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SARS-CoV 3C-Like Protease Inhibitors of some Newly Synthesized Substituted Pyrazoles and Substituted Pyrimidines Based on 1-(3-Aminophenyl)-3-(1*H*-indol-3-yl)prop-2-en-1-one

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ABSTRACT
A series of several substituted pyrazole and pyrimidine derivatives were synthesized based on 1-(3-aminophenyl)-3-(1*H*-indol-3-yl)prop-2-en-1-one (3) as starting material, which was obtained from the reaction of 3-indolaldehyde with 3-aminoacetophenone. Treatment of chalcone 3 with hydrazine hydrate or phenylhydrazine gave the corresponding pyrazole derivatives 4 and 5. Cyclization of 3 with hydroxylamine hydrochloride, thiosemicarbazide or hydrazine hydrate/acetic acid afforded the corresponding cyclized products 6-8, respectively. The reaction of 3 with guanidine hydrochloride gave the corresponding aminopyrimidine derivative 9, which was reacted with 1, 1-carbonyldiimidazole or p-flourobenzaldehyde afforded compounds 10 and 11. Condensation of 3 with urea in the presence of sodium ethoxide gave pyrimidinone derivative 12. Finally, reaction of 9 with 1, 3-indanedione afforded compound 13, which was treated with cyclopentanone or 3, 4-dimethoxybenzaldehyde to give the corresponding arylidine derivatives 14 and 15. The synthesized derivatives were tested against SARS CoV 3C-like protease and were founded active.

Key words: Synthesis, pyrazole derivatives, pyrimidine, SARS CoV 3C-like protease

INTRODUCTION

The synthesis and the chemistry of the pyrazole nucleus have been reported and have a broad spectrum in biological and pharmacological activities (Aly *et al*., 1994; El-Emary and Bakhite, 1999; Jung *et al*., 2004; Chen *et al*., 2006; Saikia *et al*., 2006). The heterocyclic pyrazole compounds has been used as; antipyretic, analgesic and anti-inflammatory drug (Sukuroglu *et al*., 2005), antimalarial (Cunico *et al*., 2006), antitumor (Naito *et al*., 2002), antibacterial, antifungal (Akbas *et al*., 2005), antiparasitic (Rathelot *et al*., 2002) and antiviral uses (Ding *et al*., 1994). In addition, pyrimidine derivatives have significant biological activity and anti-prostate cancer (Bahashwan *et al*., 2014), anti-inflammatory (Ouf *et al*., 2008), bactericidal (Hossan *et al*., 2012) and anxiolytic activities (Wagner *et al*., 2004). Recently, design, synthesis and pharmacological properties of heteroaryl pyrimidine derivatives (Khan *et al*., 2015) and used as CB_2 cannabinoid receptor partial agonists (Tabrizi *et al*., 2013). In view of these observations and in continuation of our previous work in heterocyclic chemistry, we synthesized some new heterocyclic compounds containing the pyrimidine moiety and tested their biological activity.

MATERIALS AND METHODS

Chemistry: Melting points were determined in open glass capillary tubes with an Electro Thermal Digital melting point

apparatus (model: IA9100) and are uncorrected. Elemental microanalysis for carbon, hydrogen and nitrogen (Microanalytical Unit, NRC) was found within the acceptable limits of the calculated values. Infrared spectra (KBr) were recorded on a Nexus 670 FTIR Nicolet, Fourier Transform infrared spectrometer. Proton nuclear magnetic resonance (^{1}H NMR) spectra were run in DMSO-d_6 on Jeol 500 MHz instruments. Mass spectra were run on a MAT Finnigan SSQ 7000 spectrometer, using the Electron Impact (EI) technique. Analytical Thin Layer Chromatography (TLC) was performed on silica gel aluminum sheets, 60 F_{254} (E. Merck).

Synthesis of 1-(3-aminophenyl)-3-(1*H*-indol-3-yl)prop-2-en-1-one (3): "To a mixture of 3-indolaldehyde (0.01 mole) and 3-aminoacetophenone (0.01 mole), sodium hydroxide solution (10 mL, 4%) was added portion wise and stirring was continued for 5 h. The obtained yellow precipitate was filtered off, washed with 5% aqueous hydrochloric acid, dried and recrystallized from ethanol to give the title compound 3. Yield 62%, mp. 126-128°C, IR (KBr): n = 3469 (NH), 3370 (NH_2) and 1668 (C = O). The ^{1}H-NMR: δ = 5.34 (s, 2H, NH_2 exchangeable with D_2O), 6.82 (d, 1H, CH, J = 16Hz), 7.10-8.29 (m, 9H, Ar-H), 7.53 (d, 1H, CH, J = 16 Hz) and 9.95 (s, 1H, NH exchangeable with D_2O). The ^{13}C-NMR: δ = 112.7, 113.7, 116.5, 118, 118.4, 121.4, 121.8, 122.5, 123, 123.8, 124.7, 129.5, 137.5, 138, 139, 149.4 (14C, Ar-C+2C, C = C) and at 190 (1C, C = O). The MS (EI, 70 eV): m/z (%) = 262 [M^+, 99], 117 (100%), as base peak. The $C_{17}H_{14}N_2O$ (262.30): calcd. C, 77.84; H, 5.38; N, 10.68; found C, 77.74; H, 5.30; N, 10.60".

Synthesis of 3-(4,5-dihydro-5-(1H-indol-3-yl)-1H-pyrazol-3-yl)benzenamine (4): "To a solution of chalcone (3) (0.01 mole) in absolute ethanol (30 mL), hydrazine hydrate (0.01 mole) was added. The reaction mixture was refluxed for 5 h and then left overnight in refrigerator. The formed pale yellow precipitate was filtered off, dried and crystallized from ethanol to give the title compound (4). Yield 71%, mp. 254-256°C. IR (KBr): n = 3411 (NH), 3373 (NH_2) and 3206 (NH) cm^{-1}. The ^{1}H-NMR: δ = 2.18 (d, 2H, CH_2), 5.25 (s, 2H, NH_2 exchangeable with D2O), 6.66 (t, 1H, CH), 6.95-8.35 (m, 9H, Ar-H), 8.92 (s, 1H, NH exchangeable with D_2O) and 11.75 (s, 1H, NH exchangeable with D_2O). The ^{13}C-NMR: δ = 56, 56.9 (2C, Aliph-C), at 112, 112.6, 115, 116, 121, 122.8, 123.4, 125.9, 129.3, 132.5, 137.5, 139, 149.5, 156, 157.7 (14C, Ar-C+1C, C = N). The MS (EI, 70 eV): *m/z* (%) = 276 [M^+, 100] and as base peak. The $C_{17}H_{16}N_4$ (276.33): calcd. C, 73.89; H, 5.84; N, 20.27; found C, 73.80; H, 5.80; N, 20.20".

Synthesis of 3-(4,5-dihydro-5-(1H-indol-3-yl)-1-phenyl-1H-pyrazol-3-yl)benzenamine (5): "To a solution of chalcone (3) (0.01 mole) in absolute ethanol (30 mL) and glacial acetic acid (10 mL), phenyl hydrazine (0.01 mole) was added. The reaction mixture was refluxed for 6 h and left overnight in refrigerator. The precipitated solid was filtered off, dried and recrystallized from ethanol to give the title compound (5). Yield 72%, mp. 135-137°C. The IR (KBr): n = 3407(NH) and 3160 (NH_2). The ^{1}H-NMR: δ = 3.82 (d, 2H, CH_2), 5.71 (s, 2H, NH_2 exchangeable with D_2O), 6.05 (t, 1H, CH), 6.88-7.95 (m, 14H, Ar-H) and 11.05 (s, 1H, NH exchangeable with D_2O). ^{13}C-NMR: δ = 22, 56 (2C, Aliph-C), 111, 111.9, 112, 113.2, 116, 118.5, 119, 122, 124.1, 124.9, 129, 129.4, 133.5, 137, 145.5 (14C, Ar-C, 1C, C = N) and 146.2, 149.4, 150, 170 (4C, Phenyl-C). The MS (EI, 70 eV): *m/z* (%) = 352 [M^+, 10]. The $C_{23}H_{20}N_4$ (352.43): calcd. C, 78.38; H, 5.72; N, 15.90; found C, 78.30; H, 5.65; N, 15.81".

Synthesis of 3-(5-(1H-Indol-3-yl)-4,5-dihydroisoxazol-3-yl)benzenamine (6): "A mixture of chalcone (3) (0.01 mole), hydroxylamine hydrochloride (0.01 mole) and anhydrous potassium carbonate (0.02 mole) in absolute ethanol (30 mL) was refluxed for 10 h. After cooling, the reaction mixture was poured onto cold water, the precipitate formed was filtered off, dried and recrystallized from ethanol to give the title compound (6). Yield 62%, mp. 193-195°C. The IR (KBr): n = at 3386 (NH) and at 3156 (NH_2). The ^{1}H-NMR: δ = 5.52 (d, 2H, CH_2), 5.96 (s, 2H, NH_2 exchangeable with D_2O), 6.75 (t, 1H, CH), 7.02-8.24 (m, 9H, Ar-H), 11.27 (s, 1H, NH exchangeable with D_2O). The ^{13}C-NMR: δ = 28, 56 (2C, aliph-C), 110.2, 112.4, 114, 115.2, 116, 117.5, 118, 122.8, 128.6, 130, 134.5, 136.3, 139, 143.2, 159 (14C, Ar-C+1C, CN). The MS (EI, 70 eV): *m/z* (%) = 277 [M^+, 100]. The $C_{17}H_{15}N_3O$ (277.32): calcd. C, 73.63; H, 5.45; N, 15.15; found C, 73.52; H, 5.38; N, 15.10".

Synthesis of 3-(3-aminophenyl)-5-(1H-indol-3-yl)-4,5-dihydropyrazol-1-carbothioamide (7): "To a solution of chalcone (3) (0.01 mole) in absolute ethanol (30 mL), sodium hydroxide (0.02 mole) and thiosemicarbazide (0.01 mole) were added. The reaction mixture was refluxed for 6 h and left to cool at 0°C overnight. The formed solid was filtered off, dried and recrystallized from dioxane to give the title compound (7). Yield 64%, mp. >300°C. The IR (KBr): n = 3453 (NH), 3350 (NH_2) and 3242 (NH_2). The ^{1}H-NMR: δ = 5.04 (d, 2H, CH_2), 5.28 (s, 2H, NH_2 exchangeable with D_2O), 5.33 (s, 2H, NH_2 exchangeable with D_2O), 6.15-6.38 (t, 1H, CH), 6.61-8.62 (m, 9H, Ar-H) and 10.99 (s, 1H, NH exchangeable with D_2O). MS (EI, 70 eV): *m/z* (%) = 335 [M^+, 100]. The $C_{18}H_{17}N_5S$ (335.43): calcd. The C, 64.45; H, 5.11; N, 20.88; S, 9.56; found C, 64.34; H, 5.02; N, 20.80; S, 9.50".

Synthesis of 1-(3-(3-aminophenyl)-5-(1H-indol-3-yl)-4,5-dihydropyrazol-1-yl)ethanone (8): "A mixture of chalcone

3 (0.01 mole) and hydrazine hydrate (0.01 mole) in glacial acetic acid (20 mL) was refluxed for 3 h. After cooling, the reaction mixture was dropped on iced-water. The obtained solid precipitate was filtered off, washed with water, dried and recrystallized from methanol to give the title compound (8). Yield 82%, mp. 88-90°C. IR (KBr): n = 3350 (NH), 3201 (NH_2), 1696 (C = O). The ^{1}H-NMR: δ = 1.86 (s, 3H, CH_3), 3.35 (d, 2H, CH_2), 5.70 (s, 1H, NH_2 exchangeable with D_2O), 6.12 (t, 1H, CH), 6.95-7.86 (m, 9H, Ar-H), 9.70 (s, 1H, NH of indol exchangeable with D_2O). The ^{13}C-NMR: δ = 20, 22, 50 (3C, Aliph-C), 111.3, 112.6, 114, 116.2, 119.5, 120.9, 121, 122.3, 126.4, 128, 129.7, 131.5, 136.6, 143.9, 155.1 (14C, Ar-C+1C, C = N), 169 (1C, C = O). The MS (EI, 70 eV): *m/z* (%) = 318 [M^+, 75]. The $C_{19}H_{18}N_4O$ (318.37): calcd. C, 71.68; H, 5.70; N, 17.60; found C, 71.60; H, 5.62; N, 17.51".

Synthesis of 6-(3-aminophenyl)-1,6-dihydro-4-(1H-indol-3-yl)pyrimidin-2-amine (9): "To a boiling solution of chalcone (3) (0.01 mole) in ethanolic sodium hydroxide (50 mL, 4%), guanidine hydrochloride (0.01 mole) was added. The reaction mixture was refluxed for 5 h and then allowed to cool, the formed solid was filtered off , dried and recrystallized from ethanol to give the title compound (9). Yield 87%, mp. 156-158°C. The IR (KBr): n = 3407 (NH), 3365 (NH), 3168 (NH_2), 3109 (NH_2). The ^{1}H-NMR: δ = 6.05 (s, 2H, NH_2 exchangeable with D_2O), 6.42 (s, 1H, NH_2 exchangeable with D_2O), 6.85 (d, 1H, CH pyrimidine), 6.93 (d, 1H, CH pyrimidine), 7.01-8.72 (m, 9H, Ar-H), 10.72 (s, 1H, NH of exchangeable with D_2O), 12.16 (s, 1H, NH of exchangeable with D_2O). The ^{13}C-NMR: δ = 32.2 (1C, C-N), 111.8, 113, 118.3, 118.9, 120, 121.2, 121.5, 122.7, 123.8, 124, 124.5, 127.2, 135.5, 137.4, 137.7, 138.9 (14C, Ar-C+2C, C = C), 185 (1C, -C-NH_2). The MS (EI, 70 eV): *m/z* (%) = 301 [M^+, 12] and 64 (100) as base peak. The $C_{18}H_{17}N_5$ (303.36): calcd. C, 71.27; H, 5.65; N, 23.09; found C, 71.18; H, 5.55; N, 23.00".

Synthesis of N-(di(1H-pyrazol-1-yl)methylene)-4-(3-aminophenyl)-1, 6-dihydro-6-(1H-indol-3-yl) pyrimidin-2-amine (10): "To a solution of compound (9) (0.01 mole) absolute ethanol (30 mL) and glacial acetic acid (10 mL), 1,1-carbonyldiimidazole (0.01 mole) was added. The reaction mixture was refluxed for 8 h, left overnight at 0°C. The solid formed was filtered off, dried and crystallized from ethanol to give the title compound (10). Yield 72%, mp. 128-130°C. The IR (KBr): n = 3407 (NH), 3275 (NH), 3160 (NH_2). The ^{1}H-NMR: δ = 3.47, 4.57 (m, 6H, CH of pyrazoline), 6.06 (s, 2H, NH_2 exchangeable with D_2O), 6.85 (d, 1H, CH pyrimidine), 6.95 (d, 1H, CH pyrimidine), 7.14-8.66 (m, 9H, Ar-H), 10.76 (s, 1H, NH exchangeable with D_2O), 12.13 (s, 1H, NH exchangeable with D_2O). The ^{13}C-NMR: δ = 32.4 (1C, C-N), 111.6, 112.9, 113.4, 114.7, 118.3, 118.9, 119.8, 120.6 121.3, 121.5, 122.2, 122.8, 123.6, 123.9, 124.4, 127.3, 129.4, 131.4, 137.2, 137.5, 138.4 (14 Ar-C+2C, 2C = N+2C, C = C+3C, 2-pyrazole symmetric 6C). MS (EI, 70 eV): *m/z* (%) = 447 [M^+, 8] and 59 (100), base peak. The $C_{25}H_{21}N_9$ (447.19): calcd. C, 67.10; H, 4.73; N, 28.17; found C, 67.00 H, 4.65; N, 28.10".

Synthesis of N-(4-fluorobenzylidene)-4-(3-aminophenyl)-1,6-dihydro-6-(1H-indol-3-yl)pyrimidin-2-amine (11): "To a solution of compound (9) (0.01 mole) in absolute ethanol (25 mL), 4-flourobenzaldehyde (0.01 mole) was added. The reaction mixture was refluxed for 5hr. and after cooling, the solid formed was filtered off, dried and crystallized from ethanol to give the title compound (11). Yield 83%, mp. 175-177°C. The IR (KBr): n = 3410 (NH), 3325 (NH), 3280 (NH_2). The ^{1}H-NMR: δ = 5.70 (s, 2H, NH_2 exchangeable with D_2O), 6.85 (d, 1H, CH pyrimidine), 6.95 (d, 1H, CH pyrimidine), 7.09-8.84 (m, 13H, 12Ar-H+1CH aryl), 10.83 (s, 1H, NH exchangeable with D_2O), 12.13 (s, 1H, NH exchangeable with D_2O). MS (EI, 70 eV): *m/z* (%) = 409 [M^+, 6] and 64 (100), base peak. The $C_{25}H_{20}FN_5$ (409.46): calcd. C, 73.33; H, 4.92; N, 17.10; found C, 73.20; H, 4.84; N, 17.00".

Synthesis of 6-(3-aminophenyl)-4-(1H-indol-3-yl)pyrimidine-2-(1H)-one (12): "To a solution prepared from sodium metal (0.01 mole) in absolute ethanol (30 mL), chalcone (1) (0.01 mole) and urea (0.01 mole) were added. The reaction mixture was refluxed for 6 h, left to cool, dropped on iced-water and neutralized with diluted hydrochloric acid. The formed precipitate was filtered off, dried and recrystallized from methanol to give the title compound (12). Yield 74%, mp. 170-172°C. IR (KBr): n = 3450 (NH), 3345 (NH), 3264 (NH_2), 1647 (C = O). The ^{1}H-NMR: δ = 5.43 (s, 2H, NH_2 exchangeable with D_2O), 6.78 (s, 1H, CH of pyrimidinone), 7.26-8.09 (m, 9H, Ar-H), 9.94 (s, 1H, NH exchangeable with D_2O), 11.89 (s, 1H, NH exchangeable with D_2O). The ^{13}C-NMR: δ = 113.2, 113.9, 114.5, 115.2, 115.9, 117.3, 120.1, 121.9, 123.2, 125.4, 130.5, 133.6, 137.1, 137.2, 138.8, 139.6, 148.7 (14 Ar-C+1C, C = N+2C, C = C), 191 (1C, C = O). MS (EI, 70 eV): *m/z* (%) = 302 [M^+, 68]. The $C_{18}H_{14}N_4O$ (302.33): calcd. C, 71.51; H, 4.67; N, 18.53; found C, 71.42; H, 4.60; N, 18.45".

Synthesis of 3-(4-(3-aminophenyl)-1,6-dihydro-6-(1H-indol-3-yl)pyrimidin-2-ylimino)-2,3-dihydroin-den-1-one (13): "To a solution of compound (9) (0.01 mole) absolute ethanol (30 mL), 1,3-indanedione (0.01 mole) was added. The reaction mixture was refluxed for 2 h, a green precipitate was formed after 30 min. The solid formed was filtered off, dried and recrystallized from dioxane to give the title compound (13). Yield 87%, mp. 278-280°C. IR (KBr): n = 3256 (NH), 3215 (NH), 3147 (NH_2), 1713 (C = O). The ^{1}H-NMR: δ = 3.43 (s, 2H, CH_2), 7.33 (s, 2H, NH_2 exchangeable with D_2O), 7.37

(d, 1H, CH pyrimidine), 7.45 (d, 1H, CH pyrimidine), 7.61-8.18 (m, 13H, Ar-H), 9.64 (s, 1H, NH exchangeable with D_2O), 12.75 (s, 1H, NH exchangeable with D_2O). The ^{13}C-NMR: δ = 49.8 (1C, C-N), 56.7 (1C, C-aliph.), 111.2, 111.5, 112.8, 113.5, 115.4, 118.6, 118.9, 121.2, 121.7, 122.4, 122.6, 123.1, 124.3, 128.5, 128.9, 133.2, 135.4, 135.6, 135.9, 136.3, 138.7, 140.2, 141.9, 147.2 (20C, Ar-H+2C, 2C = N+2C, C = C), 191 (1C, C = O). MS (EI, 70 eV): *m/z* (%) = 431 [M^+, 1] and 273 (100), base peak. The $C_{27}H_{21}N_5O$ (431.49): calcd. C, 75.16; H, 4.91; N, 16.23; found C, 75.10; H, 4.82; N, 16.15".

Synthesis of 3-(4-(3-aminophenyl)-1,6-dihydro-6-(1H-indol-3-yl)pyrimidin-2-ylimino)-2-cyclopentylidene-2,3-dihydroinden-1-one (14): "A mixture of compound (13) (0.01 mole) and cyclopentanone (0.01 mole) in glacial acetic acid (30 mL) was refluxed for 6 h. After cooling, the reaction mixture was poured on ice-water. The precipitated solid was filtered off, dried and recrystallize from ethanol to give the title compound (14). Yield 77%, mp. 193-195°C. IR (KBr): n = 3412 (NH), 3375 (NH), 3160 (NH_2) and 1716 (C = O) cm^{-1}. The ^{1}H-NMR: δ = 2.19 (m, 2H, CH_2), 2.61 (m, 2H, CH_2), 4.04 (t, 2H, CH_2), 5.64 (t, 2H, CH_2), 6.85 (s, 2H, NH_2 exchangeable with D_2O), 6.92 (d, 1H, CH pyrimidine), 7.19 (d, 1H, CH pyrimidine), 7.34-8.25 (m, 13H, Ar-H), 9.65 (s, 1H, NH exchangeable with D_2O), 12.82 (s, 1H, NH, exchangeable with D_2O). The $C_{32}H_{27}N_5O$ (497.59): calcd. C, 77.24; H, 5.47; N, 14.07; found C, 77.16; H, 5.40; N, 14.00".

Synthesis of 2-(3,4-dimethoxybenzylidene)-3-(4-(3-aminophenyl)-1,6-dihydro-6-(1H-indol-3-yl) pyrimidin-2-ylimino)-2,3-dihydroinden-1-one (15): "A mixture of compound 13 (0.01 mole) and 3,4-dimethoxybenzaldehyde (0.01 mole) in a mixture of absolute ethanol (30 mL) and glacial acetic acid (15 mL) was refluxed for 3 h. The solid formed was filtered off, dried and crystallize from dioxane/methanol to give the title compound (15). Yield 84%, mp. 207-209°C. IR (KBr): n = 3415 (NH), 3373 (NH), 3260 (NH_2) and 1725 (C = O) cm^{-1}. ^{1}H-NMR: δ = 3.72 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 6.61 (s, 2H, NH_2, exchangeable with D_2O), 6.76 (d, 1H, CH pyrimidine), 6.86 (d, 1H, CH pyrimidine), 6.96-8.80 (m, 17H, 16 Ar-H+1 CH aryl), 9.85 (s, 1H, NH, exchangeable with D_2O) and 12.81 (s, 1H, NH exchangeable with D_2O). The ^{13}C-NMR: δ = 35.8 (1C, C-N), 56.2, 56.5 (2C, 2OCH_3.), 110.3, 112.2, 112.6, 113.1, 113.7, 114.4, 116.2, 118.4, 120.4, 120.8, 122.2, 122.6, 122.7, 124.1, 125.6, 126.6, 127.8, 128.5, 129.9, 134.3, 135.1, 135.3, 135.6, 135.9, 136.5, 138.8, 139.6, 141.2, 142.1, 146.6, 149.3, 154.5 (26C, Ar-H+4C, 2C = C+2C, 2C = N) and 196.8 (1C, C = O). MS (EI, 70 eV): *m/z* (%) = 580 [M^+, 8]. The $C_{36}H_{29}N_5O_3$ (579.65): calcd. C, 74.59; H, 5.04; N, 12.08; found C, 74.50; H, 5.00; N, 12.00".

SARS-CoV 3C-like protease inhibitors

Biology enzyme assays (Liu *et al*., 2014): "The sequence of SARS-Cov 3CL^{pro} cloned into the pGEX-6p-1 vector was transformed into Escherichia coli BL21 (DE3) cells. The recombinant protein with GST-tag was purified by GST-glutathione affinity chromatography and ion-exchange column chromatography. The resulting purified protein was of high purity (>95%) as, judged by SDS-PAGE analysis and the concentration was 0.5 μM. The buffer contained 50 mM Tris–HCl (pH 7.3) and 1 mM EDTA. The substrate synthesized in Shanghai Biological Engineering Company was dissolved in DMSO, with 0.8 mM liquid storage for use. The SARS CoV 3CL^{pro} inhibition assays were conducted by Fluorescence Resonance Energy Transfer (FRET). The natural substrate amino acid sequence (AVLQSGFRKK) of SARS-CoV 3CL^{pro} started with the MCA fluorescent group and connected the Dnp fluorescence quenching group with penultimate K. The screening system was as follows: The final concentrations of SARS-CoV 3CL^{pro}, substrate and compound were 0.5 μM, 16 μM and 1 mM, respectively. The settled concentrations of protein, compounds were preheated at 37°C and oscillated and the substrate was added to the mixture above. The excitation/emission light was 320/405 nm and the test was carried out every 3 sec for 60 times. Drawing curves, the maximum value of the negative control curve slope was V_0 and the largest compound curve slope was V_1. The inhibition ratio was defined as 1-V_1/V_0 and IC_{50} value was obtained by the equation: $V_0/V = 1+[I]/IC_{50}$, where V_0 is the initial rate of the reaction without inhibitor, V is the initial rate of reaction with the inhibitor at various concentrations and [I] is the inhibitor concentration".

RESULTS AND DISCUSSION

Chemistry: In the present study, we report the synthesis of several substituted pyrazole and pyrimidine derivatives based on 1-(3-aminophenyl)-3-(1*H*-indol-3-yl)prop-2-en-1-one (3), which was obtained from reaction of 3-indolaldehyde with 3-aminoacetophenone. Treatment of chalcone 3 with hydrazine hydrate or phenylhydrazine in refluxing ethanol gave the corresponding substituted pyrazole derivatives 4 and 5, respectively. Cyclization of 3 with hydroxylamine hydrochloride in the presence of alcoholic potassium carbonate afforded the corresponding isoxazole derivative 6. Also, compound 3 was treated with hydrazine hydrate in the presence of glacial acetic acid or with thiosemicarbazide in the presence of sodium hydroxide to afford the corresponding N-substituted pyrazole derivatives 7 and 8, respectively (Fig. 1).

The reaction of (3) with guanidine in the presence of sodium hydroxide gave the corresponding aminopyrimidine derivative 9, which was reacted with 1, 1-carbonyldiimidazole or p-flourobenzaldehyde afforded the corresponding pyrimidine derivatives 10 and 11, respectively. Condensation of 3 with urea in the presence of sodium ethoxide gave the corresponding pyrimidinone derivatives 12 (Fig. 2).

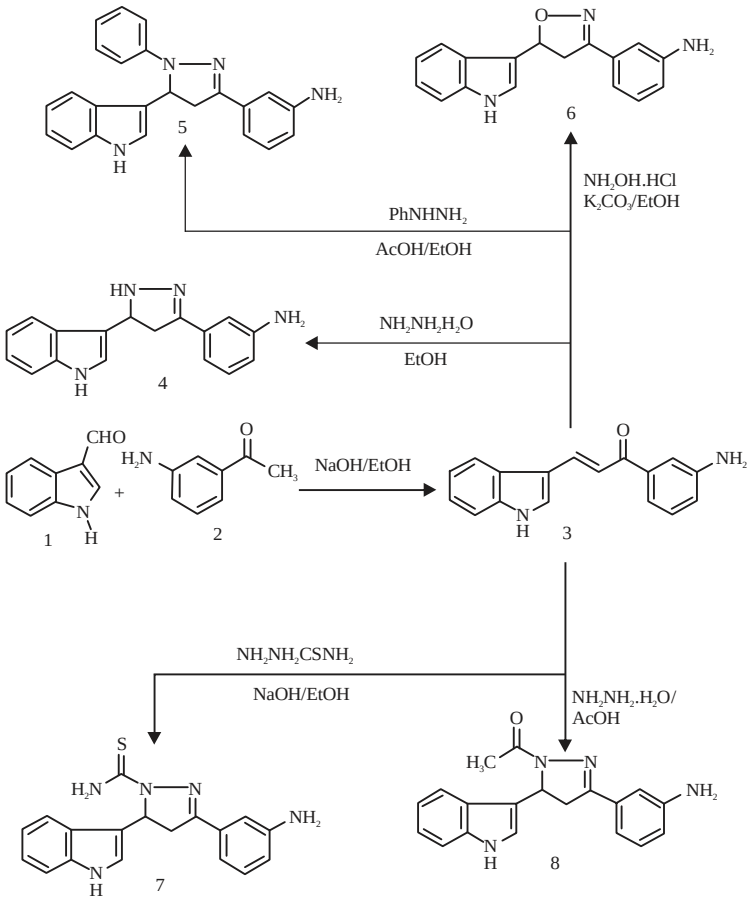

Fig. 1: Synthetic route for compounds 3-8

Finally, reaction of 9 with 1,3-indanedione in refluxing ethanol afforded the corresponding compound 13, which was treated with cyclopentanone or 3, 4-dimethoxybenzaldehyde to give the corresponding hydrazones 14 and 15, respectively (Fig. 3).

Biological activities: Biologically active of novel chalcone derivatives of 1-(4-(((1H-indol-3-yl)methylene)amino)phenyl)-3-aryl prop-2-en-1-one have been prepared and showed potent antibacterial activities (Subhashini *et al*., 2015). Also various derivatives of 3-(4,5-dihydropyrazolyl)-indoles viz 2-(4-substituted phenyl)-3-(5-(4-substituted phenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl)-1H-indole were synthesized and have anti-inflammatory activities (Chavan *et al*., 2010). A series of b-carboline hybrids bearing a substituted phenyl and a chalcone/(N-acetyl)-pyrazole moiety at the C1 and C3 positions, respectively, was designed, synthesized and evaluated for anti-cancer activity. These new hybrid molecules showed significant cytotoxic activity, with IC_{50} values ranging from 2-80 μM (Kamal *et al*., 2014). 1,3-Diphenyl-2-propenone derivatives having cytotoxic, anti bacterial activities were also prepared (Ahmad and Bano, 2011).

SARS-CoV 3C-like protease inhibitors activities (Liu *et al*., 2014): SARS-CoV is the causative agent of Severe Acute Respiratory Syndrome (SARS). The SARS coronavirus 3C-like proteinase is a good target for the treatment of Severe Acute Respiratory Syndrome (SARS). Due to the lack of clinical used drugs for the treatment of SARS, the discovery of inhibitors for SARS coronavirus 3C-like proteinase that can potentially be optimized as drugs appears to be a long sought property.

The Severe Acute Respiratory Syndrome (SARS) is a serious life-threatening and strikingly mortal respiratory illness caused by SARS-CoV. The SARS-CoV which was contained a chymotrypsin-like main protease analogous to that of the main picornavirus protease, $3CL^{pro}$. The $3CL^{pro}$ plays a pivotal role in the viral replication cycle and is a potential target for SARS inhibitor development. A series of isatin derivatives as, possible SARS-CoV 3CLpro inhibitors was designed, synthesized and evaluated by in vitro protease assay using, fluorogenic substrate peptide, in which several showed potent inhibition against the $3CL^{pro}$ (Liu *et al*., 2014).

The newly synthesized derivatives considered as a drug bioisoster for the previously prepared isatins, so this is a good

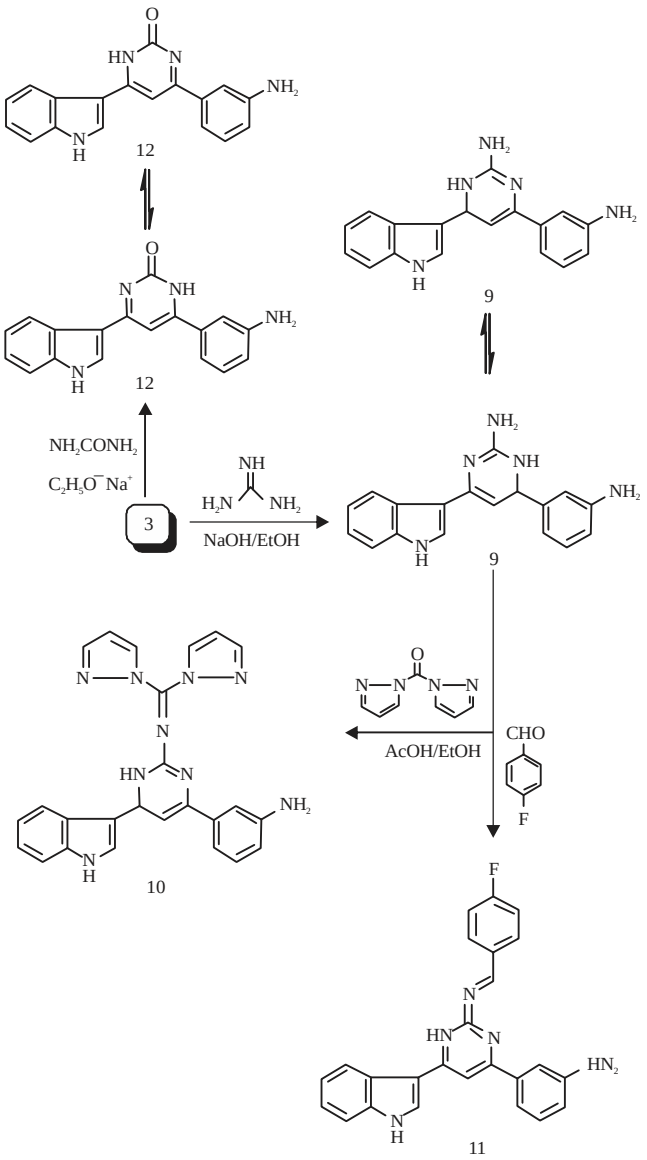

Fig. 2: Synthetic route for compounds 9-12

biological rational upon which the newly synthesized compounds where built and evaluated for SARS-CoV 3C-like protease inhibitors activities.

The newly synthesized compounds were tested for their SARS-CoV 3C-like protease inhibitors activities via determining the IC_{50} of the tested compounds using, Biological enzyme assays. All the tested compounds showed potent SARS-CoV 3C-like protease inhibitors activities (Table 1) and the descending potency order was 14, 12, 4, 13, 9, 10, 8, 5, 7, 6, 11 and 3.

Statistical analysis: Statistical comparison of the difference between control group and treated groups was done by one-way ANOVA and Duncan's multiple comparison test *$p<0.05$.

Table 1: SARS-CoV 3C-like protease inhibitors activities for some synthesized compounds (3-14)

Comp. No.	IC_{50} (lM)
3	0.98±0.00097
4	0.18±0.000017
5	0.59±0.00035
6	0.70±0.00054
7	0.69±0.000450
8	0.57±0.000360
9	0.38±0.000270
10	0.45±0.000150
11	0.96±0.000740
12	0.14±0.000015
13	0.23±0.000160
14	0.12±0.0000127

Values were calculated from the mean values of data from three separate experiments, All results are significant different from control values at $p \leq 0.005$, All results are significant different from reference standard values at $p \leq 0.005$

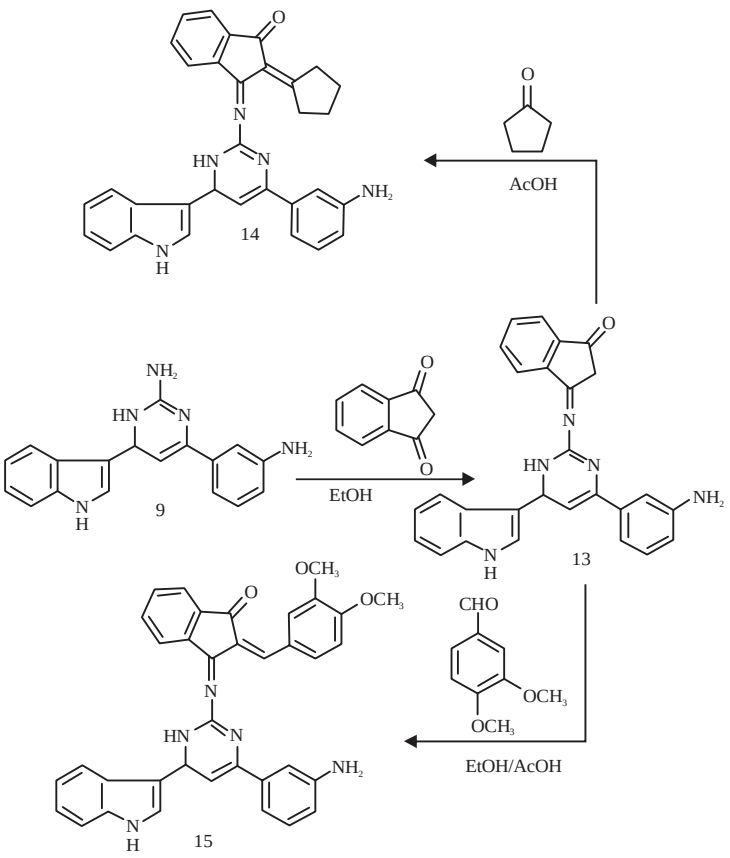

Fig. 3: Synthetic route for compounds 13-15

CONCLUSION

All the tested compounds showed potent SARS-CoV 3C-like protease inhibitors activities and correlation between the potencies of the tested compounds and their chemical structure calumniated on the following structural activities relationships assumptions.

Structure activity relationship:

- 2, 3-Dihydroinden-1-one is essential for potent activities
- Pyrimidine and pyrazole building units are essential for potent activities but the pyrimidine provides more active compounds
- Isoxazole and opening chain building units' provides less active compounds

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