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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

A convenient synthesis, reactions and biological studies of some novel selenolo[2,3-*c*]pyrazole compounds as antimicrobial and anti-inflammatory agents

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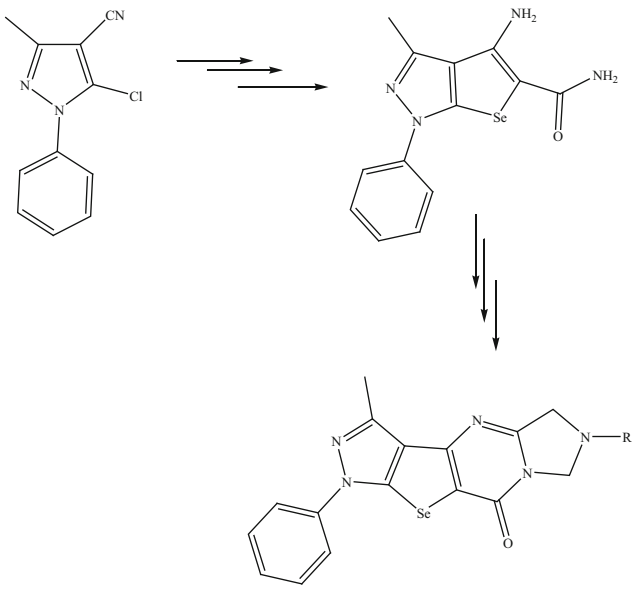
Abstract 5-Chloro-3-methyl-1-phenylpyrazole-4-carbonitrile **3** was reacted with selenium in the presence of sodium borohydride and chloroacetamide to afford selanyl acetamide **5**, which underwent *Thorpe–Ziegler* cyclization upon heating with sodium ethoxide to give the novel synthesized 4-amino-3-methyl-1-phenyl-1*H*-selenolo[2,3-*c*]pyrazole-5-carboxamide compound **(6)**. The latter compound was used as a versatile precursor for synthesis of other heterocyclic rings, namely pyrimidine, imidazopyrimidine and thiadiazinopyrimidine fused to selenolo[2,3-*c*]pyrazole moiety. The newly synthesized compounds and their derivatives were characterized by elemental and spectral analysis (IR, ^{1}H NMR, ^{13}C NMR and mass spectrometric analyses). Furthermore, some of these synthesized compounds were screened against various pathogenic bacterial and fungal strains. The results demonstrate that most of the synthesized compounds possess a significant antibacterial activity against gram-positive and gram-negative bacteria. Also, some of these compounds showed a remarkable antifungal activity, especially *Candida albicans*. On the other hand, some of the synthesized compounds possess high anti-inflammatory activity using carrageenan-induced rat paw edema assay compared with indomethacin.

Graphical Abstract The present work discussed synthesis of new selenolo[2,3-*c*]pyrazoles fused to other heterocyclic rings, namely pyrimidine, imidazopyrimidine and thiadiazinopyrimidine. Some of the synthesized compounds showed remarkable antibacterial, antifungal and anti-inflammatory activities.



Keywords Synthesis · Reactions · Selenolopyrazole · Pyrimidine · Antimicrobial activity · Anti-inflammatory activity

Introduction

Pyrazoles/*N*-arylpyrazoles are very interesting class of heterocyclic compounds which were considered as scaffolds of many efficacious biologically active agents such as antitumor (Barnes *et al.,* 2001; Altintop *et al.,* 2014; Baraldi *et al.,* 2002; Mercep *et al.,* 2004), anti-inflammatory

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(Smith *et al.,* 2001; Raffa *et al.,* 2010; Maggio *et al.,* 2001; Cardia *et al.,* 1998; Mullican *et al.,* 1993; Ochi *et al.,* 2001), hypoglycemic (Maekawa *et al.,* 2004), antithrombotic (Qiao *et al.,* 2004), adenosine receptor antagonist (Baraldi *et al.,* 2003), kinase inhibitory activity (Brown *et al.,* 2004), insecticidal (Stevensons *et al.,* 2004), ulcerogenic (Ochi *et al.,* 2001), neuropeptide Y receptor type 5 (Stamford and Wu, 2004) and thrombopoietin mimetics activities (Heerding 2004). Members of this class of compounds have also been investigated for their local anesthetic, antiarrhythmic (Iovu *et al.,* 2000), herbicidal (Vicentini *et al.,* 1999), antiviral (Storer *et al.,* 1999; El-Sabbagh *et al.,* 2009), immunosuppressant (Wang *et al.,* 1998), anticonvulsant (El-Sabbagh *et al.,* 2009), antidepressant (Abdel-Aziz *et al.,* 2009) and antimicrobial activities (Lee *et al.,* 2001; Sridhar *et al.,* 2004; Abdel-Gawad *et al.,* 2003; Mamolo *et al.,* 2001; Soliman *et al.,* 2001; Mishriky *et al.,* 2001). On the other hand, selenium has attracted the attention of many scientists working in a variety of fields due to its dietary antioxidant activity and is an essential component of active sites of several enzymes (Richardson *et al.,* 2006). Literature survey revealed that the most representative biological activity among fused pyrazoles with five-membered heterocycles such as selenolopyrazole is the antitumor activity (Raffa *et al.,* 2015; Chou *et al.,* 2010).

In the present work, we synthesized new heterocyclic rings such as imidazopyrimidine and thiazdiazinopyrimidine fused to selenolopyrazole moiety. Imidazopyrimidines are important scaffolds in medicinal chemistry, as they exhibit a broad spectrum of biological activity such as anticancer (Cosimelli *et al.,* 2014), antitubercular (Margiotta *et al.,* 2007), antiviral (Gueiffier *et al.,* 1996), antimicrobial (Rival *et al.,* 1992), antifungal (Rival *et al.,* 1991), anti-inflammatory (Vidal *et al.,* 2001), parasiticidal activity (Jiricek *et al.,* 2014), calcium channel blockers (Sanfilippo *et al.,* 1988) and benzodiazepine receptor agonists (Tully *et al.,* 1991). Also, the pyrimidothiadiazines have been described as nucleoside analogues, (Haruo *et al.,* 1978) anti-inflammatory, hypotensive and diuretic agents (Tatehiko *et al.,* 1978a, b).

The biological activities of the previous compounds persuaded us to study the antimicrobial and anti-inflammatory activities of selenolo[2,3-*c*]pyrazole and pyrimido[4′,5′:4,5]selenolo[2,3-*c*]pyrazole derivatives.

Materials and methods

Chemistry

All melting points are uncorrected and measured on a Fisher-John apparatus. IR spectra were recorded (KBr, wafer technique) with a Perkin-Elmer 1430 Spectrophotometer. ^{1}H NMR and ^{13}CNMR spectra were obtained on a Varian EM-390 MHz (90 MHz) and Bruker 400 MHz spectrometers in $CDCl_3$, DMSO-d_6 and CF_3CO_2D using Me_4Si as internal standard, and chemical shifts are expressed as ppm. Mass spectrometric analysis was measured on a Joel-JMS 600 mass spectrometer. Analytical data were obtained on elemental analyzer system GmbH-VarioEL V.3 microanalyzer in the central laboratory of Assiut University, and all results were found to be in an acceptable range (±0.20). The substituted pyrazole compounds **1–3** were synthesized according to the literature procedure (Haider *et al.,* 2005), with m.p. 140–142 °C for compound **1**. Some of the synthesized compounds in this work were chosen and screened in vitro for their antimicrobial activity against some strains of bacteria and fungi. The antifungal and antibacterial activities of tested compounds were evaluated by the reported method (Kwon-Chung and Bennett, 1992) using 0.005 % (100 µg/2 mL) concentration of selected compounds in DMSO as a solvent. The inhibition zone (mm) was compared with levofloxacin and clotrimazole as a reference for antibacterial and antifungal activities, respectively. The results of anti-inflammatory effect of some synthesized compounds 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 computer Prism program for Windows version 3.0 (GraphPad software, Inc, San Diego CA, USA). The significant difference between groups was accepted at $P < 0.05$ *, 0.01** or 0.001***, and the data were expressed as mean ± standard error (SE).

5-Chloro-3-methyl-1-phenyl-1H-pyrazole-4-carbaldehydoxime (2) A mixture of the chloro-aldehyde **1** (11 g, 0.05 mol) and hydroxylamine hydrochloride (4.20 g, 0.06 mol) in ethanol (100 mL) in the presence of fused sodium acetate (5 g, 0.06 mol) as basic catalyst, was refluxed for 2 h. The solid product, which was formed on cooling and poured into ice-water mixture, was recrystallized from ethanol as white crystals (93.40 %, 11 g) yield; mp 130–132 °C; IR (KBr) ν_{max} 3220 (br OH), 3030 (CH aromatic), 2950 (CH aliphatic), 1620 (C=N), 1340 (C–N st), 720 (C–Cl) cm^{-1}; ^{1}H NMR (δ ppm, $CDCl_3$, 90 MHz): 2.70 (3H, s, CH_3), 7.20–7.60 (5H, m, ArH), 8.90 (1H, s, CH=N), 10.80 (1H, s, OH); ^{13}C NMR (δ ppm, $CDCl_3$, 100 MHz): 12.8 (CH_3 pyrazole), 98.4 (C1), 118.3 (C6, C10: Ph), 125.5 (C8 Ph), 126.8 (C7, C9: Ph), 136.1 (C11), 140.4 (C3), 143.5 (C2); Anal. Calcd. for $C_{11}H_{10}ClN_3O$ (235.67): C, 56.06; H, 4.28; Cl; 15.04; N, 17.83. Found: C, 56.00; H, 4.35; Cl, 14.95; N, 18.00.

5-Chloro-3-methyl-1-phenyl-1H-pyrazole-4-carbonitrile (*3*) A solution of oxime compound **2** (11 g, 0.047 mol) in

acetic anhydride (30 mL) was refluxed for 6 h. The solid product which formed on pouring ice-water mixture was recrystallized from ethanol as brownish-white crystals (88.5 %, 9 g) yield; mp 118–120 °C. IR (KBr, cm^{-1}) ν_{max} 3030 (CH aromatic), 2950 (CH aliphatic), 2210 (C≡N), 1625 (C=N), 1340 (N–Ph st), 725 (C–Cl) cm^{-1}; ^{1}H NMR (δ ppm, $CDCl_3$, 90 MHz): 2.50 (3H, s, CH_3), 7.5 (5H, m, ArH); ^{13}C NMR (δ ppm, $CDCl_3$, 100 MHz): 12.7 (CH_3 pyrazole), 95.2 (C1), 110.8 (C11), 118.3 (C6, C10 Ph), 125.4 (C8 Ph), 126.8 (C7, C9 Ph), 136.7 (C5), 141.2 (C3), 143.8 (C2); Anal. Calcd. for $C_{11}H_8ClN_3$ (217.66): C, 60.70; H, 3.70; Cl, 16.29; N, 19.31. Found: C, 60.65; H, 3.84; Cl, 16.35; N, 19.25.

2-(4-Cyano-3-methyl-1-phenyl-1H-pyrazol-5-ylselanyl)acetamide (***5***) This compound was prepared by adding (4 g, 0.05 mol) of selenium metal to absolute ethanol (60 mL) in a two-necked flask in an ice bath, and then, sodium borohydride (3 g, 0.08 mol) was added in small portions till all selenium metal dissolved. The reaction mixture was added to (10.85 g, 0.05 mol) of chlorocyanopyrazole **3** with stirring for 1 h., and then, the reaction mixture was refluxed for 2 h followed by cooling at this stage, and the sodium selenate salt **4** was formed. (4.70 g, 0.05 mol) of chloroacetamide was added to the reaction mixture with stirring and then was left overnight. The solid precipitate which was formed by stirring and washing with water was recrystallized from ethanol as white crystals (55 %, 11 g) yield); mp 120–122 °C. IR (KBr, cm^{-1}) ν_{max} 3450, 3350 (NH_2), 3030 (CH aromatic), 2920, 2850 (CH aliphatic), 2210 (CN), 1660 (CO amide), 1635 (N–H amide), 1620 (C=N), 1335 (N–Ph st); ^{1}H NMR (δ ppm, $CDCl_3$, 400 MHz): 2.50 (3H, s, CH_3), 3.36 (2H, s, CH_2), 7.14 (2H, s, NH_2 disappeared by D_2O), 7.14–7.57 (5H, m, ArH); ^{13}C NMR (δ ppm, $CDCl_3$, 100 MHz): 13.8 (C6: CH_3 pyrazole), 32.2 (C14: CH_2), 100.5 (C4), 114.4 (C13: CN), 126.4 (C8, C12 Ph), 129.5 (C10 Ph), 129.7 (C9, C11 Ph), 134.8 (C7), 139.1 (C3), 153.0 (C5), 169.8 (C15: CO). Anal. Calcd. for $C_{13}H_{12}N_4OSe$ (319.23): C, 48.90; H, 3.79; N, 17.55. Found: C, 49.00; H, 3.65; N, 17.60.

4-Amino-3-methyl-1-phenyl-1H-selenolo[2,3-c]pyrazole-5-carboxamide (***6***) A mixture of selanyl compound **5** (3.20 g, 0.01 mol) in sodium ethoxide was refluxed for 10 min. The solid product which formed on cooling was recrystallized from dioxane as a white needle in (62.50 %, 2 g) yield; mp 222–224 °C. IR (KBr, cm^{-1}) ν_{max} 3380, 3310, 3180 ($2NH_2$), 3030 (CH aromatic), 1630 (CO amide), 1590 (C=N), 1340 (N–Ph st); ^{1}H NMR (δ ppm, DMSO-d_6, 400 MHz): 2.58 (3H, s, CH_3), 6.82 (2H, s, $CONH_2$), 7.00 (2H, s, NH_2 pyrazole), 7.22–7.65 (5H, m, ArH); ^{13}CNMR (δ ppm, DMSO-d_6, 100 MHz): 13.1 (C6: CH_3 pyrazole), 96.7 (C3a), 117.3 (C9, C13 Ph), 125.8 (C11: Ph), 126.3 (C10, C12 Ph), 130.4 (C5), 136.6 (C8), 139.6 (C3), 145.6 (C4), 145.8 (C6a), 169 (C14: CO); EIMS m/z 320 [M^++1] (17), 319 $[M]^+$ (100), 318 $[M]^+$-1 (9), 317 $(M)^+$-2 (26), 302 $[M]^+$-NH_3 (27), 301 $[M]^+$-H_2O (16), 274 $[M]^+$-$HCONH_2$ (11); Anal. Calcd. for $C_{13}H_{12}N_4OSe$ (319.23): C, 48.91; H, 3.79; N, 17.55. Found: C, 48.87; H, 3.70; N, 17.60.

4-(2-Chloroacetyl amino)-3-methyl-1-phenyl-1H-selenolo[2,3-c]pyrazole-5-carboxamide (***7***) A solution of aminocarboxamide compound **6** (1 g, 0.003 mol) and chloroacetyl chloride (0.40 mL, 3.5 mmol) in dioxane (30 mL) was heated on water bath at 60–70 °C for 2 h. The solid product which was obtained by cooling and pouring on diluted sodium carbonate solution was recrystallized from ethanol as white crystals in (81 %, 1.00 g) yield; mp: 250–252 °C; IR (KBr, cm^{-1}) ν_{max} 3380, 3300, 3180 (NH, NH_2), 3050 (CH aromatic), 2920, 2840 (CH aliphatic), 1680, 1630 (2C=O amide), 1620 (C=N), 1345 (N–Ph st); ^{1}H NMR (δ ppm, $CDCl_3$, 90 MHz): 2.50 (3H, s, CH_3), 4.50 (2H, s, CH_2), 6.80 (s, 2H, NH_2), 7.45–7.80 (5H, m, ArH), 10.40 (1H, s, NH); ^{13}CNMR (δ ppm, $CDCl_3$, 100 MHz): 14.3 (C7, CH_3 pyrazole), 42.5 (C16, CH_2), 98.1 (C3a), 117.8 (C9, C13 Ph), 126.0 (C11 Ph), 127.2 (C10, C12 Ph), 130.4 (C8), 136.1 (C5), 145.9 (C6a), 140.2 (C3), 164.7 (C15: NHCO), 168.9 (C18: $CONH_2$); EIMS m/z 395 $[M]^+$ (100); Anal. Calcd. for $C_{15}H_{13}ClN_4O_2Se$ (395.71): C, 45.53; H, 3.31; Cl, 8.96; N, 14.16. Found: C, 45.60; H, 3.26; Cl, 9.10; N, 14.25.

5-Chloromethyl-3-methyl-1-phenyl-pyrazolo[3′,4′:5,4]selenolo[2,3-e]pyrimidin-7(**6H**)*-one* (***8***): *method A* (3.20 g, 0.01 mol) of chloroacetyl amino compound **7** in acetic anhydride (20 mL) was refluxed for 2 h. The solid product which formed on cooling was recrystallized from dioxane as greenish white crystals in (78.5 %, 3 g) yield; mp: 308–310 °C.

Method B A mixture of amino carboxamide compound **6** (3.32 g, 0.01 mol) and chloroacetyl chloride (3 mL, 0.038 mol) was heated on water bath at 60–70 °C for 3 h, then poured into cold water (100 mL), and neutralized with sodium carbonate solution (10 %). The solid product formed was recrystallized from dioxane. It was obtained as a greenish white crystal in (3.0 g, 77 %) yield; mp: 308–310 °C; IR (KBr, cm^{-1}) ν_{max} 3500–3400 (br NH), 3030 (CH aromatic), 2900, 2850 (CH aliphatic), 1645 (CO amide), 1620 (C=N), 1340 (N–Ph st), 725 (C–Cl). ^{1}H NMR (δ ppm, DMSO-d_6, 400 MHz): 2.60 (3H, s, CH_3), 4.61 (2H, s, CH_2), 7.35–7.70 (5H, m, ArH), 13.60 (1H, s, NH); ^{13}C NMR (δ ppm, DMSO-d_6, 100 MHz), (100 MHz): δ = 13.2 (C9: CH_3 pyrazole), 43.2 (C16: CH_2Cl), 94.0 (C3a), 118.1 (C11, C15 Ph), 122.0 (C13 Ph), 126.7 (C12, C14 Ph), 126.8 (C10), 130.5 (C7a), 139.2 (C3), 146.4 (C8a), 150.6 (C5), 156.4 (C3b), 162.5 (C7: CO

pyrimidine); EIMS m/z 377 $[M]^+$ (100); Anal. Calcd. for $C_{15}H_{11}ClN_4OSe$ (377.69): C, 47.70; H, 2.94; Cl, 9.39; N, 14.83. Found: C, 47.75; H, 3.00; Cl, 9.26; N, 15.00.

3-Methyl-1-phenyl-5-alkyl(arylamino)methyl-pyrazolo[3′,4′:5,4]selenolo[2,3-e]pyrimidin-7(**6H**)*-one* (***9a–f***): *general procedure* A mixture of chloromethyl compound **8** (1 g, 2.65 mmol) and the corresponding amine (2.80 mmol) was refluxed in ethanol (20 mL) in the presence of few drops of triethyl amine for 4 h. The solid product which separated out during reflux was recrystallized from the proper solvent.

3-Methyl-1-phenyl-5-phenylaminomethyl-pyrazolo[3′,4′:5,4]selenolo[2,3-e]pyrimidin-7(**6H**)*-one* (***9a***): *obtained by the reaction with aniline* The solid product formed was recrystallized from dioxane as white crystals in (0.90 g, 78 %) yield; mp: 288–289 °C; IR (KBr, cm^{-1}) ν_{max} 3400, 3350 (2NH st), 1650 (CO pyrimidine), 1620 (C=N), 1560 (N–H amide deformation), 1340, 1310 (2N–Ph st); ^{1}H NMR (δ ppm, DMSO-d_6, 400 MHz): 2.60 (3H, s, CH_3), 4.34 (2H, s, CH_2), 6.18 (1H, s, NHPh), 6.58–7.74 (10H, m, ArH), 12.61 (1H, s, NH pyrimidine); ^{13}C NMR (δ ppm, DMSO-d_6, 100 MHz): 13.2 (C9: CH_3 pyrazole), 43.9 (C16: CH_2NH), 98.0 (C3a), 111.3 (C19, C23: NHPh), 116.8 (C21: NHPh), 118.4 (C11, C15: Ph pyrazole), 126.1 (C13: Ph pyrazole), 127.2 (C20, C22), 127.8 (C12, C14 Ph pyrazole), 130.5 (C10), 137.2 (C7a), 140.3 (C3), 140.6 (C18), 146.1 (C8a), 148.7 (C5), 153.2 (C3b), 162.0 (C7: CO pyrimidine); EI-MS m/z 434 $[M]^+$ (100 %). Anal. Calcd. for $C_{21}H_{17}N_5OSe$ (434.36): C, 58.07; H, 3.94; N, 16.12. Found: C, 57.95; H, 4.00; N, 16.00.

3-Methyl-1-phenyl-5-(p-chlorophenylaminomethyl)-pyrazolo[3′,4′:5,4]selenolo[2,3-e] pyrimidin-7(**6H**)*-one* (***9b***): *obtained by the reaction with p-chloroaniline* The solid product formed was recrystallized from dioxane as white crystals in (74 %, 0.92 g) yield; mp: 272–274 °C;IR (KBr, cm^{-1}) ν_{max} 3300, 3220 (2NH st), 1660 (CO pyrimidine), 1625 (C=N), 1570 (N–H amide deformation), 1350, 1300 (2N–Ph st); ^{1}H NMR (δ ppm, DMSO-d_6, 90 MHz): 2.85 (3H, s, CH_3 pyrazole), 4.50 (2H, s, CH_2), 6.95 (1H, s, NH *p*-chlorophenyl), 7.30–7.85 (9H, m, ArH), 11.30 (1H, s, NH pyrimidine); ^{13}C NMR (δ ppm, DMSO-d_6, 100 MHz): 13.4 (C9: CH_3 pyrazole), 48.0 (C16: CH_2NH), 98.2 (C3a), 112.8 (C19, C23: PhCl-*p*), 117.2 (C21), 118.4 (C11, C15: Ph pyrazole), 126.2 (C13: Ph pyrazole), 127.3 (C20, C22: PhCl-*p*), 127.8 (C12, C14: Ph pyrazole), 130.4 (C10), 137.3 (C7a), 140.8 (C3), 142.6 (C18), 146.5 (C8a), 148.8 (C5), 153.3 (C3b), 162.2 (C7: CO pyrimidine). EI-MS m/z 468 $[M]^+$ (86 %). Anal. Calcd. for $C_{21}H_{16}ClN_5OSe$ (468.81): C, 53.83; H, 3.44; Cl, 7.57; N, 14.94. Found: C, 54.00; H, 3.62;Cl, 7.62; N, 15.25.

3-Methyl-1-phenyl-5-(p-tolylaminomethyl)-pyrazolo[3′,4′:5,4]selenolo[2,3-e]pyrimidin-7(**6H**)*-one* (***9c***): *obtained by the reaction with p-toluidine* The solid product formed was recrystallized from dioxane as white crystals in (80 %, 0.95 g) yield; mp: 260–262 °C; IR (KBr, cm^{-1}) ν_{max} 3400, 3350 (2NH st), 1665 (CO pyrimidine), 1620 (C=N), 1570 (N–H amide deformation), 1345, 1290 (2N–Ph st) cm^{-1}; ^{1}H NMR (δ ppm, DMSO-d_6, 400 MHz): 2.13 (s, 3H, CH_3 *p*-tolyl), 2.61 (s, 3H, CH_3 pyrazole), 4.30 (s, 2H, CH_2), 5.90 (s, 1H, NH *p*-tolyl), 6.60–7.74 (m, 9H, ArH), 12.56 (s, 1H, NH pyrimidine); ^{13}C NMR (δ ppm, DMSO-d_6, 100 MHz): 13.2 (C9: CH_3 pyrazole), 20.5 (C24: CH_3 *p*-tolyl), 46.6 (C16: CH_2), 97.9 (C3a), 113.4 (C19, C23: PhCH$_3$-*p*), 118.2 (C21), 120.6 (C11, C15: Ph pyrazole), 125.8 (C13: Ph pyrazole), 126.9 (C20, C22: PhCH$_3$-*p*), 129.8 (C12, C14: Ph pyrazole), 130.6 (C10), 139.3 (C18: PhCH$_3$-*p*), 146.0 (C7a), 146.5 (C3), 147.1 (C8a), 151.0 (C5), 159.7 (C3b), 164.4 (C7: CO pyrimidine); EI-MS m/z 447 $[M^+$-1] (53.6 %). Anal. Calcd. for $C_{22}H_{19}N_5OSe$ (448.39): C, 58.93; H, 4.27; N, 15.62. Found: C, 59.00; H, 4.35; N, 15.55.

3-Methyl-1-phenyl-5-(p-anisylaminomethyl)pyrazolo[3′,4′:5,4]selenolo[2,3-e]pyrimidin-7(**6H**)*-one* (***9d***): obtained by the reaction with *p*-anisidine The solid product formed was recrystallized from dioxane as white crystals in (82 %, 1.01 g) yield; mp: 238–240 °C; IR (KBr, cm^{-1}) ν_{max} 3420 (br NH st), 3350 (s NH st), 1660 (CO pyrimidine), 1625 (C=N), 1565 (N–H amide deformation), 1340, 1300 (2N–Ph st); ^{1}H NMR (δ ppm, CF_3CO_2D, 90 MHz): 3.30 (3H, s, CH_3 pyrazole), 4.25 (3H, s, OCH_3), 5.35 (2H, s, CH_2), 7.35–7.90 (9H, m, *p*-sub anisyl, ArH pyrazole) ppm. ^{13}C NMR (δ ppm, DMSO-d_6, 100 MHz): 13.3 (C9: CH_3 pyrazole), 24.6 (C25: CH_3 *p*-anisyl), 44.9 (C16: CH_2), 98.2 (C3a), 114.5 (C19, C23: PhOCH$_3$-*p*), 119.3 (C21: PhOCH$_3$-*p*), 120.8 (C11, C15: Ph pyrazole), 126.1 (C13: Ph pyrazole), 127.6 (C20, C22: PhOCH$_3$-*p*), 130.0(C12, C14: Ph pyrazole), 130.7 (C10), 139.3 (C18: NHPhOCH$_3$-*p*), 146.8 (C7a), 146.8 (C3), 147.4 (C8a), 152.4 (C5), 158.1 (C3b), 164.9 (C7: CO pyrimidine); Anal. Calcd. for $C_{22}H_{19}N_5O_2Se$ (464.39): C, 56.90; H, 4.12; N, 15.08. Found: C, 56.84; H, 4.00; N, 14.95.

3-Methyl-1-phenyl-(5-piperidin-1-ylmethyl)-pyrazolo[3′,4′:5,4]selenolo[2,3-e]pyrimidin-7(**6H**)*-one* (***9e***): *obtained by the reaction with piperidine* The solid product formed was recrystallized from ethanol as greenish white crystals in (81 %, 0.86 g) yield; mp: 196–198 °C; IR (KBr, cm^{-1}) ν_{max} 3480 (NH st), 3030 (CH aromatic), 2920, 2900, 2850 (CH aliphatic), 1650 (C=O pyrimidine), 1630 (C=N), 1560 (N–H amide deformation), 1345 (N–Ph), 1200 (C–N piperidine); ^{1}H NMR (δ ppm, $CDCl_3$, 90 MHz): 1.35, 1.80 (6H, m, $(CH_2)_3$ piperidine), 2.65 (3H, s, CH_3 pyrazole), 2.80 (4H, m, $(CH_2)_2$N piperidine), 3.75 (2H, s, CH_2N), 7.45–7.90

(5H, m, ArH), 11.50 (1H, s, NH); ^{13}C NMR (δ ppm, $CDCl_3$, 100 MHz) 12.8 (C9: CH_3 pyrazole), 22.4 (C20: piperidine), 24.2 (C19, C21 piperidine), 40.8 (C18, C22 piperidine), 44.3 (C16: CH_2N), 97.8 (C3a), 118.4 (C11, C15: Ph pyrazole), 112.6 (C13: Ph pyrazole), 126.5 (C12, C14:Ph pyrazole), 128.8 (C10: Ph pyrazole), 136.8 (C7a), 139.7 (C3), 144.9 (C8a), 149.4 (C5), 152.3 (C3b), 163.4 (C7: CO pyrimidine). Anal. Calcd. for $C_{20}H_{21}N_5OSe$ (426.38): C, 56.34; H, 4.96; N, 16.43. Found: C, 56.40; H, 5.00; N, 16.37.

3-Methyl-1-phenyl-(5-morpholin-4-ylmethyl)-pyrazolo[3′,4′:5,4]selenolo[2,3-e]pyrimidin-7(**6H**)*-one* (***9f***): *obtained by the reaction with morpholine* The solid product formed was recrystallized from ethanol as green crystals in (80 %, 0.85 g) yield; mp: 206–208 °C; IR (KBr, cm^{-1}) ν_{max} 3450 (NH st), 3020 (CH aromatic), 2950, 2900, 2850 (CH aliphatic), 1645 (C=O pyrimidine), 1625 (C=N), 1560 (N–H amide deformation), 1340 (N–Ph st), 1190 (C–N morpholine). ^{1}H NMR (δ ppm, $CDCl_3$, 90 MHz): 2.50 (3H, s, CH_3), 2.70–2.85 (4H, m, $(CH_2)_2N$ morpholine), 3.50–3.80 (4H, m, $(CH_2)_2O$ morpholine), 3.90 (2H, s, CH_2N), 7.20–7.80 (5H, m, ArH), 11.20 (s, 1H, NH); ^{13}C NMR (δ ppm, $CDCl_3$, 100 MHz): 13.0 (C9: CH_3 pyrazole), 51.3 (C18, C22: $(CH_2)_2N$ morpholine), 55.8 (C16: CH_2), 65.0(C19, C21: $(CH_2)_2O$ morpholine), 98.4 (C3a), 118.6 (C11, C15: Ph), 122.4 (C13: Ph pyrazole), 126.8 (C12, C14: Ph), 128.4 (C10), 136.6 (C7a), 139.7 (C3), 144.8 (C8a), 149.0 (C5), 152.3 (C3b), 162.8 (C7: CO pyrimidine); EI-MS m/z 428[M]$^+$ (100 %). Anal. Calcd. for $C_{19}H_{19}N_5O_2Se$ (428.36): C, 53.28; H, 4.47; N, 16.35. Found: C, 53.35; H, 4.55; N, 16.46.

3-Methyl-1,6-diaryl-5,7-dihydroimidazo[3,4-b]pyrazolo[3′,4′:5,4]selenolo[2,3-e]pyrimidin-9-one (***10a–d***)

General procedure

Method A A mixture of *N*-aryl amino derivatives **9a–d** (1 g, 2.2 mmol) was dissolved in warm ethanol (20 mL) and then, formaldehyde (2 mL, 0.07 mol, 35 %) was added dropwise with stirring for 15 min. The reaction mixture was heated for 2 h at 50–60 °C. The solid product which separated out during reflux was recrystallized from chloroform.

Method B The *N*-aryl amino derivatives **9a–d** (1 g, 2.2 mmol) was dissolved in dioxane (20 mL), and then, formaldehyde (2 mL, 0.07 mol, 35 %) was added dropwise with stirring for 15 min. The reaction mixture was stirred for additional 2 h at 50–60 °C. The solid product which separated out on hot was recrystallized from chloroform.

3-Methyl-1,6-diphenyl-5,7-dihydroimidazo[3,4-b]pyrazolo[3′,4′:5,4]selenolo[2,3-e]pyrimidin-9-one (***10a***) The solid product formed by using method **B** was recrystallized from chloroform as white crystals in (34 %, 0.35 g) yield; mp: 334–336 °C; IR (KBr, cm^{-1}) ν_{max} 3050 (CH aromatic), 2920, 2850 (CH aliphatic), 1685 (CO pyrimidine), 1630 (C=N), 1345, 1310 (2N–Ph st); ^{1}H NMR (δ ppm, DMSO-d_6, 400 MHz): 2.51 (3H, s, CH_3), 5.54 (2H, s, CH_2N), 5.86 (2H, s, NCH_2N imidazole), 6.98–7.72 (10H, m, ArH); ^{13}C NMR (δ ppm, DMSO-d_6, 100 MHz): 12.8 (C11: CH_3 pyrazole), 52.5, 58.8 (C5, C7: $2CH_2$ imidazole), 100.6 (C3a), 114.5 (C19, C23: Ph imidazole), 118.8 (C13, C17: Ph pyrazole), 126.4 (C15: Ph pyrazole), 127.2 (C20, C22: Ph imidazole), 127.9 (C21), 128.6 (C14, C16: Ph pyrazole), 131.0 (C12), 136.2 (C18: Ph imidazole), 138.5 (C9a), 142.0 (C3), 147.8 (C10a), 152.2 (C3b), 162.2 (C4a), 165.4 (C9: CO pyrimidine). EI-MS m/z 446 [M] $^+$ (55 %). Anal. Calcd. for $C_{22}H_{17}N_5OSe$ (446.37): C, 59.20; H, 3.84; N, 15.69. Found: C, 59.25; H, 3.78; N, 15.62.

3-Methyl-1-phenyl-6-(p-chlorophenyl)-5,7-dihydroimidazo[3,4-b]pyrazolo[3′,4′:5,4]selenolo [2,3-e]pyrimidin-9-one (***10b***) The solid product formed by using method **A** was recrystallized from chloroform as white crystals in (30 %, 0.31 g) yield; mp: 328–330 °C; IR (KBr, cm^{-1}) ν_{max} 3040 (CH aromatic), 2930, 2850 (CH aliphatic), 1680 (CO pyrimidine), 1625 (C=N), 1350, 1315 (2N–Ph st); ^{1}H NMR (δ ppm, CF_3CO_2D, 90 MHz): 3.20 (3H, s, CH_3), 5.20 (2H, s, C–CH_2–N imidazole), 6.00 (2H, s, NCH_2N imidazole), 7.35–7.90 (9H, m, ArH); ^{13}C NMR (δ ppm, DMSO-d_6, 100 MHz): 12.6 (C11, CH_3 pyrazole), 52.6, 58.8 (C5, C7: $2CH_2$ imidazole), 101.3 (C3a), 113.4 (C19, C23: PhCl-*p*), 118.6 (C13, C17: Ph pyrazole), 122.7 (C20, C22: PhCl-*p*), 126.5 (C15), 128.4 (C21: PhCl-*p*), 128.8 (C14, C16 Ph pyrazole), 131.5 (C12), 138.3 (C18: PhCl-*p*), 139.0 (C9a), 142.4 (C3), 146.9 (C10a), 153.0 (C3b), 162.8 (C4a), 166.4 (C9: CO). Anal. Calcd. for $C_{22}H_{16}ClN_5OSe$ (480.82): C, 54.96; H, 3.35; N, 14.57. Found: C, 55.10; H, 3.54; N, 14.65.

3-Methyl-1-phenyl-6-(p-tolyl)-5,7-dihydroimidazo[3,4-b]pyrazolo[3′,4′:5,4]selenolo[2,3-e] pyrimidin-9-one (***10c***) The solid product formed by using method **B** was recrystallized from chloroform as white crystals in (58 %, 0.60 g) yield; mp: 308–310 °C; IR (KBr, cm^{-1}) ν_{max} 3045 (CH aromatic), 2940, 2840 (CH aliphatic), 1675 (CO pyrimidine), 1620 (C=N), 1340, 1310 (2N–Ph st); ^{1}H-NMR (δ ppm, DMSO-d_6, 400 MHz): 1.46 (3H, s, CH_3 p-tolyl), 2.25 (3H, s, CH_3 pyrazole), 4.68 (2H, s, CH_2N imidazole), 5.47 (2H, s, NCH_2N imidazole), 6.55–7.66 (9H, m, ArH); ^{13}C–NMR (δ ppm, DMSO-d_6, 100 MHz): 12.7 (C11: CH_3 pyrazole), 22.5 (C24: CH_3 *p*-tolyl), 52.4, 57.4 (C5, C7: $2CH_2$ imidazole), 100.4 (C3a), 112.6 (C19, C23: PhMe-*p*), 119.6 (C13, C17 Ph pyrazole), 121.5 (C20, C22: PhMe-*p*), 127.8 (C21: PhMe-*p*), 128.3 (C15), 129.8 (C14, C16 Ph pyrazole), 130.0 (C12), 136.1 (C18 PhMe-*p*), 139.8 (C9a), 142.6 (C3), 145.3 (C10a), 152.6 (C3b), 162.3 (C4a), 165.9 (C9, CO pyrimidine); EI-MS m/z [M + 1]$^+$ 461 (100 %).

Anal. Calcd. for $C_{23}H_{19}ClN_5OSe$ (460.40): C, 60.00; H, 4.16; N, 15.21. Found: C, 60.10; H, 4.24; N, 15.32.

3-Methyl-1-phenyl-6-(*p*-anisyl)-5,7-dihydroimidazo[3,4-b] pyrazolo[3′,4′:5,4]selenolo[2,3-*e*] pyrimidin-9-one (***10d***) The solid product formed by using method **B** was recrystallized from chloroform as white crystals (61 %, 0.60 g) yield; mp: 312–314 °C; IR: 3030 (CH aromatic), 2950, 2850 (CH aliphatic), 1670 (CO pyrimidine), 1625 (C=N), 1340, 1310 (2N–Ph st). ^{1}H NMR (δ ppm, CF_3CO_2D, 90 MHz): 3.00 (s, 3H, CH_3 pyrazole), 3.90 (s, 3H, OCH_3), 5.20 (s, 2H, NCH_2 imidazole), 6.00 (s, 2H, NCH_2N imidazole), 7.25–7.90 (m, 9H, ArH). ^{13}C–NMR (δ ppm, DMSO-d_6, 100 MHz): 13.2 (C11, CH_3 pyrazole), 24.8 (C25: CH_3 *p*-anisyl), 55.6, 58.8 (C5, C7: $2CH_2$ imidazole), 102.3 (C3a), 114.8 (C19, C23: PhOMe-*p*), 120.4 (C13, C17 Ph pyrazole), 122.2 (C20, C22: PhOMe-*p*), 127.8 (C21: $PhOCH_3$-*p*), 129.8 (C15), 130.3 (C14, C16 Ph pyrazole), 131.5 (C12), 136.8 (C18: PhOMe-*p*), 140.1 (C9a), 142.8 (C3), 146.2 (C10a), 153.6 (C3b), 162.8 (C4a), 166.2 (C9: CO pyrimidine); Anal. Calcd. for $C_{23}H_{19}N_5O_2Se$ (476.40): C, 57.99; H, 4.02; N, 14.70. Found: C, 58.00; H, 4.10; N, 14.75.

3-Methyl-1-phenyl-7-oxopyrazolo[3′,4′:5,4]selenolo[2,3-*e*] pyrimidin-5-thione (***11***) A mixture of amino carboxamide compound **6** (0.72 g, 0.002 mol) and carbon disulfide (2 mL, 0.026 mol) was refluxed on steam bath for 5 h. in the presence of anhydrous pyridine (2 mL). The solid product which separated out on hot was recrystallized from ethanol–dioxane mixture (3:1) as yellow crystals in (61.7 %, 0.5 g) yield; mp 340–342 °C; IR (KBr, cm^{-1}) ν_{max} 3300, 3100 (2NH), 3030 (CH aromatic), 2900, 2850 (CH aliphatic), 1665 (CO pyrimidine), 1240 (C=S pyrimidine), 1350 (N–Ph); ^{1}H NMR (δ ppm, DMSO-d_6, 400 MHz): 2.50 (3H, s, CH_3), 7.41–7.70 (5H, m, ArH), 12.76 (1H, s, NHCO), 13.00 (1H, s, NHCS); ^{13}C NMR (δ ppm, DMSO-d_6, 100 MHz): 13.2 (C9: CH_3 pyrazole), 66.8 (C3a), 118.5 (C11, C15: Ph), 127.3 (C13: Ph), 130.6 (C12, C14: Ph), 138.4 (C10: Ph), 139.8 (C7a), 142.1 (C3), 146.4 (C8a), 152.9 (C3b), 192.9 (C7: CO pyrimidine), 198.4 (C5: CS pyrimidine). EI-MS m/z 361 $[M]^+$ (52.5). Anal. Calcd. for $C_{14}H_{10}N_4OSSe$ (361.29): C, 46.54; H, 2.79; N, 15.51; S, 8.87. Found: C, 46.44; H, 2.85; N, 15.40; S, 8.90.

1,7-Diphenyl-3-methylpyrazolo[3″,4″:5′,4′]selenolo[2′,3′:5,6]pyrimido[2,3-b][1,3,5]thiadiazin-10(9H)-one (***12***) A mixture of the thione compound **11** (0.36 g, 0.001 mol), formaldehyde (2 mL, 0.07 mol, 35 %) and aniline (0.2 mL, 0.002 mol) was refluxed in ethanol (20 mL) in the presence of few drops of acetic acid (0.2 mL) for 30 min. The solid precipitate which separated out on hot was recrystallized from dioxane as yellow crystals in (55 %, 0.30 g) yield; mp: >360 °C. IR (KBr, cm^{-1}) ν_{max} 3030 (CH aromatic), 2920, 2820 (CH aliphatic), 1660 (CO pyrimidine), 1345, 1310 (2N–Ph); ^{1}H NMR (δ ppm, DMSO-d_6, 90 MHz): 2.80 (3H, s, CH_3), 5.50, 5.80 (4H, 2s, $2CH_2$ thiadiazine), 7.25–7.90 (10H, m, ArH); ^{13}C–NMR (δ ppm, DMSO-d_6, 100 MHz): 14.1 (C12: CH_3 pyrazole), 50.3, 64.4 (C6, C8: $2CH_2$ thiadiazine), 98.8 (C3a), 118.6 (C22: Ph thiadiazine), 120.2 (C14, C18: Ph pyrazole), 121.6 (C20, C24: Ph thiadiazine), 123.4 (C21, C23: Ph thiadiazine), 126.8 (C16: Ph pyrazole), 129.8 (C15, C17: Ph pyrazole), 130.6 (C13: Ph pyrazole), 138.4 (C10a), 140.2 (C19: Ph thiadiazine), 150.9 (C3b), 156.4 (C11a), 159.8 (C3), 162.3 (C4a), 165.6 (C10: CO pyrimidine); EI-MS m/z: 478 $[M]^+$(100); Anal. Calcd. for: $C_{22}H_{17}N_5OSSe$ (478.44): C, 55.23; H, 3.58; N, 14.64; S, 6.70. Found: C, 55.30; H, 3.52; N, 14.71; S, 6.64.

Ethyl (1-phenyl-3-methyl-7-oxopyrazolo[3′,4′:5,4]selenolo[2,3-e]pyrimidin-5-yl)acetate (***13***) A mixture of amino carboxamide compound **6** (0.50 g, 0.0015 mol) and diethyl malonate (1 mL, 6 mmol) in acetic acid (5 mL) was refluxed for 1 h. The solid product which separated out on hot was recrystallized from dioxane as brownish-white needles in (67 %, 0.50 g) yield; mp 270–272 °C; IR (KBr, cm^{-1}) ν_{max} 3150 (NH), 3030 (CH aromatic), 2920, 2850 (CH aliphatic), 1740 (CO ester), 1650 (CO pyrimidine), 1340 (N–Ph), 1150 (C-O st); ^{1}H NMR (δ ppm, DMSO-d_6, 400 MHz): 1.22–1.26 (3H, t, $J = 12.58$ Hz, CH_3 ester), 2.51 (3H, s, CH_3 pyrazole), 3.84 (2H, s, CH_2), 4.16–4.20 (2H, q, $J = 8.40$ Hz, CH_2 ester), 7.39–7.73 (5H, m, ArH), 12.81 (1H, s, NH); ^{13}C NMR (δ ppm, DMSO-d_6, 100 MHz): 13.18 (C9: CH_3 pyrazole), 14.51 (C19: CH_3 ester), 41.17 (C16: CH_2CO), 61.49 (C18: CH_2 ester), 98.8 (C3a), 118.18 (C11, C15: Ph), 120.94 (C13: Ph), 126.86 (C12, C14: Ph), 130.54 (C10: Ph), 136.2 (C7a), 139.26 (C3), 146.40 (C8a), 150.96 (C5), 155.09 (C3b), 159.57 (C7: CO pyrimidine), 168.47 (CO ester); Anal. Calcd. for $C_{18}H_{18}N_4O_3Se$ (417.33): C, 51.81; H, 4.35; N, 13.43. Found: C, 52.00; H, 4.22; N, 13.54.

Biological evaluation

Antimicrobial activity

The fungal and bacterial strains are obtained from the culture collection of Assiut University Mycological Center (AUMC). The fungal strains were grown in sterilized 9-cm Petri dishes containing Sabouraud's dextrose agar (SDA) supplemented with 0.05 % chloramphenicol to suppress bacterial contamination (Al-Doory 1980). From these cultures, agar disks (10 mm diameter) containing spores and hyphae were transferred aseptically to screw-topped vials containing 20 mL sterile distilled water. After thorough shaking, 1-mL samples of the spore suspension were pipetted into sterile Petri dishes, followed by the addition

Table 1 Antibacterial inhibition of the tested compounds (zone of inhibition in mm)

Bacteria strains	Sample no.											
	5 (0.157 μmol)	6 (0.157 μmol)	7 (0.126 μmol)	8 (0.132 μmol)	**9a** (0.115 μmol)	**9b** (0.107 μmol)	**9c** (0.112 μmol)	**9d** (0.108 μmol)	**9e** (0.117 μmol)	**9f** (0.117 μmol)	Ref (Levofloxacin) (0.138 μmol)	
											Sensitivity	Value
Staphylococcus aureus (+ve) AUMC No. B-54	R (8)	I (14)	I (12)	I (11)	I (14)	I (12)	S (22)	I (14)	R (6)	R (8)	I	14
Streptococcus pneumoniae (+ve)	R (5)	I (10)	I (10)	I (12)	I (10)	I (13)	S (24)	I (10)	R (0)	R (9)	I	13
Clostridium difficile (+ve)	R (8)	S (16)	R (4)	S (20)	I (10)	I (10)	S (18)	I (12)	R (8)	R (0)	S	15
Esherichia coli (−ve) AUMC No. B-53	S (24)	R (0)	I (10)	R (0)	R (0)	R (8)	I (12)	S (20)	R (6)	R (8)	S	17
Klebsiella pneumoniae (−ve)	S (22)	R (7)	I (12)	R (6)	R (0)	R (8)	I (14)	S (18)	R (6)	R (8)	I	14
Pseudomonas aeruginosa (−ve) AUMC No. B-73	I (14)	R (8)	I (10)	R (0)	R (6)	R (7)	I (11)	I (12)	R (8)	R (6)	S	15

The amount added in each pore 50 μL

These compounds may show some antibacterial activity in which *S* means sensitive and its value was more than 14, *I* means intermediate and its value was between 10 and 14, *R* means resistant and its value was less than 10

Ref* = Levofloxacin as antibacterial standard

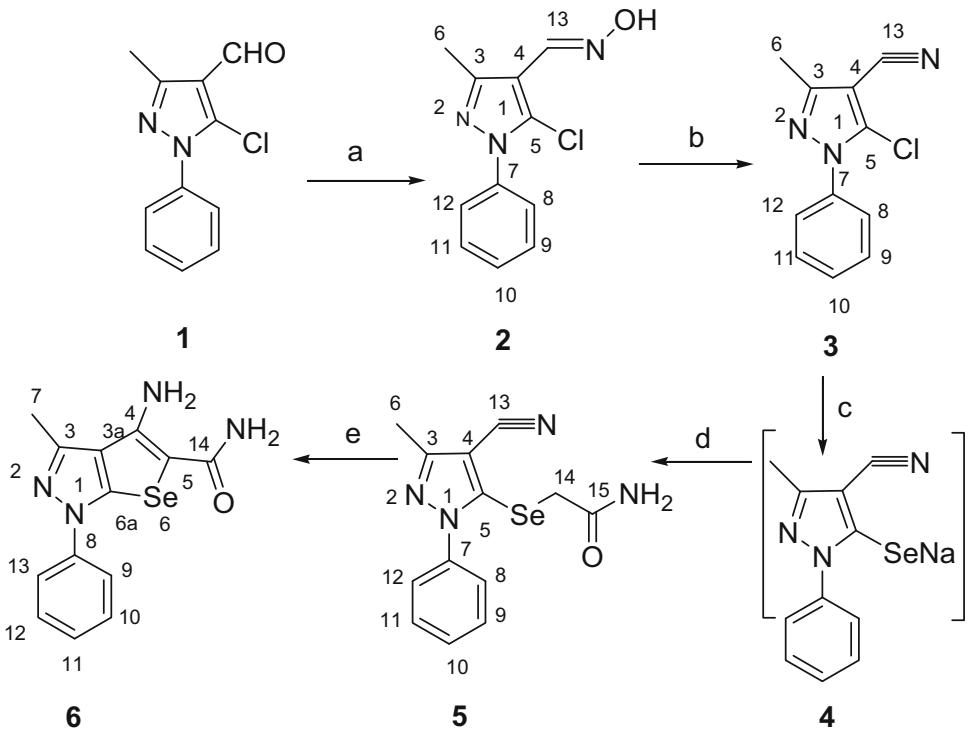
Table 2 Antifungal inhibition of the tested compounds (zone of inhibition in mm)

Fungal strains	Sample no.											
	5 (0.157 µmol)	6 (0.157 µmol)	7 (0.126 µmol)	8 (0.132 µmol	**9a** (0.115 µmol)	**9b** (0.107 µmol)	**9c** (0.112 µmol)	**9d** (0.108 µmol)	**9e** (0.117 µmol)	**9f** (0.117 µmol)	Ref** (Clotrimazole) (0.145 µmol)	
											Sensitivity	Value
Candida albicans AUMC No. 418	S (16)	I (12)	I (10)	I (10)	S (18)	R (0)	S (20)	S (18)	S (18)	S (17)	S	20
Trichophyton rubrum AUMC No. 1804	R(6)	R (0)	I (10)	R (0)	R (8)	R (4)	R (6)	I (12)	R (0)	S (28)	S	36
Aspergillus flavus AUMC No. 5451	R(0)	R (0)	R (8)	R (8)	R (6)	R (0)	R (0)	S (36)	I (10)	I (12)	S	44
Fusarium oxysporum AUMC No. 209	R(6)	R (6)	I (13)	I (11)	R (8)	R (9)	R (8)	R (0)	S (20)	I (14)	S	28
Scopulariopsis brevicaulis AUMC No. 729	I(14)	R (8)	I (10)	S (18)	R (8)	R (0)	R (0)	I (10)	S (20)	I (14)	S	20
Geotrichum candidum AUMC No. 226	R(8)	R (8)	R (0)	R (6)	I (12)	R (7)	I (13)	I (11)	S (18)	S (22)	S	24

The amount added in each pore 50 µL

These compounds may show some antifungal activity in which *S* means sensitive and its value was more than 14, *I* means intermediate and its value was between 10 and 14, *R* means resistant and its value was less than 10

Ref** = Clotrimazole as antifungal standard



Scheme 1 Synthesis of aminoselenolopyrazole carboxamide 6. Reagents and conditions: **a** H_2NOH, AcONa, EtOH; **b** Ac_2O, reflux; **c** Se, $NaBH_4$, EtOH; **d** $ClCH_2CONH_2$; **e** EtONa, EtOH

of 15 mL liquefied SDA medium which was then left to solidify. The bacterial strains were grown on nutrient agar (NA). The tested compounds (**5**, **6**, **7**, **8** and **9a–f**) and reference compound (levofloxacin) were dissolved in DMSO to give 2.0 % concentration. The specific names and numbers are indicated in Tables 1 and 2. Only 3 bacterial strains were kindly provided by the Microbiology Department, Faculty of Medicine, Assiut University. Antifungal and antibacterial activities were determined according to the method reported by Kwon-Chung and Bennett (Kwon-Chung and Bennett 1992) using 5-mm-diameter filter wells loaded with 50 µL of the solution under investigation (2.0 %), and the inoculated plates were incubated at 30 °C.

Anti-inflammatory activity

Anti-inflammatory activity screening for the synthesized compounds **9a–e** was measured in vivo using carrageenan-induced rat paw edema assay in comparison with indomethacin as reference drug (Winter *et al.,* 1962; Adeyemi *et al.,* 2002). The test is based on the pedal inflammation in rat paws 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 of all the groups; the tested compounds were dissolved in distilled water with sonication. Male adult albino rats (120–150 g) were divided into six groups; each group contains three animals. The thickness of the rat paw edema was measured by a Vernier caliper (SMIEC, China). The tested compounds **9a–e** at doses 10 mg/kg of body weight were injected to five groups of rats, while the last group was treated with indomethacin drug as a positive control. Paw thickness was measured just before and after the carrageenan injection (negative control). The difference between the thicknesses of the two paws was taken as an indication of edema and measured at 0.5, 1, 2, 3, 4 and 5 h after injection of the tested compounds.

Results and discussion

Chemistry

The synthetic methods for target compound **6** are illustrated in Scheme 1. Reaction of 5-chloro-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde **(1)** with hydroxyl amine hydrochloride in ethanol in the presence of sodium acetate afforded the corresponding oxime **2**. The latter was dehydrated upon heating with acetic anhydride into the corresponding pyrazole carbonitrile derivative **3**. The latter compound was elucidated on the basis of TLC, m.p. and IR spectrum which revealed the disappearance of absorption band at 3320 cm^{-1} characteristic for OH of oxime and appearance of band at 2210 cm^{-1} for CN group. Incorporating of selenium metal fused to pyrazole ring was achieved through reduction of selenium by sodium borohydride in ethanol to afford the intermediate sodium salt **4**

which formed in situ and not isolated. The selanyl acetamide **5** was formed by adding chloroacetamide to the sodium selenate salt **4** in situ. The selanyl acetamido compound underwent *Thorpe–Ziegler* cyclization upon heating with ethanolic sodium ethoxide to give 4-amino selenolo[2,3-*c*]pyrazole carboxamide (**6**). The latter compound was established by IR, ^{1}H NMR and mass spectra. IR revealed disappearance of absorption band at 2210 cm^{-1} for CN group in compound **6** and appearance of absorption bands at 3380, 3310 and 3180 cm^{-1} characteristic for 2NH$_2$ groups and at 1630 cm^{-1} for CO amide. ^{1}H NMR in DMSO-d$_6$ showed two signals at δ 6.80 and 7.00 ppm for 2NH$_2$ groups. ^{13}C NMR showed signal at δ 169 characteristic for CO amidic group. Mass spectrum of compound **6** showed a peak at 319.77 (100 %) as a molecular ion peak and base peak (Scheme 1).

Reaction of **6** with chloroacetyl chloride in dioxane followed by neutralization with sodium carbonate solution afforded the chloroacetyl amino **7**, which underwent ring closure using acetic anhydride to afford the chloromethyl pyrimido compound **8**. The latter compound can be obtained by heating the amino carboxamide **6** with excess chloroacetyl chloride upon heating with steam bath followed by neutralizing the reaction mixture using diluted sodium carbonate solution. The structure of the produced compound **8** was elucidated on the basis of IR, ^{1}H NMR and mass spectra. IR spectrum revealed the disappearance of absorption bands at 3380, 3300, 3180 cm^{-1} for (NH, NH$_2$) in compound **7** and appearance of broad absorption band at 3500–3400 cm^{-1} for (NH) and absorption band at 1645 cm^{-1} for (CO). ^{1}H NMR spectrum of compound **8** in (DMSO-d$_6$) revealed appearance of signals at δ 4.61 ppm characteristic for CH$_2$ and at δ 13.60 ppm for NH. ^{13}C NMR showed signal at δ 162.5 ppm characteristic for CO group of pyrimidine. Also compound **8** was confirmed by mass spectrum which showed a molecular ion peak at (m/z) 377.40 (100 %) which was in agreement with the postulated structure.

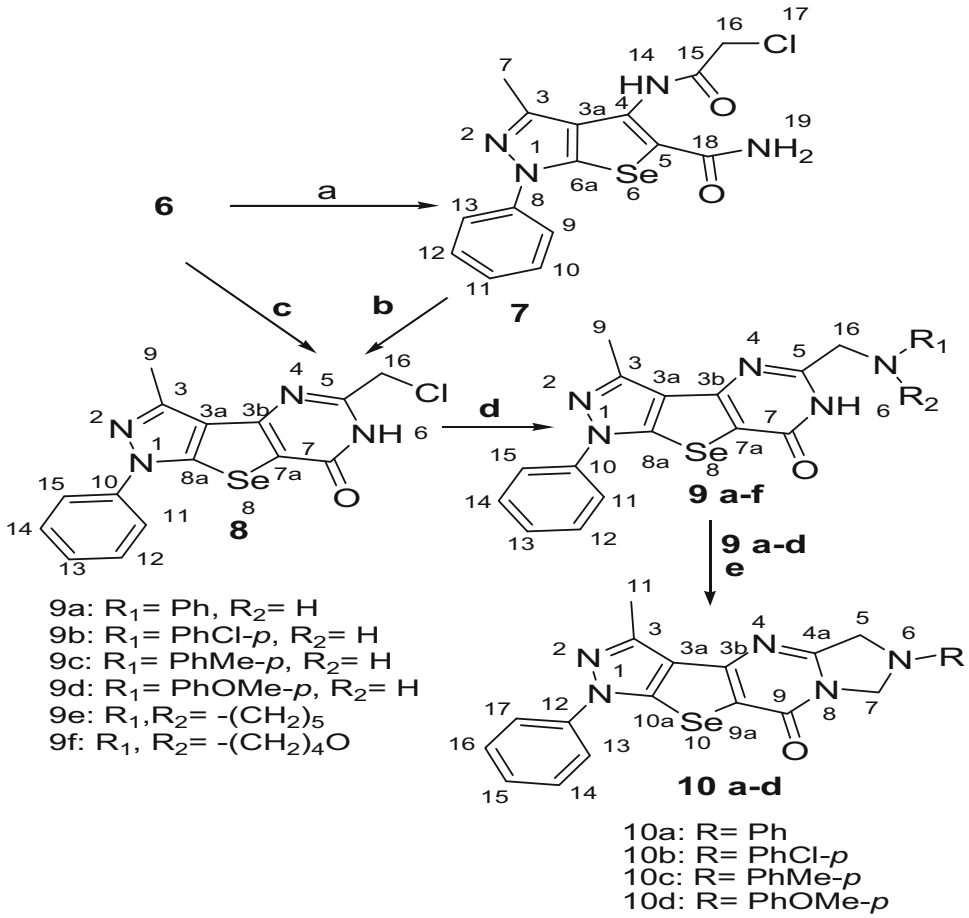
The chloromethyl derivative **8** underwent nucleophilic substitution reactions with various primary and secondary amines in refluxing ethanol to afford the alkyl(aryl) aminomethylpyrimido derivatives **9a–f**. Formation of compounds **9a–f** was established by elemental and spectral analysis. IR spectrum of compound **9c** showed two absorption bands at 3400, 3350 cm^{-1} according to 2NH groups, while compound **9e** showed absorption band at 3480 cm^{-1} for one NH group. ^{1}H NMR spectrum of compound **9c** showed two singlet signals at δ 2.13 and 2.61 ppm for 2 CH$_3$ groups of *p*-tolyl and pyrazole rings, respectively, multiplet signals at δ 6.60–7.74 ppm characteristic for aromatic protons and singlet signal at δ 12.56 ppm for NH group, while ^{1}H NMR of compounds **9e** in (CDCl$_3$) showed multiplet signals at δ 1.35, 1.80 and 2.80 ppm characteristic for aliphatic protons of piperidinyl group and showed singlet signal at δ 11.50 ppm for (NH) group. ^{13}C NMR in CDCl$_3$ showed signals at δ 22.4, 24.2 and 40.8 characteristic for aliphatic carbons of piperidine ring in addition to the signal at δ 163.4 characteristic for CO of pyrimidine.

Treatment of compounds **9a–d** with formaldehyde in refluxed ethanol or by stirring in dioxane under Mannich conditions afforded imidazopyrimidoselenolopyrazole derivatives **10a–d**. Compounds **10a–d** were confirmed on the basis of analytical and spectral data. IR spectrum of compound **10a** revealed the disappearance of absorption bands at 3400, 3350 cm^{-1} characteristic for 2NH groups and appearance of absorption bands at 1685 cm^{-1} for C=O. ^{1}H NMR spectrum of compounds **10a** in DMSO-d$_6$ showed two singlet signals at δ 5.54 and 5.86 ppm characteristic for 2 CH$_2$ imidazole groups. ^{13}C NMR of compound 10a in DMSO-d$_6$ showed two signals at δ 52.5 and 58.8 characteristic for 2 CH$_2$ groups of imidazole and signal at δ 165.4 for CO pyrimidine (Scheme 2).

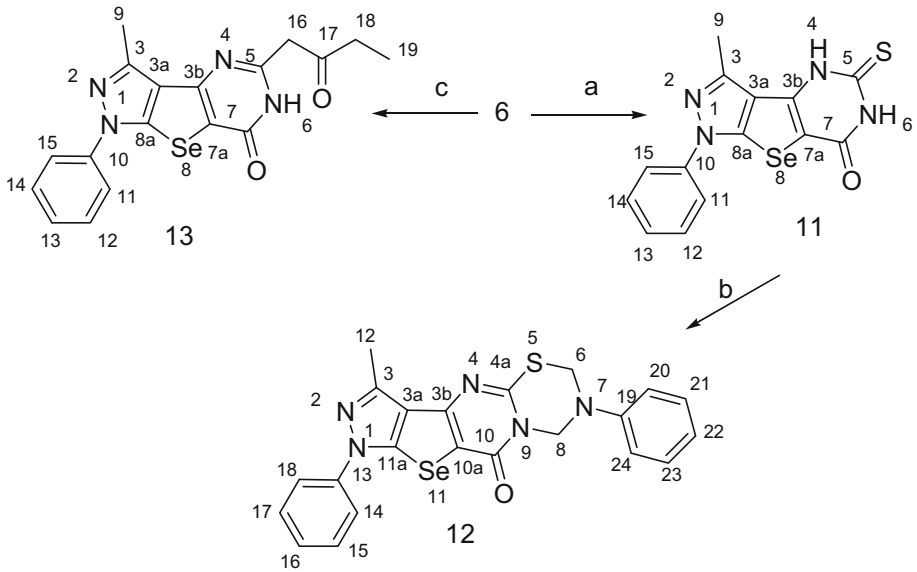
Heating of compound **6** with carbon disulfide in pyridine on steam bath for 10 h afforded the pyrimidine thione **11** which underwent cyclization under double Mannich conditions using formaldehyde and aniline in refluxed ethanol in the presence of few drops of acetic acid to give a new heterocyclic system namely 1,7-diphenyl-3-methyl[1,3,5]thiadiazino[2″,3″:2′,1′]pyrimido[4′,5′:4,5]selenolo[2,3-*c*]pyrazole-10(9H)-one **12**. The structure of compound **12** was established by TLC, mp, elemental and spectral data. IR spectrum showed absorption bands at 1660 cm^{-1} for (CO pyrimidine). ^{1}H NMR spectrum in (DMSO-d$_6$) showed two singlet signals at δ 5.50 and 5.80 ppm characteristic for 2CH$_2$ groups. Mass spectrum of compound **12** showed a peak at 478.00 as a molecular ion peak and a base peak. The reaction of compound **6** with diethyl malonate in acetic acid afforded Schiff's base followed by elimination of ethanol to give pyrimidoselenolopyrazole acetic acid ethyl ester compound **13**. Formation of compound **13** was established on the basis of spectral analysis. IR spectrum showed absorption bands at 3150 cm^{-1} for (NH) and at 1740, 1650 cm^{-1} for CO ester and CO amide, respectively. ^{1}HNMR in (DMSO-d$_6$) showed triplet and quartet signals at δ 1.22–1.26 and 4.16–4.20 ppm characteristic for CH$_2$CH$_3$ ester group. ^{13}C NMR showed signals at 159.57 and 168.47 for C=O amide and C=O ester, respectively (Scheme 3).

The suggested mechanism for proving structure of compound **12** contains the following steps. The first step includes condensation between two molecules of aniline and formaldehyde to afford the corresponding Schiff's base **a**. Next, a nucleophilic attack of the thiole group of the tautomerized form of compound **11** to the imino group of the Schiff's base a was carried out to afford intermediate **c** which underwent second nucleophilic addition of NH to a second molecule of formaldehyde to give intermediate

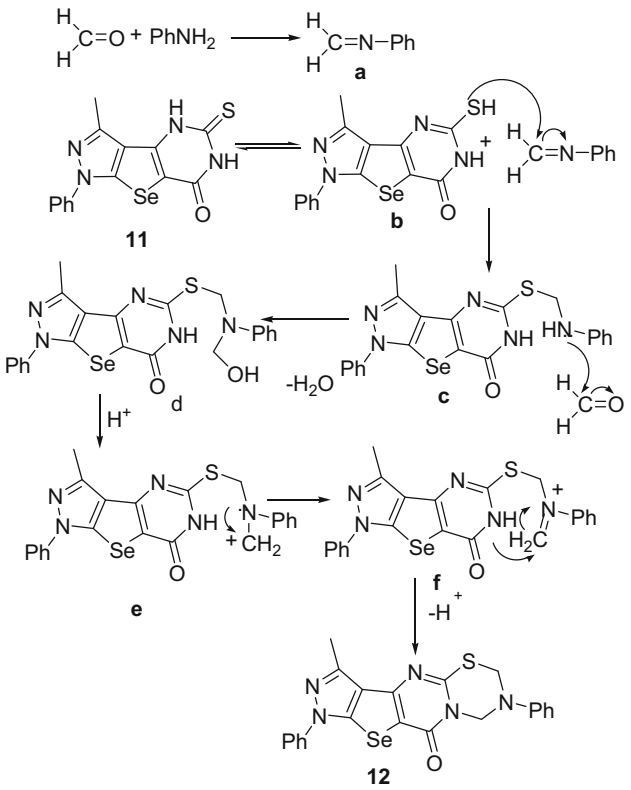
Scheme 2 Chloroacetylation of amino carboxamide followed by nucleophilic substitution to amines. Reagents and conditions: **a** $ClCH_2COCl$/dioxane; **b** Ac_2O, reflux; **c** $ClCH_2COCl$, fusion; **d** R_1R_2NH/EtOH; **e** HCHO/dioxane, stirring



Scheme 3 Reaction of amino carboxamide **6** with carbon disulfide followed by double Mannich reaction. Reagents and conditions: **a** CS_2/pyridine; **b** HCHO, $PhNH_2$/EtOH, AcOH; **c** $CH_2(CO_2Et)_2$/AcOH



alcohol **d**. The latter intermediate **d** losses water molecule in the presence of acidic medium to form the corresponding carbocation **e**. Finally, the lone pair of unshared electrons of NH group of pyrimidine attacks the carbocation with loss of a proton to afford the thiadiazino compound **12**. The suggested mechanism was described (Scheme 4).



Scheme 4 The suggested reaction mechanism for formation of thiadiazinopyrimidoselenolopyrazole **12**

Pharmacology

Antimicrobial activity

Structure–Action Relationship (SAR) of the tested compounds Some tested compounds showed high antibacterial and antifungal activities. In case of antibacterial activity, we found that pyrazolyl selanyl acetamide **5** showed high antibacterial activity against some strains of gram (−ve) bacteria such as *Esherichia coli* and *Klebsiella*, and its values (24 and 22 mm) are higher than levofloxacin (17 and 14 mm) and moderate activity against *Pseudomonas aeruginosa* (14). Ring closure of selanylacetamide **5** to afford amino carboxamide **6** decreases the antibacterial activity against gram (−ve) strains in spite of increasing activity against gram (+ve) bacteria such as *Clostridium* (16 mm). The chloroacetyl amino selenolopyrazole **7** showed moderate antibacterial activity against most strains of bacteria (10–12 mm), while the antibacterial activity of chloromethyl pyrimido compound **8** increased especially against *Clostridium* in which its value (20 mm) is higher than the reference drug (15 mm). Substitution of chlorine atom in chloromethyl compound **8** with nucleophilic primary and secondary amines strongly affected antibacterial activity. The *p*-chlorophenyl **9c** and *p*-anisyl **9d** derivatives showed the highest antibacterial activity. Compound **9c** showed high antibacterial activity against all tested gram +ve strains (18–24 mm), while compound **9d** showed high antibacterial activity against most tested gram –ve bacteria, especially *Escherichia coli* (20 mm) and *Klebsiella* (18 mm) and higher than the reference drug (17 and 14 mm), respectively. On the other hand, the phenyl and *p*-chlorophenyl derivatives **9a, 9b** showed similar intermediate antibacterial activities against gram +ve bacteria (10–14 mm), while the piperidinyl and morpholinyl derivatives **9e, 9f** are resistant to all strains of bacteria (<10 mm).

In a similar manner, the pyrazolylselanyl acetamide **5** showed almost high antifungal activity, especially against *Candida albicans* (16 mm). On the other hand, the activity decreased in amino carboxamide compound **6** was found to be intermediate against *Candida albicans* (12 mm) and resistant to the rest of antifungal strains (<10 mm). In contrast to antibacterial activity, the piperidinyl and morpholinyl derivatives **9e, 9f** showed higher antifungal activities than the other aryl amino derivatives **9a–d**. Compound **9e** showed high antifungal activity against *Candida albicans* (18 mm), *Fusarium oxysporum* (20 mm), *Scopulariopsis brevicaulis* (20 mm) and *Geotrichum candidum* (18 mm), while compound **9f** showed high antifungal activity against *Candida albicans* (17 mm), *Trichophyton rubrum* (28 mm) and *Geotrichum candidum* (22 mm).

Anti-inflammatory

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 computer Prism program for Windows version 3.0 (GraphPad software, Inc, San Diago CA, USA). The significant difference between groups was accepted at $P < 0.05$ *, 0.01** or 0.001 ***

The following Table 3 represented decrease in the thickness of rat paw edema (mm) by the effect of the tested compounds with time compared to the reference drug (indomethacin).

A plot represented the decrease in thickness of paw edema inhibition in rats by the effect of indomethacin and the tested compound with time is described in Fig. 1.

The inflammatory response is represented as the time course of the percentage of the increase in paw swelling as the area under the curve response and as the percentage of paw edema inhibition. Calculation for each compound at each time point was done using the following ratio:

Table 3 Effect of pyrazoloselenolopyrimidine derivatives (**9a–e**) on carrageenan-induced paw edema in rats (zone of inhibition in mm)

Compound	The thickness of rat paw edema (mm)						
Time (h)	Negative control	Indomethacin (0.028 mmol)	**9a** (0.023 mmol)	**9b** (0.021 mmol)	**9c** (0.022 mmol)	**9d** (0.022 mmol)	**9e** (0.023 mmol)
0.5	0.717 ± 0.016	0.683 ± 0.016	0.683 ± 0.016	0.683 ± 0.016	0.700 ± 0.0	0.700 ± 0.0	0.700 ± 0.0
1	0.717 ± 0.016	0.5500 ± 0.028	0.683 ± 0.016	0.6333 ± 0.044	0.650 ± 0.028	0.700 ± 0.0	0.650 ± 0.028
2	0.717 ± 0.016	0.483 ± 0.016	0.683 ± 0.016	0.550 ± 0.028	0.633 ± 0.016	0.683 ± 0.016	0.617 ± 0.016
3	0.717 ± 0.016	0.450 ± 0.028	0.617 ± 0.016	0.483 ± 0.016	0.583 ± 0.016	0.683 ± 0.016	0.567 ± 0.044
4	0.717 ± 0.016	0.417 ± 0.016	0.483 ± 0.016	0.467 ± 0.016	0.550 ± 0.028	0.667 ± 0.016	0.550 ± 0.028
5	0.717 ± 0.016	0.383 ± 0.016	0.483 ± 0.016	0.400 ± 0.0	0.483 ± 0.016	0.633 ± 0.016	0.500 ± 0.0

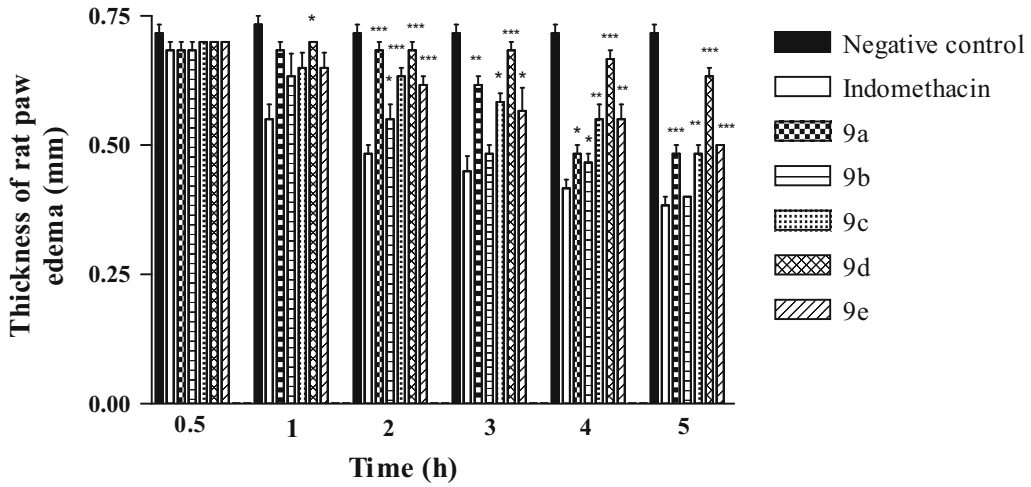


Fig. 1 A diagram represents decrease in thickness of rat paw edema of compounds **9a–e** compared with indomethacin for 5 h

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

where V_c is the volume of paw edema in negative control just after carrageenan injection and V_t. is the volume of paw edema in treated group. The following Table 4 and Fig. 2 represented increase in the percentage of paw edema inhibition for the tested compounds compared to the indomethacin.

Potency of the tested compounds was calculated relative to indomethacin "reference standard" treated group according to the following equation:

$$\text{Potency} = \frac{\text{Percentage edema inhibition of tested compound treated group}}{\text{Percentage edema inhibition of indomethacin treated group}} \quad (2)$$

From the previous results, we found that some of the tested compounds showed high anti-inflammatory activity compared with indomethacin. For example, the size of edema is the same at the control time and, after 30 min, has no significant difference in the size of edema between indomethacin and the tested compounds. As shown in Tables 4, 5 and Fig. 2, the anti-inflammatory activity of p-chlorophenyl compound 9b is higher by 1.43 times than indomethacin after 30 min, while a significant difference exists between indomethacin and *p*-anisyl **9d** which has *P* value less than 0.05 that means compound **9d** does not affect inflammation of rats after 1 h.

After 2 h, all compounds showed significant differences from indomethacin as *p*-chlorophenyl amino compound **9b** showed the lowest significance from the other tested compound, which means compound **9b** is similar in its effect to indomethacin. After 3 h., the *p*-chlorophenyl

Table 4 The increase in percentage of edema inhibition (%) of compounds **9a–e** with time (h)

Time (h)	Paw edema inhibition (%)					
	Indomethacin	**9a**	**9b**	**9c**	**9d**	**9e**
0.5	4.74	4.74	6.82	2.37	2.37	2.37
1	23.29	4.74	13.6	9.34	2.37	9.34
2	32.64	4.74	24.97	11.72	4.74	13.95
3	37.23	13.94	34.1	18.69	4.74	20.92
4	41.84	32.77	36.29	23.29	6.97	23.29
5	46.58	32.77	45.43	32.64	11.72	30.26

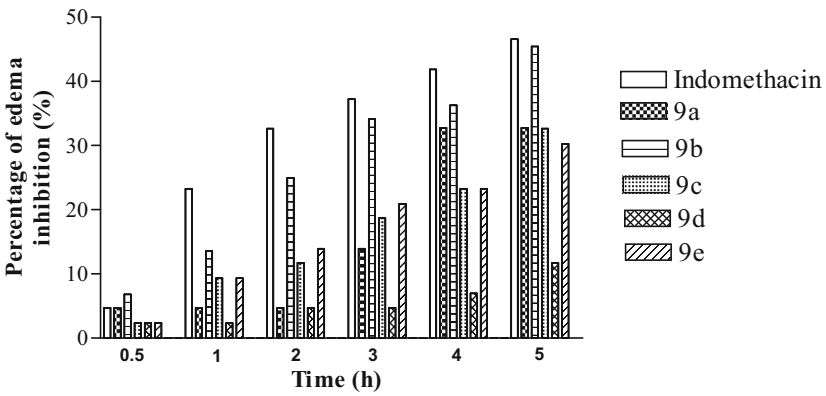


Fig. 2 A diagram represents the increase in percentage of edema inhibition (%) compounds **9a–e** with time (h)

Table 5 The potency of compounds **9a–e** relative to indomethacin

Time (h)	Potency relative to indomethacin				
	9a	**9b**	**9c**	**9d**	**9e**
0.5	1	1.43	0.50	0.50	0.50
1	0.20	0.58	0.40	0.10	0.40
2	0.14	0.76	0.36	0.14	0.42
3	0.37	0.91	0.50	0.12	0.56
4	0.78	0.86	0.56	0.17	0.56
5	0.70	0.97	0.70	0.25	0.65

amino compound **9b** showed no significance from indomethacin, while the p-tolyl amino derivative **9c** and the piperidinyl compound **9e** showed low significance ($P < 0.05$). After 4 h., the phenyl amino **9a** and *p*-chlorophenyl amino **9b** showed low significant difference from the reference compound ($P < 0.05$). After 5 h., the *p*-chlorophenyl amino **9b** showed no significance which means that compound **9b** has the highest anti-inflammatory effect of the tested compounds.

In summary, the present work focused on the synthesis of new selenolo[2,3-*c*]pyrazole compounds fused to other heterocyclic rings namely pyrimidine, imidazopyrimidine and thiadiazinopyrimidine. Some of the synthesized compounds were screened against some strains of bacteria and fungi. The antimicrobial activities results demonstrated that the most of the tested compounds showed antibacterial and antifungal activities. On the other hand, the aryl aminomethyl pyrimidoselenolo pyrazolone derivatives especially compounds **9b** then **9a** showed high anti-inflammatory activity compared with indomethacin. Consequently, this degree of biological activity displayed by the novel synthesized compounds encourages us to achieve more active derivatives in the ongoing studies.

Conclusion: the antibacterial, antifungal and anti-inflammatory activity of novel selenolo[2,3-c]pyrazoles

In this work, we synthesized the amino selenolo[2,3-c]pyrazole carboxamide compound **6** by an innovative method and used it as a versatile precursor for synthesis of other new heterocyclic rings fused to selenolopyrazole moiety. Some of the newly synthesized compounds were tested against some strains of bacteria and fungi in addition to examining the anti-inflammatory activity for these compounds in which some of them showed promising results as antibacterial, antifungal and anti-inflammatory agents.

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Compliance with ethical standards

Conflict of interest The authors declared no conflict of interest.

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