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DESIGN AND SYNTHESIS OF 3-ETHYL 5-METHYL 2-((2- SUBSTITUTED AMINOETHOXY)METHYL)-4-(2-CHLOROPHENYL)-6-METHYL-1,4-DIHYDROPYRIDINE-3,5-DICARBOXYLATE ANALOGUES AS ANTI-TUBERCULAR AND ANTI-BACTERIAL AGENTS

Gutala Jeevan Raghavendra* and V Siddaiah

Department of Organic chemistry & FDW, Andhra University, Vishakapatnam-530003
E-mail: jeevan.gutala@gmail.com

Abstract:
A series of eleven novel 3-ethyl 5-methyl 2-((2- substituted aminoethoxy)methyl)-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate analogues were synthesized, characterized (^{1}H NMR, ^{13}C NMR and MS) and screened for their *in vitro* anti-tubercular activity against $MTBH_{37}Rv$ strain and anti-bacterial activity against *Pseudomonas aeruginosa* (gram-negative), *Escherichia coli*(gram-negative), *Bacillus subtilis* (gram-positive),*Staphylococcus aureus* (gram-positive). Many of these compounds exhibited MIC values in the range 12.5-50µg/mL against *Mycobacterium tuberculosis* $H_{37}R_V$. Compounds **3i** and **3j** were found to be the active with an MIC of 12.5µg/mL. Compound **3f** has exhibited moderate activity with MIC of 25µg/mL. Compounds **3i** exhibited good anti-bacterial activity against *Pseudomonas aeruginosa* with 8mm, *Bacillus subtilis* with 9mm of inhibition.

Keywords: Anti-tubercular activity, Anti-bacterial activity, Amlodipine, Amide

Introduction

Tuberculosis (TB) is the leading airborne contagious disease caused by *Mycobacterium tuberculosis* (MTB). Tuberculosis (TB) stills a major health risk, affecting millions of people and number of deaths throughout the world. The World Health Organization (WHO) has reported that TB causes 1.8 million deaths, including 0.4 million deaths among people with HIV in 2015[I]. Worldwide in 2015, an estimated 480 000 new cases of multidrug-resistant TB (MDR-TB) and an additional 100000 people with rifampicin-resistant TB (RR-TB) who were also newly eligible for MDR-TB treatment. In 2015, an estimated 9.5% of people with MDR-TB had extensively drug resistant TB (XDR-TB) (WHO Report 2016). Although TB is curable and treatable at early stages, its drug-susceptible form requires at least six months of therapy with, first-line anti-TB drugs isoniazid (**1**), pyrazinamide (**2**) and ethambutol (**3**) [**Figure 1**].

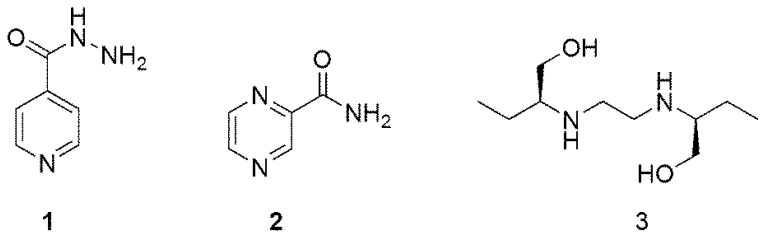


Figure 1: Anti-tubercular agents

From past few decades, many scientists have designed, synthesized a number of new drugs, and derivatives of old drugs, in an attempt to find new treatments for tuberculosis. Antibiotics are most important weapons in fighting bacterial infections[II-III].

Amlodipine (AML) (**4**) is a dihydropyridine L-type calcium channel blocker and also it exhibit remarkable antibacterial activity against several gram-positive and gram-negative strains. AML is the most powerful of the cardiovascular drugs with antibacterial activity [IV] and also have anti-inflammatory activity. AML is decrease ischemia-reperfusion injury by improving the oxidative status in ileum ischemia-reperfusion induced rabbits and beneficial in the treatment of rhinosinusitis[V-VI]. AML acts as anti bacterial agent, combination with streptomycin against several Gram-positive and Gram-negative bacterial strains *invitro* as well as *invivo*in murine salmonellosis model. AML exhibits bactericidal activity against *Listeria monocytogenes* and *Staphylococcus aureus* and it has able to cure highly virulent bacterial and parasitic infections [VII-VIII].

Recently, studies showed that 1,4-dihydropyridine derivatives with lipophilic groups have significant anti-tubercular activity against M. tuberculosis H37Rv [IX-X]. Desai *et al*., synthesized 1,4-dihydropyridine-3,5-dicarbamoyl derivatives (**5**) and screened for their anti-tubercularactivity against *M. Tuberculosis* H37Rv[XI-XII]. These studies suggest 2nd position with chloro on phenyl ring exhibited >90% inhibition against H37Rv comparable to other substituted phenyls[XIII]. Trivedi *et al*., synthesized novel *N*-aryl-1,4-dihydropyridines as potential anti-tubercular agents. Among the synthesized compoundsdiethyl 1-(2-chlorophenyl)-1,4-dihydro-2,6-dimethyl-4-(1,3-diphenyl-1*H*-pyrazol-4-yl)pyridine-3,5-dicarboxylate (**6**)derivative was more potent than first line anti-tubercular drug isoniazid and showed relatively low cytotoxicity [XIV]. 1,4-dihydropyridine analogues have depicted in **Figure 2**.

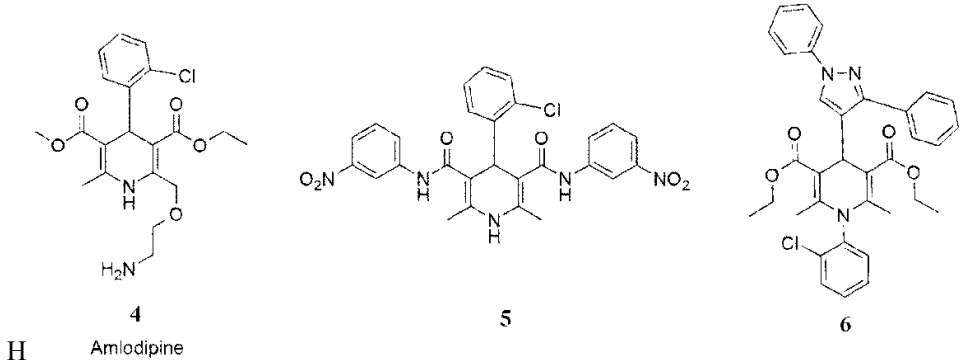


Figure 2: 1,4-dihydropyridine analogues

In order to realize the structure–antitubercular activity relationship, we decided to prepare some novel 1,4-dihydropyridine derivatives and determined their inhibitory activity against *M. tuberculosis* H37Rv and antibacterial activity against gram positive and gram negative

bacterial strains. Based on previous reports we designed and synthesized amlodipine analogues then evaluated their bio activity.

Experimental sections:

Chemistry

All reagents were purchased from commercial sources and used with further purification wherever necessary. All reactions were monitored by analytical thin layer chromatography (TLC) performed on E-Merck 0.25 mm pre coated silica gel aluminum plates (60 F254) using mixture of pet ether and ethyl acetate. Visualization of the spots on TLC plates was achieved by exposure to UV light. Column chromatography was performed using silica gel (Acme, 100-200mesh). Solvents were dried and purified by distillation prior to use. Solvents for chromatography (Pet ether and ethyl acetate) were distilled prior to use. Evaporations were carried out under reduced pressure on Heidolph rotary evaporator. Melting points were obtained using Stuart SMP30 system and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on Avance-III 400MHz (400 MHz for ^{1}H, 100 MHz for ^{13}C), in $CDCl_3$. Chemical shifts have been expressed in parts per million (δ) relative to tetramethylsilane (δ = 0.0) as an internal standard and coupling constants (*J*) in Hertz.

Biology

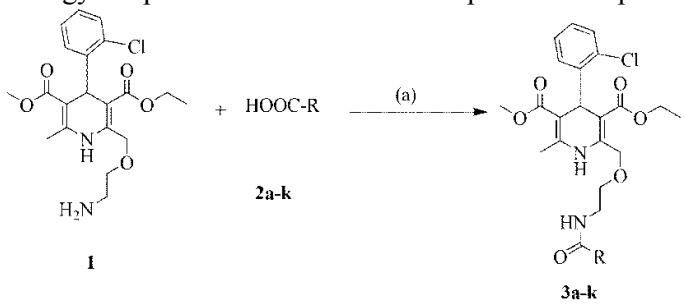
Anti Bacterial Activity

Synthetic compounds were tested against the strains for their inhibitory activity by well plate or disc diffusion method. Nutrient broth used for inoculum preparation and Muller-Hinton agar media have been used for screening the antimicrobial activity. The wells were created in Mueller Hinton solid agar medium with well puncture10, Trypton soy agar or Nutrient agar. The media has been pre-inoculated with test organisms. The standard inoculum size is of 1-2 x 108 CFU/ml of bacteria for inoculating diffusion plates which is equal to McFarland 0.5 turbidity standard. After that all the test compounds were inoculated into the wells and plates incubated at 37°C for 24 hrs. Each test was carried out in triplicate with controls. Microbial growth was determined by measuring the diameter zone of inhibition with the help of scale.

Results and discussion

Chemistry

The synthetic strategy adopted to obtain the title compounds is depicted in **Scheme 1**.



Reagents and Conditions: EDC.HCl (2.0 eq), HOBt (0.1eq), Et_3N (3eq), CH_2Cl_2, RT, 12h.

A series of 3-ethyl-5-methyl-2-((2-substituted acetamidoethoxy) methyl)-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate derivatives**3a-k** of amlodipine derivatives were prepared by using coupling reagents EDC.HCl and HOBt. In general ^{1}H NMR of all the title compounds displayed singlet in the range of 8.7-7.7 ppm corresponding to amide proton. A broad singlet due to the proton of amine in the range of 5.2-6.5 ppm. A sharp singlet resonated in the range of 3.4-3.7 ppm due to methoxy protons. Another sharp singlet in the range of 2.3-2.7 ppm due to the methyl protonsobserved. Methyl protons in ethyl group

resonated as triplet in the range of 1.1-1.25 ppm. Further, the structure of the title compounds were substantiated from ^{13}C NMR and ESI MS respectively. All the compounds were evaluated for their anti-tubercular activity and the results are summarized in **Table 1**.

Antitubercular activity

The compounds, **3a-k**, were tested for anti-tubercular activity against $MTBH_{37}Rv$ strain. The active compounds exhibited MIC in the range 12.5-50µg/mL. Compounds **3i** and **3j** were the most active compounds with MIC 12.5µg/mL (Table 1). Compound **3f** was exhibited moderate activity with MIC of 25 µg/mL.

The SAR study revealed that introducing electron withdrawing at various positions on phenyl ring exhibited moderate anti-tubercular activity. Introduction of electron donating group on the phenyl ring (**3f**) alter the activity spectrum. Interestingly, indole substitution with aliphatic chain (**3i** and **3j**, MIC 12.5 µg/mL) enhanced the activity by two fold as compared to **3f**. The enhanced activity of **3i** and **3j** might be attributed to the presence of indole group. These encouraging results further pave the way to explore different substituents on the indole group.

The compounds, **3a-k**, were tested for *Pseudomonas aeruginosa* (gram-negative), *Escherichia coli* (*E. coli*) (gram-negative), *Bacillus subtilis* (gram-positive), *Staphylococcus aureus* (gram-positive). Based on SAR study chloro containing derivatives (**3d**) exhibited moderate activity against *Pseudomonas aeruginosa* (7 mm) and *Staphylococcus aureus* (7 mm). Indole substitution with aliphatic chain derivative (**3i**) exhibited good activity against *Pseudomonas aeruginosa* (8 mm) and *Bacillus subtilis* (9 mm). The increasing aliphatic chain of indole was not impact on antibacterial activity.

Conclusion

In conclusion, this work has revealed the synthesis, and *in vitro* anti-tubercular and antibacterial activity of the new amlodipine derivatives. Amongst, the synthesized compounds, compounds**3i** and **3j** were found to be the active with an MIC of **12.5 µg/mL**. Compound **3f** was exhibited moderate activity with MIC of **25 µg/mL**. Compound**3i** exhibited good antibacterial activity against *Pseudomonas aeruginosa* with 8mm, *Bacillus subtilis* with 9mm of inhibition. The anti-tubercular and antibacterial activity SAR profile suggests that tailoring indole by means of appropriate substituent's or functional groups might provide an insight to obtain the lead compound. From the anti-tuberculararand antibacterial activity SAR summary, it is marked that introducing indole or in combination at thepara position of phenyl ring would give worthwhile information for further lead molecule to developanti-tubercular and antibacterial agent.

Experimental section

Chemistry

General procedure for the preparation of3-ethyl 5-methyl 2-((2-substituted acetamidoethoxy)methyl)-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate derivatives (3a-k)

To a solution of amlodipine (0.4891 mmol) in dry DCM (2mL), triethylamine (0.28mL, 2.054 mmol), EDC.HCl (225mg, 1.1738 mmole), HOBt (7.9mg, 0.05869) and various acids (0.5869 mmole) were added at RT under N_2 atmosphere. After the reaction was complete, as indicated by TLC, reaction mixture was diluted with 20 mL of water. The compound was extracted with CH_2Cl_2 (3×5mL). The organic layers were collected, washed with saturated brine solution, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The resultant crude was purified by column chromatography [ethyl acetate (10-50%) in hexane] to get the title compounds.

(E)-3-ethyl 5-methyl 4-(2-chlorophenyl)-6-methyl-2-((2-(3-(3-nitrophenyl)acrylamido)ethoxy)methyl)-1,4-dihydropyridine-3,5-dicarboxylate (3a)
Pale yellow solid; yield: 87%, m.p. 146-148 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.39 (s, 1H), 8.21 (d, *J* = 8.34 Hz, 1H), 7.80-7.73 (m, 2H), 7.59 (d, *J* = 8.14 Hz, 1H), 7.41 (d, *J* = 8.34 Hz, 1H), 7.25-21 (m, 2H), 7.18 (t, *J* = 8.14 Hz, 1H), 7.11 (t, *J* = 8.34 Hz, 1H), 6.58 (d, *J* = 6.5 Hz, 1H), 6.08 (s,1H), 5.41 (s, 1H),4.81-4.62 (q, 2H), 4.07-4.01 (m, 2H), 3.79-3.64 (m, 4H), 3.59 (s, 3H), 2.38 (s, 3H), 1.18 (t,*J* = 4.75 Hz, 3H).^{13}C NMR (101 MHz, $CDCl_3$) δ 168.00, 167.19, 165.36, 148.67, 145.69, 144.81, 144.14, 138.99, 136.44, 134.12, 132.33, 131.46, 129.99, 129.23, 127.38, 126.84, 124.13, 123.37, 121.56, 103.87, 101.72, 70.47, 68.08, 59.86, 50.78, 39.78, 37.16, 19.35, 14.23. ESI-MS (m/z): calcd. for $C_{29}H_{30}ClN_3O_8$ 583.24, found 584.28 $[M + H]^+$.

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References:

I. WHO Report 2016.Global Tuberculosis Report. Geneva: WHO, 2016 http://www.who.int/tb/publications/factsheetglobal.pdf.
II. Spigelman, M.; Gillespie, S. *Lancet,* **2006,** *367,* 945–947.
III. Vanden, B. J.; Kibiki, G.S.; Kisanga, E.R.; Boeree, M.J.; *Antimicrob Agents Chemother,* **2009**, *53*, 849–862.
IV. Mazumdar, K.; Asok Kumar, K.; Dutta, N.K.; Int J Antimicrob Agents, **2010**, *36,*295-302.
V. Kumar, K. A.; Ganguly, K.; Mazumdar, K.; Dutta,N. K.; Dastidar, S.G.; Chakrabarty, A.N.; *ActaMicrobiol Pol,* **2003**, *52,* 285-292.
VI. Coskun, A.K.; Gunal, A.; Halici, Z.; Oral, A.; Seyrek, M.; Bayir, Y.; *Eurasian J Med,* **2011**, *43*, 33-38.
VII. Arzu, T.; Mukadder, K.; Muhammed, Y.; Elif, P.; Hakan, U.; Zekai, H.; Secil, N.P.; Braz J Otorhinolaryngolhttp://dx.doi.org/10.1016/j.bjorl.2016. 08.006.
VIII. Mazumdar, K.; Ashok, K, K,; Dutta, N.K.; *International Journal of Antimicrobial Agents* **2010,** *36*, 295–302.
IX. Khoshnevizadeh, M.; Edraki, N.; Javidnia, K.; Alborzi, A.; Pourabbas, B.; Mardenah, J.; Miri, R. *Bioorg Med Chem.* **2009,** *17*, 1579-1586.
X. Manvar, A.T.; Pissurlenkar, R.R.; Virsodia, V.R.; Upadhyay, K.D.; Manvar, D.R.; Mishra, A.K.; Acharya, H. D.; Parecha, A.R.; Dholakia, C.D.; Shah, A.K.; Coutinho, E.C. *MolDivers* **2010,** *14,* 285-305.
XI. Kharkar, P.S.; Desai, B.; Gaveria, H.; Varu. B.; Loriya, R.; Naliapara, Y.; Shah, A.; Kulkarni, V. M. *J Med Chem,* **2002,** 45:4858-4867.
XII. Sirisha, K, Achaiah, G.; Reddy, V. M.; *Arch Pharm,* **2010,** *6,* 342-352.
XIII. Bhavik, D.; Dinesh. S.; Yogesh, N.; Anamik, S.; Anil, K.S.; *Bioorganic & Medicinal Chemistry*, **2001,** *9,* 1993–1998.
XIV. Trivedi ,A.R.; Dodiya, D.K.; Dholariya, B.H.; Kataria, V. B.; Bhuva, V.R.; Shah, V. H.;. *Bioorganic & Medicinal Chemistry Letters,* **2011**, *21*, 5181–5183.

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