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Synthesis of Novel and Diverse 1, 4-dihydropyridine Analogues and their Antimicrobial and Anticancer Activities

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ABSTRACT

Novel diverse 1, 4-dihydropyridine analogues were prepared from cyclization method. Synthesized compounds were characterized from IR, ^{1}H-NMR, ^{13}C-NMR, mass spectral, elemental analysis and mass spectral fragmentation method. The reaction was performed using ordinary condensation type, which enabled to easy work-up and good yield. Synthesized compounds (1-4) were screened for antimicrobial activity. Among these compounds (4) (MIC:8 μg mL^{-1}) has highly antibacterial activity against *E. coli* compared with standard Ciprofloxacin and compound (4) (MIC: 4 μg mL^{-1}) has highly antifungal active against *C. albicans* compared with Clotrimazole.The synthesized compounds have been screened for preliminary anti-cancer activity against HepG2 (Liver), Hela (Cervical) and MCF-7 (Breast) cancer cells. The compound (4) is highly active against HepG2, MCF-7 and Hela (Cervical) and these have been selected for advanced preclinical development. Activity has been compared with standard drug. Structure Activity Relationship (SAR) has also been discussed in this study.

Key words: 1, 4-dihydropyridine derivatives, oxadiazole ring, triazole ring, cyclization method, antimicrobial activity, anti-cancer activity

INTRODUCTION

Development of multidrug resistance is a major therapeutic obstacle in chemotherapy of cancer (Davis and Davis, 1979). Circumvention of the multidrug resistance is thus a critical step to improve cancer chemotherapy. Calcium channel blockers like dexniguldipine, nicardipine, manidipine, pranidipine, efonidipine, benidipine, barnidipine, azelnidipine, azelnidipine (Fig. 1) and others have been reported to successfully overcome drug resistance *in vitro* and *in vivo* (Tsuruo *et al*., 1983; Kessel and Wilberding, 1985). The 1, 4-dihydropyridine (DHP) derivatives known as calcium channel antagonists, are used for various treatment such as antihypertensive (Wenzel *et al*., 2000) anticonvulsant (Kumar *et al*., 2010) analgesic activity (Agudoawu *et al*., 1999) and however, mightpose a therapeutic problem because of their strong vasodilator activity (Honda *et al*., 1983; Cairo *et al*., 1989). Consequently a substance which has strong ability in overcoming anticancer drug resistance but no calcium antagonistic activity would be of value in cancer chemotherapy.

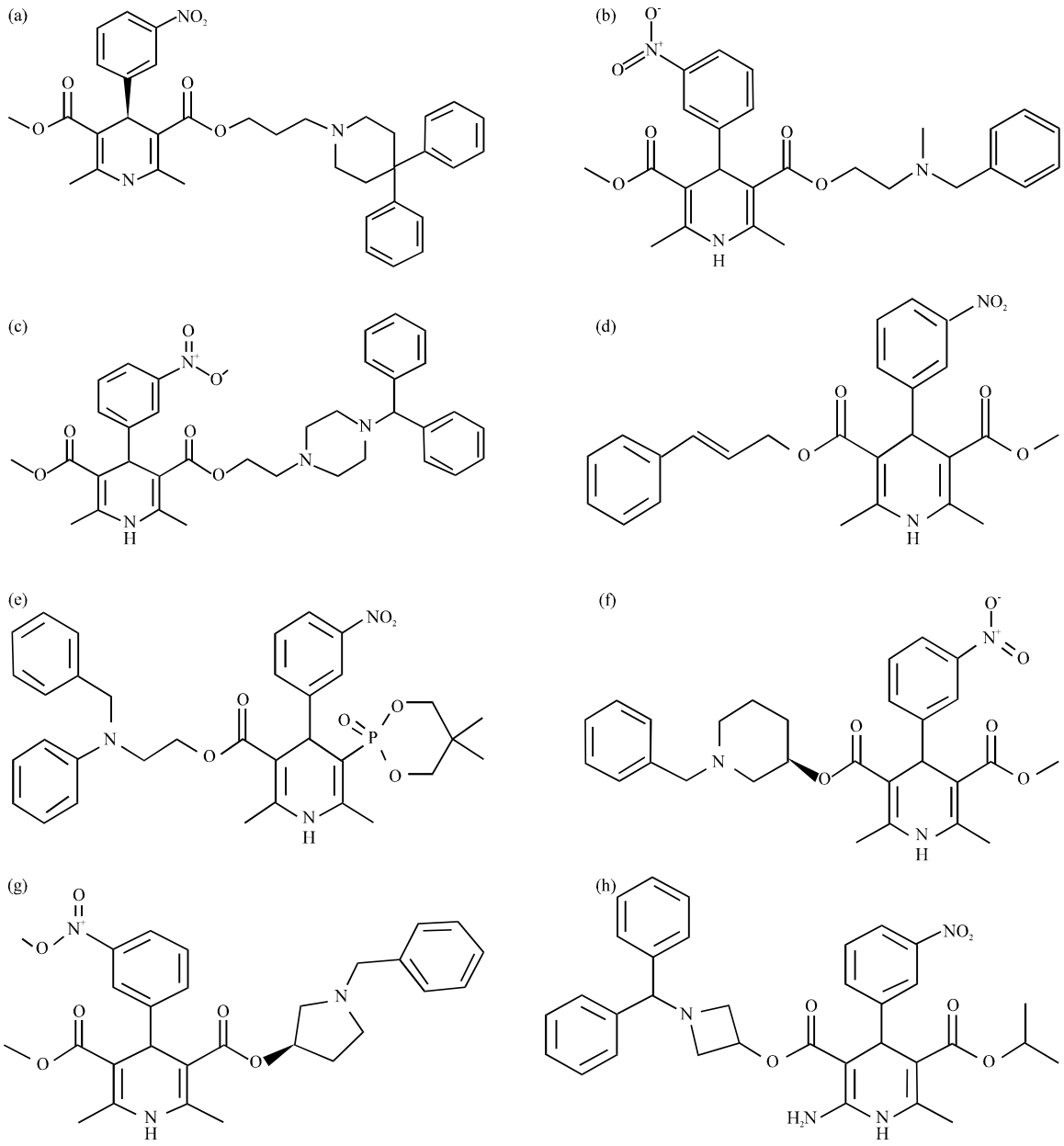

Fig. 1(a-h): Various mustidruge calcium channel blockers, (a) Dexniguldipine, (b) Nicardipine, (c) Manidipine, (d) Pranidipine, (e) Efonidipine, (f) Benidipine, (g) Barnidipine and (h) Azelnidipin

The 1, 3, 4-oxadiazoles and 1, 2, 4-triazoleare display a broad spectrum of biological activities (Girges, 1994) and in particularly those incorporating the N-C-S linkage as in the skeleton exhibit a broad spectrum of antimicrobial activity (Holla *et al*., 2006).

We have recently reported (Kumar *et al*., 2011a) that some multicompound 1, 4-dihydro pyridine derivatives with anti cancer aganist human cancer cell line (MCF-7, Hela and Hep) and antimicrobial activity (Kumar *et al*., 2011b). In this study, we have reported newly synthesized

multi compound 1, 4-dihydropyridine connected with 1, 3, 4-oxadiazoles and 1, 2, 4-triazole derivatives for improve the ability of anticancer and antimicrobal effects and with specified structural activity features with corresponding bilogical screnning.

MATERIALS AND METHODS

Chemistry: Melting points were recorded in open capillary tubes and were uncorrected. The IR spectra (Kbr) was recorded in KBr on a Shimadzu 8201pc (4000-400 cm^{-1}). The ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker DRX-300 MHZ. The elemental analysis (C, H and N) were recorded using an elementer analyzer model (Varian EL III). The purity of the compounds was checked by Thin Layer Chromatography (TLC) with silica gel plates.

4-(furan-2-yl)-2, 6-dimethyl-1, 4-dihydropyridine-3, 5-dicarbohydrazide) (Compound 2): A mixture of compound (1) (0.01 mol, 3.19 g) and hydrazine hydrate (99.99%, 0.02 mol, 0.8 mL) in methanol, it was heated under reflux for 8 h and poured into crushed ice , the obtained solid was filtered, washed with cooled water and purified by ethanol. The progress of reaction was monitored by TLC.

5, 5'-[4-(furan-2-yl)-2, 6-dimethyl-1, 4-dihydropyridine-3, 5-diyl] bis (1, 3, 4-oxadiazole-2-thiol) (Compound 3): A mixture of compound (2) (0.1 mol, 2.9 g), carbon disulphide (0.02 mol, 1.2 mL) and potassium hydroxide (0.02 mol, 1.12 g) in methanol (30 mL), it was heated under reflux for hot water bath for 8 h. On completion of the reaction it was triturated with ether and the obtained solid was filtered and recrystallised by methanol. The progress of reaction was monitored by TLC.

5, 5'-[4-(furan-2-yl)-2, 6-dimethyl-1, 4-dihydropyridine-3, 5-diyl] bis(4-amino-4H-1, 2, 4-triazole-3-thiol) (Compound 4): A mixture of compound (3) (0.01 mol, 3.75 g) and hydrazine hydrate (0.02 mol, 0.8 mL) in methanol, it was heated under reflux on water bath for 7 h. It was cooled to room temperature and poured into crushed ice, the obtained solid was filtered, dried and purified by using ethanol. The progress of reaction was monitored by TLC.

IR (cm^{-1}): The 3347.07 (NH), 3241.81 (NH_2), 1487.07 (C = N), 2781.79 (furyl CH-str), 922.07 (CH), 2982.17 (SH) ^{1}H-NMR (300MHz, DMSO-d_6): δ = 4.58 (s, 1H, 4-CH), 2.05 (s, 3H, 2-CH_3), 2.18 (s, 3H, 6-CH_3), 8.58 (s, 1H,NH), 6.45-6.49 (d,3H,furyl), 5.67 (s, 4H, 3,5 triazole-NH_2), 12.85 (s, 2H, 3, 5-SH) ^{13}C-NMR (300 MHz, DMSO-d_6): 166.66 (2×C SH), 148.11 (C_5-C), 151.86-141.13 (Furyl), 130.77 (2, 6-C-CH_3), 110.36 (3, 5-C-C), 44.03 (4C), 13.94 (2, 6-C-CH_3) MS: (EI) m/z 404.01 (M^+ + 1, 13%), 339.35 (14.21%), 309.32 (12.02%), 283.31 (28.26%), 229.35 (10.02%), 203.28 (100%), 175.22 (30.54%), 147.17 (70%).

Biological evaluation:

***In vitro* antibacterial screening:** The compounds (1-4) were evaluated for their *in vitro* antibacterial activity against *Escherichia coli* (MTCC-739), *Pseudomonas aeruginosa* (MTCC-2435), *Micrococcus luteus* (MTCC-106), *Enterococcus feacalis*, *Streptococcus epidermidis*, *Bacillus* spp, *Klebsiella pneumoniae* (recultured) and *Staphylococcus aureus* (MTCC-96) by disc diffusion method (Bauer *et al.*, 1966) was performed using Mueller-Hinton agar (Hi-Media) medium. Ciprofloxacin was used as a standard. Each compound was tested at concentration 100 μg mL^{-1} in DMSO. The zone of inhibition (mm) was measured after 24 h incubation at 37°C.

***In vitro* antifungal screening:** The compounds (1-4) were evaluated for their *in vitro* antifungal activity such as *Aspergillus niger*, *Candia albicans*, *Microsporum audouinii* and *Cryptococcus neoformans* (recultured) using disc diffusion method (Verma *et al*., 1998) with sabouraud's dextrose agar (Hi-Media). Clotrimazole was used as a standard. Each compound was tested at a concentration of 100 μg mL^{-1} in DMSO. The zone of inhibition (mm) was measured incubated at 37°C. Compounds, Ciprofloxacin and Clotrimazole dissolved in dimethylsulphoxide at concentration of 120 μg mL^{-1}. The twofold dilutions of the solution were prepared (64, 32 ...., 0.5 μg mL^{-1}). The microorganism suspensions at 106 CFU mL^{-1} (Colony Forming Unit/mL) concentrations were inoculated to the corresponding wells. The plates were incubated at 36°C at 24 h. The Minimum Inhibitory Concentration (MIC) was noted by observing the lowest concentration of the drug at which there was no visible growth.

Anti-cancer activity: The newly synthesized compounds (1-4) were screened for their anticancer activity according to the procedure suggested (Scudiero *et al*., 1988). Compounds (1-4) were submitted for the three celllines with one dose of primary anticancer assay with a concentration of 100 μm for 48 h (MTT anticancer assay). The three cell lines used in the present investigation were HepG2 (Liver), Hela (Cervical) and MCF-7 (Breast). In this current protocol, each cell line was pre-incubated on microtiter plate. There sults for each test are reported as percentage of the growth of the treated cells when compared to the untreated control cells. The compounds that reduce the growth any one of the cell lines to approximately 32% or less were evaluated as having anti tumor activity. The 0.1 mL of the cell suspension (containing 5×10^6 cells/100 μL) and 0.1 mL of the test solution (6.25-100 μg 1% DMSO such that the final concentration of DMSO in media was less than 1%) were added to the 27 well plates and kept in a 5% CO_2 incubator at 37°C for 72 h. The blank contained only cell suspension and control wells contained 1% DMSO and cell suspension. After 72 h, 20 μL of MTT was added and kept in the CO_2 incubator for 2 h followed by addition of 100 μL propanol. The plate was covered with aluminum foil to protect it from light. Then the 27 well plates were kept in arotary shaker for 10-20 min. After 10-20 min, the 27 well plates were processed on an ELISA reader for absorption at 562 nm.

RESULTS AND DISCUSSION

Chemistry: The analytical data are presented in Table 1. The compound (diethyl 4-(furan-2-yl)-2, 6-dimethyl-1, 4-dihydropyridine-3, 5-dicarboxylate) (1) was synthesized from Hantzsch method (Kumar *et al*., 2011a), compound (2) (4-(furan-2-yl)-2, 6-dimethyl-1,4-dihydro pyridine-3, 5-dicarbohydrazide) was prepared from compound (1) reacted with hydrazine hydrate by hydrazinolysis method (Fig. 2). Compound (3) (5, 5'-[4-(furan-2-yl)-2, 6-dimethyl-1, 4-dihydropyridine-3, 5-diyl] bis (1, 3, 4-oxadiazole-2-thiol) prepared from compound (2) reacted

Table 1: Physicochemical data of compounds (1-4)

					Elemental analysis calculated (Found)			
Compounds	Yield (%)	M.P (°C)	M.W	M.F	C	H	N	S
1	57	158	319.35	$C_{17}H_{21}NO_5$	63.94 (63.91)	63.94 (63.91)	4.31 (4.410)	-
2	55	168	291.30	$C_{13}H_{17}N_5O_3$	53.60 (53.62)	5.58 (5.530)	24.04 (24.01)	-
3	47	171	375.42	$C_{15}H_{13}N_5OS_2$	49.09 (49.04)	4.38 (4.400)	17.89 (17.94)	16.38 (16.41)
4a	45	200	403.48	$C_{15}H_{17}N_9OS_2$	44.65 (44.68)	4.25 (4.280)	31.24 (31.27)	15.89 (15.94)

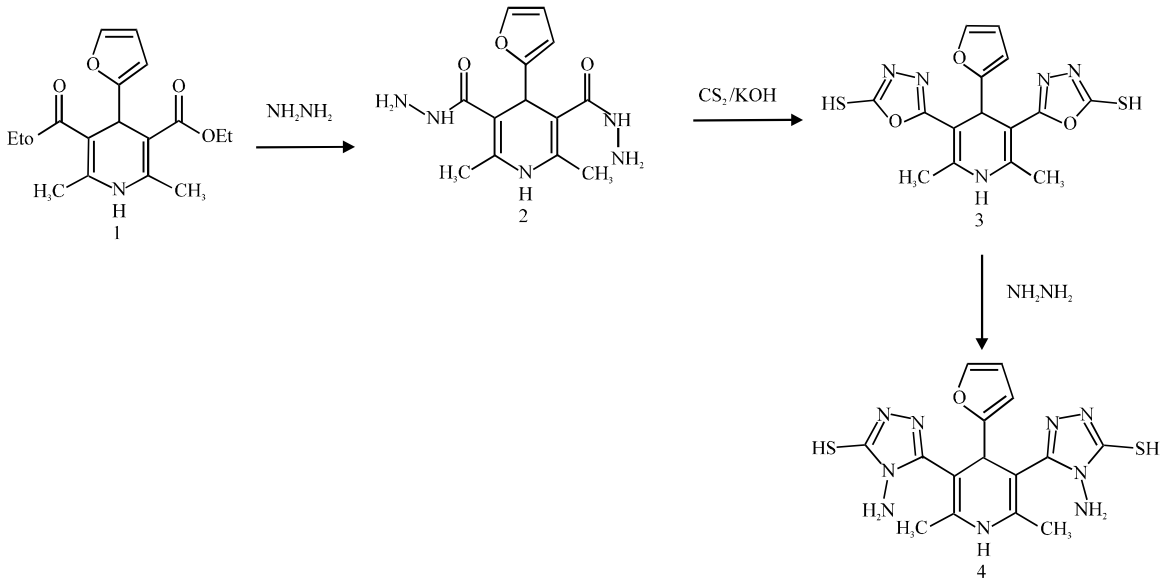

Fig. 2: Synthetic route of compounds (1-4)

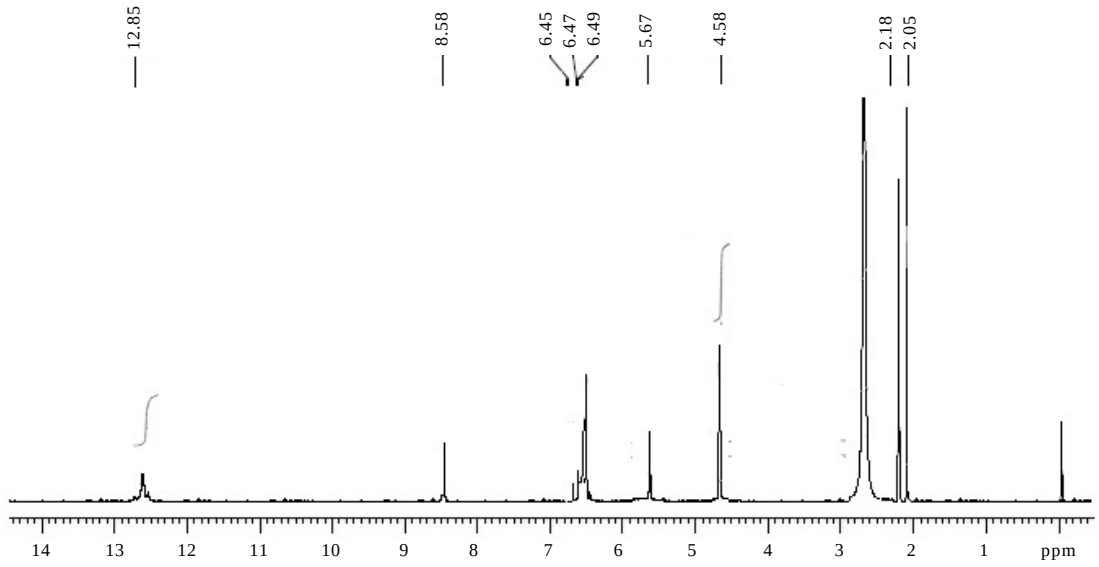

Fig. 3: ^{1}H NMR spectrum of compound 4

with CS_2 and KOH by cyclocondensation method. The compounds (1-4) were synthesized by the method described in literature (Hadizadeh *et al*., 2002; Aydogan *et al*., 2002). The IR spectra of the compound (4) shows that absorption bands at 2781.79 and 2982.17 cm^{-1} corresponding to furyl C-H and -SH, respectively. The ^{1}H NMR spectrum of compound (4) shows that signals at δ 4.58, 8.58 and 12.85 corresponding to the 3, 5-NH_2, NH and SH protons, respectively. ^{1}H-NMR spectrum of compound (4) showed in Fig. 3. The ^{13}C NMR spectrum of compound (4) shows that peaks at δ 166.66 and 44.6 corresponding to the C-SH and 4C carbons, respectively. ^{13}C-NMR spectrum of

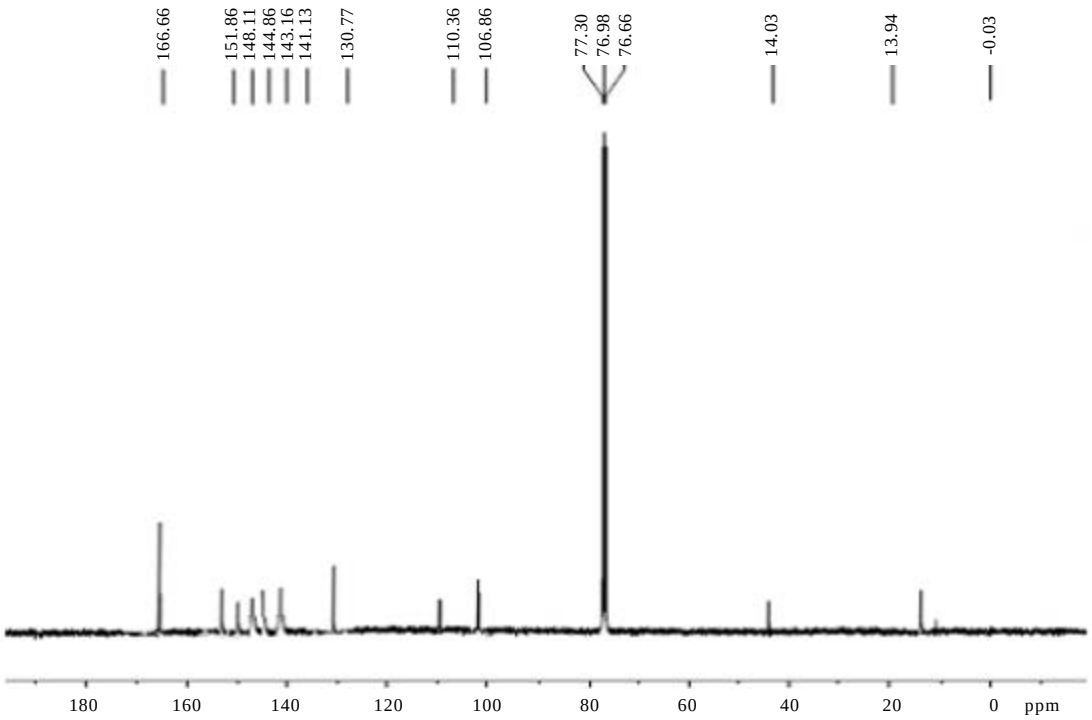

Fig. 4: ^{13}C NMR spectrum of compound 4

Table 2: Antibacterial activity of compounds (1-4)

Compounds	*E. coli* (MTCC-739)	*P. aeruginosa* (MTCC-24350)	*M. luteus* (MTCC-106)	*S. aureus* (MTCC-2940)
1	15	26	10	08
2	14	21	12	-
3	8	15	9	09
4	20	12	-	-
Standard	18	27	20	16

Zone of inhibition was measured in mm at concentration of 100 μg mL^{-1}, Ciprofloxacin was used as the standard

compound (4) showed in Fig. 4. The mass spectrum (EI) of the compound (4) shows that molecular ion peak at m/z 404.01 (M^+ + 1) and 13% relative abundance, corresponding to the molecular weight of the compound (4). Mass spectra and fragmentation of compound (4) showed in Fig. 5 and 6.

Antibacterial activity: The compounds (1-4) were screened for antibacterial activity. The synthesized compound (4) (MIC: 8 μg mL^{-1}) is highly active than the standard (Ciprofloxacin MIC: 16 μg mL^{-1}) against *E. coli*, the compound (1) has equipotent activity against *P. aeruginosa* stain compared to standard ciprofloxacin (MIC: 0.5 μg mL^{-1}). Other compounds are significantly active at concentration 100 μg mL^{-1} the zones of inhibition (mm) values are summarized in Table 2 and 3.

Antifungal activity: The compounds (1-4) were screened for the antifungal activity. The compound 4 (MIC: 4 μg mL^{-1}) is highly active compared with standard

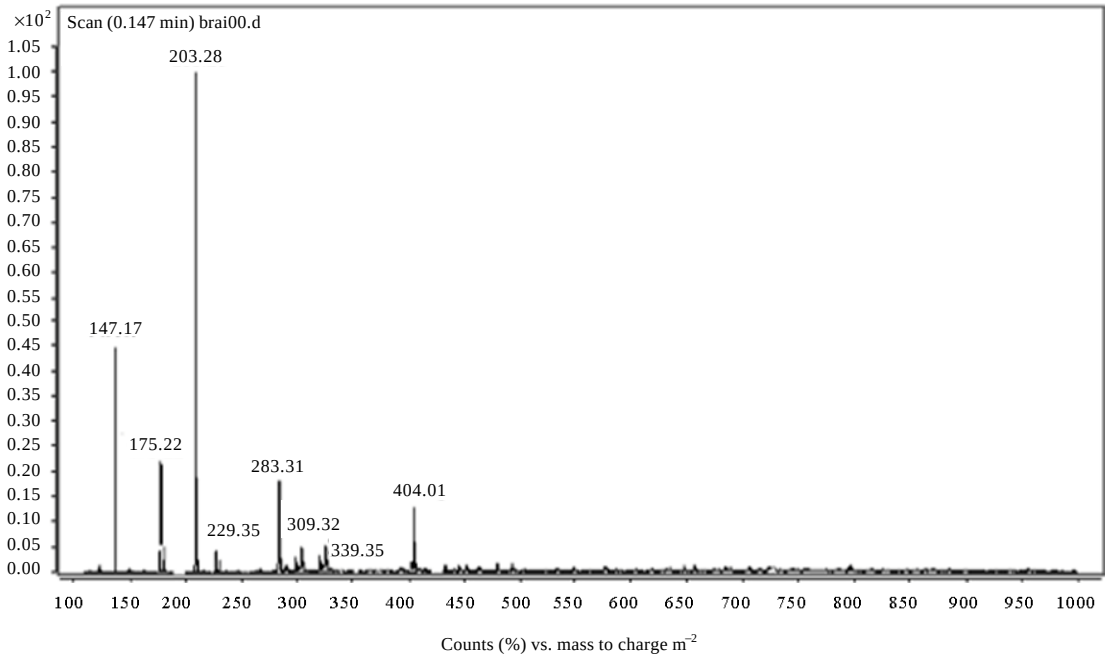

Fig. 5: Mass spectrum of compound 4

Table 3: Minimum inhibitory concentrations (MIC, μg mL^{-1}) of compounds (1-4)

	Minimam Inhibitory Concentration (MIC, μg mL^{-1})[a]							
	Antibacterial activity				Antifungal activity			
Compounds no.	*E. coli*	*P. aeruginose*	*M. luteus*	*S. aureus*	*A. niger*	*C. albicans*	*C. neoformans*	*M. audouinii*
1	32	0.5	62	62	-	62	32	16
2	32	1.0	32	-	32	-	62	-
3	62	32.0	62	62	-	62	-	32
4	8	62.0	-	-	-	4	8	8
Ciprofloxacin	16	0.5	8	32	-	-	-	-
Clotrimazole	-	-	-	-	4	8	16	16

Table 4: Antifungal activity of compounds (1-4)

Compounds	*A. niger* (ATCC 16404)	*C. albicans* (ATCC 10231)	*C. neoformans* (ATCC 24067)	*M. audouinii* (ATCC 10008)
1	-	8	12	18
2	10	-	9	-
3	-	14	-	17
4	-	20	16	19
Standard	22	18	15	16

Zone of inhibition was measured in mm at concentration of 100 μg mL^{-1}, Clotrimazole was used as the standard

(Clotrimazole MIC: 8 μg mL^{-1}) against *C. albicans* and highly activie agaist *C. neoformans* and *M. audouinii* fungal strain also. The fungal zones of inhibition (mm) values are summarized in Table 3 and 4.

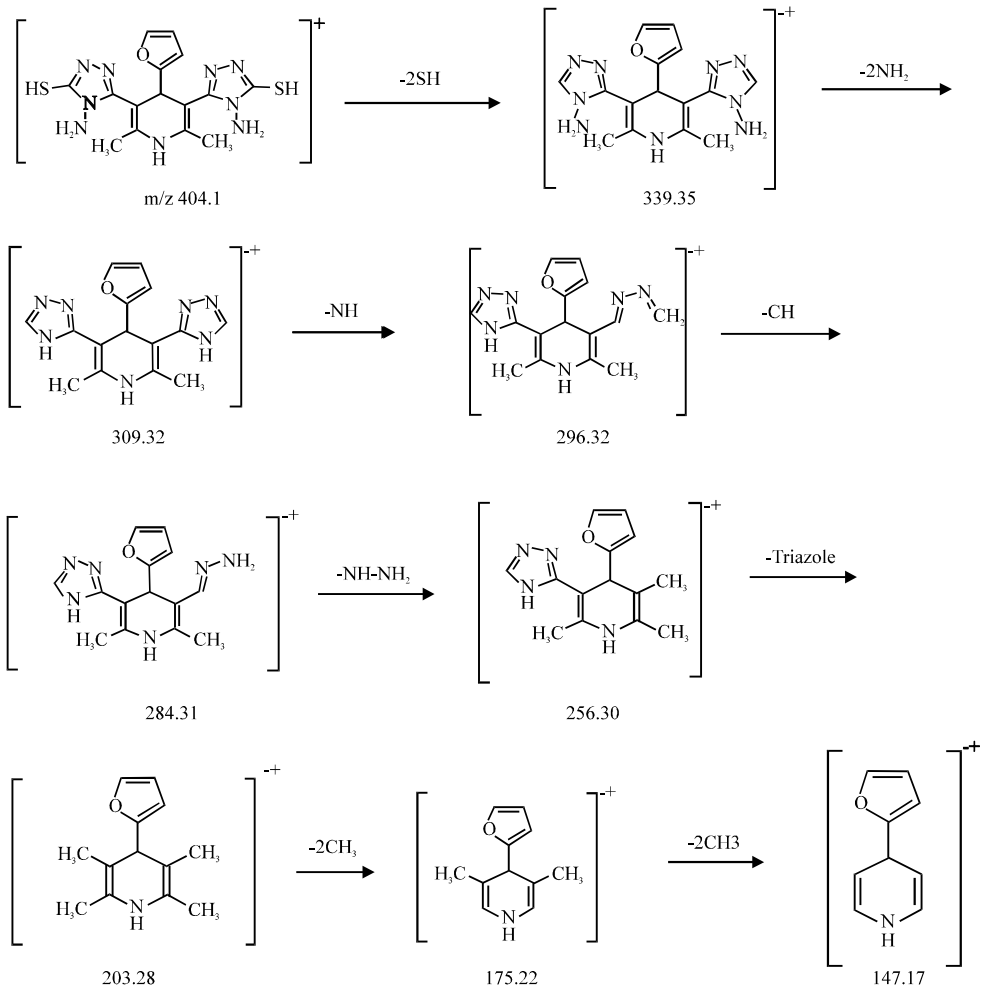

Fig. 6: Mass spectral fragmentation of compound 4

Table 5: Anticnacer activity of compounds (1-4)

	HepG2			MCF-7			HeLa		
Compounds	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}
1	16.2	29.1	88.3	22.9	46.8	>100.0	22.6	48.4	>100.0
2	15.3	24.8	81.2	20.1	45.1	>100.0	21.0	47.2	>100.0
3	10.2	20.1	64.1	14.1	40.1	87.2	15.2	38.1	74.1
4	6.3	15.3	51.2	5.2	20.1	71.2	8.1	17.1	65.3

Anticancer: Compounds (1-4) were found to be active in the preliminary anti-cancer screening studies. The compounds were tested against the three cell lines of liver, cervical, breast cancer types. Their GI_{50}, TGI and LC_{50} values were determined. The result of the screening was expressed in terms of GI_{50} growth inhibitor concentration. Table 5 shows that the compound (4) has highly active against HepG2 (liver) , Hela and MCF7 cancer cell line for the reason that low Growth of inhibition (GI_{50}) at 6.2, 5.2 and 8.1 μm compared to other compounds (1, 2 and 3).

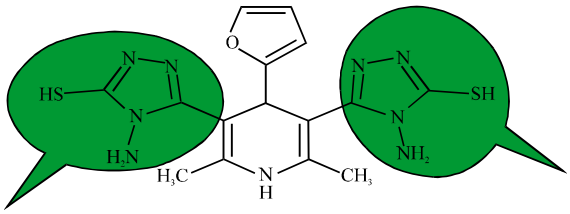

Fig. 7: Structure activity relationships

Structure activity relationship: From the results of antimicrobial and anticancer activities, we discussed structure activity relationships (Fig. 7).

The compound (4) is highly active against *E. coli* (MIC, 8 μg mL^{-1}) due to presence of triazole ring connecting with 1, 4-dihydropyridine ring. The compound (4) is highly active against *P. aeruginosa* (MIC, 0.5 μg mL^{-1}) due to presence of triazole ring connecting with 1, 4-dihydropyridine ring. The compound (4) is highly active against *C. albicans* (MIC, 4 μg mL^{-1}) due to presence of triazole ring connecting with 1, 4-dihydropyridine ring. Compounds (1-4) responded by anticancer activity also, activity range measured from Total Growth Inhibition (TGI), which is represented by Hep G2 (Liver), Hela (Cervical) and MCF-7 cancer cell lines. Anticancer activity of the compound (4) shows Total Growth Inhibition (TGI) reached at 15.3, 20.1 and 17.1 μm corresponding to HepG2 (Liver), Hela (Cervical) and MCF-7 cancer cell lines due to presence of triazole ring connecting with 1, 4-dihydropyridine ring.

CONCLUSION

This study describes by new 1, 4-dihydropyridine with triazole derivatives synthesized from cyclization method. Antimicrobial activity of compounds (1-4) out of the compound (4) exert potent antibacterial and antifungal activity, this compound may possible be used as lead compounds for developing new antimicrobial agent. The methodology was previously reported, but target molecules are new, by its use a wide variety of couplet two heterocyclic compounds could be reached in matter of days and its could be used screening for biological activities. The compound (4) has highly active aganist HepG2 (Liver), Hela (Cervical) and MCF-7 cell line. Therefore, we founded some important detail about biological properties of triazole with 1, 4-dihydropyridine derivatives (1-4), this compounds could be beneficial for anticancer drug synthesis.

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