Bioorganic & Medicinal Chemistry 19 (2011) 1950–1958

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

A rational approach for the design and synthesis of 1-acetyl-3,5-diaryl-4,5-dihydro(1*H*)pyrazoles as a new class of potential non-purine xanthine oxidase inhibitors

Kunal Nepali [a], Gurinderdeep Singh [a], Anil Turan [b], Amit Agarwal [c], Sameer Sapra [a], Raj Kumar [a,*], Uttam C. Banerjee [c], Prabhakar K. Verma [b], Naresh K. Satti [d], Manish K. Gupta [a], Om P. Suri [a], K. L. Dhar [a]

[a] Laboratory for Drug Design and Synthesis, Department of Pharmaceutical Chemistry, Indo-Soviet Friendship (ISF) College of Pharmacy, Moga-142 001, Punjab, India
[b] Department of Pharmaceutical Sciences, Maharshi Dayanand University, Rohtak, Haryana, India
[c] Department of Pharmaceutical Technology Biotechnology (PTBT), National Institute of Pharmaceutical Education and Research (NIPER), S. A. S. Nagar, Sec 67, 160 062 Punjab, India
[d] Division of Natural Product Chemistry, Indian Institute of Integrative Medicine (IIIM), CSIR, Jammu Tawi 180 001, India

ARTICLE INFO

Article history:
Received 5 January 2011
Revised 27 January 2011
Accepted 28 January 2011
Available online 23 February 2011

Keywords:
1-Acetyl-3,5-diaryl-4,5-dihydro(1*H*)-pyrazoles
Xanthine oxidase
Non-purine xanthine oxidase inhibitors
Molecular docking

ABSTRACT

Xanthine oxidase is a complex molybdoflavoprotein that catalyses the hydroxylation of xanthine to uric acid. Fifty three analogues of 1-acetyl-3,5-diaryl-4,5-dihydro(1*H*)pyrazoles were rationally designed and synthesized and evaluated for in vitro xanthine oxidase inhibitory activity for the first time. Some notions about structure activity relationships are presented. Six compounds **41**, **42**, **44**, **46**, **55** and **59** were found to be most active against XO with IC_{50} ranging from 5.3 μM to 15.2 μM. The compound **59** emerged as the most potent XO inhibitor (IC_{50} = 5.3 μM). Some of the important interactions of **59** with the amino acid residues of active site of XO have been figured out by molecular modeling.

1. Introduction

Catalytic oxidative hydroxylation of purine substrates (hypoxanthine and xanthine) by the action of an enzyme xanthine oxidase (XO), a versatile molybdoflavoprotein to produce uric acid and subsequent reduction of oxygen at the flavin centre with the generation of reactive oxygen species (ROS), either as superoxide anion radical or hydrogen peroxide is well documented.[1–3] The excess level of XO and hence its metabolites such as uric acid and ROS lead to many diseases like gout or at least symptoms of diseases like oxidative damage to the tissues.[2] There is an overwhelming acceptance that XO serum levels are increased in various pathological states like hepatitis, inflammation, ischemia-reperfusion, cancer and aging.[2,3] Therefore the selective inhibition of XO may result in broad spectrum chemotherapeutic for gout, cancer, inflammation and oxidative damage.[2–5] Several purine analogues such as allopurinol,[3,4] (Fig. 1) 2-alkylhypoxanthines (hypoxanthine analogues),[6] pterin and 6-formylpterin (pteridine analogues)[7] were developed as potent XO inhibitors. Nagamatsu et al.[8,9] reported 2-substituted 7*H*-pyrazolo [4,3-*e*]-1,2,4-triazolo-[1,5-*c*]-pyrimidines (**1**) as more potent XO inhibitors than allopurinol.

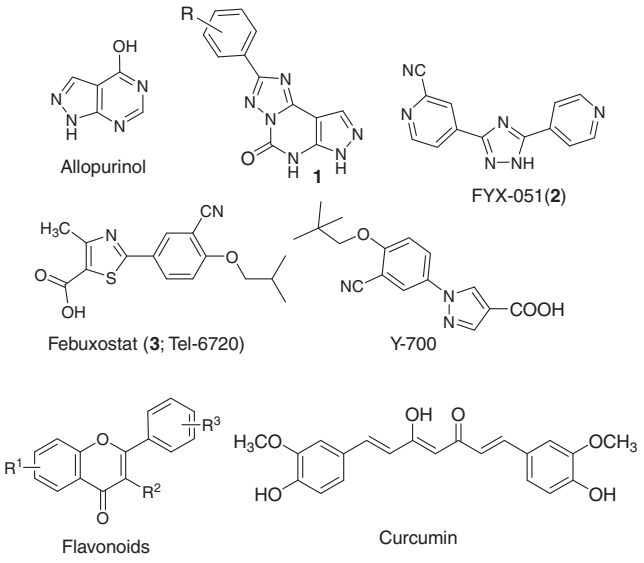


Figure 1. Some reported XO inhibitors.

* Corresponding author. Tel./fax: +91 1636 236564.
E-mail address: raj.khunger@gmail.com (R. Kumar).

0968-0896/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2011.01.058

However the interactions of purine analogue XO inhibitors on activities of purine and pyrimidine metabolism enzymes like guanine deaminase, HGPRT (hypoxanthine–guanine phosphoribosyl transferase), PNP (purine nucleoside phosphorylase), OPRT (orotate phospho-ribosyl transferase) and OMPDC (orotidine-5-monophosphate decarboxylase) leading to the hypersensitivity, (Stevens-Johnsons) a syndrome characterized by fever, skin rash, hepatitis, leukocytosis with eosinophilia and worsening renal function induced in some of the patients[2–4] encouraged the researchers to focus on XO inhibitors with structurally diverse and novel non-purine isosteres[10] such as febuxostat (**3**), a thiazole derivative,[11] flavonoids,[12] Y-700, a 1-phenylpyrazole derivative,[13] FYX-051(**2**), a 3,5-diaryltriazole derivative[14] and curcumin[15] (Fig. 1).

It was thought worthwhile to rationally design compounds derived from 1-acetyl-3,5-diaryl-4,5-dihydro(1*H*)pyrazoles (**7–59**) keeping in view the shape and structural features of **1** and **2** (Fig. 2), based on the following considerations: (i) possess all the important groups/functionalities such as two aromatic or heteroaromatic rings joined by a central linker which is essentially to be a five-membered heterocyclic ring and a site for hydroxylation near molybdenum metal, (ii) based on the space filled molecular orbital models, the carbamoyl of **1** is exactly placed as in the designed compounds, (iii) they are easy to synthesize and (iv) are derived from non-purine based skeleton so as to exclude their possibility being converted, like allopurinol, to unnatural nucleotides.

1-Acetyl-3,5-diaryl-4,5-dihydro(1*H*)pyrazoles have previously been reported to exhibit a variety of biological activities such as inhibitors of monoamine oxidases, swine kidney oxidase and bovine serum amine oxidases,[16] anticancer[17] via binding to P-glycoprotein,[17b] anti-Helicobactor pylori,[18] antiviral,[19] antibacterial via inhibition of FabH,[20] and anti-inflammatory.[21] In the present paper, we describe the synthesis of non-purine 1-acetyl-3,5-diaryl-4,5-dihydro(1*H*)pyrazoles (**7–59**) without ignoring shape and their structure–activity relationships as a new class of XO inhibitors for the first time.

2. Results and discussion

2.1. Synthesis of 1-acetyl-3,5-diaryl-4,5-dihydro(1*H*)pyrazoles

Claisen–Schmidt condensation of a series of aryl ketones (**4**) with aryl aldehydes (**5**) in ethanol yielded several 1,3-diarypropenones (**6**; 71–93%) which on further treatment with hydrazine hydrate in presence of acetic acid under reflux afforded the target acetylpyrazolines (Fig. 3; **7–59**; 75–90%) after recrystallisation in ethanol (Scheme 1). The products were characterized by using spectroscopic techniques such as IR and NMR. The compound **60** was synthesised by condensation of chalcone with hydrazine hydrate in methanol under reflux.

2.2. Biological evaluation of synthesized compounds for xanthine oxidase inhibitory activity

In vitro screening of the 1-acetyl-3,5-diaryl-4,5-dihydro(1*H*)pyrazoles (**7–59**) using bovine milk xanthine oxidase (grade 1, ammonium sulfate suspension, Sigma–Aldrich) enzymatic assay was performed as described in the literature.[22] Each compound was tested in triplicate. Among the series of 53 compounds, six compounds **41**, **42**, **44**, **46**, **55** and **59** were found to be most active against XO with IC_{50} ranging from 5.3 μM to 15.2 μM (Table 1). The inhibitory activity (IC_{50} = 5.3 μM) of the most potent compound **59** was found to be comparable to that of allopurinol (IC_{50} = 8.3 μM).

2.3. Structure–activity relationship (SAR)

Some important structure–activity relationships emerged from these studies: (a) replacement of Ph on ring A with 1-naphthyl enhances the XO inhibitory activity (Table 1; compare **7**, **8**, **11**, **12**, **13**, **14** with **26**, **29**, **33**, **36**, **34** and **38**, respectively), (b) substitution of 1-naphthyl on ring A with 2-furyl further potentiates inhibitory activity (compare **26**, **27**, **28**, **29**, **31**, **32**, **33**, **34**, **35**, **36**, **37** and **38** with **40**, **44**, **42**, **47**, **41**, **50**, **52**, **51**, **53**, **54**, **55** and **56**, respectively), (c) change of Ph on ring B with 1-naphthyl was not desirable (compare **26** with **39** and **40** with **57**), however a drastic increase in the activity was observed when ring B was substituted with 4-pyridyl (compare **40** with **59**), (d) on ring B, in general deactivating groups such as nitro and halogens potentiate the XO inhibitory activity (compare **26** with **27**, **28**, **30**, **31**, and **37**; **40** with **41**, **42** and **44–46**), whereas activating groups such methoxy and *N*,*N*-dimethyl result in dilution of the activity (compare **7** with **8**, **9**, **11–14**; **26** with **29**, **32–36** and **38**) except in few cases (compare **40** with **47–49**), and finally (e) compound **60** was found to be inactive (IC_{50} >100) highlighting the role of *N*-acetyl group. Compound **58** could not be tested due to its poor solubility. These observations helped us to formulate a basic pharmacophore as shown below with the structural features required for XO inhibitory activity.

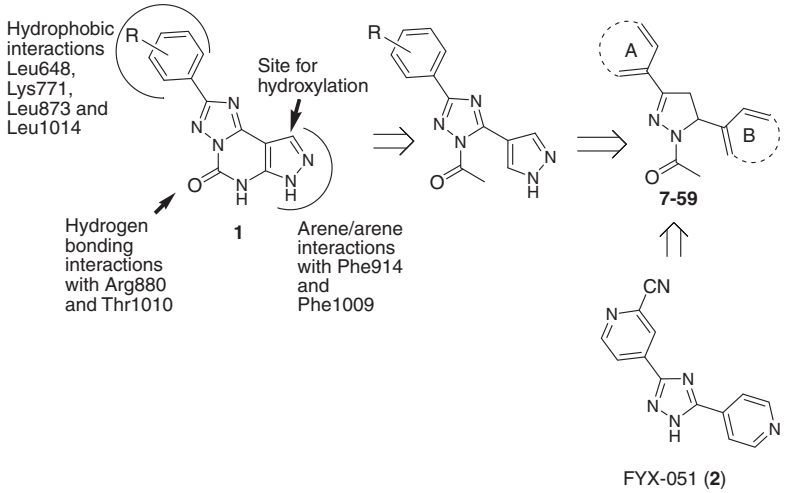
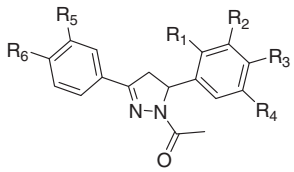
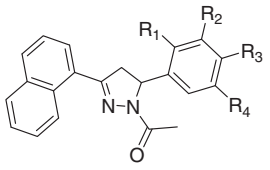


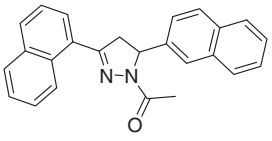
Figure 2. A rational approach for the design of target compounds (**7–59**).



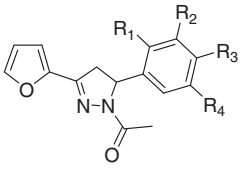
7, $R_1, R_2, R_3, R_4, R_5, R_6 = H$
8, $R_3 = OCH_3$; $R_1, R_2, R_4, R_5, R_6 = H$
9, $R_3 = N(CH_3)_2$; $R_1, R_2, R_4, R_5, R_6 = H$
10, $R_1, R_2 = Cl$; $R_3, R_4, R_5, R_6 = H$
11, $R_2, R_3 = OCH_3$; $R_1, R_4, R_5, R_6 = H$
12, $R_2 = OCH_3$, $R_3 = OH$; $R_1, R_4, R_5, R_6 = H$
13, $R_2, R_3, R_4,= OCH_3$; $R_1, R_5, R_6 = H$
14, $R_2+ R_3 = O\text{-}CH_2\text{-}O$; $R_1, R_4, R_5, R_6 = H$
15, $R_1, R_2, R_3, R_4, R_5 = H$; $R_6 = Br$
16, $R_1, R_2, R_4, R_5 = H$; $R_3 = OCH_3$; $R_6 = Br$
17, $R_2, R_3 = OCH_3$; $R_1, R_4, R_5 = H$; $R_6 = Br$
18, $R_2, R_3, R_4,= OCH_3$; $R_1, R_5 = H$; $R_6 = Br$
19, $R_1, R_2 = Cl$; $R_3, R_4, R_5 = H$; $R_6 = Br$
20, $R_2+ R_3 = O\text{-}CH_2\text{-}O$; $R_1, R_4, R_5 = H$; $R_6 = Br$
21, $R_1, R_2, R_3, R_4, R_6 = H$; $R_5 = NO_2$
22, $R_1, R_2, R_4, R_6 = H$; $R_3 = OCH_3$; $R_5 = NO_2$
23, $R_2, R_3 = OCH_3$; $R_1, R_4, R_6 = H$; $R_5 = NO_2$
24, $R_1, R_2 = Cl$; $R_3, R_4, R_6 = H$; $R_5 = NO_2$
25, $R_2+ R_3 = O\text{-}CH_2\text{-}O$; $R_1, R_4, R_6 = H$; $R_5 = NO_2$



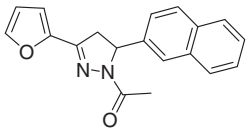
26, $R_1, R_2, R_3, R_4 = H$
27, $R_3 = NO_2$; $R_1, R_2, R_4 = H$
28, $R_2 = NO_2$, $R_1, R_3, R_4 = H$
29, $R_3 = OCH_3$; $R_1, R_2, R_4 = H$
30, $R_2 = Br$; $R_1, R_3, R_4 = H$
31, $R_1 = NO_2$; $R_2, R_3, R_4 = H$
32, $R_1, R_4 = OCH_3$; $R_2, R_3 = H$
33, $R_2, R_3 = OCH_3$; $R_1, R_4 = H$
34, $R_2, R_3, R_4,= OCH_3$; $R_1 = H$
35, $R_1, R_3, R_4,= OCH_3$; $R_2 = H$
36, $R_2 = OCH_3$; $R_3 = OH$; $R_1, R_4 = H$
37, $R_1, R_2 = Cl$; $R_3, R_4 = H$
38, $R_2+ R_3 = O\text{-}CH_2\text{-}O$; $R_1, R_4 = H$



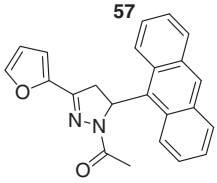
39



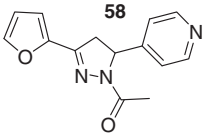
40, $R_1, R_2, R_3, R_4 = H$
41, $R_1 = NO_2$; $R_2, R_3, R_4 = H$
42, $R_2 = NO_2$, $R_1, R_3, R_4 = H$
43, $R_2 = OH$, $R_1, R_3, R_4 = H$
44, $R_3 = NO_2$; $R_1, R_2, R_4 = H$
45, $R_3 = Br$; $R_1, R_2, R_4 = H$
46, $R_3 = Cl$; $R_1, R_2, R_4 = H$
47, $R_3 = OCH_3$; $R_1, R_2, R_4 = H$
48, $R_3 = N(CH_3)_2$; $R_1, R_2, R_4 = H$
49, $R_3 = CH_3$; $R_1, R_2, R_4 = H$
50, $R_1, R_4 = OCH_3$; $R_2, R_3 = H$
51, $R_2, R_3, R_4,= OCH_3$; $R_1 = H$
52, $R_2, R_3 = OCH_3$; $R_1, R_4 = H$
53, $R_1, R_3, R_4,= OCH_3$; $R_2 = H$
54, $R_2 = OCH_3$, $R_3 = OH$; $R_1, R_4 = H$
55, $R_1, R_2 = Cl$; $R_3, R_4 = H$
56, $R_2+ R_3 = O\text{-}CH_2\text{-}O$; $R_1, R_4 = H$



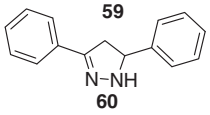
57



58

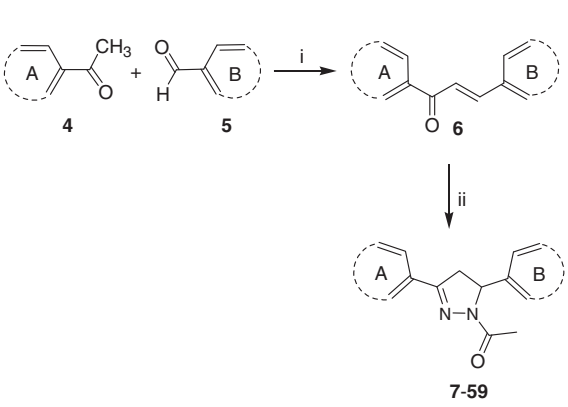


59

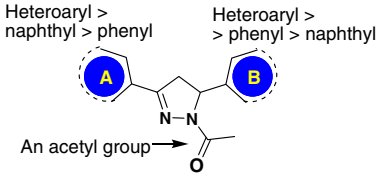


60

Figure 3. Chemical structures of the synthesized compounds.



Scheme 1. Reagents and conditions: (i) 5% NaOH, ethanol, rt, 1 h, 71–93%; (ii) $NH_2NH_2{\cdot}H_2O$, CH_3COOH, reflux, 4 h, 75–90%.



3. Molecular modeling

In order to theoretically investigate the recognition process of *R*- and *S*-enantiomers of the most potent identified XO inhibitor **59**, (Fig. 4) flexible docking experiments using GOLD software[23] were performed assuming that it gets accommodated into the salicylic acid XO active site.[24] The docking study showed that *S*-enantiomer of **59** fits well in the XO binding cavity. The binding site residues and overall binding mode have been found to be similar to those

Table 1
In vitro screening of the synthesized compounds (**7**–**60**) for XO inhibitory activity

Compound	XO inhibitory activity (IC_{50}, μM)[a]
7	61.4
8	76.2
9	73.6
10	55.1
11	83.7
12	81.2
13	86.4
14	70.8
15	71.3
16	66.5
17	64.1
18	74.9
19	51.7
20	66.3
21	79.1
22	73.3
23	75.2
24	58
25	76.1
26	41.3
27	32.2
28	36.4
29	43.5
30	38.7
31	37.2
32	44.8
33	45.6
34	49.5
35	46.2
36	45.7
37	34.2
38	47
39	>100
40	26.4
41	14.2
42	13.1
43	27.2
44	12.4
45	17.6
46	15.2
47	21.3
48	23
49	19.2
50	27.3
51	28.7
52	29.4
53	30.2
54	26.8
55	14.8
56	28.1
57	91.4
58	NT[b]
59	5.3
60	>100
Allopurinol	8.3

[a] Values are means of three experiments.
[b] NT; not tested.

observed with **1**,[9] fabuxostat[11] and salicylic acid.[24] The planar pyridyl ring of the inhibitor aligned parallel to the Phe914 ring at a distance of 3.56 Å, but the two aromatic rings showed only marginal overlap (Fig. 4). This arrangement of energetically favorable arene/arene interactions[25] has also been seen in the co-crystal structure of XO with salicylate and fabuxostat.[11,24] This conservation argues for an important role in stabilizing the binding positions of aromatic substrates and might well represent one of the key features of the substrate recognition (Fig. 4b). The carbonyl group of the inhibitor gets positioned near the oxygen atom of free hydroxyl group of Ser876 (d = 2.26 Å), while H-atom of the hydroxyl projects away from the carbonyl group. The furan ring of the inhibitor gets positioned in the cavity formed by Leu648, Leu873, Lys771 and Asn786 and involved in weak electrostatic interactions. The pyridyl ring of the inhibitor gets oriented towards the dioxothiomolybdenum (MOS) moiety at a distance of 4.76 Å. This indicates that it may block the activity of the enzyme sufficiently enough to prevent the substrate from binding to the active site. The docked conformation of *R*-enantiomer was differing from the *S*-enantiomer. In case of *R*-enantiomer, the pyridyl ring projects away from the dioxothiomolybdenum (MOS) moiety, carbonyl group gets oriented in opposite direction as compared to *S*-enantiomer. The interaction energy of *S*-enantiomer as calculated by GOLD score is 36.13 vis-a-vis 19.29 for *R*-enantiomer. The favourable binding conformation and higher interaction energy of *S*-enantiomer suggests its prevailing role in XO inhibitory activity.

4. Conclusions

We have designed, synthesised and assessed in vitro XO inhibitory activity of 53, 1-acetyl-3,5-diaryl-4,5-dihydro(1*H*)pyrazoles, out of which 38 were found as new. SAR study revealed that nature of rings A and B and nature of substituent(s) on the rings A and B greatly affect the XO inhibitory activity. Rings A and B in particular, heteroaryls such as furan or pyridyl and an *N*-acetyl group are critical for the higher XO inhibitory activity. Docking simulations were performed to position most active compound **59** into the XO active site to determine the probable binding conformation and the results confirmed that the compound was a potential inhibitor of XO and were in agreement with the previous reported studies. Further lead modification on **59** such as synthesis of compounds with diverse permutation and combinations on ring A and B is under progress and will be published in due course.

5. Experimental

The reagents were purchased from Sigma–aldrich, Loba and CDH, India and used without further purification. All yields refer to isolated products after purification. Products were characterized by comparison with authentic samples and by spectroscopic data (IR, ^{1}H NMR, ^{13}C NMR spectra). The spectra were measured in $CDCl_3$ relative to TMS (0.00 ppm). IR (KBr pallets) spectra were recorded on a Fourier transform infrared (FT-IR) Thermo spectrophotometer. Melting points were determined in open capillaries and were uncorrected.

5.1. Typical experimental procedure for the synthesis of 1,3-diarylpropenones (6)

An aqueous solution of sodium hydroxide (20%, 10 mL) was added slowly to the stirring solution of the appropriate aryl aldehyde (1 mmol) and appropriate acetophenone (1 mmol) in methanol (20 mL) in 100 mL conical flask. The stirring was continued for 6 h, keeping the temperature of reaction mixture between 20 and 25 °C. The reaction mixture was then poured into ice cold water (100 mL). It was then neutralized with hydrochloric acid (5%). A yellow solid was obtained after filtration which was recrystallized from ethanol and was used further for the next step.

5.2. Typical experimental procedure for the synthesis of 1-acetyl-3,5-diaryl-4,5-dihydro(1*H*)pyrazoles (7)

A mixture of chalcone (1 mmol) in 4 mL of acetic acid and hydrazine monohydrate 80% (4 mmol) was refluxed for 4 hours. The mixture was then poured onto ice-water (25 mL) mixture to get crude pyrazoline derivative, which was then purified by recrystallisation from ethanol to afford pure **7**. The remaining reactions were carried out following these general procedures. In each

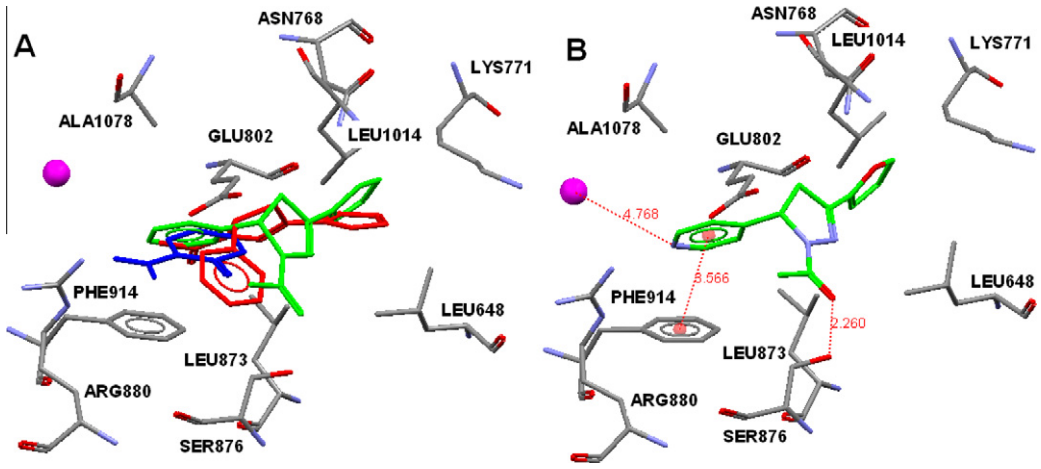


Figure 4. (A) Docking conformation of *R* and *S* isomers of **59** at salicylic acid binding site of XO and, (B) binding interactions of *S* isomer with amino acid residues.

occasion, the spectral data (FTIR, ^{1}H NMR and ^{13}C NMR) of known compounds such as 1-acetyl-5-(4-methoxyphenyl)-3-phenyl-4,5-dihydro-(1*H*)-pyrazole (**8**),[17a] 1-acetyl-3-(4-bromophenyl)-5-(3,4,5-trimethoxyphenyl)-4,5-dihydro-(1*H*)-pyrazole (**18**),[17a] 1-(4,5-dihydro-3-(naphth-1-yl)-5-phenyl-1*H*-pyrazol-1-yl)ethanone (**26**),[26] 1-(4,5-dihydro-3-(naphth-1-yl)-5-(4-nitrophenyl)-1*H*-pyrazol-1-yl)ethanone **(27)**,[26] 1-(3-(furan-2-yl)-4,5-dihydro-5-phenylpyrazol-1-yl)ethanone (**40**),[27] 1-(3-(furan-2-yl)-4,5-dihydro-5-(3-nitrophenyl)pyrazol-1-yl)ethanone **(42)**,[28] 1-(3-(furan-2-yl)-4,5-dihydro-5-(3-hydroxyphenyl)pyrazol-1-yl)ethanone **(43)**,[29] 1-(3-(furan-2-yl)-4,5-dihydro-5-(4-nitrophenyl)pyrazol-1-yl)ethanone **(44)**,[30] 1-(3-(furan-2-yl)-4,5-dihydro-5-(4-bromophenyl)-pyrazol-1-yl)ethanone **(45)**,[29] 1-(3-(furan-2-yl)-4,5-dihydro-5-(4-chlorophenyl)pyrazol-1-yl)ethanone **(46)**,[28] 1-(3-(furan-2-yl)-4,5-dihydro-5-(4-methoxyphenyl)pyrazol-1-yl)ethanone **(47)**,[28] 1-(3-(furan-2-yl)-4,5-dihydro-5-(4-*N*,*N*-dimethylaminophenyl)pyrazol-1-yl)ethanone **(48)**,[28] 1-(3-(furan-2-yl)-4,5-dihydro-5-(4-methylphenyl)pyrazol-1-yl)ethanone **(49)**[30] and 1-(5-(anthracen-10-yl)-3-(furan-2-yl)-4,5-dihydropyrazol-1-yl)ethanone (**58**)[27] were found to be identical with those reported in the literature. The physical data of thirty eight new compounds (**9–17**, **19–25**, **28–39**, **41**, **50–57**, **59**) are provided below.

5.2.1. 1-Acetyl-5-(4-dimethylaminophenyl)-3-phenyl-4,5-dihydro(1*H*)pyrazole (9)

Pale yellow solid; yield 79%; mp: 137–139 °C; IR (KBr): 1644 (C=O), 1613 (C=N) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$, TMS = 0): δ = 7.76–7.73 (m, 2H), 7.43–7.40 (m, 3H), 7.11 (d, *J* = 8.7 Hz, 2H), 6.65 (d, *J* = 8.7 Hz, 2H), 5.52 (dd, *J* = 11.6 and 4.4 Hz, 1H), 3.69 (dd, *J* = 17.6 and 11.5 Hz, 1H), 3.17 (dd, *J* = 17.7 and 4.5 Hz, 1H), 2.90 (s, 6H), 2.39 (s, 3H); Anal. Calcd for $C_{19}H_{21}N_3O$: C, 74.24; H, 6.89; N, 13.67. Found: C, 74.21; H, 6.96; N, 13.88.

5.2.2. 1-Acetyl-5-(2,3-dichlorophenyl)-3-phenyl-4,5-dihydro-(1*H*)pyrazole (10)

Pale yellow solid; yield 82%; mp: 163–165 °C; IR (KBr): 1667 (C=O), 1596 (C=N) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$, TMS = 0): δ = 7.74–7.71 (m, 2H), 7.43–7.36 (m, 4H), 7.18–7.13 (t, *J* = 7.8 Hz, 1H), 6.97 (d, *J* = 7.5, 1H), 5.92 (dd, *J* = 11.8 and 4.9 Hz, 1H), 3.88 (dd, *J* = 17.8 and 11.8 Hz, 1H), 3.04 (dd, *J* = 17.7 and 4.8 Hz, 1H), 2.49 (s, 3H). Anal. Calcd for $C_{17}H_{14}Cl_2N_2O$: C, 61.28; H, 4.23; N, 8.41. Found: C, 61.20; H, 4.33; N, 8.63.

5.2.3. 1-Acetyl-5-(3,4-dimethoxyphenyl)-3-phenyl-4,5-dihydro-((1*H*)pyrazole (11)

Pale yellow solid; yield 78%; mp: 123–125 °C; IR (KBr): 1663 (C=O), 1592 (C=N) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$, TMS = 0): δ = 7.75 (m, 2H), 7.44 (m, 3H), 6.77 (m, 3H), 5.54 (dd, *J* = 11.5 and 4.5 Hz, 1H), 3.86–3.69 (m, 7H), 3.17 (dd, *J* = 17.9 and 4.4 Hz, 1H), 2.43 (s, 3H). Anal. Calcd for $C_{19}H_{20}N_2O_3$: C, 70.35; H, 6.21; N, 8.64. Found: C, 70.52; H, 6.38; N, 8.50.

5.2.4. 1-Acetyl-5-(4-hydroxy-3-methoxy)-3-phenyl-4,5-dihydro-((1*H*)pyrazole (12)

Creamish yellow solid; yield 88%; mp: 197–199 °C; IR (KBr): br 3262 (OH), 1640 (C=O), 1594 (C=N) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$, TMS = 0): δ = 7.76–7.75 (m, 2H), 7.44–7.36 (m, 3H), 6.96 (br s, 1H), 6.82–6.66 (m, 3H), 5.54–5.50 (m, 1H), 3.84 (s, 3H), 3.72 (dd, *J* = 17.5 and 11.8 Hz, 1H), 3.16 (dd, *J* = 17.8 and 4.3 Hz, 1H), 2.41 (s, 3H). Anal. Calcd for $C_{18}H_{18}N_2O_3$: C, 69.66; H, 5.85; N, 9.03. Found: C, 70.0; H, 5.66; N, 9.38.

5.2.5. 1-Acetyl-5-(3,4,5-trimethoxyphenyl)-3-phenyl-4,5-di-(hydro(1*H*)pyrazole (13)

Pale yellow solid; yield 90%; mp: 135–137 °C; IR (KBr): 1650 (C=O), 1587 (C=N) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$, TMS = 0): δ = 7.77–7.74 (m, 2H), 7.45–7.43 (m, 3H), 6.43 (s, 2H), 5.53 (dd, *J* = 11.8 and 4.6 Hz, 1H), 3.82–3.70 (m, 10H), 3.17 (dd, *J* = 17.7 and 4.8 Hz, 1H), 2.46 (s, 3H). Anal. Calcd for $C_{20}H_{22}N_2O_4$: C, 67.78; H, 6.26; N, 7.90. Found: C, 68.04; H, 6.17; N, 8.10.

5.2.6. 1-(5-(Benzo[*d*][1,3]dioxol-6-yl)-4,5-dihydro-3-phenyl-(pyrazol-1-yl)ethanone (14)

Pale yellow solid; yield 82%; mp: 129–131 °C; IR (KBr): 1662 (C=O), 1588 (C=N) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$, TMS = 0): δ = 7.76–7.73 (m, 2H), 7.44 (m, 3H), 6.74 (s, 2H), 6.69 (s, 1H), 5.92 (s, 2H), 5.51 (dd, *J* = 11.9 and 4.1 Hz, 1H), 3.72 (dd, *J* = 17.5 and 11.5 Hz, 1H), 3.14 (dd, *J* = 17.7 and 3.6 Hz, 1H), 2.42 (s, 3H). Anal. Calcd for $C_{18}H_{16}N_2O_3$: C, 70.12; H, 5.23; N, 9.09. Found: C, 70.28; H, 5.44; N, 9.12.

5.2.7. 1-Acetyl-3-(4-bromophenyl)-5-phenyl-4,5-dihydro(1*H*)-(pyrazole (15)

Pale yellow solid; yield 78%; mp: 125–127 °C; IR (KBr): 1664 (C=O), 1590 (C=N) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$, TMS = 0): δ = 7.57 (m, 4H), 7.34–7.20 (m, 5H), 5.59 (dd, *J* = 11.8 and 4.7 Hz, 1H), 3.72 (dd, *J* = 17.6 and 11.9 Hz, 1H), 3.12 (dd, *J* = 17.6 and 4.7 Hz, 1H), 2.41 (s, 3H). Anal. Calcd for $C_{17}H_{15}BrN_2O$: C, 59.49; H, 4.41; N, 8.16. Found: C, 59.38; H, 4.77; N, 8.25

5.2.8. 1-Acetyl-3-(4-bromophenyl)-5-(4-methoxyphenyl)-4,5-dihydro(1*H*)pyrazole (16)

Pale yellow solid; yield 83%; mp: 132–134 °C; IR (KBr): 1656 (C=O), 1588 (C=N) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$, TMS = 0):

δ = 7.61–7.53 (m, 4H), 7.14 (d, J = 8.1 Hz, 2H), 6.84 (d, J = 8.4 Hz, 2H), 5.54 (dd, J = 11.3 and 3.5 Hz, 1H), 3.76 (s, 3H), 3.70–3.64 (m, 1H), 3.11 (dd, J = 17.6 and 3.8 Hz, 1H), 2.39 (s, 3H). Anal. Calcd for $C_{18}H_{17}BrN_2O_2$: C, 57.92; H, 4.59; N, 7.51. Found: C, 57.84; H, 4.71; N, 7.62.

5.2.9. 1-Acetyl-3-(4-bromophenyl)-5-(3,4-dimethoxyphenyl)-4,5-dihydro(1*H*)pyrazole (17)

Pale yellow solid; yield 80%; mp: 137–139 °C; IR (KBr): 1660 (C=O), 1591 (C=N) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, TMS = 0): δ = 7.63–7.54 (m, 4H), 6.82–6.74 (m, 3H), 5.54 (dd, J = 11.9 and 4.7 Hz, 1H), 3.85 (s, 3H), 3.84 (s, 3H), 3.70 (dd, J = 17.7 and 11.7 Hz, 1H), 1H, 3.13 (dd, J = 17.7 and 4.8 Hz, 1H), 2.41 (s, 3H). Anal. Calcd for $C_{19}H_{19}BrN_2O_3$: C, 56.59; H, 4.75; N, 6.95. Found: C, 56.37; H, 5.03; N, 6.89.

5.2.10. 1-Acetyl-3-(4-bromophenyl)-5-(2,3-dichlorophenyl)-4,5-dihydro(1*H*)pyrazole (19)

Pale yellow solid; yield 90%; mp: 177–179 °C; IR (KBr): 1660 (C=O), 1592 (C=N) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, TMS = 0): δ = 8.53 (t, J = 18 Hz, 1H), 8.29–8.26 (m, 1H), 5.05 (d, J = 7.8 Hz, 1H), 7.62 (t, J = 7.9 Hz, 1H), 7.40 (dd, J = 7.9 and 1.4 Hz, 1H), 7.17 (t, J = 7.8 Hz, 1H), 6.97 (d, J = 7.8 Hz, 1H), 5.98 (dd, J = 12 and 5.1 Hz, 1H), 3.93 (dd, J = 17.9 and 12.2 Hz, 1H), 3.10 (dd, J = 17.7 and 5.1 Hz, 1H), 2.51 (s, 3H). Anal. Calcd for $C_{17}H_{13}BrCl_2N_2O$: C, 49.55; H, 3.18; N, 6.80. Found: C, 49.15; H, 3.32; N, 6.70.

5.2.11. 1-(5-(Benzo[*d*][1,3]dioxol-6-yl)-3-(4-bromophenyl)-4,5-dihydropyrazol-1-yl)ethanone (20)

Pale yellow solid; yield 86%; mp: 175–177 °C; IR (KBr): 1650 (C=O), 1597 (C=N) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, TMS = 0): δ = 7.61–7.54 (m, 3H), 7.26 (s, 1H), 6.76–6.67 (m, 3H), 5.92 (s, 2H), 5.54–5.48 (m, 1H), 3.76–3.64 (m, 1H), 3.15–3.06 (m, 1H), 2.40 (s, 3H). Anal. Calcd for $C_{18}H_{15}BrN_2O_3$: C, 55.83; H, 3.90; N, 7.23. Found: C, 55.55; H, 4.21; N, 7.56.

5.2.12. 1-Acetyl-3-(3-nitrophenyl)-5-phenyl-4,5-dihydro(1*H*)-(pyrazole (21)

Pale brownish solid; yield 81%; mp: 130–132 °C; IR (KBr): 1665 (C=O), 1591 (C=N) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, TMS = 0): δ = 8.54 (s, 1H), 8.27 (d, J = 8.1 Hz, 1H), 8.08 (d, J = 8.7 Hz, 1H), 7.62 (t, J = 7.8 Hz, 1H), 7.36–7.21 (m, 5H), 5.66 (dd, J = 11.8 and 3.5 Hz, 1H), 3.81 (dd, J = 17.6 and 11.8 Hz, 1H), 3.21 (dd, J = 17.7 and 3.3 Hz, 1H), 2.46 (s, 3H). Anal. Calcd for $C_{17}H_{15}N_3O_3$: C, 66.01; H, 4.89; N, 13.58. Found: C, 66.37; H, 4.93; N, 13.64.

5.2.13. 1-Acetyl-5-(4-methoxyphenyl)-3-(3-nitrophenyl)-4,5-dihydro(1*H*)pyrazole (22)

Pale brownish solid; yield 79%; mp: 118–120 °C; IR (KBr): 1664 (C=O), 1592 (C=N) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, TMS = 0): δ = 8.53 (s, 1H), 8.25 (d, J = 7.5 Hz, 1H), 8.08 (d, J = 7.5 Hz, 1H), 7.61 (t, J = 7.9 Hz, 1H), 7.15 (d, J = 8.4 Hz, 2H), 6.84 (d, J = 8.4 Hz, 2H), 5.60 (dd, J = 11.6 and 4.1 Hz, 1H), 3.82–3.73 (m, 1H), 3.75 (s, 3H), 3.19 (dd, J = 17.7 and 4.4 Hz, 1H), 2.43 (s, 3H). Anal. Calcd for $C_{18}H_{17}N_3O_4$: C, 63.71; H, 5.05; N, 12.38. Found: C, 63.63; H, 4.79; N, 12.49.

5.2.14. 1-Acetyl-5-(3,4-dimethoxyphenyl)-3-(3-nitrophenyl)-4,5-dihydro(1*H*)pyrazole (23)

Pale brownish solid; yield 90%; mp: 153–155 °C; IR (KBr): 1660 (C=O), 1592 (C=N) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, TMS = 0): δ = 8.55 (s, 1H), 8.29 (d, J = 7.5 Hz, 1H), 8.09 (d, J = 8.1 Hz, 1H), 7.64 (t, J = 8.0 Hz, 1H), 6.81–6.76 (m, 3H), 5.61 (d, J = 11.7 Hz, 1H), 3.87–3.75 (m, 7H), 3.26–3.19 (m, 1H), 2.47 (s, 3H). Anal. Calcd for $C_{19}H_{19}N_3O_5$: C, 61.78; H, 5.18; N, 11.38. Found: C, 61.57; H, 5.28; N, 11.45.

5.2.15. 1-Acetyl-5-(2,3-dichlorophenyl)-3-(3-nitrophenyl)-4,5-dihydro(1*H*)pyrazole (24)

Pale brownish solid; yield 83%; mp: 161–163 °C; IR (KBr): 1668 (C=O), 1596 (C=N) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, TMS = 0): δ = 8.53 (s, 1H), 8.28 (d, J = 7.8 Hz, 1H), 8.05 (d, J = 6.9 Hz, 1H), 7.63 (t, J = 7.4 Hz, 1H), 7.42–6.96 (m, 3H), 5.98 (dd, J = 11.7 and 4.8 Hz, 1H), 3.93 (dd, J = 17.8 and 12.4 Hz, 1H), 3.10 (dd, J = 17.8 and 4.6 Hz, 1H), 2.51 (s, 3H). Anal. Calcd for $C_{17}H_{13}Cl_2N_3O_3$: C, 53.99; H, 3.46; N, 11.11. Found: C, 53.61; H, 3.79; N, 11.08.

5.2.16. 1-(5-(Benzo[*d*][1,3]dioxol-6-yl)-4,5-dihydro-3-(3-nitro-(phenyl)pyrazol-1-yl)ethanone (25)

Pale brownish solid; yield 79%; mp: 197–199 °C; IR (KBr): 1660 (C=O), 1591 (C=N) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, TMS = 0): δ = 8.52 (s, 1H), 8.27 (d, J = 8.1 Hz, 1H), 8.07 (d, J = 7.2 Hz, 1H), 7.62 (t, J = 8.1 Hz, 1H), 6.66–6.76 (m, 3H), 5.92 (s, 2H), 5.57 (dd, J = 12.0 and 4.5 Hz, 1H), 3.77 (dd, J = 17.7 and 12.0 Hz, 1H), 3.18 (dd, J = 17.9 and 4.7 Hz, 1H), 2.44 (s, 3H). Anal. Calcd for $C_{18}H_{15}N_3O_5$: C, 61.19; H, 4.28; N, 11.89. Found: C, 61.27; H, 4.33; N, 11.79.

5.2.17. 1-(4,5-Dihydro-3-(naphth-1-yl)-5-(3-nitrophenyl)-1*H*-pyrazol-1-yl)ethanone (28)

White solid; yield 79%; mp: 124–126 °C; IR (KBr): 1659 (C=O), 1590 (C=N) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, TMS = 0) δ = 9.27 (d, J = 8.7 Hz, 1H), 8.14 (m, 2H) 7.92 (m, 2H), 7.58 (m, 6H), 5.66 (dd, J = 4.8 Hz and 12 Hz, 1H), 4.06 (dd, J = 12 Hz and 17.7 Hz, 1H), 3.37 (dd, J = 4.8 and 17.7 Hz, 1H), 2.54 (s,3H,); ^{13}C (75 MHz, $CDCl_3$, TMS = 0) δ = 169.15, 154.16, 148.69, 143.86, 134.16, 131.94, 131.62, 130.51, 130, 128.90, 128.44, 127.93, 127.26, 126.51, 124.74, 122.77, 120.94, 58.25, 44.72, 22.11. Anal. Calcd for $C_{21}H_{17}N_3O_3$: C, 70.18; H, 4.77; N, 11.69. Found: C, 70.23; H, 4.89; N, 11.80.

5.2.18. 1-(4,5-Dihydro-5-(4-methoxyphenyl)-3-(naphth-1-yl)-1*H*-pyrazol-1-yl)ethanone (29)

White solid; yield 89%; mp: 151–153 °C; IR (KBr): 1663 (C=O), 1578 (C=N) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, TMS = 0) δ = 9.27 (d, J = 8.7 Hz, 1H), 7.88 (d, J = 8.1 Hz, 2H),7.65 (m, 1H), 7.53–7.58 (m, 2H), 7.44 (t, J = 7.8 Hz, 1H), 7.21 (d, J = 8.7 Hz, 2H), 6.84 (d, J = 8.7 Hz, 2H), 5.52 (dd, J = 4.2 and 11.7 Hz, 1H), 3.90 (dd, J = 11.7 and 17.4 Hz, 1H), 3.74 (s, 3H), 3.32 (dd, J = 4.2 and 17.4 Hz, 1H), 2.49 (s,3H); ^{13}C (75 MHz, $CDCl_3$, TMS = 0) δ = 168.73, 158.97, 154.31, 134.06, 134.03, 131.10, 130.49, 128.75, 128.23, 127.79, 127.64, 126.92, 126.59, 126.30, 124.73, 114.20, 58.22, 55.18, 44.77, 22.19. Anal. Calcd for $C_{22}H_{20}N_2O_2$: C, 76.72; H, 5.85; N, 8.13. Found: C, 76.80; H, 5.78; N, 8.09.

5.2.19. 1-(5-(3-Bromophenyl)-4,5-dihydro-3-(naphth-1-yl)-1*H*-pyrazol-1-yl)ethanone (30)

White solid; yield 82%; mp: 130–132 °C; IR (KBr): 1662 (C=O), 1590 (C=N) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, TMS = 0) δ = 9.26 (d, J = 8.70 Hz, 1H), 7.89 (d, J = 8.10 Hz, 2H), 7.67 (dd, J = 1.5 and 6.9 Hz, 1H), 7.56–7.63 (m, 2H), 7.35–7.46 (m, 3H), 7.14–7.22 (m, 2H), 5.49 (dd, J = 4.8 and 12 Hz, 1H), 3.90 (dd, J = 12 and 17.7 Hz, 1H), 3.28 (dd, J = 4.8 and 17.7 Hz, 1H), 2.51 (s, 3H); ^{13}C (75 MHz, $CDCl_3$, TMS = 0) δ = 168.83, 154.16, 144.05, 134.05, 131.32, 130.76, 130.46, 128.79, 128.69, 128.34, 127.74, 127.41, 126.53, 126.36, 124.70, 124.26, 122.92, 58.19, 44.72, 22.11. Anal. Calcd for $C_{21}H_{17}BrN_2O$: C, 64.13; H, 4.36; N, 7.12. Found: C, 64.29; H, 4.24; N, 7.12.

5.2.20. 1-(4,5-Dihydro-3-(naphth-1-yl)-5-(2-nitrophenyl)-1*H*-pyrazol-1-yl)ethanone (31)

White solid; yield 79%; mp: 208–210 °C; IR (KBr): 1656 (C=O), 1579 (C=N) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, TMS = 0) δ = 9.28 (d,

J = 8.7 Hz, 1H), 8.12 (d, *J* = 8.1 Hz, 1H), 7.91 (d, *J* = 7.8 Hz, 2H), 7.54–7.70 (m, 4H), 7.36–7.48 (m, 3H), 6.13 (dd, *J* = 5.1 and 12 Hz, 1H), 4.27 (dd, *J* = 12 and 18 Hz, 1H), 3.36 (dd, *J* = 5.1 Hz and 18 Hz, 1H), 2.57 (s, 3H); ^{13}C (75 MHz, $CDCl_3$, TMS = 0) δ = 168.90, 154.94, 147.17, 137.04, 134.34, 134.11, 131.53, 130.47, 128.90, 128.60, 128.47, 127.81, 127.31, 126.51, 126.41, 126.38, 125.47, 124.78, 55.90, 45.11, 22.05. Anal. Calcd for $C_{21}H_{17}N_3O_3$: C, 70.18; H, 4.77; N, 11.69. Found: C, 70.23; H, 5.08; N, 11.69.

5.2.21. 1-(4,5-Dihydro-5-(2,5-dimethoxyphenyl)-3-(naphth-1-yl)-1*H*-pyrazol-1-yl)ethanone (32)

White solid; yield 78%; mp: 163–165 °C; IR (KBr): 1664 (C=O), 1585 (C=N) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$, TMS = 0) δ = 9.25 (d, *J* = 8.4 Hz, 1H), 7.86 (d, *J* = 7.5 Hz, 2H), 7.64 (*t*, *J* = 7.2 Hz, 1H), 7.54 (m, 2H) ,7.41 (t, *J* = 7.8 Hz, 1H), 6.69–6.82 (m, 3H), 5.79 (dd, *J* = 4.2 Hz and 11.7 Hz, 1H), 3.91(dd, *J* = 11.7 Hz and 17.4 Hz, 1H), 3.79 (s, 3H), 3.70 (s, 3H), 3.20 (dd, *J* = 4.2 Hz and 17.4 Hz, 1H), 2.55 (s, 3H). ^{13}C (75 MHz, $CDCl_3$, TMS = 0) δ = 168.73, 155.15, 153.60, 150.27, 133.99, 130.92, 130.47, 130.36, 128.66, 128.11, 127.93, 127.51, 126.60, 126.20, 124.67, 112.63, 112.13, 111.83, 55.93, 55.54, 54.55, 44.01, 22.14. Anal. Calcd for $C_{23}H_{22}N_2O_3$: C, 73.78; H, 5.92; N, 7.48. Found: C, 73.44; H, 6.19; N, 7.69.

5.2.22. 1-(4,5-Dihydro-5-(3,4-dimethoxyphenyl)-3-(naphth-1-yl)-1*H*-pyrazol-1-yl)ethanone (33)

White solid; yield 77%; mp: 118–120 °C; IR (KBr): 1663 (C=O), 1591 (C=N) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$, TMS = 0) δ = 9.26 (d, *J* = 8.4 Hz, 1H), 7.86 (d, *J* = 7.8 Hz, 2H), 7.66 (t, *J* = 7.2 Hz, 1H), 7.50–7.56 (m, 2H), 7.42 (t, *J* = 7.8 Hz, 1H), 6.75–6.83 (m, 3H), 5.49 (dd, *J* = 4.2 Hz and 11.7 Hz, 1H), 3.89 (dd, *J* = 11.7 Hz and 17.4 Hz, 1H), 3.82 (s, 3H) , 3.78 (s, 3H), 3.31(dd, *J* = 4.2 Hz and 17.4 Hz, 1H), 2.51 (s, 3H); ^{13}C (75 MHz, $CDCl_3$, TMS = 0) δ = 168.53, 154.21, 149, 148.25, 134.32, 133.84, 130.91, 130.27, 128.58, 128.06, 127.52, 127.44, 126.32, 126.11, 124.56, 117.40, 111.30, 108.95, 58.33, 55.62, 44.62, 22.00. Anal. Calcd for $C_{23}H_{22}N_2O_3$: C, 73.78; H, 5.92; N, 7.48. Found: C, 74.04; H, 5.79; N, 7.52.

5.2.23. 1-(4,5-Dihydro-5-(3,4,5-trimethoxyphenyl)-3-(naphth-1-yl)-1*H*-pyrazol-1-yl)ethanone (34)

White solid; yield 76%; mp: 135–137 °C; IR (KBr): 1653 (C=O), 1592 (C=N) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$, TMS = 0) δ = 9.23(d, *J* = 8.7 Hz, 1H), 7.90 (d, *J* = 8.1 Hz, 2H), 7.55–7.68 (m, 3H), 7.46 (t, *J* = 7.5 Hz, 1H), 6.50 (s, 2H) , 5.51 (dd, *J* = 4.2 and 11.7 Hz, 1H), 3.93 (dd, *J* = 11.7 Hz and 17.4 Hz, 1H), 3.81 (s, 9H), 3.35 (dd, *J* = 4.2 Hz and 17.4 Hz, 1H), 2.54 (s, 3H); ^{13}C (75 MHz, $CDCl_3$, TMS = 0) δ = 168.89, 154.53, 153.57, 137.56, 137.35, 134.04, 131.16, 130.45, 128.77, 128.21, 127.62, 126.33, 126.30, 124.73, 102.37, 60.62, 58.99, 56.00, 45.04, 22.12. Anal. Calcd for $C_{24}H_{24}N_2O_4$: C, 71.27; H, 5.98; N, 6.93. Found: C, 71.55; H, 6.58; N, 6.98.

5.2.24. 1-(4,5-Dihydro-5-(2,3,4-trimethoxyphenyl)-3-(naphth-1-yl)-1*H*-pyrazol-1-yl)ethanone (35)

White solid; yield 82%; mp: 120–122 °C; IR (KBr): 1661 (C=O), 1587 (C=N) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$, TMS = 0) δ = 9.29 (d, *J* = 8.7 Hz, 1H), 7.89 (d, *J* = 8.1 Hz, 2H), 7.65 (t, *J* = 7.8 Hz, 1H), 7.54–7.58 (m, 2H), 7.45 (t, *J* = 7.8 Hz, 1H), 6.87 (d, *J* = 8.7 Hz, 1H), 6.60 (d, *J* = 8.7 Hz, 1H), 5.54 (dd, *J* = 4.8 Hz and 12.00 Hz, 1H), 3.90 (dd, *J* = 12.00 and 17.4 Hz, 1H), 3.94 (s, 3H), 3.86 (s, 3H), 3.81 (s, 3H), 3.30 (dd, *J* = 4.81 Hz and 17.4 Hz, 1H), 2.51 (s, 3H); ^{13}C (75 MHz, $CDCl_3$, TMS = 0) δ = 168.74, 155.02, 153.25, 150.79, 142.27, 134.05, 130.97, 130.54, 128.73, 128.22, 127.96, 127.55, 126.61, 126.23, 124.77, 121.17, 107.23, 60.73, 60.67, 55.90, 54.92, 44.42, 22.26. Anal. Calcd for $C_{24}H_{24}N_2O_4$: C, 71.27; H, 5.98; N, 6.93. Found: C, 71.33; H, 5.82; N, 7.21.

5.2.25. 1-(4,5-Dihydro-5-(4-hydroxy-3-methoxyphenyl)-3-(naphth-1-yl)-1*H*-pyrazol-1-yl)ethanone (36)

White solid; yield 83%; mp: 182–184 °C; IR (KBr): 3443 (O–H), 1662 (C=O), 1578 (C=N) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$, TMS = 0) δ = 9.26 (d, *J* = 8.7 Hz, 1H), 7.92 (d, *J* = 8.1 Hz, 2H), 7.56–7.71 (m, 3H), 7.49 (t, *J* = 8.1 Hz, 1H), 6.83 (m, 3H), 5.49 (dd, *J* = 4.2 Hz and 11.7 Hz, 1H), 3.94 (dd, *J* = 11.7 Hz and 17.4 Hz, 1H), 3.90 (s, 3H). 3.34 (dd, *J* = 4.2 Hz and 17.4 Hz, 1H), 2.49 (s, 3H). Anal. Calcd for $C_{22}H_{20}N_2O_3$: C, 73.32; H, 5.59; N, 7.77. Found: C, 73.61; H, 5.25; N, 7.69.

5.2.26. 1-(5-(2,3-Dichlorophenyl)-4,5-dihydro-3-(naphth-1-yl)-1*H*-pyrazol-1-yl)ethanone (37)

White solid; yield 89%; mp: 115–117 °C; IR (KBr): 1666 (C=O), 1585 (C=N) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$, TMS = 0) δ = 9.25 (d, *J* = 8.44 Hz, 1H), 7.89 (d, *J* = 7.20 Hz, 2H), 7.52–7.66 (m, 3H), 7.24–7.46 (m, 2H), 7.04–7.16 (m, 2H), 5.90 (d, *J* = 7.8 Hz, 1H), 4.05 (d, *J* = 12.9 and 16.8 Hz, 1H) 3.23 (d, *J* = 16.8 Hz, 1H), 2.58 (s, 3H). ^{13}C (75 MHz, $CDCl_3$, TMS = 0) δ = 168.91, 154.73, 140.78, 134.08, 133.76, 131.42, 130.46, 130.10, 129.52, 128.85, 128.46, 127.77, 127.73, 127.36, 126.49, 126.39, 124.74, 124.02, 57.08, 43.84, 22.10. Anal. Calcd for $C_{21}H_{16}Cl_2N_2O$: C, 65.81; H, 4.21; N, 7.31. Found: C, 65.79; H, 4.54; N, 7.70.

5.2.27. 1-(5-(Benzo[*d*][1,3]dioxol-5-yl)-4,5-dihydro-3-(naphth-1-yl)-1*H*-pyrazol-1-yl)ethanone (38)

White solid; yield 77%; mp: 126–128 °C; IR (KBr): 1666 (C=O), 1578 (C=N) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$, TMS = 0) δ =9.27(d, *J* = 8.4 Hz, 1H), 7.88 (d, *J* = 8.1 Hz, 2H), 7.54(m, 5H), 6.76 (m, 2H), 5.87 (s, 2H) , 5.47 (dd, *J* = 3.9 and 11.7 Hz, 1H), 3.87 (dd, *J* = 17.4 and 11.7 Hz, 1H), 3.28 (dd, *J* = 3.9 and 17.4 Hz, 1H) , 2.50 (s, 3H). ^{13}C (75 MHz, $CDCl_3$, TMS = 0) δ = 168.83, 154.36, 147.99, 146.93, 135.78, 134.00, 131.16, 130.41, 128.72, 128.25, 127.65, 127.57, 126.52, 126.28, 124.67, 119.01, 108.41, 105.95, 100.99, 58.49, 44.84, 22.13. Anal. Calcd for $C_{22}H_{18}N_2O_3$: C, 73.73; H, 5.06; N, 7.82. Found: C, 73.92; H, 5.28; N, 7.69.

5.2.28. 1-(4,5-Dihydro-5-(naphth-2-yl)-3-(naphth-1-yl)-1*H*-pyrazol-1-yl)ethanone (39)

White solid; yield 75%; mp: 142–144 °C; IR (KBr): 1664 (C=O), 1587 (C=N) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$, TMS=0) δ = 9.32(d, *J* = 8.4 Hz, 1H), 7.89 (d, *J* = 8.1 Hz, 2H),7.74–7.81(m, 4H), 7.64–7.70 (m, 1H)7.52–7.59 (m, 2H), 7.35–7.45(m, 4H), 5.71 (dd, *J* = 4.5 Hz and 11 Hz, 1H), 3.96 (dd, *J* = 11.7 and 17.4 Hz, 1H), 3.39 (dd, *J* = 4.5 Hz and 17.4 Hz, 1H), 2.54 (s, 3H); ^{13}C (75 MHz, $CDCl_3$, TMS = 0) δ = 168.89, 154.38, 139.04, 134.07, 133.29, 132.85, 131.21, 130.51, 129.03, 128.80, 128.34, 127.92, 127.72, 127.65, 127.58, 126.62, 126.35, 126.22, 125.89, 124.75, 124.58, 123.39, 58.92, 44.86, 22.22. Anal. Calcd for $C_{25}H_{20}N_2O$: C, 82.39; H, 5.53; N, 7.69. Found: C, 82.68; H, 5.37; N, 7.72.

5.2.29. 1-(3-(Furan-2-yl)-4,5-dihydro-5-(2-nitrophenyl)pyrazol-1-yl)ethanone (41)

White solid; yield 78%; mp: 144–146 °C; IR (KBr): 1667 (C=O), 1590 (C=N) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$, TMS = 0) δ = 8.12 (d, *J* = 8.0 Hz, 1H), 7.26–7.60 (m, 4H), 6.75 (d, *J* = 3.4 Hz, 1H), 6.51 (dd, *J* = 1.75 and 3.39 Hz, 1H), 6.12 (dd, J = 5.1 and 11.9 Hz, 1H), 3.99 (dd, *J* = 11.9 and 18.2 Hz, 1H), 3.08 (dd, *J* = 5.1 and 18.2 Hz, 1H), 2.45 (s, 3H); ^{13}C (75 MHz, $CDCl_3$, TMS = 0) δ = 168.88, 147.04, 146.52, 146.09, 144.97, 136.72, 134.38, 128.56, 126.43, 125.50, 113.12, 112.03, 56.68, 42.28, 21.83. Anal. Calcd for $C_{15}H_{13}N_3O_4$: C, 60.20; H, 4.38; N, 14.04. Found: C, 60.18; H, 4.24; N, 14.32.

5.2.30. 1-(3-(Furan-2-yl)-4,5-dihydro-5-(2,5-dimethoxyphenyl)-(pyrazol-1-yl)ethanone (50)

Yellow solid; yield 80%; mp: 101–106 °C; IR (KBr): 1658 (C=O), 1586 (C=N) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$, TMS = 0) δ = 7.53 (d,

J = 1.24 Hz, 1H), 6.68–6.84 (m, 3H), 6.58 (d, *J* = 2.75 Hz, 1H), 6.48 (dd, *J* = 1.77 and 3.41 Hz, 1H), 5.79 (dd, *J* = 4.49 and 11.77 Hz, 1H), 3.81 (s, 3H), 3.71(s, 3H), 3.63 (dd, *J* = 11.77 and 17.67 Hz, 1H), 2.94 (dd, *J* = 4.49 and 17.67 Hz, 1H), 2.45 (s, 3H). Anal. Calcd for $C_{17}H_{18}N_2O_4$: C, 64.96; H, 5.77; N, 8.91. Found: C, 65.16; H, 5.81; N, 9.24.

5.2.31. 1-(3-(Furan-2-yl)-4,5-dihydro-5-(3,4,5-trimethoxyphenyl)(pyrazol-1-yl)ethanone (51)

Yellow solid; yield 86%; mp: 155–156 °C; IR (KBr): 1661 (C=O), 1590 (C=N) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, TMS = 0) δ = 7.57 (d, *J* = 1.28 Hz, 1H), 6.74 (d, *J* = 3.42 Hz, 1H), 6.52 (dd, J = 1.81 Hz and 3.46 Hz, 1H), 6.41 (s, 2H), 5.50 (dd, *J* = 4.72 and 11.86 Hz, 1H), 3.85 (s, 6H), 3.82 (s, 3H), 3.69 (dd, *J* = 11.86 Hz and 17.78 Hz, 1H), 3.08 (dd, *J* = 4.72 and 17.78 Hz, 1H), 2.44 (s, 3H). ^{13}C (75 MHz, $CDCl_3$, TMS = 0) δ = 168.96, 153.65, 146.77, 145.72, 144.81, 137.33, 112.80, 111.98, 103.26, 60.72, 59.73, 56.12, 42.27, 21.98. Anal. Calcd for $C_{18}H_{20}N_2O_5$: C, 62.78; H, 5.85; N, 8.13. Found: C, 62.89; H, 6.07; N, 8.24.

5.2.32. 1-(3-(Furan-2-yl)-4,5-dihydro-5-(3,4-dimethoxyphenyl)-pyrazol-1-yl)ethanone (52)

Yellow solid; yield 82%; mp: 123–125 °C; IR (KBr): 1663 (C=O), 1587 (C=N) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, TMS = 0) δ = 7.57 (s, 1H), 6.77–6.73 (m, 4H), 6.53 (t, *J* = 1.64 Hz, 1H), 5.52 (dd, *J* = 4.51 and 11.80 Hz, 1H), 3.85 (s, 3H), 3.84 (s, 3H), 3.69 (dd, *J* = 11.80 and 17.63 Hz, 1H), 3.09 (dd, *J* = 4.51 and 17.63 Hz, 1H), 2.41 (s, 3H); ^{13}C (75 MHz, $CDCl_3$, TMS = 0) δ = 168.86, 149.33, 148.62, 146.95, 145.60, 144.72, 134.25, 117.65, 112.57, 111.94, 111.57, 109.11, 59.33, 55.95, 42.12, 21.09. Anal. Calcd for $C_{17}H_{18}N_2O_4$: C, 64.96; H, 5.77; N, 8.91. Found: C, 64.82; H, 6.01; N, 8.89.

5.2.33. 1-(3-(Furan-2-yl)-4,5-dihydro-5-(2,4,5-trimethoxyphenyl)pyrazol-1-yl)ethanone (53)

Yellow solid; yield 81%; mp: 112–114 °C; IR (KBr): 1658 (C=O), 1582 (C=N) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, TMS = 0) δ = 7.55 (d, *J* = 1.41 Hz, 1H), 6.70 (d, *J* = 3.37 Hz, 1H), 6.59 (s, 1H), 6.50 (m, 2H), 5.73(dd, *J* = 4.69 and 11.76 Hz, 1H), 3.86 (s, 3H), 3.81(s, 3H), 3.78 (s, 3H), 3.64(dd, *J* = 11.76 and 17.61 Hz, 1H), 2.98 (dd, *J* = 4.69 and 17.61 Hz, 1H), 2.43 (s, 3H); ^{13}C (75 MHz, $CDCl_3$, TMS = 0) δ = 168.78, 150.49, 149.23, 147.14, 146.49, 144.59, 143.21, 120.84, 112.49, 111.85, 110.63, 98.16, 56.74, 56.43, 56.25, 55.30, 41.30, 22.00. Anal. Calcd for $C_{18}H_{20}N_2O_5$: C, 62.78; H, 5.85; N, 8.13. Found: C, 62.82; H, 5.73; N, 8.08.

5.2.34. 1-(3-(Furan-2-yl)-4,5-dihydro-5-(4-hydroxy-3-methoxyphenyl)pyrazol-1-yl)ethanone (54)

Yellow solid; yield 85%; mp: 221–223 °C; IR (KBr): 3430 (O–H) 1658 (C=O), 1587 (C=N) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, TMS = 0) δ = 7.55 (m, 2H), 6.82–6.46 (m, 4H), 6.53 (dd, *J* = 1.8 and 3.3 Hz, 1H), 5.47 (dd, J = 4.5 and 11.7 Hz, 1H), 3.84 (s, 3H), 3.68 (dd, *J* = 11.7 and 17.7 Hz, 1H), 3.08 (dd, *J* = 4.5 and 17.7 Hz, 1H), 2.38 (s, 3H); ^{13}C (75 MHz, $CDCl_3$, TMS = 0) δ = 168.44, 147.00, 146.51, 145.51, 145.34, 144.42, 132.91, 117.68, 114.98, 112.45, 111.64, 108.70, 59.00, 55.63, 41.86, 21.65. Anal. Calcd for $C_{16}H_{16}N_2O_4$: C, 63.99; H, 5.37; N, 9.33. Found: C, 63.87; H, 5.43; N, 9.29.

5.2.35. 1-(3-(Furan-2-yl)-4,5-dihydro-5-(2,3-dichlorophenyl)-pyrazol-1-yl)ethanone (55)

Yellow solid; yield 81%; mp: 111–113 °C; IR (KBr): 1663 (C=O), 1583 (C=N) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, TMS = 0): δ = 7.55 (d, *J* = 1.07 Hz, 1H), 7.38 (dd, *J* = 1.2 and 7.97 Hz, 1H), 7.16 (t, *J* = 7.88 Hz, 1H), 6.96 (d, *J* = 7.6 Hz, 1H), 6.73 (d, *J* = 3.37 Hz, 1H), 6.50 (dd, *J* = 1.70 and 3.40 Hz, 1H), 5.89 (dd, *J* = 4.69 and 11.83 Hz, 1H), 3.81 (dd, J = 11.83 and 17.77 Hz, 1H), 2.97 (dd, *J* = 4.69 and 17.77 Hz, 1H), 2.49(3H, s); ^{13}C (75 MHz, $CDCl_3$, TMS = 0) δ = 168.88, 146.58, 145.91, 144.86, 140.45, 133.76, 129.59, 127.72, 123.97, 112.87, 111.97, 57.77, 41.06, 21.85. Anal. Calcd for $C_{15}H_{12}Cl_2N_2O_2$: C, 55.75; H, 3.74; N, 8.67. Found: C, 55.54; H, 3.91; N, 8.51.

5.2.36. 1-(5-(Benzo[*d*][1,3]dioxol-5-yl)-3-(furan-2-yl)-4,5-dihydropyrazol-1-yl)ethanone (56)

Brown solid; yield 79%; mp: 150–152 °C; IR (KBr): 1659 (C=O), 1586 (C=N) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, TMS = 0): δ = 7.56 (d, *J* = 1.13 Hz, 1H), 6.68–6.77 (m, 4H), 6.51 (dd, J = 1.78 and 3.46 Hz, 1H), 5.92 (s, 2H), 5.48 (dd, J = 4.60 and 11.74 Hz, 1H), 3.66 (dd, *J* = 11.74 and 17.70 Hz, 1H), 3.05 (dd, *J* = 4.60 and 17.70 Hz, 1H), 2.39 (s, 3H); ^{13}C (75 MHz, $CDCl_3$, TMS = 0) δ = 168.83, 148.11, 147.09, 146.87, 145.55, 144.77, 135.58, 119.10, 112.64, 111.96, 108.50, 105.99, 101.14, 59.32, 42.12, 22.02. Anal. Calcd for $C_{16}H_{14}N_2O_4$: C, 64.42; H, 4.73; N, 9.39. Found: C, 64.28; H, 4.84; N, 9.52.

5.2.37. 1-(3-(Furan-2-yl)-4,5-dihydro-5-(naphthalen-2-yl)-pyrazol-1-yl)ethanone (57)

Brown solid; yield 75%; mp: 144–146 °C; IR (KBr): 1659 (C=O), 1586 (C=N) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, TMS = 0): δ = 7.79 (m, 3H), 7.68 (s, 2H), 7.57 (d, *J* = 1.31 Hz, 1H), 7.45–7.25 (m, 2H), 6.74 (d, *J* = 3.40 Hz, 1H), 6.52 (dd, J = 1.85 and 3.44 Hz, 1H), 5.74 (dd, *J* = 4.57 and 11.91 Hz, 1H), 3.77 (dd, J = 11.91 and 17.66 Hz, 1H), 3.16 (dd, J = 4.59 and 17.66 Hz, 1H), 2.44(s, 3H); ^{13}C (75 MHz, $CDCl_3$, TMS = 0) δ = 168.90, 146.92, 145.59, 144.77, 138.74, 133.36, 132.96, 129.07, 128.01, 127.65, 126.30, 126.00, 124.50, 123.40, 112.66, 111.96, 59.71, 42.21, 22.01. Anal. Calcd for $C_{19}H_{16}N_2O_2$: C, 74.98; H, 5.30; N, 9.20. Found: C, 75.01; H, 5.25; N, 9.18.

5.2.38. 1-(3-(Furan-2-yl)-4,5-dihydro-5-(pyridin-4-yl)pyrazol-1-yl)ethanone (59)

Brown solid; yield 78%; mp: 98–100 °C; IR (KBr): 1652 (C=O), 1558 (C=N) cm^{-1}; ^1H NMR ($CDOD_3$, 400 MHz, δ, TMS = 0): δ = 8.40 (m, 2H), 7.60 (d, *J* = 1.6 Hz, 1H), 7.20 (m, 2H), 6.83 (d, *J* = 3.6 Hz, 1H), 6.49 (dd, *J* = 1.6 and 3.6 Hz, 1H), 5.48 (dd, *J* = 5.2 and 12.00 Hz, 1H), 3.78 (dd, *J* = 12.00 and 18.00 Hz, 1H), 3.01(dd, *J* = 5.2 and 18.00 Hz, 1H), 2.29 (s, 3H); ^{13}C ($CDCl_3$, 100 MHz, δ, TMS = 0) 170.77, 151.58, 149.27, 147.03, 146.43, 145.28, 121.05, 113.29, 111.76, 58.71, 41.32, 18.28. Anal. Calcd for $C_{14}H_{13}N_3O_2$: C, 65.87; H, 5.13; N, 16.46. Found: C, 65.93; H, 5.29; N, 16.41.

5.3. Biology

5.3.1. Xanthine oxidase assay

Bovine milk xanthine oxidase (grade 1, ammonium sulfate suspension, Sigma–Aldrich) activity was assayed spectrophotometrically by measuring the uric acid formation at 293 nm[22] using a Hitachi U-3010 UV–visible spectrophotometer at 25 °C. The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.6), 75 μM xanthine and 0.08 units of xanthine oxidase. Inhibition of xanthine oxidase activity by various inhibitors was measured by following the decrease in the uric acid formation at 293 nM at 25 °C. The enzyme was preincubated for 5 min, with test compound, dissolved in DMSO (1% v/v), and the reaction was started by the addition of xanthine. Final concentration of DMSO (1% v/v) did not interfere with the enzyme activity. All the experiments were performed in triplicate and values were expressed as means of three experiments.

5.4. Molecular docking

The coordinates of bovine milk XO complexed with salicylic acid were obtained from protein data bank (PDB entry: 1fiq).[24]

The *R* and *S* isomers of **59** were drawn in ChemDraw and subjected to energy minimization in the MOPAC module, using the AM1 procedure for closed shell systems, implemented in the CS Chem3D Ultra.[31] The ligands were docked in to the active site of XO using the GOLD 4.0.1 (Cambridge Crystallographic Data Center, Cambridge, UK)[23] Gold performs genetic algorithm based ligand docking to optimize the conformation of ligand at the receptor binding site. It utilizes GoldScore fitness function to evaluate the various conformations of ligand at the binding site and comprises of four components: protein–ligand hydrogen bond energy, protein–ligand van der Waals (vdw) energy, ligand internal vdw energy, and ligand torsional strain energy. Each isomer was docked 10 times and each pose was ranked according to its GoldScore fitness function. The conformations with highest score were selected for discussion.[32]

Acknowledgment

We thank Mr. Parveen Garg, Chairman of the College for providing facilities to carry out the present research work.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.01.058.

References and notes

1. Stockert, A. L.; Shinde, S. S.; Anderson, R. F.; Hille, R. *J. Am. Chem. Soc.* **2002**, *124*, 14554.
2. Borges, F.; Fernandes, E.; Roleira, F. *Curr. Med. Chem.* **2002**, *9*, 195.
3. Hille, R. *Eur. J. Inorg. Chem.* **2006**, 1913.
4. Pacher, P.; Nivorozhkin, A.; Szabó, C. *Phamacol. Rev.* **2006**, *58*, 87.
5. (a) Elion, G. B.; Callahan, S.; Nathan, H.; Bieber, S.; Rundles, R. W.; Hitching, G. H. *Biochem. Pharmacol.* **1963**, *12*, 85; (b) Tamta, H.; Thilagavathi, R.; Chakraborti, A. K.; Mukhopadhyay, A. K. *J. Enzyme Inhib. Med. Chem.* **2005**, *20*, 317.
6. (a) Biagi, G.; Giorgi, I.; Pacchini, F.; Livi, O.; Scartoni, V. *IL Pharmaco* **2001**, *51*, 809; (b) Brien, D. E.; Springer, R. H.; Albert, T. N. A.; Senga, K.; Miller, J. P.; Streeter, D. G. *J. Heterocycl. Chem.* **1985**, *22*, 601.
7. Oettl, K.; Reibneggar, G. *Biochim. Biophys. Acta* **1999**, *1430*, 387.
8. (a) Nagamatsu, T.; Fujita, T.; Endo, K. *Perkin* **2000**, *1*, 33; (b) Nagamatsu, T.; Ukai, M.; Yoneda, F.; Brown, D. J. *Chem. Pharm. Bull.* **1985**, *33*, 3113; (c) Nagamatsu, T.; Yamasaki, H. *J. Chem. Soc., Chem. Commun.* **1995**, *19*, 2041; (d) Nagamatsu, T.; Yamasaki, H.; Akiyama, T.; Hara, S.; Mori, K.; Kusakabe, H. *Synthesis* **1999**, *4*, 655; (e) Nagamatsu, T.; Fujita, T. *Chem. Commun. (Cambridge)* **1999**, *16*, 1461.
9. Ali, H. I.; Fujita, T.; Akaho, E.; Nagamatsu, T. *J. Comput. Aided Mol. Des.* **2010**, *24*, 57.
10. (a) Baldwin, J. J.; Kasinger, P. A.; Novello, F. C.; Sprague, J. M.; Duggan, D. E. *J. Med. Chem.* **1975**, *18*, 895; (b) Baldwin, J. J.; Lumma, P. K.; Novello, F. C.; Ponticello, G. S.; Sprague, J. M.; Duggan, D. E. *J. Med. Chem.* **1977**, *20*, 1189; (c) Osada, Y.; Tsuchimoto, M.; Fukushima, H.; Takahashi, K.; Kondo, S.; Hasegawa, M.; Komoriya, K. *Eur. J. Pharmacol.* **1993**, *241*, 183.
11. Okamoto, K.; Eger, B. T.; Nishino, T.; Kondo, S.; Pai, E. F.; Nishino, T. *J. Biol. Chem.* **2003**, *278*, 1848.
12. (a) Cos, P.; Ying, L.; Calomme, M.; Hu, J. P.; Cimanga, K.; Poel, B. V.; Pieters, L.; Vlietinck, A. J.; Berghe, D. V. J. *Nat. Prod.* **1998**, *61*, 71; (b) Hoorn, D. E.; Xijveidt, R. J.; Leeuwen, P. A.; Hofman, Z.; Rabet, L.; De Bont, D. B.; Van Norren, K. *Eur. J. Pharmacol.* **2002**, *451*, 111.
13. Ishibuchi, S.; Morimoto, H.; Oe, T.; Ikebe, T.; Inoue, H.; Fukunari, A.; Kamezawa, M.; Yamada, I.; Naka, Y. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 879.
14. Okamoto, K.; Matsumoto, K.; Hille, R.; Eger, B. T.; Pai, E. F.; Nishino, T. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 7931.
15. Shen, L.; Ji, H. F. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5990.
16. Manna, F.; Chimenti, F.; Bolasco, A.; Secci, D.; Bizzarri, B.; Befani, O.; Turini, P.; Mondovi, B.; Alcaro, S.; Tafi, A. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3629.
17. (a) Congiu, C.; Onnis, V.; Vesci, L.; Castorina, M.; Pisano, C. *Bioorg. Med. Chem.* **2010**, *18*, 6238; (b) Manna, F.; Chimenti, F.; Fioravanti, R.; Bolasco, A.; Secci, D.; Chimenti, P.; Ferlini, C.; Scambia, G. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4632.
18. Chimenti, F.; Bizzarri, B.; Manna, F.; Bolasco, A.; Secci, D.; Chimenti, P.; Granese, A.; Rivanera, D.; Lilli, D.; Scaltrito, M. M.; Brenciaglia, M. I. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 603.
19. El-Sabbagh, O. I.; Baraka, M. M.; Ibrahim, S. M.; Pannnecouque, C.; Andrei, G.; Snoeck, R.; Balzarini, J.; Rashad, A. A. *Eur. J. Med. Chem.* **2009**, *44*, 3746.
20. Lv, P. C.; Sun, J.; Luo, Y.; Yang, Y.; Zhu, H. L. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4657.
21. Girisha, K. S.; Kalluraya, B.; Narayana, V.; Padamshree, P. *Eur. J. Med. Chem.* **2010**, *45*, 4640.
22. (a) Escribano, J.; Gracia-Canovas, F. *Biochem. J.* **1988**, *254*, 829; (b) Takano, Y.; Hase-Aoki, K.; Horiuchi, H.; Zhao, L.; Kasahara, Y.; Kondo, S.; Becker, M. A. *Life Sci.* **2005**, *76*, 1835.
23. GOLD, *Evaluation Version 4.0.1*; Cambridge Crystallographic Data Centre: Cambridge, UK, 2009.
24. Enroth, C.; Eger, B. T.; Okamoto, K.; Nishino, T.; Nishino, T.; Pai, E. F. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 10723.
25. Burley, S. K.; Petsko, G. A. *Science* **1985**, *229*, 23.
26. Regaila, H. A. A.; El-Bayouki, K.; Hammad, M. *Egypt. J. Chem.* **1977**, *20*, 157.
27. Grimm, J. B.; Wilson, K. J.; Witter, D. J. *J. Org. Chem.* **2009**, *74*, 6390.
28. Bhendkar, A. H.; Doshi, A. G.; Raut, A. W. *Oriental J. Chem.* **2003**, *19*, 721.
29. Lévai, A.; Jekö, J. *J. Heterocycl. Chem.* **2006**, *43*, 111.
30. Kabli, R. A.; Khalaf, A. A.; Zimaity, M. T.; Khalil, A. M.; Kaddah, A. M.; Al-Rifaie, H. A. *J. Indian Chem. Soc.* **1991**, *68*, 47.
31. ChemDraw Ultra 6.0 and Chem3D Ultra, Cambridge Soft Corporation, Cambridge, USA.
32. See Supplementary data.