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MICROWAVE ASSISTED SYNTHESIS, CHARACTERIZATION, MOLECULAR DOCKING AND ANTIMICROBIAL EVALUATION OF 4-NITROCINNAMIDE ANALOGUES

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Keywords:

Molecular Docking, Cinnamides, Acylhydrazones, Antimicrobial activity, MIC and FAB protein

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ABSTRACT: A series of novel 2-Benzamido-3-(4-nitrophenyl)-N'-(substituted) benzylidene-acrylohydrazide (4a-l) analogues were designed by incorporating the few pharmacophoric fragments to enhance the activity profile of molecules. Title compounds were synthesized by the microwave irradiation of α-Benzamido-(4-nitro)-cinnamahydrazide (3) reveals with aromatic/ heteroaromatic aldehydes under acidic conditions, characterized by IR, ^{1}H & ^{13}C NMR, Mass and evaluated for antimicrobial activity by using *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis* and *Candida albicans* strains. Compounds 4e and 4k exhibited significant protection against bacterial strains (MIC: 3.12-50µg/ml; 3.12-100µg/ml respectively) and compound 4h showed better activity (MIC: 12.5 µg/ml) against fungal strain. And also molecular docking interactions with FAB protein for evaluating the antimicrobial activity was done by using XP GLIDE module of Schrodinger suite and this study highlights, all the analogues exhibited significant affinity towards the 5BNM (FAB Protein) have good docking scores (-4.51 to -8.78) than standard drug ciprofloxacin (-4.74). Of the 12 new analogues, compound 4e was identified as most active ligand with good activity profile against bacterial strains and also have higher binding affinity with target.

INTRODUCTION: The search for new antimicrobial agents is the challenging area for researchers because of dramatic increasing the resistance of microbial pathogens and it is desirable to find the drugs with improved potency and wider activity spectrum.

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In recent years, cinnamic acid derivatives such as cinnamides exhibited a variety of biological activities such as antioxidant, antitumor, antimicrobial, antitubercular, anti-inflammatory, antifungal activity [1 - 5] and are often used as promising precursor for the development of new, highly effective drugs. However, the reactive center (vinyl fragment) of cinnamides was significantly affected by substituent present at various positions of the benzene nucleus [6 - 7]. On the other hand scientific literature is more and more focused on acyl hydrazones are well known class of compounds with diverse biological activities such as antitubercular, anticancer, antioxidant,

antimicrobial activities [8-11]. In addition to the above facts, some reports showed the importance of nitro substituted antimicrobial drugs for treating urinary tract infections [12-13]. In new drug development studies, hybridization of different pharmacophores in the same frame may lead to development of new compounds having higher biological activity.

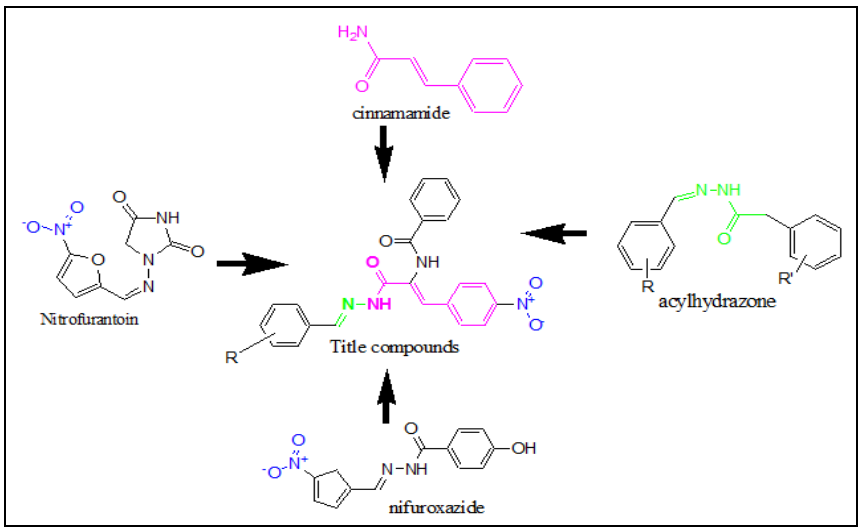


FIG. 1: DESIGN STRATEGY ADOPTED FOR DESIGNING OF TITLE COMPOUNDS (4a-l)

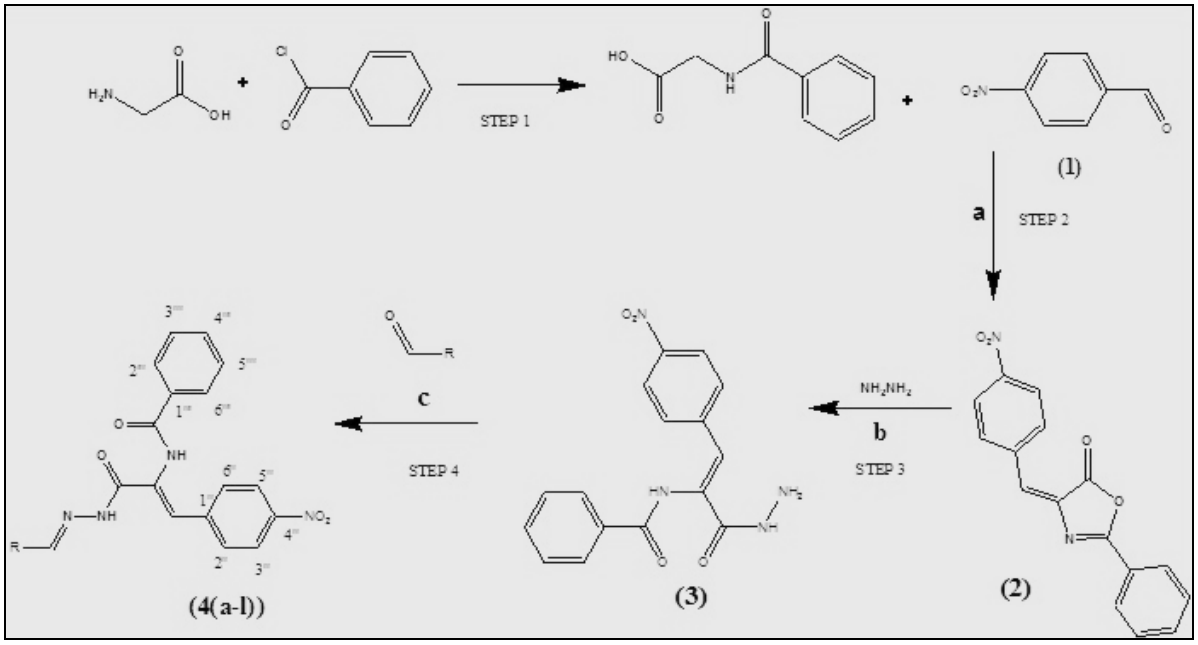
In light of the above facts, we planned to synthesize nitro substituted cinnamides linked with the other scaffold acylhydrazone, in hope of obtaining additional antimicrobial spectrum for the treatment of microbial infections and the study was further extended to known the interaction modes of ligand with target assessed by Moleculardocking studies and also predict the molecular properties, toxicity and drug likeness score using *in-silico* tools.

MATERIALS AND METHODS: All the melting points were determined in open capillaries using Thermonik precision melting point cum boiling point apparatus model C-PMB-2 and are uncorrected. The progress of reactions was monitored by pre-coated TLC plates (E. Merck Kieselgel 60 F254) and the IR spectra were recorded using Perkin-Elmer 1760 spectro photometer.^{1}H NMR and ^{13}C NMR spectra were recorded using Bruker Advance 400 MHz spectrometer in DMSO-d6, using Tetramethyl silane (TMS) as an internal standard. Elemental analyses (C, H, and N) were performed using Perkin Elmer model 240 C analyzer. Reagents and solvents were purchased from Sigma and were used without further purification.

Synthesis of 4-(4-nitro)-benzylidene – 2 - phenyl oxazol-5-ones (2): Synthesis of 4-(4-nitro) benzylidene-2-phenyloxazol-5-ones was done accordance with the previously reported method [14].

Synthesis of α-Benzamido-(4-nitro)-cinnama hydrazide (3): 4-(4-Nitro)-benzylidene-2-phenyl oxazol-5-ones **2** (0.01 mmol) was stirred with a solution of hydrazine hydrate (0.02 mmol) in ethanol (25ml) for 30 minutes. The deep yellow colour of oxazolone immediately turns to light yellow color product, which was filtered, washed and purified by recrystallization from methanol [15]. FT-IR (ν_{max}, cm-^{1}): 3288 (NH_2), 3166 (N-H), 1642 (C=O amide), 1601 (C=N) 1513, 1336 (NO_2). ^{1}HNMR (400 MHz, DMSO-d6) δ(ppm): 4.43 (s, 2H, NH_2), 7.1 (s, 1H, C= C-H), 7.49-8.20 (m, 9H, Ar C-H), 9.68 (s, 1H, CO-NH-C), 10.02 (s, 1H, CO-NH-N).

Synthesis of 2-Benzamido-3-(4-nitrophenyl)-N'-(substituted) benzylidene - acrylohydrazide (4a-l): Equimolar ratios of α-benzamido-(4-nitro)-cinnamahydrazide **(3)** and substituted aromatic aldehydes (0.01 mol) were transferred in 250 ml of conical flask containing 45 ml of absolute ethanol and catalytic amount of glacial acetic acid. The resulting mixture was subjected to microwave irradiation at 140 W with 10 sec interval for about 60 - 180 seconds. The reaction was monitored by TLC, allowed to cool at room temperature, filtered and purified by recrystallization from methanol.



SCHEME: (a): $(CH_3CO)_2$ O/ZINC OXIDE AND ETHANOL; (b): ABSOLUTE ETHANOL, STIRRING; (c): MICRO WAVE IRRADIATION AT 140 WATTS

S. no	Com code	R
1	4a	Phenyl
2	4b	4-Methoxy phenyl
3	4c	4-Dimethyl amino phenyl
4	4d	4-Nitro phenyl
5	4e	4-Hydroxy, 3-methoxy phenyl
6	4f	4-Chloro phenyl
7	4g	3,4-Dimethoxy phenyl
8	4h	2,4-Di chloro phenyl
9	4i	Styryl
10	4j	3-Indolyl
11	4k	4-Hydroxy phenyl
12	4l	2-Chloro phenyl

2-Benzamido-N'-benzylidene-3-(4-nitro) -phenyl acrylohydrazide (4a): Pale yellow crystals, m.p. 160-162ºC, yield 74%. FT-IR (ν_{max}, cm^{-1}): 3210 (N-H), 3000 (Ar C-H), 1637 (C=O amide), 1571 (C=N), 1476 (Ar C=C), 1513, 1336 (NO_2). ^{1}HNMR (400 MHz, DMSO- d_6) δ(ppm): 7.16 (s, 1H, HC=C), 7.5-8.26 (m, 14H, Ar C-H), 8.42 (s, 1H, HC=N), 10.30 (s, 1H, CO-NH), 11.87 (s, 1H, CO-NH-N). ^{13}C NMR(100 MHz, DMSO-d_6) δ(ppm): 166 (C-1), 127 (C-2), 123 (C-3), 163(-NH-CO-),145 (N=CH), 132 (C-1′), 128 (C-2′ and C-6′), 129 (C-3′ and C-5′), 131(C-4′), 140 (C-1″), 127 (C-2″ and C-6″),121 (C-3″ and C-5″),147 (C-4″), 133 (C-1 ‴), 127(C-2 ‴ and C-6 ‴), 128 (C-3 ‴ and C-5 ‴), 131(C-4 ‴). EI-MS m/z: 414 (M^+).

2-Benzamido-3-(4-nitrophenyl) - N'- (4-methoxy benzylidene)-acrylohydrazide(4b): Yellow crystals, m.p. 180-182ºC, yield 63%. FT-IR (ν_{max}, cm^{-1}): 3316 (N-H), 3047 (Ar C-H), 2902 (C-H of OCH_3), 1647 (C=O amide),1565 (C=N), 1509,1338 (NO_2); ^{1}HNMR (400 MHz, DMSO- d_6) δ(ppm): 3.81 (s, 3H, OCH_3), 7.0-8.2 (m, 13H, Ar C-H), 7.14 (s, 1H, HC=C), 8.63 (s, 1H, HC=N), 10.27 (s, 1H, CO-NH), 11.7 (s, 1H, CO-NH-N); Mass(m/z): 444 (M^+).

2-Benzamido-3-(4-nitrophenyl) - N'- (4-dimethyl aminobenzylidene)-acrylohydrazide (4c): Dark yellow crystals, m.p. 220-223ºC, yield 65%. FT-IR (ν_{max}, cm^{-1}): 3302 (N-H), 3102 (Ar C-H), 2901 (C-H of CH_3), 1645 (C=O amide), 1582 (N=C), 1514, 1335 (NO_2). ^{1}HNMR (400 MHz, DMSO- d_6) δ(ppm): 2.9 (s, 6H, $N(CH_3)_2$), 7.0-8.2 (m, 13H, Ar C-H), 7.16 (s, 1H, HC=C), 8.65 (s, 1H, HC=N), 10.11 (s, 1H, CO-NH), 11.2 (s, 1H, CO-NH-N).

2-Benzamido - 3 - (4-nitrophenyl) - N' -(4-nitro benzylidene)-acrylohydrazide (4d): Pale yellow solid, m.p. 191-194ºC, yield 78%. FT-IR (ν_{max}, cm^{-}

[1]): 3237 (N-H), 3043(Ar C-H), 1639 (C=O amide), 1478 (Ar C=C), 1592 (N=C), 1515, 1341 (NO_2). [1]HNMR (400 MHz, DMSO- d_6) δ(ppm): 7.17 (s, 1H, C=C-H), 7.5-8.3 (m, 13H, Ar C-H), 8.51 (s, 1H, HC=N), 10.34 (s, 1H, CO-NH-), 12.1 (s, 1H, CO-NH-N).

2-Benzamido-3-(4-nitrophenyl)-N' - (4-hydroxy, 3 - methoxybenzylidene) – acrylohydrazide (4e): Half white solid, m.p. 210-212ºC, yield 67%. FT-IR (ν_{max}, cm^{-1}): 3363 (OH), 3233 (N-H), 3002 (Ar C-H), 1665 (C=O amide), 1600 (C=N). [1]HNMR (400 MHz, DMSO- d_6) δ(ppm): 3.80 (s, 3H, OCH_3), 5.3 (s, 1H, OH), 6.9-8.2 (m, 12H, Ar C-H), 7.17 (s, 1H, C=C-H), 8.62 (s, 1H, HC=N), 10.07 (s, 1H, CO-NH-), 11.9 (s, 1H, CO-NH-N). [13]C NMR(100 MHz, DMSO-d_6) δ(ppm): 167 (C-1), 127 (C-2), 122 (C-3), 163 (-NH-CO-), 144 (N=CH), 126 (C-1′), 115 (C-2′), 150 (C-3′), 149 (C-4′), 115 (C-5′), 123 (C-6′), 141 (C-1″), 127 (C-2″ and C-6″), 121 (C-3″ and C-5″),147 (C-4″), 133 (C-1 ‴), 126 (C-2 ‴ and C-6 ‴), 127 (C-3 ‴ and C-5 ‴), 132(C-4 ‴), 56 (-OCH_3). EI-MS m/z: 460 (M^+).

2-Benzamido-3-(4-nitrophenyl) - N' - (4-chloro benzylidene)-acrylohydrazide (4f): Pale yellow solid, m.p. 159-161ºC, yield 75%. FT-IR (ν_{max}, cm^{-1}): 3288 (N-H), 3048 (Ar C-H), 1647 (C=O amide), 1481 (Ar C=C), 1591 (N=C), 1511, 1336 (NO_2), 631 cm^{-1} (C-Cl). [1]HNMR (400 MHz, DMSO- d_6) δ(ppm): 7.1 (s, 1H, C=C-H), 7.5-8.26 (m, 13H, Ar C-H), 8.4 (s, 1H, HC=N), 10.3 (s, 1H, CO-NH-), 11.93 (s, 1H, CO-NH-N).

2-Benzamido-3-(4-nitrophenyl) - N' - (3, 4-di methoxy benzylidene)-acrylohydrazide (4g): Yellow solid, m.p.170-172 ºC, yield 73%. FT-IR (ν_{max}, cm^{-1}): 3228 (N-H), 3066 (Ar C-H), 2991 (C-H in CH_3), 1636 (C=O amide), 1601 (C=N), 1505 (C=C). [1]HNMR (400 MHz, DMSO- d_6) δ(ppm): 3.73 (s, 6H, $(OCH_3)_2$), 6.92-8.21 (m, 12H, Ar C-H), 7.13 (s, 1H, HC=C), 8.3 (s, 1H, HC=N), 10.05 (s, 1H, CO-NH), 11.20 (s, 1H, CO-NH-N). [13]C NMR(100 MHz, DMSO-d_6) δ(ppm): 165 (C-1), 126 (C-2), 123 (C-3), 162 (-NH-CO-),144 (N=CH), 126 (C-1′), 114 (C-2′), 148 (C-3′), 149 (C-4′), 116 (C-5′), 121(C-6′), 141 (C-1″), 127 (C-2″ and C-6″),122 (C-3″ and C-5″),146 (C-4″), 134 (C-1 ‴), 127 (C-2 ‴ and C-6 ‴), 128 (C-3 ‴ and C-5 ‴), 132 (C-4 ‴), 55 (-OCH_3). EI-MS m/z: 474 (M^+).

2-Benzamido-3-(4-nitrophenyl) - N' - (2, 4- di chloro benzylidene)-acrylohydrazide (4h): Off white crystals, m.p. 163-165ºC, yield 79%. FT-IR (ν_{max}, cm^{-1}): 3292 (N-H), 2989 (Ar C-H), 1643 (C=O amide), 1585 (N=C), 1469 (Ar C=C), 1514, 1340 (NO_2), 642 (C-Cl). [1]HNMR (400 MHz, DMSO- d_6) δ(ppm): 7.19 (s, 1H, C=C-H), 7.5-8.2 (m, 12H, Ar C-H), 8.7 (s,1H, HC=N), 10.3 (s, 1H, CO-NH-), 12.1 (s, 1H, CO-NH-N).

2-Benzamido-3-(4-nitrophenyl) - N' - (3-Phenyl allylidene)-acrylohydrazide (4i): Dark yellow solid, m.p.181-183ºC, yield 77%. FT-IR (ν_{max}, cm^{-1}): 3231 (N-H), 3057 (Ar C-H), 1630 (C=O amide), 1609 (C=N), 1505 (Ar C=C). [1]HNMR (400 MHz, DMSO- d_6) δ(ppm): 6.9-8.19 (m, 16H, Ar C-H, -CH=CH of cinnamoyl), 7.18 (s, 1H, C=C-H), 8.17-8.19 (d, 1H, HC=N), 10.14 (s,1H, CO-NH), 11.79 (s, 1H, CO-NH-N).

***N'*-(Indol-3-yl-methylene) - 2-Benzamido – 3 -(4-nitrophenyl)-acrylohydrazide(4j):** Brown crystals, m.p. 196-198 ºC, yield 66%. FT-IR (ν_{max}, cm^{-1}): 3176 (N-H of Indolyl), 3056 (Ar C-H), 1640 (C=O amide), 1579 (N=C), 1458 (Ar C=C), 1517, 1347 (NO_2). [1]HNMR (400 MHz, DMSO- d_6) δ (ppm): 7.15 (s, 1H, C=C-H), 7.16-8.25 (m, 14H, Ar C-H), 8.36 (s, 1H, HC=N), 10.9 (s, 1H, CO-NH-), 12.15 (s, 2H, CO-NH-N, NH of Ind).

2-Benzamido-3-(4-nitrophenyl) - N' - (4-hydroxy benzylidene)-acrylohydrazide (4k): Off white crystals, m.p. 174-177ºC, yield 67%. FT-IR (ν_{max}, cm^{-1}):3371 (OH), 3275 (N-H), 3078 (Ar C-H), 1644 (C=O amide), 1481 (Ar C=C), 1581 (N=C), 1520, 1331 (NO_2). [1]HNMR (400 MHz, DMSO- d_6) δ(ppm): 7.17 (s, 1H, C=C-H), 7.48-8.14 (m, 13H, Ar C-H), 8.53 (s, 1H, HC=N), 10.15 (s, 1H, CO-NH-), 11.98 (s, 1H, CO-NH-N). [13]C NMR(100 MHz, DMSO-d_6) δ(ppm): 165 (C-1), 127 (C-2), 123 (C-3), 163 (-NH-CO-), 145 (N=CH), 125 (C-1′), 128 (C-2′ and C-6′), 115 (C-3′ and C-5′), 158 (C-4′), 141 (C-1″), 127 (C-2″ and C-6″), 120 (C-3″ and C-5″),146 (C-4″), 134 (C-1 ‴), 126 (C-2 ‴ and C-6 ‴), 128 (C-3 ‴ and C-5 ‴), 132 (C-4 ‴); EI-MS m/z: 430 (M^+).

2-Benzamido-3-(4-nitrophenyl) - N' - (2-chloro benzylidene)-acrylohydrazide (4l): Light yellow crystals, m.p. 168-171ºC, yield 82%. FT-IR (ν_{max}, cm^{-1}): 3198 (N-H), 3051 (Ar C-H), 1640 (C=O

amide), 1481 (Ar C=C), 1577 (N=C), 1518, 1329 (NO_2), 643 cm^{-1} (C-Cl). ^{1}HNMR (400 MHz, DMSO- d_6) δ(ppm): 7.13 (s, 1H, C=C-H), 7.57-8.29 (m, 13H, Ar C-H), 8.3 (s, 1H, HC=N), 10.1 (s, 1H, CO-NH-), 11.72 (s, 1H, CO-NH-N). ^{13}C NMR (100 MHz, DMSO-d_6) δ(ppm): 166 (C-1), 127 (C-2), 123 (C-3), 164(-NH-CO-),145 (N=CH), 133 (C-1′), 134 (C-2′), 128 (C-3′), 132(C-4′), 126 (C-5′), 130 (C-6′), 140 (C-1″), 128 (C-2″ and C-6″), 121 (C-3″ and C-5″), 147 (C-4″), 133 (C-1 ‴), 127 (C-2 ‴ and C-6 ‴), 128 (C-3 ‴ and C-5 ‴), 131 (C-4 ‴). EI-MS m/z: 448 (M^+).

Antimicrobial Activity: All the title compounds were assayed *in-vitro* for antibacterial activity against two Gram positive (*Bacillus subtilis, Staphylococcus aureus*), two Gram negative bacterial strains (*Escherichia coli, Pseudomonas aeruginosa*) and one fungal strain (*Candida albicans*). The MIC (Minimum inhibitory concentration) was determined by using twofold serial dilution method [16]. Ciproflaxin and miconazole were used as reference standards to compare the antibacterial and antifungal activities, respectively. All the title compounds and standard drugs were dissolved in dimethylsulfoxide (stock solution 5 mg/mL). Further dilutions were prepared to get 200, 100, 50, 25, 12.5, 6.25, and 3.125 µg/ml concentrations and control also maintained at the same dilution contains only solvent. The MIC values were obtained from the lowest concentration of the test compound where the tubes remain clear, indicating that the bacterial growth was completely inhibited at this concentration.

Molecular Docking: Molecular docking of compounds (**4a-l**) with the 3D X-ray crystal structure of *E.coli* FAB protein (β-ketoacyl-acyl carrier protein synthase III) retrieved from the PDB (Protein Data Bank) incorporated with inhibitor was accessed to predict active site residue. The 3D structure of target (PDB ID: 5BNM) was imported in to maestro v 9.0 and receptor grid of 20x20x20 A° was generated for the 5BNM around the centroid of respective active site using GLIDE (Schrodinger). All the ligands, standard drug were embedded in to the generated grid of FAB protein to access their binding affinities.

***In-silico* ADME:** In the present study molecular properties of compounds **(4a-l)** were calculated by using Molinspiration online tool [17] and drug likeness score, toxicity profile calculated by OSIRIS program [18] in order to predict the compounds drug likeness score. The %ABS was calculated according to the formula [19].

$$\%ABS = 109-(0.345 \times TPSA)$$

RESULTS AND DISCUSSION:

Chemistry: The synthesis of 2-Benzamido-3-(4-nitrophenyl)-N'-(substituted benzylidene)-acrylo hydrazide **(4a-l)** were carried out in four steps under green protocols by the action of different aromatic aldehydes on α-Benzamido-(4-nitro)-cinnamahydrazide **(3)** in acidic conditions by microwave irradiation (**Scheme**) with high purity and less reaction time. Compounds **(3)** has been obtained from intermediate compound (**2**) by the action of hydrazine hydrate, act as strong nucleophile attacks the oxazole ring at highly susceptible carbonyl site and opens the ring.

The IR spectra of the compounds **(4a-l)** showed NH and C=O stretching bands between 3176 - 3391 cm^{-1} and 1630 - 1665 cm^{-1} respectively indicates the formation of title compounds. Appearance of two more bands between 1335 - 1517 cm^{-1} region indicates the presence of nitro substitution in title compounds.

In addition to the above ^{1}H NMR spectra revealed the formation of title compound by the appearance of singlet between δ11.20-12.15 regions (due to the NH of CO-NH-N=) and absence of singlet at δ 4.43 (due to free NH_2 of –CO-N-NH_2) present in compound **(3)**. This is supported by the presence singlet in the region of δ 8.17 - 8.70 due to the protons of CH=N groups and showed the multiplet between δ 6.9-8.3 regions indicates the deshielding of aromatic protons by nitro group. Additional support for the structures of title compounds was provided by ^{13}C NMR spectra reveals two peaks around δ 163-166 and a peak between δ 55-56 assigned for carbonyl group of amide and methoxy group respectively. The peaks resonated at δ 114-158 is due to the presence of aromatic carbons and also appearance of signal around δ 145 indicates the presence of C=N linkage. All the title compounds conformed by the appearance of molecular ion peaks between m/z 414-483.

Antimicrobial Activity: The antimicrobial results reveals compound **4e** (4-hydroxy, 3-methoxy-phenyl) showed pronounced antibacterial activity (MIC: 3.12 µg/ml) than standard ciprofloxacin (MIC: 6.25 µg/ml) against Gram negative strain (*E.coli, P.aeruginosa*) due to the presence of methoxy group ortho to the hydroxyl group [20]. **Table 1** reveals, none of the title compounds exhibits higher activities (MIC between 6.25-200 µg/ml) against two Gram positive strains compared with standard (3.12 µg/ml) and few compounds **4e** (12.5 µg/ml), **4h** (6.25 µg/ml) exhibited considerable activity against *S. aureus* and *B. subtilis* compared with standard drug. The SAR data indicates the antimicrobial activity profile of title compounds were altered by the nature of substitution on phenyl ring such as EDG enhances the activity than EWG substituted derivatives (**4e>4d** towards *E.coli*) and this finding was supported by the previous results [13, 20]. It was anticipated that, the introduction of one more electron withdrawing group enhances the activity of title compounds but the results were in contrast to the expectations. From the antifungal data we identify compound **4h** (12.5µg/ml) only displayed good activities comparable with standard (6.25 µg/ml) it might be due to the presence of second halogen elevates the log p values responsible for better activity [21] and remaining all showed lesser activity.

Molecular Docking: Further, experimental observations were followed up with molecular docking studies and the data highlights all the compounds showed potent *E coli* FabH inhibition than standard (-4.74) except few compounds (**4c**, **4d**) (**Table 1**). Among all compound **4k** (-8.78) which is the most potent FabH inhibitor forms four hydrogen bond (2.38, 1.74, 2.10, 1.83ºA) with target (**Fig. 2**), also have lowest binding energy -72.74 Kcal/mol. From the binding modes of title compounds with target protein, we understood Asn 247, His 244, Cys112, Phe 304, Asn 274, Arg 151, Gly 209 amino acid residues constitute the active site of enzyme, depicted in **Fig. 2** and **Fig. 3**. The docking results strengthened that antibacterial activities of the synthetic compounds were probably correlated to their FabH inhibitory activities and compound **4k**, **4e** are the potent inhibitors of FabH.

TABLE 1: ANTIMICROBIAL ACTIVITY AND DOCKING SCORES OF COMPOUNDS (4a-l)

		MIC (µg/ml)						Docking scores with FAB protein			
Sno	**Code**	**EC**	**PA**	**SA**	**BS**	**CA**	**NHB**	**HB (ºA)**	**AA**	**DS**	**BE (kcal/mol)**
1	4a	12.5	25	25	100	100	1	1.94	Asn 247	-7.02	-64.48
2	4b	50	100	100	50	50	2	2.35	His 244	-6.35	-69.34
								1.75	Asn 247		
3	4c	50	100	200	200	100	1	2.41	Asn 247	-4.66	-52.45
4	4d	100	200	200	200	50	0	-	-	-4.19	-50.12
5	4e	3.12	3.12	12.5	12.5	50	3	2.26	Cys 112	-7.47	-76.38
								1.86	Phe 304		
								2.38	Asn247		
6	4f	100	100	200	100	50	1	2.43	Asn 247	-5.14	-52.14
7	4g	100	50	200	100	50	3	2.49	Cys 112	-6.38	-71.97
								2.10	Asn 247		
								2.59	Asn 274		
8	4h	25	25	200	6.25	12.5	1	2.18	Asn 247	-5.73	-61.13
9	4i	100	200	100	200	100	0	-	-	-6.64	-68.24
10	4j	100	200	200	100	100	1	1.90	Asn 247	-6.36	-71.76
								2.16	Arg 151		
11	4k	3.12	6.25	25	50	100	4	2.38	Cys 112	-8.78	-72.74
								1.74	Phe 304		
								2.10	His 244		
								1.83	Asn 247		
12	4l	12.5	12.5	25	12.5	25	2	1.86	Gly 209	-7.36	-74.82
								2.56			
13	Std	6.25	6.25	3.12	3.12	-	0	0	0	-4.74	-55.98
14	Std	-	-	-	-	6.25	-	-	-	-	-

EC: *E.coli*; **PA**: *Pseudomonas aeruginosa*; **SA**: *Staphylococcus aureus*; **BS***: Bacillus subtilis*; **CA**: *Candida albicans*; **NHB:** No of hydrogen bonds; **HD:** Hydrogen bond distance; **AA:** Amino acid; **DS:** Docking score; **BE:** Binding free energy

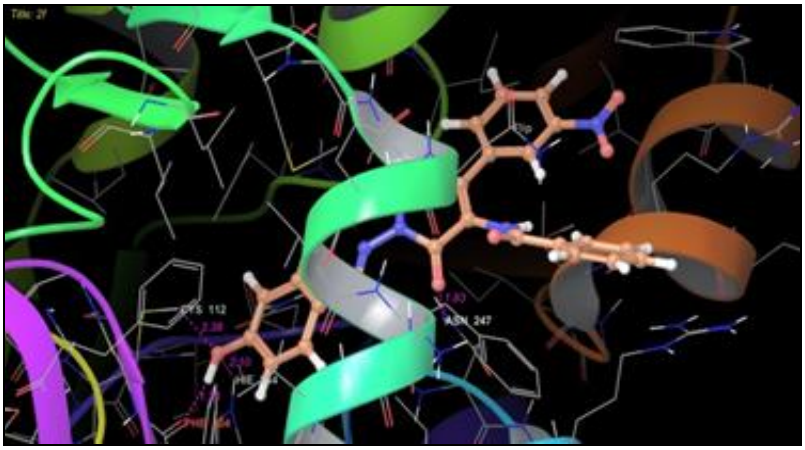


FIG. 2: DOCKING INTERACTION OF 4K WITH 5BNM

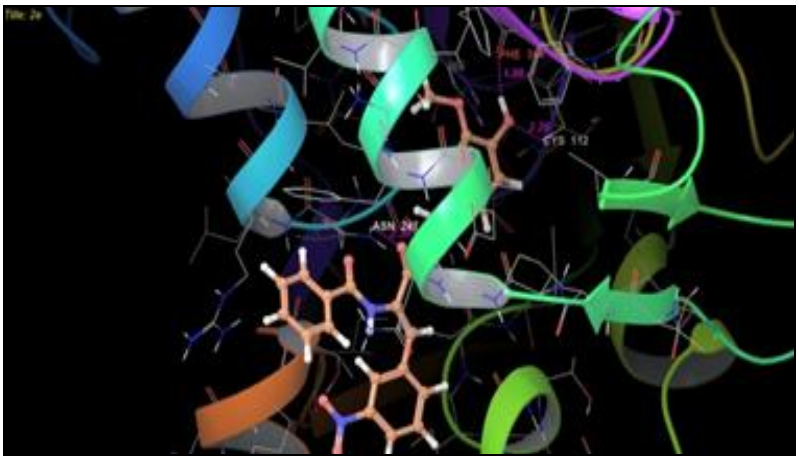


FIG. 3: DOCKING INTERACTION OF 4E WITH 5BNM

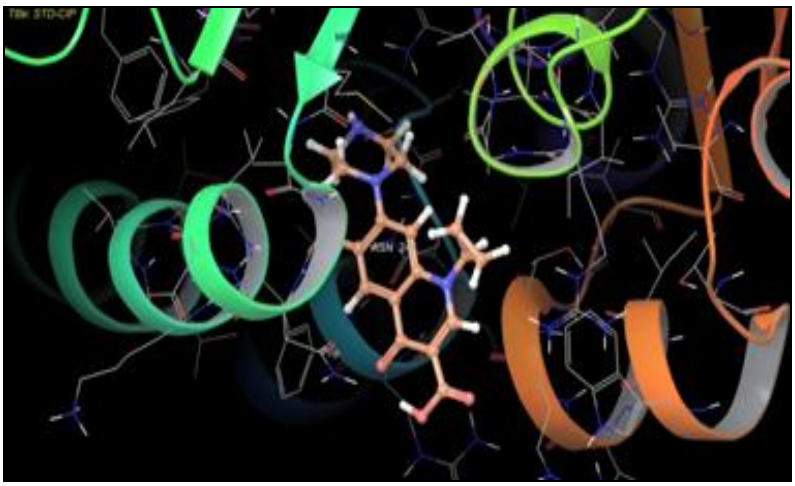


FIG. 4: DOCKING INTERACTION OF STANDARD WITH 5BNM

***In-silico* ADME:** All the compounds obeyed Lipinski rule of five is important for assessing of compounds oral bioavailability. It is clear from the **Table 2** that log P values of all the compounds found to be in the acceptable criteria (3.96-5.90) and TPSA (Total polar surface area) is another key property that has been linked to bioavailability. It was found that all the compounds showed TPSA between 116 -136 predict good oral bioavailability except compound **4d**, **4e** and it is important to note all compounds have %absorption between 53-69. The results reveals all compounds have 1.95-1.28 as Druglikeness score and devoid from toxicity except compound **4c** (**Table 2**).The results of *in-silico* data indicates that, these compounds may have the potential to become a lead compound.

TABLE 2: MOLECULAR DESCRIPTORS OF TITLE COMPOUNDS (4a-l)

S. no.	C code	Log P	Log S	TPSA	% abs	n-Hba	n-hbd	n-ROTB	Mw	Mu	Tu	Ir	Re	Dl
1	4a	4.62	-5.92	116	69	8	2	7	414	G	G	G	G	1.29
2	4b	4.68	-5.94	125	66	9	2	8	444	G	G	G	G	1.28
3	4c	4.73	-5.96	119	68	9	2	8	457	G	R	G	G	1.95
4	4d	4.58	-6.38	162	53	11	2	8	459	G	G	G	G	1.29
5	4e	3.96	-5.64	145	59	10	3	8	460	G	G	G	G	1.28
6	4f	5.30	-6.66	116	69	8	2	7	448	G	G	G	G	1.33
7	4g	4.27	-5.96	134	63	10	2	9	474	G	G	G	G	1.28
8	4h	5.90	-7.39	116	69	8	2	7	483	G	G	G	G	1.33
9	4i	4.85	-6.21	116	69	8	2	8	440	G	G	G	G	1.30
10	4j	4.77	-6.45	132	63	9	3	7	453	G	G	G	G	1.44
11	4k	4.14	-5.63	136	62	9	3	7	430	G	G	G	G	1.29
12	4l	5.25	-6.66	116	60	8	2	7	448	G	G	G	G	1.33

Log P: Lipophilicity; **Log S**: Solubility; **TPSA**: Total polar surface area; **n-Hba**: No of hydrogen bond acceptors; **n-Hbd**: No of hydrogen bond donars; **n-ROTB**: No of rotatable bonds; **Mw**: Molecular weight; **Mu**: Mutagenic; **Tu**: Tumerigenic; **Ir**: Irritant; **Re**: Reproductive effect; **Dl**: Druglikeness; **G:** No Risk; **R:** High Risk.

CONCLUSION: A new series of 4-nitro-cinnamamide analogues were synthesized with good yields, shorter reaction time by using microwave technique and screened for antimicrobial and docking studies. It could be seen clearly from our studies compound 4e, 4k exhibited good antimicrobial activity due to the presence of important pharmacophore features responsible for more affinity of ligand with the active site of target and also need further mechanistic studies for optimization of their properties.

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