Abstract

Background: Continuing our interest in preparing of new heterocyclic compounds and examining their various biological activities, this work was designed to prepare new condensed and non-condensed heterocyclic compounds 9a-c, 10a-c, 11a-c, 13a-c and 14a-c were synthesized starting with pyrimidine-2-thiones 4a-c.

Results: Thiazolo[3,2-a]pyrimidines 9a-c were synthesized by S-alkylation of pyrimidine-2-thiones, 4a-c, internal cyclization in alkaline medium with ammonia, condensation with benzaldehyde and finally reaction with hydroxylamine hydrochloride.[1,2,4]thiadiazolo[4,5-a]pyrimidines 11a-c were formed by heating of the 4a-c with benzoylcholride to afford 10a-c followed by reaction with sodium hypochlorite, ammonia and sodium hydroxide. Cyclocondensation of 4a-c with ethyl acetoacetate or formic acid yielded pyrazol-3-ones 13a-c or [1,2,4] triazolo[4,3-a]pyrimidines 14a-c, respectively Elements analysis, IR, 1H-NMR, 13C-NMR and mass spectra were used to validate the structures of newly synthesized heterocycles. Screening of the selected compounds 4a, 6a, 7a, 9a, 10a, 13a and 14a against colon carcinoma cell lines (HCT-116) and hepatocellular carcinoma cell lines (HepG-2).

Conclusions: Elements analysis, IR, 1H-NMR, 13C-NMR and mass spectra were used to validate the structures of newly synthesized heterocycles. Screening of the selected compounds 4a, 6a, 7a, 9a, 10a, 13a and 14a against colon carcinoma cell lines (HCT-116) and hepatocellular carcinoma cell lines (HepG-2) showed that compound 10a exhibited the most cytotoxic, while compounds 4a, 6a and 14a exhibited considerable cytotoxic activity.

Keywords: Pyrimidine-2-thiones, [1,2,4]thiadiazolo[4,5-a], Pyrazol-3-ones, [1, 2, 4] triazolo[4,3-a], HCT-116; HepG-2

Introduction

Continuing our interest in preparing of new heterocyclic compounds and examining their various biological activities [1–6], this work was designed to prepare new derivatives of condensed and non-condensed five-membered rings with pyrimidine. Pyrimidine derivatives have aroused the interest of researchers in recent years, as they have demonstrated a wide variety of biological activities such as antibacterial [6], antiallergic, antihypertensive [7] and antitumor activity [5, 8], along with their cardio-pulmonary and bronchodilating effect [9]. It has been observed that the substitution of the benzene ring in pyrimidine derivatives with heterocyclic moieties such as pyrrole and thiophene shows some biological activities such as anti-proliferative and anti-inflammatory activities [10–12]. In addition, the pyrazolopyrimidine and [1, 2,4]triazolopyrimidine derivatives have antimicrobial, antioxidant, antimalarial, analgesic and antitumor activities [13–17]. Most classes of heterocyclic compound have been studied to show their role as strong and chelating ligands with most of transition metals as electron rich sites [1, 18, 19], this point is important in forming novel metal-complexes to be used in different industrial,
pharmaceutical and medicinal applications. This study aimed to synthesize and investigate a new heterocyclic class that has an important role in biological behavior based on its structure.

**Results and discussion**

**Chemistry**

The reaction of 2-acetyl-1-methylpyrrole 1 with a series of 5-substituted-thiophene-2-carbaldehyde 2a-c in alcoholic sodium hydroxide afforded a new series of chalcones 3a-c as shown in Scheme 1 [20]. Melting points, yield % and IR spectral data of compounds 3a-c are included in the Additional file 1. The known 3,4-dihydro-1H-pyrimidine-2-thiones 4a-c nuclei taken as the key synthons for this work were synthesized by cyclcondensation of chalcones 3a-c with thiourea in the presence of alcoholic potassium hydroxide, Scheme 1 [21]. The structure of compounds 4a-c was established by their elemental analysis data and their IR spectra which showed two characteristic bands at ύ (3364–3394) and (3215–3275) cm⁻¹ for the two NH groups. The 1H-NMR spectra of compounds 4a-c indicated the chemical shifts (δ) at (3.36–3.56) corresponding to the protons of NCH₃, (4.76–4.94) for H-4 of pyrimidine, (6.08–6.13) for H-5 of pyrimidine, (6.87–7.56) for aromatic protons of pyrrole, (7.51–8.19) for aromatic protons of thiophene and two D₂O exchangeable singlet peaks at (8.89–10.08) ppm for 2NH groups. The 13C-NMR of compound 4a indicated a group of signals at 39.57 for NCH₃, 65.37 for C-4 of pyrimidine, 108.30 for C-5 of pyrimidine and a characteristic signal at 176.13 ppm for C=S group, (for more details see the experimental section).

S-alkylation, instead of N-alkylation was performed by heating 3,4-dihydro-1H-pyrimidine-2-thione 4a-c with ethyl chloroacetate to produce ethyl 1,6-dihydro-pyrimidin-2-ylsulfanylacetate 6a-c, Scheme 2 [22]. The structure of the compounds 6a-c was mainly confirmed from the 13C-NMR spectrum of compound 6a which showed two characteristic signals at 163.74 and 169.87 ppm for C=N and C=O with absence of C=S group signal. The IR spectra of the compounds 6a-c exhibited stretching bands at (3222–3271) and (1722–1739) cm⁻¹ for NH and C=O groups. The 1H-NMR of the compounds 6a-c contained a set of peaks for ethyl, NCH₃, SCH₂, pyrimidine, pyrrole, thiophene and NH protons, (for more details see the experimental section). The internal cyclization of dihydropyrimidine esters 6a-c took place in an alkaline medium using ammonia affording the corresponding thiazolo[3,2-a]pyrimidin-3-ones 7a-c. The structure of the compounds 7a-c was confirmed by the disappearance of the NH signals in both the IR and 1H-NMR spectra of these compounds, along with the disappearance of ethyl protons in the 1H-NMR spectra compared to those in the compounds 6a-c. In order to build up a fused heterocyclic to the compounds 7a-c, the compounds 7a-c were condensed with benzaldehyde in the presence of freshly prepared sodium acetate to give the corresponding 2-Benzylidenethiazolo[3,2-a]pyrimidin-3-ones 8a-c. Heating under reflux of the compounds 8a-c with hydroxylamine hydrochloride in the presence of freshly prepared sodium acetate yielded the corresponding isoxazolo[5′,4′:4,5]thiazolo[3,2-a]pyrimidine 9a-c [23]. The mass spectrum of 8-(5-Chloro-thiophen-2-yl)-6-(1-methyl-1H-pyrrol-2-yl)-3-phenyl-2,3-dihydro-8H-isoxazolo[5′,4′:4,5]thiazolo[3,2-a]pyrimidine 9c has
Scheme 2  Scheme of preparation of compounds 6a-c, 7a-c, 8a-c and 9a-c
molecular ion peaks at 452 and 454 with in a ratio of 3:1 which is consistent with the molecular formula and the existence of chlorine isotopes of compound 9c. Also the spectral data of the compounds 9a-c indicated the presence of NH group at (3207–3233) cm\(^{-1}\) and (9.98–10.73) ppm for the IR and \(^{1}\)H-NMR spectra, respectively.

The second path way of this work was heating the key synthons 4a-c under reflux with benzoyl chloride and a few drops of triethylamine to provide compounds 10a-c, Scheme 3. The structure of the compounds 10a-c was elucidated by their correct elemental analysis and spectral data, where the IR spectra showed two characteristic bands at (3224–3363) and (1682–1697) cm\(^{-1}\) for the NH and C=O groups, respectively. \(^{1}\)H-NMR spectra of the compounds 10a-c showed a characteristic signal at (10.98–11.42) ppm for NH group, along with two characteristic signals at 169.55 and 181.16 ppm for C=O and C=S groups in the \(^{13}\)C-NMR spectrum of the compound 10a. The reaction of the compounds 10a-c with sodium hypochlorite, ammonia and sodium hydroxide passed through the formation of non-isolate intermediates sulphenyl chloride and sulphenamide which underwent an intramolecular dehydration to produce the corresponding [1, 2, 4]thiadiazolo[4,5-a]pyrimidine 11a-c, as shown in Scheme 3 [24]. The elemental analysis of the compounds 11a-c is consistent with their molecular formula. IR spectra indicated the disappearance of the C=O groups, and the \(^{13}\)C-NMR spectrum of the product 11a also showed the disappearance of the C=S group with two new signals appearing at 150.11 and 156.34 ppm for the two C=N groups which suggesting the formation of thiadiazole ring.

The third part of this work was designed to synthesise the non-condensed system namely: pyrimidopyrazol-3-ones 13a-c and fused heterocycles compounds triazolo[4,3-a]pyrimidines 14a-c. Hydrazinolysis of pyrimidine-2-thiones 4a-c with hydrazine hydrate under reflux gave the corresponding hydrazino compounds 12a-c, Scheme 4. The structures of 12a-c were established on the basis of their elemental analysis and spectral data, the IR spectra of the compounds 12a-c showed absorption bands at (3376–3173) cm\(^{-1}\) for NH2 and NH groups. \(^{1}\)H-NMR spectra indicated \(D_{2}O\) exchangeable singlet signals at (4.46–4.65), (8.79–9.23) and (9.85–10.44) ppm corresponding to the NH2, NH of pyrimidine and NH of hydrazine, respectively. Cyclocondensation of the hydrazinopyrimidine compounds 12a-c with ethyl acetoacetate in acetic acid gave the corresponding [pyrimidin-2-yl]-2,4-dihydro-pyrazol-3-ones 13a-c. IR spectra of compounds 13a-c revealed absorption bands at (3178–3208) cm\(^{-1}\) for NH group and at (1683–1691) cm\(^{-1}\) for C=O group. The low frequency of the C=O group in the IR spectra for the compounds 13a-c is due to internal H-bonding as shown in Fig. 1 leads to C=O lengthening and as a result the C=O frequency decreases. \(^{1}\)H-NMR spectra of the compounds 13a-c indicated three singlet signals at (1.75–1.89), (2.73–3.09) and (3.36–3.51) ppm for pyrazolyl CH\(_{3}\), pyrazolyl CH\(_{2}\) and NCH\(_{3}\), respectively along with \(D_{2}O\) exchangeable singlet signals at (9.38–10.51) ppm corresponding to the NH of pyrimidine. Finally cyclocondensation of 12a-c with formic acide gave the corresponding [1, 2, 4]triazolo[4,3-a]pyrimidine 14a-c, the chemical structure of the compounds 14a-c was suggested by their elemental analysis and through the disappearance of the NH and NH\(_{2}\) signals in both \(^{1}\)H-NMR spectra of 12a-c.

**Anticancer activity**

**Anticancer activity discussion**

In this study, selected compounds 4a, 6a, 7a, 9a, 10a, 13a and 14a were tested for potential cytotoxicity using the Mossman [25], Gangadevi and Muthumary [26] methods for anticancer activity against colon carcinoma cells lines (HCT-116) and hepatocellular carcinoma cells lines (HepG-2) using Vinblastine drug as standard. Data on antitumor activity were represented by the cytotoxic effect of the selected compounds. The inhibitory activities of the tested compounds against colon carcinoma cells (HCT-116) and hepatocellular carcinoma cells lines (HepG-2) were calculated by dissolving the selected compounds in DMSO and diluting with saline to appropriate volume using different concentrations of the samples (50, 25, 12.5, 6.25, 3.125 and 1.56 µg mL\(^{-1}\)), and the cell viability (percent) of the studied compounds was determined using a colorimetric technique, Tables 1, 2. From Tables 1, 2, inhibitory concentration fifty (IC\(_{50}\)) which corresponds to the concentration necessary for 50% inhabitation of cell viability was calculated, Table 3. Screening of the selected compounds against human colon carcinoma cancer cell lines and hepatocellular carcinoma cells lines revealed that the compound 2-thioxo-3,6-dihydro-2H-pyrimidin-1-yl]-phenyl-methanone 10a was the most active among the group of selected compounds with IC\(_{50}\) (10.72 and 18.95) µM in both human colon carcinoma cancer cell lines and hepatocellular carcinoma cells lines, respectively, Table 3, Figs. 2, 3. Meanwhlie, compounds 4a, 6a and 14a exhibited considerable cytotoxic action with IC\(_{50}\) values ranging from (20.88–31.92) µM in human colon carcinoma cancer cell lines and from (35.22–42.63) µM in hepatocellular carcinoma cells lines. Furthermore, compounds 7a, 9a and 13a were showed weak cytotoxic action with IC\(_{50}\) ranging from (38.32–54.01) µM in human colon carcinoma cancer cell lines and from (56.55–86.33) µM µg in hepatocellular carcinoma cells lines. The data showed that compounds containing a thio group (C=S) such as compounds 4a
Scheme 3  Scheme of preparation of compounds 10a-c and 11a-c

- 4a-c
- 10a-c
- 11a-c

a: X = H,  b: X = CH₃,  c: X = Cl
and 10a exhibit the highest cytotoxic activity and this activity increase with the inclusion of a polar group such as the carbonyl group (C=O). The IC$_{50}$ values also show that increased toxicity necessitates larger doses in the case of hepatocellular carcinoma cell lines compared to human colon carcinoma cancer cell lines. As a result, we recommended that the synthesized compound, particularly 2-thioxo-3,6-dihydro-2H-pyrimidin-1-yl]-phenyl-methanone 10a, be used in the formulation of antibiotics as drugs to increase the sensitivity of antibiotics that stimulate cancer treatment and cause apoptosis in human colon carcinoma.

**In vitro studies**

Human colon cancer (HCT-116) cells and hepatocellular carcinoma (HepG-2) cell lines were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). The cells were cultured in RPMI-1640 media.
supplemented with 10% inactivated fetal calf serum and 50 g/mL gentamycin. The cells were kept in a humid environment with 5% CO₂, 37 °C, and sub-cultured two to three times. Cytotoxic tests on the selected compounds 4a, 6a, 7a, 9a, 10a, 13a and 14a: Monolayers of 10,000 cells adhered to the bottom of wells in a 96-well microtiter plate cultured for 24 h at 37 °C in a humidified incubator with 5% CO₂. The monolayers were then rinsed with sterile phosphate buffered saline (0.01 M pH 7.2), and the cells were incubated at 37 °C with 100 µL of various dilutions of the tested compounds or Vinblastine drug as a control. Six wells were utilized for each concentration of the tested compound, whereas control cells were produced in the absence of the tested compounds. Every 24 h, the observation was performed under an inverted microscope, and the number of (viable) surviving cells was counted by coloring cells with crystal violet, followed by cell lysis with glacial acetic acid (33%), and recording the absorbance at 495 nm, taking into account that the absorption of the untreated cell is 100%.

The following Eq. (1) was used to compute the percentage of cell viability.

\[
\text{Cell viability} \% = \left(1 - \frac{OD_t}{OD_c}\right) \times 100
\]

where ODt denotes the mean optical density of test compound-treated wells and ODc denotes the mean optical density of untreated (control) cells. The 50% inhibitory concentration (IC₅₀), which is the concentration required

| Sample conc. (µg ml⁻¹) | Cell viability (%) |
|------------------------|-------------------|
|                        | 4a    | 6a    | 7a    | 9a    | 10a   | 13a   | 14a   | Vinblastine standard |
|------------------------|-------|-------|-------|-------|-------|-------|-------|----------------------|
| 50.000                 | 16.38 | 26.18 | 29.78 | 32.08 | 14.27 | 35.18 | 21.73 | 12.98                |
| 25.000                 | 19.51 | 37.22 | 41.05 | 43.03 | 17.01 | 44.91 | 27.15 | 16.08                |
| 12.500                 | 27.13 | 46.34 | 50.68 | 52.76 | 23.99 | 54.45 | 37.72 | 20.74                |
| 6.250                  | 48.44 | 70.17 | 74.29 | 77.85 | 44.13 | 59.90 | 52.89 | 39.34                |
| 3.125                  | 58.63 | 85.23 | 83.55 | 85.49 | 52.47 | 88.11 | 63.31 | 48.60                |
| 1.560                  | 75.93 | 91.02 | 94.47 | 96.11 | 66.74 | 98.22 | 80.84 | 59.31                |
| 0.000 (DMSO)           | 100.00| 100.00| 100.00| 100.00| 100.00| 100.00| 100.00| 100.00               |

| Table 2 Evaluation of cytotoxicity of some chosen synthesized compounds against hepatocellular carcinoma cells lines (HepG-2) |
|---------------------------------------------------------------|
| Sample conc. (µg ml⁻¹) | Cell viability (%) |
|------------------------|-------------------|
|                        | 4a    | 6a    | 7a    | 9a    | 10a   | 13a   | 14a   | Vinblastine standard |
|------------------------|-------|-------|-------|-------|-------|-------|-------|----------------------|
| 50.000                 | 23.34 | 30.79 | 34.92 | 37.26 | 19.85 | 39.61 | 28.60 | 15.12                |
| 25.000                 | 32.67 | 40.42 | 43.43 | 48.67 | 24.57 | 52.16 | 39.09 | 17.33                |
| 12.500                 | 41.82 | 52.87 | 56.33 | 61.15 | 35.87 | 66.03 | 44.88 | 24.18                |
| 6.250                  | 60.09 | 69.32 | 61.38 | 68.71 | 52.49 | 73.52 | 62.94 | 46.87                |
| 3.125                  | 72.57 | 84.17 | 86.89 | 88.34 | 61.22 | 89.56 | 76.14 | 55.18                |
| 1.560                  | 86.53 | 94.37 | 97.09 | 98.03 | 78.86 | 99.12 | 88.25 | 73.98                |
| 0.000 (DMSO)           | 100.00| 100.00| 100.00| 100.00| 100.00| 100.00| 100.00| 100.00               |
Experimental

Materials and methods

The prepared compounds’ melting points are uncorrected and were determined with MEL TEMP II equipment. A Perkin-Elmer FTIR spectrophotometer was used to record the IR spectra (KBr). The NMR spectrum, including 1H NMR and 13C NMR, was registered on a Bruker spectrometer (400 MHz for 1H NMR and 100 MHz for 13C NMR) in DMSO-d6 as solvent using tetramethyl-silane (TMS) as internal reference standard. Chemical shift values are expressed in parts per million (ppm) and are abbreviated as follows: (s) for singlet signals, (d) for doublet signals, (t) for triplet signals and (m) for multiplet signals. The NMR spectra were obtained at Kafr Elsheikh University’s Faculty of Science. Elements microanalyses were carried out at El-azhr University’s Micro Analytical Center. At Cairo University’s Micro Analytical Unit, mass spectra were collected using a DI analysis Shimadzu QP-2010 plus mass spectrometer. TLC analytical silica gel plate 60 F254 was used to track the success of the chemical reaction and the purity of the compounds.

Chemistry

General method for synthesis of chalcone 3a-c

3-acetyl-1-methylpyrrole 1 (10 mmol, 1.23 g) was added dropwise to 100 mL of 60% aqueous ethanol solution of sodium hydroxide (30 mmol, 1.20 g) in an ice bath with stirring for 30 min. The 5-substituted thiophene-2-carbaldehyde 2a-c (10 mmol) was added dropwise over 15 min followed by stirring for 3 h in the ice bath. The reaction mixture was left overnight in a refrigerator, the separated solid was filtered, washed with water, dried and recrystallized from ethanol to give the corresponding 1-(1-Methyl-1H-pyrrol-2-yl)-3-(5-substituted-thiophen-2-yl)-propen- one 3a-c. Melting points, yield % and IR spectral data of compounds 3a-c were collected in Table 1.

General method for synthesis of 6-(1-Methyl-1H-pyrrol-2-yl)-4-(5-substituted-thiophen-2-yl)-3,4-dihydro-1H-pyrimidine-2-thione 4a-c

A mixture of chalcone 3a-c (10 mmol), thiourea (0.76 g, 10 mmol) and potassium hydroxide (0.85 g, 15 mmol) was heated in 50 mL of absolute ethanol under reflux for 7 h. The reaction mixture was allowed to cool, neutralized with diluted hydrochloric acid, filtrated and washed with water and then the product recrystallized from ethanol to give the corresponding 4a-c compounds.

| Compounds | IC50 (μM) |
|-----------|----------|
|           | HCT-116  |
| 4a        | 20.88 ± 1.88 |
| 6a        | 31.92 ± 3.67 |
| 7a        | 42.42 ± 5.54 |
| 9a        | 38.32 ± 4.01 |
| 10a       | 10.72 ± 0.83 |
| 13a       | 50.01 ± 6.09 |
| 14a       | 26.26 ± 3.09 |
| Vinblastine standard | 3.61 ± 0.43 |

**Table 3** IC50 (μM) of some chosen synthesized compounds on colon carcinoma cells (HCT-116) and hepatocellular carcinoma cells lines (HepG-2)

**Fig. 2** Represent a comparison of IC50 (μM) for compounds 4a, 6a, 7a, 9a, 10a, 13a, 14a and Vinblastine against colon carcinoma cells (HCT-116)

**Fig. 3** Represent a comparison of IC50 (μM) for compounds 4a, 6a, 7a, 9a, 10a, 13a, 14a and Vinblastine against hepatocellular carcinoma cells lines (HepG-2)
DMSO-d6) δ 3.42 (S, 3H, NCH3), 4.76 (d, 1H, H-4 of pyrimidine, J = 7.7 Hz), 6.33–7.31 (m, 7H, Ar–H protons), 8.89 (s, br, H, 1NH, D2O exchangeable), 9.78 ppm (br, 1H, NH, D2O exchangeable). 13C NMR (100 MHz, DMSO-d6) δ 39.57 (NCH3), 65.37 (C-4 of pyrimidine), 108.30 (C-5 of pyrimidine), 119.86, 120.15, 124.58, 127.12, 128.66, 130.18 (8 C of aryl carbons), 141.18 (C-6 of pyrimidine), 176.13 ppm (C=S). Anal. Calcd for C13H13N3S2Cl (309.84): C, 50.35; H, 3.87; N, 13.56. Found: C, 50.21; H, 3.90; N, 13.48.

**General method for synthesis of Ethyl [4-(1-Methyl-1H-pyrrol-2-yl)-6-(5-substituted-thiophen-2-yl)-1,6-dihydro-pyrimidin-2-ylsulfanyl]acetate 6a-c**

A solution of 3,4-dihydro-1H-pyrimidine-2-thione 4a-c (5 mmol) and 8 mL of ethyl chloroacetate was heated in 50 mL of absolute ethanol on a water bath for 6 h. The reaction mixture was cooled, neutralized, filtrated and washed with ethyl acetate and then the product was recrystallized from ethanol to give the corresponding ethyl ester 6a-c.

**Ethyl[4-(1-Methyl-1H-pyrrol-2-yl)-6-(5-chlorothiophen-2-yl)-1,6-dihydro-pyrimidin-2-ylsulfanyl]acetate 6a**

Applying the previous general preparation method pale yellow powder was obtained. Yield (1.34 g, 74%); mp (138–140 °C); IR (KBr) νmax: 3222 (NH), 1722 (C=O), 1631 (C=N), 1571 cm⁻¹ (C=C); ¹H NMR (400 MHz, DMSO-d6) δ 1.27 (t, 3H, CH3 of ethyl group, J = 6.9 Hz), 3.48 (s, 3H, NCH3), 3.91 (q, 2H, OCH₂J = 7.1 Hz), 4.15 (s, 2H, SCH2) 4.93 (d, 1H, H-4 of pyrimidine), 6.27 (d, 1H, H-5 of pyrimidine, J = 6.0 Hz), 6.84 (d, 1H, H-3 of pyrrole, J = 5.6 Hz), 7.18 (dd, 1H, H-4 of pyrrole), 7.28 (d, 1H, H-5 of pyrrole, J = 6.9 Hz), 7.47 (d, 1H, H-3 of thiophene, J = 5.18 Hz), 7.73 (dd, 1H, H-4 of thiophene, 8.04 (d, 1H, H-5 of thiophene, J = 6.1 Hz), 8.58 ppm (br, 1H, NH, D2O exchangeable). ¹³C NMR (100 MHz, DMSO-d6) δ 41.42 (NCH3), 46.52 (SCH2), 65.17 (C-4 of pyrimidine), 120.09, 123.51, 126.11, 128.69 (4 C of pyrrole) 136.13, 138.21, 139.79, 142.32 (4 C of thiophene), 163.74 (C=O). Anal. Calcd for C18H19N3O2S2 (361.48): C, 56.43; H, 5.18; N, 14.51. Found: C, 58.11; H, 5.14; N, 14.43.

**Ethyl [4-(1-Methyl-1H-pyrrol-2-yl)-6-(5-thiophen-2-yl)-1,6-dihydro-pyrimidin-2-ylsulfanyl]acetate 6b**

Yellow powder was obtained according to the previous general procedure. Yield (1.46 g, 78%); mp (147–149 °C); IR (KBr) νmax: 3262 (NH), 1739 (C=O), 1624 (C=O), 1602 cm⁻¹ (C=C); ¹H NMR (400 MHz, DMSO-d6) δ 1.34 (t, 3H, CH3 of ethyl group, J = 6.5 Hz), 2.46 (s, 3H, CH3 of thiophene), 3.58 (s, 3H, NCH3), 4.04 (q, 2H, OCH₂J = 7.1 Hz), 4.23 (s, 2H, SCH2) 4.99 (d, 1H, H-4 of pyrimidine), 6.61 (s, 1H, H-5 of pyrimidine, J = 6.1 Hz), 6.93 (d, 1H, H-3 of pyrrole, J = 5.3 Hz), 7.1 (dd, 1H, H-4 of pyrrole), 7.23 (d, 1H, H-5 of pyrrole, J = 7.0 Hz), 7.38 (d, 1H, H-3 of thiophene, J = 5.3 Hz), 7.89 (d, 1H, H-4 of thiophene, J = 6.3 Hz), 8.48 ppm (br, 1H, NH, D2O exchangeable). Anal. Calcd for C18H21N3O2S2 (375.51): C, 57.52; H, 5.60; N, 11.18. Found: C, 57.43; H, 5.55; N, 11.13.

**Ethyl [6-(5-Chlorothiophen-2-yl)-4-(1-methyl-1H-pyrrol-2-yl)-3,4-dihydro-1H-pyrimidine-2-thione 4c**

After recrystallization according to the previous general procedure, pale orange powder was obtained. Yield (1.62 g, 82%); mp (157–157 °C); IR (KBr) νmax: 3271 (NH), 1732 (C=O), 1624(=C=N), (1601–1603) cm⁻¹ (C=C); ¹H NMR (400 MHz, DMSO-d6) δ 1.18 (t, 3H, CH3 of ethyl group, J = 7.7 Hz), 2.46 (s, 2H, OCH₂J = 7.1 Hz), 4.23 (s, 2H, SCH2), 4.99 (d, 1H, H-4 of pyrimidine), 6.61 (s, 1H, H-5 of pyrimidine, J = 6.1 Hz), 6.93 (d, 1H, H-3 of pyrrole, J = 5.3 Hz), 7.1 (dd, 1H, H-4 of pyrrole), 7.23 (d, 1H, H-5 of pyrrole, J = 7.0 Hz), 7.38 (d, 1H, H-3 of thiophene, J = 5.3 Hz), 7.89 (d, 1H, H-4 of thiophene, J = 6.3 Hz), 8.48 ppm (br, 1H, NH, D2O exchangeable). Anal. Calcd for C18H21N3O2S2 (375.51): C, 57.52; H, 5.60; N, 11.18. Found: C, 57.43; H, 5.55; N, 11.13.
able). Anal. Calcd for C_{17}H_{18}N_{3}O_{2}S_{2}Cl (395.93): C, 51.52; H, 4.54; N, 10.61. Found: C, 51.47; H, 4.49; N, 10.57.

**General method for synthesis of 7-(1-Methyl-1H-pyrrol-2-yl)-5-(5-substitued-thiophen-2-yl)-5H-thiazolo[3,2-a]pyrimidin-3-one 7a-c**

A solution of ethyl dihydro-pyrimidin-2-yl sulfan-yl-acetate 6a-c (5 mmol) in 20 mL of absolute ethanol was treated with ammonia until alkaline (pH > 7) with stirring for 30 min at room temperature. The reaction mixture was allowed to evaporate, the residue was washed with water, dried and recrystallized from n-hexane/ethanol to give thiazolo[3,2-a]pyrimidin-3-one 7a-c at good yields.

7-(1-Methyl-1H-pyrrol-2-yl)-5-thiophene), 7.89 ppm (d, 1H, H-5 of thiophene, J = 7.8 Hz), 6.87 (d, 1H, H-3 of pyrrole, J = 6.9 Hz), 7.31 (dd, 1H, H-4 of pyrrole), 7.39 (d, 1H, H-5 of pyrrole, J = 6.9 Hz), 7.73 (d, 1H, H-3 of thiophene, J = 7.3 Hz), 8.09 ppm (d, 1H, H-4 of thiophene, J = 7.6 Hz). (m/z): 349 (M', 349, M + 2', 351) (3:1) ratio. Anal. Calcd for C_{13}H_{12}N_{2}O_{2}S_{2}Cl (349.86): C, 51.45; H, 3.43; N, 12.00. Found: C, 51.39; H, 3.40; N, 11.89.

**General method for synthesis of 2-Benzylidene-7-(1-methyl-1H-pyrrol-2-yl)-5-(5-substitued-thiophen-2-yl)-5H-thiazolo[3,2-a]pyrimidin-3-one 8a-c**

A solution of 5H-thiazolo[3,2-a]pyrimidin-3-one 7a-c (5 mmol), benzaldehyde (0.53 g, 5 mmol), and freshly prepared sodium acetate (0.41 g, 5 mmol) in 20 mL of glacial acetic acid-acetic anhydride mixture (1:1) was heated under reflux for 5 h. The reaction mixture was left to cool down and poured into ice water, filtered and recrystallized from n-hexane/ethanol to give the corresponding compounds 7a-c at good yields.
2 - Benzylidene - 5 - ( 5 - chloro - thio - phen - 2 - yl ) - 7 - ( 1 - methyl - 1H - pyr - rol - 2 - yl ) - 5H-thiazolo[3,2-a]pyrimidine 9b

73%); mp (177–179 °C); IR (KBr) ν max: 3207 (NH), 1627 (3:1) ratio. Anal. Calcd for C22H16N3OS2 Cl (437.97): C, 60.28; H, 3.65; N, 9.59. Found: C, 60.19; H, 3.58; N, 9.53.

General method for synthesis of 6-(1-methyl-1H-pyrrol-2-yl)-8-(5-substituted-thiophen-2-yl)-3-phenyl-2,3-dihydro-8H-isoxazolo[5',4',5'-thiazolo[3,2-a]pyrimidine 9a-c

A solution of compound 7a-c (5 mmol), hydroxylamine hydrochloride (0.35 g, 5 mmol), and freshly prepared sodium acetate (0.41 g, 5 mmol) in 20 mL of glacial acetic acid was heated under reflux for 8 h. The reaction mixture was left to cool down and poured into ice water, filtered and recrystallized from ethyl acetate to give the corresponding compounds 9a-c at good yields.

6 - (1 - methyl - 1H - pyrrol - 2 - yl ) - 3 - phenyl - 8-(thiophen-2-yl)-2,3-dihydro-8H-isoxazolo[5',4',5'-thiazolo[3,2-a]pyrimidine 9a

After applying the previous procedure a pale yellow powder was obtained. Yield (1.46 g, 70%); mp (172–174 °C); IR (KBr) ν max: 3225 (NH), 1617 (C=O), 1595 cm−1 (C=C); 1H NMR (400 MHz, DMSO-d6) δ 3.38 (3H, 6.68 (d, 1H, H-5 of pyrimidine), 6.70 (d, 1H, H-4 of pyrimidine), 2.19 (s, 3H, CH3 of thiophene), 4.10 ppm (m, 3H of pyrrole). (418.59): C, 63.08; H, 4.30; N, 13.38. Found: C, 62.97; H, 4.28; N, 13.34.

8-(5-Chloro-thiophen-2-yl)-6-(1-methyl-1H-pyrrol-2-yl)-2-thioxo-3,6-dihydro-2H-pyrimidin-1-yl)-phenyl-methanone 10a-c

A solution of 3,4-dihydro-1H-pyrimidine-2-thione 4a-c (5 mmol), benzoyl chloride (1.40 g, 10 mmol), and few drops of triethylamine in 25 mL of ethanol was heated under reflux for 4 h. The reaction mixture was left to cool down and poured into ice water, filtered and recrystallized from ethanol to afford compounds 10a-c.

[4-(1-Methyl-1H-pyrrol-2-yl)-6-thiophen-2-yl-2-thioxo-3,6-dihydro-2H-pyrimidin-1-yl]-phenyl-methanone 10a

Applying the previous general preparation method yellow powder was obtained. Yield (1.54 g, 81%); mp (161–163 °C); IR (KBr) ν max: 3224 (NH), 1682 (C=O), 1605–1547 cm−1 (C=C); 1H NMR (400 MHz, DMSO-d6) δ 3.38 (3H, 6.68 (d, 1H, H-5 of pyrimidine), 6.70 (d, 1H, H-4 of pyrimidine), 2.19 (s, 3H, CH3 of thiophene), 4.10 ppm (m, 3H of pyrrole). (418.59): C, 63.08; H, 4.30; N, 13.38. Found: C, 62.97; H, 4.28; N, 13.34.
7-[(1-Methyl-1H-pyrrol-2-yl)-2-thiao-3,6-dihydro-2H-pyrimidin-1-yl]-phenyl-methanone 10c According to the previous general procedure a yellow powder was obtained. Yield (1.82 g, 88%); mp (178–180) °C; IR (KBr) νmax: 3263 (NH), 1697 (C=O), (1615−1602) cm−1 (C=C); 1H NMR (400 MHz, DMSO-d6) δ 3.61 (S, 3H, NCH3), 5.73 (d, 1H, H-4 of pyrimidine, J=6.1 Hz), 6.23 (d, 1H, H-5 of pyrimidine, J=6.9 Hz), (3.34–8.18) (m, 10H, of aryl protons), 11.42 ppm (br, 1H, NH, D2O exchangeable). (m/z): 413 (M+, 413, M+2, 415) (3:1) ratio. Anal. Calcld for C20H15N4S2Cl (410.94): C, 64.46; H, 4.53; N, 14.54.

7-[(1-Methyl-1H-pyrrol-2-yl)-5-(5-substituted-thiophen-2-yl)-3-phenyl-5H-[1,2,4]thiadiazolo[4,5-a]pyrimidine 11a-c A solution of compounds 4a-c (3 mmol), 10% sodium hypochlorite (10 mL), 10 mL NH4OH and 10% of NaOH (10 mL) was heated under reflux for 3 h. The reaction mixture was left to cool down and poured into ice water, filtered and recrystallized from ethanol to obtain colored compounds 11a-c.

General method for synthesis of 7-[(1-Methyl-1H-pyrrol-2-yl)-5-(5-substituted-thiophen-2-yl)-3-phenyl-5H-[1,2,4]thiadiazolo[4,5-a]pyrimidine 11a According to the previous general preparation method, pale yellow powder was obtained. Yield (0.73 g, 64%); mp (124–126) °C; IR (KBr) νmax: 1624, 1631 (2C=O), (1615–1602) cm−1 (C=C); 1H NMR (400 MHz, DMSO-d6) δ 3.58 (S, 3H, NCH3), 5.64 (d, 1H, H-4 of pyrimidine, J=6.5 Hz), 6.21 (d, 1H, H-5 of pyrimidine, J=6.3 Hz), (3.74–8.18) (m, 12H, of pyrrole thiophene and phenyl). 13C NMR (100 MHz, DMSO-d6) δ 53.76 (NCH3), 65.13 (C-4 of pyrimidine), 118.34, 120.15, 124.66, 127.13, 130.87, 133.23, 137.22, 139.88, 141.22 (17C, C-arlyl), 150.11, 153.34 ppm (2C=N). Anal. Calcld for C20H16N3OS2Cl (413.95): C, 57.89; H, 4.38; N, 11.67. Found: C, 57.89; H, 4.38; N, 11.68.

General method for synthesis of 7-[(1-Methyl-1H-pyrrol-2-yl)-5-(5-substituted-thiophen-2-yl)-3-phenyl-5H-[1,2,4]thiadiazolo[4,5-a]pyrimidine 11b A yellow powder of 10b was obtained according to the aforementioned preparation method. Yield (0.70 g, 60%); mp (129–131) °C; IR (KBr) νmax: 1619, 1627 (2C=N), (1610–1602) cm−1 (C=C); 1H NMR (400 MHz, DMSO-d6) δ 2.31 (s, 3H, CH3 of thiophene), 3.41 (S, 3H, NCH3), 5.42 (d, 1H, H-4 of pyrimidine, J=8.1 Hz), 6.19 (d, 1H, H-4 of pyrimidine, J=6.2 Hz), 6.43 (d, 1H, H-3 of pyrimidine, J=6.8 Hz), 7.06 (dd, 1H, H-4 of pyrimidine), 7.17 (d, 1H, H-5 of pyrimidine, J=6.8 Hz), (7.31–8.17) ppm (m, 7H, of thiophene and phenyl). Anal. Calcld for C21H18N4S2 (390.53): C, 64.53; H, 4.61; N, 14.34. Found: C, 64.46; H, 4.53; N, 14.54.

General method for synthesis of [4-(1-Methyl-1H-pyrrol-2-yl)-6-(5-substituted-thiophen-2-yl)-1,6-dihydro-pyrimidin-2-yl]-hydrazine 12a-c A mixture of pyrimidine-2-thione 4a-c (5 mmol) and 10 mL hydrazine hydrate in 30 mL ethanol was refluxed for 6 h. The reaction mixture was allowed to cool and poured onto ice water. After filtration the crystallization took place from ethanol to obtain the corresponding hydrazine derivatives 12a-c.
[4-[(1-Methyl-1H-pyrrol-2-yl)-6-(5-methyl-thiophen-2-yl)-1,6-dihydro-pyrimidin-2-yl]-hydrazine 12b According to the previous general preparation method, yellow powder was obtained. Yield (1.07 g, 75%); mp (174–176) °C; IR (KBr) νmax (3367–3181) cm⁻¹ (C=N); 1H NMR (400 MHz, DMSO-d6) δ 1.89 (s, 3H, CH₃ of thiophene), 2.89 (s, 2H, CH₂ of pyrazol), 3.63 (s, 3H, NCH₃), 5.94 (d, 1H, H-4 of pyrimidine, J=5.4 Hz), 6.44 (d, 1H, H-5 of pyrimidine, J=7.9 Hz), 6.73 (d, 1H, H-3 of pyrrole, J=6.5 Hz), 7.31 (dd, 1H, H-4 of pyrrole), 7.51 (d, 1H, H-5 of pyrrole, J=6.4 Hz), 7.48 (d, 1H, H-3 of thiophene, J=5.3 Hz), 7.82 (dd, 1H, H-4 of thiophene), 8.17 (d, 1H, H-5 of thiophene, J=6.8 Hz), 9.38 ppm (s, br, H, 1NH of pyrimidine, D₂O exchangeable). 13C NMR (100 MHz, DMSO-d6) δ 25.54 (CH₃), 49.34 (NCH₃), 69.75 (C-4 of pyrimidine), 119.19, 121.33, 125.55, 127.32, 130.78, 135.59, 138.84, 145.77 (11 C of aryl C), 158.73, 160.22 (2C=C). Anal. Calcd for C₁₇H₁₄N₅SCl (307.80): C, 50.68; H, 4.55; N, 22.74. Found: C, 50.63; H, 4.49; N, 22.68.

5-Methyl-2-[4-([(1-methyl-1H-pyrrol-2-yl)-6-(5-methyl-thiophen-2-yl)-1,6-dihydro-pyrimidin-2-yl]-2,4-dihydro-pyrazol-3-one 13a-c Brown crystals were obtained according to the previous general preparation method. Yield (1.12 g, 75%); mp (205–207) °C; IR (KBr) νmax (3208) cm⁻¹ (C=N); 1H NMR (400 MHz, DMSO-d6) δ 1.82 (s, 3H, CH₃ of pyrazol), 3.09 (s, 2H, CH₂ of pyrazol), 3.51 (s, 3H, NCH₃), 5.29 (d, 1H, H-4 of pyrimidine, J=6.1 Hz), 6.11 (d, 1H, H-5 of pyrimidine, J=6.9 Hz), 6.88 (d, 1H, H-3 of pyrrole, J=7.5 Hz), 7.24 (dd, 1H, H-4 of pyrrole), 7.52 (d, 1H, H-5 of pyrrole, J=6.1 Hz), 7.71 (d, 1H, H-3 of thiophene, J=6.3 Hz), 8.23 (d, 1H, H-4 of thiophene, J=7.2 Hz), 10.32 ppm (s, br, H, 1NH of pyrimidine, D₂O exchangeable). (m/z): 373 (M⁺, 373, M+2⁺, 375) (3:1) ratio. Anal. Calcd for C₁₇H₁₆N₅O₂Cl (373.86): C, 54.57; H, 4.28; N, 18.72. Found: C, 54.48; H, 4.22; N, 18.66.

5-Methyl-2-[4-([(1-methyl-1H-pyrrol-2-yl)-6-thiophen-2-yl]-1,6-dihydro-pyrimidin-2-yl]-2,4-dihydro-pyrazol-3-one 13a According to the previous general preparation method, dark yellow crystals were obtained. Yield (0.99 g, 73%); mp (196–198) °C; IR (KBr) νmax (3176) (NH), 1692 (C=O), 1637, 1625 (2 C=C). 1H NMR (400 MHz, DMSO-d6) δ 1.89 (s, 3H, CH₃ of pyrazol), 2.89 (s, 2H, CH₂ of pyrazol), 3.63 (s, 3H, NCH₃); 5.94 (d, 1H, H-4 of pyrimidine, J=5.4 Hz), 6.44 (d, 1H, H-5 of pyrimidine, J=7.9 Hz), 6.73 (d, 1H, H-3 of pyrrole, J=6.5 Hz), 7.31 (dd, 1H, H-4 of pyrrole), 7.51 (d, 1H, H-5 of pyrrole, J=6.4 Hz), 7.48 (d, 1H, H-3 of thiophene, J=5.3 Hz), 7.82 (dd, 1H, H-4 of thiophene), 8.17 (d, 1H, H-5 of thiophene, J=6.8 Hz), 9.38 ppm (s, br, H, 1NH of pyrimidine, D₂O exchangeable).
mixture was allowed to cool and poured onto ice water. After filtration, the obtained product was dried and recrystallized from ethanol to afford the corresponding [1, 2, 4]-triazolo[4,3-a]pyrimidine 14a-c.

7-(1-Methyl-1H-pyrrol-2-yl)-5-thiophen-2-yl-1,5-dihydro-[1,2,4]triazolo[4,3-a]pyrimidine 14a. According to the previous general preparation method, brown crystals were obtained. Yield (0.74 g, 65%); mp (174–176 °C); IR (KBr) ν max: (3275) (NH), 1643, 1632 (2 C=N), (1608–1598) cm⁻¹ (C=C); ¹H NMR (400 MHz, DMSO-d6) δ 3.37 (S, 3H, NCH₃), 5.08 (d, 1H, H-4 of pyrimidine, J = 6.0 Hz), 6.23 (d, 1H, H-5 of pyrimidine, J = 7.3 Hz), (6.81–7.53) (m, 3H, of thiophene), 8.42 ppm (s, H-3 of triazole), 12.83 ppm (s, br, H, 1NH of trizole, D₂O exchangeable). Anal. Calcd for C₁₅H₁₅N₅S: C, 60.53; H, 5.04; N, 23.54. Found: C, 60.51; H, 4.59; N, 21.94.

7-(1-Methyl-1H-pyrrol-2-yl)-5-(5-methyl-thiophen-2-yl)-7-(1-methyl-1H-pyrrole)-1,5-dihydro-[1,2,4]triazolo[4,3-a]pyrimidine 14b. Brown crystals were obtained according to the previous general preparation method. Yield (0.81 g, 68%); mp (174–176 °C); IR (KBr) ν max: (3251) (NH), 1637, 1626 (2 C=N), (1613–1598) cm⁻¹ (C=C); ¹H NMR (400 MHz, DMSO-d6) δ 3.42 (S, 3H, of pyrrole, (7.89–8.11) (m, 3H, of thiophene), 7.61 (d, 1H, H-3 of thiophene, J = 7.3 Hz), 6.11 (d, 1H, H-5 of pyrimidine, J = 7.8 Hz), 5.92 (m, 1H, of triazole), 12.83 ppm (s, br, H, 1NH of trizole, D₂O exchangeable). Anal. Calcd for C₁₅H₁₅N₅S: C, 59.16; H, 4.59; N, 22.03. Found: C, 58.72; H, 3.73; N, 21.96.

Conclusion
New condensed and non-condensed heterocyclic compounds based on pyrimidine-2-thiones 4a-c were synthesized. The first synthetic path way took place through S-alkylation of pyrimidine-2-thiones 4a-c followed by reaction with ammonia to produce the corresponding triazolo[3,2-a]pyrimidin-3-ones 7a-c which underwent condensation with benzaldehyde followed by heating under reflux with hydroxylamine afforded the corresponding isoxazolo [5’,4’,5]triazolo[3,2-a]pyrimidine 9a-c. The second path way of this work was the heating of the key synthons 4a-c with benzoyl chloride followed by reaction with sodium hypochlorite, ammonia and sodium hydroxide to produce [1, 2, 4]thiadiazolo[4,5-a]pyrimidine 11a-c. A final route of this work was the hydrazinolysis of 4a-c followed by the cyclocondensation with ethyl acetocetate or formic acid to produce pyrazol-3-ones 13a-c or [1, 2, 4]triazolo[4,3-a]pyrimidine 14a-c, respectively. All newly synthesized heterocyclic structures were confirmed using various tools including, elemental analysis, IR, ¹H-NMR, ¹³C-NMR and mass spectra. Screening of the selected compounds 4a, 6a, 7a, 9a, 10a, 13a and 14a against colon carcinoma cells lines (HCT-116) and hepatocellular carcinoma cells lines (HepG-2) showed that the compound 2-thioxo-3,6-dihydro-2H-pyrimidin-1-yl]-phenyl-methanone 10a was the most active among the group of selected compounds, meanwhile, compounds 4a, 6a, 7a and 14a exhibited considerable cytotoxic action, furthermore, compounds 7a, 9a and 13a were showed weak cytotoxic action. These results encourage us to suggest that the compound 10a be used in the formulation of antibiotics as a medication to improve the sensitivity of antibiotics that stimulate cancer therapy and cause apoptosis in both human colon carcinoma cancer and hepatocellular carcinoma.

Supplementary Information
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Additional file 1: a) Figures illustrating the IR spectra of compounds 4a, 6a, 7a and 9a-13a. b) Tables contain elemental analysis for all prepared compounds. c) Table contain’s melting points, yield % and IR spectral data of compounds 3a-c.

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Authors’ contributions
EMA: prepared the newly organic compounds under study, proved the chemical structures of the prepared compounds using various spectroscopic methods, wrote the main manuscript text, prepared figures, tables, reviewed
and approved the final manuscript. DE: Wrote, reviewed and approved the final manuscript. Both authors read and approved the final manuscript.

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Declarations

Ethics approval and consent to participate
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Consent for publication
Declarations

Corresponding author
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