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ORIGINAL PAPER

Thiochromene candidates: design, synthesis, antimicrobial potential and in silico docking study

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Abstract

New sets of thiochromenes hybridized with five-membered rings as pyrazole and oxazole and/or six-membered ring as pyrimidine and thiopyrimidine were constructed. The novel constructed compounds were evaluated for their antimicrobial potential against *B. subtilis* and *S. aureus* as examples of gram-positive-bacteria, *E. coli* and *P. aeruginosa* as examples of gram-negative bacteria in addition to *A. flavus* and *C. albicans* as fungal strains. The results recorded promising antimicrobial potential with inhibition zone diameter range from 8 to 25 mm against the tested bacteria. Regarding antifungal activity, all the screened compounds revealed antifungal effect against *A. flavus* except thiochromene derivative **4** with zone of inhibition ranged from 9 to 16 mm. Moreover, all compounds recorded moderate to high antifungal potential towards *C. albicans* (ZI range = 8–19 mm) except thioxopyrazolothiochromene derivative **6** which did not exhibit any effect. To suggest mode of action of these candidates as antimicrobials, in silico docking study was carried out inside dihydropteroate synthase enzyme. Compound **8c** recorded the best binding energy score (−5.47 kcal/mol) forming good fitting within DHPS active site.

Keywords Dihydropteroate synthase · Thiochromene · Antimicrobial · In silico study · Antimicrobial

Introduction

One of the most real risks to global health is multidrug resistance emergence by pathogenic microbes [1, 2]. A wide variety of biochemical mechanisms account for bacterial resistance including mutational modification of target protein, drug's enzymatic inactivation, prevention of drug access to the targeted enzyme [3, 4]. Furthermore, misuse of antibiotics speeds up the emergence of multidrug resistant bacterial species [5]. So there is still a great demand for development of new antimicrobials to overcome the emergence and development of novel chemotherapeutic agents to combat the emergence and increasing spread of resistant pathogens. Dihydropteroate synthase enzyme (DHPS) is an essential enzyme for microbes which catalyzes the formation of 7,8-dihydropteroate—a precursor of tetrahydrofolate—formed through reacting *p*-aminobenzoic acid and 7,8-dihydropterin pyrophosphate [6–8]. Folate analogues are essential cofactors for nucleic acid and protein biosynthesis in all cells [9]. Whereas humans get folates from diet, almost all microbes biosynthesize folate de novo [10]. So, targeting DHPS is considered as a rational for design selective antimicrobial agents. Thiochromene-based heterocycles recorded versatile biological importance as HIF hydroxylase inhibitor [11], dopamine D3 receptor agonist [12, 13], anticancer [14], herbicide [15], antimicrobial [16, 17] and HIV protease inhibitor [18]. Prompted by all the previous facts and our researches concerned with designing and preparing bioactive agents [19–30], we are encouraged to synthesize some novel thiochromene derivatives and evaluating these novel compounds for their antibacterial potential against *B. subtilis*, *S. aureus*, *E. coli* and *P. aeruginosa* and antifungal potential towards *A. flavus* and *C. albicans*. In addition, in silico docking study was carried out for the synthesized compounds to display the probability of these compounds to suppress DHPS enzyme.

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Results and discussion

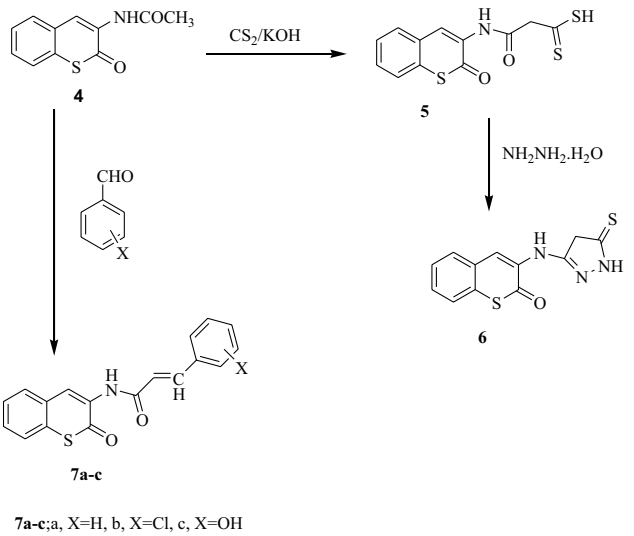
Chemistry

In the present work, the target compounds were constructed as illustrated in Schemes 1, 2 and 3. The starting compound **4** was prepared via reacting mercaptobenzaldehyde (**1**), acetamide (**2**) and ethyl chloroacetate (**3**) in the presence of piperidine in a catalyst (Scheme 1).

Reacting compound **4** with carbon disulphide and potassium hydroxide in ethanol afforded the propanedithioic acid derivative **5** (Scheme 2). ^{1}HNMR spectrum of this compound **5** displayed the disappearance of methyl protons at 1.91 ppm in addition to the appearance of two singlet signals at 2.71 and 13.25 ppm referred to CH_2 and SH groups. Moreover, in ^{13}CNMR the presence of a peak at 229.78 ppm referred to C = S confirmed the structure of compound **5**. Cyclizing the propanedithioic acid **5** with hydrazine hydrate in ethanol afforded thioxopyrazole derivative **6** in 68% yield. ^{1}HNMR of the product **6** revealed the presence of single signal exchanged with D_2O at 9.99 ppm attributed to NH proton and disappearance of SH proton at 13.25 ppm of the precursor **5**. The chalcone derivatives **7a-c** were prepared from reacting the acetamide derivative **4** with the appropriate aldehydes in presence of piperidine as a catalyst. The ^{1}HNMR spectra of these chalcones **7a-c** recorded the presence of CH = CH and additional aromatic protons in range of 6.93–8.45 ppm.

Cyclizing chalcones **7a-c** with hydrazine hydrate in acetic acid afforded acetylpyrazole derivatives **8a-c** in good yield (Scheme 3). Chemical structure of these pyrazoles was elucidated by spectral and elemental analysis. ^{1}HNMR showed the presence of a singlet signal at 2.12 ppm referred to CH_3 protons, in addition to doublet and triplet peaks at 3.47 and 5.03 ppm attributed to CH_2 and CH protons.

On the other hand, the phenylpyrazole derivatives **9a-c** were constructed from treating the chalcones with phenylhydrazine in DMF using piperidine as a catalyst. ^{1}HNMR chart of compound **9a** recorded the appearance of doublet and triplet peaks at 3.48 and 5.24 ppm referred to CH_2 and CH protons in addition to the presence of 15 aromatic protons at 6.92–8.35 ppm. Reacting chalcones **7a-c** with hydroxylamine afforded isoxazole derivatives **10a-c** in good yields. Pyrimidine derivatives **11a-c** and thiopyrimidine derivatives **12a-c** were constructed from reacting chalcones **7a-c** with urea and/or thiourea. These target compounds **10a-c**, **11a-c** and **12a-c** were confirmed by spectral and elemental analyses.

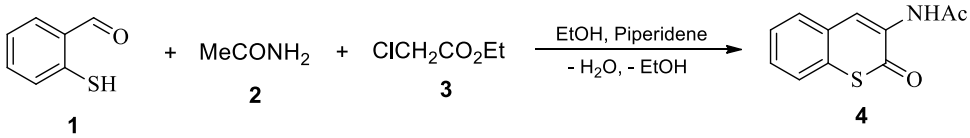


Scheme 2 Synthesis of target compounds **5, 6** and **7a-c**

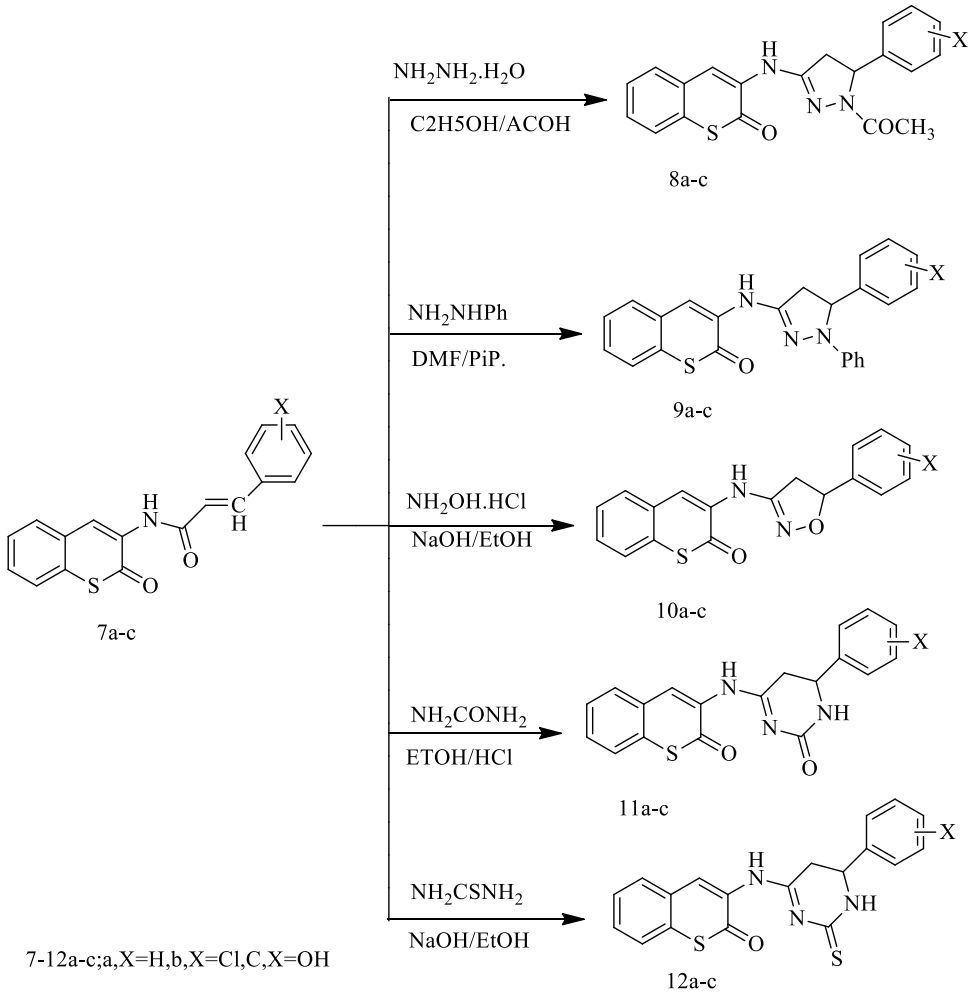
Antimicrobial potential

All the twenty novel prepared compounds **4-12a-c** were screened in vitro for their antibacterial activity against two gram-positive bacterial strains as *B. subtilis* and *S. aureus* and gram-negative bacterial strains as *E. coli* and *P. aeruginosa* using ampicillin as a standard at concentration 100 ppm adapting disc-diffusion technique. The results are listed in Table 1 which revealed that all the tested compounds exhibited antimicrobial potential with inhibition zone diameter ranged from 8 to 25 mm towards all tested bacterial strains. Concerning antimicrobial potential against *B. subtilis*, the pyrazolothiochromene derivative **9c** (ZI = 25 mm) and pyrimidiothiochromene derivative **11c** (ZI = 25 mm) recorded comparable antimicrobial potential to that recorded by ampicillin (ZI = 26 mm). Moreover, the phenylpyrazole derivatives **9a-c** (ZI = 16–25 mm) displayed higher antimicrobial potential towards *B. subtilis* than acetylpyrazole analogues **8a-c** (ZI = 13–18 mm). Moreover, pyrimidiothiochromene candidates **11a-c** (ZI = 14–25 mm) recorded higher antibacterial effect than thiopyrimidine analogues **12a-c** (12–15 mm). Furthermore, the pyrazolothiochromene **8c** (ZI = 19 mm) was the most active antibacterial agent towards *S. aureus* followed by thiochromene derivative **7b** (ZI = 17 mm) and then thiochromenes **4**, **7c** and pyrimidothiochromenes **11a** and **11c** (ZI = 14 mm). Moreover, the acetylpyrazole derivative

Scheme 1 Synthesis of starting material **4**



Scheme 3 Synthesis of target compounds **5, 6** and **7a-c**



8c (ZI = 24 mm), phenylpyrazole **9b** (ZI = 23 mm) and oxazole derivative **10b** (ZI = 24 mm) recorded similar antibacterial potential towards *E. coli* as displayed by ampicillin (ZI = 25 mm). The thioxopyrazole derivative **6** was found to be the least active antibacterial agent towards all the tested bacteria with zone of inhibition range from 8 to 9 mm. The antifungal potential of the target compounds **4-12a-c** towards *A. flavus* and *C. albicans* was screened using amphotericin B as a standard. From the obtained results, all the screened compounds revealed antifungal effect against *A. flavus* except thiochromene derivative **4** with zone of inhibition ranged from 9 to 16 mm. Besides, thiochromene candidate **7c** (ZI = 16 mm), acetylpyrazole **8b** (ZI = 15 mm) and oxazole derivatives **9c** and **10b** (ZI = 14 mm) assigned similar antifungal potential to *A. flavus* as shown by amphotericin B (ZI = 15 mm). While the antifungal potential against *C. albicans* revealed that all compounds recorded moderate to high antifungal potential (ZI range = 8–19 mm) except thioxopyrazolothiochromene derivative **6** which did not exhibit any effect. The highest antifungal activity to *C. albicans* was recorded by phenylpyrazole derivatives **9a** (ZI = 18 mm) and **9b** (ZI = 19 mm) followed by **9c** (ZI = 16 mm) and then compounds **8b, 10a** and **10b** (ZI = 14 mm).

In Silico docking study

To predict the mode of action of the newly constructed compounds **6-12a-c** as antimicrobial agents, molecular docking study was conducted on these compounds inside DHPS enzyme using MOE 2005.06. Crystal structure of DHPS with the cocrystallized ligand (pyrimido [4,5-*c*]pyridazine derivative) was downloaded from protein data bank (PDB: 4DAI). Outcomes from docking the target compounds are explained in Table 2.

Compound **8c** showed good fitting within DHPS revealing three hydrogen bonding interactions with Asp101, Arg254 and Ser218 through binding with NH, thiochromene C = O and OH moieties. Moreover, compound **8c** formed arene cation interactions with Lys220 and Arg254 through binding with phenyl moiety (Fig. 1).

The oxazolothiochromene derivative **10c** recorded binding energy score = -5.09 kcal/mol forming four hydrogen

Table 1 Antimicrobial evaluation of the synthesized compounds using the disc-diffusion method

Compound No	Gram-positive bacteria		Gram-negative bacteria		Fungi	
	B. subtilis	*S. aureus*	*E. coli*	*P. aeruginosa*	*A. flavus*	*C. albicans*
Control (DMSO)	0	0	0	0	0	0
Ampicillin	26	21	25	26	–	–
Amphotericin B	–	–	–	–	15	19
4	10	14	12	14	0	11
6	9	9	8	8	11	0
7a	14	11	10	14	10	13
7b	13	17	16	17	12	12
7c	15	14	15	19	16	13
8a	10	13	17	20	9	13
8b	11	12	16	17	15	14
8c	14	19	24	15	13	12
9a	16	13	18	11	16	18
9b	17	12	23	10	13	19
9c	25	10	17	9	14	16
10a	13	19	18	13	11	14
10b	15	11	24	15	14	14
10c	18	10	10	11	12	10
11a	14	14	15	13	13	9
11b	17	13	17	17	14	11
11c	25	14	16	10	12	12
12a	12	15	15	11	10	9
12b	13	12	16	14	13	8
12c	15	10	13	15	12	10

bonds as follows: (1) Asp101 with NH, (2) Arg254 with thiochromene C = O, (3) Asn120 with thiochromene C = O and (4) Arg219 with OH via H_2O molecule (Fig. 2).

One the other hand, compound **11c** showed binding with Glu79, Lys104 and Asp101 though hydrogen bonding with hydroxyl and amino groups, besides arene cation interaction with Arg254 and Lys220 aminoacids (Fig. 3).

Experimental

Chemistry

Reagents were purchased from Sigma Aldrich (Bayouni Trading Co. Ltd., Al-Khobar, Saudi Arabia) and used without further purification. Reaction progress was monitored by TLC on silica gel pre-coated F254Merck plates. Spots were visualized by ultraviolet irradiation. Melting points were determined on a Gallenkamp electrothermal melting point apparatus and are uncorrected. IR spectra were recorded as potassium bromide disc using Bruker-Vector 22 FTIR Spectrophotometer. The NMR spectra were recorded with a Varian Mercury VXR-300 NMR spectrometer at 300 and 75 MHz for ^{1}H and ^{13}C NMR spectra, respectively, using DMSO-d_6 as solvents. Mass spectra were recorded on a Hewlett Packard MS-5988 spectrometer at 70 eV. Elemental analyses were carried out at the Micro-analytical Center of Cairo University, Giza, Egypt.

N-(2-Oxo-2*H*-thiochromen-3-yl)acetamide (4)

A mixture of acetamide (0.59 g, 0.01 mol) and ethyl chloroacetate (1.22 g, 0.01 mol) in ethanol (30 mL), piperidine (0.5 ml) as catalyst and mercaptobenzaldehyde (1.38 g, 0.01 mol) was heated under reflux for 7–8 h, and its progress was checked by TLC. The formed precipitate was filtered, dried and crystallized from ethanol/DMF (3:1) to give the expected product (**1**) (65% yield) as green crystals. mp 200 – 202 °C.; IR (KBr): ν (cm^{-1}) 1660, 1718 (2C = O), 3400 (N–H); ^{1}H NMR (DMSO – d_6): δ 1.91 (s, 3H, CH_3), 7.32–8.19 (m, 5H, aromatic protons), 12.3 (br. s, 1H, N – H); ^{13}C NMR (DMSO – d_6): δ 24.5, 124.0, 125.6, 137.0, 128.6, 130.5, 137.4, 121.3, 138.0 (C = C), 170.5 and 187.8 (2C = O): ms (*m/z*, %): 219.0 (M^+, 55%). Anal. Calcd for $C_{11}H_9NO_2S$ (219.26): C, 60.26; H, 4.14; N, 6.39; S, 14.62%. Found: C, 60.21; H, 4.13; N, 6.02; S, 14.57%.

Table 2 Docking results for the target compounds within DHPS

Target No	E. Score Kcal/mol	Hydrogen bonds number	Distance (Å) from Amino acid		Binding group
6	−3.99	1	1.92	Lys220	Pyrazole N
7a	−3.59	2	2.34 1.76	Arg254 Asp101	Thiochromene C=O NH
7b	−3.88	2	1.87 2.29	Lys220 Arg254	C=O Thiochromene C=O
7c	−4.79	3	2.38 1.98 3.88	Arg254 Thr102 Glu78	Thiochromene C=O OH OH
8a	−4.06	3	2.66 2.79 3.09	Arg254 Arg254 Lys220	Pyrazole N Acetyl C=O Thiochromene
8b	−3.87	2	1.96 2.49	Arg254 Arg254	Acetyl C=O Pyrazole N
8c	-5.47	3	2.34 3.18 2.84	Asp101 Arg254 Ser218	NH Thiochromene C=O OH
9a	−4.94	1	2.98	Asp101	NH
9b	−5.13	3	2.58 2.98 3.06	Arg254 Asn120 Asp101	Thiochromene C=O Thiochromene C=O NH
9c	−5.04	2	2.49 3.09	Asp101 Lys104	NH OH
10a	−4.89	2	2.99 3.09	Arg254 Lys220	Thiochromene C=O Oxazole N
10b	−5.17	1	3.99	Asp101	NH
10c	−5.09	4	2.563.35 2.88 2.19	Asp101 Arg254 Asn120 Arg219	NH Thiochromene C=O Thiochromene C=O OH
11a	−4.89	3	2.98 2.95 3.01	Asp101 Arg254 Arg254	NH C=O Pyrimidine N
11b	−4.88	2	2.90 2.89	Asp101 Arg254	Pyrimidine N Pyrimidine C=O
11c	−5.44	3	3.25 2.19 2.93	Glu79 Lys104 Asp101	OH OH NH
12a	−3.94	1	2.49	Asp101	NH
12b	−4.35	2	2.73 3.26	Arg254 Lys220	Thiopyrimidine N Thiochromene C=O
12c	−4.55	2	2.18 2.94	Asp184 Gly216	OH OH
Cocrystallized ligand	−5.59	6	2.89 2.76 3.03 3.052.952.83	Asn120 Asn120 Asp184 Asp184 Lys220 IIe187	Pyrimidine N NH2 NH2 Pyrimidine NH Pyridazine C=O Pyrimidine C=O

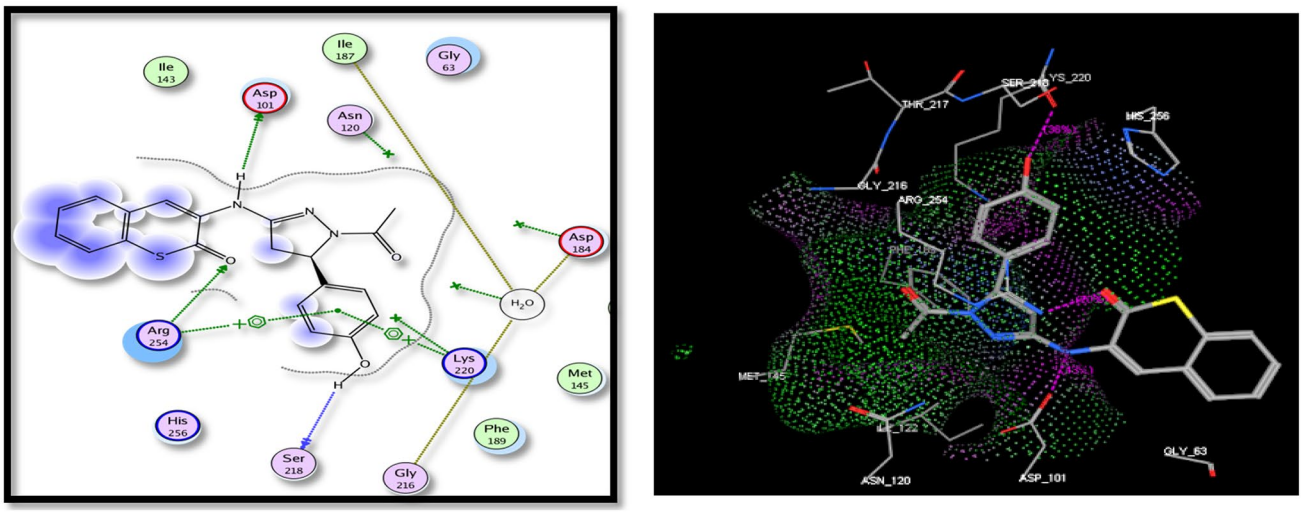


Fig. 1 Binding mode of compound **8c** within DHPS enzyme (PDB, code: 4DAI). **a** 2D interaction, **b** 3D interaction

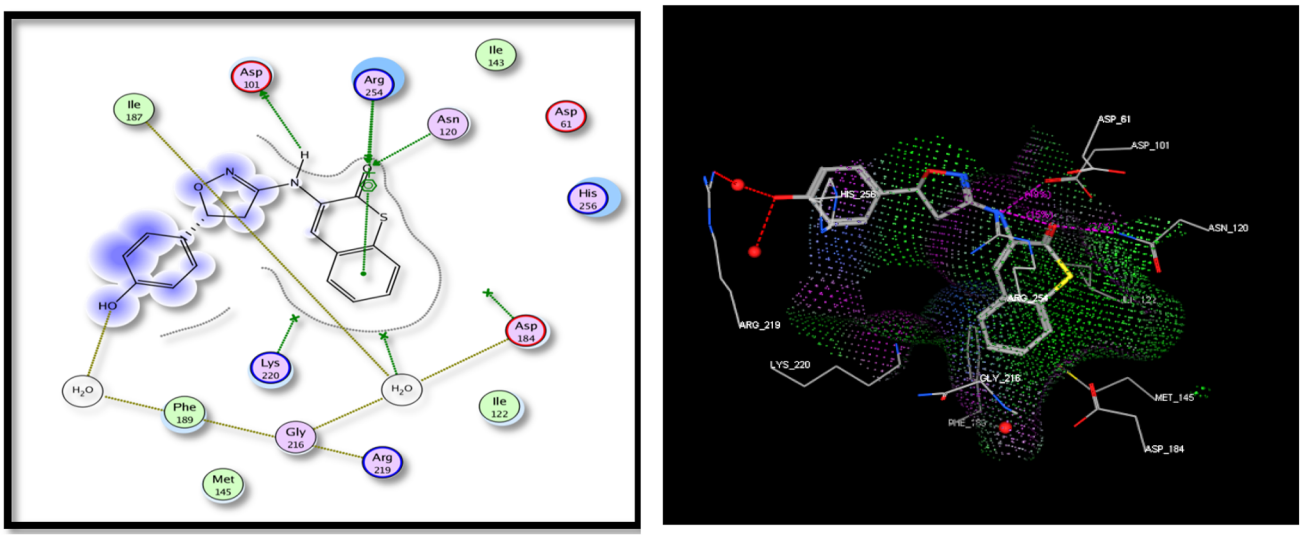


Fig. 2 Binding mode of compound **10c** within DHPS enzyme (PDB, code: 4DAI). **a** 2D interaction, **b** 3D interaction

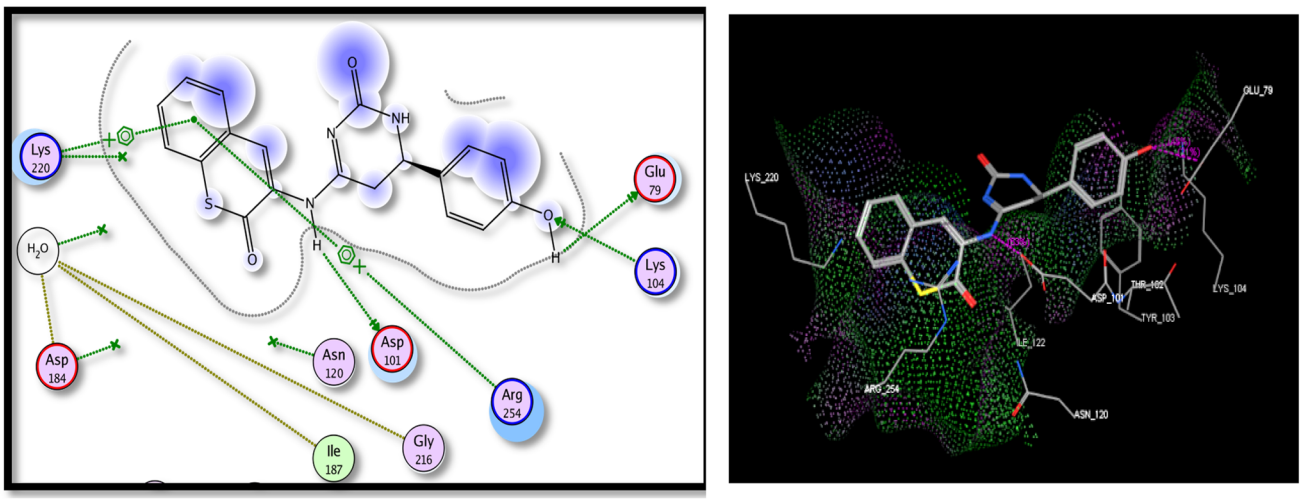


Fig. 3 Binding mode of compound **11c** within DHPS enzyme (PDB, code: 4DAI). **a** 2D interaction, **b** 3D interaction

3-Oxo-3-((2-oxo-2*H*-thiochromen-3-yl)amino)propanedithioic acid (5)

A mixture of **4** (2.19 g, 0.01 mol) and carbon disulfide (15 mL) in ethanol (30 mL) and KOH was heated in reflux for 7–8 h. The solid product formed on hot was filtered, washed with MeOH, dried and crystallized from MeOH-DMF (3:1) to afford **5.** (60% yield) as brown crystals. mp 183–185 °C; IR (KBr): ν (cm^{-1}) 1660, 1718 (2C = O), 3100–3400 (NH); ^{1}H NMR (DMSO – d_6): δ 2.71 (s, 2H, CH_2), 7.28–8.19 (m, 5H, aromatic protons), 12.25 (br. s, 1H, NH), 13.25 (s, 1H, SH); ^{13}C NMR (DMSO – d_6): δ 62.7, 117.9, 121.3, 124.0, 125.6, 137.0, 128.6, 130.5, 137.4, 138.0, 138.3, 148.2, 168.3, 187.5 and 229.7: ms (*m/z*, %): 295.0 (M^+, 40%). Anal. Calcd for: $C_{12}H_9NO_2S_3$ (295.40): C, 48.79; H, 3.07; N, 4.74; S, 32.56%. Found: C, 48.74; H, 3.02; N, 4.68; S, 32.53%.

3-((5-Thioxo-4,5-dihydro-1*H*-pyrazol-3-yl) amino)-2*H*-thiochromen-2-one (6)

To solution of **5** (0.300 g, 1 mmol) in EtOH, hydrazine hydrate (0.5 mL) was added. The mixture was refluxed for 6 h. The solid product so obtained after cooling was collected by filtration and crystallized from EtOH to afford **12** in (68% yield) as orange crystals. mp 270–273 °C; IR (KBr): ν (cm^{-1}) 1660, 1718 (2C = O), 3100–3400 (2NH); ^{1}H NMR (DMSO – d_6): δ 1.56 (s, 2H, CH_2), 7.38–8.19 (m,

5H, aromatic protons), 9.99 (s, 1H, NH), 12.25 (br. s, 1H, NH); ^{13}C NMR (DMSO – d_6): δ 50.4, 124.0, 125.6, 137.0, 128.6, 130.5, 137.4, 121.3, 138.1, 106.6, 138.3, 118.4, 150.3, 157.5, 187.5 and 201.7: ms (*m/z*, %): 275.0 (M^+, 40%). Anal. Calcd for: $C_{12}H_9N_3OS_2$ (275.35): C, 52.34; H, 3.29; N, 15.26; S, 23.29%. Found: C, 52.36; H, 3.34; N, 15.32; S, 23.35%.

General synthesis of compounds 7a-c

A solution of **4** (0.01 mol) in absolute ethanol (30 mL) was treated with different aromatic aldehydes (0.01 mol) in the presence of piperidine as catalyst. The reaction mixture was heated under reflux for 4–6 h. (monitored by TLC). The solvent was then evaporated under reduced pressure and the residue treated with ice/water acidified by HCl. The solid product was collected and crystallized from the proper solvent to afford **7a-c**.

N-(2-Oxo-2*H*-thiochromen-3-yl)cinnamamide (7a)

(64% yield) as white crystals. mp 115–117 °C; IR (KBr): ν (cm^{-1}) 1660, 1718 (2C = O), 3100–3400 (NH); ^{1}H NMR (DMSO – d_6): δ 6.95 (s, 1H, CH), 7.23–8.45 (m, 11H, aromatic protons & O = C–C<u>H</u>), 12.23 (br. s, 1H, NH); ^{13}C NMR (DMSO – d_6): δ 124.0, 125.6, 137.0, 128.6, 130.5, 137.4, 121.3, 138, 106.6, 138.3, 128.5, 135.4, 109.5, 141.7, 165.5 and 187.5: ms (*m/z*, %): 307.0 (M^+, 25%). Anal. Calcd for: $C_{18}H_{13}NO_2S$ (307.37): C, 70.34; H, 4.26; N, 4.56; S, 10.43%. Found: C, 70.30; H, 4.21; N, 4.54; S, 10.38%.

3-(4-Chlorophenyl)-*N*-(2-oxo-2*H*-thiochromen-3-yl) acrylamide (7b)

(55% yield) as grey crystals. mp 135–137 °C; IR (KBr): ν (cm^{-1}) 1660, 1718 (2C = O), 3100–3400 (NH); ^{1}H NMR (DMSO – d_6): δ 6.98 (s,1H, CH), 7.28–8.10 (m, 10H, aromatic protons & O = C–C<u>H</u>), 12.23 (br. s, 1H, NH); ^{13}C NMR (DMSO – d_6): δ 124.0, 125.6, 137.0, 128.6, 130.5, 137.4, 121.3, 138.1, 106.6, 138.3, 128.5, 135.4, 109.5, 141.7, 165.5 and 187.5: ms (*m/z*, %): 341.0 (M^+, 25%). Anal. Calcd for: $C_{18}H_{12}ClNO_2S$ (341.81): C, 63.25; H, 3.54; Cl, 10.37; N, 4.10; S, 9.38%. Found: C, 63.28; H, 3.59; Cl, 10.42; N, 4.15; S, 9.41%.

3-(4-Hydroxyphenyl)-*N*-(2-oxo-2*H*-thiochromen-3-yl)acrylamide (7c)

(58% yield) as yellow crystals. mp 167–169 °C; IR (KBr): ν (cm^{-1}) 1660, 1718 (2C = O), 3100–3400 (NH,OH); ^{1}H NMR (DMSO – d_6): δ 6.93 (s,1H, CH), 7.03–8.18 (m, 10H, aromatic protons & O = C–C<u>H</u>), 10.26 (s, 1H, OH), 12.23 (br. s, 1H, NH); ^{13}C NMR (DMSO – d_6): δ 124.0, 125.6, 137.0, 128.6, 130.5, 137.4, 121.3, 138.3, 106.6, 138.3, 128.5, 135.4, 158.1, 109.5, 141.7, 165.5 and 187.5 (2C = O): ms (*m/z*, %): 323.0 (M^+, 30%). Anal. Calcd for: $C_{18}H_{13}NO_3S$ (323.37): C, 66.86; H, 4.05; N, 4.33; S, 9.92%. Found: C, 66.89; H, 4.07; N, 4.37; S, 9.96%.

Preparation of compounds 8a-c

A solution of compound **7a-c** (0.01 mol) and hydrazine hydrate (0.01 mol) in hot ethanol (30 ml) containing few drop of catalytic amount of glacial acetic acid (3 drops) was refluxed for 8–10 h and estimated by (TLC) till completion, and then the reaction was filtered while hot and the solvent was then evaporated under reduced pressure and the residue was heated with petroleum ether and then treated with ice water and crystalized from methanol to provide **8a-c**.

3-((1-Acetyl-5-phenyl-4,5-dihydro-1*H*-pyrazol-3-yl) amino)-2*H*-thiochromen-2-one (8a)

(58% yield) as yellow crystals. mp 167–169 °C; IR (KBr): ν (cm^{-1}) 1660, 1718 (2C = O), 3100–3400 (NH); ^{1}H NMR (DMSO – d_6): δ 2.12 (s, 3H, CH_3), 3.47 (d, 2H, CH_2), 5.03 (t, 1H, CH), 7.48–8.29 (m, 10H, aromatic protons), 12.23 (br. s, 1H, NH); ^{13}C NMR (DMSO – d_6): δ 23.6, 40.0, 63.9, 124.0, 125.6, 137.0, 128.6, 130.5, 137.4, 121.3, 138.7, 106.6, 138.3, 128.5, 135.4, 118.3, 150.8 (C = C), 168.8 and 187.5 (2C = O): ms (*m/z*, %): 363.0 (M^+, 30%). Anal. Calcd for: $C_{20}H_{17}N_3O_2S$ (363.43): C, 66.10; H, 4.71; N, 11.56; S, 8.82%. Found: C, 66.13; H, 4.76; N, 11.60; S, 8.87%.

3-((1-Acetyl-5-(4-chlorophenyl)-4,5-dihydro-1*H*-pyrazol-3-yl)amino)-2*H*-thiochromen-2-one (8b)

(63% yield) as reddish brown crystals. mp 215–217 °C; IR (KBr): ν (cm^{-1}) 1660, 1718 (2C = O), 3100–3400 (NH); ^{1}H NMR (DMSO – d_6): δ 2.12 (s, 3H, CH3), 3.47 (d, 2H, CH2), 5.03 (t, 1H, CH), 7.3–8.1 (m, 9H, aromatic protons), 12.25 (br. s, 1H, NH); ^{13}C NMR (DMSO – d_6): δ 23.6, 40.0, 63.9, 124.0, 125.6, 137.0, 128.6, 130.5, 137.4, 121.3, 138.6, 106.6, 138.3, 128.5, 135.4, 118.3, 150.8, 168.8 and 187.5: ms (*m/z*, %): 397.0 (M^+, 35%). Anal. Calcd for: $C_{20}H_{16}ClN_3O_2S$ (397.88): C, 60.37; H, 4.05; Cl, 8.91; N, 10.56; S, 8.06%. Found: C, 60.39; H, 4.09; Cl, 8.95; N, 10.62; S, 8.12%.

3-((1-Acetyl-5-(4-hydroxyphenyl)-4,5-dihydro-1*H*-pyrazol-3-yl)amino)-2*H*-thiochromen-2-one (8c)

69% yield) as greenish crystals. mp 245–247 °C; IR (KBr): ν (cm^{-1}) 1660, 1718 (2C = O), 3100–3400 (NH,OH); ^{1}H NMR

(DMSO – d_6): δ 2.12 (s, 3H, CH_3), 3.47 (d, 2H, CH_2), 5.03 (t, 1H, CH), 7.3–8.1 (m, 9H, aromatic protons), 10.25 (s, 1H, OH), 12.25 (br. s, 1H, NH); ^{13}C NMR (DMSO – d_6): δ 23.6, 40.03, 63.9, 124.0, 125.6, 137.0, 128.6, 130.5, 137.4, 121.3, 138.6, 106.6, 138.34, 128.5, 135.4, 118.3, 150.8, 168.8 and 187.5: ms (*m/z*, %): 379.0 (M^+, 35%). Anal. Calcd for: $C_{20}H_{17}N_3O_3S$ (379.43): C, 63.31; H, 4.52; N, 11.07; S, 8.45%. Found: C, 63.34; H, 4.57; N, 11.11; S, 8.48%.

Synthesis of compounds 9a-c

A solution of **(7a-c)** (0.01 mol) and phenylhydrazine (0.01 mol) in dimethylformamide (30 mL) was prepared, and piperidine was used as catalyst. The mixture was refluxed and heated for 10-11 h (controlled by TLC). The resulting product was subject to reduced pressure to remove solvent and then poured into cold water and crystallized from methanol to provide (**9a-c**).

3-((1,5-Diphenyl-4,5-dihydro-1*H*-pyrazol-3-yl) amino)-2*H*-thiochromen-2-one (9a)

(66% yield) as red crystals. mp 188–190 °C; IR (KBr): ν (cm^{-1}) 1660 (C = O), 3100–3400 (NH); ^{1}H NMR (DMSO – d_6): δ 3.48 (d, 2H, CH_2), 5.24 (t, 1H, CH), 6.92–8.35 (m, 15H, aromatic protons), 12.23 (br. s, 1H, NH); ^{13}C NMR (DMSO – d_6): δ 23.6, 40.0, 63.9, 124.0, 125.6, 137.0, 128.6, 130.5, 137.4, 121.3, 138.8, 106.6, 138.3, 128.5, 135.4, 118.3, 150.8, 187.5: ms (*m/z*, %): 397.0 (M^+, 26%). Anal. Calcd for: $C_{24}H_{19}N_3OS$ (397.49): C, 72.52; H, 4.82; N, 10.57; S, 8.07%. Found: C, 72.53; H, 4.85; N, 10.62; S, 8.13%.

3-((5-(4-Chlorophenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazol-3-yl)amino)-2H-thiochromen-2-one (9b)

(72% yield) as reddish brown crystals. mp 210–212 °C; IR (KBr): ν (cm^{-1}) 1660 (C = O), 3100–3400 (NH); ^{1}H NMR (DMSO – d_6): δ 3.48 (d, 2H, CH2),5.24(t,1H,CH), 6.99–8.41 (m, 14H, aromatic protons), 12.23 (br. s, 1H, NH); ^{13}C NMR (DMSO – d_6): δ 40.0, 63.9, 124.0, 125.6, 137.0, 128.6, 130.5, 137.4, 121.3, 138.9, 106.6, 138.3, 128.5, 135.4, 118.3, 150.8, 187.5: ms (*m/z*, %): 431.0 (M^+, 26%). Anal. Calcd for: $C_{24}H_{18}ClN_3OS$ (431.94): C, 66.74; H, 4.20; Cl, 8.21; N, 9.73; S, 7.42%. Found: C, 66.72; H, 4.16; Cl, 8.17; N, 9.69; S, 7.39%.

3-((5-(4-Hydroxyphenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazol-3-yl)amino)-2*H*-thiochromen-2-one (9c)

(76% yield) as reddish brown crystals. mp 220–222 °C; IR (KBr): ν (cm^{-1}) 1660 (C = O), 3100–3400 (NH, OH); ^{1}H NMR (DMSO – d_6): δ 3.48 (d, 2H, CH_2), 5.24 (t, 1H, CH), 7.15–8.25 (m, 14H, aromatic protons), 10.22 (s, 1H, OH), 12.23 (br. s, 1H, NH); ^{13}CNMR (DMSO – d_6): δ 40.0, 63.9, 124.0, 125.6, 137.0, 128.6, 130.5, 137.4, 121.3, 138.9, 106.6, 138.3, 128.5, 135.4, 118.3, 150.8, 187.5 (C = O): ms (m/z, %): 413.0 (M + , 35%). Anal. Calcd for: $C_{24}H_{19}N_3O_2S$ (413.49): C, 69.71; H, 4.63; N, 10.16; S, 7.75%. Found: C, 69.72; H, 4.66; N, 10.20; S, 7.78%.

Preparation of compounds 10a-c

A solution of **(7a-c)** (0.01 mol) and hydroxylamine hydrochloride in ethanol (30 ml) containing catalytic amount of sodium hydroxide was prepared. The mixture was refluxed and heated for 10-12 h and then filtered hot; the solvent was evaporated and the remaining boiled with petroleum ether (60–80). The residue was poured with ice water and the solid collected and crystalized from ethanol to give (**10a-c**).

3-((5-Phenyl-4, 5-dihydroisoxazol-3-yl) amino)-2*H*-thiochromen-2-one (10a)

(54% yield) as orange crystals. mp 166–168 °C; IR (KBr): ν (cm^{-1}) 1660 (C = O), 3100–3400 (NH); ^{1}H NMR (DMSO – d_6): δ 3.41 (d, 2H, CH_2), 5.96 (t, 1H, CH), 7.25–8.64 (m, 10H, aromatic protons), 12.25 (br. s, 1H, NH); ^{13}C NMR (DMSO – d_6): δ 30.1, 80.8, 124.0, 125.6, 137.0, 128.6, 130.5, 137.4, 121.3, 138.9, 106.6, 138.3, 128.5, 135.4, 118.3, 150.8, 166.0, 187.5: ms (*m/z*, %): 322.0 (M^+, 26%). Anal. Calcd for: $C_{18}H_{14}N_2O_2S$ (322.38): C, 67.06; H, 4.38; N, 8.69; S, 9.95%. Found: C, 67.07; H, 4.42; N, 8.74; S, 9.98%.

3-((5-(4-Chlorophenyl)-4,5-dihydroisoxazol-3-yl) amino)-2*H*-thiochromen-2-one (10b)

(54% yield) as orange crystals. mp 166–168 °C; IR (KBr): ν (cm^{-1}) 1660 (C = O), 3100–3400 (NH); ^{1}H NMR (DMSO – d_6): δ 3.41(d, 2H, CH2), 5.96 (t, 1H, CH), 6.98–8.76 (m, 9H, aromatic protons), 12.25 (br. s, 1H, NH); ^{13}C NMR (DMSO – d_6): δ 30.1, 80.8, 124.0, 125.6, 137.0, 128.6, 130.5, 137.4, 121.3, 138.8, 106.6, 138.34, 128.55, 135.4, 118.3, 150.8, 166.0, 187.5: ms (*m/z*, %): 356.0 (M^+, 16%). Anal. Calcd for: $C_{18}H_{13}ClN_2O_2S$ (356.83): C, 60.59; H, 3.67; Cl, 9.94; N, 7.85; S, 8.99%. Found: C, 60.57; H, 3.60; Cl, 9.90; N, 7.80; S, 8.93%.

3-((5-(4-Hydroxyphenyl)-4, 5-dihydroisoxazol-3-yl) amino)-2*H*-thiochromen-2-one (10c)

(67% yield) as red crystals. mp 146–148 °C; IR (KBr): ν (cm^{-1}) 1660 (C = O), 3100–3400 (NH,OH); ^{1}H NMR (DMSO – d_6): δ 3.41 (d, 2H, CH_2), 5.96 (t, 1H, CH),

6.88–8.67 (m, 9H, aromatic protons), 10.23 (s, 1H, OH), 12.25 (br. s, 1H, NH); ^{13}C NMR (DMSO – d_6): δ 30.1, 80.8, 124.0, 125.6, 137.0, 128.6, 130.5, 137.4, 121.3, 138.9, 106.6, 138.3, 128.5, 135.4, 118.3, 150.8, 166.0, 187.5: ms (*m/z*, %): 338.0 (M^+, 40%). Anal. Calcd for: $C_{18}H_{14}N_2O_3S$ (338.38): C, 63.89; H, 4.17; N, 8.28; S, 9.48%. Found: C, 63.92; H, 4.21; N, 8.33; S, 9.53%.

4-((2-Oxo-2*H*-thiochromen-3-yl)amino)-6-phenyl-5,6-dihydropyrimidin-2(1*H*)-one (17a)

(50% yield) as deep brown crystals. mp 216–218 °C; IR (KBr): ν (cm^{-1}) 1718, 1660 (2C = O), 3100–3400 (2NH); ^{1}H NMR (DMSO – d_6): δ 2.21 (d, 2H, CH_2), 5.95 (t, 1H, CH),7.3–8.1 (m, 10H, aromatic protons), 11.24 (s, 1H, NH), 12.25 (br. s, 1H, NH); ^{13}C NMR (DMSO – d_6): δ 40.2, 45.6, 124.0, 125.6, 137.0, 128.6, 130.5, 137.4, 121.3, 138.6, 106.6, 138.3, 128.5, 135.4, 118.3, 150.8, 156.9, 160.0, 187.5: ms (*m/z*, %): 349.0 (M^+, 25%). Anal. Calcd for: $C_{19}H_{15}N_3O_2S$ (349.41): C, 65.31; H, 4.33; N, 12.03; S, 9.18%. Found: C, 65.38; H, 4.39; N, 12.07; S, 9.22%.

6-(4-Chlorophenyl)-4-((2-oxo-2*H*-thiochromen-3-yl)amino)-5,6-dihydropyrimidin-2(1*H*)-one(17b)

(50% yield) as deep brown crystals. mp 216–218 °C; IR (KBr): ν (cm^{-1}) 1718,1660 (2C = O), 3100–3400 (2NH); ^{1}H NMR (DMSO – d_6): δ 2.21(d, 2H, CH2), 5.95 (t, 1H, CH),7.3–8.1 (m, 10H, aromatic protons), 11.24 (s, 1H, NH), 12.25 (br. s, 1H, NH); ^{13}C NMR (DMSO – d_6): δ 40.2, 45.8, 124.0, 125.6, 137.0, 128.6, 132.5, 137.4, 121.3, 138.8, 106.6, 138.3, 128.5, 135.4, 118.3, 150.8, 156.9, 160.0, 187.5: ms (*m/z*, %): 383.0 (M^+, 20%). Anal. Calcd for: $C_{19}H_{14}ClN_3O_2S$ (383.85): C, 59.45; H, 3.68; Cl, 9.24; N, 10.95; S, 8.35%. Found: C, 59.47; H, 3.72; Cl, 9.29; N, 10.99; S, 8.38%.

6-(4-Hydroxyphenyl)-4-((2-oxo-2*H*-thiochromen-3-yl)amino)-5,6-dihydropyrimidin-2(1*H*)-one (17c)

(50% yield) as deep brown crystals. mp 234–236 °C; IR (KBr): ν (cm^{-1}) 1718, 1660 (2C = O), 3100–3400 (2NH, OH); ^{1}H NMR (DMSO – d_6): δ 2.21 (d, 2H, CH_2), 5.95 (t, 1H, CH), 7.3–8.1 (m, 10H, aromatic protons), 10.22(s, 1H, OH), 11.24 (s, 1H, NH), 12.25 (br. s, 1H, NH); ^{13}C NMR (DMSO – d_6): δ 40.2, 45.8, 124.0, 125.6, 137.0, 128.6, 132.5, 137.4, 121.3, 138.7, 106.6, 138.3, 128.5, 135.4, 118.3, 150.8, 156.8, 160.0, 187.5: ms (*m/z*, %): 365.0 (M^+, 28%). Anal. Calcd for: C19H15N3O3S (365.41): C, 62.45; H, 4.14; N, 11.50; S, 8.78%. Found: C, 62.46; H, 4.19; N, 11.56; S, 8.80%.

3-((6-Phenyl-2-thioxo-1,2,5,6-tetrahydropyrimidin-4-yl)amino)-2*H*-thiochromen-2-one(18a)

(68% yield) as yellowish crystals. mp 233–235 °C; IR (KBr): ν (cm^{-1}) 1660 (2C = O), 3100–3400 (2NH); ^{1}H NMR (DMSO – d_6): δ 2.11(d,2H,CH2),4.23(t,1H,CH),7.3–8.1 (m, 10H, aromatic protons), 11.24(s, 1H, NH), 12.25 (br. s, 1H, NH); ^{13}C NMR (DMSO – d_6): δ 40.2, 54.1, 124.0, 125.6, 137.0, 128.6, 130.5, 137.4, 121.3, 138.9, 106.6, 138.3, 128.5, 135.4, 118.3, 150.8, 156.8, 160.0, 187.5: ms (*m/z*, %): 365.0 (M^+, 38%). Anal. Calcd for: $C_{19}H_{15}N_3OS_2$ (365.47): C, 62.44; H, 4.14; N, 11.50; O, 4.38; S, 17.55%. Found: C, 62.47; H, 4.15; N, 11.56; O, 4.42; S, 17.58%.

3-((6-(4-chlorophenyl)-2-thioxo-1,2,5,6-tetrahydropyrimidin-4-yl)amino)-2H-thiochromen-2-one(18b)

(50% yield) as deep brown crystals. mp 216–218 °C; IR (KBr): ν (cm^{-1}) 1718, 1660 (2C = O), 3100–3400 (2NH); ^{1}H NMR (DMSO – d_6): δ 2.21 (d, 2H, CH_2), 5.95 (t, 1H, CH), 7.3–8.1 (m, 10H, aromatic protons), 11.24 (s, 1H, NH), 12.25 (br. s, 1H, NH); ^{13}C NMR (DMSO – d_6): δ 40.2, 45.8, 124.0, 125.6, 137.0, 128.6, 132.5, 137.4, 121.3, 138.8, 106.6, 138.3, 128.5, 135.4, 118.3, 150.8, 156.9, 160.0, 187.5: ms (*m/z*, %): 383.0 (M^+, 20%). Anal. Calcd for: $C_{19}H_{14}ClN_3O_2S$ (383.85): C, 59.45; H, 3.68; Cl, 9.24; N, 10.95; S, 8.35%. Found: C, 59.47; H, 3.72; Cl, 9.29; N, 10.99; S, 8.38%.

3-((6-(4-Hydroxyphenyl)-2-thioxo-1,2,5,6-tetrahydropyrimidin-4-yl)amino)-2H-thiochromen-2-one (18c)

(60% yield) as Reddish brown crystals. mp 225–227 °C; IR (KBr): ν (cm^{-1}) 1660 (C = O), 3100–3400 (2NH, OH); ^{1}H NMR (DMSO – d_6): δ 2.22 (d, 2H, CH2), 5.97 (t, 1H, CH), 7.3–8.1 (m, 10H, aromatic protons), 10.23 (s, 1H, OH), 11.24 (s, 1H, NH), 12.25 (br. s, 1H, NH); ^{13}C NMR (DMSO – d_6): δ 40.2, 45.8, 124.0, 125.6, 137.0, 128.6, 132.5, 137.4, 121.3, 138.9, 106.6, 138.3, 128.5, 135.4, 118.3, 150.8, 156.9, 160.0: ms (*m/z*, %): 383.0 (M^+, 20%). Anal. Calcd for: $C_{19}H_{15}N_3O_2S_2$ (381.47): C, 59.82; H, 3.96; N, 11.02; S, 16.81%. Found: C, 59.85; H, 3.98; N, 11.06; S, 16.84%.

Antimicrobial potential

Zone of inhibition technique is adapted for estimating antimicrobial potential for the target compounds against *P. aeruginosa*, *B. subtilis*, *S. aureus* and *E. coli* as examples of

bacterial species besides, *C. albicans* and *A. flavus* as used fungal strains [31].

Docking study

In silico docking study had been carried out using molecular operating environment (MOE, version 2005.6, Canada). The isolated crystal structures of DHPS and protease enzyme active sites were obtained from protein data bank (4DAI). Chemical structures of the novel candidates were built by MOE builder and minimized by force field MMFF94x. Docking of the cocrystallized ligand was performed to get its root mean standard deviation (RMSD), energy score and interactions with the amino acids. Preparing of the target candidates for docking was done through their 3D structure built by MOE. Some procedures were done before docking including 3D protonation of the structure, running conformational analysis and choosing the conformer of least energy and adapting the same docking protocol used with the ligand. The outcomes were obtained from docking study such as binding score, hydrogen bond numbers, binding groups and distance from aminoacids (Table 2).

Conclusion

In conclusion, we constructed novel derivatives of thiochromenes mixed with other heterocycle such as pyrazole **6, 8a-c** and **9a-c** and oxazole **10a-c**, pyrimidine **11a-c** and/or thiopyrimidine **12a-c**. All the thiochromene candidates were screened for their antibacterial potential towards *B. subtilis*, *S. aureus*, *E. coli* and *P. aeruginosa* and their antifungal activity against *A. flavus* and *C. albicans*. From the obtained results, thiochromene derivatives **8c** (ZI = 24 mm), **9b** (ZI = 23 mm) and **10b** (ZI = 24 mm) exhibited antibacterial potential towards *E. coli* similar to that shown by ampicillin (ZI = 25 mm). Regarding the antimicrobial activity towards *S. aureus*, compound **8c** (ZI = 19 mm) was the most active followed by **7b** (ZI = 17 mm) and then thiochromenes **4**, **7c**, **11a** and **11c** (ZI = 14 mm). All the tested compounds showed antifungal activity against *A. flavus* (ZI = 9–16 mm) except compound 4, while the antifungal potential against *C. albicans* revealed that all compounds recorded moderate to high antifungal potential (ZI range = 8–19 mm) except thioxopyrazolothiochromene derivative **6** which did not exhibit any effect. In silico docking study had been performed within dihydropteroate synthase (DHPS) to predict the binding mode of the novel compounds. The outcomes of this study displayed the ability of these compounds to bind with DHPS; in particular, compound **8c** recorded excellent fitting within the enzyme forming three hydrogen bonds with Asp101, Arg254 and Ser218 amino acids.

In silico docking study was carried out inside dihydropteroate synthase enzyme. Compound **8c** recorded the best binding energy score (−5.47 kcal/mol) forming good fitting within DHPS active site.

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