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Research Article

SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF SOME SCHIFF BASES OF 2-AMINO-N-(P-ACETAMIDOPHENYL CARBOXAMIDO)- 4,5,6,7-TETRAMETHYLENE THIOPHENES

MONICA ARORA*, J.SARAVANAN, S.MOHAN, SHIVAJI BHATTACHARJEE

Dept. of Pharmaceutical Chemistry, PES College of Pharmacy, Bangalore 560050, India. Email: monicaarora15@gmail.com

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ABSTRACT

The novel 2-amino-N-(p-acetamidophenyl carboxamido)-4,5,6,7-tetramethylene thiophenes was synthesized by using versatile Gewald Reaction and the parent compound [SM-2] was reacted with different substituted aryl aldehydes to obtain the series of compound [SM-2a-l].

All the new title compounds were characterized by spectral data and were screened for antibacterial activity against two Gram-positive bacteria i.e. *Staphylococcus aureus* & *Bacillus subtilus* and two Gram-negative bacteria i.e. *Escherichia coli* & *Klebsiella pneumoniae* using Ampicillin and Norflaxacin as standard, each at a concentration of 50µg/0.1ml, adapting agar diffusion method. The compounds were also screened for their antifungal activity against two pathogenic fungi i.e. *Candida albicans* and *Aspergillus niger* using Miconazole nitrate as standard at a concentration of 50µg/0.1ml, adapting agar diffusion method.

Keywords: Schiff bases, Aromatic aldehydes, 2-amino-3-carboxamido-4, 5, 6, 7 tetramethylene thiophene, Anti microbial activity.

INTRODUCTION

Thiophene derivatives have been known for pharmacological activities having various therapeutic applications. The benzo [b] thiophenes often present in pharmacologically active compounds, the literature survey also indicates that compounds having The benzo [b] thiophenes nucleus possess broad range of pharmacological activities namely antimicrobial [1], anti-inflammatory [2], CNS depressant activity [3], antifungal activity [4], analgesic [5], anti-tumor [6], alkaline phosphatase inhibitor [7].

Similarly Schiff Bases derivatives also have been reported to possess an array of biological activities therefore attracts interest both for synthetic and biological point of view. Schiff base exhibits antimicrobial [8], ulcerogenic [9], anti-HIV [10], anticonvulsant [11], CNS depressant activities [12]. Generally, In Pharmaceutical field new drugs are discovered by molecular modification of the lead compound of established activity. So far, a range of new thiophenes have been synthesized and screened for their biological activity. The encouraging results prompted us design and prepare new 2-amino benzo [b] thiophenes by adaptation of well known and versatile Gewald reaction [13] and their Schiff bases where in two moieties incorporating heterocycles are linked together through azomethine (-CH=N-) grouping and to study their anti microbial activity.

MATERIAL AND METHODS

Chemicals and Instrument

Ammonia(25%), ethyl cyanoacetate, p-amino acetophenone, acetic anhydride, sulphur, diethyl amine, 4'-dimethyl amino benzaldehyde, 3',4'5'-trimethoxy benzaldehyde, 3',4'-dimethoxy benzaldehyde, 4'-methoxy benzaldehyde, 4'-hydroxy 3'-methoxy benzaldehyde, 4'-hydroxy benzaldehyde, 2'-nitro benzaldehyde, 3'- nitro benzaldehyde, 4'-nitro benzaldehyde, 2'-chloro benzaldehyde, 2'-hydroxy benzaldehyde, 4'-chloro benzaldehyde, 4' methyl benzaldehyde were obtained from local dealer.

Analytical TLC was performed on Silica plates- GF254 (Merck) with visualization by UV or iodine vapors. Melting points were determined in open capillaries on a Thermonic Melting point apparatus and are uncorrected. The IR spectra (KBr, λ Max, cm^{-1}) were run on Perkin Elmer FTIR Spectrophotometer. 1H-NMR (in$CDCl_3$ / DMSO-d6) spectra were recorded using AMX-400 with TMS as internal standard. MS spectra were recorded on Brucker DPX 200. Elemental analyses were performed on Carlo Erba 1108 elemental analyzer and were within ± 0.4% of theoretical values. All the chemicals used were of analytical grade.

Preparation of p-acetamido cyano acetanilide

Condensation of equimolar p-amino acetanilide and ethyl cyano acetate at a temperature of 160-170^{0}C for 8 hrs and cooled overnight to yield the 2-cyano-N-(o-fluorophenyl)-acetamide. Yield 55 %.

Preparation of 2-amino-N-(p-acetamidophenyl carboxamido)-4, 5, 6, 7-tetramethylene thiophenes [SM-2]

A mixture of Cyclohexanone (4.12ml, 0.04mol), p-acetamido cyano acetanilide (8.68gm, 0.04 mol), ammonium acetate (1gm) and glacial acetic acid (2 ml) in benzene (100 ml) was refluxed for 12 hours in Dean Stark apparatus with continuous separation of water. After 12 hours the reaction mixture was cooled. On cooling the liquid reaction mixture turned to fine crystalline solid which was employed for further reaction.

To a mixture of the above crude intermediate, sulphur (0.04 mol) in ethanol (40 ml) and diethyl amine (4.0 ml) was added drop wise with stirring. The mixture was stirred for 4 hours at 45–50 °C, Excess benzene was distilled off, chilled overnight the reaction mixture turned to be fine crystalline solid which was filtered, washed with ethanol to yield crystalline solids. Recrystallized from alcohol. Yield 45 %.

General method for synthesis of 2- [(substituted benzylidene) amino]-N-(p-acetamidophenyl carboxamido)-4, 5, 6, 7-tetramethylene thiophene Schiff Base (SM-2a-2l)

A mixture of the starting compound **(SM-2)** (0.005 M) and the required substituted aryl aldehydes (0.005 M) in ethanol (30 ml) and catalytic amount of glacial acetic acid (2 ml) was subjected to reflux for 2 hours, the reaction mixture was allowed to cool. Solid obtained was filtered, washed with ethanol, dried and recrystallized using DMF: Water in a ratio of (4:1) to get the pure title compounds (SM-2a-2l).

2-(4-(dimethylamino benzylideneamino)-N-(4-acetanilido) 4,5,6,7-tetra methylene thiophene-3-carboxamide (SM-2a)

M.P. 130^{0}C; yield: 57%; MS: 460(100%), 362(60%), 155(70%), 229(50%);

IR max cm^{-1}: 3473.22 (-NH); 3124.69 (Ar-CH); 2824.43 (CH_2); 2972.16 (Ali-CH); 1622.65 (C=O); 1599.03 (-NH bend); 1500.65 (Ar C=C); 1650.11(HC=N); 854.00(C-N); 755.69(C-S)

^{1}HNMR($CDCl_3$):δ=8.3(1H,s,N=CH);8.2(2H,s.CONH),7.6(4H,m,aromatic);7.37.5(4H,m,aromatic) ;2.3(3H,s,CH_3); 2.6(6H,s,of 2CH_3); 2.8(8H,m of CH_2 of cyclohexane)

[13]C NMR: 20.2-25.6 (4C of cyclohexane ring) 23(3C,CH_3 of CONH) 40.53(C of CH_3);118-148(C aromatic ring);145(2C of thiophene ring);130(C,thiophene ring);160(C,thiophene ring), 161(2C,CONH);163(C,N=CH)

Elemental analysis: C- 67.8%, H-6.13%, N-12.16%, O-6.94%, S-6.96%

2-(4-hydroxy benzylidene amino)-N-(4-acetanilido)-4,5,6,7-tetra methylene thiophene-3-carboxamide (SM-2b)

M.P. 120^{0}C; yield: 53%; MS: 433(100%), 362(60%), 155(70%), 229(50%);

IR max cm-1: 3499.78 (-OH) 3473.22 (-NH); 3124.69 (Ar-CH); 2824.43 (CH2); 2972.16 (Ali-CH); 1622.65 (C=O); 1599.03 (-NH bend); 1500.65 (Ar C=C); 1650.11(HC=N); 854.00(C-N); 755.69 (C-S)

^{1}HNMR($CDCl_3$):δ=8.5(1H,s,N=CH);8.3(2H,s,CONH),7.6(2H,m,aromatic) ;7.3-7.5(2H,m,aromatic) 7.4-7.3(2H,m,aromatic);7.3-7.2(2H,m,aromatic); 5.2(1H,s,OH);2.3(3H,s,CH_3);1.9-2.1(8H,m of CH_2 of cyclohexane)

[13]C NMR:20.23-24.6 (4C of cyclohexane ring) 23(3C,CH_3 of CONH);120-140(C aromatic ring); 145(C of thiophene ring);127(C,thiophene ring);120(C,thiophene ring)164(C,thiophene ring), 165(2C,CONH);167(C,N=CH);168 (C attached to OH)

Elemental analysis: C- 66.4%, H-5.35%, N-9.69%, O-11.07%, S-7.4%

2-(2-hydroxy benzylidene amino)-N-(4-acetanilido)-4,5,6,7-tetra methylene thiophene-3-carboxamide (SM-2c)

M.P. 110^{0}C; yield: 47%; MS: 433(100%), 250(50%), 155(70%), 319(80%) 241(60%)

IR max cm^{-1}: 3433.32 (-OH); 3383.07 (-NH); 3195.51 (Ar-CH); 2808.09 (CH_2); 2911.179(Ali-CH); 1647.56 (C=O); 1521.99 (-NH bend); 1487.97 (Ar C=C); 1669.01(HC=N); 876.09(C-N); 777.99(C-S)

^{1}HNMR($CDCl_3$):δ=8.6(1H,s,N=CH);8.3(2H,s,CONH),7.5(2H,m,aromatic);7.3-7.5(2H,m,aromatic) 7.4-7.3(2H,m,aromatic);7.3-7.2(2H,m,aromatic); 5.0(1H,s,OH);2.3(3H,s,CH_3);2.0-2.2(8H,m of CH_2 of cyclohexane)

[13]C NMR:21.02-23.00 (4C of cyclohexane ring) 23(3C,CH_3 of CONH);120-140(C aromatic ring);145(C of thiophene ring);127(C,thiophene ring);120(C,thiophene ring)164(C,thiophene ring),165(2C,CONH);167(C,N=CH);165(C attached to OH)

Elemental analysis: C- 66.49%, H-5.35%, N-9.69%, O-11.07%, S-7.4%

2-(2-nitro benzylidene amino)-N-(4-acetanilido)-4,5,6,7-tetra methylene thiophene-3-carboxamide (SM-2d)

M.P. 110^{0}C; yield: 44%; MS: 462(100%), 250(50%), 353(60%), 319(80%) 229(50%) 151(70%)

IR max cm^{-1}: 3283.74 (-NH); 3074.23 (Ar-CH); 2808.09 (CH_2); 2941.19(Ali-CH); 1677.58 (C=O); 1600.09 (-NH bend); 1479.56 (Ar C=C); 1668.42(HC=N); 816.19(C-N); 734.65(C-S); 1360.09(N-O of NO_2)

^{1}HNMR($CDCl_3$): δ=8.7(1H,s,N=CH);8.5(2H,s,CONH),7.6-7.7(2H,m, aromatic) 7.4-7.6(4H,m, aromatic);7.3-7.2(2H,m,aromatic); 2.3(3H,s,CH_3);2.3-2.6(8H,m of CH_2 of cyclohexane)

[13]C NMR: 20.2-24.0 (4C of cyclohexane ring) 23(3C,CH_3 of CONH);119-141(C aromatic ring); 149(C aromatic attached to NO_2) 145(C of thiophene ring);127(C,thiophene ring);120(C,thioph- enes ring)164(C,thiophene ring),165(2C,CONH);167(C,N=CH)

Elemental analysis: C- 62.32%, H-4.79%, N-12.11%, O-13.11%, S-6.93%

2-(3-nitro benzylidene amino)-N-(4-acetanilido)-4,5,6,7-tetra methylene thiophene-3-carboxamide (SM-2e)

M.P. 107^{0}C; yield: 46%; MS: 462(100%), 382(70%), 353(60%), 340(50%) 229(30%)

IR max cm^{-1}: 3282.71 (-NH); 3065.13 (Ar-CH); 2908.09 (CH_2); 2941.19(Ali-CH); 1657.11 (C=O); 1612.01 (-NH bend); 1499.97 (Ar C=C); 1633.43(HC=N); 878.51(C-N); 758.51(C-S); 1352.12(N-O of NO_2)

^{1}HNMR($CDCl_3$): δ=8.9(1H,s,N=CH);8.7(2H,s,CONH),7.6-7.7 (2H,m, aromatic) 7.4-7.6(2H,m, aromatic);7.3-7.2(2H,m,aromatic); 7.1-7.2(2H,m,aromatic); 2.1(3H,s,CH_3);2.4-2.8(8H,m of CH_2 of cyclohexane)

[13]C NMR: 22.2-25.0 (4C of cyclohexane ring) 23(3C,CH_3 of CONH);120-141(C aromatic ring); 149(C aromatic attached to NO_2) 147(C of thiophene ring);129(C,thiophene ring); 119 (C, thiop hene ring)163(C,thiophene ring),165(2C,CONH);169(C,N=CH)

Elemental analysis: C- 62.32%, H-4.79%, N-12.11%, O-13.84%, S-6.93%

2-(3, 4-dimethoxy benzylidene amino)-N-(4-acetanilido)-4,5,6,7-tetra methylene thiophene-3-carboxamide (SM-2f)

M.P. 121^{0}C; yield: 54%; MS: 477(100%), 340(70%), 365(60%), 287(50%)

IR max cm^{-1}: 3272.01 (-NH); 3043.11 (Ar-CH); 2921.96 (CH_2); 2999.01(Ali-CH); 1650.12(C= O); 1665.21 (-NH bend); 1474.39 (Ar C=C); 1645.43(HC=N); 1243.12(OCH_3); 873.61(C-N); 750.55 (C-S)

^{1}HNMR($CDCl_3$): δ=9.0(1H,s,N=CH);8.8(2H,s,CONH),7.6-7.7(2H,m,aromatic) 7.4-7.6(2H,m, aromatic);7.3-7.2(2H,m,aromatic); 2.1(3H,s,CH_3); 3.8(6H,s,OCH_3); 2.4-2.8(8H,m of CH_2 of cyclohexane)

[13]C NMR: 22.2-25.0 (4C of cyclohexane ring) 23(3C,CH_3 of CONH); 56.53(C,OCH_3); 55.8 (C, OCH_3);122-143(C aromatic ring); 148(C of thiophene ring);130(C,thiophene ring);120 (C,thio phene ring)165(C,thiophene ring),166(2C,CONH);168(C,N=CH)

Elemental analysis: C- 65.39%, H-5.7%, N-8.1%, O-13.4%, S-6.73%

2-(4 hydroxy -3-methoxy benzylidene amino)-N-(4-acetanilido)-4,5,6,7-tetra methylene thiophene-3-carboxamide (SM-2g)

M.P. 107^{0}C; yield: 54%; MS: 463(100%), 353(60%), 365(60%), 229(50%), 151(70%);

IR max cm^{-1}: 3433.32 (-OH); 3278.21 (-NH); 3063.91 (Ar-CH); 2932.06 (CH_2); 2955.11(Ali-CH); 1654.21(C=O); 1615.61 (-NH bend); 1514.14 (Ar C=C); 1650.77 (HC=N); 1253.21 (OCH_3); 877.43(C-N); 754.35 (C-S)

^{1}HNMR($CDCl_3$): δ=9.1(1H,s,N=CH);8.8(2H,s,CONH),7.6-7.7(2H,m,aromatic) 7.5-7.7(2H,m, aromatic);7.3-7.2(3H,m,aromatic); 2.0(3H,s,CH_3); 3.7(3H,s,OCH_3);5.1(1H,s,OH); 2.4-2.8(8H,m of CH_2 of cyclohexane)

[13]C NMR: 21.2-23.6 (4C of cyclohexane ring) 23.5(3C,CH_3 of CONH);57.23(C,OCH_3);118-140(C aromatic ring); 145(C of thiophene ring);129(C,thiophene ring);120(C,thiophene ring) 165(C,thiophene ring),164.1(2C,CONH); 160.3(C attached to OH);164.1(C,N=CH);

Elemental analysis: C- 64.78%, H-5.4%, N-9.06%, O-13.81%, S-6.92%

2-(2-chloro benzylidene amino)-N-(4-acetanilido)-4,5,6,7-tetra methylene thiophene-3-carboxamide (SM-2h)

M.P. 115^{0}C; yield: 48%; MS: 451(100%), 340(85%), 365(60%), 229(70%),151(40%);

IR max cm^{-1}: 3286.74 (-NH); 3074.32 (Ar-CH); 2942.79 (CH_2); 2985.90(Ali-CH); 1675.00 (C=O); 1630.33 (-NH bend); 1490.14 (Ar C=C); 1668.42 (HC=N); 815.15(C-N); 735.65 (C-S); 641.98 (C-Cl)

^{1}HNMR($CDCl_3$): δ=8.5(1H,s,N=CH);8.4(2H,s,CONH);7.9(2H,m,aromatic) 7.5-7.8(4H,m,aroma tic);7.5-7.2(2H,m,aromatic); 1.9(3H,s,CH_3); 2.4-2.8(8H,m of CH_2 of cyclohexane)

[13]C NMR: 21.5-23.5 (4C of cyclohexane ring) 23.5 (3C,CH_3 of CONH); 118-140(C aromatic ring) ;146(C of thiophene ring);128(C,thiophene ring);119(C,thiophene ring)164(C,thiophene ring), 165.5(2C,CONH); 167.3(C attached to Cl);164.1(C,N=CH)

Elemental analysis: C- 63.78%, H-4.91%, Cl 7.84% N-9.30%, O-7.08%, S-7.09%

2-(4-chloro benzylidene amino)-N-(4-acetanilido)-4,5,6,7-tetra methylene thiophene-3-carboxamide (SM-2i)

M.P. 119^{0}C; yield: 52%; MS: 451(100%), 352(60%), 229(50%), 151(70%);

IR max cm^{-1}: 3216.18 (-NH); 3072.34 (Ar-CH); 2922.16 (CH_2); 2985.60(Ali-CH); 1668(C=O); 1625.86 (-NH bend); 1527.24 (Ar C=C); 1656.68 (HC=N); 821.89(C-N); 789.51(C-S); 697.71(C-Cl)

^{1}HNMR($CDCl_3$): δ=8.7(1H,s,N=CH);8.45(2H,s,CONH);7.5-7.8(3H,m, aromatic) 7.3-7.5 (3H,m,aromatic);7.1-7.3(2H,m,aromatic); 2.3(3H,s,CH_3); 2.5-2.9(8H,m of CH_2 of cyclohexane)

[13]C NMR: 20.5-23.5 (4C of cyclohexane ring) 22.5 (3C,CH_3 of CONH); 118-140(C aromatic ring) ;145.5(C of thiophene ring);128.5(C,thiophene ring);118(C,thiophene ring)166(C,thiophene ring), 167(2C,CONH); 168.6(C attached to Cl);163(C,N=CH)

Elemental analysis: C- 63.78%, H-4.91%, Cl-7.84% N-9.30%, O-7.08%, S-7.09%

2-(4-methoxy benzylidene amino)-N-(4-acetanilido)-4,5,6,7-tetra methylene thiophene-3-carboxamide (SM-2j)

M.P. 120^{0}C; yield: 54%; MS: 463(100%), 353(60%), 365(60%), 229(50%), 151(70%);

IR max cm^{-1}: 3282.41 (-NH); 3056.22 (Ar-CH); 2900.00 (CH_2); 2923.32(Ali-CH); 1667.32 (C= O); 1634.12 (-NH bend); 1523.14 (Ar C=C); 1633.43 (HC=N); 1223.18(OCH_3); 878.52(C-N); 754.35 (C-S)

^{1}HNMR($CDCl_3$): δ=8.7(1H,s,N=CH);8.5(2H,s,CONH),7.7-7.6(3H,m, aromatic) 7.5-7.3(2H,m, aromatic);7.2-7.1(3H,m,aromatic); 2.2(3H,s,CH_3); 3.9(3H,s,OCH_3); 2.3-2.7(8H,m of CH_2 of cyclohexane)

[13]C NMR: 21.2-23.6 (4C of cyclohexane ring) 23.5(3C,CH_3 of CONH);55.53(C,OCH_3);118-141(C aromatic ring); 145.5(C of thiophene ring);128(C,thiophene ring);121(C,thiophene ring) 164(C,thiophene ring),165.5(2C,CONH); 167.6(C,N=CH)

Elemental analysis: C- 67.9%, H-5.63%, N-9.39%, O-10.72%, S-7.16%

2-(4-nitro benzylidene amino)-N-(4- acetanilido)-4, 5, 6, 7- tetra methylene thiophene-3-carboxamide (SM-2k)

M.P. 106^{0}C; yield: 44%; MS: 462(100%), 382(70%), 340(70%), 365(60%) 287(50%)

IR max cm^{-1}: 3282.71 (-NH); 3065.13 (Ar-CH); 2908.09 (CH_2); 2941.19(Ali-CH); 1657.11 (C= O); 1612.01 (-NH bend); 1499.97 (Ar C=C); 1633.43(HC=N); 878.51(C-N); 758.51(C-S); 1360.12 (N-O of NO_2)

^{1}HNMR($CDCl_3$): δ=8.8(1H,s,N=CH);8.65(2H,s,CONH),7.7-7.5(2H,m, aromatic) 7.4-7.6(4H,m, aromatic);7.3-7.2(2H,m,aromatic); 2.1(3H,s,CH_3);2.4-2.8(8H,m of CH_2 of cyclohexane)

[13]C NMR: 22.2-25.0 (4C of cyclohexane ring) 23(3C,CH_3 of CONH);119-140(C aromatic ring); 148.5(C aromatic attached to NO_2) 147(C of thiophene ring);129(C,thiophene ring) ;119 (C,thio phene ring)163(C,thiophene ring),165(2C,CONH);169(C,N=CH)

Elemental analysis: C- 62.32%, H-4.79%, N-12.11%, O-13.84%, S-6.93%

2-(4-(dimethylamino benzylideneamino)-N-(4-acetanilido) 4,5,6,7-tetra methylene thio phene-3-carboxamide (SM-2l)

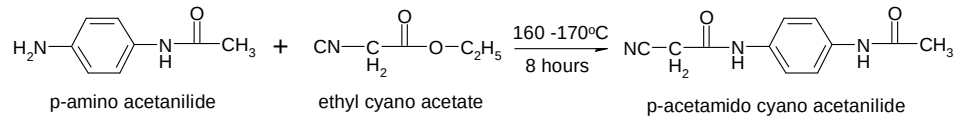
M.P. 123^{0}C; yield: 48%; MS: 431(100%), 348(70%), 243(80%), 111(30%);

IR max cm^{-1}: 3256.22 (-NH); 3084.69 (Ar-CH); 2824.34 (CH_2); 2954.87 (Ali-CH); 1629.36 (C=O); 1605.05 (-NH bend); 1587.54 (Ar C=C); 1650.11(HC=N); 873.08(C-N); 754.55(C-S)

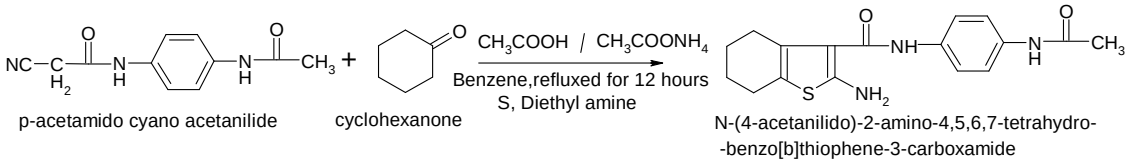
^{1}HNMR($CDCl_3$):δ=8.3(1H,s,N=CH);8.2(2H,s.CONH),7.6(4H,m,aromati c);7.37.5(4H,m,aromatic) ;2.3(3H,s,CH_3); 2.0 (3H,s,methyl); 2.7(8H,m of CH_2 of cyclohexane)

[13]C NMR: 20.2-25.6 (4C of cyclohexane ring) 23(3C,CH_3 of CONH) 24.5(C of CH_3);118-148(C aromatic ring);145(2C of thiophene ring);130(C,thiophene ring);160(C,thiophene ring),161 (2C, CONH);163(C,N=CH)

Elemental analysis: C- 69.58%, H-5.84%, N-9.74%, O-7.41%, S-7.43%



p-amino acetanilide ethyl cyano acetate p-acetamido cyano acetanilide



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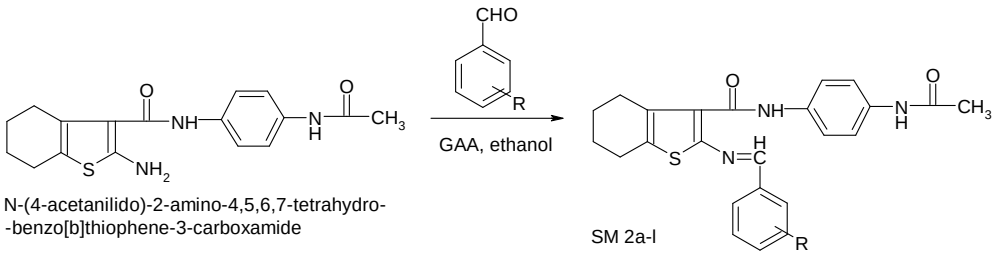

N-(4-acetanilido)-2-amino-4,5,6,7-tetrahydro--benzo[b]thiophene-3-carboxamide SM 2a-l

Fig. 1: General Experimental scheme for the synthesis of (SM-2a-l)

R = 4'-dimethyl amino,4'-hydroxy, 2'-hydroxy,2'-nitro, 3'-nitro, 3',4'- dimethoxy, 4'-hydroxy 3-methoxy, 2'-chloro, 4'-chloro, 4'-methoxy,4'-nitro, 4'-methyl

Table 1: Physical Data SM-2a-2l

Compound No.	R	M.P	% Yield	R *f*
SM-2a	4,-dimethyl amino	130	57	0.64
SM-2b	4'-hydroxy	120	53	0.78
SM-2c	2'-hydroxy	110	47	0.67
SM-2d	2'-nitro	110	44	0.65
SM-2e	3'-nitro	107	46	0.62
SM-2f	3',4'- di methoxy	121	54	0.51
SM-2g	4'-hydroxy 3'-methoxy	107	54	0.70
SM-2h	2'-chloro	115	48	0.59
SM-2i	4'-chloro	119	52	0.68
SM-2j	4'- methoxy	120	54	0.71
SM-2k	4'-nitro	106	44	0.55
SM-2l	4'-methyl	130	48	0.62

Study of antibacterial activity by agar diffusion method

In our current study, the antimicrobial activity was carried out by the agar diffusion method [14]. Here responses of organisms to the synthesized compounds were measured and compared with the response of the standard reference drug. The standard reference drugs used in the present work were **Ampicillin** and **Norfloxacin**.

Microorganisms

The four microorganisms used were *Klebsiella* sp (Gram –ve), *Escherichia coli* (Gram–ve), *Staphylococcus aureus* (Gram+ve) and *Bacillus subtilis* (Gram+ve).

Preparation of Mueller Hinton agar media

The beef infusion was taken in a 1000 ml beaker and made up the volume to 1000 ml with water. To this mixture known quantities of beef infusion, agar, starch and amino acids were added and dissolved by heating the mixture. The pHwas adjusted to7.3. Finally the media was sterilized by autoclaving at 121ºC for 15 minutes at 15-PSI pressure. Afterwards the mixture was cooled to 45ºC and then inoculums were added to the above cooled media, mixed properly and poured into the sterile petri dishes for solidifying. Bores were made on the medium using sterile borer. 0.1 ml of test solution and standard solution at a concentration of 50 µg/0.1 ml were taken. Two standards (Ampicillin and Norfloxacin) were maintained with same concentration in each plate and a control having only DMF in one plate, then the petri dishes were incubated at 37 ºC for 24 hrs and zones of inhibition were observed and measured. The averages of three readings were recorded.

Preparation of test solutions

Each test compound was dissolved in DMF to get a concentration of 500 µg/ml. This concentration was used for testing antibacterial activity.

Study of Antifungal Activity by Agar Diffusion Method

In our current study, the antifungal activity was carried out by the agar diffusion method. Here responses of organisms to the synthesized compounds were measured and compared with the response of the standard reference drug. The standard reference drug used in the present work was **Miconazole nitrate.**

Microorganisms

The two microorganisms used were *Candida albicans* and *Aspergillus niger*.

Preparation of test solutions

Each test compound was dissolved in DMSO to get a concentration of 500 µg/ ml. This concent- ration was used for testing antifungal activity.

Procedure

The pathogenic fungi were isolated by inoculating infected sample into corn meal agar. The inoculated plates were incubated at 28ºC for 3 days. The colonies thus formed were then selected for further testing. The Sabouraud's Agar medium was prepared for the organisms *Candida albicans* and *Aspergillus niger*. The medium was sterilized and the plates were prepared. Bores were made on the agar plates using sterile borer. 0.1 ml of test and standard solutions at a concentration of 50 µg/0.1ml were taken. The standard (miconazole nitrate) was maintained with same concentration in each plate and a control having only DMSO in one plate. The plates were inoculated using the selected colonies by swabbing and incubated at 28ºC for 3 days. After incubation the results were analyzed by measuring the zones of inhibition.The results were interpreted by comparing with the standard miconazole nitrate. The average of two readings was recorded.

Table 3: Antimicrobial activity of 2- [(substituted benzylidene)amino]-N-(p-acetamidophenyl carboxamido)-4,5,6,7-tetra methylene thiophene (SM-2a-2l)

Compound Code	*S.aureus*	*B.subtilus*	*E.coli*	*K.pneumonia*	*A.niger*	*C.albicans*
4'- dimethyl amino benzaldehyde	12	14	12	--	15	--
4'-hydroxy benzaldehyde	21	18	16	18	17	10
2'-hydroxy benzaldehyde	22	16	19	16	17	09
2'-nitro benzaldehyde	13	12	11	10	12	--
3'-nitro benzaldehyde	12	11	10	10	11	--
3'4'dimethoxy benzaldehyde	15	14	--	14	14	--
4' hydroxy 3'methoxy benzaldehyde	20	16	19	15	18	09
2' –chloro benzaldehyde	21	18	18	19	18	09
4' –chloro benzaldehyde	23	19	20	17	19	11
4' –methoxy benzaldehyde	14	14	15	17	17	09
4' –nitro benzaldehyde	14	12	13	--	14	--
4' –methyl benzaldehyde	13	13	12	11	13	--
Ampicillin	22	17	24	18	--	--
Norfloxacin	33	28	26	28	--	--
Miconazole	--	--	--	--	30	27

Table 2: Antimicrobial activity of 2-amino-N-(p-acetamidophenyl carboxamido)-4,5,6,7-tetramethylene thiophenes [SM-2]

Compound Code	*S.aureus*	*B.subtilus*	*E.coli*	*K.pneumonia*	*A.niger*	*C.albicans*
SM-2	16	15	13	13	16	--
Ampicillin	22	17	24	18	--	--
Norfloxacin	23	28	26	28	--	--
Miconazole	--	--	--	--	30	27

RESULTS

Twelve derivatives were synthesized and their structure was confirmed by IR, NMR, mass spectrum and elemental analysis.

All the twelve derivatives were subjected to Anti microbial activity by Agar Diffusion Method (Table2) & (Table 3) shows the results of anti microbial activity, while Table 1 shows the physiochemical parameters.

From the antibacterial activity results it was observed that both electron donating and electron withdrawing groups on the aldehydic phenyl ring of the compounds influenced the activity. Among the drugs tested for antimicrobial activities SM-2b,SM-2c,SM-2g,SM-2h,SM-2i exhibited potent activity by showing zone of inhibition ranging from 14mm-23mm.All these drugs showed potent activity against *Staphylococcus aureus* with high zone of inhibition. All other drug showed drugs showed moderate inhibititory properties against the test organism. Against *Candida albicans* none of the test drugs showed significant activity. Standard drugs, Ampicillin, Norfloxacin and Mecanazole nitrate exhibited potent inhibitory properties against test organism.

DISCUSSION

The formation of the starting compound was confirmed by IR spectra where it shows $-NH_2$ peak at 3454.0 cm^{-1}. The NMR spectrum shows a peak at δ (ppm) = 5.2 of free NH_2 group in the compound. The IR spectra of all the Schiff bases (SM- 2a-l) show the disappearance of $-NH_2$ peak and the appearance of –N=CH (Imine) peak at a range of 1690-1640 cm^{-1}, which clearly suggest the formation of the expected compounds. The NMR spectra of the compounds show sharp singlet peak at δ (ppm) =8.9-8.1 of –N=CH (Imine-H) which also further confirm the formation of the compounds of the series. The title compounds were also confirmed by Mass spectra. In Anti microbial activity it is seen from the table 2 and table 3 that all the derivatives having moderate to mild activity whereas the derivatives (SM-2b, SM-2c, SM-2g, SM-2h, SM-2i) having electron donating group on the benzylidene ring shows comparable activity with the standard drugs.

CONCLUSION

The title compounds (SM-2a-l) were prepared from the starting compound 2-amino-N-(p-acetamidophenyl carboxamido)-4,5,6,7-tetramethylene thiophenes [SM-2] and screened for Anti microbial activity. Among all compounds it was found that derivatives such as (SM-2b, SM-2c, SM-2g, SM-2h, SM-2i) were showing comparable activity when compared to standard drugs.

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