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Solution-Phase Synthesis of Small Schiff Bases Combinatorial Library with Potential Antitubercular Activity

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Abstract

A model of small combinatorial library composed from five hydrazides **(1-5)** and five carbonyl derivatives **(A-E)** has been designed to formally generate subliberaries of 10 mixtures, $\mathbf{M_1}$-$\mathbf{M_{10}}$ comprising of 25 Schiff bases. Components of the designed liberary, **1-5(A-E)**, have been synthesized by solution-phase method and evaluated for their antitubercular activity against four *Mycobacterium* strains. Variable anti-TB activity was revealed with the investigated mixtures and maximum activity shown by $\mathbf{M_2}$ and $\mathbf{M_7}$ at a concentration of 50µg/ml. Individual compounds included in the library were synthesized and their anti-TB activity were determined. Compound **2B**, predicted from the intersection of the active mixtures of $\mathbf{M_2}$ and $\mathbf{M_7}$, was the sole one that revealed anti-TB activity with the same MIC of the reference drug isoniazid (INH, 12.5 µg/ml). Other predicted compounds **1B, 3B** and **1C-3C**predicted from orthogonal cross matching of the less active subliberaries $\mathbf{M_1}$- $\mathbf{M_3}$, $\mathbf{M_7}$ and $\mathbf{M_8}$ were devoid of anti-TB activity at the tested concentrations when challenged in the pure state.

Keywords: Small combinatorial library, Heteroaryl hydrazides, Schiff Bases, Solution-Phase Synthesis, Antitubercular Activity

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Introduction

Tuberculosis (TB) is the most prevalent infectious disease worldwide and a leading killer caused by a single infectious agent [1]. According to World Health Organization (WHO) report, TB currently infects over 2 billion people worldwide, almost one-third of the world's population, with 30 million new cases reported each year [2]. This intracellular infection accounts for at least 3 million deaths annually, a life lost to TB every 15 seconds [2,3]. Drugs for treating TB have been available for over half a century, and yet the incidence of disease worldwide continues to

rise year by year. Moreover, the resurgence of TB in industrialized countries and worldwide increases in the prevalence of *Mycobacterium avium* complex (MAC) infections in immuno-compromised hosts (often accompanied by other bacterial infections) as well as the appearance of multidrug resistance (MDR) strains of TB have prompted the quest for new antimycobacterial agents, lacking cross-resistance with known antituberculous agents [4].

Development of resistance to existing drugs is a constantly growing phenomenon that has concerned researchers throughout the world, and now has reached alarming levels for TB. This combined with the recent decline in the development of new drugs to combat them can be anticipated to lead to infectious diseases lacking ready treatment regimens [5]. Consequently, the search for new antimycobacterial agents is currently very urgent, as TB has become the major emerging opportunistic infection [6]. Fortunately, combinatorial chemistry is one of the most rapidly developing field in the pharmaceutical industry in recent years [7,8]. It has become an essential tool, both in the discovery and the development of new drugs. The advent of this chemistry techniques, have altered the face of medicinal chemistry forever and a very substantial literature has been developed in a short time. Thus, the generation and use of combinatorial chemical libraries for the identification of novel chemical leads or for the optimization of a promising lead candidate has emerged as a potentially powerful method for the acceleration of the drug discovery process [9]. Accordingly, as a contribution to the anti-TB drug development using combinatorial library synthesis, design and synthesis of a small mixture-based Schiff base library for screening of anti-TB activity was undertaken in the current work. The design of Schiff base combinatorial library is based on the hypothesis that hydrazone derivatives undergo hydrolysis to their parent hydrazides and the corresponding carbonyl compounds and their activity are related to the hydrazide type [10].

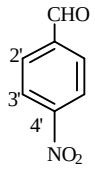
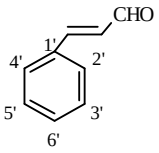
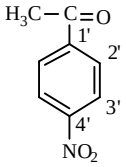
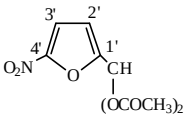
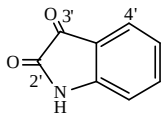
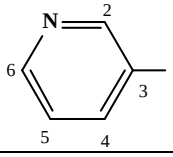
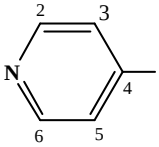
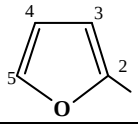
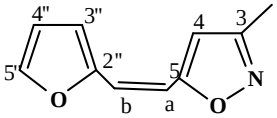
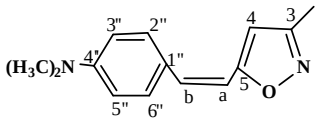
Results and Discussion

Small molecule libraries have been rapidly accessed using a variety of methodologies and techniques developed for use in solid-phase, as well as solution-phase synthesis. Although solid-phase has been at forefront of combinatorial chemistry, parallel solution-phase synthesis is an interesting alternative approach. The advantages characterizing solution-phase synthesis include validation time, facility of manipulations and the diversity of reactions that can be performed [11]. A solution-phase library approach is an attractive choice if reactions provide high yield production and removable byproducts [12]. The current mixture-based combinatorial library was designed in accordance to literature survey for anti-TB activity of Schiff bases. A two-component coupling reaction for library synthesis was selected. This is the simplest reaction type upon which combinatorial libraries have been based and it has been applied widely [13]. Hydrazone formation was also used as the base of library synthesis as it generates only water as a byproduct. These libraries are composed of five hydrazides **(1-5)** and five carbonyl derivatives (aldehydes, ketones or esters) **(A-E)** to formally generate 25 hydrazones as shown in the matrix displayed by table 1.

Cells in the library displayed by the 5x5 matrix serve as visual aids in determining contents of each mixture and in deconvoluting them. The first column and row represent the starting materials for the reaction carried out and the rest represent the synthesized compounds. The

matrix describes also ten mixtures produced as the last column and row representing mixtures $\mathbf{M_1}$-$\mathbf{M_5}$ and $\mathbf{M_6}$-$\mathbf{M_{10}}$, respectively.

Table 1. Building blocks and the designed Schiff base combinatorial library

Compd. No	**Y** **X-CONHNH$_2$**						
		A	**B**	**C**	**D**	**E**	
1		1A	1B	1C	1D	1E	**M_1**
2		2A	2B	2C	2D	2E	**M_2**
3		3A	3B	3C	3D	3E	**M_3**
4		4A	4B	4C	4D	4E	**M_4**
5		5A	5B	5C	5D	5E	**M_5**
		M_6	**M_7**	**M_8**	**M_9**	**M_{10}**	

As depicted in fig. 1 each of the sublibraries **(M_1-M_5)** was prepared by refluxing at a time an ethanolic solution containing 5 equivelent of the hydrazide **(1-5)** with stiochiometric and equimolar mixture of the 5 carbonyl derivatives **(A-E)** in presence of glacial acetic acid. The yielded sublibraries **(M_1-M_5)** consist of mixture of five Schiff bases, **1(A-E), 2(A-E), 3(A-E), 4(A-E)** and **5(A-E)**. It is worthy to mention that each sublibrary has the fixed $\mathbf{X_i}$ while containing all possible **Y** subsituents (carbonyl derivatives). By this way, variation of biological activities of each sublibrary will reveal the contribution of **X** moiety. In the same way, second set of sublibraries was prepared; however, ethanolic solution containing 5 equivelent of the carbonyl derivatives **(A-E)** was refluxed with equimolar mixture of the 5 hydrazides **(1-5)** to give the corresponding sublibraries **(M_6-M_{10})**. Combining information coming from the biological screening of the mixtures by orthogonal intersection of the two sets of sublibraries, the best $\mathbf{X_i}$ and $\mathbf{Y_i}$ combination can be identified.

Set 1: M_1-M_5

$$5\ X_i\text{-}CONHNH_2 + \sum_{Y=A}^{Y=E} Y \longrightarrow \sum_{Y=A}^{Y=E} X_i\text{-}CONHN{=}Y \qquad i = 1\text{-}5$$

Set 2: M_6-M_{10}

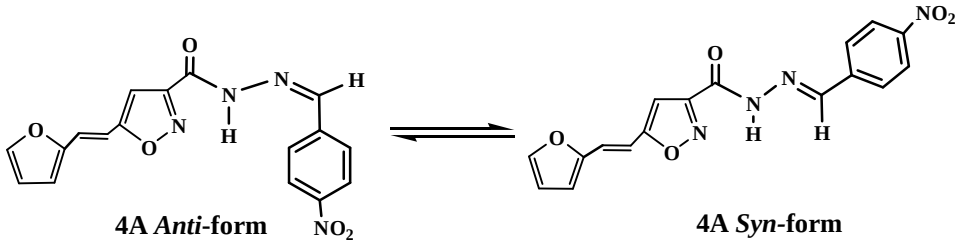
$$\sum_{X=1}^{X=5} X\text{-}CONHNH_2 + 5\ Y_i \longrightarrow \sum_{X=1}^{X=5} X\text{-}CONHN{=}Y_i \qquad i = A\text{-}E$$

Figure 1. Synthesis of the sublibrary sets 1 and 2.

Individual compounds shown in table 1 were synthesized in order to prove the validity of the technique used for evaluation of their anti-TB activities in mixtures. Synthesis of these targets was achieved by the classical method reported for hydrazide preparation. Thus, refluxing the corresponding carbonyl derivatives with the hydrazide derivatives in ethanol in the presence of glacial acetic acid afforded the desired products. Structures of the synthesized compounds were confirmed by elemental methods of analyses, table 3, and spectral data, tables 4. A common pattern was observed in the IR for these Schiff bases assigned for the –CONH- group around 3100-3300 cm^{-1} and stretching vibration at about 1676-1659 cm^{-1} assigned for NH and CO functionality. Characteristic bands of nitro group around 1530 cm^{-1} (*asym*) and 1330 cm^{-1} (*sym*) were shown by compounds **(1A-5A**, **1C-5C** and **1D-5D)**, table 4. Moreover, an interesting observation appeared in the IR spectra of the isatin derivatives **1E-5E** where a broad absorption bands shown at high ύ values 3390- 3420 cm^{-1} attributed to contribution to enolic OH and NH groups. This observation is in consistence with similar reported compounds containing isatin moiety [14]. The enolic character in this series was further confirmed by the ^{1}H-NMR of compound **5E** at 500MHz. Two humps centered at 14 and 13.6 ppm were assigned to amide-iminol structures, respectively [15].

-C(O)-NH- $\rightleftharpoons$ **-C(OH)=N-**

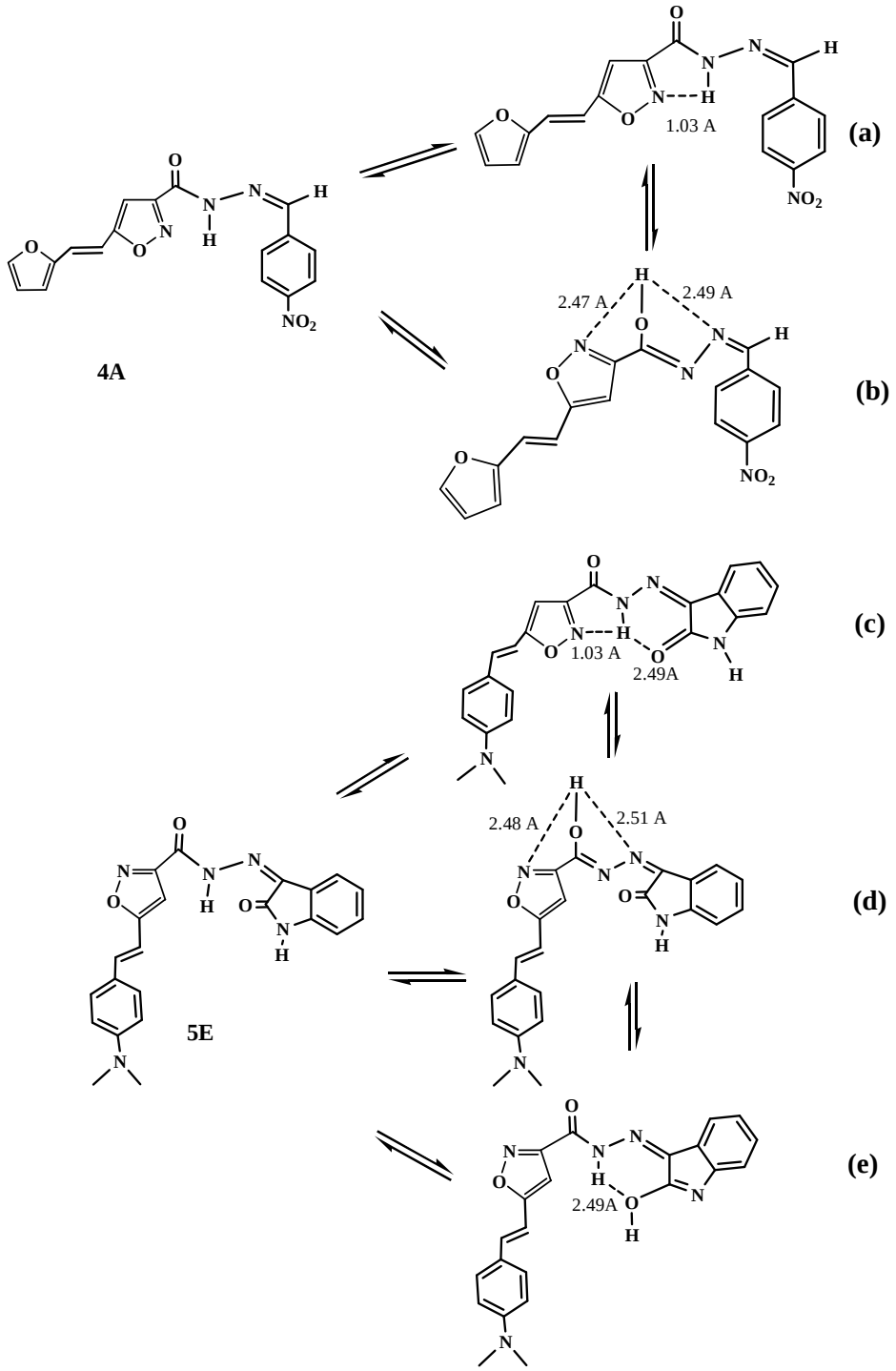
Furthermore, high resolution ^{1}H-NMR of compound **4A** revealed signals that can be interpreted based on probability of conformational isomerism of azomethine π-bond and amide-iminol tautomerism. Signal at 13.5 ppm assigned to NH of the *anti*-form while the signal at 12.5 ppm was assigned to NH of the *syn*-form, Scheme 1.



4A *Anti*-form **4A *Syn*-form**

Scheme 1 . *anti*- and *syn*-forms of 4A.

It was reported that the energy barrier between *syn* and *anti* isomers of Shciff bases is reported to be low so that isolation of the discrete isomers is not usually possible [16]. Moreover, upfield signal at 12 ppm was assigned to iminol OH proton.



Scheme 2. Amide-iminol forms of compound 4A and 5E

It was reported that only the amide form was prominent structure for N-benzoyl-α-phenylethyl amine and its derivatives (but not the iminol form) [15]. However, a mixture of amide-iminol

structures **(a-e)** was observed by the examination of the ^{1}H-NMR at 500MHz of the isoxazoles (compounds **4A** and **5E**). This may be attributed to the gain of energy enhanced by the intramolecular hydrogen bonding between the iminol OH and the nitrogen of isoxazole ring [17]. Extended conjugation affected through attachment of the isoxazole ring to an aromatic system via a vinyl bridge may contribute to more stabilization of the probable iminol form as illustrated by Scheme 2. *J* values of high resolution ^{1}H-NMR spectra of compounds **2B**, **4A** and **5E** were around 16.5-19 Hz. This indicates conserved *trans* configuration of the vinyl moiety found in their precursors.

As a general pattern, in the current series of Schiff bases, the most downfield peak at 11.3-14.3 ppm was assigned to the amidic -CONH proton. However, in case of possible existence of tautomerism 2 signals were detected where the upfield shifted one was assigned to the enolic -C(OH)=NH- proton.

Table 2. Anti-TB evaluation results of the synthesized combinatorial mixtures

Mixture	MIC (µg/mL)	Mixture	MIC (µg/mL)
M_1	100	**M_6**	NA
M_2	50	**M_7**	50
M_3	100	**M_8**	100
M_4	NA	**M_9**	NA
M_5	NA	**M_{10}**	NA

NA: no activity at 100µg/mL

The ten mixtures, **M_1-M_{10}**, of the synthesized library were tested for their anti-TB activity against four *Mycobacterium* strains: *M. intercellulari, M.xenopi, M. cheleneoi* and *M. smegmatis* according to the protocol mentioned in experimental section. Control experiments were done using a growth media free from the investigated compounds and results are shown in table 2 and illustrated by fig. 2.

Variable anti-TB activity was observed within the investigated mixtures **M_1-M_{10}**.The mixtures **M_2** and **M_7** are the most active ones while **M_1-M_3** and **M_8** exhibited modest activity. **M_4-M_6**, **M_9** and **M_{10}** did not show inhibitory potential at the highest concentration tested. Indexed method of analysis of the prepared small library was applied to elucidate the active components in mixtures. Intersection of the active rows **M_1-M_3** and columns **M_7** and **M_8** allowed the location of the most probably active contributors, **1B-3B** and **1C-3C** in these mixtures. In order to confirm the reliability of the predictions from the crossing procedure, all twenty five Schiff bases were synthesized and challenged in the neat form against the same *Mycobacterium* strains under the same conditions. Surprisingly, compound **2B**, predicted from the intersection of **M_2** and **M_7**, is the sole one in the neat form that revealed anti-TB activity with the same MIC value as that of

the reference drug INH (12.5 µg/mL), whereas the located compounds **1B, 3B** and **1C-3C** were inactive even at the highest tested concentration. Relatively weak activity of the mixtures **M_1, M_3,** and **M_8** may be attributed to the additive contribution of these components that are effective at higher concentrations than that tested.

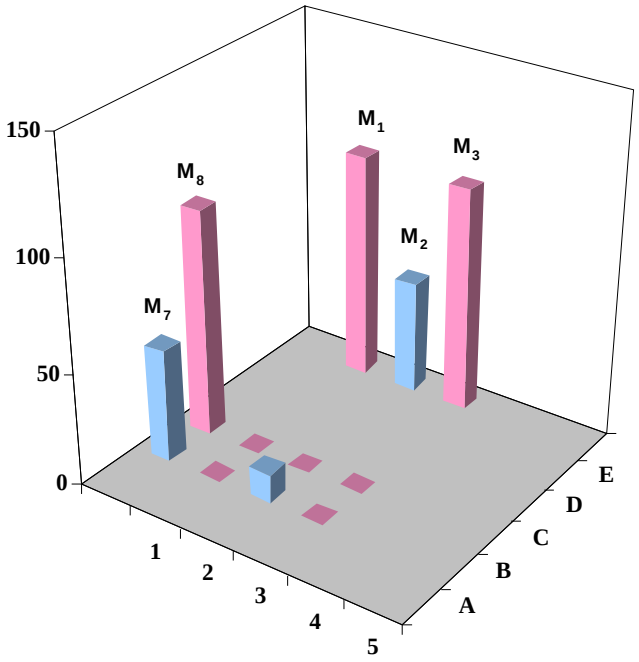


Figure 2. Orthogonal method of analysis of the anti-TB activity of the combinatorial library

The figures indicate the hydrazides while the letters indicate the carbonyl building blocks in the mixtures M_i of the combinatorial library.The back and left columns show inhibitory concentration levels of the mixtures. The floor shows the anticipated active compounds deduced from the orthogonal matching of the mixtures.

Materials and Methods

Building blocks **A-C**, **E** and **1** of the designed library were obtained commercially; however, the other building blocks **D** and **2** of the library were synthesized according to the reported literature [18,19]. All other chemicals used were of commercially available reagent grade and were used without further purification. Furfural and the solvents, however, were distilled before used.

Melting Points were determined using electrothermal apparatus (Stuart Scientific, England), and were uncorrected. IR-Spectra were recorded as KBr disk using Shimadzu IR 400-91527 Spectrophotometer (Shimadzu Corp., Kyoto, Japan), and the data are given in ύ (cm^{-1}). ^{1}H-NMR Spectra were determined in DMSO-d_6 and recorded either on Varian EM-360L, NMR Spectrophotometer (60 MHz) (Varian, palo Alto, CA, USA) or on Bruker EM-300L, NMR Spectrophotometer (500 MHz). The chemical shifts are given in δ (ppm) values relative to tetramethylsilane. Elemental Analyses were performed at the central laboratory of Assiut

University on "Analytischer Funktionstest vario EL Fab-Nr. 11982027" (Germany) and the results were within ±0.4% of the theoretical values.

Preparation of combinatorial mixtures

Synthesis of Schiff base mixtures ***M_1- M_5 (set 1)***

To ethanolic solutions of the individual **hydrazides**, **1-5,** (5 mmol) and the mixed five **carbonyl derivatives**, **A-E**, (1 mmol each), glacial acetic acid (1 ml) was added and the reaction mixtures were refluxed for 7 h. The mixtures were left overnight at room temperature. The resulting precipitates were filtered, washed with minimal amount of ethanol and dried without further purification to afford mixtures **M_1- M_5**.

Synthesis of Schiff base mixtures ***M_6- M_{10} (set 2)***

To ethanolic solutions of the individual **carbonyl derivative, A-E**, (5 mmol) and the mixed five **hydrazides**, **1-5**, (1 mmol each), glacial acetic acid (1 ml) was added and the reaction mixtures were refluxed for 7 h. The mixtures were left overnight at room temperature. The resulting precipitates were filtered, washed with minimal amount of ethanol and dried without further purification to afford mixtures **M_6- M_{10}**

General procedure for synthesis of hydrazones ***1-5(A-E)***

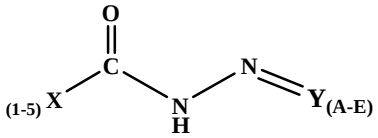
A mixture of acid hydrazide (**1-5**) (5 mmol) and appropriate carbonyl derivative **(A-E)** (5 mmol) in ethanol (25 ml) was refluxed in presence of glacial acetic acid (1 ml) for appropriate time. The reaction mixture was cooled and the precipitated solid was filtered and crystallized from appropriate solvent. Physicochemical constants of the prepared derivatives were given in tables 2-6; while IR and ^{1}H-NMR spectral data of the synthesized compounds are summarized in tables 3 and 4.

Evaluation of Antitubercular activity of the synthesized library and the individual compounds ***1B-3B*** *and* ***1C-3C****.*

Antitubercular activity was performed at the research center, College of Pharmacy, King Saud University, Saudi Arabia.. The tested Mycobacterium tuberculosis strains are *M. intercellulari* (ATCC 35743), *M.xenopi* (ATCC 14470), *M. cheleneoi* (ATCC 35751) *and M. smegmatis* (ATCC 35797) using Rist and Grosset proportion method (agar dilution method) [23].

The mixtures **M_1-M_{10}** compounds **1B-3B, 1C-3C** and **INH** were solubilized in DMSO at a concentration of 1 mg/mL. Appropriate aliquot of each solution was diluted with 10% molten agar to give concentrations of 100, 50 and 25 μg/ml. The agar and the compound solution were mixed thoroughly and the mixture was poured into Petri-dishes on a level surface to result in an agar depth of 3 to 4 mm and allowed to harden. The incula were prepared by growing overnight culture in Muller-Hinton broth. The cultures were diluted 1:100. Tested organisms were streaked in a radial pattern and plates were incubated at 35 ˚C for 48 hr and the minimum inhibitory concentration (MIC) of the tested compounds were determined. Experiment using the tested strains in a media free from investigated compounds was also carried out and the results are given in table 2.

Table 3. Physicochemical properties of the Schiff bases



Compd No.	X	Y	Reaction time (h)	M.p.(°C) (Cryst. Solvent)	Yield (%)	M.Formula (M.wt.)	Micoanalysis (%) Calcd./ Found		
							C	H	N
1A	**3-Pyridyl**	**4-Aminobenzylidene**	5	204-6 Ethanol	93	$C_{13}H_{10}N_4O_3$ (270.2)	57.78 57.56	3.73 3.60	20.73 20.42
1B		**3-Phenylallylidene**	6	253 Ethanol	90	$C_{15}H_{13}N_3O$ (251.3)	71.70 71.34	5.21 4.86	16.72 16.65
1C		**4-Nitrophenyl Ethylidene**	5	205-7 Aq.ethanol	93	$C_{14}H_{12}N_4O_3$. 0.5 H_2O [a] (293.3)	57.34 57.24	4.47 4.29	19.10 19.20
1D		**5-Nitro(furan-2-yl)methylene**	4	234 Ethanol	95	$C_{12}H_{11}N_4O_4$ (260.2)	50.77 50.43	3.10 2.98	21.53 21.25
1E		**Indoline-2-one-3-ylidene**	4	281-3 (reported 283) [b] Ethanol	92	$C_{14}H_{10}N_4O_2$ (266.3)	63.15 62.80	3.79 3.55	21.01 20.89
2A	**4-Pyridyl**	**4-Aminobenzylidene**	6	269-71 (reported 269) [c] 50% Glaecial acetic acid	94	$C_{13}H_{10}N_4O_3$ (270.2)	57.78 58.08	3.73 3.91	20.73 21.00
2B		**3-Phenylallylidene**	5	200 (reported 201) [b] Ethanol	91	$C_{15}H_{13}N_3O$ (251.3)	71.70 71.58	5.21 4.83	16.72 16.62
2C		**4-Nitrophenyl Ethylidene**	6	272 Ethanol	85	$C_{14}H_{12}N_4O_3$ (284.3)	59.15 58.72	4.25 4.39	19.71 19.53
2D		**5-Nitro(furan-2-yl)methylene**	4	245 (as reported)[d] Ethanol	91	$C_{12}H_{11}N_4O_4$ (260.2)	50.77 50.82	3.10 3.34	21.53 21.92
2E		**Indoline-2-one-3-ylidene**	4	293 (reported 293-5) [e] Aq.methanol	96	$C_{14}H_{10}N_4O_2$ (266.3)	63.15 63.50	3.79 3.40	21.04 21.03
3A	**Furan-2-yl**	**4-Aminobenzylidene**	3.5	268 (reported 268-9) [f] Ethanol	95	$C_{12}H_9N_3O_4$ (259.2)			
3B		**3-Phenylallylidene**	5	236 Ethanol	70.8	$C_{14}H_{12}N_3O_2$ (240.3)	69.99 69.60	5.03 4.57	11.66 11.66

Table 3. (Cont.)

Compd No.	X	Y	Reaction time (h)	M.p.(°C) (Cryst. Solvent)	Yield (%)	M.Formula (M.wt.)	Micoanalysis (%) Calcd./ Found		
							C	H	N
3C	**Furan-2-yl**	**4-Nitrophenyl Ethylidene**	3.5	240 Dioxane	75	$C_{13}H_{11}N_3O_4$ (273.2)	57.14 57.27	4.06 4.00	15.38 14.96
3D		**5-Nitro(furan-2-yl)methylene**	5	231-3 (reported 233-5) [a] Dioxane	85	$C_{10}H_7N_3O_5$ (249.2)			
3E		**Indoline-2-one-3-ylidene**	4	290 Dioxane / Ethanol	98	$C_{13}H_9N_3O_3$ (255.2)	61.18 61.39	3.55 3.73	16.46 16.66
4A	**5-[2-(Furan-2-yl)vinyl] isoxazole-3-yl**	**4-Aminobenzylidene**	6	250 Dioxane	76	$C_{17}H_{12}N_4O_5$ (352.1)	57.96 57.46	3.43 3.21	15.90 16.19
4B		**3-Phenylallylidene**	7	210 Ethanol	80	$C_{19}H_{15}N_3O_3$ (333.3)	68.46 68.34	4.54 4.17	12.61 12.63
4C		**4-Nitrophenyl Ethylidene**	7	243-5 Dioxane	87	$C_{18}H_{14}N_4O_5$ (366.3)	59.02 58.59	3.85 3.56	15.29 15.02
4D		**5-Nitro(furan-2-yl)methylene**	5	265 Aq.ethanol	83	$C_{15}H_{10}N_4O_6$ (342.3)	52.64 52.36	2.94 2.61	16.37 16.24
4E		**Indoline-2-one-3-ylidene**	3	270 Ethyl acetate	77	$C_{18}H_{12}N_4O_4$ (348.3)	62.07 61.54	3.47 3.25	16.09 15.96
5A	**5-[2-(4-dimethylamino phenyl)vinyl] isoxazole-3-yl**	**4-Aminobenzylidene**	5	>300 Ethanol	97	$C_{21}H_{19}N_5O_4$ (405.4)	62.22 62.40	4.72 4.51	17.27 17.67
5B		**3-Phenylallylidene**	5	263 Ethanol	82	$C_{23}H_{24}N_4O_2$ (386.5)	71.11 71.43	6.23 6.04	14.42 14.87
5C		**4-Nitrophenyl Ethylidene**	6	285 Dioxane	91	$C_{22}H_{21}N_5O_4$ (419.4)	63.00 62.77	5.05 4.72	16.70 16.33
5D		**5-Nitro(furan-2-yl)methylene**	5	>300 Ethanol	89	$C_{19}H_{17}N_5O_5$ (395.4)	57.72 58.02	4.33 4.65	17.71 17.62
5E		**Indoline-2-one-3-ylidene**	5	294-6 Ethanol	93	$C_{22}H_{19}N_5O_3$ (401.4)	65.83 65.55	4.77 4.73	17.45 17.80

a. Determined by TGA. b. Reference 20 c. Reference 21
d. Reference 22 e. Reference 23 f. Reference 24

Table 4. IR and ^{1}H-NMR spectral data of the Schiff bases

Compd. No	IR (cm^{-1})	^{1}H-NMR *
1[a]	3185, 1663, 1608, 1568, 1332, 850 , 812,690	7.8-8.2 (2H, m; C2'-H, C6'-H), 8.4-9 (5H, m; C3'-H, C5'-H, pyridyl protons), 9.2 (1H, d, J ~ 5; N=CH), 9.6 (1H, s; C2-H), 12.1 (1H, br. s; CONH) [a]
1B	3215, 1666, 1610**,** 956, 818,653 , 755, 705	7.3 (2H, d; -CH=CH), 7.6-8.2 (6H, m; phenyl protons, C5-H), 8.5-8.8 (2H, m; C4-H, C6-H), 9.1 (1H, d, J ~ 5; N=CH), 9.5 (1H, s; C2-H), 12.5 (1H, br. s; CONH) [a]
1C	3195, 1662, 1607, 1547,1332, 849, 812, 690	2.5 (3H, s; -CH_3), 7.8-8.2 (6H, m, C5-H, phenyl protons), 9.2 (1H, d, J ~ 5; C6-H), 9.6 (1H, s; C2-H), 11.9 (1H, br. s; CONH) [a]
1D	3225, 1669, 1606, 1542,1330**,** 803, 688	7.5 (1H, d, J ~ 4; C3'-H), 7.8-8.3 (2H, m; C4'-H, C5-H), 8.5-8.9 (2H, m; C4-H, N=CH), 9.1 (1H, m; C6-H), 9.6 (1H, s; C2-H), 13 (1H, br. s; CONH) [a]
1E	3195, 1664, 1609, 3390, 1688**,** 818, 691	7.1-8.2 (5H, m; C5-H, C4'-H, C5'-H, C6'-H, C7'-H), 8.5-8.9 (1H, m; C4-H), 9.2-9.8 (2H, m; C6-H, C2-H), 12 (1H, br.s, cyclic NH, exchangable with D_2O), 14.1 (1H, br. s; CONH) [a]
2[a]	3220, 1660, 1608, 1568, 1332, 850, 839	8.2-8.95 (6H, m; phenyl protons, C3-H, C5-H), 9 (1H, s; N=CH), 9.2 (2H, d, J ~ 6; C2-H, C6-H), 12.95 (1H, br. s; NH) [a]
2B	3220, 1659, 1610, 958 , 835,756, 705	7 (2H, d, J ~ 16.5; -CH=CH), 7.3-7.4 (3H, m; C4'-H, C3'-H, C5'-H), 7.5-7.6 (2H, m; C2'-H, C6'-H), 7.8 (2H, d, J ~ 5.5; C3-H, C5-H), 8.2 (1H, m; J ~ 6; N=CH), 8.7 (2H, d, J ~ 5; C2-H, C6-H), 11.9 (1H, br. s; NH) [b]
2C	3175, 1662,1607,1547, 1332, 848,838	Not determined [c]
2D	3225, 1660, 1606,1542, 1330, 838	7.6 (1H, d, J ~ 4; C3'-H), 7.9-8.3 (2H, m; C3-H, C5-H), 8.6 (2H, d, J ~ 8; C2-H, C6-H), 9.2 (1H, d, J ~ 5; C4'-H), 9.6 (1H, s; N=CH), 12.8 (1H, br. s; CONH) [a]
2E	3390, 3215, 1664,1609, 1688, 832	7.1-8 (4H, m; C4'-H, C5'-H, C6'-H, C7'-H), 8.2 (2H, d, J ~ 6; C3-H, C5-H), 9.2-9.4 (2H, m; C2-H, C6-H), 11.9 (1H, br. s; cyclic NH), 14.6 (1H, br. s; CONH) [a]
3[a]	3185,1670, 1619,1587, 1333, 833	Not determined [c]
3B	3215, 1661,1618, 974, 746, 690	7.2 (1H, m; C4-H), 7.7-7.9 (2H,d; CH=CH), 8-8.4 (6H, m; C3-H, phenyl protons), 8.8-9 (1H, m, C5-H), 9-9.2 (1H, t; N=CH), 12.7 (1H, br. s; CONH) [a]
3C	3195, 1670, 1621, 1552,1318, 843	Not determined [c]
3D	3225, 1675, 1624, 1553,1337	7.5-8 (1H, m; C4-H), 8-8.3 (2H, m; C3-H, C3'-H), 8.4-8.6 (2H, m; C5-H, C4'-H), 9.6 (1H, s; N=CH), 12.6 (1H, br. s; CONH, exchangeable with D_2O) [a]
3E	3135, 1674, 1622,1674	7.2-8 (7H, m; C4-H, C4'-H, C5'-H, C6'-H, C7'-H, C3-H, C5-H), 9.6 (1H, br. s; cyclic NH, exchangable with D_2O), 11.3 (1H, br. s; CONH) [a]
4[a]	3300, 1663, 1625, 1543, 1329, 962 , 833	6.6-6.8 (6H, m; furan protons), 7 (2H, d, J ~ 17.5; Ar-CH=CH), 7.4 (2H, d, J ~ 19, Ar-CH=CH), 7.7 (1H, s; C4-H), 7.8 (1H, s; C4-H), 7.9-8.4 (4H, m; phenyl protons), 8.3 (4H, s; 4N=CH), 8.6 (2H, s; 2N=CH), 12 (1H, br. s; OH), 12.5 (1H, br.s; CONH *syn*-form.), 13.5 (1H, br.s; CONH *anti*-form) [b]
4B	3240, 1663,1634, 975,739,701	6.6 (1H, t; -CH=CH-), 6.8 (1H, d, J ~ 3.4; C4"-H), 6.9-7.1 (4H, m; -CH=CH-, C3"-H, Ar-CH=CH, C5"-H), 7.3-7.4 (4H, m; C4'-H, C3'-H, C5'-H, Ar-CH=CH), 7.6 (2H, d, J ~ 7.5; C2'-H, C6'-H), 7.8 (1H, s; C4-H), 8.2 (1H, q, J ~ 7.5; N=CH), 12.1 (1H, s; CONH) [b]
4C	3115, 1662, 1625, 1574,1335, 951,846	2.6 (3H, s; -CH_3), 6.9-7. (1H, m, C4"-H), 7.1-7.2 (1H, m; C3"-H), 7.2 (2H, d, J ~ 10 ; Ar-CH=CH), 7.6 (1H, s; C5"-H), 8.1-8.8 (1H, s, C4-H), 8.2 (4H, m; C2'-H, C6'-H, C3'-H, C5'-H), 11.4 (1H, br.s; CONH) [a]
4D	3235, 1674, 1628, 1539,1343, 953	7.1-7.3 (2H, m; furan protons), 7.5-7.7 (2H, m; Ar-CH=CH, C3'-H),7.7-8 (2H, m; Ar-CH=CH, C4'-H), 8.2-8.4 (2H, m; C4-H, C5"-H), 9.2 (1H, s; N=CH), 13.7 (1H, br.s; CONH) [a]

Table 4. (Cont.)

Compd. No	IR (cm^{-1})	^{1}H-NMR *
4E	3415, 3295, 1664, 1627, 1688, 976	Not determined [c]
5[a]	3185, 1673,1594,1509, 1327, 964, 807	3.1 (6H, s; $(CH_3)_2N$), 7-8.1 (6H, m; C3''-H, C5''-H, Ar-CH=CH, C-2'-H, C6'-H), 8.4-9.1 (4H, m; C3'-H, C5'-H, C2''-H, C6''-H), 9.3 (1H, s; C4-H), 12.8 (1H, d, J ~ 5; N=CH), 14 (1H, br.s; CONH) [a]
5B	3205,1665, 1596,975, 804, 741,714	3.2 (6H, s; $(CH_3)_2N$), 7.2-7.8 (6H, m; -CH=CH-, Ar-CH=CH, C4'-H, C3''-H, C5''-H), 7.9-8.4 (7H, m; Ar-CH=CH, C3'-H, C5'-H, C2'-H, C6'-H, C2-H, C6-H), 8.9 (1H, s; N=CH), 12.5 (1H, s; C4-H), 14.2 (1H, br.s; CONH) [a]
5C	3180,1673, 1596, 1536,1334, 967, 848	3.2 (3H, s; $-CH_3$), 3.65 (6H, s; $(CH_3)_2N$), 7.1-7.4 (4H, m; C3''-H, C5''-H, C2''-H, C6''-H), 8.05 (2H, d, J ~ 8; Ar-CH=CH), 8.6-9.1 (4H, m; C2'-H, C6'-H, C3'-H, C5'-H), 11.3 (1H, s, C4-H), 14.3 (1H, br.s; CONH) [a]
5D	3165, 1676,1593,1509,1339, 964, 849	3.2 (6H, s; $(CH_3)_2N$), 7.1-7.8 (8H, m; Ar-CH=CH, phenyl and furan protons), 9.2 (1H, s; N=CH), 13.15 (1H, s, C4-H), 14.3 (1H, br.s; CONH) [a]
5E	3395, 3260, 1667,1593, 1689, 954	2.9 (6H, s; $(CH_3)_2N$), 6.7 (2H, d, J ~ 8.5; C3''-H, C5''-H), 6.8 (1H, d, J ~ 16.5; Ar-CH=CH), 6.9 (2H, d, J ~ 8; C2''-H, C6''-H), 7-7.1 (1H, m; C2'-H), 7.2 (1H, d, J ~ 16.5; Ar-CH=CH), 7.3-7.4 (3H, m; C3'-H, C4'-H, C4-H), 7.6 (1H, d, J ~ 7; C1'-H), 11.2 (1H, br.s; enolic OH), 13..5 (1H, br.s; iminol OH), 14 (1H, br.s; CONH) [b]

* The numbering of carbon centres follows the order that in the table 1.
a. Run at 60MHz, b Run at 500MHz, c Insoluble in the available solvents

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