Medicinal Chemistry Research (2018) 27:2297–2311
https://doi.org/10.1007/s00044-018-2236-3

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Design, synthesis and identification of novel substituted isothiochromene analogs as potential antiviral and cytotoxic agents

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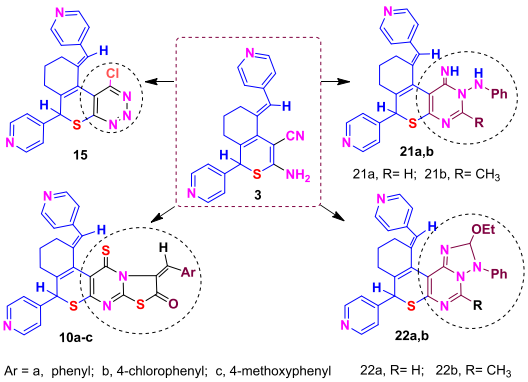
Received: 30 January 2018 / Accepted: 17 August 2018 / Published online: 24 August 2018

Abstract

In this study, we present the synthesis of novel isothiochromenes, thiazolidonone, thiazinone, arylidines, triazoles, and pyrimidinone compounds based on the starting material 3-amino- isothiochromene-4-carbonitrile **3**. The chemical structures were confirmed using spectroscopic methods and elemental analyses. These compounds were screened for their in vitro antiviral and antitumor activities. Compounds **10a-c** and **22a-b** showed activity against herpes simplex virus-1 (HSV-1) and human immunodeficiency virus-1 (HIV-1). Compounds **15** and **21a-b** exhibited activity against various types of cancer cell lines.

Graphical Abstract

Compounds **22a-b** and **10a-c** showed to be active against herpes simplex virus-1 (HSV-1) and virus-1 (HIV-1). Also, the products **21a-b** and **15** proved to a wide extent antitumor activity



Keywords Isothiochromenes · Thiazolopyrimidines · Thiazines · 1, 2, 3-triazine · Triazolopyrimidines · Pyrrolopyrimidines · Antiviral · Antitumor activity

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00044-018-2236-3) contains supplementary material, which is available to authorized users.

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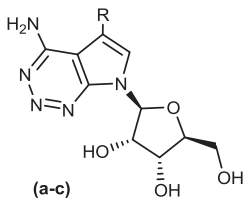
Introduction

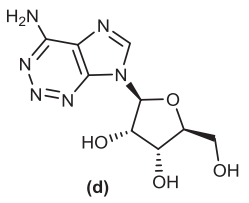
Many biological activities in the human body are modulated by heterocyclic compounds such as nucleic acids, hormones, and neurotransmitters, etc. Heterocycles also play a crucial role in drug discovery where they are involved in many therapeutic areas. One of those heterocyclic compounds, the pyrimidine moiety, is an essential component of many classes of drugs, such as antineoplastic (Uramustine), antibacterial (Trimethoprim), antifungal (Flucytosine), and antivirals (Broxuridine) (Selvam et al. 2012; Abu-Hashem and Hussein 2015).

Substituted thiopyrans are heterocycles that exhibit an extensive range of biological activities such as anti-inflammatory (Rogier et al. 2001), antibacterial (Brown et al. 2002), effective antagonists at α1- adrenoreceptor (Quaglia et al. 2002), and dopamine D3 receptor-selective agonists (Van Vliet et al. 2000). Furthermore, isothiochromene-based compounds are reported to exhibit antitubercular, anti-HIV-1 (Bennani et al. 2007), anticancer and antitrypanosomal activities (Kaminskyy et al. 2014)

Also, thiazolopyrimidine compounds are shown to have activities as acetylcholinesterase inhibitors (Zhi et al. 2008). Meanwhile, thiazine-based compounds are known to have antimicrobial (Ram et al. 2013), antiviral (Galal et al. 2011), anti-inflammatory, analgesic, and ulcerogenic activities (Vijay 2011). Likewise, 1, 2, 4-triazolopyrimidine derivatives displayed numerous pharmacological activities as antimicrobial (Abu-Hashem et al. 2017), anti-inflammatory, analgesic (Sung and Lee 1992), and antimalarial (Havaldar and Patil 2008) agents. Also, 1, 2, 3-triazine is an interesting class of heterocyclic compounds. They are known to have pharmacological activity and may be considered as lead molecules for the development of future drugs (Kumar et al. 2014) for examples: Tubercidin (**a**) is used to preventing the growth of bacteria. Toyocamycin (**b**) is recognized as antibiotic and antineoplastic. Sangivamycin (**c**) is active against leukemia, lung carcinoma, against colon, and gall-bladder cancer in humans. 2-Aza-adenosine (**d**) exhibits the highest cytotoxicity against epidermoid carcinoma cells (Migawa et al. 2005), (Fig. 1).



Tubercidin (a), R= H
Toyocamycin (b), R= CN
Sangivamycin (c), R= $CONH_2$



(2*S*,3*S*,4*R*,5*S*)-2-(4-amino-7*H*-imidazo [4,5-*d*][1,2,3]triazin-7-yl)-5-(hydroxy-methyl) tetrahydrofuran-3,4-diol

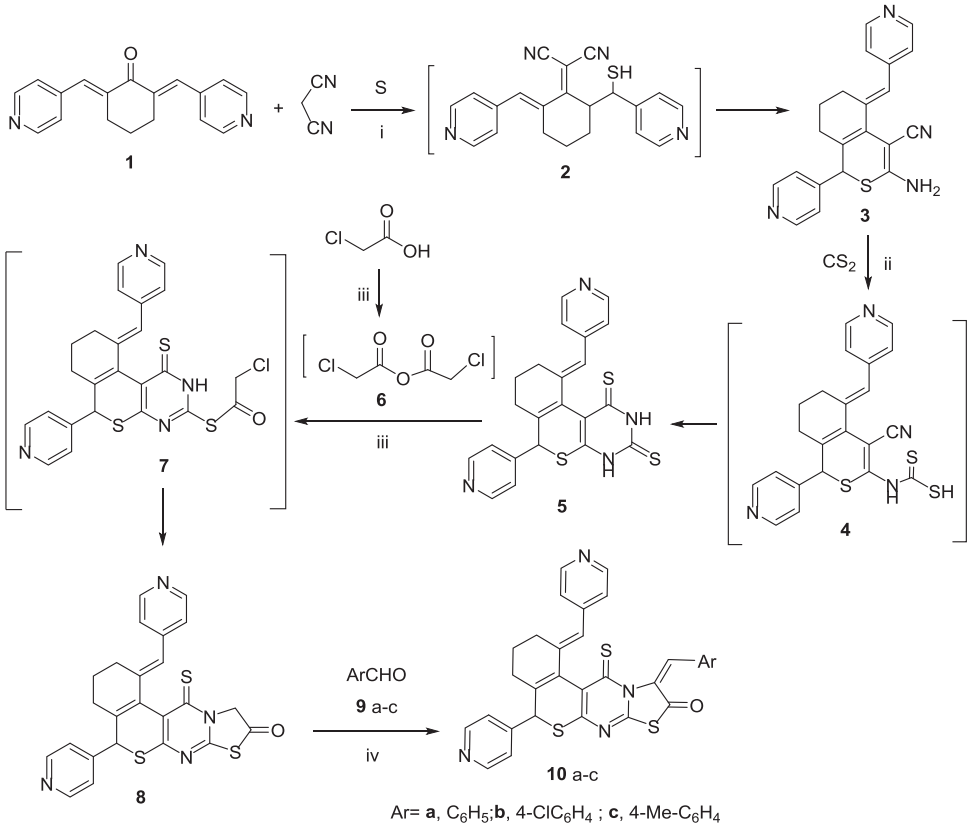
Fig. 1 Biologically active 1, 2, 3-triazine derivatives

Pyrrolopyrimidines are a valuable group of bioactive compounds showing antivirals, antiprotozoal, antibacterial and anticancer (De Coen et al. 2016), anti-hyperglycemic activities (Mohamed et al. 2014) and anti-cancer agents (Dholakia et al. 2015). Based on the potential biological activities of these heterocycles we synthesized new pyrimidine-, thiazolopyrimidine-, pyrimidothiazine-, triazine-, triazolo- pyrimidine-, pyrrolopyrimidine, and pyridine-based compounds incorporating isothiochromene in order to investigate their antiviral and cytotoxic activities. Thus, it is a real challenge to combine the aforementioned rings together in a molecular framework to make use of the additive effect of these rings toward the antiviral and cytotoxic activities.

Results and discussion

Chemistry

The synthetic strategies, adopted to obtain the poly-functional substituted isothiochromenes, are shown in Schemes 1–4. Compound **3** was prepared via Gewald reaction (Gewald et al. 1966) of 2, 6-bis (pyridin-4-ylmethylene) cyclohexan-1-one **1** (Somers-Edgar et al. 2009) with malononitrile and elemental sulfur in ethanol containing diethylamine. The IR spectrum of compound **3** showed absorption bands at ν 3420 (NH_2), 2212 (CN) cm^{-1}. Furthermore, the ^1H NMR spectrum of **3** exhibited two singlet signals at δ 4.62, 6.28, and 8.23 ppm for one proton (C_1-H) of the thiopyran ring and the amino group (as D_2O exchangeable) and the methine proton respectively. Moreover, its mass spectrum revealed the ion peak at *m/z* 358 (M^+, 100 %), which agrees with the molecular formula ($C_{21}H_{18}N_4S$). Compound **3** reacted with carbon disulfide in ethanol and in the presence of aqueous potassium hydroxide according to the reported condition by (Rashad et al. 2005) to afford the pyrimidine-1, 3(2*H*)-dithione (**5**) through the intermediate **4**. The IR spectrum of compound **5** revealed the absorption bands at ν 3395 (NH), 1620 (C=N) and 1355, 1370 cm^{-1} (2 C=S). Moreover, its ^1H NMR spectrum exposed two singlet signals at δ 9.19 and 9.21 ppm due to (2NH). Subsequently, compound **5** reacted with chloroacetic acid in the presence of Ac_2O/AcOH to give the isothiochromeno[3,4-d]thiazolo[3,2-a]pyrimidin-9(10*H*)-one **8**, the formation of **8** can be explained via nucleophilic addition of the thiol group of 1, 3(2*H*)-dithione **5** to in-situ formed chloroacetic anhydride **6**, followed by cyclization of the formed intermediate **7**. Knovenagel condensation of compounds **8** with the appropriate aromatic aldehydes derivatives namely, benzaldehyde, or 4-chlorobenzaldehyde or 4-methoxybenzaldehyde afforded the isothiochromeno [3,4-d]thiazolo[3,2-a]pyrimidin-9(10*H*)-one **10a-c**. The IR

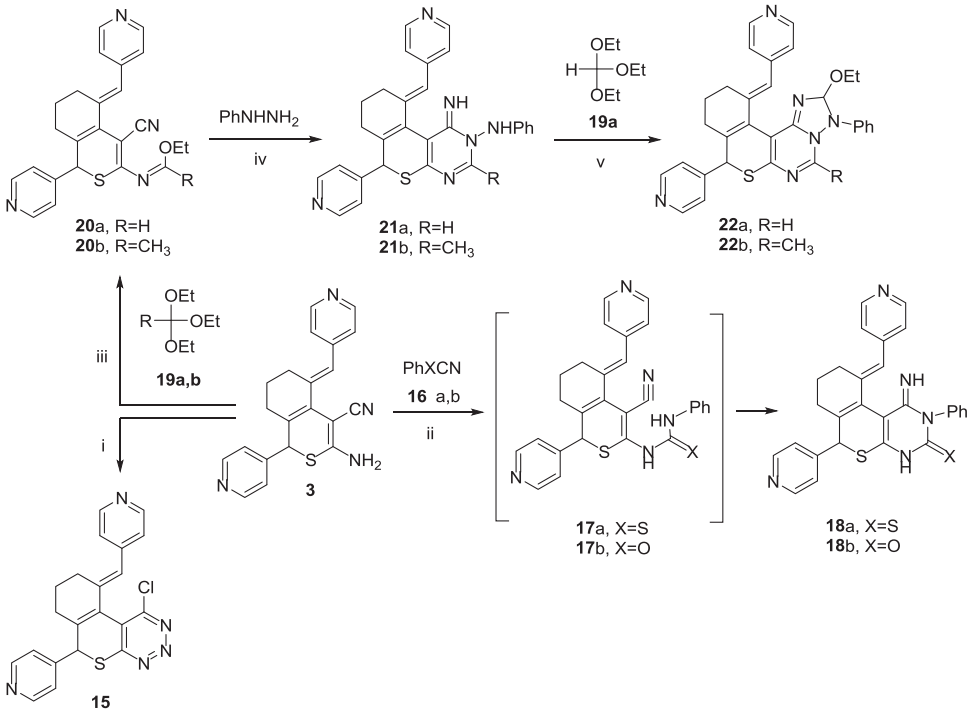


Scheme 1 Synthesis of thiazolopyrimidines **8** and **10**a–c. Reagents and conditions: (i) Et_2NH/EtOH, reflux, 80–90 °C, 7–9 h. (ii) KOH, stirring, 13–15 h. (iii) Ac_2O/AcOH, reflux, 7–10 h. (iv) AcOH, reflux, 12–14 h

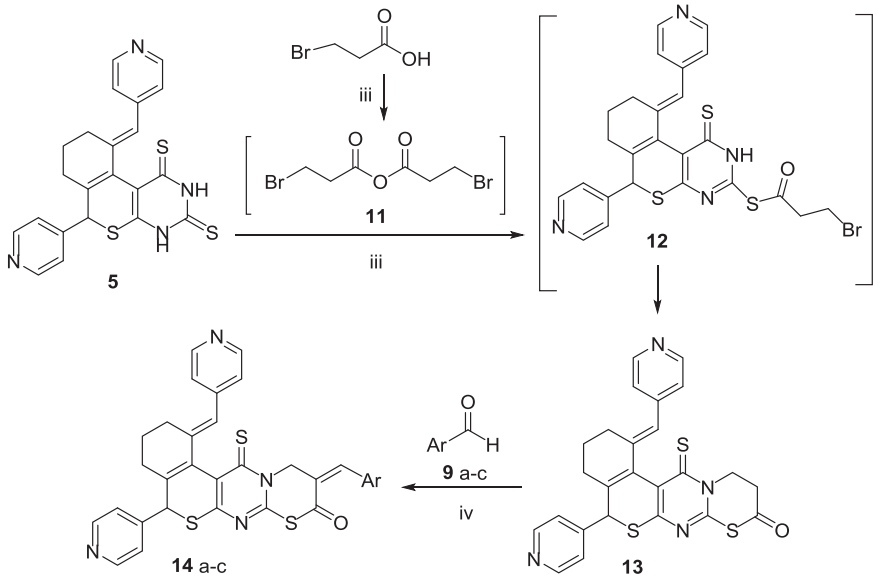
spectra of compounds **8** displayed a characteristic absorption band at ν 1710 due to the carbonyl group of thiazolone ring respectively. The ^{1}H NMR spectrum of **8** exhibited multiplet signals at δ 1.19–1.69 ppm corresponding to six protons of cyclohexene ($3CH_2$) and two singlet signals 4.18 and 8.07 ppm due to two protons of the thiazolidinone moiety (CH_2) and the methine proton of thiopyran ring, respectively. Furthermore, the ^{1}H-NMR spectra of **10a-c** displayed the disappearances of the singlet signal for two protons of the thiazolidinone moiety (CH_2) (Scheme 1).

Similarly, 10-(sub.-benzylidene)-5-(pyridin-4-yl)-1-(pyridin-4-ylmethylene)-13-thioxo-1, 3, 4, 5, 10, 11-hexahydro-2*H*,9*H*,13*H*-isothiochromeno[3',4':4,5]pyrimido[2,1-b] [1,3]thiazin-9-one **14a-c**, were prepared via the reaction of **5** with 3-bromopropanoic acid, followed by the condensation of the formed pyrimido[2,1-b][1,3] thiazin-9-one derivative **13** with the same aldehydes **9**a–c. The IR spectrum of compound **13** revealed a characteristic absorption bands at ν 1718 cm^{-1} due to the carbonyl group of the thiazinone ring. Furthermore, the mass spectra of **14a–c** showed the molecular ion peaks at m/z 576 (M^+, 98 %), 611 (M^+, 100 %), 606 (M^+, 90 %), respectively (Scheme 2).

The treatment of 2-aminoisothiochromene **3** with sodium nitrite solution in the presence of the concentrated hydrochloric acid generated the 1-chloro-6*H*-isothiochromeno [3,4-d][1,2,3]triazine **15**, ^{1}H NMR spectrum of **15** displayed two signals at δ 4.42 (s, 1H, thiopyran), and δ 8.23 (s, 1H, C=CHAr), respectively. Its mass spectrum showed the molecular ion peak at m/z 405 (M^+, 100 %), which is in agreement with the molecular formula ($C_{21}H_{16}ClN_5S$). Furthermore, the 3*H*-isothio- chromeno[3,4-d]pyrimidine-3-thione **18a** and the 3*H*-isothiochromeno[3,4-d]pyrimidin-3-one **18b** were prepared via refluxing of compound **3** with phenylisothiocyanate **16a** or phenyl isocyanate **16b** in of pyridine, respectively through the formation of the intermediate **17a,b**. IR spectra of **18a** and **18b** indicated the presence of three absorption bands at ν 3250, 3220, and 1275 cm^{-1} corresponding to 2NH and (CS) groups. The ^{1}H NMR spectrum of compound **18a** exhibited two characteristic broad singlet signals at δ 10.58 and 10.62 corresponding to the two protons of 2NH groups. Moreover, condensation of **3** with triethyl orthoformate **19a** or triethyl orthoacetate **19b** in refluxing acetic anhydride gave the ethylformimidate derivative (**20a**) and the ethylacetimidate (**20b**), respectively. Compounds **20a** or **20b** undergo cyclic condensation upon treating with phenylhydrazine in dioxane affording the 1*H*-isothiochromeno[3,4-d]pyrimidin-2(6*H*)-amines (**21**a) and (**21b**) respectively. The ^{1}H NMR spectrum of compound (**21**a) displayed two broad singlet signals at δ 9.35 and 10.58 ppm due to (2NH) protons. The



Scheme 3 Synthesis of 1-chloro-6*H*-isothiochromeno[3,4-d][1,2,3]triazine **15**, 3*H*-isothiochromeno[3,4-d]pyrimidine **18a, b** and 8*H*-isothiochromeno[4,3-e][1,2,4]triazolo[1,5-c] pyrimidine **22a**, and **22b**. Reagents and conditions: (i) $NaNO_2$, HCl/ AcOH, stirring, 4 h. (ii) pyridine, reflux, 10–12 h. (iii) Ac_2O, reflux, 10–12 h. (iv) dioxane, reflux, 6–8 h. (v) reflux, 4 h

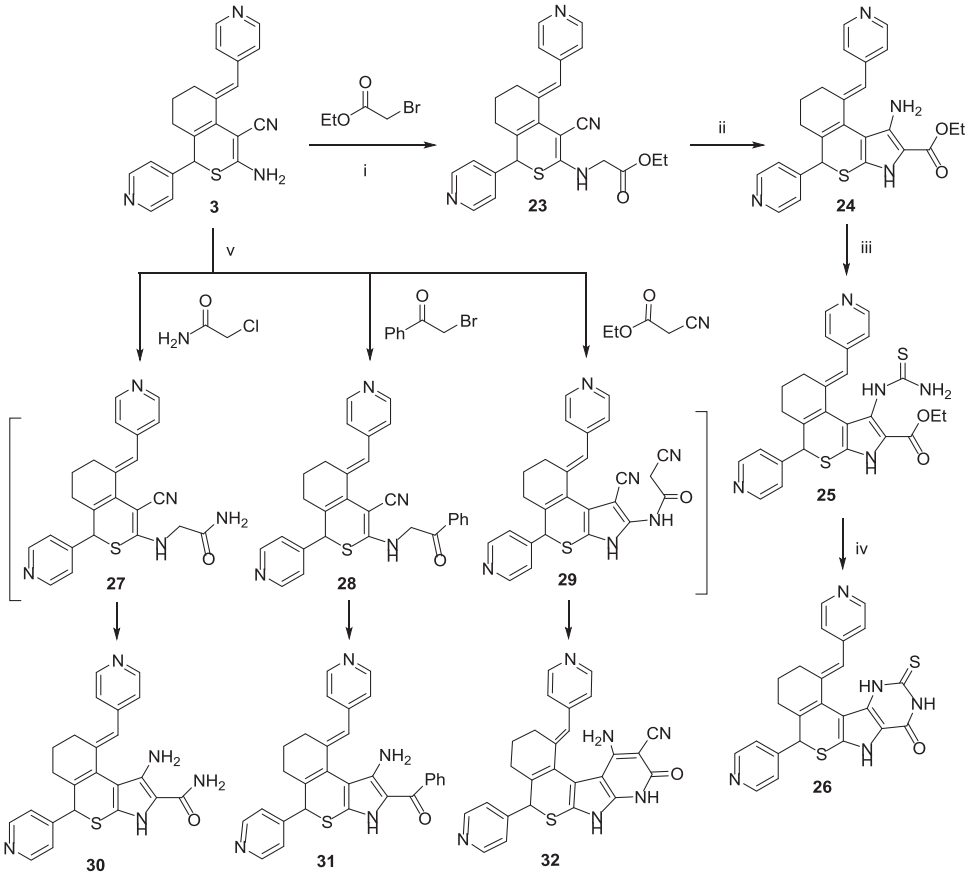


Ar= **a**, C_6H_5;**b**, 4-ClC_6H_4 ; **c**, 4-Me-C_6H_4

Scheme 2 Synthesis of pyrimido[2,1-b][1,3] thiazin-9-ones **13** and, **14**a–c. Reagents and conditions: (i) Ac_2O/AcOH, reflux, 7–10 h. (ii) AcOH, reflux, 12–14 h

mass spectra of **20a, 20b, 21**a, and **21**b revealed the molecular ion peaks at *m/z* 414 (M^+, 100 %), 428 (M^+, 90 %), 476 (M^+, 100 %), 490 (M^+, 88 %). Subsequently, compounds **21a**, and **21b** underwent cyclization upon heating under reflux in excess of triethyl orthoformate **19**a to achieve the 8*H*-isothiochromeno[4,3-e][1,2,4]triazolo[1,5-c]pyrimidine **22a**, and **22b**, respectively. ^{1}H NMR spectrum of **22a** showed triplet and quartet signals at δ 1.31 and δ 4.22 ppm due to the ethyl group, and four singlet signals at δ 4.72 (s, 1H, thiopyran), 6.34 (s, 1H, CHOEt, triazole ring), 8.13 (s, 1H, C=CHAr), and 8.84 (s, 1H, N=CH), (Scheme 3)

The cyclocondensation of compound (**3**) with ethyl bromoacetate in dry acetone and anhydrous potassium carbonate gave the ethyl glycinate **23**, which underwent cyclization to the corresponding ethylisothiochromeno[3,4-b]pyrrole-2-carboxylate **24** upon refluxing in dimethylformamide. The structure of **23** and **24** was recognized through



Scheme 4 Synthesis of pyrrolo [3,2-d]pyrimidine **26** and **32**, and isothiochromeno[3,4-b] pyrrole **30** and **31**. Reagents and conditions: (i) K_2CO_3, Acetone, reflux, 10 h. (ii) K_2CO_3, DMF, reflux, 8 h. (iii) KSCN, HCl, reflux, 7 h. (iv) EtOH/EtONa, reflux, 10 h. (v) EtOH/ EtONa, reflux, 10–12 h

the spectral and elemental analysis. The IR spectrum of **24** exhibited three characteristic absorption bands at ν 3405, 3280 and 1735 cm^{-1} due to the presence of NH_2, NH and carbonyl group. Hence, compound **24** reacted with potassium thiocyanate to give the ethylisothiochromeno [3,4-b] pyrrole-2-carboxylate **25**, which cyclized via refluxing in ethanol in the presence of sodium ethoxide to achieve the corresponding isothiochromeno[4′,3′:4,5] pyrrolo[3,2-d] pyrimidin-8(2*H*)-one **26**. The ^{1}H-NMR spectra of (**26**) revealed three broad singlet signals at δ 10.35, 12.32, 13.13 ppm due to the presence of 3NH groups. The mass spectra of compounds **25** and **26** indicated the molecular ion peaks at *m/z* 503 (M^+, 90 %), 457 (M^+, 99 %), Finally, the treatment of compound **3** with 2-chloroacetamide or phenacyl bromide or ethyl cyanoacetate was refluxed in sodium ethoxide solution afforded the corresponding isothiochromeno [3,4-b]pyrrole-2-carboxamide **30**, isothiochromeno[3,4-b]pyrrol-2-yl) phenyl methanone **31** and isothiochromeno[3,4-b]pyridine-2-carbonitrile **32**, respectively. IR spectrum of compound **30** exposed the absorption band at ν 3410-3250 (br) and 1675 cm^{-1} due to the $2NH_2$, NH, and the carbonyl groups. Meanwhile, the ^{1}H-NMR spectrum of **31** showed two characteristic singlet signals at δ 6.43 and δ 12.67 ppm corresponding to the two protons of NH_2 and the one proton of NH groups. Furthermore, the ^{13}C-NMR of **32** showed signals at δ 15.7, 21.6, 31.2 ppm for three carbon atoms of ($3CH_2$, cyclohexene), 65.6, 88.9 ppm for two carbon atoms of thiopyran ring, 94.1 ppm for one carbon atom of pyridine ring, 102.4 ppm for one carbon atom of cyano group, 111.9 ppm for one carbon atom of (CH), 165.7 ppm for one carbonyl group, and 172.1 ppm for one carbon atom of (C-NH_2). The MS of **30**, **31**, and **32** showed the molecular ion peaks at *m/z* 415 (M^+, 90 %), 476 (M^+, 95 %), 425 (M^+, 92 %), respectively (Scheme 4).

Biological screening

In vitro anti-Herpes Simplex-1 Virus activity

The in vitro Anti-Herpes Simplex-1 Virus (HSV-1) for the isothiochromene derivatives were evaluated with the method reported by (El-Subbagh et al. 2000; Hufford et al. 1991) where Aphidicolin (0.001 µg/mL) was used as a positive control. The results in (Table 1) revealed that compounds **10a, 10b, 10c, 14b, 22a**, and **22b** displayed the highest activity among the tested compounds where they decrease the number of viral plaques by 82, 88, 85, 80, 90, and 95 % respectively. The remarkable anti-HSV-1 activity

Table 1 In vitro Anti-Herpes Simplex-1 Virus (HSV-1) activity of the new compounds

Compounds	% Reduction[a]	MAC[b]		IC_{50}[c]	
		µg/mL	µM	µg/mL	µM
3	20	0.124	0.77	0.80	0.72
5	36	0.072	0.53	0.57	0.70
8	60	0.031	0.26	0.35	0.40
13	58	0.034	0.28	0.39	0.42
10a	82	0.007	0.13	0.20	0.22
10b	88	0.004	0.10	0.16	0.20
10c	85	0.006	0.12	0.18	0.21
14a	75	0.015	0.16	0.23	0.25
14b	80	0.014	0.14	0.21	0.23
14c	78	0.013	0.15	0.22	0.24
15	49	0.052	0.43	0.50	0.60
18a	45	0.061	0.45	0.52	0.65
18b	40	0.066	0.50	0.55	0.68
20a	21	0.092	0.71	0.75	0.69
20b	25	0.085	0.62	0.70	0.60
21a	50	0.041	0.35	0.48	0.55
21b	55	0.035	0.31	0.44	0.50
22a	90	0.003	0.08	0.14	0.19
22b	95	0.002	0.05	0.12	0.17
23	30	0.075	0.54	0.60	0.75
24	62	0.029	0.24	0.33	0.38
25	73	0.017	0.18	0.24	0.28
26	70	0.019	0.19	0.25	0.30
30	68	0.021	0.20	0.27	0.31
31	65	0.025	0.21	0.29	0.35
32	28	0.081	0.58	0.65	0.80
Aphidicolin[d]	**100**	**0.001**	**0.03**	**0.10**	**0.15**

[a]Percent (%) reduction in the number of viral plaques

[b]Minimum antiviral concentration (minimum molar concentration proved to be lethal to the viral population)

[c]Cytotoxicity (compound concentration caused 50% loss of the monolayer present around the viral plaques)

[d]Positive control

Table 2 In vitro Anti-Human Immunodeficiency-1 Virus (HIV-1) activity of the new compounds

Compounds	IC_{50}[a] (µM)	EC_{50}[b] (µM)	TI_{50}[c] (IC_{50}/EC_{50})
3	>200.0	d	d
5	>200.0	d	d
8	72.6	d	d
10a	>200.0	71.9	>5.08
10b	>200.0	71.6	>5.05
10c	>200.0	71.3	>5.02
13	85.5	d	d
14a	20.2	d	d
14b	10.5	d	d
14c	14.1	d	d
15	>200.0	d	d
18a	>200.0	d	d
18b	>200.0	d	d
20a	>200.0	d	d
20b	>200.0	d	d
21a	115.1	d	d
21b	101.4	d	d
22a	>200.0	70.2	>4.04
22b	>200.0	69.8	>4.01
23	>200.0	d	d
24	55.2	d	d
25	26.8	d	d
26	32.6	d	d
30	40.5	d	d
31	45.8	d	d
32	>200.0	d	d
AZT[e]	**33.8**	**0.0005**	**48.79**

[a]50% Inhibitory concentration (molar concentration of compounds which cause 50% inhibition of cell growth)

[b]50% Effective concentration (molar concentration of compounds which cause 50% protection against HIV cytopathic effects)

[c]Therapeutic index (the ratio of 50% inhibitory concentration to 50% effective concentration for each of the active compounds)

[d]Inactive compounds

[e]Positive control, azidothymidine (AZT)

of these compounds may be attributable to the presence of the 3-(4-substitutedbenzylidene)-5-thioxo-3*H*-thiazolo[3,2-a]pyrimidin-2(5*H*)-one, 3-(4-chlorobenzylidene)-6-thioxo-pyrimido[2,1-b][1,3]thiazin-2(6*H*)-one, and 2-ethoxy-5-substituted-3-phenyl-[1,2,4]triazolo[1,5-f]pyrimidine moieties, respectively (El-Subbagh et al. 2000).

In vitro anti-Human Immunodeficiency-1 Virus activity

The effect of the newly synthesized isothiochromene and the azidothymidine (AZT) drug on acutely and persistently infected (T4) cell lines in vitro were evaluated using the method reported by (Chen et al. 1992; Weislow et al. 1989). The method used to estimate the anti-Human Immunodeficiency-1 Virus (HIV-1) effectiveness is intended to discover agents acting at any phase of the virus procreative cycle. The result in (Table 2) displayed that the compounds **10a-c, 22a**, and **22b** have moderate activity against (HIV-1) cytopathic effect. Whereas; compounds **3, 5, 8, 13, 14a, 14b, 14c**, **15**, **18a**, **18b, 20a**, **20b**, **21a**, **21b**, **23, 24, 25, 26, 30, 31**, and **32** are inactive.

Table 3 Cytotoxic activity of the new compounds against different human cancer cell lines

In vitro cytotoxicity IC_{50} (μM)				
Compounds	KB[a]	CNE2[a]	MCF-7[a]	MGC-803[a]
3	>50	>50	>50	>50
5	13.7 ± 1.1	15.1 ± 1.5	15.2 ± 1.2	15.4 ± 1.4
8	42.5 ± 1.7	45.1 ± 2.1	44.8 ± 2.7	46.8 ± 2.5
10a	38.5 ± 2.4	39.3 ± 2.1	38.7 ± 2.3	40.6 ± 2.5
10b	34.1 ± 1.4	35.3 ± 1.1	33.9 ± 1.5	35.2 ± 2.2
10c	36.9 ± 2.1	37.1 ± 1.9	36.5 ± 1.8	38.4 ± 2.3
13	40.4 ± 1.4	42.2 ± 2.3	41.1 ± 2.1	43.2 ± 2.4
14a	23.4 ± 1.1	24.5 ± 1.6	25.6 ± 1.9	26.1 ± 1.3
14b	16.5 ± 1.3	17.9 ± 1.7	18.6 ± 1.4	18.9 ± 1.8
14c	19.8 ± 1.6	20.2 ± 1.5	21.4 ± 1.3	22.5 ± 1.6
15	11.9 ± 1.1	13.3 ± 1.2	13.1 ± 1.3	12.4 ± 1.5
18a	13.1 ± 1.5	14.2 ± 1.2	14.6 ± 1.4	14.8 ± 1.1
18b	13.4 ± 1.3	14.7 ± 1.4	14.8 ± 1.6	14.9 ± 1.6
20a	46.8 ± 1.2	49.4 ± 1.7	49.6 ± 2.1	> 50
20b	44.6 ± 1.8	47.9 ± 1.5	47.2 ± 2.2	48.6 ± 2.6
21a	11.7 ± 1.4	12.9 ± 1.3	12.8 ± 1.5	12.2 ± 1.6
21b	11.5 ± 1.1	12.6 ± 1.2	12.4 ± 1.3	11.9 ± 1.4
22a	12.5 ± 1.4	13.8 ± 1.3	13.9 ± 1.5	13.2 ± 1.6
22b	12.2 ± 1.1	13.5 ± 1.2	13.4 ± 1.3	12.9 ± 1.4
23	48.5 ± 1.5	>50	>50	>50
24	>50	>50	>50	>50
25	49.4 ± 1.3	>50	>50	>50
26	12.8 ± 1.2	14.1 ± 1.3	14.3 ± 1.5	14.5 ± 1.4
30	>50	>50	>50	>50
31	>50	>50	>50	>50
32	13.9 ± 1.8	15.3 ± 1.4	15.6 ± 1.6	15.8 ± 1.3
5-Fluorouracil	**11.2 ± 1.3**	**12.5 ± 1.1**	**12.1 ± 1.4**	**11.6 ± 1.2**

[a]KB cells are oral carcinoma cells, CNE2 cells are nasopharyngeal carcinoma cells, MCF-7 cells are breast adenocarcinoma cells, and MGC-803 cells are gastric carcinoma cells.

Antitumor screening (in vitro cytotoxicity)

The isothiochromene derivatives were tested for their in vitro cytotoxicity using the standard MTT method (Mosmann 1983; Denizot and Lang 1986; Thabrew et al.1997) against the human oral carcinoma cells (KB), nasopharyngeal carcinoma cells (CNE2), breast adenocarcinoma cells (MCF-7), and gastric carcinoma cells (MGC-803). The MTT method is based on the reduction of soluble 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2*H*-tetrazolium bromide. The result in (Table 3) revealed that the isothiochromenes **15**, **21a**, **21b**, **22a**, and **22b** exhibited high cytotoxicity against all carcinoma cell lines as follows; the KB (IC_{50} 11.9, 11.7, 11.5, 12.5, and 12.2 μM), the CNE2 (IC_{50} 13.3, 12.9, 12.6, 13.8, and13.5 μM), the MCF-7 (IC_{50} 13.1, 12.8, 12.4, 13.9, and 13.4 μM), the MGC-803 (IC_{50} 12.4, 12.2, 11.9, 13.2, and 12.9 μM), respectively. Also, some compounds as; **5, 18a**, **18b**, **26**, and **32** showed moderate cytotoxicity against the carcinoma cell lines and the rest of the compounds showed weak cytotoxicity activities.

Structural activity relationship

By comparing the observed antiviral and cytotoxic activities of the isothiochromenes obtained in this study to their structures, the (SAR's) were postulated. (**I**) the presence of the isothiochromen moiety may be necessary for the broad spectrum of the antiviral and cytotoxicity activity. (**II**) 8*H*-isothiochromeno[4,3-e][1,2,4]triazolo[1,5-c] pyrimidines **22a-b** exhibited high in vitro anti-herpes simplex -1 virus activity and moderate anti-human immunodeficiency-1 virus activity. The 1, 2, 4 triazole template, either fused with a pyrimidine ring (Abu-Hashem et al. 2017) or not (Sung and Lee 1992; Havaldar and Patil 2008) display remarkable biological activity, thus the noticeable behavior of **22a-b** may be attributed to the presence of the triazolo[1,5-c] pyrimidine moiety. (**III**) Compound **10a–c** and **14a–c** are more potent than compound **8** and **13**: a comparison of the corresponding structures leads to the conclusion that this fact may be attributed to the presence of the arylidene moiety in the more active molecules. (**IV**) Compounds **15, 21a-b**, and **22a-b** exhibited high cytotoxicity against all carcinoma cell lines. The 1, 2, 3-triazine scaffold from **15** has a potent biological activity (Kumar et al. 2014). The iminopyrimido moiety from compounds **21** was already identified as presenting in vitro antitumor activity against human breast cancer cells MCF-7 (El-Ashmawy et al. 2013). Thus, the remarkable antineoplastic activity of the compounds may be attributable to the presence of the iminopyrimidine, chlorotriazine, and triazolo-pyrimidine moieties, respectively.

Conclusions

The objective of the present study is to synthesize and evaluate the antiviral and cytotoxic activities of some new isochromene-based compounds with the hope of discovering new structure leads serving as antiviral or antitumor agents. The data revealed that isothiochromenotriazolopyrimidines **22a**,**b** possess promising in vitro antiviral activity against HSV-1 and HIV-1. While isothiochromenotriazine **15** and isothiochromenopyrimidin-2(6*H*)-amine**21a**, **21b** showed wonderful cytotoxic activity against carcinoma cell lines when compared to the 5-fluorouracil drug.

Experimental

Materials, equipment's, and methods

All materials used were obtained from Sigma Aldrich (Saint Lewis, USA). The melting points are in degree centigrade (uncorrected) and were determined on Gallenkamp electric melting point apparatus. TLC analysis was carried out on silica gel 60 F_{254} precoated aluminum sheets. The IR spectra were recorded (KBr) on a Perkin–Elmer 1430 spectrometer (λ, cm^{-1}) in National Research Center, Egypt. ^1H and ^{13}C-NMR spectra were measured on JEOL-ECA 500 and JEOL JNM-LA-400 FT NMR Spectrometers at 500/400, 125/100 MHz, respectively, using tetramethylsilane (TMS) as an internal reference and DMSO-d_6 as the solvent at the Microanalytical Center in National Research Center, Egypt. The mass spectra (EI) were recorded on GCMS-QP 1000 EX (Shimadzu) at National Research Center, Egypt. Elemental analyses (C, H, and N) were carried out at the Microanalytical Center in National Research Center, Egypt. The elemental analyses were found to agree favorably with the calculated values. The synthesized compounds were tested in vitro for their antiviral activity using HSV-1, HIV-1 and cytotoxicity activity at the Pharmacology Unit, Department of Pharmacognosy, College of Pharmacy, University of Mansoura, Mansoura, Egypt and National Cancer Institute, Cairo University, Cairo, Egypt.

Synthesis of 3-amino-1-(pyridin-4-yl)-5-(pyridin-4-ylmethylene)-5, 6, 7, 8-tetrahydro-1H-isothiochromene-4-carbonitrile (3)

A mixture of 2, 6-bis(pyridin-4-ylmethylene) cyclohexan-1-one (2.76 g, 0.01 mol), malononitrile (0.66 g, 0.01 mol), sulfur (0.32 g, 0.01 mol), and diethylamine (0.01 mol) in ethanol (30 mL) was heated at 80–90 °C for 7–9 h, then the mixture was left for 36 h at 0 °C. The formed product was collected by filtration, washed with ethanol (25 mL), dried and crystallized from absolute methanol, as yellow crystals (90%), mp 290–292 °C. IR (ν, cm^{-1}) KBr: 3420 (br. NH_2), 3050 (CH-aryl), 2952 (CH-aliph), 2212 (CN), 1624 (C=N). ^1H NMR (DMSO-d_6, ppm) δ 1.57–2.23 (m, 6H, $3CH_2$, cyclohexene), 4.62(s, 1H, thiopyran), 6.28 (brs, NH_2, D_2O exchangeable),7.56–7.96 (m, 8H, Ar-H), 8.23 (s, 1H, C=CHAr), ^{13}C NMR (DMSO-d_6) δ 15.6, 21.5, 31.2 (3 C, CH_2), 65.5, 88.9 (2 C, thiopyran),111.9 (CN), 116.2 (1 C, C=CHAr), 121.4, 124.8, 130.2, 130.8, 130.9, 131.5, 136.1, 147.7, 158.3 (13 C, Ar-C), 165.6 (C-NH_2); MS (70 eV, %) *m/z* 358 (M^+, 100 %); Anal. Calc. (Found) for $C_{21}H_{18}N_4S$ (358.46): C, 70.36 (70.45); H, 5.06 (5.14); N, 15.63(15.72); S, 8.94(8.51).

Synthesis of 6-(pyridin-4-yl)-10-(pyridin-4-ylmethylene)-4, 6, 7, 8, 9, 10-hexahydro-1H-isothio chromeno[3, 4-d] pyrimidine-1,3 (2H)-dithione (5)

A mixture of compound **3** (3.58 g, 0.01 mol), and carbon disulfide (excess 15 mL) was heated under reflux in ethanolic potassium hydroxide solution for 15 h. The solution was allowed to cool to 0 °C for 5 h; the solid precipitate was filtered off, washed with water (30 mL), dried and crystallized from dimethylformamide to give (**5**) as a yellow crystals (85%); mp 315–317 °C. IR (ν, cm^{-1}) KBr: 3395 (brs, NH), 3055 (CH-aryl), 2950 (CH-aliph), 1620 (C=N), 1355, 1370 (2 C=S).^1H NMR (DMSO-d6, ppm) δ 1.26–2.35 (m, 6H, $3CH_2$, cyclohexene), 4.29 (s,1H, thiopyran),7.45–8.02 (m, 8H, Ar-H), 8.27 (s, 1H, C=CHAr), 9.19, 9.21 (brs, 2NH, D_2O exchangeable), ^{13}C NMR (DMSO-d_6) δ 16.8, 22.5, 32.5 (3 C, CH_2), 60.2, 90.8 (2 C, thiopyran), 114.7 (1 C, C=CHAr), 122.9, 123.8, 132.5, 133.9, 142.4, 145.5, 146.8, 148.7, 149.1,158.6 (14 C, Ar–C), 168.5, 185.4 (2 C, 2 C=S); MS (70 eV, %) *m/z* 434 (M^+, 100 %); Anal. Calc. (Found) for $C_{22}H_{18}N_4S_3$ (434.59): C, 60.80 (60.75); H, 4.17 (4.25); N, 12.89 (12.94); S, 22.13 (22.06).

Synthesis of 5-(pyridin-4-yl)-1-(pyridin-4-ylmethylene)-12-thioxo-1,3,4,5,7,7a-hexa- hydro-2H,12H-isothiochromeno [3,4-d]thiazolo[3,2-a]pyrimidin-9(10H)-one (8) and 5-(pyridin-4-yl)-1-(pyridin-4-ylmethylene)-13-thioxo-1,3,4,5,7,7a,10,11-octahydro-2H,9H,13H-isothiochromeno [3′,4′:4,5] pyrimido[2,1-b][1,3]thiazin-9-one (13)

General method: A mixture of compound **5** (4.34 g, 0.01 mol), and chloroacetic acid (0.93 g, 0.01 mol) or β-bromopropionic acid (1.52 g, 0.01 mol) was heated under reflux in a solution of glacial acetic acid and acetic anhydride (8 ml, 3:1 V) in the presence of sodium acetate anhydrous (0.82 g, 0.01 mol) for 7–10 h. The reaction mixture was into crashed ice water. The formed precipitate was washed with water and filtered off, dried, and recrystallized from the suitable solvent to give **8** and **13** respectively.

Compound **8**: Yellowish crystals, yield (75%), mp 328–330 °C, crystallized from EtOH; IR (ν, cm^{-1}) KBr: 3050 (CH-aryl), 2954 (CH-aliph), 1710 (C=O), 1624 (C=N), 1325 (C=S), ^1H NMR (DMSO-d6, ppm) δ 1.19–1.69 (m, 6H, $3CH_2$, cyclohexene), 4.18 (s, 2H, CH_2, thiazolidinone), 4.62 (s, 1H, thiopyran), 7.10–7.74 (m, 8H, Ar-H), 8.07 (s, 1H, C=CHAr), ^{13}C-NMR (DMSO-d_6) δ 15.7, 21.6, 31.2 (3 C, CH_2), 47.7 (1 C, thiopyran), 89.1 (1 C, CH_2, thiazole),111.8 (1 C, C=CHAr), 116.2, 121.5, 124.8, 130.3, 131.5, 135.8, 136.1, 136.9, 140.1, 140.2, 140.3, 140.5 (16 C,Ar-C), 165.6 (1 C, C=O), 172.5 (1 C, C=S); MS (70 eV, %) *m/z* 474 (M^+, 100 %); Anal. Calc. (Found) for

$C_{24}H_{18}N_4OS_3$ (474.62): C, 60.74 (60.78); H, 3.82 (3.75); N, 11.80 (11.90); S, 20.26 (20.20).

Compound **13**: Yellow crystals, yield (70%), mp 338–340 °C, crystallized from MeOH; IR (ν, cm^{-1}) KBr: 3058 (CH-aryl), 2960 (CH-aliph), 1718 (C=O), 1621 (C=N), 1322 (C=S). ^{1}H NMR (DMSO-d6, ppm) δ 1.01 (t, 2H, J = 3.70 Hz, $\underline{CH_2}CH_2S$, thiazine), 1.75–2.41 (m, 6H, $3CH_2$, cyclohexene), 3.13 (t, 2H, J = 3.74 Hz, $CH_2\underline{CH_2}S$, thiazine), 4.51 (s, 1H, thiopyran), 7.05–8.07 (m, 8H, Ar-H), 8.09 (s, 1H, C=$\underline{CH}$Ar),^{13}C NMR (DMSO-d_6) δ 17.9, 23.6, 30.5 (3 C, CH_2), 55.8 (1 C, thiopyran), 48.5 $\underline{C}H_2CH_2S$, thiazine, 50.5 ($CH_2\underline{C}H_2S$, thiazine), 113.9 (1 C, C=$\underline{C}$HAr), 123.8, 124.5, 128.4, 132.7, 133.9, 143.5, 145.6, 147.8, 149.4, 149.9, 155.7, 158.7 (16 C,Ar-C),169.2 (1 C, $\underline{C}$=O), 180.1 (1 C, $\underline{C}$=S); MS (70 eV, %) *m/z* 488 (M^+, 100 %); Anal. Calc. (Found) for $C_{25}H_{20}N_4OS_3$ (488.64): C, 61.45 (61.54); H, 4.13 (4.18); N, 11.47 (11.40); S, 19.68 (19.61).

Synthesis of 10-(4-substitute-benzylidene)-5-(pyridin-4-yl)-1-(pyridin-4-ylmethylene)-12-thioxo-1,3,4,5-tetrahydro -2H,12H-isothiochromeno[3,4-d]thiazolo[3,2-a]pyrimidin-9 (10H)-one(10a-c) and 10-(4-substituted-benzylidene)-5-(pyridin-4-yl)-1-(pyridin-4-ylmethylene)-13-thioxo-1,3,4,5,10, 11-hexahydro-2H,9H,13H-isothiochromeno [3′,4′:4,5]pyrimido[2,1-b][1,3] thiazin-9-one (14a-c)

General method: A mixture of compound **8** (4.74 g, 0.01 mol) or compound **13** (4.88 g, 0.01 mol), and aromatic aldehyde derivatives, namely: benzaldehyde (1.06 g, 0.01 mol) or 4-chloro- benzaldehyde (1.40 g, 0.01 mol) or 4-methoxybenzaldehyde (1.36 g, 0.01 mol), respectively was refluxed in a solution of glacial acetic acid (10 mL) and anhydrous sodium acetate anhydrous (0.82 g, 0.01 mol) for 12–14 h. The reaction mixture was into crashed ice water and the formed precipitate product was collected by filtration and crystallized from the appropriate solvent to afford **10a–c** and **14a–c**, respectively.

10-(benzylidene)-5-(pyridin-4-yl)-1-(pyridin-4-ylmethylene)-12-thioxo-1, 3, 4, 5-tetrahydro-2H, 12H-isothiochromeno [3,4-d]thiazolo[3,2-a]pyrimidin-9(10H)-one(10a)

Yellowish crystals, yield (60%), mp > 340 °C, crystallized from EtOH; IR (ν, cm^{-1}) KBr: 3060 (CH-aryl), 2958 (CH-aliph), 1720 (C=O),1625(C=N), 1328 (C=S), ^{1}H NMR (DMSO-d6, ppm) δ 1.19–1.69 (m, 6H, $3CH_2$, cyclohexene), 4.72 (s, 1H, thiopyran),7.10–8.06 (m, 13H, Ar-H), 8.13 (s, 1H, C=$\underline{CH}$Ar), 8.23 (s, 1H, C=$\underline{CH}$Ar),^{13}C NMR (DMSO-d_6) δ 18.7, 23.6, 33.2 (3 C, CH_2), 44.9 (1 C, thiopyran), 110.5, 112.8 (2 C, 2 C=C$\underline{H}$Ar), 123.4, 124.6, 127.2, 127.9, 128.2, 128.5, 133.2, 133.9, 134.9, 144.1, 146.3, 147.5, 149.2, 149.9, 151.5, 155.4, 158.9 (23 C, Ar–C), 179.8 (1 C, $\underline{C}$=O), 186.2(1 C, C=S); MS (70 eV, %) *m/z* 562 (M^+, 100 %); Anal. Calc. (Found) for $C_{31}H_{22}N_4OS_3$ (562.72): C, 66.17 (66.24); H, 3.94 (3.90); N, 9.96 (9.92); S, 17.09 (17.15).

10-(4-chlorobenzylidene)-5-(pyridin-4-yl)-1-(pyridin-4-ylmethylene)-12-thioxo-1,3,4,5-tetrahydro-2H,12H-isothiochromeno[3,4-d]thiazolo[3,2-a]pyrimidin-9(10H)-one (10b)

Yellow crystals, yield (65%), mp > 340 °C, crystallized from hexane; IR (ν, cm^{-1}) KBr: 3064 (CH-aryl), 2960 (CH-aliph), 1628(C=N), 1330 (C=S), 1724 (C=O).^{1}H NMR (DMSO-d6, ppm) δ 1.21–1.73 (m, 6H, $3CH_2$, cyclohexene), 4.62 (s, 1H, thiopyran),7.10–7.95(m, 12H, Ar-H), 8.09 (s, 1H, C=$\underline{CH}$Ar), 8.18 (s, 1H, C=$\underline{CH}$Ar),^{13}C NMR (DMSO-d_6) δ 15.6, 21.6, 31.2 (3 C, CH_2), 65.6 (1 C, thiopyran), 107.8, 111.9 (2 C, 2 C=$\underline{CH}$Ar), 116.8, 118.2, 121.5, 124.8, 130.2, 130.8, 130.9, 131.5, 136.1, 141.7, 143.6, 147.7, 149.5, 153.6, 158.4, 165.7, 168.2 (23 C, Ar–C), 173.8 (1 C, $\underline{C}$=O), 177.9 (1 C, $\underline{C}$=S); MS (70 eV, %) *m/z* 597 (M^+, 90 %); Anal. Calc. (Found) for $C_{31}H_{21}ClN_4OS_3$ (597.17): C, 62.35 (62.40); H, 3.54 (3.59); N, 9.38 (9.33); S, 16.11 (16.18).

10-(4-methoxybenzylidene)-5-(pyridin-4-yl)-1-(pyridin-4-ylmethylene)-12-thioxo-1,3,4,5-tetra hydro-2H,12H-isothiochromeno[3,4-d]thiazolo[3,2-a]pyrimidin-9(10H)-one (10c)

Brownish crystals, yield (62%), mp > 340 °C, crystallized from benzen; IR (ν, cm^{-1}) KBr: 3059 (CH-aryl), 2957 (CH-aliph), 1722 (C=O), 1626 (C=N), 1328 (C=S), ^{1}H NMR (DMSO-d6, ppm) δ 1.22–1.75 (m, 6H, $3CH_2$, cyclohexene), 3.85 (s,3H, CH_3), 4.67 (s, 1H, thiopyran),7.12–7.94 (m, 12H, Ar-H), 8.08 (s, 1H, methine proton), 8.15 (s, 1H, C=$\underline{CH}$Ar),^{13}C NMR (DMSO-d_6) δ 19.1, 23.4, 31.3 (3 C, CH_2), 44.9 (1 C, thiopyran), 56.7(1 C,OCH_3), 120.8, 121.9 (2 C, 2CH C=$\underline{C}$HAr), 122.8, 123.1, 124.2, 127.4, 129.1, 130.4, 133.5, 134.6, 144.2, 146.7, 147.5, 149.5, 149.8, 150.3, 156.7, 158.1, 159.5 (23 C, Ar–C), 169.2 (1 C, $\underline{C}$=O), 175.5 (1 C, $\underline{C}$=S); MS (70 eV, %) *m/z* 592 (M^+, 95 %); Anal. Calc. (Found) for $C_{32}H_{24}N_4O_2S_3$ (592.75): C, 64.84 (64.90); H, 4.08 (4.12); N, 9.45 (9.49); S, 16.23 (16.28).

10-(benzylidene)-5-(pyridin-4-yl)-1-(pyridin-4-ylmethylene)-13-thioxo-1,3,4,5,10,11-hexahydro-2H,9H,13H-isothiochromeno[3′,4′:4,5]pyrimido[2,1-b] [1,3]thiazin-9-one (14a)

Yellow crystals, yield (70%), mp > 340 °C, crystallized from dioxane; IR (ν, cm^{-1}) KBr: 3065 (CH-aryl), 2960 (CH-aliph), 1730 (C=O), 1629(C=N), 1335 (C=S), ^{1}H

NMR (DMSO-d6, ppm) δ 1.25–2.31 (m, 6H, $3CH_2$, cyclohexene), 4.29 (s, 2H, $\underline{CH_2}N$, thiazine), 4.41 (s, 1H, thiopyran), 7.06–7.95 (m, 13H, Ar–H), 8.07 (s, 1H, C=$\underline{CH}$Ar), 8.15 (s, 1H, C=$\underline{CH}$Ar),^{13}C NMR (DMSO-d_6) δ 20.2, 24.6, 28.8 (3 C, CH_2), 45.2 (1 C, thiopyran), 58.2 (1 C, $\underline{C}H_2N$, thiazine), 121.2, 123.1 (2 C, 2 C=$\underline{C}$HAr), 123.1, 124.4, 127.1, 127.9, 128.6, 128.8, 133.3, 133.8, 135.4, 141.2, 144.7, 146.1, 147.3, 149.5, 149.8, 155.6, 159.1 (23 C, Ar–C), 168.5 (1 C, $\underline{C}$=O), 177.4 (1 C, $\underline{C}$=S); MS (70 eV, %) *m/z* 576 (M^+, 98 %); Anal. Calc. (Found) for $C_{32}H_{24}N_4OS_3$ (576.75): C, 66.64 (66.60); H, 4.19 (4.12); N, 9.71 (9.78); S, 16.68 (16.62).

10-(4-chlorobenzylidene)-5-(pyridin-4-yl)-1-(pyridin-4-ylmethylene)-13-thioxo-1,3,4,5,10,11-hexa hydro-2H,9H,13H-isothiochromeno[3′,4′:4,5]pyrimido[2,1-b][1,3]thiazin-9-one (14b)

Yellowish crystals, yield (75%), mp > 340 °C, crystallized from methanol; IR (ν, cm^{-1}) KBr: 3062 (CH-aryl), 2958 (CH-aliph), 1735 (C=O), 1625 (C=N), 1338 (C=S). ^{1}H NMR (DMSO-d6, ppm) δ 1.18–2.25 (m, 6H, $3CH_2$, cyclohexene), 4.30 (s, 2H, $\underline{CH_2}N$, thiazine), 4.33 (s, 1H, thiopyran), 7.21–7.99 (m, 12H, Ar–H), 8.01 (s, 1H, C=$\underline{CH}$Ar), 8.26 (s, 1H, C=$\underline{CH}$Ar),^{13}C NMR (DMSO-d_6) δ 19.8, 24.7, 28.6 (3 C, CH_2), 45.5 (1 C, thiopyran), 57.8 (1 C, CH_2N, thiazine), 120.6, 123.3 (2 C, C=$\underline{C}$HAr), 123.2, 124.5, 127.6, 128.9, 129.1, 133.1, 133.4, 133.8, 135.2, 140.7, 144.4, 146.6, 147.5, 149.6, 149.9, 156.2, 159.5 (23 C, Ar–C),168.2 (1 C, $\underline{C}$=O), 178.2 (1 C, $\underline{C}$=S); MS (70 eV, %) *m/z* 611 (M^+, 100 %); Anal. Calc. (Found) for $C_{32}H_{23}ClN_4OS_3$ (611.19): C, 62.89 (62.80); H, 3.79 (3.72); N, 9.17 (9.11); S, 15.74 (15.78).

10-(4-methoxybenzylidene)-5-(pyridin-4-yl)-1-(pyridin-4-ylmethylene)-13-thioxo-1,3,4,5,10,11-hexahydro-2H,9H,13H-isothiochromeno[3′,4′:4,5]pyrimido[2,1-b][1,3]thiazin-9-one (14c)

Yellow crystals, yield (71%), mp > 350 °C, crystallized from DMF; IR (ν, cm^{-1}) KBr: 3060 (CH-aryl), 2955 (CH-aliph), 1738 (C=O), 1628(C=N), 1340 (C=S).^{1}H NMR (DMSO-d6, ppm) δ 1.20–2.28 (m, 6H, $3CH_2$, cyclohexene), 3.80 (s,3H, CH_3), 4.29 (s, 2H, $\underline{CH_2}N$, thiazine), 4.41 (s, 1H, thiopyran), 7.22–7.97 (m, 12H, Ar-H), 8.05 (s, 1H, C=$\underline{CH}$Ar), 8.22 (s, 1H, C=$\underline{CH}$Ar),^{13}C NMR (DMSO-d_6) δ 24.8, 30.8, 30.9 (3 C, CH_2), 58.3 (1 C, thiopyran), 58.5 (1 C, OCH_3), 58.9 (1 C, $\underline{C}H_2N$, thiazine), 115.8, 115.9 (2 C, C=$\underline{C}$HAr), 116.1, 121.5, 124.8, 130.3, 130.8, 130.9, 131.5, 136.1, 140.1, 140.2, 147.7, 152.6, 152.8, 158.3, 165.5, 165.6 (23 C, Ar–C), 168.5 (1 C, $\underline{C}$=O), 180.1 (1 C, $\underline{C}$=S); MS (70 eV, %) *m/z* 606 (M^+, 90 %); Anal. Calc. (Found) for $C_{33}H_{26}N_4O_2S_3$ (606.78): C, 65.32 (65.38); H, 4.32 (4.40); N, 9.23 (9.29); S, 15.85 (15.91).

Synthesis of 1-chloro-6-(pyridin-4-yl)-10-(pyridin-4-ylmethylene)-7, 8, 9, 10-tetrahydro-6H-isothiochromeno [3, 4- d] [1, 2, 3] triazine (15)

To a cool solution of compound **3** (3.58 g, 0.01 mol) in a mixture of acetic acid (15 mL) and conc. hydrochloric acid (10 mL), a sodium nitrite solution 10% (2 mL) was added with stirring for 15 min. The stirring was continued at (1–5 ° C) for 4 h. The formed precipitate was filtered off, dried and crystallized from DMF to give compound **15**. Yellow crystals, yield (78%), mp 325–327 °C (dec.); IR (ν, cm^{-1}) KBr: 3065 (CH-aryl), 2952 (CH-aliph), 1625(C=N). ^{1}H NMR (DMSO-d_6, ppm) δ 1.57–2.23 (m, 6H, $3CH_2$, cyclohexene), 4.42 (s,1H, thiopyran), 7.56–7.96 (m, 8H, Ar–H), 8.23 (s, 1H, C=$\underline{CH}$Ar), ^{13}C NMR (DMSO-d_6) δ 15.6, 21.6, 31.2 (3 C, CH_2), 65.6 (1 C, thiopyran), 1119 (1 C, C=$\underline{C}$HAr), 120.7, 121.5, 124.9, 130.2, 130.8, 130.9, 131.5, 136.1, 143.2, 147.7, 154.1, 158.4 (16 C, Ar–C); MS (70 eV, %) *m/z* 405 (M^+, 100 %); Anal. Calc. (Found) for $C_{21}H_{16}ClN_5S$ (405.90): C, 62.14 (62.20); H, 3.97 (3.90); N, 17.25 (17.20); S, 7.90 (7.85).

Synthesis of 1-imino-2-phenyl-6-(pyridin-4-yl)-10-(pyridin-4-ylmethylene)-1, 2, 4, 6, 7, 8, 9, 10-octahydro-3H-isothiochromeno[3,4-d]pyrimidine-3-thione (18a) and 1-imino-2-phenyl-6-(pyridin-4-yl)-10-(pyridin-4-ylmethylene)-1,2,4,6,7,8,9,10-octahydro-3H-isothiochromeno[3,4-d] pyrimidin-3-one (18b)

General method: A mixture of compound **3** (3.58 g, 0.01 mol) and phenylisothiocyanate (1.2 mL, 0.01 mol) or phenylisocyanate (1.1 mL, 0.01 mol) in pyridine (30 mL) was refluxed for 10–12 h. The reaction mixture was left to cool, acidified with dilute hydrochloric acid; the formed product precipitate was filtered off, dried, and recrystallized from the suitable solvent to give **18a** and **18b**, respectively.

Compound **18a**: Recrystallized from THF as yellow crystals in 68% yield, mp > 340 °C (dec.); IR (ν, cm^{-1}) KBr: 3250, 3220 (2NH), 3050 (CH-aryl), 2958 (CH-aliph), 1628 (C=N), 1275 (C=S). ^{1}H NMR (DMSO-d_6, ppm) δ 1.19–2.28 (m, 6H, $3CH_2$, cyclohexene), 4.72 (s,1H, thiopyran), 7.01–8.01 (m, 13H, Ar–H), 8.05 (s, 1H, C=$\underline{CH}$Ar), 10.58 (br. s, 1H, NH, D_2O exchangeable), 10.62 (br. s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 15.9, 21.8, 31.5 (3 C, $3CH_2$), 67.4, 91.6 (2 C, thiopyran), 112.8 (1 C, C=$\underline{C}$HAr),119.4, 122.2, 128.5, 129.3, 132.7, 133.6, 138.4, 144.6, 146.9, 147.4, 149.1, 149.9, 151.5 (20 C, Ar–C), 159.5 (1 C, $\underline{C}$=NH), 181.2 (1 C, $\underline{C}$=S); MS (70 eV, %) *m/z* 493 (M^+, 99%); Anal. Calc. (Found) for $C_{28}H_{23}N_5S_2$

(493.65): C, 68.13 (68.20); H, 4.70 (4.78); N, 14.19 (14.25); S, 12.99 (12.92).

Compound **18b**: Recrystallized from Toluene as yellowish crystals in 66% yield, mp > 350 °C (dec.); IR (ν, cm^{-1}) KBr: 3255, 3222 (2NH), 3051 (CH-aryl), 2962 (CH-aliph), 1670 (C=O), 1629 (C=N).^{1}H NMR (DMSO-d_6, ppm) δ 1.20–2.30 (m, 6H, 3CH_2, cyclohexene), 4.74 (s,1H, thiopyran), 7.02–7.99 (m, 13H, Ar–H), 8.06 (s, 1H, C=CHAr), 10.60 (br. s, 1H, NH, D_2O exchangeable), 10.65 (br. s, 1H,NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 15.6, 21.5, 31.2(3 C, 3CH_2), 65.5, 88.9 (2 C, thiopyran),111.9 (1 C, C=CHAr), 116.3, 118.5, 121.6, 124.8, 130.3, 130.8, 130.9, 131.6, 136.2, 139.9, 144.3, 147.8, 152.5, 153.6 (20 C, Ar–C), 158.2 (1 C, C=NH), 160.2 (1 C, C=O); MS (70 eV, %) *m/z* 477 (M^+, 100 %); Anal. Calc. (Found) for $C_{28}H_{23}N_5OS$ (477.59): C, 70.42 (70.50); H, 4.85 (4.80); N, 14.66 (14.61); S, 6.71 (6.78).

Synthesis of Ethyl-N-(4-cyano-1-(pyridin-4-yl)-5-(pyridin-4-ylmethylene)-5,6,7,8-tetrahydro-1H-isothio- chromen-3-yl) formimidate (20a) and ethyl-N-(4-cyano-1-(pyridin-4-yl)-5-(pyridin-4-yl methylene)-5, 6, 7, 8-tetrahydro-1H-isothiochromen-3-yl) acetimidate (20b)

General method: A mixture of compound **3** (3.58 g, 0.01 mol) and triethylorthoformate (1.66 mL, 0.01 mol) or triethylorthoacetate (1.83 mL, 0.01 mol) in acetic anhydride (30 mL) was heated under reflux for 10–12 h (TLC control). The solution was cooled overnight to room temperature and then concentrated. The precipitated solid was filtered off and recrystallized to yields **20a** and **20b** respectively.

Compound **20a**: Crystallized from dioxane, yellow crystals in 80% yield, mp 260–262 °C (dec.); IR (ν, cm^{-1}) KBr: 3055 (CH-aryl), 2960 (CH-aliph), 2210(CN), 1622 (C=N). ^{1}H NMR (DMSO-d_6, ppm) δ 1.29 (t, $J = 5.55$ Hz, 3H, CH_3), 1.32–2.43 (m, 6H, 3CH_2, cyclohexene), 4.23 (q, $J = 5.60$ Hz, 2H, CH_2), 4.72 (s,1H, thiopyran), 7.01–7.98 (m, 8H, Ar-H), 8.03 (s, 1H, C=CHAr), 8.05 (s, 1H, N=CHO); ^{13}C NMR (DMSO-d_6) δ 20.2 (1 C, CH_3), 24.4, 25.9, 28.1 (3 C, CH_2), 46.5 (1 C, thiopyran), 66.2 (1 C, OCH_2), 98.8 (1 C, thiopyran), 118.7(1 C, CN),121.1 (1 C, C=CHAr), 123.6, 124.8, 133.7, 134.6, 145.1, 146.7, 147.5, 149.4, 149.9, 153.4 (14 C, Ar–C), 155.8(1 C, N=CHOEt); MS (70 eV, %) *m/z* 414 (M^+, 100 %); Anal. Calc. (Found) for $C_{24}H_{22}N_4OS$ (414.53): C, 69.54 (69.62); H, 5.35 (5.40); N, 13.52 (13.58); S, 7.73 (7.81).

Compound **20b**: Crystallized from benzene, yellowish crystals in 82% yield, mp 273–275 °C (dec.); IR (ν, cm^{-1}) KBr: 3058 (CH-aryl), 2964 (CH-aliph), 2212 (CN), 1620 (C=N). 1H NMR (DMSO-*d6*, ppm) δ 1.21 (s,1H,CH_3),1.33 (t, $J = 5.58$ Hz, 3H, $_{CH3}$), 1.36–2.45 (m, 6H, 3CH_2, cyclohexene), 4.10 (q, $J = 5.62$ Hz,2H, OCH_2CH_3), 4.70 (s,1H, thiopyran), 7.04–7.99 (m, 8H, Ar-H), 8.06 (s, 1H, C=CHAr); ^{13}C NMR (DMSO-*d6*) δ 19.5 (1 C, CH_2CH_3), 21.9 (1 C, N=CCH_3), 23.6, 25.2, 27.8 (3 C, CH_2), 47.1 (1 C, thiopyran), 68.4 (1 C,OCH_2), 99.2 (1 C, thiopyran), 118.2 (1 C, CN), 121.5 (1 C, C=CHAr), 123.3, 124.5, 133.2, 134.1, 145.3, 146.6, 147.4, 149.2, 149.6, 152.1 (14 C, Ar–C), 164.5 (1 C, N=COEt); MS (70 eV, %) m/z 428 (M^+, 90 %); Anal. Calc. (Found) for $C_{25}H_{24}N_4OS$ (428.55): C, 70.07 (70.10); H, 5.65 (5.71); N, 13.07 (13.12); S, 7.48 (7.55).

Synthesis of 1-imino-N-phenyl-6-(pyridin-4-yl)-10-(pyridin-4-ylmethylene)-7,8,9,10-tetrahydro-1H-isothiochromeno [3,4-d]pyrimidin-2(6H)-amine (21a) and 1-imino-3-methyl-N-phenyl-6-(pyridin-4-yl)-10-(pyridin-4-ylmethylene)-7,8,9,10-tetrahydro-1H-isothiochromeno [3,4-d]pyrimidin-2 (6H)-amine (21b)

General method: A suspension of compound **20a** (4.14 g, 0.01 mol) or **20b** (4.28 g, 0.01 mol) and phenylhydrazine (1.08 g, 0.01 mol) in dioxane (30 mL) was refluxed for 6–8 h. The solid product which formed was filtered off, washed with water, dried in air and recrystallized from the suitable solvent to afford **21a** and **21b** respectively.

Compound **21a**: Crystallized from methanol, yellowish crystals in 78% yield, mp > 340 °C (dec.); IR (ν, cm^{-1}) KBr: 3210, 3190 (2NH), 3054 (CH-aryl), 2956 (CH-aliph), 1626(C=N). ^{1}H NMR (DMSO-d_6, ppm) δ 1.30–2.21 (m, 6H, 3CH_2, cyclohexene), 4.75 (s, 1H, thiopyran), 7.15–7.95 (m, 13H, Ar-H), 8.05 (s, 1H, C=CHAr), 8.30 (s, 1H, N=CH), 9.35 (br. s, 1H, NH, D_2O exchangeable), 10.58 (br. s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 23.8, 26.2, 28.5 (3 C, 3CH_2), 48.1, 108.2 (2 C, thiopyran),120.9 (1 C, C=CHAr), 121.8, 122.5, 123.4, 124.6, 130.5, 133.1, 134.8, 144.1, 145.3, 146.8, 147.5, 147.9, 149.4, 149.7, 154.2 (21 C, Ar-C),159.3 (1 C, C=NH); MS (70 eV, %) *m/z* 476 (M^+, 100 %); Anal. Calc. (Found) for $C_{28}H_{24}N_6S$ (476.60): C, 70.56 (70.50); H, 5.08 (5.15); N, 17.63 (17.68); S, 6.73(6.80).

Compound **21b**: Crystallized from DMF, yellow crystals in 74 % yield, mp > 350 °C (dec.); IR (ν, cm^{-1}) KBr: 3215, 3195 (2NH), 3052 (CH-aryl), 2954 (CH-aliph), 1628(C=N). ^{1}H NMR (DMSO-d_6, ppm) δ 1.29–2.10 (m, 6H, 3CH_2, cyclohexene), 2.92(s, 3H, CH_3), 4.72 (s,1H, thiopyran), 7.21–8.01 (m, 13H, Ar-H), 8.03 (s, 1H, C=CHAr), 9.28 (br. s, 1H, NH, D_2O exchangeable), 10.52 (br. s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 20.4 (1 C,CH_3), 23.5, 26.6, 28.2 (3 C, 3CH_2), 46.3,105.5 (2 C,thiopyran), 120.6 (1 C, C=CHAr), 121.5, 122.7, 123.2, 124.4, 129.8, 133.5, 134.7, 144.4, 145.8, 146.5, 147.4, 149.3, 149.6, 153.9, 156.2 (21 C, Ar–C),157.9 (1 C, C=NH); MS (70 eV, %) *m/z* 490 (M^+, 88 %); Anal. Calc. (Found) for $C_{29}H_{26}N_6S$ (490.63): C, 70.99 (70.90); H, 5.34 (5.40); N, 17.13 (17.20); S, 6.53(6.61).

Synthesis of 2-ethoxy-3-phenyl-8-(pyridin-4-yl)-12-(pyridin-4-ylmethylene)-2,3,9,10,11,12-hexahydro-8H-isothiochromeno[4,3-e][1,2,4]triazolo[1,5-c]pyrimidine (22a) and 2-ethoxy-5-methyl-3-phenyl-8-(pyridin-4-yl)-12-(pyridin-4-ylmethylene)-2,3,9,10,11,12-hexahydro-8H-isothiochromeno[4,3-e][1,2,4]triazolo[1,5-c]pyrimidine (22b)

General method: A solution of compounds **21a** (4.76 g, 0. 01 mol) or **21b** (4.90 g, 0. 01 mol) in triethylorthoformate (10 mL) was refluxed for 2–4 h. The reaction mixture was poured into crashed ice water; the formed precipitated was collected via filtration and recrystallized to afford **22a** and **22b** respectively.

Compound **22a**: Crystallized from chloroform, yellow crystals in 75 % yield, mp >340 °C (dec.); IR (ν, cm^{-1}) KBr: 3050 (CH-aryl), 2952 (CH-aliph), 1622(C=N). ^{1}H NMR (DMSO-d_6, ppm) δ 1.31 (t, $J = 5.60$ Hz, 3H, $CH_2\underline{CH_3}$), 1.33–2.43 (m, 6H, $3CH_2$, cyclohexene), 4.22 (q, $J = 5.65$ Hz, 2H, OCH_2), 4.72 (s,1H, thiopyran), 6.34 (s,1H,$\underline{CH}$OEt, triazole ring), 7.01–7.55 (m, 13H, Ar–H), 8.13 (s, 1H, C=$\underline{CH}$Ar), 8.84 (s, 1H, N=$\underline{CH}$); ^{13}C NMR (DMSO-d_6) δ 18.5(1 C, CH_3), 22.6, 25.8, 27.2 (3 C, $3CH_2$), 66.5 (1 C, $O\underline{C}H_2CH_3$), 69.7, 190.4 (2C, thiopyran), 112.2 (1 C, C=$\underline{C}$HAr), 121.5, 122.4, 123.5, 124.4, 127.2, 130.3, 133.6, 134.5, 142.8, 144.3, 145.9, 146.7, 149.4, 149.7, 150.1, 152.4, 155.2, (23 C, Ar–C); MS (70 eV, %) *m/z* 532 (M^+, 100 %); Anal. Calc. (Found) for $C_{31}H_{28}N_6OS$ (532.67): C, 69.90 (69.98); H, 5.30 (5.25); N, 15.78 (15.72); S, 6.02 (6.10).

Compound **22b**: Crystallized from ethanol, yellowish crystals in 73% yield, mp >340 °C (dec.); IR (ν, cm^{-1}) KBr: 3053 (CH-aryl), 2954 (CH-aliph), 1621(C=N).^{1}H NMR (DMSO-d_6, ppm) δ 1.30 (t, $J = 5.64$ Hz, 3H, CH_3), 1.35–2.44 (m, 6H, $3CH_2$, cyclohexene), 3.92 (s,3H,CH_3), 4.20 (q, $J = 5.69$ Hz,2H, CH_2), 4.70 (s,1H, thiopyran), 6.32 (s,1H, triazole ring), 7.04–7.59 (m, 13H, Ar–H), 8.11 (s, 1H, C=$\underline{CH}$Ar),^{13}C NMR (DMSO-d_6) δ 15.7, 21.6(2 C,2CH_3), 31.2, 31.3, 31.9, 38.6 (4 C, $4CH_2$), 65.6, 88.9 (2 C, thiopyran), 111.9 (1 C, C=$\underline{C}$HAr), 116.2, 121.4, 124.8, 130.2, 130.8, 130.9, 131.5, 136.1, 137.2, 138.1, 140.4, 142.5, 144.2, 147.7, 150.1, 152.5, 159.3 (23 C, Ar–C); MS (70 eV, %) *m/z* 546 (M^+, 90 %); Anal. Calc. (Found) for $C_{32}H_{30}N_6OS$ (546.69): C, 70.30 (70.38); H, 5.53 (5.59); N, 15.37 (15.32); S, 5.86 (5.80).

Synthesis of Ethyl-(4-cyano-1-(pyridin-4-yl)-5-(pyridin-4-ylmethylene)-5, 6, 7, 8-tetrahydro-1H-isothiochromen-3-yl) glycinate (23)

A mixture of compound **3** (3.58 g, 0.01 mol), ethyl bromoacetate (1.11 mL, 0.01 mol) and anhydrous potassium carbonate (1.38 g, 0.01 mol) in dry acetone (35 mL) was refluxed for 8–10 h. The reaction mixture was allowed to cool to room temperature and water (30 mL) was added, then the solid that separated was filtered off, dried and crystallized from diethyl ether to give **23**, yellow crystals in 88 % yield, mp 240–242 ° C (dec.); IR (ν, cm^{-1}) KBr: 3250 (NH), 3055 (CH-aryl), 2960 (CH-aliph), 2215 (CN), 1730 (C=O), 1630 (C=N).^{1}H NMR (DMSO-d_6, ppm) δ 1.23 (t, 3H, $J = 4.81$ Hz, CH_3), 1.70–2.43 (m, 6H, $3CH_2$, cyclohexene), 4.03 (s, 2H, NHCO$\underline{CH_2}$), 4.21 (q, 2H, $J = 4.88$ Hz, OCH_2), 4.72 (s,1H, thiopyran), 7.11–7.96 (m, 8H, Ar–H), 8.24 (s, 1H, C=$\underline{CH}$Ar), 9.12 (br. s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 15.2 (1 C, $\underline{C}H_3$), 23.5, 26.8, 27.9 (3 C, $3CH_2$), 44.1 (1 C, NHCO$\underline{C}H_2$), 47.5 (1 C, thiopyran), 64.3 (1 C, O$\underline{C}H_2$), 78.6 (1 C, thiopyran), 112.2 (1 C, C=$\underline{C}$HAr), 115.5 (1 C, CN), 122.4, 124.8, 133.6, 134.8, 144.1, 146.3, 147.7, 149.1, 149.5, 165.2 (14 C, Ar–C), 168.9 (1 C, $\underline{C}$=O); MS (70 eV, %) *m/z* 444 (M^+, 100 %); Anal. Calc. (Found) for $C_{25}H_{24}N_4O_2S$ (444.55): C, 67.55 (67.50); H, 5.44 (5.49); N, 12.60 (12.68); S, 7.21 (7.27).

Synthesis of Ethyl-1-amino-5-(pyridin-4-yl)-9-(pyridin-4-ylmethylene)-3, 5, 6, 7, 8, 9-hexahydroisothiochromeno [3, 4-b] pyrrole-2-carboxylate (24)

A solution of compound **23** (4.44 g, 0.01 mol), and anhydrous potassium carbonate (1.38 g, 0.01 mol) in dimethylformamide (30 mL) was refluxed for 5–8 h and then left to cool to room temperature and water (25 mL) was added. The formed precipitate was filtered off, dried and crystallized from ethyl acetate to give **24**, yellow crystals, yield (85%), mp 250–252 °C (dec.); IR (ν, cm^{-1}) KBr: 3405 (NH_2), 3280 (NH), 3058 (CH-aryl), 2962 (CH-aliph), 1735 (C=O), 1632 (C=N).^{1}H NMR (DMSO-d_6, ppm) δ 1.23 (t, 3H, $J = 4.80$ Hz, CH_3),1.68–2.42 (m, 6H, $3CH_2$, cyclohexene), 4.22 (q, 2H, $J = 4.85$ Hz, OCH_2), 5.24 (s,1H, thiopyran), 6.27 (br. s, 2H, NH_2, D_2O exchangeable), 7.10–7.95 (m, 8H, Ar–H), 8.23 (s, 1H, C=$\underline{CH}$Ar), 10.10 (br. s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 15.5 (1 C, CH_3), 21.8, 25.6, 30.7 (3 C, $3CH_2$), 52.8 (1 C, OCH_2), 65.1, 92.6 (2 C, thiopyran), 111.8 (1 C, C=$\underline{C}$HAr), 118.9, 122.6, 124.5, 130.8, 133.7, 135.9,143.5, 144.2, 146.8, 147.6, 149.3,149.9 (16 C, Ar–C), 162.4 (1 C, $\underline{C}$=O); MS (70 eV, %) *m/z* 444 (M^+, 92 %); Anal. Calc. (Found) for $C_{25}H_{24}N_4O_2S$ (444.55): C, 67.55 (67.60); H, 5.44 (5.40); N, 12.60 (12.55); S, 7.21 (7.16).

Synthesis of Ethyl-5-(pyridin-4-yl)-9-(pyridin-4-ylmethylene)-1-thioureido-3, 5, 6, 7, 8, 9-hexahydroisothiochromeno[3, 4-b] pyrrole-2-carboxylate (25)

A mixture of **24** (4.44 g, 0.01 mol) and 10 % HCl (10 mL) was refluxed with potassium thiocyanate (0.97 g, 0.01 mol) for 5–7 h. The reaction mixture was allowed to cool to room temperature. The formed solid was collected by filtration, washed with water, dried and crystallized from xylene to

give **25**, Yellow crystals, yield (80%), mp 302–304 °C (dec.); IR (ν, cm^{-1}) KBr: 3412 (NH_2), 3300 (NH), 3056 (CH-aryl), 2968 (CH-aliph), 1740 (C=O), 1631(C=N). ^{1}H NMR (DMSO-d_6, ppm) δ 1.23 (t, 3H, $J = 4.79$ Hz, CH_3), 1.68–2.42 (m, 6H, $3CH_2$, cyclohexene), 4.22 (q, 2H, $J =$ 4.87 Hz, CH_2), 5.82 (s,1H, thiopyran), 6.28 (br. s, 2H, NH_2, D_2O exchangeable), 7.10–7.95 (m, 8H, Ar-H), 8.23(s, 1H, C=CHAr), 9.17 (br. s, 1H, NH, D_2O exchangeable), 9.32 (br. s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 15.8 (1 C, CH_3), 21.6, 27.5, 31.2 (3 C, $3CH_2$), 53.9 (1 C, OCH_2), 66.3, 90.5 (2 C, thiopyran), 112.5 (1 C, C=CHAr), 119.6, 122.4, 124.7, 131.1, 133.5, 135.7, 143.2, 144.8, 146.7, 147.5, 149.2, 149.6 (16 C, Ar–C), 163.5 (1 C, C=O), 181.2 (1 C, C=S); MS (70 eV, %) *m/z* 503 (M^+, 90 %); Anal. Calc. (Found) for $C_{26}H_{25}N_5O_2S_2$ (503.64): C, 62.01 (62.10); H, 5.00 (5.09); N, 13.91 (13.97); S, 12.73 (12.80).

Synthesis of 5-(pyridin-4-yl)-1-(pyridin-4-ylmethylene)-10-thioxo-1,3,4,5,7,9,10,11-octahydro isothiochromeno [4′,3′:4,5]pyrrolo[3,2-d]pyrimidin-8 (2H)-one (26)

A solution of **25** (5.03 g, 0.01 mol) in ethanolic sodium ethoxide (0.23 g of sodium metal in 30 mL ethanol) was stirred under reflux for 8–10 h. After cooling, the reaction mixture was neutralized with 10% HCl and the solid formed was collected by filtration, washed with water, dried and then crystallized from dioxane to give **26**. Yellow crystals, yield 78%, mp > 340 °C (dec.); IR (ν, cm^{-1}) KBr: 3355 (3NH), 3062 (CH-aryl), 2954 (CH-aliph), 1678 (C=O), 1628 (C=N). ^{1}H NMR (DMSO-d_6, ppm) δ 1.75–2.25 (m, 6H, $3CH_2$, cyclohexene), 4.30 (s,1H, thiopyran), 7.11–7.93 (m, 8H, Ar-H), 8.12(s, 1H, C=CHAr), 10.35 (br. s, 1H, NH, D_2O exchangeable), 12.32 (br. s, 1H, NH, D_2O exchangeable), 13.13 (br. s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 15.7, 21.6, 31.2 (3 C, $3CH_2$), 89.1, 111.8 (2 C, thiopyran), 116.1 (1 C, C=CHAr), 118.1, 121.9,124.8, 128.1, 131.5, 135.8, 136.1, 142.1, 148.2, 152.1, 154.2, 159.8 (16 C, Ar-C), 165.6 (1 C, C=O),172.5 (1 C, C=S); MS (70 eV, %) *m/z* 457 (M^+, 99 %); Anal. Calc. (Found) for $C_{24}H_{19}N_5OS_2$ (457.57): C, 63.00 (63.10); H, 4.19 (4.12); N, 15.31 (15.38); S, 14.01 (14.10).

Synthesis of 1-amino-5-(pyridin-4-yl)-9-(pyridin-4-ylmethylene)-3,5,6,7,8,9-hexa- hydroisothio- chromeno[3,4-b]pyrrole-2-carboxamide(30); (1-amino-5-(pyridin-4-yl)-9-(pyridin-4-ylmethylene)-3,5,6,7,8,9-hexahydroisothiochromeno[3,4-b]pyrrol-2-yl)(phenyl) methanone (31) and 1-amino-3-oxo-6-(pyridin-4-yl)-10-(pyridin-4-ylmethylene)-4,6,7,8,9,10-hexahydro-3H -isothiochromeno[3,4-b]pyridine-2-carbonitrile (32)

General method: A mixture of **3** (3.58 g, 0.01 mol), and the appropriate active methylene compounds namely, 2-chloroacetamide (0.93 g, 0.01 mol) or phenacyl bromides (1.99 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol), was stirred in (40 mL) of anhydrous ethanol containing sodium ethoxide (3%) for 3–4 h, and then was refluxed with stirring for 10–12 h. The reaction mixture was cooled and poured into ice water. The solid formed was filtered off, dried, and recrystallized from suitable solvent to give **30, 31** and **32** respectively.

Compound **30**: The compound was obtained from the reaction of **3** (3.58 g, 0.01 mol) and 2-chloroacetamide (0.93 g, 0.01 mol), as a white crystals, crystallized from dioxane (70%), mp 348–350 °C. IR (ν, cm^{-1}) KBr: 3410–3250 broad ($2NH_2$ and NH), 3055 (CH-aryl), 2970 (CH-aliph), 1675 (C=O), 1625 (C=N).^{1}H NMR (DMSO-d_6, ppm) δ 1.85–2.27 (m, 6H, $3CH_2$, cyclohexene), 4.63 (s,1H, thiopyran), 6.42, 6.45 (2 s, $2NH_2$, D_2O exchangeable), 7.25–7.98 (m, 8H, Ar–H), 8.02 (s, 1H, C=CHAr), 12.68 (br., 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 18.5, 22.4, 30.8 (3 C, 3 CH_2), 62.1, 94.3,110.5 (3C, thiopyran), 112.7 (1 C, C=CHAr), 122.7, 124.2, 132.6, 133.7, 135.9, 143.4, 145.6, 147.1, 148.4, 149.1, 149.5 (15 C, Ar–C), 164.6 (1 C, C=O); MS (70 eV, %) *m/z* 415 (M^+, 90 %); Anal. Calc. (Found) for $C_{23}H_{21}N_5OS$ (415.52): C, 66.48 (66.40); H, 5.09 (5.15); N, 16.85 (16.77); S, 7.72 (7.65).

Compound **31**: The compound was obtained from the reaction of **3** (3.58 g, 0.01 mol) and phenacyl bromides (1.99 g, 0.01 mol), as a yellowish crystals, crystallized from DMF (73%), mp > 340 °C. IR (ν, cm^{-1}) KBr: 3400–3300 broad (NH_2 and NH), 3058 (CH-aryl), 2965 (CH-aliph), 1690 (C=O), 1627(C=N).^{1}H NMR (DMSO-d_6, ppm) δ 1.80–2.26 (m, 6H, $3CH_2$, cyclohexene), 4.10 (s,1H, thiopyran), 6.43 (s, NH_2, D_2O exchangeable), 7.10–7.99 (m, 13H, Ar–H), 8.03 (s, 1H, C=CHAr), 12.67(br., 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 17.1, 21.4, 30.9 (3 C, 3 CH_2), 59.5, 90.4, 98.8 (3C, thiopyran),111.1 (1 C, C=CHAr),122.4, 124.5,128.6, 129.5, 130.8, 132.9, 133.1, 135.2, 136.7, 144.1, 146.2, 147.5, 149.1, 149.5, 149.9 (21 C, Ar–C), 167.1 (1 C, C=O); MS (70 eV, %) *m/z* 476 (M^+, 95 %); Anal. Calc. (Found) for $C_{29}H_{24}N_4OS$ (476.60): C, 73.08 (73.14); H, 5.08 (5.14); N, 11.76 (11.70); S, 6.73 (6.78).

Compound **32**: The compound was obtained from the reaction of **3** (3.58 g, 0.01 mol) and ethyl cyanoacetate (1.13 g, 0.01 mol), as a yellow crystals, crystallized from methanol (75%), mp > 340 °C. IR (ν, cm^{-1}) KBr: 3405–3280 broad (NH_2 and NH), 3054 (CH-aryl), 2960 (CH-aliph), 2210 (CN), 1676 (C=O), 1624(C=N).^{1}H NMR (DMSO-d_6, ppm) δ 1.75–2.25 (m, 6H, $3CH_2$, cyclohexene), 4.25 (s,1H, thiopyran), 6.60 (s, NH_2, D_2O exchangeable), 7.57–7.98 (m, 8H, Ar-H), 8.12 (s, 1H, C=CHAr),12.32 (br., 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 15.7, 21.6, 31.2 (3 C, $3CH_2$), 65.6, 88.9 (2 C, thiopyran), 94.1 (1 C, pyridine),102.4 (1 C, CN), 111.9 (1 C, C=CHAr), 116.3,

121.5, 124.8,130.3,130.8, 130.9,131.5, 136.2, 147.7, 158.4 (14 C, Ar–C), 165.7 (1 C, C=O), 172.1(1 C, C–NH_2); MS (70 eV, %) *m/z* 425 (M^+, 92 %); Anal. Calc. (Found) for $C_{24}H_{19}N_5OS$ (425.51): C, 67.75 (67.79); H, 4.50 (4.58); N, 16.46 (16.40); S, 7.53 (7.48).

Acknowledgements This work was supported by the Photochemistry Department (Heterocyclic Unit); Chemical Industries Research Division, National Research Center, Dokki, Giza 12622, Egypt.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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