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Discovery of selective and potent anti-tubercular activity in arylpentane-1,4-diones

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Anti-tubercular activity (MIC 50µg/mL against Mtb H37Rv) have been reported in hispolons, which possess α, β-unsaturated-1,3-diketone functional group. In the pursuit to optimize structure and bioactivity, various substituted arylpentane-1,4-diones **S1**-**8** have been synthesised. Upon screening, the test compounds show negligible antimicrobial activity against the tested organisms at 100 µg/mL but show antitubercular activity against *Mycobacterium tuberculosis* H37Rv strain with an MIC 0.8 to 25µg/mL. Compounds having a halogen at the *ortho* position on the aromatic ring show highest potency (MIC 0.8µg/mL), implying a relation between the position of the halogen atom and anti-tubercular activity.

Keywords: Stetter reaction, 1,4-diketones, antitubercular activity, MABA assay, docking

Hispolons are metabolites derived from mushrooms like *Inonotushispidus*, *Phellinuslinteus* and *Phellinusigniarius* among others[1–3]. They have an α, β unsaturated 1,3-diketo group conjugated with an aromatic ring. Hispolons have been reported to have anti microbial[4], anti-viral, anti oxidant[4], anti inflammatory[5] and anti cancer[6–9]. The anti tubercular properties of hispolons and their analogs were recently reported by our group[10]. Here, we observed that potency increased from 25µg/mL to 1.6µg/mL with saturation of the double bonds (Figure 1). As hispolons exhibit keto-enoltautomerism, conjugation of the 'enol' with double bond may render the molecule rigid. Hence, hydrogenation of the double bond might have enhanced structural flexibility and better fitment into the receptor pocket meant for biosynthesis of fatty acid, which are highly flexible.

Further, the docking studies on hispolons and dihydrohispolons predicted the β-keto acyl synthase enzyme (mtFabH), an essential enzyme for mycolic acid biosynthesis, as a probable enzyme target. Mycolic acids are an essential component of the cell wall of tubercle bacilli and it needs β-keto acyl synthase – II (mtFabH) for initiation of their biosynthesis.[11–15]. Hence, we pursued the synthesis of arylpentane-1,4-dionesas structural analogues of dihydrohispolons, and screened for anti TB activity.

Results and Discussion

Chemistry

The compounds under discussion were synthesized using conventional Stetter reaction conditions (Scheme I)[16,17]. Appropriate aromatic aldehydes (1mmol) were treated with methyl-vinyl ketone (3mmol) in the presence of 3-benzyl-5-(2-hydroxyethyl)-4-methylthiazolium chloride (0.12mmol) as a catalyst in the presence of triethylamine (1.5mmol) and sodium acetate (0.12mmol) using dimethylformamide as a solvent. The reaction mixture was refluxed under nitrogen for a period of 8- 12 hours. The reaction progress was monitored by TLC. Upon completion, the reaction was quenched by adding water. The mixture was extracted with three successive quantities (75 mL) of ethyl acetate. The combined ethyl acetate fractions were dried over anhydrous sodium sulphate and concentrated under vacuum. The concentrate was

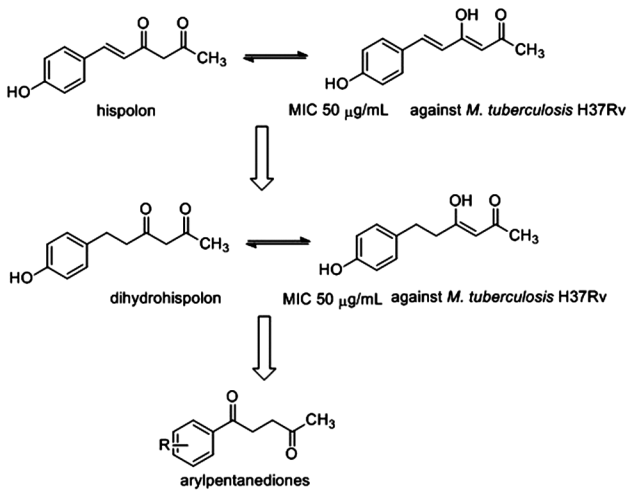
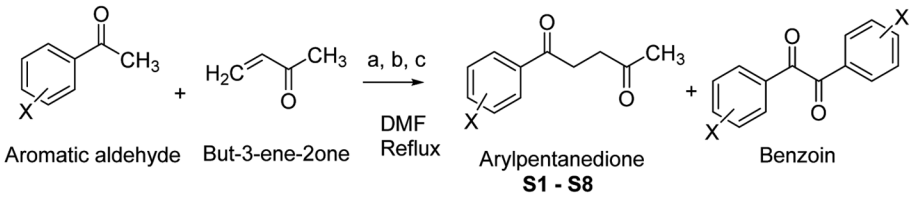


Figure 1 — Design of arylpentane diones from dihydrohispolons



Scheme I — The Stetter reaction

subjected to column chromatography using mixtures of hexane and ethyl acetate to obtain the desired compounds in a pure form.

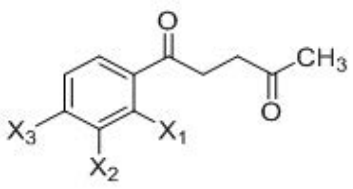
The purified compounds were characterized by IR, NMR and mass spectra; *in vitro* studies were initiated after confirmation of the structure of the compounds. In the synthesis, using one part of aldehyde to three parts of but-3-ene-2-one in dimethylformamide as solvent gave optimal yields. Here, we observed formation of the corresponding benzoin as a by product in 10 – 15% yield.

The structures of the synthesized compounds were established by spectral analysis. The presence of major infrared absorption peaks in the region of 1715 $cm^{-1}$and 1680 to 1690 cm^{-1} due to the presence of carbonyl groups; in 3000 to 3100 cm^{-1} region due to presence of aromatic –CH indicated the formation of the desired compounds. The ^{13}C NMR spectrum of the sample showed presence of two strong carbonyl peaks at δ 196.83 and 207.15. The presence of a sharp singlet at δ 2.1(3H, s, CH_3), and two mutually coupled triplets in the regions between δ 2.5 to 3.5 (2H, t, CH_2, J = 6.4 Hz) ascertained the presence of pentane-2,4-dione moiety in the structure. Eight analogues of arylpentanediones having halogens in *ortho*, *meta* and *para* positions have been synthesized.

Antimicrobial assay

Antimicrobial studies were performed by agar well diffusion method[18,19] to test bacterial susceptibility of the synthesized compounds at different concentrations. Antibacterial activity was tested against *Staphylococcus aureus* (Gram positive) and *Escherichia coli* (Gram negative) using rifampicin as the standard and methanol as the control. Antifungal activity was tested against *Aspergillusniger* and *Candida albicans*, using ketaconazole as the standard and methanol as control. The synthesized compounds did not inhibit the tested bacteria and fungi at concentrations ≤ 100 µg/mL.

Table I — List of synthesized compounds with MIC values



Compd	X_1	X_2	X_3	MIC value in MABA assay (µg/mL)
S1	H	H	H	6.25
S2	Cl	H	H	0.8
S3	F	H	H	0.8
S4	Br	H	H	0.8
S5	Cl	H	Cl	0.8
S6	H	F	F	12.5
S7	H	Br	H	12.5
S8	H	H	Cl	12.5

Anti-tubercular assay

Anti-tubercular activity was recorded by Micro-plate Alamar Blue Assay (MABA)[20-22] against *Mycobacterium tuberculosis* H37Rv strain. The final drug concentrations tested were 100, 50, 25, 12.5, 6.25, 3.125, 1.6 and 0.8 µg/mL. Development of a blue color was regarded as susceptibility of the organism to the compound. Development of a pink color was regarded as resistance to the compound. Minimum inhibitory concentration was defined as the lowest concentration which facilitated color change from pink to blue. The tested compounds have shown anti-tubercular activity in the range 0.8 to 12.5 µg/mL.

Compounds S-2, 3, 4 and 5; having a halogen atom in the *ortho* position showed highest potency with MIC 0.8 µg/mL (Table I); which is four times more potent than the remaining synthesized compounds. To study the role of halogen in structure activity

relationship, molecular interactions and the possible role of this substitution on upsurge in potency, we conducted molecular docking simulations with mtFabH enzyme, the target enzyme for hispolons. The compounds were screened by using docking score (ΔG, kcal/mol), ligand efficiency[23,24] (binding affinity contribution per a non-hydrogen atom), hydrogen bonding and pose analysis.

The catalytic site of FabH enzyme includes the amino acids Cys^{112}, His^{244}, Asn^{274}; essential for ligand binding[25]. In docking simulations (Table II), the O-halo substituted compounds docked snugly into the receptor pocket in "head first" mode with the terminal ketone showing strong interactions with binding site amino acids Arg^{249}, Asn^{247}, Ala^{246}, Phe^{213} and Gly^{209}at the entry point of the enzyme and Asn^{274} of the catalytic site (Figure 2). Further, these compounds also have shown very good ligand efficiency of < -0.5 Kcal/Mol/Atom. However, the remaining compounds showed strong interactions with the catalytic site amino acids but failed to interact with the entry point amino acids. This could be the underlying reason for observed anti-tubercular activity pattern in our tested compounds.

Experimental Section

All the reagents used are of reagent grade. The reactions were monitored with silica gel TLC using appropriate mobile phase. Visualization of the spots was done with the help of UV at 254 nm. Melting points were recorded using EZMELT 120 (Stanford Research Systems, USA) and are uncorrected. IR spectral data were recorded on Bruker ALPHA-T FTIR system using appropriate method. NMR spectral data was documented on Bruker-400 MHz system in an appropriate deuterated solvent using TMS as internal standard. Pure cultures of the experimental organisms were obtained from the biotechnology lab of Andhra University College of Pharmaceutical Sciences.

Physical constants and characterization of 1-phenylpentane-2,5-dione, S1: Yellow colored oily liquid. Yield 30%. IR (ZnSe, cm^{-1}): (CH bending) 1447.70, (C=O str) 1733.98, 1685.42, (Ar C-H str) 3787.41; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.97 (2H, d, J = 7.6 Hz, Ar-H), 7.50 (3H, m, J = 7.2 Hz, Ar-H), 3.227 (2H, t, J = 6.4 Hz, CH_2), 2.88 (2H, t, J = 6.4 Hz, CH_2), 2.25 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 30.16 (CH_3) , 32.65(CH_2), 37.15(CH_2), 128.30(CH), 129.16(CH), 133.67(CH), 136.94(CH), 190.03(C=0), 207.59(C=0).

Physical constants and characterization of 1-(2-chlorophenyl)pentane-2,5-dione, S2: Light yellow colored oily liquid. Yield 75%. IR (ZnSe, cm^{-1}): (CH bending) 1431.17, (C=O str) 1714.12, 1688.10, (Ar C-H str) 3075.62; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.58 (1H, dd, Ar-H), 7.323 – 7.439 (3H, m, Ar-H), 3.209 (2H, t, J = 6.4 Hz, CH_2), 2.913 (2H, t, J = 6.4 Hz, CH_2), 2.25 (3H, t, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 29.90(CH_3), 36.66(CH_2), 37.51(CH_2), 126.94(CH), 129.21(CH), 130.50(CH), 130.83(CH), 131.76(CH), 139.05(CH), 201.66(C=0), 206.90(C=0).

Physical constants and characterization of 1-(2-fluorophenyl)pentane-2,5-dione, S3: Yellow colored oily liquid. Yield 72%. IR (ZnSe, cm^{-1}): (CH bending) 1451.79, (C=O str) 1734.90, 1688.50, (Ar C-H str) 2985.57; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.92 – 7.86 (1H, dt, Ar-H), 7.58 – 7.50 (1H, m, Ar-H), 7.265

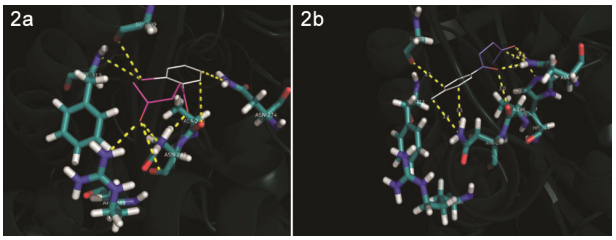


Figure 2 — Docking interactions of S2 (**2a**) and S8 (**2b**) with the enzyme mtFabH

Table II — Results of Docking studies on mtFabH enzyme

S.No.	Compd	Ligand Efficiency (kCal/ Mol/atom)	MIC against *Mycobacterium tuberculosis*H37Rv (µg/mL)	Ligand – Receptor interactions
1	**S-1**	−0.32	6.25	Asn-274
2	**S-2**	−0.52	0.8	Arg-249, Asn-247, Ala-246
3	**S-3**	−0.51	0.8	Arg-249, Asn-247, Ala-246, Phe- 213 and Gly-209
4	**S-4**	−0.51	0.8	Asn-274, Arg-249, Asn-247, Ala-246, Asn-247, Gly-209
5	**S-5**	−0.48	0.8	Asn-274,Ala-246, His-244, Phe-213, Gly-209
6	**S-6**	−0.36	12.5	Ala-246, His-244, Gly-209, Ala-176
7	**S-7**	−0.36	12.5	Ala-246, His-244, Gly-209, Ala-176
8	**S-8**	−0.36	12.5	Ala-246, His-244, Gly-209, Ala-176

– 7.227 (1H, m, Ar-H), 7.19 – 7.13 (1H, m, Ar-H), 3,32 – 3.27 (2H, m, CH_2), 2.89 (2H, t, J = 6.1 Hz, CH_2), 2.2 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 30.06(CH_3), 37.07-37.34(CH_2-CH_2), 116.82(CH), 124.42(CH), 130.61(CH), 134.59(CH), 160.85(CH), 163.38(CH), 196.76(C=0), 207.16(C=0).

Physical constants and characterization of 1-(2-bromophenyl)pentane-2,5-dione, S4: Yellow colored oily liquid, yield – 75%. IR (ZnSe, cm^{-1}): (CH bending) 1434.17, (C=O str) 1714.12, 1688.10, (Ar C-H str) 3075.62; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.59 – 7.55 (1H, m Ar-H), 7.50 (1H, dd, Ar-H), 7.39-7.32 (1H, m,Ar-H), 7.31-7.23 (1H, m,Ar-H),3.140 (2H, t, J = 6.4 Hz, CH_2), 2.872 (2H, t, J = 6.4 Hz, CH_2), 2.2 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 29.89(CH_3), 36.35(CH_2), 37.38(CH_2), 118.50(CH), 127.45(CH), 128.76(CH), 131.63(CH),133.62 (CH), 141.29(CH), 202.47(C=0), 206.86(C=0).

Physical constants and characterization of 1-(2, 4-dichlorophenyl)pentane-2,5-dione, S5: Yellow colored oily liquid. Yield 95%. IR (ZnSe, cm^{-1}): (CH bending) 1446.43, (C=O str) 1701.60, (Ar C-H str) 3787.41; ^{1}H NMR (400 MHz, DMSO): δ 7.963 – 8.01 (1H,m, Ar-H), 7.87 (1H, s, Ar-H), 7.55 – 7.62 (1H, m, Ar-H), 3.20 (2H, t, J = 6.1 Hz,CH_2), 2.80 (2H, t, J = 6.1 Hz, CH_2), 2.15 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 25.07(CH_3), 31.78 (CH_2), 32.82 (CH_2),122.58 (CH) , 125.62 (CH), 125.73 (CH), 127.24 (CH), 132.42 (CH), 132.62 (CH), 195.57 (C=0), 202.03 (C=0).

Physical constants and characterization of 1-(3, 4-difluorophenyl)pentane-2,5-dione, S6: Pale yellow crystals. Yield 95%. m.p.48.9 – 52.4°C; IR (KBr, cm^{-1}): (CH bending) 1469.40, (C=O str) 1702, (Ar C-H str) 3068.17; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.88 – 7.72 (2H, m, Ar-H), 7.33 – 7.22 (1H, m, Ar-H), 3.23 (2H, t, J = 6.2 Hz, CH_2), 2.92 (2H, t, J = 6.2 Hz, CH_2), 2.28 (3H, s, CH_3);^{13}C NMR (100 MHz, $CDCl_3$): δ 30.02(CH_3), 32.24(CH_2), 36.99(CH_2), 117.28(CH), 117.41(CH), 117.59(CH), 125.04(CH), 125.08(CH), 125.12(CH), 196.03(C=0), 206.99(C=0).

Physical constants and characterization of 1-(3-bromophenyl)pentane-2,5-dione, S7: Yellow colored oily liquid. Yield 72%. IR (ZnSe, cm^{-1}): (CH bending) 1431.17, (C=O str) 1714.12, 1688.10, (Ar C-H str) 3075.62; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.101 (1H, dd,Ar-H), 7.90 (1H, d,J = 7.6 Hz, Ar-H), 7.69 (1H, d, J = 7.6 Hz, Ar-H), 3.230 (2H, t, J = 6.4 Hz, CH_2), 2.90 (2H, t, J = 6.4 Hz, CH_2), 2.2 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 30.02(CH_3), 32.44(CH_2), 36.96(CH_2), 122.93(CH), 126.59(CH), 130.21(CH), 131.12 (CH), 135.98(CH), 138.36(CH), 197.20(C=0), 207.07(C=0).

Physical constants and characterization of 1-(4-chlorophenyl)pentane-2,5-dione, S8: White color crystals. Yield 90%. m.p.56.2 – 58.4°C; IR (KBr, cm^{-1}): (CH bending) 1488.05, (C=O str) 1716, 1735, (Ar C-H str) 3064.66; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.957 (2H, d, J = 8.4 Hz, Ar-H), 7.472 (2H, d, J = 8.4 Hz, Ar-H), 3.261 (2H, t, J = 6.4 Hz, CH_2), 2.916 (2H, t, J = 6.4 Hz, CH_2), 2.283 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 30.07(CH_3), 32.35(CH_2), 37.01(CH_2), 128.92(CH), 129.48(CH), 134.98(CH), 139.63(CH), 197.33(C=0), 207.14(C=0).

Conclusion

The eight arylpentanediones showed activity comparable to that of hispolons, underlining the need for further exploration of their stability, safety and efficacy. The presence of a halogen atom at the *ortho* position of the aromatic ring has a significantly positive influence on their anti-tubercular activity. These experimental results have been in concurrence with the results of docking simulations on the target enzyme mtFabH. These molecules may serve as possible leads for identification of safe, selective and potent anti-tubercular agents.

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