Molecular Docking of Some Novel Pyrazolone Derivatives

Ahmad Farouk Eweas1,* , Ahmed OH El-Nezhawy2,*, Rehaf Fawzy Abdel-Rahman4 and Ayman R Baiuomy4,5

1Medicinal Chemistry Department, National Research Center, Dokki, Cairo, Egypt
2Department of Chemistry of Natural and Microbial Products, National Research Center, Dokki, Cairo, Egypt
3Pharmaceutical Chemistry Department, College of Pharmacy, Taif University, Taif, KSA
4Pharmacy Department, National Research Center, Dokki, Cairo, Egypt
5Faculty of Medicine and Medical Sciences, Taif University, KSA

Abstract

A novel series of 1,5-dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one derivatives were synthesized. The synthesis started with the important building block 3, which was prepared via coupling of from 2-(bis (methylthio) methylene) malononitrile 1 with 4-amino-1,5-dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one 2. The 5-amino pyrazole derivatives 4-8 were prepared from the cyclocondensation of 3 with the appropriate sulfonohydrazides and pyridine-4-carboxyl derivative correspondingly pyrazole derivatives 10, 11, 12 and 13. Condensation 2 and 1-isothiocyanato-4-methyl benzene 14 yielded 15 which was refluxed with malonic acid to yield 15-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-2-thioxo-3-(2methylphenyl) dihydroprymidin-4,6(1H,5H)-dione (16). All tested compounds showed analgesic and anti-inflammatory activities in comparison to the reference standard drugs tramadol, acetyl salicylic acid and indomethacin. Maximum protection against the thermal stimulus was observed at 90 min following the administration of the compound (5) (105.8%), which was statistically significant comparable to the reference drug tramadol (148.7%). Compounds (5, 6, 11 and 13) revealed their maximal analgesic effect after 60 min (68.5%, 77.5%, 84.6% and 89.7%, respectively), then their effect started to decrease. In addition, derivatives 10, 12 and 16 showed anti-inflammatory activity after 4 hours, which was greater than that of the reference drug indomethacin and reached the maximum effect at the 2nd h. Additionally, a molecular docking study was performed against the COX enzyme using the Molsoft ICM 3.8 software.

Keywords: Pyrazole; Anti-inflammatory; Analgesic; Indomethacin; Molecular docking

Introduction

The anti-inflammatory properties of Nonsteroidal anti-inflammatory drugs (NSAIDs) have been attributed to their ability to inhibit the enzyme cyclooxygenase (COX) enzymes, which catalyzes the formation of arachidonic acid (AA) to prostaglandins H2 (PGH2) [1-4]. Many of (NSAIDs) have a wide clinical use in the treatment of acute or chronic inflammation [5,6]. There are two isomers of cyclooxygenase (COX); COX-1 and COX-2 [7]. These isomers are poorly distinguishable by most of the classical NSAIDs. They actually inhibit COX-1 extensively; COX-1 has housekeeping functions, including low-level production of gastro protective PGs, besides COX-2, leading to gastrointestinal injury, suppression of Thrombocox A2 (TXA2) formation and platelet aggregation. The combination of these interactions is probably the reason for gastrointestinal bleeding as the most serious complication of these drugs [8]. To prevent or decrease these side effects, a current strategy consists of designing selective COX-2 inhibitors with an improved gastric safety profile. The improved safety profile of COX-2 inhibitors may allow the use of these new agents for long-term prophylactic use in certain chronic diseases. This has led intense efforts in search for potent and selective COX-2 inhibitors, which could provide anti-inflammatory drugs with fewer risks. Several classes of compounds having selective COX-2 inhibitory activity have been reported in the literature such as SC-558 and celecoxib. Pyrazole, pyrazoline, and pyrazoline ring systems found in many non-steroidal anti-inflammatory drugs have been used for clinical application as NSAIDs like celecoxib [9] antipyrine, phenylbutazone, ramifenazone and famprofazone. Antipyrine is the first pyrazoline derivative used as an NSAIDS like celecoxib [9] antipyrine, phenylbutazone, ramifenazone and famprofazone. Antipyrine is the first pyrazoline derivative used as an anti-inflammatory drug.

heterocyclic compounds having a broad spectrum of application in the field of medicinal chemistry [14]. Pyrazole derivatives were found to exhibit anti-inflammatory [15-17], analgesic [18], antitumor [19,20], antiviral [21,22], anticonvulsant [23] and antimicrobial activities [22,24]. The importance of pyrazole derivatives as antimicrobial agents attracted attention after the discovery of the natural pyrazole C-glycoside pyrazofurin which demonstrated a broad spectrum of antimicrobial activities [25]. Appreciation with the well-documented anti-inflammatory and analgesic properties associated with these heterocyclic cores and as part of our continuing work in the area of drug discovery including anti-inflammatory and analgesic compounds [26-28], herein we report the synthesis of new pyrazolone derivatives in combination with pyrazole and dihydroprynidinidine scaffold, in addition to heteroaryl and aryl pyrazole derivatives. The analgesic and anti-inflammatory activities of all novel compounds were investigated utilizing the acetic acid-induced writhing test and the carrageenan-induced hind paw edema test, respectively. Furthermore, a molecular docking study was carried out for the most potent anti-inflammatory new compounds against COX-1 and COX-2 crystal structures in an attempt to understand their binding mode to both enzymes in comparison to the reference drug indomethacin.

*Corresponding author: Ahmad Farouk Eweas, Medicinal Chemistry Department, National Research Center, Dokki, Cairo, Egypt, Tel: 00966559058923, E-mail: eweas1@gmail.com

Received September 03, 2015; Accepted October 20, 2015; Published October 15, 2015

Citation: Eweas AF, El-Nezhawy AOH, Abdel-Rahman RF, Baiuomy AR (2015) Design, Synthesis, In Vivo Anti-inflammatory, Analgesic Activities and Molecular Docking of Some Novel Pyrazolone Derivatives. Med chem 5: 458-466. doi:10.4172/2161-0444.1000301

Copyright: © 2015 Eweas AF, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
Materials and Methods

Chemistry

All chemicals were purchased from common commercial suppliers and used without further purification. All reactions were carried out under argon with dry solvents. Also all reactions were monitored by TLC carried out on Merck silica gel-coated plastic sheets (60 F254) by using UV light as visualizing agent. Thin layer chromatography (TLC) was performed on silica gel 60 F254 plastic plates (E.Merck, layer thickness 0.2 mm). Detection was achieved by treatment either with a solution of 20 g of ammonium molybdate and 0.4 g of cerium (IV) sulfate in 400 ml of 10% H2SO4 or with 15% H2SO4, and heating at 150°C. Melting points were determined on a Gallenkamp melting point apparatus and were uncorrected. IR spectra (KBr) were recorded on a Perkin-Elmer 1650 spectrophotometer, NRC. 'H and 13C NMR were determined on a Varian Mercury (300 MHz) spectrometer (Varian, UK) and the chemical shifts were expressed in δ ppm relative to TMS as an internal reference. Faculty of science, Cairo University. Mass spectra were recorded on Thermo Finnigan LCQ Advantage spectrometer in ESI mode, I Spray Voltage 4.8 kV. Microanalyses were performed at the Micro analytical Center of Cairo University.

2-(((1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)amino)(methylthio)methylene)malononitrile 3 [1] (3): A mixture of 2-(bis(methylthio)methylene)malononitrile 1 (10 mmol) and 4-amino-1,5-dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one 2 (10 mmol) in ethanol (20 ml) was heated under reflux for 12h (under TLC control). The reaction mixture was cooled, poured into ice-water and the solid formed was filtered off and crystallized from methanol to give product of type 2-(((1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)amino)(methylthio)methylene)malononitrile 3.

General method for preparation of 4-8

A mixture of compound (3) (10 mmol) and benzenesulfonylhydrazide, 4-methylbenzene-sulfonohydrazide, 4-bromobenzenesulfonylhydrazide, 2-thiouracil-5-sulfonylhydrazide or pyridine-4-carboxylhydrazide respectively (10 mmol) in toluene (30 ml) was refluxed for 8-12 h. The solid obtained after cooling was filtered off, dried on suction, and crystallized from ethanol to give products of type 4-8.

5-amino-3-((1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)amino)-1-(phenylsulfonyl)-1H-pyrazole-4-carbonitrile (4): Yield 75%, m.p. 295-297°C, IR (KBr, cm–1): 3547, 3425 (NH), 3030-3019 (C-H aromatic), 2218 (CN), 1627 (C=N), 1322, 1215 (-N=SO2); 'H NMR (DMSO-d6) δ 2.40 (s, 3H, CH3), 3.70 (s, 3H, N-CH3), 6.90-8.20 (m, 10 H, aromatic), 9.10, 10.10 and 11.70 (s, 3H, NH), NH exchangeable with D2O); MS (m/z) M+ at m/z (449) (7%); Anal. Calc. for C21H18N3O5S: C, 47.64; H, 3.30; Br, 15.02; N, 18.50; S, 6.14.

5-amino-3-((1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)amino)-1-((4-bromophenyl)sulfonyl)-3-((1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)amino)-1H-pyrazole-4-carbonitrile (5): Yield 71%, m.p. 265-267°C, IR (KBr, cm–1): 3547, 3425 (NH), 3030, 3019 (C-H aromatic), 2208 (CN), 1630 (C=C), 1322, 1215 (-N=SO2); 'H NMR (DMSO-d6) δ 2.20 (s, 3H, CH3), 3.73 (s, 3H, CH3), 3.70 (s, 3H, N-CH3), 6.90-8.20 (m, 9H, aromatic), 9.10, 10.20, 11.50 (s, 3H, NH), NH exchangeable with D2O); MS (El) m/z (%): 463.5 (10, M+); Anal. Calc. for C21H17BrN3O5S: C, 57.09; H, 4.73; N, 21.74; S, 6.70.

5-amino-1-((4-bromophenyl)sulfonyl)-3-((1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)amino)-1H-pyrazole-4-carbonitrile (6): Yield 79%, m.p. 252-254°C; IR (KBr, cm–1): 3447, 3345 (NH), 3030, 3025 (C-H aromatic), 2214 (CN), 1621 (C=C), 1322, 1215 (-N=SO2); 'H NMR (DMSO-d6) δ 2.30 (s, 3H, CH3), 3.74 (s, 3H, N-CH3), 6.42-8.30 (m, 9H, aromatic), 9.10,10.01, 11.42 (s, 3H, NH), NH exchangeable with D2O); MS (El) m/z (%): 528.3 (9, M+); Anal. Calc. for C21H17BrN3O5S: C, 47.74; H, 4.33; Br, 15.12; N, 18.56; S, 6.07. Found: C, 47.64; H, 3.30; Br, 15.02; N, 18.50, S, 6.14.

5-amino-3-((1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)amino)-1-((4-oxo-2-thioxo-1,2,3,4-tetrahydroprymidin-5-yl)sulfonyl)-1H-pyrazole-4-carbonitrile (7): Yield 75%; m.p. >300°C; IR (KBr, cm–1): 3427, 3315 and 3225 (NH), 3030, 3019 (C-H aromatic), 2212(CN), 1622 (C=N),1322, 1215 (-N=SO2); 'H NMR (DMSO-d6) δ 2.41 (s, 3H, CH3), 3.72 (s, 3H, N-CH3), 6.90-8.21 (m, 5H, aromatic, C=CH of thiouracil), 9.41,10.10,11.22, 11.40 and 12.41 (s, 5H, NH3, NH exchangeable with D2O); MS (El) m/z (%): 552.4 (9, M+); Anal. Calc. for C21H15N4O5S: C, 47.74; H, 3.30; Br, 15.02; N, 21.74; S, 6.70.

Figure 1: Some reported pyrazole-containing anti-inflammatory agents.
m/z (%): 499.5 (16, M+); Anal. Calcd. for C_{19}H_{16}N_{2}O_{5}S (499.53): C, 45.68; H, 3.43; N, 25.24; S, 12.89. Found: C, 45.60; H, 3.31; N, 25.20; S, 12.89.

5-amino-3-[(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-amino]-1-(pyridin-4-ylcarbonyl)-1H-pyrazole-4-carboxitride (8): Yield 80%; m.p. 275-277°C; IR (KBr, cm⁻¹): 3474, 3365 and 3286 (NH, NH), 3030-3019 (C–H aromatic), 2218 (CN), 1672, 1665 (2 CO), 1620 (C=N); H NMR (DMSO-d₆) δ 2.52 (s, 3H, CH₃), 3.85 (s, 3H, N–CH₃), 6.30-8.41 (m, 9H, aromatic), 9.12-10.00, 11.40 (s, 3H, NH, NH exchangeable with D₂O); MS (EI) m/z (%): 414 (11, M+); Anal. Calcd. for C₁₉H₁₆N₃O₅S (414.28): C, 46.80; H, 3.32; N, 29.74. Found: C, 46.60; H, 3.42; N, 29.74.

**General method for preparation of 10 and 12**

An equimolar amounts of 2-(bis(methylthio)methylene)propanenitrile 1 (10 mmol) and pyridine-4-carboxylic acid or 4-methylbenzenesulfonohydrazide (10 mmol) and 2-4 drops of triethylamine in (25 ml) methanol was heated for 8 h. The reaction mixture was cooled, poured onto ice-water and the solid formed was collected by filtration, dried under suction and crystallized from ethanol absolute to give (10 or 12).

5-amino-3-(methylsulfonyl)-1-(pyridin-4-ylcarbonyl)-1H-pyrazole-4-carboxitride (10): Yield 75%; m.p. >300°C; IR (KBr, cm⁻¹): 3427 and 3359 (NH), 3058 and 3026 (C–H aromatic), 2218 (CN), 1676 (CO); H NMR (DMSO-d₆) δ 2.72 (s, 3H, S–CH₃), 4.10-4.22 (bs, 2H, NH, exchangeable with D₂O), 7.90 (d, j = 4.3, 2H, pyridyl), 8.70 (d, j = 4.2, 2H, pyridyl); MS (EI) m/z (%): 259 (18, M+); Anal. Calcd. for C₁₉H₁₆N₃O₅S (259.29): C, 50.95; H, 3.50; N, 27.01; S, 12.37. Found: C, 50.80; H, 3.58; N, 26.91; S, 12.22.

**General method for preparation of 11 and 13**

An equimolecular amounts of (ethoxy-methylidene) propanenitrile 9 (10 mmol) and pyridine-4-carboxylic acid or 4-methylbenzenesulfonohydrazide (10 mmol) and 4-6 drops of triethylamine in (25 ml) methanol was heated for 8hrs. The reaction mixture was cooled, poured onto ice-water and the solid formed was collected by filtration, dried under suction and crystallized from ethanol absolute to give (11 or 13).

5-amino-1-(pyridin-4-ylcarbonyl)-1H-pyrazole-4-carboxitride (11): Yield 75%; m.p. 300°C; IR (KBr, cm⁻¹): 3420 and 3368 (NH), 3030 and 3016 (C–H aromatic), 2212 (CN), 1320, 1225 (–N=SO₂), H NMR (DMSO-d₆) δ 2.30 (s, 3H, CH₃), 2.79 (s, 3H, S–CH₃), 4.30-4.45 (bs, 2H, NH, exchangeable with D₂O), 7.50, 7.77 (m, 6H, aromatic); MS (EI) m/z (%): 308 (26, M+); Anal. Calcd. for C₂₁H₂₀N₄O₅S (308.38): C, 46.74; H, 3.92; N, 18.17; S, 20.80. Found: C, 46.65; H, 3.79; N, 18.10; S, 20.89.

**Materials and methods**

**Animals:** Albino mice and rats used in this experiment were obtained from the Animal House Colony at the National Research Centre (NRC), Egypt. Albino mice of both sexes (25-30 g b. wt) and Wistar rats of both sexes (150-200 g b. wt) were utilized. All animals were housed under standard conditions and were reserved in polyethylene cages under standard conditions (temperature 25±3, and relative humidity 60 ± 10%) of natural 12 h light and dark cycle with free access to food and water. Animals were allowed to adapt to the laboratory environment for one week before experimentation. Mice and rats will be used only once in this study. All animal procedures were performed after approval from the Ethics Committee of The National Research Centre- Egypt and in accordance with the recommendations of the proper care and use of laboratory animals.

**Pharmacological assay**

Animals: Albino mice and rats used in this experiment were obtained from the Animal House Colony at the National Research Centre (NRC), Egypt. Albino mice of both sexes (25-30 g b. wt) and Wistar rats of both sexes (150-200 g b. wt) were utilized. All animals were housed under standard conditions and were reserved in polyethylene cages under standard conditions (temperature 25±3, and relative humidity 60 ± 10%) of natural 12 h light and dark cycle with free access to food and water. Animals were allowed to adapt to the laboratory environment for one week before experimentation. Mice and rats will be used only once in this study. All animal procedures were performed after approval from the Ethics Committee of The National Research Centre- Egypt and in accordance with the recommendations of the proper care and use of laboratory animals.

**Analytical activity:** Analytical activity of the selected Compounds was carried out in mature Albino mice (25-30 g body weight) by using two different models.

a. Central analgesic activity (Hot plate test): The central analgesic activity of the selected Compounds was tested in mice as described by Turner [29] using hot-plate apparatus. Seventy two mice were divided into 12 groups of 6 animals each. Mice of the 1st (normal control) and 2nd (reference one) groups were treated orally with the vehicle (5 ml/kg) and tramadol (40 mg/kg, orally). Animals of the 3rd till the 12th groups were orally given the selected Compounds at doses of (20 mg/kg, p.o.). One h post-medication, mice were placed individually on a hot plate maintained at 53 ± 0.5°C. The time taken by the animals to lick the fore or hind paw or jump out of the place was taken as the reaction time for the thermal stimulus. The reaction time was measured at 30, 60 and 90 min after treatment. The cutoff time for the response to the thermal stimulus was set at 60 Sec. to avoid tissue damage to the mouse paws. All drugs were dissolved in DMSO (20 mg/kg, orally), except tramadol (dissolved in DMSO, 40 mg/kg, orally).

b. Peripheral analgesic activity (Writhing test): The peripheral analgesic activity of the selected Compounds was determined in mice as described by Collier [30]. Seventy two mice were divided into 12 groups of 6 animals each. Mice of the 1st (normal control) and 2nd (reference one) groups were treated orally with the vehicle (5 ml/kg)
and acetyl salicylic acid (150 mg/kg), respectively. Animals of the 3rd
till the 12th groups were orally given the selected Compounds at doses
of (20 mg/kg, p.o.). After 30 min of medication, writhing was induced
by an intraperitoneal injection of acetic acid (0.7% aqueous solution)
in a dose of 10 ml/kg b.w.t. All drugs were dissolved in DMSO (20 mg/
kg, orally), except acetyl salicylic acid (was dissolved in DW, 150 mg/
kg, orally). Mice were then placed in transparent boxes and the number
of writhes per animal was counted for 20 min after acetic acid injection
and expressed as the percentage of protection using the following ratio:

Protection (%)= [Control mean - Treated mean/ Control mean] × 100

Anti-inflammatory activity: Carrageenan-induced mouse paw edema model: The anti-inflammatory testing was performed according
to the method of Winter [31] in Wistar rats. Paw edema was induced
in rats by subcutaneous (s.c.) injection of 0.1 ml of 1% (w/v) carrageenan
in distilled water in the sub-plantar region of their left hind paws. A
group of six rats was left without any treatment, but orally given a
respective volume of the solvent (DMSO), and was kept as control. The selected Compounds were administered at doses (20 mg/kg, p.o.).
Indomethacin (20 mg/kg, p.o.) was used as a reference drug. The paw
volumes of the rats were measured using plethysmometer, before and
and after injection of 1% carrageenan at different time intervals (1, 2, 3 and
4 h). Edema and inhibition rates of each group were calculated at the
above-mentioned time intervals as follows:

Edema (%)=[Vt-Vo/Vo] × 100
Inhibition (%)=[Ec-Et/Ec] × 100

Where, Vo is the volume before carrageenin injection [8], Vt is the
volume at t hour after carrageenin injection [8], Ec is the edema rate
of the control group, and Et is the edema rate of the treated group. All
drugs were dissolved in DMSO (20 mg/kg, orally), except indomethacin
(was dissolved in DW).

Statistical analysis: Statistical analysis of results, was done using
analytical software named SPSS statistics 17.0, Release (Aug. 23, 2008),
Chicago, USA.

Molecular docking

All docking studies were performed using "Internal Coordinate Mechanics (Molsoft ICM 3.8)". A set of three compounds 10, 12 and 16
designed to inhibit cyclooxygenases was compiled and 3D structures
were constructed using Chembio3D ultra 13.0 software [Molecular Modelling and Analysis; Cambridge Soft Corporation, USA (2013)].
They were then energetically minimized by using MOPAC (semi empirical quantum mechanics), Job Type with 100 iterations and
minimum RMS gradient of 0.01, and saved as MDL Mol File (*.mol). The X-ray crystallographic structures of COX-1 (PDB: 3KK6) in
complex with celecoxib and COX-2 complexed with a non-selective inhibitor, Indomethacin (PDB: 4COX) were obtained from the Protein
Data Bank http://www.rcsb.org.

Results and Discussion

Chemistry

The crucial building block 2-(((1,5-Dimethyl-3-oxo-2-phenyl-
2,3-dihydro-1H-pyrazol-4-yl)amino) (methylthio) methylene)
malononitrile 3 was prepared from 2-(bis(methylthio) methylene)
malononitrile 1 and 4-amino-1,5-dimethyl-2-phenyl-1,2-dihydro-3H-
pyrazol-3-one 2 in ethanol under reflux according to the procedure
described in the literature [32] (Scheme 1).

The 5-aminoazopyrazole derivatives 4-8 were prepared from the
cyclocondensation of 2-(((1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-
1H-pyrazol-4-yl)amino) (methylthio) methylene)malononitrile 3
with benzenesulfonylhydrazide, 4-methylbenzene-sulfonohydrazide,
4-bromobenzenesulfonohydrazide, 2-thiourcil-5-sulfonohydrazide or
pyridine-4-carboxyhydrazide respectively in toluene and refluxed for 8-12h (Scheme 2). The chemical structures of 4-8 were established
based on their spectral data and elemental analysis. The IR spectra of 4-8 showed bands between 3550 cm⁻¹ and 3400 cm⁻¹ for NH and
NH₂ groups respectively, 2200 cm⁻¹ for CN and 1215 cm⁻¹ for (-N-SO₂-).
¹H NMR spectra showed singlet signals with δ values between 9.12 ppm
and 11.40 for NH, and NH exchangeable with D₂O. The structures of 4-8 were supported by their mass spectrometry (MS) results, which
showed molecular ions corresponding to the molecular formulas of compounds 4-8.

Cyclocondensation of 2-(bis(methylthio)methylene) propanedinitrile 1 with pyridine-4-carboxyhydrazide and
4-methylbenzenesulfonylhydrazide in refluxing methanol containing
a catalytic amount of triethylamine, yielded the corresponding
pyrazole derivatives 10, 11. In addition Cyclocondensation of ethoxy-
methylene) propan- dinitrile 9 with pyridine-4-carboxyhydrazide or
4-methylbenzene sulfono hydrazide in refluxing methanol containing
a catalytic amount of triethylamine yielded the corresponding pyrazole
derivatives 12, 13 as demonstrated in Scheme 3. The structures of new
pyrazole derivatives 10-13 were established based on their spectral data
and elemental analysis.

Condensation of 4-amino-1,5-dimethyl-2-phenyl-1,2-dihydro-
3H-pyrazol-3-one 2 and 1-isothiocyanato-4-methylbenzene 14
in toluene under reflux yielded 1-(1,5-dimethyl-3-oxo-2-phenyl-2,3-
dihydro-1H-pyrazol-4-yl)-3-(2-methylphenyl)thiourea 15 which
was refluxed with malonic acid for 10 h to afford 1-(1,5-dimethyl-3-oxo-
2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-2-thiozo-3-(2-methylphenyl)
dihydroprymidine-4,6(1H,5H)-dione 16 (Scheme 4). The proposed
structure of (16) was established based on spectral data and elemental
analysis and spectral data. The IR spectra of (16) showed bands 1715,
1685 and 1639 cm⁻¹. ¹C NMR spectra showed singlet

Scheme 1: Reagents and conditions: (a) ethanol, reflux, 12h.
signals with δ value 3.90 for methylene of dihydropyrimidine ring. Mass spectra showed molecular ion peak at m/z 420.

**Pharmacology**

**Analgesic activity:**

- **a. Central analgesic activity (Hot plate test):**
  All tested compounds as well as the reference drug tramadol significantly prolonged the reaction time against the thermal stimulus as compared to the control one after 30, 60 and 90 min of administration (Table 1). Maximum protection against the thermal stimulus was observed at 90 min following the administration of the compound (5) (105.8%), which was statistically significant comparable to the reference drug tramadol (148.7%). As shown in Table 1, compounds (5, 6, 11 and 13) revealed...
Intra-plantar injection of carrageenan in rats led to increase in paw edema in rats in comparison to indomethacin, as a reference drug.

The selected compounds were evaluated for their possible anti-inflammatory activities in a rat model of carrageenan-induced paw edema. Table 3 shows the effect of selected compounds (4, 5, 6, 7, 8, 10, 11, 12, 13 and 16) on carrageenan-induced paw edema in rats in comparison to indomethacin, as a reference drug. Intraplantar injection of carrageenan in rats led to increase in paw volume denoting edema in the control non-treated group as shown in Table 3. It was noticed that compounds (6, 8, 10, 11, 12 and 16) in oral doses of 20 mg/kg significantly decreased the paw edema rate all over the four hours in comparison to the control non-treated group. The anti-inflammatory potencies of selected compounds were calculated by comparing their inhibition rate at different time intervals; with those obtained from animals receiving indomethacin, as standard anti-inflammatory drug. Administration of indomethacin significantly decreased the carragenin-induced edema starting from the first hour and it was persistent till the end of the experiment. The inhibitory effect of indomethacin on paw edema was 32.78, 26.35, 29.02, and 27.45% at the 1st, 2nd, 3rd and 4th hour, respectively. It was noticeable that compound 13 failed to decrease inflammation all over the experimental period. Moreover, the compounds (7 and 5) failed to decrease inflammation at the 1st hour, while compound 4 failed to decrease inflammation at the 1st and 2nd hours. It is noteworthy to mention that the derivatives 10, 12, and 16 showed anti-inflammatory potency after 4 hours greater than that of indomethacin and reached the maximum effect at the 2nd h.

**Molecular docking:** In an attempt to understand both the anti-inflammatory and analgesic data on a structural basis, molecular docking studies were carried out using Molsoft ICM 3.8 software. The aim of the flexible docking calculations is prediction of correct binding geometry for each binder. The scoring functions and hydrogen bonds formed with the surrounding amino acids of the receptor. The new compounds which scores the highest anti-inflammatory activates 10, 12, and 16 were docked against the active site of COX-1 and COX-2 enzymes. Indomethacin was also docked against both COX-1 and COX-2 enzymes. The scoring functions of the complexes were calculated from minimized ligand protein complexes. The new geometry for each binder. The scoring functions and hydrogen bonds formed with the surrounding amino acids of the receptor. The new compounds which scores the highest anti-inflammatory activates 10, 12, and 16 were docked against the active site of COX-1 and COX-2 enzymes.

The analgesic activity of the tested compounds after 90 min, as compared to the reference drug tramadol, arranged in descending order, were 105.8, 83.7, 80.5, 79.4, 69.4, 69.4, 68.8, 65.5, 44.8 and 37.0% compared to the controls. Data are shown as mean ± SEM.

### Table 1: Central analgesic activity of compounds (4, 5, 6, 7, 8, 10, 11, 12, 13 and 16) in mice.

| Group   | 0 min Reaction Time [25] | 30 min Reaction Time [25] | Protection (%) | 60 min Reaction Time [25] | Protection (%) | 90 min Reaction Time [25] | Protection (%) |
|---------|--------------------------|---------------------------|----------------|---------------------------|----------------|---------------------------|----------------|
| Control | 14.0 ± 1.02              | 15.2 ± 1.30†              | 0              | 15.6 ± 0.71†              | 0              | 15.4 ± 0.48†              | 0              |
| 4       | 15.0 ± 1.28              | 23.5 ± 1.43†              | 54.6           | 24.9 ± 1.61†              | 50.6           | 27.8 ± 1.26†              | 80.5           |
| 5       | 15.8 ± 1.14              | 25.5 ± 0.44*              | 67.7           | 26.3 ± 0.38*              | 68.5           | 31.7 ± 0.68†              | 105.6          |
| 6       | 14.6 ± 1.18              | 24.0 ± 0.70†              | 57.8           | 27.7 ± 1.22†              | 77.5           | 28.6 ± 0.80†              | 83.7           |
| 7       | 13.3 ± 0.40              | 17.5 ± 0.40†              | 15.1           | 20.9 ± 0.62†              | 33.9           | 21.1 ± 1.05†              | 37             |
| 8       | 15.7 ± 0.78              | 21.0 ± 1.97†              | 38.1           | 23.3 ± 1.74†              | 49.3           | 26.1 ± 1.59†              | 69.4           |
| 10      | 14.8 ± 1.22              | 21.9 ± 1.76†              | 44             | 23.6 ± 1.85†              | 51.2           | 26.0 ± 1.51†              | 68.8           |
| 11      | 16.0 ± 0.61              | 23.7 ± 1.46†              | 55.9           | 28.8 ± 1.56*              | 84.6           | 26.1 ± 1.92†              | 69.4           |
| 12      | 14.3 ± 0.90              | 22.8 ± 1.51†              | 50             | 24.5 ± 1.50*              | 57             | 25.5 ± 1.78†              | 65.5           |
| 13      | 14.6 ± 1.22              | 25.7 ± 2.01†              | 69             | 29.6 ± 2.34†              | 89.7           | 30.4 ± 1.64†              | 79.4           |
| 16      | 14.0 ± 1.24              | 20.9 ± 1.28†              | 37.2           | 23.5 ± 0.94*              | 50.6           | 22.3 ± 1.79†              | 44.8           |
| tramadol| 13.6 ± 0.73              | 29.6 ± 1.00*              | 94.7           | 31.6 ± 0.94*              | 102.5          | 38.3 ± 1.47*              | 148.7          |

* P< 0.05: Statistically significantly from control (Dunnett’s test).
† P< 0.05: Statistically significant from tramadol (Dunnett’s test).

**Table 2:** Peripheral analgesic activity (Writhing test): The selected compounds showed significant reduction in the number of writhes (Table 2). The most active compounds (4, 6, 7, 8, 10, 12 and 13) showed significant analgesic activity (85.3, 89.1, 90.3, 80.0, 76.5, 89.7 and 83.8%) which was greater than that of the reference drug aspirin (71.5%).

**Anti-Inflammatory Activity:** The selected compounds were evaluated for their possible anti-inflammatory activities in a rat model of carrageenan-induced paw edema. Their maximal analgesic effect after 60 min (68.5%, 77.5%, 84.6% and 89.7%, respectively), then the activity of compounds 11 and 13 started to decrease. Compounds (5 and 13) revealed the most prominent analgesic effect after 30 min (67.7% and 69.0%).

The maximal anti-inflammatory effect after 60 min (68.5%, 77.5%, 84.6% and 89.7%, respectively), then the activity of compounds 11 and 13 started to decrease. Compounds (5 and 13) revealed the most prominent anti-inflammatory effect after 30 min (67.7% and 69.0%).

Table 3. It was noticed that compounds (6, 8, 10, 11, 12 and 16) in oral doses of 20 mg/kg significantly decreased the inflammation starting from the first hour and it was persistent till the end of the experiment. The anti-inflammatory effect of indomethacin on paw edema was 32.78, 26.35, 29.02, and 27.45% at the 1st, 2nd, 3rd and 4th hour, respectively. It was noticeable that compound 13 failed to decrease inflammation all over the experimental period. Moreover, the compounds (7 and 5) failed to decrease inflammation at the 1st hour, while compound 4 failed to decrease inflammation at the 1st and 2nd hours. It is noteworthy to mention that the derivatives 10, 12, and 16 showed anti-inflammatory potency after 4 hours greater than that of indomethacin and reached the maximum effect at the 2nd h.

**Molecular docking:** In an attempt to understand both the anti-inflammatory and analgesic data on a structural basis, molecular docking studies were carried out using Molsoft ICM 3.8 software. The aim of the flexible docking calculations is prediction of correct binding geometry for each binder. The scoring functions and hydrogen bonds formed with the surrounding amino acids of the receptor. The new compounds which scores the highest anti-inflammatory activates 10, 12, and 16 were docked against the active site of COX-1 and COX-2 enzymes. Indomethacin was also docked against both COX-1 and COX-2 enzymes. The scoring functions of the complexes were calculated from minimized ligand protein complexes. The docking results revealed that all the tested compounds showed

### Scheme 4: Reagents and conditions: (a) toluene, reflux, 5 h 15 (b) malonic acid, acetyl chloride, reflux, 10 h 16.

**Citation:** Eweas AF, El-Nezhawy AOH, Abdel-Rahman RF, Baiuomy AR (2015) Design, Synthesis, In Vivo Anti-inflammatory, Analgesic Activities and Molecular Docking of Some Novel Pyrazolone Derivatives. Med chem 5: 458-466. doi:10.4172/2161-0444.1000301
The newly synthesized pyrazolone derivatives were found to possess potent analgesic and anti-inflammatory activities. The results showed that the central analgesic potencies of the tested compounds indicated non-selective inhibition of both COX-1 and COX-2 enzymes. Results of their interaction energies with COX-1 and COX-2 are shown in Table 4.

The docking results revealed that all tested compounds 10, 12 and 16 bound nicely to the active site of both cyclooxygenase enzymes I and II, scoring binding energies ranging from -26.89 to -46.21 Kcal/mol for COX-I, and -30.96 to -43.44 Kcal/mol for COX-II. Compared to -14.76 and -26.36 Kcal/mol for indomethacin reference drug for both COX-I and COX-II respectively, details of the interactions and H-bonding between tested compounds and COX enzymes are listed in (Figures 2a-2d and 3a-3d). The ICM score values show good agreement with predicted binding affinities obtained by molecular docking studies as verified by pharmacological screening.

Conclusion

In this work, we have prepared a series of new pyrazolone derivatives in combination with pyrazole and dihydropyrimidinone scaffold, as well as heteroaryl and aryl pyrazole derivatives. The analgesic and anti-inflammatory activities were investigated for the title compounds utilizing the acetic acid-induced writhing test and the carrageenan-induced hind paw edema test, respectively.

The newly synthesized pyrazolone derivatives were found to possess potent analgesic and anti-inflammatory activities. The results showed that the central analgesic potencies of the tested compounds

| Group | 1 hour | 2 hours | 3 hours | 4 hours |
|-------|--------|---------|---------|---------|
| Edema rate (%) | Potency (%) | Edema rate (%) | Potency (%) | Edema rate (%) | Potency (%) | Edema rate (%) | Potency (%) |
| Control | 68.0 ± 2.51 | 68.0 ± 2.51 | 68.0 ± 2.51 | 68.0 ± 2.51 |
| 4 | 43.0 ± 4.15b (9.07) | 43.0 ± 4.15b (9.07) | 43.0 ± 4.15b (9.07) | 43.0 ± 4.15b (9.07) |
| 5 | 43.0 ± 4.15b (9.07) | 43.0 ± 4.15b (9.07) | 43.0 ± 4.15b (9.07) | 43.0 ± 4.15b (9.07) |
| 6 | 43.0 ± 4.15b (9.07) | 43.0 ± 4.15b (9.07) | 43.0 ± 4.15b (9.07) | 43.0 ± 4.15b (9.07) |
| 7 | 43.0 ± 4.15b (9.07) | 43.0 ± 4.15b (9.07) | 43.0 ± 4.15b (9.07) | 43.0 ± 4.15b (9.07) |
| 8 | 43.0 ± 4.15b (9.07) | 43.0 ± 4.15b (9.07) | 43.0 ± 4.15b (9.07) | 43.0 ± 4.15b (9.07) |
| 9 | 43.0 ± 4.15b (9.07) | 43.0 ± 4.15b (9.07) | 43.0 ± 4.15b (9.07) | 43.0 ± 4.15b (9.07) |
| 10 | 43.0 ± 4.15b (9.07) | 43.0 ± 4.15b (9.07) | 43.0 ± 4.15b (9.07) | 43.0 ± 4.15b (9.07) |
| 11 | 43.0 ± 4.15b (9.07) | 43.0 ± 4.15b (9.07) | 43.0 ± 4.15b (9.07) | 43.0 ± 4.15b (9.07) |
| 12 | 43.0 ± 4.15b (9.07) | 43.0 ± 4.15b (9.07) | 43.0 ± 4.15b (9.07) | 43.0 ± 4.15b (9.07) |
| 13 | 43.0 ± 4.15b (9.07) | 43.0 ± 4.15b (9.07) | 43.0 ± 4.15b (9.07) | 43.0 ± 4.15b (9.07) |
| 14 | 43.0 ± 4.15b (9.07) | 43.0 ± 4.15b (9.07) | 43.0 ± 4.15b (9.07) | 43.0 ± 4.15b (9.07) |
| 15 | 43.0 ± 4.15b (9.07) | 43.0 ± 4.15b (9.07) | 43.0 ± 4.15b (9.07) | 43.0 ± 4.15b (9.07) |
| 16 | 43.0 ± 4.15b (9.07) | 43.0 ± 4.15b (9.07) | 43.0 ± 4.15b (9.07) | 43.0 ± 4.15b (9.07) |

Each value in parenthesis indicates the percentage inhibition rate, *P<0.05: Statistically significantly from control (Dunnett’s test). ǂP<0.05: Statistically significant from acetyl salicylic acid (Dunnett’s test).

Table 2: Peripheral analgesic activity of compounds (4, 5, 6, 7, 8, 9, 10, 11, 12, 13 and 16) in mice. Peripheral pain was induced in Albino mice by acetic acid as detailed in the Materials and Methods section. Animals were treated with the test and control compounds and the analgesic activity was determined after 30, 60 and 90 min and compared to the controls. Data are shown as mean ± SEM.

| Group | 1 hour | 2 hours | 3 hours | 4 hours |
|-------|--------|---------|---------|---------|
| Edema rate (%) | Potency (%) | Edema rate (%) | Potency (%) | Edema rate (%) | Potency (%) | Edema rate (%) | Potency (%) |
| Control | 68.0 ± 2.51 | 68.0 ± 2.51 | 68.0 ± 2.51 | 68.0 ± 2.51 |
| 10 | 26.0 ± 2.32a (-39.09) | 26.0 ± 2.32a (-39.09) | 26.0 ± 2.32a (-39.09) | 26.0 ± 2.32a (-39.09) |
| 11 | 33.0 ± 2.53 (-22.54) | 33.0 ± 2.53 (-22.54) | 33.0 ± 2.53 (-22.54) | 33.0 ± 2.53 (-22.54) |
| 12 | 28.1 ± 2.39a (-34.14) | 28.1 ± 2.39a (-34.14) | 28.1 ± 2.39a (-34.14) | 28.1 ± 2.39a (-34.14) |
| 13 | 48.8 ± 4.03b (14.64) | 48.8 ± 4.03b (14.64) | 48.8 ± 4.03b (14.64) | 48.8 ± 4.03b (14.64) |
| 14 | 29.3 ± 2.11a (-31.14) | 29.3 ± 2.11a (-31.14) | 29.3 ± 2.11a (-31.14) | 29.3 ± 2.11a (-31.14) |
| 15 | 28.6 ± 2.39a (-32.78) | 28.6 ± 2.39a (-32.78) | 28.6 ± 2.39a (-32.78) | 28.6 ± 2.39a (-32.78) |
| 16 | 28.6 ± 2.39a (-32.78) | 28.6 ± 2.39a (-32.78) | 28.6 ± 2.39a (-32.78) | 28.6 ± 2.39a (-32.78) |

Table 4: Interaction energies of compounds Indomethacin, 10, 12 and 16 with the COX-1 and COX-2 enzymes.
after 90 min, as compared to tramadol, arranged in descending order, were 105.8, 83.7, 80.5, 79.4, 69.4, 69.4, 68.8, 65.5, 44.8 and 37.0% in 5, 6, 4, 13, 8, 11, 10, 12, 16 and 7, respectively. In the acetic acid induced writhing test the selected Compounds showed significant reduction in the number of writhes. The compounds (4, 6, 7, 8, 10, 12 and 13) showed analgesic activity in a percent % (85.3, 89.1, 90.3, 80.0, 76.5, 89.7 and 83.8) which was greater than that of acetyl salicylic acid (71.5%). The selected Compounds were evaluated for their possible anti-inflammatory effects in a rat model of carrageenan-induced paw edema. It was noticed that compounds, (6, 8, 10, 11, 12, and16) in oral doses of 20 mg/kg significantly decreased the paw edema rate all over

Figure 2a: Binding mode of the original ligand indomethacin into binding site of COX-I, showing two H-bonds between H21 of Gln203 with CO of the Carboxyl group of Indomethacin and H2 of His204 with O of the Carbonyl group of Indomethacin.

Figure 2b: Binding mode of compound 10 into binding site of COX-I, showing one H-bond between OH of Tyr130 with NH of the amino group in compound 10.

Figure 2c: Binding mode of compound 12 into binding site of COX-I, showing two H-bonds between NH2 of amide group in Gln461 with O of the sulfoxide group in 12 and one H-bond between O of the carboxyl group in Cys41 with NH of the amino group in 12. In addition to one H-bond between NH of the amino group in Gln44 with N of the CN group in 12.

Figure 2d: Binding mode of compound 16 into binding site of COX-I, showing one H-bond between NH of the amino group in Cys47 with O of the carbonyl group in the pyrazole ring in 16.

Figure 3a: Binding mode of the original ligand indomethacin into binding site of COX-II, showing H-bond between H of OH in Ser530 with O of the carbonyl group in Indomethacin. In addition to two H-bonds between H of the phenolic OH in Tyr385 with CO of the carboxyl group in Indomethacin and O of the phenolic OH in Tyr385 with H of the carboxyl group in Indomethacin.

Figure 3b: Binding mode of compound 10 into binding site of COX-II, showing three H-bonds between Ser530 with NH2 and O of the carbonyl group in 10. In addition to a fourth H-bond between H of the phenolic OH in Tyr385 with O of the carbonyl group in 10.

Figure 3c: Binding mode of compound 12 into binding site of COX-II, showing six H-bonds between Gln203 with N of CN, Trp387 with O1, O2 and N2 in 12, H-bond between Thr206 with H5 in 12. In addition to a H-bond between Tyr385 with H6 of compound 12.
and pharmacological study of ethyl 1-methyl-5-(substituted 3,4-di-hydro-4-oxoquinazolin-3-yl)-1H-pyrazole-4-acetates. Eur J Med Chem 36: 737-742.

16. Abdel-Aziz HA, Al-Rashood KA, ElTahir KEH, Suddekk GM (2014) Synthesis of N-benzensulfonamide-1H-pyrazoles bearing arylsulfonyl moiety: Novel celecoxib analogs as potent anti-inflammatory agents. European journal of medicinal chemistry 80: 416-422.

17. Khalil NA, Ahmed EM, Mohamed KO, Nissan YM, Zaitone SA (2014) Synthesis and biological evaluation of new pyrazolone-pyridazine conjugates as anti-inflammatory and analgesic agents. Bioorg Med Chem 22: 2080-2089.

18. Kumar A, Sharma S, Bajaj K, Bansal D, Sharma D, et al. (2003) Synthesis and anti-inflammatory, analgesic, ulcerogenic, and cyclooxygenase activities of novel quinazolinyl-7-2-pyrazolines. Indian J Chem B 42: 1979-1984.

19. Radzikowska E, Onish K and Chojak K (1995) 1077 Prospective assessment of cancer incidence and anti-phylline metabolism. European Journal of Cancer 31: S225.

20. Khanduja KL, Dogra SC, Kaushal S, Sharma RR (1984) The effect of anti-cancer drugs on pharmacokinetics of anti-phylline in vitamin A deficiency. Biochem Pharmacol 35: 551-560.

21. Mahmoud M, Abdel-Kader R, Hassanein M, Saleh S, Botros S (2007) Anti-phylline clearance in comparison to conventional liver function tests in hepatitis C virus patients. Eur J Pharmacol 569: 222-227.

22. Evstropov A, Yavorovskaya V, Vorob’ev E, Khudonogova Z, Gritsenko L, et al. (1992) Synthesis and antiviral activity of anti-phylline derivatives. Pharmaceutical Chemistry Journal 26: 428-430.

23. Michon V, du Penhoat CH, Tom Bret F, Gillardin J, Lepage F, et al. (1995) Preparation, structural analysis and anticonvulsant activity of 3-and 5-aminopyrazole N-benzoyl derivatives. European journal of medicinal chemistry 30: 147-155.

24. Isip E, Toroglu S and Kayralidiz A (2008) Syntheses, characterization, antimicrobial and genotoxic activities of new Schiff bases and their complexes. Transition metal chemistry 33: 953-960.

25. Comber RN, Grey RJ, Secrist JA 3rd (1991) Acyclic analogues of pyrazofurin: syntheses and antiviral evaluation. Carbohydr Res 216: 441-452.

26. Eweas A, El-Nezhawy AO, Gaballah ST, Radwan MA, Baioumy AR, Abdel-Salam OM (2009) Structure-based design of benzimidazole sugar conjugates: synthesis, SAR and in vivo anti-inflammatory and analgesic activities. Med Chem 5: 558-569.

27. Eweas AF, El-Nezhawy AO, Baioumy AR and Awad MM (2013) Design, synthesis, anti-inflammatory, analgesic screening, and molecular docking of some novel 2-pyridyl (3H)-quinazolin-4-one derivatives. Medicinal Chemistry Research 22: 1011-1020.

28. El-Nezhawy AO, Baioumy AR, Hassaan FS, Isamal AK, Omar HA (2013) Design, synthesis and pharmacological evaluation of omeprazole-like agents with anti-inflammatory activity. Bioorg Med Chem 21: 1661-1670.

29. Turner RA (2013) Screening methods in pharmacology. Elsevier.

30. Collier HO, Dinneen LC, Johnson CA, Schneider C (1968) The abdominal distention reaction and its suppression by analgesic drugs in the mouse. Br J Pharmacol Chemother 32: 295-310.

31. Winter CA, Risley EA, Nuss GW (1962) Carragenen-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc Soc Exp Biol Med 111: 544-547.

32. Fathalla OA, Zaki ME, Swelam SA, Nofal SM, el-Eraky WI (2003) Facile synthesis of fused pyrazolo[1,5-a]pyrimidinepyrazolo [1,5-a]brazines and N-sulphonamidopyrazoles as anti-inflammatory. Acta Pol Pharm 60: 51-60.