molbank

MDPI

Short Note

1-[5-(4-Ethoxyphenyl)-3-(naphthalen-1-yl)-4,5-dihydro-1*H*-pyrazol-1-yl]ethan-1-one

Sangeetha Karanth [1], Badiadka Narayana [1,*], Jayashree Avvadukkam [1] and Balladka Kunhanna Sarojini [2]

[1] Department of Studies in Chemistry, Mangalore University, Mangalagangothri-574199, Karnataka, India; sangeethakudupu@gmail.com (S.K.); shree.aj31chem6@gmail.com (J.A.)
[2] Department of Industrial Chemistry, Mangalore University, Mangalagangothri-574199, Karnataka, India; bksaroj35@gmail.com
* Correspondence: nbadiadka@yahoo.co.uk; Tel.: +824-228-7262

Received: 23 May 2018; Accepted: 17 June 2018; Published: 19 June 2018

Abstract: The cyclization reaction of 3-(4-ethoxyphenyl)-1-(naphthalen-1-yl)prop-2-en-1-one (**1**) using hydrazine hydrate in acetic acid medium culminates, resulting in the title compound 1-[5-(4-ethoxyphenyl)-3-(naphthalen-1-yl)-4,5-dihydro-1*H*-pyrazol-1-yl]ethan-1-one (**2**). The structure of the newly synthesized compound is determined by IR, ^{1}H-NMR, ^{13}C-NMR and mass spectral data. In order to evaluate the putative molecular interactions, the title compound (**2**) is docked against the active site of Cytochrome P_{450} 14α-sterol demethylase (CYP51) from *Mycobacterium tuberculosis*.

Keywords: pyrazoline; cytochrome P_{450}; molecular docking

1. Introduction

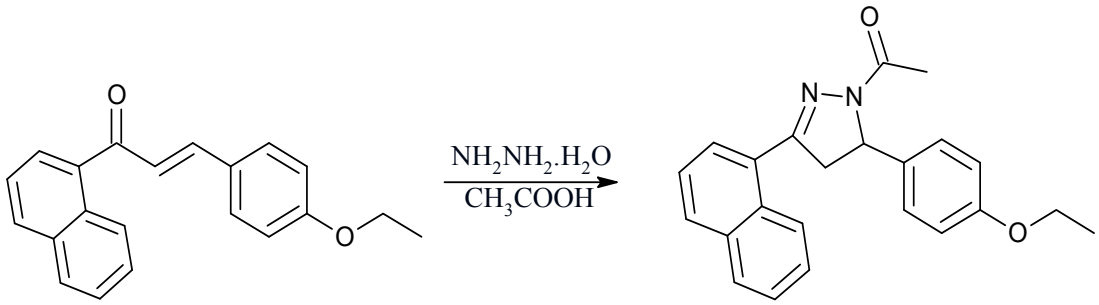
One of the most convenient synthetic methodologies employed for the synthesis of pyrazoline derivatives involves the cyclization of α,β-unsaturated ketones with hydrazine and its derivatives in the presence of a suitable cyclizing agent such as acetic acid. Compounds containing pyrazoline pharmacophore have been reported to display pronounced anti-diabetic [1], anti-tubercular [2], antidepressant [3], anticonvulsant [4], antimicrobial [5], anti-inflammatory [6,7], anticancer [8–10] and antiamoebic [11] properties. In view of developing relevant pharmacological activities using pyrazoline tethered heterocyclic compounds, the design and synthesis of the title compound were undertaken to test its biological potency.

Molecular docking is a very powerful computational tool which determines the extent of interactions of the ligand bound to the active site of the enzyme. Cytochrome P_{450} (CYP) are proteins belonging to the class of hemoproteins, with heme embodied as a cofactor. These enzymes are associated with the metabolism of potentially active compounds such as drugs and products of endogenous metabolism. In bacteria, their function is attributed to its role in specialized metabolite biosynthetic pathways. Cytochrome P_{450} 14α-sterol demethylases being imperative enzymes in sterol biosynthesis, prove to be the suitable target for evaluating the potency of newly synthesized molecules [12]. One of the legitimate drug targets for cytochrome $P_{450,}$ being fluconazole bound CYP51 from *Mycobacterium tuberculosis* (MTCYP51), has been selected for the evaluation of its interactions with the synthesised compound.

2. Results

The schematic pathway for the synthesis of the title compound (**2**) is outlined in Scheme 1. The compound 3-(4-ethoxyphenyl)-1-(naphthalen-1-yl)prop-2-en-1-one (**1**) on reaction with hydrazine hydrate in glacial acetic acid medium, underwent cyclization in reflux condition to yield the title

compound (**2**). Compound (**1**) was in turn synthesized by the base-catalysed Claisen-Schmidt condensation of 1-(naphthalen-1-yl)ethan-1-one and 4-ethoxybenzaldehyde. The structure and purity of the newly synthesised compound was confirmed by elemental analysis, IR, ^{1}H-NMR, ^{13}C-NMR and mass spectral data.



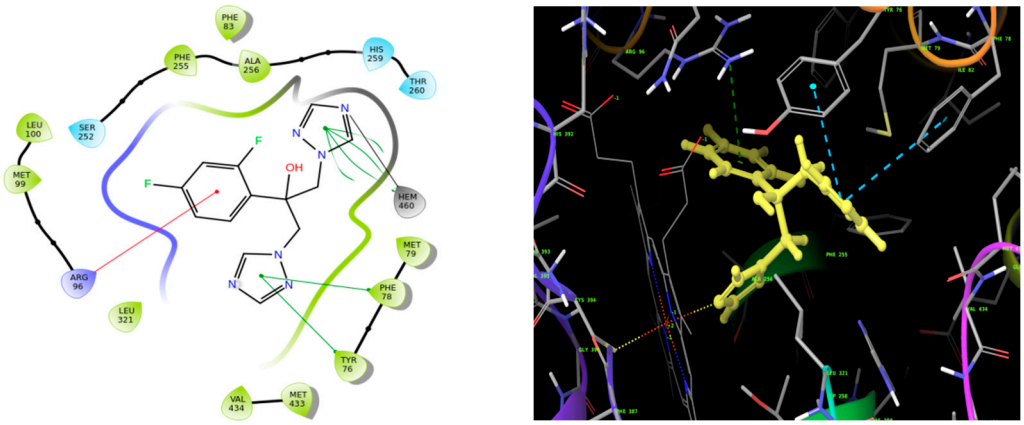
(**1**) (**2**)

Scheme 1. Synthesis of 1-[5-(4-ethoxyphenyl)-3-(naphthalen-1-yl)-4,5-dihydro-1*H*-pyrazol-1-yl]ethan-1-one.

Molecular Docking

In order to obtain a comprehensive insight into the molecular interaction with the target protein, the title compound was docked within the generated target grid of the protein MTCYP51. The active pocket of the protein embodies Hem 460 and amino acid residues Arg 96, Phe 78, Tyr 76, Leu 100, Met 99, Met 79, Met 433, Val 434, Ser 252, Phe 255, Ala 256, Phe 83, His 259 and Thr 260. Among the two triazole rings in the fluconazole, one is involved in distinct π-π stacking interaction with Tyr 76 and Phe 78 residues, whereas the other displays π-π stacking interactions and metal coordination with the Hem 460 in the active site of the enzyme. Furthermore, the 2,4-disubstituted phenyl ring of the drug also displays a π-cation interaction with the Arg 96 residue of the active site.

The receptor grid generated, docked by the title compound displayed π-π stacking and π-cation interactions with the amino acid residues of the protein. The glide docking score of the synthesised compound in comparison with the standard drug fluconazole is presented in Table 1, followed by Figures 1 and 2, which exemplifies the 2D (Figures 1a and 2a) and 3D (Figures 1b and 2b) docking poses of the standard drug fluconazole and compound (**2**) in the active pocket of MTCYP51 respectively.



(**a**) (**b**)

Figure 1. (**a**) 2D and (**b**) 3D docking pose of the standard drug fluconazole with MTCYP51.

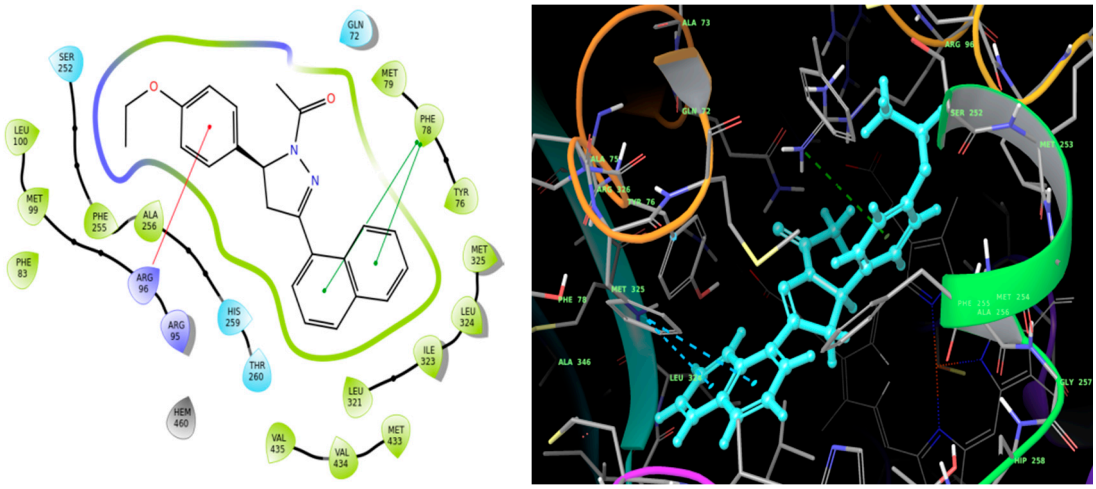


Figure 2. (**a**) 2D and (**b**) 3D docking pose of the compound (**2**) with MTCYP51.

Table 1. Docking score of the compound (**2**) and the bound ligand fluconazole.

Ligand	Docking Score (kcal/mol)
Compound (**2**)	−7.501
Fluconazole	−5.823

3. Discussion

The IR spectrum of compound (**2**) exhibited absorption band at 1641 cm^{-1}, corresponding to the C=N group of the pyrazoline ring. The absorption band due to C=O stretching vibrations appeared at the high frequency region of 1722 cm^{-1}. The appearance of a forked absorption band at 2910 cm^{-1} accounted for the presence of aliphatic C-H stretch. Similarly, a band seen at 3035 cm^{-1} suggested the presence of Ar-H vibrations. In addition to this, =C-O-C- stretching due to the ethoxy substituent resulted in an absorption band at 1203 cm^{-1}.

The ^{1}H-NMR spectrum of the compound displayed characteristic system of AMX pattern due to the pyrazoline moiety. The two diastereotopic methylene protons H_A and H_M at position 4 of the pyrazoline ring displayed two doublet of doublets at 3.36 ppm (J_{AM} = 17.6 Hz, J_{AX} = 4.4 Hz) and 3.96 ppm (J_{MA} = 8.4 Hz, J_{MX} = 2.5 Hz) respectively, whereas the methine proton H_X at position 5 of the ring showed doublet of doublets at 5.73 ppm (J_{XA} =11.6 Hz, J_{XM} = 4.4 Hz). The protons of the acetyl –CH_3 resonated as a singlet at 2.50 ppm, whereas the methyl and the methylene protons of the ethoxy substituent displayed peaks at 1.39 ppm and 3.98 ppm respectively, the former resonating as a triplet and the latter as a quartet. The eleven aromatic protons appeared as a multiplet in the region 6.83–7.92 ppm.

The ^{13}C-NMR spectrum of the title compound exhibited signals at 168.8 and 154.4 ppm accounting for the C=O and C=N carbons respectively. Signals for the C-4 and C-5 carbons of the pyrazoline ring appeared at 58.3 ppm and 63.4 ppm respectively. Carbon of the –CH_3 group of the acetyl group attached to the ring resonated at 22.2 ppm, whereas that of the ethoxy substituent displayed a signal at 14.8 ppm. The signal at 158.4 ppm was exhibited by the aromatic carbon directly attached to the O atom of the ethoxy group, whereas the signal at 44.9 ppm accounts for the –CH_2 carbon of the ethoxy substituent. Mass spectrum of the compound confirmed the molecular weight with the molecular ion peak m/z at 359.15 (M^+ + 1), which corresponded to the molecular formula $C_{23}H_{22}N_2O_2$. Although two optical isomers are possible for the title compound on account of its chiral centre, no attempt was made to separate these isomers.

Molecular docking between the title compound (**2**) and the target protein MTCYP51 showed entrenched π-π stacking and π-cation interactions, thus depicting the enzyme-ligand binding. The two 6-membered rings of the naphthyl group exhibited two π-π stacking interactions with the same Phe 78 residue of the active site of the protein. In addition to these interactions, the phenyl ring containing the ethoxy group, displayed π-cation interaction with the Arg 96 residue of the protein. Furthermore, the title compound also showed a better glide score in comparison with the standard drug fluconazole.

4. Materials and Methods

All reagents and chemicals were purchased from Sigma-Aldrich India (Bangalore, India) and utilised without further purification. The melting point was determined using an open capillary tube (Shiv Scientific Stores (Regd.), Delhi, India) and was uncorrected. The purity of the compound was monitored by thin layer chromatography using Merck (Darmstadt, Germany) silica gel 60 F_{254} coated aluminium plates. IR spectrum was recorded on Shimadzu-FTIR Infrared spectrometer (Shimadzu, Kyoto, Japan) (υ_{max} in cm^{-1}). ^{1}H-NMR (400 MHz) spectrum was recorded on a Bruker 400 MHz high resolution multinuclear FT-NMR spectrometer (Bruker BioSpin AG, Fällanden, Switzerland), with 5 mm PABBO BB-1H tubes, whereas the ^{13}C-NMR (100 MHz) spectrum was recorded in $CDCl_3$ solvent at 100 MHz with tetramethylsilane (TMS) as the internal standard. Mass spectrum was obtained using Shimadzu LCMS-8030 mass spectrometer (Shimadzu, Kyoto, Japan). Elemental analysis was carried out by using VARIO EL-III (Elemental AnalysensystemeGmBH, Langenselbold, Germany). The structure of the protein was downloaded directly from the Protein Data Bank, (PDB ID: 1EA1), followed by optimization by the removal of water molecules. The computational calculations were performed using Schrödinger Software Suite 2015-2 with hardware 2× Intel Xeon 1.9 GHz ES-2420/6C/15MB Cache RAM 6× 4Gb DDR-3 1333 MHz ECC RDIMM 4× 500 Gb Graphics Card NvidiaQuadro 600 machine. The ligand was prepared using LigPrep and fluconazole was kept as a reference ligand. The grid was generated for the protein, and the title compound was docked into the active site. The ligand-enzyme interaction studies and the docking figures were generated using Schrödinger Glide and the 2D structure was drawn using Schrödinger maestro 10.2. (Schrödinger, New York, NY, USA).

Synthesis of 1-[5-(4-ethoxyphenyl)-3-(naphthalen-1-yl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one

3-(4-Ethoxyphenyl)-1-(naphthalen-1-yl)prop-2-en-1-one (302 mg, 1 mmol) which was synthesised according to the reported experimental protocol [13], was treated with hydrazine hydrate (0.1 mL, 2.05 mmol) and refluxed for 5 h in glacial acetic acid medium. The completion of the reaction was determined by thin layer chromatography, after which the reaction mixture was cooled to room temperature and plunged into crushed ice. The precipitated solid was filtered, dried and recrystallised using ethanol to obtain the title compound as cream coloured needles with the yield of 82% (294 mg).

Melting point: 98–100 °C; MS: m/z = 359.15 (M^+ + 1); IR: υ_{max}(cm^{-1}), 3022 (Ar-H), 2910 (Al-H), 1722 (C=O), 1641 (C=N), 1203 (Ar-O-C- stretch); ^{1}H-NMR (400 MHz, $CDCl_3$): ppm, 1.39 (t, 3H, -CH_3, J = 6.8 Hz), 2.50 (s, 3H, -C(O)$\underline{CH}_3$), 3.36 (dd, 1H, pyrazoline H_A, J_{AM} = 17.6 Hz, J_{AX} = 4.4 Hz), 3.96 (dd, 1H, pyrazoline H_M, J_{MA} = 8.4 Hz, J_{MX} = 2.5 Hz), 3.98 (q, 2H, -CH_2, J = 6.8 Hz) 5.73 (dd, pyrazoline H_X, J_{XA} = 11.6 Hz, J_{XM} = 4.4 Hz), 6.83–7.92 (m, 11H, Ar-H) ; ^{13}C-NMR (100 MHz, $CDCl_3$): ppm, 14.8 (-CH_3), 22.2 (C(O)$\underline{C}H_3$), 44.9 (-CH_2), 58.3 (C-4 of pyrazoline ring), 63.4 (C-5 of pyrazoline ring), 114.8, 124.7, 126.3, 126.6, 126.9, 127.6, 127.8, 128.2, 128.7, 130.5, 131.1, 133.9, 134.1, 154.4 (C=N), 158.4, 168.8 (C=O); Elemental analysis: Calculated for $C_{23}H_{22}O_2N_2$, C, 77.07%; H, 6.19%; N, 7.82%. Found: C, 77.01%; H, 6.12%; N, 7.74%

5. Conclusions

In this study, a simple and straight-forward method for the synthesis of pyrazoline derivative is reported. The structure of the compound obtained is confirmed by spectroscopic data. A conjoint docking evaluation of the compound with the MTCYP51 protein is also carried out to investigate

the potency of the compound to act as a ligand to the target protein. The docking results indicated the presence of π-π stacking and π-cation interactions, as well as an augmented docking score of the title compound in comparison with the standard drug fluconazole, throws light on the possible pharmacological utility of the title compound.

Supplementary Materials: IR, ^{1}H-NMR, ^{13}C-NMR and mass spectral data are available online.

Author Contributions: S.K. and J.A. performed the experiments and docking studies; B.K.S. analysed the data; B.N. guided throughout the research work.

Funding: This research was funded by DST and UGC through fellowship.

Acknowledgments: The authors are thankful to Division of Biochemistry, Mangalore University, for docking facilities, DST-PURSE, Mangalore University for Mass spectral data and IISc Bangalore, for NMR data. S.K. thanks DST for INSPIRE Fellowship and J.A. thanks UGC for SRF-Fellowship. S.K. and J.A. thank Mangalore University for research facilities.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Ahn, J.H.; Kim, H.M.; Jung, S.H.; Kang, S.K.; Kim, K.R.; Rhee, S.D.; Yang, S.D.; Cheon, H.G.; Kim, S.S. Synthesis and DP-IV inhibition of cyano-pyrazoline derivatives as potent anti-diabetic agents. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4461–4465. [CrossRef] [PubMed]
2. Ali, M.A.; Shaharyar, M.; Siddiqui, A.A. Synthesis, structural activity relationship and anti-tubercular activity of novel pyrazoline derivatives. *Eur. J. Med. Chem.* **2007**, *42*, 268–275. [CrossRef] [PubMed]
3. Prasad, Y.R.; Rao, A.L.; Prasoona, L.; Murali, K.; Kumar, P.R. Synthesis and antidepressant activity of some 1,3,5-triphenyl-2-pyrazolines and 3-(2″-hydroxy naphthalene-1″-yl)-1,5-diphenyl-2-pyrazolines. *Bioorg. Med. Chem. Lett.* **2005**, *22*, 5030–5034. [CrossRef] [PubMed]
4. Bhandari, S.; Tripathi, A.C.; Saraf, S.K. Novel 2-pyrazoline derivatives as potential anticonvulsant agents. *Med. Chem. Res.* **2013**, *22*, 5290–5296. [CrossRef]
5. Salian, V.V.; Narayana, B.; Sarojini, B.K.; Kumar, M.S.; Nagananda, G.S.; Byrappa, K.; Kudva, A.K. Spectroscopic, single crystal X-ray, Hirshfeld, in vitro and in silico biological evaluation of a new series of potent thiazole nucleus integrated with pyrazoline scaffolds. *Spectrochim. Acta A Mol. Biomol.* **2017**, *174*, 254–271. [CrossRef] [PubMed]
6. Chandra, T.; Garg, N.; Lata, S.; Saxena, K.K.; Kumar, A. Synthesis of substituted acridinyl pyrazoline derivatives and their evaluation for anti-inflammatory activity. *Eur. J. Med. Chem.* **2010**, *45*, 1772–1776. [CrossRef] [PubMed]
7. Bansal, E.; Srivastava, V.K.; Kumar, A. Synthesis and anti-inflammatory activity of 1-acetyl-5-substituted aryl-3-(β-aminonaphthyl)-2-pyrazolines and β-(substituted aminoethyl) amidonaphthalenes. *Eur. J. Med. Chem.* **2001**, *36*, 81–92. [CrossRef]
8. Havrylyuk, D.; Zimenkovsky, B.; Vasylenko, O.; Zaprutko, L.; Gzella, A.; Lesyk, R. Synthesis of novel thiazolone-based compounds containing pyrazoline moiety and evaluation of their anticancer activity. *Eur. J. Med. Chem.* **2009**, *44*, 1396–1404. [CrossRef] [PubMed]
9. Shaharyar, M.; Abdullah, M.M.; Bakht, M.A.; Majeed, J. Pyrazoline bearing benzimidazoles: Search for anticancer agent. *Eur. J. Med. Chem.* **2010**, *45*, 114–119. [CrossRef] [PubMed]
10. Havrylyuk, D.; Zimenkovsky, B.; Vasylenko, O.; Gzella, A.; Lesyk, R. Synthesis of new 4-thiazolidinone-, 4-pyrazoline-, and isatin-based conjugates with promising antitumor activity. *J. Med. Chem.* **2012**, *55*, 8630–8641. [CrossRef] [PubMed]
11. Abid, M.; Azam, A. Synthesis and antiamoebic activities of 1-*N*-substituted cyclised pyrazoline analogues of thiosemicarbazones. *Bioorg. Med. Chem.* **2005**, *13*, 2213–2220. [CrossRef] [PubMed]

12. Bano, S.; Alam, M.S.; Javed, K.; Dudeja, M.; Das, A.K.; Dhulap, A. Synthesis, biological evaluation and molecular docking of some substituted pyrazolines and isoxazolines as potential antimicrobial agents. *Eur. J. Med. Chem.* **2015**, *95*, 96–103. [CrossRef] [PubMed]
13. Kumar, S.; Jayashree, A.; Narayana, B.; Sarojini, B.K.; Kotai, L.; Anthal, S.; Kant, R. Synthesis and crystal structure of (*E*)-3-(4-butoxyphenyl)-1-(naphthalen-1-yl)-prop-2-en-1-one. *Eur. Chem. Bull.* **2016**, *12*, 501–504.

Sample Availability: Samples of the compounds are available from the authors.