*Section A-Research paper*
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# *In vitro* antimicrobial, *in vivo* anti-inflammatory and molecular docking of novel *(E)*-N'-(furan-2-ylmethylene) furan-2-carbohydrazide

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**Abstract:** A novel hydrazide-hydrazone derivative, *(E)*-N'-(furan-2-ylmethylene)furan-2-carbohydrazide, was synthesized by simple synthesis using methanol as a solvent with a high yield. The proton NMR spectra confirmed the formation of (*E)*-N'-(furan-2-ylmethylene) furan-2-carbohydrazide. The compound was investigated for antimicrobial activities against *Escherichia coli, Staphylococcus aureus,* and *Candida albicans via* a zone of inhibition assay. The molecule was then examined for anti-inflammatory activity using the paw edema method and showed appreciable performance. The molecule was further analyzed for antimicrobial and anti-inflammatory activities against 6S6M and COX5 receptors, respectively. Four possible types of stable orientations were observed and the interactions are investigated.

**Keywords**: hydrazide-hydrazone, docking studies, anti-microbial, anti-inflammatory, inhibition test.

## 1. Introduction

Noticeably increasing rates of cases pertaining to multi-drug-resistant microbial infections have become very difficult to diagnose and treat effectively. Hence, it becomes a serious health care difficulty. Particularly, the occurrence of multi-drug-resistant strains of bacteria of Gram-negative pathogens like *Enterococcus* and *Staphylococcus aureus* is the main reason

behind this serious healthcare problem [1-4]. In addition to that, an obviously increasing number of systematic fungus infections in human, chemotherapeutic cancer patients and organ transplantation, receiving long-standing antimicrobial drug treatment, also because of suppressed immune system in the AIDS (acquired immune deficiency syndrome) patients becoming defenseless to fungal infections like aspergillosis, candidiasis and also cryptococcosis [5]. In order to get control these health problems, it is very important to design and develop brand-new antimicrobial agents, which are non-toxic with better effectiveness is required. Out of several routes, one among them is to discover new chemotherapeutic agents by modifying the chemical structure of already available medicines which results in the formation of a less toxic moiety for the human body [6].

One among them is the use of hydrazide-hydrazone derivatives, which are playing a vital role in the field of medicinal and organic chemistry due to the presence of azomethine group. The same group connected with carbonyl group is responsible for various pharmaceutical applications [7]. Apart from this over the years that have attracted huge number of researchers due to its promising biological activities, that includes anticancer [8-10], antimicrobial [11-14], antiviral [15], anticonvulsant [12] and antituberculosis activities [16-18]. Few hydrazide-hydrazone compounds were considered as drug molecules and they were also used in clinics, such as furazolidone, nitrofurazone and nitrofurantoin [19].

Herein, the above-mentioned fact motivated us to study the antimicrobial and anti-inflammatory effects of the prepared novel hydrazide–hydrazone moiety. The most important routine to prepare hydrazide-hydrazone derivatives is to heat an appropriate amount of hydrazide of carboxylic acids or with tetracarboxylic acids with various aldehydes or ketones in different organic solvents such as methanol, ethanol, or butanol [20-23]. In this research, the hydrazide–hydrazone derivative was prepared by normal routine synthetic procedure by the dissolution of furan-2-carbohydrazide and furan-2-carbaldehyde in methanol to yield ***(E)-N'-(furan-2-ylmethylene) furan-2-carbohydrazide,*** for the first of its time. The synthesized compound was recrystallized and structurally characterized by proton nuclear magnetic resonance ($^1$H-NMR) spectroscopy.

The anti-microbial and anti-inflammatory effects were evaluated against one gram-negative bacteria (*Escherichia Coli* ATCC 25922), one gram-positive bacteria (*Staphylococcus aureus* ATCC 25923) and one yeast-like pathogenic fungus (*Candida albicans* ATCC 22972) *via*

zone of inhibition (ZOI) assay. *In vivo* anti-inflammatory tests were performed using carrageenan-induced paw edema method. The antimicrobial activity and anti-inflammatory activities were further investigated by molecular docking studies considering 6S6M receptor and COX5 enzyme, respectively.

## 2. Materials and methods

### 2.1 Materials

All the solvents and reagents such as methanol, tetrahedrafuran (THF), furan-2-carbohydrazide and furan-2-carbaldehyde, dimethyl sulfoxide (DMSO) used in this study were purchased from Sigma-Aldrich Pvt. Ltd. India and used without any prior purification. Cation-adjusted Mueller Hinton broth (CAMHB), Mueller Hinton Agar (MHA) (Cat# M1657), Sabouraud's dextrose broth (SDB) (Cat# MH-033) and Sabouraud's dextrose agar (SDA) (Cat# M063) were from HiMedia Laboratories Pvt. Ltd, India. Ciprofloxacin was from sigma Aldrich and Ketoconazole from TCI chemicals. Bacterial and fungal strains, *Staphylococcus aureus* ATCC 25923, *Escherichia Coli* ATCC 25922, and *Candida albicans* ATCC 22972 were procured form NCBS, Pune. Plethysmometer for anti-inflammatory studies was procured from IITC Life science.

### 2.2 Synthesis of *(E)*-N'-(furan-2-ylmethylene) furan-2-carbohydrazide.

The furan-2-carbohydrazide (0.126g, 1.0 mM) and furan-2-carbaldehyde (0.096g, 1.0 mM) were dissolved in methanol and refluxed for 2 h. The obtained reaction mixture was poured into ice cold distilled water and kept for 30 min at room temperature. The precipitate formed was filtered and air dried and recrystallized using THF. Schematic representation of the same is provided in figure 1. Here after, for the convenience, the compound will be uttered as "OO" in the manuscript.

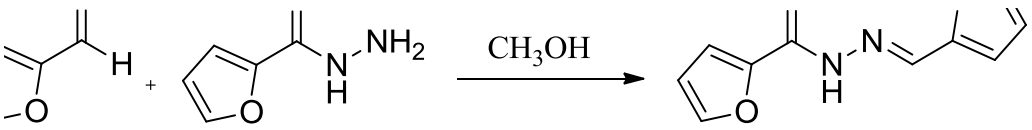



**Figure 1: Scheme for synthesis of *(E)*-N'-(furan-2-ylmethylene) furan-2-carbohydrazide.**

### 2.3 NMR spectroscopy

The structure of hydrazide-hydrazone derivative was confirmed by $^{1}$H-NMR, SA-Varian 400MHz NMR spectrometer with TMS as internal standard and *d6*-DMSO as a solvent. The values of chemical shifts are expressed in ppm.

### 2.4 Antimicrobial activities

The ZOI assay was performed in the disc diffusion plate method. The discs were made from sterile Whatman filter paper by punching with sterile punching machine and for each disc different concentration of test compound (32, 16, 8, and 4 µg/ml) was made by adding 10 µl of different concentration and allowed to dry (RT for 30 min) same procedure was done for both the vehicle and positive control. The dried discs were placed on MHA and SDA plates containing a lawn of *Staphylococcus aureus, Escherichia coli, and Candida albicans*, and the plates were incubated at 37°C for 24 h. Post incubation plates were examined for zones, the assay was done in triplicate. The ZOI was measured Post incubation; the zone of inhibition (diameter mm) around the discs was measured and recorded.

### 2.5 *In vivo* anti-inflammatory studies

*In vivo* anti-inflammatory activity of synthesised compound was examined by carrageenan-induced paw edema method. Male Swiss albino mice were divided into six groups with each six animals in the group. **Group I**: control, animals fed with DM water 10 ml/kd p.o/day; **Group II**: animals fed with indomethacin (standard drug), 10 mg/kg; **Group III to Group VI**: animals were administered with 10, 25, 50 and 100 mg/kg of synthesized derivative, respectively. All animals were overnight fasted and deprived of water before starting the experiment. The initial paw volume was measured by dipping the paw in Plethysmometer till marked. After recording the initial paw volume, animals were applied with the respective drug. After 30 min of drug application, a 25 µl injection of 1 % of carrageenan in saline into the sub-plantar region of the right hind paws. The paw volume was recorded at 1, 2, 4, 6, and 24 hours. The edema was calculated by subtracting the initial paw volume of the respective animal at different time points.

### 2.6 Molecular docking studies

The crystal structures of 6S6M (human LL37(17-29) antimicrobial peptide and COX5 (uninhibited mouse cuclooxygenase-2 (prostaglandin synthase-2)) were obtained from protein

data bank, http://doi.org/10.2210/pdb6S6M/pdb and http://doi.org/10.2210/pdb5COX/pdb, respectively [24]. The crystal structures of water molecules and all other molecules were removed using PyMOL. The target receptors were docked with OO using UCSF Chimera, version 1.11.2 and AutoDock Vina to obtain docking scores. The docked complexes were analysed using Discovery Studio 3.1 visualizer.

## 3. Results and discussions

### 3.1 Chemistry

$H^1$ NMR: Solvent *d6*-DMSO peaks (s, 3.3 ppm and s, 2.5 ppm); -C=O (s, 12.5 ppm), -NH (s, 8.69 ppm), aromatic protons (7.41 to 8.2 ppm). Yield:92.70%. The respective proton NMR spectrum is provided in figure 2.

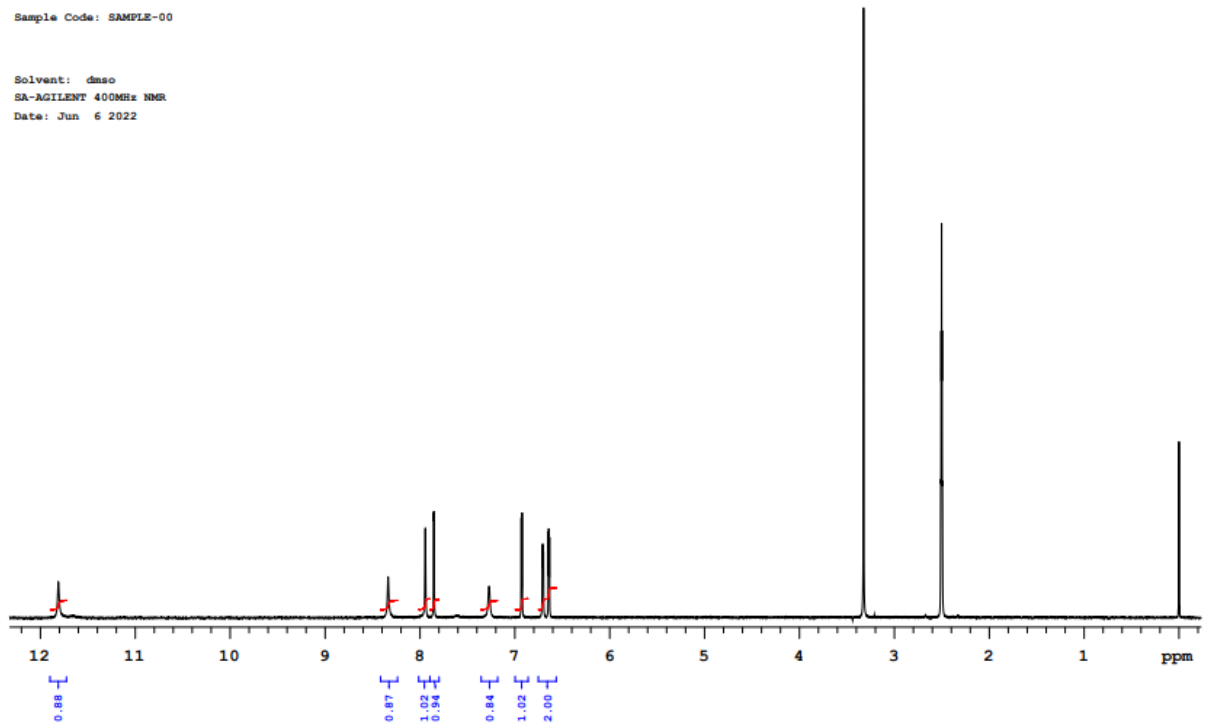



**Figure 2: Proton NMR spectrum of *(E)*-N'-(furan-2-ylmethylene)furan-2-carbohydrazide**

### 3.2 Biological properties

The antibacterial activity and antifungal activity of *(E)*-N'-(furan-2-ylmethylene) furan-2-carbohydrazide was investigated against *E. coli*, *S. aureus* and the fungus *C. albicans*.

Ciprofloxacin and ketocanozole were considered as antibacterial and antifungal standards (positive controls), respectively, for comparison. The results of ZOI assay performed via disc diffusion plate method are presented in figure 3 and figure 4. The results comprehensively showed microplates of all vehicle control (VC), positive control (PC) and compound OO for both *S. aureus and E. coli*. The ZOI for PC of A. aureus was concentration dependent and maximum ZOI was observed for greater concentration (0.5 µg/ml) and minimal ZOI was obtained for 0.06 µg/ml. however, in case of compound OO, the ZOI was observed only at greater concentration i.e., 32 µg/ml and was found to be 12.6 mm, conferring antibacterial activity against *S. aureus*. In case of *E. coli*, the PC showed good antibacterial activity and the compound OO showed 11.8 mm ZOI at all the considered four concentrations (4, 8, 16 and 32 µg/ml) indicating lack of activity for *E. coli*. Lukasz and Anna had investigated the nitrofurazone analogues with the hydrazide-hydrazone group for both gram +ve and gram –ve bacteria, and interestingly, for most of the derivatives gram +ve bacteria were greatly susceptible and the gram –ve bacteria were comparatively less susceptible [13], for which the present work is in accordance with.

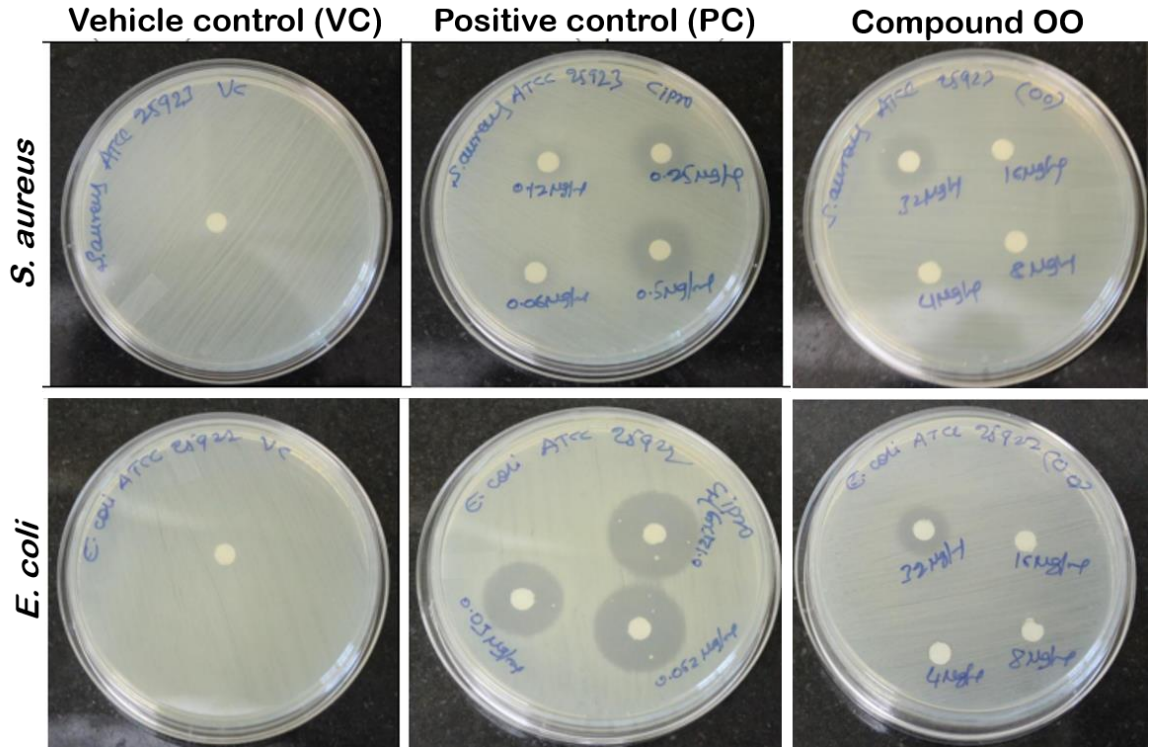
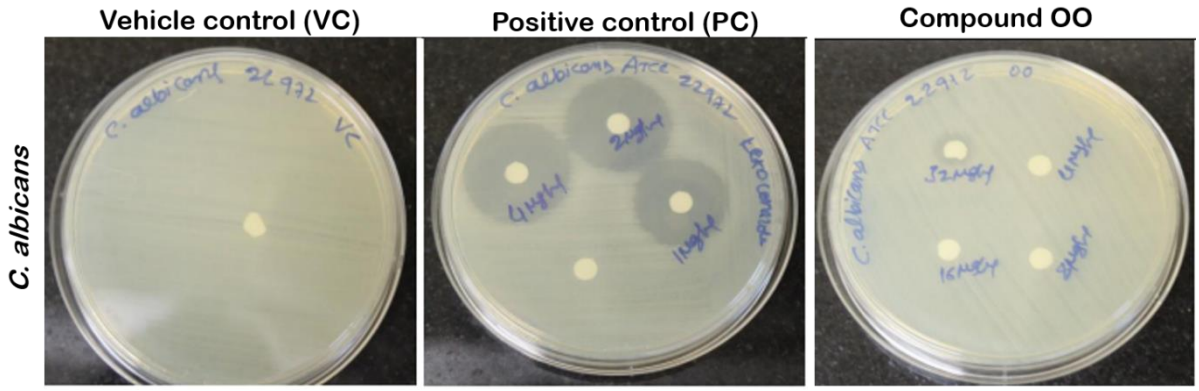



**Figure 3: Results of antibacterial activity of synthesized compound OO against *S. aureus* and *E. coli***

The microplates of antifungal activity, vehicle control, positive control and compound OO presented in figure 2 reveal that, the PC katocanozole was active even at lower concentrations. However, for the synthesized compound OO, the ZOI or the antifungal activity was observed at 32 µg/ml, is of 6.7 mm. The revisit to literature confirms that, the presence of -NH-N=C- is the major responsible moiety for the antibacterial and antifungal properties of hydrazide-hydrazone derivatives [25, 26].

**Figure 4: Results of antifungal activity of synthesized compound OO against *C. albicans***



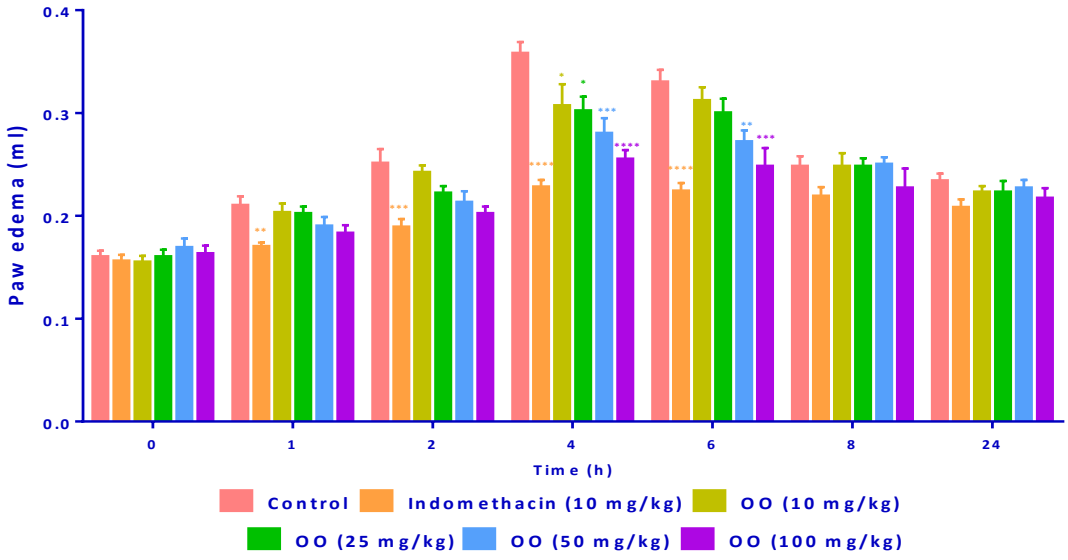




**Figure 5: Anti-inflammatory activity of different doses of OO variants in carrageenan-induced inflammation in mice**

### 3.3 *In vivo* anti-inflammatory activity

The model was conducted to evaluate the anti-inflammatory potential of the synthesized compound in the carrageenan-induced inflammation model. Sub plantar injection of 25 µL of 1% carrageenan to the mice's hind paw produced a progressive increment of paw thickness that reached its maximum value at 4 of induction in control (figure 5). All the groups showed approximately 0.16 ± 0.01 ml of paw edema at 0 h. For all the other groups, including control and standard groups, maximum paw edema was observed at $4^{th}$ h. After $4^{th}$ hour, the paw edema decreased significantly. All test doses of OO variants (10, 25, 50, and 100 mg/kg) produced statistically significant inhibition of paw thickness starting ($p < 0.05$, $p < 0.01$, $p < 0.001$and $p < 0.0001$) persisted till the sixth hour of observation after post carrageenan induction as compared to the control mice.

### 3.4 Docking studies

#### 3.4.1 Antimicrobial

To investigate upon the interesting features of prepared novel molecule, antimicrobial performance was further examined via molecular docking studies. Frequently considered LL-37 active core, residues 17–29 (6S6M receptor), an antimicrobial human antimicrobial peptide is used in the study as a receptor. Present docking study helps to understand the possible interactions between the antimicrobial peptide and the compound OO. Possible four types of interactions were observed and found stable. The 3D interactions and 2D image of the respective interactions are presented in figure 6(a-d). The possible interactions observed in each binding sites and the amino acid residues involved in the same are presented in Table 1. The amino acids ASP, ALA, PHE, PRO, ILE are majorly involved in the interactions with carbon hydrogen bonds, Pi-Pi T-shaped, Pi-Anion, Pi-Alkyl, conventional hydrogen bonds and Pi-sigma bonds are the found interaction types which are provided in detail in the Table 1. The docking scores were of -4.5, -4.4, -4.2 and -4.0 were observed for interactions 1, 2, 3 and 4, respectively. The negative values confirmed the stable interactions. Two aromatic furan rings, N=N bond, carbonyl group and the electron donating oxygen heteroatom in the five membered ring are the responsible groups for the possible interactions.

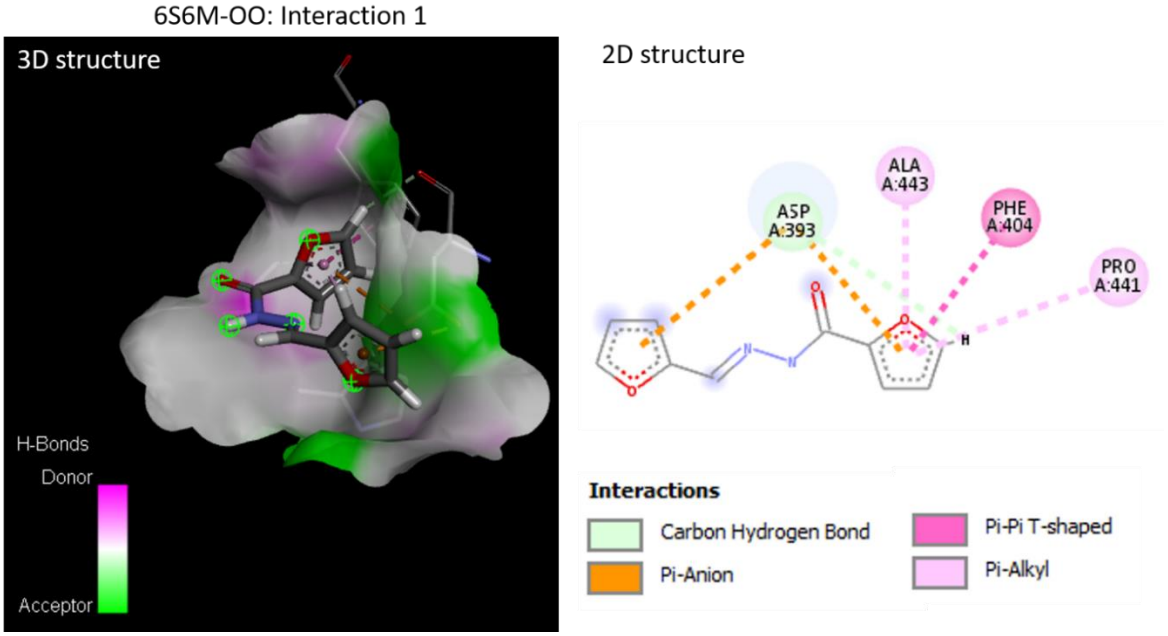



**Figure 6a: 3D and 2D structures of *(E)*-N'-(furan-2-ylmethylene) furan-2-carbohydrazide interacting with 6S6M receptor (Interaction 1)**

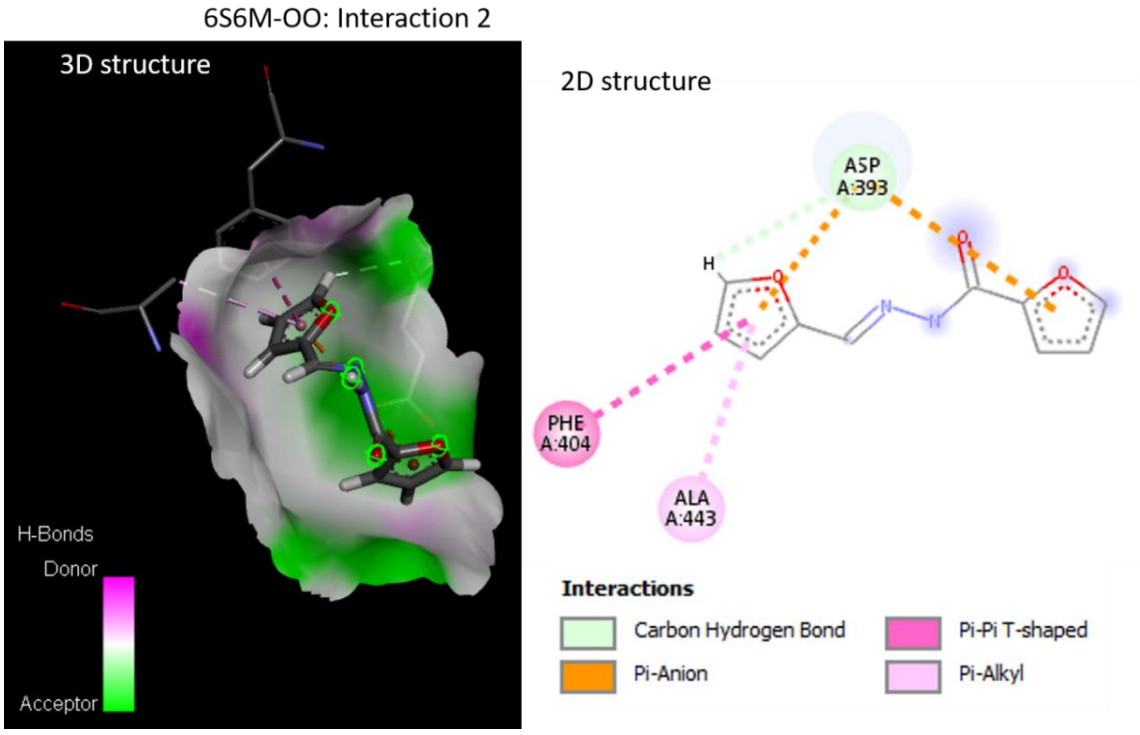



**Figure 6b: 3D and 2D structures of *(E)*-N'-(furan-2-ylmethylene) furan-2-carbohydrazide interacting with 6S6M receptor (Interaction 2)**

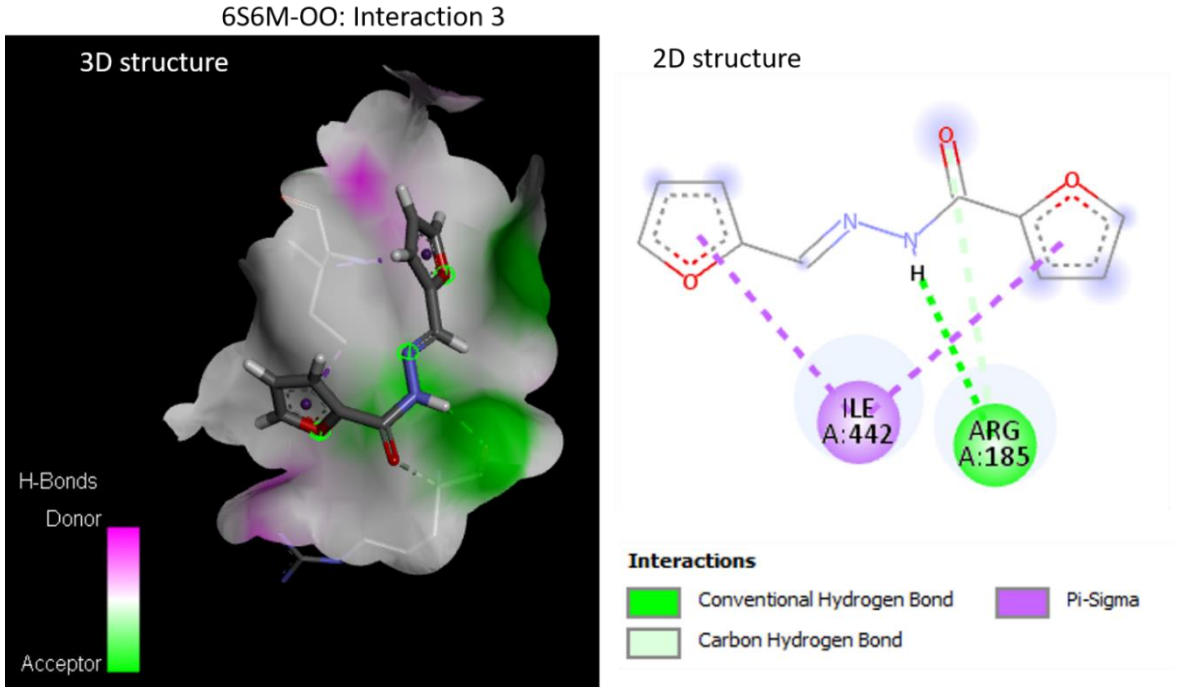



**Figure 6c: 3D and 2D structures of *(E)*-N'-(furan-2-ylmethylene) furan-2-carbohydrazide interacting with 6S6M receptor (Interaction 3)**

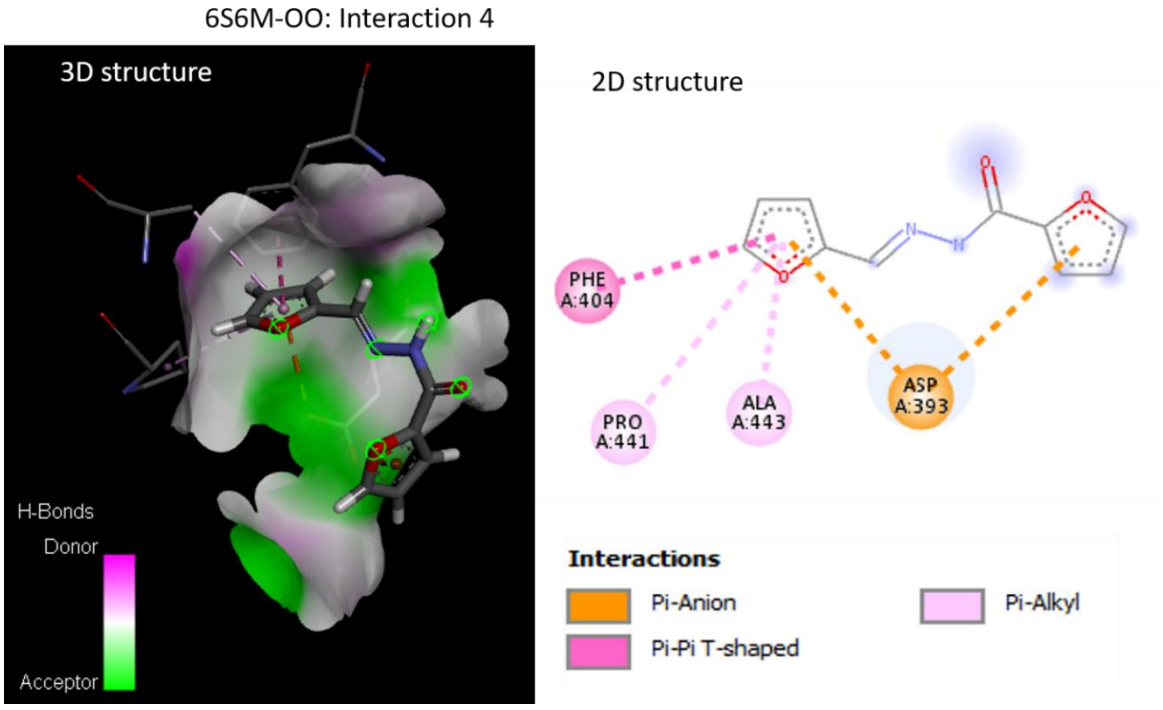



**Figure 6d: 3D and 2D structures of *(E)*-N'-(furan-2-ylmethylene) furan-2-carbohydrazide interacting with 6S6M receptor (Interaction 4)**

**Table 1: Summery of *(E)*-N'-(furan-2-ylmethylene) furan-2-carbohydrazide and 6S6M receptor interactions**

| **Interaction** | **Docking score** (Kcal/mol) | **Amino acids and respective interactions** |
|---|---|---|
| 1 | -4.5 | PHE (Pi-Pi T-Shaped)<br>ASP (Pi-Anion, Carbon hydrogen bond)<br>ALA (Pi-Alkyl)<br>PRO (Pi-Alkyl) |
| 2 | -4.4 | PHE (Pi-Pi T-Shaped)<br>ASP (Pi-Anion, Carbon hydrogen bond)<br>ALA (Pi-Alkyl) |
| 3 | -4.2 | ARG (Conventional hydrogen bond, Carbon hydrogen bond)<br>ILE (Pi-Sigma) |
| 4 | -4.0 | PHE (Pi-Pi T-Shaped)<br>ASP (Pi-Anion)<br>ALA (Pi-Alkyl)<br>PRO (Pi-Alkyl) |

### 3.4.2 Anti-inflammatory

The activity of synthesized *(E)*-N'-(furan-2-ylmethylene) furan-2-carbohydrazide was investigated for anti-inflammatory activity against COX5 enzyme and the results are presented in Table 2 and figure 7 (a-d) (both 3D and 2D diagrams indicating the interactions). ILE, LYS, ALA, ASP, PRO, ARG and PHE are the amino acid residues of COX5 receptor are found interacting with the synthesized compounds. As explained in the antimicrobial docking study, similar bonds are observed in the obtained conformations and are summarized in Table 2. The docking scores for interactions 1, 2, 3 and 4 are found to be -4.5, -4.2, -4.0 and -4.0, respectively. The presence of hydrogen donor, acceptor groups in the receptors, aromatic segments in the receptor and ligand structure are attributed for the obtained docking scores.

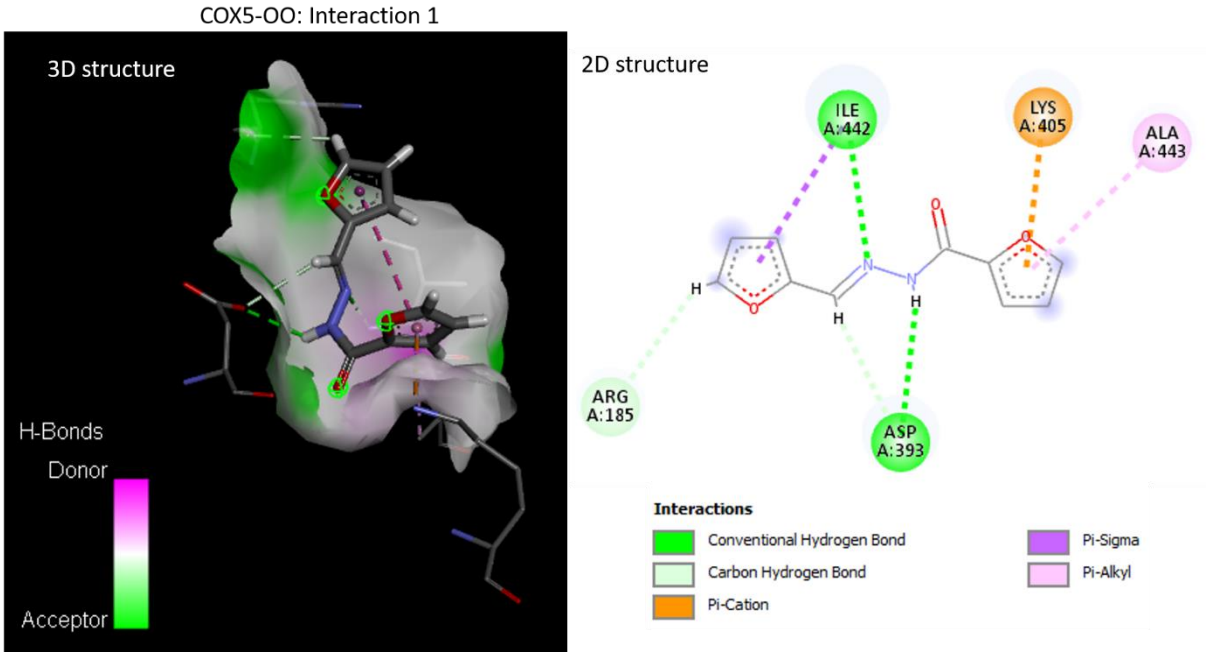



**Figure 7a: 3D and 2D structures of *(E)*-N'-(furan-2-ylmethylene) furan-2-carbohydrazide interacting with COX5 receptor (Interaction 1)**

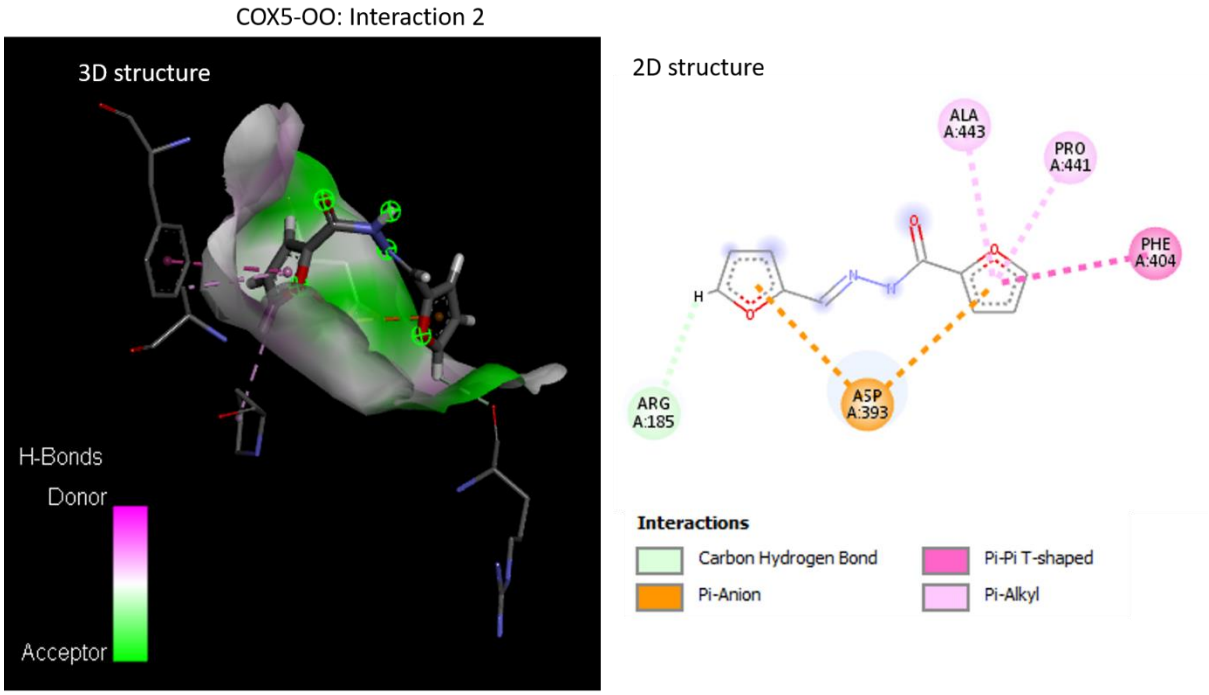



**Figure 7b: 3D and 2D structures of *(E)*-N'-(furan-2-ylmethylene) furan-2-carbohydrazide interacting with COX5 receptor (Interaction 2)**

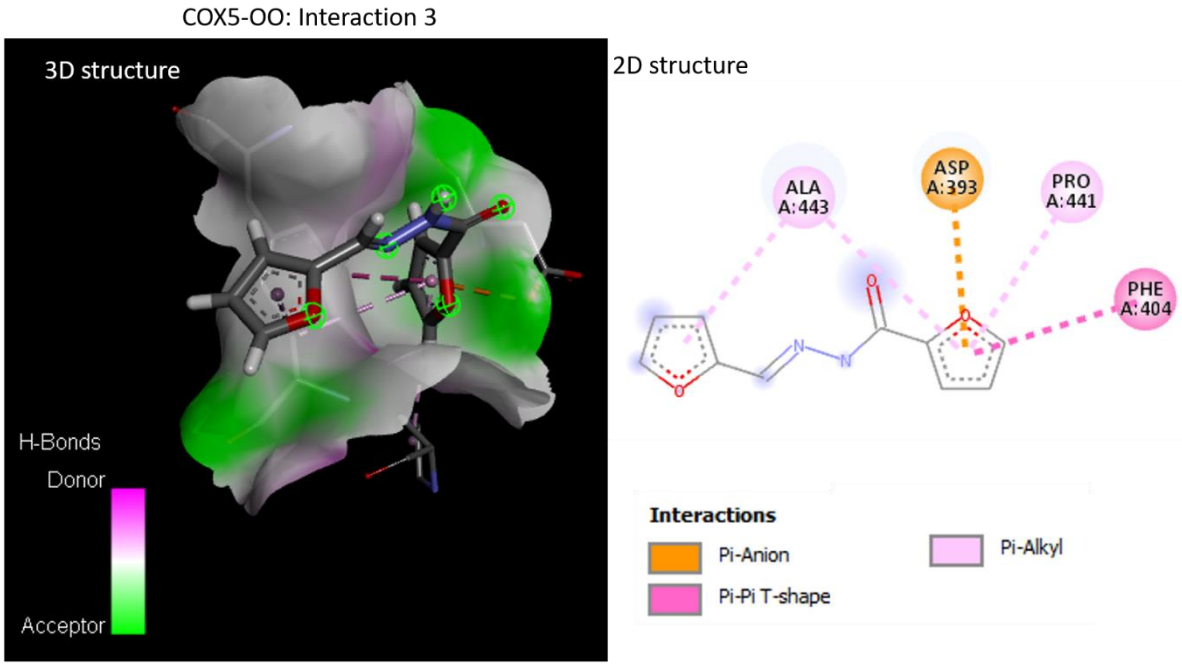



**Figure 7c: 3D and 2D structures of *(E)*-N'-(furan-2-ylmethylene) furan-2-carbohydrazide interacting with COX5 receptor (Interaction 3)**

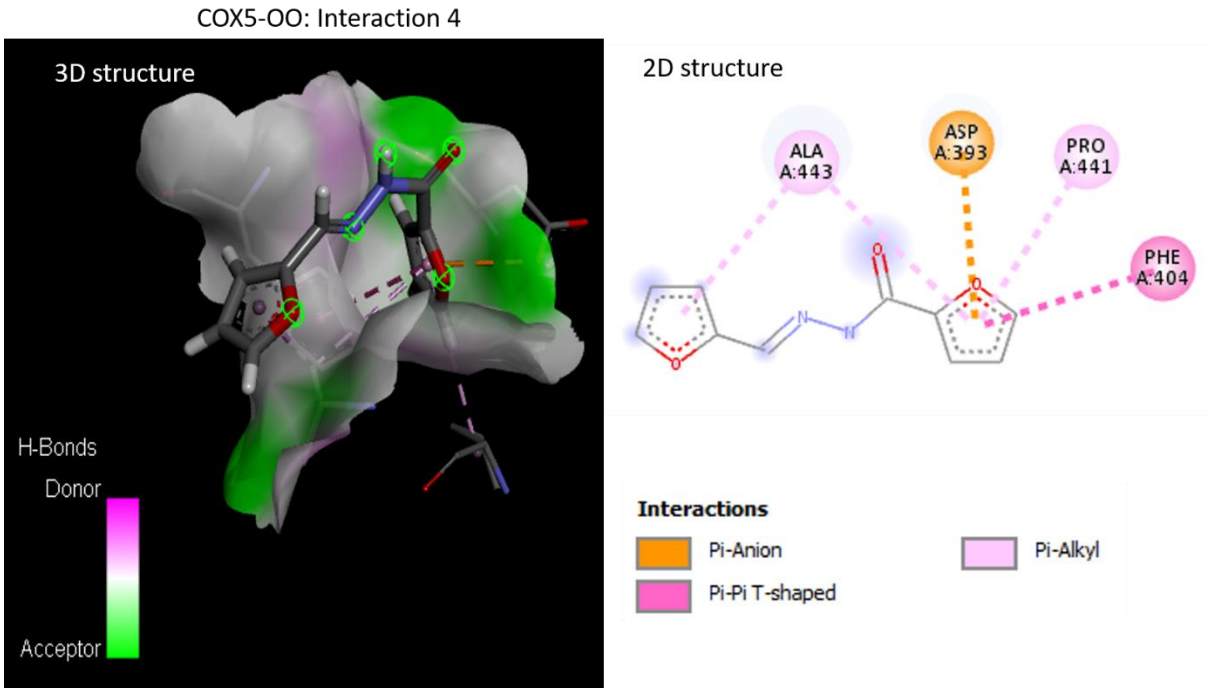



**Figure 7d: 3D and 2D structures of *(E)*-N'-(furan-2-ylmethylene) furan-2-carbohydrazide interacting with COX5 receptor (Interaction 4)**

**Table 2: Summery of *(E)*-N'-(furan-2-ylmethylene) furan-2-carbohydrazide and COX5 receptor interactions**

| Interaction | Docking score (Kcal/mol) | Amino acids and respective interactions |
|---|---|---|
| 1 | -4.5 | ILE (Conventional hydrogen bond, Pi-Sigma bond)<br>LYS (Pi-Cation)<br>ALA (Pi-Alkyl)<br>ASP (Conventional hydrogen bond, Carbon hydrogen bond)<br>ARG (Carbon hydrogen bond) |
| 2 | -4.4 | ALA (Pi-Alkyl)<br>ASP (Pi-Anion)<br>PRO (Pi-Alkyl)<br>ARG (Carbon hydrogen bond)<br>PHE (Pi-Pi T-shaped) |
| 3 | -4.2 | ALA (Pi-Alkyl)<br>ASP (Pi-Anion)<br>PRO (Pi-Alkyl)<br>PHE (Pi-Pi T-shaped) |
| 4 | -4.0 | ALA (Pi-Alkyl)<br>ASP (Pi-Anion)<br>PRO (Pi-Alkyl)<br>PHE (Pi-Pi T-shaped) |

## 4. Conclusions

A synthesized novel *(E)*-N'-(furan-2-ylmethylene) furan-2-carbohydrazide presented a proton NMR peak for hydrazide-hydrazone group at 12.5 ppm confirming the formation of the molecule. The molecule examined for antibacterial and antifungal activities showed a better result at 32 µg/ml under ZOI assay. ZOI for *S. aureus*, *E. coli* and *C. albicans* were of 12.6, 11.2 and 6.7 mm indicating feasible zone of inhibition, however lesser than that of ciprofloxacin and ketoconazole. The anti-inflammatory activity was significant, maximum paw edema was observed at $4^{th}$ h and that reduced after $4^{th}$ h. The molecular docking confirmed four possible orientations for both antimicrobial and anti-inflammatory receptors. For both activities the docking score varied from -4.0 to 4.5 kcal/mol indicating the acceptable antimicrobial and anti-inflammatory activities. The results suggest that, the

synthesized novel compound could be further examined for cytotoxicity tests, *in vitro* and *in vivo* anti-inflammatory activities.

## References

1. Poole, K., *Multidrug resistance in Gram-negative bacteria.* Current opinion in microbiology, 2001. **4**(5): p. 500-508.
2. Abbanat, D., M. Macielag, and K. Bush, *Novel antibacterial agents for the treatment of serious Gram-positive infections.* Expert Opinion on Investigational Drugs, 2003. **12**(3): p. 379-399.
3. Goossens, H., *European status of resistance in nosocomial infections.* Chemotherapy, 2005. **51**(4): p. 177-181.
4. Coates, A., et al., *The future challenges facing the development of new antimicrobial drugs.* Nature reviews Drug discovery, 2002. **1**(11): p. 895-910.
5. Gurkok, G., N. Altanlar, and S. Suzen, *Investigation of antimicrobial activities of indole-3-aldehyde hydrazide/hydrazone derivatives.* Chemotherapy, 2009. **55**(1): p. 15-19.
6. Moellering Jr, R.C., *Discovering new antimicrobial agents.* International journal of antimicrobial agents, 2011. **37**(1): p. 2-9.
7. Rollas, S. and Ş. Güniz Küçükgüzel, *Biological activities of hydrazone derivatives.* Molecules, 2007. **12**(8): p. 1910-1939.
8. Nasr, T., S. Bondock, and M. Youns, *Anticancer activity of new coumarin substituted hydrazide–hydrazone derivatives.* European journal of medicinal chemistry, 2014. **76**: p. 539-548.
9. He, H., et al., *Synthesis, antitumor activity and mechanism of action of novel 1, 3-thiazole derivatives containing hydrazide–hydrazone and carboxamide moiety.* Bioorganic & medicinal chemistry letters, 2016. **26**(14): p. 3263-3270.
10. Nasr, T., et al., *Novel hydrazide-hydrazone and amide substituted coumarin derivatives: Synthesis, cytotoxicity screening, microarray, radiolabeling and in vivo pharmacokinetic studies.* European journal of medicinal chemistry, 2018. **151**: p. 723-739.
11. Backes, G.L., D.M. Neumann, and B.S. Jursic, *Synthesis and antifungal activity of substituted salicylaldehyde hydrazones, hydrazides and sulfohydrazides.* Bioorganic & medicinal chemistry, 2014. **22**(17): p. 4629-4636.

12. Küçükgüzel, S.G., et al., *Synthesis and biological activities of diflunisal hydrazide–hydrazones.* European journal of medicinal chemistry, 2003. **38**(11-12): p. 1005-1013.
13. Popiołek, Ł. and A. Biernasiuk, *Synthesis and investigation of antimicrobial activities of nitrofurazone analogues containing hydrazide-hydrazone moiety.* Saudi Pharmaceutical Journal, 2017. **25**(7): p. 1097-1102.
14. Rollas, S., N. Gulerman, and H. Erdeniz, *Synthesis and antimicrobial activity of some new hydrazones of 4-fluorobenzoic acid hydrazide and 3-acetyl-2, 5-disubstituted-1, 3, 4-oxadiazolines.* Il Farmaco, 2002. **57**(2): p. 171-174.
15. Şenkardeş, S., et al., *Synthesis of novel diflunisal hydrazide–hydrazones as anti-hepatitis C virus agents and hepatocellular carcinoma inhibitors.* European journal of medicinal chemistry, 2016. **108**: p. 301-308.
16. Velezheva, V., et al., *Synthesis and antituberculosis activity of indole–pyridine derived hydrazides, hydrazide–hydrazones, and thiosemicarbazones.* Bioorganic & Medicinal Chemistry Letters, 2016. **26**(3): p. 978-985.
17. Pavan, F.R., et al., *Thiosemicarbazones, semicarbazones, dithiocarbazates and hydrazide/hydrazones: Anti–Mycobacterium tuberculosis activity and cytotoxicity.* European Journal of Medicinal Chemistry, 2010. **45**(5): p. 1898-1905.
18. Bedia, K.-K., et al., *Synthesis and characterization of novel hydrazide–hydrazones and the study of their structure–antituberculosis activity.* European journal of medicinal chemistry, 2006. **41**(11): p. 1253-1261.
19. Pham, V.H., et al., *Synthesis and bioactivity of hydrazide-hydrazones with the 1-adamantyl-carbonyl moiety.* Molecules, 2019. **24**(21): p. 4000.
20. Bala, S., et al., *Hydrazones as promising lead with diversity in bioactivity-therapeutic potential in present scenario.* Int J Pharm Sci Rev Res, 2013. **18**(1): p. 65-74.
21. Popiołek, Ł., A. Biernasiuk, and A. Malm, *Synthesis and antimicrobial activity of new 1, 3-thiazolidin-4-one derivatives obtained from carboxylic acid hydrazides.* Phosphorus, Sulfur, and Silicon and the related elements, 2015. **190**(2): p. 251-260.
22. Popiołek, Ł., et al., *Synthesis, Dissociation Constants, and Antimicrobial Activity of Novel 2, 3- Disubstituted- 1, 3- thiazolidin- 4- one Derivatives.* Journal of Heterocyclic Chemistry, 2016. **53**(2): p. 393-402.

23. Popiołek, Ł., A. Biernasiuk, and A. Malm, *Design, synthesis, and in vitro antimicrobial activity of new furan/thiophene- 1, 3- benzothiazin- 4- one hybrids.* Journal of Heterocyclic Chemistry, 2016. **53**(2): p. 479-486.
24. Morris, G.M., et al., *AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility.* Journal of computational chemistry, 2009. **30**(16): p. 2785-2791.
25. Popiołek, Ł., *Hydrazide–hydrazones as potential antimicrobial agents: overview of the literature since 2010.* Medicinal Chemistry Research, 2017. **26**(2): p. 287-301.
26. Mohareb, R.M., D.H. Fleita, and O.K. Sakka, *Novel Synthesis of Hydrazide-Hydrazone Derivatives and Their Utilization in the Synthesis of Coumarin, Pyridine, Thiazole and Thiophene Derivatives with Antitumor Activity.* Molecules, 2011. **16**(1): p. 16-27.