Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications.

Novel pyrrole derivatives as selective CHK1 inhibitors: design, regioselective synthesis and molecular modeling

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4. Experimental section

All data pertaining to the experimental procedures and techniques adopted for the chemical synthesis of the target compounds, their biological screening and molecular modeling are included in the ESI.

4.1. Chemistry

4.1.1. General

All melting points were measured on a Gallenkamp melting point apparatus using open capillary and are uncorrected. The infrared spectra were recorded in potassium bromide disks on a PyeUnicam SP 3300 and Shimadzu FT IR 8101 PC infrared spectrophotometers. The NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer. ^{1}H NMR (300 MHz) and ^{13}C NMR (75.46 MHz) were run in deuterateddimethylsulphoxide (DMSO-d_6) or deuterated chloroform ($CDCl_3$) as indicated. Chemical shifts were related to that of the solvent. Mass spectra were recorded on a Shimadzu GCMS-QP 1000 EX mass spectrometer at70 e.V. Elemental analyses were carried out at the MicroanalyticalCenter of Cairo University, Giza, Egypt.

3-Acetyl-2-methyl-4,5-diphenyl-1*H*-pyrrole (**1**),[1] hydrazonoyl chlorides **4a-j**[2-5] were prepared following the procedures reported in the literature.

4.1.1.1. 3-(Dimethylamino)-1-(2-methyl-4,5-diphenyl-1*H*-pyrrol-3-yl)prop-2-en-1-one (2): A mixture of the acetylpyrrole**1** (5.50 g, 20 mmol) and dimethylformamide-dimethylacetal (DMF-DMA) (2.66 mL, 20 mmol) in dry xylene (30 mL) was refluxed for 6h, then allowed to cool. The orange yellow precipitate was filtered off, washed with petroleum ether (60/80 ^{o}C), dried and crystallized from ethanol/DMF to afford compound **2**. Yield 87%; mp.204-206 ^{o}C. IR (KBr) $\tilde{\nu}$ cm^{-1}: 3189 (NH), 1693 (C=O). ^{1}H NMR (DMSO-d_6) δ: 1.75 (s, 3H, CH_3), 2.49 (s, 6H, $2CH_3$), 5.76(d, 1H, CH, *J* = 12.2 Hz), 7.11-7.37 (m, 10H, ArH's), 7.59 (d, 1H, CH, *J* = 12.2 Hz), 11.60 (br., s, 1H, NH, D_2O exchangeable). MS *m/z* (%): 331 (M^++1, 25), 330 (M^+, 60), 286 (100), 260 (82), 230 (65), 77 (78). Anal. Calcd. for$C_{22}H_{22}N_2O$ (330.42): C, 79.97; H, 6.71; N, 8.48 %. Found: C, 79.78; H, 6.82; N, 8.30%.

4.1.1.2. 1-(2-Methyl-4,5-diphenyl-1*H*-pyrrol-3-yl)-3-morpholinoprop-2-en-1-one (3): A solution of the enaminone **2** (3.30g, 10 mmol) and morpholine (0.84 mL, 10 mmol) in ethanol (15 mL) was heated under reflux condition for 4 h. The reaction mixture was concentrated under reduced pressure. The solid product obtained upon cooling was filtered off and crystallized from ethanol/DMF to give compound **3**. Yield 76%; mp: 167-169 ^{o}C. IR (KBr) $\tilde{\nu}$ cm^{-1}: 2190 (NH), 1669 (C=O). ^{1}H NMR (DMSO-d_6) δ: 1.74 (s, 3H, CH_3), 2.92-3.84 (m, 8H, $4CH_2$), 5.77 (d, 1H, CH, *J* = 12.3 Hz), 7.32-7.61 (m, 10H, ArH's), 7.82 (d, 1H, CH, *J* = 12.3 Hz), 11.62 (br., s, 1H, NH, D_2O exchangeable). MS *m/z* (%): 372 (M^+, 64), 286 (100), 237 (43), 198 (28), 172 (59), 140 (68), 112 (35), 77 (78). Anal. Calcd. for$C_{24}H_{24}N_2O_2$ (372.46): C, 77.39; H, 6.49; N, 7.52%. Found: C, 77.24; H, 6.25; N, 7.27%.

4.1.1.3. Synthesis of (1,3-disubstituted-1*H*-pyrazol-4-yl)(2-methyl-4,5-diphenyl-1*H*-pyrrol-3-yl) methanone 7a-j.

General procedure: To a mixture of enaminone derivative **2** (0.330g, 10 mmol) and the appropriate hydrazonoyl chloride **4-j** (1 mmol) in benzene (20 mL), an equivalent amount of triethylamine was added. The reaction mixture was heated under reflux for 8 h. The solvent was distilled off at reduced pressure and the residual brown viscous liquid was triturated with

methanol. The resulting solid was collected by filtration washed thoroughly with ethanol, dried and finally crystallized from ethanol to afford the corresponding pyrazole derivatives **7a-j**.The synthesized compounds together with their physical and spectral data are listed below.

4.1.1.3.a.1-(4-(2-Methyl-4,5-diphenyl-1*H*-pyrrole-3-carbonyl)-1-phenyl-1*H*-pyrazol-3-yl)-ethanone (7a): Yield 77%; mp: 170-172 ^{o}C. IR (KBr) $\tilde{\nu}$ cm^{-1}: 2248 (NH), 1702, 1632 (2C=O). ^{1}H NMR (DMSO-d_6) δ: 1.82 (s, 3H, CH_3), 2.26 (s, 3H, CH_3), 7.19-7.44 (m, 15H, ArH's), 8.43 (s, 1H, py H-5), 11.66 (br., s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ: 13.7 (CH_3), 34.3 (CH_3), 116.1, 117.5, 117.7, 118.7, 122.0, 125.6, 126.70, 126.0, 126.5, 126.8, 128.1, 128.2, 128.9, 129.6, 129.7, 130.7, 132.1, 135.1, 137.0 (Ar-C), 176.8 (C=O), 194.3 (C=O). MS *m/z* (%): 446 (M^+ + 1, 54), 445 (M^+, 89), 362 (92), 304 (54), 255 (100), 214 (66), 154 (94), 80 (79). Anal. Calcd. for$C_{29}H_{23}N_3O_2$ (445.51): C, 78.18; H, 5.20; N, 9.43%. Found: C, 78.10; H, 5.31; N, 9.29%.

4.1.1.3.b. 1-(1-(4-Chlorophenyl)-4-(2-methyl-4,5-diphenyl-1*H*-pyrrole-3-carbonyl)-1*H*-pyrazol-3-yl) ethanone (7b): Yield 72%; mp: 194-196 ^{o}C. IR (KBr) $\tilde{\nu}$ cm^{-1}: 2240 (NH), 1708, 1631 (2C=O). ^{1}H NMR (DMSO-d_6) δ: 1.87 (s, 3H, CH_3), 2.31 (s, 3H, CH_3), 7.21-7.48 (m, 14H, ArH's), 8.46 (s, 1H, py H-5), 11.68 (br., s, 1H, NH, D_2O exchangeable). MS *m/z* (%): 481(M^+ + 2, 8), 479 (M^+, 25), 460 (92), 343 (100), 236 (88), 203 (72), 108 (91), 64 (92). Anal. Calcd. for$C_{29}H_{22}ClN_3O_2$ (479.96): C, 72.57; H, 4.62; N, 8.75%. Found: C, 72.44; H, 4.57; N, 8.68%.

4.1.1.3.c. 1-(4-(2-Methyl-4,5-diphenyl-1*H*-pyrrole-3-carbonyl)-1-(p-tolyl)-1*H*-pyrazol-3-yl)-ethanone (7c): Yield 76%; mp: 178-180 ^{o}C. IR (KBr) $\tilde{\nu}$ cm^{-1}: 2239 (NH), 1703, 1631 (2C=O). ^{1}H NMR (DMSO-d_6) δ: 1.84 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 2.48 (s, 3H, CH_3), 7.18-7.44 (m, 14H, ArH's), 8.44 (s, 1H, py H-5), 11.64 (br., s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ: 13.7 (CH_3), 14.7 (CH_3), 116.4, 119.3, 120.4, 121.5, 121.9, 122.1, 123.5, 124.1, 125.4, 127.4, 127.6, 127.8, 128.1, 129.7, 129.0, 130.4, 134.1, 138.9, 143.8 (Ar-C), 169.5 (C=O), 194.3 (C=O). MS *m/z* (%): 459 (M^+, 56), 406 (34), 327 (100), 203 (65), 108 (52), 77 (86). Anal. Calcd. for$C_{30}H_{25}N_3O_2$ (459.54): C, 78.41; H, 5.48; N, 9.14%. Found: C, 78.49; H, 5.32; N, 9.01%.

4.1.1.4.a.Ethyl 4-(2-methyl-4,5-diphenyl-1*H*-pyrrole-3-carbonyl)-1-phenyl-1*H*-pyrazole-3-carboxylate (7d): Yield 78%; mp: 177-179 ^{o}C.IR (KBr) $\tilde{\nu}$ cm^{-1}: 2248 (NH), 1728, 1630 (2C=O). ^{1}H NMR ($CDCl_3$) δ: 1.28 (t, 3H, CH_3, *J* = 7.2 Hz), 1.75 (s, 3H, CH_3), 4.20 (q, 2H, CH_2, *J* = 7.2 Hz), 7.12-7.69 (m, 15H, ArH's), 8.60 (s, 1H, pyrazole H-5), 11.59 (br., s, 1H, NH, D_2O exchangeable). MS *m/z* (%): 476 (M^+ + 1, 53), 475 (M^+, 88), 450 (100), 343 (94), 229 (95), 179 (87), 102 (79), 62 (91). Anal. Calcd. for$C_{30}H_{25}N_3O_3$ (475.54): C, 75.77; H, 5.30; N, 8.84%. Found: C, 75.87; H, 5.13; N, 8.55%.

4.1.1.4.b. Ethyl 1-(4-chlorophenyl)-4-(2-methyl-4,5-diphenyl-1*H*-pyrrole-3-carbonyl)-1*H*-pyrazole-3-carboxylate (7e): Yield 74%; mp: 212-214 ^{o}C. IR (KBr) $\tilde{\nu}$ cm^{-1}: 2286 (NH), 1727, 1645 (2C=O). ^{1}H NMR ($CDCl_3$) δ: 1.26 (t, 3H, CH_3, *J* = 7.2 Hz), 1.82 (s, 3H, CH_3), 4.27 (q, 2H, CH_2, *J* = 7.2 Hz), 7.19-7.66 (m, 14H, ArH's), 8.60 (s, 1H, pyrazole H-5), 11.57 (br., s, 1H, NH, D_2O exchangeable). MS *m/z* (%): 511 (M^+ + 2, 7), 509 (M^+, 23), 446 (93), 327 (88), 277 (89), 184 (81), 111 (95), 75 (83), 56 (100). Anal. Calcd. for$C_{30}H_{24}ClN_3O_3$ (509.98): C, 70.65; H, 4.74; N, 8.24%. Found: C, 70.44; H, 4.79; N, 8.12%.

4.1.1.4.c. Ethyl 4-(2-methyl-4,5-diphenyl-1*H*-pyrrole-3-carbonyl)-1-(p-tolyl)-1*H*-pyrazole-3-carboxylate (7f): Yield 74%; mp: 202-204 ^{o}C. IR (KBr) $\tilde{\nu}$ cm^{-1}: 2281 (NH), 1723, 1648 (2C=O). ^{1}H NMR ($CDCl_3$) δ: 1.24 (t, 3H, CH_3, *J* = 7.2 Hz), 1.75 (s, 3H, CH_3), 2.32 (s, 3H, CH_3), 4.25 (q, 2H, CH_2, *J* = 7.2 Hz), 7.12-7.82 (m, 14H, ArH's), 8.58 (s, 1H, pyrazole H-5), 11.59 (br., s, 1H, NH, D_2O exchangeable). MS *m/z* (%): 489 (M^+, 53), 430 (73), 295 (85), 229 (74), 172 (73), 91 (100).Anal. Calcd. for$C_{31}H_{27}N_3O_3$ (489.56): C, 76.05; H, 5.56; N, 8.58%. Found: C, 76.01; H, 5.50; N, 8.46%.

4.1.1.5.a. 4-(2-Methyl-4,5-diphenyl-1*H*-pyrrole-3-carbonyl)-N,1-diphenyl-1*H*-pyrazole-3-carboxamide (7g): Yield 70%; mp: 246-268 ^{o}C. IR (KBr) $\tilde{\nu}$ cm^{-1}: 3381, 3242 (2NH), 1668, 1643 (2C=O). ^{1}HNMR (DMSO-d_6) δ: 1.78 (s, 3H, CH_3), 7.16-7.49 (m, 20H, ArH's), 8.48 (s, 1H, pyrazole H-5), 11.59 (br., s, 1H, NH, D_2O-exchangeable), 11.91 (br., s, 1H, NH, D_2O-exchangeable). MS *m/z* (%): 523 (M^+ + 1, 8), 522 (M^+, 62), 456 (97), 337 (87), 250 (87), 203 (94), 124 (100), 103 (95),

77 (97). Anal. Calcd. for$C_{34}H_{26}N_4O_2$ (522.60): C, 78.14; H, 5.01; N, 10.72%. Found: C, 78.10; H, 5.22; N, 10.53%.

4.1.1.5.b. 1-(4-Chlorophenyl)-4-(2-methyl-4,5-diphenyl-1*H*-pyrrole-3-carbonyl)-N-phenyl-1*H*-pyrazole-3-carboxamide (7h): Yield 80%; mp: 253-255 ^{o}C.IR (KBr) $\tilde{\nu}$ cm^{-1}: 3364, 3249 (2NH), 1680, 1634 (2C=O). ^{1}H NMR (DMSO-d_6) δ: 1.79 (s, 3H, CH_3), 7.27-7.68 (m, 19H, ArH's), 8.43 (s, 1H, pyrazole H-5), 11.54 (br., s, 1H, NH, D_2O-exchangeable), 11.98 (br., s, 1H, NH, D_2O-exchangeable). MS *m/z* (%): 559 (M^+, 6), 557 (M^+, 19), 476 (88), 272 (22) 180 (8), 141 (25), 77 (100). Anal. Calcd. for$C_{34}H_{25}ClN_4O_2$ (557.04): C, 73.31; H, 4.52; N, 10.06%. Found: C, 73.17; H, 4.58; N, 9.87%.

4.1.1.5.c. 4-(2-Methyl-4,5-diphenyl-1*H*-pyrrole-3-carbonyl)-N-phenyl-1-(p-tolyl)-1*H*-pyrazole-3-carboxamide (7i): Yield 74%; mp: 212-214 ^{o}C.IR (KBr) $\tilde{\nu}$ cm^{-1}: 3341, 3244 (2NH), 1675, 1630 (2C=O). ^{1}H NMR (DMSO-d_6) δ: 1.76 (s, 3H, CH_3), 2.36 (s, 3H, CH_3), 7.21-7.63 (m, 19H, ArH's), 8.40 (s, 1H, pyrazole H-5), 11.53 (br., s, 1H, NH, D_2O-exchangeable), 11.90 (br., s, 1H, NH, D_2O-exchangeable). MS *m/z* (%): 536 (M^+, 15), 456 (100), 343 (42), 244 (54), 77 (78). Anal. Calcd. for$C_{35}H_{28}N_4O_2$ (536.62): C, 78.34; H, 5.26; N, 10.44 %. Found: C, 78.39; H, 5.16; N, 10.23%.

4.1.1.6. (1,3-Diphenyl-1*H*-pyrazol-4-yl)(2-methyl-4,5-diphenyl-1*H*-pyrrol-3-yl)methanone (7j): Yield 68%; mp: 227-228 ^{o}C. IR (KBr) $\tilde{\nu}$ cm^{-1}: 2234 (NH), 1634 (C=O). ^{1}H NMR (DMSO-d_6) δ: 1.83 (s, 3H, CH_3), 7.21-7.41 (m, 20H, ArH's), 8.52 (s, 1H, pyrazole H-5), 11.56 (br., s, 1H, NH, D_2O-exchangeable); ^{13}C-NMR (DMSO-d_6) δ: 13.7(CH_3), 115.7, 116.8, 117.4, 118.3, 122.3, 123.1, 125.2, 125.5, 125.7, 126.1, 126.8, 127.9, 128.1, 128.2, 129.3, 129.6, 130.0, 130.9, 133.2, 134.1, 137.0, 138.4, 143.4 (Ar-C), 194.4 (C=O). MS *m/z* (%): 479 (M^+, 49), 247 (26), 116 (58), 77 (100). Anal. Calcd. for $C_{33}H_{25}N_3O$ (479.57): C, 82.65; H, 5.25; N, 8.76%. Found: C, 82.47; H, 5.15; N, 8.54%.

4.1.1.7. Synthesis of pyrazolo[3,4-d]pyridazines 10a-f.

General Procedure: Hydrazine hydrate (80%, 2 mL) was added to a solution of the appropriate compounds **7a-f** (5 mmol) in EtOH (10 mL). The reaction mixture was heated under reflux for 2 h, concentrated under reduced pressure, and diluted with water. The precipitate obtained was filtered off, washed with ice-cold water, dried and crystallized from EtOH. The synthesized pyrazolo[3,4-*d*]pyridazines **10a-f** together with their physical and spectral data are listed below.

4.1.1.7.a.7-Methyl-4-(2-methyl-4,5-diphenyl-1*H*-pyrrol-3-yl)-2-phenyl-2*H*-pyrazolo[3,4-d]-pyridazine (10a): Yield 75%; mp: 264-268 ^{o}C. IR (KBr) $\tilde{\nu}$ cm^{-1}: 2237 (NH). ^{1}H NMR (DMSO-d_6) δ: 1.86 (s, 3H, CH_3), 2.40(s, 3H, CH_3), 7.19-7.49 (m, 15H, ArH's), 8.60 (s, 1H, pyrazole-H-5), 11.56 (br., s, 1H, NH, D_2O-exchangeable); ^{13}C-NMR (DMSO-d_6) δ: 13.3, 18.4 (CH_3), 114.5, 115.3 117.7, 117.8, 120.2, 124.5, 127.6, 127.9, 128.2, 128.4, 128.1, 128.9, 129.2, 129.6, 130.3, 130.7, 132.1, 135.1, 137.0, 142.4, 145.2 (Ar-C). MS *m/z* (%): 441 (M^+, 100), 284 (57), 103 (48), 77 (72). Anal. Calcd. for$C_{29}H_{23}N_5$ (441.53): C, 78.89; H, 5.25; N, 15.86%. Found: C, 78.70; H, 5.13; N, 15.63 %.

4.1.1.7.b.2-(4-Chlorophenyl)-7-methyl-4-(2-methyl-4,5-diphenyl-1*H*-pyrrol-3-yl)-2*H*-pyrazolo-[3,4-d]pyridazine (10b): Yield 73%; mp: 273-275 ^{o}C.IR (KBr) $\tilde{\nu}$ cm^{-1}: 2238 (NH). ^{1}H NMR (DMSO-d_6) δ: 1.82 (s, 3H, CH_3), 2.37 (s, 3H, CH_3), 7.20-7.44 (m, 14H, ArH's), 8.75 (s, 1H, pyrazole-H-5), 11.63 (br., s, 1H, NH, D_2O-exchangeable). MS *m/z*: 477 (M^+ + 2, 31), 475 (M^+, 100), 284 (43), 244 (62), 104 (62), 77 (73). Anal. Calcd. for$C_{29}H_{22}ClN_5$ (475.97): C, 73.18; H, 4.66; N, 14.71%. Found: C, 73.10; H, 4.59; N, 14.49%.

4.1.1.7.c.7-Methyl-4-(2-methyl-4,5-diphenyl-1*H*-pyrrol-3-yl)-2-(p-tolyl)-2*H*-pyrazolo[3,4-d]-pyridazine (10c): Yield 76%; mp: 267-269 ^{o}C. IR (KBr) $\tilde{\nu}$ cm^{-1}: 2239 (NH). ^{1}H NMR (DMSO-d_6) δ: 1.84 (s, 3H, CH_3), 2.24 (s, 3H, CH_3), 2.46 (s, 3H, CH_3), 7.24-7.47 (m, 14H, ArH's), 8.68 (s, 1H, py H-5), 11.61 (br., s, 1H, NH, D_2O-exchangeable). MS *m/z*: 455 (M^+, 100), 284 (54), 244 (43), 103 (38), 77 (76). Anal. Calcd. for$C_{30}H_{25}N_5$ (455.55): C, 79.10; H, 5.53; N, 15.37%. Found: C, 79.03; H, 5.67; N, 15.18%.

4.1.1.8.a. 4-(4,5-Diphenyl-1*H*-pyrrol-3-yl)-2-phenyl-2*H*-pyrazolo[3,4-d]pyridazin-7-ol (10d): Yield 73%; mp: 254-256 ^{o}C. IR (KBr) $\tilde{\nu}$ cm^{-1}: 3439 (OH), 2279 (NH). ^{1}H NMR (DMSO-d_6) δ: 1.84 (s, 3H, CH_3), 2.96 (br., s, 1H, OH, D_2O-exchangeable), 7.17-7.46 (m, 15H, ArH's), 8.63 (s, 1H, pyrazole H-5), 11.60 (br., s, 1H, NH, D_2O exchangeable). MS *m/z* (%): 429 (M^+, 100), 398 (38), 295 (54), 180 (41), 103 (32), 77 (94). Anal. Calcd. for$C_{27}H_{19}N_5O$ (429.47): C, 75.51; H, 4.46; N, 16.31%. Found: C, 75.44; H, 4.65; N, 16.23%.

4.1.1.8.b. 2-(4-Chlorophenyl)-4-(4,5-diphenyl-1*H*-pyrrol-3-yl)-2*H*-pyrazolo[3,4-d]pyridazin-7-ol (10e): Yield 72%; mp: 280-282 ^{o}C. IR (KBr) $\tilde{\nu}$ cm^{-1}: 3437 (OH), 3234 (NH). ^{1}H NMR (DMSO-d_6) δ: 1.87 (s, 3H, CH_3), 3.12 (br., s, 1H, OH, D_2O-exchangeable), 7.25-7.56 (m, 14H, ArH's), 8.70 (s, 1H, pyrazole H-5), 11.64 (br., s, 1H, NH, D_2O-exchangeable). MS *m/z* (%): 465 (M^+ + 2, 30), 463 (M^+, 100), 412 (17), 332 (29), 114 (38), 103 (56), 77 (72). Anal. Calcd. for$C_{27}H_{18}ClN_5O$ (463.92): C, 69.90; H, 3.91; N, 15.10%. Found: C, 69.96; H, 3.75; N, 14.93%.

4.1.1.8.c 4-(4,5-Diphenyl-1*H*-pyrrol-3-yl)-2-(p-tolyl)-2*H*-pyrazolo[3,4-d]pyridazin-7-ol (10f): Yield 75%; mp: 257-158 ^{o}C. IR (KBr) $\tilde{\nu}$ cm^{-1}: 3454 (OH), 3246 (NH). ^{1}H NMR (DMSO-d_6) δ: 1.78 (s, 3H, CH_3), 2.49 (s, 3H, CH_3), 3.11 (br., s, 1H, OH, D_2O exchangeable), 7.25-7.47 (m, 14H, ArH's), 8.62 (s, 1H, pyrazole-H-5), 11.61 (br., s, 1H, NH, D_2O exchangeable). MS *m/z* (%): 443 (100), 412 (35), 326 (82), 214 (56), 103 (61), 77 (68). Anal. Calcd. for$C_{28}H_{21}N_5O$ (443.50): C, 75.83; H, 4.77; N, 15.79%. Found: C, 75.63; H, 4.69; N, 15.69%.

4.1.1.9. Alternative synthesis of pyrazole 7j.

To a mixture of enaminone **3** (0.372 g, 1 mmol) and N'-phenylbenzohydrazonoyl chloride (**7j**) (0.230 g, 1 mmol) in dry benzene (20 mL), an equivalent amount of triethylamine was added. The reaction mixture was heated under reflux for 10 h. The solvent was removed under reduced pressure. The residual brown viscous liquid was taken in methanol. The resulting solid was collected by filtration washed with ethanol, dried and finally crystallized from DMF to afford a product which was identical in all aspects (m.p., mixed m.p. and spectra) with that obtained from reaction of **2** with **7j** but in 70% yield.

4.1.1.10. Synthesis of *N*-(6-(2-methyl-4,5-diphenyl-1*H*-pyrrol-3-yl)-2-oxo-2*H*-pyran-3-yl)benzamide (14): A solution of enaminone **2** (0.330 g, 1 mmol) and hippuric acid (**11**) (0.17 g,1 mmol) in acetic anhydride (20 mL) was heated under reflux for 2 h. The reaction mixture was concentrated under reduced pressure. The solid product obtained upon cooling was filtered off and recrystallized from DMF to yielded compound **14** in 79% yield, mp: 276-278 ^{o}C. IR (KBr) $\tilde{\nu}$ cm^{-1}: 3298, 3288 (2NH), 1706, 1648 (2C=O). ^{1}H NMR (DMSO-d_6) δ: 1.82 (s, 3H, CH_3), 6.74 (d, 1H, *J* = 7.6 Hz*,* pyran-H5), 6.78 (d, 1H, *J* = 7.6 Hz*,* pyran-H4),7.19-7.61 (m, 15H, ArH's), 9.43 (br., s, 1H, NH, D_2O exchangeable), 11.66 (br., s, 1H, NH, D_2O exchangeable). MS *m/z* (%): 447 (M^+ + 1, 73), 446 (M^+, 98), 418 (83), 344 (79), 276 (91), 218 (91), 105 (89), 77 (100).Anal. Calcd. for $C_{29}H_{22}N_2O_3$ (446.50): C, 78.01; H, 4.97; N, 6.27. Found: C, 78.08; H, 4.68; N, 6.05%.

4.1.1.11. Synthesis of derivatives 16 and 18.

General Procedure: To a stirred solution of enaminone derivative **2** (0.330 g, 1 mmol) in glacial acetic acid (20 mL), p-benzoquinone **15** or 1,4-naphthoquinone **17** (1 mmol) was added, then the resulting mixture was stirred for 6 h at room temperature. The solvent was evaporated under reduced pressure, and the solid product obtained was filtered off and recrystallized from DMF to afford a pure solid of **16** and **18**, respectively.

4.1.1.11.a. (5-Hydroxybenzofuran-3-yl)(2-methyl-4,5-diphenyl-1*H*-pyrrol-3-yl)methanone (16): Yield 73%; mp: 246-248 ^{o}C. IR (KBr) $\tilde{\nu}$ cm^{-1}: 3368 (OH), 3269 (NH), 1629 (C=O). ^{1}H NMR (DMSO-d_6) δ: 1.75 (s, 3H, CH_3), 3.18 (br., s, 1H, OH, D_2O exchangeable), 7.02-7.79 (m, 13H, ArH's), 8.72 (s, 1H, furan-H-2), 11.66 (br., s, 1H, NH, D_2O exchangeable). MS *m/z* (%): 394 (M^++1, 55), 393 (M^+, 99), 370 (100), 238 (88), 174 (96), 133 (96), 114 (96), 69 (80**)**. Anal. Calcd. ror $C_{26}H_{19}NO_3$ (393.43): C, 79.37; H, 4.87; N, 3.56%. Found: C, 79.30; H, 4.96; N, 3.25%.

4.1.1.11.b. (5-Hydroxynaphtho[1,2-b]furan-3-yl)(2-methyl-4,5-diphenyl-1H-pyrrol-3-yl)-methanone (18): Yield 70%; mp: 230-232 ^{o}C. IR (KBr) $\tilde{\nu}$ cm^{-1}: 3432 (OH), 3187 (NH), 1631 (C=O). ^{1}H NMR (DMSO-d_6) δ: 1.82 (s, 3H, CH_3),3.18 (br., s, 1H, OH, D_2O exchangeable), 7.04-7.97 (m,

15H, ArH's), 8.66 (s, 1H, furan-H-2), 11.65 (br., s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ: 13.7 (CH_3), 118.4, 118.5, 119.2, 119.8, 122.0, 126.0, 126.50, 128.1, 128.2, 129.5, 130.7, 132.2, 135.9, 136.6, 136.9, 137.7, 137.9, 138.1, 138.8, 139.4, 141.3, 141.8, 144.6, 145.2 (Ar-C), 194.4 (C=O). MS *m/z* (%): 444 (M^+ + 1, 82), 443 (M^+, 100), 386 (100), 321 (87), 212 (88), 113 (77), 77 (76), 58 (92). Anal. Calcd. for $C_{30}H_{21}NO_3$ (443.49): C, 81.25; H, 4.77; N, 3.16%. Found: C, 81.38; H, 4.79; N, 3.02%.

4.2. Biology

4.2.1. Kinase screening at 30 µM to determine % kinase inhibition: Microfluidic mobility shift assays to determine kinase inhibition were carried out using ProfilerPro assay kit (Caliper LifeSciences).The kit contained an enzyme plate, a substrate plate, reconstitution buffer, 1M DTT, protease inhibitor cocktail and termination buffer. Test compounds were added as a solution in DMSO, at the appropriate concentration. The peptide phosphorylation assay was carried out as per the manufacturer's instructions. The electrophoretic separation and analysis of the phosphorylated and non-phosphorylated peptides was carried out on a 4-sipper chip using a LabChipEZReader II (Caliper LifeSciences). Results were expressed as % inhibition of peptide phosphorylation relative to control wells for 0% (no test compound) and 100% (no ATP) inhibition. A plate layout was used to accommodate up to 12 test compounds in each assay run. The pan-kinase inhibitor H-89 (30 µM) was incorporated as a test compound in each separate assay plate run.

4.2.2. Determination of IC_{50} for EGFR and VEGFR-2 kinase activity by ELISA: The assay was performed in 96-well plates pre-coated with 20 µgmL^{-1} poly (Glu,Tyr) 4:1 (Sigma) as a substrate. In each well, 85 µL of an 8 µM ATP solution and 10 µL of the test compounds (at varying concentrations) were added. 0.1% (v/v) DMSO was sued as a negative control. Experiments at each concentration were performed in triplicate. The reaction was initiated by adding 5 µL of VEGFR-2 or EGFR kinase. After incubation for 1 h at 37 °C, the plate was washed three times with PBS containing 0.1% Tween 20 (T-PBS). Next, 100 µL of anti-phosphotyrosine (PY99; 1:100 dilution) antibody was added. After 1 h of incubation at room temperature, the plate was

washed three times. Goat anti-mouse IgG horseradish peroxidase (100 µL; 1:2000 dilution) diluted in T-PBS containing 5 mg mL^{-1} BSA was added. The plate was reincubated at room temperature for 1 h, and washed as before. Finally, 100 µL of developing solution (0.03% H_2O_2, 2 mg mL^{-1} o-phenylenediamine in citrate buffer 0.1 M, pH 5.5) was added and incubated at room temperature until color emerged. The reaction was terminated by the addition of 100 µL of 2 M H_2SO_4, and absorbance at 492 nm (A492) was measured using a multiwell spectrophotometer (VERSAmax™). IC_{50} values were calculated in GraphPad Prism5 using a non-linear regression fit of the log[inhibitor] *vs* response with variable slope equation.

4.2.3. Determination of IC_{50} for CHK1 kinase activity by ELISA: CHK1 kinase activity was measured in a microfluidic assay that monitored the separation of a phosphorylated product from its substrate. The assay was run on an EZ Reader II (Caliper Life Sciences Ltd, Runcorn, UK) using separation buffer (#760367 Caliper LS) containing CR-8 (500 nM, #760278, Caliper LS). An ECHOR 550 (LabcyteInc™) acoustic dispenser was used to generate duplicate 8 point dilution curves directly into 384 polypropylene assay plates (Greiner Bio-One, Gloucestershire, UK). For each compound a 50 µM stock concentration in 100% DMSO was used. The total amount of DMSO dispensed per well was 250 nL to give a final assay concentration of 2.5% DMSO and compound concentrations in the range 0.5-1000 nM. To this assay plate, 6 pL CHK1 (2 nM final concentration, in-house protein preparation), 2 pL peptide 10 (5-FAM-KKKVSRSGLYRSPSMPENLNRPR-COOH, 1.5 pM final concentration, #760354 Caliper LS) and 2 pL ATP (90 pM final concentration) all diluted in kinase buffer (HEPES 50 mM, NaN_3 0.02%, BSA 0.01%, sodium orthovanadate 0.1 mM, DTT 1 mM, $MgCl_2$ 2 mM, Tween20 0.1%) were added. The plate was sealed and centrifuged (1 min, 1000 rpm) before incubation for 1 h at room temperature. The reaction was stopped by the addition of separation buffer (90 pL). The plate was read on an EZ Reader II, using a 12-sipper chip (#760404, CaliperLS) with instrument settings of -1.5 psi and 1750 ΔV. The percentage conversion of product from substrate was generated automatically and the percentage inhibition was calculated relative to blank wells (containing no enzyme and 2.5% DMSO) and total wells (containing all reagents and 2.5% DMSO). IC_{50} values were calculated in GraphPadPrism5 using a non-linear regression fit of the

log (inhibitor) *vs* response with variable slope equation. The enzyme reaction was carried out in 96-well polypropylene plates (Greiner).

4.3. Docking study

Since the main goal of this part of the study was to perform docking to better understand the possible binding affinity and mode between the synthesized compounds as candidate ligands and their respective receptor, CHK1 protein structure obtained from the protein databank (PDB ID: 2YEX) was chosen for the docking study. During the docking procedure, the default settings were applied utilizing MOE v10.2008. CHK1 protein structure was first repaired and then appropriately protonated in the presence of ligand using the Protonate 3D process in MOE and then used directly for docking. The candidate ligands were prepared by performing a systematic conformational search associated with energy minimization. The lowest energy conformer for each compound was utilized for performing the docking. The experimental structure of the "template" ligand is then deleted, leaving the candidate ligand docked to the protein. Docking was performed employing the default parameters using Triangle Matcher placement method with London dG scoring was used for the docking runs, and the 30 docking poses were retained by the software were individually examined for final choice of the best docking pose. The best docking conformer for each candidate ligand was chosen based on a set of parameters *viz*. the S score reflecting the ligand's binding energy, energy of placement, energy of conformation and ligand-receptor binding interactions.

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