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Novel Heterocycles via 2-Cyano-*N*-arylacetamide Synthesis with Docking Studies of Novel Heterocycles as Antimicrobial Agents Utilizing 2-Cyano-*N*-arylacetamide

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2-CYANO-*N*-arylacetamide reagent is used in a straight forward synthesis of various interesting nitrogenous heterocycles such as iminocoumarine, thiazole, dihydropyridine and imidazole or benzoimidazole. Molecular docking studies are described to show the most active compounds as antimicrobial agents. Also, Biological testing of these compounds for their antimicrobial activities against some strains of Gram bacteria and strains of fungi has been carried out.

Keywords: Antimicrobial activities, 2-Cyano-*N*-arylacetamide, Iminocoumarine, Thiazole and Dihydropyridine.

Introduction

Heterocyclic nitrogenous moieties can be considered as a potential building block in a wide variety of biologically and pharmacologically active compounds and as they are important in drug discovery.[1-3] 2-Cyanoacetamide moieties have found as vital synthons in the synthesis of many active biological heterocyclic systems such as quinoxalinones, pyridines, thiazoles and pyrimidines.[4-9] So, our interested work herein is focusing on using *N*-(4-substitutedphenyl)-2-cyanoacetamide **1a-c** as valuable synthons in the synthesis of simple active heterocyclic compounds and evaluate the resulted compounds as antimicrobial agents against strains of *Escherichia coli* (Gram negative bacteria) and *Staphylococcus aureus* (Gram positive bacteria) by the disc diffusion method [10, 11] together with strains of fungi *Aspergillus flavus* and *Candida albicans* [12] by the agar diffusion method.

Since the cyanoacetamide derivative **1** has two nucleophilic sites; the active methylene group which takes part in condensation and substitution reactions and the amide nitrogen. Also, they have three electrophilic sites; the carbonyl of amide and the cyano function group, which react with bidentate reagents (Fig. 1). In the same manner, cyanoacetamide derivative **1** has HN-CO and CH_2CN together with acetyl group opens a wide opportunity for further utilizing it as a good starting material in the syntheses of many vital heterocyclic compounds (Fig. 1) [13-15]. The direct reaction between amines and methylcyanoacetate afforded the desired compounds **1a-c**. [16, 17]

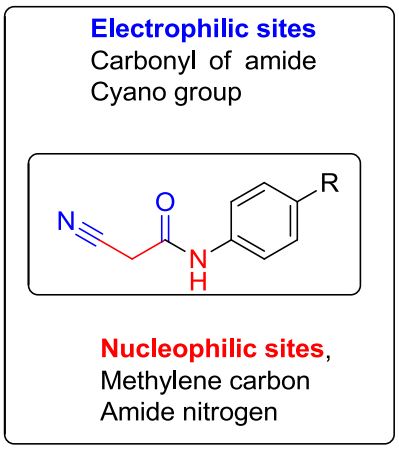

Fig.1. Starting material; *N*-(4-substitutedphenyl)-2-cyanoacetamide 1a-c.

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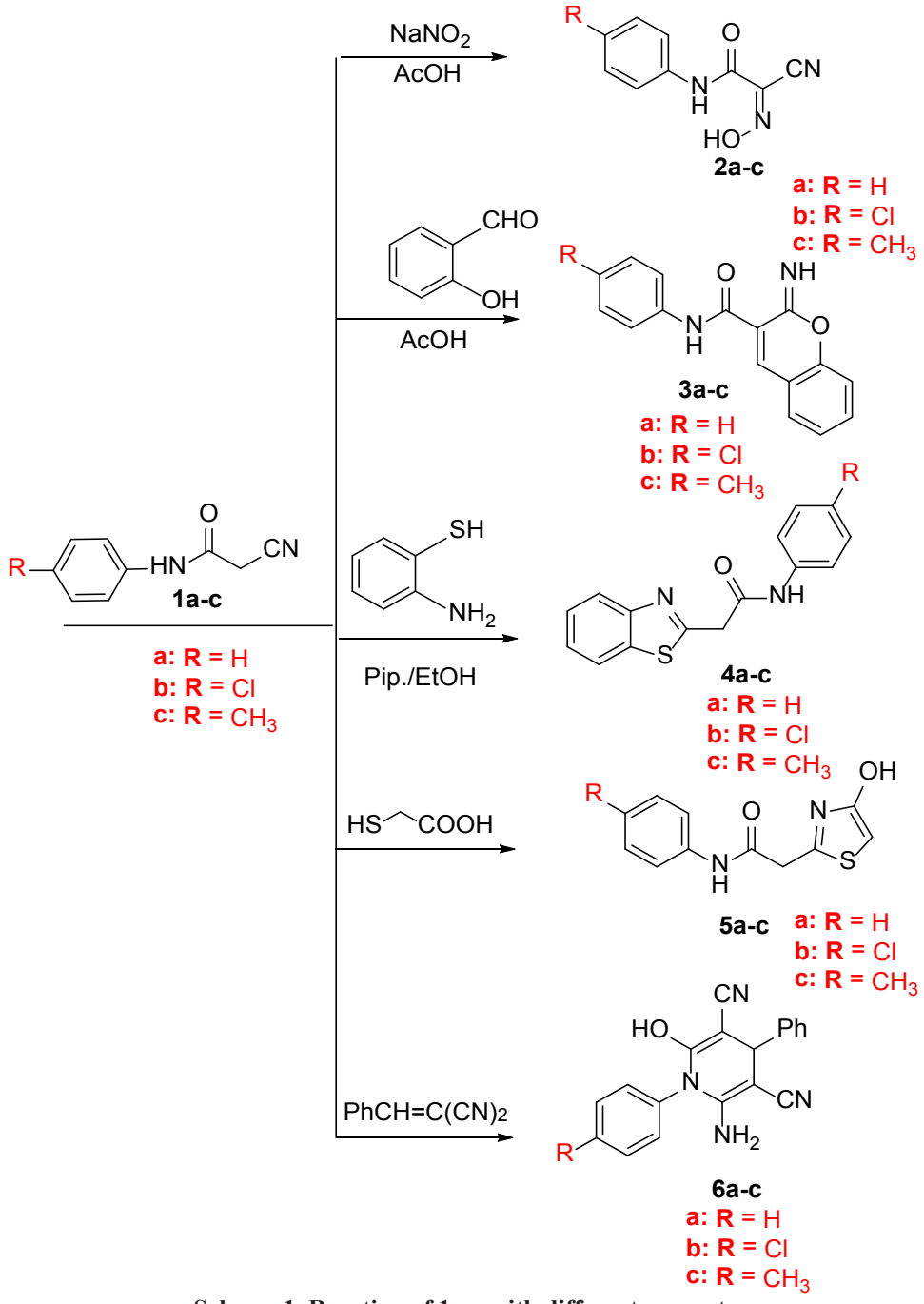
Results and Discussion

Chemistry

Herein, we utilized *N*-(4-substitutedphenyl)-2-cyanoacetamide derivatives **1a-c** of a simple manner in the synthesis of various derivatives of heterocyclic compounds such as the corresponding hydroxyimino **2a-c**, 2-imino-chromene-3-carboxamide **3a-c**, 2-(Benzo[*d*] thiazole **4a-c**, 4-hydroxythiazol-2-yl)acetamide **5a-c**, and 1,4-dihydropyridine-3,5-dicarbonitrile **6a-c** derivatives (Scheme 1).

Compounds **1a-c** reacted with sodium nitrite in acetic acid through nitrosation process [18] afforded the corresponding hydroxyimino derivatives **2a-c**, respectively (Scheme 1). The structures of **2a-c** are proven according to full sets of analytical and spectroscopic data (*cf. Experimental*).



Scheme 1. Reaction of 1a-c with different reagents.

Treatment of **1a-c** with salicylaldehyde in boiling acetic acid gave the corresponding 2-imino-chromene-3-carboxamide derivatives **3a-c** in a moderate yield. The most important features of structures **3a-c** are the presence of imino group at 3284 cm^{-1} with absence of CN group at region 2000-2300 cm^{-1} in IR spectrum, while signals of chromene H-4 at 8.66 ppm and imino proton (D_2O exchangeable) at 13.40 ppm in ^1H NMR spectra (*cf. Experimental*).

When compounds **1a-c** were reacted with *O*-amino-thiophenol under basic conditions, 2-benzothiazolyl derivatives **4a-c** were obtained, the disappearance of CN group in the IR spectrum with appearance the benzothiazolyl protons as a multiplet at 7.12-8.23, 7.23-8.53 and 7.26-8.62 ppm in ^1H NMR of **4a-c** supported the suggested structures. Moreover, when compounds **1a-c** was allowed to react with thioglycolic acid (as a second bidentate ligand) the same results were obtained to yield compounds **5a-c** in satisfactory yields.

A better success was achieved to obtained the pyridine derivative, when compounds **1a-c** were reacted with benzylidene malononitrile in basic medium, compound **6a-c** exists in the enolic form, which was stabilized by the hydrogen bond (Scheme 1, *cf. Experimental*).

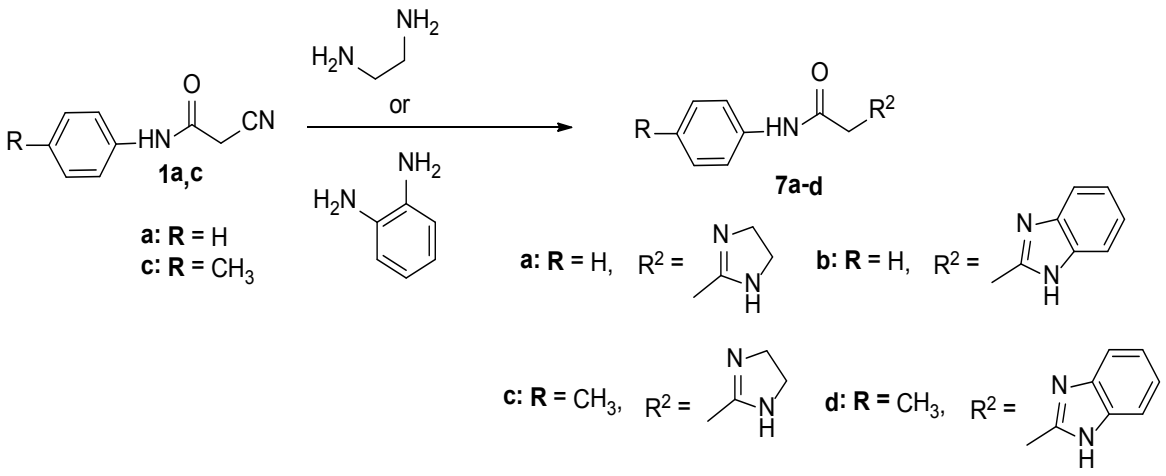
Finally, when one mole equivalent of **1a,c** reacted with one mole equivalent of ethylenediamine or with *o*-phenylenediamine in the presence of carbon disulfide, gave the corresponding imidazole derivatives **7a-d** (Scheme 2). Spectroscopic and analytical analyses elucidated the structures of compounds **7a-d** (*cf*. Experimental).

Molecular docking

Auto Dock tools were applied to explain the antimicrobial features of drugs and favor the experimental products. The ligands (guest) executed with tow protein receptors (host): **(3 t88)** and **(5aez)** by molecular docking (Fig. 2-9). The energies and distance from best mode for the docking procedure were studied, proteins were sending to yasara to minimized proteins energy. According to computation, a strong interaction with all receptors with comparable results was illustrated through 3D plots (Fig. 2-9). This indicated to mutable capacity for the hydrogen-bonding interaction by multi-central group's internal docking ligands. All proteins have inter hydrogen bonding [19-21]. The manner of interaction internal the docking molecules pocket was proved by three dimensional plots (Fig. 2-9). So, the ligands interacted with amino acids of proteins through hydrogen bonds as follows:

For 3 t88 protein, amino acid of protein reacted with ligands by H-bond as follows:

Compound **3a** ligand with **3t88** protein we find interaction with amino acids, hydrogen bond and energy; 3t88-A1/A/SER`84/O (hydrogen bond length = 2.2 Å), 3t88-A1/A/GLY`86/O (hydrogen bond length = 3.2 Å), 3t88-A/ASP`163/H (hydrogen bond length = 3.3 Å), 3t88-A1/A/GLY`153/O (hydrogen bond length = 2.3 Å) and 3t88-A1/A/PHE`162/O (hydrogen bond length = 3.1 Å), with binding energy = −7.3kcal mol^{-1} (Fig. 2).



Scheme 2. Reaction of 1a,c with ethylenediamine or *o*-phenylenediamine .

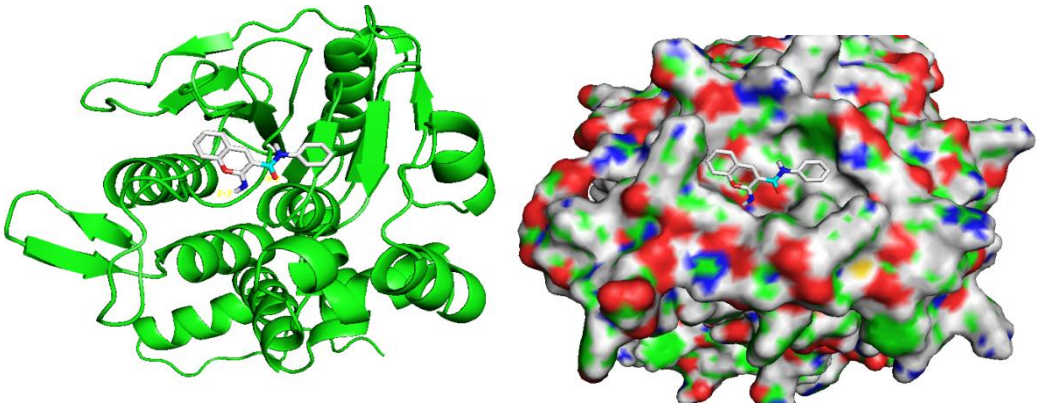


Fig. 2. Three dimensional plot of interaction of (3a) ligand with 3 t88 receptor.

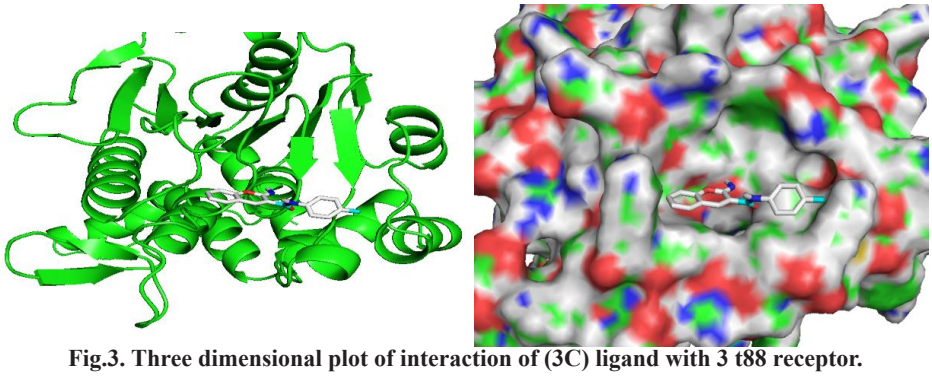


Fig.3. Three dimensional plot of interaction of (3C) ligand with 3 t88 receptor.

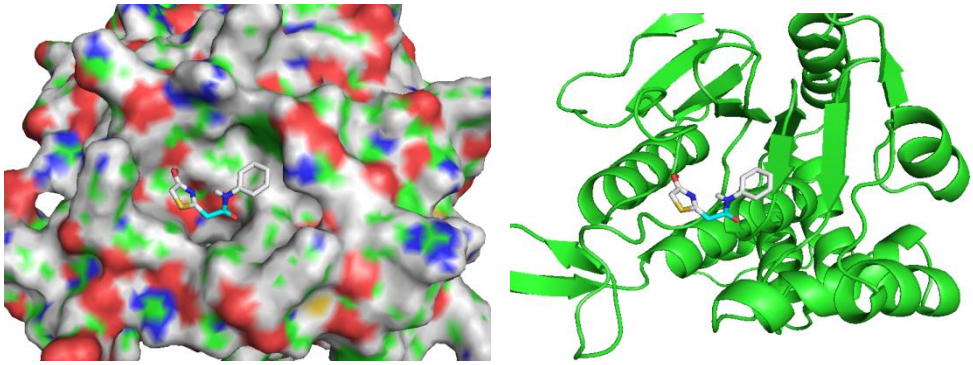


Fig.4.Three dimensional plot of interaction of (°a) ligand with 3 t88 receptor.

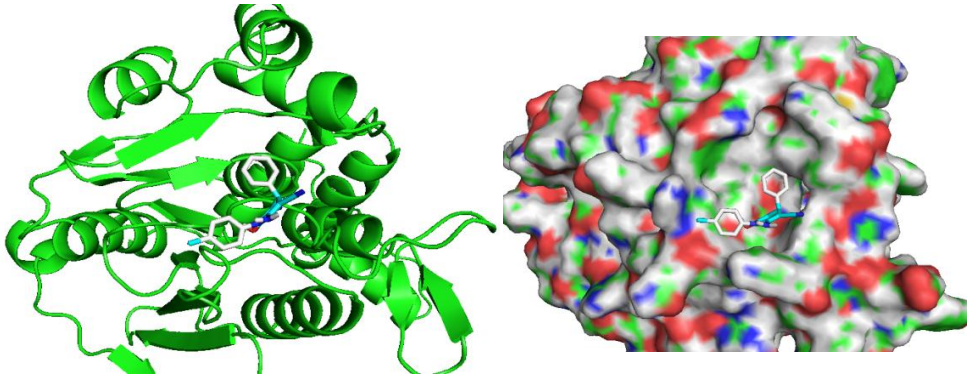


Fig.5.Three dimensional plot of interaction of (6b) ligand with 3 t88 receptor.

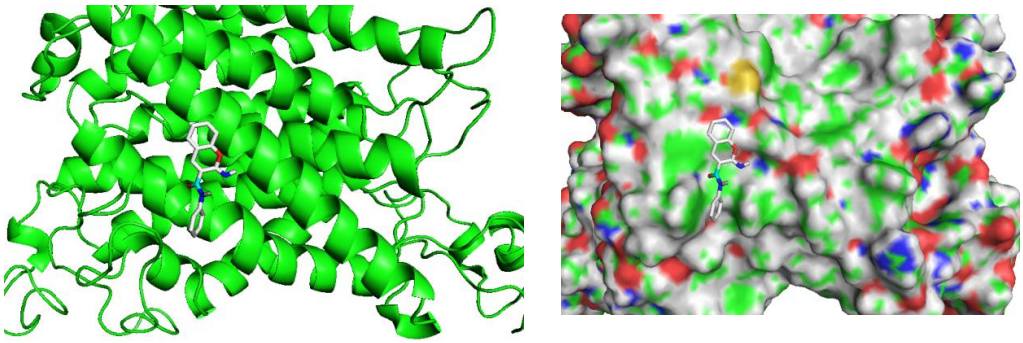


Fig. 6. Three dimensional plot of interaction of (3a) ligand with 5aez receptor.

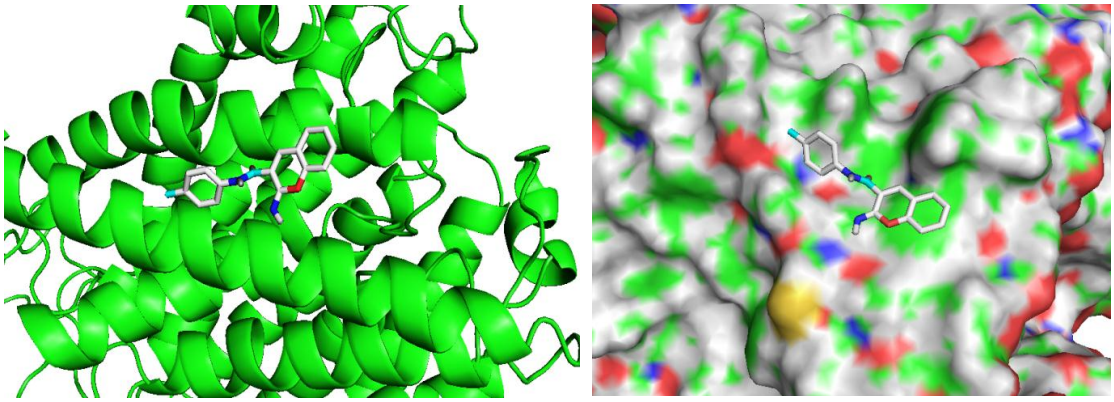


Fig. 7. Three dimensional plot of interaction of (3C) ligand with 5aez receptor.

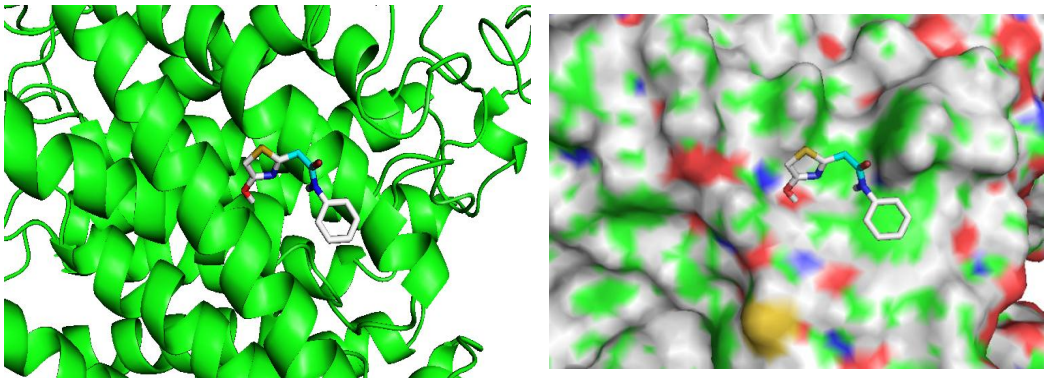


Fig. 8. Three dimensional plot of interaction of (5a) ligand with 5aez receptor.

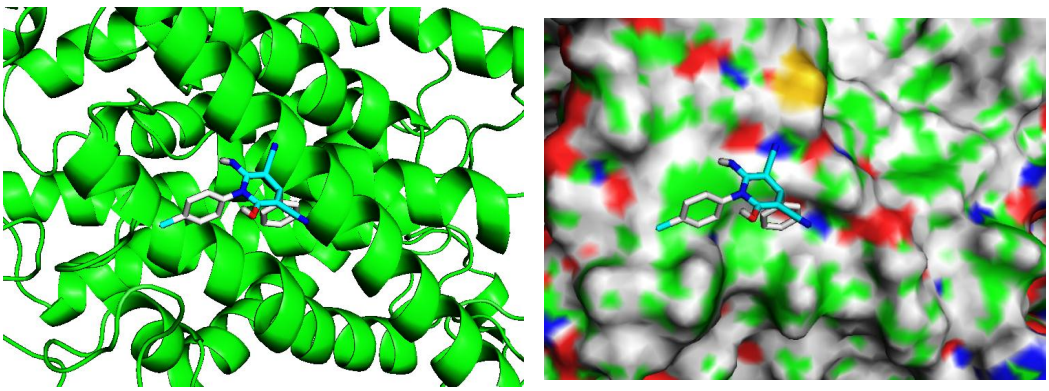


Fig. 9. Three dimensional plot of interaction of (6b) ligand with 5aez receptor.

For **3C** ligand with **3t88** protein we find interaction with amino acids, hydrogen bond and energy; 3t88-A1/A/THR`155/HG1 (hydrogen bond length = 2 Å), 3t88-A1/A/SER`84/O (hydrogen bond length = 2.5 Å) and 3t88-A1/A/SER`84/HG (hydrogen bond length = 2.8 Å), with binding energy = −6.7 kcal mol^{-1} (Fig. 3).

For **5a** ligand with **3t88** protein we find interaction with amino acids, hydrogen bond and energy; 3t88- A1/A/ARG`45/NH1 (hydrogen bond length = 3.2 Å), 3t88-A1/A/THR`155/HG1 (hydrogen bond length = 2.3 Å) and 3t88- A1/A/VAL`44/O (hydrogen bond length = 2.4 Å), with binding energy = −5.8 kcal mol^{-1} (Fig. 4).

For Compound **6b** ligand with **3 t88** protein, the amino acids, hydrogen bond and energy; 3t88-A1/A/SER`84/HG (hydrogen bond length = 2.2 Å), 3t88-A1/A/GLY`86/O (hydrogen bond length = 2.9 Å), 3t88-A1/A/GLY`86/O (hydrogen bond length = 2.8 Å) and 3t88-A1/A/TRP`15/HH2 (hydrogen bond length = 3.1 Å), with binding energy = −6.5 kcal mol^{-1}.

As we see from above results that the interactions of ligands with amino acids of proteins are ordered as follows: **3a**> **6b**> **3C**> **5a** and this are congruent with the experimental result.

For 5aez protein, the amino acids, hydrogen bond and energy

For **3a** ligand with **5aez** protein we find interaction with amino acids, hydrogen bond and energy; 5aez-A/TRP`172/HE1 (hydrogen bond length = 3.1 Å), 5aez-A/TRP`394/HZ3 (hydrogen bond length = 2.3 Å) and 5aez-A/ALA`390/HB1 (hydrogen bond length = 3.3 Å), with binding energy = − 6.7 kcal mol^{-1} (Fig. 6).

For **3C** ligand with **5aez** protein we find interaction with amino acids, hydrogen bond and energy; 5aez-A/THR`397/OG1 (hydrogen bond length = 3.1 Å) and 5aez-A/CYS`389/O (hydrogen bond length = 2.9 Å) with binding energy = −6.7 kcal mol^{-1} (Fig. 7).

For **5a** ligand with **5aez** protein we find interaction with amino acids, hydrogen bond and energy; 5aez-A/ALA`390/O (hydrogen bond length = 2.7 Å), 5aez-A/ALA`393/HN (hydrogen bond length = 2.2Å) with binding energy = −5.5 kcal mol^{-1} (Fig. 8).

For **6b** ligand with **5aez** protein we find interaction with amino acids, hydrogen bond and energy; 5aez-A1/A/SER`84/O (hydrogen bond length = 2.9 Å), 5aez - A1/A/SER`84/OG (hydrogen bond length = 2.2 Å) and 5aez - A1/A/GLY`86/O (hydrogen bond length = 2.8 Å), with binding energy = −6.1 kcal mol^{-1} (Fig. 9).

Also, as we see from above results that the interactions of ligands with amino acids of proteins are ordered as follows: **3a**> **6b**> **3C**> **5a** and this are congruent with the experimental result.

Antimicrobial activity [6-8]

The newly synthesized compounds were screened for their antibacterial activity against *Escherichia coli* (Gram negative bacteria) and *Staphylococcus aureus* (Gram positive bacteria) by the disc diffusion method, and for their antifungal activity against *Aspergillus flavus* and *Candida albicans* in DMSO by the agar diffusion method.

As shown in Table 1, the synthesized compounds showed moderate to excellent antifungal activity (ranged from 11-14 mm) in comparison with *Amphotericin B* as a reference drug (18mm, diameter of the clear zoon of inhibition), while most of them showed inactivity against *Aspergillus flavus* (Table 1).

Also, the most active derivatives against *Escherichia coli* (Gram negative bacteria) and *Staphylococcus aureus* (Gram positive bacteria) are **3a**> **6b**> **3C**= **5a** in comparison with *Tetracycline* as a reference drug (32mm or 29mm, diameter of the clear zoon of inhibition) (Table 1).
The results revealed that most of the tested compounds exhibited good antibacterial and antifungal activities compared with the standards (Table 1). Iminocoumarine [22], thiazole and dihydropyridine skeletons present in compounds **3a**, **5a** and **6b** respectively are considered to be the reason for their higher remarkable antimicrobial activity [19-21] (Table 1).

Experimental

All chemicals were supplied by either Fluka or Aldrich chemical companies and were used without further purification. All melting points are uncorrected and were taken in open capillary tubes using Electrothermal apparatus 9100. Elemental microanalyses were carried out at Microanalytical Unit, Central Services Laboratory, National Research Centre, Dokki, Giza, Egypt, using Vario Elementar and were found within ±0.4% of the theoretical values. FT-IR spectra were recorded with a Perkin-Elmer Frontier. Routine NMR

spectra were recorded at room temperature on a Bruker Avance TM 400 spectrometer as solutions in dimethyl sulfoxide (DMSO-d_6). All chemical shifts are quoted in δ relative to the trace resonance of protonated dimethyl sulfoxide (δ2.50 ppm), DMSO (δ39.51 ppm). The mass spectra were measured with a GC Finnigan MAT SSQ-7000 mass spectrometer. Follow up of the reactions and checking the purity of the compounds were made by TLC on silica gel-precoated aluminum sheets (Type 60, F 254, Merck, Darmstadt, Germany) and the spots were detected by exposure to UV lamp at λ_{254} nanometer for few seconds. The chemical names given for the prepared compounds are according to the IUPAC system. The reported yields are based upon pure materials isolated by crystallization. Solvents were dried/purified according to conventional procedures.

General method for the preparation of 2a-c

Hydrochloric acid (1.1 mmol) was added to an ice cooled stirred solution of compounds **1a**, **1b** or **1c** (10 mmol) in 20 ml Dioxane. An aqueous solution of sodium nitrite (0.69g, 10 mmol) was added portion-wise to the reaction mixture and the temperature was kept between 0-5°C. Stirring was continued for further an hour. The precipitate formed after the addition of water was collected, washed with water, dried and finally recrystallized from ethanol.

N-hydroxy-2-oxo-2-(phenylamino)acetimidoyl cyanide (2a)

Yield: 77%; m.p. 160-162°C. IR (KBr, cm^{-1}): 3365 (OH), 2210 (CN), 1668 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.80-7.97 (m, 6H, Ar-H and NH), 11.01 (brs., 1H, OH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.2 (C=O), 148.9, 124.5, 123.7, 122.4, 116.3 (aromatic, C-H), 106.9 (CN). MS (*m/z*): M^+ 189 (77%). Analysis for $C_9H_7N_3O_2$ (189.17). Calced.: % C, 57.14; H, 3.73; N, 22.21. Found: % C, 57.20; H, 3.70; N, 22.26.

2-((4-Chlorophenyl)amino)-N-hydroxy-2-oxoacetimidoyl cyanide (2b)

Yield: 60%; m.p. 123-125°C. IR (KBr, cm^{-1}): 3364 (OH), 3270 (NH), 2215 (CN), 1680 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.82-7.89 (m, 5H, Ar-H and NH), 10.89 (brs., 1H, OH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.1(C=O), 147.6, 124.6, 124.2, 122.4, 116.3 (aromatic, C-H), 106.9 (CN). MS (*m/z*): M^+ 223 (24%). Analysis for $C_9H_6ClN_3O_2$ (223.62). Calced.: % C, 48.34; H, 2.70; N, 18.79. Found: % C, 48.39; H, 2.65; N, 18.75.

TABLE 1. Antimicrobial activity* of some newly prepared compounds .

Sample	*Escherichia coli* (G^-)	*Staphylococcus aureus* (G^+)	*Aspergillus flavus*	*Candida albicans*
Tetracycline	32	29	-	-
Amphotericin B	-	-	16	18
2a	13	15	0.0	11
2b	15	12	0.0	11
2c	12	11	0.0	14
3a	22	20	12	13
3b	15	15	10	13
3c	16	18	11	14
4a	14	12	0.0	12
4b	15	12	0.0	11
4c	14	12	0.0	13
5a	16	16	10	13
5b	12	12	9	12
5c	14	12	7	13
6a	15	12	10	11
6b	20	21	13	13
6c	15	15	10	13
7a	15	14	0.0	10
7b	14	15	10	10
7c	15	12	0.0	11
7d	12	15	10	12

* The diameter of the clear zoon of inhibition is measured (mm).

N-Hydroxy-2-oxo-2-(p-tolylamino)acetimidoyl cyanide (2c)

Yield: 56 %; m.p.: 133-135 ºC. IR (KBr, cm^{-1}): 3370 (OH), 3286 (NH), 2220 (CN), 1667 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.34 (s, 3H, CH_3), 6.95-8.01 (m, 5H, Ar-H and NH), 10.95 (brs., 1H, OH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 180.3 (C=O), 148.6, 124.7, 124.6, 122.5, 116.7 (aromatic, C-H), 106.5 (CN), 20.3 (CH_3). MS (*m/z*): M^+ 237 (24%). Analysis for $C_{10}H_9N_3O_2$ (203.20): Calced.: % C, 59.11; H, 4.46; N, 20.68. Found: % C, 59.06; H, 4.53; N, 20.58.

General method for the preparation of 3a-c

A solution of **1a**, **1b** or **1c** (10 mmol) and salisaldehyde (1.22g, 10 mmol) in 20 ml acetic acid was refluxed for 6 h. The solid formed after cooling, filtered off, washed with ethanol and recrystallized from acetic acid.

2-Imino-N-phenyl-2H-chromene-3-carboxamide (3a)

Yield: 75 %; m.p.: 157-159 ºC. IR (KBr, cm^{-1}): 3284 (NH), 1667 (C=O), 1610 (C=N). ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.53 (brs., 2H, NH), 8.03-7.11 (m, 9H, Ar-H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.1 (C=O), 153.3 (C-O), 152.4, 136.7, 131.8, 129.03, 129.0, 125.0, 124.9, 122.5, 119.9, 117.0 (aromatic, C-H). MS (*m/z*): M^+ 237 (24%). Analysis for $C_{10}H_9N_3O_2$ (203.20): Calced.: % C, 72.72; H, 4.58; N, 10.60. Found: % C, 72.68; H, 4.64; N, 10.55.

N-(4-Chlorophenyl)-2-imino-2H-chromene-3-carboxamide (3b)

Yield: 68 %; m.p.: 186-188 ºC. IR (KBr, cm^{-1}): 3287 (NH), 1665 (C=O), 1623 (C=N). ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.33 (s, 2H, NH), 8.02-7.11 (m, 8H, Ar-H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.5 (C=O), 153.9 (C-O), 152.5, 136.2, 131.8, 129.9, 128.9, 125.0, 123.0, 120.9, 119.5, 117.1 (aromatic, C-H). MS (*m/z*): M^+ 298 (58%). Analysis for $C_{16}H_{11}ClN_2O_2$ (298.72): Calced.: % C, 64.33; H, 3.71; N, 9.38. Found: % C, 64.38; H, 3.65; N, 9.43.

2-Imino-N-p-tolyl-2H-chromene-3-carboxamide (3c)

Yield: 50 %; m.p.: 118-120 ºC. IR (KBr, cm^{-1}): 3259 (NH), 1659 (C=O), 1620 (C=N). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.35 (s, 3H, CH_3), 4.38 (brs., 1H, NH), 8.03-7.10 (m, 8H, Ar-H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.3 (C=O), 153.6, 152.4, 136.0, 134.8, 131.8, 129.3, 129.0, 125.06, 120.5, 120.2, 119.9, 119.5, 117.0 (aromatic, C-H), 21.2 (CH_3). MS (*m/z*): M^+ 278 (33 %). Analysis for $C_{17}H_{14}N_2O_2$ (278.31): Calced.: % C, 73.37; H, 5.07; N, 10.07. Found: % C, 73.29; H, 5.14; N, 10.13.

General method for the preparation of 4a-c

A mixture of **1a**, **1b** or **1c** (10 mmol), *O*-aminothiophenol (1.25g, 10 mmol) in 20 ml ethanol in the presence of few drops piperidine was boiled under reflux for 8 h. The reaction mixture was cooled, poured onto ice cooled water, the solid was precipitated, filtered off, washed with water and recrystallized from ethanol.

2-(Benzo[d]thiazol-2-yl)-N-phenylacetamide (4a)

Yield: 64 %; m.p.: 189-190 ºC. IR (KBr, cm^{-1}): 3176 (NH), 1693 (C=O), 1579 (C=N). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 3.96 (s, 2H, CH_2), 8.06-7.23 (m, 9H, Ar-H), 8.07 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ = 176.5 (C=O), 167.0 (S-C=N), 152.4, 137.7, 136.3, 129.3, 127.1, 123.9, 123.2, 123.2, 122.2, 121.5 (aromatic, C-H), 43.1 (CH_2). MS (*m/z*): M^+ 268 (32). Analysis for $C_{15}H_{12}N_2OS$ (268.33): Calced.: % C, 67.14; H, 4.51; N, 10.44. Found: % C, 67.20; H, 4.46; N, 10.37.

2-(Benzo[d]thiazol-2-yl)-N-(4-chlorophenyl) acetamide (4b)

Yield: 55 %; m.p.: 193-195 ºC. IR (KBr, cm^{-1}): 3194 (NH), 1676 (C=O), 1580 (C=N). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 4.01 (s, 2H, CH_2), 8.05-7.37 (m, 9H, Ar-H), 8.10 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-*d6*) δ 176.4 (C=O), 167.0 (S-C=N), 152.3, 136.5, 136.3, 129.4, 128.6, 127.0, 123.9, 123.4, 122.2 (aromatic, C-H), 43.2 (CH_2). MS (*m/z*): M^+ 302 (36%). Analysis for $C_{15}H_{11}ClN_2OS$ (302.78): Calced.: % C, 59.50; H, 3.66; N, 9.25. Found: % C, 59.65; H, 3.58; N, 9.31.

2-(Benzo[d]thiazol-2-yl)-N-p-tolylacetamide (4c)

Yield: 78 %; m.p.: 201-203 ºC. IR (KBr, cm^{-1}): 3205 (NH), 1669 (C=O), 1576 (C=N). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 2.33 (s, 3H, CH_3), 3.96 (s, 2H, CH_2), 8.04-7.22 (m, 9H, Ar-H), 8.09 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-*d6*) δ 176.4 (C=O), 167.2 (S-C=N), 152.5, 136.8, 136.3, 133.1, 129.5, 127.9, 123.1, 122.7, 119.3 (aromatic, C-H), 43.2 (CH_2), 21.3 (CH_3). MS (*m/z*): M^+ 282 (46%). Analysis for $C_{16}H_{14}N_2OS$ (282.36): Calced.: % C, 68.06; H, 5.00; N, 9.92. Found: % C, 68.13; H, 5.07; N, 9.86.

General method for the preparation of 5a-c

A solution of compounds **1a, 1b or 1c** (10 mmol) and thioglycolic acid (0.92g, 10 mmol) in 20 ml acetic acid was heated under reflux for 8-10 h. Then, the reaction mixture was cooled, poured onto ice cooled water, the precipitate formed, filtered

off, washed with water, dried and recrystallized from acetic acid.

2-(4-Hydroxythiazol-2-yl)-N-phenylacetamide (5a)

Yield: 57 %; m.p.: 270-272 ºC. IR (KBr, cm^{-1}): 3340 (OH), 3189 (NH), 1650 (C=O). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 3.96 (s, 2H, CH2), 6.01 (s, 1H, thiazole H-5), 7.12-7.80 (m, 6H, Ar-H, NH), 12.54 (s, 1H, OH). ^{13}C NMR (100 MHz, DMSO-*d6*) δ 176.8 (C=O), 170.1 (S-C=N), 137.7, 129.1, 123.7, 121.6 (aromatic, C-H), 81.5 (CH, thiazole), 43.5 (CH_2). MS (*m/z*): M^+ 234 (75%). Analysis for $C_{11}H_{10}N_2O_2S$ (234.27): Calced.: % C, 56.39; H, 4.30; N, 11.96. Found: % C, 56.44; H, 4.26; N, 11.87.

N-(4-Chlorophenyl)-2-(4-hydroxythiazol-2-yl) acetamide (5b)

Yield: 73 %; m.p.: 210-212 ºC. IR (KBr, cm^{-1}): 3344 (OH), 3185 (NH), 1654 (C=O). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 1.78 (s, 1H, OH), 4.01 (s, 2H, CH_2), 6.03 (s, 1H, thiazole H-5), 7.23-7.88 (m, 5H, Ar-H, NH). ^{13}C NMR (100 MHz, DMSO-*d6*) δ 176.2 (C=O), 170.3 (S-C=N), 136.2, 129.4, 128.6, 122.2 (aromatic, C-H), 81.4 (CH, thiazole), 43.7 (CH_2). MS (*m/z*): M^+ 268 (65%). Analysis for $C_{11}H_9ClN_2O_2S$ (268.72): Calced.: % C, 49.17; H, 3.38; N, 10.42. Found: % C, 49.08; H, 3.47; N, 10.36.

2-(4-Hydroxythiazol-2-yl)-N-p-tolylacetamide (5c)

Yield: 62 %; m.p.: 170-172 ºC. IR (KBr, cm^{-1}): 3376 (OH), 3193 (NH), 1669 (C=O). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 2.21 (s, 3H, CH_3), 4.40 (s, 2H, CH_2), 6.06 (s, 1H, thiazole H-5), 6.52-7.90 (m, 5H, Ar-H, NH). ^{13}C NMR (100 MHz, DMSO-*d6*) δ 176.7 (C=O), 170.2 (S-C=N), 136.7, 133.3, 129.3, 119.3 (aromatic, C-H), 81.6 (CH, thiazole), 43.9 (CH_2), 21.4 (CH_3). MS (*m/z*): M^+ 248 (44%). Analysis for $C_{12}H_{12}N_2O_2S$ (248.30): Calced.: % C, 58.05; H, 4.87; N, 11.28. Found: % C, 58.12; H, 4.78; N, 11.35..

General method for the preparation of 6a-c

A mixture of compounds **1a, 1b** or **1c** (10 mmol) and benzylidene malononitrile (1.54g, 10 mmol) in 20 ml ethanol in presence of few drops of piperidine was refluxed for 6 h. Then the reaction mixture was cooled, poured onto ice-cooled water and acidified with diluted hydrochloric acid. The precipitate formed, filtered off, washed with water, dried and recrystallized from acetic acid.

2-Amino-6-hydroxy-1,4-diphenyl-1,4-dihydropyridine-3,5-dicarbonitrile (6a)

Yield: 57 %; m.p.: 270-272 ºC. IR (KBr, cm^{-1}): 3360 (OH), 3220 (NH_2), 2221, 2231 (CN). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 2.20 (brs., 2H, NH_2), 2.60 (s, 1H, OH), 4.81 (s, 1H, CH), 6.67-7.37 (m, 10H, Ar-H). ^{13}C NMR (100 MHz, DMSO-*d6*) δ 159.7, (C-OH), 158.9, (C-OH), 140.5, 139.6, 129.2, 128.7, 127.7, 127.1, 126.6, 122.9 (aromatic, C-H), 119.1, (CN), 73.9 (*C*-CN), 69.6 (*C*-CN), 39.6 (pyridine, CH). MS (*m/z*): M^+ 314 (54 %). Analysis for $C_{19}H_{14}N_4O$ (314.35): Calced.: % C, 72.60; H, 4.49; N, 17.82. Found: % C, 72.54; H, 4.56; N, 17.73.

2-Amino-1-(4-chlorophenyl)-6-hydroxy-4-phenyl-1,4-dihydropyridine-3,5-dicarbonitrile (6b)

Yield: 69 %; m.p.: 243-245 ºC. IR (KBr, cm^{-1}): 3365 (OH), 3225 (NH_2), 2220, 2230 (CN). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 3.81 (brs., 2H, NH_2), 4.20 (s, 1H, OH), 4.75 (s, 1H, CH), 6.88-7.37 (m, 9H, Ar-H). ^{13}C NMR (100 MHz, DMSO-*d6*) δ 159.8, (C-OH), 158.6, (C-OH), 140.2, 139.1, 129.0, 128.2, 127.5, 127.0, 126.2, 122.3, (aromatic, C-H), 119.2, (CN), 73.8 (*C*-CN), 69.2 (*C*-CN), 39.5 (pyridine, CH). MS (*m/z*): M^+ 348 (35 %). Analysis for $C_{19}H_{13}ClN_4O$ (348.79): Calced.: % C, 65.43; H, 3.76; 10.16. Found: % C, 65.39; H, 3.67; 10.20.

2-Amino-6-hydroxy-4-phenyl-1-p-tolyl-1,4-dihydropyridine-3,5-dicarbonitrile (6c)

Yield: 75 %; m.p.: 217-219 ºC. IR (KBr, cm^{-1}): 3360 (OH), 3220 (NH_2), 2195, 22190 (CN). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 2.36 (s, 3H, CH_3), 2.47 (s, 1H, OH), 2.72 (brs., 2H, NH_2), 4.61 (s, 1H, CH), 6.70-7.35 (m, 9H, Ar-H). ^{13}C NMR (100 MHz, DMSO-*d6*) δ 160.8 (C-OH), 159.6 (C-OH), 141.2, 140.1, 130.0, 129.2, 128.5, 127.0, 126.2, 122.3 (aromatic, C-H), 118.2 (CN), 72.8 (*C*-CN), 69.2(*C*-CN), 38.5 (pyridine, CH), 20.3 (CH_3). MS (*m/z*): M^+ 328 (45 %). Analysis for $C_{20}H_{16}N_4O$ (328.37): Calced.: % C, 73.15; H, 4.91; N, 17.06. Found: % C, 73.21; H, 4.85; N, 17.11.

Reaction of 1a,c with diamine

To a mixture of compounds **1a,c** (0.002 mol) and ethylenediamine or *o*-phenylenediamine (5 mL) carbon disulfide (1 mL) was added dropwise with stirring at room temperature. The reaction mixture was heated on water bath for 10 h. The formed precipitate was filtered off, dried and recrystallized from ethanol to give compound **7a-d**.

2-(4,5-Dihydro-1H-imidazol-2-yl)-N-phenylacetamide (7a)

Red crystals in 55% yield: mp 145-147 ºC (ethanol). IR (KBr, cm^{-1}): 3110 (NH), 1635 (C=O), 1559 (C=N). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 7.90 (s, 1H, NH), 7.51-7.11 (m, 5H, CH_{arom}), 3.92, 3.47 (2t, 4H, 2 CH_2), 3.30 (s, 2H, CH_2), 1.41 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-*d6*) δ 172.5 (C=O), 158.0 (C=N, imidazole), 138.6, 129.5, 123.2, 121.8 (aromatic, C-H), 54.2, 45.2, 33.5 (3 CH_2). MS (*m/z*): M^+ 203 (25%). Analysis for $C_{11}H_{13}N_3O$ (203.11). Calced: % C, 65.01; H, 6.45; N, 20.68. Found: % C, 65.05; H, 6.41; N, 20.72.

2-(1H-benzo[d]imidazol-2-yl)-N-phenylacetamide (7b)

Pale red crystals in 50% yield: mp 200-202 ºC (ethanol). IR (KBr, cm^{-1}): 3100 (NH), 1630 (C=O), 1560 (C=N). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 7.74 (s, 1H, NH), 7.62-7.09 (m, 9H, CH_{arom}), 6.96 (s, 1H, NH), 3.69 (s, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.2 (C=O), 150.1 (C=N, imidazole), 138.2, 137.1, 129.2, 123.3, 122.5, 121.5, 118.2, 114.6 (aromatic, C-H), 39.5 (CH_2). MS (*m/z*): M^+ 251 (15%). Analysis for $C_{15}H_{13}N_3O$ (251.11). Calced: % C, 71.70; H, 5.21; N, 16.72. Found: % C, 71.74; H, 5.18; N, 16.75.

2-(4,5-Dihydro-1H-imidazol-2-yl)-N-(p-tolyl) acetamide (7c)

Pale red crystals in 35% yield: mp 150-152 ºC (ethanol). IR (KBr, cm^{-1}): 3110 (NH), 1640 (C=O), 1550 (C=N). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 7.89 (s, 1H, NH), 7.45-7.23 (m, 4H, CH_{arom}), 3.92, 3.47 (2t, 4H, 2 CH_2), 3.30 (s, 2H, CH_2), 2.34 (s, 3H, CH_3), 1.41 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-*d6*) δ 172.2 (C=O), 158.1 (C=N, imidazole), 138.2, 129.2, 123.3, 121.5 (aromatic, C-H), 54.2, 45.2, 33.5 (3 CH_2), 21.5 (CH_3). MS (*m/z*): M^+ 217 (35%). Analysis for $C_{12}H_{15}N_3O$ (217.12). Calced: % C, 66.34; H, 6.96; N, 19.34. Found: % C, 66.38; H, 6.93; N, 19.30.

2-(1H-benzo[d]imidazol-2-yl)-N-(p-tolyl) acetamide (7d)

Brown crystals in 46% yield: mp 170-172 ºC (ethanol). IR (KBr, cm^{-1}): 3130 (NH), 1630 (C=O), 1550 (C=N). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 7.88 (s, 1H, NH), 7.60-7.21 (m, 8H, CH_{arom}), 7.01 (s, 1H, NH), 3.64 (s, 2H, CH_2), 2.33 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-*d6*) δ 172.7 (C=O), 150.8 (C=N, imidazole), 138.5, 137.0, 129.7, 123.5, 122.4, 121.3, 118.4, 114.5 (aromatic, C-H), 39.5 (CH_2), 22.9 (CH_3). MS (*m/z*): M^+ 265 (35%). Analysis for $C_{16}H_{15}N_3O$ (265.12). Calced: % C, 72.43; H, 5.70; N, 15.84. Found: % C, 72.40; H, 5.77; N, 15.80.

Molecular docking

AutoDock tools 4.2, docking computations applying Gasteiger partial charges added to ligand (designed drug) atoms were used. On the ligand–protein pattern, the calculations were executed. Non-polar hydrogen atoms were conjoined, and rotatable bonds were clarified. After the addition of fundamental hydrogen atoms, Kollman united atom type charges and salvation parameters the AutoDock tools were applied [15]. Determining van der Waals and electrostatic terms was performed by AutoDock parameter set- and distance-dependent dielectric functions, respectively. Simulative docking was executed by Solis & Wets local search method and Lamarckian genetic algorithm [16, 17]. Incidentally, initial position, orientation and torsions of the ligand molecule were set. All rotatable torsions were omitted during docking. Ten different runs were used to derive each docking experiment, which was set to block after an ultimate of 250 000 energy estimations. The size was established to 150. Throughout the study, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were utilized.

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Conflict of Interest:

The authors have no conflict of interest

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