Synthesis of Thiophene-Based Flavone Schiff Base Derivatives and a Comparison of Biological Activities with Furanflavone Analogs

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ABSTRACT: *In this investigation, a series of thiophene-based flavone Schiff base derivatives were synthesized starting from phloroglucinol. Initially, the acetophenone derivative of phloroglucinol, 2-hydroxy-4,6-di-O-methyl phloroglucinol* ***(3)*** *was prepared by acetylation followed by selective O-methylation. Later, compound* ***3*** *was condensed with thiophene aldehyde to obtain respective thiophene chalcone and was cyclized with I_2/DMSO to get the corresponding thiophene-based flavones intermediate. In the final stage, the titled Schiff base derivatives were obtained by formylation at the 8^{th} position of the flavone skeleton followed by condensation with various amines. All the synthesized compounds were tested for their antibacterial and anticancer activities. Among the tested compounds, compound* ***8h*** *(3-Cl, 4-NO_2) showed good to excellent antibacterial activity on four microorganisms. Further, the compounds were compared with corresponding Schiff's base analogs of furan-flavone Schiff's bases.*

KEYWORDS: *Thiophene flavones; 5-Membered heterocyclic aldehydes; Schiff bases; Anticancer; Antibacterial.*

INTRODUCTION

In 5-membered heterocycles, thiophene and its substitutes were found to be important structural units in many pharmaceutically active molecules [1-5]. On the other hand, flavonoids are attractive targets in organic synthesis due to their occurrence in a wide range of natural products with potential biological significance[6]. Further, they have been classified in the neutraceutical group and are found in a broad range of plant species including mango, kaju (*Anacardium occidentale*), custard apple (*Annona Reticulata*), green tea, grapes, etc [7- 9]. Flavonoids show a wide range of biological activities such as antiviral [10-12], anticancer [13,14], anti-inflammatory [15,16], antimicrobial [17-20], and antioxidant [21,22] properties. Moreover, they are structurally distinct and possess a variety of biological activities [23-25]. These studies increased the interest of medicinal chemists to further study flavonoids as lead molecules to treat various diseases. Recently, researchers are paying attention to flavonoid bioactivities like free radicals' scavenging ability and protection against the peroxidation of lipids [26,27].

It has been established that flavonoids such as kaempferol,

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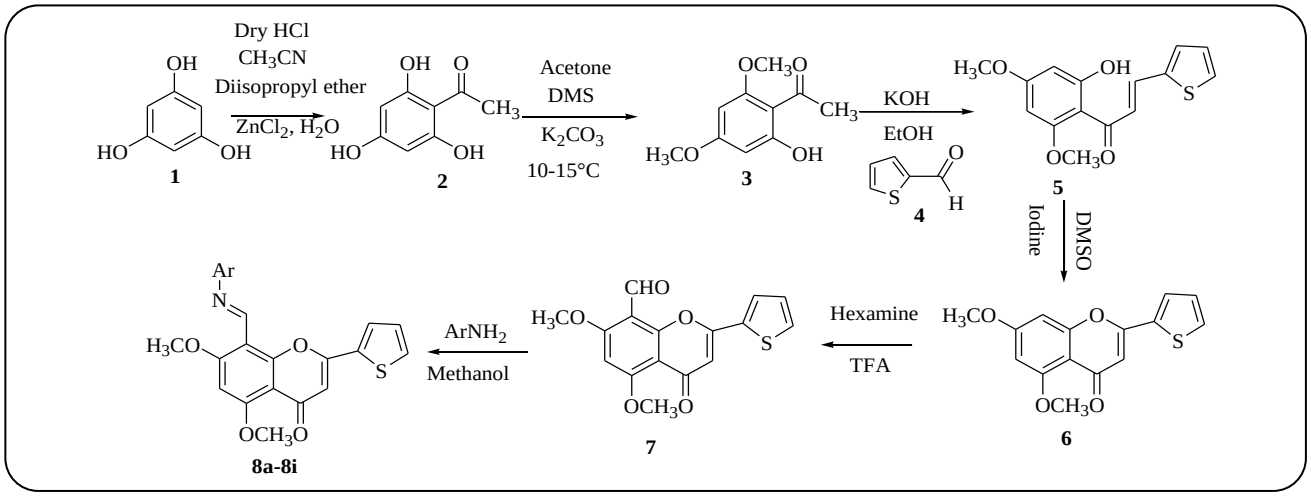


Scheme 1: Synthetic route for thiophene flavone Schiff base derivatives.

luteolin, apigenin, chrysin, and baicalein have the capability of inhibiting α-glucosidase activity [28,29]. Similarly, the Schiff base analogs generally formed by condensation of a primary amine and an aldehyde [30,31] were also well known for their biological activities [32-36]. In the search of our research towards biologically active flavonoid group compounds [12,23-25,37], a new structurally distinct chrysin analog was aimed here by replacing the ring B of the flavone skeleton with five-membered heterocyclic rings such as furan and thiophene. Further, the 8th position of the newly synthesized heterocyclic flavones was methylated to develop Schiff's base moiety using various aromatic amines. In our recent report [37], the work on furan-based flavone Schiff bases towards potential antibacterial compounds was reported, but as per the knowledge of the authors, the thiophene-based flavone Schiff bases were not reported so far. So, in this investigation, the authors aimed to undergo the synthesis of thiophene flavone Schiff bases as shown in Scheme 1, to study and compare the biological activities of furan flavones.

EXPERIMENTAL SECTION

Materials and equipment

The ^{1}H NMR spectra were recorded on BrukerAvance AV 400 MHz NMR spectrometer and ^{13}C NMR spectra were recorded on BrukerAvance AV 100 MHz NMR spectrometer. Mass studies were performed on LC-MS system equipped with Agilent 1100 series LC/ MSD detector and 1100 series Agilent HPLC pump. Normal phase silica gel (ACME, 100-200 mesh) was used for column chromatography. Silica gel pre-coated plates (AlugramSil G/UV254) were used for thin-layer chromatography. The plates were eluted with a solvent system containing hexane/ethyl acetate combination and visualized by immersing the plate in sulfuric acid/methanol reagent followed by heating at 110 °C. The solvents and other chemicals used were of AR grade and were procured from Qualigens Fine Chemicals, Mumbai (India).

Preparation of dimethyl phloroacetophenone (3)

A mixture of well-dried phloroglucinol (5g, 39.68 mmol), anhydrous acetonitrile (7.3 mL, 139 mmol), diisopropyl ether (16.76 mL, 119 mol), and finely powdered and fused zinc chloride (0.99 g, 7.2 mmol) was cooled in an ice&salt mixture at 0 °C under stirring for about 7 h, by passing dry HCl gas. The flask was then allowed to cool in an ice chest overnight in the refrigerator. Later decanting the diisopropyl ether a bulky orange-yellow precipitate was separated and the precipitate was washed with diisopropyl ether (25 mL). The solid was transferred into a round bottom flask and added 500 mL of distilled water and refluxed under stirring for 2 h at 100 °C. Finally, the mixture was cooled to room temperature and left overnight. Pale yellow needles were observed and the product was filtered and dried under a vacuum oven at 120 °C. The yield of compound **2** was 6.4 g and the product structure was confirmed with literature data [36]. Later, a mixture of phloroacetophenone (**2**) (6 g, 35.6 mmol), acetone (30 mL, 404 mmol), and potassium carbonate (14.78 g, 107 mmol) was charged under continuous stirring at room temperature. Further, with the help of the addition funnel dimethyl sulfate

(9.9 g, 2.2 eq, 78.4 mmol) was added dropwise at 10-15 °C for about 3 h. TLC (20% EtOAc in hexane) showed no traces of starting material but observed traces of trimethoxyacetophenone, and after completion of the reaction, the reaction mixture was poured into ice-cold water (50 mL) and stirred for 3 h. A crude solid material was obtained on filtration and washed with cold water and dried well. The yield obtained from compound **3** was found to be 5.9 g and the product structure was confirmed in the literature report [37].

*Synthesis of 3-(Thiophene)-2-yl-1-(2-hydroxy-4,6-dimethoxyphenyl)-prop-2-en-1-one (**5**)*

A solution of 1-(2-hydroxy-4,6-dimethoxy-phenyl)-ethanone (**3**) [5.0g, 25.50mmol] and thiophenaldehyde (28.05 mmol] (**4**) in ethanolic potassium hydroxide (2.8 g in 6 mL ethanol, 50.10 mmol) were stirred for about 24 h at room temperature and the progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was poured into ice-cold water (50 mL) and neutralized with 4 M HCl, a yellow precipitate was obtained. The precipitate was washed with water repeatedly and filtered using a Buckner funnel to yield compound **5**.

1-(2-hydroxy-4,6-dimethoxyphenyl)-3-thiophene-2-yl-pro-2en-1-one (**5**): Yield was 6.0 g, 81% and the compound was characterized by spectral data. ^{1}H NMR(400 MHz, DMSO-d_6)δ: 13.62 (1H, s), 7.9 (1H, d, *J* = 0.8 Hz) 7.56 (1H, d, *J* = 15.6 Hz),7.58 (1H, d, *J* = 15.6 Hz), 7.03 (1H, d, *J* = 3.6 Hz), 6.69-6.67 (1H,dd, *J* = 1.6 ,3.2 Hz), 6.16 (1H, d, *J* = 2.0 Hz), 6.13 (1H, d, *J* = 2.4 Hz), 3.82 (3H, s); ^{13}CNMR(100 MHz, DMSO-d_6)δ: 191.4, 165.7, 165.5, 161.8, 151.1, 146.2, 129.3, 124.0, 116.9, 113.0, 106.1, 93.9, 91.1, 56.1, 55.6; LC-MS (m/z)*:* 291 (M+H).

*Synthesis of 2-(thiophene)-2-yl-5,7-dimethoxychromen-4-one (**6**)*

A solution of thiophenylchalcone (**5**, 18.24 mmol) in DMSO (10 mL), a catalytic amount of iodine was added. The reaction mixture was stirred for 12 h at reflux temperature. The reaction progress was monitored by TLC. After completion of the reaction, the mixture was poured into a solution of hypo and was extracted by using ethyl acetate (2×25 mL). The solvent was dried over anhydrous sodium sulphate and concentrated under a vacuum.

The yield of 2-thiophene-2-yl-5,7-dimethoxy chromen-4-one (**6**) was 3.7 g, 70.56 % and the product structure was confirmed by their spectral analysis.^{1}HNMR(400 MHz, $CDCl_3$) *δ:* 4.06 (3H, s), 4.08 (3H, s), 6.38 (1H, s), 6.56 (1H, s), 6.72 (1H, s), 7.18 (1H, dd, *J* = 3.6, 4.4 Hz), 7.55 (1H, d, *J* = 4.8 Hz), 7.94 (1H, d, *J* = 4.0 Hz); ^{13}CNMR (100 MHz, $CDCl_3$)δ: 188.9, 164.3, 163.8, 162.6, 158.4, 146.3, 128.4, 123.2, 113.6, 108.4, 94.6, 57.4, 56.2; LC-MS (*m/z)*: 289.0 (M+H).

*Synthesis of 2-(theiphene)-2-yl-5,7-dimethoxy-4-oxo-4H-chromene-8-carbaldehyde (**7**)*

A solution of 2-(Thiophene)-2-yl-5,7-dimethoxychromen-4-one (**6**, 11.02 mmol) in trifluoroacetic acid (30 mL) added hexamine (3.09 g, 22.04 mmol) and stirred for 24 h at 110 °C. The reaction progress was monitored by TLC. After completion of the reaction, the reaction mixture was poured into ice water and extracted with ethyl acetate. The organic layer was washed with 10% $NaHCO_3$ solution, water, and brine solution. The organic layer was dried over anhydrous sodium sulfate and concentrated in a vacuum.

The yield of 2-thiophene-2-yl-5,7-dimethoxy-4-oxo-4*H*-chromene-8-carbaldehyde (**7**) obtained was 2.04 g, 57.75 %, and the product structure was confirmed by their spectral analysis. ^{1}HNMR(400 MHz, $CDCl_3$) *δ*: 4.06 (3H, s), 4.08 (3H, s), 6.38 (1H, s), 6.56 (1H, s), 7.18 (1H, dd, *J* = 3.6, 4.4 Hz), 7.55 (1H, d, *J* = 4.8 Hz), 7.94 (1H, d, *J* = 4.0 Hz), 10.56 (1H, s); ^{13}C NMR(100 MHz, DMSO-d_6)δ: 194.8, 180.4, 163.2, 161.8, 160.3, 158.9, 147.2, 138.4, 129.7, 114.2, 106.3, 94.7, 58.2, 57.6; LC-MS (*m/z*): 317 (M+H).

*The general method for synthesis of thiophene flavone Schiff base derivatives(**8a-8i**)*

To a solution of 2-thiophene-2-yl-5,7-dimethoxychromen-4-onecarbaldehyde (**7**, 1 mmol) in methanol (5 mL) added amine (1 mmol). The reaction mixture was allowed to stir for 48 h. The reaction progress was monitored by TLC. After completion of the reaction, the organic layer was subjected to concentration and the crude compound was adsorbed on silica gel and eluted through the column with ethyl acetate-hexane in 1:4).

Procedure for antibacterial activity

This was studied using the agar well diffusion method [38]. This bacterial culture 24 h old was used for analysis. About 0.3 mL of each bacterial suspension was added to sterile Petri plates and to these plates molten state nutrient agar medium was also poured by using the pour plate method. After complete solidification, wells were bored with a sterile cock borer of 6 mm in diameter. The present samples were prepared by dissolving 500 μg in 1 mL DMSO. Wells were filled with 100 μL of the sample. The plates were incubated at 37 °C for 24 h. After incubation, the diameter of the zone of inhibition was measured. For each sample and bacterial species, triplicates were maintained. Streptomycin standard antibiotic with a concentration of 10 μg/mL DMSO was used as a positive control.

Procedure for anticancer activity

The compounds were tested on HCT116 (humancolon cancer cell line), PANC-1 (Pancreatic cancer cell line), and SKBR3 (human breast cancer cell line)using MTT cell proliferation assay method [39]. HCT116, PANC-1and SKBR3 cell line was obtained from National Centre for Cell Science (NCCS), Pune (India) and cultivated in Dulbecco's modified Eagle's medium (DMEM) (Sigma Life Science, USA) containing 10 % fetal bovine serum (FBS). The cells (2,000 cells per well) were seeded in a 96-well microplate containing 100 μL of DMEM complete medium per well and incubated at 37 ºC with 5 % CO_2. The cells were treated with different concentrations of compounds for up to 72 h for every 24-hour interval. Controls were maintained with 0.5 % DMSO. After 72 h of treatment, 5 μL of MTT (3-(4,5-dimethyl- thiazol-2-yl)-2,5-diphenyltetrazolium bromide) reagent (R&D Systems, USA) along with 45 µL of phenol red-free DMEM (Sigma Life Science, USA) without FBS was added to each well, and plates were incubated at 37 ºC with 5 % CO_2 for 4 h. Thereafter, 50 μL of solubilization buffer (R&D Systems, USA) was added to each well to dissolve the colored formazan crystals produced by the reduction of MTT. After 24 h, the optical density was measured at 550 nm using a microplate reader (Bio-Rad, USA).

RESULTS AND DISCUSSION

The targeted compounds were synthesized (**Scheme1**) starting from phloroglucinol (**1**), following the methodology reported by us recently for furan flavone Schiff base analogs [37]. Initially, the 2-hydroxy-4,6-*O*-dimethylphloroglucinol (**3**) was prepared by acetylation of phloroglucinol followed by selective methylation. Acetophenone (**3**) and thiophene aldehyde (**4**) were condensed in the presence of potassium hydroxide in ethanol at room temperature overnight yielding corresponding chalcone (**5**) with good yield. The cyclization of chalcone was carried out using a catalytic amount of iodine in dimethyl sulphoxide at 70 ºC, which yielded the corresponding flavone derivative (**6**). The formylated flavone (**7**) was then synthesized by the reaction of hexamine in trifluoroacetic acid and the formylated flavones were condensed with different aromatic amines in methanol at room temperatures to yield flavone Schiff base derivatives (**8a-8i**). All the synthesized compounds were well characterized with their ^{1}H & ^{13}C NMR and mass spectral data. Further, the formation of final compounds **8a-8i** was clearly observed in ^{1}H NMR spectral data showing the disappearance of the aldehydic proton of compound **7** at δ 10.56 ppm and the imine-related peaks at δ 7-8 ppm which are merged/overlapped with some aromatic protons. Similarly, the aldehydic carbon of ^{13}C also disappeared in the ^{13}C NMR spectra of compounds **8a-8i**, and its imine corresponding peak was observed at δ=160-165 ppm. The structural details and yields of all the products were summarized in **Table-1**.

In thiophene Schiff base derivatives compounds with no substitution *i.e* simple aniline derivative (**8a**) yielded 83 % and compounds with nitro substitution **8b**, **8c,** and **8h** yielded around 70-76%. The above results clearly indicate that compounds with no substitution or with nitro substituent gave high yields. In respect of furan flavones Schiff base analogs, the percentage of yields obtained [37] in the furan flavone Schiff base aromatic amine with no substitution *i.e.* free aniline derivative also yielded a high percentage 84 % as in thiophene analog (**8a**) and the compound with substitutions 4-chloro-3-nitro yielded in 83 %. Compounds with 4-fluoro and 4-nitro derivatives also yielded a moderate yield of ≥ 75%. So, the yields were almost similar in both analogs.

The present synthesized compounds were screened for their antibacterial activity on three Gram-positive and three Gram-negative organisms namely *Escherichia coli* (MTCC 1687), *Pseudomonas aeruginosa* (MTCC 1688), *Proteus Vulgaris* (MTCC 426), and *Bacillus megaterium*

Table 1: Thiopheneflavone Schiff base derivatives.

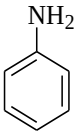
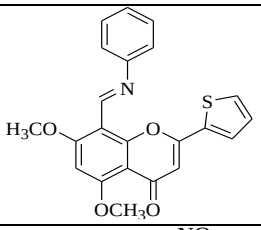
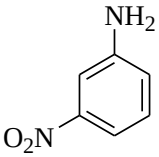
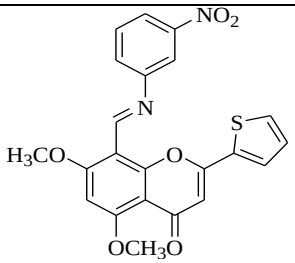
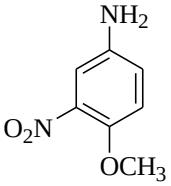
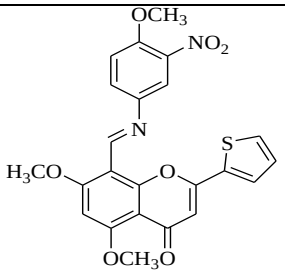
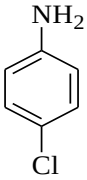
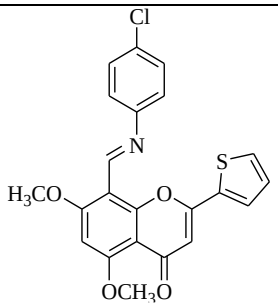
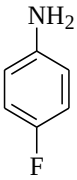
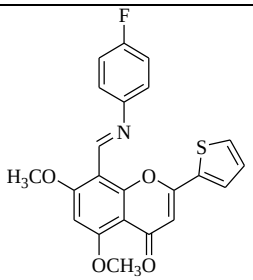
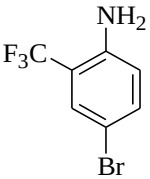
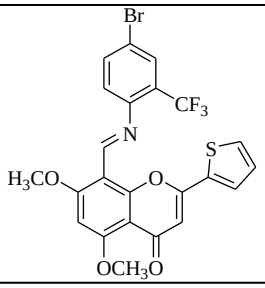
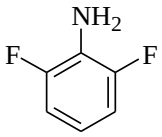
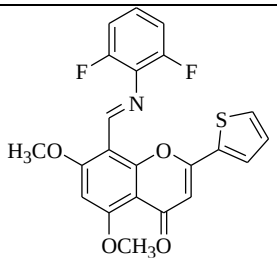
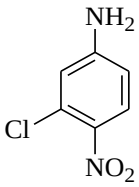
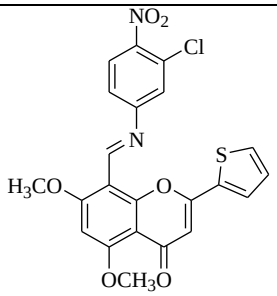
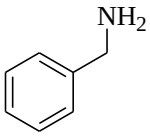
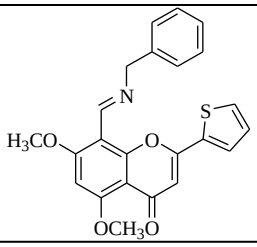
C.No	Amine	Product	Yield (%)
8a			83
8b			76
8c			72
8d			68
8e			64
8f			62

Table 1: Thiopheneflavone Schiff base derivatives. (Continuation).

C.No	Amine	Product	Yield (%)
8g			60
8h			70
8i			78

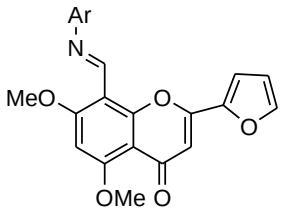
(MTCC 42), *Streptococcus mutans* (MTCC 497), and *Staphylococcus aureus* (MTCC 737) by using the agar well diffusion method [38], and results are summarized in **Table 2**.

The compound **8h** (3-Cl, 4-NO_2) showed good to excellent antibacterial activity on four organisms namely *Escherichia coli* MTCC 1687 (zone of inhibition compound/streptomycin; 12.33/8.0 mm), *Pseudomonas aeruginosa* MTCC 1688 (zone of inhibition compound/streptomycin; 7.0/7.0 mm), *Bacillus megaterium* MTCC 428 (zone of inhibition compound/streptomycin; 13.0/7.0 mm) and *Staphylococcus aureus* MTCC 737 (zone of inhibition compound/streptomycin; 7.33/6.0 mm) when compared to the standard streptomycin. Compounds with nitro substitutions **8b, 8c**, and **8g** also showed good antibacterial activity on some tested organisms. The compound **8e** with *para*-Fluoro substitution also showed significant activity against MTCC 428 strain. It is clearly shown that thiophene Schiff base derivatives with nitro functional group possess excellent to good antibacterial activities.

All the synthesized compounds were also screened for their anticancer activity on three cancer cell lines namely HCT116 (human colon cancer cell line), PANC-1 (Pancreatic cancer cell line) and SKBR3 (human breast cancer *cell* line). By using MTT cell proliferation assay method [39]. The compounds were tested at 200 μg/mL and the IC_{50} values were estimated and are reported in **Table 3**. Moreover, a series of furan flavones Schiff base compounds (**Fig. 1**) were synthesized and confirmed as per our recent report [37] and were also studied on the same anticancer cell lines and reported in **Table 3** for comparison. Observing the activity results given in **Table 3** on three cancer cell lines it clearly indicated that compounds **6b** and **6d-6i** of furan flavone Schiff base derivatives showed moderate anticancer activity upto 200 μg/mL, and the remaining compounds did not show activity up to 200 μg/mL. Results obtained for the thiophene flavone Schiff base derivatives **8d, 8f**, and **8i** showed moderate activity, and the remaining thiophene derivatives did not show IC_{50} value up to 200 μg/mL. Among these three compounds, **8i** showed the highest activity on HCT-116,

Table 2: Antibacterial activity results of the synthesized compounds.

Sample	Zone of inhibition in mm at 10 μg/mL concentration					
	Escherichiacoli MTCC 1687	Pseudomonas aeruginosa MTCC 1688	Proteus vulgaris MTCC 426	Bacillus megaterium MTCC 428	Streptococcus mutans MTCC 497	Staphylococcus aureus MTCC 737
8a	2.00±0.00	4.33±0.57	0.00±0.00	2.00±0.00	0.00±0.00	0.00±0.00
8b	6.20±0.57	2.73±0.00	6.25±0.57	3.28±0.57	5.00±1.00	5.23±0.57
8c	5.60±0.57	6.38±0.57	6.10±0.57	3.30±0.57	4.00±1.00	2.66±0.57
8d	2.33±0.57	3.33±0.57	0.00 ±0.00	2.33±0.57	0.00±0.00	0.00±0.00
8e	6.25±0.57	4.40±1.00	3.91±0.57	7.00±1.00	4.21±1.00	3.63±0.57
8f	2.33±0.57	5.00±0.00	3.33±0.57	2.33±0.57	0.00±0.00	5.66±0.57
8g	6.34±0.57	6.52±1.00	5.60±1.00	4.035±0.57	3.80±1.00	5.50±0.57
8h	12.33±0.57	7.00±1.00	4.00±1.00	13.00±1.00	2.33±0.57	7.33±0.57
8i	2.62±0.57	5.23±0.57	3.00 ±1.00	4.21±0.57	0.00±0.00	3.32±1.00
Streptomycin	8.00±0.00	7.00±0.00	7.00±0.00	7.00±0.00	8.00±0.00	6.00±0.00



6a-l

6a: o-NO_2, p-Cl; **6b**: p-NO_2; **6c**: m,p-tri-F; **6d**: p-F; **6e**: p-CH_3CO-; **6f**: p-Cl;
6g: o,p-di-Cl; **6h**: o-CF_3, p-Br, **6i**: o-di-Cl; **6j**: o-OMe, m-NO_2, **6k**: o-di-F; **6l**: Ph

Fig. 1: Structures of furan-flavone analogs.

8f showed the highest activity on PANC-1 and **8d** showed promising inhibitory activity of cell growth on SKBR3 cell line at 200 μg/mL (**Fig. 2**), Finally, analyzing the results of both furan and thiophene flavone Schiff base derivatives reveals that these compounds did not show potent activity on tested anticancer cell lines. The moderate activity of compounds prompted more potent molecules as earlier reports were also confirmatory with the anticancer potential of flavones in various cancer cell lines [40].

SAR studies

In thiophene analogs, the compounds with electron-withdrawing groups –NO_2 at *m*&*p*- positions (in **8b, 8c**, and **8h**) increased the zone of inhibitions compared to the simple phenyl group on the phenyl nucleus (**8a**). Moreover compound **8h** having *p*-Nitro and *m*-Chloro substitutions showed a maximum zone of inhibitions on MTCC-428(13.0±1) and MTCC-1687(12.33±0.53) compared to standard streptomycin, followed by MTCC-737 (7.33±0.57) and MTCC-1688(7.00±1.00).

In the case of halogens at the *p*-position, the compound **8e** with F-group at the *p*-position showed a reasonably good zone of inhibitions compared to **8d** and **8f** with *p*-Chloro and *p*-Bromin in most microorganisms. In particular, compound **8e** showed an equal zone of inhibitions with standard streptomycin (7.00±1.00). The compound **8g** having two *o*-Fluorine atoms also showed a good zone of inhibitions in most organisms except MTCC-428 & 497.

However, as per our reported data [37], in the case of furan analogs the compound with nitro substitution at *p*-position of the amine group (**6b**) showed a better zone of inhibition compared to nitro substitution in the*m*-position along with *o*-methoxy group (**6j**) towards MTCC-1688 and MTCC-426 compared to standard streptomycin. The other microorganisms did not show much effect compared to the standard.

Table 3:Anticancer activity results of all the synthesized compounds at different concentrations (200 µg/mL).

Sample	HCT116	PANC-1	SKBR3
ThiopheneflavoneSchiff base derivatives			
8a	>200	>200	>200
8b	>200	>200	>200
8c	>200	>200	>200
8d	162.6	150	157
8e	>200	>200	>200
8f	162.3	135	172.2
8g	>200	>200	>200
8h	>200	>200	>200
8i	151.7	>140.4	148
FurfuralflavoneSchiff base derivatives*			
6a	>200	>200	>200
6b	165	153	170
6c	>200	>200	>200
6d	157.4	160.5	151.3
6e	153.8	136.7	156
6f	163.1	174	176.1
6g	167.1	122.3	185
6h	165.0	182.4	167.3
6i	179.2	171	189.3
6j	>200	>200	>200
6k	>200	>200	>200
6l	>200	>200	>200
Paclitaxol	8.2	24.8	31.4

Note: The compounds **6a-l represent the furfural heterocyclic flavones Schiff bases synthesized as per Ref.37.*

In the case of halogen substitutions, the compound **6a** with *p*-Chloro substitution along with *o*-Nitro substitution showed a good zone of inhibition compared to the *p*-Chloro compound (**6f**) towards MTCC-1687 and MTCC-428, but interestingly the zone of inhibition is reversed in respect of the organisms MTCC-1688 and MTCC-426. However, compound **6g** having *o*-Chloro substituent along with *p*-Chloro did not show many zones of inhibition, but compound **6i** with two *o*-Chloro substituents showed equal zone of inhibitions with standard and the others not. It is important to note that the compound **6d** with *p*-Choloro substitution showed a good zone of inhibitions compared to **6c** with trifluoro substitution at *m*- and *p*- and **6k** with two *o*-fluoro substitutions in respect of organisms MTCC-1688, MTCC-426, and particularly MTCC-737.

Spectral data

8-((E)-(phenylimino)methyl)-2-(thiophene-2-yl)-5,7-dimethoxy-4H-chromen-4-one (**8a**)

Yield 324 mg, 83 %; M.P: 172-174 °C; ^{1}HNMR (400 MHz, $CDCl_3$) δ:8.04 (1H, d, J = 3.6 Hz), 7.52 (1H, d, J = 4.4 Hz), 7.45-7.34 (5H, m), 7.23-7.19 (1H, m), 6.57 (1H, m), 6.38 (1H, m) 3.821 (3H, s); ^{13}CNMR (100 MHz,

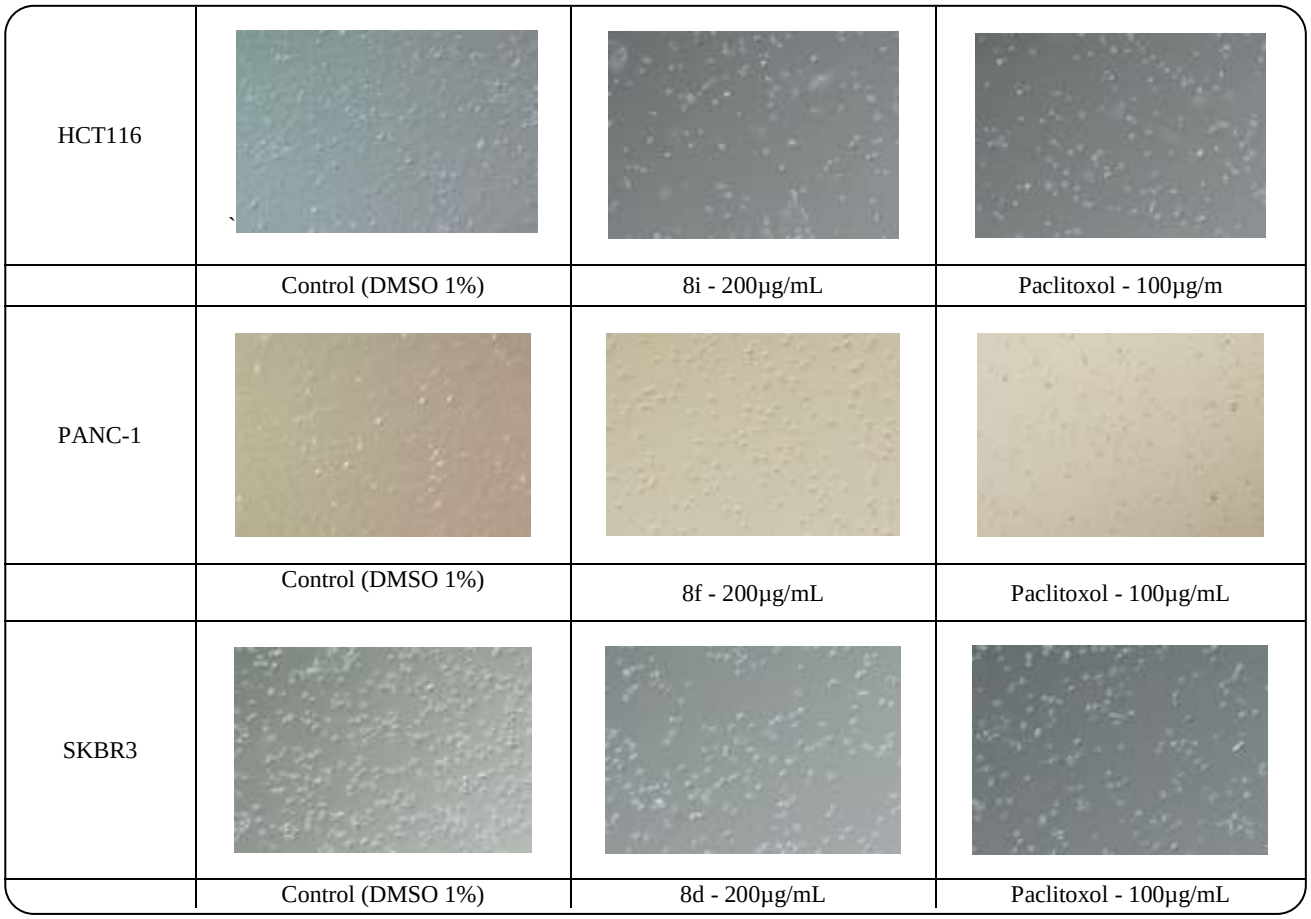


Fig. 2: Inhibitory activity of compound 8d on cell proliferation at 200µg/mL, negative control (DMSO 1%), positive control (Paclitaxol - 100µg/ml). The cell lines such as HCT 116, SKBR3 and Panc1 were employed.

$CDCl_3$)δ: 178.4, 164.7, 162.8, 161.2, 160.9, 159.7, 157.4 156.7, 130.2, 129.3, 128.7, 128.5, 118.93, 115.6, 106.6, 96.6, 93.2, 91.9, 56.2, 53.1; LC-MS(*m/z)*:392 (M+H).

8-((E)-(3-nitrophenylimino)methyl)-2-(thiophene-2-yl)-5,7-dimethoxy-4H-chromen-4-one **(8b**)

Yield 331 mg, 76 %; M.P: 194-195 °C; ^{1}H NMR(400 MHz, $CDCl_3$)δ: 8.22 (1H, s), 8.05 (2H, m), 7.61-7.55 (1H, m), 6.62-6.52 (2H, m), 6.41-6.38 (1H, s), 4.08 (3H, s), 4.01 (3H, s); ^{13}C NMR(100 MHz, $CDCl_3$) δ: 180.4, 163.2, 161.7, 160.3, 159.6, 153.6, 138.6, 134.4, 126.5, 124.2, 113.2, 112.5, 105.4, 93.7, 57.8, 57.4; LC-MS (*m/z)*: 435 (M-H).

8-((E)-(3-nitro-4-methoxyphenylimino)methyl)-2-(thiophene-2-yl)-5,7-dimethoxy-4H-chromen-4-one (**8c**)

Yield 335 mg, 72 %; M.P: 197-199 °C; ^{1}H NMR (400 MHz, $CDCl_3$)δ: 8.04-7.90 (3H, m), 7.82-7.80 (3H, m), 7.67(1H, s) 6.64-6.56 (1H, m), 6.39 (1H, s), 4.08 (3H, s), 3.94(3H, s); ^{13}C NMR(100 MHz, $CDCl_3$) δ: 184.2, 165.2, 163.6, 161.4, 160.3, 159.7, 147.4, 145.6, 144.52, 138.3, 119.69, 111.80, 106.8, 93.4, 58.1, 57.6, 56.2; LC-MS (*m/z)*: 465 (M-H).

8-((E)-(4-chlorophenylimino)methyl)-2-(thiophene-2-yl)-5,7-dimethoxy-4H-chromen-4-one (**8d**)

Yield 289 mg, 68 %; M.P: 184-186 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.58-8.25 (1H, m), 8.07-8.02 (1H, m), 7.55-7.37 (2H, m), 7.24 (1H, m), 7.06 (2H, s), 6.62(1H, s), 6.50-6.43 (1H, m), 3.96 (6H, s); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 191.96, 164.1, 162.6, 161.2, 160.3, 157.5, 147.1, 132.3, 129.8, 123.2, 121.4, 114.8, 105.6, 92.8, 56.4, 56.2; LC-MS (*m/z)*: 426.0 (M+H).

8-((E)-(4-fluorophenylimino)methyl)-2-(thiophene-2-yl)-5,7-dimethoxy-4H-chromen-4-one (**8e**)

Yield 261 mg, 64 %; M.P: 188-189 °C. ^{1}H NMR (400 MHz, DMSO-d_6)δ: 8.01-7.95 (1H, m), 7.70-7.60 (1H, m), 7.21-7.17 (3H, m), 6.86 (1H, s), 6.66-6.59 (1H, m),

3.92 (3H, s), 3.82 (3H, s). ^{13}C NMR (100 MHz, DMSO-d_6)δ: 185.38, 164.3, 163.2, 161.6, 160.1, 153.6, 142.3, 140.9, 130.8, 128.8, 125.0, 116.9, 116.73, 105.9, 88.3, 56.4, 56.1; LC-MS (*m/z)*: 410.0 (M+H).

8-((E)-(2-trifluoromethyl-4-bromophenylimino)methyl)-2-(thiophene-2-yl)-5,7-dimethoxy-4H-chromen-4-one (**8f**)

Yield 333 mg, 62 %;M.P: 208-210 °C.^{1}HNMR (400 MHz, DMSO-d_6) δ:8.05-7.96 (3H, m), 7.73-7.59 (2H, m), 7.33-7.00 (2H, m) 6.68-6.44 (1H, m), 5.76 (1H, s), 4.03 (6H, s); ^{13}CNMR (100 MHz, DMSO-d_6)δ: 185.94, 164.3, 164.2, 161.1, 160.3, 148.7, 141.3, 135.31, 130.52, 128.6, 117.6, 115.4, 107.2, 91.6, 56.4, 56.1; LC-MS (*m/z)*: 540 (M+2H).

8-((E)-(2,6-difluorophenylimino)methyl)-2-(thiophene-2-yl)-5,7-dimethoxy-4H-chromen-4-one (**8g**)

Yield 256 mg, 60 %; M.P: 194-196 °C. ^{1}HNMR (400 MHz, $CDCl_3$)δ: 8.95-8.61 (2H, m), 8.25-8.03 (1H, m), 7.57-7.51 (1H, m), 7.16-7.11 (1H, m), 6.93-6.89 (2H, m), 6.67-6.60 (1H, m), 6.45-6.35 (1H, m), 4.08 (3H, s), 4.01 (3H, m); ^{13}C NMR (100 MHz, DMSO-d_6)δ: 183.2, 164.3, 161.7, 160.9, 160.1, 158.1, 154.3, 143.2, 138.4, 135.3, 133.3, 133.2, 128.3, 125.4, 119.6, 118.3, 112.1, 108.4, 94.3, 56.155.9; LC-MS (*m/z)*: 426 (M-H).

8-((E)-(3-chloro-4-nitrophenylimino)methyl)-2-(thiophene-2-yl)-5,7-dimethoxy-4H-chromen-4-one (**8h**)

Yield 329 mg, 70 %; M.P: 206-208 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.89 (1H, s), 7.84 (1H, s), 7.74 (1H, s), 7.58 (1H, d, J = 8.0 Hz), 7.53 (1H, d, J = 2.8 Hz),7.42 (1H, d, J = 6.4 Hz), 7.13 (1H, s), 6.60 (1H, s), 6.44 (1H, s), 4.08 (3H, s) 4.06 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$)δ: 178.4, 164.6, 164.2, 157.67, 157.18, 156.19, 153.23, 134.73, 132.43, 130.28, 129.07, 128.41, 126.04, 117.29, 109.45, 107.61, 105.59, 91.18, 56.69, 56.45; LC-MS (*m/z)*: 471.0 (M+H).

8-((E)-(4-benzylphenylimino)methyl)-2-(thiophene-2-yl)-5,7-dimethoxy-4H-chromen-4-one(**8i**)

Yield 315 mg, 78 %; M.P: 176-178 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.85 (1H, s), 7.99 (2H, d, J = 3.6 Hz), 7.55 (1H, d, J = 4.4 Hz), 7.38-7.32 (5H, m), 7.30-7.28 (2H, m), 7.19-7.17 (1H, m), 6.52 (1H, s), 4.55-4.51 (2H, m), 3.82 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 183.4, 163.2, 161.9, 161.4, 160.2, 158.4, 156.3, 145.3, 138.4, 134.2, 128.5, 120.1, 117.1, 110.3, 107.4, 93.2, 56.4, 56.1, 47.2; LC-MS (*m/z)*: 406 (M+H).

CONCLUSIONS

In this report, structurally distinct heterocyclic flavone Schiff base derivatives were synthesized by replacing ring B of chrysin skeleton with thiophene (**8a-i**); further, also synthesized furan analogs (**6a-l**; reported recently by us [35] for comparative study. The yields of both analogs were found to be almost similar. Further, the developed heterocyclic flavones Schiff bases were screened for their antibacterial and anticancer activity. Compound **8h** (3-Cl, 4-NO_2) showed excellent to good antibacterial activity on four organisms tested; the remaining compounds also showed good to moderate activity on tested organisms. A comparative study was reported between furan and thiophene analogs in respect of both antimicrobial and anticancer activities. The concept may be utilized for the study of other flavonoids with the same or other diverse heterocyclic rings.

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