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ANTICANCER EVALUATION AND SYNTHESIS OF STYRYLDIHYDROPYRIMIDINE VIA BRONSTED ACID-CATALYZED VINYLOGOUS ALDOL REACTION

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ABSTRACT
Vinylogous aldol condensation on some dihydropirimidine (DHPM) derivatives has been successfully performed using *p*-toluenesulfonic acid as a Brønsted acid catalyst. The pure products could be obtained without further purification. Employing this method, 20 styryldihydropyrimidine derivatives have been successfully synthesized wherein 19 of them are new compounds. The structures of the reaction products were elucidated by spectroscopic methods (IR, NMR, MS), while their cytotoxic activities were determined toward two cancer cell lines HeLa and MCF-7. Some compounds showed high activity better than doxorubicin as a positive control.
Keywords: Vinylogous Aldol Condensation, Brønsted Acid Catalyst, Dihydropyrimidinone, Cancer Cells, Cytotoxic
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INTRODUCTION

Derivatives of 3,4-dihydropyrimidine-2-one (DHPM) as a product of Biginelli reaction have been already known to possess various interesting pharmacological activities, such as antimicrobial[1,2], antioxidant[3,4], antiinflammation[5,6], antitubercular[7,8], antimalarial[9], antihypertension[10,11], anticancer[12,13], and antiretroviral[14]. Structure modification of DHPM derivatives whether to enhance or explore their new pharmacological activity belongs to an attractive interest. One possibility of DHPM structure modification is the addition of styryl substituent to furnish styryl heterocyclic derivatives, which is styryl-DHPM. Recently, styryl-DHPM derivatives catch particular interest due to their broad attractive pharmacological activities such as antimicrobial[15], antimalarial, antiretroviral[16], antiinflammation[17], anticancer[18,19], and antihypertensive.[20] Therefore, the synthesis of styryl-DHPM becomes an attractive topic to be explored and developed. Styryl substituent on DHPM is generally attached at the C-4 or C-6 position. Usually, a DHPM derivative possessing styryl substituent at C-4 is synthesized by Biginelli reaction using cinnamaldehyde derivative as substrate.[18] Whereas, DHPM derivative with styryl substituent at C-6 is made by aldol reaction between DHPM with aromatic aldehyde by utilizing nucleophilicity of vinylogous methyl at C-6. Although the nucleophilicity of this vinylogous methyl is relatively weak, a lot of articles available report various reactions of vinylogous methyl with various electrophiles such as halogenation[21-24], alkylation[24-26], Claisen condensation followed by cyclization[26], and vinylogous aldol condensation.[27-30] Differing from simple aldol condensation, vinylogous aldol condensation on DHPM compound is relatively more difficult. Therefore developing this reaction method is such a challenging theme. Synthesis of styryl-DHPM derivative using vinylogous condensation was reported firstly by Kappe and Falsone (2001) as a side product of Biginelli reaction using *p*-toluenesulfonic acid (PTSA) as catalyst and xylene as the solvent, although they were not sure.[31] Zhang *et al*. (2014) reported the synthesis of styryl-DHPM using amide-attaching DHPM and Lewis acid catalyst ($FeCl_3.6H_2O$) in acetonitrile.[27] Furthermore in the year 2015, they successfully developed a synthesis method of styryl-DHPM in a one-pot pseudo-4-components reaction.[28] However, unfortunately, the synthesis protocol failed to synthesize styryl-DHPM derivatives from DHPM containing ester moiety. Mondal *et al.* (2018) claimed that DHPM with ester moiety can be transformed into styryl-DHPM in the same reaction condition but need more amount of catalyst.[30] Previously, Suwito *et al.* (2017) reported the

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formation of styryl-DHPM from Biginelli reaction with PTSA as a catalyst in ethanol but failed to achieve an efficient reaction condition.[29] Besides the utilization of acid, the base catalyst can also be used for the synthesis of this derivative. Unfortunately, the use of the strong base as NaOH or KOH is only suitable for the synthesis of styryl-DHPM without an ester group because a strong base leads to ester hydrolysis.[15-17] Organic bases such as pyridine can also be used to avoid ester hydrolysis, but there is no further study on whether this reaction condition is efficient for various aldehydes.[20] Therefore, the use of an acid catalyst is appropriate to avoid undesired side reactions. In this paper, we report the synthesis of styryl-DHPM derivatives from ester-DHPM using PTSA as a Brønsted acid catalyst. The advantage of this method is that all the products were obtained without further purification. Moreover, we also report anti-proliferation activities of the prepared compounds toward HeLa and MCF-7 cancer lines.

EXPERIMENTAL

Material

All chemicals used in the research were provided by E. Merck (Darmstadt-Germany) and Sigma-Aldrich (St Louis, Missouri, United State of America) with the pro-synthesis grade for reactants, and pro-analysis grade for solvents. They were used without prior purification. The purity of the reaction products and reaction progress was controlled by thin layer chromatography using silica gel plate GF_{254} (E. Merck, Darmstadt, Germany), eluted with suitable eluent systems, and the spots were observed using a UV lamp (254 nm).

Instrumentation

Mass spectra were recorded on HRESIMS QTOF micrOTOF-Q II (Bruker Daltonics, Billerica, MA, USA) and ESI-MS TSQ Vantage Triple-Stage Quadrupole Mass Spectrometer (Thermo Scientific, Waltham, MA, USA). FTIR spectra were recorded on an IRTracer-100 (Shimadzu, Kyoto, Japan) spectrophotometer using the diffuse-reflectance method with KBr powder. All spectra of ^{1}H-NMR (400 MHz) and ^{13}C-NMR (APT, 101 MHz) were recorded on JEOL JNM-ECA400 (JEOL Ltd., Tokyo, Japan) using $CDCl_3$ or DMSO-d_6 as a solvent and internal standard.

General Procedure

All of the DHPM derivatives used as precursors were prepared using Biginelli reaction under reflux and PTSA as catalyst and ethanol as solvent.[35] Synthesis of the target molecules was conducted as follows, 0.5 mmol of DHPM derivatives, 1 mmol aromatic aldehyde, and some PTSA were placed in 25 mL three neck round bottom flask, dissolved in 3 mL acetonitrile, then refluxed. The reaction progress was monitored by TLC. After completion of the reaction, the reaction mixture was cooled down and precipitated by cold methanol-water. The precipitate was then filtered off, dried, and tested its purity using TLC. The molecular structure was then elucidated by spectroscopy methods (FTIR, HRESIMS or ESIMS, and NMR). The results and structure characterization of the prepared compounds are briefly displayed below.

Ethyl (E)-4-(2,4-dimethoxyphenyl)-6-(2,5-dimethoxystyryl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (TM1)

It was prepared by following the procedure as described using 20 mol% catalysts, yellow solid (69.2 mg; 30%), R_f = 0.46 ($CHCl_3$: EtOAc = 3:1); MS(ESI) $[M+Na]^+$ calcd. for $C_{25}H_{28}N_2O_7$ 491, found 491; IR $\tilde{\nu}$ (KBr, cm^{-1}): 3273, 3211, 3100, 2954, 1697, 1684, 1628, 1611, 1587, 1221, 1088; ^{1}H-NMR (400 MHz, $CDCl_3$) δ_H (ppm): 8.15 (d, J = 16.9 Hz, 1H), 7.38 (d, J = 16.9 Hz, 1H), 7.20 (d, J = 2.8 Hz, 1H), 7.02 (d, J = 8.4 Hz, 1H), 7.01 (s, 1H), 6.88 (dd, J = 8.9, 2.8 Hz, 1H), 6.84 (d, J = 8.9 Hz, 1H), 6.47 (d, J = 2.3 Hz, 1H), 6.37 (dd, J = 8.4, 2.3 Hz, 1H), 5.75 (m, 2H), 4.12 (m, 2H), 3.85 (s, 3H), 3.83 (s, 3H), 3.81 (s, 3H), 3.78 (s, 3H), 1.17 (t, J = 7.1 Hz, 3H); ^{13}C-NMR (101 MHz, $CDCl_3$) δ_C (ppm): 165.7 (C), 160.8 (C), 158.0 (C), 153.8 (C), 153.3 (C), 152.1 (C), 144.6 (C), 127.9 (CH), 127.5 (CH), 125.2 (C), 122.2 (C), 120.1 (CH), 116.3 (CH), 112.4 (CH), 111.8 (CH), 103.9 (CH), 101.0 (C), 98.9 (CH), 60.4 (CH_2), 56.2 (CH_3), 56.0 (CH_3), 55.5 (CH_3), 55.5 (CH_3), 50.1 (CH), 14.4 (CH_3).

Ethyl (E)-4-(2,4-dimethoxyphenyl)-6-(2-methoxystyryl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (TM2)

It was prepared by following the procedure as described using 20 mol% catalysts, reddish yellow solid (54.1 mg; 25%); R_f = 0.50 ($CHCl_3$: EtOAc = 3:1); MS(ESI) $[M+H]^+$ calcd. for $C_{24}H_{26}N_2O_6$ 439, found 439; IR $\tilde{\nu}$ (KBr, cm^{-1}): 3264, 3103, 2955, 1697, 1686, 1630, 1587, 1502, 1225, 1089; ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H (ppm): 9.18 (s, 1H), 7.98 (d, *J* = 16.8 Hz, 1H), 7.57 (d, *J* = 16.8 Hz, 1H), 7.52 (dd, *J* = 7.7, 1.4 Hz, 1H), 7.34 (t, *J* = 7.7 Hz, 1H), 7.29 (s, 1H), 7.07 (d, *J* = 8.4 Hz, 1H), 7.00 (t, *J* = 8.4 Hz, 1H), 6.99 (d, *J* = 8.4 Hz, 1H), 6.56 (d, *J* = 2.3 Hz, 1H), 6.46 (dd, *J* = 8.4, 2.3 Hz, 1H), 5.50 (d, *J* = 3.2 Hz, 1H), 3.99 (m, 2H), 3.87 (s, 3H), 3.79 (s, 3H), 3.74 (s, 3H), 1.08 (t, *J* = 7.1 Hz, 3H). ^{13}C-NMR (101 MHz, DMSO-d_6) δ_C (ppm): 165.2 (C), 160.0 (C), 157.5 (C), 157.3 (C), 152.7 (C), 145.3 (C), 130.3 (CH), 129.7 (CH), 127.7 (CH), 127.6 (CH), 124.8 (C), 123.7 (C), 120.8 (CH), 120.5 (CH), 111.7 (CH), 104.5 (CH), 100.8 (C), 98.5 (CH), 59.5 (CH_2), 55.6 (CH_3), 55.5 (CH_3), 55.2 (CH_3), 48.7 (CH), 14.0 (CH_3).

Ethyl (E)-4-(2,5-dimethoxyphenyl)-6-(2,5-dimethoxystyryl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (TM3)

It was prepared by following the procedure as described using 40 mol% catalysts, yellow solid (165.8 mg; 71%), R_f = 0.5 ($CHCl_3$: EtOAc = 2:1); HRMS (ESI) $[M+Na]^+$ calcd. For $C_{25}H_{28}N_2O_7$ 491.1794, found 491.1782; IR $\tilde{\nu}$ (KBr, cm^{-1}): 3321, 3092, 2953, 1701, 1682, 1634, 1593, 1497, 1221, 1101; ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H (ppm): 9.22 (s, 1H), 7.95 (d, *J* = 16.7 Hz, 1H), 7.53 (d, *J* = 16.7 Hz, 1H), 7.37 (s, 1H), 7.08 (d, *J* = 3.0 Hz, 1H), 7.01 (d, *J* = 9.1 Hz, 1H), 6.94 (d, *J* = 8.9 Hz, 1H), 6.93 (dd, *J* = 9.1, 3.1 Hz, 1H), 6.83 (dd, *J* = 8.9, 3.1 Hz, 1H), 6.66 (d, *J* = 3.1 Hz, 1H), 5.53 (d, *J* = 3.3 Hz, 1H), 4.01 (m, 2H), 3.81 (s, 3H), 3.76 (s, 3H), 3.75 (s, 3H), 3.66 (s, 3H), 1.10 (t, *J* = 7.1 Hz, 3H). **^{13}C-NMR** (101 MHz, DMSO-d_6) δ_C (ppm): 165.2 (C), 153.3 (C), 152.9 (C), 152.7 (C), 151.8 (C), 150.7 (C), 145.4 (C), 132.3 (C), 129.8 (CH), 125.4 (C), 120.8 (CH), 115.6 (CH), 114.2 (CH), 113.1 (CH), 112.3 (CH), 112.2 (CH), 112.0 (CH), 100.6 (C), 59.6 (CH_2), 56.2 (CH_3), 55.9 (CH_3), 55.5 (CH_3), 55.3 (CH_3), 49.2 (CH), 14.0 (CH_3).

Ethyl (E)-4-(2,5-dimethoxyphenyl)-6-(2,3-dimethoxystyryl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (TM4)

It was prepared by following the procedure as described using 40 mol% catalysts, yellow solid (121.5 mg; 52%), R_f = 0.52 (CH_2Cl_2: EtOAc = 3:1); HRMS (ESI) $[M+Na]^+$ calcd. for $C_{25}H_{28}N_2O_7$ 491.1794, found 491.1795; IR $\tilde{\nu}$ (KBr, cm^{-1}): 3242, 3090, 2941, 1697, 1682, 1628, 1593, 1503, 1229, 1101; ^{1}H-NMR (400 MHz, $CDCl_3$) δ_H (ppm): 8.21 (d, *J* = 16.9 Hz, 1H), 7.38 (d, *J* = 16.9 Hz, 1H), 7.30 (dd, *J* = 8.0, 1.3 Hz, 1H), 7.11 (s, 1H), 7.07 (t, *J* = 8.0 Hz, 1H), 6.91 (dd, *J* = 8.0, 1.3 Hz, 1H), 6.82 (d, *J* = 8.8 Hz, 1H), 6.76 (dd, *J* = 8.8, 2.9 Hz, 1H), 6.72 (d, *J* = 2.9 Hz, 1H), 5.80 (s, 2H), 4.12 (m, 2H), 3.88 (s, 3H), 3.86 (s, 3H), 3.85 (s, 3H), 3.71 (s, 3H), 1.16 (t, *J* = 7.1 Hz, 3H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ_C (ppm): 165.5 (C), 153.7 (C), 153.1 (C), 153.0 (C), 151.1 (C), 147.9 (C), 144.9 (C), 130.7 (C), 129.7 (C), 127.7 (CH), 124.3 (CH), 121.0 (CH), 118.5 (CH), 114.0 (CH), 113.2 (CH), 112.4 (CH), 111.4 (CH), 100.7 (C), 61.4 (CH_3), 60.4 (CH_2), 56.0 (CH_3), 55.9 (CH_3), 55.8 (CH_3), 50.4 (CH), 14.3 (CH_3).

Ethyl (E)-4-(2,5-dimethoxyphenyl)-6-(2-methoxystyryl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (TM5)

It was prepared by following the procedure as described using 40 mol% catalysts, yellow solid (151.3 mg; 69%), R_f = 0.51 (CH_2Cl_2: EtOAc = 2:1); HRMS (ESI) $[M+Na]^+$ calcd. for $C_{24}H_{26}N_2O_6$ 461.1689, found 461.1683; IR $\tilde{\nu}$ (KBr, cm^{-1}): 3231, 3092, 2937, 1701, 1686, 1628, 1595, 1501, 1229, 1097; ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H (ppm): 9.22 (s, 1H), 7.96 (d, *J* = 16.7 Hz, 1H), 7.56 (d, *J* = 16.7 Hz, 1H), 7.53 (d, *J* = 7.5 Hz, 1H), 7.36 (s, 1H), 7.33 (t, *J* = 8.3, 1H), 7.07 (d, *J* = 8.3 Hz, 1H), 7.00 (t, *J* = 7.5 Hz, 1H), 6.94 (d, *J* = 8.9 Hz, 1H), 6.82 (dd, *J* = 8.9, 3.0 Hz, 1H), 6.65 (d, *J* = 3.0 Hz, 1H), 5.52 (d, *J* = 3.2 Hz, 1H), 4.00 (m, 2H), 3.86 (s, 3H), 3.75 (s, 3H), 3.65 (s, 3H), 1.08 (t, *J* = 7.1 Hz, 3H). ^{13}C-NMR (101 MHz, DMSO-d_6) δ_C (ppm): 165.3 (C), 157.4 (C), 153.0 (C), 152.7 (C), 150.8 (C), 145.6 (C), 132.4 (C), 130.4 (CH), 130.0 (CH), 127.7 (CH), 124.7 (C), 120.9 (CH), 120.4 (CH), 114.2 (CH), 112.3 (CH), 112.1 (CH), 111.8 (CH), 100.5 (C), 59.6 (CH_2), 56.0 (CH_3), 55.7 (CH_3), 55.4 (CH_3), 49.3 (CH), 14.1 (CH_3).

Ethyl (E)-4-(2,5-dimethoxyphenyl)-6-(4-fluorostyryl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (TM6)

It was prepared by following the procedure as described using 40 mol% catalysts, yellow solid (132.6 mg; 62%), R_f = 0.54 (CH_2Cl_2: EtOAc = 2:1); HRMS (ESI) $[M+Na]^+$ calcd. for $C_{23}H_{23}FN_2O_5$ 449.1489, found 449.1480; IR $\tilde{\nu}$ (KBr, cm^{-1}): 3248, 3115, 3092, 2953, 1684, 1636, 1599, 1499, 1231; ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H (ppm): 9.12 (s, 1H), 7.89 (d, J = 16.7 Hz, 1H), 7.57 (dd, J = 8.5, 5.8 Hz, 2H), 7.44 (d, J = 16.7 Hz, 1H), 7.41 (s, 1H), 7.26 (t, J = 8.5 Hz, 2H), 6.94 (d, J = 8.6 Hz, 1H), 6.82 (dd, J = 8.6, 2.6 Hz, 1H), 6.65 (d, J = 2.6 Hz, 1H), 5.53 (d, J = 2.8 Hz, 1H), 4.01 (m, 2H), 3.75 (s, 3H), 3.65 (s, 3H), 1.09 (t, J = 7.0 Hz, 3H). ^{13}C-NMR (101 MHz, DMSO-d_6) δ_C (ppm): 165.2 (C), 162.4 (d, $^1J_{CF}$ = 247.5 Hz, C), 152.9 (C), 152.5 (C), 150.7 (C), 144.9 (C), 133.2 (CH), 132.6 (d, $^4J_{CF}$ = 4.0 Hz, C), 132.4 (C), 129.2 (d, $^3J_{CF}$ = 9.1 Hz, CH), 119.5 (CH), 116.0 (d, $^2J_{CF}$ = 21.2 Hz, CH), 114.2 (CH), 112.3 (CH), 112.2 (CH), 100.7 (C), 59.7 (CH_2), 56.0 (CH_3), 55.3 (CH_3), 49.4 (CH), 14.0 (CH_3).

Ethyl (E)-6-(4-chlorostyryl)-4-(2,5-dimethoxyphenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (TM7)

It was prepared by following the procedure as described using 40 mol% catalysts, yellow solid (56 mg; 25%), R_f = 0.46 (CH_2Cl_2: EtOAc = 2:1); HRMS (ESI) $[M+Na]^+$ calcd. for $C_{23}H_{23}ClN_2O_5$ 465.1193, found 465.1174; IR $\tilde{\nu}$ (KBr, cm^{-1}): 3219, 3111, 3092, 2963, 1701, 1686, 1634, 1597, 1493, 1232, 1097; ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H (ppm): 9.14 (s, 1H), 7.95 (d, J = 16.7 Hz, 1H), 7.54 (d, J = 8.7 Hz, 2H), 7.49 (d, J = 8.7 Hz, 2H), 7.44 (d, J = 16.7 Hz, 1H) 7.43 (s, 1H), 6.94 (d, J = 8.9 Hz, 1H), 6.82 (dd, J = 8.9, 3.1 Hz, 1H), 6.66 (d, J = 3.1 Hz, 1H), 5.54 (d, J = 3.2 Hz, 1H), 4.01 (m, 2H), 3.75 (s, 3H), 3.65 (s, 3H), 1.09 (t, J = 7.1 Hz, 3H). ^{13}C-NMR (101 MHz, DMSO-d_6) δ_C (ppm): 165.1 (C), 152.9 (C), 152.4 (C), 150.7 (C), 144.7 (C), 135.0 (C), 133.4 (C), 133.0 (CH), 132.3 (C), 129.0 (CH), 128.8 (CH), 120.4 (CH), 114.2 (CH), 112.3 (CH), 112.2 (CH), 101.0 (C), 59.7 (CH_2), 56.0 (CH_3), 55.3 (CH_3), 49.5 (CH), 14.0 (CH_3).

Ethyl(E)-6-(2,5-dimethoxystyryl)-4-(4-hydroxy-3-methoxyphenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (TM8)

It was prepared by following the procedure as described using 60 mol% catalysts, pale yellow solid (140.3 mg; 62%); R_f = 0.50 ($CHCl_3$: EtOAc = 1:5); MS(ESI) $[M+H]^+$ calcd. for$C_{24}H_{26}N_2O_7$ 455, found 455; IR $\tilde{\nu}$ (KBr, cm^{-1}): 3242, 3325, 3094, 2938, 1703, 1684, 1632, 1595, 1528, 1221, 1096; ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H (ppm): 9.23 (s, 1H), 8.94 (s, 1H), 7.91 (d, J = 16.7 Hz, 1H), 7.72 (s, 1H), 7.51 (d, J = 16.7 Hz, 1H), 7.05 (d, J = 3.0 Hz, 1H), 7.00 (d, J = 9.0 Hz, 1H), 6.92 (dd, J = 9.0, 3.0 Hz, 1H), 6.87 (d, J = 2.0 Hz, 1H), 6.73 (d, J = 8.2 Hz, 1H), 6.67 (dd, J = 8.2, 2.0 Hz, 1H), 5.17 (d, J = 3.4 Hz, 1H), 4.08 (qd, J = 7.0, 1.2 Hz, 2H), 3.81 (s, 3H), 3.74 (s, 6H), 1.17 (t, J = 7.1 Hz, 3H). ^{13}C-NMR (101 MHz, DMSO-d_6) δ_C (ppm): 165.3 (C), 153.3 (C), 152.7 (C), 151.8 (C), 147.3 (C), 145.9 (C), 144.6 (C), 135.3 (C), 129.86 (CH), 125.4 (C), 121.0 (CH), 118.3 (CH), 115.6 (CH), 115.3 (CH), 113.1 (CH), 112.4 (CH), 110.9 (CH), 102.3 (C), 59.7 (CH_2), 56.2 (CH_3), 55.6 (CH_3), 55.5 (CH_3), 53.5 (CH), 14.2 (CH_3).

Ethyl (E)-4-(4-hydroxy-3-methoxyphenyl)-2-oxo-6-styryl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (TM9)

It was prepared by following the procedure as described using 60 mol% catalysts, pale yellow solid (105.2 mg; 53%); R_f = 0.55 ($CHCl_3$: EtOAc = 1:4); MS(ESI) $[M+H]^+$ calcd. for $C_{22}H_{22}N_2O_5$ 395, found 395; IR $\tilde{\nu}$ (KBr, cm^{-1}): 3238, 3123, 3271, 2965, 1676, 1601, 1518, 1238; ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H (ppm): 9.18 (s, 1H), 8.99 (s, 1H), 7.94 (d, J = 16.7 Hz, 1H), 7.78 (s, 1H), 7.52 (d, J = 7.3 Hz, 2H), 7.46 (d, J = 16.7 Hz, 1H), 7.43 (t, J = 7.3 Hz, 2H), 7.36 (t, J = 7.3 Hz, 1H), 6.86 (d, J = 1.8 Hz, 1H), 6.73 (d, J = 8.1 Hz, 1H), 6.68 (dd, J = 8.1, 1.8 Hz, 1H), 5.19 (d, J = 3.3 Hz, 1H), 4.09 (q, J = 7.1 Hz, 2H), 3.74 (s, 3H), 1.18 (t, J = 7.1 Hz, 3H). ^{13}C-NMR (101 MHz, DMSO-d_6) δ_C (ppm): 165.4 (C), 152.7 (C), 147.4 (C), 146.0 (C), 144.2 (C), 136.1 (C), 135.4 (C), 134.6 (CH), 129.1 (CH), 129.1 (CH), 127.2 (CH), 119.7 (CH), 118.4 (CH), 115.4 (CH), 110.9 (CH), 102.5 (C), 59.9 (CH_2), 55.6 (CH_3), 53.7 (CH), 14.2 (CH_3).

Ethyl (E)-6-(4-fluorostyryl)-4-(4-hydroxy-3-methoxyphenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (TM10)

It was prepared by following the procedure as described using 60 mol% catalysts, pale yellow solid (90.7 Mg; 44%); R_f = 0.57 ($CHCl_3$: EtOAc = 1:4); MS(ESI) $[M+H]^+$ calcd. for $C_{22}H_{21}FN_2O_5$ 413, found 413; IR

$\tilde{\nu}$ (KBr, cm^{-1}): 3237, 3123, 3277, 3092, 2982, 1697, 1674, 1645, 1599, 1512, 1234, 1101; ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H (ppm): 9.16 (s, 1H), 8.99 (s, 1H), 7.87 (d, J = 16.8 Hz, 1H), 7.78 (s, 1H), 7.56 (dd, J = 8.6, 5.6 Hz, 2H), 7.44 (d, J = 16.8 Hz, 1H), 7.27 (t, J = 8.6 Hz, 2H), 6.86 (d, J = 1.8 Hz, 1H), 6.73 (d, J = 8.2 Hz, 1H), 6.67 (dd, J = 8.2, 1.8 Hz, 1H), 5.18 (d, J = 3.3 Hz, 1H), 4.08 (q, J = 7.1 Hz, 2H), 3.73 (s, 3H), 1.17 (t, J = 7.1 Hz, 3H). ^{13}C-NMR (101 MHz, DMSO-d_6) δ_C (ppm): 165.3 (C), 162.5 (d, $^1J_{CF}$ = 248.5 Hz, C), 152.6 (C), 147.4 (C), 146.0 (C), 144.2 (C), 135.3 (C), 133.4 (CH), 132.7 (d, $^4J_{CF}$ = 3.0 Hz, C), 129.2 (d, $^3J_{CF}$ = 8.1 Hz, CH), 119.6 (CH), 118.4 (CH), 116.0 (d, $^2J_{CF}$ = 21.2 Hz, CH), 115.4 (CH), 110.9 (CH), 102.4 (C), 59.8 (CH_2), 55.6 (CH_3), 53.6 (CH), 14.2 (CH_3).

Ethyl (E)-6-(2,5-dimethoxystyryl)-4-(4-fluorophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (TM11)

It was prepared by following the procedure as described using 40 mol% catalysts, yellow solid (91 mg; 43%), R_f = 0.49 (CH_2Cl_2: EtOAc = 2:1); HRMS (ESI) $[M+Na]^+$ calcd. For $C_{23}H_{23}FN_2O_5$ 449.1489, found 449.1467; IR $\tilde{\nu}$ (KBr, cm^{-1}): 3225, 3094, 2943, 1707, 1695, 1634, 1599, 1495, 1231, 1096; ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H (ppm): 9.33 (s, 1H), 7.90 (d, J = 16.7 Hz, 1H), 7.84 (s, 1H), 7.54 (d, J = 16.7 Hz, 1H), 7.32 (dd, J = 8.8, 5.5 Hz, 2H), 7.18 (t, J = 8.8 Hz, 2H), 7.06 (d, J = 3.0 Hz, 1H), 7.01 (d, J = 9.1 Hz, 1H), 6.93 (dd, J = 9.1, 3.0 Hz, 1H), 5.25 (d, J = 3.5 Hz, 1H), 4.06 (q, J = 7.1 Hz, 2H), 3.81 (s, 3H), 3.73 (s, 3H), 1.14 (t, J = 7.1 Hz, 3H). ^{13}C-NMR (101 MHz, DMSO-d_6) δ_C (ppm): 165.2, 161.4 (d, $^1J_{CF}$ = 244.4 Hz, C), 153.3 (C), 152.5 (C), 151.8 (C), 145.2 (C), 140.6 (d, $^4J_{CF}$ = 3.0 Hz, C), 130.3 (CH), 128.2 (d, $^3J_{CF}$ = 8.1 Hz, CH), 125.3 (C), 120.9 (CH), 115.72 (CH), 115.2 (d, $^2J_{CF}$ = 22.2 Hz, CH), 113.1 (CH), 112.3 (CH), 101.7 (C), 59.8 (CH_2), 56.1 (CH_3), 55.5 (CH_3), 53.2 (CH), 14.1 (CH_3).

Ethyl (E)-6-(2,3-dimethoxystyryl)-4-(4-fluorophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (TM12)

It was prepared by following the procedure as described using 40 mol% catalysts, yellow solid (54.3 mg; 25%), R_f = 0.56 (*n*-hexane: EtOAc = 2:3); HRMS (ESI) $[M+Na]^+$ calcd. for $C_{23}H_{23}FN_2O_5$ 449.1489, found 449.1480; IR $\tilde{\nu}$ (KBr, cm^{-1}): 3221, 3088, 2974, 1695, 1630, 1599, 1508, 1229, 1096; ^{1}H-NMR (400 MHz, $CDCl_3$) δ_H (ppm): 8.08 (d, J = 16.9 Hz, 1H), 7.79 (s, 1H), 7.46 (d, J = 16.9 Hz, 1H), 7.33 (dd, J = 8.5, 5.3 Hz, 2H), 7.24 (dd, J = 7.9, 1.0 Hz, 1H), 7.05 (t, J = 7.9 Hz, 1H), 6.99 (t, J = 8.5 Hz, 2H), 6.89 (d, J = 7.9 Hz, 1H), 6.03 (s, 1H), 5.48 (d, J = 2.2 Hz, 1H), 4.13 (q, J = 7.1 Hz, 2H), 3.86 (s, 3H), 3.86 (s, 3H), 1.20 (t, J = 7.1 Hz, 3H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ_C (ppm): 165.4 (C), 162.5 (d, $^1J_{CF}$ = 248.5 Hz, C), 153.1 (C), 153.1 (C), 147.8 (C), 143.3 (C), 139.5 (C), 129.8 (C), 128.6 (d, $^3J_{CF}$ = 8.1 Hz, CH), 128.4 (CH), 124.4 (CH), 121.0 (CH), 118.5 (CH), 115.8 (d, $^2J_{CF}$ = 21.2 Hz, CH), 113.1 (CH), 103.2 (C), 61.6 (CH_3), 60.6 (CH_2), 56.0 (CH_3), 55.4 (CH), 14.3 (CH_3).

Ethyl (E)-4-(4-fluorophenyl)-6-(2-methoxystyryl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (TM13)

It was prepared by following the procedure as described using 40 mol% catalysts, yellow solid (119 mg; 60%), R_f = 0.51 (CH_2Cl_2: EtOAc = 3:1); HRMS (ESI) $[M+Na]^+$ calcd. for $C_{22}H_{21}FN_2O_4$ 419,1383, found 419,1374; IR $\tilde{\nu}$ (KBr, cm^{-1}): 3219, 3090, 2953, 1699, 1631, 1599, 1508, 1230, 1097; ^{1}H-NMR (400 MHz, $CDCl_3$) δ_H (ppm): 8.05 (d, J = 16.9 Hz, 1H), 7.61 (dd, J = 7.7, 1.4 Hz, 1H), 7.43 (d, J = 16.9 Hz, 1H), 7.37 – 7.27 (m, 3H), 7.00 (t, J = 8.5 Hz, 2H), 6.95 (t, J = 7.3 Hz, 1H), 6.90 (d, J = 8.4 Hz, 1H), 5.94 (s, 1H), 5.48 (d, J = 2.2 Hz, 1H), 4.14 (q, J = 7.1 Hz, 2H), 3.87 (s, 3H), 1.21 (t, J = 7.1 Hz, 3H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ_C (ppm): 165.4 (C), 162.5 (d, $^1J_{CF}$ = 248.5 Hz, C), 157.6 (C), 152.8 (C), 143.4 (C), 139.5 (d, $^4J_{CF}$ = 2.0 Hz, C), 130.8 (CH), 128.6 (CH), 128.6 (d, $^3J_{CF}$ = 8.1 Hz, CH), 127.2 (CH), 124.5 (C), 121.0 (CH), 120.0 (CH), 115.8 (d, $^2J_{CF}$ = 21.2 Hz, CH), 111.2 (CH), 102.8 (C), 60.6 (CH_2), 55.7 (CH_3), 55.4 (CH), 14.3 (CH_3).

Ethyl (E)-4-(4-fluorophenyl)-6-(4-fluorostyryl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (TM14)

It was prepared by following the procedure as described using 40 mol% catalysts, yellow solid (75.2 mg; 39%), R_f = 0.56 (CH_2Cl_2: EtOAc = 3:1); HRMS (ESI) $[M+Na]^+$ calcd. for $C_{21}H_{18}F_2N_2O_3$ 407.1183, found 407.1174; IR $\tilde{\nu}$ (KBr, cm^{-1}): 3240, 3096, 2982, 1693, 1639, 1599, 1508, 1231, 1097; ^{1}H-NMR (400 MHz, $CDCl_3$) δ_H (ppm): 8.35 (s, 1H), 7.95 (d, J = 16.7 Hz, 1H), 7.49 (dd, J = 8.6, 5.4 Hz, 2H), 7.29 (dd, J = 8.5,

5.4 Hz, 2H), 7.14 (d, *J* = 16.7 Hz, 1H), 7.02 (t, *J* = 8.6 Hz, 2H), 6.97 (t, *J* = 8.5 Hz, 2H), 6.24 (s, 1H), 5.44 (d, *J* = 2.7 Hz, 1H), 4.12 (q, *J* = 7.1 Hz, 2H), 1.19 (t, *J* = 7.1 Hz, 3H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ_C (ppm): 165.3 (C), 163.4 (d, $^1J_{CF}$ = 250.5 Hz, C), 162.5 (d, $^1J_{CF}$ = 248.5 Hz, C), 153.5 (C), 143.3 (C), 139.4 (d, $^4J_{CF}$ = 3.0 Hz, C), 133.4 (CH), 132.1 (d, $^4J_{CF}$ = 3.0 Hz, C), 129.4 (d, $^3J_{CF}$ = 8.1 Hz, CH), 128.6 (d, $^3J_{CF}$ = 8.1 Hz, CH), 119.7 (CH), 116.0 (d, $^2J_{CF}$ = 22.2 Hz, CH), 115.8 (d, $^2J_{CF}$ = 21.2 Hz, CH), 102.9 (C), 60.6 (CH_2), 55.3 (CH), 14.3 (CH_3).

Ethyl (E)-6-(4-chlorostyryl)-4-(4-fluorophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (TM15)

It was prepared by following the procedure as described using 40 mol% catalysts, yellow solid (100.5 g; 50%), R_f = 0.56 (*n*-hexane: EtOAc = 2:3); MS (ESI) $[M+H]^+$ calcd. for $C_{21}H_{18}ClFN_2O_3$ 401, found 401; IR $\tilde{\nu}$ (KBr, cm^{-1}): 3238, 3123, 3094, 2982, 1697, 1639, 1603, 1508, 1232, 1097, 816; ^{1}H-NMR (400 MHz, $CDCl_3$) δ_H (ppm): 8.43 (s, 1H), 7.99 (d, *J* = 16.7 Hz, 1H), 7.43 (d, *J* = 8.5 Hz, 2H), 7.29 (d, *J* = 8.5 Hz, 2H), 7.28 (dd, *J* = 8.6, 5.7 Hz, 2H), 7.12 (d, *J* = 16.7 Hz, 1H), 6.97 (t, *J* = 8.6 Hz, 2H), 6.26 (s, 1H), 5.43 (d, *J* = 2.8 Hz, 1H), 4.12 (q, *J* = 7.1 Hz, 2H), 1.19 (t, *J* = 7.1 Hz, 3H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ_C (ppm): 165.2 (C), 162.6 (C) (d, $^1J_{CF}$ = 248.5 Hz, C), 153.5 (C), 143.1 (C), 139.4 (d, $^4J_{CF}$ = 4.0 Hz, C), 135.2 (C), 134.4 (C), 133.3 (CH), 129.2 (CH), 128.8 (CH), 128.6 (d, $^3J_{CF}$ = 8.1 Hz, CH), 120.4 (CH), 115.9 (d, $^2J_{CF}$ = 21.2 Hz, CH), 103.2 (C), 60.7 (CH_2), 55.3 (CH), 14.3 (CH_3).

Ethyl (E)-4-(4-chlorophenyl)-6-(2,4-dimethoxystyryl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (TM16)

It was prepared by following the procedure as described using 40 mol% catalysts, brownish yellow solid (50.4 mg; 23%), R_f = 0.56 ($CHCl_3$: EtOAc = 3:2); MS (ESI) $[M+H]^+$ calcd. for $C_{23}H_{23}ClN_2O_5$ 443, found 443; IR $\tilde{\nu}$ (KBr, cm^{-1}): 3223, 3092, 2978, 1705, 1691, 1630, 1605, 1574, 1236, 1103, 826; ^{1}H-NMR (400 MHz, $CDCl_3$) δ_H (ppm): 7.96 (d, *J* = 16.8 Hz, 1H), 7.54 (d, *J* = 8.6 Hz, 1H), 7.35 (d, *J* = 16.8 Hz, 1H), 7.30 – 7.20 (m, 4H), 6.49 (dd, *J* = 8.6, 2.3 Hz, 1H), 6.43 (d, *J* = 2.3 Hz, 1H), 6.06 (s, 1H), 5.45 (d, *J* = 2.8 Hz, 1H), 4.13 (q, *J* = 7.1 Hz, 2H), 3.85 (s, 3H), 3.82 (s, 3H), 1.21 (t, *J* = 7.2 Hz, 3H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ_C (ppm): 165.5 (C), 162.3 (C), 159.0 (C), 153.0 (C), 144.0 (C), 142.2 (C), 133.9 (C), 129.0 (CH), 128.6 (CH), 128.4 (CH), 128.2 (CH), 117.5 (C), 117.3 (CH), 105.6 (CH), 101.6 (C), 98.4 (CH), 60.5 (CH_2), 55.7 (CH_3), 55.6 (CH_3), 55.3 (CH), 14.7 (CH_3).

Ethyl (E)-4-(4-chlorophenyl)-6-(3-methoxystyryl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (TM17)

It was prepared by following the procedure as described using 40 mol% catalysts, brownish white solid (113.9 mg; 55%); R_f = 0.61 ($CHCl_3$: EtOAc = 3:2); MS (ESI) $[M+H]^+$ calcd. for $C_{22}H_{21}ClN_2O_4$ 413, found 413; IR $\tilde{\nu}$ (KBr, cm^{-1}): 3225, 3088, 2978, 1707, 1693, 1638, 1603, 1473, 1233, 1099, 777; ^{1}H-NMR (400 MHz, $CDCl_3$) δ_H (ppm): 8.31 (s, 1H), 7.99 (d, *J* = 16.6 Hz, 1H), 7.27 – 7.23 (m, 5H), 7.17 – 7.07 (m, 2H), 7.03 (s, 1H), 6.86 (dd, *J* = 8.2, 2.2 Hz, 1H), 6.37 (s, 1H), 5.41 (d, *J* = 2.4 Hz, 1H), 4.12 (q, *J* = 7.1 Hz, 2H), 3.80 (s, 3H), 1.21 (t, *J* = 7.2 Hz, 3H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ_C (ppm): 165.3 (C), 160.0 (C), 153.6 (C), 143.4 (C), 142.1 (C), 137.2 (C), 134.6 (CH), 133.9 (C), 129.9 (CH), 129.1 (CH), 128.2 (CH), 120.3 (CH), 120.1 (CH), 115.1 (CH), 112.9 (CH), 102.7 (C), 60.7 (CH_2), 55.4 (CH_3), 55.3 (CH), 14.3 (CH_3).

Ethyl (E)-4-(4-chlorophenyl)-2-oxo-6-styryl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (TM-18)

It was prepared by following the procedure as described using 40 mol% catalysts, brownish yellow solid (73.6 g; 38%), R_f = 0.44 ($CHCl_3$: EtOAc = 3:2); MS (ESI) $[M+H]^+$ calcd. for $C_{21}H_{19}ClN_2O_3$ 383, found 383; IR $\tilde{\nu}$ (KBr, cm^{-1}): 3235, 3098, 2982, 1690, 1636, 1599, 1097, 767; ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H (ppm): 9.27 (s, 1H), 7.93 (d, *J* = 16.7 Hz, 1H), 7.89 (s, 1H), 7.52 (d, *J* = 7.3 Hz, 2H), 7.48 (d, *J* = 16.7 Hz, 1H), 7.43 (t, *J* = 7.3 Hz, 2H), 7.42 (d, *J* = 8.5 Hz, 2H), 7.36 (t, *J* = 7.3 Hz, 1H), 7.31 (d, *J* = 8.5 Hz, 2H), 5.26 (d, *J* = 3.4 Hz, 1H), 4.07 (q, *J* = 7.0 Hz, 2H), 1.15 (t, *J* = 7.1 Hz, 3H). ^{13}C-NMR (101 MHz, DMSO-d_6) δ_C (ppm): 165.0 (C), 152.3 (C), 144.9 (C), 143.2 (C), 135.9 (C), 135.0 (CH), 132.0 (C), 129.1 (CH), 129.0 (CH), 128.5 (CH), 128.2 (CH), 127.1 (CH), 119.5 (CH), 101.5 (C), 59.9 (CH_2), 53.4 (CH), 14.0 (CH_3).

Ethyl (E)-4-(4-chlorophenyl)-6-(4-fluorostyryl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (TM19)

It was prepared by following the procedure as described using 40 mol% catalysts, pale yellow solid (113.9 mg; 57%); R_f = 0.57 ($CHCl_3$: EtOAc = 3:2); MS (ESI) $[M+H]^+$ calcd. for $C_{21}H_{18}ClFN_2O_3$ 401, found 401; IR $\tilde{\nu}$ (KBr, cm^{-1}): 3229, 3094, 2980, 1703, 1694, 1636, 1599, 1508, 1230, 1097, 827; ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H (ppm): 9.28 (s, 1H), 7.92 (s, 1H), 7.86 (d, J = 16.7 Hz, 1H), 7.57 (dd, J = 8.7, 5.6 Hz, 2H), 7.46 (d, J = 16.7 Hz, 1H), 7.43 (d, J = 8.6 Hz, 2H), 7.31 (d, J = 8.6 Hz, 2H), 7.27 (t, J = 8.7 Hz, 2H), 5.26 (d, J = 3.4 Hz, 1H), 4.07 (q, J = 7.1 Hz, 2H), 1.15 (t, J = 7.1 Hz, 3H). ^{13}C-NMR (101 MHz, DMSO-d_6) δ_C (ppm): 165.1 (C), 162.6 (d, $^1J_{CF}$ = 248.5 Hz, C), 152.4 (C), 145.0 (C), 143.3 (C), 133.9 (CH), 132.6 (d, $^4J_{CF}$ = 3.0 Hz, C), 132.1 (C), 129.3 (d, $^3J_{CF}$ = 9.1 Hz, CH), 128.6 (CH), 128.3 (CH), 119.5 (CH), 116.1 (d, $^2J_{CF}$ = 22.2 Hz, CH), 101.5 (C), 60.0 (CH_2), 53.5 (CH), 14.1 (CH_3).

Ethyl (E)-4-(4-chlorophenyl)-6-(4-chlorostyryl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (TM20)

It was prepared by following the procedure as described using 100 mol% catalysts, yellow solid (85 mg; 41%), R_f = 0.56 ($CHCl_3$: EtOAc = 3:2); IR $\tilde{\nu}$ (KBr, cm^{-1}): 3231, 3096, 2980, 1694, 1638, 1593, 1489, 1094, 818; ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H (ppm): 9.29 (s, 1H), 7.92 (s, 1H), 7.92 (d, J = 16.8 Hz, 1H), 7.54 (d, J = 8.7 Hz, 2H), 7.49 (d, J = 8.7 Hz, 2H), 7.46 (d, J = 16.8 Hz, 1H), 7.43 (d, J = 8.5 Hz, 2H), 7.30 (d, J = 8.5 Hz, 2H), 5.26 (d, J = 3.4 Hz, 1H), 4.07 (q, J = 7.1 Hz, 2H), 1.15 (t, J = 7.1 Hz, 3H). ^{13}C-NMR (101 MHz, DMSO-d_6) δ_C (ppm): 165.0 (C), 152.3 (C), 144.8 (C), 143.2 (C), 134.9 (C), 133.7 (CH), 133.6 (C), 132.0 (C), 129.1 (CH), 128.8 (CH), 128.6 (CH), 128.2 (CH), 120.3 (CH), 101.7 (C), 60.0 (CH_2), 53.4 (CH), 14.1 (CH_3).

Cytotoxicity Assay

Breast cancer cell lines MCF-7 and cervix cancer cell lines HeLa were provided from the American Type Culture Collection (ATCC) collection of the Laboratory of Parasitology, Faculty of Medicine-Public Health-and Nursury, Gadjah Mada University, Yogyakarta, Indonesia. Cells were cultured routinely in RPMI-1640 free of phenol red and added with 10% Fetal Bovine Serum (FBS) and antibiotic peniciline-streptomycine. Cells were cultured at 37 °C in a 5% CO_2 atmosphere. In vitro Cytotoxicity test was performed employing 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay following the available protocol. This assay is based on the reduction of MTT into purple formazan by mitochondrial dehydrogenase.[36] Briefly, cells were cultured in 96 well-plate at a density of 1×10^4 cells/well for 24 h. Subsequently, tested compounds (TM1–TM20) was added in various concentration, DMSO as a control, and doxorubicin as a positive control, incubated for 24 h. After the addition of 0.5% MTT solution (10% of medium volume), incubation was continued for a further 4 h at 37°C/5% CO_2. Next, stop solution (0.04N HCl in isopropanol) was added to each well with the same volume and then its absorbance at 570 (peak) and 630 nm (base) was measured. All of the results were compared to the absorbance of control which represents 100% viability. Procentual cell viability was calculated using the formula $[A_{experiment}/A_{control}]$ x 100%. Cytotoxicity is reported as IC_{50} (concentration that inhibits 50% of cell proliferation). The experiment was conducted in triplicate.

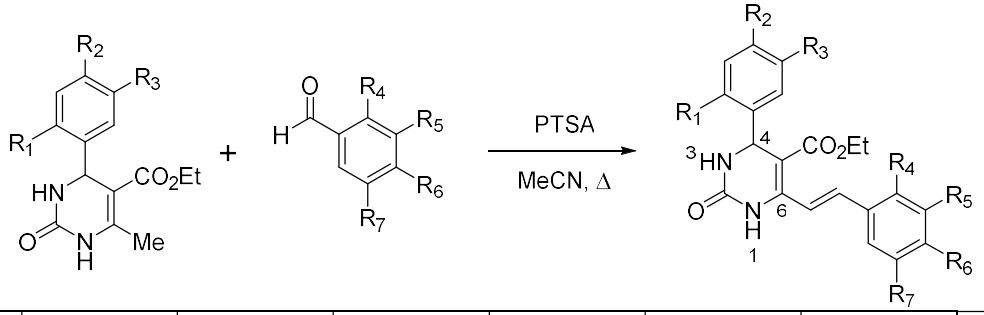
RESULTS AND DISCUSSION

Structure Characterization

In this research, we developed a method to synthesize 6-styryl-DHPM derivatives from DHPM derivatives using vinylogous aldol condensation. Although this is not a new method, the use of Brønsted acid to catalyze this reaction was not been explored previously. The choice of PTSA as a catalyst is due to its known capacity as a catalyst for this reaction.[32] The use of acetonitrile as a solvent has been proven to be suitable as a medium for the reaction.[27,28,30] Therefore as the first step we do optimization of aldehyde and catalyst amount ratio (Table-1). In addition, we observed also the effect of a substituent on the reaction yield (substituent at the aromatic ring of DHPM and the aromatic aldehyde). The reaction and molecular structure of the prepared compounds are displayed in Scheme-1.

The structure of all prepared compounds was determined using FTIR, NMR, and HR(ESI) or ESI-MS. Based on FTIR spectra, the existence of N–H amide bond, C–H sp^2, C–H sp^3, C=O of unsaturated ester,

C=C aromatic and C–O ester in all prepared compounds were proven by vibration bands consecutively at a range of ṽ 3321–3219, 3103–3088, 2982–2937, 1707–1693, 1695–1674, 1645–1473, 1103–1088 cm^{-1}. The ^{1}H-NMR spectrum of all prepared compounds showed a consistent pattern, which is two singlet signals at the range of δ_H 9.22–9.12 and 7.92–7.29 ppm (in DMSO-d_6) or 8.43–7.01 and 7.03–5.75 ppm (in $CDCl_3$) representing proton of N-1 and N-3. Then, doublet signals at δ_H 5.80–5.17 ppm represent the proton of C-4.



Molecule	R_1	R_2	R_3	R_4	R_5	R_6	R_7
TM1	OMe	OMe	H	OMe	H	H	OMe
TM2	OMe	OMe	H	OMe	H	H	H
TM3	OMe	H	OMe	OMe	H	H	OMe
TM4	OMe	H	OMe	OMe	OMe	H	H
TM5	OMe	H	OMe	OMe	H	H	H
TM6	OMe	H	OMe	H	H	F	H
TM7	OMe	H	OMe	H	H	Cl	H
TM8	H	OH	OMe	OMe	H	H	OMe
TM9	H	OH	OMe	H	H	H	H
TM10	H	OH	OMe	H	H	F	H
TM11	H	F	H	OMe	H	H	OMe
TM12	H	F	H	OMe	OMe	H	H
TM13	H	F	H	OMe	H	H	H
TM14	H	F	H	H	H	F	H
TM15	H	F	H	H	H	Cl	H
TM16	H	Cl	H	OMe	H	OMe	H
TM17	H	Cl	H	H	OMe	H	H
TM18	H	Cl	H	H	H	H	H
TM19	H	Cl	H	H	H	F	H
TM20	H	Cl	H	H	H	Cl	H

Scheme-1: Chemical Reaction and Molecular Structure of the Prepared styryl-DHPM

These data were also supported by ^{13}C-NMR (APT) data which exhibited the existence of C-2, C-4, C-5, and C-6 signals that appeared consecutively at a range of δ_C 153.7–152.3, 55.4–48.7, 103.2–100.5, and 145.9–143.1 ppm. These data showed the existence of the dihidropyrimidinone ring of all target molecules.[29] The alkene with *trans* configuration for all molecules was proven by two doublet signals at a range of δ_H 8.21–7.12 ppm which coupled each other with a coupling constant of 16.6-16.9 Hz. The HR(ESI) or ESI-MS spectrum showed suitability between observed with a calculated molecular mass of the prepared compounds.

Effect of Aldehyde and Catalyst Ratio

Firstly, we study the effect of three various ratios of DHPM and aldehyde 1:1, 1:2, and 1:3 consecutively. The reaction did not proceed perfectly if we used an equimolar ratio (1:1) because DHPM always remains in the reaction mixture, while the ratio of 1:3 led to elongation reaction time with a lower yield than of ratio of 1:2. Therefore the ratio of DHPM and aldehyde of 1:2 is suitable for this reaction, which is consistent to the previous report.[27,30] The results obtained were then used for the optimization of catalyst amount. However, we could not find the optimum amount of catalyst to be applied generally, so we used four

different optimizations to find the suitable condition for a wide variation of reactants. To find the optimal condition for the reaction between DHPM and aldehyde with strong electron donating groups, the reaction of synthesis TM1 was used. For phenolic DHPM, the reaction of synthesis TM9 was used. For the reaction between DHPM and aldehyde with moderate electron withdrawing or donating groups, the reaction of synthesis TM17 was used. For the reaction between DHPM and aldehyde with an electron-withdrawing group, the reaction of synthesis TM20 was used. In the product formation using DHPM with strong electron donating substituent (DHPM attaching 2,4-dimethoxy aromatic substituent) optimum condition was achieved by using 20 mol% catalysts. The use of more catalyst amounts leads to the formation of side products that is difficult to be separated. The optimum yield was obtained if we used 40 mol% catalysts for DHPM composed with moderate electron donating or withdrawing substituents (2,5-dimethoxy, 4-fluoro, and 4-chloro), whereas DHPM with phenolic substituent needed 60 mol% catalysts to get optimum yield. For the reaction which used DHPM and aldehyde with the electron-withdrawing group, 100 mol% catalysts should be used. Based on the facts, it is obvious that this reaction is strongly affected by the substituents attached to the aromatic ring of DHPM.

Table-1: Data of Reaction Optimization Based on Substrate Ratio and Catalyst Amount

Entry	Amount of catalyst (mol%)	Substrate ratio (DHPM: Aldehyde)	Time (hour)	Yield (%)
1[a]	0	1:2	> 300	No reaction
2[a]	20	1:2	117	46
3[a]	40	1:1	> 300	incomplete
4[a]	40	1:2	17	55
5[a]	40	1:3	37	44
6[a]	60	1:2	92	42
7[b]	10	1:2	> 300	incomplete
8[b]	20	1:2	30	30
9[b]	40	1:2	19	complex mixture
10[c]	20	1:2	185	24
11[c]	40	1:2	119	34
12[c]	60	1:2	38	53
13[c]	80	1:2	48	37
14[d]	40	1:2	358	21
15[d]	100	1:2	175	41
16[d]	200	1:2	46	complex mixture

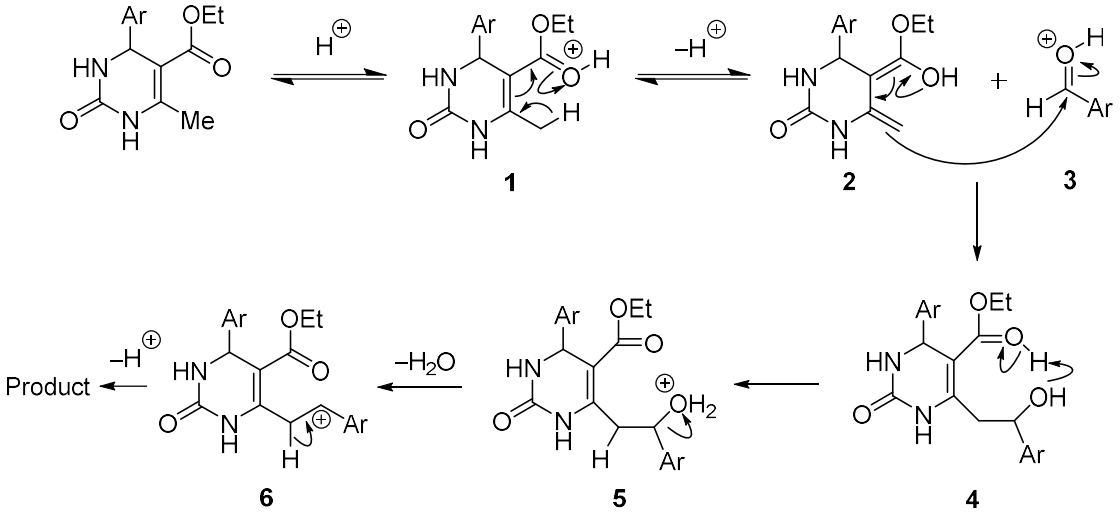
(a)using reaction of synthesis TM17; (b) using reaction of synthesis TM1; (c) using reaction of synthesis TM9; (d) using reaction of synthesis TM20.

Effect of Substituent on DHPM Aromatic and Aldehyde Aromatic Ring

After optimization, we performed reactions using aromatic aldehydes with various substituents, and the results are presented in Table-2. According to the data, the best reaction results were achieved if DHPM and aromatic aldehyde with electron withdrawing group were used. However, this reaction condition did not apply to the reaction between DHPM and aldehyde with a 2,4-dimethoxy substituent which gave a low yield (TM1, TM2, and TM6). We assumed that the existence of an electron-rich aromatic ring is susceptible to electrophilic substitution usually proceeding in acid conditions to form various side products. We thought the side product formed because of the nature of the rich electron aromatic system prone to attack aldehyde in acid condition with Friedel-Crafts type reaction.[33] In the reaction using aldehydes and DHPM possessing electron-withdrawing substituents (TM18, TM19, and TM20) needed a long time (93-216 hours).

The role of PTSA as a Brønsted acid catalyst can be seen in the proposed reaction mechanism (Scheme-2). PTSA could protonate the carbonyl in ester and aldehyde, promoting enolization to form enol intermediate 2 and highly electrophilic cation 3. The two intermediates react with the nucleophilic addition mechanism to form alcohol intermediate 4. By proton transfer, or protonated by PTSA, intermediate 4 was then converted to intermediate 5 which can be dehydrated to form carbocation 6. The stability of carbocation 6 influenced the reaction rate so which affected the yield of the product. The carbocation 6 is then deprotonated to form the product. This elimination reaction followed the unimolecular elimination (E1)

mechanism. According to the facts obtained, the aldol-vinylogous reaction proceeded in the cationic intermediate, therefore the presence of the electron-donating group is very important to stabilize the intermediate and transition state during the reaction.[34]



Scheme-2: Plausible Proposed Reaction Mechanism of Formation of styryl-DHPM

This synthesis method can be applied for the synthesis using phenolic DHPM substrate. However, as we tried a reaction using phenolic aldehyde (vanillin), no reaction was observed. Similarly, by using aldehyde with an amine group (N, N-dimethylaminobenzaldehyde) we obtained a mixture product that was difficult to be separated. Briefly, this reaction method is suitable for the synthesis of styryldihydropyrimidinone using a substrate possessing moderate electron withdrawing or donating groups.

Table-2: Time and Yield of the Synthesis of Target Molecules (TM1 - TM20).

Molecule	Time (hours)	Yield (%)	Molecule	Time (hours)	Yield (%)
TM1[a]	30	30	TM11[b]	52	43
TM2[a]	60	25	TM12[b]	100	25
TM3[b]	15	71	TM13[b]	8	60
TM4[b]	10	52	TM14[b]	110	39
TM5[b]	13	69	TM15[b]	26	50
TM6[b]	30	62	TM16[b]	17	23
TM7[b]	39	25	TM17[b]	17	55
TM8[c]	23	62	TM18[b]	93	38
TM9[c]	38	53	TM19[b]	216	57
TM10[c]	73	44	TM20[d]	175	41

(a)using catalyst ratio of 20 mol%; (b) using catalyst ratio of 40 mol%; (c) using catalyst ratio of 60 mol%; (d) using catalyst ratio of 100 mol%.

Biological Activity

All the prepared compounds were then tested for their cytotoxicity towards two cancer cell lines HeLa and MCF-7 using doxorubicin as a positive control. The cytotoxicity was reported as IC_{50} and tabulated in Table 4. Almost all of the compounds showed no activity against HeLa cell lines. Only five compounds exhibited cytotoxicity, TM17, TM18, and TM19 showed weak activity (IC_{50} = 79.5–86.7 μM), while TM9 and TM10 showed moderate activity with IC_{50} values of 29.8 and 45.2 μM respectively. Almost all of the tested compounds exhibited strong cytotoxicity activity against MCF-7 cancer cell lines. 13 compounds showed strong activity (IC_{50} = 3,8–27,3 μM), and 3 compounds showed very strong cytotoxicity (IC_{50} < 1 μM), those were TM7, TM17, and TM18 with consecutively IC_{50} values of 46, 92, and 0.764 nM. Based on the data obtained, it is observed that the methoxy group attached to both aromatic rings decreased the cytotoxicity toward both cancer cells. The existence of chloro and hydroxy groups played an important role. For HeLa cell lines, compounds exhibiting good activity possess chlor or fluor substituents in the styryl

aromatic ring and hydroxy group in the aromatic ring attached in dihydropyrimidine. For MCF-7 cell lines, the existence of substituents in the aromatic ring of the styryl fragment gave no significant effect on activity, while the substituent of the aromatic ring attaching to the dihydropyridine ring plays an important role in the activity. In most cases, compounds with halogen and hydroxy substituents of the aromatic ring attached to the dihydropyrimidine ring showed very good activity. The results are similar to the results of Soumyanarayanan *et al*. (2012) who reported that DHPM with 4-chlorophenyl substituent possesses high cytotoxicity toward HeLa and HepG2 cancer cell lines.[13] Based on the data, it can be concluded that polar substituents (halogen and hydroxy) attached in both aromatic rings play a very important role in cytotoxicity activity against both cancer cell lines.

Table-4: Anticancer Activity of the Prepared Compounds Against HeLa and MCF-7 Cancer Cell Lines

Molecule	IC_{50} (μM)	
	HeLa	MCF-7
TM1	> 100	> 100
TM2	> 100	> 100
TM3	> 100	> 100
TM4	> 100	> 100
TM5	> 100	> 100
TM6	> 100	6,8 ± 0,3
TM7	> 100	0,046 ± 0,02
TM8	> 100	16,4 ± 2,0
TM9	29,8 ± 2,1	3,8 ± 0,6
TM10	45,2 ± 4,9	3,9 ± 1,5
TM11	> 100	19,0 ± 3,1
TM12	> 100	10,0 ± 1,5
TM13	> 100	27,3 ± 11,9
TM14	> 100	6,2 ± 2,9
TM15	> 100	9,3 ± 1,0
TM16	> 100	3,8 ± 0,6
TM17	79,5 ± 4,6	0,092 ± 0,05
TM18	> 100	0,000764 ± 0,000782
TM19	86,7 ± 29	7,71 ± 0,2
TM20	85,1 ± 8,4	9,6 ± 0,3
Doxorubicin	1,97 ± 0,78	45,0 ± 5,7

CONCLUSION

In conclusion, we have synthesized successfully a series of 6-styryldihydropyrimidine using PTSA as a Brønsted acid catalyst. The best results were obtained in the reaction involving DHPM or aldehyde possessing electron-donating substituents, this method was tolerant toward DHPM with a hydroxyl group. Cytotoxicity test showed that 4-hydroxy and 4-chloro substituents of the aryl group attached at the DHPM ring play an important role in cytotoxic activity.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

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