DOI 10.1007/s11094-018-1729-1
Pharmaceutical Chemistry Journal, Vol. 51, No. 11, February, 2018 (Russian Original Vol. 51, No. 11, November, 2017)

SYNTHESIS OF ANALOGS OF TRANS-FAGARAMIDE AND THEIR CYTOTOXIC ACTIVITY

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Original article submitted May 28, 2017.

A series of 30 compounds were synthetized inspired by active *trans*-fagaramide structure skeleton. On this synthetic platform, 18 compounds were achieved via Knoevenagel condensation using maleic acid and piperonal, followed by peptide coupling with various amines, giving an average yield of 54%. Subsequently, nine compounds were obtained by palladium-mediated Heck coupling with an average yield of 79%. In addition, cytotoxic activity was evaluated against cardiomyoblast H9c2, breast adenocarcinoma MCF7, hepatocellular carcinoma HepG2, and glioblastoma U-87 cells. The results revealed two aryl halogen-substituted compounds moderately active against H9c2 and MCF7 with IC_{50} values > 50 μM. One functionalized coumarin showed inhibitory activity against H9c2 (IC_{50} > 50 μM). In contrast, *p*-aminophenyl-β-monosubstituted *trans*-fagaramide was found to inhibit MCF7 (IC_{50} > 50 μM) without showing toxicity against H9c2 cells.

Keywords: *trans*-fagaramide; Knoevenagel condensation; peptide coupling; palladium-mediated Heck coupling; cytotoxic activity.

1. INTRODUCTION

Trans-fagaramide, a natural amide specifically found in the *Zanthoxylum* genus [1, 2], is well known for its previously studied activity against cancer, cardiac affections, rheumatism and as antifungal agent [3, 4]. Earlier studies of *trans*-fagaramide biological effects revealed ovicidal activity [5, 6] and moderate cytotoxicity against selected cancer cell lines. Fundamentally, *trans*-fagaramide is formed by a methylenedioxyphenyl group and an α,β-unsaturated amide [7], which have inspired scientists to continue looking for active molecules structurally related to *trans*-fagaramide skeleton.

Preceding reports showed considerable number of active compounds against certain cancer cells, containing structural units of *trans*-fagaramide [8]. In particular, some piperine analogs having the skeleton structure of *trans*-fagaramide were synthesized and evaluated for their activity against MCF-7 breast cancer and Hela cervix, showing significant activity against both human cancer cell types [9]. The identification and characterization of FL118 molecule [10] showed it to be an active compound capable of selectively inhibiting multiple cancer survival and proliferation-associated anti-apoptotic proteins (survivin, Mcl-1, XIAP, clAP2) (Fig. 1).

In this context, this paper describes the elaboration of a synthetic platform of 30 analogs of *trans*-fagaramide generated via Knoevenagel condensation, peptide coupling, Fischer esterification, and palladium-mediated Heck coupling. Thus, some modifications in the *trans*-fagaramide chemical structure may lead effective synthetic candidates toward the determination of optimized and efficient drugs against cancer cells.

2. RESULTS AND DISCUSSION

2.1 Synthesis of trans-Fagaramide and Its Analogs by Knoevenagel Condensation, Peptide Coupling and Fischer Esterification

The synthesis of *trans*-fagaramide (compound **2**) and its analogs were performed in two steps with an overall yield of 56%. First, Knoevenagel condensation between commercially malonic acid and piperonal was used to afford the required precursor **1** (cinnamic acid) at 75% yield in the presence of excess basic solvents (pyridine and piperidine)

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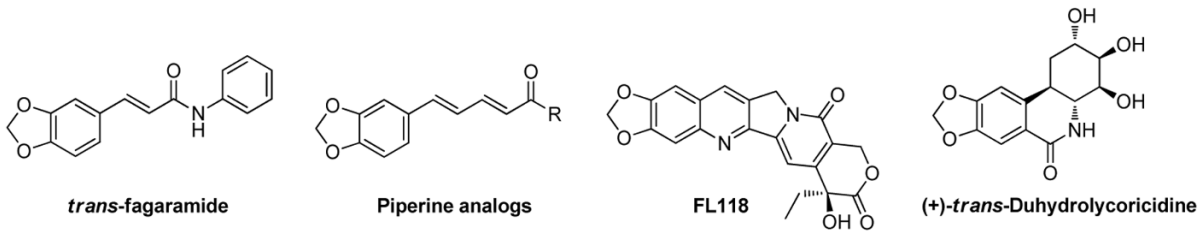


Fig. 1. Examples of active analogs of *trans*-fagaramide [7 – 10].

[12, 13]. Compound **1** was further functionalized by its activation using *N*,*N*′-diisopropylcarbodiimide (DIC); *N*,*N*,*N*′,*N*′-tetramethyl-O-(benzotriazol-1-yl)uranium tetrafluoroborate (TBTU), and hydroxybenzotriazole (HOBt) as coupling agents [14, 15], which allowed the obtaining of compounds **2 – 20** by following a peptide coupling reaction employing various mono-substituted amines (Schemes 1a and 1b). In order to obtain the optimum reaction conditions, TBTU (well documented as a highly potent amide coupling reagent [16]) and compound **1** were reacted in equal amounts to avoid the formation of excess urea derivatives. These derivatives are more or less soluble in organic solvents as well as in aqueous phases, which makes them difficult to eliminate [17]. However, this removal was easily achieved by the work-up shown in the experimental part of this paper so as to afford 18 analogs of *trans*-fagaramide without further purification.

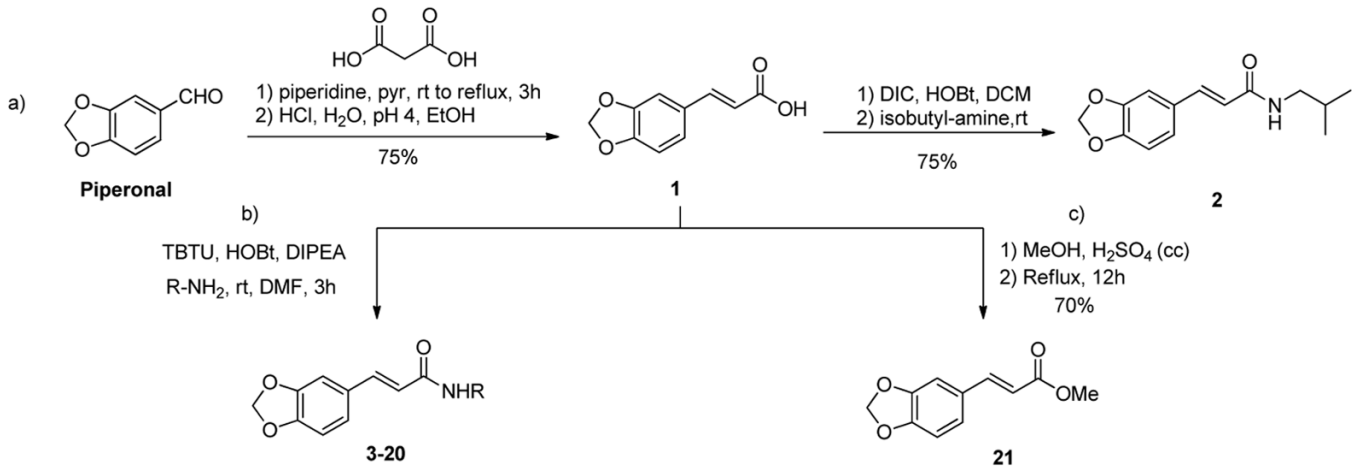
Compound **21** was obtained by Fischer esterification between compound **1** (cinnamic acid) and methanol in the presence of H_2SO_4 (acid catalyst) with an overall yield of 53% (Scheme 1c).

Analogs of *trans*-fagaramide were obtained by peptide coupling at an average yield of 54% within 20 – 92% range (Fig. 2).

2.2. Synthesis of trans-Fagaramide Analogs by Palladium-Mediated Heck Coupling

Subsequently, compounds **22 – 30** were achieved by palladium-mediated Heck coupling, another powerful tool in organic synthesis, which contributes in the formation of carbon – carbon and carbon – heteroatom bonds [18]. First, compounds **2** and **21** were coupled successfully, generating the corresponding eight products as mixtures of stereoisomers. The formation of aryl-substituted olefins were performed by using various mono-substituted aryl iodides, which reacted with the correspond olefin at elevated temperatures in DMF. The coupling reaction was catalyzed by $Pd(OAc)_2$ in the presence of tetrabutylammonium bromide (TBABr) as a phase-transfer-catalyst (Scheme 2) [18]. In contrast to other compounds, compound **26** contains a coumarin unit in its chemical structure, which is formed via the internal attack of the hydroxyl group from the benzene ring to the carbonyl group.

Compound **26** (yield, 66%) was obtained as a white solid. The rest of the compounds were obtained as mixtures of stereoisomers at an average yield of 80% within an interval of 68 – 96% (Fig. 3).



Scheme 1. (a, b) Synthesis of trans-fagaramide (**2**) and its analogs (**3 – 20**) by peptide coupling; (c) synthesis of compound **21** by Fischer esterification.

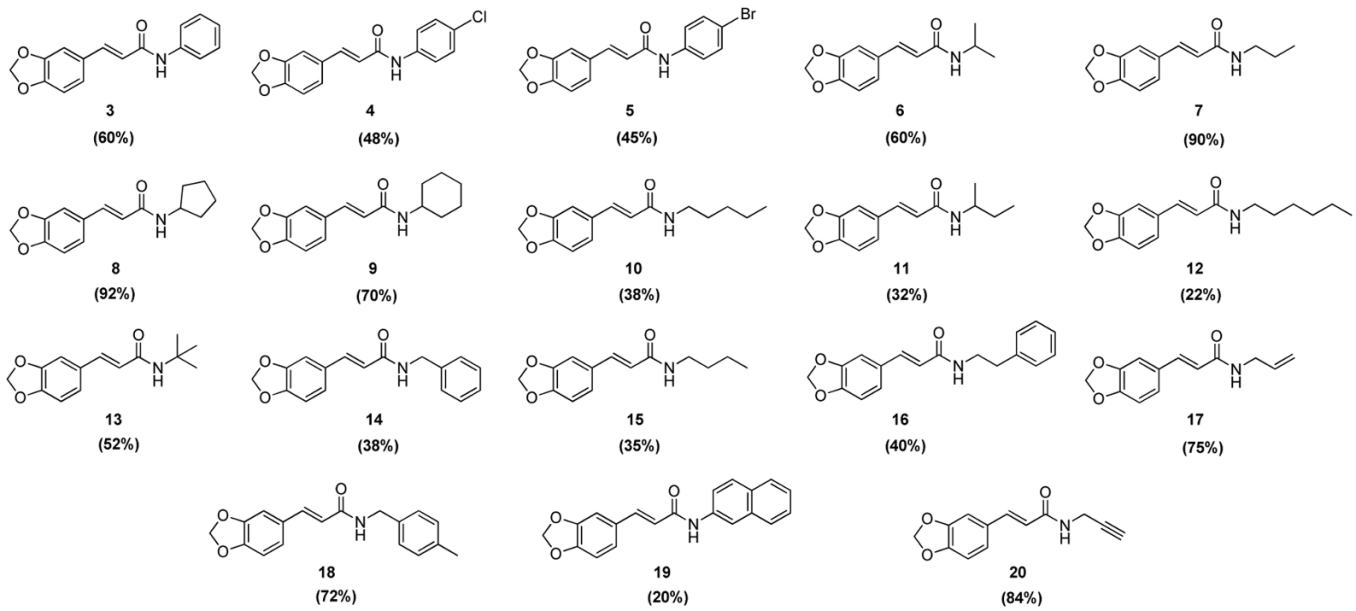


Fig. 2. Analogs of *trans*-fagaramide synthesized by peptide coupling.

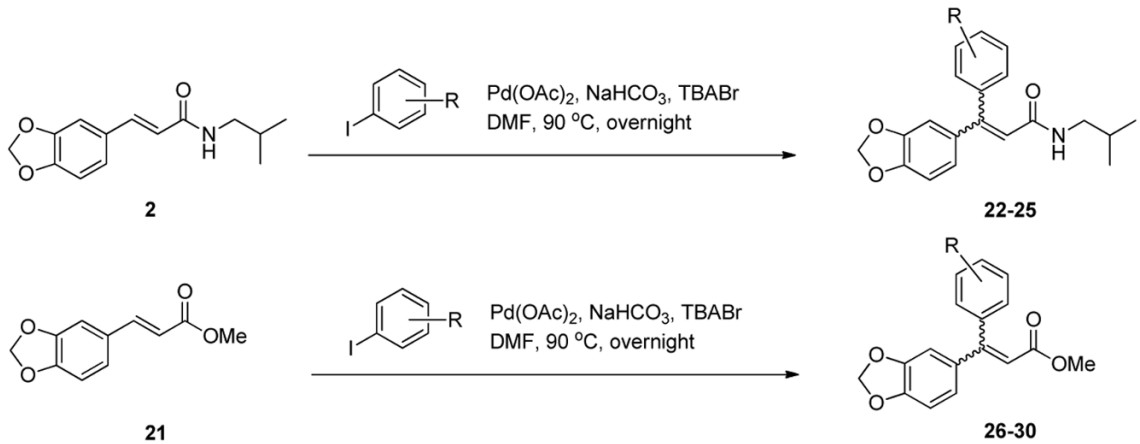
2.3. Biological Testing

The synthesized analogs of *trans*-fagaramide were tested for their cytotoxic activity against breast adenocarcinoma MCF7, hepatocellular carcinoma HepG2, glioblastoma U-87 cells as well as normal H9c2 cells. The results presented in Table 1 are expressed as drug concentrations inhibiting fifty percent of cell growth (IC_{50}). Capsaicin was used as a positive control with IC_{50} ranging from 1 to 100 μM. The viability of cells was measured using the resazurin assay [19, 20]. Compounds **4** and **5** reduced the cellular viability of H9c2 and MCF7, with IC_{50} over 50 μM. This result showed that the anticancer activity was related to the presence of halo-substitued aryl structure. Likewise, compounds **25** and **26** exhibited an inhibition activity against MCF7 and H9c2, respectively, with IC_{50} above 50 μM. (Table 1). The rest of compounds are not mentioned because of their lack of activity in these cells lines.

3. EXPERIMENTAL SECTION

3.1 General

All solvents (Acros Organics or Aldrich) were used without additional purification. The isolation and purification were monitored by thin layer chromatography (TLC) on silica gel plate (Merck 60 F254) and by column chromatography using silica gel (Silica Gel 60, particle size



Scheme 2. Synthesis of compounds **22 – 30** by palladium-mediated Heck coupling.

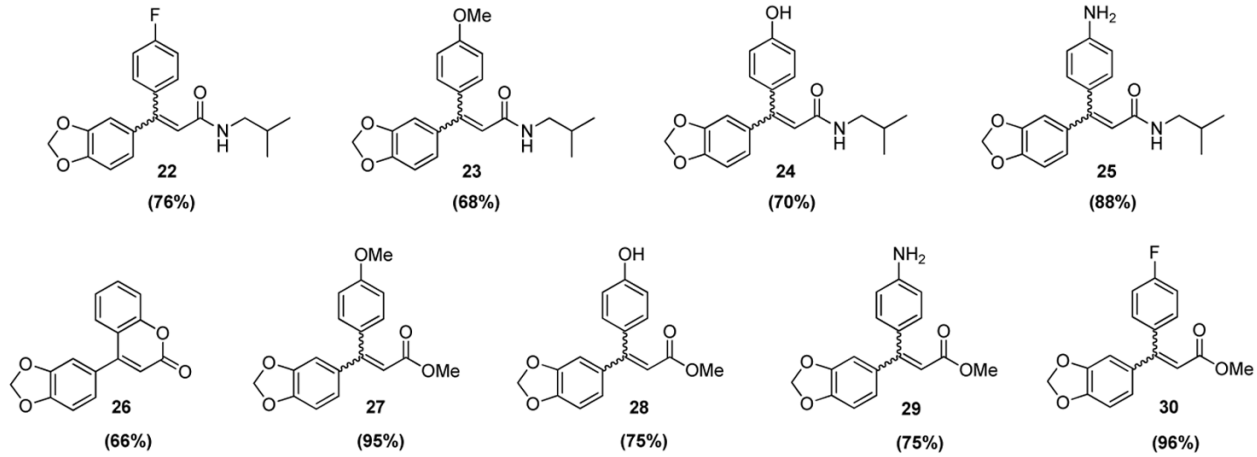


Fig. 3. Structures of *trans*-fagaramide analogs **22 – 30** synthesized by palladium-mediated Heck coupling.

0.063 – 0.200 mm). Compounds obtained by extraction were analyzed and characterized in $CDCl_3$ by ^1H NMR at 300 MHz, ^1H NMR at 600 MHz, and ^{13}C NMR at 150 MHz on a Varian Inova AS600 spectrometer. The molecular ions were protonated as $[M+H]^+$ for the confirmation of their empirical formula. The notations used for the spectral analysis are as follows: s (singlet), d (doublet), dd (doublet of doublets), m (multiplet). Chemical shifts are reported in δ (ppm) using TMS as the internal standard; the coupling constants (*J*) were measured in Hz. Infrared spectra were recorded on a JASCO FT/IR-410 spectrophotometer.

3.2. Synthesis and Characterization of Compound 1 and Synthetic trans-Fagaramide

(E)-3-(Benzo[d][1,3]dioxol-5-yl)acrylic acid (1). To a solution of piperonal (2.3 g, 15 mmol), malonic acid (3.1 g, 30 mmol) and pyridine (5 mL, 61 mmol), piperidine (50 mL, 0.48 mmol) was slowly added drop by drop and the final reaction mixture was stirred at room temperature for 45 min. Then the reaction was heated at 100°C for 3 h. The mixture was cooled and the excess of pyridine was neutralized by the addition of 1 M HCl (20 mL), so that a white precipitate appeared between pH 4 – 5. The precipitated acid was filtered, washed with cold EtOAc, and recrystallized from saturated hot ethanol. The filtrate was dried under vacuum in a desiccator for 48 h to afford 11.55 mmol (75%) of compound **1** as white crystals; ^1H-NMR ($(CD_3)_2SO$, 600 MHz; δ, ppm): 7.51 (1H, d, J = 16.0 Hz, benzene-C*H*), 7.36 (1H, d, J = 1.6 Hz, *Ha*), 7.15 (1H, dd, J = 1.6 Hz, J = 8.2 Hz, *Hb*), 6.94 (1H, d, J = 8.2 Hz, *Hc*), 6.40 (1H, d, J = 16.0 Hz, C*H*CO), 6.07 (2H, s, OCH_2O); ^{13}C-NMR ($(CD_3)_2SO$, 150 MHz; δ, ppm): 167.8 (*C*OOH), 149.1, 148.0, 128.7, 124.6, 108.5, 106.7 (arom), 143.9 (*C*HCH), 117.1 (*C*HCO), 101.6 (OCH_2O); ESI^+-HRMS (*m/z*): 193.0497 $[M + H]^+$ (calcd. for $C_{10}H_9O_4$, 193.0495).

(E)-3-(Benzo[d][1,3]dioxol-5-yl)-N-isobutylacrylamide, synthetic *trans*-fagaramide (2). Compound **1** (2.5 g, 13 mmol) in dry DCM (130 mL) was purged with N_2 and DIC (2.0 mL, 13 mmol) was added, followed sequentially by HOBt (175.7 mg, 1.3 mmol) and isobutylamine (1.3 mL, 13 mmol). The mixture was stirred at room temperature for 24h (monitored by TLC) and then concentrated under vacuum to remove most of the CH_2Cl_2. The crude material was diluted with EtOAc and washed successively with distilled water in the presence of hexane (1/4 of the volume of EtOAc), then with saturated potassium bisulfate ($KHSO_4$), and sodium bicarbonate ($NaHCO_3$). The organic phase was dried over anhydrous sodium sulfate (Na_2SO_4), filtered, and concentrated. The crude material was purified by column chromatography on silica gel (eluent: hexane/EtOAc, 100:0 to 75:25) to afford 9.75 mmol (75%) of compound **2** as white crystals; ^1H-NMR ($CDCl_3$, 600 MHz; δ, ppm): 7.53 (1H, d, J = 15.9 Hz, benzene-C*H*), 7.0 (1H s, H_a), 6.97 (1H, d,

TABLE 1. Cytotoxic Activity* of *trans*-Fagaramide Analogs against Cardiomyoblast H9c2, Breast Adenocarcinoma MCF7, Hepatocellular Carcinoma HepG2, and Glioblastoma U-87

Compound	IC_{50} (μM)			
	H9c2	MCF7	HepG2	U-87
Capsaicin	>50	N/A	37 ± 10	>50
4	>50	>50	N/A	N/A
5	>50	>50	N/A	N/A
25	N/A	>50	N/A	N/A
26	>50	N/A	N/A	N/A

* Each value represents the mean ± SD from at least two individual experiments performed in triplicate. Statistical significance was calculated for $p < 0.05$; RM ANOVA on ranks and post hoc Student–Newman–Keuls method; N/A = not assessed (compounds with IC_{50} higher than 100 μM were considered inactive).

J = 8.2 Hz, H_b), 6.78 (1H, d, J = 8.2 Hz, H_c), 6.22 (1H, d, J = 15.9 Hz, C*H*CO), 5.98 (2H, s, OCH_2O), 5.63 (1H, s, N*H*), 3.20 (2H, t, CH_2), 1.13 (1H, m, C*H*), 0.94 (6H, d, CH_3); ^{13}C-NMR ($CDCl_3$, 150 MHz; δ, ppm): 166.0 (*C*ONH), 149.0, 148.2, 129.3, 123.8, 108.5, 106.3 (arom), 140.6 (*C*HCH), 118.8 (CH*C*O), 101.4 (O*C*H$_2$O), 47.0 (*C*H$_2$), 28.6 (*C*H), 20.1 (*C*H$_3$); ESI$^+$-HRMS (*m/z*): 248.1281 $[M + H]^+$ (calcd. for $C_{14}H_{18}NO_3$, 248.1281.

3.3 General Procedure of Peptide Coupling

To a solution of compound **1** (1 eq.) in dry DMF (5 mL) at room temperature, TBTU (167.0 mg, 0.52 mmol) and HOBt (35.16 mg, 0.26 mmol) were added while the solution was stirred for 30 min. Then, the corresponding amine (1.5 eq.) and *N*,*N*-diisopropylethylamine (DIPEA) (0.52 mL, 3.12 mmol) were added sequentially and the reaction mixture was stirred at room temperature for 2.5 h and concentrated under reduced pressure. The crude product was dissolved in EtOAc and washed sequentially with distilled water in the presence of hexane (1/4 of the volume of EtOAc) and then with saturated $KHSO_4$ and $NaHCO_3$. The organic phase was dried over anhydrous Na_2SO_4 and the filtrate was evaporated to dryness *in vacuo*.

(E)-3-(Benzo[d][1,3]dioxol-5-yl)-N-phenylacrylamide (3). Compound **1** (100 mg, 0.52 mmol) was used as reagent for the peptide coupling with aniline (89 μL, 0.78 mmol) to afford 0.31 mmol (60%) of compound **3** as white solid; ^{1}H-NMR ($CDCl_3$, 300 MHz; δ, ppm): 7.93 (1H, d, J = 16 Hz, benzene-CH), 7.67 – 7.58 (5H, m, arom), 7.53 – 7.06 (3H, m, arom), 6.65 (1H, d, J = 16 Hz, CHCO), 6.27 (2H, s, OCH$_2$O); ^{13}C-NMR ($CDCl_3$, 75 MHz; δ, ppm): 165.0 (CONH), 149.5, 148.4, 138.2, 120.1, 108.7, 106.6 (arom), 142.3 (CHCH), 118.2 (CH$_2$CO) 101.1 (OCH$_2$O), 137.8, 129.2, 124.4, 120.1 (arom); ESI$^+$-HRMS (*m/z*): 268.096 $[M + H]^+$ (calcd. for $C_{13}H_{14}NO_3$, 248.1281).

(E)-3-(Benzo[d][1,3]dioxol-5-yl)-N-(4-chlorophenyl)-acrylamide (4). Compound **1** (100 mg, 0.52 mmol) was used as reagent for the peptide coupling with 4-chloroaniline (99.5 mg, 0.78 mmol) to afford 0.29 mmol (56%) of compound **4** as white solid; ^{1}H-NMR ($CDCl_3$, 300 MHz; δ, ppm): 7.66 (1H, d, J = 15.8 Hz, benzene-CH), 7.58 – 7.29 (4H, m, arom), 7.33 (1H, s, NH), 7.03 – 6.80 (3H, m, arom), 6.35 (1H, d, J = 15.8 Hz, CHCO), 6.01 (2H, s, OCH$_2$O); ^{13}C-NMR ($CDCl_3$, 75 MHz; δ, ppm): 174.6 (CONH), 148.5, 148.0, 129.2, 121.3, 108.8, 106.5, 101 (arom), 142.8 (CHCH), 136.8, 133.3, 129.0, 121.3 (arom), 117.8 (CHCO), 101.7 (OCH$_2$O); ESI$^+$-HRMS (*m/z*): 302.0586 $[M + H]^+$ (calcd. for $C_{16}H_{13}ClNO_3$, 302.0578).

(E)-3-(Benzo[d][1, 3]dioxol-5-yl)-N-(4-bromophenyl)-acrylamide (5). Compound **1** (100 mg, 0.52 mmol) was used as reagent for the peptide coupling with 4-bromoaniline (134.2 mg, 0.78 mmol) to afford 0.23 mmol (45%) of compound **5** as white solid; ^{1}H-NMR ($CDCl_3$, 300 MHz; δ, ppm): 7.66 (1H, d, J = 15.7 Hz, benzene-CH), 7.53 – 7.43 (4H, m, arom), 7.33 (1H, s, NH), 7.03 – 6.80 (3H, m, arom), 6.35 (1H, d, J = 15.7 Hz, CHCO), 6.01 (2H, s, OCH$_2$O); ^{13}C-NMR ($CDCl_3$, 75 MHz; δ, ppm): 165.5 (CONH), 148.5, 148.0, 128.9, 122.9, 108.8, 106.5 (arom), 142.2 (CHCH), 132.2, 130.3, 124.6, 121.6 (arom), 118.9 (CHCO), 101.7 (OCH$_2$O); ESI$^+$-HRMS (*m/z*): 346.0078 $[M + H]^+$ (calcd. for $C_{16}H_{13}BrNO_3$, 346.0073).

(E)-3-(Benzo[d][1,3]dioxol-5-yl)-N-isopropylacrylamide (6). Compound **1** (100 mg, 0.52 mmol) was used as reagent for the peptide coupling with isopropylamine (64 μL, 0.78 mmol) to afford 0.31 mmol (60%) of compound **6** as white solid; ^{1}H-NMR ($CDCl_3$, 300 MHz; δ, ppm): 7.51 (1H, d, J = 16.2 Hz, benzene-CH), 6.98 – 6.77 (3H, m, arom), 6.18 (1H, d, J = 16.2 Hz, CHCO), 5.98 (2H, s, OCH$_2$O), 5.45 (1H, s, NH), 4.21 (1H, m, CH), 1.22 (6H, d, CH$_3$); ^{13}C-NMR ($CDCl_3$, 75 MHz; δ, ppm): 165.3 (CONH)), 140.6 (CHCH), 149.1, 148.3, 129.5, 123.9, 108.7, 106.4 (arom), 119.2 (CHCO), 101.5 (OCH$_2$O), 41.7 (NHCH), 23.0 (CH$_3$); ESI$^+$-HRMS (*m/z*): 234.1136 $[M + H]^+$ (calcd. for $C_{13}H_{16}NO_3$, 234.1125).

(E)-3-(Benzo[d][1,3]dioxol-5-yl)- N-propylacrylamide (7). Compound **1** (100 mg, 0.52 mmol) was used as reagent for the peptide coupling with propylamine (64 μL, 0.78 mmol) to afford 0.47 mmol (90%) of compound **7** as white solid; ^{1}H-NMR ($CDCl_3$, 300 MHz; δ, ppm): 7.53 (1H, d, J = 15.7 Hz, benzene-CH), 6.98 – 6.77 (3H, m, arom), 6.23 (1H, d, J = 15.7 Hz, CHCO), 5.98 (2H, s, OCH$_2$O), 5.70 (1H, s, NH), 3.33 (2H, m, NHCH$_2$), 1.60 (2H, m, NHCH$_2$CH$_2$), 0.95 (3H, m, CH$_3$); ^{13}C-NMR ($CDCl_3$, 75 MHz; δ, ppm): 166.2 (CONH), 149.1, 148.3, 129.5, 123.9, 108.6, 106.4 (arom), 140.7 (CHCH), 119.0 (CHCO), 101.5 (OCH$_2$O), 41.6 (NHCH$_2$), 23.1 (NHCH$_2$CH$_2$) 11.6 (CH$_3$); ESI$^+$-HRMS (m/z): 233.1266 $[M + H]^+$ (calcd. for $C_{13}H_{16}NO_3$, 233.1285).

(E)-3-(Benzo[d][1,3]dioxol-5-yl)-N-cyclopentylacrylamide (8). Compound **1** (100 mg, 0.52 mmol) was used as reagent for the peptide coupling with cyclopentylamine (80 μL, 0.78 mmol) to afford 0.48 mmol (92%) of compound **8** as white solid; ^{1}H-NMR ($CDCl_3$, 300 MHz; δ, ppm): 7.52 (1H, d, J = 15.3 Hz, benzene-C*H*), 6.98 – 6.77 (3H, m, arom), 6.18 (1H, d, J = 15.3 Hz, C*H*CO), 5.98 (2H, s, OCH_2O), 5.66 (1H, s, N*H*), 4.33 (1H, m, NHC*H*), 2.07 (2H, m, CH_2CH$_2$CH$_2$), 1.66 (4H, m, CH$_2$CH_2CH_2), 1.44 (2H, m, CH_2CH$_2$CH$_2$); ^{13}C-NMR ($CDCl_3$, 75 MHz; δ, ppm): 165.7 (*C*ONH), 149.1, 148.3, 129.5, 123.9, 108.7, 106.4 (arom), 140.6 (*C*HCH), 119.1 (*C*HCO), 101.5 (O*C*H$_2$O), 51.5 (NH*C*H), 33.4 (*C*H$_2$CH$_2$CH$_2$*C*H$_2$), 23.7 (CH$_2$*C*H$_2$*C*H$_2$CH$_2$); ESI$^+$-HRMS (*m/z*): 260.1293 $[M + H]^+$ (calcd. for $C_{15}H_{18}NO_3$, 260.1281).

(E)-3-(Benzo[d][1,3]dioxol-5-yl)-N-cyclohexylacrylamide (9). Compound **1** (100 mg, 0.52 mmol) was used as reagent for the peptide coupling with cyclohexylamine (90 μL, 0.78 mmol) to afford 0.36 mmol (70%) of compound **9** as white solid; ^{1}H-NMR ($CDCl_3$, 300 MHz; δ, ppm): 7.51 (1H,

d, J = 15.6 Hz, benzene-C*H*), 6.99 – 6.77 (3H, m, arom), 6.18 (1H, d, J = 15.6 Hz, *CH*CO), 5.98 (2H, s, OCH_2O), 5.46 (1H, s, N*H*), 3.90 (1H, m, NHC*H*), 2.01 – 1.15 (10H, m, cyclohexyl); ^{13}C-NMR ($CDCl_3$, 75 MHz; δ, ppm): 165.1 (*C*ONH), 149.1, 148.3, 129.5, 123.9, 108.7, 106.4 (arom), 140.5 (*C*HCH), 119.3 (*C*HCO), 101.5 (OCH_2O), 48.4 (NH*C*H), 33.4 (*C*H_2CH$_2$CH$_2$CH$_2$*C*H$_2$), 25.7 (CH$_2$CH$_2$*C*H$_2$CH$_2$CH$_2$CH$_2$), 25.0 (CH$_2$*C*H$_2$CH$_2$*C*H$_2$CH$_2$CH$_2$); ESI$^+$-HRMS (*m/z*): 274.1438 $[M + H]^+$ (calcd. for $C_{16}H_{20}NO_3$, 274.1438).

(E)-3-(Benzo[d][1,3]dioxol-5-yl)-N-pentylacrylamide (10). Compound **1** (100 mg, 0.52 mmol) was used as reagent for the peptide coupling with n-amylamine (90 µL, 0.78 mmol) to afford 0.2 mmol (38%) of compound **10** as white solid; ^{1}H-NMR ($CDCl_3$, 300 MHz; δ, ppm): 7.53 (1H, d, J = 15.7 Hz, benzene-C*H*), 6.94 – 6.77 (3H, m, arom), 6.20 (1H, d, J = 15.7 Hz, C*H*CO), 5.98 (2H, s, OCH_2O), 5.63 (1H, s, N*H*), 3.37 (1H, m, NHCH_2), 1.56 (2H, m, NHCH$_2$CH_2), 1.34 (4H, m, NHCH$_2$CH$_2$CH_2CH_2), 0.90 (3H, m, CH_3) ^{13}C-NMR ($CDCl_3$, 75 MHz; δ, ppm): 166.1 (*C*ONH), 149.1, 148.3, 129.5, 123.9, 108.7, 106.5 (arom), 140.7 (*C*HCH), 119.0 (*C*HCO), 101.6 (OCH_2O), 39.9 (NH*C*H$_2$), 29.5 (NHCH$_2$*C*H$_2$), 29.3 (*C*H$_2$CH$_2$CH$_3$), 22.5 (*C*H$_2$CH$_3$), 14.1 (*C*H$_3$); ESI$^+$-HRMS (*m/z*): 262.1426 $[M + H]^+$ (calcd. for $C_{15}H_{20}NO_3$, 262.1438).

(E)-3-(Benzo[d][1,3]dioxol-5-yl)-N-sec-butylacrylamide (11). Compound **1** (100 mg, 0.52 mmol) was used as reagent for the peptide coupling with sec-butylamine (80 µL, 0.78 mmol) to afford 0.17 mmol (32%) of compound **11** as white solid; ^{1}H-NMR ($CDCl_3$, 300 MHz; δ, ppm): 7.52 (1H, d, J = 15.5 Hz, benzene-C*H*), 6.98 – 6.77 (3H, m, arom), 6.20 (1H, d, J = 15.5 Hz, C*H*CO), 5.98 (2H, s, OCH_2O), 5.43 (1H, s, N*H*), 4.05 (1H, m, NHC*H*), 1.51 (2H, m, NHCHCH_2), 1.18 (3H, m, NHCHCH_3), 0.93 (3H, m, NHCHCH$_2$CH_3); ^{13}C-NMR ($CDCl_3$, 75 MHz; δ, ppm): 165.5 (*C*ONH), 149.0, 148.3, 129.5, 123.8, 108.6, 106.4 (arom), 140.6 (*C*HCHCO), 119.3 (*C*HCO), 101.5 (OCH_2O), 45.9 (NH*C*H), 29.9 (NHCH*C*H$_2$), 20.7 (NHCH*C*H$_3$), 10.5 (CHCH$_2$*C*H$_3$); ESI$^+$-HRMS (*m/z*): 248.1273 $[M + H]^+$; (calcd. for $C_{14}H_{18}NO_3$, 248.1281).

(E)-3-(Benzo[d][1,3]dioxol-5-yl)-N-hexylacrylamide (12). Compound **1** (100 mg, 0.52 mmol) was used as reagent for the peptide coupling with hexylamine (110 µL, 0.78 mmol) to afford 0.17 mmol (33%) of compound **12** as white solid; ^{1}H-NMR ($CDCl_3$, 300 MHz; δ, ppm): 7.53 (1H, d, J = 15.3 Hz, benzene-C*H*), 6.99 – 6.77 (3H, m, arom), 6.20 (1H, d, J = 15.3 Hz, C*H*CO), 5.98 (2H, s, OCH_2O), 5.62 (1H, s, N*H*), 3.37 (2H, m, NHCH_2), 1.52 (2H, m, NH$_2$CH$_2$CH_2), 1.31 (6H, m, NH$_2$CH$_2$CH$_2$ CH_2CH_2CH_2), 0.88 (3H, m, CH$_2$CH_3); ^{13}C-NMR ($CDCl_3$, 75 MHz; δ, ppm): 166.1 (*C*ONH), 149.1, 148.3, 129.5, 123.9, 108.7, 106.4 (arom), 140.7 (*C*HCH), 119.0 (*C*HCO), 101.6 (OCH_2O), 39.9 (NH*C*H$_2$), 31.6 (NHCH$_2$*C*H$_2$), 29.8 (NHCH$_2$CH$_2$*C*H$_2$), 26.8 (*C*H$_2$CH$_2$CH$_3$), 22.7 (*C*H$_2$CH$_3$), 14.2 (*C*H$_3$); ESI$^+$-HRMS (*m/z*): 276.1602 $[M + H]^+$ (calcd. for $C_{16}H_{22}NO_3$, 276.1594).

(E)-3-(Benzo[d][1,3]dioxol-5-yl)-N-tert-butylacrylamide (13). Compound **1** (100 mg, 0.52 mmol) was used as reagent for the peptide coupling with t-butylamine (82 µL, 0.78 mmol, 1.5 equiv) to afford 0.27 mmol (52%) of compound **13** as white solid; ^{1}H-NMR ($CDCl_3$, 300 MHz; δ, ppm): 7.47 (1H, d, J = 15.8 Hz, benzene-C*H*), 6.97 – 6.76 (3H, m, arom), 6.15 (1H, d, J = 15.8 Hz, *CH*CO), 5.98 (2H, s, OCH_2O), 5.44 (1H, s, N*H*), 1.42 pm (9H, s, CH_3); ^{13}C-NMR ($CDCl_3$, 75 MHz; δ, ppm): 165.5 (*C*ONH), 149.0, 148.3, 129.5, 123.8, 108.7, 106.4 (arom), 140.1 (*C*HCH), 120.2 (*C*HCO), 101.5 (OCH_2O), 51.6 (*C*(CH$_3$)$_3$), 29.0 (C(*C*H$_3$)$_3$); ESI$^+$-HRMS (*m/z*): 248.1285 $[M + H]^+$ (calcd. for $C_{14}H_{18}NO_3$, 248.1281).

(E)-3-(Benzo[d][1,3]dioxol-5-yl)-N-benzylacrylamide (14). Compound **1** (100 mg, 0.52 mmol) was used as reagent for the peptide coupling with benzylamine (90 µL, 0.78 mmol) to afford 0.18 mmol (38%) of compound **14** as white solid; ^{1}H-NMR ($CDCl_3$, 300 MHz; δ, ppm): 7.58 (1H, d, J = 15.7 Hz, benzene-C*H*), 7.35 – 7.26 (5H, m, arom), 6.99 – 6.78 (3H, m, arom), 6.24 (1H, d, J = 15.7 Hz, *CH*CO), 5.99 (2H, s, OCH_2O), 5.87 (1H, s, N*H*), 4.57 (2H, d, NHCH_2); ^{13}C-NMR ($CDCl_3$, 75 MHz; δ, ppm): 166.0 (*C*ONH), 149.2, 148.4, 129.3, 124.0, 108.7, 106.5 (arom), 138.4, 128.9, 128.1, 127.7 (arom), 141.3 (*C*HCH), 118.5 (*C*HCO), 101.6 (OCH_2O), 44.0 (NH*C*H$_2$); ESI$^+$-HRMS (*m/z*): 282.1141 $[M + H]^+$ (calcd. for $C_{17}H_{16}NO_3$, 282.1125).

(E)-3-(Benzo[d][1,3]dioxol-5-yl)-N-butylacrylamide (15). Compound **1** (100 mg, 0.52 mmol) was used as reagent for the peptide coupling with n-butylamine (80 µL, 0.78 mmol) to afford 0.18 mmol (35%) of compound **15** as white solid; ^{1}H-NMR ($CDCl_3$, 300 MHz; δ, ppm): 7.53 (1H, d, J = 15.8 Hz, benzene-C*H*), 6.99 – 6.77 (3H, m, arom), 6.21 (1H, d, J = 15.8 Hz, C*H*CO), 5.98 (2H, s, OCH_2O), 5.61 (1H, s, N*H*), 3.38 (2H, m, NHCH_2), 1.52 (2H, m, NHCH$_2$CH_2), 1.35 (2H, m, CH_2CH$_3$), 0.88 (3H, t, CH$_2$CH_3); ^{13}C-NMR ($CDCl_3$, 75 MHz; δ, ppm): 166.1 (*C*ONH), 149.1, 148.3, 129.5, 123.9, 108.7, 106.4 (arom), 140.7 (*C*HCH), 119.0 (*C*HCO), 101.5 (OCH_2O), 39.6 (NH*C*H$_2$), 31.9 (*C*H$_2$CH$_2$CH$_3$), 20.3 (*C*H$_2$CH$_3$), 13.9 (*C*H$_3$); ESI$^+$-HRMS (*m/z*248.1269): $[M + H]^+$ (calcd. for $C_{14}H_{18}NO_3$, 248.1281).

(E)-3-(Benzo[d][1,3]dioxol-5-yl)-N-phenethylacrylamide (16). Compound **1** (100 mg, 0.52 mmol) was used as reagent for the peptide coupling with phenetylamine (100 µL, 0.78 mmol) to afford 0.21 mmol (40%) of compound **16** as white solid; ^{1}H-NMR ($CDCl_3$, 300 MHz; δ, ppm): 7.53 (1H, d, J = 15.7 Hz, benzene-C*H*), 7.36 – 7.21 (5H, m, arom), 6.97 – 6.77 (3H, m, arom), 6.14 (1H, d, J = 15.7 Hz, C*H*CO), 5.98 (2H, s, OCH_2O), 5.57 (1H, s, N*H*), 3.65 (2H, m, NHCH_2), 2.89 (2H, m, CH_2-benzene) ^{13}C-NMR ($CDCl_3$, 75 MHz; δ, ppm): 166.1 (*C*ONH), 149.1, 148.4, 129.3, 124.0, 108.7, 106.5 (arom), 140.9 (*C*HCH), 139.0, 129.0, 128.8, 126.7 (arom*)* 118.8 (*C*HCO), 101.6 (OCH_2O), 40.9 (NH*C*H$_2$), 35.8 (*C*H$_2$- benzene); ESI$^+$-HRMS (*m/z*): 296.1294 $[M + H]^+$ (calcd. for $C_{18}H_{18}NO_3$, 296.1281).

(E)-N-allyl-3-(Benzo[d][1,3]dioxol-5-yl)acrylamide (17). Compound **1** (100 mg, 0.52 mmol) was used as reagent for the peptide coupling with allylamine (60 μL, 0.78 mmol) to afford 0.39 mmol (75%) of compound **17** as white solid; ^{1}H-NMR ($CDCl_3$, 300 MHz; δ, ppm): 7.55 (1H, d, J = 15.5 Hz, benzene-C*H*), 6.99 – 6.77 (3H, m, arom), 6.25 (1H, d, J = 15.5 Hz, *CH*CO), 5.98 (2H, s, OCH_2O), 5.90 (1H, m, *CH*CH_2), 5.85 (1H, s, N*H*), 5.20 (2H, m, CHCH_2), 4.01 (2H, m, NHCH_2) ^{13}C-NMR ($CDCl_3$, 75 MHz; δ, ppm): 166.0 (*C*ONH), 149.2, 148.4, 129.3, 124.0, 108.6, 106.5 (arom), 141.1 (*C*HCH), 134.3 (*C*HCH_2), 118.6 (*C*HCO), 116.7 (CH*C*H_2), 101.6 (O*C*H_2O), 42.3 (NH*C*H_2); ESI$^+$-HRMS (*m/z*): 232.0974 $[M + H]^+$ (calcd. for $C_{13}H_{14}NO_3$, 232.0968).

(E)-3-(Benzo[d][1,3]dioxol-5-yl)-N-(4-methylbenzyl)-acrylamide (18). Compound **1** (100 mg, 0.52 mmol) was used as reagent for the peptide coupling with methylbenzylamine (100 μL, 0.78 mmol) to afford 0.37 mmol (72%) of compound **18** as white solid; ^{1}H-NMR ($CDCl_3$, 300 MHz; δ, ppm): 7.58 (1H, d, J = 15.8 Hz, benzene-C*H*), 7.23 – 7.14 (4H, m, arom), 6.99 – 6.78 (3H, m, arom), 6.21 (1H, d, J = 15.8 Hz, C*H*CO), 5.99 (2H, s, OCH_2O), 5.79 (H, s, N*H*), 4.52 (2H, d, NHCH_2), 2.34 (3H, s, CH_3); ^{13}C-NMR ($CDCl_3$, 75 MHz; δ, ppm): 165.9 (*C*ONH), 149.2, 148.4, 129.6, 124.0, 108.7, 106.5 (arom), 141.2 (*C*HCH), 137.5, 135.4, 129.3, 128.1 (arom), 118.6 (*C*HCO), 101.6 (O*C*H_2O), 43.8 (NH*C*H_2), 21.2 (*C*H_3); ESI$^+$-HRMS (*m/z*): 296.1281 $[M + H]^+$ (calcd. for $C_{18}H_{18}NO_3$, 296.1281).

(E)-3-(Benzo[d][1,3]dioxol-5-yl)-N-(naphthalen-2-yl)-acrylamide (19). Compound **1** (100 mg, 0.52 mmol) was used as reagent for the peptide coupling with 2-naphtylamine (112 mg, 0.78 mmol) to afford 0.18 mmol (35%) of compound **19** as white solid; ^{1}H-NMR ($CDCl_3$, 300 MHz; δ, ppm): 8.32 (1H, s, N*H*), 7.79 – 7.39 (7H, m, arom), 7.70 (1H, d, J = 15.3 Hz, benzene-C*H*), 7.01 – 6.76 (3H, m, arom), 6.45 (1H, d, J = 15.3 Hz, C*H*CO), 5.99 (2H, s, OCH_2O); ^{13}C-NMR ($CDCl_3$, 75 MHz; δ, ppm): 164.5 (*C*ONH), 149.5, 148.8, 129.1, 124.4, 108.7, 106.6 (arom), 142.4 (*C*HCH), 135.7, 134.0, 130.8, 128.9, 127.9, 127.7, 126.6, 125.2, 120.3, 117.3 (napthalene), 119.0 (*C*HCO), 101.6 (O*C*H_2O); ESI$^+$-HRMS (*m/z*): 318.1114 $[M + H]^+$ (calcd. for $C_{20}H_{17}NO_3$, 318.1125).

(E)-3-(Benzo[d][1,3]dioxol-5-yl)-N-(prop-2-yn-1-yl)acrylamide (20). Compound **1** (100 mg, 0.52 mmol) was used as reagent for the peptide coupling with propargylamine (50 μg, 0.78 mmol) to afford 0.41 mmol (80%) of compound **21** as white solid; ^{1}H-NMR ($CDCl_3$, 300 MHz; δ, ppm): 7.57 (1H, d, J = 15.2 Hz, benzene-C*H*), 6.99 – 6.78 (3H, m, arom), 6.23 (1H, d, J = 15.2 Hz, C*H*CO), 5.99 (2H, s, OCH_2O), 5.85 (1H, s, N*H*), 4.18 (2H, m, NHCH_2), 2.26 (1H, m, CH_3); ^{13}C-NMR ($CDCl_3$, 75 MHz; δ, ppm): 165.8 (*C*ONH), 149.4, 148.4, 129.1, 124.2, 108.7, 106.5 (arom), 141.8 (*C*HCH), 117.8 (*C*HCO), 101.6 (O*C*H_2O), 79.7 (*C*CH), 71.9 (C*C*H) 29.6 (NH*C*H_2); ESI$^+$-HRMS (*m/z*): 230.0808 $[M + H]^+$ (calcd. for $C_{13}H_{12}NO_3$, 230.0812).

3.4. General Procedure of Fischer Esterification

(E)-Methyl 3-(benzo[d][1,3]dioxol-5-yl)acrylate (21). To a solution of compound **1** (2.0 g, 10.4 mmol) in dry methanol (25 mL) was added $H_2SO_{4(cc)}$ dropwise and stirred for some minutes. The mixture was heated with reflux for 12 h, cooled to room temperature, and concentrated under pressure. The crude material was diluted with EtOAc, and washed with aqueous $NaHCO_3$ and brine. The organic phase was dried over anhydrous Na_2SO_4, concentrated, and recrystallized from saturated hot Hex/EtOAc (1/4) to afford 7.37 mmol (70%) of compound **21** as white crystals; ^{1}H-NMR ($CDCl_3$, 300 MHz; δ, ppm): 7.58 (d, 1H, J = 16.3 Hz, benzene-C*H*), 7.01 (s, 1H, *Ha*), 6.99 (d, 1H, J = 8.0 Hz, *Hb*), 6.80 (d, 1H, J = 8.0 Hz, *Hc*), 6.26 (d, 1H, J = 16.3 Hz, C*H*CO), 5.99 (s, 2H, OCH_2O), 3.78 (s, 3H, CH_3); ^{13}C-NMR ($CDCl_3$, 75 MHz; δ, ppm): 167.7 (*C*ONH), 149.7, 148.5, 128.9, 124.5, 108.7, 106.6 (arom), 144.7 (*C*HCH), 115.8 (*C*HCO), 101.4 (O*C*H_2O), 51.7 (*C*H_3); ESI$^+$-HRMS (*m/z*): 208.0675 $[M + H]^+$ (calcd. for $C_{11}H_{11}O_4$, 208.0686).

3.5. General Procedure of Platinum-Mediated Heck Reaction

To a solution of compound **2** or **21** (100 mg, 1 eq.) dissolved in anhydrous DMF (3 mL) under nitrogen atmosphere were sequentially added $Pd(AcO)_2$ (catalytic amount), the appropriate aryl iodide (0.97 mmol, 2 eq.), tetrabutylammonium bromide (TBABr) (161.2 mg, 1 eq.), and $NaHCO_3$ (122.2 mg, 3 eq.). The reaction mixture was stirred at room temperature for 15 min under nitrogen atmosphere and then heated at 105 °C overnight (monitored by TLC). After cooling to room temperature, the reaction mixture was concentrated under reduced pressure and the crude product was dissolved in EtOAc and washed with distilled water in the presence of hexane (1/4 of the volume of EtOAc). The organic layer was washed sequentially with saturated $KHSO_4$ (10 mL), $NaHCO_3$ (10 mL), and brine (10 mL), dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure.

Benzo[d][1,3]dioxol-5-yl)-3-(4-fluorophenyl)-N-isobutylacrylamide (22). Compound **2** was used as reagent with 4-fluoro iodobenzene. The crude material was purified by column chromatography on silica gel using the eluting mixture hex/Et_2O/EtOAc (10:0:0 to 6:1:3), to afford 0.31 mmol (76%) of a mixture of stereoisomers *cis/trans* (3:1) of compound **22** as a white solid; ^{1}H-NMR ($CDCl_3$, 300 MHz; δ, ppm): 7.28 – 6.88 (7H, m, arom), 6.54 (1H, s, CC*H*), 6.08 (2H, s, OCH_2O), 4.22 (1H, s, N*H*), 3.02 (2H, m, CH_2), 2.08 (1H, m, C*H*), 0.87 (6H, d, CH_3 (2x)); ^{13}C-NMR ($CDCl_3$, 75 MHz; δ, ppm): 166.7 (*C*OO), 162.1, 148.5, 148.1, 138.7, 131.3, 129.6, 121.9, 121.1, 115.8, (arom), 152.2 (*C*CHCO) 108.3 (C*C*HCO), 101.2 (O*C*H_2O), 48,1 (CH_2), 29.2 (*C*H)

and 20.1 (CH_3); ESI$^+$-HRMS (*m/z*): 299.2999 $[M + H]^+$ (calcd. for $C_{17}H_{15}O_5$, 299.3001).

Benzo[d][1,3]dioxol-5-yl)-N-isobutyl-3-(4-methoxyphenyl)acrylamide (23). Compound **2** was used as reagent with 4-iodoanisol. The crude material was purified by column chromatography on silica gel using the eluting mixture hex/EtOAc (gradient: 5:1 to 1:1), to afford 0.24 mmol (68%) of a mixture of stereoisomers *cis/trans* (1:1) of compound **23** as a white solid; ^{1}H-NMR ($CDCl_3$, 300 MHz; δ, ppm): 7.19 – 6.73 (7H, m, arom), 6.25 (1H, s, CC*H*), 5.99 (2H, s, OCH_2O), 5.35 (1H, s, N*H*), 2.97 (2H, m, CH_2), 2.04 (1H, m, C*H*), 0.85 (6H, d, CH_3 (2x)); ^{13}C-NMR ($CDCl_3$, 75 MHz; δ, ppm): 164.2 (*C*OO), 160.1, 148.6, 148.0, 135.2, 131.3, 129.6, 121.1, 115.8, 108.5 (arom), 153.1 (*C*CHCO) 108.4 (C*C*HCO), 101.2 (OCH$_2$O), 55,6 (OCH$_3$), 47.9 (*C*H$_2$), 28.9 (*C*H) and 20.1 (*C*H$_3$); ESI$^+$-HRMS (*m/z*): 354.17 $[M + H]^+$ (calcd. for $C_{21}H_{24}O_4$, 354.17)

Benzo[d][1,3]dioxol-5-yl)-3-(4-hydroxyphenyl)-N-isobutylacrylamide (24). Compound ***2*** was used as reagent with 4-iodophenol. The crude material was purified by column chromatography on silica gel using the eluting mixture hex/Tol/EtOAc (5:1:1 to 5:1:4), to afford 0.28 mmol (70%) of a mixture of stereoisomers *cis/trans* of compound **24** as a white solid; ^{1}H-NMR ($CDCl_3$, 300 MHz; δ, ppm): 7.26 – 6.71 (7H, m, arom), 6.23 (1H, s, CC*H*), 5.97 (2H, s, OCH_2O), 5.48 (1H, s, N*H*), 2.98 (2H, m, CH_2), 1.58 (1H, m, C*H*), 0.78 (6H, d, CH_3 (2x); ^{13}C-NMR ($CDCl_3$, 75 MHz; δ, ppm): 166.7 (*C*OO), 157.0, 148.2, 134.1, 132.2, 131.3, 124.6, 121.1, 115.8, 109.8 (arom), 153.1 (*C*CHCO) 108.4 (C*C*HCO), 101.2 (OCH$_2$O), 55.6 (OCH$_3$), 47.9 (*C*H$_2$), 28.9 (*C*H) and 20.1 (*C*H$_3$); ESI$^+$-HRMS (*m/z*): 340.1541 $[M + H]^+$ (calcd. for $C_{20}H_{22}NO_4$, 340.1543)

4-Aminophenyl)-3-(benzo[d][1,3]dioxol-5-yl)-N-isobutylacrylamide (25). Compound **2** was used as reagent with 4-iodoaniline. The crude material was purified by column chromatography on silica gel using the eluting mixture hex/EtOAc (50:0 to 1:1), to afford 0.35 mmol (88%) of a mixture of stereoisomers *cis/trans* of compound **25** as a white solid; ^{1}H-NMR ($CDCl_3$, 300 MHz; δ, ppm): 7.08 – 6,59 (7H, m, arom), 6.20 (1H, s, CC*H*), 5.97 (2H, s, OCH_2O), 5.36 (1H, s, N*H*), 2.96 (2H, m, CH_2), 1.52 (1H, m, C*H*), 0.76 (6H, d, CH_3 (2x)); ^{13}C-NMR ($CDCl_3$, 75 MHz; δ, ppm): 166.7 (*C*OO), 149.1, 148.2, 134.2, 132.2, 131.3, 124.6, 121.1, 115.8, 109.8 (arom), 153.1 (*C*CHCO) 108.4 (C*C*HCO), 101.2 (OCH$_2$O), 55.6 (OCH$_3$), 47.9 (*C*H$_2$), 28.9 (*C*H) and 20.1 (*C*H$_3$); ESI$^+$-HRMS (*m/z*): 170.0887 $[M + H]^+$ (calcd. for $C_{20}H_{24}N_2O_3$, 170.0888)

Benzo[d][1,3]dioxol-5-yl)-2H-chromen-2-one (26). Compound **21** was used as reagent with 2-iodophenol. The crude material was purified by column chromatography on silica gel using the eluting mixture hexane /Et$_2$O/EtOAc (100:0:0 to 70:10:5) to afford 0.19 mmol (35%) of compound **26** as white solid; ^{1}H-NMR ($CDCl_3$, 300 MHz; δ, ppm): 7.57 – 7.21 (7H, m, arom), 6.34 (1H, s, CC*H*), 6.03 (2H, s, OCH_2O); ^{13}C-NMR ($CDCl_3$, 75 MHz; δ, ppm): 160.9 (*C*OO), 154.3, 149.1, 148.2, 132.0, 129.0, 127.1, 124.3, 122.8, 119.1, 117.5, 109.0, 108.9 (arom), 155.3 (*C*CHCO) 115.0 (C*C*HCO), 101.8 (OCH$_2$O); ESI$^+$-HRMS (*m/z*267.0658): $[M + H]^+$ (calcd. for $C_{16}H_{11}O_4$, 267.0652).

Methyl 3-(benzo[d][1,3]dioxol-5-yl)-3-(4-methoxyphenyl)acrylate (27). Compound **21** was used as reagent with 4-iodoanisol. The crude material was purified by column chromatography on silica gel using the eluting mixture hexane/Tol/EtOAc (50:0:0 to 40:5:5), to afford 142 mg (95%) of a mixture of stereoisomers *cis/trans* (3:1) **27** as white solid; ^{1}H-NMR ($CDCl_3$, 300 MHz; δ, ppm): 7.16 – 6.69 (7H, m, arom), 6.24 (1H, s, CC*H*), 6.00 (2H, s, OCH_2O), 3.86 (3H, s, OCH_3), 3,63 (3H, s, OCH_3); ^{13}C-NMR ($CDCl_3$, 75 MHz; δ, ppm): 163.9 (*C*OO), 158.3, 148.2, 148.1, 133.6, 129.6, 124.7, 121.9, 120.1, 116.5, 109.0, (arom), 153.1 (*C*CHCO) 111.3 (C*C*HCO), 101.4 (OCH$_2$O), 55, 2 (OCH$_3$), 52,9 (OCH$_3$); ESI$^+$-HRMS (*m/z*): 313.1083 $[M + H]^+$ (calcd. for $C_{18}H_{17}O_4$, 313.1071).

Methyl 3-(benzo[d][1,3]dioxol-5-yl)-3-(4-hydroxyphenyl)acrylate (28). Compound **21** was used as reagent with 4-iodophenol. The crude material was purified by column chromatography on silica gel using the eluting mixture hexane/EtOAc (50:0 to 1:1), to afford 0.36 mmol (75%) of a mixture of stereoisomers *cis/trans* (2:1) of compound **28** as white solid; ^{1}H-NMR ($CDCl_3$, 300 MHz; δ, ppm): 7.22 – 6,68 (7H, m, arom), 6.22 (1H, s, CC*H*), 5.98 (2H, s, OCH_2O), 5.17 (1H, s, O*H*), 3.64 (3H, s, OCH_3); ^{13}C-NMR ($CDCl_3$, 75 MHz; δ, ppm): 166.9 (*C*OO), 157.2, 147.8, 133.7, 132.7, 130.3, 123.3, 115.4, 114.7, 100.1 (arom), 147.8 (*C*CHCO) 108.1 (C*C*HCO), 101.3 (OCH$_2$O), 54.4 (OCH$_3$); ESI$^+$-HRMS (*m/z*): 299.2901 $[M + H]^+$ (calcd. for $C_{17}H_{17}O_4$, 299.2903).

Methyl 3-(4-aminophenyl)-3-(benzo[d][1,3]dioxol-5-yl)acrylate (29). Compound **21** was used as reagent with 4-iodoaniline. The crude material was purified by column chromatography on silica gel using the eluting mixture hexane/EtOAc (50:0 to 35:15), to afford 0.36 mmol (75%) of a mixture of stereoisomers *cis/trans* (2:1) of compound **29** as white solid; ^{1}H-NMR ($CDCl_3$, 300 MHz; δ, ppm): 7.26 – 6.58 (7H, m, arom), 6.21 (1H, s, CC*H*), 5.99 (2H, s, OCH_2O), 3.86 (2H, s, N*H*), 3.63 (3H, s, OCH_3); ^{13}C-NMR ($CDCl_3$, 75 MHz; δ, ppm): 166.5 (*C*OO), 148.7, 147.8, 133.5, 132.7, 128.9, 123.3, 121.3, 114.3, (arom), 152.8 (*C*CHCO) 108.3 (C*C*HCO), 101.3 (OCH$_2$O), 54.4 (OCH$_3$); ESI$^+$-HRMS (*m/z*): 298.1086 $[M + H]^+$ (calcd. for $C_{17}H_{16}NO_4$, 298.1074).

Methyl 3-(benzo[d][1,3]dioxol-5-yl)-3-(4-fluorophenyl)acrylate (30). Compound **21** was used as reagent with 4-fluoro iodobenzene. The crude material was purified by column chromatography on silica gel using the eluting mixture hexane/Et$_2$O/EtOAc (40:0:0 to 40:5:2), to afford 0.46 mmol (96%) of a mixture of stereoisomers *cis/trans* of compound **30** as white solid; ^{1}H-NMR ($CDCl_3$, 300 MHz; δ, ppm): 7.26 – 6.75 (7H, m, arom), 6.26 (1H, s, CC*H*), 5.99

(2H, s, OC*H*$_2$O), 3.61 (3H, s, OCH$_3$); ^{13}C-NMR ($CDCl_3$, 75 MHz; δ, ppm): 166.5 (*C*OO), 162.1, 148.0, 148.1, 139.0, 131.5, 129.8 137.3, 129.8, 121.9, 115.8 (arom), 130.2 (*C*CHCO) 108.6 (C*C*HCO), 101.2 (O*C*H$_2$O), 52.3 (O*C*H$_3$); ESI$^+$-HRMS (*m/z*): 301.0878 $[M + H]^+$ (calcd. for $C_{17}H_{14}FO_4$, 301.0871).

3.6. Cytotoxic Activity Evaluation on MCF7, U-87, HepG2 and H9c2 Cells

3.6.1. Cell Culture. The human primary glioblastoma cell line U-87 was grown in Eagle's minimal essential medium (EMEM) supplemented with 10% (v/v) fetal bovine serum (FBS) and 1 mM sodium pyruvate. The human breast cancer cell line MCF7 was grown in EMEM supplemented with 10% (v/v) FBS, 100 units/mL penicillin/streptomycin, 1 mM sodium pyruvate, 0.01 mg/mL insulin and 10 nM estradiol. The human perpetual cell line HepG2 was grown in EMEM supplemented with 10% (v/v) FBS, 100 units/mL penicillin/streptomycin and 1 mM sodium pyruvate. The rat cardiomyoblast cell line H9c2 was grown in Dulbecco's Modified Eagle's Medium (DMEM) high glucose supplemented with 10% (v/v) FBS and 100 units/mL penicillin/streptomycin. All cell lines were cultured at 37°C with 5% CO_2 and split when reached 70 – 80% confluences.

3.6.2. Cell Viability Assay. U87, MCF7, HepG2 and H9c2 cells were seeded in black wall clear bottom 96-well plates (treated with tissue culture) at a density of 5,000 cells/well (100 μL/cell). After 24 h incubation at 37°C in a 5% CO_2 incubator, the cell medium was removed and replaced with serum-free medium in the absence or presence of test compounds. The compounds were dissolved in DMSO and the final concentration of DMSO in the culture media was maintained below 1% (v/v). Cells were incubated another 48h at 37°C in 5% CO_2 and the cell viability was measured using the resazurin reduction assay [21]. The cell viability was expressed as percentage (%) of that in the control calculated using the ratio of the fluorescence intensity of treated sample to the vehicle control. The fluorescence was measured on an automated 96-well Fluoroskan Ascent FL plate reader (Labsystems) using an excitation wavelength of 530 nm and an emission wavelength of 590 nm. the cytotoxicity was expressed as the drug concentration inhibiting cell growth by 50% (IC_{50}), averaged for each experiment in triplicate. Results are presented as mean valued with standard deviation.

4. CONCLUSION

The synthesis of *trans*-fagaramide was achieved by Knoevenagel condensation using maleic acid and piperonal, followed by peptide coupling with isobutyl amine giving an overall yield of 56%. This synthetic platform has been used as starting material for the elaboration of a chemical library of 28 analogs of *trans*-fagaramide generated via Fischer esterification, palladium-mediated Heck couplings, and peptide coupling. Compounds **3 – 20** (18 compounds) were synthesized by peptide coupling at an average yield of 54%. Compound **21** was obtained by Fischer esterification with a yield of 70%. The synthesis of compounds **22 – 30** was afforded by palladium-mediated Heck coupling: compound **26** was obtained with 66% yield as a white solid, and the rest of compounds were obtained as mixtures of stereoisomers with an average yield of 80%. The synthesized compounds were elucidated using 1D spectroscopy (^{1}H-NMR and ^{13}C-NMR), 2D spectroscopy (COSY and HMQC), and mass spectrometry. Furthermore, all compounds were screened on cancer cells and in toxicity experiments against breast adenocarcinoma MCF7, hepatocellular carcinoma HepG2, glioblastoma U-87 as well as normal cells H9c2. Compounds **4** and **5** showed IC_{50} values above 50 ìM against H9c2 and MCF7. Likewise, compounds **25** and **26** exhibited inhibition activity at IC_{50} values above 50 ìM against MCF7 and H9c2, respectively. The rest of compounds showed no activity against these cell lines. The lack of activity in most compounds makes proper structure – activity assessment not possible.

AUTHOR CONTRIBUTIONS

M. Barrera did all the synthesis as a part of her Master Thesis and wrote the paper. T. C. Shiao contributed to the interpretation of spectra. P. T. Nguyen and S. Bourgault performed evaluation of the cytotoxic activity. R. Roy designed thematics and supervised all experiments presented in the paper.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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