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Discovery and synthesis of 2-amino-1-methyl-1H-imidazol-4(5H)-ones as GPCR ligands; an approach to develop breast cancer drugs via GPCR associated PAR1 and PI3Kinase inhibition mechanism

S.R. Ashok[a], M.K. Shivananda[a,*], A. Manikandan[b,**], R. Chandrasekaran[c]

[a] Dept of Studies & Research in Chemistry, Tumkur University, Tumkur 572102, Karnataka, India
[b] Dept. of Biotech, Vellore Institute of Technology (VIT), Vellore 632014, Tamil Nadu, India
[c] Sai Supreme Chemicals, Gummidipoondi, Chennai 601201, Tamil Nadu, India

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ABSTRACT

Efforts were taken to synthesis and characterize 2-amino-1-methyl-1H-imidazole-4(5H)-one derivatives (**4a-u**) through a four-step reaction. The achieved compounds in remarkable yield have characterized through standard analytical techniques such as FTIR, LC-MS, NMR, HRMS, and elemental analysis. Present study mainly aimed to evaluate **4a-u** as G protein-coupled receptors (GPCR). In the mechanism, stimulation of phosphoinositide 3-kinase (PI3K) and Akt (protein kinase B) is a general reaction activated by a series of membrane-bound receptors such as GPCR. Protease-activated receptor-1 (PAR1) is a subfamily of related GPCR, which triggered by the division of fragment of its extracellular domain. Therefore, molecular docking is done to ensure the inhibition of PAR1 and PI3Kinase. PI3Kinase is a chief enzyme in the development of breast cancer via the Akt/mTOR pathway. Thus, *in vitro* PI3Kinase inhibition and anti-breast cancer studies has also done to screen medicinally important compounds among (**4a-u**). Based on the best binding affinity, *in vitro* relative % activity and IC_{50} values, compounds **4a**, **4g**, **4i**, **4n**, and **4u** were screened for further preclinical studies in animal model evaluations.

1. Introduction

Small molecule drugs are discoverable through conventional and modern drug discovery techniques [1–3]. Imidazole-based drugs are ubiquitous since they are most effective against a wide range of drug targets [4]. They are efficacious towards various cancers [4] and active inhibitors against protein kinases [5]. Imidazole-based antifungal and antimycobacterial agents have shown remarkable activities in the recent past decade [6]. In this study, we have approached a four-step synthesis of 2-amino-1-methyl-1H-imidazole-4(5H)-one derivatives (**4a-u**). The final confirmed structures of these compounds were subjected to a series of *in silico* preliminary bioactivity screening in order to adjudge their most suitable and efficacious bioactivity (Fig. 1) to be executed. Free online tools such as Molinspiration (www.molinspiration.com), PASSonline (www.pharmaexpert.ru/passonline/) and MedChem designer (www.simulations-plus.com). All results obtained from these tools were centralized with correlations between the most favorably predicted bioactivity. From the four repeated prediction results, the compounds have shown favorable prediction values against G protein-coupled receptors (GPCRs) ligand, Enzyme inhibition, and Kinase inhibition.

GPCRs establish receptors from enormous protein families that are recognized from the external molecules of the corresponding cell environment and induces internal signal transduction pathways and simultaneously also stimulating the other accompanying cellular processes [7,8]. GPCRs also recognized as the heptahelical receptors, G protein-linked receptors (GPLR), and Serpentine receptors. GPCRs also was known as the seven-(pass)-transmembrane domain receptors (since they are Coupling with G proteins), and 7TM receptors (because they pass through the cell membrane seven times) [8]. GPCRs are highly associated and intricate in several diseases, in fact, GPCRs being the drug target of about 34% of all recent drug discovery researches [9–11]. GPCRs are involved majorly in the cAMP and PI3Kinase signal pathways [12]. Protease-Activated Receptors (PARs), a sub-family of GPCRs, often extremely expressed in platelets (thrombocytes), myocytes (muscle cell), endothelial cells, and neurons (nerve cells) [13].

* Corresponding author.
** Corresponding author.
E-mail addresses: shivanmk@yahoo.com (M.K. Shivananda), mailtomicromani@gmail.com (A. Manikandan).

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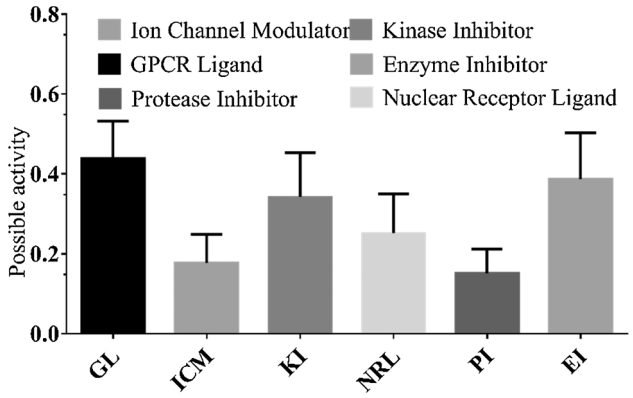


Fig. 1. Bioactivity prediction results.

PAR1-4 are the members of the seven-transmembrane GPCR superfamily, and are almost expressed all over the body and responsible for various diseases including inflammation and cancers [14,15]. Therefore, the present study targeted PAR1 and PI3Kinase inhibition studies along with anti-breast cancer investigations for 2-amino-1-methyl-1H-imidazole-4(5H)-one derivatives (**4a-u**) (Fig. 2).

2. Materials and methods

All organic chemicals and solvents have procured from Sigma-Aldrich, Merck, Spectrochem, and AVRA. Solvents and common reagents used as without doing any further purification. For thin-layer chromatography (TLC) analysis, Merck pre-coated plates (silica gel 60 F254) used and the spots visualized under UV light. Merck silica gel 60 (230–400 mesh) was used for flash column chromatography and the eluting solvents are indicated in the procedures. Melting point (mp) determinations were performed using OptiMelt automated melting point system and are uncorrected. ^{1}H NMR (300 MHz) and ^{13}C NMR (300 MHz) spectra were recorded on Bruker-300 MHz instrument. Mass spectra recorded on Agilent ion trap MS and Infrared (IR) spectra recorded on Perkin Elmer FT-IR spectrometer. HPLC analysis of all the compounds carried out on an Agilent 1260 series HPLC with an Agilent Extend-C18 column.

*2.1. General procedure for the synthesis of title compounds, 2-amino-1-methyl-1H-imidazole-4(5H)-one derivatives (**4a-u**)*

*2.1.1. General procedure for the synthesis of ethyl 1H-indole-5-carboxylate (**1**)*

To a 1000 ml three neck RB flask indole-4-carboxylic acid (1 eq, 1 mol), ethanol (10 V) were added. The reaction mass was stirred for 10 mins at 20–30 °C. Thionyl chloride (2.0 eq, 2 mol) added slowly on stirring condition and heated the reaction mass to reflux. The obtained clear solution stirred and refluxed further for 2 h. The reaction mass cooled to 20–30 °C and stirred for another 10 mins. 10 V of MTBE (Methyl tert-butyl ether) added as anti-solvent and stirred for 30 mins. The off-white solid material filtered and washed with 2 V of MTBE. The material was suction dried till no MLR and the obtained mass was dried further under vacuum at 50–60 °C for 6 h to get ethyl 1H-indole-5-carboxylate **(1)** with ≈90% yield. (-^{1}H NMR (300 MHz, DMSO-d_6) δ: 11.49 (S, 1H, −NH), 8.29 (S, 1H, Ar-H), 7.76–7.73 (d, J = 9 Hz 1H, Ar−H), 7.49–7.47 (m, 2H, Ar−H), 6.61 (S, 1H, Ar-H), 4.32–4.30 (quat, J = 6 Hz, 2H, $-OCH_2$) and 1.35–1.32 (t, J = 3 Hz and J = 6 Hz, 3H, CH_3)).

*2.1.2. General procedure for the synthesis of N-substituted-ethyl-indole-5-carboxylate (**2a-o**)*

To a 100 ml single neck, RB flask, ethyl 1H-indole-5-carboxylate (**1**) (1 eq, 1 mol), DCM (20 V), tetra butyl ammonium hydrogen sulfate (0.010 eq) was added and the reaction mass was stirred for 5mins at 20–30 °C. Then, Potassium hydroxide powder (3.0 eq) added to the

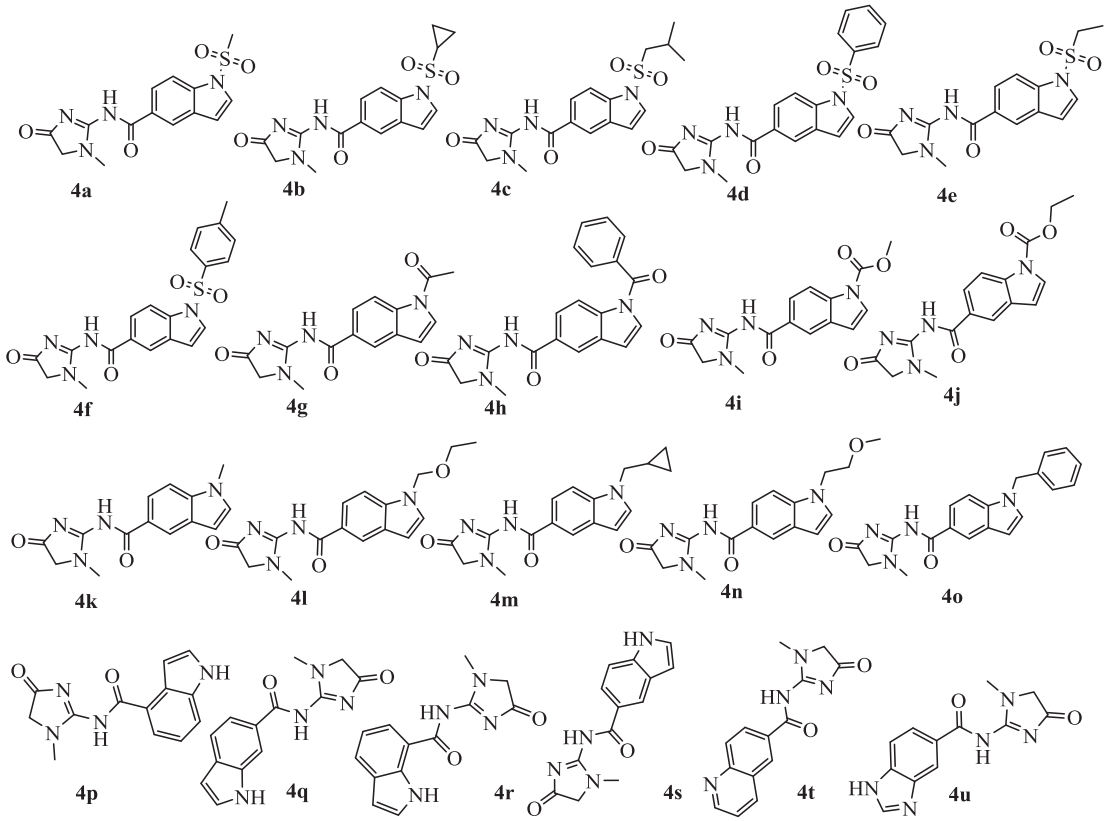


Fig. 2. Proposed final structures of 2-amino-1-methyl-1H-imidazole-4(5H)-one derivatives.

clear solution and stirred at 0–5 °C for 10mins. After that, the respective alkyl halides/sulfonyl halides or carbamates (1.5–2.0 eq) were added and the reaction mass stirred for reaction completion at 20–30 °C. The reaction completion was confirmed over TLC/LCMS. After the completion of the reaction, the mass was filtered out using a hyflo bed and washed with 2 V of DCM. Further, the filtered content dried over anhydrous sodium sulfate and the volatiles distilled off under reduced pressure to get the crude gummy/semi-solid material. These crude materials were purified using column chromatography with Silica as the stationary phase and EtOAc: *n*-Heptane as the mobile phase to get pure products (**2a-o**). The pure material thus obtained in a range of 60–90 % yield of the target product.

*2.1.3. General procedure for the synthesis of N-substituted-ethyl-indole-5-carboxylic acid (**3a-o**)*

To a 100 ml single neck RB flask, *N*-substituted-ethyl-indole-5-carboxylate (1 eq), THF: MeOH: H_2O (1:1:0.5) (20 V) were added and the reaction mass stirred for 5mins at 20–30 °C. Lithium hydroxide (5.0 eq) added and stirred for further 4–6 h at 20–30 °C until the reaction completion confirmed by TLC/LCMS. Upon the reaction completion, the reaction mass was concentrated under vacuum and the residual material was diluted with 20 V of water. The pH was adjusted to 3–4 using 3 N HCI and the product was extracted using ethyl acetate (2 × 15 V). The separated ethyl acetate layer washed with water (10 V), dried over anhydrous sodium sulfate, and concentrated under vacuum. The obtained products (**3a-o**) dried thoroughly under vacuum and taken for coupling reaction without further purification.

*2.1.4. Synthesis of 2-amino-1-methyl-1H-imidazole-4(5H)-one (creatinine) derivatives (**4a-u**)*

Compounds (**4a-u**) synthesized from *N*-substituted-4-indole carboxylic acids (**3a-o**) and derivatives (**4p-u**) from heterocyclic carboxylic acids using standard amidation reaction. To a 100 ml single neck RB flask, (*N*-substituted-4-indole carboxylic acids (**3a-u**)) (1 eq/1mol), creatinine (1.5 eq/1.5 mol), HATU (1 eq/1 mol) and DMF (8–12 V) were added under nitrogen atmosphere and stirred for 5mins at 20–30 °C. Upon a clear solution formed, DIPEA (2 eq or 2 mol) was added and the reaction mass stirred again for 12–16 h at 20–30 °C. After 12–16 h, TLC/LCMS confirmed the completion of the reaction. Ice-cold water (20 volumes) was added slowly to the reaction mixture under stirring condition. The product was extracted with dichloromethane (2 × 10 volumes). The combined dichloromethane extract washed with (10 volumes) water and the organic layer separated then dried over anhydrous sodium sulfate. Further, the volatiles was distilled off under reduced pressure to get the crude product. The crude material further purified using column chromatography with silica as the stationary phase and MDC: MeOH as the mobile phase to get pure products (**4a-u**) with 60–88% yield.

*2.1.5. N-(1-methyl-4-oxo-4,5-dihydro-1H-imidazol-2-yl)-1-(methylsulfonyl)-1H-indole-5-carboxamide (**4a**)*

1-(Methylsulfonyl)-1H-indole-5-carboxylic acid was used to prepare **4a**. Melting point: 128–130 °C. IR (KBr): 3360 (*amide N—H* stretch), 3205 (*aromatic C—H* stretch), 2903 (*—CH* stretch), 1624 (amide *—C═O* stretch), 1671 (*aromatic C═C* stretch), 1268 (*—C—N* stretch), 1242 (*—S═O* stretch) and 721 (*S—C* stretch) Cm^{-1}; 1H NMR (300 MHz, DMSO-d_6) *δ*: 11.31 (s, 1H), 8.58 (s, 1H), 8.24–8.21 (d, *J* = 9, 1H), 7.92–7.89 (d, *J* = 9, 1H), 7.68–7.67 (d, *J* = 3, 1H), 6.99–6.98 (d, *J* = 3, 1H, 4.16 (s, 2H, 3.15 (s, 3H), 2.91 (s, 3H, CH_3). ^{13}C NMR *δ*: 174.78 (*—CH_2CONH_2*), 172.34 (—CCONH); 158.72, 137.12, 130.29, 128.73, 126.08, 123.79, 112.78 and 108.93 (Aromatic C), 51.78 (—NCH_2), 48.86 (—NCH_3) and 32.35 (-SCH_3) ppm. LC-MS: 335.05 (M + 1). Yield: 88%; Anal calcd for: $C_{14}H_{14}N_4O_4S$; Elemental Analysis calculated: C, 50.29; H, 4.22; N, 16.76; S, 9.59; found: C, 50.30; H, 4.23; N, 16.77; S, 9.59; HRMS Calculated 334.3500 [M +] *m/z*, Found 334.3504.

*2.1.6. 1-(Cyclopropylsulfonyl)-N-(1-methyl-4-oxo-4,5-dihydro-1H-imidazol-2-yl)-1H-indole-5-carboxamide (**4b**)*

1-(Cyclopropylsulfonyl)-1H-indole-5-carboxylic acid was used to prepare 4b. Melting point: 124–126 °C. IR (KBr): 3352 (*amide N—H* stretch), 3195 (*aromatic C—H* stretch), 2909 (*—CH* stretch), 1616 (amide *—C═O* stretch), 1656 (*aromatic C═C* stretch), 1234 (*-C—N* stretch), 1208 (*—S═O* stretch) and 717 (*S—C* stretch) Cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) *δ*: 11.34 (s, 1H), 8.59 (s, 1H), 8.26–8.23 (d, *J* = 9, 1H), 8.0–7.97 (d, *J* = 9, 1H), 7.74–7.73 (d, *J* = 3, 1H), 7.03–7.02 (d, *J* = 3, 1H), 4.16 (s, 2H), 3.20 (m, 4H), 1.33–1.32 (d, *J* = 3, 2H), 1.15–1.13 (d, *J* = 3, 2H). ^{13}C NMR *δ*: 174.78 (*—CH_2CONH_2*), 172.33 (—CCONH); 158.69, 137.02, 132.73, 130.38, 128.45, 123.72, 121.18, 112.94 and 109.21 (Aromatic C), 51.74 (*—CH_3NCH_2CO)*, 31.54 (—NHCH_3), 30.98 (-SCHCH2CH2), 6.30 (—CCH_2CH_2), ppm. LC-MS: 361.05 (M + 1). Yield: 72%; Anal calcd for: $C_{16}H_{16}N_4O_4S$; Elemental Analysis calculated: C, 53.32; H, 4.48; N, 15.55; S, 8.90; found: C, 53.32; H, 4.48; N, 15.56; S, 8.90; HRMS Calculated 360.3880 [M +] *m/z*, Found 360.3882.

*2.1.7. 1-(Isobutylsulfonyl)-N-(1-methyl-4-oxo-4,5-dihydro-1H-imidazol-2-yl)-1H-indole-5-carboxamide (**4c**)*

1-(Isobutylsulfonyl)-1H-indole-5-carboxylic acid was used to prepare 4c. Melting point: 118–120 °C. IR (KBr): 3346 (*amide N—H* stretch), 3210 (*aromatic C—H* stretch), 2923 (*—CH* stretch), 1642 (amide *—C═O* stretch), 1668 (*aromatic C═C* stretch), 1244 (*-C—N* stretch), 1230 (*—S═O* stretch) and 740 (*S—C* stretch) Cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) *δ*: 11.34 (s, 1H), 8.59 (s, 1H), 8.26–8.23 (d, *J* = 9, 1H,), 7.96–7.93 (d, *J* = 9, 1H), 7.74–7.73 (d, *J* = 3, 1H), 7.03–7.02 (d, *J* = 3, 1H), 4.16 (s, 2H), 3.59–3.57 (d, *J* = 6, 2H), 3.20 (s, 3H), 2.08–1.98 (t, *J* = 3, 1H), 0.99–0.96 (d, *J* = 9, 6H). ^{13}C NMR *δ*: 174.77 (*—CH_2CONH_2*), 172.30 (—CCONH); 158.69, 136.86, 132.75, 130.27, 128.23, 126.08, 123.77, 112.69 and 109.08 (Aromatic C), 60.98 (*—CH_3NCH_2CO)*, 51.74 (—NHCH_3), 30.97 (—SCH2CH), 24.78 (—SCH2CHCH3) and 22.10 (—CCH_3CH_3), ppm. LC-MS: 377.1 (M + 1). Yield: 68%; Anal calcd for: $C_{17}H_{20}N_4O_4S$; Elemental Analysis: calculated: C, 54.24; H, 5.36; N, 14.88; S, 8.52; found: C, 54.24; H, 5.36; N, 14.89; S, 8.52; HRMS Calculated 376.4310 [M +] *m/z*, Found 376.4310.

*2.1.8. 1-(Phenylsulfonyl)-N-(1-methyl-4-oxo-4,5-dihydro-1H-imidazol-2-yl)-1H-indole-5-carboxamide (**4d**)*

1-(Phenylsulfonyl)-1H-indole-5-carboxylic acid was used to prepare 4d. Melting point: 130–132 °C. IR (KBr): 3332 (*amide N—H* stretch), 3245 (*aromatic C—H* stretch), 2939 (*—CH* stretch), 1663 (amide *—C═O* stretch), 1646 (*aromatic C═C* stretch), 1280 (*-C—N* stretch), 1265 (*—S═O* stretch) and 786 (*S—C* stretch) Cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) *δ*: 11.32 (s, 1H), 8.49 (s, 1H), 8.24–8.21 (d, *J* = 9, 1H), 8.08–8.06 (d, *J* = 6, 3H), 7.95–7.93 (d, *J* = 6, 1H), 7.77–7.72 (m, 1H), 7.67–7.62 (m, 2H), 7.03–7.02 (d, *J* = 3, 1H), 4.14 (s, 2H) and 3.20 (s, 3H). ^{13}C NMR *δ*: 174.54 (*—CH_2CONH_2*), 172.29 (—CCONH); 158.70, 137.40, 136.56, 135.27, 133.16, 130.73, 130.38, 128.30, 127.20, 126.36, 123.73, 112.96 and 110.68 (Aromatic C), 51.74 (—NCH_2) and 30.95 (—NCH_3) ppm. LC-MS: 397.1 (M + 1). Yield: 74.1%; Anal calcd for: $C_{19}H_{16}N_4O_4S$; Elemental Analysis calculated: C, 57.57; H, 4.07; N, 14.13; S, 8.09; found: C, 57.57; H, 4.08; N, 14.14; S, 8.09; HRMS Calculated 396.4210 [M +] *m/z*, Found 396.4213.

*2.1.9. 1-(Ethanesulfonyl)-N-(1-methyl-4-oxo-4,5-dihydro-1H-imidazol-2-yl)-1H-indole-5-carboxamide (**4e**)*

1-(Ethanesulfonyl)-1H-indole-5-carboxylic acid was used to prepare 4e. Melting point: 125–127 °C. IR (KBr): 3390 (*amide N—H* stretch), 3223(*aromatic C—H* stretch), 2946 (*—CH* stretch), 1645 (amide *—C═O* stretch), 1665 (*aromatic C═C* stretch), 1266 (*-C—N* stretch), 1253 (*—S═O* stretch) and 768 (*S—C* stretch) Cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) *δ*: 11.29 (s, 1H), 8.55 (s, 1H), 8.21–8.18 (d, *J* = 9, 1H), 7.91–7.88 (d, *J* = 9, 1H), 7.68–7.67 (d, *J* = 3, 1H), 6.98–6.97 (d, *J* = 3,

1H), 4.14 (s, 2H), 3.70–3.63 (d, *J* = 6, 2H), 3.15 (s, 3H), 1.09–1.04 (d, *J* = 9, 3H). ^{13}C NMR *δ*: 174.75 (−*CH$_2$CONH$_2$*), 172.30 (−CCONH); 158.69, 137.07, 130.25, 128.66, 126.03, 123.75, 112.75 and 108.89 (Aromatic C), 51.75 (−NCH$_2$), 48.84 (−NCH$_3$). 30.98 (-SCH$_2$CH$_3$) and 8.24 (-SCH$_2$CH$_3$) ppm. LC-MS: 371.1 (M + Na). Yield: 77.6%; Anal calcd for: $C_{15}H_{16}N_4O_4S$; Elemental Analysis calculated: C, 51.72; H, 4.63; N, 16.08; S, 9.20; found: C, 51.72; H, 4.64; N, 16.09; S, 9.21; HRMS Calculated 348.3770 [M+] *m/z*, Found 348.3774.

2.1.10. N-(1-methyl-4-oxo-4,5-dihydro-1H-imidazol-2-yl)-1-tosyl-1H-indole-5-carboxamide (4f)

1-Tosyl-1H-indole-5-carboxylic acid was used to prepare 4f. Melting point: 119–121 °C. IR (KBr): 3380 (*amide N−H* stretch), 3228(*aromatic C−H* stretch), 2931 (*−CH* stretch), 1652 (amide *−C=O* stretch), 1675 (*aromatic C=C* stretch), 1252 (*−C−N* stretch), 1270 (*−S=O* stretch) and 791 (*S−C* stretch) Cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) *δ*: 11.26 (s, 1H), 8.44 (s, 1H), 8.18–8.15 (d, *J* = 9, 1H), 8.00–7.88 (m, 4H), 7.40–7.36 (m, 2H), 6.96 (s, 1H), 4.10 (s, 2H), 3.12 (s, 3H) and 2.31 (s, 3H). ^{13}C NMR *δ*: 174.56 (*−CH$_2$CON*), 172.28 (−CCONH); 158.69, 146.17, 136.54, 134.51, 133.07, 130.78, 130.71, 128.31, 127.25, 126.27, 123.68, 112.97 and 110.52 (Aromatic C), 51.74 (−NCH$_2$) 30.96 (−NCH$_3$) and 21.49 (−CCH$_3$) ppm. LC-MS: 411.3 (M + 1). Yield: 80.8%; Anal calcd for: $C_{20}H_{18}N_4O_4S$; Elemental Analysis calculated: C, 58.53; H, 4.42; N, 13.65; S, 7.81; found: C, 58.53; H, 4.43; N, 13.64; S, 7.82; HRMS Calculated 410.1049 [M+] *m/z*, Found 410.1048.

2.1.11. 1-Acetyl-N-(1-methyl-4-oxo-4,5-dihydro-1H-imidazol-2-yl)-1H-indole-5-carboxamide (4g)

1-Acetyl-1H-indole-5-carboxylic acid was used to prepare 4 g. Melting point: 112–114 °C. IR (KBr): 3412 (*amide N−H* stretch), 3201(*aromatic C−H* stretch), 2920 (*−CH* stretch), 1624 (amide *−C=O* stretch), 1634 (*aromatic C=C* stretch) and 1256 (*-C−N* stretch) Cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) *δ*: 11.27 (S, 1H, −NH), 8.47 (S, 1H, Ar-H), 8.38–8.35 (d, J = 9 Hz, 1H, Ar-H), 8.17–8.14 (d, J = 9 Hz, 1H, Ar-H), 7.93 (S, 1H, Ar-H), 6.88 (S, 1H, Ar-H), 4.11 (S, 2H, CH$_2$), 3.14 (S, 3H, CH$_3$) and 2.51 (S, 3H, CH$_3$). ^{13}C NMR *δ*: 174.93 (*−CH$_2$CON*), 172.31 (−CH3CON), 170.23(−CCONH), 158.62, 137.36, 130.50, 128.73, 126.32, 123.03, 115.59 and 109.23 (Aromatic C), 51.73 (−NCH$_2$), 30.98 (−NCH$_3$) and 24.35 (−NCOCH$_3$) ppm. LC-MS: 321.05 (M + Na). Yield: 68.2%; Anal calcd for: $C_{15}H_{14}N_4O_3$; Elemental Analysis calculated: C, 60.40; H, 4.73; N, 18.78; O, 16.09; found: C, 60.40; H, 4.74; N, 18.78; O, 16.09; HRMS Calculated 298.3020 [M+] *m/z*, Found 298.3022.

2.1.12. 1-Benzoyl-N-(1-methyl-4-oxo-4,5-dihydro-1H-imidazol-2-yl)-1H-indole-5-carboxamide (4h)

1-Benzoyl-1H-indole-5-carboxylic acid was used to prepare 4 h. Melting point: 120–122 °C. IR (KBr): 3419 (*amide N−H* stretch), 3224(*aromatic C−H* stretch), 2916 (*−CH* stretch), 1645 (amide *−C=O* stretch), 1618 (*aromatic C=C* stretch) and 1241 (*-C−N* stretch) Cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) *δ*: 11.30 (s, 1H), 8.53 (s, 1H), 8.33–8.30 (d, *J* = 9, 1H), 8.23–8.20 (d, *J* = 9, 1H), 7.81–7.60 (m, 5H), 7.48–7.47 (d, *J* = 3, 1H), 6.90–6.89 (d, *J* = 3, 1H), 4.12 (s, 2H) and 3.16 (s, 3H). ^{13}C NMR *δ*: 174.83 (*−CH$_2$CON*), 172.31 (−CHCON), 168.81(−CCONH), 158.68, 137.94, 134.22, 133.29, 132.73, 130.81, 129.60, 129.23, 126.35, 123.22, 115.55 and 109.50 (Aromatic C), 51.75 (−NCH$_2$) and 31.01 (−NCH$_3$) ppm. LC-MS: 361.1 (M + 1). Yield: 78.4%; Anal calcd for: $C_{20}H_{16}N_4O_3$; Elemental Analysis calculated: C, 66.66; H, 4.48; N, 15.55; O, 13.32; found: C, 66.66; H, 4.48; N, 15.55; O, 13.33; HRMS Calculated 360.3730 [M+] *m/z*, Found 360.3732.

2.1.13. Methyl-5-((1-methyl-4-oxo-4,5-dihydro-1H-imidazol-2-yl) carbamoyl)-1H-indole-1-carboxylate (4i)

1-(Methoxycarbonyl)-1H-indole-5-carboxylic acid was used to prepare 4i. Melting point: 119–120 °C. IR (KBr): 3442 (*amide N−H* stretch), 3220(*aromatic C−H* stretch), 2911 (*−CH* stretch), 1702 (ester *−C=O* stretch), 1631 (amide *−C=O* stretch), 1611 (*aromatic C=C* stretch), 1241 (*−C−N* stretch) and 1109 (*C−O* stretch) Cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) *δ*: 11.28 (s, 1H), 8.49 (s, 1H), 8.20–8.13 (t, *J* = 9, 2H), 7.79–7.78 (d, *J* = 3, 1H), 6.89–6.88 (d, *J* = 3, 1H), 4.11 (s, 2H), 4.02 (s, 3H), 3.15 (s, 3H). ^{13}C NMR *δ*: 174.89 (*−CH$_2$CON*), 172.30(−CCONH), 158.62, 151.28, 137.18, 132.54, 130.33, 127.33, 126.12, 123.37, 114.42 and 109.23 (Aromatic C), 54.77 (−NCH$_2$), 51.73 (OCH$_3$) and 30.98 (−NCH$_3$) ppm. LC-MS: 337.05 (M + Na). Yield: 61.1%; Anal calcd for: $C_{15}H_{14}N_4O_4$; Elemental Analysis calculated: C, 57.32; H, 4.49; N, 17.83; O, 20.36; found: C, 57.32; H, 4.49; N, 17.84; O, 20.37; HRMS Calculated 314.3010 [M+] *m/z*, Found 314.3012.

2.1.14. Ethyl-5-((1-methyl-4-oxo-4,5-dihydro-1H-imidazol-2-yl) carbamoyl)-1H-indole-1-carboxylate (4j)

1-(Ethoxycarbonyl)-1H-indole-5-carboxylic acid was used to prepare 4j. Melting point: 115–117 °C. IR (KBr): 3440 (*amide N−H* stretch), 3224 (*aromatic C−H* stretch), 2921 (*−CH* stretch), 1743 (ester *−C=O* stretch), 1632 (amide *−C=O* stretch), 1619(*aromatic C=C* stretch), 1245 (*−C−N* stretch) and 1145 (*C−O* stretch) Cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) *δ*: 11.28 (s, 1H), 8.49 (s, 1H), 8.20–8.12 (t, *J* = 9, 2H), 7.79–7.77 (d, *J* = 3, 1H), 6.88–6.87 (d, *J* = 3, 1H), 4.51–4.43 (t, *J* = 9, 2H), 4.11 (s, 2H), 3.15 (s, 3H) and 1.43–1.39 (t, *J* = 6, 3H). ^{13}C NMR *δ*: 174.92 (*−CH$_2$CON*), 172.29(−CCONH), 158.62 (−NCOO), 150.74, 137.16, 132.50, 127.32, 126.11, 123.36, 114.46 and 109.12 (Aromatic C), 63.98 (−OCH$_2$CH$_3$) 51.72 (−NCH$_2$), 30.97 (−NCH$_3$) and 14.59 (OCH$_2$CH$_3$) ppm. LC-MS: 351.05 (M + Na). Yield: 74.4%; Anal calcd for: $C_{16}H_{16}N_4O_4$; Elemental Analysis calculated: C, 58.53; H, 4.91; N, 17.06; O, 19.49; found: C, 58.53; H, 4.91; N, 17.07; O, 19.48; HRMS Calculated 328.3280 [M+] *m/z*, Found 328.3284.

2.1.15. 1-Methyl-N-(1-methyl-4-oxo-4,5-dihydro-1H-imidazol-2-yl)-1H-indole-5-carboxamide (4k)

1-Methyl-1H-indole-5-carboxylic acid was used to prepare compound 4 k. Melting point: 110–112 °C. IR (KBr): 3329 (*amide N−H* stretch), 3201 (*aromatic C−H* stretch), 2864 (*−CH* stretch), 1601 (amide *−C=O* stretch), 1629 (*aromatic C=C* stretch) and 1271 (*−C−N* stretch) Cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) *δ*: 11.24 (s, 1H), 8.49 (s, 1H), 8.03–8.00 (d, *J* = 9, 1H), 7.47–7.40 (m, 2H), 6.57 (s, 1H), 4.09 (s, 2H), 3.82 (s, 3H), 3.14 (s, 3H). ^{13}C NMR *δ*: 176.05 (*−CH$_2$CON*), 172.24 (−CCONH), 158.18, 131.31, 128.65, 127.91, 123.48, 123.01, 109.40 and 102.38 (Aromatic C), 51.61 (−NCH$_2$), 33.10 (−CH$_2$NCH$_3$) and 30.92 (−CHNCH$_3$) ppm. LC-MS: 271.2 (M + 1). Yield: 69.2%; Anal calcd for: $C_{14}H_{14}N_4O_2$; Elemental Analysis calculated: C, 62.21; H, 5.22; N, 20.73; O, 11.84; found: C, 62.21; H, 5.22; N, 20.74; O, 11.84; HRMS Calculated 270.2920 [M+] *m/z*, Found 270.2973.

2.1.16. 1-(Ethoxymethyl)-N-(1-methyl-4-oxo-4,5-dihydro-1H-imidazol-2-yl)-1H-indole-5-carbox-amide (4l)

1-(Ethoxymethyl)-1H-indole-5-carboxylic acid was used to prepare **4l**. Melting point: 119–121 °C. IR (KBr): 3346 (*amide N−H* stretch), 3254(*aromatic C−H* stretch), 2871 (*−CH* stretch), 1623 (amide *−C=O* stretch), 1651 (*aromatic C=C* stretch), 1280 (*−C−N* stretch) and 1154 (*-C−O−C* stretch) Cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) *δ*: 11.27 (s, 1H), 8.51 (s, 1H), 8.06–8.03 (d, *J* = 9, 1H), 7.61–7.55 (m, 2H), 6.64 (s, 1H), 5.59 (s, 2H), 4.09 (s, 2H,), 3.44–3.37 (m, 2H), 3.14 (S, 3H), 1.07–1.03 (d, *J* = 6, 3H). ^{13}C NMR *δ*: 175.83 (−CH$_2$CON), 172.26 (−CCONH); 158.28, 138.62, 130.77, 129.60, 128.57, 123.55, 123.47, 110.05 and 103.57 (Aromatic C), 75.66 (*−NCH$_2$O)*, 63.72 (*−CH$_3$NCH$_2$CO)*, 51.62 (*−OCH$_2$CH$_3$)*, 30.91 (−NHCH$_3$) and 15.27 (*−OCH$_2$CH$_3$)* ppm. LC-MS: 315.10 (M + 1). Yield: 73.4%; Anal calcd for: $C_{16}H_{18}N_4O_3$; Elemental Analysis calculated: C, 61.14; H, 5.77; N, 17.82; O, 15.27; found: C, 61.14; H, 5.77; N, 17.83; O, 15.26; HRMS Calculated 314.3450 [M+] *m/z*, Found 314.3454.

2.1.17. 1-(Cyclopropylmethyl)-N-(1-methyl-4-oxo-4,5-dihydro-1H-imidazol-2-yl)-1H-indole-5-carboxamide (4 m)

1-(Cyclopropylmethyl)-1H-indole-5-carboxylic acid was used to prepare 4 m. Melting point: 124–126 °C. IR (KBr): 3382 (*amide N—H* stretch), 3221(*aromatic C—H* stretch), 2869 (*—CH* stretch), 1622 (amide *—C═O* stretch), 1667 (*aromatic C═C* stretch) and 1276 (*—C—N* stretch) Cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ: 11.24 (s, 1H), 8.49 (s, 1H), 8.01–7.98 (d, J = 9, 1H), 7.55–7.51 (m, 2H), 6.59 (s, 1H), 4.08 (s, 4H), 3.14 (s, 3H), 1.25 (s, 1H) and 0.53–0.40 (m, 4H). ^{13}C NMR δ: 176.03 (—CH_2<u>C</u>ON), 172.25 (—C<u>C</u>ONH); 158.18, 138.31, 130.20, 128.62, 127.99, 123.52, 123.97, 109.61 and 102.54 (Aromatic C), 51.60 (—N<u>C</u>H_2CH3), 50.31 (—<u>C</u>H_3NCH_2), 30.91 (—N<u>C</u>H_2) 12.03 (—<u>C</u>CH_2CH_2) and 4.16 (—C<u>C</u>H_2<u>C</u>H_2) ppm. LC-MS: 311.10 (M + 1). Yield: 76.4%; Anal calcd for: $C_{16}H_{18}N_4O_3$; Elemental Analysis calculated: C, 61.14; H, 5.77; N, 17.82; O, 15.27; found: C, 61.15; H, 5.82; N, 17.83; O, 15.28; HRMS Calculated 310.3520 [M +] *m/z*, Found 310.3524.

2.1.18. 1-(2-Methoxyethyl)-N-(1-methyl-4-oxo-4,5-dihydro-1H-imidazol-2-yl)-1H-indole-5-carboxamide (4n)

1-(2-Methoxyethyl)-1H-indole-5-carboxylic acid was used to prepare 4n. Melting point: 117–119 °C. IR (KBr): 3392 (*amide N—H* stretch), 3230 (*aromatic C—H* stretch), 2891 (*—CH* stretch), 1642 (amide *—C═O* stretch), 1654 (*aromatic C═C* stretch), 1267 (*—C—N* stretch) and 1104 (*-C—O—C* stretch) Cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ: 11.30 (s, 1H), 8.54 (s, 1H), 8.06–8.03 (d, J = 9, 1H), 7.58–7.48 (m, 2H), 6.63 (s, 1H), 4.41 (s, 2H), 4.12 (s, 2H), 3.72 (s, 2H), 3.27 (s, 3H), 3.19 (s, 3H). ^{13}C NMR δ: 176.02 (—CH_2<u>C</u>ON), 172.25 (—C<u>C</u>ONH); 158.19, 138.50, 130.27, 128.71, 127.98, 123.47, 122.96, 109.66 and 102.60 (Aromatic C), 71.47 (—N<u>C</u>H_2CH_2), 58.54 (—CH_3N<u>C</u>H_2CO), 51.60 (—<u>C</u>H_2OCH_3), 45.98 (—NH<u>C</u>H_3) and 30.91 (—CH_2<u>O</u><u>C</u>H_3) ppm. LC-MS: 315.10 (M + 1). Yield: 76.9%; Anal calcd for: $C_{16}H_{18}N_4O_3$; Elemental Analysis calculated: C, 50.29; H, 4.22; N, 16.76; O, 19.14; S, 9.59; found: C, 50.29; H, 4.23; N, 16.76; O, 19.13; S, 9.59; HRMS Calculated 314.3450 [M +] *m/z*, Found 314.3453.

*2.1.19. 1-Benzyl-N-(1-methyl-4-oxo-4,5-dihydro-1H-imidazol-2-yl)-1H-indole-5-carboxamide (**4o**)*

1-Benzyl-1H-indole-5-carboxylic acid was used to prepare compound **4o**. Melting point: 126–128 °C. IR (KBr): 3429 (*amide N—H* stretch), 3285(*aromatic C—H* stretch), 2897 (*—CH* stretch), 1622 (amide *—C═O* stretch), 1682 (*aromatic C═C* stretch) and 1321 (*—C—N* stretch) Cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ: 11.25 (s, 1H), 8.50 (s, 1H), 7.99–7.96 (d, J = 9, 1H), 7.59–7.48 (m, 2H), 7.31–7.23 (m, 5H), 6.64 (S, 1H), 5.46 (s, 2H), 4.08 (s, 2H) and 3.13 (s, 3H). ^{13}C NMR δ: 175.93 (—CH_2<u>C</u>ON), 172.26 (—C<u>C</u>ONH); 158.21, 138.48, 138.30, 130.82, 129.04, 128.94, 128.23, 127.89, 127.53, 123.58, 123.19, 109.89 and 103.08 (Aromatic C), 51.60 (—N<u>C</u>H_2CO), 49.50 (-N<u>C</u>H_2Ph) and 30.91 (—N<u>C</u>H_3) ppm. LC-MS: 347.10 (M + 1). Yield: 80.0%; Anal calcd for: $C_{20}H_{18}N_4O_2$; Elemental Analysis calculated: C, 69.35; H, 5.24; N, 16.17; O, 9.24; found: C, 69.35; H, 5.25; N, 16.17; O, 9.25; HRMS Calculated 346.3900 [M +] *m/z*, Found 346.3901.

*2.1.20. N-(1-methyl-4-oxo-4,5-dihydro-1H-imidazol-2-yl)-1H-indole-4-carboxamide (**4p**)*

1H-indole-4-carboxylic acid was used to prepare **4p**; Melting point: 109–111 °C. IR (KBr): 3351 (*N—H* stretch), 3236 (*amide N—H* stretch), 3259 (*aromatic C—H* stretch), 2881 (*—CH* stretch), 1601 (amide *—C═O* stretch), 1632 (*aromatic C═C* stretch) and 1165 (*—C—N* stretch) Cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ: 11.30 (s, 2H), 8.03–8.01 (d, J = 9, 1H), 7.61–7.58 (d, J = 9, 1H), 7.46 (s, 1H), 7.26 (s, 1H), 7.18–7.14 (t, J = 6, 1H), 4.09 (s, 2H) 3.14 (s, 2H). ^{13}C NMR δ: 176.81 (—CH_2<u>C</u>ON), 172.31 (—C<u>C</u>ONH); 158.17, 137.36, 128.64, 127.66, 127.16, 123.09, 120.41, 115.85 and 103.67 (Aromatic C), 51.61 (—N<u>C</u>H_2CO) and 30.99 (—N<u>C</u>H_3)ppm. LC-MS: 257.05 (M + 1). Yield: 82.0%; Anal calcd for: $C_{13}H_{12}N_4O_2$; Elemental Analysis calculated: C, 60.93; H, 4.72; N, 21.86; O, 12.49; found: C, 60.93; H, 4.72; N, 21.86; O, 12.49; HRMS Calculated 256.2650 [M +] *m/z*, Found 256.2652.

2.1.21. N-(1-methyl-4-oxo-4,5-dihydro-1H-imidazol-2-yl)-1H-indole-6-carboxamide(4q)

1H-indole-6-carboxylic acid was used to prepare compound **4q**. Melting point: 111–113 °C. IR (KBr): 3362 (*N—H* stretch), 3231 (*amide N—H* stretch), 3253(*aromatic C—H* stretch), 2889 (*—CH* stretch), 1612 (amide *—C═O* stretch), 1641 (*aromatic C═C* stretch) and 1175 (*—C—N* stretch) Cm^{-1}; ^1H NMR (DMSO-d_6) δ: 11.38 (s, 1H), 11.26 (s, 1H), 8.32 (s, 1H), 7.88–7.85 (d, J = 9, 1H), 7.58–7.54 (m, 2H), 6.49 (s, 1H), 4.09 (s, 2H), 3.13 (s, 2H). ^{13}C NMR δ: 176.03 (—CH_2<u>C</u>ON), 172.25 (—C<u>C</u>ONH); 158.24, 135.82, 131.01, 130.44, 128.88, 120.63, 119.54, 113.95 and 101.79 (Aromatic C), 51.63 (—N<u>C</u>H_2CO) and 30.89 (—N<u>C</u>H_3) ppm. LC-MS: 257.10 (M + 1). Yield: 82.4%; Anal calcd for: $C_{13}H_{12}N_4O_2$; Elemental Analysis calculated: C, 60.93; H, 4.72; N, 21.86; O, 12.49; found: C, 60.93; H, 4.72; N, 21.87; O, 12.49; HRMS Calculated 256.2650 [M +] *m/z*, Found 256.2654.

2.1.22. N-(1-methyl-4-oxo-4,5-dihydro-1H-imidazol-2-yl)-1H-indole-7-carboxamide (4r)

1H-indole-7-carboxylic acid was used to prepare compound **4r**. Melting point: 112–114 °C. IR (KBr): 3359 (*N—H* stretch), 3224 (*amide N—H* stretch), 3243 (*aromatic C—H* stretch), 2884 (*—CH* stretch), 1609 (amide *—C═O* stretch), 1635 (*aromatic C═C* stretch) and 1169 (*—C—N* stretch) Cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ: 11.27 (s, 1H), 10.99 (s, 1H), 8.04–8.02 (d, J = 6, 1H), 7.79–7.77 (d, J = 6, 1H), 7.42 (s, 1H), 7.12–7.08 (t, J = 6, 1H), 6.53 (s, 1H), 4.14 (s, 2H), 3.17 (s, 2H). ^{13}C NMR δ: 176.09 (—CH_2<u>C</u>ON), 172.24 (—C<u>C</u>ONH); 158.57, 135.32, 129.50, 126.80, 125.28, 124.61, 120.35, 118.80 and 101.76 (Aromatic C), 51.90 (—N<u>C</u>H_2CO) and 31.22 (—N<u>C</u>H_3) ppm. LC-MS: 257.10 (M + 1). Yield: 83.6%; Anal calcd for: $C_{13}H_{12}N_4O_2$; Elemental Analysis calculated: C, 60.93; H, 4.72; N, 21.86; O, 12.49; found: C, 60.93; H, 4.72; N, 21.86; O, 12.49; HRMS Calculated 256.2650 [M +] *m/z*, Found 256.2650.

2.1.23. N-(1-methyl-4-oxo-4,5-dihydro-1H-imidazol-2-yl)-1H-indole-5-carboxamide(4 s)

1H-indole-5-carboxylic acid was used to prepare **4s**. Melting point: 116–118 °C. IR (KBr): 3356 (*N—H* stretch), 3213 (*amide N—H* stretch), 3261(*aromatic C—H* stretch), 2872 (*—CH* stretch), 1621 (amide *—C═O* stretch), 1626 (*aromatic C═C* stretch) and 1151 (*-C—N* stretch) Cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ: 11.35 (s, 1H), 11.25 (s, 1H, —NH), 8.50 (s, 1H), 7.99–7.96 (d, J = 9, 1H), 7.43–7.40 (m, 2H), 6.57 (s, 1H), 4.08 (s, 2H), 3.13 (s, 2H). ^{13}C NMR δ: 176.15 (—CH_2<u>C</u>ON), 172.25 (—C<u>C</u>ONH); 158.13, 138.55, 128.60, 127.58, 126.96, 123.26, 123.05, 111.08 and 103.00 (Aromatic C), 51.59 (—N<u>C</u>H_2CO) and 30.89 (—N<u>C</u>H_3)ppm. LC-MS: 257.10 (M + 1). Yield: 80.5%; Anal calcd for: $C_{13}H_{12}N_4O_2$; Elemental Analysis calculated: C, 60.93; H, 4.72; N, 21.86; O, 12.49; found: C, 60.93; H, 4.72; N, 21.86; O, 12.49; HRMS Calculated 256.2650 [M +] *m/z*, Found 256.2653.

2.1.24. N-(1-methyl-4-oxo-4,5-dihydro-1H-imidazol-2-yl)quinoline-6-carboxamide (4 t)

quinoline-6-carboxylic acid was used to prepare **4t**. Melting point: 117–119 °C. IR (KBr): 3382 (*N—H* stretch), 3254 (*amide N—H* stretch), 3251(*aromatic C—H* stretch), 2859 (*—CH* stretch), 1613 (amide *—C═O* stretch), 1601 (*aromatic C═C* stretch) and 1163 (*—C—N* stretch) Cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ: 11.35 (s, 1H), 8.99 (s, 1H), 8.84 (s, 1H), 8.58–8.55 (d, J = 9, 1H), 8.48–8.45 (d, J = 9, 1H), 8.09–8.06 (d, J = 9, 1H), 7.62–7.59 (m, 1H), 4.14 (s, 2H), 3.19 (s, 2H). ^{13}C NMR δ: 174.07 (—CH_2<u>C</u>ON), 172.35 (—C<u>C</u>ONH); 159.01, 152.54, 149.64, 137.95, 135.33, 129.69, 129.18, 127.68, and 122.40 (Aromatic C), 51.58 (—N<u>C</u>H_2CO) and 31.10 (—N<u>C</u>H_3) ppm. LC-MS: 269.05 (M + 1). Yield: 83.9%; Anal calcd for: $C_{14}H_{12}N_4O_2$; Elemental Analysis calculated: C, 62.68; H, 4.51; N, 20.88; O, 11.93; found: C, 62.68; H, 4.51; N, 20.89; O, 11.94; HRMS Calculated 268.2760 [M +] *m/z*, Found 268.2761.

*2.1.25. N-(1-methyl-4-oxo-4,5-dihydro-1H-imidazol-2-yl)-1H-benzo[d] imidazole-5-carboxamide (**4u**)*

1H-benzo[*d*]imidazole-5-carboxylic acid was used to prepare **4u**. Melting point: 113–115 °C. IR (KBr): 3367 (*N—H* stretch), 3242 (*amide N—H* stretch), 3267(*aromatic C—H* stretch), 2871 (*—CH* stretch), 1629 (amide *—C═O* stretch), 1613 (*aromatic C═C* stretch) and 1124 (*—C—N* stretch) Cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) *δ*: 11.30 (s, 1H), 8.48 (s, 1H), 8.37 (s, 1H), 8.11–8.08 (d, *J* = 9, 1H), 7.65–7.62 (d, *J* = 9, 1H), 4.10 (s, 2H), 3.13 (s, 2H). ^{13}C NMR *δ*: 175.39 ($-CH_2\underline{C}ON$), 172.31 ($-C\underline{C}ONH$); 158.46, 144.40, 141.50, 138.1, 131.57, 123.86, 117.83 and 114.78 (Aromatic C), 51.67 ($-N\underline{C}H_2CO$) and 30.91 ($-N\underline{C}H_3$) ppm. LC-MS: 258.10 (M + 1). Yield: 86.7%; Anal calcd for: $C_{12}H_{11}N_5O_2$; Elemental Analysis calculated: C, 56.03; H, 4.31; N, 27.22; O, 12.44; found: C, 56.04; H, 4.32; N, 27.22; O, 12.44; HRMS Calculated 257.2530 [M+] *m/z*, Found 257.2534.

2.2. Connotation among PARs and PI3Kinase – And the assay protocol PI3Kinase inhibition activity of **4a-u**

Protease-Activated Receptors (PARs) mostly expressed on the exterior region of almost all cell types and intracellular signal transducer enzyme, PI3Kinase, play an important role in regulating the functional reactions in haemopoietic cells such as neutrophils, eosinophils, mast cells, monocytes, and B- and *T*-cells that are highly involving in the cancer development [16]. Accordingly, a chemical entity that inhibiting PI3 Kinases would be the ideal drug target against cancers, especially breast cancer due to the high involvement of PI3Kinase in it. Therefore, the inhibitory potentials of 2-amino-1-methyl-1H-imidazole-4(5H)-one derivatives (**4a-u**) has evaluated by means of Glutathione-coated 96 well plate assay [17–20]. Pictilisib (GDC-0941) was commercially obtained and served as the standard drug. The kinase and 2-amino-1-methyl-1H-imidazole-4(5H)-ones **4a-u** were pre-incubated about 10–15 min before adding the substrate (PIP2 (Phosphatidylinositol biphosphate)). 5X kinase reaction buffer (5 µL) was added after adding 5 µL/well of PIP2 substrate to corresponding wells. Millipore water was added to each well to bring the final volume to 25 µL/well. Then the reaction mix was incubated at temperature for 1 h. The absorbance read at 450 nm. As the result different, the relative % activity to b-PIP3 was calculated by means of the following formula,

$$\%\text{inhibition} = \frac{\text{OD of samples (buffer, kinase \& inhibitors)}}{\text{OD of B—PIP3 average}} \times 100$$

2.3. Molecular docking evaluations

Molecular docking study was carried out using Autodock version 4.2.6 (MGL Tools 1.5.6 was used as the Graphical User Interface) [21,22]. Argus lab 2.0 was used to cleaning the receptor protein and the ligands **4a-u**. Based on the predicted biological activity values (Fig. 1), medicinal value assessments of 2-amino-1-methyl-1H-imidazole-4(5H)-one derivatives (**4a-u**) towards GPCR ligand (through PAR1 inhibition) and PI3Kinase inhibition was measured via molecular docking studies since they are mainly involved in cancer development. PAR1 is the characteristic adherent component of the family of GPCRs that ease cellular events to thrombin and associated kinases and proteases. To get more insight into the mode of binding interactions to GPCR and PI3-Kinase, the ligands structures (**4a-u**) was docked into the active site of GPCR and PI3Kinase. The PDB X-ray crystallography structures of PAR1, PDB ID: 2GNJ and PI3Kinase, PDB ID: 3HHM.

2.4. Anti-breast cancer studies - Michigan cancer foundation-7 (MCF-7) cell lines

Anti-breast cancer experimentations by means of Michigan Cancer Foundation-7 (MCF-7) cell culture widely used for emerging novel diagnostic examinations and new treatments for breast cancer [23]. The early passage MCF-7 cell lines used in this study were established and cultivated as described earlier [17–20]. Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) which was supplemented with 10% Fetal Bovine Serum (FBS), 100 µg/ml streptomycin and (100U) 20 µg/ml penicillin. Cells were incubated at 37 °C in 5% CO_2 atmosphere. For the anti-proliferative activity comparison to normal and cancerous cells, the normal breast epithelial cells (MCF-10) in addition to MCF-7 cells. These cells were cultured in 1:1 combination of DMEM and Ham's F12 medium with 20 mg/ml of epidermal growth factor (EGF), 0.01 mg/ml insulin, 100 µg/ml cholera toxins, 500 µg/ml hydrocortisone, and 5% chelex treated horse serum were added. For the biological activity assessments, 1 ml of homogenized cell suspension of both normal and cancerous cells dispensed in each well of a separate microtiter plate and kept in a desiccator underneath 5% CO_2 atmosphere. Further, the cells observed under the inverted microscope after 48 h of incubation and assessed for their physiological and morphological changes if any. 0.05 ml of the compounds **4a-u** along with the standard (Doxorubicin) dissolved in 4.95 ml of DMSO to get a working concentration of 1 mg/ml. This working concentration was set freshly and filtered over a 0.45-µm filter prior to bioassay.

2.5. MTT assay for anticancer evaluations

To execute the MTT assay, about 5000 cells were seeded in a Glutathione-coated 96-well flat-bottom titer plate and incubated for 24, 48, and 72 h at 37 °C in 5% CO_2 atmosphere. Different aliquots of compounds (**4a-u**) (25–250 µg/mL) were added and incubated at room temperature. After the incubation, the medium was removed from corresponding wells of the working plate. The wells were washed several times with Phosphate Buffer Solution; Further, 100 µL of the working MTT dye in DMEM media was added and incubated for 120 min. With this, the MTT lysis buffer (100 µL) was included and incubation was continued for 4 hrs more. The absorbance was measured at 570 nm and the cell viability was calculated using the following formula,

$$\text{Cell viability (\%)} = \text{Mean OD/Control OD} \times 100\%$$

2.6. Hydrogen peroxide (H_2O_2) radical scavenging evaluation of **4a-u**

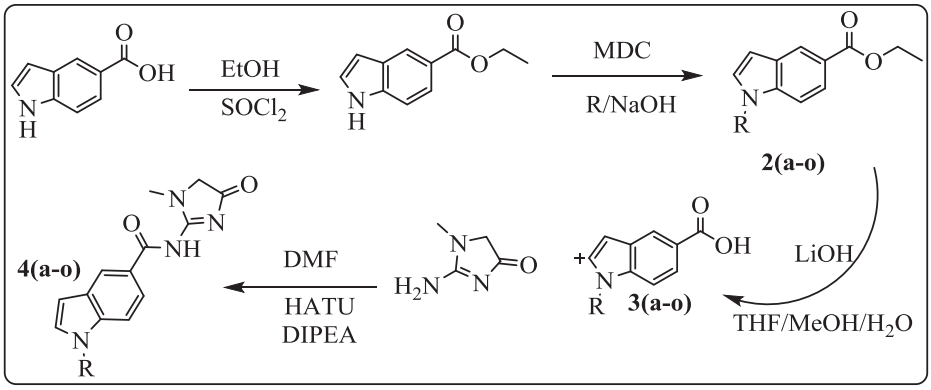
The connection between free radicals and breast cancer is well known. Recent researches suggest that free radicals are playing a vital role in breast cancer development [24,25]. Antioxidant properties of **4a-u** assessed by H_2O_2 scavenging assay [26]. The assay performed in triplicate with Ascorbic acid as standard. Compounds **4a-u** and Ascorbic acid assessed with a range of concentration from 25 to 250 µg/mL and incubated by adding 0.6 ml of fresh H_2O_2 (40 mM) at 37 °C for 60 min. Ascorbic acid used as positive control. Absorbance at 230 nm was recorded to determine the relative H_2O_2 % scavenging rate of compounds **4a-u**·H_2O_2 dissolved in phosphate buffer read at 230 nm taken as control OD. The relative % scavenging of H_2O_2 and Ascorbic acid was determined by the following formula:

$$\%\ \ H_2O_2\ \ \text{reducing activity} = [OD_0 - OD_1/OD_0] \times 100$$

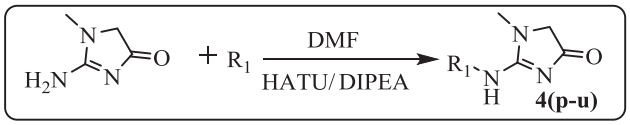
where OD_0 is the optical density (OD) of the control and OD_1 is the OD of **4a-u**.

2.7. In-vitro thrombolytic valuations to exemplify PAR1 inhibitory potentials of **4b** and **4g**

In order to evaluate the PAR1 inhibition potentials possessed by compounds **4b** and **4g**, thrombolytic activity assessment was executed [26]. Protease-activated receptors (PARs) belong to the GPCR family that proteolytically activated by numerous proteases. Stimulus of fibrinolysis through plasmin (infusion of tissue plasminogen) activator



Scheme 1. The synthetic route for the preparation of title compounds (**4a-o**).



Scheme 2. The synthetic route for the preparation of title compounds (**4p-u**).

enabling thrombolysis. The coagulation and fibrinolytic systems contribute to malignancy by increasing angiogenesis, tumor growth, tumor invasion, and tumor metastasis [27]. PAR intermediated crosstalk between coagulation and fibrinolysis has been studied broadly and well-known [27]. Coagulation proteases utilize their tumor-triggering activities by initiating PAR-1 and PAR-2 [28,29].

For *in vitro* thrombolysis assessment, 100 µL (30,000 I.U) of suspension of streptokinase prepared and used as stock solution. Streptokinase and Millipore water were used as thrombolytic standard and non-thrombolytic control, correspondingly. Each healthy volunteers from various age groups donated 5 ml of blood (human, n = 4). The collected blood was transferred to each of the Eppendorf tubes (750 µL) to obtain clots at room temperature. Serum was articulated by not upsetting the clot formed. The most active compounds **4b** and **4g** were administered at different concentrations and tubes were incubated at room temperature for 2 h and the fluid development was perceived. To measure the serum amount produced, the weight of blood comprising tubes were weighed before and after the removal of serum. The assessment was repeated up to four times. The differences in weight measured before and after clot lysis specified as a percentage.

2.8. Statistical analysis

All results were expressed as a percentage increase or decrease with respect to control values. All results were compared by performing one-way ANOVA with Dunnett's post-test. GraphPad Prism version 7.1 for Windows, GraphPad Software, USA (www.graphpad.com) was used for statistical analysis. A difference was considered statistically significant if $p \leq 0.05$. The 50% inhibitory concentration (IC_{50}) was calculated from the dose-response curve obtained by plotting percentage inhibition versus concentration.

3. Results and discussions

In this study, initially, we have tried for the −NH substitution of creatinine coupled indole derivative (N-(1-methyl-4-oxo-4,5-dihydro-1H-imidazole-2-yl)-1H-indole-5-carboxamide (**4s**) but due to poor solubility of the compound in solvents reaction did not complete and percentage of product formation was less. In order to get the good quality and yield of the desired compound, a four-step reaction was explored at normal room temperature without using any external energy for reaction completion and achieved the high pure 2-amino-1-methyl-1H-imidazole-4(5H)-one derivatives (**4a-u**) with remarkable yields according to Schemes 1 and 2. The first step is esterification of indole carboxylic acid by using standard esterification reaction with thionyl chloride in ethanol as a solvent to get corresponding acid ester. After esterification, the product was easily isolated with full yield by adding MTBE as anti-solvent and after drying, the product was completely free from excess thionyl chloride used in the reaction.

The second step is the *N*-substitution of indole carboxylic acid ester with substituting reagents in presence of a base and this was a very challenging step in the concern of isolation of product due to the sensitivity of *N*-substituted indole ester to aqueous media in presence of a base. However the same issue was successfully addressed by opting MDC as solvent and NaOH powder as a base, this combination along with catalyst TBAF resulted in very ease of isolation method due to immiscibility of NaOH powder in MDC. After reaction completion, NaOH powder removed by celite filtration and product contained MDC layer washed with water in the absence of a base.

Finally resulted in the high recovery of product with remarkable yield. The third step is the hydrolysis of *N*-substituted indole carboxylic acid ester without disturbing the *N*-substitution of indole by using the mild base as LiOH in presence protic solvent mixture MeOH:THF: water (1:1:0.5). The fourth step is acid amine coupling by using standard coupling agent HATU with the well-known base as diisopropylethylamine (DIPEA) in best protic solvent DMF to get the title compounds. The achieved compounds in remarkable yield have characterized through standard analytical techniques such as LC-MS, NMR, HRMS, and elemental analysis. Table 1–3 are displaying the details of reaction optimization of compounds **4a-u**. Indole N−H substitution reaction using different substituting reagents was optimized by using methylsulfonyl chloride as substituting reagent with different bases, solvents, catalysts and different temperature ranges to get intermediate compounds 2a with high yield (Table 1).

Ethyl ester hydrolysis reaction of *N*-substituted indole carboxylate compounds **2a-o** was optimized by using ethyl 1-(methylsulfonyl)-1H-indole-5-carboxylate (**2a**) compound with different bases, solvents, solvent mixtures, solvent mixtures with varied composition and different temperature ranges to get intermediate compounds **3a** with high yield (Table 2). The coupling reaction of *N*-substituted indole carboxylic acid compounds **3a-o** and Creatinine (2-amino-1-methyl-1H-imidazole-4(5H)-one) was optimized by using 1-(methylsulfonyl)-1H-indole-5-carboxylic acid (**3a**) compound with different bases, solvents and different temperature ranges to get final pure compounds **4a** with high yield (Table 3).

3.1. Results of molecular docking studies

In molecular docking studies, the results of PAR1 has attained the maximum requirement, but not up to the level of PI3Kinase results. With a binding affinity range of 0.25–1.75 µM inhibitory constant (*ki*)

Table 1
Optimization of reaction conditions for the synthesis of intermediates (**2a-o**).

Entry	Base	Catalyst	Solvent	T °C	Time	% of Yield *
1	TEA	–	THF	5–10	2.00 h	40
2	TEA	–	THF	25–30	2.00 h	45
3	TEA	–	THF	25–30	2.00 h	35
4	TEA	TBAF[a]	THF	25–30	2.00 h	50
5	TEA	TBAB[b]	THF	25–30	2.00 h	40
6	TEA	TBAF	Methyl THF	25–30	2.00 h	48
7	TEA	TBAF	MDC	25–30	2.00 h	65
8	TEA	TBAF	MDC	10–15	2.00 h	68
9	DIPEA	TBAF	MDC	10–15	2.00 h	70
10	NaOH	TBAF	MDC	10–15	2.00 h	85
11	NaOH powder	TBAF	MDC	10–15	2.00 h	95
12	KOH powder	TBAF	MDC	10–15	2.00 h	93
13[a]	NaOH powder	TBAF	MDC	20–30	2.00 h	95
14	NaOH powder	TBAF	THF	20–30	2.00 h	89
15	NaOH powder	TBAF	Toluene	20–30	2.00 h	76

13[a] The optimized reaction conditions for indole N−H substitution reaction.
* The yields are mentioned for the isolated products.
[a] Tetra-*n*-butylammonium fluoride.
[b] Tetra-*n*-butylammonium bromide.

values, all the compounds were able to inhibit PAR1. Therefore, we took only the PI3Kinase inhibition results to discuss elaborately here. The lowest binding affinity and best-docked poses had taken as the key measurements in order to screen a compound as efficacious towards the proposed biological activity.

The molecular mechanistic values such as least binding energy (kcal/mol), ligand efficiency, and inhibitory constant (*ki*) have retrieved and based on those values the compounds **4a-u** were ranked. Top five compounds among **4a-u** had screened for further *in vitro* studies. In the results, the remarkable binding affinity of all compounds has recognized through the established binding energy values (−7.20 to −12.17 kcal/mol) and the inhibitory constant values (1.35–0.085 μM) (Fig. 3 & B). Excellent molecular mechanistic values are shown by the compounds **4b**, **4g**, **4i**, **4n,** and **4u** enforced us to screen them for further *in vitro* investigations to unveil their PI3Kinase inhibitory potentials.

3.2. PI3Kinase inhibition and relevant molecular interactions of *4a-u* to PI3Kinase

The inhibition potential of present study compounds **4b**, **4g**, **4i**, **4n** and **4u** that has screened from molecular docking studies based on molecular mechanistic values assessed through competitive ELISA method. Four repeated assays were done (n = 4). The obtained results statistically optimized and mentioned with ± SD.

In the results, almost all compounds showed equal or more activity than the standard drug Pictilisib (Fig. 4A–C). Compounds **4b** and **4g** dominated for the activity while comparing the standard Pictilisib (78.14 ± 2.64 relative % activity and IC_{50} 0.78 ± 0.02) by attaining the highest relative percentage activity (84.16 ± 2.12 and 87.94 ± 1.89 respectively) and lowest inhibitory constant (IC_{50} 0.75 ± 0.02 and 0.5 ± 0.02). Fig. 4D illustrates the anticipated interaction to establish the highest relative percentage activity with the lowest inhibitory constant by the compound **4g**. The study results also used to screen these two compounds for further anticancer studies in breast cancer cell lines and their antioxidant potentials.

Table 2
Optimization of reaction conditions for the preparation of intermediates **3a-o**.

Entry	Base	Solvent	Solvent Composition	T °C	Time	% of Yield*
1	NaOH	MeOH	1	5–10	4.00 h	NA
2	NaOH	MeOH	1	25–35	4.00 h	NA
3	NaOH	MeOH	1	Reflux	4.00 h	NA
4	NaOH	THF	1	5–10	4.00 h	NA
5	NaOH	THF	1	25–35	4.00 h	NA
6	NaOH	THF	1	Reflux	4.00 h	NA
7	NaOH	Water:MeOH	1:1	25–35	4.00 h	NA
8	NaOH	THF:MeOH	1:1	25–35	4.00 h	NA
9	NaOH	THF:MeOH:water	1:1:1	25–35	4.00 h	30
10	NaOH	THF:MeOH:water	1:1:1	5–10	4.00 h	NA
11	KOH	THF:MeOH:water	1:1:1	5–10	4.00 h	NA
12	KOH	THF:MeOH:water	1:1:1	25–35	4.00 h	25
13	LiOH	THF:MeOH:water	1:1:1	25–35	1.00 h	65
14 [a]	**LiOH**	**THF:MeOH:water**	**1:1:0.5**	**25–35**	**2.00 h**	**96**
15	LiOH	THF:MeOH:water	1:1:0.5	5–10	10.0 h	NA

14[a]The optimized reaction conditions for Ethyl ester hydrolysis reaction of *N*-substituted indole carboxylate compounds; NA – incomplete reaction.
* The yields are mentioned for the isolated products.

Table 3
Optimization of reaction conditions for the preparation of final compounds **4a-u**.

Entry	Base	Solvent	Coupling reagents	T °C	Time	% of Yield*
1	TEA	THF	DCC[c]/HOBt[d]	25–35	6.00 h	NA
2	TEA	THF	DCC/HOBt	Reflux	6.00 h	NA
3	TEA	THF	EDC.HCl[e]/HOBt	Reflux	6.00 h	NA
4	TEA	THF	HATU[f]	25–35	6.00 h	NA
5	DIPEA	THF	HATU	25–35	6.00 h	NA
6[a]	**DIPEA**	**DMF**	**HATU**	**25–35**	**6.00 h**	**88**
7	DIPEA	DMA	HATU	25–35	6.00 h	82
8	DIPEA	DMF	HBTU[g]	25–35	6.00 h	80
9	DIPEA	DMF	EDC.HCl/HOBt	25–35	6.00 h	61

6[a] The optimized reaction conditions for the preparation of final compounds. phosphate.
* The yields are mentioned for the isolated products. NA- Incomplete reactions.
[c] Dicyclohexylcarbodiimide.
[d] Hydroxybenzotriazole.
[e] N′-ethyl carbodiimide hydrochloride.
[f] (1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-*b*]pyridinium3-oxid hexafluoro
[g] 3-[Bis(dimethylamino)methyliumyl]-3*H*-benzotriazol-1-oxide hexafluorophosphate.

3.3. Anticancer study results

Compounds **4b** and **4g** screened based on strong PI3Kinase inhibition used to assess their anti-cancer potentials against breast-cancer cell lines. We have also assessed the cytotoxicity of these compounds against normal breast epithelial cells in order to have the activity variations between these two different but same environmental type cells. Expectedly, compound **4g** showed excellent anti-proliferative effects than the standard doxorubicin. At the same time **4b** also equally active as **4g**. Only a negligible variation of result values found between **4b** and **4g**. The calculated relative percentage activity was excellent for **4b** (85.25 ± 1.98) and **4g** (87.25 ± 1.84) while comparing with Doxorubicin (80.18 ± 2.75).

The IC_{50} values were 0.48 ± 0.05, 0.25 ± 0.02 and 1.05 ± 0.15 for **4b**, **4g** and Doxorubicin respectively. From these results, the ability or potential to act as an effective PI3Kinase inhibitor or breast cancer therapeutics by **4b** and **4g** had recognized. Fig. 5 illustrates the timely evaluated anticancer study result variations between the cancerous (MCF-7) and normal breast epithelial cells. Remarkably, there was no necrosis or adverse effects found on normal epithelial cells (MCF-10) even up to eight-hour observation. However, necrosis was observed on cancerous cells from 30 min of incubation after treating the compounds **4b** and **4g**.

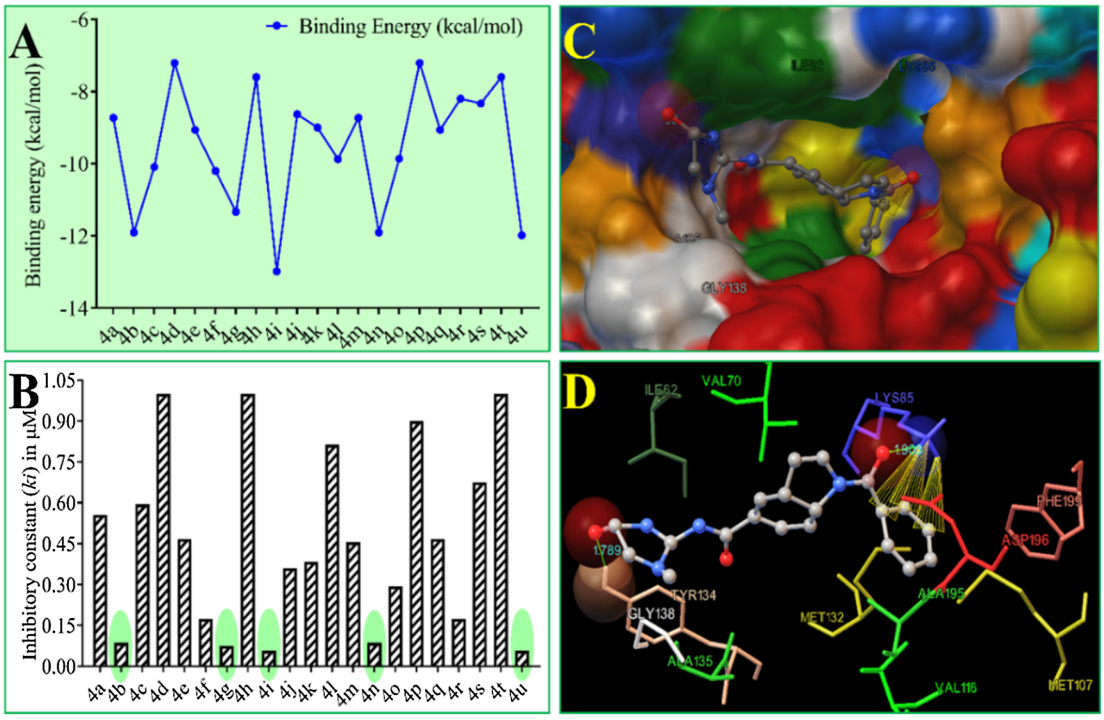


Fig. 3. Molecular docking study results. **Note**: 4A. Binding energy values (kcal/mol) of **4a-u**; 4B. Obtained inhibitory constant (*ki*) values of **4a-u** (best compounds are highlighted oval shape); 4C. Best docked pose (compound **4g** into the binding pocket of PI3Kinase PDB ID: 3HHM); 4D. Molecular interaction of PI3Kinase and compound **4g** (Stick and balls = **4g**; sticks only = amino acid residues of PI3Kinase).

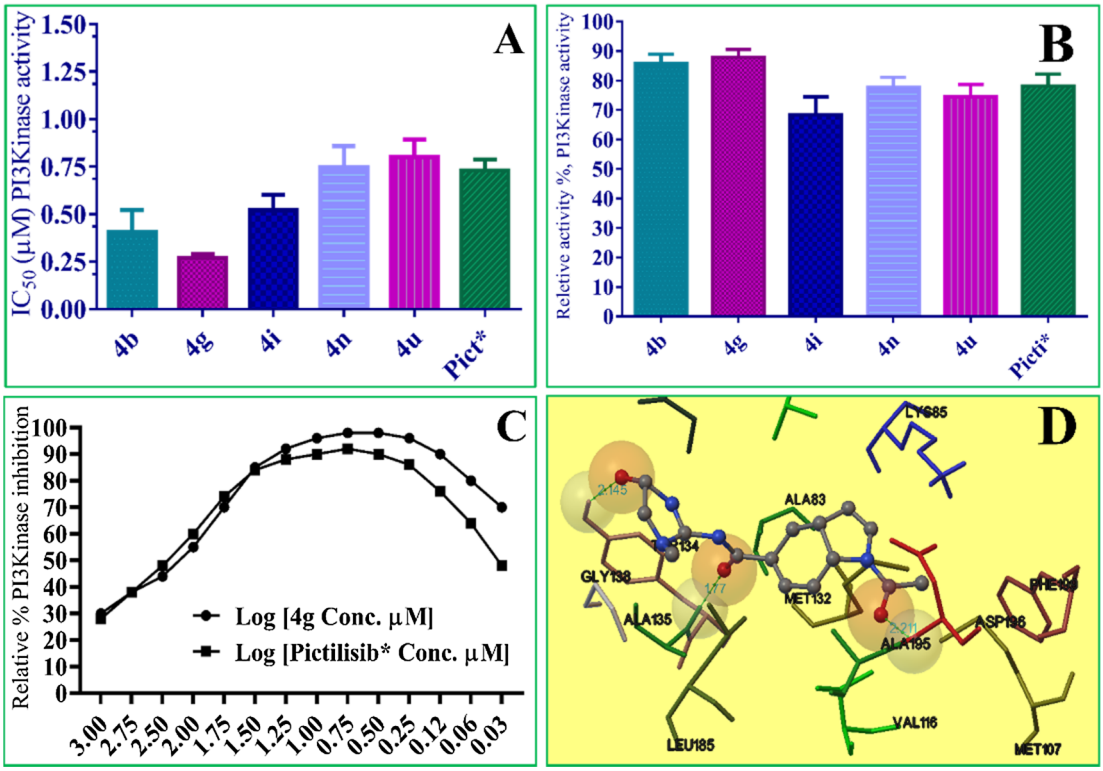


Fig. 4. Results of PI3kinase inhibition activity of present study compounds **4b**, **4g**, **4i**, **4n,** and **4q**. **Note**: 3A. Relative percentage activity in comparison to Doxorubicin; 3B. Inhibitory constant values (IC_{50} values); 3C. Dose-response curve of compound **4g** and Doxorubicin.

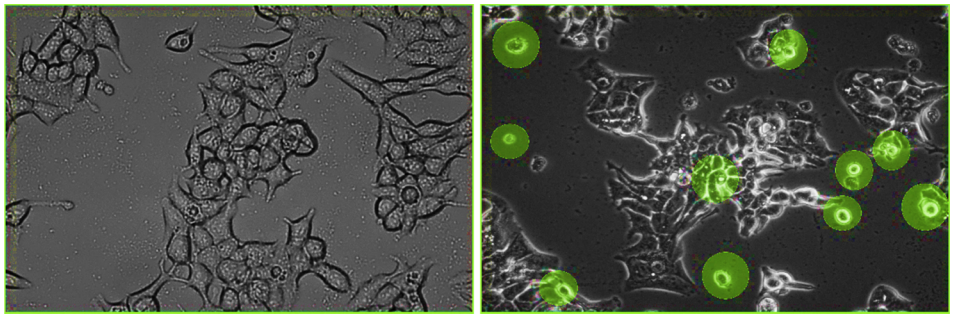


Fig. 5. The appearance of normal breast epithelial cells (MCF-10) (left) and cancerous breast epithelial cells after 2 h after treating compound **4g**.

*3.4. Results of radical scavenging effects of compounds **4b**, **4g**, and Ascorbic acid*

Excellent free radical scavenging potential observed for compounds **4b** and **4g** while comparing Ascorbic acid. Scavenging potential of **4g** was almost 98.42 ± 1.76%, **4a** 96.30 ± 1.85, Ascorbic acid 86.24 ± 2.46 and the IC50 was 54 nM, 62 nM and 1 μM for **4g**, **4b** and Ascorbic acid respectively.

3.5. Results of PAR1 inhibition through thrombolytic assay

Thrombin is one of the vital factors and playing a major role in the advancement of numerous diseases. In the current approach, the clot lysis activity of compounds **4b** and **4g**, assessed for PAR1 inhibition through thrombolytic effects. Subsequently administering **4b**, **4g**, and streptokinase (standard), the dry weight of the Eppendorf tube with clotted blood measured as the result by removing serum released due to the thrombin lysis activity by both compounds and standard in every 15 min. In the results, compounds, **4b** (92.15 ± 1.28% lysed) and **4g** (96.25 ± 1.42% lysed) were established most active thrombolytic effects. They were able to lyse the whole blood-clot within 15–30 min after the treatment while streptokinase (standard) (87.12 ± 1.72% lysed) utilized more than 1 h to the whole completion of lysis. Furthermore, it was observed that there was no variations or differences in clot lysis activity on the variedly collected blood samples collected from three set of age group (18–24 (n = 4); 25–35 (n = 4); 36–40 (n = 4)) and 41 above (n = 4).

4. Conclusion

In conclusion, 21-member family of, four-step involved 2-amino-1-methyl-1H-imidazole-4(5H)-one derivatives (**4a-u**) synthesized in very good yield and characterized through conventional analytical techniques. As indicated through preliminary bioactivity predictions, these compounds evaluated as PAR1, PI3Kinase inhibitors, and anticancer agents through various established techniques. In the end, compounds **4b** and **4g** found to have remarkable activities against proposed biological activities and screened for future pre-clinical studies over animal models.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2019.02.048.

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