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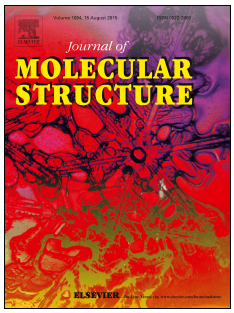

Synthesis of novel Schiff bases using green chemistry techniques; antimicrobial, antioxidant, antiurease activity screening and molecular docking studies

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Synthesis of novel Schiff bases using green chemistry techniques; antimicrobial, antioxidant, antiurease activity screening and molecular docking studies

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Abstract

Schiff base derivatives were synthesized in this study via conventional, microwave irradiation and ultrasound sonication methods. Optimization conditions were examined for several parameter such as solvent, reaction time and yield. After determining the optimization conditions, the compounds were synthesized by using ultrasound sonication. The structures of the synthesized compounds were examined by spectral data, and the antiurease, antioxidant and antimicrobial activities of the Schiff bases derivatives were investigated due to the imine group (-C=N-) and promising results were obtained. The enzyme inhibitory potentials of these compounds were further validated through molecular docking studies. Also, *In Silico* ADME prediction studies were calculated for compounds.

Keywords: Ultrasound sonication, microwave irradiation, biological activity, ADME prediction.

1. Introduction

Schiff bases present a crucial group of organic compounds in many aspects and they have a wide variety of biological activities such as antibacterial, anti-inflammatory, antifungal, antimalarial, antitubercular and anti-urease [1-10]. Schiff bases containing the imine (-C=N-) group are formed by the reaction of an aldehyde or ketone with a primary amine and imine group offer for this kind of compounds is important for their biological activities. Therefore, this moiety has been extensively explored for the development of new bioactive compounds [11, 12].

Green chemistry techniques have attracted in many areas, especially in the field of synthetic organic chemistry. Microwave and ultrasound mediated organic synthesis have become an increasingly used techniques for the production of new molecules [13]. They usually cause shorter reaction time, high reaction yield and purity. The main advantage of thesemethods is the almost instantaneous 'in-core' heating of compounds in a homogenous and selective conduct [14].

Urease, which posses nickel ions is the first crystallized and an important enzyme used in agriculture and medicine industry. The urease enzyme allows rapid formation of ammonia and carbamic acid by hydrolyzing the urea [15]. However, at the same time, the by-products resulting from the reaction lead to an increase in pH which is responsible for the adverse effects of urease activity in human health, leading to diseases such as gastric ulcers, stomach cancer. The urease causes in pathologies by *Helicobacter pylori* (HP), by helping the bacteria to stand at low pH of the stomach during colonization. Thus, it plays a vital role in the pathogenesis of the gastric as well as peptic ulcers which may cause cancer [16,17]. Additionally, urease not only causes kidney stones formations [18], but also engages in the growth of urolithiasis, pyelonephritis and hepatic encephalopathy [19]. In agriculture, during urea fertilization, high urease activity results in significant environmental and economic losses by discharge of abnormally huge amounts of ammonia in atmosphere. This also leads to plant damage by depriving them from essential nutrients, secondary ammonia toxicity and increase in pH of the soil [20]. Urease inhibition, therefore, has been identified as first line of treatment of diseases caused by ureolytic bacteria [21]. To remove these adverse effects, it is interesting to control the urease activity by the use of inhibitors [22]. The -C=N- imine bond in Schiff bases plays a unique role in conferring broad-spectrum biological activities to these compounds. The electrophilic carbon and nucleophilic nitrogen in -C=N- imine bond provides excellent binding opportunities with different nucleophiles and electrophiles, thereby inhibiting targeted diseases, enzymes or DNA replication.

Antioxidants are generally hydrogen donors or electron donors to the reactive site in neutralizing free radicals. Antioxidants are extensively studied for their capacity for protect organism and cell from damage that is induced by oxidative stress. Scientists in many different disciplines become more interested in new compounds, either synthesized or obtained from natural sources that could provide active components to prevent or reduce the impact of oxidative stress on cell [23-25]. The scavenging activity of different organic compounds can be assessed using DPPH, hydrogen peroxide, superoxide anion radical. Many organic compounds already reported before have showed very good antioxidant capacity, thus

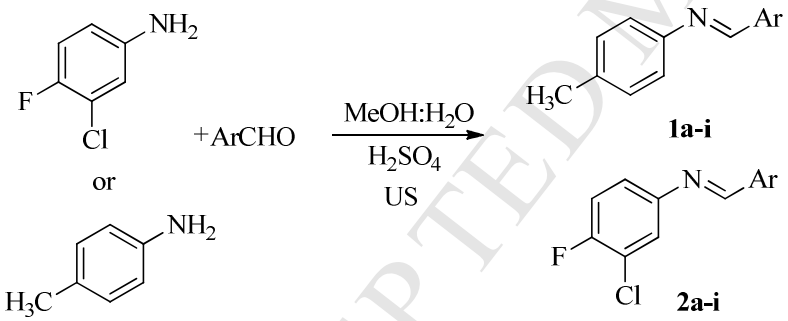
it is important to understand the mode of action and efficiency of these antioxidants. There are large numbers of natural and synthetic antioxidants which have been explored, and their antioxidant capacity has been assessed by different methods.

In this paper, we reported the synthesis of basic Schiff base derivatives using green chemistry techniques which are microwave irradiation and ultrasound sonication. Antimicrobial, antioxidant and urease inhibition studies were investigated and carried out molecular docking studies of newly synthesized compounds. At the same time, *In Silico* ADME prediction studies were calculated for all compounds.

2. Results and Discussion

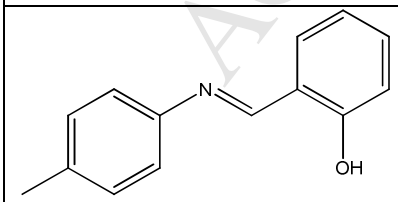
2.1. Chemistry

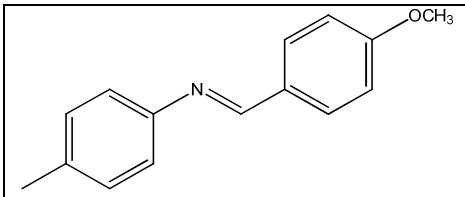
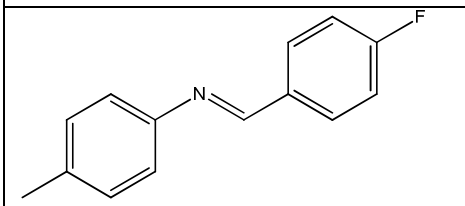
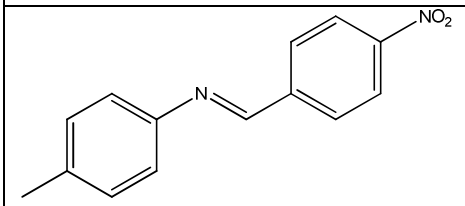
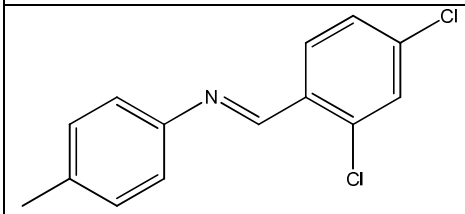
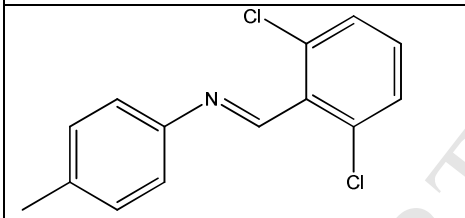
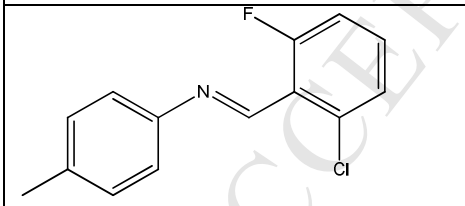
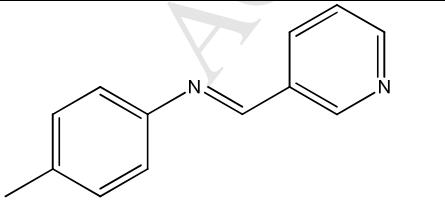
In the present study, the ecofriendly synthesis, urease enzyme inhibition, antioxidant activity screening and molecular docking studies of new simple Schiff base derivatives were contemplated. The structures of newly synthesized compounds were established on spectral data ^{1}H NMR, ^{13}C NMR, FT IR and MS spectra. Eighteen aromatic Schiff bases were synthesized (Table 1) and characterized.

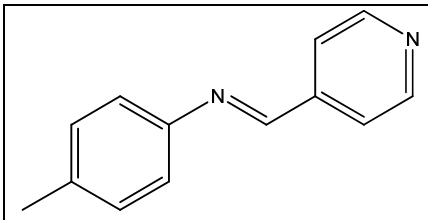
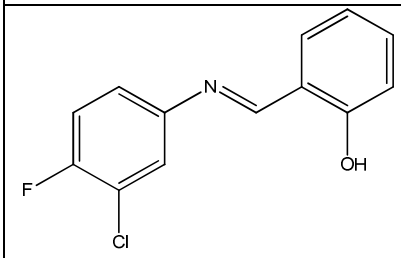
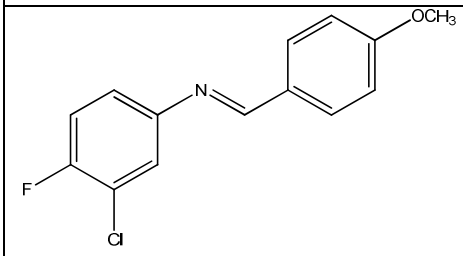
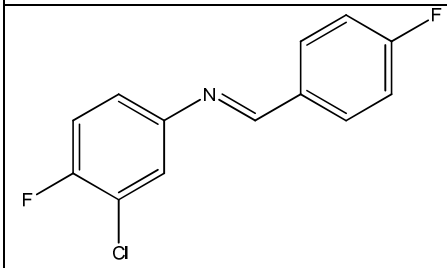
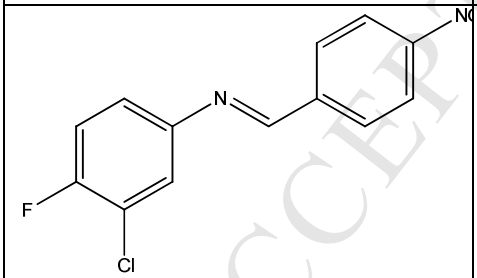
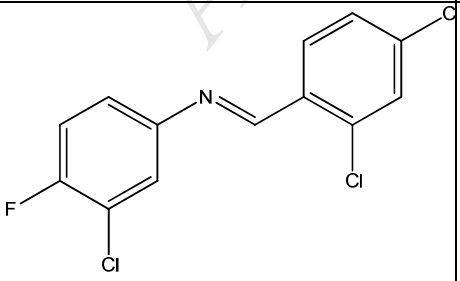


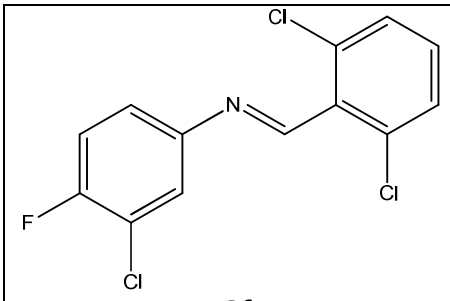
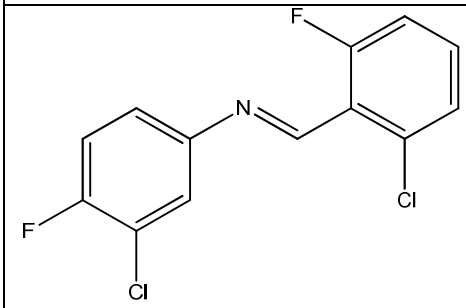
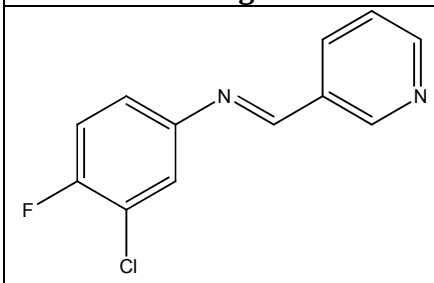
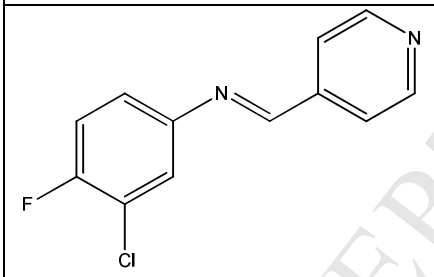
Scheme 1. Preparation of Schiff bases

Table 1. Molecular structure, melting point and yield of synthesized compounds

Compound	Melting Point (^{o}C)	Yield (%)
 **1a**	94-95	92[a] 97[b] 98[c]

 **1b**	96-97	87[a] 92[b] 96[c]
 **1c**	73-75	89[a] 93[b] 96[c]
 **1d**	124-126	81[a] 86[b] 89[c]
 **1e**	93-94	86[a] 90[b] 93[c]
 **1f**	74-76	84[a] 89[b] 90[c]
 **1g**	93-94	85[a] 90[b] 92[c]
 **1h**	60-61	90[a] 93[b] 96[c]

 **1i**	107-108	89[a] 92[b] 94[c]
 **2a**	137-139	87[a] 91[b] 94[c]
 **2b**	90-91	89[a] 92[b] 93[c]
 **2c**	79-81	87[a] 89[b] 92[c]
 **2d**	148-150	86[a] 88[b] 91[c]
 **2e**	139-141	85[a] 87[b] 91[c]

 **2f**	159-161	86[a] 89[b] 90[c]
 **2g**	95-96	84[a] 87[b] 91[c]
 **2h**	93-95	90[a] 93[b] 95[c]
 **2i**	80-82	89[a] 91[b] 94[c]

[a]Conventioanl Method, [b]Microwave Irradiation Method, [c]Ultrasound sonication method

Initially, to optimize the solvent effect for this reaction, 4-methyl aniline (1 mmol), 4-nitrobenzaldehyde (1 mmol), H_2SO_4 (3-4 drops) and for solvents four solvents were tried; ethanol, tetrahydrofurane, MeOH:H_2O (3:1) and acetonitrile in conventional method. The reaction completed in 3 hours (the progress of reaction was determined by TLC) and it was determined that the solvent was effective on the reaction (Table 2). Accordingly, methanol:water (3:1) mixture is the most effective solvent.

Table 2. Solvent effect on reaction yield

Compound No.	**Solvent**	**Time (h)**	**Yield (%)**

1d	Ethanol	3	67
1d	THF	3	72
1d	**Methanol:H_2O**	**3**	**81**
1d	Acetonitrile	3	56

After determination of the solvent, the time and yield of this reaction were examined by applying the green chemistry techniques to this reaction. For this purpose, we used two green chemistry techniques; Microwave irradiation and ultrasound sonication. To optimize reaction conditions in microwave method, the same reagents were chosen as model reaction and microwave (MW) irradiation was implemented at same power (100 W), different time and temperature (the progress of reaction was monitored by TLC) (Table 3). The highest reaction yield (86%) was obtain in this method 100 W, 100 ^{o}C and 3 min. In this optimization method, after 100 ^{o}C the reaction yield decreased from 86 to 82%.

Table 3. Optimization conditions of the reaction in Microwave Irradiation

Entry	Solvent	Power (W)	Temp. (^{o}C)	Time (min)	Yield (%)
1	MeOH:H_2O	100	50	5	76
2	MeOH:H_2O	100	75	3	81
3	**MeOH:H_2O**	**100**	**100**	**3**	**86**
4	MeOH:H_2O	100	100	3	82
5	MeOH:H_2O	100	150	5	78
6	MeOH:H_2O	100	200	3	73

The second ecofriendly method were applied ultrasound soication. Again we also optimized the conditions in US. By changing the parameters of temperature and time, we determined in which conditions the reaction takes place with the highest yield. As a result of the optimization study, it was determined that the maximum yield (89%) was 50 ^{o}C and 1.5 min (Table 4).

When all the results are examined, it was realized that the green chemistry techniques are superior to the conventional method. This reaction, which took place in 180 minutes in the conventional method, took place in 3 min in the microwave irradiated method and 1.5 min in the ultrasound sonication method. At the same time, when the reaction yield is evaluated in these three methods, the highest reaction yield was obtained by the ultrasonic method.

Table 4. Optimization condtions of the reaction in Ultrasound Sonication

Entry	Solvent	Temp (^{o}C)	Time (min)	Yield (%)
1	MeOH:H_2O	25	3	75

2	MeOH:H_2O	25	1.5	77
3	MeOH:H_2O	50	3	82
4	**MeOH:H_2O**	**50**	**1.5**	**89**
5	MeOH:H_2O	75	3	83
6	MeOH:H_2O	75	3	80

In the FT IR spectra of the azomethine groups in the Schiff bases, HC=N bands are observed at 1600-1650 cm^{-1} [26-31]. The ^{1}H NMR spectra of all synthesized compounds were recorded. The appearance of singlet proton of the azomethine group at 8.53-9.06 ppm in the ^{1}H NMR spectrum supported the structures of Schiff bases obtained. The protons of the hydroxyl group in the compounds **1a** and **2a** were observed at 13.23 and 12.60 ppm, respectively. Aromatic protons were resonated in the region 6.95-8.77 ppm. In ^{13}C NMR spectra of new Schiff bases, the carbon atom (HC=N) were observed between 151.56 and 165.04 ppm. Another spectroscopic evidence supporting the constructions of Schiff bases obtained is the [M], [M+1] and [M+2] ion signals observed in the mass spectra of the compounds. The compounds having imine group may exist as *E*/*Z* geometrical isomers about –C=N double bond and cis–trans amide conformers (Scheme 2). According to the literature, the compounds containing imine bond are present in higher percentage in dimethyl-D6 sulfoxide solution in the form of geometrical *E* isomer about –C=N double bond. The Z isomer can be stabilized in less polar solvents by an intramolecular hydrogen bond [32, 33]. In the present study, the spectral data were obtained in dimethyl-$_{d6}$ sulfoxide solution and no signal belonging to *Z* isomer was observed. On the other hand, the cis–trans conformers of *E* isomer were present in the dimethyl-d_6 sulfoxide solution of compounds 1a-i and 2a-i.

2.2. Antimicrobial activity screening

The synthesized compounds were screened for their antimicrobial activity using the disk diffusion method and the results obtained were showed in Table 5. Amphicilin and Gentamicin were used as standart antibiotic. All synthesized compounds showed antimicrobial activity against test microorganisms. Among these, **1d**, **1e** and **2a** exhibited good-modarate activity against *S. Aureus*, all compounds except **1b**, **1h** and **2g** showed good-modarate activity against *E. faecalis*.**1a**, **1d**, **2a** and **2d** exhibited good-modarate activity against *E. coli, P. aeruginosa, K. pneumoniae, A. haemolyticus*. Among the compounds, **2e** exhibited excellent activity against all test microorganisms with the MIC values varying 0.5–4 µg/mL.

Table 5. Screening forantimicrobial activity of newly synthesized compounds

Comp. No	Microorganisms and Minimal Inhibition Concentration (µg/mL)					
	Sa	Ef	Ec	Pa	Kp	Ah
1a	4	16	2	8	1	2
1b	8	32	4	16	8	8
1c	16	16	8	16	8	4
1d	2	8	2	2	0.5	4
1e	2	4	4	4	1	1
1f	8	8	16	8	16	8
1g	16	8	8	4	8	8
1h	32	32	16	8	16	16
1i	32	16	32	32	8	8
2a	2	4	2	4	2	2
2b	4	8	32	16	16	16
2c	8	16	16	32	16	32
2d	4	8	1	2	2	2
2e	1	4	0.5	1	0.5	1
2f	4	16	8	16	8	8
2g	8	32	16	16	16	8
2h	16	16	32	16	16	32
2i	32	16	32	8	16	16
Amp.	1.56	12.25	-	-	-	-
Gen.	-	-	0.78	1.56	0.39	0.78

Sa: *Staphylococcus aureus* ATCC 25923, Ef: *Enterococcus faecalis* ATCC 29212, Ec: *Escherichiacoli* ATCC 25922, Pa: *Pseudomonas aeruginosa* ATCC 27853, Kp: *Klebsiella pneumoniae* ATCC 13883, Ah: *Acinetobacter haemolyticus* ATCC 19002. Amp.: Amphicilin, Gen.: Gentamicin

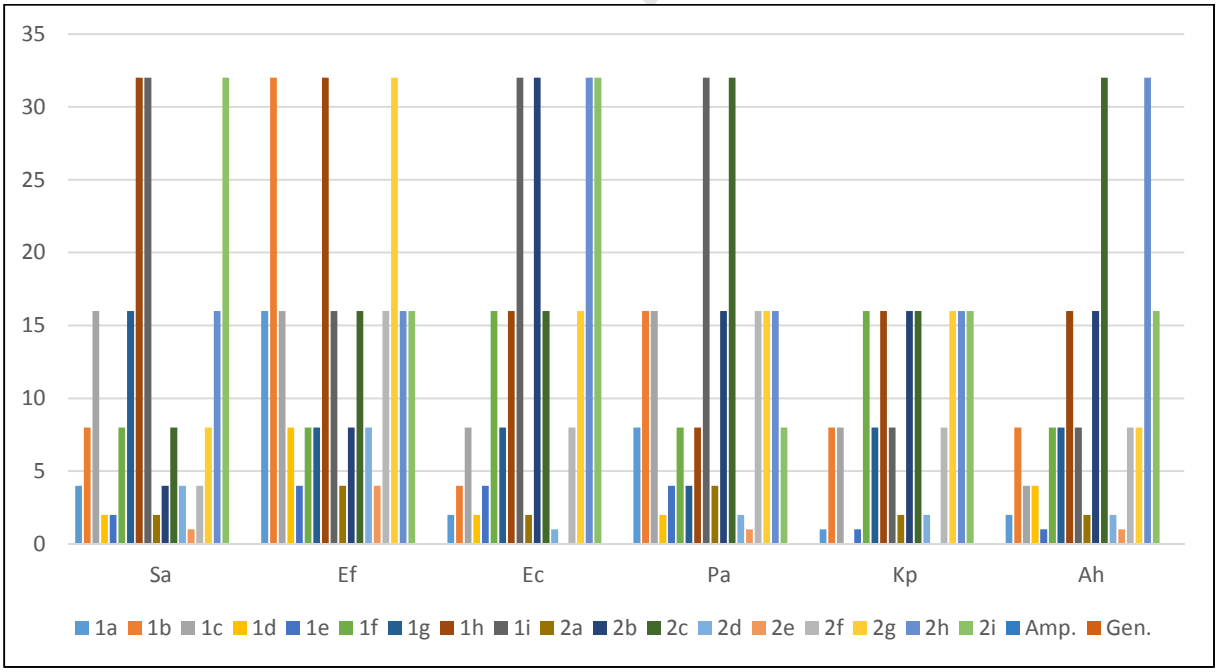


Figure 1. Antimicrobial activity of the synthesized compounds.

2.3. Antioxidant capacity

The newly synthesized Schiff base analogues of 4-methyl aniline and 3-chloro,4-fluoro aniline were evaluated for their antioxidant capacity by DPPH, CUPRAC and FRAP (Table 6). When the antioxidant results of the synthesized compounds were examined, it was determined that the Schiff base derivatives obtained from 3-chloro-4-fluoro aniline showed better activity. The compounds **2c**, **2f** and **2b** exhibited good-moderate activities with IC_{50} values 0,15±0,01, 0,19±0,00 and 0,22±0,00 µM/mL, respectively in DPPH assay much better than standart Trolox (IC_{50}=0,04±0,00). For FRAP and CUPRAC assays, the same compounds **2c**, **2f** and **2b**showed best activities with the values 4486,91±18,22, 4225,13±9,67 and 4155,32±14,78 (for FRAP), 8887,63±12,12, 8880,98±23,55 and 8156,13±15,65 (for CUPRAC). On the basis of the above observation, compounds having –F (fluorine), –2,6-Cl (chlororine) and $–OCH_3$ (methoxy) groups in the phenyl ring (**2c**, **2f** and **2b**) were found to be the most potent antioxidants. Schiff bases containing fluorine, methoxy and chlorine groups showed higher activity in this study, while it was expected that antioxidant activities of hydroxyl group-containing structures would be expected to be higher, and electron-withdrawing groups would have lower activity [34]. **2b** has a fluorine atom in *para* position on phenyl ring and **2c** has methoxy group in *para* position on phenyl ring. **2f** has chlorine atom in *ortho* and *para* position on phenyl ring. Other compounds also displayed remarkable antioxidant activity for FRAP, CUPRAC and DPPH.

2.4. Urease inhibition assay

Obtained compounds which are Schiff base derivatives were evaluated against Jack bean urease in vitro. At first, the compounds were screened at a concentration of 1 mM. Compounds with greater than 50% inhibition for full characterization were also selected. All of compounds were potent inhibitors of Jack bean urease. Thiourea was selected and used as the standart compound for the assay, its affinity is also comprising in Table 6. The synthesized compounds carry various functional groups in the phenyl ring came from aldehyde. All results were represented in Table 6. The compounds displayed excellent inhibitory activity in milimolar range. Second series (**2a-i**) of synthesized compunds showed better inhibition than first series (**1a-i**). Among the tested compounds **2c** was the most vigoroues having 0,19±0,02 IC_{50} value. Other potent compounds were **2f** and **2b** having IC_{50} values 0,21±0,02 and 0,25±0,02, respectively. The other compounds also exhibted better activity than thiourea used as standart against Jack bean urease with IC_{50} values ranging from 0,42±0,02 to 8,46±0,02. The anti-urease activities of the synthesized compounds vary due to

the functional groups present in both the amine and the aldehyde group [35]. The activity of schiff bases obtained using toluidine as amine was found to be less active than those elicited using 3-chloro-4-fluoroaniline. Furthermore, the functional groups in the para position of the aldehydes used are more active than the ortho and meta positions.

Table 6. Antioxidant capacity (AC) values and anti-urease activity of 18 synthesized novel compounds

Compound	FRAP (µmol TE/g)	DPPH IC_{50}	CUPRAC (µmol TE/g)	Urease Inh. (IC_{50})
1a	322.25±7.18	6.12±0.04	613.13±34.34	8.46±0.02
1b	407.33±1.12	4.91±0.03	1866.62±15.12	5.41±0.02
1c	369.37±6.21	5.29±0.06	1186.74±12.66	6.14±0.02
1d	433.51±4.68	4.83±0.01	2001.32±6.76	5.36±0.02
1e	447.91±7.27	4.61±0.02	2156.47±21.54	4.18±0.02
1f	359.55±5.18	5.86±0.03	986.85±8.19	7.12±0.02
1g	488.48±6.14	4.45±0.06	2574.63±5.10	1.24±0.02
1h	376.57±3.17	5.18±0.00	1425.74±9.98	5.53±0.02
1i	470.16±8.11	4.48±0.06	2345.45±5.43	3.13±0.02
2a	2728.62±10.14	1.01±0.04	6875.13±14.10	1.13±0.02
2b	4155.32±14.78	0.22±0.00	8156.13±15.65	0.25±0.02
2c	4486.91±18.22	0.15±0.01	8887.63±12.12	0.19±0.02
2d	3640.49±16.45	0.42±0.01	7645.13±7.44	0.52±0.02
2e	3858.64±12.25	0.36±0.02	7854.46±16.81	0.48±0.02
2f	4225.13±9.67	0.19±0.00	8880.98±23.55	0.21±0.02
2g	3448.52±13.43	0.51±0.03	7312.42±27.76	0.64±0.02
2h	3243.46±19.11	0.67±0.02	7125.69±5.34	0.89±0.02
2i	4015.71±11.18	0.29±0.00	8012.56±15.21	0.42±0.02
Trolox		0.04±0.00		
Thiourea				12.02±0.06

2.5. *In Silico* ADME prediction study

Table 7. Pharmacokinetic parameters important for good oral bioavailability of synthesized compound 1a-i and 2a-i.

Entry	% ABS	TPSA (A^2)	n-ROTB	MV	MW	miLog *P*	n-ON acceptors	n-OHNH donors	Nviolations	Drug Likeness Model Score
	-	-	-	-	≤500	≤5	<10	<5	≤1	
1a	97.75	32.59	2	203.29	211.26	3.86	2	1	0	-0.47
1b	101.54	21.60	3	220.82	225.29	3.98	2	0	0	-0.51
1c	104.74	12.36	2	200.21	213.25	4.08	1	0	0	-0.54
1d	88.92	58.19	3	218.61	240.26	3.88	4	0	0	-0.53
1e	104.74	12.36	2	222.34	264.15	5.20	1	0	1	-0.52
1f	104.74	12.36	2	222.34	264.15	5.18	1	0	1	-0.47
1g	104.74	12.36	2	213.74	247.70	4.67	1	0	0	-0.61
1h	100.28	25.26	2	191.12	196.25	2.68	2	0	0	-0.43
1i	100.28	25.26	2	191.12	196.25	2.63	2	0	0	-0.48
2a	97.75	32.59	2	205.20	249.67	4.18	2	1	0	-0.46

2b	101.54	21.60	3	222.72	263.70	4.30	2	0	0	-0.51
2c	104.74	12.36	2	202.11	251.66	4.40	1	0	0	-0.49
2d	88.92	58.19	3	220.51	278.67	4.20	4	0	0	-0.52
2e	104.74	12.36	2	224.25	302.56	5.52	1	0	1	-0.47
2f	104.74	12.36	2	224.25	302.56	5.50	1	0	1	-0.43
2g	104.74	12.36	2	215.65	286.11	4.99	1	0	0	-0.53
2h	100.28	25.26	2	193.02	234.66	3.00	2	0	0	-0.41
2i	100.28	25.26	2	193.02	234.66	2.95	2	0	0	-0.46
Thiourea		52.05	0	63.07	76.12	-0.46	2	4	0	-3.78

% ABS: percentage absorption, TPSA: topological polar surface area, n-ROTB: number of rotatable bonds, MV: molecular volume, MW: molecular weight, miLog P: logarithm of partition coefficient of compound between n-octanol and water, n-ON acceptors: number of hydrogen bond acceptors, n-OHNH donors: number of hydrogen bonds donors.

Many biologically active compounds fail to achieve the clinic because of their inadequate absorption, distribution, metabolism, and elimination (ADME) parameters. For this reason, a computational study of synthesized compounds 1a-i and 2a-i werestudied for evaluation of ADME properties and value obtained is represent in Table 7. Polar surface area (TPSA), number of rotatable bonds (n-ROTB), molecular volume (MV), and Lipinski's rule of five were calculated using Molinspiration online property calculation toolkit [36]. When the results are examined, all the synthesized compounds showed excellent % absorption. Moreover, none of the compounds trespass on Lipinski's rule of five and thus showing potential utility of series for improving the compound with good drug like properties. A compound probably likely to be developed as an orally active drug candidate should demonstrate no more than one violation of the following four criteria: logP (octanol-water partition coefficient) ≤5, molecular weight ≤500, number of hydrogen bond acceptors ≤10 and number of hydrogen bond donors ≤5 [37]. All the synthesized compounds complied the standart for orally active drug and therefore, these compounds can be further advanced as oral drug candidates. The results of this in silico ADME prediction analysis propose that the obtained compounds follow the computational assessment and thus indicate a pharmacologically active framework that should be considered on progressing further potential hits. Drug-likeness model score (a combined effect of physico-chemical properties, pharmacokinetics and pharmacodynamics of a compound and is displayed by a numerical value) was calculated by Molinspiration software (http://www.molinspiration.com) for the 18 synthesized compounds. The best drug-likeness score was found to be -0.41 and -0.43 for compounds **2h** and **2f**, respectively.

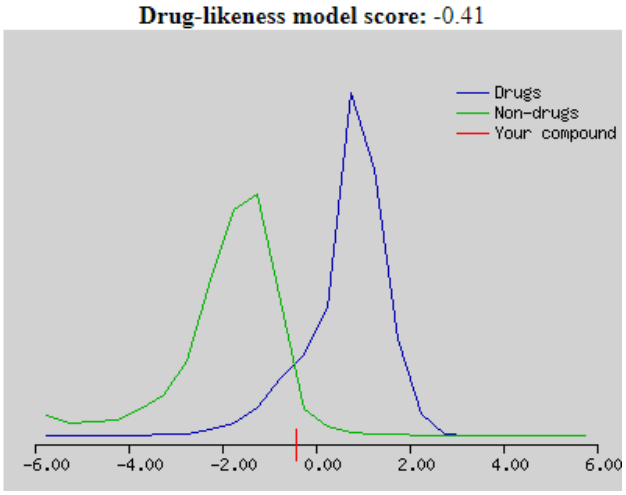


Figure 2. Drug likeness score of compound **2h**

2.6. Molecular docking studies

According to the X-ray crystallographic structure of Urease (PDB ID:1E9Y),main binding site has been determined around Ni atoms including Ni3001, Ni3002, His221, Asp362, Ala365 [www.rcsb.org]. It has been reported that acetohydroxamic acid interacts with active site in the gorge concordantly binding site [38]. Analyses of binding modes of the acetohydroxamic acid indicate that the carbonyl, amino and hydroxyl groups are in H-bonds with His221, Asp362, Ala365 in binding cavity [39]. The formation of hydrogen bond between the nitrogen atom in the imine group of compound **2c** and Cys321 showed binding to the active site. The pyridine of compound **2i** has a position in the gorge to interact with His221 by doing a hydrogen bond. **2c, 2f** and **2i** interacted with nickel, especially Ni3001. Docking studies were performed for the most active compounds **2c** and **2f** interaction modes with enzyme active sites were determined. Docking studies revealed that there is a strong interaction between the active sites of Helicobacter Pylori Urease enzyme and these compounds. Ideally, a Helicobacter Pylori Urease inhibitor is expected to effectively interact with these sites (Figure 3-4) (Table 8). The binding mode was produced by AutoDock and showed by Maestro (Figure 5).

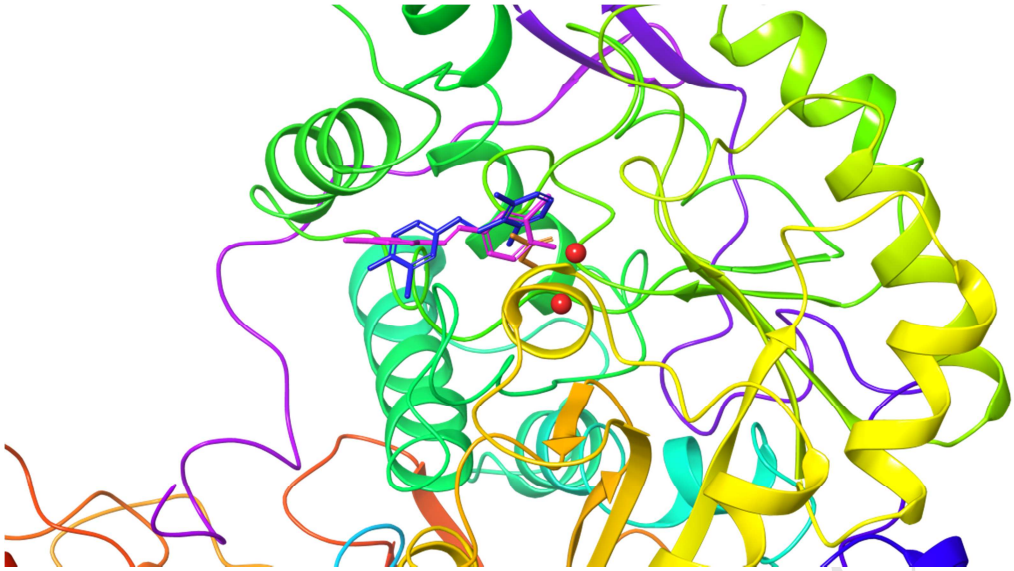


Figure 3. Compounds **2c** (pink) and **2f** (blue) are presented into the Helicobacter pylori urease (PDB ID: 1E9Y) binding cavity. Acetohydroxamic acid (orange), Ni^{+2} atoms (NI3001, NI3002) are shown as sphere (red). For clarity, receptor residues are shown as cartoon.

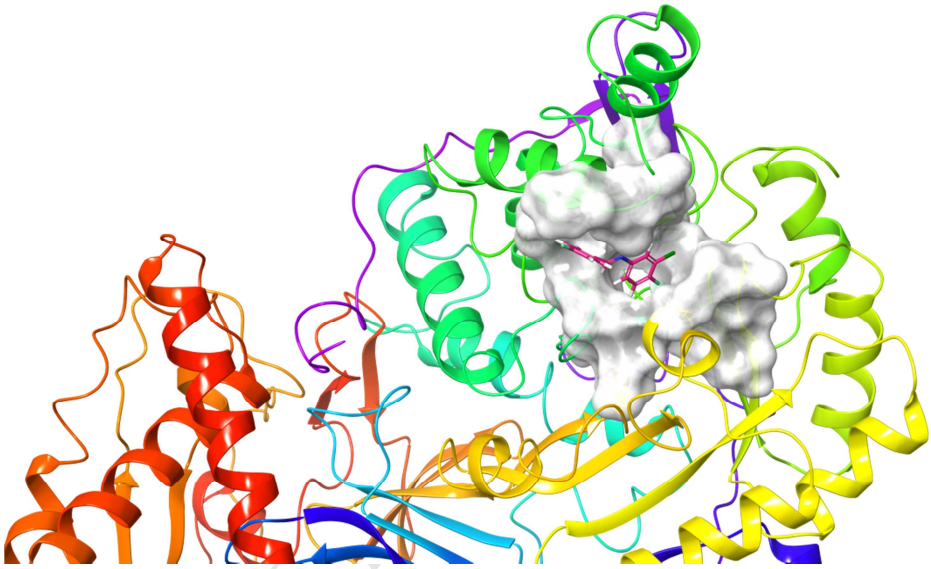


Figure 4. Compound 2c (pink sticks) in the binding site (molecular surface rendered) of urease

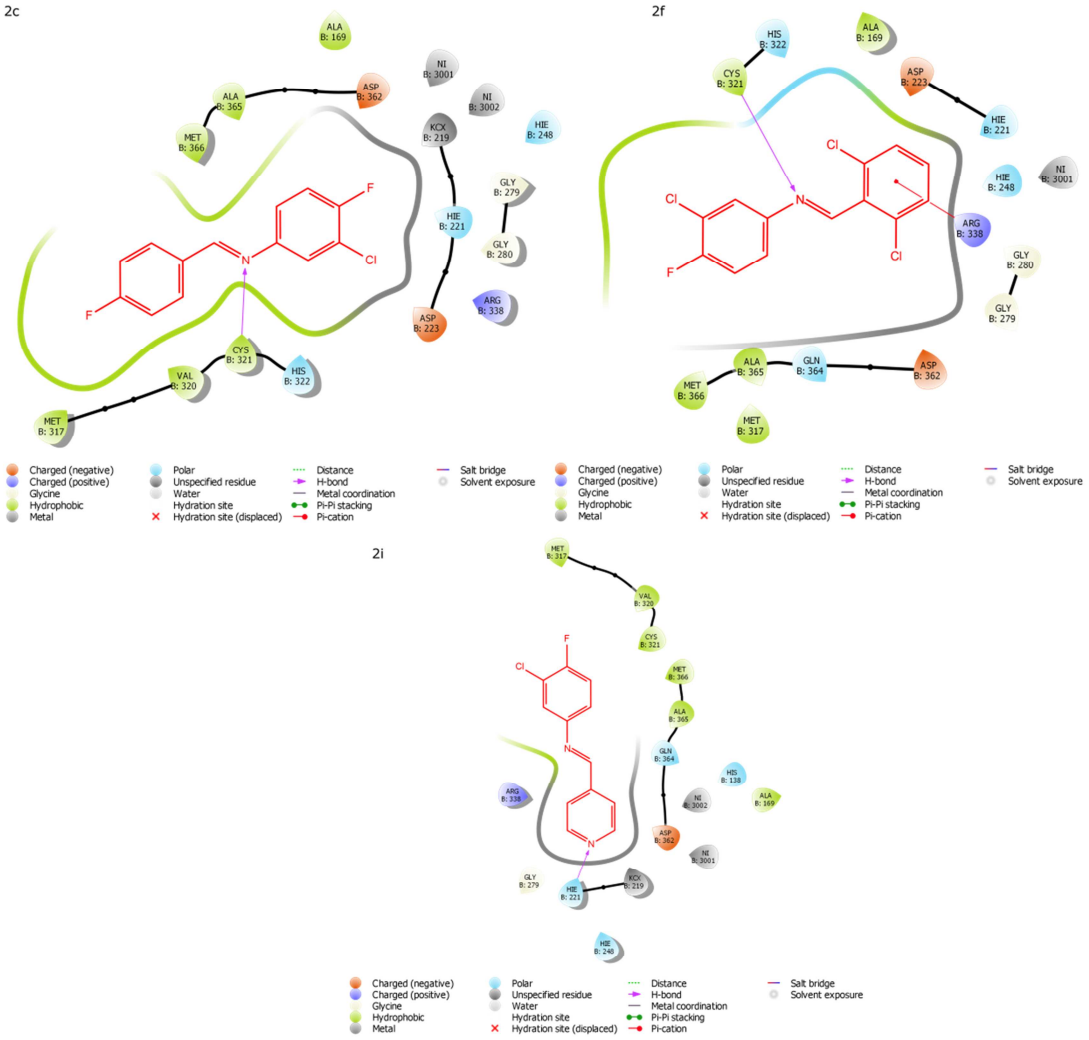


Figure 5. 2D interaction diagram from Glide for some compounds

Table 8. Molecular docking binding scores of some compounds**,** within the Helicobacter pylori urease (PDB ID: 1E9Y) active site. Residues participating in hydrogen bonds and Pi-cation contacts with the compounds are shown.

Comp.	Estimated Free Energy of Binding (kcal/mol)	Estimated Inhibition Constant, Ki, Temp. = 298.15 K	Residue	
			H-bond	Pi-cation
2c	-5.93	45.33 uM	Cys321	-
2f	-6.02	38.49 uM	Cys321	Arg338
2i	-5.77	58.96 uM	His221	-

uM: micromolar

3. Experimental

All the chemicals were purchased from *FlukaChemie AG Buchs* (Switzerland) and used without further purification. Melting points of the synthesized compounds were determined in open capillaries on a Büchi B-540 melting point apparatus and are uncorrected. Reactions were monitored by thin-layer chromatography (TLC) on silica gel 60 F254 aluminium sheets. The mobile phase was ethyl acetate: diethyl ether (1:1), and detection was made using UV light. FT-IR spectra were recorded using a *Perkin Elmer* 1600 series FTIR spectrometer. ^{1}H NMR and ^{13}C NMR spectra were registered in DMSO-d_6 on a *BRUKER AVENE II* 400 MHz NMR Spectrometer (400.13 MHz for ^{1}H and 100.62 MHz for ^{13}C). The chemical shifts are given in ppm relative to Me_4Si as an internal reference, *J* values are given in Hz. Microwave and ultrasound mediated syntheses were carried out using monomod CEM-Discover microwave apparatus and BandelinSonorex Super RK102H ultrasonic bath, respectively. The Mass spectra were obtained on a *Quattro LC-MS* (70 eV) Instrument. Compound **1a** and **2a** are known [40, 41]. In literature, **1a** and **2a** was synthesized by microwave irradiation and obtained 97%, 79% yield in 1.5 minutes and 6 minutes, respectively (melting points of compounds **1a** and **2a** are 93-94 and 138^{o}C, respectively).

3.1. General method for the synthesis of compounds 1a-i and 2a-i

Method 1. The amine compounds (10 mmol) (4-methyl aniline or 3-chloro-4-fluoro aniline) was added in a solution of suitably substituted benzaldehyde (10 mmol) in methanol:H_2O (3:1) (10 ml). And conc. sulphuric acid was dropped in catalytic amount to the solution. The reaction mixture was refluxed for 3-5 hours (The reaction progress was followed by TLC). After evaporating the solvent under reduced pressure, a solid was obtained. The obtained compound was recrystallized from an appropriate solvent to afford the desired product.

Method 2. The solution of suitably substituted benzaldehyde (10 mmol) and amine compound (10 mmol) in methanol:H_2O (3:1) (10 mL) and conc. H_2SO_4 (3-4 drops) was irradiated in closed vessels at 100 ^{o}C, 100 W, for 20 min. (The reaction progress was followed by TLC). After evaporating the solvent under reduced pressure, a solid was obtained. The obtained compound was recrystallized from an appropriate solvent to afford the desired product.

Method 3. The solution of suitably substituted benzaldehyde (10 mmol) and amine compound (10 mmol) in methanol:H_2O (3:1) (10 mL) and conc. H_2SO_4 (3-4 drops) was sonicated at 50 ^{o}C for 20 min. (The reaction progress was followed by TLC). After

evaporating the solvent under reduced pressure, a solid was obtained. The obtained compound was recrystallized from an appropriate solvent to afford the desired product.

(*E*)-2-((p-tolylimino)methyl)phenol (1a)

FT-IR (υ_{max},cm^{-1}): 3052 (ar-CH), 1613 (C=N). ^{1}H NMR (DMSO-d_6, δppm): 2.35 (s, 3H,CH_3), 6.95-7.00 (m, 2H, arH), 7.28 (d, 2H, *J*=8.0 Hz, arH), 7.34 (d, 2H, *J*=8.0 Hz, arH), 7.39-7.43 (m, 1H, arH), 7.64 (d, 1H, *J*=8.0 Hz, arH), 8.96 (s, 1H, CH), 13.23 (s, 1H, OH). ^{13}C NMR (DMSO-d_6, δppm): 21.08 (CH_3), arC: [117.02 (CH), 119.55 (CH), 119.78, 121.69 (2CH), 130.42 (2CH), 132.94 (CH), 133.54 (CH), 136.99, 145.86, 163.03], 160.74 (CH). EI MS *m/z* (%): 212.25 ($[M+1]^+$, 100).

3.1.1. (*E*)-*N*-(4-methoxybenzylidene)-4-methylaniline (1b)

FT-IR (υ_{max},cm^{-1}): 3007 (ar-CH), 1621 (C=N), 1217 (C-O). ^{1}H NMR (DMSO-d_6, δppm): 2.34 (s, 3H,CH_3), 3.84 (s, 3H, OCH_3), 7.07 (d, 2H, *J*=8.0 Hz, arH), 7.18 (q, 4H, *J*=16.0 Hz, arH), 7.88 (d, 2H, *J*=8.0 Hz, arH), 8.53 (s, 1H, CH). ^{13}C NMR (DMSO-d_6, δppm): 21.02 (CH_3), 55.83 (OCH_3),arC: [114.70 (2CH), 121.31 (2CH), 129.53, 130.11 (2CH), 130.78 (2CH), 135.30, 149.58, 162.24], 159.39 (CH). EI MS *m/z* (%):226.18 ($[M+1]^+$, 100).

3.1.2. (*E*)-*N*-(4-fluorobenzylidene)-4-methylaniline (1c)

FT-IR (υ_{max},cm^{-1}): 3027 (ar-CH), 1625 (C=N). ^{1}H NMR (DMSO-d_6, δppm): 2.33 (s, 3H,CH_3), 7.21 (q, 4H, *J*=24.0 Hz, arH), 7.35 (t, 2H, *J*=16.0 Hz, arH), 8.00 (q, 2H, *J*=16.0 Hz, arH), 8.62 (s, 1H, CH).^{13}C NMR (DMSO-d_6, δppm): 21.03 (CH_3), arC: [116.22 (CH), 116.44 (CH), 121.42 (2CH), 130.15 (2CH), 131.25and 131.34 (d, *J*=9.0 Hz, 2CH), 133.32, 135.87, 149.09, 163.12 and 165.59 (d_{C-F}, *J*=247.0 Hz)], 158.86 (CH). EI MS *m/z* (%): 214.24 ($[M+1]^+$, 100).

3.1.3. (*E*)-4-methyl-*N*-(4-nitrobenzylidene)aniline (1d)

FT-IR (υ_{max},cm^{-1}): 3084 (ar-CH), 1623 (C=N), 1505 and 1337 (-NO_2). ^{1}H NMR (DMSO-d_6, δppm): 2.34 (s, 3H,CH_3), 7.27 (t, 4H, *J*=16.0 Hz, arH), 8.17 (d, 2H, *J*=8.0 Hz, arH), 8.35 (d, 2H, *J*=8.0 Hz, arH), 8.81 (s, 1H, CH). ^{13}C NMR (DMSO-d_6, δppm): 21.10 (CH_3), arC: [121.78 (2CH), 124.46 (2CH), 129.96 (2CH), 130.28 (2CH), 137.05, 142.17, 148.36, 149.18], 158.18 (CH). EI MS *m/z* (%): 241.28 ($[M+1]^+$, 100).

3.1.4. (*E*)-*N*-(2,4-dichlorobenzylidene)-4-methylaniline (1e)

FT-IR (υ_{max},cm^{-1}): 3064 (ar-CH), 1617 (C=N). ^{1}H NMR (DMSO-d_6, δppm): 2.34 (s, 3H,CH_3), 7.25 (s, 4H, arH), 7.57 (d, 1H, *J*=8.0 Hz, arH), 7.78 (s, 1H, arH), 8.16 (d, 1H, *J*=8.0 Hz, arH), 8.83 (s, 1H, CH). ^{13}C NMR (DMSO-d_6, δppm): 21.08 (CH_3), arC: [121.60 (2CH), 128.50

(CH), 129.68 (CH), 130.00 (CH), 130.02 (CH), 130.52 (2CH), 132.25, 136.10, 136.85, 136.98, 148.67], 154.70 (CH). EI MS *m/z* (%): 264.14 ($[M]^+$,100), 266.03 ($[M+2]^+$, 76).

3.1.5. (*E*)-*N*-(2,6-dichlorobenzylidene)-4-methylaniline (1f)

FT-IR (υ_{max},cm^{-1}): 3083 (ar-CH), 1632 (C=N). ^{1}H NMR (DMSO-d_6, δppm): 2.33 (s, 3H,CH_3), 7.18 (d, 2H, *J*=8.0 Hz, arH), 7.24 (d, 2H, *J*=8.0 Hz, arH), 7.48 (d, 1H, *J*=4.0 Hz, arH), 7.57 (d, 2H, *J*=8.0 Hz, arH),8.69 (s, 1H, CH). ^{13}C NMR (DMSO-d_6, δppm): 21.06 (CH_3), arC: [121.20 (2CH), 129.55 (2CH), 130.26 (2CH), 132.11 (CH), 132.83, 134.45, 136.78, 148.73], 156.07 (CH). EI MS *m/z* (%):265.12 ($[M+1]^+$, 100).

3.1.6. (*E*)-*N*-(2-chloro-6-fluorobenzylidene)-4-methylaniline (1g)

FT-IR (υ_{max},cm^{-1}): 3084 (ar-CH), 1620 (C=N)). ^{1}H NMR (DMSO-d_6, δppm): 2.33 (s, 3H,CH_3), 7.18 (d, 2H, *J*=8.0 Hz, arH), 7.25 (d, 2H, *J*=8.0 Hz, arH), 7.36 (t, 1H, *J*=16.0 Hz, arH), 7.45 (d, 1H, *J*=8.0 Hz, arH), 7.55 (d, 1H, *J*=4.0 Hz, arH),8.71 (s, 1H, CH). ^{13}C NMR (DMSO-d_6, δppm): 21.04 (CH_3), arC: [116.04and 116.26 (d, *J*=22.0 Hz, CH), 121.25 (2CH), 122.70 and 122.83 (d, *J*=13.0 Hz,), 126.62 and 126.66 (d, *J*=4.0 Hz, CH), 130.27 (2CH), 133.06 and 133.16 (d, *J*=10.0 Hz, CH), 135.16 and 135.20 (d, *J*=4.0 Hz), 136.71, 149.27, 159.95 and 162.51 (d_{C-F}, *J*=256.0 Hz), 145.86, 163.03], 151.56 (CH). EI MS *m/z* (%):247.04 ($[M]^+$, 100), 249.10 ($[M+2]^+$, 69).

3.1.7. (*E*)-4-methyl-*N*-(pyridin-3-ylmethylene)aniline (1h)

FT-IR (υ_{max},cm^{-1}): 3017 (ar-CH), 1625 (C=N). ^{1}H NMR (DMSO-d_6, δppm): 2.33 (s, 3H,CH_3), 7.24 (s, 4H, arH), 7.54 (q, 1H, *J*=12.0 Hz, arH), 8.31 (d, 1H, *J*=8.0 Hz, arH), 8.69-8.72 (m, 2H, arH), 9.05 (s, 1H, CH). ^{13}C NMR (DMSO-d_6, δppm): 21.06 (CH_3), arC: [121.53 (2CH), 124.50 (CH), 130.21 (2CH), 132.13, 135.32 (CH), 136.38, 148.86, 150.78 (CH), 152.24 (CH)], 157.93 (CH).EI MS *m/z* (%): 219.23 ($[M+Na]^+$, 100).

3.1.8. (*E*)-4-methyl-*N*-(pyridin-4-ylmethylene)aniline (1i)

FT-IR (υ_{max},cm^{-1}): 3028 (ar-CH), 1622 (C=N). ^{1}H NMR (DMSO-d_6, δppm): 2.34 (s, 3H,CH_3), 7.27 (s, 4H, arH), 7.84 (d, 2H, *J*=4.0 Hz, arH), 8.70 (s, 1H,CH), 8.74 (d, 2H, *J*=4.0 Hz, arH). ^{13}C NMR (DMSO-d_6, δppm): 21.09 (CH_3), arC: [121.72 (2CH), 122.58 (2CH), 130.27 (2CH), 137.02, 143.10, 148.33, 150.90 (2CH)], 158.59 (CH).EI MS *m/z* (%): 220.19 ($[M+1+Na]^+$, 100).

3.1.9. (*E*)-2-(((3-chloro-4-fluorophenyl)imino)methyl)phenol (2a)

FT-IR (υ_{max},cm^{-1}): 3089 (ar-CH), 1614 (C=N). ^{1}H NMR (DMSO-d_6, δppm): 6.97-7.02 (m, 2H, arH), 7.42-7.52 (m, 3H, arH), 7.66 (d, 1H, *J*=4.0 Hz, arH), 7.73 (d, 1H, *J*=8.0 Hz, arH), 8.96 (s, 1H, CH), 12.60 (s, 1H, OH).^{13}C NMR (DMSO-d_6, δppm): arC: [117.12 (CH), 118.03 (CH), 119.68, 120.60 and 120.70 (d, J=10.0 Hz), 122.97 and 123.04 (d, J=7.0 Hz, CH), 123.45 (CH), 133.07 (CH), 134.11 (CH), 146.07, 155.17 and 157.62 (d_{C-F}, J=245.0 Hz), 160.59], 165.04 (CH). EI MS *m/z* (%): 128.47 (100), 250.55 ($[M+1]^+$, 94).

3.1.10. (*E*)-3-chloro-4-fluoro-*N*-(4-methoxybenzylidene)aniline (2b)

FT-IR (υ_{max},cm^{-1}): 3066 (ar-CH), 1624 (C=N). ^{1}H NMR (DMSO-d_6, δppm): 3.84 (s, 3H, OCH_3), 7.08 (d, 2H, *J*=8.0 Hz, arH), 7.25-7.28 (m, 1H, arH), 7.43 (t, 1H, *J*=16.0 Hz, arH), 7.49 (d, 1H, *J*=8.0 Hz, arH), 7.88 (d, 2H, *J*=8.0 Hz, arH), 8.57 (s, 1H, CH).^{13}C NMR (DMSO-d_6, δppm): 55.90 (OCH_3), arC: [114.78 (2CH), 117.76 (CH), 122.80 (CH), 129.03, 131.18 (2CH), 132.27 (CH), 146.77, 149.36, 154.50 and 156.93 (d_{C-F}, J=243.0 Hz), 162.66], 161.86 (CH). EI MS *m/z* (%): 156.29 (52), 252.66(45), ($[M+1]^+$, 58), 254.19 ($[M+Na]^+$, 100).

3.1.11. (*E*)-3-chloro-4-fluoro-*N*-(4-fluorobenzylidene)aniline (2c)

FT-IR (υ_{max},cm^{-1}): 3082 (ar-CH), 1629 (C=N). ^{1}H NMR (DMSO-d_6, δppm): 7.29-7.32 (m, 1H, arH),7.37 (t, 2H, *J*=16.0 Hz, arH), 7.46 (t, 1H, *J*=16.0 Hz, arH), 7.53 (d, 1H, *J*=4.0 Hz, arH), 7.99 (t, 2H, *J*=12.0 Hz, arH), 8.66 (s, 1H, CH).^{13}C NMR (DMSO-d_6, δppm):arC: [116.33 and 116.55 (d, *J*=22.0 Hz, 2CH), 117.83 (CH), 122.62 and 122.70 (d, *J*=8.0 Hz, CH), 122.94, 131.64 and 131.73 (d, *J*=9.0 Hz, 2CH), 132.83, 146.75, 148.84 and 144.88 (d, *J*=4.0 Hz), 154.78 and 157.21 (d_{C-F}, J=243.0 Hz), 163.41 and 165.90 (d_{C-F}, J=249.0 Hz)], 161.43 (CH). EI MS *m/z* (%): 252.64 ($[M+1]^+$, 100), 254.58 (36).

3.1.12. (*E*)-3-chloro-4-fluoro-*N*-(4-nitrobenzylidene)aniline (2d)

FT-IR (υ_{max},cm^{-1}): 3116 (ar-CH), 1627 (C=N), 1514 and 1347 (-NO_2). ^{1}H NMR (DMSO-d_6, δppm): 7.38-7.42 (m, 1H, arH),7.51 (t, 1H, *J*=16.0 Hz, arH), 7.65 (d, 1H, *J*=4.0 Hz, arH), 8.18 (d, 2H, *J*=8.0 Hz, arH), 8.38 (d, 2H, *J*=8.0 Hz, arH), 8.85 (s, 1H, CH). ^{13}C NMR (DMSO-d_6, δppm):arC: [117.77 and 117.99 (d, *J*=22.0 Hz, CH), 120.51 and 120.70 (d, *J*=19.0 Hz), 123.24 (CH), 124.51 (2CH), 130.26 (2CH), 131.10 (CH), 141.58, 148.13 and 148.16 (d, *J*=3.0 Hz), 149.51, 152.28 and 157.73 (d_{C-F}, J=245.0 Hz)], 160.96 (CH).EI MS *m/z* (%): 111.45 (41), 128.15 (53), 233.60 (100), 250 (50), 279.62 ($[M+1]^+$, 82).

3.1.13. (*E*)-3-chloro-*N*-(2,4-dichlorobenzylidene)-4-fluoroaniline (2e)

FT-IR (υ_{max},cm^{-1}): 3090 (ar-CH), 1615 (C=N). ^{1}H NMR (DMSO-d_6, δppm): 7.33-7.37 (m, 1H, arH),7.48 (t, 1H, *J*=16.0 Hz, arH), 7.58 (d, 2H, *J*=8.0 Hz, arH), 7.78 (s, 1H, arH), 8.13 (d,

1H, *J*=8.0 Hz, arH), 8.83 (s, 1H, CH). ^{13}C NMR (DMSO-d_6, δppm):arC: [117.77 and 117.98 (d, *J*=21.0 Hz, CH), 122.74 and 122.81 (d, *J*=7.0 Hz, CH), 123.31 (CH), 128.53 (CH), 130.09 (CH), 130.24 (CH), 131.88, 136.49, 137.54, 148.40, 155.19 and 157.63 (d_{C-F}, J=244.0 Hz)], 157.38 (CH). EI MS *m/z* (%): 302.54($[M]^+$, 99), 304.35 ($[M+2]^+$, 100).

3.1.14. (*E*)-3-chloro-*N*-(2,6-dichlorobenzylidene)-4-fluoroaniline (2f)

FT-IR (υ_{max},cm^{-1}): 3081 (ar-CH), 1635 (C=N). ^{1}H NMR (DMSO-d_6, δppm): 7.33 (s, 1H, arH),7.54 (s, 3H, arH), 7.60 (s, 2H, arH), 8.79 (s, 1H, CH). ^{13}C NMR (DMSO-d_6, δppm):arC: [117.88 and 118.10 (d, *J*=22.0 Hz, CH), 122.46 (CH), 123.00 (CH), 129.66 (2CH), 132.34, 132.59 (CH), 148.17, 154.54 (2C), 155.32 and 157.77 (d_{C-F}, J=245.0 Hz)], 158.82 (CH). EI MS *m/z* (%): 264.38 (100), 302.55($[M]^+$, 80), 304.24 ($[M+2]^+$, 76).

3.1.15. (*E*)-3-chloro-*N*-(2-chloro-6-fluorobenzylidene)-4-fluoroaniline (2g)

FT-IR (υ_{max},cm^{-1}): 3091 (ar-CH), 1629 (C=N). ^{1}H NMR (DMSO-d_6, δppm): 7.30-7.34 (m, 1H, arH), 7.39 (t, 1H, *J*=16.0 Hz, arH), 7.48 (d, 2H, *J*=8.0 Hz, arH), 7.55-7.60 (m, 2H, arH), 8.75 (s, 1H, CH). ^{13}C NMR (DMSO-d_6, δppm):arC: [116.12 and 116.34 (d, *J*=22.0 Hz, CH), 117.81 and 118.03 (d, *J*=22.0 Hz, CH),120.71, 122.43 and 122.51 (d, *J*=8.0 Hz, CH),123.00 (CH), 126.77 and 126.81 (d, *J*=4.0 Hz, CH), 133.66 and 133.77 (d, *J*=11.0 Hz, CH), 135.34, 148.84, 155.21 and 157.66 (d_{C-F}, J=245.0 Hz), 160.06 and 162.63 (d_{C-F}, J=257.0 Hz)], 156.22 (CH). EI MS *m/z* (%):286.09 ($[M]^+$, 100), 288.02 ($[M+2]^+$, 62).

3.1.16. (*E*)-3-chloro-4-fluoro-*N*-(pyridin-3-ylmethylene)aniline (2h)

FT-IR (υ_{max},cm^{-1}): 3034 (ar-CH), 1629 (C=N). ^{1}H NMR (DMSO-d_6, δppm): 7.34-7.37 (m, 1H, arH),7.49 (t, 1H, *J*=16.0 Hz, arH), 7.55-7.60 (m, 2H, arH), 8.31 (d, 1H, *J*=8.0 Hz, arH), 8.72 (d, 1H, *J*=4.0 Hz, arH), 8.76 (s, 1H, arH), 9.06 (s, 1H, CH). ^{13}C NMR (DMSO-d_6, δppm):arC: [117.70 and 117.91 (d, *J*=21.0 Hz, CH), 120.41 and 120.59 (d, *J*=18.0 Hz),122.80 and 122.88 (d, *J*=8.0 Hz, CH), 123.07 (CH), 124.56 (CH), 131.68, 135.59 (CH), 148.62 and 148.65 (d, *J*=3.0 Hz), 151.04 (CH), 152.71 (CH), 155.01 and 157.45 (d_{C-F}, J=244.0 Hz)], 160.69 (CH). EI MS *m/z* (%): 235.62 ($[M+1]^+$, 100).

3.1.17. (*E*)-3-chloro-4-fluoro-*N*-(pyridin-4-ylmethylene)aniline (2i)

FT-IR (υ_{max},cm^{-1}): 3100 (ar-CH), 1626 (C=N). ^{1}H NMR (DMSO-d_6, δppm): 7.37-7.41 (m, 1H, arH), 7.49-7.53 (m, 1H, arH), 7.64 (d, 1H, *J*=8.0 Hz, arH), 7.84 (d, 2H, *J*=4.0 Hz, arH), 8.74 (s, 1H, CH), 8.77 (d, 2H, *J*=8.0 Hz, arH), ^{13}C NMR (DMSO-d_6, δppm): arC: [117.78 and 117.99 (d, *J*=21.0 Hz, CH), 122.61 (CH), 122.74 (2CH), 123.24 (CH),135.09, 142.59, 148.11

and 148.14 (d, *J*=3.0 Hz), 151.00 (2CH), 155.29 and 157.73 (d_{C-F}, J=244.0 Hz)], 161.24 (CH). EI MS *m/z* (%): 235.58 ($[M+1]^+$, 100).

3.2. Antimicrobial activity

The test microorganisms which are gram-positive and gram-negative as follows: Sa: *Staphylococcus aureus* ATCC 25923, Ef: *Enterococcus faecalis* ATCC 29212, Ec: *Escherichiacoli* ATCC 25922, Pa: *Pseudomonas aeruginosa* ATCC 27853, Kp: *Klebsiella pneumoniae* ATCC 13883, Ah: *Acinetobacterhaemolyticus* ATCC 19002 were supplied from the Hifzissihha Institute of Refik Saydam (Ankara, Turkey) and were. Amphicilin and Gentamicin was used as standart compounds. For antimicrobial activity test, the obtained compounds were dissolved in ethanol to prepare extract stock solution of 10.000 microgram/milliliter (µg/mL). The antimicrobial activities of the compounds were quantitatively tested in the corresponding broth media by using double micro-dilution and the minimal inhibitory concentration (MIC) values (µg/mL) were detected. Mueller-Hinton broth (MH) (Difco, Detroit, MI) at pH.7.3 and buffered Yeast Nitrogen Base (Difco, Detroit, MI) at pH 7.0 were used for the assay. The micro dilution test plates were incubated for 18–24 h at 35 ^{o}C [42]. All results were presented in Table 5.

3.3. Antioxidant capacity

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity: A 100 mL: chemical solution was mixed with 1 mL of freshly prepared methanolic DPPH solution and then the reaction mixture was incubated for 30 min at room temperature in the dark and was measured at 520 nm as described by Blois [43]. The activity was given IC_{50} values.

FRAP (the ferric reducing ability of plasma): To 100 µL of each sample was added 2.9 mL freshly prepared FRAP reagent containing 300 mmol/L acetate buffer (pH 3.6), 10 mmol/L TPTZ (2,4,6-tripyridyle-s- triazine) and 20 mmol/L $FeCl.6H_2O$ in proportions of 10:1:1 (v/v/v). The mixture was incubated for 30 min at 37 ^{o}C and measured at 593 nm [44]. The values were expressed as µmol of Trolox/g.

CUPRAC (cupric ion reducing antioxidant capacity): 100 µL of each chemical solution was mixed with 900 µL bi-distilled water, 1 mL acetate buffer solution (1 mmol/L, pH: 7.0), 1 mL $CuCl_2$ (10 mmol/L) and 1 mL 7.5 mmol/L neocuproine to a final volume of 4 mL. The reaction mixture was then incubated in the dark for 30 min at room temperature, and the absorbance of the reaction mixture was measured at 450 nm against a water blank [45].

Trolox was used as the standard calibration curves, and the results were expressed as µmol Trolox equivalent per g.

3.4. Urease inhibition assay [20]

Reaction mixture including 25 µL of Jack Bean urease, 55 µL of buffer (0.01 mol/L K_2HPO_4, 1 mmol/L EDTA and 0.01 mol/L LiCl, pH 8.2) and 10 mmol/L urea were incubated with 5 µL of the test compounds at room temperature for 15 min in microtiter plates. The production of ammonia was measured following the indophenol method and was used to determine the urease inhibitory activity. The phenol reagent (45 µL, 1% w/v phenol and 0.005% w/v sodium nitroprusside) and alkali reagent (70 µL, 0.5% w/v sodium hydroxide and 0.1% v/v NaOCl) were added to each well. This mixture was incubated for 15 minutes more at 35 °C and optical density was measured at 625 nm against a blank solution including distilled water instead of enzyme. For the determination of the IC_{50} value of the extracts, activity assays were conducted at five different extract concentration and dose response curve was generated. Thiourea was used as standard inhibitor.

3.5. Molecular Docking

Ligands were energy-minimized using GAMESS [46] module for ChemOffice version Ultra 8.0.3 on an Intel®(Core™ i7-3632QM CPU @ 2.20GHz 2.20GHz) using Widows 8.1 operating system. Appropriate grid box points were determined by centering on Ni atoms for each compound. Grid box for all ligands, defined with a size of 70*70*70 $Å^3$ and a regular space of 0.375 Å, was considered for docking.

"Crystal structure of Helicobacter pylori urease in complex with acetohydroxamic acid" pdb file (PDB ID: 1E9Y) was get (www.rcsb.org) and was modified using the Maestro [47]. Subsequently, Gasteiger charges were calculated and the generated pdbqt files were saved by ADT package version 1.5.6rc3. The Lamarckian Genetic Algorithm was used in medium type so docking score and calculated Ki values were obtained using function of AutoDock 4.2 release 4.2.5.1 [48] software.

4. Conclusions

In this study, we designed ecofriendly synthesis of novel Schiff bases. In synthesis process, we applied there different method, conventional, microwave irradiation and ultasound sonication, and among these, we obtained the best conditions in ultrasound sonication method. By this method, the reaction time took 3h in conventional method decreased to 1.5 min and a

significant increase in yield. The main purpose of this study is to have a lack of studies on the synthesis of Schiff bases by ultrasonic sonication in the literature. Also, antimicrobail activity, antioxidant capacity and antiurease activity of the synthesized compounds were investigated. **2c** was the most potent compound having IC_{50}=0,19±0,02 value. In antioxidant capacity assay which are DPPH, CUPRAC and FRAP, **2c**, **2f** and **2b** exhibited the best results. In particular, almost all of the compounds exhibited excellent antiurease activity. *In silico* ADME prediction were performed and drug likeness model score showed that **2a-i** were found to have higher drug likeness model score than the compounds **1a-i**. And also, the theoretical results obtained by ADME prediction and experimental inhibition studies were overlapped. Interactions with key residues such as His221, Asp362, Ala365, NI3001, NI3002 of urease were observed. The binding mode was produced and some compounds in the series showed similar binding.

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Highlights

- Urease inhibition of newly synthesized Schiff base derivatives
- Screening antimicrobial and antioxidant activityi and *in silico* ADME prediction study of novel drug-like compounds.
- Docking studies were performed for the most active compounds and interaction modes with enzyme active sites were determined.
- Conventional, microwave and ultrasound prompted synthesis of new Schiff base derivatives.