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## Synthesis and Characterization of Novel Conformers of (*E*)-2-(3-nitro-*H*-imidazo[1,2-*a*]pyridin-2-ylthio)-*N'*-benzylideneacetohydrazide Derivatives.

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### Abstract

Eighteen (18) new N-acylhydrazones (**9a-r**) containing the imidazo[1,2-*a*]pyridine scaffold were synthesized through a seven steps reaction sequence, ending with a condensation of 2-(3-nitro-*H*-imidazo[1,2-*a*]pyridin-2-ylthio)acetohydrazide with various benzaldehyde derivatives (**8a-r**). All synthesized compounds were characterized by 1D NMR (<sup>1</sup>H and <sup>13</sup>C NMR) and 2D NMR (NOESY) spectroscopic analyses and high-resolution mass spectrometry (HRMS). The analysis of <sup>1</sup>H NMR data performed at room temperature in deuterated dimethylsulfoxide (DMSO-*d*<sub>6</sub>) revealed the presence of (*E*)-2-(3-nitro-*H*-imidazo[1,2-*a*]pyridin-2-ylthio)-*N'*-benzylideneacetohydrazide (**9a-r**) as a mixture of two conformers, namely *syn-periplanar E* (*sp E*) and *anti-periplanar E* (*ap E*). For all N-acylhydrazones that were synthesized, the *sp E*

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conformer was found to be the major form except in the case of hydrazone derived from *o*-hydroxybenzaldehyde.

**Keywords:**  $^1\text{H}$ ,  $^{13}\text{C}$ , 2D-NOESY NMR in DMSO- $d_6$ , hydrazide-hydrazones, N-acylhydrazones, imidazo[1,2-*a*]pyridine, benzaldehydes, conformational analysis

## 1-Introduction

*N*-acylhydrazones are featured by (CO–NH–N=CH) moiety, which when connected to heterocyclic scaffold, very often leads to compounds with interesting biological activities, including anticancer[1,2], antibacterial[3–5], antiviral[6,7] and antihypertensive[8] properties. The molecules based on imidazo[1,2-*a*]pyridine are heterobicyclic compounds that also exhibited antimicrobial[9–11] anti-inflammatory[12], anticancer[13][14] and antiparasitic[15] properties, among many others. When hydrazide-hydrazones are coupled with heterocyclic compounds, they lead to organic compounds with interesting biological activities[16–18]. Investigations on these class of compounds have led to the development of bioactive molecules such as Furazolidone, Nitrofurantoin and Nitrofurazon (**Figure 1**). Nitrofurazon is a drug with a topical anti-infectious bactericidal effect on skin infections and was first synthesized by Mc Calla *et al.*[19]. Nitrofurantoin is also another one, which possess antibiotic activity used to treat bacterial infections of the urinary tract [20] and first synthesized by Mcosker and Fitzpatrick[21] .

*N*-Acyldiazone is a 6  $\pi$ -electrons conjugated moiety; provided the azomethine moiety adopts the *E*, most stable, configuration[22–24]. This conjugated system may be subject to further isomerism, due to slow rotation around the N–N and N–CO[25] bonds (**Figure 2**), which potentially may give rise to four conformers.

Although the *N*-acyldiazone moiety may show traces of the *Z* configuration in some particular cases, such as presence of intramolecular hydrogen bonding, in less polar solvents, such as chloroform, the *E* isomer is the only one observed in DMSO- $d_6$ [24][26]. For example, Rubina Munir *et al.*[27] showed in their work on *N'*-(benzylidene)-2-(6-methyl-1*H*-pyrazolo[3,4-*b*]quinolin-1-yl)acetohydrazide derivatives that these compounds exist as a mixture of two conformers in a DMSO- $d_6$  solution. They also showed that the stereoisomer *E*

exists as a mixture of two conformers, due to the slow rotation around the *N*-acyl bond, with the N–H bond being in *cis* or *trans* position with respect to the carbonyl bond, leading respectively to *syn-periplanar E* (*sp E*) or *anti-periplanar E* (*ap E*) conformers. Literature data reveal that *sp E* would predominates in DMSO-*d*<sub>6</sub>[24,26]. To contribute to this topic, we have synthesized a series of new *N*-acylhydrazone derivatives containing the thio-imidazo[1,2-*a*]pyridine scaffold in order to access a new class of compounds with potential biological activities. In this article, we will focus on their synthesis and the investigation of their-structure in DMSO-*d*<sub>6</sub> by taking advantage of 1D NMR (<sup>1</sup>H and <sup>13</sup>C NMR) and 2D NMR (NOESY) spectroscopic analyses.

## 2-Results and discussion

### 2.1- Synthetic chemistry

The *N*-acylhydrazone derivatives (**9a-r**) were obtained in four steps from a 3-nitroimidazo[1,2-*a*]pyridine derivative. The choice of a powerful electron-withdrawing nitro group (NO<sub>2</sub>) at 3-position of compound (**1**) will promote the depletion of its 2-position in electron density, thus facilitating suitable aromatic nucleophilic substitution reactions. For this purpose, by reacting 2-chloro-3-nitroimidazo[1,2-*a*]pyridine (**1**) with thiourea in refluxing acetonitrile[28], we could obtain the isothiuronium salt of 3-nitro imidazo[1,2-*a*]pyridine (**3**) with a yield of 96 %. In the presence of ethyl 2-chloroacetate, under basic conditions in refluxing mixture of water and ethanol, salt (**3**) undergoes *S*-alkylation and concomitant elimination of urea leading to thioether (**5**) in satisfactory yield (73 %)[29]. Next, the treatment of ester (**5**) with hydrazine hydrate in refluxing ethanol allowed its conversion into the corresponding hydrazide (**7**) in moderate yield (52 %). Finally, a series of the desired *N*-acylhydrazone derivatives (**9a-r**) were synthesized with yields varying from 52 to 82 %, by reacting hydrazide (**7**) with various aromatic aldehydes (**8**) during 1h under reflux in methanol (**Scheme 1**). These new compounds thus synthesized were fully characterized using various analytical methods, particularly NMR that revealed the existence of a dynamic equilibrium between two isomers for each member of this series of *N*-acylhydrazones (**9**). The results of this stereochemical study will be discussed in the next section.

Reaction conditions: i) Thiourea (**2**), MeCN, reflux 48h; ii) ClCH<sub>2</sub>CO<sub>2</sub>Et (**4**), NaOH, EtOH/H<sub>2</sub>O, reflux 1h; iii) NH<sub>2</sub>-NH<sub>2</sub> (**6**), EtOH, reflux 5h; iv) Ar-COH(**8**), MeOH, reflux 1h

## 2.2- Spectral analysis

The  $^1\text{H}$  NMR spectroscopic analysis of compound (**5**) reveals the presence of a singlet at 4.12 ppm corresponding to the two methylene protons located between the sulfur atom and the ester function (**S-CH<sub>2</sub>**) (**Figure S4**). The protons of the ethoxy group from ester function appear as a quadruplet at 4.24 ppm and a triplet at 1.30 ppm (**Figure S4**). Aromatic protons appear between 7.31 and 9.39 ppm (**Figure S4**). The spectroscopic analysis  $^{13}\text{C}$  NMR of compound (**5**) allowed us to observe the carbon of the (**C=O**) ester function at 171.74 ppm. Carbon peaks of the methylene group **S-CH<sub>2</sub>** is observed at 33.04 ppm (**Figure S4**). Analysis of the  $^1\text{H}$  NMR spectrum of compound (**7**), shows the disappearance of the ethoxy group's signals, and the appearance of two broad singlets at 4.32 and 7.42 ppm corresponding to the NH protons of the **NH<sub>2</sub>** and **C(O)-NH** groups respectively (**Figure S5**). The  $^1\text{H}$  NMR spectra of all final compounds (**9a-r**) in *DMSO-d<sub>6</sub>* show featuring and significant signal duplication of three neighboring groups of the amide moiety: **NH**, **N=CH** and **S-CH<sub>2</sub>-CO** (**Figures S6-S23**). In Figure 3 are shown some selected examples of  $^1\text{H}$ -NMR spectra focusing on the aromatic and NH signals region. Moreover, systematic signal duplication was observed in the  $^{13}\text{C}$  NMR spectra (**Figures S6-S23**), for the amide carbonyl, the azomethine moiety (**N=CH**) and the thioether methylene group (**S-CH<sub>2</sub>**).

## 2.3-Stereochemical analysis of hydrazide-hydrazone (**9a-r**)

The observed duplication (with various intensities) of featuring ( $^1\text{H}$ - and  $^{13}\text{C}$ -NMR) signals (**NH**, **N=CH** and **S-CH<sub>2</sub>** groups) indicates the presence of two different isomers in the case of all our compounds (**9a-r**). Considering the *N*-acylhydrazone structure, one may ascribe this signals duplication to the geometric stereoisomerism (*Z* or *E* configuration) of the azomethine (**N=CH**) moiety, or slow (restricted) rotation around **N-N** or **N-CO** bond. Generally, reaction of primary amines, hydrazine or hydroxylamine, with aromatic aldehydes leads mainly, if not exclusively to *E* isomer of the corresponding condensation compound. Usually, in most cases imines stereoisomers show different values of their retention front on thin layer chromatography (TLC) and can be, at least partially separated by column chromatography; in terms of global dipolar moment, *E* isomer is expected to be slightly more polar than *Z* isomer. In our case, all samples analyzed by NMR show single spot TLC. Moreover, the  $^1\text{H}$ -NMR spectra of the few examples of reported *Z* isomer of particular structures of *N*-acylhydrazones are featured by a singlet around 14 ppm that corresponds to the **N-H** proton[22] the absence of a (duplicated) singlet around this chemical shift in our cases corroborates the absence of the

$Z_{(C=N)}$  isomer. Furthermore, the ratio between the pair of duplicated signals changes when spectra were recorded in  $CDCl_3$ , such ratio variation cannot be due to a  $Z/E$  interconversion which is featured by a high activation barrier that requires high temperature or photochemical activation. All these observations suggest that we were able to isolate only the  $E_{(C=N)}$  isomer[22].

The observed duplication of signals in the  $^1H$ -NMR spectra of the synthesized compounds **9a-r** is, therefore, ascribed to the existence of conformers that may arise due N–N or N–CO bond rotation. Due to steric hindrance of the acyl group (Het–SCH<sub>2</sub>–CO) and the arylidene moiety (=CH–Ar), the  $Z_{(N-N)}$  conformer is unlikely to exist. Therefore, we believe that the emergence of the duo signals in NMR spectra of the isolated compounds **9a-r** denotes rather the existence of rotamers about the amide bond (N–CO), namely *syn-periplanar*  $E_{(C=N)(N-N)}$  (*sp E*) and *anti-periplanar*  $E_{(C=N)(N-N)}$  (*ap E*) as shown in **Figure 4**[27]. To confirm this hypothesis, we decided to proceed with 2D NOESY experiments to assess the spatial proximity of  $^1H$ - $^1H$  in each isomer, particularly for NH with N=CH, and NH with SCH<sub>2</sub>.

First, we found that for the three featured duplicated signals (NH, S–CH<sub>2</sub> and N=CH) each expected diagonal signal from an isomer shows a cross-correlation in phase with the corresponding diagonal signal of the second isomer (**Figure 5**, for compound **9a**). This cross-correlation cannot be attributed to chemical exchange with solvent, since it is also observed with non-exchangeable protons (S–CH<sub>2</sub> and N=CH). The most plausible explanation of this cross-correlation is an exchange between two rotamers where a proton has a distinct chemical shift in each conformer. Additionally, with this 2D NOESY spectrum we could exclude the  $Z$  configuration of both C=N and N–N bond, and could ascribe each part of a duplicated signal to the corresponding conformer *sp E* or *ap E*. Indeed, on the NOESY (proton-proton spatial correlation) spectrum of compound **9a**, in both isomers, a strong dipolar coupling was observed between the azomethine (N=CH) proton and that of the NH group, in good agreement with an  $E_{(C=N)(N-N)}$  configuration for each conformer. The minor isomer shows a closer spatial proximity (stronger NOE) between the NH and the S-CH<sub>2</sub> groups, in good agreement with its *ap E* conformation, while no detectable NH/CH<sub>2</sub> NOE was observed in the major isomer, as expected for the *sp E* conformer (**Figure 5**).

Interestingly, a change in the conformer's ratio was observed in the case of *N*-acylhydrazone **9b** derived from 2-hydroxybenzaldehyde; in DMSO-*d*<sub>6</sub>, *ap E* conformer slightly prevails

(strong NH/CH<sub>2</sub> NOE), with also a larger chemical shift difference between the NH duo signals and reduced difference for those of the azomethine duo signal (**Figure 6**).

The slight stability increase of the *ap E* conformer in the case of **9b** might be a consequence of an intramolecular hydrogen bonding between the hydroxyl group and the azomethine nitrogen lone pair, thus forming a six-membered ring structure where the hydroxyl proton could be also involved in a second hydrogen bonding with one carbonyl lone pair, thus forming a five-membered ring (**Figure 7**). Noteworthy, in less polar solvent, *i.e.* CDCl<sub>3</sub>, the *ap E*/*sp E* ratio reaches 85/15, versus 54/46 in DMSO-*d*<sub>6</sub> where intramolecular hydrogen bonding is less favored than in chloroform.

In the absence of such an intramolecular hydrogen bonding involving both lone pairs of nitrogen and oxygen atoms, the *ap E* conformer is less stabilized, probably due to electronic repulsion between these lone pairs (**Figure 8**).

Based on this series of NMR analysis, the chemical shifts of the featuring protons (**NH**, **N=CH** and **S-CH<sub>2</sub>**) could be ascribed to each conformer of compounds **9a-r** as shown in **Table 1**. As a general trend, and as reported in literature,[27] for the methylene protons (SCH<sub>2</sub>), the deshielded signals are assigned to the *sp E* conformers, while the shielded signals to the *ap E* conformers. Conversely, for the azomethine (N=CH) and amide (H-NC=O) protons, the deshielded signals are ascribed to *ap E* conformers, and shielded signals to *sp E* conformers (**Table 1**). In the case of amide proton, this difference is probably due to stereoelectronic effect; in the *ap E* conformer there is a possible overlap between  $\sigma_{\text{N-H}}$  and  $\sigma^*_{\text{C=O}}$  orbitals resulting in a deshielding of the NH proton, such an electron transfer toward the carbonyl may result in a reduced electronic withdrawing effect of the latter towards the methylene group (S-CH<sub>2</sub>) and subsequent shielding of its protons. Such a hyperconjugation effect does not exist in the *sp E* conformer.

The conformers ratio for each *N*-acylhydrazone was assessed (**Table 2**) considering the relative intensities of each duo signals on the <sup>1</sup>H NMR, of the non-exchangeable SCH<sub>2</sub> group protons, using equation (1), where *Ianti* and *Isyn* refer to the integration intensity of this methylene group signal of each conformer on the <sup>1</sup>H NMR spectrum.

$$R_{syn} = \frac{I_{syn}}{I_{syn}+I_{anti}} \quad \text{and} \quad R_{anti} = \frac{I_{anti}}{I_{syn}+I_{anti}} \quad (1)$$

As mentioned above, the signals duplication was also observed in  $^{13}\text{C}$ -NMR spectra for three featuring carbons of final compounds (**9**): two very close peaks around 34 ppm corresponding to the  $\text{SCH}_2$  methylene carbon, the amide carbon of the *sp E* conformers appears around 169 ppm, while that of *ap E* conformers is found around 164 ppm, also duo signals are observed around 153 ppm that state for the azomethine ( $\text{C}=\text{N}$ ) carbon of both conformers of **9** (Table 3).

### 3-Conclusion

In this work, we synthesized and characterized eighteen (18) new *N*-acylhydrazones from the imidazo[1,2-*a*]pyridine scaffold after getting a carbon-sulfur bond in position-2 of the neat heterocycle. All these compounds were obtained with yields over 50 %, and their structures were confirmed by NMR spectroscopic analyses and HRMS. The *N*-acylhydrazone compounds were obtained as equilibrating mixture of two conformers: *syn-periplanar* and *anti-periplanar E* (*sp E* & *ap E*) with ratio of approximately 66 % for *sp E* vs. 34 % for *ap E*. Except in the case of *N*-acylhydrazone **9b**, which derives from 2-hydroxybenzaldehyde, where the *ap E* conformer slightly prevails probably due to additional stability resulting from intramolecular hydrogen bonding involving the 2-hydroxyl proton both with the azomethine nitrogen lone pair and the carbonyl oxygen lone pair. The predominance of this *ap E* conformer of **9b** is even more pronounced in  $\text{CDCl}_3$ , a less polar solvent that favors intramolecular hydrogen bonding much more than  $\text{DMSO-}d_6$ . Attempts to estimate the rotation barrier of this conformational equilibrium were unsuccessful; no significant change in the *syn/anti* ratio was observed, nor in chemical shifts of the duo signals of compound **9a**, while recording its  $^1\text{H}$ -NMR at 500 MHz in  $\text{DMSO-}d_6$ , at 80 °C, thus indicating that the coalescence could not be reached at 500 MHz by heating samples at reasonable temperature. This rotation barrier seems higher than for isolated amide, this may be attributed to the extended conjugation of the  $\pi$ -system of compounds **9**, that involves six  $\pi$ -electrons, plus the aromatic ring  $\pi$ -electrons, thus resulting in a larger resonance stabilization effect of both planar conformations. Variable temperature experiments at lower field (80 MHz) would be helpful in this case.

### 4-Experimental



All reagents and solvents were purchased from Sigma Aldrich and used without further purification unless otherwise noted. All anhydrous solvents, reagent grade solvents for chromatography and starting materials were purchased at the highest commercial quality from either Aldrich Chemical or Fisher Scientific. Water was distilled and purified through a Milli-Q water system (Millipore Corp., Bedford, MA). The reactions were monitored by TLC on precoated Merck 60 F254 silica gel plates and visualized using UV-Lamp (6 W, 254 nm and/or 365 nm). Sample Analysis

Unless otherwise indicated,  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded on *Bruker BioSpin Avance* spectrometers operating at proton resonance frequencies of either 300 MHz (resp. 75 MHz for  $^{13}\text{C}$ ), 400 MHz (resp. 101 MHz for  $^{13}\text{C}$ ) or 500 MHz (resp. 126 MHz for  $^{13}\text{C}$ ) using TopSpin 3.6. The probe used was a 5-mm broadband inverse (BBI) room temperature probe. The sample temperature was 298 K. The spectra were internally referenced to the residual proton solvent signal. Residual solvent peaks were taken as reference  $\text{CDCl}_3$ : 7.26 ppm,  $\text{DMSO-}d_6$ : 2.50 ppm,  $\text{Acetone-}d_6$ : 2.05 ppm. For  $^1\text{H}$  NMR assignments, the chemical shifts are given in ppm on the  $\delta$  scale. Multiplicities are described as s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), m (multiplet) and further qualified as app (apparent), br (broad signal) coupling constants,  $J$  are reported in Hz. Spectral processing was performed with Mestrenova 14.1 (mestrelab). Samples **9a**, and **9b** were analyzed with 2-D Nuclear Overhauser Effect Spectroscopy (2D-NOESY) (4 scans, 256 increments, 300 ms of mixing time, 2 s of relaxation time), and 1-D protons experiments (32 scans, 20 ppm sweep width).

HRMS were measured in the electrospray (ESI) mode on a LC-MSD TOF mass analyzer. Melting points were measured on a Thomas-Hoover capillary melting point apparatus. Abbreviations: DCM = dichloromethane;  $\text{Et}_3\text{N}$  = triethylamine; MeCN = acetonitrile, EtOAc = ethyl acetate; EtOH = ethanol. All compounds are > 98 % pure by HPLC analysis.

#### ***Synthesis methods of 2-chloro-H-imidazo[1,2-a]pyridine***

A solution of (1.5 g, 9.8 mmol) of 2-(2-iminopyridin-1(2H)yl)acetic acid **3** in 15 mL of toluene was prepared in an round bottom flask. 4 eq (3.7 mL, 39.43 mmol) of  $\text{POCl}_3$  was added dropwise to the solution. After 16 h of reaction, the mixture was allowed to cool down at room temperature during 15 minutes and then water was added. The RBF containing the reaction medium was cooled down this time at 0 °C in an ice bath and neutralized by a solution of NaOH (10 %). The reaction crude was extracted with DCM. Then organic layer was washed with a saturated NaCl, dried over anhydrous  $\text{Na}_2\text{SO}_4$ . The solvent was evaporated under *vacuo*, then a precipitate was formed and further purified by column chromatography ( $\text{SiO}_2$ , hexane/EtOAc

(60/40) yielded 1.23 g (82 %) compound 2-chloro-*H*-imidazo[1,2-*a*]pyridine as white powder. M.p : 74-76°C, <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ (ppm) 8.04 (d, *J* = 6.8 Hz, 1H, H<sub>Ar</sub>), 7.53 (d, *J* = 9.1 Hz, 1H ; H<sub>Ar</sub>), 7.49 (s, 1H ; H<sub>Ar</sub>), 7.24 – 7.17 (m, 1H ; H<sub>Ar</sub>), 6.83 (t, *J* = 6.8 Hz, 1H ; H<sub>Ar</sub>). ; <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 125.19, 117.13, 113.06 . HRMS (ESI): Calc for C<sub>7</sub>H<sub>6</sub>ClN<sub>2</sub> [M+H<sup>+</sup>] = 153.0984 Found = 153.0986

### **Synthesis methods of 2-chloro-3-nitro-*H*-imidazo[1,2-*a*]pyridine 1**

A round bottom flask immersed in ice bath containing 15 mL of H<sub>2</sub>SO<sub>4</sub>, 1.5 g (9.83 mmol) of 2-chloro-*H*-imidazo[1,2-*a*]pyridine **4** and 3.5 eq (1.6 mL, 34.40 mmol) of HNO<sub>3</sub> were added. The reaction mixture was stirred at room temperature for 3 h and followed by TLC analysis. Reaction mixture was extracted with DCM and the organic layer was dried over Na<sub>2</sub>SO<sub>4</sub>. The organic phase was evaporated under *vacuo*, dried and without any further purification to give 1.76 g (91 %) compound **5** as yellow crystals. M.p= 166-168 °C, <sup>1</sup>H NMR (400 MHz, Acetone-*d*<sub>6</sub>) δ (ppm) 9.42 (dt, *J* = 7.0, 1.1 Hz, 1H; H<sub>Ar</sub>), 7.92 – 7.79 (m, 2H; H<sub>Ar</sub>), 7.52 (td, *J* = 7.0, 1.5 Hz, 1H; H<sub>Ar</sub>). <sup>13</sup>C NMR (400 MHz, Acetone-*d*<sub>6</sub>) δ (ppm) 132.08, 117.33. HRMS (ESI): Calc for C<sub>7</sub>H<sub>5</sub>ClN<sub>2</sub>O<sub>2</sub> [M+H<sup>+</sup>] = 198.8974 Found = 198.8977

### **Synthesis methods of thiouronium imidazopyridine chloride salt 3**

In a solution of CH<sub>3</sub>CN (50 mL), 2-chloro-3-nitro-*H*-imidazo[1,2-*a*]pyridine **1** (0.2 g, 10.12 mmol), 1.2 eq of thiourea **6** (0.10 g, 12.14 mmol) were added in an round bottom flask. The mixture was heated up under reflux for 48 h. After cooling at room temperature, a precipitate was formed, filtered and washed several times with EtOAc. The compound was dried in the open air to yielded 0.26 g (96 %) as yellow crystals. M.p= 260-262 °C, <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ (ppm) 10.01 (s, 2H; NH<sub>2</sub>), 9.39 (t, *J* = 6.1 Hz, 1H, H<sub>Ar</sub>), 8.05 (d, *J* = 8.9 Hz, 1H, H<sub>Ar</sub>), 7.95 (dd, *J* = 11.9, 4.1 Hz, 1H, H<sub>Ar</sub>), 7.59 (dt, *J* = 5.3, 2.6 Hz, 1H, H<sub>Ar</sub>), 7.25 – 7.08 (m, 2H, NH<sub>2</sub>). <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>) δ (ppm) 164.43, 145.65, 141.93, 133.82, 128.59, 118.36, 117.80. HRMS (ESI): Calc for C<sub>9</sub>H<sub>10</sub>ClN<sub>5</sub>O<sub>2</sub>S [M+H<sup>+</sup>] = 274.4124 Found = 274.4128

### **Synthesis methods of 2-(3-nitro-*H*-imidazo[1,2-*a*]pyridin-2-ylthio)ethyl acetate (5)**

In a flask containing 10 mL of EtOH, were added 3.8 mmol of thiouronium imidazopyridine chloride salt **7**. This mixture was carried out under reflux. Then an aqueous solution of NaOH (23 μM) was added. The reaction was allowed to warm up under reflux again during 5 min, then 3eq (1.14 mmol) of ethyl 2-chloroacetate (**4**) were added. The reaction stayed likewise during 1 h and followed by TLC analysis until a new spot appears. The reaction mixture was extracted with DCM and then the organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated under *vacuo*. A further purification by column chromatography (SiO<sub>2</sub>, hexane /EtOAc (60/40))

yielded compounds **5** as pale-yellow powder. M.p = 128-130 °C. Yield = 73 %, <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ(ppm) 9.44 – 9.37 (m, 1H; H<sub>Ar</sub>), 7.66 – 7.59 (m, 2H; H<sub>Ar</sub>), 7.24 – 7.15 (m, 1H; H<sub>Ar</sub>), 4.24 (q, *J* = 7.1 Hz, 2H; CH<sub>2</sub>), 4.12 (s, 2H; S-CH<sub>2</sub>), 1.30 (t, *J* = 7.1 Hz, 3H; CH<sub>3</sub>). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ(ppm) 168.75, 152.89; 145.97; 131.55; 127.68; 116.86; 115.75; 61.90, 33.04, 14.17. HRMS (ESI): Calc for [M+H]<sup>+</sup> C<sub>11</sub>H<sub>12</sub>N<sub>3</sub>O<sub>4</sub>S = 282.0635 Found = 282.0633

**Synthesis methods of 2-(1.8a-dihydro-3-nitro-imidazo[1.2-a]pyridin-2-ylthio)acetohydrazide (7)**

In a flask containing 25 mL of absolute ethanol, a mixture of 14.70 mmol of ethyl 2-(1.8a-dihydro-3-nitroimidazo[1.2-a]pyridin-2-ylthio)acetate and 58.8 mmol (4eq) of hydrated hydrazine were added. Then the mixture was put under reflux for 4 h. Following the reaction by TLC, at the end of the reaction, ice water was added and allowed to stir continuously at room temperature. A precipitate was obtained, filtered, dried with Mg<sub>2</sub>SO<sub>4</sub> and then recrystallized in ethanol to give 2-(1.8a-dihydro-3-nitroimidazo[1.2-a]pyridin-2-ylthio)acetohydrazide with a yield of 52% as an orange powder. M.p = 232-234 °C. Yield = 52 %, <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ(ppm) 9.39 – 9.26 (m, 2H), 7.87 – 7.70 (m, 2H), 7.49 (s, 1H, NH), 4.03 (s, 2H, NH<sub>2</sub>). <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>) δ(ppm) 166.46, 152.88, 145.80, 133.05, 128.09, 116.40, 32.66. HRMS (ESI): Calc for [M+H]<sup>+</sup> C<sub>9</sub>H<sub>10</sub>N<sub>5</sub>O<sub>3</sub>S = 268.0345 Found = 268.0342

**General procedure for the synthesis of (E)-2-(3-nitroH-imidazo[1.2-a]pyridin-2-ylthio)-N'-benzylideneacetohydrazide (9a-r)**

1.23 mmol of 2-(1.8a-dihydro-3-nitroimidazo[1.2-a]pyridin-2-ylthio)acetohydrazide and 1.23 mmol (1 eq) of benzaldehyde derivative were dissolved in methanol. The reaction mixture was refluxed for 1h. After cooling to room temperature, the precipitate formed was filtered, dried and then purified by recrystallization in a water/ethanol mixture (1/1) to give 2-(1.8a-dihydro-3-nitroimidazo[1.2-a]pyridin-2-ylthio)-N'-benzylideneacetohydrazide with yields between 56 and 86%.

**(E)-2-(3-nitro-H-imidazo[1.2-a]pyridin-2-ylthio)-N'-benzylideneacetohydrazide (9a)**

Orange powder. M.p = 232-234 °C; Yield = 79 %, <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ (ppm) 11.77<sub>ap</sub>, 11.67<sub>sp</sub> (s, 1H; NH), 9.34<sub>(sp+ap)</sub> (dt, *J* = 6.9, 1.1 Hz, 1H; H<sub>Ar</sub>), 8.22<sub>ap</sub>, 8.05<sub>sp</sub> (s, 1H; N=CH), 7.90 – 7.77<sub>(sp+ap)</sub> (m, 2H; H<sub>Ar</sub>), 7.71<sub>(sp+ap)</sub> (td, *J* = 3.8 ;2.2 Hz, 2H; H<sub>Ar</sub>), 7.51 – 7.34<sub>(sp+ap)</sub> (m, 4H; H<sub>Ar</sub>), 4.63<sub>sp</sub>, 4.22<sub>ap</sub> (s, 2H, S-CH<sub>2</sub>). <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>) δ (ppm) 169.39<sub>sp</sub>, 164.18<sub>ap</sub> (C=O), 153.54<sub>sp</sub>, 153.15<sub>ap</sub> (N=CH), 147.44, 146.23, 144.16, 134.53, 134.48,

133.52<sub>sp</sub>, 133.46<sub>ap</sub> (C<sub>Ar</sub>), 130.60<sub>sp</sub>, 130.44<sub>ap</sub> (C<sub>Ar</sub>), 129.29, 128.65<sub>sp</sub>, 128.40<sub>ap</sub> (C<sub>Ar</sub>), 127.58, 127.34, 11697<sub>sp</sub>, 116.87<sub>ap</sub> (C<sub>Ar</sub>), 116.79, 33.95<sub>ap</sub>, 33.08<sub>sp</sub> (S-CH<sub>2</sub>), HRMS (ESI):Calc for [M+Na<sup>+</sup>] C<sub>16</sub>H<sub>13</sub>N<sub>5</sub>NaO<sub>3</sub>S = 378.0521. Found = 378.0525

**(E)-N'-(2-hydroxybenzylidene)-2-(3-nitro-H-imidazo[1.2-a]pyridin-2-ylthio)acetohydrazide (9b)**

Brown powder, M.p = 238-240 °C, Yield = 69 %, <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ (ppm) 11.64<sub>sp</sub> (s, 1H ; OH), 11.14<sub>ap</sub>, 11.01<sub>sp</sub> (s, 1H; NH), 10.13<sub>ap</sub> (s, 1H, OH), 9.35<sub>(sp-ap)</sub> (m, 1H; H<sub>Ar</sub>), 8.44<sub>ap</sub>, 8.36<sub>sp</sub> (s, 1H; N=CH), 7.90 – 7.77<sub>(sp-ap)</sub> (m, 4H ; H<sub>Ar</sub>), 7.70<sub>(sp-ap)</sub> (ddd, *J* = 8.1, 4.8, 1.6 Hz, 1H, H<sub>Ar</sub>), 7.54 (dd, *J* = 8.0, 1.7 Hz, 1H ; H<sub>Ar</sub>), 7.49 – 7.35 (m, 2H; H<sub>Ar</sub>), 7.35 – 7.20 (m, 2H, H<sub>Ar</sub>), 7.03 – 6.80 (m, 5H, H<sub>Ar</sub>), 4.61<sub>sp</sub>, 4.23<sub>ap</sub>(s, 2H, S-CH<sub>2</sub>). <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>) δ (ppm) 169.03<sub>sp</sub>, 164.07<sub>ap</sub> (C=O), 163.28<sub>ap</sub>, 159.10<sub>sp</sub> (C-OH), 157.73, 156.89, 153.54<sub>sp</sub>, 153.06<sub>ap</sub> (N=CH), 147.65, 146.24, 141.78, 133.72, 133.53, 133.47, 131.94, 131.72, 131.32, 129.68, 128.54, 126.86, 120.51, 120.09, 119.91, 119.84, 119.08, 118.64, 116.99, 116.80, 116.64, 33.67<sub>ap</sub>, 33.12<sub>sp</sub> (S-CH<sub>2</sub>), HRMS (ESI): Calc for [M+H<sup>+</sup>] C<sub>16</sub>H<sub>14</sub>N<sub>5</sub>O<sub>4</sub>S = 372.0843. Found = 372.0839

**(E)-2-(3-nitro-H-imidazo[1.2-a]pyridin-2-ylthio)-N'-(4-fluorobenzylidene)acetohydrazide (9c)**

Orange powder. M.p= 266-268 °C. Yield = 79 %. <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ (ppm) 11.79<sub>ap</sub>, 11.68<sub>sp</sub>,(s, 1H. NH), 9.35<sub>(sp+ap)</sub> (d, *J* = 6.8 Hz, 1H, H<sub>Ar</sub>), 8.22<sub>ap</sub>, 8.05<sub>sp</sub> (s, 1H, N=CH), 7.89 – 7.70<sub>(sp+ap)</sub> (m, 4H, H<sub>Ar</sub>), 7.42<sub>(sp+ap)</sub> (s, 1H, H<sub>Ar</sub>), 7.28<sub>(sp+ap)</sub> (t, *J* = 8.6 Hz, 2H, H<sub>Ar</sub>), 4.62<sub>sp</sub>, 4.21<sub>ap</sub> (s, 2H, S-CH<sub>2</sub>). <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>) δ (ppm) 168.00<sub>sp</sub>, 162.67<sub>ap</sub> (C=O), 153.11<sub>sp</sub>, 152.73<sub>ap</sub> (N=CH), 145.83, 142.59, 133.09, 130.74, 129.43<sub>sp</sub>, 129.32<sub>ap</sub> (C<sub>Ar</sub>), 129.17<sub>sp</sub>, 129.06<sub>ap</sub> (C<sub>Ar</sub>), 128.27<sub>sp</sub>, 128.00<sub>ap</sub> (C<sub>Ar</sub>), 116.57<sub>sp</sub>, 116.48<sub>ap</sub> (C<sub>Ar</sub>), 116.38, 116.12, 115.82, 33.50<sub>ap</sub>, 32.60<sub>sp</sub> (S-CH<sub>2</sub>), HRMS (ESI):Calc for [M+Na<sup>+</sup>] C<sub>16</sub>H<sub>12</sub>FN<sub>5</sub>NaO<sub>3</sub>S = 396.0452. Found = 396.0456

**(E)-2-(3-nitro-H-imidazo[1.2-a]pyridin-2-ylthio)-N'-(4-methylbenzylidene)acetohydrazide(9d)**

Khaki powder, M.p = 242-244 °C, Yield = 55 %, <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ (ppm) 11.72<sub>ap</sub>, 11.62<sub>sp</sub> (s, 1H, NH), 9.36 – 9.27<sub>(sp+ap)</sub> (m, 1H, H<sub>Ar</sub>), 8.17<sub>ap</sub>, 8.00<sub>sp</sub> (s, 1H. N=CH), 7.89 – 7.75<sub>(sp+ap)</sub> (m, 2H, H<sub>Ar</sub>), 7.57<sub>(sp+ap)</sub> (dd, *J* = 8.2; 2.2 Hz, 2H, H<sub>Ar</sub>), 7.46 – 7.34<sub>(sp+ap)</sub> (m, 1H, H<sub>Ar</sub>), 7.23<sub>(sp+ap)</sub> (d, *J* = 7.9 Hz, 2H, H<sub>Ar</sub>), 4.61<sub>sp</sub>, 4.20<sub>ap</sub> (s, 2H, S-CH<sub>2</sub>), 2.32<sub>(sp+ap)</sub> (s, 3H, CH<sub>3</sub>). <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>) δ (ppm) 168.19<sub>sp</sub>, 162.97<sub>ap</sub> (C=O), 152.56<sub>sp</sub>, 152.15<sub>ap</sub> (N=CH), 146.39, 145.18, 143.15, 139.38<sub>ap</sub>, 139.18<sub>sp</sub> (C<sub>Ar</sub>), 132.45<sub>ap</sub>, 132.39<sub>sp</sub> (C<sub>Ar</sub>). 130.80<sub>ap</sub>, 130.75<sub>sp</sub> (C<sub>Ar</sub>), 128.84, 127.61<sub>sp</sub>, 127.36<sub>ap</sub> (C<sub>Ar</sub>), 126.51<sub>ap</sub>, 126.27<sub>sp</sub> (C<sub>Ar</sub>), 115.91, 115.81<sub>sp</sub>, 115.74<sub>ap</sub>

(C<sub>Ar</sub>), 32.97<sub>ap</sub>, 32.10<sub>sp</sub> (S-CH<sub>2</sub>), 20.47<sub>(sp+ap)</sub> (CH<sub>3</sub>). HRMS (ESI): Calc for [M+Na<sup>+</sup>] C<sub>17</sub>H<sub>15</sub>N<sub>5</sub>NaO<sub>3</sub>S = 392.0774. Found = 392.0778

***(E)-2-(3-nitro-H-imidazo[1.2-a]pyridin-2-ylthio)-N'-(4-nitrobenzylidene)acetohydrazide (9e)***

Yellow powder, M.p = 224-225 °C, Yield = 80 %, <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ (ppm) 12.08<sub>ap</sub>, 11.98<sub>sp</sub> (s, 1H; NH), 9.36<sub>(sp+ap)</sub> (dt, *J* = 6.9, 1.1 Hz, 1H; H<sub>Ar</sub>), 8.31<sub>ap</sub>, 8.20<sub>sp</sub> (s, 1H; N=CH), 8.22<sub>(sp+ap)</sub> (d, *J* = 32.6 Hz, 1H, H<sub>Ar</sub>), 7.98<sub>(sp+ap)</sub> (dd, *J* = 8.8, 6.5 Hz, 2H; H<sub>Ar</sub>), 7.87 – 7.81<sub>(sp+ap)</sub> (m, 2H; H<sub>Ar</sub>), 7.45 – 7.40 (m, 1H; H<sub>Ar</sub>), 4.68<sub>sp</sub>, 4.26<sub>ap</sub> (s, 2H; S-CH<sub>2</sub>), <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>) δ (ppm) 169.79<sub>sp</sub>, 164.71<sub>ap</sub> (C=O), 153.37<sub>sp</sub>, 153.04<sub>ap</sub> (N=CH), 148.35<sub>sp</sub>, 148.23<sub>ap</sub> (C<sub>Ar</sub>), 146.24, 144.94, 141.78, 140.82, 133.50, 128.70<sub>sp</sub>, 128.53<sub>ap</sub> (C<sub>Ar</sub>), 128.30, 124.56, 116.92<sub>sp</sub>, 116.82<sub>ap</sub> (C<sub>Ar</sub>), 33.95<sub>ap</sub>, 32.99<sub>sp</sub> (S-CH<sub>2</sub>). HRMS (ESI): Calc for [M+Na<sup>+</sup>] C<sub>16</sub>H<sub>12</sub>N<sub>6</sub>NaO<sub>5</sub>S = 423.0624. Found = 423.0627.

***(E)-2-(3-nitro-H-imidazo[1.2-a]pyridin-2-ylthio)-N'-(4-methoxybenzylidene)acetohydrazide (9f)***

Tan powder. M.p = 234-236 °C, Yield = 67 %, <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ (ppm) 11.66<sub>ap</sub>, 11.55<sub>sp</sub>, (s, H, NH), 9.33<sub>(sp+ap)</sub> (dd, *J* = 6.8, 1.4 Hz, H, H<sub>Ar</sub>), 8.15<sub>ap</sub>, 7.99<sub>sp</sub> (s, 1H, N=CH), 7.90 – 7.76<sub>(sp+ap)</sub> (m, 2H, H<sub>Ar</sub>), 7.63<sub>(sp+ap)</sub> (dd, *J* = 8.8, 1.9 Hz, 2H, H<sub>Ar</sub>), 7.48 – 7.35 (m, 1H, H<sub>Ar</sub>), 6.98 (dd, *J* = 8.8, 1.9 Hz, 2H, H<sub>Ar</sub>), 4.60<sub>sp</sub>, 4.19<sub>ap</sub> (s, 2H, S-CH<sub>2</sub>), 3.79<sub>(sp+ap)</sub> (s, 3H, O-CH<sub>3</sub>). <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>) δ (ppm) 168.67<sub>sp</sub>, 163.43<sub>ap</sub> (C=O), 160.89<sub>sp</sub>, 160.74<sub>ap</sub> (C<sub>Ar</sub>), 153.18<sub>sp</sub>, 152.76<sub>ap</sub> (N=CH-), 146.87, 145.79, 143.58, 133.07, 133.02, 128.76, 128.48<sub>sp</sub>, 128.21<sub>ap</sub> (C<sub>Ar</sub>), 127.96, 126.63, 116.52<sub>sp</sub>, 116.42<sub>ap</sub> (C<sub>Ar</sub>), 116.36, 114.34, 55.33 (O-CH<sub>3</sub>), 33.54<sub>ap</sub>, 32.68<sub>sp</sub> (S-CH<sub>2</sub>), HRMS (ESI): Calc for [M+H<sup>+</sup>] C<sub>17</sub>H<sub>16</sub>N<sub>5</sub>O<sub>4</sub>S = 386.1152. Found = 386.1155

***(E)-2-(3-nitro-H-imidazo[1.2-a]pyridin-2-ylthio)-N'-(4-hydroxy-3-methoxybenzylidene)acetohydrazide (9g)***

Tan powder. M.p = 202-204 °C, Yield = 52 %, <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ (ppm) 11.61<sub>ap</sub>, 11.50<sub>sp</sub> (s, 1H ; NH), 9.56<sub>(sp-ap)</sub> (d, *J* = 5.4 Hz, 1H ; OH), 9.34<sub>(sp-ap)</sub> (dt, *J* = 6.9, 1.1 Hz, 1H ; H<sub>Ar</sub>), 8.09<sub>ap</sub>, 7.93<sub>sp</sub> (s, 1H ; N=CH), 7.86 – 7.76 (m, 2H, H<sub>Ar</sub>), 7.42 (ddt, *J* = 8.4, 6.7, 3.0 Hz, 1H ; H<sub>Ar</sub>), 7.28 (dd, *J* = 10.1, 1.9 Hz, 1H , H<sub>Ar</sub>), 7.08 (td, *J* = 8.3, 1.9 Hz, 1H , H<sub>Ar</sub>), 6.81 (d, *J* = 8.1 Hz, 1H ; H<sub>Ar</sub>), 4.60<sub>sp</sub>, 4.18<sub>ap</sub> (s, 2H, S-CH<sub>2</sub>), 3.79 (s, 3H ; OCH<sub>3</sub>). <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>) δ (ppm) 169.02<sub>sp</sub>, 163.75<sub>ap</sub> (C=O), 153.71<sub>sp</sub>, 153.22<sub>ap</sub> (N=CH), 149.47, 149.25, 148.44<sub>sp</sub>, 147.95<sub>ap</sub> (C<sub>Ar</sub>), 146.24, 144.48, 133.54<sub>sp</sub>, 133.48<sub>ap</sub> (C<sub>Ar</sub>), 128.68, 128.42, 125.95<sub>sp</sub>, 125.90<sub>ap</sub> (C<sub>Ar</sub>), 122.56, 121.73, 116.97<sub>sp</sub>, 116.86<sub>ap</sub> (C<sub>Ar</sub>), 116.76, 116.02<sub>sp</sub>, 115.87<sub>ap</sub>

(C<sub>Ar</sub>), 110.03, 109.57, 56.01<sub>sp-ap</sub> (OCH<sub>3</sub>), 33.98<sub>ap</sub>, 33.15<sub>sp</sub> (S-CH<sub>2</sub>), HRMS (ESI): Calc for [M+Na<sup>+</sup>] C<sub>17</sub>H<sub>15</sub>N<sub>5</sub>NaO<sub>5</sub>S = 424.3878. Found = 424.3875

***(E)-2-(3-nitro-H-imidazo[1.2-a]pyridin-2-ylthio)-N'-(4-hydroxybenzylidene)acetohydrazide (9h)***

Yellow powder, M.p = 248-250 °C, Yield = 79 %, <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ (ppm) 11.58<sub>ap</sub>, 11.46<sub>sp</sub> (s, 1H, NH), 9.92<sub>(sp+ap)</sub> (s, 1H, OH), 9.39 – 9.30 (m, 1H, H<sub>Ar</sub>), 8.10<sub>ap</sub>, 7.94<sub>sp</sub> (s, 1H, N=CH), 7.91 – 7.76<sub>(sp+ap)</sub> (m, 2H, H<sub>Ar</sub>), 7.53<sub>(sp+ap)</sub> (dd, *J* = 8.7, 2.5 Hz, 2H, H<sub>Ar</sub>), 7.48 – 7.36<sub>(sp+ap)</sub> (m, 1H, H<sub>Ar</sub>), 6.86 – 6.76<sub>(sp+ap)</sub> (m, 2H, H<sub>Ar</sub>), 4.59<sub>sp</sub>, 4.19<sub>ap</sub> (s, 2H, S-CH<sub>2</sub>). <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>) δ (ppm) 168.50<sub>sp</sub>, 163.23<sub>ap</sub> (C=O), 159.45<sub>sp</sub>, 159.29<sub>ap</sub> (C<sub>Ar</sub>), 153.18<sub>sp</sub>, 152.76<sub>ap</sub> (N=CH), 147.24, 145.77, 143.96, 133.04<sub>sp</sub>, 132.98<sub>ap</sub> (C<sub>Ar</sub>), 128.89, 128.61, 128.12, 128.45<sub>sp</sub>, 127.93<sub>ap</sub> (C<sub>Ar</sub>), 125.04, 116.48, 116.39<sub>sp</sub>, 116.36<sub>ap</sub> (C<sub>Ar</sub>), 115.71, 33.52<sub>ap</sub>, 32.68<sub>sp</sub> (S-CH<sub>2</sub>), HRMS (ESI): Calc for [M+Na<sup>+</sup>] C<sub>16</sub>H<sub>13</sub>N<sub>5</sub>NaO<sub>4</sub>S = 394.3573. Found = 394.3575

***(E)-2-(3-nitro-H-imidazo[1.2-a]pyridin-2-ylthio)-N'-(4-cyanobenzylidene)acetohydrazide (9i)***

Yellow powder, M.p = 260-261 °C, Yield = 65 %, <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ (ppm) 12.02<sub>ap</sub>, 11.92<sub>sp</sub> (s, 1H, NH), 9.36<sub>(sp+ap)</sub> (dt, *J* = 6.9, 1.1 Hz, H, H<sub>Ar</sub>), 8.28<sub>ap</sub>, 8.10<sub>sp</sub> (s, 1H, N=CH), 7.90 (d, *J* = 5.6 Hz, 4H, H<sub>Ar</sub>), 7.86 – 7.82 (m, 2H, H<sub>Ar</sub>), 7.43 (dt, *J* = 6.9, 4.2 Hz, 1H, H<sub>Ar</sub>), 4.66<sub>ap</sub>, 4.24<sub>sp</sub> (s, 2H, S-CH<sub>2</sub>). <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>) δ (ppm) 169.26<sub>sp</sub>, 164.08<sub>ap</sub> (C=O), 152.92<sub>sp</sub>, 152.58<sub>ap</sub> (N=CH), 145.75, 144.94, 141.70, 138.52, 133.03, 132.76<sub>sp</sub>, 132.73<sub>ap</sub> (C<sub>Ar</sub>), 128.23<sub>sp</sub>, 127.96<sub>ap</sub> (C<sub>Ar</sub>), 127.67<sub>sp</sub>, 127.47<sub>ap</sub> (C<sub>Ar</sub>), 118.68(CN), 116.43<sub>sp</sub>, 116.33<sub>ap</sub> (C<sub>Ar</sub>), 111.76, 33.48<sub>ap</sub>, 32.46<sub>sp</sub> (S-CH<sub>2</sub>). HRMS (ESI): Calc for [M+H<sup>+</sup>] C<sub>17</sub>H<sub>13</sub>N<sub>6</sub>O<sub>3</sub>S = 381.0642. Found = 381.0639

***(E)-2-(3-nitro-H-imidazo[1.2-a]pyridin-2-ylthio)-N'-(4-(dimethylamino)benzylidene)acetohydrazide (9j)***

Orange powder, M.p = 240-242 °C, Yield = 72 %, <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ(ppm) 11.48<sub>ap</sub>, 11.41<sub>sp</sub> (s, 1H, NH), 9.35<sub>ap+sp</sub> (dt, *J* = 6.9, 1.2 Hz, 1H, H<sub>Ar</sub>), 8.06<sub>ap</sub>, 7.91<sub>sp</sub> (s, 1H, N=CH), 7.87 – 7.79<sub>sp+ap</sub> (m, 2H, H<sub>Ar</sub>), 7.50<sub>sp+ap</sub> (d, *J* = 8.6 Hz, 2H, H<sub>Ar</sub>), 7.41<sub>sp+ap</sub> (td, *J* = 6.5, 2.1 Hz, 1H, H<sub>Ar</sub>), 6.72<sub>sp+ap</sub> (dd, *J* = 9.1, 2.5 Hz, 2H, H<sub>Ar</sub>), 4.58<sub>sp</sub>, 4.17<sub>ap</sub> (s, 2H, S-CH<sub>2</sub>), 2.95<sub>sp+ap</sub> (s, 6H, N(CH<sub>3</sub>)<sub>2</sub>). <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>) δ(ppm) 168.97<sub>sp</sub>, 163.78<sub>ap</sub>(C=O), 153.11<sub>sp</sub>, 152.73<sub>ap</sub>(N=CH), 145.83, 142.59, 133.09, 130.74, 129.43<sub>sp</sub>, 129.31<sub>ap</sub> (C<sub>Ar</sub>), 129.17, 129.06, 128.27<sub>sp</sub>, 128.00<sub>ap</sub> (C<sub>Ar</sub>), 116.57<sub>sp</sub>, 116.48<sub>ap</sub> (C<sub>Ar</sub>), 116.38, 116.11, 115.82, 33.50<sub>ap</sub>, 32.60<sub>sp</sub> (S-CH<sub>2</sub>), 30.75(N(CH<sub>3</sub>)<sub>2</sub>). HRMS (ESI): Calc for [M+H<sup>+</sup>] C<sub>18</sub>H<sub>19</sub>N<sub>6</sub>O<sub>3</sub>S = 399.1113. Found = 399.1109

***(E)-2-(3-nitro-H-imidazo[1.2-a]pyridin-2-ylthio)-N'-(4-chlorobenzylidene)acetohydrazide(9k)***

Khaki powder. M.p = 256-258 °C. Yield = 76%. <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ (ppm) 11.86<sub>ap</sub>, 11.75<sub>sp</sub> (s, 1H, NH), 9.39 – 9.31 (m, 1H, H<sub>Ar</sub>), 8.22<sub>ap</sub>, 8.04<sub>sp</sub> (s, 1H, N=CH), 7.87 – 7.81<sub>(sp+ap)</sub> (m, 2H, H<sub>Ar</sub>), 7.73<sub>(sp+ap)</sub> (m, 2H, H<sub>Ar</sub>), 7.50<sub>(sp+ap)</sub> (d, *J* = 8.5 Hz, 2H, H<sub>Ar</sub>), 7.42<sub>(sp+ap)</sub> (m, 1H, H<sub>Ar</sub>), 4.63<sub>sp</sub>, 4.22<sub>ap</sub> (s, 2H, CH<sub>2</sub>). <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>) δ (ppm) 169.23<sub>sp</sub>, 164.03<sub>ap</sub> (C=O), 153.29<sub>sp</sub>, 152.92<sub>ap</sub> (N=CH), 146.03<sub>sp</sub>, 145.86<sub>ap</sub> (C<sub>Ar</sub>), 142.61, 134.82<sub>sp</sub>, 134.64<sub>ap</sub> (C<sub>Ar</sub>), 133.34<sub>sp</sub>, 133.28<sub>ap</sub> (C<sub>Ar</sub>), 129.20<sub>sp</sub>, 129.17<sub>ap</sub> (C<sub>Ar</sub>), 129.02<sub>sp</sub>, 128.79<sub>ap</sub> (C<sub>Ar</sub>), 128.49<sub>sp</sub>; 128.22<sub>ap</sub> (C<sub>Ar</sub>), 116.77, 116.68<sub>sp</sub>, 116.60<sub>ap</sub> (C<sub>Ar</sub>), 33.75<sub>ap</sub>, 32.81<sub>sp</sub> (CH<sub>2</sub>). HRMS (ESI): Calc for [M+H<sup>+</sup>] C<sub>16</sub>H<sub>13</sub>ClN<sub>5</sub>O<sub>3</sub>S = 390.0261. Found = 390.0258

***(E)-N'-(3-bromo-4-hydroxybenzylidene)-2-(3-nitro-H-imidazo[1.2-a]pyridin-2-ylthio)acetohydrazide (9l)***

Yellow powder, M.p = 186-188 °C, Yield = 68 %, <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ (ppm) 11.68<sub>ap</sub>, 11.56<sub>sp</sub> (s, 1H; NH), 10.78<sub>(sp-ap)</sub> (s, 1H, OH), 9.35 (dt, *J* = 6.9, 1.1 Hz, 1H, H<sub>Ar</sub>), 8.07<sub>ap</sub>, 7.91<sub>sp</sub> (s, 1H, N=CH), 7.89 – 7.78 (m, 3H, H<sub>Ar</sub>), 7.54 (dt, *J* = 8.4, 2.2 Hz, 1H, H<sub>Ar</sub>), 7.43 (m, 1H, H<sub>Ar</sub>), 7.00 (d, *J* = 8.4 Hz, 1H, H<sub>Ar</sub>), 4.61<sub>sp</sub>, 4.20<sub>ap</sub> (s, 2H, S-CH<sub>2</sub>), <sup>13</sup>C NMR (101 MHz, DMSO-*d*<sub>6</sub>) δ (ppm) 169.14<sub>sp</sub>, 163.94<sub>ap</sub> (C=O), 156.15<sub>(sp-ap)</sub> (C-OH), 153.59<sub>sp</sub>, 153.23<sub>ap</sub> (N=CH), 146.09, 142.87, 133.48, 132.03, 131.57, 128.69, 128.42<sub>sp</sub>, 128.25<sub>ap</sub> (C<sub>Ar</sub>), 127.26, 116.98, 116.88<sub>sp</sub>, 116.81<sub>ap</sub> (C<sub>Ar</sub>), 110.36<sub>sp</sub>, 110.21<sub>ap</sub> (C<sub>Ar</sub>), 33.92<sub>ap</sub>, 33.08<sub>sp</sub> (S-CH<sub>2</sub>), HRMS (ESI): Calc for [M+H<sup>+</sup>] C<sub>16</sub>H<sub>13</sub>BrN<sub>5</sub>O<sub>4</sub>S = 449.8382. Found = 449.8386

***(E)-N'-(3,5-dichlorobenzylidene)-2-(3-nitro-H-imidazo[1.2-a]pyridin-2-ylthio)acetohydrazide (9m)***

Tan powder. M.p = 260-262 °C. Yield = 81 %. <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ (ppm) 11.90 (s, 1H, NH), 9.35<sub>(sp-ap)</sub> (dt, *J* = 6.9, 1.1 Hz, 1H, H<sub>Ar</sub>), 8.56<sub>ap</sub>, 8.38<sub>sp</sub> (s, 1H, N=CH), 7.99<sub>(sp-ap)</sub> (dd, *J* = 21.5, 8.6 Hz, 1H, H<sub>Ar</sub>), 7.86 – 7.80 (m, 2H, H<sub>Ar</sub>), 7.72 (t, *J* = 2.4 Hz, 1H, H<sub>Ar</sub>), 7.50 (dd, *J* = 8.5, 2.1 Hz, 1H, H<sub>Ar</sub>), 7.42 (dt, *J* = 6.7, 4.2 Hz, 1H, H<sub>Ar</sub>), 4.64<sub>sp</sub>, 4.22<sub>ap</sub> (s, 2H, S-CH<sub>2</sub>). <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>) δ (ppm) 169.60<sub>sp</sub>, 164.51<sub>ap</sub> (C=O), 153.39<sub>sp</sub>, 153.04<sub>ap</sub> (N=CH), 146.23, 142.29, 139.15, 135.46, 134.32<sub>sp</sub>, 134.17<sub>ap</sub> (C<sub>Ar</sub>), 133.53, 130.88, 129.89, 128.68, 128.52, 117.01<sub>sp</sub>, 116.92<sub>ap</sub> (C<sub>Ar</sub>), 116.80, 33.87<sub>ap</sub>, 32.97<sub>sp</sub> (S-CH<sub>2</sub>). HRMS (ESI): Calc for [M+H<sup>+</sup>] C<sub>16</sub>H<sub>12</sub>Cl<sub>2</sub>N<sub>5</sub>NaO<sub>3</sub>S = 424.0112. Found = 424.0115

***(E)-2-(3-nitro-H-imidazo[1.2-a]pyridin-2-ylthio)-N'-(4-(4-methylbenzyloxy)benzylidene)acetohydrazide (9n)***

Khaki powder; M.p = 216-218 °C; Yield = 72 %; <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ (ppm) 11.65<sub>ap</sub>, 11.55<sub>sp</sub> (s, 1H, NH), 9.33<sub>(sp+ap)</sub> (dt, *J* = 6.9, 1.1 Hz, 1H, H<sub>Ar</sub>), 8.15<sub>ap</sub>, 7.98<sub>sp</sub> (s, 1H,

N=CH-), 7.88 – 7.75<sub>(sp+ap)</sub> (m, 2H, H<sub>Ar</sub>), 7.62<sub>(sp+ap)</sub> (dd,  $J = 8.8, 2.2$  Hz, 2H, H<sub>Ar</sub>), 7.40<sub>(sp+ap)</sub> (ddd,  $J = 6.6, 5.4, 2.9$  Hz, 1H, H<sub>Ar</sub>), 7.33<sub>(sp+ap)</sub> (d,  $J = 7.7$  Hz, 2H, H<sub>Ar</sub>), 7.18<sub>(sp+ap)</sub> (d,  $J = 7.8$  Hz, 2H, H<sub>Ar</sub>), 7.05<sub>(sp+ap)</sub> (dd,  $J = 8.9, 2.2$  Hz, 2H, H<sub>Ar</sub>), 5.08<sub>(sp+ap)</sub> (s, 2H, O-CH<sub>2</sub>), 4.60<sub>sp</sub>, 4.19<sub>ap</sub> (s, 2H, S-CH<sub>2</sub>), 2.29<sub>sp</sub>, 2.08<sub>ap</sub> (s, 3H, CH<sub>3</sub>), <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>) δ (ppm) 168.65<sub>sp</sub>, 163.39<sub>ap</sub> (C=O), 159.99<sub>sp</sub>, 159.82<sub>ap</sub> (C<sub>Ar</sub>), 153.15<sub>sp</sub>, 152.74<sub>ap</sub> (N=CH-), 146.80, 145.77, 143.48, 137.20, 133.69, 133.05<sub>sp</sub>, 132.99<sub>ap</sub> (C<sub>Ar</sub>), 129.01, 128.71, 128.44, 128.20, 127.94<sub>sp</sub>, 127.87<sub>ap</sub>, 126.76, 116.49, 116.39<sub>sp</sub>, 116.33<sub>ap</sub> (C<sub>Ar</sub>), 115.18, 69.27, 33.52<sub>ap</sub>, 32.63<sub>sp</sub> (S-CH<sub>2</sub>), 20.79. HRMS (ESI): Calc for [M+H<sup>+</sup>] C<sub>24</sub>H<sub>22</sub>N<sub>5</sub>O<sub>4</sub>S = 476.0956. Found = 476.0952

***(E)-2-(3-nitro-H-imidazo[1.2-a]pyridin-2-ylthio)-N'-(4-(4-methoxybenzyloxy)benzylidene)acetohydrazide (9o)***

Orange powder; M.p = 208-210 °C ; Yield = 72 % ; <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ (ppm) 11.66<sub>ap</sub>, 11.56<sub>sp</sub> (s, 1H, NH), 9.40 – 9.31<sub>(sp+ap)</sub> (m, 1H, H<sub>Ar</sub>), 8.16<sub>ap</sub>, 7.99<sub>sp</sub> (s, 1H, N=CH), 7.90 – 7.77<sub>(sp+ap)</sub> (m, 2H, H<sub>Ar</sub>), 7.64<sub>(sp+ap)</sub> (dd,  $J = 8.8, 3.1$  Hz, 2H, H<sub>Ar</sub>), 7.49 – 7.34<sub>(sp+ap)</sub> (m, 3H, H<sub>Ar</sub>), 7.06<sub>(sp+ap)</sub> (dd,  $J = 8.8 ; 2.0$  Hz, 2H, H<sub>Ar</sub>), 6.95<sub>(sp+ap)</sub> (d,  $J = 8.5$  Hz, 2H, H<sub>Ar</sub>), 5.06<sub>(sp+ap)</sub> (s, 2H, O-CH<sub>2</sub>), 4.61<sub>sp</sub>, 4.20<sub>ap</sub> (s, 2H, S-CH<sub>2</sub>), 3.75<sub>(sp+ap)</sub> (s, 3H, O-CH<sub>3</sub>). <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>) δ (ppm) 169.11<sub>sp</sub>, 163.84<sub>ap</sub> (C=O), 160.34<sub>sp</sub>, 159.55<sub>ap</sub> (C<sub>Ar</sub>), 153.61<sub>sp+ap</sub> (N=CH-), 146.25, 143.96, 133.47, 130.09, 129.17, 129.06, 128.91, 128.70, 127.18, 116.82, 115.67, 114.32, 69.62, 55.58, 33.08<sub>sp+ap</sub> (S-CH<sub>2</sub>). HRMS (ESI): Calc for [M+Na<sup>+</sup>] C<sub>24</sub>H<sub>21</sub>N<sub>5</sub>NaO<sub>5</sub>S = 514.1108. Found = 514.1105

***(E)-2-(3-nitro-H-imidazo[1.2-a]pyridin-2-ylthio)-N'-(4-(benzylthio)benzylidene)acetohydrazide (9p)***

Khaki powder; M.p = 250-252 °C; Yield = 72 %. <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ (ppm) 11.77<sub>ap</sub>, 11.62<sub>sp</sub> (s, 1H, NH), 9.35<sub>(sp+ap)</sub> (d,  $J = 6.8$  Hz, 1H, H<sub>Ar</sub>), 8.17<sub>ap</sub>, 8.00<sub>sp</sub> (s, 1H, N=CH-), 7.91 – 7.80<sub>(sp+ap)</sub> (m, 2H, H<sub>Ar</sub>), 7.66 – 7.56<sub>(sp+ap)</sub> (m, 2H, H<sub>Ar</sub>), 7.46-7.39<sub>(sp+ap)</sub> (m, 5H, H<sub>Ar</sub>), 7.34 – 7.27<sub>(sp+ap)</sub> (m, 2H, H<sub>Ar</sub>), 7.27 – 7.23<sub>(sp+ap)</sub> (m, 1H, H<sub>Ar</sub>), 4.62<sub>sp</sub>, 4.31<sub>ap</sub> (s, 2H, S-CH<sub>2</sub>), 4.39<sub>sp</sub>, 4.21<sub>ap</sub> (s, 2H, S-CH<sub>2</sub>). <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>) δ (ppm) 168.80<sub>sp</sub>, 163.57<sub>ap</sub> (C=O), 153.07<sub>sp</sub>, 152.69<sub>ap</sub> (N=CH-), 146.35<sub>sp</sub>, 145.76<sub>ap</sub> (C<sub>Ar</sub>), 143.10, 139.10<sub>sp</sub>, 138.82<sub>ap</sub> (C<sub>Ar</sub>), 137.15<sub>sp</sub>, 137.09<sub>ap</sub> (C<sub>Ar</sub>), 133.00, 131.32, 129.85, 128.86<sub>sp</sub>, 128.83<sub>ap</sub> (C<sub>Ar</sub>), 128.53<sub>sp</sub>, 128.44<sub>ap</sub> (C<sub>Ar</sub>), 128.22, 127.96, 127.66, 127.54<sub>sp</sub>, 127.51<sub>ap</sub> (C<sub>Ar</sub>), 127.27<sub>sp</sub>, 127.16<sub>ap</sub> (C<sub>Ar</sub>), 126.43, 116.50<sub>sp</sub>, 116.33<sub>ap</sub> (C<sub>Ar</sub>), 35.92, 33.49<sub>ap</sub>, 32.58<sub>sp</sub> (S-CH<sub>2</sub>). HRMS (ESI): Calc for [M+Na<sup>+</sup>] C<sub>23</sub>H<sub>19</sub>N<sub>5</sub>NaO<sub>3</sub>S<sub>2</sub> = 500.1021. Found = 500.1025

***(E)-2-(3-nitro-H-imidazo[1.2-a]pyridin-2-ylthio)-N'-((thiophen-2-yl)methylene)acetohydrazide (9q)***



Yellow powder ; M.p = 232-234 °C ; Yield = 80 % ,  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ )  $\delta$  (ppm) 11.70<sub>ap</sub>, 11.65<sub>sp</sub> (s, 1H; NH), 9.35<sub>(sp+ap)</sub> (dd,  $J = 6.9, 1.4$  Hz, 1H, H<sub>Ar</sub>), 8.43<sub>ap</sub>, 8.23<sub>sp</sub> (s, 1H; N=CH), 7.90 – 7.68<sub>(sp+ap)</sub> (m, 2H, H<sub>Ar</sub>), 7.72 – 7.60<sub>(sp+ap)</sub> (m, 1H ; H<sub>Ar</sub>). 7.44 (m, 2H, H<sub>Ar</sub>), 7.13 (dd,  $J = 5.1, 3.6$  Hz, 1H, H<sub>Ar</sub>), 4.54<sub>sp</sub>, 4.19<sub>ap</sub> (s, 2H; S-CH<sub>2</sub>).  $^{13}\text{C}$  NMR (75 MHz, DMSO- $d_6$ )  $\delta$  (ppm) 168.98<sub>sp</sub>, 163.98<sub>ap</sub> (C=O), 153.56<sub>sp</sub>, 153.13<sub>ap</sub> (N=CH), 146.25, 142.59, 139.32<sub>sp</sub>, 139.26<sub>ap</sub> (C<sub>Ar</sub>), 139.15, 133.54<sub>sp</sub>, 133.47<sub>ap</sub> (C<sub>Ar</sub>), 131.60, 131.11, 129.50, 129.06, 128.66, 128.44<sub>sp</sub>, 128.34<sub>ap</sub> (C<sub>Ar</sub>), 116.98, 116.88<sub>sp</sub>, 116.80<sub>ap</sub> (C<sub>Ar</sub>), 33.99<sub>ap</sub>, 32.92<sub>sp</sub> (S-CH<sub>2</sub>). HRMS (ESI) : Calc for [M+H<sup>+</sup>] C<sub>14</sub>H<sub>12</sub>N<sub>5</sub>O<sub>3</sub>S<sub>2</sub> = 362.0264. Found = 362.0267

***(E)-2-(3-nitro-H-imidazo[1.2-a]pyridin-2-ylthio)-N'-((furan-2-yl)methylene)acetohydrazide (9r)***

Yellow powder; M.p = 224-225 °C ; Yield = 81 % ;  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ )  $\delta$  (ppm) 11.73<sub>ap</sub>, 11.64<sub>sp</sub> (s, 1H, NH), 9.34<sub>(sp+ap)</sub> (dt,  $J = 6.9, 1.2$  Hz, 1H, H<sub>Ar</sub>), 8.11<sub>ap</sub>, 7.94<sub>sp</sub> (s, 1H, N=CH), 7.91 – 7.76<sub>(sp+ap)</sub> (m, 3H, H<sub>Ar</sub>), 7.42<sub>(sp+ap)</sub> (ddd,  $J = 8.7, 5.7, 2.6$  Hz, 1H, H<sub>Ar</sub>), 6.95 – 6.87<sub>(sp+ap)</sub> (m, 1H, H<sub>Ar</sub>), 6.62<sub>(sp+ap)</sub> (td,  $J = 3.7, 1.8$  Hz, 1H, H<sub>Ar</sub>), 4.55<sub>sp</sub>, 4.19<sub>ap</sub> (s, 2H, S-CH<sub>2</sub>).  $^{13}\text{C}$  NMR (75 MHz, DMSO- $d_6$ )  $\delta$  (ppm) 168.68<sub>sp</sub>, 163.66<sub>ap</sub> (C=O), 153.14<sub>sp</sub>, 152.69<sub>ap</sub> (N=CH), 149.21<sub>sp</sub>, 149.02<sub>ap</sub> (C<sub>Ar</sub>), 145.83, 145.28<sub>sp</sub>, 145.12<sub>ap</sub> (C<sub>Ar</sub>), 136.80, 133.93, 133.10<sub>sp</sub>, 133.03<sub>ap</sub> (C<sub>Ar</sub>), 128.22, 116.54<sub>sp</sub>, 116.45<sub>ap</sub> (C<sub>Ar</sub>), 116.35, 113.94<sub>sp</sub>, 113.84<sub>ap</sub> (C<sub>Ar</sub>), 112.23, 33.50<sub>ap</sub>, 33.01<sub>sp</sub> (S-CH<sub>2</sub>). HRMS (ESI): Calc for [M+H<sup>+</sup>] C<sub>14</sub>H<sub>12</sub>N<sub>5</sub>O<sub>4</sub>S = 346.0368. Found = 346.0365.

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### **Conflict of interest**

The authors declare no conflicts of interest regarding the publication of this paper

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## References

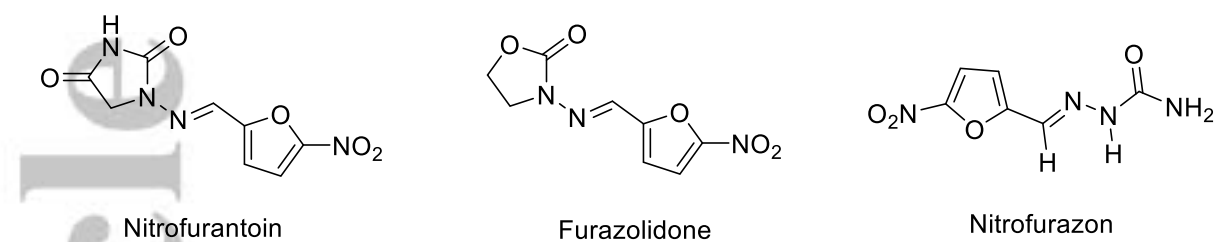
1. Nasr, T., Bondock, S., and Youns, M. (2014) Anticancer activity of new coumarin substituted hydrazide-hydrazone derivatives. *Eur. J. Med. Chem.*, **76**, 539–548.
2. Morcoss, M., Abdelhafez, E.S., Abdel-Rahman, H., Abdel-Aziz, M., and Abou El-Ella, D. (2020) Novel Benzimidazole/Hydrazone Derivatives as Promising Anticancer Lead Compounds: Design, Synthesis, and Molecular Docking Study. *J. Adv. Biomed. Pharm. Sci.*, **3** (2), 45–52.
3. Ajani, O.O., Iyaye, K.T., Audu, O.Y., Olorunshola, S.J., Kuye, A.O., and Olanrewaju, I.O. (2018) Microwave Assisted Synthesis and Antimicrobial Potential of Quinoline-Based 4-Hydrazide-Hydrazone Derivatives. *J. Heterocycl. Chem.*, **55** (1), 302–312.
4. Saidugari, S., and Rao, L. (2017) Synthesis, characterization and antibacterial activity of (E)-4-((3-methyl-4-(methylsulfonyl)pyridin-2-yl)methoxy)-N<sup>2</sup>-(substitutedbenzylidene)benzohydrazide derivatives. *Indian J. Chem.*, **56** (February), 177–182.
5. Morjan, R.Y., Mkadmh, A.M., Beadham, I., Elmanama, A.A., Mattar, M.R., Raftery, J., Pritchard, R.G., Awadallah, A.M., and Gardiner, J.M. (2014) Antibacterial activities of novel nicotinic acid hydrazides and their conversion into N-acetyl-1,3,4-oxadiazoles Dedicated to Professor Abdel-Rahman S. Ferwanah on the occasion of his retirement. *Bioorganic Med. Chem. Lett.*, **24** (24), 5796–5800.
6. Pelin, Ç., Küçükgülzel, G., and Rollas, S. (2010) Synthesis and evaluation of antiviral , antitubercular and anticancer activities of some novel thioureas derived from 4-aminobenzohydrazide hydrazones. *Marmara Pharm. J.*, **13**, 13–20.
7. Senkardes, S., Kaushik-Basu, N., Durmaz, I., Manvar, D., Basu, A., Atalay, R., and Guniz Kucukguzel, S. (2016) Synthesis of novel diflunisal hydrazide-hydrazones as anti-hepatitis C virus agents and hepatocellular carcinoma inhibitors. *Eur. J. Med. Chem.*, **108**, 301–308.
8. Leal, C.M., Pereira, S.L., Kümmerle, A.E., Leal, D.M., Tesch, R., De Sant'Anna, C.M.R., Fraga, C.A.M., Barreiro, E.J., Sudo, R.T., and Zapata-Sudo, G. (2012) Antihypertensive profile of 2-thienyl-3,4-methylenedioxybenzoylhydrazone is mediated by activation of the A2A adenosine receptor. *Eur. J. Med. Chem.*, **55**, 49–57.
9. Li, L., Li, Z., Liu, M., Shen, W., Wang, B., Guo, H., and Lu, Y. (2016) Design, synthesis

- and antimycobacterial activity of novel imidazo[1,2-a]pyridine amide-cinnamamide hybrids. *Molecules*, **21** (1), 1–14.
10. Kumar, S., Sharma, N., Maurya, I.K., Bhasin, A.K.K., Wangoo, N., Brandão, P., Félix, V., Bhasin, K.K., and Sharma, R.K. (2016) Facile synthesis, structural evaluation, antimicrobial activity and synergistic effects of novel imidazo[1,2-a]pyridine based organoselenium compounds. *Eur. J. Med. Chem.*, **123**, 916–924.
  11. Klenc, J., Raux, E., Barnes, S., Sullivan, S., Duszynska, B., Bojarski, A.J., and Streckowski, L. (2009) Synthesis of 4-Substituted 2- ( 4-Methylpiperazino ) pyrimidines and Quinazoline Analogs as Serotonin 5-HT 2A Receptor Ligands. *J. Heterocycl. Chem.*, **46** (6), 1259–1265.
  12. Ge, X., Feng, Z., Xu, T., Wu, B., Chen, H., Xu, F., Fu, L., Shan, X., Dai, Y., Zhang, Y., and Liang, G. (2016) A novel imidazopyridine derivative, X22, attenuates sepsis-induced lung and liver injury by inhibiting the inflammatory response in vitro and in vivo. *Drug Des. Devel. Ther.*, **10**, 1947–1959.
  13. Chitti, S., Singireddi, S.R., Santosh Kumar Reddy, P., Trivedi, P., Bobde, Y., Kumar, C., Rangan, K., Ghosh, B., and Sekhar, K.V.G.C. (2019) Design, synthesis and biological evaluation of 2-(3,4-dimethoxyphenyl)-6 (1,2,3,6-tetrahydropyridin-4-yl)imidazo[1,2-a]pyridine analogues as antiproliferative agents. *Bioorganic Med. Chem. Lett.*, **29** (18), 2551–2558.
  14. Reddy Gangireddy, M., Mantipally, M., Gundla, R., Nayak Badavath, V., Paidikondala, K., and Yamala, A. (2019) Design and Synthesis of Piperazine-Linked Imidazo[1,2-a]pyridine Derivatives as Potent Anticancer Agents. *ChemistrySelect*, **4** (46), 13622–13629.
  15. Chezal, J.M., Paeshuyse, J., Gaumet, V., Canitrot, D., Maisonial, A., Lartigue, C., Gueiffier, A., Moreau, E., Teulade, J.C., Chavignon, O., and Neyts, J. (2010) Synthesis and antiviral activity of an imidazo[1,2-a]pyrrolo[2,3-c]pyridine series against the bovine viral diarrhoea virus. *Eur. J. Med. Chem.*, **45** (5), 2044–2047.
  16. Rozada, A.M.F., Rodrigues, F.A.V., Sampiron, E.G., Seixas, F.A.V., Basso, E.A., Scodro, R.B.L., Kioshima, É.S., and Gauze, G.F. (2019) Novel 4-methoxynaphthalene-N-acylhydrazones as potential agents for the treatment of paracoccidioidomycosis and tuberculosis co-infection. *Future Microbiol.*, **14** (7), 587–598.
  17. Velezheva, V., Brennan, P., Ivanov, P., Kornienko, A., Lyubimov, S., Kazarian, K., Nikonenko, B., Majorov, K., and Apt, A. (2016) Synthesis and antituberculosis activity of indole-pyridine derived hydrazides, hydrazide-hydrazones, and thiosemicarbazones.

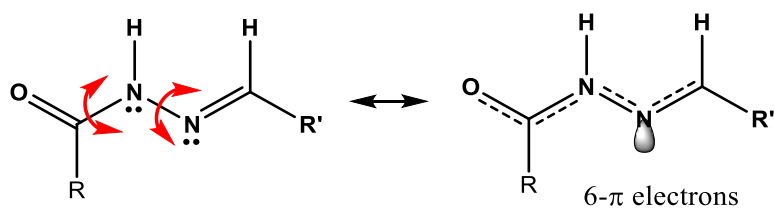
- Bioorganic Med. Chem. Lett.*, **26** (3), 978–985.
18. De Miranda, A.S., Bispo, W., Da Silva, Y.K.C., Magna Suzana Alexandre-Moreira, De Paula Castro, R., Sabino, J.R., Lião, L.M., Lima, L.M., and Barreiro, E.J. (2012) Design, synthesis, antinociceptive and anti-inflammatory activities of novel piroxicam analogues. *Molecules*, **17** (12), 14126–14145.
  19. McCalla, D.R., Reuvers, A., and Kaiser, C. (1970) Mode of Action of Nitrofurazone. *J. Bacteriol.*, **104** (3), 1126–1134.
  20. Munoz-Davila, M.J. (2014) Role of Old Antibiotics in the Era of Antibiotic Resistance. Highlighted Nitrofurantoin for the Treatment of Lower Urinary Tract Infections. *Antibiotics*, **3**, 39–48.
  21. Mc Osker, C.C., and Fitzpatrick, P.M. (1994) Nitrofurantoin: Mechanism of action and implications for resistance development in common uropathogens. *J. Antimicrob. Chemother.*, **33**, 23–30.
  22. Reis, D.C., Despaigne, A.A.R., Da Silva, J.G., Silva, N.F., Vilela, C.F., Mendes, I.C., Takahashi, J.A., and Beraldo, H. (2013) Structural studies and investigation on the activity of imidazole-derived thiosemicarbazones and hydrazones against crop-related fungi. *Molecules*, **18** (10), 12645–12662.
  23. Ramzan, A., Siddiqui, S., Irfan, A., Al-Sehemi, A.G., Ahmad, A., Verpoort, F., Chughtai, A.H., Khan, M.A., Munawar, M.A., and Basra, M.A.R. (2018) Antiplatelet activity, molecular docking and QSAR study of novel N'-arylmethylidene-3-methyl-1-phenyl-6-p-chlorophenyl-1H-pyrazolo[3,4-b] pyridine-4-carbohydrazides. *Med. Chem. Res.*, **27** (2), 388–405.
  24. Wyrzykiewicz, E., Błaszczuk, A., and Turowska-Tyrk, I. (2000) N-(E)-2-Stilbenyloxymethylenecarbonyl substituted hydrazones of ortho, meta and para hydroxybenzaldehydes. *Bull. Polish Acad. Sci. Chem.*, **48** (3), 213–229.
  25. Palla, G., Predieri, G., Domiano, P., Vignali, C., and Turner, W. (1986) Conformational behaviour and E/Z isomerization of N-acyl and N-aroilydrazones. *Tetrahedron*, **42** (13), 3649–3654.
  26. Syakaev, V. V., Podyachev, S.N., Buzykin, B.I., Latypov, S.K., Habicher, W.D., and Kononov, A.I. (2006) NMR study of conformation and isomerization of aryl- and heteroarylaldehyde 4-tert-butylphenoxyacetylhydrazones. *J. Mol. Struct.*, **788** (1–3), 55–62.
  27. Munir, R., Javid, N., Zia-Ur-rehman, M., Zaheer, M., Huma, R., Roohi, A., and Athar, M.M. (2021) Synthesis of novel n-acylhydrazones and their c-n/n-n bond

- conformational characterization by nmr spectroscopy. *Molecules*, **26** (16), 1–18.
28. Oleynik, A.S., Pevneva, N.Y.U., Kandalintseva, N. V, Prosenko, A.E., Khoshchenko, O.M., and Dushkin, M.I. (2008) Synthesis and Biological Activity of Hydrophilic Alkylphenols. *Chem. Sustain. Dev.*, **16**, 551–556.
29. Ibatullin, F.M., Selivanov, S.I., and Shavva, A.G. (2001) A general procedure for conversion of S-glycosyl isothiourea derivatives into thioglycosides, thiooligosaccharides and glycosyl thioesters. *Synthesis (Stuttg.)*, **2001** (3), 419–422.

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**Figure 1:** Structures of commercial drug hydrazide-hydrazone based molecules

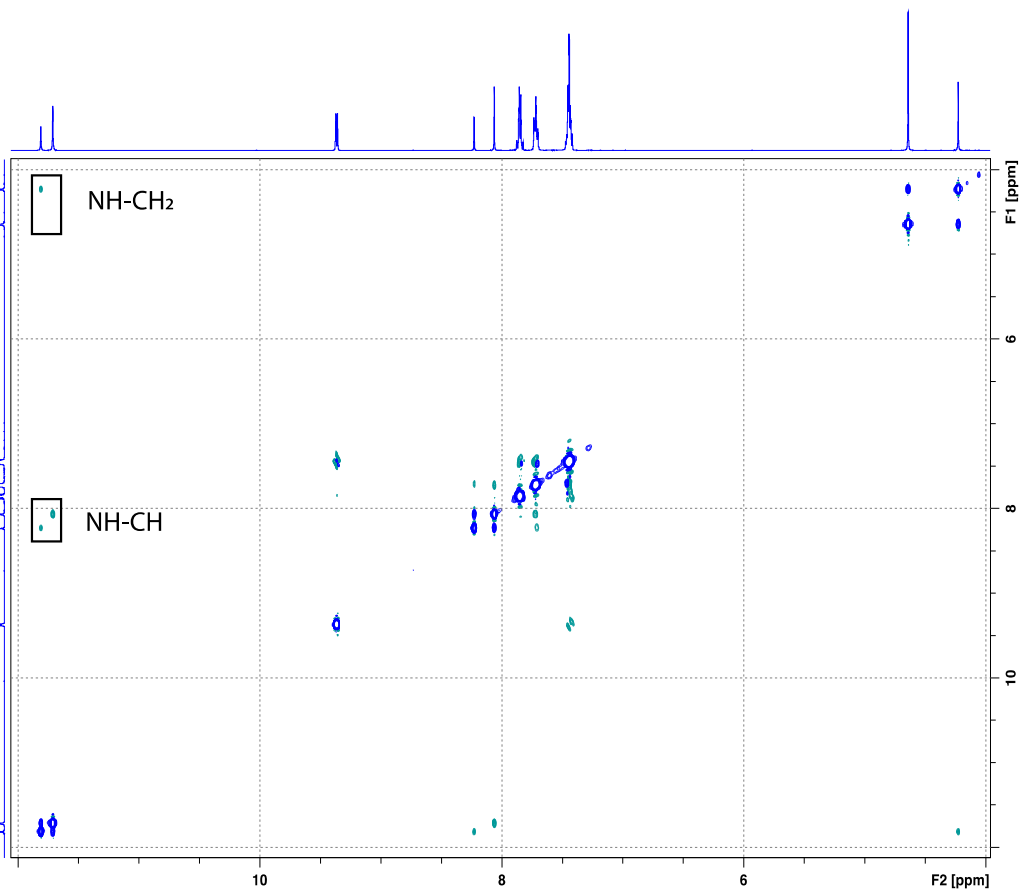


**Figure 2:** Four possible rotamers of the *N*-acylhydrazone moiety

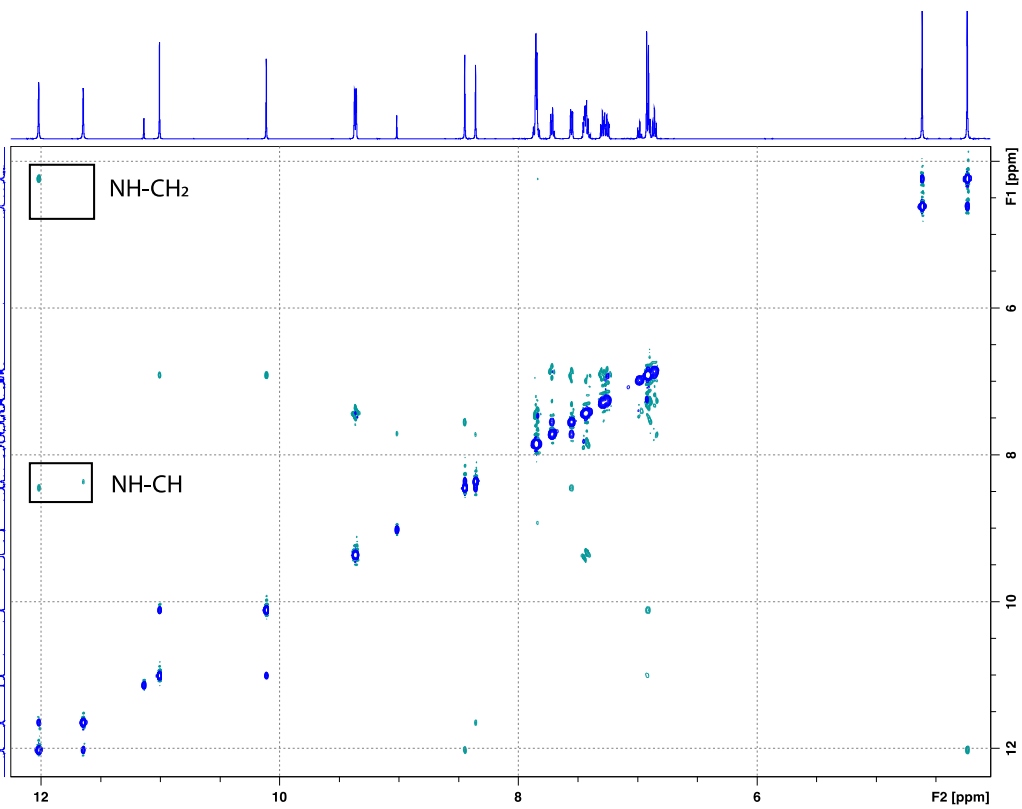
**Figure 4:** Structure of conformers *syn*- and *anti-periplanar*  $E_{(C=N)(N-N)}$  of *N*-acylhydrazones

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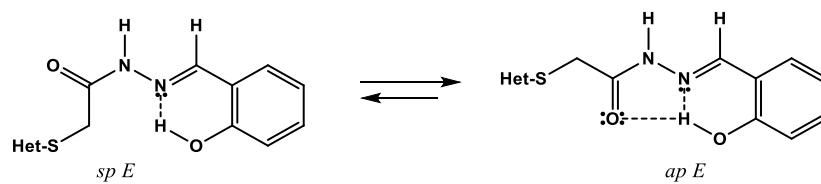




**Figure 5:** NOESY Spectrum NMR of compound **9a**

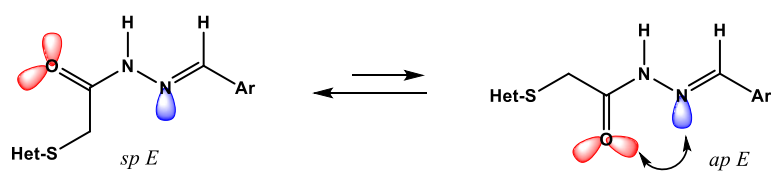


**Figure 6:** NOESY Spectrum NMR of compound **9b** in DMSO-*d*<sub>6</sub>

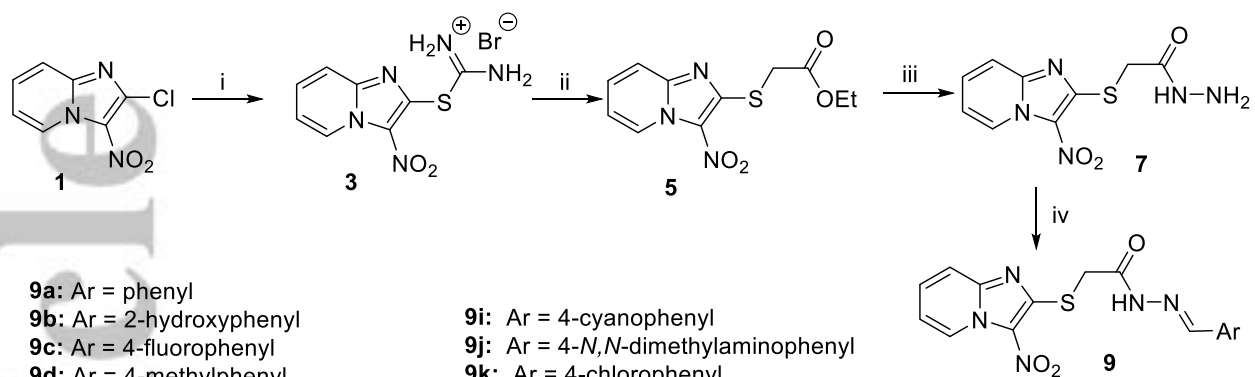


**Figure 7:** Putative explanation for the slight predominance of the *ap E* conformer of

**9b**



**Figure 8:** Possible explanation for the difference of stability of *ap E* and *sp E* conformers



**9a:** Ar = phenyl

**9b:** Ar = 2-hydroxyphenyl

**9c:** Ar = 4-fluorophenyl

**9d:** Ar = 4-methylphenyl

**9e:** Ar = 4-nitrophenyl

**9f:** Ar = 4-methoxyphenyl

**9g:** Ar = 4-hydroxy-3-methoxyphenyl

**9h:** Ar = 4-hydroxyphenyl

**9i:** Ar = 4-cyanophenyl

**9j:** Ar = 4-*N,N*-dimethylaminophenyl

**9k:** Ar = 4-chlorophenyl

**9l:** Ar = 3-brom-4-hydroxyphenyl

**9m:** Ar = 3,5-dichlorophenyl

**9n:** Ar = 4-methylbenzyloxyphenyl

**9o:** Ar = 4-methoxybenzyloxyphenyl

**9p:** Ar = benzylthiophenyl

**9q:** Ar = thiophen-2-yl

**9r:** Ar = furan-2-yl

**Scheme 1:** Synthesis of N-acylhydrazones (**9a-r**)

**Table 1: Chemical shifts of compounds 9a-r**

Compound	Chemical Shift $\delta$ (ppm)						Ar Nature
	C(O)-NH		N=CH		CH <sub>2</sub> -CO		
	syn-	anti-	syn-	anti-	syn-	anti-	
9a	11.67	11.77	8.05	8.22	4.62	4.21	C <sub>6</sub> H <sub>5</sub>
9b	11.01	11.14	8.36	8.44	4.60	4.22	2-OH-C <sub>6</sub> H <sub>4</sub>
9c	11.68	11.79	8.05	8.22	4.61	4.23	4-F-C <sub>6</sub> H <sub>4</sub>
9d	11.62	11.72	8.00	8.17	4.61	4.21	4-Me-C <sub>6</sub> H <sub>4</sub>
9e	11.98	12.08	8.20	8.31	4.68	4.36	4-NO <sub>2</sub> -C <sub>6</sub> H <sub>4</sub>
9f	11.55	11.66	7.99	8.15	4.60	4.19	4-MeOH-C <sub>6</sub> H <sub>4</sub>
9g	11.52	11.61	7.93	8.09	4.61	4.19	4-OH,3-MeOH-C <sub>6</sub> H <sub>3</sub>
9h	11.48	11.58	7.94	8.10	4.59	4.19	4-OH-C <sub>6</sub> H <sub>4</sub>
9i	11.92	12.02	8.10	8.28	4.66	4.22	4-CN-C <sub>6</sub> H <sub>4</sub>
9j	11.40	11.48	7.91	8.06	4.60	4.20	4-N(CH <sub>3</sub> ) <sub>2</sub> -C <sub>6</sub> H <sub>4</sub>
9k	11.75	11.86	8.04	8.22	4.63	4.22	4-Cl-C <sub>6</sub> H <sub>4</sub>
9l	11.58	11.68	7.91	8.07	4.60	4.17	3-Br-4-OH-C <sub>6</sub> H <sub>3</sub>
9m	11.90	-	8.38	8.56	4.63	4.27	3,4-Cl <sub>2</sub> -C <sub>6</sub> H <sub>3</sub>
9n	11.55	11.65	7.98	8.15	4.60	4.19	4-Me-C <sub>6</sub> H <sub>4</sub> O-C <sub>6</sub> H <sub>4</sub>
9o	11.56	11.66	7.99	8.16	4.61	4.20	4-MeOH-C <sub>6</sub> H <sub>4</sub> O-C <sub>6</sub> H <sub>4</sub>
9p	11.66	11.77	8.00	8.17	4.62	4.31	4-C <sub>6</sub> H <sub>4</sub> S-C <sub>6</sub> H <sub>4</sub>
9q	11.65	11.70	8.23	8.43	4.54	4.19	C <sub>5</sub> H <sub>3</sub> S
9r	11.63	11.73	7.94	8.11	4.55	4.19	C <sub>5</sub> H <sub>3</sub> O

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**Table 2:** Estimated ratio *sp E/ap E* for compounds **9a-r** in DMSO-*d*<sub>6</sub>

Compound	syn- conformer		anti- conformer		Ratio (%) (syn-: anti-)
	$\delta$ SCH <sub>2</sub> (ppm)	Height	$\delta$ SCH <sub>2</sub> (ppm)	Height	
9a	4.62	1.21	4.21	0.71	63 : 37
9b	4.60	0.90	4.22	1.04	46 : 54
9c	4.62	0.96	4.21	0.60	61 : 39
9d	4.61	1.25	4.21	0.67	65 : 35
9e	4.60	1.14	4.19	0.68	63 : 37
9f	4.60	1.21	4.19	0.70	63 : 37
9g	4.61	1.16	4.19	0.76	60 : 40
9h	4.59	1.29	4.19	0.69	65 : 35
9i	4.66	1.24	4.22	0.60	67 : 32
9j	4.60	1.14	4.20	0.77	60 : 40
9k	4.66	1.23	4.22	0.62	67 : 33
9l	4.60	1.35	4.17	0.83	62 : 38
9m	4.63	1.14	4.21	0.71	62 : 38
9n	4.60	1.19	4.19	0.68	64 : 36
9o	4.29	1.61	4.20	0.70	70 : 30
9p	4.60	1.04	4.20	0.59	64 : 36
9q	4.54	1.26	4.19	0.57	69 : 31
9r	4.55	1.24	4.19	0.58	68 : 32

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**Table 3:** Carbon Chemical shifts of the different 9a-r compounds

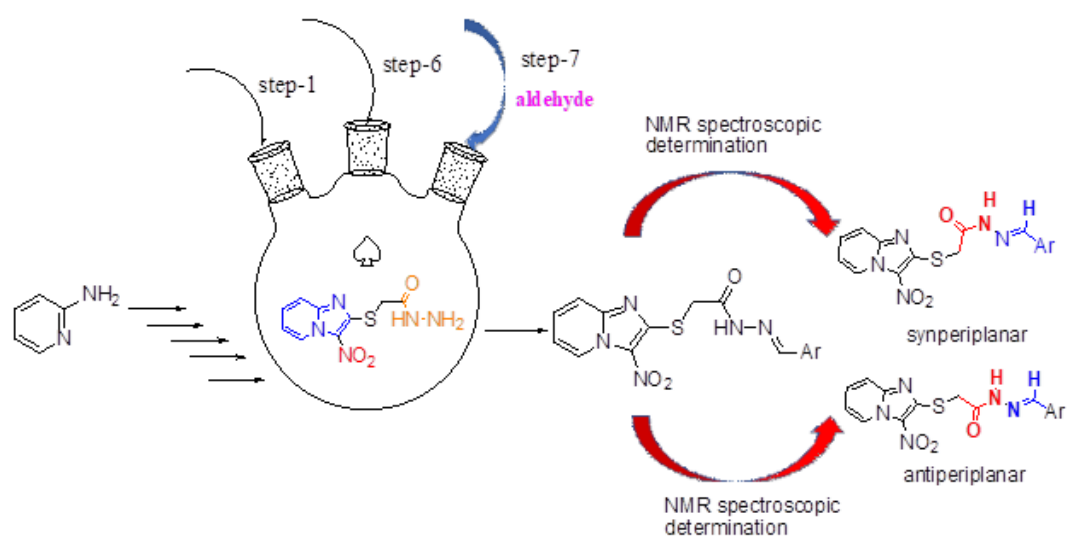
Compound	Chemical Shifts $\delta$ (ppm)						
	C(O)-NH		N=CH		CH <sub>2</sub> -CO		Ar
	syn-	anti-	syn-	anti-	syn-	anti-	
9a	169.39	164.18	153.54	153.15	33.08	33.95	C <sub>6</sub> H <sub>5</sub>
9b	169.03	164.07	153.54	153.06	33.12	33.65	2-OH-C <sub>6</sub> H <sub>4</sub>
9c	168.00	162.67	153.11	152.73	32.60	33.50	4-F-C <sub>6</sub> H <sub>4</sub>
9d	168.19	162.97	152.56	152.15	33.10	32.97	4-Me-C <sub>6</sub> H <sub>4</sub>
9e	169.79	164.71	153.37	153.04	32.99	33.95	4-NO <sub>2</sub> -C <sub>6</sub> H <sub>4</sub>
9f	168.67	163.43	153.18	152.76	32.68	33.54	4-MeOH-C <sub>6</sub> H <sub>4</sub>
9g	169.02	163.75	153.71	153.22	33.15	33.98	4-OH,3-MeOH-C <sub>6</sub> H <sub>3</sub>
9h	168.50	163.23	153.18	152.76	32.68	33.52	4-OH-C <sub>6</sub> H <sub>4</sub>
9i	169.23	164.08	152.92	152.58	32.46	33.48	4-CN-C <sub>6</sub> H <sub>4</sub>
9j	168.97	163.78	153.11	152.73	32.60	33.50	4-N(CH <sub>3</sub> ) <sub>2</sub> -C <sub>6</sub> H <sub>4</sub>
9k	169.23	164.03	153.29	152.92	32.81	33.75	4-Cl-C <sub>6</sub> H <sub>4</sub>
9l	169.14	163.94	153.59	153.23	33.08	33.92	3-Br-4-OH-C <sub>6</sub> H <sub>3</sub>
9m	169.60	164.51	153.39	153.04	32.97	33.87	3,4-Cl <sub>2</sub> -C <sub>6</sub> H <sub>3</sub>
9n	168.65	163.39	153.15	152.74	32.63	33.52	4-Me-C <sub>6</sub> H <sub>4</sub> O-C <sub>6</sub> H <sub>4</sub>
9o	169.11	163.84	153.61	-*	33.08	-*	4-MeOH-C <sub>6</sub> H <sub>4</sub> O-C <sub>6</sub> H <sub>4</sub>
9p	168.80	163.57	153.07	152.69	32.58	33.49	4-C <sub>6</sub> H <sub>4</sub> S-C <sub>6</sub> H <sub>4</sub>
9q	168.98	163.98	153.56	153.13	33.92	33.99	C <sub>5</sub> H <sub>3</sub> S
9r	168.68	163.66	153.14	152.69	33.01	33.50	C <sub>5</sub> H <sub>3</sub> O

-\* No signal duplication was observed.

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