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Synthesis and Evaluation of 4-Alkyl /Aryl/Heteroaryl 3, 5-Bis-N-(4-Chloro Phenyl) Carbamoyl 2, 6-Dimethyl 1, 4-Dihydropyridines

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Abstract
A new carbamoyl series of 1,4-dihydropyridines were synthesized from the condensation reaction of N-(4-chlorophenyl) acetoacetamide, ammonium acetate and appropriate aliphatic, aromatic or hetero aromatic aldehyde by conventional and microwave irradiation methods. The synthesized compounds were purified and confirmed by IR, ^{1}HNMR and Mass spectral data. The methods employed have been compared in terms of yields, reaction times. All the experimental conditions in MWI method, when compared to conventional, are easy simple, eco-friendly and the reactions are rapid and high yielding. The synthesized compounds have been evaluated for their cytotoxic activity against MCF-7 (breast cancer) HeLA (cervical cancer), and HT-29 (colon cancer) cell lines and antimicrobial activity against Staphylococcus aureus Escherichia coli and Candida albicans. Among the series compounds containing heterocyclic as carbamoyl derivatives exhibited more cytotoxic and antimicrobial activities, remaining compounds could exhibit some degree of cytotoxicity but not at all comparable with that of the standard.

Keywords
1,4-Dihydropyridines, Calcium channel blockers, Microwave synthesis, Thin layer Chromatography, Antimicrobial activity, Cytotoxicity.

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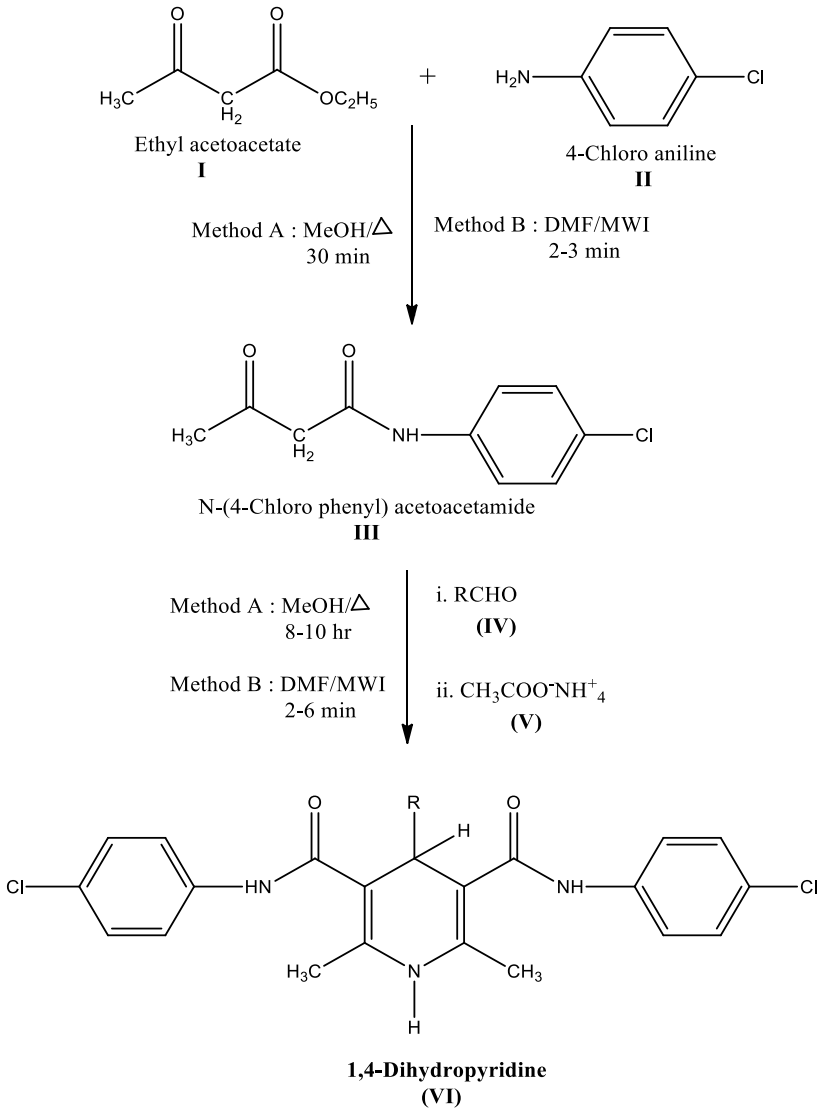
INTRODUCTION
1,4-Dihydropyridines (DHPs) are the known class of therapeutic agents to treat angina and hypertension, as calcium channel blockers [1,2]. Several of their derivatives are also reported to exhibit a variety of biological and pharmacological activities, viz., antitumor [3] antidiabetic [4], antioxidant [5], anti-inflammatory [6], anticoagulant [7], anticonvulsant [8] and antimicrobial [9]. Hence, this field has-ever-growing importance resulting in the development scores of DHPs. Therefore, it has been considered worthwhile to synthesize some new DHPs by two different procedures, i.e., conventional and microwave irradiation [10] methods for comparison,

to characterize the new DHPs by their analytical and spectral (IR, ^{1}H NMR and Mass) data.

The new DHPs, have been synthesized by a modified and improvised Hantzsch one-pot synthesis starting with N-(4-chloro phenyl) acetoacetamide with an appropriate aliphatic, aromatic or hetero aromatic aldehydes and ammonium acetate in conventional method and as well as rapid microwave irradiation method **(Scheme-1).** The synthesized DHPs were purified and characterized as 4-Alkyl/ aryl/ heteroaryl-3, 5-bis-N-(4-chlorophenyl) carbamoyl-2, 6-dimethyl-1, 4-dihydropyridines. Physical data of 1,4-dihydropyridines are presented in **Table-1.**

MATERIALS AND METHODS

The conventional and microwave assisted experimental procedures are given as general methods. The melting points were determined in open capillaries using Toshnwal melting point apparatus. Infra-red spectra of the compounds were recorded in KBr pellet using Shimadzu FTIR-8700 spectrometer, ^{1}H NMR spectra on omega-500 MHz spectrometer using TMS as internal standard and mass spectra by the direct inlet method on VG micro mass 7070 H spectrometer operating at 70 ev.



Scheme-I

Experimental

A. Synthesis of N-(4-chloro phenyl) acetoacetamide (III)

a. Conventional method

Ethyl acetoacetate **(I; 0.05 mole)** and 4-chloro aniline **(II; 0.05 mole)** were taken into a round bottomed flask (250 ml) and dissolved in alcohol (25 ml) and added catalytic amount of potassium ter-butoxide. The reaction mixture was heated under reflux for 30 min, on a hot water bath. Alcohol was removed to a possible extent by distillation under reduced pressure and cooled. The resultant product was triturated with ice cold water, filtered, washed with cold water and dried. It was purified by recrystallization from aqueous methanol to get a pure crystalline solid.

b. Microwave irradiation method

An equimolar **(0.05 mole each)** mixture of ethyl acetoacetate and 4-chloro aniline was taken into a beaker and dissolved in 10 ml of dimethyl form amide. Add catalytic amount of potassium ter-butoxide while shaking thoroughly. A funnel was hanged in the beaker and covered with a watch glass. The reaction mixture was subjected to the microwave irradiation at 480 watts for 2 min in microwave oven at a pulse rate of 30 secs, each. The resultant product was triturated with crushed ice, filtered, washed with cold water and dried. The product was further purified by recrystallization from aqueous methanol to get a pure colourless, crystalline solid.

B. Synthesis of 4-Alkyl/ aryl/ heteroaryl-3,5-bis-N(4-chlorophenyl) carbamoyl-2,6-di-methyl-1,4-dihydropyridines

a. Conventional method:

N-(4-Chloro phenyl) acetoacetamide **(III; 0.05 mole)** and an appropriate aliphatic, aromatic or hetero aromatic aldehyde **(IV; 0.025 mole)** were taken into a RB flask and dissolved in methanol (25 ml) by shaking. To this solution, ammonium acetate **(V; 0.025 mole)** was added while shaking and the reaction mixture was heated under reflux for 10-12 hr on a hot water-bath. Alcohol was removed to a possible extent by distillation under reduced pressure and the residue was cooled. The product was filtered, washed with small portions of petroleum ether and dried. It was purified by recrystallization from hot alcohol.

b. Microwave irradiation method:

N-(4-Chloro phenyl) acetoacetamide **(III; 0.05 mole)** was taken into a beaker and dissolved in dimethyl form amide (10 ml). An appropriate aliphatic, aromatic or hetero aromatic aldehyde **(IV; 0.025 mole)** was added to the above solution while shaking followed by the addition of ammonium acetate **(V; 0.025 mole)**. The reaction mixture was irradiated in a microwave oven at 480 watts for 2-5 min. The mixture was cooled and poured onto crushed ice (100g), while stirring. The resultant product was filtered, washed with cold water and dried. It was purified by recrystallization from alcohol.

Spectral characterization data

Spectral characterization data of N-(4-chloro phenyl) acetoacetamide (III)

IR (KBr, Cm^{-1}) υ: 3346 (-NH, carbamoyl), 3028 (C-H, aromatic) 2836 (C-H, aliphatic), 1642 (C=O, ketone), 1608 (C=C, aromatic) and 1102 (C-Cl, aromatic).

^{1}H NMR ($CDCl_3$, 300 MHz, ppm) δ: 2.38 (s, 3H, -CH_3CO), 3.60 (s, 2H, -$COCH_2$), 9.64 (s, 1H, D_2O exchangeable, -CONH) and 6.98 to 7.40 (m, 4H, Ar-H of aromatic).

Mass spectrum of the compound exhibited its molecular ion (M^+) at m/z 211.

Spectral characterization data of 4-Phenyl-3,5-bis-N-(4-chlorophenyl) carbamoyl-2,6-dimethyl-1,4-dihydropyridine (VIc).

IR (KBr, Cm^{-1}) υ: 3160 (-NH, carbomoyl), 3326 (-NH, DHP) 3052 (C-H, aromatic), 2986 (C-H, aliphatic), 1674 (-C=O, carbamoyl), 1630 (C=C, aromatic) and 1087 (C-Cl, aromatic).

^{1}H NMR ($CDCl_3$, 300 MHz, ppm) δ: 2.40 (s, 6H, -CH_3 at C_2 & C_6 of DHP), 4.90 (s, 1H, -NH of DHP), 5.22 (s, 1H, -CH at C_4 of DHP), and 6.94 to 7.62 (m, 13H, Ar-H at C_3, C_4 & C_5 of DHP) 8.30 (d, 2H, D_2O exchangeable, -CONH at C_3 & C_5 of DHP).

Mass spectrum: of the compound exhibited its molecular ion (M^+) at m/z 490.

Antimicrobial activity

All the synthesized of compounds (**VIa–j)** were evaluated for antimicrobial activity by cup-plate [11] method against Staphylococcus aureus, Escherichia coli and Candida albicans. Ciprofloxacin and Clotrimazole were taken as standard drug (10 µg/ml) for antibacterial and antifungal activities. The bacterial zones of inhibition values (mm) are given in **Table 2**.

Anticancer activity

All the synthesized compounds (**VIa-j**) were evaluated for cytotoxic activity against the MCF-7 (breast cancer) HeLA (cervical cancer), and HT-29 (colon cancer) cells using the MTT assay method [12]. The inhibitory potencies (IC_{50}) are listed in **Table-3**.

RESULTS AND DISCUSSION

4-Alkyl/aryl/heteroaryl-3,5-bis-N-(4-chloro phenyl) carbamoyl-2,6-dimethyl-1,4-dihydropyridines **(VI a-j)** synthesized by two methods. A significant increase in percentage yields with a shorter reaction times have been recorded in MWI method, when compared with conventional methods which involves longer

reaction times under refluxing conditions with moderate yields. The reactions are also easy, simple and eco-friendly.

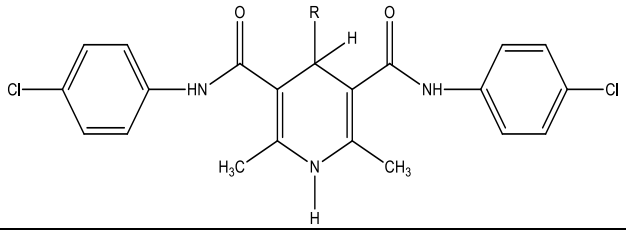
Table 2 represents the antimicrobial activity data. It reveals that compound **VIj, VIg & VIh** were found to be more active against *S. aureus* organism. Compound **VId, VIh & VIj** were found to be more active against *E.coli.* The synthesized compounds were also screened for their antifungal activity, a compound **VIj, VIh, VId & VI g** were showed higher activity against *C. albicans*. Remaining compounds exhibited mild to moderate antimicrobial activity.

Table 3 represents the anticancer activity data. Among the series compounds **VIj, Vii, VIg & VIh** were found to be more active against all the types of cancer cell lines. Remaining compounds exhibited mild to moderate anticancer activity. The compounds with alkyl substitution exhibited least anticancer activity among the series. The anticancer activity of all the compounds is not at all comparable to that of the standard (Cis-platin) employed in the present investigation; their activity is many folds inferior or low to that of the cis-platin.

CONCLUSIONS

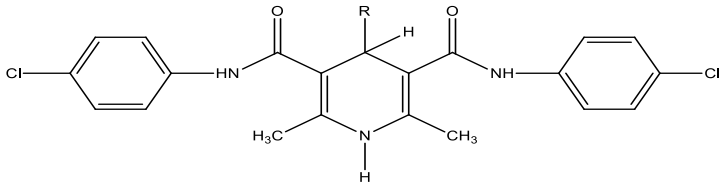
The present work was intended for the synthesis and characterization of some new dihydropyridines by conventional and MWI methods. The methods are easy simple, eco-friendly and the reactions are rapid and high yielding. All the synthesized compounds were characterized by physical, analytical and spectral data. The synthesized compounds were screened for their antimicrobial and anticancer activities. The compounds substituted with heterocycles, pyridyl, electron donating groups like methoxy and hydroxyl and halogen (Cl) substituted derivatives were found to be more active.

Table 1: Physical and analytical data of 4-Alkyl/aryl/heteroaryl-3,5-bis-N-(4-chloro phenyl) carbamoyl- 2,6-dimethyl-1,4-dihydropyridines (VIa-j)



Compound Code	R	Mol.Formula	Mol.Wt	Method-A (% Yield)	Method-B (% Yield)	m.p(°C)
VIa	H	$C_{21}H_{19}N_3O_2Cl_2$	415	49	75	148-150
VIb	CH_3	$C_{22}H_{21}N_3O_2Cl_2$	429	52	84	164-166
VIc	C_6H_5	$C_{27}H_{23}N_3O_2Cl_2$	491	48	76	184-186
VId	4-ClC_6H_4	$C_{27}H_{22}N_3O_2Cl_3$	525	58	88	212-214
VIe	4-NO_2 C_6H_4	$C_{27}H_{22}N_4O_4Cl_2$	536	56	84	168-170
VIf	4-$CH_3C_6H_4$	$C_{28}H_{25}N_3O_2Cl_2$	505	58	82	184-186
VIg	4-OHC_6H_4	$C_{27}H_{23}N_3O_3Cl_2$	507	52	69	190-192
VIh	3,4,5-$(OCH_3)_3$ C_6H_2	$C_{30}H_{29}N_3O_5Cl_2$	581	64	88	224-226
VIi	2_Furyl	$C_{25}H_{21}N_3O_2Cl_2$	481	62	91	188-190
VIj	2-Pyridyl	$C_{26}H_{22}N_4O_2Cl_2$	493	59	87	164-166

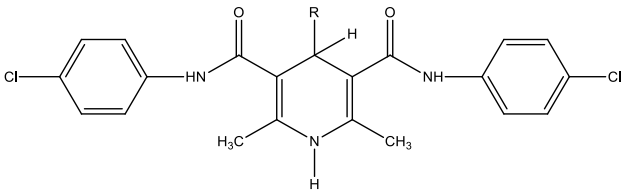
Table 2: Antimicrobial activity of 4-Alkyl/aryl/heteroaryl-3,5-bis-N-(4-chloro phenyl) carbamoyl- 2,6-dimethyl-1,4-dihydropyridines (VIa-j)



Compound	-R	Antibacterial	Antifungal	
		S.aureus (Gram +ve)	*E.coli* (Gram -ve)	*C. albicans*
VIa	-H	6	7	7
VIb	$-CH_3$	5	8	8
VIc	$-C_6H_5$	7	10	7
VId	$-C_6H_4$.Cl-4	10	14	13
VIe	$-C_6H_4.NO_2$ -4	9	9	10
VIf	$-C_6H_4.CH_3$ -4	8	7	9
VIg	C_6H_4. OH -4	12	10	12
VIh	C_6H_2 .3,4,5-$(OCH_3)_3$	11	11	13
VIi	2-Furyl	9	10	10
VIj	2-Pyridyl	13	12	14
Ciprofloxacin	-	**24**	**26**	-
Clotrimazole	-	-	-	22

(Concentration of the test compound: 100 µg/cup; Zone of inhibition in mm)

Table 3: Anticancer activity of 4-Alkyl/aryl/heteroaryl-3,5-bis-N-(4-chloro phenyl) carbamoyl- 2,6-dimethyl-1,4-dihydropyridines (VIa-j)



Compound	-R	IC_{50} µM		
		MCF-7	HeLA	HT-29
Via	-H	>100	96.8	>100
VIb	$-CH_3$	95.3	98.1	88.9
VIc	$-C_6H_5$	82.6	92.1	72.8
VId	$-C_6H_4$.Cl-4	78.4	81.3	71.5
VIe	$-C_6H_4.NO_2$ -4	65.4	70.4	77.4
VIf	$-C_6H_4.CH_3$-4	58.9	76.2	64.8
VIg	C_6H_4. OH -4	52.3	68.8	62.3
VIh	C_6H_2 .3,4,5-$(OCH_3)_3$	56.8	72.2	61.2
VIi	2-Furyl	49.4	60.8	60.2
VIj	2-Pyridyl	44.2	61.8	59.7
Cisplatin	-	**12.7**	**14.70**	**8.56**

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