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RESEARCH ARTICLE

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Synthesis, biological evaluation, and molecular docking studies of new pyrazol-3-one derivatives with aromatase inhibition activities

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A new series derived from 4-(2-chloroacetyl)-1,2-dihydro-1,5-dimethyl-2-phenyl-3H-pyrazol-3-one was synthesized, characterized and its pharmacological activity toward aromatase enzyme inhibition was screened and compared to the reference native ligand letrozole. The most active compound of the series was **16**, showing IC_{50} value of 0.0023 ± 0.0002 μM compared to letrozole with IC_{50} of 0.0028 ± 0.0006 μM. In addition, compounds **26** and **36** exhibit good inhibition activities close to letrozole with IC_{50} values 0.0033 ± 0.0001 and 0.0032 ± 0.0003 μM, respectively. Moreover, molecular docking studies were conducted to support the findings.

KEYWORDS

aromatase activity inhibitors, IC_{50}, letrozole, molecular docking studies, pyrazol-3-one

The majority of hormone-dependent cancers such as breast cancer are related to estrogen hormone. Estrogen was found to play a key role in the progression and metastasis of such tumors.[1,2] Tamoxifen represents one of the treatments for hormone-dependent cancers by blocking the binding of estrogen to the estrogen receptor.[3–5] However, resistance to tamoxifen therapy was developed and consequently, the search for an alternative treatment protocol has become a crucial matter.[6] Inhibition of estrogen synthesis represents another promising therapeutic approach causing the body to produce less estrogen through the inhibition of aromatase enzyme.[7–9] Aromatase is an enzyme found in the liver and is responsible for the conversion of the androgens, androstenedione, and testosterone into the estrogens, estrone, and estradiol.[10,11] Several generations of aromatase inhibitors were developed, and letrozole was found to be a highly potent competitor of tamoxifen as estrogen blocker (Figure 1).[12–16]

Diverse pharmacological activities have been associated with pyrazole-containing heterocyclic compounds with several applications in the field of medicinal chemistry and pharmaceuticals. Compounds with pyrazole subunit show potent anticancer,[17–22] antibacterial,[23] and antifungal[24] activities. Phenazone and ampyrone are two potent drugs showing a 3-pyrazolone ring in their structure that is believed to contribute to their effective pharmacological behavior (Figure 1). Phenazone drug is used as analgesic, non-steroidal anti-inflammatory drug,[25] whereas ampyrone shows antioxidant and anti-inflammatory activity.[26] Other 3-pyrazolone drugs were reported to reduce fever, treat arthritis,[27,28] be used in veterinary medicine,[29] and also to show anti-estrogenic activity,[21,22] This anti-estrogenic pharmacological activity stimulated our attention to synthesize a new series of heterocyclic systems containing the 3-pyrazolone core and examine their biological activity as aromatase enzyme inhibitors. For this purpose, we synthesized 4-(2-chloroacetyl)-1,2-dihydro-1,5-dimethyl-2-phenyl-3H-pyrazol-3-one[30] which was then converted to 1,2-dihydro-1,5-dimethyl-2-phenyl-4-thiocyanatoacetyl-3H-pyrazol-3-one (**2**), the main precursor of this series of functionalized 3-pyrazolones.

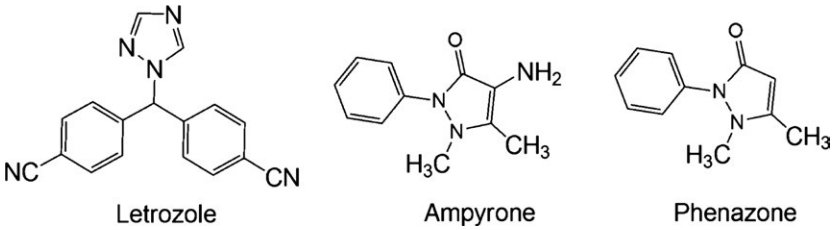


FIGURE 1 The structure of letrozole, ampyrone and phenazone.

1 | EXPERIMENTAL PROTOCOL

All melting points were measured on a Gallenkamp melting point apparatus. The infrared spectra were recorded in potassium bromide disks on a Pye Unicam SP 3300 (Pye Unicam Ltd., Cambridge, UK) and Shimadzu FT IR 8101 PC (Schimadzu, Tokyo, Japan) infrared spectrophotometers. The NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer. ^{1}H (300 MHz) and ^{13}C NMR (75 MHz) were run in deuterated chloroform ($CDCl_3$) or dimethylsulfoxide (DMSO-d6). Chemical shifts were related to that of the solvent. Mass spectra were recorded on a Shimadzu GCMS-QP 1000 EX mass spectrometer (Schimadzu) at 70 eV. Elemental analyses were carried out at the Microanalytical Center of Cairo University, Giza, Egypt.

1.1 | 1,2-Dihydro-1,5-dimethyl-2-phenyl-4-thiocyanatoacetyl-3H-pyrazol-3-one (2)

To a solution of 4-(2-chloroacetyl)-1,2-dihydro-2,3-dimethyl-1-phenylpyrazol-5-one (**1**) (26.45 g, 0.1 mol) in ethanol was added potassium thiocyanate (97 g, 0.1 mol). The reaction mixture was refluxed for 1 h then allowed to cool. The reaction mixture poured onto cold water with continuous stirring. The solid product was collected by filtration, washed with water, dried, and finally recrystallized from ethanol to afford the corresponding product in 90% yield, m.p.: 145–147 °C, IR (KBr) ν (cm^{-1}) = 2152 (SCN), 1643, 1633 (2C=O), 1589 (C=N), MS, m/z; 287 (M^+, 80%), 215 (90%), 187 (18%), 100 (10%), 77 (20%), ^{1}HNMR (DMSO-d_6) δ (ppm) = 2.49 (s, 3H), 3.36 (s, 3H), 4.5 (s, 2H), 7.36–7.59 (m, 5H). For $C_{14}H_{13}N_3SO_2$ (287.34), Calcd.: C: 58.52; H: 4.56; N: 14.62; S: 11.16%. Found: C: 58.45; H: 4.65; N: 14.62; S: 11.18%.

1.2 | 6-[4-(1,2-Dihydro-1,5-dimethyl-2-phenyl-3-oxo-3H-pyrazol-4-yl)-1,2,3,4-tetrahydro-1,3-oxathiine-2-imine (4)

A solution of compound **2** in pyridine was refluxed for 1 h then allowed to cool. The reaction mixture was diluted with water. The solid product was collected by filtration, washed by water, and dried. Recrystallization from ethanol afforded oxathiine-2-imine derivative **4** in 60% yield, m.p.: 208–210 °C, IR (KBr) ν (cm^{-1}) = 3449 (NH), 1663 (C=O), 1597 (C=N). ^{1}HNMR (DMSO-d_6) δ (ppm) = 2.49 (s, 3H), 3.36 (s, 3H), 7.1 (s, 1H, NH/D_2O-exchangeable), 7.36–7.59 (m, 5H, ArH), 8.1 (s, 1H, CH-oxathiole). MS, m/z = 288 (M^+ + 1), 287 (M^+), 215, 187, 77, 56. For $C_{14}H_{13}N_3SO_2$, (287.34) Calcd.: C: 58.52; H: 4.56; N: 14.62; S: 11.16%. Found: C: 58.45; H: 4.65; N: 14.62; S: 11.18%.

1.3 | 4-[4-(1,2-Dihydro-1,5-dimethyl-2-phenyl-3-oxo-3H-pyrazol-4-ylcarbonyl)]-5-phenylamino-1,3-dithiole-2-imine (7)

To a stirred solution of potassium hydroxide (0.56 g, 0.01 mmol) in dimethylformamide (20 mL) was added 1,2 -dihydro-1,5-dimethyl-2-phenyl-4-thiocyanatoacetyl-3H-pyrazol-3-one (**2**) (2.87 g, 0.01 mmol). Phenyl isothiocyanate (1.35 g, 0.01 mmol) was added to the produced mixture after stirring for 30 min. Stirring was continued for 6 h and then poured over crushed ice containing hydrochloric acid. The formed product was filtered off, washed with water, dried, and finally recrystallized from DMF/water to give 1,3-dithiole-2-imine derivative **7** in 70% yield, m.p.: 197–199 °C, IR (KBr) ν (cm^{-1}) = 3449, 3419 (2NH), 1643, 1634 (2C=O), MS, m/z; 442(M^+), 388, 256, 215, 177, 77, 56, ^{1}HNMR (DMSO-d_6) δ (ppm) = 2.57 (s, 3H), 3.33 (s, 3H), 7.1 (s, 1H, NH/D_2O-exchangeable), 7.3–7.49 (m, 10H, ArH), 7.8 (s, 1H, NH/D_2O-exchangeable). For $C_{21}H_{18}N_4O_2S_2$ (422.53), Calcd.: C: 59.70; H: 4.29; N: 13.26; S: 15.18%. Found: C: 59.88; H: 4.34; N: 13.28; S: 15.38%.

1.4 | 2-[4-(1,2-Dihydro-1,5-dimethyl-2-phenyl-3-oxo-3H-pyrazol-4-yl)]-1,3,4-thidiazole[3,2-a]-1,3-quinazoline-2-imine (10a)

To a cold solution of compound **2** (2.87 g, 0.01 mmol) in ethanol (50 mL) and sodium acetate trihydrate (5 g) was added the diazonium salt solution of anthranilonitrile (1.18 g, 0.01 mmol), while stirring over a period of 30 min. After the addition was complete, the reaction was stirred for further 3 h at 0–5 °C and left to stand in an ice box for 12 h then diluted with water. The solid so formed was filtered

off, washed with water, and dried. Recrystallization from DMF afforded thidiazole[3,2-a]-1,3-quinazoline-2-imine derivative **10a** in 70% yield, m.p.: 262–264 °C, IR (KBr) ν (cm^{-1}) = 3425 (NH), 1643, 1633 (2C=O), ^{1}HNMR (DMSO-d_6) δ (ppm) = 2.61 (s, 3H), 3.2 (s, 3H), 4.5 (s, 1H), 7.35–7.56 (m, 9H, ArH). For $C_{21}H_{16}N_6O_2S$, (416.46) Calcd.: C: 60.57; H: 3.87; N: 20.18; S: 7.70%. Found: C: 60.57; H: 3.99; N: 19.99; S: 7.55%.

1.5 | 2-[4-(1,2-Dihydro-1,5-dimethyl-2-phenyl-3-oxo-3H-pyrazol-4-yl]-1,3,4 -thiazolo[3,2-a]-1,3-quinazolin-2-one (10b)

1.5.1 | Method A

To a cold solution of compound **2** (2.87 g, 0.01 mmol) in ethanol (50 mL) and sodium acetate trihydrate (5 g) was added the diazonium salt solution of methyl anthranilate (1.51 g, 0.01 mmol), while stirring over a period of 30 min. After the addition was complete, the reaction mixture was stirred for further 3 h at 0–5 °C and left to stand in an ice box for 12 h then diluted with water. The solid that formed was filtered off, washed with water, and dried. Recrystallization from DMF afforded thidiazole [3,2-a]-1,3-quinazoline-2-one derivative **10b** in 70% yield, m.p.: 270–272 °C, IR (KBr) ν (cm^{-1}) = 1710, 1690, 1639 (3C=O), 1598, 1590 (2C=N); ^{1}HNMR (DMSO-d_6) δ (ppm) = 2.7 (s, 3H), 3.3 (s, 3H), 7.39–8.2 (m, 9H, ArH). For $C_{21}H_{15}N_5O_3S$ (417.45), ^{13}C NMR (DMSO-d_6) δ (ppm) = 13.8 (CH_3), 30.4 (CH_3), 114.8, 122.5, 122.7, 125.9, 126.5, 126.8, 128.0, 128.2, 128.6, 128.9, 129.7, 130.7, 135.3, 142.5 (Ar-C), 162.8, 163.5. 164.8 (3C=O) ppm. Calcd.: C: 60.42; H: 3.62; N: 16.78; S: 7.68%. Found: C: 60.44; H: 3.42; N: 16.87; S: 7.68%.

1.5.2 | Method B

To a cold solution of compound **10a** (12.48 g, 30 mmol) in ethanol (20 mL) was added hydrochloric acid (37%, 5 mL). The reaction mixture was refluxed for 4 h then left to cool, and the precipitate so formed was collected by filtration, washed with water, and dried. Recrystallization from dioxane afforded compound which is identical in all respects (m.p., mixed m.p., and spectra) with that obtained by method A above.

1.6 | 1-[4-(1,2-Dihydro-1,5-dimethyl-2-phenyl-3-oxo-3H-pyrazol-4-yl)-3-ethoxy-2-thiocyanatopropenone (11)

In a 250 mL three-necked round-bottomed flask, fitted with air condenser and thermometer were placed triethyl orthoformate (29.6 g, 200 mmol). The flask was immersed in an oil bath heated to 145–150 °C and then compound **2** (5.74 g, 200 mmol) was added portion wise over a period of 1 h and heating was continued for an additional 1 h. The reaction flask was removed from the oil bath, left to cool, and triturated with water. The solid product was filtered off, washed with water several times, and dried. Recrystallization from ethanol afforded the corresponding product thiocyanato-3-ethoxypropenone derivative **11** in 80% yield, m.p.: 286–287 °C, IR (KBr) ν (cm^{-1}) = 2152 (SCN), 1643, 1633 (2C=O), MS, m/z; 343 (M^+), 328, 287, 215, 187, 77, 56. ^{1}HNMR (DMSO-d_6) δ (ppm) = 1.36 (t, 3H). 2.49 (s, 3H), 3.3 (s, 3H), 3.9 (q, 2H), 6.9 (s, 1H), 7.35–7.59 (m, 5H, ArH). ^{13}C NMR (DMSO-d_6) δ (ppm) = 13.8, 15.2, 33.4 ($3CH_3$), 56.3 (CH_2),117.8, 122.5, 125.9, 130.7, 145.3, 152.5 (Ar-C), 125.1 (CH=O), 126.3 (C=), 136.8 (SCN). 164.8 (C=O). For $C_{17}H_{17}N_3SO_3$ (343.41), Calcd.: C: 59.46; H: 4.99; N: 12.24; S: 9.34%. Found: C: 59.51; H: 5.01; N: 12.34; S: 9.36%.

1.7 | 1-Phenyl-3-[4-(1,2-Dihydro-1,5-dimethyl-2-phenyl-3-oxo-3H-pyrazol-4-ylcarbonyl]-4-thiocyanatopyrazole (13)

To a solution of thiocyanato-3-ethoxypropenone derivative **11** (3.43 g, 0.01 mmol) in ethanol (20 mL) was added phenylhydrazine hydrate (1.26 g, 0.01 mmol). The reaction mixture was refluxed for 2 h then cooled, and the solid precipitate was filtered off, washed with water, and dried. Recrystallization from ethanol afforded the corresponding product derivative thiocyanatopyrazole **13** in 70% yield, m.p.: 256–257 °C, IR (KBr) ν (cm^{-1}) = 2052 (SCN), 1627 (C=O), 1597 (C=N). ^{1}HNMR (DMSO-d_6) δ (ppm) = 2.49 (s, 3H), 3.36 (s, 3H), 5.8 (s, 1H, CH-pyrazole), 7.36–7.59 (m, 10H, ArH). ^{13}C NMR (DMSO-d_6) δ (ppm) = 14.8 (CH_3), 34.4 (CH_3), 117.8, 122.5, 124.7, 125.9, 126.8, 128.0, 128.2, 128.6, 128.9, 129.7, 130.7, 135.3, 142.5 (Ar-C), 136.8 (SCN). 164.8 (C=O). MS; m/z: 417.13 (100.0%), 418.13 (24.9%), 419.12 (4.5%), For $C_{22}H_{19}N_5O_2S$ (417.13), Calcd.: C: 63.29; H: 4.59; N: 16.78; S: 7.68%. Found: C: 63.31; H: 4.58; N: 16.78; S: 7.70%.

1.8 | 3-[4-(1,2-Dihydro-1,5-dimethyl-2-phenyl-3-oxo-3H-pyrazol-4-ylcarbonyl] isoxazole (16)

To a solution of thiocyanato-3-ethoxypropenone derivative **11** (3.43 g, 0.01 mmol) in ethanol (20 mL) was added hydroxylamine hydrochloride (0.69 g, 0.01 mmol) in presence of equivalent amount of potassium carbonate. The reaction mixture was heated under reflux for 2 h then left to cool. The precipitate was filtered off, washed with water, and dried. Recrystallization from ethanol afforded the corresponding product isoxazole derivative **16** in 65% yield, m.p.: 223–225 °C, IR (KBr) ν (cm^{-1}) = 20 525 (SCN), 1641 (C=O). ^{1}HNMR (DMSO-d_6) δ (ppm) = 2.49 (s, 3H), 3.36 (s,

3H), 6.3 (s, 1H, CH-isooxazole), 7.36–7.59 (m, 5H, ArH). MS; m/z: 314.08 (80.0%), 315.09 (16.5%), 316.08 (14.8%), 315.08 (2.3%), For $C_{15}H_{14}N_4O_2S$ (314.08), Calcd.: C: 57.31; H: 4.49; N: 17.82; O: 10.18; S: 10.20%. Found: C: 57.31; H: 4.49; N: 17.82; O: 10.18; S: 10.20.

1.9 | 2-Hydroxy-4-([4-(1,2-dihydro-1,5-dimethyl-2-phenyl-3-oxo-3H-pyrazol-4-yl) thiazole (25)

A mixture of compound **2** (2.87 g, 10 mmol), sulfuric acid (5 mL), and acetic acid (5 mL) was refluxed for 30 min then cooled. The solid product was filtered off, washed with water, and dried. Recrystallization from ethanol afforded thiazole derivative **25** in 70% yield, m.p.: 222–224 °C, IR (KBr) ν (cm^{-1}) = 3418 (OH), 1643 (C=O). ^{1}HNMR (DMSO-d_6) δ (ppm) = 2.49 (s, 3H), 3.36 (s, 3H), 6.1 (s, 1H, CH-thiazole), 9.1 (s, 1H, OH/D_2O-exchangeable), 7.36–7.59 (m, 5H, ArH). MS; m/z: 287.07 (70.0%), 288.08 (15.4%), 289.08 (11.6%). For $C_{14}H_{13}N_3O_2S$ (287.07), Calcd.: C: 58.52; H: 4.56; N: 14.62; S: 11.16%. Found: C: 58.44; H: 4.66; N: 14.62; S: 11.17%.

1.10 | 2-Chloro-4-[4-(1,2-dihydro-1,5-dimethyl-2-phenyl-3-oxo-3H-pyrazol-4-yl) thiazole (26)

A solution of compound **25** (10 mmol) in ether (30 mL) was cooled to 10–15 °C and saturated with dry hydrochloric acid gas during 1.5–2 h. After the reaction was completed, the solid product was filtered off, washed with ether, and recrystallized from dioxane to give compound **26** in 63% yield, m.p.: 216–217 °C, IR (KBr) ν (cm^{-1}) = 1643 (C=O), 1587 (C=N). MS; m/z: 305.04 (60.0%), 306.04 (17.1%). For $C_{14}H_{12}ClN_3OS$ (305.04), Calcd.: C: 54.99; H: 3.96; Cl: 11.59; N: 13.74; S: 10.49%. Found: C: 54.99; H: 3.98; Cl: 11.69; N: 13.70; S: 10.50%.

1.11 | 3-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-5H-thiazolo[2,3-b] quinazolin-5-one (21)

1.11.1 | Method A

To a solution of 4-thiocyanatopyrazolone **2** (2.87 g, 10 mmol) in ethanol (20 mL) was added methyl anthranilate hydrobromide (3.32 g, 10 mmol). The mixture was refluxed for 6–8 h. The solid that formed was collected by filtration, washed with water, and dried. Recrystallization from DMF afforded 9-(4-(1,2-dihydro-1,5-dimethyl-2-phenyl-3-oxo-3H-pyrazol-4-yl)thiazolo[1,2-a]quinazolin-3-one in 66% yield, m.p.: 260–262 °C, IR (KBr) ν (cm^{-1}) = 1643, 1633 (2C=O) 1587 (C=N). MS; m/z: 388.10 (60.0%), 389.10 (25.1%), 390.10 (7.5%). ^{1}HNMR (DMSO-d_6) δ (ppm) = 2.49 (s, 3H), 3.36 (s, 3H), 6.2 (s, 1H, CH-thiazole), 7.26–7.79 (m, 9H). ^{13}C NMR (DMSO-d_6) δ (ppm) = 14.8 (CH_3), 34.8 (CH_3), 117.8, 122.5, 124.7, 125.9, 126.8, 128.0, 128.2, 128.6, 128.9, 129.7, 130.7, 135.3, 137.3, 139.7 142.5 (Ar-C), 162.8, 163.5 (2C=O) For $C_{21}H_{16}N_4O_2S$ (388.45), Calcd.: C: 64.93; H: 4.15; N: 14.42; S: 8.25%. Found: C: 65.01; H: 4.15; N: 14.34; S: 8.25%.

1.11.2 | Method B

A mixture of 2-chloro-4-[4-(1,2-dihydro-1,5-dimethyl-2-phenyl-3-oxo-3H-pyrazol-4-yl)thiazole (**26**) (3.88 g, 0.01 mmol) and methyl anthranilate hydrobromide (3.32 g, 0.01 mmol) in presence of phenol (4 mL) was heated into an oil bath at 150–180 °C for 7 h then left to cool. The precipitated solid was filtered off, washed with water, dried, and recrystallized from acetic acid to afford a product identical in all respects (m.p., mixed m.p., and spectra) with that obtained from reaction of compound **2** with methyl anthranilate hydrobromide as in method A above.

1.12 | 1-[4-(1,2-Dihydro-1,5-dimethyl-2-phenyl-3-oxo-3H-pyrazol-4-yl)]-3-(4-methoxyphenyl)-2-thiocyanatopropenone (30)

To a solution of compound **2** (2.87 g, 0.01 mmol)in ethanol (20 mL) was added p-methoxy benzaldehyde (1.36 g, 0.01 mmol) in presence of few drops of piperidine as catalyst. The reaction mixture was refluxed for 3 h then cooled. The solid product so formed was collected by filtration, washed with ethanol, and dried. Recrystallization from ethanol afforded compound **30** in 90% yield, m.p.: 255 °C, IR (KBr) ν (cm^{-1}) = 2152 (SCN), 1643, 1633 (2C=O), 1589 (C=N), ^{1}HNMR (DMSO-d_6) δ (ppm) = 2.49 (s, 3H), 2.6 (s, 3H), 3.2 (s, 3H), 3.4 (s, 1H), 4.5 (s, 1H), 7.3–7.56 (m, 10H, ArH). MS; m/z: 387.16 (100.0%), 388.16 (26.3%), 389.17 (3.0) %. For $C_{23}H_{21}N_3O_3$ (387.16), Calcd.: C: 71.30; H: 5.46; N: 10.85%. Found: C: 71.30; H: 5.46; N: 10.85%.

1.13 | 5-(*p*-Methoxyphenylmethylene)-6-[4-(1,2-dihydro-1,5-dimethyl-2-phenyl-3-oxo-3H-pyrazol-4-yl)]-1,2,4-triazine (34)

To a solution of compound **30** (3.87 g, 0.01 mmol) in ethanol (20 mL) was added hydrazine hydrate (1 g, 20 mmol) in presence of a catalytic amount of piperidine. The mixture was refluxed for 4 h; the solid product so formed was collected by filtration, washed with ethanol, dried, and recrystallized from ethanol to afford 60% yield of **34**, m.p.: 252–254 °C, IR (KBr) ν (cm^{-1}) = 1643 (C=O), 1587 (C=N). MS; m/z: 387.17 (100.0%), 388.17 (25.7%), 389.18 (2.8%). ^{1}HNMR

(DMSO-d_6) δ (ppm) = 2.49 (s, 3H), 2.6 (s, 3H), 3.8 (s, 3H), 4.5 (s, 1H), 7.3–7.56 (m, 10H, ArH). ^{13}C NMR (DMSO-d_6): δ (ppm) = 13.8, 33.4. 52.8 ($3CH_3$), 117.8, 122.5, 124.7, 125.9, 126.8, 128.0, 128.2, 128.9, 129.7, 130.7, 135.3, 137.3, 142.5 (Ar-C), 126.8 (CH=), 164.8 (C=O). For $C_{22}H_{21}N_5O_2$ (387.17), Calcd.: C: 68.20; H: 5.46; N: 18.08%. Found: C: 68.21; H: 5.47; N: 18.10%.

2 | RESULTS AND DISCUSSION

2.1 | Chemistry

Reaction of 4-(2-chloroacetyl)-1,2-dihydro-2,3-dimethyl-1-phenylpyrazol-5-one (**1**) with potassium thiocyanate, in refluxing ethanol, afforded a single product in good yield which was identified as 1,2-dihydro-1,5-dimethyl-2-phen yl-4-thiocyanatoacetyl-3H-pyrazol-3-one (**2**) (Scheme 1). The structure of compound **2** was inferred from its elemental analysis and spectral data. Thus, the IR spectrum of compound **2** revealed a band at 2152 cm^{-1} assignable to SCN group, in addition to two carbonyl absorption bands at 1643 and 1633 cm^{-1}. Its ^{1}HNMR spectrum displayed two singlet signals at 2.49 and 3.36 ppm due to two methyl protons and another singlet signal at 4.2 ppm due to methylene protons, whereas its mass spectrum exhibited a peak at m/z 287 corresponding to its molecular ion.

Heating of compound **2** in ethanol at reflux temperature afforded a single product which was analyzed correctly for $C_{14}H_{13}N_3SO_2$ and identified as 2-imino-5-[4-(1,2-dihydro-1, 5-dimethyl-2-phenyl-3-oxo-3H-pyrazol-4-yl)-4H-1,3-oxathiin e (**4**). The structure of the isolated product was established on the basis of its elemental analysis and spectral data. For example, the IR spectrum of compound **3** revealed the presence of NH absorption band at 3095 cm^{-1} and the presence of only one carbonyl bands at 1663 cm^{-1} in addition to the absence of SCN band in its corresponding region. Its mass spectrum displayed a peak at m/z 287 corresponding to its molecular ion (Scheme 1).

Treatment of compound **2** with phenyl isothiocyanate, in dimethylformamide in the presence of potassium hydroxide, at room temperature, afforded the corresponding potassium salt **5** that was converted into the non-isolable intermediate **6** upon treatment with dilute hydrochloric acid. The latter compound **6** underwent intramolecular cyclization to afford the corresponding imino-1,3-dithiole derivative **7**. The structure of the isolated product was confirmed on the basis of its elemental analysis and spectral data. For example, its IR spectrum revealed two absorption bands at 3449 and 3419 cm^{-1} due to two NH groups and two carbonyl absorption bands at 1643 and 1634 cm^{-1}. Its ^{1}HNMR exhibited two singlet signals at 2.57 and 3.33 ppm due to two methyl protons and another two singlet signals at 7.1 and 9.78 ppm assigned to two NH protons, whereas its mass spectrum displayed a peak at m/z 442 corresponding to its molecular ion.

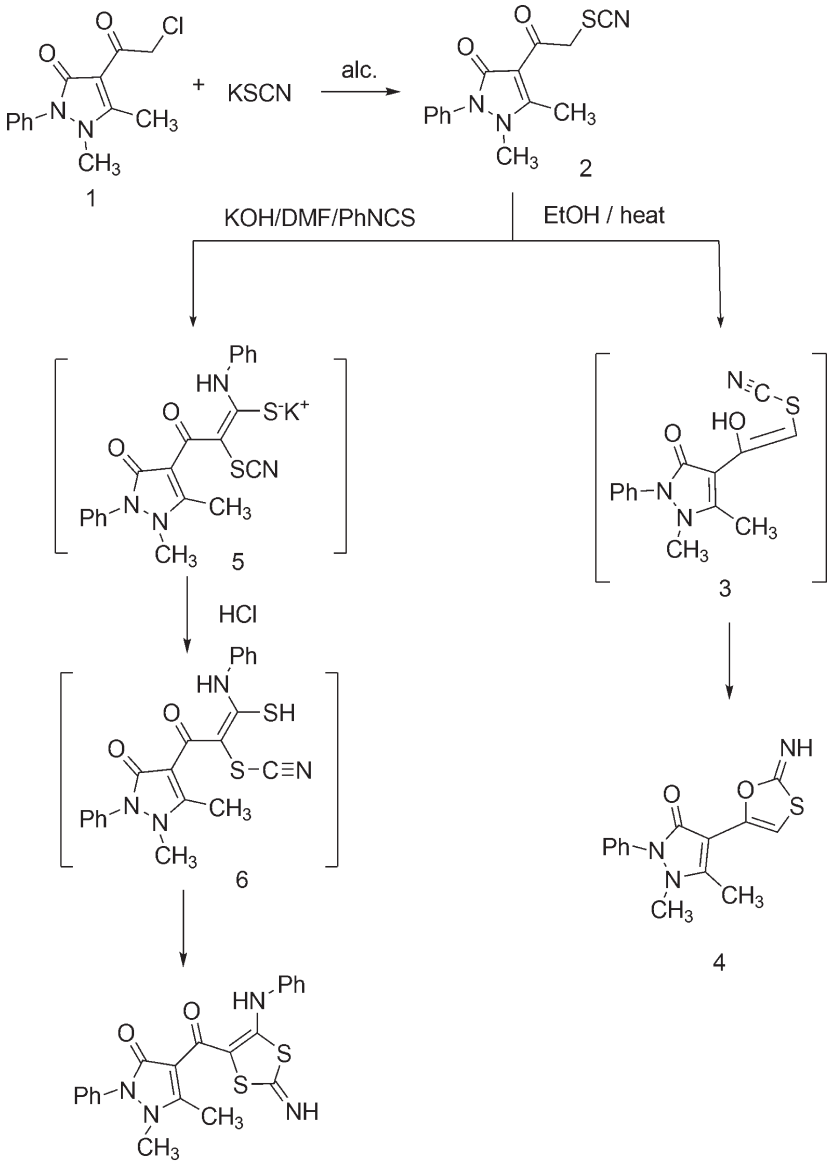
2-[4-(1,2-Dihydro-1,5-dimethyl-2-phenyl-3-oxo-3H-pyr azol-4-yl]-1,3,4-thiadiazolo[3,2-a]-1,3-quinazoline-2-imine **10a** was obtained via treatment of compound **2** with diazotized anthranilonitrile in ethanol, in the presence of sodium acetate at 0–5 °C (Scheme 2). The structure of the latter product was supported by elemental analysis, IR, and NMR data. For example, the IR spectrum showed a stretching NH band at 3425 cm^{-1} and two carbonyl absorption bands at 1643 and 1633 cm^{-1}. Its ^{1}HNMR spectrum exhibited a singlet signal at 4.5 ppm due to NH proton.

In a similar manner, when compound **2** was treated with diazotized anthranilic acid or methyl anthranilate, it afforded one and the same product identified as 2-[4-(1, 2-dihydro-1,5-dimethyl-2-phenyl-3-oxo-3H-pyrazol-4-yl] -2,3,4-thiadiazolo[3,2-a]-1,3-quinazoline-2-one **10b**. The IR spectrum of **10b** revealed three carbonyl absorption bands at 1710, 1690, and 1639 cm^{-1}, and its mass spectrum showed a peak corresponding to its molecular ion. Chemical confirmation of the structure of **10b** was also established on basis of an alternative synthetic pathway from **10a** by refluxing in ethanolic hydrochloric acid solution (Scheme 2).

Fusion of compound **2** with triethyl orthoformate afforded a single product identified as 1-[4-(1,2-dihydro-1,5-dimethyl-2-phenyl-3-oxo-3H-pyrazol-4-yl)]-2-thiocyanato-3-ethoxyprop enone (**11**) on the basis of its elemental analysis and spectral data. For example, its IR spectrum showed absorption band at 2152 cm^{-1} due to SCN group, in addition to two carbonyl absorption bands at 1643 and 1633 cm^{-1}. The ^{1}HNMR showed a triplet signal at 1.21 ppm due to the methyl protons and a quartet signal at δ 4.49 ppm assigned to the methylene protons and a singlet signal at 7.91 ppm due to the methine proton. In addition, the mass spectrum showed a peak at m/z 343 corresponding to its molecular ion.

Treatment of **11** with phenylhydrazine in refluxing ethanol, in the presence of catalytic amount of piperidine, afforded a single product identified as 3-[4-(1,2-dihydro-1,5-dimet hyl-2-phenyl-3-oxo-3H-pyrazol-4-ylcarbonyl]-4-thiocyanato pyrazole (**13**). The structure of the isolated product was confirmed on the basis of elemental analysis and spectral data. For example, its IR spectrum showed a strong absorption band at 2052 cm^{-1} due to SCN group and a carbonyl absorption band at 1627 cm^{-1}. Also, the absence of IR bands corresponding to NH_2, NH, and a second carbonyl group excluded the possible structures 12 and 14.

Reaction of compound **11** with hydroxylamine hydrochloride, in refluxing ethanol in presence of equivalent amount of potassium carbonate, afforded the corresponding isoxazole derivative **16** in moderate yield. Confirmation of the structure of the product was based on elemental analysis and spectral data. For example, its IR spectrum revealed an absorption band at 2052 cm^{-1} assigned to SCN group, in addition to a carbonyl absorption band at 1641 cm^{-1}.

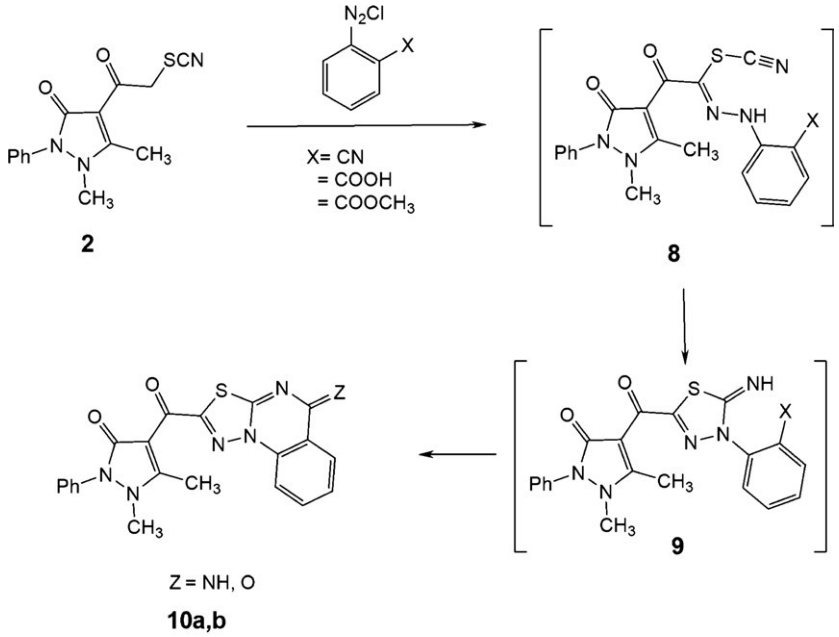


SCHEME 1

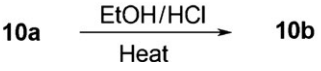
When compound **2** was treated with the hydrobromide salt of methyl anthranilate in refluxing ethanol, it afforded a compound identified as 2-[4-(1,2-dihydro-1,5-dimethyl-2-phenyl-3-oxo-3H-pyrazol-4-yl]-1,3,4-thiazolo[3,2-a]-1,3-quinazoline-2-one (**21**) (Scheme 5). The structure of the latter product was confirmed on the basis of its elemental analysis and spectral data. The IR spectrum showed two carbonyl absorption bands at 1643 and 1633 cm^{-1}, whereas, its mass spectrum showed a molecular ion peak at m/z at 388 corresponding to its molecular ion. These data provided an additional support for the assigned structure.

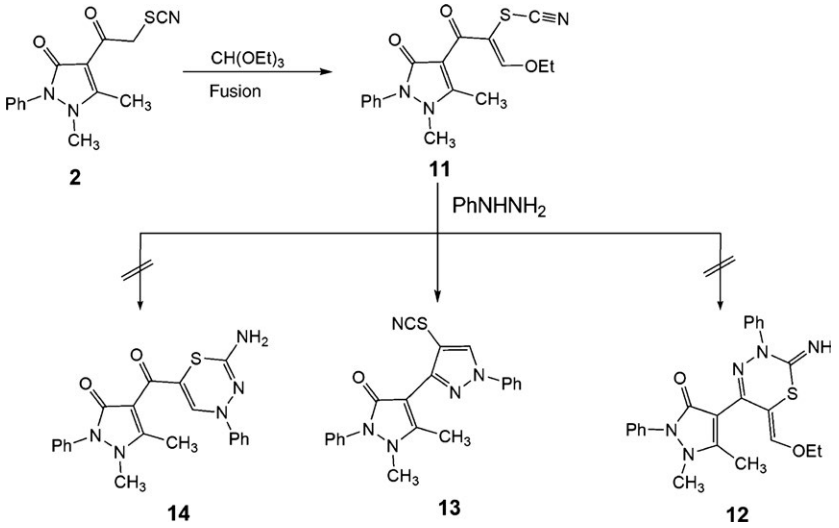
The structure of the isolated product was proven to be a linear **21** rather than the angular isomer **23**; this was supported by an alternative chemical synthesis of compound **21** via the reaction of the methyl anthranilate hydrobromide with 2-chloro-4-[4-(1,2-dihydro-1,5-dimethyl-2-phenyl-3-oxo-3H-pyrazol-4-yl]-1,3-thiazole (**26**) that afforded a compound identical in all aspects (m.p., mixed m.p., spectra, and TLC) to that of **21** (Scheme 6).

1-[4-(1,2-dihydro-1,5-dimethyl-2-phenyl-3-oxo-3H-pyrazol-4-yl)]-3-(4-methoxyphenyl)-2-thiocyanatopropenone (**30**) was prepared via condensation reaction of compound **2** with *p*-anisaldehyde, in refluxing ethanol, in presence of catalytic amount of piperidine to afford a single product (Scheme 7). The structure of the isolated product was supported on the basis of its elemental analysis and spectral data. For example, its IR spectrum showed two carbonyl absorption bands at 1643 and 1633 and SCN absorption at 2152 cm^{-1}; its ^{1}HNMR revealed three singlet signals at 2.49, 2.6, and 3.2 ppm due to three methyl protons, in addition to singlet signal at 4.5 ppm due to methine proton, whereas its mass spectrum showed a peak at m/z 387 corresponding to its molecular ion. Compound



SCHEME 2





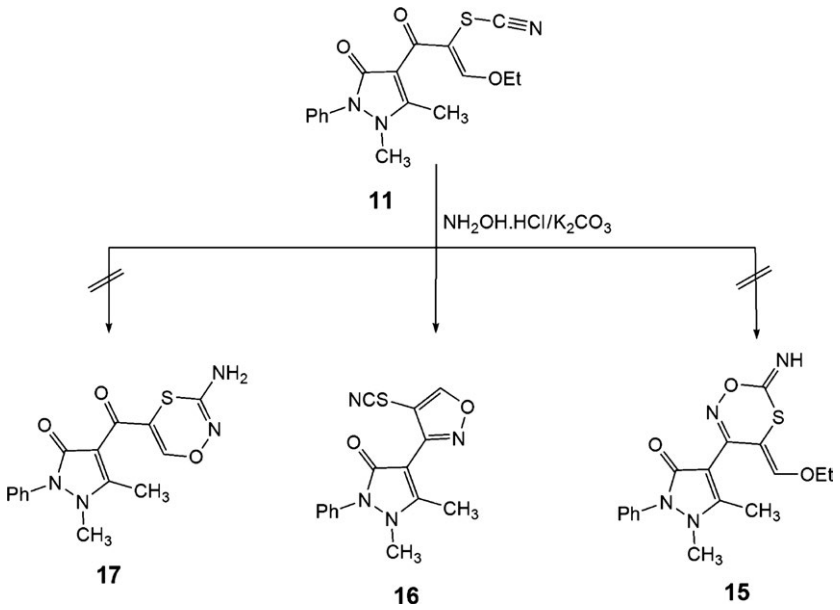
SCHEME 3

30 reacted with hydrazine hydrate in refluxing ethanol to afford the corresponding 1,2,4-triazine derivative **34** as confirmed by its elemental analysis and spectral data (see Section 1).

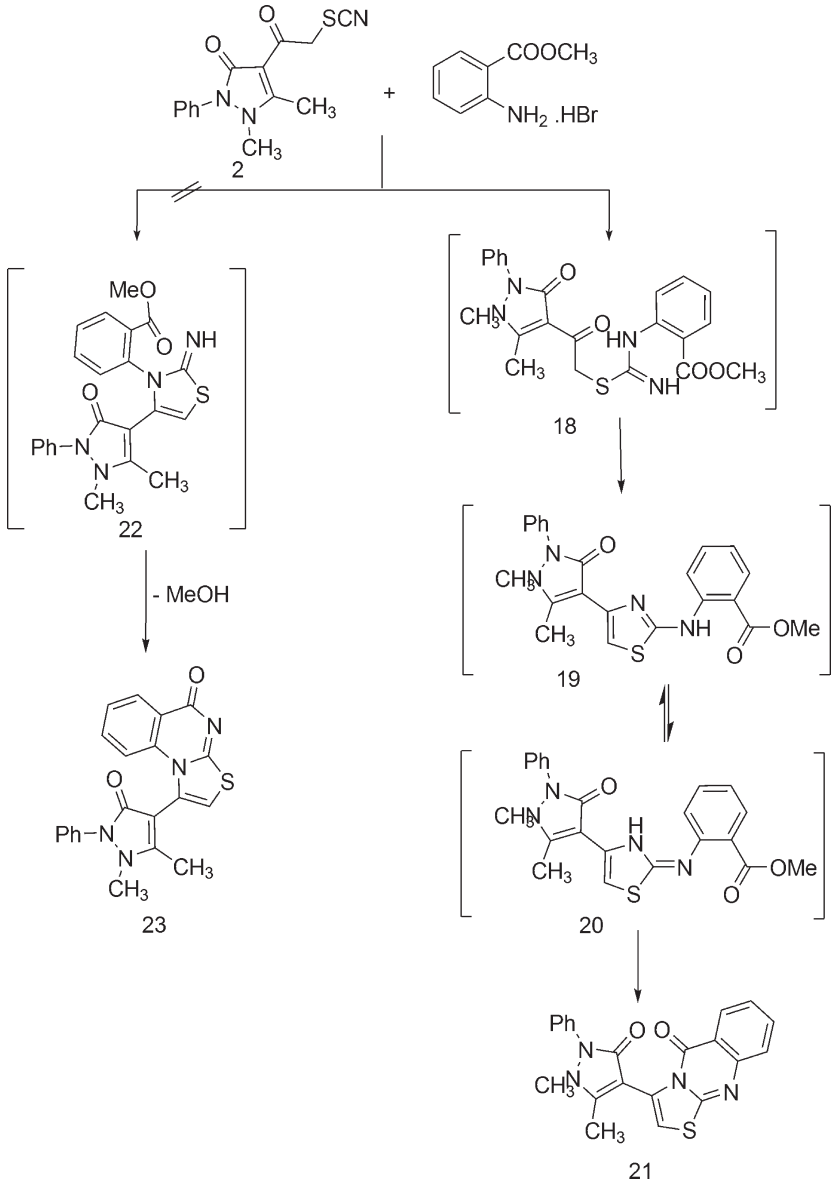
3 | PHARMACOLOGICAL EVALUATION

3.1 | Aromatase inhibition activity assay

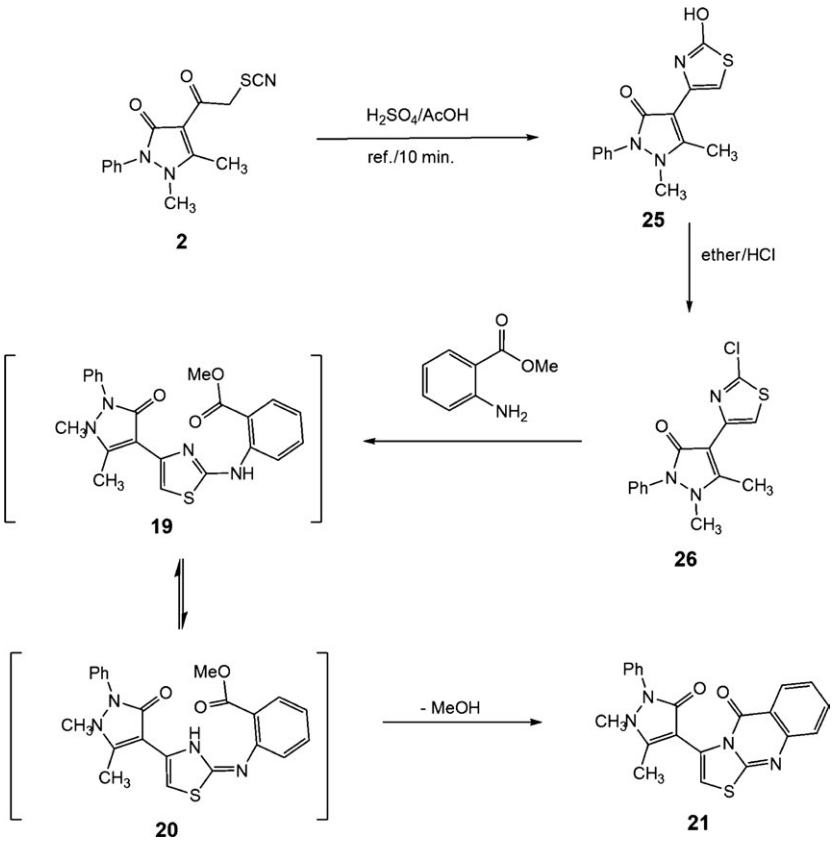
Inhibitory potencies of compounds toward aromatase enzyme were determined according to an established procedure using a commercially available aromatase test kit from BD Gentest. This fluorescence-based assay measures the rate at which recombinant human aromatase (baculovirus/insect cell-expressed) converts the substrate 7-methoxy-trifluoromethy lcoumarin (MFC) into a fluorescent product 7-ethynyl-triflu oromethylcoumarin (HFC; λex = 409 nm, λem = 530 nm) in a NADPH regenerating system. Briefly, concentrated stock solutions of test compounds were prepared in acetonitrile. Hundred microliters of samples containing serial dilutions of test compounds (dilution factor of 3 between samples) and cofactor mixture (0.4 U/mL glucose-6-phosphate dehydrogenase; 16.2 μM $NADP^+$; 825 μM $MgCl_2$; 825 μM glucose-6-phosphate; 50 μM citrate buffer, pH 7.5) was prepared in a 96-well plate. After incubating the plate for 10 min at 37 °C,



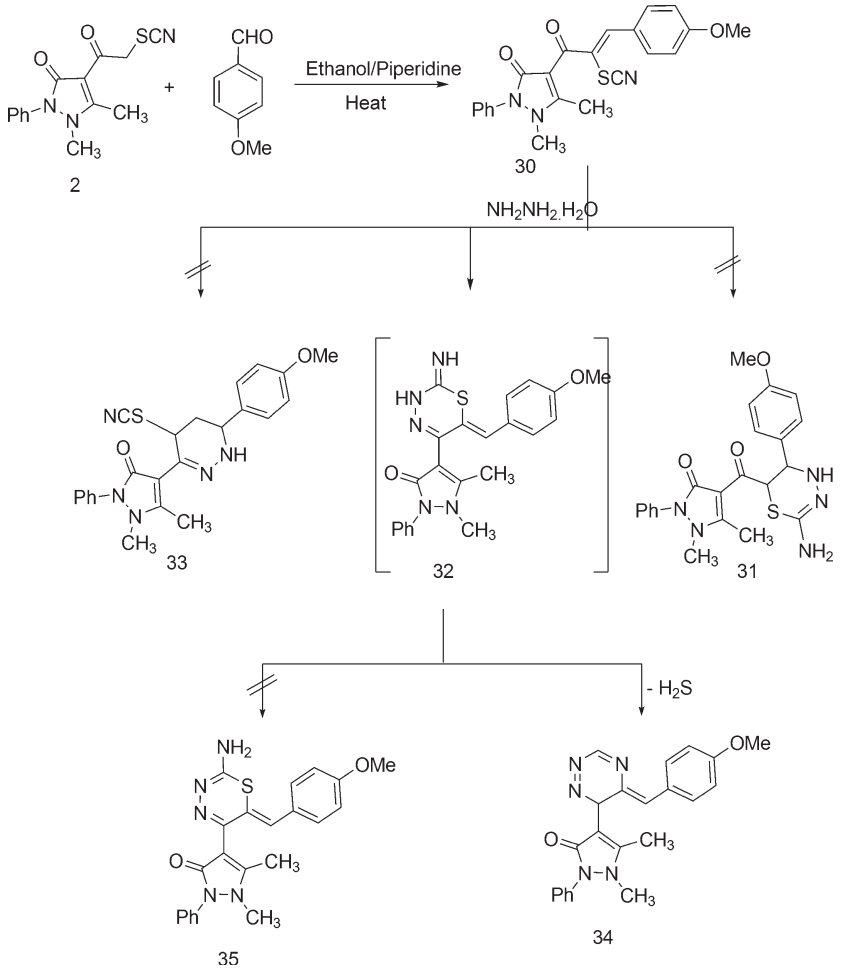
SCHEME 4



SCHEME 5



SCHEME 6



SCHEME 7

100 μL of an aromatase/P450 reductase/substrate solution (105 μg protein/mL enzyme; 50 μM MFC; 20 mM phosphate buffer, pH 7.4) was added to each well. The plate was covered and incubated for 30 min at 37 °C. Seventy-five microliters of 0.5 M Tris base was then added to stop the reaction, and the fluorescence of the formed de-methylated MFC was measured with a plate reader (Spectra Max Gemini; Molecular Devices, Sunnyvale, CA, USA). Fluorescence intensities, which were proportional to the amount of reaction product generated by aromatase, were graphed as a function of inhibitor concentration and then fit to a 3-parameter logistic function. Inhibitory potencies were expressed in terms of the IC_{50} value, the inhibitor concentration necessary to reduce the enzyme activity by half. Each experiment was performed at least in triplicate. The aromatase inhibition activity of the synthesized compounds was tested and summarized (Table 1). As seen in Table 1, the most active compound of the series was **16**, showing IC_{50} value of 0.0023 ± 0.0002 μM compared to the native ligand letrozole with IC_{50} of 0.0028 ± 0.0006 μM. In addition, compounds **26** and **36** exhibit good inhibition activities close to letrozole with IC_{50} values 0.0033 ± 0.0001 and 0.0032 ± 0.0003 μM, respectively.

TABLE 1 Aromatase inhibition activity of the synthesized compounds and the native ligand letrozole

Compound	IC_{50} (μM)
2	0.0067 ± 0.0002
4	0.0054 ± 0.0002
7	0.0046 ± 0.0001
10a	0.0041 ± 0.0001
10b	0.0039 ± 0.0002
13	0.0037 ± 0.0002
16	**0.0023 ± 0.0002**
21	0.0034 ± 0.0001
25	0.0035 ± 0.0002
26	**0.0033 ± 0.0001**
36	**0.0032 ± 0.0003**
Letrozole	**0.0028 ± 0.0006**

3.2 | Molecular modeling and molecular dynamics studies

Protein structure of aromatase with its equilibrium molecular dynamic simulation is obtained directly from protein data bank, as the molecular dynamics simulation of the protein was performed to relax to its equilibrium conformation. The protein co-ordinates have been downloaded from Protein Data Bank website (PDB ID: 4GL7) with 2 ligands (PDB ID: OXJ) (6α,8α)-6-(pent-2-yn-1-yloxy)androsta-1,4-diene-3,17-dione $C_{24}H_{30}O_3$, (PDB ID: HEM) protoporphyrin IX containing Fe. The water molecules and all other substructures including HEAM (protoporphyrin IX containing Fe) were removed. The hydrogen atoms were added to make the system closer to reality, Kollman united atom charges and salvation parameters were added, and the energy of the protein was minimized using the Swiss pdb-viewer 4.1.0.

It was found that bulky compounds are difficult to enter the binding site as it needed to rotate its side chains to fit the cavity entrance, as by analyzing the binding site of aromatase by PDBsum (31) explained that aromatase binding site volumes occupy 1525.92 Å and the binding site diameter when measured by PYMOL was 3.24 Å. Traditional approaches to Pyrazolone development focus on inhibiting aromatase by binding to its active site. It may be worthwhile to consider the development of drugs that may sterically block access of natural substrate to the entrance of the binding site. From other previous studies, it was observed that the catalytic binding residue is M374 (methionine 374). The structures of newly synthesized pyrazolone compound **16** exhibited the lowest IC_{50} value (0.0023 ± 0.0002 μM) compared to the native

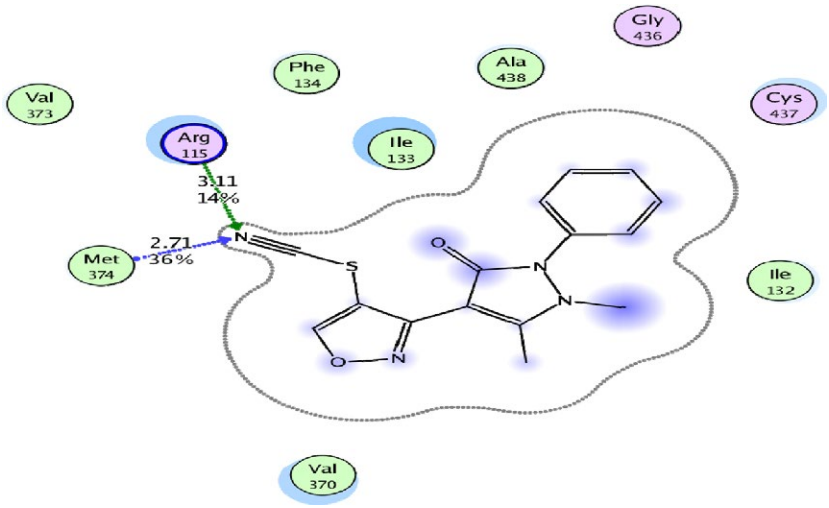


FIGURE 2 MOE docking showing the interaction between Methionine 374 with the nitrogen atom of the thiocyanate group in the ligand **16** which is 2.71 Å and 36%..

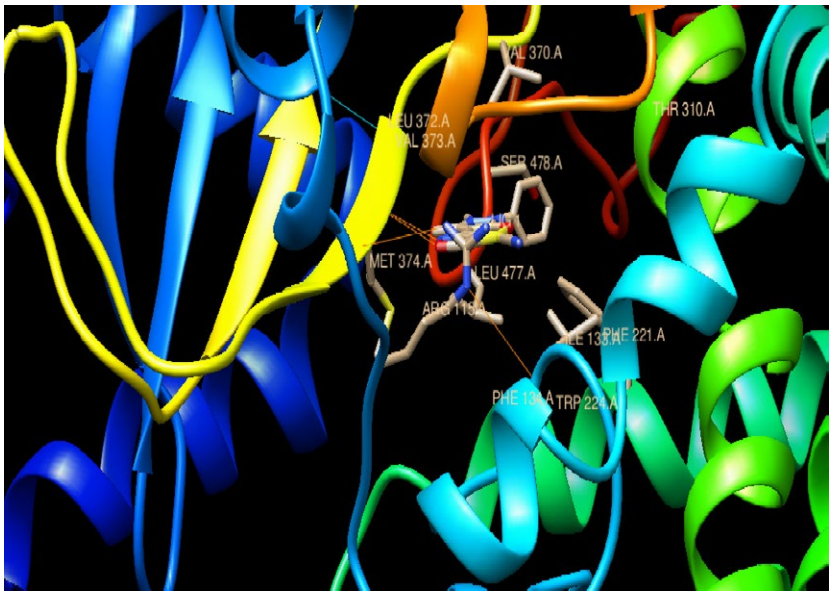


FIGURE 3 Autodock and visualized by UCSF Chimera showing the Hydrogen bond between Methionine and the ligand **16**.

ligand letrozole IC_{50} (0.0028 ± 0.0006 μM). The structures were built with CHEMSKETCH software (Advanced Chemistry Development, Inc., Toronto, ON, Canada) and minimized by the MOE 2008.10. (Macrovision corporation 2830 De la cruz Blvd Santa Clara, CA, USA) Next, the ligand structures were prepared for docking by merging non-polar hydrogen atoms, adding Gasteiger partial charges, and defining rotatable bonds. The energy-optimized ligand (**16**) was docked into the binding site in the protein using MOE 2008.10 and AUTODOCK 4 (Molecular Graphics Laboratory, The Scripps Research Institute, La Jolla, CA, USA) to elucidate the binding mode with aromatase. The maximum distance between hydrogen bond donor and acceptor was set to 3.5 Å. And all the parameters were set as default values. By this tool, we can determine all type of interactions as hydrogen bond, π–π interaction, and cation–π interaction. Finally docking algorithm files were run on CYGWIN-I and CYGWIN-II for analysis and visualization of result by UCSF CHIMERA, Regents of the University of California, USA (Figures 2 and 3).

Furthermore, the free energies of binding (ΔG_b) and inhibition constants (K_i) as calculated by AUTODOCK are summarized (Table 2). Docking calculations were performed with AUTODOCK, version 4.2 using the Lamarckian genetic algorithm. A grid box size of 50 × 64 × 78 Å points with a grid spacing of 0.375 Å was generated using AutoGrid. The grid was centered at *x*, *y*, *z* co-ordinates of 85.363, 61.426, and 42.824, which was reported as the binding site residues AUTODOCK parameter set- and distance-dependent dielectric functions were used for calculating the van der Waals and the electrostatic terms, respectively. The initial position, orientation, and torsions of the ligand molecules were set randomly. The docked compound was derived from 10 independent docking runs that were set to terminate after a maximum of 2.5×10^6 energy evaluations with mutation rate of 0.02 and crossover rate of 0.8. The search for low-energy binding orientations was performed by Lamarckian genetic algorithm using a translational step of 0.2 Å, a quaternion step of 5 Å, and a torsion step of 5 Å. To validate the accuracy of the docking system, the nature substrate androstenedione was re-docked to aromatase and its orientation with respect to the crystal structure was determined.

TABLE 2 The approximate free energy of binding (ΔG_b) and inhibition constants (K_i) calculated by AUTODOCK

Lowest binding energy	−7.95 kcal/mol
Mean binding energy	−7.91 kcal/mol
Estimated free energy of binding	−7.95 kcal/mol
Estimated inhibition constant, K_i [temperature = 298.15 K]	1.48 μM
Final intermolecular energy	−8.25 kcal/mol
vdW + H bond + desolvation energy	−8.19 kcal/mol
Electrostatic energy	−0.06 kcal/mol
Final total internal energy	−0.55 kcal/mol
Torsional free energy	+0.30 kcal/mol
Unbound system's energy	−0.55 kcal/mol
RMSD from reference structure	106.718 Å

Statistical mechanical analysis calculated by AUTODOCK.
Partition function, $Q = 10.13$ at temperature, $T = 298.15$ K.
Free energy, $A \sim -1372.15$ kcal/mol at temperature, $T = 298.15$ K.
Internal energy, $U = -7.91$ kcal/mol at temperature, $T = 298.15$ K.
Entropy, $S = 4.58$ kcal/mol/K at temperature, $T = 298.15$ K.

4 | CONCLUSION

In conclusion, a new series derived from 4-(2-chloroacetyl)-1,2-dihydro-2,3-dimethyl-1-phenylpyrazol-5-one was synthesized and characterized, and its pharmacological

activity toward aromatase enzyme inhibition was screened and compared to the reference native ligand letrozole (IC_{50} of 0.0028 ± 0.0006μM). Moreover, molecular docking studies were conducted to support the findings. One of the synthesized compounds (**16**) was found to be more potent inhibitor (IC_{50} value of 0.0023 ± 0.0002μM) than the reference letrozole. In addition, two other compounds (**26** and **36**) exhibited good inhibition activities close to letrozole with IC_{50} values 0.0033 ± 0.0001 and 0.0032 ± 0.0003μM, respectively.

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