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Pyrazole derivatives as potent inhibitors of c-Jun N-terminal kinase: Synthesis and SAR studies

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ABSTRACT

Mitogen activated protein kinases including c-Jun N-terminal kinase play an indispensable role in inflammatory diseases. Investigation of reported JNK-1 inhibitors indicated that diverse heterocyclic compounds bearing an amide group rendered potent JNK-1 inhibitory activity which prompted us to synthesize new JNK-1 inhibitors containing a pyrazole heterocyclic group. A DABCO mediated 1,3-dipolar cycloaddition reaction in neat resulted in pyrazole carboxylic acid which was converted to desired amides. Upon confirmation of the structures, all the compounds were screened for JNK-1 inhibitory activity and in vivo anti-inflammatory activity. Several synthesized analogues have exhibited JNK-1 inhibitory activity less than 10 μM, in particular compounds **9c**, **10a** and **10d** were found to be potent among all the compounds.

1. Introduction

The c-Jun N-terminal kinases (JNK), a group of conserved family of serine/threonine mitogen activated protein kinases, are involved in vital regular physiological functions in human life. Three distinct genes *Jnk1*, *Jnk2*, and *Jnk3* have been identified which encode for ten different JNKs with varying size from 46 kDa to 55 kDa and show more than 80% sequence similarity.[1,2] JNK-1 and 2 isoforms expressed ubiquitously while JNK-3 expressed in brain and testis.[3] JNKs are activated by proinflammatory cytokines such as TNF-α and IL-Iβ as well as environmental stress, such as anisomycin, UV irradiation, hypoxia, and osmotic shock.[4,5] Members of the JNK family (JNK-1, 2 and 3) act as an integrated unit for multiple intracellular biochemical signals governing a wide variety of cellular processes such as proliferation, apoptosis, migration and transcriptional regulation.[6] Like other MAP kinases, JNKs are activated by dual specific serine/threonine mitogen activated protein kinase kinase-4 and 7 (MAPKK4 and MAPKK7) by phosphorylation at Thr-183 and Tyr-185. Both, MAPKK-4 and 7 are further activated by MAPKKK including ASK1 and TAK-13, depending up on nature of stimulus and the cell lineage.[7–11] Phosphorylated JNKs further activate downstream substrates including numerous transcription factors. JNKs are well known for their ability to activate transcriptional factor c-Jun by phosphorylation at both Ser-63 and Ser-73 within the transactivation domain of the transcription factor c-Jun.[12] c-Jun, is a component of activator protein-1(AP-1) complex, which is an important transcription factor that is activated by JNK mediated phosphorylation.[13] The other transcription factors that are activated by JNK are ATF2 and Elk-1.[14] Activated ATF2 heterodimerizes with c-Jun and stimulates the expression of c-Jun gene where as activated Elk-1 is involved in induction of the c-fos gene that induces heterodimer formation of AP-1 with c-Jun.[15] Activation of ATF2 and c-Jun transcription factors activate the promoters of matrix metallo proteinase genes and c-Jun itself resulting in expression of matrix metallo proteinases that degrade type II collagen in rheumatoid arthritis. Thus JNKs play a key role in autoimmune and inflammatory diseases including arthritis, neurological, metabolic diseases and cancer.[16,17]

Numerous compounds with different heterocyclic rings have been reported to possess JNK-1 inhibitory activity including pyrazole, indazole, pyridine and benzotriazole scaffold[18,19] and few of the reported molecules are in advanced stage of clinical trials Figure 1a. Our group is actively involved in design and development of p38 MAP kinase inhibitors, now intended to expand the p38 kinase drug discovery to JNK-1 which is also a known validated target for inflammatory diseases.[20–25]

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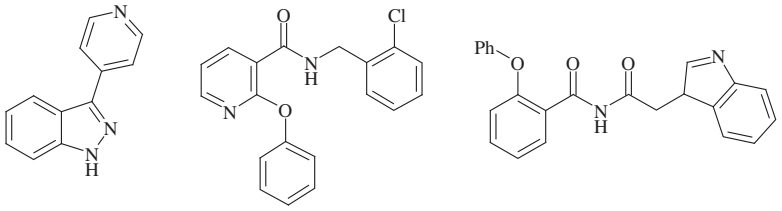


Figure 1a. JNK-1 inhibitors bearing indazole, pyridine and Indole scaffold.

Compound **D1**, 3-(4-pyridyl)-indazole was identified as novel scaffold for JNK inhibition with pIC_{50} value 5.8 from high through put screening,[26] compound **D2** resulted from modification of ATP competitive inhibitors and retained the activity. Compound **D3** was discovered as a new class of potent and selective inhibitor of JNK3.[27] An X-ray crystal structure of **D4**, a potent JNK inhibitor bound to the ATP binding site of JNK-1[28,29] revealed that the phenoxy group binds in the pocket occupied by the ribose of the competitive binder ATP. The amide present in **D4** is acting as a H-bond acceptor making a key interaction with the hinge-region through Met111 (2.8 Å). In view of the above reports, we designed substituted pyrazoles (the general structure, **8a–i**, **9a–e** and **10a–h**) with phenyl pyrazole and various alkyl, aryl, aralkyl and heteryl moieties attached via amide linker as shown in Figure 1b. This manuscript aimed to present synthesis, JNK-1 inhibitory and anti-inflammatory activity of substituted pyrazoles.

2. Material and methods

All the solvents were purchased from local vendors and are purified before being used and chemicals were purchased from M/s Sigma Aldrich, S. D. Fine chemicals and Loba. Pre-coated silica gel F254 (Merck) plates were employed for thin layer chromatography and column chromatography was performed using silica gel 60–120 mesh. Melting points were recorded in open glass capillaries using Polmon melting point apparatus and are uncorrected. Infrared spectra were recorded on Shimadzu FT IR spectrophotometer in KBr pellet. Mass spectra obtained on VG-7070H mass spectrometer. ^{1}H NMR spectra were recorded at 300 MHz on a Bruker Avance NMR spectrometer in $CDCl_3$ (δ 7.26) or DMSO-d_6 (δ 2.49).

2.1. Synthetic methodology

2.1.1. Procedure for preparation of ethyl 5-phenylpyrazole-3-carboxylate 3

To a mixture of phenyl acetylene **1** (0.1 g, 1 mmol) and ethyl diazoacetate **2** (0.114 g) was added DABCO (1,4-diazabicyclo[2.2.2]octane) (0.011 g, 0.1 mmol) and stirred for 6 h at ambient temperature. Upon completion of the reaction which was monitored by TLC, the crude reaction product was purified by passing through Silica gel using 20% ethyl acetate in pet. ether to afford compound **3**.

2.1.2. Procedure for preparation of 5-phenylpyrazole-3-carboxylic acid 4

A solution of the ester **3** (0.1 g, 0.46 mmol) in MeOH (4 mL) was treated with 4 N NaOH (4 mL) and stirred at rt for 30 min. Later methanol was removed in vacuo and adjusted to pH 2–3 with aq 1 N HCl and extracted with EtOAc. The organic layer was dried over anhydrous sodium sulfate and evaporated to get acid **4**.

2.1.3. General procedure for preparation of N-substituted 5-phenylpyrazole-3-carboxamides 8a–i, 9a–e and 10a–h

A solution of acid **4** (0.09 g, 0.47 mmol) in dry DMF (2 mL) treated sequentially with amine (**5a–i**, **6a–e** and **7a–h**; 0.517 mmol) and triethylamine (0.94 mmol) was stirred under a N_2 atmosphere for 15 min, later TBTU (0.56 mmol) was added and reaction mixture refluxed for 4–10 h. The reaction mixture was quenched with aq satd NH_4Cl solution (10 mL). After 10 min, it was diluted with $CHCl_3$ (2 × 10 mL) and washed with water (10 mL), $NaHCO_3$ solution (10 mL) and brine (10 mL). The organic layers were dried over anhydrous sodium sulfate, evaporated and the residue purified by

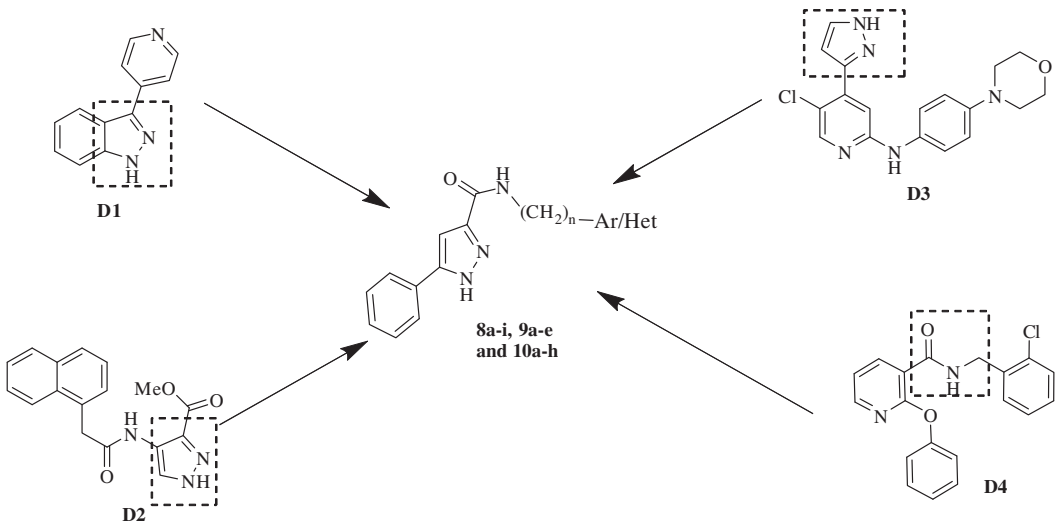


Figure 1b. The design of pyrazole amides **8a–i**, **9a–e** and **10a–h**.

column chromatography using 30% ethyl acetate in pet. ether to afford corresponding amides **8a–i**, **9a–e** and **10a–h**.

2.2. Pharmacological screening methodology

2.2.1. JNK-1 Inhibitory activity

A cell line based in vitro method was followed to screen the compounds for JNK-1 inhibitory activity. COS cells upon transfection with FLAG-tagged JNK-1 were exposed to 0.5 M sorbitol for 30 min to elicit maximal JNK-1 activation and then FLAG-JNK was immune precipitated with GST-ATF2. The immune precipitate was pretreated by test compound and incubated in buffer B. During this incubation period phosphorylation takes place and this phosphorylated substrate is separated by SDS–PAGE, visualized by autoradiography and quantitated by Cerenkov counting method using the kinase profiler service according to the manufacturer procedure.

2.2.2. Anti-inflammatory activity

The method described by Winter et al., was followed to screen the anti-inflammatory activity of synthesized compounds. Suspension of test compounds were administered orally one hour later 1% carrageenan was injected into subplantar region of right paw of rats in all groups and paw volume was monitored at different time intervals using digital plethysmometer (UGO Basil, Italy). The percentage inhibition of paw volume for each test group is calculated using following equation.[30–32]

$$\text{Percentage of inhibition}\,(\%) = [1 - \text{Volume in mL (test compound)} / \text{Volume in mL (control)}] \times 100.$$

2.3. Molecular docking studies

2.3.1. Dataset preparation

A dataset comprising of 22 phenyl pyrazolyl amides were drawn with all possible tautomers and were minimized with OPLS-2005 force field using water as solvent in the GB/SA continuum solvation model.[33] The extended cutoff includes contribution of van der Waals interaction (8.0), electrostatic (20), and hydrogen bond (4.0). The minimization of molecules was carried out using Polak–Ribiere Conjugate Gradient (PRCG) method with maximum of 5000 iterations. The extensive conformational search was carried out with Mixed torsional/Low-mode sampling method with the use of 100 steps per rotatable bond, maximum number of steps 1000, energy window for saving structures with 5.02 kcal/mol, eliminate the redundant conformers with maximum atom deviation cut-off 0.5 Å and saved 100 structures for each search. The generated conformers (1177) further subjected to selection of specific tautomeric states (554) that are fit to hinge region of JNK1 and other tautomeric states were removed. The selected conformers were used for molecular docking study.

2.3.2. Protein preparation

The crystal structure of human JNK1 (PDB ID: 2GMX) has been selected for the docking studies. The 2GMX structure was prepared by adjusting bond orders, tautomers and adding hydrogen atoms using protein preparation wizard of Schrödinger software graphical user interface Maestro v9.3.[34] Further the protein was minimized by OPLS-2005 force field with converge heavy atoms to RMSD 0.3 Å relative to original protein structure.

2.3.3. Docking studies

In the first step, the docking study has been carried out with most potent compound (**10a**) and its conformers with prepared 2GMX crystal structure using Glide module of Schrödinger suite.[35] The prepared protein structure (2GMX) was used for grid generation using the default value of protein atom scaling (1.0) within a cubic box centered on the co-crystal ligand. The constraints in hinge region with carbonyl backbone in Glu109, –NH backbone moiety in Met111 were imposed and extra precision (XP) flexible docking of ligands was carried out with default value of ligand atom scaling (0.8). The post docking minimization has been carried out and maximum of 10 poses per ligand was saved. The best prioritized docked complex structure (**10a**) was used for grid generation using the above discussed default parameters and subjected to XP refinement docking for all the series of Phenyl pyrazole amide compounds. The obtained docked complex structures were analyzed and the compounds were prioritized by using docking score, interactions with active site residues.

2.4. Compound characterization

2.4.1. Ethyl 5-phenyl-3-pyrazole carboxylate 3

IR (KBr) (ν, cm^{-1}): 3141 (N—H str.), 2990 (C—H aromatic str.), 2928 (CH_2-str.), 1722 (C=O str.), 1571 (N—H bnd.); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.57 (d, 2H, Ar-H, J = 7.9 Hz), 7.40–7.22 (br m, 3H, Ar-H), 7.10 (s, 1H, Ar-H), 4.34 (q, 2H, CH_2, J = 7.1 Hz), 1.2–1.4 (t, 3H, CH_3, J = 6.9 Hz); ESI-MS (m/z): 217 $[M+H]^+$.

2.4.2. 5-Phenyl-3-pyrazole carboxylic acid 4

IR (KBr) (ν, cm^{-1}): 3332 (OH str.), 3137 (C—H aromatic str.), 2923 (CH_2 str.), 1730 (C=O str.), 1568 (N—H bnd.); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.56 (d, 1H, Ar-H), 7.50–7.40 (m, 3H, Ar-H), 7.10 (d, 2H, Ar-H), 2.10 (br s, 1H, OH); ESI-MS (m/z): 188 $[M+H]^+$.

2.4.3. *N*-Phenyl-5-phenyl-3-pyrazole carboxamide 8a

IR (KBr) (ν, cm^{-1}): 3375 (N—H str.), 1742 (C=O-str.), 1659 (N—H bnd.); ^{1}H NMR (300 MHz, $CDCl_3$): δ 13.30 (br s, 1H, NH), 9.21 (br s, 1H, NH) 8.10–7.70 (m, 4H, Ar-H), 7.62–7.21 (m, 6H, Ar-H), 7.10 (s, 1H, Ar-H); ESI-MS (m/z): 264 $[M+H]^+$.

2.4.4. *N*-(2,5-Dichlorophenyl)-5-phenyl-3-pyrazole carboxamide 8b

IR (KBr) (ν, cm^{-1}): 3300 (N—H str.), 1665 (C=O-str.), 1580 (N—H bnd.); ^{1}H NMR (500 MHz, $CDCl_3$, 298 K): δ 13.5 (br s, 1H, NH), 11.2 (br s, 1H, NH), 10.1 (br s, 1H, NH), 7.7 (d, 2H, Ar-H, J = 6.9 Hz), 7.5–7.3 (br m, 3H, Ar-H), 6.7 (s, 1H, CH=C), 2.5 (s, 2H, NH), 1.3 (s, 9H, *t*-butyl); MS (ESI) MS (ESI) m/z: 355 $[M+Na]^+$.

2.4.5. *N*-(4-Nitrophenyl)-5-phenyl-1*H*-pyrazole-3-carboxamide 8c

IR (KBr) (ν, cm^{-1}): 3421 (N—H str.), 3070 (C—H-aromatic str.), 1790 (C=O str.), 1610 (N—H bnd.); ^{1}H NMR (400 MHz, $CDCl_3$, 298 K): δ 7.80–7.90 (m, 4H, Ar-H), 7.50–7.20 (m, 5H, Ar-H), 7.0 (s, 1H, Ar-H); MS (ESI) m/z: 309 $[M+H]^+$.

2.4.6. *N*-(4-Methoxyphenyl)-5-phenyl-3-pyrazolecarboxamide 8d

IR (KBr) (ν, cm^{-1}): 3287 (N—H str.), 3005 (C—H-aromatic str.), 2928 (CH_3 str.), 1606 (C=O-str.), 1507 (N—H bnd.); ^{1}H NMR (400 MHz, $CDCl_3$, 298 K): δ 13.51 (s, 1H, NH), 9.4 (s, 1H, NH), 7.72 (d, 2H, Ar-H, J = 7.3 Hz), 7.70–7.21 (m, 5H, Ar-H), 6.82 (d, 3H, Ar = H, J = 8.7 Hz), 3.9 (s, 3H, OCH_3); MS (ESI) m/z: 294 $[M+H]^+$; Anal. Calcd for $C_{17}H_{15}N_3O_2$: C, 69.61; H, 5.15. Found: C, 69.50; H, 5.02.

2.4.7. *N*-(4-Hydroxyphenyl)-5-phenyl-3-pyrazole carboxamide 8e

IR (KBr) (ν, cm^{-1}): 3408 (N—H str.), 3192 (C—H aromatic, str.), 1730 (C=O str.), 1582 (N—H bnd.); ^{1}H NMR (400 MHz, $CDCl_3$,

298 K): δ 7.5 (d, 2H, Ar-H), 7.4–7.3 (m, 3H, Ar-H), 7 (s, 1H, CH═C), 6.7 (br m, 1H, NH), 2.8 (m, 1H, CH), 0.8 (m, 2H, CH_2), 0.6 (m, 2H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$, 278 K): δ 128.3 (3C), 127.8 (2C), 125, 102, 40, 21.7, 5.9 (2C); MS (ESI) *m*/*z* 280 $[M+H]^+$.

2.4.8. *N*-(4-Bromophenyl)-5-phenyl-3-pyrazole carboxamide 8f

IR (KBr) (*v*, cm^{-1}): 3360 (N–H str.), 3189 (C—H-aromatic, str.), 1602 (C═O-str.), 1547 (N—H bnd.); ^{1}H NMR (400 MHz, $CDCl_3$, 298 K): δ 8.1 (d, 1H, Ar-H, *J* = 8.3 Hz), 7.8 (d, 1H, Ar-H, *J* = 7.5 Hz), 7.7 (d, 1H, Ar-H, *J* = 8.3 Hz), 7.5 (d, 3H, Ar-H, *J* = 6.7 Hz), 7.4–7.2 (br m, 7H, Ar-H), 7.1 (d, 1H, NH), 6.9 (s, 1H, CH═C), 6 (q, 1H, CH, *J* = 7.3 Hz), 1.7 (d, 3H, CH_3, *J* = 6.7 Hz); ^{13}C NMR (75 MHz, $CDCl_3$, 278 K): δ 160.9, 145.9, 138.1, 133.8, 130.9, 128.9 (3C), 128.6 (3C), 128.2, 126.4, 125.7, 125.4, 125.1, 123.2, 122.5, 103.1, 44.6, 21; MS (ESI) *m*/*z*: 342 $[M]^+$, 344 $[M+H]^+$.

2.4.9. *N*-(3-Chloro-4-fluorophenyl)-5-phenyl-3-pyrazole carboxamide 8g

IR (KBr) (*v*, cm^{-1}): 3360 (N—H str.), 3189 (C—H-aromatic, str.), 2922 (CH_2 str.), 1663 (C = 0 str.), 1528 (N—H bnd.); ^{1}H NMR (400 MHz, $CDCl_3$, 298 K): δ 13.3 (s, 1H, NH), 9.6 (s, 1H, NH), 7.9 (d, 1H, Ar-H, *J* = 1.5 Hz), 7.7 (d, 2H, Ar-H, *J* = 8 Hz), 7.6 (br d, 1H, Ar-H, *J* = 7.1 Hz), 7.3 (t, 2H, Ar-H, *J* = 8 Hz), 7.2 (d, 1H, Ar-H, *J* = 7.1 Hz), 7.12 (t, 2H, Ar-H, *J* = 8.9 Hz); MS (ESI) *m*/*z*: 337 $[M+Na]^+$; Anal. Calcd for $C_{16}H_{11}ClFN_3O$: C, 60.87; H, 3.51. Found: C, 60.77; H, 3.40.

2.4.10. *N*-Mesityl-5-phenyl-3-pyrazole carboxamide 8h

IR (KBr) (*v*, cm^{-1}): 3360 (N—H str.), 3189 (C—H-aromatic, str.), 2922 (CH3, str.), 1658 (C═O-str.), 1568 (N—H bnd.); ^{1}H NMR (400 MHz, $CDCl_3$, 298 K): δ 8.16 (m, 1H, Ar-H), 8.14 (m, 1H, Ar-H), 7.50 (m, 2H, Ar-H), 7.42 (m, 1H, Ar-H), 6.81 (m, 2H, Ar-H), 6.7 2 (s, 1H, Ar-H), 2.40 (s, 3H, CH_3), 2.30 (s, 6H, 2. CH_3); MS (ESI) *m*/*z*: 306 $[M+H]^+$.

2.4.11. *N*-(3-Acetylphenyl)-5-phenyl-3-pyrazole carboxamide 8i

IR (KBr) (*v*, cm^{-1}): 3316 (N—H str.), 3193 (C—H-aromatic, str.), 2924 (CH, aliphatic, str.), 1686 (C═O-str.), 1604 (N—H bnd.); ^{1}H NMR (400 MHz, $CDCl_3$, 298 K): δ 8.10 (m, 1H, Ar-H), 8.0 (m, 1H, Ar-H), 7.80–7.60 (m, 3H, Ar-H), 7.50–7.20 (m, 4H, Ar-H), 7.15 (s, 1H, Ar-H), 2.60 (s, 3H, CH_3); MS (ESI) *m*/*z*: 328 $[M+Na]^+$.

2.4.12. *N*-Benzyl-5-phenyl-3-pyrazole carboxamide 9a

IR (KBr) (*v*, cm^{-1}): 3449 (N—H str.), 3053 (C—H aromatic), 2926 (CH, aliphatic, str.), 1665 (C═O-str.), 1614 (N—H bnd.); ^{1}H NMR (500 MHz, $CDCl_3$, 298 K); δ 8.1 (d, 2H, Ar-H, *J* = 8.3 Hz), 7.62 (d, 2H, Ar-H, *J* = 8.3 Hz), 7.52 (t, 3H, Ar-H, *J* = 7.5 Hz), 7.4–7.32 (m, 3H, Ar-H), 7.2 (s, 1H, NH), 2.35 (s, 1H, CH_2); MS (ESI) *m*/*z*: 300 $[M+Na]^+$; Anal. Calcd $C_{17}H_{15}N_3O$: C, 73.63; H, 5.45. Found: C, 73.51; H, 5.37.

2.4.13. *N*-(3,4-Dichlorobenzyl)-5-phenyl-3-pyrazole carboxamide 9b

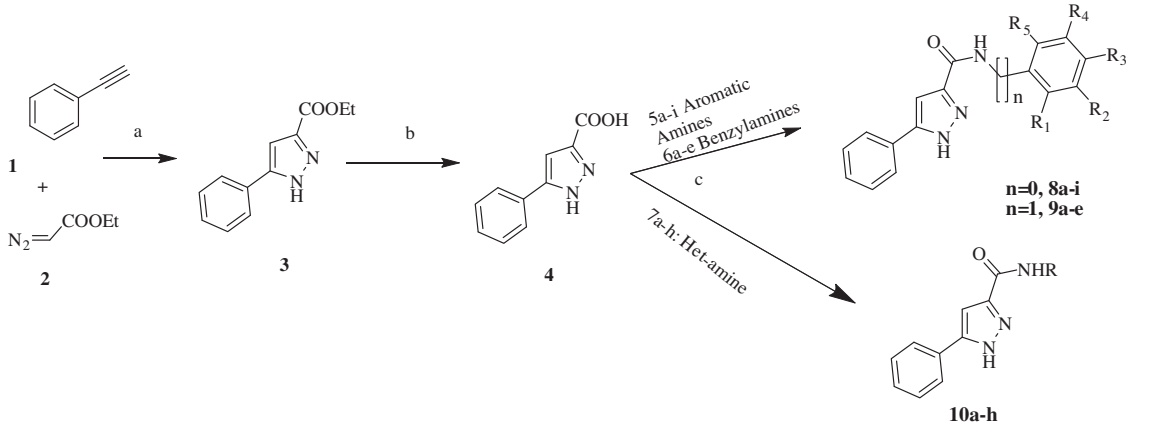
IR (KBr) (*v*, cm^{-1}): 3410 (N—H str.), 2900 (CH, aliphatic, str.), 1755 (C═O-str.), 1611 (NH bnd.); ^{1}H NMR (400 MHz, $CDCl_3$, 298 K): δ 7.95 (d, 1H, Ar-H, *J* = 1.5 Hz), 7.86 (d, 1H, Ar-H, *J* = 1.7 Hz), 7.71–7.60 (dd, 1H, Ar-H, *J* = 1.7 Hz), 7.62 (s, 1H, Ar-H), 7.50 (t, 1H, Ar-H, *J* = 1.8 Hz), 7.46 (s, 1H, NH), 7.40 (s, 1H, Ar-H), 7.40–7.30 (br m, 2H, Ar-H), 7.10 (dd, 1H, Ar-H, *J* = 2 Hz), 4.70 (s, 2H, CH_2); MS (ESI) *m*/*z*: 347 $[M+H]^+$; Anal. Calcd for $C_{17}H_{13}Cl_2N_3O$: C, 58.98; H, 3.78. Found: C, 58.89; H, 3.67.

2.4.14. *N*-(4-Fluorobenzyl)-5-phenyl-3-pyrazole carboxamide 9c

IR (KBr) (*v*, cm^{-1}): 3325 (N—H str.), 3127 (C—H-aromatic, str.), 2928 (CH_2 str.), 1663 (C═O-str.), 1586 (N—H bnd.); ^{1}H NMR (400 MHz, $CDCl_3$, 298 K): δ 8.10 (m, 1H, Ar-H), 7.82 (m, 1H, Ar-H), 7.81 (m, 1H, Ar-H), 7.82–7.62 (m, 3H, Ar-H), 7.53–7.42 (m, 3H, Ar-H), 6.40 (s, 1H, Ar-H); MS (ESI) *m*/*z*: 318 $[M+Na]^+$.

2.4.15. *N*-(4-Methoxybenzyl)-5-phenyl-3-pyrazole carboxamide 9d

IR (KBr) (*v*, cm^{-1}): 3341 (N—H str.), 3215 (C—H aromatic, str.), 2923 (CH_2 str.), 1636 (C═O str.), 1539 (N—H bnd.); ^{1}H NMR (400 MHz, $CDCl_3$, 298 K): δ 9.12 (t, 1H, NH, *J* = 5.2 Hz), 8.80 (t, 1H, NH, *J* = 6 Hz), 7.80 (t, 2H, Ar-H, *J* = 7.3 Hz), 7.50–7.20 (m, 4H, Ar-H), 7.0 (s, 1H, Ar-H), 6.91 (s, 2H, Ar-H), 6.82 (d, 1H, Ar-H, *J* = 8.3 Hz), 4.42 (dd, 2H, CH_2, *J* = 5.8, 6.4 Hz), 3.71 (s, 3H, OCH_3); MS (ESI) *m*/*z*: 308 $[M+H]^+$; Anal. Calcd for $C_{18}H_{17}N_3O_2$: C, 70.34; H, 5.58. Found: C, 70.23; H, 5.47.



R=Alkyl or Aryl Group

Reagents and conditions:
(a) DABCO
(b) NaOH, MeOH, rt, 30 min
(c) RNH_2, Et_3N, cat. TBTU, dry DMF, reflux. N_2

5a-i substituted anilines
a: aniline
b:2,5-dichloro aniline
c: 4-nitroaniline
d: 4-methoxyaniline
e: 4-hydroxyaniline
f: 4-bromoaniline
g: 4-chloroaniline
h: 2,4,6-trimenthyl anilie
i: 3-acetyl aniline

6a-e: substiuted benzylamines
a: benzylamine
b:3,4-dichloro benzylamine
c: 4-fluoro benzylamine
d: 3-methoxybenzylamine
e: 3,4-dimethoxy benzylamine

7a-h: Het-amines
a: 5-methyl-furan-2-yl amine
b: 5-(4-methoxy)phenyl-isoxazol-5-yl amine
c: 5-t-butyl-isoxazol-5-yl amine
d: 2-mercaptobenzimidazol-5-yl amine
e: 2-thiazolyl amine
f: cyclopropyl amine
g: 1-(2-naphthyl) ethyl amine
h: 2-pyridinyl amine

Scheme 1. Synthesis of pyrazole containing JNK-1 inhibitors.

2.4.16. *N*-(3,4-Dimethoxybenzyl)-5-phenyl-3-pyrazole carboxamide 9e

IR (KBr) (ν, cm^{-1}): 3221 (N—H str.), 3027 (C—H-aromatic, str.), 2967 (CH, aliphatic, str.), 1646 (C=O-str.), 1595 (N—H bnd.); ^1H NMR (400 MHz, $CDCl_3$, 298 K): δ 9.0 (t, 1H, NH, J = 5.2 Hz), 8.70 (t, 1H, NH, J = 6 Hz), 7.80 (t, 2H, Ar-H, J = 7.3 Hz), 7.50–7.20 (m, 3H, Ar-H), 7.0 (s, 1H, Ar-H), 6.90 (s, 2H, Ar-H), 6.80 (d, 1H, Ar-H, J = 8.3 Hz), 4.40 (dd, 2H, Ar-CH_2, J = 5.8, 6.4 Hz), 3.70 (s, 3H, OCH_3); MS (ESI) m/z: 360 $[M+Na]^+$.

2.4.17. *N*-(Furan-2-yl)methyl-5-phenyl-3-pyrazole carboxamide 10a

IR (KBr) (ν, cm^{-1}): 3420 (N—H str.), 3129 (C—H aromatic str.), 2925 (CH_2-str.), 1640 (C=O str.), 1546 (N—H bnd.); ^1H NMR (300 MHz, $CDCl_3$, 298 K): δ 7.58 (d, 2H, Ar-H, J = 7.1 Hz), 7.45–7.29 (br m, 3H, Ar-H), 7.21 (1H, Ar-H), 7.0 (s, 1H, Ar-H), 6.30 (dd, 2H, Ar-H, J = 4.1 Hz), 2.0 (br s, 1H, NH); MS (ESI) m/z: 268 $[M+H]^+$, 290 $[M+Na]^+$; Anal. Calcd for $C_{15}H_{13}N_3O_2$: C, 67.40; H, 4.90. Found: C, 67.30; H, 4.70.

2.4.18. *N*-(4-(4-Methoxyphenyl)isoxazol-3-yl)-5-phenyl-1*H*-pyrazole-3 10b

IR (KBr)(ν, cm^{-1}): 3320 (N—H str.), 3039 (C—H aromatic, str.), 2829 (CH_3 str.), 1695 (C=O-str.), 1642 (N—H bnd.); ESI-MS (m/z): 361 $[M+H]^+$.

2.4.19. *N*-(5-*t*-Butyl-2,5-dihydroisoxazol-3-yl)-5-phenyl-3-pyrazole carboxamide 10c

IR (KBr) (ν, cm^{-1}): 3375 (N—H str.), 3124 (C—H aromatic, str.), 2923 (CH_2 str.), 1742 (C=O str.), 1595 (N—H bnd.); ^1H NMR (500 MHz, $CDCl_3$, 298 K): δ 13.82 (br s, 1H, NH), 11.46 (br s, 1H, NH), 10.21 (br s, 1H, NH), 7.70 (d, 2H, Ar-H, J = 6.9 Hz), 7.53–7.31 (br m, 4H, Ar-H), 6.78 (s, 1H, Ar-H), 2.5 (s, 1H, CH), 1.3 (s, 9H, t-butyl); MS (ESI) m/z: 311 $[M]^+$.

2.4.20. *N*-(2-Mercaptobenzimidazol-5-yl)-5-phenyl-3-pyrazole carboxamide 10d

IR (KBr) (ν, cm^{-1}): 3285 (N—H str.), 3052 (C—H aromatic str.), 1739 (C=O str.), 1649 (N—H bnd.); ^1H NMR (400 MHz, $CDCl_3$, 298 K): δ 9.10 (br s, 1H, NH), 8.71 (br s, 1H, NH), 7.71 (br t, 2H, Ar-H), 7.50–7.20 (br m, 5H, Ar-H), 6.91–6.70 (br m, 2H, Ar-H); MS (ESI) m/z: 335 $[M+H]^+$; Anal. Calcd for $C_{16}H_{13}N_5O_5$: C, 59.43; H, 4.05. Found: C, 59.33; H, 4.0.

2.4.21. 5-Phenyl-*N*-(thiazol-2-yl)-3-pyrazole carboxamide 10e

IR (KBr) (ν, cm^{-1}): 3376 (N—H str.), 3216 (C—H aromatic, str.), 1730 (C=O-str.), 1680 (N—H bnd.); ^1H NMR (400 MHz, $CDCl_3$, 298 K): δ 9 (t, 1H, NH, J = 5.2 Hz), 8.7 (t, 1H, NH, J = 6 Hz), 7.8 (t, 2H, Ar-H, J = 7.3 Hz), 7.5–7.2 (m, 4H, Ar-H), 7 (s, 1H, Ar-H); MS (ESI) m/z: 271 $[M+H]^+$.

2.4.22. *N*-Cyclopropyl-5-phenyl-3-pyrazole carboxamide 10f

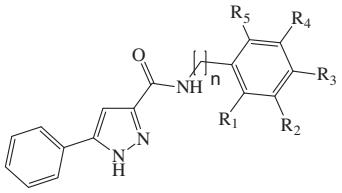
IR (KBr) (ν, cm^{-1}): 3400 (N—H str.), 3127 (C—H-aromatic, str.), 2921 (CH_2 str.), 1648 (C=O str.), 1539 (N—H bnd.); ^1H NMR (400 MHz, $CDCl_3$, 298 K): δ 7.68 (d, 2H, Ar-H), 7.44–7.32 (m, 3H, Ar-H), 7.0 (s, 1H, Ar-H), 6.71 (br m, 1H, NH), 2.88 (m, 1H, CH), 0.89 (m, 2H, CH_2), 0.65 (m, 2H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$, 278 K): δ 128.3 (3C), 127.8 (2C), 125, 102, 40, 21.7, 5.9 (2C); MS (ESI) m/z: 250 $[M+Na]^+$; Anal. Calcd for $C_{13}H_{13}N_3O$: C, 68.70; H, 5.77. Found: C, 68.59; H, 5.67.

Table 1
Physical properties of pyrazole compounds

Compd no	Mol. for.	Mol. wt.	Yield (%)	Mp (°C)	R_f	c·Log P[a]
8a	$C_{16}H_{13}N_3O$	263.30	78.5	134–136	0.32	3.715
8b	$C_{16}H_{11}C_{l2}N_3O$	332.19	46.2	152–154	0.34	4.412
8c	$C_{16}H_{12}N_4O_3$	308.30	76.0	122–124	0.47	3.6512
8d	$C_{17}H_{15}N_3O_2$	293.33	48.5	130–132	0.42	3.68874
8e	$C_{16}H_{13}N_3O_2$	279.30	86.4	132–134	0.46	3.048
8f	$C_{16}H_{12}BrN_3O$	342.20	92.4	210–212	0.36	4.66816
8g	$C_{16}H_{11}ClFN_3O$	315.74	67.5	190–192	0.38	4.69272
8h	$C_{18}H_{17}N_3O$	305.37	54.9	174–176	0.45	4.063
8i	$C_{18}H_{15}N_3O_2$	305.34	77.6	111–113	0.44	3.31822
9a	$C_{17}H_{15}N_3O$	277.33	91.2	161–163	0.41	3.894
9b	$C_{17}H_{13}C_{l2}N_3O$	346.22	79.3	198–200	0.35	5.2
9c	$C_{17}H_{14}FN_3O$	295.32	68.6	182–184	0.54	4.037
9d	$C_{18}H_{17}N_3O_2$	307.36	72.4	115–117	0.41	3.813
9e	$C_{19}H_{19}N_3O_3$	337.38	66.8	242–244	0.34	3.552
10a	$C_{15}H_{13}N_3O_2$	267.29	72.4	201–203	0.36	3.07
10b	$C_{2O}H_{16}N_4O_3$	360.12	58.0	245–247	0.43	3.428
10c	$C_{17}H_{20}N_4O_2$	312.37	65.0	190–192	0.40	3.09148
10d	$C_{17}H_{13}N_5OS$	335.39	92.0	240–242	0.58	4.2042
10e	$C_{13}H_{10}N_4OS$	270.31	91.6	202–204	0.56	2.51174
10f	$C_{13}H_{13}N_3O$	227.27	89.0	128–130	0.33	2.51
10g	$C_{22}H_{19}N_3O$	341.42	61.0	136–138	0.35	5.377
10h	$C_{15}H_{12}N_4O$	264.29	89.0	198–200	0.38	2.6482

[a] Calculated using ChemBio 3D Ultra.

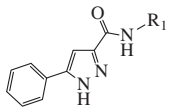
Table 2
Inhibition results for compounds **8a–i** and **9a–e** against JNK-1



Compound	n	R_1	R_2	R_3	R_4	R_5	IC_{50}[a] μM
8a	0	H	H	H	H	H	9.6
8b	0	H	Cl	H	H	Cl	6.2
8c	0	H	H	NO_2	H	H	9.8
8d	0	H	H	OCH_3	H	H	8.1
8e	0	H	H	OH	H	H	NT
8f	0	H	H	Br	H	H	5.9
8g	0	H	H	Cl	F	H	3.1
8h	0	CH_3	H	CH_3	H	CH_3	11.4
8i	0	H	$COCH_3$	H	H	H	6.9
9a	1	H	H	H	H	H	9.1
9b	1	H	H	Cl	Cl	H	5.8
9c	1	H	H	F	H	H	2.9
9d	1	H	H	OCH_3	H	H	6.1
9e	1	H	H	OCH_3	OCH_3	H	5.8

[a] IC_{50} in μM, SEM ± 0.2, NT—Not tested against the isolated JNK-1.

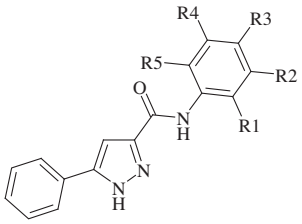
Table 3
Inhibition results for compounds **10a–h** against JNK-1



Compd	R_1	IC_{50}[a]
10a	Furan-2yl methyl	2.8
10b	5-(4-Methoxyphenyl)isoxazol-3-yl	3.2
10c	5-*t*-Butylisoxazol-3-yl	NT[b]
10d	2-Mercaptobenzimidazol-5-yl	2.9
10e	2-Thiazolyl	6.8
10f	Cyclopropyl	6.2
10g	1-(1-Naphthyl)ethyl	NT[b]
10h	2-Pyridinyl	8.9

[a] IC_{50} in μM, SEM ± 0.2.
[b] NT—Not tested against the isolated JNK-1.

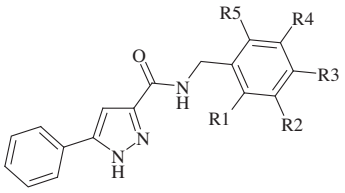
Table 4
Anti-inflammatory activity of compounds **8a–i**



Compd no	R_1	R_2	R_3	R_4	R_5	Mean paw volume (mL) ± SEM and % inhibition			
						1 h	2 h	3 h	4 h
8a	H	H	H	H	H	0.52 ± 0.059 32.48**	0.45 ± 0.051** 35.02	0.35 ± 0.042* 45.64	0.26 ± 0.032* 30.58
8b	H	Cl	H	H	Cl	0.52 ± 0.065* 31.02	0.51 ± 0.057** 40.16	0.51 ± 0.050** 45.47	0.38 ± 0.033** 18.90
8c	H	H	NO_2	H	H	0.50 ± 0.069* 89.24	0.42 ± 0.054* 94.31	0.36 ± 0.041* 92.56	0.22 ± 0.025* 68.70
8d	H	H	OCH_3	H	H	0.56 ± 0.057** 33.3	0.18 ± 0.042* 79.54	0.14 ± 0.027* 81.08	0.07 ± 0.011* 66.44
8e	H	H	OH	H	H	0.17 ± 0.035** 22.11	0.42 ± 0.038* 43.24	0.055 ± 0.012** 23.28	0.063 ± 0.016** 16.64
8f	H	H	Br	H	H	0.53 ± 0.045 42.16*	0.45 ± 0.054* 46.31	0.52 ± 0.035* 51.4	0.36 ± 0.022* 38.22
8g	H	H	Cl	F	H	0.29 ± 0.037* 68.81	0.20 ± 0.028* 72.7	0.073 ± 0.018* 90.54	0.054 ± 0.011* 64.22
8h	CH_3	H	CH_3	H	CH3	0.40 ± 0.047* 53.65	0.47 ± 0.027* 45.6	0.43 ± 0.035* 44.24	0.28 ± 0.016* 26.8
8i	H	$COCH_3$	H	H	H	0.55 ± 0.063* 41.3	0.54 ± 0.057** 56.7	0.56 ± 0.053* 74.32	0.39 ± 0.032* 56.8

NT—Not determined.
* p <0.01.
** p <0.05.

Table 5
Anti-inflammatory activity of compounds **9a–e**



Compd no	R_1	R_2	R_3	R_4	R_5	Mean paw volume (mL) ± SEM and % inhibition			
						1 h	2 h	3 h	4 h
9a	H	H	H	H	H	0.60 ± 0.079** 35.48	0.41 ± 0.050* 53.4	0.38 ± 0.045* 48.64	0.22 ± 0.018* 25.66
9b	H	H	Cl	Cl	H	0.51 ± 0.047* 45.16	0.49 ± 0.098* 44.31	0.54 ± 0.033* 27.02	0.31 ± 0.014* 10.11
9c	H	H	F	H	H	0.55 ± 0.038* 67.86	0.23 ± 0.019* 70.27	0.21 ± 0.020* 75.27	0.14 ± 0.010* 54.66
9d	H	H	H	OCH_3	H	0.54 ± 0.067* 41.93	0.53 ± 0.058** 39.77	0.50 ± 0.050** 32.43	0.28 ± 0.032** 16.46
9e	H	H	OCH_3	OCH_3	H	0.42 ± 0.054* 54.83	0.47 ± 0.027* 46.59	0.42 ± 0.038* 43.24	0.26 ± 0.019* 24.80

NT—Not determined.
* p <0.01.
** p <0.05.

2.4.23. *N*-(1-(Naphthalen-2-yl)ethyl)-5-phenyl-3-pyrazole carboxamide 10g

IR (KBr) (ν, cm^{-1}): 3400 (N—H str.), 3127 (C—H aromatic, str.), 2921 (CH_2 str.), 1646 (C=O str.), 1539 (N—H bnd.); ^1H NMR (400 MHz, $CDCl_3$, 298 K): δ 8.40 (d, 1H, Ar-H, J = 8.3 Hz), 7.56 (d, 1H, Ar-H, J = 8.3 Hz), 7.54 (d, 1H, Ar-H, J = 7.5 Hz), 7.40–7.20 (m, 8H, Ar-H), 7.10 (d, 1H, NH), 6.90 (s, 1H, CH=C), 6.20 (q, 1H, CH, J = 7.3 Hz), 1.72 (d, 3H, CH_3, J = 6.7 Hz);^{13}C NMR (75 MHz, $CDCl_3$, 278 K): δ 160.9, 145.9, 138.1, 133.8, 130.9, 128.9 (3C), 128.6 (3C), 128.2, 126.4, 125.7, 125.4, 125.1, 123.2, 122.5, 103.1, 44.6, 21; MS (ESI) m/z: 364 $[M+Na]^+$; HRMS (ESI+): m/z $[M+Na]^+$; calcd for $C_{22}H_{19}N_3O$: C, 77.40; H, 5.61. Found: C, 77.29; H, 5.55.

2.4.24. 5-Phenyl-*N*-(pyridin-2-yl)-3-pyrazole carboxamide 10h

IR (KBr) (ν, cm^{-1}): 3376 (N—H str.), 3024 (C—H Aromatic str.), 1730 (C=O-str.), 1664 (N—H bnd.); ^1H NMR (400 MHz, $CDCl_3$, 298 K): δ 8.2 (m, 1H, Ar-H), 8.2 (m, 1H, Ar-H), 7.9–7.6 (m, 4H, Ar-H), 7.1 (m, 2H, Ar-H, NH), 7.5–7.2 (m, 2H, Ar-H), 9.2 (br s, 1H, NH); MS (ESI) *m*/*z*: 265 $[M+Na]^+$.

3. Results and discussions

3.1. Chemistry

The title compounds were synthesized by adopting appropriate synthetic methodologies as shown in Scheme 1 and their physical properties are listed in Table 1. The key intermediate **3** was synthesized by the 1,3-dipolar cycloaddition reaction of phenylacetylene (dipolarophile) and ethyldiazoacetate (dipole). This reaction was mediated by solid catalyst DABCO resulting in compound **3** in a neat reaction. Despite a high yielding reaction, the product was purified over silica using 20% ethyl acetate. Compound **3** upon alkaline hydrolysis and subsequent acidification resulted in corresponding acid **4**. Further, compound **4** was treated with various amines (**5a**–**h**, **6a**–**i** and **7a**–**e**) in presence of coupling agent and base yielded expected amides (**8a**–**i**, **9a**–**e** and **10a**–**h**), which were purified by passing through silica gel using 30% ethyl acetate in petroleum ether as eluent.

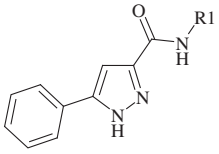
IR spectrum of compound **3** (KBr) divulged intense absorption band at 2950 cm^{-1} accounting for methyl group, band for carbonyl group of ester appeared at 1664 cm^{-1} and a peak for NH was observed at 3230 cm^{-1}. ^1H NMR spectrum of compound **3** confirmed the cyclization of phenylacetylene with diazonium compound, all the aromatic protons of 5-phenyl group resonated in the range of δ 7.7–7.2 as one doublet at δ 7.7 and another at δ 7.2 as triplet. C4 proton of pyrazole was observed little downfield at 7.0 ppm and a broad singlet at δ 7.6 is resultant of NH resonance. A quartet peak at δ 4.3 is due to CH_2 protons and a triplet found at 1.3 ppm is accounted for methyl protons. The molecular ion peak [*m*/*z*] at 216.10 in mass spectrum of compound **3** found to be in conformity with the molecular formula of the assigned structure.

Hydrolysis of compound **3** led to formation of 3-pyrazole carboxylic acid derivative 4 which showed presence of a broad IR absorption band peaking at 3433 cm^{-1} accounted for OH group of carboxylic acid apart from a band at 1704 cm^{-1} for carbonyl group stretching. ^1H NMR of compound **4** also confirmed the hydrolysis of **3** by the appearance of a broad singlet at 2.1 ppm due to OH proton. The absence of IR frequencies for methyl and methylene group deduced completion of hydrolysis and this was also supported by the absence of corresponding peaks in ^1H NMR. IR spectrum of all the amides demonstrated presence of a medium intense band at ~3200 cm^{-1} due to stretching vibration of amide NH. ^1H NMR spectra of these amide derivatives supported the formation of amide bond by showing a signal between 8.0–9.0 for the amide proton and by the absence of the broad peak due to carboxylic acid OH at 2.1 ppm. Structures assigned for the synthesized compounds were further ascertained based on the *m*/*z* values of individual compounds and elemental analysis (C, H and N) data.

3.2. JNK-1 inhibitory activity

All the twenty two molecules (**8a**–**i**, **9a**–**e** and **10a**–**h**) were screened for in vitro JNK-1 inhibitory activity as listed in Tables 2 and 3. Compound **10a** was found to be the most potent among all the synthesized compounds with IC_{50} value of 2.8 μM. Three compounds **8e**, **10c** and **10g** have not been tested for JNK-1 inhibitory activity and one compound **8h** showed mild JNK-1 inhibitory activity with IC_{50} value of 11.9 μM where as, rest of the compounds inhibited JNK-1 with IC_{50} values less than 10 μM. Unsubstituted phenyl compound **8a** demonstrated significant inhibitory activity with IC_{50} of 9.6 μM and substitution at R3 with groups like halogens helped in improving the activity as compound **8f** with

Table 6
Anti-inflammatory activity of compounds **10a**–**h**



Compd	R_1	Mean paw volume (mL) ± SEM and % inhibition			
		1 h	2 h	3 h	4 h
10a	2-Methyl furanyl	0.25 ± 0.042*	0.16 ± 0.27*	0.065 ± 0.026*	0.033 ± 0.016*
		73.11	81.81	91.89	66.33
10b	3-(4-Methoxy phenyl)isoxazolyl	0.29 ± 0.037*	0.20 ± 0.028*	0.073 ± 0.018*	0.058 ± 0.012*
		68.81	72.72	90.54	71.66
10c	5-*t*-Isoxazolyl	0.27 ± 0.019*	0.25 ± 0.027*	0.42 ± 0.024*	0.51 ± 0.031*
		68.60	70.60	52.8	41.7
10d	2-Mercapto benzimidazol-5-yl	0.05 ± 0.037*	0.04 ± 0.0032*	0.062 ± 0.021*	0.045 ± 0.015*
		94.62	95.45	91.62	74.15
10e	2-Thiazolyl	0.21 ± 0.026*	0.23 ± 0.017*	0.068 ± 0.084*	0.041 ± 0.044*
		45.31	51.06	54.65	31.40
10f	Cyclopropyl	0.28 ± 0.028*	0.13 ± 0.023*	0.11 ± 0.028*	0.06 ± 0.008*
		69.89	85.22	88.13	55.87
10g	1-(l-Naphthyl) ethyl	0.36 ± 0.020*	0.23 ± 0.019*	0.19 ± 0.021*	0.11 ± 0.014*
		61.29	73.86	74.32	50.64
10h	2-Pyridinyl	0.56 ± 0.057**	0.18 ± 0.042*	0.14 ± 0.027*	0.08 ± 0.01*
		33.3	79.54	81.08	64.26
Diclofenac sodium (50 mg/kg)		23**	36**	47**	51**

NT—Not determined.
* p <0.01.
** p <0.05.

bromine group showed IC_{50} value of 5.9 μM and compound **8g** though contains fluoro group at R4 along with chloro at R3 was the most potent among the series (**8a**–**h**). Surprisingly methoxy substituted compound **8d** was found to be more potent than **8a** this indicates that not only steric property but also electronic properties may also contribute towards JNK-1 inhibitory activity. Lone meta substituted compound **8i** (R2 = $COCH_3$) was found to show superior activity over unsubstituted analog **8a** and similarly 2,5-dichloro analog **8b** demonstrated enhanced activity over **8a**.

In order to ascertain influence of a linker/spacer (—CH_2—) between the amide nitrogen and substituted phenyl group, a series of N-substituted benzyl **9a**–**e** ($n = 1$) were synthesized and screened for JNK-1inhibitory activity. Among this series, compound **9c** with fluoro group at R3 was found to be the most potent with IC_{50} value of 2.9 μM and unsubstituted compound **9a** was found to be least active in the series with IC_{50} of 9.1 μM, however it is more potent than **8a**. Dichloro and dimethoxy analogs (**9b** and **9e**) showed less activity (IC_{50} = 5.8 μM) when compared to **9c**. Overall substitutions over benzyl ring improved activity.

Further, compounds with N-heterocyclic rings (**10a**–**h**) were synthesized and evaluated for the JNK-1 inhibitory activity with an expectation of improved potency. Compound **10a** with *N*-methyl-2-furanyl substitution exhibited maximum potency against JNK-1 inhibition with IC_{50} of 2.8 μM. Compound **10d** with 2-mercaptobenzimidazol-5-yl ring showed equipotent activity (IC_{50} = 2.9 μM) when compared to **9c**. The rest four **10b**, **10e**, **10f** and **10h** demonstrated superior activity over the phenyl and benzyl analogs (**8a** and **9a**). The 4-methoxyphenylisoxazol-3-yl analog **10b** showed significant JNK-1 inhibitory activity (IC_{50} = 3.2 μM), where as substitution with cyclopropyl and thiazole rings (**10e** and **10f**) resulted in drastic decrease in potency with IC_{50} values of 6.2 and 6.8 μM, respectively. A lone compound with six member heterocyclic ring, that is, 2-pyridyl analog (**10h**) was found to be slightly better than **8a** and **9a**.

Table 7
Docking parameters (kcal/mol) and physicochemical properties of phenyl pyrazolamide series

S. no	gscore	evdw	ecoul	Energy	HBA	HBD	RB
8a	−7.97	−36.65	−4.22	−40.86	2	2	3
8b	−7.27	−23.36	−2.62	−25.98	2	2	3
8c	−6.69	−15.78	−2.58	−18.36	2	2	4
8d	−8.04	−39.19	−3.41	−42.6	3	2	4
8e	−8.58	−37.33	−6.14	−43.47	3	3	3
8f	−6.37	−38.08	−3.04	−41.12	2	2	3
8g	−8.4	−38.96	−3.2	−42.16	2	2	3
8h	−7.91	−18.05	−4.55	−22.61	2	2	3
8i	−8.24	−40.29	−3.87	−44.16	3	2	4
9a	−8.92	−40.08	−4.79	−44.87	2	2	4
9b	−9.41	−44.58	−4.4	−48.98	2	2	4
9c	−8.94	−40.08	−5.3	−45.37	2	2	4
9d	−9.15	−41.33	−4.7	−46.03	3	2	5
9e	−9.23	−43.04	−5.27	−48.31	4	2	6
10a	−8.73	−38.47	−5.53	−44.01	3	2	4
10b	−7.24	−39.46	−4.53	−43.99	5	2	5
10c	−8.09	−38.41	−4.63	−43.04	4	2	4
10d	−8.29	−42.43	−4.33	−46.76	2	4	3
10e	−7.56	−38.28	−4.61	−42.89	3	2	3
10f	−8.2	−35.09	−4.1	−39.19	2	2	3
10g	−9.25	−46.61	−3.98	−50.6	2	2	4
10h	−7.85	−37.28	−4.47	−41.75	3	2	3

MW, molecular weight; RB, rotatable bond; HBA, hydrogen bond acceptor; HBD, hydrogen bond donor.

3.3. In vivo antiinflammatory activity

Encouraging results of in vitro JNK-1 inhibitory activity of all synthesized compounds prompted us to evaluate them for

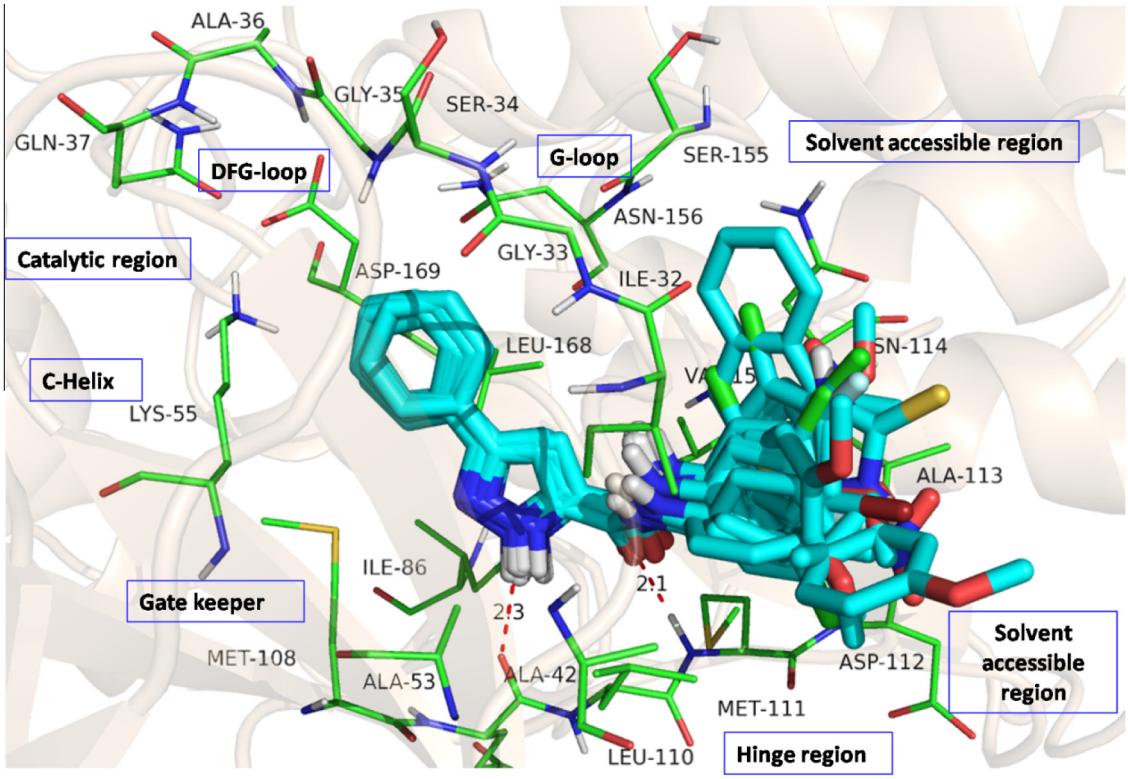


Figure 2. Binding modes all 22 phenyl pyrazoloamide series of compounds (cyan) in the active site of JNK1 and the hydrogen bond interactions are shown in red color dotted lines, the important residues are shown in green color and protein cartoon is represented in wheat color.

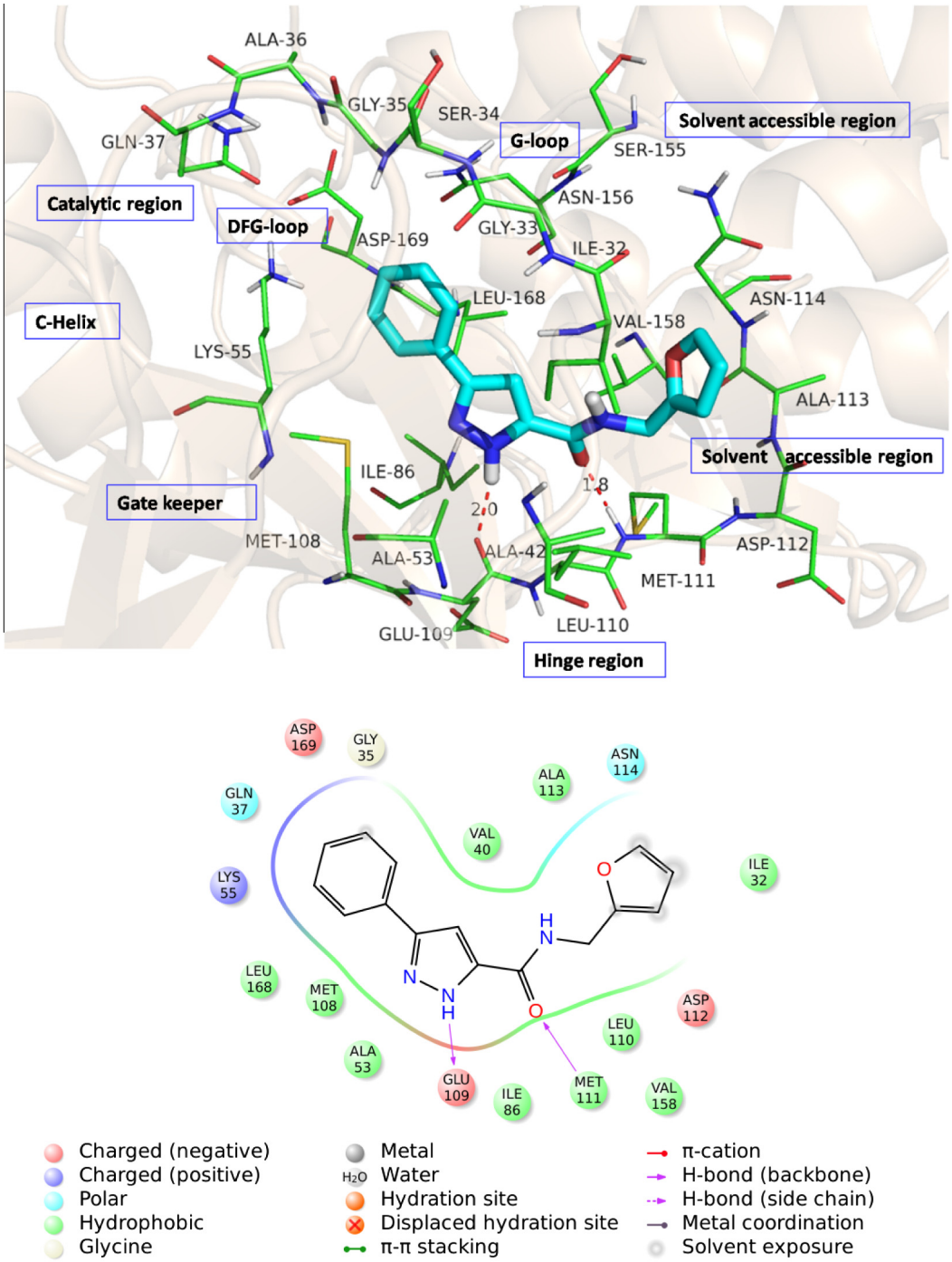
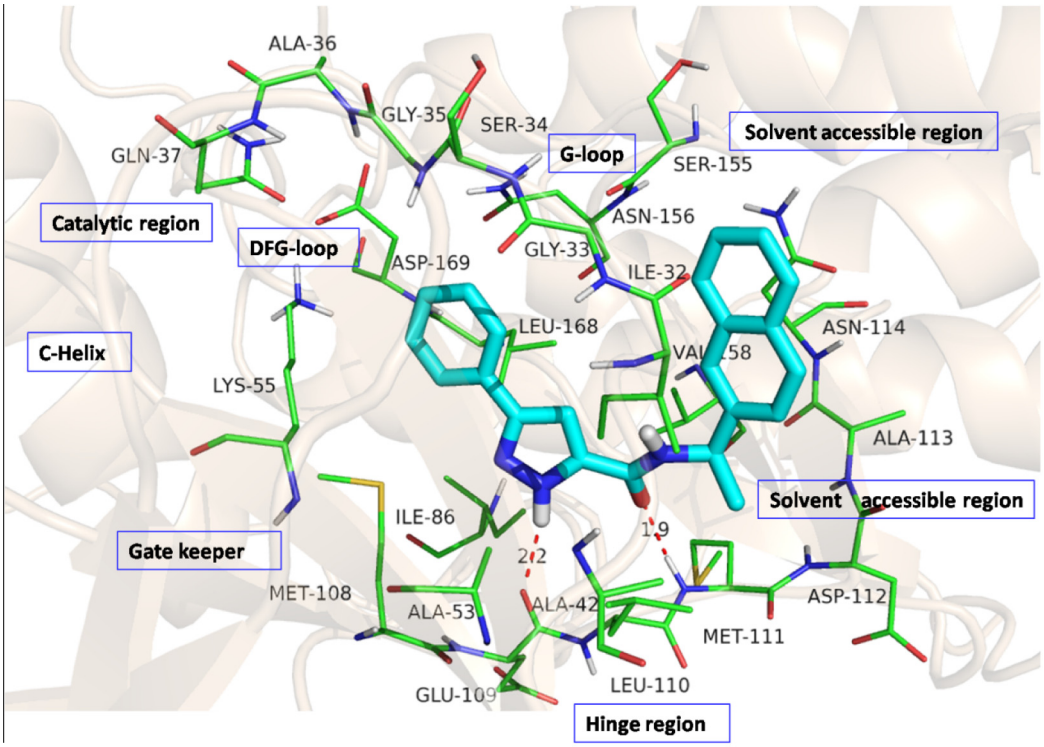


Figure 3. The probable binding mode of most potent compound (**10a**, cyan) in the active site of JNK1 and the hydrogen bond interactions are shown in red color dotted lines, the important residues are shown in green color and protein cartoon is represented in wheat color.

in vivo antiinflammatory activity in carrageenan induced rat paw edema and the results are listed in Tables 4–6. Nearly all compounds exhibited significant antiinflammatory activity and peak paw protection was observed at the 3rd hour of carrageenan challenge. For compounds **8c**, **8e** and **9a** the peak in vivo activity was observed at the 2nd hour of carrageenan injection and rest of the compounds **8h**, **9b**, **9d** and **9e** the apex of the activity was noticed at the 1st hour and activity gradually decreased with respect to time duration. Compounds **8c** and **10d** were found to be the most potent among all the tested compounds. Compound **10d** showed 95.45% paw protection at the second hour of carrageenan challenge which could be attributed to its JNK-1 inhibitory activity. Interestingly, compound **8c** with lesser JNK-1 inhibitory activity also exhibited almost equal potency (94.31% protection) at the 2nd hour when compared with **10d**. In similar fashion, most potent compound in the in vitro activity **10a** demonstrated 91.89% activity at the 3rd hour. Substitution on aromatic ring as in **8a–i** resulted in augmentation of activity in maximum compounds, with exception **8b** and **8e** demonstrated further inferior activity when compared to unsubstituted compound **8a**. Compound **8c**, which showed much less in vitro JNK-1 inhibition (IC_{50} = 9.8 μM), exhibited potent activity in in vivo assay with 92.56% inhibition of rat paw edema at 3rd hour. *N*-Benzyl derivatives **9a–e** were found to be less potent when compared with substituted *N*-phenyl analogs



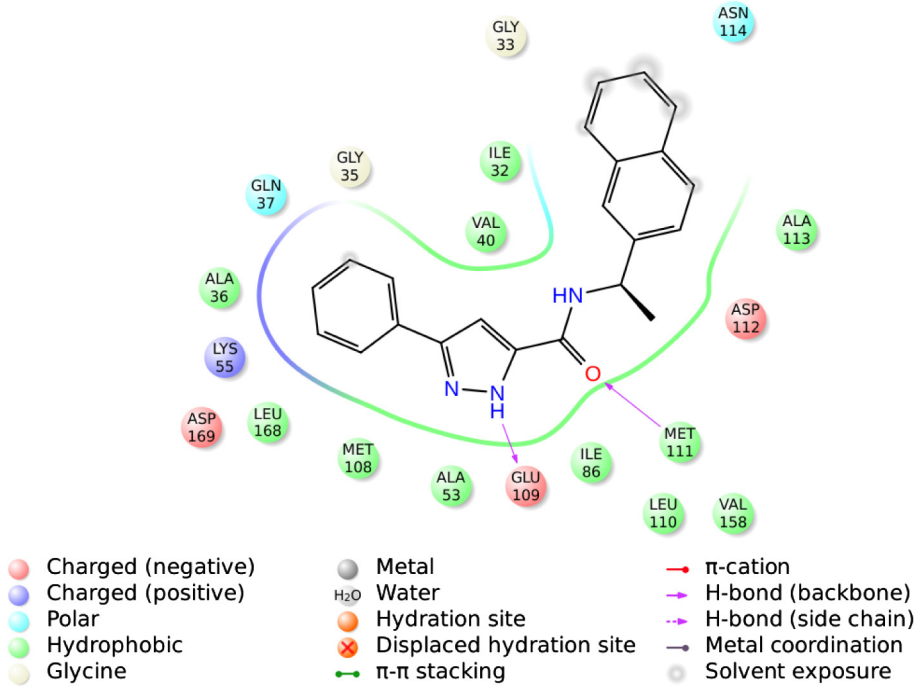


Figure 4. The probable binding mode of least potent compound (**10g**, cyan) in the active site of JNK1 and the hydrogen bond interactions are shown in red color dotted lines, the important residues are shown in green color and protein cartoon is represented in wheat color.

8a–**i**. Compound **9c** the most potent JNK-1 inhibitor (IC_{50} = 2.9 μM) among all the compounds, showed only moderate in vivo activity (75.27% inhibition at 3rd hour). However this compound **9c** was the most potent among the *N*-benzyl derivatives (**9a**–**i**).

In order to explore further influence of substituents, N-heterocyclic compounds **10a**–**h** have also been subjected for in vivo activity. This series of compounds were proved to be superior in in vitro activity among the three series. Two of the most active compounds **10a** and **10d** have also exhibited potent antiinflammatory activity with greater than 90% paw edema inhibition at the 3rd hour of carrageenan administration and even **10b** showed similar activity profile.

3.4. Molecular docking

All the twenty two compounds have been docked into human JNK1 target. With understanding of available JNK-1 inhibitors

profile, the pyrazole derivatives **8a–i**, **9a–e** and **10a–h** seem to be ATP competitive inhibitors. The compounds were docked into hinge region that connected both N-terminal lobe and C-terminal region. The active site was divided into hinge region (Glu109, Leu110, Met111), gate keeper (Met108), catalytic triad region (Lys55, Asp169, Glu73), G-loop region (Gly33, Ser34, Gly35), and DFG-loop region (Asp169, Phe170, Gly171). An attempt has been made to analyze the probable binding modes, key active site interactions for the most potent (**10a**), least active (**10g**), compound with best docked score (**9b**) and compound with least docked score (**8f**). All the docked compounds found to retain hinge backbone interactions with Glu109 and Met111 in which the pyrazole nitrogen showed hydrogen bond interaction with carbonyl backbone Glu109 and carbonyl moiety of amide showed hydrogen bond interaction with NH of Met111 backbone residue. The phenyl ring in compound **10a** accommodated near the catalytic triad residues Lys55, Asp169, Glu73 and showed hydrophobic interactions with Val40, Leu168. The pyrazole ring accommodated in close proximity to hydrophobic residues Ile32, Leu110, Val158. The docking scores have been listed in Table 7 and docking poses of all the molecules, most active and least active have been portrayed in Figures 2–4.

4. Conclusions

Twenty two new pyrazole heterocyclic group containing compounds have been synthesized and screened against JNK-1 in the current study. In a neat 1,3-dipolar cycloaddition reaction, phenylacetylene was reacted with ethyldiazoacetate in presence DABCO, the ester that resulted was hydrolyzed and converted into desired amides using suitable primary amines. All compounds, except three showed JNK-1 inhibitory activity with IC_{50} of less than 10 μM. Among the *N*-phenyl and *N*-benzyl derivatives halogen substitution enhanced the JNK-1 inhibitory activity as witnessed in compounds **9c** and **8g**. Further different heterocyclic amines were used to synthesize compounds **10a–h**, compounds **10a**, **10b** and **10d** were found to possess potent JNK-1 inhibitory activity. In the in vivo antiinflammatory activity, compounds **8c** and **8g** demonstrated potent activity at the third hour of the observation and compounds **10a**, **10b**, **10f** and **10h** showed over 80% paw edema protection.

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