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Article

A Convenient Synthesis, Reactions and Biological Activity of Some New 6*H*-Pyrazolo[4',3':4,5]thieno[3,2-*d*][1,2,3]triazine Compounds as Antibacterial, Anti-Fungal and Anti-Inflammatory Agents

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We describe here the design and synthesis of novel pyrazolothienotriazine compounds based on diazotization followed by cycloaddition reactions of 4-amino-3-methyl-1-phenyl-1*H*-thieno[2,3-*c*]pyrazol-5-carbonitrile with sodium nitrite in the presence of concentrated HCl in acetic acid. The produced chloropyrazolothienotriazine underwent nucleophilic substitution reactions with various primary and secondary amines including sulfa drugs to afford the *N*-substituted aminopyrazolothienotriazines. Hydrazinolysis of the chlorotriazine with hydrazine hydrate afforded the hydrazinotriazine, which was used as a versatile precursor for synthesis of other compounds. The chemical structures of the newly synthesized compounds were confirmed on the basis of elemental and spectral analyses containing Fourier transform infrared spectroscopy (FTIR), ^{1}H and ^{13}C nuclear magnetic resonance (NMR) and mass spectrometry. Some of the synthesized compounds showed high antibacterial and anti-fungal activities. Also, most of the tested compounds exhibited high anti-inflammatory activity compared with indomethacin using carrageenan induced rat paw edema assay.

Keywords: pyrazole, thienopyrazole, pyrazolothienotriazine, synthesis, antimicrobial activity, anti-inflammatory activity

Introduction

Pyrazoles and condensed pyrazoles are very important class of heterocyclic compounds which were considered as important scaffolds in medicinal chemistry due to their wide range of pharmacological activities; the most biological activities are anti-inflammatory,[1-5] antimicrobial,[6,7] antioxidant,[8] anticancer,[9-11] fungicidal,[10] and antiviral activities.[10,12,13] Some thienopyrazoles are used for inhibiting PDE 7 (phosphodiesterase 7) selectively, which is responsible for allergy, immunological and inflammatory diseases.[14] Bindi *et al*.[15] reported a series of thienopyrazoles to demonstrate their activities as a potent inhibitor for aurora kinase. Some members of this class of compounds have also been investigated for their local anesthetic, antiarrhythmic,[16] herbicidal,[17] molluscicidal properties,[18] and for antiviral[19] and immunosuppressant activities.[20]

Structurally simple 5-amino-1-*tert*-butyl pyrazole-4-carboxamide **A** was found to inhibit p56 Lck[16] (Figure 1). 5-Amino-1-(4-methylphenyl)pyrazole **B** has been tested as an NPY5 antagonist.[17] 5-Amino-4-benzoyl-3-methylthio-1-(2,4,6-trichlorophenyl)pyrazole **C** has been reported as a potent corticotrophin-releasing factor-1 (CRF-1) receptor antagonist.[18]

5-Amino-1-(2,6-dichloro-4-(trifluoromethyl)phenyl)-4-(3-methoxyphenyl)-3-methylthio-pyrazole **D** has been described as a potent GABA (gamma-aminobutyric acid) inhibitor with selectivity towards insect *versus* mammalian receptors.[19] The simple *N*-phenyl amide of 5-amino-1,3-dimethyl pyrazole-4-carboxylic acid **E** has been shown to exhibit antifungal activity.[21] The 5-amino-1-pyrazinyl-3-carboxamido pyrazole derivative **F** has been recently reported as a potent antibacterial agent with a very broad spectrum (Figure 1).[22]

Recently, components of the mitotic machinery have been targeted in an attempt to develop novel anticancer agents. These include critical signaling kinases such as the Aurora, polo-like kinases (PLK), and the cyclin-dependent kinases (CDK). Compound **G** (AZD1152) is the first Aurora B selective inhibitor to enter clinical trials[23] (Figure 2). Aurora B facilitates proper bipolar end-on microtubule (MT)-kinetochore attachment,[24] participates in spindle assembly checkpoint (SAC) signaling,[25] and mediates

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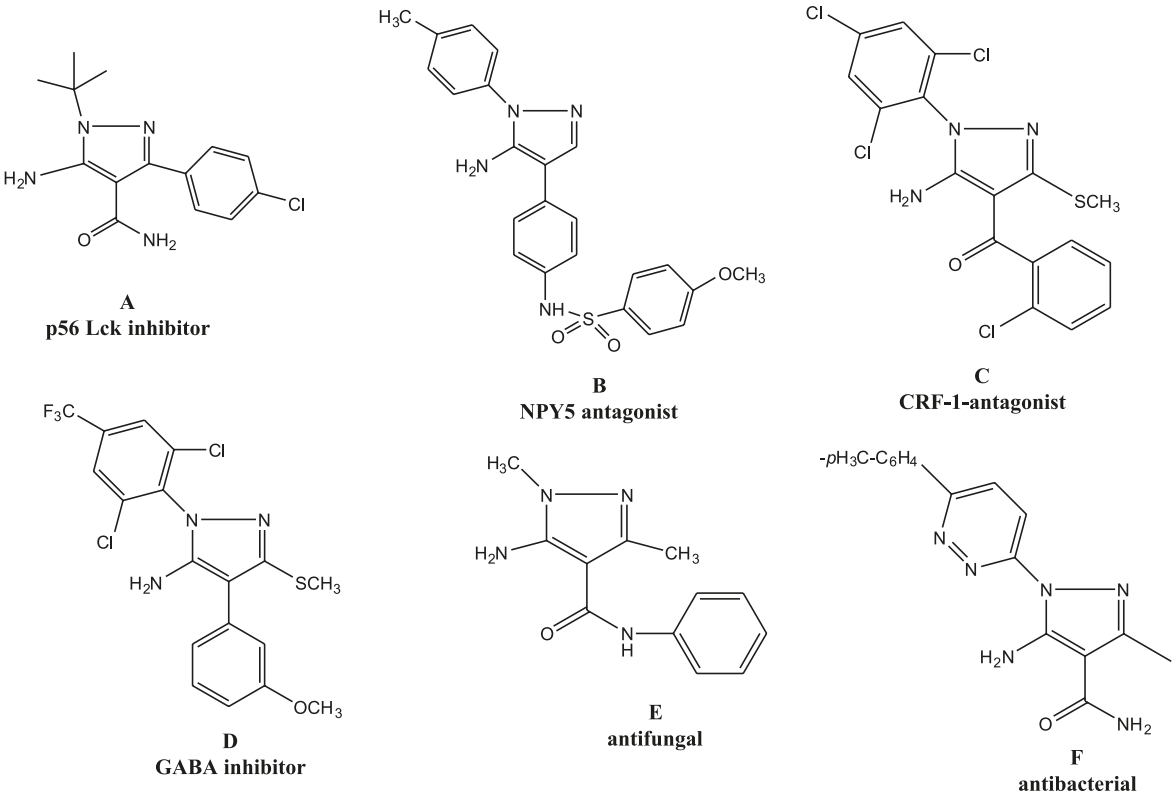


Figure 1. Pharmacology active 5-aminopyrazoles.

chromosome condensation and cohesion.[26] Also, Aurora B relocalizes to the central spindle during late anaphase and to the mid-body during telophase, thereby facilitating cytokinesis.[27] Chemical perturbation of Aurora kinases has proven invaluable in parsing the temporal and spatial functions of each isoform and assessing the therapeutic potential in inhibiting kinase activity in the context of cancer.

On the other hand, thieno[2,3-*c*]pyrazoles have created great interest in medicinal chemistry due to their broad spectrum of antitumor, antiviral, antimicrobial and anti-inflammatory activities.

In the light of the previous biological importance of pyrazoles and thienopyrazoles, and in continuation of our work for synthesis of new thieno[2,3-*c*]pyrazoles,[28-30] we have synthesized a series of novel pyrazolothienotriazines **6**-**14**. Literature survey revealed that the pyrazolothienotriazine system has not been previously synthesized. Therefore, as a result of resistance of some bacterial and fungi strains to the existing antimicrobial therapy, we got interested in the search for syntheses of more effective agents. In addition, non-steroidal anti-inflammatory drugs (NSAIDs), which are widely used for reducing pain and swelling associated with inflammation, represent a research area of continuous development. Hence, the suspected promising biological activities of the pyrazolothienotriazine compounds encouraged us to study the *in vitro* anti-microbial and *in vivo* anti-inflammatory activities of some pyrazolothienotriazine heterocycles in comparison with the standard drugs. The obtained results from biological screening demonstrated

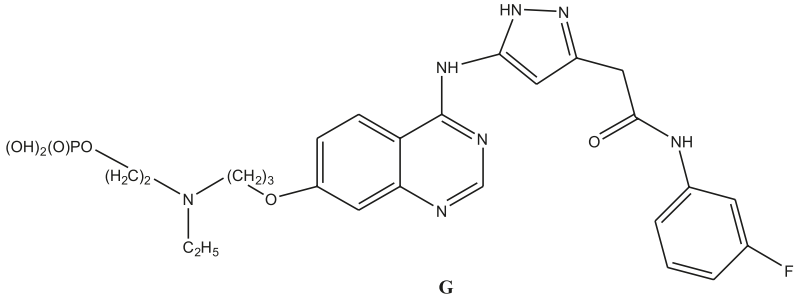


Figure 2. Anticancer agent AZD 1152.

that most of pyrazolothienotriazine compounds revealed promising antibacterial, antifungal and anti-inflammatory activities, which can be used as potential antibacterial, antifungal and anti-inflammatory drugs.

Experimental

All the required chemicals were purchased from Merck, Sigma-Aldrich and Loba chemical companies. The melting points were uncorrected and recorded on a Gallen Kamp electric melting point apparatus. The elemental analyses were carried out at the Micro Analytical Center of Chemistry Department, Assiut University, Egypt. The Fourier transform infrared (FTIR) spectra were recorded using potassium bromide disks on a FT-IR 8201 PC Shimadzu. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were obtained on Varian Mercury VX-300 NMR (300 MHz) and Bruker (400 MHz) spectrometers in $CDCl_3$ and DMSO-d_6 using tetramethylsilane (Me_4Si) as internal standard and chemical shifts were expressed as ppm. Mass spectra were measured on a Jeol-JMS 600 spectrometer at the Regional Center for Mycology & Biotechnology, Al-Azhar University, Cairo, Egypt. All reactions were monitored by thin layer chromatography (TLC) technique on silica gel coated aluminum sheets (silica gel 60 F_{254}, Merck). The chloropyrazolecarbonitrile compound (**1**) was prepared according to the literature procedure.[31] Numbering of carbon atoms used in ^{13}C NMR analyses for compounds **9a**, **9c**, **13** and **14** is shown in Figure 3.

5-(Cyanomethylthio)-3-methyl-1-phenyl-1*H*-pyrazole-4-carbonitrile (**4**)

To a stirred suspension of finely powdered sulfur (4.00 g, 0.125 mol) in absolute ethanol (60 mL) in an ice bath, sodium borohydride (4.00 g, 0.105 mol) was added in small portions until all sulfur powder dissolved. Chlorocyanopyrazole **1** (10.00 g, 46 mmol) was added with stirring for additional 1 h. The reaction mixture was heated under reflux at 100 °C for 4 h, followed by cooling. At this stage, the non-isolated sulfanyl sodium salt **3** was formed. After reflux was completed, chloroacetonitrile (3.50 mL, 46 mmol) was added to the reaction mixture and was left overnight with stirring. The solid precipitate formed on cooling was filtered, dried and recrystallized from ethanol as white crystals in 85% (10.00 g) yield; mp 78-80 °C; FTIR (KBr) ν / cm^{-1} 3035 (CH aromatic), 2985-2925 (CH aliphatic), 2275, 2227 (2CN); ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.85 (s, 3H, CH_3), 3.85 (s, 2H, CH_2), 7.60-7.40 (m, 5H, ArH); MS (EI, 70 eV) *m/z* 254.40 $[M]^+$, 239.24 $[M - CH_3]^+$, 177.05 $[M - Ph]^+$; anal. calcd. for $C_{13}H_{10}NO_4S$ (254.32): C, 61.40; H, 3.96; N, 22.03; S, 12.61%. Found: C, 61.23; H, 4.20; N, 21.98; S, 12.75%.

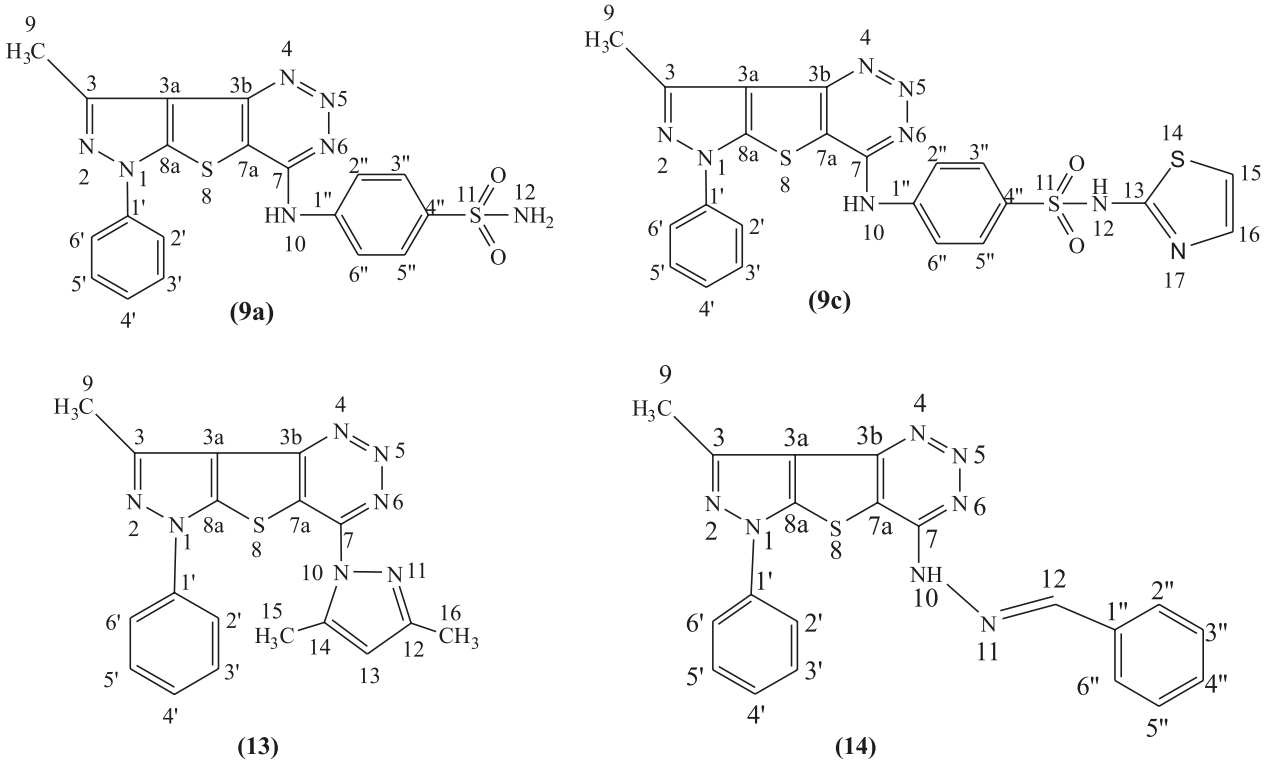


Figure 3. Carbon numbering of compounds **9a**, **9c**, **13** and **14**.

4-Amino-3-methyl-1-phenyl-1*H*-thieno[2,3-*c*]pyrazole-5-carbonitrile (**5**)

Pyrazole **4** (4.00 g, 16 mmol) was heated under reflux at 100 °C in ethanolic sodium ethoxide solution (prepared from 0.50 g of finely divided sodium metal in 20 mL of absolute ethanol) for 10 min. The solid precipitate, which separated out during reflux, was filtered, dried and recrystallized from ethanol:dioxane mixture (2:1) as white crystals in 75% (3.00 g) yield; mp 198-200 °C; FTIR (KBr) ν / cm^{-1} 3455, 3359, 3229 (NH_2), 3045 (CH aromatic), 2950, 2890 (CH aliphatic), 2183 (CN); ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.50 (s, 3H, CH_3), 7.00 (s, 2H, NH_2), 7.30-7.60 (m, 5H, ArH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 13 (C7: CH_3 pyrazole), 71.50 (C5), 107.50 (C3a), 116.50 (C9: CN), 122 (C2', C6' aromatic), 126 (C4' aromatic), 127 (C6a), 130.50 (C3', C5' aromatic), 138.50 (C1' aromatic), 144 (C3), 148 (C4); MS (EI, 70 eV) *m/z* 255.81 $[M + 1]^+$, 254.33 $[M]^+$; anal. calcd. for $C_{13}H_{10}N_4S$ (254.32): C, 61.40; H, 3.96; N, 22.03; S, 12.61%. Found: C, 61.50; H, 4.03; N, 21.99; S, 12.65%.

4-Chloro-8-methyl-6-phenyl-6*H*-pyrazolo[4',3':4,5]thieno[3,2-*d*][1,2,3]triazine (**6**)

To a stirred solution of the thienopyrazole **5** (1.30 g, 5 mmol) in a mixture of acetic acid (10 mL) and concentrated HCl 37% (7 mL) at 0-5 °C, sodium nitrite solution (0.40 g in 4 mL H_2O, 10%) was added drop wise within 5 min. After the addition was completed, the reaction mixture was stirred for additional 3 h. The solid product formed was collected, dried and recrystallized from ethanol as orange crystals in 51% (0.78 g) yield; mp 160-162 °C; FTIR (KBr) ν / cm^{-1} 2950 (CH aliphatic), 3047 (CH aromatic) and 1593 (C=N); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.90 (s, 3H, CH_3), 7.40-7.80 (m, 5H, ArH); MS (EI, 70 eV) *m/z* 303 $[M + 2]^+$, 301 $[M]^+$, 266 $[M - Cl]^+$; anal. calcd. for $C_{13}H_8ClN_5S$ (301.75): C, 51.75; H, 2.67; Cl, 11.75; N, 23.21; S, 10.62%. Found: C, 51.79; H, 2.63; Cl, 11.79; N, 23.27; S, 10.52%.

8-Methyl-6-phenyl-4-(*p*-substituted phenylamino)-6*H*-pyrazolo[4',3':4,5]thieno[3,2-*d*][1,2,3] triazines (**7a-c**, **8a-c** and **9a-c**)

General procedure

To a stirred solution of the chlorotriazine **6** (0.25 g, 0.83 mmol) in absolute ethanol (10 mL), the corresponding primary or secondary amine (2 mmol) and triethylamine (0.1 mL) were added, The reaction mixture was gently refluxed for 2 h. The solid precipitate, which separated out during reflux, was filtered, dried and recrystallized from the proper solvent.

8-Methyl-6-phenyl-4-phenylamino-6*H*-pyrazolo[4',3':4,5]thieno[3,2-*d*][1,2,3]triazine (**7a**)

Obtained by the reaction with aniline. The solid product formed was recrystallized from ethanol:dioxane mixture (2:1) as pale violet crystals in 34% (0.10 g) yield; mp 100-102 °C; FTIR (KBr) ν / cm^{-1} 3421 (NH), 2900 (CH aliphatic), 3048 (CH aromatic), 1596 (C=N); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.20 (s, 3H, CH_3), 7.10-7.80 (m, 10H, ArH), 9.00 (s, 1H, NH); MS (EI, 70 eV) *m/z* 357 $[M - 1]^+$ (6.36%); anal. calcd. for $C_{19}H_{14}N_6S$ (358.42): C, 63.67; H, 3.94; N, 23.45; S, 8.94%. Found: C, 63.63; H, 3.91; N, 23.42; S, 9.04%.

8-Methyl-6-phenyl-4-(*p*-tolylamino)-6*H*-pyrazolo[4',3':4,5]thieno[3,2-*d*][1,2,3]triazine (**7b**)

Obtained by the reaction with *p*-toluidine. The solid product formed was recrystallized from ethanol:dioxane mixture (2:1) as greenish white crystals in 78% (0.24 g) yield; mp 128-130 °C; FTIR (KBr) ν / cm^{-1} 3377 (NH), 3030 (CH aromatic), 2922 (CH aliphatic), 1597 (C=N); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.30 (s, 3H, CH_3 pyrazole), 2.40 (s, 3H, CH_3 *p*-tolyl), 7.00-7.80 (m, 9H, ArH), 8.80 (s, 1H, NH); anal. calcd. for $C_{20}H_{16}N_6S$ (372.45): C, 64.50; H, 4.33; N, 22.56; S, 8.61%. Found: C, 64.55; H, 4.28; N, 22.60; S, 8.57%.

4-(*p*-Anisylamino)-8-methyl-6-phenyl-6*H*-pyrazolo[4',3':4,5]thieno[3,2-*d*][1,2,3]triazine (**7c**)

Obtained by the reaction with *p*-anisidine. The solid product formed was recrystallized from ethanol:dioxane mixture (2:1) as faint brown crystals in 52% (0.17 g) yield; mp 80-82 °C; FTIR (KBr) ν / cm^{-1} 3400 (NH), 2900 (CH aliphatic), 1595 (C=N), 1244 (C–O); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.70 (s, 3H, CH_3 pyrazole), 3.90 (s, 3H, CH_3 *p*-anisyl), 7.30-7.80 (m, 9H, ArH), 8.55 (s, 1H, NH); anal. calcd. for $C_{20}H_{16}N_6OS$ (388.45): C, 61.84; H, 4.15; N, 21.64; O, 4.12; S, 8.25%. Found: C, 61.94; H, 4.25; N, 21.58; S, 8.18%.

8-Methyl-6-phenyl-4-(piperidin-1-yl)-6*H*-pyrazolo[4',3':4,5]thieno[3,2-*d*][1,2,3]triazine (**8a**)

Obtained by the reaction with piperidine. The solid product formed was collected and recrystallized from ethanol:dioxane mixture (2:1) as faint brown crystals in 71% (0.20 g) yield; mp 100-102 °C; FTIR (KBr) ν / cm^{-1} 2852, 2932 (CH aliphatic), 3010 (CH aromatic), 1595 (C=N); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.60 (m, 2H, CH_2: C4 piperidinyl), 2.50 (s, 3H, CH_3 pyrazole), 2.78 (m, 4H, $2CH_2$: C3, C5 piperidinyl), 3.94 (m, 4H, $2CH_2$: C2, C6 piperidinyl), 7.19-7.72 (m, 5H, ArH); anal. calcd. for $C_{18}H_{18}N_6S$ (350.44): C, 61.69; H, 5.18;

N, 23.98; S, 9.15%. Found: C, 61.75; H, 5.15; N, 23.84; S, 9.26%.

8-Methyl-4-(morpholin-4-yl)-6-phenyl-6*H*-pyrazolo[4',3':4,5]thieno[3,2-*d*][1,2,3]triazine (**8b**)

Obtained by the reaction with morpholine. The solid product formed was recrystallized from ethanol:dioxane mixture (2:1) as faint brown crystals in 52% (0.15 g) yield; mp 108-110 °C; FTIR (KBr) ν / cm^{-1} 3010 (CH aromatic), 2920, 2854 (CH aliphatic), 1596 (C=N); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.70 (s, 3H, CH_3), 2.70-2.80 (m, 4H, $(CH_2)_2$N morpholinyl), 3.80-4.20 (m, 4H, $(CH_2)_2$O morpholinyl), 7.30-7.90 (m, 5H, ArH); MS (EI, 70 eV) *m/z* 352 $[M]^+$ (42%), 266 [M – morpholine]$^+$ (16%); anal. calcd. for $C_{17}H_{16}N_6OS$ (352.42): C, 57.94; H, 4.58; N, 23.85; S, 9.10%. Found: C, 57.87; H, 4.45; N, 23.84; S, 9.20%.

8-Methyl-6-phenyl-4-(piperazin-1-yl)-6*H*-pyrazolo[4',3':4,5]thieno[3,2-*d*][1,2,3]triazine (**8c**)

Obtained by the reaction with piperazine. The solid product formed was collected, dried and recrystallized from ethanol:dioxane mixture (2:1) as violet crystals in 43% (0.13 g) yield; mp 140-142 °C; FTIR (KBr) ν / cm^{-1} 3020 (CH aromatic), 2922 (CH aliphatic), 1596 (C=N), 3434 (NH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.50 (s, 1H, NH piperidine), 2.80 (s, 3H, CH_3), 3.20 (m, 4H, 2$\underline{CH_2}$NH: C_3, C_5 piperazine), 4.00 (m, 4H, 2$\underline{CH_2}$N: C_2, C_4 piperazine), 7.40-7.90 (m, 5H, ArH); anal. calcd. for $C_{17}H_{17}N_7S$ (351.43): C, 58.10; H, 4.88; N, 27.90; S, 9.12%. Found: C, 58.17; H, 4.95; N, 27.84; S, 9.04%.

4-((8-Methyl-6-phenyl-6*H*-pyrazolo[4',3':4,5]thieno[3,2-*d*][1,2,3]triazin-4-yl)amino)-*p*-benzene sulfonamide (**9a**)

Obtained by the reaction with sulfanilamide. The solid product formed was filtered and recrystallized from ethanol:dioxane mixture (2:1) as yellowish white crystals in 28% (0.10 g) yield; mp 122-124 °C; FTIR (KBr) ν / cm^{-1} 3400, 3370 and 3200 (NH, NH_2), 3080 (CH aromatic), 2920 (CH aliphatic), 1595 (C=N), 1443 (SO_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.50 (s, 3H, CH_3), 5.90 (s, 2H, NH_2), 6.90-7.70 (m, 9H, ArH), 8.70 (s, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.50 (C9: CH_3 pyrazole), 104 (C7a), 106 (C3a), 114.50 (C2", C6": benzene sulfonamide), 118 (C2', C6': Ph pyrazole), 124 (C4': Ph pyrazole), 126.50 (C3b), 128 (C8a), 129 (C3', C5': Ph pyrazole), 131 (C3", C5": benzene sulfonamide), 133 (C4": benzene sulfonamide), 138 (C1': Ph pyrazole), 144 (C1": benzene sulfonamide), 147 (C3), 155 (C7); anal. calcd. for $C_{19}H_{15}N_7O_2S_2$ (437.50): C, 52.16; H, 3.46; N, 22.41; S, 14.66%. Found: C, 52.13; H, 3.56; N, 22.34; S, 14.54%.

N-Carbamimidoyl-4-((8-methyl-6-phenyl-6*H*-pyrazolo[4',3':4,5]thieno[3,2-*d*][1,2,3]triazin-4-yl)amino)-*p*-benzenesulfonamide (**9b**)

Obtained by the reaction with sulfaguanidine. The solid product formed was recrystallized from ethanol:dioxane mixture (2:1) as faint brown crystals in 57% (0.23 g) yield; mp 144-146 °C; FTIR (KBr) ν / cm^{-1} 3435, 3400, 3344, 3223 (NH_2, 3NH), 2910 (CH aliphatic), 3050 (CH aromatic), 1597 (C=N), 1442 (SO_2); ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.50 (s, 3H, CH_3), 5.55 (s, 1H, SO_2NH), 6.50 (s, 2H, NH_2), 7.30-7.60 (m, 9H, ArH), 8.35 (s, 1H, C=NH), 9.10 (s, 1H, NH phenyl); anal. calcd. for $C_{20}H_{17}N_9O_2S_2$ (479.54): C, 50.09; H, 3.57; N, 26.29; S, 13.37%. Found: C, 50.17; H, 3.68; N, 26.25; S, 13.33%.

4-((8-Methyl-6-phenyl-6*H*-pyrazolo[4',3':4,5]thieno[3,2-*d*][1,2,3]triazin-4-yl)amino)-*N*-thiazol-2-yl)-*p*-benzenesulfonamide (**9c**)

Obtained by the reaction with sulfathiazole. The solid product formed was recrystallized from ethanol:dioxane mixture (2:1) as faint brown crystals in 58% (0.25 g) yield; mp 138-140 °C; FTIR (KBr) ν / cm^{-1} 3360, 3320 (NH), 3060 (CH aromatic), 2909 (CH aliphatic), 1595 (C=N), 1425 (SO_2); ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.50 (s, 3H, CH_3), 6.50, 6.70 (2d, *J* 3.20 Hz, 2H, 2CH thiazolyl), 7.20-7.70 (m, 9H, ArH), 8.80 (s, 1H, NH phenyl), 12.40 (s, 1H, SO_2NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 13 (C9: CH_3 pyrazole), 101.50 (C7a), 104 (C3a), 114 (C17), 117 (C2", C6": benzene sulfonamide), 119 (C2', C6': Ph pyrazole), 123.50 (C4': Ph pyrazole), 126 (C3b), 128 (C8a), 130 (C3', C5': Ph pyrazole), 132 (C4": benzene sulfonamide), 133 (C3", C5": benzene sulfonamide), 136 (C16), 138 (C1': Ph pyrazole), 142 (C1": benzene sulfonamide), 147 (C3), 150 (C7), 166 (C14); anal. calcd. for $C_{22}H_{16}N_8O_2S_3$ (520.60): C, 50.76; H, 3.10; N, 21.52; S, 18.47%. Found: C, 50.67; H, 3.15; N, 21.54; S, 18.40%.

4-Hydrazino-8-methyl-6-phenyl-6*H*-pyrazolo[4',3':4,5]thieno[3,2-*d*][1,2,3]triazine (**10**)

A suspension of the chlorotriazine compound **6** (0.25 g, 0.83 mmol) and hydrazine hydrate (2.00 mL, 0.04 mol) was gently heated in absence of solvent under neat conditions for 5 min, then absolute ethanol (10 mL) was added. The reaction mixture was refluxed for additional 2 h. The solid precipitate formed after cooling was filtered, dried and recrystallized from ethanol as faint brown crystals in 80% (0.20 g) yield; mp 240-242 °C; FTIR (KBr) ν / cm^{-1} 3463, 3361 and 3255 (NH, NH_2), 2917, 2848 (CH aliphatic), 3030 (CH aromatic), 1596 (C=N); ^{1}H NMR

(300 MHz, DMSO-d_6) δ 2.60 (s, 3H, CH_3), 5.80 (s, 2H, NH_2), 7.35-7.80 (m, 5H, ArH), 8.55 (s, 1H, NH); MS (EI, 70 eV) *m/z* 297 $[M]^+$ (51.89%); anal. calcd. for $C_{13}H_{11}N_7S$ (297.34): C, 52.51; H, 3.73; N, 32.98; S, 10.78%. Found: C, 52.63; H, 3.82; N, 32.84; S, 10.71%.

7-Methyl-9-phenyl-9*H*-pyrazolo[4',3':4,5]thieno[2,3-*e*][1,2,4]triazolo[4,3-*c*][1,2,3]triazine (**11**)

A mixture of the hydrazino compound **10** (1.00 g, 3.30 mmol) and triethylorthoformate (3 mL) in the presence of few drops of acetic acid (0.5 mL) were refluxed for 1 h. The solid product that separated out during reflux was filtered, dried and recrystallized from acetic acid as faint brown crystals in 78% (0.80 g) yield; mp > 360 °C; FTIR (KBr) ν / cm^{-1} 2924 (CH aliphatic), 3046 (CH aromatic), 1596 (C=N); ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.60 (s, 3H, CH_3), 7.40-7.80 (m, 5H, ArH), 12.30 (s, 1H, CH triazole); anal. calcd. for $C_{14}H_9N_7S$ (307.34): C, 54.71; H, 2.95; N, 31.90; S, 10.43%. Found: C, 54.67; H, 2.85; N, 31.94; S, 10.54%.

7-Methyl-9-phenyl-2,9-dihydro-3*H*-pyrazolo[4',3':4,5]thieno[2,3-*e*][1,2,4]triazolo[4,3-*c*][1,2,3] triazine (**12**)

A solution of the hydrazino compound **10** (0.50 g, 1.70 mmol) and carbon disulfide (1 mL) in pyridine (2 mL) was heated on a steam bath for 8 h. The solid precipitate, which separated out upon heating, was recrystallized from ethanol as green needles in 66% (0.38 g) yield; mp > 360 °C; FTIR (KBr) ν / cm^{-1} 3400 (NH), 2950 (CH aliphatic), 3020 (CH aromatic), 1660 (C=N); ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.50 (s, 3H, CH_3), 7.40-7.80 (m, 5H, ArH), 9.80 (s, 1H, NH); anal. calcd. for $C_{14}H_9N_7S_2$ (339.40): C, 49.55; H, 2.67; N, 28.89; S, 18.89%. Found: C, 49.67; H, 2.69; N, 28.80; S, 18.84%.

4-(3,5-Dimethyl-1*H*-pyrazol-1-yl)-8-methyl-6-phenyl-6*H*-pyrazolo[4',3':4,5]thieno[3,2-*d*][1,2,3] triazine (**13**)

To a stirred solution of hydrazinotriazine **10** (0.71 g, 2.30 mmol) in 95% ethanol (20 mL), acetyl acetone (0.20 mL, 2.00 mmol) was added. The reaction mixture was heated under refluxed for 3 h to the point where the precipitate was formed. The solid precipitate, which separated out during reflux, was recrystallized from ethanol as faint brown crystals in 85% (0.73 g) yield; mp 248-250 °C; FTIR (KBr) ν / cm^{-1} 2921, 2850 (CH aliphatic), 3010 (CH aromatic), 1596 (C=N); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.30 (s, 3H, CH_3 pyrazole), 2.55 (s, 3H, CH_3 pyrazolyl), 2.75 (s, 3H, CH_3 pyrazolyl), 6.40 (s, 1H, CH pyrazolyl), 7.30-7.70 (m, 5H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 10 (C16: CH_3 pyrazolyl), 13 (C9: CH_3 pyrazole), 15 (C15: CH_3 pyrazolyl), 110 (C3a), 114.50 (C13: CH pyrazolyl), 118 (C2', C6': aromatic), 122 (C7a), 124 (C4': aromatic), 126 (C3b), 128.50 (C8a), 132 (C3', C5': aromatic), 137 (C1': aromatic), 142 (C14), 147 (C3), 150 (C12), 163.50 (C7); MS (EI, 70 eV) *m/z* 361 $[M]^+$ (100%); anal. calcd. for $C_{18}H_{15}N_7S$ (361.43): C, 59.82; H, 4.18; N, 27.13; S, 8.87%. Found: C, 59.77; H, 4.15; N, 27.19; S, 8.89%.

4-(2-Benzylidenehydrazino)-8-methyl-6-phenyl-6*H*-pyrazolo[4',3':4,5]thieno[3,2-*d*][1,2,3] triazine (**14**)

To a stirred solution of hydrazinotriazine **10** (0.70 g, 2.30 mmol) in ethanol (20 mL), benzaldehyde (1.00 mL, 10 mmol) and piperidine (0.2 mL) were added, the reaction mixture was heated under reflux for 3 h. The solid product, which separated out during reflux, was recrystallized from dioxane as yellow crystals in 74% (0.67 g) yield; mp 210-212 °C; FTIR (KBr) ν / cm^{-1} 3400 (NH), 3070 (CH aromatic), 2924 (CH aliphatic), 1597 (C=N); ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.50 (s, 3H, CH_3), 7.35-7.75 (m, 10H, ArH), 8.30 (s, 1H, CH benzylidene), 9.75 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 13 (C9: CH_3 pyrazole), 102 (C7a), 106 (C3a), 110 (C2', C6': Ph pyrazole), 115 (C4': Ph pyrazole), 119 (C3b), 124 (C8a), 126 (C3", 5": benzylidene), 128 (C2", C6": benzylidene), 132 (C3', C5': Ph pyrazole), 134 (C4": benzylidene), 140 (C1": benzylidene), 142.50 (C1': Ph pyrazole), 145 (C12), 147.50 (C3), 152 (C7); anal. calcd. for $C_{20}H_{15}N_7S$ (385.45): C, 62.32; H, 3.92; N, 25.44; S, 8.32%. Found: C, 62.37; H, 3.85; N, 25.39; S, 8.39%.

Biological activity tests

In vitro antibacterial assay

All microorganisms used were obtained from the culture collection of Microbiology Department, Faculty of Medicine, Assiut University. Activities of several synthesized compounds against a number of Gram-negative bacterial strains (*Haemophilus influenzae*, *Escherichia coli* and *Pseudomonas aeruginosa*) and a number of Gram-positive bacterial strains (*Streptococcus pneumoniae*, *Bacillus cereus* and *Bacillus subtilis*) were investigated using 5 mL solution of the tested compounds **8a-c** in DMSO as a solvent. The synthesized compounds were initially screened by a maximum concentration of 100 μg mL^{-1} in DMSO and a series of antibiotic drugs as references namely: clindamycin, streptomycin, gentamycin, levofloxacin, moxifloxacin and gemifloxacin. The sterile medium (nutrient agar medium, 15 mL) in each Petri dish

was uniformly smeared with cultures of Gram-positive and Gram-negative bacteria. Antibacterial activity of the tested compounds were determined according to the disc diffusion method reported by Kwon-Chung and Bennett[32] using 5 mm diameter filter paper discs loaded with 50 µL of the solution under investigation. The minimum inhibitory concentration (MIC) of each compound was taken as the lowest concentration (mg mL^{-1}) that did not give any visible bacteria growth. The plates were incubated at 37 ± 2 °C for 24 h and the zone of inhibition was determined and listed in Table 1.

In vitro antifungal assay

The fungal strains (*Candida albicans*, *Penicillium* sp., *Aspergillus fumigatus*, *Geotrichum candidum*, *Syncephalastrum racemosum* and *Trichophyton rubrum*) were obtained from some cases of human dermatophytosis (Assiut University Mycological Center, AUMC). The fungal strains were grown in sterilized 9-cm Petri dishes containing Sabouraud dextrose agar (SDA) supplemented with 0.05% of chloramphenicol to suppress bacterial contamination.[33] From these cultures, agar disks (10 mm diameter) containing spores were transferred aseptically to screw-topped vials containing 20 mL sterile distilled water. After shaking, 1 mL samples of the spore suspension were pipetted into sterile Petri dishes, followed by the addition of 15 mL liquefied SDA medium and then left to solidify. The tested compounds **8a**-**c** and the reference compound (ketoconazole) were dissolved in DMSO to give a concentration of 100 µg mL^{-1}. Antifungal activity was determined according to the disc diffusion method reported by Kwon-Chung and Bennett[32] using 5-mm diameter filter paper discs loaded with 50 µL of the solution under investigation (2.0%) and the inoculated plates were incubated at room temperature for 4 days. MIC of each compound was taken as the lowest concentration (mg mL^{-1}) that did not give any visible fungi growth. The zone of inhibition was determined and listed in Table 2.

In vivo anti-inflammatory activity

Anti-inflammatory activity for the newly synthesized compounds **7a**-**c**, **9b** and **9c** were measured *in vivo* using carrageenan-induced rat paw edema assay in comparison with indomethacin as a reference drug.[34,35] The test is

Table 1. Antibacterial activity (inhibition zone and minimum inhibitory concentration (MIC)) of compounds **8a**-**c**

Bacteria strain	Inhibition zone (MIC / (mg mL^{-1})) / mm			
	8a	**8b**	**8c**	Reference antibacterial agent
Haemophilus influenzae (–)	14 (4.0)	13 (5.0)	19 (4.0)	clindamycin 20 (4.0)
Escherichia coli (–)	18 (8.0)	21 (6.0)	24 (5.0)	streptomycin 26 (5.0)
Pseudomonas aeruginosa (–)	19 (4.0)	23 (4.0)	25 (4.0)	gentamycin 28 (4.0)
Streptococcus pneumoniae (+)	13 (4.0)	19 (4.0)	20 (5.0)	levofloxacin 20 (4.0)
Bacillus cereus (+)	16 (6.0)	22 (4.0)	25 (4.0)	moxifloxacin 28 (4.0)
Bacillus subtilis (+)	19 (4.0)	14 (6.0)	19 (4.0)	gemifloxacin 25 (4.0)

The amount added of the tested compounds **8a**-**c** and/or the reference antibacterial agent in each pore is 50 µg mL^{-1}.

Table 2. Antifungal activity (inhibition zone and minimum inhibitory concentration (MIC)) of compounds **8a**-**c**

Fungal strain	Inhibition zone (MIC / (mg mL^{-1})) / mm			
	8a	**8b**	**8c**	Ketoconazole
Candida albicans	14 (4.0)	17 (4.0)	19 (5.0)	21 (4.0)
Penicillium sp.	13 (5.0)	12 (5.0)	19 (3.0)	18 (4.0)
Aspergillus fumigatus	13 (6.0)	21 (4.0)	17 (4.0)	22 (4.0)
Geotrichum candidum	16 (5.0)	15 (6.0)	17 (5.0)	18 (5.0)
Syncephalastrum racemosum	17 (5.0)	14 (5.0)	14 (6.0)	19 (4.0)
Trichophyton rubrum	17 (5.0)	14 (5.0)	14 (6.0)	19 (4.0)

The amount added of the tested compounds **8a**-**c** and/or ketoconazole in each pore is 50 µg mL^{-1}.

based on the pedal inflammation in rat paw induced by sub plantar injection of 100 μL of 1% freshly prepared solution of carrageenan in distilled water into the right-hind paws of each rat for all the groups; the tested compounds were dissolved in distilled water with sonication. Male adult albino rats (150-200 g) were divided into six groups; each group containing three animals. The thickness of the rat paw edema was measured by a vernier caliper (SMIEC, China). Animals of groups A, B and C, were treated with a single dose of the tested compound, group D was treated with indomethacin drug. Paw thickness were measured just before the carrageenan injection, that is, at "0 hour" and then at 30 min, 1, 2, 3, 4, and 5 h after carrageenan injection. Increasing in paw thickness was measured as a difference in the paw thickness at "0 hour" and at respective hours. The edema was expressed as a mean reduction in paw thickness (mm) after treatment with tested compounds. The percentage of edema inhibition was calculated from the mean effect in the control and treated animals according to the following equation 1:

$$\text{Edema inhibition (\%)} = (1 - V_t/V_c) \times 100 \quad (1)$$

where V_t is the increase in paw volume of test and V_c is the increase in paw volume of control group of rats.

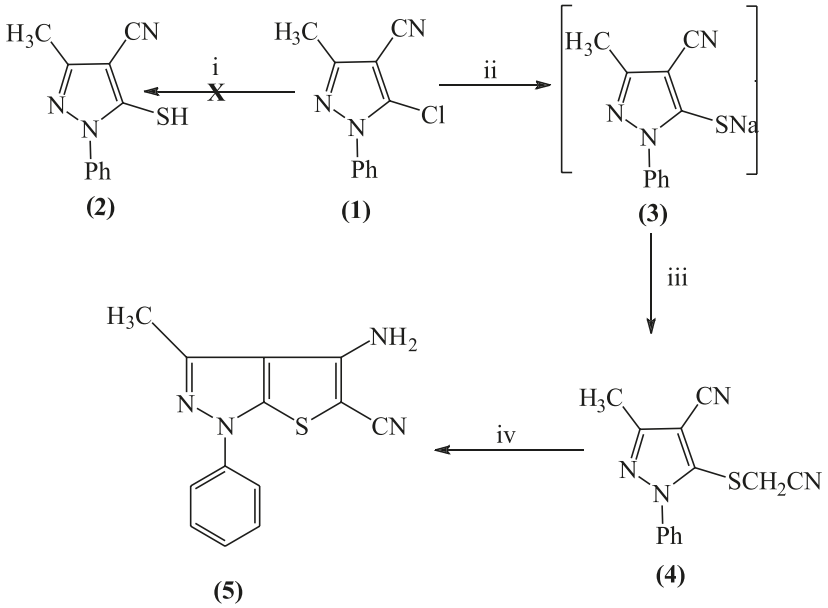
Statistical analysis

The results were analyzed by one way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test as a post-test. These analyses were carried out using GraphPad Prism software.[36] The significant differences between groups were accepted at $p < 0.05$*, 0.01** or 0.001***, and the data were expressed as a mean ± standard error (SE).

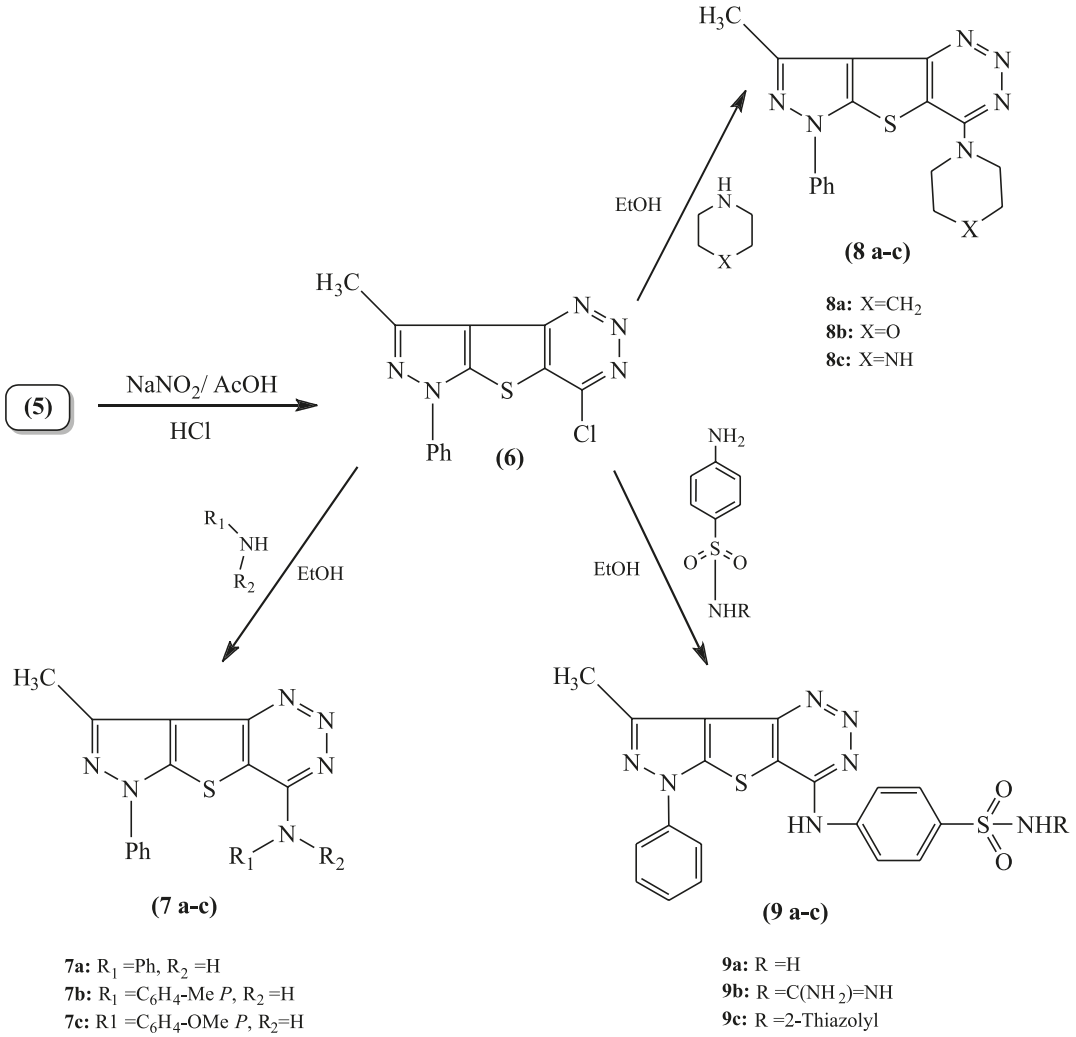
Results and Discussion

In the present work and in continuation of our program for synthesis of novel pyrazolothienotriazine heterocycles that exhibits biological importance, our synthesis is commenced with the preparation of the required substrate starting material **5**, which is a useful intermediate for synthesis of fused pyrazolothienotriazines. 4-Amino-3-methyl-1-phenyl-1*H*-thieno[2,3-*c*]pyrazole-5-carbonitrile (**5**) was synthesized by a new method according to literature procedure[28-30] (Scheme 1). All attempts to displace the chloride ion by the thiol group in the previously prepared 5-chloro-3-methyl-1-phenyl-1*H*-pyrazole-4-carbonitrile (**1**) by the reaction with thiourea in ethanol, as with other moieties, to obtain 5-mercapto-3-methyl-1-phenyl-1*H*-pyrazole-4-carbonitrile (**2**) failed, giving the chloropyrazole carbonitrile starting material **1**. The previous results forced us to search for another method to prepare the target *o*-aminothienopyrazole carbonitrile compound **5**. The desired results were achieved by the reaction of elemental sulfur with chloropyrazole **1** in the presence of sodium borohydride to give the non-isolated sulfanyl sodium salt **3**, which was subjected to react *in situ* with chloroacetonitrile to afford the pyrazolesulfanyl acetonitrile derivative **4**. The latter compound underwent Thorpe-Ziegler cyclization by heating in ethanolic sodium ethoxide solution to afford the amino thienopyrazolecarbonitrile **5**. The chemical structure of compound **5** was elucidated on the basis of its elemental and spectral data. IR spectrum revealed appearance of absorption band at 3455, 3359 and 3229 cm^{-1} due to NH_2 group. ^1H NMR spectrum showed two singlet signals at δ 2.50 and 7.00 ppm, characteristic of CH_3 and NH_2 groups, respectively. ^{13}C NMR spectrum displayed signals at δ 13 and 116.50 ppm attributed to CH_3 and CN groups, respectively. Also, mass spectrum displayed a peak at *m/z* 254.33 as a molecular ion peak.

Diazotization of the *o*-aminothienopyrazole carbonitrile **5** with sodium nitrite solution (10%) in a mixture of acetic acid and concentrated HCl, at room temperature, afforded the newly synthesized chloropyrazolothienotriazine **6**. The chemical structure of chlorotriazine **6** was confirmed by IR, ^1H NMR and mass spectra. IR spectrum of compound **6** revealed disappearance of absorption bands characteristic of NH_2 and CN groups and appearance of absorption band at 1593 cm^{-1} for C=N group. ^1H NMR spectrum of **6** exhibited disappearance of a singlet signal at δ 7.00 ppm for NH_2. Also, the mass spectrum of compound **6** displayed a peak at *m/z* 301, particular of a molecular ion peak. Furthermore, the chloride ion in compound **6** underwent nucleophilic substitution reactions with various primary and secondary amines upon heating in absence of solvent under neat conditions for a short time, followed by reflux in ethanol to give the *N*-substituted aminopyrazolothienotriazine derivatives **7**-**9** (Scheme 2). Assignment of the chemical structures for the newly synthesized compounds **7**-**9** were proved from their elemental and spectral analyses. IR spectrum of the phenylamino compound **7a** represented absorption band at 3421 cm^{-1} attributed to NH group. ^1H NMR spectrum showed a singlet signal at δ 9.00 ppm for NH group. Also, IR spectrum of piperidinyl compound **8a** showed absorption bands at 2852 and 2932 cm^{-1} for CH aliphatic of piperidine. ^1H NMR spectrum represented multiplet signals at δ 1.60-3.94 ppm for five CH_2 groups of piperidine. Moreover, IR spectrum of benzenesulfonamide **9a** displayed absorption band at 3330 and 3217 cm^{-1} for NH and NH_2 groups, and absorption band at 1444 cm^{-1} for SO_2 group. ^1H NMR spectrum revealed two singlet signals at



Scheme 1. Synthesis of 4-amino-3-methyl-1-phenyl-1*H*-thieno[2,3-*c*]pyrazole-5-carbonitrile (**5**). Reagents and conditions: (*i*) H_2NCSNH_2/EtOH, reflux 3 h; (*ii*) S/$NaBH_4$/EtOH, stirring in an ice bath 1 h; (*iii*) $ClCH_2CN$/ EtOH, reflux 2 h, stirring overnight; (*iv*) EtONa/ EtOH, Δ 10 min.



Scheme 2. Nucleophilic substitution of the chlorotriazine **6** with various primary and secondary amines affording the *N*-substituted triazine compounds **7**-**9**.

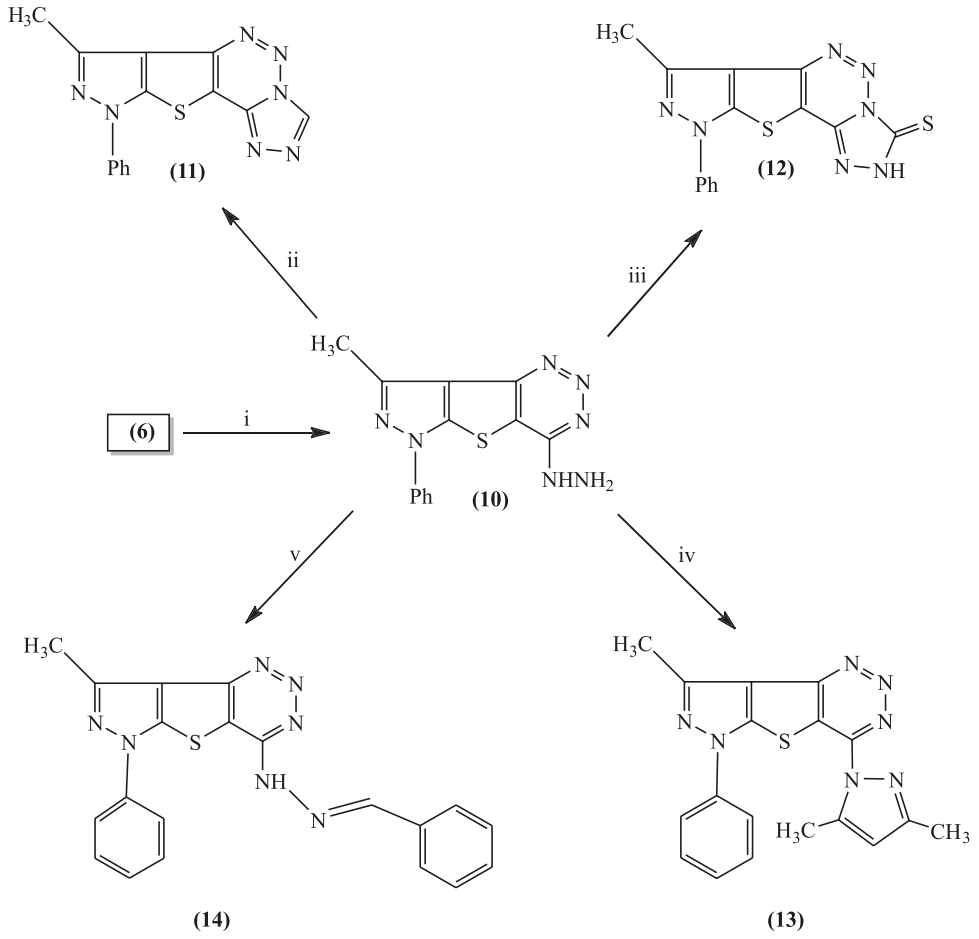
δ 5.90 and 8.70 ppm attributed to NH_2 and NH groups, respectively. ^{13}C NMR spectrum of compound **9a** revealed signals at δ 114-144 ppm, characteristic of 12 carbon atoms of the two phenyl rings.

Consequently, hydrazinolysis of the chlorotriazine compound **6** with hydrazine hydrate upon heating under neat conditions for a short time, followed by addition of ethanol, furnished the hydrazinopyrazolothienotriazine **10**. The latter compound **10** was used as a versatile precursor for synthesis of other heterocyclic rings attached or fused to pyrazolothienotriazine ring system to afford compounds **11**-**14** (Scheme 3). Thus, the reaction of hydrazino compound **10** with triethylorthoformate, in presence of a catalytic amount of acetic acid, afforded the pyrazolothienotriazolotriazine **11**. Also, nucleophilic addition of NH_2 group of hydrazino compound **10** to carbon disulfide, followed by elimination of H_2S, yielded the corresponding triazolotriazinethione derivative **12**. On the other hand, condensation of **10** with acetyl acetone and benzaldehyde gave the corresponding dimethylpyrazolyl **13** and the benzylidenehydrazinotriazine (Schiff's base) **14**, respectively. Formation of compounds **11**-**14** were assigned by both elemental and spectral analyses. IR spectrum of the triazolothione **12** displayed absorption band at 3400 cm^{-1} for NH group, while IR spectrum of **13** revealed disappearance of absorption bands of NH and NH_2 groups. ^1H NMR spectrum of **12** showed a singlet signal at δ 9.80 ppm due to NH group. ^1H NMR spectrum of **13** showed two singlet signals at δ 2.55 and 2.75 ppm, characteristic of $2CH_3$ groups of pyrazolyl ring, in addition to a singlet signal at δ 6.40 ppm for CH pyrazole. ^{13}C NMR spectrum of the dimethyl pyrazolyl compound **13** represented signals at δ 10 and 15 ppm, attributed to the two methyl groups of pyrazolyl ring, in addition to a signal at δ 114.50 ppm for CH pyrazolyl group.

Biological activities

Antibacterial activity

Antibacterial activity was determined according to the disc diffusion method reported by Kwon-Chung and



Scheme 3. Synthesis and reactions of the chlorotriazine compound **10** with various reagents forming triazolotriazines **11** and **12**, pyrazolyl **13** and benzylidenehydrazinopyrazolothienotriazine **14**. Reagents, conditions and yields: (*i*) NH_2NH_2, fusion 5 min, then reflux in EtOH, 2 h, 80%; (*ii*) $CH(OEt)_3$/AcOH, reflux, 1 h, 78%; (*iii*) CS_2/pyridine, reflux on steam bath, 8 h, 66%; (*iv*) Ac_2CH_2/EtOH, reflux, 3 h, 85%; (*v*) PhCHO/EtOH, piperidine, reflux, 3 h, 74%.

Bennett.[32] The antibacterial screening was measured by the average diameter of the inhibition zones, expressed in mm, and presented in Table 1. It was observed that all the tested compounds **8a-c** exhibited a significant antibacterial activity. The piperazinyl compound **8c** showed the highest antibacterial activity against all strains of bacteria (*Haemophilus influenzae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, *Bacillus cereus* and *Bacillus subtilis*), which its inhibition zones (19-25 mm) were very close to the reference antibiotics zones (20-28 mm). Also, the morpholinyl compound **8b** was very effective against *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae* and *Bacillus cereus* and showed comparable activity with the standard references. At the same time, compound **8b** exhibited a moderate activity against *Haemophilus influenzae* and *Bacillus subtilis*. The piperidinyl compound **8a** revealed a moderate activity against all the tested strains of bacteria as well as the lowest activity among the tested compounds.

Antifungal activity

Antifungal activity was determined according to the disc diffusion method reported by Kwon-Chung and Bennett.[32] The antifungal activity of compounds **8a-c** were reported as zones of inhibition and were summarized in Table 2. It revealed that compound **8c** exhibited the highest activity against *Candida albicans*, *Penicillium* sp., *Geotrichum candidum* and *Trichophyton rubrum*, which its inhibition zones (17-22 mm) were very close to ketoconazole ones (18-23 mm). The morpholinyl compound **8b** displayed a high antifungal activity against *Aspergillus fumigatus* as well as a moderate activity against the rest of the fungal strains. Furthermore, compound **8a** showed a high activity against *Geotrichum candidum* and *Syncephalastrum racemosum*. Also, the piperidinyl compound **8a** showed a moderate activity against *Candida albicans*, *Penicillium* sp. and *Aspergillus fumigatus* (Table 2).

Anti-inflammatory activity

The results of anti-inflammatory activity assessment for some of the newly synthesized compounds were summarized in Tables 3 and 4, and they were also presented in Figures 4 and 5. The results were analyzed by one way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test as a post-test. These analyses were carried out using GraphPad Prism software.[36] From the previous results shown in Tables 3 and 4, and Figures 4 and 5, we found that the anti-inflammatory activity of the *p*-substituted phenylaminotriazines **7b** and **7c**, and the substituted benzene sulfonamide compounds **9b** and **9c**, displayed the same effect as indomethacin after 30 min. The *p*-anisyl amino **7c** showed the highest effect of the tested compounds and very close to the effect of indomethacin after 1 h. After 3 h, the *p*-tolyl amino**7b** and the *p*-anisylamino **7c** represented a significant anti-inflammatory activity compared to indomethacin. After 4 and 5 h of treatment, the *p*-tolyl **7b**, *p*-anisyl **7c** and the *N*-carbamimidolyl **9b** revealed the highest anti-inflammatory activity and their effects were very close to the effect of indomethacin. Moreover, the phenylamino compound **7a** and the thiazolyl *p*-benzenesulfonamide **9c** represented low effect on the inflammation on rats during the period of experiment. From the previous results, we can conclude that the *p*-tolyl and *p*-anisylamino compounds **7b**, **7c** and the *N*-carbamimidolyl **9b** were the best anti-inflammatory agents among the tested compounds compared to indomethacin as a reference anti-inflammatory drug.

Conclusions

In the present work, we have provided an easy access for synthesis of novel tricyclic pyrazolothienotriazine **6**,

Table 3. Anti-inflammatory activity of compounds **7a-c**, **9b** and **9c** using acute carrageenan-induced paw edema in rats (statistical analysis)

Compound	Thickness of rat paws edema (mean ± SE)[a,b,c] / mm					
	30 min	1 h	2 h	3 h	4 h	5 h
7a	0.70 ± 0.00	0.65 ± 0.03	0.67 ± 0.02	0.58 ± 0.02	0.48 ± 0.02	0.47 ± 0.02
7b	0.68 ± 0.02	0.62 ± 0.02	0.52 ± 0.02	0.42 ± 0.02	0.42 ± 0.02	0.40 ± 0.00
7c	0.68 ± 0.02	0.60 ± 0.03	0.47 ± 0.02	0.42 ± 0.02	0.42 ± 0.02	0.40 ± 0.00
9b	0.68 ± 0.02	0.62 ± 0.02	0.57 ± 0.04	0.43 ± 0.02	0.42 ± 0.02	0.40 ± 0.00
9c	0.68 ± 0.02	0.63 ± 0.02	0.62 ± 0.03	0.47 ± 0.02	0.48 ± 0.02	0.42 ± 0.02
Indomethacin	0.68 ± 0.02	0.60 ± 0.03	0.45 ± 0.03	0.38 ± 0.02	0.37 ± 0.02	0.37 ± 0.02
Control	0.73 ± 0.02	0.75 ± 0.00	0.75 ± 0.00	0.77 ± 0.02	0.77 ± 0.02	0.77 ± 0.02

[a]Dose 20 μmol kg^{-1}; [b]n = 6; [c]statistically significant for the indomethacin at $p < 0.05$. SE: standard error.

Table 4. Paw edema inhibition for compounds **7a-c**, **9b** and **9c**

Compound	Anti-inflammatory activity (inhibition) / %					
	30 min	60 min	120 min	180 min	240 min	300 min
7a	4.11	13.33	10.67	24.68	37.66	38.96
7b	6.85	17.33	30.67	45.45	45.45	48.05
7c	6.85	20.00	37.33	45.45	45.45	48.05
9b	6.85	17.33	24.00	44.16	45.45	48.05
9c	6.85	16.00	17.33	38.96	37.66	45.45
Indomethacin	6.85	20.00	40.00	50.65	51.95	51.95

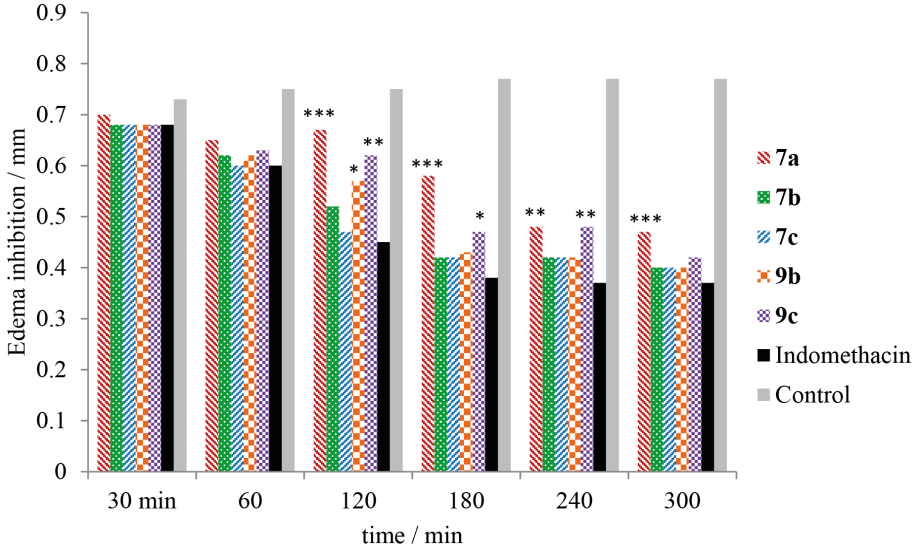


Figure 4. The relationship between paw edema inhibition with time.

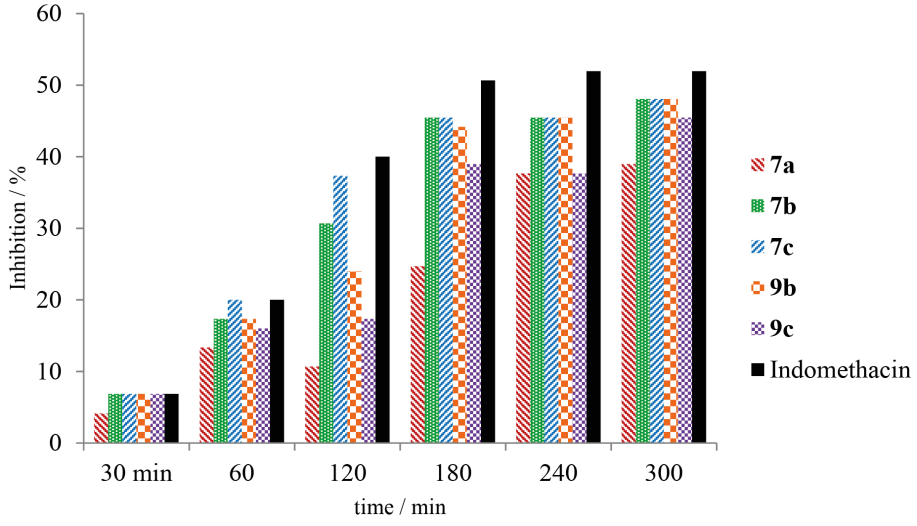


Figure 5. The percentage of edema inhibition with time.

which was used as a versatile precursor for the synthesis of *N*-alkyl(aryl)amino triazines **7-9** and building new heterocyclic ring systems namely: triazole and pyrazole, attached or fused to the pyrazolothienotriazine moiety.

The results of antimicrobial activities assays demonstrated that the tested compounds **8a-c** represented significant antibacterial and antifungal activities. On the other hand, the *p*-tolylamino**7b**, *p*-anisyl amino **7c** and

the *N*-carbamimidolyl **9b** showed the highest anti-inflammatory activities compared to indomethacin. From the previous results, we found that most of the examined novel pyrazolothienotriazines exhibited promising antibacterial, antifungal and anti-inflammatory activities, which can be used as potential antibacterial, antifungal and anti-inflammatory drugs.

Supplementary Information

Supplementary information (FTIR, ^{1}H NMR, ^{13}C NMR and mass spectral analyses) is available free of charge at http://jbcs.sbq.org.br as PDF file.

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References

1. Amir, M.; Shikha, K.; *Eur. J. Med. Chem.* **2004**, *39*, 535.
2. Bekhit, A. A.; Ashour, H. M.; Abdel Ghany, Y. S.; Bekhit, A. D.; Baraka, A.; *Eur. J. Med. Chem.* **2008**, *43*, 456.
3. Palaska, E.; Sahin, G.; Kelicen, P.; Demirdamar, R.; Altinok, G.; *Chem. Abstr.* **2001**, *135*, 326954a.
4. Palaska, E.; Sahin, G. K. P.; Durlu, N. T.; Altinok, G.; *Farmaco* **2002**, *57*, 101.
5. Sauzem, P. D.; Machado, P.; Rubin, M. A.; Sant'Anna, G. S.; Faber, H. B.; de Souza, A. H.; Mello, C. F.; Beck, P.; Burrow, R. A.; Bonacorso, H. G.; Zanatta, N.; Martins, M. A. P.; *Eur. J. Med. Chem.* **2008**, *43*, 1237.
6. El-Sayed, W. A.; Flefel, E. M.; Morsy, E. M. H.; *Pharma Chem.* **2012**, *4*, 23.
7. Ragavan, R. V.; Vijayakumar, V.; Kumari, N. S.; *Eur. J. Med. Chem.* **2010**, *45*, 1173.
8. Padmaja, A.; Payani, T.; Dinneswara, R. G.; Padmavathi, V.; *Eur. J. Med. Chem.* **2009**, *44*, 4557.
9. El-borai, M. A.; Rizk, H. F.; Abd-Aal, M. F.; El-Deeb, I. Y.; *Eur. J. Med. Chem.* **2012**, *48*, 92.
10. Riyadh, S. M.; Farghaly, T. A.; Abdallah, M. A.; Abdalla, M. M.; Abd El-Aziz, M. R.; *Eur. J. Med. Chem.* **2010**, *45*, 1042.
11. Vujasinovic, I.; Paravic-Radicevic, A.; Mlinaric-Majerski, K. K.; Brajsa, K.; Bertosa, B.; *Bioorg. Med. Chem.* **2012**, *20*, 2101.
12. Genin, M. J.; Biles, C.; Keiser, B. J.; Poppe, S. M.; Swaney, S. M.; Tarplay, W. G.; Yagi, Y.; Romero, D. L.; *J. Med. Chem.* **2000**, *43*, 1034.
13. Rashad, A. E.; Hegab, M. I.; Abdel-Megeid, R. E.; Fathalla, N.; Abdel-Megeid, F. M. E.; *Eur. J. Med. Chem.* **2009**, *44*, 3285.
14. Inoue, H.; Murafuji, H.; Hayashi, Y.; *US pat. 2009/0131413 A1* **2009**.
15. Bindi, S.; Fancelli, D.; Alli, C.; Berta, D.; Bertrand, J. A.; Cameron, A. D.; Cappella, P.; Carpinelli, P.; Cervi, G.; Croci, V.; Anello, M. D.; Forte, B.; Giorgini, M. L.; Marsiglio, A.; Moll, J.; Pesenti, E.; Pittalà, V.; Pulici, M.; Sirtori, F. R.; Roletto, F.; Soncini, Ch.; Storici, P.; Varasi, M.; Volpi, D.; Zugnoni, P.; Vianello, P.; *Bioorg. Med. Chem.* **2010**, *18*, 7113.
16. Iovu, M.; Zalaru, C.; Dumitrascu, F.; Draghici, C.; Cristea, E.; *Farmaco* **2000**, *55*, 362.
17. Vicentini, C. B.; Manfrini, M.; Mazzanti, M.; Scatturin, A.; Romagnoli, C.; Mares, D.; *Arch. Pharm.* **1999**, *332*, 337.
18. Nawwar, G. A.; Swellem, R. H.; Ibrahim, A. M.; *Arch. Pharm. Res.* **1994**, *17*, 66.
19. Storer, R.; Ashton, C. J.; Baxter, A. D.; Hann, M. M.; Mar, C. L.; Mason, A. M.; Mo, C. L.; Meyers, P. L.; Noble, S. A.; Penn, C. R.; Weir, N. G.; Woods, J. M.; Coe, P. L.; *Nucleosides Nucleotides* **1999**, *18*, 203.
20. Wang, A. X.; Xie, Q.; Lane, B.; Mollison, K. W.; Hseih, G. C.; March, K.; Sheets, M. P.; Luly, J. R.; Coghlan, M. J.; *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2787.
21. Huppatz, J. L.; *Aust. J. Chem.* **1985**, *38*, 221.
22. Davis, P. D.; Davis, J. M.; Moffat, D. F. C.; *WO pat. 9740019 (A1)* **1997**.
23. Kordik, C. P.; Luo, C.; Zanoni, B. C.; Lovenberg, T. W.; Wilson, S. J.; Vaidya, A. H.; Crooke, J. J.; Rosenthal, D. I.; Reitz, A. B.; *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2287.
24. Lampson, M. A.; Renduchitala, K.; Khodjakov, A.; Kapoor, T. M.; *Nat. Cell. Biol.* **2004**, *6*, 232.
25. Santaguida, S.; Vernieri, C.; Villa, F.; Ciliberto, A.; Musacchio, A.; *EMBO J.* **2011**, *30*, 1508.
26. Giet, R.; McLean, D.; Descamps, S.; Lee, M. J.; Raff, J. W.; Prigent, C.; Glover, D. M.; *J. Cell. Biol.* **2002**, *156*, 437.
27. Terada, Y.; Tatsuka, M.; Suzuki, F.; Yasuda, Y.; Fujita, S.; Otsu, M.; *EMBO J.* **1998**, *17*, 667.
28. Kamal El-Dean, A. M.; Zaki, R. M.; Abdulrazzaq, A. Y.; *Russ. J. Bioorg. Chem.* **2015**, *41*, 97.
29. Sayed, A. H.; Zaki, R. M.; Kamal El-Dean, A. M.; Abdulrazzaq, A. Y.; *Toxicol. Rep.* **2015**, *2*, 1445.
30. Zaki, R. M.; Kamal El-Dean, A. M.; Abdulrazzaq, A. Y.; *J. Chin. Chem. Soc.* **2015**, *62*, 1121.
31. Haider, N.; Farghaly, A.; Al-Mekhlali, N.; El-Kashef, H.; *J. Chem. Res.* **2005**, 761.
32. Kwon-Chung, K. J.; Bennett, J. E. In *Medical Mycology*; Kwon-Chung, K. J.; Bennett, J. E., eds.; Lea & Febiger: Philadelphia, PA, USA, 1992, p. 81-102.
33. Al-Doory, Y.; *Laboratory Medical Mycology*, vol. 20; Lea & Febiger: Philadelphia, 1980, p. 219.

34. Winter, C. A.; Risley, E. A.; Nuss, G. W.; *Proc. Soc. Exp. Biol. Med.* **1962**, *111*, 544.
35. Adeyemi, O. O.; Okpo, S. O.; Ogunti, O. O.; *Fitoterapia* **2002**, *73*, 375.
36. *GraphPad Prism*, version 3.0; GraphPad Software, Inc., San Diego, CA, USA, 1999.

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