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SYNTHESIS, ANTIBACTERIAL ACTIVITY AND MOLECULAR PROPERTIES PREDICTION OF SOME PYRIDAZIN-3-ONE DERIVATIVES

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ABSTRACT: We have synthesized some new pyridazine-3-one derivatives and characterized by means of IR, NMR & mass spectral data. Compounds were evaluated for *in-vitro* antibacterial activity by disc diffusion method and further subjected to molecular properties prediction and drug-likeness by Molinspiration software. All compounds were found in compliance with Lipinski 'Rule of Five' and presented a good drug-likeness score with no violations. Compound I and IV having substitutent (6-phenyl, 6-chloro phenyl) has documented good antibacterial activity against *S. aureus & S. epidermidis*. Compound III & V (4'-chloro-benzylidine, 4'-methoxy-benzylidine) has reported good activity against *P. aeruginosa & E. coli*.

INTRODUCTION: Many potent drugs derived from synthetic as well as natural sources, commonly in practice contain nitrogen in the heterocyclic ring system. Based on the observation of natural products having potent pharmacological activity, attempts with successful results are in progress for more than 150 years now, regarding the synthesis of new nitrogen based heterocycles with the hope to obtain more potent drugs and in many cases the results have been quite rewarding.

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Considerable interest has been attracted towards the pyridazine-3-one scaffold and its derivatives due to their widespread applications such as anticonvulsant activity [1], antimicrobial activity [2], antiviral activity [3], anticancer [4] and analgesic activity [5].

MATERIALS AND METHODS*:* Melting points were determined on Perfit melting point apparatus by open capillary method and are uncorrected. UV spectra were recorded on Beckman DU-64 spectrophotometer in methanol. The IR spectra were recorded as KBr pellets on Hitachi-270 spectrophotometer. The Proton Resonance Spectra (^{1}H-NMR) were recorded on Bruker DRX-400 spectrometer (Chemical Shift in ppm) in $CDCl_3$/DMSO as solvent and TMS as internal standard. The purity of the compound was checked by TLC using different solvent systems

All the solvents and reagents used were of laboratory grade (LR). All the reactions were monitored by TLC using Toluene : Ethyl Acetate: Formic Acid (5:4:1) as solvent system. Iodine chamber was used for visualization of TLC spots. Anhydrous sodium sulphate or potassium carbonate was used for drying various solvents.

The physicochemical parameter and bioactivity of the compounds were calculated by Molinspiration software.

Procedure for synthesis:

Step 1- Synthesis of Benzoyl Propanoic Acid derivatives: After suspending anhydrous Aluminium Chloride (12 g) in dry substituted benzene (100ml) under anhydrous conditions, the contents were refluxed on a water bath. Succinic anhydride (14 g) was then added in small proportion through condenser with continuous stirring. Stirring and heating were continued for 6 hours and left for overnight at room temperature. The contents were then poured into ice-cold hydrochloric acid (2.5% v/v). The aqueous solution was concentrated to a small volume by evaporating on a water bath to obtain the crude compounds. Yield 72%, m.p.125-126^{o}C.

Step 2- Synthesis of 6-substituted-phenyl-4,5-dihydro-pyridazine-3(2H)-one derivatives: The Benzoyl Propanoic Acid derivatives (1gm) in methanol added with Hydrazine Hydrate (1ml) and added Sodium acetate (0.5 g) refluxed at least for 10 hours. After completion of the reaction, methanol was distilled off and then the concentrate was poured into ice-cold water. Solid was formed, which was recrystallized with alcohol to get the compound. Yield 50%, m.p. 251-252^{0}C.

Step 3- Synthesis of 4-arylidine-6-substituted-phenyl-4,5-dihydropyridazine-3(2H)-one derivatives (I-VI): The compound (1gm) was refluxed for 10 hours with aromatic aldehydes (1 ml) in methanol containing sodium hydroxide (0.5g). Contents were concentrated, then poured into ice-cold water, filtered and recrystallized to get the crystals.

Molecular properties prediction and drug-likeness by Molinspiration [6]:

Molecular properties prediction: Physicochemical parameter such as TPSA, MW, Drug Likeness & MiLogP (octanol/water partition coefficient) is calculated by the methodology developed by Molinspiration software. These parameters play a vital role in generation and determination of bioactivity of chemical entity.

Drug Likeness: Drug likeliness is a qualitative means of analysis to check whether the given molecule is a drug or not and it is defined as a complex balance of various molecular properties and structural features which determine whether a particular molecule is similar to the known drugs. Activity of all test compounds and standard drug (Ampicillin) were analyzed under six criteria of known successful drug activity in areas of GPCR ligand, ion channel modulator, kinase inhibitor, nuclear receptor ligand, protease inhibitor and enzyme inhibitor by the molinspiration software.

Antibacterial Activity: All the compounds synthesized were screened *in vitro* for anti-bacterial activity against Gram positive bacteria *Staphylococcus aureus* (MTCC 737) and *Staphylococcus epidermidis* (MTCC 3615) and Gram negative bacteria- *Escherichia coli* (MTCC 1687) and *Pseudomonas aeruginosa* (MTCC 424) using disc diffusion method at 30 µg/ml concentration, ampicillin (30 µg/ml) was taken as standard. Dimethyl sulphoxide (1%, DMSO) was used a control [7].

RESULT:

Chemistry: The synthesis of final 4-arylidine-6-substituted-phenyl-4,5-dihydropyridazine-3(2H)-one derivatives **(I-VI)** was achieved by refluxing dry substituted benzene, succinic anhydride in the presence of anhyd. aluminium chloride to yield benzoyl propionic acid derivatives. Then this was refluxed with hydrazine hydrate to yield 6-substituted-phenyl-4,5-dihydro-pyridazin-3(2H)-one derivatives as an intermediate. This intermediate was refluxed with aromatic aldehydes to afford final compounds **(I-VI), Figure 1**. All the synthesized compounds were characterized by the IR, NMR spectra in **Table 1**.

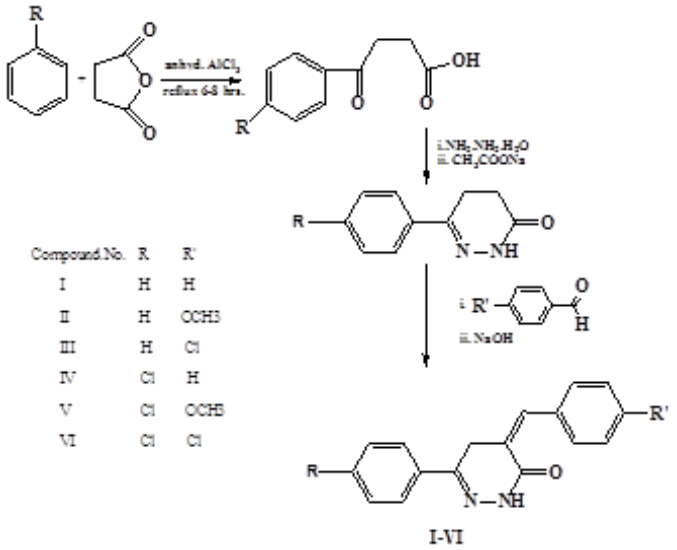

FIGURE 1: REACTION SCHEME

TABLE 1: SPECTRAL DATA FOR INDIVIDUAL COMPOUNDS

IR wave number ν_{max} (in cm^{-1})	^1H NMR δ (in ppm)
I 3400 (Ar-H), 3350 (NH stretch), 2862 (C-H stretch), 1642.09 (C=O stretch), 1603.52 (heterocyclic ring), 1258.32 (C-N stretch).	12.2 (s, 1H, NH), 10.04 (s, 1H, aromatic-H), 7.91-7.89 (d, 2H,aromatic-H), 7.57-7.55 (t, 2H, aromatic-H), 7.53-7.37 (t, 4H, aromatic-H), 7.33 (d, 2H, aromatic-H) 7.27 (t, 1H, CH methine) 4.03 (d, 2H, CH_2), 1.78 (s, 1H, NH).
II 3400 (Ar-H), 3435 (NH strech), 2831 (C-H strech), 1682.59 (C=O stretch),1602.56 (heterocyclic ring), 1509.99 (C-O-CH_3), 1255.32 (C-N strech), 1165.76 (OCH_3 strech).	9.78 (s, 1H, NH), 8.496 (s, 1H, aromatic-H), 7.75-7.73 (d, 2H, aromatic-H), 7.69-7.67 (t, 2H, aromatic-H), 7.19 (t, 1H, aromatic-H), 6.95-6.93 (d, 2H, aromatic-H), 6.77 (t, 1H, CH methine), 4.45 (d, 2H,CH_2), 4.44 (s, 3H, OCH_3), 2.498, s, 1H, NH)
III 3400 (Ar-H), 3444 (NH str), 2857 (C-H str), 1698.98 (C=O stretch), 1602.56 (heterocyclic ring),1484.92 (C-Cl strech),1292.07 (C-N stretch).	10.21 (s, 1H, NH), 8.62 (s, 1H, aromatic-H), 7.78 (d, 2H, aromatic-H), 7.64 (t, 2H, aromatic-H), 7.43 (t, 2H, aromatic-H), 7.09 (t, 1H, aromatic-H), 7.01 (d, 2H, aromatic-H), 7.27 (t, 1H, methine), 4.38 (d, 2H,CH_2), 1.77 (s, 1H, NH)
IV 3402 (Ar-H), 3435 (NH strech), 2831 (C-H strech), 1682.59 (C=O stretch), 1602.56 (heterocyclic ring), 1255.3 (C-N strech).	8.68 (s, 1H, N-H), 5.25 (s,1H, aromatic-H), 7.476 (d,2H, aromatic-H), 7.37 (t, 2H, aromatic-H), 7.43 (d, 2H, aromatic-H), 7.27 (t, 1H, aromatic-H), 7.45 (d, 2H, aromatic-H), 3.92 (t, 1H, aromatic-H), 1.26 (d, 2H, CH_2), 0.89 (s, 1H, NH).
V 3402 (Ar-H), 3435 (NH strech), 2831 (C-H strech), 1682.59 (C=O stretch), 1602.56 (heterocyclic ring), 1509.99 (C-O-CH_3), 1255.32 (C-N strech), 1166.72 (OCH_3 strech)	9.78 (s, 1H, NH), 8.49 (s, 1H, aromatic-H), 7.75-7.73 (d, 2H, aromatic-H), 6.95-6.93 (d, 2H, aromatic-H), 7.69-7.67 (d, 2H, aromatic-H), 6.88-6.86 (d, 2H, aromatic-H), 3.81 (t, 1H, CH methine), 2.49 (d, 2H, CH_2), 3.05 (s, 3H, OCH_3), 2.06 (s, 1H, NH).
VI 3400 (Ar-H), 3444 (NH str), 2857 (C-H str), 1698.98 (C=O stretch), 1602.56 (heterocyclic ring), 1489.79 (C-Cl str), 1292.07 (C-N str)	8.62 (s, 1H, NH), 7.80(s, 1H, aromatic-H), 7.78 (d, 2H, aromatic-H), 7.45-7.33 (d, 4H, aromatic-H), 7.57-7.52 (d, 2H, aromatic-H), 4.68 (t, 1H, CH methine), 2.04-2.00 (d, 2H, CH_2), 1.44 (s, 1H, NH).

Molinspiration Calculation & Bioactivity score: Predicted results of compounds **I-VI** in terms of molecular properties (molecular weight, MilogP and TPSA, nrotb, volume etc.) are valued in **Table 2**.

Drug likeness prediction of compounds **I-VI** along with the standard compound Ampicillin was tabulated in **Table 3**.

Antibacterial activity: The synthesized compounds exhibited weak to significant in-vitro antibacterial activities as compared to standard ampicillin as shown in **Table 4**.

TABLE 2: MOLINSPIRATION CALCULATION FOR SYNTHESIZED COMPOUNDS (I-VI)

Compound	MilogP	TPSA	Natoms	MW	nON	nOHNH	Nviolation	Nrotb	Volume
I	3.181	41.124	20	264.33	3	2	0	2	249.445
II	3.238	50.359	22	294.35	4	2	0	3	274.991
III	3.851	41.125	21	298.77	3	2	0	2	262.981
IV	3.851	41.125	21	298.77	3	2	0	2	262.981
V	3.916	50.359	23	328.79	4	2	0	3	288.526
VI	4.537	41.125	22	333.21	3	2	0	2	276.516
Ampicillin	-0.873	112.729	24	349.71	7	4	0	4	298.868

TABLE 3: DRUG LIKENESS CALCULATION BY MOLINSPIRATION

Compound Code	GPCR ligand	Ion Channel Modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
I	-0.16	-0.24	-0.44	-0.55	-0.33	-0.30
II	-0.14	-0.31	-0.39	-0.45	-0.30	-0.33
III	-0.12	-0.23	-0.41	-0.52	-0.33	-0.32
IV	-0.12	-0.23	-0.41	-0.52	-0.33	-0.32
V	-0.13	-0.30	-0.38	-0.44	-0.31	-0.35
VI	-0.09	-0.22	-0.37	-0.46	-0.28	-0.31
Ampicillin	0.04	-0.47	-0.71	-0.61	0.87	0.25

TABLE 4: ZONE OF INHIBITION (ZOI) OF SYNTHESIZED COMPOUNDS (I-VI)

S. No.	Derivative	Zone of inhibition (in mm)			
		S. aureus	*S. epidermidis*	*P. aeruginosa*	*E. coli*
1	I	11	12	7	7
2	II	9	11	-	6
3	III	7	8	12	13
4	IV	10	12	7	6
5	V	7	8	13	12
6	VI	-	-	8	10
7	Ampicillin	17	16	15	16

DISCUSSION: FTIR spectra of all synthesized compounds nearer to 3400 cm^{-1} attributable to Aromatic-H, whereas absorption bands at 3435 cm^{-1} confirms the presence of NH group. Narrow absorption frequency of C=O bond occurs at 1660cm^{-1}, and broad band nearer to 1600 cm^{-1} indicative of CH in aromatic ring. A band at 1258.32 cm^{-1} showed C-N stretch. ^{1}H-NMR predicted NH at 8-12 ppm & another NH at 1.7-2.5 ppm, CH benzene at 6.6-7.98 ppm (Table 1).

Compound **I and IV** having substitutent (6-phenyl, 6-chloro phenyl) has documented good antibacterial activity against *S. aureus* (zone of inhibition=11, 10mm respectively), *S. epidermidis* (ZOI=12, 12mm) and less activity against *Gram*-Negative bacteria. Compound **III & V** (4'-chloro-benzylidine, 4'-methoxy-benzylidine**)** has reported less to least activity against S. *aureus* (ZOI=7, 7mm respectively), *S. epidermidis* (ZOI=8, 8mm) and good activity against *P. aeruginosa* (ZOI=12, 13mm respectively), *E. coli* (ZOI=13, 12mm respectively).

Compound **VI** (6-chloro-phenyl, 4'-chloro-benzylidine) has shown moderate antibacterial activity against Gram-Negative bacteria and completely inactive for Gram-Positive bacteria. In case of compound **II** with having 4'-methoxy-benzylidine substituent has shown moderate activity.

According to Lipinski rule of five[8], most "drug-like" molecules have logP <= 5, molecular weight <= 500, number of hydrogen bond acceptors <= 10, and number of hydrogen bond donors <= 5. Molecules violating more than one of these rules may have problems with bioavailability [9]. On close inspection of Table 3, all molecules were found in compliance with Lipinski rule of five recommendations for new chemical entity to have good bioavailability with no violations.

The Milog P valve of all compounds were found below five, suggest that the molecules have good permeability across the cell membrane which in turn is needed for generation of bioactivity.

In respect of TPSA, all the compounds were within the limit i.e. 160 Å which implies that molecules are fulfilling the optimal requirement for drug absorption.

On comparing the relative activity scores of Ampicillin with **I-VI** compounds utilizing the above discussed six drug classes, all the compounds were showed expected similar bioactivity especially in case of ion channel modulator, kinase inhibitor and nuclear receptor ligand. Different substitution pattern on core bioactive scaffold pyridazine-3-one were able to induce marked effect on antibacterial activity. Compounds **I, IV** have proven significant activity against Gram positive bacteria and **III, V** showed significant activity against Gram negative bacteria.

On the basis of effective pharmacomodulation and further optimization, present work may be fruitful for ongoing work in antibacterial chemotherapy.

REFERENCES:

1. Guan LP, Xian-Qing XS, Quan YC,Quan ZS: Synthesis and anticonvulsant activity of a new 6-alkoxy-[1,2,4]triazolo[4,3-b]pyridazine. European Journal of Medicinal Chemistry 2010; 45(5): 1746–1752.
2. Kandile NG, Mohamed MI, Zaky H, Mohamed HM. Novel pyridazine derivatives: Synthesis and antimicrobial activity evaluation. European Journal of Medicinal Chemistry 2009; 44(5): 1989–1996.
3. Wang Z,Wang M,Yao X,Li Y, Tan J Wang, Qiao W, Geng Y, Liu Y, Wang Q. Design, synthesis and antiviral activity of novel pyridazines. European Journal of Medicinal Chemistry. 2012; 54: 33–41.
4. Won YH, Park MS. Synthesis and anticancer activities of new 3-allylthio-6-(mono or disubstituted) aminopyridazines. Arch. Pharm. Res. 2010; 33(2):189-96.
5. Wies1aw Malinka W, Redzicka A, Wiesek MJ, Filipek B, Dyba1 M, Karczmarzyk Z, Urbanczyk-Lipkowska Z, Kalicki P. Derivatives of pyrrolo[3,4- d]pyridazinone, a new class of analgesic agents. European Journal of Medicinal Chemistry 2011; 46: 4992- 4999.
6. Molinspiration cheminformatics [homepage on the internet], Nova ulica, SK-900 26 Slovensky Grob, Slovak Republic; [cited 2012 July 3], Available from http://www.molinspiration.com.
7. Shrinivasan D, Sangeetha N, Suresh T, Lakshmanaperumalsamy P. J. Ethanophamacol. 2001;74: 217-220
8. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Adv. Drug Delivery Rev. 1997; 23(1-3): 4-25.
9. Veber D.F., Johnson S.R., Cheng H.Y., Smith B.R., Ward K.W., Kopple K.D. Molecular Properties That Influence the Oral Bioavailability of Drug Candidates J. Med. Chem. 2002; 45(12): 2615-2623.

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