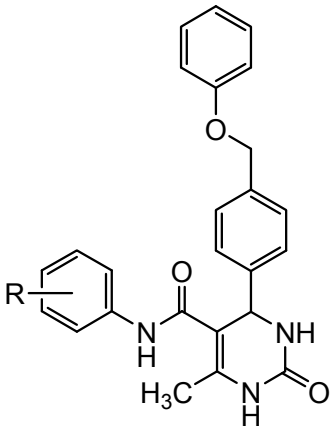
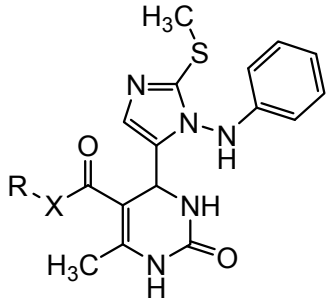


Figure 67. Arylamide derivatives of 1,2,3,4-THPMs possessing antimicrobial activity

Maddila *et al.* provided a series of pyrimidine derivatives fused to a thiazolidinone ring. These compounds were screened against gram-negative bacteria (*E. coli* and *P. aeruginosa*), gram-positive bacteria (*B. subtillis* and *S. aureus*) and fungi (*C. albicans* and *A. niger*). All the molecules displayed moderate to good antibacterial and antifungal activity. The halogenated and amino derivatives exhibited higher potency (131) Figure 69.

The same research group reported molecules which could be considered acyclic analogues of the above compounds. The antibacterial screening data revealed that all the tested compounds showed moderate to good bacterial inhibition. Compound **64** exerted ZIs of 25, 20, 29, 23 and 18 mm at 6.5 µg/mL concentration against *E. coli*, *S. aureus*, *P. aeruginosa*, *S. pyogenes* and *K. pneumonia*, respectively (Figure 70). The corresponding data for Ciprofloxacin, used as the standard, were 30, 24, 33, 25 and 23 mm, respectively, at the same concentration,. The prepared compounds were also screened for their antifungal activity and proved to be moderate to good antifungals against a wide variety of fungi. For instance, **64** emerged as a strong compound against *A. flavus*, *A. fumigatus*, *C. albicans*, *Penicillium marneffei* and *Trichophyton mentagrophytes*, with zones of inhibition (in millimeters) of 22, 22, 20, 22 and 25 at 6.5 µg/mL, respectively (Amphotericin: 21, 25, 19, 25 and 23 at the same concentration, respectively) (132).

Other thiazolo[3,2-*a*]pyrimidine derivatives have been shown to possess significant antimicrobial activity. Compound **65a**, for example, had ZI at 100µg/mL of 16, 14 and 15 (mm) against *B. cereus, S. aureus* and *E. coli*, respectively. Compound **65b** demonstrated strong antifungal activity against *C. albicans* (Zone of inhibition at 100µg/mL = 15 mm). The zones of inhibition at 100µg/mL for the reference drug, Tetracycline, were 21 mm against *B. cereus* and *S. aureus* and 22 against *E. coli*. Miconazole inhibited *C. albicans* growth with a 19 mm zone of inhibition at 100 µg/mL (133) Figure 71.



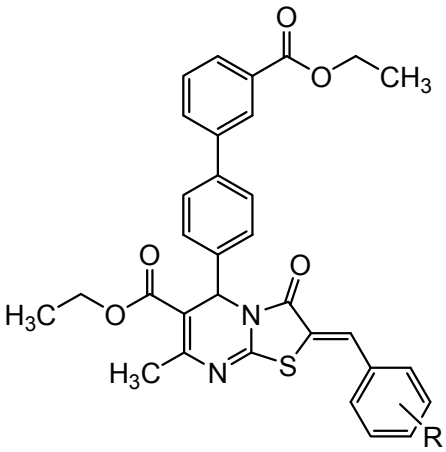
			MIC (µg/mL)			
	X	**R**	***E. coli***	***S. aureus***	***A. niger***	***C. albicans***
62a	NH	4-ClC_6H_4	512	>512	64	32
62b	O	C_2H_5	256	>512	128	64
Ciprofloxacin			0.25	0.5	-	-
Ketoconazol			-	-	4	2

Figure 68. Chemical structure and antimicrobial activity of **62a** and **62b**

Nagarajaiah *et al.* reported an antimicrobial activity for thiazolo[3,2-*a*]pyrimidines with no substitution on the thiazolo part of the fused ring against *S. aureus, E. coli* and *C. albicans* that was similar to that of Gentamicin and Nystatin (134) (Figure 72).

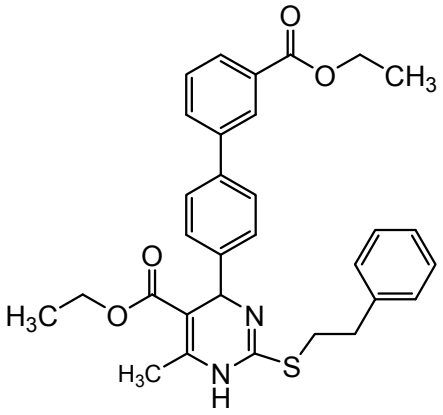
Akbari *et al.* explained the antimicrobial activity of arylcarboxamide derivatives of thiazolo[3,2-*a*]pyrimidines by the fact that pyrimidine analogues (such as compound **67**) possessed higher antimicrobial activity than thiazolopyrimidines (compound **68**) due to the free C=S group (135) (Figure 73).

The antibacterial and antifungal activities of carbohydrazide derivatives of THPMs were investigated in 2012. When they were screened against the gram negative bacteria *E. coli* and *P. aeruginosa*, the gram positive bacteria *B. subtilis* and *S. aureus* and two fungal organisms, *A. niger* and *C. albicans*, the molecules revealed weak activity against all bacterial strains, while compounds **69a** and **69b** showed good activity against *A. niger* and *C. albicans*, respectively (136) Figure 74.



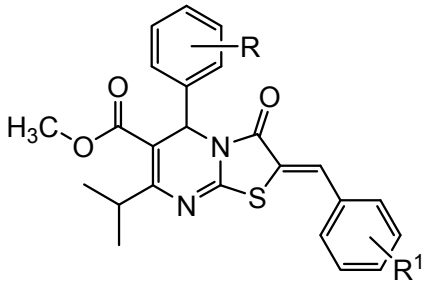
		Zone of inhibition at 25 µg/mL (mm)					
	R	***E. coli***	***P. aeruginosa***	***B. subtilis***	***S. aureus***	***C. albicans***	***A. niger***
63a	*p*-Cl	13.16	12.15	13.42	14.60	11.48	12.16
63b	*p*-$N(CH_3)_2$	14.72	11.79	14.18	13.07	13.44	11.23
Streptomycin		12.78	13.36	12.14	15.39	-	-
Amphotericin-B		-	-	-	-	12.89	14.89

Figure 69. Antimicrobial activity of pyrimidine derivatives fused to a thiazolidinone ring



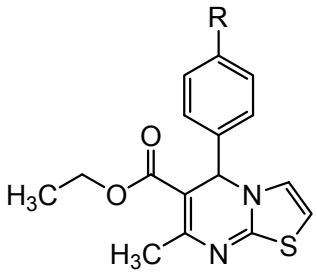
64

Figure 70. Chemical structure of **64**



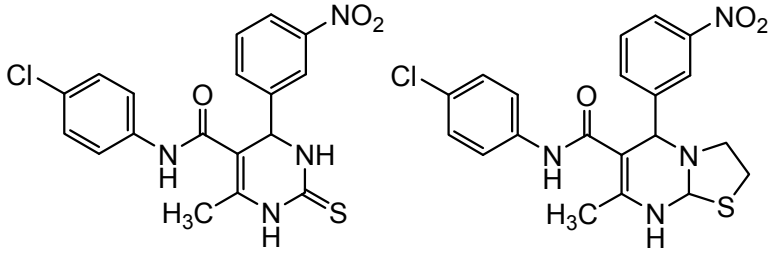
	R	**R^1**
65a	*p*-Cl	*p*-OCH_3
65b	m-NO_2	*o*-Cl

Figure 71. Chemical structures of some thiazolo[3,2-*a*]pyrimidines



		Zone of inhibition at 50 μg/disc (mm)		
	R	***E. coli***	***C. albicans***	***S. aureus***
66a	OCH_3	15	15.66	-
66b	OH	-	-	18
Gentamicin (10 μg/disc)		26	-	30
Nystatin (10 μg/disc)		-	26	-

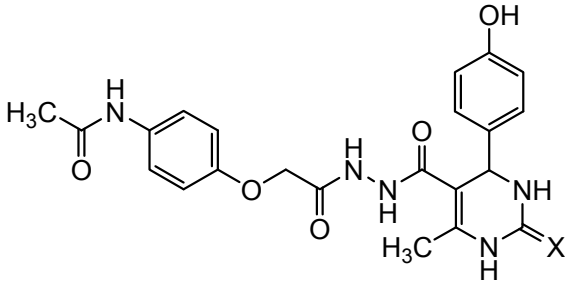
Figure 72. Antimicrobial activity of thiazolo[3,2-*a*]pyrimidines



67 **68**

	Zone of inhibition at 200 μg/mL (mm)				
	S. aureus	***S. epidermidis***	***E. coli***	***P. aeruginosa***	***C. albicans***
67	22.9	26	20	24.5	22.5
68	21.2	18.5	17.5	20	22
Ciprofloxacin	30	26	21	28	-
Griseofulvin	28	22	23	27	23

Figure 73. Antimicrobial activity of arylcarboxamide derivatives of 1,2,3,4-THPMs and thiazolo[3,2-*a*]pyrimidines



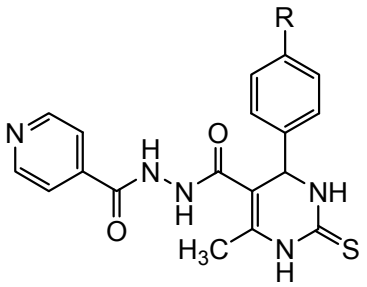
		Zone of inhibition at 100 μg/mL (mm)	
	X	***A. niger***	***C. albicans***
69a	S	21	-
69b	NH	-	21

Figure 74. Antifungal activities of carbohydrazide derivatives of 1,2,3,4-THPMs

Other hydrazide derivatives have shown a range of antimicrobial activities against gram positive and gram negative bacteria (Figure 75). Strong electron-withdrawing atoms (F, Cl) were responsible for the good activity of the molecules. Among the compounds reported, **70a** and **70b** were more potent than Norfloxacin (137).

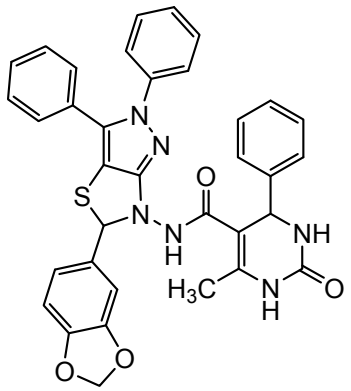
Some more complicated carbohydrazide derivatives of THPMs were tested by Alsharifi *et al.* in 2012 for their antimicrobial activity. The results indicated that some of these compounds exhibit good fungicidal and antibacterial activity (138) Figure 76.

Compounds **72a** and **72b** shown in Figure 77 with a heteroaromatic ring at the C-5 position of the tetrahydropyrimidine scaffold showed promising antibacterial activity against *Streptococcus pneumonia* and *E. coli* (mean ZI of 15 mm for both) compared with standard drugs Ofloxacin (mean ZI=19 mm) and Levofloxacin (mean ZI=16 mm). The presence of NO_2 and OCH_3 at *para* position of the C-4 moiety was responsible for providing this good antibacterial activity to the molecules (139).



		MIC (μmol/mL)	
	R	***B. subtilis***	***E. coli***
70a	*p*-Cl	0.0111	0.0120
70b	*p*-F	0.0112	0.0119
Norfloxacin		0.0124	0.0237

Figure 75. Chemical structures and antibacterial activities of **70a** and **70b**



	MIC (μg/mL)			
	B. subtilis	***E. coli***	***S. aueus***	***P. picketti***
71	26.24	17.60	20.35	22.25
Tetracyclie	23	14	17.85	18.50

	Zone of inhibition at 1000 ppm (%)					
	T. longifusus	***C. albicans***	***A. flavus***	***M. canis***	***F. solani***	***C. glabrata***
71	88	72	78	61	79	91
Miconazole	90	90	90	90	90	90
Amphotericin	90	90	90	90	90	90

Figure 76. Chemical structure and antimicrobial activities of **71**

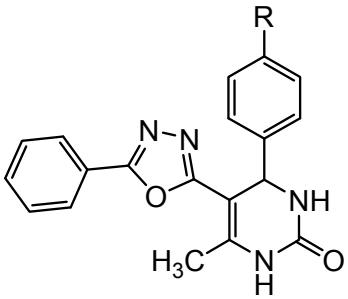
Recently, Elumalai *et al.* evaluated the antimicrobial activities of the acetazolamide cyclocondensed 1,2,3,4-tetrahydropyrimidines shown in Figure 78. All the compounds displayed potent antimicrobial activity. Compounds containing *p*-chlorophenyl or *p*-fluorophenyl moiety at the C-4 position and S or O atoms at the C-2 of the dihydropyrimidine ring showed antibacterial activity against both gram positive (*B. subtilis*) and gram negative (*E. coli*) bacteria (140).

El-Fattah *et al.* investigated the antimicrobial activity of some C-5 unsaturated ketone derivatives of the THPM scaffold. These compounds were seen to have weak to moderate antimicrobial activity, with **74** showing the highest activity against all the tested strains, with zones of 7, 10, 10, 9, 12, 11, 8 and 10 mm at 100 µg/mL against *E. coli*, *S. typhimurium, L. monocytogenes, S. aureus, P. arginosus, B. cereus, C. albicans* and *A. flavus*, respectively. Tobramycin (10 µg/mL), which was used as standard antibacterial, showed zones of inhibition of 20, 18, 20, 19, 18 and 19 mm against *E. coli*, *S. typhimurium, L. monocytogenes, S. aureus, P. aeruginosa* and *B. cereus*, respectively. Flucanazole (25 µg/mL), which was used as standard antifungal, had zones of inhibition=17 and 16 mm against *C. albicans* and *A. flavus*, respectively (141) Figure 79.

An unusual C-5 substitution on the 1,2,3,4-tetrahydropyrimidine scaffold (Figure 80) led to weak antifungal and antibacterial compounds (142).

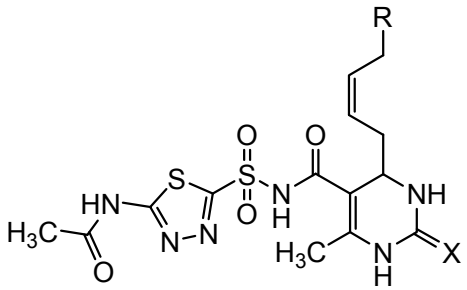
ANTICANCER ACTIVITY

In the search for novel anticancer drugs 1,2,3,4-tetrahydropyrimidine derivatives have also been considered as promising cytotoxic agents. Among these compounds, **76a** possessed specific cytotoxicity against colon carcinoma cells. Compounds **76b** and **76c** proved to be non-cytotoxic inhibitors of carcinogen metabolic activators (Cyp), inducers of glutathione-S-transferase (GST) activity, scavengers of $^{\bullet}OH$ and $ROO^{\bullet}$; and inhibitors of DNA fragmentation. They can therefore be regarded as active anti-initiation and multi-potent tumor blocking agents (143) Figure 81.



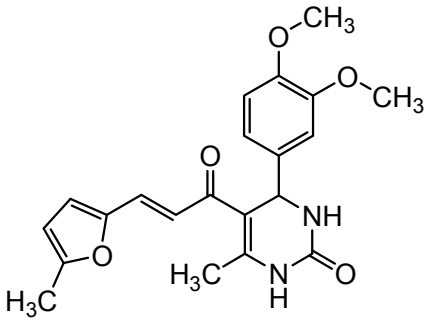
	R
72a	NO_2
72b	OCH_3

Figure 77. 1,2,3,4-THPMs with heteroaromatic ring at the C-5 position of the scaffold



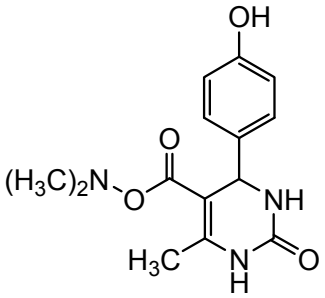
			MIC (µmol/mL)	
	X	**R**	***B. subtilis***	***E. coli***
73a	O	Cl	0.0129	0.0134
73b	S	F	0.0114	0.0121
Norfloxacin			0.0128	0.0132

Figure 78. Antimicrobial activities of the acetazolamide cyclocondensed 1,2,3,4-THPMs



74

Figure 79. Chemical structure of an 1,2,3,4-THPM containing unsaturated ketone moiety at C-5



	Zone of inhibition at 250 μg/mL (mm)	
	S. aureus	***E. coli***
75	0	6
Ciprofloxacin	31	27

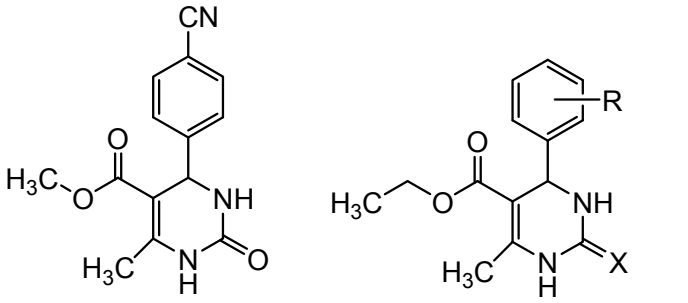
Figure 80. Chemical structure and antibacterial activity of compound of **75**

Chromone derivatives, which also possess anticancer activities, were introduced into the structure of cytotoxic THPMs, while Raju *et al.*explained the cytotoxicity of a series of 4*H*-chromen-1,2,3,4-tetrahydropyrimidine-5-carboxylate derivatives against three different human cancer cell lines (144). Compound **72** shown in Figure 82, with a cytotoxicity percentage of 47.6, 56 and 60.6 against lung (A549), CNS (SK-N_SH) and cervical (Hela) cancer cell lines was more effective than the standard drug, Doxorubicin (cytotoxicity percentage = 55.0, 31.8 and 86.5 against A549, SK-N_SH and Hela, respectively) (145). This compound was the most active amongst the studied compound.

The anticancer activity of a series of THPMs against human breast cancer (MCF-7) and colon cancer (HCT 116) cell lines was reported in 2012. Amongst the studied compounds, molecules **78a** and **78b** were found to be the most effective against MCF-7 and HCT-116, respectively (Figure 83). **78a** had an IC_{50} of 2.5 μg/mL while the standard drug, 5-fluorouracil (5-FU) had an IC_{50} of 0.67 μg/mL against MCF-7. **78b**, with an IC_{50} of 5 μg/mL, was more potent than 5-FU (IC_{50} = 6 μg/mL against HCT-116). According to a structure-activity investigation, the presence of the electron-withdrawing group on the phenylamino moiety was the enhancing factor in the anticancer activity of the synthesized compounds against HCT-116. On the other hand electron-releasing groups located on the phenylamino moiety increased the anticancer activity against the MCF-7 cancer cell line. Also, the existence of an oxo moiety at the C-2 position of the tetrahydropyrimidine ring improved the anticancer activity against both tested cell lines, although a thioxo moiety did not significantly improve the anticancer activity (146).

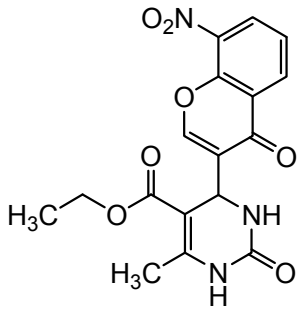
Prashantha Kumar *et al.* reported the *in vitro* anticancer activity of a series of 5-arylcarboxamide Biginelli pyrimidines against MCF-7 human breast cancer cells. Compounds with a cinnamoyl moiety at the C-4 position of the THPM ring (**79a** and **79b**) exhibited major activity against MCF-7 cell lines. The presence of furan and pyridine moieties at the same position provided potential anticancer activity to the compounds (**79c**, **79d** and **79e**). Based on 3D-QSAR studies, it was concluded that the 1,2,3,4-tetrahydropyrimidine scaffold was the basic requirement for the cytotoxicity of this class of compounds (147) Figure 84.

THPMs with a substituted pyrazole at C-4 position were also subjected to *in vitro* anticancer activity evaluation against the MCF-7 cell line. Excellent inhibition of MCF-7 growth (70.6% and 63.7%) at 10 μM concentration was observed for **80a** and **80c**, respectively. Structure-activity relationship studies revealed that compounds containing thioxo moiety at C-2 position were more potent than the oxo analogues, which contrasts with the observations of Sharma *et al*. concerning the SAR of these compounds (146). The SAR results also revealed that the compounds with chlorine (Cl) at R^1 in the series (**80a**, **80b** and **80c**) were more potent than compounds with no substitution at this position (**80d**). The structures of the compounds are illustrated in Figure 85.



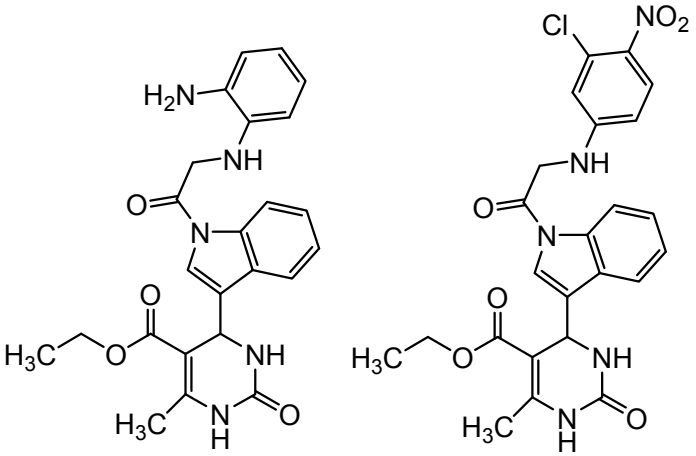
		R	X
76a	**76b**	*p*-OH , *m*-OCH_3	O
	76c	COOH	S

Figure 81. Chemical structures of anticancer tetrahydropyrimidine carboxylate esters



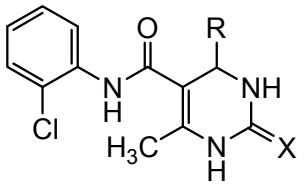
77

Figure 82. 1,2,3,4-THPM containing chromone moiety at C-4 position



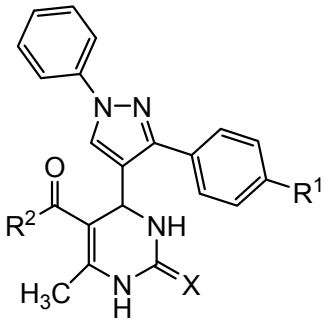
78a **78b**

Figure 83. Chemical structures of **78a** and **78b**



	R	X	% Cytotoxicity
79a	Cinnamoyl	S	71
79b	Cinnamoyl	O	79
79c	Furan-2-yl	S	76
79d	Furan-2-yl	O	69
79e	Pyridin-4-yl	S	72

Figure 84. Anticancer activity of some 5-arylcarboxamide Biginelli pyrimidines against MCF-7



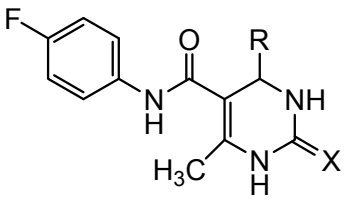
	X	R^1	R^2	GI_{50} (μM)
80a	S	Cl	OC_2H_5	33.2
80b	S	Cl	*p*-$CH_3C_6H_4NH$	43.5
80c	O	Cl	*p*-$CH_3OC_6H_4NH$	69.7
80d	O	H	*p*-$CH_3OC_6H_4NH$	-

Figure 85. Chemical structures and anticancer activity of 1,2,3,4-THPMs with a substituted pyrazole at C-4 position

These results also suggest that replacing the alkyl ester moiety (R^2) with lipophilic carbamoyl influences the GI_{50} values against MCF-7 breast cancer cells (13).

Desai *et al.* investigated the correlation between anticancer activity against different cancer cell lines of several substituted 1,2,3,4-tetrahydropyrimidine derivatives and molecular descriptors based on quantitative structure-activity relationships. Weak correlation was observed between the physicochemical parameters and anticancer activity against leukemia, lung, and colon cancer cell lines, and a very good correlation between the descriptors and anticancer activity in the case of a breast cancer cell line. The results suggested that less lipophilic, less bulky and electron-withdrawing substituents may increase the potency against the breast cancer cell line. A positive influence of lipophilic and electronic parameters and a negative influence of steric parameters was observed when descriptors were correlated with anticancer activity against a prostate cancer cell line. The correlation between the activity against the CNS cancer cell line and molecular descriptors showed that decreasing the lipophilicity and electron density while increasing the bulk of the substituent will increase the potency (148).

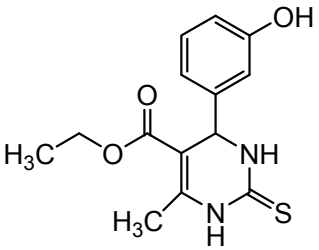
The cytotoxicity activity of some 5-arylcarboxamide THPMs against *Vero cells* has also reported (149). Weak to high cytotoxicity was claimed for the compounds with the general structure of Figure 86:



R	X
Aryl	S
Heteroaryl	O
Cinnamyl	

Figure 86. Arylamide derivatives of 1,2,3,4-THPMs possessing anticancer activity

Tetrahydropyrimidinone derivatives have been suggested as human kinesin Eg5 inhibitors. Monastrol (ethyl 4-(3-hydroxyphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate) is known as a low molecular weight, cell-permeable molecule, and has inspired a research line for the development of potentially new anticancer drugs (150) Figure 87.



Monastrol

Figure 87. Chemical structure of Monastrol

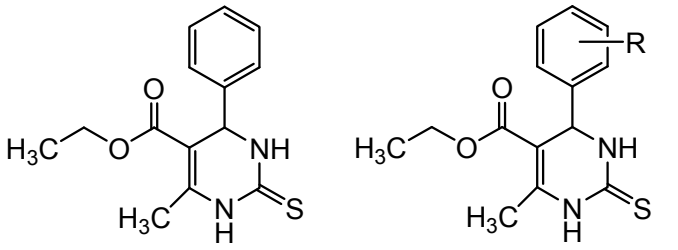
This compound specifically affects cell division (mitosis) by means of a mechanism that does not involve the binding to tubulin, unlike the natural Vinca and Taxane alkaloids and Epothilones. It has been established that the activity of Monastrol is based on the specific and reversible inhibition of the motility of mitotic kinesin Eg5, a motor protein required for bipolar spindle formation during mitosis (151, 152). Investigations showed that Monastrol was more potent than its deoxy derivative; therefore, the hydroxyl group in the aromatic ring is essential for the cytotoxic activity. However, the presence of the hydroxyl group seems not to be the best option, as can be observed *ortho*-methoxy, *para*-methoxy and *meta* and *para*-methylenedioxy, substituents were all more potent than Monastrol (153) Figure 88.

Soumyanarayanan *et al.* screened the cytotoxic activity of compounds and suggested that compounds **81a** and **81b** (Figure 89) were the most potent against the HepG2 cell line, with IC_{50} = 124 and 120 μg/mL, respectively. They also introduced **81a** as the most potent compound against the Hela cell line (IC_{50} = 187 μg/mL). Based on the biological results, it was anticipated that substitution of electron-withdrawing substituents, such as chlorine, at the *para* position of the C-4 phenyl ring may be essential for the ligand-receptor interaction. SAR analysis revealed that compounds with weakly basic pyrrolidine and piperidine substitutions in the C-5 side chain of these THPMs reduce the anticancer activity. In contrast, morpholine was not tolerated at this position and hence resulted in a decrease in IC_{50}. Modeling the molecular interactions of these compounds with kinesin Eg5 protein revealed that the positioning of a hydrogen bond donor/acceptor on the C-4 phenyl ring plays a critical role in the inhibition of this enzyme (154).

CONCLUSIONS AND PERSPECTIVES

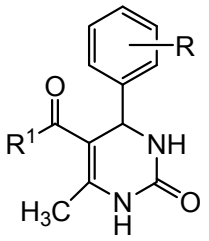
A detailed study of 1,4-dihydropyridine and 1,2,3,4-tetrahydropyrimidine scaffolds points to some remarkable structural features that mark them as potential lead compounds for chemotherapeutic applications.

The nature of the groups substituted at C-3, C-4 and C-5 positions of 1,4-DHPs proved to be important for antitubercular, antimicrobial and anticancer activities of the derivatives of this scaffold. The structures of the active derivatives confirm that the lipophilicity of the molecule is an important physicochemical property that affects potency. An adequate degree of lipophilicity seems to be necessary for the chemotherapeutic properties of the compounds.



R
o-OCH_3
p-OCH_3
m,p-methylenedioxy

Figure 88. Structures of some anticancer 1,2,3,4-THPM carboxylate esters



	R	R^1
81a	*p*-Cl	pyrrolidine
81b	*p*-Cl	piperidine
81c	H	morpholine

Figure 89. 1,2,3,4-THPMs with pyrrolidine and piperidine substituents in the C-5 side chain

The presence of bulk substituents at C-3 and C-5 positions of 1,4-DHPs, whether aromatic or heteroaromatic, increases the chemotherapeutic potency. Chain substitution at both positions also leads to antimycobacterial and antimicrobial compounds that are as potent as heteroaromatic substituted 1,4-DHPs.

Asymmetric 1,4-DHPs bearing both a carboxylate ester group and a carboxamide moiety, as well as symmetric DHPs with carboxylate ester groups in both C-3 and C-5 positions, represent potent antitubercular agents. Asymmetric 3,5-dicarboxamide 1,4-dihydropyridines with two different dicarboxamide derivatives were seen to be weaker antitubercular agents than symmetric DHPs**.** Both symmetric and asymmetric compounds with at least one bulk group were seen to have effective cytotoxicity.

The C-4 substituent has a determinant effect on antitubercular, antimicrobial and anticancer activities. In almost all the reported 1,4-dihydropyridines, the C-4 substituent is a (substituted) phenyl ring. Substitution on this ring is important for the antitubercular, antifungal, antibacterial and MDR-inhibiting activities. Replacement of the phenyl ring in C-4 position by heteroaromatic rings leads to compounds with high antitubercular, antimicrobial and anticancer activities. The substitution of medium-length (up to six carbons) aliphatic chains or cycloaliphatic groups at C-4 may provide key information about the SAR of this position.

N-1 substitution of 3,5-phenylcarboxamide and 3,5-alkylcarboxylate esters of 1,4-DHPs decreases both the antitubercular and antimicrobial potency of the compound.

Aromatic pyridine derivatives have not yet been investigated for their antitubercular and antibacterial activities. Investigation into the effect of aromatization on these biological properties is recommended. Aromatized Hanztsch pyridines were anticancer agents as potent as 1,4-dihydropyridines.

The C-2 and C-6 positions have not been subjected to SAR studies. Alterations of the substitutents at these positions and exploring the effect of the hydrophobic/hydrophilic nature and also the steric limitations of these substituents will increase our knowledge of the structural requirements for chemotherapeutic properties of the compound.

As regards the structural and biological similarities between 1,4-dihydropyridines and 1,2,3,4-tetrahydropyrimidins described above, most

of the conclusions provided for the structure-activity relationship are applicable to Biginelli THPMs.

Antitubercular, antimicrobial and anticancer activities are affected by the properties of the C-2, C-4 and C-5 substituents of 1,2,3,4-THPMs. Here again, bulk and lipophilic substituents, whether aromatic or heteroaromatic, increase the chemotherapeutic potency.

Oxo or thioxo atoms substituted on the C-2 position of the 1,2,3,4-tetrahydropyrimidine both exist in the structure of the active compounds. Lipophilic substitutents at this position lead to improved antibacterial activity. Such substituents should also be investigated for antitubercular and anticancer derivatives.

Evaluation of other lipophilic moieties - aliphatic, cycloaliphatic, aromatic or heteroaromatic - located at N-3 and N-1 positions is recommended.

The importance of the C-4 substituent concerning its chemotherapeutic properties is the same as that deduced in the case of 1,4-DHPs. Again, medium-length (up to six carbons) aliphatic chains or cycloaliphatic groups substituted at C-4 are worthy of further investigation for their structure-activity relationships.

Most of the 1,2,3,4-THPMs studied for their potential antitubercular activity bear C-5 carboxamide moieties, although carboxylate ester derivatives proved to be potent antitubercular agents. Thus, there seems to be a need for more investigation into carboxylate ester derivatives. Conversely, carboxamide derivatives need to be investigated more for their antimicobial properties. Both carboxamide and carboxylate ester derivatives have been confirmed as potent anticancer agents.

Alterations of the substitutents at C-6 position will develop the knowledge of structure-chemotherapeutic properties relationships established for 1,2,3,4-tetrahydropyrimidine scaffold.

The aromatization of Biginelli pyrimidines does not seem to add to their antitubercular properties and perhaps needs more investigation. The possible effect of this structural change on antitubercular and anticancer activity of the compounds needs to be clarified.

Determination of exact targets for the antitubercular, antimicrobial and anticancer properties of 1,4-dihydropyridines and 1,2,3,4-tetrahydropyrimidines will pave the way for the rational structure-based design of novel chemotherapeutic derivatives. To the best of our knowledge, there are only two reports of modeling the molecular interactions of chemotherapeutically active 1,4-DHPs. In the first of these, *Mycobacteriun tuberculosis* enoyl reductase as a possible target for the antimycobacter 1,4-dihydropyridine-3,5-dicarboxamides was subjected to molecular docking and molecular dynamics simulation studies. The second report was a molecular docking study of anticancer 1,4-dihydropyridines in MRP1-NBD1 protein. Molecular interactions of 1,2,3,4-tetrahydropyrimidines were modeled against human kinesin Eg5 protein for Monastrol, the FDA approved anticancer agent (154).

Among the ligand-based computer aided drug design approaches, the QSAR and 3D-QSAR methods provide valuable information about the structural features of the biologically active molecules (QSAR) and the binding features (3D-QSAR) of their possible targets. Only a few QSAR studies concerning anticancer 1,4-DHPs, antimicrobial and anticancer 1,2,3,4-THPMs are available in the literature. The same is true for the 3D-QSAR studies. Antitubercular 1,4-DHPs, antitubercular and antimicrobial 1,2,3,4-THPMs have only been submitted to 3D-QSAR investigations. The lack of such research is obvious in the field of chemotherapeutic 1,4-DHP and 1,2,3,4-THPM compounds.

Thus, there is a clear need for computer-aided investigations to determine biological target(s) among those possibly present in the biochemical pathways involved, and for specific laboratory assays to experimentally determine such target(s). The structural features of the biologically active compounds and the binding features of their target active sites also need clarification.

ACKNOWLEDGMENT

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ABBREVIATIONS

ABC	ATP-binding cassette
1,4-DHP	1,4-dihydropyridine
3D-QSAR	3D-quantitative structure–activity relationship
DXR	Doxorubicine
GST	Glutathione-S-transferase
MCR	Multi-component reaction
MDR	Multi-drug resistance
MRP1	Multidrug resistance protein 1
Pgp	P-glycoprotein
QSAR/QSPKR	Quantitative structure-activity/pharmacokinetics relationship
RS	Resistant cells
SAR	Structure-activity relationship
TB	Tuberculosis
1,2,3,4-THPM	1,2,3,4-Tetrahydropyrimidine
TS	Tumor specific
VCR	Vincristine
VP	Verapamil
ZI	Zone of inhibition