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Short Communication

Synthesis of new series of 1-Aryl-1,4-dihydro-4-oxo-6-methyl pyridazine-3-carboxylic acid as potential antibacterial agents

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Abstract

New series of 1-aryl-1,4-dihydro-4-oxo-6-methyl pyridazine-3-carboxylic acid has been synthesized and the structures of the new compounds were established on the basis of ^{1}H-NMR, mass (ES/MS), elemental analysis and IR spectral data. In vitro antibacterial activity (MIC activity) was evaluated and compared with standard drugs ciprofloxacin, sparfloxacin and trovafloxacin. Most of the compounds in the series have shown very interesting antibacterial activity against both Gram-positive and Gram-negative organisms. In this paper, we describe studies leading to identification of antibacterial agents incorporating novel pyridazine ring surrogate. In a gratifying result, the initial pyridazine-3-carboxylic acid analogues prepared were found to exhibit in vitro antibacterial activity approaching that of corresponding fluoroquinolone progenitor.

Keywords: Pyridazine-3-carboxylic acids; Antibacterial activity; Minimum inhibitory concentration (μg/ml); Gram-positive and Gram-negative bacteria; Pharmacophore; Quinolone antibiotic analogues

1. Introduction

Several antibiotics have been prescribed and found to be effective on various infectious disorders. However, the appearance of multidrug resistant Gram-positive bacteria, in particular, methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant Enterococci (VRE) is causing a serious menace. Moreover, the emergence of vancomycin-resistant MRSA can be anticipated in foreseeable future. For the treatment of these intractable infections, a new anti-infectious agent is needed.

The synthetic antibiotics include the sulfa drugs, nitrofuran derivative, pyridine-carboxylic acid analogues, fluoroquinolones and oxazolidinones. The semi-synthetic antibiotics include the penicillins, cephalosporins, tetracyclines and macrolides. Among them, the sulfa drugs, nitrofuran derivatives, penicillins and tetracyclines are scarcely used in clinical therapy.

Quinolone antibiotics are widely prescribed drugs because of their safety, good tolerance, broad antibacterial spectrum and less resistance [1–4]. Macrolide antibiotics, including erythromycin and related compounds, continue to be an important therapeutic class against Gram-positive organisms, with second generation macrolides such as clarithromycin and azithromycin being widely prescribed due to their efficacy, safety and lack of serious side effects [5]. Oxazolidinone antibacterial agents [6] are newer class of synthetic antibacterial agents with activity against Gram-positive bacteria. Linezolid [7] is well known as first promising candidate of oxazolidinone and works effectively against numerous serious Gram-positive human pathogens caused by MRSA and VRE.

Herein, we have described the synthesis of new series of 1-aryl-1,4-dihydro-4-oxo-6-methyl pyridazine-3-carboxylic acid as novel class of synthetic antibacterials along with their in vitro biological activity (minimum inhibitory concentration (MIC) activity).

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2. Chemistry

The synthesis of 1-aryl-1,4-dihydro-4-oxo-6-methyl pyridazine-3-carboxylic acid is outlined in Scheme 1.

2.1. Synthesis of 4-pyridazones [8]

Azo dyes derived from substituted 1,2-pyran-2,4 (3) diones, gradually fade in color when heated in dilute alkaline solution. For example, when an aqueous alkaline solution of 6-methyl-3-*p*-nitrophenyl-azo-1,2-pyran-2,4(3) dione was refluxed for 1–2 h with equimolar amount of sodium hydroxide, a good yield of yellow carboxylic acid resulted, as a result of rearrangement of the original azo molecule.

The conversion of azo compounds to pyridazones may be carried out about equally well in either aqueous alcohol or water alone. The alkali used may be sodium hydroxide, sodium carbonate or sodium bicarbonate. The reaction proceeds satisfactorily when excessive amounts of sodium bicarbonate are used though too much of sodium hydroxide may have a harmful effect on yields.

The azo compounds required as starting material were easily prepared from 1,2-pyran-2,4(3) diones and the appropriate diazo compounds in either alkaline or slightly acidic solution. The corresponding pyridazones are most conveniently prepared without isolation or purification of azo intermediates.

Thus alkaline hydrolysis of 6-alkyl-3-aryl-azo-1,2-pyran-2,4(3)-dione results in cleavage of pyran-2,4(3) dione nucleus followed by rearrangement to 1,4-dihydro-6-alkyl-1-aryl-4-oxo-3-pyridazine carboxylic acid (4-pyridazones).

2.2. Synthesis of 1-aryl-1,4-dihydro-4-oxo-6-methyl pyridazine-3-carboxylic acid

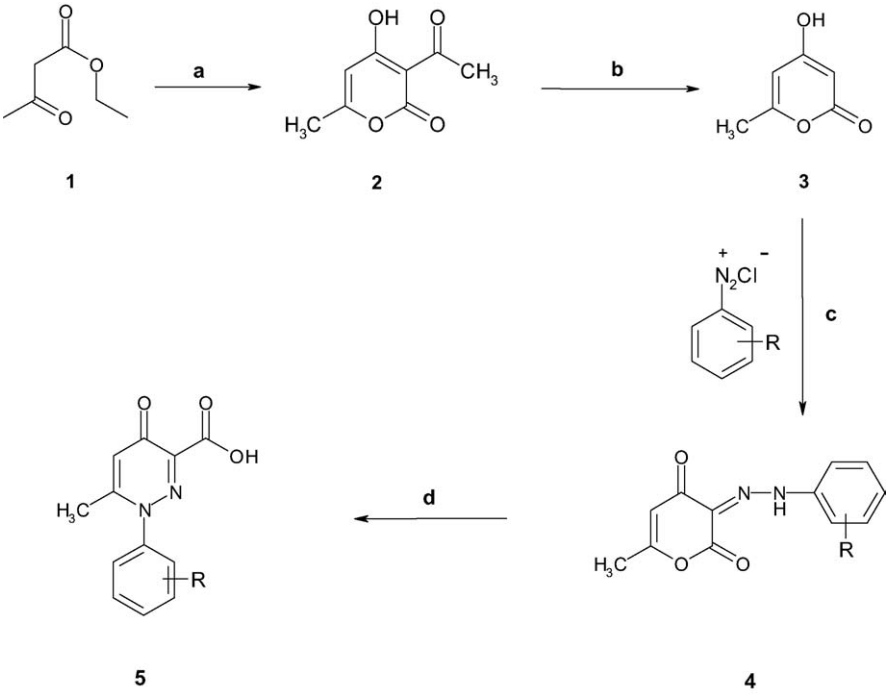
3-Acetyl-4-hydroxy-6-methyl-2H-pyran-2-one (dehydroacetic acid) (**2**) prepared from ethylacetoacetate (**1**) [9], on refluxing with Conc. H_2SO_4 gives 4-hydroxy-6-methyl-2-pyrone (**3**) [9]. This on coupling with diazonium chloride, prepared by conventional diazotization technique from an amine of the formula R-NH_2 (where R is phenyl or substituted phenyl) gave hydrazone (**4**), which on treatment with an aqueous acid yields pyridazine-3-carboxylic acid (**5**) [8–10].

The following new series of 1-(aryl substituted)-1,4-dihydro-4-oxo-6-methyl pyridazine-3-carboxylic acid has been synthesized and all the compounds were characterized by ^{1}H-NMR, electron spray ionization mass spectra (ES-MS), elemental analysis, IR and MP (Table 1).

Also HPLC method development was established for assessment of purity of each compound and each of the compound showed excellent purity of about + 98% (Table 1).

3. Results and discussion

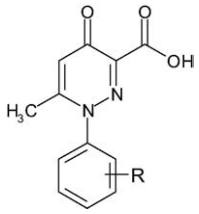
The new 1-(aryl substituted)-1,4-dihydro-4-oxo-6-methyl pyridazine-3-carboxylic acid analogues prepared above were



[a] (a) Reflux , 7 hrs . , 53 % . (b) 90 % H_2SO_4 , Reflux , 1 hr. , 90 % .
(c) Na_2CO_3 / H_2O , 0 - 10 ° C (d) i. Reflux 3 hrs . ii. Conc . HCl , 40 - 70 % .

Scheme 1.

Table 1
Physical and spectral data of 1-(aryl substituted)-1,4-dihydro-4-oxo-6-methyl pyridazine-3-carboxylic acid (**5a–i**)



Serial number	Compounds	R	Molecular formula	Molecular weight	Yield (%)	M.p. (°C)	HPLC RT (min)	HPLC purity (%)
1	**5-a**	4-F	$C_{12}H_9FN_2O_3$	248	68	158–60	11.72	99.68
2	**5-b**	4-CN	$C_{13}H_9N_3O_3$	255	35	218–20	10.34	98.97
3	**5-c**	4-CF_3	$C_{13}H_9F_3N_2O_3$	298	40	210–12	11.46	99.37
4	**5-d**	3-F	$C_{12}H_9FN_2O_3$	248	76	190–91	11.69	99.95
5	**5-e**	2,4-Difluoro	$C_{12}H_8F_2N_2O_3$	266	56	181–83	12.42	98.91
6	**5-f**	3,5-Difluoro	$C_{12}H_8F_2N_2O_3$	266	45	220–22	12.31	99.85
7	**5-g**	3,4-Difluoro	$C_{12}H_8F_2N_2O_3$	266	42	207–09	12.52	99.67
8	**5-h**	4-COOH	$C_{13}H_{10}N_2O_5$	274	40	217–18	9.03	97.10
9	**5-i**	4-$CONH_2$	$C_{13}H_{11}N_3O_4$	273	45	214–15	5.27	99.92

Serial number	Compounds	^{1}H-NMR (200 MHz, $CDCl_3$)	Mass (ES/MS) *m/z* (M + H)
1	**5-a**	δ 2.40(s,3H), 6.95(s,1H), 7.35 (dd,2H), 7.50(dd,2H), 15.40(bs,1H)	249
2	**5-b**	δ 2.35(s,3H), 6.90(s,1H), 7.60, (dd,2H), 7.90(dd,2H), 15.10(bs,1H)	256
3	**5-c**	δ 2.35(s,3H), 6.90(s,1H), 7.60(dd,2H), 7.90(dd,2H), 15.20(bs,1H)	299
4	**5-d**	δ 2.40(s,3H), 6.90(s,1H), 7.20(bm,3H), 7.60(m,1H), 15.30(bs,1H)	249
5	**5-e**	δ 2.30(s,3H), 6.90(s,1H), 7.10(bm,2H), 7.50(m,1H), 15.20(bs,1H)	267
6	**5-f**	δ 2.40(s,3H), 6.95(s,1H), 7.05(bm,3H), 15.10(bs,1H)	267
7	**5-g**	δ 2.30(s,3H), 6.95(s,1H), 7.30(bm,3H), 15.20(bs,1H)	267
8	**5-h**	δ 2.20(s,3H), 7.00(s,1H), 7.70(dd,2H), 8.20(dd,2H), 15.20(bs,1H)	275
9	**5-i**	δ 2.30(s,3H), 7.10(s,1H), 7.70(dd,2H), 8.20(dd,2H), 15.20(bs,1H)	274

Elemental analysis data were obtained for all the compounds and was within the limit of ± 0.4% of the calculated value.

tested in vitro versus a panel of Gram-positive and Gram-negative clinical isolates. MIC was determined by standard agar dilution method as per NCCLS guidelines and the values are shown in Table 2. The data of ciprofloxacin, sparfloxacin and trovafloxacin were used as reference standards.

Most of the compounds in the series exhibited excellent and promising in vitro antibacterial activity against both Gram-positive and Gram-negative strains, except for *Pseudomonas aeruginosa* where all the tested compounds were inactive (MIC > 32 μg/ml) in comparison with ciprofloxacin, sparfloxacin and trovafloxacin as reference standards.

Compounds **5-b** and **5-h** have shown MIC of 0.06–4 μg/ml against *S. aureus*. Compounds **5-b** and **5-g** have shown MIC of 0.03–4 and 0.06–8 μg/ml, respectively, against *Escherichia coli*. Compounds **5-b** and **5-h** have shown MIC of 0.06–4 μg/ml against *S. epidermis*. Compounds **5-b** and **5-d** have shown MIC of 0.25–8 μg/ml against *Klebsiella* species while compounds **5-e** and **5-f** have shown MIC of 0.06–16 and 0.125–16 μg/ml, respectively, against *E. faecalis*. All the tested compounds have moderate activity (MIC = 1–32 μg/ml) against *E. faecium*. Rest of the compounds in the series also had excellent antibacterial activity against the strains tested in comparison with ciprofloxacin, sparfloxacin and trovafloxacin.

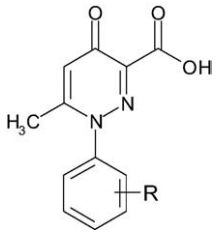
With the results in hand an extensive study survey of differently substituted derivatives of above class was undertaken in order to increase the spectrum of antibacterial activity.

4. Conclusion

In summary, we have described the genesis and synthesis of antibacterially active 1-(aryl substituted)-1,4-dihydro-4-oxo-6-methyl pyridazine-3-carboxylic acid derivatives. Notably, the analogues **5-b** with a para cyano phenyl substituent, **5-e** with a 2,4-difluoro phenyl substituent, **5-f** with a 3,5-difluoro phenyl substituent, **5-g** with a 3,4-difluoro phenyl substituent and **5-h** with a para carboxy group phenyl substituent, in particular, have exhibited superior MIC activity against *S. aureus*, *E. coli*, *S. epidermis* and *E. faecalis* as compared to ciprofloxacin, sparfloxacin and trovafloxacin.

Since this preliminary series of pyridazine-3-carboxylic acid has shown good activity against the tested organisms, the possibility remains that more divergent structural modifications might improve the spectrum of antibacterial activity. In connection with this notion, we point out the pharmacophoric similarity of this pyridazine-3-carboxylic acid series relative to fluoroquinolone counterpart and suggest that further study of these agents is warranted.

Table 2
MIC (μg/ml) of 1-(aryl substituted)-1,4-dihydro-4-oxo-6-methyl pyridazine-3-carboxylic acid (**5a–i**)



Compounds	*P. aeruginosa* (*N* = 26)[a]			*S. aureus* (*N* = 80)[b]			*E. coli* (*N* = 54)[c]			*S. epidermis* (*N* = 33)		
	MIC 50	MIC 90	Range	MIC 50	MIC 90	Range	MIC 50	MIC 90	Range	MIC 50	MIC 90	Range
5-a	Inactive (> 32)			2	4	0.25–16	0.25	4	0.06–8	2	4	0.25–16
5-b	″			0.25	1	0.06–4	0.06	2	0.03–4	0.25	1	0.06–4
5-c	″			1	8	0.5–32	0.5	4	0.25–8	1	8	0.5–32
5-d	″			0.50	2	0.12–8	0.12	4	0.06–8	0.50	2	0.12–8
5-e				1	4	0.12–2	0.12	2	0.06–8	1	4	0.12–2
5-f	″			2	8	0.25–16	0.25	2	0.12–8	2	8	0.25–16
5-g	″			2	8	0.25–32	0.25	2	0.06–8	2	8	0.25–32
5-h	″			0.125	4	0.06–4	0.12	4	0.25–16	0.125	4	0.06–4
5-I	″			1	8	0.12–16	0.12	4	0.25–16	1	8	0.12–16
Cipro.	0.25	1	0.12–4	1	4	0.5–8	0.007	0.015	0.003–0.03	1	4	0.5–8
Spar.	1.0	2	0.25–4	1	2	0.5–4	0.007	0.03	0.003–0.03	1	2	0.5–4
Trova.	0.25	1	0.06–4	0.5	1	0.25–4	0.002	0.015	0.003–0.03	0.5	1	0.25–4
Compounds	*Klebsiella* sp. (*N* = 24)			*E. faecium* (*N* = 16)			*E. faecalis* (*N* = 24)					
	MIC 50	MIC 90	Range	MIC 50	MIC 90	Range	MIC 50	MIC 90	Range			
5-a	2	8	0.5–16	8	> 32	2–> 32	1	8	0.25–16			
5-b	1	4	0.25–8	4	> 16	2–> 32	0.5	4	0.25–16			
5-c	2	8	0.5–16	8	> 32	2–>32	1	8	0.5–8			
5-d	1	4	0.25–8	4	> 16	1–> 32	0.5	4	0.12–8			
5-e	2	4	0.5–8	8	> 16	2–> 32	0.25	4	0.06–8			
5-f	4	16	1–16	16	> 32	4–> 32	1	4	0.125–8			
5-g	4	16	1–16	16	> 32	4–> 32	2	16	0.25–16			
5-h	4	16	1–16	16	> 32	8–> 32	2	8	0.5–16			
5-i	2	8	0.5–16	8	> 16	2–> 32	1	4	0.5–8			
Cipro.	0.01	0.02	0.01–0.5	16	> 16	NA	2	8	0.5–16			
Spar.	0.01	0.02	0.01–0.25	> 16	> 16	NA	1	4	0.5–8			
Trova.	0.025	0.1	0.06–0.4	> 16	> 16	NA	1	2	0.5–4			

N = number of strains tested.
[a] *P. aeruginosa* ATCC 27853.
[b] *S. aureus* ATCC 25923.
[c] *E. coli* ATCC 25922.

5. Experimental section

Melting points were determined on Quality Precise apparatus and are uncorrected. ^{1}H-NMR spectra were recorded on Varian Gemini 200 MHz spectrometer. Chemical shifts are reported in δ units (ppm) relative to TMS as internal standard. ES-MS were recorded on Water-Micromass Quattro-II spectrometer. HPLC analysis were carried out on Shimadzu instrument with SPD-10A UV detector and YMC Pack AM.25 cm column. IR spectra were recorded on Varian spectrometer. All the solvents and reagents used were of AR grade and were used without further purification.

5.1. Part A:- Preparation of 3-acetyl-4-hydroxy-6-methyl-2H-pyran-2-one (dehydroacetic acid) (2) [9]

Hundred grams (780 mmol) of freshly vacuum distilled ethylacetoacetate (**1**) and 0.5 g of sodium bicarbonate are heated until the liquid has reached to 200–210 °C. The time for heating was usually 7–8 h, during which period 27 g of distillate boiling at 72 °C (mostly ethanol) was collected and the color of the reaction mixture becomes dark brown. The resulting dehydroacetic acid was distilled under reduced pressure (the dehydroacetic acid was collected at 140 °C–12 mm).

The yield of dehydroacetic acid melting at 104–110 °C is 34 g (53%). A purer product m.p. 108 °C was secured in 80% by recrystallization from ethanol using 2 ml/g of material.

M.p. 107–108 °C; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 2.05 (s, 3H), 2.25 (s, 3H), 5.90 (dd, 1H); MS (ES) *m/z* 169 (M + H, 100%).

5.2. Part B:- De-acetylation of dehydroacetic acid to 6-methyl-2H-pyran-2,4-(3H)-dione (3) [9]

Fifty grams (290 mmol) of dehydroacetic acid (**2**) was dissolved in 150 g of dilute 90% H_2SO_4. The mixture was then heated upto 130 °C and maintained at the same temperature for about 15 min. The flask was then rapidly cooled and the contents were poured into 200 ml of ice-cold water. The precipitated solid was collected by filtration, washed with 2 × 15 ml cold water and dried completely to get off-white solid as desired compound with yield of 36 g (90%).

M.p. 188–190 °C; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 2.20 (s, 3H), 5.60 (dd, 1H), 5.90 (dd, 1H); MS (ES) *m/z* 127 (M + H, 100%); IR (cm^{-1}) 2900, 1680, 1600, 1310, 1265, 1005, 850, 530.

*5.3. Part C:- General procedure for synthesis of 1-(aryl substituted)-1,4-dihydro-4-oxo-6-methyl pyridazine-3-carboxylic acid (compound **5a–5i**) [8–10]*

4-Hydroxy-6-methyl-2-pyrone (**3**) (0.5 g, 3.96 mmol) was dissolved in 20 ml water and 2.24 g (21.03 mmol) of sodium carbonate was added to the suspension to make the solution alkaline.

In a separate flask, 4.20 mmol of required substituted aniline was dissolved in 10 ml 6 N HCl and cooled to 0 °C. A solution of 0.35 g (5.04 mmol) of sodium nitrite in 5 ml water was added at 0 °C and the resulting diazonium chloride solution was added dropwise to stirred pyrone solution while maintaining the temperature at 5–10 °C and pH at 10–12.

The resulting hydrazone was refluxed for 3 h at 110 °C and then neutralized with acetic acid upto pH 7. A small amount of charcoal was added and again refluxed for 30 min. The solid was filtered in hot, the filtrate cooled to 0–5 °C and treated with conc. HCl. The precipitated solid was collected by filtration, washed well with water and dried completely to get white to off-white solid as desired compound.

All the compounds were characterized by ^{1}H-NMR, IR, ES-MS and MP. The purity was determined by HPLC (YMC Pack AM.25 cm column, Mobile phase as 0.05% TFA in acetonitrile, flow rate of 1.25 ml/min, run time of 30 min and UV detection at 254 nm) (Table 1).

*5.3.1. Compound **5-a**:- 1-(4-Fluoro phenyl)-1,4-dihydro-4-oxo-6-methyl pyridazine-3-carboxylic acid*

^{1}H-NMR (200 MHz, $CDCl_3$) δ 2.40 (s, 3H), 6.95 (s, 1H), 7.35 (dd, 2H), 7.50 (dd, 2H), 15.40 (bs, 1H); MS (ES) *m/z* 249 (M + H, 100%).

*5.3.2. Compound **5-b**:- 1-(4-Cyano phenyl)-1,4-dihydro-4-oxo-6-methyl pyridazine-3-carboxylic acid (**5-b**)*

^{1}H-NMR (200 MHz, $CDCl_3$) δ 2.35 (s, 3H), 6.90 (s, 1H), 7.60 (dd, 2H), 7.90 (dd, 2H), 15.10 (bs, 1H); MS (ES) *m/z* 256 (M + H, 100%).

*5.3.3. Compound **5-c**:- 1-(4-Trifluoromethyl phenyl)-1,4-dihydro-4-oxo-6-methyl pyridazine-3-carboxylic acid*

^{1}H-NMR (200 MHz, $CDCl_3$) δ 2.35 (s, 3H), 6.90 (s, 1H), 7.60 (dd, 2H), 7.90 (dd, 2H), 15.20 (bs, 1H); MS (ES) *m/z* 299 (M + H, 100%).

*5.3.4. Compound **5-d**:- 1-(3-Fluoro phenyl)-1,4-dihydro-4-oxo-6-methyl pyridazine-3-carboxylic acid*

^{1}H-NMR (200 MHz, $CDCl_3$) δ 2.40 (s, 3H), 6.90 (s, 1H), 7.20 (bm, 3H), 7.60 (m, 1H), 15.30 (bs, 1H); MS (ES) *m/z* 249 (M + H, 100%).

*5.3.5. Compound **5-e**:- 1-(2,4-Difluoro phenyl)-1,4-dihydro-4-oxo-6-methyl pyridazine-3-carboxylic acid*

^{1}H-NMR (200 MHz, $CDCl_3$) δ 2.30 (s, 3H), 6.90 (s, 1H), 7.10 (bm, 2H), 7.50 (m, 1H), 15.20 (bs, 1H); MS (ES) *m/z* 267 (M + H, 100%).

*5.3.6. Compound **5-f**:- 1-(3,5-Difluoro phenyl)-1,4-dihydro-4-oxo-6-methyl pyridazine-3-carboxylic acid*

^{1}H-NMR (200 MHz, $CDCl_3$) δ 2.40 (s, 3H), 6.95 (s, 1H), 7.05 (bm, 3H), 15.10 (bs, 1H); MS (ES) *m/z* 267 (M + H, 100%).

*5.3.7. Compound **5-g**:- 1-(3,4-Difluoro phenyl)-1,4-dihydro-4-oxo-6-methyl pyridazine-3-carboxylic acid*

^{1}H-NMR (200 MHz, $CDCl_3$) δ 2.30 (s, 3H), 6.95 (s, 1H), 7.30 (bm, 3H), 15.20 (bs, 1H); MS (ES) *m/z* 267 (M + H, 100%).

*5.3.8. Compound **5-h**:- 1-(4-Carboxy-phenyl)-1,4-dihydro-4-oxo-6-methyl pyridazine-3-carboxylic acid*

^{1}H-NMR (200 MHz, DMSO-d_6) δ 2.20 (s, 3H), 7.00 (s, 1H),7.70 (dd, 2H), 8.20 (dd, 2H), 15.20 (bs, 1H); MS (ES) *m/z* 275 (M + H, 100%).

*5.3.9. Compound **5-i**:- 1-(4-Carbamoyl-phenyl)-1,4-dihydro-4-oxo-6-methyl pyridazine-3-carboxylic acid*

^{1}H-NMR (200 MHz, DMSO-d_6) δ 2.30 (s, 3H), 7.10 (s, 1H), 7.70 (dd, 2H), 8.20 (dd, 2H), 15.20 (bs, 1H); MS (ES) *m/z* 274 (M + H, 100%).

5.4. Part D:- MIC determination

5.4.1. Bacterial isolates

The strains were collected from major hospitals of India during the period 2003–2004 and were identified by standard laboratory procedures.

MIC was determined by standard agar dilution method as per NCCLS guidelines (M7-A5 January 2000). Strains were

grown in tryptic soya broth (TSB, HiMedia, India) for 18–24 h. The overnight grown cultures were diluted appropriately so that the final density is approximately 10^7 CFU/ml. A portion of this diluted broth was transferred to seed block of a multipoint inoculator (AQS Manufacturing, UK). The inoculating pins were standardized to inoculate 1–2 μl of this broth, so that the final CFU was 1×10^4–5×10^4 CFU per spot. The inoculated plates were allowed to stand until the moisture in the inoculum spot has been absorbed in the media.

The inoculated plates were inverted and incubated for 18–24 h at 35 °C in an ambient air incubator (Newtronic, India). After the completion of incubation period, the plates were read visually.

MIC was defined as the lowest concentration which inhibited the growth of strain completely. Drug free plates were used to ensure the growth of strains. ATCC strain of *S. aureus*, *E. coli* and *P. aeruginosa* were used as quality control.

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