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Synthesis and biological screening of 1,4-dihydropyridine derivatives containing benzothiazolyl moiety

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ABSTRACT

A series of N-(6-nitrobenzothiazolyl)-2,3,5,6-tetrasubstituted-4-(aryl)-1,4-dihydropyridines were synthesized by reaction of 2-amino-6-nitrobenzothiazole, aromatic aldehyde and active methylene compound in methanol by conventional, as well as, microwave irradiation (solvent free and solid support) methods. The microwave irradiation technique gives better yield in shorter reaction time. Among solid supported microwave irradiation better yields are obtained in acidic alumina as compared to silica, neutral alumina, and basic alumina. All compounds were tested for antibacterial and antifungal activities and results have been compared with standard drugs. Entomological activities and Antiproliferative activity of some synthesized compound were also tested. The results showed that a change in the substitution pattern in 1,4-dihydropyridine derivatives may cause a marked effect on their antimicrobial activity.

Key Words: Benzothiazole, 1,4-Dihydropyridine, MW synthesis, Antimicrobial activity, Antiproliferative activity.

INTRODUCTION

Heterocyclic compounds containing nitrogen and sulphur heteroatoms are useful material in drug research [1-4]. In addition to this Benzothiazole ring present in various marine or terrestrial natural compounds, which have useful biological activities [5-8]. Benzothiazoles comprise a class of therapeutic compounds that exert a wide and broad spectrum of biological activities such as antimicrobial [9-10], anticancer [11-12], antifungal [13], antihelmintic [14], antileishmanial [15], anticonvulsant [16], antirheumatic [17-18], anti-inflammatory [19-21], antiallergic [22], insecticides [23-26] and herbicidal [27-29] activities. 2-(4-Aminophenyl) benzothiazole [30-31] comprises a novel mechanistic class of antitumor agents. Similarly dihydropyridine chemistry is

of interest from the point of view of pure research on heterocyclic compounds and also from a biological point of view [32]. 1,4-dihydropyridines (1,4-DHPs) possess different pharmacological activities such as anticancer [33], antidiabetic [34], antianginal[35], bronchodilating [36], neurotropic [37], antiallergic [38], anti-inflammatory [39], acaricidal, insecticidal, bactericidal, herbicidal [40] and other pharmacological activities [41]. These are used extensively in the treatment of angina pectoris, hypertension and arrhythmia [42] and some cardiovascular disorder. Several new derivatives of 1,4-DHP have been produced and pharmacologically evaluated in order to find drugs with better pharmacological properties [43]. So pharmacology of 1,4-DHP derivatives is at the eve of a novel boom.

In view of the exhaustive literature survey investigated by us about the biological profile of benzothiazole and 1,4-DHPs prompted us to couple these moieties to synthesis some benzothiazolodihydropyridine derivatives with the hope of achieving enhanced biological activities. 2-Amino-6-nitrobenzothiazole **1** was treated with active methylene compounds (ethylacetoacetate, diethylmalonate, acetylacetone) and **2** aromatic aldehydes (benzaldehyde, p-hydroxybenzaldehyde**,** p-dimethylaminobenzaldehyde, p-methoxybenzaldehyde) **3** on steam bath for 2-3 hours and refluxed in methanol for 10-15 hours. The above described method apparently suffer from disadvantages such as prolonged refluxing, use of volatile organic solvents, waste effluent and low to moderate yields, cumbersome work –up procedure, create pollution to the environment and lack of selectivity in the presence of other functional groups.

Looking to these drawback of conventional methods we are reporting a novel environmentally benign approach using a facile, microwave synthesis of title compounds carried out by solvent free method, and various solid supports like silica gel, alumina basic, alumina neutral, and alumina acidic. The present work deals with the synthesis of N-(6-nitrobenzothiazolyl)-2,3,5,6-tetrasubstituted-4-(aryl)-1,4-dihydropyridines followed by antiproliferative activity. Antimicrobial susceptibility test (AST) against *Lactobacillus sp., Pseudomonas aeruginosa, Staphylococcus aureus, Micrococcus leutius, Kocuria rosea, Aspergillus niger, Aspergillus candidus* using standard methods and comparison with standard drugs. All the synthesized derivatives were also screened for their entomological activity.

MATERIALS AND METHODS

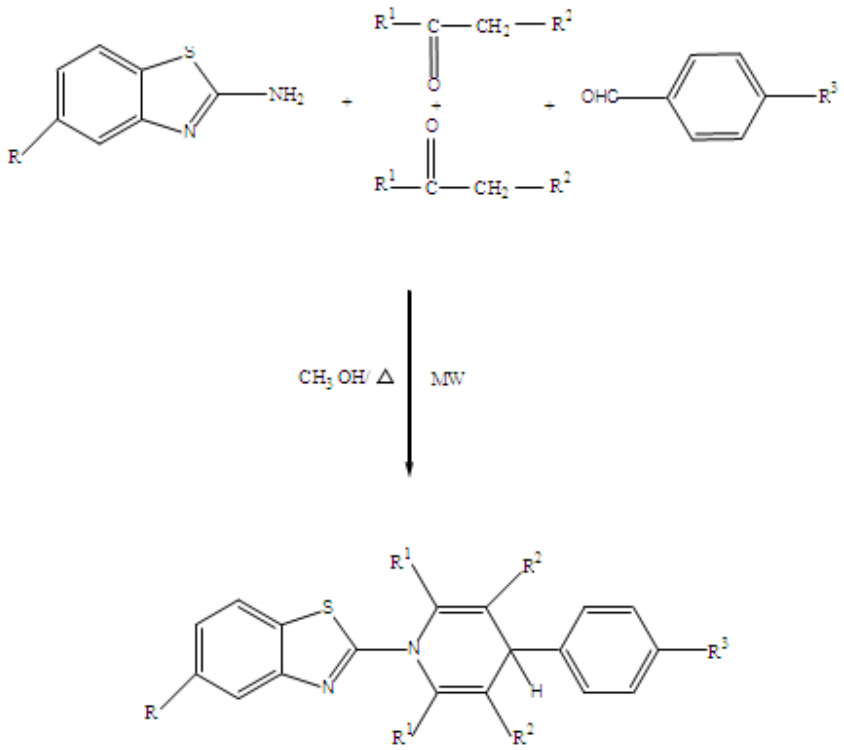
2. 1 Chemistry

Reagent grade chemicals were used without further purification. The substrates and solvents were used as received. All the melting points are taken in open capillaries an uncorrected. The purity of synthesized compounds was checked by Thin Layer Chromatographic studies. IR spectra were scanned on FT IR Perkin Elmer (Spectrum RX1) spectrophotometer (υ in cm^{-1}) using KBr disc. ^1H NMR was recorded in $CDCl_3$ with tetramethylsilane (TMS) as the internal standard at 300 MHz on a Bruker DRTX-300 spectrophotometer. The chemical shifts are reported as parts per million (ppm). Fast atom bombardment mass spectra (FABMS) were recorded at room temperature on a Jeol SX-102/DA-6000 mass spectrophotometer/data system using Argon/Xenon (6 kV, 10mA) as the FAB gas. The accelerating potential was 10kV. Microwave synthesis was carried out in a "Q-pro-M Modified Microwave system". The elemental analysis of compounds was performed on a Carlo Erba-1108 elemental analyzer.

2.1.1General procedure for the synthesis of N-(6-nitrobenzothiazolyl)-2,3,5,6-tetrasubstituted-4-(aryl)-1,4-dihydropyridines.

2.1.2. Conventional Method

A reaction mixture of 2-amino-6-nitrobenzothiazole (0.1 mole), aromatic aldehyde (0.1 mole) and active methylene compound (0.2 mole) was heated (without solvent) on steam bath for 2-3 hours. After elimination of water, methanol (25mL) was added directly to the reaction mixture and refluxed for 10-15 hours. Then the reaction mixture was poured into the ice water, the solid mass separated was extracted with diethyl ether (50mL) and dried over magnesium sulphate, recrystallised from methanol (**scheme 1.**) **Table 1**.



where
R=NO_2; R_1 = CH_3, OC_2H_5; R_2 = C_2H_5OCO, CH_3CO ; R_3 = H, $(CH_3)_2N$, OCH_3, OH

Scheme 1. Synthesis of N-(6-nitrobenzothiazolyl)-2,3,5,6-tetrasubstituted–4-(aryl)-1,4-dihydropyridines.

2.1.3. Microwave method A reaction mixture of 2-amino-6-nitrobenzothiazole (0.01mole), aromatic aldehyde (0.01 mole) and active methylene compound (0.02 mole) was taken in a round bottom flask. The round bottom flask was placed in a microwave oven and irradiated under a low power 160 Watt and monitored by TLC using Benzene: DMF (7:3). To ensure the reproducibility every reaction is carried out five times in microwave. The reaction mixture was cooled at room temperature and extracted with diethyl ether (10mL), dried over magnesium sulphate gave pure product and recrystallised from methanol **Table 1**.

2.1.4. Solid supported microwave synthesis

To a mixture of 2-amino-6-nitrobenzothiazole (0.01 mol), aromatic aldehyde (0.01 mole) and active methylene compound (0.02 mole) in a round bottom flask, solid support alumina (acidic /basic /neutral) and silica gel were mixed thoroughly in a mortar then the reaction mixture was transferred to a round bottom flask and irradiated under a low power 160 Watt in microwave oven at 30 seconds intervals of specified times. On completion of reaction as monitored by TLC using Benzene: DMF (7:3). To ensure the reproducibility every reaction is carried out five times in microwave. The reaction mixture was cooled at room temperature and extracted with diethyl ether (10mL), dried over magnesium sulphate which gave pure product and recrystallised from methanol **Table 2.**

Spectral and microanalysis data of **(4a-l):**

N-(6-nitrobenzothiazolyl)-3,5-dicarbethoxy-2,6-dimethyl-4-(p-dimethylaminophenyl)-1,4-dihydropyridine (4a).

^{1}H-NMR: 2.27 (s, 6H, dihydropyridine (DHP), CH_3), 4.93 (s, 1H, DHP), 6.47-8.42 (m, 7H, Ar-H), 1.33 (t, J=7.2 Hz, 6H, DHP, CH_2CH_3), 4.20 (q, J=7.2 Hz, 4H, DHP, CH_2), 2.88 (s, 6H, $N(CH_3)_2$), IR (cm^{-1}): ν_{max} 1058, 1215, 1138, 1596, 1545, 1457, 2974, 1660, 1701; Mass: 550 (M^+); Elemental Anal. Calcd. (CHNS) for $C_{28}H_{30}N_4O_6S$: C, 61.08, H, 5.49, N, 10.18, S, 5.82, Found: C, 65.4, H, 6.41, N, 3.60, S, 5.84.

N-(6-nitrobenzothiazolyl)-3,5-dicarbethoxy-2,6-diethoxy-4-(p-dimethylaminophenyl)-1,4-dihydropyridine (4b).

^{1}H-NMR: 4.96 (s, 1H, DHP), 6.47-8.43 (m, 7H, Ar-H), 1.30 (t, J=7.2 Hz, 6H, $DHPCOOCH_2CH_3$), 4.19 (q, J=7.1 Hz, 4H, $DHPCOOCH_2$), 2.84 (s, 6H, $N(CH_3)_2$), 1.25 (t, J=7.2 Hz, 6H, DHP OCH_2CH_3), 4.01 (q, J=7.2 Hz, 4H, $DHPOCH_2$) IR (cm^{-1}): ν_{max} 1058, 1215, 1138, 1596, 1545, 1457, 2974, 1670, 1692; Mass: 610 (M^+); Elemental Anal. Calcd. (CHNS) for $C_{30}H_{34}N_4O_8S$: C, 59.00, H, 5.61, N, 9.17, S, 5.25, Found: C, 63.03, H, 6.42, N, 6.87, S, 5.25.

N-(6-nitrobenzothiazolyl)-3,5-dicarbethoxy-2,6-dimethyl-4-(4-methoxyphenyl)-1,4-dihydropyridine (4c).

^{1}H-NMR: 2.30 (s, 6H,DHP, CH_3), 4.94 (s, 1H, DHP), 6.45-8.14 (m, 7H, Ar-H), 1.30 (t, J=7.2 Hz, 6H DHP, CH_2CH_3), 4.20 (q, J=7.2 Hz, 4H, DHP, CH_2), 3.75 (s, 3H, Ar-OCH_3), IR (cm^{-1}): ν_{max} 1057, 1209, 1545, 1460, 1596, 1665, 1688; Mass: 537 (M^+); Elemental Anal. Calcd. (CHNS) for $C_{27}H_{27}N_3O_7S$: C, 60.32, H, 5.06, N, 7.82, S, 5.96, Found: C, 64.87, H, 6.0, N, 5.20, S, 5.96.

N-(6-nitrobenzothiazolyl)-3,5-dicarbethoxy-2,6-dimethyl-4-(4-hydroxyphenyl)-1,4-dihydropyridine (4d).

^{1}H-NMR: 2.18 (s, 6H,DHP, CH_3), 4.96 (s, 1H, DHP), 6.49-8.16 (m, 7H, Ar-H), 1.32 (t, J=7.0 Hz, 6H, DHP, CH_3), 4.22 (q, J=6.9 Hz, 4H, DHP, CH_2), 5.02 (s, 1H, Ar-OH), IR (cm^{-1}): ν_{max} 1057, 1209, 1545, 1460, 1596, 1665, 1688; Mass: 523 (M^+); Elemental Anal. Calcd. (CHNS) for $C_{26}H_{25}N_3O_7S$: C, 59.05, H, 4.81, N, 8.03, S, 6.12, Found: C, 64.34, H, 5.78, N, 5.34, S, 6.14

N-(6-nitrobenzothiazolyl)-3,5-dicarbethoxy-2,6-diethoxy-4-(4-hydroxyphenyl)-1,4-dihydropyridine (4e).

^{1}H-NMR: 4.93 (s, 1H, DHP), 6.47-8.13 (m, 7H, Ar-H), 1.29 (t, J=7.2 Hz, 6H, DHP, $COOCH_2CH_3$), 4.20 (q, J=7.2 Hz, 4H, DHP, $COOCH_2$), 5.02 (s, 1H, Ar-OH), 1.25 (t, 6H, DHP, OCH_2CH_3), 4.03 (q, 4H, DHP, OCH_2) IR (cm^{-1}): ν_{max} 1058, 1214, 1137, 1594, 1543, 1456, 2975, 3575, 1650, 1689; Mass: 583 (M^+); Elemental Anal. Calcd. (CHNS) for $C_{28}H_{29}N_3O_9S$: C, 57.63, H, 5.01, N, 7.20, S, 5.49, Found: C, 61.82, H, 5.86, N, 4.80, S, 5.49.

N-(6-nitrobenzothiazolyl)-3,5-dicarbethoxy-2,6-diethoxy-4-(phenyl)-1,4dihydropyridine (4f).

^{1}H-NMR: 4.96 (s, 1H, DHP), 6.47-8.14 (m, 8H, Ar-H), 1.29 (t, J=7.1 Hz, 6H, DHP, $COOCH_2CH_3$), 4.18 (q, J=7.1 Hz, 4H, DHP, $COOCH_2$), 1.22 (t, J=7.2 Hz, 6H, DHP, OCH_2CH_3), 4.02 (q, J=7.2 Hz, 4H, DHP, OCH_2). IR (cm^{-1}): ν_{max} 1058, 1209, 1349, 1596, 1545, 1467, 2973, 1694, 1675; 1670; Mass: 567 (M^+); Elemental Anal. Calcd. (CHNS) for $C_{28}H_{29}N_3O_8S$: C, 59.25, H, 5.15, N, 7.40, S, 5.65, Found: C, 63.58, H, 6.04, N, 4.93, S, 5.65

N-(6-nitrobenzothiazolyl)-3,5-diacetyl-2,6-dimethyl-4-(p-dimethoxyphenyl)-1,4-dihydropyridine (4g).

^{1}H-NMR: 2.20 (s, 6H, DHP, CH_3), 4.98 (s, 1H, DHP), 6.48-8.13 (m, 7H, Ar-H), 2.32 (s, 6H, DHP, $COCH_3$), 3.75 (s, 3H, Ar-OCH_3), IR (cm^{-1}): ν_{max} 1056, 1209, 1545, 1460, 1596, 1664, 1694; Mass: 477 (M^+); Elemental Anal. Calcd. (CHNS) for $C_{25}H_{23}N_3O_5S$: C, 62.88, H, 4.85, N, 8.80, S, 6.71, Found: C, 68.04, H, 5.91, N, 5.87, S, 6.72

N-(6-nitrobenzothiazolyl)-3,5-dicarbethoxy-2,6-dimethyl-4-(phenyl)-1,4dihydropyridine (4h).

^{1}H-NMR: 2.35 (s, 6H, DHP, CH_3), 4.96 (s, 1H, DHP), 6.46-8.12 (m, 8H, Ar-H), 1.28 (t, J=7.2 Hz, 6H, DHP, CH_3), 4.17 (q, 4H, DHP, CH_2), IR (cm^{-1}): ν_{max} 1057, 1213, 1137, 1593, 1456, 2971, 1670, 1696; Mass: 507 (M^+); Elemental Anal. Calcd. (CHNS) for $C_{26}H_{25}N_3O_6S$: C, 61.53, H, 4.96, N, 8.28, S, 6.32, Found: C, 66.37, H, 5.96, N, 5.52, S, 6.82.

N-(6-nitrobenzothiazolyl)-3,5-diacetyl-2,6-dimethyl-4-(p-hydroxyphenyl)-1,4-dihydropyridine (4i).

^{1}H-NMR: 2.23 (s, 6H, DHP, CH_3), 4.98 (s, 1H, DHP), 6.48-8.14 (m, 7H, Ar-H), 2.33 (s, 6H, DHP, $COCH_3$), 5.02 (s, 1H, Ar-OH), IR (cm^{-1}): ν_{max} 1056, 1214, 1137, 1594, 1543, 1454, 2975, 3573, 1660, 1691; Mass: 463 (M^+); Elemental Anal. Calcd. (CHNS) for $C_{24}H_{21}N_3O_5S$: C, 62.19, H, 4.57, N, 9.07, S, 6.92, Found: C, 66.50, H, 5.60, N, 6.04, S, 6.91.

N-(6-nitrobenzothiazolyl)-3,5-diacetyl-2,6-dimethyl-4-(phenyl)-1,4-dihydropyridine (4j).

^{1}H-NMR: 2.18 (s, 6H, DHP, CH_3), 4.97 (s, 1H, DHP), 6.47-8.14 (m, 8H, Ar-H), 2.32 (s, 6H, DHP, $COCH_3$),). IR (cm^{-1}): ν_{max} 1056, 1200, 1342, 1534, 1542, 1452, 2972, 1665, 1695; Mass: 447 (M^+); Elemental Anal. Calcd. (CHNS) for $C_{24}H_{21}N_3O_4S$: C, 64.42, H, 4.73, N, 9.39, S, 7.16, Found: C, 69.90, H, 5.80, N, 6.24, S, 7.15

N-(6-nitrobenzothiazolyl)-3,5-diacetyl-2,6-dimethyl-4-(p-dimethylaminophenyl)-1,4-dihydropyridine (4k).

^{1}H-NMR: 2.30 (s, 6H, DHP, CH_3), 4.95 (s, 1H, DHP), 6.47-8.12 (m, 7H, Ar-H), 2.32 (s, 6H, DHP, $COCH_3$), 2.86 (s, 6H, $N(CH_3)_2$), IR (cm^{-1}): ν_{max} 1056, 1212, 1135, 1593, 1542, 1456, 2971,

1670, 1693; Mass: 490 (M^+); Elemental Anal. Calcd. (CHNS) for $C_{26}H_{26}N_4O_4S$: C, 63.66, H, 5.34, N, 11.42, S, 6.54, Found: C, 68.60, H, 6.32, N, 8.54, S, 6.52.

N-(6-nitrobenzothiazolyl)-3,5-dicarbethoxy-2,6-diethoxy-4-(p-methoxyphenyl)-1,4-dihydropyridine (4l).

^{1}H-NMR: 4.97 (s, 1H, DHP), 6.47-8.12 (m, 7H, Ar-H), 1.32 (t, J=7.2 Hz, 6H, DHP, $COOCH_2CH_3$), 4.22 (q, 4H, DHP, $COOCH_2$), 3.75 (s, 3H, Ar-OCH_3), 1.24 (t, 6H, DHP, OCH_2CH_3), 4.02 (q, 4H, DHP, OCH_2). IR (cm^{-1}): ν_{max} 1057, 1210, 1136, 1594, 1543, 1456, 2973, 1701, 1664; Mass: 596 (M^+); Elemental Anal. Calcd. (CHNS) for $C_{29}H_{31}N_3O_9S$: C, 58.28, H, 5.23, N, 7.03, S, 5.36, Found: C, 62.35, H, 6.02, N, 4.65, S, 5.32.

Table 1. Preparation of N-(6-nitrobenzothiazolyl)-2,3,5,6-tetrasubstituted-4-(aryl)-1,4-Dihydropyridines

Compounds	R1	R2	R3	M.P. ^{0}C	Conventional heating		Microwave (Neat reaction)	
					Time (Hrs.)	Yield (%)	Time (Sec.)	Yield (%)
4a	Me	O=COEt	$N(Me)_2$	232	20.0	52	200	75
4b	OEt	O=COEt	$N(Me)_2$	190	20.0	520	180	70
4c	Me	O=COEt	OMe	207	20.0	50	160	68
4d	Me	O=COEt	OH	188	20.0	53	200	70
4e	OEt	O=COEt	OH	194	20.0	50	190	70
4f	OEt	O=COEt	H	192	20.0	57	170	75
4g	Me	CO Me	OMe	210	20.0	45	100	70
4h	Me	O=COEt	H	180	20.0	60	190	75
4i	Me	CO Me	OH	220	20.0	50	180	65
4j	Me	COMe	H	224	20.0	55	160	67
4k	Me	COMe	$N(Me)_2$	183	20.0	48	170	69
4l	OEt	O=COEt	OMe	215	20.0	55	160	62

2.1.5 BIOLOGICAL SECTION

Antimicrobial activity

All the synthesized compounds were tested for their antibacterial activity against various bacteria, *Lactobacillus sp., Pseudomonas aeruginosa, Staphylococcus aureus, Micrococcus leutius, Kocuria rosea,* and antifungal activity against *Aspergillus niger and Aspergillus candidus* using paper disc method. Muller Hinton Agar (Hi-Media Pvt. Ltd. Mumbai, India) was used to culture the test bacteria and Potato Dextrose Agar was used to culture fungi. The microbial culture were grown at 37^0C for 8 hours and then appropriately diluted with sterile 0.8% saline solution. The concentration of test drugs was kept 200 µg/mL in DMF. Standard drugs Novobiocine, Gentamycin, Kanamycin, Amikacin (for antibacterial) and Ampicilline (for antifungal) were used for comparison. The antimicrobial activity was evaluated by measuring the zone of growth inhibition around disc of test organism **Table 3.**

Entomological activities

We have screened all synthesized compounds for their contact toxicity, stomach toxicity, Antifeedant activity and Acaricidal activity against *Spodoptera litura* .

Antifeedant activity

The antifeedant activity of these compounds was also carried out by leaf dip method [46-47], using fourth instars larvae of *Spodoptera litura*. The leaf discs of about 25 cm^2 were prepared and dipped for thirty seconds in solution of the test compounds. The leaf discs were air-dried to evaporate the excess acetone and offered for feeding. The insects were allowed to feed for 24 hrs. After 24 hrs leaf area uneaten was measured by using leaf area meter. The difference between leaf area provided and the leaf area uneaten is taken as amount of leaf area consumed. The feeding inhibition was calculated and used for calculation of effective concentration (EC_{50}/LD_{50}) using Maximum likelihood programmer (MLP) 3.01. The results of antifeedant activity are summarized in **Table 4**.

Acaricidal activity

The acaricidal activity of these compounds was carried out by leaf dip method [46-47]. Leaf discs of Mulberry (5 cm^2 diameter) were dipped in test solution for 30 seconds. Now air dried the leaf discs to remove the excess of acetone and placed over wet cotton in Petri plate. The adult female mites were released on treated leaf discs and mortality data were recorded after 48 hours. Mites released on leaf treated only with acetone and tween 20 emulsifier served as control. The mortality data was used for calculation of LC_{50}/LD_{50} using Maximum Likelihood Programmer (MLP) 3.01.The results of acaricidal activity are summarized in **Table 5.**

Antiproliferative activity

MTT Assay Method

The human breast cancer cell line (MCF-7) was co-incubated with the test compounds at 1µ g/ml doses for 96 hours and the all growth count was measured by MTT assay as described below [48-52]. 17β-estradiol and culture medium was kept as positive and negative control, respectively. The cell proliferation activity was carried out to estimate the effect of test compounds on the growth of tumor cells *in vitro*. A measurement of cell viability and proliferation forms the basis for this *in vitro* assay. The reduction of tetrazolium salt is now widely accepted as reliable way to examine cell proliferation.

Experimental

The yellow colored tetrazolium MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium-bromide] is reduced by metabolically active cells partially by the action of dehydrogenase enzymes to generate reducing equivalent such as NADH and NADPH. The resulting intracellular purple color zones was solubilized and quantified by spectrophotometer method. When metabolic events lead to necrosis or apoptosis in cell the MTT method measures the cell viability. The assay gives low background absorbance values in the absence or necrosis of the cells. The MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium-bromide] was dissolved in PBS at a concentration of 5 mg/ml. The 50 µl of the MTT solution was added to each well of the 96 well culture plate containing 100 µl culture medium and incubated at 37^0C for 4 hrs.

The medium was then removed carefully without disturbing the purple colored formazon crystals. Subsequently, 50 µl of Dimethyl Sulphoxide (DMSO) was added to each well and mixed thoroughly to dissolve the crystals of formazon. The plates were then read on a micro plate reader at a wavelength of 670 nm. The readings were presented as OD (optical density).

For observing cell proliferative activity on MCF-7 cell line, 17β-estradiol as positive control and culture medium is negative control were used. The results of antiproliferative activity of synthesized compounds were summarized in **Table 6** and **7**.

RESULTS AND DISCUSSION

Synthesis of title compounds by conventional methods suffer from long reaction time, moderate yields, tedious workup (Table 1), and requirement of large quantity of solvent associated with conventional method is another problem. A relatively more versatile yet simplified procedure was created, in which 2-amino-6- nitrobenzothiazole, aromatic aldehydes and active methylene compounds could react without using any solvent (without, as well as, with solid support). Microwave synthesis has received attention as new strategy for organic synthesis due to the fact that many reactions seem to proceed with much alacrity under such conditions as opposed to the corresponding thermal-assisted reaction [53]. The strategy worked well, affording the desired product in improved yields and in significantly lower reaction time (Tables 1 and 2). In microwave promoted reactions, solid supports like silica gel, alumina basic, alumina neutral and alumina acidic has been used, and it is found that the acidic alumina is the best solid support in the present investigation. The structures of all the synthesized compounds **4a**-**4l** were established on the basis of spectroscopic and analytical data. The elemental analysis (C, N and H) found for all the condensed products were in close agreement with the calculated values, the infrared (IR) spectrum of compounds **4** display two characteristic bands at 1622 and 1698 cm-1 due to C=C, and CO2 stretching, respectively. The ^{1}H nuclear magnetic resonance (NMR) spectrum of compounds **4** and its derivatives exhibit characteristic signals at δ 4.90-4.98 and δ 4.21 due to DHP proton and DHPCOOCH$_2$, respectively. Similarly the mass spectra of the 1,4 dihydropyridine derivatives revealed a molecular ion peak at m/z values corresponding to the molecular weight of the target compound. Thus on the basis of spectral data all the products **4a**-**4l** have been identified.

3.1. Antibacterial Activity

The antibacterial activity of all the synthesized compounds were tested *in-vitro* against pathogenic microbial strains, *Lactobacillus spp., Pseudomonas aeruginosa, Staphylococcus aureus, Micrococcus lutius* and *Kocuria rosea* and the results were compared with some standard drugs like novobiocine, gentamycin, kanamycin and amkacin. In case of *Lactobacillus spp*. Compounds **4a, 4b** and **4k** exhibit higher activity. In case of *Pseudomonas aeruginosa* compounds **4b, 4k** and **4a** show higher activity than the rest of the compounds. The inhibition of compounds **4a, 4b** and **4f** was higher in case of *Staphylococcus aureus* while rest of the were moderately active. In case of *Micrococcus lutius* and *Kocuria rosea* compounds **4b, 4a** and **4k** show higher efficacy than the rest of the compounds. The activity results clearly indicate that the compounds **4a**, **4b** and **4k** along with **4a**, **4f** show higher activity against the bacterial strains in comparison to the rest of the compounds. The presence of nitro groups in compounds plays an important role in activity, while in compounds presence ethoxy group, along with nitro group, justify the activity. It may be found that the nitro group present on the phenyl ring generally forms complexes with metaloenzymes, particularly those which are responsible in basic physiology such as cytochrome oxidase. These compounds may react with the peptidoglycan layer of the bacterial cell wall and damage it by penetrating in such a manner that the phenyl ring gets entered inside the cell by puncturing it, followed by bacterial cell death [54]. Sometimes

these compounds when present in low concentrations may cause bacteriostatic conditions which slow down the growth of bacteria.

3.2. Antifungal Activity

The antifungal activity of all these compounds were carried out against two pathogenic fungal strains *Aspergillus niger* and *Aspergillus candidus* using Ampicilin as a standard. It was found that compounds **4a, 4b, 4g** and **4j** show potent activity. Compound **4a** exhibit higher efficacy against both fungal strains. The higher efficacy of this compound may be due to the presence of nitrogen and sulphur content (containing a lone pair of electrons), presence of two methyl groups along with a nitro group on the phenyl ring. These compounds generally damage the fungal strains by puncturing the cell wall similar to the mechanism proposed for bacteria. The water and lipid solubility also increases the activity due to presence of polar groups in the molecules [55] **(Table 3)**.

3.3. Entomological Activity.

The newly synthesized compounds were also screened out for their entomological activity (antifeedant and acaricidal) against *Spodoptera litura* (an insect which damages the Indian agriculture crops) and *Tetranychus urticae* of mites (damage house goods) respectively.

3.3.1. Antifeedant activity.

The Antifeedant activity of the newly synthesized compounds was tested by a leaf dip method against larvae of *Spodoptera litura*. The results clearly indicate that the compounds show higher, moderate and less antifeedant activity against the larvae of the insect. Compounds **4g**, **4k**, **4h** and **4l** show higher activity, compounds **4c**, **4i**, **4j**, **4d** show moderate activity while the rest of the compounds exhibit lower activity as seen by their LC_{50}/LD_{50} results. The results clearly show that the presence of nitro and methyl groups on the aromatic ring enhance the activity. The presence of $N(Me)_2$ group as R_3 in the side ring also plays an important role in activity. It may be found that these compounds may cause a spasm condition in insects by interacting with the active site of the enzyme responsible for nervous breakdown in insects [56] **(Table 4).**

3.3.2. Acaricidal Activity.

The acaricidal activity of these compounds was performed by the same method, as in the case of antifeedant activity, against *Tetranychus urticae*, a species of mite using acetone as a standard. The results obtained clearly show that compound **4e** shows the highest acaricidal activity with respect to the other compounds. The higher activity of this compound is due to the presence of three polar groups in the molecules which enhance the water and lipid solubility of this compound. It is reported that the compounds which are easily soluble in polar solvent have higher activity against microbes and insect pests or mites [57]. Compounds **4j**, **4i**, **4f** and **4k** shows moderate activity and the rest of the compounds show lower to moderate activity against the mites. Besides solubility, the presence of a lone pair of electrons on the nitrogen and sulphur along with the phenyl ring, having varying side groups, may also be responsible for variation in activity **(Table5).**

Table 2. Comparitative study of synthesized compounds (4a- l) under solid supported Microwave irradiation.

Compounds	Solid supported microwave irradiation							
	Silica		Alumina basic		Alumina neutral		Alumina acidic	
	Time (Sec.)	Yield (%)	Time (Sec.)	Yield (%)	Time (Sec.)	Yield (%)	Time (Sec.)	Yield (%)
4a	230	63	280	55	130	85	60	97
4b	210	60	240	55	130	82	65	90
4c	190	58	230	45	140	80	55	89
4d	230	60	280	50	130	78	55	87
4e	220	60	270	55	120	80	85	91
4f	200	67	260	48	120	85	75	93
4g	190	60	240	55	110	89	65	87
4h	220	70	280	45	110	82	65	89
4i	210	56	270	50	100	80	75	90
4j	190	62	240	45	120	81	85	91
4k	200	60	220	45	130	81	95	94
4l	190	58	230	50	120	83	65	93

Table 3. Antimicrobial activities of compounds 4a-4l.

	Antibacterial activity[a]					Antifungal activity[a]	
Compounds	**B1**	**B2**	**B3**	**B4**	**B5**	**F1**	**F2**
4a	17.0	21.0	20.0	25.0	20.0	20.0	22.0
4b	18.0	24.0	19.0	27.0	22.0	22.0	24.0
4c	12.0	18.0	17.0	21.0	17.0	16.0	17.0
4d	9.0	14.0	13.0	15.0	14.0	15.0.	14.0
4e	8.0	12.0	12.0	14.0	12.0	11.0	13.0
4f	12.0	19.0	18.0	21.0	17.0	14.0	16.0
4g	11.0	16.0	17.0	18.0	16.0	18.0	18.0
4h	15.0	20.0	19.0	23.0	18.0	12.0	18.0
4i	10.0	18.0	17.0	16.0	15.0	16.0	16.0
4j	10.0	15.0	14.0	16.0	15.0	18.0	20.0
4k	16.0	23.0	18.0	24.0	19.0	13.0	19.0
4l	9.0	13.0	16.0	15.0	14.0	6.0	20.0
Benzothiazole	2.0	6.0	5.0	3.0	1.0	6.0	5.0
Blank	0.0	0.0	10.0	0.0	0.0	0.0	0.0
Novobiocine	7.0	20.0	22.0	3.5	22.0	-	-
Gentamycin	19.0	24.0	18.0	35.0	24.0	-	-
Kanamycin	5.0	11.0	18.0	25.0	22.0	-	-
Amikacin	16.0	25.0	18.0	32.0	24.0	-	-
Ampicilline	-	-	-	-	-	30.0	32.0

[a] *Data represent zones of inhibition (mm)*
B1 *= Lactobacillus sp.* ***B2*** *= Pseudomonas aeruginosa* ***B3*** *= Staphylococcus aureus* ***B4*** *= Micrococcus leutius* ***B5*** *= Kocuria rosea* ***F1*** *= Aspergillus candidus* ***F2*** *= Aspergillus niger.*

Table 4. Antifeedant activity of compounds 4a-4l.

Compounds	Fiducial Limits	Slop ±	Chi. Sq. (3)	LC_{50}/LD_{50} At 24 hrs.
4a	0.82–3.45	0.82±0.10	0.46 (3)	1.38
4b	0.67–1.72	1.04±0.11	0.68 (3)	1.00
4c	0.44–0.86	1.05±0.10	0.37 (3)	0.60
4d	0.62–1.43	1.08±0.13	1.06 (3)	0.88
4e	0.87–2.36	1.09±0.12	0.80 (3)	1.26
4f	0.72–2.45	0.96±0.13	0.24 (3)	1.17
4g	0.34–0.50	1.29±0.14	3.42 (3)	0.40
4h	0.36–0.67	1.02±0.11	0.70 (3)	0.45
4i	0.45–1.08	0.88±0.10	1.72 (3)	0.67
4j	0.49–0.80	1.52±0.13	2.59 (3)	0.60
4k	0.31–0.53	1.03±0.11	5.37 (3)	0.42
4l	0.27–0.58	0.98±0.12	0.28 (3)	0.45

Table 5. Acaricidal activity of compounds 4a-4l.

Compounds	Fiducial Limits	Slop ±	Chi. Sq. (3)	LC_{50}/LD_{50} At 24 hrs.
4a	0.14–0.35	0.78±0.86	1.74 (3)	0.19
4b	0.15–0.27	0.90±0.06	2.15 (3)	0.19
4c	0.19–0.39	0.10±0.07	8.29 (3)	0.24
4d	0.16–0.34	0.12±0.07	7.55 (3)	0.26
4e	0.09–0.14	1.30±0.09	14.30 (3)	0.10
4f	0.12–0.28	0.72±0.05	6.17(3)	0.18
4g	0.16–0.40	0.98±0.09	6.98 (3)	0.16
4h	0.36–1.92	0.70±0.06	3.60 (3)	0.75
4i	0.10–0.29	0.70±0.05	8.54 (3)	0.17
4j	0.15–0.36	0.85±0.08	1.76 (3)	0.22
4k	0.09–0.23	0.88±0.70	9.18 (3)	0.17
4l	0.08–0.12	0.80±0.07	15. 85 (3)	0.48

Table 6 Antiproliferative activity data of N-(6-nitrobenzothiazolyl)-2,3,5,6-tetrasubstituted-4-(aryl)-1,4-dihydropyridines against human hepatoma cell line (hepatic cancer).

Compounds	Cell X 10^4	Activity	Cell line
4a	8.52±0.66	+	Hep-G-2
4b	9.17±0.70	+	Hep-G-2
4c	8.97±0.82	+	Hep-G-2
4d	7.35±0.66	+	Hep-G-2
4e	7.68±0.34	+	Hep-G-2
4f	9.36±0.76	+	Hep-G-2
4g	9.58±0.60	++	Hep-G-2
4h	8.62±0.80	+	Hep-G-2
4i	8.87±0.44	+	Hep-G-2
Control	11.21±1.01	–	Hep-G-2

Table 7 Antiproliferative activity data of N-(6-substitutedbenzothiazolyl)-2,3,5,6-tetrasubstituted-4-(aryl)-1,4-dihydropyridines against human breast adinocarcinoma cell line (breast cancer).

Compounds	Cell X 10^4	Activity	Cell line
4a	10.08±0.94	-	MCF-7
4b	9.24±0.64	+	MCF-7
4c	8.32±0.92	+	MCF-7
4d	9.24±1.05	+	MCF-7
4e	8.17±0.90	++	MCF-7
4f	9.21±0.26	+	MCF-7
4g	9.13±0.72	+	MCF-7
4h	9.29±0.78	+	MCF-7
4i	9.17±0.80	+	MCF-7
Control -ve	10.08±1.01	–	MCF-7
Control +ve	40.26±3.23	–	MCF-7

CONCLUSION

We have developed an economical, solvent free, very efficient microwave assisted protocol for the synthesis of N-(6-nitrobenzothiazolyl)-2,3,5,6-tetrasubstituted-4-(aryl)-1,4-dihydropyridines which can be a viable alternative to their conventional synthesis. The synthetic protocol has the inherent potential for future drug synthesis. The 1,4-dihydropyridine derivatives (**4a**-**4l**) showed potent antimicrobial activity against various bacterial and fungal strains. The maximum antibacterial and antifungal activity was exhibited by **4a**, **4b** and **4k**. The presence of substituent on the phenyl ring along with a variation in solubility plays a significant role in determining the antimicrobial activity of the compounds in comparison to other substituents [58-60]. The compounds also show potent antifeedant and acaricidal activity against *Spodoptera litura* and *Tetranychus urticae*, respectively, where solubility of the compounds plays a significant role [61]. From the results, it is clear that these compounds would be better used in drug development to combat bacterial and fungal infections, and would be better used as pesticides in the future as well.

MTT assay method used for Antiproliferative activity against Human hepatoma cell line (hepatic cancer) and Human breast adinocarcinoma cell line (breast cancer) and their results were summarized in **Table 6 and 7**. Transporting a drug selectively to the malignant cells without damaging the cells of the host organism is the desirable goal in the drug design for anticancer therapy. This task is made difficult because the morphological and biochemical differences between the malignant and normal cells are often minute. However, It is evident that all compounds are slightly to moderately active against these cell lines. These results prompted us that change in substitution pattern in N-Benzothiazolyl-1,4-dihydropyridine derivatives may cause marked effect on their antiproliferative activity.

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