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Diaryloxy methano phenanthrenes: a new class of antituberculosis agents☆

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Dedicated to Professor Goverdhan Mehta on the occasion of his 60th birthday

Abstract—A new series of diaryloxy methano phenanthrenes were prepared through tertiary-aminoalkylations of [(methoxy-phenyl)-phenanthren-9-yl-methyl]-phenols obtained from Friedel–Crafts alkylations on (methoxy-phenyl)-phenanthren-9-yl-methanols. These series of compounds were evaluated against *Mycobacterium tuberculosis* $H_{37}R_v$ and showed the desired activity in the range of 6.25 μg/mL in vitro. One of the compound **12j** protects the mice from the challenge of *M. tuberculosis* in vivo, as 30% of the mice were survived at treatment of 50 mg/kg body weight.

1. Introduction

Tuberculosis, a leading infectious disease caused by *Mycobacterium tuberculosis* is major concern of death in the world today.[1] It is estimated that worldwide 100 million people are infected annually. Approximately 10 million develop the disease, with 5 million of these progressing to the infectious stage and ultimately 3 million dying. Even though improved methods of prevention, detection, diagnosis and modern treatment have greatly reduced the number of people getting infected and dying from it, the emergence of multi-drug-resistant (MDR) strains, its synergy with global human immunodeficiency virus (HIV) and many socioeconomic problem has led to declare this disease as 'global emergency' by WHO (World Health Organization). In spite of modern sophisticated developments in improved methods of diagnosis, prevention and treatment, the gloomy picture of this disease is still alarming.[2,3] Resistance has been described for all first-line drugs (isoniazid, rifampin, pyrazinamide, ethambutol and streptomycin) and for several second-line and newer drugs (ethionamide, fluoroquinolones, macrolides, nitroimidazopyrans).[4] The search for more effective agents against *M. tuberculosis* (MT) and *M. avium complex* (MAC) is ongoing in an attempt to enhance survival and reduce morbidity, as proven by the high number of patents of new antituberculous agents recently published.[5,6]

Because of this, there is an urgent need for anti-TB drugs with improved properties such as enhanced activity[7,8] against MDR strains, reduced toxicity, shortened duration of therapy, rapid mycobactericidal mechanism of action and the ability to penetrate host cells and exert anti-mycobacterial effects in the intracellular environment.[9,10]

Diaryl naphthalenes are known for a number of biological activities such as anti-inflammatory, antichagasic etc. This class of molecule is known to alter the cellular machinery processes. Biphenyl methanone derivatives are known to possess antimycobacterial activity. Keeping that in mind, we were prompted to see the effect of diaryloxy methano phenanthrenes on the growth of *M. tuberculosis* as this class of compounds possess enough hydrophobicity, a requirement for good antituberculosis agents.[11] Thus we have identified **1** as a potential target

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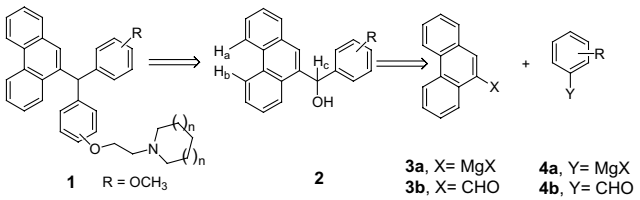


Scheme 1. Retrosynthesis of target compounds.

molecule for antituberculosis agents. Retrosynthetic analysis of **1** leads to compound **2** as the precursor, which can be obtained from nucleophilic addition of Grignard reagent **3a**, **4a** onto carbaldehyde **4b**, **3b**, respectively (Scheme 1).

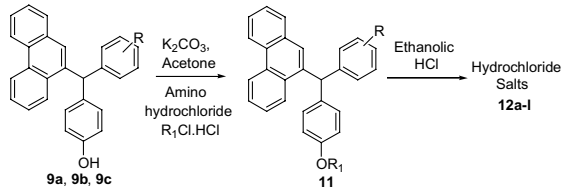
2. Results and discussion

2.1. Chemistry

The compounds were synthesized essentially following the steps as depicted below. The reaction between Grignard reagent **3a**, **4a** and aldehyde **4b**, **3b** in dry THF furnished the carbinol[7] **2a**–**c** in 70% isolated yield. IR frequency at 3431 cm^{-1} indicated the presence of hydroxyl group in **2a**–**c**. The characteristic peaks of **2a**–**c** at δ 8.66, 8.62 and 6.39 ppm in its ^1H NMR spectra were assigned for phenanthrene (H_a, H_b) and benzylic methine proton (H_c), respectively. ^{13}C NMR and mass spectral data (molecular ion peak at 314 amu) further confirmed the structural identity of **2a**–**c** (Scheme 2).

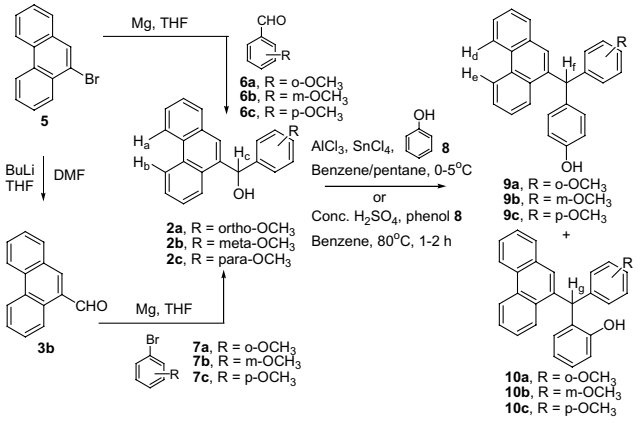
Friedel–Crafts alkylation of the compounds **2a**–**c** with phenol **8** in the presence of $AlCl_3$ and $SnCl_4$ led to enantiomeric mixtures of **9a**–**c** as major and **10a**–**c** as minor products. The compound **9a**–**c** was characterized from its deshielded doublet aromatic protons H_d, H_e (δ 8.68 and 8.62 ppm) and singlet methine proton H_f (δ 6.11 ppm) resonances, respectively. Whereas in ^1H NMR of **10a**–**c**, the singlet methine proton H_g appears at δ 6.37 ppm due to −I inductive effect of *ortho*-hydroxy group of the benzene nucleus. The structural identity of **9a**–**c** and **10a**–**c** were further confirmed by their ^{13}C NMR and mass spectrum fragmentation analysis. In this competitive reaction, the generation of compound **9a**–**c** and **10a**–**c** could be attributed to the fact that Friedel–Crafts alkylation occurs via attack of phenol at both *ortho* and *para* position of the benzene nucleus with the carbinol **2a**–**c**.

The reaction between **9a**–**c** with different alkylaminohydrochloride chains in the presence of K_2CO_3 and acetone led to the formation of compounds **11a**–**l** in good yields (Scheme 3). Similarly, compounds **10a**–**c** also on reaction with alkylaminohydrochlorides gave **13a**–**d** in respectable yields (Scheme 4). Diaryloxy methano phenanthrenes **12a**–**l** and **14a**–**d** were prepared as aminohydrochloride salts through treating their corresponding amines **11a**–**l** and **13a**–**d** with ethanolic HCl. All the hydrochloride salts were good crystalizable solid compounds.

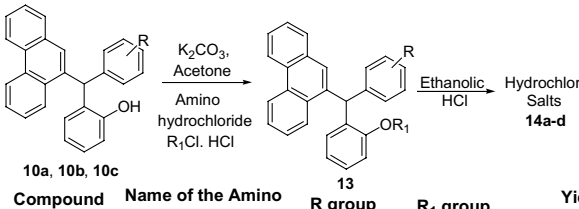


Compound No.	Name of the Amino hydrochloride Chain R_1Cl. HCl	R group	R_1 group	Yields (%)
12a	$ClCH_2CH_2N(CH_3)_2$.HCl	o-OCH_3	$CH_2CH_2N(CH_3)_2$	67
12b	$ClCH_2CH_2N(CH_2CH_3)_2$.HCl	o-OCH_3	$CH_2CH_2N(CH_2CH_3)_2$	62
12c	$ClCH_2CH_2N$ .HCl	o-OCH_3	CH_2CH_2N	72
12d	$ClCH_2CH_2N$ .HCl	o-OCH_3	CH_2CH_2N .HCl	70
12e	$ClCH_2CH_2N(CH_3)_2$.HCl	m-OCH_3	$CH_2CH_2N(CH_3)_2$	77
12f	$ClCH_2CH_2N(CH_2CH_3)_2$.HCl	m-OCH_3	$CH_2CH_2N(CH_2CH_3)_2$	77
12g	$ClCH_2CH_2N$ .HCl	m-OCH_3	CH_2CH_2N .HCl	72
12h	$ClCH_2CH_2N$ .HCl	m-OCH_3	CH_2CH_2N .HCl	70
12i	$ClCH_2CH_2N(CH_3)_2$.HCl	p-OCH_3	$CH_2CH_2N(CH_3)_2$	93
12j	$ClCH_2CH_2N(CH_2CH_3)_2$.HCl	p-OCH_3	$CH_2CH_2N(CH_2CH_3)_2$	83
12k	$ClCH_2CH_2N$ .HCl	p-OCH_3	CH_2CH_2N .HCl	80
12l	$ClCH_2CH_2N$ .HCl	p-OCH_3	CH_2CH_2N .HCl	77

Scheme 3. Synthesis of phenolic derivatives **12a**–**l**.

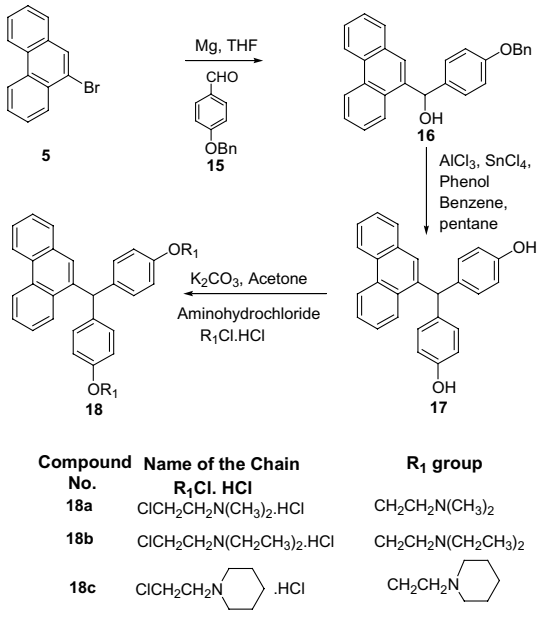


Scheme 2. Synthesis of carbinols.



Compound No.	Name of the Amino hydrochloride Chain R_1Cl. HCl	R group	R_1 group	Yield (%)
14a	$ClCH_2CH_2N(CH_3)_2$.HCl	p-OCH_3	$CH_2CH_2N(CH_3)_2$	80
14b	$ClCH_2CH_2N(CH_2CH_3)_2$.HCl	p-OCH_3	$CH_2CH_2N(CH_2CH_3)_2$	92
14c	$ClCH_2CH_2N$ .HCl	p-OCH_3	CH_2CH_2N	78
14d	$ClCH_2CH_2N$ .HCl	p-OCH_3	CH_2CH_2N .HCl	65

Scheme 4. Synthesis of phenolic derivatives **14a**–**d**.



Scheme 5. Synthesis of phenolic derivatives **18a–c**.

While the mono-aminohydrochloride salts **14a–l** and **14a–d** are active against *M. tuberculosis*, we became interested in synthesizing their bis-aminohydrochloride salts and evaluate their antitubercular activity. Towards this objective, the Grignard reagent **3a** derived from **5** was reacted with benzyloxy benzaldehyde **15** to furnish the carbinol **16**. The identity of **16** was characterized through ^{1}H and ^{13}C NMR data analysis. Upon Friedel–Crafts reaction on **16**, the bis-phenolic derivative **17** was obtained with disappearance of benzyl group. The compound **17** was reacted with three aminohydrochlorides to furnish the bis-phenolic derivatives **18a–c** (Scheme 5). The compounds were evaluated against *M. tuberculosis* $H_{37}R_v$ in vitro and the results are shown below.

3. Biology

3.1. Determination of activity in vitro and in vivo

The activity of the compounds against *M. tuberculosis* $H_{37}R_v$ was determined by agar micro dilution technique and standard BACTEC radiometric growth assay.[12] (details are described in experimental section). The activity of the compounds **12a–l**, **14a–d** and **18a–c** in vitro are shown in Table 1.

All the alkylaminohydrochloride derivatives (**12a–l**, **14a–d** and **18a–c**) displayed activity against *M. tuberculosis* with minimum inhibitory concentrations (MIC) ranging from 6.25 to 25 μg/mL. These compounds were synthesized to evaluate the effect of *ortho*, *meta* and *para* methoxy substituents of benzene ring on the central methano-phenanthrene nucleus and also the effect of increasing the alkyl substituents of nitrogen ring on antitubercular activity. It is noteworthy that all the diaryloxy methano phenanthrenes with *para* methoxy substituents except in case of **12i** showed desired activity with MIC 6.25 μg/mL in comparison to other *ortho* and *meta* substituted methoxy derivatives possibly due to better exposition of *p*-methoxy groups in the hydrophobicity of central pharmacophore skeleton. Effect of difference in *ortho* and *meta* methoxy substituents on MIC value was marginal. Increasing the alkyl chain on nitrogen resulted into better activity. Compound **12i** with dimethyl substituents on nitrogen is having MIC 12.5 (BACTEC method) whereas **12j**, **12k** and **12l** with diethyl, pyrrolidine and piperidine rings, respectively, are having MIC 6.25 (BACTEC method). Interestingly, increasing the alkylaminohydrochloride chains on the central pharmacophore gave better results. Compounds **18a–c** with bis-alkylaminohydrochloride chains at the *para* position of the benzene ring are having MIC 6.25 evaluated in both methods (Agar microdilution and BACTEC). Thus, methanophenanthrenes with *para*-methoxy group on one benzene nucleus and alkylaminohydrochloride chains on other benzene nucleus gave better antitubercular activity in vitro. On the basis of MIC, three compounds (**12j**, **12k**, **12l**) were selected for further evaluation of their activity. Out of them, compound **12j** was selected as a representative to evaluate its activity in vivo.

Table 1. In vitro antituberculosis activity of **12a–l**, **14a–d** and **18a–c** against *M. tuberculosis* $H_{37}R_v$

Compound no.	MIC (μg/mL), Agar micro dilution method	MIC (μg/mL), BACTEC method
12a	12.5	12.5
12b	12.5	12.5
12c	12.5	12.5
12d	12.5	12.5
12e	—	—
12f	12.5	12.5
12g	25.0	12.5
12h	12.5	12.5
12i	25.0	12.5
12j	6.25	6.25
12k	6.25	6.25
12l	6.25	6.25
14a	25.0	25.0
14b	25.0	25.0
14c	12.5	12.5
14d	12.5	12.5
18a	6.25	6.25
18b	6.25	6.25
18c	6.25	6.25
SPAR	0.75	1.00
Isoniazid (INH)	0.05	0.025

— means not active at 25 μg/mL.

Forty inbred female AKR mice, weighing 18–20 g were infected i.v. via lateral vein with 10^7 colony forming units of *M. tuberculosis* $H_{37}R_v$. Mice were divided into four groups after two days comprising 10 mice each. One group received the aqueous suspension of the test agent **12j** by oral route daily for 14 days at the dose of 50 mg/kg body weight. The second group received sparfloxacin at 25 mg/kg body weight (ED_{90}) for 14 days orally. The third group received the test compound **12j** at the dose of 100 mg/kg body weight whereas the fourth group served as control receiving no drug. Mice were

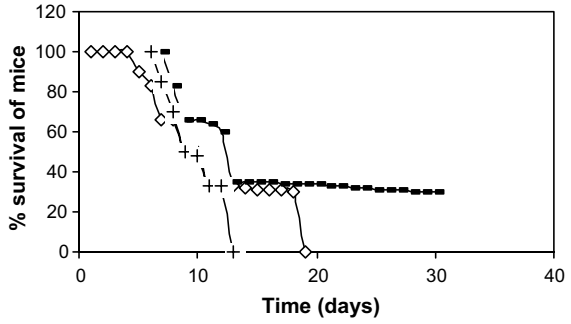


Figure 1. Plot of % survival of mice. (◇) No drug; (+) 100 mg/kg of **12j**; (–) 50 mg/kg of **12j**.

observed for 35 days. Antitubercular activity was measured by general health of the mice, mean survival time, lesions in lung and spleen, load of bacilli (Fig. 1).

All the mice in the untreated group died of tuberculosis within 20 days of receiving infection. These mice were weak with ruffled hairs. The mean survival time (MST) was 13.7 days. The lungs were full of tubercles and spleen was enlarged. The load of the bacilli was estimated around 3×10^7/g of lung. All the mice treated with sparfloxacin survived with normal appearance of lung and spleen. Hardly few acid-fast bacilli were seen in lung smears under microscope.

In the group treated with 50 mg/kg dose of **12j**, 30% of the mice survived. The survival rate was not significantly different from the control group up to day 18. However, the number of survivors in the 50 mg/kg dose group was significantly greater than zero on day 19 onwards when compared by the test of proportion ($p < 0.05$). The mean survival time (MST) was found to be 18.5 days, hence MST increased compared to that of control group (13.7 days). There were few tubercles in the lung and the spleen was looking normal in the surviving mice. The load of the bacilli in the lungs of surviving mice was significantly less (approx. 10^3–7×10^3 bacilli/g of lung). However, it was surprising that increasing the dose of the compound from 50 to 100 mg/kg could not give better protection against *M. tuberculosis* infection. The mice receiving 100 mg/kg of the test compound **12j** died earlier than the control (untreated infected mice). The mice were weak and there were tubercles in the lung. Microscopic examination of the lung smear showed a large number of acid-fast bacilli (AFB) as those seen in the lung of control group of mice. It, therefore, appears that in *M. tuberculosis* infected mice, the compound may have become toxic. It is already known that mice with tubercular infection are prone to immunological disorders and loss immune function.[13] This observation suggest that flaring up of the infection is much faster due to toxicity of the compound in immunologically weak mice at 100 mg/kg dose and protection in mice at 50 mg/kg dose appears to be due to the fine balance between toxicity and efficacy of the compound. In 25 mg/kg dose group, infection load was the same as that in the untreated control group (data not shown). This may be due to inefficacy of the compound at a lower dose.

4. Conclusion

The analysis of in vitro and in vivo data for the compounds **12a**–**l** and **18a**–**c** clearly suggests that these class of compounds are indeed antitubercular. The compound **12j** seems to protect the mice from the challenge of *M. tuberculosis*, as 30% of mice were survived and this protection is dose dependent. Since the mice receiving 100 mg/kg dose were not protected and there was no inhibition of growth of infection, the logical assumption was that the immune system of the mice was compromised by treatment of the compound for several days. This class of compounds appears to have antitubercular activity. We are reporting for the first time that diaryloxy methano phenanthrenes might be a suitable pharmacophore for developing novel antitubercular agents. Compounds containing biphenyl and related hydrophobic groups are known to act through fatty-acid biosynthesis and offer antitubercular activity.[14] Our compounds also bear the similar chemical moieties. Therefore, it can reasonably be assumed that this class of compounds having related hydrophobicity might be acting through inhibition of fatty acid biosynthesis of *M. tuberculosis*. A rational and logical design of a compound retaining the antitubercular activity without toxicity may be a favourable molecule. Synthesis of the compounds and their biological evaluation towards this direction is currently underway.

5. Experimental details

5.1. Representative Grignard reaction

To a solution of bromoanisole **7a**–**c** (3.6 mL, 29.1 mmol) in dry THF (15 mL) was added activated magnesium (0.79 g, 32.98 mmol) and was stirred at room temperature under dry nitrogen for 2 h. To Grignard reagent thus formed was added phenanthrene-9-carbaldehyde **3b** (2 g, 9.7 mmol) in THF (15 mL) and stirring was continued for another 3–4 h. The reaction mixture was quenched by gradual addition of saturated NH_4Cl (~10 mL) and THF was removed in vacuo. The mixture was extracted thrice with ethyl acetate, washed with brine and dried over sodium sulfate. It was concentrated and charged over silica gel. Elution with 10% ethylacetate in hexane furnished carbinol product **2a**–**c**.

5.1.1. (4-Methoxy-phenyl)-phenanthren-9-yl-methanol. Pale yellow solid, **2c** (2.57 g, 85%), mp 70 °C, IR (neat): 3409, 1597, 1485, 1256, 1045, 760 cm^{-1}, ^1H NMR ($CDCl_3$, 200 MHz): δ 8.66 (d, 1H, $J = 8.3$ Hz), 8.62 (d, 1H, $J = 8.3$ Hz), 7.94 (s, 1H), 7.90 (d, 1H, $J = 8$ Hz), 7.86 (d, 1H, $J = 8$ Hz), 7.69–7.40 (m, 4H), 7.28 (d, 2H, $J = 8.8$ Hz), 6.78 (d, 2H, $J = 8.6$ Hz), 6.39 (s, 1H), 3.70 (s, 3H), 2.50 (bs, 1H); ^{13}C NMR: δ 159.6, 137.4, 135.5, 131.8, 131.2, 130.7, 130.1, 129.4, 129.0, 127.1, 126.9, 126.6, 125.5, 125.3, 123.5, 122.8, 114.4, 73.9, 55.6; MS (EI): *m/z* 314 (M^+); Anal. Calcd for $C_{22}H_{18}O_2$: C, 84.05; H, 5.77. Found: C, 84.57; H, 5.62.

5.1.2. (3-Methoxy-phenyl)-phenanthren-9-yl-methanol. Pale yellow solid, **2b** (2.5 g, 85%), mp 123 °C, IR (neat):

3409, 1599, 1488, 1256, 1045, 760 cm^{-1}, ^1H NMR ($CDCl_3$, 200 MHz): δ 8.66 (d, 1H, $J = 8.6$ Hz), 8.62 (d, 1H, $J = 8.4$ Hz), 7.98 (d, 1H, $J = 8$ Hz), 7.87 (s, 1H), 7.84 (d, 1H, $J = 8$ Hz), 7.66–7.40 (m, 4H), 7.19 (t, 1H, $J = 8$ Hz), 6.98 (s, 1H), 6.96 (d, 1H, $J = 8.4$ Hz), 6.76 (d, 1H, $J = 8$ Hz), 6.39 (s, 1H), 3.69 (s, 3H), 2.56 (bs, 1H); MS: 314 (M^+); Anal. Calcd for $C_{22}H_{18}O_2$: C, 84.05; H, 5.77. Found: C, 83.94; H, 6.00.

5.1.3. (2-Methoxy-phenyl)-phenanthren-9-yl-methanol. Pale yellow solid, **2a** (3.2 g, 70%), mp 162 °C, IR (neat): 3262, 1593, 1456, 1237, 1035 cm^{-1}, ^1H NMR ($CDCl_3$, 200 MHz): δ 8.69 (d, 1H, $J = 8.6$ Hz), 8.64 (d, 1H, $J = 8.6$ Hz), 7.97 (s, 1H), 7.92 (d, 1H, $J = 8.6$ Hz), 7.87 (d, 1H, $J = 8.4$ Hz), 7.70–7.40 (m, 4H), 7.23 (t, 1H, $J = 7.8$ Hz), 7.0–6.7 (m, 3H), 6.95 (s, 1H), 3.90 (s, 3H), 3.10 (d, 1H, $J = 4.2$ Hz); ^{13}C NMR: δ 132.0, 131.5, 130.6, 130.5, 129.6, 129.4, 129.0, 127.09, 127.04, 126.9, 126.5, 125.5, 125.3, 123.5, 122.8, 121.3, 68.6, 56.0; MS: 314 (M^+); Anal. Calcd for $C_{22}H_{18}O_2$: C, 84.05; H, 5.77. Found: C, 84.25; H, 5.98.

5.1.4. (4-Benzyloxy-phenyl)-phenanthren-9-yl-methanol. Pale yellow solid, **16** (1.32 g, 87%), mp 131 °C, ^1H NMR ($CDCl_3$, 200 MHz): δ 8.72 (d, 1H, $J = 8.4$ Hz), 8.67 (d, 1H, $J = 8.4$ Hz), 8.01 (s, 1H), 7.93 (d, 2H, $J = 8.6$ Hz), 7.66–7.40 (m, 4H), 7.40–7.29 (m, 7H), 6.92 (d, 2H, $J = 8.6$ Hz), 6.50 (d, 1H, $J = 2.8$ Hz), 5.02 (s, 2H), 2.30 (d, 1H, $J = 3.8$ Hz); MS: 373 (M^+); Anal. Calcd for $C_{28}H_{22}O_2$: C, 86.13; H, 5.68. Found: C, 86.93; H, 5.99.

5.2. Representative Friedel–Crafts reaction

To a solution of carbinol **2a**–**c** (6.0 g, 19.11 mmol) taken in dry benzene (60 mL) and pentane (120 mL) at room temperature was gradually added phenol (2.37 mL, 28.66 mmol) and the reaction mixture was stirred at 0 °C for 15 min. $AlCl_3$ (2.56 g, 19.11 mmol) was added followed by $SnCl_4$ (4.56 mL, 24.84 mmol) and stirring was continued for another 1 h. Water was slowly added and the mixture was extracted with ethylacetate. The organic layer was washed with water, brine and dried over anhydrous Na_2SO_4. It was concentrated to give an oily residue that was charged over silica gel. Elution with 15% ethylacetate in hexane furnished the compound **9a**–**c**, **10a**–**c**.

5.3. Alternative procedure

To a solution of carbinol **2a**–**c** (2.85 g, 9.07 mmol) and phenol (1.28 g, 13.61 mmol) in dry benzene (40 mL) catalytic amount of conc. H_2SO_4 was added and the reaction mixture was refluxed at 80 °C for 1 h. It was cooled to room temperature, treated with saturated $NaHCO_3$ and extracted with ethylacetate. The organic layer was washed with water and dried over anhydrous Na_2SO_4. Column chromatography over silica gel and elution with 15% ethyl acetate in hexane furnished the desired compound.

5.3.1. 4-[(4-Methoxy-phenyl)-phenanthren-9-yl-methyl]-phenol. Pale yellow solid, **9c** (2.4 g, 32.2%) mp 64 °C, IR (neat): 3391, 3017, 2928, 1605, 1506, 1449, 1245, 1033, 833, 753 cm^{-1}, ^1H NMR ($CDCl_3$, 200 MHz): δ 8.68 (d, 1H, $J = 8.2$ Hz), 8.62 (d, 1H, $J = 8.2$ Hz), 8.01 (d, 1H, $J = 7.9$ Hz), 7.72–7.45 (m, 5H), 7.14 (s, 1H), 7.04 (d, 2H, $J = 8.6$ Hz), 6.98 (d, 2H, $J = 7.6$ Hz), 6.83 (d, 2H, $J = 7.7$ Hz), 6.70 (d, 2H, $J = 8$ Hz), 6.11 (s, 1H), 5.08 (bs, 1H), 3.74 (s, 3H); MS: 390 (M^+); Anal. Calcd for $C_{28}H_{22}O_2$: C, 86.13; H, 5.68. Found: C, 86.18; H, 5.99.

5.3.2. 4-[(3-Methoxy-phenyl)-phenanthren-9-yl-methyl]-phenol. Pale yellow solid, **9b** (1.92 g, 49%). mp 70 °C, IR (neat): 3400, 1599, 1488, 1259, 756 cm^{-1}, ^1H NMR ($CDCl_3$, 200 MHz): δ 8.71 (d, 1H, $J = 8.2$ Hz), 8.64 (d, 1H, $J = 8.2$ Hz), 8.02 (d, 1H, $J = 7.9$ Hz), 7.70–7.48 (m, 5H), 7.22 (d, 1H, $J = 8$ Hz), 7.16 (s, 1H), 7.02 (d, 2H, $J = 8.2$ Hz), 6.80–6.73 (m, 5H), 6.15 (s, 1H), 4.73 (s, 1H), 3.71 (s, 3H); MS: 390 (M^+); Anal. Calcd for $C_{28}H_{22}O_2$: C, 86.13; H, 5.68. Found: C, 86.68; H, 5.66.

5.3.3. 4-[(2-Methoxy-phenyl)-phenanthren-9-yl-methyl]-phenol. Pale yellow solid, **9a** (3 g, 40%), mp 80 °C, IR (neat): 3390, 1500, 1241, 758 cm^{-1}, ^1H NMR ($CDCl_3$, 200 MHz): δ 8.70 (d, 1H, $J = 8.2$ Hz), 8.64 (d, 1H, $J = 8.2$ Hz), 8.01 (d, 1H, $J = 8$ Hz), 7.69–7.43 (m, 5H), 7.27–7.18 (m, 2H), 7.15 (s, 1H), 7.02 (d, 2H, $J = 8.4$ Hz), 6.96–6.70 (m, 4H), 6.53 (s, 1H), 4.70 (bs, 1H), 3.74 (s, 3H); MS: 390 (M^+); Anal. Calcd for $C_{28}H_{22}O_2$: C, 86.13; H, 5.68. Found: C, 86.75; H, 5.63.

5.3.4. 2-[(4-Methoxy-phenyl)-phenanthren-9-yl-methyl]-phenol. Pale yellow solid, **10c** (0.8 g, 12%), mp 80 °C, IR (neat): 3391, 3017, 2928, 1605, 1506, 1449, 1245, 1033, 833, 753 cm^{-1}, ^1H NMR ($CDCl_3$, 200 MHz): δ 8.72 (d, 1H, $J = 8$ Hz), 8.65 (d, 1H, $J = 8$ Hz), 8.01 (d, 1H, $J = 8$ Hz), 7.65–7.40 (m, 5H), 7.24–7.08 (m, 3H), 7.14 (s, 1H), 6.88–6.78 (m, 5H), 6.37 (s, 1H), 4.81 (bs, 1H), 3.78 (s, 3H); MS: 390 (M^+); Anal. Calcd for $C_{28}H_{22}O_2$: C, 86.13; H, 5.68. Found: C, 86.28; H, 6.00.

5.3.5. 4-[(4-Hydroxy-phenyl)-phenanthren-9-yl-methyl]-phenol. Dark orange solid, **17** (1.2 g, 52%), mp 112 °C, IR (neat): 3323, 1602, 1505, 1441, 1365, 1230, 1170 cm^{-1}, ^1H NMR ($CDCl_3$, 200 MHz): δ 8.72 (d, 1H, $J = 8.2$ Hz), 8.65 (d, 1H, $J = 8.2$ Hz), 8.02 (d, 1H, $J = 9$ Hz), 7.69–7.48 (m, 5H), 7.14 (s, 1H), 7.01 (d, 4H, $J = 8.2$ Hz), 6.75 (d, 4H, $J = 8.2$ Hz), 6.12 (s, 1H), 4.67 (bs, 2H); ^{13}C NMR: 154.4, 139.1, 136.4, 131.8, 131.6, 131.2, 130.2, 129.1, 128.7, 127.0, 126.8, 126.5, 125.6, 123.4, 122.7, 115.7, 109.9, 52.2; MS: 376 (M^+); Anal. Calcd for $C_{27}H_{20}O_2$: C, 86.14; H, 5.36. Found: C, 86.45; H, 5.78.

5.4. Representative chain reaction

A mixture of compounds **9a**–**c** (1 g, 2.564 mmol), anhydrous K_2CO_3 (1.77 g, 12.82 mmol), 1-(2-chloroethyl)-piperidine hydrochloride (0.708 g, 3.84 mmol) and dry acetone (50 mL) was refluxed for 7 h. K_2CO_3 was filtered off and acetone was removed. The residue was diluted with water and extracted with ethyl acetate. The organic layer was washed with water, brine and dried over anhydrous Na_2SO_4. Column chromatography over silica gel

and elution with 35% ethylacetate in hexane furnished the compound. The product was dissolved in absolute ethanol (25 mL) and ethanolic HCl was added dropwise till the pH of the mixture was acidic. Ethanol was removed in vacuo. The residue was recrystallized from a mixture of absolute ethanol and dry ether to give compound as hydrochloride salt.

5.4.1. 1-(2-{4-[(4-Methoxy-phenyl)-phenanthren-9-yl-methyl]-phenoxy}-ethyl)-piperidine. White solid, **11l** (1 g, 77%), mp 80 °C (HCl salt), IR (neat): 2929, 1507, 1246, 755 cm^{-1}, ^1H NMR ($CDCl_3$, 200 MHz): δ 8.71 (d, 1H, $J = 8$ Hz), 8.65 (d, 1H, $J = 8$ Hz), 8.03 (d, 1H, $J = 8$ Hz), 7.65–7.48 (m, 5H), 7.15 (s, 1H), 7.07 (d, 4H, $J = 8.2$ Hz), 6.82 (d, 4H, $J = 8.6$ Hz), 6.14 (s, 1H), 4.08 (t, 2H, $J = 6$ Hz), 3.78 (s, 3H), 2.77 (t, 2H, $J = 6$ Hz), 2.51 (m, 4H), 1.60 (m, 4H), 1.45 (m, 2H); MS: 502 (M^+); Anal. Calcd for HCl salt, $C_{35}H_{36}ClNO_2$: C, 78.12; H, 6.74; N, 2.66. Found: C, 78.26; H; 6.56; N, 2.49.

5.4.2. 1-(2-{4-[(4-Methoxy-phenyl)-phenanthren-9-yl-methyl]-phenoxy}-ethyl)-pyrrolidine. White solid, **11k** (1 g, 80%), mp 90 °C (HCl salt), IR (neat): 2926, 1507, 1246, 756 cm^{-1}, ^1H NMR ($CDCl_3$, 200 MHz): δ 8.72 (d, 1H, $J = 7.6$ Hz), 8.65 (d, 1H, $J = 7.8$ Hz), 8.03 (d, 1H, $J = 7.2$ Hz), 7.69–7.45 (m, 5H), 7.14 (s, 1H), 7.06 (d, 4H, $J = 8.8$ Hz), 6.82 (d, 4H, $J = 8.8$ Hz), 6.14 (s, 1H), 4.08 (t, 2H, $J = 6$ Hz), 3.79 (s, 3H), 2.89 (t, 2H, $J = 6$ Hz), 2.62 (m, 4H), 1.79 (m, 4H); MS: 488 (M^+); Anal. Calcd for HCl salt, $C_{34}H_{34}ClNO_2 \cdot H_2O$: C, 77.92; H, 6.54; N, 2.67. Found: C, 78.22; H, 6.78; N, 2.27.

5.4.3. 1-(2-{4-[(4-Methoxy-phenyl)-phenanthren-9-yl-methyl]-phenoxy}-ethyl)-dimethyl-amine. White solid, **11i** (1.1 g, 93%), mp 110 °C (HCl salt), IR (Neat): 3440, 1633, 769 cm^{-1}, ^1H NMR ($CDCl_3$, 200 MHz): δ 8.72 (d, 1H, $J = 8$ Hz), 8.65 (d, 1H, $J = 8.2$ Hz), 8.03 (d, 1H, $J = 8$ Hz), 7.69–7.44 (m, 5H), 7.14 (s, 1H), 7.05 (d, 4H, $J = 8$ Hz), 6.83 (d, 4H, $J = 8$ Hz), 6.14 (s, 1H), 4.04 (t, 2H, $J = 6$ Hz), 3.78 (s, 3H), 2.71 (t, 2H, $J = 6$ Hz), 2.32 (s, 6H); MS: 462 (M^+); Anal. Calcd for HCl salt, $C_{32}H_{32}ClNO_2$: C, 77.17; H, 6.48; N, 2.81. Found: C, 77.87; H, 6.75; N, 2.69.

5.4.4. Diethyl-(2-{4-[(4-methoxy-phenyl)-phenanthren-9-yl-methyl]-phenoxy}-ethyl)-amine. White solid, **11j** (1.05 g, 83%), mp 85 °C (HCl salt), IR (Neat): 2927, 1507, 1244, 759 cm^{-1}, ^1H NMR ($CDCl_3$, 200 MHz): δ 8.73 (d, 1H, $J = 8$ Hz), 8.66 (d, 1H, $J = 8$ Hz), 8.06 (d, 1H, $J = 8.2$ Hz), 7.94–7.49 (m, 5H), 7.17 (s, 1H), 7.09 (d, 4H, $J = 8.4$ Hz), 6.84 (d, 4H, $J = 8.4$ Hz), 6.16 (s, 1H), 4.05 (t, 2H, $J = 6$ Hz), 3.78 (s, 3H), 2.89 (t, 2H, $J = 6$ Hz), 2.66 (q, 4H), 1.08 (t, 6H); ^{13}C NMR: δ 139.3, 136.3, 131.9, 131.8, 131.0, 130.0, 129.1, 128.8, 127.0, 126.9, 126.4, 125.7, 123.4, 122.7, 114.8, 114.2, 66.8, 55.6, 52.2, 48.2, 12.2; MS: 490 (M^+); Anal. Calcd for HCl salt, $C_{34}H_{36}ClNO_2$: C, 77.62; H, 6.92; N, 2.66. Found: C, 77.64; H, 6.47; N, 2.60.

5.4.5. 1-(2-4-[(3-Methoxy-phenyl)-phenanthren-9-yl-methyl]-phenoxy-ethyl)-pyrrolidine. White solid, **11g** (450 mg, 72%), mp 60 °C (HCl salt), IR (neat): 2927, 1219, 761 cm^{-1}, ^1H NMR ($CDCl_3$, 200 MHz): δ 8.72 (d, 1H, $J = 8$ Hz), 8.65 (d, 1H, $J = 7.8$ Hz), 8.01 (d, 1H, $J = 8.2$ Hz), 7.71 (d, 1H, $J = 7.6$ Hz), 7.66–7.45 (m, 4H), 7.20 (d, 1H, $J = 8$ Hz), 7.15 (s, 1H), 7.04 (d, 2H, $J = 8$ Hz), 6.84 (s, 1H), 6.82–6.70 (m, 4H), 6.16 (s, 1H), 4.08 (t, 2H, $J = 6$ Hz), 3.71 (s, 3H), 2.89 (t, 2H, $J = 6$ Hz), 2.63 (m, 4H), 1.80 (m, 4H); MS: 488 (M^+); Anal. Calcd for HCl salt, $C_{34}H_{34}ClNO_2$: C, 77.92; H, 6.54; N, 2.67. Found: C, 77.62; H, 6.78; N, 2.27.

5.4.6. 1-(2-{4-[(3-Methoxy-phenyl)-phenanthren-9-yl-methyl]-phenoxy}-ethyl)-piperidine. White solid, **11h** (450 mg, 70%), mp 90 °C (HCl salt), IR (neat): 2927, 1652, 1505, 1219, 761 cm^{-1}, ^1H NMR ($CDCl_3$, 200 MHz): δ 8.71 (d, 1H, $J = 8.2$ Hz), 8.64 (d, 1H, $J = 8.2$ Hz), 8.03 (d, 1H, $J = 8$ Hz), 7.70–7.40 (m, 5H), 7.22 (d, 1H, $J = 7.8$ Hz), 7.17 (s, 1H), 7.06 (d, 1H, $J = 8$ Hz), 7.02 (d, 1H, $J = 8.2$ Hz), 6.84 (s, 1H), 6.80–6.67 (m, 4H), 6.16 (s, 1H), 4.07 (t, 2H, $J = 6$ Hz), 3.70 (s, 3H), 2.75 (t, 2H, $J = 6$ Hz), 2.52–2.46 (m, 4H), 1.70–1.56 (m, 4H), 1.46–1.44 (m, 2H); MS: 488 (M^+); Anal. Calcd for HCl salt, $C_{35}H_{36}ClNO_2$: C, 78.12; H, 6.74; N, 2.60. Found: C, 78.50; H, 6.99; N, 2.94.

5.4.7. (2-{4-[(3-Methoxy-phenyl)-phenanthren-9-yl-methyl]-phenoxy}-ethyl)-dimethylamine. White solid, **11e** (460 mg, 77%), mp 70 °C (HCl salt), IR (neat): 2926, 1604, 1460, 1244, 758 cm^{-1}, ^1H NMR ($CDCl_3$, 200 MHz): δ 8.71 (d, 1H, $J = 8$ Hz), 8.64 (d, 1H, $J = 8$ Hz), 8.03 (d, 1H, $J = 8$ Hz), 7.70–7.43 (m, 5H), 7.22 (d, 1H, $J = 8.6$ Hz), 7.16 (s, 1H), 7.06 (d, 1H, $J = 8.8$ Hz), 7.02 (d, 1H, $J = 8.6$ Hz), 6.86 (s, 1H), 6.83–6.68 (m, 4H), 4.02 (t, 2H, $J = 6$ Hz), 3.71 (s, 3H), 2.72 (t, 2H, $J = 6$ Hz), 2.33 (s, 6H); MS: 462 ($M^+ - 1$); Anal. Calcd for HCl salt, $C_{32}H_{32}ClNO_2$: C, 77.17; H, 6.48; N, 2.81. Found: C, 76.84; H, 6.12; N, 2.58.

5.4.8. Diethyl-(2-{4-[(3-methoxy-phenyl)-phenanthren-9-yl-methyl]-phenoxy}-ethyl)-amine. White solid, **11f** (448 mg, 77%), mp 65 °C (HCl salt), IR (neat): 2926, 1602, 1241, 1046, 758 cm^{-1}, ^1H NMR ($CDCl_3$, 200 MHz): δ 8.71 (d, 1H, $J = 8$ Hz), 8.64 (d, 1H, $J = 8.2$ Hz), 8.03 (d, 1H, $J = 8$ Hz), 7.70–7.40 (m, 5H), 7.22 (d, 1H, $J = 8.2$ Hz), 7.16 (s, 1H), 7.06 (d, 1H, $J = 8.4$ Hz), 7.02 (d, 1H, $J = 8.4$ Hz), 6.84 (s, 1H), 6.82–6.70 (m, 4H), 6.16 (s, 1H), 4.02 (t, 2H, $J = 6$ Hz), 3.71 (s, 3H), 2.86 (t, 2H, $J = 6$ Hz), 2.61 (q, 4H, $J = 6$ Hz), 1.05 (t, 2H, $J = 7.2$ Hz); MS: 490 (M^+); Anal. Calcd for HCl salt, $C_{34}H_{36}ClNO_2$: C, 77.62; H, 6.92; N, 2.66. Found: C, 77.83; H, 6.39; N, 2.13.

5.4.9. 1-(2-{4-[(2-Methoxy-phenyl)-phenanthren-9-yl-methyl]-phenoxy}-ethyl)-piperidine. White solid, **11d** (450 mg, 70%), mp 90 °C (HCl salt), IR (neat): 2930, 1604, 1242, 758 cm^{-1}, ^1H NMR ($CDCl_3$, 200 MHz): δ 8.70 (d, 1H, $J = 8.2$ Hz), 8.64 (d, 1H, $J = 8.2$ Hz), 8.01 (d, 1H, $J = 8$ Hz), 7.70–7.43 (m, 5H), 7.23 (d, 1H, $J = 8$ Hz), 7.15 (s, 1H), 7.05 (d, 2H, $J = 8.2$ Hz), 6.92 (d, 1H, $J = 8.2$ Hz), 6.88–6.78 (m, 4H), 6.54 (s, 1H), 4.11 (t, 2H, $J = 6$ Hz), 3.73 (s, 3H), 2.86 (t, 2H, $J = 6$ Hz), 2.61 (t, 4H, $J = 6$ Hz), 1.66 (m, 4H), 1.47 (m, 2H); MS: 502 ($M^+ - 1$); Anal. Calcd for HCl salt,

$C_{35}H_{36}ClNO_2$: C, 78.12; H, 6.74; N, 2.60. Found: C, 78.81; H, 6.38; N, 2.76.

5.4.10. 1-(2-{4-[(2-Methoxy-phenyl)-phenanthren-9-yl-methyl]-phenoxy}-ethyl)-pyrrolidine. White solid, **11c** (450 mg, 72%), mp 97 °C (HCl salt), IR (neat): 3017, 2929, 1505, 1218, 758 cm^{-1}, ^1H NMR ($CDCl_3$, 200 MHz): δ 8.69 (d, 1H, $J = 8.2$ Hz), 8.64 (d, 1H, $J = 8.4$ Hz), 8.01 (d, 1H, $J = 8$ Hz), 7.70–7.43 (m, 5H), 7.24 (d, 1H, $J = 8$ Hz), 7.15 (s, 1H), 7.05 (d, 2H, $J = 8.4$ Hz), 6.91 (d, 1H, $J = 8.2$ Hz), 6.90–6.75 (m, 4H), 6.54 (s, 1H), 4.07 (t, 2H, $J = 6$ Hz), 3.73 (s, 3H), 2.88 (t, 2H, $J = 6$ Hz), 2.65–2.56 (m, 4H), 1.79–1.77 (m, 4H), MS: 488 (M^+); Anal. Calcd for HCl salt, $C_{34}H_{34}ClNO_2$: C, 77.92; H, 6.54; N, 2.67. Found: C, 77.44; H, 6.43; N, 2.38.

5.4.11. (2-{4-[(2-Methoxy-phenyl)-phenanthren-9-yl-methyl]-phenoxy}-ethyl)-dimethylamine. White solid, **11a** (400 mg, 67%), mp 95 °C (HCl salt), ^1H NMR ($CDCl_3$, 200 MHz): δ 8.69 (d, 1H, $J = 8.4$ Hz), 8.63 (d, 1H, $J = 8.4$ Hz), 8.02 (d, 1H, $J = 8$ Hz), 7.70–7.45 (m, 5H), 7.22 (d, 1H, $J = 8.2$ Hz), 7.15 (s, 1H), 7.05 (d, 2H, $J = 8.6$ Hz), 6.95–6.80 (m, 5H), 6.54 (s, 1H), 4.03 (t, 2H, $J = 6$ Hz), 3.72 (s, 3H), 2.70 (t, 2H, $J = 6$ Hz), 2.33 (s, 6H), MS: 462 (M^+-1), IR (neat): 2930, 1600, 1496, 1461, 1240, 761 cm^{-1}; Anal. Calcd for HCl salt, $C_{32}H_{32}ClNO_2$: C, 77.17; H, 6.48; N, 2.81. Found: C, 76.85; H, 6.88; N, 2.34.

5.4.12. Diethyl-(2-{4-[(2-methoxy-phenyl)-phenanthren-9-yl-methyl]-phenoxy}-ethyl)-amine. White solid, **11b** (390 mg, 62%), mp 78 °C (HCl salt), IR (neat): 3019, 1216, 765 cm^{-1}, ^1H NMR ($CDCl_3$, 200 MHz): δ 8.70 (d, 1H, $J = 8.2$ Hz), 8.64 (d, 1H, $J = 8.2$ Hz), 8.02 (d, 1H, $J = 8$ Hz), 7.69–7.42 (m, 5H), 7.25 (d, 1H, $J = 8$ Hz), 7.15 (s, 1H), 7.05 (d, 2H, $J = 8.4$ Hz), 6.91 (d, 1H, $J = 8.2$ Hz), 6.90–6.78 (m, 4H), 6.54 (s, 1H), 4.01 (t, 2H, $J = 6$ Hz), 3.73 (s, 3H), 2.85 (t, 2H, $J = 6$ Hz), 2.62 (q, 4H, $J = 7$ Hz), 1.05 (t, 6H); MS: 490 (M^+-1); Anal. Calcd for HCl salt, $C_{34}H_{36}ClNO_2$: C, 77.62; H, 6.92; N, 2.66. Found: C, 77.17; H, 6.88; N, 2.66.

5.4.13. Diethyl-(2-{2-[(4-methoxy-phenyl)-phenanthren-9-yl-methyl]-phenoxy}-ethyl)-amine. White solid, **13b** (250 mg, 91%), mp 150 °C (HCl salt), IR (neat): 2942, 1241, 756 cm^{-1}, ^1H NMR ($CDCl_3$, 200 MHz): δ 8.70 (d, 1H, $J = 8.4$ Hz), 8.64 (d, 1H, $J = 8.4$ Hz), 8.03 (d, 1H, $J = 8.2$ Hz), 7.68–7.47 (m, 5H), 7.22 (d, 1H, $J = 6.8$ Hz), 7.16 (s, 1H), 7.08 (d, 2H, $J = 8.2$ Hz), 6.91 (d, 1H, $J = 8$ Hz), 6.83–6.78 (m, 4H), 6.54 (s, 1H), 3.95 (t, 2H, $J = 6$ Hz), 3.78 (s, 3H), 2.53 (t, 2H, $J = 6$ Hz), 2.36 (q, 4H), 0.8 (t, 6H), MS: 490 (M^+-Cl^-); Anal. Calcd for HCl salt, $C_{34}H_{36}ClNO_2$: C, 77.62; H, 6.92; N, 2.66. Found: C, 76.20; H, 6.80; N, 2.39.

5.4.14. 1-(2-{2-[(4-Methoxy-phenyl)-phenanthren-9-yl-methyl]-phenoxy}-ethyl)-piperidine. White solid, **13d** (450 mg, 65%), mp 110 °C (HCl salt), IR (neat): 2938, 1244, 754 cm^{-1}, ^1H NMR ($CDCl_3$, 200 MHz): δ 8.60 (d, 1H, $J = 8.2$ Hz), 8.56 (d, 1H, $J = 8.1$ Hz), 7.95 (d, 1H, $J = 8.2$ Hz), 7.60–7.40 (m, 5H), 7.17 (d, 1H, $J = 6.8$ Hz), 7.08 (s, 1H), 7.01 (d, 2H, $J = 8.6$ Hz), 6.84–6.71 (m, 5H), 6.45 (s, 1H), 3.96 (t, 2H, $J = 6$ Hz), 3.70 (s, 3H), 2.43 (t, 2H, $J = 6.2$ Hz), 2.09–2.08 (m, 4H), 1.21–1.18 (m, 6H), MS: 502 (M^+-Cl^-); Anal. Calcd for HCl salt, $C_{35}H_{36}ClNO_2$: C, 78.12; H, 6.74; N, 2.66. Found: C, 78.91; H, 6.99; N, 2.87.

5.4.15. 1-(2-{2-[(4-Methoxy-phenyl)-phenanthren-9-yl-methyl]-phenoxy}-ethyl)-pyrrolidine. White solid, **13c** (390 mg, 78%), mp 80 °C (HCl salt), IR (neat): 2938, 1244, 754 cm^{-1}, ^1H NMR ($CDCl_3$, 200 MHz): δ 8.67 (d, 1H, $J = 8.2$ Hz), 8.63 (d, 1H, $J = 8.0$ Hz), 8.00 (d, 1H, $J = 8.2$ Hz), 7.65–7.48 (m, 5H), 7.18 (d, 1H, $J = 6.8$ Hz), 7.05 (s, 1H), 7.02 (d, 2H, $J = 8.2$ Hz), 6.93–6.80 (m, 5H), 6.54 (s, 1H), 4.03 (t, 2H, $J = 6$ Hz), 3.78 (s, 3H), 2.63 (t, 2H, $J = 6$ Hz), 2.25 (m, 4H), 1.45 (m, 4H), MS: 488 (M^+-Cl^-); Anal. Calcd for HCl salt, $C_{34}H_{34}ClNO_2$: C, 77.92; H, 6.54; N, 2.67. Found: C, 77.98; H, 6.29; N, 2.41.

5.4.16. 1-(2-{2-[(4-Methoxy-phenyl)-phenanthren-9-yl-methyl]-phenoxy}-ethyl)-dimethylamine. White solid, **13a** (380 mg, 80%), mp 210 °C (HCl salt), IR (neat): 2938, 1244, 754 cm^{-1}, ^1H NMR ($CDCl_3$, 200 MHz): δ 8.67 (d, 1H, $J = 8.2$ Hz), 8.64 (d, 1H, $J = 8.0$ Hz), 8.01 (d, 1H, $J = 8.2$ Hz), 7.65–7.48 (m, 5H), 7.22 (d, 1H, $J = 6.9$ Hz), 7.15 (s, 1H), 7.08 (d, 2H, $J = 8.0$ Hz), 6.93–6.78 (m, 5H), 6.54 (s, 1H), 3.97 (t, 2H, $J = 6$ Hz), 3.78 (s, 3H), 2.39 (t, 2H, $J = 6$ Hz), 2.05 (s, 6H), MS: 462 (M^+-Cl^-); Anal. Calcd for HCl salt, $C_{32}H_{32}ClNO_2$: C, 77.17; H, 6.48; N, 2.81. Found: C, 76.90; H, 6.98; N, 2.76.

5.4.17. Bis-1-(2-{2-[(4-methoxy-phenyl)-phenanthren-9-yl-methyl]-phenoxy}-ethyl)-dimethylamine. White solid, **18a** (200 mg, 48%), mp 192 °C (HCl salt), IR (neat): 2930, 2862, 2822, 2773, 1461, 1245 cm^{-1}, ^1H NMR ($CDCl_3$, 200 MHz): δ 8.71 (d, 1H, $J = 8.4$ Hz), 8.65 (d, 1H, $J = 8.4$ Hz), 8.01 (d, 1H, $J = 8.2$ Hz), 7.70–7.46 (m, 5H), 7.14 (s, 1H), 7.04 (d, 4H, $J = 8.2$ Hz), 6.82 (d, 4H, $J = 8.2$ Hz), 6.13 (s, 1H), 4.03 (t, 4H, $J = 6.2$ Hz), 2.71 (t, 4H, $J = 6.2$ Hz), 2.34 (s, 12H), MS: 519 (M^+); Anal. Calcd for HCl salt, $C_{35}H_{40}Cl_2N_2O_2$: C, 71.06; H, 6.81; N, 4.74. Found: C, 71.66; H, 6.71; N, 4.99.

5.4.18. Bis-diethyl-(2-{2-[(4-methoxy-phenyl)-phenanthren-9-yl-methyl]-phenoxy}-ethyl)-amine. White solid, **18b** (215 mg, 47%), mp 220 °C (HCl salt), IR (neat): 2970, 2932, 2820, 1604, 1506, 1243, 1042, 758 cm^{-1}, ^1H NMR ($CDCl_3$, 200 MHz): δ 8.71 (d, 1H, $J = 8.2$ Hz), 8.65 (d, 1H, $J = 8.0$ Hz), 8.03 (d, 1H, $J = 8.2$ Hz), 7.70–7.46 (m, 5H), 7.14 (s, 1H), 7.04 (d, 4H, $J = 8.2$ Hz), 6.82 (d, 4H, $J = 8.2$ Hz), 6.13 (s, 1H), 4.01 (t, 4H, $J = 6.2$ Hz), 2.85 (t, 4H, $J = 6.2$ Hz), 2.61 (q, 8H, $J = 6.2$ Hz), 1.05 (t, 12H, $J = 6.2$ Hz), MS: 575 (M^+); Anal. Calcd for HCl salt, $C_{39}H_{48}Cl_2N_2O_2$: C, 72.32; H, 7.47; N, 4.32. Found: C, 72.91; H, 7.99; N, 4.65.

5.4.19. Bis-1-(2-{4-[(3-methoxy-phenyl)-phenanthren-9-yl-methyl]-phenoxy}-ethyl)-piperidine. White solid, **18c** (98 mg, 30%), mp 146 °C (HCl salt), IR (neat): 2929, 2853, 2783, 1505, 1242, 1036, 758 cm^{-1}, ^1H NMR ($CDCl_3$, 200 MHz): δ 8.71 (d, 1H, $J = 8.0$ Hz), 8.65 (d,

1H, $J = 8.2$ Hz), 8.03 (d, 1H, $J = 8.2$ Hz), 7.70–7.46 (m, 5H), 7.14 (s, 1H), 7.04 (d, 4H, $J = 8.2$ Hz), 6.82 (d, 4H, $J = 8.4$ Hz), 6.13 (s, 1H), 4.08 (t, 4H, $J = 6.2$ Hz), 2.76 (t, 4H, $J = 6.2$ Hz), 2.52–2.48 (m, 8H), 1.60–1.56 (m, 8H), 1.45–1.42 (m, 4H), MS: 600 (M^+); Anal. Calcd for HCl salt, $C_{41}H_{48}Cl_2N_2O_2$: C, 73.31; H, 7.20; N, 4.17. Found: C, 73.69; H, 7.98; N, 3.99.

5.5. Antimycobacterial activity

Agar Micro dilution method: Drug susceptibility and determination of MIC of the test compounds/drugs against *M. tuberculosis* $H_{37}R_v$ were performed by agar micro dilution method where serial twofold dilutions of each test compound were added into 7H10 agar and *M. tuberculosis* $H_{37}R_v$ was used as test organism. MIC is the concentration of the compound that completely inhibits the growth and colony forming ability of *M. tuberculosis*.

In 24 well plate, 3 mL middle brook 7H11 agar medium with OADC supplement is dispensed in each well. The test compound is added to the middle brook medium agar before in duplicate so that final concentration of test compound in each well is 25, 12.5, 6.25, 3.125 and 1.56 μg/mL, respectively. The known CFU of $H_{37}R_v$ culture was dispensed on top of agar in each well in negative pressure biosafety hood. The plates are then incubated at 37 °C/5% CO_2 incubator. The concentration at which complete inhibition of colonies was observed was taken as MIC of test drug.

BACTEC method: Stock solution of the test compounds prepared in DMSO at 1 mg/mL was sterilized by passage through 0.22 m filters. Fifty microliters were added to 4 mL radiometric 7H12 Broth (BACTEC 12B; Becton Dickinson Diagnostic Instrument System US) to achieve final concentrations. Controls received 50 μL DMSO. Isoniazid and rifampin (Sigma Chemical Co. St. Louis, MO) were included as positive drug control. In BACTEC method, 10^4 to 10^5 CFU/mL of *M. tuberculosis* $H_{37}R_v$ was inoculated in 4 mL fresh BACTEC 12B broth containing the test compounds. An additional control was inoculated with 1:100 dilution of the inoculum to represent 1% of the bacterial population (10^2 to 10^3 CFU mL). The vials were incubated at 37 °C and GI readings were recorded daily until the GI in 1:100 control had reached 30. The concentration of the drug producing final GI reading lower than those in 1:100 control was considered to have inhibited more than 90% of the bacteria and was defined as the MIC.

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References and notes

1. Dolin, P. J.; Raviglione, M. C.; Kochi, A. *Bull. WHO* **1994**, *72*, 213.
2. Daffe, M.; Draper, P. *Adv. Microb. Physiol.* **1998**, *39*, 131.
3. Barry, C. E., III; Mdluli, K. *Trends Microbiol.* **1996**, *4*, 275.
4. Brennan, P. J.; Nikaido, H. *Ann. Rev. Biochem.* **1995**, *64*, 29.
5. Young, D. B.; Duncan, K. *Ann. Rev. Microbiol.* **1995**, *49*, 641; Schaeffer, M. L.; Khoo, K. H.; Besra, G. S.; Chatterjee, D.; Brennan, P. J.; Belisle, J. T.; Inamine, J. M. *J. Biol. Chem.* **1999**, *274*, 31625; Collins, L.; Franzblau, S. G. *Antimicrob. Agents Chemother.* **1997**, *41*, 1004; Saita, H.; Tomioka, H.; Sato, K.; Yamani, T.; Yamashita, K.; Hosol, K.; Hidaka, T. *Antimicrob. Agents Chemother.* **1991**, *35*, 542.
6. Minnikin, D. E. In *The Biology of the Mycobacteria*; Ratedge, C., Stanford, J., Eds.; Academic: San Diego, 1982; p 95; Farmer, P.; Bayona, J.; Becerra, M.; Furin, J.; Henry, C.; Hiatt, H.; Kim, J. Y.; Mitnick, C.; Nardell, E.; Shin, S. *Int. J. Tuberc. Lung Dis.* **1998**, *2*, 869; Chopra, I.; Brennan, P. *Tubercle Lung Dis.* **1998**, *78*, 89.
7. Lee, R. E.; Smith, M. D.; Nash, R. J.; Griffiths, R. C.; McNeil, M.; Grewal, R. K.; Yan, W.; Besra, G. S.; Brennan, P. J.; Fleet, G. W. J. *Tetrahedron Lett.* **1997**, *38*, 6733; Mehta, G.; Panda, G. *Tetrahedron Lett.* **1997**, *38*, 2145.
8. C.A. no. 90: 152534y Wojtowicz, M.; Wienlawski, W. *Acta Pol. Pharm.* **1978**, *35*, 37; C.A. no. 89: 110253e Wojtowicz, M.; Wienlawski, W. *Acta Pol. Pharm.* **1977**, *34*, 575.
9. Reynolds, R. C.; Bansal, N.; Rose, J.; Friedrich, J.; Suling, W. J.; Maddry, J. A. *Carbohydr. Res.* **1999**, *317*, 164; Gonzalez, F.; Lesage, S.; Perlin, A. S. *Carbohydr. Res.* **1975**, *42*, 267.
10. Pathak, R.; Shaw, A. K.; Bhaduri, A. P.; Chandrasekhar, K. V. G.; Srivastava, A.; Srivastava, K. K.; Chaturvedi, V.; Srivastava, R.; Srivastava, B. S.; Arora, S.; Sinha, S. *Bioorg. Med. Chem.* **2002**, *10*, 1695; Hirata, N.; Yamagiwa, Y.; Kamikawa, T. J. *Chem. Soc. Erkin. Trans.* **1991**, *1*, 2279; Tripathi, R. P.; Tripathi, R.; Tiwari, V. K.; Bala, L.; Sinha, S.; Srivastava, B. S. *Eur. J. Med. Chem.* **2002**, *37*, 773.
11. deSouza, A. O.; Alderete, J. B.; Schimidt, F.; Sato, D. N.; Duran, N. *Drug Res.* **1999**, *49*(II), 1025–1029; De Conti, R.; Gimenez, S. M. N.; Haun, M. *Eur. J. Med. Chem.* **1996**, *31*, 1; Klopman, G.; Fercu, D.; Jacob, J. *J. Chem. Phys.* **1996**, *204*, 181; Karash, N.; Terzioglu, N.; Gursoy, A.; Arzneim, F. *Drug Res.* **1998**, *48*(II), 758.
12. Siddiqi, S. In *Clinical Microbiology Handbook*; ASM: Washington, DC, 1992; Vol. 1.
13. Venkatachalem, T. K.; Qazi, S.; Samuel, P.; Uckun, F. M. *Bioorg. Med. Chem.* **2003**, *11*(6), 1095–1105; Hussaini, I. M.; Zhang, Y. H.; Lysiak, J. J.; Shen, T. Y. *Acta Pharmacol. Sin.* **2000**, *21*(10), 897–904; Bamias, G.; Sugawara, K.; Pagnini, C.; Cominelli, F. *Curr. Opin. Investig. Drugs* **2003**, *4*(11), 1279–1286.
14. Senior, S. J.; Illarionov, P. A.; Gurcha, S. S.; Campbell, I. B.; Schaeffer, M. L.; Minnikin, D. E.; Besra, G. S. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3685–3688; Rugutt, J. K.; Ruggutt, K. J. *Nat. Prod. Lett.* **2002**, *16*(2), 107–113; Sikkema, J.; deBont, J. A. M.; Poolman, B. *Microbiol. Rev.* **1995**, *59*(2), 201–222; Kanaly, R. A.; Harayama, S. *J. Bacteriol.* **2000**, *182*(8), 2059–2067.