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Antitubercular agents. Part 1: Synthesis of phthalimido- and naphthalimido-linked phenazines as new prototype antitubercular agents

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Abstract—The preparation and antitubercular properties of a series of phthalimido- and naphthalimido-linked phenazines are described. Some of these new compounds inhibited the growth of *Mycobacterium tuberculosis* ATCC 27294, *Mycobacterium avium* ATCC 49601, *Mycobacterium intracellulare* ATCC 13950 and some clinical isolates.

Tuberculosis is generally considered as the most important chronic communicable disease in the world.[1] The disease continues to be one of the major health problems not only in India but also in other South Asian countries.[2] In the last decade the increase of tuberculosis coinciding with the AIDS epidemic has resulted in additional drug resistant isolates of *Mycobacterium tuberculosis*.[3] The researchers of drug resistant tuberculosis have generated a renewal of interest in a strategic search for prototype leads.[4,5]

Many studies have attempted in the past to develop new antitubercular compounds to resolve some of the problems with the currently available antitubercular drugs. Phenazines have been initially developed as drugs for mycobacterial infections like tuberculosis and leprosy.[6] Clofazimine, the lead compound in this series has contributed significantly towards the control of leprosy, particularly for dapsone-resistant bacteria.[7,8] Clofazimine has also shown chemical efficacy in tuberculosis that is caused by multiple drug resistant strains in particular. Some of the newer phenazine analogues such as B746 and B4157 have not only showed increased antimycobacterial activity but also produced less skin pigmentation, which is the main drawback of these compounds.[9,10] In this context a programme was launched in this laboratory to synthesize new analogues of phenazines by linking them to phthalimido and naphthalimido moieties through bis alkyl piperazines. The piperazine system has been incorporated in the linker spacer keeping in mind the need to improve the solubility of such new analogues. Phthalimido and naphthalimido type of compounds have been reported in the literature to possess antimicrobial activity.[11]

These new conjugates of phenazine with phthalimido and naphthalimido moieties have been evaluated for their in vitro antitubercular activity. There are some reports on the synthesis of the phenazine analogues in the literature.[12] However, the method employing 2-chloronitrobenzene as the starting material has been utilized for the preparation of piperazine linked phenazine ring system (**6**) by a modified procedure.

Synthesis of phthalimido- and naphthalimido-linked phenazine conjugates **8a**–**h** has been carried out by employing *N*-(*p*-chlorophenyl)-*o*-phenylenediamine **4**, which has been obtained by reacting *o*-chloronitrobenzene **1** with *p*-chloroaniline **2** in presence of K_2CO_3 in DMF, the product **3** obtained is then reduced to **4** using $SnCl_2{\cdot}2H_2O$ in methanol. The oxidation of **4** in presence of $FeCl_3{\cdot}6H_2O$ gave the product **5**, which on refluxing

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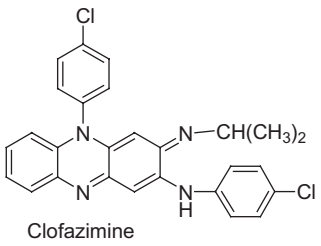
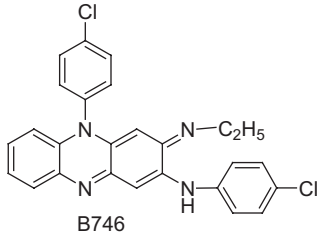
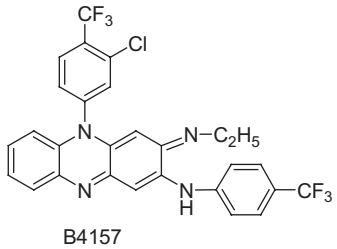


Clofazimine



B746



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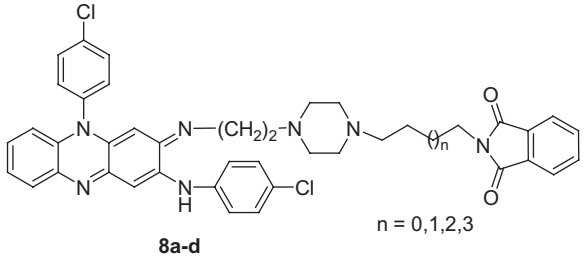
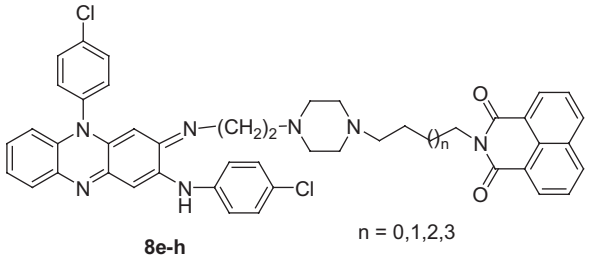


n = 0,1,2,3

8a-d



n = 0,1,2,3

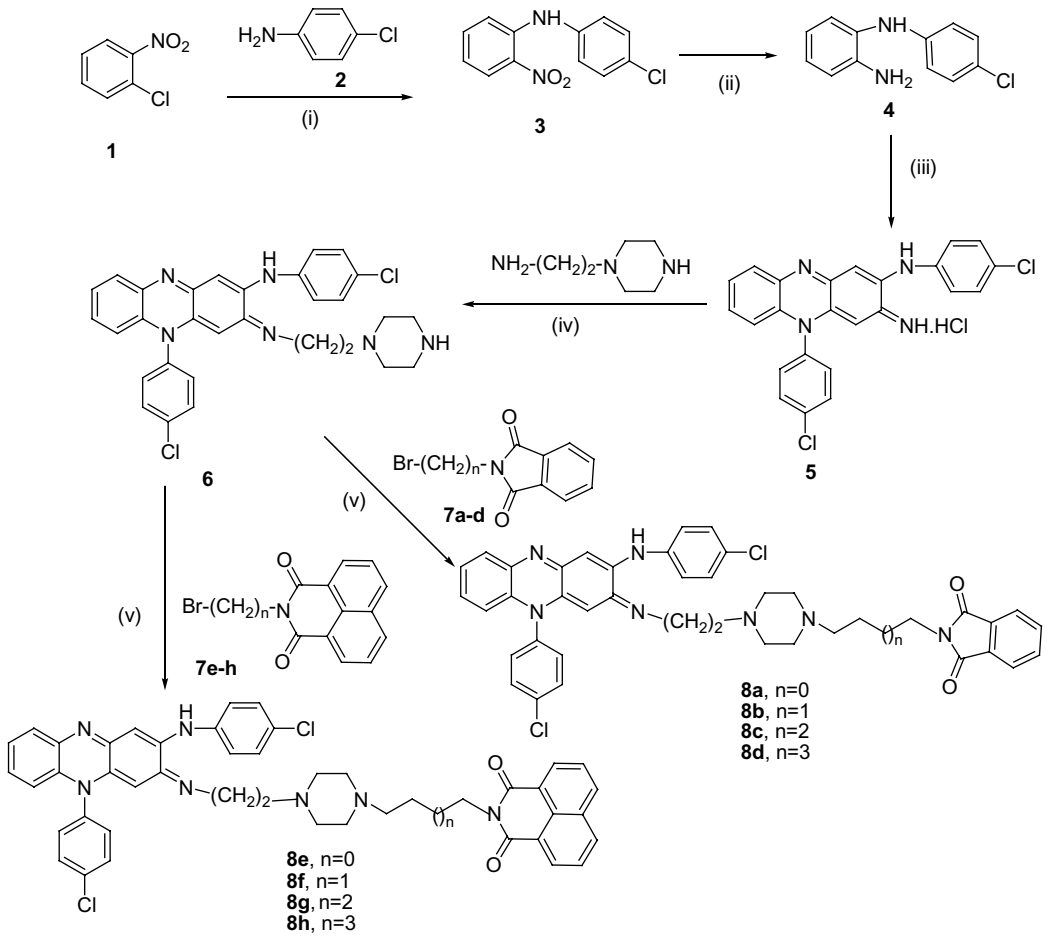
8e-h

with 1-(2-aminoethyl)piperazine in dioxane gave the product **6**, this upon refluxing with **7a**–**h** in acetonitrile in presence of K_2CO_3 gave the desired products **8a**–**h**[13–15] as illustrated in Scheme 1.

All the compounds (**8a**–**h**) were evaluated for antimycobacterial activity against three different mycobacteria (*M. tuberculosis* ATCC 27294, *M. avium* ATCC 49601 and *M. intracellulare* ATCC 13950) species at a concentration of 50, 25 and 12.5 μg by diffusion assay (Table 1). Compounds **8c**, **8d** and **8h** demonstrated good to mild inhibition of the mycobacterium cultures. The active compounds were then assayed for determination of minimum inhibitory concentration (MIC) against a variety of *M. tuberculosis* clinical isolates (drug sensitive and resistant) in agar dilution assay as per the NCCLS-M24-T2 recommendations. Briefly, 10 serial twofold dilutions of the compound/standard drug were prepared in DMSO and incorporated into Middlebrook7H10 agar medium. Individual *M. tuberculosis* isolates at concentration of 1×10^7 CFU/mL were spotted (3–5 μL/spot) on to the media plates. The plates were sealed and incubated at 37 °C for 3–4 weeks. Minimum inhibitory concentration (MIC) was recorded as the highest dilution of the compounds that completely inhibited the growth.

Compounds **8c** and **8d** having phthalimide linked phenazines were the most active compounds with an MIC value of 1.0 μg/mL against the sensitive strain of *M. tuberculosis* and an MIC value of 1.0–4.0 μg/mL against drug resistant clinical isolates of *M. tuberculosis*. Compound **8h** having naphthalimide linked phenazines demonstrated poor activity (MIC 8.0–16.0 μg/mL) against sensitive and resistant strains of *M. tuberculosis*. None of the compounds were found to have significant activity against *M. avium* and *M. intracellulare* cultures.

In conclusion, a novel series of antimycobacterial compounds have been designed and synthesized that demonstrated significant activity (**8c** and **8d**) against drug sensitive and resistant *M. tuberculosis* cultures. The antimycobacterial activity of these two compounds were better than isoniazid on drug resistant clinical isolates of *M. tuberculosis*. These findings clearly indicate that phthalimide–phenazine conjugates linked through piperazine moiety side-armed with alkane spacers exhibit good antimycobacterial activity compared to their



Scheme 1. Reagents and conditions: (i) K_2CO_3, DMF, reflux, 24 h; (ii) $SnCl_2$, MeOH, reflux, 2 h; (iii) $FeCl_3{\cdot}6H_2O$, HCl; (iv) dioxane, reflux, 5 h; (v) K_2CO_3, CH_3CN, reflux, 48 h.

Table 1. Antimycobacterial activity of compounds **8a**–**h**

Phenazine hybrid	MIC (μg/mL)				
	M.tuberculosis $H_{37}Rv$ ATCC 27294	*M. tuberculosis* clinical isolates		*M. avium* ATCC 49601	*M. intracellulare* ATCC 13950
		Sensitive	Resistant		
8a	>16.0	>16.0	>16.0	>16.0	>16.0
8b	>16.0	>16.0	>16.0	>16.0	>16.0
8c	**1.0**	**1.0–4.0**	**1.0–4.0**	**16.0**	**8.0**
8d	**1.0**	**1.0–2.0**	**1.0–4.0**	**16.0**	**8.0**
8e	>16.0	>16.0	>16.0	>16.0	>16.0
8f	>16.0	>16.0	>16.0	>16.0	>16.0
8g	>16.0	>16.0	>16.0	>16.0	>16.0
8h	**16.0**	**8.0–16.0**	**8.0–16.0**	**>16**	**>16**
Isoniazid	0.25	0.125–0.25	8.0–>16.0	>16.0	8.0

naphthalimido counterparts. Additionally, as the alkane spacer between the piperazine and phthalimide moieties increase from three to five and six there is enhancement in the activity. This investigation indicates that there is potential to design and synthesize such type of hybrids for the development of new antitubercular compounds.

Acknowledgements

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13. Typical procedure for the synthesis of **8a**–**h**: To a stirred solution of **6** (1 mmol) in acetonitrile was added **7a**–**h** (1.2 mmol) and K_2CO_3 (5 mmol) and the resulting mixture was refluxed for 48 h. After completion of the reaction, the reaction mixture was diluted with ethyl acetate and filtered on a celite bed to afford the crude product this was further purified by column chromatography on neutral alumina using ethylacetate/methanol (9:1) as eluent gave the pure product **8a**–**h** in 80% yield.
14. Selected spectral data for compound **8c**: ^{1}H NMR (300 MHz $CDCl_3$) δ 1.44–1.55 (m, 4H), 1.7–1.8 (m, 2H), 2.2–2.5 (m, 8H), 2.8–3.0 (m, 4H), 3.62–3.65 (m, 2H), 3.75–3.80 (m, 2H), 5.6 (s, 1H), 6.3 (s, 1H), 6.8–7.1 (m, 4H), 7.25 (d, J = 7.4Hz, 2H), 7.45 (d, J = 6.9 Hz, 2H), 7.6–7.8 (m, 4H), 7.95–8.20 (m, 4H), 9.5 (br s, 1H); MS (FAB) 759 $[M]^+$.
15. Selected spectral data for compound **8h**: ^{1}H NMR (300 MHz $CDCl_3$) δ 1.42–1.53 (m, 2H), 1.6–1.8 (m, 6H), 2.3–2.6 (m, 8H), 2.9–3.0 (m, 2H), 3.45–3.55 (m, 2H), 3.8–3.9 (m, 2H), 5.56 (s, 1H), 6.22 (s, 1H), 6.55–6.65 (m, 4H), 6.92–7.15 (m, 4H), 7.44–7.55 (m, 4H), 8.23 (d, J = 7.8 Hz, 2H), 8.56 (d, J = 7.0 Hz, 2H), 9.5 (br s, 1H); MS (FAB) 823 $[M]^+$.