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Original article

Schiff bases of indoline-2,3-dione (isatin) derivatives and nalidixic acid carbohydrazide, synthesis, antitubercular activity and pharmacophoric model building

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ABSTRACT

Tuberculosis (TB) remains among the world's great public health challenges. Worldwide resurgence of TB is due to two major problems: the AIDS epidemic, which started in the mid-1980s, and the outbreak of multidrug resistant (MDR) TB. Thus, there is an urgent need for anti-TB drugs with enhanced activity against MDR strains. In recent years, Schiff bases of 1H-indole-2,3-diones are reported to exhibit anti-TB activity. On the other hand, several quinolone antibacterial agents have been examined as inhibitors of TB, as well as other mycobacterial infections. Accordingly, the current work involved design and synthesis of Schiff bases of nalidixic acid carbohydrazide and isatin derivatives (**5**,**6a**–**f** and **7**,**8a**–**c**). Structures of the synthesized derivatives were confirmed on the bases of spectral methods of analyses. Anti-TB activity of the synthesized derivatives was investigated against four *Mycobacterium* strains: *Mycobacterium intercellulari*, *Mycobacterium xenopi*, *Mycobacterium cheleneo* and *Mycobacterium smegmatis*. Modest anti-TB activity was observed within the investigated compounds, however, compound **5f** revealed potent anti-TB activity with MIC 0.625 μg/ml, which is 20 times greater than the reference drug isoniazid, INH, (MIC = 12.5 μg/ml). A hypothetical pharmacophore model was built using Molecular Operating Environment (MOE) program and 10 compounds structurally related to the synthesized ones with reported anti-TB activity. The Pharmacophoric model built revealed the necessity of the following pharmacophoric features for anti-TB activity: aromatic center, hydrogen bond acceptor/metal ligator center, hydrogen bond donor center and aromatic center/hydrophobic area. Theses features were consistent with the found anti-TB activity of the tested compounds.

1. Introduction

Although drugs for treatment of tuberculosis (TB) have been available for nearly 50 years, TB remains a global health crisis, killing 2–3 million people annually and for a global economic toll of $12 billion each year [1]. The recent emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis resulted in a major setback in the global fight against TB. The prevailing situation is made worse by the continuous increase in the number of immune-compromised patients living with HIV who are more prone to TB and other bacterial infections [2–4]. These results have further stimulated research efforts globally [5]. No new drugs have been developed specifically against mycobacteria since the 1960s and only within the last few years have some promising drug candidates emerged [6,7]. Thus, more than ever, there is an urgent need to develop new anti-TB drugs to combat the spread of TB, particularly in its hard-to-kill multidrug-resistant, persistent and latent forms.

There are two basic approaches to develop a new drug for TB:

i Synthesis of new analogues, modifications or derivatives of existing compounds for shortening and improving TB treatment [8].
ii Searching for novel structures, that the TB organism has never been presented with before, for the treatment of MDR-TB [9].

To pursue this goal, the current work is directed to synthesize derivatives starting from chemical moieties reported to have potential antimycobacterial activity. Indoline-2,3-dione (isatin) derivatives are reported to show antitubercular activities [8,10]. Moreover, it was recently reported that indoline-2-one structural scaffold as potent DNA gyrase inhibitors [11]. DNA gyrase is a bacterial enzyme that catalyzes the introduction of negative supercoils in a closed-circular DNA using the energy of the ATP hydrolysis. Since it is found only in

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prokaryotes and is vital for their survival, it has become an attractive target for antibacterial agents [12]. Accordingly isatin is a versatile lead molecule for designing of potential antitubercular agent. In view of the antitubercular property of this pharmacophore, it was envisaged that its combined effect with an active moiety may result in increased antitubercular activity [13]. In recent years, Schiff and Mannich bases of isatins are reported to exhibit anti-TB activity. Investigation of the structure–activity relationships in 1*H*-indoline-2,3-dione derivatives revealed that 5-halogenation, N-alkylation, and *N*-Mannich bases were effective in causing a marked enhancement in the activity against various bacteria [14]. Accordingly, design of the Schiff's bases of 5-bromo-1-substitued and/or 1-substitued isatin (alkyl, aralkyl or Mannich bases) and nalidixic acid carbohydrazide was undertaken. Evaluation of the anti-TB activity of the synthesized Schiff's bases together with the estimation of their pharmacophoric model using Molecular Operating Environment (MOE) software were of great interest.

2. Results and discussion

2.1. Chemistry

The designed Schiff's bases were obtained through 2 steps reaction as described in Scheme 1. The first step involved N-substitution of isatin (**1a**) or 5-bromoisatin (**1b**) to obtain the corresponding 1-alkyl, 1-benzyl and 1-hydroxymethyl derivatives. 1-Alkyl or 1-benzylisatin derivatives, **2** and **3**(**a**–**e**), were achieved in very good yields by reaction of convenient alkyl halide or benzyl chloride with **1a** and **1b** respectively in DMF using 1.5 equivalent of K_2CO_3 at 80 °C [13]. However, 1-hydroxymethyl isatin derivatives (**2f** and **3f**), were obtained in a good yield by the reaction of appropriate isatin with 40% formaldehyde in water [13]. The second step is the condensation of the 1-substituted isatins with nalidixic acid carbohydrazide (**4**). The later prepared according to a reported procedures [15]. The prepared derivatives were confirmed by spectral methods of analysis and were consistent with the proposed structures.

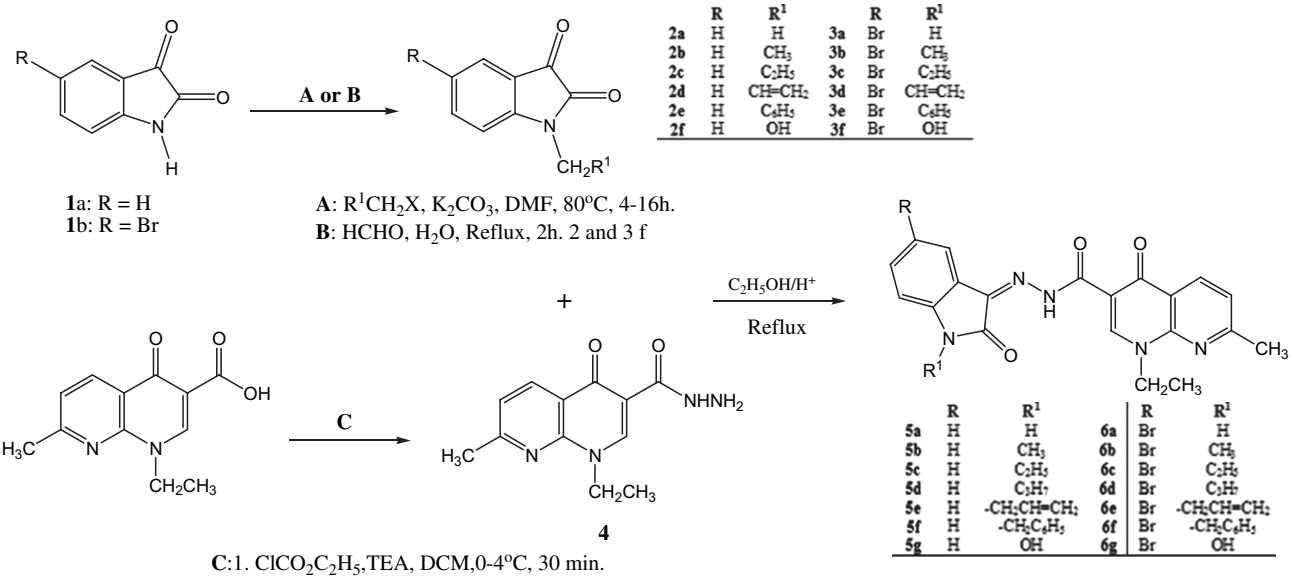
Mannich bases on the other hand were obtained from the reaction of the convenient Schiff's bases **5a** or **6a** with formaline and appropriate secondary amine, Scheme 2. Unfortunately the crystalline products obtained were insoluble in the available solvents, the phenomena that hindered the structural verification of these products by NMR. However, the mass spectra of these compounds were in agreement with their structures. An interesting observation appeared in the IR spectra of the Mannich derivatives, **7** and **8**(**a**–**c**), where distinguished absorption bands appeared around 2813.70–2940.54 cm^{-1} attributed to N–CH_2–N. This observation is consistent with similar reported compounds [16], and is another evidence for the formation of the Mannich bases.

2.2. Antitubercular screening of the target compounds

The synthesized compounds, **5**(**a**–**g**), **6**(**a**–**g**), **7**(**a**–**c**) and **8**(**a**–**c**), were evaluated for their antimycobacterial activity *in vitro* against four Mycobacterium strains: *Mycobacterium intercellulari* (ATCC 35743), *Mycobacterium xenopi* (ATCC 14470), *Mycobacterium chelaneo* (ATCC 35751) and *Mycobacterium smegmatis* (ATCC 35797) by agar dilution method according to the protocol described in the experimental section similar to that recommended by the National Committee for Clinical Laboratory Standards (NCCLS) for the determination of MIC [17]. Isoniazid (INH) was used as a reference drug and control experiments were done using a growth media free from drugs or the tested compounds. Results of the *in vitro* antitubercular activity of the tested compounds along with the standard drug for comparison are given in Table 1. The data of the antitubercular activity screening revealed that with the exception of compound **5f** all the other tested compounds did not show any considerable activity. The lonely active compound, **5f** was found to be 20 times more potent than the first line antitubercular drug INH *in vitro*. It is worthy to mention that the preliminary antimycobacterial evaluation results showed that unlike *N*-alkyl derivatives of isatin, its Mannich bases, **7** and **8**(**a**–**c**), are completely devoid of anti-TB activity. Most probably this observation may be attributed to the poor solubility of these derivatives in DMSO.

2.3. Pharmacophore generation

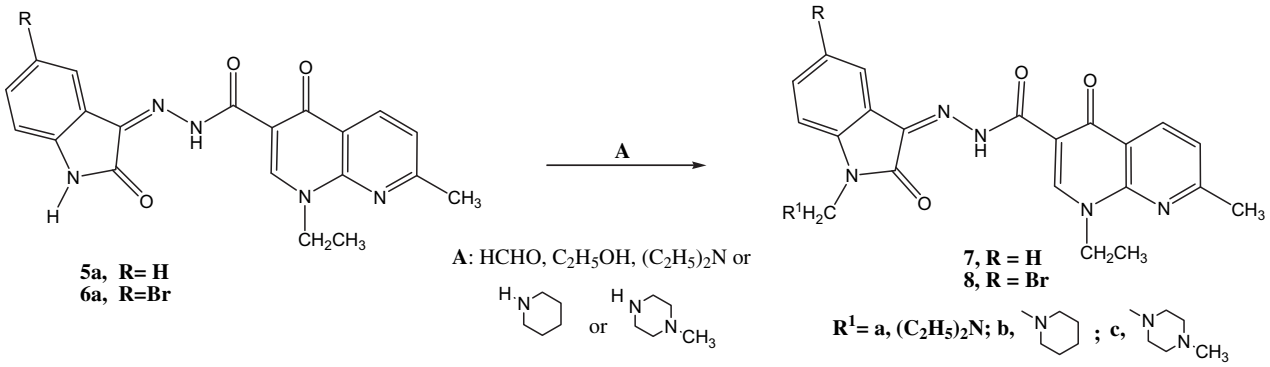
The aim of this approach is to generate and assess a pharmacophore model (hypothesis) derived from compounds of reported anti-TB activity [18]. Three steps are usually employed for 3D pharmacophore-based applications: First, it is necessary to



	R	R1		R	R1
2a	H	H	3a	Br	H
2b	H	CH_3	3b	Br	CH_3
2c	H	C_2H_5	3c	Br	C_2H_5
2d	H	$CH=CH_2$	3d	Br	$CH=CH_2$
2e	H	C_6H_5	3e	Br	C_6H_5
2f	H	OH	3f	Br	OH

	R	R1		R	R1
5a	H	H	6a	Br	H
5b	H	CH_3	6b	Br	CH_3
5c	H	C_2H_5	6c	Br	C_2H_5
5d	H	C_3H_7	6d	Br	C_3H_7
5e	H	$-CH_2CH=CH_2$	6e	Br	$-CH_2CH=CH_2$
5f	H	$-CH_2C_6H_5$	6f	Br	$-CH_2C_6H_5$
5g	H	OH	6g	Br	OH

Scheme 1.



Scheme 2.

Table 1
In vitro anti-TB activity of the synthesized compounds.

Compound	MIC (μg/ml)/Mycobacterium strain			
	M. intercellulari	*M. Xenopi*	*M. cheleneo*	*M. smegmatis*
5a	NA	NA	NA	100
5b	200	NA	NA	200
5c	200	200	200	200
5f	0.625	0.625	0.625	0.625
6a	NA	NA	NA	200
6c	100	100	NA	NA
6f	50	50	50	50
INH	12.5	12.5	12.5	12.5

NA: no activity at 200 μg/ml

generate 3D structures of training set molecules of known biological activity. This is generally achieved using automated 3D structure generation programs that translate 2D molecular descriptions to 3D ones. Second, the assignment of the pharmacophoric features. Finally, the application of a method for undertaking conformational searching of databases for new structures matching the generated pharmacophoric features [19]. These steps will be expounded in the building of a hypothetical pharmacophoric model for certain reported anti-TB compounds and testing the synthesized compounds **5**(**a**–**g**), **6**(**a**–**g**), **7**(**a**–**c**) and **8**(**a**–**c**). Chemical Computing Group's Molecular Operating Environment (MOE) is used for pharmacophore building. In MOE, 3D pharmacophore queries can contain locations of the features or chemical groups as well as restrictions on shape. Restriction on shape can be imposed by specifying the included and/or excluded volume areas. Furthermore, a consensus query from not one but a set of aligned molecules can be used for the 3D pharmacophore database search which provides high control. Consequently, both partial and systemic matching as well as flexible matching rules are offered [20]. The program expresses the degree of mapping of a given compound to a generated hypothetical model in term of rmsd value, which in turn correlated with compound's activity. Rmsd value refers to the root of the mean square distance between the query features and their matching ligand target points [21].

2.3.1. Generating a query

The developed query can be based on one conformation of the most active molecule. Alternatively, it can be based on an alignment of several active molecules (training set), a method that is used here. Such an alignment can be obtained by using MOE's flexible alignment, and all conformations of the molecules were considered for the alignment [21]. A hypothetical pharmacophore model was built using MOE program and 10 compounds structurally related to the synthesized ones with reported anti-TB activity as it was published recently in W. S. Abdel-Aal et al [18]. The classical pharmacophoric features include hydrogen bond acceptors (Acc), hydrogen bond donors (Don), charged or ionizable groups (Cat and Ani), hydrophobic (Hyd), metal ligator (ML) and/or aromatic rings (Aro) together with geometrical constraints like distances, angles and dihedral angles. To carry out pharmacophore analyses, relevant features in a molecule need to be identified. The initial pharmacophoric query was performed and it is composed of 4 features, as given in Table 2 and illustrated graphically in Fig. 1.

Table 2
Pharmacophoric and structure features of the training set.

Pharmacophoric features	Structure features
F1: Aro	*p*-substituted phenyl, 4-pyridyl
F2: Acc/ML	C=O
F3: Don	CONH–N=
F4: Aro/Hyd	Methyl, cyclohexyl, *p*-substituted phenyl, 4-pyridyl, 1-morpholinyl, 4-nitro-five membered heterocycles (furan or thiophene)

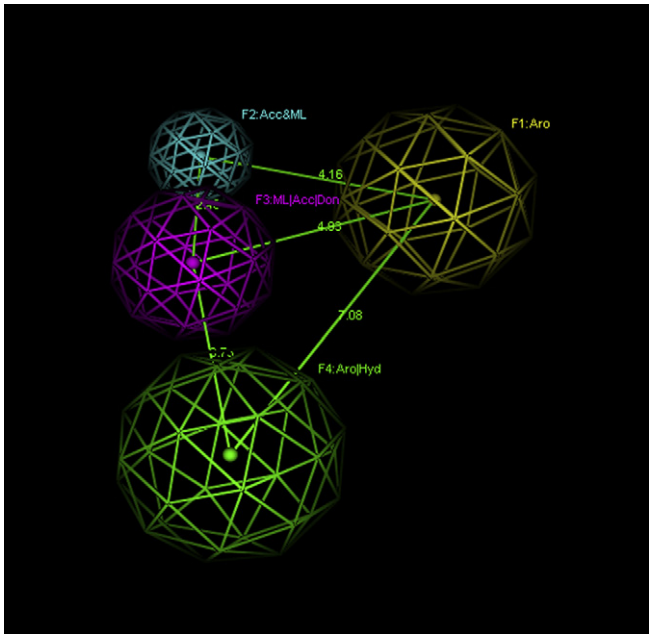


Fig. 1. Pharmacophore features and distances.

Table 3
Rmsd values of the hit set and their anti-TB activity (MIC).

Compound	MIC (μg/ml)	Rmsd
5a	100	0.5995
5b	200	0.7413
5c	200	0.7410
5d	NA	0.7405
5e	NA	0.7404
5f	**0.625**	**0.2479**
5g	NA	0.7412
6a	200	0.7431
6b	NA	0.7420
6c	100	0.7417
6d	NA	0.7414
6e	NA	0.7409
6f	**50**	**0.2508**
6g	NA	0.7416
7a	NA	0.7407
7b	NA	0.7411
7c	NA	0.7408
8a	NA	0.7411
8b	NA	0.7403
8c	NA	0.7415
INH	**12.5**	**0.2890**

2.3.2. Consensus query searching

A search of the test set using this new consensus query resulted in 2 hits, 5f and 6f. Results are listed in Table 3 and revealed that the lower the rmsd values the higher inhibitory activity. In these hits, the amidic moiety CONH–N=C fitted the region of the Don, F3, while the amidic carbonyl group fitted the region of the Acc/ML, F2. Superimposition of compound 5f, the most active compounds, with the pharmacophoric model is illustrated by Fig. 2. 5f has rmsd value of 0.2479 and MIC 0.625 μg/ml. In addition to F1 and F4, fitting the isatin aligned to the hydrophobic/aromatic feature F1 and the naphthyridine ring fitted to the aromatic feature F4. On the other hand, partially matching compounds to the pharmacophoric model is displayed by Fig. 3. Compound **6a**, with MIC 200 μg/ml, was selected as a representative example of the practically poor matching compounds showed fitting only to F1 and F4 features of the pharmacophoric model, extent of deviation of fitting from the other features was reflected by increased rmsd value.

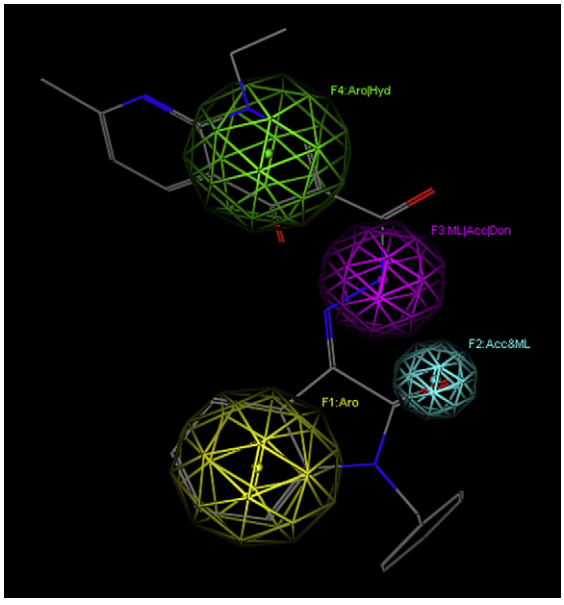


Fig. 2. Superposition of 5f (MIC 0.625 μg/ml) with the query.

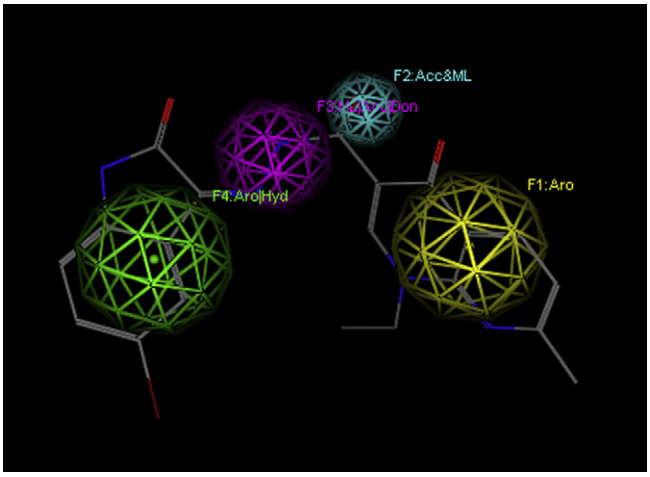


Fig. 3. Inadequate fitting of 6a (MIC 200 μg/ml) with the pharmacophore.

From these data it can be concluded that the bulky N_1-benzyl group in compounds **5f** and **6f** result in change of their orientation therefore, the two compounds have very good fit with the pharmacophore, similar to what was obtained with the reference drug INH. Nonetheless, the observed modest anti-TB activity of compound **6f** (MIC 50 μg/ml) may be attributed to the steric hindrance of bromine atom with the fitting of benzene ring of isatin into the aromatic pocket of the receptor.

3. Conclusion

In the current work a promising anti-TB lead compound (**5f**) of the Schiff bases of isatin derivatives and nalidixic acid carbohydrazide has been emerged and a pharmacophoric features have been recognized. Consequently, further optimization of the anti-TB activity for these derivatives are requested and are currently in progress.

4. Experimental

4.1. General notes and methods

Melting Points were determined on Stuart melting point apparatus (Stuart Scientific, England) and were uncorrected. Precoated silica gel plates, 60G F254, obtained from Merck, Darmstadt (Germany) and were used for thin layer chromatography (TLC). Spots were visualized by using either UV-lamp at 254 nm or iodine. Infrared (IR) Spectra were recorded as KBr disk using Perkin Elmer FT–IR apparatus at the research center, College of Pharmacy, King Saud University, Saudi Arabia. The data are given in ν_{max} (cm^{-1}). NMR Spectra were determined in either DMSO-d_6 or $CDCl_3$ and recorded on Bruker NMR Spectrophotometer (500 MHz) at the research center, College of Pharmacy, King Saud University, Saudi Arabia. The chemical shifts are expressed as δ values (ppm) relative to tetramethylsilane (TMS) as internal standard. The J constant was given in (Hz). Mass spectra were taken on a Varian 320 – MS spectrometer at the research center, College of Pharmacy, King Saud University, Saudi Arabia. Mass spectral data were given as m/z (intensity%).

The anti-TB screening was carried out at the research center, College of Pharmacy, King Saud University, Saudi Arabia.

Pharmacophore building was carried out using "Molecular Operating Environment (MOE) version 2006.08", Chemical Computing Group Inc., 1010 Sherbrooke Street West, Suite 910, Montreal, H3A 2R7, Canada. Program operated under "Windows

XP" operating system installed on an Intel Pentium IV PC with a 2.8 MHz processor and 512 RAM.

Building blocks **2**(**a**–**f**), **3**(**a**–**f**) and **4** were prepared according to the reported procedures [13,15]. Compounds **2**(**a**–**f**), **3a** and **4** were described in literature, melting points and spectral data for these compounds are consistent with the reported ones [13,15,22–24]. Data that were not found in the literature are given in the following section:

4.1.1. 5-Bromo-1-ethylindolin-2,3-dione (**3b**)

Yield: 97%; m.p. 230–231 °C (from EtOH). IR ν_{max}/cm^{-1}: 1120.33 (C–Br), 1475.76 (C–N), 1604.31, 1684.02 (C=O amidic), 1750.22 (C=O), 2853.34, 2879.63, 2922.01 (C–H aliphatic), 3095.24 (C–H aromatic). ^{1}H NMR δ_H(DMSO-d_6): 1.20 (3H, t, J = 7, NCH_2CH_3), 3.19 (2H, q, J = 6.25, NCH_2CH_3), 6.69 (1H, d, J = 9, C_7H), 7.41 (1H, dd, J_1 = 2, J_2 = 9, C_6H), 7.66 (1H, bs, C_4H). ^{13}C NMR δ_C (DMSO-d_6): 14.74 (NCH_2CH_3), 37.01 (NCH_2CH_3), 104.43 (C_4), 113.98 (C_7), 116.35 (C_6), 136.67 (C_{3a}), 136.74 (C_{7a}), 150.30 (C_5), 169.04 (C_2), 200.80 (C_3). MS m/z(%): 117.0 (100%), 156.0 (4.04%), 183.0 (6.04%), 184.0 (75.96%), 211.0 (0.58%), 225.0 (12.67%), 253.0 (56.40%), 254.0 (3.15%), 255.0 (50.23%), 256.0 (1.66%).

4.1.2. 5-Bromo-1-propylindolin-2,3-dione (**3c**)

Yield: 92%; m.p. 127–130 °C(from EtOH). IR ν_{max}/cm^{-1}: 1114.72 (C–Br), 1469.52 (C–N), 1608.51, 1676.06 (C=O amidic), 1735.58 (C=O), 2928.24 (C–H aliphatic), 3095.24 (C–H aromatic). ^{1}H NMR δ_H($CDCl_3$): 0.99 (3H, bs, $NCH_2CH_2CH_3$), 1.72 (2H, bs, $NCH_2CH_2CH_3$), 3.68 (2H, bs, $NCH_2CH_2CH_3$), 6.83 (1H, bs, C_7H), 7.68 (2H, bs, C_6 and C_4H). ^{13}C NMR δ_C ($CDCl_3$): 11.28 ($NCH_2CH_2CH_3$), 20.55 ($NCH_2CH_2CH_3$), 41.95 ($NCH_2CH_2CH_3$), 111.92 (C_4), 116.37 (C_7), 118.74 (C_6), 128.10 (C_{3a}), 140.49 (C_{7a}), 149.83 (C_5), 157.46 (C_2), 182.42 (C_3). MS m/z(%): 85.0 (0.80%), 156.0 (10.25%), 183.0 (11.67%), 184.0 (56.49%), 210.0 (100%), 211.0 (46.77%), 225.0 (9.52%), 267.0 (67.18%), 268.0 (8.70%), 269.0 (65.62%), 270.0 (8.27%).

4.1.3. 1-Allyl-5-bromoindolin-2,3-dione (**3d**)

Yield: 98%; m.p. 87–90 °C(from EtOH). IR ν_{max}/cm^{-1}: 904.69, 995.35, 1647.62 (C–H allyl), 1125.41 (C–Br), 1471.42 (C–N), 1606.55, 1674.30 (C=O amidic), 1742.47 (C=O), 2854.00, 2927.23 (C–H aliphatic), 3096.98 (C–H aromatic). ^{1}H NMR δ_H($CDCl_3$): 4.38 (2H, d, J = 5, $NCH_2CH{=}CH_2$), 5.35 (2H, d, J = 4, $NCH_2CH{=}CH_2$), 5.81–5.88 (1H, m, $NCH_2CH{=}CH_2$), 6.83 (1H, d, J = 8, C_7H), 7.70 (1H, dd, J_1 = 1.5, J_2 = 8, C_6H), 7.74 (1H, bs, C_4H). ^{13}C NMR δ_C ($CDCl_3$): 42.62 ($NCH_2CH{=}CH_2$), 112.57 (C_4), 116.67 (C_7), 118.78 ($NCH_2CH{=}CH_2$), 118.94 (C_6), 128.14 ($NCH_2CH{=}CH_2$), 130.00(C_{3a}), 140.47 (C_{7a}), 149.52 (C_5), 157.15 (C_2), 182.00 (C_3). MS m/z(%): 82.0 (1.15%), 130.0 (100%), 156.0 (10.78%), 183.0 (6.07%), 184.0 (13.43%), 225.0 (1.61%), 265.0 (45.92%), 266.0 (7.26%), 267.0 (43.59%), 268.0 (5.40%).

4.1.4. 1-Benzyl-5-bromoindoline-2,3-dione (**3e**)

Yield: 98%; m.p. 105–108 °C(from EtOH). IR ν_{max}/cm^{-1}: 1126.18 (C–Br), 1470.07 (C–N), 1610.60, 1652.97 (C=O amidic), 1731.81 (C=O), 2854.11, 2928.24 (C–H aliphatic), 3032.05, 3063.44 (C–H aromatic). ^{1}H NMR δ_H(DMSO-d_6): 4.91 (2H, s, NCH_2-ph), 6.92 (1H, d, J = 8, C_6H), 7.01–7.58 (6H, m, C_6H and phenyl protons), 7.74 (1H, d, J = 11.5, C_4H). ^{13}C NMR δ_C (DMSO-d_6): 43.50 (NCH_2-ph), 111.06 (C_4), 113.63, 127.18, 127.79, 129.10, 135.67, 135.98 (phenyl carbons), 115.55 (C_7), 120.02 (C_6), 128.02 (C_{3a}), 140.09 (C_{7a}), 149.71 (C_5), 158.40 (C_2), 182.02 (C_3). MS m/z(%): 90.0 (3.72%), 91.0 (100%), 156.0 (1.45%), 183.0 (0.71%), 184.0 (0.96%), 210.0 (0.73%), 225.0 (2.63%), 315.0 (14.86%), 316.0 (2.97%), 317.0 (15.01%).

4.1.5. 5-Bromo-1-hydroxymethylindolin-2,3-dione (**3f**)

Yield: 75%; m.p. 128–131 °C(from AcOEt). IR ν_{max}/cm^{-1}: 1088.73 (C–Br), 1258.51 (C–O), 1474.80 (C–N), 1607.10, 1730.65 (C=O), 2976.19, 2904.76 (C–H aliphatic), 3057.62 (C–H aromatic), 3367.14 (O–H); ^{1}H NMR δ_H(DMSO-d_6): 5.09 (2H, d, J = 5.5, NCH_2OH), 6.45 (1H, bs, NCH_2OH), 7.22 (1H, d, J = 10, C_7H), 7.75 (1H, s, C_4H), 7.86 (1H, d, J = 10, C_6H). ^{13}C NMR δ_C (DMSO-d_6): 63.59 (NCH_2OH), 114.37 (C_4), 115.77 (C_7), 119.57 (C_6), 127.24 (C_{3a}), 140.44 (C_{7a}), 149.64 (C_5), 157.67 (C_2), 182.74 (C_3). MS m/z(%): 43.8 (100%), 209.6 (1.07%), 224.7 (6.83%), 255.2 (0.53%), 256.6 (2.28%), 257.8 (1.96%), 258.7 (1.18%).

4.2. Synthesis of Schiff's bases of isatin and its derivatives and carbohydrazide of nalidixic acid **5**(**a**–**g**) and **6**(**a**–**g**)

4.2.1. General procedures

A stirred mixture of appropriate isatin or its derivative, 1a, 1b, 2 (a–f) and 3 (a–f), (0.5 mmol) and 4 (0.12 g, 0.5 mmol) in EtOH acidified with 4 drops of glacial acetic acid was refluxed for a specified time (4–13 h) and the reaction progress monitored by TLC. The reaction mixture was then concentrated, cooled and filtered. The filtrate was either crystallized by the appropriate solvent or washed thoroughly with ethanol.

4.2.2. 1-Ethyl-1,4-dihydro-7-methyl-4-oxo-N′-(2-oxoindolin-3-ylidene)-1,8-naphthyrid-ine-3-carbohydrazide (**5a**)

Yield: 79%; m.p. 301–305 °C (charring, from EtOH). IR ν_{max}/cm^{-1}: 1441.31 (N–N), 1463.87 (C–N), 1541.37, 1575.84 (C=N), 1611.45, 1679.04 (C=O amidic), 2940.18, 2963.99 (C–H aliphatic), 3062.91 (C–H aromatic), 3348.62 (N–H). MS m/z(%): 103.0 (16.00%), 104.0 (30.00%), 118.0 (12.60%), 131.0 (25.10%), 132.0 (20.60%), 133.0 (4.90%), 145.0 (6.40%), 146.0 (4.90%), 160.0 (16.40%), 161.0 (2.80%), 173.0 (4.70%), 187.0 (45.60%), 188.0 (60.80%), 203.0 (1.80%), 215.0 (100%), 231.0 (7.60%), 246.0 (0.40%), 347.0 (34.20%), 374.0 (0.90%), 375.0 (14.20%), 376.0 (3.00%), 377.0 (0.50%).

4.2.3. 1-Ethyl-1,4-dihydro-7-methyl-N′-(1-methyl-2-oxoindolin-3-ylidene)-4-oxo-1,8-naphthyridine-3-carbohydrazide (**5b**)

Yield: 57%; m.p. 312 °C (from DCM). IR ν_{max}/cm^{-1} 1441.53 (N–N), 1467.98 (C–N), 1541.32 (C=N), 1611.82, 1682.67 (C=O amidic), 2934.16, 2957.97 (C–H aliphatic), 3056.73 (C–H aromatic), 3446.32 (N–H). ^{1}H NMR δ_H($CDCl_3$): 1.57 (3H, t, J = 7.25, $N_1'CH_2CH_3$), 2.71 (3H, s, $C_7'CH_3$), 3.35 (3H, s, N_1CH_3), 4.62 (2H, q, J = 7, $N_1CH_2CH_3$), 6.89 (1H, d, J = 7.5, C_7H), 7.14 (1H, t, J = 7.75, C_5H), 7.33 (1H, d, J = 8, C_4H), 7.40 (1H, t, J = 8.75, C_6H), 7.90 (1H, d, J = 7.5, C_6'H), 8.86 (1H, d, J = 8, C_5'H), 9.05 (1H, s, C_2'H), 15.38 (1H, s, CONH). ^{13}C NMR δ_C ($CDCl_3$): 15.28 ($N_1'CH_2CH_3$), 25.15 ($C_7'CH_3$), 25.69 (N_1CH_3), 47.07 ($N_1'CH_2CH_3$), 108.53 (C_7), 112.01 (C_5'), 120.49 (C_{3a}), 120.64 (C_6'), 121.49 (C_{4a}'), 121.96 (C_5), 122.97 (C_4), 131.09 (C_3'), 137.01 (C_3), 137.25 (C_6), 143.80 (C_2'), 148.68 (C_{8a}'), 148.73 (C_{7a}), 160.24 (C_2), 163.32 (C_7'), 163.37 (C_9'), 176.32 (C_4'). MS m/z(%): 43.8 (100%), 103.3 (17.68%), 104.8 (3.12%), 131.4 (5.10%), 143.9 (0.68%), 147.0 (8.19%), 162.7 (2.82%), 176.6 (2.71%), 230.2 (1.14%), 231.3 (5.46%), 244.7 (1.32%), 388.9 (0.91%), 391.3 (1.00%).

4.2.4. 1-Ethyl-N′-(1-ethyl-2-oxoindolin-3-ylidene)-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carbohydrazide (**5c**)

Yield: 75%; m.p. 278 °C(from DCM). IR ν_{max}/cm^{-1}: 1442.21 (N–N), 1464.90, 1493.12 (C–N), 1508.40, 1571.72 (C=N), 1610.72, 1690.69 (C=O amidic), 2977.65 (C–H aliphatic), 3055.48 (C–H aromatic), 3447.34 (N–H). ^{1}H NMR δ_H($CDCl_3$): 1.34 (3H, t, J = 7, $N_1CH_2CH_3$), 1.56 (3H, t, J = 7.25, $N_1'CH_2CH_3$), 2.69 (3H, s, $C_7'CH_3$), 3.91 (2H, q, J = 7, $N_1CH_2CH_3$), 4.61 (2H, q, J = 7, $N_1'CH_2CH_3$), 6.90 (1H, d, J = 7.5, C_7H), 7.12 (1H, t, J = 7.5, C_5H), 7.32 (1H, d, J = 8, C_4H), 7.38 (1H, t, J = 7.5, C_6H), 7.90 (1H, d, J = 7.5, C_6'H), 8.86 (1H, d, J = 8, C_5'H), 9.04 (1H, s, C_2'H), 15.36 (1H, s, CONH). ^{13}C NMR δ_C ($CDCl_3$): 12.95 ($N_1CH_2CH_3$), 15.24 ($N_1'CH_2CH_3$), 25.25 ($C_7'CH_3$), 34.35 (NCH_2CH_3), 46.99 ($N_1'CH_2CH_3$), 108.51 (C_7), 112.03 (C_5'), 120.60 (C_{3a}), 120.75 (C_6'), 121.45 (C_{4a}'), 122.09 (C_5), 122.76 (C_4), 130.99 (C_3'),

137.04 (C_3), 137.39 (C_6), 142.99 (C_2'), 148.69 (C_{8a}'), 148.89 (C_{7a}'), 158.89 (C_2), 163.20 (C_7'), 163.30 (C_9'), 176.55 (C_4'). MS *m*/*z*(%): 42.8 (100%), 101.2 (2.26%), 103.9 (10.98%), 117.8 (1.05%), 130.2 (1.30%), 132.8 (21.22%), 143.8 (1.64%), 147.0 (10.58%), 160.0 (2.69%), 161.0 (30.02%), 174.1 (2.59%), 186.5 (9.66%), 188.3 (2.90%), 189.3 (0.51%), 203.2 (2.58%), 214.9 (1.58%), 230.8 (0.81%), 245.3 (1.53%), 333.7 (0.65%), 346.8 (2.65%), 375.2 (2.49%), 401.8 (0.50%), 404.4 (1.82%), 405.6 (0.64%).

4.2.5. 1-Ethyl-1,4-dihydro-7-methyl-4-oxo-N′-(2-oxo-1-propylindolin-3-ylidene)-1,8-naphthyridine-3-carbohydrazide (**5d**)

Yield: 237–240%; m.p. 278 °C(from DCM). IR ν_{max}/cm^{-1}: 1444.11 (N–N), 1463.45, 1487.09 (C–N), 1508.12, 1541.77, 1575.71 (C=N), 1608.34, 1674.71 (C=O amidic), 2927.70 (C–H aliphatic), 3023.81 (C–H aromatic), 3446.34 (N–H). ^{1}H NMR δ_H($CDCl_3$): 1.02 (3H, t, J = 7.5, $N_1CH_2CH_2CH_3$), 1.57 (3H, t, J = 7, $N_1'CH_2CH_3$), 1.77–1.82 (2H, m, $N_1CH_2CH_2CH_3$), 2.70 (3H, s, $C_7'CH_3$), 3.81 (3H, t, J = 7.5, $N_1CH_2CH_2CH_3$), 4.62 (2H, q, J_1 = 14.5, J_2 = 21.5, $N_1'CH_2CH_3$), 6.90 (1H, d, J = 8, C_7H), 7.12 (1H, t, J = 7.5, C_5H), 7.33 (1H, d, J = 8, C_4H), 7.37 (1H, t, J = 7.75, C_6H), 7.90 (1H, d, J = 7.5, C_6'H), 8.87 (1H, d, J = 8, C_5'H), 9.05 (1H, s, C_2'H), 15.35 (1H, s, CO*NH*). ^{13}C NMR δ_C ($CDCl_3$): 11.41 ($N_1CH_2CH_2CH_3$), 15.27 ($N_1'CH_2CH_3$), 20.96 ($N_1CH_2CH_2CH_3$), 25.1 ($C_7'CH_3$), 41.37 ($N_1CH_2CH_2CH_3$), 47.07 ($N_1'CH_2CH_3$), 108.83 (C_7), 112.15 (C_5'), 120.00 (C_{3a}), 120.60 (C_6'), 121.49 (C_{4a}'), 122.04 (C_5), 122.73 (C_4), 131.00 (C_3'), 137.04 (C_3), 137.31 (C_6), 143.34 (C_2'), 148.68 (C_{8a}'), 148.74 (C_{7a}), 160.16 (C_2), 163.28 (C_7'), 167.00 (C_9'), 176.35 (C_4'). MS *m*/*z*(%): 43.8 (100%), 102.6 (25.57%), 104.0 (12.03%), 118.0 (11.08%), 130.6 (7.05%), 131.7 (13.99%), 145.8 (6.04%), 146.9 (18.76%), 160.1 (11.55%), 172.7 (4.94%), 175.5 (0.41%), 187.4 (1.60%), 188.3 (4.64%), 203.4 (1.49%), 214.9 (2.93%), 229.4 (0.48%), 230.6 (1.53%), 246.7 (3.32%), 346.7 (2.94%), 375.4 (2.03%), 417.0 (10.45%), 419.5 (3.76%).

4.2.6. N′-(1-Allyl-2-oxoindolin-3-ylidene)-1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carbohydrazide (**5e**)

Yield: 58%; m.p. 281 °C(from DCM). IR ν_{max}/cm^{-1}: 910.91, 1018.21, 1656.20 (C–H allyl), 1441.89 (N–N), 1474.89 (C–N), 1542.64 (C=N), 1608.58, 1679.16 (C=O amidic), 2891.68, 2963.11 (C–H aliphatic), 3053.19 (C–H aromatic), 3447.34 (N–H). ^{1}H NMR δ_H($CDCl_3$): 1.56 (3H, t, J = 7.25, $N_1'CH_2CH_3$), 2.68 (3H, s, $C_7'CH_3$), 4.48 (2H, d, J = 5, $N_1CH_2CH{=}CH_2$), 4.60 (2H, q, J_1 = 14.5, J_2 = 21.5, $N_1'CH_2CH_3$), 5.25 (1H, d, J = 10.5, $N_1CH_2CH{=}HCH$), 5.30 (1H, d, J = 17.5, $N_1CH_2CH{=}HCH$), 5.85–5.90 (1H, m, $N_1CH_2CH{=}CH_2$), 6.87 (1H, d, J = 8, C_7H), 7.12 (1H, t, J = 7.75, C_5H), 7.30–7.36 (2H, m, C_4H and C_6H), 7.89 (1H, d, J = 7.5, C_6'H), 8.84 (1H, d, J = 8, C_5'H), 9.04 (1H, s, C_2'H), 15.34 (1H, s, CO*NH*). ^{13}C NMR δ_C ($CDCl_3$): 15.26 ($N_1'CH_2CH_3$), 25.13 ($C_7'CH_3$), 42.03 ($N_1CH_2CH{=}CH_2$), 47.08 ($N_1'CH_2CH_3$), 109.48 (C_7), 112.07 (C_5'), 118.15 ($N_1CH_2CH{=}CH_2$), 120.54 (C_{3a}), 120.59 (C_6'), 121.49 (C_{4a}'), 121.94 (C_5), 122.93 (C_4), 130.98 (C_3'), 131.17 ($N_1CH_2CH{=}CH_2$), 137.96 (C_3), 136.99 (C_6), 143.00 (C_2'), 148.66 (C_{8a}'), 148.75 (C_{7a}), 159.84 (C_2), 163.25 (C_7'), 163.39 (C_9'), 176.34 (C_4'). MS *m*/*z*(%): 41.0 (100%), 103.0 (9.97%), 104.9 (3.51%), 118.2 (2.68%), 131.6 (5.40%), 145.1 (9.68%), 160.1 (12.79%), 172.3 (1.20%), 184.5 (17.98%), 186.9 (0.51%), 188.2 (9.02%), 203.1 (1.41%), 214.9 (0.53%), 230.3 (0.67%), 231.1 (0.66%), 246.5 (3.72%), 330.9 (1.69%), 347.0 (1.36%), 414.3 (2.29%), 415.7 (4.34%), 417.2 (5.52%).

4.2.7. N′-(1-Benzyl-2-oxoindolin-3-ylidene)-1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carbohydrazide (**5f**)

Yield: 55%; m.p. 292 °C(from DCM). IR ν_{max}/cm^{-1} 1441.03 (N–N), 1466.24, 1489.30 (C–N), 1510.87, 1541.11 (C=N), 1611.46, 1679.65 (C=O amidic), 2926.03 (C–H aliphatic), 3058.36 (C–H aromatic), 3446.27 (N–H). ^{1}H NMR δ_H($CDCl_3$) 1.58 (3H, t, J = 6.5, $N_1'CH_2CH_3$), 2.73 (3H, s, $C_7'CH_3$), 4.63 (2H, q, J = 7.5, $N_1'CH_2CH_3$), 5.08 (2H, s, N_1CH_2ph), 6.76 (1H, d, J = 8, C_7H), 7.12 (1H, t, J = 7.5, C_5H), 7.29–7.39 (7H, m, C_4H, C_6H and phenyl protons), 7.93 (1H, d, J = 7.5, C_6'H), 8.88 (1H, d, J = 8.5, C_5'H), 9.07 (1H, s, C_2'H), 15.42 (1H, s, CO*NH*). ^{13}C NMR δ_C (DMSO-d_6): 15.38 ($N_1'CH_2CH_3$), 25.19 ($C_7'CH_3$), 43.44 (N_1CH_2ph), 47.07 ($N_1'CH_2CH_3$), 110.03 (C_7), 110.94 (C_5'), 120.01 (C_{3a}), 120.37 (C_6'), 121.43 (C_{4a}'), 121.92 (C_5), 122.27 (C_4), 127.39, 127.83 128.93, 132.68, 135.73, 135.84 (phenyl carbons), 131.39 (C_3'), 136.45 (C_3), 137.69 (C_6), 144.03 (C_2'), 148.48 (C_{8a}'), 149.37 (C_{7a}), 163.12 (C_2), 163.72 (C_7'), 164.24 (C_9'), 176.55 (C_4'). MS *m*/*z*(%): 43.8 (100%), 90.7 (1.21%), 103.0 (6.91%), 104.1 (8.54%), 117.0 (7.58%), 131.2 (0.78%), 132.8 (22.71%), 145.8 (3.19%), 159.8 (8.66%), 173.1 (1.85%), 188.4 (2.32%), 201.9 (1.30%), 210.2 (6.92%), 214.5 (1.80%), 230.3 (1.16%), 231.4 (0.99%), 238.7 (0.54%), 246.8 (22.17%), 332.2 (3.31%), 346.2 (2.25%), 464.6 (0.45%), 465.8 (2.71%), 466.9 (2.25%).

4.2.8. 1-Ethyl-1,4-dihydro-N′-(1-hydroxymethyl-2-oxoindolin-3-ylidene)-7-methyl-4-oxo-1,8-naphthyridine-3-carbohydrazide (**5g**)

Yield: 83%; m.p. 275–278 °C(charring, from EtOH). IR ν_{max}/cm^{-1} 1206.10 (C–O), 1440.81 (N–N), 1464.06, 1484.97 (C–N), 1540.76, 1576.16 (C=N), 1610.04, 1678.43 (C=O amidic), 1695.95 (C=O), 2936.28, 2960.09 (C–H aliphatic), 3058.24 (C–H aromatic), 3340.74 (O–H and N–H). MS *m*/*z*(%): 57.0 (100%), 103.0 (22.30%), 104.0 (35.00%), 118.0 (7.60%), 131.0 (23.40%), 132.0 (24.90%), 145.0 (6.50%), 160.0 (12.80%), 163.2 (1.04%), 173.0 (5.70%), 175.7 (0.97%), 187.0 (49.50%), 188.0 (60.10%), 190.0 (1.30%), 203.0 (1.60%), 215.0 (100%), 230.3 (0.56%), 231.0 (2.80%), 246.6 (0.27%), 332.5 (0.10%), 347.0 (37.70%), 375.0 (12.90%), 403.9 (0.13%), 405.2 (0.20%), 406.1 (0.01%), 407.1 (0.15%).

4.2.9. N′-(5-Bromo-2-oxoindolin-3-ylidene)-1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carbohydrazide (**6a**)

Yield: 99%; m.p. 362 °C(from DMF). IR ν_{max}/cm^{-1} 1111.32 (C–Br), 1441.92 (N–N), 1466.03 (C–N), 1542.34 (C=N), 1609.54, 1664.04 (C=O amidic), 1666.42 (C=O nalidixic acid hydrazide), 2935.12, 2982.74 (C–H aliphatic), 3095.24 (C–H aromatic), 3447.18 (N–H). ^{1}H NMR δ_H(, DMSO-d_6): 1.44 (3H, m, $N_1'CH_2CH_3$), 2.71 (3H, s, $C_7'CH_3$), 4.64 (2H, m, $N_1'CH_2CH_3$), 6.91 (1H, dd, J_1 = 8.5, J_2 = 8, C_7H), 7.52 (1H, d, J = 7.5, C_6'H), 7.59 (1H, d, J = 7.5, C_6H), 8.59 (1H, s, C_4H), 8.64 (1H, d, J = 8, C_5'H), 9.28 (1H, s, C_2'H), 13.74 (1H, s, NH), 14.96 (1H, s, CO*NH*). ^{13}C NMR δ_C (DMSO-d_6): 15.48 ($N_1'CH_2CH_3$), 25.30 ($C_7'CH_3$), 46.00 ($N_1'CH_2CH_3$), 112.00 (C_4), 113.77 (C_5'), 115.01 (C_7), 120.00 (C_6'), 121.00 (C_{4a}'), 122.00 (C_6), 123.86 (C_{3a}), 133.89 (C_3'), 136.56 (C_{7a}), 137.40 (C_3), 146.00 (C_2'), 148.00 (C_{8a}'), 148.99 (C_5), 159.98 (C_2), 163.00 (C_7'), 165.00 (C_9'), 176.00 (C_4'). MS *m*/*z*(%): 45.0 (100%), 132.1 (1.21%), 145.1 (3.51%), 160.0 (6.29%), 183.3 (1.14%), 186.8 (1.95%), 187.9 (4.09%), 202.3 (11.43%), 208.0 (26.57%), 213.6 (0.55%), 215.7 (5.55%), 222.8 (3.16%), 245.7 (1.01%), 411.0 (0.48%), 426.5 (4.08%), 435.9 (3.32%), 452.9 (7.31%), 454.8 (0.56%), 456.0 (2.17%).

4.2.10. N′-(5-Bromo-1-methyl-2-oxoindolin-3-ylidene)-1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carbohydrazide (**6b**)

Yield: 59%; m.p. 369–372(charring, from DMF). IR ν_{max}/cm^{-1}: 1101.83 (C–Br), 1441.98 (N–N), 1478.41 (C–N), 1543.16 (C=N), 1611.30, 1679.50 (C=O), 2924.59 (C–H aliphatic), 3011.90, 3071.43 (C–H aromatic), 3446.70 (N–H). MS *m*/*z*(%): 40.2 (100%), 131.9 (8.48%), 145.6 (1.63%), 159.0 (0.92%), 172.8 (3.20%), 183.7 (1.35%), 188.3 (3.80%), 197.7 (3.40%), 203.3 (0.76%), 210.0 (3.12%), 214.6 (1.11%), 225.6 (5.49%), 231.3 (0.61%), 239.5 (1.37%), 245.1 (1.26%), 254.9 (2.81%), 425.1 (1.18%), 452.6 (0.93%), 466.7 (1.16%), 470.2 (1.49%).

4.2.11. N′-(5-Bromo-1-ethyl-2-oxoindolin-3-ylidene)-1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carbohydrazide (**6c**)

Yield: 93%; m.p. 331 °C(from EtOH). IR ν_{max}/cm^{-1}: 1106.76 (C–Br), 1441.59 (N–N), 1473.49 (C–N), 1543.54 (C=N), 1608.48,

1682.08 (C=O), 2922.00 (C–H aliphatic), 3071.43 (C–H aromatic), 3447.43 (N–H). ^{1}H NMR δ_H(DMSO-d_6): 1.15–1.25 (3H, m, $N_1CH_2CH_3$), 1.43 (3H, m, $N_1'CH_2CH_3$), 2.69 (3H, s, $C_7'CH_3$), 3.80 (2H, m, $N_1CH_2CH_3$), 4.62 (2H, m, $N_1'CH_2CH_3$), 7.19 (1H, d, J = 15, C_7H), 7.54 (1H, d, J = 10, C_6'H), 7.62 (1H, m, C_6H), 7.70 (1H, s, C_4H), 8.62 (1H, d, J = 15, C_5'H), 9.15 (1H, s, C_2'H), 15.00 (1H, s, CO*NH*). MS m/z(%): 91.0 (100%), 145.0 (1.57%), 187.0 (1.07%), 188.0 (0.87%), 197.0 (1.17%), 203.0 (0.90%), 210.0 (0.44%), 253.0 (0.44%), 482.0 (0.44%).

4.2.12. *N′-(5-Bromo-2-oxo-1-propylindolin-3-ylidene)-1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carbohydrazide (**6d**)*

Yield: 97%; m.p. 314 °C(from DMF). IR ν_{max}/cm^{-1} 1109.32 (C–Br), 1442.28 (N–N), 1472.23 (C–N), 1542.15 (C=N), 1608.34, 1680.70 (C=O), 2933.60, 2981.22 (C–H aliphatic), 3047.60 (C–H aromatic), 3446.84 (N–H). ^{1}H NMR δ_H($CDCl_3$): 1.00 (3H, t, J = 7.25, $N_1CH_2CH_2CH_3$), 1.57 (3H, t, J = 7.25, $N_1'CH_2CH_3$), 1.75–1.80 (2H, m, $N_1CH_2CH_2CH_3$), 2.71 (3H, s, $C_7'CH_3$), 3.79 (2H, t, J = 7.5, $N_1CH_2CH_2CH_3$), 4.62 (2H, q, J_1 = 14.5, J_2 = 21.5, $N_1'CH_2CH_3$), 6.78 (1H, d, J = 8, C_7H), 7.33 (1H, d, J = 8, C_6'H), 7.48 (1H, dd, J_1 = 2, J_2 = 2, C_6H), 8.05 (1H, s, C_4H), 8.86 (1H, d, J = 8.5, C_5'H), 9.05 (1H, s, C_2'H), 15.36 (1H, s, CO*NH*). ^{13}C NMR δ_C ($CDCl_3$): 11.37 ($N_1CH_2CH_2CH_3$), 15.27 ($N_1'CH_2CH_3$), 20.91 ($N_1CH_2CH_2CH_3$), 25.15 ($C_7'CH_3$), 41.47 ($N_1CH_2CH_2CH_3$), 47.13 ($N_1'CH_2CH_3$), 110.27 (C_4), 111.96 (C_5'), 115.61 (C_7), 120.62 (C_6'), 121.54 (C_{4a}'), 122.38 (C_6'), 124.84 (C_{3a}), 133.30 (C_3'), 135.87 (C_{7a}), 137.02 (C_3), 142.02 (C_2'), 148.66 (C_{8a}'), 148.84 (C_5), 159.72 (C_2), 163.29 (C_7'), 163.45(C_9'), 176.38 (C_4'). MS m/z(%): 43.1 (100%), 133.2 (12.82%), 146.8 (1.55%), 160.0 (4.87%), 172.7 (1.21%), 182.4 (2.15%), 187.7 (0.48%), 188.9 (2.35%), 196.8 (3.35%), 203.2 (2.07%), 209.7 (4.64%), 215.1 (0.93%), 231.9 (0.86%), 246.0 (0.76%), 256.0 (6.25%), 266.3 (1.88%), 281.0 (2.65%), 410.7 (1.02%), 426.1 (1.09%), 455.3 (2.61%), 497.5 (2.45%).

4.2.13. *N′-(1-Allyl-5-bromo-2-oxoindolin-3-ylidene)-1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carbohydrazide (**6e**)*

Yield: 92%; m.p. 309 °C(from DCM). IR ν_{max}/cm^{-1}: 916.82, 976.37, 1645.18 (C–H allyl), 1114.64 (C–Br), 1443.27 (N–N), 1474.15 (C–N), 1542.13 (C=N), 1609.47, 1679.86 (C=O amidic), 2935.56, 2983.18 (C–H aliphatic), 3071.42 (C–H aromatic), 3447.53 (N–H). ^{1}H NMR δ_H($CDCl_3$): 1.57 (3H, t, J = 10, $N_1'CH_2CH_3$), 2.71 (3H, s, $C_7'CH_3$), 4.48 (2H, d, J = 2.5, $N_1CH_2CH{=}CH_2$), 4.61 (2H, q, J_1 = 9, J_2 = 9.5, $N_1'CH_2CH_3$), 5.28 (2H, m, $N_1CH_2CH{=}CH_2$), 5.80–5.90 (1H, m, $N_1CH_2CH{=}CH_2$), 6.78 (1H, d, J = 7, C_7H), 7.35 (1H, d, J = 8, C_6'H), 7.46 (1H, d, J = 7, C_6H), 8.06 (1H, s, C_4H), 8.86 (1H, d, J = 7.5, C_5'H), 9.05 (1H, s, C_2'H), 15.38 (1H, s, CO*NH*). ^{13}C NMR δ_C ($CDCl_3$): 15.28 ($N_1'CH_2CH_3$), 25.16 ($C_7'CH_3$), 42.10 ($N_1CH_2CH{=}CH_2$), 47.15 ($N_1'CH_2CH_3$), 110.96 (C_4), 111.91 (C_5'), 115.89 (C_7), 118.38 ($N_1CH_2CH{=}CH_2$), 120.61 (C_6'), 121.59 (C_{4a}'), 122.33 (C_6), 124.78 (C_{3a}), 130.82 ($N_1CH_2CH{=}CH_2$), 133.31 (C_3'), 135.60 (C_{7a}), 136.98 (C_3), 141.67 (C_2'), 148.66 (C_{8a}'), 148.86 (C_5), 159.41 (C_2), 163.29 (C_7'), 163.49 (C_9'), 176.39 (C_4'). MS m/z(%): 40.9 (100%), 130.7 (0.75%), 145.8 (9.38%), 160.0 (0.73%), 173.3 (1.80%), 181.5 (4.35%), 183.6 (4.31%), 196.7 (0.75%), 203.0 (1.07%), 211.5 (4.11%), 213.7 (1.36%), 229.7 (2.94%), 244.5 (1.80%), 251.6 (2.26%), 264.9 (7.54%), 280.5 (12.88%), 410.8 (1.42%), 453.5 (2.76%), 492.6 (1.92%), 493.8 (0.73%), 495.7 (1.90%), 496.8 (0.84%).

4.2.14. *N′-(1-Benzyl-5-bromo-2-oxoindolin-3-ylidene)-1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carbohydrazide (**6f**)*

Yield: 99%; m.p. 323 °C(from DCM). IR ν_{max}/cm^{-1}: 1115.18 (C–Br), 1441.62 (N–N), 1472.18 (C–N), 1541.43 (C=N), 1608.23, 1683.65 (C=O amidic), 2869.64, 2934.07 (C–H aliphatic), 3047.62 (C–H aromatic), 3447.36 (N–H). ^{1}H NMR δ_H($CDCl_3$): 1.57 (3H, bs, $N_1'CH_2CH_3$), 2.70 (3H, bs, $C_7'CH_3$), 4.61 (2H, bs, $N_1'CH_2CH_3$), 5.04 (2H, bs, N_1CH_2ph), 6.61 (1H, bs, C_7H), 7.20–7.50 (7H, m, C_6'H, C_6H and phenyl protons), 8.04 (1H, bs, C_4H), 8.84 (1H, bs, C_5'H), 9.05 (1H, bs, C_2'H), 15.40 (1H, s, CO*NH*). ^{13}C NMR δ_C ($CDCl_3$): 15.22 ($N_1'CH_2CH_3$), 25.10 ($C_7'CH_3$), 43.57 (N_1CH_2ph), 47.10 ($N_1'CH_2CH_3$), 111.08 (C_4), 111.97 (C_5'), 115.97 (C_7), 120.61 (C_6'), 121.53 (C_{4a}'), 122.46 (C_6), 124.71 (C_{3a}), 127.41, 127.88, 128.87, 129.50, 134.99, 135.25 (phenyl carbons), 133.28 (C_3'), 135.51 (C_{7a}), 136.97 (C_3), 141.61 (C_2'), 148.67 (C_{8a}'), 148.85 (C_5), 159.76 (C_2), 163.24 (C_7'), 163.46 (C_9'), 176.38 (C_4'). MS m/z(%): 40.6 (100%), 91.0 (14.15%), 131.5 (5.68%), 143.7 (1.92%), 159.9 (1.84%), 173.5 (0.70%), 181.9 (1.36%), 182.9 (7.39%), 186.9 (0.84%), 198.2 (1.13%), 203.3 (2.15%), 209.0 (9.74%), 231.1 (1.34%), 246.3 (2.51%), 300.4 (0.90%), 315.5 (2.46%), 330.4 (1.74%), 410.2 (4.26%), 454.6 (1.28%), 544.1 (2.23%), 546.6 (2.23%).

4.2.15. *N′-(5-Bromo-1-hydroxymethyl-2-oxoindolin-3-ylidene)-1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carbohydrazide (**6g**)*

Yield: 75%; m.p. 315–318 °C(charring, from EtOH). IR ν_{max}/cm^{-1} 1111.70 (C–Br), 1253.95 (C–O), 1441.20 (N–N), 1465.56 (C–N), 1541.85 (C=N), 1609.19, 1684.10 (C=O amidic), 2939.91, 2987.53 (C–H aliphatic), 3047.62 (C–H aromatic), 3357.14 (O–H), 3452.38 (N–H). MS m/z(%): 132.0 (48.10%), 145.0 (16.20%), 160.0 (34.30%), 173.0 (10.00%), 182.0 (3.30%), 183.0 (6.20%), 187.0 (61.40%), 188.0 (97.10%), 198.0 (8.10%), 203.0 (2.90%), 215.0 (100%), 231.0 (12.40%), 425.0 (10.00%), 453.0 (5.20%).

4.3. Synthesis of Mannich bases **7(a–c)** and **8(a–c)**

Mixture of appropriate Schiff's base **5a** or **6a** (0.5 mmol) and 40% formaldehyde (0.02 g, 0.75 mmol) was stirred at RT in EtOH (20 ml). Appropriate secondary amine (0.75 mmol) was added and stirring was continued for overnight and the reaction progress checked by TLC (DCM:MeOH 95:5 v/v). The solvent was concentrated and the precipitate was filtrated and washed thoroughly with aqueous ethanol.

4.3.1. *N′-(1-((Diethylamino)methyl)-2-oxoindolin-3-ylidene)-1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carbohydrazide (**7a**)*

Yield: 51%; m.p. 380–385 °C (charring, from EtOH). IR ν_{max}/cm^{-1} 1440.63 (N–N), 1464.14 (C–N), 1505.53, 1540.30, 1576.47 (C=N), 1610.14, 1677.05 (C=O amidic), 1696.19 (C=O), 2885.59, 2933.15 (N–CH_2–N), 2980.77 (C–H aliphatic), 3057.33 (C–H aromatic), 3339.77 (N–H). MS m/z(%): 57.0 (100%), 72.0 (8.80%), 86.9 (14.75%), 102.0 (20.60%), 103.0 (32.40%), 104.0 (57.40%), 118.0 (47.10%), 131.0 (48.50%), 132.0 (35.30%), 144.8 (1.96%), 160.0 (4.40%), 173.1 (1.33%), 187.0 (48.50%), 188.0 (41.20%), 203.0 (7.40%), 215.1 (3.08%), 218.1 (1.86%), 230.3 (0.42%), 231.3 (1.62%), 246.1 (0.32%), 331.0 (8.80%), 346.7 (0.59%), 375.0 (22.10%), 459.2 (0.09%), 460.0 (10.30%), 461.0 (4.40%).

4.3.2. *1-Ethyl-1,4-dihydro-7-methyl-N′-(1-((4-methylpiperazin-1-yl)methyl)-2-oxoindolin-3-ylidene)-4-oxo-1,8-naphthyridine-3-carbohydrazide (**7b**)*

Yield: 59%; m.p. > 400 °C(from EtOH). IR ν_{max}/cm^{-1} 1440.07 (N–N), 1469.00 (C–N), 1505.69, 1540.21, 1576.22 (C=N), 1610.00, 1676.66 (C=O amidic), 1696.29 (C=O), 2861.54, 2932.97 (N–CH_2–N), 2980.59 (C–H aliphatic), 3056.63 (C–H aromatic), 3338.77 (N–H). MS m/z(%): 103.0 (36.80%), 104.0 (73.50%), 118.0 (15.40%), 131.0 (42.60%), 132.0 (55.90%), 145.0 (22.10%), 160.0 (31.60%), 173.0 (13.20%), 187.0 (95.60%), 188.0 (95.60%), 215.0 (100%), 231.0 (0.70%), 347.0 (50.00%), 375.0 (19.10%), 486.5 (0.02%), 487.4 (0.15%), 488.3 (0.01%), 489.8 (0.06%).

*4.3.3. 1-Ethyl-1,4-dihydro-7-methyl-4-oxo-N′-(2-oxo-1-((piperidin-1-yl)methyl)indolin-3-ylidene)-1,8-naphthyridine-3-carbohydrazide (**7c**)*

Yield: 59%; m.p. > 400 °C (from EtOH). IR ν_{max}/cm^{-1}: 1440.24 (N–N), 1464.02 (C–N), 1505.60, 1540.25, 1576.30 (C=N), 1610.02, 1676.80 (C=O amidic), 1696.19 (C=O), 2892.92, 2940.54 (N–CH_2–N), 2964.35 (C–H aliphatic), 3056.59 (C–H aromatic), 3338.90 (N–H). MS *m*/*z*(%): 84.0 (3.20%), 98.1 (1.69%), 103.0 (18.50%), 104.0 (23.30%), 113.9 (1.49%), 118.0 (5.70%), 131.0 (20.70%), 132.0 (19.70%), 145.0 (5.50%), 160.0 (10.40%), 173.0 (4.10%), 187.0 (35.90%), 188.0 (53.00%), 203.0 (1.40%), 215.0 (100%), 230.9 (0.39%), 231.0 (4.30%), 243.1 (0.18%), 247.0 (0.70%), 258.0 (0.34%), 331.0 (0.70%), 347.0 (32.80%), 375.0 (10.50%), 471.3 (0.05%), 472.4 (0.11%), 473.9 (0.12%).

*4.3.4. N′-(1-((Diethylamino)methyl)-5-bromo-2-oxoindolin-3-ylidene)-1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carbohydrazide (**8a**)*

Yield: 75%; m.p. 398–400 °C (charring, from EtOH). IR ν_{max}/cm^{-1}: 1111.50 (C–Br), 1441.83 (N–N), 1465.92 (C–N), 1542.86 (C=N), 1608.49, 1664.23 (C=O amidic), 1685.11 (C=O), 2833.33, 2880.95 (N–CH_2–N), 2928.57 (C–H aliphatic), 3029.73, 3101.16, 3148.78 (C–H aromatic), 3447.19 (N–H). MS *m*/*z*(%): 72.0 (6.30%), 85.0 (3.10%), 102.0 (16.30%), 132.0 (31.30%), 145.0 (9.40%), 160.0 (33.10%), 173.0 (3.10%), 187.0 (49.40%), 188.0 (81.30%), 196.0 (11.30%), 215.0 (100%), 231.0 (15.60%), 425.0 (13.10%), 538.4 (0.51%), 539.5 (1.28%).

*4.3.5. N′-(5-Bromo-1-((4-methylpiperazin-1-yl)methyl)-2-oxoindolin-3-ylidene)-1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carbohydrazide (**8b**)*

Yield: 52%; m.p. > 400 °C(from EtOH). IR ν_{max}/cm^{-1}: 1110.75 (C–Br), 1440.87 (N–N), 1464.68 (C–N), 1543.07 (C=N), 1607.82, 1664.29 (C=O amidic), 1688.10 (C=O), 2837.73, 2861.54 (N–CH_2–N), 2909.16, 2956.78 (C–H aliphatic), 3047.62, 3119.05, 3147.64 (C–H aromatic), 3474.35 (N–H). MS *m*/*z*(%): 43.9 (100%), 99.0 (2.18%), 113.1 (1.35%), 129.0 (2.96%), 132.0 (13.10%), 145.0 (8.30%), 160.0 (12.90%), 173.0 (5.00%), 181.0 (2.20%), 183.0 (1.40%), 187.0 (44.70%), 188.0 (48.40%), 196.0 (3.00%), 203.0 (2.90%), 210.0 (1.30%), 215.0 (62.86%), 230.1 (0.23%), 231.0 (4.30%), 247.0 (0.40%), 323.8 (0.03%), 337.3 (0.04%), 351.4 (0.05%), 412.0 (0.90%), 426.4 (2.30%), 453.0 (2.80%), 566.2 (0.01%), 567.0 (0.06%), 568.2 (0.05%).

*4.3.6. N′-(5-Bromo-2-oxo-1-((piperidin-1-yl)methyl)indolin-3-ylidene)-1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carbohydrazide (**8c**)*

Yield: 62%; m.p. > 400 °C(from EtOH). IR ν_{max}/cm^{-1}: 1111.40 (C–Br), 1441.42 (N–N), 1465.81 (C–N), 1543.38 (C=N), 1608.78, 1664.28 (C=O amidic), 1688.09 (C=O), 2813.70, 2861.32 (N–CH_2–N), 2932.75, 2980.37 (C–H aliphatic), 3071.43, 3119.05, 3149.30 (C–H aromatic), 3461.68 (N–H). MS *m*/*z*(%): 85.0 (2.30%), 99.0 (2.50%), 115.0 (14.10%), 132.0 (27.00%), 145.0 (10.40%), 160.0 (27.90%), 173.0 (8.20%), 182.0 (3.10%), 183.0 (2.00%), 187.0 (45.50%), 188.0 (62.30%), 197.0 (2.00%), 203.0 (3.90%), 210.0 (2.30%), 215.0 (100%), 230.0 (1.40%), 231.0 (4.10%), 246.0 (2.00%), 338.0 (1.40%), 426.0 (3.50%), 454.0 (3.70%).

4.4. Evaluation of anti-TB activity of the synthesized compounds

The anti-TB activity of the synthesized compounds **5**(**a**–**g**), **6**(**a**–**g**), **7**(**a**–**c**) and **8**(**a**–**c**) was carried out using the agar dilution method [13,17]. The synthesized compounds **5**(**a**–**g**), **6**(**a**–**g**), **7**(**a**–**c**) and **8**(**a**–**c**) were solubilized in DMSO at a concentration of 1 mg/ml. Appropriate amount of each was diluted first with 10% molten agar to give a concentration of 200 μg/ml. Further dilutions for the active compounds were done to give 100, 50, 25, 12.5, 6.25 and up to 0.625 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 3–4 mm and allowed to harden. The incula were prepared by growing overnight culture in Muller–Hinton broth. The cultures were diluted 1:100. Test organisms were streaked in a radial pattern and plates were incubated at 35 °C for 48 h. Control experiment consisted of the tested TB strains, DMSO and the growing media were treated in the same manner. Complete suspension of growth was observed prior to declaring the compound to be active. Results are given in Table 1.

4.5. Pharmacophore building

The procedures followed for pharmacophore building explained in the following points [21].

1. Flexible alignment operated for the 10 compounds as a training set by the following steps: a. The selected compounds were built using the builder interface of the MOE program; b. Open the Flexible Alignment panel. The Flexible mode was selected as it enables all-atom flexibility (and hence conformational search) during the generation of molecular alignments; c. The output of flexible alignment contain the following data:

U The average strain energy of the molecules in the alignment in kcal/mol.

F The total mutual similarity score of the configuration.

S The alignment score of the configuration. Lower values are intended to indicate better alignments.

2. Copy the aligned structures having the lowest S value to MOE window.
3. Create a pharmacophore query for the aligned training set molecules using the Pharmacophore Query Editor.
4. The generated model is subjected to the systemic test set conformational search using the Pharmacophore Search.
5. The program generate annotations for all ligand conformations in the test set database using the Pharmacophore Preprocessor by the currently selected PCH (Polarity–Charge–Hydrophobicity) pharmacophore scheme.
6. Modify the query using consensus query method and search the same database again.
7. The program expresses the degree of mapping of a given compound to a generated hypothetical model in term of rmsd value.

Results are shown in Table 3.

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