Bioorganic & Medicinal Chemistry xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Development of acridine derivatives as selective *Mycobacterium tuberculosis* DNA gyrase inhibitors

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ARTICLE INFO

Article history:
Received 4 November 2015
Revised 29 December 2015
Accepted 7 January 2016
Available online xxxx

Keywords:
Tuberculosis
DNA gyrase
Acridine derivatives

ABSTRACT

In this study we have designed *p*-phenylene diamine linked acridine derivative from our earlier reported quinoline–aminopiperidine hybrid MTB DNA gyrase inhibitors with aiming more potency and less cardiotoxicity. We synthesized thirty six compounds using four step synthesis from 2-chloro benzoic acid. Among them compound 4-chloro-*N*-(4-((2-methylacridin-9-yl)amino)phenyl)benzenesulphonamide (**6**) was found to be more potent with MTB DNA gyrase super coiling IC_{50} of 5.21 ± 0.51 μM; MTB MIC of 6.59 μM and no zHERG cardiotoxicity at 30 μM and 11.78% inhibition at 50 μM against mouse macrophage cell line RAW 264.7.

1. Introduction

Tuberculosis (TB) is an air-borne contagious disease that infects majorly lungs and caused by *Mycobacterium tuberculosis* (MTB) bacillus. Resistance to powerful first-line antibiotic drugs is making worst situation for the treatment of TB resulting in prolonged illness caused to severe death rate. Further emergence of more resistance strains like multi-drug resistance TB (MDR-TB), extensively drug resistance TB (XDR-TB) and totally drug resistance TB (TDR-TB) are major concerns to public health and scientific communities worldwide, signifies the importance of development of new, safer and more efficacious inhibitors/drugs to prevent TB. According to 2015 WHO Global TB reports[1] revealed that approximately 9 million people get infected with this MTB bacillus, 1.5 million died from the TB also revealed that the existence of MDR-TB in almost all countries. From past 40 years the only drug has been approved by US-FDA was bedaquiline (SIRTURO/TMC-207) for treatment of drug sensitive as well as MDR-TB[2] which acts on an ATP synthase enzyme even its showing adverse effects like QT prolongation, increased mortality.[3] Still there are no available drugs in the market that treat effectively and shorten the current treatment regimen. All these factors necessitate the design of new antimycobacterial agents with fewer side effects, more effective and synthetically feasible ones for the development of efficient anti-TB drugs.

Presently, we focused on to develop new MTB DNA gyrase inhibitors.[4–8] DNA gyrase is a type II topoisomerase that introduces negative supercoiling of native bacterial DNA, maintains its topology, plays a major role in DNA replication for its survival and its absence in eukaryotic cell makes interesting target for developing potential new anti-TB drugs.[9] DNA gyrase not only involves in supercoiling of DNA but also helps in relaxation, cleavage and catenation activities. DNA gyrase is a tetrameric holo enzyme with two A and B subunits together forming a heterodimer structure (A2B2 complex). Whereas Gyr A involves in nicking and ligation of DNA, on the other hand, Gyr B portion plays an important role in ATPase activity acts as power house in DNA replication process. Fluoroquinolones are the well-known DNA gyrase inhibitors; they were still in clinical practise that target Gyr A portion specifically. Novobiocin was licenced during 1960's is the one which acts as competitive inhibitor of ATPase activity of Gyr B protein portion of DNA gyrase enzyme.[10] The potency of Novobiocin is considerably higher than the fluoroquinolones that also acts on DNA gyrase but it was withdrawn because of its adverse side effects and poor pharmacological properties. Aminopiperidine based novel bacterial topoisomerase inhibitors (NBTIs) inhibitors targets MTB DNA gyrase enzyme and they also possess cardiac toxicity.[5,11] In this work we modify our earlier reported quinoline–aminopiperidine linker hybrid MTB DNA gyrase molecules[5] with considerable cardiotoxicity into acridine-*p*-phenylenediamine linker and evaluated its biological properties.

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http://dx.doi.org/10.1016/j.bmc.2016.01.011
0968-0896/

2. Results and discussion

2.1. Designing of the molecules

The chemical structures of NBTIs comprise a bicyclic hetero aromatic left-hand side (LHS) ring, a mono- or bicyclic hydrophobic right-hand side (RHS) ring and a middle aminopiperidine linker part (Fig. 1). As per literature, it is reported that aminopiperidines having a bicyclic aromatic moiety, generally show potent broad-spectrum antibacterial activity, but usually suffer from potent hERG inhibition leading to hERG or cardiotoxicity.[12,13] In order to overcome this toxicity issues, we have made an attempt to synthesize tricyclic hetero aromatic acridine as LHS with *p*-phenylenediamine based linker replacing the aminopiperidine as shown in Figure 1 and check its efficacy and cardiotoxicity profile.

2.2. Chemistry

The titled compounds were synthesized by a series of reactions that has been shown in Figure 2. Starting with simple 2-chlorobenzoicacid (**1**), it was reacted with different *para*-substituted anilines using stille coupling conditions, that is, Copper, DMF (*N*,*N* Dimethylformamide) heating the reactants at 140 °C for 6 h resulting in formation of 2-(*p*-tolyl/4-chlorophenyl/phenylamino)benzoic acid derivatives (**2a**–**c**). Further these **2a**–**c** intermediates were cyclized intra molecularly in refluxing phosphorousoxy chloride ($POCl_3$) for 6 h to get corresponding 9-chloro-2-substituted acridine derivatives (**3a**–**c**). The formed acridine intermediates (**3a**–**c**) were reacted with *p*-phenylenediamine using microwave conditions at 130 °C, PTSA (*para* toluenesulphonic acid) as catalyst in methanol for 1 h resulting in formation of N^1-(2-methyl/2-chloro/simple acridin-9-yl)benzene-1,4-diamine derivatives (**4a**–**c**). These amine intermediates were further reacted with different substituted aryl sulfonylchorides and aryl isothio/iso cyanates to get desired final sulphonamide (**5**–**16**) and thio/urea (**17**–**28**, **29**–**40**) derivatives. All the reactions were monitored by thin layer chromatography. Purity of the synthesized compounds was checked using LC–MS, structure characterized by ^{1}H NMR, ^{13}C NMR and elemental analysis. In the spectra (^{1}H NMR and ^{13}C NMR), the signals of the respective protons of the synthesized derivatives were verified on the basis of their chemical shifts, multiplicities, and coupling constants. The elemental analysis results were within ±0.4% of the theoretical values.

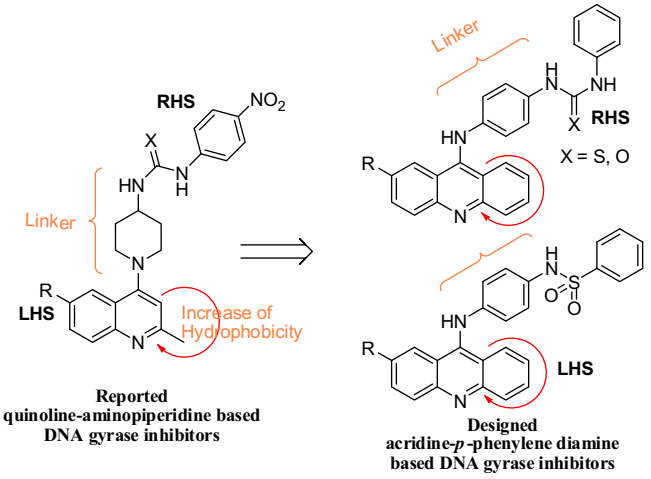


Figure 1. Design of compounds.

2.3. Biological evaluation

In the preliminary screening all the thirty six synthesized compounds were subjected to screening for their biological inhibition studies using MTB DNA gyrase supercoiling assay kit (Inspiralis, Norwich, UK). Novobiocin and Moxifloxacin was used as positive control in these assays, as it had been shown to be a potent inhibitor of DNA supercoiling of mycobacterial DNA gyrase (Table 1). Among the compounds tested, all compounds showed activity at <50 μM concentration, twenty six compounds showed IC_{50} value of <25 μM, ten compounds showed IC_{50} value of ⩽10 μM. Compound 4-chloro-*N*-(4-((2-methylacridin-9-yl)amino)phenyl)benzenesulphonamide (**6**) emerged as more potent with MTB DNA gyrase super coiling IC_{50} of 5.21 ± 0.51 μM (Fig. 3); and was ~2 times more potent than standard antitubercular DNA gyrase inhibitor Moxifloxacin [IC_{50} of 11.2 ± 0.36 μM].

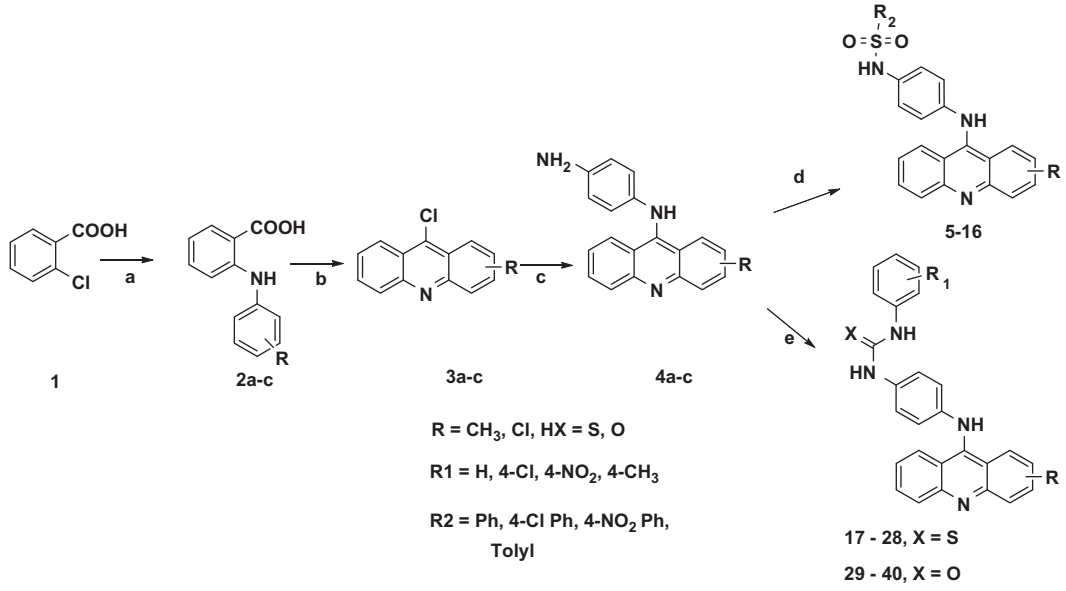
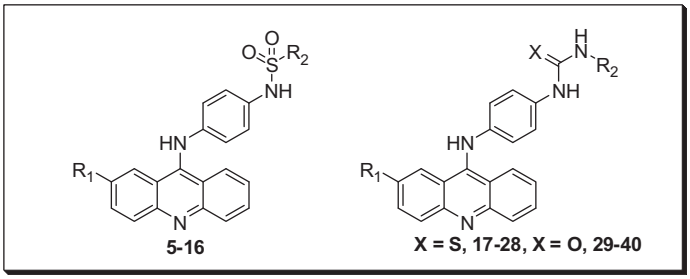


Figure 2. Synthetic protocol for the entitled substituted *N*-[4-(substituted-9-acridinyl)amino]phenyl analogues. Reagents and conditions: (a) (sub) aniline, Cu powder, DMF, 140 °C, 6 h; (b) $POCl_3$ reflux 6 h; (c) 1,4-phenylenediamine, *p*-toluenesulfonic acid, MeOH, MW 13 °C, 1 h; (d) (sub) alkyl/aryl sulfonyl chlorides, Et_3N, DMF, 0 to rt, 3 h; (e) (sub) aryl isothiocyanate/isocyanate, Et_3N, 80 °C, 8 h.

Table 1
In vitro biological evaluation of the acridine derivatives



Compd	R_1	R_2	MTB supercoiling assay (IC_{50} in μM)	MTB MIC (μM)	Cytotoxicity at 50 μM (% Inhib.)
5	CH_3	Phenyl	5.86 ± 0.35	28.44	39.57
6	CH_3	4-Chlorophenyl	5.21 ± 0.51	6.59	11.78
7	CH_3	4-Nitrophenyl	8.34 ± 0.59	12.90	26.98
8	CH_3	4-Toluyl	9.8 ± 0.33	27.56	27.81
9	Cl	Phenyl	9.2 ± 0.28	27.17	14.46
10	Cl	4-Chlorophenyl	12.4 ± 0.66	25.08	14.19
11	Cl	4-Nitrophenyl	8.1 ± 0.61	24.75	30.39
12	Cl	4-Toluyl	22.8 ± 0.42	>52.75	38.54
13	H	Phenyl	15.1 ± 0.59	14.69	42.06
14	H	4-Chlorophenyl	23.8 ± 1.24	27.18	34.21
15	H	4-Nitrophenyl	22.4 ± 0.29	24.64	34.12
16	H	4-Toluyl	9.3 ± 0.65	28.44	28.44
17	CH_3	Phenyl	6.3 ± 0.58	7.20	10.81
18	CH_3	4-Chlorophenyl	11.2 ± 0.83	26.70	26.00
19	CH_3	4-Nitrophenyl	24.8 ± 0.29	>52.13	28.83
20	CH_3	4-Toluyl	26.9 ± 0.48	55.73	34.99
21	Cl	Phenyl	29.3 ± 0.34	27.47	11.69
22	Cl	4-Chlorophenyl	31.5 ± 0.81	>51.08	6.70
23	Cl	4-Nitrophenyl	28.8 ± 1.94	>50.00	30.37
24	Cl	4-Toluyl	33.9 ± 1.44	>53.30	25.51
25	H	Phenyl	8.2 ± 0.29	14.86	29.24
26	H	4-Chlorophenyl	8.4 ± 0.44	13.74	12.54
27	H	4-Nitrophenyl	13.1 ± 0.59	13.43	20.80
28	H	4-Toluyl	31.1 ± 0.54	>57.53	27.41
29	CH_3	Phenyl	22.1 ± 0.82	29.87	24.78
30	CH_3	4-Chlorophenyl	11.4 ± 0.64	27.60	25.86
31	CH_3	4-Nitrophenyl	7.4 ± 0.29	13.48	33.94
32	CH_3	4-Toluyl	29.6 ± 0.55	57.80	43.11
33	Cl	Phenyl	23.6 ± 1.82	28.48	27.67
34	Cl	4-Chlorophenyl	11.8 ± 0.34	13.20	17.12
35	Cl	4-Nitrophenyl	7.3 ± 0.43	6.46	45.88
36	Cl	4-Toluyl	12.5 ± 0.59	13.80	29.20
37	H	Phenyl	32.6 ± 0.61	>61.81	30.45
38	H	4-Chlorophenyl	10.4 ± 0.46	28.48	26.25
39	H	4-Nitrophenyl	26.8 ± 1.59	27.81	19.25
40	H	4-Toluyl	13.1 ± 0.88	14.93	18.37
		Novobiocin	46 ± 10 nM	>50	NT
		Isoniazid	>50	0.66	NT
		Rifampicin	>50	0.23	NT
		Moxifloxacin	11.2 ± 0.36	1.26	1.26

NT indicates not tested.

Further we tested these compounds for their in vitro activity using *Mycobacterium smegmatis* (MS) GyrB ATPase assay as ATPase activity of GyrB protein from MTB was found to be very low when compared to MS. None of the compounds were shown any appreciable inhibition in GyrB assay indicates that these molecules specifically interact with GyrA subunit of DNA gyrase like other reported NBTIs. These molecules were more also selective towards MTB DNA gyrase only and did not inhibit DNA supercoiling activity of *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* DNA gyrases. These molecules are structurally similar to *m*-Amsacrine (Fig. 4), an acridine derivative, act as a eukaryotic topoisomerase II poison[14] was also reported as MTB topoisomerase I inhibitor with IC_{50} of 10 μM.[15] As synthesized molecules were structurally related to *m*-Amsacrine we also tested our compounds MTB topoisomerase 1 relaxation assay[16] and none of the compounds showed any inhibition at 50 μM. This result indicates the importance of methoxyl group present in the phenyl ring linker of *m*-Amsacrine.

With respect to structure activity relationship we have not found good correlation in SAR. To describe SAR in general; we have prepared twelve sulphonamides, urea and thiourea and in general order of activity sulphonamides (**5**–**16**) > thiourea (**17**–**28**) > urea (**29**–**40**). Among sulphonamides derivatives, we prepare three derivatives with H, Cl, and CH_3 groups at 2nd position of acridine moiety (LHS). Among them methyl derivatives (**5**–**8**) showed better activity (IC_{50} ranges from 5.21 ± 0.51 to 9.88 ± 0.33 μM) followed by chloro derivatives (**9**–**12**) with IC_{50} ranges from 8.1 ± 0.61 to 22.8 ± 0.42 μM and then unsubstituted acridine derivatives (**13**–**16**) with IC_{50} ranges from 9.3 ± 0.65 to 23.8.8 ± 1.24 μM (except compound **16**). Among compounds **5**–**16** we also prepared H, Cl,

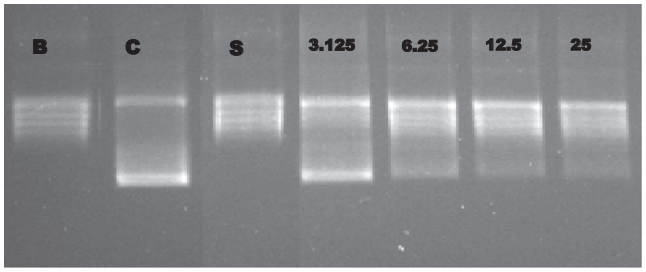


Figure 3. Picture depicting the supercoiling assay of compound **5**. B—relaxed DNA; C—relaxed DNA + enzyme; S—relaxed DNA + enzyme + Novobiocin; 25, 12.5, 6.25, 3.125—relaxed DNA + enzyme + compd **5** at different concentrations.

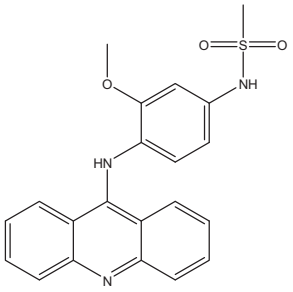


Figure 4. Structure of *m*-Amsacrine.

and CH_3 groups in the phenyl ring of RHS; among them the order of activity H > NO_2 > CH_3 and Cl groups at the 4th position of RHS phenyl ring.

The compounds were further screened for their in vitro antimycobacterial activity against *M. tuberculosis* H37Rv by microplate alamar blue assay method. Isoniazid, Rifampicin and Moxifloxacin were used as a positive control and for comparison. The minimum inhibitory concentration (MIC) was determined for each compound which was measured as the minimum concentration of compound required to completely inhibit the bacterial growth. Compounds **6**, **7**, **13**–**15**, **17**, **21**, **27**, **29**, **33**–**36**, and **39**–**40** showed good correlation between the MTB DNA gyrase super coiling assay IC_{50} and in vitro MTB MIC (Table 1); whereas other compounds showed more than two times higher than DNA gyrase super coiling IC_{50}; this might be because of less penetration of these molecules towards the MTB cell wall or efflux pump present in the cytoplasmic membrane of MTB may pump out these compounds efficiently. Among thirty six compounds tested twelve compounds showed MIC < 20 μM and three compounds (**6**, **17**, and **35**) showed MTB MIC of <10 μM.

The safety profile of the synthesized compounds were also accessed by testing their in vitro cytotoxicity against RAW 264.7 cells at 50 μM concentration using (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Selection of mouse macrophage cell line RAW 264.6 is based to check the toxicity in macrophages; as MTB resides in its. Percentage inhibitions of cells are reported in Table 1. Most of the tested compounds demonstrated good safety profile with very low inhibitory potential (<50%).

The most potent compound **6** was further examined for hERG channel inhibition by elucidating arrhythmogenic potential on Zebrafish ether-a-go-go-related gene (zERG) which was orthologus to the human ether-a-go-go-related gene (hERG).[17] This method has significant advantages over current conventional animal models include ethical issues, low compound requirement and cost of experiment. Compound was treated starting from 1 μM to 30 μM concentrations with 0.1% DMSO as a vehicle. The compound was analysed for the heart rate variations and AV ratio using a protocol

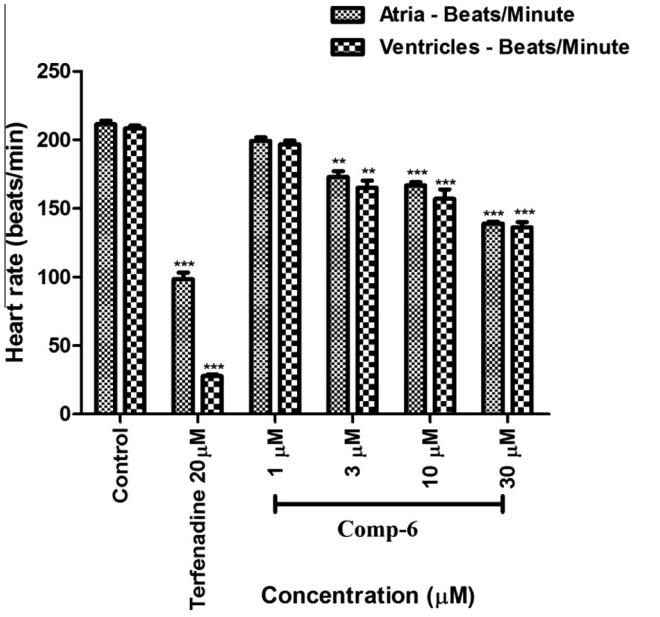


Figure 5. Mean (±S.E.M.) of the heart rates of atria and ventricles of compound **6** treatment groups ($^{*}p$ <0.05, $^{**}p$ <0.01 and $^{***}p$ <0.001). Statistical significance was analysed comparing control group versus treated groups.

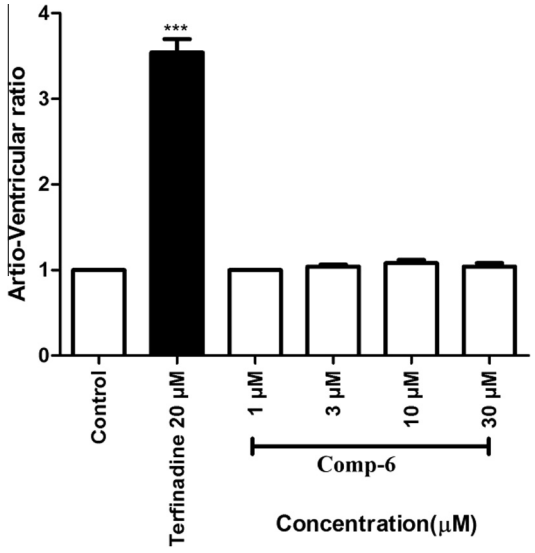


Figure 6. Mean (±S.E.M.) score of atrio ventricular ratio of compound **6** treatment groups ($^{*}p$ <0.05, $^{**}p$ <0.01 and $^{***}p$ <0.001). Statistical significance was analysed comparing control group versus all groups.

described in more detail in the Section 4. The compound was found to be safe when compared to positive control (20 μM terfenadine), not showing any significant cardiotoxicity until 30 μM (Figs. 5 and 6). Significant changes in the heart rate as well as AV ratio were also not observed, compared to control group making them relatively safe. In our earlier study with aminopiperidine based MTB DNA gyrase molecules[5] at 30 μM was toxic to fish and died. To conclude replacement of aminopiperidine linker with *p*-phenylenediamine linker reduces cardiac toxicity.

3. Conclusion

In conclusion, we identified a new class of acridine derivatives that selectively inhibit MTB DNA gyrase enzyme with promising

attributes of synthetic accessibility, and anti-TB activity. With new anti-TB agents desperately needed, we believe that the class of MTB DNA gyrase inhibitors reported in this work, would be interesting as potential leads for further development.

4. Experimental section

4.1. Chemistry

All commercially available chemicals and solvents were used without further purification. Melting points of the synthesized compounds were determined by Buchi B-540 open capillary instrument and were uncorrected. The homogeneity of the compounds was monitored by TLC (Thin layer chromatography) on silica gel 40 F254 coated on aluminium plates, visualized by UV or iodine chamber and $KMnO_4$ treatment. All ^1H and ^{13}C NMR spectra were recorded on a Bruker AM-300 (300.12 MHz, 75.12 MHz) NMR spectrometer and BrukerBioSpin Corp, Germany, respectively. Molecular weights of the synthesized compounds were checked by SHIMADZU LCMS-2020 series in ESI mode. Chemical shifts are reported in ppm (δ) with reference to the internal standard TMS. The signals were designated as follows: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; m, multiplet. Elemental analyses were carried out on ElementarVario MICRO CUBE CHN Analyser.

4.1.1. General procedure for the synthesis of 2-(*p*-tolylamino)/2-((4-chlorophenyl)amino)/2-(phenylamino)benzoic acid intermediates (2a–c)

To a stirred solution of 2-chlorobenzoic acid (5 g, 32 mmol) in 20 mL of DMF (*N*,*N*-dimethylformamide) potassium carbonate (7.51 g, 54.4 mmol) corresponding aniline (38.4 mmol) and copper powder (0.81 g, 12.8 mmol) were added and heated up to 140 °C for 6 h. Progress of reaction was monitored by thin layer chromatography after completion of the reaction, reaction mass was filtered through celite, celite bed was washed with ethyl acetate. The combined organic layer was evaporated under reduced pressure, obtained crude was purified by flash column chromatography using 5–20% ethyl acetate: hexane as eluent in 100–200 mesh silica gel to get the corresponding phenylamino benzoic acid derivatives in good yield. The intermediates (**2a**–**c**) were confirmed by mass analysis (ESI mode) and proceed to next step.

4.1.1.1. 2-(*p*-Tolylamino) benzoic acid (2a). Pale brown solid; yield 77%; ESI-MS was found at 228.47 $(M+H)^+$.

4.1.1.2. 2-((4-Chlorophenyl) amino) benzoic acid (2b). Off-white solid; yield 78%; ESI-MS was found at 248.78 $(M+H)^+$.

4.1.1.3. 2-(Phenylamino) benzoic acid (2c). Off-white solid; yield 75%; ESI-MS was found at 212.32 $(M-H)^-$.

4.1.2. Preparation of 9-chloroacridine derivatives from substituted phenylamino benzoic acid intermediates (3a–c)

The phenyl amino benzoic acid derivatives (47 mmol) (**2a**–**c**) were cyclized intra molecularly to 9-chloroacridine derivatives (**3a**–**c**) by refluxing them in 60 mL of $POCl_3$ for 4 h and the reaction was monitored by TLC. After completion, the excess $POCl_3$ was removed by rota evaporator under reduced pressure to the crude reaction mass crushed ice was added, pH was adjusted ~7 by adding saturated bicarbonate solution. The solid separated was filtered, dried and purified by flash column chromatography using 5–10% ethyl acetate: hexane as eluent in 60–120 mesh silica gel to get the corresponding 9-chloroacridine derivatives in good yield. These intermediates were confirmed by mass analysis (ESI mode) and proceed to next step.

4.1.2.1. 9-Chloro-2-methylacridine (3a). Pale brown solid; yield 71%; ESI-MS was found at 228.64 $(M+H)^+$.

4.1.2.2. 2,9-Dichloroacridine (3b). Pale brown solid; yield 74%; ESI-MS was found at 249.34 $(M+H)^+$.

4.1.2.3. 9-Chloroacridine (3c). Pale orange solid; yield 69%; ESI-MS was found at 228.47 $(M+H)^+$.

4.1.3. Preparation of *N*-(2-methoxy-4-nitrophenyl)acridin/2-methylacridine-9-amine intermediates (4a–b)

The 9-chloroacridine derivatives (**3a**–**c**) (10 mmol) (**3a**–**b**) were further reacted with *p*-phenylenediamine (1.8 g, 10.7 mmol) under microwave conditions in methanol using PTSA (*para*-toluene sulphonic acid) as a catalyst (5–10 mg) at 130 °C for 1 h, reaction was monitored by TLC. After completion of reaction, methanol was evaporated under vacuum the crude was dissolved in ethyl acetate, washed with saturated bicarbonate solution. The organic layer was dried over anhydrous sodium sulphate and evaporated under reduced pressure obtained crude was further purified by flash column chromatography in 60–120 silica gel using 10–35% ethyl acetate: hexane as eluent to get an orange to yellow solid in moderate yields.

4.1.3.1. N^1-(2-Methylacridin-9-yl)benzene-1,4-diamine (4a). Brown solid; yield 69%; ESI-MS was found at 300.38 $(M+H)^+$.

4.1.3.2. N^1-(2-Chloroacridin-9-yl)benzene-1,4-diamine (4b). Pale brown solid; yield 68%; ESI-MS was found at 318.89 $(M-H)^-$.

4.1.3.3. N^1-(2-Chloroacridin-9-yl)benzene-1,4-diamine (4c). Pale brown solid; yield 70%; ESI-MS was found at 286.42 $(M+H)^+$.

4.1.4. Preparation of *N*-(3-methoxy-4-((acridin/2-methylacridin-9-yl)amino)phenyl)methane/substituted benzene sulphonamide derivatives (5–16)

For the preparation of desired sulphonamide derivatives (**5**–**16**) corresponding acridine amine derivatives (**4a**–**c**) (0.6 mmol) were taken in (3 mL) DMF to this triethylamine (1.38 mmol) and corresponding substituted alkyl/aryl sulphonyl chlorides (0.6 mmol) were added at 0 °C and allowed to stir at room temperature for 3 h. After completion of reaction crushed ice was added to the crude reaction mass the solid precipitated was filtered, dried and further purified by flash column chromatography in 100–200 mesh silica gel using 5–10% methanol: dichloromethane as eluent to get desired sulphonamide derivatives (**5**–**16**) in good yields.

4.1.4.1. *N*-(4-((2-Methylacridin-9-yl)amino)phenyl)benzenesulphonamide (5). Yellow solid; yield 68%; mp 236–238 °C; ^1H NMR (DMSO-d_6): δ_H. 2.35 (s, 3H, CH_3), 6.41 (m, 4H, Ar-H), 7.52–7.83 (m, 10H, Ar-H), 8.01–8.19 (m, 2H, Ar-H), 10.69 (s, 1H, Ar-NH-Ar), 10.96 (s, 1H, Ar-NH-S). ^{13}C NMR (DMSO-d_6): δ_C. 149.9, 142.7, 140.4(2C), 136.6(2C), 132.7(3C), 130.5, 129.6(3C), 128.5, 128.0(3C), 122.2, 121.4, 119.8, 118.9(2C), 117.7(2C), 108.5, 22.4. EI-MS *m*/*z* (Calcd for $C_{26}H_{21}N_3O_2S$: 439.14); found: 440.19 $(M+H)^+$. Anal. Calcd for $C_{26}H_{21}N_3O_2S$: C, 71.25; H, 4.82; N, 9.56. Found: C, 71.19; H, 4.79; N, 9.54.

4.1.4.2. 4-Chloro-*N*-(4-((2-methylacridin-9-yl)amino)phenyl) benzenesulphonamide (6). Pale yellow solid; yield 71%; mp 245–247 °C; ^1H NMR (DMSO-d_6): δ_H. 2.32 (s, 3H, CH_3), 6.36 (m, 4H, Ar-H), 7.53–7.87 (m, 9H, Ar-H), 8.02–8.22 (m, 2H, Ar-H), 10.68 (s, 1H, Ar-NH-Ar), 10.94 (s, 1H, Ar-NH-S). ^{13}C NMR (DMSO-d_6): δ_C. 150.0, 142.8, 140.2, 138.5(2C), 136.7(2C), 132.2(2C),

130.6, 130.0(3C), 129.4(2C), 128.5(2C), 122.1, 121.4, 119.8, 118.9 (2C), 118.7(2C), 108.3, 22.3. EI-MS *m/z* (calcd for $C_{26}H_{20}ClN_3O_2S$: 473.10); found: 472.14 $(M-H)^-$. Anal. Calcd for $C_{26}H_{20}ClN_3O_2S$: C, 65.89; H, 4.25; N, 8.87. Found: C, 66.05; H, 4.27; N, 8.85.

4.1.4.3. *N*-(4-((2-Methylacridin-9-yl)amino)phenyl)-4-nitrobenzenesulphonamide (7). Brown gammy; yield 69%; ^{1}H NMR (DMSO-d_6): δ_H. 2.36 (s, 3H, CH_3), 6.42 (m, 4H, Ar-H), 7.53–7.97 (m, 6H, Ar-H), 8.14–8.41 (m, 5H, Ar-H), 10.67 (s, 1H, Ar-NH-Ar), 10.88 (s, 1H, Ar-NH-S). ^{13}C NMR (DMSO-d_6): δ_C. 151.9, 149.7, 146.3, 142.6, 140.1, 139.5(2C), 132.0(2C), 130.3(2C), 128.8(2C), 128.0(2C), 124.9(2C), 122.2, 121.5, 120.0, 119.2(2C), 117.9(2C), 108.4, 22.6. EI-MS *m/z* (Calcd for $C_{26}H_{20}N_4O_4S$: 484.12); found: 485.31 $(M+H)^+$. Anal. Calcd for $C_{26}H_{20}N_4O_4S$: C, 64.45; H, 4.16; N, 11.56. Found: C, 64.54; H, 4.18; N, 11.54.

4.1.4.4. 4-Methyl-*N*-(4-((2-methylacridin-9-yl)amino)phenyl) benzenesulphonamide (8). Yellow solid; yield 66%; mp 252–254 °C; ^{1}H NMR (DMSO-d_6): δ_H. 2.32 (s, 6H, CH_3), 6.37 (m, 4H, Ar-H), 7.39–7.83 (m, 9H, Ar-H), 8.01–8.23 (m, 2H, Ar-H), 10.67 (s, 1H, Ar-NH-Ar), 10.93 (s, 1H, Ar-NH-S). ^{13}C NMR (DMSO-d_6): δ_C. 149.9, 142.7, 140.1, 138.3, 137.2, 136.4(2C), 132.6(2C), 130.7(2C), 130.1(2C), 129.0(2C), 128.5(2C), 121.9(2C), 119.8, 118.9(2C), 117.7(2C), 108.3, 22.6(2C). EI-MS *m/z* (Calcd for $C_{27}H_{23}N_3O_2S$: 453.15); found: 454.32 $(M+H)^+$. Anal. Calcd for $C_{27}H_{23}N_3O_2S$: C, 71.50; H, 5.11; N, 9.26. Found: C, 71.72; H, 5.09; N, 9.23.

4.1.4.5. *N*-(4-((2-Chloroacridin-9-yl)amino)phenyl)benzenesulphonamide (9). Orange solid; yield 63%; mp 181–183 °C; ^{1}H NMR (DMSO-d_6): δ_H. 6.41 (m, 4H, Ar-H), 7.61–7.79 (m, 9H, Ar-H), 7.99–8.17 (m, 3H, Ar-H), 10.71 (s, 1H, Ar-NH-Ar), 10.90 (s, 1H, Ar-NH-S). ^{13}C NMR (DMSO-d_6): δ_C. 149.3, 143.8, 140.4(2C), 136.6, 132.3, 131.9(2C), 131.3(2C), 130.7, 129.8(2C), 128.3, 127.9 (3C), 122.7, 121.4, 119.2(3C), 117.8(2C), 109.9. EI-MS *m/z* (Calcd for $C_{25}H_{18}ClN_3O_2S$: 459.08); found: 458.11 $(M-H)^-$. Anal. Calcd for $C_{25}H_{18}ClN_3O_2S$: C, 65.28; H, 3.94; N, 9.14. Found: C, 65.45; H, 3.92; N, 9.16.

4.1.4.6. 4-Chloro-*N*-(4-((2-chloroacridin-9-yl)amino)phenyl) benzenesulphonamide (10). Yellow solid; yield 71%; mp 189–191 °C; ^{1}H NMR (DMSO-d_6): δ_H. 6.39 (m, 4H, Ar-H), 7.59–7.81 (m, 8H, Ar-H), 8.01–8.22 (m, 3H, Ar-H), 10.67 (s, 1H, Ar-NH-Ar), 10.89 (s, 1H, Ar-NH-S). ^{13}C NMR (DMSO-d_6): δ_C. 149.2, 143.9, 140.2, 138.4(2C), 136.7, 132.0(2C), 131.7(2C), 130.4, 129.8(2C), 129.4(2C), 128.2(2C), 122.9, 121.6, 119.3(3C), 117.9(2C), 109.7. EI-MS *m/z* (Calcd for $C_{25}H_{17}Cl_2N_3O_2S$: 493.04); found: 494.25 $(M+H)^+$. Anal. Calcd for $C_{25}H_{17}Cl_2N_3O_2S$: C, 60.73; H, 3.47; N, 8.50. Found: C, 60.92; H, 3.46; N, 8.53.

4.1.4.7. *N*-(4-((2-Chloroacridin-9-yl)amino)phenyl)-4-nitrobenzenesulphonamide (11). Brown gammy; yield 67%; ^{1}H NMR (DMSO-d_6): δ_H. 6.43 (m, 4H, Ar-H), 7.63–7.97 (m, 5H, Ar-H), 8.07–8.41 (m, 6H, Ar-H), 10.57 (s, 1H, Ar-NH-Ar), 10.92 (s, 1H, Ar-NH-S). ^{13}C NMR (DMSO-d_6): δ_C. 152.8, 149.3, 146.5, 143.9, 140.3, 136.7, 132.2(2C), 131.5(2C), 130.5, 129.0(2C), 128.4(2C), 124.8(2C), 122.9, 121.5, 119.1(3C), 117.7(2C), 109.9. EI-MS *m/z* (Calcd for $C_{25}H_{17}ClN_4O_4S$: 504.07); found: 505.31 $(M+H)^+$. Anal. Calcd for $C_{25}H_{17}ClN_4O_4S$: C, 59.47; H, 3.39; N, 11.10. Found: C, 59.62; H, 3.40; N, 11.12.

4.1.4.8. *N*-(4-((2-Chloroacridin-9-yl)amino)phenyl)-4-methylbenzenesulphonamide (12). Yellow solid; yield 69%; mp 214–216 °C; ^{1}H NMR (DMSO-d_6): δ_H. 2.35 (s, 3H, CH_3), 6.41 (m, 4H, Ar-H), 7.39–7.78 (m, 8H, Ar-H), 7.99–8.19 (m, 3H, Ar-H), 10.49 (s, 1H, Ar-NH-Ar), 10.91 (s, 1H, Ar-NH-S). ^{13}C NMR (DMSO-d_6): δ_C. 149.5, 143.8, 140.4, 128.3, 137.5, 136.8, 132.3(2C), 131.7 (2C), 130.4, 129.9(2C), 128.7(2C), 128.0(2C), 122.8, 121.4, 119.3 (3C), 117.6(2C), 109.7, 22.0. EI-MS *m/z* (Calcd for $C_{26}H_{20}ClN_3O_2S$: 473.10); found: 472.05 $(M-H)^-$. Anal. Calcd for $C_{26}H_{20}ClN_3O_2S$: C, 65.89; H, 4.25; N, 8.87. Found: C, 65.61; H, 4.27; N, 8.85.

4.1.4.9. *N*-(4-(Acridin-9-ylamino)phenyl)benzenesulphonamide (13). Orange solid; yield 72%; mp 187–189 °C; ^{1}H NMR (DMSO-d_6): δ_H. 6.36 (m, 4H, Ar-H), 7.56–7.94 (m, 11H, Ar-H), 8.19 (m, 2H, Ar-H), 10.56 (s, 1H, Ar-NH-Ar), 10.89 (s, 1H, Ar-NH-S). ^{13}C NMR (DMSO-d_6): δ_C. 150.4, 142.9(2C), 140.3, 136.5, 132.2, 130.6(2C), 129.8(2C), 128.5(3C), 127.8(2C), 126.9(2C), 121.5(2C), 118.8(2C), 117.6(2C), 115.4(2C). EI-MS *m/z* (Calcd for $C_{25}H_{19}N_3O_2S$: 425.12); found: 426.29 $(M+H)^+$. Anal. Calcd for $C_{25}H_{19}N_3O_2S$: C, 70.57; H, 4.50; N, 9.88. Found: C, 70.76; H, 4.48; N, 9.85.

4.1.4.10. *N*-(4-(Acridin-9-ylamino)phenyl)-4-chlorobenzenesulphonamide (14). Pale yellow solid; yield 77%; mp 220–222 °C; ^{1}H NMR (DMSO-d_6): δ_H. 6.42 (m, 4H, Ar-H), 7.61–7.96 (m, 10H, Ar-H), 8.15 (m, 2H, Ar-H), 10.53 (s, 1H, Ar-NH-Ar), 10.91 (s, 1H, Ar-NH-S). ^{13}C NMR (DMSO-d_6): δ_C. 150.2, 142.8(2C), 138.4 (2C), 136.3, 130.5(2C), 129.7(2C), 129.4(2C), 128.6(3C), 126.9(2C), 121.4(2C), 119.0(2C), 117.8(2C), 115.5(2C). EI-MS *m/z* (Calcd for $C_{25}H_{18}ClN_3O_2S$: 459.08); found: 460.15 $(M+H)^+$. Anal. Calcd for $C_{25}H_{18}ClN_3O_2S$: C, 65.28; H, 3.94; N, 9.14. Found: C, 65.46; H, 3.96; N, 9.11.

4.1.4.11. *N*-(4-(Acridin-9-ylamino)phenyl)-4-nitrobenzenesulphonamide (15). Brown solid; yield 68%; mp 187–189 °C; ^{1}H NMR (DMSO-d_6): δ_H. 6.39 (m, 4H, Ar-H), 7.57–7.93 (m, 6H, Ar-H), 8.10–8.41 (m, 6H, Ar-H), 10.49 (s, 1H, Ar-NH-Ar), 10.87 (s, 1H, Ar-NH-S). ^{13}C NMR (DMSO-d_6): δ_C. 152.7, 150.5, 146.2, 142.9(2C), 136.6, 130.4(2C), 128.8(2C), 128.2(3C), 127.0 (2C), 124.7(2C), 121.5(2C), 119.1(2C), 117.9(2C), 115.3(2C). EI-MS *m/z* (Calcd for $C_{25}H_{18}N_4O_4S$: 470.10); found: 469.08 $(M-H)^-$. Anal. Calcd for $C_{25}H_{18}N_4O_4S$: C, 63.82; H, 3.86; N, 11.91. Found: C, 63.65; H, 3.88; N, 11.94.

4.1.4.12. *N*-(4-(Acridin-9-ylamino)phenyl)-4-methylbenzenesulphonamide (16). Brown solid; yield 75%; mp 206–208 °C; ^{1}H NMR (DMSO-d_6): δ_H. 2.36 (s, 3H, CH_3), 6.41 (m, 4H, Ar-H), 7.43–7.92 (m, 10H, Ar-H), 8.21 (m, 2H, Ar-H), 10.53 (s, 1H, Ar-NH-Ar), 10.97 (s, 1H, Ar-NH-S). ^{13}C NMR (DMSO-d_6): δ_C. 150.4, 142.8(2C), 138.3, 137.5, 136.8, 130.5(2C), 129.9(2C), 129.0(2C), 128.6(3C), 127.0(2C), 121.4(2C), 119.1(2C), 117.8(2C), 115.4(2C), 22.0. EI-MS *m/z* (Calcd for $C_{26}H_{21}N_3O_2S$: 439.14); found: 440.31 $(M+H)^+$. Anal. Calcd for $C_{26}H_{21}N_3O_2S$: C, 71.05; H, 4.82; N, 9.56. Found: C, 71.24; H, 4.84; N, 9.52.

4.1.5. Preparation of 1-(3-methoxy-4-((acridin/2-methylacridin-9-yl)amino)phenyl)-3-phenylthiourea/ urea derivatives (17–40)

For the preparation of desired thiourea (**17–28**) and urea (**29–40**) derivatives corresponding acridin amine derivative (**4a–c**) (0.6 mmol), triethylamine (1.38 mmol) (Et_3N) and substituted phenyl isothiocyanate/isocyanate (0.6 mmol) were dissolved in ethanol and heated up to 80 °C for 8 h reaction was monitored by TLC, after completion of the reaction the solid precipitated was filtered, dried and recrystallized from ethanol to get the desired corresponding thiourea (**17–28**) and urea (**29–40**) derivatives.

4.1.5.1. 1-(4-((2-Methylacridin-9-yl)amino)phenyl)-3-phenylthiourea (17). Orange solid; yield 67%; mp 120–122 °C; ^{1}H NMR (DMSO-d_6): δ_H. 2.36 (s, 3H, CH_3), 6.24–6.79 (m, 5H, Ar-H), 7.21–7.81 (m, 9H, Ar-H), 8.01–8.19 (m, 2H, Ar-H), 8.67 (s, 2H, Ar-NH), 10.54 (s, 1H, Ar-NH-Ar). ^{13}C NMR (DMSO-d_6): δ_C. 180.3, 149.7, 142.4(2C), 139.9, 138.8, 135.6, 131.7(2C), 130.1(2C), 129.5(2C), 128.9(2C), 127.7(3C), 126.8(2C), 122.0, 121.2, 119.7, 118.3(2C),

108.5, 22.2. EI-MS *m*/*z* (Calcd For $C_{27}H_{22}N_4S$: 434.16); found: 435.09 $(M+H)^+$. Anal. Calcd for $C_{27}H_{22}N_4S$: C, 74.63; H, 5.10; N, 12.89. Found: C, 74.81; H, 5.08; N, 12.85.

4.1.5.2. 1-(4-Chlorophenyl)-3-(4-((2-methylacridin-9-yl)amino) phenyl)thiourea (18). Yellow solid; yield 68%; mp 138–140 °C; ^{1}H NMR (DMSO-d_6): δ_H. 2.32(s, 3H, CH_3), 6.19–6.56 (m, 6H, Ar-H), 7.25–7.84 (m, 7H, Ar-H), 8.0–8.17 (m, 2H, Ar-H), 8.71 (s, 2H, Ar-NH), 10.61 (s, Ar-NH-Ar). ^{13}C NMR (DMSO-d_6): δ_C. 180.1, 149.8, 142.5(2C), 140.0, 137.2, 135.7, 134.1, 132.0(2C), 131.5(2C), 130.1(2C), 129.8(2C), 128.8, 127.9(3C), 121.9, 121.0, 120.2, 118.4(2C), 108.3, 22.1. EI-MS *m*/*z* (Calcd for $C_{27}H_{21}ClN_4S$: 468.12); found: 469.01 $(M+H)^+$. Anal. Calcd for $C_{27}H_{21}ClN_4S$: C, 69.14; H, 4.51; N, 11.95. Found: C, 69.28; H, 4.55; N, 11.97.

4.1.5.3. 1-(4-((2-Methylacridin-9-yl)amino)phenyl)-3-(4-nitrophenyl)thiourea (19). Pale orange solid; yield 65%; mp 131–133 °C; ^{1}H NMR (DMSO-d_6): δ_H. 2.35 (s, 3H, CH_3), 6.23–6.67 (m, 6H, Ar-H), 7.58–7.89 (m, 6H, Ar-H), 8.03–8.18 (m, 3H, Ar-H), 8.57 (s, 2H, Ar-NH), 10.61 (s, 1H, Ar-NH-Ar). ^{13}C NMR (DMSO-d_6): δ_C. 180.3, 150.1, 145.0, 144.4(2C), 142.1(2C), 136.7, 131.9, 130.2 (2C), 128.9, 127.8(2C), 127.0, 126.3, 125.6(2C), 124.9(2C), 124.5, 122.0, 121.7, 118.4(2C), 1.8.8, 22.3. EI-MS *m*/*z* (Calcd for $C_{27}H_{21}N_5O_2S$: 479.14; found: 478.25 $(M-H)^-$. Anal. Calcd for $C_{27}H_{21}N_5O_2S$: C, 67.62; H, 4.41; N, 14.60. Found: C, 67.43; H, 4.39; N, 14.56.

4.1.5.4. 1-(4-((2-Methylacridin-9-yl)amino)phenyl)-3-(*p*-tolyl) thiourea (20). Orange solid; yield 63%; mp 138–140 °C; ^{1}H NMR (DMSO-d_6): δ_H. 2.36 (s, 6H, CH_3), 6.26–6.94 (m, 8H, Ar-H), 7.51–7.95 (m, 6H, Ar-H), 8.16 (m, 1H, Ar-H), 8.59 (s, 2H, Ar-NH), 10.63 (s, 1H, Ar-NH-Ar). ^{13}C NMR (DMSO-d_6): δ_C. 180.0, 149.8, 142.3(2C), 140.1, 137.7, 135.9(2C), 132.2(2C), 130.5(2C), 129.8 (2C), 129.0, 127.9(2C), 126.8(2C), 121.7, 121.3(2C), 119.6, 118.2 (2C), 108.4, 22.5(2C). EI-MS *m*/*z* (Calcd for $C_{28}H_{24}N_4S$: 448.17); found: 449.19 $(M+H)^+$. Anal. Calcd for $C_{28}H_{24}N_4S$: C, 74.97; H, 5.39; N, 12.49. Found: C, 75.18; H, 5.37; N, 12.45.

4.1.5.5. 1-(4-((2-Chloroacridin-9-yl)amino)phenyl)-3-phenylthiourea (21). Orange solid; yield 66%; mp 108–110 °C; ^{1}H NMR (DMSO-d_6): δ_H. 6.22–6.35 (m, 4H, Ar-H), 6.85–7.18 (m, 3H, Ar-H), 7.62–7.97 (m, 7H, Ar-H), 8.07–8.21 (m, 2H, Ar-H), 8.55 (s, 2H, Ar-NH), 10.48 (s, 1H, Ar-NH-Ar). ^{13}C NMR (DMSO-d_6): δ_C. 180.1, 149.3, 143.7, 142.4, 140.2, 139.0, 132.3(2C), 131.9(2C), 130.5, 129.7(2C), 129.2(2C), 128.0(2C), 127.7, 127.1(2C), 122.8, 121.4, 119.2, 118.5(2C), 109.7. EI-MS *m*/*z* (Calcd for $C_{26}H_{19}ClN_4S$: 454.10); found: 453.01 $(M-H)^-$. Anal. Calcd for $C_{26}H_{19}ClN_4S$: C, 68.64; H, 4.21; N, 12.31. Found: C, 68.82; H, 4.19; N, 12.34.

4.1.5.6. 1-(4-((2-Chloroacridin-9-yl)amino)phenyl)-3-(4-chlorophenyl)thiourea (22). Brown solid; yield 66%; mp 135–137 °C; ^{1}H NMR (DMSO-d_6): δ_H. 6.19–6.55 (m, 6H, Ar-H), 7.21–7.75 (m, 6H, Ar-H), 8.01–8.19 (m, 3H, Ar-H), 8.76 (s, 2H, Ar-NH), 10.57 (s, 1H, Ar-NH-Ar). ^{13}C NMR (DMSO-d_6): δ_C. 180.5, 149.1, 143.9, 142.3, 140.0, 137.2, 134.4, 132.0(2C), 131.8(2C), 131.4(2C), 130.1, 129.6(2C), 128.9, 127.7(2C), 127.3, 122.8, 121.2, 118.9, 118.4(2C), 109.9. EI-MS *m*/*z* (Calcd for $C_{26}H_{18}C_{l2}N_4S$:488.06); found: 489.12 $(M+H)^+$. Anal. Calcd for $C_{26}H_{18}C_{l2}N_4S$: C, 63.81; H, 3.71; N, 11.45. Found: C, 63.62; H, 3.73; N, 11.49.

4.1.5.7. 1-(4-((2-Chloroacridin-9-yl)amino)phenyl)-3-(4-nitrophenyl)thiourea (23). Orange solid; yield 68%; mp 142–144 °C; ^{1}H NMR (DMSO-d_6): δ_H. 6.22–6.67 (m, 6H, Ar-H), 7.61–7.89 (m, 5H, Ar-H), 8.02–8.18 (m, 4H, Ar-H), 8.56 (s, 2H, Ar-NH), 10.64 (s, 1H, Ar-NH-Ar). ^{13}C NMR (DMSO-d_6): δ_C. 180.3, 150.1, 145.3, 144.5(2C), 142.3(2C), 132.9, 132.0, 130.8, 130.1, 129.2, 127.9(2C), 126.4(2C), 125.2(2C), 124.8(2C), 124.5, 122.0(2C), 118.4(2C), 108.7. EI-MS *m*/*z* (Calcd for $C_{26}H_{18}ClN_5O_2S$: 499.09); found: 500.21 $(M+H)^+$. Anal. Calcd for $C_{26}H_{18}ClN_5O_2S$: C, 62.46; H, 3.63; N, 14.01. Found: C, 62.64; H, 3.61; N, 14.05.

4.1.5.8. 1-(4-((2-Chloroacridin-9-yl)amino)phenyl)-3-(*p*-tolyl) thiourea (24). Pale orange solid; yield 70%; mp 149–153 °C; ^{1}H NMR (DMSO-d_6): δ_H. 2.36 (s, 3H, CH_3), 6.23–6.95 (m, 8H, Ar-H), 7.65–8.14 (m, 7H, Ar-H), 8.63 (s, 2H, Ar-NH), 10.71 (s, 1H, Ar-NH-Ar). ^{13}C NMR (DMSO-d_6): δ_C. 180.5, 149.2, 143.9, 142.5, 140.3, 237.8, 136.2, 132.0(2C), 131.5(2C), 130.5, 129.9(2C), 129.2, 127.8(2C), 127.4, 126.9(2C), 122.7, 121.4, 118.9, 118.3(2C), 109.7, 21.8. EI-MS *m*/*z* (Calcd for $C_{27}H_{21}ClN_4S$: 468.12); found: 467.05 $(M-H)^-$. Anal. Calcd for $C_{27}H_{21}ClN_4S$: C, 69.14; H, 4.51; N, 11.95. Found: C, 69.33; H, 4.49; N, 11.92.

4.1.5.9. 1-(4-(Acridin-9-ylamino)phenyl)-3-phenylthiourea (25). Brown solid; yield 67%; mp 156–158 °C; ^{1}H NMR (DMSO-d_6): δ_H. 6.25–6.78 (m, 5H, Ar-H), 7.23–7.71 (m, 8H, Ar-H), 7.97–8.15 (m, 4H, Ar-H), 8.69 (s, 2H, Ar-NH), 10.71 (s, 1H, Ar-NH-Ar). ^{13}C NMR (DMSO-d_6): δ_C. 180.2, 150.4, 143.0(2C), 142.5, 139.1, 130.3(2C), 129.7(2C) 129.0(2C), 128.4(2C), 127.9(2C), 127.0 (2C), 126.8(2C), 121.4(2C), 118.6(2C), 115.3(2C). EI-MS *m*/*z* (Calcd for $C_{26}H_{20}N_4S$: 420.14); found: 421.28 $(M+H)^+$. Anal. Calcd for $C_{26}H_{20}N_4S$: C, 74.26; H, 4.79; N, 13.32. Found: C, 74.04; H, 4.83; N, 13.34.

4.1.5.10. 1-(4-(Acridin-9-ylamino)phenyl)-3-(4-chlorophenyl) thiourea (26). Orange solid; yield 65%; mp 147–149 °C; ^{1}H NMR (DMSO-d_6): δ_H. 6.19–6.56 (m, 6H, Ar-H), 7.27–7.774 (m, 6H, Ar-H), 7.99–8.18 (m, 4H, Ar-H), 8.82 (s, 2H, Ar-NH), 10.61 (s, 1H, Ar-NH-Ar). ^{13}C NMR (DMSO-d_6): δ_C. 180.4, 150.5, 142.9(2C), 142.5, 137.3, 134.2, 131.8(2C), 130.4(2C), 129.7(2C), 129.1, 128.5 (2C), 127.9(2C), 126.7(2C), 121.4(2C), 118.3(2C), 115.5(2C). EI-MS *m*/*z* (Calcd for $C_{26}H_{19}ClN_4S$: 454.10); found: 455.03 $(M+H)^+$. Anal. Calcd for $C_{26}H_{19}ClN_4S$: C, 68.64; H, 4.21; N, 12.31. Found: C, 68.83; H, 4.23; N, 12.28.

4.1.5.11. 1-(4-(Acridin-9-ylamino)phenyl)-3-(4-nitrophenyl) thiourea (27). Orange solid; yield 67%; mp 159–161 °C; ^{1}H NMR (DMSO-d_6): δ_H. 6.22–6.65 (m, 6H, Ar-H), 7.61–7.92 (m, 6H, Ar-H), 8.04–8.19 (m, 4H, Ar-H), 8.78 (s, 2H, Ar-NH), 10.73 (s, 1H, Ar-NH-Ar). ^{13}C NMR (DMSO-d_6): δ_C. 180.2, 150.4, 145.0, 144.5, 142.9(2C), 142.6, 130.4(2C), 129.1, 128.3(2C), 127.9(2C), 127.0 (2C), 125.3(2C), 124.9(2C), 121.5(2C), 118.2(2C), 115.6(2C). EI-MS *m*/*z* (Calcd for $C_{26}H_{19}N_5O_2S$: 465.13); found: 466.21 $(M+H)^+$. Anal. Calcd for $C_{26}H_{19}N_5O_2S$: C, 67.08; H, 4.11; N, 15.04. Found: C, 67.26; H, 4.13; N, 15.08.

4.1.5.12. 1-(4-(Acridin-9-ylamino)phenyl)-3-(*p*-tolyl)thiourea (28). Brown solid; yield 64%; mp 190–192 °C; ^{1}H NMR (DMSO-d_6): δ_H. 2.36 (s, 3H, CH_3), 6.23–6.95 (m, 8H, Ar-H), 7.62–7.91 (m, 6H, Ar-H), 8.21 (m, 2H, Ar-H), 8.79 (s, 2H, Ar-NH), 10.66 (s, 1H, Ar-NH-Ar). ^{13}C NMR (DMSO-d_6): δ_C. 180.3, 150.4, 142.6 (3C), 137.7, 136.2, 130.6(2C), 129.8(2C), 128.9, 128.2(2C), 127.6 (2C), 126.9(2C), 126.7(2C), 121.5(2C), 118.3(2C), 115.5(2C), 21.7. EI-MS *m*/*z* (Calcd for $C_{27}H_{22}N_4S$: 434.16); found: 435.19 $(M+H)^+$. Anal. Calcd for $C_{27}H_{22}N_4S$: C, 74.63; H, 5.10; N, 12.89. Found: C, 74.85; H, 5.08; N, 12.92.

4.1.5.13. 1-(4-((2-Methylacridin-9-yl)amino)phenyl)-3-phenylurea (29). Brown gummy; yield 69%; ^{1}H NMR (DMSO-d_6): δ_H. 2.31 (s, 3H, CH_3), 6.65 (m, 2H, Ar-H), 7.22–7.95 (m, 13H, Ar-H), 8.19 (d, J = 8.1 Hz, 1H, Ar-H), 8.79 (s, 2H, Ar-NH), 10.58 (s, 1H, Ar-NH-Ar). ^{13}C NMR (DMSO-d_6): δ_C. 153.5, 149.7, 142.3(2C), 140.1(2C), 135.6, 132.0(2C), 130.4, 129.9(2C), 129.3(2C), 128.6, 127.4, 122.9(2C), 122.2(2C), 122.0, 121.3, 119.9, 118.4(2C), 108.6,

22.3. EI-MS *m/z* (Calcd For $C_{27}H_{22}N_4O$: 418.18); found: 417.06 $(M-H)^-$. Anal. Calcd For $C_{27}H_{22}N_4O$: C, 77.49; H, 5.30; N, 13.39. Found: C, 77.25; H, 5.32; N, 13.35.

4.1.5.14. 1-(4-Chlorophenyl)-3-(4-((2-methylacridin-9-yl) amino)phenyl)urea (30). Pale yellow solid; yield 66%; mp 163–165 °C; ^{1}H NMR (DMSO-d_6): δ_H. 2.35 (s, 3H, CH_3), 6.59 (m, 2H, Ar-H), 7.41–7.96 (m, 12H, Ar-H), 8.21 (d, J = 7.8 Hz, 1H, Ar-H), 8.84 (s, 2H, Ar-NH), 10.58 (s, 1H, Ar-NH-Ar). ^{13}C NMR (DMSO-d_6): δ_C. 153.6, 149.7, 142.3(2C), 140.1, 138.3, 135.8, 133.9, 132.1(2C), 130.5, 130.0(2C), 129.7(2C), 127.5, 122.9(2C), 122.1, 121.4(2C), 121.3, 119.8, 118.6(2C), 108.3, 22.5. EI-MS *m/z* (Calcd for $C_{27}H_{21}ClN_4O$: 452.14); found: 453.23 $(M+H)^+$. Anal. Calcd for $C_{27}H_{21}ClN_4O$: C, 71.60; H, 4.67; N, 12.37. Found: C, 71.79; H, 4.65; N, 12.41.

4.1.5.15. 1-(4-((2-Methylacridin-9-yl)amino)phenyl)-3-(4-nitrophenyl)urea (31). Brown gummy; yield 61%; ^{1}H NMR (DMSO-d_6): δ_H. 2.32 (s, 3H, CH_3), 6.62 (m, 2H, Ar-H), 7.42–7.89 (m, 10H, Ar-H), 8.19–8.27 (m, 3H, Ar-H), 8.79 (s, 2H, Ar-NH), 10.61 (s, 1H, Ar-NH-Ar). ^{13}C NMR (DMSO-d_6): δ_C. 153.4, 149.8, 146.1, 144.3(2C), 142.0(2C), 136.9, 131.8, 130.5(3C), 127.3, 126.1, 124.7(3C), 122.9(2C), 122.2, 121.6, 120.3(2C), 118.5(2C), 108.7, 22.2. EI-MS *m/z* (Calcd for $C_{27}H_{21}N_5O_3$: 463.16); found: 462.14 $(M-H)^-$. Anal. Calcd for $C_{27}H_{21}N_5O_3$: C, 69.97; H, 4.57; N, 15.11. Found: C, 70.16; H, 4.55; N, 15.08.

4.1.5.16. 1-(4-((2-Methylacridin-9-yl)amino)phenyl)-3-(*p*-tolyl) urea (32). Pale orange gummy; yield 67%; ^{1}H NMR (DMSO-d_6): δ_H. 2.32 (s, 6H, CH_3), 6.64 (m, 2H, Ar-H), 7.23–7.61 (m, 9H, Ar-H), 7.80–8.21 (m, 4H, Ar-H), 8.78 (s, 2H, Ar-NH), 10.68 (s, 1H, Ar-NH-Ar). ^{13}C NMR (DMSO-d_6): δ_C. 153.6, 149.7, 142.3(2C), 140.1, 137.3(2C), 135.8, 132.0(2C), 130.7, 130.1(2C), 129.6(2C), 127.8, 123.0(2C), 122.1(3C), 121.3, 119.8, 118.4(2C), 108.3 22.5 (2C). EI-MS *m/z* (Calcd for $C_{28}H_{24}N_4O$: 432.20); found: 433.12 $(M+H)^+$. Anal. Calcd for $C_{28}H_{24}N_4O$: C, 77.75; H, 5.59; N, 12.95. Found: C, 77.51; H, 5.61; N, 12.99.

4.1.5.17. 1-(4-((2-Chloroacridin-9-yl)amino)phenyl)-3-phenylurea (33). Brown solid; yield 68%; mp 168–170 °C; ^{1}H NMR (DMSO-d_6): δ_H. 6.62 (m, 2H, Ar-H), 7.23–7.62 (m, 9H, Ar-H), 7.81–8.22 (m, 5H, Ar-H), 8.68 (s, 2H, Ar-NH), 10.71 (s, 1H, Ar-NH-Ar). ^{13}C NMR (DMSO-d_6): δ_C. 153.4, 149.3, 143.9, 142.1, 140.4 (2C), 132.2(2C), 131.8(2C), 130.5, 129.9, 129.3(3C), 127.7, 122.9 (3C), 122.3(2C), 121.4, 119.0, 118.5(2C), 109.8. EI-MS *m/z* (Calcd for $C_{26}H_{19}ClN_4O$: 438.12); found: 439.25 $(M+H)^+$. Anal. Calcd for $C_{26}H_{19}ClN_4O$: C, 71.15; H, 4.36; N, 12.77. Found: C, 70.98; H, 4.34; N, 12.81.

4.1.5.18. 1-(4-((2-Chloroacridin-9-yl)amino)phenyl)-3-(4-chlorophenyl)urea (34). Brown solid; yield 69%; mp 234–236 °C; ^{1}H NMR (DMSO-d_6): δ_H. 6.59 (m, 2H, Ar-H), 7.41–7.79 (m, 10H, Ar-H), 9.01–8.23 (m, 3H, Ar-H), 8.65 (s, 2H, Ar-NH), 10.59 (s, 1H, Ar-NH-Ar). ^{13}C NMR (DMSO-d_6): δ_C. 153.5, 149.1, 143.8, 142.0, 140.1, 138.3, 133.9, 132.3(2C), 131.7(2C), 130.3(2C), 129.4(2C), 127.5, 122.9(3C), 121.6(3C), 119.1, 118.4(2C), 109.8. EI-MS *m/z* (Calcd for $C_{26}H_{18}Cl_2N_4O$: 472.09); found: 473.17 $(M+H)^+$. Anal. Calcd for $C_{26}H_{18}Cl_2N_4O$: C, 65.97; H, 3.83; N, 11.84. Found: C, 66.19; H, 3.81; N, 11.86.

4.1.5.19. 1-(4-((2-Chloroacridin-9-yl)amino)phenyl)-3-(4-nitrophenyl)urea (35). Brown gummy; yield 68%; ^{1}H NMR (DMSO-d_6): δ_H. 6.64 (m, 2H, Ar-H), 7.43–7.89 (m, 9H, Ar-H), 8.13–8.26 (m, 4H, Ar-H), 8.76 (s, 2H, Ar-NH), 10.67 (s, 1H, Ar-NH-Ar). ^{13}C NMR (DMSO-d_6): δ_C. 153.7, 150.5, 146.1, 144.0(2C), 142.3(2C), 132.8, 132.0, 140.9, 130.3(2C), 126.7(2C), 124.9(3C), 123.0(2C), 12.3(2C), 120.5(2C), 118.3(2C), 108.8. EI-MS *m/z* (Calcd for $C_{26}H_{18}ClN_5O_3$: 483.11); found: 482.05 $(M-H)^-$. Anal. Calcd for $C_{26}H_{18}ClN_5O_3$: C, 64.53; H, 3.75; N, 14.47. Found: C, 64.71; H, 3.73; N, 14.44.

4.1.5.20. 1-(4-((2-Chloroacridin-9-yl)amino)phenyl)-3-(*p*-tolyl) urea (36). Yellow solid; yield 62%; mp 253–255 °C; ^{1}H NMR (DMSO-d_6): δ_H. 2.25 (s, 3H, CH_3), 6.62 (m, 2H, Ar-H), 7.23–7.59 (m, 8H, Ar-H), 7.81–8.15 (m, 5H, Ar-H), 8.79 (s, 2H, Ar-NH), 10.68 (s, 1H, Ar-NH-Ar). ^{13}C NMR (DMSO-d_6): δ_C. 153.6, 149.2, 144.1, 142.3, 140.5, 137.4(2C), 132.2(3C), 131.1, 130.3(2C), 129.8(2C), 127.7, 123.0(3C), 122.2(2C), 121.3, 119.0, 118.5(2C), 109.9, 21.7. EI-MS *m/z* (Calcd for $C_{27}H_{21}ClN_4O$: 452.14); found: 453.45 $(M+H)^+$. Anal. Calcd for $C_{27}H_{21}ClN_4O$: C, 71.60; H, 4.67; N, 12.37. Found: C, 71.58; H, 4.65; N, 12.41.

4.1.5.21. 1-(4-(Acridin-9-ylamino)phenyl)-3-phenylurea (37). Orange solid; yield 65%; mp 162–164 °C; ^{1}H NMR (DMSO-d_6): δ_H. 6.65 (m, 2H, Ar-H), 7.19–7.59 (m, 9H, Ar-H), 7.81–8.15 (m, 6H, Ar-H), 8.79 (s, 2H, Ar-NH), 10.51 (s, 1H, Ar-NH-Ar). ^{13}C NMR (DMSO-d_6): δ_C. 153.4, 150.2, 142.9(2C), 142.0, 139.8, 130.3(3C), 120.5(3C), 128.3(2C), 127.0(2C), 123.2(2C), 122.1(2C), 121.5(2C), 118.1(2C), 115.4(2C). EI-MS *m/z* (Calcd for $C_{26}H_{20}N_4O$: 404.16); found: 405.29 $(M+H)^+$. Anal. Calcd for $C_{26}H_{20}N_4O$: C, 77.21; H, 4.98; N, 13.85. Found: C, 77.45; H, 5.01; N, 13.82.

4.1.5.22. 1-(4-(Acridin-9-ylamino)phenyl)-3-(4-chlorophenyl) urea (38). Brown solid; yield 68%; mp 241–243 °C; ^{1}H NMR (DMSO-d_6): δ_H. 6.59 (m, 2H, Ar-H), 7.41–7.92 (m, 12H, Ar-H), 8.19 (m, 2H, Ar-H). ^{13}C NMR (DMSO-d_6): δ_C. 153.6, 150.4, 143.1 (2C), 141.9, 138.2, 133.8, 130.5(3C), 129.5(2C), 128.2(2C), 127.0 (2C), 122.9(2C), 121.4(2C), 121.0(2C), 118.3(2C), 115.5(2C). EI-MS *m/z* (Calcd for $C_{26}H_{19}ClN_4O$: 438.12); found: 437.01 $(M-H)^-$. Anal. Calcd for $C_{26}H_{19}ClN_4O$: C, 71.15; H, 4.36; N, 12.77. Found: C, 70.95; H, 4.38; N, 12.74.

4.1.5.23. 1-(4-(Acridin-9-ylamino)phenyl)-3-(4-nitrophenyl) urea (39). Brown solid; yield 62%; mp 252–254 °C; ^{1}H NMR (DMSO-d_6): δ_H. 6.62 (m, 2H, Ar-H), 7.43–7.94 (m, 10H, Ar-H), 8.15–8.27 (m, 4H, Ar-H), 8.73 (s, 2H, Ar-NH), 10.77 (s, 1H, Ar-NH-Ar). ^{13}C NMR (DMSO-d_6): δ_C. 153.5, 150.3, 146.1, 144.3, 142.9 (2C), 141.7, 130.5(3C), 128.3(2C), 127.0(2C), 124.8(2C), 123.1(2C), 121.6(2C), 120.4(2C), 118.5(2C), 115.3(2C). EI-MS *m/z* (Calcd for $C_{26}H_{19}N_5O_3$: 449.15); found: 450.45 $(M+H)^+$. Anal. Calcd for $C_{26}H_{19}N_5O_3$: C, 69.48; H, 4.26; N, 15.58. Found: C, 69.68; H, 4.27; N, 15.53.

4.1.5.24. 1-(4-(Acridin-9-ylamino)phenyl)-3-(*p*-tolyl)urea (40). Brown gummy; yield 64%; ^{1}H NMR (DMSO-d_6): δ_H. 2.31 (s, 3H, CH_3), 6.64 (m, 2H, Ar-H), 7.19–7.74 (m, 10H, Ar-H), 7.98–8.15 (m, 4H, Ar-H), 8.67 (s, 2H, Ar-NH), 10.59 (s, 1H, Ar-NH-Ar). ^{13}C NMR (DMSO-d_6): δ_C. 153.4, 150.2, 142.9(2C), 142.1, 137.3(2C), 130.5(2C), 129.9(3C), 128.3(2C), 127.0(2C), 122.8(2C), 122.1(2C), 121.6(2C), 118.3(2C), 115.6(2C), 21.9. EI-MS *m/z* (Calcd for $C_{27}H_{22}N_4O$: 418.18); found: 419.27 $(M+H)^+$. Anal. Calcd for $C_{27}H_{22}N_4O$: C, 77.49; H, 5.30; N, 13.39. Found: C, 77.25; H, 5.32; N, 13.43.

4.2. Biological activity

4.2.1. MTB DNA super coiling assay

Supercoiling assay was performed using MTB DNA supercoiling assay kits (Inspiralis Limited, Norwich). Briefly, the assay was done in 30 μL reaction volume for 30 min at 37 °C in an assay buffer containing 50 mM HEPES. KOH (pH 7.9), 6 mM magnesium acetate, 4 mM DTT, 1 mM ATP, 100 mM potassium glutamate, 2 mM sper-

midine and 0.05 mg/mL of albumin. During the assay 2 U of DNA gyrase was incubated with 0.5 μg of relaxed pBR322 in the assay buffer for 30 min, then the reaction was quenched by the addition of equal volume of 30 mL of chloroform/isoamylalcohol (24:1) and STEB buffer, with a brief vortex and followed by centrifugation. The products were analysed by electrophoresis on 1% agarose gels after staining with ethidium bromide. Using Image lab software (Bio-rad), intensity of bands were measured and analysed to determine the enzyme inhibition by relative band intensity.

4.2.2. In vitro MTB screening

Two-fold serial dilutions of each test compound/drug were prepared and incorporated into Middle-brook 7H11 agar medium with oleic acid, albumin, dextrose, and catalase (OADC) growth supplement to get final concentrations of 50, 25, 12.5, 6.25, 3.13, 1.56, and 0.78 μg/mL. Inoculum of *M. tuberculosis* H37Rv ATCC 27294 was prepared from fresh Middlebrook 7H11 agar slants with OADC (Difco) growth supplement adjusted to 1 mg/mL (wet weight) in Tween 80 (0.05%) saline diluted to 10^{-2} to give a concentration of $\sim 10^7$ cfu/mL. Five microlitres of this bacterial suspension was spotted onto 7H11 agar tubes containing different concentrations of the drug as discussed above. The tubes were incubated at 37 °C, and final readings (as MIC in μg/mL) were determined after 28 days. The MIC is defined as the minimum concentration of compound required to give complete inhibition of bacterial growth. This method is similar to that recommended by the National Committee for Clinical Laboratory Standards for the determination of MIC in triplicate.

4.2.3. In vitro cytotoxicity screening

Some compounds were further examined for toxicity in a RAW 264.7 cell line at the concentration of 50 μM. After 72 h of exposure, viability was assessed on the basis of cellular conversion of MTT into a formazan product using the Promega Cell Titer 96 non-radioactive cell proliferation assay.

4.2.4. zERG channel inhibition screening

All experiments were performed as per the guidelines published by the National Institutes of Health for care and use of zebrafish. In brief, male and female fishes were maintained separately in the re-circulatory system with 14 h light and 10 h dark cycle, with 28 °C as optimum temperature. Breeding was carried out using 2 females: 3 males in a separate breeding cage. Embryos were collected into petridishes and allowed to grow in the incubator in E3 medium at 28 °C temperature. On the 3rd day embryos were removed and washed. Stock solution of the drug was prepared in 100% DMSO and the working concentrations were prepared accordingly by serial dilutions. 6 embryos were transferred into each well of 24 well plate along with 250 μl of 0.1% DMSO. Each well was then added with 250 μl of working concentration of the drug. Embryos were allowed to incubate at the optimum temperature for 4 h. After 4 h of incubation, embryos were treated with tricaine and immediately used for reading the heart rate. 30 heart beats were counted for atrium and ventricle per embryo and the corresponding time was noted, from which number of heart beats per minute was calculated by the formula: $1800/X$ = beats/minute (where X = time in seconds).

Acknowledgements

DS acknowledges University Grants Commission, New Delhi, India for UGC Research Award. Authors also thankful to J.P.S. Devi for her assistance in MTB screening.

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