Heliyon 5 (2019) e02233

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.heliyon.com

Synthesis of novel Schiff bases using 2-Amino-5-(3-fluoro-4-methoxyphenyl) thiophene-3-carbonitrile and 1,3-Disubstituted pyrazole-4-carboxaldehydes derivatives and their antimicrobial activity

Divyaraj Puthran [a,b], Boja Poojary [a,*], Nikil Purushotham [a], Nandam Harikrishna [c], Soukhyarani Gopal Nayak [a], Vinuta Kamat [a]

[a] Department of Studies in Chemistry, Mangalore University, Mangalagangothri, Karnataka, 574199, India
[b] Solara Active Pharma Sciences, No. 120 A&B, Industrial Area, Baikampady, New Mangalore, Karnataka, 575011, India
[c] Alivira Animal Health, Plot No.104-109,112,113 JNPC, Special Economic Zone, Paravada Mandal, Visakhapatnam, Andhra Pradesh-531019, India

ARTICLE INFO

Keywords:
Organic chemistry
Gewald synthesis
1-(3-fluoro-4-methoxyphenyl)ethanone
Vilsmeier-Haack reaction
Schiff bases
Anti-microbial

ABSTRACT

2-Amino-5-(3-fluoro-4-methoxyphenyl)thiophene-3-carbonitrile have been synthesized from 1-(3-fluoro-4-methoxyphenyl)ethanone, malononitrile, a mild base and sulfur powder using Gewald synthesis technique and the intermediate was treated with 1,3-disubstituted pyrazole-4-carboxaldehyde to obtain the novel Schiff bases. 1,3-disubstituted pyrazole-4-carboxaldehyde derivatives have been synthesized by Vilsmeier-Haack reaction in the course of a multi-step reaction. The structure of novel compounds were established on the basis of their elemental analyses IR, ^{1}H NMR, ^{13}C NMR, and mass spectral data and then screened for their *in vitro* antimicrobial activity. Among them 5a, 5c, 5f and 5h showed excellent activity when compared to other derivatives. Remaining derivatives showed moderate activity.

1. Introduction

The compounds haulage azomethine functional group (–C=N-) which are accepted as Schiff's base have projected significance in medicinal and pharmaceutical fields due to their application in synthesis of versatile organic intermediates and their broad range of biological activities, such as antimycobacterial [1], antitubeculosis [2], anticancer [3], analgesic [4] and anti-inflammatory [5], anticovulsunt [6, 7], antibacterial and antifungal [8, 9].

On the other hand, compounds containing pyrazole pharmacophore are having their application in healing of inflammation and inflammation linked disorders, such as arthritis [10]. Pyrazole derivatives are the subject of many research studies due to their extensive potential biological activities such as antimicrobial [11], antiviral [12], antitumor [13], antihistaminic [14], antidepressant [15], insecticides [16], and fungicides [17].

Alternatively, the thiophene nucleus has been familiar as an essential part in the synthesis of heterocyclic compounds which shows potential pharmacological characteristics. A widespread multiplicity of therapeutic applications of thiophene derivatives has been surveyed in the literature [18, 19, 20, 21]. Thiophene moiety and their derivatives are known as best pharmacophore in the diabetes mellitus [22], antimicrobial [23], hepatitis *B virus* inhibitors [24], cholesterol inhibitors [25], antiviral [26], antitumor [27] and antitubercular agents [28].

The Chemistry of fused pyrazolo- and thieno-pyrazole moieties has drawn great attention due to their pharmacological importance as reported earlier [29, 30, 31].M. G. Prabhudeva *et al,* in 2018 reported the synthesis of bioactive thiophene-pyrazole conjugates and screened further for their antimicrobial and anti-oxidant properties. Their biological evaluation studies showed that compounds containing chloro substitution only in the thiophene ring exhibited excellent inhibition against all the tested organisms in comparison with that of the standard drug ciproflaxin [32].

Key synthons namely, thiophene, pyran, thiazole and some fused heterocyclic derivatives were been synthesized and further studied for antitumor activity against three human tumour cells lines, namely breast adenocarcinoma (MCF-7), non-small cell lung cancer (NCI–H460) and CNS cancer (SF-268) by Mohareb *et al,* in 2012 [33].

In view of the above mentioned literature and as a part of our nonstop efforts in the direction of the growth of more potent antimicrobial agents we reported the synthesis of Schiff bases which contain substituted thiophene hybridized with 1,3-disubstituted pyrazole-4-carbaldehydes

* Corresponding author.
E-mail address: bojapoojary@gmail.com (B. Poojary).

https://doi.org/10.1016/j.heliyon.2019.e02233
Received 27 May 2019; Received in revised form 6 July 2019; Accepted 1 August 2019

derivatives. Based on the literature and in continuation of our interest we have designed new candidates that may be value in potent, selective and less toxic antimicrobial agents.

For this reason, we hereby present the synthesis, spectral studies and biological evaluation of the combination of two heterocyclic compounds such as thiophene and substituted pyrazoles derivatives. We have also studied the bacterial and fungal growth inhibition of the compounds and it was thought that the above mentioned heterocyclic rings together in a molecular framework have additive effect on antibacterial and antifungal activities.

2. Result and discussion

2.1. Chemistry

The first step of synthetic method involves a convenient, commercial and simple process for the synthesis of desired product [1-(3-fluoro-4-methoxyphenyl)ethylidene]propanedinitrile (2) using 1-(3-fluoro-4-methoxyphenyl)ethanone (1), malononitrile and ammonium acetate. IR analysis of compound **2** showed the peak at 2834 cm^{-1} is due to (C–H) group. Presence of (C=C) and (C=N) group recorded at 1512 cm^{-1} and 1000 cm^{-1} respectively. Then (C≡N) group band was observed at 2750 cm^{-1}. The ^{1}H NMR spectrum of compound **2** in DMSO-*d6* showed singlet at δ 2.51 ppm due to the presence of three protons of $–CH_3$ group. Singlet at δ 3.95 ppm was assigned to three protons of $–OCH_3$ protons and multiplet showed in the range of δ 7.35–7.71 was assigned to aromatic proton. Three protons of the aromatic group appeared as a multiplet in the range of δ 7.61–7.71 ppm. Then the mass spectrum of compound **2** showed a molecular ion peak at m/z = 215.06 [M+1]. This in turn, confirmed the formation of a compound having the molecular formula $C_{12}H_9FN_2O$.

Then [1-(3-fluoro-4-methoxyphenyl)ethylidene]propanedinitrile (2) was converted to 2-amino-5-(3-fluoro-4-methoxyphenyl)thiophene-3-carbonitrile (3) in presence of sulphur powder and sodium bicarbonate using tetrahydrofuran as a solvent.

Formation of compound **3** was confirmed by recording their IR, ^{1} H NMR, ^{13}C NMR, and mass spectra. IR analysis of compound **3** showed the peak at 3200 cm^{-1} is due to (N–H) stretchig. The existence of (C=C) and (C–N) group observed at 1624 cm^{-1} and 1276 cm^{-1} respectively. Finally, (C≡N) group showed at 2202 cm^{-1}. The ^{1}H NMR spectrum of compound **3** in DMSO-*d6* showed singlet at δ 3.87 ppm which was due to the presence of three protons of $–OCH_3$ group and then singlet at δ 7.65 ppm was assigned to aromatic protons. Protons appeared in the range of δ 7.21–7.28 ppm and δ 7.34–7.41 ppm was assigned to remaining five protons of the aromatic group. Then the mass spectrum of compound **3** showed a molecular ion peak at m/z = 249.1 [M+1]. This, in turn, confirmed the formation of a compound having the molecular formula $C_{12}H_9FN_2OS$.

The vital pyrazole skeleton *i.e.* 1,3-disubstituted pyrazole-4-carbaldehydes (**4a-o)** were synthesized by Vilsmeier-Haack reaction in moderate good yields and confirmed by IR and NMR [34].

2-Amino-5-(3-fluoro-4-methoxyphenyl)thiophene-3-carbonitrile (3) in presence of 1,3-disubstituted pyrazole-4-carbaldehydes **4(a-o)** and catalytic amount glacial acetic acid in ethanol media to resulted derivatives **5(a-o)** with reasonably excellent yield.

The synthetic scheme for the preparation of compounds **5(a-o)** was presented in Fig. 1.

IR analysis of compound **5h** showed the peak at 3427 cm^{-1} is due to (C–H) group. Presence of (C≡N) and (C=N) group observed at 2400 cm^{-1} and 1496 cm^{-1} respectively. Then the (C=C) group observed at 1408 cm^{-1}. At the end, IR analysis (C–C) and (N=N) group observe at 1095 cm^{-1} and 1438 cm^{-1} respectively. The ^{1}H NMR spectrum of **5h** in DMSO-*d6* showed singlet at δ 3.91 ppm and it was assigned to three protons of $–OCH_3$ group and then triplet showed at at δ 7.22 ppm was assigned to aromatic proton with a coupling constant J = 4.0 Hz. Multiplet showed in the range of δ 7.30–7.45 ppm, δ 7.52–7.75 ppm was assigned to remaining seven protons of aromatic group. Doublet observed at δ 8.02 ppm with a coupling constant was assigned to two protons of aromatic group. Three more singlets for aromatic protons were observed at δ 8.85 ppm, δ 9.44 ppm and δ 10.1 ppm. Then the mass spectrum of compound **5h** showed a molecular ion peak at m/z = 517.03 [M+1]. This, in turn, confirmed the formation of a compound having the molecular formula $C_{26}H_{16}ClFN_4OS_2$. The spectral data of other derivatives **5(a-o)** was explained in the supplementary data. The physical properties of synthesized compounds are listed in Table 3.

Where, Ar = C_6H_5, 4-Cl-C_6H_4, 4-F-C_6H_4, 3-Br-C_6H_4, 4-Br-C_6H_4, 4-OCH_3-C_6H_4, 4-CH_3-C_6H_4, 3-thienyl, 4-C_6H_5–C_6H_4, R = H, Cl.

2.2. Antimicrobial studies

Inhibitory zone (diameter) mm of synthesized compounds against tested bacterial and fungal strains by well diffusion method.

Synthesized compounds were tested for the antimicrobial activity against two Gram-positive (*S. aureus*), (*B. subtilis)* and a Gram-negative (*E. coli*) bacterial strains along with a fungal strain (*C. albicans*) by the zone of inhibition (ZOI) method. The zone of inhibition was noted to be in the range of 15–41 mm. From Table 1 it was concluded that the compounds containing 4-OCH_3 and 4-Cl substituent on *N*-phenyl ring of pyrazole moiety showed comparable activity to that of the standard ciprofloxacin. In the case of Gram-negative *E. coli* compounds **5a, 5f** and **5h** showed significant activity with ZOI of 40, 38 and 37 mm. Then in case of Gram-positive (*S. aureus*), bacterial strain compounds containing 4-F and 4-Cl substituents on *N*-phenyl ring of pyrazole moiety showed significant activity to that of the standard Ciprofloxacin with ZOI value 40 mm. Further moving on to Gram-positive bacterial strain (*B. Subtilis)*, substituent containing 3-thienyl, 4 biphenyl and 4-OMe groups found to

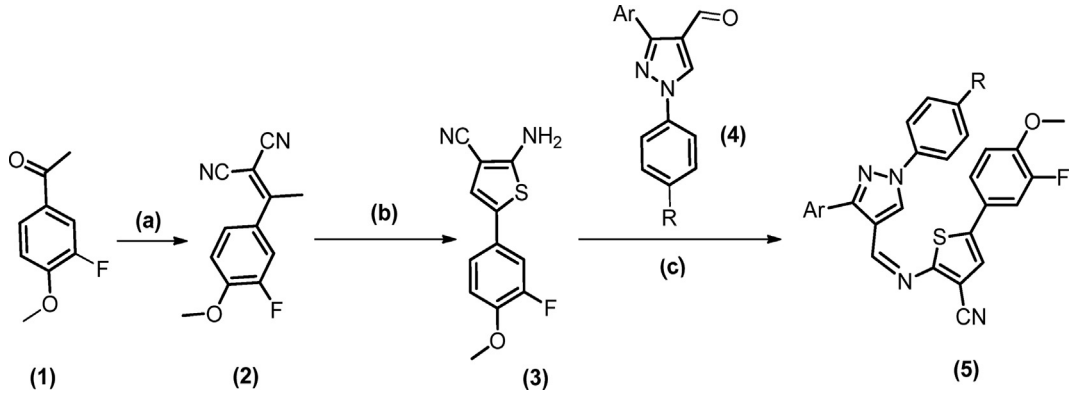

Fig. 1. Reagents and conditions: (a) Malononitrile, ammonium acetate, acetic acid, toluene, reflux (b) THF, sulphur powder, sodium bicarbonate, H_2O (c) 1,3-disustituted pyrazole-4-carboaldehydes, ethanol, catalytic amount of acetic acid.

Table 1
Zone of inhibition (mm) of the compounds 5(a-o) against the tested microorganisms.

Compound	*E. coli*	*S. aureus*	*B. subtilis*	*C. albicans*
5a	**40**	36	**40**	21
5b	32	30	27	19
5c	**38**	**40**	32	15
5d	32	15	35	23
5e	37	35	38	19
5f	33	**38**	**39**	28
5g	23	31	32	27
5h	**37**	**40**	**38**	**32**
5i	31	34	27	17
5j	28	21	37	19
5k	34	18	39	23
5l	33	37	20	19
5m	21	23	32	25
5n	25	28	32	32
5o	32	34	27	26
Ciprofloxacin	41	44	42	–
Fluconazole	–	–	–	34

be excellent activity when compared with standard with ZOI value 38, 39 and 41 mm respectively. 3-thienyl, and 4-biphenyl substituted compound **5h** and **5f** were active with a ZOI of 32 and 28 mm with respect to Fluconazole standard *against* fungal strain (*C. albicans*).

Table 2 represents the results of the quantitative assay of the antimicrobial and antifungal activities of the novel compounds, a concentration of lower value μg/mL represents an enormously strong effect and a higher μg/mL concentration represents a reasonable effect. The tested compounds offered an antimicrobial activity at concentrations among 31.25 and >125 μg/mL.

Few of the tested compounds exhibited the antibacterial and antifungal activity. It was worth to be noticed the good antimicrobial activity was showed by the synthesized derivatives. Compound **5a** (Ar-4-OCH_3, R-4-Cl), **5e** (Ar-4-Cl, R-4-Cl) and **5g** (Ar-4-Br, R-4-Cl) were showed MIC values from 31.25 to >125 μg/mL against *B. subtilis*. Further compounds **5c** (Ar-4-F, R-4-Cl) and **5m** (Ar-4-Me, R-4-Cl) showed inhibition against *C. albicans* (MIC values in the range of 62.5 to >125 μg/mL). Halogen substituted pyrazoles containing thiophene Schiff bases derivatives showed good inhibition activity against both bacterial and antifungal strains. The activity against *S. aureus* was moderate with the tested compounds **5g** (Ar-4-Br, R-4-Cl) where it exhibited MIC value ranging from 62.5 to >125 μg/mL. The activity against *E. coli* was noteworthy, the tested compounds **5a** (Ar-4-OCH_3, R-4-Cl), **5g** (Ar-4-Br, R-4-Cl), and **5m** (Ar-4-Me, R-4-Cl) exhibiting MIC values ranging from 31.5 to >125 μg/mL.

3. Conclusion

In the current work, 2-amino-5-(3-fluoro-4-methoxyphenyl)thiophene-3-carbonitrile containing Schiff bases derivatives were synthesized and characterized by FT-IR, NMR, Mass spectroscopy and elemental analysis. Further synthesized compounds were evaluated for their antibacterial and antifungal activities. The newly synthesized thiophene and pyrazole hybridized heterocyclic compounds exhibited moderate antibacterial activity against *S. aureus (NCIM 5021), B. subtilis (NCIM 2063)*, and *E. coli (NCIM 2574),* and significant antifungal activity against *C. albicans (NCIM 3100)*. It can be concluded that these classes of compounds **3a, 3c, 3e** and **3g** certainly holds great promise towards good active leads in the medicinal chemistry. A further study to acquire more information concerning pharmacological activity is in progress.

Table 2
Minimum inhibitory concentration (MIC) determination.

Compound	MIC (*μg/ml*)			
	E. coli	*S. aureaus*	*B. subtilis*	*C. albicans*
5a	62.5	–	31.25	–
5c	–	125	–	62.5
5e	125	125	31.25	–
5g	31.5	62.5	>125	–
5m	62.5	>125	–	>125
Ciprofloxacin	3.95	2	2	–
Fluconazole	–	–	–	2

4. Experimental

4.1. General information

Solvents and reagents were obtained from commercial sources and used without purification. Recrystallization technique was used for purification instead of column chromatography with appropriate solvents. Melting point was determined by open capillary method and was uncorrected. IR spectrum was obtained in KBr disc on a Shimadzu FT-IR 157 spectrometer. NMR spectra were recorded on a Bruker WH_200 (400 MHz) in DMSO-d_6 as solvent and TMS as an internal standard. Chemical shifts and coupling constants were expressed as ppm (δ) and Hz (*J*) respectively. Mass spectrum was recorded on a Jeolsx 102/Da-600 mass spectrometer/Data system using argon/xenon (6 kV, 10mA) as the FAB gas. The accelerating voltage was 10 kV and spectra were recorded at room temperature. The elemental analyses (CHN) were performed using VARIO EL-III (Elemental Analyse system GmBH). The progresses of the reactions were monitored by TLC on pre-coated silica gel G plates. All the spectral data of newly synthesized compound were consistent with proposed structure.

4.2. General procedure for of [1-(3-fluoro-4-methoxyphenyl)ethylidene] propanedinitrile (2)

To a solution of 1-(3-fluoro-4-methoxyphenyl)ethanone (1.0 mol) in dry toluene was added malononitrile (1.1 mol). Ammonium acetate (0.1 mol) and (0.2 mol) acetic acid. The reaction mixture was heated under reflux using Dean- Stark water separator until water ceased to be collected. Reaction mixture maintained for 24–28 h, with reflux condition followed by TLC monitoring [Hexene:EtOAc, 4.5:0.5 (v/v)]. Then solid product obtained was crystallized from ethyl acetate and toluene mixture to give (75% yield).

4.3. General procedure for of 2-Amino-5-(3-fluoro-4-methoxyphenyl) thiophene-3-carbonitrile (3)

[1-(3-fluoro-4-methoxyphenyl)ethylidene]propanedinitrile (1.0 mol) and sulfur powder (1.3 mol) were suspended in tetrahydrofuran (25 mL) and warmed to an internal temperature of 35 °C. A solution of sodium bicarbonate (1.4 mol) in 5 mL of water was added over 1 h. The mixture was stirred for 24 h. After completion of reaction quenched to ice-cold water then filtered and washed with water. Then crude product was recrystallized with toluene and ethyl acetate mixture.

4.4. General procedure for 2-[{(substituted phenyl-1H-pyrazol-4-yl) methylene}amino]-5-(3-fluoro-4-methoxyphenyl)thiophene-3-carbonitrile derivatives 5(a-o)

1,3-disustituted pyrazole-4-carboaldehydes (1.1 mol) and of 2-amino-5-(3-fluoro-4-methoxyphenyl)thiophene-3-carbonitrile (1.0 mol) were dissolved in warm ethanol 20 mL containing glacial acetic acid (0.50 mL). The reaction mixture was refluxed for 5–7 h. The major part of the product precipitated while hot. The solid formed on cooling was filtered, washed with hot ethanol and dried under vacuum to give respective Schiff bases respectively. Crude material was purified with ethyl acetate recrystallization (The physical properties of synthesized compounds are listed below in Table 3).

Table 3
Characteristic data of 2-[{(substituted phenyl-1*H*-pyrazol-4-yl)methylene} amino]-5-(3-fluoro-4-methoxyphenyl)thiophene-3-carbonitrile derivatives 5(a-o).

Compound	Ar	R_1	% Yield	Melting Point (°C)
5a	4-OCH_3-C_6H_4	4-Cl	55	247–248
5b	4- H	4-Cl	54	211–215
5c	4-F-C_6H_4	4-Cl	60	233–235
5d	3-Br-C_6H_4	4-Cl	43	238–240
5e	4-Cl-C_6H_4	4-Cl	41	242–245
5f	4- C_6H_5–C_6H_4	4-H	53	>250
5g	4-Br-C_6H_4	4-Cl	42	219–222
5h	3-thienyl	4-Cl	46	248–251
5i	4- C_6H_5	4-H	53	215–217
5j	4-CH_3-C_6H_4	4-H	57	212–215
5k	4-Cl-C_6H_4	4-H	56	215–217
5l	4-F-C_6H_4	4-H	61	201–203
5m	4-CH_3-C_6H_4	4-Cl	52	210–212
5n	4-OCH_3-C_6H_4	4-H	57	205–209
5o	4-Br-C_6H_4	4-H	55	196–199

4.4.1. [1-(3-fluoro-4-methoxyphenyl)ethylidene]propanedinitrile (2)

Reddish orange colour solid; 82.08%; Mp. 118–119 °C; IR (KBr, υ_{max}, cm^{-1}): 2834 (C–H), 2750 (C≡N, 1512 (C=C), 1000 (C–C). ^{1}H NMR (400 MHz; DMSO-d_6, δ ppm) δ 2.55 (s, 3H, –CH_3) 3.95 (s, 3H, –OCH_3), 7.35–7.71 (m, 1H, Ar–H), 7.61–7.71 (m, 2H, Ar–H); ^{13}C NMR (100 MHz; DMSO-d_6, δ ppm): δ 24.2 (CH_3), 56.8 (O–CH_3), 82.6 (C≡N), 113.9 (Ar–C), 114.1, 114.3, 116.2, 116.4, 126.0, 126.1, 128.4, 128.5, 150.1, 150.8, 150.93, 152.5, 175.1; ESI-MS (*m/z*): 215.06 $[M-H]^-$; Anal. calcd. for $C_{12}H_9FN_2O$: C, 66.66; H, 4.20; N,12.96. Found: C, 66.67; H, 4.23; N, 12.94.

4.4.2. 2-Amino-5-(3-fluoro-4-methoxyphenyl)thiophene-3-carbonitrile (3)

Orange colour solid; 82.08%; Mp. 118–119 °C; IR (KBr, υ_{max}, cm^{-1}): (N–H) 3200, (C≡N) 2202, (C=C) 1624, (C–N) 1276 cm^{-1}; ^{1}H NMR (400 MHz; DMSO-d_6, δ ppm) 3.87 (s, 3H, –OCH_3), 6.52 (s, 1H, Ar–H), 7.21–7.28 (m, 3H, Ar–H), 7.34–7.41 (m, 2H, Ar–H); ^{13}C NMR (100 MHz; DMSO-d_6, δ ppm): 56.5 (O–CH_3), 83.3 (C≡N), 105.1 (Ar–C), 114.4, 114.7, 114.4, 114.9, 117.1, 123.5, 123.6, 127.9, 137.2, 137.2, 147.2, 147.3, 150.51, 152.9, 166.9; ESI-MS (*m/z*): 247.03 $[M-H]^-$; Anal. calcd. for $C_{12}H_9FN_2OS$: C, 58.05; H, 3.65; N, 11.28. Found: C, 58.04; H, 3.66; N, 11.29.

4.4.3. 2-[{(1-(4-chlorophenyl)-3-(4-methoxyphenyl)-1H-pyrazol-4-yl) methylene}amino]-5-(3-fluoro-4-methoxyphenyl)thiophene-3-carbonitrile (5a)

Yellow colour solid; 55.04% Mp. 247–248 °C IR (KBr, υ_{max}, cm^{-1}): (C–H) 3387, (C≡N) 2222, (C=N) 1602, (C=C) 1249, (C–C) 1020, (N=N) 1444; ^{1}H NMR (400 MHz; DMSO-d_6, δ ppm) δ 3.84 (s, 3H, –OCH_3), 3.91 (s, 3H, –OCH_3), 7.07–7.10 (m, 2H, Ar–H), 7.33 (t, 1H, Ar–H, $J = 8.0$ Hz), 7.47–7.66 (m, 4H, Ar–H). 7.90–8.09 (m, 4H, Ar–H), 8.68 (s, 1H, Ar–H), 9.27–9.33 (s, 1H, Ar–H), 9.33 (s, 1H, Ar–H); ^{13}C NMR (100 MHz; DMSO-d_6, δ ppm): 55.7 (O–CH_3), 56.6 (O–CH_3), 114.4 (Ar–C), 114.5, 114.6, 115.3, 115.5, 118.4, 119.0, 121.3, 122.6, 124.1, 124.2, 130.1, 130.5, 130.8, 132.1, 132.2, 135.6, 137.9, 155.6, 160.4,160.6,185.0; ESI-MS (*m/z*): 541.08 $[M-H]^-$; Anal. calcd. for $C_{29}H_{20}ClFN_4O_2S$: C, 64.14; H, 3.71; N, 10.32. Found: C, 64.14; H, 3.71; N, 10.32.

4.4.4. 2-[{(1-(4-chlorophenyl)-3-phenyl-1H-pyrazol-4-yl)methylene} amino]-5-(3-fluoro-4-methoxyphenyl)thiophene-3-carbonitrile (5b)

Yellow colour solid; 53.88%; Mp. 211–215 °C. IR (KBr, υ_{max}, cm^{-1}): (C–H) 3387, (C≡N) 2212, (C=N) 1615, (C=C) 1239, (C–C) 1025, (N=N) 1434: ^{1}H NMR (400 MHz; DMSO-d_6, δ ppm) δ 3.91 (s, 3H, –OCH_3), 7.29–7.43 (m, 5H, Ar–H), 7.48–7.53 (m, 4H, Ar–H), 7.55–7.80 (m, 4H, Ar–H). 8.42 (s, 1H, Ar–H), 9.11 (s, 1H, Ar–H); ^{13}C NMR (100 MHz; DMSO-d_6, δ ppm): δ 56.3 (O–CH_3), 84.1 (C≡N), 114.3 (Ar–C), 114.5, 116.5, 118.5, 120.5, 123.5, 125.4, 130.8, 130.5, 131.8, 132.6, 136.5, 138.9, 156.5, 161.4, 183.3; ESI-MS (*m/z*): 511.17 $[M-H]^-$; Anal. calcd. for $C_{28}H_{18}ClFN_4OS$: C,65.56; H, 3.54; N,10.92. Found: C, 65.55; H, 3.51; N, 10.94.

4.4.5. 2-[{(1-(4-chlorophenyl)-3-(4-fluorophenyl)-1H-pyrazol-4-yl) methylene}amino]-5-(3-fluoro-4-methoxyphenyl)thiophene-3-carbonitrile (5c)

Yellow colour solid; 60.09%; Mp. 233–235 °C. IR (KBr, υ_{max}, cm^{-1}): (C–H) 3429, (C≡N) 2212, (C=N) 1504, (C=C) 1276, (C–C) 1220, (N=N) 1440: ^{1}H NMR (400 MHz; DMSO-d_6, δ ppm): 3.91 (s, 3H, –OCH_3), 7.30–7.37 (m, 3H, Ar–H), 7.47–7.67 (m, 5H, Ar–H), 8.06–8.14 (m, 4H, Ar–H), 8.71 (s, 1H, Ar–H), 9.31 (s, 1H, Ar–H). ^{13}C NMR (100 MHz; DMSO-d_6, δ ppm): 56.6 (O–CH_3), 84.5 (C≡N), 103.5 (Ar–C), 114.6, 115.5, 115.8, 116.0, 119.2, 121.3, 130.1, 131.7, 131.8, 155.3, 165.3; ESI-MS (*m/z*): 529.06 $[M-H]^-$; Anal. calcd. for $C_{28}H_{17}ClF_2N_4O$: C, 63.34; H, 3.23; N, 10.55. Found: C, 63.37; H, 3.25; N, 10.53.

4.4.6. 2-[{(3-(3-bromophenyl)-1-(4-chlorophenyl)-1H-pyrazol-4-yl) methylene}amino]-5-(3-fluoro-4-methoxyphenyl)thiophene-3-carbonitrile (5d)

Yellow colour solid; 42.81%; Mp. 238–240 °C. IR (KBr, υ_{max}, cm^{-1}): (C–H) 3287, (C≡N) 2242, (C=N) 1622, (C=C) 1241, (C–C) 1038, (N=N) 1441, (C–Cl) 731; ^{1}H NMR (400 MHz; DMSO-d_6, δ ppm): 3.91 (s, 3H, –OCH_3), 7.31–7.47 (m, 6H, Ar–H), 7.51–7.80 (m, 5H, Ar–H), 7.79 (s, 1H, Ar–H). 8.49 (s, 1H, Ar–H), 9.21 (s, 1H, Ar–H); ^{13}C NMR (100 MHz; DMSO-d_6, δ ppm): 55.6 (O–CH_3), 83.4 (C≡N), 115.4 (Ar–C), 115.1, 116.3, 118.8, 122.3, 123.8, 125.8, 126.2, 130.5, 130.8, 133.1, 135.9, 137.1, 154.7, 161.5, 162.6, 184.3; ESI-MS (*m/z*): 588.98 $[M-H]^-$; Anal. calcd. for $C_{28}H_{17}BrClFN_4OS$: C, 56.82; H, 2.90; N, 9.47. Found: C, 56.85; H, 2.93; N, 9.50.

4.4.7. 2-[{(1,3-bis(4-chlorophenyl)-1H-pyrazol-4-yl)methylene}amino]-5-(3-fluoro-4-methoxyphenyl)thiophene-3-carbonitrile (5e)

Yellow colour solid; 41.36%; Mp. 242–245 °C. IR (KBr, υ_{max}, cm^{-1}): (C–H) 3182, (C≡N) 2124, (C=N) 1582, (C=C) 1217, (C–C) 1012, (N=N) 1404, (C–Cl) 723; ^{1}H NMR (400 MHz; DMSO-d_6, δ ppm): 3.91 (s, 3H, –OCH_3), 7.31–7.48 (m, 4H, Ar–H), 7.50–7.61 (m, 6H, Ar–H), 8.01 (d, 2H, Ar–H, $J = 8.0$ Hz). 8.51 (s, 1H, Ar–H), 9.21 (s, 1H, Ar–H); ^{13}C NMR (100 MHz; DMSO-d_6, δ ppm): 56.8 (O–CH_3), 84.1 (C≡N), 115.5 (Ar–C), 115.8, 116.1, 118.5, 119.9, 121.1, 122.9, 124.5, 130.6, 130.9, 132.4, 132.9, 135.2, 138.8, 154.6, 161.4, 160.1; ESI-MS (*m/z*): 545.04 $[M-H]^-$; Anal. calcd. for $C_{28}H_{17}Cl_2FN_4OS$: C, 61.43; H, 3.13; N, 10.23. Found: C, 61.41; H, 3.14; N, 10.25.

4.4.8. 2-[{(3-([1,1′-biphenyl]-4-yl)-1-phenyl-1H-pyrazol-4-yl)methylene} amino]-5-(3-fluoro-4-methoxyphenyl)thiophene-3-carbonitrile (5f)

Yellow colour solid; 52.74%; Mp. > 250 °C. IR (KBr, υ_{max}, cm^{-1}): (C–H) 3182, (C≡N) 2462, (C=N) 1679, (C=C) 1300, (C–C) 1004, (N=N) 1497; ^{1}H NMR (400 MHz; DMSO-d_6, δ ppm) δ 3.78 (s, 3H, –OCH_3), 6.68–8.00 (m, 19H, Ar–H). ^{13}C NMR (100 MHz; DMSO-d_6, δ ppm): 56.2 (O–CH_3), 84.2 (C≡N), 118.9 (Ar–C), 119.6, 126.5, 127.0, 127.1, 127.4, 128.4, 129.4, 130.0, 130.2, 164.5; ESI-MS (*m/z*): 589.10 $[M+H]^+$; Anal. calcd. for $C_{34}H_{22}ClFN_4OS$: C, 69.32; H, 3.76; N, 9.51. Found: C, 69.31; H, 3.77; N, 9.54.

4.4.9. 2-[{(3-(4-bromophenyl)-1-(4-chlorophenyl)-1H-pyrazol-4-yl) methylene}amino]-5-(3-fluoro-4-methoxyphenyl)thiophene-3-carbonitrile (5g)

Yellow colour solid; 41.59%; Mp. 219–222 °C. IR (KBr, υ_{max}, cm^{-1}): (C–H) 3162, (C≡N) 2347, (C=N) 1601, (C=C) 1320, (C–C) 1114, (N=N) 1403; ^{1}H NMR (400 MHz; DMSO-d_6, δ ppm) δ 3.93 (s, 3H, –OCH_3), 7.29–7.46 (m, 6H, Ar–H), 7.51–7.82 (m, 5H, Ar–H), 7.80 (s, 1H, Ar–H). 8.48 (s, 1H, Ar–H), 9.17 (s, 1H, Ar–H); ^{13}C NMR (100 MHz; DMSO-d_6, δ ppm): δ 56.8 (O–CH_3), 83.4 (C≡N), 103.2 (Ar–C), 104.3, 114.5, 114.7, 115.8, 115.9, 117.5, 117.4, 120.4,120.8, 123.9, 124.6, 126.9, 128.4, 130.1, 132.9, 133.9, 134.7, 137.9, 139.4, 146.8, 147.9, 150.5, 153.4,

167.1; ESI-MS (*m/z*): 588.58 $[M-H]^-$; Anal. calcd. for $C_{28}H_{17}BrClFN_4OS$: C, 56.8; H, 2.90; N, 9.47. Found: C, 56.83; H, 2.91; N, 9.48.

4.4.10. 2-[{(1-(4-chlorophenyl)-3-(thiophen-3-yl)-1H-pyrazol-4-yl) methylene}amino]-5-(3-fluoro-4-methoxyphenyl)thiophene-3-carbonitrile (5h)

Yellow colour solid; 46.41%; Mp. 248–251 °C. IR (KBr, υ_{max}, cm^{-1}): (C–H) 3427, (C≡N) 2400, (C=N) 1496, (C=C) 1408, (C–C) 1095, (N=N) 1438; ^{1}H NMR (400 MHz; DMSO-d_6, δ ppm) δ 3.91–3.84 (s, 3H, –OCH_3), 7.22 (t, 1H, Ar–H, $J = 4.0$ Hz), 7.30–7.45 (m, 2H, Ar–H), 7.52–7.75 (m, 5H, Ar–H), 8.02 (d, 2H, Ar–H, $J = 12.0$ Hz), 8.85 (s, 1H, Ar–H), 9.44 (s, 1H, Ar–H), 10.10 (s, 1H, Ar–H); ^{13}C NMR (100 MHz; DMSO-d_6, δ ppm): δ 56.5 (O–CH_3), 56.6 (O–CH_3), 83.4 (C≡N), 103.7 (Ar–C), 105.1, 114.4, 114.6, 114.7, 114.9, 115.3, 115.5, 115.6, 118.6, 118.7, 121.1,121.2, 123.5, 124.2, 126.5, 126.6, 128.4, 130.1, 130.5, 132.3, 133.9, 134.7, 137.2, 137.6, 139.4, 147.6, 147.8, 147.9, 150.6, 153.0, 168.1; ESI-MS (*m/z*): 519.10 $[M + H]^+$; Anal. calcd. for $C_{26}H_{16}ClFN_4OS_2$: C, 60.17; H, 3.11; N, 10.79. Found: C, 60.19; H, 3.14; N, 10.81.

4.4.11. 2-[{(1,3-diphenyl-1H-pyrazol-4-yl)methylene}amino]-5-(3-fluoro-4-methoxyphenyl)thiophene-3-carbonitrile (5i)

Yellow colour solid; 53.12%; Mp. 215–217 °C. IR (KBr, υ_{max}, cm^{-1}): (C–H) 3143, (C≡N) 2411, (C=N) 1623, (C=C) 1312, (C–C) 1024, (N=N) 1456; ^{1}H NMR (400 MHz; DMSO-d_6, δ ppm) δ 3.91–3.84 (s, 3H, –OCH_3), 7.30–7.45 (m, 5H, Ar–H), 7.50–7.61 (m, 4H, Ar–H), 7.63–7.85 (m, 5H, Ar–H), 8.45 (s, 1H, Ar–H), 8.90 (s, 1H, Ar–H); ^{13}C NMR (100 MHz; DMSO-d_6, δ ppm): δ 56.7 (O–CH_3), 84.2 (C≡N), 104.5 (Ar–C), 104.9, 114.4, 115.6, 115.9, 116.6, 119.2, 119.8, 121.5, 121.5, 130.5, 131.9, 132.8, 156.3, 166.5; ESI-MS (*m/z*): 477.21 $[M-H]^-$; Anal. calcd. for $C_{28}H_{19}FN_4OS$: C, 70.28; H, 4.00; N,11.71. Found: C, 70.25; H, 4.03; N, 11.74.

4.4.12. 5-(3-fluoro-4-methoxyphenyl)-2-[{(1-phenyl-3-(p-tolyl)-1H-pyrazol-4-yl)methylene}amino]thiophene-3-carbonitrile (5j)

Yellow colour solid; 56.56%; Mp. 212–215 °C. IR (KBr, υ_{max}, cm^{-1}): (C–H) 3117, (C≡N) 2262, (C=N) 1631, (C=C) 1320, (C–C) 1024, (N=N) 1497; ^{1}H NMR (400 MHz; DMSO-d_6, δ ppm) δ 2.35 (s, 3H, –CH_3), 3.91 (s, 3H, –OCH_3), 7.28 (d, 2H, Ar–H, $J = 8.0$ Hz), 7.33–7.49 (m, 2H, Ar–H), 7.52–7.71 (m, 4H, Ar–H), 7.63–7.85 (m, 5H, Ar–H), 8.49 (s, 1H, Ar–H), 9.01 (s, 1H, Ar–H); ^{13}C NMR (100 MHz; DMSO-d_6, δ ppm): δ 21.5 (CH_3), 56.6 (O–CH_3), 84.1 (C≡N), 103.5 (Ar–C), 114.6, 115.5, 115.8, 116.0, 119.2, 121.3, 130.1, 131.7, 131.8, 155.3, 165.3; ESI-MS (*m/z*): 491.24 $[M-H]^-$; Anal. calcd. for $C_{29}H_{21}FN_4OS$: C, 70.71; H, 4.30; N, 11.37. Found: C, 70.74; H, 4.33; N, 11.35.

4.4.13. 2-[{(3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-4-yl)methylene} amino]-5-(3-fluoro-4-methoxyphenyl)thiophene-3-carbonitrile (5k)

Yellow colour solid; 55.82%; Mp.215–217 °C. IR (KBr, υ_{max}, cm^{-1}): (C–H) 3112, (C≡N) 24132, (C=N) 1651, (C=C) 1323, (C–C) 1105, (N=N) 1411; ^{1}H NMR (400 MHz; DMSO-d_6, δ ppm) δ 3.93 (s, 3H, –OCH_3), 7.28–7.41 (m, 5H, Ar–H), 7.49–7.54 (m, 4H, Ar–H), 7.55–7.80 (m, 3H, Ar–H), 7.83 (d, 1H, Ar–H, $J = 8.0$ Hz), 8.42 (s, 1H, Ar–H), 9.11 (s, 1H, Ar–H); ^{13}C NMR (100 MHz; DMSO-d_6, δ ppm): δ 56.5 (O–CH_3), 83.4 (C≡N), 103.7 (Ar–C), 105.1, 114.9, 115.3, 115.6, 118.7,121.2, 123.5, 126.5, 128.9, 130.5, 132.7, 134.7, 137.5, 137.7, 139.8, 147.2, 148.2, 149.7, 151.6, 152.1, 153.2, 167.5; ESI-MS (*m/z*): 511.47 $[M-H]^-$; Anal. calcd. for $C_{28}H_{18}ClFN_4OS$: C, 65.56; H, 3.54; N, 10.92. Found: C, 65.55; H, 3.52; N, 10.95.

4.4.14. 5-(3-fluoro-4-methoxyphenyl)-2-[{(3-(4-fluorophenyl)-1-phenyl-1H-pyrazol-4-yl)methylene}amino]thiophene-3-carbonitrile (5l)

Yellow colour solid; 60.81%; Mp.201–203 °C. IR (KBr, υ_{max}, cm^{-1}): (C–H) 3122, (C≡N) 2383, (C=N) 1659, (C=C) 1343, (C–C) 1125, (N=N) 1429: ^{1}H NMR (400 MHz; DMSO-d_6, δ ppm): 3.92 (s, 3H, –OCH_3), 7.33–7.45 (m, 5H, Ar–H), 7.49–7.55 (m, 4H, Ar–H), 7.59–7.83 (m, 4H, Ar–H). 8.46 (s, 1H, Ar–H), 9.17 (s, 1H, Ar–H); ^{13}C NMR (100 MHz; DMSO-d_6, δ ppm): 56.4 (O–CH_3), 83.6 (C≡N), 114.1 (Ar–C), 114.9, 115.8, 118.5, 119.9, 121.5, 122.8, 124.9, 130.8, 131.1, 132.5, 132.9, 135.9, 137.8, 154.8, 160.9, 185.1; ESI-MS (*m/z*): 495.23 $[M-H]^-$; Anal. calcd. for $C_{28}H_{18}F_2N_4OS$: C, 67.73; H, 3.65; N, 11.28. Found: C, 67.71; H, 3.67; N, 11.26.

4.4.15. 2-[{(1-(4-chlorophenyl)-3-(p-tolyl)-1H-pyrazol-4-yl)methylene} amino]-5-(3-fluoro-4-methoxyphenyl)thiophene-3-carbonitrile (5m)

Yellow colour solid; 52.38%; Mp.210–212 °C. IR (KBr, υ_{max}, cm^{-1}): (C–H) 3122, (C≡N) 2433, (C=N) 1643, (C=C) 1334, (C–C) 1115, (N=N) 1425; ^{1}H NMR (400 MHz; DMSO-d_6, δ ppm): 2.35 (s, 3H, –CH_3), 3.91 (s, 3H, –OCH_3), 7.28–7.35 (m, 3H, Ar–H), 7.41–7.63 (m, 5H, Ar–H), 8.02–8.09 (m, 4H, Ar–H), 8.65 (s, 1H, Ar–H), 9.23 (s, 1H, Ar–H); ^{13}C NMR (100 MHz; DMSO-d_6, δ ppm): 56.6 (O–CH_3), 83.8 (C≡N), 104.5(Ar–C), 114.9, 115.8, 116.8, 117.9, 119.8, 124.3, 130.9, 131.5 131.8, 156.3, 158.9, 165.3; ESI-MS (*m/z*): 525.15 $[M-H]^-$; Anal. calcd. for $C_{29}H_{20}ClFN_4OS$: C,66.09; H, 3.83; N, 10.63. Found: C, 66.11; H, 3.85; N, 10.66.

4.4.16. 5-(3-fluoro-4-methoxyphenyl)-2-[{(3-(4-methoxyphenyl)-1-phenyl-1H-pyrazol-4-yl}methylene]amino)thiophene-3-carbonitrile (5n)

Yellow colour solid; 57.35%; Mp.205–209 °C. IR (KBr, υ_{max}, cm^{-1}): (C–H) 3125, (C≡N) 2422, (C=N) 1662, (C=C) 1344, (C–C) 1131, (N=N) 1429 cm^{-1}; ^{1}H NMR (400 MHz; DMSO-d_6, δ ppm): 3.91 (s, 3H, –OCH_3), 3.93 (s, 3H, –OCH_3), 7.08 (d, 2H, Ar–H, $J = 8.0$ Hz), 7.25–7.51 (m, 5H, Ar–H), 7.62–7.73 (m, 6H, Ar–H), 8.61 (s, 1H, Ar–H), 9.13 (s, 1H, Ar–H); ^{13}C NMR (100 MHz; DMSO-d_6, δ ppm): 55.9 (O–CH_3), 56.8 (O–CH_3), 82.9 (C≡N), 114.5(Ar–C), 114.8, 115.5, 115.6, 118.4, 119.9, 121.5, 122.9, 124.2, 130.4, 130.9, 132.7, 132.5, 135.9, 136.8, 154.9, 161.4, 160.7; ESI-MS (*m/z*): 507.12 $[M-H]^-$; Anal. calcd. for $C_{29}H_{21}FN_4O_2S$: C, 68.49; H, 4.16; N, 11.02. Found: C, 68.51; H, 4.15; N, 11.04.

4.4.17. 2-[{(3-(4-bromophenyl)-1-phenyl-1H-pyrazol-4-yl)methylene} amino] -5-(3-fluoro-4-methoxyphenyl)thiophene-3-carbonitrile (5o)

Yellow colour solid; 54.91 %; Mp.196–199 °C. IR (KBr, υ_{max}, cm^{-1}): (C–H) 3354, (C≡N) 2215, (C=N) 1605, (C=C) 1257, (N=N) 1435, (C–C) 1011: ^{1}H NMR (400 MHz; DMSO-d_6, δ ppm): 3.91 (s, 3H, –OCH_3), 7.13 (d, 2H, Ar–H, $J = 8.0$ Hz), 7.32–7.56 (m, 5H, Ar–H), 7.64–7.79 (m, 6H, Ar–H), 8.66 (s, 1H, Ar–H), 9.21 (s, 1H, Ar–H); ^{13}C NMR (100 MHz; DMSO-d_6, δ ppm): 56.6 (O–CH_3), 84.5 (C≡N), 103.5 (Ar–C), 105.9, 114.1, 116.5, 116.8, 118.2, 119.5, 122.3, 128.9, 129.7, 130.9, 131.9, 132.8, 155.3, 154.6, 165.3; ESI-MS (*m/z*): 555.02, $[M-H]^-$; Anal. calcd. for $C_{28}H_{18}BrFN_4OS$: C,60.33; H, 3.25; N, 10.05. Found: C, 60.32; H, 3.27; N, 10.08.

4.5. Antimicrobial activity

Well diffusion method under NCCLS document M62-A7 protocols was followed for the antimicrobial assay. Three bacterial strains have chosen for the study namely *Escherichia coli (E. coli), Staphylococcus aureus (S. Aureus) and Bacillus subtilis (B. Subtilis)*. The bacterial strains were maintained on Muller-Hinton (MH) agar medium. Ciprofloxacin was used as standard antibacterial drug. *Candida albicans (C. Albicans)* was considered for the antifungal study and Fluconazole was used as standard drug for antifungal studies. 10 mg/mL solutions of the target compounds were prepared in dimethyl sulfoxide for the screening.

The isolates were inoculated in saline solution and incubated at 37 °C for 4 h. The inoculum was adjusted to 0.5 McFarland standards (1.5 × 108 CFU/mL). Sterile swabs were used in the process. The bacteria were seeded on Mulleur Hinton Agar plates performing lawn culture. Wells were punched in the agar plate using a sterile borer. 50 μL of each 3% stock solution the compound was dispensed in the wells. Culture plates were incubated and inhibition zones were measured.

4.5.1. Minimum inhibitory concentration (MIC) determination

MIC was determined by performing serial dilutions. Compounds

suspension was prepared by dissolving 0.1 g of the compound in 10 mL of DMSO respectively. This represents 10 mg/mL and was then serially diluted. Tube containing only growth medium and inoculated organism was considered as control without any compound in it. The tubes were incubated at 37 °C for 24 h. Subculture was done from each tube on nutrient agar (including the control) and incubated at 37 °C for 24 h.

Declarations

Author contribution statement

Divyaraj Puthran, Boja Poojary, Nikil Purushotham, Soukhyarani Gopal Nayak, Vinuta Kamat: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Nandam Harikrishna: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing interest statement

The authors declare no conflict of interest.

Additional information

Supplementary content related to this article has been published online at https://doi.org/10.1016/j.heliyon.2019.e02233.

Acknowledgements

One of the authors, Divyaraj Puthran, is grateful to the management of Solara Active Pharma sciences Ltd., New Mangalore, for supporting the research work and Central Instrumentation Facility, MIT Manipal for providing IR, ^{1}H NMR and ^{13}C NMR spectral facilities.

References

[1] J. Patole, D.S. Shingnapurkar, Padhye, C. Ratledge, Schiff base conjugates of *p*-aminosalicylic acid as antimycobacterial agents, Bioorg. Med. Chem. Lett. 16 (2006) 1514–1517.

[2] M.J. Hearn, M.H. Cynamon, Design and synthesis of antituberculars: preparation and evaluation against Mycobacterium tuberculosis of an isoniazid Schiff base, J. Antimicrob. Chemother. 53 (2004) 185–191.

[3] S. Ren, R. Wang, K. Komatsu, P. Bonaz-Krause, Y. Zyrianov, C.E. McKenna, C. Csipke, Z.A. Tokes, E.J. Lien, Synthesis, biological evaluation, and quantitative structure-activity relationship analysis of new Schiff bases of hydroxysemicarbazide as potential antitumor agents, J. Med. Chem. 45 (2002) 410–419.

[4] S.V. Bhandari, K.G. Bothara, M.K. Raut, A.A. Patil, A.P. Sarkate, V.J. Mokale, Design, synthesis and evaluation of antiinflammatory, analgesic and ulcerogenicity studies of novel S-substituted phenacyl-1,3,4-oxadiazole-2-thiol and Schiff bases of diclofenac acid as nonulcerogenic derivatives, Bioorg. Med. Chem. 16 (2008) 1822–1831.

[5] S.K. Sridhar, S.N. Pandeya, J.P. Stables, A. Ramesh, Anticonvulsant activity of hydrazones, Schiff and Mannich bases of isatin derivatives, Eur. J. Pharm. Sci. 16 (2002) 129–132.

[6] J.P. Kalpana, B.M. Raizon, M. Desarmenien, P. Feltz, P.M. Headley, P. Worms, K.G. Lloyd, G. Bartholini, New anticonvulsants: Schiff bases of y-aminobutyric acid and y-aminobutyramide, J. Med. Chem. 23 (1980) 702–704.

[7] L. Shi, H. Ge, S. Tan, H. Li, Y. Song, H. Zhu, R. Tan, Synthesis and antimicrobial activities of Schiff bases derived from 5-chloro-salicylaldehyde, Eur. J. Med. Chem. 42 (2007) 558–564.

[8] S. Imran, M. Taha, N.H. Ismail, K.M. Khan, F. Naz, M. Hussain, S. Tauseef, Synthesis of novel bisindolylmethane Schiff bases and their antibacterial activity, Molecules 19 (2014) 11722–11740.

[9] J. John Talley, J. Donald Rogier Jr., Both of St. Louis, MO, Pt No: 5 434, G.D. Searle & Co., Skokie, 1995, p. 178.

[10] E.V. Pimenova, E.V. Voronina, Antimicrobial activity of pyrazoles and pyridazines obtained by intreraction of 4-aryl-3-arylhydrazono-2,4-dioxobutanoic acids and their esters with hydrazines, Pharm. Chem. J. 35 (2001) 18–20.

[11] S.L. Janus, A.Z. Magdif, B.P. Erik, N. Claus, Synthesis of triazenopyrazole derivatives as potential inhibitors of HIV-1, Monatsh. Chem. 130 (1999) 1167–1173.

[12] H. Park, K. Lee, S. Park, B. Ahn, J. Lee, H.Y. Cho, Identification of antitumor activity of pyrazole oxime ethers, Bioorg. Med. Chem. Lett. 15 (2005) 3307–3312.

[13] I. Bouabdallah, L.A. M'barek, A. Zyad, A. Ramadan, I. Zidane, A. Melhaoui, Anticancer effect of three pyrazole Derivatives, Nat. Prod. Res. 20 (2006) 1024–1030.

[14] I. Yildirim, N. Ozdemir, Y. Akçamur, M. Dinçer, O. Andaç, 4-Benzoyl-1,5-diphenyl-1H-pyrazole-3-carboxylic acid methanol solvate, Acta Cryst E61 (2005) o256–o258.

[15] D.M. Bailey, P.E. Hansen, A.G. Hlavac, E.R. Baizman, J. Pearl, A.F. Defelice, M.E. Feigenson, 3,4-Diphenyl-Iff-pyrazóle-1 -propanamine antidepressants, J. Med. Chem. 28 (1985) 256–260.

[16] C.K. Chu, S.J. Cutler, Chemistry and antiviral activities of acyclonucleosides, J. Heterocycl. Chem. 23 (1986) 289–319.

[17] Y.N. Mabkhot, N.A. Kaal, S. Alterary, S.S. Al-Showiman, A. Barakat, H.A. Ghabbour, W. Frey, Synthesis, *in-vitro* antibacterial, antifungal, and molecular modeling of potent anti-microbial agents with a combined pyrazole and thiophene pharmacophore, Molecules 20 (2015) 8712–8729.

[18] S. Sakamaki, E. Kawanishi, Y. Koga, Y. Yamamoto, C. Kuriyama, Y. Matsushita, K. Ueta, S. Nomura, Synthesis and biological evaluation of thiophene-C-glucosides as sodium-dependent glucose cotransporter 2 inhibitors, Chem. Pharm. Bull. 61 (2013) 1037–1043.

[19] A. Chaudhary, K.K. Jha, S. Kumar, Biological diversity of thiophene: a review, J. Adv. Sci. Res. 3 (2012) 03–10.

[20] F.E.M. El-Baih, H.A.S. Al-Blowy, H.M. Al-Hazimi, Synthesis of some thienopyrimidine derivatives, Molecules 11 (2006) 498–513.

[21] F.F. Lepifre, G. R Lena, V. Autier, M. Kergoat, L.R. Faveriel, C.G. Charon, S.N. Raynal, A.M. Audet, United States Patent Application 20150197530, 2015.

[22] A.M. Vega, I. Aldana, M.M. Robbani, E. Fernandez, The synthesis of 11*H*-1,2,4-triazolo [4,3-b] pyridazino [4,5-b] indoles, 11*H*-tetrazolo [4,5- b] pyridazino [4,5-b] indoles and 1,2,4-triazolo [3,4-f]-1,2,4-triazino [4,5-a]indoles, J. Heterocycl. Chem. 17 (1980) 77–80.

[23] Y.N. Mabkhot, F. Alatibi, N.N.E. El-Sayed, N.A. Kheder, S.S. Al-Showiman, Synthesis and structure-activity relationship of some new thiophene-based heterocycles as potential antimicrobial agents, Molecules 21 (2016) 1036.

[24] H. Chen, W. Wang, G. Wang, L. Shi, M. Gu, Y. Ren, L. Hou, P. He, F. Zhu, X. Zhong, W. Tang, J. Zuo, F. Nan, Rational design and synthesis of 2,2-bisheterocycle tandem derivatives as non-nucleoside hepatitis B virus inhibitors, Med. Chem. 3 (2008) 1316–1321.

[25] J.P. Gotteland, C. Loubat, B. Planty, D. Junquero, A. Delhon, S. Halazy, Sulfonamide derivatives of benzylamine block cholesterol biosynthesis in HepG2 cells :A new type of potent squalene epoxidase inhibitors, Bioorg. Med. Chem. Lett. 8 (1998) 1337–1342.

[26] N.A. Hassan, A.S. Shawali, K.M. Abu-Zied, D.A. Osman, Synthesis and antiviral evaluation of some new thieno[2,3-d]pyrimidine S-glycosides, J. Appl. Sci. Res. 9 (2013) 833–842.

[27] J.A. Valderrama, O. Espinoza, J. Rodriguez, C. Theoduloz, Synthesis and antitumor evaluation of thiophene analogs of kigelinone, Lett. Org. 6 (2009) 278–281.

[28] M.K. Parai, G. Panda, V. Chaturvedi, Y.K. Manju, S. Sinha, Thiophene containing triarylmethanes as antitubercular agents, Bioorg. Med. Chem. Lett 18 (2008) 289–292.

[29] K.H. Huang, J.M. Veal, P.R. Fadden, J.W. Rice, J. Eaves, J.P. Strachan, A.F. Barabasz, B.E. Foley, T.E. Barta, W. Ma, et al., Discovery of novel 2-amino-benzamide inhibitors of heat shock protein 90 as potent, selective and orally active antitumor agents, J. Med. Chem. 52 (2009) 4288–4305.

[30] J.Z. Chandanshive, B.F. Bonini, W. Tiznado, C.A. Escobar, J. Caballero, C. Femoni, M. Fochi, M.C. Franchini, 1,3-Dipolar cycloaddition of nitrile imines with cyclic α-β-Unsaturated ketones: a regiochemical route to ring-fused pyrazoles, Eur. J. Org. Chem. (2011) 4806–4811.

[31] J.Z. Chandanshive, B.F. Bonini, D. Gentili, M. Fochi, L. Bernardi, M.C. Franchini, Regiocontrolled synthesis of ring-fused thieno[2,3-c]pyrazoles through 1,3-dipolar cycloaddition of nitrile imines with sulfur-based acetylenes, Eur. J. Org. Chem. 33 (2010) 6440–6447.

[32] M.G. Prabhudevaa, N. Renukab, K.A. Kumara, Synthesis of thiophene-pyrazole conjugates as potent antimicrobial and radical scavengers, Curr. Chem. Lett. 7 (2018) 73–80.

[33] R.M. Mohareb, N.N.E. El-Sayed, M.A. Abdelaziz, Uses of cyanoacetylhydrazine in heterocyclic synthesis: novel synthesis of pyrazole derivatives with anti-tumor activities, Molecules 17 (2012) 8449–8463.

[34] N. Harikrishna, A.M. Isloor, K. Ananda, A. Obaid, H. Fun, Synthesis, antitubercular and antimicrobial activity of 1'-(4-chlorophenyl) pyrazole containing 3,5-disubstituted pyrazoline derivatives, New J. Chem. 37 (2013) 1–3.