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# Synthesis, Free Radical Scavenging Activity, Antimicrobial And Molecular Docking Studies Of Novel Pyrazine 2-Carboxylic Acid Derivatives Of Piperazines.

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**ABSTRACT**

A novel series of pyrazine-2-carboxylic acid derivatives were synthesized using substituted pyrazine 2-carboxylic acids with various piperazines in the presence of T3P (propyl phosphonic anhydride) as a coupling reagent. The synthetic approach, a possible reaction mechanism and the analytical data of the synthesized compounds are presented and these compounds were analyzed using M.P, LC-MS, $H^1$NMR, and FT-IR. Further, the synthesized compounds were evaluated for their antioxidant (ABTS and DPPH method) and antimicrobial activities(agar well diffusion method) Among the synthesized pyrazine-2- carboxylic acid derivatives (3-aminopyrazin-2-yl)(4-(6-aminopyrimidin-4-yl)piperazin-1-yl)methanone (**P10**) exhibited good antioxidant activity as well as moderate antimicrobial activity where in, (4-(6-aminopyrimidin-4-yl)piperazin-1-yl)(5-methylpyrazin-2-yl)methanone (**P4**) depicted highest antimicrobial activity alone. In molecular docking studies, although all the molecules showed good inhibition with GlcN-6-P synthase, the **P4** showed higher docking score. So, it can be predicted as inhibition of GlcN-6-P synthase may be responsible for antibacterial activity of the synthesized piperazine derivatives. The results obtained prompt us in further studies for characterizing these derivatives for their diverse biological activities.

**Keywords:** Antioxidant, Antimicrobial, Coupling reagent, Pyrazine-2-carboxylic acid, Piperazines, T3P.

**Abbreviations:** T3P, propyl phosphonic anhydride; EDCI, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide; HOBt, 1-hydroxybenzotriozole; DCC, Dicyclohexylcarbodiimide;HBTU, 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate; TBTU, N, N, N', N'-Tetramethyl-O-(benzotriazol-1-yl)uroniumtetrafluoroborate; ABTS, 2, 2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical; DPPH, (1, 1-diphenyl-2-picrylhydrazyl) radical.

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## INTRODUCTION

Pyrazines are a vital class of heterocyclic compounds present in nature and are also synthesized in the laboratory since 1876 [1-3]. Different synthetic methods have been adopted to prepare these biologically important heterocyclic derivatives [4-7]. The usual acid-amine coupling reagents used in the synthesis of heterocyclic compounds are EDCI (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide andHOBt (1-hydroxybenzotriozole) [8-10], DCC (Dicyclohexylcarbodiimide) [11], HBTU (1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate) [12], TBTU (N,N,N',N'-Tetramethyl-O-(benzotriazol-1-yl)uroniumtetrafluoroborate) [13] etc. In comparison with these coupling agents T3P (propyl phosphonic anhydride) delivers outstanding advantages such as low toxicity, easy handling, water solubility or hydrophilicity. When T3P was used in coupling reactions, there are no byproducts and hence easy purification of products with high yield and low epimerization [14-17].

Piperazines are used as building blocks in the synthesis of pyrazines due its therapeutic activity. This can be attributed to its conformational flexibility and the polar nitrogen atom in piperazine ring which confers bioactivity to molecules their by enhancing favorable interaction with macromolecules. Inclusion of both biologically active components pyrazines and piperazines in target molecules were found to be interesting for biological studies [18]. In particular, pyrazine derivatives exhibit wide range of pharmaceutical activities as anti-inflammatory [19], anticancer [20], antidiabetic [18] and diuretic [21]. It is noteworthy that, several substituted pyrazine derivatives demonstrated a marked inhibiting effect on tuberculosis (TB) [22]. Pyrazinamide (PZA) a derivative of pyrazine is an important anti- tuberculosis agent prescribed for the treatment of tuberculosis. Various pyrazine derivatives and pyrazinamide analogs were also studied for their high antimicrobial activity [23].

Microbial diseases are now more frequent and being still challenging to diagnose clinically. Due to the growing number of outbreaks of infections in hospitals and other patient care areas, it becomes essential to design a new drug molecule which can combat various infections. The drugs are primarily useful in situations where in, the normal host defense cannot be relied upon to kill or destroy the disease causing microorganisms. Since past two decades, a number of different classes of antimicrobial agents have been invented. Although there is a discovery of several synthetic and semi synthetic antibacterial compounds, such as sulfonamides, quinolones, chloramphenicol, penicillins, cephalosporins, tetracyclines, macrolides, oxazolidinones [24-30] and antifungal agents such as fluconazole, ketoconazole and miconazole, including amphotericin B [31-35], there has been a significant progress in this field. In spite of discovering the new efficient drugs and the studies made on antibacterial and antifungal therapies, the synthesis of competent new antimicrobial drugs is still a promising field of research.

Free radicals cause chronic pathogenesis leading to cancer, coronary heart disease, Alzheimer's disease, atherosclerosis and inflammation. The compounds have antioxidative activity mainly due to their redox potential, which exhibits an important role in absorbing and neutralizing free radicals [36, 37]. In recent times the application of antioxidants in the field of medicine has gained the interest in treating chronic diseases due to oxidative stress. Oxidative stress in humans can be induced by a various environmental factors including UV stress, pathogen invasion and oxygen shortage. Herbal and synthetic antioxidant compounds with high free radical scavenging activity are receiving increased attention in the field of medicine and biopharma research [38].

To the best of our knowledge synthesis of pyrazine-2-carboxylic acid derivatives of piperazines are not reported from T3P (proplyphosphonic anhydride), which is a well known acid-amine coupling reagent. Hence in this work, an attempt has been made to synthesize derivatives of pyrazine-2-carboxylic acids from T3P as an acid-amine coupling agent. The prepared novel pyrazine-2-carboxylic acid- heterocyclic compounds were characterized by MP, LC-MS, $^{1}$HNMR, FT-IR and investigated further for their antioxidant and antimicrobial properties. Further in molecular docking studies the molecules showed good inhibition with GlcN-6-P synthase substantiating its antibacterial activity.

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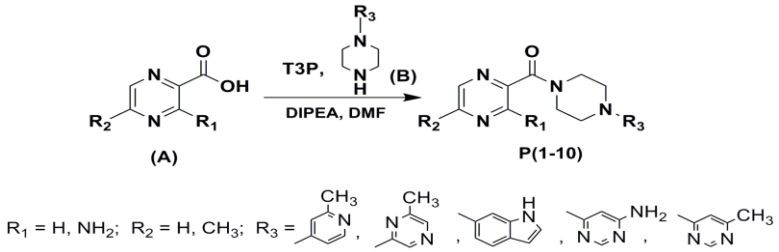
## MATERIALS AND METHODS

### Chemicals and instrumentation

The chemicals were purchased from commercial sources (Aldrich, Merck). Melting points were determined using Labfit melting point apparatus and are uncorrected. The IR spectra recorded on a Nicolet Spectrometer with KBr disks. The $^{1}$HNMR spectra were measured in DMSO-$d_6$ solution on a Bruker Advance (400 MHz) Spectrometer. The chemical shifts (δ) are given in ppm (parts per million), related to tetramethylsilane (TMS) as an internal standard and coupling constants (*J*) are reported in Hertz (Hz). Mass spectra for compounds were taken on a VG-Analytical Autospec-Q instrument. The reactions were monitored by thin layer chromatography (TLC). The solvents systems used for eluting the compounds P(1-10) in column chromatography are Chloroform/Methanol (9:1).

Novel pyrazine-2-carboxylic acid derivatives of piperazines were synthesized from substituted pyrazine-2-carboxylic acids (A) which includes 5-methylpyrazine-2-carboxylic, 3-amino pyrazine-2-carboxylic acid with N-heteroarylpiperazenes (B) using T3P as a coupling reagent as shown in Figure 1.

**Figure 1: General scheme for the preparation of pyrazene-2-carboxylic acids derivatives**
**DIPEA = Diisopropylethyl amine**




Initially, five different N-heteroarylpiperazine derivatives were prepared using Pd catalyst as shown in Figure 2. The reagents N-Bocpiperazine or tert-butyl piperazine-1-carboxyate (1.0 mmol), substituted aromatic bromo compounds (1.5 mmol) and dioxane (anhydrous, 15 mL), were sequentially charged into a clean reaction vessel fitted with a reflux condenser and nitrogen inlet adapter. NaOt-Bu (1.33 mmol), 2-(di-tert-butylphosphino) biphenyl (0.02 mmol) and $Pd(OAc)_2$ (0.01 mmol) were added in the presence of nitrogen gas. The mixture was heated to 100 $^{o}$C for 12 h. Next, the reaction mixture was diluted with $H_2O$ and extracted with EtOAc. The organic layer was concentrated under diminished pressure and the residue was purified by silica gel chromatography. The products Boc-N-hetero arylpiperazine compounds (1-5) were obtained as white solids (80-90% yield). The resulting compounds (1-5) treated with HCl-dioxane to afford N-hetero aryl piperazine hydrochloride salts (A-E), which were recrystallized using hexane and taken as such for the next step without further purification.

**Figure 2: Scheme ofPd-catalysed preparation of N-heteroarylpiperazine compounds**

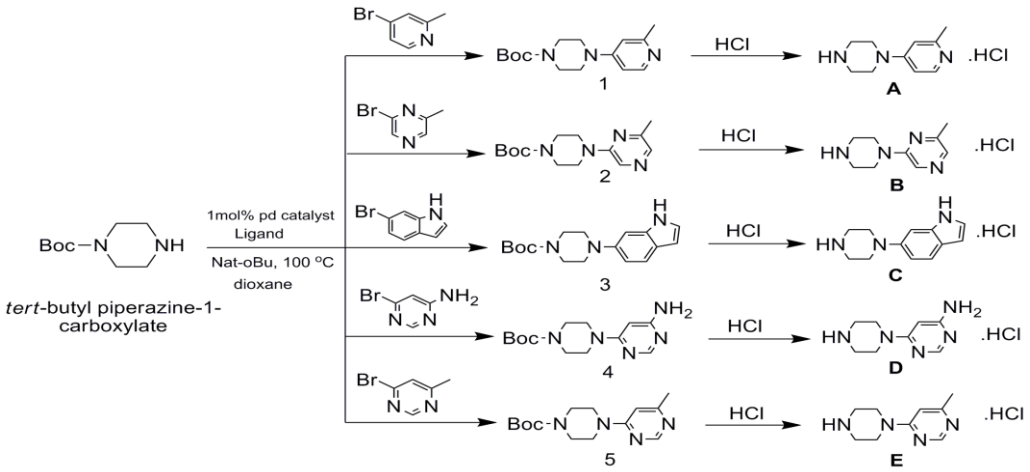


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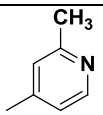
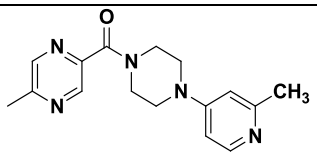
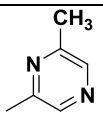
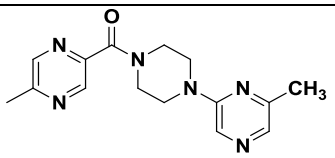
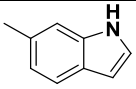
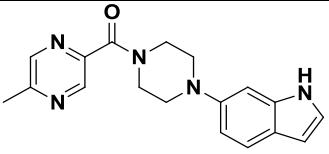
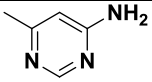
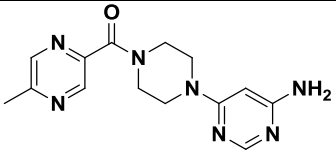
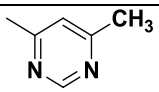
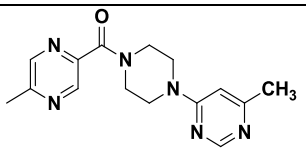
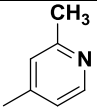
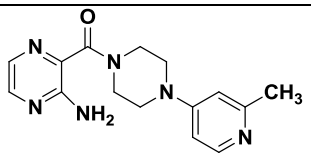
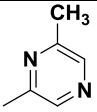
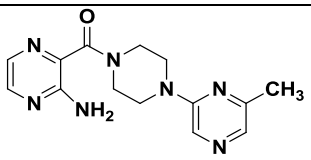
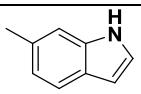
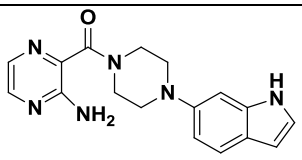
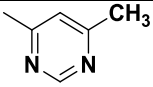
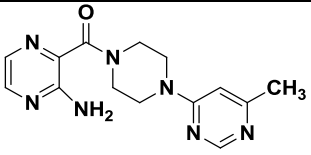
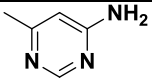
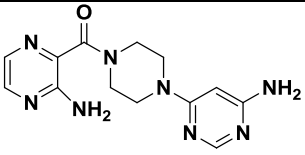
Table 1: **A series of pyrazine 2- carboxylic acid derivatives (P1-P10).**

| *Compounds* | *$R_1$* | *$R_2$* | *$R_3$* | *Structure of the target compounds* |
|---|---|---|---|---|
| **P1** | H | $CH_3$ |  |  |
| **P2** | H | $CH_3$ |  |  |
| **P3** | H | $CH_3$ |  |  |
| **P4** | H | $CH_3$ |  |  |
| **P5** | H | $CH_3$ |  |  |
| **P6** | $NH_2$ | H |  |  |
| **P7** | $NH_2$ | H |  |  |
| **P8** | $NH_2$ | H |  |  |
| **P9** | $NH_2$ | H |  |  |
| **P10** | $NH_2$ | H |  |  |

In a typical synthesis of pyrazine 2-carboxylic acid derivatives, T3P (1.3 mmol) was added drop wise to a stirred suspension of substituted pyrazine-2-carboxylic acid (i.e 5-methylpyrazine-2-carboxylic, 3-amino pyrazine-2-carboxylic acid, 1.0 mmol), N-heteroarylpiperazine hydrochloride (A-E, 1.1 mmol), and diisopropylethyl amine (3.0 mmol) in DMF (10 mL) in an inert nitrogen atmosphere. Further, the reaction mixture was stirred at room temperature for 30 min. After the completion of the reaction, it was diluted with water and extracted into ethyl acetate. The obtained product was dried over $Na_2SO_4$, filtered and the solvent was removed using rotavapor. The crude product was purified by silica gel chromatography, eluting with DCM/MeOH (9:1). The structural details of the obtained compounds are listed in table 1.

**Spectroscopic data**

**(5-methylpyrazin-2-yl)(4-(2-methylpyridin-4-yl)piperazin-1-yl)methanone (P1) -** Off-white solid. Yield 80%, M.P- 110 $^{o}$C , IR (cm$^{-1}$): 2922, 2850, 1676, 1650, 1645, 1638, 1630, 1258, 1226, 1015, 1005. $^{1}$HNMR (400 MHz, DMSO-$d_6$) δ: 2.31 (3H, s), 2.5 (3H, s), 3.51-3.32 (6H, m), 3.74 (2H, t, *J* = 4.56 Hz), 6.65-6.63 (1H, m), 6.70 (1H, d, *J* = 2.36 Hz), 7.77 (1H, d, *J* = 2.56 Hz), 8.05 (2H, t, *J* = 2.52 Hz), MS m/z: 298 (M+H)$^{+}$.

**(5-methylpyrazin-2-yl)(4-(6-methylpyrazin-2-yl)piperazin-1-yl)methanone (P2) -** Pale yellow solid. Yield 86 %, M.P - 82 $^{o}$C; IR (cm$^{-1}$): 2922, 2855, 1647, 1577, 1526, 1438, 1252, 1151, 997. $^{1}$HNMR (400MHz, DMSO-$d_6$) δ: 2.30 (3H, s), 2.55 (3H, s), 3.58 (4H, d, *J* = 1.64 Hz), 3.69-3.66 (2H, m), 3.75 (2H, d, *J* = 2.88 Hz), 7.76 (1H, s), 8.11(1H, s), 8.57 (1H, d, *J* = 0.92 Hz), 8.75 (1H, d, *J* =1.44 Hz), MS m/z: 299 (M+H)$^{+}$.

**(4-(1H-indol-6-yl) piperazin-1-yl)(5-methylpyrazin-2-yl)methanone (P3) -** Brown solid. Yield 74%, M.P – 177 $^{o}$C; IR (cm$^{-1}$): 2812, 1628, 1578, 1458, 1282, 1234, 1008. $^{1}$HNMR (400MHz, DMSO-$d_6$) δ: 2.55 (3H, s), 3.10 (2H, d, *J* = 4.44 Hz), 3.21 (2H, t, *J* = 4.68 Hz), 3.68 (2H, t, *J* = 4.72 Hz), 3.90 (2H, t, *J* = 4.44 Hz), 6.46 (2H, t, *J* = 7.36 Hz), 6.97 (1H, t, *J* = 7.6 Hz), 7.05 (1H, d, *J* = 8.08 Hz), 7.25 (1H, t, *J* = 2.72 Hz), 8.58 (1H, d, *J* = 0.72 Hz), 8.75 (1H, d, *J* =1.32 Hz), 11.05 ( 1H, br s), MS m/z: 322 (M+H)$^{+}$.

**(4-(6-aminopyrimidin-4-yl) piperazin-1-yl)(5-methylpyrazin-2-yl)methanone (P4) –** Yellow solid. Yield 92%, M.P – 187 $^{o}$C, IR (cm$^{-1}$): 3135, 2854, 1638, 1593, 1489, 1439, 1219, 1162, 1008. $^{1}$HNMR (400 MHz, DMSO-$d_6$) δ: 2.55 (3H, s), 3.46 (2H, t, *J* = 2.04 Hz), 3.57-3.52 (4H, m), 3.72 (2H, t, *J* = 4.48 Hz), 5.59 (1H, d, 0.68 Hz), 6.25 (2H, s), 7.95(1H, d, *J* = 0.68 Hz), 8.57 (1H, d, *J* = 0.96 Hz), 8.74(1H, d, *J* =1.4 Hz), MS m/z: 300 (M+H)$^{+}$.

**(5-methylpyrazin-2-yl)(4-(6-methylpyrimidin-4-yl)piperazin-1-yl)methanone (P5)–** Yellow solid. Yield 82%, M.P - 133 $^{o}$C, IR (cm$^{-1}$): 2885, 2920, 1636, 1603, 1498, 1438, 1341, 1260, 1234, 1008. $^{1}$HNMR (400 MHz, DMSO-$d_6$) δ: 2.25 (3H, s), 2.55 (3H, s), 3.57 (2H, t, *J* = 3.12 Hz), 3.73-3.62 (6H, m), 6.72 (1H, s), 8.40 (1H, t, *J* = 3.36 Hz), 8.57 (1H, d, *J* = 0.88 Hz), 8.75 (1H, d, *J* = 1.4 Hz), MS m/z: 300 (M+H)$^{+}$.

**(3-aminopyrazin-2-yl)(4-(2-methylpyridin-4-yl)piperazin-1-yl)methanone (P6) -** Off white solid. Yield 85%, Mp – 110 $^{o}$C, IR (cm$^{-1}$): $^{1}$HNMR (400 MHz, DMSO-$d_6$) δ: 2.39 (3H, s), 3.53-3.27 (6H, m), 3.63 (2H, t, *J* = 4.56 Hz), 6.50 (2H, s), 6.75-6.60 (1H, m), 6.78 (1H, d, *J* = 2.36 Hz), 7.8 (1H, d, *J* = 4.4 Hz), 8.15 (2H, m), MS m/z: 299.3 (M+H)$^{+}$.

**(3-aminopyrazin-2-yl)(4-(6-methylpyrazin-2-yl)piperazin-1-yl)methanone (P7) –** Off white solid. Yield 79 %, M.P - 103 $^{o}$C, IR (cm$^{-1}$): 3288, 2854, 1628, 1529, 1438, 1256, 1152, 998, $^{1}$HNMR (400 MHz, DMSO-$d_6$) δ: 2.29 (3H, s), 3.56-3.52 (4H, m), 3.73-3.66 (4H, m), 6.54 (2H, s), 7.77 (1H, d, *J* = 6.28 Hz), 7.78 (1H, s), 8.05 (1H, d, *J* = 2.56Hz), 8.1 (1H, s). MS m/z: 300.3 (M+H)$^{+}$.

**(4-(1H-indol-6-yl) piperazin-1-yl)(3-aminopyrazin-2-yl)methanone (P8) –**Yellow solid.Yield 85%, M.P – 195 $^{o}$C, IR (cm$^{-1}$): 3340, 1593, 1475, 1441, 1367, 1281, 1234, 1152, 1004, $^{1}$HNMR (400 MHz, DMSO-$d_6$) δ: 3.09 (2H, d, *J* = 4.4 Hz), 3.22 (2H, t, *J* = 4.6 Hz), 3.60 (2H, d, *J* = 4.6 Hz), 3.87(2H, d, *J* = 4.88), 6.45-6.42 (2H, m), 6.52 (2H, s), 6.97 (1H, t, *J* = 7.6 Hz), 7.04 (1H, d, *J* = 8.08 Hz), 7.25 (1H, t, *J* = 2.76 Hz), 7.78 (1H, d, *J* = 2.56 Hz), 8.04 (1H, d, *J* = 2.6 Hz), MS m/z: 323.3 (M+H)$^{+}$.

**(3-aminopyrazin-2-yl)(4-(6-methylpyrimidin-4-yl)piperazin-1-yl)methanone (P9) –** White solid. Yield 82%, M.P – 112 $^{o}$C; IR (cm$^{-1}$); 3302, 3189, 1597, 1499, 1439, 1343, 1279, 1153, 1007, $^{1}$HNMR (400 MHz, DMSO-$d_6$) δ: 2.25 (3H, s), 3.49 (2H, d, *J* = 5.32 Hz), 3.74-3.62 (6H, m), 6.55 (2H, s), 6.71 (1H, s), 7.77 (1H, d, *J* = 2.52 Hz), 8.05 (1H, d, *J* =2.56 Hz), 8.39 (1H, s), MS m/z: 300.0 (M+H)$^{+}$.

**(3-aminopyrazin-2-yl)(4-(6-aminopyrimidin-4-yl)piperazin-1-yl)methanone (P10) -** White solid. Yield 83%, M.P – 200 $^{o}$C, IR ($cm^{-1}$): 3301, 3189, 1597, 1445, 1237, 1153, 1008, 984, $^{1}$HNMR (400 MHz, DMSO-$d_6$) δ: 3.59-3.45 (6H, m), 3.69 (2H, d, *J* = 5.56 Hz), 5.59 (1H, d, *J* = 0.6 Hz), 6.25 (2H, s), 6.53 (2H, s), 7.77 (1H, d, *J* = 2.56 Hz), 7.95 (1H, d, *J* = 0.44 Hz), 8.04 (1H, d, *J* =2.6 Hz), MS m/z: 301.2 $(M+H)^{+}$.

**Evaluation of antioxidant properties of different synthesized compounds:**

**Free radical scavenging activity on 1, 1-diphenyl-2-picrylhydrazyl (DPPH).**

Sample compounds and standard ascorbic acid (1 $mgmL^{-1}$) at various concentrations (10-50 $\mu gmL^{-1}$) were taken and the volume was adjusted to 100 µL with methanol. Five milliliter of a 0.1 mM methanolic solution of DPPH was added and shaken vigorously. The tubes were incubated at 27$^{o}$C for 20 min and the absorbance was measured at 517 nm and the experiment was performed in triplicate [39]. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the formula.

DPPH radical scavenging activity % = [(Control OD – Sample OD)/ Control OD)] *100

**ABTS (2, 2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)) radical** cation decolorization **assay**

ABTS radical cation ($ABTS^{+}$) is produced when ABTS (7 mM) reacts with 2.45 mM ammonium persulfate and the mixture further incubated in dark at room temperature for 12-16 h before use. Sample compounds and standard ascorbic acid (1 $mgmL^{-1}$) at various concentrations (10-50 $\mu gmL^{-1}$) were taken and the volume was adjusted to 500 µL with DMSO along with another 500 µL of DMSO serves as blank. 300 µL of ABTS solution was added; the final volume was made up with ethanol to make 1 mL of the solution and was allowed to stand in dark for 30 min at RT. The absorbance was read at 745 nm and the experiment was performed in triplicate [40]. Radical cation decolorization activity was expressed as the inhibition percentage of cations by the sample and was calculated using the following formula.

ABTS radical scavenging activity % = [(Control OD – Sample OD)/ Control OD)] *100

**Evaluation of antimicrobial activity of different synthesized compounds:**

The bacterial and fungal pathogenic clinical isolates maintained in Centre for Emerging Technologies, Jain University, Bangalore, were used as a source for anti microbial activity studies. The test bacterial pathogens included are *Escherichia coli* (*E.coli*), *Pseudomonas aeruginosa*(*P. aeruginosa*), *Bacillus subtilis*(*B. subtilis*), *Staphylococcus aureus*(*S. aureus*) and *Candida albicans*(*C. albicans*). All tests were performed in a Muller-Hinton broth for the bacterial stains and sabouraud dextrose broth for the *C. albicans*. All the microbes were incubated overnight and the final concentration was adjusted to $2x10^{6}$ CFU/mL for the bacteria and $2x10^{5}$CFU for *C. albicans*. The concentrations of 3.125 $\mu gmL^{-1}$, 6.25 $\mu gmL^{-1}$, 12.5$\mu gmL^{-1}$, 25 $\mu gmL^{-1}$, 50 $\mu gmL^{-1}$ and 100 $\mu gmL^{-1}$ of each synthesized compound dissolved in methanol were used in the study against five microorganisms. Microorganisms were inoculated into Muller-Hinton broth along with the compounds and incubated at 37$^{o}$C for 24-48 h. The microbial growth was determined by the absorbance at 600 nm using a universal microplate reader. Gentamycin sulphate was used as a standard. The lowest concentration of the compounds completely inhibiting the growth of bacteria and fungi compared against standard antibiotic was recorded as minimum inhibitory concentration [41].

**Patchdock**

It is a prediction server, whose algorithm is inspired by object identification and image sequestration, the techniques commonly employed in computer vision. Patchdock is one of the resourceful algorithms working both for protein-small molecule and protein-protein docking purposes. The reason behind the high Patchdock efficiency is the fast transformational search apart from spatial pattern detection techniques involved in this algorithm [42, 43]. The surface of particular molecule is divided into patches on the basis of their surface shape. These patches usually seen are convex, concave or flat surface ones which are detected by segmentation algorithm. All patches are filtered except those containing hot spot residues [44]. The recognized patches in the previous step are matched by using Geometric Hashing [45] and Pose-Clustering

matching techniques. Patching is done concave versus convex and flat versus any patch [46]. The final step further filters those complexes with unacceptable penetrations of receptor atoms with ligand atoms and the acceptable complexes are ranked according to geometric shape complementarily score.

The structure of synthesized ligands P1, P2, P3, P4, P5, P6, P7, P8, P9, P10 was drawn by using ChemSketch while the structure of standard gentamycin was retrieved from PubChem (CID 3467) [47]. Structures were cleaned, explicit hydrogen atoms were added and 3D structure optimization was performed by same tool. The structures were exported as mol2 and later converted into PDB format using UCSF Chimera [48].

**Receptors**

The structure of glucosamine-6-phosphate synthase i.e. GlcN-6-P synthase was retrieved from Protein Data Bank (http://www.rcsb.org/pdb/home/home.do) with PDB ID: 1GDO. The unnecessary heteroatoms and chains were removed based on the available evidences in the literature.

**Molecular Docking**

The selected receptor and ligands were docked by uploading each receptor and ligand in sequential manner in Patch Dock server keeping the format of both as PDB. As our docking involved protein and small molecule we kept the value of Clustering RMSD at 1.5 Å. The results were retrieved by providing email address at appropriate place before submitting the docking job. Upon completion of docking many top scoring solutions are generated from which we take only the complex ranking highest in the hierarchy.

**Rendering the docked complex**

The receptor ligand complex is opened in UCSF Chimera for providing a three dimensional view [48]. This clearly shows ligand occupying the active site groove of the protein.

## RESULTS AND DISCUSSION

**Chemistry**

Fig. 1 shows the condensation of commercially available (5-Me, 3-$NH_2$) pyrazine 2-carboxylic acids (A) and N-hetero aryl piperazine hydrochlorides (B) in presence of base DIPEA (Diisopropyl ethylamine) and coupling reagent T3P (the water scavenger) to obtained a pyrazine 2-carboxylic acid derivatives P (1-10) in good yield (79-92%).

**Figure 3: Outline of the formation amide bond and regeneration of T3P**

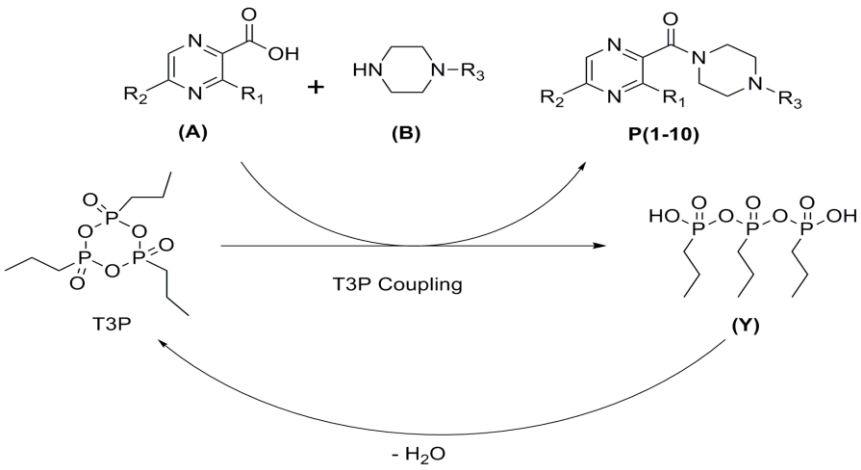


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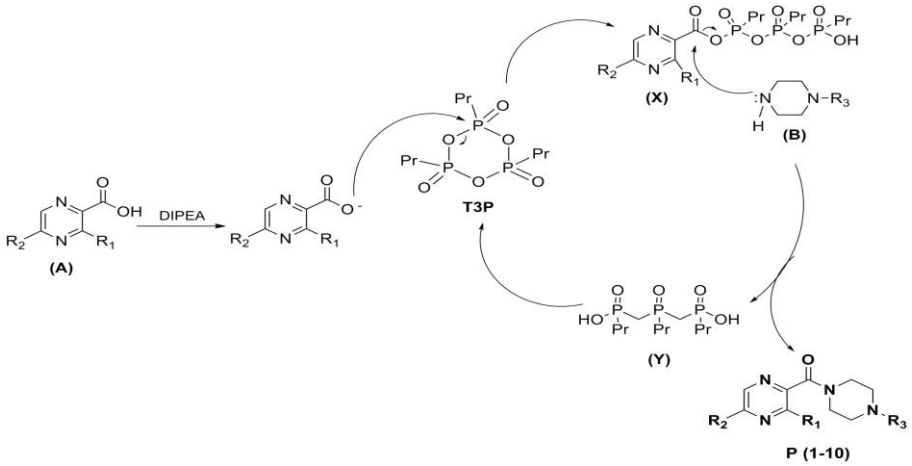
**Figure 4: Mechanism of amide bond formation in presence of T3P**




A possible participation T3P during the formation of amide bond follows the general mechanism shown in Fig. 3 and 4. T3P converts the oxygen of a carboxylic acid into an ionic leaving group through intermediate (X), which in turn, promotes the formation of product P (1-10). The open hydrated T3P (Y) produced during the process cyclizes to regenerate the catalyst (T3P), and the catalytic cycle then continues by the regeneration of T3P in the process.

**Antioxidant Activity**

The synthesized compounds at different concentrations were tested for DPPH and ABTS free radical scavenging activity. The compound (3-aminopyrazin-2-yl)(4-(6-aminopyrimidin-4-yl)piperazin-1-yl)methanone (P10) showed the highest percentage of antioxidant activity and $IC_{50}$ values of 71.708 % and 60.375 $\mu gmL^{-1}$for DPPH assay. ABTS assay showed the highest percentage of antioxidant activity and $IC_{50}$ values of37.548 % and 6.53 $\mu gmL^{-1}$when compared to the other synthesized compounds (Table 2, Fig. 5 & 6). The antioxidant activity of the compounds is related to their electron or hydrogen radical releasing abilities to DPPH so that they become stable diamagnetic molecules. This may be the reason for the higher or lower antioxidant activity [49]. Dubuisson et al., (2004) [2] have also reported good antioxidant activity of synthesized aminopyrazine derivatives which is also similar to our study. Biological activities of a list of piperazine and pyrazine derivatives have been reported by Meher et al., (2013) [18]. Bheru et al., (2013) [50]have also illustrated the anticancer, antibacterial, antifungal, antioxidant and SAR studies of synthesized novel (E)-3-aryl-1-(3-alkyl-2-pyrazinyl)-2-propenone. According to Jean-Francois et al., (2001) [51] derivatives of 2, 6-Diamino-3, 5-diaryl-1, 4-pyrazine have exhibited good antioxidant activity. The synthesized compound showing highest activity can be used in the field of medicine and it might have the anti-cancerous property [37].

**Table 2: Percentage of scavenging activity and $IC_{50}$ value of different synthesized compounds against different assays.**

| Compounds | DPPH % of scavenging | ABTS % of scavenging | DPPH $IC_{50}$ (µg/mL) | ABTS $IC_{50}$ (µg/mL) |
|---|---|---|---|---|
| **Std** | $77.984^{a}$ | $15.0552^{b}$ | $10.11^{a}$ | $30.624^{b}$ |
| **P1** | $61.896^{c}$ | $1.644^{c}$ | $1730^{k}$ | $2875^{i}$ |
| **P2** | $61.864^{c}$ | $1.532^{c}$ | $1662^{j}$ | $24273^{j}$ |
| **P3** | $62.418^{c}$ | $1.958^{c}$ | $533^{f}$ | $1364.833^{c}$ |
| **P4** | $62.016^{c}$ | $1.422^{c}$ | $968.33^{g}$ | $2586.157^{g}$ |
| **P5** | $61.48^{c}$ | $1.292^{c}$ | $1074.54^{i}$ | $2465.7^{f}$ |
| **P6** | $58.46^{d}$ | $1.336^{c}$ | $514^{e}$ | $2734.22^{h}$ |
| **P7** | $58.716^{d}$ | $1.092^{c}$ | $392.91^{d}$ | $48596^{k}$ |
| **P8** | $58.742^{d}$ | $1.098^{c}$ | $1061.25^{h}$ | $1910.96^{d}$ |
| **P9** | $59.878^{d}$ | $1.972^{c}$ | $78.96^{c}$ | $2118.43^{e}$ |
| **P10** | $71.708^{b}$ | $37.548^{a}$ | $60.375^{b}$ | $6.53^{a}$ |

**Note:** Mean of 15 replicate. Mean values with different superscripts ($^{a, b, c, d, e, f, g, h, i, j, k}$) differ significantly at P<0.01 by Tukey (HSD) test

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**Figure 5: ABTS scavenging activity of different compounds and its $IC_{50}$ (µg/mL)**

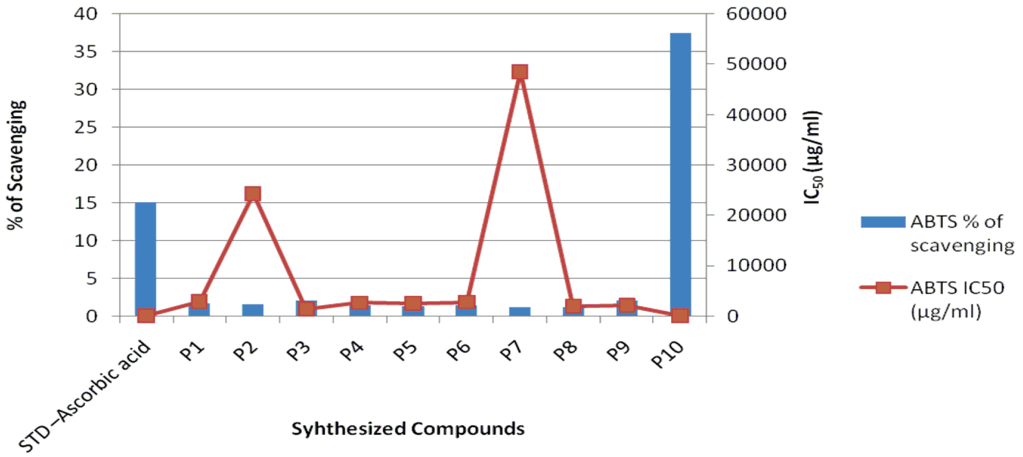
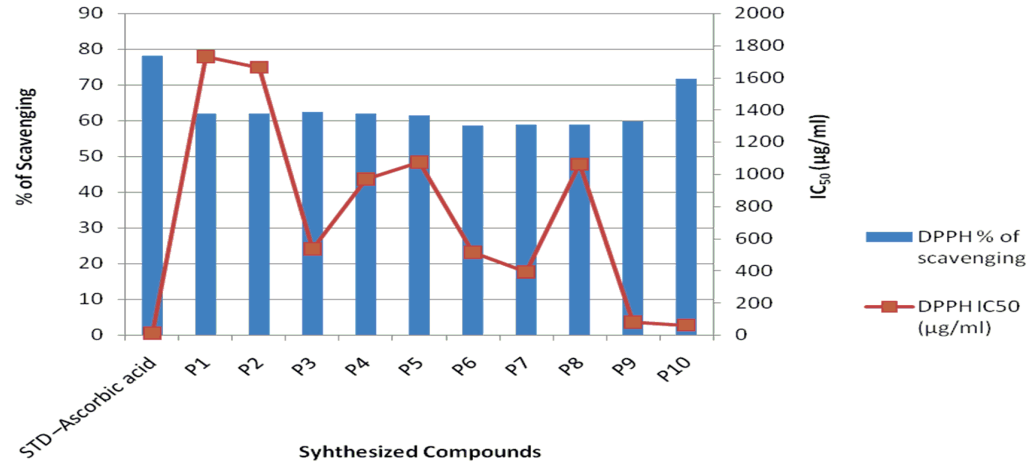



**Figure 6: DPPH scavenging activity of different compounds and its $IC_{50}$ (µg/mL)**




**Antimicrobial activity**

All synthesized pyrazine-2-carboxylic acid derivatives exhibited good antimicrobial activity against five clinical isolates *E. coli*, *P. aeruginosa*, *B. subtilis*, *S. aureus* and *C. albicans* as shown in Table 3. Compound 'P10' and 'P4' was found to be best for *C. albicans* with a MIC value of 3.125µgmL$^{-1}$. In case of gram negative bacteria i.e., *E. coli* compounds such as 'P3', 'P4', 'P7' and 'P9' were found to be better with a MIC value of 50 µgmL$^{-1}$ concentrations. *P. aeruginosa*, a gram negative bacterium was sensitive (25 µgmL$^{-1}$) to compounds 'P6', 'P7', 'P9' and 'P10'. Almost all the ten compounds were effective against *B. subtilis* with a MIC value of 25 µgmL$^{-1}$. *S. aureus* a causative agent of sepsis/ skin infection was found to be sensitive to compounds 'P2' and 'P4' (6.25 µgmL$^{-1}$). The data revealed that, although all the compounds (P1-P10) showed comparably good antimicrobial activities, the (4-(6-aminopyrimidin-4-yl) piperazin-1-yl) (5-methylpyrazin-2-yl) methanone (P4) showed much more potent inhibitory activity towards all the pathogenic stains used in the experiment. In an earlier study, Chandrakant and Naresh, (2004) [19] have also reported the antimicrobial activity of synthesized pyrazine containing thiazolines and thiazolidinones. According to Bheru et al. (2013) [50], the ethyl pyrazinechalcones have been depicted to posses considerable antibacterial activity when compared to ethylpyrazinechalcones. Barbora et al., (2014) [49] have also demonstrated the antimycobacterial activity of synthesized pyrazinamide. According to Henryk et al., (2005) [52] the compound Spiro-piperidin-1, 1'-1H-pyrazolo [3, 4-b] pyrazin-3-ylamine chloride exhibited highest antibacterial activity against three of the bacterial strains tested at lower concentrations. It seems that the presence of free amino group that is present in pyrazine or pyrimidine ring would have contributed to the antimicrobial activity of both '(4-(6-aminopyrimidin-4-yl) piperazin-1-yl)(5-methylpyrazin-2-yl) methanone (P4)' and (3-aminopyrazin-2-yl)(4-(6-aminopyrimidin-4-yl)piperazin-1-yl)methanone (P10) [50].

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**Table 3: Minimum inhibition concentration (MIC) in µg/mL of synthesized compounds against tested bacterial strains by micro dilution method.**

| *Compounds* | *Test microorganisms* | | | | |
|---|---|---|---|---|---|
| | *E.coli* | *P.auruginosa* | *B.subtilis* | *S.aureus* | *C.albicans* |
| **P1** | 100 | 100 | 50 | 50 | 6.25 |
| **P2** | 100 | 100 | 25 | 6.25 | 6.25 |
| **P3** | 50 | 50 | 25 | 50 | 6.25 |
| **P4** | 50 | 50 | 25 | 6.25 | 3.125 |
| **P5** | 100 | 100 | 25 | 50 | 6.25 |
| **P6** | 100 | 25 | 25 | 100 | 6.25 |
| **P7** | 50 | 25 | 25 | 25 | 6.25 |
| **P8** | 100 | 50 | 50 | 25 | 6.25 |
| **P9** | 50 | 25 | 25 | 50 | 6.25 |
| **P10** | 100 | 25 | 25 | 25 | 3.125 |
| **Std** | 0.39 | 0.78 | 0.39 | 0.39 | 0.78 |

**'Std'-** standard Gentamycin sulphate, MIC values expressed in µg/mL

**Docking studies**

The docking studies predicted that the compound P4 shows highest binding affinity among all the newly synthesized compounds against glucosamine-6-phosphate synthase but less compared to the approved drug gentamycin. P4 showed a geometry shape complementarity score (PatchDock Score) of 4142 and atomic contact energy value of -267.35compared to standard gentamycin where the geometry shape complementarity score was found to be 3814 against glucosamine-6-phosphate synthase. Though the PatchDock Score was found to high in almost all the test compounds compared to gentamycin, gentamycin displayed the highest atomic contact energy value of -422.23 compared to newly synthesized compounds where it remained below -300 in almost all the cases. Table 4 gives intricate details about the PatchDock Score and atomic contact energy value of all the ligands including standard gentamycin against glucosamine-6-phosphate synthase. Greater value of Score and highest negative value of ACE reflects strong affinity between ligand and receptor and viceversa [53]. Our docking studies showed compound P4 very close to and thereby possibly interacting with residues Asp123, Gly99, His97, Thr76, Trp73, and Arg74 (Fig. 7). Similar residues were reported by earlier groups with other inhibitors targeting glucosamine-6-phosphate synthase thereby validating our docking accuracy [54]. Our results correlated well with the in vitro findings. The view of ligands bound to their receptors is as given in Fig. 8.

**Table 4: Geometric shape complementarity score and atomic contact energy values of variousinhibitors against glucosamine-6-phosphate synthase.**

| Ligand | Receptor | PatchDock Score | ACE |
|---|---|---|---|
| Gentamycin | Glucosamine-6-phosphate synthase | 3814 | -422.23 |
| P1 | | 4222 | -156.64 |
| P2 | | 4394 | -165.76 |
| P3 | | 4458 | -232.11 |
| P4 | | 4142 | **-267.35** |
| P5 | | 4124 | -242.12 |
| P6 | | 3932 | -182.08 |
| P7 | | 3964 | -176.05 |
| P8 | | 4068 | -188.50 |
| P9 | | 3828 | -187.71 |
| P10 | | 3846 | -180.12 |

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**Figure 7: Docked complex of pyrazine 2-carboxylic acid derivatives (P1-P10) and glucosamine-6- phosphate synthase. Std – Gentamycin.**

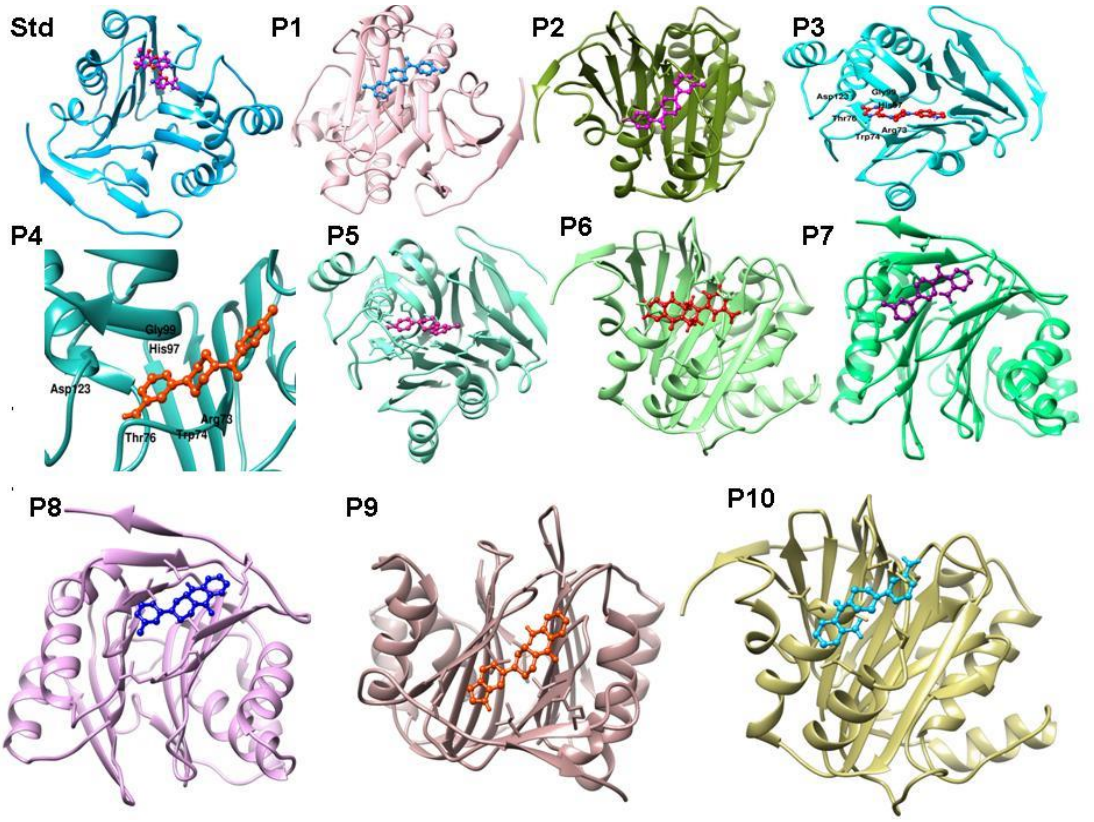
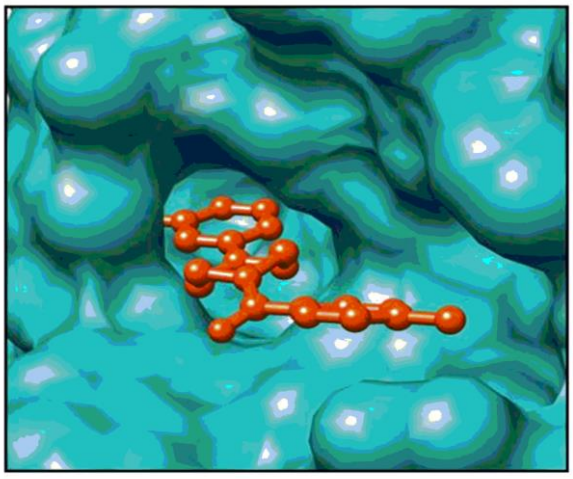



**Figure 8: Compound pyrazine 2-carboxylic acid derivative (P4) into the active site groove of glucosamine-6-phosphate synthase**



**CONCLUSION**

In summary, ten new pyrazine 2-carboxylic acid derivatives were synthesized with good percentage yield using the commercially available, inexpensive T3P as a coupling regent. The preference of T3P has been proven to be an excellent catalyst to access the amide bond synthesis. Unlike other coupling reagents, the use of catalytic amount of T3P is economical which makes T3P as a first choice in the synthesis of high value added products (particularly in large scale synthesis). Further these pyrazine 2- carboxylic acid derivatives were characterized by different analytical techniques and screened for antioxidant and antimicrobial activities. Among the ten new pyrazine 2-carboxylic acid derivatives, (4-(6-aminopyrimidin-4-yl) piperazin-1-yl)(5-methylpyrazin-2-yl)methanone (P4), (3-aminopyrazin-2-yl)(4-(6-aminopyrimidin-4-yl) piperazin-1-yl) methanone(P10) was found to be the best candidate exhibiting high antioxidant property, indicating that it can

be a promising active component in the formulations of cosmetics and also in the field of biomedical applications. With regard to antimicrobial studies, among all the synthesized compounds, (4-(6-aminopyrimidin-4-yl) piperazin-1-yl) (5-methylpyrazin-2-yl) methanone (P4) showed highest antimicrobial activity, which was also confirmed by docking studies, where in P4 showed higher docking score with GlcN-6-P synthase. So, it can be predicted that as an inhibition of GlcN-6-P synthase may be responsible for antibacterial activity of the synthesized pyrazine -2 carboxylic acid derivatives. The results obtained are prompting for further studies on modification in their structures and characterizing their diverse biological activities.

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