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Research Article

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Synthesis, Characterization and Biological activity of Novel 6-Methyl-2-oxo-N-(4-(Piperidin-1-yl) Phenyl)-1,2,3,4-Tetrahydropyrimidine-5-Carboxamide Derivatives

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ABSTRACT

Novel Biginelli dihydropyrimidines were prepared using 4-fluoro nitrobenzene and Piperidine in presence of K2CO3 in DMSO solvent, followed by reduction with Raney Ni and hydrazine hydrate. Then, this amine was reacted with ethyl acetoacetate in Xylene at high temperature and isolated 3-oxo-N-(4-(piperidin-1-yl) phenyl) butanamide as a scaffold, This scaffold was finally react with urea and different aldehyde in presence of p-toluene sulphonic acid as an efficient catalyst to isolate titled compound derivatives. The chemical structures of synthesized compounds were confirmed by 1H-NMR, FT-IR and Mass spectral analysis. The synthesized compounds were screened for potential Antimicrobial, Antifungal and Antimalerial activity.

Keywords: Biginelli condensation; Dihydropyrimidines; Antimicrobial; Antimalerial

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INTRODUCTION

The purpose of this study was to synthesize substituted 3, 4-dihydropyrimidin-2(1H)-ones (DHP) and to evaluate them for their antibacterial and antifungal activities. These compounds were synthesized by cyclo condensation reaction among substituted aromatic aldehyde, active Methylene compounds and urea in the presence catalytically amount of p-toluene sulphonic acid (pTSA). In the past decades, a broad range of biological effects, including antiviral , anti tumor, antibacterial and anti-inflammatory activities has been ascribed to these partly reduced pyrimidine derivatives. More recently, DHP have emerged as, for e.g., orally active antihypertensive agents.

In recent years Dihydropyrimidinones (DHP) and their derivatives have occupied an important position in natural and synthetic organic chemistry because of their wide range of biological activities, such as antioxidant, anti-inflammatory, anthelminic, antimicrobial, [1-3] ant tuberculoses [4] and anticancer. Antihypertensive agents, antagonists and neuropeptide Y (NPY) antagonists, explains the widespread presence of studies related to the “Biginelli compounds in the specialized literature,[5] not to mention the fact that several marine alkaloids, with interesting biological activities as well, include the DHPM motif in their structures.[6] Apart from subtle modifications from the original design,[7] the Biginelli reaction takes place by mixing an aromatic aldehyde, substituted or unsubstituted urea or thiourea and an active 1,3-dicarbonyl compound under appropriate conditions [8]

Among the wide range of compounds tested as potential anticancer agents, pyrimidine and fused pyrimidine derivatives comprise an important class of therapeutic agents. They were reported as antitumor, [9-11] antimicrobial, [12] anti-inflammatory,[13] anti HIV,[14] antinociceptive,[15,16] and antioxidant agents.[17],

In 1893, P Biginelli reported on the acid catalyzed cyclo-condensation reaction of ethyl acetoacetate, benzaldehyde and urea. The reaction was carried out by simply heating a mixture of the three components dissolved in ethanol with a catalytic amount of hydrochloric acid at reflux temperature. The product of this novel one-pot, three components synthesis that precipitated on cooling of the reaction mixture was identified as

3,4-dihydropyrimidin-2*(1H)*-one and this reaction is known as "Biginelli reaction", or "Biginelli Condensation", or as "Biginelli dihydropyrimidines synthesis"

EXPERIMENTAL SECTION

Materials and Methods

All the reagents were of commercial grade and used after purification. The synthesis of all compounds was carried out as per the general reaction scheme mentioned in figure 1. Melting points were determined in open capillary tubes and are uncorrected. Progress of reaction was monitored by TLC (Merck silica gel PF254 plates) of 0.5 mm thickness and spots were located by iodine and UV light. IR spectra were recorded on Shimadzu FT-IR-8400 instrument. Mass spectra were recorded on Shimadzu GCMS-QP-2010 model using Direct Injection Probe technique. 1H-NMR was determined in CDCl3/DMSO-d6 solution on a Bruker Ac 400 MHz spectrometer data.

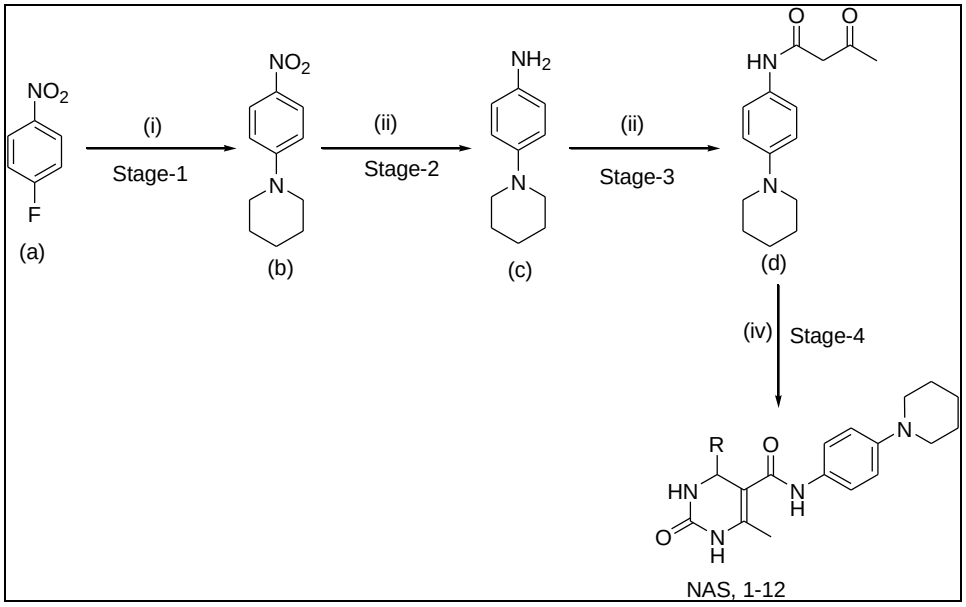


Figure 1: General reaction scheme

(i) Piperidine, K2CO3, DMSO, 55°C, 2h. (ii) Raney Ni, NH2-NH2, Ethanol, 30°C, 30 min. (iii) Ethyl acetoacetate, Xylene, 160°C, 30 min (iv) Aromatic aldehyde, pTSA, Ethanol, 70°C, 12h.

General Procedure for Synthesis of Novel N-(4-(8-methoxy-2-oxo-2H-chromen-3-yl) thiazol-2-yl) amide derivatives

Step-1: 1-(4-nitrophenyl) Piperidine [18] (b)

To a solution of 4-Fluoronitrobenzene (**a**) (10 g, 70.92 mmol) in DMSO (40 ml), potassium carbonate (14.68 g, 106.38 mmol) and Piperidine (7.23g, 85.10 mmol) were added and the mixture was stirred at 55° C for 2 hr. The progress of reaction was monitored by TLC using Ethyl acetate: Hexane (2:8) as Mobil phase. After completion of reaction, reaction mass poured in water and solid precipitate was filtrated out. (**B**, 13.89g) yield 95%.

Step-2: 4-(piperidin-1-yl) aniline[19] (c)

13.89g (67.34 mmol) 1-(4-nitrophenyl) Piperidine (**b**) was dissolved in Ethanol (70 ml) and to this solution was added Raney Ni (1.40 g). Then, Hydrazine hydrate was added drop wise in the reaction mass controlling the temperature to 25-35°C. Reaction progress was monitored by TLC using Ethyl acetate: Hexane (2:8) as Mobil phase. After completion of reaction, mass was filtrated through celite bed and distilled out under reduced pressure to get 4-(piperidin-1-yl) aniline (**c**, 8.90 g) 75% yields.

Step-3: 3-oxo-N-(4-(piperidin-1-yl) phenyl) butanamide [20] (d)

A solution of 4-(piperidin-1-yl) aniline (**c**, 8.90 g, 50.49 mmol) in Xylene (70 ml) was added to a solution of ethyl acetoacetate (26.7 g 205.16) in Xylene (30 ml) at 140-150°C over the period of 30 min. After completion of addition, the mass was refluxed for further 10-15 minutes. Reaction progress was monitored by TLC using

Ethyl acetate: Hexane (2:8) as Mobil phase. After completion of reaction, the reaction mass was cooled to 10-15°C and filtrated to isolate the solid product and recrystallized in ethanol to get desired pure product (**d**, 6.70 g) having 50.9% yield.

Step-4: General procedure for 6-methyl-2-oxo-N-(4-(piperidin-1-yl) phenyl)-1,2,3,4-tetrahydropyrimidine-5-carboxamide derivative[21] (NAS, 1-12)

To solution of 3-oxo-N-(4-(piperidin-1-yl) phenyl) butanamide (d) (0.250 g 1.15 mmol) in 5 ml ethanol were added appropriate aldehyde (1.20 mmol), Urea (0.08 g 1.38 mmol) and catalytic amount of p-toluene sulphonic acid, then the reaction mixture was heated to 75-80° for 12 h. Reaction progress was monitored by TLC using Methylene dichloride: Methanol (9.5:0.5) as Mobil phase. After completion of reaction, the mass was cooled to 0-5°C. The precipitated mass was filtered, washed with Ethanol to isolate the pure product derivatives as mentioned in Table-1.

The spectral data

NAS -1

LCMS (ESI) m/z (M+H**)**: 397.4. IR (cm-1): 3450 (NH), 3277 (N-H), 2935(CH3), 1672 (NHC=O), 1517 (C-N), 1442, 1411 (ArH), 1H-NMR (DMSO-d6**)** δ: 1.09-1.24 (m, 6H), 2.23 (s, 3H), 3.96-4.02 (m, 4H), 5.05 (s, 1H), 6.60-6.85, (m, 4H), 7.07-7.11 (m, 3H), 7.69 (s, 1H), 9.16 (s, 1H), 9.41(s, 1H), mp 207-209°C.

NAS -2

LCMS (ESI) m/z (M+H**)**: 409.5. IR (KBr, cm-1): 3421 (NH), 3213 (N-H), 2953(-CH3), 1680 (NH-C=O), 1514 (C-N), 1477, 1421 (ArH), 1H-NMR (DMSO-d6**)** δ: 1.52-1.95 (m, 6H), 2.05 (s, 3H), 3.42-3.52 (m, 4H), 5.43 (s, 1H), 7.03-7.13, (m, 4H), 7.35-7.41, (m, 4H), 7.71(s, 1H), 8.88 (s, 1H), 9.88 (s, 1H), mp 213-214°C.

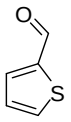
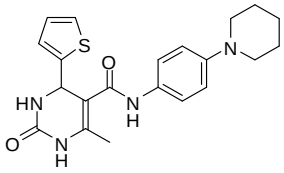
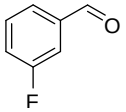
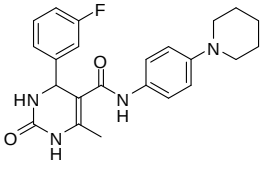
NAS -4

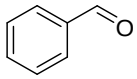
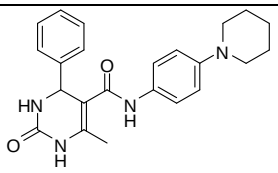
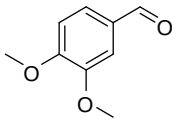
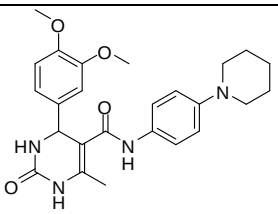
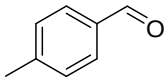
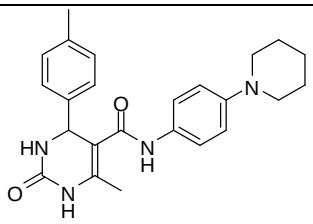
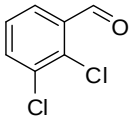
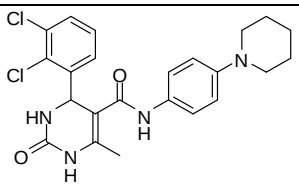
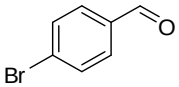
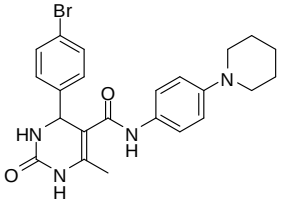
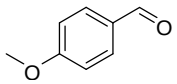
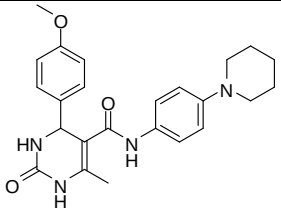
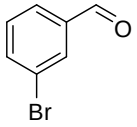
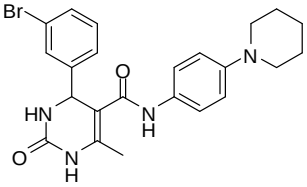
LCMS (ESI) m/z (M+H**)**: 451.7. IR (cm-1): 3438 (NH), 3232 (N-H), 2991(-CH3), 1676 (NH-C=O), 1517 (C-N), 1411, 1392 (ArH), 1H-NMR (DMSO-d6**)** δ: 1.49-1.59 (m, 6H), 2.01 (s, 3H), 3.00-3.03 (m, 4H), 3.68 (s, 3H), 3.70 (s, 3H), 5.33 (s, 1H), 6.79-6.92, (m, 5H), 7.37, (d, *j*=9.2, 2H),7.46, (s, 1H), 8.60 (s, 1H), 9.31(s, 1H), mp 202-204°C.

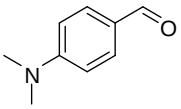
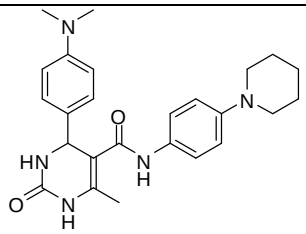
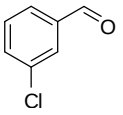
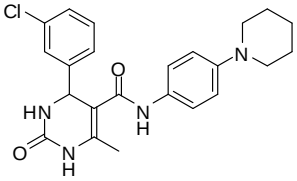
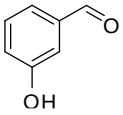
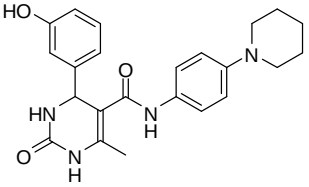
NAS -5

LCMS (ESI) m/z (M+H**)**: 405.64. IR (cm-1): 3406 (NH), 3280 (N-H), 2972(-CH3), 1670 (NH-C=O), 1517 (C-N), 1452, 1411 (ArH), 1H-NMR (DMSO-d6**)** δ: 1.49-1.59 (m, 6H), 2.01 (s, 3H), 2.25 (s, 3H), 3.00-3.03 (m, 4H), 5.34 (s, 1H), 6.81, (d, *j*=8.4, 2H), 7.10-7.16, (m, 4H), 7.35-7.37, (m, 2H), 7.48 (s, 1H), 8.61 (s, 1H), 9.31 (s, 1H), mp 212-214°C.

Table 1: 6-methyl-2-oxo-N-(4-(piperidin-1-yl) phenyl)-1,2,3,4-tetrahydropyrimidine-5-carboxamide derivative

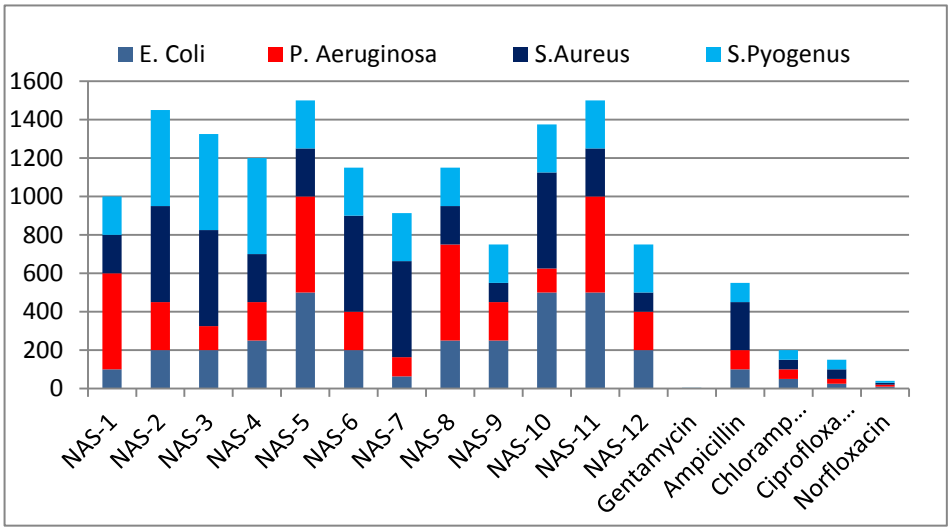
Sr No	Name	Acid chloride	Structure	Mol. wt	Qty-(mg)	Yield
1	NAS-1			396.5	100	26.2%
2	NAS -2			408.4	195	49.7%

Sr No	Name	Acid chloride	Structure	Mol. wt	Qty-(mg)	Yield
3	NAS -3			390.4	40	10.7%
4	NAS -4			450.5	120	27.7%
5	NAS -5			404.5	130	33.4%
6	NAS -6			459.3	200	45.3%
7	NAS -7			469.3	180	39.8%
8	NAS -8			420.5	50	12.4%
9	NAS -9			469.3	105	23.3%

Sr No	Name	Acid chloride	Structure	Mol. wt	Qty-(mg)	Yield
10	NAS -10			433.5	160	38.4%
11	NAS -11			424.9	120	29.4%
12	NAS -12			406.4	150	38.4%

BIOLOGICAL EVALUATION

All the synthesized compounds were screened for their potential antibacterial, antifungal and antimalerial activity. The *in vitro* antibacterial and antifungal activity was conducted with representative strains of Gram-negative bacteria (*Escherichia Coli*, *Pseudomonas Aeruginosa*) and Gram-positive bacteria (*Staphylococcus Aureus*, *Streptococcus Pyogenus*). *Candida Albicans*, *Aspergillus Niger and Aspergillus Clavatus* were used as representative strains for antifungal activity. The Gentamycine, Ampicillin, Chloramphenicol, Ciprofloxacin and Norfloxacin were used as standard antibacterial drugs for the comparison. While, Nystatin and Greseofulvin were used as standard antifungal drugs. The antimalerial activity was studied using *Plasmodium facliparum*. The results of antimicrobial activity are summarized in table 2 and 3. While, table 4 summarize the results of antimalerial activity.



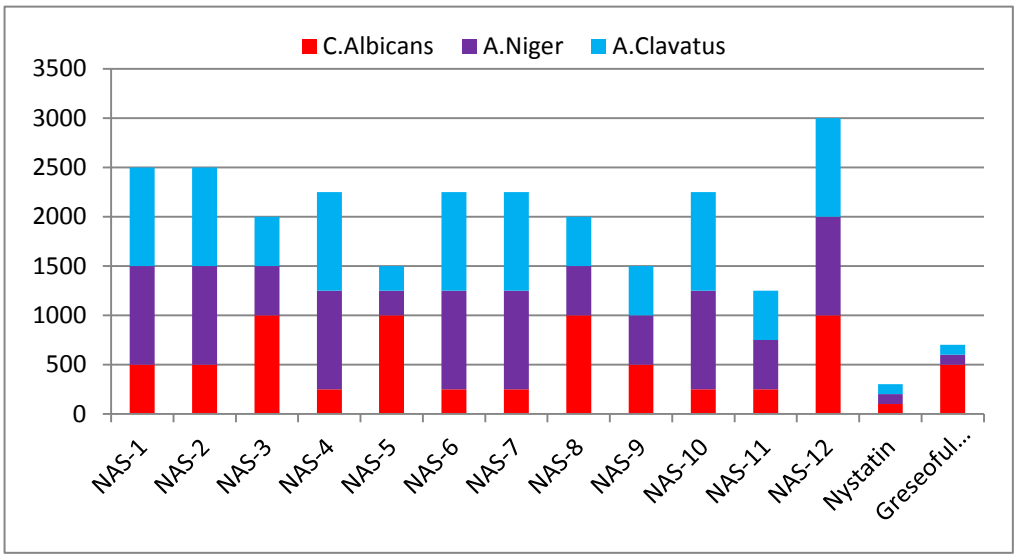
Graph-1 Antibacterial Activity of NAS 1-12

Table 2: Antibacterial Activity, Minimum Inhibition Concentration (MIC)

Compound Code	*E.Coli* MTCC 443	*P.Aeruginosa* MTCC 1688	*S.Aureus* MTCC 96	*S.Pyogenus* MTCC 442
NAS-1	**100**	500	**200**	200
NAS-2	200	250	500	500
NAS-3	200	125	500	500
NAS-4	250	200	**250**	500
NAS-5	500	500	**250**	250
NAS-6	200	200	500	250
NAS-7	**62.5**	**100**	500	250
NAS-8	250	500	**200**	200
NAS-9	250	200	**100**	200
NAS-10	500	125	500	250
NAS-11	500	500	250	250
NAS-12	200	200	**100**	250
Gentamycine	0.05	1	0.25	0.5
Ampicillin	**100**	**100**	**250**	**100**
Chloramphenicol	50	50	50	50
Ciprofloxacin	25	25	50	50
Norfloxacin	10	10	10	10

Table 3: Antifungal Activity, Minimum Inhibition Concentration (MICa)

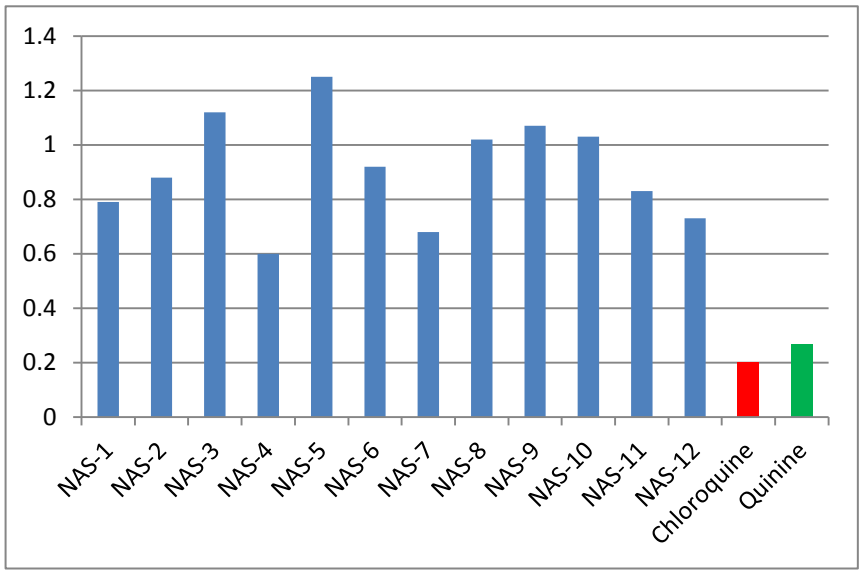
Compound Code	C.Albicans MMCC227	A. Niger MMCC282	A.Clavatus MMCC1323
NAS-1	**500**	1000	1000
NAS-2	**500**	1000	1000
NAS-3	>1000	500	500
NAS-4	**250**	1000	>1000
NAS-5	>1000	250	250
NAS-6	**250**	1000	1000
NAS-7	**250**	>1000	>1000
NAS-8	1000	500	500
NAS-9	**500**	500	500
NAS-10	**250**	>1000	>1000
NAS-11	**250**	500	500
NAS-12	1000	1000	1000
Nystatin	100	100	100
Greseofulvin	**500**	100	100



Graph-2 Antifungal Activity of NAS 1-12

Table 4: Antimalerial Activity against *Plasmodium facliparum*, Minimum Inhibition Concentration (MIC)

Compound Code	Mean IC50 Values (µg/ml)
NAS-1	0.79
NAS-2	0.88
NAS-3	1.12
NAS-4	0.6
NAS-5	1.25
NAS-6	0.92
NAS-7	0.68
NAS-8	1.02
NAS-9	1.07
NAS-10	1.03
NAS-11	0.83
NAS-12	0.73
Chloroquine	0.020 (µg/ml)
Quinine	0.268 (µg/ml)



Graph-3 Antimalerial Activity of NAS 1-12 against *Plasmodium facliparum*

RESULTS AND DISCUSSION

Aiming to adopt simpler reaction conditions for syntheses of new biologically active heterocyclic compounds, we have established convenient and practical methodology for the preparation of a variety 6-methyl-2-oxo-N-(4-(piperidin-1-yl) phenyl)-1,2,3,4-tetrahydropyrimidine-5-carboxamide derivative. The reaction of commercially available 4-fluoro nitrobenzene and Piperidine in presence of K2CO3 in DMSO solvent lead to compound (**b**) which was reduced with Raney Ni and hydrazine hydrate to produce amine **c**, this amine (**c**) was reacted with ethyl acetoacetate in Xylene at high temperature and isolated 3-oxo-N-(4-(piperidin-1-yl) phenyl) butanamide as a scaffold, This scaffold was finally react with Urea and different aldehyde in presence of p-toluene sulphonic acid as an efficient catalyst to isolate ~~title~~ compound **d**. This compound **d** was further reacted with different aldehyde to result in novel series of amide compounds as listed in Table-1.

All the new synthesized compounds were characterized by 1H NMR, IR and LCMS/Mass spectra analysis. The presence of characteristics absorptions at 3421 and 3213 cm-1 in the infrared spectra confirms the presence of NH of amide derivative. The presence of absorbance band at 1670 cm-1 in IR spectra confirms the presence of amide Carbonyls moiety of carboxamide heterocyclic. In addition to these, presence of characteristics bands in 1H NMR, IR and LCMS/Mass spectra confirm the structure of synthesized compounds.

All newly synthesized compounds were screened for their potential antibacterial, antifungal and antimalerial activity. Among all, NAS-1 and NAS-7 were found to possess equipotent activity to Ampicillin (MIC=100 µg/mL) against E.Coli and P.Aeruginosa (gram -ve strains) while NAS-4 and NAS-5 were found to be equipotent to Ampicillin against S.Aureus (gram +ve) while compound NAS-1, NAS-8, NAS-9 and NAS-12 were found to be more efficient than Ampicillin (MIC=250 µg/mL) against S.Aureus. All the newly synthesized

compounds were not shown good antibacterial activity against S.Pyogenus (gram +ve). Overall, compound NAS-7 possessed moderate to good antibacterial activity. On the antifungal activity side, compound NAS-1, NAS-2 and NAS-9 were found be equipotent to Greseofulvin (MIC=500 μg/mL) while compound NAS-4, NAS-6, NAS-7, NAS-10 and NAS-11were found to be more potent against C.Albicans; rest other synthesized compounds were not shown good antifungal activity and less potent than standard drugs. On antimalerial screening, compound NAS-4, NAS-7 and NAS-12 found to possess active against *Plasmodium facliparum.*

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