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Short communication

Effect of substituents on diarylmethanes for antitubercular activity☆

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Abstract

Aminoalkyl derivatives of diarylmethanes were prepared using Grignard, Friedel–Crafts arylation and aminohydrochloride chain formation reactions. These series of compounds were evaluated against *Mycobacterium tuberculosis* $H_{37}R_v$ and showed the activity in the range of 6.25–25 μg/mL. Effect of heteroaryl, anthracenyl and phenanthrene groups on diarylmethane pharmacophores for antitubercular activity is described.

Keywords: Antitubercular agents; Diarylmethanes; Phenanthrenes

Tuberculosis (TB) is a bacterial infection caused by *Mycobacterium tuberculosis* and is one of the major causes of worldwide death [1]. It has been estimated that approximately eight million people develop active disease and two million die of the disease every year. However, there has been resurgence in the incidence of TB since 1985 and in 1993 WHO declares TB as a global emergency [2]. The renaissance of TB is related to the lack of the suitable therapeutic agents, emergence of HIV AIDS epidemic and the development of drug resistant strain [3]. However, no new drug has been introduced in the market after the discovery of rifampicin [4].

Therefore there is an imperative need to develop novel antitubercular drugs for the management of tuberculosis, i.e. having new mechanism of action and also will be able to minimize the chances of MDR strains with shorter duration of therapy. In the recent years several new classes of compounds such as derivatives of biphenyl methanones [5], benzothiadiazine [6], oxazolidinones [7], azoles [8], benzylpurines[9], fluoroquinolones [10], benzo[*h*]chromene [11] and benzo[*a*]anthracenes [11] have been reported as antitubercular agents. Recently we have also developed a new type of antitubercular agents based on substituted diarylmethane scaffolds containing good amount of hydrophobicity. In this context, the synthesis and antitubercular activity of aminoalkyl and 2-hydroxy-aminoalkyl derivatives of phenanthrene substituted diarylmethanes have been reported [12–15]. Therefore as a consequence of above facts and in continuation of our previous work, we herein report the synthesis and antitubercular activity of aminoalkyl derivatives of diarylmethanes and substituted diarylmethanes.

Initially synthesis of aminoalkyl derivatives of unsubstituted diarylmethane derivatives was carried out. The reaction of Grignard reagent **3** derived from 4-bromoanisole **2** with 4-benzyloxybenzaldehyde **1** furnished carbinol **4** which was dehydrogenated under H_2, Pd/C conditions. It was interesting to find that hydroxyl functionality of **4** disappearing along with deprotection of benzyl group to furnish diarylmethane **5**. The reaction of **5** with two alkylamine hydrochloride chains in the presence of K_2CO_3 and acetone led to the formation of

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compounds **6a** and **6b** in 66% and 71% yields, respectively (Scheme 1). By treatment of amines **6a** and **6b** with ethanolic hydrogen chloride the corresponding salts 6**a**·HCl and **6b**·HCl were prepared (Scheme 1).

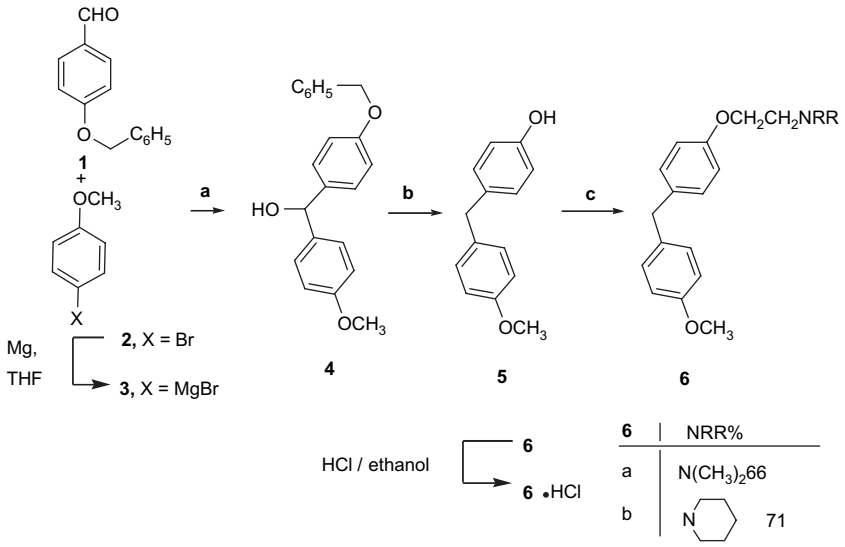
Further to synthesize aminoalkyl derivatives of substituted diarylmethanes, Grignard reagent **3** was treated with commercially available substituted heteroaryl or anthracenyl carbaldehydes to furnish carbinols **7**, **8**, **9** and **10** as enantiomeric mixtures after column chromatography. Subsequent Friedel–Crafts arylation of carbinols **7**, **8**, **9** and **10** with phenol in the presence of conc. H_2SO_4 or anhydrous $AlCl_3$ provided **11**, **12**, **13** and **14** by nucleophilic attack of phenol through *para* carbon atom of benzene ring. The reaction of **11**, **12**, **13** and **14** with 1-(2-chloroethyl)-alkylamine hydrochlorides in the presence of K_2CO_3 and acetone led to the formation of aminoalkyl derivatives **15a–e**, **16a–d**, **17** and **18a–d** in good yields, respectively (Scheme 2 and Table 1). The ethanolic HCl salts of **15a–e**, **16a–d, 17** and **18a–d** were found to be active against *M. tuberculosis* $H_{37}R_v$ in vitro with MIC in the range of 6.25, 12.5 and 25 μg/mL (Table 3). Further, by comparing the activity results of all diarylmethane derivatives i.e. pyridine, indole, anthracene and phenanthrene, it was observed that phenanthrene substituted diarylmethane derivatives are active pharmacophore and were selected for optimizing antitubercular activity, and thus further synthetic transformations were performed.

Since solution phase synthesis of phenolic derivatives was active with MIC ranging from 6.25 to 25 μg/mL against *M. tuberculosis* Tables 2 and 3), we wanted to explore the synthetic methodology over solid phase to obtain hydroxy substituted aminoalkyl derivatives. Towards this objective, Grignard reagent obtained from 9-bromo phenanthrene **19** was reacted with 4-benzyloxy-benzaldehyde to furnish the carbinol **20** (87 %) which on treatment with phenol and conc. H_2SO_4 gave **22** as major product and **21** as minor one. Reaction of **22** with TBDMSCl/imidazole in DCM gave silyl derivative **23** which on hydrogenation over Pd/C gave the phenolic derivative **24**. Compound **24** was loaded on 2-chlorotritylchloride resin (0.82 mmol/gm) in pyridine to furnish **25** and TBDMS group on **25** was cleaved on solid support through treatment with TBAF in THF to give **26** as phenolic derivative. Reaction between **26** with three alkylaminohydrochloride chains in presence of K_2CO_3 and DMF over resin furnished the compound **27** which after cleavage with 1% TFA in DCM gave the final hydroxy substituted phenolic derivatives **28a–c** in good yields and purity, Scheme 3.

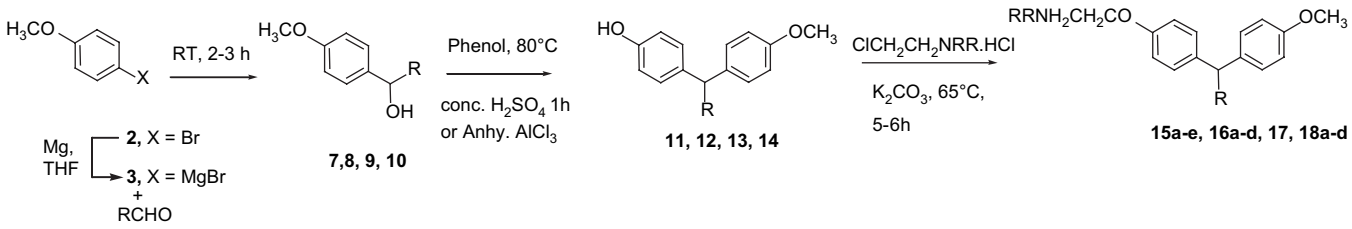
A closer look into the structure–activity relationship in these compounds shows that aminoalkyl derivatives of diarylmethane with indole **15a–e**, anthracenyl **17**, phenanthrenyl rings **18a–d** and **28a–c** showed the antitubercular activity with MIC ranging from 6.25 to 25 μg/mL. However, aminoalkyl derivatives of diarylmethane **6a–b** and pyridine substituted diarylmethanes **16a–d** exhibited MIC higher than 25 μg/mL. It is interesting to note that increasing the bulkiness of the substituents on diarylmethane resulted in better activity. Compound **6a–b** without substituent are having lower order of activity whereas the activity increases in case of **15a–e**, **17**, **18a–d** and **28a–c** with indole, anthracene and phenanthrene rings as substituents. It is noteworthy that activity was partially dependent on the alkyl moiety on nitrogen atom. Compounds like **15a** having dimethylaminoalkyl chain is having MIC > 25 (MABA method) but **15b**, **15c**, and **15e** having diethylamine, pyrrolidine and azepane ring are having MIC 12.5 whereas **15d** having piperidine ring has MIC 6.25 μg/mL. Thus, compounds having medium size aminoalkyl moiety gave better antitubercular activity.

Cytotoxicity of **15d**, **18b–d** in VERO cell line as well as mouse bone marrow macrophages at different concentrations beginning from 10× MIC of the compounds was assayed and is expressed as inhibitory concentration (IC_{50}) [18]. On the basis of IC_{50} values, selectivity index (SI) of **18b** and **18c** was found to be 20, indicating that these compounds can be identified as useful leads (Table 4).

All the compounds synthesized (**6a–b**, **15a–e**, **16a–d**, **17**, **18a–d** and **28a–c)** were evaluated for their antitubercular



Scheme 1. Reagents and conditions: (a) Mg, THF, RT, 2 h, 73%; (b) Pd/C, H_2, RT, 1 h, 95%; (c) $ClCH_2CH_2NRR·HCl$, K_2CO_3, acetone, 66–71%.



Scheme 2.

activity against *M. tuberculosis* $H_{37}R_v$ through Micro alamar blue assay (MABA) [16], agar microdilution technique [17] and standard BACTEC radiometric growth assay [17] and their results are shown below (Table 3).

In conclusion we have reported the synthesis of the aminoalkyl derivatives of diarylmethane and substituted diarylmethanes through Grignard, Friedel–Crafts arylation and aminohydrochloride chain formation reactions and these compounds were active in the range of 6.25–25 μg/mL against *M. tuberculosis* $H_{37}R_v$. Indole and phenanthrene substituted diarylmethane derivatives showed promising activity. All these results suggest that it will be interesting to prepare the analogs of active molecules for finding new compounds that possess better activity and bioavailability.

1. Selected spectral data

*1.1. (4-Benzyloxy-phenyl)-(4-methoxy-phenyl)-methanol **4***

To a solution of 4-bromoanisole **2** (8.2 mL, 70.74 mmol) in dry THF (30 mL) was added activated magnesium (1.93 g, 80.2 mmol) and was stirred at room temperature under dry nitrogen for 2 h. To Grignard reagent thus formed was added 4-benzyloxybenzaldehyde **1** (5 g, 23.58 mmol) in THF (10 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 sulphate. It was concentrated and charged over silica gel. Elution with 10% ethyl acetate in hexane furnished carbinol product **4** (5.5 g, 73.3%) as white solid, m.p. 93 °C. IR (KBr): 3402, 1592, 1585, 1365, 1145, 780 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ 7.43–7.24 (m, 9H), 6.94–6.83 (m, 4H), 5.75 (s, 1H), 5.03 (s, 2H), 3.77 (s, 3H), 3.11 (bs, 1H). MS: 320 (M^+). Anal. Calcd for ($C_{21}H_{20}O_3$): C, 78.73; H, 6.29. Found: C, 78.79; H, 6.35.

*1.2. 4-(4-Methoxy-benzyl)-phenol **5***

The compound **4** (0.5 g, 0.84 mmol) was hydrogenated over 10% Pd/C (0.05 g) and after usual work-up and purification furnished **5** (0.32 g, 95.8%) as white semi-solid. IR (neat): 3390, 1510, 1258, 759 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ 7.03–6.93 (m, 4H), 6.78–6.64 (m, 4H), 3.80 (s, 2H), 3.72 (s, 3H). MS: 214 (M^+). Anal. Calcd for ($C_{14}H_{14}O_2$): C, 78.48; H, 6.59. Found C, 78.42; H, 6.45.

*1.3. {2-[4-(4-Methoxy-benzyl)-phenoxy]-ethyl}-dimethylamine **6a***

A mixture of compound **5** (0.22 g, 1.028 mmol), anhydrous K_2CO_3 (0.71 g, 5.14 mmol), 1-(2-chloroethyl)-dimethylamine hydrochloride (0.284 g, 1.54 mmol) and dry acetone (20 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 basic alumina and elution with 35% ethyl acetate in hexane furnished compound **6a** (0.2 g, 66.6%) as yellow semi-solid. IR (neat): 2928, 1501, 1256, 759 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ 7.10–7.04 (m, 4H), 6.85–6.79 (m, 4H), 4.05 (t, 2H, $J = 7$ Hz), 3.85 (s, 2H), 3.77 (s, 3H), 2.74 (t, 2H, $J = 7$ Hz), 2.34 (s, 6H). MS: 286 ($M^+ + H$). Anal. Calcd for ($C_{18}H_{23}NO_2$): C, 75.76; H, 8.12; N, 4.91. Found: C, 75.72; H, 8.18; N, 4.95.

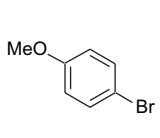
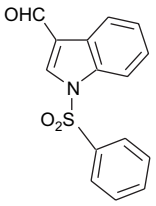
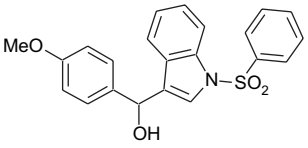
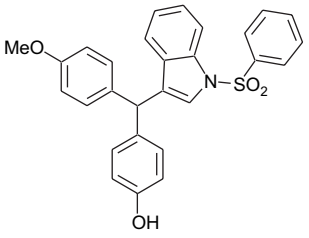
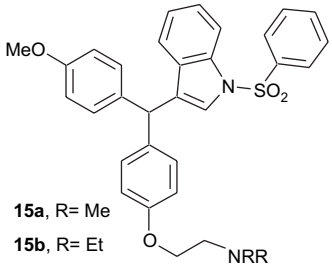
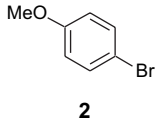
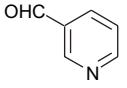
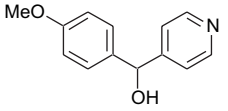
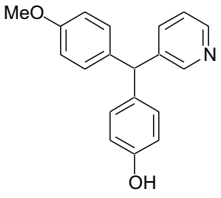
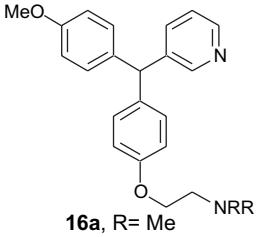
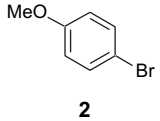
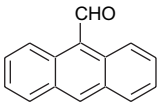
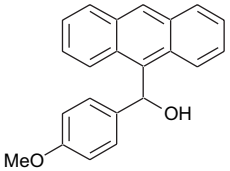
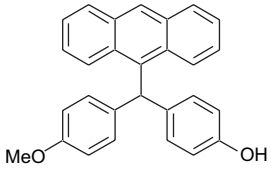
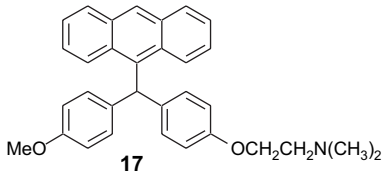
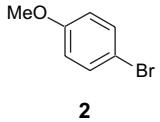
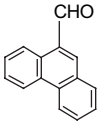
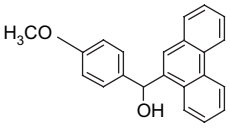
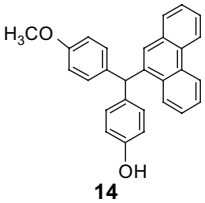
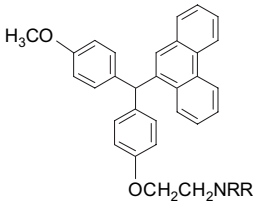
*1.4. 1-{2-[4-(4-Methoxy-benzyl)-phenoxy]-ethyl}-piperidine **6b***

As described for **6a**, compound **5** (0.2 g, 1.02 mmol), K_2CO_3 (0.71 g, 5.14 mmol) and 1-(2-chloroethyl)-piperidine hydrochloride (0.22 g, 1.54 mmol) furnished **6b** (0.19 g, 71.4%) as light yellow semi-solid. IR (neat): 3440, 1635, 1245, 770 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ 7.10–7.04 (m, 4H), 6.85–6.79 (m, 4H), 4.07 (t, 2H, $J = 7$ Hz), 3.85 (s, 2H), 3.79 (s, 3H), 2.75 (t, 2H, $J = 7$ Hz), 2.52–2.47 (m, 4H), 1.62–1.55 (m, 4H), 1.45–1.43 (m, 2H). MS: 326 ($M^+ + H$). Anal. Calcd for ($C_{21}H_{27}NO_2$): C, 77.50; H, 8.36; N, 4.30. Found: C, 77.59; H, 8.35; N, 4.35.

*1.5. (1-Benzenesulfonyl-1H-indol-3-yl)-(4-methoxy-phenyl)-methanol **7***

As described for **4**, compound **2** (1.31 mL, 10.52 mmol) in dry THF (25 mL), magnesium (0.31 g, 12.842 mmol) and 1-benzenesulfonyl-1*H*-indole-3-carbaldehyde (2.0 g, 7.01 mmol) in THF (5 mL) furnished **7** (1.98 g, 72%) as brown semi-solid. IR (neat): 3402, 2927, 1607, 1508, 1247, 1175, 1032, 767 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ 7.94–7.84 (m, 3H), 7.47–7.25 (m, 9H), 6.85 (m, 2H), 5.95 (s, 1H), 3.79 (s, 3H). MS: 393 (M^+).

Table 1
Synthesis of aminoalkyl derivatives of substituted diarylmethane derivatives **15a–e**, **16a–d**, **17** and **18a–d** from carbinols **7**, **8**, **9** and **10**, respectively

Entry	Bromo compound	RCHO	Carbinol	Alkylated product	Alkylated product with chain
1	 **2**		 **7**	 **11**	 **15a**, R= Me **15b**, R= Et **15c**, R-R= $(CH_2)_4$ **15d**, R-R= $(CH_2)_5$ **15e**, R-R= $(CH_2)_6$
2	 **2**		 **8**	 **12**	 **16a**, R= Me **16b**, R= Et **16c**, R-R= $(CH_2)_4$ **16d**, R-R= $(CH_2)_5$
3	 **2**		 **9**	 **13**	 **17**
4	 **2**		 **10**	 **14**	 **18a**, R=Me **18b**, R= Et **18c**, R-R= $(CH_2)_4$ **18d**, R-R= $(CH_2)_5$

*1.6. (4-Methoxy-phenyl)-pyridin-3yl-methanol **8***

As described for **4**, compound **2** (3.50 mL, 28.00 mmol) in dry THF (30 mL), magnesium (0.896 g, 37.34 mmol) and pyridine 3-carboxaldehyde (1.75 mL, 18.67 mmol) in THF (5 mL) furnished **8** (1.8 g, 45%) as white solid, m.p. 70 °C. IR (KBr): 3402, 1592, 1585, 1365, 1145, 780 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ 8.43 (d, 1H, $J = 1.6$ Hz), 8.29 (dd, 1H, $J_1 = 1.3$ Hz, $J_2 = 4.8$ Hz), 7.66 (d, 1H, $J = 7.8$ Hz), 7.26–7.15 (m, 3H), 6.84 (d, 2H, $J = 8.6$ Hz), 5.74 (s, 1H),

Table 2
Solid phase synthesis of aminoalkyl derivatives of diaryloxy methanophenanthrenes

Compound	Alkyl chain	Yield (%)
28a	$ClCH_2CH_2N(CH_3)_2 \cdot HCl$	70
28b	$ClCH_2CH_2N(CH_2CH_3)_2 \cdot HCl$	72
28c	$ClCH_2CH_2N(CH_2)_5 \cdot HCl$	76

3.76 (s, 3H). MS: 215 (M^+). Anal. Calcd for ($C_{13}H_{13}NO_2$): C, 72.54; H, 6.09; N, 6.51. Found: C, 72.59; H, 6.05; N, 6.84.

*1.7. Anthracen-9-yl-(4-methoxy-phenyl)-methanol **9***

As described for **4**, compound **2** (16.15 g, 1.86 mmol) in dry THF (20 mL), magnesium (2.06 g, 8.6 mmol) and anthracene-9-carbaldehyde (5.94 g, 2.8 mmol) in THF (25 mL) furnished **9** (6.0 g, 66%) as yellow semi-solid. IR (neat): 3510, 2362, 1604, 1507, 1242, 1169, 732 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 8.46 (s, 1H), 8.36 (d, 2H, $J = 9$ Hz), 8.03 (d, 1H, $J = 7.8$ Hz), 8.01 (d, 1H, $J = 9$ Hz), 7.47–7.34 (m, 6H), 7.27 (d, 1H, $J = 9$ Hz), 6.79 (d, 2H, $J = 10$ Hz), 3.74 (s, 3H), 2.64 (d, 1H, $J = 5.4$ Hz). MS: 314 (M^+). Anal. Calcd for ($C_{22}H_{18}O_2$): C, 89.05; H, 5.77. Found: C, 89.12; H, 5.64.

*1.8. 4-[(1-Benzenesulfonyl-1H-indol-3-yl)-(4-methoxy-phenyl)-methyl]-phenol **11***

To a solution of carbinol **7** (1.77 g, 4.51 mmol) and phenol (0.52 mL, 6.32 mmol) in dry benzene (20 mL) was added aluminium trichloride (0.601 g, 4.51 mmol) and the mixture was heated at 80 °C for 1 h. After cooling, the reaction mixture was neutralized with saturated aq. $NaHCO_3$ and extracted with ethyl acetate. The concentrated extract was subjected to column chromatography on silica gel and elution with 15% ethyl acetate in hexane furnished **11** (1.31 g, 62%) as brown viscous oil. IR (neat): 3431, 2368, 1712, 1591, 1510, 1170, 1105, 1025, 769 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ 7.95 (d, 1H, $J = 8.2$ Hz), 7.77 (d, 2H, $J = 8.6$), 7.29–7.21 (m, 3H), 6.99–6.72 (m, 12H), 5.39 (s, 1H), 4.95 (bs, 1H), 3.79 (s, 3H). MS: 469 (M^+).

Table 3
In vitro antituberculosis activity of **6a–b**, **15a–e**, **16a–d**, **17**, **18a–d** and **28a–c** against *M. tuberculosis* $H_{37}R_v$

Compound	MIC (μg/mL)		
	MABA	Microdilution	BACTEC
6a	>25	>25	ND
6b	>25	>25	ND
15a	25	>12.5	ND
15b	12.5	12.5	ND
15c	12.5	12.5	ND
15d	6.25	6.25	6.25
15e	12.5	12.5	12.5
16a	>25	>25	ND
16b	>25	>25	12.5
16c	>25	>25	12.5
16d	>25	>25	12.5
17	12.5	12.5	ND
18a	25	25.0	12.5
18b	6.25	6.25	6.25
18c	6.25	6.25	6.25
18d	6.25	6.25	6.25
28a	25	12.5	12.5
28b	25	12.5	12.5
28c	25	12.5	12.5

ND means not done in that particular test system.

*1.9. 4-[(4-Methoxy-phenyl)-pyridin-3-yl-methyl]-phenol **12***

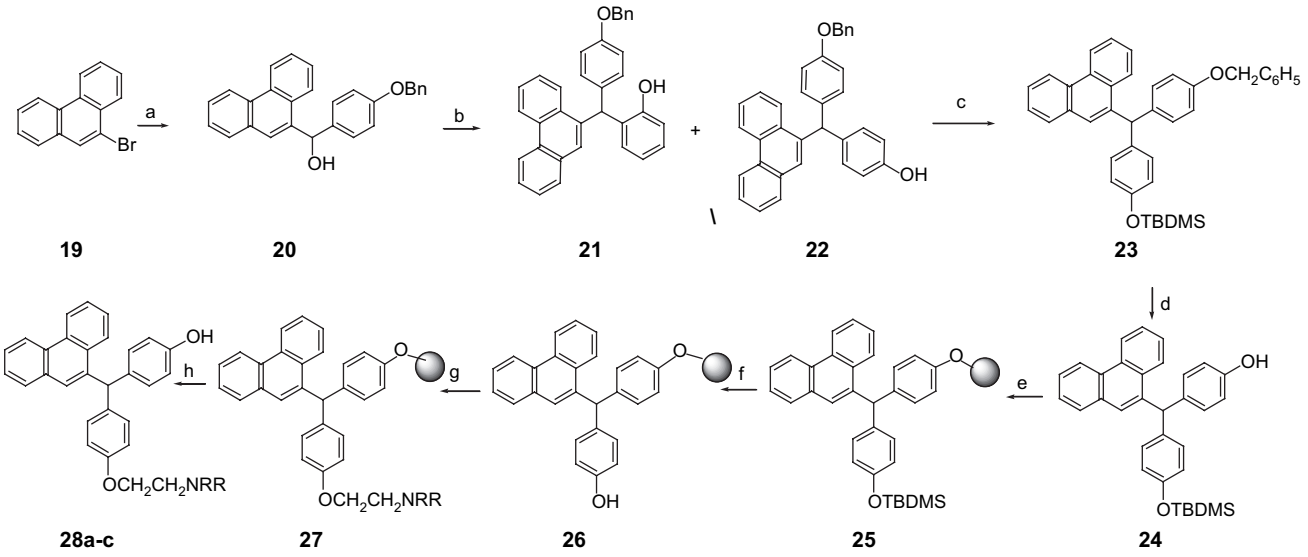
To a solution of carbinol **8** (2.6 g, 12.09 mmol) and phenol (1.59 g, 16.93 mmol) in dry benzene (30 mL) was added aluminium trichloride (1.61 g, 12.09 mmol) and then the mixture was heated at 80 °C for 5 h. After cooling, the reaction mixture was neutralized with saturated aq. $NaHCO_3$ and extracted with ethyl acetate. The concentrated extract was subjected to column chromatography on silica gel and elution with 40% ethyl acetate in hexane furnished **12** (1.8 g, 51.3%) as dark yellow viscous oil. IR (neat): 3431, 1605, 1507, 1443, 1245, 1172, 1028 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 8.43–8.37 (m, 2H), 7.44–7.39 (m, 1H), 7.27–7.24 (m, 1H), 7.00 (d, 2H, $J = 8.6$ Hz), 6.89–6.73 (m, 6H), 5.41 (s, 1H), 3.76 (s, 3H). MS: 292 ($M^+ + H$). Anal. Calcd for ($C_{19}H_{17}NO_2$): C, 78.33; H, 5.88; N, 4.81. Found: C, 78.56; H, 4.96; N, 4.75.

*1.10. 4-[9-Anthryl (4-methoxy-phenyl)methyl]phenol **13***

As described for **11**, carbinol **9** (2.85 g, 9.07 mmol) and phenol (1.28 g, 13.61 mmol) in dry benzene (40 mL) furnished **13** (0.3 g, 15%) as a brown solid, m.p. 78 °C. IR (KBr): 3431, 1605, 1507, 1443, 1245, 1172, 1028 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 8.44 (s, 1H), 8.14 (d, 2H, $J = 9$ Hz), 8.00 (d, 2H, $J = 8.5$ Hz), 7.45–7.20 (m, 4H), 7.14–6.90 (m, 4H), 6.97 (s, 1H), 6.77 (d, 2H, $J = 8$ Hz), 6.69 (d, 2H, $J = 8$ Hz), 3.75 (s, 3H). MS: 390 (M^+). Anal. Calcd for ($C_{28}H_{22}O_2$): C, 86.13; H, 5.68. Found: C, 86.31; H, 5.78.

*1.11. (2-{4-[(1-Benzenesulfonyl-1H-indol-3-yl)-(4-methoxy-phenyl)-methyl]-phenoxy}-ethyl)-dimethylamine **15a***

As described for **6a**, compound **11** (0.175 g, 0.373 mmol), K_2CO_3 (0.257 g, 1.86 mmol), 1-(2-chloroethyl)-dimethylamine hydrochloride (0.064 g, 0.447 mmol) furnished **15a** (0.16 g, 81%) as orange viscous liquid. IR (neat): 3394, 2361, 1592, 1463, 1242, 1033, 768 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ 7.96 (s, 1H), 7.28–6.71 (m 16H), 6.46 (s, 1H), 5.48 (s, 1H), 3.96 (t, 2H, $J = 5.2$ Hz), 3.70 (s, 3H), 2.65 (t, 2H, $J = 5.2$ Hz), 2.26 (s, 6H). MS: 540 (M^+).



Scheme 3. Reagents and conditions: (a) Mg, 4-benzyloxybenzaldehyde, THF, RT, 2 h, 87%; (b) phenol, cat. H_2SO_4, benzene, 1 h, 52% (*ortho*/*para* = 3:7); (c) TBDMSCl, imidazole, DCM, 3–5 h, 87%; (d) 5% Pd/C, H_2, ethyl acetate, 1–2 h, 87%; (e) tritylchloride resin, pyridine, 50 °C, 8 h, 98%; (f) TBAF, THF, 0.5–1 h, 90%; (g) $ClCH_2CH_2NRR \cdot HCl$, K_2CO_3, dry DMF, 48 h, then 1% TFA, DCM, 70–76%.

1.12. (2-{4-[(1-Benzenesulfonyl-1H-indol-3-yl)-(4-methoxy-phenyl)-methyl]-phenoxy}-ethyl)-diethylamine ***15b***

As described for **6a**, compound **11** (0.175 g, 0.373 mmol), K_2CO_3 (0.257 g, 1.86 mmol) and 1-(2-chloroethyl)-diethylamine hydrochloride (0.0765 g, 0.44 mmol) furnished **15b** (0.165 g, 78%) as yellow viscous oil. IR (neat): 3413, 2926, 2360, 1607, 1507, 1245, 1033, 768 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ 7.96 (d, 1H, $J = 8.2$ Hz), 7.75 (d, 2H, $J = 8.5$ Hz), 7.55–7.32 (m, 3H), 7.18–6.71 (m, 12H), 5.42 (s, 1H), 3.97 (t, 2H, $J = 5.9$ Hz), 3.70 (s, 3H), 2.79 (t, 2H, $J = 5.9$ Hz), 2.58–2.55 (m, 4H), 0.99 (t, 4H, $J = 7$ Hz). MS: 569 ($M^+ + H$).

1.13. 1-Benzenesulfonyl-3-{(4-methoxy-phenyl)-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-methyl}-1H-indole ***15c***

As described for **6a**, compound **11** (0.175 g, 0.373 mmol), K_2CO_3 (0.257 g, 1.86 mmol) and 1-(2-chloroethyl)-pyrrolidineamine hydrochloride (0.075 g, 0.447 mmol) furnished **15c** (0.140 g, 67%) as brown viscous liquid. IR (neat): 3413, 2926, 2360, 1607, 1507, 1245, 1033, 768 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ 7.96 (d, 1H, $J = 8.2$ Hz), 7.77 (d, 2H, $J = 8.5$ Hz), 7.65–7.31 (m, 3H), 7.09–6.08 (m, 12H), 5.39 (s, 1H), 4.08 (t, 2H, $J = 6.0$ Hz), 3.78 (s, 3H), 2.89 (t, 2H, $J = 5.9$ Hz), 2.61–2.45 (m, 4H), 1.92–1.81 (m, 4H). MS: 567 ($M^+ + H$).

Table 4
The cytotoxicity values and corresponding selectivity index (SI) of selected compounds

Compound	Cytotoxicity value (IC_{50})	Selectivity index
15d	62	10
18b	125	20
18c	125	20
18d	62	10

1.14. 1-Benzenesulfonyl-3-{(4-methoxy-phenyl)-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-methyl}-1H-indole ***15d***

As described for **6a**, compound **11** (0.175 g, 0.373 mmol), K_2CO_3 (0.257 g, 1.86 mmol) and 1-(2-chloroethyl)-piperidine hydrochloride (0.088 g, 0.44 mmol) furnished **15d** (0.15 g, 71%) as yellow viscous liquid. IR (neat): 3431, 2933, 2363, 1603, 1509, 1371, 1248, 1177, 753, 580 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ 7.89 (d, 1H, $J = 8.2$ Hz), 7.71 (d, 2H, $J = 8.5$ Hz), 7.38–7.35 (m, 3H), 7.01–6.71 (m, 12H), 5.32 (s, 1H), 4.00 (t, 2H, $J = 6.1$ Hz), 3.71 (s, 3H), 2.69 (t, 2H, $J = 6.1$ Hz), 2.45–2.40 (m, 4H), 1.55–1.48 (m, 4H), 1.21–1.14 (m, 2H). MS: 581 ($M^+ + H$).

1.15. 3-[[4-(2-Azepan-1-yl-ethoxy)-phenyl]-(4-methoxy-phenyl)-methyl]-1-benzenesulfonyl-1H-indole ***15e***

As described for **6a**, compound **11** (0.175 g, 0.373 mmol), K_2CO_3 (0.257 g, 1.869 mmol) and 1-(2-Chloro-ethyl)-azepane (0.088 g, 0.44 mmol) furnished **15e** (0.151 g, 69%) as brown viscous liquid. IR (neat): 3432, 2928, 2362, 1596, 1352, 766 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ 7.97 (d, 1H, $J = 8.2$ Hz), 7.79 (d, 2H, $J = 8.5$ Hz), 7.59–7.41 (m, 3H), 7.11–6.81 (m, 12H), 5.41 (s, 1H), 4.06 (t, 2H, $J = 6$ Hz), 3.80 (s, 3H), 2.96 (t, 2H, $J = 6$ Hz), 2.82–2.79 (m, 4H), 1.69–1.61 (m, 8H). MS: 595 ($M^+ + H$).

*1.16. (2-{4-[(4-Methoxy-phenyl)-pyridin-3-yl-methyl]-phenoxy}-ethyl)-dimethylamine **16a***

As described for **6a**, compound **12** (0.2 g, 0.687 mmol), K_2CO_3 (0.474 g, 3.43 mmol) and 1-(2-chloroethyl)-dimethylamine hydrochloride (0.149 g, 1.03 mmol) furnished **16a** as yellow viscous oil (0.185 g, 74.59%). IR (neat): 3440, 1635, 1245, 770 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ 8.33–8.30 (m, 2H), 7.25 (d, 1H, $J = 7.8$ Hz), 7.08–7.04 (m, 1H), 6.90–6.68 (m, 8H), 5.32 (s, 1H), 3.91 (t, 2H, $J = 6$ Hz), 3.63 (s, 3H), 2.59 (t, 2H, $J = 6$ Hz), 2.20 (s, 6H). MS: 363 ($M^+ + H$). Anal. Calcd for ($C_{23}H_{26}N_2O_2$): C, 76.21; H, 7.23; N, 7.73. Found C, 76.35; H, 7.34; N, 7.85.

*1.17. Diethyl-(2-{4-[(4-methoxy-phenyl)-pyridin-3-yl-methyl]-phenoxy}-ethyl)-amine **16b***

As described for **6a**, compound **12** (0.2 g, 0.687 mmol), K_2CO_3 (0.474 g, 3.43 mmol) and 1-(2-chloroethyl)-diethylamine hydrochloride (0.176 g, 1.03 mmol) furnished **16b** as yellow viscous oil (0.170 g, 65.38%). IR (neat): 3440, 1635, 1245, 770 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ 8.37–8.33 (m, 2H), 7.29 (d, 1H, $J = 7.7$ Hz), 7.18–6.72 (m, 9H), 5.35 (s, 1H), 3.97 (t, 2H, $J = 6$ Hz), 3.68 (s, 3H), 2.78 (t, 2H, $J = 6$ Hz), 2.55 (q, 4H, $J = 7.1$ Hz), 1.00 (t, 6H, $J = 7.2$ Hz). MS: 391 ($M^+ + H$). Anal. Calcd for ($C_{25}H_{30}N_2O_2$): C, 76.89; H, 7.74; N, 7.17. Found C, 76.75; H, 7.64; N, 7.29.

*1.18. 3-{(4-Methoxy-phenyl)-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-methyl}-pyridine **16c***

As described for **6a**, compound **12** (0.2 g, 0.687 mmol), K_2CO_3 (0.474 g, 3.43 mmol) and 1-(2-chloroethyl)-pyrrolidine hydrochloride (0.174 g, 1.03 mmol) furnished **16c** as yellow viscous oil (0.165 g, 62.26%). IR (neat): 3440, 1635, 1245, 770 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ 8.32–8.30 (m, 2H), 7.27 (d, 1H, $J = 7.8$ Hz), 7.07–6.67 (m, 9H), 5.31 (s, 1H), 3.95 (t, 2H, $J = 6$ Hz), 3.61 (s, 3H), 2.75 (t, 2H, $J = 6$ Hz), 2.49–2.47 (m, 4H), 1.65–1.58 (m, 4H). MS: 389 ($M^+ + H$). Anal. Calcd for ($C_{25}H_{28}N_2O_2$): C, 77.29; H, 7.26; N, 7.21. Found C, 77.35; H, 7.34; N, 7.09.

*1.19. 3-{(4-Methoxy-phenyl)-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-methyl}-pyridine **16d***

As described for **6a**, compound **12** (0.2 g, 0.687 mmol), K_2CO_3 (0.474 g, 3.43 mmol) and 1-(2-chloroethyl)-piperidine hydrochloride (0.19 g, 1.03 mmol) furnished **16d** as yellow viscous oil (0.18 g, 65.2%). IR (neat): 3440, 1635, 1245, 770 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ 8.36–8.31 (m, 2H), 7.29 (d, 1H, $J = 7.8$ Hz), 7.19–6.72 (m, 9H), 5.35 (s, 1H), 4.02 (t, 2H, $J = 6$ Hz), 3.67 (s, 3H), 2.71 (t, 2H, $J = 6$ Hz), 2.48–2.43 (m, 4H), 1.56–1.49 (m, 4H), 1.37–1.39 (m, 2H). MS: 403 ($M^+ + H$). Anal. Calcd for ($C_{26}H_{30}N_2O_2$): C, 77.58; H, 7.51; N, 6.96. Found C, 77.65; H, 7.60; N, 6.85.

*1.20. (2-{4-[Anthracen-9-yl-(4-methoxy-phenyl)-methyl]-phenoxy}-ethyl)-dimethylamine **17***

As described for **6a**, compound **13** (0.25 g, 0.641 mmol), K_2CO_3 (0.443 g, 3.2 mmol) and 1-(2-chloroethyl)-dimethylamine hydrochloride (0.138 g, 0.961 mmol) furnished **17** (0.2 g, 67%) as brown semi-solid. IR (neat): 3437, 3019, 2930, 1635, 1510, 1216, 761 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ 8.26 (d, 1H, $J = 8$ Hz), 8.21–7.90 (m, 2H), 7.73 (d, 1H, $J = 8$ Hz), 7.15–6.62 (m, 13H), 5.00 (s, 1H), 4.24 (t, 2H, $J = 6.2$ Hz), 3.77 (s, 3H), 2.84 (t, 2H, $J = 6.2$ Hz), 2.39 (s, 6H). MS: 462 ($M^+ + H$). Anal. Calcd for ($C_{32}H_{31}NO_2$): C, 83.26; H, 6.77; N, 3.03. Found: C, 83.29; H, 6.85; N, 2.95.

*1.21. (4-Benzyloxy-phenyl)-phenanthren-9-yl-methanol **20***

As described for **4**, compound **19** (2 g, 7.78 mmol) in dry THF (30 mL), magnesium (0.205 g, 8.55 mmol) and **1** (0.825 g, 3.89 mmol) in THF (5 mL) furnished **20** (1.32 g, 87%) as yellow semi-solid. IR (neat): 3452, 1597, 1582, 1367, 1148, 785 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ 8.71–8.69 (m, 2H), 8.01–7.89 (m, 3H), 7.65–7.49 (m, 4H), 7.38–7.25 (m, 7H), 6.93 (d, $J = 8.5$ Hz, 2H), 6.50 (s, 1H), 5.02 (s, 2H), 2.31 (bs, 1H). MS: 390 (M^+). Anal. Calcd for ($C_{28}H_{22}O_2$): C, 86.13; H, 5.68. Found C, 86.54; H, 6.73.

*1.22. 2-[(4-Benzyloxy-phenyl)-phenanthren-9-yl-methyl]-phenol **21***

As described for **11**, carbinol **20** (2.0 g, 5.01 mmol) and phenol (0.63 g, 7.6 mmol) in dry benzene (30 mL) furnished **21** (0.360 mg, 30%) as dark orange solid, m.p. 90 °C. IR (KBr): 2926, 1628, 1453, 1259, 1051, 785 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ 8.66 (d, 1H, $J = 8.2$ Hz), 8.62 (d, 1H, $J = 8$ Hz), 7.99 (d, 1H, $J = 8$ Hz), 7.57–7.37 (m, 10H), 7.16 (d, 1H, $J = 7.8$ Hz), 7.14–7.04 (m, 3H), 7.14 (d, 2H, $J = 8.4$ Hz), 7.00 (s, 1H), 6.96 (d, 2H, $J = 8.4$ Hz), 6.37 (s, 1H), 4.98 (s, 2H), 4.87 (bs, 1H). MS: 466 (M^+). Anal. Calcd for ($C_{34}H_{26}O_2$): C, 87.52; H, 5.62. Found: C, 87.25; H, 5.73.

*1.23. 4-[(4-Benzyloxy-phenyl)-phenanthren-9-yl-methyl]-phenol **22***

As described for **11**, carbinol **20** (2.0 g, 5.01 mmol) and phenol (0.63 g, 7.6 mmol) in dry benzene (30 mL) furnished **22** (0.849 g, 70%) as dark orange solid, m.p. 85 °C. IR (KBr): 2932, 1621, 1443, 1252, 1041, 790 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ 8.70 (d, 1H, $J = 8.2$ Hz), 8.64 (d, 1H, $J = 8$ Hz), 8.01 (d, 1H, $J = 8$ Hz), 7.58–7.32 (m, 10H), 7.22 (d, 1H, $J = 7.8$ Hz), 7.15–7.06 (m, 3H), 7.07 (s, 1H), 6.88 (d, 2H, $J = 8.4$ Hz), 6.73 (d, 2H, $J = 8.4$ Hz), 6.12 (s, 1H), 5.01 (s, 2H). MS: 466 (M^+). Anal. Calcd for ($C_{34}H_{26}O_2$): C, 87.52; H, 5.62. Found: C, 87.59; H, 6.70.

*1.24. {4-[(4-Benzyloxy-phenyl)-phenanthren-9-yl-methyl]-phenoxy}-tert-butyl-dimethyl-silane **23***

To a solution of **22** (0.500 g, 1.07 mmol), and imidazole (0.109 g, 1.60 mmol) in dry DCM (10 mL), TBDMSCl (0.241 g, 1.60 mmol) was added and stirred at room temperature for 8 h. After extraction and column chromatography on silica gel, **22** furnished the silyl derivative **23** (0.540 g, 87%) as white semi-solid. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 8.60 (d, 1H, $J = 8.2$ Hz), 8.57 (d, 1H, $J = 8.2$ Hz), 7.88 (d, 1H, $J = 8$ Hz), 7.45–7.15 (m, 8H), 7.06 (s, 1H), 6.92 (d, 2H, $J = 8.4$ Hz), 6.88 (d, 2H, $J = 8.2$ Hz), 6.81 (d, 2H, $J = 8.1$ Hz), 6.71 (d, 2H, $J = 8.6$ Hz), 6.57 (d, 2H, $J = 8.4$ Hz), 5.95 (s, 1H), 4.83 (s, 2H), 0.78 (s, 9H), 0.09 (s, 6H). MS: 580 (M^+). Anal. Calcd for ($C_{40}H_{40}O_2Si$): C, 82.71; H, 6.94. Found: C, 83.00; H, 6.83.

*1.25. 4-{[4-(tert-Butyl-dimethyl-silanyloxy)-phenyl]-phenanthren-9-yl-methyl}-phenol **24***

The silyl derivative **23** (0.490 g, 0.84 mmol) was hydrogenated over Pd/C (0.015 g) and after usual work-up and purification furnished **24** (0.360 g, 87%) as pale yellow semi-solid. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 8.61 (d, 1H, $J = 8.2$ Hz), 8.57 (d, 1H, $J = 8.2$ Hz), 7.88 (d, 1H, $J = 8$ Hz), 7.45–7.18 (m, 5H), 6.93 (s, 1H), 6.85–6.78 (m, 4H), 6.59–6.53 (m, 4H), 5.93 (s, 1H), 4.30 (s, 1H), 0.79 (s, 9H), 0.01 (s, 6H). MS: 490 (M^+). Anal. Calcd for ($C_{33}H_{34}O_2Si$): C, 80.77; H, 6.98. Found: C, 80.89; H, 6.90.

2. Loading of 24 on resin

A mixture of compound **24** (0.200 g, 0.40 mmol), 2-chlorotritylchloride resin (0.24 g, 0.20 mmol) and pyridine (5 mL) was refluxed at 50 °C for 8 h. The solvent was removed and the resin beads were washed with pyridine, methanol, DCM and ether. Treatment of few resin beads **25** with 1% TFA in DCM gave the authentic starting material **24**.

3. Cleavage of TBDMS group of 25 with TBAF on resin

The resin bound compound **25** (0.030 g, 0.024 mmol) was treated with 1 M TBAF in THF (0.036 mL, 0.036 mmol) at room temperature for 24 h. The solvent was removed and the resin beads were washed with pyridine, methanol, DCM and ether. Treatment of few resin beads **26** with 1% TFA in DCM gave 4-[(4-hydroxy-phenyl)-phenanthren-9-yl-methyl]-phenol as dark orange solid, m.p. 112 °C. IR (KBr): 3323, 1602, 1505, 1441, 1365, 1230, 1170 cm^{-1}. ^{1}H 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: δ 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.

4. Representative chain reaction on solid phase and cleavage from resin beads

A mixture of compound **26** (0.06 g, 0.049 mmol), anhydrous K_2CO_3 (0.203 g, 1.47 mmol), aminohydrochloride chain (1.47 mmol) and dry DMF (15 mL) was refluxed for 48 h. K_2CO_3 was filtered off and DMF was removed. The resin beads were washed with DMF, methanol, water, methanol, DCM and ether. It was dried under vacuo. Treatment of whole resin beads **27** with 1% TFA in DCM gave the hydroxy substituted alkylamine derivatives **28a–c**.

*4.1. 4-{[4-(2-Dimethylamino-ethoxy)-phenyl]-phenanthren-9-yl-methyl}-phenol **28a***

Dark yellow semi-solid. IR (neat): 3323, 2937, 1623, 1529, 1460, 1249, 1052 cm^{-1}. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 8.60 (d, 1H, $J = 8.2$ Hz), 8.58 (d, 1H, $J = 8.2$ Hz), 7.98 (d, 1H, $J = 8$ Hz), 7.60–7.41 (m, 5H), 7.06 (s, 1H), 6.96–6.90 (m, 4H), 6.70–6.66 (m, 4H), 6.04 (s, 1H), 4.08 (t, 2H, $J = 7$ Hz), 2.82 (t, 2H, $J = 7$ Hz), 2.42 (s, 6H). MS: 448 ($M^+ + H$). Anal. Calcd for ($C_{31}H_{29}NO_2$): C, 83.19; H, 6.53; N, 3.13. Found: C, 83.25; H, 6.63; N, 3.12.

*4.2. 4-{[4-(2-Diethylamino-ethoxy)-phenyl]-phenanthren-9-yl-methyl}-phenol **28b***

Dark yellow semi-solid. IR (neat): 3420, 2972, 1613, 1502, 1473, 1240, 1031 cm^{-1}. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 8.62 (d, 1H, $J = 8.2$ Hz), 8.60 (d, 1H, $J = 8.2$ Hz), 8.00 (d, 1H, $J = 8$ Hz), 7.64–7.51 (m, 5H), 7.01 (s, 1H), 7.05–6.97 (m, 4H), 6.80–6.73 (m, 4H), 6.11 (s, 1H), 4.08 (t, 2H, $J = 7$ Hz), 2.84 (t, 2H, $J = 7$ Hz), 2.75 (q, 4H, $J = 7$ Hz), 1.11 (t, 6H, $J = 7$ Hz). MS: 476 ($M^+ + H$). Anal. Calcd for ($C_{33}H_{33}NO_2$): C, 83.33; H, 6.99; N, 2.94. Found: C, 83.25; H, 6.93; N, 2.99.

*4.3. 4-{Phenanthren-9-yl-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-methyl}-phenol **28c***

Dark yellow semi-solid. IR (neat): 3395, 2935, 1610, 1513, 1453, 1247, 1076 cm^{-1}. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 8.62 (d, 1H, $J = 8.2$ Hz), 8.60 (d, 1H, $J = 8.2$ Hz), 8.02 (d, 1H, $J = 8$ Hz), 7.70–7.51 (m, 5H), 7.10 (s, 1H), 7.00–6.90 (m, 4H), 6.80–6.70 (m, 4H), 6.09 (s, 1H), 4.08 (t, 2H, $J = 7$ Hz), 2.79 (t, 2H, $J = 7$ Hz), 2.53 (t, 4H, $J = 7$ Hz), 1.67–1.60 (m, 6H). MS: 488 ($M^+ + H$). Anal. Calcd for ($C_{34}H_{33}NO_2$): C, 83.74; H, 6.82; N, 2.87. Found: C, 83.63; H, 6.90; N, 2.98.

5. Antimycobacterial activity

5.1. 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 microdilution method where serial two-fold

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. 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.

5.2. 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, 10^4–10^5 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 (10^2–10^3 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.

6. Micro alamar blue assay (MABA)

M. tuberculosis, $H_{37}R_a$ was used as a suitable surrogate for the virulent $H_{37}R_v$ strain. The standard antitubercular agents Rifamycin, isoniazid, *p*-aminosalicylic acid, ethambutol and ethionamide were taken as positive controls. A compound is considered active only if it shows inhibition $\geq 90\%$.

6.1. Cytotoxicity evaluation

Cytotoxicity of the compound(s) was checked by cell proliferation assay using VERO cells. In the assay number of viable cells are determined colorimeterically with a reagent containing a tetrazolium compound (MTS, Owen's reagent) and an electron-coupling reagent (PES, phenazine ethosulphate). The MTS is bioreduced (by NADPH or NADH produced by dehydrogenase enzyme in live cells) into a coloured formazen that is soluble in tissue culture medium.

VERO cells (104 cells/well/0.1 mL MEM containing antibiotics and 10% FBS) were seeded in 96-well tissue culture plate. After 24 h incubation (37 °C, 5% CO_2) medium was replaced with fresh medium (5% FBS and no antibiotic) containing different concentrations of test compound/known toxic compound/DMSO. After 24 h incubation (37 °C, 5% CO_2) 20 μL MTS reagent (Promega Kit) is added and absorbance is read after 2 h at 490 nm. Absorbance shown by DMSO containing wells is taken as 100% survivors. A compound is considered toxic if it causes ≥50% inhibition at concentration 10-fold higher than its MIC (minimum concentration which completely inhibits growth of *M. tuberculosis* $H_{37}R_v$).

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