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SYNTHESIS AND ANTI-MICROBIAL AGENTS OF NOVEL (E)-N'-4, 4'-DIFLUORO-CYCLOHEXANECARBOXYLIC ACID (SUBSTITUTED-BENZYLIDENE)-HYDRAZIDE DERIVATIVES

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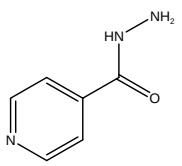
ABSTRACT

A series of (E)-N'-(substituted-benzylidene)cyclohexanehydrazide derivatives were synthesized by coupling of 4,4'-diflouro cyclohexane carboxylic acid hydrazide with different aromatic aldehydes in the presence of ammonium acetate, acetic acid in catalytic amount, methanol as solvent at room temperature. All the synthesized compounds were confirmed and characterized by using various spectral technique like IR, H1NMR, C13NMR, and mass spectral studies. All the synthesized hydrazone derivatives were screened for anti-bacterial activities by disc diffusion methodagainst *Staphylococcusareus,Bacillusmegaterium (Gram+ve);E.coli and Pseudomonasaeruginosa(Gram-ve)bacrerial strains,*andanti-fungalactivity against Candidaalbicans, Rizopusmicrosporus var. oligosporus. The activity studies of all the synthesized compounds against bacterial strains in both gram positive and gram negative activity against bacterial strains revealed that the compounds 5a,5c,5d,5f,5g,5h,5i,5j,5k,5l and 5oshows moderate *activity against Pseudomonas aeruginosa MTCC No. 1034compared with standard pencillin.off all the above compounds 5g and 5k have highest activity compared to all the synthesized compounds in gram negative in* 100µg/ml/mm concentration. Activity against Fungi *Rizopusmicrosporus var. oligosporus* (MTCC No: 2785) revealed that the compounds 5a,5h,5j and 5n have highest activity compared to all the synthesized compounds in 100µg/ml/mm concentration. This study proved that 4,4'difluoro cyclohexane hydrazone substituted benzilidenes synthesized by condencing 4,4'-diflouro cyclohexane carboxylic acid hydrazide with different aromatic aldehydes displayed moderate to potent activity.

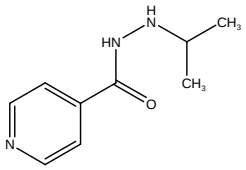
KEYWORDS: N-substituted benzylidene derivatives, cyclohexane hydrazide, anti bacterial activity, anti fungal activity.

INTRODUCTION

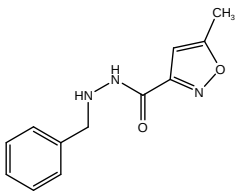
Hydrazones have wide interest because of their diverse biological applications. Hydrazones possess various biological activities like antimicrobial,[1-18] antitumor,[19-21] antiviral, anti-hypertensive, anti-convulsant, anti-inflammatory, antidepressant, analgesic, antiplatelets, vasodilator, anti coagulant ,anti- tubercular, anti-HIV, analthamintic, antidiabatic,trypanocidal and protozoal activities(Ali et al., 2012:Kumar et al., 2010).hydrazide-hydrazones are not only intermediates but they are also effective organic compounds. Some effective compounds like Iproniazide and Isocarboxazide.Isoniazid is used in the treatment of tuberculosis(TB)and also showed antidepressant effect.



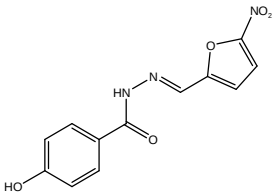
Isoniazid



Iproniazide



Isocarboxazide



Nifuroxazide

Another effective hydrazide hydrazones is **Nifuroxazide**, which is used as an intestinal antiseptic (Negi et al., 2012; Rollas and Kucukguzel. 2007). The 2-Chloroquinolinyl hydrazone derivative are anticonvulsants and ribavirin hydrazone derivatives are anticancer (Liu et al., 2009), hydrazones of indane-1, 3-dione are anticoagulant and antimicrobial activity (Jubie et al., 2010), 4-arylhydrazono-2- pyrazoline-5-one derivatives are anti-TB activity (Guniz et al., 2007). Some hydrazide hydrazones were active against Mtb H37Rv between the concs of 0.78-6.25µg/ml (Kaymakçıoglu, et al., 2006). A series of hydrazide-hydrzones reported antidepres-sant, sedative and analgesic activities (Mohareb et al., 2010).

The synthesis and the importance of hydrazides were studied by many researchers shows various biological, medical and industrial activities. Hydrazone derivatives are molecules

containing highly reactive azomethine group (-CO-NH-N=CH) and thus useful in new drug development[22] Due to the growth of population and changes in climatic conditions several new diseases are likely to affect the human beings. So, there is a continuous need for the synthesis of new bio-logically active organic compounds by using a fast and efficient approach which may act as potential antimicrobial agents. Based on the higher bio-reactivity of hydra-zones, we have synthesized novel hydrazones.[23-26] The anti-bacterial studies were effectively done for newly synthesized hydrazones by standard disc diffusion method[27] with different concentrations.

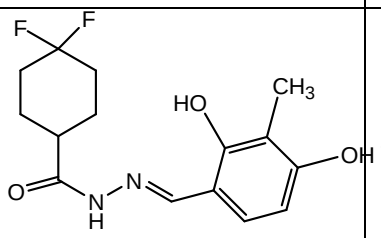
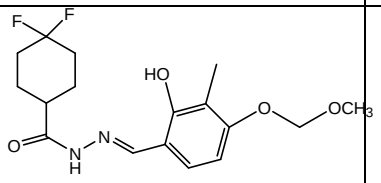
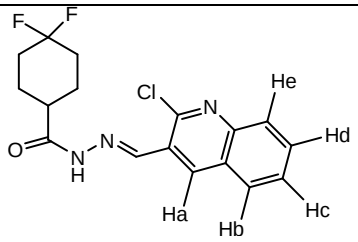
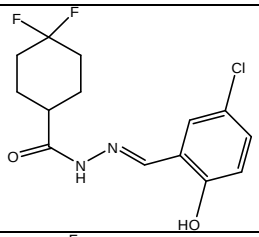
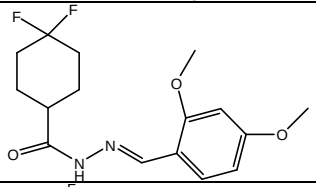
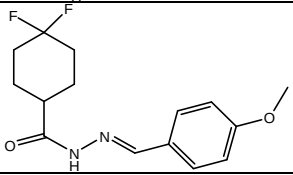
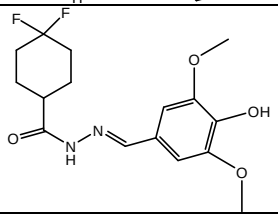
CHEMISTRY

The synthesis of target compounds were carried out in scheme-(1). 4,4-difluoro cyclohexane carboxylic acid esterifies with methanol in the presence of sulfuric acid yields methyl ester of 4,4-difluoro cyclohexane carboxylic acid and it is treated with hydrazine hydrate refluxed in methanol to get 4,4-diflouro cyclohexane carboxylic acid hydrazide. It is coupled with aromatic aldehydes in methanol, acetic acid along with catalytic amount of ammonium acetate at room temperature for overnight. My expected target compounds are 3-(4,4-difluoro-cyclohexyl)-5-phenyl substituted-4H-1,2,4-triazoles[28-30],butunexpectedly(E)-N'-(substituted-benzylidene)cyclohexane carboxylic acid hydrazide derivatives were formed and was confirmed by H^1NMR, C^{13} NMR,MASS and FT-IR spectral techniques. By the usage of acetic acid triazoles were not formed and formed as Schiff bases. From the H1NMR spectra the structures of synthesized compounds 5(a-o) were confirmed on the basis of the fact that the aldehydic proton which was visible at 10.55 in the starting compounds (4) disappeared and new signal due to the azomethine**(-CH=N)** group appeared at values between 7.56-8.2 ppm in all the compounds the **CONH** protons appearing as singlet's resonated at values between 8.5-9.0 ppm.The **4xCH2** protons of cyclohexane centered at 1.6-2.3 ppm as multiplets and 1**CH** proton centered at value 3.2-2.4ppm as multiplets by integrating in one proton.

The FT-IR spectra of compounds **5(a-o)** showened absorption bands at 1670-1645cm-1 due the presence of **-C=O** functional group ,while the bands observed at 1630-1585 cm-1 corresponding with **-C=N** linkage and 3180-3450 cm-1 observed due to the-NH-group. The absorption peak at2840-2970 cm-1 was due to the **-CH**-linkage and the band appearing at 3000-3100cm-1 ,**C-F** absorption appear s at 1111-1115cm-1 in the IR spectrum of the

compound represented as aromatic protons. The synthetic conditions and melting points of the newly synthesized compounds are summarized in table-1.

Table-1:

S.no	Entry	Code	Molecular formula	Structure	m.p	Mass	%yield
1	5a	NFC/A	$C_{15}H_{18}N_2O_3F_2$		238-241°C	312	95.23
2	5b	NFC/B	$C_{17}H_{22}N_2O_4F_2$		167-170°C	356	89.28
3	5c	NFC/CQC	$C_{17}H_{16}N_3OClF_2$		232-235°C	351	92.30
4	5d	NFC/CSA	$C_{14}H_{15}N_2O_2F_2Cl$		232-234°C	316	85.22
5	5e	NFC/DMB	$C_{16}H_{20}N_2O_3F_2$		148-150°C	326	95.23
6	5f	NFC/AN	$C_{15}H_{18}N_2O_2F_2$		152-155°C	296	87.50
77	5g	NFC/SY	$C_{16}H_{20}N_2O_4F_2$		231-233°C	342	91.30

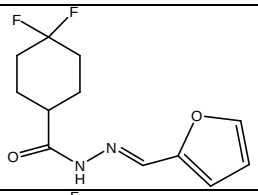
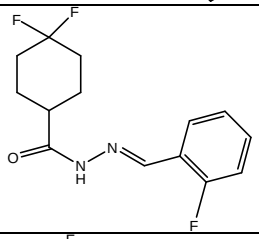
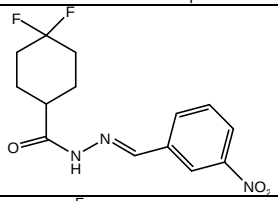
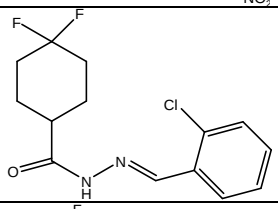
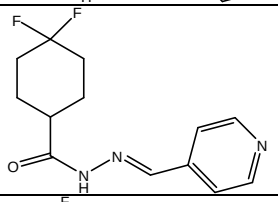
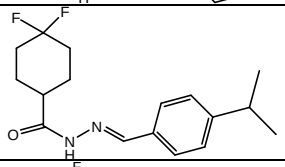
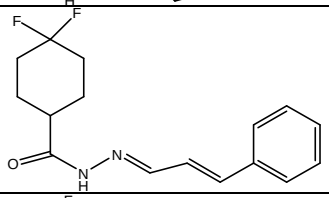
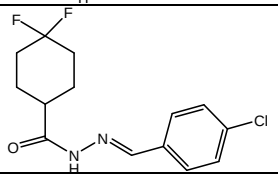
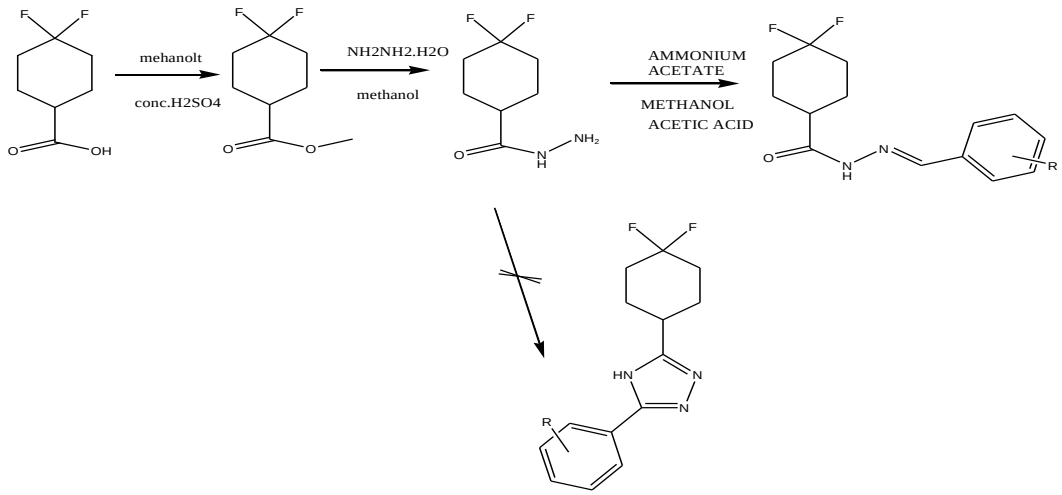
8	5h	NFC/FF	$C_{12}H_{14}N_2O_2F_2$		190-193°C	256	85.71
9	5i	NFC/2F	$C_{14}H_{15}N_2OF_3$		165-167°C	284	90
10	5j	NFC/3-NO2	$C_{14}H_{15}N_3O_3F_2$		178-180°C	308	90.90
11	5k	NFC/2-Cl	$C_{14}H_{15}N_2OF_2Cl$		180-182°C	300	95.23
12	5l	NFC/PY	$C_{13}H_{24}N_3OF_2$		187-189°C	267	93.33
13	5m	NFC/4-ISO	$C_{17}H_{22}N_2OF_2$		115-117°C	308	76.02
14	5n	NFC/CINN	$C_{16}H_{18}N_2OF_2$		195-197°C	292	85.36
15	5o	NFC/4-Cl	$C_{14}H_{15}N_2OF_2Cl$		200-202°C	300	89.28

Table-1: list of synthesized compounds

MATERIALS AND METHODS

The synthesized compounds were determined in open glass capillaries on stuart SMP10 melting point apparatus and were uncorrected.the purity of the sample checked by (TLC) thin layer chromatography.silicagel plates DC kieselgel 0.25mm,60 F_{254} precoated sheets obtained from merck,and spots were visualised by iodine vapours/ultraviolet light as visualising

agent.the IR spectra were obtained with IR AFFINITY-1 FTIR Shimadzu spectro meter . H^1NMR spectra (δ,ppm) were recorded in $CDCl_3$ and DMSO-d_6 on a varian-mercury 300MHZ spectro meter using TMS as internal reference. C^{13}NMR spectra were recorded in CDCl3 and DMSO-d_6 on a Bruker Avance II 400 spectrometer at 400MHZ using TMS as an internal reference. Mass spectra were recorded on a shimadzu . All compounds were routinely checked by TLC on silica gel plates using chloroform:m (9:1 V/V) as solvent system and the developed plates were visualized by UV light, iodine vapour and KMnO4 solution. The detailed scheme of synthesis has been shown in **Scheme 1**.



Scheme-1: synthesis of 4, 4'-cyclohexane carboxylic acid hydrazones

General Procedure For The Synthesis of 4,4'-Cyclohexane Carboxylic Acid Hydrazones: The substituted benzaldehydes (0.01mol)was reacted with methyl ester of 4,4'-cycloxane carboxylic acid (0.01mol)in methanol and glacial acetic acid with catalytic amount of ammonium acetate at room temperature for 20hours . after completion of reaction was confirmed by thin layer chromatography. The reaction mixture was poured in ice water and neutralized with ammonia solution. Filtered and dried the solid at 40˚c in hot air oven. percentage of yield for all the synthesized compounds in the range from 76-96%.

1.(E)-N'-4,4'-Difluoro-cyclohexanecarboxylicacid(3,5-dihydroxy-4-methyl-benzylidene)-hydrazide(NFC-A)(5a):IR(cm-^{1}):3240,2960,2929,2870,2854,1662,1627,1502,1419,1371,1288, 1265,1232,1111,1070, 1033, 956,910,796,713.H^1NMR:(300MHz,DMSO-d6,ppm):δ9.25 (s,1H.-OH),9.14(s,1H,-OH),8.208(s,1H,-NH-),7.459(s,1H,=CH)6.898-6.836(t,1H,Ar-H,J_1=9.82Hz,J_2

=8.876Hz)6.481-6.426(t,1H,-Ar-H,J=8.12Hz),2.30-2.33(m,1H,-CH,J=8.309Hz),2.12(s,3H,CH_3), 1.824-2.2m,8H, 4X CH2, J_1=8.12Hz ,J_2 = 11.51Hz) .

2.(E)-N'-4,4'-Difluoro-cyclohexane carboxylic acid(4-ethoxy-2-hydroxy-3-methyl-benzylideme)-hydrazide(NFC-B)(5b):IR(cm-^{1}):2935,1654,1622,16081494,1417,1359,1247, 1228, 1153,1105, 1060,995,954,921,783,748**.** H^1NMR:(300MHz,DMSO-d6,ppm):8.238(S,1H,-NH),8.04 (S,1H,-OH),7.5(S,1H,-N=CH),7.034-6.973(t,1H,Ar-H-J_1=9.35Hz,J_2=9.064Hz),6.6712-6.618(t,1H,Ar-H, J=8.1 2 Hz),5.22(S,2H,-OCH_2OCH_3),3.482(S,3H,-OCH_3),2.334-2.375(m,1H,-CH,cyclohexyl),2.123(s,3H,-Ar-CH_3),1,823-2.16(m,8H,4XCH_2,cyclohexyl)

3.(E)-N'-4,4'-Difluoro-cyclohexane carboxylic acid(2-chloro-quinolin-3-yl-methylene)-hydrazide(NFC-CQC) (5c): IR(cm-^{1}):3233,3066,2939,2920,2872 ,2854,1668,1593, 1560,1548, 1400,1342,1327,1263,1226,1111,1051, 950,754. H^1NMR:(300MHz,DMSO-d6,ppm):8.55(s,1H.-NH),8.47(S,1H,-Ar-H_a,J6.23Hz),8.121-8.123(d,1H,Ar-H_b,J=8.15Hz),7.671-7.678(t,1H,Ar-H_d, J=9.014Hz),7.52(s,1H,-N=CH),7.34-7.328(dt,1H,Ar-H_C,J=8.12Hz),2.35-2.38(m,1H,-CH, cyclohexyl),1.87-2.21(m,8H,4xCH_2,cyclohexyl).

4.(E)-N'-4,4'-Difluoro-cyclohexane carboxylic acid(5-chloro-2-hydroxy-benzylidene)-hydrazide (NFC-CSA) (5d):IR(cm-^{1}):3236,3207,3057,2939,1662,1608, 1539,1477,1371,1342, 1265,1203 ,1105, 1035,1008 ,960, 947,881, 800,709. H^1NMR:(300MHz,DMSO-d6,ppm):8.45(S,1H,-NH),8.12(S,1H,-N=CH),7.623(S,1H,Ar-Ha),7.121-7.127 (dd,1H,Ar-Hb,J=6.78Hz),6.602-6.618(dd,1H,Ar-H,J=8.23Hz,J=2.72Hz),5.75(brs,1H,-OH),2.36-2.398(m,1H,-CH,cyclohexyl), 2.25-1.979 (m.8H,4xCH2,cyclohexyl).

5.(E)-N'-4,4'-Difluoro-cyclohexane carboxylic acid(2,4-dimethoxy-benzylidene)-hydrazide(NFC-DMB)(5e): IR(ῡ,cm^1) : 3209,3078,2941,2922,2846,1651,1598,1558, 1458,1375, 1313,1271,1207,1159 ,1109,1031, 943 . H^1NMR:(500MHz, CDCl3,ppm): δ8.665 (S,1H,-NH-N=),8.065(S,1H,-N=C-H),7.769-7.784(S,Ar-H- ,1H,J=8.69 Hz), 6.54-6.56 (dd, 1H, J=6.4Hz),6.452-6.448(d,1H,J=2.136Hz), 3.85(s,6H, -OCH3),3.23-3.28(m,1H, cyclohexyl), 1.597-2.17 (m,8H,cyclohexyl).

6.(E)-N'-4,4'-Difluoro-cyclohexane carboxylic acid(4-methoxy-benzylidene)-hydrazide (NFC-AN)(5f):IR(cm-^{1}):3244,3082,2966,2841, 1654,1604, 1554, 1508,1448,1367, 1303,

1249,1205,1172, 1111,1024,941,835,671.H^1NMR:(300MHz,DMSO-d6,ppm):8.45(s,1H,-NH),8.17(S,1H,-N=CH) ,7.572-7.579(d,2H,Ar-H_b,J=6.237Hz),6.87-6.882(d,2H,Ar-H_a, J=8.25 Hz) ,3.75(s,3H,-OCH3), 2.38-2.352(m,1H,-CH, cyclohexyl),2.21-1.89(m,8H, 4xCH2 ,cyclohexyl).

7.(E)-N'-4,4'-Difluoro-cyclohexane carboxylic acid(4-hydroxy-3,5-dimethoxy-benzylidene)-hydrazide(NFC/SY)(5g):IR(cm^1):3228,3078,2941,1651,1585,1548,1512,1458,1427,1367,1323,1273,1253,1199,1107,1039,937,678.**H^1NMR**:(500MHz,CDCl3,ppm):δ8.43(s,1H,-NH-N=), 7.603(s,1H,=CH),6.878(s,2H,Ar-H),5.757(brs,1H,OH), 3.942(s.6H,2X-OCH3),3.19-3.25(m,1H,-CH,cyclohexyl),1.79-2.25(m,8H, 4XCH2,cyclohexyl), Mass(ESI)m/z: 343 (M+1).

8.(E)-N'-4,4'-Difluoro-cyclohexane carboxylic acid furan-2-yl-methylene-hydrazide(NFC-FF)(5h):IR(cm-^{1}):3244,3078,2976,2857,1657,1612,1548,1505,1447,1312,1225,1177,1111,1023, 954,674.H^1NMR:(300MHz,DMSO-d6,ppm):8.47(s,1H,-NH),8.21(S,1H,-N=CH),7.47(d,1H=3.23 Hz),6.95(dd,2H,H_b&H_c of furon,J_1=2.13Hz .J_2=6.85 Hz) ,2.38(m,1H,-CH,cyclohexyl),2.05-1.67 (m,8H,4 xCH_2, cyclohexyl)

9.(E)-N'-4,4'-Difluoro-cyclohexane carboxylic acid(2-fluoro-benzylidene)-hydrazide(NFC-2F(5i):IR(cm^1):3213,3178,3078,2956,2939,2872,1658,1606,1554,1514,1485,1450,1359,1238, 1211,11114,1066,1037,943,765,684.H^1NMR:(300MHz,DMSO-d6,ppm):8.23(S,1H,-NH),8.10 (S,1H,-N=CH),7.6(dd,1H,Ar-H,J_1=2.13Hz,J_2=6.85Hz),7.27(dt,2H,Ar-H,J_1=2.13Hz,J_2=8.23Hz, J_3=2.15Hz)7.32(d,1H,Ar-H,J=8.23Hz),2.38(m,1H,-CH,cyclohexyl),2.25-1.89(m,8H,4xCH_2, cyclohexyl).

10.(E)-N'-4,4'-Difluoro-cyclohexane carboxylic acid(3-nitro-benzylidene)-hydrazide(NFC-3NO2)(5j):IR(cm-^{1})**:**3186,3086,2939,2866,1668,1606,1525,1404,1344,1265,1230,1105,960,806 ,738.H^1NMR:(300MHz,DMSO-d6,ppm):δ8.42(s,1H,-NH-N=C),8.346(S,1H,Ar-H),8.24(S,1H, =CH),8.097-8.125(d,1H,J=8.12Hz,Ar-H)8.034-7.884(dt,1H,Ar-

H,J_1=7.931,J_2=7.742Hz,J_3=14.73 Hz),7.46-7.54(m,1H,J=7.931Hz,Ar-H),3.19-3.22(m,1H,-CH,cyclohexyl),1.80-2.34(m,8H, 4XCH_2, cyclohexyl)

11.(E)-N'-4,4'-Difluoro-cyclohexane carboxylic acid(2-chloro-benzylidene)-hydrazide(NFC-2-Cl)(5k):IR(cm-^{1}):3201,3064,2943,1668,1593,1566,1440,1367,1226,1203,1107,1037,958,752, 698.H^1NMR:(300MHz,DMSO-d6,ppm):8.43(S,1H,-NH),8.17(S,1H,-N=CH),7.67(dd,1H,Ar-H , J1=2.13Hz,J2=6.85Hz),7.27(dt,2H,Ar-H,J_1=2.13Hz,J_2=8.23Hz,J_3=2.15Hz)7.32(d,1H,Ar-H, J=8.23Hz),2.38(m,1H,-CH,cyclohexyl),2.20-1.86(m,8H, 4xCH_2,cyclohexyl).

12.(E)-N'-4,4'-Difluoro-cyclohexane carboxylic acidpyridine-4-yl-methylene-hydrazide (NFC-Py)(5l):IR(cm-1) :3233,3088,2960,2945,2870,1664,1595, 1516,1398,1344, 1224, 1109, 952,800.Mas:m/z:268(M+1);H^1NMR: (300 M Hz , DMSO-d6,ppm):δ8.626-8.613(d,2H,py-H , J=4.532Hz),7.926(s,1H,-NH),7.508-7.521(d,2H,J=6043Hz),3.30-3.26(m,1H,-CH-,cycliohexyl),1.799-2.221(m,8H,cyclohexyl).

13.(E)-N'-4,4'-Difluoro-cyclohexane carboxylic acid(4-isopropyl-benzylidene)-hydrazide (NFC-4-ISO)(5m): IR(cm-^{1})3176,2966,2939,2895,2899,2779,1656,1608,1568,1508,1446,1365, 1267, 1232,1211, 1111,1035,1012, 954, 832,723.H^1NMR:(500MHz,CDCl3,ppm):8.719(s,1H,-NH-), 7.721 (s,1H,=CH),7.27-7.294(d,2H,J=7.931Hz,Ar-H) 7.583-7.556(d,2H,J=8.12Hz,Ar-H)4.087-4.1548 (m,1H,J=7.176Hz,-CH(CH_3)$_2$),2.968-2.924(m,1H,J=6.987Hz,-CH-cyclohexyl),1.631-2.04(m,8H, 4XCH_2,cyclohexyl) 1.26-1.282(d,6H,2XCH_3,J=6.609Hz)

14. (E)-N'-4,4'-Difluoro-cyclohexane carboxylic acid(3-phenyl-allylidene)-hydrazide(NFC-CINN)(5n):IR(cm-1):3240,3057,2941,2872,1656,1625,1543,1448,1369,1357,1201,1180,1111, 1039,1004,974,956,754.Mass:m/z:293(M+1).H^1NMR:(300MHz,DMSO-d6,ppm):8.24(S,1H,-NH),7.659-7.65(d,1H,-N=CH-CH=CH-Ar,J=8.17Hz),7.108-7.325(m,5H,Ar-H,J_1=8.25Hz, J_2=2.13Hz),6.59-6.61(d,1H,-N=CH-CH=CH-Ar),5.723-5.731(t,1H,-N=CH-CH=CH-Ar), 2.38(m,1H,-CH,cyclohexyl),2.17-1.67 (m,8H, 4xCH2, cyclohexyl)

15.(E)-N'-4,4'-Difluoro-cyclohexane carboxylic acid(4-chloro-benzylidene)-hydrazid(NFC-4-Cl)(5o):IR(cm-^{1}):3205,2937,2870,1662,1602,1544,1489,1226,1197,1103,958,823,804. H^{1}NMR:(300MHz,DMSO-d6,ppm):8.43(S,1H,-NH),8.13(S,1H,-N=CH),7.58-7.593(dd,2H,Ar-H,J_1=2.13Hz,J_2=6.85Hz),7.28(dd,2H,Ar-H,J_1=2.13Hz,J_2=6.85Hz),2.40(m,1H,-CH,cyclohexyl), 2.268-1.88(m,8H ,4xCH2,Cyclohexyl).

Antimicrobial testing

The compounds synthesized were screened for their antimicrobial activity by Disc Diffusion Method. In this method the sensitivity of the compounds is measured by determining the zone of inhibition after placing the paper disc dipped in solution of compounds, on LBS agar medium, which was previously inoculated with test organism. These results were compare with the zone of inhibition produced after placing disc dipped in the solution of standard antibiotic. The diameter of zone of inhibition is directly proportional to antimicrobial activity of the compound. The size of zone of inhibition depends on rate of antibiotic diffusion, rate of bacterial growth and incubation condition, concentration of organism.

Materials used

- Sterilized Petri dishes
- Sterilized test tubes and watch glasses.
- Micropipette and micro-tips.
- Cotton swabs

Test organism used in the study

- **Bacterial cultures used:**

1. *Escherichia coli* –Gram negative bacteria
2. *Pseudomonasaeruginosa*–Gram negative bacteria
3. *Staphylococcus areus*– Gram positive bacteria
4. *Bacillus megaterium*– Gram positive bacteria.

SUBCULTURE: One day prior to the testing, the organisms obtained from the laboratory stock were subculture into sterile nutrient broth and incubated at 37 º C for 18-24h. The culture growth thus obtained was used as inoculums for the antibacterial testing.

- **Fungal Cultures used**

1. *Candida albicansand*
2. *Rizopusmicrosporus var. oligosporus*

SUBCULTURE: Two days before the testing the culture is prepared by inoculating the fungus from master culture into potato dextrose medium an incubated for 48 h at room temperature.

Drugs Control

1. Penicillin(antibacterial)
2. Griseofulvin (antifungal)

Concentration: all the test compounds were tested at 250 µg/ml.

Solvent: Methanol.

Preparation of paper discs: Paper disk of 6mm diameter and 2mm thickness was used for the test. These disks were sterilized by autoclaving at 121º C (15 lbs psi) for 15 minutes.

Preparation of culture medium: Culture media, provides all essential nutrients for the growth of microorganism. Luria Broth (LB) Agar was used to inoculate bacterial strains and PDA (Potassium-dextrose agar) medium used for fungal strains.

Composition of LB broth agar medium

Ingredients	Gms/Litre
Tryptone	10gm
Beef extract	5 gm
Sodium chloride	10gm
Agar	15 gm
Distill water	Make up to 1000ml

Composition of PDA medium

Ingredients	Gms/Litre
Potato, infusion form	200gm
Dextrose	20gm
Agar	15
Distill water	Make upto 1000ml

Nutrient media thus prepared was sterilized by autoclave at 121º C for 20 mins at 15 lbs pressure.

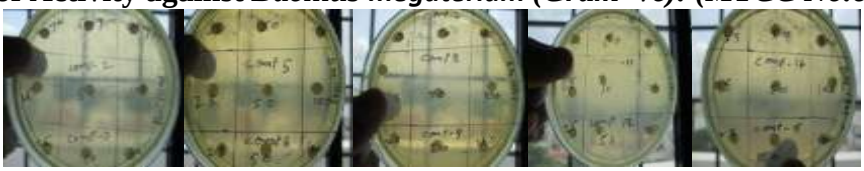
Procedure

Petri dishes were filled to depth of 3-4mm with a nutrient agar medium. This poured medium was allowed to set and then inoculated with susceptible test organism culture using cotton swab under aseptic conditions under laminar air flow unit. Each plate was divided into six equal positions along the diameter. Each portion was used to place one disk. Five disk of each sample was placed on five portions using sterilized forceps. Two disks were placed one each with ciprofloxacin disk and a disk impregnated with the solvent. The petri dishes were incubated at 37°C for 24 h for bacterial culture and incubated for 28 ºC for 4 days for fungal culture. Diameter of the zone of inhibition was measured and the results are shown in Table. The diameter obtained for the test samples were compared with that produced by standards. Diameter of the zone of inhibition was measured in mm.

Table-2: activity results of anti bacterial strains.

Compound no	Entry	(Gram+ve)						(Gram-ve)					
		Activity against Bacillus megaterium (MTCC No.6544)			Activity against Staphylococcus aureus(MTCC No.3160)			Activity against E.coli (MTCC No. 42)			Activity against Pseudomonas aeruginosa MTCC No. 1034)		
		25 µg/ml/ mm	50 µg/ml/ mm	100 µg /ml/mm	25 µg/ ml/mm	50 µg/ ml/mm	100 µg /ml/mm	25 µg /ml/mm	50 µg/ml/ mm	100 µg/ ml/mm	25 µg /ml/ mm	50 µg /ml/mm	100 µg /ml/mm
1	5a	14	15	16	-	-	-	-	-	23	9	28	27
2	5b	15	16	20	11	12	15	-	-	-	6	14	14
3	5c	-	16	17	-	0	15	-	-	-	7	22	27
4	5d	12	15	23	-	16	21	10	23	28	6	20	22
5	5e	16	18	26	-	-	-	-	0	0	6	18	20
6	5f	-	16	20	-	-	15	-	11	16	6	22	25
7	5g	-	-	16	-	-	20	-	0	17	7	28	30
8	5h	16	18	23	12	20	21	-	23	25	7	20	22
9	5i	15	17	23	-	-	-	-	-	21	6	22	25
10	5j	20	25	30	-	15	16	-	-	-	0	22	27
11	5k	-	13	20	13	16	21	-	-	-	15	33	38
12	5l	-	16	23	-	16	20	-	-	-	7	22	25
13	5m	16	18	23	11	21	23	10	-	30	-	-	20
14	5n	15	23	32	-	16	20	-	-	28	-	14	20
15	5o	-	14	15	12	20	23	-	-	23	8	25	26
Pencillin	standard	17	39	48	14	36	40	15	35	42	12	36	43

Images of Activity against *Bacillus megaterium (Gram+ve): (MTCC No.6544)*



Images of Activity against *Staphylococcus areus(Gram+ve): (MTCC No. 3160)*

Images of Activity against *E.coli (Gram-ve)(MTCC No. 42)*

Images of Activity against *Pseudomonasaeruginosa (Gram-ve) MTCC No. 1034)*

Fig-1: Images of activity against bacterial srains of the synthesized compounds

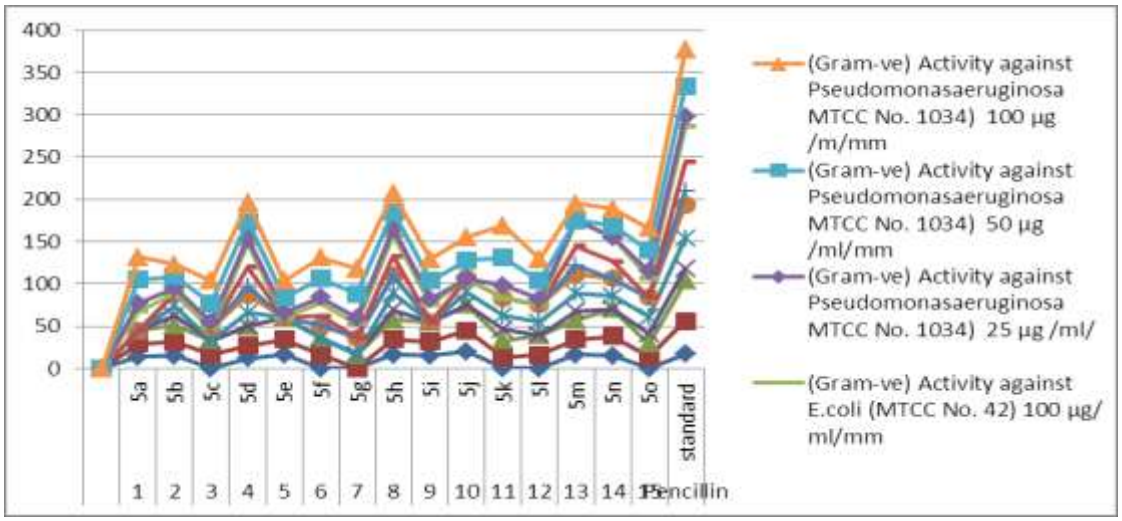


Fig-2: the pictorial representation of tested compounds activity against bacterial strains.

Table-3: activity results of fungal strains

Compound number	Entry	Activity against Fungi Candia Albicans :(MTCC No.: 3017)			Activity against Fungi Rizopus microsporus var. oligosporus: :(MTCC No: 2785)		
		25µg/ml/mm	50µg/ml/mm	100µg/ml/mm	25µg/ml/mm	50µg/ml/mm	100µg/ml/mm
1	5a	13	16	17	-	18	25
2	5b	13	15	17	-	-	-
3	5c	-	-	20	-	-	8

4	5d	-	-	-	-	16	24
5	5e	-	-	-	-	-	-
6	5f	-	-	-	-	-	15
7	5g	-	-	17	-	19	21
8	5h	-	-	18	17	20	27
9	5i	-	-	12	-	21	18
10	5j	-	-	-	-	18	26
11	5k	-	-	17	-	-	-
12	5l	-	-	16	-	-	-
13	5m	-	-	-	-	9	15
14	5n	-	-	-	-	21	29
15	5o	-	-	-	-	17	23
Griseofulvin	standard	14	18	23	19	22	33

Images of Activity against Fungi Candida albicans: (MTCC No. 3017)

Images of activity against Fungi Rizopus microsporus (MTCC No 2785)

Fig-3: Images of activity against fungal strains of tested compounds

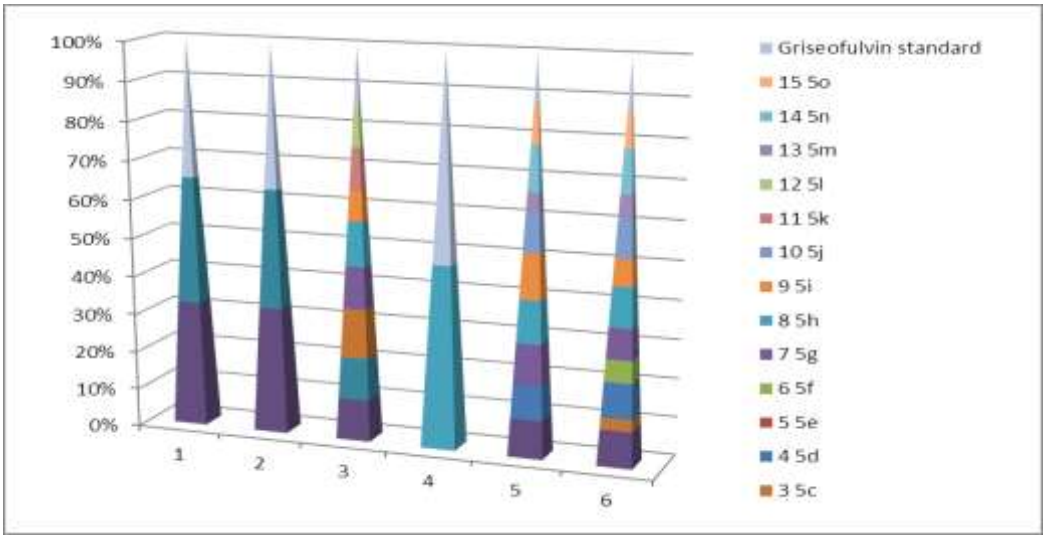


Fig-4: The pictorial representation anti fungal activity of the synthesized compounds

CONCLUSION

The activity studies of all the synthesized compounds against bacterial strains in both gram positive and gram negative activity against bacterial strains revealed that the compounds

5a,5c,5d,5f,5g,5h,5i,5j,5k,5l and 5oshows moderate *activity against Pseudomonas aeruginosa MTCC No. 1034compared with standard pencillin.off all the above compounds 5g and 5k have highest activity compared to all the synthesized compounds in gram negative in* 100µg/ml/mm concentration. Activity against Fungi *Rizopus microsporus var. oligosporus* (MTCC No: 2785) revealed that the compounds 5a,5h,5j and 5n have highest activity compared to all the synthesized compounds in 100µg/ml/mm concentration.

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REFERENCES

1. Abadi AH, Eissa AAH, Hassan GS. Synthesis of novel 1, 3, 4-trisubstituted pyrazole derivatives and their evaluation as antitumor and antiangiogenic agents. *Chem. Pharm. Bull.*, 2003; *51*: 838-844.
2. Abdel-Aal MT, El-Sayed WA, El-Ashry EH. Synthesis and antriviral evaluation of some sugar arylglycinoylhydrazones and their oxadiazoline derivatives. *Arch. Pharm. Chem., Life Sci.* 2006; *339*: 656-663.
3. Abdel-Wahab FB, Awad AEG, Badria AF. Synthesis, antimicrobial, antioxidant, anti-hemolytic and cytotoxic evaluation of new imidaz-ole-based heterocycles, *Eur. J. Med. Chem*, 2011; 46: 1505-1511.
4. Abdel-Wahab FB, Khidre ER, Awad AEG. Regioselective synthesis and antimicrobial activities of some novel aryloxyacetic acid deriva-tives, *Eur. J. Med. Chem.*, 2012; 50: 55-62.
5. Abu-Surrah AS, Safieh KAA, Ahmad IM, Abdalla MY, Ayoub MT, Qaroush AK, Abu-Mahtheieh AM. New palladium (II] complexes bearing pyrazole-based Schiff base ligands: Synthesis, characteriza-tion and cytotoxicity, *Eur. J. Med. Chem.*, 2010; 45: 471-475.
6. Ajani OO, Obafemi, C.A, Nwinyi, O.C, Akinpelu, D.A. Microwave assisted synthesis and antimicrobial activity of 2-quinoxalinone-3-hydrazone derivatives, *Bioorg. Med. Chem.*, 2010; 18: 214-221.
7. Ali, A, Fisara, P, Freemont, J.A, Kyi, S, Meyer, A.G, Andrew, G, Riches, A.G, Sargent, R.M, Sawutz, D.G, Turner, K.A, Winzenberg, KN, Yang, Q. Discovery of

ectoparasiticidal hydrazono-trifluoromethanesulfonanilides, *Bioorg. Med. Chem. Lett.*, 2010; 20: 649-652.

8. Ali, A, Mohammad, T, Davood, Abbas, S. Synthesis and analgesic activity of N-aryl hydrazone derivatives of mefenmic acid. *J Pharm Pharmaceut Sci.*, 2005; 8(3): 419-25.
9. Ali, M.R, Marella, A, Alam, M.T, Naz, R, Akhter, M, Shaquiquzzaman, M, Saha, R, Tanwar, O, Alam, M.M, Hooda, J. Re-view of Biological Activities of Hydrazones. *Indonesian J. Pharm.*, 2012; 23(4): 193-202.
10. Al-Macrosaur, L.Q, Dayam, R, Taheri, L, Witvrouw, M, Debyser, Z, Neamati, N. Discovery of novel non-cytotoxic salicylhydrazine con-taining HIV-1 integrase Inhibitors. *Bioorg. Med. Chem. Lett.* 2007; 17: 6472-75.
11. Al-Said, M.S, Bashandy, M.S, Al-qasoumi, S.I, Ghorab, M.M. Anti-breast cancer activity of some novel 1,2-dihydropyridine, thiophene and thiazole derivatives, *Eur. J. Med. Chem.*, 2011; 46: 137-141.
12. Angelusiua, M.V, Barbuceanu, S-F, Draghicic, C, Almajan, G.L. New Cu(II), Co(II), Ni(II) complexes with aroyl- hydrazone based ligand. Synthesis, spectroscopic characterization and in vitro antibacterial evaluation. *Eur. J. Med. Chem.*, 2010; 45: 2055–62.
13. Aponte, J.C, Vaisberg, A.J, Castillo, D, Gonzalez, G, Estevez, Y, Arevalo, J, Quiliano, M, Zimic, M, Verastegui, M, Malaga, E, Juan, R.H.G, Bustamante, B, Tarleton, R.L, Wang, Y, Franzblau, S.G, Pau-li, G.F, Sauvain, M, Hammond, G.B. Trypanoside, antituberculosis, leishmanicidal, and cytotoxic activities of tetrahydrobenzothienopyrimidines, *Bioorg. Med. Chem.*, 2010; 18: 2880-2886.
14. Aslam, M.A.S, Mahmood, S, Shahid, M, Saeed, A, Iqbal, J. Synthesis, biological assay in vitro and molecular docking studies of new Schiff base derivatives as potential urease inhibitors, *Eur. J. Med. Chem*., 2011; 46: 5473-5479.
15. Belskaya, N.P, Dehaen, W, Bakulev, V.A. Synthesis and properties of hydrazones bearing amide, thioamide and amidine functions. *ARKIVOC*, 2010; 1: 275-332.
16. Bernardino, A, Gomes, A., Charret, K, Freitas, A, Machado, G, Can-to-Cavalheiro, M, Leon, L, Amaral, V. Synthesis and leishmanicidal activities of 1-(4-X-phenyl)-N′-((4-Yphenyl) methylene)-1H-pyrazole-4-carbohydrazides *Eur. J. Med. Chem*. 2006; *41*: 80-87.
17. Bijev A. New heterocyclic hydrazones in the search for antitubercular agents: Synthesis and in vitro evaluations. *Lett.Drug Des. Discov.* 2006; *3*: 506-512.

18. Caffrey, C.R, Schanz, M, Nkemgu-Njinkeng, J, Brush, M, Hausell, E, Cohen, F.E, Flaherty, T.M, Mckerrow, J.H, Steverding, D. Screening of acyl hydrazide proteinase inhibitors for antiparasitic activity against Trypanosomabrucei. Int. J. Antimicrob. Agents. 2002; 19: 227-251.
19. L. Savini, L. Chiasserini, V. Travagli, C. Pellerano, E. Novellino, S. Consentino and M. B. Pisano, "New α-(N)- Heterocyclichydrazones: Evaluation of Anticancer, Anti- HIV and Antimicrobial Activity," European Journal of Medicinal Chemistry, 2004; 39(2): 113-122. doi:10.1016/j.ejmech.2003.09.012.
20. A. M. El-Hawash, W. A. E Abdel and M. A. El-Dewe- llawy, "Cyanoacetic Acid Hydrazones of 3-(and 4-)Acetyl- pyridine and Some Derived Ring Systems as Potential Antitumor and Anti-HCV Agents," Archiv der Pharmazie, 2006; 339(1): 14-23. doi:10.1002/ardp.200500161.
21. M. T. Cocco, C. Congiu, V. Lilliu and V. Onnis, "Syn-thesis and in Vitro Antitumoral Activity of New Hy-drazinopyrimidine-5-carbonitrile Derivatives," Bioorga- nic & Medicinal Chemistry, Vol. 14, No. 2, 2006, pp. 366-372. doi:10.1016/j.bmc.2005.08.012.
22. O. A. Olayinka, A. O. Craig, C. N. Obinna and A. A. David, "Microwave Assisted Synthesis and Antimicrobial Activity of 2-Quinoxalinone-3-hydra-zone Derivatives," Bioorganic & Medicinal Chemistry, 2010; 18(1): 214-221. doi:10.1016/j.bmc.2009.10.064.
23. P. C. Lima, L. M. Lima, K. C. Silva, P. H. Leda, A. L. P. Miranda, C. A. M. Fraga and E. J. Barreiro, "Synthesis and Analgesic Activity of Novel N-Acylarylhydrazones and Isosters, Derived from Natural Safrole," European Journal of Medicinal Chemistry, 2000; 35(2): 187-203. doi:10.1016/S0223-5234(00)00120-3.
24. G. U. Salgin, K. N. Gokham, O. Gostal, Y. Koysal, E. Kilici, S. Isik, G. Aktay and M. Ozalp, "1-Acylthiose- micarbazides, 1,2,4-Triazole-5(4H)-thiones, 1,3,4-Thia- diazoles and Hydrazones Containing 5-Methyl-2-Benzo- xazolinones: Synthesis, Analgesic-Anti-Inflammatory and Antimicrobial Activities," Bioorganic & Medicinal Che- mistry, 2007; 15(17): 5738-5751. doi:10.1016/j.bmc.2007.06.006.
25. A. R. Todeschini, A. L. Miranda, C. M. Silva, S. C. Par-rini and E. J. Barreiro, "Synthesis and Evaluation of An-algesic, Anti-Inflammatory and Antiplatelet Properties of New 2-Pyridylarylhydrazone Derivatives," European Jour- nal of Medicinal Chemistry, 1998; 33(3): 189-199. doi:10.1016/S0223-5234(98)80008-1.
26. G. A. Silva, L. M. M. Costa, F. C. B. Brito, A. L. P Miranda, E. J. Barreiro and C. A. M. Fraga, "New Class of Potent Antinociceptive and Antiplatelet 10H-Pheno- thiazine-1-

Acylhydrazone Derivatives," Bioorganic & Medicinal Chemistry, 2004; 12(12): 3149-3158. doi:10.1016/j.bmc.2004.04.009.

27. A. Espinel-Ingroff, "Standardized Disk Diffusion Method for Yeasts," Clinical Microbiology Newsletter, Vol. 29, No. 13, 2007, pp. 97-100. doi:10.1016/j.clinmicnews.2007.06.001.
28. Pravina B.Piste, Shubhangi P.Waghamale,"Green synthesis of triazole derivatives with pyridine moiety",Int.J.Pharm.Sci.Rev.Res., 2013; 22(2): 46-49.
29. S.Janardhan,G.Balaswamy and M.Sarangapani,"Synthesis of some biologically potent novel 5-(5-substituted phenyl)-4H-1,2,4-triazole-3-yl-1,3-Benzoxazoles"Rasayan.J.Chem. 2011; 4(3): 588-93.
30. SD Joshi,UA Moore,VH Kulkarni,"Synthesis,antimicrobia and cytotoxic activity of new heterocyclic hybrids based on 2,5-dimethyl pyrrole and pyrrole scaffolds, Indian Journal Of Pharmaceutical Sciences, 2013; 75(3): 310-323.