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Antitubercular activity of some new Azomethine compounds

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Abstract

Derivatives of 4-amino salicylic acid were prepared and characterised by FTIR, ^{1}HNMR and^{13}CNMRspectra. They were screened for antitubercular activity against *Mycobacterium tuberculosis* by disc well method. 4-amino salicylic acid was used as standard drug for antitubercular activity.

Keywords: 4-amino salicylic acid, antitubercular activity and *Mycobacterium tuberculosis*.

Introduction

Organic and inorganic molecules like sulfanilamide, streptomycin, penicillin, dapsone, benzocaine, phenylethylamine, $KMnO_4$, H_2O_2, and $AgNO_3$ etc. possess wide range of biological and pharmacological activities[1]. Much attention has been paid during the last century to nitrogen, sulphur and oxygen moieties associated with a broad spectrum of activities such as antibacterial[2], antifungal[3], antitubercular[4], antileprosy[5], anti-inflammatory[6], anti-cancer[7]etc, The demand for a new chemotherapeutic agents is substantially high in the last century due to increased resistance towards various available antibiotics. An attempt has been made to prepare a new derivatives of 4-amino salicylic acid. Our present study deals with the condensation of 4-amino salicylic acid and ethyl-4-amino benzoate with 4-chloro benzaldehyde, 4-bromo benzaldehyde, benzophenone, acetophenone, vanillin, nitrovanillin, in the presence of ethanol and acetic acid to afford corresponding azomethine derivatives[7-13]such as(I)2-ydroxy-4-((2-hydroxy-3, 5-diiodobenzylidene)amino) benzoic acid, (II)4-((4-bromobenzylidene) amino)-2-hydroxybenzoic acid, (III)4-((4-chlorobenzylidene) amino)-2-hydroxybenzoic acid, (IV)4-((5-bromo-2-hydroxybenzylidene) amino)-2 hydroxybenzoic acid, (V)2-hydroxy-4-(((5-methylfuran-2-yl)methylene) amino)benzoic acid, (VI)2-hydroxy-4-((4-hydroxy-3-methoxy-5-nitrobenzlidene)amino)benzoic acid, (VII)2-hydroxy-4-((3-hydroxybenzylidene) amino) benzoic acid, (VIII)4-((3-carboxy- 4-hydroxy benzylidenne)amino)-2-hydroxybenzoic acid, and (IX)2-hydroxy-4-((3,4,5-trimethoxybennzylidene) amino)benzoic acid. The new azomethine compounds are characterized by spectral data such as FTIR, ^{1}HNMR, ^{13}CNMR and also analytical and physical methods. The azomethine compounds are screened for antitubercular activity against strains of *Mycobacterium tuberculosis*.

Materials and Methods

Materials

All the reagents used were of AR grade. Commercially available rectified spirit was dried over anhydrous quicklime for 24 hours, filtered and distilled before use (BP 78^{o}C). Dimethylsulphoxide (sigma) and N,N-dimethylformamide (sigma) were used as such.

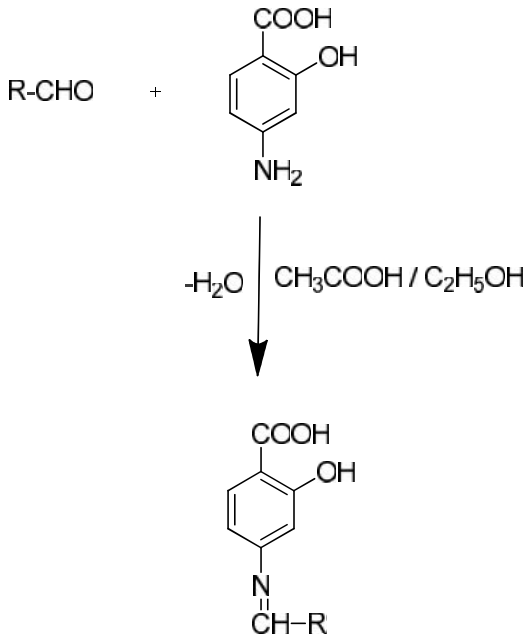
4-amino salicylic acid, ethyl-4-amino benzoate, 4-chlorobenzaldehyde, 4-bromobenzaldehyde, 3-hydroxybenzaldehyde, 3,5-diiodosalicylaldehyde, 5-formyl salicylic acid, 5-nitrovaniline, 5-bromosalicylaldehyde, 3,4,5-trimethoxy benzaldehyde and 5-methyl-2-furaldehyde were purchased from Alfa Aesar.

Instruments

Melting points were determined using Elico melting point apparatus. Elemental analysis were performed using Elementar Vario EL III. IR spectra of the compounds were recorded with KBr pellets with carry 630 FTIR Spectrometer in the 4000-400 cm^{-1}range. The ^{1}HNMR and ^{13}CNMRspectra were recorded on a Bruker 400 MHz FT- PMR Spectrometer.

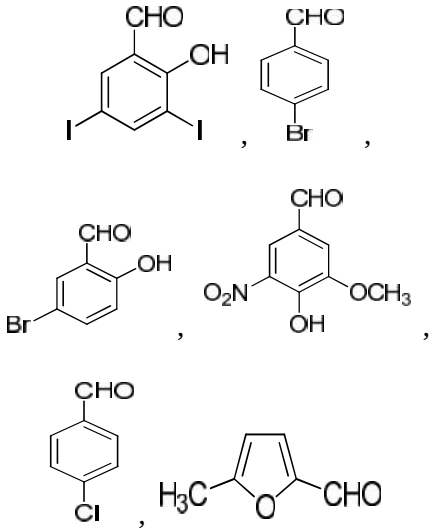
General preparation of derivatives of 4-amino salicylic acid.

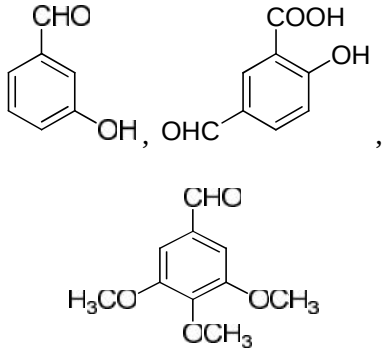
All the azomethine compounds of derivatives of 4-amino salicylic acid were prepared[8]by the following scheme - 1



Scheme.1

Where R-CHO =





Preparation of (I)2-hydroxy-4-((2-hydroxy-3, 5-diiodobenzylidene)amino)benzoic acid

25ml of ethanolic solution of 4-amino salicylic acid (1.53g 0.01mol) was added to 25ml of ethanolic solution of 3,5-diiodosalicylaldehyde (3.73g 0.01mol). Then three drops of conc. $H_2SO_4$was added to the reaction mixture. The reaction mixture was heated and refluxed for about 5 hours at 90°C. The resulting solution was further concentrated by water bath. The product 2-hydroxy-4-((2-hydroxy-3, 5-diiodobenzylidene)amino)benzoic acid obtained was precipitated, cooled and collected after filtration. The precipitate was purified by washing with distilled water and then with ethanol. The 2-hydroxy-4-((2-hydroxy-3, 5-diiodobenzylidene)amino)benzoic acid was again recrystallized in ethanol and then dried over vacuum desiccator.

Preparation of (II)4-((4-bromobenzylidene)amino)-2-hydroxybenzoic acid

Three grams of 4-amino salicylic acid (0.02mol) was mixed with 3.6g of 4-bromobenzaldehyde(0.02mol) and was grained well in acidic acid medium at room temperature. The mixture was transferred into hundred milliliter Round Bottom flask and was refluxed for six hours in oil bath. The solid product 4-((4-bromobenzylidene)amino)-2-hydroxybenzoic acid was filtered and washed with ethanol and recrystallized in DMSO and then dried over vacuum desiccator.

Preparation of (III) 4-((4-chlorobenzylidene)amino)-2-hydroxybenzoic acid

A mixture of 4-chlorobenzaldehyde (1.8g, 0.01mol) and 4-amino salicylic acid (1.53g, 0.01mol) was grained in a mortar with a pestle made of porcelain for 10 minutes. The mixture turned pasty after few minutes of grainding. It was grained till brown Colour product appears. The mixture was left overnight. The resultant product 4-((4-chlorobenzylidene)amino)-2-hydroxybenzoic acid was recrystallized using ethanol and then dried over vacuum desiccator.

Preparation of (IV)4-((5-bromo-2-hydroxybenzylidene)amino)-2-hydroxybenzoic acid

Equimolar quantities of 0.01 mole of p-aminosalicylic acid (1.53g,0.01mm) and4-bromo benzaldehyde (1.85g,0.01mm) were dissolved in 20 ml of DMSO and 3 drop of glacial acetic acid was added and refluxed for 3 hours. After completion of the reaction (monitored by TLC) , some solvent was distilled out, the reaction mixture was poured on ice cold water and the solid 4-((5-bromo-2-hydroxybenzylidene)amino)-2-hydroxybenzoic acid came out which was filtered and then recrystallized by DMSO and then dried over vacuum desiccator.

Preparation of (V) 2-hydroxy-4-(((5-methylfuran-2-yl)methylene)amino)benzoic acid

2-hydroxy-4-(((5-methylfuran-2-yl)methylene)amino) benzoic acid was prepared from equimolar quantity of 4-amino salicylic acid(1.53g,0.01mol) and 5-methyl-2-furaldehyde (1.10g,0.01mol) in 30 ml of methanol were heated at 70^{o}C on water bath for 4-hrs in presence of few drops of glacial acetic acid. The crude product were obtained after removal of methanol under reduced pressure. The products were recrystallized from methanol and then dried over vacuum desiccator.

Preparation of (VI)2-hydroxy-4-((4-hydroxy-3-methoxy-5-nitrobenzlidene)amino)benzoic acid

A mixture of nitro vanillin1.95g(0.01mol) and 4-amino salicylic acid1.53g(0.01mol) were grained with a pestle in an open mortar at room temperature for 3 minutes. To this reaction mixture sulphuric acid 2 dropsand 20ml DMF were added and grained for 5 minutes. On completion of reaction as monitored by TLC, the light greenish-colored solid 2-hydroxy-4-((4-hydroxy-3-methoxy-5-nitrobenzlidene)amino)benzoic acid was separated out. The obtained solid was isolated by simple Buchner filtration and was recrystallized from DMF and then dried over vacuum desiccator.

Preparation of (VII) 2-hydroxy-4-((3-hydroxybenzylidene)amino) benzoic acid

The 2-hydroxy-4-((3-hydroxybenzylidene) amino) benzoic acid was prepared by stirring a methanolic solution of paraaminosalicylic acid (1.53g,0.01mol) with 3-hydroxy benzaldehyde (1.22g,0.01mol) in 1:1 stoichiometric ratio at room temperature over 24hours. The precipitate obtained were filtered and washed with methanol and recrystallized from methanol and then dried over vacuum desiccator.

Preparation of (VIII)4-((3-carboxy- 4-hydroxybenzylidenne)amino)-2-hydroxybenzoic acid

4-amino salicylic acid(1.53g,0.01mol) was dissolved in 5ml of hot glacial acetic acid, 1.66g (0.01mol) of 5-formyl salicylic acid was dissolved in 5ml of glacial acetic acid and were mixed. The reaction mixture was refluxed with stirring for 5 hours. The mixture was allowed to cool, and poured onto ice. The crude solid 4-((3-carboxy- 4-hydroxybenzylidenne)amino)-2-hydroxybenzoic acid was filtered off and washed with distilled water, then re-crystallized from acetic acid and then dried over vacuum desiccator.

Preparation of (IX)2-hydroxy-4-((3,4,5-trimethoxybennzylidene)amino)benzoic acid

To the hot solution of 4-amino salicylic acid 1.53g (0.01mol)in minimum amount of dimethyl formamide, and the DMF solution of 3,4,5-trimethoxy benzaldehyde1.96g (0.01mol) were mixed. The reaction mixture was refluxed for 3 hrs. The brown coloured solid mass 2-hydroxy-4-((3,4,5-trimethoxybennzylidene)amino)benzoic acid was formed during refluxing. The crude product was cooled, filtered, washed with ethanol, ether and re-crystallized from DMF and then dried over vacuum desiccator.

Antitubercular susceptibility test by Disc Well Technique

Principle

Disc impregnated with known concentration of antitubercular drug are placed on an agar plate that has been inoculated uniformly over the entire plate with a culture of the bacterium to be tested. The plate is incubated for 18 to 24 hours at 37^{o}C. During this period, the antimicrobial agent diffuses through the agar and may prevent the growth of the organism. Effectiveness of susceptibility is proportional to the diameter of the inhibition zone around the disc. Organisms which grow up to the edge of the disc are resistant.

Procedure

The plate was labeled with the name of the culture, sample and standard at the bottom of the plate. Then sterile cotton swab on a wooden applicator stick was dipped into the bacterial suspension. Excess fluid was removed by rotating the swab and rubbed gently over the plate to obtain uniform distribution of the inoculums. The sterile disc was held on the inoculated plate with the help of micropipette. The sample was leveled in the sterile disc and incubated at 37^{o}C in an incubator. After incubation, the diameter of the zone of inhibition of growth was measured[9].

Table.1 Observation of antitubercular activity.

Observation	Report
Inhibition zone > 15mm	**Highly active**
Inhibition zone > 10mm	**Moderatively active**
Inhibition zone > 5mm	**Slightly active**
Inhibition zone ≤ 5mm	**Inactive**

Results and Discussion

The physical and analytical data of the derivatives(I)2-hydroxy-4-((2-hydroxy-3, 5-diiodobenzylidene) amino)benzoic acid, (II)4-((4-bromobenzylidene) amino)-2-hydroxybenzoic acid, (III)4-((4-chlorobenzylidene) amino)-2-hydroxybenzoic acid, (IV)4-((5-bromo-2-hydroxybenzylidene)amino)-2-hydroxybenzoic acid, (V)2-hydroxy-4-(((5-methylfuran-2-yl)methylene) amino)benzoic acid, (VI)2-hydroxy-4-((4-hydroxy-3-methoxy-5-nitrobenzlidene)amino) benzoic acid,

(VII)2-hydroxy-4-((3-hydroxybenzylidene) amino) benzoic acid, (VIII)4-((3-carboxy- 4-hydroxy benzylidenne)amino)-2-hydroxybenzoic acid, and (IX)2-hydroxy-4-((3,4,5-trimethoxybenzylidene) amino)benzoic acid are given in table.2.

(I)2-hydroxy-4-((2-hydroxy-3, 5-diiodobenzylidene)amino)benzoic acid.

FTIR (cm^{-1})=(-CH=N)1591,(-OH)3388, (-C-I)538,(-C=O)1663

^{1}H NMR δ(ppm) =.(-CH=N)8.39, (-OH)5.35, (-COOH)11.0

^{13}C NMR δ (ppm) = (-CH=N)160.0, (-C-OH)163.5, (-COOH)171.8, (-C-I)83.8

(II)4-((4-bromobenzylidene) amino)-2-hydroxybenzoic acid.

FTIR (cm^{-1}) =(-CH=N)1607, (-OH)3097, (-C-Br)544, (-C=O)1670

^{1}H NMR δ (ppm) =(-CH=N)8.39, (-OH)5.35, (-COOH)11.0

^{13}C NMR δ (ppm) = (-CH=N)160.0, (-C-OH)163.5, (-COOH)171.8, (-C-Br)125.4

(III)4-((4-chlorobenzylidene) amino)-2-hydroxybenzoic acid.

FTIR (cm^{-1}) = (-CH=N)1593, (-OH)3376, (-C-Cl)722,(-C=O)1627

^{1}H NMR δ (ppm) =.(-CH=N)8.39, (-OH)5.35, (-COOH)11.0

^{13}C NMR δ (ppm) = (-CH=N)160.0, (-C-OH)163.5, (-COOH)171.8, (-C-Cl)136.6

(IV)4-((5-bromo-2-hydroxybenzylidene)amino)-2-hydroxybenzoic acid.

FTIR (cm^{-1}) = (-CH=N)1601, (-OH)3373, (-C-Br)552,(-C=O)1622

^{1}H NMR δ (ppm) = (-CH=N)8.39, (-OH)5.35, (-COOH)11.0

^{13}C NMR δ (ppm) = (-CH=N)160.0, (-C-OH)163.5, (-COOH)171.8, (-C-Br)110.5

(V)2-hydroxy-4-(((5-methylfuran-2-yl)methylene)amino)benzoic acid.

FTIR (cm^{-1}) = (-CH=N)1600, (-OH)3380,($-CH_3$)2852,(-C=O)1627

^{1}H NMR δ (ppm) =.(-CH=N)7.50, (-OH)5.35, (-COOH)11.0, ($-CH_3$)2.30

^{13}C NMR δ (ppm) = (-CH=N)144.9, (-C-OH)163.5, (-COOH)171.8, ($-CH_3$)13.4

(VI)2-hydroxy-4-((4-hydroxy-3-methoxy-5-nitrobenzlidene)amino)benzoic acid.

FTIR (cm^{-1}) = (-CH=N)1602, (-OH)3359,(-C=O)1623,($-OCH_3$)1511,($-NO_2$)1382

^{1}H NMR δ (ppm) = (-CH=N)8.39, (-OH)5.35, (-COOH)11.0, ($-CH_3$)3.83

^{13}C NMR δ (ppm) = (-CH=N)160.0, (-C-OH)140.3, (-COOH)171.8, ($-CH_3$)56.1, ($-C-NO_2$)138.1

(VII)2-hydroxy-4-((3-hydroxybenzylidene)amino)benzoic acid.

FTIR (cm^{-1}) = (-CH=N)1592 (-OH)3373,(-C=O)1615

^{1}H NMR δ (ppm) = (-CH=N)8.39, (-OH)5.35, (-COOH)11.0

^{13}C NMR δ (ppm) = (-CH=N)160.0, (-C-OH)158.6, (-COOH)171.8,

(VIII)4-((3-carboxy-4-hydroxybenzylidene)amino)-2-hydroxybenzoic acid.

FTIR (cm^{-1}) = (-CH=N)1604, (-OH)3196(-C=O)1625

^{1}H NMR δ (ppm) = (-CH=N)8.39, (-OH)5.35, (-COOH)11.0

^{13}C NMR δ (ppm) = (-CH=N)160.0, (-C-OH)164.5, (-COOH)171.8

(IX)2-hydroxy-4-((3,4,5-trimethoxybennzylidene)amino)benzoic acid.

FTIR (cm^{-1}) = (-CH=N)1591,(-OH)3372(C=O)1629,(-OCH_3)1502,(-COOH)

^{1}H NMR δ (ppm) = (-CH=N)8.39, (-OH)5.35, (-COOH)11.0, (-CH_3)3.83

^{13}C NMR δ (ppm) = (-CH=N)160.0, (-OH)163.5, (-COOH)171.8, (-CH_3)56.1

Table.2 The physical and analytical data of TB drugs

Derivatives of TB drugs	M. Weight	Colour	M.P	Yield	Elemental analysis						
					C	H	O	N	Br	I	Cl
$C_{14}H_9I_2NO_4$(I)	509.86	Reddish brown	174	75 %	33.03	1.78	12.57	2.75	---	49.86	
$C_{14}H_{10}BrNO_3$(II)	320.14	Brown	154	69 %	52.52	3.15	14.99	44.38	24.96	--	
$C_{14}H_{10}ClNO_3$(III)	275.69	Brown	136	78 %	60.99	3.66	17.41	5.08	--	--	12.86
$C_{14}H_{10}BrNO_4$(IV)	336.14	Brown	140	80 %	50.02	3.00	19.04	4.17	23.77	--	--
$C_{13}H_{11}NO_4$(V)	245.23	Brown	153	68 %	63.67	4.52	26.10	5.17	--	--	--
$C_{15}H_{12}N_2O_7$(VI)	332.26	Brown	149	87 %	54.22	3.64	33.71	8.43	--	--	--
$C_{14}H_{11}NO_4$(VII)	257.24	Brown	139	70 %	65.37	4.31	24.88	5.44	--	--	--
$C_{15}H_{11}NO_6$(VIII)	301.25	Wine red	140	85 %	59.80	3.68	31.87	4.65	--	--	--
$C_{17}H_{17}NO_6$(IX)	331.32	Greenish brown	144	81 %	61.63	5.17	28.97	4.23	--	--	--

The observed values of FTIR, ^{1}HNMR, ^{13}CNMR, physical and analytical data (Table. 2) of derivatives of 4-amino salicylic acid I-IX consistent with reported values in the literature[10,13]. These values confirm the presence of -CH=N- , -C=O , -OCH_3, -CH_3, -NO_2 , -C-Cl , -C-Br , -C-I , -OH groups and the compound structure of scheme. 1 in the azomethine compounds.

Antitubercular activity

Antitubercular activity of all azomethine compounds are determined by Agar well diffusion method[11,15], as recommended by the national committee for clinical laboratory standards against *mycobacterium tuberculosis* at 25,50,75,100µg/ml concentration in dimethyl sulfoxide(DMSO) solvent.

The results are compared with standard drug 4-amino salicylic acid. The fresh culture of *Mycobacterium tuberculosis* are obtained by inoculating bacteria and nutrient. They are incubated at 37^0C for 24 hours. This culture mixed with nutrient agar media is poured into petridishes under aseptic conditions. After solidification of media, bores are made by using sterile cork borer (8mm diameter). Into these cups standard drug and synthesized drugs are introduced, the plates are placed in refrigirator at10^oC for proper diffusion of drugs into media. After 2 hours, the petriplates were transferred to incubator and maintained at 37±20^oC for 24 hours. After the incubation period, the petriplates were observed for zone of inhibition. The results are evaluated by comparing the zone of inhibition by the azomethine compounds with standard drug.

Table. 3 Antitubercular activity of TB drugs

SAMPLES	Zone of inhibition (mm/ml)				
	25 µl	50 µl	75 µl	100 µl	Control
$C_{14}H_9I_2NO_4$(I)	20	24	26	30	15
$C_{14}H_{10}Br\ NO_3$(II)	22	23	28	33	15
$C_{14}H_{10}\ Cl\ NO_3$(III)	15	17	20	23	15
$C_{14}H_{10}Br\ NO_4$(IV)	25	27	30	38	15
$C_{13}H_{11}NO_4$(V)	20	24	27	30	15
$C_{15}H_{12}N_2O_7$(VI)	14	16	18	20	15
$C_{14}H_{11}NO_4$(VII)	21	23	26	29	15
$C_{15}H_{11}NO_6$(VIII)	24	27	30	33	15
$C_{17}H_{17}NO_6$(IX)	12	14	16	20	15

Antitubercular activity of (I)2-hydroxy-4-((2-hydroxy-3, 5-diiodobenzylidene)amino)benzoic acid ($C_{14}H_9I_2NO_4$)(Fig.1), (II)4-((4-bromobenzylidene) amino)-2-hydroxybenzoic acid($C_{14}H_{10}Br\ NO_3$)(Fig.2), (III)4-((4-chlorobenzylidene) amino)-2-hydroxy benzoic acid($C_{14}H_{10}ClNO_3$) (Fig.3), (IV)4-((5-bromo-2-hydroxybenzylidene)amino)-2 hydroxy benzoic acid($C_{14}H_{10}BrNO_4$) (Fig.4), (V)2-hydroxy-4-(((5-methylfuran-2-yl)methylene) amino) benzoic acid ($C_{13}H_{11}NO_4$)(Fig.5), (VI)2-hydroxy-4-((4-hydroxy-3-methoxy-5-nitrobenzlidene)amino) benzoic acid ($C_{15}H_{12}N_2O_7$) (Fig.6), (VII)2-hydroxy-4-((3-hydroxy benzylidene) amino) benzoic acid($C_{14}H_{11}NO_4$) (Fig.7), (VIII)4-((3-carboxy- 4-hydroxybenzylidenne) amino)-2-hydroxybenzoic acid($C_{16}H_{14}NO_6$)(Fig.8), and (IX)2-hydroxy-4-((3,4,5-trimethoxybennzylidene) amino) benzoic acid($C_{17}H_{17}NO_6$)(Fig.9) are shown in the following figure 1-9.


Fig. 1


Fig. 2


Fig. 3


Fig. 4


Fig. 5


Fig. 6


Fig. 7


Fig. 8


Fig. 9

The antitubercular activity of azomethine compounds I-IX (table. 3 and figure 1-9) clearly indicate that all the compounds (I-IX) highly inhibit the growth of *Mycobacterium tuberculosis.*

The nature of bonding and structure of azomethine organic compounds are elucidated by the elemental analysis, melting point, FTIR, ^{1}HNMR ,^{13}CNMR,spectral analysis, chromatography and molar ratio methods. In accordance with the data obtained in the present investigation, it is found that the antitubercular activity of (I)2-hydroxy-4-((2-hydroxy-3, 5-diiodobenzylidene)amino)benzoic acid, (II)4-((4-bromobenzylidene) amino)-2-hydroxybenzoic acid, (III)4-((4-chlorobenzylidene) amino)-2-hydroxybenzoic acid, (IV)4-((5-bromo-2-hydroxybenzylidene)amino)-2 hydroxybenzoic acid, (V)2-hydroxy-4-(((5-methylfuran-2-yl)methylene) amino)benzoic acid, (VI)2-hydroxy-4-((4-hydroxy-3-methoxy-5-nitrobenzlidene)amino)benzoic acid, (VII)2-hydroxy-4-((3-hydroxybenzylidene) amino) benzoic acid, (VIII)4-((3-carboxy- 4-hydroxybenzylidenne)amino)-2-hydroxybenzoic acid, and (IX)2-hydroxy-4-((3,4,5-trimethoxybennzylidene) amino)benzoic acid have highly inhibited the growth[14] of *Mycobacterium tuberculosis.*

Conclusion

The derivatives of 4-amino salicylic acid(I)2-hydroxy-4-((2-hydroxy-3, 5-diiodobenzylidene)amino)benzoic acid, (II)4-((4-bromobenzylidene) amino)-2-hydroxybenzoic acid, (III)4-((4-chlorobenzylidene) amino)-2-hydroxybenzoic acid, (IV)4-((5-bromo-2-hydroxybenzylidene)amino)-2 hydroxybenzoic acid, (V)2-hydroxy-4-(((5-methylfuran-2-yl)methylene) amino)benzoic acid, (VI)2-hydroxy-4-((4-hydroxy-3-methoxy-5-nitrobenzlidene)amino)benzoic acid, (VII)2-hydroxy-4-((3-hydroxybenzylidene) amino) benzoic acid, (VIII)4-((3-carboxy- 4-hydroxybenzylidenne)amino)-2-hydroxybenzoic acid, and (IX)2-hydroxy-4-((3,4,5-trimethoxybennzylidene) amino)benzoic acid were prepared by the condensation of 4-chlorobenzaldehyde, 4-bromobenzaldehyde, 3-hydroxybenzaldehyde, 3,5-diiodosalicylaldehyde, 5-formyl salicylic acid, 5-nitrovaniline, 5-bromosalicylaldehyde, 3,4,5-trimethoxy benzaldehyde and 5-methyl-2-furaldehyde with 4-amino salicylic acid and were screened against *Mycobacterium tuberculosis.* It was shown that the growth of *Mycobacterium tuberculosis* were highly inhibited by the derivatives of 4-amino salicylic acid I-IX.

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