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Antimelanomal Activities of some Newly Synthesized Pyrrolotriazolopyrimidines and Pyrrolotetrazolopyrimidines and their Derivatives

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ABSTRACT

A series of pyrrolo[2,3-d]pyrimidine, pyrrolotriazolopyrimidine and pyrrolotetrazolopyrimidine derivatives 2-13 were synthesized using 2-amino-1-(3-(trifluoromethyl)phenyl)-4,5-bis (4-methoxy-phenyl)-1H-pyrrole-3-carbonitrile 1 as starting material. The biological rational upon which the newly synthesized compounds was built was their structural similarity with some pyrrole derivatives that having antimelanomal, anti-tetrahymena and some kinases inhibitor activities. Some of the newly synthesized compounds showed antimelanomal, anti-tetrahymena and some kinases inhibitor activities of the 124 kinases panel.

Key words: Pyrrolopyrimidines, pyrrolotriazolopyrimidines, antiproliferative activities, antimelanomal

INTRODUCTION

In the previous studies, some of substituted pyridines, pyrimidines and their derivatives were synthesized and screening of pharmacological activities (Amr *et al*., 2006, 2009; Amr and Abdulla, 2006). Pyrrolo[2,3-d]pyrimidine derivatives have exhibited a range of biological activities, such as anti-ocular hypertension (Harrison *et al*., 2009), antitumor (Jung *et al*., 2009; McHardy *et al*., 2010), antiviral (Ivanov *et al*., 2008), enzyme inhibitors (Supuran *et al*., 1998), cytotoxic (Mangalagiu *et al*., 2001), anti-inflammatory (Mohamed *et al*., 2007), antiallergic (Nagashima *et al*., 2009) and antimicrobial agents (Mohamed *et al*., 2009). Recently, some new substituted pyrimidine derivatives have been synthesized, which exhibit pharmacological activities (Bahashwan *et al*., 2012), such as antiviral (Khalifa *et al*., 2013), antiparkinsonian (Al-Harbi *et al*., 2013), anti-inflammatory (Said *et al*., 2015; Khayyat *et al*., 2015; Hussain *et al*., 2015) and androgenic-anabolic activities (Abdalla *et al*., 2014). In view of these observations and as continuation of our previous study on pyrimidine chemistry, we synthesized some new compounds containing pyrimidine nuclei and tested their antiproliferative activities comparable to sorafenib as reference drugs.

MATERIALS AND METHODS

Chemistry: Melting points were determined and are uncorrected in an Electro Thermal Digital melting point apparatus IA9100 (Shimadzu, Tokyo, Japan). Elemental microanalysis was determined in Micro-analytical Unit, National Research Center, Egypt. Infrared spectra were recorded on a Nexus 670 FTIR Fourier Transform infrared spectrometer (Nicolet, Norwalk, CT, USA). ^{1}H-NMR-500 MHz and ^{13}C-NMR-125 MHz spectra were run in DMSO-d_6 on a JEOL 500 MHz instrument (Tokyo, Japan). MS were run

on a MAT Finnigan SSQ 7000 spectrometer (Madison, WI, USA), using the Electron Impact technique (EI).

Synthesis of 2-amino-1-(3-(trifluoromethyl)phenyl)-4,5-bis (4-methoxyphenyl)-1H-pyrrole-3-carbonitrile 1: A mixture of benzoin (2-hydroxy-1,2-diphenylethanone) (2.72 g, 0.01 mol), 3-trifluoromethylaniline (1.23 g, 0.01 mol) and concentrated hydrochloric acid (6-8 drops) in toluene (50 mL) was heated under reflux for 6 h. After cooling, malononitrile (0.66 mg, 0.01 mol) was added, followed by a catalytic amount (1.5 mL) of pyridine was added portion wise. The reaction mixture was refluxed until a solid was formed. The solvent was evaporated under reduced pressure and the residue was recrystallized from methanol to give the title compound 1. Yield 82%, m.p. 82-86°C; IR (KBr): ν = 3380 (NH_2), 2227 (C = N) cm^{-1}. The ^{1}H-NMR: δ = 3.80 (s, 6H, $2OCH_3$), 5.70-5.90 (b, 2H, NH_2, exchangeable with D_2O), 6.80-7.70 (m, 12H, Ar-H). ^{13}C-NMR: δ = 57.00 (2C, $2OCH_3$), 117.00 (1C, CN), 124.20 (1C, CF_3), 116.00, 116.10, 128.05, 128.15, 128.50, 128.65, 125.10, 160.25 (12C, 2Ph-C), 113.25, 122.50, 124.90, 130.10, 131.50, 142.56 (6C, Ph-C), 115.56, 118.50, 126.42, 127.20 (4C, pyrrole-C). MS (EI, 70 eV): m/z (%) = 463 [M^+, 18]. $C_{26}H_{20}F_3N_3O_2$ (463.45): calcd. C, 67.38; H, 4.35; N, 9.07; found. C, 67.30; H, 4.30; N, 9.00.

Reaction of 1 with triethylorthoformate: Synthesis of 2-(ethoxymethyleamino)-4,5-bis(4-methoxy-phenyl)-1-[3-(trifluoromethyl)phenyl]-1H-pyrrole-3-carbonitrile 2: To a solution of compound 1 (0.01 mol) in acetic anhydride (10 mL), triethylorthoformate (5 mL) was added. The reaction mixture was heated under reflux for 5 h, then, it was evaporated under reduced pressure. The obtained residue was washed by water and the product was extracted with ethyl acetate, dried over anhydrous sodium sulfate, filtered off and evaporated under reduced pressure to give the title compound (2) as oil product in pure form. Yield 80%, IR (KBr): ν = 3086 (CH-Aliph), 2226 (C ≡ N) cm^{-1}. ^{1}H-NMR: δ = 1.30 (t, 3H, CH_3), 3.68 (q, 2H, CH_2), 3.80 (s, 6H, $2OCH_3$), 6.70-7.60 (m, 12H, Ar-H), 8.05 (s, 1H, CH-O). ^{13}C-NMR: δ = 15.00 (1C, CH_3), 57.02 (2C, $2OCH_3$), 62.45 (1C, CH_2), 117.20 (1C, CN), 124.35 (1C, CF_3), 115.85, 116.00, 128.02, 128.10, 128.52, 128.60, 125.12, 160.28 (12C, 2Ph-C), 160.12 (1C, CH-O), 113.35, 122.58, 124.86, 130.12, 131.51, 142.52 (6C, Ph-C), 115.55, 120.58, 126.48, 127.18 (4C, pyrrole-C). MS (EI, 70 eV): m/z (%) = 520 [M^+, 32]. $C_{29}H_{24}F_3N_3O_3$ (519.52): calcd. C, 67.05; H, 4.66; N, 8.09; found. C, 66.95; H, 4.60; N, 8.00.

Synthesis of 4-amino-5,6-bis(4-methoxyphenyl)-7-[3-(trifluoromethyl)phenyl]pyrrolo[2,3-d]-pyrimidine 3: A mixture of 1 (3 g) and formamide (30 mL) was heated under reflux for 2 h. After cooling, the resulting solution was diluted with water and the obtained precipitate was filtered off, washed with water, dried and crystallized from ethanol to give the title compound (3). Yield, 65%, m.p. 166-168°C. IR (KBr): ν = 3390-3270 (NH_2) cm^{-1}. ^{1}H-NMR: δ = 3.80 (s, 6H, $2OCH_3$), 5.82 (s, 2H, NH_2, exchangeable with D_2O), 6.80-7.60 (m, 12H, Ar-H), 8.10 (s, 1H, CH-pyrimidine). ^{13}C-NMR: δ = 57.00 (2C, $2OCH_3$), 124.30 (1C, CF_3), 115.92, 116.08, 128.10, 128.22, 128.68, 128.72, 125.18, 160.45 (12C, 2Ph-C), 112.94, 123.65, 124.84, 130.16, 131.50, 142.35 (6C, Ph-C), 117.54, 123.45, 126.40, 154.25 (4C, pyrrol-C), 157.52, 158.05 (2C, pyrimidine-C). MS (EI, 70 eV): m/z (%) = 490 [M^+, 16]. $C_{27}H_{21}F_3N_4O_2$ (490.48): calcd. C, 66.12; H, 4.32; N, 11.42; found. C, 66.01; H, 4.22; N, 11.30.

Synthesis of 7-(3-(trifluoromethyl)phenyl)-5,6-bis(4-methoxyphenyl)-3H-pyrrolo[2,3-d]pyrimidine-4(7H)-one 4: A solution of 1 (3 g) in formic acid (40 mL, 85%) was heated under reflux for 6 h. After cooling, the reaction mixture was poured into ice-water, the precipitated solid was filtered off, washed with water, dried and crystallized from ethanol to give the title compound (4). Yield 75%, m.p. 264-266°C. IR (KBr): ν = 3298 (NH), 1680 (C = O) cm^{-1}. ^{1}H-NMR: δ = 3.76 (s, 6H, 2 OCH_3), 6.75-7.90 (m, 12H, Ar-H), 8.10 (s, 1H, CH-pyrimidine), 8.78 (s, 1H, NH, exchangeable with D_2O). ^{13}C-NMR: δ = 57.05 (2C, $2OCH_3$), 124.28 (1C, CF_3), 115.90, 116.00, 127.95, 128.24, 128.60, 128.74, 125.16, 160.48 (12C, 2Ph-C), 113.75, 124.78, 124.80, 131.05, 131.95, 141.82 (6C, Ph-C), 117.50, 123.40, 126.15, 134.50 (4C, pyrrol-C), 147.92 (1C, pyrimidine-C), 162.12 (1C, C = O). MS (EI, 70 eV): m/z (%) = 491 [M^+, 42]. $C_{27}H_{20}F_3N_3O_3$ (491.46): calcd. C, 65.98; H, 4.10; N, 8.55; found. C, 65.87; H, 4.00; N, 8.50.

Synthesis of thiosemicarbazide derivatives 5a,b: A mixture of 3 (0.01 mol) and the appropriate isothiocyanates, namely, methylisothiocyanate or phenylisothiocyanate (0.03 mol) in dimethyl-formamide (10 mL) in the presence of triethylamine (few drops) was heated at 80°C (water bath) for 15 h. After cooling, the reaction mixture was diluted with aqueous methanol. The separated solid product was filtered off, dried, washed with water and crystallized from the proper solvent to give the title compounds 5a,b, respectively.

N-Methyl-N`-[4,5-bis(4-methoxyphenyl)-7-[3-(trifluoromethyl)phenyl)pyrrolo[2,3-d]pyrimidine]-thiourea 5a: Yield 76%, m.p. 128-130 ºC (MeOH). IR (KBr): ν = 3398, 3356 (2 NH), 1248 (C = S) cm^{-1}. ^{1}H-NMR: δ = 2.38 (s, 3H, CH_3), 3.78 (s, 6H, $2OCH_3$), 6.80-7.90 (m, 12H, Ar-H), 8.10 (s, 1H, CH-pyrimidine), 10.50, 11.25 (2s, 2H, 2 NH, exchangeable with D_2O). The ^{13}C-NMR: δ = 34.16 (1C, CH_3), 57.05 (2C, $2OCH_3$), 124.45 (1C, CF_3), 115.98, 116.02, 125.18, 128.14, 128.28, 128.62, 128.75, 160.53 (12C, 2Ph-C), 112.90, 123.72, 124.80, 130.10, 131.54, 142.30 (6C, Ph-C), 117.65, 123.62, 126.41, 153.28 (4C, pyrrol-C), 156.52, 156.78 (2C,

pyrimidine-C), 172.56 (1C, C = S). MS (EI, 70 eV): m/z (%) = 564 [M^+, 12]. $C_{29}H_{24}F_3N_5O_2S$ (563.59): calcd. C, 61.80; H, 4.29; N, 12.43; S, 5.69; found. C, 61.69; H, 4.20; N, 12.33; S, 5.60.

N-Phenyl-N`-[4,5-bis(4-methoxyphenyl)-7-[3-(trifluoromethyl)phenyl)pyrrolo[2,3-d]pyrimidine] thiourea 5b: Yield 72%, m.p. 218-220 ºC (MeOH); IR (KBr): ν = 3392, 3350 (2 NH), 1234 (C = S) cm^{-1}. The ^{1}H-NMR: δ = 3.75 (s, 6H, 2 OCH_3), 6.62-7.56 (m, 17H, Ar-H), 8.05 (s, 1H, CH-pyrimidine), 10.65, 11.12 (2s, 2H, 2 NH, exchangeable with D_2O). The MS (EI, 70 eV): m/z (%) = 626 [M^+, 8]. $C_{34}H_{26}F_3N_5O_2S$ (625.66): calcd. C, 65.27; H, 4.19; N, 11.19; S, 5.12; found. C, 65.18; H, 4.10; N, 11.10; S, 5.06.

Synthesis of 4-chloro-5,6-bis(4-methoxyphenyl)-7-[3-(trifluoromethyl)phenyl]pyrrolo[2,3-d]-pyrimidine 6: A mixture of 4 (3 g) and phosphorous oxychloride (30 mL) was heated under reflux for one hour, after cooling, poured onto crushed ice. The obtained solid was separated by filtration, washed with water, dried and crystallized from ethanol to give the title compound (6). Yield 65%, m.p. 138-40 ºC. ^{1}H-NMR: δ = 3.82 (s, 6H, 2 OCH_3), 6.80-7.70 (m, 12H, Ar-H), 8.30 (s, 1H, CH-pyrimidine). ^{13}C-NMR: δ = 57.02 (2C, 2OCH_3), 124.44 (1C, CF_3), 115.92, 116.01, 125.22, 128.15, 128.26, 128.65, 128.70, 160.36 (12C, 2Ph-C), 112.84, 123.60, 124.87, 130.25, 131.51, 142.36 (6C, Ph-C), 117.66, 123.58, 125.98, 152.25 (4C, pyrrol-C), 150.52, 157.05 (2C, pyrimidine-C). MS (EI, 70 eV): m/z (%) = 510 [M^+, 14]. $C_{27}H_{19}ClF_3N_3O_2$ (509.90): calcd. C, 63.60; H, 3.76; Cl, 6.95; N, 8.24; found. C, 63.49; H, 3.70; Cl, 6.90; N, 8.18.

Synthesis of 5,6-bis(4-methoxyphenyl)-4-hydrazino-7[3-(trifluoromthyl)phenyl]pyrrolo[2,3-d]pyrimidine 7: A mixture of 6 (0.01 mol) and hydrazine hydrate (0.5 mol, 80%) in absolute ethanol (20 mL) was heated under reflux for 3 h. After cooling, the obtained solid product was filtered off, washed with water, dried and crystallized from ethanol to give the title compound (7). Yield 65%, m.p. 150-152 ºC. IR (KBr): ν = 3386-3342 (NH_2, NH) cm^{-1}. ^{1}H-NMR: δ = 3.76 (s, 6H, 2OCH_3), 6.30 (s, 2H, NH_2, exchangeable with D_2O), 6.80-7.40 (m, 12H, Ar-H), 8.32 (s, 1H, CH-pyrimidine), 8.56 (2s, 2H, 2 NH, exchangeable with D_2O). ^{13}C-NMR: δ = 56.92 (2C, 2OCH_3), 124.12 (1C, CF_3), 115.95, 116.12, 125.25, 128.16, 128.22, 128.64, 128.71, 160.38 (12C, 2Ph-C), 112.95, 123.62, 124.85, 130.26, 131.55, 142.48 (6C, Ph-C), 117.65, 123.54, 125.95, 153.02 (4C, pyrrole-C), 154.48, 167.16 (2C, pyrimidine-C). MS (EI, 70 eV): m/z (%) = 505 [M^+, 44]. $C_{27}H_{22}F_3N_5O_2$ (505.49): calcd. C, 64.15; H, 4.39; N, 13.85; found. C, 64.05; H, 4.30; N, 13.78.

Synthesis of 7,8-bis(4-methoxyphenyl)-9-[3-trifluoromethyl)phenyl]pyrrolo[3,2-e]tetrazolo[1,5-c]-pyrimidine 8: To a solution of 7 (0.01 mol) in acetic acid (50 mL, 55%), sodium nitrite solution (0.15 mol in 5 mL H_2O) was added. The reaction mixture was stirred at room temperature for 15 min. The separated solid product was filtered off, washed with water, dried and crystallized from n-butanol to give the title compound (8). Yield 60%, m.p. >300°C. ^{1}H-NMR: δ = 3.80 (s, 6H, 2OCH_3), 6.86-7.70 (m, 12H, Ar-H), 8.18 (s, 1H, CH-pyrimidine). The MS (EI, 70 eV): m/z (%) = 516 [M^+, 24]. $C_{27}H_{19}F_3N_6O_2$ (516.47): calcd. C, 62.79; H, 3.71; N, 16.27; found. C, 62.70; H, 3.62; N, 16.20.

Synthesis of 7,8-bis(4-methoxyphenyl)-9-[3-(trifluoromethyl)phenyl]pyrolo[3,2-e]-1,2,4-triazolo-[4,3-c]pyrimidine 9: A mixture of hydrazide derivative 7 (0.01 mol) and formic acid (20 mL) was heated under reflux for 1.5 h. The reaction mixture was evaporated under reduced pressure till dryness. The obtained residue was triturated with isopropanol and the obtained solid product was filtered off, dried and crystallized from n-butanol to give the title compound (9). Yield 65%, m.p. 298-300°C. ^{1}H-NMR: δ = 3.78 (s, 6H, 2OCH_3), 6.80-7.75 (m, 12H, Ar-H), 9.08 (s, 1H, CH-pyrimidine), 9.80 (s, 1H, triazole-H). ^{1}H-NMR: δ = 57.02 (2C, 2OCH_3), 124.16 (1C, CF_3), 116.05, 116.18, 125.28, 128.10, 128.28, 128.65, 128.70, 160.44 (12C, 2Ph-C), 112.90, 123.68, 124.80, 130.22, 131.58, 142.45 (6C, Ph-C), 117.67, 123.50, 125.90, 152.87 (4C, pyrrol-C), 143.48 (1C, pyrimidine-C), 140.54, 154.34 (2C, triazole-C). MS (EI, 70 eV): m/z (%) = 510 [M^+, 22]. $C_{28}H_{20}F_3N_5O_2$ (509.90): calcd. C, 65.24; H, 3.91; N, 13.59; found. C, 65.15; H, 3.88; N, 13.50.

Synthesis of 4-(3,5-dimethylpyrazol-1-yl)-5,6-bis(4-methoxyphenyl)-7-[3-(trifluoromethyl)-phenyl]-pyrrolo[2,3-d]pyrimidine 10: A mixture of 7 (0.5 g) and acetyl acetone (5 mL) was heated on steam-bath for 5 h. The reaction mixture was evaporated under reduced pressure till dryness and the remained residue was triturated with cold methanol. The obtained solid product was filtered off, dried and crystallized from methanol to give the title compound (10). "Yield 55%, m.p. 288-90ºC. ^{1}H-NMR: δ = 3.30, 3.40 (2s, 6H, 2CH_3), 3.79 (s, 6H, 2OCH_3), 6.60-7.80 ((m, 12H, Ar-H), 8.32 (s, 1H, CH-pyrimidine), 11.15 (s, 1H, CH-pyrazole). ^{13}C-NMR: δ = 13.54, 18.95 (2C, 2CH_3), 56.98 (2C, 2OCH_3), 124.42 (1C, CF_3), 115.95, 116.15, 125.21, 128.13, 128.20, 128.69, 128.76, 160.34 (12C, 2Ph-C), 112.98, 123.65, 124.82, 130.29, 131.56, 142.44 (6C, Ph-C), 117.64, 123.52, 125.98, 153.15 (4C, pyrrol-C), 106.76, 145.15, 148.85 (3C, pyrazole-C), 158.40, 168.46 (2C, pyrimidine-C). MS (EI, 70 eV): m/z (%) = 570 [M^+, 10]. $C_{32}H_{26}F_3N_5O_2$ (569.58): calcd. C, 67.48; H, 4.60; N, 12.30; found. C, 67.40; H, 4.51; N, 12.20".

Reaction of 4-chloropyrrolopyrimidine 6 with active methylenes: Synthesis of compounds 11 and 12: A mixture of 6 (0.01 mol), malononitrile or ethylcyanoacetate

(0.012 mol) and potassium hydroxide (0.5 g in 1 mL H_2O) in dimethylsulfoxide (15 mL) was heated while stirring on water bath at 90-100ºC for 1 h. After cooling, the reaction mixture was diluted by cold water and acidified with dilute acetic acid. The separated solid products were filtered off, washed with water, dried and crystallized from butanol to give the corresponding derivatives 11 and 12, respectively.

4-(Cyanoethoxycarbonyl)methyl-5,6-bis(4-methoxyphenyl)-7-[3-(trifluormethyl)phenyl]pyrrolo-[2,3-d]pyrimidine 11: Yield 65%, m.p. 158-160ºC. IR (KBr): n = 2232 (CN), 1745 (CO, ester) cm^{-1}. ^{1}H-NMR: δ = 1.30 (t, 3H, CH_3), 3.60 (q, 2H, CH_2), 3.80 (s, 6H, $2OCH_3$), 4.72 (s, 1H, CH), 6.70-7.60 (m, 12H, Ar-H), 8.15 (s, 1H, CH-pyrimidine). ^{13}C-NMR: δ = 14.85 (1C, CH_3), 36.86 (1C, CH), 57.00 (2C, $2OCH_3$), 61.56 (1C, CH_2), 124.32 (1C, CF_3), 117.02 (1C, CN), 115.96, 116.12, 125.34, 128.18, 128.34, 128.56, 128.72, 160.38 (12C, 2Ph-C), 112.98, 123.68, 124.85, 130.28, 131.54, 142.46 (6C, Ph-C), 117.75, 123.64, 125.92, 152.98 (4C, pyrrole-C), 156.58, 165.35 (2C, pyrimidine-C), 164.45 (1C, CO ester). MS (EI, 70 eV): m/z (%) = 587 [M^+, 8]. $C_{32}H_{25}F_3N_4O_4$ (586.56): calcd. C, 65.52; H, 4.30; N, 9.55; found. C, 65.40; H, 4.21; N, 9.50.

4-(Dicyanomethyl-5,6-bis(4-methoxyphenyl)-7-[3-(trifluoromthyl)phenyl]yrrolo[2,3-d]pyrimidine 12: Yield 72%, m.p. 278-280ºC. IR (KBr): ν = 2232, 2226 (2 CN) cm^{-1}. ^{1}H-NMR: δ = 3.75 (s, 6H, $2OCH_3$), 4.79 (s, 1H, CH), 6.80-7.60 (m, 12H, Ar-H), 8.25 (s, 1H, CH-pyrimidine). MS (EI, 70 eV): m/z (%) = 539 [M^+, 6]. $C_{30}H_{20}F_3N_5O_2$ (539.50): calcd. C, 66.79; H, 3.74; N, 12.98; found. C, 66.70; H, 3.68; N, 12.90.

Synthesis of thiosemicarbazide derivatives (13a, b): A mixture of hydrozide 7 (0.01 mol) and the appropriate isothiocyanate, namely, methylisothiocyanate or phenylisothiocyanate (0.012 mol) in methylene chloride (10 mL) was left overnight at room temperature with stirring. The obtained precipitate products was filtered off, dried and crystallized from methanol to give the corresponding thiosemicarbazide derivatives 13a, b, respectively.

N-Methyl-2-[4,5-bis(4-methoxyphenyl)-7-[3-(trifluoromethyl)phenyl)pyrrolo[2,3-d]pyrimidine]-hydrazinethioamide 13a: Yield 72%, m.p. 170-172°C. IR (KBr): ν = 3400, 3370 (NH), 1252 (C = S) cm^{-1}. ^{1}H-NMR: δ = 2.42 (s, 3H, CH_3), 3.76 (s, 6H, $2OCH_3$), 6.80-7.60 (m, 12H, Ar-H), 8.16 (s, 1H, CH-pyrimidine), 8.76, 10.34, 11.12 (3s, 3H, 3 NH, exchangeable with D_2O). MS (EI, 70 eV): m/z (%) = 579 [M^+, 5]. $C_{29}H_{25}F_3N_6O_2S$ (578.60): calcd. C, 60.20; H, 4.36; N, 14.52; S, 5.54; found. C, 60.05; H, 4.30; N, 14.45; S, 5.47.

N-Phenyl-2-[4,5-bis(4-methoxyphenyl)-7-[3-(trifluoromethyl)phenyl)pyrrolo[2,3-d]pyrimidine] hydrazinethioamide 13b: Yield 65%, m.p. 143-145ºC. IR (KBr): ν = 3405, 3365 (NH), 1248 (C = S) cm^{-1}. ^{1}H-NMR: δ = 3.76 (s, 6H, 2 OCH_3), 6.76-7.62 (m, 17H, Ar-H), 8.15 (s, 1H, CH-pyrimidine), 8.78, 10.25, 11.10 (3s, 3H, 3 NH, exchangeable with D_2O). MS (EI, 70 eV): m/z (%) = 641 [M^+, 18]. C34H27F3N6O2S (640.67): calcd. C, 63.74; H, 4.25; N, 13.12; S, 5.00; found. C, 63.62; H, 4.20; N, 13.05; S, 4.92.

Pharmacological screening

Anti-proliferative activities of tested compounds on A375P: A375P cells were obtained from American Type Culture Collection (ATCC, Rockville, MD, US) and maintained in a DMEM supplemented with 10% FBS (Welgene) and 1% penicillin/streptomycin in a humidified atmosphere with 5% CO_2 maintained at 37°C. A375P cells taken from culture substrate with 0.05% trypsin-0.02% EDTA and plated at a density of 5×10^3 cells/well in 96 well plates and then incubated at 37°C for 24 h in a humidified atmosphere with 5% CO_2 prior to treatment of various concentration (three fold serial dilution, 12 points) of tested compounds. The A375P cell viability was assessed via the conventional MTT reduction assay. MTT assays carried out with CellTiter 96® (Promega) according to the manufacturer's instructions. The absorbance at 590 nm was recorded using Shimadzu spectrophotometer. The IC_{50} was calculated using GraphPad Prism 4.0 software.

Action on tetrahymena: The tetrahymena strain was obtained from National Research center Giza, Cairo, Egypt.

Determination of Minimum Protozoacidal Concentrations (MPC): Stock solutions of the agents made in DMSO at a concentration of 5120 µg mL^{-1}. Benzalkonium chloride (stock in water) was included as control. Water was used as dilutant producing doubling concentrations of the agents at 128–4 µg mL^{-1}. These intermediate dilutions (50 µL) were pipetted in triplicate into a 96-well, Nunc round-bottomed microtiter plate system (Thermo Fischer Scientific, USA). Addition of 50 L of the inoculum gave the final tested concentration range (2-64 µg mL^{-1}) and maximally 1.25% DMSO. A positive control (no agent) and a negative control (with-out Tetrahymena) tests were also included. Tetrahymena was grown on NNA seeded with a thick pasteurized suspension of *E. coli* for 48 h under a humidified atmosphere in dark place at 22±2°C. After incubation, the protozoa were harvested and washed as previously described (Otterholt and Charnock, 2011) and resuspended in pasteurized *E. coli* (corresponding to a 0.5 MacFarland standard) at 1×104 cells mL^{-1}. After incubation for 48 h at 22±2°C, wells were examined for motile cells using an inverted microscope. This approach enabled the whole content of the well to be

visualized. The estimated Minimum Protozoacidal Concentration (MPC; 48 h) was the lowest concentration at which no motile cells were seen.

After examination in the microscope, the whole content of wells was transferred to culture dishes containing NNA/pasteurized *E. coli*. Cultures were examined over a 7 day period with an inverted microscope to see if a cell population developed. The MPC value measured (MPC; 7 days) was the lowest concentration that prevented the development of even a single viable cell in the 7 day period. Each test was performed in triplicate and the results were averaged to give the MPC value.

Kinase profiling: Compounds were profiled utilizing a panel of 124 protein kinases in the MRC National Centre for Protein Kinase Profiling Service at the University of Dundee (http://www.kinase-screen.mrc.ac.uk). The compounds were tested *in vitro*, in duplicate, at a concentration of 25 nM for further details of the methodology (Bain *et al*., 2007).

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 (*$p<0.05$).

RESULTS AND DISCUSSION

Chemistry: The 2-amino-1-(3-(trifluoromethyl)phenyl)-4,5-bis (4-methoxy-phenyl)-1H-pyrrole-3-carbonitrile 1 was prepared by treating of benzoin with 3-trifluoromethylaniline and malononitrile in the presence of concentrated hydrochloric acid. The reaction of cyano amino derivative I with triethylorthoformate in the presence of acetic anhydride afforded the corresponding compound 2. Also, 1 was cyclized with formamide or formic acid by refluxing to give the corresponding amino pyrrolopyrimidine 3 and pyrrolopyrimidine one derivatives 4, respectively. Treatment of aminopyrimidine derivative 3 with isothiocyanate derivatives in the presence of triethylamine afforded the corresponding thiosemicarbazide derivatives 5a, b, respectively (Fig. 1).

In addition, refluxing of compound 4 with phosphorous oxychloride afforded the corresponding chloropyrimidine derivative 6, which was hydrazinolysis with hydrazine hydrate to give the corresponding hydrazinopyrimidine derivative 7. Cyclization of hydrazine compound 7 with glacial acetic acid in the presence of sodium nitrite or formic acid gave the corresponding tetrazolopyrimidine 8 and triazolopyrimidine 9, respectively. But, compound 7 was

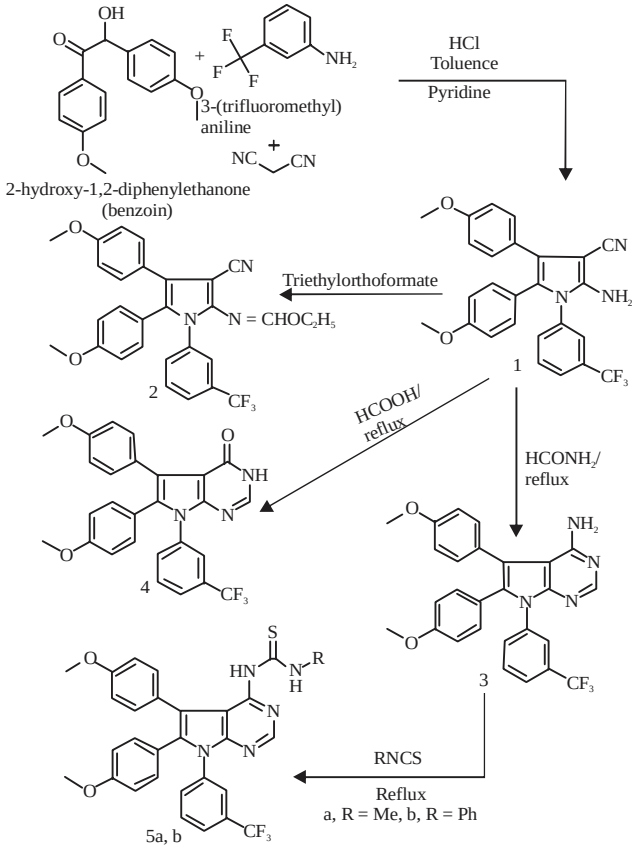

Fig. 1: Synthetic route for compounds 1-5

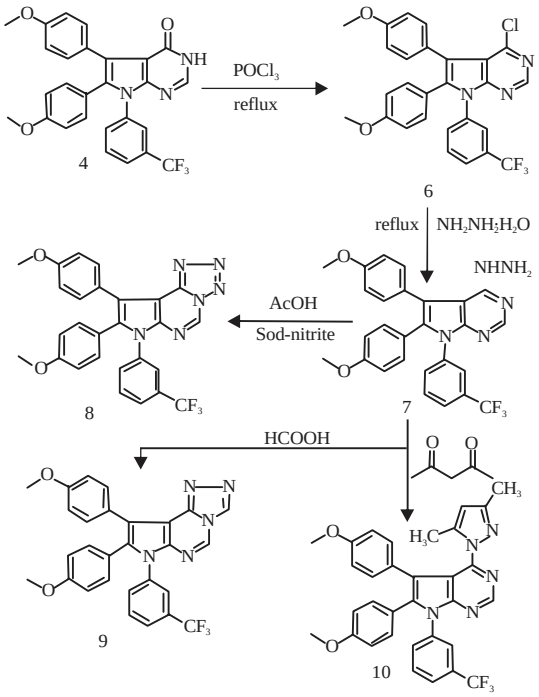

Fig. 2: Synthetic route for compounds 6-10

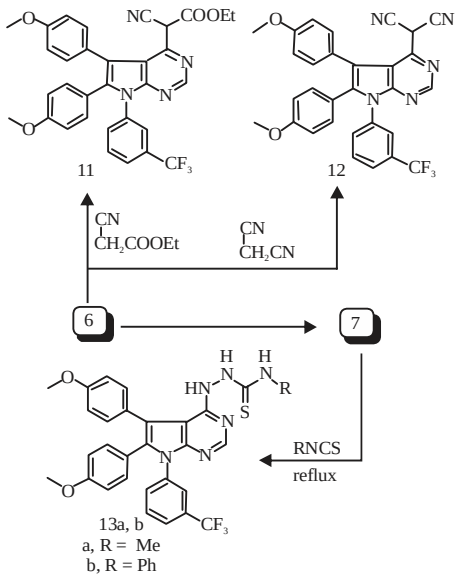

Fig. 3: Synthetic route for compounds 11-13

reacted with acetylacetone by refluxing to give the corresponding dimethylpyrazolopyrimidine derivative 10 (Fig. 2).

The reaction of chloro compound 6 with active methylene compounds, namely, ethylcyano acetate or malononitrile in the presence of potassium hydroxide in hot dimethylformamide to give cyano ethyl ester pyrimidine 11 and dicyanopyrimidine 12 derivatives, respectively. Finally, treatment of hydrazine derivative 7 with methyl- or phenyl- isothiocyanate afforded the corresponding N-thiosemicarbazide pyrimidine derivatives 13a, b, respectively (Fig. 3).

Table 1: Antimelanomal activity of the tested compounds against human melanoma cell line A375

Compound No.	A375P (IC_{50}, μM)
3	12.43
4	13.45
5a	14.54
5b	16.78
6	17.89
7	10.02
8	8.55
9	9.65
10	9.89
11	20.23
12	22.54
13a	23.45
13b	25.27
Sorafenib	5.00

Pharmacological activities: A series 6-aryl-pyrrolo[2,3-d] pyrimidine-4-amines were synthesized and some of which are epidermal growth factor tyrosine kinase inhibitors, these compounds were evaluated for their protozoal toxicity using an environmental tetrahymena strain as model organism. The protozoacidal activity of these compounds was very potent (Kaspersen *et al*., 2012).

A series of diaryl ureas having a pyrrolo[2,3-d]pyrimidine were synthesized and were evaluated for their *in vitro* antiproliferative activities against human melanoma cell line. Some of thee of these compounds were exhibited potent activity against A375 (Jung and Oh, 2008).

Due to structural similarity of our newly synthesized compounds and the last mentioned compounds, the newly synthesized compounds were evaluated for their protozoal toxicity using an environmental tetrahymena strain as model organism, *in vitro* antiproliferative activities against human melanoma and finally for their kinase inhibitor activities against 124 panel kinases.

***In vitro* antimelanoma activity:** The antiproliferative activities of the newly synthesized derivatives against human melanoma cell line A375 was given in Table 1 using Sorafenib as the reference standard. All the newly synthesized compounds showed potent antimelanomal activities against the melanomal cell line A 375, this clear from determining the IC_{50} of these compounds. The descending order of the antimelanomal activities was 8, 9, 10, 7, 3, 4, 5a, 5b, 6, 11, 12, 13a and 13b. All the tested compounds still less active than the positive reference standard Sorafenib (Gray-Schopfer *et al*., 2007; Eisen *et al*., 2006).

Toxicity towards tetrahymena: The newly synthesized tested compounds showed potent anti-tetrahymena activities this demonstrated via determining their MPC at 48 and 72 h. The

Table 2: Activity of the tested compounds against tetrahymena

	MPC (μg mL^{-1})	
Compound No.	48 h[a]	72 h[a]
3	8.54	13.73
4	9.63	14.64
5a	10.74	15.77
5b	11.85	17.88
6	12.74	18.99
7	7.16	12.65
8	4.36	7.77
9	5.46	8.76
10	6.25	9.74
11	13.86	19.88
12	15.95	20.78
13a	22.98	32.69
13b	34.99	44.50

[a]MPC values were determined via the averaging of 3 parallel measurements, MPC: Minimum protozoacidal concentration

Table 3: Kinases inhibitor activities of the newly synthesized compounds

	Remaining kinase activity (%) at 25 nM								
Compound No.	PKCa	JNK2	TrkA	ERK1	IKKe	MAPKAP-K3	CAMK1	MINK1	VEGFR
3	33.34	34.34	46.63	35.67	46.35	19.2	24.56	17.54	32.42
4	34.32	40.524	47.73	56.54	47.54	21.32	26.54	17.93	56.42
7	30.45	27.53	45.54	33.89	45.46	17.34	23.78	17.33	27.53
8	22.43	23.76	33.55	24.67	21.98	12.67	18.34	16.67	20.87
9	28.54	25.75	40.46	29.45	34.80	13.45	22.97	16.55	24.75
10	29.67	26.64	44.35	31.23	44.79	15.32	24.90	17.44	26.64

anti-tetrahymena descending activity order 8, 9, 10, 7, 3, 4, 5a, 5b, 6, 11, 12, 13a and 13b. All the tested newly synthesized compounds have protozoacidal activity with respect to as shown in Table 2.

Kinase inhibitor activity: The kinases inhibitor activities of the some newly synthesized compounds against 124 panel kinases culminated on that the newly synthesized compounds inhibited only PKCa, JNK2, TrkA, ERK1, MAPKAP-K3, CAMK1, MINK1 and VEGFR only and as shown in Table 3.

CONCLUSION

Compounds 8, 9, 10, 7, 3, 4, 5a, 5b, 6, 11, 12, 13a and 13b showed potent antimelanomal activities against the melanomal cell line A 375, anti-tetrahymena activities and kinases inhibitor activities against PKCa, JNK2, TrkA, ERK1, MAPKAP-K3, CAMK1, MINK1 and VEGFR, Carful examination of the relation of the chemical structures of the tested compounds and their pattern of activities was revealed on the following SAR points:

- Pyrrolopyrimidine ring essential for activities
- Tetraoko moiety provided the potent activities followed by triazole ones followed by pyrazole ones
- Hydrazine and free amino groups provide modern patterns of activities
- Thiourea gives the least potent compounds

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