How to Cite:
More, V. S., Chumbhale, D. C., Rangari, N. T., Mishra, P. A., Jagnar, A. B., & Kadam, J. N. (2022). Synthesis, analgesic and anti-inflammatory activities of some pyrazolo[3,4-c]pyrazole derivatives. *International Journal of Health Sciences*, *6*(S2), 1218–1231. https://doi.org/10.53730/ijhs.v6nS2.5177

Synthesis, Analgesic and Anti-Inflammatory Activities of Some Pyrazolo[3,4-c]Pyrazole Derivatives

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***Abstract*---**Historically, heterocyclic compounds containing Nitrogen, and their derivatives have been invaluable as a source of therapeutic agents. Pyrazole, with two nitrogen atoms and aromatic character, provides diverse functionality and stereochemical complexity in a five-membered ring structure. In Knorr pyrazole synthesis, diimine compound gets deprotonated to regenerate the acid catalyst and provide the final pyrazole product. Formation of pyrazole derivatives from hydrazines, hydrazides, semicarbazides, thiosemicarbazide and aminoguanidines by condensation with 1,3-dicarbonyl compounds is possible. As fused pyrazoles are reported to be well known pharmacophores, this has motivated to synthesize some of the

International Journal of Health Sciences ISSN 2550-6978 E-ISSN 2550-696X © 2022.
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Manuscript submitted: 09 Nov 2021, Manuscript revised: 18 Feb 2022, Accepted for publication: 25 March 2022

pyrazolopyrazole derivatives by using hydrazine hydrate, thiosemicarbazide and semicarbazide. A series of 3-(aryl)-4-methyl-3a,6-dihydropyrazolo[3,4-c]pyrazole-2(3H)-carboxamides (IVa3-e3), 3-(aryl)-4-methyl-3a,6-dihydropyrazolo[3,4-c]pyrazole-2(3H)-carbothioamide (IVa2-e2) and 4-(aryl)-3-methyl-1,3a,4,5-tetrahydropyrazolo[3,4-c]pyrazoles (IVa1-e1) were synthesized by conventional method where fused pyrazopyrazoles were prepared. All the compounds were synthesized with good yield (56-81 %) and characterized by IR, 1H NMR spectral data and C, H, N elemental analysis. All the synthesized compounds exhibited analgesic and anti-inflammatory activities.

***Keywords*---**analgesic, anti-inflammatory, knorr pyrazole synthesis, pyrazole, pyrazolo[3,4-c]pyrazole.

Introduction

Pyrazole refers to the significant [1] class of simple aromatic ring organic compounds of the heterocyclic series characterized by a 5-membered ring structure composed of three carbon atoms and two nitrogen atoms in adjacent positions [2- 4]. Being so composed and having pharmacological effects on humans, they are classified as alkaloids, although they are rare in nature. In 1959, the first natural pyrazole, 1-pyrazolyl- alanine, was isolated from seeds of watermelons. Nitrated-pyrazole-based energetic compounds have attracted wide publicity in the field of energetic materials [5]. The term pyrazole was given by Ludwig Knorr in 1883. Researchers synthesized pyrazole scaffold and studied their applications in Alzheimer's Disease and Parkinson's Disease Treatment [6]. Formation of pyrazole derivatives from hydrazines, hydrazides, semicarbazides and aminoguanidines by condensation with 1,3-dicarbonyl compounds is possible. Pyrazole derivatives have a long history of application in agrochemicals and pharmaceutical industry as herbicides and active pharmaceuticals. Thienopyrazole Moieties are also proved effective [7]. Pyrazoles have been the recent target of numerous methodologies, mostly due to their prevalence as scaffolds in drug discovery programs as antimicrobials [8- 9] as HMG-CoA reductase inhibitors, as inhibitors of HIV-1 reverse transcriptase and synthesis in particular of bioactive compounds and reactions in different media. The pyrazole ring is present as the core in a variety of leading drugs such a Celebrex, Viagra or Rimonabant. Pyrazoles are found to possess anti-inflammatory [10- 11] and analgesic [10, 12] activitives. Many attempts were made by researchers to find out able potent pyrazolo-pyrazole derivatives [13-16] and applications [17] of pyrazoles and related compounds to enhance the biological activity.

Materials and Methods

Well dried apparatus was used to conduct the reactions requiring anhydrous conditions. Laboratory reagent grade solvents and reagents used and purified by distillation and crystallization wherever necessary. Open capillary method was used for determining melting points of newly synthesized compounds. The final products were purified by recrystalization and purity was checked by micro TLC.

The IR spectra of the compounds were recorded on JASCO FT/IR-5300 spectrometer using KBr pressed pellet. ^{1}H NMR spectra were recorded in a BRUKER DPX-200MHz spectrometer using TMS as internal standard. Perkin Elmer 2400 elemental analyzer was used for analysis of C, H and N which were found within ± 0.4 % of the theoretical values.

Synthetic scheme

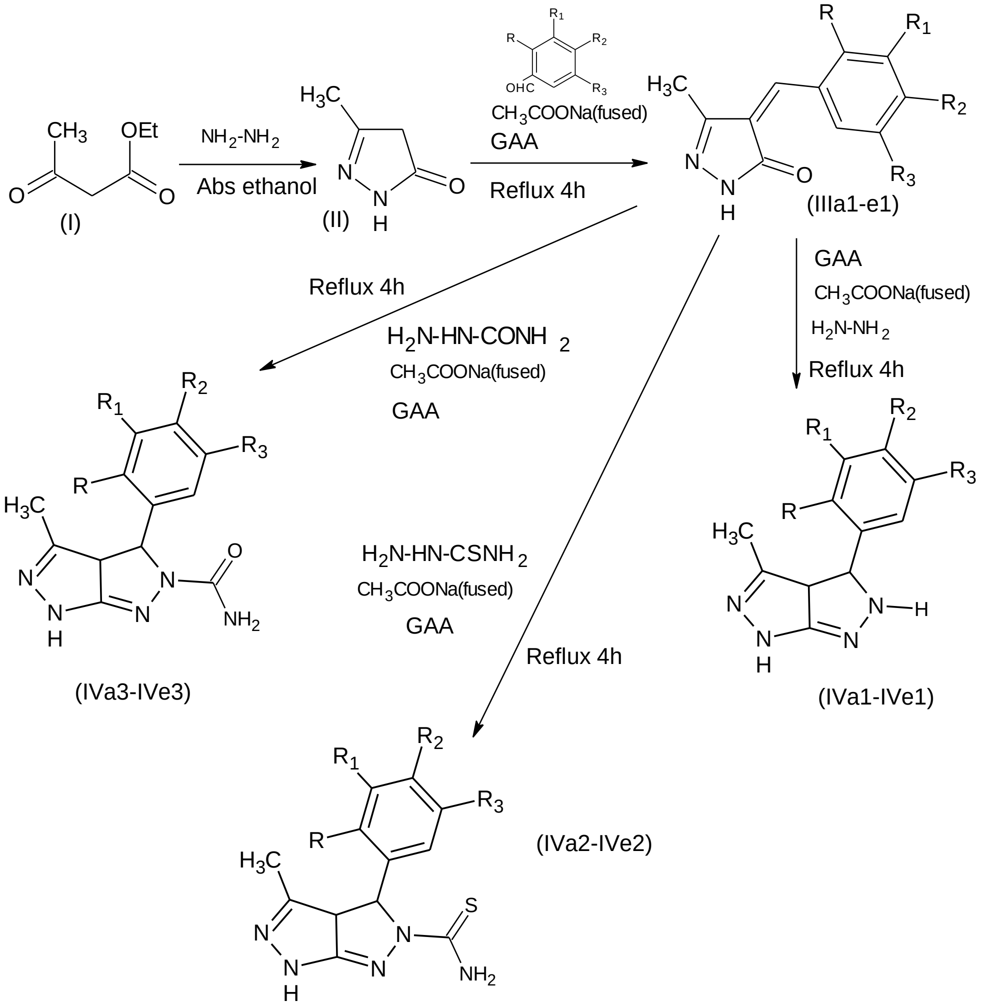

Figure1. Scheme of synthesis

As shown in figure1, a series of 3-(aryl)-4-methyl-3a,6-dihydropyrazolo[3,4-c]pyrazole-2(3H)-carboxamides (IVa3-e3) was prepared by the reaction between (4E)-4-arylidene-5-methyl-2,4–dihydro-3H-pyrazol-3-ones (IIIa1-e1) and semicarbazide refluxed in acetic acid in presence of anhydrous sodium acetate. A series of 3-(aryl)-4-methyl-3a,6-dihydropyrazolo[3,4-c]pyrazole-2(3H)-carbothioamide (IVa2-e2) was prepared by the reaction between compounds (IIIa1-e1) and thiosemicarbazide refluxed in ethanol in presence of anhydrous sodium acetate. A series of 4-(aryl)-3-methyl-1,3a,4,5-tetrahydropyrazolo[3,4-

c]pyrazoles (IVa1-e1) was prepared by the reaction between compounds (IIIa1-e1) and hydrazine hydrate, refluxed in ethanol in presence of anhydrous sodium acetate

Conventional method for the synthesis of 5-methyl-2,4 dihydro-3*H*-pyrazol-3-one (II)

Ethylacetoacetate (1.3g, 0.01mol) was placed in a conical flask and stirred magnetically during the slow dropwise addition of solution of hydrazine hydrate (98%,0.5 ml, 0.01 mol) in absolute ethanol (1ml) and temperature of about 60^0 C was maintained, a crystalline deposit was separated. After stirring for 1 h at room temp, the reaction mixture was cooled in an ice bath to complete recrystalisation, filtered, washed with ice-cold ethanol, dried, m.p.222^0 C. Yield 0.88g,90%. [18]

General procedure for the synthesis of (4E)-4-arylidene-5-methyl-2,4–dihydro-3*H*-pyrazol-3-ones (IIIa1-e1)

A mixture of 5-methyl-2,4 dihydro-3*H*-pyrazol-3-one (II) (0.98g,0.01mol), appropriate aldehyde (0.01 mol), anhydrous sodium acetate (0.82g,0.01mol) and glacial acetic acid (40ml), was heated under reflux on heating mantle for 4 hours, cooled to room temperature and poured in an ice cold water, filtered, washed with water and recrystalised from methanol/ glacial acetic acid. The yield and m. p. were reported. [19- 20]

General procedure for the Synthesis of compounds 4-(aryl)-3-methyl-1,3a,4,5-tetrahydropyrazolo[3,4-c]pyrazoles (IVa1-e1)

To a mixture of compounds (IIIa1-e1) (0.01 mol) and hydrazine hydrate (0.01 mol) in 50 ml of ethanol, anhydrous sodium acetate (0.01 mol) was added and refluxed for 4 hours. The product was poured in a mixture of crushed ice and water, filtered, dried and recrystallized from ethanol/ glacial acetic acid [20- 21].

General procedure for the Synthesis of compounds 3-(aryl)-4-methyl-3a,6-dihydropyrazolo[3,4-c]pyrazole-2(3H)-carbothioamide (IVa2-e2)

To a mixture of compounds (IIIa1-e1) (0.01 mol) and thiosemicarbazide (0.01 mol) in 40 ml of ethanol, anhydrous sodium acetate (0.01 mol) was added and refluxed for 6 hours. Reaction mixture was cooled and poured in a mixture of crushed ice and water, filtered, dried and recrystallized from ethanol/ glacial acetic acid [20-21].

General procedure for the Synthesis of 3-(aryl)-4-methyl-3a,6-dihydropyrazolo[3,4-c]pyrazole-2(3H)-carboxamides (IVa3-e3)

A mixture of compounds (IIIa2-b2) (0.01 mol) and semicarbazide (0.01 mol) was refluxed in glacial acetic acid (40 ml) in presence of anhydrous sodium acetate (0.01 mol) for 6 hours. Reaction mixture was cooled to room temperature and poured in a mixture of crushed ice and water, filtered, dried and recrystallized from ethanol/ glacial acetic acid [20- 21].

Biological activity

Acute toxicity studies: - The LD50 values of synthesized compounds have been determined by the Karber's method [22- 23]. Analgesic activity: - The synthesized compounds were assessed for the analgesic activity using Wistar Albino mice of either sex. Analgesic activity was measured by acetic acid induced writhings method [24- 25]. Control group received vehicle (1 mL, 0.25 % CMC solution). Standard drug used was aspirin (100 mg/kg). Six groups of animals were pretreated with the synthesized compounds and two groups were pretreated with standard and vehicle, respectively. Under similar conditions, after 0.5 h they were injected with 1 % (v/v) acetic acid (1 mL/100 g body weight, i.p.) and number of abdominal contractions, trunk twist response and extension of hind limbs as well as number of animals showing such response during 5 min were recorded. Mean writhings scores in all groups were calculated. Anti-inflammatory activity: - Anti-inflammatory activity of synthesized compounds was studied by carrageenan-induced rat paw oedema method [24- 25]. The newly synthesized compounds were evaluated for their anti-inflammatory activity in Male Albino Wistar rats (150-200 gm). Carrageenan (Sigma, St. Louis, USA) was used in the study and Indomethacin (Recon Ltd, Bangalore) was used as the standard drug.

Characterization of 3-methyl-4-phenyl-1,3a,4,5-tetrahydropyrazolo[3,4-*c*]pyrazole (IVa1)

The compound IVa1 with melting point 209-211 ^{0} C was analyzed for $C_{11}H_{12}N_4$. The IR spectrum of the compound by KBr method is given in figure 5.29. It exhibits intense bands at 3352 cm^{-1}(aromatic N-H str), 3102 cm^{-1} (aromatic C-H str), 2908 cm^{-1} (C-H str in CH_3), 1585 cm^{-1} and 1617cm^{-1} (C=C and C=N), 1328 cm^{-1} (C-N str), 1049 cm^{-1} , 753 cm^{-1} (monosubstituted benzene ring). The ^{1}H NMR spectrum in $CDCl_3$ is given in figure 5.30. It shows peaks at δ: 2.148 (d, 1H, C3a -H), 3.91 (d, 1H, C4-H), 7.27- 7.40 (m, 5H, Ar-H), 7.022 (s, 1H, pyrazoline N-H) and 1.949 (s, 3H,-CH_3). Elemental analysis for composition of C, H and N is given as calculated: C(65.98 %) H(6.04 %) N(27.98 %) found: C(65.95 %) H(6.01 %) N(27.96 %). The data confirms the structure of the compound.

Characterization of 4-(2-chlorophenyl)-3-methyl-1,3a,4,5 tetrahydropyrazolo [3,4*c*] pyrazole (IVb1)

The compound IVb1 with melting point 198-201^0 C was analyzed for $C_{11}H_{11}ClN_4$. The IR spectrum of the compound by KBr method is given in figure 5.31. It exhibits intense bands at 3274 cm^{-1}(aromatic N-H str), 3102 cm^{-1} (aromatic C-H str), 2992 cm^{-1} (C-H str in CH_3), 1536cm^{-1} and 1656 cm^{-1} (C=C and C=N), 1344 cm^{-1} (C-N str), 1000 cm^{-1}, 750 cm^{-1} (1,2-disubstituted benzene ring). The ^{1}H NMR spectrum in $CDCl_3$ is given in figure 5.32. It shows peaks at δ: 2.412 (d, 1H, C3a -H), 3.91 (d, 1H, C4-H), 7.45- 7.72 (m, 4H, Ar-H), 7.074 (s, 1H, pyrazoline N-H) and 1.940 (s, 3H,-CH_3). Elemental analysis for composition of C, H and N is given as calculated: C(56.30 %) H(4.72%) N(23.87%) found: C(56.27%) H(4.73 %) N(23.85%). The data confirms the structure of the compound.

Characterization of 2-(4-methyl-2,3,3a,6-tetrahydropyrazolo[3,4-*c*]pyrazol-3-yl)phenol (IVc1)

The compound IVc1 with melting point 212-215^0 C was analyzed for $C_{11}H_{12}N_4O$. The IR spectrum of the compound by KBr method is given in figure 5.33. It exhibits intense bands at 3412 cm^{-1} (O-H str), 3286 cm^{-1}(aromatic N-H str), 3034 cm^{-1} (aromatic C-H str), 2921cm^{-1} (C-H str in CH_3), 1519 cm^{-1} and 1620 cm^{-1} (C=C and C=N), 1286 cm^{-1} (C-N str),1037 cm^{-1}, 788 cm^{-1} (1,2-disubstituted benzene ring). The ^{1}H NMR spectrum in $CDCl_3$ is given in figure 5.34. It shows peaks at δ: 9.68 (s, 1H, OH), 2.54 (d, 1H, C3a -H), 4.97 (d, 1H, C4-H), 7.22- 7.47 (m, 4H, Ar-H), 6.832 (s, 1H, pyrazoline N-H) and 2.035 (s, 3H,-CH_3). Elemental analysis for composition of C, H and N is given as calculated: C(61.10%) H(5.59%) N(25.91%) found: C(61.07%) H(5.61%) N(25.94%). The data confirms the structure of the compound.

Characterization of 4-(2,4-dichlorophenyl)-3-methyl-1,3a,4,5-tetrahydropyrazolo[3,4-*c*]pyrazole (IVd1)

The compound IVd1 with melting point 226-228^0 C was analyzed for $C_{11}H_{10}Cl_2N_4$. The IR spectrum of the compound by KBr method is given in figure 5.35. It exhibits intense bands at 3282 cm^{-1}(aromatic N-H str), 3073 cm^{-1} (aromatic C-H str), 2921cm^{-1} (C-H str in CH_3), 1581 cm^{-1} and 1615 cm^{-1} (C=C and C=N),1328 cm^{-1} (C-N str), 1123 cm^{-1}, 817 cm^{-1} (1,2,4-trisubstituted benzene ring). The ^{1}H NMR spectrum in $CDCl_3$ is given in figure 5.36. It shows peaks at δ: 2.432 (d, 1H, C3a - H), 3.991 (d, 1H, C4-H), 7.04- 7.75 (m, 3H, Ar-H), 6.9 (s, 1H, pyrazoline N-H) and 2.090 (s, 3H,-CH_3). Elemental analysis for composition of C, H and N is given as calculated: C(49.09%) H(3.75%) N(20.82%) found: C(49.04%) H(3.72%) N(20.81%). The data confirms the structure of the compound.

Characterization of *N,N*-dimethyl-4-(4-methyl-2,3,3a,6-tetrahydropyrazolo[3,4-*c*] pyrazol-3-yl) aniline (IVe1)

The compound IVe1 with melting point 241-243^0 C was analyzed for $C_{13}H_{17}N_5$. The IR spectrum of the compound by KBr method is given in figure 5.37 It exhibits intense bands at 3365 cm^{-1}(aromatic N-H str), 3167 cm^{-1} (aromatic C-H str), 2924 cm^{-1} (C-H str in CH_3), 1507 cm^{-1} and 1611 cm^{-1} (C=C and C=N), 1251 cm^{-1} (C-N str),1089 cm^{-1}, 828 cm^{-1} (1,4-disubstituted benzene ring). The ^{1}H NMR spectrum in $CDCl_3$ is given in figure 5.38. It shows peaks at δ: 2.409 (d, 1H, C3a - H), 3.933 (d, 1H, C4-H), 6.71- 7.10 (m, 4H, Ar-H), 7.02 (s, 1H, pyrazoline N-H), 3.028 (s, 6H,-N $(CH_3)_2$) and 2.091 (s, 3H,-CH_3). Elemental analysis for composition of C, H and N is given as calculated: C(64.17 %) H(7.04%) N(28.78%) found: C(64.16%) H (7.00%) N(28.81%). The data confirms the structure of the compound.

Characterization of 4-methyl-3-phenyl-3a,6-dihydropyrazolo[3,4-*c*]pyrazole-2(3*H*) carbothioamide (IVa2)

The compound IVa2 with melting point 201-204^0 C was analyzed for $C_{12}H_{13}N_5S$. The IR spectrum of the compound by KBr method is given in figure 5.39. It exhibits intense bands at 3289 cm^{-1}(aromatic N-H str), 3090 cm^{-1} (aromatic C-H str), 2863 cm^{-1} (C-H str in CH_3), 1510 cm^{-1} and 1623 cm^{-1} (C=C and C=N), 1326 cm^{-1} (C-N str), 1207 cm^{-1} (C=S str), 1051 cm^{-1} , 825 cm^{-1} (monosubstituted benzene ring). The ^{1}H NMR spectrum in $CDCl_3$ is given in figure 5.40. It shows peaks at δ: 3.91(d, 1H, C3 -H), 2.10 (d, 1H, C4-H), 7.27- 7.40 (m, 5H, Ar-H), 7.02 (s, 1H, pyrazoline N-H), 9.503 (bs, 2H,-$CSNH_2$) and 1.946 (s, 3H,-CH_3). Elemental analysis for composition of C, H and N is given as calculated: C(55.58%) H(5.05%) N(27.01%) found: (55.61%) H(5.07%) N(27.05%). The data confirms the structure of the compound.

Characterization of 3-(2-chlorophenyl)-4-methyl-3a,6-dihydropyrazolo[3,4-*c*]pyrazole-2(3*H*)-carbothioamide (IVb2)

The compound IVb2 with melting point 198-201^0 C was analyzed for $C_{12}H_{12}ClN_5S$. The IR spectrum of the compound by KBr method is given in figure 5.41. It exhibits intense bands at 3330 cm^{-1}(aromatic N-H str), 3147 cm^{-1} (aromatic C-H str), 2912 cm^{-1} (C-H str in CH_3), 1520 cm^{-1} and 1622 cm^{-1} (C=C and C=N), 1328 cm^{-1} (C-N str), 1133 cm^{-1} (C=S str), 1051 cm^{-1}, 825 cm^{-1} (1,2-disubstituted benzene ring). The ^{1}H NMR spectrum in $CDCl_3$ is given in figure 5.42. It shows peaks at δ: 3.91(d, 1H, C3 -H), 2.31 (d, 1H, C4-H), 7.21- 7.72 (m, 5H, Ar-H), 6.92 (s, 1H, pyrazoline N-H), 9.502 (bs, 2H,-$CSNH_2$) and 2.029 (s, 3H,-CH_3). Elemental analysis for composition of C, H and N is given as calculated: C(49.06%) H(4.12%) N(23.84%) found: C(49.08%) H(4.15%) N(23.79%). The data confirms the structure of the compound.

Characterization of 3-(2-hydroxyphenyl)-4-methyl-3a,6-dihydropyrazolo[3,4-*c*]pyrazole-2(3*H*)-carbothioamide (IVc2)

The compound IVc2 with melting point 212-215^0 C was analyzed for $C_{12}H_{13}N_5OS$. The IR spectrum of the compound by KBr method is given in figure 5.43. It exhibits intense bands at 3499 cm^{-1} (O-H str), 3216 cm^{-1}(aromatic N-H str), 3114 cm^{-1} (aromatic C-H str), 2922 cm^{-1} (C-H str in CH_3), 1509 cm^{-1} and 1614 cm^{-1} (C=C and C=N), 1268 cm^{-1} (C-N str), 1268 cm^{-1} (C=S str), 1037 cm^{-1}, 748 cm^{-1} (1,2-disubstituted benzene ring). The ^{1}H NMR spectrum in $CDCl_3$ is given in figure 5.44. It shows peaks at δ: 9.70 (s, 1H, OH), 4.04 (d, 1H, C3 -H), 2.31 (d, 1H, C4-H), 6.87- 7.44 (m, 4H, Ar-H), 7.09 (s, 1H, pyrazoline N-H), 6.49 (s, 2H,-$CSNH_2$) and 2.029 (s, 3H,-CH_3). Elemental analysis for composition of C, H and N is given as calculated: C(52.35%) H (4.76%) N(25.44%) found: C(52.34%) H(4.76%) N(25.43%). The data confirms the structure of the compound.

Characterization of 3-(2,4-dichlorophenyl)-4-methyl-3a,6-dihydropyrazolo[3,4-*c*]pyrazole-2(3*H*)-carbothioamide (IVd2)

The compound IVd2 with melting point 226-228^0 C was analyzed for $C_{12}H_{11}Cl_2N_5S$. The IR spectrum of the compound by KBr method is given in figure 5.45. It exhibits intense bands at 3328 cm^{-1}(aromatic N-H str), 3082 cm^{-1} (aromatic C-H str), 2913 cm^{-1} (C-H str in CH_3), 1482 cm^{-1} and 1600 cm^{-1} (C=C and C=N), 1323 cm^{-1} (C-N str), 1272 cm^{-1} (C=S str),1108 cm^{-1}, 781 cm^{-1} (1,2,4-trisubstituted benzene ring). The ^{1}H NMR spectrum in $CDCl_3$ is given in figure 5.46. It shows peaks at δ: 3.91(d, 1H, C3 -H), 2.40 (d, 1H, C4-H), 7.04- 7.75 (m, 3H, Ar-H), 76.90 (s, 1H, pyrazoline N-H), 9.720 (bs, 2H,-$CSNH_2$) and 2.028 (s, 3H,-CH_3). Elemental analysis for composition of C, H and N is given as calculated: C(43.91%) H(3.38%) N(21.34%) found: C(43.90%) H(3.37%) N(21.35 %). The data confirms the structure of the compound.

Characterization of 3-[4-(dimethylamino)phenyl]-4-methyl-3a,6-dihydropyrazolo[3,4-*c*]pyrazole-2(3*H*)-carbothioamide (IVe2)

The compound IVe2 with melting point 241-243^0 C was analyzed for $C_{14}H_{18}N_6S$. The IR spectrum of the compound by KBr method is given in figure 5.47 It exhibits intense bands at 3305 cm^{-1}(aromatic N-H str), 3110 cm^{-1} (aromatic C-H str), 3007 cm^{-1} (C-H str in CH_3), 1507 cm^{-1} and 1611 cm^{-1} (C=C and C=N), 1326 cm^{-1} (C-N str), 1251 cm^{-1} (C=S str), 1015 cm^{-1}, 828 cm^{-1} (1,4-disubstituted benzene ring). The ^{1}H NMR spectrum in $CDCl_3$ is given in figure 5.48. It shows peaks at δ: 3.91(d, 1H, C3 -H), 2.41 (d, 1H, C4-H), 6.71- 7.10 (m, 4H, Ar-H), 7.02 (s, 1H, pyrazoline N-H), 9.72 (bs, 2H,-$CSNH_2$), 3.062 (s, 6H,-N $(CH_3)_2$) and 2.028 (s, 3H,-CH_3). Elemental analysis for composition of C, H and N is given as calculated: C(55.61%) H(6.00%) N(27.79%) found: C(55.60%) H(6.10%) N(27.80%). The data confirms the structure of the compound.

Characterization of 4-methyl-3-phenyl-3a,6-dihydropyrazolo[3,4-*c*]pyrazole-2(3*H*)-carboxamide (IVa3)

The compound IVa3 with melting point 210-213^0 C was analyzed for $C_{12}H_{13}N_5O$. The IR spectrum of the compound by KBr method is given in figure 5.49. It exhibits intense bands at 3317 cm^{-1}(aromatic N-H str), 3122 cm^{-1}(aromatic C-H str), 2993 cm^{-1} (C-H str in CH_3), 1707 cm^{-1} (C=O), 1557 cm^{-1} and 1610 cm^{-1} (C=C and C=N), 1320 cm^{-1} (C-N str), 1092 cm^{-1} , 820 cm^{-1} (monosubstituted benzene ring). The ^{1}H NMR spectrum in $CDCl_3$ is given in figure 5.50. It shows peaks at δ: 2.10 (d, 1H, C3a -H), 3.916 (d, 1H, C3-H), 7.27- 7.40 (m, 5H, Ar-H), 7.02 (s, 1H, pyrazoline N-H), 6.21 (bs, 2H,-$CONH_2$) and 1.948 (s, 3H,-CH_3). Elemental analysis for composition of C, H and N is given as calculated: C(59.25%) H(5.39%) N(28.79%) found: C(59.23 %) H(5.40%) N(28.79%). The data confirms the structure of the compound.

Characterization of 3-(2-chlorophenyl)-4-methyl-3a,6-dihydropyrazolo[3,4-*c*]pyrazole-2(3*H*)-carboxamide (IVb3)

The compound IVb3 with melting point 198-201^0 C was analyzed for $C_{12}H_{12}ClN_5O$. The IR spectrum of the compound by KBr method is given in figure 5.51. It exhibits intense bands at 3428 cm^{-1}(aromatic N-H str), 3118 cm^{-1} (aromatic C-H str), 2992 cm^{-1} (C-H str in CH_3), 1706 cm^{-1} (C=O), 1482 cm^{-1} and 1682 cm^{-1} (C=C and C=N), 1278 cm^{-1} (C-N str), 1108 cm^{-1}, 733 cm^{-1} (1,2-disubstituted benzene ring). The ^{1}H NMR spectrum in $CDCl_3$ is given in figure 5.52. It shows peaks at δ: 2.49 (d, 1H, C3a -H), 4.91 (d, 1H, C3-H), 7.21- 7.72 (m, 4H, Ar-H), 7.07 (s, 1H, pyrazoline N-H), 6.21 (bs, 2H,-$CONH_2$) and 1.940 (s, 3H,-CH_3). Elemental analysis for composition of C, H and N is given as calculated: C(51.90%) H(4.36%) N(25.22%) found: C(51.92%) H (4.37%) N(25.23%). The data confirms the structure of the compound.

Characterization of 3-(2-hydroxyphenyl)-4-methyl-3a,6-dihydropyrazolo[3,4-*c*] pyrazole-2(3*H*)-carboxamide (IVc3)

The compound IVc3 with melting point 212-215 ^{0} C was analyzed for $C_{12}H_{13}N_5O_2$. The IR spectrum of the compound by KBr method is given in figure 5.53. It exhibits intense bands at 3511 cm^{-1} (O-H str), 3246 cm^{-1}(aromatic N-H str), 3114 cm^{-1}(aromatic C-H str), 2930 cm^{-1} (C-H str in CH_3), 1701 cm^{-1} (C=O), 1539 cm^{-1} and 1638 cm^{-1} (C=C and C=N), 1261 cm^{-1} (C-N str), 1107 cm^{-1}, 748cm^{-1} (1,2-disubstituted benzene ring). The ^{1}H NMR spectrum in $CDCl_3$ is given in figure 5.54. It shows peaks at δ: 9.68 (s, 1H, OH), 2.53 (d, 1H, C3a -H), 4.93 (d, 1H, C3-H), 7.22- 7.47 (m, 4H, Ar-H), 6.83 (s, 1H, pyrazoline N-H), 6.42 (bs, 2H,-$CONH_2$) and 2.03 (s, 3H,-CH_3). Elemental analysis for composition of C, H and N is given as calculated: C(55.59%) H(5.05%) N (27.01%) found: C(55.58 %) H(5.03 %) N(27.03%). The data confirms the structure of the compound.

Characterization of 3-(2,4-dichlorophenyl)-4-methyl-3a, 6-dihydropyrazolo[3,4-*c*] pyrazole-2(3*H*)-carboxamide (IVd3)

The compound IVd3 with melting point 226-228^{0} C was analyzed for $C_{12}H_{11}Cl_2N_5O$. The IR spectrum of the compound by KBr method is given in figure 5.55. It exhibits intense bands at 3318 cm^{-1}(aromatic N-H str), 3127 cm^{-1} (aromatic C-H str), 2987 cm^{-1} (C-H str in CH_3), 1707 cm^{-1} (C=O), 1512 cm^{-1} and 1602 cm^{-1} (C=C and C=N), 1382 cm^{-1} (C-N str), 1098 cm^{-1}, 751 cm^{-1} (1,2,4-trisubstituted benzene ring). The ^{1}H NMR spectrum in $CDCl_3$ is given in figure 5.56. It shows peaks at δ: 2.50 (d, 1H, C3a -H), 4.90 (d, 1H, C3-H), 7.04- 7.75 (m, 3H, Ar-H), 6.90 (s, 1H, pyrazoline N-H), 6.20 (bs, 2H,-$CONH_2$) and 2.29 (s, 3H,-CH_3). Elemental analysis for composition of C, H and N is given as calculated: C(46.17%) H(3.55%) N(22.44%) found: C(46.16%) H(3.53%) N(22.43%). The data confirms the structure of the compound.

Characterization of 3-[4-(dimethylamino)phenyl]-4-methyl-3a,6-dihydropyrazolo[3,4-*c*]pyrazole-2(3*H*)-carboxamide (IVe3)

The compound IVe3 with melting point 241-243 ^{0} C was analyzed for $C_{14}H_{18}N_6O$. The IR spectrum of the compound by KBr method is given in figure 5.57. It exhibits intense bands at 3268 cm^{-1}(aromatic N-H str), 3060 cm^{-1} (aromatic C-H str), 2891cm^{-1} (C-H str in CH_3), 1700 cm^{-1} (C=O), 1505 cm^{-1} and 1636 cm^{-1} (C=C and C=N), 1300 cm^{-1} (C-N str), 755 cm^{-1} (1,4-disubstituted benzene ring). The ^{1}H NMR spectrum in $CDCl_3$ is given in figure 5.58. It shows peaks at δ: 2.41 (d, 1H, C3a -H), 3.90 (d, 1H, C3-H), 6.71- 7.10 (m, 4H, Ar-H), 7.02 (s, 1H, pyrazoline N-H), 6.15 (bs, 2H,-$CONH_2$), 3.028 (s, 6H,-N $(CH_3)_2$) and 2.091 (s, 3H,-CH_3). Elemental analysis for composition of C, H and N is given as calculated: C(58.73%) H(6.34%) N(29.35%) found: C(58.71%) H(6.34%) N(29.36%). The data confirms the structure of the compound.

Results and Discussion

All the synthesized compounds were evaluated for their Physical constants and biological activity. Physical constants found for the synthesized compounds are sown in table1, ED50 values are shown in table 2. Analgesic activity is shown in table 3 and anti-inflammatory activity is shown in table 4. The structures of synthesized compounds were in agreement with elemental analysis and IR and NMR spectral data. All the synthesized compounds exhibited analgesic and anti-inflammatory activities. Compound IVd1, IVd2, IVd3, IVb1, IVb2 and IVb3 showed significant analgesic and anti-inflammatory activity.

Table 1
Physical constants of compounds (IVa1-e1, IVa2-e2 and IVa3-e3)

Compound	Recrystalization Solvent	% yield	m. p. (^{0}c)	Molecular formula	Molecular weight	*Rf
IVa1	Ethanol	59	209-211	$C_{11}H_{12}N_4$	200.239	0.66
IVb1	Glacial acetic acid + Ethanol(1:1)	68	198-201	$C_{11}H_{11}ClN_4$	234.684	0.44
IVc1	Glacial acetic acid	63	212-215	$C_{11}H_{12}N_4O$	216.239	0.64
IVd1	Glacial acetic acid	81	226-228	$C_{11}H_{10}Cl_2N_4$	269.129	0.59
IVe1	Ethanol	78	241-243	$C_{13}H_{17}N_5$	243.307	0.43
IVa2	Ethanol	56	201-204	$C_{12}H_{13}N_5S$	259.330	0.66
IVb2	Glacial acetic acid	73	198-201	$C_{12}H_{12}ClN_5S$	293.775	0.42
IVc2	Ethanol	61	212-215	$C_{12}H_{13}N_5OS$	275.329	0.60
IVd2	Glacial acetic acid	76	226-228	$C_{12}H_{11}Cl_2N_5S$	328.220	0.62
IVe2	Ethanol	73	241-243	$C_{14}H_{18}N_6S$	302.397	0.46
IVa3	Ethanol	64	210-213	$C_{12}H_{13}N_5O$	243.264	0.65
IVb3	Glacial acetic acid	68	198-201	$C_{12}H_{12}ClN_5O$	277.709	0.43
IVc3	Glacial acetic acid	71	212-215	$C_{12}H_{13}N_5O_2$	259.263	0.64
IVd3	Glacial acetic acid	58	226-228	$C_{12}H_{11}Cl_2N_5O$	312.154	0.58
IVe3	Ethanol	70	241-243	$C_{14}H_{18}N_6O$	286.332	0.39

R_f value was determined in benzene: acetone (1:1)

Table 2:
ED50 values of synthesized compounds (IVa1-e1, IVa2-e2 and IVa3-e3)

Sr. No.	Compound	ED_{50} (mg/kg)
1.	IVa1	150
2.	IVb1	135
3.	IVc1	148
4.	IVd1	132
5.	IVe1	118
6.	IVa2	148
7.	IVb2	131
8.	IVc2	146
9.	IVd2	128
10.	IVe2	113
11.	IVa3	145
12.	IVb3	126
13.	IVc3	145
14.	IVd3	130
15.	IVe3	119

Table 3
Analgesic activity of compounds (IVa1-e1, IVa2-e2 and IVa3-e3)

Sr. No.	Design of treatment (Groups)	Dose (mg/kg, p.o.)	Number of writhings in 5 minutes	% Inhibition
	Control(CMC, 0.25%, 1ml)	-	180.00 ±0.607	-
	Aspirin	100	41.83 ±0.477**	76.76
1.	Compound IVa1	150	79.62±0.595**	55.76
2.	Compound IVb1	135	66.00±0.321**	63.33
3.	Compound IVc1	148	73.33±0.693**	59.26
4.	Compound IVd1	132	54.33±0.705**	69.81
5.	Compound IVe1	118	71.00±0.378**	60.55
6.	Compound IVa2	148	80.33±0.608**	55.37
7.	Compound IVb2	131	64.33±0.548**	64.26
8.	Compound IVc2	146	75.33±0.611**	58.15
9.	Compound IVd2	128	57.33±0.433**	68.15
10.	Compound IVe2	113	70.33±0.493**	60.92
11.	Compound IVa3	145	81.62±0.595**	54.65
12.	Compound IVb3	126	66.33±0.272**	63.15
13.	Compound IVc3	145	74.00±0.513**	58.88
14.	Compound IVd3	130	54.66±0.504**	69.63
15.	Compound IVe3	119	71.66±0.503**	60.18

Values are expressed as mean ± SEM, N=6
When compared with control, *= P< 0.05, **= P< 0.01, ***= P< 0.001
(One way ANOVA followed by Dunnett's multiple comparison test)

Table 4
Antiinflammatory activity of compounds IVa1-e1, IVa2-e2 and IVa3-e3)

Sr. No.	Design of treatment (Groups)	Dose (mg / kg, p.o.)	Change in paw edema at the end of 3h (mm)	% Inhibition
	Control (CMC, 0.25%,1ml)	-	0.85±0.0067	-
	Indomethacin	10	0.22±0.0060**	74.11
1.	Compound IVa1	153	0.52±0.0043**	38.82
2.	Compound IVb1	131	0.40±0.0041**	52.94
3.	Compound IVc1	144	0.49±0.0069**	42.35
4.	Compound IVd1	129	0.31±0.0035**	63.52
5.	Compound IVe1	118	0.44±0.0031**	48.23
6.	Compound IVa2	146	0.56±0.0026**	34.11
7.	Compound IVb2	126	0.41±0.0052**	51.76
8.	Compound IVc2	149	0.47±0.0052**	44.70
9.	Compound IVd2	121	0.33±0.0017**	61.17
10.	Compound IVe2	116	0.42±0.0046**	50.58
11.	Compound IVa3	143	0.51±0.0034**	40.00
12.	Compound IVb3	121	0.39±0.0060**	54.11
13.	Compound IVc3	147	0.53±0.0020**	37.64

14.	Compound IVd3	133	0.36±0.0017**	57.64
15.	Compound IVe3	121	0.39±0.0020**	54.11

Values are expressed as mean ± SEM, N=6
When compared with control,*= P< 0.05, **= P< 0.01, ***= P< 0.001
(One-Way ANOVA followed by Dennett's multiple comparison test)

Conclusion

Synthesis of new chemical entity incorporating the same active pharmacophore to another namely pyrazole in a single molecular framework was successfully carried out. Conventional synthesis of new series of pyrazolo-pyrazoles, characterization of synthesized compounds by spectral methods viz. Infra Red, Nuclear Magnetic Resonance spectroscopy, elemental analysis and screening for the analgesic and anti-inflammatory activity were the major highlights of the research work. pyrazolo-pyrazoles, pyrazolo-pyrazole carbothiomides and pyrazolo-pyrazole carboxamides were synthesized by conventional method. The yield was quantitative. The synthesized compounds were found to give analgesic and anti-inflammatory activities and are believed to exert various other activities such as antimicrobial, anticonvulsant, CNS depressant, ulcerogenic and anthelmintic.

Acknowledgment

Authors are thankful to Amrutvahini College of pharmacy, Sangamner, Maharashtra and PRIST University, Thanjavur, Tamilnadu, India for providing all necessary research facilities for the research work.

Conflict of interest: Nil
Financial support: n. A.

Ethics statement

The prior permission of Institutional Animal Ethics Committee [IAEC Registration No. 1153/PO/Re/S/08/CPCSEA (present), 1153/PO/ac/08/CPCSEA (previous)] was taken before conducting acute toxicity studies, analgesic and anti-inflammatory activity on animals.

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