

Synthesis, Biochemical Characterization, and Theoretical Studies of Novel β -Keto-enol Pyridine and Furan Derivatives as Potent Antifungal Agents

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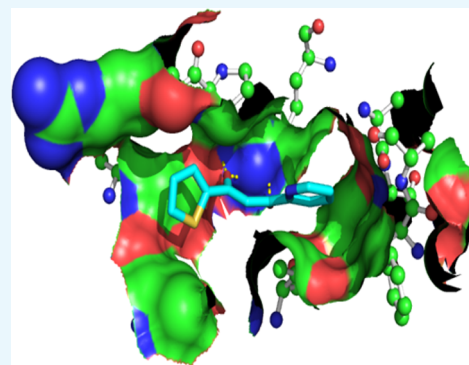


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ABSTRACT: In the present study, we report the design and synthesis of new derivatives of the β -keto-enol grafted on pyridine and furan moieties (L_1 – L_{11}). Structures of compounds were fully confirmed by Fourier transform infrared spectroscopy (FT-IR), ^1H NMR, ^{13}C NMR, electrospray ionization/liquid chromatography-mass spectrometry (ESI/LC-MS), and elemental analysis. The compounds were screened for antifungal and antibacterial activities (*Escherichia coli*, *Bacillus subtilis*, and *Micrococcus luteus*). *In vitro* evaluation showed significant fungicidal activity for L_1 , L_4 , and L_5 against fungal strains (*Fusarium oxysporum f.sp. albedinis*) compared to the reference standard. Especially, the exceptional activity has been demonstrated for L_1 with $\text{IC}_{50} = 12.83 \mu\text{g/mL}$. This compound and the reference benomyl molecule also showed a correlation between experimental antifungal activity and theoretical predictions by Petra/Osiris/Molinspiration (POM) calculations and molecular coupling against the Fgb1 protein. The highest inhibition of bacterial growth for L_1 is due to its strongest binding to the target protein. This report may stimulate the further synthesis of examples of this substance class for the development of new drugs.



1. INTRODUCTION

Fungal infection is a common disease that causes a serious growing threat to mankind including high morbidity and mortality every year around the world,^{1–3} especially among immune-compromised patients.^{4,5} Therefore, it is urgent to discover novel antifungal inhibitors, particularly those with new action modes, with low toxicity and bioavailability. Furthermore, it should be safer and more effective against sensitive and drug-resistant fungi. In recent years, the fused heterocyclic compounds containing bridgehead nitrogen- or oxygen-donor atoms have attracted great interest due to their biological activities and chemical medicinal properties. Indeed, pyridine and furan derivatives have been recently reported to possess pharmacological potential as fungicidal,^{6,7} anti-inflammatory,^{8,9} antiviral,^{10,11} antibacterial,^{12,13} and even anticancer agents.^{14,15}

On the other hand, heterocyclic compound bearing β -keto-enol functionality has been a fruitful source of inspiration for pharmaceutical and medicinal industries due to their widespread potential biological activities and their versatile utility in the world of medicinal chemistry,^{16,17} such as antioxidant,¹⁸ anti-inflammatory,¹⁹ anticancer,²⁰ and antifungal²¹ activities. In this context, β -keto-enol functionality was found in numerous natural products such as the curcumin and its derivatives (Figure 1), which have spurred numerous studies in medicinal chemistry.^{22–24} Some compounds have even been approved

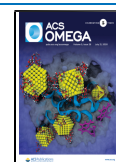
for treating patients with human immunodeficiency virus (HIV).^{25,26}

Recently, several analogic compounds containing the β -keto-enol pharmacophore have been designed and synthesized such as the keto-enol tetrazoles and triazoles as anti-HCV agents,²⁷ the triazolyl-keto-enol calix[4]arene as a potent integrase strand transfer inhibitor,²⁸ coumarinyl chalcones as highly selective agents for breast cancer cell lines,²⁹ etc. On the other hand, virtual screening combined with other computational methods, such as ligand–target Docking, can be very useful to confirm biological activities.^{30,31} While the classic drug development process is very expensive and tedious, computational methods are highly efficient and economical.³² Modern drug designing extensively relies on computer-assisted techniques like ligand-based drug design (LBDD) and structure-based drug design (SBDD) approaches. Molecular docking is a contemporary approach under the umbrella of SBDD. Molecular docking is highly useful to identify the

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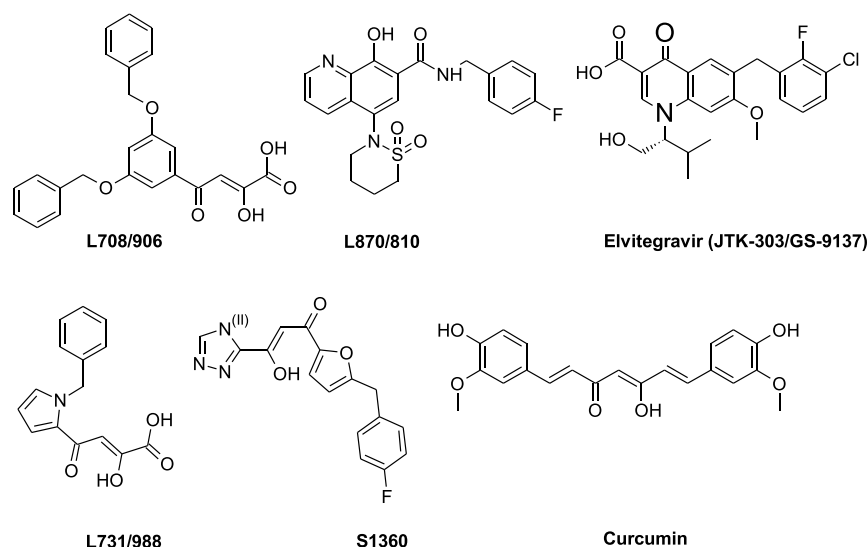
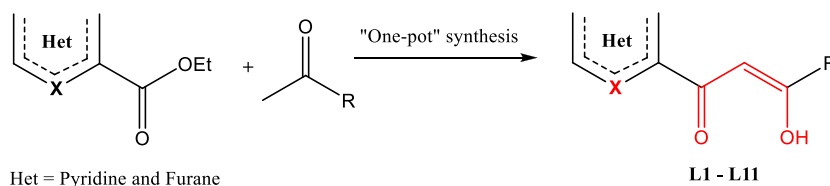


Figure 1. Molecular structures of some drugs containing keto-enol functionality.

Scheme 1. Synthetic Route for the Compounds L_1 – L_{11}



structural/pharmacophoric features that govern the bioactivity profile of a molecule. The identification of a pharmacophoric pattern is then used for future optimizations. This approach is highly economical, time saving, and result oriented. Considering all these observations, and in continuation of our previous work on biologically important β -keto-enol heterocyclic compound, we report herein a new series of β -keto-enol group embedded with heterocyclic moieties such as pyridine and furan derivatives. Their antifungal activities were *in vitro* evaluated and correlated by Petra/Osiris/Molinspiration (POM) calculations and molecular coupling against the Fgb1 protein.

2. RESULTS AND DISCUSSION

2.1. Synthesis. The synthetic route of the target compounds (L_1 – L_{11}) was carried out following a Claisen condensation under mild conditions (Scheme 1).^{21,33–41} Indeed, a solution of ethyl heterocycle-2-carboxylate was added to a suspension of sodium in toluene, and then ketone derivatives were added at 0 °C. After neutralization of the generated keto-enolate salts to pH = 5.5 using acetic acid, the obtained residue was filtered through silica using CH_2Cl_2 /MeOH as an eluent to afford the title products in acceptable yields. These products are presumably in anticonformation isomeric forms as already demonstrated for similar products analyzed too by single-crystal X-ray diffraction.^{21,33–41}

Intramolecular hydrogen bonding is the main factor that governs the structure of keto-enol tautomers in solution.³⁷ In fact, the stable β -keto-enol form is generally present in large amount due to the conjugation effect of the enol with the carbonyl group and the presence of an intramolecular hydrogen bond (Scheme 1).³⁸ ^1H NMR spectra of (L_1 – L_{11}) exhibited peaks at δ = 6.20–8.10 ppm assigned to the vinylic

proton ($-(\text{OH})\text{C}=\text{CH}-$) of the enolic tautomer. In addition, a very small peak at δ = 3.14–4.87 ppm for the methylene protons ($-\text{CO}-\text{CH}_2-\text{CO}-$) of the β -diketones tautomer form was observed. Indeed, according to the ^1H NMR integration of signals, the parent β -diketones exist almost exclusively in the enol form (>90%), and only a trace of the keto form is detected. ^{13}C NMR signals assigned with the aid of distortionless enhancement by polarization transfer (DEPT) experiments were also performed to confirm the previous result. Indeed, one signal at around δ = 92–95 ppm was observed for the vinylic proton ($-\text{CH}=\text{C}-(\text{OH})-$) of the enolic forms. Also, a very small negative signal of ketone form ($-\text{CO}-\text{CH}_2-\text{CO}-$) was observed on the DEPT-135 spectra at around δ = 176–184 ppm. Representative NMR and mass spectra are given in Supporting Information (SI) Figures S1–S20. The target products were obtained in majority in the β -keto-enol tautomer form (90%), whereas the remaining 10% represents the β -diketone tautomer form. This trend is confirmed by single-crystal X-ray diffraction, which systematically showed the β -keto-enol in this substance class, along with a mesomeric effect.^{21,33–41} We recall that there are two driving forces responsible for the formation of keto-enol isomers in solution:⁴² (i) the electronic force, in which the formation of the enol isomer is controlled by the electronegativity of the R and R' substituents fixed on the β -diketone ($\text{R}'\text{COCH}_2\text{COR}$) and (ii) the resonance force (mesomeric effect), which has priority over the inductive effect when one or two substituents R and R' are aromatic.

2.2. Pharmacology. The compounds (L_1 – L_{11}) were evaluated for their antifungal activity against *Fusarium oxysporum* and two Gram-positive (strains, viz., *Bacillus subtilis* and *Micrococcus luteus*). A complementary screening was performed too against two Gram-negative bacterial strains

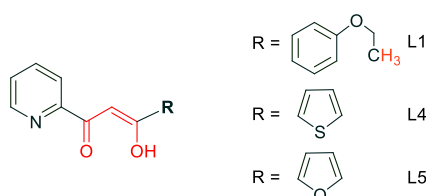
Table 1. Structures and Anti-Fusarium Activities of L₁–L₁₁

Compound	Structure	Volume with Drawn (μL)	Diameter of the strain (cm) ^a	Inhibition (%)	IC ₅₀ (μg. mL ⁻¹)
L ₁		50	2	60	12.83
		200	1.3	74	
		500	0.2	96	
L ₂		50	5	0	NS ^b
		200	3.5	30	
		500	2.3	54	
L ₃		50	5	0	NS
		200	3.6	28	
		500	2.5	50	
L ₄		50	2.7	46	17
		200	0.5	90	
		500	0	100	
L ₅		50	5	0	36
		200	0.8	84	
		500	0	100	
L ₆		50	5	0	NS
		200	3.8	24	
		500	3.2	36	
L ₇		50	4	20	NS
		200	3.2	36	
		500	2.5	50	
L ₈		50	3.8	24	NS
		200	2.7	46	
		500	1.3	74	
L ₉		50	3.9	22	NS
		200	2.8	44	
		500	1.7	66	
L ₁₀		50	4.2	16	NS
		200	3.7	26	
		500	2.1	58	
L ₁₁		50	4	20	NS
		200	3.8	24	
		500	2.3	54	

^aIn the presence of the tested compounds (cm). ^bNS = not significant.

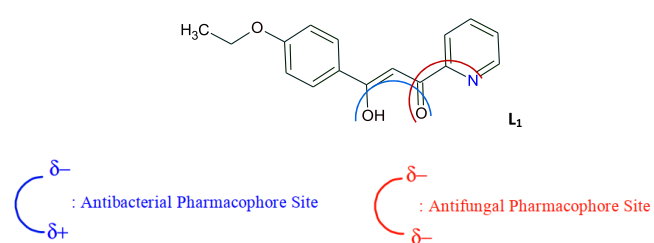
(bacterium, viz., *Escherichia coli*) but very weak or absence of antibacterial activity was noticed. Only small inhibition areas were observed compared to the gentamicin standard. These results can be interpreted by the lack of pharmacophore sites that can serve as potential and specific features for inhibiting bacterial growth.⁴³ In contrast, most of these molecules have strong antifungal activity against FOA, depending on their dose (Table 1). Their activity largely depends on the activity relationships of the structure (SAR) and shows an interesting influence of the substitution pattern by observing the influence of the R substituent (Figure 2).

Indeed, L₄ with the thiophene group has an IC₅₀ = 17 μg/mL, whereas L₁, which contains the ethoxyphenyl, has a decreased IC₅₀ = 12.83 μg/mL. Replacement of thiophene by a

Figure 2. R substituent for L₁, L₄, and L₅.

furan group (L₅) results however in an increased IC₅₀ = 34 μg/mL.

It is known that the negative charges of the keto and pyridine groups contribute positively to the antifungal activity, rather the antibacterial activity.⁴³ This is consistent with the antifungal mode of action of compounds with pharmacophore sites (X_{δ-}...Y_{δ+}) (Figure 3). Indeed, the fungal activity of L₁ is

Figure 3. Antibacterial and antifungal pharmacophore sites for L₁.

very substantial and decreases slightly in the case of L₄ because of ethoxyphenyl groups. The greater activity of the pharmacophore site is due to its physicochemical properties and its ability to penetrate the envelope of fungal cells and reach its cellular site of action.

It is worth noting that the present results are the best among previously tested analogues against the FOA fungus.^{21,33} In

Table 2. Osiris Calculations of Toxicity Risks of L₁–L₁₁

Molecule	MW	Toxicity Risks ^[a]				Osiris calculations ^[b]			
		MUT	TUM	IRRI	REP	cLogP	LogS	DL	DS
L ₁	269					1.99	-2.92	-0.87	0.27
L ₂	303					2.38	-3.44	-0.28	0.17
L ₃	226					0.70	-1.83	1.44	0.52
L ₄	231					1.52	-2.61	3.46	0.55
L ₅	215					0.84	-2.28	1.24	0.30
L ₆	231					1.44	-2.5	-1.91	0.52
L ₇	264					2.98	-4.66	0.44	0.28
L ₈	204					0.98	-2.74	0.60	0.46
L ₉	244					1.72	-3.07	1.25	0.80
L ₁₀	220					1.65	-3.07	2.55	0.87
L ₁₁	228					2.13	-3.40	0.11	0.67
Benomyl	290					2.82	-3.89	-2.51	0.21

^aHighly toxic (red), slightly toxic (orange), and not toxic (green). MW, molecular weight; MUT, mutagenic; TUM, tumorigenic; IRRI, irritant; and REP, reproductive effective. ^bSol: solubility, DL: druglikeness, DS: Drug-Score.

Table 3. Molinspiration Calculations of (L₁–L₁₁) and Benomyl

molecule	molinspiration calculations ^a				drug-likeness ^b					
	TPSA	NONH	NV	VOL	GPCRL	ICM	KI	NRL	PI	EI
L ₁	59.42	2	0	248.06	-0.52	-0.31	-0.67	-0.46	-0.45	0.12
L ₂	50.19	1	0	223.60	-0.69	-0.32	-0.78	-0.79	-0.65	0.11
L ₃	63.08	1	0	201.56	-0.49	-0.20	-0.60	-0.66	-0.41	0.31
L ₄	50.19	1	0	196.43	-0.92	-0.65	-1.00	-1.14	-0.84	-0.00
L ₅	63.33	1	0	187.28	-0.84	-0.64	-1.10	-1.12	-0.95	-0.02
L ₆	50.19	1	0	196.43	-0.69	-0.28	-0.83	-0.82	-0.65	0.17
L ₇	50.44	1	0	235.43	-0.67	-0.44	-0.80	-0.59	-0.66	-0.07
L ₈	63.58	1	0	173.01	-1.27	-0.86	-1.49	-1.40	-1.33	-0.35
L ₉	59.67	1	0	216.98	-1.03	-0.67	-1.20	-0.93	-1.06	-0.29
L ₁₀	50.44	1	0	182.15	-1.54	-1.15	-1.66	-1.64	-1.55	-0.57
L ₁₁	50.44	1	0	208.00	-1.14	-0.72	-1.34	-1.08	-1.18	-0.35
benomyl	85.26	2	0	264.53	0.36	0.21	0.33	-0.68	-0.04	0.28

^aTPSA, total molecular polar surface area; NONH, number of OH...N and O...NH interactions; NV, number of violation of five Lipinsky rules; and VOL, volume. ^bGPCRL, GPCR ligand; ICM, ion-channel modulator; KI, kinase inhibitor; NRL, nuclear receptor ligand, PI, protease inhibitor; and EI, enzyme inhibitor.

connection with a pronounced biological interest in heterocyclic compounds, this study suggests that the nature of the R substituent (Figure 2) should be considered seriously to be able to tune properties and determine the structural activity ratio for this new class of antifungal agent.

2.3. Computational Studies. **2.3.1. POM Analyses of Compounds.** The theoretical toxicity risks of L₁–L₁₁, which were calculated using the POM program,⁴⁴ show that some of our compounds are nontoxic and can be used as therapeutic agents (Table 2). Besides, the assessment of the mutagenicity of the free drug assumes that the structures of the compounds examined are not mutagenic and have a low risk compared to synthetic drugs. The standards for irritant and reproductive effects are related.

The hydrophilicity of each compound was evaluated by the cLogP value because the absorption is known to be strongly influenced by this parameter. Our results show that all compounds have cLogP values that are within acceptable

standards, with the highest value of 2.98 for L₇, and may be effective against multiple biological targets as shown in Table 3. Therefore, the good absorption of test compounds L₁–L₁₁ may be attributed to their good solubility.⁴⁵ In addition, Table 3 shows the similarity of the drug of compounds L₁–L₁₁ in the area of incompatibility with known standard medicinal products (benomyl for fungi). The Drug-Score (DS) combines the risks of toxicity, cLogP, LogS (S = solubility), molecular weight (MW), and toxicity into a useful value that can be used to assess the overall potential of a compound to qualify as a drug. Overall, L₁–L₁₁ are considered as good candidates, in terms of the bioactivity score prediction, against regular human receptors such as GPCR ligand, ion-channel modulator, kinase inhibitor, nuclear receptor ligand, protease inhibitor, and enzyme inhibitor. Compounds L₁–L₁₁ are subject to important tautomerism/mesomerism chemical processes leading to the regeneration of combined pharmacophore sites, as shown in Figure 3 for L₁. In addition, Figure 3 shows that L₁ is more

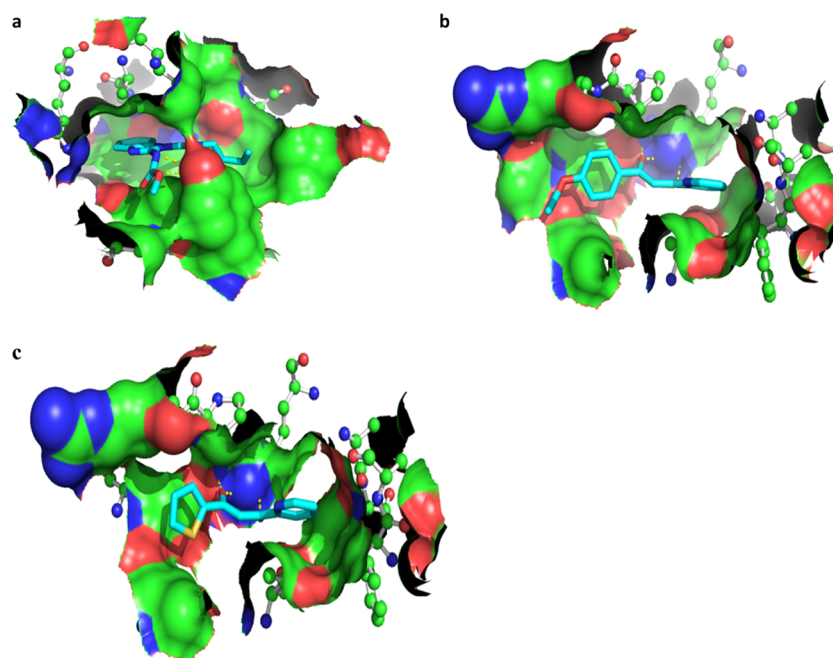


Figure 4. Binding conformations predicted from the ADVina runs for benomyl (a), L_1 (b), and L_4 (c) bound to β -tubulin of *F. oxysporum*. The protein (Fgb1) binding site is shown by the molecular surface and colored according to the protein atomic composition. All protein residues are drawn as ball-and-stick models. Hydrogen bonds are visualized as dashed lines. The ligand molecules are depicted in sticks, and hydrogen atoms were removed to enhance clarity.

likely to inhibit fungal activity because its pharmacodynamic sites are more specific than multiple substitution sites. POM analyses show the crucial role and influence of substituents on bioactivity, which indicate unfavorable structural parameters in the actual design of the drug: a stronger substitution does no longer guarantee efficiency in bioactivity.

2.3.2. Molecular Docking and Molecular Dynamics Simulations. Two best-performing molecules (L_1 and L_4) as well as benomyl as a positive control, presenting minimum IC_{50} values, were further evaluated in molecular docking experiments to predict their binding affinity to β -tubulin of *F. oxysporum* (Figure 4 and Table 4). This *in silico* experimental

Table 4. Binding Free Energies (ΔG_{bind} , ΔG_{GBSA} , ΔG_{PBBSA}), Dissociation Constants (K_d), and Minimum Inhibitory Concentrations IC_{50} Capable of Inhibiting *F. oxysporum* Growth at 50% for the Analyzed Substances

molecule	ΔG_{bind} (kcal/mol)	K_d (μM)	IC_{50} ($\mu\text{g/mL}$)	ΔG_{GBSA} (kcal/mol)	ΔG_{PBBSA} (kcal/mol)
L_1	−7.0	7.08	12.83	−31.01	−2.76
L_4	−6.5	16.23	17.0		
benomyl	−7.2	5.05	5.0	−41.38	−11.45

design takes into consideration tubulin-binding properties of benomyl as an antimetabolic antifungal agent and the information about the receptor-binding site, which was previously validated with the co-crystallized ligand taxol.^{46,47} As an outcome, the Gibbs free energy of binding (ΔG_{bind}) and K_d values were determined to accurately predict the minimum inhibitory concentration (MIC) values obtained from the MIC assay, which shows a good correlation between the experimental and theoretical data (Table 4). In particular, the L_1 compound represented the stronger binding ($\Delta G_{\text{bind}} = -7.0$ kcal/mol and $K_d = 7.08$ μM) to β -tubulin compared to L_4 ($\Delta G_{\text{bind}} = -6.5$ kcal/mol and $K_d = 16.23$ μM), which correlates with their IC_{50}

values. On the other hand, the 50 ns MD simulations of L_1 and benomyl compounds bound to β -tubulin confirmed the previous results of molecular docking and antifungal assay, where the reference substance achieved the highest inhibition of bacterial growth due to its strongest binding to the target protein (Table 4).

3. CONCLUSIONS

We have designed and synthesized a different new set of new β -keto-enol pyridine and furan derivatives. They were characterized by elemental analysis, ^1H NMR, ^{13}C NMR, Fourier transform infrared spectroscopy (FT-IR), and electrospray ionization/liquid chromatography-mass spectrometry (ESI/LC-MS). Most of the newly synthesized compounds demonstrated appropriate antifungal activities that were evaluated *in vitro*. In this context, we noticed that L_1 exhibited excellent antifungal activity at $IC_{50} = 12.83$ $\mu\text{g/mL}$ concentration against tested fungicide strains; which is even equal to benomyl fungicide reference. These results exhibited by such β -keto-enol group pyridine and furan derivatives are encouraging and may stimulate further studies for the development of new drugs.

4. MATERIALS AND METHODS

4.1. General Information. All chemical reagents used in this study were of analytical grade (Aldrich, purity >99%). Melting points were measured with a BUCHI 510 mp apparatus. ^1H and ^{13}C NMR spectra were recorded on a Bruker AC 300 spectrometer operating at 300 MHz for proton and 75.47 for carbon nuclei. Molecular weights were determined using a JEOL JMS DX-300 mass spectrometer. Elemental analysis was performed by CNRST Rabat. Infrared (IR) spectra were acquired on a Shimadzu infrared spectrophotometer using KBr disks. X-ray diffraction data collection was carried out on the four-circle Oxford Xcalibur

diffractometer (Mo K α radiation, $\lambda = 0.71073$ Å). The *in vitro* antifungal and antibacterial activities were tested by the agar diffusion technique.

4.2. General Procedure for the Synthesis of β -Keto-enol Heterocycles. The β -keto-enol pyridine and furan derivatives were prepared using our previously reported procedure.^{21,33–35} Briefly, aryl methyl ketones (17.39 mmol) were added at 0 °C to a mixture of sodium (0.4 g, 17.39 mmol) and ethyl heterocycle-2-carboxylate (13.23 mmol) in toluene (50 mL). The mixture was kept under stirring at room temperature for 2 days. The resulting solid was treated thoroughly with toluene and neutralized with acetic acid to pH of 5.5. The organic layer obtained after extraction with CH₂Cl₂ was dried over anhydrous sodium sulfate and concentrated *in vacuo*. The obtained residue was filtered through silica using CH₂Cl₂/MeOH as an eluant followed by recrystallization from methanol (95%) to give the desired products L₁–L₁₁ as solids in acceptable yields.

4.2.1. 3-(4-Ethoxyphenyl)-3-hydroxy-1-(pyridin-2-yl)prop-2-en-1-one (L₁). Brown powder; yield: 45% (1.3 g); mp = 198–199 °C; $R_f = 0.5$ (CH₂Cl₂/MeOH 7/3)/silica. IR (KBr, cm⁻¹): $\nu(\text{OH}) = 3444$; $\nu(\text{C}=\text{O}) = 1599$; $\nu(\text{enolic C}=\text{C}) = 1549$; ¹H NMR (DMSO-*d*₆): δ 1.31 (t, 3H, CH₂–CH₃); 3.14 (s, 0.1H, keto, CH₂); 4.06 (q, 2H, O–CH₂); 6.96 (d, 2H, Ar-H_{3,5}); 7.49 (s, 0.9H, enol, CH); 7.73 (t, 1H, Py-H _{β}); 7.78 (t, 1H, Py-H _{δ}); 7.84 (m, 2H, Ar-H_{2,6}); 8.03 (t, 1H, Py-H γ); 8.75 (d, 1H, Py-H α); ¹³C NMR (DMSO-*d*₆): δ 14.94 (1C, CH₂–CH₃); 48.24 (1C, keto, CH₂); 63.90 (1C, OCH₂); 92.87 (1C, enol, CH); 114.64 (2C, Ar-C_{3,5}); 123.25 (1C, Py-C δ); 130.12 (2C, Ar-C_{2,6}); 138.14 (1C, Py-C γ); 150.11 (1C, Py-C α); 182.16 (1C, C–OH); 186.77 (1C, C=O); Anal. Calcd. for C₁₆H₁₅NO₃: C 71.36, H 5.61, N 5.20. Found C 71.32, H 5.58, N 5.18; M.S: *m/z*, 270.01 (M + H)⁺.

4.2.2. 3-(4-Bromophenyl)-3-hydroxy-1-(pyridin-2-yl)prop-2-en-1-one (L₂). Brown powder; yield: 31% (0.9 g); mp = 236–238 °C; $R_f = 0.73$ (CH₂Cl₂/MeOH 8/2)/silica. IR (KBr, cm⁻¹): $\nu(\text{OH}) = 3442$; $\nu(\text{C}=\text{O}) = 1596$; $\nu(\text{enolic C}=\text{C}) = 1554$; ¹H NMR (DMSO-*d*₆): δ 3.54 (s, 0.1H, keto, CH₂); 5.71 (s, 0.9H, enol, CH); 7.68 (m, 2H, Ar-H_{2,6}); 7.74 (m, 1H, Py-H _{β}); 7.77 (m, 1H, Py-H γ); 7.84 (m, 2H, Ar-H_{3,5}); 8.08 (t, 1H, Py-H δ); 8.79 (d, 1H, Py-H α); ¹³C NMR (DMSO-*d*₆): δ 48.24 (1C, keto, CH₂); 95.93 (1C, enol, CH); 122.25 (1C, Py-C δ); 125.35 (1C, Py-C β); 125.64 (1C, Ar-C₄); 128.56 (2C, Ar-C_{3,5}); 131.44 (1C, Ar-C₁); 132.10 (2C, Ar-C_{2,6}); 137.14 (1C, Py-C γ); 149.61 (1C, Py-C α); 167.13 (2C, Py-C ϵ); 182.24 (1C, C–OH); 184.46 (1C, C=O); Anal. Calcd. for C₁₄H₁₀BrNO₂: C 55.29, H 3.31, Br 26.27, N 4.61; Found C 55.30, H 3.32, Br 26.24, N 4.64; M.S: *m/z*, 305 (M + H)⁺.

4.2.3. 3-Hydroxy-1,3-di(pyridin-2-yl)prop-2-en-1-one (L₃). Brown powder; yield: 59% (2.5 g); mp = 108–110 °C; $R_f = 0.82$ (CH₂Cl₂/MeOH 6/4)/silica. IR (KBr, cm⁻¹): $\nu(\text{OH}) = 3451$; $\nu(\text{C}=\text{O}) = 1617$; $\nu(\text{enolic C}=\text{C}) = 1568$; ¹H NMR (CDCl₃-*d*): δ 4.87 (s, 0.1H, keto, CH₂); 7.37 (dd, 2H, Py-H _{β}); 8.79 (td, 2H, Py-H _{γ}); 8.07 (t, 2H, Py-H _{δ}); 8.10 (s, 1H, enol, C–H); 8.68 (dd, 2H, Py-H α); ¹³C NMR (CDCl₃-*d*): δ 48.32 (1C, keto, CH₂); 94.56 (1C, enol, C–H); 121.97–122.14 (2C, Py-C δ); 126.39–127.20 (2C, Py-C β); 136.90 (2C, Py-C γ); 148.90–149.52, (2C, Py-C α); 152.43 (2C, Py-C ϵ); 184.44 (1C, C=O); 196.97 (1C, C–OH); Anal. Calcd. for C₁₃H₁₀N₂O₂: C 69.02, H 4.46, N 12.38; Found C 69.04, H 4.43, N 12.36; M.S: *m/z*, 227.16 (M + H)⁺.

4.2.4. 3-Hydroxy-1-(pyridin-2-yl)-3-(thiophen-2-yl)prop-2-en-1-one (L₄). Colorless crystals; yield: 38% (1.5 g); mp = 94–

96 °C; $R_f = 0.13$ (CH₂Cl₂/MeOH, 9/1)/silica. IR (KBr, cm⁻¹): $\nu(\text{OH}) = 3426$; $\nu(\text{C}=\text{O}) = 1622$; $\nu(\text{enolic C}=\text{C}) = 1514$. ¹H NMR (CDCl₃-*d*): δ 4.80 (s, 0.1H, keto, CH₂); 7.18 (dd, 1H, Th-H _{β}); 7.39 (s, 1H, enol, C–H); 7.43 (dd, 1H, Py-H γ); 7.67 (dd, 1H, Py-H _{β}); 7.86 (td, 1H, Py-H _{δ}); 7.92 (dd, 1H, Th-H γ); 8.11 (dt, 1H, Th-H α); 8.71 (dd, 1H, Py-H α); ¹³C NMR (CDCl₃-*d*): δ 49.10 (1C, keto, CH₂); 94.12 (1C, enol, C–H); 122.06 (1C, Py-C δ); 126.28 (1C, Th-C β); 128.53 (1C, Py-C β); 131.33 (1C, Th-H γ); 133.36 (1C, Th-C α); 137.20 (1C, Th-C ϵ); 142.59 (1C, Py-C γ); 149.54 (1C, Py-C α); 151.89 (1C, Py-C ϵ); 177.99 (1C, C=O); 184.16 (1C, C–OH); Anal. Calcd. for C₁₂H₉NO₂S: C 62.32; H 3.92; N 6.06; Found: C 61.79; H 3.96; N 6.09; M.S: *m/z*, 232.02 (M + H)⁺.

4.2.5. 3-(Furan-2-yl)-3-hydroxy-1-(pyridin-2-yl)prop-2-en-1-one (L₅). Yellow powder; yield: 30% (0.98 g); mp = 102–104 °C; $R_f = 0.6$ (CH₂Cl₂/MeOH 9/1)/silica. IR (KBr, cm⁻¹): $\nu(\text{OH}) = 3428$; $\nu(\text{C}=\text{O}) = 1625$; $\nu(\text{enolic C}=\text{C}) = 1515$. ¹H NMR (CDCl₃-*d*): δ 4.69 (s, 0.1H, keto, CH₂); 6.58 (dd, 1H, Fu-H _{β}); 7.29 (dd, 1H, Fu-H γ); 7.37 (s, 0.9H, enol, C–H); 7.41 (dd, 1H, Py-H _{β}); 7.63 (dd, 1H, Fu-H α); 7.84 (td, 1H, Py-H γ); 8.09 (td, 1H, Py-H _{δ}); 8.69 (dd, 2H, Py-H α); ¹³C NMR (CDCl₃-*d*): δ 48.52 (1C, keto, CH₂); 93.82 (1C, enol, C–H); 112.79 (1C, Fu-C β); 116.67 (1C, Fu-H γ); 122.01 (1C, Py-C δ); 126.33 (1C, Py-C β); 126.33 (1C, Py-C γ); 137.15 (1C, Fu-C α); 149.56 (1C, Py-C α); 151.18 (1C, Py-C ϵ); 151.96 (1C, Fu-C ϵ); 178.73 (1C, C=O); 179.58 (1C, C–OH); Anal. Calcd. for C₁₂H₉NO₃: C 66.97, H 4.22, N 6.51; Found: C 66.99, H 4.20, N 6.54; MS *m/z*: 216 [M + H]⁺.

4.2.6. 3-Hydroxy-1-(pyridin-2-yl)-3-(thiophen-3-yl)prop-2-en-1-one (L₆). Yellow powder; yield: 42% (1.32 g); mp = 79–80 °C; $R_f = 0.37$ (CH₂Cl₂/MeOH 9/1)/silica. IR (KBr, cm⁻¹): $\nu(\text{OH}) = 3424$; $\nu(\text{C}=\text{O}) = 1625$; $\nu(\text{enolic C}=\text{C}) = 1514$. ¹H NMR (CDCl₃-*d*): δ 4.77 (s, 0.1H, keto, CH₂); 7.38 (s, 0.9H, enol, C–H); 7.40 (m, 1H, Th-H α); 7.44 (dd, 1H, Py-H γ); 7.65 (dd, 1H, Py-H ϵ); 7.87 (td, 1H, Th-H _{β}); 8.15 (dt, 1H, Py-H _{δ}); 8.20 (dd, 1H, Th-H ϵ); 8.71 (dd, 1H, Py-H α); ¹³C NMR (DMSO-*d*₆): δ 49.65 (1C, keto, CH₂); 94.52 (1C, enol, C–H); 122.16 (1C, Py-C δ); 126.43 (1C, Py-C β); 126.70 (2C, Th-C α , Th-C β); 130.72 (1C, Th-C ϵ); 137.20 (1C, Py-C γ); 139.68 (1C, Py-C α); 149.41 (1C, Th-C γ); 152.55 (1C, Py-C ϵ); 182.01 (1C, C–OH); 182.77 (1C, C=O); Anal. Calcd. for C₁₂H₉NO₂S: C 62.32, H 3.92, N 6.06; Found: C 62.29, H 3.94, N 6.09; MS *m/z*: 232.06 [M + H]⁺.

4.2.7. 1-(Furan-2-yl)-3-hydroxy-3-(naphthalene-1-yl)prop-2-en-1-one (L₇). Yellow powder; yield: 28% (0.79 g); mp = 162–164 °C; $R_f = 0.24$ (CH₂Cl₂)/silica. IR (KBr, cm⁻¹): $\nu(\text{OH}) = 3434$; $\nu(\text{C}=\text{O}) = 1629$; $\nu(\text{enolic C}=\text{C}) = 1533$. ¹H NMR (CDCl₃) δ 3.35 (s, 0.1H, keto CH₂); 6.27 (s, 0.9H, enol, C–H); 7.56 (m, 1H, Fu-H _{β}); 7.62 (d, 1H, Fu-H α); 8.08 (d, 2H, Ar-H_{1,6}); 8.20 (m, 1H, Ar-H₄); ¹³C NMR (CDCl₃) δ 49.50 (1C, keto CH₂); 94.72 (1C, C–H, enol); 113.40 (1C, Fu-C β); 114.04 (2C, Ar-C_{3,5}); 122.74 (1C, Fu-C γ); 124.66 (1C, Ar-C₁); 125.63 (1C, Ar-C₄); 125.91 (1C, Ar-C₉); 145.92 (1C, Fu-C α); 178.52 (1C, C=O); 180.55 (1C, C–OH); Anal. Calcd. for C₁₇H₁₂O₃: C 77.26, H 4.58; Found: C 77.26, H 4.58; MS *m/z*: 265 [M + H]⁺.

4.2.8. 1,3-Di(Furan-2-yl)-3-hydroxyprop-2-en-1-one (L₈). Yellow powder; yield: 31% (0.82 g); mp = 68–70 °C; $R_f = 0.42$ (CH₂Cl₂)/silica. IR (KBr, cm⁻¹): $\nu(\text{OH}) = 3448$; $\nu(\text{C}=\text{O}) = 1640$; $\nu(\text{enolic C}=\text{C}) = 1532$. ¹H NMR (DMSO-*d*₆) δ 4.43 (s, 0.1H, keto, CH₂); 6.69 (s, 0.9H, enol, C–H); 6.73 (dd, 1H, Fu₂-H _{β}); 6.76 (dd, 1HFu₂-H _{β}); 7.46 (dd, 1H, Fu₂-H γ);

7.54 (dd, 1H, $\text{Fu}_\text{c}\text{-H}_\gamma$); 8.00 (dd, 1H, $\text{Fu}_\text{c}\text{-H}_\alpha$); 8.02 (dd, 1H, $\text{Fu}_\text{c}\text{-H}_\alpha$); ^{13}C NMR ($\text{DMSO}-d_6$) δ 49.23 (1C, keto, CH_2); 92.21 (1C, C-H, enol); 113.33 (1C, $\text{Fu}_\text{c}\text{-C}_\gamma$); 113.62 (1C, $\text{Fu}_\text{c}\text{-C}_\gamma$); 117.39 (1C, $\text{Fu}_\text{c}\text{-C}_\beta$); 120.52 (1C, $\text{Fu}_\text{c}\text{-C}_\beta$); 148.42 (1C, $\text{Fu}_\text{c}\text{-C}_\alpha$); 148.99 (1C, $\text{Fu}_\text{c}\text{-C}_\alpha$); 149.51 (1C, $\text{Fu}_\text{c}\text{-C}_\delta$); 152.01 (1C, $\text{Fu}_\text{c}\text{-C}_\delta$); 174.44 (1C, $\text{C}=\text{O}$); 182.62 (1C, C-OH); Anal. Calcd. for $\text{C}_{11}\text{H}_8\text{O}_4$: C 77.26, H 4.58; Found: C 77.23, H 4.56; MS m/z : 205.08 $[\text{M} + \text{H}]^+$.

4.2.9. 1-(Furan-2-yl)-3-hydroxy-3-(4-methoxyphenyl)-prop-2-en-1-one (L_9). Yellow powder; yield: 43% (0.82 g); mp = 174–176 °C; R_f = 0.22 ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 9/1)/silica. IR (KBr, cm^{-1}): $\nu(\text{OH})$ = 3430; $\nu(\text{C}=\text{O})$ = 1626; $\nu(\text{enolic C}=\text{C})$ = 1533. ^1H NMR (CDCl_3) δ 3.36 (s, 0.1H, keto CH_2); 3.86 (s, 3H, Ar- OCH_3); 6.73 (s, 0.9H, enol, C-H); 6.98 (m, 1H, $\text{Fu}_\text{c}\text{-H}_\beta$); 7.46 (d, 2H, Ar- $\text{H}_{3,5}$); 7.58 (d, 1H, $\text{Fu}_\text{c}\text{-H}_\gamma$); 7.97 (m, 1H, Ar- $\text{H}_{2,6}$); 8.02 (d, 1H, $\text{Fu}_\text{c}\text{-H}_\alpha$); ^{13}C NMR (CDCl_3) δ 54.69 (1C, Ar- OCH_3); 49.50 (1C, keto CH_2); 94.43 (1C, C-H, enol); 113.40 (1C, $\text{Fu}_\text{c}\text{-C}_\beta$); 114.04 (2C, Ar- $\text{C}_{3,5}$); 122.74 (1C, $\text{Fu}_\text{c}\text{-C}_\gamma$); 129.43 (3C, Ar- $\text{C}_{1,2,6}$); 131.54 (1C, $\text{Fu}_\text{c}\text{-C}_\alpha$); 178.52 (1C, $\text{C}=\text{O}$); 181.84 (1C, C-OH); Anal. Calcd. for $\text{C}_{14}\text{H}_{12}\text{O}_4$: C 68.85, H 4.95; Found: C 68.84, H 4.93; MS m/z : 245 $[\text{M} + \text{H}]^+$.

4.2.10. 1-(Furan-2-yl)-3-hydroxy-3-(thiophen-2-yl)prop-2-en-1-one (L_{10}). Yellow powder; yield: 28% (0.84 g); mp = 72–74 °C; R_f = 0.69 (CH_2Cl_2)/silica. IR (KBr, cm^{-1}): $\nu(\text{OH})$ = 3445; $\nu(\text{C}=\text{O})$ = 1639; $\nu(\text{enolic C}=\text{C})$ = 1546. ^1H NMR ($\text{MeOD}-d_4$) δ 4.52 (s, 0.1H, keto, CH_2); 6.66 (dd, 1H, $\text{Fu}_\text{c}\text{-H}_\beta$); 6.70 (s, 0.9H, enol, C-H); 7.20 (dd, 1H, Th- H_β); 7.26 (dd, 1H, $\text{Fu}_\text{c}\text{-H}_\gamma$); 7.78 (dd, 1H, Th- H_γ); 7.79 (dd, 1H, $\text{Fu}_\text{c}\text{-H}_\alpha$); 7.89 (dd, 1H, Th- H_α); ^{13}C NMR ($\text{MeOD}-d_4$) δ 46.91 (1C, keto, CH_2); 92.65 (1C, C-H, enol); 112.51 (1C, $\text{Fu}_\text{c}\text{-C}_\gamma$); 115.44 (1C, $\text{Fu}_\text{c}\text{-C}_\beta$); 128.36 (1C, Th- C_γ); 130.76 (1C, Th- C_β); 132.89 (1C, Th- C_α); 141.06 (1C, $\text{Fu}_\text{c}\text{-C}_\alpha$); 146.78 (1C, Th- C_δ); 149.75 (1C, $\text{Fu}_\text{c}\text{-C}_\delta$); 172.37 (1C, $\text{C}=\text{O}$); 180.70 (1C, C-OH); Anal. Calcd. for $\text{C}_{11}\text{H}_8\text{O}_3\text{S}$: C 59.99, H 3.66; Found: C 59.96, H 3.63; MS m/z : 221 $[\text{M} + \text{H}]^+$.

4.2.11. 1-(Furan-2-yl)-3-hydroxy-3-*p*-tolylprop-2-en-1-one (L_{11}). Brown powder; yield: 32% (0.72 g); mp = 102–103 °C; R_f = 0.73 ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 5/5)/silica. IR (KBr, cm^{-1}): $\nu(\text{OH})$ = 3431; $\nu(\text{C}=\text{O})$ = 1621; $\nu(\text{enolic C}=\text{C})$ = 1531. ^1H NMR (CDCl_3) δ 2.34 (s, 3H, Ar- CH_3); 3.37 (s, 0.1H, keto CH_2); 7.29 (s, 0.9H, enol, C-H); 7.26 (t, 1H, $\text{Fu}_\text{c}\text{-H}_\beta$); 7.52 (d, 1H, $\text{Fu}_\text{c}\text{-H}_\gamma$); 7.57 (d, 2H, Ar- $\text{H}_{3,5}$); 7.64 (d, 1H, $\text{Fu}_\text{c}\text{-H}_\alpha$); 7.81 (t, 1H, Ar- H_4); ^{13}C NMR (CDCl_3) δ 21.58 (1C, Ar- CH_3); 49.50 (1C, keto CH_2); 94.56 (1C, C-H, enol); 112.55 (1C, $\text{Fu}_\text{c}\text{-C}_\gamma$); 117.62 (1C, $\text{Fu}_\text{c}\text{-C}_\beta$); 128.48 (2C, Ar- $\text{C}_{3,5}$); 129.59 (3C, Ar- $\text{C}_{2,4,6}$); 143.50 (1C, $\text{Fu}_\text{c}\text{-C}_\alpha$); 178.52 (1C, $\text{C}=\text{O}$); 180.84 (1C, C-OH); Anal. Calcd. for $\text{C}_{14}\text{H}_{12}\text{O}_3$: C 73.67, H 5.30; Found: C 73.64, H 5.33; MS m/z : 229 $[\text{M} + \text{H}]^+$.

4.3. Biological Evaluation. **4.3.1. Antifungal Assay.** We determined the *in vitro* antifungal potential of the 11 compounds against the pathogenic fungus *F. oxysporum f.sp. albedinis* (FAO) using the agar diffusion technique.⁴⁸ Different volumes (50, 200, and 500 μL) of a DMSO solution of the compounds were tested to prepare Petri dishes with different concentrations with papa dextrose agar medium (PDA). Subsequently, 6 mm diameter disks of the microorganism (FAO) were placed in the middle of these Petri dishes. After incubation at 28 °C for 7 days, the inhibition percentages were calculated and the semi-maximal inhibitory concentration (IC_{50}) was determined using a nonlinear regression algorithm

of the percentage dose inhibition graph. Benomyl was used as a standard drug (positive control).

4.3.2. Antibacterial Test. The antibiotic effect of L_1 – L_{11} was evaluated against a Gram-negative bacterium, viz., *E. coli*, and two Gram-positive strains, viz., *B. subtilis* and *Micrococcus luteus*, according to the recommendations of the National Clinical Laboratory Standards Committee (NCCLS). For the tests, compounds were dissolved in dimethylsulfoxide (DMSO) and sterile WHATMAN paper disks (6 mm in diameter) were impregnated with different volumes of each compound and then placed in the middle of the Petri dishes containing the culture media (Muller–Hinton agar) previously inoculated with cultures during the night of the target strains. After 24 h of incubation at 37 °C, the diameter of the zones of inhibition around each disk was measured. Gentamicin was used as a standard drug.

4.4. Computational Studies. **4.4.1. POM Analyses of Compounds.** The POM program is an efficient bioinformatics platform that can process virtually all organic compounds and most organometallic ones, leading to the identification of their pharmacophore sites. The POM program also leads to its optimization based on geometric, physicochemical parameters of each site and electronic load distribution of the corresponding heteroatoms X, Y, and Z.^{49,50}

4.4.1.1. Osiris Calculations. The prediction results are valued and color-coded. Properties with a high risk of undesirable effects such as mutagenicity or poor intestinal absorption are shown in red, while a green color indicates a drug conformation behavior. The hydrophilicity of the series (L_1 – L_{11}) has been expressed in terms of the $\text{cLog } P$ value, where P is a partition coefficient of the molecule between *n*-octanol and water. It has been established that absorption is greatly affected by hydrophilicity. In particular, when $\text{cLog } P$ is greater than 5, absorption decreases. This intratemplate effect is in favor of the constitution of an important antifungal/antiviral pharmacophore site $\text{N}\cdots\text{O}$ as hypothesized by the POM theory.^{51,52}

4.4.1.2. Molinspiration Calculations. The bioactivity scores (BS) of L_1 – L_{11} were calculated for different parameters such as TPSA, $\text{NH}\cdots\text{O}$ or N-HO interaction and molecular volume and compared with some standard medications. All of the mentioned parameters were calculated with the Molinspiration online software (www.molinspiration.com), which predicted a moderate biological activity for the synthesized compounds (L_1 – L_{11}).

4.4.2. Molecular Docking and Molecular Dynamics Simulations. The ADVina algorithm was implemented to perform the molecular docking of the hit and reference molecules (benomyl) to the binding site of *F. oxysporum* β -tubulin.⁵³ The protein three-dimensional (3D) structure, namely, *Fusarium oxysporum* Guanine nucleotide-binding protein β (**Fgb1**), was generated using the SWISS-MODEL server,⁵⁴ as a fully automated protein homology modeling workflow. The docking grids with a 3D size of 22.5 Å were used in the study. The binding site was determined according to the information obtained from the α -tubulin-taxol crystallized complex (PDB ID: 1JFF) with Cartesian coordinates of 332.24 Å (*x*-axis), 424.26 Å (*y*-axis), and 323.41 Å (*z*-axis). The PyMOL molecular imaging software and the in-house Python data processing scripts were utilized to calculate the dissociation constants (K_d) from the ΔG_{bind} (Gibbs free energy of binding) values, generate high-quality graphics, and analyze the results (PyMOL Molecular Graphics System, Version

1.7.2.1, Schrödinger, LLC). All molecular dynamics (MD) simulations were performed using the AMBER 16 package,⁵⁵ with the FF99SB and GAFF force fields for the protein and its ligands. The systems were solvated with the TIP3P water models and neutralized by adding the Na⁺ ions using the tLEap input script available from the AmberTools package. Long-range electrostatic interactions were modeled *via* the particle-mesh Ewald method.⁵⁶ The SHAKE algorithm,⁵⁷ was applied to constrain the length of covalent bonds, including the hydrogen atoms. Langevin thermostat was implemented to equilibrate the temperature of the system at 300 K. A 2.0 fs time step was used in all of the MD setups. For the minimization and equilibration phases, 50 000 steps and 1 ns period were used, respectively. Finally, 50 ns classical MD simulations, with no constraints were performed for each of the protein-ligand complexes using the molecular mechanics combined with the Poisson-Boltzmann (MM-PBSA) or generalized Born (MM-GBSA) surface area term.^{58,59} The MM-PBSA/GBSA solvation models were applied as a postprocessing end-state method to calculate the free energies of molecules in the solution employing the optimized python script (MM-PBSA.py).

■ ASSOCIATED CONTENT

■ Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsomega.0c02365>.

Mass, ¹H NMR, and ¹³C spectra of ligands used in this work (PDF)

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Notes

The authors declare no competing financial interest.

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