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Original Research Article

Synthesis, characterization, antimicrobial activity and molecular docking studies of combined pyrazol-barbituric acid pharmacophores

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Abstract

Purpose: *To synthesize, and determine the antibacterial activity and binding mode of new pyrazol-barbituric acid derivatives in a search for new antimicrobial agents.*

Methods: *One-pot multi-component reaction of aldehyde derivatives, barbituric acid and 3-methyl-1-phenyl-1H-pyrazol-5(4H)-one in the presence of $NHEt_2$ to afford Michael adduct was carried out. The reaction was carried out in water and afforded new heterocycles in a one-step fashion, with expedient work-up and high yield without extraction and purification steps. The synthesized compounds were evaluated for antimicrobial activity using agar disc diffusion. Molecular docking approach via MOE-Dock program was applied to predict the binding interactions of some of the new pyrazol-barbituric acid derivatives against six different target proteins downloaded from Protein Data Bank.*

Results: *A series of pyrazole-barbituric acid derivatives were successfully synthesized and characterized. The synthesized compounds showed moderate to very good antibacterial activity against S. aureus ATCC 29213 and E. faecalis ATCC29212, as well as also antifungal activity against Candida albicans ATCC 10400*

Conclusion: *A series of pyrazole-barbituric acid derivatives has been synthesized and some of them display antimicrobial activities.*

Keywords: *Pyrazole, Barbituric acid, Pyrazole-barbituric acid derivatives, Antimicrobial activity, Molecular docking*

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INTRODUCTION

Multicomponent reactions (MCRs) are one of the most powerful research protocol for generation of complex polyfunctionalized molecules using convergent one-pot transformations [1-7]. In addition, multicomponent reactions in green solvent such as water are of considerable interest. Nitrogen-containing compounds have been known to have a tremendous potential application in chemistry. Besides providing great biological properties, the nitrogen atoms are able to act as donors and find applications in the construction of supramolecular blocks. In this context, Pyrazole derivatives are of particular interest because of their pharmacological profile

[8-10] such as cyclooxygenase 2 inhibitors (e.g., celecoxib, SC-558, and tepoxalin) (e.g., Fig 1) [11,12] and reduction in obesity for example cannabinoid-1 inverse agonists (e.g., rimonabant) [13].

In particular, fused pyrazoles with other privileged scaffolds possess divergent pharmacological activities [14], they are also useful in the field of luminophores and fluorescence applications [14-20].

Recently, Barakat *et al* [21-23], synthesized and evaluated some novel zwitterionic adducts derived from pyrimidine-2,4,6-trione which possess anti-oxidant activity. In this context, we have synthesized a new series of pyrazole-pyrimidine trione using one pot fashion for the construction of new heterocycles. Their anti-microbial properties and molecular docking were also investigated.

EXPERIMENTAL

All chemical reagents were purchased from Sigma-Aldrich. IR spectra were measured as CsI pellets on Perkin-Elmer, FT-IR Spectrometer, Spectrum 1000. NMR spectra were recorded on a Jeol-400 NMR spectrometer. ^{1}H-NMR (400 MHz), and ^{13}C-NMR (100 MHz) were run in either deuterated chloroform ($CDCl_3$) or deuterated dimethylsulphoxide (DMSO-d_6). Chemical shifts (δ) are referred in terms of ppm and J -coupling constants are given in Hz. Mass spectra were carried out on a Jeol JMS-600 H equipment. Elemental analysis was carried out on Perkin-Elmer 2400 Elemental Analyzer; CHN mode. All melting points were measured on a Gallenkamp melting point apparatus in open glass capillaries and are uncorrected.

General method for the synthesis of 4a-o (GP1)

A mixture of aldehyde **1** (1.5 mmol), 1,3-dimethylpyrimidine-2,4,6(1H,3H,5H)-trione **2**, (1.5 mmol), 3-methyl-1-phenyl-1H-pyrazol-5(4H)-one (1.5 mmol) and Et_2NH (1.5 mmol, 155 µL) in 3 mL of degassed H_2O was stirred at room temperature for 1–5 h. The completion of the reaction was monitored by TLC. The solid product was filtered, washed with ether (3 × 20 mL), and dried to afford pure product **4a-o**.

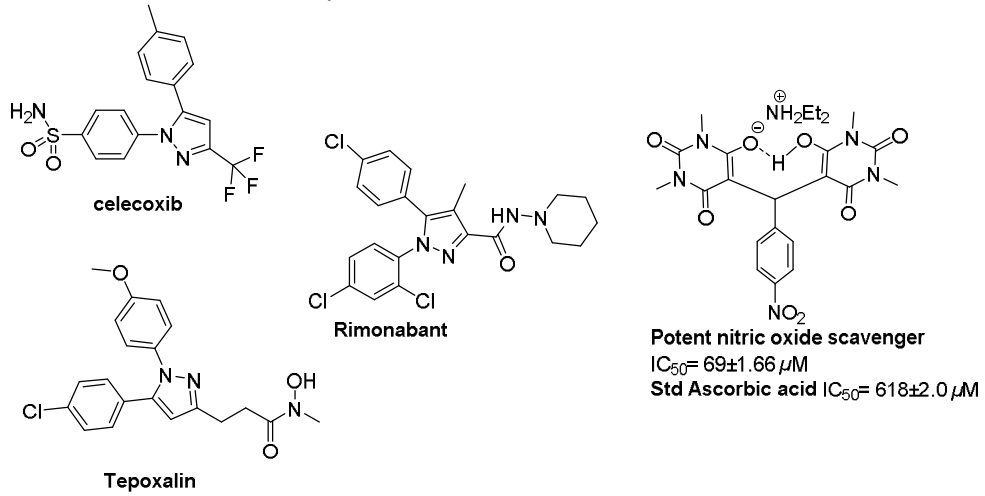
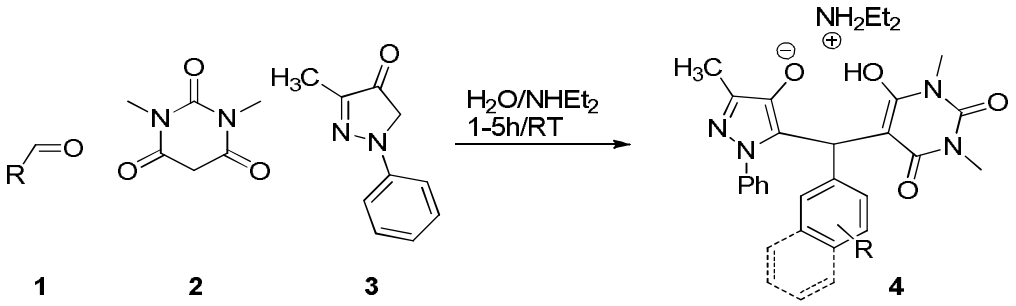


Figure 1: Biologically active pyrazole and barbituric acid scaffolds



Scheme-1: Protocol for the synthesis of **4a-o**

Table 1: Different *S*-substituted alkyl groups for **4a-o**

#	4	R	Yield (%)[b]
1	**4a**	Ph	96
2	**4b**	*p*-ClPh	93
3	**4c**	*p*-CH_3Ph	94
4	**4d**	*m*-CH_3Ph	93
5	**4e**	*p*-BrPh	91
6	**4f**	*m*-BrPh	88
7	**4g**	*p*-NO_2Ph	92
8	**4h**	*m*-NO_2Ph	90
9	**4i**	*p*-CH_3OPh	89
10	**4j**	*p*-FPh	92
11	**4k**	*p*-CF_3Ph	89
12	**4l**	2,4-Cl_2Ph	90
13	**4m**	2,6-Cl_2Ph	87
14	**4n**	2-Naphthaldehyde	89
15	**4o**	Thiophene	85

[a]*All reactions were carried out with aldehyde* ***1****(1.5 mmol), 1,3-dimethylpyrimidine-2,4,6(1H,3H,5H)-trione* ***2,*** *(1.5 mmol), 3-methyl-1-phenyl-1H-pyrazol-5(4H)-one (1.5 mmol) and* $NHEt_2$ *(1.5 mmol) in water (1.5 mL) for 1 - 5 h;* [b] *yield of isolated product*

The different *S*-substituted alkyl groups for **4a-o** are provided in Table 1

Antimicrobial assay

The initial screening of antimicrobial activity and minimum inhibitory concentration (MIC) determination for the tested compounds were performed by cup plate method and broth dilution method respectively with different strains (BSAC, 2015). Fifteen synthesized compounds were screened for their antimicrobial activity against six bacterial standard strains; three gram-positive (*Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, and *Bacillus subtilis* ATCC 10400) and three Gram-negative (*Escherichia coli* ATCC 25922, *Proteus vulagris* ATCC 6380, and *Pseudomonas aeruginosa* ATCC 27857) and one unicellular fungi (yeast) standard strain) *Candida albicans* ATCC 2091). The tested compounds were dissolved in dimethyl sulfoxide (DMSO) to obtain 5120 mg/mL stock solution.

Three Gram-positive and three Gram-negative bacterial strains and fungi were grown in Cation Adjustment Mueller-Hinton (CAMH) broth (Merck®, Darmstadt, Germany) while *C. albicans* strain was grown in Sabauraud Dextrose Broth (SDB) to mid-log phase. The suspension was diluted 1:100 in CAMH broth to obtain 1 x 10^6 CFU/mL. This suspension was swabbed on a CAMH agar plate (Merck®, Darmstadt, Germany) and allowed to dry completely. Mueller-Hinton Agar and Sabauraud Dextrose Agar were used for bacteria and fungi respectively. Four wells (7 mm in diameter) were made in agar plate using cork borer. A 1 mL of stock solution (5120 mg/mL) was 2-fold diluted in 1 mL DMSO to obtain 2560 mg/mL. A 100 µL (256 µg) of the tested compound was poured in the well using calibrated pipette. The plates were kept in refrigerator at 4 ^oC for half an hour to allow diffusion of the compound in the agar. Then, the plates were incubated at 37 ^oC for 24 h. After incubation period, the diameter of the inhibition zone was measured and recorded in mm by aid of ruler. Ciprofloxacin (10 µg/cup) and fluconazole (10 µg/mL) were used as positive controls for antibacterial and antifungal activity, respectively. The experiment was carried out in duplicate and the mean diameter taken [24].

Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) was determined for the compounds that showed antimicrobial activity by cup plate method. Briefly, 2 mL of CAMH broth (for bacterial strains) and 2 mL of SAB (for fungal strain) was dispensed into 7 mL Peju sterile tubes. For each compound, 14 tubes were used. Tube nos. 13 and 14 were used as positive growth control (no test compound) and negative control for medium sterility (no microorganism), respectively. A 1 mL aliquot of the stock solution (5120 mg/mL) was 10-fold diluted in 9 mL CAMH to obtain 512 mg/mL. A 2 mL aliquot of the test compounds (512 mg/mL) was pipetted into the first tube and mixed well. Thereafter, 2 mL was withdrawn from the 1st tube and added to the 2nd tube to make a two-fold dilution. This procedure was repeated down to the 12th tube a concentration of 0.125 mg/mL was obtained. Two millilitres were discarded from the 12th tube. A volume of 2 mL of inoculum (1 x 10^6 CFU/mL) was added to each tube except tube no. 14 to give a final strength of 1 x 10^6 CFU/mL. Ciprofloxacin and fluconazole were used as positive control for antibacterial and antifungal assay, respectively. The inoculated tubes were incubated at 37 ^oC for 20 h. After the incubation period, the results of MIC were recorded manually and interpreted according to the guidelines of British Society of Antimicrobial Chemotherapy (BSAC)"[24].

Statistical analysis

All computations were executed in triplicate and statistical analysis was performed with Microsoft Excel 2010. The results are expressed as mean ± SEM (n = 3). Minimum inhibitory concentration (MIC) was computed with suitable dilutions (5120 - 512 µg/well) for each sample and results

calculated using EZ-Fit software (Perrella Scientific Inc, Amherst, USA)" [24].

Molecular modeling and docking data

Molecular docking simulation is an efficient tool, used to predict binding mode of ligands within target proteins binding pockets. In order to computationally identify anti-fungal and anti-bacterial (Gram positive) targets for these newly synthesized compounds (**4a-4o**), six different targets proteins were downloaded from the Protein Data Bank [25], i.e., dihydrofolate reductase (DHFR) (PDB ID 4HOF), secreted aspartic protease (PDB ID 3Q70), and N-myristoyl transferase (PDB ID 1IYL) were chose as anti-fungal targets from *Candida albicans*, whereas dihydrofolate reductase (PDB ID 3FYV), gyrase B (PDB ID 4URM) and sortase A (PDB ID 2MLM) were selected from *S. aureus* as antibacterial targets. On the basis of docking score and interactions of these compounds against all the targets, only two targets, DHFR from *C. albicans* and gyrase B from *S. aureus*, were selected as good docking scores and interactions were observed for the synthesized compounds using MOE 2013 [25].

Before docking experiment, two dimensional (2D) structures of all the compounds were modelled on builder implemented in MOE and then their three dimensional (3D) conformation were generated by MOE. The structure of target proteins were prepared, protonated, charged and minimized using MOE. Using the default parameters of docking in MOE, i.e., TMA (Triangle Matcher Algorithm) with London dG and GBVI/WSA dG as rescoring functions were used to develop 30 binding poses for each ligand. All the docking observations along with scoring and different conformations of compounds were stored in the mdb output files.

RESULTS

The desired zwitterion derivatives **4a-o** [24,25] bearing different substituents showed excellent yield (up to 96 %) as shown in Scheme 1. The preparation of **4a-o** was ensued via cascade Aldol-Michael addition of N,N-dimethyl barbituric acid, 3-methyl-1-phenyl-1H-pyrazol-5(4H)-one and aldehyde mediated by aquoues $NHEt_2$. Notably, a variety of functional groups such as hydroxyl, methoxy and chloro were tolerated under our new reaction protocol. The chemical structures of all the synthesized compounds were deduced with the aid of physical and spectroscopic methods.

5-((6-Hydroxy-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)(phenyl)methyl)-3-methyl-1-phenyl-1H-pyrazol-4-olate (4a)

4a was prepared according to (**GP1**) from benzaldehyde yielding orange materials (yield 96%). m.p: 116 ^{o}C; IR (CsI, cm^{-1}): 3449, 3060, 2988, 1661, 1581, 1501, 1426, 1367;^{1}H-NMR (400 MHz, DMSO-d_6): δ 14.48 (s, 1H, OH), 7.33-7.09 (m, 10H, Ph), 5.52 (s, 1H, benzyl-H), 3.36 (m, 6H, CH_3), 2.88(q, 4H, J = 7.3Hz, CH_2CH_3), 2.19 (s, 3H, CH_3), 1.13(t, 6H, J = 7.3Hz, CH_2CH_3); ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 198.0, 174.8, 164.0, 163.6, 163.2, 151.4, 146.9, 138.0, 128.8, 127.9, 127.5, 126.9, 125.9, 121.9, 121.8, 91.2, 65.8, 42.1, 12.6, 12.2, 10.7; Anal. for $C_{27}H_{33}N_5O_4$; calcd C, 65.97; H, 6.77; N, 14.25;Found: C, 65.98; H, 6.76; N, 14.24; LC/MS (ESI): 492 $[M]^+$.

5-((4-Chlorophenyl)(6-hydroxy-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)methyl)-3-methyl-1-phenyl-1H-pyrazol-4-olate (4b)

4b was prepared according to (**GP1**) from *p*-cholorbenzaldehyde yielding rose materials (yield 93%). m.p: 162 ^{o}C; IR (CsI, cm^{-1}): 3444, 3045, 2987, 2721, 2495, 1679, 1579, 1502, 1487, 1370;^{1}H-NMR (400 MHz, $CDCl_3$): δ 17.62 (s, 1H, OH), 8.45 (bs, NH, $NHEt_2$), 7.25-7.13 (m, 10H, Ph), 5.46 (s, 1H, benzyl-H), 3.17 (m, 6H, CH_2CH_3), 2.48(q, 4H, J = 7.3Hz, CH_2CH_3), 2.16 (s, 3H, CH_3), 1.03(t, 6H, J = 7.3Hz, CH_2CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 198.2, 174.8, 164.0, 163.6, 163.2, 151.4, 139.7, 139.1, 131.4, 128.3, 127.8, 96.8, 91.2, 44.1, 42.1, 34.2, 28.6, 12.4, 12.3, 11.3; Anal. for $C_{27}H_{32}ClN_5O_4$; calcd C, 61.65; H, 6.13; Cl, 6.74; N, 13.31; Found: C, 61.66; H, 6.14; Cl, 6.75; N, 13.29; LC/MS (ESI): 527 $[M]^+$.

5-((6-Hydroxy-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)(p-tolyl)methyl)-3-methyl-1-phenyl-1H-pyrazol-4-olate (4c)

4c was prepared according to (**GP1**) from *p*-toulaldehyde yielding orange materials (yield 94%). m.p: 147 ^{o}C; IR (CsI, cm^{-1}): 3432, 2983, 2716, 2490, 1683, 1578, 1501, 1362; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 14.31 (s, 1H, OH), 9.94 (bs, NH, $NHEt_2$), 7.35-7.00 (m, 9H, Ph), 5.27 (s, 1H, benzyl-H), 3.30(t, 6H, J = 7.3Hz, CH_2CH_3), 2.42 (s, 3H, CH_3), 2.24(s, 3H, CH_3), 2.16(s, 3H, CH_3), 0.86 (t, 6H, J = 7.3Hz, CH_2CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 192.5, 163.1, 157.4, 152.4, 152.2, 147.4, 146.0, 144.6, 139.8, 139.7, 130.4, 129.4, 128.8, 128.6, 119.9, 119.6, 102.5, 91.5, 41.2, 31.2, 12.8, 11.1Anal. for $C_{28}H_{35}N_5O_4$; calcd C, 66.51; H, 6.98; N, 13.85; Found: C,

66.52; H, 6.99; N, 13.83; ; LC/MS (ESI): 506 $[M]^+$.

5-((6-Hydroxy-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)(m-tolyl)methyl)-3-methyl-1-phenyl-1H-pyrazol-4-olate (4d)

4d was prepared according to (**GP1**) from *m*-toulaldehyde yielding red materials (yield 93%). m.p: 98 ^oC; IR (CsI, cm^{-1}): 3449, 3043, 2987, 2734, 2509, 1681, 1581, 1501, 1426, 1369; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 17.40 (s, 1H, OH), 9.97 (bs, NH, $NHEt_2$), 7.65-7.07 (m, 9H, Ph), 5.45 (s, 1H, benzyl-H), 2.42(t, 4H, J = 7.3Hz, CH_2CH_3), 2.34 (s, 3H, CH_3), 2.24(s, 3H, CH_3), 2.22(s, 3H, CH_3), 0.83 (t, 6H, J = 7.3Hz, CH_2CH_3); ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 192.5, 163.1, 157.4, 152.4, 152.2, 147.4, 146.0, 144.6, 139.8, 139.7, 130.4, 129.4, 128.8, 128.6, 119.9, 119.6, 102.5, 91.5, 41.4, 21.5, 12.8, 11.1Anal. for $C_{28}H_{35}N_5O_4$; calcd C, 66.51; H, 6.98; N, 13.85; Found: C, 66.53; H, 6.98; N, 13.85; LC/MS (ESI): 506 $[M]^+$.

5-((4-Bromophenyl)(6-hydroxy-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)methyl)-3-methyl-1-phenyl-1H-pyrazol-4-olate (4e)

4e was prepared according to (**GP1**) from *p*-bromobenzaldehyde yielding orange materials (yield 91%). m.p: 104 ^oC; IR (CsI, cm^{-1}): 3451, 2988, 2737, 2508, 1677, 1581, 1502, 1429, 1371; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 14.33 (s, 1H, OH), 9.96 (bs, NH, $NHEt_2$), 7.82 (d, 2H, J = 7.3Hz, Ph), 7.35-7.07 (m, 7H, Ph), 5.46 (s, 1H, benzyl-H), 2.89(t, 4H, J = 7.3Hz, CH_2CH_3), 3.51 (s, 6H, CH_3), 2.18(s, 3H, CH_3), 1.12 (t, 6H, J = 7.3Hz, CH_2CH_3); ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 192.5, 163.1, 157.4, 152.4, 152.2, 147.4, 146.0, 144.6, 139.8, 139.7, 130.4, 129.4, 128.8, 128.6, 119.9, 119.6, 102.5, 91.5, 41.2, 31.2, 12.8, 11.1; Anal. for $C_{27}H_{32}BrN_5O_4$; calcd C, 56.85; H, 5.65; Br, 14.01; N, 12.28; Found: C, 56.85; H, 5.64; Br, 14.05; N, 12.29; LC/MS (ESI): 571 $[M]^+$.

5-((3-Bromophenyl)(6-hydroxy-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)methyl)-3-methyl-1-phenyl-1H-pyrazol-4-olate (4f)

4f was prepared according to (**GP1**) from *m*-bromobenzaldehyde yielding orange materials (yield 88%). m.p: 163 ^oC; IR (CsI, cm^{-1}): 3452, 2989, 2736, 2510, 1678, 1584, 1502, 1429, 1371; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 14.36 (s, 1H, OH), 9.95 (bs, NH, $NHEt_2$), 7.89 (d, 1H, J = 7.3Hz, Ph), 7.31-7.12 (m, 8H, Ph), 5.51 (s, 1H, benzyl-H), 2.87(t, 4H, J = 7.3Hz, CH_2CH_3), 3.50 (s, 6H, CH_3), 2.15(s, 3H, CH_3), 1.12 (t, 6H, J = 7.3Hz, CH_2CH_3); ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 192.5, 163.1, 157.3, 152.4, 152.1, 148.3, 147.4, 146.0, 140.4, 139.7, 130.0, 129.4, 128.8, 128.6, 123.4, 119.9, 119.6, 102.5, 91.5, 41.2, 31.2, 12.9, 11.1; Anal. for $C_{27}H_{32}BrN_5O_4$; calcd C, 56.85; H, 5.65; Br, 14.01; N, 12.28; Found: C, 56.83; H, 5.64; Br, 14.04; N, 12.30; LC/MS (ESI): 571 $[M]^+$.

5-((6-Hydroxy-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)(4-nitrophenyl)methyl)-3-methyl-1-phenyl-1H-pyrazol-4-olate (4g)

4g was prepared according to (**GP1**) from *p*-nitrobenzaldehyde yielding paige materials (yield 92%). m.p: 195 ^oC; IR (CsI, cm^{-1}): 3453, 3062, 2989, 2507, 1678, 1585, 1513, 1454, 1346; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 17.53 (s, 1H, OH), 10.15 (bs, NH, $NHEt_2$), 8.03 (d, 2H, J = 7.3Hz, Ph), 7.57 (d, 2H, J = 7.3Hz, Ph), 7.57-7.25(m, 5H, Ph), 5.57 (s, 1H, benzyl-H), 3.33 (s, 6H, CH_3), 3.32(t, 4H, J = 7.3Hz, CH_2CH_3), 2.08(s, 3H, CH_3), 0.96(t, 6H, J = 7.3Hz, CH_2CH_3);^{13}C-NMR (100 MHz, DMSO-d_6): δ 192.7, 163.1, 157.3, 152.4, 152.1, 148.3, 147.4, 146.0, 140.4, 139.7, 130.0, 129.4, 129.0, 128.6, 123.4, 119.9, 119.6, 102.5, 91.5, 42.0, 28.5, 12.9, 11.1; Anal. for $C_{27}H_{32}N_6O_6$; calcd C, 60.44; H, 6.01; N, 15.66; Found: C, 60.44; H, 6.02; N, 15.67; LC/MS (ESI): 537 $[M]^+$.

5-((6-Hydroxy-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)(3-nitrophenyl)methyl)-3-methyl-1-phenyl-1H-pyrazol-4-olate (4h)

4h was prepared according to (**GP1**) from *m*-nitrobenzaldehyde yielding orange materials (yield 90%). m.p: 116 ^oC; IR (CsI, cm^{-1}): 3451, 2990, 2508, 1677, 1583, 1526, 1348; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 14.10 (s, 1H, OH), 10.11(bs, NH, $NHEt_2$), 7.60 (d, 1H, J = 7.3Hz, Ph), 7.60-7.15 (m, 8H, Ph), 5.59 (s, 1H, benzyl-H), 3.33 (s, 6H, CH_3), 2.50(t, 4H, J = 7.3Hz, CH_2CH_3), 2.23(s, 3H, CH_3), 1.00 (t, 6H, J = 7.3Hz, CH_2CH_3);^{13}C-NMR (100 MHz, DMSO-d_6): δ 192.5, 163.1, 156.3, 152.4, 152.1, 148.3, 146.9, 138.9, 129.4, 128.8, 128.6, 125.9, 122.5, 122.3, 122.1, 121.1, 102.5, 91.5, 42.0, 34.3, 28.7, 12.7, 11.3; Anal. for $C_{27}H_{32}N_6O_6$; calcd C, 60.44; H, 6.01; N, 15.66; Found: C, 60.45; H, 6.02; N, 15.65; LC/MS (ESI): 537$[M]^+$.

5-((6-Hydroxy-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)(4-methoxyphenyl)methyl)-3-methyl-1-phenyl-1H-pyrazol-4-olate (4i)

4i was prepared according to (**GP1**) from anisaldehyde yielding orange materials (yield 89%). m.p: 105 ^{o}C; IR (CsI, cm^{-1}): 3455, 2998, 273, 2502, 1681, 1584, 1556, 1499, 1430, 1361, 1268;^{1}H-NMR (400 MHz, DMSO-d_6): δ 14.50(s, 1H, OH), 8.68 (bs, NH, $NHEt_2$), 7.76 (d, 2H, J = 7.3Hz, Ph), 7.41 (d, 2H, J = 7.3Hz, Ph), 7.19-7.08 (m, 5H, Ph), 5.51 (s, 1H, benzyl-H), 3.85 (s, 6H, CH_3), 2.28(s, 3H, CH_3), 3.44(t, 4H, J = 7.3Hz, CH_2CH_3), 2.28(s, 3H, CH_3), 1.06 (t, 6H, J = 7.3Hz, CH_2CH_3); ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 192.5, 163.5, 161.8, 157.4, 151.7, 147.9, 146.1, 138.4, 136.8, 136.8, 128.8, 128.7, 126.2, 120.2, 118.3, 114.3, 113.4, 104.6, 91.5, 55.6, 41.9, 31.2, 18.5, 15.1, 13.1, 11.8; Anal. for $C_{28}H_{35}N_5O_5$; calcd C, 64.47; H, 6.76; N, 13.43; Found: C, 64.47; H, 6.76; N, 13.43; LC/MS (ESI): 552 $[M]^+$.

5-((4-Fluorophenyl)(6-hydroxy-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)methyl)-3-methyl-1-phenyl-1H-pyrazol-4-olate (4j)

4j was prepared according to the general procedure (**GP1**) from *p*-flurobenzaldehyde yielding orange materials (yield 92%). m.p: 108 ^{o}C; IR (CsI, cm^{-1}): 3452, 3064, 2991, 2739, 2511, 16800, 1582, 1503, 1455, 1370; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 14.40(s, 1H, OH), 9.95 (bs, NH, $NHEt_2$), 7.86 (d, 2H, J = 7.3Hz, Ph), 7.36 (d, 2H, J = 7.3Hz, Ph), 7.36-6.96 (m, 5H, Ph), 5.49 (s, 1H, benzyl-H), 3.50 (s, 6H, CH_3), 2.87(t, 4H, J = 7.3Hz, CH_2CH_3), 2.17(s, 3H, CH_3), 1.12 (t, 6H, J = 7.3Hz, CH_2CH_3); ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 191.8, 163.5, 161.8, 157.3, 152.4, 152.2, 147.4, 146.0, 140.1, 139.7, 128.7, 128.6, 128.5, 123.7, 119.9, 119.5, 102.9, 91.7, 41.9, 31.5, 28.5, 12.8, 11.11; Anal. for $C_{27}H_{32}FN_5O_4$; calcd C, 63.64; H, 6.33; F, 3.73; N, 13.74; Found: C, 63.65; H, 6.35; F, 3.70; N, 13.75; LC/MS (ESI): 510 $[M]^+$.

5-((6-Hydroxy-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)(4-(trifluoromethyl)phenyl)methyl)-3-methyl-1-phenyl-1H-pyrazol-4-olate (4k)

4k was prepared according to (**GP1**) from *p*-trifluromethylbenzaldehyde yielding orange materials (yield 89%). m.p: 171 ^{o}C; IR (CsI, cm^{-1}): 3452, 3063, 2992, 2510, 1664, 1582, 1502, 1430, 1326; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 17.40 (s, 1H, OH), 10.09(bs, NH, $NHEt_2$), 7.54 (d, 1H, J = 7.3Hz, Ph), 7.42-7.25 (m, 8H, Ph), 5.57 (s, 1H, benzyl-H), 3.31 (s, 6H, CH_3), 2.89(t, 4H, J = 7.3Hz, CH_2CH_3), 2.29(s, 3H, CH_3), 0.88 (t, 6H, J = 7.3Hz, CH_2CH_3);^{13}C-NMR (100 MHz, DMSO-d_6): δ 192.5, 163.1, 156.3, 152.4, 152.1, 148.3, 146.9, 138.7, 128.8, 128.7, 128.0, 127.3, 125.8, 124.8, 122.1, 119.5, 102.5, 91.5, 41.5, 34.3, 28.2, 12.6, 11.0; Anal. for $C_{28}H_{32}F_3N_5O_4$; calcd C, 60.10; H, 5.76; F, 10.19; N, 12.52; Found: C, 60.11; H, 5.75; F, 10.21; N, 12.54; LC/MS (ESI): 560 $[M]^+$.

5-((2,4-Dichlorophenyl)(6-hydroxy-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)methyl)-3-methyl-1-phenyl-1H-pyrazol-4-olate (4l)

4l was prepared according to (**GP1**) from 2,4-dicholrobenzaldehyde yielding orange materials (yield 90%). m.p: 109 ^{o}C; IR (CsI, cm^{-1}): 3450, 3064, 2989, 2735, 2509, 1680, 1583, 1501, 1456, 1376; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 14.30 (s, 1H, OH), 10.40(bs, NH, $NHEt_2$), 7.53 (d, 1H, J = 7.3Hz, Ph), 7.27-7.09 (m, 7H, Ph), 5.48 (s, 1H, benzyl-H), 3.31 (s, 3H, CH_3), 3.18 (s, 3H, CH_3), 2.36(t, 4H, J = 7.3Hz, CH_2CH_3), 2.27(s, 3H, CH_3), 0.89 (t, 6H, J = 7.3Hz, CH_2CH_3);^{13}C-NMR (100 MHz, DMSO-d_6): δ 188.7, 163.1, 156.9, 146.9, 138.5, 133.7, 132.2, 131.7, 129.1, 129.0, 128.9, 128.8, 126.6, 125.8, 122.2, 119.1, 102.7, 91.4, 41.2, 32.4, 28.22, 12.7, 11.0, 10.9; Anal. for $C_{27}H_{31}C_{l2}N_5O_4$; calcd C, 57.86; H, 5.57; Cl, 12.65; N, 12.50; Found: C, 57.85; H, 5.57; Cl, 12.62; N, 12.53; LC/MS (ESI): 561 $[M]^+$.

5-((2,6-Dichlorophenyl)(6-hydroxy-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)methyl)-3-methyl-1-phenyl-1H-pyrazol-4-olate (4m)

4m was prepared according to (**GP1**) from 2,6-dicholrobenzaldehyde yielding yellow materials (yield 97%). m.p: 88 ^{o}C; IR (CsI, cm^{-1}): 3449, 2988, 2787, 2507, 1677, 1582, 1499, 1431, 1370; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 14.30 (s, 1H, OH), 10.40(bs, NH, $NHEt_2$), 7.53 (d, 1H, J = 7.3Hz, Ph), 7.36-7.21 (m, 7H, Ph), 5.77 (s, 1H, benzyl-H), 3.07 (s, 6H, CH_3), 2.87(t, 4H, J = 7.3Hz, CH_2CH_3), 1.96(s, 3H, CH_3), 1.13 (t, 6H, J = 7.3Hz, CH_2CH_3);^{13}C-NMR (100 MHz, DMSO-d_6): δ 188.7, 163.1, 156.9, 146.9, 138.5, 133.7, 132.2, 131.7, 129.1, 129.0, 128.9, 128.8, 126.6, 125.8, 122.2, 119.1, 102.7, 91.4, 41.2, 32.4, 28.22, 12.7, 11.0, 10.9; Anal. for $C_{27}H_{31}C_{l2}N_5O_4$; calcd C, 57.86; H, 5.57; Cl, 12.65; N, 12.50; Found: C, 57.87; H, 5.58; Cl, 12.63; N, 12.52; LC/MS (ESI): 561$[M]^+$.

5-((6-Hydroxy-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)(naphthalen-2-yl)methyl)-3-methyl-1-phenyl-1H-pyrazol-4-olate (4n)

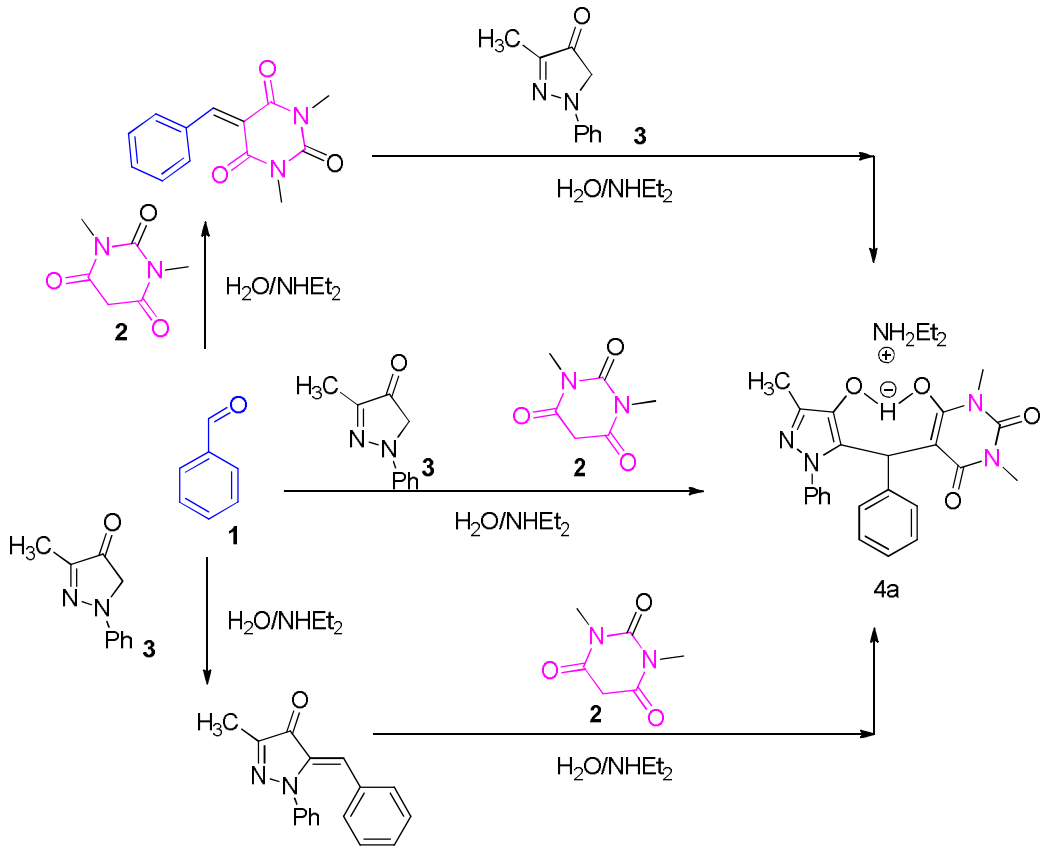
4n was prepared according to (**GP1**) from naphthaldehyde yielding orange materials (yield 89%). m.p: 94 ^{o}C; IR (CsI, cm^{-1}): 3448, 3054, 2988, 2735, 2507, 1681, 1851, 1502, 1427, 1368; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 14.38(s, 1H, OH), 10.15 (bs, NH, $NHEt_2$), 7.64-7.23 (m, 12H, Ph), 5.69 (s, 1H, benzyl-H), 3.23 (s, 6H, CH_3), 2.22(t, 4H, J = 7.3Hz, CH_2CH_3), 1.97(s, 3H, CH_3), 0.58 (t, 6H, J = 7.3Hz, CH_2CH_3); ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 192.5, 164.6, 156.5, 152.7, 147.1, 129.6, 129.2, 128.8, 128.2, 127.4, 125.7, 121.9102.9, 91.7, 41.2, 34.2, 28.3, 14.4, 12.8, 10.8, 10.10.6; Anal. for $C_{31}H_{35}N_5O_4$; calcd C, 68.74; H, 6.51; N, 12.93; Found: C, 68.74; H, 6.50; N, 12.90; LC/MS (ESI):542 $[M]^+$.

5-((6-hydroxy-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)(thiophen-2-yl)methyl)-3-methyl-1-phenyl-1H-pyrazol-4-olate (4o)

4o was prepared according to (**GP1**) from thiophenldehyde yielding red materials (yield 85%). m.p: 103 ^{o}C; IR (CsI, cm^{-1}): 3451, 3079, 2988, 2740, 2504, 1682, 1582, 1500, 1413, 1336, 1304, 1210; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 14.30 (s, 1H, OH), 8.70 (bs, NH, $NHEt_2$), 7.98(d, 2H, J = 7.3Hz, thiophene), 7.59 (d, 1H, J = 7.3Hz, thiophene),7.98-7.07 (m, 6H, Ph), 5.67 (s, 1H, benzyl-H), 3.16 (s, 6H, CH_3), 2.33(t, 4H, J = 7.3Hz, CH_2CH_3), 2.20(s, 3H, CH_3), 0.77 (t, 6H, J = 7.3Hz, CH_2CH_3); ^{13}C-NMR (100 MHz, DMSO-d_6): δ = 192.8, 162.3, 156.4, 150.2, 146.6, 140.9, 138.7, 138.4, 138.3, 136.8, 136.5, 128.9, 128.8, 124.9, 121.9, 119.1, 104.1, 91.4, 41.5, 30.6, 28.3, 13.1, 12.4, 10.8; Anal. for $C_{25}H_{31}N_5O_4S$; calcd C, 60.34; H, 6.28; N, 14.07; S, 6.44.; Found: C, 60.34; H, 6.27; N, 14.10; S, 6.45; LC/MS (ESI): 498 $[M]^+$.

The desired compounds **4a-o** were synthesized in one step fashion in high yield. The chemical structure was assigned via different spectroscopic tools including NMR, IR, MS and CHN elemental analysis.

A possible mechanism for the tandem Aldol-Michael reaction is shown in scheme 2. In the first step of the reaction, olefin is produced by Aldol condensation between aryl aldehyde **1** and either **2** or **3** promoted by diethyl amine (DEA). The Michael addition occurred in the second step *via* addition of enolate into olefin to afford the final desired products **4a-o**.



Scheme 2: Probable mode of tandem Aldol- Michael reaction

Antimicrobial activity

Results of the biological activity are shown in Table 2 and are expressed in mm inhibition. All the compounds exhibited very good activity against Gram-positive bacteria and fungi. The most promising compound against *C. albicans* was **4j**. Compounds **4a-o** had no activity against Gram-negative bacteria including *Escherichia coli* ATCC 25922, *Proteus vulagris* ATCC 6380, and *Pseudomonas aeruginosa* ATCC 27857.

DISCUSSION

Visual inspection of the binding mode of these newly synthesized compounds were carried out to determine the promising anti-fungal and anti-bacterial (gram-positive) agents.

As shown for the *in vitro* observations, the docking results confirmed the anti-fungal and gram positive anti-bacterial activity of these compounds, especially **4j** and **4c**, revealed good interactions against the two target proteins (Fig. 2). Although, compounds **4h**, **4i**, **4l**, **4n** and **4o** have some sort of activity but they did not show good interactions against the target proteins (4HOF and 4URM) like compound **4j** and **4c**. Moreover, molecular docking of **4j** against 4HOF showed that three hydrogen bonds and one arene-cation interaction with the active site residues Thr58, Lys57 and Arg56 respectively of protein (Figure 2a), Alternatively, docking simulation with gyrase B (PDB ID: 4URM) revealed that the carbonyl oxygen of compound **4j** was involved in hydrogen bonding with active site residues Ile86 and Gly85 (Fig. 2b). In case of compound **4c**, good interactions were observed with the active site residues of target protein 4HOF (Figure 2c) and 4URM (Figure 2d). The orientation of the compound **4j** and **4c** in the active site of the target proteins are represented in Figure 3. Overall our docking results showed that all the synthesized compounds, particularly compounds **4j** and **4c** revealed significant hydrogen bonds and hydrophobic interactions with the important active site residues of 4HOF and 4URM and are the promising anti-fungal and anti-bacterial agents respectively.

CONCLUSION

In conclusion, a new series of Michael adducts combined pyrazol-barbituric acid pharmacophore are synthesized and characterized. The synthesized products were examined against antimicrobial activity and also the molecular docking was investigated.

Table 2: Antimicrobial activity and minimal inhibitory concentrations of the compounds that show antimicrobial activity

Tube no.	Compound no.	Gram positive bacteria						Yeast	
		S. aureus ATCC 29213		*E. faecalis* ATCC29212		*B. subtilis* ATCC10400		*C. albicans* ATCC 2091	
		CPM (mm)	*MIC (mg/L)*	*CPM (mm)*	*MIC (mg/L)*	*CPM (mm)*	*MIC (mg/L)*	*CPM (mm)*	*MIC (mg/L)*
1	***4a***	12	32	12	16	11	32	14	32
2	***4b***	12	32	14	32	11	32	14	32
3	***4c***	13	32	13	32	12	32	18	8
4	***4d***	14	32	15	16	14	16	14	32
5	***4e***	15	32	12	64	11	64	14	32
6	***4f***	14	32	13	32	13	32	15	16
7	***4g***	14	32	14	32	13	32	15	16
8	***4h***	15	32	15	32	14	32	12	128
9	***4i***	15	32	19	32	15	16	13	64
10	***4j***	14	32	14	16	15	32	17	8
11	***4k***	12	32	12	32	11	32	16	16
12	***4l***	10	>128	Nil	>128	13	32	10	>128
13	***4m***	12	32	12	32	14	16	16	16
14	***4n***	13	64	11	64	12	64	14	32
15	***4o***	13	64	11	64	14	16	15	16
Standard	***Ciprofloxacin***	27	≤0. 25	24	≤0.25	25	≤0.25	ND	ND
	Fluconazole	ND	ND	ND	ND	ND	ND	28	0.5

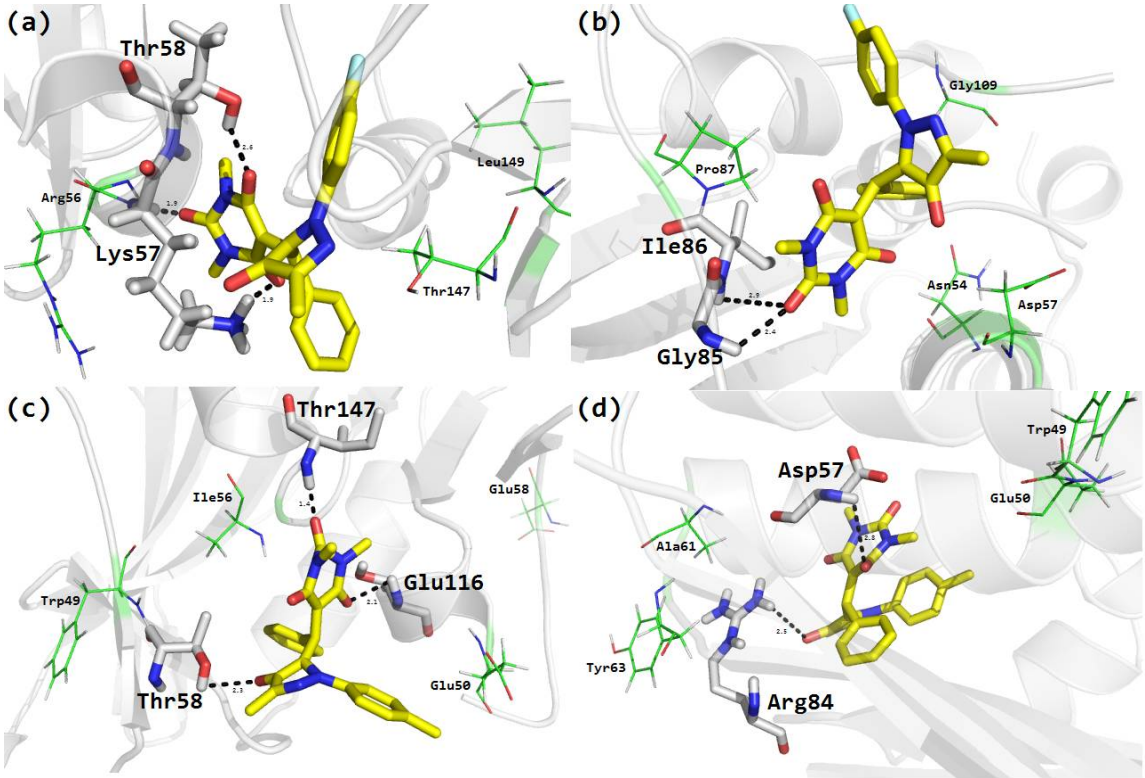


Figure 2: (a) Molecular docking conformation of compound **4j** in the active site of 4HOF; (b) Molecular docking conformation of compound **4j** in the active site of 4URM; (c) Molecular docking conformation of compound **4c** in the active site of 4HOF; (d) Molecular docking conformation of compound **4c** in the active site of 4URM

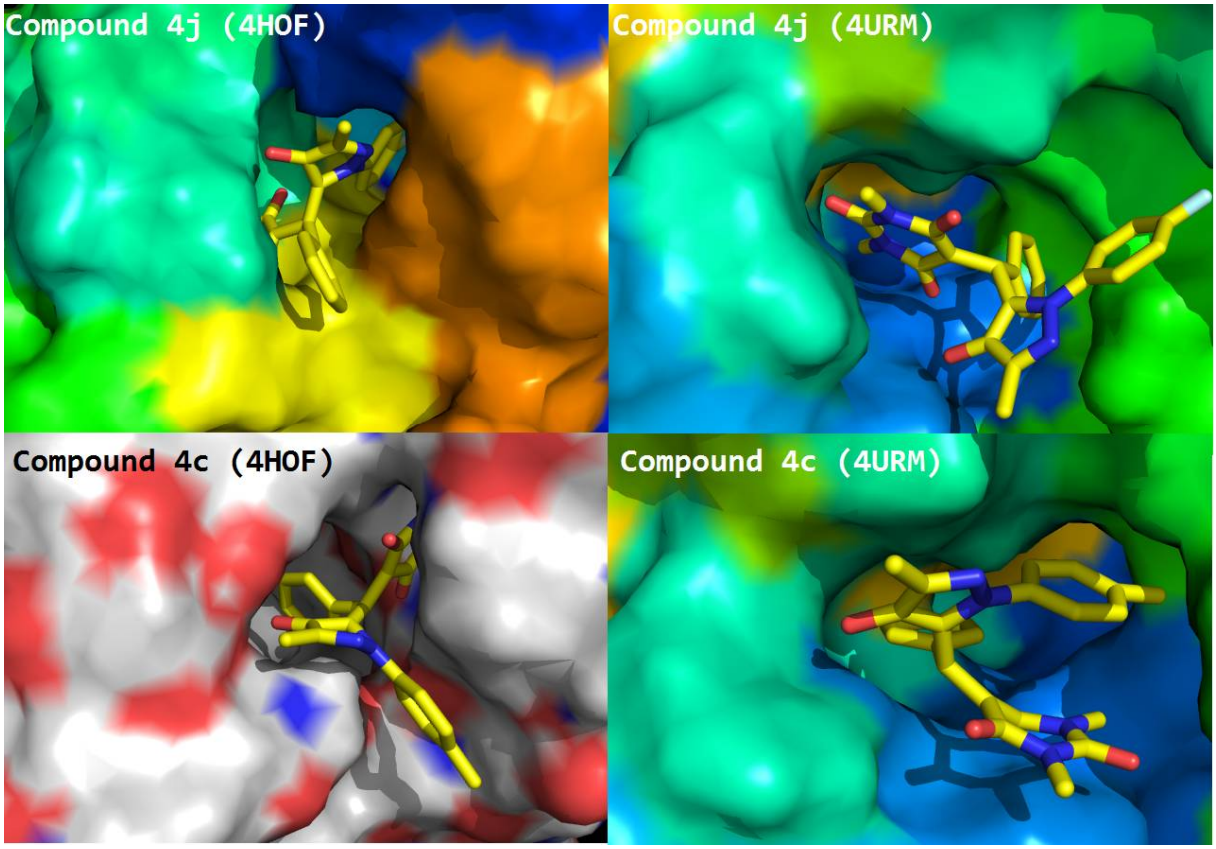


Figure 3: Binding orientation of compound **4j** and **4c** in the active sites of 4HOF and 4URM

DECLARATIONS

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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