ORIGINAL ARTICLE

Plumbagin analogs-synthesis, characterization, and antitubercular activity

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J. Adv. Pharm. Technol. Res.

ABSTRACT

Considering the emerging problem of drug resistance in tuberculosis, there is an urgent need of development of new analogs that are useful in curing drug resistant tuberculosis. In India, tuberculosis continues to remain one of the most pressing health problems. India is the highest tuberculosis burden country in the world, accounting one fifth of global incidence - estimated 2.0-2.5 million cases annually. In 2011, approximately 8.7 million new cases of tuberculosis and 1.4 million people die from tuberculosis each year worldwide. Current antitubercular therapies are successful against normal tuberculosis but it is not suitable for drug resistant tuberculosis. In this study Plumbagin analogs, obtained from *Plumbago zeylanica* (Family-*Plumbaginaceae*), have been synthesized. Out of the various synthesized analogs, the antitubercular activity of compound a and b was evaluated using standard H_{37}Rv and S, H, R, and E sensitive M tuberculosis strains using LRF assay method. Compound a showed strong activity against both standard H_{37}Rv and S, H, R and E sensitive *M. tuberculosis* strains as compared to standard Rifampicin. The other compounds are proved to be more active against standard H_{37}Rv and S, H, R and E sensitive *M. tuberculosis* strain as compared to Rifampicin.

Key words: Ethambutol (R), isoniazid (h), plumbagin, rifampicin (r)

INTRODUCTION

Tuberculosis is an airborne disease caused by the bacterium *Mycobacterium tuberculosis*. *M. tuberculosis* and seven other related mycobacterial species (*M. bovis, M. africanum, M. microti, M. caprae, M. pinnipedii, M. canetti* and *M. mungi*) together form *M. tuberculosis* complex. Most but not all species have been found to cause disease in humans.[1] Multidrug-resistant TB (MDR TB) is caused by organisms resistant to the most effective anti-TB drugs, isoniazid and rifampin. These drugs are considered first-line drugs and are used to treat most persons with TB disease. Extensively drug-resistant TB (XDR TB) is a relatively rare type of drug-resistant TB. XDR TB is resistant to isoniazid and rifampin, plus any fluoroquinolone and at least one of three injectable second-line drugs (i.e. amikacin, kanamycin, or capreomycin).[2]

The multidrug resistance, reinfection, and latent infection has become the major cause of concern for the treatment of tuberculosis in all over the world. This together with the problem of the interactions of the current tuberculosis drugs with the antiretroviral drugs taken by HIV positive people, means that there is an urgent need for new tuberculosis drugs.[3]

Plants containing 1,4-naphthoquinone derivatives were shown promising antitubercular activity with novel mode of action and their synthetic derivatives. Plumbagin have naphthoquinone moiety which inhibits menaquinone pathway which is essential for the growth of *M. tuberculosis* and *M. smegmatis*.[4]

Esters of 5-hydroxyl group of Plumbagin have been reported to have activity against Mycobacterium species.[5]

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Access this article online

Quick Response Code:

Website: www.japtr.org

DOI: 10.4103/2231-4040.126984

MATERIALS AND METHODS

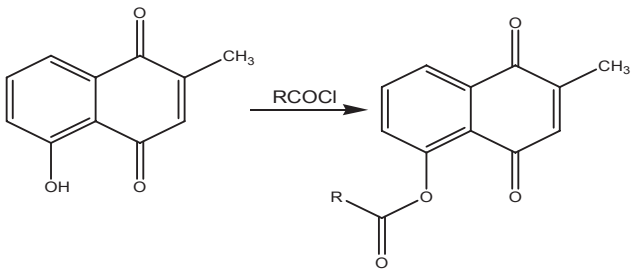
Materials

Plumbagin was purchased from Sigma-Aldrich, Germany. 1-Naphtholyl chloride, 2-Naphtholyl chloride, Hexanoyl chloride, and Pyrazinamide were purchased from Sigma-Aldrich, Germany. Isoniazid and Ethionamide provided by Themis medicare as a gift samples. The IR spectra were recorded on a IR spectrometer Carry 630. The 1H NMR spectra have been recorded on Bruker 400 Avance Fourier Transform Spectrometer operating at 400 mega hertz in deuterated dimethylsulfoxide (DMSO) with all shifts referred to internal tetramethylsilane (TMS). The mass spectra were recorded on a LCMS Agilent Technology model 6520. All other reagents used in synthesis as well as analysis were of synthetic grade and analytical grade, respectively.

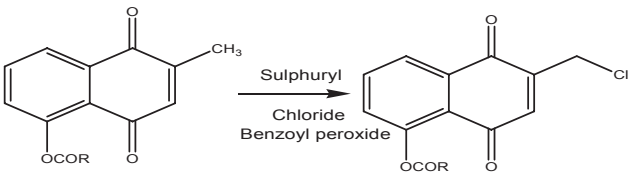
Methods

Plumbagin derivatives have been synthesized as per the following scheme given in the following

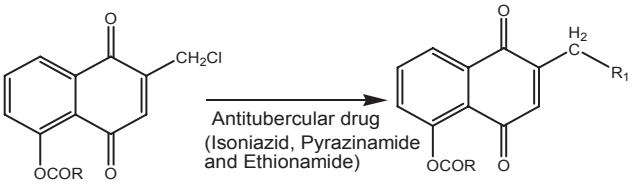
a. Treatment with Acyl chloride



b. Chlorination of methyl group of plumbagin



c. Condensation with antitubercular drugs



Where R = 1-Naphthoyl chloride, 2-Naphthoyl chloride, Hexanoyl chloride

R_1 = Ethionamide, Pyrazinamide, Isoniazid

The steps involved in synthesis of plumbagin derivatives included

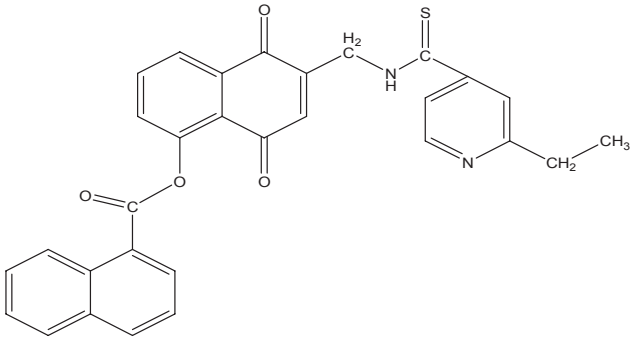
Step 1: Plumbagin was dissolved in dichloromethane and pyridine at 0°C and reaction was maintained in an ice-bath. Solution was stirred for 5 min. Acid chlorides were added dropwise to the reaction mixture at 0°C. Reaction mixture further stirred for 3 h at room temperature.

After 3 h, reaction mixture was diluted with dichloromethane and washed with water and brine solution. Products were separated using separating funnel. Oily product obtained. Reaction was confirmed by an IR spectrum.

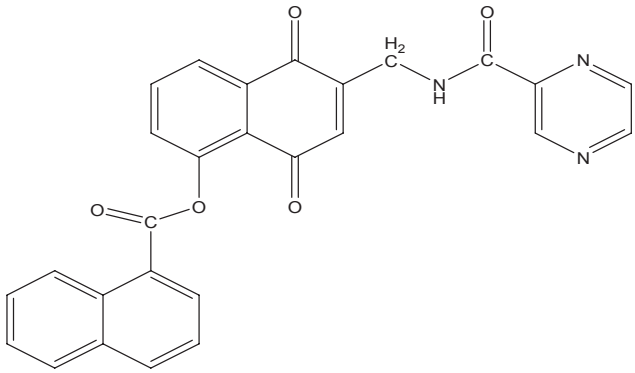
Step 2: To the step 1 product, ethanol was added. Further, sulfuryl chloride and benzoyl peroxide were added to the reaction mixture. Stirred it for 2 h at room temperature and dried it in oven at 50°C. Reaction was confirmed by an IR spectrum.

Step 3: To the step 2 product, ethanol and anti-tubercular drug were added. Refluxed for 3 h and dried it in an oven at 50°C. Final product was confirmed by an IR spectrum and TLC both.

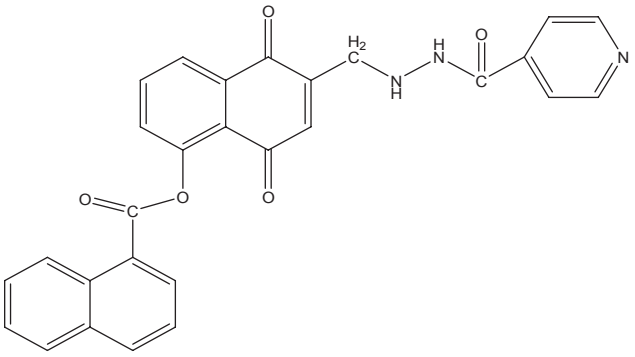
Compound a: 2-(2′-Ethylisonicotinoylthiamido)-methyl-5-(1′-naphthoyl)-oxy-1,4- naphthoquinone



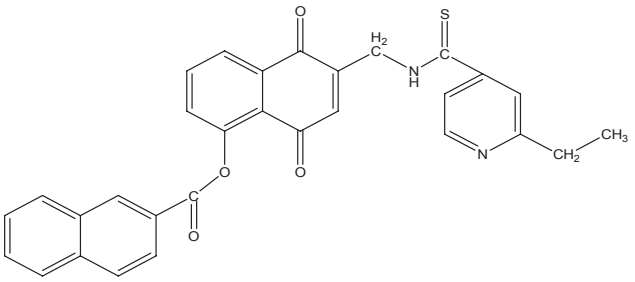
Compound b: 2-(Pyrazinecarboxamido)-methyl-5-(1′-naphthoyl)-oxy-1,4- naphthoquinone



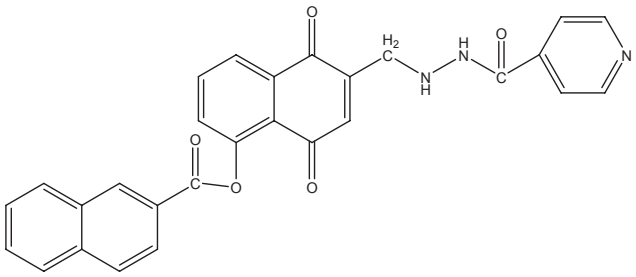
Compound c: 2-(2′-Isonicotinoylhydrazino)-methyl-5-(1′-naphthoyl)-oxy-1,4- naphthoquinone



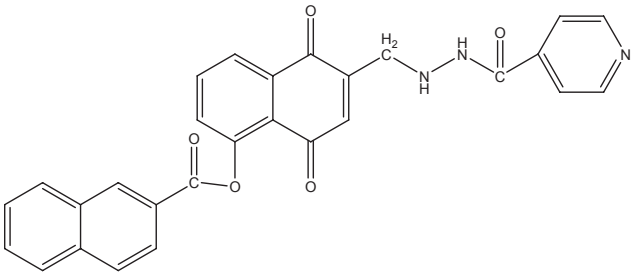
Compound d: 2-(2′-Ethylisonicotinoylthiamido)-methyl -5- (2′-naphthoyl) -oxy-1,4-naphthoquinone



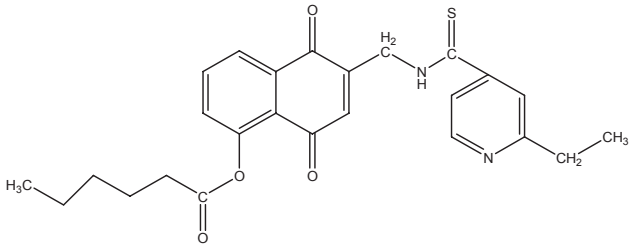
Compound e: 2-(Pyrazinecarboxamido)-methyl-5-(2′-naphthoyl) -oxy-1,4-naphthoquinone



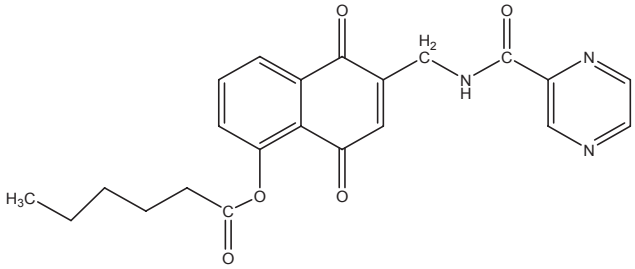
Compound f: 2-(2′-Isonicotinoylhydrazino)-methyl-5-(2′-naphthoyl) -oxy-1,4-naphthoquinone



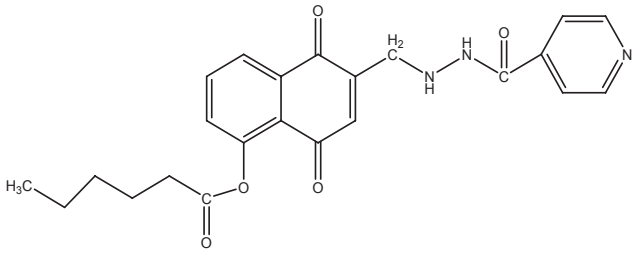
Compound g: 2-(2′-Ethylisonicotinoylthiamido)-methyl -5-(n-hexanoyl) -oxy-1,4-naphthoquinone



Compound h: 2-(Pyrazinecarboxamido)-methyl-5-(n-hexanoyl)-oxy-1,4-naphthoquinone



Compound i: 2-(2′-Isonicotinoylhydrazino)-methyl-5-(n-hexanoyl) -oxy-1,4-naphthoquinone



Compound a

This was synthesized using above scheme. R_f 0.44 (Silica Gel G, n-hexane: EtOAc, 10:2); IR (cm^{-1}) 3067 (Aromatic C-H), 2985 (Aliphatic-CH_2), 1678 (Aryl C = C), 1631 (C = O), 1452 (Aromatic ring stretch); (400 MHz, DMSO-d6), dH ppm: 2.469 (1H, s), 3.025-3.006 (4H, q, J = 7.6 Hz), 1.258-1.240 (3H, t, J = 7 Hz), 6.998 (1H, m), 7.126 (1H, m), 7.254 (1H, m), 7.468 (1H, m), 7.536 (1H, m), 7.552 (1H, m), 8.076 (1H, m), 7.969 (1H, s), 7.900 (1H, s), 8.605 (1H, s); m/z 507.13(M+).

Compound b

This was synthesized using above scheme. R_f 0.46 (Silica Gel G, hexane: EtOAc, 10:2); IR $(cm)^{-1}$, 3423 (Aromatic C-H), 2969 (Aliphatic-CH_2), 1674 (Aryl C = C), 1596 (C = O), 1169 (Amide C = O); (400 MHz, DMSO-d6), dH ppm: 2.469 (1H, s), 7.993–7.973 (2H, d, J = 8 Hz), 7.629 (1H, m), 7.326 (1H, m), 7.609 (1H, m), 7.591 (1H, m), 7.574 (1H, m), 7.555 (1H, m), 8.241 (1H, m), 7.845 (1H, s); m/z 464.12(M+).

Compound c

This was synthesized using above scheme. R_f 0.43 (Silica Gel G, hexane: EtOAc, 10:2); IR $(cm)^{-1}$ 3216 (Aromatic C-H),

2121(Aliphatic-CH_2), 1659 (Aryl C = C), 1544 (C = O), 1413 (Aromatic ring stretch), 1287 (Amide C = O); (400 MHz, DMSO-d6), dH ppm: 2.469 (1H, s), 7.700-7.687 (2H, d, J = 7.8 Hz), 7.763-7.748 (2H, d, J = 6 Hz), 7.443 (1H, m), 7.462 (1H, m), 7.481 (1H, m), 7.531 (1H, m), 7.551 (1H, m), 7.566 (1H, m), 7.583 (1H, m); m/z not clear.

Compound d

This was synthesized using above scheme. R_f 0.41 (Silica Gel G, n-hexane: EtOAc, 10:2); IR (cm^{-1}) 3052 (Aromatic C-H), 2821 (Aliphatic-CH_2), 1683(Aryl C = C), 1428 (Aromatic ring stretch), 1240 (C = S); (400 MHz, DMSO-d6), dH ppm: 3.691-3.673 (4H, q, J = 6.8), 1.067-1.049 (3H, t, J = 6.41 Hz), 2.469 (1H, s), 7.923 (1H, s), 7.903 (1H, s), 6.839 (1H, s); m/z 501.13(M+).

Compound e

This was synthesized using above scheme. R_f 0.38 (Silica Gel G, hexane: EtOAc, 10:2); IR (cm^{-1}) 3421(Aromatic C-H), 3090 (Aliphatic-CH_2), 1702 (Aryl C = C), 1672 (Aromatic C = O), 1432(Aromatic ring stretch), 1173(Amide C = O); (400 MHz, DMSO-d6), dH ppm: 8.235 (1H, s), 2.468 (1H, s), 7.91 (1H, m), 7.89 (1H, m), 7.83 (1H, m), 7.204 (1H, m), 7.077 (1H, m), 6.949 (1H, m), 8.238 (1H, m); m/z 464.12(M+).

Compound f

This was synthesized using above scheme. R_f 0.44 (Silica Gel G, hexane: EtOAc, 10:2); IR (cm^{-1}) 3197(Aromatic C-H), 2519 (Aliphatic-CH_2), 1682 (Aryl C = C), 1659 (Aromatic C = O), 1413 (Aromatic ring stretch), 1283 (Amide C = O); (400 MHz, DMSO-d6), dH ppm: 9.153 (1H, s), 8.824-8.818 (2H, m, J = 0.36 Hz), 8.63 (1H, s), 6.957 (1H, s), 2.469-2.324 (2H, q, J = 8.7 Hz); m/z not clear.

Compound g

This was synthesized using above scheme. R_f 0.50 (Silica Gel G, n-hexane: EtOAc, 10:2); IR (cm^{-1}) 3391(Aromatic C-H), 2117 (Aliphatic-CH_2), 1640 (Aryl C = C), 1443(Aromatic ring stretch), 1190 (C = S); (400 MHz, DMSO-d6), dH ppm: 2.469 (1H, s), 1.069-1.052 (3H, t, J = 6.7 Hz), 1.197-1.181 (3H, t, J = 6.4 Hz), 3.724-3.707 (5H, p, J = 6.8 Hz), 6.979 (1H, s), 7.107 (1H, s), 7.235 (1H, s); m/z 451.16(M+).

Compound h

This was synthesized using above scheme. R_f 0.47 (Silica Gel G, hexane: EtOAc, 10:2); IR (cm^{-1}) 3427(Aromatic C-H), 2933(Aliphatic-CH_2), 1665(Aryl C = C), 1598 (Aromatic C = O), 1460(Aromatic ring stretch), 1169 (Amide C = O); (400 MHz, DMSO-d6), dH ppm: 2.469 (1H, s), 1.210-1.201 (3H, t, J = 3.6Hz), 1.191-1.178 (3H, t, J = 5.2 Hz), 2.145 (1H, s), 7.847 (1H, s),7.949-7.933 (2H, d, J = 6.4 Hz); m/z 408.15(M+).

Compound i

This was synthesized using above scheme. R_f 0.52 (Silica Gel G, hexane: EtOAc, 10:2); IR (cm^{-1}) 3197(Aromatic C-H), 1626(Aryl C = C), 1551(Aromatic C = O), 1413(Aromatic ring stretch), 1063(Amide C = O); (400 MHz, DMSO-d6), dH ppm: 2.469 (1H, s), 1.077-1.050 (3H, t, J = 10.8Hz), 1.190-1.180 (3H, t, J = 4 Hz), 7.697-7.683 (2H, d, J = 5.6 Hz), 7.783-7.768 (2H, d, J = 6 Hz); m/z not clear.

Mycobacterial growth inhibitory assay

Luciferase Reporter Phage (LRF) Assay

Standard strain H_{37}Rv, a clinical sensitive *M. tuberculosis* strain and a clinical isolate S, H, R, and E sensitive *M.tuberculosis* strain were grown in Middlebrook 7H complete medium with and without extracts of samples for 3 days at 37°C. Luciferase Reporter Phage Assay was done using concentrations of 50 and 100 µg/ml of samples. Rifampicin was included as an assay control and DMSO as the solvent control. LRP phage AETRC21 was added and the samples were incubated for four hours. Equal volume of the cell phage mixture was mixed with 0.3 Mm D-Luciferin in 0.05 M sodium citrate buffer of pH 4.5 and light output was immediately measured as RLU (Relative light units) in the luminometer at 10 s integration. Compounds exhibiting a reduction of 50% or more in RLU in the test vials compared to that of the control were considered to have antimycobacterial activity. These LRP assays offer an elegant means of detecting viable mycobacteria and provide a rapid tool for drug susceptibility screening.

RESULTS AND DISCUSSION

Plumbagin analogs and its ester were synthesized. Plumbagin has been referred to possess antitubercular activity. Accordingly, various newer analogs have been synthesized and tested for their antitubercular activity. Compounds were synthesized by treating Plumbagin with 1-Naphthoyl chloride, 2-Naphthoyl chloride and Hexanoyl chloride. Further the synthesized Plumbagin derivatives were condensed with antitubercular drugs- Isoniazid, Pyrazinamide, and Ethionamide. IR spectra of synthesized compounds exhibit a band in the region IR spectra confirmed the formation of product. Further, formations of compounds were confirmed by Proton NMR and Mass Spectra. Calculating log *P* values revealed that among synthesized compounds Ethionamide analogs of Plumbagin possess best Antitubercular activity. Thus, 1-Naphthoyl chloride and Hexanoyl chloride analogs of plumbagin synthesized by condensation of Ethionamide were sent to National Institute for Research in Tuberculosis (ICMR), Chennai for screening of Antitubercular activity. The compounds were screened in both standard H_{37}Rv and clinical isolate S, H, R, and E sensitive *M. tuberculosis* strain with taken Rifampicin as a standard drug. Compounds b showed better Antitubercular activity as compared to Rifampicin against standard H_{37}Rv *M. tuberculosis* strain while Compounds showed alone best Antitubercular activity as compared to Rifampicin against clinical isolate S, H, R, and E sensitive *M. tuberculosis* strain. Out of various compounds, compounds a (1-Naphthoyl

Table 1: Percentage reductions in relative light units (RLU) by 50 µg/ml against $H_{37}Rv$ Standard and Clinical isolate: S, H, R, and E sensitive Mycobacterium tuberculosis

Compound code	$H_{37}Rv$ Standard	Clinical isolate: S, H, R and E sensitive
A	97.99	99.03
B	0	7.78
Rifampicin (2 µg/ml)	97.54	16.93

RLU: Relative light units

Table 2: Percentage reductions in relative light units (RLU) by 100 µg/ml against $H_{37}Rv$ Standard and Clinical isolate: S, H, R, and E sensitive Mycobacterium tuberculosis

Compound code	$H_{37}Rv$ Standard	Clinical isolate: S, H, R, and E sensitive
A	98.23	99.05
B	49.87	8.67
Rifampicin (2 µg/ml)	97.54	16.93

RLU: Relative light units

chloride-Ethionamide derivative of Plumbagin) was found to be most effective in clinical isolate: S, H, R and E sensitive *M. tuberculosis* [Tables 1 and 2].

CONCLUSION

Based on the computational studies, all the synthesized compounds must possess Antitubercular activity. The Antitubercular activity showed that compounds can cure tuberculosis against standard $H_{37}Rv$ *M. tuberculosis* strain but compounds (approx. 98% reduction) have more potential to cure tuberculosis against clinical isolate S, H, R and E sensitive *M. tuberculosis* strain as compared to Rifampicin (approx 16% reduction). Thus, compounds will be beneficial for multidrug resistant tuberculosis (MDR TB) and extensively drug-resistant tuberculosis (XDR TB).

ACKNOWLEDGMENTS

The authors thank Mr. K.K. Jain, who helped us in procurement of drug samples. The authors acknowledge with thanks Dr. Saumya Swaminathan and Dr. Prabhu Seeniwasan (National Institute for Research in Tuberculosis, Chennai) for carrying out Antimycobacterial Activity.

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How to cite this article: Nayak N, Bajpai M, Razdan B. Plumbagin analogs-synthesis, characterization, and antitubercular activity. J Adv Pharm Technol Res 2014;5:28-32.

Source of Support: Nil, **Conflict of Interest:** Nil.