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ORIGINAL ARTICLE

1st Heterocyclic Update

Multicomponent reactions for synthesis of bioactive polyheterocyclic ring systems under controlled microwave irradiation

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Antitumor activity

Abstract The multi-component reaction of 1-benzothiopyran-4-ones with heterocyclic amines and dimethylformamide-dimethylacetal (DMFDMA) in DMF at 150 °C under controlled microwave heating afforded novel poly-heterocyclic ring systems. Also, reaction of 3-dimethylaminomethylene-1-benzothiopyran-4-one with activemethylene derivatives was investigated. The structure of all products was established on the bases of spectral data and elemental analyses and alternative synthesis if possible. The prepared compounds were screened for their antitumor activity against HCT-116 "colon" cancer cell line and some derivatives showed promising activity.

1. Introduction

Multi-component reactions (MCR), an important class of organic tandem reactions, are one-pot processes with at least three components to form a single product, which incorporates most or even all of the starting materials (Hulme and Gore, 2003; Orru and De Greef, 2003; Domling and Ugi, 2000; Nair et al., 2003; Zhu, 2003; Domling, 2002). The huge interest for such multi-component reactions during the last years has been oriented toward developing combinatorial chemistry procedures, because of their high efficiency and convenience of these reactions in comparison with multistage procedures. Hence, most of the scientific efforts have been focused on the development of multi-component procedures to prepare diverse heterocyclic compound libraries (Orru and De Greef, 2003). Also, the utility of MCR under microwave irradiation in the synthesis of heterocyclic compounds enhanced the reaction rates and improved the regioselectivity (Andrade et al., 2008; Lidstrom et al., 2001; Bortolini et al., 2008; El Ashry and Kassem, 2006; Sadek et al., 2012). 1-Benzothiopyran-4-ones, also known as thiochromen-4-one, are an important class of heterocycles. They serve as key intermediate for the synthesis of bioactive heterocyclic ring systems (Dawood et al., 2001; Al-Nakib et al., 1990, 1991; Ares et al., 1996; Wang et al., 1996). Recently, we have concentrated much of our recent work in the preparation of bioactive nitrogen-containing

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heterocycles (Farghaly et al., 2012; Abdel Hafez et al., 2010; Farghaly and Abdalla, 2009; Riyadh and Farghaly, 2012; Farghaly and Hassaneen, 2013; Farghaly and Mahmoud, 2013; Gomha et al., 2013; Gomha and Abdel-Aziz, 2013; Gomha and Khalil, 2012), and have already described simple and efficient procedures to prepare interesting molecules with biological properties. We describe here the preparation, through multi-component reactions under microwave heating, of new poly-heterocyclic ring systems incorporating of benzothiopyrane moiety that have not been reported hitherto in addition to the study of the effects of the newly synthesized compounds on the growth of human colon cancer cell line (HCT-116).

2. Experimental

2.1. Chemistry

Melting points were measured on an Electrothermal IA 9000 series digital melting point apparatus. IR spectra were recorded in potassium bromide disks on Pye Unicam SP 3300 and Shimadzu FTIR 8101 PC infrared spectrophotometers. NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer operating at 300 or 400 MHz for ^{1}H-NMR and run in deuterated dimethylsulfoxide (DMSO-d_6). Chemical shifts were related to that of the solvent. Mass spectra were recorded on a Shimadzu GCMS-QP1000 EX mass spectrometer at 70 eV. Elemental analyses were measured by using a German made Elementar vario LIII CHNS analyzer. 3-Dimethylaminomethylene-1-benzothipyran-4-one **9** was prepared as previously reported in the respective literature (Bruno et al., 1999).

2.1.1. Reaction of 1-benzothiopyran-4-one (*1*) with heterocyclic amines *2*, *10*–*14* and DMF-DMA

Method A: A solution of appropriate heterocyclic amine derivatives **2**, **10**–**14** (1 mmol), 1-benzothiopyran-4-one (**1**) (1 mmol) and dimethylformamide dimethylacetal (DMF-DMA) (1 mmol) in dry dimethylformamide (10 mL) was heated under reflux in a Milestone Microwave Labstation at 150 °C for 10–15 min (TLC). After concentration and cooling to room temperature, the resulting solid product so formed was collected by filtration, washed well with EtOH, dried and recrystallized from EtOH.

Method B: To a solution of **9** (1.095 g, 0.005 mol) in acetic acid (20 ml) was added (0.005 mol) of the appropriate heterocyclic amines (**2**, **10**–**14** or **20**). The mixture was heated under reflux in a Milestone Microwave Labstation at 150 °C for 5 min. After concentration and cooling to room temperature, the precipitated product was collected by filtration, washed well with EtOH, dried and recrystallized from EtOH to give compounds **5**, **15**–**19** or **21**, respectively. The products **5**, **15**–**19** and **21** together with their physical constants are listed below.

*2.1.1.1. 6H-Thiochromeno[3,4-e][1,2,4]triazolo[1,5-a]pyrimidine (**5**).* Yellow solid, (82% yield); mp 185–187 °C; IR (KBr) υ 3057, 2970, 1595, 1496, 1263, 1234 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.20 (s, 2H, CH_2), 7.45–7.64 (m, 4H, Ar-H), 8.75 (s, 1H, pyrimidine-H), 8.96 (s, 1H, triazole-H); MS, *m*/*z* (%) 240 (M^+, 100), 239 (83), 200 (63), 69 (58); Anal. calcd for $C_{12}H_8N_4S$ (240.28): C, 59.98; H, 3.36; N, 23.32; found: C, 59.84; H, 3.23; N, 23.15%.

*2.1.1.2. 10-Phenyl-6H-thiochromeno[3,4-e]pyrazolo[1,5-a]pyrimidine (**15**).* Yellow solid, (83% yield); mp 168–170 °C; IR (KBr) υ 3047, 2901, 1593, 1499, 1463, 1234 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.12 (s, 2H, CH_2), 7.35 (s, 1H, pyrazole-H), 7.41–8.10 (m, 9H, Ar-H), 8.62 (s, 1H, pyrimidine-H); MS, *m*/*z* (%) 316 ($M^+ + 1$, 24), 315 (M^+, 100), 314 (89), 211 (20), 185 (17), 145 (14), 140 (17), 102 (12), 77 (66), 76 (27), 75 (13); Anal. calcd for $C_{19}H_{13}N_3S$ (315.39): C, 72.36; H, 4.15; N, 13.32; found: C, 72.19; H, 4.01; N, 13.09%.

*2.1.1.3. 10-Cyano-6H-thiochromeno[3,4-e]pyrazolo[1,5-a]pyrimidine (**16**).* Yellow solid, (76% yield); mp 218–220 °C; IR (KBr) υ 3052, 2974, 2220 (CN), 1602, 1514, 1446, 1372, 1239 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.17 (s, 2H, CH_2), 7.43–7.65 (m, 4H, Ar-H), 8.85 (s, 1H, pyrimidine-H), 8.91 (s, 1H, pyrazole-H); MS, *m*/*z* (%) 265 ($M^+ + 1$, 18), 2264 (M^+, 92), 263 (100), 262 (12), 236 (11), 171 (7), 140 (9), 105 (11), 92 (6), 69 (15); Anal. calcd for $C_{14}H_8N_4S$ (264.31): C, 63.62; H, 3.05; N, 21.20; found: C, 63.48; H, 2.98; N, 21.07%.

*2.1.1.4. 6H-thiochromeno[3,4-e]tetrazolo[1,5-a]pyrimidine (**17**).* Yellow solid, (80% yield); mp 138–140 °C; IR (KBr) υ 3052, 2968, 1594, 1498, 1261, 1230 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.31 (s, 2H, CH_2), 7.39–8.49 (m, 4H, Ar-H), 8.71 (s, 1H, pyrimidine-H); MS, *m*/*z* (%) 242 ($M^+ + 1$, 21), 241 (M^+, 100), 240 (55), 212 (33),186 (87), 159 (39), 103 (33), 102 (25), 84 (63), 76 (25), 75 (28); Anal. calcd for $C_{11}H_7N_5S$ (241.27): C, 54.76; H, 2.92; N, 29.03; found: C, 54.58; H, 2.76; N, 28.94%.

*2.1.1.5. 6H-thiochromeno[3,4-e]benzimidazo[1,2-a]pyrimidine (**18**).* Orange crystals, (88% yield); mp 255–256 °C; IR (KBr) υ 3051, 2968, 1450, 1299 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.06 (s, 2H, CH_2), 7.18–8.15 (m, 8H, Ar-H), 8.85 (s, 1H, pyrimidine-H); MS, *m*/*z* (%) 290 ($M^+ + 1$, 36), 289 (M^+, 100), 288 (92), 287 (61), 145 (30), 144 (35), 102 (22), 77 (22), 76 (20), 75 (22); Anal. calcd for $C_{17}H_{11}N_3S$ (289.36): C, 70.56; H, 3.83; N, 14.52; found: C, 70.38; H, 3.64; N, 14.37%.

*2.1.1.6. 6H-9,11-dimethyl-thiochromeno[3,4-e]pyrido[3′,2′:3,4]pyrazolo[1,5-a]pyrimidine (**19**).* Yellow solid, (82% yield); mp 227–229 °C; IR (KBr) υ 3072, 2965, 1624, 1577, 1503, 1406, 1289, 1225 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.91 (s, 3H, CH_3), 2.64 (s, 3H, CH_3), 4.27 (s, 2H, CH_2), 7.0–7.36 (m, 4H, Ar-H), 7.58 (s, 1H, pyridine-H), 8.91 (s, 1H, pyrimidine-H); MS, *m*/*z* (%) 319 ($M^+ + 1$, 30), 318 (M^+, 100), 317 (62), 316 (32), 289 (8), 158 (16), 140 (6), 115 (11), 75 (7); Anal. calcd for $C_{18}H_{14}N_4S$ (318.40): C, 67.90; H, 4.43; N, 17.60; found: C, 67.78; H, 4.39; N, 17.45%.

*2.1.1.7. 1,2,3,4,7-Pentahydro-3-thioxo-thiochromeno[4′,3′:4,5]pyrido[2,3-d]pyrimidine-1-one (**21**).* Yellow solid, (88% yield); mp 352–354 °C; IR (KBr) υ 3423 (br, 2NH), 3058, 2978, 1714 (C = O), 1643, 1579, 1430, 1259 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.19 (s, 2H, CH_2), 7.36–7.46 (m, 4H, Ar-H), 8.31 (s, 1H, pyrimidine-H), 12.0 (s, 1H, NH), 12.60 (s, 1H, NH),; MS, *m*/*z* (%) 300 ($M^+ + 1$, 24), 299 (M^+, 100), 298 (95), 297 (26), 239 (21), 211 (28), 210 (15), 185 (12), 140 (16), 120 (12), 106 (16), 105 (12), 92 (12), 76 (10); Anal. calcd for $C_{14}H_9N_3OS_2$ (299.37): C, 56.17; H, 3.03; N, 14.04; found: C, 56.39; H, 3.15; N, 14.24%.

*2.1.2. Reaction of enaminone (**9**) with activemethylene*

To a solution of **9** (1.10 g, 0.005 mol) in acetic acid (20 ml) was added activemethylene derivatives (**24**–**27**) (0.005 mol) in the presence of ammonium acetate anhydrous (0.5 g). The mixture was heated under reflux in a Milestone Microwave Labstation at 150 °C for 2 min. After concentration and cooling to room temperature, the precipitated product was collected by filtration, washed well with EtOH, dried and recrystallized from EtOH to give compounds (**28**–**31**). The products (**28**–**31**) together with their physical constants are listed below.

*2.1.2.1. 3-Cyano-1H-thiochromeno[4,3-b]pyridin-2(5H)-one (**28a**).* Yellow solid, (88% yield); mp 140–142 °C; IR (KBr) υ 3353 (NH), 3055, 2891, 2211 (CN), 1639 (C≡O), 1584, 1429, 1406, 1358, 1258 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 4.09 (s, 2H, CH_2), 6.88–7.60 (m, 4H, Ar-H), 7.87 (s, 1H, pyridine-H), 12.40 (s, 1H, NH); MS, *m*/*z* (%) 240 (M^+, 53), 239 (53), 238 (60), 151 (40), 149 (87), 105 (33), 104 (60), 83 (53), 77 (27), 71 (100); Anal. calcd for $C_{13}H_8N_2OS$ (240.28): C, 64.98; H, 3.36; N, 11.66; found: C, 64.73; H, 3.18; N, 11.43%.

*2.1.2.2. 3-Benzoyl-1H-thiochromeno[4,3-b]pyridin-2(5H)-one (**28b**).* Yellow solid, (84% yield); mp 153–155 °C; IR (KBr) υ 3428 (NH), 3053, 2892, 1642 (C≡O), 1582, 1437, 1359, 1325, 1256 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 4.18 (s, 2H, CH_2), 7.22–7.84 (m, 9H, Ar-H), 7.93 (s, 1H, pyridine-H), 9.48 (s, 1H, NH); MS, *m*/*z* (%) 320 ($M^+ + 1$, 14), 319 (M^+, 31), 318 (24), 317 (45), 285 (52), 164 (35), 147 (59), 104 (48), 85 (41), 77 (51), 76 (45), 73 (100); Anal. calcd for $C_{19}H_{13}NO_2S$ (319.38): C, 71.45; H, 4.10; N, 4.39; found: C, 71.28; H, 4.01; N, 4.18%.

*2.1.2.3. Ethyl 2-methyl-5H-thiochromeno[4,3-b]pyridine-3-carboxylate (**29**).* Yellow solid, (82% yield); mp 120–122 °C; IR (KBr) υ 3056, 2893, 1732 (C≡O), 1584, 1429, 1406, 1359, 1258 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.35 (t, J = 7 Hz, 3H, CH_3), 2.78 (s, 3H, CH_3), 4.14 (s, 2H, CH_2), 4.36 (q, J = 7 Hz, 2H, CH_2), 7.32–7.43 (m, 9H, Ar-H), 8.17 (s, 1H, pyridine-H); MS, *m*/*z* (%) 286 ($M^+ + 1$, 19), 285 (M^+, 57), 284 (55), 256 (38), 149 (60), 104 (15), 84 (30), 77 (38), 57 (100); Anal. calcd for $C_{16}H_{15}NO_2S$ (285.36): C, 67.34; H, 5.30; N, 4.91; found: C, 67.17; H, 5.21; N, 4.74%.

*2.1.2.4. Indeno[2,1-e]thiochromeno[4,3-b]pyridin-8(6H)-one (**30**).* Yellow solid, (82% yield); mp 152–154 °C; IR (KBr) υ 3059, 2972, 1707 (C≡O), 1582, 1440, 1315, 1262 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 3.92 (s, 2H, CH_2), 7.23–8.05 (m, 8H, Ar-H), 8.83 (s, 1H, pyridine-H); MS, *m*/*z* (%) 301 (M^+, 44), 300 (25), 283 (31), 282 (44), 255 (56), 190 (56), 162 (50), 135 (69), 105 (69), 85 (56), 77 (69), 55 (100); Anal. calcd for $C_{19}H_{11}NOS$ (301.36): C, 75.72; H, 3.68; N, 4.65; found: C, 75.54; H, 3.56; N, 4.42%.

*2.1.2.5. Chromeno[3,4-e]thiochromeno[4,3-b]pyridin-6(8H)-one (**31**).* Yellow solid, (86% yield); mp 144–146 °C; IR (KBr) υ 3052, 2971, 1727 (C≡O), 1595, 1490, 1412, 1328, 1264 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 4.32 (s, 2H, CH_2), 7.30–7.72 (m, 8H, Ar-H), 8.57 (s, 1H, pyridine-H); MS, *m*/*z* (%) 318 ($M^+ + 1$, 22), 317 (M^+, 80), 316 (100), 315 (49), 136 (21), 75 (11); Anal. calcd for $C_{19}H_{11}NO_2S$ (317.36): C, 71.91; H, 3.49; N, 4.41; found: C, 71.75; H, 3.22; N, 4.29%.

*2.1.3. Synthesis of compounds **32a**,**b***

To a solution of the enaminone **9** (0.219 g, 1 mmol) in EtOH (10 mL) was added hydrazine hydrate (1 mL) or phenylhydrazine (1 mL) and the mixture was heated under reflux in a Milestone Microwave Labstation at 150 °C for 2 min. The reaction mixture was acidified by HCl/ice mixture and the formed product was filtered and crystallized from EtOH to give the respective 2,4-dihydrothiochromeno[4,3-c]pyrazole (**32a**) [mp 168–169 °C, lit. mp 168–170 °C] (Ramalingam et al., 1977) and 2-phenyl-2,4-dihydrothiochromeno[4,3-c]pyrazole (**32b**) [mp 169–171 °C, lit. mp 169–171 °C] (Ramalingam et al., 1977) in excellent yields.

*2.1.4. Synthesis of 4H-thiochromeno[4,3-c]isoxazole (**33**)*

Hydroxylamine hydrochloride (0.189 g, 1 mmol) was added to a mixture of enaminone (**3**) (0.219 g, 1 mmol) and sodium ethoxide solution [prepared from sodium metal (0.023 g, 1 mmole) in ethanol (10 mL)]. The mixture was heated under reflux in a Milestone Microwave Labstation at 150 °C for 3 min. After cooling the solution to room temperature, it was poured onto water. The solid product was filtered and crystallized from ethanol to give isoxazole **33** [mp 71–73 °C, lit. mp 71–73 °C] (Ramalingam et al., 1977).

2.1.5. Evaluation of the antitumor activity

The antitumor activity was evaluated on carcinoma cell lines, namely HCT-116 cell. The cell line was grown as monolayers in growth medium supplemented with 10% inactivated fetal calf serum and 50 µg/ml gentamycin. The monolayers of 10,000 cells adhered at the bottom of the wells in a 96-well microtiter plate (Falcon, NJ, USA) incubated for 24 h at 37 °C in a humidified incubator with 5% CO_2. The monolayers were then washed with sterile phosphate buffered saline (0.01 M pH 7.2) and simultaneously the cells were treated with 100 µl from different dilutions of tested compound in a fresh maintenance medium and incubated at 37 °C. A control of untreated cells was made in the absence of the tested compound. Three wells were used for each concentration of the test sample. Every 24 h the observation under the inverted microscope was made. The number of the surviving cells was determined by staining the cells with crystal violet followed by cell lysing using 33% glacial acetic acid and read the absorbance at 590 nm using ELISA reader after well mixing. The absorbance values from untreated cells were considered as 100% proliferation and the percentage of viability was calculated as $[1-(ODt/ODc)] \times 100\%$ where ODt is the mean optical density of wells treated with the tested compounds and ODc is the mean optical density of untreated cells.

3. Results and discussion

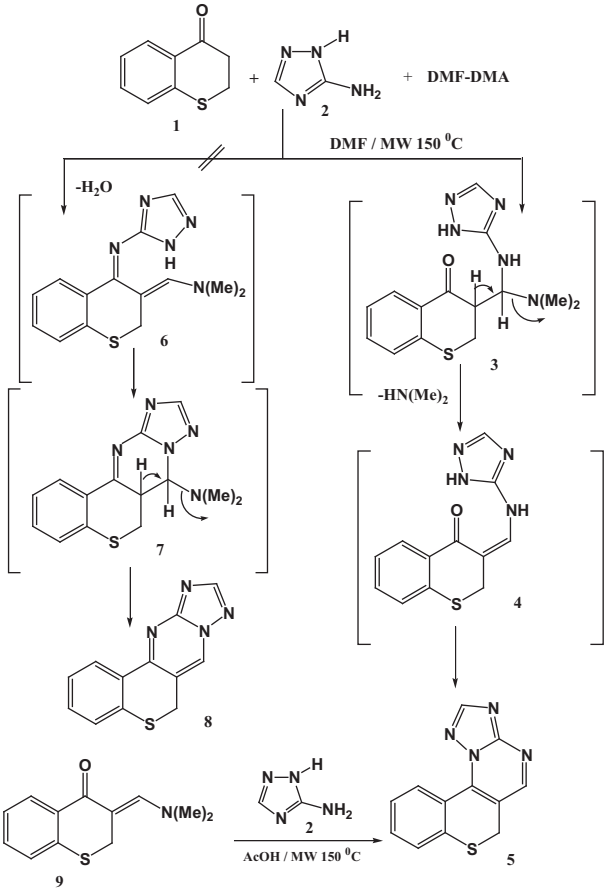
3.1. Chemistry

Multi-component reaction of benzothiopyrane 1,3-aminotriazole **2** and dimethylformamide-dimethylacetal (DMF-DMA) in DMF under microwave irradiation at 150 °C for 10 min. afforded compound **5** rather than its isomeric structure **8** (Scheme 1). The conformation of compound **5** was established on the bases of spectral data (Ms, IR, ^1H NMR) and elemental analyses. The mass spectrum of the reaction product **5** showed

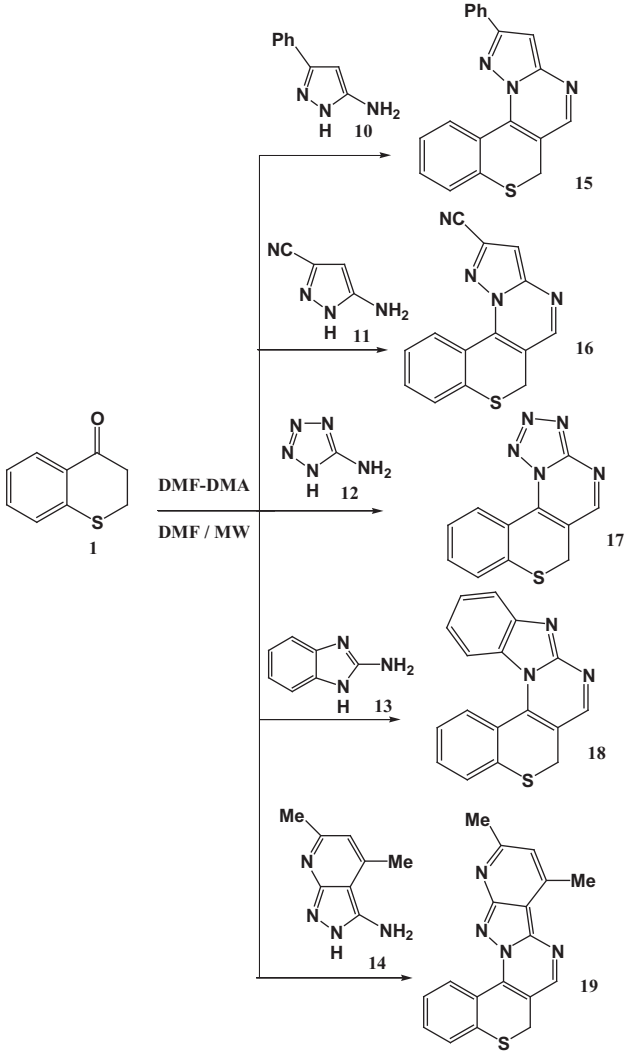
a molecular ion peak m/z = 240 (100%). Its ^{1}H NMR spectrum revealed a singlet signal at δ 8.75 ppm corresponding to one proton at C7 (Farghaly et al., 2010) which was consistent with the isomeric structure **5**, while chemical shift of the proton at C5 of the other isomer **8** is downfield at δ 9.09 (Al-Qalaf et al., 2009). Furthermore, alternative synthesis of compound **5** was achieved *via* condensation of benzothiopyrane **1** with dimethylformamide dimethylacetal (DMFDMF) to give compound **9** (Bruno et al., 1999), and treatment of the product **9** with 3-aminotriazole **2** under the same reaction condition to yield authentic product **5** (Scheme 1). In addition, literature reports explained that, enaminone of the active methylene compound was formed firstly then nucleophilic attack of the amino group of the heterocyclic amine on the double bond of the enaminone with concurrent elimination of the dimethylamine to form intermediate **4** rather than condensation of water molecule in intermediate **6** (Al-Saleh et al., 2005; Dawood, 2005). Also, this mechanism was established by X-ray crystallographic analysis in another report (Farghaly et al., 2010). On the basis of these findings, structure **8** was discarded and the isolated product from the studied reaction was assigned structure **5** (Scheme 1).

With this result in hand, we went on to study the scope of such multi-component reactions with several heterocyclic amine derivatives **10**–**14** and 1-benzothiopyran-4-one **1** and dimethylformamide-dimethylacetal (DMF-DMA) under the same reaction conditions, afforded in each case the corresponding tetra-heterocyclic ring systems **15**–**17** and penta-heterocyclic ring systems **18** and **19**, respectively (Scheme 2). The structure of compounds **15**–**19** was established on the bases of spectral data (Ms, IR, ^{1}H NMR) and elemental analyses (see Experimental part).

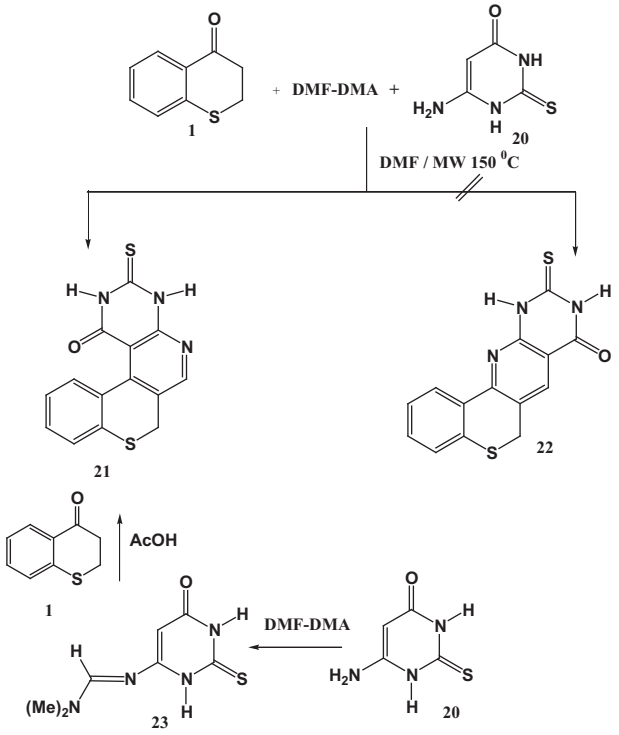
By the same way reaction of 6-amino-2-thioxopyrimidin-4(3*H*)-one (**20**) with 1-benzothiopyran-4-one **1** and dimethylformamide-dimethylacetal (DMF-DMA) in DMF under microwave irradiation at 150 °C for 15 min. gave compound **21** or its isomeric structure **22**. The ^{1}H NMR spectrum of the product revealed a singlet signal at 8.31 ppm assigned for pyridine-2H proton not pyridine-4H proton (Sadek et al., 2012; Farghaly et al., 2010; Quiroga et al., 2002) which was consistent with the isomeric structure **21** rather than the isomeric structure **22**. Compound **21** was also obtained by an alternative route: synthesis of compound **21** was achieved *via* condensation of 6-amino-2-thioxopyrimidin-4(3*H*)-one (**20**) with dimethylformamide dimethylacetal (DMFDMF) to give compound **23** (Hassaneen and Abdallah, 2003), and treatment of the product **23** with 1-benzothiopyran-4-one **1** to yield authentic product **21** (Scheme 3).



Scheme 1 MCR of benzothiopyranone 1, aminotriazole 2 and DMF-DMA

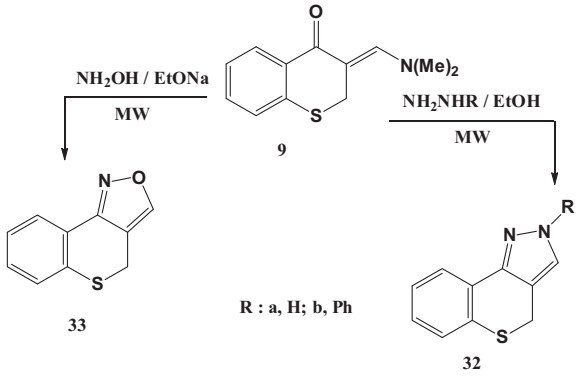


Scheme 2 MCR of benzothiopyranone 1 , heterocyclic amines 10-14 and DMF-DMA



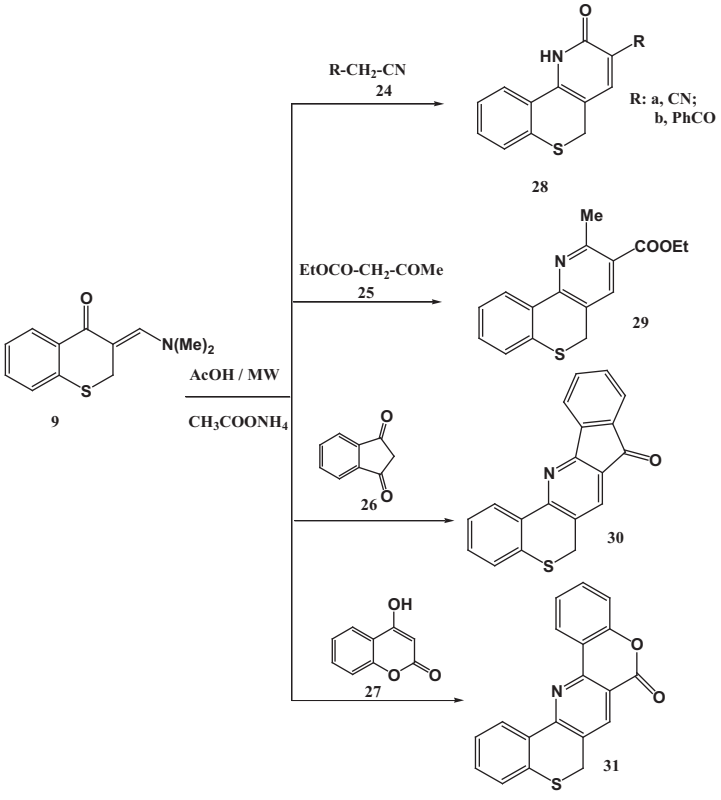
Scheme 3 MCR of benzothiopyranone 1, 6-amino-2-thioxopyrimidine-4(3H)-one 20 and DMF-DMA

Also, we were interested to investigate the multi-component reactions of the activemethylene derivatives with 1-benzothiopyran-4-one and DMF-DMA, but all attempts to isolate pure single products failed. So, we carried the reaction of enaminone **9** with activemethylene under microwave irradiation. Reaction of enaminone **9** with activemethylene derivatives **24**–**27** in glacial acetic acid in the presence of ammonium acetate gave poly-heterocyclic ring systems **28**–**31**, respectively (Scheme 4). The structure of the products was assigned based on the spectral data and elemental analyses. For example mass spectrum of compound **31** revealed molecular ion peak at m/z 317 (80%) and its ^{1}H NMR spectrum showed a characteristic signal at δ 8.57 ppm assignable to pyridine-2H proton. Its IR spectra showed one carbonyl group band at 1727 cm^{-1}.



Scheme 5 Synthesis of compounds 32 and 33.

Finally, reaction of enaminone **9** with hydrazine derivatives or hydroxyl amine under microwave heating at 150 °C afforded thiochromeno[4,3-c]pyrazole derivatives **32a**,**b** and



Scheme 4 Reaction of enaminone 9 with activemethylene.

Table 1 Cytotoxicity activity of the prepared compounds against colon cancer cell line HCT-116 cell line.

Compd. No	IC_{50} (μg/ml)
5	8.8
9	4.6
15	3.6
16	45.5
18	14.6
19	28.3
21	31.6
28a	2.6
28b	5.3
29	29.4
30	49.2
Doxorubicin	0.469

thiochromeno[4,3-c]isoxazole **33**, respectively (Scheme 5). It is worthily mentioned that compounds **32** and **33** had been prepared by Ramalingam et al. (Ramalingam et al., 1977) *via* a different method.

3.2. Pharmacology

3.2.1. Anticancer activity

The in vitro anti-tumor activity of the tested compounds was evaluated at Regional Center for Mycology and Biotechnology at Al-Azhar University, Cairo, Egypt. Eleven compounds were tested for their in vitro antitumor activity against HCT-116 "colon" cancer cell line. Doxorubicin was used as a reference drug and showed IC_{50} = 0.469 μg/ml. The most active compounds **28a**, **15**, **9**, **28b**, **5**, **18**, revealed IC_{50} < 20 μg/ml against HCT-116 (Table 1). 3-Dimethylaminomethylene-1-benzothipyran-4-one **9** showed good activity with IC_{50} = 4.6 μg/ml against HCT-116. Fused benzothiopyrane with the pyridine ring in compound **28a** increases the activity (IC_{50} = 2.6 μg/ml) while, the replacement of the cyano group in compound **28a** with the benzoyl group in compound **28b** decreases the activity (IC_{50} = 5.3 μg/ml). On the other hand, fused benzothiopyrane with pyrazolo[1,5-*a*]pyrimidine in compound **15** increases the activity with IC_{50} = 3.6 μg/ml against HCT-116. The other derivatives have activity less than 3-dimethylaminomethylene-1-benzothipyran-4-one **9**.

4. Conclusion

In this context, we describe an efficient MCR for the synthesis of polyheterocyclic ring systems under microwave irradiation. The anti-tumor activity of some of the prepared compounds against colon cancer cell line HCT-116 was evaluated. The results demonstrate that benzothiopyrane fused with pyridine or pyrazolopyrimidine derivatives showed good activity against colon cancer cell line (HCT-116 cell line).

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