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Research Article

SYNTHESIS OF 2-SUBSTITUTED AMINO-3-CARBOXAMIDO-4, 5, 6, 7-TETRAMETHYLENE THIOPHENE DERIVATIVES AND THEIR ANTI-MICROBIAL ACTIVITY

[A]P. SIDDARTHA KUMAR*, [B]SUNIL JUNAPUDI, [C]SRIKANTH GURRALA, [D]RAMBABU BATHINI

[a]Department of Pharmaceutical Chemistry, Balaji Institute of Pharmaceutical Sciences, Lakenepalli, Narsampet, Warangal 506331, [b]Department of Pharmaceutical Chemistry, Anurag Pharmacy College, Kodad, [c]Department of Pharmaceutical Chemistry, Gland institute of Pharmaceutical Sciences, Kothapet, Shangri-la, Narsapur, Medak- 502313, [d]Department of Pharmacolgy, Mother Teresa College of Pharmacy, Ghatkesar, Hyderabad, Andhra Pradesh, India. Email: Steev.g99@gmail.com, sunilpharma49@gmail.com

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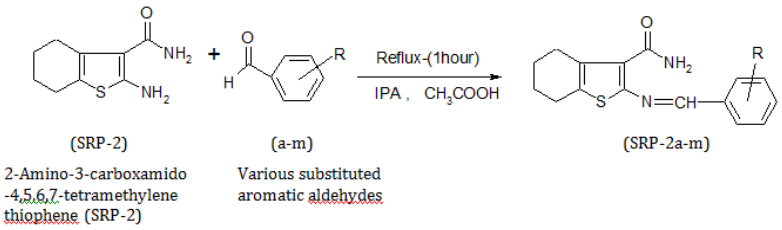
ABSTRACT

The conventional methodology was adopted to synthesize the titled compounds. The synthesis of titled compounds from starting compound i.e 2-amino-3-carboxamido-4,5,6,7 tetramethylene thiophene (SRP-2) was prepared from cyanoacetamide (SRP-1) by condensation with cyclohexanone in the presence elemental sulphur and a basic catalyst diethyl amine in ethanol to form (SRP-2). A set of thirteen azomethine derivatives (Schiff bases) were synthesized by reacting 2-amino-3-carboxamido-4,5,6,7-tetramethylene thiophene (SRP-2) in isopropyl alcohol with various substituted aromatic aldehydes involving glacial acetic acid as a catalyst. The title compounds (SRP-2a-m) were screened for their 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 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, Cyano acetamide

INTRODUCTION

Thiophene has exhibited an array of biological activities ranging from anti-microbial[1-2], anti-tumour[3] and anti-inflammatory activity[4]. Among the antimicrobial agents thiophene derivatives are known to have a promising activity. Few to name are Cephalothin, Cephalorodine and Cefoxitin. In Pharmaceutical field new drugs are discovered by molecular modification of the lead compound of established pharmacological activity. In the current literature survey, it has been observed that drug designed by molecular modification is more rational and productive foundation of new drug, consequently the need to synthesize new molecule as potential medicinal agent is more relevant today. So far various new thiophenes have been synthesized and screened in our laboratories for antimicrobial activity. The enthusiastic results prompted us to continue the investigation. So, an attempt was made to synthesize new substituted thiophenes as anti-bacterial agents adapting Gewald reaction.[5-7] Hence the synthesis of 2-amino-3-carboxamido-4,5,6,7-tetra methylenethiophene (SRP-2) was carried out. The different derivatives of the parent compound SRP-2 was achieved by using different aryl aldehyde to obtain a series of Schiff Bases (SRP-2a-m).

MATERIAL AND METHODS

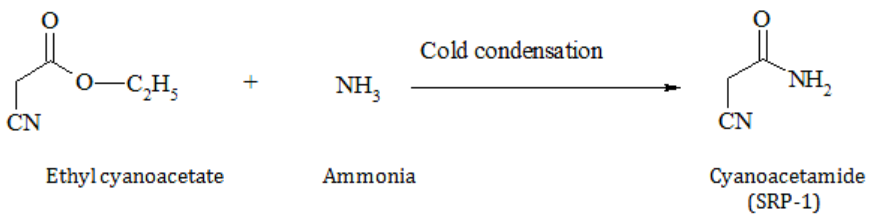
Experimental

Melting points were determined by using Precision melting point apparatus in open capillaries and are uncorrected. The purity of the compounds was checked by TLC on silica gel G plates using benzene and ethanol (9:1) solvent system and Ultraviolet lamp and iodine chambers used as a visualizing agent. IR-spectra were recorded using KBr pellets on a SHEMADZU 8000 series spectro-photometer. ^{1}H-NMR spectra on BRUKER 400 MHz Spectrophotometer using DMSO as solvent and TMS as internal standard (chemical shift values expressed in ppm).

Procedure

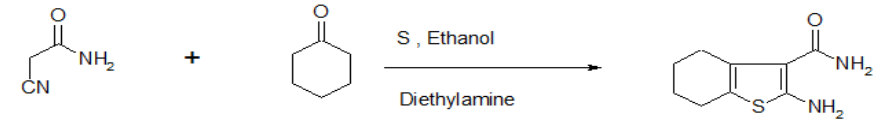
Step-1: Synthesis of Cyanoacetamide (SRP-1)

A mixture of concentrated Aq. Ammonia (150ml, *d* 0.88 Mol) and Ethylcyano acetate (200gm, 1.77 Mol) was taken in 250ml Iodine flask. The mixture was exothermic and cloudy Later it turns to clear mixture and was kept on the ice bath for 1 hour. White Cyanoacetamide was filtered rapidly, dried in air and recrystallized with 95% of Ethanol.



Synthesis of 2-amino-3-carboxamido-4,5,6,7-tetramethylene thiophene (SRP-2)

A mixture of Cyanoacetamide (3.2gm, 0.04 Mol), (4.0ml, 0.04 Mol) of Cyclohexanone , Sulphur (1.28gm, 0.04 Mol) and 30 ml of Ethanol was taken in conical flask and stirred at 45-50 ºC. Once the temperature was attained, 4ml of Diethylamine was added dropwise until Sulphur completely went in. The reaction mixture was kept overnight in refrigerator. The obtained crystals was filtered, dried and recrystsllized with Ethanol.



Cyanoacetamide (SRP-1) | Cyclohexanone | SRP-2

General method for the syntheses of 2-substituted benzylidene imino-3-carboxamido-4, 5, 6, 7 tetramethylene thiophenes (Schiff bases) SRP-2a-2m

A mixture of the starting compound (SPR-2) (0.005 Mol) and the required aryl aldehydes (0.005 Mol) in isopropyl alcohol (30 ml) and catalytic amount of glacial acetic acid (2 ml) was subjected to reflux for 1 hour. Then cooled to room temperature. The solid separated was filtered, washed with isopropyl alcohol. The synthesiszed derivatives were recrystallized by depending upon their solubility in Iso propyl alcohol or benzene, or ethnol.

Study of Antibacterial Activity by Agar Diffusion Method

In our current study, the antibacterial 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 drugs. The standard reference drug used was Ampicillin (Std-I).

Microorganisms

The four microorganisms used were *Staphylococcus aureus* (Gram-positive), *Bacillus subtilus* (Gram-positive), *Escherichia coli* (Gram-negative) and *Klebsiella pneumoniae* (Gram-negative).

Preparation of Nutrient agar media

A mixture of known quantities of peptone, meat extract, sodium chloride, dextrose and agar was dissolved in 1000 ml of distilled water by heating. The pH was adjusted to 7.4. Finally the medium was sterilized by autoclaving at 121ºC for 15 minutes at 15 lb pressure per square inch. Afterwards the mixture was cooled to 45ºC and then inoculums were added to the above cooled medium, mixed properly and poured into the sterile petridishes for solidifying. Bores were made on the medium using sterile borer. 0.1 ml of test and standard solutions at a concentration of 50 g/0.1ml were taken. Standard (ampicillin) were maintained with same concentration in each plate and a control having only DMSO in one plate. Then the petridishes were incubated at 37ºC for 24 hours and zones of inhibition were observed and measured. The average of three readings was recorded.

Preparation of test solutions

Each test compound was dissolved in DMSO 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 concentration was used for testing antifungal activity.

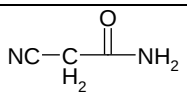
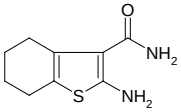
Procedure

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.

RESULTS AND DISCUSSION

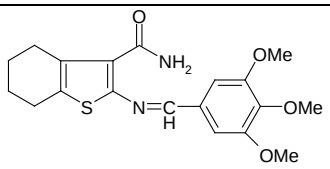
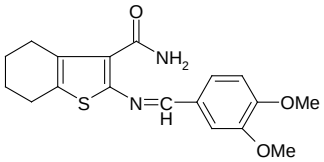
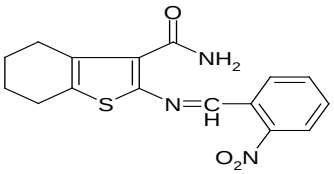
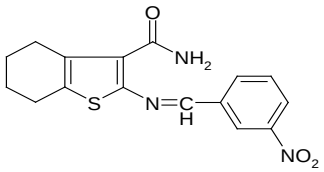
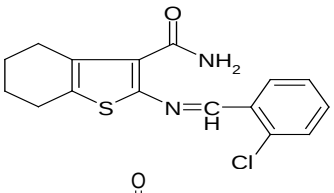
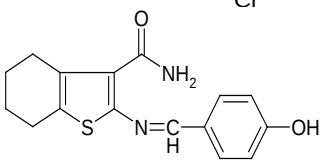
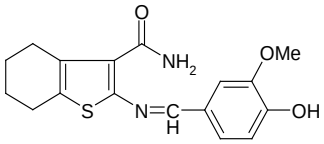
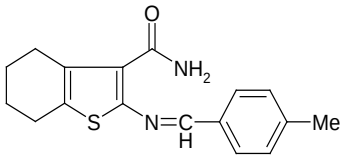
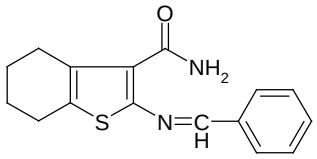
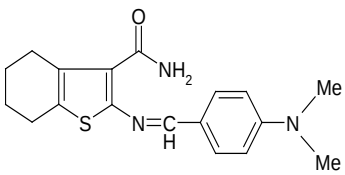
From the literature survey it reveals that 2-substituted amino-3-carboxamido-4,5,6,7-tetramethylene thiophene derivatives have been reported for number of pharmacological activities and some molecules have shown significant activities and some compounds shows moderate and good activities. Here we have synthesized some novel thiophene analogues and screened them for their anti-bacterial and anti-fungal activities and the results are as follows. The physiochemical parameters of compounds SRP-1 & SRP-2 was shown in Table no-1.

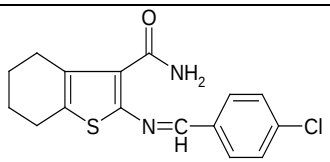
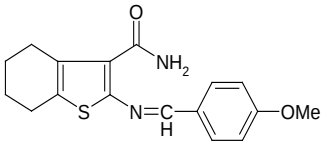
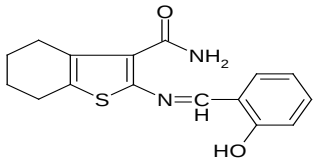
Table 1: The physiochemical parameters of compounds SRP-1 & SRP-2

Sr. No.	Comp. No.	Structure	Chemical Name	M.W. (g)	M. P. (ºC)	% Yield
1	SRP-1		cyano acetamide	80	119	45.99
2	SRP-2		2-amino-3-carboxamido-4,5,6,7-tetramethylene thiophene	196	186	50.00

The physiochemical parameters of 2-(substituted benzylidene) imino-3- carboxamido-4,5,6,7-tetramethylene thiophenes (Schiff bases SRP-2a-m) were shown in Table no -2.

Table 2: The physiochemical parameters of Schiff bases SRP-2a-m

Sr. No.	Comp. No.	Structure	Chemical Name	M.W. (g)	M. P. (°C)	% Yield
1	SRP-2a		2-[(3',4',5'-trimethoxy benzylidene) imino]-3-carboxamido-4,5,6,7-tetra methylene thiophene	372	186	73.17
2	SRP-2b		2-[(3'4'- dimethoxy benzylidene) imino]-3-carboxamido-4,5,6,7-tetramethylene thiophene	344	192	64.07
3	SRP-2c		2-[(2'-nitrobenzylidene) imino]-3-carboxamido-4,5,6,7-tetra methylene thiophene .	329	228	70.25
4	SRP-2d		2-[(3'-nitrobenzylidene) imino]-3-carboxamido-4,5,6,7-tetra methylene thiophene	329	218	61.18
5	SRP-2e		2-[(2'-chloro benzylidene)imino]-3-carboxamido -4,5,6,7-tetramethylene thiophene	318	210	61.96
6	SRP-2f		2-[(4'-hydroxy benzylidene) imino]-3-carboxamido-4,5,6,7-tetramethylene thiophene	300	274	66.16
7	SRP-2g		2-[(4'-hydroxy-3'-methoxybenzylidene) imino]-3-carboxamido -4,5,6,7-tetramethylene thiophene	330	238	65.75
8	SRP-2h		2-[(4'-methyl benzylidene) imino]-3-carboxamido-4,5,6,7-tetramethylene thiophene	298	192	69.84
9	SRP-2i		2-[(benzylidene) imino]-3-carboxamido-4,5,6,7-tetra methylene thiophene .	284	222	45.36
10	SRP-2j		2-[(4'-dimethylamino benzylidene) imino]-3-carboxamido-4,5,6,7-tetramethylene thiophene	313	262	51.66

11	SRP-2k		2-[(4'-chlorobenzylidene) imino]-3-carboxamido-4,5,6,7-tetramethylene thiophene	318	210	52.35
12	SRP-2l		2-[(4'-methoxy benzylidene) imino]-3-carboxamido-4,5,6,7-tetramethylene thiophene	314	218	45.36
13	SRP-2m		2-[(2'-hydroxybenzylidene) imino]-3-carboxamido-4,5,6,7-tetramethylene thiophene	304	218	53.66

Spectral data of 2-(substituted benzylidene) imino-3- carboxamido-4, 5, 6, 7-tetramethylene thiophenes (Schiff bases SRP-2a-m)

1. 2-[(3',4',5'-trimethoxy benzylidene) imino]-3-carboxamido-4,5,6,7-tetra methylene thiophene (compound-SRP-2a):
   λ_{max} (nm): 335, IR (KBr): 3523 (-NH_2); 3383 (Aro-CH); 2924 (Ali-CH); 1639(C=O); 1600 (C=N); 1275(C-O); 1116(C-N); 746(C-S).
2. 2-[(3'4'- dimethoxy benzylidene) imino]-3-carboxamido-4,5,6,7-tetramethylene thiophene (compound-SRP-2b):
   λ_{max} (nm): 338, IR (KBr): 3523 (-NH_2); 3383 (Aro-CH); 2924 (Ali-CH); 1639(C=O); 1600 (C=N); 1275(C-O); 1116(C-N); 746(C-S).
3. 2-[(2'-nitrobenzylidene) imino]-3-carboxamido-4,5,6,7-tetra methylene thiophene (compound-SRP-2c):
   λ_{max} (nm): 340, IR (KBr): 3389 (-NH_2); 1636 (C=O); 1532 (N=O); 1342 (C-O); 738(C-S). ^{1}HNMR (DMSO): 8.89 s, 1H, N=CH e); 8.30 (s,2H, CH ArH phenyl ring, b'c'); 7.7-7.6 (m,2H,CH,ArH, phenylring, a'd'); 5.62 (s,2H, NH_2, amino,f) ; [2.97 (t, 2H, CH_2, a); 2.75 (t, 2H, CH_2, d); 1.85 (t,4H, $2CH_2$, ring, b, c-). Cyclohexanone]
4. 2-[(3'-nitrobenzylidene) imino]-3-carboxamido-4,5,6,7-tetra methylene thiophene (Compound-SRP-2d):
   λ_{max} (nm): 342, IR (KBr): 3389 (-NH_2); 1636(C=O); 1532 (C=N); 1342(C-N); 738(C-S).
5. 2-[(2'-chloro benzylidene)imino]-3-carboxamido -4,5,6,7-tetramethylene thiophene (compound-SRP-2e):
   λ_{max} (nm): 349, IR (KBr): 3383 (-NH_2); 3187 (Aro-CH); 2935 (Ali-CH); 1641 (C=O); 1588 (C=N); 808(C-N).
6. 2-[(4'-hydroxy benzylidene) imino]-3-carboxamido-4,5,6,7-tetramethylene thiophene (compound-SRP-2f):
   λ_{max} (nm): 357, IR (KBr): 3400 (-NH_2); 3101 (Aro-CH); 2935 (Ali-CH); 1653(C=O); 1560 (C=N); 1177(C-N); 834(C-S).
7. 2-[(4'-hydroxy-3'-methoxybenzylidene) imino]-3-carboxamido -4,5,6,7-tetramethylene thiophene (compound-SKP-2g):
   λ_{max} (nm): 361, IR (KBr): 3535 (-NH_2); 3170 (Aro-CH); 2924 (Ali-CH); 1627(C=O); 1543 (C=N); 1298(C-O); 1267(C-N); 749(C-S).
8. 2-[(4'-methyl benzylidene) imino]-3-carboxamido-4,5,6,7-tetramethylene thiophene (compound-SKP-2h):
   λ_{max} (nm): 371, IR (KBr): 3372 (-NH_2); 3187 (Aro-CH); 2935 (Ali-CH); 1625(C=O); 1599 (C=N); 1090(C-N); 813(C-S).
9. 2-[(benzylidene) imino]-3-carboxamido-4,5,6,7-tetra methylene thiophene (compound-SKP-2i):
   λ_{max} (nm): 366, IR (KBr): 3383 (-NH_2); 3170 (Aro-CH); 2918 (Ali-CH); 1641(C=O); 1613 (C=N); 1298(C-O); 1127(C-N); 746(C-S).
10. 2-[(4'-dimethylamino benzylidene) imino]-3-carboxamido-4,5,6,7-tetramethylene thiophene (compound-SKP-2j):
    λ_{max} (nm): 373, IR (KBr): 3417 (-NH_2); 3299 (Aro-CH); 2991 (Ali-CH); 1653(C=O); 1586 (C=N); 1275(C-O); 1172(C-N); 777(C-S). ^{1}HNMR (DMSO): 9.08 (s, 1H, N=CH e); 7.66 (d,2H, CH ArH phenyl ring, b'c');6.72 (d,2H,CH,ArH, phenylring, a'd'); 5.52 (s,2H, NH_2, amino,f) 3.1 (s,6H, $2CH_3$, g, h); [2.98 (s, 2H, CH_2, a); 2.7 (s, 2H, CH_2, d); 2.4 (t,4H, $2CH_2$, ring, b, c-). Cyclohexanone].
11. 2-[(4'-chlorobenzylidene) imino]-3-carboxamido-4,5,6,7-tetramethylene thiophene (compound-SKP-2k):
    λ_{max} (nm): 384, IR (KBr): 3383 (-NH_2); 3187 (Aro-CH); 2935 (Ali-CH); 1641 (C=O); 1588 (C=N); 808(C-N). ^{1}HNMR (DMSO): 8.85 (s, 1H, N=CH e); 7.72 (d,2H, CH ArH phenyl ring, b'c'); 7.45 (d,2H,CH,ArH, phenylring, a'd'); 5.60 (s,2H, NH_2, amino,f); [2.98 (s, 2H, CH_2, a); 2.7 (s, 2H, CH_2, d); 1.8 (t,4H, $2CH_2$, ring, b, c-). Cyclohexanone].
12. 2-[(4'-methoxy benzylidene) imino]-3-carboxamido-4,5,6,7-tetramethylene thiophene (compound-SKP-2l):
    λ_{max} (nm): 381, IR (KBr): 3339 (-NH-); 2927 (Ali-CH); 1669 (C=O); 1566 (C= N); 1220(C-O); 1086 (C-N amine); 824 (C-N). ^{1}HNMR (DMSO): 8.84 s, 1H, N=CH e); 7.77 (d,2H, CH ArH phenyl ring, b'c'); 6.99 (d,2H,CH,ArH, phenylring, a'd'); 5.60 (d,2H, NH_2, amino,f) 3.9 (s,3H, OCH_3, g); [2.99 (s, 2H, CH_2, a); 2.7 (s, 2H, CH_2, d); 1.83 (t,4H, $2CH_2$, ring, b, c-). Cyclohexanone].
13. 2-[(2'-hydroxybenzylidene) imino]-3-carboxamido-4,5,6,7-tetramethylene thiophene (compound-SKP-2m):
    λ_{max} (nm): 391, IR (KBr): 3400 (-NH_2); 3249 (Aro-CH); 2935 (Ali-CH); 1653 (C=O); 1644 (C=N); 1090(C-N);752(C-S) .

Anti Bacterial Activity

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. But aldehydic phenyl ring containing electron withdrawing group has shown promising result. Among all the compounds tested, SRP-2k with 4'-chloro substitution at R was found to be most active against both Gram-positive and Gram-negative bacteria. The remaining compounds of both the series exhibited mild to moderate activities when compared to the standards.

Anti Fungal Activity

The antifungal screening results also suggest that the test compounds showed mild to moderate activity against *A.niger* only but no significant activity against *C.albicans* compared to the standard employed.

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