

Docking Studies, Synthesis, and Evaluation of Antioxidant Activities of *N*-Alkylated, 1,2,4-Triazole, 1,3,4-Oxa-, and Thiadiazole Containing the Aminopyrazolopyridine Derivatives

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ABSTRACT

Synthesis of some 1,3,4- thia-, oxadiazol and 1,2,4 triazole incorporated the biologically active and the pyrazolopyridine derivative. Molecular modeling and docking of the active compounds into AKRIC3 complexed with its bound inhibitor indomethacin using Molsoft ICM 3.4-8C program were performed in order to predict the affinity and orientation of the synthesized compounds at the active site.

Keywords: Pyrazolopyridine; Biologically Active; Molecular Modeling and Inhibitor

1. Introduction

1*H*-Pyrazolo[3,4-*b*]pyridines comprise a very interesting class of compounds because of their significant and versatile biological and pharmacological activities, such as antimalarial [1] antiproliferative [2] antimicrobial [3-5] inhibition of cyclin-dependent kinases [6] and cardiovascular [7-9] antiviral [10-12] and antileishmanial [13] activities. Pyrazole fused pyridines and pyrimidines are known to possess a wide range of biological activity. Specifically pyrazolopyridines exhibit antitubercular, anxiolytic [14].

It has been reported that certain compounds bearing 1,3,4-oxa-, thiadiazole, and 1,2,4-triazole nucleus possess significant anti-inflammatory activity [15,16]. Also, in view of these reports and in continuation of our recent work on the pyrazolo[3,4-*b*]pyridine derivatives, to synthesize a new heterocyclic compounds [17,18], we reported here the synthesis of a number of new alkylated 4,6-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridine-3-amine, formation of 1,2,4-triazoles, 1,3,4-oxadiazole, and 1,3,4-thiadiazoles.

Design of drug targets containing two carboxylic groups appropriately attached to the opposite sides of the aromatic fragment (e.g., naphthalene ring) as shown by our synthesized compounds to interact with the active

site hydrophobic pocket while the first carboxylate may occupy the oxyanion hole and the second forms H-bonds with oxygen of coenzyme's diphosphate moiety will be a good rationale for potent inhibitors.

2. Material and Methods

2.1. Experimental

Melting points were determined on a Buchi melting point. IR spectra were recorded with a Perkin-Elmer model 1720 FTIR (KBr), ¹H NMR spectra were recorded with Bruker AC 250 FT NMR spectrometer at 250 MHz with TMS as an internal standard. EIMS and FABMS spectra were recorded with a Finnigan MAT 312 = AMD. The microanalyses were performed at the microanalytical unit, Cairo University.

Ethyl 2-(3-amino-4,6-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridin-1-yl)acetate (2). To a stirred suspension of pyrazolopyridine **1** (0.81 g, 5 mmol) in dry DMF (10 mL) containing K₂CO₃ (0.67 g, 5 mmol), the ethyl chloroacetate (0.61 g, 5 mmol) was added dropwise. The reaction mixture was stirred at room temperature for an additional 13 h and then poured into ice cold water with stirring. The obtained solid product was collected by the filtration, washed with water and recrystallized from ethanol to afford the pale yellow crystals of ester **2**. (0.77 g, 95%), m.p. 127°C - 129°C. IR (KBr, ν_{\max} , cm⁻¹): 3316 (NH₂),

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2923, 2853 (CH aliphatic), 1722 (C=O), 1596 (C=N). ¹H NMR (CDCl₃), δ , ppm: 1.13 (3H, t, $J = 7$ Hz, CH₃CH₂), 4.22 (2H, q, $J = 13$ Hz, CH₃CH₂), 2.70 (3H, s, CH₃), 2.79 (3H, s, CH₃), 5.09 (2H, s, CH₂), 6.57 (1H, s, H-5), 7.14 (2H, bs, NH₂). Mass spectrum, m/z (I, %): 249 [M⁺+1] (35), 248 [M⁺] (100), 161 (70), 131 (16). Found, %: C 58.05; H 6.50; N 22.57. C₁₂H₁₆N₄O₂ Calculated, %: C 57.99; H 6.22; N 22.32.

2-(3-Amino-4,6-dimethyl-1H-pyrazolo[3,4-b] pyridin-1-yl) acetohydrazide (3). To a suspension of ester 2 (1.24 g, 5 mmol) in ethanol (15 ml), an excess of hydrazine hydrate (4 ml) was added. The reaction mixture was refluxed for 4 h, cooled, the solid product was collected by filtration, dried, and recrystallized from methanol to give the pale yellow crystals of compound 3 (0.91 g, 77%). mp 150°C - 152°C IR (KBr) (cm⁻¹), $\nu = 3343 - 3202$ (NHNH₂), 2938, 2892 (CH aliphatic), 1660 (C=O), 1589 (C=N). ¹H NMR (DMSO-d₆), δ , ppm: 2.45 (3H, s, CH₃), 2.51 (3H, s, CH₃), 4.98 (2H, s, CH₂), 5.12 (2H, bs, NH₂NH), 6.54 (1H, s, CH-5), 6.79 (2H, bs, NH₂), 9.16 (1H, bs, NH₂NH). Mass spectrum, m/z (I, %): 234 [M⁺] (8), 252 (25), 219 (23), 175 (100). Found, %: C 51.27; H 6.02; N 35.88. C₁₀H₁₄N₆O Calculated, %: C 51.13; H 5.88; N 35.47.

5-((3-amino-4,6-dimethyl-1H-pyrazolo[3,4-b] pyridin-1-yl)methyl)-1,3,4-oxadiazole-2-thiol (4). Hydrazid 3 (0.94 g, 4 mmol) and CS₂ (0.3 g, 4 mmol) were added to a solution of KOH (0.22 g, 4 mmol) in water (10 ml) and ethanol (10 ml). The reaction mixture was refluxed for 4 h. After evaporation under reduced pressure, a solid was obtained. This was dissolved in water and acidified with conc. HCl. The precipitate was filtered, washed with water, and recrystallized from methanol to give colorless powder, yield 0.72 g, 65%, mp 230°C - 233°C (MeOH). IR spectrum (thin layer), ν , cm⁻¹: 2784, 2725 (S-H), 1566 (C=N). ¹H NMR spectrum (300 MHz, DMSO-d₆), δ , ppm (J , Hz): 2.45 (3H, s, CH₃), 2.52 (3H, s, CH₃), 4.18 (s, 2H, CH₂), 6.74 (1H, s, CH-5), 6.87 (2H, bs, NH₂) 13.82 (brs, 1H, SH). ¹³C NMR spectrum (75.5 MHz, DMSO-d₆), δ , ppm: 15.13, 19.62 (2 CH₃), 33.30 (CH₂), 115.12, 125.17, 128.59, 128.79, 129.37, 129.72, 135.84, 144.28 (Ar-C). Mass spectrum (EI, 70 ev), m/z (I_{rel} , %): 277 [M⁺+1] (2), 276 [M⁺] (20), 194 (18), 161 (24), 129 (22), 44 (74), 28 (100). Found, %: C, 47.81, H, 4.38, N, 30.41. C₁₁H₁₂N₆OS. Calculated, %: C, 47.32; H, 4.31; N, 30.29.

4,6-dimethyl-1-((5-(alkylthio)-1,3,4-oxadiazol-2-yl)methyl)-1H-pyrazolo[3,4-b]pyridin-3-amine (5a-c). To a stirred suspension of oxadiazol thion 4 (1mmol) in dry DMF (10 ml), sodium hydride (0.04 g, 1 mmol, 60% dispersion oil) was added. When liberation of hydrogen had ceased (1.5 h), the appropriate alkyl reagent (ethyl-, methyl iodide and benzylchloride) was added in dropwise, and the reaction mixture was stirred at room tem-

perature for 10 - 15 h. The solvent was removed under reduced pressure and the residue was triturated with cold water with stirring for 2 - 5 h, the products was filtered off, deride and recrystallized with ethanol to give 5a-c.

1-((5-(Ethylthio)-1,3,4-oxadiazol-2-yl)methyl)-4,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-3-amine (5a). Colorless crystals, yield 0.19 g 61%, mp 139°C -140°C; ¹H NMR spectrum (300 MHz, CDCl₃), δ , ppm (J , Hz): 0.93 (3H, t, $J = 7.0$ Hz, CH₃CH₂), 2.45 (3H, s, CH₃), 2.53 (3H, s, CH₃), 3.11 (2H, q, $J = 7.1$, SCH₂CH₃), 4.87 (2H, s, N-CH₂), 6.88 (1H, s, H-5), 7.02 (2H, bs, NH₂). Mass spectrum (EI, 70 ev), m/z (I_{rel} , %): 304 [M⁺] (20), 290 [M-CH₃], 148 (100). Found, %: C, 51.30; H, 5.30; N, 27.16. C₁₃H₁₆N₆OS. Calculated, %: C, 51.15; H, 5.14; N, 27.09.

4,6-Dimethyl-1-((5-(methylthio)-1,3,4-oxadiazol-2-yl)methyl)-1H-pyrazolo[3,4-b]pyridin-3-amine (5b). Colorless crystals, yield 0.24 g, 77%, mp 160°C - 162°C; ¹H NMR spectrum (300 MHz, CDCl₃), δ , ppm (J , Hz): 2.33 (3H, s, CH₃), 2.49 (3H, s, SCH₃), 2.54 (3H, s, CH₃), 4.55 (2H, s, N-CH₂), 6.67 (1H, s, H-5), 7.25 (2H, bs, NH₂). Mass spectrum (EI, 70 ev), m/z (I_{rel} , %): 290 (M⁺, 43), 148 (100). Found, %: C, 49.64; H, 4.86; N, 28.95. C₁₂H₁₄N₆OS. Calculated, %: C, 49.45; H, 4.52; N, 28.65.

1-((5-(benzylthio)-1,3,4-oxadiazol-2-yl)methyl)-4,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-3-amine (5c). Colorless powder, yield 0.31 g, 78%, mp 202°C - 204°C; ¹H NMR spectrum (300 MHz, CDCl₃), δ , ppm (J , Hz): 2.53, 2.64 (6H, 2s, 2CH₃), 4.40 (2H, s, SCH₂Ph), 4.55 (2H, s, CH₂), 6.32 (1H, s, H-5), 6.67 - 7.47 (5H, m, Ph). Mass spectrum (EI, 70 ev), m/z (I_{rel} , %): 366 [M⁺], 289 (55), 275 (34), 91 (100). Found, %: C, 59.00; H, 4.95; N, 22.93. C₁₈H₁₈N₆OS. Calculated, %: C, 58.87; H, 4.34; N, 22.86.

2-(2-(3-Amino-4,6-dimethyl-1H-pyrazolo[3,4-b] pyridin-1-yl)acetyl)-N-phenylhydrazinecarbothioamide (6). To suspension of acid hydrazide 3 (2.34 g, 10 mmol) in absolute ethanol (20 mL), PhNCS (1.35 g, 10 mmol) was added. The reaction mixture was heated under reflux for 6 h. The product that separated on cooling was filtered off, washed with ethanol dried and recrystallized from methanol to give a colorless crystals of compound 6, yield, 3.46 g, 94%, m.p. 173°C - 175°C. IR (KBr, ν_{max} , cm⁻¹): 3336 - 3244 (NH, NH₂), 4035, 2978, 2886 (CH aliphatic), 1697 (CONH), 1593 (C=N), 1195 - 1130 (C=S). ¹H NMR (CDCl₃), δ , ppm: 2.55 (3H, s, CH₃), 2.68 (3H, s, CH₃), 4.95 (2H, s, CH₂), 6.98 (1H, s, H-5), 7.93-8.54 (7H, m, NH, ph), 8.77 (1H, s, N=CH), 9.07 (1H, bs, CSNH), 10.43 (1H, bs, CONH). Mass spectrum, m/z (I, %): 270 [M⁺+1, 20], 269 [M⁺, 70], 192 (35), 240 [M⁺] (100), 161 (70), 131 (16). Found, %: C 55.27; H 5.18; N 26.54. C₁₇H₁₉N₇OS Calculated, %: C 55.33; H 5.15; N 26.57.

5-((3-amino-2-yl-methyleneamino)-4,6-dimethyl-1H

-pyrazolo[3,4-b]pyridin-1-yl)methyl)-N-phenyl-1,3,4-thiadiazol-2-amine (7). The phenylhydrazinecarbothioamide **6** (1.34 g, 5 mmol) was added gradually with stirring to cold conc. H₂SO₄ (10 ml) during 10 min. The mixture was further stirred for another 2 h in an ice bath. The solid separated out was filtered, washed with water, dried and recrystallized from methanol to give a yellow crystals of compound **7**, yield, (2.95 g, 84%), m.p. 190°C - 192°C. IR (KBr, ν_{\max} , cm⁻¹): 3316 - 3285 (NH), 2955 - 2933 (CH aliphatic), 1598 (C=N). ¹H NMR (CDCl₃), δ , ppm: 2.51 (3H, s, CH₃), 2.64 (3H, s, CH₃), 5.33 (2H, s, CH₂), 6.33 (1H, s, H-5), 7.21 (2H, bs, NH₂), 7.55 - 7.76 (6H, m, ph), 11.55 (1H, bs, NHPh). Mass spectrum, m/z (I, %): 352 [M⁺+1] (35), 351 [M⁺] (100), 161 (70), 131 (16). Found, %: C 58.01; H 4.88; N 27.90. C₁₇H₁₇N₇S Calculated, %: C 58.17; H 4.74; N 27.72.

5-((3-amino-2-yl-methyleneamino)-4,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-1-yl)methyl)-4-phenyl-4H-1,2,4-triazole-3-thiol (8). A suspension of thiosemicarbazide **8** (2.68 g, 10 mmol) in ethanol (50 ml) was dissolved in 4N aqueous sodium hydroxide (50 ml), resulting in the formation a clear solution. The reaction mixture was refluxed for 4 h on water bath, concentration, cooled, and filtered. The pH of the filtrate was adjusted between 5-6 with acetic acid and kept aside for 2 h. The solid separated out was filtered, washed with water, dried and recrystallized with ethanol to give white powder of compound **8**, (2.35 g, 67%), m.p. 217°C - 219°C. IR (KBr, ν_{\max} , cm⁻¹): (NH₂), 3022 (CH aromatic), 2955-2933 (CH aliphatic), 2786 - 2715 (SH), 1638 (C=N). ¹H NMR (CDCl₃), δ , ppm: 2.50 (3H, s, CH₃), 2.61 (3H, s, CH₃), 5.39 (2H, s, CH₂), 6.23 (1H, s, H-5), 7.22 - 7.75 (8H, m, ph, NH₂), 12.05 (1H, bs, SH). Mass spectrum, m/z (I, %): 352 [M⁺+1] (35), 351 [M⁺] (69), 160 (35), 131 (100). Found, %: C 58.01; H 4.88; N 27.90. C₁₇H₁₇N₇S Calculated, %: C 58.28; H 4.95; N 27.66.

5-((3-amino-4,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-1-yl)methyl)-N-phenyl-1,3,4-oxadiazol-2-amine (9): Mercuric oxide (2.37 g, 11 mmol) was added to solution of thiosemicarbazide **6** (2.69 g, 10 mmol) in methanol (20 mL) and the resulting mixture was refluxed for 3 h. The precipitate mercuric sulfide was filtered off and washed with hot methanol. The filtrate on cooling gave a solid product which was filtered, dried and recrystallized from methanol to give a yellow crystals of oxadiazol **9** (2.78 g, 83%), m.p. 210°C - 212°C. IR (KBr, ν_{\max} , cm⁻¹): 3230 - 3205 (NH, NH₂), 3055 (CH aromatic), 2944 - 2910 (CH aliphatic), 1635 (C=N). ¹H NMR (CDCl₃), δ , ppm: 2.29 (3H, s, CH₃), 2.50 (3H, s, CH₃), 5.28 (2H, s, CH₂), 6.13 (1H, s, H-5), 7.05 - 7.20 (8H, m, ph, NH₂), 10.12 (1H, bs, NHPh). Mass spectrum, m/z (I, %): 336 [M⁺+1] (35), 335 [M⁺] (100), 147 (70), 128 (16). Found, %: C 60.96; H 5.25; N 29.32. C₁₇H₁₇N₇O Calculated, %: C 60.88; H 5.11; N 29.24.

2.2. Molecular Modeling Studies

2.2.1. Generation of Ligand and Enzyme Structures

The crystal structure of AKR1C3 complexed with its bound inhibitor Indomethacin was downloaded through the Protein Data Bank PDB/ RCSB site and saved as *.pdb file [18].

A set of novel N-Alkylated, 1,2,4-triazole, 1,3,4-oxa-, thiadiazole and complexes containing the aminopyrazolopyridine derivatives were designed to inhibit AKR1C3. All compounds were built in ChemDraw Ultra version 8.0.3 and their energy minimized through Chem3D Ultra version 8.0.3/ MM2, Jop Type: minimum RMS Gradient of 0.100, and saved as MDL MolFile (*.mol).

2.2.2. Docking Using Molsoft ICM 3.4-8C Program

The novel energy-minimized Indomethacin analogues were docked into the active site of AKR1C3 crystal structure using ICM-Pro software version 3.4 - 8 C. ICM-Pro scores the binding of a ligand to a receptor based upon the comparison of a series of small molecule/protein interactions that have been reported in the PDB database. A rigid receptor/flexible ligand approach was adopted that uses five potential energy maps combining hydrophobicity, electrostatics, hydrogen bond formation, and two van-der-Waals parameters. In all cases, the program's default parameters were used.

3. Results and Discussion

The starting material 4,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-3-amine (**1**) was prepared according to the reported method [19,20], which was alkylated, after its treatment with anhydrous potassium carbonate in dry *N,N*-dimethylformamide, with ethyl-2-chloroacetate to give Ethyl 2-(3-amino-4,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-1-yl)acetate (**2**) in good yield. The ¹H NMR spectrum of derivative **2** showed that *N*-CH₂ of the *N*-alkylated product appeared as a triplet at δ 1.13 and quartet at δ 4.22 ppm respectively. The latter derivative **2** was treated with hydrazine hydrate, in ethanol on boiling, to afford the corresponding acid hydrazid. 2-(3-Amino-4,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-1-yl) acetohydrazide (**3**) was cyclized on treating with carbon disulfide, in basic medium, to give the 5-((3-amino-4,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-1-yl)methyl)-1,3,4-oxadiazole-2-thiol (**4**) which showed the broad singlet at δ 13.82 ppm corresponding to the SH group in its H¹ NMR, the latter oxadiazole **4** was also elucidated via its alkylation reactions with some alkylation agents such as methyl, ethyl iodide and benzyl chloride to afford the S-alkyl derivatives **5a-c**. These alkylated derivatives were elucidated by the MS spectra which showed the molecular ion peaks corresponding to its molecular weights (**Scheme 1**).

The acetohydrazide **3** was treated with phenylisothio-

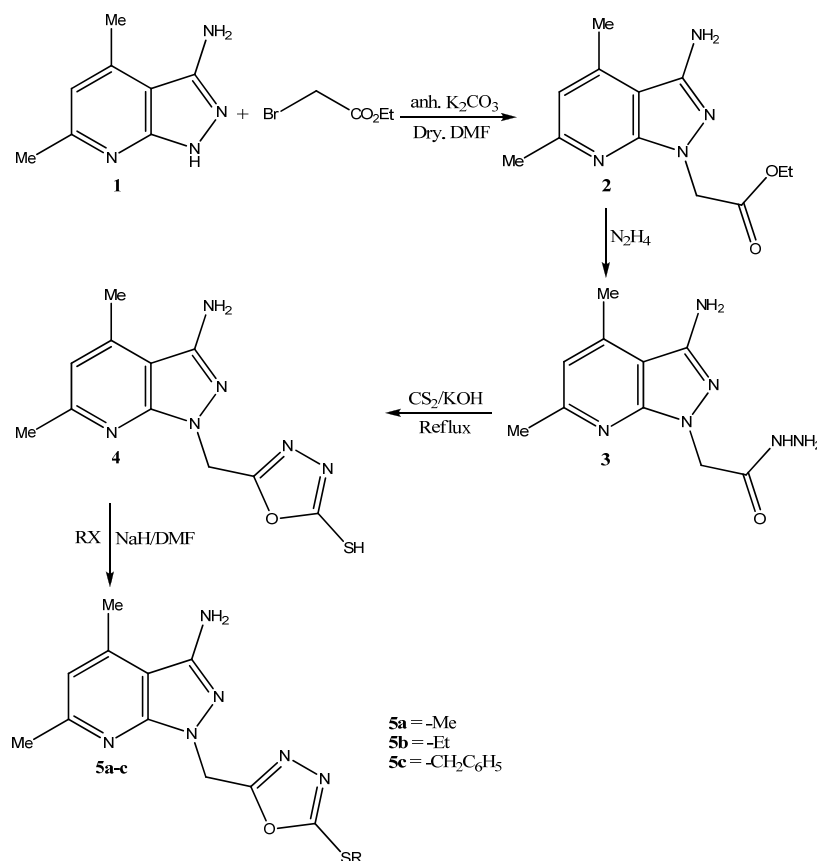
cyanate to give the corresponding phenylhydrazinecarbothioamide derivative **6**, which was elucidated, besides the ^1H NMR and IR, by the mass spectrum which showed the molecular ion peak at m/z 270 corresponding to $[\text{M}^+ + 1]$, m/z . When the thiosemicarbazide **6** was reacted with concentrated sulfuric acid, 1,3,4-thiadiazole derivative **7** was obtained which showed in its ^1H NMR spectrum of the broadband of the NHPh at δ 11.55 ppm. The formation of the thiadiazole ring, under such acidic conditions, is due to the loss of nucleophilicity of N -4 as a result of its protonation leading to an increase in the nucleophilicity of the sulfur atom toward the attack of the carbonyl carbon as shown in **Scheme 2**. On the other hand, when the cyclization of **6** was carried out under basic conditions, the nucleophilicity of N -4 was enhanced and affording cyclization with carbonyl carbon atom to afford 1,2,4-triazole derivative in 67% yield. In the treatment of thiosemicarbazide **6** with mercuric oxide, the cyclization was performed, affording the 1,3,4-oxadiazole derivative **9**. The method of cyclization includes desulfurization by HgO . The ^1H NMR of triazole **8** showed a broad singlet at δ 12.05 ppm corresponding to the SH group. The oxadiazole derivative **9** was elucidated besides, NMR, elemental analysis, by the mass spectrum which showed a $[\text{M}^+]$ peak, in agreement with

its molecular formula (**Scheme 3**).

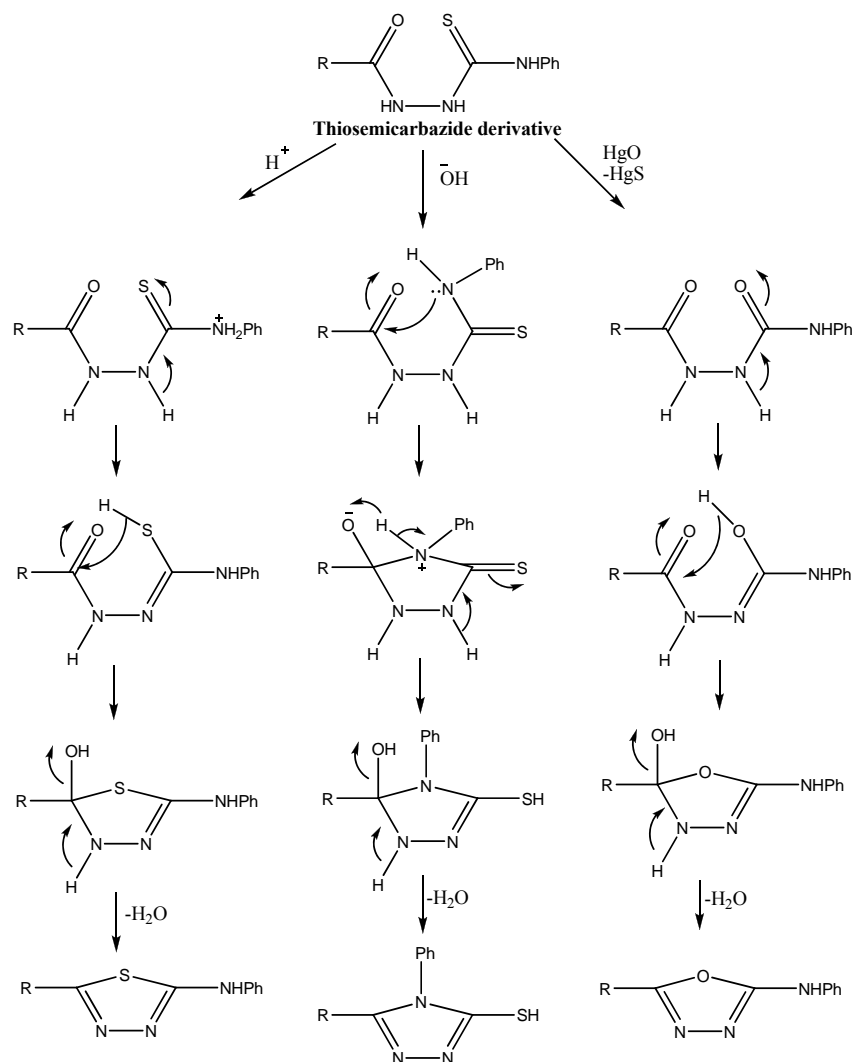
Molecular Modeling Studies

To pre-assess the anti-tumorigenic behavior of our N -Alkylated, 1,2,4-triazole, 1,3,4-oxa-, thiadiazole and complexes containing the aminopyrazolopyridine derivatives **5(a-c)** and (**7-9**) on a structural basis, automated docking studies were carried out using MOLSOFT ICM 3.4 - 8C program [21]. The scoring functions and hydrogen bonds formed with the surrounding amino acids are used to predict their binding modes, their binding affinities and orientation of these compounds at the active site of AKR1C3 enzyme. The protein-ligand complex was constructed based on the X-ray structure (PDB entry 1S2A) AKR1C3 with its bound inhibitor indomethacin [22].

The scoring functions of the compounds were calculated from minimized ligand protein complexes. The X-ray crystal structure of AKR1C3 reveals a substrate-binding site that consists mainly of: hydrophobic aromatic amino acid side chains (Tyr24, Tyr55, Leu54, Trp227, and Phe306). An oxyanion hole, which is located at the bottom of the hydrophobic pocket, is formed by active site tyrosine (Tyr55), histidine (His117), and the coenzymes nicotinamide ring [21].



Scheme 1. Formation of the oxadiazole derivatives.



Scheme 2. Schematic investigation according to the condition applied.

In our investigation, the 3D-coordinates in X-ray crystal structure of AKR1C3 in complex with the ligand, indomethacin (PDB entry 1S2A) [21] was used as the receptor model in AKR1C3 docking simulation. The docked model of indomethacin with AKR1C3 (**Figure 1**) was consistent with the previously reported X-ray analysis [22] and revealed the following binding mode: The carbonyl oxygen is far away from Tyr 55 or His 117 to H-bond directly. Instead, the carboxylate group points toward and interacts with the oxygen atoms O_{1n}, O_{2n} from the nicotinamide half of the NADP⁺ diphosphate moiety, forming two hydrogen bonds, and additional H-bond if formed between indomethacin O₂ of COOH and He₂ of Glu 222.

Our active compounds **5(a-c)** and **(7-9)** when modeled in the active site of AKR1C3 enzyme (**Table 1, Figures 2,3**) revealed strong binding affinities. Their binding energies were -67.08 , -88.58 , -96.73 , -103.82 , -88.74 and -97.28 Kcal/mol, respectively compared to -80.45 of

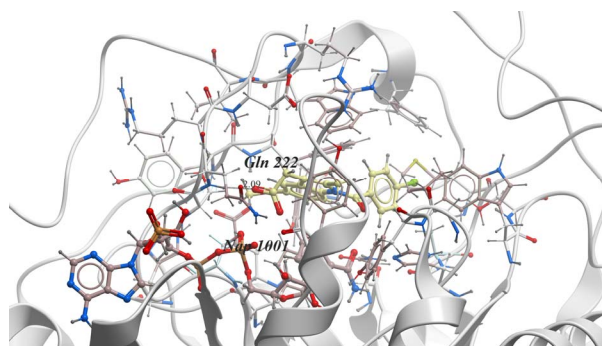
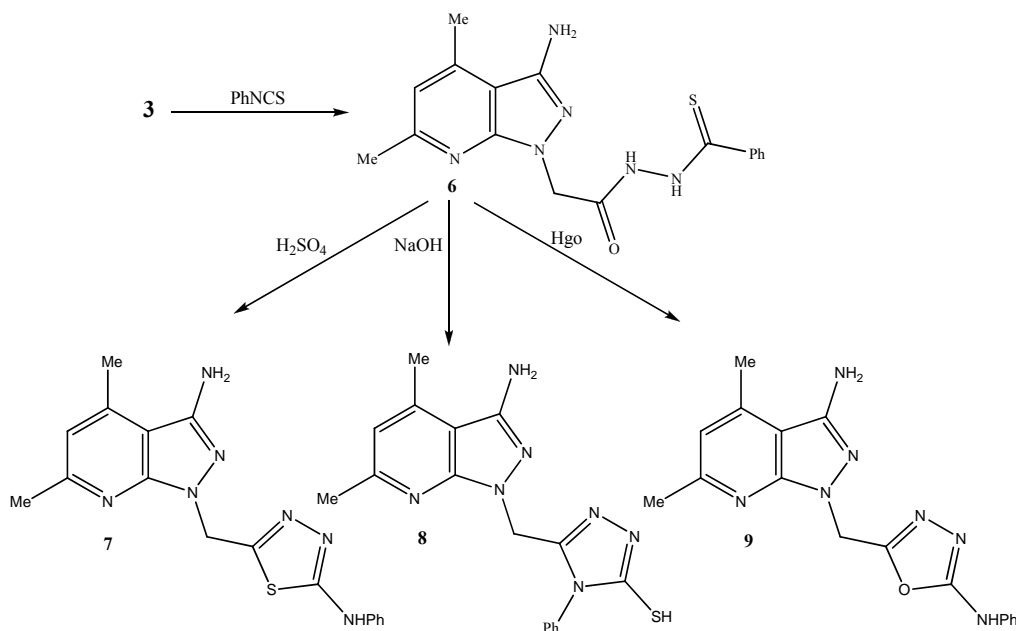


Figure 1. The proposed binding mode of original ligand indomethacin into the active binding site of AKR1C3 active site. It has ICM score of -80.45 , it forms three hydrogen bonds, two of them between H of COOH and O_{1n}, O_{2n} of Phosphate moiety of NADP. And another hydrogen bond between O₂ of COOH and He₂ of amino acid Glu222....

indomethacin. It is interesting to point out that compounds **5(a-c)** and **(7-9)** are found to be very promising

Table 1. Docking of compounds on AKR1C3.

Compound number	ICM score (ΔG)	No of Hydrogen bonds	Atom of ligand involved	Amino acid residues forming the hydrogen bonds	Bond length (Å)			
Indomethacin	-80.45	3	H of COOH	Phosphate moiety of NADP....O1n	2.36			
			H of COOH	Phosphate moiety of NADP....O2n	1.57			
			O2 of COOH	Glu222...He2	2.09			
5a	-67.08	3	m of M n3	Arg226...hh21	2.18			
			m of M h2	Tyr24...oh	1.79			
			m of M h3	Tyr24...oh	2.68			
			m of M n5	Ala25...hn	2.75			
			m of M o1	Arg31...hh11	2.14			
			m of M o1	Arg31...hh12	1.58			
5b	-88.58	13	m of M n5	Arg31...hh21	2.73			
			m of M n4	Arg226...hh12	1.51			
			m of M h2	Tyr24...oh	1.86			
			m of M h05	Ala25...o	1.91			
			m of M h04	Tyr55...o	2.07			
			m of M n4	Tyr24...hh	2.74			
			m of M n4	Arg226...hh12	1.50			
5c	-96.73	7	m of M n1	Arg226...hh21	1.98			
			m of M n4	Arg226...hh21	2.54			
			m of M h2	Tyr24...oh	1.34			
			m of M h3	Tyr24...oh	2.22			
			m of M h04	Ser129...og	2.37			
			m of M n5	Ala25...hn	1.59			
			m of M n5	Arg31...hh12	2.53			
			m of M n6	Arg31...hh12	1.69			
			m of M n7	Arg31...hh21	1.55			
			m of M n7	Arg31...hh22	2.54			
			m of M n4	Arg226...hh12	1.45			
			m of M n4	Arg226...hh21	1.76			
7	-103.82	12	m of M h03	Tyr24...oh	1.46			
			m of M h3	Tyr24...oh	2.21			
			m of M h12	Ala25...o	2.62			
			m of M h12	Pro26...o	2.80			
			m of M h05	Tyr55...o	1.44			
			m of M n5	Glu222... He2	2.06			
			m of M n6	Lys270...hz1	2.00			
			m of M n7	Lys270...hz1	2.59			
			m of M n5	Lys270...hz2	2.60			
			m of M n6	Lys270...hz2	1.83			
			m of M n6	Lys270...hz3	1.70			
			8	-88.74	19	m of M n7	Lys270...hz3	2.22
m of M n2	Phosphate moiety of NADP.... h01	2.18						
m of M n7	Phosphate moiety of NADP.... h01	1.40						
m of M n6	Phosphate moiety of NADP.... h10	2.45						
m of M h03	Ser221...o	2.65						
m of M h71	Phosphate moiety of NADP.... o3b	1.80						
m of M h02	Phosphate moiety of NADP.... o2x	2.78						
m of M n5	Glu222...he21	2.39						
9	-97.28	4				m of M n7	Glu222...he21	1.55
						m of M h04	Glu222...he12	1.50
						m of M h2	Tyr24...oh	2.40



Scheme 3. crystallization of the thiosemicarbazole derivative.

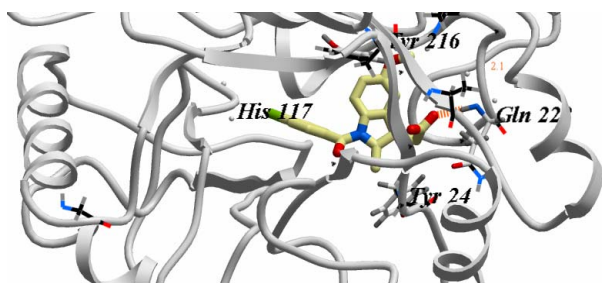


Figure 2. The proposed binding mode of 5a into the active binding site of AKR1C3 active site. It has ICM score of -67.08 , it forms three hydrogen bonds, two of them between O2 of OH and He2 and He3 of Tyr24 and Glu58...One hydrogen bond between O3 of SO₂ and Hh1 of amino acid Arg 226. And hydrogen bond between O4 of SO₂ and H1n1 of Phosphate moiety of NADP....

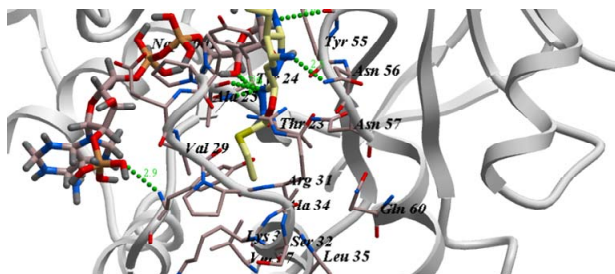


Figure 3. The proposed binding mode of 5b into the active binding site of AKR1C3 active site. It has ICM score of -88.58 , it forms 13 hydrogen bonds, one between O1 of OCH₃ and Ha1 of Phosphate moiety of NADP. Two hydrogen bonds between O4 of SO₂ and Hd2 of Arg31 and H7n7 of Amide of Nicotinamide ring ... three hydrogen bonds, two of them between O2 of OH and He2 and He3 of Tyr24 and Another two hydrogen bonds is between H14, H15 of benzene ring of sulfonamide and He2 of amino acid Tyr55.

AKR1C3 inhibitors, as they take advantage of their ability to make H bond with the amino acid present in the oxyanion hole (Tyr 55 and Glu222) in the presence of the coenzyme's nicotinamide with the aromatic residues located in the hydrophobic pocket, and at the same time to NADP⁺ diphosphate.

Using Molsoft ICM 3.4 - 8C program, molecular modelling and docking studies of the synthesized compounds into ARK1C3 complexed with its bound inhibitor indomethacin (1S2A) were performed in order to predict the binding affinities and orientations of these compounds at the active site. The ICM score values of 5(a-c) and (7-9) were -67.08 , -88.58 , -96.73 , -103.82 , -88.74 and -97.28 Kcal/mol, respectively compared to -80.45 of indomethacin. These derivatives will encourage researchers to help to design future anticancer agents with therapeutic potentials.

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