Journal of Pharmaceutical Research International

33(28B): 220-231, 2021; Article no.JPRI.68692
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Design, Synthesis, Characterization, Computational Study and *In-vitro* Antioxidant and Anti-inflammatory Activities of Few Novel Pyrazol-3-one Derivatives

Aziz Unnisa[1], B. Anupama[2*], Humera Banu[3], Syeda B. Fatima[3], K. N. V. Chenchu Lakshmi[4] and Rahamat Unissa[5]

[1]Department of Pharmaceutical Chemistry, College of Pharmacy, University of Hail, Hail, Saudi Arabia.
[2]Department of Pharmaceutical Chemistry, KVSR Siddhartha College of Pharmaceutical Sciences, Andhra Pradesh, India.
[3]Department of Clinical Nutrition, College of Applied Medicine, University of Hail, Hail, Saudi Arabia.
[4]Department of Pharmaceutical Chemistry, QIS College of Pharmacy, Andhra Pradesh, India.
[5]Department of Pharmaceutics, College of Pharmacy, University of Hail, Hail, Saudi Arabia.

Authors' contributions

This work was carried out in collaboration among all authors. Authors AU and BA designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors HB and SBF managed the analyses of the study. Authors RU and KNVCL managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i28B31556
Editor(s):
(1) Rafik Karaman, Al-Quds University, Palestine.
Reviewers:
(1) Estabraq A. Mahmoud, College of Education for pure Sciences Ibn Al-Haitham, University of Baghdad, Iraq.
(2) Josie Naomi Iyeyasu, State University of Campinas, Brazil.
Complete Peer review History: http://www.sdiarticle4.com/review-history/68692

Original Research Article

Received 10 March 2021
Accepted 13 May 2021
Published 14 May 2021

ABSTRACT

Aim: To design, synthesize and perform computational study on a few Novel pyrazol-3-one derivatives.
Study design: Experimental study.
Methodology: A series of 6-aryl substituted pyrimidine azodyes were synthesized by coupling phenyl pyrimidine 2-amine with different aromatic amines. The synthetic compounds were screened for their in-vitro antioxidant and anti-inflammatory activities. The Computational study of

**Corresponding author: E-mail: anurochi8@gmail.com;*

designed compounds was done by OCHEM, Molinspiration cheminformatics, Datawarrior, and Swiss ADME. DPPH assay was used to determine the antioxidant activity and heat hemolysis method for anti-inflammatory activity.

Results: Molinspiration, Data Warrior, Ochem which are helpful to predict molecule general properties, bioactive scores, toxicity, and drug-likeness. Data Warrior results inferred that the compounds possess moderately active towards mutagenic (compound 2, 11), reproductive (compound 6, 7, 8), and highly active towards Tumorogenic (compound 2) toxicities. OCHEM results showed that most of the synthesized compounds were found to be non-inhibitors of all the subtypes of cytochrome P450 except compound 8. All compounds under this study were effective scavengers of free radicals except the compounds 1, 2, 6, 10. *Invitro* Anti-inflammatory studies have shown that the compounds (6, 7, 8, 9, 13) active toward heat hemolysis.

Conclusion: The synthesized compounds were comprehensively studied and targets were identified rendering them as lead molecules for further development of newer agents with greater efficacy and safety.

Keywords: Pyrazol-3-one derivatives; molinspiration; data warrior; ochem; swiss ADME.

1. INTRODUCTION

Heterocyclic compounds are cyclic compounds that have atoms of at least two different elements as members of their ring [1]. A pyrazolone is a group of heterocyclic compounds having great importance because of their broad spectrum of biological activities and their wide-ranging use as synthetic tools. Pyrazolone is a five-membered lactam ring containing two adjacent nitrogens and a keto group in its structure [2].

Pyrazolone is an interesting template for medicinal, and combinatorial chemistry because of its simple synthesis and wide range of biological activities which include The literature review revealed the studies conducted for various pharmacological activities of pyrazoles such as cytotoxic activity [3,4,5], antimicrobial activity [6,7,8], antioxidant activity [3,9], anti-Inflammatory activity [10,11], analgesic activity [12,13], Sars-Corona virus 3c-Like protease inhibitors [14], hypoglycemic activity [15,16], antiviral activity [17,18]. Pyrazolone was first reported by Knorr in 1883 who also reported its derivative- Antipyrine which was approved for clinical use. Many other NSAIDs"s like oxyphenbutazone, phenylbutazone, propyphenazone, aminophenazone, etc. contain pyrazolone as their basic ring and are widely used as anti-inflammatory, analgesic, and antipyretic drugs. However, the clinical use of pyrazolones is not free of controversies because of their association with serious side effects like gastrointestinal irritation, ulceration, etc. but their benefits and a range of clinical applications have kept the interest of the research community alive.

Given these observations, the present study was aimed to synthesize newer (4E)-2-acetyl-4-benzylidene-5-methyl-2,4-dihydro-3H-pyrazole-3-one derivatives. In-silico evaluation serves as a key tool in computer-assisted drug design to predict ADME properties by using Swiss ADME and investigate the targets by Swiss target prediction for the designed pyrazolone derivatives. Additionally, Lipinski parameters and BOILED egg (Brain or Intestinal Estimate permeation method) were also evaluated for the synthesis of titled compounds

2. EXPERIMENTAL DETAILS

2.1 Procedure for the Synthesis of (4E)-2-acetyl-4-benzylidene-5-methyl-2, 4-dihydro-3H-Pyrazol-3-one derivatives (Compounds 1-13)

2.1.1 The method employed was one-pot synthesis (Knovenegal condensation)

An equimolar mixture of hydrazine dichloride, ethyl acetoacetate, acetic anhydride, acetic acid, and aromatic aldehyde was taken in a conical flask and stirred with a magnetic stirrer. The temperature of the reaction mixture was maintained at 60º c. The reaction mixture formed after continuous stirring for 30 mins was transferred into a beaker containing crushed ice and neutralized with sodium bicarbonate. The product was precipitated and the precipitate was filtered, washed, dried, and weighed. The obtained products were recrystallized with methanol. The progress of the reaction was assessed by thin-layer chromatography and the purity of the compounds was ensured by melting points and thin-layer chromatography. The compounds synthesized were characterized by spectroscopic techniques [18].

2.2 Characterisation of Synthesised (4E)-2-acetyl-4-benzylidene-5-methyl-2,4-dihydro-3H-pyrazol-3-one Derivatives

2.2.1 Compound-1 (4E)-2-acetyl-5-methyl-4-[(2-nitrophenyl) methylidene]-2,4-dihydro-3H-pyrazol-3-one

Molecular Formula: $C_{13}H_{11}N_3O_4$, Molecular Weight: 273.24, Melting Point: 160^0c, Percentage yield: 69.8%, Elemental Composition: C (57.14%) H (4.06%) N (15.38%) O (23.42%), Lipinski rule: yes, Rf value: 0.7, IR data: FTIR (γ max, cm-1), 1702(-C=O stretch), 1552(-NO_2), 1519(-C=N), 1200(-C-N stretch).

2.2.2 Compound 2 (4E)-2-acetyl-5-methyl-4-[(3-nitrophenyl) methylidene]-2,4-dihydro-3H-pyrazol-3-one

Molecular Formula: $C_{13}H_{11}N_3O_4$, Molecular Weight: 273.24, Melting Point: 170^0c, Percentage yield: 71.2%, Elemental Composition: Composition: C (57.14%) H (4.06%) N (15.38%) O (23.42%), Lipinski rule: yes, Rf value: 0.65, IR data: FTIR (γ max, cm-1), 1785(-C=O stretch), 1523(-NO_2), 1346(-C=N), 1199(-C-N stretch).

2.2.3 Compound 3: (4E)-2-acetyl-5-methyl-4-[(3,4,5-trimethoxyphenyl) methylidene]-2,4-dihydro-3H-pyrazol-3-one

Molecular Formula: $C_{16}H_{18}N_2O_5$, Molecular Weight: 318.32, Melting Point: 130^0c, Percentage yield: 89%, Elemental Composition: C (60.37%) H (5.70%) N (8.80%) O (25.13%), Lipinski rule: yes, Rf value: 0.7, IR data: FTIR (γ max, cm-1), 1738 (-C=O stretch), 1501 (-NO_2), 1339 (-C=N), 1119 (-C-N stretch).

2.2.4 Compound 4: (4E)-2-acetyl-4-[(3,4-dimethoxyphenyl) methylidene]-5-methyl-2,4-dihydro-3H-pyrazol-3-one:

Molecular Formula: $C_{15}H_{16}N_2O_4$, Molecular Weight: 288.3, Melting Point: 150^0c, Percentage yield: 78%, Lipinski rule: yes, Elemental Composition: C (62.49%) H (5.59%) N (9.72%) O (22.20%), IR data: FTIR (γ max, cm-1), 1681(-C=O stretch), 1574(-NO_2), 1266(-C=N), 1134(-C-N stretch), NMR data: 7.232-7.588 (aromatic protons), 6.609 (C=CH), 3.292-3.545 (OCH_3)1.16-1.42 (methyl), Mass Spectrum: base peak 203 m/z.

2.2.5 Compound 5: (4E)-2-acetyl-4-[(4-hydroxyphenyl) methylidene]-5-methyl-2,4-dihydro-3H-pyrazol-3-one

Molecular Formula: $C_{13}H_{12}N_2O_3$, Molecular Weight 244.25, Melting Point: 160^0c, Percentage yield: 57%, Elemental Composition: C (62.49%) H (5.59%) N (9.72%) O (22.20%), Lipinski rule: yes, IR data: FTIR (γ max, cm-1), 1649 (-C=O stretch), 1273 (-NO_2), 1159 (-C=N), 1026 (-C-N stretch).

2.2.6 Compound 6: (4E)-2-acetyl-4-[(3-methoxyphenyl) methylidene]-5-methyl-2,4-dihydro-3H-pyrazol-3-one:

Molecular Formula: $C_{14}H_{14}N_2O_3$, Molecular Weight: 258.27, Melting Point: 60^0c, Percentage yield: 87%, Elemental Composition: C (65.11%) H (5.46%) N (10.85%) O (18.58%), Lipinski rule: yes, Rf value: 0.7, IR data: FTIR (γ max, cm-1), 3360 (-O-H Stretch), 1692 (-C=O), 1510 (-C=N), 1452 (-C=C), 1257 (-C-N)

2.2.7 Compound 7: (4E)-2-acetyl-4-benzylidene-5-methyl-2,4-dihydro-3H-pyrazol-3-one

Molecular Formula: $C_{13}H_{12}N_2O_2$, Molecular Weight: 228.25, Melting Point: 160^0c

Percentage yield: Elemental Composition: C (68.41%) H (5.30%) N (12.27%) O (14.02%), Lipinski rule: yes, Rf value: 0.7, IR data: FTIR (γ max, cm-1), 3031(-C-H Stretch), 1713 (-C=O),1532 (-C=N), 1495 (-C=C), 1254 (-C-N stretch).

2.2.8 Compound 8: (4E)-2-acetyl-5-methyl-4-[(2Z)-3-phenylprop-2-en-1-ylidene]-2,4-dihydro -3H-pyrazol-3-one

Molecular Formula: $C_{15}H_{14}N_2O_2$, Molecular Weight: 254.28, Melting Point: 110^0c, Percentage yield: 77%, Elemental Composition: C (70.85%) H (5.55%) N (11.02%) O (12.58%), Lipinski rule: yes, Rf value: 0.6, IR data: FTIR (γ max, cm-1), 1642(-C=O), 1598 (-C=N), 1453 (-C=C), NMR data: 7.168-7.585 (aromatic protons), 1.40 (methyl), Mass Spectrum: base peak 208 m/z.

2.2.9 Compound 9: (4Z)-2-acetyl-4-[(furan-2-yl) methylidene]-5-methyl-2,4-dihydro-3H-pyrazol-3-one

Molecular Formula: $C_{11}H_{10}N_2O_3$, Molecular Weight: 218.21, Melting Point: 90^0c, Percentage

yield: 67%, Elemental Composition: C (60.55%) H (4.62%) N (12.84%) O (22.00%), Lipinski rule: yes, Rf value: 0.7, IR data: FTIR (γ max, cm-1), 1702 (-C=O), 1631 (-C=N), 1503 (-C=C), 1217 (-C-O-C).

2.2.10 Compound 10: (4E)-2-acetyl-4-{[4-(dimethylamino) phenyl]methylidene}-5-methyl-2,4-dihydro-3H-pyrazol-3-one

Molecular Formula: $C_{15}H_{17}N_3O_2$, Molecular Weight: 271.31, Melting Point: 120^0c, Percentage yield: 86%, Elemental Composition: C (66.40%) H (6.32%) N (15.49%) O (11.79%), Lipinski rule: yes, Rf value: 0.8, IR data: FTIR (γ max, cm-1), 1785 (-C=O stretch), 1523 (-NO2), 1346 (-C=N), 1199 (-C-N stretch).

2.2.11 Compound 11: (4E)-2-acetyl-4-[(2-hydroxyphenyl) methylidene]-5-methyl-2,4-dihydro-3H-pyrazol-3-one

Molecular Formula: $C_{13}H_{12}N_2O_3$, Molecular Weight: 244.25, Melting Point: 125^0c, Percentage yield: 75%, Elemental Composition: C (63.93%) H (4.95%) N (11.47%) O (19.65%), IR data: FTIR (γ max, cm-1), 3360(-O-H Stretch), 1692 (-C=O), 1510 (-C=N), 1452 (-C=C), 1257 (-C-N).

2.2.12 Compound 12: (4Z)-2-acetyl-5-methyl-4-[(thiophen-2-yl) methylidene]-2,4-dihydro-3H-pyrazol-3-one

Molecular Formula: C11H10N2O2S, Molecular Weight: 234.27, Melting Point: 75^0c, Percentage yield: 67%, Elemental Composition: C (56.39%) H (4.30%) N (11.96%) O (13.66%) S (13.69%), IR data: FTIR (γ max, cm-1), 1739 (-C=O stretch), 1523 (-NO2), 1368 (-C=N), 1036 (-C-N stretch).

2.2.13 Compound 13: (4E)-2-acetyl-4-[(4-hydroxy-3-methoxyphenyl) methylidene]-5-methyl-2,4-dihydro-3H-pyrazol-3-one

Molecular Formula: $C_{14}H_{14}N_2O_4$, Molecular Weight: 274.27, Melting Point: 130^0c, Percentage yield: 91%, Elemental Composition: C (63.93%) H (4.95%) N (11.47%) O (19.65%), IR data: FTIR (γ max, cm-1), 1721 (-C=O stretch), 1407 ($-NO_2$), 1262 (-C=N), 1139 (-C-N stretch).

2.3 Procedure for Prediction of Physico-Chemical Properties and *In-silico* Evaluation of (4E)-2-acetyl-4-benzylidene-5-methyl-2,4-dihydro-3H-pyrazol-3-one Derivatives [19,20]

2.3.1 Molinspiration [18]

Molinspiration offers a broad range of Cheminformatics software tools supporting molecule manipulation and processing, including SMILES and SD file conversion, normalization of molecules, generation of tautomer's, molecule fragmentation, calculation of various molecular properties needed in QSAR, molecular modeling and drug design, high-quality molecule depiction, molecular database tools supporting substructure and similarity searches. Our products support also fragment-based virtual screening, bioactivity prediction, and data visualization.

Molinspiration tools are written in Java, therefore can be used practically on any computer platform. The software is used for the calculation of important molecular properties like logP, polar surface area, Number of hydrogen bond donors, Number of hydrogen bond acceptors, Number of rotatable bonds, Volume, Number of violations from the rule of five. It is used to predict bioactive scores for the most important drug targets like GPCR ligand, Kinase inhibitors, Ion channel modulators, nuclear receptors, Protease inhibitors, Enzyme inhibitors.

2.3.2 Data warrior [21]

Data Warrior combines dynamic graphical views and interactive row filtering with chemical intelligence. Scatter plots, box plots, bar charts, and pie charts not only visualize numerical or category data but also show trends of multiple scaffolds or compound substitution patterns. Chemical descriptors encode various aspects of chemical structures, e.g. the chemical graph, chemical functionality from a synthetic chemist's point of view, or 3-dimensional pharmacophore features. These allow for fundamentally different types of molecular similarity measures, which can be applied for many purposes including row filtering and the customization of graphical views.

Data Warrior supports the enumeration of combinatorial libraries as the creation of evolutionary libraries. Compounds can be clustered and diverse subsets can be picked. Calculated compound similarities can be used for

multidimensional scaling methods, e.g. Kohonen nets. Physicochemical properties can be calculated, structure-activity relationship tables can be created and activity cliffs be visualized.

2.3.3 OCHEM [22]

The Online Chemical Modeling Environment is a unique and web-based platform on the Web that aims to automate and simplify the typical steps required for QSAR modeling. The platform consists of two major subsystems: the database of experimental measurements and the modeling framework. The database is user-contributed and contains a set of tools for easy input, search, and modification of thousands of records.

The OCHEM database is based on the wiki principle and focuses on data quality and verification. The database is tightly integrated with the modeling framework, which supports all the steps required to create a predictive model: data search, calculation, and selection of a vast variety of molecular descriptors, application of machine learning methods, validation, analysis of the model, and assessment of the applicability domain.

2.3.4 Swiss ADME [23]

A free web tool to evaluate pharmacokinetics, drug-likeness, and medicinal chemistry friendliness of small molecules. The software used in the processes of drug discovery and development where a large number of molecular structures are evaluated according to very diverse parameters to steer the selection of which chemicals to synthesize, test, and promote, with the final goal to identify those with the best chance to become an effective medicine for the patients.

The ADME parameters (for Absorption, Distribution, Metabolism, and Excretion) can be evaluated separately by dedicated methods. It has been demonstrated that early estimation of ADME in the discovery phase reduces drastically the fraction of pharmacokinetics-related failure in the clinical phases. Computer models have been fostered as a valid alternative to experimental procedures for the prediction of ADME, especially at initial steps, when investigated chemical structures are numerous but the availability of compounds is scarce.

The Swiss ADME web tool is freely accessible and meant for user-friendly submission and easy analysis of the results, also for a non-expert in CADD. Swiss ADME includes different input methods, computation for multiple molecules, and the possibility to display, save and share results per individual molecule or through global intuitive and interactive graphs. Finally, Swiss ADME is integrated into the Swiss Drug Design workspace

Swiss target Prediction has been primarily developed for identifying targets of molecules known to be bioactive. Swiss target Prediction will suggest some targets, based on the assumption that if the molecule is active, it will likely bind to some protein. For molecules with unknown bioactivity, this assumption is not valid per the molecule may not bind to any protein, in which case all predicted targets are false positives. In particular, inactive compounds can sometimes exhibit good similarity with active molecules if they have been obtained by modifying an active compound at some key position that was crucial for its interactions. This is a known limitation of ligand-based approaches when applied to any kind of compounds and therefore target predictions should be interpreted with care in the absence of indication of bioactivity.

2.4 *In-Vitro* Study of the Pharmacological Activities

2.4.1 Anti-inflammatory activity: (By Heat hemolysis method) [24,25]

In-Vitro Anti-Inflammatory Activity was performed using the human red blood cell (HRBC) membrane stabilization method. The blood was collected from a healthy human volunteer who had not taken any non-steroidal anti-inflammatory drugs for 2 weeks before the experiment and mixed with saline solution and centrifuged at 3,000 rpm. Various concentrations of compounds were prepared (100,250 and 500 µg/ml) using methanol to obtain 3ml and 3 ml of HRBC suspension were added. It was incubated at 60℃ for 30 min and centrifuged at 3,000 rpm for 20 min. and the hemoglobin content of the supernatant solution was estimated on a UV spectrophotometer at 560 nm. Aspirin (100 and 250,500 µg/ml) was used as the reference standard and HRBC was used as control.

The % inhibition of hemolysis was determined using the below equation.

%Inhibition = (O.D of Control-O. D of the test)/ (O.D of control)

2.4.2 Antioxidant activity using 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) free radical scavenging assay [26]

0.1 mM DPPH solution was prepared in methanol. The test samples (1-13) were dissolved in methanol. The concentrations of test solutions were 2.5, 5, 7.5, and 10 µg/ml. 1 mg/ml solutions of Ascorbic acid (1ml) were prepared in methanol and add equal volumes of DPPH solution (1ml). This mixture was treated as a standard solution. DPPH solution was treated as control. Equal volumes of test solution (1ml) and DPPH solution (1ml) were transferred into 2 ml centrifuges and incubated for 30 minutes at 37^0 c in dark conditions. After incubation absorption was measured at 517 nm using a visible spectrophotometer. Percentage inhibition of test samples (1-13) was determined in comparison with the control by using the following formula:

% inhibition = (O.D of Control-O. D of test)/ (O.D of control) X 100

IC-50 values were determined from the % Inhibition and were tabulated.

3. RESULTS AND DISCUSSION

The titled compounds (4E)-2-acetyl-4-benzylidene-5-methyl-2, 4-dihydro-3H-pyrazol-3-one derivatives (C1-C13) were synthesized by one-pot condensation of ethyl acetoacetate, hydrazine dichloride, and aromatic aldehydes under acidic condition. Thirteen compounds were synthesized by condensation reaction (figure 1) and were characterized by IR, NMR spectroscopic methods. The IR spectra revealed the presence of CH stretching, carbonyl peak in the range 1650-1700^{-1}, C=N peak between 1500-1550 cm^{-1}. NMR spectra revealed the presence of aromatic protons and the presence of C=C protons in the region between 6.5-8 ppm.

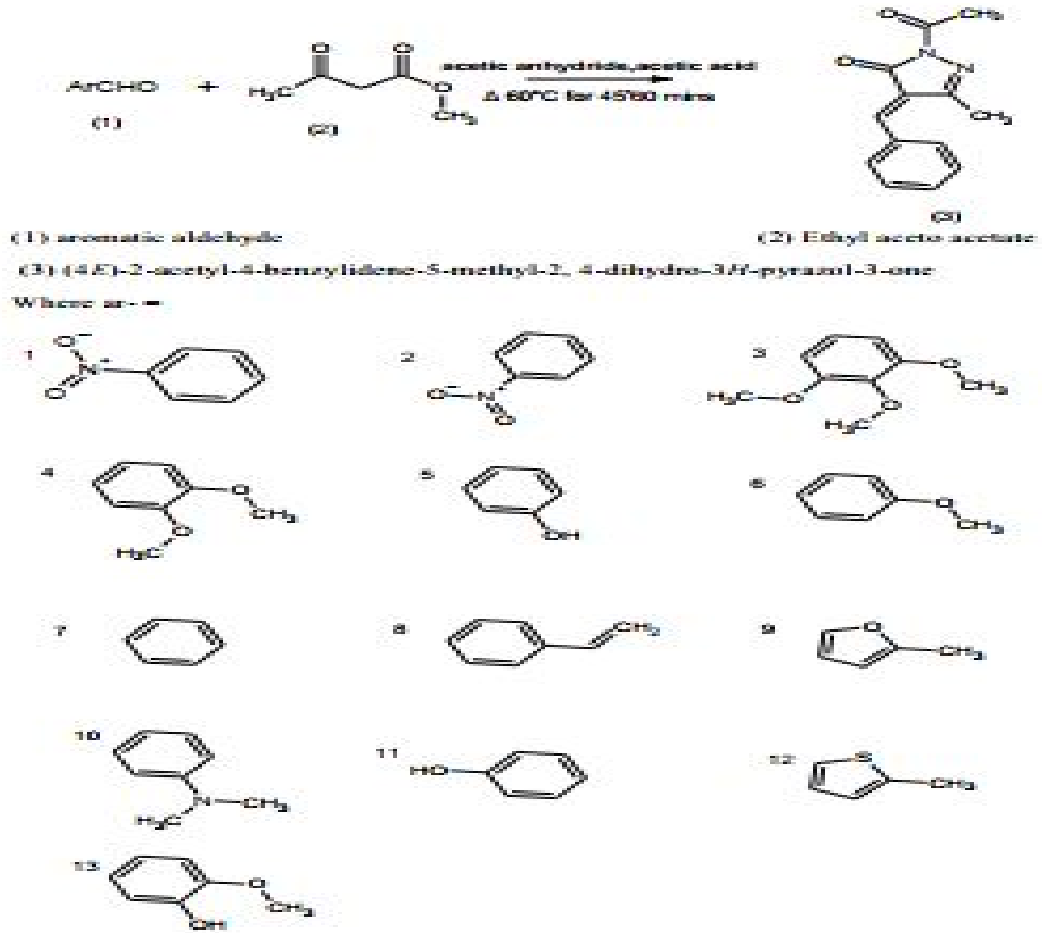

Fig. 1. Scheme and structures of the synthesized (4E)-2-acetyl-4-benzylidene-5-methyl-2, 4-dihydro-3H-pyrazol-3-one derivatives

From the in-silico evaluation of the synthesized compounds, it was observed that compounds obey Lipinski rule of five with high saturation and lower values of flexibility, size, polarity, and solubility and found to be active inhibitors' of kinase, GPCR ligand, and enzyme targets.

The concept of a BOILED egg representing the Brain or Intestina L Estimate D permeation method (Fig. 2) represented that the synthesized compounds can penetrate the blood-brain barrier and are not substrates of PgP carrier.

The derivatives synthesized were evaluated by three software's: Molinspiration, Data Warrior, Ochem which are helpful to predict molecule general properties, bioactive scores, toxicity, and drug-likeness.

The Molinspiration predicted bioactive scores for the titled compounds on the most important drug targets like GPCR ligand, Kinase inhibitors, Ion channel modulators, nuclear receptors, Protease inhibitors, Enzyme inhibitors are given in Table 1.

DATAWARRIOR results inferred that the compounds possess moderately active towards mutagenic (compound 2, 11), reproductive (compound 6, 7, 8), and highly active towards Tumorogenic (compound 2) toxicities (Figs. 3-5).

From the OCHEM results tabulated below in the table. 2, it may be predicted that the drugs might produce their action by acting on sites other than receptor sites or by inhibiting enzyme targets. Most of the synthesized compounds were found to be non-inhibitors of all the subtypes of cytochrome P450 except compound 8 (inhibitors of CYP2C19 & CYP1A2) remaining are non-inhibitors of subtypes.

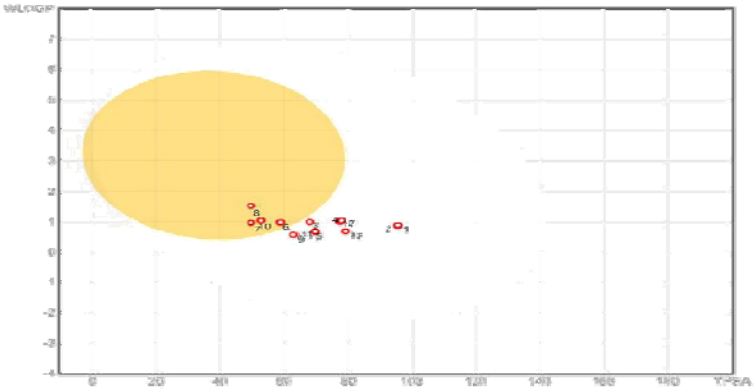

Fig. 2. Brain or intestinal estimated permeation method (BOILED egg) (1-13)

Table 1. Molinspiration Bio activity score (1-13)

Compound	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
1	-1.06	-1.41	-0.59	-0.87	-1.03	-0.55
2	-1.02	-1.43	-0.49	-0.80	-1.06	-0.56
3	-0.71	-1.27	-0.23	-0.61	-0.77	-0.39
4	-0.81	-1.38	-0.31	-0.64	-0.89	-0.43
5	-0.95	-1.43	-0.39	-0.65	-1.05	-0.40
6	-0.97	-1.52	-0.44	-0.76	-1.04	-0.49
7	-1.12	-1.58	-0.54	-0.96	-1.19	-0.53
8	-0.79	-1.18	-0.44	-0.75	-0.86	-0.35
9	-1.49	-2.00	-0.91	-1.51	-1.59	-0.85
10	-0.81	-1.37	-0.27	-0.65	-0.90	-0.42
11	-1.10	-1.46	-0.47	-0.75	-1.12	-0.42
12	-1.37	-1.86	-0.69	-1.53	-1.43	-0.70
13	-0.84	-1.39	-0.31	-0.61	-0.97	-0.39

**If a bioactivity score of more than 0.00 is most likely to possess biological activities*
**If values -0.50 to 0.00 are expected to be moderately active*
**If the score is less than -0.50 it is presumed to be inactive*

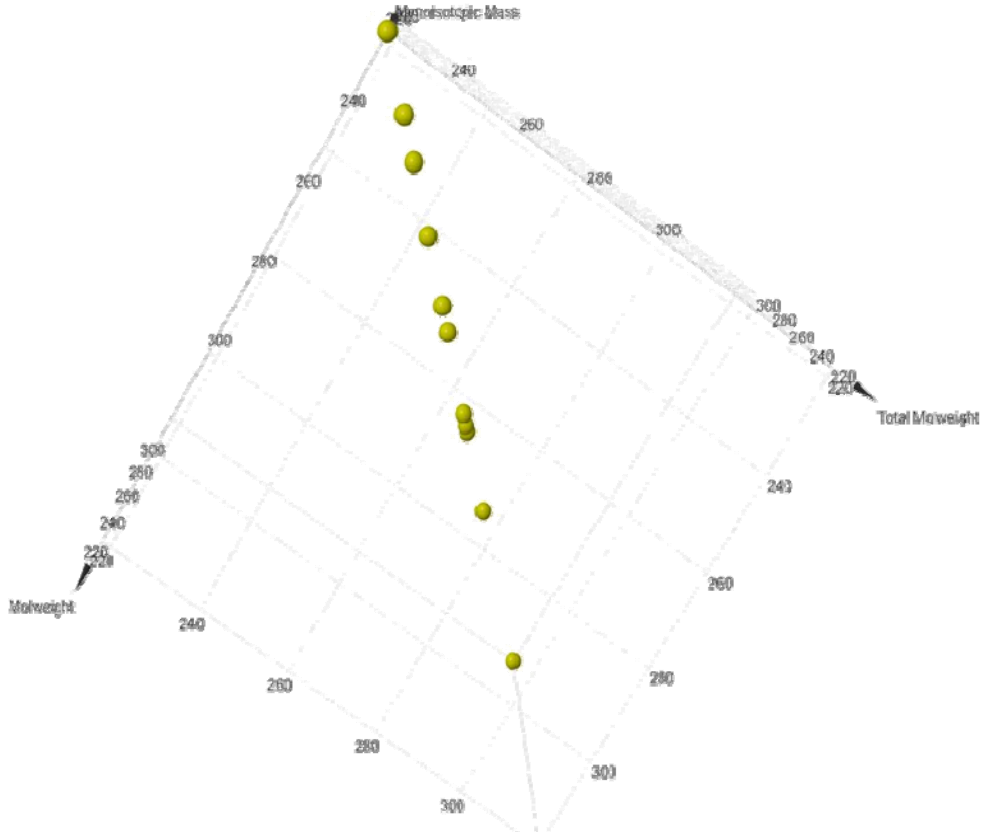

Fig. 3. 3D GRID view of mol. weight; monoisotopic vs total mol. weight (C1-C13)

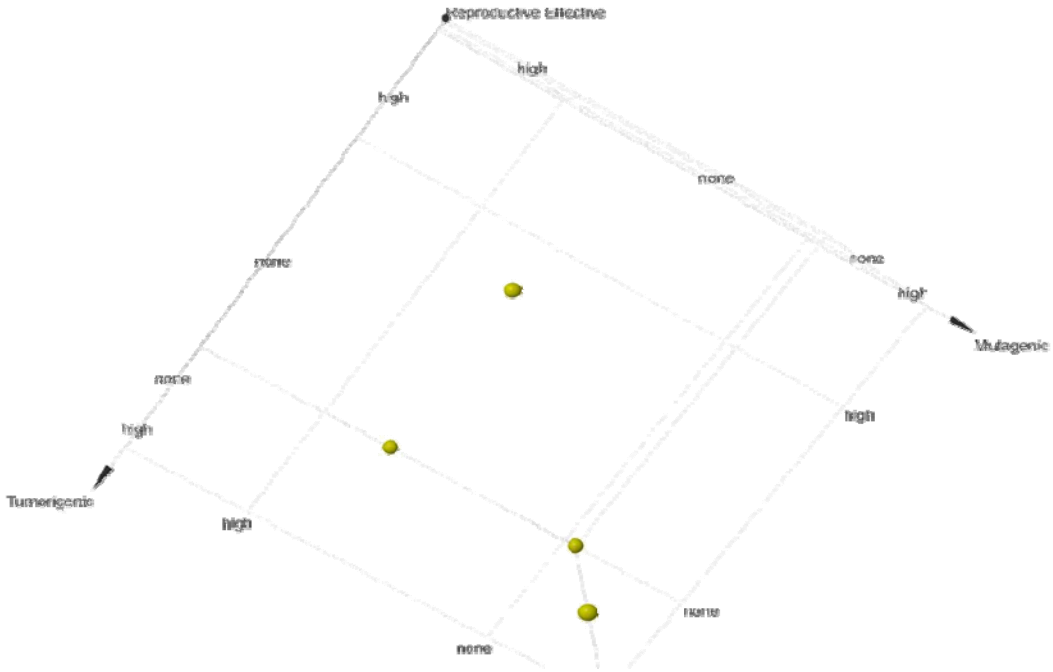

Fig. 4. 3D GRID view of tumorigenic; mutagenic vs. reproductive effect (C1-C13)

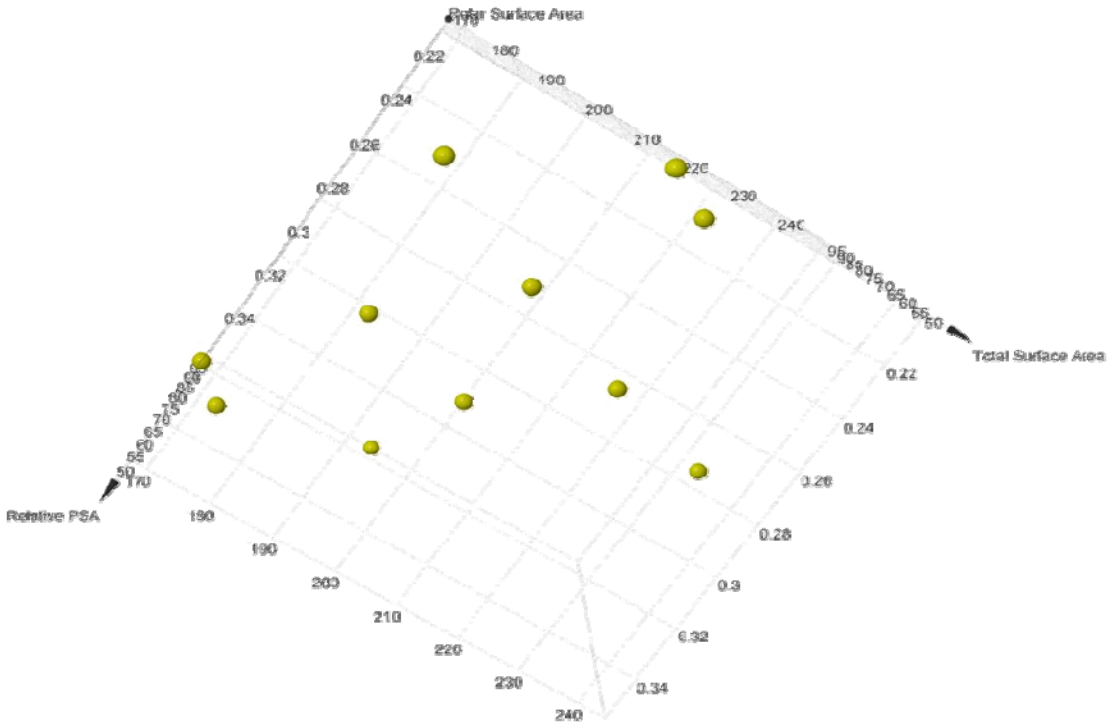

Fig. 5. 3D GRID view of relative PSA; PSA vs. TSA (C1-C13)

Table 2. Ochem: Online chemical modelling (1-13)

Compound	AMES	CYP3A4	CYP2D6	CYP2C19	CYP2C9	CYP1A2
1	Active	-	-	-	-	-
2	Active	-	-	-	-	-
3	Active	-	-	-	-	-
4	Active	-	-	-	-	-
5	Active	-	-	-	-	-
6	Active	-	-	-	-	-
7	Active	-	-	-	-	-
8	Active	-	-	+	-	+
9	Active	-	-	-	-	-
10	Active	-	-	-	-	-
11	Active	-	-	-	-	-
12	Active	-	-	-	-	-
13	Active	-	-	-	-	-

+ Inhibitor, - Non-inhibitor

The scavenging effect of a chemical by the DPPH radical assay method is a quick and reliable parameter to assess the in vitro antioxidant activity. All compounds under this study were effective scavengers of free radicals except the compounds 1, 2, 6, 10. The result has shown that the free radical scavenging activity of these compounds was concentration-dependent. It could be seen that all compounds of the present study are in agreement with Lipinski's Rule of Five, which is important for the further development of these synthesized drugs and their analogs. Furthermore, the replacement of the ring with aromatic phenol sharply enhanced the antioxidant potency. The antioxidant efficacy depends strongly on its reducing property, the compounds 11, 12 might have the higher reducing potential. Due to this extra stabilization, radicals obtained from compounds 11 and 12 would have a higher aptitude to trap free radicals at a faster rate than the other similar type of molecules [26].

Table 3. Results obtained from anti-oxidant activity assay

Compound	2.5 µg/ml	5 µg/ml	7.5 µg/ml	10 µg/ml	IC50 values
Std	37.39	66.08	53.04	25.21	3.93
1	98.26	89.56	96.52	93.04	-
2	87.82	91.30	91.30	91.30	-
3	39.13	40.86	44.34	43.47	18.80
4	46.95	40	45.21	48.69	17.7
5	46.08	50.43	48.69	50.43	8.67
6	48.69	42.60	34.78	50.43	-
7	49.56	48.69	50.43	50.43	7.52
8	43.47	51.30	51.30	47.82	9.17
9	54.78	53.91	52.17	51.30	12.48
10	50.43	53.91	49.56	53.04	-
11	46.95	39.13	26.95	28.69	0.81
12	52.17	49.56	53.04	53.91	0.028
13	53.04	49.56	52.17	50.43	12.5

Table 4. Results obtained from *In-vitro* anti-inflammatory studies

Compound	%Inhibition		
	100 µg/ml	250 µg/ml	500 µg/ml
Control		0.3	
Std	61.2	60.6	56.6
1	79.3	88.3	62.3
2	63.3	71.6	52
3	78	84.3	71
4	83.3	71.6	84
5	79	84.6	-
6	61.6	83	85
7	87.3	88.6	89.6
8	86.6	83.3	91
9	88.3	91	94.3
10	87.6	94	92.3
11	78.3	58.3	25.6
12	89.6	89.6	88.3
13	89.67	93	93.3

The results obtained from anti-inflammatory *In-vitro* studies have shown that the compounds (6, 7, 8, 9, 13) active toward heat hemolysis (Table 4).

4. CONCLUSION

The findings of the study inferred the design, functioning, and target identification of synthesized compounds rendering them as lead molecules for further development of newer agents with greater efficacy and safety.

ACKNOWLEDGEMENTS

This research has been funded by the scientific research deanship at the University of Ha'il – Saudi Arabia through project number RG-191339.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

DISCLAIMER

The reagents and solvents used for this research are commonly and predominantly used ones in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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