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Original article

Synthesis, antimicrobial, antimycobacterial and structure–activity relationship of substituted pyrazolo-, isoxazolo-, pyrimido- and mercaptopyrimidocyclohepta[*b*]indoles

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ABSTRACT

A new class of heterocycles, specifically substituted pyrazolo-, isoxazolo- and pyrimidocyclohepta[*b*]indoles, has been prepared by condensation of substituted 7-(hydroxymethylene)-7,8,9,10-tetrahydrocyclohepta[*b*]indol-6(5*H*)-ones with hydrazine hydrate, hydroxylamine hydrochloride, phenylhydrazine, urea and thiourea, respectively. The structures of the compounds were established by IR, ^{1}H NMR, ^{13}C NMR, mass spectral analysis, X-ray diffraction, and the compounds have been screened for in vitro antimicrobial and antimycobacterial against Mycobacterium tuberculosis H37Rv (MTB). Among the compounds screened, five substances were found to have an MIC of 3.12 μg/ml or greater against MTB. Structure–activity relationship (SAR) analyses and in silico drug relevant properties (HBD, HBA, PSA, c Log P, M.wt) confirmed that the compounds are potential lead compounds for future drug discovery studies.

1. Introduction

The indole moiety is probably the most widely spread nitrogen heterocycle in nature. It is for example an essential part of the amino acid tryptophan and the neurotransmitter serotonin, and the indole scaffold is also found in a manifold of naturally occurring plant based alkaloids. The biological importance of indole heterocylces and, directly associated with this, their pharmacological and medical potential, have made indoles extremely attractive and rewarding research targets and have motivated countless researchers to study their synthesis and pharmacological properties [1]. Cyclohepta[*b*]indoles, a sub-class of the indoles, represent an important part of many naturally occurring alkaloids, such as ervatamine [2], 20-epiervatamine [3], methuenine [4], 16-episilicine [5], ervitsine [6], caulersine [7] and homoarcyriaflavin [8], all compounds with highly interesting pharmacological properties (Fig. 1). The biological activities of cyclohepta[*b*]indoles cover a wide spectrum and include for example antitumour, antibiotic and anti inflammatory activities [9–13]. Motivated by these observations, we had earlier designed several compounds with a heterocyclic ring attached to a cyclohept[*b*]indole moiety and evaluated their pharmacological activities [14].

Tuberculosis is an infection caused by *Mycobacterium tuberculosis* and is the leading cause of infectious disease mortality in the world [15]. Around 1.86 billion people, that is, 32% of the world's population, is infected [16] with *M. tuberculosis* (MTB). The World Health Organization estimates that about 8 million people get newly infected with tuberculosis (TB) each year and that nearly 2 million people die from tuberculosis each year [16,17], that is, 5000 people every day [18]. HIV positive patients are especially susceptible to MTB with a 50-fold risk increase over HIV negative patients [19,20]. Similarly, the rate of progression of latent TB to the active disease in HIV positive patients is higher than for non-HIV infected individuals. It is pertinent to note that no new drug against tuberculosis has been developed in over 30 years. The increasing resistance of MTB strains against one or more first line TB drugs such as isoniazid [21], pyrazinamide [22] and rifampicin [23] has recently intensified the urgent need to develop new and more efficient drugs for the treatment of mycobacterial infections and this is the subject of numerous recent studies [24,25]. This manuscript presents the results of detailed investigations on the synthetic potential of the protocol in the construction of pyrazolo-,

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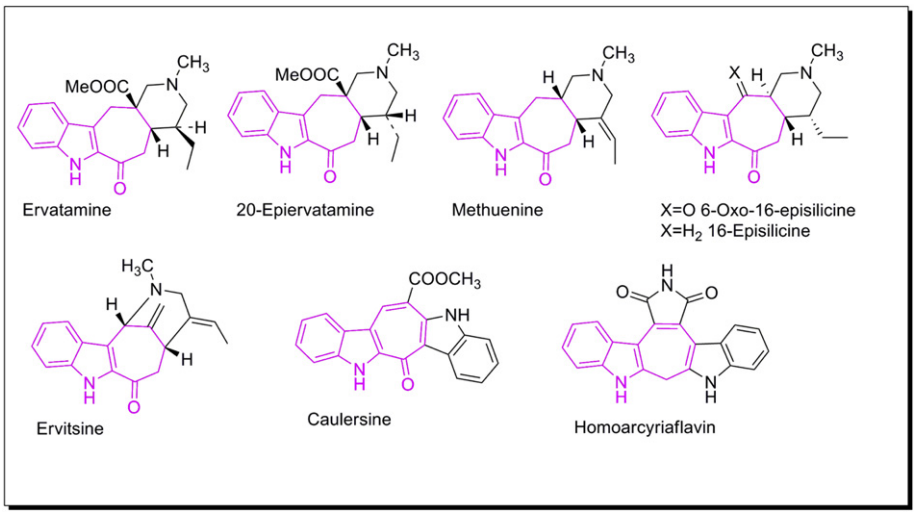
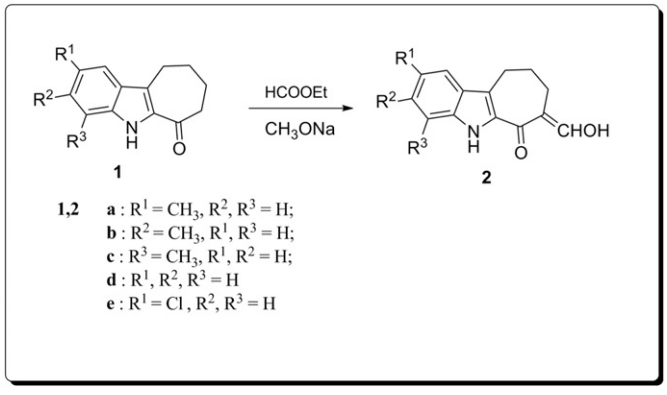


Fig. 1. Representative naturally occurring cyclohepta[*b*]indole alkaloids.

isoxazolo-, and pyrimidocyclohepta[*b*]indoles, and their potential activity against mycobacterial infections. The synthesized compounds were subjected to antimicrobial activity and preliminary antitubercular screening against *M. tuberculosis* H37Rv (MTB). Structure–activity relationships (SAR) were studied to explore their biological activities using pharmacological parameters such as c Log P, hydrogen bond acceptors (HBAs) and hydrogen bond donors (HBDs), which were calculated using computational software packages. Since the compounds are considered for oral delivery, they were also submitted to the analysis of Lipinski rule of five [26].

2. Chemistry

As part of our ongoing studies on the development of facile methods for the synthesis of organic compounds from readily available starting materials, specifically 7-(hydroxymethylene)-7,8,9,10-tetrahydrocyclohepta[*b*]indol-6(5*H*)-ones (**2**), which can be obtained by Claisen condensation of 7,8,9,10-tetrahydrocyclohepta[*b*]indol-6(5*H*)-ones (**1**) with ethylformate in the presence of sodium methoxide as shown in (Scheme 1). We would like to describe here the reaction of these readily available compounds (**2**) with hydrazine hydrate, hydroxylamine hydrochloride, phenylhydrazine, urea and thiourea leading to highly-substituted pyrazolo-, isoxazolo-, pyrimido- and mercaptopyrimidocyclohepta[*b*]indoles in one-pot addition reactions to form a library of new pyrazolo-, isoxazolo-, pyrimido- and mercaptopyrimidocyclohepta[*b*]indoles (Scheme 2). IR, ^{1}H NMR and ^{13}C NMR data, microanalyses and X-ray diffraction studies were used to ascertain the structures of all the compounds.



Scheme 1. Synthesis of 7-hydroxymethylene-7,8,9,10-tetrahydrocyclohepta[*b*]indol-6(5*H*)-ones **2**.

3. Biology

3.1. Antimicrobial activity

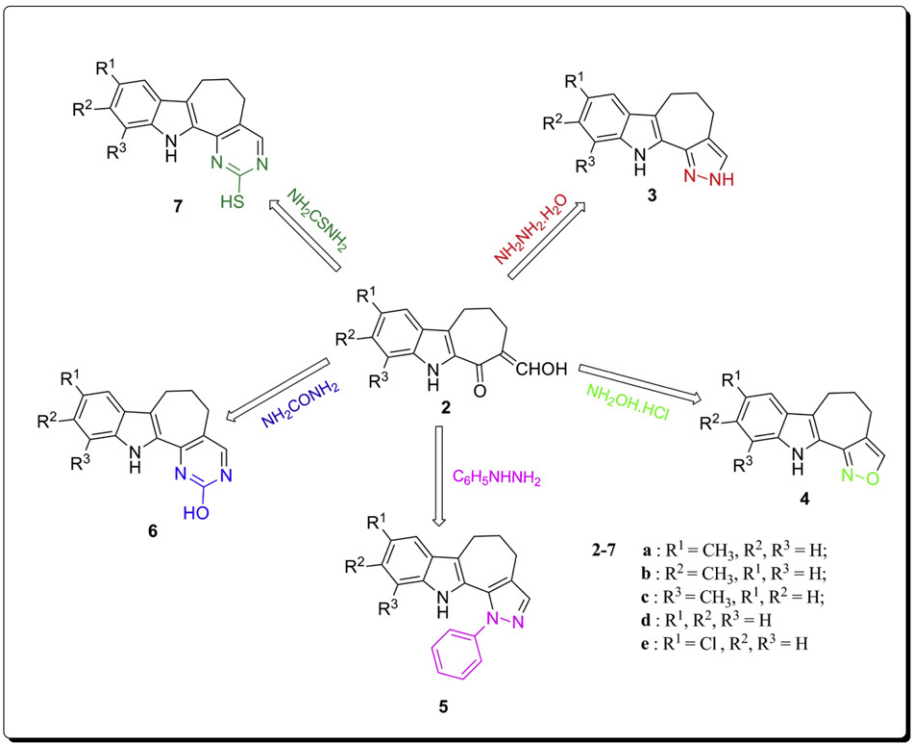
Compounds **2**–**7** were tested for in vitro antimicrobial activity against the Gram positive bacteria *Staphylococcus aureus* (NCIM No. 5021), *Bacillus subtilis* (NCIM No. 2063), the Gram negative bacteria *Klebsiella pneumoniae* (NCIM No. 2957) and *Proteus vulgaris* (NCIM No. 2027), and the fungi *Fusarium solani* (NCIM No. 1330), *Curvularia lunata* (NCIM No. 716) and *Aspergillus niger* (NCIM No. 596). The primary screening was carried out by the agar disc-diffusion method [27] using nutrient agar medium. For the most active compounds, **3e**, **4e**, **5e, 6e** and **7e**, the minimum inhibitory concentrations against the same microorganisms used in the preliminary screening were measured using a microdilution susceptibility method [28]. Chloramphenicol and ketoconazole were used as control drugs. The observed data on the antimicrobial activity of the compounds and control drugs are given in Tables 1 and 2, and in Tables S1, S2&S3 in the supplementary material.

3.2. Antimycobacterial activity

The antimycobacterial activities of five different series of compounds **2**–**7** were measured against *M. tuberculosis* H37Rv (ATCC 25618) using a resazurin microtitre assay (REMA) at different concentrations (50, 25, 12.5, 6.25, 3.12 and 1.5 μg/ml) and are shown in Table 2. Among the five series investigated, compounds **4e**, **5e** and **6e** have shown good activity compared to the other derivatives with an MIC (Minimum Inhibitory Concentration) value of 3.12 μg/mL.

4. Results and discussion

We have synthesized pyrazolo-, isoxazolo-, and pyrimidocyclohepta[*b*]indoles by the reaction of compounds **2** with appropriate nucleophiles. As a model reaction, we initially



Scheme 2. Synthesis of substituted pyrazolo-, isoxazolo-, pyrimido- and mercaptopyrimidocyclohepta[*b*]indoles **3**–**7**.

investigation the reaction of 7,8,9,10-tetrahydrocyclohepta[*b*]indol-6(5*H*)-one (**2**) with ethylformate (Scheme 1). The structures of the products were deduced from their elemental analysis data, and from their IR, mass, ^{1}H NMR and ^{13}C NMR spectra. The IR spectrum of compound **2a** exhibits sharp and strong bands at 3370 cm^{-1} and 1611 cm^{-1} due to the presence of hydroxyl (overlapped with NH) and carbonyl groups respectively. In the ^{1}H NMR spectrum –OH and olefinic –CH signals appear as two doublets at δ 15.58 ($J = 8.04$ Hz) and δ 7.85 ($J = 8.04$ Hz) and the carbazole NH is found as a singlet at δ 8.88, which suggests **2a** to be a hydroxymethylene compound. The aromatic protons appear in the expected regions. The structure of **2a** was confirmed by single crystal X-ray analysis (Fig. 2). In the next step we expanded the range of nucleophiles and investigated the construction of pyrazolo-, isoxazolo- and pyrimidocyclohepta[*b*]indoles from the precursor, 7-hydroxymethylene-7,8,9,10-tetrahydrocyclohepta[*b*]indol-6(5*H*)-ones (**2**) upon reaction with hydrazine hydrate, hydroxylamine hydrochloride, phenylhydrazine, urea and thiourea as shown in Scheme 2.

We first focussed our attention towards the synthesis of pyrazolocyclohepta[*b*]indoles **3** from 7-hydroxymethylene-7,8,9,10-tetrahydrocyclohepta[*b*]indol-6(5*H*)-ones (**2**) upon reaction with hydrazine hydrate. The proton NMR spectrum of **3a** shows the appearance of two broad singlets at δ 9.36 and δ 7.39 which account for indole–NH and pyrazolo–NH protons respectively. The aromatic protons appear in the region δ 7.49–7.11. A three proton singlet at δ 2.45 accounts for the methyl protons at the C_8 position. Mass spectrum and elemental analysis data support the molecular formula as $C_{15}H_{15}N_3$. The identities of the other compounds **3b** to **3i** were established in the same way with all spectroscopic data readily assignable. Subsequently, 7-hydroxymethylene-7,8,9,10-tetrahydrocyclohepta[*b*]indol-6(5*H*)-ones (**2**) was heated to reflux with hydroxylamine hydrochloride, phenylhydrazine, urea and thiourea in glacial acetic acid to afford isoxazolo- (**4**), pyrazolo- (**5**), pyrimido- (**6**) and mercaptopyrimidocyclohepta[*b*]indoles (**7**) (Scheme 2). The structures of the products were deduced from their elemental analysis data, and from their IR, mass, ^{1}H NMR and ^{13}C NMR spectra. For compounds **5a** and **5e** the structures were confirmed by single crystal X-ray diffraction analysis (Fig. 3 and Fig. S1).

4.1. Biological results

The results of preliminary antibacterial testing of compounds **2**–**7** are, shown in the supplementary material (Figs. S2–S4), reveal that compounds **2e** display excellent activity against Gram positive

Table 1
Minimum inhibitory concentration (MIC), μg/ml of **3e**, **4e**, **5e** and **6e**.

Compound	Minimum inhibitory concentrations, MIC (μg/ml)						
	S. aureus	*B. subtilis*	*K. pneumoniae*	*P. vulgaris*	*F. solani*	*C. lunata*	*A. niger*
3e	100	100	100	100	200	200	200
4e	50	50	100	200	100	100	100
5e	25	25	50	50	200	100	100
6e	50	50	100	100	100	50	50
7e	50	50	100	100	100	50	50
Chloramphenicol	6.25	6.25	12.5	12.5	–	–	–
Ketoconazole	–	–	–	–	12.5	6.25	6.25

Table 2
Antimycobacterial activity of **2**–**6** (*M. tuberculosis*).

Compound	Minimum inhibitory concentrations, MIC (μg/ml)					
	50 μg/ml	25 μg/ml	12.5 μg/ml	6.25 μg/ml	3.12 μg/ml	1.5 μg/ml
2a	+	+	−	−	−	−
2b	−	−	−	−	−	−
2c	+	−	−	−	−	−
2d	+	−	−	−	−	−
2e	+	+	−	−	−	−
3a	+	−	−	−	−	−
3b	+	+	−	−	−	−
3c	+	+	−	−	−	−
3d	−	−	−	−	−	−
3e	+	+	+	−	−	−
4a	+	−	−	−	−	−
4b	+	+	−	−	−	−
4c	+	+	−	−	−	−
4d	−	−	−	−	−	−
4e	+	+	+	+	+	−
5a	−	−	−	−	−	−
5b	+	+	−	−	−	−
5c	+	+	−	−	−	−
5d	−	−	−	−	−	−
5e	+	+	+	+	+	−
6a	+	−	−	−	−	−
6b	−	−	−	−	−	−
6c	−	−	−	−	−	−
6d	−	−	−	−	−	−
6e	+	+	+	+	+	−
7a	+	−	−	−	−	−
7b	+	−	−	−	−	−
7c	+	−	−	−	−	−
7d	−	−	−	−	−	−
7e	+	+	−	−	−	−

bacteria (inhibitory zone >28 mm) and good activity against Gram negative bacteria (inhibitory zone >25 mm). Compounds **3e, 4e** and **5e** exhibit the largest activity against Gram positive bacteria (inhibitory zone >31 mm) and good activity against Gram-negative bacteria (inhibitory zone >26 mm). Compound **6** shows moderate to high activity towards gram positive bacteria (21–29 mm) and moderate activity (19–24 mm) towards gram negative bacteria. Compound **7** displays only low activity against both bacteria. All the test compounds inhibited spore germination of tested fungi. The test compounds exhibited relatively high inhibitory activity against *F. solani*, *C. lunata*, and a slightly lower activity against *A. niger*. The bar graph representation of antibacterial activity and antifungal activity of the most potent compounds is shown in Figs. 4 and 5. The compounds that have pyrazole, isoxazole, pyrimidine units display a pronounced antimicrobial activity. The MIC values were determined as the lowest concentration that completely inhibited visible growth of the microorganisms (Table 1). The structure–antimicrobial activity relationship of the synthesized compounds revealed that pyrazolocyclohepat[*b*]indoles and isoxazolocyclohepta[*b*]indoles display especially high activities. The maximum activity was attained with the compounds that also have a chloro substituent in the cyclohepta[*b*]indole moiety, viz. **3e**, **4e**, **5e, 6e** and **7e**.

The results of the antimicrobial screening leads to the following assumptions about the structural activity relationship (SAR):

- The substituent R = CH_3, H and Cl at the C_2-position plays a key role in varying the efficacy of antimicrobial activity.

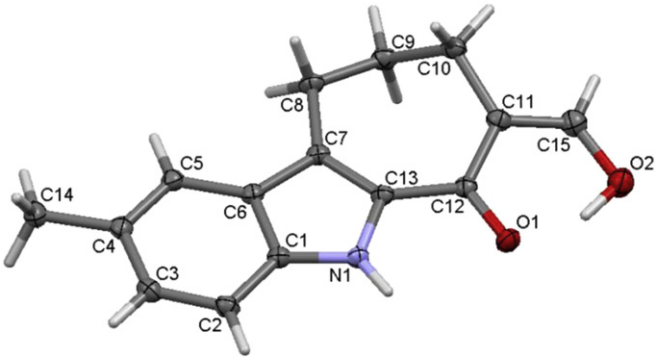


Fig. 2. X-ray crystal structure for **2a** with thermal ellipsoids at the 50% probability level.

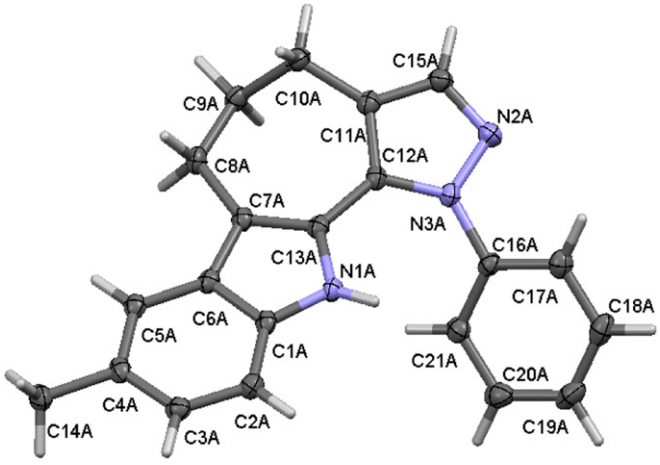


Fig. 3. X-ray crystal structure for **5a** with thermal ellipsoids at the 50% probability level. Only one of the two crystallographically independent molecules is shown for clarity.

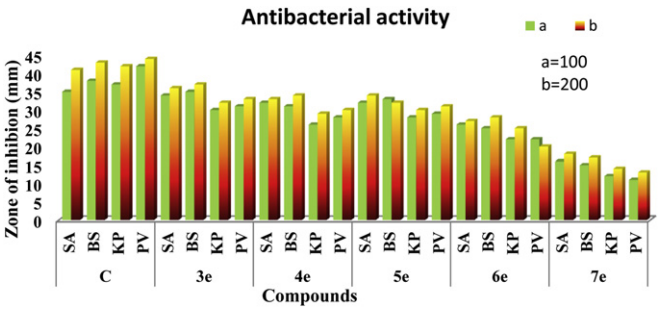


Fig. 4. Representative antibacterial activity of most potent compound.

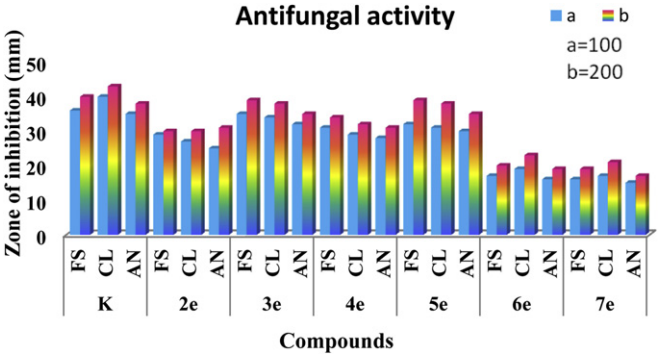


Fig. 5. Representative antifungal activity of most potent compound.

- The pyrazole moiety [29] in pyrazolocyclohepta[*b*]indoles positively influences in the antibacterial effectiveness, inducing good antimicrobial activity against all the four bacterial pathogens and three antifungal pathogens when compared to all other compounds.
- The introduction of electron withdrawing group at the C_2 position also led to a significant increase of activity, especially when compared to an electron donating CH_3 group. The role of electron withdrawing group in improving antimicrobial activities had been reported in the literature [30].
- The presence of a pyrimidine or mercaptopyrimidine moiety does induce only a moderate improvement of antimicrobial activity against all pathogens but does not lead to a major improvement in antibacterial activity.
- Compounds with an unsubstituted cyclohepta[*b*]indole ring showed the least activity at only 100 μg/ml.

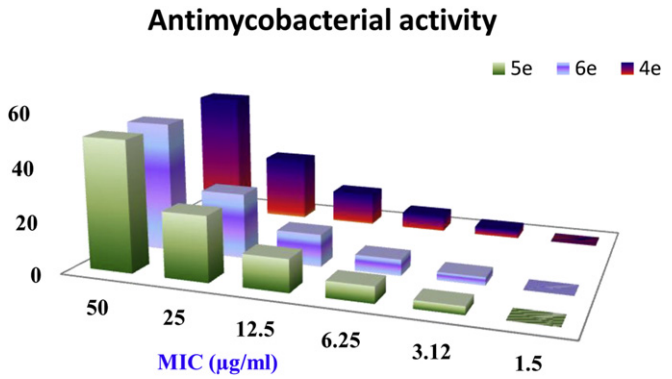


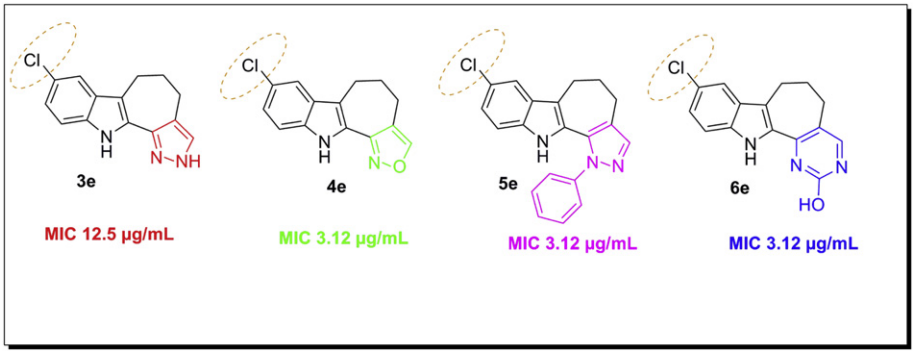
Fig. 6. Representative antimycobacterial activity of most potent compound.

4.2. In vitro antimycobacterial activity

Among the series of pyrazolo-, isoxazolo-, pyrimido- and mercaptopyrimidocyclohepta[*b*]indoles, **4e**, **5e** and **6e** have shown good activity compared to the other derivatives with MIC values of 3.12 μg/mL (Table 2). This major difference in the anti-TB activity between the pyrimido- and mercaptopyrimidocyclohepta[*b*] indoles could be attributed to two major stereoelectronic factors: first, the electronegativity of sulphur is lower than that of oxygen, and therefore mercaptopyrimidocyclohepta[*b*]indoles might not be able to undergo the required dipolar interaction and, secondly, the relatively larger size of sulphur could alter the binding affinity of mercaptocyclohepta[*b*]indoles.

In the hydroxymethylene series, compounds **2a**, **2b**, **2c**, **2d**, **2e** display only weak anti-tuberculosis activity with inhibition concentrations of 50–25 μg/mL. The introduction of a pyrazole ring system into the hydroxymethylene series leads to similarly active compounds **3a**, **3b**, **3c** with MIC 50–25 μg/mL, and **3e** with 50–12.5 μg/mL. In the isoxazolecyclohepta[*b*]indole derivatives **4a–e, 4e** exhibits the greatest potency with a minimal MIC concentration of 3.12 μg/mL. In the other pyrazole series (compounds **5**), the insertion of a phenyl ring at the N_1 position enhances the anti-tuberculosis activity for **5e** (50–3.12 μg/mL) as shown in Scheme 3. A bar graph representation of the anti-mycobacterial activity of the most potent compounds is shown in Fig. 6.

The present study investigated the effects of the substituention patterns of the synthesized cyclohepta[*b*]indoles on the anti-mycobacterial activity. From the results the following structure–activity relationships can be derived:



Scheme 3. Role of substituents in increasing the efficacy of antimycobacterial activity.

- Compounds with a pyrazole skeleton did generally show excellent antimycobacterial activity [31,32]. The presence of a chlorine atom on the pyrazolocyclohepta[*b*]indole moiety **3e** led to an improvement in the activity with respect to non-halogenated **3a** and **3d.**
- Compounds **4e, 5e** and **6e** bearing isoxazole, pyrazole (having N_1-phenyl) and pyrimidine moieties proved to be very active with MIC = 3.12 μg/mL. The presence of only R = H, on the other hand, was detrimental for the observed activity.
- The lipophilicity of the compounds may also play a role on the effectiveness of the compounds tested.

4.3. In silico pharmacological property and SAR study

To qualify the compounds as drug candidates, they were analyzed by the parameters set by Lipinski's rule of five, i.e. HBD, HBA, PSA, c Log P, and molecular weight, using the Osiris property explorer. The c Log P is the important physiochemical property indicating the lipophilicity and the ability of a molecule to cross biological membranes. According to Lipinski's rule of five a c Log *P* value below 5 is sufficient for a compound to be a potential drug candidate. The synthesized compounds showed a marginal lipophilicity within the range of 4.0–5.0. The molecular weight property of a compound is related to its in vivo administration. All the synthesized compounds have a molecular weight within the suggested range. The compounds showed to have HBA (Hydrogen Bond Acceptor) values below 10 and HBD (Hydrogen Bond Donor) values below 5, which is also within the limits set by Lipinski's rule. A compound with a polar surface area (PSA) larger than 140 A^2 is thought to have low oral bioavailability. All compounds in this study have PSA values substantially smaller than this upper limit. Interestingly all compounds studied also feature good drug likeness values. Compounds **2a**, **2d**, **2e**, **3a**, **3d**, **3e**, **4a**, **4d** and **6d** show overall good drug score values, calculated by combination of all parameters. Drug toxicity is a factor of great importance for a potential commercial drug, since a significant number of drugs are rejected in clinical trials based on their high toxicity profile. The toxicity of the compounds is calculated in terms of primary irritation, mutagenic, tumorigenic, and reproductive effects. All the compounds were confirmed as non-mutagenic and therefore were biologically safe for intake.

5. Conclusion

The newly synthesized heterocycles pyrazolo-, isoxazolo-, pyrimido-, and mercaptopyrimidocyclohepta[*b*]indoles were prepared from 7-hydroxymethylene-7,8,9,10-tetrahydrocyclohepta[*b*]indol-6(5*H*)-ones by cyclocondensation with appropriate nucleophiles. The maximum activity was observed for compounds having a chloro substituent in the cyclohepta[*b*]indole moiety. All these new cyclohepta[*b*]indole analogues were evaluated for their in vitro antimycobacterial activity against *M. tuberculosis* H37Rv (MTB) by the resazurin microtitre assay (REMA). Among the 30 synthesized compounds screened, **3e**, **4e**, **5e** and **6e** (MIC 3.12 μg/mL) were found to be the most active antitubercular agents. Bioavailability and initial toxicity tests of the compounds indicate that the compounds have properties that make them suitable for further testing as potential drug candidates.

6. Experimental

Melting points (M.p) were determined on a Mettler FP 51 apparatus (Mettler Instruments, Switzerland) and are uncorrected. They are expressed in degree centigrade (°C). IR spectra were recorded on a Schimadzu FTIR-8201PC spectrophotometer (Schimadzu, Japan) using KBr discs. ^{1}H NMR spectra ware recorded on a Bruker AMX 400 spectrometer (400 MHz) at IISc, Bangalore and on a Bruker AMX 500 (500 MHz) spectrometer at IIT, Chennai, using tetramethylsilane (TMS) as an internal reference. The chemical shifts are expressed in parts per million (ppm). Microanalyses were performed on a Vario EL III model CHNS analyser (Vario, Germany) at the Department of Chemistry, Bharathiar University. Diffraction data for compounds **2a**, **5a** and **5e** were collected on a Bruker AXS SMART APEX CCD diffractometer at 100 K using monochromatic Mo Kα radiation with the omega scan technique. Compound and **5a** and **5e** both crystallized with two crystallographically independent molecules and both are non-merohedrally twinned structures. Complete cif files for compounds **2a**, **5a** and **5e** with details of the structures (including description of the twinning) were deposited with the Cambridge Crystallographic Data Centre, CCDC Deposit # 841550–841552. These data may be obtained free of charge by emailing: www.ccdc.cam.ac.uk/data_request/cif. The purity of the products was tested by TLC with plates coated with silica gel-G with petroleum ether, ethyl acetate and methanol as developing solvents. 1-Oxo-2,3,4,5,10-hexahydrocyclohepta[*b*]indole was prepared by a reported procedure [33]. All chemicals were purchased from Aldrich, Bangalore, India.

6.1. Chemistry

6.1.1. General procedure for the synthesis of 7-(hydroxymethylene)-7,8,9,10-tetrahydrocyclohepta[b]indol-6(5H)-ones (2)

An appropriate 7,8,9,10-tetrahydrocyclohepta[*b*]indol-6(5*H*)-one (**1**, 0.005 mol) was added in small portions over a period of 5 min to a vigorously stirred mixture of sodium methoxide (2.5 g of sodium in 25 ml of methanol), and ethylformate (15 ml) cooled to 0 °C on an ice bath. The mixture was stirred on the ice bath for another half an hour and then allowed to stand at room temperature for 24 h. At the end of the period, ice and water were added to the yellow solid mass which was then acidified with cold concentrated hydrochloric acid. The precipitate obtained was filtered and dried. The brown solid separated out was then purified by column chromatography over silica gel using petroleum ether : ethyl acetate as the eluant (99:1) to yield the corresponding 7-(hydroxymethylene)-7,8,9,10-tetrahydrocyclohepta[*b*]indol-6(5*H*)-one (**2**).

*6.1.1.1. 7-(Hydroxymethylene)-2-methyl-7,8,9,10-tetrahydrocyclohepta[b]indol-6(5H)-one (**2a**).* Yellow solid; M.p. 183 °C; Yield: 1.060 g (88%); IR (KBr, cm^{-1}) ν_{max}: 3370, 2922, 1611; ^{1}H NMR ($CDCl_3$, 500 MHz) (ppm) δ: 2.03–2.09 (m, 2H, C_9–2H), 2.44 (s, 3H, C_2–CH_3), 2.51–2.54 (m, 2H, C_{10}–2H), 3.10–3.13 (m, 2H, C_8–2H), 7.17 (d d, 1H, C_3–H, J_o = 8.75 Hz, J_m = 1.36 Hz), 7.26 (d, 1H, C_4–H, J = 8.75 Hz), 7.38 (d, 1H, C_1–H, J = 1.50 Hz), 7.85 (d, 1H, C_7–CH, J = 8.04 Hz), 8.88 (bs, 1H, N_5–H), 15.58 (d, 1H, C_7–CHO, J = 8.04 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) (ppm) δ: 21.37 (C_2–CH_3), 25.83 (C_8), 27.36 (C_9), 28.49 (C_{10}), 111.40 (C_4), 112.86 (C_7), 120.08 (C_1), 124.18 (C_{10a}), 128.54 (C_{10b}), 128.64 (C_3), 129.49 (C_2), 130.04 (C_{4a}), 130.37 (C_{5a}), 173.27 (CHO), 182.37 (C=O); MS: *m*/*z* (%) 241 (100) [M^+]; Anal. Calcd. for: $C_{15}H_{15}NO_2$: C, 74.67; H, 6.27; N, 5.81. Found: C, 74.60; H, 6.22; N, 5.80%.

*6.1.1.2. 7-(Hydroxymethylene)-3-methyl-7,8,9,10-tetrahydrocyclohepta[b]indol-6(5H)-one (**2b**).* Yellow solid; M.p. 129 °C; Yield: 1.063 g (86%); IR (KBr, cm^{-1}) ν_{max}: 3364, 2921, 1618; ^{1}H NMR ($CDCl_3$, 500 MHz) (ppm) δ: 2.03–2.07 (m, 2H, C_9–2H), 2.45 (s, 3H, C_3–CH_3), 2.50–2.53 (m, 2H, C_{10}–2H), 3.09–3.12 (m, 2H, C_8–2H), 7.16 (d, 1H, C_2–H, J = 8.50 Hz), 7.28 (s, 1H, C_4–H), 7.36 (d, 1H, C_1–H, J = 8.50 Hz), 7.85 (d, 1H, C_7–CH, J = 8.04 Hz), 8.86 (bs, 1H, N_5–H),

15.57 (d, 1H, C_7–CHO, J = 8.04 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) (ppm) δ: 21.51 (C_3–CH_3), 25.43 (C_8), 27.23 (C_9), 28.54 (C_{10}), 111.52 (C_4), 112.74 (C_7), 119.11 (C_1), 121.38 (C_2), 14.09 (C_{10a}), 128.53 (C_3), 128.73 (C_{10b}), 130.18 (C_{4a}), 130.55 (C_{5a}), 174.09 (CHO), 182.41 (C=O); MS: m/z (%) 241 (100) [M^+]; Anal. Calcd. for: $C_{15}H_{15}NO_2$: C, 74.67; H, 6.27; N, 5.81. Found: C, 74.61; H, 6.26; N, 5.85%.

*6.1.1.3. 7-(Hydroxymethylene)-4-methyl-7,8,9,10-tetrahydrocyclohepta[b]indol-6(5H)-one (**2c**).* Yellow solid; M.p. 148 °C; Yield: 0.988 g (82%); IR (KBr, cm^{-1}) ν_{max}: 3369, 2932, 1619; ^{1}H NMR ($CDCl_3$, 500 MHz) (ppm) δ: 2.03–2.09 (m, 2H, C_9–2H), 2.49 (s, 3H, C_4–CH_3), 2.50–2.53 (m, 2H, C_{10}–2H), 3.12–3.15 (m, 2H, C_8–2H), 7.04 (t, 1H, C_2–H, J = 8.00 Hz), 7.13 (d, 1H, C_3–H, J = 8.00 Hz), 7.45 (d, 1H, C_1–H, J = 8.00 Hz), 7.82 (d, 1H, C_7–CH, J = 8.00 Hz), 8.89 (bs, 1H, N_5–H), 15.56 (d, 1H, C_7–CHO, J = 8.00 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) (ppm) δ: 16.51 (C_4–CH3), 25.56 (C_8), 27.23 (C_9), 28.47 (C_{10}), 111.98 (C_7), 116.37 (C_1), 120.81 (C_3), 123.51 (C_4), 124.12 (C_{10a}), 125.86 (C_2), 128.31 (C_{10b}), 130.21 (C_{4a}), 130.43 (C_{5a}), 174.00 (CHO), 182.93 (C=O); MS: m/z (%) 241 (100) [M^+]; Anal. Calcd. for: $C_{15}H_{15}NO_2$: C, 74.67; H, 6.27; N, 5.81. Found: C, 74.62; H, 6.21; N, 5.88%.

*6.1.1.4. 7-(Hydroxymethylene)-7,8,9,10-tetrahydrocyclohepta[b]indol-6(5H)-one (**2d**).* Yellow solid; M.p. 159 °C; Yield: 0.964 g (85%); IR (KBr, cm^{-1}) ν_{max}: 3374, 2925, 1610; ^{1}H NMR ($CDCl_3$, 500 MHz) (ppm) δ: 2.03–2.09 (m, 2H, C_9–2H), 2.50–2.53 (m, 2H, C_{10}–2H), 3.12–3.15 (m, 2H, C_8–2H), 7.05 (d t, 1H, C_2–H, J_o = 8.00 Hz, J_m = 2.00 Hz), 7.29 (d t, 1H, C_3–H, J_o = 8.00 Hz, J_m = 2.00 Hz), 7.38 (d, 1H, C_4–H, J = 8.00 Hz), 7.49 (d, 1H, C_1–H, J = 8.00 Hz), 7.82 (d, 1H, C_7–CH, J = 8.00 Hz), 8.88 (b s, 1H, N_5–H), 15.49 (d, 1H, C_7–CHO, J = 8.00 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) (ppm) δ: 25.83 (C_8), 27.67 (C_9), 28.83 (C_{10}), 111.28 (C_4), 112.10 (C_7), 118.78 (C_1), 120.42 (C_2), 121.58 (C_3), 123.92 (C_{10a}), 128.28 (C_{10b}), 130.31 (C_{4a}), 130.55 (C_{5a}), 173.71 (CHO), 182.83 (C=O); MS: m/z (%) 227 (100) [M^+]; Anal. Calcd. for: $C_{14}H_{13}NO_2$: C, 73.99; H, 5.77; N, 6.16. Found: C, 73.94; H, 5.73; N, 6.19%.

*6.1.1.5. 2-Chloro-7-(hydroxymethylene)-7,8,9,10-tetrahydrocyclohepta[b]indol-6(5H)-one (**2e**).* Yellow solid; M.p. 167 °C; Yield: 1.004 g (80%); IR (KBr, cm^{-1}) ν_{max}: 3373, 2927, 1614; ^{1}H NMR ($CDCl_3$, 500 MHz) (ppm) δ: 2.05–2.10 (m, 2H, C_9–2H), 2.53–2.55 (m, 2H, C_{10}–2H), 3.08–3.11 (m, 2H, C_8–2H), 7.29 (d, 1H, C_4–H, J = 8.00 Hz), 7.30 (d, 1H, C_3–H, J_o = 8.00 Hz, J_m = 2.00 Hz), 7.58 (d, 1H, C_1–H, J = 8.00 Hz), 7.92 (d, 1H, C_7–CH, J = 8.50 Hz), 8.99 (bs, 1H, N_5–H), 15.49 (d, 1H, C_7–CHO, J = 8.50 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) (ppm) δ: 25.49 (C_8), 27.36 (C_9), 28.49 (C_{10}), 111.73 (C_7), 112.28 (C_4), 120.17 (C_1), 123.41 (C_3), 123.88 (C_{10a}), 128.28 (C_{10b}), 128.48 (C_2), 130.21 (C_{4a}), 130.31 (C_{5a}), 173.89 (CHO), 182.73 (C=O); MS: m/z (%) 261 (100) [M^+], 263 (30) [M + 2]; Anal. Calcd. for: $C_{14}H_{12}ClNO_2$: C, 64.25; H, 4.62; N, 5.35. Found: C, 64.21; H, 4.66; N, 5.31%.

*6.1.2. General procedure for the synthesis of 4,5,6,11-tetrahydropyrazolo[4′,3′:6,7]cyclo hepta[b]indoles (**3**)*

The reaction mixture consisting of the appropriate 7-(hydroxymethylene)-7,8,9,10-tetrahydrocyclohepta[*b*]indol-6(5*H*)-one (**2**, 0.001 mol), hydrazine hydrate (0.005 mol) was refluxed in absolute ethanol (15 ml) for 5 h. After the reaction was complete, the excess solvent was removed by distillation and the mixture was poured into ice-water, filtered, dried and purified by column chromatography over silica gel (eluting with a petroleum ether and ethylacetate mixture, 85:15) and recrystallised from ethanol to give the respective 4,5,6,11-tetrahydropyrazolo[4′,3′:6,7]cyclohepta[*b*] indoles (**3**) as yellow prisms.

*6.1.2.1. 8-Methyl-4,5,6,11-tetrahydropyrazolo[4′,3′:6,7]cyclohepta[b] indole (**3a**).* Yellow prism; M.p. 203 °C; Yield: 0.154 g (65%); IR (KBr, cm^{-1}) ν_{max}: 3270, 2923, 1629; ^{1}H NMR ($CDCl_3$, 500 MHz) (ppm) δ: 2.09–2.14 (m, 2H, C_5–2H), 2.45 (s, 3H, C_8–CH_3), 2.92–2.94 (m, 2H, C_4–2H), 3.08–3.12 (m, 2H, C_6–2H), 7.11 (d d, 1H, C_9–H, J_o = 8.00 Hz, J_m = 2.00 Hz), 7.20 (d, 1H, C_{10}–H, J = 8.00 Hz), 7.28 (d, 1H, C_7–H, J = 2.00 Hz), 7.39 (s, 1H, N_2–H), 7.49 (s, 1H, C_3–H), 9.36 (s, 1H, N_{11}–H); ^{13}C NMR ($CDCl_3$, 125 MHz) (ppm) δ: 21.99 (C_8–CH_3), 24.46 (C_4), 25.06 (C_5), 26.32 (C_6), 110.20 (C_{10}), 112.22 (C_{3a}), 117.30 (C_7), 118.29 (C_9), 120.44 (C_{6b}), 124.43 (C_{6a}), 126.99 (C_{11b}), 127.33 (C_3), 127.89 (C_8), 128.22 (C_{11a}), 134.19 (C_{10a}); MS: m/z (%) 237 (100) [M^+]; Anal. Calcd. for: $C_{15}H_{15}N_3$: C, 75.92; H, 6.37; N, 17.71. Found: C, 75.90; H, 6.34; N, 17.75%.

*6.1.2.2. 9-Methyl-4,5,6,11-tetrahydropyrazolo[4′,3′:6,7]cyclohepta[b] indole (**3b**).* Yellow prism; M.p. 223 °C; Yield: 0.151 g (64%); IR (KBr, cm^{-1}) ν_{max}: 3278, 2926, 1623; ^{1}H NMR ($CDCl_3$, 500 MHz) (ppm) δ: 2.08–2.13 (m, 2H, C_5–2H), 2.44 (s, 3H, C_9–CH_3), 2.90–2.92 (m, 2H, C_4–2H), 3.13–3.15 (m, 2H, C_6–2H), 6.94 (d, 1H, C_8–H, J = 8.00 Hz), 7.06 (s, 1H, C_{10}–H), 7.32 (s, 1H, N_2–H), 7.43 (d, 1H, C_7–H, J = 8.00 Hz), 7.49 (s, 1H, C_3–H), 9.12 (s, 1H, N_{11}–H); ^{13}C NMR ($CDCl_3$, 125 MHz) (ppm) δ: 21.53 (C_9–CH_3), 24.38 (C_4), 24.51 (C_5), 26.38 (C_6), 111.38 (C_{10}), 112.21 (C_{3a}), 118.71 (C_7), 121.31 (C_8), 124.83 (C_{6a}), 126.76 (C_{11b}), 127.12 (C_{6b}), 127.89 (C_3), 128.31 (C_9), 128.99 (C_{11a}), 134.69 (C_{10a}); MS: m/z (%) 237 (100) [M^+]; Anal. Calcd. for: $C_{15}H_{15}N_3$: C, 75.92; H, 6.37; N, 17.71. Found: C, 75.97; H, 6.31; N, 17.75%.

*6.1.2.3. 10-Methyl-4,5,6,11-tetrahydropyrazolo[4′,3′:6,7]cyclohepta[b] indole (**3c**).* Yellow prism; M.p. 189 °C; Yield: 0.158 g (67%); IR (KBr, cm^{-1}) ν_{max}: 3267, 2919, 1626; ^{1}H NMR ($CDCl_3$, 500 MHz) (ppm) δ: 2.09–2.13 (m, 2H, C_5–2H), 2.49 (s, 3H, C_{10}–CH_3), 2.91–2.93 (m, 2H, C_4–2H), 3.08–3.10 (m, 2H, C_6–2H), 7.11 (t, 1H, C_8–H, J = 8.25 Hz), 7.21 (d, 1H, C_9–H, J = 8.25 Hz), 7.37 (s, 1H, N_2–H), 7.42 (d, 1H, C_7–H, J = 8.25 Hz), 7.49 (s, 1H, C_3–H), 9.26 (b s, 1H, N_{11}–H); ^{13}C NMR ($CDCl_3$, 125 MHz) (pppm) δ: 16.71 (C_4–CH_3), 24.33 (C_4), 24.47 (C_5), 26.78 (C_6), 112.56 (C_{3a}), 116.31 (C_7), 120.51 (C_{6b}), 120.83 (C_9), 123.33 (C_8), 123.51 (C_{10}), 123.99 (C_{6a}), 126.69 (C_{11b}), 127.88 (C_3), 129.01 (C_{11a}), 134.48 (C_{10a}); MS: m/z (%) 237 (100) [M^+]; Anal. Calcd. for: $C_{15}H_{15}N_3$: C, 75.92; H, 6.37; N, 17.71. Found: C, 75.96; H, 6.32; N, 17.76%.

*6.1.2.4. 4,5,6,11-Tetrahydropyrazolo[4′,3′:6,7]cyclohepta[b]indole (**3d**).* Yellow prism; M.p. 195 °C; Yield: 0.144 g (65%); IR (KBr, cm^{-1}) ν_{max}: 3267, 2919, 1634; ^{1}H NMR ($CDCl_3$, 500 MHz) (ppm) δ: 2.09–2.13 (m, 2H, C_5–2H), 2.91–2.94 (m, 2H, C_4–2H), 3.09–3.11 (m, 2H, C_6–2H), 7.05 (d t, 1H, C_8–H, J_o = 8.50 Hz, J_m = 2.00 Hz), 7.20 (d t, 1H, C_7–H, J_o = 8.50 Hz, J_m = 2.00 Hz), 7.24 (d, 1H, C_{10}–H, J = 8.50 Hz), 7.35 (s, 1H, N_2–H), 7.41 (d, 1H, C_7–H, J = 8.50 Hz), 7.48 (s, 1H, C_3–H), 9.21 (b s, 1H, N_{11}–H); ^{13}C NMR ($CDCl_3$, 125 MHz) (ppm) δ: 24.47 (C_4), 24.73 (C_5), 26.83 (C_6), 111.29 (C_{10}), 112.49 (C_{3a}), 118.51 (C_7), 120.38 (C_{6b}), 120.78 (C_8), 121.71 (C_9), 124.01 (C_{6a}), 126.93 (C_{11b}), 128.01 (C_3), 129.09 (C_{11a}), 134.19 (C_{10a}); MS: m/z (%) 223 (100) [M^+]; Anal. Calcd. for: $C_{14}H_{13}N_3$: C, 75.31; H, 5.87; N, 18.82. Found: C, 75.35; H, 5.83; N, 18.87%.

*6.1.2.5. 8-Chloro-4,5,6,11-tetrahydropyrazolo[4′,3′:6,7]cyclohepta[b] indole (**3e**).* Yellow prism; M.p. 213 °C; Yield: 0.154 g (60%); IR (KBr, cm^{-1}) ν_{max}: 3268, 2929, 1631; ^{1}H NMR ($CDCl_3$, 500 MHz) (ppm) δ: 2.09–2.14 (m, 2H, C_5–2H), 2.91–2.93 (m, 2H, C_4–2H), 3.10–3.11 (m, 2H, C_6–2H), 7.10 (d d, 1H, C_9–H, J_o = 8.25 Hz, J_m = 2.00 Hz), 7.19 (d, 1H, C_{10}–H, J = 8.25 Hz), 7.29 (d, 1H, C_7–H, J_m = 2.00 Hz), 7.39 (s, 1H, N_2–H), 7.49 (s, 1H, C_3–H), 9.37 (s, 1H, N_{11}–H); ^{13}C NMR ($CDCl_3$, 125 MHz) (ppm) δ: 24.53 (C_4), 24.91 (C_5), 26.84 (C_6), 112.41 (C_{3a}), 112.71 (C_{10}), 120.31 (C_7), 120.41 (C_{6b}), 123.32 (C_9), 124.03 (C_{6a}), 126.81 (C_{11b}), 128.03 (C_3), 128.41 (C_8), 129.11 (C_{11a}), 133.89 (C_{10a}); MS: m/z (%) 257 (100) [M^+], 259 (30) [M + 2]; Anal. Calcd. for: $C_{14}H_{12}ClN_3$: C, 65.25; H, 4.69; N, 16.30. Found: C, 65.20; H, 4.61; N, 16.32%.

*6.1.3. General procedure for the synthesis of 4,5,6,11-tetrahydroisoxazolo[4′,3′:6,7] cyclohepta[b]indoles (**4**)*

The reaction mixture consisting of the appropriate 7-(hydroxymethylene)-7,8,9,10-tetrahydrocyclohepta[*b*]indol-6(5*H*)-one (**2**, 0.001 mol), hydroxylamine hydrochloride (0.005 mol) and glacial acetic acid (5 ml) was refluxed at 120 °C for 4 h. After completion of the reaction it was then cooled and poured onto crushed ice, the solid thus separated out was collected by filteration, washed with water, dried and purified by column chromatography over silica gel (eluting with a petroleum ether and ethyl acetate mixture, 85:15) and recrystallised from ethanol to give the corresponding 4,5,6,11-tetrahydroisoxazolo[4′,3′:6,7] cyclohepta[*b*] indoles (**4**).

*6.1.3.1. 8-Methyl-4,5,6,11-tetrahydroisoxazolo[4′,3′:6,7]cyclohepta[b] indole (**4a**).* Yellow prism; M.p. 204 °C; Yield: 0.138 g (58%); IR (KBr, cm^{-1}) ν_{max}: 3323, 2925, 1616; ^1H NMR ($CDCl_3$, 500 MHz) (ppm) δ: 2.07–2.13 (m, 2H, C_5–2H), 2.45 (s, 3H, C_8–CH_3), 2.82–2.85 (m, 2H, C_4–2H), 3.11–3.12 (m, 2H, C_6–2H), 7.09 (d d, 1H, C_9–H, J_o = 8.00 Hz, J_m = 2.00 Hz), 7.29 (d, 1H, C_{10}–H, J = 8.00 Hz), 7.35 (d, 1H, C_7–H, J_m = 2.00 Hz), 8.09 (s, 1H, C_3–H), 8.43 (b s, 1H, N_{11}–H); ^{13}C NMR ($CDCl_3$, 125 MHz) (ppm) δ: 21.48 (C_8–CH_3), 22.49 (C_4), 23.22 (C_5), 25.31 (C_6), 110.98 (C_{10}), 114.43 (C_{3a}), 116.31 (C_{6a}), 118.78 (C_7), 125.63 (C_{6b}), 125.81 (C_9), 129.03 (C_{11a}), 129.18 (C_8), 133.78 (C_{10a}), 151.73 (C_3), 158.38 (C_{11b}); MS: *m*/*z* (%) 238 (100) [M^+]; Anal. Calcd. for: $C_{15}H_{14}N_2O$: C, 75.61; H, 5.92; N, 11.76. Found: C, 75.60; H, 5.93; N, 11.74%.

*6.1.3.2. 9-Methyl-4,5,6,11-tetrahydroisoxazolo[4′,3′:6,7] cyclohepta [b]indole (**4b**).* Yellow prism; M.p. 199 °C; Yield: 0.133 g (56%); IR (KBr, cm^{-1}) ν_{max}: 3325, 2927, 1612; ^1H NMR ($CDCl_3$, 500 MHz) (ppm) δ: 2.12–2.17 (m, 2H, C_5–2H), 2.56 (s, 3H, C_9–CH_3), 2.87–2.90 (m, 2H, C_4–2H), 3.11–3.12 (m, 2H, C_6–2H), 7.23 (d d, 1H, C_8–H, J_o = 8.00 Hz, J_m = 2.00 Hz), 7.35 (d, 1H, C_7–H, J = 8.00 Hz), 7.57 (d, 1H, C_{10}–H, J = 2.00 Hz), 8.14 (s, 1H, C_3–H), 8.59 (b s, 1H, N_{11}–H); ^{13}C NMR ($CDCl_3$, 125 MHz) (ppm) δ: 21.50 (C_9–CH_3), 22.50 (C_4), 23.85 (C_5), 25.31 (C_6), 111.31 (C_{10}), 114.52 (C_{3a}), 116.31 (C_{6a}), 118.31 (C_7), 121.78 (C_8), 125.71 (C_{6b}), 128.71 (C_9), 129.41 (C_{11a}), 133.38 (C_{10a}), 51.54 (C_3), 158.27 (C_{11b}); MS: *m*/*z* (%) 238 (100) [M^+]; Anal. Calcd. for: $C_{15}H_{14}N_2O$: C, 75.61; H, 5.92; N, 11.76. Found: C, 75.64; H, 5.96; N, 11.73%.

*6.1.3.3. 10-Methyl-4,5,6,11-tetrahydroisoxazolo[4′,3′:6,7] cyclohepta [b]indole (**4c**).* Yellow prism; M.p. 219 °C; Yield: 0.130 g (55%); IR (KBr, cm^{-1}) ν_{max}: 3326, 2920, 1613; ^1H NMR ($CDCl_3$, 500 MHz) (ppm) δ: 2.11–2.16 (m, 2H, C_5–2H), 2.54 (s, 3H, C_{10}–CH_3), 2.85–2.89 (m, 2H, C_4–2H), 3.10–3.12 (m, 2H, C_6–2H), 7.25 (t, 1H, C_8–H, J = 8.00 Hz), 7.38 (d, 1H, C_9–H, J = 8.00 Hz), 7.56 (d, 1H, C_7–H, J = 8.00 Hz), 8.14 (s, 1H, C_3–H), 8.58 (b s, 1H, N_{11}–H); ^{13}C NMR ($CDCl_3$, 125 MHz) (ppm) δ: 16.58 (C_{10}–CH_3), 22.60 (C_4), 23.94 (C_5), 25.29 (C_6), 112.37 (C_7), 114.66 (C_{3a}), 116.52 (C_{6a}), 118.68 (C_9), 120.39 (C_{10}), 124.18 (C_8), 125.90 (C_{6b}), 129.14 (C_{11a}), 134.58 (C_{10a}), 151.60 (C_3), 158.30 (C_{11b}); MS: *m*/*z* (%) 238 (100) [M^+]; Anal. Calcd. for: $C_{15}H_{14}N_2O$: C, 75.61; H, 5.92; N, 11.76. Found: C, 75.65; H, 5.95; N, 11.71%.

*6.1.3.4. 4,5,6,11-Tetrahydroisoxazolo[4′,3′:6,7]cyclohepta[b]indole (**4d**).* Yellow prism; M.p. 196 °C; Yield: 0.132 g (59%); IR (KBr, cm^{-1}) ν_{max}: 3329, 2918, 1612; ^1H NMR ($CDCl_3$, 500 MHz) (ppm) δ: 2.12–2.17 (m, 2H, C_5–2H), 2.87–2.90 (m, 2H, C_4–2H), 3.11–3.12 (m, 2H, C_6–2H), 7.09 (d, 1H, C_8–H, J = 8.00 Hz), 7.29 (d t, 1H, C_9–H, J_o = 8.00 Hz, J_m = 2.00 Hz), 7.38 (d, 1H, C_{10}–H, J = 8.00 Hz), 7.56 (d, 1H, C_7–H, J = 8.00 Hz), 8.14 (s, 1H, C_3–H), 8.58 (b s, 1H, N_{11}–H); ^{13}C NMR ($CDCl_3$, 125 MHz) (ppm) δ: 22.53 (C_4), 23.71 (C_5), 25.23 (C_6), 111.28 (C_{10}), 114.70 (C_{3a}), 116.44 (C_{6a}), 118.31 (C_7), 120.31 (C_8), 121.41 (C_9), 125.41 (C_{6b}), 129.15 (C_{11a}), 133.89 (C_{10a}), 151.49 (C_3), 158.41 (C_{11b}); MS: *m*/*z* (%) 224 (100) [M^+]; Anal. Calcd. for: $C_{14}H_{12}N_2O$: C, 74.98; H, 5.39; N, 12.49. Found: C, 74.92; H, 5.34; N, 12.44%.

*6.1.3.5. 8-Chloro-4,5,6,11-tetrahydroisoxazolo[4′,3′:6,7]cyclohepta[b] indole (**4e**).* Yellow prism; M.p. 201 °C; Yield: 0.144 g (56%); IR (KBr, cm^{-1}) ν_{max}: 3328, 2922, 1611; ^1H NMR ($CDCl_3$, 500 MHz) (ppm) δ: 2.09–2.14 (m, 2H, C_5–2H), 2.92–2.94 (m, 2H, C_4–2H), 3.08–3.12 (m, 2H, C_6–2H), 7.23 (d d, 1H, C_9–H, J_o = 8.00 Hz, J_m = 2.00 Hz), 7.34 (d, 1H, C_{10}–H, J = 8.00 Hz), 7.57 (d, 1H, C_7–H, J = 2.00 Hz), 8.14 (s, 1H, C_3–H), 8.56 (b s, 1H, N_{11}–H); ^{13}C NMR ($CDCl_3$, 125 MHz) (ppm) δ: 22.23 (C_4), 23.69 (C_5), 25.41 (C_6), 112.21 (C_{10}), 114.57 (C_{3a}), 116.38 (C_{6a}), 120.21 (C_7), 123.33 (C_9), 125.81 (C_{6b}), 128.38 (C_8), 129.21 (C_{11a}), 138.99 (C_{10a}), 151.38 (C_3), 158.37 (C_{11b}); MS: *m*/*z* (%) 258 (100) [M^+], 260 (100) [M + 2]; Anal. Calcd. for: $C_{14}H_{11}ClN_2O$: C, 65.00; H, 4.29; N, 10.83. Found: C, 65.06; H, 4.23; N, 10.88%.

*6.1.4. General procedure for the synthesis of 1-phenyl-4,5,6,11-tetrahydropyrazolo [4′,3′:6,7]cyclohepta[b]indole (**5**)*

The reaction mixture consisting of the appropriate 7-(hydroxymethylene)-7,8,9,10-tetrahydrocyclohepta[*b*]indol-6(5*H*)-one (**2**, 0.001 mol), phenylhydrazine (0.005 mol) and glacial acetic acid (5 ml) was refluxed at 120 °C for 10 h. After completion of reaction it was then cooled and poured onto crushed ice, the solid thus separated out was filtered, washed with water, dried and purified by column chromatography over silica gel (elutanting with a petroleum ether and ethyl acetate mixture, 97:3) to give the respective 1-phenyl-4,5,6,11-tetrahydro pyrazolo[4′,3′:6,7]cyclohepta[*b*]indole (**5**).

*6.1.4.1. 8-Methyl-1-phenyl-4,5,6,11-tetrahydro pyrazolo[4′,3′:6,7] cyclohepta[b]indole (**5a**).* Yellow solid; M.p. 201 °C; Yield: 0.203 g (65%); IR (KBr, cm^{-1}) ν_{max}: 3234, 2925, 1592; ^1H NMR ($CDCl_3$, 500 MHz) (ppm) δ: 2.11–2.18 (m, 2H, C_5–2H), 2.42 (s, 3H, C_8–CH_3), 2.90–2.93 (m, 2H, C_4–2H), 3.13–3.16 (m, 2H, C_6–2H), 6.88 (d, 1H, C_{10}–H, J = 8.50 Hz), 6.94 (d d, 1H, C_9–H, J_o = 8.5 Hz, J_m = 1.5 Hz), 7.08 (b s, 1H, N_{11}–H), 7.30 (s, 1H, C_7–H), 7.49 (s, 1H, C_3–H), 7.51–7.57 (m, 5H, C_2'-, C_3'-, C_4'-, C_5'- & C_6'-H); ^{13}C NMR ($CDCl_3$, 125 MHz) (ppm) δ: 21.28 (C_8–CH_3), 24.28 (C_4), 25.83 (C_5), 26.41 (C_6), 110.85 (C_{10}), 116.39 (C_{6a}), 119.50 (C_{3a}), 120.18 (C_7), 122.01 (C_{11b}), 125.88 (C_9), 127.58 (C_{6b}), 127.73 (C_2' & C_6'), 129.00 (C_4'), 129.19 (C_3' & C_5'), 129.31 (C_8), 133.42 (C_{11a}), 134.38 (C_{10a}), 140.27 (C_3), 140.53 (C_1'); MS: *m*/*z* (%) 313 (100) [M^+]; Anal. Calcd. for: $C_{21}H_{19}N_3$: C, 80.48; H, 6.11; N, 13.41. Found: C, 80.42; H, 6.16; N, 13.45%.

*6.1.4.2. 9-Methyl-1-phenyl-4,5,6,11-tetrahydro pyrazolo[4′,3′:6,7] cyclohepta[b]indole (**5b**).* Yellow solid; M.p. 196 °C; Yield: 0.215 g (69%); IR (KBr, cm^{-1}) ν_{max}: 3236, 2922, 1596; ^1H NMR ($CDCl_3$, 400 MHz) (ppm) δ: 2.11–2.18 (m, 2H, C_5–2H), 2.41 (s, 3H, C_9–CH_3), 2.90–2.93 (m, 2H, C_4–2H), 3.14–3.17 (m, 2H, C_6–2H), 6.81 (s, 1H, C_{10}–H), 6.91 (d, 1H, C_8–H, J = 8.80 Hz), 7.04 (b s, 1H, N_{11}–H), 7.40 (d, 1H, C_7–H, J = 8.80 Hz), 7.50 (s, 1H, C_3–H), 7.54–7.57 (m, 5H, C_2'-, C_3'-, C_4'-, C_5'- & C_6'-H); ^{13}C NMR ($CDCl_3$, 100 MHz) (ppm) δ: 20.89 (C_9–CH_3), 24.21 (C_4), 25.93 (C_5), 26.37 (C_6), 111.21 (C_{10}), 116.50 (C_{6a}), 118.61 (C_7), 119.52 (C_{3a}), 121.48 (C_8), 121.73 (C_{11b}), 127.16 (C_2' & C_6'), 127.58 (C_{6b}), 128.58 (C_9), 129.32 (C_4'), 129.41 (C_3' & C_5'), 133.73 (C_{11a}), 133.99 (C_{10a}), 140.32 (C_3), 140.73 (C_1'); MS: *m*/*z* (%) 313 (100) [M^+]; Anal. Calcd. for: $C_{21}H_{19}N_3$: C, 80.48; H, 6.11; N, 13.41. Found: C, 80.45; H, 6.12; N, 13.46%.

*6.1.4.3. 10-Methyl-1-phenyl-4,5,6,11-tetrahydro pyrazolo[4′,3′:6,7] cyclohepta[b]indole (**5c**).* Yellow solid; M.p. 219 °C; Yield: 0.197 g

(63%); IR (KBr, cm^{-1}) ν_{max}: 3233, 2922, 1590; ^1H NMR ($CDCl_3$, 500 MHz) (ppm) δ: 2.00 (s, 3H, C_{10}–CH_3), 2.11–2.17 (m, 2H, C_5–2H), 2.93–2.96 (m, 2H, C_4–2H), 3.14–3.17 (m, 2H, C_6–2H), 6.89 (d, 1H, C_9–H, J = 8.00 Hz), 6.99 (t, 1H, C_8–H, J = 8.00 Hz), 7.07 (b s, 1H, N_{11}–H), 7.36 (d, 1H, C_7–H, J = 8.00 Hz), 7.50 (s, 1H, C_3–H), 7.55–7.61 (m, 5H, C_2'-, C_3'-, C_4'-, C_5'- & C_6'-H); ^{13}C NMR ($CDCl_3$, 125 MHz) (ppm) δ: 15.55 (C_{10}–CH_3), 24.16 (C_4), 25.88 (C_5), 26.43 (C_6), 116.29 (C_7), 116.46 (C_{6a}), 119.61 (C_{3a}), 120.00 (C_9), 121.67 (C_{11b}), 123.31 (C_8), 123.35 (C_{10}), 127.35 (C_2' & C_6'), 127.57 (C_{6b}), 129.48 (C_4'), 129.64 (C_3' & C_5'), 133.43 (C_{11a}), 134.82 (C_{10a}), 140.59 (C_3), 140.91 (C_1'); MS: m/z (%) 313 (100) [M^+]; Anal. Calcd. for: $C_{21}H_{19}N_3$: C, 80.48; H, 6.11; N, 13.41. Found: C, 80.43; H, 6.14; N, 13.43%.

*6.1.4.4. 1-Phenyl-4,5,6,11-tetrahydro pyrazolo[4′,3′:6,7]cyclohepta[b] indole (**5d**).* Yellow solid; M.p. 243 °C; Yield: 0.188 g (63%); IR (KBr, cm^{-1}) ν_{max}: 3234, 2925, 1592; ^1H NMR ($CDCl_3$, 500 MHz) (ppm) δ: 2.12–2.19 (m, 2H, C_5–2H), 2.90–2.93 (m, 2H, C_4–2H), 3.14–3.17 (m, 2H, C_6–2H), 6.88 (d t, 1H, C_8–H, J_o = 8.50 Hz, J_m = 2.00 Hz), 6.99 (d t, 1H, C_9–H, J_o = 8.50 Hz, J_m = 2.00 Hz), 7.09 (d, 1H, C_{10}–H, J = 8.50 Hz), 7.15 (b s, 1H, N_{11}–H), 7.39 (d, 1H, C_7–H, J = 8.50 Hz), 7.49 (s, 1H, C_3–H), 7.55–7.60 (m, 5H, C_2'-, C_3'-, C_4'-, C_5'- & C_6'-H); ^{13}C NMR ($CDCl_3$, 125 MHz) (ppm) δ: 24.17 (C_4), 25.76 (C_5), 26.31 (C_6), 111.28 (C_{10}), 116.38 (C_{6a}), 118.38 (C_7), 119.63 (C_{3a}), 120.48 (C_8), 121.58 (C_9), 121.66 (C_{11b}), 127.21 (C_{6b}), 127.28 (C_2'& C_6'), 129.50 (C_4'), 129.87 (C_3'& C_5'), 133.18 (C_{11a}), 134.12 (C_{10a}), 140.32 (C_3), 140.79 (C_1'); MS: m/z (%) 299 (100) [M^+]; Anal. Calcd. for: $C_{20}H_{17}N_3$: C, 80.24; H, 5.72; N, 14.04. Found: C, 80.28; H, 5.76; N, 14.07%.

*6.1.4.5. 8-Chloro-1-phenyl-4,5,6,11-tetrahydro pyrazolo[4′,3′:6,7] cyclohepta[b]indole (**5e**).* Yellow solid; M.p. 234 °C; Yield: 0.233 g (70%); IR (KBr, cm^{-1}) ν_{max}: 3237, 2924, 1595; ^1H NMR ($CDCl_3$, 500 MHz) (ppm) δ: 2.11–2.17 (m, 2H, C_5–2H), 2.92–2.94 (m, 2H, C_4–2H), 3.10–3.13 (m, 2H, C_6–2H), 6.89 (d, 1H, C_{10}–H, J = 8.00 Hz), 7.05 (d d, 1H, C_9–H, J_o = 8.00 Hz, J_m = 2.00 Hz), 7.18 (b s, 1H, N_{11}–H), 7.48 (d, 1H, C_7–H, J_m = 2.00 Hz), 7.52 (s, 1H, C_3–H), 7.55–7.56 (m, 5H, C_2'-, C_3'-, C_4'-, C_5'- & C_6'-H); ^{13}C NMR ($CDCl_3$, 125 MHz) (ppm) δ: 24.54 (C_4), 25.81 (C_5), 26.41 (C_6), 112.28 (C_{10}), 116.23 (C_{6a}), 119.54 (C_{3a}), 120.11 (C_7), 121.70 (C_{11b}), 123.41 (C_9), 126.98 (C_{6b}), 127.37 (C_2' & C_6'), 128.58 (C_8), 129.31 (C_4'), 129.63 (C_3' & C_5'), 133.11 (C_{11a}), 133.99 (C_{10a}), 140.38 (C_3), 140.86 (C_1'); MS: m/z (%) 333 (100) [M^+], 335 (31) (M + 2); Anal. Calcd. for: $C_{20}H_{16}ClN_3$: C, 71.96; H, 4.83; N, 12.59. Found: C, 71.92; H, 4.86; N, 12.54%.

*6.1.5. General procedure for the synthesis of 2-hydroxy-5,6,7,12-tetrahydropyrimido [5′,6′:6,7]cyclohept[b]indoles (**6**)*

A mixture of the appropriate 7-(hydroxymethylene)-7,8,9,10-tetrahydrocyclohepta [*b*]indol-6(5*H*)-one (**2**, 0.001 mol) and urea (0.002 mol) was refluxed in glacial acetic acid (6 ml) at 120 °C for 5 h. After completion of the reaction it was then poured into crushed ice with stirring. The solid thus separated was filtered, dried and purified by column chromatography over silica-gel using a petroleum ether and ethyl acetate mixture (75:25) as the solvent system to yield the corresponding 2-hydroxy-5,6,7,12-tetrahydropyrimido [5′,6′:6,7]cyclohepta[*b*]indoles (**6**) as a yellow solid.

*6.1.5.1. 2-Hydroxy-9-methyl-5,6,7,12-tetrahydropyrimido[5′,6′:6,7] cyclohepta[b]indole (**6a**).* Yellow solid; M.p. 220 °C; Yield: 0.185 g (70%); IR (KBr, cm^{-1}) ν_{max}: 3438, 3265, 1602; ^1H NMR ($CDCl_3$, 500 MHz) (ppm) δ: 2.09–2.13 (m, 2H, C_6–2H), 2.45 (s, 3H, C_9–CH_3), 2.92–2.94 (m, 2H, C_5–2H), 3.08–3.12 (m, 2H, C_7–2H), 7.10 (d, 1H, C_{10}–H, J = 8.00 Hz), 7.21 (d, 1H, C_{11}–H, J = 8.00 Hz), 7.32 (s, 1H, C_8–H), 7.49 (s, 1H, C_4–H), 9.19 (b s, 1H, N_{12}–H), 11.30 (s,1H, C_2-OH); ^{13}C NMR ($CDCl_3$, 125 MHz) (ppm) δ: 21.48 (C_9–CH_3), 24.24 (C_5), 25.41 (C_6), 26.38 (C_7), 110.98 (C_{11}), 116.41 (C_{7a}), 119.17 (C_{4a}), 120.11 (C_8), 125.82 (C_{10}), 126.68 (C_{12b}), 127.38 (C_{7b}), 129.31 (C_9), 133.48 (C_{12a}), 134.56 (C_{11a}), 140.37 (C_4), 159.17 (C_2); MS: m/z (%) 265 (100) [M^+]; Anal. Calcd. for: $C_{16}H_{15}N_3O$: C, 72.43; H, 5.70; N, 15.84. Found: C, 72.40; H, 5.74; N, 15.89%.

*6.1.5.2. 2-Hydroxy-10-methyl-5,6,7,12-tetrahydropyrimido[5′,6′:6,7] cyclohepta[b]indole (**6b**).* Yellow solid; M.p. 246 °C; Yield: 0.188 g (71%); IR (KBr, cm^{-1}) ν_{max}: 3437, 3263, 1608; ^1H NMR ($CDCl_3$, 500 MHz) (ppm) δ: 2.10–2.13 (m, 2H, C_6–2H), 2.44 (s, 3H, C_{10}–CH_3), 2.90–2.92 (m, 2H, C_5–2H), 3.12–3.14 (m, 2H, C_7–2H), 6.98 (d, 1H, C_9–H, J = 8.00 Hz), 7.04 (s, 1H, C_{11}–H), 7.39 (d, 1H, C_8–H, J = 8.00 Hz), 7.49 (s, 1H, C_4–H), 9.21 (b s, 1H, N_{12}–H), 11.34 (s,1H, C_2-OH); ^{13}C NMR ($CDCl_3$, 125 MHz) (ppm) δ: 21.39 (C_{10}–CH_3), 24.25 (C_5), 25.43 (C_6), 26.41 (C_7), 111.28 (C_{11}), 116.24 (C_{7a}), 118.71 (C_8), 119.18 (C_{4a}), 121.48 (C_9), 126.86 (C_{12b}), 127.31 (C_{7b}), 128.51 (C_9), 133.48 (C_{12a}), 134.68 (C_{11a}), 140.44 (C_4), 158.98 (C_2); MS: m/z (%) 265 (100) [M^+]; Anal. Calcd. for: $C_{16}H_{15}N_3O$: C, 72.43; H, 5.70; N, 15.84. Found: C, 72.43; H, 5.75; N, 15.80%.

*6.1.5.3. 2-Hydroxy-11-methyl-5,6,7,12-tetrahydropyrimido[5′,6′:6,7] cyclohepta[b]indole (**6c**).* Yellow solid; M.p. 213 °C; Yield: 0.193 g (73%); IR (KBr, cm^{-1}) ν_{max}: 3429, 3336, 1603; ^1H NMR ($CDCl_3$, 500 MHz) δ: 2.09–2.13 (m, 2H, C_6–2H), 2.48 (s, 3H, C_{11}–CH_3), 2.90–2.93 (m, 2H, C_5–2H), 3.08–3.10 (m, 2H, C_7–2H), 7.11 (t, 1H, C_9–H, J = 8.00 Hz), 7.21 (d, 1H, C_{10}–H, J = 8.00 Hz), 7.41 (d, 1H, C_8–H, J = 8.00 Hz), 7.48 (s, 1H, C_4–H), 9.26 (bs, 1H, N_{12}–H), 11.36 (s,1H, C_2-OH); ^{13}C NMR ($CDCl_3$, 125 MHz) (ppm) δ: 16.55 (C_{11}–CH_3), 24.16 (C_5), 25.88 (C_6), 26.43 (C_7), 116.29 (C_{7a}), 116.46 (C_8), 119.68 (C_{4a}), 120.88 (C_{10}), 123.35 (C_9), 123.64 (C_{11}), 126.48 (C_{12b}), 127.58 (C_{7b}), 133.41 (C_{12a}), 134.12 (C_{11a}), 140.91 (C_4), 159.31 (C_2); MS: m/z (%) 265 (100) [M^+]; Anal. Calcd. for: $C_{16}H_{15}N_3O$: C, 72.43; H, 5.70; N, 15.84. Found: C, 72.48; H, 5.73; N, 15.87%.

*6.1.5.4. 2-Hydroxy-5,6,7,12-tetrahydropyrimido[5′,6′:6,7]cyclohepta [b]indole (**6d**).* Yellow solid; M.p. 189 °C; Yield: 0.188 g (75%); IR (KBr, cm^{-1}) ν_{max}: 3432, 3262, 1605; ^1H NMR ($CDCl_3$, 500 MHz) (ppm) δ: 2.08–2.13 (m, 2H, C_6–2H), 2.91–2.93 (m, 2H, C_5–2H), 3.09–3.11 (m, 2H, C_7–2H), 7.04 (d t, 1H, C_9–H, J_o = 8.50 Hz, J_m = 2.00 Hz), 7.21 (d t, 1H, C_{10}–H, J_o = 8.50 Hz, J_m = 2.00 Hz), 7.26 (d, 1H, C_{11}–H, J = 8.50 Hz), 7.40 (d, 1H, C_8–H, J = 8.50 Hz), 7.45 (s, 1H, C_4–H), 9.24 (b s, 1H, N_{12}–H), 11.32 (s, 1H, C_2-OH); ^{13}C NMR ($CDCl_3$, 125 MHz) (ppm) δ: 24.28 (C_5), 25.63 (C_6), 26.31 (C_7), 111.28 (C_{11}), 116.24 (C_{7a}), 118.51 (C_8), 119.18 (C_{4a}), 120.38 (C_9), 121.63 (C_{10}), 126.42 (C_{12b}), 127.41 (C_{7b}), 133.28 (C_{12a}), 134.40 (C_{11a}), 140.53 (C_4), 159.11 (C_2); MS: m/z (%) 251 (100) [M^+], 252 (30) [M + 2]; Anal. Calcd. for: $C_{15}H_{13}N_3O$: C, 71.70; H, 5.21; N, 16.72. Found: C, 71.74; H, 5.26; N, 16.75%.

*6.1.5.5. 9-Chloro-2-hydroxy-5,6,7,12-tetrahydropyrimido[5′,6′:6,7] cyclohepta[b]indole (**6e**).* Yellow solid; M.p. 197 °C; Yield: 0.199 g (70%); IR (KBr, cm^{-1}) ν_{max}: 3430, 3268, 1602; ^1H NMR ($CDCl_3$, 500 MHz) (ppm) δ: 2.08–2.14 (m, 2H, C_6–2H), 2.90–2.93 (m, 2H, C_5–2H), 3.09–3.11 (m, 2H, C_7–2H), 7.10 (d, 1H, C_{10}–H, J = 8.00 Hz), 7.18 (d, 1H, C_{11}–H, J = 8.00 Hz), 7.29 (s, 1H, C_8–H), 7.49 (s, 1H, C_4–H), 9.25 (bs, 1H, N_{12}–H), 11.25 (s,1H, C_2-OH); ^{13}C NMR ($CDCl_3$, 125 MHz) (ppm) δ: 24.29 (C_5), 25.43 (C_6), 26.23 (C_7), 112.12 (C_{11}), 116.21 (C_{7a}), 119.20 (C_{4a}), 120.18 (C_8), 123.42 (C_{10}), 126.40 (C_{12b}), 126.98 (C_{7b}), 128.41 (C_9), 133.24 (C_{12a}), 133.98 (C_{11a}), 140.38 (C_4), 159.18 (C_2); MS: m/z (%) 285 (100) [M^+]; Anal. Calcd. for: $C_{15}H_{12}ClN_3O$: C, 63.05; H, 4.23; N, 14.71. Found: C, 63.09; H, 4.26; N, 14.75%.

*6.1.6. General procedure for the synthesis of 2-mercapto-5,6,7,12-tetrahydropyrimido [5′,6′:6,7]cyclohept[b]indoles (**7**)*

A mixture of the appropriate 7-(hydroxymethylene)-7,8,9,10-tetrahydrocyclohepta[*b*]indol-6(5*H*)-one (**2**, 0.001 mol) and thiourea (0.002 mol) was refluxed in glacial acetic acid (6 ml) at 120 °C for 5 h. After completion of the reaction it was then poured into crushed

ice with stirring. The solid thus separated was filtered, dried and purified by column chromatography over silica-gel using petroleum ether and ethylacetate mixture (75:25) as the solvent system to yield the corresponding 2-mercapto-5,6,7,12-tetrahydropyrimido [5′,6′:6,7]cyclohept[*b*]indole (**7**) as a yellow solid.

*6.1.6.1. 2-Mercapto-9-methyl-5,6,7,12-tetrahydropyrimido[5′,6′:6,7] cyclohepta[b]indole (**7a**).* Yellow solid; M.p. 247 °C; Yield: 0.196 g (70%); IR (KBr, cm^{-1}) ν_{max}: 3261, 2947, 1154; ^1H NMR ($CDCl_3$, 500 MHz) (ppm) δ: 2.06–2.13 (m, 2H, C_6–2H), 2.44 (s, 3H, C_9–CH_3), 2.81–2.83 (m, 2H, C_5–2H), 3.10–3.11 (m, 3H, C_7–2H, SH), 7.07 (d, 1H, C_{10}–H, J = 8.25 Hz), 7.30 (d, 1H, C_{11}–H, J = 8.25 Hz), 7.56 (s, 1H, C_8–H), 7.81 (s, 1H, C_4–H), 9.27 (b s, 1H, N_{12}–H); ^{13}C NMR ($CDCl_3$, 125 MHz) (ppm) δ: 21.48 (C_9–CH_3), 24.63 (C_4), 25.78 (C_5), 26.51 (C_7), 111.61 (C_{11}), 116.18 (C_{7a}), 119.47 (C_{4a}), 120.10 (C_8), 126.48 (C_{12b}), 128.01 (C_{7b}), 128.68 (C_{10}), 129.43 (C_9), 133.51 (C_{12a}), 135.01 (C_{11a}), 140.58 (C_4), 166.42 (C_2); MS: *m*/*z* (%) 281 (100) [M^+]; Anal. Calcd. for: $C_{16}H_{15}N_3S$: C, 68.30; H, 5.37; N, 14.93; S, 11.40. Found: C, 68.35; H, 5.33; N, 14.91; S, 11.44%.

*6.1.6.2. 2-Mercapto-10-methyl-5,6,7,12-tetrahydropyrimido [5′,6′:6,7]cyclohepta[b]indole (**7b**).* Yellow solid; M.p. 203 °C; Yield: 0.193 g (69%); IR (KBr, cm^{-1}) ν_{max}: 3262, 2944, 1155; ^1H NMR ($CDCl_3$, 500 MHz) (ppm) δ: 2.12–2.17 (m, 2H, C_6–2H), 2.56 (s, 3H, C_{10}–CH_3), 2.86–2.90 (m, 2H, C_5–2H), 3.10–3.13 (m, 3H, C_7–2H, SH), 7.06 (s, 1H, C_{11}–H), 7.23 (d, 1H, C_9–H, J = 8.00 Hz), 7.34 (d, 1H, C_8–H, J = 8.00 Hz), 7.82 (s, 1H, C_4–H), 9.28 (b s, 1H, N_{12}–H); ^{13}C NMR ($CDCl_3$, 125 MHz) (ppm) δ: 21.41 (C_{10}–CH_3), 24.68 (C_5), 25.71 (C_6), 26.48 (C_7), 111.28 (C_{11}), 116.30 (C_{7a}), 118.71 (C_8), 119.48 (C_{4a}), 121.43 (C_9), 126.54 (C_{12b}), 127.18 (C_{7b}), 128.49 (C_{10}), 133.49 (C_{12a}), 134.89 (C_{11a}), 140.73 (C_4), 165.99 (C_2); MS: *m*/*z* (%) 281 (100) [M^+]; Anal. Calcd. for: $C_{16}H_{15}N_3S$: C, 68.30; H, 5.37; N, 14.93; S, 11.40. Found: C, 68.32; H, 5.35; N, 14.95; S, 11.43%.

*6.1.6.3. 2-Mercapto-11-methyl-5,6,7,12-tetrahydropyrimido[5′,6′:6,7] cyclohepta[b]indole (**7c**).* Yellow solid; M.p. 234 °C; Yield: 0.196 g (70%); IR (KBr, cm^{-1}) ν_{max}: 3324, 2915, 1158; ^1H NMR ($CDCl_3$, 500 MHz) (ppm) δ: 2.11–2.16 (m, 2H, C_6–2H), 2.55 (s, 3H, C_{11}–CH_3), 2.84–2.88 (m, 2H, C_5–2H), 3.09–3.12 (m, 3H, C_7–2H, SH), 7.23 (t, 1H, C_9–H, J = 7.50 Hz), 7.36 (d, 1H, C_{10}–H J = 7.50 Hz), 7.54 (d, 1H, C_8–H, J = 7.50 Hz), 7.80 (s, 1H, C_4–H), 9.18 (b s, 1H, N_{12}–H); ^{13}C NMR ($CDCl_3$, 125 MHz) (ppm) δ: 16.57 (C_{11}–CH_3), 24.76 (C_5), 25.87 (C_6), 26.49 (C_7), 116.29 (C_{7a}), 116.96 (C_8), 119.69 (C_{4a}), 120.89 (C_{10}), 123.35 (C_9), 123.84 (C_{11}), 126.88 (C_{12b}), 127.55 (C_{7b}), 133.45 (C_{12a}), 134.52 (C_{11a}), 140.58 (C_4), 168.88 (C_2); MS: *m*/*z* (%) 281 (100) [M^+]; Anal. Calcd. for: $C_{16}H_{15}N_3S$: C, 68.30; H, 5.37; N, 14.93; S, 11.40. Found: C, 68.35; H, 5.32; N, 14.97; S, 11.46%.

*6.1.6.4. 2-Mercapto-5,6,7,12-tetrahydropyrimido[5′,6′:6,7]cyclohepta [b]indole (**7d**).* Yellow solid; M.p. 210 °C; Yield: 0.197 g (74%); IR (KBr, cm^{-1}) ν_{max}: 3256, 2947, 1154; ^1H NMR ($CDCl_3$, 500 MHz) (ppm) δ: 2.12–2.16 (m, 2H, C_6–2H), 2.86–2.90 (m, 2H, C_5–2H), 3.10–3.12 (m, 3H, C_7–2H, SH), 7.08 (d t, 1H, C_9–H, J_o = 8.80 Hz, J_m = 2.00 Hz), 7.28 (d t, 1H, C_{10}–H, J_o = 8.80 Hz, J_m = 2.00 Hz), 7.37 (d, 1H, C_{11}–H, J = 8.80 Hz), 7.55 (d, 1H, C_8–H, J = 8.80 Hz), 7.84 (s, 1H, C_4–H), 9.20 (b s, 1H, N_{12}–H); ^{13}C NMR ($CDCl_3$, 125 MHz) (ppm) δ: 24.28 (C_5), 25.83 (C_6), 26.48 (C_7), 111.78 (C_{11}), 116.38 (C_{7a}), 118.73 (C_8), 119.63 (C_{4a}), 120.56 (C_9), 121.48 (C_{10}), 126.83 (C_{12b}), 127.41 (C_{7b}), 133.51 (C_{12a}), 134.83 (C_{11a}), 140.61 (C_4), 165.98 (C_2); MS: *m*/*z* (%) 267 (100) [M^+]; Anal. Calcd. for: $C_{15}H_{13}N_3S$: C, 67.39; H, 4.90; N, 15.72; S, 11.99. Found: C, 67.33; H, 4.95; N, 15.76; S, 11.94%.

*6.1.6.5. 9-Chloro-2-mercapto-5,6,7,12-tetrahydropyrimido[5′,6′:6,7] cyclohepta[b]indole (**7e**).* Yellow solid; M.p. 264 °C; Yield: 0.207 g (69%); IR (KBr, cm^{-1}) ν_{max}: 3268, 2943, 1158; ^1H NMR ($CDCl_3$, 500 MHz) (ppm) δ: 2.12–2.16 (m, 2H, C_6–2H), 2.86–2.90 (m, 2H, C_5–2H), 3.09–3.12 (m, 3H, C_7–2H, SH), 7.06 (d, 1H, C_{10}–H, J = 8.00 Hz), 7.24 (d, 1H, C_{11}–H, J = 8.00 Hz), 7.48 (s, 1H, C_8–H), 7.83 (s, 1H, C_4–H), 9.25 (b s, 1H, N_{12}–H); ^{13}C NMR ($CDCl_3$, 125 MHz) (ppm) δ: 24.38 (C_5), 25.43 (C_6), 26.49 (C_7), 112.48 (C_{11}), 116.47 (C_{7a}), 119.71 (C_{4a}), 120.31 (C_8), 123.12 (C_{10}), 126.83 (C_{12b}), 127.73 (C_{7b}), 128.51 (C_9), 133.83 (C_{12a}), 134.98 (C_{11a}), 140.71 (C_4), 165.76 (C_2); MS: *m*/*z* (%) 301 (100) [M^+], 301 (100) [M + 2]; Anal. Calcd. for $C_{15}H_{12}ClN_3S$: C, 59.70; H, 4.01; N, 13.92; S, 10.62. Found: C, 59.75; H, 4.04; N, 13.97; S, 10.65%.

6.2. Biological assays

6.2.1. Cells

Bacterial strains *S. aureus, B. subtilis, E. coli, K. pneumoniae* and fungi *F. solani, C. lunata* and *A. niger* were obtained from the National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory, Pune, India.

6.2.2. Antibacterial and antifungal assays

Preliminary antimicrobial activities of compounds **2**–**7** were tested by the Agar disc-diffusion method. Sterile filter paper discs (6 mm diameter) moistened with the test compound solution in DMSO of the specific concentration 100 μg and 200 μg/disc were carefully placed on the agar culture plates that had been previously inoculated separately with the microorganisms. The plates were incubated at 37 °C and the diameter of the growth inhibition zones were measured after 24 h in case of bacteria and after 48 h in case of fungi. All determinations were made in triplicate for each compound. The average of three independent readings for each organism was recorded. The MICs of the compound assays were carried out using the microdilution susceptibility method. Chloramphenicol was used as the reference antibacterial agent. Ketoconazole was used as the reference antifungal agent. The test compounds, chloramphenicol and ketoconazole were dissolved in DMSO at concentration of 800 μg/mL and two-fold dilutions of the solutions were prepared (400, 200, 100, 6.25 μg/mL). The microorganism suspensions were inoculated to the corresponding wells. The plates were incubated at 36 °C for 24 and 48 h for bacteria and fungi, respectively. The minimum inhibitory concentrations of the compounds were recorded as the lowest concentration of each chemical compound in the tubes with no turbidity (i.e. no growth) of inoculated bacteria/fungi.

6.3. Antimycobacterial screening by resazurin microtitre assay (REMA)

Compounds **2**–**7** were screened for their antimycobacterial activity on *M. tuberculosis* H37Rv (ATCC 25618), a virulent laboratory strain of the causative agent of tuberculosis, by the resazurin microtitre assay (REMA) [34,35]. Two 1 μl-loops of bacteria grown on Löwenstein-Jensen media were suspended in 3 ml Middlebrook 7H9 medium in 5 ml sterile glass vials containing glass beads. The bacterial suspension was homogenised using an ultrasound waterbath and thereafter adjusted to a McFarland turbidity of 1.0 followed by 1:20 dilution in 7H9 medium. From this culture, 100 μl was added to 100 μl medium in the wells of a 96-well plate containing the compounds at final concentrations of 50, 25, 12.5, 6.25, 3.12 and 1.5 μg/ml. Two growth controls (GC) with DMF and a control for sterility, containing only the 7H9 medium, were included. Rifampicin at 1 μg/ml served as an internal control. The microtiter plate was sealed in plastic bags and incubated at 37 °C for 7 days. Thirty microlitres of 0.02% resazurin (Sigma) solution was added to one of the two GCs and the plate was again incubated for

48 h. Resazurin, which is blue in its oxidised state, turns pink when reduced by the metabolic activity of viable cells. The MIC was defined as the lowest concentration of the test molecule that prevented the change of colour of resazurin from blue to pink.

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Appendix. Supplementary information

Supplementary data related to this article can be found online at doi:10.1016/j.ejmech.2011.10.046.

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